PICO

2024

Celebrating 20 Years of the ER-C

EIGHTH CONFERENCE ON FRONTIERS OF ABERRATION CORRECTED ELECTRON MICROSCOPY

celebrating

20 years of the ER-C

Kasteel Vaalsbroek

April 21st – April 25th

2024

Conference Organizers

Rafal Dunin-Borkowski (Jülich)

Joachim Mayer (Aachen)

Carsten Sachse (Jülich)

Genevieve Wilbs (Jülich)

PICO 2024

Celebrating 20 Years of the ER-C

The organisers kindly acknowledge the support of PICO2024 from the following sponsors:

The cover displays a high-resolution micrograph of an interface between polar $LaVO_3$ (top) and nonpolar $SrTiO_3$ (bottom) recorded along the ‹110› zone axis of $SrTiO_3$ under NCSI conditions by Lei Jin. Upward shifts of every second oxygen column with respect to the adjacent vanadium columns (fourth row from the bottom) give evidence for an interface reconstruction. Local variations in the 90° elastic domain structures in $LaVO_3$ become evident when analysing the angular variation of -O-V-O-V-O- chains ranging from 164° on the left (verso) to about 180° on the right (front cover).

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Preface

PICO2024 – the eighth Conference on Frontiers of Aberration Corrected Electron Microscopy, celebrating 20 years of the ER-C – will be held in Kasteel Vaalsbroek from April 21st – April 25th, 2024. The event is hosted by the Ernst Ruska-Centre for Microscopy and Spectroscopy with Electrons (Forschungszentrum Jülich GmbH and RWTH Aachen University) and supported by FEI Electron Optics BV (Part of Thermo Fisher Scientific), JEOL (Germany) GmbH, AMETEK GmbH BU EMT (EDAX/Gatan), Hitachi High-Tech Europe GmbH, CEOS GmbH, DECTRIS AG, TESCAN GROUP a.s., Quantum Detectors Ltd., NanoMEGAS, condenZero AG, Bruker Nano GmbH, DENSsolutions B.V., Protochips Inc., CryoCloud B.V., CryoSol-World, Carl Zeiss Microscopy Deutschland GmbH, and Vitrotem B.V.

The conference has attracted ca. 250 participants from 27 countries throughout the world and the programme committee has put together an oral programme including 58 scientific lectures including four keynote speeches and three guest of honour sessions. Further contributions are scheduled for poster presentations.

The organisers hope that you will have a wonderful time at Kasteel Vaalsbroek and the chance to discuss with new collaborators and meet old friends.

Rafal Lukasz Borkowski

Joachim Mayer

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Kasteel Vaalsbroek

April 21st – April 25th

2024

PROGRAMME

PICO2024 Conference Program April 21st – 25th

Time	Sunday April 21st 2024	Time	Monday April 22nd 2024	Time	Tuesday April 23rd 2024	Time	Wednesday April 24th 2024	Time	Thursday April 25th 2024
		07:00	Breakfast	07:00	Breakfast	07:00	Breakfast	07:00	Breakfast
		08:30	Chris Russo	08:30	Gerald Kothleitner / Petra Wendler	08:30	David Smith / Olivia Pfeil-Gardiner	08:30	HOTEL CHECK-OUT
		09:05	Henning Stahlberg	09:05	Odile Stéphan / Irene Vercellino	09:05	Joanne Etheridge / Arne Möller	09:05	David Muller
		09:40	Jürgen Plitzko	09:40	Ondrej Krivanek / Taiana de Oliveira	09:40	Ute Kaiser / Gunnar Schröder	09:40	Sarah Haigh
		10:15	Coffee Break	10:15	Coffee Break	10:15	Coffee Break	10:15	Coffee Break
		10:45	Roger Wepf	10:45	Christoph Koch / Bettina Böttcher	10:45	Frances Ross / Kay Grünewald	10:45	Raynald Gauvin
		11:20	Max Haider	11:20	Jian-Min Zuo / Ardan Patwardhan	11:20	Eva Olsson / Niko Grigorieff	11:20	Johan Verbeeck
12:00	CONFERENCE REGISTRATION	11:55	**Flash Presentations**	11:55	Xiaoyan Zhong / Alexis Rohou	11:55	Thomas Kelly / Katharina Hipp	11:55	Vincenzo Grillo
		12:30	Lunch	12:30	Lunch	12:30	Lunch	12:30	Lunch
		13:30	**LAUDATIO** Sandra Van Aert	13:30	**LAUDATIO** Elena Orlova	13:30	**LAUDATIO** Nestor Zaluzec	13:30	Knut Müller-Caspary
		13:40	**GUEST OF HONOR** Dirk Van Dyck	13:40	**GUEST OF HONOR** Marin van Heel	13:40	**GUEST OF HONOR** Hamish Fraser	14:05	Marc Willinger
14:30	HOTEL CHECK-IN	14:15	Sandra Van Aert	14:15	Elena Orlova	14:15	Nestor Zaluzec	14:40	**Closing Remarks**
		14:50	Fu-Rong Chen	14:50	Holger Stark	14:50	Rajarshi Banerjee	15:00	**Campus Tour**
15:00	Welcome Reception	15:25	**Commercial Presentation** JEOL	15:25	**Commercial Presentation** TFS Life Science	15:25	**Commercial Presentation** TFS Material Science	15:00	DEPARTURE
16:00	**Opening Remarks**	15:40	Coffee Break	15:40	Coffee Break	15:40	Coffee Break		
16:15	Robert Sinclair	16:10	Christian Kisielowski	16:10	Bruno Klaholz	16:10	Michael Mills		
16:50	Yūichi Ikuhara	16:45	Axel Lubk	16:45	Timothy Grant	16:45	David McComb		
17:25	Peter Peters	17:20	Angus Kirkland	17:20	Jan Pieter Abrahams	17:20	Dipankar Banerjee		
18:00	Dierk Raabe	17:55	**Trade Pitches** CRYOSOL QUANTUM DETECTORS NANOMEGAS TESCAN	17:55	**Trade Pitches** PROTOCHIPS AMETEK VITROTEM CRYOCLOUD	17:55	**Trade Pitches** DECTRIS DENSSOLUTIONS CONDENZERO HITACHI		
18:35	Dinner Group 1	18:15	Dinner Group 1	18:15	Dinner Group 1	18:15	Wine Reception & Best Poster Award		
19:15	Dinner Group 2	19:15	Dinner Group 2	19:15	Dinner Group 2				
20:00	**Networking Event**	20:00	**Poster Session A/B/C/D**	20:00	**Poster Session E/F/G/H**	20:00	**Conference Dinner**		

SUNDAY APRIL 21ST, 2024

12:00 – 16:00	CONFERENCE REGISTRATION "Woonkamer" castle ground floor
14:30 – 23:00	HOTEL CHECK-IN hotel reception desk
15:00 – 16:00	WELCOME RECEPTION "Binnenplaats" in front of the castle

SESSION A: KEYNOTE PRESENTATIONS
"Morettipaviljoen" castle ground floor

16:00 – 16:15	**A0**	OPENING REMARKS Rafal Dunin-Borkowski, Forschungszentrum Jülich GmbH (Germany), Joachim Mayer, RWTH Aachen University (Germany), and Carsten Sachse, Forschungszentrum Jülich GmbH (Germany)
16:15 – 16:50	**A1**	APPLICATION OF ADVANCED TEM FOR MEDICAL STUDIES: EARLY CANCER DETECTION AND ALZHEIMER'S DISEASE Robert Sinclair, Stanford University (USA)
16:50 – 17:25	**A2**	ATOMIC-SCALE DYNAMIC OBSERVATIONS OF GRAIN BOUNDARY PHENOMENA IN OXIDES Yuichi Ikuhara, University of Tokyo (Japan)
17:25 – 18:00	**A3**	VITROJET: ICE THICKNESS CONTROL AND MEASUREMENT FOR TIME-EFFICIENT SINGLE PARTICLE STRUCTURE DETERMINATION Peter Peters, Maastricht University (NL)
18:00 – 18:35	**A4**	HYDROGEN-BASED IRON OXIDE REDUCTION FOR GREEN STEEL MAKING STUDIED BY ATOMPROBE TOMOGRAPHY AND ELECTRON MICROSCOPY Dierk Raabe, Max-Planck Institut für Eisenforschung (Germany)

18:35 – 19:15	DINNER GROUP 1 “Kruidentuin” restaurant
19:15 – 20:00	DINNER GROUP 2 “Kruidentuin” restaurant
20:00 – 22:00	NETWORKING EVENT “Morettipaviljoen” castle ground floor

MONDAY APRIL 22ND, 2024

07:00 – 08:30 BREAKFAST
"Kruidentuin" restaurant

SESSION B
"Morettipaviljoen" castle ground floor

08:30 – 09:05 **B1** MOLECULAR STRUCTURE DETERMINATION AT 100 KEV AND POTENTIAL IMPROVEMENTS IN CRYOEM IN SITU INCLUDING CHROMATIC ABERRATION CORRECTION AND LIQUID HELIUM COOLING

Christopher Russo, MRC Laboratory of Molecular Biology (UK)

09:05 – 09:40 **B2** LOW-DOSE CRYO-ELECTRON PTYCHOGRAPHY OF PROTEINS AT SUB-NANOMETER RESOLUTION

Henning Stahlberg, EPFL (Switzerland)

09:40 – 10:15 **B3** SERIAL-LIFT-OUT: THE PATH TO THE MOLECULAR ANATOMY OF WHOLE ORGANISMS BY CRYO-ET

Jürgen Plitzko, Max-Planck-Institute of Biochemistry (Germany)

10:15 – 10:45 COFFEE BREAK

10:45 – 11:20 **B4** ADVANCED TOOLS FOR THE PRESERVATION AND PREPARATION OF SENSITIVE, PRECIOUS, OR NATIVE SAMPLES

Roger Wepf, University of Queensland (Australia)

11:20 – 11:55 **B5** ONGOING INSTRUMENTAL DEVELOPMENTS FOR ANALYTICAL SCIENCES

Maximilian Haider, CEOS GmbH (Germany)

FLASH PRESENTATIONS
"Morettipaviljoen" castle ground floor

11:55 – 12:30 As another new highlight we introduce Flash Presentations. All Junior Scientists (students and postdocs during the first three years after receiving their PhD) were invited to apply for a slot in this new format where they can give a sneak peak to their poster contents. Our selection committee chose 7 contributions which will be introduced in 3 min oral presentations (no questions, max. 1 slide).

12:30 – 13:30 LUNCH
"Kruidentuin" restaurant

SESSION C: GUEST OF HONOR I – DIRK VAN DYCK
"Morettipaviljoen" castle ground floor

13:30 – 13:40 **C0** LAUDATIO ON DIRK VAN DYCK

Sandra Van Aert, University of Antwerpen (Belgium)

13:40 – 14:15 **C1** **3D ATOMIC RESOLUTION DYNAMICS FOR NANO-CRYSTALLINE AND MOIRE MATERIALS**

Dirk Van Dyck, University of Antwerpen (Belgium)

14:15 – 14:50 **C2** 3D ATOMIC STRUCTURES OF NANOPARTICLES ESTIMATED FROM SINGLE PROJECTION STEM DATA

Sandra Van Aert, University of Antwerpen (Belgium)

14:50 – 15:25 **C3** DESIGN OF AN ELECTRON MACH-ZEHNDER INTERFEROMETER FOR INTERACTION FREE ELECTRON MICROSCOPE

Fu-Rong Chen, University of Hong Kong (China)

15:25 – 15:40 **ST1** LATEST TECHNIQUES USING ABERRATION CORRECTORS

Shigeyuki Morishita, JEOL Ltd. (Japan)

15:40 – 16:10 COFFEE BREAK

16:10 – 16:45 **C4** SELF-COHERENCE AND INELASTIC SCATTERING

Christian Kisielowski, Lawrence Berkeley National Laboratory (USA)

16:45 – 17:20 **C5** INELASTIC HOLOGRAPHY AND COHERENCE

Axel Lubk, TU Dresden (Germany)

17:20 – 17:55 **C6** MAKING EVERY ELECTRON COUNT: STRATEGIES FOR ELECTRON PTYCHOGRAPHYAT LOW FLUENCE

Angus Kirkland, University of Oxford (UK)

17:55 – 18:00 **SP01** VITROJET: INDUSTRIALIZING THE CRYO-EM WORKFLOW

René Henderikx, CryoSol-World (NL)

18:00 – 18:05 **SP02** THE IMPORTANCE OF AN OPEN CAMERA SYSTEM DEMONSTRATED WITH WIDE-RANGING APPLICATIONS OF MERLINEM, HYBRID PIXEL DIRECT DETECTOR

Matúš Krajňák, Quantum Detectors Ltd. (UK)

18:05 – 18:10 **SP03** IN SITU DYNAMICAL STUDIES (LIQUID/GAS/OXIDATION/STRAINING & PHASE MAPS) USING 4D-SPED AND PIXELATED DETECTORS

Athanasios Galanis, NanoMEGAS SPRL (Belgium)

18:10 – 18:15 **SP04** ENHANCING AND ENABLING FAST AND INTUITIVE CHARACTERIZATION OF MATERIALS BY ADVANCED METHODS OF PRECESSION-ASSISTED ELECTRON DIFFRACTION

Dirk van der Wal, TESCAN GROUP (Czech Republic)

18:15 – 19:15 DINNER GROUP 1
"Kruidentuin" restaurant

19:15 – 20:00 DINNER GROUP 2
"Kruidentuin" restaurant

PLEASE NOTE THAT POSTER SESSIONS A / B / C / D WILL RUN IN PARALLEL

POSTER SESSION A

"Vaalsbroekerhof" castle ground floor

20:00 – 22:00 See Poster Program

POSTER SESSION B

"Jachtkelder" castle ground floor

20:00 – 22:00 See Poster Program

POSTER SESSION C

"Oude Keuken" castle ground floor

20:00 – 22:00 See Poster Program

POSTER SESSION D

"Tuinkamer" castle first floor

20:00 – 22:00 See Poster Program

TUESDAY APRIL 23RD, 2024

please note

- ✓ sessions DA & DB will run in parallel

07:00 – 08:30 BREAKFAST
"Kruidentuin" restaurant

SESSION DA
"Morettipaviljoen" castle ground floor

08:30 – 09:05 **DA1** EXPLORING CONTEMPORARY MATERIALS THROUGH MULTI-MODAL STEM TECHNIQUES

Gerald Kothleitner, TU Graz (Austria)

09:05 – 09:40 **DA2** RECENT ADVANCES IN STEM SPECTROSCOPY USING SYNCHRONIZED ELECTRON AND PHOTON BEAMS

Odile Stéphan, Université Paris-Saclay (France)

09:40 – 10:15 **DA3** ATOMIC-RESOLUTION SECONDARY ELECTRON IMAGING AND ULTRA-HIGH ENERGY RESOLUTION EELS

Ondrej Krivanek, Arizona State University (USA)

10:15 – 10:45 COFFEE BREAK

10:45 – 11:20 **DA4** FAST ELECTRONS OBSERVE ATOMS IN MOTION

Christoph Koch, Humboldt-Universität zu Berlin (Germany)

11:20 – 11:55 **DA5** THE GOLDEN AGE OF ELECTRON DIFFRACTION

Jian-Min Zuo, University of Illinois (USA)

11:55 – 12:30 **DA6** SPATIALLY-RESOLVED ELECTRON MAGNETIC CIRCULAR DICHROISM AND ITS APPLICATION ON INTERFACIAL MAGNETISM BY ACHROMATIC TRANSMISSION ELECTRON MICROSCOPE

Xiaoyan Zhong, City University of Hong Kong (China)

12:30 – 13:30 LUNCH
"Kruidentuin" restaurant

please note

- ✓ sessions DA & DB will run in parallel

SESSION DB
"Vaalsbroekerhof" castle ground floor

08:30 – 09:05	**DB1**	VISUALIZING ELECTRONS BY SINGLE PARTICLE CRYO-EM Petra Wendler, University of Potsdam (Germany)
09:05 – 09:40	**DB2**	SCAF1 DRIVES THE ASSEMBLY AND COMPOSITIONAL DIVERSITY OF MAMMALIAN RESPIRATORY SUPERCOMPLEXES Irene Vercellino, Forschungszentrum Jülich GmbH (Germany)
09:40 – 10:15	**DB3**	CRYO-EM AND SBDD AT ASTRAZENECA Taiana Maia de Oliveira, AstraZeneca R&D (UK)
10:15 – 10:45		COFFEE BREAK
10:45 – 11:20	**DB4**	LYMPHOSTATIN IN AN INACTIVE TRANSPORT FORM Bettina Böttcher, University of Würzburg, (Germany)
11:20 – 11:55	**DB5**	EMPIAR: THE PLEASURE AND PAIN OF BUILDING A BIG IMAGE ARCHIVE Ardan Patwardhan, European Bioinformatics Institute (UK)
11:55 – 12:30	**DB6**	TECHNOLOGY DEVELOPMENT IN THE PURSUIT OF CHALLENGING CRYOEM TARGETS: RIGID FABS AND SOFT LANDING OF HYDRATED SINGLE PARTICLES Alexis Rohou, Genetech Inc. (USA)
12:30 – 13:30		LUNCH "Kruidentuin" restaurant

SESSION E: GUEST OF HONOR II – MARIN VAN HEEL
"Morettipaviljoen" castle ground floor

13:30 – 13:40	**E0**	LAUDATIO ON MARIN VAN HEEL Elena Orlova, University of London (UK)
13:40 – 14:15	**E1**	**HOW CRYO-EM IS CHANGING THE LAWS OF PHYSICS** **Marin van Heel, Brazilian Centre for Research in Energy and Materials (Brazil)**
14:15 – 14:50	**E2**	STRUCTURAL STUDIES OF PHAGES IN MODERN ELECTRON MICROSCOPY Elena Orlova, University of London (UK)
14:50 – 15:25	**E3**	TBD Holger Stark, MPI for Multidisciplinary Sciences (Germany)
15:25 – 15:40	**ST2**	TOWARDS HIERARCHICAL MULTIMODAL CRYO-ELECTRON MICROSCOPY FOR CELL BIOLOGY Sagar Khavnekar, Thermo Fisher Scientific (Life Science) (NL)
15:40 – 16:10		COFFEE BREAK
16:10 – 16:45	**E4**	FROM 2D LOCAL CLASSIFICATIONS TO 3D FOCUSED CLASSIFICATIONS & REFINEMENTS: PUSHING RESOLUTION TO BELOW 2 Å AND UNDERSTANDING FUNCTIONAL STATES BY ADVANCED CRYO-EM Bruno Klaholz, Centre for Integrative Biology (France)
16:45 – 17:20	**E5**	NEW METHODOLOGIES FOR PREPARING AND IMAGING CRYO-EM SAMPLES Tim Grant, University of Wisconsin-Madison (USA)
17:20 – 17:55	**E6**	SINGLE MOLECULE ELECTRON DIFFRACTION Jan Pieter Abrahams, Basel University (Switzerland)

17:55 – 18:00	**SP05**	ADVANCES WITHIN IN SITU TEM WORKFLOWS FOR RENEWABLE ENERGY APPLICATIONS David Nackashi, Protochips Inc. (USA)
18:00 – 18:05	**SP06**	NEW APPLICATIONS OF DIRECT DETECTION, ELECTRON COUNTING CAMERAS FOR MATERIALS SCIENCE Saleh Gorji, Ametek GmbH, BU EMT (Gatan / EDAX) (Germany)
18:05 – 18:10	**SP07**	REVOLUTIONIZING SAMPLE PREPARATION: VITROTEM'S NAIAD SYSTEM FOR EFFICIENT GRAPHENE LIQUID CELL ASSEMBLY Sina Sadighikia, vitroTEM (NL)
18:10 – 18:15	**SP08**	CRYOCLOUD: A CLOUD-NATIVE CRYO-EM DATA ANALYSIS PLATFORM FOR INCREASED ACCESSIBILITY, THROUGHPUT AND COLLABORATION Robert Englmeier, CryoCloud (NL)
18:15 – 19:15		DINNER GROUP 1 “Kruidentuin” restaurant
19:15 – 20:00		DINNER GROUP 2 “Kruidentuin” restaurant

PLEASE NOTE THAT POSTER SESSIONS E / F / G / H WILL RUN IN PARALLEL

POSTER SESSION E

"Vaalsbroekerhof" castle ground floor

20:00 – 22:00 See Poster Program

POSTER SESSION F

"Jachtkelder" castle ground floor

20:00 – 22:00 See Poster Program

POSTER SESSION G

"Oude Keuken" castle ground floor

20:00 – 22:00 See Poster Program

POSTER SESSION H

"Tuinkamer" castle first floor

20:00 – 22:00 See Poster Program

WEDNESDAY APRIL 24TH, 2024

please note

- ✓ sessions FA & FB will run in parallel

07:00 – 08:30 BREAKFAST
"Kruidentuin" restaurant

SESSION FA
"Morettipaviljoen" castle ground floor

08:30 – 09:05 **FA1** RECENT DEVELOPMENTS IN ULTRAWIDE-BANDGAP SEMICONDUCTORS
David Smith, Arizona State University (USA)

09:05 – 09:40 **FA2** 4D-STEM IN REAL SPACE – MECHANISMS AND APPLICATIONS
Joanne Etheridge, Monash University (Australia)

09:40 – 10:15 **FA3** FINDINGS FROM THE HAPPY MARRIDGE BETWEEN LOW-VOLTAGE TEM AND LOW-DIMENSIONAL MATERIALS
Ute Kaiser, Ulm University (Germany)

10:15 – 10:45 COFFEE BREAK

10:45 – 11:20 **FA4** PROBING MATERIALS TRANSFORMATIONS BY TARGETING INDIVIDUAL ATOMIC COLUMNS
Frances Ross, Massachusetts Institute of Technology (USA)

11:20 – 11:55 **FA5** IN SITU ELECTRON MICROSCOPY OF 2D MATERIALS
Eva Olsson, Chalmers University of Technology (Sweden)

11:55 – 12:30 **FA6** TOWARD ATOMIC-SCALE ANALYTICAL TOMOGRAPHY
Thomas Kelly, Steam Instruments, Inc. (USA)

12:30 – 13:30 LUNCH
"Kruidentuin" restaurant

please note

- ✓ sessions FA & FB will run in parallel

SESSION FB
"Vaalsbroekerhof" castle ground floor

08:30 – 09:05	**FB1**	3D ELEMENTAL MAPPING IN SINGLE-PARTICLE RECONSTRUCTIONS BY EELS Olivia Pfeil-Gardiner, Max Planck Institute of Biophysics (Germany)
09:05 – 09:40	**FB2**	CRYO-EM OF ABC TRANSPORTER UNDER THE INFLUENCE OF NUCLEOTIDES, SUBSTRATES AND LIPIDS Arne Möller, University of Osnabrück (Germany)
09:40 – 10:15	**FB3**	CRYO-EM OF AB FIBRILS FROM MOUSE MODELS FIND TG-APPARCSWE FIBRILS RESEMBLE THOSE FOUND IN SPORADIC ALZHEIMER'S DISEASE PATIENTS Gunnar Schröder, Forschungszentrum Jülich GmbH (Germany)
10:15 – 10:45		COFFEE BREAK
10:45 – 11:20	**FB4**	TBD Kay Grünewald, Universität Hamburg (Germany)
11:20 – 11:55	**FB5**	FIB MILLING DAMAGE IN BIOLOGICAL SAMPLES Nikolaus Grigorieff, University of Massachusetts (USA)
11:55 – 12:30	**FB6**	STRUCTURAL ANALYSES OF BROWN ALGAE Katharina Hipp, Max Planck Institute for Biology Tübingen (Germany)
12:30 – 13:30		LUNCH "Kruidentuin" restaurant

SESSION G: GUEST OF HONOR III – HAMISH FRASER
"Morettipaviljoen" castle ground floor

13:30 – 13:40	**G0**	LAUDATIO ON HAMISH FRASER Nestor Zaluzec, University of Chicago (USA)
13:40 – 14:15	**G1**	**COUPLING PHYSICAL METALLURGY AND ELECTRON MICROSCOPY FOR 54 YEARS** **Hamish Fraser, Ohio State University (USA)**
14:15 – 14:50	**G2**	CHALLENGES AND STRATEGIES FOR MICROSCOPY AND MICROANALYSIS OF ENERGY AND QUANTUM MATERIALS IN MODERN ANALYTICAL TEM/STEM INSTRUMENTS Nestor Zaluzec, University of Chicago (USA)
14:50 – 15:25	**G3**	ARE OMEGA PRECIPITATES ALWAYS BAD FOR TITANIUM ALLOYS? Rajarshi Banerjee, University of North Texas (USA)
15:25 – 15:40	**ST3**	VISUALIZING CHEMISTRY AT THE ATOMIC SCALE! Maarten Wirix, Thermo Fisher Scientific, Materials & Structural Analysis (NL)
15:40 – 16:10		COFFEE BREAK
16:10 – 16:45	**G4**	ACCELERATED MATURATION OF MATERIALS BY COUPLING CHARACTERIZATION AND COMPUTATIONAL MODELING: HAMISH FRASER'S PIONEERING VISION Michael Mills, Ohio State University (USA)
16:45 – 17:20	**G5**	ELECTRON ENERGY-LOSS SPECTROSCOPY OF "HARD" MATERIALS David McComb, Ohio State University (USA)
17:20 – 17:55	**G6**	SOME APPLICATIONS OF ELECTRON MICROSCOPY TO SEMICONDUCTOR MATERIALS Dipankar Banerjee, Indian Institute of Science (India)

17:55 – 18:00 **SP09** FAST 4D STEM: HIGHLIGHTS OF THE ARINA HYBRID-PIXEL DETECTOR

Daniel Stroppa, DECTRIS Ltd. (Switzerland)

18:00 – 18:05 **SP10** A VERSATILE MULTI-CONTACT ENVIRONMENTAL HOLDER WITH A REMOVABLE TIP FOR OPERANDO TEM ACROSS MULTIPLE PLATFORMS

Robert Endert, DENSsolutions (NL)

18:05 – 18:10 **SP11** LIQUID HELIUM TEM SAMPLE HOLDER: SWIFT COOL-DOWN AND LONG HOLDING TIME

Denys Sutter, condenZero AG (Switzerland)

18:10 – 18:15 **SP12** IN-SITU STEM WITH SURFACE SENSITIVITY

Felix von Cube, Hitachi High-Tech Europe GmbH (Germany)

18:15 – 20:00 WINE RECEPTION
& BEST POSTER AWARD
“Binnenplaats” in front of the castle

20:00 – 22:00 CONFERENCE DINNER
castle – see seating plan

THURSDAY APRIL 25TH, 2024

07:00 – 08:30		BREAKFAST "Kruidentuin" restaurant
08:30 – 09:05		HOTEL CHECK-OUT hotel reception desk

SESSION H
"Morettipaviljoen" castle ground floor

09:05 – 09:40	**H1**	ELECTRON PTYCHOGRAPHY COMES OF AGE David Muller, Cornell University (USA)
09:40 – 10:15	**H2**	ADVANCING UNDERSTANDING OF NANOCATALYSTS WITH IN SITU STEM IMAGING Sarah Haigh, University of Manchester (UK)
10:15 – 10:45		COFFEE BREAK
10:45 – 11:20	**H3**	TOWARDS QUANTITATIVE MAPS OF LITHIUM IN THE ELECTRON MICROSCOPE Raynald Gauvin, McGill University (Canada)
11:20 – 11:55	**H4**	GENTLE INTERROGATION OF NANOSCALE OBJECTS Johan Verbeeck, University of Antwerpen (Belgium)
11:55 – 12:30	**H5**	"SPOOKY" QUANTUM AND CLASSICAL USES OF SHAPED ELECTRON BEAMS Vincenzo Grillo, Institute of Nanosciences CNR-S3 (Italy)
12:30 – 13:30		LUNCH "Kruidentuin" restaurant

13:30 – 14:05	**H6**	STRUCTURE RETRIEVAL BY PARAMETRIZED INVERSE MULTISLICE Knut Müller-Caspary, Ludwig-Maximilians-Universität München (Germany)
14:05 – 14:40	**H7**	DYNAMIC INTERPLAY BETWEEN METAL NANOPARTICLES AND OXIDE SUPPORT UNDER REDOX CONDITIONS Marc Willinger, Technical University Munich (Germany)
14:40 – 15:00		CLOSING REMARKS Rafal Dunin-Borkowski, Forschungszentrum Jülich GmbH (Germany), Joachim Mayer, RWTH Aachen University (Germany), and Carsten Sachse, Forschungszentrum Jülich GmbH (Germany)
15:00 – 18:00		CAMPUS TOUR Forschungszentrum Jülich
15:00		DEPARTURE

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Kasteel Vaalsbroek

April 21st – April 25th

2024

ABSTRACTS

APPLICATION OF ADVANCED TEM FOR MEDICAL STUDIES: EARLY CANCER DETECTION AND ALZHEIMER'S DISEASE

Robert Sinclair[1*], Yitian Zeng[1], Steven J. Madsen[1], Victor Warlop Piers de Raveschoot[1], Yi Cui[1], Sanjiv S. Gamhir[2], Philip S. DiGiaorno[3] and Michael M. Zeineh[2]

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While electron microscopy has long played a significant role in the understanding and treatment of human medical issues, there are few studies which have utilized the full power of modern transmission electron microscopes (TEM) to address important problems. This paper describes the application of an aberration-corrected, monochromated TEM-STEM for the use of nanotechnology in early cancer detection and for analyzing mineral deposits in the brain tissue of Alzheimer's Disease (AD) patients. Critical to this type of study is the collaboration between active medical researchers with their counterparts developing advanced TEM imaging and spectroscopic techniques.

For early cancer detection, our collaborators have developed a triple-modality gold nanoparticle structure [1] for surface enhanced Raman spectroscopy detection of small tumors, and have successfully designed an endoscopic device employed to utilize this approach [2]. As this procedure is centered on the influence of the gold nanoparticle surface plasmons to amplify a Raman signal by many orders of magnitude, we have used low loss STEM-EELS (electron energy loss spectroscopy) to correlate the plasmon energies with the Raman signal enhancement as a function of nanoparticle parameters such as shape, size, coating etc. [3]. This allows us to design superior nanoparticles for the cancer detection. Likewise, we have used monochromated STEM-EELS to determine the chemical state of iron oxide deposits in the brain tissue of advanced AD patients (i.e. ferrous versus ferric) as it has been speculated that the presence of ferrous iron species can contribute appreciably to the development of this disease [e.g. 4]. Of importance here is the availability of representative human tissue samples and the specimen preparation approach to achieve the advanced TEM characterization. Our belief is that the combination of the techniques commonly applied in physical science TEM studies can greatly benefit advances in medical science, under the right circumstances [5].

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ATOMIC-SCALE DYNAMIC OBSERVATIONS OF GRAIN BOUNDARY PHENOMENA IN OXIDES

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Materials properties of oxides depend on the dynamic behavior under various conditions such as temperature, stress, atmosphere. For example, mechanical properties of polycrystals are influenced by the interaction of grain boundary (GB), dislocation and crack propagation. On the other hand, oxides processing is often influenced by ion diffusion at high temperatures. So far, many atomic scale observations have been performed for understanding fracture, deformation, diffusion and so on, but these experiments were mostly carried out statically, and the dynamic behavior is still not well understood yet. In addition, these dynamic behaviors often occur at GBs. In this study, in-situ nanoindentation experiments were conducted for $SrTiO_3$ and Al_2O_3 bicrystals inside (S)TEM to understand their deformation and fracture process at GBs. For observing ion diffusion along GBs, electron irradiation technique was used to enhance GB migration in Al_2O_3. Ti-doped Al_2O_3 bicrystals were also used for the ex-situ experiments to investigate atomistic GB diffusion mechanism as a model system.

For observing dislocation-GB interaction, various types of GBs including CSL (Coincidence Site Lattice) GBs and low angle tilt and twist GBs for $SrTiO_3$ and Al_2O_3 were systematically prepared. Various phenomena such as dislocation pile-up at GBs, jog formation, jog-drag motion, deformation twinning were dynamically observed by these experiments. The dislocation-GB interaction and its dependence on the GB characters, the propagation mechanism of deformation twinning will be discussed in detail. In addition, the crack propagation behavior of Zr-doped Al_2O_3 GB was directly observed, and the as-fractured wall surfaces were characterized by Cs corrected STEM. It was found that the crack propagated in zigzag manner within the segregated Zr atom layers. The relationship of the atomic-scale crack propagation path and the grain boundary structure will be discussed in detail.

GB migration also plays an important role in considering the sintering behavior and the high temperature mechanical properties in oxides. But, it has been still unclear as to how the GB migration proceeds at atomic scale. Recently, we have found that GB migration behavior in Al_2O_3 can be precisely controlled by the aid of the high-energy electron beam irradiation. We applied this electron beam technique to directly visualize the atomistic GB migration as a stop motion movie. It was revealed that the GB migration is processed by a cooperative shuffling of atoms in GB ledges along specific routes. It is demonstrated that GB migration is facilitated by the GB structural transformations between low-energy GB structures.

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VITROJET: ICE THICKNESS CONTROL AND MEASUREMENT FOR TIME-EFFICIENT SINGLE PARTICLE STRUCTURE DETERMINATION

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Through many exciting developments, cryo-electron microscopy (EM) has emerged as a powerful technique to elucidate the 3D structure of macromolecular complexes. This knowledge provides valuable insight in understanding their functions and is the foundation for developing novel medicines. Sample preparation is an essential step in cryo-EM: for studying macromolecular in their native state, a specimen needs to be cooled so rapidly that ice crystals are unable to form and cause damage. Trapping the molecules in amorphous ice is called vitrification. Traditionally, vitrification was performed by manually plunging the sample on an EM-grid in liquid ethane. In the late 1990s the first automated plunge-vitrification instruments were developed. With the increasing demand on cryo-EM, sample preparation has become the major bottleneck that limits the true potential of cryo-EM. Though automated cryo-EM microscopes are common nowadays, plunge vitrification does not offer sufficient cooling capacity to cope with the added thermal mass of the sturdy ring-shaped in which EM-grids need to be mounted to enable the automated handling. Consequently, mounting the fragile EM-grid in the ring-shaped support needs to be performed manually under cryogenic conditions post vitrification, severely limiting throughput and further reducing reproducibility.

We developed the VitroJet, a fully automated sample preparation solution for cryo-EM in which after highly reproducible thin layer deposition of the sample onto the EM-grid, two simultaneous jets of liquid ethane are targeted onto the center of the EM-grid. This raises cooling capacity, and results in increased cooling rates that ensure successful vitrification of samples on EM-grids that are already mounted in the ring-shaped support. Consequently, EM-grids do not need to be post-mounted anymore, further improving reproducibility of the results. Targeting optimal ice thickness plays a crucial role to predictably achieve high resolution in cryo-EM. Up till now, thickness screening and selecting holes for data collection is done in the electron microscope, squandering valuable beam time. In the Vitrojet ice thickness can be controlled using the printing velocity and standoff distance from pin to grid. Both a theoretical model and experimental validation demonstrate that lowering velocity or distance results in thinner ice, where ice thickness is confirmed in the electron microscope by the energy filter method. Using the optical camera integrated in the VitroJet, we developed a method to determine thickness of buffer-suspended holes on an EM grid during sample preparation (Fig. 1). Dependent on the thickness, the error is below ± 20 nm in the range between 0 - 70 nm down to ± 10 nm in the thinnest ice regions (10 - 40 nm). Both ice thickness control and measurement allow users to optimize layer thickness on-the-fly during grid preparation, without the need of cryo-EM. Moreover, the ice thickness estimation can be used to target holes for data collection based on this optical image. Our test case demonstrates that when comparing 30 nm to 70 nm ice, 3.7 times less particles are required to reach a resolution of 3 Å (Fig. 2). This proves how these methods enable speeding up the entire workflow and using microscope infrastructure for data collection more efficiently. The automated approach enables users to efficiently optimize sample conditions and obtain new and exciting macromolecular structures.

HYDROGEN-BASED IRON OXIDE REDUCTION FOR GREEN STEEL MAKING STUDIED BY ATOMPROBE TOMOGRAPHY AND ELECTRON MICROSCOPY

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Steel has forged the backbone of modern civilization. While it enables sustainability via its high recyclability rates or when used in wind turbines or magnets, its primary production has strong negative impacts on the environment (1). This is because iron is extracted from its oxides through redox reactions that use fossil carbon carriers as reductants, producing about 2 tons of CO_2 per ton of steel produced. This qualifies steel production as the biggest single emitter of greenhouse gas, with about 8% of all CO_2 emissions on the planet (1,2).
Alternatives to fossil reductants are hydrogen, electrons and radicals in a plasma.
The lecture, therefore, deals with key mechanisms of sustainable hydrogen- and ammonia-based direct reduction and hydrogen-based plasma reduction of iron oxides into iron studied from a near-atomistic perspective using atom probe tomography and electron microscopy (3-5). We primarily present results from cryogenic-atom probe tomography to study the quasi in-situ evolution of iron oxide in the solid and gas phases of the direct reduction of iron oxide by deuterium gas at 700°C. We observed several atomic-scale characteristics, including, D_2 accumulation at the reaction interface; formation of a core (wüstite)-shell (iron) structure; inbound diffusion of D through the iron layer and partitioning of D among phases and defects; outbound diffusion of oxygen through the wüstite and/or through the iron to the next free available inner/outer surface; and the internal formation of heavy nano-water droplets at nano-pores.

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MOLECULAR STRUCTURE DETERMINATION AT 100 KEV AND POTENTIAL IMPROVEMENTS IN CRYOEM *IN SITU* INCLUDING CHROMATIC ABERRATION CORRECTION AND LIQUID HELIUM COOLING.

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High-resolution transmission electron cryomicroscopy (cryoEM) of biological structures is undergoing unprecedented growth. Advances in the last ten years were based on technical developments in specimen preparation techniques and supports, electron optics, microscope stability, source brightness, detector efficiency, and computer-based image processing and structure analysis. Together, these advances have underpinned exponential growth in the popularity of cryoEM and the expansion of cryoEM facilities around the world. I will describe some of the factors that have been important in driving growth in cryoEM, discuss new developments that could provide further improvements, such as lower specimen temperature [1], chromatic aberration correction [2] and phase plates [3], but also some others that might not be so useful.

Alongside the fascinating technical developments that increase the power of cryoEM, there exists a desperate need for more affordable, entry-level transmission electron cryo-microscopes. It seems likely that cryoEM in structural biology might head in two, increasingly separate directions: 1. lower energy, high-resolution microscopes for single particle analysis and 2. higher energy instruments for tomography and *in situ* imaging, with the possibility of economy and luxury models to cater for all. I will also discuss our latest work [4, 5] to identify the critical features for affordable, entry-level single-particle cryoEM, with examples of structures determined at minimal cost and effort.

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LOW-DOSE CRYO-ELECTRON PTYCHOGRAPHY OF PROTEINS AT SUB-NANOMETER RESOLUTION

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Cryo-transmission electron microscopy (cryo-EM) of frozen hydrated specimens is an efficient method for the structural analysis of purified biological molecules. However, for particles smaller than 50kDa, single particle cryo-EM often fails due to the limited imaging contrast of individual micrographs. For dose-resilient samples often studied in the physical sciences, electron ptychography – a coherent diffractive imaging technique using 4D scanning transmission electron microscopy (4D-STEM) – has recently demonstrated resolution down to tens of picometers for thin specimens imaged at room temperature.

Here we applied ptychographic data analysis to frozen hydrated single protein particles, reaching sub-nanometer resolution 3D reconstructions. We employed low-dose cryo-EM with an aberration-corrected, convergent electron beam to collect 4D-STEM data for our reconstructions. The high speed of the electron detector allowed us to record large datasets of electron diffraction patterns with substantial overlaps between the interaction volumes of adjacent scan position, from which the scattering potentials of the samples were iteratively reconstructed. The reconstructed micrographs show strong contrast enabling the reconstruction of the structure of apoferritin protein at up to 5.8 Å resolution. Ptychography is a promising tool to study the structure of smaller frozen hydrated protein particles, and, once combined with electron tomography tilt series, bears the potential to provide unique insight into the ultrastructure of vitrified biological tissue.

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SERIAL-LIFT-OUT: THE PATH TO THE MOLECULAR ANATOMY OF WHOLE ORGANISMS BY CRYO-ET

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Within less than a decade, focused ion beam (FIB) milling in combination with cryo-electron tomography (cryo-ET) has become routinely used in structural cell biology to achieve molecular resolution of cells or cell extracts. However, for larger entities such as smaller organisms like *Caenorhabditis elegans* or for tissue samples, the use of FIB technology, namely cryo-lift-out, has been limited and by and large has not been very practicable [1, 2].
A new key concept is "serial lift-out" that we have recently introduced [3]. This method, reminiscent of serial sectioning of embedded material at room temperature, modifies the lift-out process to expose the biology of frozen hydrated material by creating a series of lamellae, yet compared to classical serial sectioning now at molecular resolution. A series of lamellae are obtained in steps as small as 1 micrometer from a larger extracted volume (i.e. a block of ice 180x40x25 micrometers). In this way, multiple lamellae are produced with a single lift-out, which increases throughput and the imageable volume for tomography. However, in contrast to serial sectioning approaches, the loss of material in this process is much higher at around 80% (i.e. a 1 micrometer section is reduced to a 200 nm lamella). In previous lift-out processes, though, more than 99% of the starting material had to be removed. The game changer is that any area within an organism can be targeted, that lamellae can be obtained from different angles or orientations and finally larger and numerous lamellae can be generated in a timely manner, containing more of the underlying biology. Provided, however, that the sample has been adequately vitrified.
With further progress in this methodology and technology, one can imagine that cell and even developmental biologists will also adopt our somewhat extravagant approach. The latter, however, only once all remaining technical hurdles have been overcome.
In this lecture we will not only present and demonstrate serial lift-out, but also take the practitioner's perspective and discuss the current limitations, stumbling blocks and the strategy we are pursuing to overcome at least some of the influencing factors. Factors that include a reproducible process for vitrification (i.e., high-pressure freezing – HPF or “waffle” approaches [4]), simple, fully automated or machine learning routines for targeting (i.e. cryo-CLEM) and FIB milling (i.e. lift-out or going even “lift-less”) and finally methods that could at best annotate and mine the tomographic data in an unsupervised and efficient manner. Although there are now novel instruments (e.g. plasma FIBs) and some alternative or adapted methods that allow insight into the molecular anatomy, it is clear that more development work is required to make access to samples at this scale common practice.

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ADVANCED TOOLS FOR THE PRESERVATION AND PREPARATION OF SENSITIVE, PRECIOUS, OR NATIVE SAMPLES

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From the dawn of electron microscopy (EM), pioneers like Bodo von Borries and Ernst Ruska were warned by their senior colleagues, primarily physicists, about the potential damage electrons could inflict on samples and modification electrons may cause to the their native state of samples. Despite these warnings, they persevered in their quest to construct the first electron microscope using pico-meter waves. Their determination has led us to the advanced analytical capabilities currently available today and why we can meet today. Despite the availability of modern multimillion-dollar analytical tools, researchers often need extra help preserving and preparing samples. This has become an "artwork" and a dedicated skillset of highly skilled "craftsmen/women" hardly recognized for their skills outside our facilities. These sample challenges can stem from the sample's sensitivity to our environment, its specific state, or the energy impact during preparation and analysis. Currently, no universal toolset can protect the native state of a sample and prepare it with minimal artefacts.

Over the past five years, the Centre for Microscopy and Microanalysis (CMM) has successfully refurbished four sites, won grants, and installed various new instruments in different research fields. This has led to a multi-user/multimodal operation across five different sites with more than 55'000h analytical beam time, from 720-750 users supporting 270-300 publications per year. However, we have observed that many client demands could not be met with the existing infrastructure due to the lack of the most advanced instrumentation or because some sample handling and preparation options were unavailable from instrument providers.

In response to these challenges, we have established an R&D team and local workshop resources to develop new tools, processes, and workflows. Over the last five years, we have initiated several projects with industry partners, including: *Cryo UHV and HV transfer (MicroSol), Cryo-XPS and cryo-SIMS with a cryo loading dock (MicroSol), Cryo CLEM – ENZEL (Consortia with DELMIC), Cryo micro-ED upscaling old TEM's, Triple beam FIB/SEM NX5000 (Hitachi), Inert-gas transfer Glovebox docks, electro-chemical liquid holder for HF5000 and HT7700 (Hitachi), TEM side entry Autoloader (NewPro, Hitachi and JEOL), high-throughput Nano-Crystallisation under Oil and Nano Crystal Growth using Deoptimisation, Cryo-sectioning for Maldi Imaging MS (TimsTofPro L2 – Bruker), Autoloader for DESI MRT Imaging MS (Waters), Improved lateral resolution on Maldi and Desi Imaging MS equipment, along two new capabilities and sites.*

After establishing a vibrant environment at CMM, the focus has moved towards consolidating and optimizing these modalities and laboratory sites, with an emphasis on inter-instrumental connectivity and high-quality sample preparation for a wide range of research collaborations. This requires careful consideration of "common/standard" sample carriers that allow for automated sample exchange between inter-linkable tools. This presentation will provide examples illustrating why these sample preparation and handling tools are essential. We will also discuss the requirements for an interchangeable sample carrier that allows for correlative measurements, "protective" sample manipulation and automated sample handling and a call for collaboration and resources to help realising a sample preparation/handling tool box matching the investment efforts of the "primary" tools.

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ONGOING INSTRUMENTAL DEVELOPMENTS FOR ANALYTICAL SCIENCES

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EELS acquisitions and elemental maps are taking a major part of the applications of advanced electron microscopes and, therefore, the overall performance of the equipment needed for these applications plays an important role for the usability of these instruments. For this purpose we developed over the years a high resolution energy filter and spectrometer (CEFID) and currently we are in the process of developing a novel ground potential monochromator (GP-MC). One of the figures of merit of spectrometers in combination with a monochromator, for example, is the attainable energy resolution and the available beam current. Which, of course, also depends on the brightness of the electron source.

From the electron optical point of view the design of the spectrometer as well as the monochromator are important for their flexibility and the attainable energy resolution. The overall stability of the whole acquisition system mainly depends on the mechanical design and the set-up of the power supplies. In contrast to the high stability, also fast electronics is needed in order to allow a fast switching of various ranges of the EELS spectrum. All involved components, from the high tension tank down to the recording system, are important and each one is contributing to the signal-to-noise ratio one will finally observe in the acquired data.

An additional important tool is the available software to align and to control the various modules of the system. Software routines are also needed to acquire and to inspect the data on the flight. For elemental mapping, the recorded data can be optimized in order to enhance the element specific signal by such routines. Furthermore, with the implementation of available external data like the generalized oscillator strength important properties of the object under investigation can be extracted more robustly.

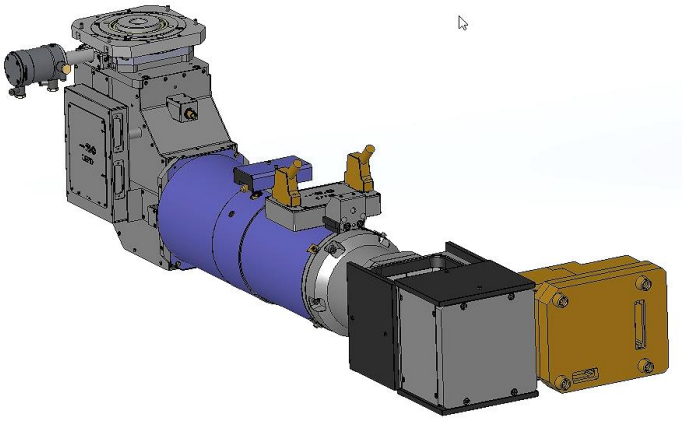

Fig. 1: Drawing of the CEOS ENERGY FILTER IMAGING DEVICE

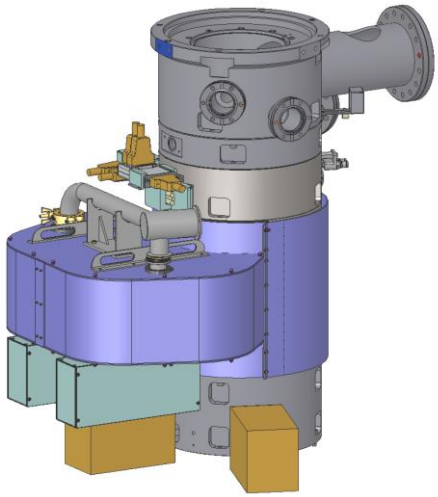

Fig. 2: Sketch of the novel GP-MC

Acknowledgments

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3D ATOMIC RESOLUTION DYNAMICS FOR NANO-CRYSTALLINE AND MOIRE MATERIALS

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It is well understood that material properties are encoded in their three-dimensional arrangements and dynamics under external stimuli. The present 3D reconstruction methods based on broad-beam are usually based on the de-coding of an in-line hologram containing well-resolved projected atom columns. With the broad-beam mode, the focal series images can be recorded with electron dose rate about one order magnitude lower, since the reconstructed exit wave function can gain back the signal from contribution of all through focal images. For a crystalline material, the 3D atomic information of was determined from the maximum propagation intensity of the atomic column wave relative to the vacuum wave and/or refined by the big-bang method.[1, 2, 3] The dynamics is obtained from tracking the 3D atom positions deduced from time-resolved exit wave functions reconstructed from sub-sets of defocus series of images[3].

For helix materials, such as carbon nanotube (CNT) and moire materials, such as twist graphene, isolated atomic columns can not be well-resolved due to the fact that most of the projected spacings between atoms are beyond the resolution of the TEM, instead that the moiré pattern formed by the rolled-up graphene layer is clearly visible between two intense bright lines. Each bright spot in the images may be associated with a cluster of atoms in the projection view. Here, we introduce a genetic evolution 3D method, simulation annealing (SA)-energy minimization (EM), to quantitatively describe single-atom dynamics at 3D in helix and moire materials composed of not well defined atom columns. Basically, in this 3D method, the atoms are moved randomly through a large search space of (x, y, z) coördinates under constraint of the bonding energy to bring simulated image close to the experimental one. The modification of atom position will be accepted based on the maximum likelihood criterion. The detail algorithm of ASA-EM will be presented in the meeting. Example of a reconstructed 3D CNT structure is given in Figure 1.

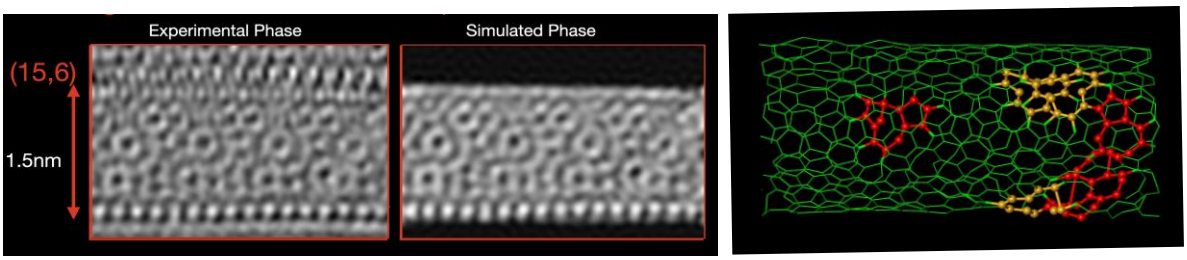

Fig. 1 (a) experimental phase (b) simulated phase from (c) reconstructed 3D structure using SA-EM

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3D ATOMIC STRUCTURES OF NANOPARTICLES ESTIMATED FROM SINGLE PROJECTION STEM DATA

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The properties of nanomaterials are highly dependent on their 3D structure and composition. Consequently, a thorough quantification by aberration corrected TEM is of great importance. In quantitative ADF STEM, images are treated as datasets from which structure parameters are determined by comparison with image simulations, by using parameter estimation-based methods, and more recently by applying deep learning. A quantitative interpretation of atomic resolution ADF STEM images enables one to determine the positions of the atomic columns, the atomic column composition, and the number of atoms in an atomic column.

For homogeneous nanocrystals, the number of atoms can be counted from ADF STEM images using so-called scattering cross-sections (SCS) which correspond to the total intensity of electrons scattered by a single atomic column [1]. However, for mixed columns, all types of elements will contribute differently to the image intensities thus significantly complicating a quantitative interpretation. Progress has been made to extend atom counting from homogeneous to heterogeneous materials by combining ADF STEM with EDX [2]. In this framework, an iterative weighted least squares minimization algorithm is applied to count the number of atoms of each element by matching experimental STEM and EDX SCSs to simulated values. Although the method can be used to unscramble elements even when the difference in atomic number is only one, EDX measurements may not always be the most viable option for beam-sensitive materials due to the substantial electron dose needed to achieve a sufficiently high signal-to-noise ratio in the EDX elemental maps. As an alternative, multimode atomic resolution ADF STEM is investigated, involving the simultaneous analysis of multiple ADF STEM images [3,4]. Moreover, the information-richness and dose efficiency of novel 4D STEM techniques will be explored, alongside evaluating their quantitative abilities.

Atom counts can be used to create an initial atomic model which serves as an input for energy minimization to obtain a relaxed 3D reconstruction. In order to avoid that purely computational energy minimization approaches result in a close local minimum where the reconstructed structure may deviate from the experimental observation, a Bayesian genetic algorithm has been developed incorporating a priori information concerning the finite atom-counting precision and neighbor-mass relations [5]. The method enables reliable 3D reconstructions of beam-sensitive nanoparticles during dynamical processes from images acquired with sufficiently low incident electron doses. Furthermore, quantitative measurements of the coordination numbers of the surface atoms of catalytic nanoparticles at high temperatures and in gaseous environments [5,6], can be provided [7].

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DESIGN OF AN ELECTRON MACH-ZEHNDER INTERFEROMETER FOR INTERACTION FREE ELECTRON MICROSCOPE

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As proposed[1], the key components of an interaction-free electron microscope are a barn door and multiple-interrogation Mach-Zenhder which performs beam splitting and coupling. The concept of interaction-free measurement (IFM) stems from a quantum bomb tester proposed by Elitzur and Vaidman utilizing Mach-Zehnder interferometer,[2]. A Mach-Zenhder interferometer is composed of two beam splitters (BS1 and BS2) and two mirrors (M1 and M2). The first beam splitter splits the incident beam into two branches termed sample beam Ψ_S and reference beam Ψ_r (Fig. 1a). However, the second beam splitter BS2 also plays a role as a beam coupler that couples partially split beams in both sample and reference beam arms to interference each other. For a single Mach-Zehnder interferometer, with an ideal absorber in the sample beam path the quantum efficiency of "no interaction" is 25%[2] (Fig. 1b). To increase the quantum efficiency of "no interaction" to a level close to 100%, a multi-interrogation interferometer called Quantum Zeno Resonator (QZR) is proposed in the optics. IFMs have been demonstrated experimentally with photons, neutrons, and neutral atoms and recently a flexible two-grating electron interferometer is constructed in a conventional transmission electron microscope to demonstrate electron interaction-free measurements with an efficiency of 14±1% for single interrogation[3].

A perfect Mach-Zehnder interferometer does two actions: beam splitting and coupling at entrance and exit of the interferometer. Here, we report a double-well pseudo potential using the high frequency oscilating quadrupole field resemble to a hybrid dipole and hexapole field as a beam splitter/ coupler for a 15keV electron microscope. An example of the electron beam ray trajectory is shown in figure 1.

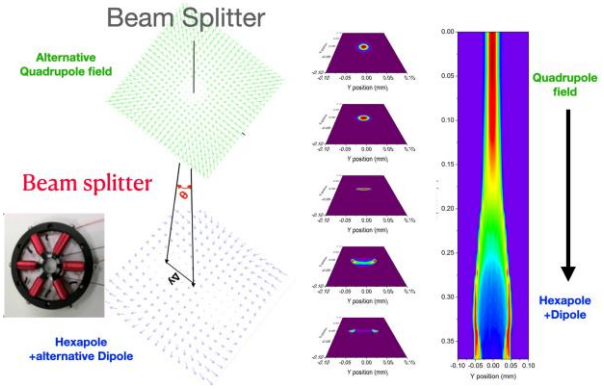

Fig. 1 a ray trajectory calculation for a dipole/ hexapole beam splitter

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LATEST TECHNIQUES USING ABERRATION CORRECTORS

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Atomic-resolution observations have been routine works due to spherical aberration correctors [1]. By correcting higher-order geometrical aberrations as well as spherical aberration, the highest resolution has been improved [2] and atomic-resolution observation became possible even at low acceleration voltages [3]. Besides the improvement of the conventional STEM/TEM resolution, the spherical aberration correctors have contributed to dedicated optical systems. The latest techniques using aberration correctors will be presented.

The first technique is the correction of large spherical aberration, which is useful for observation in a magnetic field free environment. Conventional objective lenses have spherical aberration coefficients (C_s) of less than several mm, but spherical aberration correctors can correct even larger spherical aberration. When an objective lens is turned off to suppress the magnetic field on a specimen, another lens closest to the specimen is generally used as an objective lens though the C_s of such a lens is very large, about several meters. By correcting this C_s, the aberration correction range was extended and an information limit of 1 nm or less was obtained. Also, in the case of an objective lens developed for magnetic-field-free atomic-resolution observation, the focal length and chromatic aberration coefficient are as small as a few mm, but the spherical aberration coefficient is as large as several tens of mm. By correcting this C_s as well, a resolution of less than 1 Å was achieved under a magnetic field free environment [4].

The second is dedicated optical systems that uses the corrector for purposes other than resolution improvement in conventional modes. The hexapole corrector consists of multipoles and transfer lenses. By turning off the aberration correction function, the lenses of the correctors can be used for other purposes. This enables a parallel beam scanning mode, which is useful for momentum-resolved EELS [5]. In another case, by using the triple hexapole corrector as the double hexapole corrector, a tilt-scan averaged DPC STEM is also available, which is important for reducing diffraction contrast in DPC images [6].

We also developed an aberration measurement technique using convolutional neural network for automating the alignment of the corrector [7], and investigated designs to correct higher-order geometrical aberrations [8]. The development of technologies related to aberration correctors is expected to open up further possibilities for microscopic observation.

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SELF-COHERENCE AND INELASTIC SCATTERING

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The characterization of nano-materials at atomic resolution by aberration-corrected electron microscopy has reached the point where, arguably, all atoms of the Periodic Table of Elements can be spectroscopically identified and localized in 3D. For decades, Dirk van Dyck helped guiding the development and applications of such tools by establishing suitable theories.[1,2] Specifically, work at the National Center for Electron Microscopy in Berkeley, USA, benefitted from procedures to reconstruct electron exit wave functions [3,4], the development of a S-state model for electron channeling [5], and suggestions that electron scattering should be inelastic [6]. They contributed to the development of discrete tomography [7] and a fully quantitative description of atomic structures in 3D including electron beam-induced atom dynamics [8]. Looking forward, new principles and experiments are explored by embracing the time domain [9] and by considering how beam-sample interactions relate to coherence in the Heisenberg Limit [10]. Targeted experiments including diffraction [11] and energy filtered transmission electron microscopy (EFTEM) [10] suggest that Coulomb scattering is always coherent-inelastic and causes energy-dependent self-interferences. They create wave packages that obey the time-dependent Schrödinger equation. Due to a reciprocal dependence on energy losses, their width - characterized by a self-coherence length or self-coherence time - shrinks to reach typical lattice constant values at energy losses of a few 100 eV. In these circumstances it is experimentally shown that the ability gets lost to coherently illuminate crystal unit cells. For electron energy losses beyond 1000 eV the wave package width's reach atomic dimensions so that they appear particle-like. Consequently, atomic resolution observations inevitably include pulse-like wave propagations that stimulate structural dynamics at any electron energy loss, which allows predicting the critical accumulated doses of radiation hard and soft matter equally well, for example.[11] The emerging picture is consistent with results from inelastic electron holography and the measured "delocalization" of wave functions. Further, the principle can be reasonably expanded to include other interactions.**

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INELASTIC HOLOGRAPHY AND COHERENCE

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Predominantly, holography measures phase shifting effects occurring under elastic interaction. By energy transfer to other degrees of freedom, inelastic scattering ejects some electrons from their primary wave, i.e., the elastic wave, where their loss dampens the amplitude. Of course, the inelastic electrons are still in the beam, but now they travel in a wave newborn by the inelastic process. The question arises, whether and how coherence properties change in this process, i.e., whether these electrons contribute to a detected image or an EELS-spectrum at reduced degree of coherence depending on the structure of the inelastic degrees of freedom. For understanding, most decisive are the coherence properties both inside the newborn waves and with respect to other newborn waves and the pristine elastic one, for the whole manifold of excitations occurring. Here we discuss the prominent limiting cases of interaction with a coherent state and with a two-level system. With primary electrons considered as plane waves, [1] shows more details and examples. However, treating an electron as a Gaussian wave packet in real space, it loses the property of sharply defined energy; instead, it represents a coherent energy spectrum, the broader the narrower in real space. Calculations show that, after inelastic interaction, coherence is preserved the better, the smaller the energy transfer remains within the Gaussian energy spectrum.

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MAKING EVERY ELECTRON COUNT: STRATEGIES FOR ELECTRON PTYCHOGRAPHYAT LOW FLUENCE

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Acquisition of a ptychographic dataset typically requires the collection of a series of far field diffraction patterns as a function of probe position at the specimen plane. This can then be used to recover the complex specimen object function using either iterative or non-iterative algorithms. Importantly, ptychography is an inherently dose efficient technique, enabling effective the reconstruction of the exit wavefunction of radiation sensitive objects.

For applications in the life sciences cryo-electron ptychography holds much promise particularly when used with a defocused probe to scan across a specimen with highly overlapped probe positions. This can be applied in a variant of conventional single particle analysis to provide 3D structures taking advantage of the known resolution variation of the effective ptychographic transfer function with convergence angle to provide wide spatial frequency bandwidth transfer. This geometry also allows datasets from wide fields of view to be collected that are suitable for studies of biologically relevant structures in a low concentration cellular context.

Ultimately the resolution of reconstructions of radiation sensitive samples is limited by radiation damage which inherently scales with electron fluence and in general most Ptychographic datasets have extremely low signal to noise. Methods to overcome this will be discussed including sparse scan geometries optimised on the basis of diffusion equations and the use of neural networks for processing of the raw input data. For the latter accurate centring of the bright field disk, data denoising and deconvolution of the detector MTF provides a typical 3-4 X enhancement of the SNR.

Finally, regularised and Fourier Ptychography as alternative data acquisition and processing strategies will be discussed.

VITROJET: INDUSTRIALIZING THE CRYO-EM WORKFLOW

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Single particle cryo-electron microscopy (cryo-EM) has become a widely adopted method in structural biology over the past decade, since it eliminates the need for crystallization. This technique enables visualization of individual particles making it suitable in a wide variety of projects, including ones that exhibit conformational heterogeneity. The resolution revolution was a leap forward that resulted in the ability to resolve structures down to atomic resolution, increasing the demand on samples and personnel.

CryoSol-World developed the VitroJet (Fig. 1), moving cryo-EM sample preparation into the new era of high quality and high-throughput. Patented technologies include sample deposition by pin-printing and jet-vitrification, providing a reproducible basis to obtain high quality grids. The system is fully automated to enhance throughput and enable unattended sample preparation for a wide variety of samples. Operation through an intuitive user interface and the ability to use pre-clipped autogrids makes it suitable for novice practitioners (Fig. 2). The flexibility to use any grid type and minimum sample volume is essential to guarantee suitability for many projects at this moment and in the future, unlocking the vitrification revolution.

With the optical ice thickness measurement integrated in the VitroJet, samples can already be screened before entering the microscope. The ability to target grids, squares and even holes based on the optical image makes the grids ready-to-collect. This decreases microscope time on atlas making, low and high magnification screening, using beam time much more time efficient.

Fig. 1: VitroJet system for automated cryo-EM sample preparation

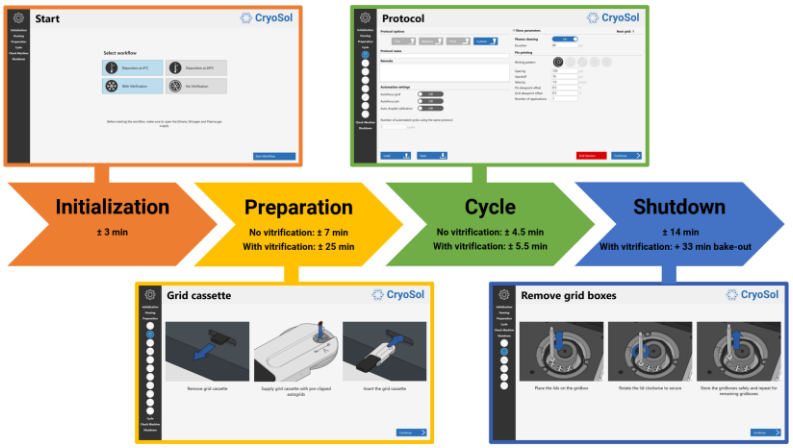

Fig. 2: VitroJet workflow to automatically prepare high quality samples for high-throughput cryo-EM.

THE IMPORTANCE OF AN OPEN CAMERA SYSTEM DEMONSTRATED WITH WIDE-RANGING APPLICATIONS OF MERLINEM, HYBRID PIXEL DIRECT DETECTOR

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The MerlinEM has gained widespread acceptance in the transmission electron microscopy (TEM) community due to its versatility and adaptable integration with various systems. Quantum Detectors enhanced the system, focusing on scanning transmission electron microscopy (STEM) providing live 4D-STEM analysis (Fig. 1). Recently, Quantum Detectors also redesigned the retractable version of the MerlinEM platform, MerlinEM RDP, which now fits more microscopes and is shaping the future generation of hybrid pixel detectors (Fig. 2). In partnership with Amsterdam Scientific Instruments, Quantum Detectors now provides hybrid pixel detectors using Timepix3 technology. This consolidation brings together both Medipix3 and Timepix3 CERN technologies, offering frame-based and event-based electron counting detectors from a single source.

Open data formats and remote operation capabilities are at the core of these offerings. These are essential in exploring new applications and improving established techniques, such as imaging electromagnetic fields [1] and ptychographic imaging [2]. Rapid data streaming to third-party software has been utilised for live ptychography [3] and integrated centre of mass imaging [4], while collaborations with commercial partners like NanoMegas (scanning precession electron diffraction toolkit), CEOS and Université Paris-Saclay (spectrometer systems) enhanced capabilities of TEM instrumentation.

The openness of the detectors has fostered community collaboration, exemplified by projects like LiberTEM [6], pyXem [7] and others. These initiatives facilitate sharing data processing routines, accelerating the development of innovative methods for extracting sample information, and addressing a wider range of specimens more efficiently.

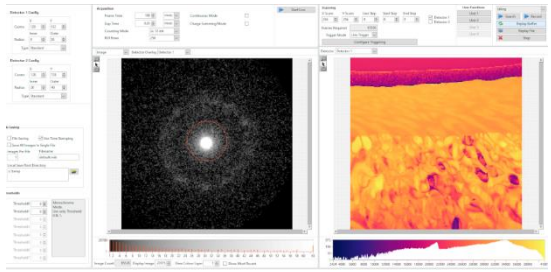

Fig. 1: 4D-STEM interface for MerlinEM

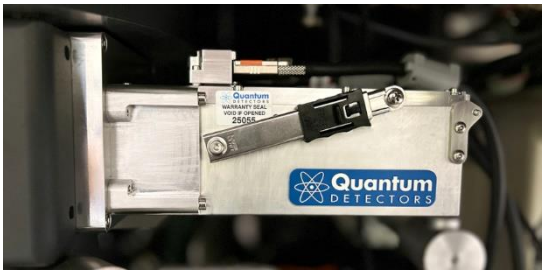

Fig. 2: The new MerlinEM RDP

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IN SITU DYNAMICAL STUDIES (LIQUID/GAS/OXIDATION/STRAINING & PHASE MAPS) USING 4D-SPED AND PIXELATED DETECTORS

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Automatic crystal orientation & phase mapping (ASTAR technique) can be applied in a variety of materials [1, 2] applying 4D-SPED "scanning Precession Electron Diffraction" where beam precession (to improve ED patterns quality [3]) in TEM is used in synchronization with beam scanning. ASTAR technique has significant spatial resolution down to 1-3 nm (in case of FEG-TEM). Local crystallographic orientation/phases are identified through an original algorithm that compares experimental ED spot patterns with pre-calculated (simulated) templates for all possible orientations and/or phases [4].

In addition, *in situ* methods have been extensively applied towards dynamical studies. Combination of 4D-SPED ASTAR with the new generation of pixelated detectors, allows the use of extremely low electron dose with significantly fast acquisition times (500-1000fps). Combining pixelated detectors (like QD & ASI Medipix, Dectris, etc) with NanoMEGAS 4D-SPED ASTAR system, has allowed in situ dynamical studies.
As an example , we have obtained orientation /texture maps on Au colloidal nanoparticles in water by monitoring local orientation changes with time. On the other hand , using a gas-cell holder we have studied Zr metal oxidation mechanism by monitoring local orientation/phase changes using ASTAR and strain mapping. The latter implies that we can actually perform corrosion studies in situ for a wide range of materials .

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ENHANCING AND ENABLING FAST AND INTUITIVE CHARACTERIZATION OF MATERIALS BY ADVANCED METHODS OF PRECESSION-ASSISTED ELECTRON DIFFRACTION

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Structural analysis of materials and nanoscale devices is dramatically changing with the introduction of the new approach to advanced electron diffraction techniques by using the new TESCAN TENSOR microscope. Performance and usability of analytical 4D-STEM techniques is not anymore compromised by the legacy design of conventional TEM microscopes that were not intrinsically designed for analytical STEM applications. The new STEM-dedicated design integrates state-of-the-art components such as a large direct electron detector with hybrid pixel technology, electron beam precession, electrostatic beam blanker and large dual EDS detectors. Moreover, ultra-high vacuum engineering guarantees unrestricted investigation of samples due to negligible hydrocarbon contamination from the column. Full integration of all these components facilitates their precise synchronization, true multi-modal data acquisition, and improvement in the performance and overall throughput of STEM, EDS, and 4D-STEM measurements.

Controlled from a single user interface that provides seamless user experience, acquired data are processed and analyzed on-the-fly and visualized almost in real time, making sample characterization an interactive experience instead of batch data acquisition and later post-processing. This novel approach to analytical STEM measurements has enabled simplification of sample analysis workflows that do not require TEM imaging and switching between TEM and STEM modes anymore. Advanced automation of system alignments in the background and fine guided adjustments of STEM imaging, STEM analysis and 4D-STEM nanobeam settings enable user experience comparable to SEM and FIB/SEM instruments. Users can therefore focus and spend precious instrument time on interaction with the specimen, whilst minimizing time spent on alignments of the TEM microscope. The set of collected nanoscale characterization data and results can thus be easily extended to structural information provided by the 4D-STEM and 3D-ED techniques, while data quality and accuracy of results is boosted by fully integrated beam precession.

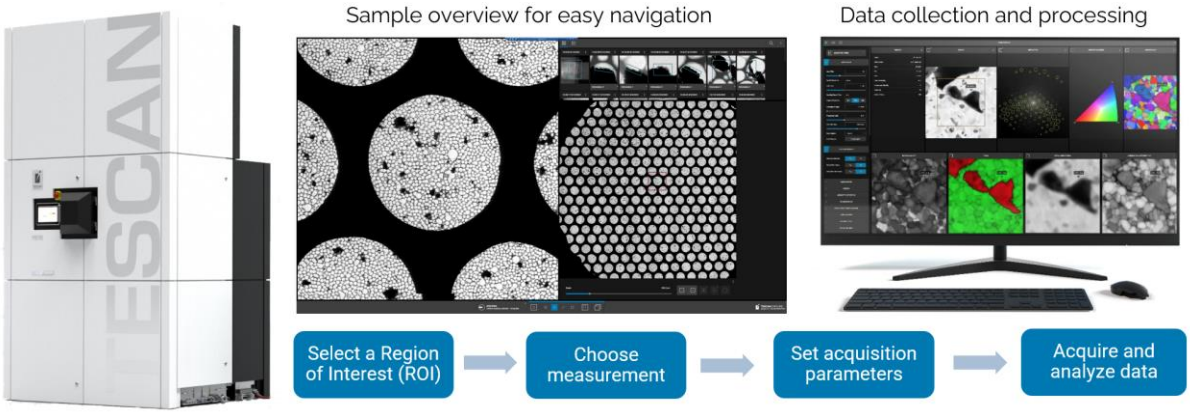

Fig. 1: Streamlined sample analysis workflow using the novel multimodal analytical electron diffraction microscope, the TESCAN TENSOR

EXPLORING CONTEMPORARY MATERIALS THROUGH MULTI-MODAL STEM TECHNIQUES

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At the heart of solving today's key societal issues - such as environmental preservation, the quest for renewable energy sources, and advancements in healthcare - lie advanced material systems. The intricate relationship between the structure, properties, and functions of these materials necessitates a detailed atomic-level analysis, integrating assessments of their structural, compositional, and electronic characteristics. Multi-modal STEM can illuminate essential aspects such as defects, interface behaviors, and the placement of dopants - crucial for the material's performance and is key for decoding the complex behaviors of materials across various conditions.

Part of the contribution is intended to highlight some recent findings related to energy materials. In a study, we explored phase transitions in lithium iron phosphate (LFP), mechanisms of lithium-ion transport, and the uneven distribution of lithiation within LFP. That way, a diffusion coefficient for lithium ions could be derived, providing experimental validation for Funke's ion transport jump relaxation model for the first time. Another investigation deals with the formation of a complex hydride within helium bubbles in neutron-irradiated beryllium. Analytical spectroscopy and simulations showed strong (hydride) hydrogen bonding to the basal planes of hexagonal helium bubbles, alongside detected elements such as aluminum, silicon, and magnesium at the bubble interfaces. The complex hydride compound exhibits a remarkably high decomposition temperature, and its formation provides new insights into metal-hydrogen interactions [1]. Secondly, we present insights into the complex interplay between structural and electronic properties at oxide interfaces and look at porous oxide materials that incorporate ions, molecules and clusters into their channels. For one, we visualize the formation of a two-dimensional electron gas and Ti^{3+} defect states at the $TiO_2/LaAlO_3$ interface using atomic-scale EELS and DPC analysis. We also analyze the three-dimensional distribution of individual cesium atoms within the channels of a beryl crystal [2].

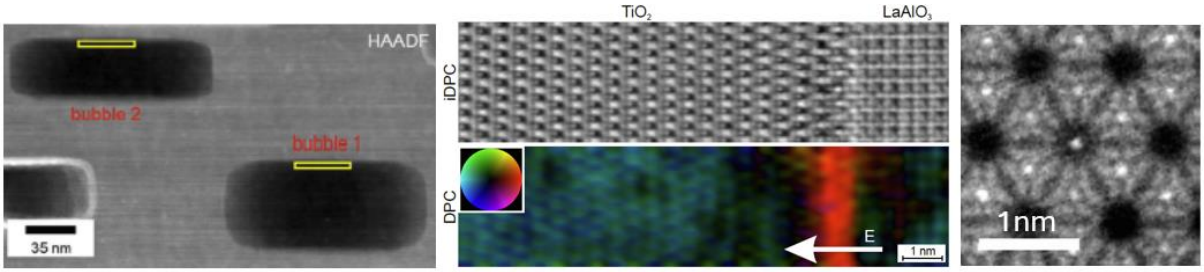

Fig. 1: (left) He bubbles within neutron irradiated beryllium. (middle) 2D electron gas at the TiO_2/LAO interface. (right) Cs atoms in the channel of beryl.

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RECENT ADVANCES IN STEM SPECTROSCOPY USING SYNCHRONIZED ELECTRON AND PHOTON BEAMS

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The field of nano-optics using free electron beams in an electron microscope is blooming, with applications from plasmons, phonons or excitons mapping at near atomic resolution, to quantum optics. These results have been boosted by constant disruptions in EM technology – monochromation, fs sources of pulsed electrons, high efficiency light injection and detection system in the EM - and theory – introduction of optics or concepts of optics in the realm of EM, such as EMLDOS or quantum statistics, to name a few. In particular, the combination of free electrons and light has recently been utilized to achieve a remarkable level of simultaneous spatial and spectral resolution and for accessing new physical information. Next to cathodoluminescence, photon-induced near-field electron microscopy and stimulated electron energy-gain (and loss) spectroscopy have emerged, leveraging this fruitful combination. The new detectors such as the Timepix3 direct electron detector, which provides sub-10 ns time resolution, occur as real game changers. Event-based hyperspectral acquisition schemes [1] offer an alternative approach to pulsed electron beam technology for synchronizing photon and electron beams or conducting coincidence experiments.
We will present an overview of these recent developments by focusing on two major issues in the fields of nano-optics.

- Firstly, we will describe time-correlated experiments based on coincidence measurements between inelastic electron scattering and photon emission events. Cathodoluminescence excitation (CLE) spectra (so called as the counterpart to photoluminescence excitation (PLE) spectroscopy) are constructed with EELS events that are time-correlated with a photon emission [2]. We will show how this newly developed spectroscopy can image energy transfer pathways at the nanometer scale, unveiling the fate of optical excitations, from their creation through absorption to their annihilation through emission, and providing a measure of the excitations' decay time [3].
- Secondly, we will show how Electron Energy-Gain Spectroscopy that allies the spectral resolution of lasers to the spatial resolution of free electrons is the perfect tool to probe photonic modes in high-quality factor photonic cavities, which cannot be resolved with common EM spectroscopic techniques. In such experiments, μeV spectral resolution is achieved to realize nanoscale mapping of photonic modes with quality factors as high as 10^4, relying on mode matching of a tightly focused laser beam to whispering gallery modes [4]. Such specificities should make them ideal for quantum coherent applications [5].

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ATOMIC-RESOLUTION SECONDARY ELECTRON IMAGING AND ULTRA-HIGH ENERGY RESOLUTION EELS

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Scanning transmission electron microscopy (STEM) has recently advanced in two major directions: atomic resolution imaging of surfaces using the Secondary Electron (SE) signal [1, 2], and ultra-high energy resolution electron energy loss spectroscopy (UHR-EELS) [3]. In this contribution, we review the progress at Nion/Bruker AXS.

Figure 1 shows three different types of images of graphene with B and N atomic substitutions: medium angle dark field (MAADF), SE, and an atomic-resolution elemental map obtained by EELS spectrum-imaging. All the images detect the single-atom substitutions, and provide further information. The MAADF image essentially "weighs" the atomic nuclei, the SE image allows atomic resolution to be reached on the surfaces of thick and even bulk samples, and the EELS map demonstrates that the chemical species of individual atoms can be readily identified by their energy loss signal. Using separate SE detectors allows the top and bottom surfaces of a thin sample to be imaged simultaneously, and results illustrating this capability will be shown at the meeting.

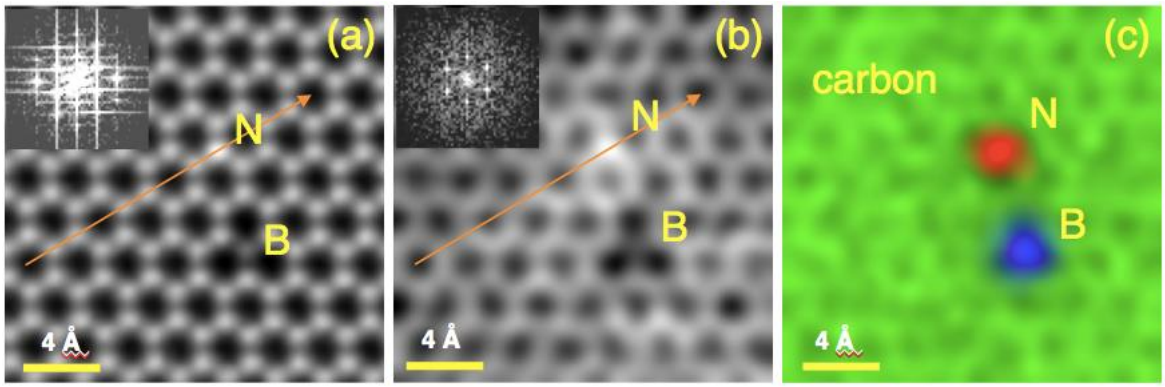

Fig. 1. a) MAADF image, b) SE image (Gaussian filtered), c) EELS elemental maps of the same area of graphene with B and N substitutions. E_o = 60 keV.

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FAST ELECTRONS OBSERVE ATOMS IN MOTION

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More than 20 years ago it was shown that proper modelling of phonons being thermally excited in a crystal yields highly quantitative electron diffraction simulations of bulk crystals [1]. More recently, it was demonstrated that quantitative fitting of 3D atomistic models to 4D-STEM data sets requires the thermal motion of the atoms to be included. Doing so, allowed the atom positions to be determined with a precision in the single pm regime [2]. We have recently shown that, even without assuming any atomistic model, the effect of thermal motion can be incorporated into iterative ptychographic reconstruction schemes, allowing the contribution of all atoms in the sample to be included, i.e. also the amorphous surface layers or contamination [3]. In a parallel development the instrumental improvement of simultaneous spatial and energy-resolution in the (S)TEM has reached a level that makes it possible to study spatial variations in the excitation of atomic vibrations [4]. Both types of information about the thermal motion of atoms are complimentary to one another, and their accessibility within the same instrument makes it possible to combine them. This talk will present recent experimental results of atomically resolved observation of variations in the phonon spectrum at different grain boundaries in silicon [5]. It will also be experimentally demonstrated that multi-object ptychography, which accounts for thermal diffuse scattering, has superior resolution and reveals spatially-resolved statistics of the motion of atoms. The correlation of both measurements on the same atomic column may yield valuable information about details of the interaction between atoms on a very local level, i.e. including individual defects which cannot be studied directly using bulk diffraction or spectroscopy methods.

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THE GOLDEN AGE OF ELECTRON DIFFRACTION

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Recent developments in electron optics and fast detectors have revolutionized the way that materials are characterized at atomic and nm scale. Rather than relying primarily on electron imaging performed inside a transmission electron microscope, increasingly, electron microscopes are used more like diffractometers for the collection of massive diffraction datasets, taking advantage of large electron scattering cross-sections and small electron probes, down to tens of picometers. By data mining collected diffraction patterns, materials microstructure can be characterized in a quantitative way. A major advantage of data-driven electron microscopy is being able to form direct images of crystal structural properties and thus solves the perennial problem of electron image interpretation [1]. Innovations in diffraction techniques and analyses are critical to realize the full potential. Here, new developments in electron diffraction analysis of crystal defects and large unit cell crystals will be described. It will be shown that cepstral transformation of diffraction patterns is a powerful method for electron diffuse scattering analysis, and the use of cepstral signals for scanning transmission electron microscopy imaging allows the detection of chemical short-range ordering and deformation microstructure in high-entropy alloys [2,3]. For radiation sensitive materials, compressive sensing with sparse sampling potentially allows the imaging of molecular crystals [4]. Large angle rocking beam electron diffraction can be collected from crystals with large unit cells using direct electron detectors for structure and bonding analysis. These capabilities are in addition to strain and orientation mapping, which have become part of standard materials characterization techniques.

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SPATIALLY-RESOLVED ELECTRON MAGNETIC CIRCULAR DICHROISM AND ITS APPLICATION ON INTERFACIAL MAGNETISM BY ACHROMATIC TRANSMISSION ELECTRON MICROSCOPE

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Magnetically coupled interfaces between materials with different magnetic orders could give rise to interesting physical phenomena due to interfacial magnetic coupling. The Exchange magnetic spring with high coercivity and high saturation properties has been achieved through the exchange interaction between a hard magnetic phase and a soft magnetic phase. Exchange spring magnet first brought up by Eckart F. Kneller [1], has been widely studied in coupled hard/soft material multilayer systems such as $DyFe_2/YFe_2$ [2]. Along with the broad range of properties and potential applications, magnetic exchange springs have their intrinsic complexities. The phenomena aroused by magnetic coupling between interfaces are usually at a few nanometers scale. Limited by the depth resolution of most magnetic characterization methods, the magnetic profiles inside each layer of the magnetic exchange spring are not yet provided.

One of the best options to push the spatial resolution of magnetic imaging lies in the electron energy-loss magnetic chiral dichroism [3], which is also called electron magnetic circular dichroism (EMCD). Physically, X-ray magnetic circular dichroism (XMCD) and EMCD share the same underlying physics in which the angular momentum transferred during X-ray absorption or inelastic electron scattering can selectively excite magnetic sublevels in atoms. In principle, EMCD can offer higher spatial resolution and greater depth sensitivity due to the short de Broglie wavelength and penetration of high-energy electrons compared to XMCD. Recently by using EMCD and achromatic electron microscopy, we can access the magnetic circular dichroism with atomic plane resolution [4].

Based on spatially resolved EMCD, we have obtained the magnetic information in the YFe_2 layer and $DyFe_2$ layer, respectively. We have observed the inversion of Fe EMCD signals in both $DyFe_2$ and YFe_2 layers. Through our spatially resolved EMCD results, we can provide spatially resolved information on the magnetic profile inside each layer. We then propose a possible spatially resolved magnetic component configuration based on our experimental results.

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VISUALIZING ELECTRONS BY SINGLE PARTICLE CRYO-EM

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Scattering of electrons by atoms in electron microscopy produces coulomb potential maps and hence EM maps reflect the charge of atoms in protein complexes. At resolution ranges between 5 Å and 10 Å, atomic scattering amplitudes are usually weaker the more negatively charged atoms are [1]. Here, we compare the cryo-EM structures of redox proteins in different oxidation states to visualize negative charges on electron accepting atoms in the electron transfer chain of the reduced map. Two redox systems are examined: *Rhodobacter capsulatus* formate dehydrogenase (FDH) and *Carboxydothermus hydrogenoformans* CO dehydrogenase acetyl-CoA synthase complex (CODH/ACS).
FDH catalyzes the reversible oxidation of formate to carbon dioxide. The electrons gained in this reaction at the active site molybdenum atom are transferred to nicotinamide adenine dinucleotide (NAD^+) via an electron-transfer pathway formed by numerous FeS clusters and flavin mononucleotide (FMN). As part of the Wood-Ljungdahl pathway, CODH/ACS catalyzes the reduction and fixation of CO_2 to acetyl-CoA. At resolutions of 3.25 Å for FDH and 2.1 Å for CODH/ACS, the difference maps between the oxidized and reduced states of the enzymes show qualitatively negative charges on the electron accepting atoms in the electron transfer chain [2]. The setup of the microscope, imaging conditions, and applications are discussed.

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SCAF1 DRIVES THE ASSEMBLY AND COMPOSITIONAL DIVERSITY OF MAMMALIAN RESPIRATORY SUPERCOMPLEXES

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The mitochondrial respiratory chain is a central component of mammalian metabolism and is constituted by four membrane-embedded complexes (CI, CII, $CIII_2$, CIV) that generate an electrochemical gradient across the membrane, necessary to fuel energy production in the form of ATP. The complexes of the mitochondrial respiratory chain are well known to associate in the membrane in high order structures named supercomplexes, but their assembly mechanism, as well as the exact composition of the high molecular weight supercomplexes remained uncertain. These aspects represent important open questions that need to be addressed to understand the currently debated physiological role of supercomplexes in metabolism. Our cryo-EM work enabled to decipher the assembly mechanism of the mammalian mitochondrial supercomplex $CIII_2CIV$, mediated by the supercomplex associated factor SCAF1. Furthermore, the cryo-EM structures of high molecular weight supercomplexes revealed that three compositionally and conformationally diverse species of respirasome exist in mammalian mitochondria and that this compositional diversity is again driven by SCAF1. Taken together, our structures clarify the landscape of mammalian mitochondrial supercomplexes and allow to hypothesize the mechanistic basis for their role in metabolism.

CRYO-EM AND SBDD AT ASTRAZENECA

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Cryo-EM has rapidly gained traction as an aid to medicinal chemistry within pharmaceutical companies, but it has also been extensively used for enlightening the molecular mode of action of important drug targets that eluded x-ray crystallography for years. In this talk, I will elaborate on the experience we have at AstraZeneca and on how the developments in the EM and Integrative Structural Biology field allowed us to understand the mechanism of action of potentially disease modifying compounds.

LYMPHOSTATIN IN AN INACTIVE TRANSPORT FORM

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The exotoxin Lymphostatin is a hallmark of pathogenic A/E Escherichia coli.. With 360 kDa it is one of the largest known toxins and outsizes the well-studied large clostridial toxins by some 1000 residues. Here we represent the first high-resolution structures of lymphostatin at some 2.3-3.5 Å resolution (Figure 1). Electron cryo microscopy and image processing revealed an intrinsically flexible particle.
We found that Lymphostatin has more functional domains than any other large bacterial toxin with known structure. The active sites of some of these domains are occluded by extensive linkers that interconnect the different domains. These linkers also fill the gaps between the domains and act as molecular glue that holds Lymphostatin in a compact form. We discovered that a central part of Lymphostatin has the typical fold of an active component found in many other bacterial toxins. This assignment was previously missed due to low sequence conservation. In particular, the active site lacks some signature residues for substrate coordination suggesting that it is catalytically inactive.

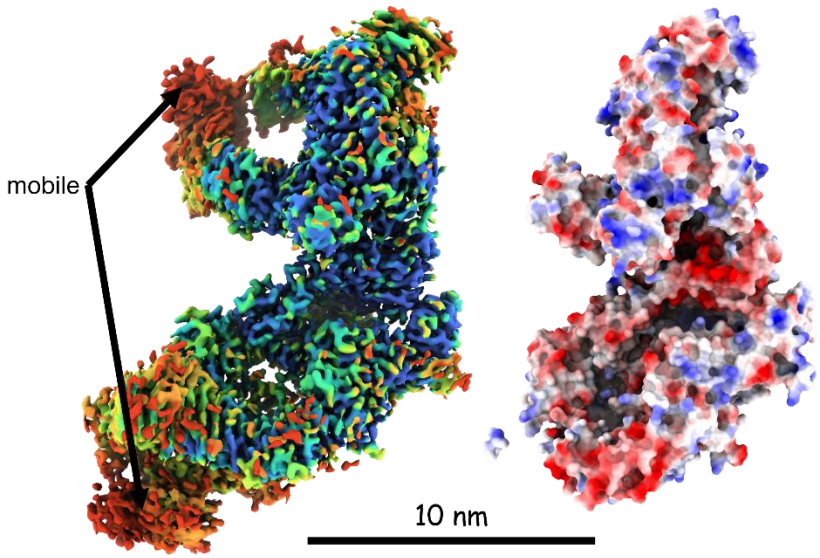

Figure 1; Surface representations of map (left) and model (right) of Lymphostatin. The map was scaled with occupy [1] to enhance the representation of the mobile regions.. The model was built automatically with Modelangelo [2] resolving most of the map apart from the mobile regions.

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EMPIAR: THE PLEASURE AND PAIN OF BUILDING A BIG IMAGE ARCHIVE

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In July 2023, we celebrated the tenth anniversary of the first release of an EMPIAR [1] entry. In this time EMPIAR has transformed from a pilot archive built on an ad hoc framework and a shoestring budget with occasional depositions to an established community archive with dedicated resources and personnel and taking regular depositions (30-40 per month on average). EMPIAR is growing at a tremendous pace. As of 31 January 2024, it contains 1533 entries, Fig. 1, totalling almost 4 petabytes of data, Fig. 2. In fact, in 2023 alone we released almost as much data as in the first eight years of EMPIAR's existence combined. EMPIAR entries comprise 2D image data related to cryoEM entries deposited to EMDB (~90%), volume EM reconstructions and data (~6%) with the remainder (~ 4%) from several other related imaging techniques. There are over 850 publications citing EMPIAR and the reuse of EMPIAR data is widespread (e.g., for training, methods development and testing, or reprocessing). The maturing status of EMPIAR has been recognised with the award of the status of ELIXIR Deposition Database (EDD) in December 2023 (https://elixir-europe.org/news/resource-announcement- 2023). EDDs are key European databases recommended for the deposition of experimental biodata. In this talk we review the history of the development of EMPIAR, showcase recent and planned developments and discuss the challenges we face in maintaining and developing EMPIAR for the future.

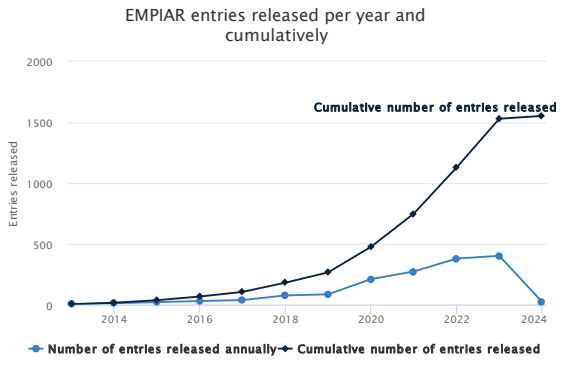

Fig. 1: Number of EMPIAR entries

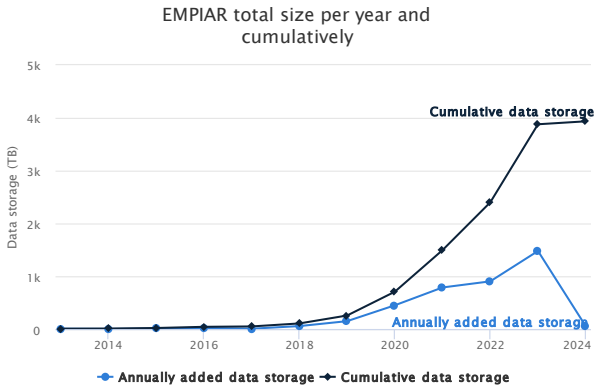

Fig. 2: EMPIAR data storage

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TECHNOLOGY DEVELOPMENT IN THE PURSUIT OF CHALLENGING CRYOEM TARGETS: RIGID FABS AND SOFT LANDING OF HYDRATED SINGLE PARTICLES

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Cryogenic electron microscopy (cryoEM) of biological macromolecules and single-particle image analysis have supercharged structural biologists' ability to help discover and design drugs against major human diseases. But limitations remain. Notably, while many proteins of importance in disease are smaller than 50 kDa, cryoEM can be extremely challenging against such targets. We are exploring multiple avenues that may make it possible to routinely solve structure of small proteins by cryoEM.

One way to circumvent the size problem altogether is to augment the mass of the target macromolecule by binding it with a high-affinity antibody fragment (Fab). Antibodies are relatively easy to obtain against any protein or peptide but unfortunately they are instrinsically flexible, often making them mediocre cryoEM tools. We have discovered a set of mutations that can be applied to any Fab to render it rigid enough for high-resolution cryoEM. We demonstrate this by solving structures of several targets as small as ~ 25 kDa.

Another avenue we are pursuing involves the deposition of nanodroplets, each containing exactly one hydrated macromolecular complex, onto otherwise dry cryoEM grids. This leads to images of our targets with extremely high contrast, which might be a promising way to enable direct structural elucidation of small targets. However, we are not yet able to perfectly preserve proteins' native states in the landed nanodroplets and the technique needs more work before it can be of use to structural biologists.

HOW CRYO-EM IS CHANGING THE LAWS OF PHYSICS

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The classical Shannon-Nyquist sampling theorem is at the basis of all measurements in the physical sciences and that includes Cryogenic-Electron-Microscopy (Cryo-EM [1]). Unfortunately, Shannon's proof of the sampling theorem is mathematically incorrect and incomplete. An important aspect of its incompleteness is the lack of symmetry between the real-space and the Fourier space treatment of the sampling problem. Symmetric sampling remedies that incompleteness [4], but in its comprehensiveness it also opens a new and wider perspective on sampling in all physical sciences. The fact that the Laws of Physics are based on continuous analytical functions implies that they cannot be measured or expressed digitally in a computer with the necessary infinite precision. Strangely enough the classical sampling theorem is a Law of Physics in that sense, a law that does not include any explicit transition from a continuous analytical input signal to an actual discrete physical measurement! This may sound like the pedantic remarks of a theoretician, but this observation comes really close to the problems that theoreticians like Erwin Schrödinger, Albert Einstein and Paul Dirac expressed, around a century ago, with respect to what was postulated to be a "measurement" ("Born rule"). in classical Quantum Mechanics (QM). With the novel technique of counting individual arriving electrons, CryoEM may have helped remove that 100-years old theoretical conundrum in QM: the measurement postulate. Measurements, are primarily the harvesting of information [2:3], under symmetric sampling protocols [4-6], both in Cryo-EM and in QM.

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STRUCTURAL STUDIES OF PHAGES IN MODERN ELECTRON MICROSCOPY

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Structural studies of biological molecular complexes have been advanced during the last decade significantly due to substantial improvements in understanding in physics of image formation in electron microscopes, developing technology for better preservation of samples under the electron beam, implementation of highly coherent sources of electrons, and eventually use of statistical methods allowing to both, significantly increase amount of the data collected, and to speed up their processing. In this talk I will demonstrate the progress in structural analysis of biological macromolecular complexes illustrated by studies of bacteriophages that are viruses selectively targeting bacteria to kill them. Modern methods of electron microscopy include examination of separate proteins, their complexes, or segments of phages. These studies represent new approaches in understanding the phage activity and how to optimize the procedures to overcome the antibiotic resistance of the host.

TOWARDS HIERARCHICAL MULTIMODAL CRYO-ELECTRON MICROSCOPY FOR CELL BIOLOGY

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Cryo-electron microscopy (cryo-EM), especially single particle analysis, has become the method of choice for determining structures of biological macromolecules. More recently, Cryo-electron tomography (cryo-ET) is emerging as an approach for cell biology to determine macromolecular structures in their native contexts and to visualise the molecular architecture of cells and tissues. This is typically from FIB-milled lamella, which are 100-200nm in thickness. One inherent limitation to this approach is that the resulting 3D tomographic reconstructions only offer a limited window into the molecular architecture of cells and tissues. While cryo block face imaging of cells and tissues offers a larger and more complete picture of cellular and tissue ultrastructure, this data lacks the resolution achieved in a 3D tomographic reconstruction from cryo-ET data and is not amenable to higher-resolution macromolecular structure determination using approaches such as subtomogram averaging. Here, we present our efforts in cellular cryo-electron tomography by large-scale collaborative endeavor to visualize the molecular architecture of *C. Reinhardtii* [1]; complemented by an exploration of whole cell cryo block face imaging [2]. Furthermore, we discuss our ongoing efforts to bridge the gap between these two imaging modalities and establish the continuum in the imaging resolution for cellular cryo-electron microscopy.

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FROM 2D LOCAL CLASSIFICATIONS TO 3D FOCUSED CLASSIFICATIONS & REFINEMENTS: PUSHING RESOLUTION TO BELOW 2 Å AND UNDERSTANDING FUNCTIONAL STATES BY ADVANCED CRYO-EM

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Cryo electron microscopy (cryo-EM) is currently moving forward at high pace towards the high-resolution analysis of macromolecular complexes. Two key factors contributing towards that are (i) new-generation cryo electron microscopes that include improved electron sources, energy filters and direct electron detectors (see our recent comparison of two 300 kV electron microscopes [1]), and (ii) new tools for advanced image processing including 3D classification methods to sort different structural states and refine sub-regions of the complexes of interest by focused refinements and thereby obtain better resolved maps and enable the analysis of different conformational and functional states [2,3,4]. Originally, the now-a-days widespread method of focused classification and refinement comes from local classifications [5], which were performed on sub-regions of the macromolecular complex of interest (bacterial ribosome) using multivariate statistical analysis (MSA), hence "local MSA" [5]. Random resampling (bootstrapping) and 3D reconstruction followed by MSA-based 3D classification then allowed to classify different structural and hence functional states [2,6]. Using localized reconstructions, such methods have also been applied to viruses [7] and implemented in various software, enabling also multibody refinements [8]. We have recently shown that an optimal mask size exists for focused refinements to perform in a stable manner and achieve high resolution [4]. Using latest-generation instrumentation [1] and advanced image processing methods [2-6] we have now succeeded in crossing the 2 Å resolution barrier on the human ribosome. Focused refinements and multibody refinements allowed obtaining a 1.9 Å resolution structure revealing many previously unseen chemical modifications of the ribosomal RNA, ions such as Zn^{2+}, K^{+} and Mg^{2+} and associated water molecules in octahedral coordination [9].

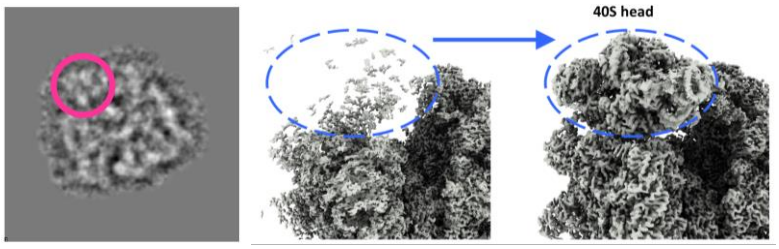

Fig. 1: Concept of local classification on a region of structural disorder [5] and map improvement by multibody refinement [4].

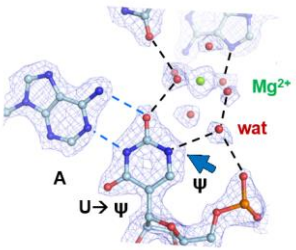

Fig. 2: Sub-2 Å resolution map of the human ribosome.

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NEW METHODOLOGIES FOR PREPARING AND IMAGING CRYO-EM SAMPLES.

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In collaboration with the group of Prof. Josh Coon, we have investigated using native mass spectrometry to land isolated protein complexes onto EM grids, subsequently solving their structure by negative stain EM [1]. I will discuss these results and our current results using the technique at cryo-temperatures [2], as well as the potential for MS-EM to provide ideal samples for single-particle cryo-EM. In addition, I will also discuss a new imaging method we are developing, “Defocus Sweep Imaging”, which, via computational microscope control, allows the microscope defocus to be changed as sample movies are recorded. This allows high-resolution close-to-focus information to be recorded at the beginning of an exposure when radiation damage is low, and lower resolution far-from-focus data to be recorded at the end of the exposure when the sample is highly damaged.

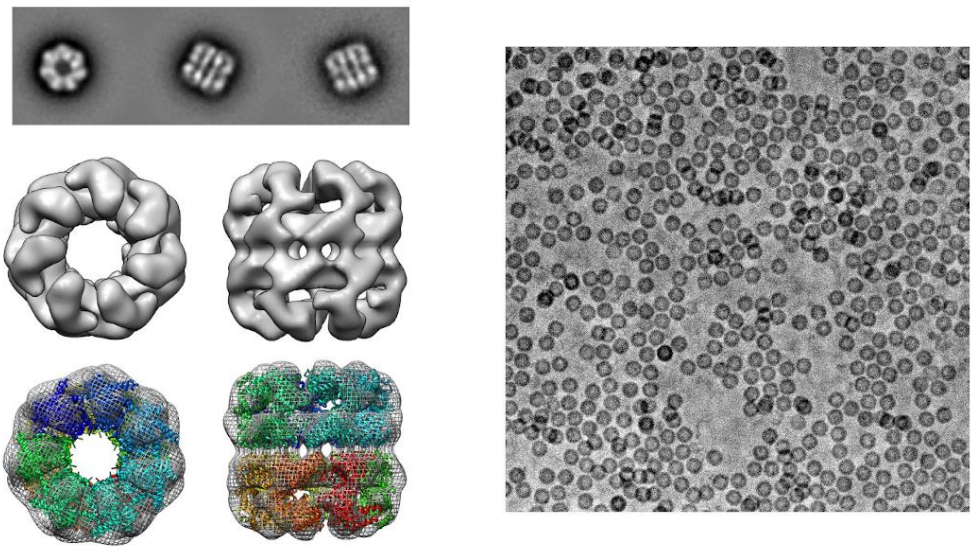

Figure 1. Negative stain reconstruction of GroEL landed via native spray mass spectrometry (left). Defocus sweep imaging of apo-ferritin demonstrating enhanced contrast (right).

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SINGLE MOLECULE ELECTRON DIFFRACTION

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Proteins undergo various conformational transitions throughout their lifecycle, that are induced by environmental factors and interactions with other biomolecules. This dynamic behavior severely impedes unraveling the mechanistic details of protein function, because only in rare cases can all the relevant conformations be observed directly with current technology. AI-driven methods, while providing promising insights into protein structure prediction from amino acid sequences, currently fall short in capturing the essence of protein dynamics. This limitation stems largely from the need for extensive datasets encompassing multi-conformational proteins, a prerequisite that is yet to be fully met. One critical obstacle in this endeavor is the difficulty in obtaining dynamical data, largely due to the inadequate signal-to-noise ratio inherent in established cryo-electron microscopy (cryo-EM) techniques. In this context, I will present a case study of a highly dynamic protein complex (fig. 1), and highlight our advances in novel methodologies. Our focus is on enhancing the signal-to-noise ratio in native electron microscopy (EM) by transitioning from traditional imaging techniques to measuring single molecule data diffraction data directly. This approach may allow a significant leap in our ability to understand and visualize protein conformations in their native state, paving the way for deeper insights into protein functionality.

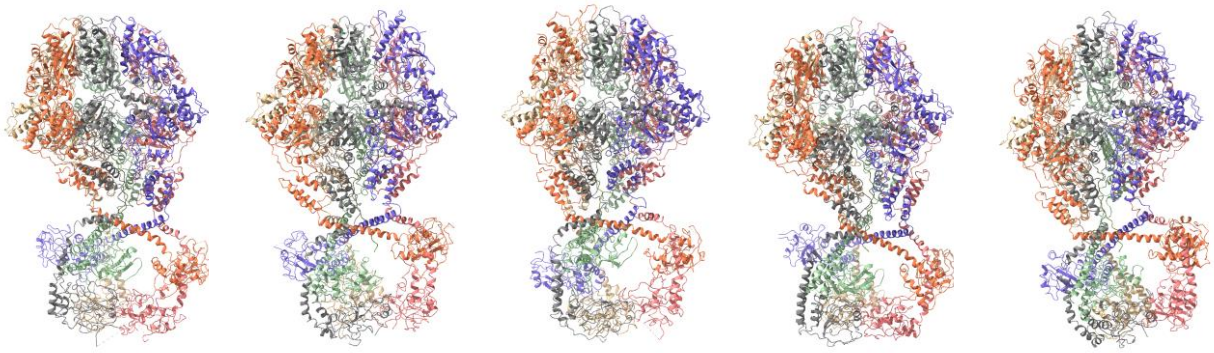

Fig. 1: Processing states of human mitochondrial Lon protease [1].

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ADVANCES WITHIN *IN SITU* TEM WORKFLOWS FOR RENEWABLE ENERGY APPLICATIONS

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Due to the intense global interest in developing technologies that support the world's renewable energy goals, a new wave of research has embraced Transmission Electron Microscopy as a critical instrument for understanding the correlation between structure and function of chemical processes [Fig 1]. To support this research, several new TEM technologies have matured into commercial products that support the development and understanding of processes including Fischer-Tropsch synthesis, CO_2 reduction and hydrogen evolution reactions. Structural studies of catalysts that facilitate these reactions have greatly benefited from new imaging techniques including iDPC and 4DSTEM as well as developments within in situ techniques that enhance resolution without compromising chemistry. Correlation with function has reached a new level of accuracy and relevance with breakthroughs in the ability to routinely generate electrochemistry [2] and mass spec data [1] from TEM samples that are directly comparable to bulk-scale reactions. In this talk, these new technologies will be presented along with several application specific examples highlighting the importance of in situ TEM for renewable energy research.

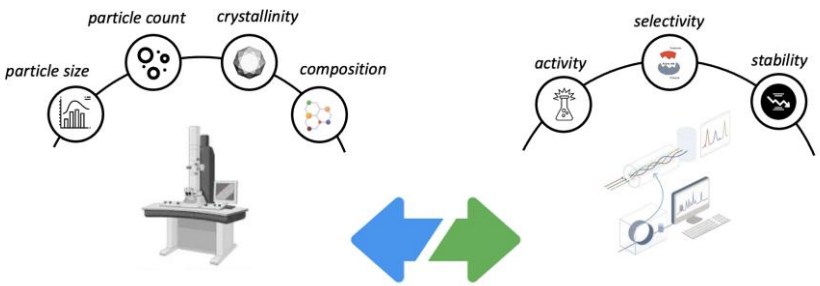

Fig. 1: Illustration of the correlation of structure versus function for gas phase reactions

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NEW APPLICATIONS OF DIRECT DETECTION, ELECTRON COUNTING CAMERAS FOR MATERIALS SCIENCE

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Direct detection cameras are changing the transmission electron microscopy landscape by delivering outstanding data quality at extremely low electron dose rates. These cameras have supercharged the cryo-EM research community and are increasingly valuable to materials scientists as research interests continually extend to more beam-sensitive materials. This presentation will review the fundamentals of direct detection sensor design, concepts of electron counting, and how these technological advancements enable low dose TEM imaging, diffraction, and in-situ experiments. Key results from a range of materials applications will be highlighted, 2-D materials, perovskites, and other beam sensitive samples. Extending direct detection technology to low-kV TEM analysis, 4D STEM, EELS, and EBSD will also be discussed.

REVOLUTIONIZING SAMPLE PREPARATION: VITROTEM'S NAIAD SYSTEM FOR EFFICIENT GRAPHENE LIQUID CELL ASSEMBLY

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Graphene Liquid Cells (GLCs) offer unparalleled opportunities for imaging samples in their native hydrated environment, facilitating atomic-resolution observations and leveraging graphene's beam scavenging properties [1]. However, the manual preparation of GLC samples poses significant challenges, leading to low yields and hindering widespread adoption. Here, we introduce VitroTEM's groundbreaking Naiad system, revolutionizing GLC assembly and streamlining the sample preparation process. The system seamlessly constructs GLCs on standard TEM grids, utilizing a layered structure comprising a bottom TEM grid coated with a monolayer graphene sheet, a droplet containing the particles of interest, and a top monolayer graphene sheet, effectively encapsulating the liquid sample [2]. Unlike traditional methods, the Naiad system prepares GLC grids within minutes, eliminating the need for arduous graphene preparation and enabling researchers to swiftly focus on sample imaging. By significantly reducing the barrier to entry for GLC experimentation, the Naiad system promises to accelerate discoveries and advancements in various fields, including biological materials [3] and nanomaterial science [4], where high-resolution imaging of liquid-phase samples is paramount for understanding complex biological systems and characterizing novel nanomaterials.

Fig. 1: VitroTEM's Naiad system for automated GLC sample preparation

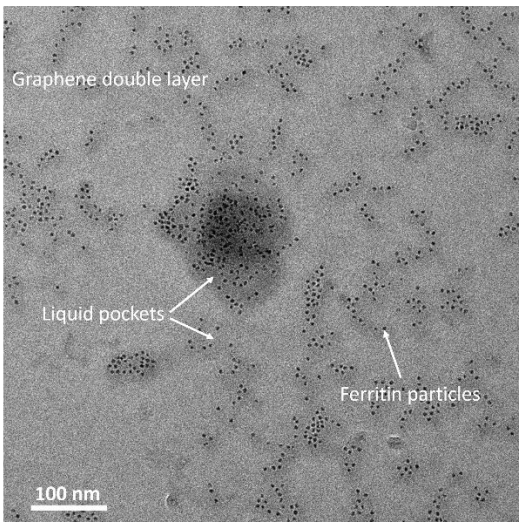

Fig. 2: Ferritin particles encapsulated in GLC pockets. The sample was prepared using the Naiad system.

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CRYOCLOUD: A CLOUD-NATIVE CRYO-EM DATA ANALYSIS PLATFORM FOR INCREASED ACCESSIBILITY, THROUGHPUT AND COLLABORATION

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CryoCloud is a cloud-native platform for cryo-EM data analysis, providing solutions for fast and secure data uploads, data storage and end-to-end data analysis. CryoCloud is accessible via an intuitive, newly designed web-application that adheres to highest security standards and can be easily accessed via a web-browser - thus CryoCloud removes the need for complex IT infrastructure setup, maintenance and upgrades. Image analysis tasks are computed by fast and scalable cloud resources that are launched upon demand and have been optimized for each job, demonstrated by the end-to-end analysis of the GLP-1 receptor to 3.4 Å resolution in 1.5 hours. Next to fast compute resources, CryoCloud provides project and data management solutions including tools for live data uploads and an archiving feature that moves datasets to cost-efficient cold storage at the click of a button, while allowing easy temporary retrieval. CryoCloud's end-to-end workflow for single particle analysis has been successfully used by scientists in industry, academic research groups and at facilities, and has been now expanded with a workflow for cryoET data analysis as well as tools for protein structure prediction and automated model building.

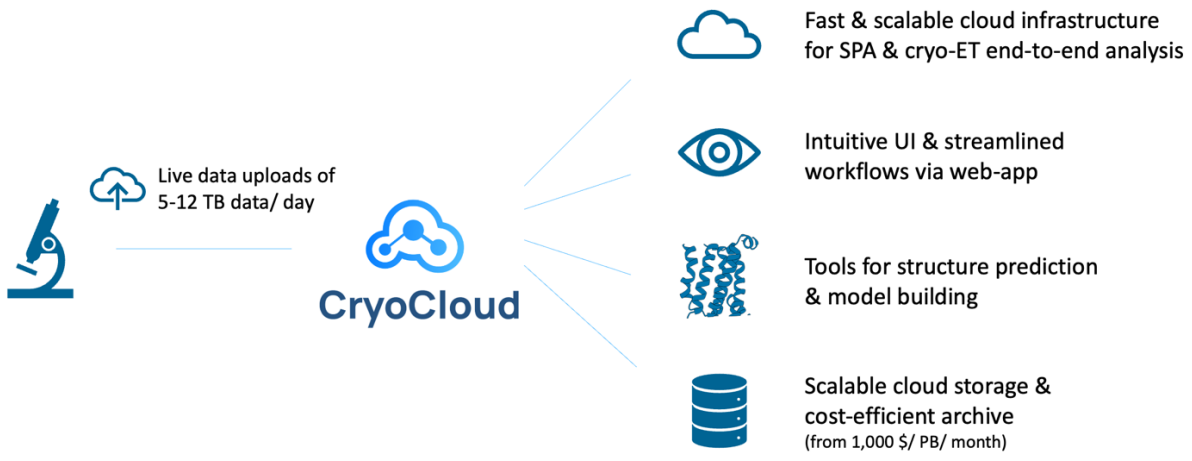

Fig. 1: CryoCloud in a nutshell: CryoCloud enables live data uploads, provides access to fast & scalable cloud infrastructure, streamlined workflows, structure prediction and modelling tools, and scalable hot & cold cloud storage.

RECENT DEVELOPMENTS IN ULTRAWIDE-BANDGAP SEMICONDUCTORS

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There is much current interest in ultrawide-bandgap (UWBG) semiconductors such as AlGaN, BN and diamond, because of their very large band-gaps, high breakdown fields and excellent thermal conductivity, which make them ideal candidates for high temperature electronic device applications, both for high power as well as RF electronics. The growth of high-quality UWBG heterostructures is critical to the fabrication of devices based on these materials but has proven to be difficult to achieve in practice due either to issues with growth or problems associated with substrate lattice mismatch that invariably give rise to interfacial dislocations and threading defects.

This talk will provide an overview of our recent structural investigations of two highly promising systems, namely, vertical GaN-on-GaN power devices, and cubic-boron nitride (c-BN) / diamond heterostructures. The growth of GaN electronic devices on GaN substrates overcomes the major obstacle of large lattice mismatch with common substrates such as SiC and sapphire, especially since freestanding GaN substrates with defect densities < 10^6/cm^2 are nowadays readily available. However, selective-area etching and regrowth as well as doping remain essential to successful fabrication of functional devices. Cubic boron nitride (a_0 = 0.3615 nm) and cubic diamond (a_0 = 0.3567 nm) are closely lattice-matched (Δa_0 ~ 0.013%) suggesting a close-to-ideal combination for device fabrication purposes. However, BN allotropes crystallize in sp^2-bonded rhombohedral (r-BN), hexagonal (h-BN), and turbostratic (t-BN) phases, as well as sp^3-bonded c-BN and the wurtzite (w-BN) phase. Cubic BN is considered theoretically to be the most stable, but mixed (t-, h- and c-) BN layers are often observed until more optimal growth conditions are established.

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4D-STEM IN REAL SPACE – MECHANISMS AND APPLICATIONS

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In 'conventional' scanning transmission electron microscopy (STEM) or 4D-STEM, the scattered electrons are detected in the diffraction plane (momentum space). In this case, we measure the change in momentum of the scattered electrons for each position of the probe on the specimen. We can equally record this signal in the image plane (coordinate or 'real' space). In this case, we measure the change in position of the scattered electrons at each position of the probe on the specimen.

Specimen information is contained in the exit wavefield. This information is the same whether it is collected in momentum or coordinate space, except that the mathematical representation is different so that the information is revealed and presented in different ways. This can mean that certain types of information may be more accessible in one space compared with the other. It can also facilitate different types of image contrast mechanisms.

Some early examples of specimen information obtained using detectors in coordinate space include 3D imaging using point detectors in confocal modes, for example [1,2], alternative approaches to generating atomic number contrast [2] and chemical mapping [3] and imaging the scattered electron probe versus position within a unit cell [4]. However, the potential of real space STEM (R-STEM) has been constrained by fixed detector geometries. Now, with the advent of relatively fast, pixelated detectors, the full scattered signal is available and new types of images can be created and information extracted.

This presentation will discuss this and other novel image contrast mechanisms in 4D-STEM and will illustrate these with applications to functional materials.

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FINDINGS FROM THE HAPPY MARRIDGE BETWEEN LOW-VOLTAGE TEM AND LOW-DIMENSIONAL MATERIALS

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For 2500 years, we have understood that the world is composed of atoms. However, it is only now that we are discovering how the precisely arranged atoms in just two dimensions result in materials with extraordinary solid-state and quantum properties. Additionally, we are uncovering that introducing well-defined atomic defects can imbue the material with new functionalities. Moreover, constructing thin layered heterostructures out of single 2D materials in a well- defined manner creates new materials that have never exist before. Withthe practical realization of aberration correction, atomically-resolved TEM/STEM has become a reality enabling the determination of the physical and chemical properties of thin structures at the level of a single atom.

We first develop a fundamental understanding of the interaction of the lower energy electron beam with the inorganic 2D crystal from atomically-resolved, voltage-dependent TEM [1] using the chromatic and spherical aberration-corrected low- voltage SALVE instrument [2-4]. We discuss specifics of sample preparation for oxygen-sensitive 2D materials [5-7] and utilize the electron beam for modifying, imaging and analyzing different 2D TMDs [7-11] and TMPTs [12,13] arranged in lateraland vertical heterostructures. Knowledge gained from imaging 2D inorganic materialsis applied to the study of 2D polymers [15, 16, 17] and differently structured 2D metal-organic frameworks (MOFs) [18, 19], allowing to gain better understanding of the main properties contributing to the electron beam resilience, pathing the way towards atomic resolution imaging of 2D organic materials [20].

Finally, basics finding are presented about the bonding nature between two atoms [20], the direct measurement of the van der Waals distance between atoms [21] and the significance of the local environment around a liquid Pt nanoparticle for its solidification [22].

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PROBING MATERIALS TRANSFORMATIONS BY TARGETING INDIVIDUAL ATOMIC COLUMNS

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The scanning transmission electron microscope is a powerful tool for modifying materials and thereby changing local electronic, magnetic, or optical properties. But tailoring materials with atomic precision is challenging, as the dose must be delivered accurately to the target location while simultaneously monitoring the reaction progress and minimizing effects on neighboring parts of the crystal. We propose that new approaches towards steering the beam and measuring its effects can enhance the opportunities for creating specific atomic configurations with useful properties. To illustrate the possibilities, we consider a material, CrSBr, that undergoes a fascinating transformation when irradiated over a broad area. CrSBr is of interest for its anisotropic magnetic and electronic properties, hosted in a van der Waals structure composed of alternating columns of Cr and S/Br atoms [1-4]. We find that irradiation of thick crystals (tens of layers thick) moves Cr in a correlated fashion, opening vertical gaps in the lattice; subsequent rebonding of the displaced Cr is calculated to create a new layered material with layer orientation and magneto/electronic anisotropies perpendicular to those of the original crystal [1]. Given the selective Cr mobility evident from these experiments, targeting individual Cr columns appears a promising pathway towards local control of magnetic texture or electronic structure. However, we find that beam steering based on machine learning is not precise enough to target Cr without affecting nearby columns. Instead, we use a strategy of "atomic lock-on" [5, 6] that combines prior knowledge of the crystal structure and analysis of circular scans to measure and compensate drift to within tens of picometers, rapidly and without additional irradiation of the target area. Once locked on, channeling keeps the beam on the column, minimizing collateral damage, and from the intensity we can measure in real time the displacement of the Cr atoms progressively out of the column. We describe defect formation in thick CrSBr crystals and generalize to other materials and to applications in spectroscopy. We conclude by discussing prospects for materials synthesis, structure/property correlations and exploration of spatially controlled transformations.

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IN SITU ELECTRON MICROSCOPY OF 2D MATERIALS

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The functionality of 2D materials structures is governed by atomic arrangement. Advanced and *in situ* electron microscopy enable site-specific studies of the coupling between atomic structure and properties. This information is crucial for the fundamental understanding of the 2D materials and the mechanisms that determine their properties and for material design. The properties can be tuned by strain that can be used to modify the properties and to induce phase transformations. The strain induced effects on atomic structure, electric field distribution, energy band gap, optical resonances and charge conduction mechanisms can be determined with at least subnano-meter spatial resolution using *in situ* electron microscopy. The spatial variation on properties including band gap, plasmon resonances and polaritons. Special specimen holders for the electron microscopes enable high spatial resolution studies of the effect of electric fields, optical illumination, mechanical strain and temperature on properties and structure. The quality of the electrical contacts to 2D materials is one of the important aspects for the evaluation of the obtained experimental data from the *in situ* experiments. This talk concerns our recent and ongoing studies of 2D structures and crucial aspects that need to be considered in order to perform advanced and in situ experiments that provide representative information about the correlation between atomic structure and properties.

TOWARD ATOMIC-SCALE ANALYTICAL TOMOGRAPHY

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If we knew the identity and location of every atom in a material, would we know everything there is to know about the material? Could we calculate the strength, electrical conductivity, color, … from this information? If such information existed, would it change how science and engineering operate? We think so but it may be decades before we have the computing power and algorithms to take full advantage. Of course, we will need to determine the atomic positions to get this all started.

Atomic-Scale Analytical Tomography (ASAT) has been defined as the determination of the correct (isotopic) identity of every atom and its precise three-dimensional (3-D) location in a structure for large volumes (>10^6 nm^3) of material. ASAT should also be able to determine the local electronic structure of these atoms which makes true 3-D atomic-structure / electronic-property relationships possible. No single technique that exists today has achieved or can achieve ASAT. However, combining transmission electron microscopy (TEM) and atom probe tomography with improved (100% quantum detection efficiency) detector technology has been identified as a path toward ASAT. Atom Probe Tomography (APT) provides mass spectral identification for each individual atom and atom positions; but lacks information about the specimen that limits the spatial resolution. The TEM provides precise information about the atom positions. This combination should achieve ASAT.

As an illustration of ASAT's promise, ASAT images of semiconductor nanostructures should reveal the 3-D position of individual dopant atoms with picometer spatial precision, the 3-D position of nuclear spin isotopes, nanometer-scale changes in strain due to structural defects and lattice mismatch, possibly the 3-D position of point defects such as vacancies, and any electronic band structure changes at all these atomic-scale features. It is expected that ASAT will be achieved through integration of TEM with EELS and APT into a single instrument with integration of 100% efficiency ion detectors. The prospective advances described in this talk are the first steps toward new tools that will meet the demands of scientists and engineers for decades to come.

3D ELEMENTAL MAPPING IN SINGLE-PARTICLE RECONSTRUCTIONS BY EELS

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With single-particle reconstructions, the three-dimensional structures of dose-sensitive macromolecules can be visualized at high spatial resolutions. In biological cryo-EM, this enables atomic modelling of protein complexes. However, no technique currently exists for elemental mapping in these complexes, leading to errors in the modelling of components which are not encoded in the amino acid sequence. These are often essential to the complex's function. Electron energy-loss spectroscopy (EELS) is a well-established technique for elemental analysis of dose-tolerant samples but is challenging for dose-sensitive samples, including biological samples, especially in a cryo-preserved state. In this talk, I will describe how we apply the reconstruction technique of single-particle analysis to EELS spectral images in order to enhance elemental signal in 3D. Parameters determined from elastic reference images enable the reconstruction of energy-loss information along the full spectrum (Fig. 1). In this way, dose-limitations can be overcome by accumulating EELS signal from many spectral images into a single reconstruction. The resulting 4D data set can be analyzed to map elements in protein complexes. We call this technique Reconstructed Electron Energy-Loss – Elemental Mapping (REEL-EM). Our proof-of-principle data demonstrate that we can reliably localize abundant elements in the reconstruction of the ion channel RyR1 and that we can apply background subtraction in 3D to remove contributions from the EELS background to the signal of elemental edges.

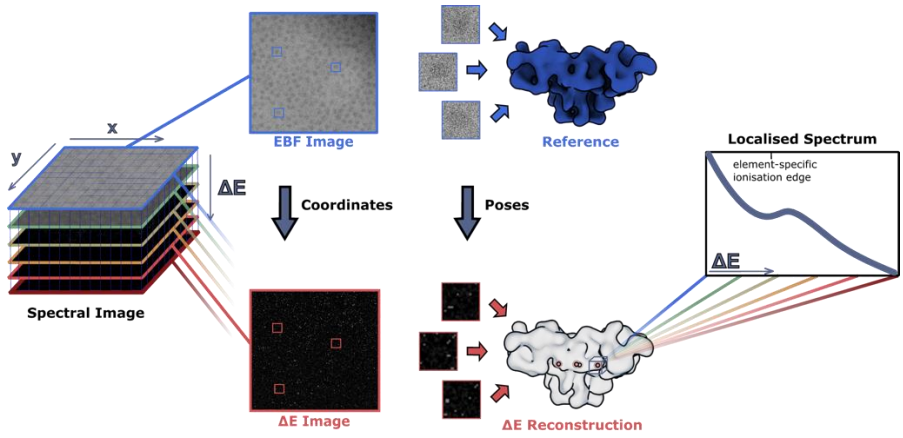

Fig. 1: Schematic of the REEL-EM workflow, showing how reconstruction parameters (coordinates and poses) from elastic bright-field (EBF) reference images can be applied to energy-loss (ΔE) images to generate a series of energy-loss reconstructions that show elemental signatures in 3D.

CRYO-EM OF ABC TRANSPORTER UNDER THE INFLUENCE OF NUCLEOTIDES, SUBSTRATES AND LIPIDS

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ATP binding cassette (ABC) transporters are a family of integral membrane proteins that actively translocate various substances across cellular membranes and are highly relevant targets for developing therapeutical drugs. Conformational dynamics and activity of ABC transporters are directly tied to the composition and local organization of the hydrophobic environment and lipid bilayer. As such, high-resolution structures of ABC transporters often display structured lipids and cholesterol at conserved positions on the transmembrane domains, indicating that they closely interact with their surrounding lipids and are immediately affected by membrane plasticity.
Despite their fundamental importance in health and disease, how their environment affects the conformational spectrum of ABC transporters and how the membrane plasticity influences their function remains unclear. Consequently, our understanding of the structural dynamics is biased by the specific hydrophobic conditions, skewing the interpretation of the data.
In my talk I will discuss our recent cryo-EM based findings of ABC transporter plasticity and the membranes impact on the conformational spectrum.

CRYO-EM OF AB FIBRILS FROM MOUSE MODELS FIND TG-APP$_{ARCSWE}$ FIBRILS RESEMBLE THOSE FOUND IN SPORADIC ALZHEIMER'S DISEASE PATIENTS

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Alzheimer's disease (AD) is the most common type of dementia, characterised by the aggregation of the amyloid-β (Aβ) protein into oligomers, fibrils and extracellular plaques. To date, there is no cure for the disease, but aggregated Aβ is a common target for therapeutics. Considering that drug development failure rate is almost 100% in AD, the structural information of different Aβ aggregates is highly valuable. A variety of mouse models are used for AD research, however, little is known about the structural differences of the aggregated Aβ when compared to humans or other *in vitro* structures. These differences might help to understand why fibril-targeting drug candidates show efficacy when tested in mouse models but often fail to show the desired effect in clinical trials. Here, we determined the structures of nine ex vivo Aβ fibrils from six different mouse models by cryogenic-electron microscopy [1]. We found novel Aβ fibril structures in the APP/PS1, ARTE10 and tg-SwDI models, whereas the human type II filament fold was found in the ARTE10, tg-APPSwe and APP23 models. The tg-APPArcSwe mice showed an Aβ fibril whose structure resembles the human type I filament found in patients with sporadic Alzheimer's disease. A detailed assessment of the Aβ fibril structure is key to the selection of adequate mouse models for the preclinical development of novel plaque-targeting therapeutics and positron emission tomography imaging tracers in Alzheimer's disease.

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FIB MILLING DAMAGE IN BIOLOGICAL SAMPLES

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Electron cryo-microscopy (cryo-EM) can be used to image macromolecular molecules and assemblies in their native, cellular environment at atomic resolution. Cells are imaged while frozen and have to be thinned to <500 nm lamellae (Fig. 1). The thinning can be achieved by focused ion beam (FIB) milling, which represents a significant advance over prior approaches because of its ease of use, scalability, and lack of large-scale sample distortions. In the present study, we measure the amount of damage FIB milling causes to a thinned sample using 2D template matching (2DTM), an approach for detecting and identifying single molecules in cryo-EM images of cells [1,2]. The signal-to-noise ratio (SNR) of the detected structure (target) is a measure of its similarity to a given molecular model (template) and can therefore also be used to assess its damage. We show that FIB-milling using a 30 keV gallium ion beam introduces a layer of variable damage that extends to a depth of 60 nm from each lamella surface (Fig. 2) [3]. This layer of damage limits the recovery of information for in situ structural biology. By accounting for both electron scattering and FIB-milling damage, we estimate an optimal lamella thickness of 90 nm for 2DTM target detection.

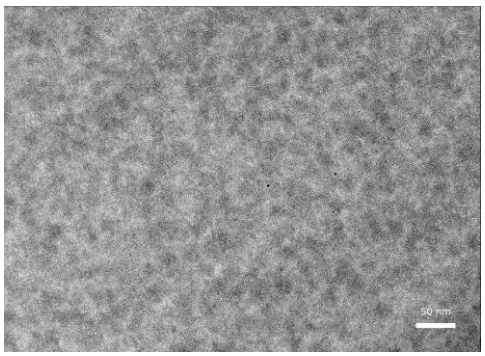

Fig. 1: An electron micrograph of the yeast cytoplasm in a 200 nm region of a lamella. Scale bar: 50 nm

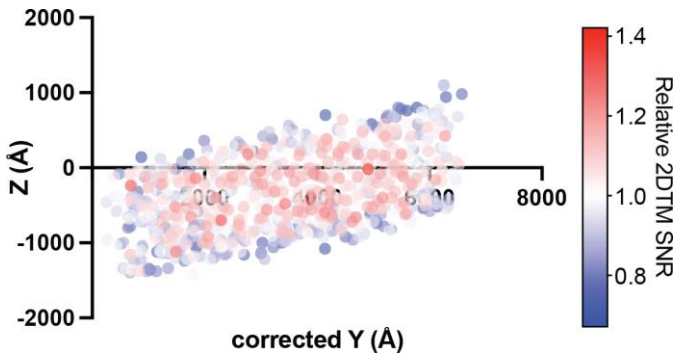

Fig. 2: Scatterplot showing the measured 2DTM SNR (color coded) of detected large ribosomal subunits in the lamella in Fig. 1 as a function of depth (Z) and position within the image along a direction perpendicular to the lamella tilt axis (corrected Y).

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STRUCTURAL ANALYSES OF BROWN ALGAE

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Brown algae belong to the stramenopiles and as such are phylogenetically distant from plants, animals and fungi. They are one of only five eukaryotic lineages that have evolved independently to develop into complex multicellular organisms. To contribute to the understanding of developmental processes that lead to the observed complex multicellularity in brown algae we have analysed *Ectocarpus siliculosus* both by scanning and transmission electron microscopy. We have compared morphologically the wild-type and a mutant that shows defects in the first cell divisions of the developing organism. Potential differences in Golgi morphology were analysed in datasets obtained by focused-ion beam scanning electron microscopy of plastic embedded samples of both the wild-type and the mutant.
As higher plants and other algae, brown algae are photosynthetically active thereby reducing carbon dioxide from the atmosphere. The chloroplast of brown algae originated from a secondary endosymbiosis event in contrast to chloroplasts from land plants. In order to obtain a better understanding of the molecular mechanisms involved in photosynthesis, we started to explore these processes structurally in Ectocarpus.

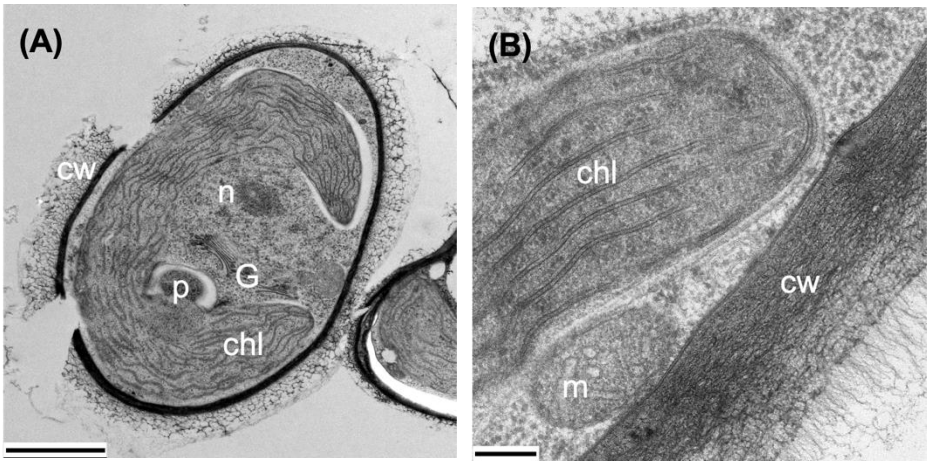

Fig. 1: (A) TEM of an ultrathin section of an Ectocarpus cell with a prominent chloroplast (chl) showing the thylakoid membranes and the pyrenoid (p). nucleus n, Golgi apparatus G, cell wall cw. (B) Details of chloroplast membranes. mitochondrium m. Bars: (A) 2 µm, (B) 200 nm.

COUPLING PHYSICAL METALLURGY AND ELECTRON MICROSCOPY FOR 54 YEARS

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As may be appreciated, significant improvements in the capabilities afforded by the various techniques of electron microscopy have occurred over the past half-century. While these various advances have been extremely useful when applied to problems in materials science, sometimes robust and “tried and tested” techniques have been somewhat forgotten. Three examples of the application of advanced techniques will be presented, all involving problems in physical metallurgy. The first involves the use of aberration-corrected STEM in the study of soft phonons in titanium alloys, while the second exploits the possibilities afforded by advances in imaging in the scanning electron microscope to solve a problem experienced over the decades regarding the application of hot isostatic pressing to the fabrication of Ni-base turbine disks (for gas turbine applications). The third example involves almost forgotten techniques, specifically diffraction contrast to identify the identity of dislocations in alloys and compounds. Here, diffraction contrast is used to characterize deformation microstructures in refractory high entropy alloys.

CHALLENGES AND STRATEGIES FOR MICROSCOPY AND MICROANALYSIS OF ENERGY AND QUANTUM MATERIALS IN MODERN ANALYTICAL TEM/STEM INSTRUMENTS

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The application of state-of-the-art analytical transmission electron microscopy to a variety of materials problems ranging from amorphous to crystalline solids, organic/inorganic interfaces, energy materials, nanoarrays and catalysis has been accepted as a essential tool in today's research. However, when applied to analysis of energy and quantum materials there are challenges in the microanalysis regime due to their sensitivity to electron beam effects/damage, particularly under microanalytical situations employing XEDS and EELS. A selection of exemplar studies from the former will be used to illustrate these challenges as well as the mitigation and analysis strategies that are being used on the Analytical PicoProbe instrument at University of Chicago / Argonne National Laboratory.

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ARE OMEGA PRECIPITATES ALWAYS BAD FOR TITANIUM ALLOYS?

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For decades the poor mechanical properties of Ti alloys were attributed to the intrinsic brittleness of the hexagonal omega phase that has fewer than 5-independent slip systems. However, what has often not been recognized is that this hexagonal omega phase is not always embrittling but can rather be a strengthening precipitate, while retaining tensile ductility in the alloy. Compositionally invariant or congruent omega precipitates typically form when the Ti alloy is rapidly cooled from the high temperature single body centered cubic (BCC) phase field to room temperature, and these precipitates are referred to as athermal omega precipitates. On annealing at low temperatures, these athermal omega precipitates are replaced by isothermal omega precipitates as they reject the BCC stabilizing solute elements in the alloy via diffusion. While omega precipitates have been observed in many Ti alloys over the past fifty years, the crystallographic and compositional nature of these nanometer scale precipitates has been quite difficult to decipher due to the available characterization techniques. With the advent of modern techniques such as aberration-corrected transmission electron microscopy (TEM), monochromated spectroscopy, and atom probe tomography (APT), it is now possible to discern the details of the lattice structure and composition of these hexagonal omega precipitates, the surrounding BCC matrix, and the intervening interface. The present study focuses on atomic scale structure, specifically addressing the nature of the shuffle leading to the transformation from BCC to hexagonal omega, and the attendant compositional variations, in a simple model binary Ti-12wt%Mo alloy. While the BCC matrix with the athermal omega precipitates retains tensile ductility, strain hardenability and TRIP/TWIP deformation mechanisms, formation of isothermal omega precipitates increases the strength substantially, while leading to a complete loss of ductility. One of the possible mechanisms relates to the change in the bonding nature within the omega precipitates as a function of composition (e.g. Mo content). This is being investigated via electron energy loss spectroscopy (EELS) and energy loss near edge structure (ELNES) by comparing athermal and isothermal omega precipitates. These investigations are being complemented with density functional theory (DFT) based first-principles and cluster expansion calculations.

VISUALIZING CHEMISTRY AT THE ATOMIC SCALE!

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The energy and environmental challenges of our modern society call for radical new chemistry and materials. Sustainable production of chemicals and fuels, improved energy efficiency of industrial processes and abatement of environmental harmful emissions are few important areas in which e.g. new catalytic reactions and materials are needed. However, as catalysis is a surface phenomenon and materials' surfaces tend to adapt dynamically to their surroundings, the ability to visualize chemical dynamics at the atomic-scale becomes key to develop fundamental understanding of structure-function relationships and novel designs of catalysts. Such insights can be gained using the environmental transmission electron microscope (ETEM), which has developed over more than three decades for uncovering gas-solid interactions with successive advances that have promoted new important breakthrough findings in heterogeneous catalysis and nanomaterials investigations in general.[1-3]

Here, we are proud to introduce the next generation in ETEM. The differentially pumped open cell gaseous capability is now integrated on the Spectra Ultra S/TEM platform, providing both nanomaterial inspection in gaseous environments and at the best atomic resolution TEM & STEM imaging. The high stability of the Spectra ETEM is based on the combination of ETEM with the Spectra Ultra enclosure. In our presentation, we will reveal details of this new ETEM platform. We will discuss the interesting technology and features enabled, and the milestones are results we achieved in this project.

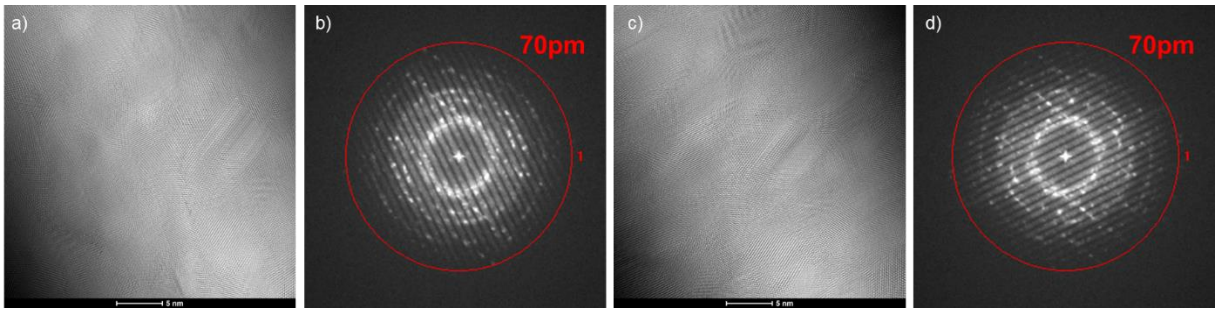

Figure 1: Information Limit of 80pm with 0.5mbar of N2 in the octagon as determined by a young fringe experiment. a) displaced images in X, b) young fringes showing 70pm transfer in X, c) displacement image in Y, d) young fringes showing 80pm transfer in Y. These experiments show the atomic resolution capability in gaseous environment on the Spectra ETEM.

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ACCELERATED MATURATION OF MATERIALS BY COUPLING CHARACTERIZATION AND COMPUTATIONAL MODELING: HAMISH FRASER'S PIONEERING VISION

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Characterization of metallic materials using electron microscopy techniques has advanced dramatically in the past several decades. Hamish Fraser been at the forefront of these advancements and their application in metallurgical topics ranging from intermetallics, powder metallurgy, titanium alloys, and high entropy alloys. While his impact on these fields has been enormous, his influence has extended well beyond these scientific advances to include mentorship of generations of graduate students and researchers, bold strategic initiatives, and creative collaborations with faculty colleagues. These many contributions have helped chart the course of the Department of Materials Science and Engineering at the Ohio State University for over three decades. Through the founding of the Center for the Maturation of Materials (CAMM), he created an early (and sustaining) example of the important synergies made possible by combining experiments and computational modeling to accelerate the advancement of materials development. Hamish was an early pioneer of this vision, which has since evolved into the subfield of Integrated Materials Science and Engineering. A specific example from my research that has been inspired by the "CAMM approach" involves the improvement of polycrystalline Ni-based superalloys that are vital materials for disks in the hot section of aerospace and land-based turbine engines. Diffraction contrast and high angle annular dark field imaging, coupled with energy dispersive spectroscopy, have all been used to fully characterize the extraordinarily complex deformation mechanisms that control the performance of these alloys at elevated temperature. For instance, microtwinning and stacking fault shearing through the strengthening precipitates are important operative mechanisms in the critical 600-800°C temperature range. These unusual, high temperature mechanisms have been understood using a range of modeling techniques from density functional theory to phase field modeling to continuum level approaches. Emerging from these studies is the discovery that a local phase transformation (LPT) phenomenon occurs commonly at the stacking faults and microtwins formed during creep of superalloys. A comprehensive modeling approach to predict alloys compositions which promote the formation of local ordered phases at these deformation faults has been developed. The localized phase transformation strengthening approach demonstrates great promise for guiding the improved performance of these important structural materials. This example is a personal testament to Hamish's vision for innovative materials development that requires advanced electron microscopy to develop the most accurate, high-fidelity characterization of materials microstructure.

ELECTRON ENERGY-LOSS SPECTROSCOPY OF "HARD" MATERIALS

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"Difficult, arduous, demanding great exertion or effort….the opposite of all that is easy" is one definition of "hard." [1] Hamish L. Fraser, a thoroughbred metallurgist, is largely associated with the study of "hard" materials, and has never shied away from tackling "hard" problems. In this contribution, I will begin with a discussion of the results of research that originated from Hamish's desire to understand if we could really trust atomically localized signals in analytical electron microscopy. Investigating this "hard" problem, in collaboration with Les Allen & others yielded insights that were critical for many subsequent materials investigations in metallurgy [2,3] In the second part of the talk, building on this foundation, I will discuss how our approach to electron energy-loss spectroscopy (EELS) evolved with the first generation of electron monochromators to allow us to tackle the "hard" problem of detecting and identifying molecular functional groups in polymers and functional materials. [4,5] In the final section, I will report on recent efforts in my group to tackle a very "hard" challenge, namely to detect and map functional groups by vibrational EELS in cryogenically frozen liquids. In all of these examples, I will discuss the developments in experimental methods, instrumentation and theory that were necessary in order to tackle head-on these "hard" problems in the manner of Hamish L. Fraser!

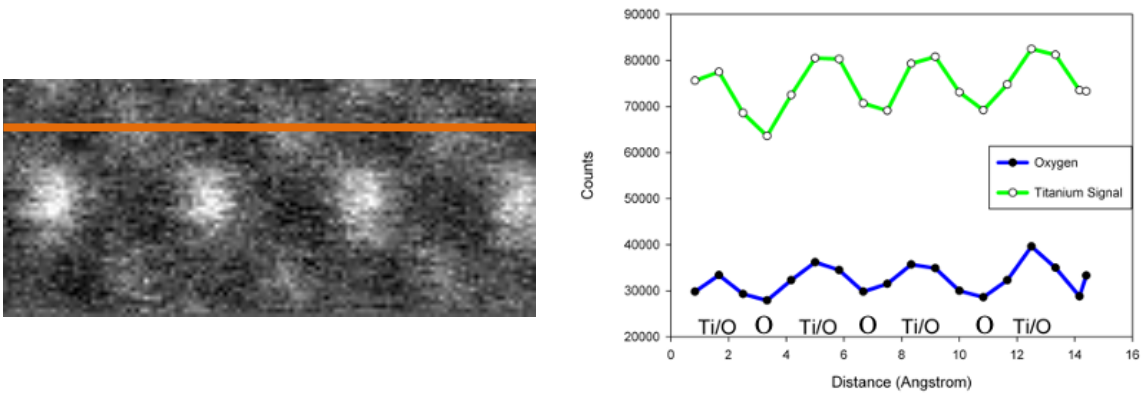

Fig. 1: EELS line scan from (001) STO monitoring the oxygen K-shell signal along successive Ti/O & O columns. The density of oxygen in both types of columns is identical but the O signal varies from column to column. [2]

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SOME APPLICATIONS OF ELECTRON MICROSCOPY TO SEMICONDUCTOR MATERIALS

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We explore the application of aberration corrected microscopy in applications involving semiconductor materials. The first example describes the control of the structure of transition layers on a sapphire substrate to form defects free n-polar GaN epilayers for high power, high frequency applications. We describe processing parameters that determine the crystallography of an AlN buffer layer as a means of obtaining the desired polarity of the epilayer. Defects in the epilayers such as inversion domains and threading dislocations are then analysed and their origin understood in terms of atomic scale steps and atomic distributions on the sapphire substrate. In a second example, we describe defects in CdTe/ZnTe films grown on a vicinal GaAs surfaces for photovoltaic applications, in particular the use of scanning moire fringe technique (SMF) to locate these defects at relatively low magnifications

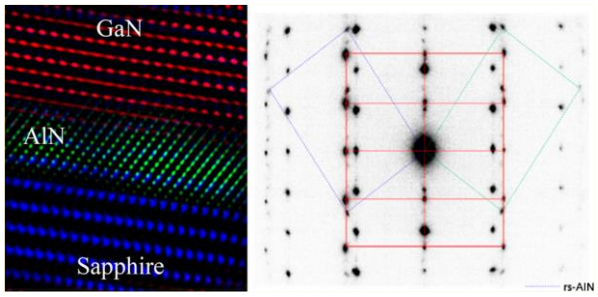

Fig. 1: Epitaxial GaN on sapphire with an AlN buffer layer

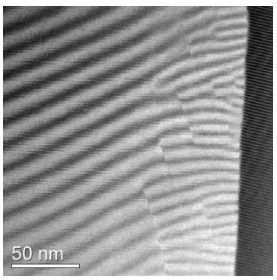

Fig. 2: A scanning moire fringe image showing defects in CdTe/ZnTe films grown on a GaAs substrate

FAST 4D STEM: HIGHLIGHTS OF THE ARINA HYBRID-PIXEL DETECTOR

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The recent evolution of direct electron detection technologies has been deeply connect to the growing interest in 4D STEM characterization approaches. Back at PICO 2022, the perspective of 4D STEM experiments with similar acquisition speed than conventional STEM imaging (i.e. 10 µs dwell time) was presented as an ongoing development using hybrid-pixel technology [1]. Since then, the ARINA detector [2] was finalized and launched as a product, and has been put to test on outstanding 4D STEM experiments by collaborators worldwide [3].

This flash talk summarizes the main development aspects supporting the ARINA detector technology, in particular the ones connected to 4D STEM data acquisition, real-time visualization and advanced data processing, and highlights the achievements of collaborators exploring it fast 4D STEM experiments.

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A VERSATILE MULTI-CONTACT ENVIRONMENTAL HOLDER WITH A REMOVABLE TIP FOR OPERANDO TEM ACROSS MULTIPLE PLATFORMS

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In situ (scanning) transmission electron microscopy ((S)TEM) experiments in liquids or gases became widely possible with the development of sandwiched MEMS-based samples carriers (e.g. Nano-Chips) – liquid Nano-Cells and gas Nano-Reactors. Various stimuli like heating or biasing in liquid and gaseous environments have been employed to study materials synthesis, secondary batteries, catalysts, resistive switching and more. The majority of the commercial in situ (S)TEM sample holders, however utilizes only four electrical contacts that limits the combination of stimuli that the user can simultaneously apply. Real life applications, like Li-ion batteries, proton exchange membranes, solid oxide fuel cells, and non-volatile memory, etc, on the other hand, require combined application of thermal and electrical stimuli at ambient environmental conditions and thus, higher number of electrical signals.

In this work we would like to present our new platform for in situ environmental (S)TEM operando experiments. The heart of this platform is a newly designed holder with Nano-Cells and Nano-Reactors for in situ liquid or gas experiments that have eight electrical contacts. The extended number of contacts empowers users to extend the applications to combined liquid heating and biasing and gas heating and biasing experiments. The design of the tip of the holder has generic design and fits different brands of TEM, which substantially improves to correlation of the same sample between different TEM platforms. Moreover, due to the modular design of the new environmental sample holder, there is no need to have two separate holders to perform environmental studies – one can be used for both; the user just needs to choose the functionality required and to add auxiliary equipment to carry out any desired experiments. We will present several experimental examples of the new environmental operando system, including metal electrodeposition as a function of the temperature and explain how our new platform can be used in correlative studies involving different experimental methods like in situ SEM, X-ray and more.

LIQUID HELIUM TEM SAMPLE HOLDER: SWIFT COOL-DOWN AND LONG HOLDING TIME

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Quantum materials display a variety of unique electronic and magnetic properties—including superconductivity, charge ordering, and topological states—which are predominantly observed at cryogenic temperatures [1, 2]. Swift progress in cryogenic scanning transmission electron microscopy (S)TEM methodologies has been achieved at liquid nitrogen (LN_2) temperatures. While LN_2-cooled side-entry sample holders and cartridge-integrated microscopes are tailored to suit the demands of the life sciences, the study of phase transitions within materials science typically necessitates adjustable temperatures with a base in the liquid helium (LHe) range [3].

Historically, LHe solutions for electron microscopes were constructed in a cryo-stage setup [4-7], achieving temperatures as low as 1.5 K utilising superfluid helium alongside LN_2-cooled shields, and thus enabling high-resolution imaging over a continuous five-hour span [8]. Despite these achievements, the preference for technically versatile side-entry holders has hindered further advancements in cryo-stage development. Presently, LHe side-entry holders are limited by considerable mechanical and thermal instability, and their base temperature holding times are short due to LHe's low latent heat and the limited cryogen storage capacity of the dewar attached to the holder. Efforts to extend cryogenic holding periods have led to integrating a commercial LHe continuous flow cryostat into a modified 60 mm pole piece gap following the removal of the objective lens [9]. This modification has facilitated temperature control within a range of 6.5 K to 400 K, maintainable over several days. However, the substantial alterations to the microscope structure and the requirement to vent the column for specimen loading present practical challenges for routine operation.

Here, we present recent innovations of a lightweight, ultra-low-temperature LHe TEM sample holder. Starting from room temperature, a base temperature of 5.2 K—measured adjacent to the specimen by a Cernox sensor—can be attained within one minute and sustained for days with a temperature stability of +/- 2.5 mK. Initially designed for X-ray diffraction studies of quantum matter in pulsed magnetic fields, condenZero, a spin-off company from the University of Zurich, has adapted their miniaturisable cryostat design for cryo-TEM usage. Collaborative efforts with the ER-C at the Research Centre Juelich have led to additional enhancements and optimisations. Here, we demonstrate the capabilities of our latest LHe cryo-TEM setup.

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IN-SITU STEM WITH SURFACE SENSITIVITY

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Advanced nanomaterials and electronic devices benefit greatly from the fusion of imaging and chemical analysis at the atomic level. The capacity of a transmission electron microscope (TEM) to conduct such studies swiftly and consistently, while gathering a broad array of analytical data, is pivotal for future technological advancements.

Hitachi's HF5000 FE-TEM, equipped with an in-house spherical aberration corrector, is engineered to deliver sub-angstrom (Å) imaging alongside high-sensitivity elemental analysis. Leveraging the fully automated aberration corrector, dual symmetrical SDD EDX, and Cs-corrected SE imaging, the HF5000 represents a significant leap in capability.

In this presentation, I will showcase various examples, spanning from simultaneous STEM and SEM observations to EDX analysis and in-situ gas and heating experiments.

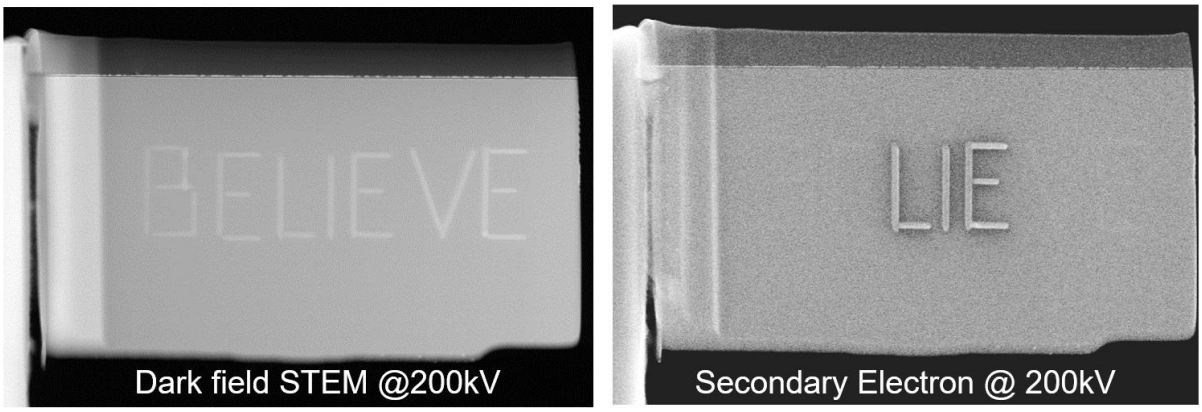

Figure 1: When surface information matters. Simultaneous SEM & STEM image of a TEM Lamella. The letters "LIE" are deposited on the front side and the letter "BE" and VE" on the backside.

ELECTRON PTYCHOGRAPHY COMES OF AGE

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With recent advances in detector technology and ptychographic algorithms to unscramble multiple scattering, the resolution of the electron microscope is now limited only by the dose to the sample, and thermal vibrations of the atoms themselves [2]. At high doses, these approaches have allowed us to image the detailed vibrational envelopes of individual atom columns as well as locating individual interstitial atoms that would be hidden by scattering of the probe with conventional imaging modes. The three-dimensional nature of the reconstruction means surface relaxations can be distinguished from the bulk structure (Figure 1), and interface roughness and step edges inside devices can be resolved – including gate-all-around transistors and Josephson junctions. Even the location of all atoms in thin amorphous films now seems within reach. The reduced sensitivity to chromatic aberrations also makes these ptychographic approaches of interest for thick biological samples. However, as the dose is reduced, so is our ability to robustly reconstruct the object. Challenges in characterizing and predicting performance will be discussed.

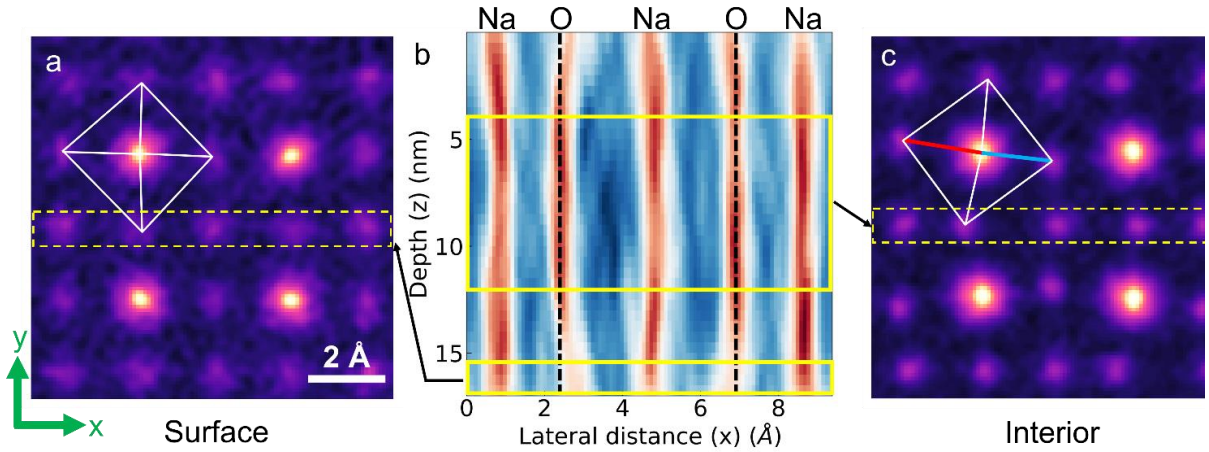

Fig. 1 Ptychographic reconstruction of ferroelectric $NaNbO_3$ showing the (a) surface and (c) interior structure of the sample. (b) Depth profile along the Na-O plane marked with a yellow dotted box in (a, c) showing the relaxation of the surface oxygen atoms back to the cubic structure of (a), with octahedral rotations present in the bulk (c). The slices summed to get the images shown in (a, c) are also labelled in (b). Blue and red solid lines are used to label the short and long in-plane Nb-O bond lengths that give rise to the ferroelectricity in a structure that would otherwise appear to be cubic by conventional imaging methods.

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ADVANCING UNDERSTANDING OF NANOCATALYSTS WITH IN SITU STEM IMAGING

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Improving the performance of metal nanocatalysts has great potential to address the current energy emergency. However, probing nanoparticle synthesis and the evolution during catalyst activation and use requires atomic spatial resolution and spectroscopy in reactive environments.

We have used in situ gas cell scanning transmission electron microscopy to investigate how the catalyst morphology is determined by the wet impregnation synthesis parameters, as well as how the starting structure and composition determines the evolution of industrial supported nanoparticle catalysts during activation heat treatment.[1] However conventional SiN windowed gas/liquid holders often suffer from limited spatial resolution. Our group have been developing in situ graphene cells for transmission electron microscopy (TEM) imaging and analysis at atomic resolution, using a combination of 2D materials like graphene to enable functionality such as liquid-liquid mixing [2]. We have used these 2D heterostructure liquid cells to investigate the dynamic processes that occur at a solid-liquid interfaces as metal ions from solution interact with a solid support at atomic resolution.[3]

While in situ TEM studies are often limited to 2D projections with limited spectroscopic information we have shown that the single particle reconstruction method, which is widely used in cryogenic TEM imaging of proteins, is a valuable means to probe the three dimensional structural evolution of inorganic nanoparticles.[4,5] This approach averages over particles present in the image with different orientation to build up a tomographic reconstruction at much lower radiation dose than is required for conventional tilt series tomography, enabling imaging for active nanoparticle systems like PtNi, used for the oxygen reduction reaction.[4,5] We have shown that this opens up the single particle reconstruction technique to allow 3D visualization at different time points during in a synthesis process or catalytic reaction.[6] This approach could be brought to the atomic scale through harnessing the improved imaging performance achievable with new in situ cell designs.

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TOWARDS QUANTITATIVE MAPS OF LITHIUM IN THE ELECTRON MICROSCOPE

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This paper will present where we are to perform quantitative EDS and EELS maps of Lithium-based materials with the Hitachi SU-9000 dedicated STEM having electron beam energies between 0.1 to 30 keV. State-of-the-art results acquired will be shown. This microscope has EELS capabilities that allow Li detection [1]. It is also equipped with the Extreme EDS system from Oxford Scientific that can detect the K_α line of Lithium [2]. The SU-9000 has a resolution of 0,22 nm in bright field STEM without aberration correctors and this allows lattice imaging. Figure 1 shows an EELS spectrum of Li_2FeSiO_4 obtained at 30 keV. Even if the X-ray K_α line was not seen for this material with the Extreme EDS detector, the ionization edges are visible for Fe, Li, and Si. Figure [2] shows *a* Li EELS jump ratio map of an Al 2099 alloy taken with the Hitachi SU-9000 at 30 keV. This map shows δ precipitates (Al_3Li) which are spheres between 5 to 20 nm and T_1 plates (AlCuLi) that have thicknesses between 2 to 6 nm. The fact that the edges are always ionized is a strong advantage for EELS since it does not matter if there is not an electron transition leading to X-ray emission, where is often the case that there are no Li K_α line with oxides cathode-based materials. Also, the emission rate in EELS is greater than about 10,000 than that of EDS owing to the fluorescence factor. The downside of EELS is the need for a transparent specimen and beam damage can also be an issue. Results obtained with a cryo-holder to minimize beam damage will be presented for Spodumene and other materials.

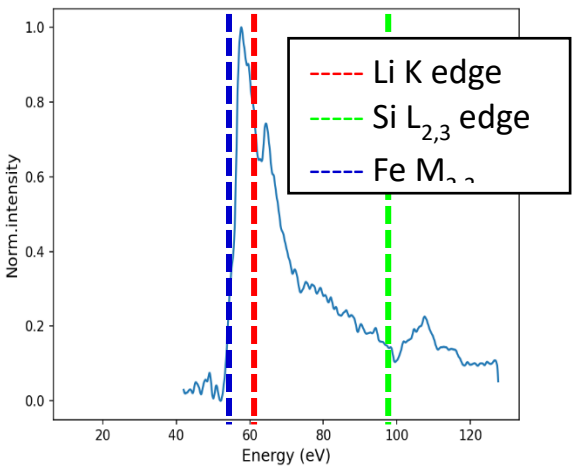

Fig. 1: EELS spectrum of Li_2FeSiO_4 taken with the Hitachi SU-9000 at 30 keV.

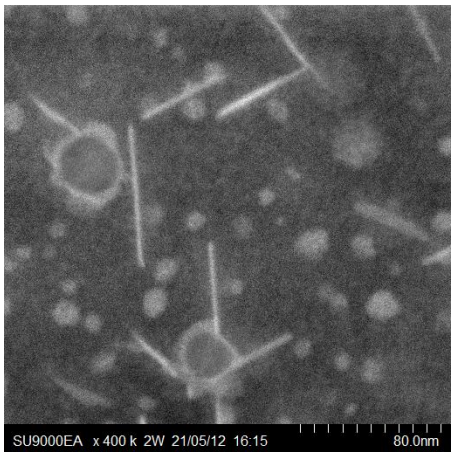

Fig. 2: Li EELS jump ratio map of an Al 2099 alloy taken with the Hitachi SU-9000 at 30 keV.

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GENTLE INTERROGATION OF NANOSCALE OBJECTS

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When we look at samples in the (electron) microscope we aim to obtain information from an object under study by exchanging energy with it. I am deeply indebted to Dirk Van Dyck for this information viewpoint on electron microscopy, which resonated with my former background in signal processing and telecommunication. Seen like this, each electron (or photon) caries a little 'bit' of information about the sample and the goal becomes obtaining as much desired information as possible with the least amount of incoming particles. This would then result in the least amount of sample damage and/or the fastest method. Interestingly, this process is limited by physical boundaries from information theory and quantum physics and the question arises just how far we currently are removed from these ultimate limits as opposed to technological limits or common experimental habits.
In this talk I will give examples of how modern evolutions in phase shaping of electron beams [1], improved detectors [2] and data extraction algorithms [3] bring us closer to these fundamental limits [4].

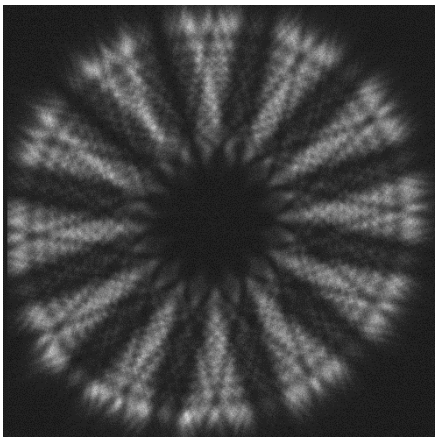

Fig. 1: Interference pattern generated with an AdaptEM 16 element programmable phase plate for electrons, showing the ability to adjust the wavefront of a coherent electron wave as a new degree of freedom in electron microscopy.

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[4] This work summarises results obtained in multiple projects. JV acknowledges funding from the Flemish Research Foundation FWO under grant nrs. G042820N and G042920N. ERC proof of concept project DLV-789598, The eBEAM project is supported by the European Union's Horizon 2020 research and innovation programme FETPROACT-EIC-07-2020: emerging paradigms and communities;The IMPRESS project has received funding from HORIZON EUROPE framework program for research and innovation under grant agreement n. 101094299.

“SPOOKY” QUANTUM AND CLASSICAL USES OF SHAPED ELECTRON BEAMS

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I will summarize the progress of our group on electron beam shaping for applications that include the orbital angular momentum sorter [1], computational ghost imaging [2] and aberration control.
Beam shaping can be useful in classical microscopy but also open a window to more quantum phenomena. Indeed I will highlight the common denominator that connects the concepts of ghost imaging, computational ghost imaging (CGI), coherence determination and inelastic interferometry. I will highlight how present and electron beam shaping technology can provide us with a new way of considering elastic and inelastic scattering. I will describe recent advances in the use of MEMS technology to produce innovative electron optical elements and an ultrafast TEM-based near field light-electron beam modulation approach [3].

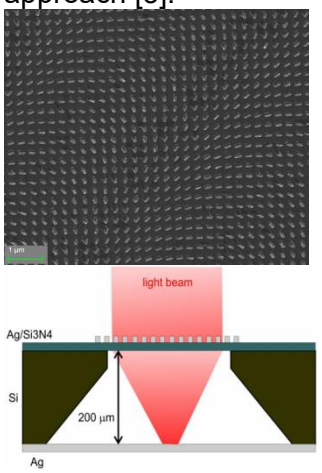

Fig. 1: Metalens system to increase numerical aperture and produce localized electron beam shaping

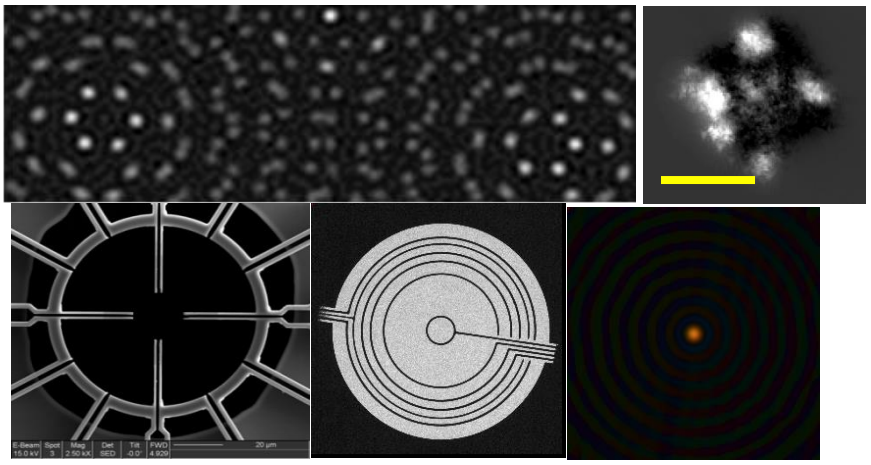

Fig. 2: Top: from left to right: Simulated CGI reconstruction of atomic resolution, experimental CGI with 1 nm resolution (scalebar is 20 nm). Bottom: MEMS design for CGI, for aberration correction and best expected probe in the order of 1Å.

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STRUCTURE RETRIEVAL BY PARAMETRIZEDINVERSE MULTISLICE

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The development of momentum-resolved STEM (4D-STEM) as to experimental performance and ptychographic approaches has paved the way for resolutions beyond the Abbe limit and the inversion of dynamical scattering [1]. Inherent to ptychography is a forward scattering model whose parameters are adjusted to match the experiments. Thick specimens cause thermal diffuse scattering (TDS), thus inverse multislice needs to incorporate frozen phonons (FP) [2]. Here, we suggest a gradient-based inversion in which this is achieved by the stringent atomistic parametrization of the specimen [3] as relativistic forward scattering demands. Phase gratings are optimized based on atom types and their equilibrium positions, and thermal displacements according to the Debye parameter. This assures physically correct potentials [4] and avoids artefacts often observed in non-regularized pixel-wise phase retrieval, treats atom positions, types and specimen temperature as differentiable parameters and includes TDS explicitly. We show that atomic number sensitivity of high-angle scattering is exploited that way, increasing chemical sensitivity. Besides the multimodal specimen, partial coherence of the illumination is incorporated similarly by incoherently averaging independent multislice simulations of ensembles of parametrized probes according to the source size and focal spread. Efficient gradient calculation within a neural network [5] delivers parameter updates despite the complex incoherent summations of probes and specimen. The method is first developed using a simulation study of a $PbZr_{0.2}Ti_{0.8}O_3$ ferroelectric, where Fig. 1 shows the retrieval of the ferroelectric displacement of an O and (ZrTi) in the same column. We then demonstrate the increased chemical sensitivity by reconstructing site occupancies, before we report the retrieval of the ferroelectric displacements in a 4D-STEM experiment of $PbZr_{0.2}Ti_{0.8}O_3$, as shown in Fig. 2. The result is compared to models neglecting TDS, and perspectives for future microscopy and methodological challenges will be outlined.

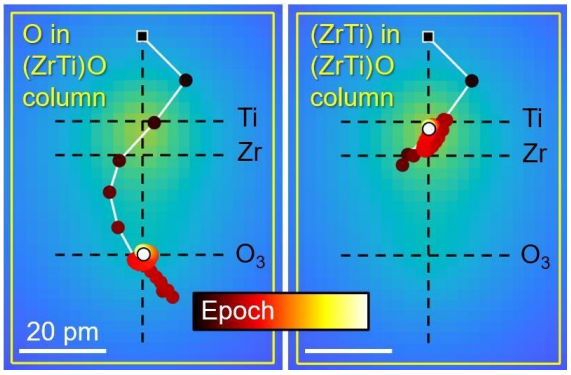

Fig. 1: Reconstruction paths for oxygen (left) and (ZrTi) (right) to obtain ferroelectric displacements. True position: dashed cross.

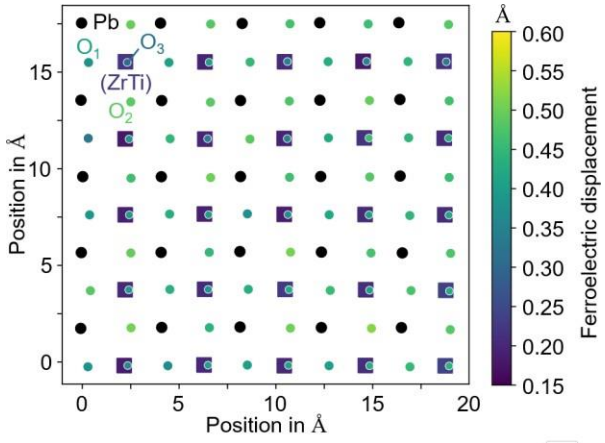

Fig. 2: Measured ferroelectric displacements in $PbZr_{0.2}Ti_{0.8}O_3$ from experimental 4D-STEM data. The precision amounts to 3 pm.

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DYNAMIC INTERPLAY BETWEEN METAL NANOPARTICLES AND OXIDE SUPPORT UNDER REDOXCONDITIONS

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As a PhD student dealing with electron microscopy, I sometimes heard chemists say: “catalysts have a memory”. This statement reflects the fact that small deviations from a defined precursor synthesis protocol and details of the activation procedure, as well as the history of reaction conditions applied, can strongly influence the performance and stability of a catalyst. In the case of noble metal nanoparticles supported on reducible supports, high-temperature reductive activation can induce the so-called SMSI state. It is characterized by the encapsulation of the metal particles by a thin layer of a reduced form of the support material. The effect, which was first observed by Tauster *et al.*[1] can be reversed through a high- temperature oxidation followed by a low-temperature reduction step.[1] The controlled induction of the SMSI state is considered in the literature as a viable means of tuning the chemisorption, selectivity, sintering and dissolution behavior of the metal particles. To investigate the behavior and structural stability of the thin encapsulation layer and its relevance under reactive conditions, we performed operando electron microscopy studies. Direct observation at the atomic scale (Figure 1) has led us to a better understanding of the material chemistry of the combined system and the various faces of metal-support interactions. We were able to see how the system adapts to the surrounding gas-phase and how dynamic and synergistic interactions can lead to the emergence of catalytic function.[2] Meanwhile, I have a better understanding of what was meant by “catalysts have a memory”.

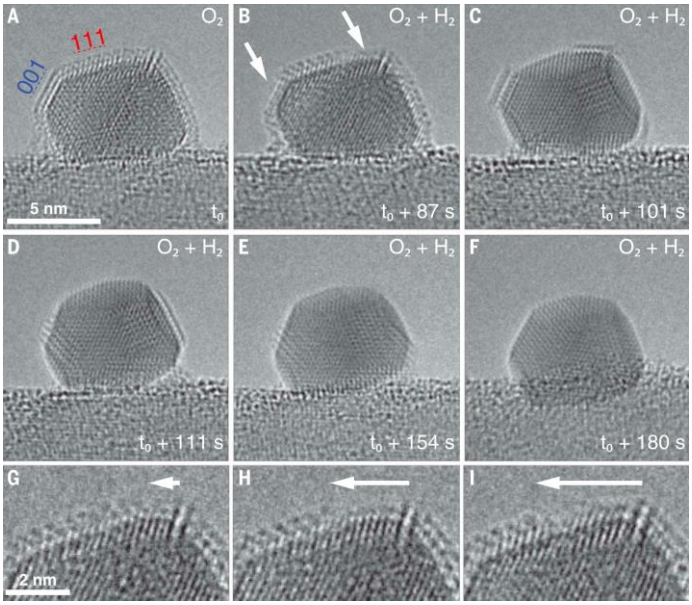

Figure 1: Morphological change upon transition into the redox-active regime.

References:

[1] S. J. Tauster, S. C. Fung, R. L. Garten, J. Am. Chem. Soc. 100, 170–175 (1978)
[2] H. Frey, A. Beck, X. Huang, J.A. Van Bokhoven, M.G. Willinger, Science 376, 982-987(2022)

PICO

2024

Celebrating 20 Years of the ER-C

EIGHTH CONFERENCE ON FRONTIERS OF ABERRATION CORRECTED ELECTRON MICROSCOPY

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Kasteel Vaalsbroek

April 21st – April 25th

2024

POSTER PROGRAMME

MONDAY APRIL 22ND, 2024

POSTER SESSION A — **"Vaalsbroekerhof" castle ground floor**

PA01 UNDERSTANDING DEGRADATION MECHANISMS IN Ni-RICH NMC CATHODE OF LITHIUM-ION BATTERIES – A MULTILAYERED MATTER

Lara Ahrens, RWTH Aachen University, (Germany)

PA02 MECHANISTIC INSIGHTS TO THE ATOMIC-SCALE COARSENING BEHAVIOR OF EXSOLVED CATALYTIC NANOPARTICLES

Dylan Jennings, Forschungszentrum Jülich GmbH (Germany)

PA03 IN-SITU TEM STUDIES OF NOVEL HYBRID NANOWIRES WITH FERROMAGNETIC SHELLS

Anna Kaleta, Polish Academy of Science (Poland)

PA04 IN SITU STUDY OF ELECTRON BEAM INDUCED ETCHING

Stefan Noisternig, University of Vienna (Austria)

PA05 CONTROLLING ACCEPTOR DOPANT SEGREGATION TO TUNE THE CONDUCTIVITY OF BARIUM ZIRCONATE-BASED PROTON CONDUCTORS

Moritz Kindelmann, Forschungszentrum Jülich GmbH (Germany)

PA06 REACTIVE METAL-SUPPORT INTERACTION OF ZINC PALLADIUM NANOPARTICLES ON ZINC OXIDE: AN ENVIRONMENTAL SCANNING TRANSMISSION ELECTRON MICROSCOPY STUDY

Ansgar Meise, Forschungszentrum Jülich GmbH (Germany)

PA07 CONVERGENT BEAM ELECTRON DIFFRACTION OF ADSORBATES ON GRAPHENE

Sara Mustafi, Paul Scherrer Institut (Switzerland)

PA08 UNRAVELING THE ENIGMA OF (Fe,Cr)2B STRUCTURE: EXPLORING ATOM COLUMN INSIGHTS AND DFT CORRELATION

Akhil G. Nair, Indira Gandhi Centre for Atomic Research (India)

PA09 DECIPHERING THE ULTRA-HIGH PLASTICITY IN METAL MONOCHALCOGENIDES

Lok Wing Wong, The Hong Kong Polytechnic University (China)

PA10 UNVEILING DEGRADATION MECHANISMS IN LAYERED Li-RICH CATHODE MATERIALS USING COMBINED IN OPERANDO NEUTRON DIFFRACTION AND 4D-STEM

Tingting Yang, Forschungszentrum Jülich GmbH (Germany)

PA11 MAPPING OF COMPOSITIONS AND OXIDATION STATES IN NANOCATALYSTS

Dalaver Anjum, Khalifa University of Science & Technology (United Arab Emirates)

PA12 ADVANCEMENTS IN AVALANCHE PHOTODIODES THROUGH THE DEVELOPMENT OF AlGaAsBi ALLOY SEMICONDUCTORS

Verónica Braza, Universidad de Cádiz (Spain)

PA13 NANOFABRICATION OF SUPERCONDUCTING AND FERROMAGNETIC STRUCTURES FOR OPERATION IN TEM

Faley Michael, Forschungszentrum Jülich GmbH (Germany)

PA14 CROSS-SECTIONAL PREPARATION OF COMPLEX ENERGY DEVICES AND THEIR CHARACTERIZATION BY ADVANCED MICROSCOPIC METHODS

Charles Ogolla, University of Siegen (Germany)

PA15 NOVEL TOPOTAXIAL EXCHANGE GROWTH OF EuInAs NANOWIRES

Lothar Houben, Weizmann Institute of Science (Israel)

PA16 IDENTIFICATION AND HEALING OF FIB INDUCED DEFECTS IN GaN USING ELECTRON HOLOGRAPHY

Keyan Ji, Forschungszentrum Jülich GmbH (Germany)

PA17 TWO-DIMENSIONAL FEW-ATOM NOBLE GAS CLUSTERS IN A GRAPHENE SANDWICH

Peter Karnthaler, University of Vienna (Austria)

PA18 ELECTROSTATIC EXTENSION OF MAGNETIC PROXIMITY EFFECT IN $La_{0.7}Sr_{0.3}MnO_3$

Qianqian Lan, Forschungszentrum Jülich GmbH (Germany)

PA19 REVEALING THE EPITAXIAL RELATION IN MOCVD GROWN 2D GaS BY STEM

Oliver Maßmeyer, Philipps-Universität Marburg (Germany)

PA20 IN SITU OFF-AXIS ELECTRON HOLOGRAPHY ANALYSIS OF III-V JUNCTIONS FOR SOLAR CELL APPLICATIONS

Vita Mergner, Forschungszentrum Jülich GmbH (Germany)

PA21 STEM ANALYSIS OF MOCVD-GROWN TWO-DIMENSIONAL WS2-MoS2 HETEROSTRUCTURE

Badrosadat Ojaghi, Philipps-Universität Marburg (Germany)

PA22 CORRELATIVE IN SITU SRnanoCT AND (S)TEM IMAGING OF BIODEGRADABLE Mg-BASED ALLOYS

Jan Reimers, Helmholtz-Zentrum Hereon (Germany)

PA23 ASSESSING THE INFLUENCE OF GROWTH INTERRUPTION STAGES ON THE SEGREGATION OF Sb IN ULTRATHIN GaAsSb LAYERS

Daniel Reyes, Universidad de Cádiz (Spain)

PA24 UNCOVERING THE NANOSCALE ARCHITECTURE OF HUMAN ENAMEL WITH ADVANCED STEM TECHNIQUES

Paul Smeets, Northwestern University (USA)

PA25 AI ENHANCED EELS USING BACKSCATTERED LOW ENERGY ELECTRONS

Rasmus Schröder, University Heidelberg (Germany)

POSTER SESSION B — **"Jachtkelder" castle ground floor**

PB01 SPIN HALL EFFECT INDUCED IN-SITU MAGNETIC MEASUREMENTS USING ELECTRON HOLOGRAPHY: A CASE STUDY WITH YIG

Joseph Vas, Forschungszentrum Jülich GmbH (Germany)

PB02 AN ELECTRON BEAM MODULATOR FOR COMPUTATIONAL GHOST IMAGING IN TEM

Lorenzo Viani, University of Modena and Reggio Emilia (Italy)

PB03 TRANSFORMATION OF INORGANIC NANOPARTICLE ELECTRON MICROSCOPY ANALYSIS

MaKayla Walker, Colorado State University (USA)

PB04 MINIATURIZED MATERIAL TESTING DEVICES FOR MULTIMODAL IN SITU MICROSCOPIC STUDIES

Matthias Weber, University of Siegen (Germany)

PB05 DIRECT IMAGING OF DYNAMIC AND RESONANT SPIN PHENOMENA: PROSPECTS FOR CATALYSIS, SPINTRONICS AND MAGNETIC CHARACTERIZATION

Benjamin Zingsem, Forschungszentrum Jülich GmbH (Germany)

PB06 MINDSHIFT: MULTIMODAL INTEGRATION FOR NANOPARTICLE DATA SCREENING USING HIGH-THROUGHPUT FRAMEWORKS IN TRANSMISSION ELECTRON MICROSCOPY

Roberto dos Reis, Northwestern University (USA)

PB07 ELECTRICAL CONTROL OF MAGNETIC PROPERTIES OF Cr2Ge2Te6 – DIRECT OBSERVATION THROUGH CRYO-LORENTZ TEM

Joachim Thomsen, University of California (USA)

PB08 VITROJET: ICE THICKNESS CONTROL AND MEASUREMENT FOR TIME-EFFICIENT SINGLE PARTICLE STRUCTURE DETERMINATION

René Henderikx, CryoSol-World (NL)

PB09 SPARSE ARRAYS FOR FOUR-DIMENSIONAL SCANNING TRANSMISSION ELECTRON MICROSCOPY

Dieter Weber, Forschungszentrum Jülich GmbH (Germany)

POSTER SESSION C	**“Oude Keuken” castle ground floor**
PC01	SARS-COV2 SPIKE PROTEIN: A FUNCTIONAL ANALYSIS OF ALL CRYO-EM STRUCTURES
	Sayan Bhakta, Brazilian Nanotechnology Nat. Lab (Brazil)
PC02	STRUCTURAL CHARACTERIZATION OF P62 HELICAL FILAMENTS IN THEIR ROLE AS SELECTIVE AUTOPHAGY CARGO RECEPTOR
	Lisa Jungbluth, Forschungszentrum Jülich GmbH (Germany)
PC03	INVESTIGATING THE PRACTICAL LIMITS OF CRYO-FIB MILLING FOR FROZEN HYDRATED/HIGH-PRESSURE FROZEN MATERIAL
	David Klebl, Max Planck Institute of Biochemistry (Germany)
PC04	BEAMSHAPING THE FIB FOR LAMELLA PREPARATION - YAY OR NAY?
	Sven Klumpe, Max Planck Institute of Biochemistry (Germany)
PC05	DYNAMICAL SCATTERING IN ICE-EMBEDDED PROTEINS IN CONVENTIONAL TRANSMISSION ELECTRON MICROSCOPY
	Max Leo Leidl, Ludwig-Maximilians-University Munich (Germany)
PC06	ADAPTING A SELENIUM NANOPARTICLE FORMING ENZYME TO PRODUCE EM CONTRAST MARKERS WITH IMPROVED SIZE CONTROL
	Tony Tien, Colorado State University (USA)
PC07	IN SITU STRUCTURAL ORGANIZATION OF THE P62 AUTOPHAGY CARGO RECEPTOR
	Sabrina Berkamp, Forschungszentrum Jülich GmbH (Germany)
PC08	REPLICATION ORGANELLE MEMBRANE REMODELING IN SARS-COV-2 INFECTION
	Kevin Boga, Forschungszentrum Jülich GmbH (Germany)
PC09	CHARGING OF VITREOUS SAMPLES IN CRYOGENIC ELECTRON MICROSCOPY MITIGATED BY GRAPHENE
	Yue Zhang, Maastricht University (NL)

POSTER SESSION D **"Tuinkamer" castle first floor**

PD01 POST-ACQUISITION ABERRATION CORRECTION IN CENTER-OF-MASS IMAGING

Zhiyuan Ding, University of Oxford (UK)

PD02 CRYO-STEM OF BIOLOGICAL MACROMOLECULES: VISUALIZATION BY INTEGRATED DIFFERENTIAL PHASE CONTRAST

Aikaterini Filopoulou, Forschungszentrum Jülich GmbH (Germany)

PD03 OPTIMUM METHODOLOGY FOR ATOMIC-RESOLUTION PTYCHOGRAPHY IN ULTRA-LOW-DOSE CONDITIONS

Hoelen L. Lalandec-Robert, University of Antwerp (Belgium)

PD04 TOWARDS SUB-NM RESOLUTION OF CRYOGENIC PTYCHOGRAPHY SINGLE-PARTICLE ANALYSIS (CRYO-EPTY SPA)

Yu Lei, University of Warwick (UK)

PD05 CONSISTENCY OF DIFFERENT PTYCHOGRAPHIC PROBE RETRIEVAL METHODS

Tizian Lorenzen, Ludwig-Maximilians-Universität Munich (Germany)

PD06 STRUCTURED ILLUMINATION ELECTRON PTYCHOGRAPHY

Peng-Han Lu, Forschungszentrum Jülich GmbH (Germany)

PD07 AUTOMATED 4D STEM CRYO-TOMOGRAPHY

Shahar Seifer, Weizmann Institute of Science (Israel)

PD08 CRYO-STEM FOR BIOLOGICAL MACROMOLECULES

Daniel Mann, Forschungszentrum Jülich GmbH (Germany)

TUESDAY APRIL 23RD, 2024

POSTER SESSION E — **"Vaalsbroekerhof" castle ground floor**

PE01 NON-CLASSICAL CRYSTALLISATION OF CEO2: LIQUIDPHASE TEM AND GAMMA-RADIATION INDUCED STUDIES

Nadezda Tarakina, Max Planck Institute of Colloids and Interfaces (Germany)

PE02 STUDY OF ATOMIC AND ELECTRONIC STRUCTURAL EVOLUTION IN NiFe2O4

Qi Wang, City University of Hong Kong, (China)

PE03 THE STATIC CORROSION OF T91 IN LEAD BISMUTH EUTECTIC WITH DIFFERENT OXYGEN LEVEL

Minyi Zhang, University of Oxford (UK)

PE04 4D-STEM AND EELS ANALYSIS OF COMPLEX C-BASED SENSOR ARCHITECTURES

Charles Ogolla, University of Siegen (Germany)

PE05 4DSTEM-IN-SEM IN EVENT DRIVEN MODE FOR IN SITU APPLICATIONS

Ujjval Bansal, Karlsruhe Institute of Technology, (Germany)

PE06 THE EFFECTS OF DIFFRACTION ON MAGNETIC IMAGING OF MONOCRYSTALLINE THIN FILMS USING STEM-DPC

Sivert Dagenborg, NTNU, (Norway)

PE07 TOWARDS ATOM COUNTING FROM FIRST-MOMENT STEM IMAGES

Yansong Hao, University of Antwerp, (Belgium)

PE08 ACHIEVING ATOMIC PRECISION 3D RECONSTRUCTIONS OF 10 NANOMETER SIZED NANOPARTICLES

Tom Stoops, University of Antwerp, (Belgium)

PE09 ELECTRON PTYCHOGRAPHY WITH AN ELECTROSTATIC PHASE PLATE FOR ELECTRONS

Francisco Vega Ibáñez, University of Antwerp, (Belgium)

PE10 RETRIEVE DOPANTS INFORMATION FROM INLINE HOLOGRAPHY

Yuxuan Zhang, City University of Hong Kong, (China)

PE11 SIMULTANEOUS MAPPING STRAIN AND ELECTROMAGNETIC FIELD AT THE NANOSCALE

Yucheng Zou, Forschungszentrum Jülich GmbH, (Germany)

PE12 MAPPING THE MAGNETIC CIRCULAR DICHROISM USING 5DSTEM

Hasan Ali, Uppsala University, (Sweden)

PE13 A NOVEL LIQUID PURGING METHOD FOR HIGH-RESOLUTION AND ANALYTICAL LIQUID PHASE TRANSMISSION ELECTRON MICROSCOPY FOR UNDERSTANDING ELECTROCHEMICAL PROCESSES

Shibabrata Basak, Forschungszentrum Jülich GmbH, (Germany)

PE14 TRUE QUANTIFICATION OF POLARIZATION AT NITRIDE INTERFACES BY ELECTRON HOLOGRAPHY

Philipp Ebert, Forschungszentrum Jülich GmbH, (Germany)

PE15 FIELD EMISSION DYNAMICS FROM ELECTRON-BEAM IRRADIATED GOLD NANOPRISMS

Kenan Elibol, Max Planck Institute for Solid State Research, (Germany)

PE16 CEFID: A FLEXIBLE PLATFORM FOR ADVANCED SPECTROSCOPIC EXPERIMENTS

Maximilian Haider, CEOS GmbH, (Germany)

PE17 ULTRATHIN MICROCHIP FOR ENVIROMENTAL TRANSMISSION ELECTRON MICROSCOPY

Xiaobing Hu, Northwestern University, (USA)

PE18 LONG-RANGE ELECTRIC FIELDS AND POTENTIALS FROM CONDUCTING AND INSULATING NEEDLES MEASURED BY OFF-AXIS ELECTRON HOLOGRAPHY AND 4D-STEM

Janghyun Jo, Forschungszentrum Jülich GmbH, (Germany)

PE19 OFF-AXIS ELECTRON HOLOGRAPHY OF ELECTRON-BEAM-INDUCED CHARGING OF A POLYSTYRENE NANOSPHERE AT LOW TEMPERATURE

Yan Lu, Forschungszentrum Jülich GmbH, (Germany)

PE20 OPTICAL DESIGNS FOR SIXTH-ORDER GEOMETRICAL ABERRATION CORRECTORS

Shigeyuki Morishita, JEOL Ltd, (Japan)

PE21 PROSPECT FOR TWO-DIMENSIONAL TRANSITION-METAL OXIDE PEROVSKITES BY ELECTRON MAGNETIC CHIRAL DICHROISM IN 3-BEAM ORIENTATION

Jie Ren, University of Hong Kong, (China)

PE22 STRUCTURAL PHASE TRANSITIONS RESOLVED BY ULTRAFAST TRANSMISSION ELECTRON MICROSCOPY

Sophie Schaible, Max Planck Institute for Multidisciplinary Sciences, (Germany)

PE23 COUNTING POINT DEFECTS AT NANOPARTICLE SURFACES BY ELECTRON HOLOGRAPHY

Michael Schnedler, Forschungszentrum Jülich GmbH, (Germany)

PE24 VISUALISING NUCLEATION AND GROWTH MECHANISMS IN SOLID AND LIQUID SOLUTIONS USING IN-SITU TRANS-MISSION ELECTRON MICROSCOPY

Govind Ummethala, Forschungszentrum Jülich GmbH, (Germany)

PE25 ADVANCING FOCUSED ION BEAM TECHNIQUES FOR ENHANCED IN-SITU MEMS-BASED SAMPLE PREPARATION

Vesna Srot, Max Planck Institute for Solid State Research, (Germany)

POSTER SESSION F — **"Jachtkelder" castle ground floor**

PF01 FULLY AUTOMATED MATERIALS CHARACTERIZATION USING CORE LOSS ELECTRON ENERGY LOSS SPECTROSCOPY AND DEEP LEARNING

Arno Annys, EMAT, University of Antwerp, (Belgium)

PF02 HOW TO USE SIMULATED DATA TO TRAIN A NEURAL NETWORK ON CLASSIFYING NANOPARTICLES IN HRTEM IMAGES BASED ON CRYSTALLINITY

Nina Gumbiowski, University of Duisburg-Essen, (Germany)

PF03 COMPUTATIONAL MODELING, SIMULATION, AND OPTIMIZATION OF MEMS PHASE PLATES USING COMSOL MULTIPHYSICS

Payam Habibzadeh Kavkani, University of Modena and Reggio Emilia, (Italy)

PF04 UNSUPERVISED MACHINE LEARNING-BASED STEM DIFFRACTION PATTERN DENOISING FOR ENHANCED GRAIN VISUALIZATION IN PHASE CHANGE MATERIALS

Karina Ruzaeva, Forschungszentrum Jülich GmbH, (Germany)

PF05 INFLUENCE OF THERMAL DIFFUSE SCATTERING ON INVERSE MULTISLICE RECONSTRUCTIONS

Ziria Herdegen, Ludwig-Maximilians-Universität München, (Germany)

PF06 ENHANCING SEMANTIC SEGMENTATION IN HIGH RESOLUTION TEM IMAGES: A COMPARATIVE STUDY OF BATCH NORMALIZATION AND INSTANCE NORMALIZATION

Bashir Kazimi, Forschungszentrum Jülich GmbH, (Germany)

PF07 AN APPROACH FOR ANALYZING 3D STRAIN DISTRIBUTION IN HYBRID AND HETERO NANOWIRES THROUGH NBED AND FEM ITERATIVE MODEL FITTING

Sławomir Kret, Instytute of Physics, (Poland)

PF08 DATA PROCESSING OF IN-SITU TEM TOWARD LIVE PROCESSING

Junbeom Park, Forschungszentrum Jülich GmbH, (Germany)

POSTER SESSION G	**“Oude Keuken” castle ground floor**
PG01	A HIGH-THROUGHPUT CRYO-CORRELATIVE LAMELLA GENERATION PIPELINE FOR IN-SITU CELLULAR STUDIES USING CRYO ELECTRON TOMOGRAPHY Deniz Daviran, Delmic B.V, (NL)
PG02	STRUCTURAL PLASTICITY OF BACTERIAL ESCRT III PROTEIN PSPA IN HIGHER ORDER ASSEMBLIE Esther Hudina, Forschungszentrum Jülich GmbH, (Germany)
PG03	HIGH RESOLUTION ANALYSIS OF SIGLEC-10 RECEPTOR BY CRYOGENIC ELECTRON MICROSCOPY Jahnavi Jangala, Institute de Biologie Structurale, (France)
PG04	STRUCTURAL AND QUANTITATIVE ANALYSIS OF VIPP1 CARPETS David Kartte, Forschungszentrum Jülich GmbH, (Germany)
PG05	AUTOMATED CLUSTERING OF HELICAL FILAMENTS Janus Lammert, Forschungszentrum Jülich GmbH, (Germany)
PG06	MEMBRANE ANALYSIS TOOLKIT – A QUANTITATIVE WAY TO ANALYSE PROTEIN LIPID MIXTURES FROM CRYO-EM IMAGES Philipp Schönnenbeck, Forschungszentrum Jülich GmbH, (Germany)
PG07	STRUCTURAL BASIS OF THE ALLOSTERIC REGULATION OF CYANOBACTERIAL GLUCOSE-6-PHOSPHATE DEHYDROGENASE BY THE REDOX SENSOR OPCA Dmitry Shvarev, Osnabrück University, (Germany)
PG08	CRYO-EM OF AMYLOID FIBRILS FORMED BY MEDICAL INSULIN Simon Sommerhage, Forschungszentrum Jülich GmbH, (Germany)
PG09	ANALYSIS OF CRYO-EM STRUCTURES OF ARBOVIRUSES Katie Cristina Takeuti Riciluca, Brazilian Nanotechnology Nat. Lab, (Brazil)

POSTER SESSION H	**“Tuinkamer” castle first floor**
PH01	ER-C 2.0
	Markus Schmitz, Forschungszentrum Jülich GmbH, (Germany)
PH02	CRYO EM USER FACILITY AT ERNST RUSKA CENTER, FORSCHUNGSZENTRUM, JULICH, GERMANY
	Saba Shahzad, Forschungszentrum Jülich GmbH, (Germany)
PH03	INCYTE – THE INTEGRATED USER FACILITY ALONG THE PROCESS CHAIN OF COMPLEX SENSOR / DEVICE DEVELOPMENT
	Benjamin Butz, University of Siegen, (Germany)
PH04	CRYOEM TECHNIQUES AT ISTA: MULTI-MODAL APPROACHES REVEAL NOVEL INSIGHTS INTO EXTRACELLULAR MATRIX, ACTIN FILAMENT STRUCTURES, AND POXVIRUS CORES
	Victor-Valentin Hodirnau, Institute of Science and Technology Austria, (Austria)
PH05	ReMade@ARI: A HUB FOR MATERIALS RESEARCH
	Marta Lipińska-Chwałek, Forschungszentrum Jülich GmbH, (Germany)
PH06	RIANA: RESEARCH INFRASTRUCTURE ACCESS IN NANOSCIENCE & NANOTECHNOLOGY
	Marta Lipińska-Chwałek, Forschungszentrum Jülich GmbH, (Germany)
PH07	INTEROPERABLE ELECTRON MICROSCOPY PLATFORM FOR ADVANCED RESEARCH AND SERVICES (IMPRESS)
	Amir Tavabi, Forschungszentrum Jülich GmbH, (Germany)
PH08	ELECTRON MICROSCOPY WITHIN THE EUROPEAN JOINT VIRTUAL LAB ON ARTIFICIAL INTELLIGENCE, DATA ANALYTICS AND SCALABLE SIMULATION (AIDAS)
	Dieter Weber, Forschungszentrum Jülich GmbH, (Germany)
PH09	THE EM GLOSSARY: A COMMUNITY EFFORT TOWARDS A HARMONISED TERMINOLOGY IN ELECTRON MICROSCOPY
	Rasmus Schröder, Universität Heidelberg, (Germany)

MONDAY APRIL 22ND, 2024

AND

TUESDAY APRIL 23RD, 2024

POSTER SESSION A / E	**SPONSORS** **"Vaalsbroekerhof" castle ground floor**
PS AE01	LATEST DEVELOPMENT IN EELS Saleh Gorji, Ametek GmbH, BU EMT (Gatan / EDAX), (Germany)
PS AE02	TBD Maximilian Haider, CEOS GmbH, (Germany)
PS AE03	FAST 4D STEM WITH ARINA HYBRID-PIXEL DETECTOR Daniel Stroppa, DECTRIS Ltd, (Switzerland)
PS AE04	LATEST INSTRUMENTATION DEVELOPMENTS FOR DYNAMIC STEM APPLICATIONS Manuel Reinhard, JEOL (Germany) GmbH, (Germany)
PS AE05	ADVANCES IN CHARACTERIZATION OF ATOMIC STRUCTURE IN MATERIALS OVER LARGE ROIS BY PRECESSION-ASSISTED 4D-STEM Dirk van der Wal, TESCAN GROUP, (Czech Republic)
PS AE06	TOWARDS RELIABLE INVESTIGATIONS OF MATERIALS IN THEIR NATIVE STATE Maria Meledina, Thermo Fisher Scientific, (NL)

POSTER SESSION B / F	**SPONSORS** **"Jachtkelder" castle ground floor**
PS BF01	STEM EDS IN SEM AND TKD COMBINED FOR ELEMENTAL AND CRYSTALLOGRAPHIC ANALYSES Meiken Falke, Bruker Nano GmbH, (Germany)

PS BF02 CRYOCLOUD: A CLOUD-NATIVE CRYOEM DATA ANALYSIS PLATFORM FOR INCREASED ACCESSIBILITY, THROUGHPUT AND COLLABORATION

Robert Englmeier, CryoCloud, (NL)

PS BF03 VITROJET: USE CASES SHOW VERSATILITY FOR GRIDS AND SAMPLES

René Henderikx, CryoSol-World, (NL)

PS BF04 STREAMLINING GRAPHENE LIQUID CELL PREPARATION: VITROTEM'S NAIAD SYSTEM

Sina Sadighikia, VitroTEM, (NL)

POSTER SESSION C / G **SPONSORS**
"Oude Keuken" castle ground floor

PS CG01 STABLE CRYOGENIC IN SITU BIASING AND HEATING SYSTEM FOR ATOMIC RESOLUTION (S)TEM

Mia Andersen, DENSsolutions, (NL)

PS CG02 A NEW WORKFLOW FOR MANAGING LARGE IN SITU DATASETS FROM HIGH FRAME RATE TEM DATA

David Nackashi, Protochips, Inc, (USA)

PS CG03 LIQUID HELIUM TEM SAMPLE HOLDER: SWIFT COOL-DOWN AND LONG HOLDING TIME

Denys Sutter, condenZero AG, (Switzerland)

POSTER SESSION D / H **SPONSORS**
"Tuinkamer" castle first floor

PS DH01 DYNAMICAL STUDIES COMBINING IN SITU 4D-SPED MAPPING AND PIXELATED DETECTORS

Athanasios Galanis, NanoMEGAS SPRL, (Belgium)

PS DH02 APPLICATION OF FRAME-BASED AND EVENT-BASED DIRECT ELECTRON DETECTORS IN ELECTRON MICROSCOPY: MERLINEM, CHEETAH M3 AND CHEETAH T3

Matúš Krajňák, Quantum Detectors Ltd, (UK)

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2024

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POSTER ABSTRACTS

UNDERSTANDING DEGRADATION MECHANISMS IN Ni-RICH NMC CATHODE OF LITHIUM-ION BATTERIES – A MULTILAYERED MATTER

Lara Ahrens[1,2*], Kilian Vettori[3], Shibabrata Basak[4], Rüdiger-A. Eichel[4], Joachim Mayer[1,2]

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[2]Forschungszentrum Jülich, Ernst Ruska-Centre for Microscopy and Spectroscopy with Electrons, Wilhelm-Johnen-Straße, 52428, Jülich, Germany
[3]Justus-Liebig-University, Physical-Chemical Institute, Heinrich-Buff-Ring, 35392, Gießen
[2]Forschungszentrum Jülich, Institute of Energy and Climate Research (IEK-9), Wilhelm-Johnen-Straße, 52428, Jülich, Germany

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Lithium-ion batteries (LIBs) have become an integral part of our everyday lives and play an important role in traffic transformation, particularly in electrical vehicles (EVs). [1,2] Anode, electrolyte and cathode are the three main components determining the performance of LIBs. Ni-rich NMC ($Li_xNi_yMn_zCo_{1-y-z}O_2$, with $x > 0$, $y > 0.6$) is one of the most promising cathode materials. [1,2] The increased Ni-content leads to a higher capacity, high energy density and lower costs, making it well-suited for long-range vehicles. However, Ni-rich NMC cathodes suffer from structural and thermal instabilities resulting in server capacity fading and a reduced lifespan. [1,2] Previous studies have shown that electrochemical degradation in form of Cathode-Electrolyte-Interface (CEI) formation as well as structural degradation manifested as phase changes, contribute to the diminished performance of Li-ion batteries. Although, there is no doubt that these phenomena appear, it is controversially discussed which of the degradation mechanism is dominate, which might be negligible and which might even have a positive impact, at least to a certain extent, on the electrochemical performance. [2-5]
In order to develop targeted material solutions and to overcome the drawbacks of capacity fading and decreased lifespan, it is important to address the issue. Therefore, we kept a set of battery half-cells under the harsh condition of 4.5V for days up to weeks to trigger degradation mechanisms in the Ni-rich cathode material. Using both low and high-resolution scanning transmission electron microscopy (STEM) and spectroscopy, this study aims to shed light on this controversy discussion.

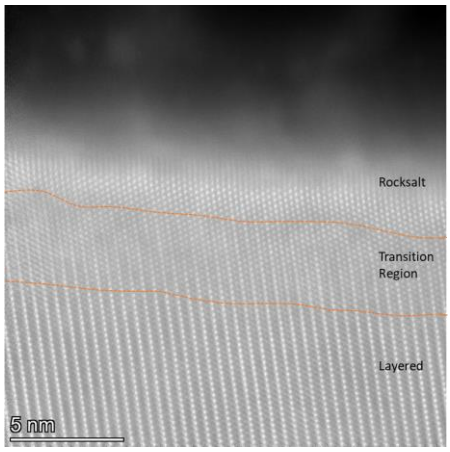

Fig. 1: HR-STEM image of layered Ni-rich NMC with reconstructed surface layer.

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MECHANISTIC INSIGHTS TO THE ATOMIC-SCALE COARSENING BEHAVIOR OF EXSOLVED CATALYTIC NANOPARTICLES

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The development of nanostructured composite materials is critical for improving the efficiency of a variety of heterogeneous catalytic processes. Metal exsolution is a promising synthesis route for the formation of oxide-supported metal catalysts, allowing for the formation of well dispersed and highly active metal nanoparticles. While exsolution is often proposed as a route for enhancing particle stability (thus leading to catalytic systems with increased efficiency and extended lifetimes), recent work has shown that there are still limitations to the thermal stability of exsolved nanoparticles. Provided this, a fundamental understanding of the factors which impact the stability of exsolved nanoparticles remains essential to the development of optimized catalytic systems. In this work, an environmental STEM is combined with a careful sample preparation process to allow for atomic-resolution secondary electron (SE) imaging during *in situ* STEM exsolution experiments.

An epitaxially grown Ni-doped strontium titanate thin film is utilized for *in situ* experiments, which has a nanostructure that consists of a Ni-doped strontium titanate matrix with embedded, heteroepitaxial NiO nanocolumns. First, exsolution from this material is characterized, providing evidence that two distinct populations of Ni nanoparticles form – those that precipitate above second-phase nanocolumns, and those which nucleate above the matrix. The dynamics of exsolved nanoparticles post-exsolution are observed by *in situ* STEM, with particular focus on the different behaviors of the two types in regards to Ostwald Ripening and particle migration. Fundamental insights to particle migration include the measurement of random-walk kinetics, along with particle shape changes during movement which contradict classical understanding of the atomistic mechanism of particle migration. Additionally, the Ostwald Ripening-correlated particle dissolution process is captured *in situ* with atomic resolution SE imaging, providing evidence for surface modification in the immediate aftermath of particle dissolution.

IN-SITU TEM STUDIES OF NOVEL HYBRID NANOWIRES WITH FERROMAGNETIC SHELLS

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Hybrid nanostructures, particularly core-shell semiconductor-ferromagnet nanowires (NWs), hold significant promise for emerging spintronic nanotechnologies [1]. This study employs Molecular Beam Epitaxy (MBE) to grow NWs with wurtzite (WZ) GaAs semiconductor cores and three types of ferromagnetic shells: I) MnAs – soft ferromagnetic metal; II) MnGa and FeGa – hard ferromagnetic metals; III) (Ga,Mn)As – diluted ferromagnetic semiconductor (Fig. 1a). Their structural characterization, i.e. core-shell interfaces, crystallographic orientation, lattice mismatch-induced strain, is conducted using Transmission Electron Microscopy (TEM).

A central focus of our research involves the thermal manipulation of (Ga,Mn)As shell (NWs III-type), using an in-situ heating TEM technique. Our findings demonstrate that annealing WZ-(Ga,Mn)As leads to the formation of tensile-strained α-MnAs nanocrystals (NCs), embedded semi-coherently in the WZ-GaAs matrix, manifesting ferromagnetic properties up to 127°C [2], in contrast to bulk α-MnAs, with a Curie temperature (T_C) ~ 40°C.

In-situ scanning TEM (STEM) results shown in Fig. 1 reveal structural changes in WZ-(Ga,Mn)As during annealing at increasing temperatures (Fig. 1b). Properly chosen STEM conditions enable the visibility of Mn-rich nanoclusters (brighter) at each stage of Mn segregation: 1) nucleation of coherent, strained WZ nanoclusters (Mn_{Ga}), 2) nanocluster phase transition from WZ-MnAs to α-MnAs, and 3) growth of semi-coherent α-MnAs NCs. Consequently, Mn precipitation is captured and advanced image processing is employed to automatically analyze NW images, providing information about size and distribution of MnAs NCs (Fig. 1c,b) for future granular GaAs:MnAs system design.

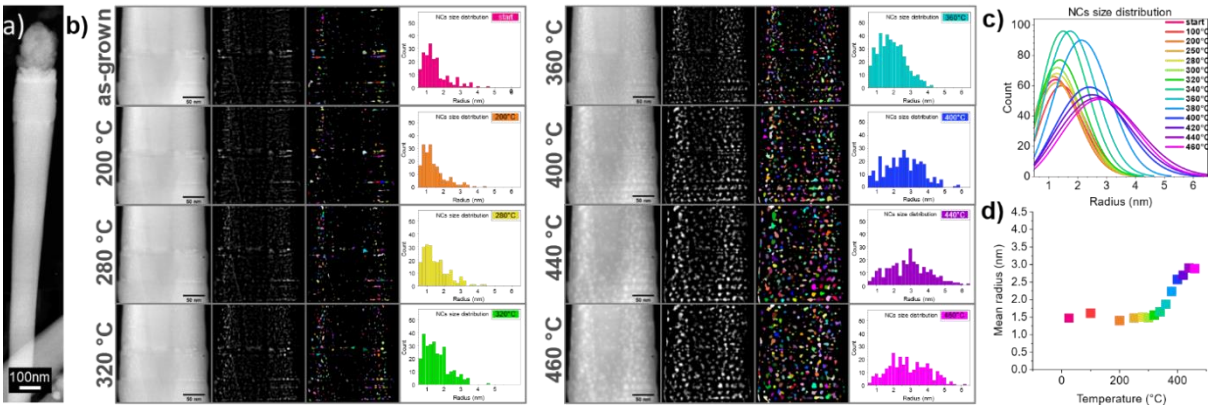

Fig. 1 In-situ annealing: a) STEM image of as-grown NW with (Ga,Mn)As shell (NW III-type), b) Exemplary in-situ experiment (NW kept at increasing temperatures for 10 minutes). Each row (temperature) contains STEM original image, image after image processing, image with segmented and color-coded NCs and their histograms, c) Normal distribution curves for each temperature. d) NCs mean radius as a function of annealing temperature.

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IN SITU STUDY OF ELECTRON BEAM INDUCED ETCHING

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Aberration corrected (scanning) transmission electron microscopy (S)TEM at low acceleration voltages is frequently used to analyse beam sensitive materials and even to manipulate them on an atomic scale [1,2]. For these applications electron beam induced etching (EBIE) is an important effect that needs to be understood to minimize or even control its impact.
We present two studies of EBIE on a 2 nm ultra-thin amorphous carbon foil in the STEM by leaking in pure oxygen into the microscope column. The studies were carried out in situ at a Nion Ultra Stem 100 having a leak valve system for the sample stage area. It is part of a system for Controlled Alteration of Nanomaterials in Vacuum down to the Atomic Scale (CANVAS) [3].
In a first study we succeeded to analyse the etching rates of the foil during imaging conditions while scanning the electron beam and acquiring high angle annular dark field (HAADF) image series. We used different vacuum levels: ultra high vacuum (UVH) of ~ 5 x 10^{-10} mbar as standard operating condition and vacuum conditions of up to 7.0 x 10^{-7} mbar when leaking in pure oxygen. The chosen acceleration voltages were 55 and 80 kV. Our results demonstrate that at low acceleration voltages EBIE can be the dominant factor for sample damage, leading to a thickness reduction depending on the oxygen concentrations in the microscope column [4]. To explain the non-trivial dependency of the etching rates on the electron flux a scanning etching model is worked out.
In a further study we focused on the interaction range of EBIE in the case of a non-contact electron beam setting at ~ 7 x 10^{-7} mbar oxygen pressure and 55 kV acceleration voltage. During this study the aberration corrected focused electron beam was parked or scanned inside nanometer sized holes made in the ultra-thin amorphous carbon foil and the observed hole growth was measured. The non-contact setting reduces diffusion effects and makes it possible to study delocalized EBIE. The measured EBIE effect extends up to ~ 5 nm from the angstrom sized electron beam. This long interaction range compared to the beam size can be explained by the deloalized inelastic scattering process [5] of beam electrons with oxygen molecules and also by the electron flux in the beam tails that suffice for EBIE.

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CONTROLLING ACCEPTOR DOPANT SEGREGATION TO TUNE THE CONDUCTIVITY OF BARIUM ZIRCONATE-BASED PROTON CONDUCTORS

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Acceptor-doped barium zirconates are of major interest as proton conducting electrolyte materials for electrochemical applications at intermediate operating temperatures. The conduction of protons through polycrystalline yttrium doped barium cerium zirconates (BZCY) is blocked by the formation of space charge regions at grain boundaries caused by a positive core charge. During high temperature sintering, the positive core charge acts as a driving force for acceptor dopant segregation to the grain boundary. Yttrium segregation to grain boundaries has been observed in sintered ceramics but the fundamental relationships between the degree of segregation and protonic conductivity are not explored. Here, we present a comprehensive study of the influence of yttrium segregation on the chemical composition and structure at grain boundaries in BZCY and its impact on electrochemical properties. We designed an out-of-equilibrium model material, that displays no observable Y segregation to the grain boundary and used it as a starting point to observe the kinetics of segregation and the induced changes in grain boundary conductivity after varied thermal histories. Furthermore, we coupled the electrochemical results derived from impedance spectroscopy to atomic resolution transmission electron microscopy. We discovered that atomically sharp acceptor segregation drastically increases the grain boundary conductivity both in the model system and reference samples processed by the industrially applied solid state reactive sintering (SSRS) route.

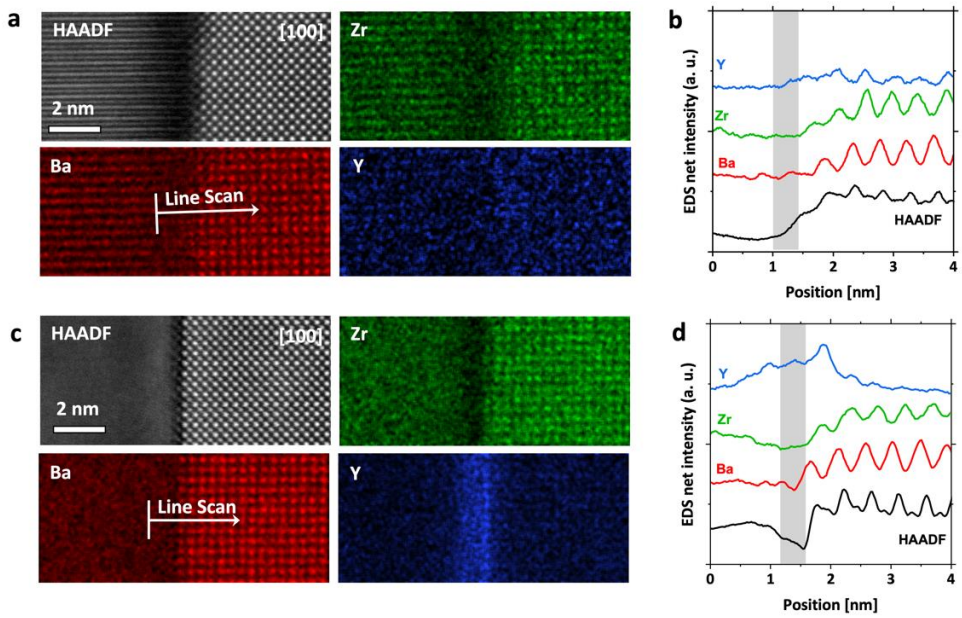

Fig. 1: HR-STEM EDS mappings of a non-equilibrium grain boundary without Y segregation (a, b) and a grain boundary after equilibration at 1600°C for 5h showing atomically sharp Y segregation.

REACTIVE METAL-SUPPORT INTERACTION OF ZINC PALLADIUM NANOPARTICLES ON ZINC OXIDE: AN ENVIRONMENTAL SCANNING TRANSMISSION ELECTRON MICROSCOPY STUDY

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Supported nanoparticles (NPs) are important in various applications, such as nanomedicine, nanotechnology, and catalysis. In general, the support enhances the utilization efficiency of metallic nanoparticles by maintaining their fine distribution and slowing down deactivation mechanisms such as sintering. Often, the interaction between particle and support during preparation leads to profound changes in the composition, morphology and catalytic performance of the system. This is referred to as Reactive Metal-Support Interaction (RMSI). [1] During the reduction of palladium oxide NPs supported on zinc oxide, RMSI is expected to be the driving force for the formation of zinc palladium alloy NPs due to the hydrogen spillover reaction. [2], [3]
In the present study, morphological and structural changes during reduction of PdO/ZnO are studied by performing *in situ* experiments using environmental scanning transmission electron microscopy in a hydrogen atmosphere. The interaction between NPs and support, as well as the reduction and transformation process of the NPs, are explored on the atomic scale.

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CONVERGENT BEAM ELECTRON DIFFRACTION OF ADSORBATES ON GRAPHENE

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Convergent beam electron diffraction (CBED) on 2D materials provides a method for high-resolution imaging of individual particles deposited on graphene. A single CBED pattern contains information of a diffraction pattern and a real-space image of the sample, where the zero-order CBED disk represents an in-line hologram of the sample [1]. The combination of the CBED pattern with holography reconstruction algorithms allows phase information to be obtained [2]. Moreover, by moving the sample along the z axis (along the probing convergent beam), CBED allows regulating the radiation dose on the sample. This renders the technique ideal for studying radiation sensitive materials and allows us to establish the resolution limits of the technique as function of the required radiation dose. In this study, by employing simulations and experiments, we investigate the optimal experimental imaging conditions for applying CBED to image adsorbates.

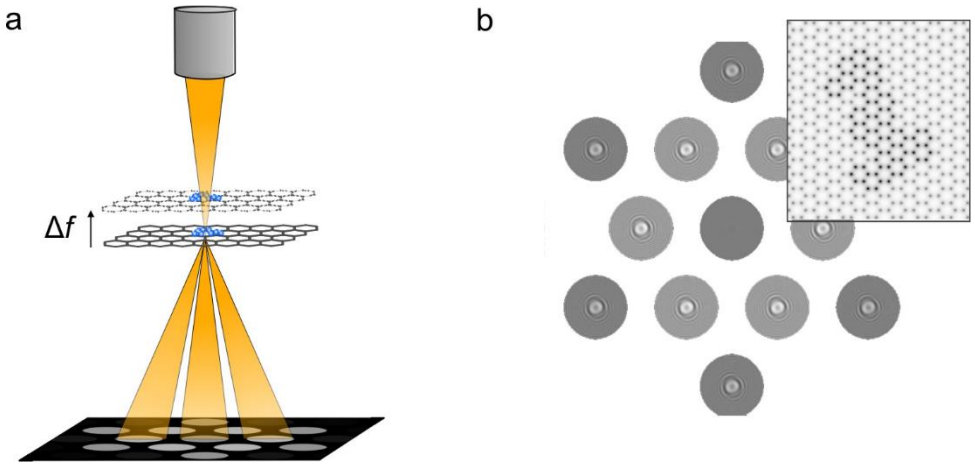

Fig. 1: (a) Sketch of the experimental scheme for CBED. Δf is the defocus. (b) Simulated CBED pattern of a carbon patch on top of graphene. Inset: The phase distribution of the transmission function.

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UNRAVELING THE ENIGMA OF $(Fe,Cr)_2B$ STRUCTURE: EXPLORING ATOM COLUMN INSIGHTS AND DFT CORRELATION

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This work narrates a comprehensive analysis of the atomistic-level characterization of M_2B in high boron (5 wt.%) P91 steel, employing probe aberration-corrected Scanning Transmission Electron Microscope (STEM) techniques. The alloy designing involved thermodynamic calculation using Thermocalc, followed by alloy synthesis through high vacuum arc melting. The material is a composite consisting of relatively softer α-Fe (ferrite) and a hard/brittle $(Fe,Cr)_2B$ (boride) as concluded from multi-scale STEM-based characterization techniques. STEM Energy-dispersive X-ray spectroscopy (XEDS) facilitated a quantitative assessment of the composition, and the stoichiometry of the borides, (Fig.1a). STEM-XEDS also demonstrated a disordered-phase structure, with Cr randomly substituting Fe in the boride lattice, resulting in $(Fe,Cr)_2B$ which is structurally tetragonal Fe_2B. The intensity variations in STEM-High Angle Annular Dark Field (HAADF) atomistic images (Fig.1b,c) were attributed to Cr substitution in Fe lattice and further confirmed using simulation (Dr. Probe). The use of STEM - Integrated Differential Phase Contrast (iDPC) provided an insight into the $(Fe,Cr)_2B$ structure, revealing the projection of Fe/Cr and B atom columns along [100] (Fig.2a,b). However, the observed delocalization of B atom columns prompted us to employ density functional theory (DFT) calculations for estimating the effect of Cr substitution on B-B bond length, which was however, found to have minimal impact. Further theoretical calculations confirmed that the energy imparted by the incident electrons (Fig.2c) during imaging caused the observed B atom delocalization. This research substantially enhances the ability to understand the impact of Cr on the Fe_2B phase and elucidates B atom delocalization induced by high-energy electron beams. Interestingly, the material has been observed to possess exceptional compressive strength. This understanding is expected to open up new avenues for applications of otherwise less utilized high B-containing steels.

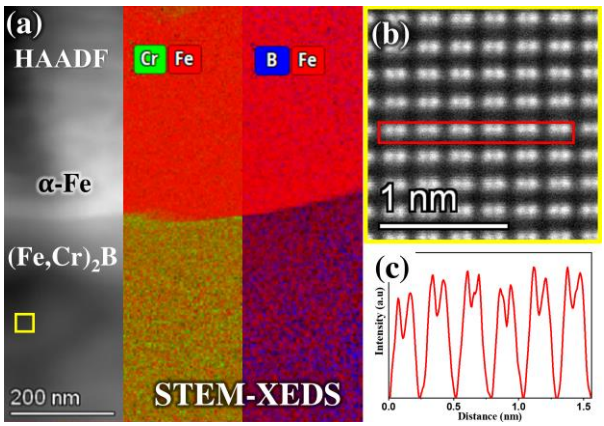

Fig. 1: (a) STEM-(HAADF+XEDS) of $(Fe,Cr)_2B$ and α-Fe, (b) HAADF showing atomic arrangement with corresponding (c) atom column contrast in $(Fe,Cr)_2B$.

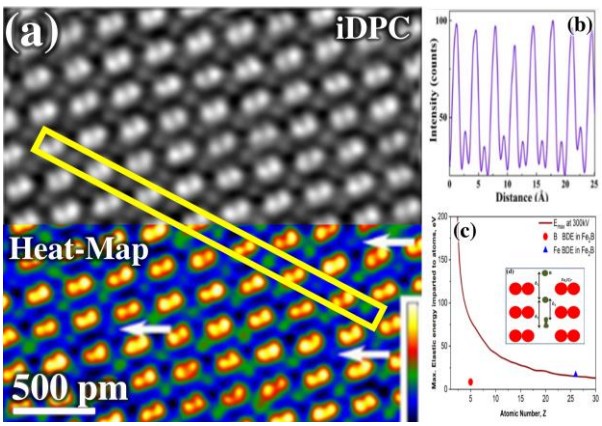

Fig. 2: (a) STEM-iDPC and heatmap showing inconsistencies in B atom position, (b) Intensity profile of (Fe,Cr) and B, (c) Energy imparted by 300 keV electron as f(z).

DECIPHERING THE ULTRA-HIGH PLASTICITY IN METAL MONOCHALCOGENIDES

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The quest for electronic devices that offer flexibility, wearability, durability, and high-performance, while simultaneously exploring new additive manufacturing technologies to enhance the plasticity and elasticity of inorganic semiconductors, has spotlighted two-dimensional (2D) van der Waals (vdW) materials as potential next-generation semiconductors. Especially noteworthy is indium selenide (InSe), which has demonstrated surprising ultra-high plasticity. The recent achievement of a high-performance ballistic transistor utilizing 2D InSe has further sparked considerable interest. In an effort to deepen our understanding of this unusual plasticity in 2D vdW materials and to explore additional inorganic plastic semiconductors, we have conducted in-depth experimental and theoretical investigations on MX (M = In, Ga; X = S, Se, Te) and MX_2 (M = Mo, W; X = S, Se, Te) vdW layered materials. We have discovered a novel and general plastic deformation mode in MX materials, which is facilitated by the synergetic effect of phase transitions, inter-layer gliding, and microcracks. This is in contrast to crystals with strong atomic bonding, such as metals and ceramics, where plasticity is primarily driven by dislocations. Via atomic-scale transmission electron microscopy, *in situ* experiments, X-ray diffractometer, Raman spectroscopy, first-principles calculations, and molecular dynamics simulations, we have unveiled that the key to generating numerous microcracks in a well-synchronized manner, while avoiding crack coalescence, growth, or macroscopic fracture, is the facile phase transitions associated with switchable stacking order and increased interlayer gliding barriers resulting from the 2H-to-3R phase transition in MX, but not in MX_2. By harnessing the 3R pinning effect, it is possible to cease the propagation of microcracks, resulting in enhanced deformability. The discovery of ultra-high plasticity and the novel phase transition mechanism combining microcrack deformation found in 2D MX materials, could be useful for the design and the development of high-performance inorganic plastic semiconductors.

UNVEILING DEGRADATION MECHANISMS IN LAYERED Li-RICH CATHODE MATERIALS USING COMBINED IN OPERANDO NEUTRON DIFFRACTION AND 4D-STEM

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Layered Li-rich nickel-cobalt-manganese (LR-NCM) oxides are promising materials for applications as cathodes in next-generation high-capacity power batteries[1]. However, the commercialization of LR-NCMs is challenging due to their cycling instability, which originates from their intrinsic structural characteristics and deterioration processes. Until now, neither their structures, which can comprise two-phase nanocomposites or only single-phase solid solutions, nor their degradation pathways, have been clarified. Recently, four-dimensional scanning transmission electron microscopy (4D-STEM), which involves recording 2D images using converged electron probes over 2D grids of probe positions, has developed rapidly. the technique allows for versatile data acquisition and precise structural analysis of material properties such as phase, strain and thickness without some of the limitations of other atomic-resolution imaging techniques, while also providing flexibility in reducing electron dose and using larger fields of view[2-3]. In contrast, neutron diffraction is considered to be the most suitable technique to deliver structural insight about Li-ion batteries, since neutrons are sensitive to Li, while retaining favorable scattering contrast to transitional metal (TM) elements and exhibiting strong penetration[4]. In particular, in operando neutron diffraction can be used to follow real-time structural evolution in Li-ion batteries non-destructively. Here, we use both techniques to explore the degradation mechanisms of LR-NCM cathode materials. We prepared a LR-NCM | graphite full cell and carried out both in operando neutron diffraction and low dose and precession-assisted 4D-STEM to map spatially-averaged structural evolution in real time over a large field of view and visualize light (O, Li) and heavy (TM) atoms simultaneously with atomic spatial resolution before, during and after cycling. Our preliminary results show that LR-NCM initially has a local partially ordered structure, which includes Li_2TMO_3 (monoclinic) and $LiTMO_2$ (trigonal) phases with multiple planar defects. The material is therefore a solid solution single phase. During cycling, several intermediate states form. Importantly, the Li-Mn ordered monoclinic phase transforms to a disordered trigonal phase, with additional defect formation after cycling, which is responsible for performance reduction. Our results provide information about the relationship between microstructure degradation and performance reduction of the cycled LR-NCM, paving the way for the development of high-performance cathodes.

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MAPPING OF COMPOSITIONS AND OXIDATION STATES IN NANOCATALYSTS

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The properties of materials change at nanoscale primarily due to very high surface-to-volume ratio and increasing the number of atoms on the surface. These reasons are important for the emergence of catalytic properties in such nano size materials or nanomaterials [1]. Today, nanomaterials are widely used as Nanocatalysts in the field of Nanochemistry [2].

In this poster presentation, elemental mappings of various Nanocatalysts by using STEM-EDS, and STEM-EELS will be presented. Along with elemental mapping, the oxidation states using STEM-EELS are mapped for 3d and 4f metal elements present in the Nanocatalysts. Elemental mapping is performed for 3d-metals based Nanocatalysts having same elements namely Cu, Zn, Al, and O but in different morphologies and synthesis methods. In the same way, 4f and 3d metal based Nanocatalysts were analyzed to generate elemental maps and oxidation states (Fig. 1). Some general rules are drawn from the presented results acquired with STEM-EDS and STEM-EELS. It is concluded from the presented results that TEM offers a powerful way for analyzing Nanocatalysts from nano to atomic scales.

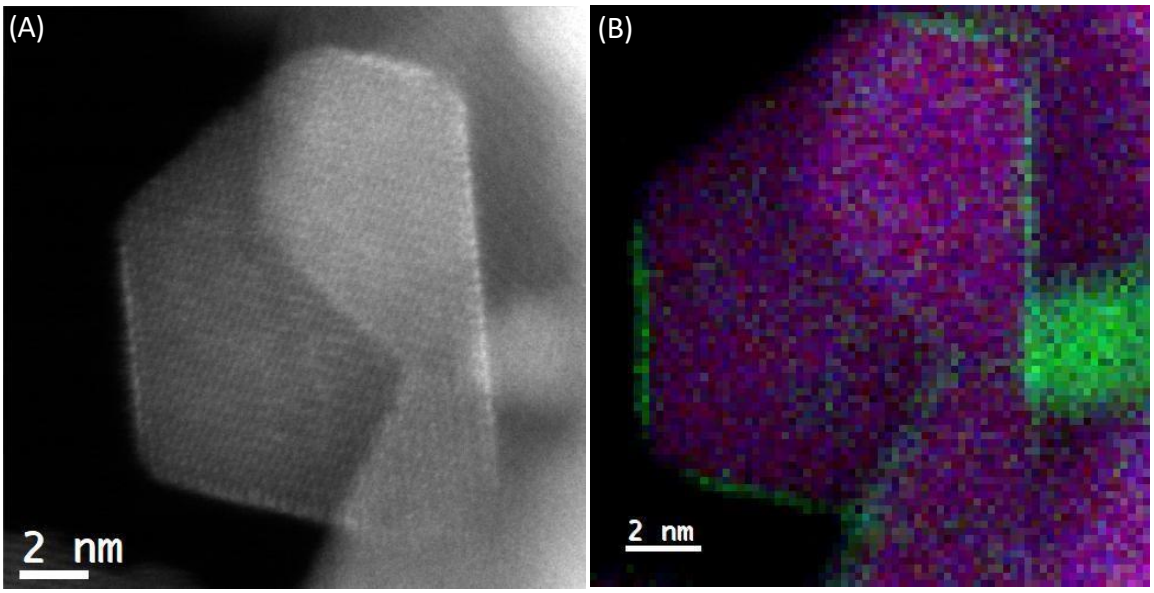

Fig. 1: STEM-EELS analysis of Ce-coated Co_2O_3 nanoparticles (NPs). (A) Aberration corrected HAADF-STEM of Ce coated Co_2O_3 NPs. (B) RGB composite of Co (red), Ce (green), and O (blue) showing one-atom thick layer of Ce atoms was found to covering a Co_2O_3 NP.

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ADVANCEMENTS IN AVALANCHE PHOTODIODES THROUGH THE DEVELOPMENT OF AlGaAsBi ALLOY SEMICONDUCTORS

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III-V semiconductor avalanche photodiodes (APDs) have a significant advantage in the fabrication of high-performance near-infrared (NIR) photodetectors [1]. They can overcome the limitations of conventional materials that require high voltage or low-temperature operating conditions. A promising approach lies in alloying GaAs with up to about 5% Bi. The significant spin-orbit splitting energy in the valence band ascribed to Bi results in a hole ionisation coefficient reduction while the electron ionisation coefficient keeps nearly unaffected [2]. Additionally, the addition of Al in GaAsBi alloys is predicted to synergistically affect these parameters. However, the growth of nanostructures with diluted Bi-containing alloys presents significant challenges. Precise management of growth temperature and atomic fluxes is required to avoid undesired phenomena such as ordering, segregation, or droplet formation. These phenomena are frequent due to the low miscibility and solubility between Bi and GaAs [3, 4]. The extra-addition of Al further complicates the growth of AlGaAsBi alloys. However, its effect is not yet fully understood.

In this work we analyse the compositional distribution and structural quality of AlGaAsBi layers used as active regions together AlGaAs barriers for APDs. The influence of different parameters, such as Al and/or Bi flux, growth temperature, and III/As ratio were examined using transmission-scanning electron microscopy, Nomarsky microscopy, and photoluminescence techniques. Results indicate a complex interaction due to the concomitant presence of Bi and Al. On one hand, increasing the Bi flux results in higher Bi content and improved AlGaAsBi/AlGaAs interface roughness. However, it also leads to the progressive formation of surface droplets, which is associated with the generation of high segregation regions. On the other hand, the Al presence undesirably enhances the Bi segregation, delaying its effective deposition into the active layer. On the contrary, lowering the temperature intensifies the incorporation of Bi, but at the expense of an increase in the crystal defects density. This work analyses the relationship between structural characteristics, growth conditions, and optical properties.

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NANOFABRICATION OF SUPERCONDUCTING AND FERROMAGNETIC STRUCTURES FOR OPERATION IN TEM

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Nanoscale superconducting (Nb, TiN, NbN and BSCCO) and ferromagnetic (Permalloy) structures were prepared on SiN, SiO_2 and SiC membranes for experiments in a transmission electron microscope (TEM) [1-4]. Exfoliated e-beam transparent $Bi_2Sr_2Ca_2Cu_3O_{10}$ (BSCCO) flakes were fixed on 4-contact chips to study correlations between temperature, electron transport properties and behavior of Abrikosov vortices. Metals Ti and Nb or nitrides TiN and NbN were combined into 3-layer heterostructures for adjusting superconducting parameters through the proximity effect. This allowed to reduce spread of parameters in ultrathin superconducting films and to fit operation temperature to the most stable temperature of the commercial TEM sample holder that was cooled using flow of liquid helium. An on-chip thermometer was used also as a heater, allowing the sample temperature to be changed in the range from 400 K to 5 K and back in a few seconds. Nanoscale superconducting quantum interference devices (nanoSQUIDs) with nanobridge Josephson junctions (nJJs) were prepared at a distance of below 200 nm from the tip of a cantilever by using bulk nanosculpturing of the substrate with a focused ion beam. The nanoSQUIDs had a sub-micrometer loop size, which limited the dimensions of the nJJs to below ~100 nm. Electron beam lithography and high selectivity reactive ion etching with pure SF_6 gas were used to pattern nJJs with a width down to 10 nm that is comparable to the coherence length in thin films of Nb and NbN and provides better reproducibility in the case of a Nb functional layer and better long term stability due to enhanced corrosion resistance in the case of NbN layer. A naturally created undercut in the Si substrate was used to prepare nanoSQUIDs on a 10-nm-thick SiO_2 membrane within 500 nm from the edge of the substrate. High-resolution TEM revealed that NbN films on SiN have a columnar structure while they observe cube-on-cube epitaxial growth on SiC membranes. Measurements revealed non-hysteretic *I(V)* characteristics of the nJJs and nanoSQUIDs, peak-to-peak quantum oscillations in the *V(B)*-characteristics of the nanoSQUIDs with an amplitude of >20 μV and a white noise spin resolution of approximately 400 $\mu_B/\sqrt{Hz}$ that was obtained without use of SQUID array preamplifier. Towards future realization of hybrid superconductor-ferromagnetic nanostructures for spintronics experiments in TEM, Permalloy nanodots and triangles with dimensions down to ~100 nm were prepared on SiN membranes and studied by Lorentz microscopy and electron holography TEM methods. These technologies are promising for the fabrication of superconducting electronics and spintronics based on nJJs with ferromagnetic nanostructures for operation inside a TEM.

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CROSS-SECTIONAL PREPARATION OF COMPLEX ENERGY DEVICES AND THEIR CHARACTERIZATION BY ADVANCED MICROSCOPIC METHODS

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Energy devices like batteries, fuel cells and solar cells are assembled from multiple functional components with different material properties. To gain a fundamental understanding of structure-property relations, to systematically contribute to performance enhancement and to unravel device degradation and failure mechanisms, high quality cross-sections of entire devices or of as large regions as possible are required. Since many devices not only consist of different classes of materials but also of liquid and/or air sensitive components, working under cryogenic and/or inert conditions is essential to conserve their pristine state. To enable scale-bridging cross-sectional characterization of those devices all the way down to the atomic level, we utilize different preparation tools. A novel self-built cryo-cutter allows us to produce cross-sections of entire devices, e.g., pouch-cells, in a quick one-step process and to observe them in their pristine state by OM even on the centimeter scale. For smaller scales (cryo-)ultramicrotomy as well as FIB are routinely applied and capable to prepare cross-sections not only for investigations by OM and SEM but also for (atomic resolution) TEM, as high-quality thin samples below 50 nm are provided. In this contribution we demonstrate the scale-bridging capabilities of those preparation techniques applied on various devices and their components in conjunction with advanced (electron) microscopic and spectroscopic methods. Examples include devices like batteries (Fig. 1) and complex PEM fuel cells as well as battery components (Fig. 2). The applied techniques are ideally suited to investigate the integrity of complete devices as well as the interfaces of individual layers and components in terms of their morphology and contact as well as the systematic identification of their composition and chemical bonding states.

Part of this work was performed at the Micro-and Nanoanalytics Facility (MNaF) of the University of Siegen.

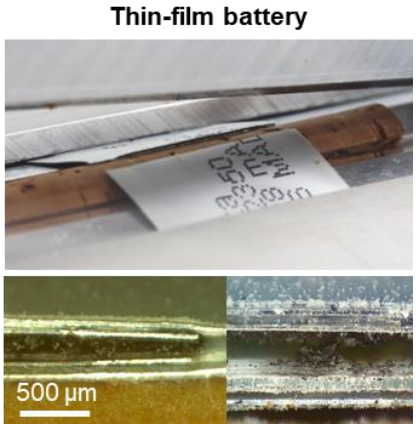

Fig. 1: Cross-sectional preparation of commercially available Li/MnO_2 thin-film battery by self-built cryo-cutter tool

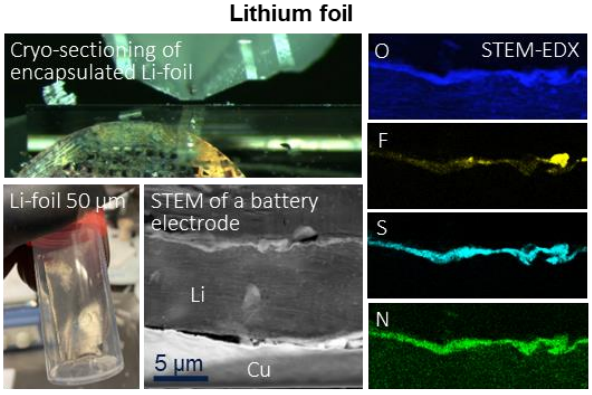

Fig. 2: Cryo-ultramicrotomy of lithium foil for battery solid electrolyte interphase research

NOVEL TOPOTAXIAL EXCHANGE GROWTH OF EuInAs NANOWIRES

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Nanomaterials bring to expression unique electronic properties that promote advanced functionality and technologies. Albeit, nanoscale growth presents paramount challenges for synthesis limiting the diversity in structures and compositions. Topotaxy is particularly valuable in nanomaterials, where nanowires and other nanostructures can be transformed into new materials with tailored characteristics; examples are the solution-based cation exchange in chalcogenides or the conversion of oxide nanowires. Here, we demonstrate the vapor-solid topotactic exchange that converts Wurtzite InAs nanowires into Zintl phase $Eu_3In_2As_4$ nanowires and Zinc-blend InAs into $Eu_5In_2As_6$. In situ evaporation of Eu and As over InAs nanowire cores in molecular beam epitaxy results in the mutual exchange of Eu from the shell and In from the core. A continuous EuInAs shell grows that gradually consumes the InAs core and converts it into a single-phase EuInAs nanowire. We examine the parameter phase space that supports topotaxial exchange, elucidate the mechanism by atomically resolved scanning transmission electron microscopy (figure 1), and provide an initial characterization of the coupled magnetic and electronic phase diagrams of the Zintl phases.

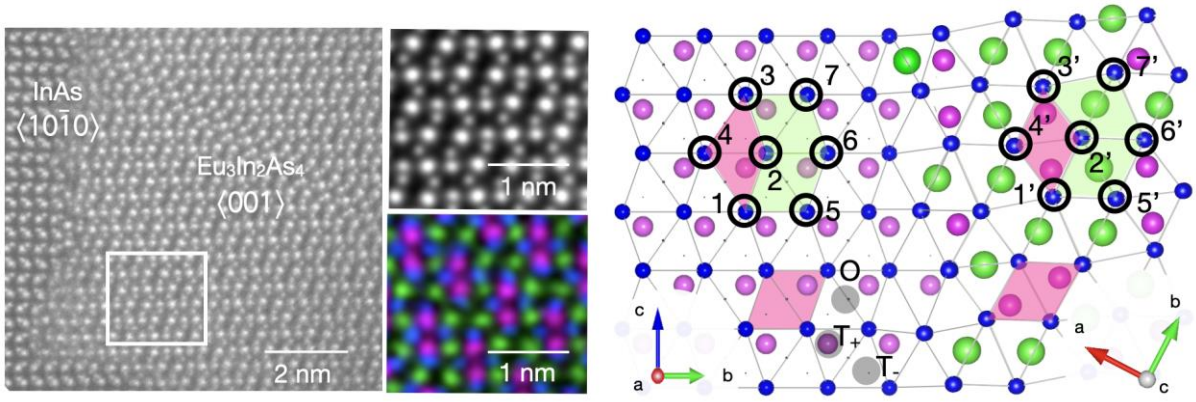

Fig. 1: Topotaxial transformation of hexagonal InAs into orthorhombic $Eu_3In_2As_4$. Left: Atomic resolution STEM images of the transformation interface between InAs and $Eu_3In_2As_4$, and EDS elemental maps of the $Eu_3In_2As_4$ (Eu: green, As: blue, In: pink). Right: Atomistic model of the InAs/EuInAs interface: $\langle 10\bar{1}0\rangle_{InAs} \parallel \langle 001\rangle_{EuInAs}$ and $[12\bar{1}0]_{InAs} \parallel [\bar{2}30]_{EuInAs}$. The edges of the polyhedral network spanned by the As sub-lattices are outlines. T_+, T_- and O mark tetrahedral and octahedral coordination sites in InAs. Pairs of tetrahedra (red filling) around In in the $Eu_3In_2As_4$ form the polyanionic InAs chains that align along the c-axis, surrounded by octahedrally coordinated Eu.

IDENTIFICATION AND HEALING OF FIB-INDUCED DEFECTS IN GaN USING ELECTRON HOLOGRAPHY

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Thermal healing of focused ion beam-implanted defects in GaN is investigated by off-axis electron holography in TEM in conjunction with self-consistent electrostatic potential and electron optical phase change simulations. Freshly FIB-prepared GaN lamellas exhibit a Fermi-level pinning of 0.57 eV above the valence band maximum (E_V). The pinning level gradually increases to about 2 eV above E_V upon annealing between 250 and 500°C and remains high after cooling down to room temperature (Figure 1). This indicates an irreversible transition from defect-induced Fermi-level pinning near the valence band toward a midgap pinning attributed to amorphous shell-crystalline core interface states (see Figure 2 for the different shell layers of the TEM lamella). Based on the measured pinning levels and the defect charge states, we identify the dominant defect type to be substitutional carbon on nitrogen sites (C_N). The irreversible thermal healing, gradually reduces the implanted C_N concentration and hence lifting the C_N-induced Fermi-level pinning. The results demonstrate that temperatures as low as about 250°C are sufficient to initiate defect healing in GaN.

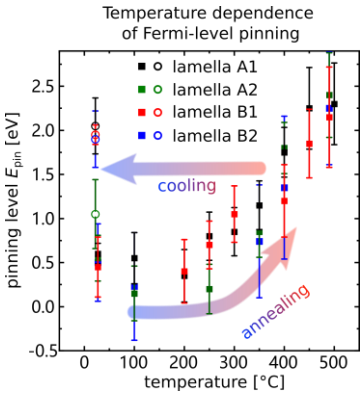

Fig. 1: Temperature dependence of surface Fermi-level pinning.

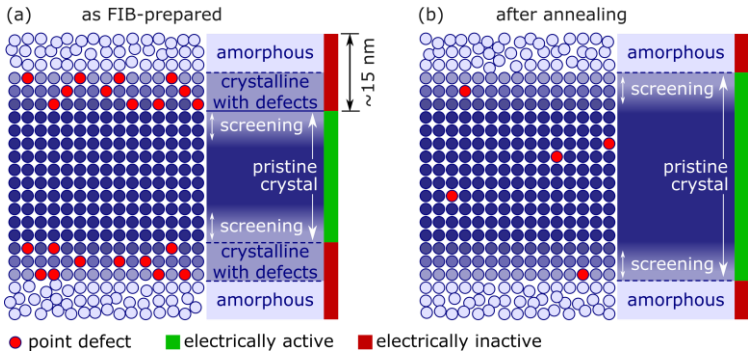

Fig. 2: FIB-induced structures of a lamella in the as-prepared (a) and healed state (b).

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TWO-DIMENSIONAL FEW-ATOM NOBLE GAS CLUSTERS IN A GRAPHENE SANDWICH

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The van der Waals atomic solids of noble gases on metals at cryogenic temperatures were the first experimental examples of two-dimensional systems [1]. Recently, such structures have also been created on surfaces under encapsulation by graphene, allowing studies at elevated temperatures through scanning tunnelling microscopy [2,3]. However, for this technique, the encapsulation layer often obscures the arrangement of the noble gas atoms.
Here we create Kr and Xe clusters in between two suspended graphene layers by irradiation with singly charged low energy ions, and uncover their atomic structure through transmission electron microscopy [4]. We show that small crystals ($N < 9$) arrange on the basis of the simple non-directional van der Waals interaction (Fig. 1). Larger crystals show some deviations, possibly enabled by deformations in the encapsulating graphene lattice. We further discuss the dynamics of the clusters within the graphene sandwich, and show that although all the Xe clusters with up to $N \approx 100$ remain solid, Kr clusters with already $N \approx 16$ turn occasionally fluid under our experimental conditions (under a pressure of ~0.3 GPa). This study opens a way for the so-far unexplored frontier of encapsulated two-dimensional van der Waals solids with exciting possibilities for fundamental condensed-matter physics research.

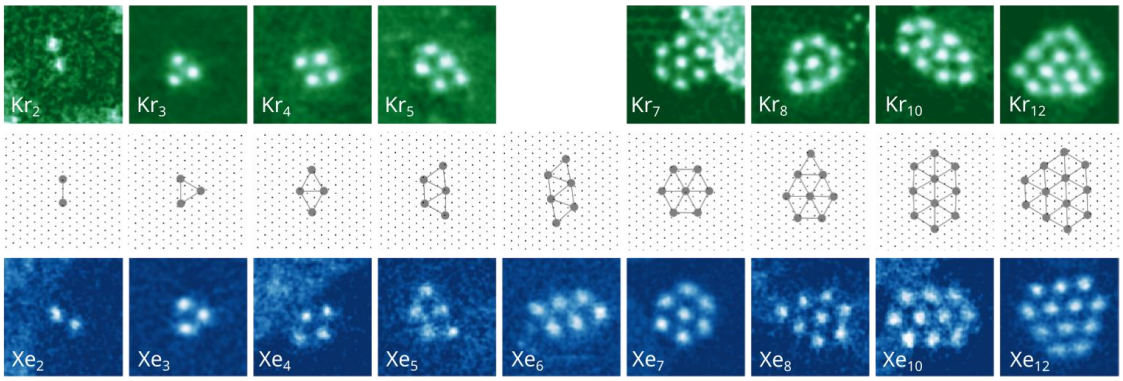

Fig. 1 Structure of small clusters. Filtered STEM-ADF images of Kr (top row) and Xe (bottom row) including all the cases where the experimentally observed structures correspond to that with the lowest energy according to the simulations for Kr (middle row).

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ELECTROSTATIC EXTENSION OF MAGNETIC PROXIMITY EFFECT IN $La_{0.7}Sr_{0.3}MnO_3$

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Magnetic proximity effects are typically limited to a few nanometers due to the short-range nature of the underlying magnetic interactions, such as the exchange interaction, the Dzyaloshinsky-Moriya interaction, interface states, rehybridization, and reconstruction, all of which are highly localized. Here, we use off-axis electron holography to reveal an electrostatically-induced long-range magnetic proximity effect that extends over a distance of 40 nm at a ferromagnetic/paramagnetic interface in $La_{0.7}Sr_{0.3}MnO_3$. We show that this behavior results from carrier diffusion and drift across the interface, which changes the Mn^{3+}/Mn^{4+} ratio and hence the local Curie temperature and density of magnetic moments. The unravel quantitative relationship between electrostatic properties (i.e., local hole concentration and Mn valence) and local magnetization, as well as Curie temperature, provides a fundamental understanding of the electrostatic-shaping of nanoscale magnetism. [1]

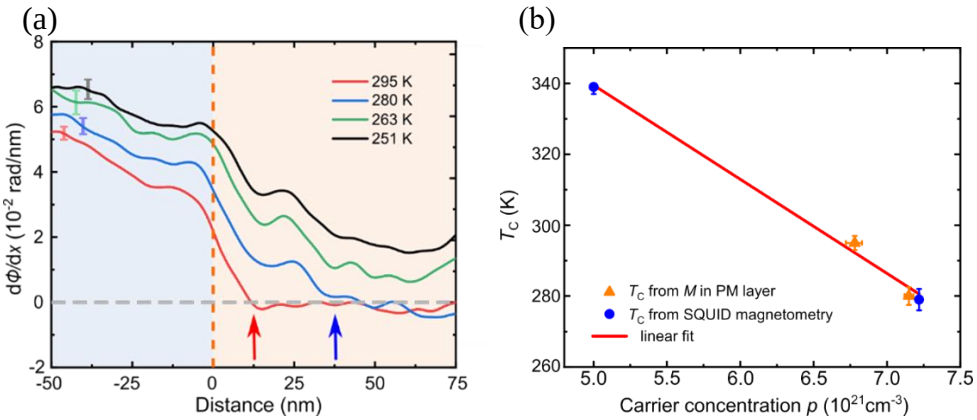

Figure 1. (a) First derivative of the magnetic contribution to the phase shift dφ/dx (left axis) plotted as a function of distance from the PM/FM interface (x = 0 nm) along [001] in the LSMO film at 295 K (red), 280 K (blue), 263 K (green) and 251 K (black). (b) Curie temperature (TC) determined using SQUID magnetometry (blue dots) and off-axis electron holography (orange triangles), plotted as a function of carrier concentration. The red fitting line is consistent with a linear relationship between TC and carrier concentration.

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REVEALING THE EPITAXIAL RELATION IN MOCVD GROWN 2D GaS BY STEM

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Two-dimensional (2D) materials have the potential to transform semiconductor technology. Their rich compositional and stacking diversity allows material properties to be tailored, enabling a wide range of device applications. In addition to the much-discussed transition metal dichalcogenides, III-VI semiconductors such as GaS can be deposited in the 2D semiconducting phase, which with its ultraviolet bandgap is promising for applications in solar blind photodiodes and light-emitting diodes.[1]
To realize wafer scale growth of GaS by metal organic chemical vapour deposition (MOCVD) on sapphire substrates, a pulsed growth mode is established. The grown structures are prepared as electron transparent focused ion beam lamellae in cross section geometry using a JEOL JB 4601 and analyzed in an aberration corrected JEOL 2200 FS STEM operated at 80 kV and 200 kV. The STEM images show the formation of an additional Ga ad layer during the nucleation step on the sapphire substrate as shown in Figure 1 a). Furthermore, we observe a distinct epitaxial relation with respect to the substrate, which shows in-plane strain. In contrast, the multilayer sample shows in-plane strain relaxation. In addition, geometric phase analysis (GPA)[2] reveals strain in the growth direction, leading to the formation of islands on top of the coalesced GaS films, which will be discussed in more detail in the contribution.

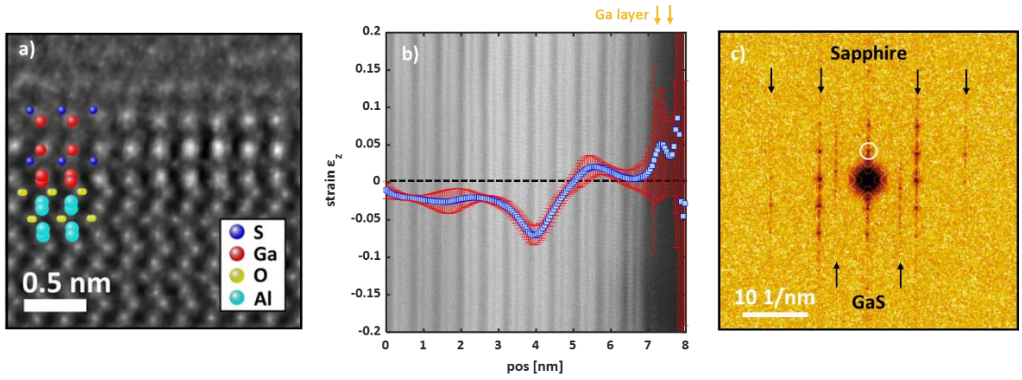

Figure 1: a) HR-STEM of the GaS monolayer sample showing the additional Ga ad layer.* b) Strain ε_Z in the growth direction derived from GPA analysis and superimposed onto the HR-STEM image of the GaS multilayer sample. c) Combined FFTs of the sapphire substrate and the GaS adlayer showing the in-plane relaxation.

** Post-processed by ABSF filter and the deep-learning method AtomSegNet.[3,4]*

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IN SITU OFF-AXIS ELECTRON HOLOGRAPHY ANALYSIS OF III-V JUNCTIONS FOR SOLAR CELL APPLICATIONS

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Multi-junction solar cells that are based on MOVPE-grown III-V compound semiconductor layers achieve the highest solar cell efficiencies. However, major challenges in epitaxial growth procedures need to be overcome to develop devices that contain III-V multinary compound interfaces, as the electrical junction and current-voltage characteristics are highly sensitive to the interface quality. In an attempt to perform comprehensive structural and electrical characterization of working photovoltaic devices with high spatial resolution, we have combined *in situ* electrical biasing in the transmission electron microscope (TEM) with optimized TEM sample preparation and contacting. Electrostatic potentials at *p-n* junctions can be measured with nm spatial resolution using off-axis electron holography (EH).

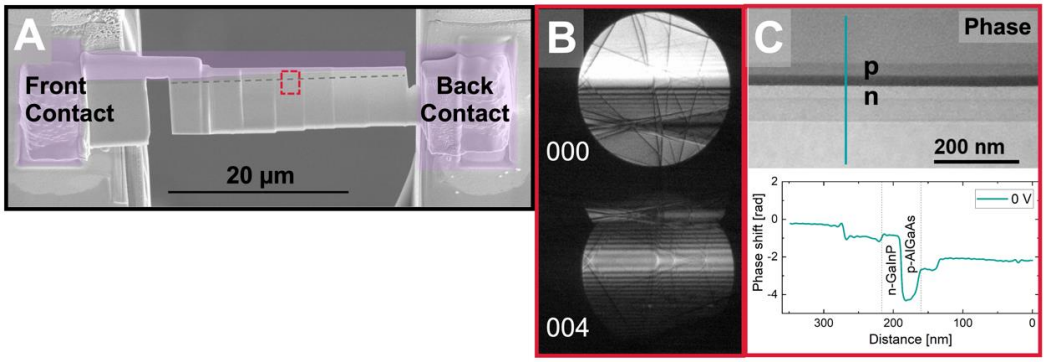

Fig. 1: A) Scanning electron microscope image of a TEM lamella with regions of different thickness prepared by FIB milling and mounted on a chip for *in situ* electrical biasing. B) STEM-CBED pattern recorded at the ROI indicated in A to derive the crystalline specimen thickness. C) Electron phase image reconstructed from an off-axis electron hologram stack recorded from the same ROI, and the corresponding line profile extracted from the phase image across the junction.

Here, we present *in situ* EH analyses of electrically-contacted p-AlGaAs/n-GaInP hetero-junctions. Electron-transparent specimens of different thickness (Fig. 1A) were studied to assess the influence of electrically inactive specimen surface layers introduced during focused ion beam (FIB) milling. The crystalline specimen thicknesses were derived from energy-filtered convergent beam electron diffraction (CBED) analysis (Fig. 1B). The TEM specimens were connected to electrical biasing chips and studied in a biasing TEM specimen holder (Protochips Aduro 500). Reliable ohmic contacting was attempted by applying a backside FIB milling procedure to preserve the pre-deposited ohmic device top contact, and by depositing different contacting materials, such as W and Pt, to the top and bottom lamella surfaces. We obtained the electron optical phase shift (proportional to projected electrostatic potential) as a function of applied bias. Fig. 1C shows a phase shift map reconstructed from an electron hologram stack acquired at zero bias and an extracted profile of the phase distribution across the junction. The results demonstrate progress toward the successful *in situ* characterization of the electrostatic potential distribution of p-n junctions in real solar cell devices.

STEM ANALYSIS OF MOCVD-GROWN TWO-DIMENSIONAL WS_2-MoS_2 HETEROSTRUCTURE

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The study of two-dimensional (2D) van der Waals (VdW) materials and their structural characterization have recently received much attention due to their unique electronic and optical properties.[1] For example, a monolayer of transition metal dichalcogenides (TMDs) has a direct band gap, while multilayers have an indirect band gap.[1] MoS_2 and WS_2 are among the two most studied 2D VdW materials and good candidates for optoelectronic applications. Their heterostructure would facilitate charge transport and recombination and improve photodetector performance.[1,2]

In this study, metal organic chemical vapor deposition (MOCVD) is used as synthesis technique to grow 2D homo- and heterostructure layers on c-plane sapphire substrates. The samples are then transferred to the transmission electron microscopy (TEM) grid for further investigations by taking advantage of the etchant free PMMA transfer method.[3] The transfer procedure is modified to maximize the yield of transfer and obtain a flat 2D layer with few defects on the TEM grid.

The Helios 5 Hydra CX PFIB at 30 kV and the aberration-corrected JEOL 2200 FS STEM at 80 kV as well as 200 kV are used for (low voltage) scanning transmission electron microscopy (LV)STEM characterization.

The high angle annular dark field (HAADF) image of the MoS_2 on WS_2 heterostructure is shown in Figure 1. The structure shows the growth of triangular structures of WS_2 and MoS_2 on an almost coalesced layer of WS_2. The cropped section shows the high-resolution image of a lateral interface between WS_2 and MoS_2 in the second layer of the heterostructure. From interpretation of the Z-contrast the WS_2 monolayer (region A), the AB- stacking of WS_2 on WS_2 (region B), MoS_2 on WS_2 (region C) can be identified. These and further investigations on the chemical composition will be discussed in the contribution.

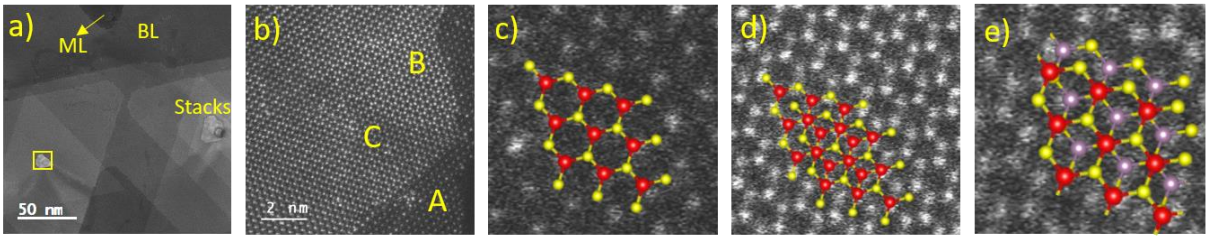

Fig. 1: a) HAADF and b) high resolution image of MoS_2 -WS_2 heterostructure, c, d, e) enlarged section of region A, B, C respectively.

References:
[1] H. Wang et. al. Chemical Society Reviews **47** 6101-6127 (2018).
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CORRELATIVE *IN SITU* SRnanoCT AND (S)TEM IMAGING OF BIODEGRADABLE Mg-BASED ALLOYS

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Magnesium-based alloys have gained significant interest as potential implant materials due to their biocompatibility and biodegradability, presenting an alternative to conventional materials like stainless steel and titanium alloys. The use of biodegradable implants aims to reduce health risks and financial costs associated with healthcare by eliminating the need for a second surgery due to implant rejection, which is unavoidable in the case of conventional implants [1]. A comprehensive understanding of the biodegradation process of magnesium alloys under physiological conditions and simulated body environments is crucial for developing application-tailored implant materials.

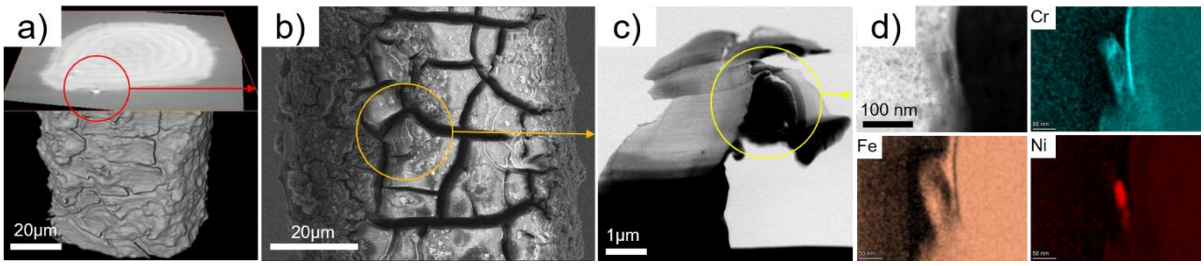

Figure 1: Correlative workflow: (a) SRnanoCT identification of ROI with impurity particle, (b) ROI identification in dual-beam FIB-SEM, (c) FIB lamella preparation within ROI, and (d) (S)TEM-EDX analysis at the particle-degradation layer interface.

In this study, a correlative workflow (see Fig. 1) was implemented to investigate the biodegradation of magnesium-based alloys at different length scales. Non-destructive *in situ* 3D imaging using synchrotron radiation nano computed tomography (SRnanoCT) was employed to study dynamic degradation processes. To complement the limitations of *in situ* SRnanoCT, additional measurements were conducted using (scanning) transmission electron microscopy ((S)TEM) and energy-dispersive X-ray spectroscopy (EDX) to provide information on chemical composition. The correlative use of these techniques aimed to achieve a comprehensive understanding of the degradation process, addressing critical challenges such as the identification of regions of interest (ROI), sample transfer, correlative preparation, and image registration.

Wire samples (80 µm diameter) of Mg-2wt.%Ag and Mg-2wt.%Gd were subjected to degradation in two simulated body environments under physiological conditions (37 °C, pH 7.4, flow rate 1 ml/min). Using a flow-cell setup for *in situ* SRnanoCT, information on degradation rates, formation and homogeneity of the degradation layer (DL), and the presence or formation of insoluble precipitates were obtained. *In situ* SRnanoCT scans were acquired every 13-30 minutes, with degradation-relevant ROIs identified and further characterized using correlative (S)TEM.

Preliminary results indicate an inhomogeneous degradation behavior, the formation of insoluble precipitates, and agglomeration of Ag at the surface. Mg-alloys with Gd-addition were found to degrade faster than Ag-containing alloys, and an increase in Ag content was observed to further accelerate the degradation rate.

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ASSESSING THE INFLUENCE OF GROWTH INTERRUPTION STAGES ON THE SEGREGATION OF Sb IN ULTRATHIN GaAsSb LAYERS.

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III-V-Sb materials are emerging as prime candidates to enhance device efficiency in the infrared spectrum due to their unique properties, such as the tunability over a wide wavelength range (from 0.1 eV to 1.8 eV) and the ability to switch between type I and type II bandgap alignments. However, the growth of very thin films, such as quantum wells or superlattices, presents significant challenges, mainly due to the pronounced segregation behavior of Sb, which enlarges both interfaces [1]. In the case of GaAsSb layers, different growth strategies have been proposed in the literature to mitigate this phenomenon, such as exposing the surface to Sb fluxes before (soaking) or As fluxes after (desorption) the deposition of the GaAsSb layer. In this work, we have quantitatively evaluated the effect of applying both growth interruption (GI) strategies, combined and separately, on the segregation of Sb in thin films with thicknesses between 1 and 20 ML. For this purpose, we have introduced AlAs markers to perform a rigorous application of the 3LKFM segregation model for the estimation of segregation parameters using HAADF and EDX techniques in STEM mode [2].
The results show that in the absence of GI (sample R), Sb profiles resemble Gaussian-like peaks whose maximum and peak position shift with increasing film thickness. In particular, the AlAs markers identify a constant delay of 5 ML of the onset of the GaAsSb layer. The use of the soaking step leads to a more effective incorporation (sample S), improving the upward composition gradient and advancing the film onset. However, desorption stages (sample D) significantly affect the Sb profiles, and only thick GaAsSb layers remain. The combination of both strategies leads to squared profiles with higher Sb contents and sharper gradients but the mechanism is much more complex than the simple addition and subtraction of Sb amounts. Segregation simulations show that the segregation energy changes during layer growth, being initially higher but stabilizing around the same value for all cases. Sb-soaked samples reach the segregation steady state much earlier than non-soaked samples.

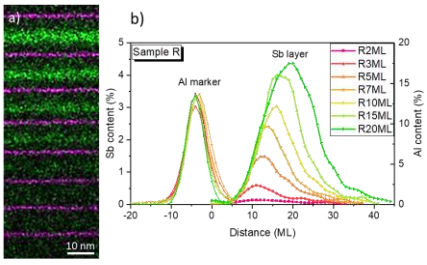

Fig. 1: a) EDX map of sample R showing Sb (green) and Al (purple) distribution. b) Composition profiles along the growth direction for different layer thicknesses.

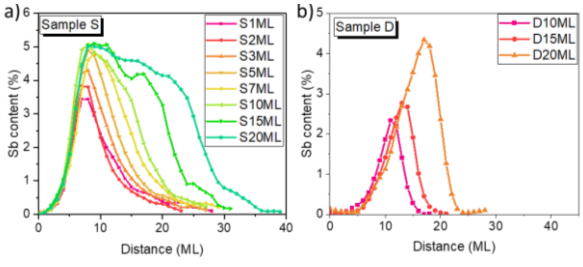

Fig. 2: Superposed composition profiles of Sb along the growth direction of samples S (a), and D (b) for different layer thicknesses.

References:
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[2] S. Flores, et al. Appl. Surf. Sci. 607 (2023) 154966.

UNCOVERING THE NANOSCALE ARCHITECTURE OF HUMAN ENAMEL WITH ADVANCED STEM TECHNIQUES

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As hardest material in the human body, tooth enamel is comprised of predominantly mineral in the form of carbonated apatite. A combination of this high mineral content with its intricate hierarchical structure across various length scales, results in a superior wear resistance from mastication forces. On the nanoscale, enamel is comprised of oblong polygon-shaped, long aspect-ratio crystallites, which contain chemically graded impurities within their apatite unit cell following a core-shell architecture [1]. Although crystallites have been reported to possess a coherent crystal structure, here, we show by using a low-dose 4D-STEM approach for this beam-sensitive material, that there are in fact rotations and tilts within individual crystallites by carefully analyzing focused ion beam-prepared enamel sections with crystallites either oriented approx. parallel (Fig. 1) or perpendicular (Fig. 2) the long (*c*-)axis. In addition to quantifying orientation between crystallites using the Automated Crystal Orientation Mapping (ACOM) approach implemented in py4DSTEM [2], we discover a non-uniformal 3D crystallite strain distribution for these sparse datasets. These findings have important implications for the nanoscale structure-property relationship in enamel, which may aid in provision of (nanoscale) toughening mechanisms and/or the prevention of improved crack initiation and propagation.

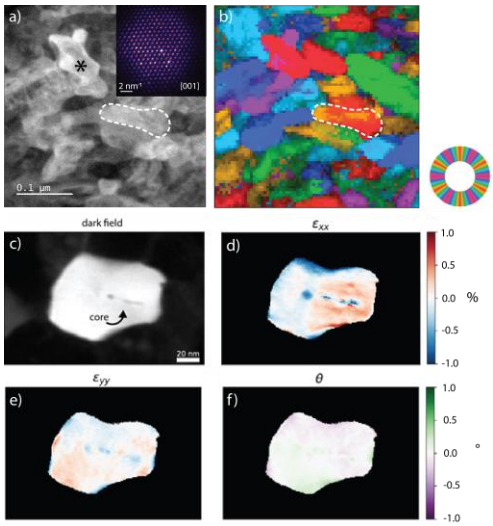

Fig. 1: a) Dark field image of multiple enamel crystallites viewed (close) down the long direction ([001]). Inset: Diffraction pattern of * in a). b) ACOM in-plane rotation (6-fold symmetry). Dashed region shows rotation within a crystallite. c) Dark field image of a single crystallite, with associated strain maps (d-e) and rotation variation (f).

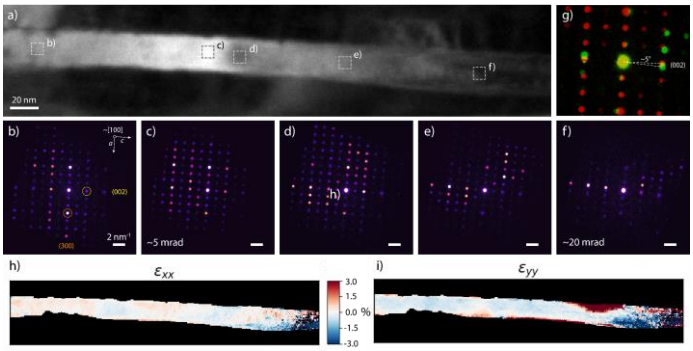

Fig. 2: Crystallographic orientation and strain variation illustrated along the long axis (~[100]) of a human enamel crystallite. a) Dark field image, b-f) diffraction patterns from areas indicated in squared boxes in a), g) overlay of diffraction pattern b) (red) and f) (green) showing rotation along the *c*-axis, h-i) associated strain maps in x and y direction.

References:
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AI ENHANCED EELS USING BACKSCATTERED LOW ENERGY ELECTRONS

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In transmission electron microscopy (TEM) electron energy loss spectroscopy (EELS) is a well-established high resolution analytical characterization technique for a large range of materials and applications. However, the required sample dimension (i.e. < 100 nm thickness) is a limiting factor and often makes sample preparation rather challenging. Furthermore, beam-induced sample degradation at typical TEM electron energies is hampering the observation of organic materials. These obstacles can be overcome by applying EELS to backscattered electrons (BSE) using an ultra-low voltage scanning electron microscope (SEM). For our studies we use a prototype of an aberration corrected, electron-spectroscopic SEM (Zeiss Delta-SEM [1]). We show that for electron energies below 500 eV (as far down as 10 eV) the image contrast and imaging quality is significantly improved – in particular for low-dose imaging of organic materials – while retaining high spatial resolution (< 1 nm), with high surface sensitivity and significantly reduced beam-damage.

For the first time we can identify the excitation of states (e.g. plasmon excitations) in a SEM by backscattered electron energy loss spectroscopy (bsEELS) and utilize this signal for material characterization. As initial model system we analyze the spectral data obtained from graphene on silicon wafer (SiO_2), materials where TEM-EELS measurements can serve as a reference [2,3]. Fig 1 shows measured bsEEL spectra together with a correlating naïve model fit derived from TEM-EELS data. Although the energy resolution of our prototype is limited (about 5 eV), we find a good agreement of the SEM spectral data to e.g. the known characteristic surface plasmon signal of graphene.

For two tetra-phenyl cumulene model materials with distinct UV/Vis absorption peaks it was possible to obtain the deconvoluted bsEELS spectra applying a convolutional neural network trained on the SiO_2-spectrum, which has no excitation peaks below the 9eV bandgap (cf. Fig. 1c). These deconvolution spectra are currently cross-checked against experimental high-resolution EELS spectra.

Acknowledgements: We thank R. Tykwinski (Alberta) for the cumulene materials and P. Tegeder (Heidelberg) for high-resolution EELS control measurements. Research is funded by the German Research Foundation via the Excellence Cluster '3D Matter Made to Order'.

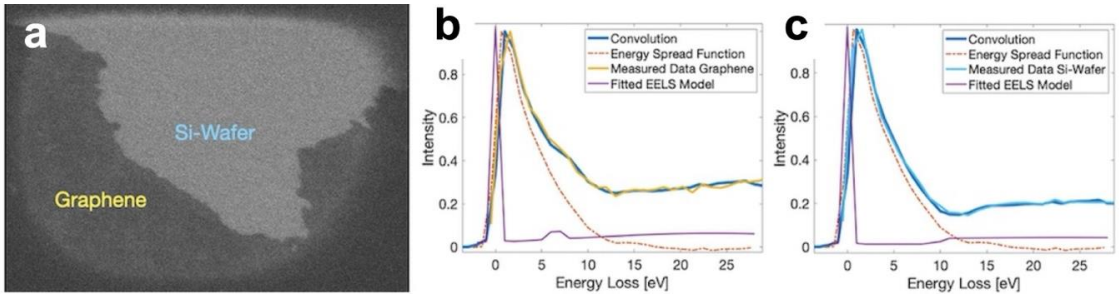

Fig. 1: a) BSE image of a graphene flake on Si-wafer. b) for graphene and c) for SiO2 show the expected EEL spectra from known TEM measurements (purple, [2] and [3]) and a naïve model fit (convolution of TEM data with the energy spread function of the DELTA, blue).

References:

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SPIN HALL EFFECT INDUCED IN-SITU MAGNETIC MEASUREMENTS USING ELECTRON HOLOGRAPHY: A CASE STUDY WITH YIG

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We present magnetic imaging in a transmission electron microscopy (TEM) using Lorentz imaging and Electron Holography. We investigate the switching of magnetic domains in an in-plane magnetic material -Yttrium Iron Garnet (YIG) using spin currents injected using spin Hall Effect in an adjacent platinum layer. The orientation of the spin was modified by changing the sample geometry to investigate the magnetic switching of vortices and domain walls. The interaction with the injected spin and electron phase measured was studied using electron holography. The pure electron phase change with spin current was studied by removing the contributions from mean inner potential and phase change from magnetization. We observed a time averaged domain wall oscillation induced by spin current injection using Electron Holography.

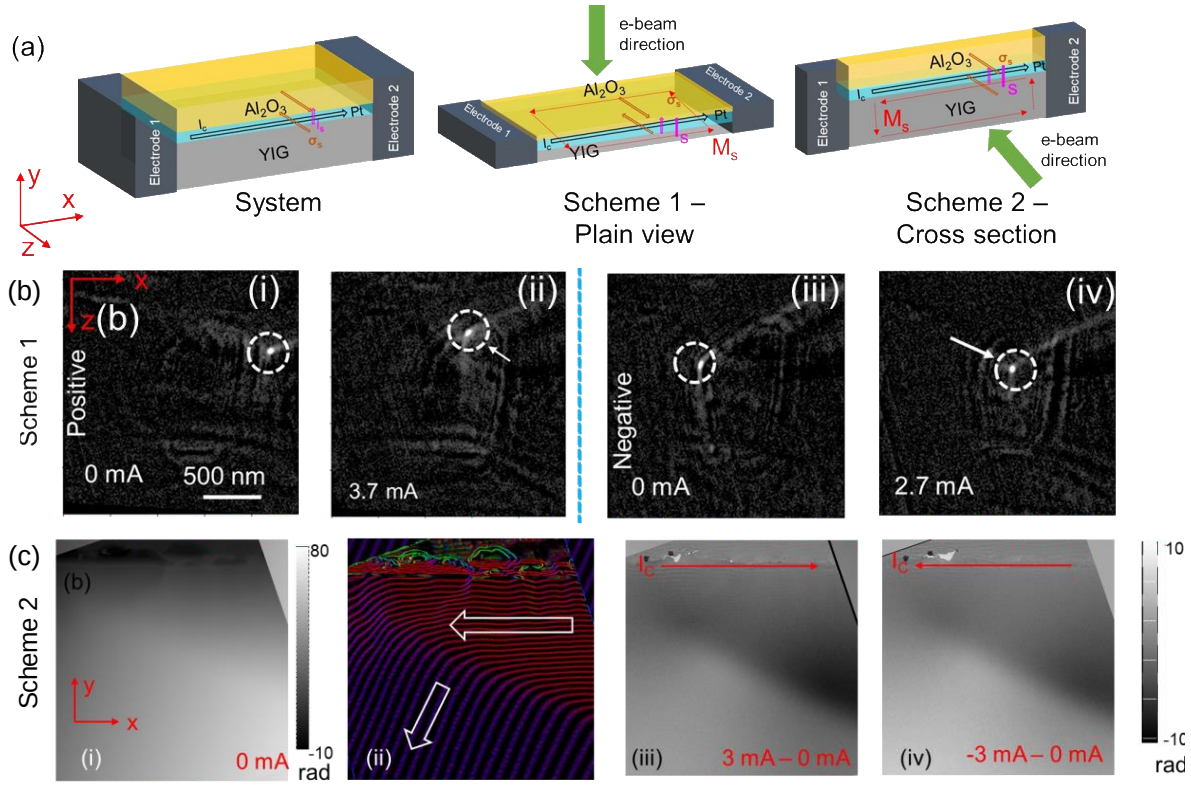

Figure 1. (a) Different schemes of YIG/Pt used for the study, (b) switching of magnetic vortex using spin currents and (c) domain wall oscillations observed in electron holography.

References:
[1] E. Tyukalova, J. V. Vas, et al. , Acc. Chem. Res. 54, 3125–3135 (2021).

AN ELECTRON BEAM MODULATOR FOR COMPUTATIONAL GHOST IMAGING IN TEM

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The modern aberration-corrected Transmission Electron Microscope (TEM) is one of the most sought-after scientific instruments in modern material science and life science laboratories since it allows to image and characterize samples with the highest lateral resolution. [1–3] Since aberration correctors are quite costly and complex, research groups continue to study other methods to increase the lateral resolution in TEMs.
If the optical system is well characterized and the aberrations that act on the illumination are known and quantified it is possible to overcome the aberration-limit via a Computational Ghost Imaging (CGI) [4,5] scheme adapted to TEMs. In CGI, the image of the sample is computationally recovered by illuminating the sample with a series of known structured beams and collecting the integrated transmitted intensity via a single-pixel bucket detector. If the image formation is linear, it is possible to computationally recover the sample transfer function by combining the intensity pattern of the structured illumination and the transmitted signal through an inversion algorithm.
The structured beams can be generated via a novel electron modulator that we designed, and then crafted using MEMS technology. At the moment, we are working on improving a CGI experimental setup that uses this device for beam shaping and the HAADF as the single-pixel detector.

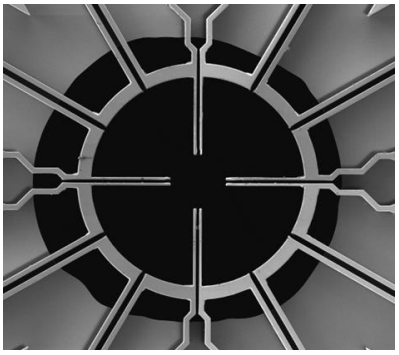

Fig. 1: Electron beam modulator, detail of the aperture

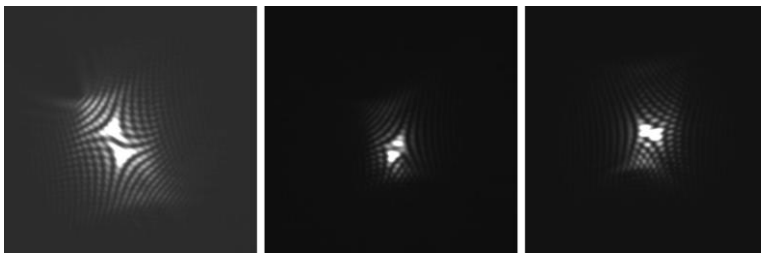

Fig. 2: Images of real structured probes generated by the device in far field

References:
[1] H. H. Rose, Historical Aspects of Aberration Correction, J. Electron Microsc. (Tokyo). 58, 77 (2009).
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TRANSFORMATION OF INORGANIC NANOPARTICLE ELECTRONMICROSCOPY ANALYSIS

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Routine characterization of inorganic nanoparticles (iNPs) via transmission electron microscopy (TEM) imaging is primitive (analyzing at most a few hundred particle images by hand), compared to the cryo-EM methods developed for biomacromolecule analysis over the past decade (which allow 10^3-10^6 particle images to be collected and analyzed). We are working to adapt biological cryo-EM methods for routine characterization of iNPs as well as the total determination of iNP structures (including their ligand shells/interfacial layers). Both goals are facilitated by automated data acquisition software (e.g., SerialEM). Expected outcomes include: (1) recommendations to the iNP community for improved protocols for iNP data collection and analysis; (2) total structural determination of iNPs, including their organic ligand shells. Total structures will be determined by adapting biologically developed single particle analysis (SPA) software (e.g., cryoSPARC) for 3D structural determination. As developmental cryo-EM targets, we propose to investigate iNPs with high symmetry axes (e.g., gold nanorods) or alternatively anisotropic iNPs with confined spaces in which ligand movement is restricted (e.g., gold dendrimers). We will present initial results from our attempts to adapt biological cryo-EM methods to improve iNP characterization and determine iNP structure.

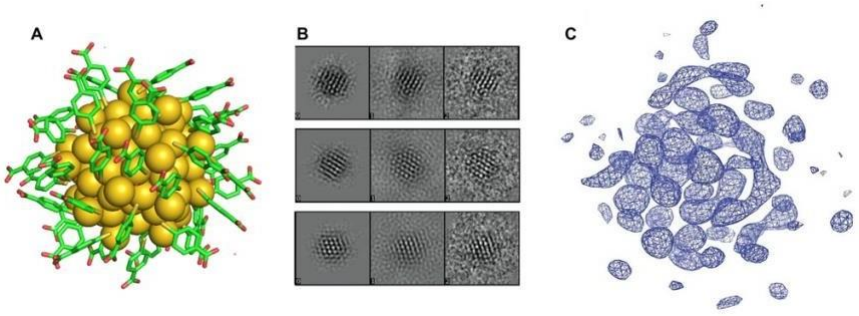

Fig. 1: A) X-ray crystal structure of $Au_{102}(pMBA)_{44}$. B) Class averages and C) electron density of Au_{68}, the first SPA of an iNP.[1,2]

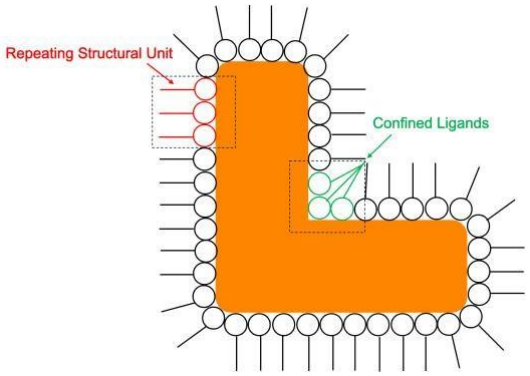

Fig. 2: A cartoon of a hypothetical anisotropic iNP where ligands are either in confined spaces (green) or a part of a repeating structural unit (red).

References:
[1] P.D. Jadzinsky et al., Science **318**, 430-433 (2007).
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MINIATURIZED MATERIAL TESTING DEVICES FOR MULTIMODAL IN SITU MICROSCOPIC STUDIES

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To contribute to the understanding of failure and fatigue, we examine the local microstructural strain/stress peaking as well as crack initiation/propagation by in situ methodology. Moreover, systematic errors in load/displacement of the measuring setup may be overcome by highly localized strain measurements, e.g. high-resolution EBSD in conjunction with image correlation. This requires in situ material tests, and we aim at two complementary goals. One is to contribute to alloy development and to optimize microstructure of selected alloys determining the macroscopic mechanical properties.
The second is to validate modern microstructure-based FEM simulations and to develop termination criteria for classical FEM simulations, e.g. for crack initiation. Therefore, the Mechanical Engineering Department develops in situ testing machines for static and dynamic tests. Latest developments are a portable, miniaturized 3-point bending machine for use in FIB/SEM/XRD (Fig. 1 a). Thos enable the direct observation of microstructural changes like crack initiation/propagation, as well as phase transformations, e.g. in steels. As an example, the bending machine (750g, 100x100x50mm) with independent load/displacement sensors allows for massive plastic deformation at loads up to 4kN. Our solution includes a user-friendly interface to lower the entry barriers for beginners (Fig. 1 c). In this contribution, we highlight the possibilities to observe dynamics of crack initiation/propagation, phase transformations, and the local evolution of the microstructure during testing of materials like Al alloys and austenitic steel. Electron backscatter diffraction (EBSD) is employed to in detail understand the crystallographic structure adjacent to the crack (Fig. 1 b). Direct image correlation is used to examine local strain peaking. Future plans involve establishing high-resolution EBSD instrumentation and data analysis for highly localized strain/stress/GND mapping as well as corresponding XRD data during a single experiment.

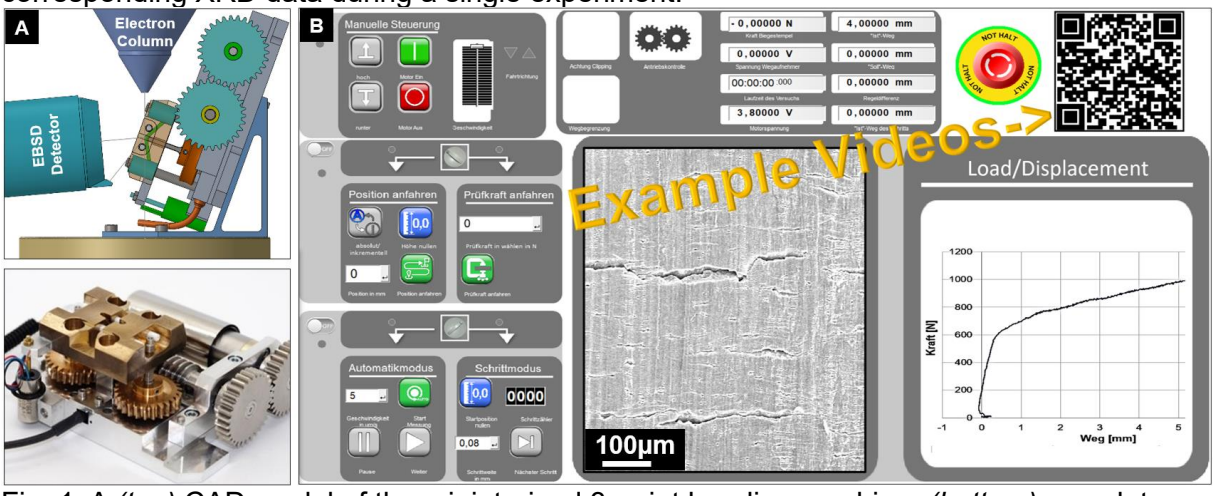

Fig. 1: A *(top)* CAD model of the miniaturized 3-point bending machine, *(bottom)* complete set-up after development, B) Graphical User Interface showing exemplary measurement in progress

Acknowledgements: We acknowledge use of the DFG-funded Micro-and Nanoanalytics Facility (MNaF) at the University of Siegen (INST 221/131-1).

DIRECT IMAGING OF DYNAMIC AND RESONANT SPIN PHENOMENA: PROSPECTS FOR CATALYSIS, SPINTRONICS AND MAGNETIC CHARACTERIZATION

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We present direct Transmission Electron Microscopy (TEM) imaging results of spin-waves, electron-spin resonance, and spin-hall scattering. By applying a combination of bright field imaging and time-resolved imaging using a delay line detector (DLD), we are able to image the domain wall motion, vortex gyration and spatially map the ground state as well as higher harmonics in ferromagnetic resonance spectroscopy of individual magnetic nanodots. By monitoring the beam deflection in defocused bright-field and diffraction images, we are able to directly monitor the cavitronic coupling of spin-resonance eigenstates in electron spin resonance of paramagnetic Diphenyl-Pikryl-Hydrazyl (DPPH) and ferromagnetic magnetite (Fe3O4). While current time-resolved techniques with sufficient magnetic contrast are limited to a picosecond time resolution, we extend the available toolset to much shorter timesteps by defining a technique to resolve the magnetic dynamics in reciprocal time, i.e., frequency and real-space by using stationary bright field imaging.

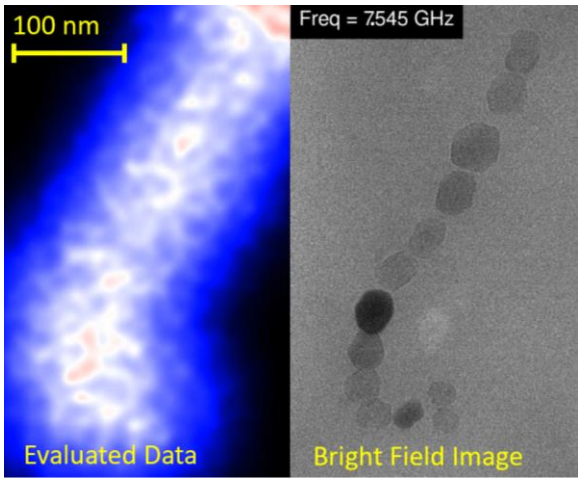

Fig. 1: Spin wave amplitude distribution off resonance (left) and bright field image (right).

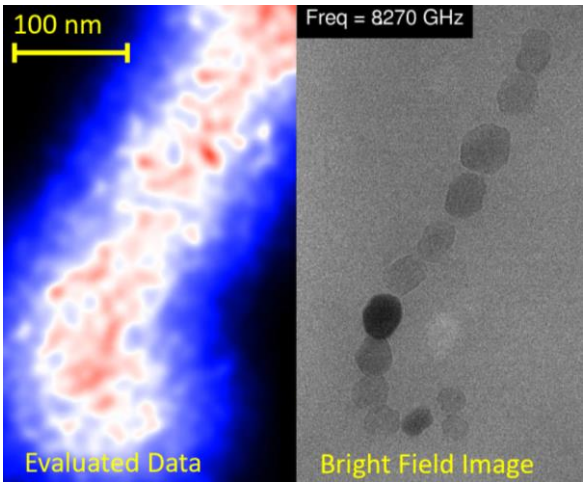

Fig. 2: Spin wave amplitude distribution in resonance (left) and bright field image (right).

MINDSHIFT: MULTIMODAL INTEGRATION FOR NANOPARTICLE DATA SCREENING USING HIGH-THROUGHPUT FRAMEWORKS IN TRANSMISSION ELECTRON MICROSCOPY.

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The rapidly advancing field of materials science has ushered in the era of nanoparticle megalibraries [1], offering immense potential for diverse applications. To truly harness the capabilities of these megalibraries, apt characterization tools, attuned to their vast synthetic throughput, are paramount. In this work we introduce a platform aiming at achieving spatial resolution, high-fidelity spectroscopic information, and optimal throughput, all powered by advanced machine learning (ML) techniques. The main goal is to leverage multimodality of the electron microscopes to turn them into a multifaceted high-throughput tool for structural materials data acquisition. Diving deeper, beyond mere structural data, a comprehensive material characterization mandates insights into its elemental composition, a cornerstone in shaping initial megalibraries. Integrating these analyses with predictive models for ensuing syntheses is pivotal, thereby closing the experimental feedback loop. Building upon our prior works, where ML techniques were adeptly employed with 4D-STEM data [2,3], we are evolving to an advanced nD-STEM approach by enhancing our analysis with simultaneous EDS and EELS. This transition offers a full view of nanoparticle properties, despite the current challenges in EDS timing and signal quality. We are re improving EDS precision and employing new acquisition techniques, while also refining EELS for richer electronic structure data, all augmented by ML for more efficient, comprehensive insights (Fig. 1).

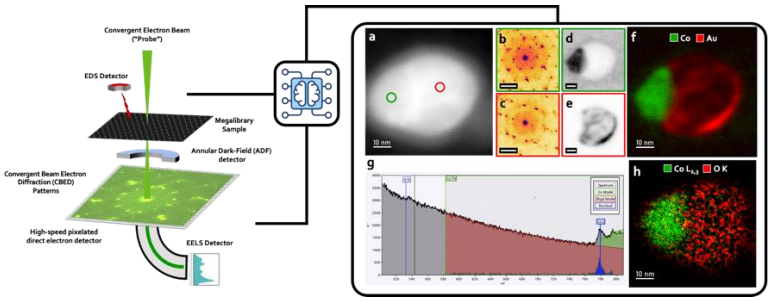

Fig. 1: *Left panel* - nD-STEM experiment setup leveraging the multimodal aspect of a S/TEM. Electron diffraction and spectroscopic information can be obtained from the same area of interest in a high-throughput fashion. *Right panel* – preliminary results obtained from a AuCo nanoparticle. a. ADF image from a AuCo nanoparticle in megalibrary. b-c electron diffraction patterns obtained from areas within the green and red circles in a. Virtual images in d and e, corresponding to Co and Au areas can be produced by integrating diffraction patterns within the dataset. f. shows a red-green image overlaying virtual images for Co and Au. G shows an EELS spectra obtained from the green circle marked in a. O K and Co L2,3 edges are visible and can be used to map out Co-rich areas as shown in h.

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ELECTRICAL CONTROL OF MAGNETIC PROPERTIES OF $Cr_2Ge_2Te_6$ – DIRECT OBSERVATION THROUGH CRYO-LORENTZ TEM

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Topological magnetic textures, such as skyrmions and domain walls, in two-dimensional magnets show promise for novel memory and quantum computing applications. Electrical control of such spin textures is important for realizing these applications as it would enable, e.g., ultra-low energy consumption and control of the magnetic states. Using Lorenz TEM, electrically contacted graphene/$Cr_2Ge_2Te_6$/graphene heterostrucures, and liquid-helium-cooling, we show that electric fields break the centrosymmetry of the $Cr_2Ge_2Te_6$ crystal, resulting in control of skyrmion chirality (Fig. 1) [1]. Furthermore, we investigate the effects of crystal thickness and surface oxidation on the magnetic properties and the structure of the magnetic textures of $Cr_2Ge_2Te_6$ [2]. These findings provide several avenues for control of magnetic properties in $Cr_2Ge_2Te_6$ through electric fields, thickness, and surface oxidation. In addition, they demonstrate the strengths of combining in-situ biasing, electrically contacted van der Waals heterostructures, and liquid helium cooling to directly observe novel physical phenomena in quantum materials.

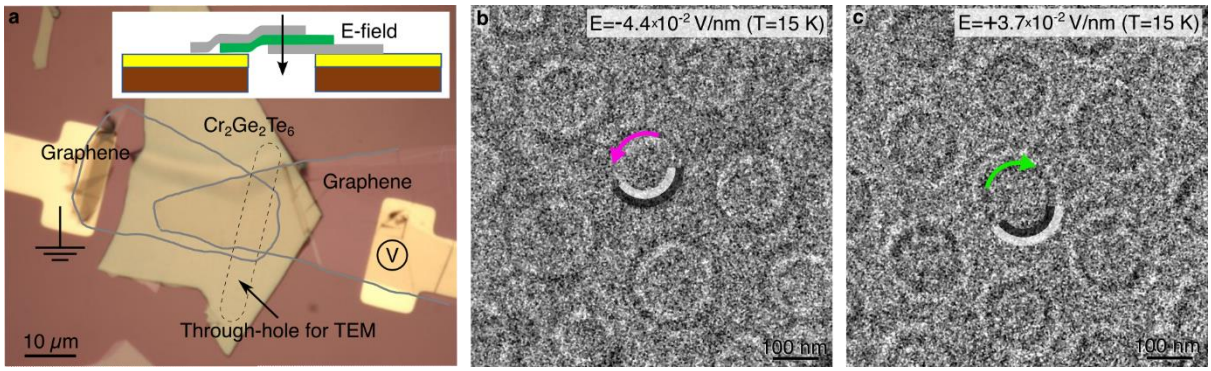

Figure 1: (a) Optical microscopy image of an electrically contacted graphene/$Cr_2Ge_2Te_6$/graphene heterostructure on a silicon/silicon nitride TEM grid with Pt electrodes. (b, c) Lorentz TEM images of the sample shown in (a) obtained at 15 K after field cooling in a magnetic field of 52 mT and with an applied electric field of (a) -44 mV/nm and (b) +37 mV/nm applied during field cooling.

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VITROJET: ICE THICKNESS CONTROL AND MEASUREMENT FOR TIME-EFFICIENT SINGLE PARTICLE STRUCTURE DETERMINATION

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Cryo-electron microscopy (cryo-EM) is increasing in popularity as a method for biomolecular structure determination. Targeting optimal ice thickness plays a crucial role to predictably achieve high resolution in cryo-EM. Up till now, thickness screening and selecting holes for data collection is done in the electron microscope, squandering valuable beam time.

The cryo-EM samples are prepared on the VitroJet, a cryo-EM sample preparation system which combines pin-printing and jet vitrification. The ice thickness can be controlled using the printing velocity and standoff distance from pin to grid. Both a theoretical model and experimental validation demonstrate that lowering velocity or distance results in thinner ice, where ice thickness is confirmed in the electron microscope by the energy filter method.

Using the optical camera integrated in the VitroJet, we developed a method to determine thickness of buffer-suspended holes on an EM grid during sample preparation (Fig. 1). Dependent on the thickness, the error is below ± 20 nm in the range between 0 - 70 nm down to ± 10 nm in the thinnest ice regions (10 - 40 nm).

Both ice thickness control and measurement allow users to optimize layer thickness on-the-fly during grid preparation, without the need of cryo-EM. Moreover, the ice thickness estimation can be used to target holes for data collection based on this optical image. Our test case demonstrates that when comparing 30 nm to 70 nm ice, 3.7 times less particles are required to reach a resolution of 3 Å (Fig. 2). This proves how these methods enable speeding up the entire workflow and using microscope infrastructure for data collection more efficiently.

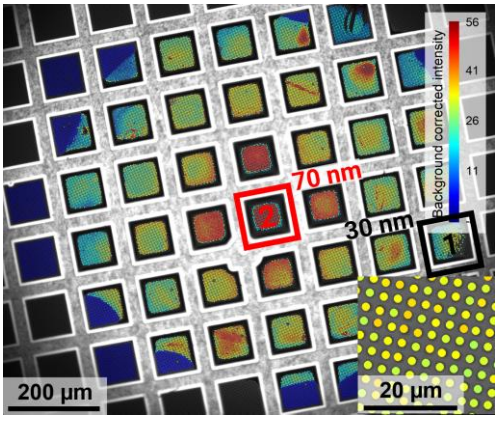

Fig. 1: VitroJet deposition image with intensity color overlay, which correlates with ice thickness

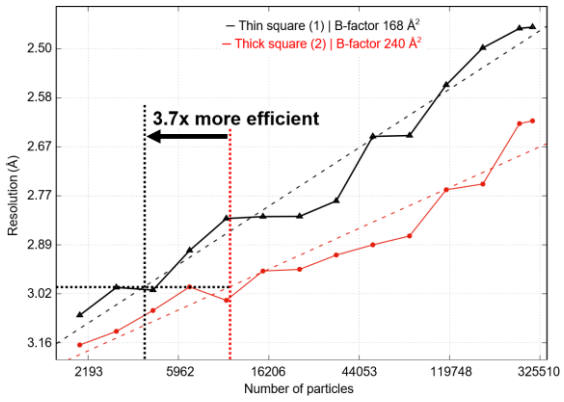

Fig. 2: Number of particles required to reach a certain resolution from a square with thick and thin ice

SPARSE ARRAYS FOR FOUR-DIMENSIONAL SCANNINGTRANSMISSION ELECTRON MICROSCOPY

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Only a few electrons may be registered per detector frame for low-dose four-dimensional scanning transmission electron microscopy (4D STEM) [1]. Event-based detectors [2, 3] like the Timepix3-based CheeTah T3 by Amsterdam Scientific Instruments (ASI) register the time and location of individual electron impacts. They can be more efficient than traditional frame-based detectors in this regime since they only record the non-zero values. To use such detectors for 4D STEM, the electron impact must be correlated to a scan position.

The CheeTah T3 allows recording precise timing of signals, such as level change of a line trigger signal, with the same clock as the electron events. That way, electron impact times can be correlated to scan positions without requiring a synchronized clock between the systems.

By converting the time stamps to scan position indices and combining them with the existing detector position indices, the data structure of a sparse array in coordinate (COO) format is created. Software libraries such as the sparse Python module allow operating on such sparse arrays with an API that is compatible with dense arrays. That way, event-based data can be processed in its compact sparse form, without re-implementing algorithms or converting data to a much larger dense format.

The COO format doesn't allow efficient extraction of individual frames, while the compressed sparse row (CSR) format includes a row index that enables this. The CSR format, however, requires that non-zero values are aggregated by frame index. The Timepix3 chip doesn't guarantee that the events are read out in the order of their time stamp. The ASI software SERVAL exploits that the arrival times are only scrambled within a short time interval, so that the events can be sorted in a small local environment. This allows creating CSR data in real time, including live streaming over network.

LiberTEM 0.11 [4] and newer implements support for reading sparse arrays in CSR format and receiving chunks of CSR data over network for live processing. A number of core routines, such as virtual detectors, are already implemented to work directly on both sparse and dense arrays. Live 4D STEM with 1 million frames per second has been demonstrated with LiberTEM-live [5]. In combination, SERVAL and LiberTEM allow using the CheeTah T3 detector in the same way as a conventional frame-based detector from a user and application developer perspective, while retaining the advantage of much smaller data size and higher frame rate. That allows the users to track sample and adjust astigmatism with live reconstructed movies from virtual detectors applied to event-driven 4D STEM dataset [5, 6].

The excellent performance of sparse array libraries, including GPU support, combined with the scalability and performance of LiberTEM, position this approach well for the upcoming Timepix4 detectors. They will increase the maximum event rate by an order of magnitude, which enables higher beam currents.

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SARS-COV2 SPIKE PROTEIN: A FUNCTIONAL ANALYSIS OF ALL CRYO-EM STRUCTURES

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Cryogenic Electron Microscopy (Cryo-EM) has been the major structural biology technique in structure based functional characterization of SARS-CoV2 corona virus and thus has contributed immensely to the vaccine development. More than one thousand Cryo-EM Spike-protein structures have thus far been deposited in the Electron Microscopy Data Bank (EMDB). Cryo-EM, being an imaging technology where linear transfer of information is fundamentally possible, enables us to harvest more information from the comparisons of independently solved structures. This type of information harvesting does not follow usual Cryo-EM workflow of structure determination. Hence, a number of initial problems needed to be sorted. For example; due to different processing approaches, the deposited structures often have a large variation in the density values which needs to be normalised carefully. Furthermore, it is necessary to bring all the deposited structures to a unified scale with respect to pixel size and box size. The pool of thousand spike proteins also contains variations of different antibodies attached, and exhibit different functional states of the trimer itself. While dealing with these initial hurdles, a significant differences in magnification of the deposited structures (relative to the claimed magnification) were diagnosed by Multivariate Statistical Analysis (MSA) of the dataset [1]–[3]. This level of magnification differences rendered the alignment in real space a problem. Three-dimensional versions of the Self-Correlation-Function (SCF) proposed decades ago [4], turned out to be the right tool to decouple the rotational alignment from the magnification correction. An iterative refinement of rotational alignments and magnification corrections, applied to an SCF version of the Spike proteins, facilitates the alignment of different spike proteins to render them comparable.

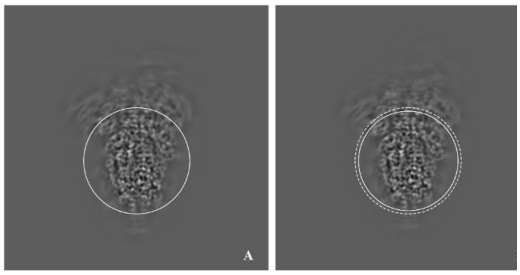

Fig. 1: MSA reveals magnification differences: Unsupervised classification along the main eigen-vector axis related to magnification differences, yields classes of spike proteins of different sizes. The continuous white circle enclosing the main features of one class-average in (A), is larger than the corresponding white circle in another class-average (B). The difference in sizes between the two class averages is indicated by the dotted white circle in (B).

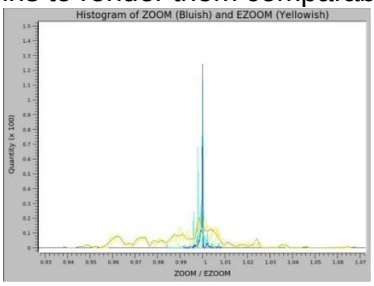

Fig. 2: Histogram of magnification differences: The yellow curve shows magnification values claimed by the authors of the structures in EMDB. A spread of ±5% of claimed magnifications was diagnosed in the database. The dark-blue curve shows the residual variations (spread of ±0.1%) in magnification of the final standardized stack of all 3D spike proteins structures in the data base. A single “4D” stack of re-calibrated 3D structures was obtained.

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[5] This research was supported by FAPESP (Brazil).

STRUCTURAL CHARACTERIZATION OF P62 HELICAL FILAMENTS IN THEIR ROLE AS SELECTIVE AUTOPHAGY CARGO RECEPTOR

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Autophagy is the process of digesting cellular material and recycling of nutrients by degradation of cargo in autolysosomes. For selective autophagy the cargo gets specifically tagged for digestion, e.g., after labeling with polyubiquitin and followed by the interaction with the selective autophagy receptor p62. p62 bridges the cellular machinery for degradation with the cargo by binding to autophagy key player Atg8 proteins like LC3B via the LIR motif and ubiquitinated cargo. Due to homo-oligomerization of p62 molecules, p62 polymers are able to establish the spatial proximity between autophagy machinery and cargo. Additionally, p62 has been shown to crosslink cargo into phase separated droplets leading to an initiation of autophagy on site. *In vitro*, purified p62 forms helical filaments. Here, we investigate how p62 filaments interact with model autophagy membranes and cargo. First, we investigate the interaction of p62 filaments mixed with SUVs decorated with LC3B using cryo electron tomography. The reconstructed tomograms were segmented and analyzed with regard to p62 filament morphology and membrane properties at interaction sites. Second, we studied p62 filaments in a cargo-rich environment by adding soluble GST-4xUbiquitin in excess molar ratio and imaging the emerging droplets with cryo electron tomography. These tomograms reveal how p62 is organized in *in vitro* phase separated droplets.

Fig. 1: Segmentation of a cryo electron tomogram of p62 filaments (yellow) with LC3B decorated SUVs (grey).

Fig. 2: Exemplary slice of a tomogram of p62 filaments in a phase separation droplet induced by excess of GST-4xUbiquitin.

INVESTIGATING THE PRACTICAL LIMITS OF CRYO-FIB MILLING FOR FROZEN HYDRATED/HIGH-PRESSURE FROZEN MATERIAL

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Focused ion beam (FIB) milling of vitrified biological samples allows the visualization of macromolecules in their native environment by cryo-electron microscopy (cryo-EM). For single cells, FIB-milled lamellae have given high-resolution insight into macromolecular structure and organization *in situ* [1]. Through recent advances in sample preparation, multi-cellular organisms and tissues have become more amenable to cryo-EM. Achieving high resolutions, however, remains challenging in these rather complex samples.

We set out to quantify the current limitations of cryo-EM in complex vitrified samples: With a modified protocol of the "waffle-method" for high-pressure freezing [2], we achieved reliable vitrification. We used Serial Lift-Out to extract a block of vitrified material, prepare slices and produce thin lamellae on a Ga-FIB instrument (Figure 1 a, b) [3]. On average, 80 % of lamellae were successfully transferred to the cryo-transmission electron microscope (TEM). To assess the resolution-limiting factors, we prepared thin lamellae (197 ± 30 nm) of high-pressure frozen, purified apoferritin. The resulting structure from 143 micrographs was resolved to 5.8 Å (Figure 1 c, d). Lamella thickness primarily limited the signal to noise ratio (SNR) and "alignability" of the particle images. Particularly for thin lamellae, FIB-induced damage also reduces the SNR close to the lamella surface. This effect can be mitigated by FIB-milling at lower energies [4], but at the cost of a broader ion beam.

By further optimizing the preparation of thin lamellae using well-established high-pressure frozen model samples, we aim to visualize the molecular landscape of tissues or small organisms at higher resolution in the future.

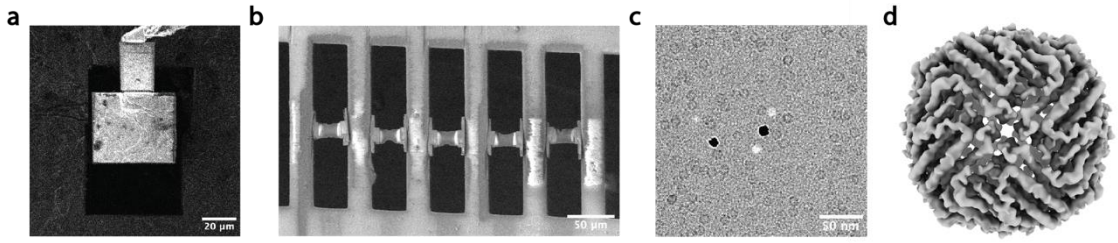

Fig. 1: Serial lift-out for high-resolution cryo-EM. (a) Ion beam image during lift-out of vitrified sample block from high-pressure frozen sample. (b) Scanning electron microscopy image of a series of polished lamellae after lift-out. (c) TEM image of high-pressure frozen and FIB-milled apoferritin sample, dark spots are Pt particles from sputter coating. (d) Apoferritin reconstruction at 5.8 Å from 1115 particles.

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BEAMSHAPING THE FIB FOR LAMELLA PREPARATION - YAY OR NAY?

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Focused ion beams are wide-spread instruments used for transmission electron microscopy sample preparation across scientific disciplines [1,2]. Generally, site-specific shaping of materials is achieved via scanning a highly focused probe across an area, leading to ablation of the material. However, the geometry milled for TEM lamella preparation is usually that of a thin foil, with the lamella's thickness being orders of magnitude smaller than its width and length. Here, we propose a change in probe geometry for milling. Instead of using a spot probe ion beam, we use the FIB stigmator as quasi-cylindrical lens to create a sheet geometry that we term 'ion knife'.

The ion knife spread the current over a larger area and render the probe dimensions anisotropic. To explore its applications in cryogenic lamella preparation, we demonstrate cellular lamella milling [2] and sectioning of Serial Lift-Out [3] volumes with the ion knife. Our initial characterization shows that FIB shaping can narrow the beam in a given direction and holds the potential to reduce material loss in Serial Lift-Out. We discuss the current limitations and hardware developments needed to unravel the potential of FIB shaping towards enabling the structural cell biologist's dream of quasi-continuous multicellular organisms at molecular resolution.

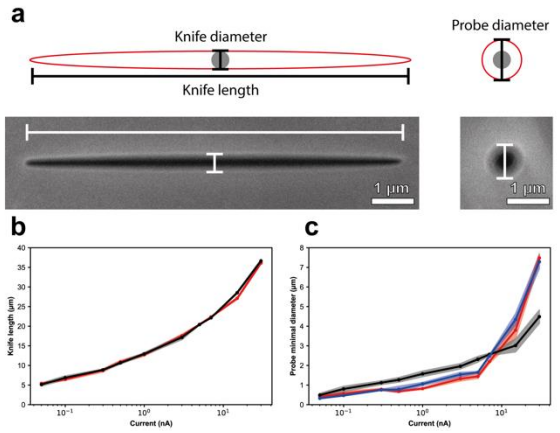

Fig. 1: Characterization of ion knife beam shapes. a) Definitions of the probe diameters and knife diameters and length for analysis. b-c) Measurements of b) knife length and c) minimal knife diameter for different currents.

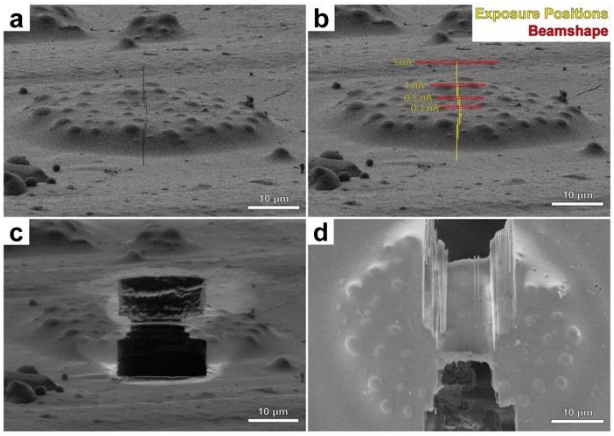

Fig. 2: Lamella milled using an ion knife. a) Cryo-FIB image of yeast cells prior to milling. b) Exposed positions with the shaped ion beam. Red ellipses indicate the beam shape. Yellow lines indicate the exposure positions. c) FIB and d) SEM image after lamella preparation.

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DYNAMICAL SCATTERING IN ICE-EMBEDDED PROTEINS IN CONVENTIONAL TRANSMISSION ELECTRON MICROSCOPY

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In the field of low-dose cryo-transmission electron microscopy (cryo-TEM), atomic resolution was reached for Apoferritin in recent years [1,2]. However, achieving atomic or near-atomic resolution for other proteins remains challenging. A more general question is whether the projection assumption (PO) and the weak phase object (WPO) assumption, implicitly or explicitly exploited during single particle 3D reconstructions, can explain some difficulties in reaching atomic resolution for other proteins. To shed light on this question, we performed multislice simulations of different proteins embedded in glass-like ice obtained by molecular dynamics to investigate the effect of multiple scattering and propagation [3].
We compare multislice simulations with the single-slice models propagated to the exit surface of the specimen (PO_{exit} and WPO_{exit}) to investigate the effect of dynamical scattering on the imaging process in cryo-TEM. Comparing the phase of the exit waves of the single-slice models with a multislice simulation in Fig. 1 shows an agreement for low to medium resolution but at near atomic resolution a detailed analysis yields fringes due to the additional propagation. Using the multislice cases as a reference for a Fourier ring correlation (FRC), the commonly used PO_{exit} models only show contrast transfer up to 0.62/Å (Fig. 2) for the largest protein (ribosom). These results suggest that for 3D reconstructions better than 1.2 Å resolution, multiple dynamical scattering should be taken into account for very high-resolution image reconstructions of biological specimens.

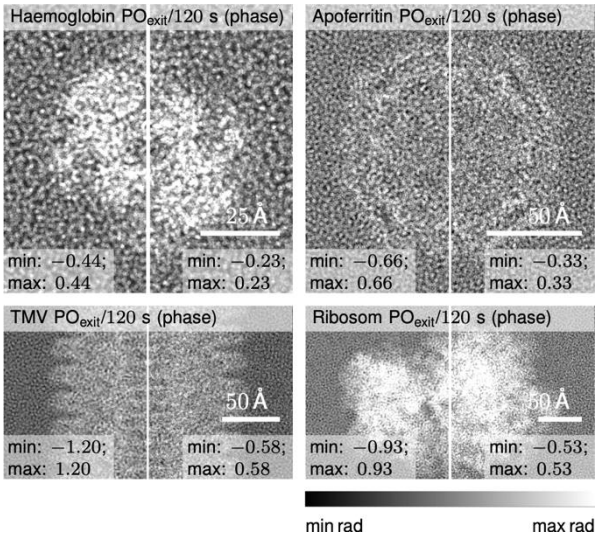

Fig. 1: Phases of the exit waves according to the phase object (left) and multislice (right) simulation for four different proteins.

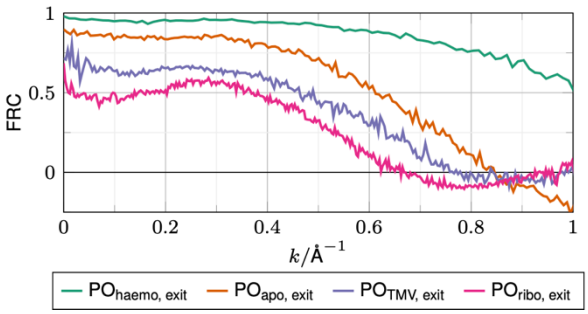

Fig. 2: FRCs between PO_{exit} and the multislice simulation for the four proteins givien in the legend.

References:
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ADAPTING A SELENIUM NANOPARTICLE FORMING ENZYME TO PRODUCE EM CONTRAST MARKERS WITH IMPROVED SIZE CONTROL

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In cellular electron microscopy, large macromolecular objects like filaments and ribosomes can be resolved. However, smaller macromolecules require additional contrast and are difficult to directly identify. The Ackerson group at Colorado State University is working on an approach to impart unique contrast to macromolecules that cannot otherwise be identified in cellular EM. The approach is based on a Glutathione Reductase-Like Metalloid Reductase (GRLMR) enzyme, which converts soluble selenium ions to high-contrast selenium nanoparticles [1]. To localize the SeNPs to the enzyme (and protein of interest), the enzyme was genetically fused to a SeNP binding peptide [2]. To better understand how the peptide can control the size of the enzymatically created SeNPs, we are varying the number of peptide repeats fused to the enzyme., as depicted in **Fig. 1**. Preliminary data has shown that size control characteristics appear to vary with different tandem repeat quantities, as shown in **Fig. 2**. Ongoing efforts include more rigorous characterization of nanoparticle sizes formed by these variants by combining higher throughput TEM data collection via SerialEM with nanoparticle classing in software like CryoSPARC. Further investigation, particularly through refining higher throughput data collection workflows, would yield information about improving particle size tunability for easily trackable EM contrast markers.

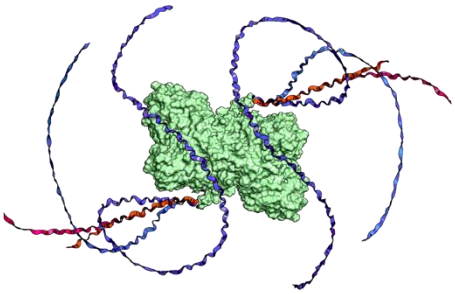

Fig. 1: AlphaFold2 structural prediction of GRLMR (green) with covalently linked SeBP tandem repeats in multiples of two (orange), three (pink), five (blue), and ten (purple).

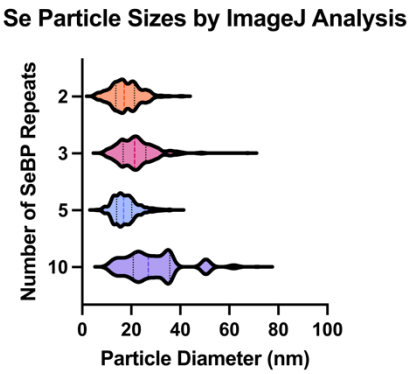

Fig. 2: Preliminary data of selenium nanoparticle sizes as a function of the number of SeBP tandem repeats. Particle sizes were determined by TEM image thresholding in ImageJ (n = 350 particles).

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IN SITU STRUCTURAL ORGANIZATION OF THE P62 AUTOPHAGY CARGO RECEPTOR

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The process of autophagy, also called cellular self-eating, is essential for the removal of aggregated proteins, malfunctioning organelles and even intact microbes. Poly-ubiquitinated cargo is recognized by cargo receptors that recruit a series of autophagy core machinery complexes and a double membrane and subsequently directed to the lysosome or vacuole for degradation. One of these cargo receptors, called p62 or SQSTM1, acts as a central hub in autophagy; it binds poly-ubiquitinated cargo, several autophagy regulators. Additionally, p62 forms polymers in vitro and phase-separates in the cytosol together with the poly-ubiquitinated cargo [1,2]. However, the exact molecular mechanism behind cargo recognition and the formation of large intracellular structures is poorly understood. We have been working to bridge this gap in knowledge by studying both the in vitro structure one of these cargo receptors as well as the in situ structural organization using cellular cryo electron tomography. To characterize these protein condensates inside the context of the cell, we have used correlative light and electron microscopy [3], super resolution confocal microscopy as well as Energy-dispersive X-ray spectroscopy, a technique commonly used in material science to do elemental analysis. Correlative light and electron microscopy allows us to positions the cells on an EM grid using micropatterning, locate p62 in individual cells using an in-chamber fluorescence microscope, before thinning the sample to ~150nm within that same cryo-FIB-SEM machine and finally imaging it in a cryo-TEM. Having all these technological improvements increased our through-out greatly, and reduced ice contamination on the cryo-lamella

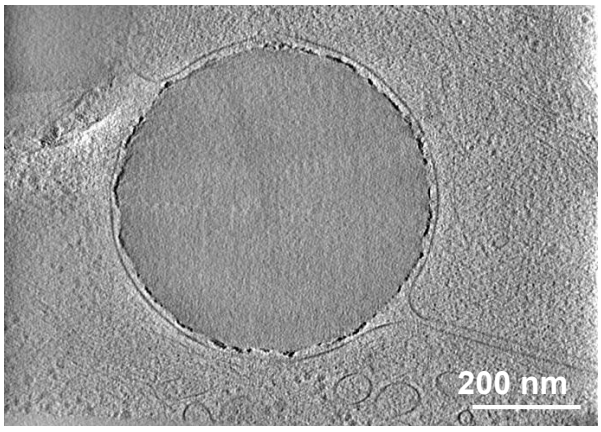

Fig. 1: Slice of a tomogram recorded on RPE1 cells expressing mCherry-p62. p62 forms an electron dense coat around a cellular lipid droplet. The function of this structure is unknown.

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REPLICATION ORGANELLE MEMBRANE REMODELING IN SARS-COV-2 INFECTION

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel, highly pathogenic positive-strand RNA virus of the *coronaviridae* family and cause of the COVID-19 pandemic. It targets cells of the respiratory system, where it releases viral RNA into the cell and induces replication [1]. Studies have shown that viral replication is, in addition to other factors, heavily dependent on some selected autophagy host factors, thereby suggesting a dependency of SARS-CoV-2 replication on parts of the autophagy machinery of the host cell [2]. Autophagy is a degradation pathway in eukaryotic cells by which large proteins, protein complexes or organelles are engulfed in a vesicle and subsequently degraded in the lysosome. Targeted cargo is encapsulated in autophagosomes, large double-membrane vesicles that emerge from the membrane of the endoplasmic reticulum [3]. Structural studies showed remarkable morphological similarities between autophagosomes and DMVs, dedicated replication organelles induced by SARS-CoV-2 infection, in which the viral RNA is replicated. Furthermore, important proteins involved in the membrane biogenesis of autophagosomes, such as the membrane-remodeling protein DFCP1, have been shown to be essential for DMV biogenesis and viral replication. This project aims towards identifying the effect of different host cell proteins such as DFCP1 on membrane biogenesis and remodeling of viral DMVs. *In situ* cryo-electron tomography is employed to study morphological changes in membrane structure in the absence of target proteins on a cellular level. To identify and localize features of interest in the frozen cell, correlative light and electron microscopy (CLEM) is used by labelling either autophagosomes or DMVs with fluorescent proteins.

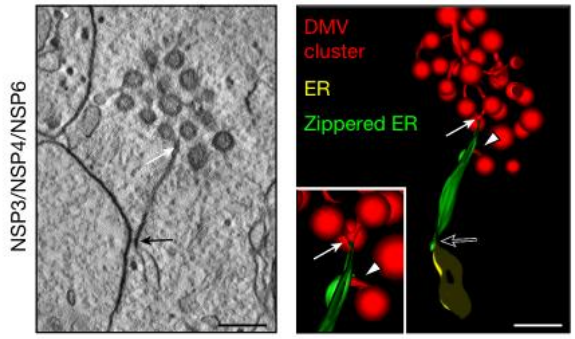

Fig. 1: DMV cluster induced by nsp3, nsp4 and nsp6

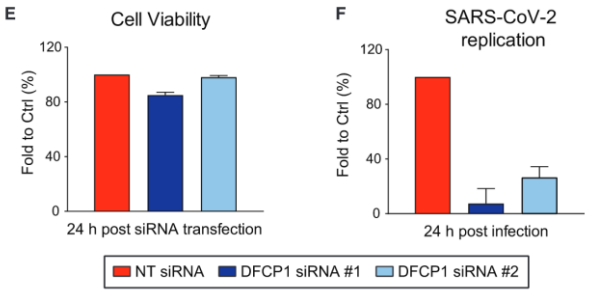

Fig. 2: SARS-CoV-2 replication is severely inhibited in the absence of DFCP1

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CHARGING OF VITREOUS SAMPLES IN CRYOGENIC ELECTRON MICROSCOPY MITIGATED BY GRAPHENE

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Cryogenic electron microscopy (cryo-EM) can be used to provide high-resolution reconstructions of macromolecules embedded in thin layers of ice, from which atomic models can be built *de novo* [1]. However, the interaction between an ionising electron beam and a sample can result in beam-induced motion and image distortion, which limit attainable resolution [2]. Sample charging is a contributing factor to beam-induced motion and image distortion and is normally alleviated by including part of the supporting conducting film within the beam-exposed region [3]. Here, we characterise electrostatic charging of vitreous samples, both in imaging and in diffraction mode. We mitigate sample charging by depositing a single layer of conductive graphene on regular EM grids (Fig. 1). We obtained high-resolution single-particle analysis (SPA) reconstructions at 2 Å resolution with the electron beam irradiating only the central parts of holes on graphene-coated grids, using data collection schemes that previously failed to produce sub-3-Å reconstructions without the use of a graphene layer. We find that SPA data obtained from graphene-coated grids exhibit a higher b-factor and reduced particle movement, when compared with data obtained without the use of a graphene layer (Fig. 2). Such mitigation of charging promises to have broad implications a wide variety of EM techniques, including SPA and cryo-tomography, as well as for the study of radiation damage and the development of future sample carriers. Furthermore, it may facilitate the exploration of more dose-efficient scanning transmission EM-based SPA techniques.

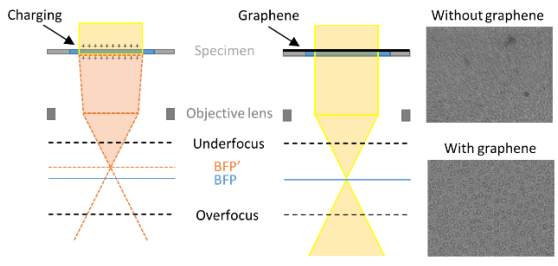

Fig. 1: Graphene-coated grids reduce charging and result in non-blurred images.

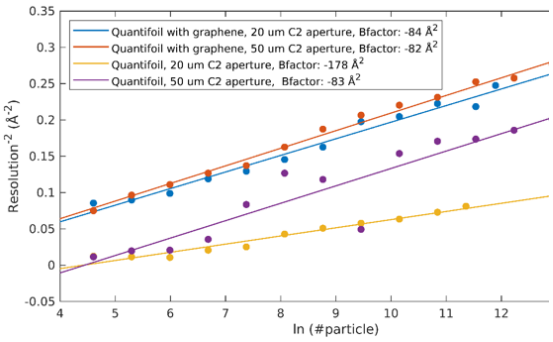

Fig. 2: Rosenthal-Henderson B-factor plot showing resolution versus number of particles for different datasets.

References:
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[3] R. M. Glaeser, Methods Enzymol 579, 19-50 (2016).

POST-ACQUISITION ABERRATION CORRECTION IN CENTER-OF-MASS IMAGING

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Center-of-mass imaging (COM) is a commonly used method in scanning transmission electron microscopes (STEM). The contrast of COM images, which is proportional to projected electric field distribution in sample plane, is calculated from the center-of-mass shift of convergence beam electron diffraction (CBED) patterns. For crystal samples, it can provide information on defects and nuclear electric field distribution at atomic level. Further, COM images are used to calculate integrated center-of-mass (iCOM, proportional to projected potential) and differential center-of-mass (dCOM, proportional to projected charge density) images [1]. However, the existing algorithm for calculating COM images from a 4DSTEM is limited to an in-focus condition, and is sensitive to aberrations, including defocus, and iCOM is sensitive to low-frequency noise.

Here we report an algorithm, which can correct residual aberrations in 4DSTEM datasets for COM and by extension iCOM/dCOM images, named Single-Side Band masked COM (SSBm-COM). As the name suggests, a mask which is similar to the mask used in Single-Side Band (SSB) ptychography [2, 3] is applied for COM image. Theoretically, if the sample can be regarded as a weak phase object, then all phase-contrast information is included in the SSB mask [4]. Therefore, this mask optimally filters the original dataset and removes pixels without information and that only contain noise. This method can be a potential solution at defocus or low-dose conditions for direct field measurement and imaging defects in thin samples.

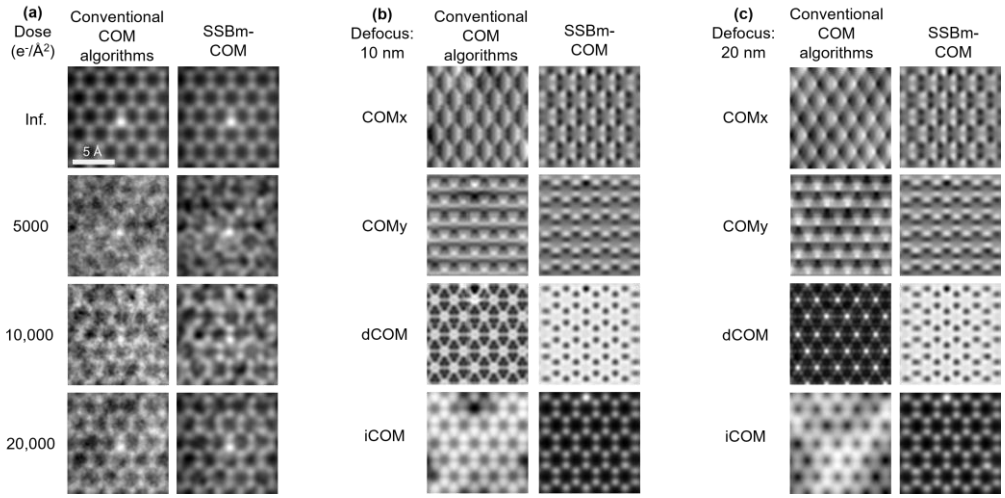

Fig. 1: Simulations of conventional COM algorithms and SSBm-COM of a monolayer graphene sample. (a) iCOM images at different dose conditions, (b) aberration correction with a defocus of 10 nm, (c) aberration correction with a defocus of 20 nm.

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CRYO-STEM OF BIOLOGICAL MACROMOLECULES: VISUALIZATION BY INTEGRATED DIFFERENTIAL PHASE CONTRAST

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Scanning transmission electron microscopy (STEM) is a well-established method for the characterization of materials. However, it is not a widely used method for imaging biological samples in cryogenic conditions. Biological specimens are dose-sensitive, often have poor contrast against ice background and can therefore be challenging to visualize. Recently, we showed that integrated Differential Phase Contrast (iDPC)-STEM can be applied on vitrified biological specimens and near atomic resolution can be determined [(1)]. In STEM the resolution is dependent on the convergence semi-angle (CSA) of the focused beam. Using the iDPC-STEM approach, the maximum contrast of the image can be achieved in focus. In this work, tobacco mosaic virus (TMV) and keyhole limpet hemocyanin (KLH) have been used as specimens at different CSAs (2.0, 3.0, 4.0 mrad). In case of TMV near-atomic resolution results are demonstrated and in the case of KLH, the theoretical and experimental challenges of the method for single particle samples are described.

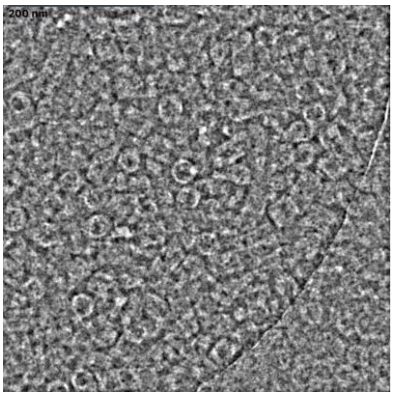

Fig. 1: KLH embedded in vitreous ice.

References:
[1] Lazić, I., Wirix, M., Leidl, M.L. et al. Single-particle cryo-EM structures from iDPC–STEM at near-atomic resolution. Nat Methods **19**, 1126–1136 (2022). https://doi.org/10.1038/s41592-022-01586-0

OPTIMUM METHODOLOGY FOR ATOMIC-RESOLUTION PTYCHOGRAPHY IN ULTRA-LOW-DOSE CONDITIONS

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Several recent developments in scanning transmission electron microscopy (STEM) have made it a viable option for the imaging of dose-sensitive specimens such as 2D materials, protein crystals and ordered nanoporous particles. Well-reasoned experimental protocols encompassing the different aspects and interests of existing methods are nevertheless still desirable, e.g. to exploit dynamics inherent to damage mechanisms [1]. In particular, the combination of a low acceleration voltage, a measurement strategy optimized for damage sites diffusion and self-healing [2], an event-driven detector [3] and a highly dose-efficient computational imaging method [4] would make it feasible to perform ultra-low-dose (<100 $e^-/Å^2$) atomic-resolution microscopy of single dose-sensitive objects (e.g. without single particle analysis). Here, we demonstrate improvements in the retrieval of the electrostatic potential of a MoS_2 specimen, using multislice-simulated electron diffraction, through the introduction of optimized contrast transfer functions (CTF), the correction of the modulation transfer function (MTF) of the detector and the partial compensation of source size and focus spread effects. We furthermore compare different sampling strategies, e.g. alternative scanning and defocused illumination, in terms of their ability to exploit the diffusion dynamics of an arbitrary beam damage process. Finally, we introduce improved analytical ptychography algorithms and paradigms on the basis of event-driven diffraction data.

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[2] D. Jannis et al. ; Ultram. **240**, 113568 (2022)
[3] D. Jannis et al. ; Ultram. **233**, 113423 (2022)
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TOWARDS SUB-NM RESOLUTION OF CRYOGENIC PTYCHOGRAPHY SINGLE-PARTICLE ANALYSIS (CRYO-EPTY SPA)

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Cryo-EM with single particle analysis (SPA) facilitates the visualization of 3D macromolecular structures at an atomic scale [1]. However, the electron sensitivity inherent in biological samples leads to low contrast in EM images. While imaging at a large defocus can improve contrast, it also limits the information transfer at high spatial frequencies with the sample [2]. Ptychography diffractive imaging, a technique capable of reconstructing phase information from diffraction patterns using an iterative algorithm known as ePIE [3] (Fig. 1), holds great promise for achieving super-resolution, high-contrast, low-dose, and 3D imaging of biological samples in vitreous ice at low doses [4]. Moreover, ptychography utilizes the entire diffraction patterns, making it particularly efficient in dose usage, especially when using direct electron detector data with a high signal-to-noise ratio at a low electron dose [5]. Using ptychography we have successfully reconstructed the 2D phase images of rotavirus at cryogenic temperatures with a dose of 5 e/Å^2 and have further demonstrated the visualization of 3D structures at nm resolution by integrating SPA [6]. To show the potential that cryogenic ptychography (cryo-EPty) and SPA to achieve atomic level resolution, here we will employ apoferritin as a benchmark sample, and implement different convergence semi-angles (CSA) to reconstruct its structure as shown in Fig. 2a-b. Subsequent SPA 3D density maps have shown resolutions of 1.1 nm and 0.8 nm, respectively. Our findings suggest that the promising capabilities for cryo-EPty combination with SPA pave the way for high-resolution 3D reconstructions of biological samples, potentially reaching atomic resolution.

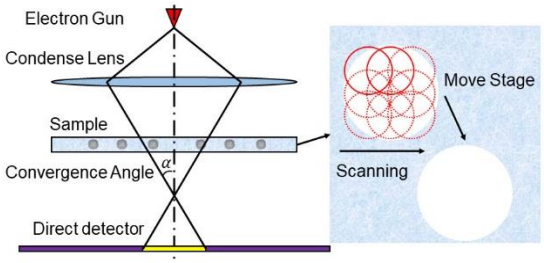

Fig. 1: Schematic diagrams of the optical configuration for ptychography

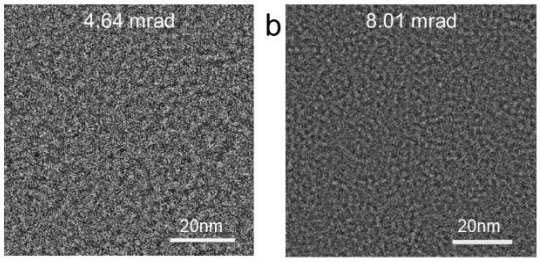

Fig. 2: Ptychography reconstructions of apoferritins using various CSAs, (a) 4.64 mrad, (b) 8.01 mrad.

References:

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CONSISTENCY OF DIFFERENT PTYCHOGRAPHIC PROBE RETRIEVAL METHODS

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Knowledge of the electron probe is an important prerequisite for a successful ptychographic reconstruction as it is otherwise impossible to deconvolve the effects of specimen and illumination. While direct schemes such as the single sideband ptychography (SSB) are able to correct for lens aberrations, these have to be known beforehand [1]. Using iterative schemes, such as the extended ptychographic iterative engine (ePIE) [2], it is possible to optimize both the specimen and the probe at the same time. However, the retrieved probe is not limited to physical solutions, and it is difficult to gauge the reliability of a retrieved probe.
To investigate the consistency and quality of the retrieved probes a 4D-STEM focal series of a 2D SnS_2 sheet was acquired with defoci between -20 and 20 nm. The datasets were first reconstructed using ePIE, and an iterative procedure was developed to fit probes to each result, parametrized by standard aberration coefficients. Figure 1 shows the reconstructed objects and the real space probes as well as the aberration function retrieved from ePIE, together with the respective fit results. For all investigated foci the agreement between retrieved and fitted probe is very good which can be recognized both in real space and in the phase plate. A plot of the defocus determined using the ePIE probe against the defocus set at the microscope shows the expected linear relationship (Figure 2). Additionally, the aberration coefficients for different foci are in very good agreement with each other throughout the series. Consequently, the probes obtained by ePIE are physically reasonable electron probes and can be fully characterized using axial lens aberrations. The fitted aberration coefficients can be used as a start for other reconstruction schemes or to align the TEM. The applicability of the found aberration coefficients to other ptychographic schemes, such as SSB or gradient based methods, will be shown additionally. Probes and specimen retrieved using different reconstruction schemes will be compared and the influence of partial coherence is discussed.

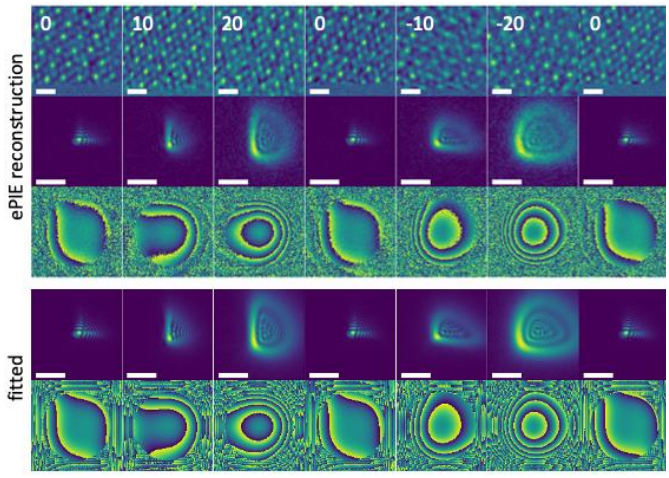

Fig. 1: **Top:** Phase grating (top) with foci (nm), probe (middle), aberration function (bottom) from ePIE. **Bottom:** Probe fit.

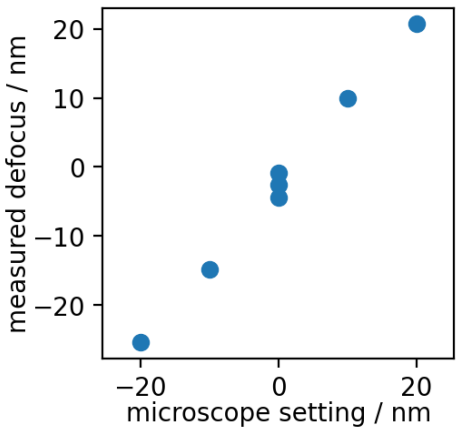

Fig. 2: Plot of the fitted defocus vs. the nominal one set at the microscope.

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STRUCTURED ILLUMINATION ELECTRON PTYCHOGRAPHY

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Ptychography measures a correlated matrix of diffraction patterns during scanning with sufficient overlap between adjoining scanned area, and reconstructs both the object and the probe with their amplitude and phase components. For a ptychographically reconstructed image, its pixel size is determined by the detector sampling in the Fourier space rather than the scanning step size in the real space [1]. This would allow a coarser scanning sampling and therefore a larger field of view with reasonable number of scan pixels or a lower dose with a practically used probe current.

In fact, ptychography has been demonstrated as a dose-efficient imaging method thanks to the reconstructed pure phase contrast image using advanced algorithms [2]. This is particularly useful for weak phase objects such as biological specimens which only contains light elements. However, a limited band of spatial frequency for a usable contrast transfer from the ptychography reconstruction will make it difficult to target biological molecules using their low spatial frequency information when trying to resolve close to atomic structures using their high spatial frequency counterpart [3]. Applying a structured illumination to the probe will bring in a mixed range of spatial frequency to the diffraction patterns and open up the contrast transfer function over a wide range of frequencies.

When it comes to large field of view phase contrast imaging, near-field ptychography, which uses a full-field illumination and measures Fresnel, instead of Fraunhofer, diffraction, has been demonstrated to be more efficient [4]. In this case, structured illumination is also of vital importance. Otherwise, each diffraction image carries the same information except for laterally shifted, and therefore the ptychography reconstruction reduces to a single phase retrieval loop. Different diffuser designs and optical setups have been tested for the near-field electron ptychography. Particularly an amplitude mask shaping between the probe-forming optics turns to be working well for a variety of applications including magnetic contrast imaging under magnetic field free condition [5]. More recent progress will be presented in this contribution.

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AUTOMATED 4D STEM CRYO-TOMOGRAPHY

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Cryogenically preserved three-dimensional electron tomography (cryo-ET) of biological samples gains increasing attention because of the possibility to image macromolecular assemblies in the cellular context [1]. Tomography based on conventional TEM with defocus phase contrast provides excellent lateral resolution but is limited in specimen thickness due to inelastic scattering and the need for energy filtering. Cryo-Scanning Transmission Electron Tomography (CSTET) [2–6] offers an alternative with specific advantages for large 3D fields of view. The accessible specimen thickness can exceed one micron. CSTET is especially useful for large or unique structures that are not amenable to sub-volume averaging. In light of the extreme sensitivity of organic samples to radiation damage, efficient use of the illumination is essential. The approach of 4D STEM is to record the entire diffraction pattern at every position of the probe using segmented or pixelated detectors. We have developed a hardware and software system for 4D STEM integration with SerialEM, which supports automated tomography and other high-level data acquisition protocols using the Opal six-channel diode detector (El-Mul Technologies) and the ultra-fast ARINA hybrid pixel detector (DECTRIS) [7,8]. Using samples of T4 bacteriophage and in-cell mitochondria, we demonstrate the variety of contrast modes that can be obtained from a single tomographic dataset. These include Differential Phase Contrast (DPC), principal component analysis (PCA), and integrated Center-Of-Mass (iCOM) contrasts, all adapted to tomography. 3D deconvolution makes an important improvement in contrast and in axial resolution [6,9,10]. In summary, we introduce automation of 4D-STEM cryo-tomography and image analysis and compare different ways to analyze the resulting data.

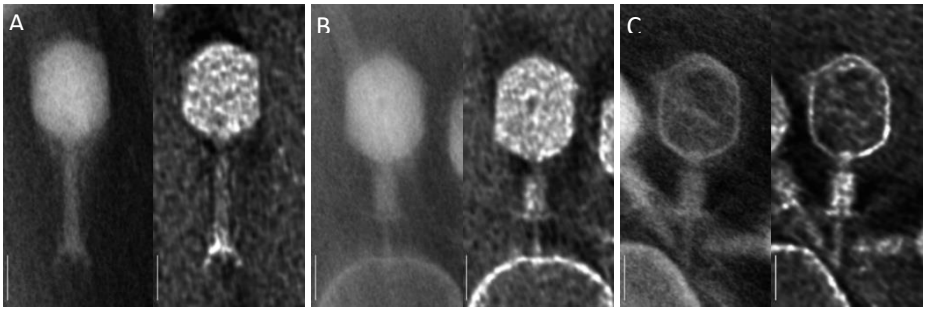

Figure 1: Different conformations of T4-Phage virions obtained by 4D-STEM cryo-tomography data using PCA2 analysis. (A) A full, (B) partial, and (C) empty virion before and after 3D-deconvolution. Scale bar is 50 nm.

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CRYO-STEM FOR BIOLOGICAL MACROMOLECULES

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Scanning transmission electron microscopy (STEM) has unique potential advantages over conventional TEM (Fig. 1), like in-focus measurements with non-oscillating contrast transfer and the ability to image µm thick samples. We have recently applied integrated differential phase contrast (iDPC-) STEM measurements to the biological macromolecules keyhole limpet hemocyanin and tobacco mosaic virus, reaching near-atomic resolution [1].
Conventional electron cryomicroscopy (Cryo-EM) uses sophisticated algorithms to deal with beam induced motion and contrast transfer. We now apply these techniques to cryo-STEM measurements, including motion correction for STEM movies and a function for contrast transfer baseline correction, continuing to improve resolution, contrast and accuracy (Fig. 2).
We furthermore applied ptychographic algorithms like SSB reconstructions to Cryo-STEM samples and show initial results of 4D-STEM measurements with a Dectris Arina detector mounted to a 300 kV Titan Krios.

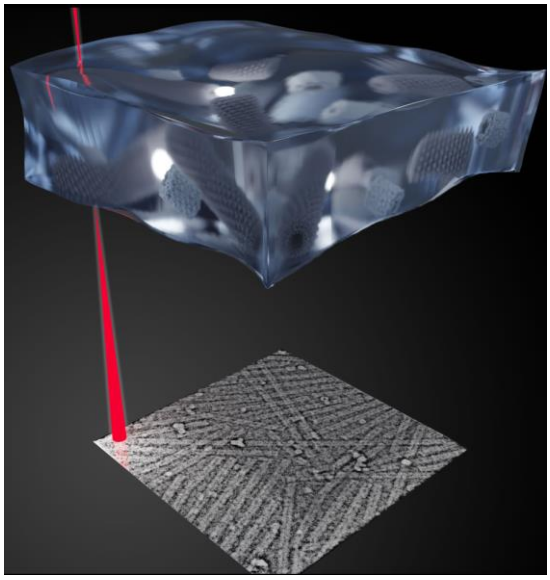

Fig. 1: Cryo-STEM illustration

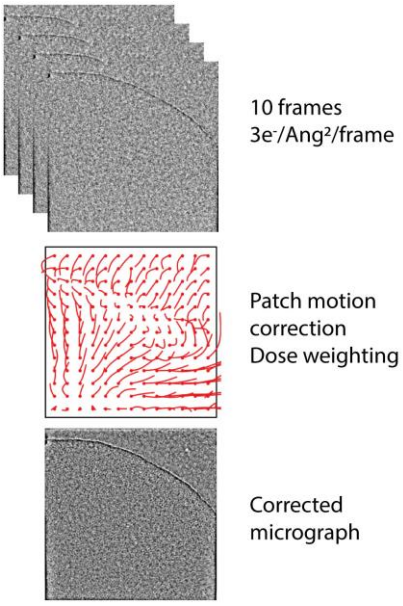

Fig. 2: Motion correction and damage mitigation via dose weighting on a frozen-hydrated apo-ferritin sample

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NON-CLASSICAL CRYSTALLISATION OF CeO_2: LIQUID-PHASE TEM AND GAMMA-RADIATION INDUCED STUDIES

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Liquid-phase electron microscopy observations have been extensively used in the last decade to perform in-situ and in-operando measurements in solutions. While many successful and thorough experiments have been carried out already, questions about (1) the extent of the influence of the electron beam on reaction pathways in liquids and (2) the effect of the confinement space of the liquid-cell holder on the studied processes are still poorly understood. In an attempt to address these questions, we ran comparative experiments in which a model radiolytic reaction (which enables precise control over the beam-induced chemistry in solution) is performed in a TEM liquid cell and in the large volume of a γ-radiation cell. As a model reaction, the radiolytic oxidation of Ce^{3+} in aqueous solution was chosen. This reaction follows a non-classical crystallization (NCC) mechanism leading to the formation of CeO_2 mesocrystals. Here it is worth mentioning that although NCC theory describes the formation of mesocrystals generally well, a detailed understanding of particular stages of growth (e.g. the mechanism of three-dimensional mutual alignment of the nanoparticles and the role of intermediates) is still missing. The radiolytic oxidation of Ce^{3+} happens in aqueous solution, without additives, at elevated temperatures and under constant dose rate, thus giving precise control over the reaction and excluding many of the parameters which would otherwise complicate the separation of different stages of growth (e.g. additives, heating).

To follow crystallization in the gamma-cell, we carried out a time-dependent ex-situ TEM study (eRDF, HRTEM) and characterized the solution at different stages of the reaction. This enabled us to identify several stages in CeO_2 mesocrystal formation: (1) the formation of amorphous hydrated Ce(IV)-hydroxides, which serve as an intermediate in the liquid-to-solid phase transformation; (2) the nucleation and growth of CeO_2 primary particles inside the intermediate; (3) the alignment of the primary particles into “pre-mesocrystals” and subsequently into mesocrystals guided by the intermediate phase.

During the liquid-phase electron microscopy experiment we systematically analyzed the effects of the applied irradiation dose rates and the induced diffusion of Ce ions on the growth mechanisms. An eight orders of magnitude higher dose rate used in an electron microscope compared to the dose rate in a γ-cell does not affect the CeO_2 particle growth pathway when homogeneous irradiation is applied. Despite the significantly higher Ce^{3+} to Ce^{4+} oxidation rate we still observed the formation of highly ordered structures (mesocrystals). The latter can only be explained by a stepwise formation of ceria particles via an intermediate phase. When irradiation is applied locally using scanning transmission electron microscopy, the higher conversion rate induces Ce-ion concentration gradients leading to the appearance of CeO_2 particles with branched morphologies (diffusion-limited growth). We found that the growth of CeO_2 particles with different morphologies can be controlled and uniquely produced by varying Ce^{3+} concentration gradients using periodic pulsed irradiation in liquid phase STEM.

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STUDY OF ATOMIC AND ELECTRONIC STRUCTURAL EVOLUTION IN $NiFe_2O_4$

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The phase transition from spinel to rock salt structure possesses significant implications for information storage and energy conversion applications. Previous studies have utilized X-ray diffraction (XRD), neutron scattering, and X-ray absorption spectroscopy (XAS) to elucidate the mechanism and pathway of this transition.[1] However, a comprehensive understanding of the transformation of local structures during this transition, particularly how local lattice and chemical environments influence the process, remains elusive due to technical constraints. Additionally, the role of local structural order and chemical environment in this transition process has not been fully elucidated.[2] To bridge this knowledge gap, our study employs advanced transmission electron microscopy (TEM) and electron energy loss spectroscopy (EELS) techniques to monitor atomic migration and electronic structure alterations in real-time during the phase transition.

This investigation leverages high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) and annular bright-field scanning transmission electron microscopy (ABF-STEM) to dynamically observe the migration of tetrahedrally-coordinated and octahedrally-coordinated Fe atoms in $NiFe_2O_4$'s spinel structure under electron beam irradiation. Through the integration of STEM image simulations with quantitative image analysis, we have unraveled the reconstruction dynamics of octahedrally-coordinated Fe/Ni atoms, providing insights into the lattice dynamics and Fe atom rearrangement pathways across varying coordination environments. Concurrently, real-time EELS spectra acquisition throughout the phase transition stages has enabled us to track the changes in the chemical valence states of Fe, Ni, and O in $NiFe_2O_4$. These findings offer a nuanced atomic and electronic level understanding, critical for advancing phase transition applications in energy and information technology fields.[3]

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[3] Acknowledge: This work was financially supported by NSFC (52171014), Science, Technologyand Innovation Commission of Shenzhen Municipality (HZQB-KCZYB-2020031, SGDX20210823104200001, JCYJ20210324134402007), Sino-German Center for Research Promotion (M-0265), RGC (C1013-23E,E-CityU101/20, CityU 11302121, CityU 11309822), ITF (ITS/365/21), European Research Council (856538, “3D MAGiC”), and CityU (9610484, 9680291, 9678288, 9610607，7020043).. The NiFeO4 film was prepared by Hideto Yanagihara from University of Tsukuba.

THE STATIC CORROSION OF T91 IN LEAD BISMUTH EUTECTIC WITH DIFFERENT OXYGEN LEVEL

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Lead-bismuth eutectic (LBE) cooled fast reactors, as one of the designs for GenIV reactors promising higher efficiency and improved safety, are drawing increasing attention. T91 steel is one of the commonly-proposed candidate structural materials for these reactors. The corrosion-resistance of materials in LBE environments remains the main critical issue for further development. Detailed understanding of the corrosion mechanisms is required to mitigate the effect of corrosion in these systems.

Oxide layer is one of the methods that may help to limit corrosion. However, according to research, there are requirements for temperature and oxygen content to form effective protection layers which may not be meet if we try to operate the reactor with higher temperatures or control the oxygen content to avoid lead/bismuth oxides forming. To further research on this, static corrosion tests with reducing (low oxygen content) and oxidising (high oxygen content) environment are investigated.

The STEM HAADF image shown in Fig.1 is an overview of a lift out from the reducing environment corroded sample which shows LBE penetrates into T91 through grains and the main process is completed by physically elements dissolution. With more detailed study, we found a thin layer of oxide (10s nm) formed at the interface of T91 and LBE. Fig. 2 presents the STEM HAADF image of oxidising environment, which shows corrosion happened along grain boundaries. Combining the results from EDX and EELS, the corroded grain boundaries are composed of Cr and Si oxides with no Pb or Bi detected. Thus, we propose the corrosion of T91 in oxidising environment is more controlled by oxidation.

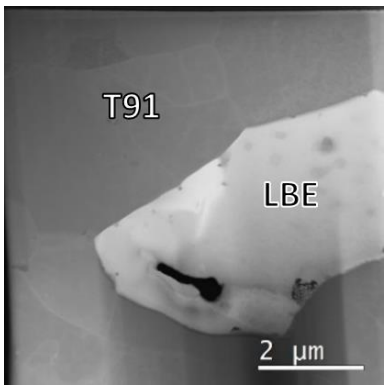

Fig. 1: STEM HAADF view of the TEM lift out foil of reducing environment corroded sample.

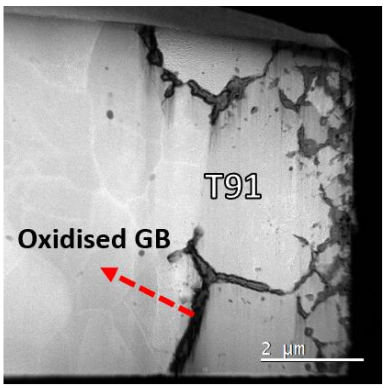

Fig. 2: STEM HAADF view of the TEM lift out foil of oxidising environment corroded sample.

4D-STEM AND EELS ANALYSIS OF COMPLEX C-BASED SENSOR ARCHITECTURES

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The conversion of high-carbon content precursors into open-porous carbon foams and coatings through thermal processes is emerging as a promising and environmentally friendly approach for various applications such as catalysis, energy utilization, and sensing [1]. In this study, we utilize a one-step laser patterning technique to locally pyrolyze doctor-bladed ink coatings on flexible PET substrates, creating highly porous, complex CO_2 sensor architectures with a thickness of approximately 50 µm. The ink formulation consists of glucose as a pore-forming agent and adenine as a nitrogen source to impart sensing functionalities. Laser treatment in an oxygen-rich environment yields flexible, highly porous sensor heterostructures with distinct regions containing nitrogen and oxygen functionalities. The laser intensity's depth-dependent attenuation results in the formation of a well-defined highly porous graphitic surface layer (acting as the electric transducer layer) and a less porous nitrogen-rich lower sensor layer connected by a thin transition region [2]. To elucidate the structure formation and the structure-functionality relationship of these sensors, we conducted a comprehensive TEM investigation on cross-sections (0.25 - 0.5 t/λ) of the entire device prepared by microtomic cross-sectioning. STEM-EELS elemental distribution maps reveal a clear differentiation in the chemical composition between the upper and lower layers of the sensor. Principal component analyses are employed to deconvolve the complex sensor structures, which comprise various crystalline and amorphous C- and N-containing phases. 4D-STEM analysis unveils the distribution of the crystalline graphitic phase, and the alignment of the graphite basal planes relative to the pore walls of the open-porous sensor. Understanding these parameters is critical for comprehending the electrical performance of the sensor (see Figure 1) [3].

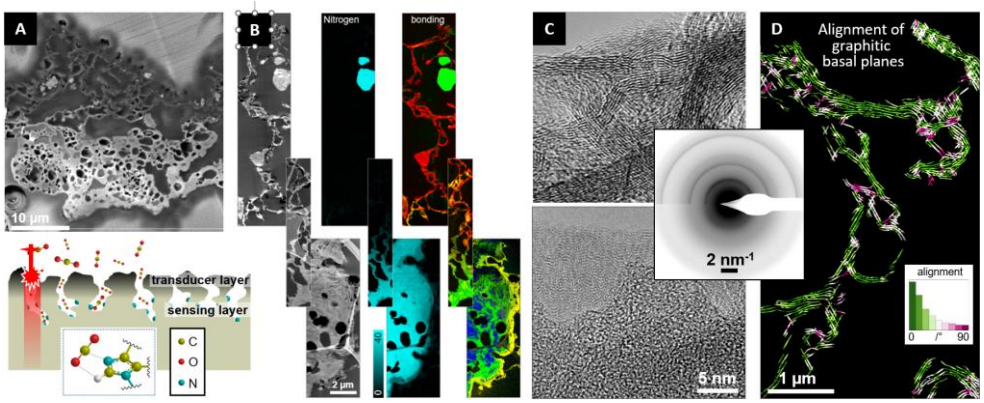

Fig. 1: A *(top)*) SEM micrograph of sensor embedded in epoxy, (*bottom) d*epth-dependent thermal laser intensity attenuation; B *(left)*) HAADF-STEM micrograph showing different sensor layers, *(middle)* STEM-EELS nitrogen distribution map, *(right)* PCA bond analysis, C) HRTEM images of sensor from transducer (top) and sensor (bottom) layers with SAED image of respective ROIs (inset); D) 4D-STEM analysis depicting misalignment angle between graphitic carbon basal plane normal relative to the pore surface normal

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4DSTEM-IN-SEM IN EVENT DRIVEN MODE FOR *IN SITU* APPLICATIONS

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In recent years, 4DSTEM has evolved as a powerful tool in transmission electron microscopy (TEM) offering insights into crystal structures, defects, and local strains [1]. With the advancements in the detector technology, there has been a paradigm shift, extending capabilities of 4DSTEM into scanning electron microscope (SEM) [2]. Characterization of 2D materials such as MoS_2 and graphene, enabling orientation mapping to have been demonstrated [3-5]. This opens a possibility of its potential application for *in situ* experiments to study, for example, deformation behavior and degradation mechanism of materials in controlled environments, that is comparatively easy to handle in SEM than in TEM. Enabling this novel technique for bulk materials offers valuable insights into material's real-world performances. This study focuses on the challenges posed by the characterization of bulk metallic materials using 4DSTEM-in-SEM. We will address the intricacies of sample preparation and optimization of the microscopic parameters. For our experimental setup, we have developed an in-house configuration (shown in Fig. 1(a)) utilizing the MiniPIX TPX3 camera from ADVACAM, with 256 × 256 pixels. The camera is installed on a heat sink constructed from aluminum alloy, connected to a water chiller to maintain the camera's temperature during operation. Fig. 1(b) illustrates the in-chamber situation of our setup. The detector screen captures events at each scanning position. Subsequently, data is processed using python scripts and stored as 4D data set. Utilizing open-source platforms like LiberTEM and py4DSTEM, virtual bright and dark-field (BF and DF) images were yielded. As an example, Fig. 1(c) shows the SEM image of a Pt thin film with nano grains. Further, virtual DF image is developed using the 4DSTEM dataset, as presented in Fig. 1(d) and (e). In conclusion, our investigation of 4DSTEM-in-SEM for bulk materials successfully addresses specific challenges, marking a significant step forward and establishing a foundation for ongoing breakthroughs in this field.

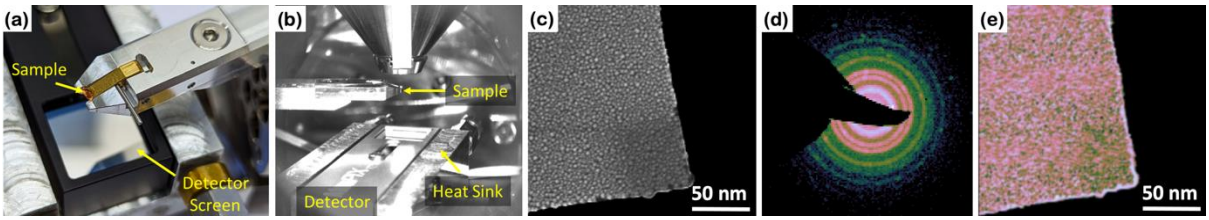

Fig. 1. (a) Sample holder and detector assembly, (b) In-chamber situation of Zeiss Merlin® during 4DSETM data collection, (c) SEM image of Pt thin film with nano grains, (d) 4D data set using data processing algorithms, and (e) virtual DF image.

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THE EFFECTS OF DIFFRACTION ON MAGNETIC IMAGING OF MONOCRYSTALLINE THIN FILMS USING STEM-DPC

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Scanning transmission electron microscopy – differential phase contrast (STEM-DPC) is a technique that enables characterization of ferromagnetic domains in materials [1]. This can be combined with other TEM techniques on the same sample area [2], and recent improvements in pixelated electron detectors have unlocked even further potential, including for use in nanomagnetic imaging [3]. However, STEM-DPC faces some challenges, especially for monocrystalline samples where diffraction can deform the electron probe and thus severely impair imaging by obscuring the magnetic contrast. An example of this is presented in Fig. 1 a), showing a STEM-DPC dataset taken from a heavily diffracting sample. Fig. 2 a) shows one of the distorted bright-field disks that cause this effect.
This work aims to give a thorough examination on the effect of diffraction and sample tilt on magnetic STEM-DPC. Using freestanding magnetic single-crystalline LSMO films [4], a systematic study has been conducted to examine the effects of diffraction on probe shape and magnetic data. These LSMO films have distinct magnetic domains and domain walls, which demonstrate how structural effects can obscure visualization of ferromagnetic domains. The research includes analyzing the effects at various tilt angles, both far away from and close to the zone axis.

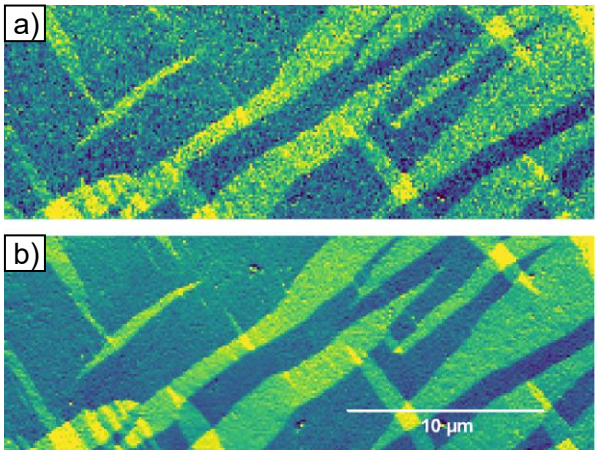

Fig. 1: STEM-DPC measured vertical deflection of probe from freestanding LSMO at a) close to a zone axis b) 7.5º tilted away.

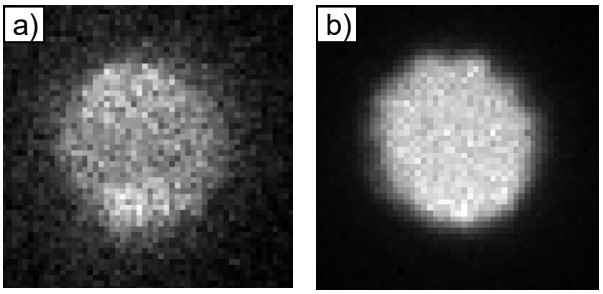

Fig. 2 Bright-field disk taken from the same position at a) close to a zone axis b) 7.5º tilted away.

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TOWARDS ATOM COUNTING FROM FIRST-MOMENT STEM IMAGES

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Over the past few years, a model-based approach has been established to extract quantitative measurements from atomic resolution HAADF STEM images [1]. In this approach, the image is modelled as a superposition of 2D Gaussian peaks, from which unknown structure parameters, such as atomic column positions, peak intensities and scattering cross-sections, can be estimated. This approach has been used to achieve accurate and precise determination of the chemical composition of materials [2] and to count the number of atoms with single atom sensitivity [3]. Atomic column intensities in HAADF STEM scale with the square of the atomic number [4] and so light columns are easily hidden by the stronger intensity from heavier columns, making simultaneously estimating structural parameters for both light and heavy atomic columns challenging. However, this limitation might be overcome by quantifying other types of STEM image via a model-based method.

Recent advances in pixelated detectors, along with the capability to collect 4D STEM datasets, offers significant flexibility in generating diverse STEM imaging modes [5]. One example is first-moment STEM images, in which the center of mass (COM) of convergent beam electron diffraction (CBED) patterns is recorded at each scan position. Typically, the COM is determined in two perpendicular directions, generating COM_x and COM_y images. In the phase object approximation, COM is proportional to the gradient of the projected potential, which is expected to scale linearly with atomic number [6]. We have performed model-based quantification on simulated $COM_{x(y)}$ images of aluminum crystals (Fig. 1), fitting to these simulated images a parametric model of a superposition of X(Y) derivatives of 2D Gaussians from which we calculate the integrated-intensities of atomic columns as a function of thickness. For aluminum, a monotonic increase is observed up to a thickness of 30 atoms (~12nm) suggesting these intensities can be used as a quantitative measure for atom-counting. Finally, the relative width, which quantifies the overlap of the integrated-intensity distribution between atomic columns with consecutive thickness, is evaluated from repetitive noise realizations of both HAADF and $COM_{x(y)}$ images. A reduced relative width indicates more precise atom-counting. These are encouraging results for performing atom counting with $COM_{x(y)}$ images [7].

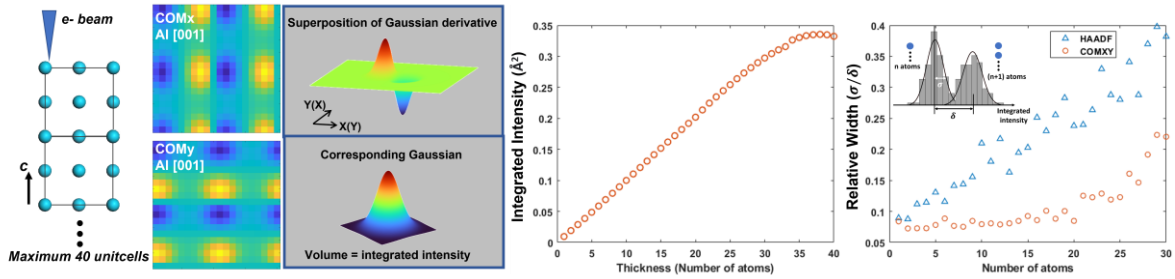

Fig 1: Model based quantification of first-moment STEM images, calculation of integrated intensity and relative width as a function thickness.

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ACHIEVING ATOMIC PRECISION 3D RECONSTRUCTIONS OF 10 NANOMETER SIZED NANOPARTICLES

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Understanding and controlling the unique properties of nanoparticles hinges on precise and accurate knowledge of their three-dimensional shape. High-angle annular dark field scanning transmission electron microscopy (HAADF-STEM) provides a powerful tool for quantitative analysis, allowing the extraction of structural information through atom-counting [1]. In HAADF-STEM, the intensities of the atomic columns can be used in a statistical analysis to count the atoms with single-atom sensitivity [2–4]. However, reconstructing the 3D structure remains challenging. Established energy minimisation schemes refine the initial guess for the 3D structure but may fall short of an accurate reconstruction by deviating from the experimental data, becoming trapped in local energy minima, or neglecting the finite precision of atom-counting methods.

The implementation of the Bayesian genetic algorithm [5] can combat these issues. This algorithm is restrained to the experimental observations in the atomic column positions and finite atom-counting precision (Fig. 1). Additionally, by generating large populations and including mutations we scan the local energy landscape for an optimal solution which balances the energy minimisation and atom-counting probabilities. However, genetic algorithms can be prohibitively computationally expensive for large nanoparticles as the number of parameters that describe the 3D shape of the nanoparticle rapidly grows. Furthermore, the simulation parameters (population size, mutation density, …) are difficult to standardise [6] as they may differ significantly for each application. To this end, we have optimised the algorithm for computational efficiency and proposed simulation parameters so that they may be used for nanoparticles of up to at least 10 nanometers in size [7].

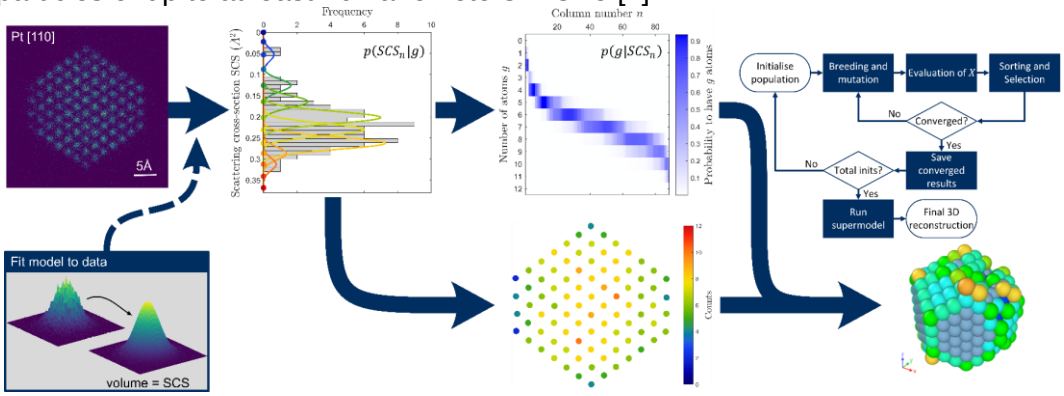

Fig. 1: The Bayesian genetic algorithm reconstructs the 3D shape of a nanoparticle based on the counts and probabilities derived from the atom-counting procedure.

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ELECTRON PTYCHOGRAPHY WITH AN ELECTROSTATIC PHASE PLATE FOR ELECTRONS

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The development of fast direct electron detectors has made electron ptychography a popular method for high-resolution, dose-efficient, phase-contrast imaging. However, this method requires dense scanning of a convergent electron probe over a small area of the sample, which may lead to a limited field of view [1]. To overcome this hurdle, recent research has suggested the use of a quasi-parallel beam illumination with amplitude/phase modulation of the electron wave [2]. We propose a new approach based on our recently developed electrostatic phase plate for electrons [3], which can improve the reconstruction by removing artifacts and improving the convergence of the reconstruction algorithm. This approach aims to expand the field of view while maintaining high resolution and dose efficiency. We provide simulation results using different combinations of amplitude and phase modulation strategies at different applied electron dose (total dose divided by the number of phase configurations). We demonstrate the viability of such amplitude and phase modulation through experimental results.

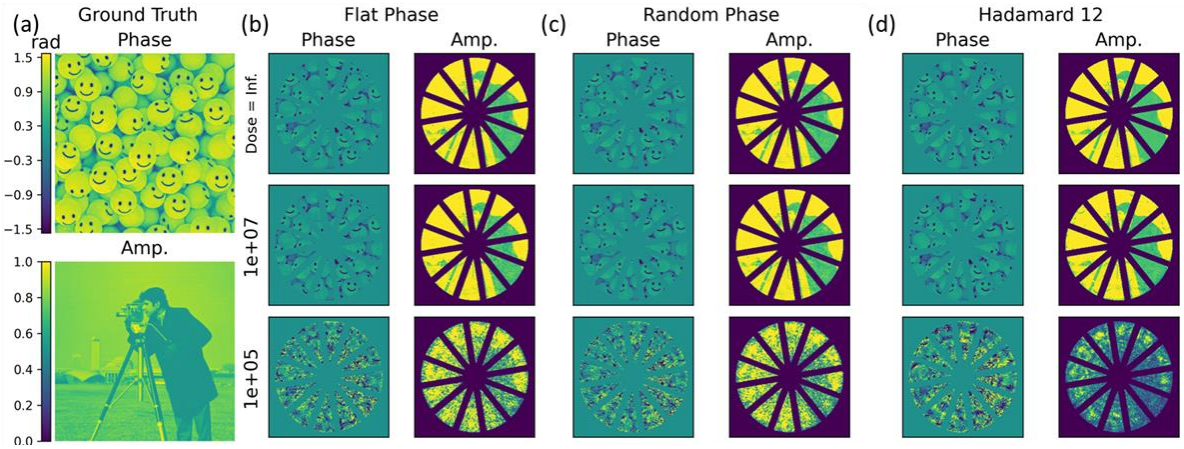

Fig. 1: Simulated near-field ptychography reconstructions with structured illumination for different illumination profiles (columns) and cumulative dose for the measurement (rows). The panels show (a) the phase (top) and amplitude (bot) ground truth, (b) the reconstruction with amplitude modulation only, (c) the reconstruction with amplitude and random phase modulation, and (d) the reconstruction with amplitude and an orthogonal set of phase profiles.

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RETRIEVE DOPANTS INFORMATION FROM INLINE HOLOGRAPHY

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Introducing dopants into nanocrystals adapts properties such as electrical conductivity and catalytic responses [1, 2]. The retrieval of crystal structure at atomic resolution can be realized by reconstructing the exit wave from a focal series of images of the high-resolution transmission electron microscopy (HRTEM), with resolution requirement attained and the dose rate acceptable for the samples [3]. In the past, the interpretation of electron channeling allowed for the complete retrieval of the three-dimensional (3D) structure of homogeneous nanocrystals [3]. However, the dopants inside the crystals affect the ordinary channeling effect and lead to the complexity of the exit wave.

The perturbation model into the classic channeling model enables a quantitative analysis of the exit wave along a particular atom column which includes a dopant, which demonstrates that the dopant not only causes a phase shift to the original s-state, which reflects the chemical information of the dopant but also brings additional states, which are in the form of spherical waves and can be analyzed through the "Big-bang scheme" [4]. The dopants' element type and depth can be extracted by separating the s-state and other states of the exit wave obtained.

Meanwhile, the interference between the s-state wavefunction and the wavefunction of the state stimulated by the dopant changes the appearance of the exit wave, which is especially observable when backpropagation is conducted on the exit wave in a vacuum. This phenomenon brings the clue to retrieve dopant information from the exit wave reconstructed from the focal series of HRTEM. Furthermore, the dynamic movements of the dopants can be tracked by the selection of image series at different time intervals, along with comparisons on the change of structure reconstructed.

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SIMULTANEOUS MAPPING STRAIN AND ELECTROMAGNETIC FIELD AT THE NANOSCALE

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Large elastic strains can be generated by external loading or epitaxy with an underlying substrate on thin films and nanomaterials [1]. This leads to new possibilities in optimizing the functional properties of materials by controlling their elastic strain fields as continuous variables. For example, in a recent study of our group, by applying a uniaxial tensile strain to a ferromagnetic Ni thin plate, its magnetic domain structure changed as a function of strain and nanoscale magnetic hardening was directly observed during *in situ* electron microscopy experiments using phase contrast imaging methods [2]. In this case, the strain is only estimated by the distance between pre-deposited markers and only as a global value, while local strain at the nanoscale would be useful to understand the possible coupling between the strain fields and the corresponding magnetic structures. Therefore, simultaneous measurement of local strain and electromagnetic fields with high spatial resolution would be highly desirable.

Four-dimensional scanning transmission electron microscopy (4D STEM), a cutting-edge technique that utilizes a pixelated electron detector to record 2D images of convergent beam electron diffractions over a 2D grid of beam positions, enables wide range applications in virtual diffraction imaging, phase and orientation mapping, etc. It also shows capabilities in high-precision strain measurement as well as electromagnetic field determination with a resolution down to the sub-nanometer scale. For nanoscale field mapping, the measured center-of-mass (CoM) shift of the bright-field (BF) disk shows linear correlation with the field, and integration of CoM (iCoM) leads to the phase change that can be compared with results from other techniques such as electron holography. For strain mapping, variations in the interspacing between a pair of diffraction disks are usually used for the quantification.

In this contribution, we will explain the idea how the strain and electromagnetic field induced contributions can be decoupled from the measured CoM of a pair of diffraction patterns in a 4D STEM dataset, as shown in Fig. 1. We will also present further results by applying this concept to several systems, including static and *in situ* experiments.

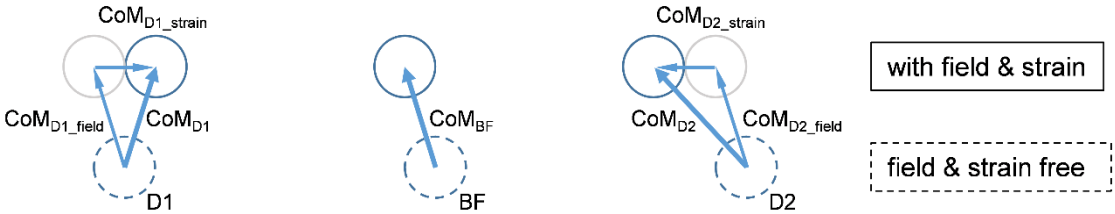

Fig. 1: Schematic illustration of how the strain and electromagnetic field induced contributions to the measured CoM can be decoupled from a pair of diffraction disks.

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MAPPING THE MAGNETIC CIRCULAR DICHROISM USING 5DSTEM

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The classical Electron magnetic circular dichroism (EMCD) [1] experiments are carried out by tilting the crystal in specific diffraction conditions and acquiring EELS spectra at conjugate scattering angles around the direct beam, guided by simulations. In the simplest case where a crystal is tilted to 2-beam condition, two EELS spectra are acquired, and this number increases for other diffraction conditions and can be as high as 16 for Fe in [110] zone axis [2]. A tight control of collection apertures both in size and angular position is important to make sure the detection of notoriously week EMCD signal. This can be challenging if the EMCD distribution is not very well known or if the EMCD experiment is carried out under zone axis conditions which is the essential experimental setup to achieve atomic resolution measurements.

With the development of direct electron detectors, it is now possible to acquire full diffraction patterns (DP) at each probe position in STEM mode, producing the so called 4DSTEM datasets. In principle, the concept of 4DSTEM can be extended to energy-filtered 4DSTEM where a narrow energy-selecting slit in the EELS spectrometer is used to acquire an energy-filtered DP (EF-DP) at each probe position. I have proposed to acquire a series of such energy-filtered 4DSTEM datasets at core energy losses in the EELS spectra to reconstruct a 5DSTEM dataset where the 5th dimension represents the energy loss. With such a dataset in hand, one has access to spatial, momentum and energy loss axes where EELS spectra at any scattering angles can be extracted by using virtual apertures in the EF-DP stacks found at each spatial pixel. This gives the user enormous freedom to adjust the aperture positions and sizes to optimize the EMCD signal strength. This would enable the detection of EMCD signals under zone axis conditions using convergent beams, leading to possible detection of EMCD with atomic resolution.

We have carried out the initial experiments and detected an EMCD signal under 2-beam condition. The experiments have been carried out on a probe and image with chromatic aberration corrected Thermofisher Titan PICO equipped with a Gatan continuum imaging filer and K3 direct detector. We will present the first results.

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A NOVEL LIQUID PURGING METHOD FOR HIGH-RESOLUTION AND ANALYTICAL LIQUID PHASE TRANSMISSION ELECTRON MICROSCOPY FOR UNDERSTANDING ELECTROCHEMICAL PROCESSES

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Liquid phase transmission electron microscopy (LPTEM) has emerged as a powerful tool, presenting enormous potential for understanding the electrochemical processes behind electrolysis, batteries, and other technologies [1].However, obtaining high-resolution structural and chemical information during these electrochemical processes remains a challenge due to the issue of liquid thickness. While utilizing a monolithic MEMS based nanoreactor with integrated electrodes can be a potential solution, it too faces limitations from an electrochemical perspective. To ensure proper ion conduction to mirror processes occurring in macroscopic cells, LPTEM studies require a thicker liquid layer, which directly contradicts the need for high-resolution information. On the other hand, obtaining high-resolution information through post-mortem studies, by disassembling a typical two-chip nanoreactor, too is not a viable solution due to possible changes due to absence of the native liquid environment. Furthermore, ideally high-resolution information is required at different stages of *in situ* experiments. These necessitate the ability to dynamically control the liquid thickness.
We recently developed a novel purging method that enables high-resolution and analytical electron microscopy studies within a liquid flow cell [2]. We anticipate that the development and application of this method will lead to the unraveling of detailed electrochemical mechanisms in electrolysis and batteries. This, in turn, will pave the way for improved efficiency and lifetime of these electrochemical conversion and storage devices, consequently accelerate the transition to a greener future.

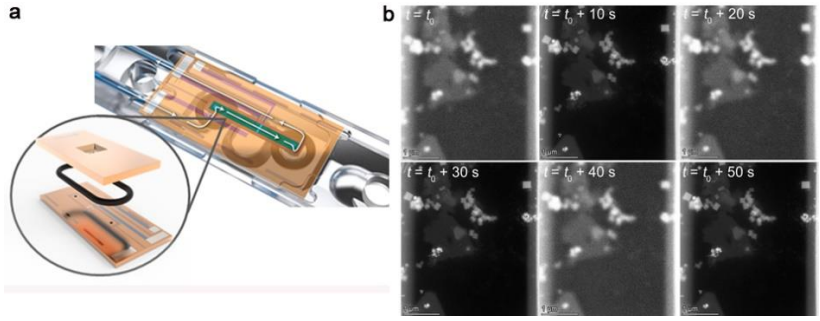

Figure: Time series of *in situ* STEM images in a LPTEM holder (a) showing the repeatable removing and refill of liquid by purging with an inlet gas pressure of 3 bar.

Acknowledgments
S. Basak, J. Park, E. Jodat, A. Karl, and R.-A. Eichel acknowledge the funding provided by the BMBF (German Research Foundation) through the project DERIEL (03HY122C).

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TRUE QUANTIFICATION OF POLARIZATION AT NITRIDE INTERFACES BY ELECTRON HOLOGRAPHY

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Electron holography offers an intriguing access to probe electrostatic potentials in semiconductor devices and thereby polarization changes at internal interfaces. However, the electron optical phase changes at interfaces are typically far off what is expected. The central reason is the presence of so-called dead layers at the surfaces of TEM lamellas. Unfortunately, the prior handling of the deal layers is rather crude and inhibits a real quantification. Recently we showed that the focused ion beam preparation of TEM lamellas leads to an implantation of carbon (C) into near surface areas. This creates a shell-in-shell structure, where the inner pristine bulk material is surrounded by an inner crystalline shell with high density of Fermi-level pinning centers (notably identified as C on nitrogen sites).[1] This is followed by an outer amorphous shell. The defect-induced Fermi level pinning defines the potential, which is screened by free carriers in the pristine core. However, the characteristics of the shells are sensitive to the exact lamella preparation recipe. Therefore, one needs to have a calibration of the surface potential within the same sample and measurement to achieve a true quantification of electron holography.

Here we illustrate that by using a defined doping structure within the same sample, one can calibrate the electrostatic potential induced by the so-called dead layer of TEM lamellas and derive subsequently quantitatively polarization and electron affinity changes at interfaces as well as charge transfer levels of implanted point defects.[2] The first achievement of true quantitative electron holography provides a deep physical insight into group-III nitride semiconductors well beyond what has been achieved thus far.

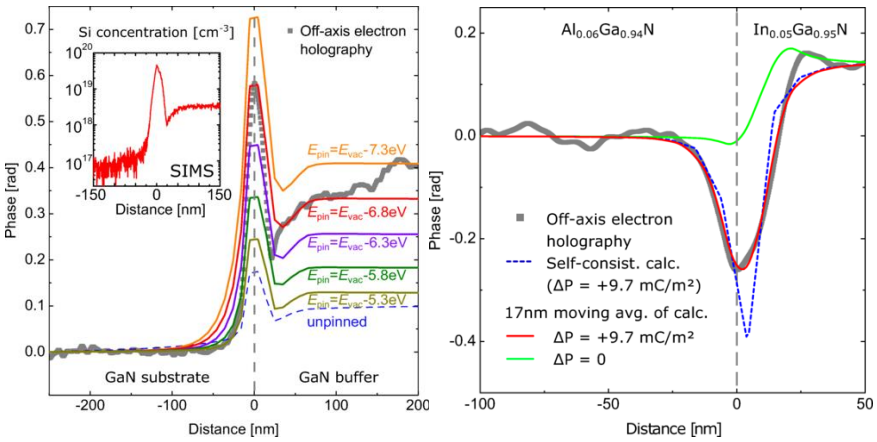

Figure (Left) Average profile of the phase change (gray symbols) across a δ-doped GaN layer measured by off-axis electron holography. The lines represent self-consistent calculations of the phase change for different surface Fermi level pinning energies. (Right) This enables a calibration for subsequent measurements and analysis.

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FIELD EMISSION DYNAMICS FROM ELECTRON-BEAM IRRADIATED GOLD NANOPRISMS

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Plasmonic metal nanoparticles (NPs) can confine electromagnetic energy to nanoscale volumes by exciting their localized surface plasmon resonances (LSPRs) [1]. This property facilitates the coupling of free-space light to the nanoscale, leading to significantly enhanced light–matter interactions that are desirable for the devices used in photocatalysis, solar energy harvesting, imaging and photodetection [2]. In addition, the plasmonic NPs are used as ultrafast electron emitters. Ultrafast electron emission from plasmonic NPs is achieved by exciting their LSPRs with a femtosecond laser [1,2]. Thus, the strong localized optical fields induced by the excitation of the LSPRs drive large photoemission currents from the NPs. Here, we fabricate plasmonic gold (Au) nanoprisms on Au electrodes patterned on SiNx membranes by electron beam lithography and irradiate these Au nanoprisms with a parallel electron beam accelerated to 200 kV to trigger the electron emission from the nanoprism tips (Fig. 1a,b). The emitted electrons are detected by directing them to an unoccupied Au electrode via a static electric field. Figures 1c-e show the simulated dipolar, breathing and edge LSPR modes of an Au nanoprism. Notably, the dipolar LSPR excited at the tips of the nanoprisms exhibits a higher scattering cross section than other higher-order LSPR modes. The strong localized field induced by the dipolar LSPR supports the electron emission at the tips of Au nanoprisms excited by the parallel electron beam. The electric field induced at the surface of nanoprisms under electron beam irradiation is imaged by 4D-STEM measurements. The electrical measurements confirm the field-driven electron emission from the Au nanoprisms irradiated under a parallel electron beam.

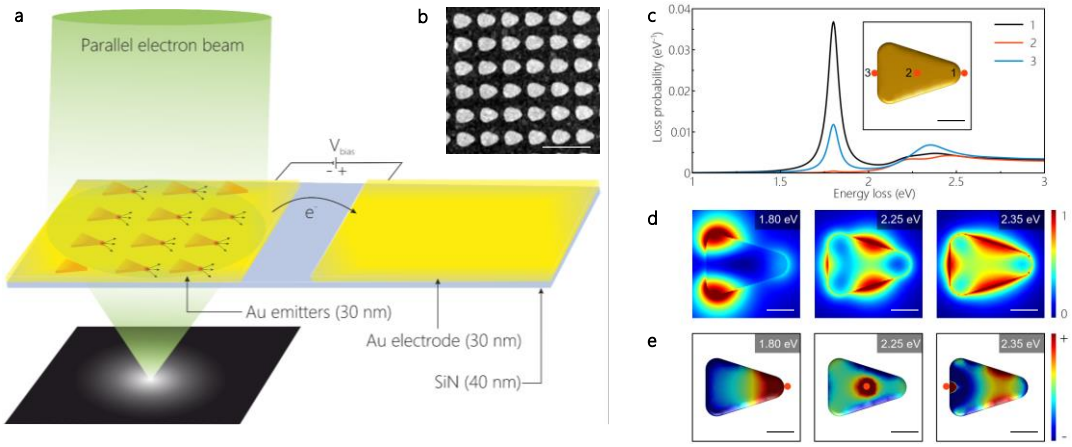

Fig. 1: (a) Schematic of the experimental setup. The distance between two Au electrodes is 3 µm. (b) HAADF-STEM image of Au nanoprisms on Au electrode. The scale bar is 400 nm. (c) Simulated EEL spectra of a Au nanoprism with a tip radius of 20.3 nm. The EEL spectra are derived at different locations on the nanoprism shown in the inset. (d) Simulated EELS maps showing dipolar (1.80 eV), breathing (2.25 eV) and edge (2.35 eV) LSPR modes, respectively. (e) Simulated surface-charge distributions for the LSPR modes shown in panel d. The scale bars on panels c, d and e are 50 nm.

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CEFID: A FLEXIBLE PLATFORM FOR ADVANCED SPECTROSCOPIC EXPERIMENTS

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The CEOS Energy Filtering and Imaging Device (CEFID) is an energy filter and spectrometer offering state-of-the-art specifications and the flexibility to implement ambitious and unprecedented experiments. The filter design comprises highly optimised and stable optics up to the energy-selecting slit, and a flexible and minimalist projective stage [1]. This gives high performance and stability, while allowing to hop between modes (imaging, spectroscopic dispersions) with little to no re-tuning. The Python/Qt-based graphical software used for the filter operation, implements interactive and automated procedures for alignments, common workflows ranging from EFTEM to 4D-STEM, and tools for on-the-fly analysis from live DFT to EELS maps computation. A wide range of detectors (Fig. 1) and scan generators from different manufacturers has already been integrated and can be used for both the tuning and data acquisition. The software is highly extendable and offers a scripting and plug-in API in python and a remote control interface for the integration into third-party software. The capability and versatility of the system make it well suited for rapidly acquiring and evaluating spectroscopic information on samples of interest [2], while its flexibility and compatibility make it a platform for complex experiments where different tools need to work in unison, such as the synchronization of acquisition with in-situ stimuli, or photon-induced near-field electron microscopy [3].

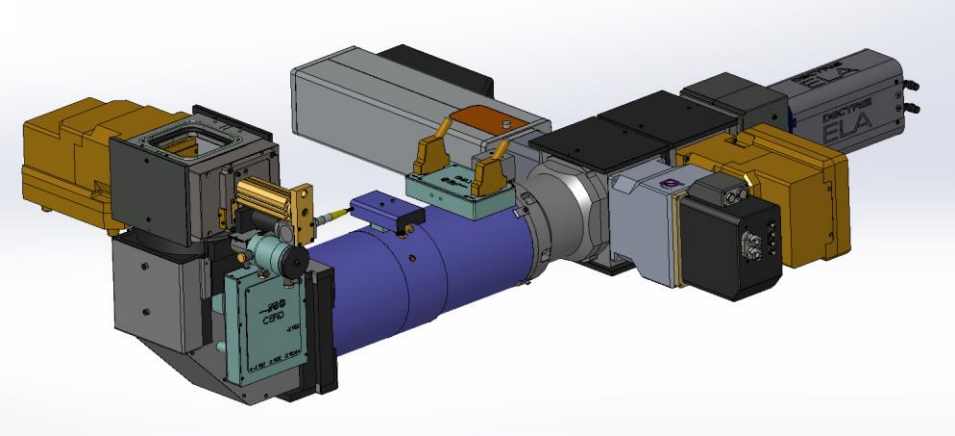

Fig. 1: Rendering of CEFID outfitted with five cameras (TVIPS XF416R, ASI Cheetah, Direct Electron DE-16 and Dectris ELA) and a retractable ADF detector developed by CEOS. Each detector has its specific strengths which make it ideal for a class of experiments.

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ULTRATHIN MICROCHIP FOR ENVIROMENTAL TRANSMISSION ELECTRON MICROSCOPY

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Close cell strategy, which creates an isolated environment to host the fluidic mediums and observed materials by using various membrane materials, is widely adopted for both liquid cell and gas cell transmission electron microscopy (TEM). Among various candidate materials such as silicon nitride (SiN_x), silicon oxide (SiO_x), graphene and transition metal dichalcogenides, SiN_x is the most popular used one due to the advantages on ease of manufacturing, reliable physical robustness at several tens of nanometers thickness, and reasonable electron transparency. As a membrane material, the thinner thickness, the better signal quality. Nowadays, the typical thickness of SiN_x is 50 nm. For a sealed nanoreactor, the total membrane thickness will be 100 nm, which is usually much thicker than that of the observed materials. Considering the additional adverse scattering from membrane bulging and the sealed fluidic media, the spatial resolution and spectral visibility of the microchip-based operando TEM are significantly reduced although the microscope itself may have very good performance. In the past decades, the figure-of-merit for the microscope has been advanced a lot due to significant updates from aberration corrector, monochromator and direct detectors. However, the experimental performance of the close cell operando TEM has not been advanced much. This thick membrane is arising as a key factor which limit the figure-of-merit of the environment TEM. In this work, we propose to use a hybrid strategy with honeycomb structure to reduce the SiN_x thickness to less than 10 nm. Meanwhile, the fabricated ultrathin membranes have excellent robustness with superior mechanical properties and electron beam resistance.

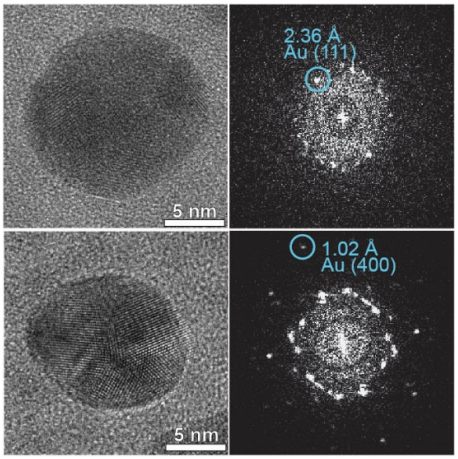

Fig. 1: Improvement of ultrathin membranes in real space high resolution TEM imaging.

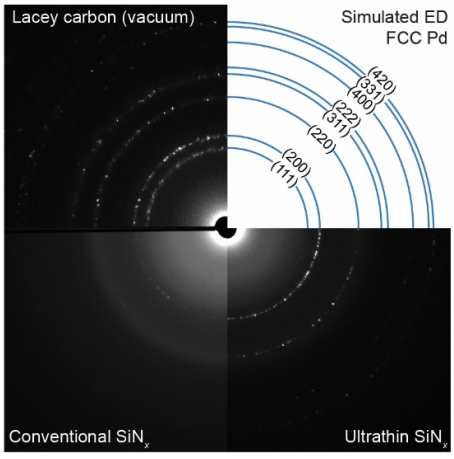

Fig. 2: Improvement of ultrathin membranes in diffraction analysis.

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LONG-RANGE ELECTRIC FIELDS AND POTENTIALS FROM CONDUCTING AND INSULATING NEEDLES MEASURED BY OFF-AXIS ELECTRON HOLOGRAPHY AND 4D-STEM

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Quantitative measurements of nanoscale and long-range electromagnetic fields are of great interest for both fundamental research and the development of next-generation electronic and spintronic devices. Information about electromagnetic fields and potentials within and outside materials can be recorded in the transmission electron microscope (TEM) using phase contrast imaging techniques, such as off-axis electron holography (OAEH) [1, 2] and four-dimensional scanning TEM (4D-STEM). OAEH measures the projected electrostatic potential field of the sample from the phase shift directly and further provides the projected (in-plane) electric field from its gradient. The 4D-STEM technique involves recording a full set of convergent beam electron diffraction (CBED) patterns from each position of the electron beam on a pixelated detector [3]. The projected (in-plane) electric field of the sample is then obtained from the CBED patterns by computing their centers of mass (COM-STEM) from the barycenters of their recorded intensities [4]. The projected electrostatic potential of the sample is then obtained by integration of the projected (in-plane) electric field (iCOM-STEM) [4, 5].

Here, we present quantitative comparisons of long-range electric field and potential measurements recorded using OAEH and 4D-STEM (COM-STEM and iCOM-STEM) from two Au needles, which were mounted in a Nanofactory STM-TEM specimen holder and separated by a distance of 200 nm. A bias of +50 V was applied between the needles. Interestingly, measurements recorded using the two techniques revealed that the projected electrostatic potential and projected (in-plane) electric field were smaller when measured using OAEH than with 4D-STEM. The difference between the measurements originates from the effect of the perturbed reference wave (PRW) when using OAEH, as the long-range electric field from the needles affects the vacuum reference wave, thereby reducing the inferred potential and field. Analytical equations with and without the inclusion of the PRW effect were used to confirm this hypothesis.

We also studied the long-range electric field originating from an unbiased needle comprising an Al_2O_3 tip on a conducting base. The insulating part of the needle was charged positively as a result of secondary electron illumination. Differences between results obtained using OAEH and 4D-STEM resulted from dynamical charging of the specimen during scanning of the electron beam when recording 4D-STEM measurements.

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OFF-AXIS ELECTRON HOLOGRAPHY OF ELECTRON-BEAM-INDUCED CHARGING OF A POLYSTYRENE NANOSPHERE AT LOW TEMPERATURE

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Electron-beam-induced specimen charging is frequently encountered in transmission electron microscopy (TEM) when studying insulating materials. Off-axis electron holography is a powerful TEM technique that can be used to map local variations in electron optical phase shift, which are in turn sensitive to electrostatic potentials and magnetic fields. In the absence of magnetic contributions to the phase shift, the recorded phase is proportional to the projected electrostatic potential within and outside the specimen. Insulating nanoparticles with simple geometries are ideal objects for the study of specimen charging in the TEM.

Figure 1 (a-g) shows a polystyrene nanosphere with a radius of ~110 nm investigated at both room temperature (RT) and 23 K in an FEI Titan G2 TEM at 300 kV. By using a model-independent approach for the quantification of the spatially-dependent projected charge density from a recorded phase image [1, 2], the amount of positive charge on the sphere at RT and at 23 K was determined to correspond to 200 and 380 *e*, respectively. Simulations (Fig. 1 (h-j)) suggest that the charge is located primarily within the volume of the sphere, rather than on its surface. Interestingly, our results suggest that at low temperature the mean inner potential of the sphere increases by ~10% from that at RT.

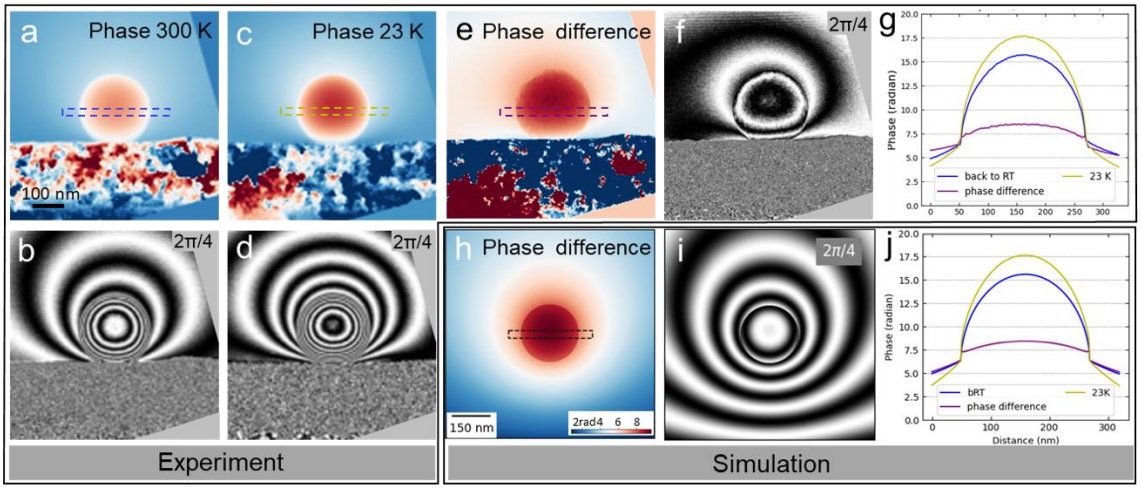

Figure 1 (a-g) Experimental results. (h-j) Simulation results. (a, c, e) Phase images at RT, 23 K and their difference, respectively. (b, d, f) Corresponding equiphase contours with a fringe spacing of 2π/4 radians. (g) Experimental phase profiles extracted from the marked regions from the results recorded at RT (blue), 23 K (yellow) and their difference (purple). (j) Corresponding phase profiles from simulations.

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OPTICAL DESIGNS FOR SIXTH-ORDER GEOMETRICAL ABERRATION CORRECTORS

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Aberration correction in transmission electron microscopy has become indispensable for the atomic-level structural analysis of materials. The most popular type of spherical-aberration correctors is a hexapole corrector, which consists of two hexapole fields connected by a transfer doublet. Using a thick hexapole field, negative spherical aberration is generated as a self-combination aberration of three-fold astigmatism. Moreover, to correct six-fold astigmatism, two types of correctors have been developed: an optimized double-hexapole corrector and a triple-hexapole corrector (delta type). Using these correctors, a correction range of approximately 70 mrad was achieved. However, in both correctors, the correction range could not be increased further because of a sixth-order three-lobe aberration (R_7) [1]. In this study, we propose two spherical-aberration correctors that can also correct R_7.

One of the proposed correctors is four-hexapole corrector. The optical design of the corrector might be described as the connection of two double-hexapole correctors. Because the direction of the R_7 in a double-hexapole corrector depends on the direction of the hexapole fields, we can cancel R_7s by connecting two double-hexapole correctors which have opposite hexapole fields. Although there is another R_7 which is generated as combination aberration of four hexapole fields, it can be controlled by changing the intensity ratio of hexapole fields. By using the four-hexapole corrector, the aberration correction range can be extended to approximately 100 mrad which is limited by seventh-order spherical aberration.

Although the proposed four-hexapole corrector can correct R_7, it has the disadvantage that its size tends to be large because of the large number of electron-optical elements. We investigated the possibility of compensation for R_7 by a two-hexapole corrector. To achieve this, we proposed a technique to correct high-order aberration using nonuniform hexapole fields. Our calculation based on a sharp cut-off field approximation shows that changing the hexapole-field intensity by approximately 5% at the center plane of the hexapole field reduces the R_7 to zero. By using this corrector, the aberration correction range can be extended to approximately 85 mrad, which is limited by chaplet aberration. Various intensity distributions of the hexapole fields can be considered in future work.

Both proposed sixth-order geometrical aberration correctors have their own advantages. The aberration correction range of the four-hexapole corrector is larger than that of the two nonuniform-hexapole corrector. Although it has less correction capability, the two nonuniform-hexapole corrector allows a reduction of the corrector hardware size so that the required room height can be reduced [2].

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PROSPECT FOR TWO-DIMENSIONAL TRANSITION-METAL OXIDE PEROVSKITES BY ELECTRON MAGNETIC CHIRAL DICHROISM IN 3-BEAM ORIENTATION

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Two-dimensional (2D) materials have gained significant attention due to their intriguing magnetic and electronic properties, presenting potential applications in various functional devices [1]. While traditional 2D van der Waals magnents like transitional metal dichalcogenides have been extensively studied, there exists a research gap in the exploration of 2D transition-metal oxide perovskites. These materials exhibit noteworthy magnetic phenomena, such as high-temperature superconductivity , colossal magnetoresistance, and multyferroicity,due to strong interactions among d-band electrons [2,3]. This study introduces the application of electron magnetic chiral dichroism (EMCD) in transmission electron microscopy (TEM) for the analysis of 2D transition-metal oxide perovskites, specifically focusing on $La_{1-x}Sr_xMnO_3$ (LSMO), a typical colossal magnetoresistance material. A comprehensive simulation of the EMCD was conducted, considering parameters including three-beam tilt angle, thickness, voltage, doping, and aperture size and positions. The findings reveal that EMCD signal was slightly influenced by doping extent and voltage. Significantly, the three-beam tilt angle emerges as a crucial factor of EMCD signals for samples with different thickness, with a higher tilt angle being preferable for 2D LSMO. Our results provide a feasibility analysis and guideline to collect 2D transition-metal oxide perovskites and further realize its atomic level magentism analysis [5].

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STRUCTURAL PHASE TRANSITIONS RESOLVED BY ULTRAFAST TRANSMISSION ELECTRON MICROSCOPY

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Ultrafast transmission electron microscopy (UTEM, Fig.1a) enables investigation of non-equilibrium dynamics in a stroboscopic laser-pulse pump/electron-pulse probe approach. In the Göttingen UTEM [1] a highly coherent beam is generated via linear photoemission from a nanometric tip emitter. This allows for combined nanometer spatial and femtosecond temporal resolution of structural dynamics [2]. Here, we investigate structural phase transitions in charge density wave (CDW) materials at unprecedented repetition rates of up to 2 MHz by means of nanobeam ultrafast electron diffraction. Specifically, we elucidate the phase switching mechanism between two CDW phases in 1*T*-TaS_2 [3]. Upon exciting the material at room temperature, we find a light-induced hexatic state coupled to a dimensional crossover preceding the formation of the thermally stable incommensurate (IC) CDW phase (Fig.1b-d). This transient state is characterized by a high density of topological defects that suppresses the translational long-range symmetry and leads to a loss of interlayer correlations, while in-plane orientational order is weakly preserved. Our results demonstrate the merits of nanoscale diffractive probing of ultrafast dynamics with high reciprocal-space resolution, paving the way for future exploration of structural dynamics in a wide range of materials by UTEM.

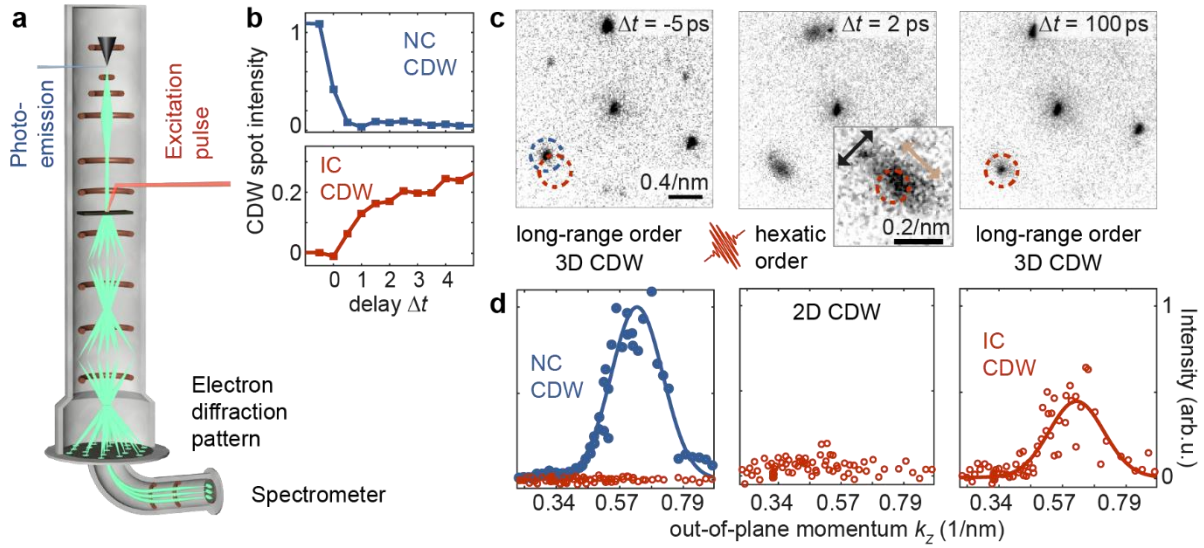

Fig. 1: **a** UTEM setup. **b** Suppression of the nearly commensurate (NC) CDW spot intensity (blue) after optical excitation and emergence of CDW spots characteristic of the IC phase (red). **c** Diffraction pattern at different temporal delays, showing the structural rotation of the NC to IC transition, and broadened spots indicative of a transient hexatic state. **d** CDW rocking curves acquired by beam tilting show the dimensional crossover into a transient 2D-state.

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COUNTING POINT DEFECTS AT NANOPARTICLE SURFACES BY ELECTRON HOLOGRAPHY

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Metal oxide nanoparticles exhibit outstanding catalytic properties, believed to be related to the presence of oxygen vacancies at the particle's surface. Hence, quantification of such point defect densities at the surfaces of metal oxide nanoparticles is vital for understanding the physical and chemical processes and for improving current and future applications. However, little quantitative information is known about concentrations of point defects inside and at surfaces of these nanoparticles, due to the challenges in achieving an atomically resolved experimental access. By employing off-axis electron holography, we demonstrate, using MgO nanoparticles as an example, a methodology that discriminates between mobile charge induced by electron beam irradiation and immobile charge associated with deep traps induced by point defects as well as distinguishes between bulk and surface point defects. Counting the immobile charge provides a quantification of the concentration of F^{2+} centers induced by oxygen vacancies at the MgO nanocube surfaces.[1]

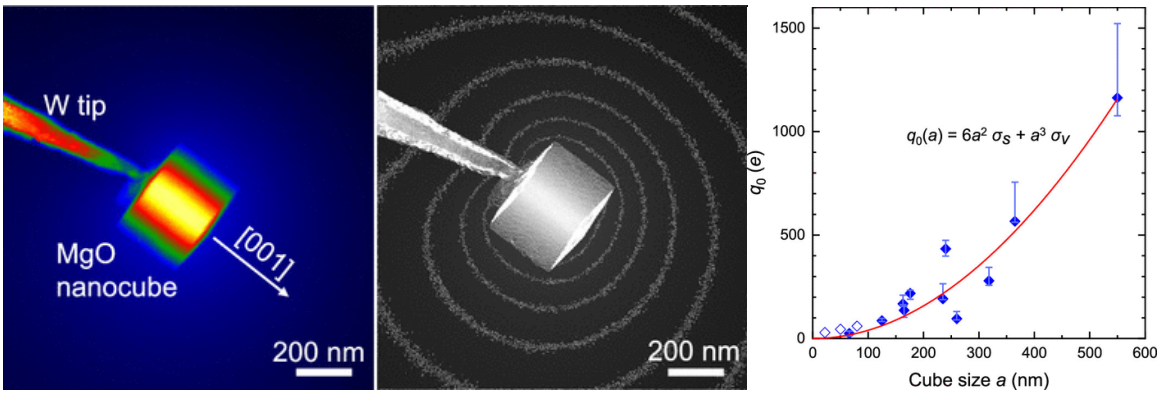

Figure (Left) Phase map of a MgO nanocube with a size of 260 nm acquired at a dose rate of 4.1 $eÅ^{-2}s^{-1}$. (Middle) Corresponding phase contours with a contour spacing of 2π/8. The electron beam direction is close to the ⟨110⟩ zone axis. (Right) Charge vs. size of the MgO nanocubes. The parabolic dependence suggests that the charge is mostly localized on the surfaces of the nanocubes.

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VISUALISING NUCLEATION AND GROWTH MECHANISMS IN SOLID AND LIQUID SOLUTIONS USING IN-SITU TRANSMISSION ELECTRON MICROSCOPY

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Transmission electron microscopy (TEM) is a powerful technique for obtaining structural and compositional information about materials across scientific disciplines[1]. *In situ* TEM has become a highly effective tool for monitoring dynamic processes in materials in real time with atomic spatial resolution in response to an external stimulus, in order to understand the underlying mechanisms[2]. Recent advances in microfabrication technology and the advent of aberration correction and highly efficient electron detectors are paving the way for new experimental possibilities[3,4,5]. Here, we describe microelectromechanical-systems-based *in situ* TEM studies, which focus on phase transformations in sputtered thin films and nucleation and growth processes through liquids. The first study elucidates spinodal decomposition in Ag-Cu alloy films, highlighting the promise of *in situ* heating TEM for understanding phase transformations in solid solutions. In the second study, a novel approach for mixing two distinct liquids within a liquid cell, which offers real time insight into industrially-relevant antisolvent crystallisation, is demonstrated.

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ADVANCING FOCUSED ION BEAM TECHNIQUES FOR ENHANCED *IN-SITU* MEMS-BASED SAMPLE PREPARATION

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This study introduces a novel and refined focused ion beam (FIB) methodology tailored for the preparation of samples on micro-electro-mechanical-system (MEMS) chips, facilitating advanced *in-situ* electrical and electro-thermal experiments within a scanning transmission electron microscope ((S)TEM). The fundamental challenge of producing contamination- and damage-free, electron-transparent lamellae is addressed with a particular focus on preserving the integrity of the samples during preparation – a prerequisite for successful (S)TEM experiments. While FIB instruments are widely employed for standard TEM sample preparation, their conventional use often introduces damage and contamination, primarily with gallium (Ga) and/or platinum (Pt). Such impurities can significantly influence the electrical properties of the investigated samples. The complexities of *in-situ* sample preparation compound these challenges, involving extraction, transfer, fixation to the MEMS device, and thinning to achieve electron transparency.

In response, our recently developed FIB sample-preparation methodology [1] minimizes attachment and detachment steps, notably reducing the reliance on Pt fixation (Fig. 1). Rigorous characterization of various sample preparation parameters and lamellae geometries has been conducted to comprehensively assess their impact on electrical measurements. Our proposed methodology advocates for the direct fixation of extracted lamellae onto the E-chip, bypassing intermediate attachment steps to the grid, thereby enhancing overall sample quality. This improvement is substantiated through high-resolution STEM imaging and advanced analytical techniques. The versatility of this approach extends to any material amenable to regular FIB preparation [1], marking a significant advancement in the field.

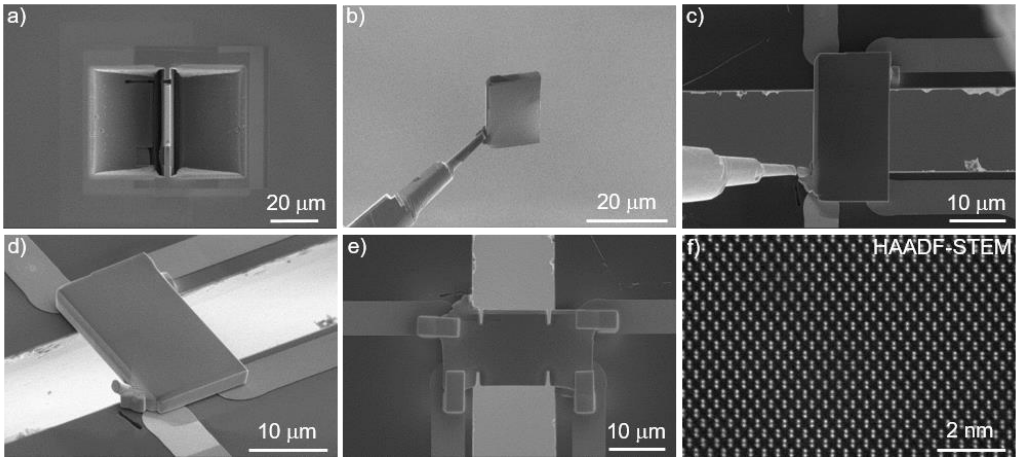

Fig. 1: Optimized FIB-based sample preparation workflow for in-situ electrical experiments. (a, b) Lamella extracted from bulk with alternative geometry. (c, d) Lamella in contact with E-chip. (e) Secure fixation to E-chip using Pt contacts. (f) HAADF-STEM image of thinned Si lamellae on E-chip.

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FULLY AUTOMATED MATERIALS CHARACTERIZATION USING CORE-LOSS ELECTRON ENERGY LOSS SPECTROSCOPY AND DEEP LEARNING

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Electron energy loss spectroscopy (EELS) is a well-established analytical technique that yields spatially resolved chemical information when combined with scanning transmission electron microscopy (STEM). Although core-loss EELS reveals chemical content in a very direct manner, conventional analysis methods such as peak finding algorithms struggle with the low signal-to-noise ratio and dominant background that are common in EELS. Therefore, identification of core-loss edges in EELS spectra usually remains a manual task for a human operator. This is not only slow and inconvenient in spectrum imaging, but also inevitably leads to experimenter's bias and reproducibility issues that can plague EELS quantification methods. Deep learning is especially suited to tackle this problem because the analysis can be learned from training data instead of having to be algorithmically predefined. In this work, a simulated dataset is introduced with 736,000 labeled EELS spectra that represents 107 K, L, M or N core-loss edges through all 80 elements from Be to Bi. The use of generic fine structures and generic low-loss regions result in sufficiently realistic spectra while keeping computational costs limited. The simulated dataset is used to train and evaluate a series of neural network (NN) architectures including convolutional NNs and attention-based transformer networks. The use of an ensemble of NNs allows for a further increase in performance and is used to demonstrate fully automated elemental mapping in a spectrum image. The element identification network is useful as a stand-alone tool, but an even more complete analysis is obtained by combining the NN with more flexible existing physical model-based tools like EELSMODEL [1]. This combination can form the basis of an entirely unsupervised quantification workflow which is urgently needed to cope with the ever-increasing amounts of data that are generated in modern STEM EELS experiments. [2][3]

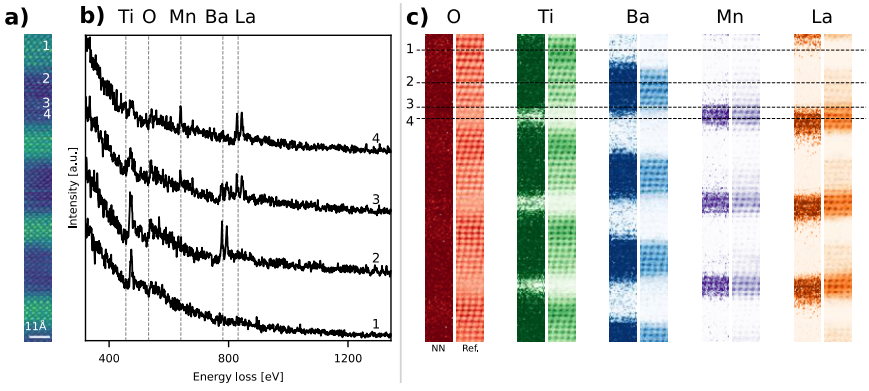

Figure 1: Fully automated elemental mapping of a $LaMnO_3/BaTiO_3/SrTiO_3$ superlattice.

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HOW TO USE SIMULATED DATA TO TRAIN A NEURAL NETWORK ON CLASSIFYING NANOPARTICLES IN HRTEM IMAGES BASED ON CRYSTALLINITY

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High-resolution transmission electron microscopy (HRTEM) is an important analytical tool in nanoparticle research as it can visualize size, shape and structure of the nanoparticles. However, analyzing these HRTEM images is often a time-consuming and tedious, mostly manual process. Machine learning tools can be used to build faster and more automated processes for image analysis. We have already facilitated a machine learning based program to analyze particles for their size and shape [1]. This tool can be expanded upon by also analyzing the particles for their crystallinity.
A main problem with machine learning is the availability and quality of training data. While real images which are usually manually labelled are the closest representation of the data the network will be applied to, it is often expensive to generate the data and the labelling is error prone. For a crystallographic classification of nanoparticles, large amounts of high-quality training data would be needed. To overcome this problem of training data scarcity, simulated data can be used. The important factor with using simulated data is to make sure that it is a good enough representation of the real data.
We tested two approaches to simulate data to use for training a neural network to distinguish nanoparticles based on their internal crystallinity. The first approach is a very simple pattern-based approach. The second approach is a more sophisticated simulation using the Dr. Probe software where we created artificial HRTEM images of nanoparticles with different crystallinity on a thin amorphous support [2]. A dataset of manually labelled real HRTEM images was used to validate the performance of the trained networks. It could be shown that more sophisticated approaches than very simple pattern approaches are needed to train a network that is applicable to real data. The network trained on the images generated using the Dr. Probe software showed an excellent performance in recognizing the internal crystallinity of nanoparticles in real data.

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COMPUTATIONAL MODELING, SIMULATION, AND OPTIMIZATION OF MEMS PHASE PLATES USING COMSOL MULTIPHYSICS

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Electrostatic phase plates have a large importance as means to shape the electron beam in a transmission electron microscope, opening avenues for diverse applications such as generating vortex beams [1], OAM sorter [2], aberration corrector phase plates, and more [3]. This contribution shows the computational modeling, simulation, and optimization of MEMS-based phase plates employing COMSOL Multiphysics. A critical aspect of this study involves the simulation, analysis, and enhancement of the proposed MEMS-based phase plate devices examining the interaction of the generated field by the phase plate with the electron beam before fabrication and experimental testing. We utilized finite element analysis, complemented by musltislice and analytical models. Beyond mere modeling of phase plates, our study explores the optimization of the proposed models to achieve an optimal match between the design and our desired outcomes. The research unfolds in two key stages: first, the COMSOL simulation of various phase plates. Subsequently, we use optimization methods, including forward simulation optimization and machine learning, to refine both the topology and parameters of the plate, thereby enhancing its functionality.

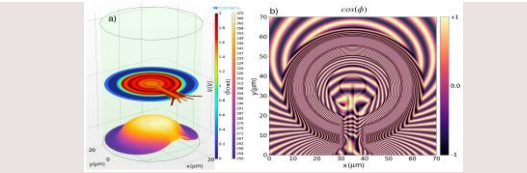

Fig. 1: a) Simulation and optimization of aberration corrector phase plate and b) 3D surface representation of the phase shift fitted to a quartic profile.

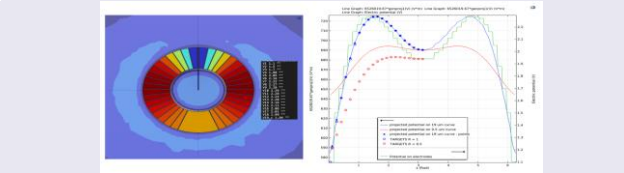

Fig. 2: a) Optimized potential of boundary electrodes for the OAM sorter. b) comparison of the optimal potentials (blue line) with the aimed profile (blue dots) near the boundary.

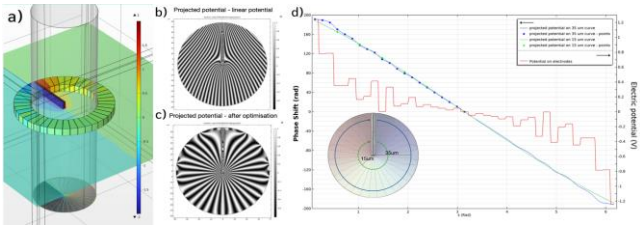

Fig. 3 a) optimal potentials and phase shift for a spiral phase plate b&c) phase before and after optimisation. d) optimal potential on boundary electrodes (red) and aimed phase shift (green and blue).

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UNSUPERVISED MACHINE LEARNING-BASED STEM DIFFRACTION PATTERN DENOISING FOR ENHANCED GRAIN VISUALIZATION IN PHASE CHANGE MATERIALS

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Phase change materials (PCM) are an emerging class of materials in which different phases of the same material may have different optical, electric, or magnetic properties and can be used as a phase change memory [1]. Phase-change memory materials, exemplified by (Ag, In)-doped Sb2Te (AIST) in this research, have several advantages, including high-speed read and write operations, non-volatility, and a long lifespan [2]. PCMs are able to switch between amorphous and crystalline phases when subjected to heat or electrical current. However, the full understanding of PCMs depends heavily on accurate characterization, often through techniques like scanning transmission electron microscopy (STEM).

In the field of materials science and nanotechnology, the analysis of STEM diffraction patterns is crucial for understanding the structural characteristics of materials, especially in the context of PCMs. Accurate interpretation of diffraction patterns is essential for crystallographic analysis, phase identification, and grain visualization in PCMs during the in-situ switching experiment. However, the analysis of STEM diffraction patterns in PCMs can be challenging due to the presence of noise and weak signals.

In this study, we present a solution to address this challenge. We propose an unsupervised machine learning (ML) approach that employs an autoencoder to denoise STEM diffraction patterns. Autoencoders are neural network architectures known for their ability to learn and represent complex data in a lower-dimensional, noise-reduced form [3]. By applying this technique, we enhance the quality of diffraction patterns, improving the signal-to-noise ratio.

Our results demonstrate a significant enhancement in the clustering and visualization [4] of crystalline grains within STEM diffraction patterns of phase change materials. By reducing noise and enhancing signal clarity, the unsupervised ML-based denoising technique allows for more precise discrimination between different crystallographic orientations and refines the identification of grain boundaries. The proposed approach paves the way for a deeper understanding of phase change behavior, aiding in designing and optimizing PCMs for various applications, from thermal energy storage to non-volatile memory technology.

The proposed denoising technique is not limited to PCM. It does not require the laborious production of specific training data and, therefore, can serve as a universal tool for STEM diffraction pattern denoising and signal enhancement.

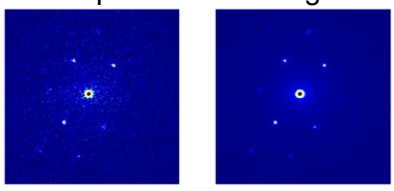

Fig. 1: The diffraction pattern before (left) and after (right) the denoising

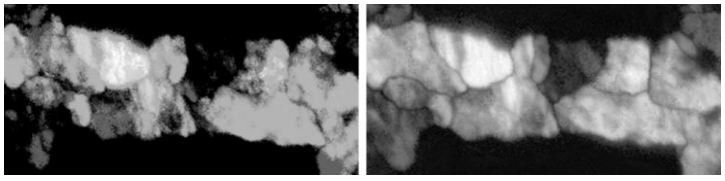

Fig. 2: The index map generated with the original (left) and the denoised (right) STEM dataset

References:
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INFLUENCE OF THERMAL DIFFUSE SCATTERING ON INVERSE MULTISLICE RECONSTRUCTIONS

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Thermal diffuse scattering (TDS) contains crucial information about specimens, e.g., their atomic structure or chemical composition. At elevated specimen thickness, TDS becomes increasingly dominant in diffraction patterns (DP), especially in high-angle dark field regions. Based on momentum-resolved STEM (4D-STEM), inverse multislice (IM) schemes [1] have enabled structure retrieval in the presence of dynamical scattering, extending the applicability of electron ptychography assuming single scattering [SSB, WDD, ePIE]. Especially the implicit or explicit consideration of TDS varies among different IM approaches, which suggests investigating its impact on reconstructed phase gratings (PG). The IM model calculates DPs by propagating the electron beam through a stack of PGs, comparing the resulting DPs with the experimental ones, and iteratively adjusting the PGs to minimize the loss. Based on frozen phonon (FP) simulations for strontium titanate as ground truth, we first consider the repeated use of one slice optimized on a pixelated grid, disabling the generation of TDS. However, Figs. 1a, b show that TDS can be created formally by artefacts in the PG. In a second study, multiple independent slices are optimized pixel-wise. An analysis of the atom positions shows a symmetric distribution around the equilibrium positions (Fig. 1c), but with a variance of only 1-4% of the theoretical mean squared thermal displacements. Artifacts in some of the PG layers indicate that TDS is partly produced by truly perturbing the translational symmetry of the atomic structure, and partly by artifacts known from the wrapped slice model. A study using different cutoff angles in diffraction space shows a direct correlation between the reconstructed diffuse intensity and the amount of artifacts in the phase grating (Fig. 2). Finally a fully parameterized atomic model including FP averaging was used during gradient-based inversion [2]. Thereby, TDS could be reconstructed accurately, and atomic potentials were obtained quantitatively, which has been observed case-dependent for the pixel-wise approaches.

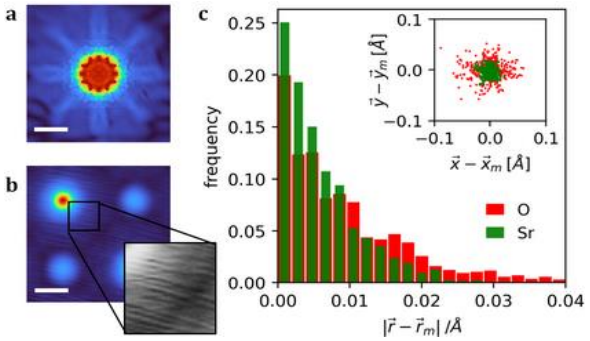

Fig. 1. **a:** Reconstructed PACBED, **b:** phase grating for a wrapped slice model. **c**: Displacement distribution Sr and O columns for multiple independent slices.

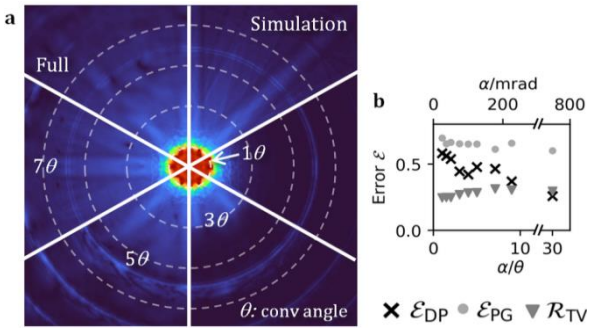

Fig. 2. **a**: PACBEDs reconstructed with different cutoff angles. **b**: Respective errors in PACBED and PG and its total variation noise R_{TV} quantifying the amount of artifacts.

References:
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ENHANCING SEMANTIC SEGMENTATION IN HIGH RESOLUTION TEM IMAGES: A COMPARATIVE STUDY OF BATCH NORMALIZATION AND INSTANCE NORMALIZATION

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This study investigates the impact of Batch Normalization (BN) and Instance Normalization (IN) on semantic segmentation models for high-resolution TEM images of gold nanoparticles. Varying batch sizes (8 and 16) during training were explored, emphasizing the distinct characteristics of BN and IN. While BN normalizes across the entire mini-batch, IN operates independently for each instance, making it more suitable for varied instances or smaller batch sizes [1, 2]. Experimental results demonstrate, as shown in Fig. 1, that IN consistently outperforms BN in capturing nuanced features. Interestingly, with a batch size of 16, IN initially lags behind but catches up, reaching parity with the performance of batch size 8. Conversely, BN exhibits minor differences in performance between the two batch sizes. These findings underscore the significance of normalization techniques, positioning IN as a promising choice, especially fors intricate structures and small batch sizes. The observed dynamics stress the importance of thoughtful normalization and batch size selection in developing robust segmentation models for electron microscopy images.

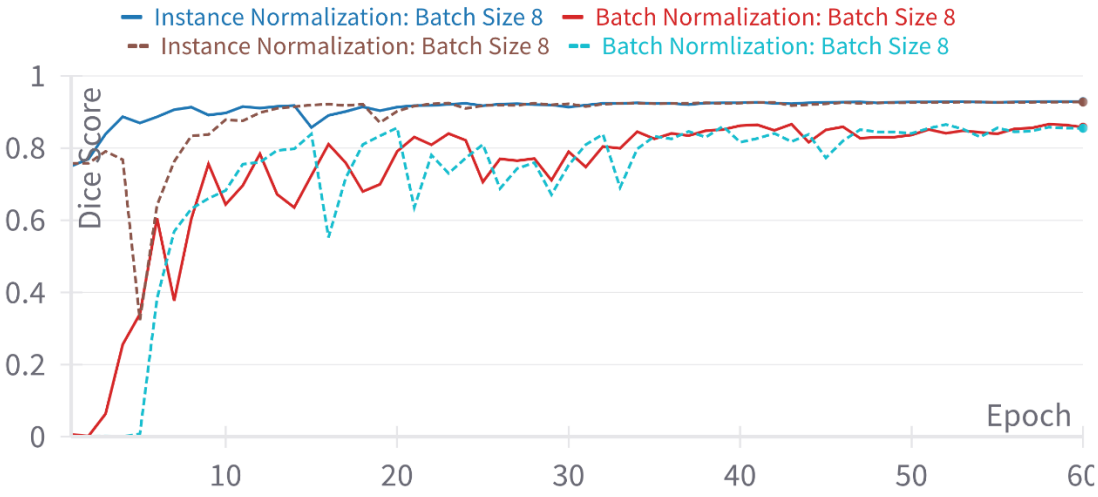

Fig. 1: Dice score on validation data for BN and IN with batch sizes of 8 and 16

References:
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AN APPROACH FOR ANALYZING 3D STRAIN DISTRIBUTION IN HYBRID AND HETERO NANOWIRES THROUGH NBED AND FEM ITERATIVE MODEL FITTING

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Our proposed methodology utilizes an image processing algorithm based on the circular Hough transform and Sobel filtering. This allows for the precise determination of the dynamic disk centers on nanobeam diffraction patterns acquired at a convergent angle of ~1mrad by the CCD camera. The internal intensity distribution of all disks is taken into consideration, enabling the generation of projected components of the lattice distortion tensor, local lattice orientations, and thickness maps. This is achieved by matching patterns to a vast library of pre-simulated templates that account for all dynamic effects of electron scattering [1].
Our approach effectively addresses challenges posed by overlapping diffraction patterns near twin boundaries and stacking faults in the core-shell ZnTe/CdZnTe nanowire (NW). In the next step, the 3D strain distribution inside the NW is estimated through iterative comparison of the Finite Element Model of NW with experimental lattice distortion components. The approach is demonstrated using the example of an elastically strained curved core-shell nanowire. Consequently, low-dose quasi-tomography data were obtained using only a single zone-axis diffraction scanning. Our method is versatile, extending its applicability to electron beam-sensitive materials where conventional tomography presents difficulties due to material damage by electron beam. [2]

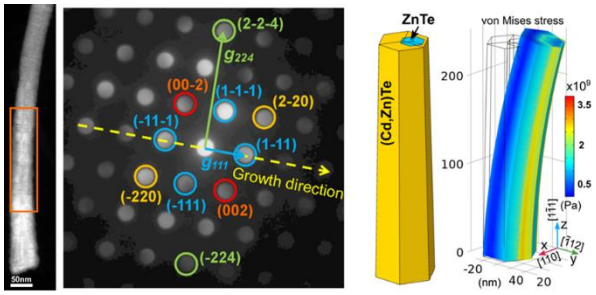

Fig. 1: Curved ZnTe/CdZnTe core-shell NWs, single NB diffraction pattern and FEM model of NW

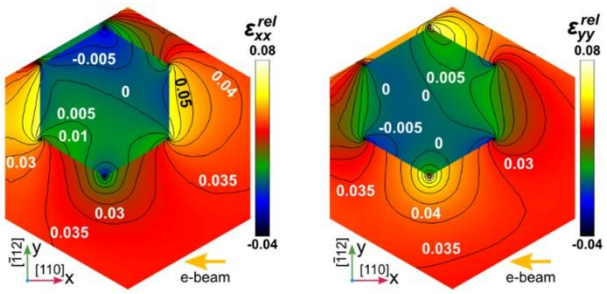

Fig. 2: Quasi tomographic reconstruction of lattice distortion tensor components exx and eyy in NW cross-section maps of relative strain, calculated using relaxed ZnTe as a reference-best fit.

References:
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DATA PROCESSING OF IN-SITU TEM TOWARD LIVE PROCESSING

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In-situ transmission electron microscopy (in-situ TEM) is an evolving technique because it enables the investigation of nanoscale phenomena with various stimuli such as heating, cooling, bias, gas, and liquid [1]. As external stimuli usually reduce the TEM data quality, many in-situ TEM studies have focused on improving the data quality by stabilizing the TEM from stimuli or post-processing the data with machine learning. However, an important point for in-situ TEM is the implantation of the target phenomena into the TEM reality, which is only available when the stimuli actively interact with the living processed information from the observed data. In this case, the processing time is more important than the processing quality. In this paper, we show two data processing cases about electrodeposition with liquid phase in-situ TEM (Fig. 1)[2-3]. First, by applying basic processing (Gaussian filtering, subtraction and thresholding), we could track the size and number of electrodeposited Cu particles and quantitatively compare the change between cycles and time series. Finally, by applying radial Fourier analysis method to 4D STEM data set, we could obtain pseudo-orientation map of electrodeposited Zn with electron diffraction patterns of each selected area. In both cases, valuable information can be extracted even though we did not use complicated algorithms or machine learning. As these procedures are simple enough to adapt to live processing during in-situ TEM, we can actively interact between stimulus condition and phenomena based on live processed information.

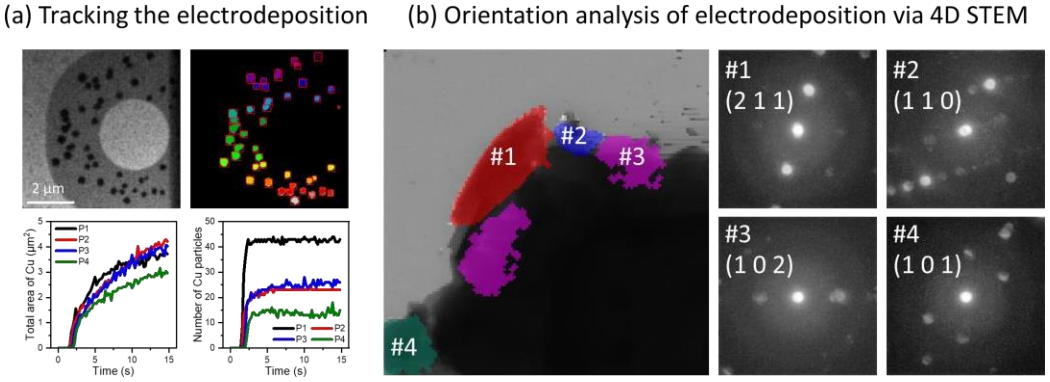

Fig. 1 Data processing of in-situ TEM. (a) Tracking size and number of Cu particles during electrodeposition. (b) Orientation analysis of electrodeposited Zn via 4D STEM analysis

Acknowledgments
J. Park, E. Jodat, A. Karl, S. Basak and R.-A. Eichel acknowledge the funding provided bythe BMBF (German Research Foundation) through the project DERIEL (03HY122C).

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A HIGH-THROUGHPUT CRYO-CORRELATIVE LAMELLA GENERATION PIPELINE FOR IN-SITU CELLULAR STUDIES USING CRYO ELECTRON TOMOGRAPHY

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In-situ cryo electron tomography (cryo-ET) is a powerful technique to study cell machinery in its near-native state. Using this technique, cellular components can be directly visualized in native context and near-atomic resolution. Nevertheless, the workflow is laborious and often requires the use of a focused ion beam (FIB) to thin down the vitrified cell to into a cryo-lamella[1]. The challenges in this lamella generation process compromise the overall success rate and throughput of the technique. Mainly:

1. Conventional cell culture procedures do not provide adequate control over the shape and positioning of the cells on TEM grids, rendering them unsuitable for FIB milling[2].
2. Leaving lamellae inside the vacuum chamber during overnight milling sessions increases the risk of in-chamber ice contamination[3,4].
3. Using a stand-alone fluorescence light microscope (FLM) to target the region of interest (ROI) introduces an unnecessary cryo-transfer step between the two microscopes and significantly increases the chance of ice contamination and devitrification of the sample. Additionally, the non-integrated nature of this ROI targeting often leads to losing the ROI in the final lamella, rendering it useless[4].

To address these challenges, we describe an improved workflow with several newly available technological upgrades:

1. Micropatterning the EM grids prior to cell culture using PRIMO: The customized patterns result in improved control over cell shape and spatial distribution over the entire grid[5].
2. Cryo-FIB milling in the presence of the CERES Ice Shield: In-chamber ice buildup on the lamellae is eradicated, increasing the number of useful lamellae at the end of an overnight batch milling session[3,4].
3. Continuous, in-chamber fluorescence detection using METEOR: The integrated cryo-FLM eliminates a major cryo-transfer step from the workflow. Moreover, continuous monitoring of the fluorescence in the ROI, significantly reduces the risk of losing it in the final lamella[6].

The impact of the improved workflow on in-situ correlative cryo-FIB milling of human RPE-1 cells was a significant increase in the number of useful lamellae generated per grid, rising from 2 – 4 to 24, thus enhancing the ability to resolve structures of interest within the cell[7].

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STRUCTURAL PLASTICITY OF BACTERIAL ESCRT-III PROTEIN PSPA IN HIGHER-ORDER ASSEMBLIE

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The phage shock protein A (PspA) is the main effector of the *Psp* response, a bacterial membrane maintenance system, and has been identified as a bacterial member of the ESCRT-III superfamily. PspA has been shown to form rod-shaped assemblies that mediate membrane tubulation while the detailed structural mechanism of how they trigger accompanying membrane fusion and fission events remain unclear. To enable various membrane interactions, ESCRT-III monomers require high structural plasticity to adapt to filament curvatures or form complex oligomer structures. Here we show the structure of 61 PspA rods by cryo-electron microscopy that exhibit a wide range of diameters and demonstrate that the addition of nucleotides influences the distribution of rod diameters. Without nucleotides, the PspA rods have narrow diameters, with ADP they shift to wider diameters, and in the presence of ATP they reach the largest diameters. The comparison of 11 individual PspA structures with different diameters shows that the structural plasticity of PspA rods requires conformational changes at three defined hinge regions. Furthermore, the length of helix α3 is shown to increase as the loop between helices α3 and α4 shortens in larger diameter rods. The deletion of this loop leads to a significant reduction in structural plasticity. Analysis of the membrane remodelling activity of PspA suggests that this structural plasticity is crucial for the ability of PspA to engulf and remodel membranes.

HIGH RESOLUTION ANALYSIS OF SIGLEC-10 RECEPTOR BY CRYOGENICELECTRON MICROSCOPY

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Siglecs, or sialic acid binding immunoglobulin-like lectins, are immunomodulatory transmembrane receptors that recognise sialylated glycans on the surface of mammalian immune cells but also of some microbial pathogens which use them to evade host immune response[1]. Each Siglec has a unique function and glycan-binding specificity, depending on sialic acid linkage and underlying glycan structure. Aberrant Siglec-sialic acid interactions lead to various pathologies including infection, autoimmune diseases and cancer.Understanding the structural basic of Siglec - ligand binding is therefore essential for designing therapeutic and diagnostic strategies against numerous diseases[1,2]. The heavily glycosylated CD24 protein, overexpressed in human cancer, promotes immune evasion by binding Siglec-10, highly expressed by tumor-associated macrophages, making CD24– Siglec-10 an innate immune checkpoint mediating anti-tumor immunity and thus a major therapeutic target [3]. In the current study, we reveal the near-atomic resolution of the Siglec- 10ECD dimers and tetramers by cryo-electron microscopy[4], with dimers potentially representing the functionally relevant assembly. Using click chemistry[5] and flow cytometry, we demonstrate functionality of purified Siglec-10ECD in binding assays with cells featuring either natural or modified sialic acid at their surface. This study is a fundamental step towards deciphering the complex mechanisms regulating host immune suppression.

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STRUCTURAL AND QUANTITATIVE ANALYSIS OF VIPP1 CARPETS

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Vesicle-inducing protein in plastids 1 (Vipp1) is a bacterial ESCRT-III homologue and essential for cell functioning [1,2]. It is known to be crucially involved in thylakoid membrane biogenesis, stabilization and/or maintenance in plants, algae and cyanobacteria [1]. It tends to form large homo-oligomeric protein complexes on its own, but also in the presence of lipids [3,4]. The various structures Vipp1 can assume in presence of lipids, and the potential mechanistic connections between them for Vipp1 to perform its functions have yet to be explored. In this experiment, a biochemically reconstituted system was used to investigate Vipp1 interactions with membranes. The focus of this study are Vipp1-Carpets, an assembly of Vipp1 into a stable structure on flexibly curved membranes. To that end, a large dataset of tomograms was collected. A deep learning approach was employed for membrane segmentation, accurate enough to additionally detect Vipp1 assemblies on membranes. The correlation between Vipp1 presence on membranes and membrane curvature was quantitatively investigated. Finally, subtomogram averaging was performed, yielding medium resolution structures of Vipp1 carpets. This allowed conclusions on the relation between Vipp1 carpets and previously described symmetrical formations Vipp1 assumed in the absence of lipids [4].

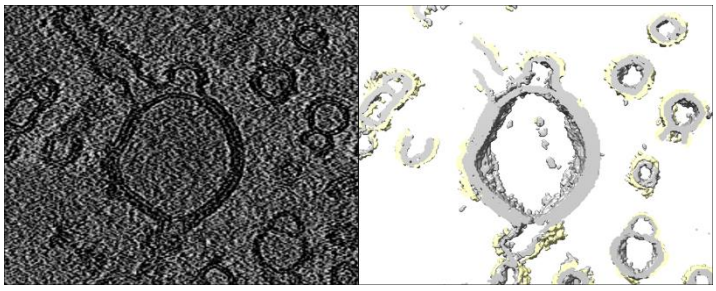

Fig. 1: Left: Raw tomogram, showing Vipp1 assemblies on membranes. Right: Segmentation, membrane (grey) and Vipp1 carpets (yellow).

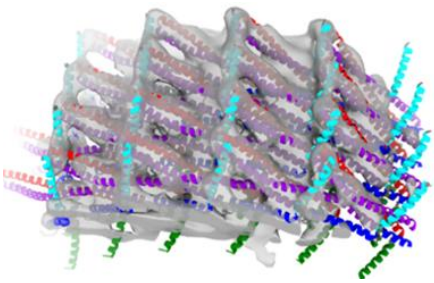

Fig. 2: Subtomogram Average of Vipp1 carpet with fitted model

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AUTOMATED CLUSTERING OF HELICAL FILAMENTS

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Recent steps towards automation have improved the quality and efficiency of the entire cryo-electron microscopy workflow, from sample preparation to image processing. Most of the image processing steps are now quite automated, but there are still a few steps which need the specific intervention of researchers. One such step is the identification and separation of helical protein polymorphs at early stages of image processing. Here, we present a new clustering approach based on analyzing partial similarities between 2D class averages. As an automated polymorph separation method, it has the potential to complement automated helical picking, which typically cannot easily distinguish between polymorphs with subtle differences in morphology, and is therefore a useful tool for the image processing and structure determination of helical proteins.

MEMBRANE ANALYSIS TOOLKIT – A QUANTITATIVE WAY TO ANALYSE PROTEIN LIPID MIXTURES FROM CRYO-EM IMAGES

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Cryo-EM is a powerful tool to study protein membrane interactions as it delivers high-resolution images of vitrified macromolecules including biological membranes. The quantitative analysis of protein-induced effects on the membrane structure is a challenging and time-consuming task as they are not amenable to established single-particle image reconstruction techniques. However, due to the high contrast of phospholipid bilayers, the images are very suitable for detailed analysis and can yield characteristic lipid features and membrane shapes. Currently, localisation of such membrane structures and the extraction of their features is often performed interactively and is, therefore, subjective and a time-consuming task. This is particularly true for larger data sets collected by automated electron microscopes because the number of available images is often too large to be analysed interactively. The here developed "membrane analysis toolkit" aims to automate the membrane analysis and facilitate quantitative comparison of different functional datasets, e.g., capturing the variations such as protein mutations and lipid compositions. The membrane structures are localized using convolutional neural networks and sequentially analysed by a variety of image processing techniques. Typical determined features are membrane thickness, curvature and the size and circularity of vesicles.

The newly developed membrane analysis toolkit provides a GUI for fast and easy analysis of any Cryo-EM micrograph containing membranes.

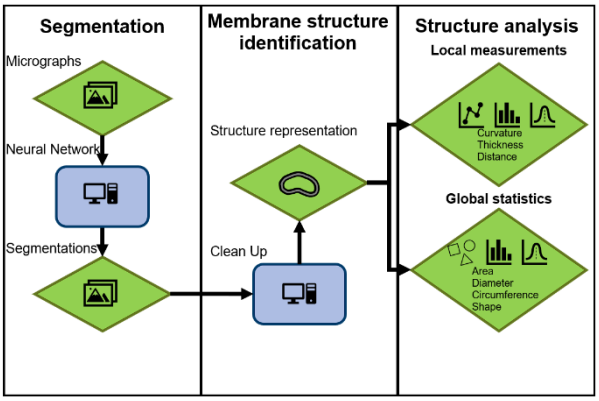

Figure 1 Workflow of the Membrane analysis toolkit

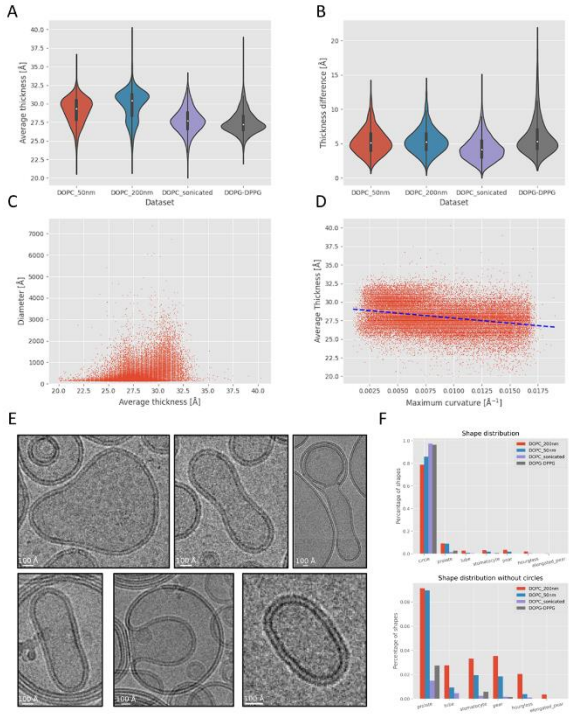

Figure 2 Example analysis of 4 different datasets. DOPC_50nm, DOPC_200nm, DOPC_sonicated, DOPG-DPPG. Analysis includes bilayer thickness, diameter, curvature and shape distribution

STRUCTURAL BASIS OF THE ALLOSTERIC REGULATION OF CYANOBACTERIAL GLUCOSE-6-PHOSPHATE DEHYDROGENASE BY THE REDOX SENSOR OPCA

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Glucose-6-phosphate dehydrogenase (G6PDH) is a key enzyme in the oxidative pentose phosphate (OPP) pathway, a fundamental route for the generation of reducing power and metabolic intermediates for biosynthetic processes [1]. In photosynthetic organisms, G6PDH is redox-regulated to prevent futile cycles while carbon is being fixed [2]. In cyanobacteria, regulation of G6PDH requires the redox sensor protein OpcA [3]. Here, we used functional analysis to show that OpcA binds G6PDH under all conditions, but complex formation enhances G6PDH activity when OpcA is oxidized and inhibits it when OpcA is reduced. To understand the molecular basis of this regulation, we determined the structures of cyanobacterial G6PDH and the G6PDH-OpcA complex by cryo-EM. OpcA binds the G6PDH tetramer and induces conformational changes in the active site of G6PDH. The redox sensitivity of OpcA is achieved through the formation of intramolecular disulfide bridges, which affect the allosteric regulation of G6PDH. Our findings unveil a novel and unique molecular mechanism governing the regulation of the OPP pathway in cyanobacteria.

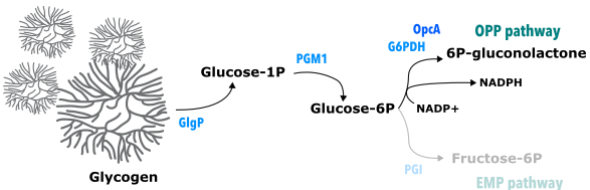

Fig. 1: Scheme of the G6PDH role in carbon catabolism

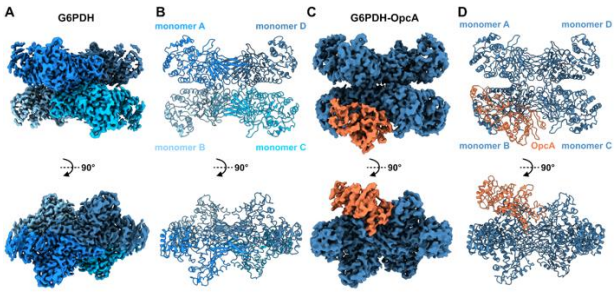

Fig. 2: Cryo-EM analysis of cyanobacterial G6PDH and G6PDH-OpcA complex

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CRYO-EM OF AMYLOID FIBRILS FORMED BY MEDICAL INSULIN

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Insulin is a peptide hormone involved in the regulation of the glucose level and is the primary drug used to treat diabetes. In recent years, global demand for medical insulin increased in the wake of a worldwide type 2 diabetes pandemic. However, insulin has the tendency to aggregate into amyloid fibrils rendering it hormonally inactive and pro-inflammatory. This instability, which is favored by higher temperatures, poses a challenge in both storage and therapeutic application especially in low-income and middle-income countries [1]. Understanding the molecular structure of insulin amyloid fibrils is valuable for developing strategies to prevent their formation. In this study insulin amyloid fibrils from human wild-type insulin and different insulin drug preparations were investigated by cryo-electron microscopy (cryo-EM), revealing a high amount of polymorphism inside each sample and different polymorphs across samples. 3D reconstructions of three different polymorphs were solved at a FSC resolution of up to 3.6 A sufficient for atomic model building. Although the exact fold of the peptide's A and B-chain differs between fibrils, certain motifs are conserved across the different structures. Those can now be targeted in the development of next-generation insulin analogues, which are less prone to aggregation and therefore improve global diabetes care.

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ANALYSIS OF CRYO-EM STRUCTURES OF ARBOVIRUSES

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Arboviruses are a group of viruses which are transmitted by arthropod vectors [1]. The group includes Chikungunya, Mayaro, Dengue, Zika, etc. [2] [3], which causes many (human) diseases throughout the world [4]. *Alphavirus* (Chikungunya and Mayaro) are enveloped viruses approximately 70 nm in diameter, with an icosahedral capsid containing positive-sense, single-stranded RNA genomes, ranging from 10 to 12 kbases [5]. The *Orthoflavivirus* (Dengue and Zika), a member of Flaviviridae family, have positive-sense RNA of approximately 9.0–13 kbases within an icosahedral structure of an approximate diameter; 40 to 60 nm [6]. Many Cryo-EM structures of these viruses have been published [7] and deposited in the Electron Microscopy Data Bank (EMDB). This database serves as a resource for harvesting information about many fundamental viral processes, such as; maturation, evolution and infection. However, this type of information harvesting from a large pool of Cryo-EM structures is hampered by different Cryo-EM workflow. We have downloaded the available Cryo-EM structures of these viruses from EMDB for a proof-of-concept test. We have thus encountered multiple challenges in carry out our experiments. The first challenge in comparing these different structures is to bring them all into a standardized digital format, in terms of: box size, pixel size, density values, alignment and magnification. We have two standardized stacks: one for Alphaviruses [***Fig. 1***] and one for Orthoflaviviruses [***Fig 2***]. The MSA classification [8] of the Alphavirus stack resulted in grouping by species and maturation stage. Our work may yield new structural insights in the life cycle of arboviruses and help combating the spread of such diseases.

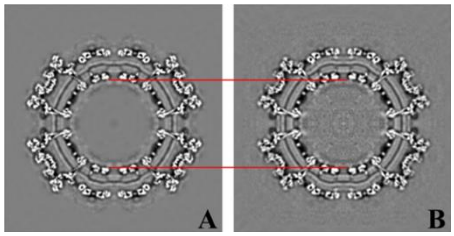

Fig. 1: Central sections of Mayaro virus (**A**) and Chikungunya virus (**B**). The red lines show that the two viruses have been scaled to the same size for multivariate comparison.

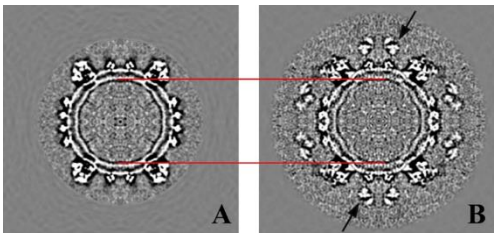

Fig. 2: Central sections of Spondweni virus (**A**) and Dengue virus (**B**) scaled to the same size of the central capsid (as indicated by the red-lines)

References***:***

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[9] This research was supported by CZI and FAPESP (2020/06062-1)

ER-C 2.0

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The Ernst Ruska-Centre for Microscopy and Spectroscopy with Electrons (ER-C) is currently coordinating a national roadmap project "ER-C 2.0" for large-scale research infrastructures in Germany [1]. This infrastructure project is based on the existing ER-C and aims for the extension of the national infrastructure for the ultra-high spatial, temporal and energy resolution characterization of atomic and molecular structures using next-generation electron microscopes. A set of new next-generation instruments will be installed at the ER-C – two main instruments "TOMO" and "BIO" will be installed in the first phase of the project and three main instruments "SPECTRO", "FEMTO" and "OPERANDO" in the second phase. With the combination of those primary instruments and correlative techniques, ER-C 2.0 will offer new opportunities for characterizing soft and biological materials, in addition to further developments in the existing focus of ER-C on inorganic materials, leading to advancement of instrumental design and cross-fertilization between materials and life science communities. The resources of ER-C 2.0 will be available to users from academia and industry in a transparent and peer-reviewed process.

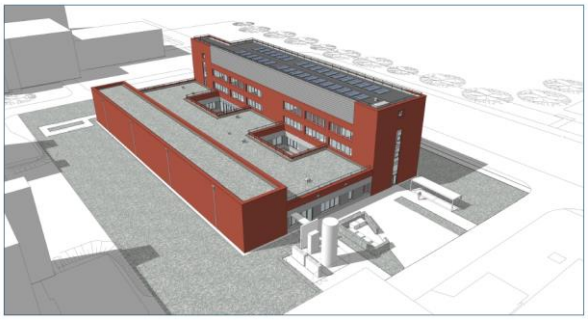

Fig. 1: New building housing the next-generation instrumentation.

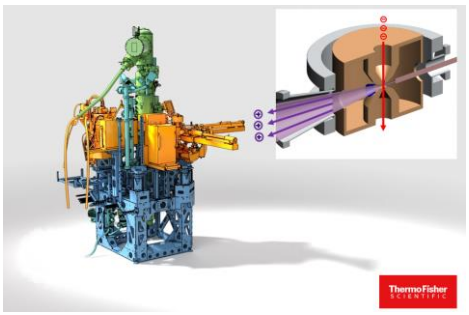

Fig. 2: TOMO tool: integrated atom probe tomography on TEM.

References:
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CRYO EM USER FACILITY AT ERNST RUSKA CENTER, FORSCHUNGSZENTRUM, JULICH, GERMANY

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The Cryo EM facility at Ernst Ruska Centre, Forschungszentrum in Julich, Germany, is a leading national user facility that provides access to advanced cryo-EM infrastructure for the global scientific community. Our state-of-the-art equipment includes the Titan Krios 300 kV, Talos Arctica 200 kV , Talos L120 and Aquilos 2 FIB-SEM, which are all capable of supporting standard structural analysis techniques such as Negative Stain, Cryo-EM Single Particle Analysis, Tomography, and Cryo-CLEM.
Our Titan Krios 300 kV, equipped with Falcon 4 and GIF-K3, has achieved impressive resolution ranging from 1.7 Å to 2 Å for benchmark Apoferritin proteins. Additionally, the Titan Krios 300 kV is equipped with the Panther detector system, which includes 4 segments and annular darkfield detectors that can be used for iDPC and HAADF-STEM imaging.

Our Aquilos 2 FIB-SEM platform has been upgraded with the METEOR system and CERES ice shield (Delmic), allowing *in-situ* fluorescence microscopy to be performed directly on samples inside the system, reducing transfer steps and associated ice contamination. This upgrade also improves the efficiency of the cryo-CLEM pipeline. In addition to our impressive imaging equipment, our facility is equipped with a range of sample preparation equipment. Along with Vitrobot Mark IV and Leica EM GP2 we have also upgraded our facility with VitroJet.

At the ER-C cryo-EM user facility, we provide access to the entire pipeline from sample preparation to image acquisition. Experienced users can operate the equipment with minimal supervision, and we are committed to teaching and training new users to be independent. Since CryoEM user facility conception we have had 50 external projects from around the globe. With ERC 2.0 project by BMBF in vision, the new building will host two new high-end tools from TFS, i.e BIO tool and Arctis, amongst other Material science TEMs.

Read more : https://er-c.org/index.php/facilities-2/life-science/

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INCYTE – THE INTEGRATED USER FACILITY ALONG THE PROCESS CHAIN OF COMPLEX SENSOR / DEVICE DEVELOPMENT

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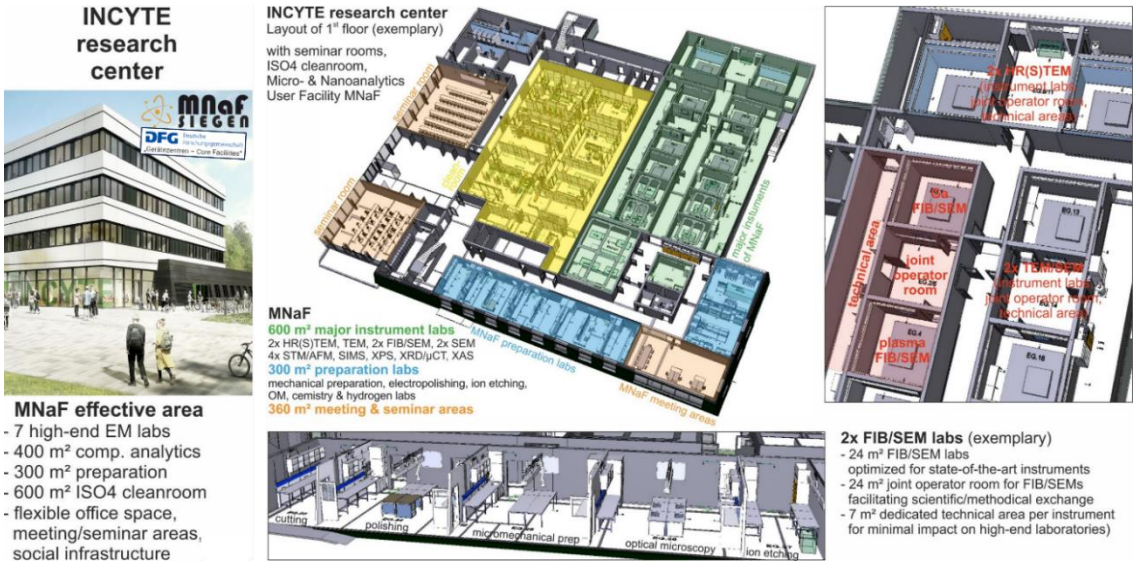
INCYTE – The new 11.000 m² large Interdisciplinary Research Center for Nanoanalytics, Nanochemistry and Cyber-physical Sensor Technologies at the Univ. of Siegen, will exploit the synergies of materials/sensor design, synthesis/fabrication, and comprehensive in-depth characterization to facilitate I) the efficient development of novel (bio)sensors and II) challenging research on highly reactive/sensitive materials and even complete devices like batteries. Therefore, the building includes comprehensive central user facilities like a 600 m² ISO4 cleanroom, S2/S1 biolabs, numerous high-end labs for advanced electron microscopy and complementary micro- and nanoanalytics (DFG MNaF facility) as well as ample space and equipment for sample preparation. Particular attention was put on the following aspects:

- As the research center is hosted by a university with educational responsibility, the building is optimized to foster exchange among the user groups and particularly between experienced users, methodical novices, and students at the major instruments.
- Even highly reactive samples may be handled and analyzed along the complete process chain under inert/vacuum/cryogenic conditions. The facilities with highly specialized equipment and dedicated office space are open to host external users.
- Data handling and processing: a future-proof fiber optic network in addition to copper-based infrastructure will connect the different building sections with an internal server room for efficient data collection/sharing, screening and high-performance evaluation.
- Cost efficiency, sustainability of current technologies and the opportunity to adapt labs to changing requirements in the future: the comprehensive pre-planning phase and an optimal interplay between all involved teams including the major user groups allowed the whole project to proceed according to schedule and to keep it within the approved budget.

The authors acknowledge the excellent collaboration of the whole planning team at the university, the Bau- und Liegenschaftsbetrieb NRW and the executing companies. We thank the state of NRW and the federal government via the DFG for funding of the building, the major instrumentation, and the initial equipment.



CRYOEM TECHNIQUES AT ISTA: MULTI-MODAL APPROACHESREVEAL NOVEL INSIGHTS INTO EXTRACELLULAR MATRIX, ACTIN FILAMENT STRUCTURES, AND POXVIRUS CORES

Victor-Valentin Hodirnau[1*], Bettina Zens[1], Florian Fäßler[1,2], Julia Datler[1], Jesse Hansen[1],Alois Schloegl[1], Stefano Elefante[1], Ludek Lovicar[1], Florian KM Schur[1]

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The Electron Microscopy Facility at ISTA provides a comprehensive array of state-of-the-art instruments for conducting advanced cryogenic electron microscopy (cryoEM) studies. The poster highlights the synergistic application of plunge freezing, high-pressure freezing, cryogenic correlative light and electron microscopy (cryo-CLEM), sample milling through focus ion beam scanning electron microscopy (FIBSEM), the lift-out technique, and transmission electron microscopy (TEM), coupled with robust computational resources. The integrated approach employed in these studies facilitates the discovery of intricate biological insights across a diverse sample spectrum, ranging from viruses and individual cells to tissue-like samples. Elusive structural details of the extracellular matrix (ECM) are revealed through a novel 3D-ECM platform, using cryo-lift out FIB milling and cryo-electron tomography (cryo-ET). Utilizing cell-derived matrices (CDMs) on EM grids, this tool faithfully mimics ECM environments, uncovering native ECM and its components in a hydrated, cellular context [1]. Investigating the actin-related protein Arp2/3 complex showcases a 7.8Å resolution structure, exposing detailed structural rearrangements and actin filament interactions [2]. For poxviruses, a cryoEM and molecular modeling approach identified trimers of the A10 cleavage product as palisade layer components, significantly revising our understanding of poxvirus core architecture [3]. The seamless combination of experimental cryoEM techniques and computational tools enhances our ability to unravel intricate details within diverse biological specimens.

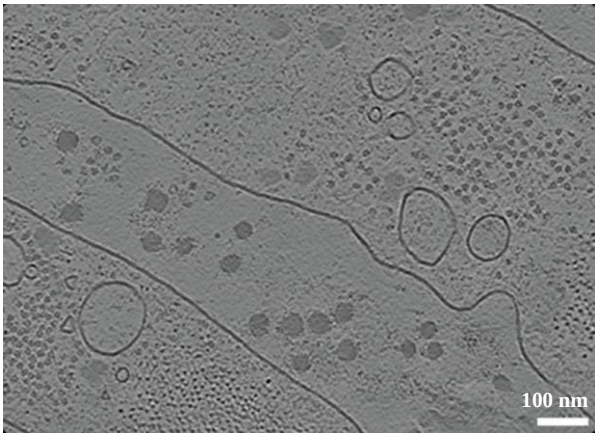

Fig. 1: An IsoNet-corrected tomogram of the CDM acquired from a cryo-lift out lamella.

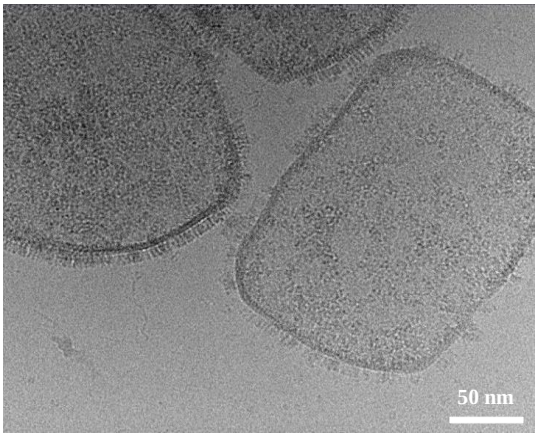

Fig. 2: SPA micrograph showing isolatedvaccinia viruses.

References:
[1] Bettina Zens et al., bioRxiv **559261** (2023).
[2] Julia Datler and Jesse Hansen et al., bioRxiv **541142** (2023).
[3] Florian Faessler et al., Nat. Commun. **11**, 6437 (2020).

ReMade@ARI: A HUB FOR MATERIALS RESEARCH

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As a result of the progressive depletion of natural resources, together with increasing waste, a cooperation aimed at shifting the global economy towards a circular economy is urgently needed. This change requires substantial research on materials that have high recycling potential, while exhibiting competitive functionalities.
In order to address this challenge, the most significant European analytical research infrastructures have joined forces in the ReMade@ARI Horizon Europe project, which provides a support hub for materials research while facilitating a step change to a circular economy.

ReMade@ARI offers comprehensive analytical services for research that focuses on the development of new materials for the circular economy [1]. The project offers coordinated access to over 50 research infrastructures across Europe, including electron microscopy facilities, synchrotrons, free electron lasers, neutron sources, high magnetic field laboratories, and ion or positron beam facilities. Senior scientists, facility experts and young researchers contribute scientific knowledge and extensive support to provide user services [1]. Particular attention is given to the implementation of comprehensive support mechanisms for researchers and developers from industry [3].

ReMade@ARI releases calls for standard access (ReMade-TNA) twice a year. Separate calls for additional modes of access that are dedicated to industry (ReMade-SME and (ReMade-IND) are released separately. The schedule of calls for different modes of access are outlined in Fig. 1.

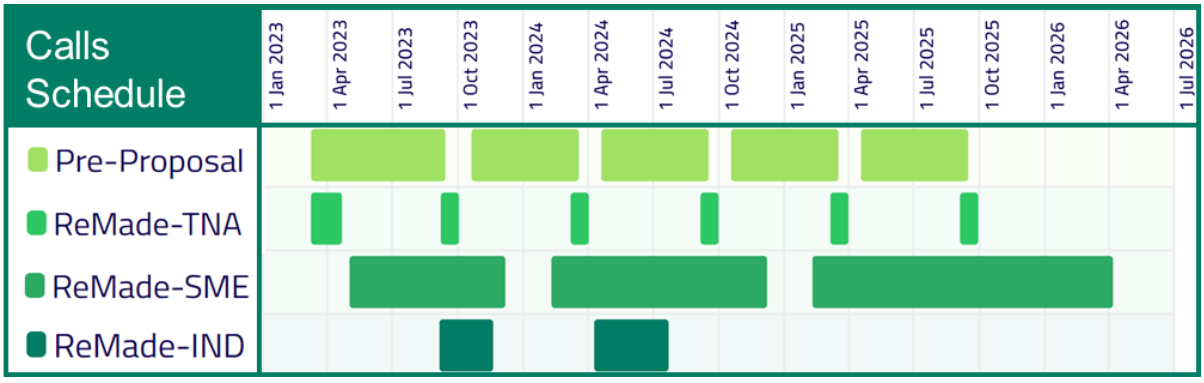

Fig. 1: Schedule of calls for access to ReMade@ARI analytical research infrastructures [4].

References:
[1] info@remade-project.eu (for general information)
[2] sciencesupport@remade-project.eu (for scientific support)
[3] industry@remade-project.eu (for industrial support)
[4] remade-project.eu

RIANA: RESEARCH INFRASTRUCTURE ACCESS IN NANOSCIENCE & NANOTECHNOLOGY

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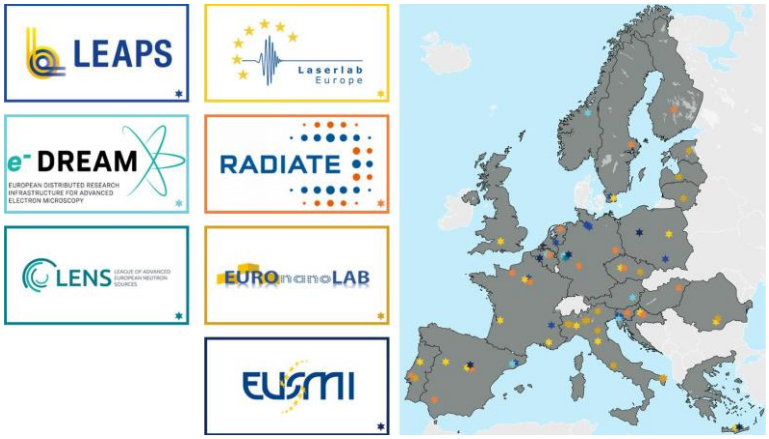
Research in the fields of nanoscience and nanotechnology is vital for a global sustainability. As the advancement in nanoscience and nanotechnology cannot be achieved without using research infrastructures (RI), the EU-funded RIANA project joined 7 European networks of top-level RIs providing access to the most advanced techniques relevant for nanofabrication, processing/synthesis, characterization and analytic as well as simulation capacity [1].
Highly customized and efficient access to 69 infrastructures, spread across 22 European countries (Fig.1), is coordinated via a single-entry point and enabled through comprehensive scientific and innovation service by senior scientists, facility experts and highly trained junior scientists. This core of RIANA is aligned to attract experienced and new users from academia or industry and will be prioritized for researchers with the brightest ideas and approach to make best use of the RI for nanoscience and nanotechnology in view of sustainability [2].

The excellence of user projects will be upheld by an independent review panel applying sharp evaluation criteria, in particular:

- scientific excellence,
- potential to technology readiness level increase
- level of cross-disciplinarity
- impact on safety for environment
- impact on nanoscience or nanotechnology.

RIANA will accept user proposals based on a “continuous call” that will be launched soon.



RIANA Networks

LEAPS: synchrotron- and free-electron-laser based photon sources
Laserlab: laser sources
e-DREAM: electron microscopy
RADIATE: ion sources
LENS: neutron sources
EuroNanoLab: clean rooms
EUSMI: soft matter research infrastructures, NMR and high-power computing

Fig. 1: The RIANA consortium encompasses 7 networks (LEAPS, Laserlab, e-DREAM, RADIATE, LENS, EuroNanoLab and EUSMI) offering access to 69 infrastructures, spread across 22 European countries [1].

References:
[1] RIANA proposal - grant agreement No. 101130652
[2] RIANA-project.eu

Funded by the European Union

INTEROPERABLE ELECTRON MICROSCOPY PLATFORM FOR ADVANCED RESEARCH AND SERVICES (IMPRESS)

Regina Ciancio[1], Rafal E Dunin-Borkowski[2], Amir H. Tavabi[2], Paul Bersans[3] and all IMPRESS partners.

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IMPRESS is an ambitious project that addresses a major evolution in transmission electron microscopy (TEM) from a laboratory technique to a broad-impact advanced analytical method that is capable of unprecedented integrated experiments and user-driven innovation. A central objective is the development of concepts and the construction of prototypes for ground-breaking new TEM instrumentation with unique flexibility and interoperability of customized sample environments, creating radically improved services for users of analytical research infrastructures (RIs) and business opportunities for small and medium-sized enterprises (SMEs).
IMPRESS will overcome the limitations imposed by state-of-the-art TEM column and component manufacturers by developing a new concept for TEM instrumentation that will be based on a standardized cartridge-based platform hosting new experiments that are currently not feasible on commercial instruments, inspired by open science principles.

Fig 1. IMPRESS aims to achieve connectivity between TEM and other characterization techniques based on open hardware and software concepts.

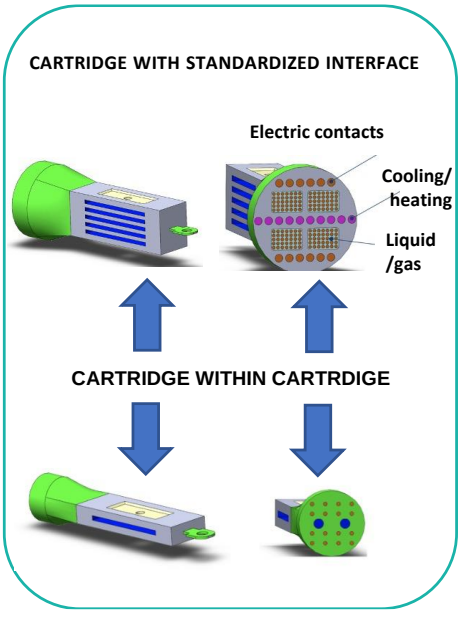

Fig 2. Schematic illustration of the proposed development in IMPRESS of cartridge in cartridge concepts and standardized interface that are compatible with larger numbers of electrical contacts, gases, liquids and light and can be inserted in different positions on a TEM column.

ELECTRON MICROSCOPY WITHIN THE EUROPEAN JOINT VIRTUAL LAB ON ARTIFICIAL INTELLIGENCE, DATA ANALYTICS AND SCALABLE SIMULATION (AIDAS)

Dieter Weber[1*], Matthew Bryan[2], Keyan Ji[1], Alexander Clausen[1], David Landers[2], Vita Mergner[1], Michael Schnedler[1], Qianqian Lan[1], David Cooper[2], Philipp Ebert[1], Rafal E. Dunin-Borkowski[1]

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AIDAS, the European Joint Virtual Lab on Artificial Intelligence, Data Analytics and Scalable Simulation, is a cooperation between the French Alternative Energies and Atomic Energy Commission (CEA) and Forschungszentrum Jülich. It covers six areas: quantum computing, extreme scale systems, extreme scale computing, brain networks, material science, as well as energy. CEA-LETI and the Ernst Ruska Centre are involved in the materials science work package, subproject “Data Express”.

First, we work on offline and live data processing for electron microscopy. This requires a highly scalable software architecture in order to support the GB/s scale data rate of current detectors. Our goal is not only high performance, but also open source software and interfaces that enable open science, open data, as well as innovative experiments at the partner institutes and elsewhere [1].

Second, this work package includes work on model-based quantitative electron microscopy that combines experiments with advanced simulations and digital twins (Figs. 1 and 2) [2, 3, 4, 5].

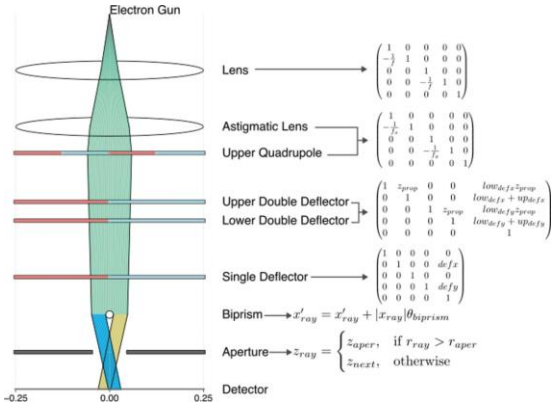

Fig. 1: Ray tracing simulation of a transmission electron microscope (TEM) [2].

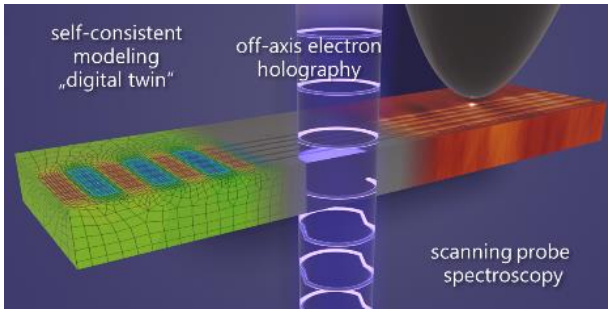

Fig. 2: Combining semiconductor simulations and correlative microscopy.

References:
[1] A. Clausen et al., Zenodo, 10.5281/zenodo.1477847 (2023).
[2] D. Landers et al., J. Appl. Crystallogr. 56, 4, 1267-1276 (2023).
[3] K. Ji et al., Appl. Phys. Express 17, 016505 (2024).
[4] Y. Wang et al., Phys. Rev. B. 24, 245304 (2020).
[5] V. Mergner et al., FEMS EUROMAT (2023)

THE EM GLOSSARY: A COMMUNITY EFFORT TOWARDS A HARMONISED TERMINOLOGY IN ELECTRON MICROSCOPY

Schröder, R.R.[1*]; Hofmann, V.[2]; Kühbach, M.[3]; Pauly, C.[4]; Aversa, R.[5]; Brockhauser, S.[3]; Azocar-Guzman, A.[2]; Joseph, R.E.[5]; Özkan, Ö.[6]; Konijnenberg, P.J.[2]; Wollgarten, M.[6]; Mannix, O.[6]; Sandfeld, S.[2]

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To be fully exploitable and 'future proof', research data needs to meet various requirements. For this, the FAIR principles – Findable, Accessible, Interoperable, and Reusable - provide a widely accepted framework.

Here, we want to focus on 'interoperability', which requires a common language for knowledge representation within interdisciplinary fields, such as electron microscopy (EM). Such semantic standards are often lacking or even controversial. In the field of EM, a number of application-level initiatives independently started developing metadata schemas, to describe experimental equipment, workflows, and analysis procedures. Semantic harmonisation of such efforts is required to ensure data interoperability in the future. As a first step, misalignment of terminology has to be addressed by mapping concepts of different scientific contexts, and user groups.

The Helmholtz Metadata Collaboration (HMC) is currently coordinating a first effort, the EM glossary group [1], to establish a documented terminology for electron and ion microscopy (IM). This community involves scientists from more than 23 institutions across Switzerland, Austria, and Germany and representatives of the FAIRmat and the MatWerk NFDI consortia.

In a remote collaborative workflow and bi-weekly online meetings, we work towards formulating consensus on terms that are commonly used in the EM and IM communities. We aim to produce concise, unpacked definitions with rich annotations in accordance with semantic best practices.

By now, we provide harmonized definitions for more than 60 terms which can be explored via a web interface [2]. In a next step, the glossary will be implemented as a machine accessible resource in the web ontology language (OWL). Both these representations are intended as a central resource to map and align application-level semantics, thereby acting as semantic glue within the field.

Interested to get involved? See [1] and send an email to hmc@fz-juelich.de to get in touch!

References:
[1] EM Glossary Group: https://go.fzj.de/EMG-repo (development repository)
[1] EM Glossary User Interface: https://go.fzj.de/EMG-UI (temporary demonstrator)

LATEST DEVELOPMENT IN EELS

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With the advent of aberration-correction, the modern scanning transmission electron microscope (STEM) can routinely achieve sub-Ångstrom resolution. Aberration corrected STEM, in conjunction with electron energy loss spectroscopy (EELS) cannot only resolve compositional changes atom-by-atom, but also changes in the projected unoccupied density of states across an atomic layer. The type and quality of the detector used at the end of energy filter is a key to realizing the full potential of EELS analysis in the TEM. The characteristics of an ideal detector include single electron sensitivity (Quantum efficiency), knowing the spatial location of each electron, elimination of background and readout noise, and a high dynamic range. Previously, detectors in electron microscopy have been based on indirect (scintillator) detection technology with a fiber-coupled CCD or CMOS sensor. In an indirect detector, the scintillator converts an incident electron into photons which are coupled through optic fibers onto the image sensor. The photons are then converted to an electrical charge and read out as a signal. Multiple scattering in the scintillator broadens the point spread function (PSF) of the detector, photon scattering in the optical fiber reduces the signal incident on the sensor, and read-out and noise of the CCD or CMOS sensor further reduces the DQE.

The alternative is a direct detection system in which electrons are directly incident on a radiation-hardened sensor, eliminating the inefficiency of electron-photon conversion process, resulting in an increase in the sensitivity of localized electron detection. Gatan's K3 camera is an example of a monolithic direct detector, with a very thin low-Z material that produces a very sharp point spread function at or above 80kV. However, the PSF in monolithic detectors starts to degrade below 80 kV. To address this, we introduced an integrated hybrid pixel detector (Gatan Stela), optimized for low kV experiments, in the GIF Continuum K3 system. Stela utilizes Dectris' hybrid pixel electron counting technology with single electron sensitivity, excellent PSF ≤ 80kV, extremely high dynamic range owing to the on-the-fly digitization, and high-speed electron counting (>16000 fps). Fig.1 shows the GIF Continuum system equipped with both K3 and Stela cameras that covers the whole range of TEM voltages from 30 to 300 kV, suitable for a large variety of materials (batteries, polymers, bio- and 2D materials to metals, semiconductors and ceramics).

Here we demonstrate, with selected application examples, how a GIF Continuum K3 and Stela, fully integrated with DigitalMicrograph software, allows for seamless high-quality data acquisition and analysis across all kVs, not only for EELS but also for energy-filtered imaging, diffraction and 4D-STEM experiments.

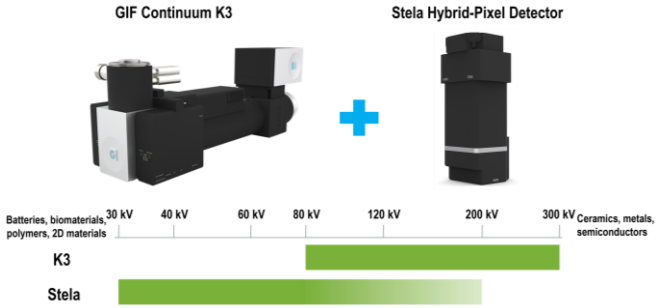

Fig.1. Integrated GIF Continuum K3 system with Stela Camera. Energy-filtered imaging and diffraction for the entire range of 30-300 kV.

FAST 4D STEM WITH ARINA HYBRID-PIXEL DETECTOR

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Electron detection technology has been evolving over the last few years and improving TEM characterization in both Materials Sciences and Life Sciences, particularly when beam-sensitive samples are involved. The hybrid-pixel detector (HPD) concept [1] has the distinctive advantage of a flexible design with respect to the sensor material and electronics, allowing the direct electron detection and counting optimization for a range of TEM experimental parameters (such as electron energy) and different applications.

Building on its successful HPD technology for X-ray detectors, DECTRIS fine-tuned its design to enable the precise detection of electrons. Its most recent development is an application-specific integrated circuit (ASIC) designed to allow read-out rates above 100 kHz and to perform electron counting up to 10 pA beam current per detector pixel with zero read-out noise [2].

ARINA detector combines this newly-designed ASIC with a flexible choice of sensor materials, an easy-to-use application programming interface (API), and a detector retraction mechanism, making it fit to most TEMs with electron energies from 30 to 300 keV and 4D STEM experiments requirements. Initial tests show that ARINA is suitable for flexible virtual STEM imaging with dwell time below 10 µs, allowing for flexible differential phase contrast (DPC) with atomic resolution (Figure 1), and electron diffraction experiments with high dynamic range for crystal phase/orientation mapping [3].

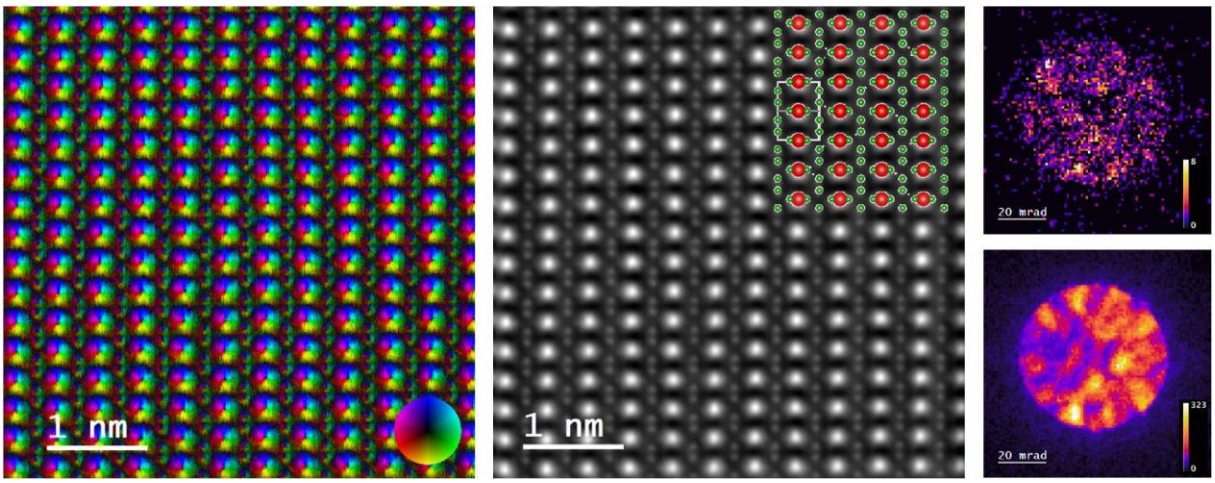

Fig 1.: a) CoM and b) iCoM images calculated after a 10 s 4D STEM measurement with a SmB6 [110] sample. c) Example diffraction patterns extracted from the same 4D STEM dataset, for a single pixel with 10 µs acquisition (above) and for a 10x10 pixels integration. Collaboration: Mingjian Wu (FAU), Elisabeth Muller and Emiliya Poghosyan (PSI).

[1] M. Bochenek et al. IEEE Trans Nucl Sci 65 (6), 1285-1291 (2018).
[2] P Zambon et al., Nucl. Inst. Meth. Phys. Res. A 1048 (2023).
[3] M Wu et al, J. Phys. Mater. 6 045008 (2023)

LATEST INSTRUMENTATION DEVELOPMENTS FOR DYNAMIC STEM APPLICATIONS

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In our survey, we present several hard- and software solutions to enable novel experimental techniques for scanning transmission electron microscopy.
First, we will report on modifications of the scanning probe system to acquire STEM images at reasonable resolutions of 512x512 pixels at a high frame rate of 25fps allowing for in-situ atomic resolution STEM imaging and spectroscopy [1]. By adding additional deflection coils, we are able to scan samples with a tilted beam. The beam conditions can be precisely controlled by defining arbitrary tilt angle waveforms. This tilt scan method, e.g. in combination with segmented detectors, will enable quantitative electromagnetic field observations for crystalline samples [2].
In addition, we will give some examples of the implementation of an ultra-fast pre-specimen electrostatic beam blanker. Since this technology allows the beam to be switched on and off many times per scanned pixel (see Fig. 1 for example), many useful applications arise. One of them is to blank the beam in the pre-scan/flyback in each scanned line to scan only the "true area" and thus reduce beam damage. This can be very useful for reducing beam damage, e.g. during long EDS mappings. The programmable scan can also be used to define the electron dose on the sample individually for each pixel ("dose painting").
The combination of this electrostatic beam blanker with a real-time signal processor paves the way for re-defining pixel intensity in STEM imaging: Intensity can now be defined by the time it takes to detect a fixed number of electrons, as opposed to the number of electrons detected in a fixed dwell time. We will show examples where this distinction will lead to significant benefits especially for low-dose STEM imaging (Fig. 2).

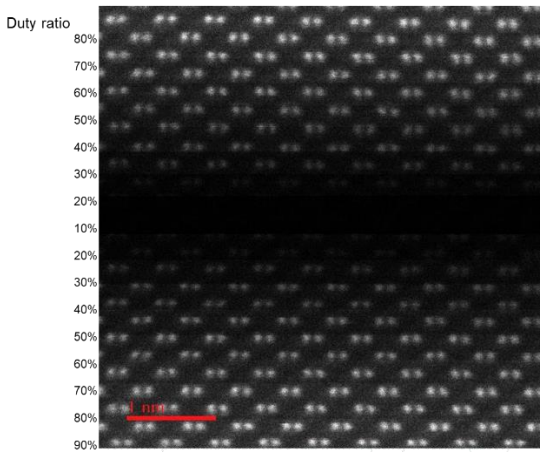

Fig. 1 HAADF image of Si [110] scanned using a pulsed beam.

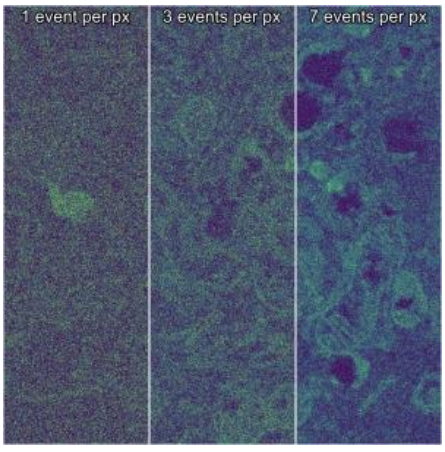

Fig. 2. Compositional image of human macrophage cells recorded with the beam blanker triggering after various event numbers per pixel.

References:
[1] R. Ishikawa et. al., Microscopy **69(4)**, 240-247 (2020)
[2] Y. Kohno et. al., Microscopy **71(2)**, 111-116 (2022)

ADVANCES IN CHARACTERIZATION OF ATOMIC STRUCTURE IN MATERIALS OVER LARGE ROIS BY PRECESSION-ASSISTED 4D-STEM

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Electron diffraction measurements, such as 3D electron diffraction (3D ED) [1] and 4D-STEM, provide structural information on materials at the atomic level. Advances in hardware and software integration of electron beam precession to analytical STEM microscope, TESCAN TENSOR, provide increased speed of data acquisition and processing, which enable and facilitate interactive sample analysis. The power of the electron diffraction techniques is demonstrated here by the determination of distribution of the stannite and enargite phases in a copper-rich sulfide ceramics that was investigated as potential thermoelectric energy harvesting material [2]. The STEM image of the sample showed elongated grains of about 100-nm wide, within which intermixing of the stannite and enargite phases was seen as planar defect bands (Fig. 1). To identify the two phases, we used the TENSOR's ability to collect precession-assisted 4D-STEM data. Firstly, it was done using a parallel beam at different tilt angles on a region of interest (ROI) using a large grid. 3D ED tilt series were then extracted and used for a structure refinement of the two phases. Secondly, using a fine grid at a single tilt angle, precession-assisted 4D-STEM was used to determine the distribution of enargite and stannite, including their orientations, in the same ROI. This identification is based on templates matching [3] generated directly from the actual crystal structure identified by 3D ED. These results illustrate how these two approaches can complement each other to gain a better understanding of the internal structure of this type of nanocomposite and to establish a link between its physical properties and its (micro-)structure.

These results were obtained as part of the European project NanED (Electron Nanocrystallography – H2020-MSCA-ITN GA956099).

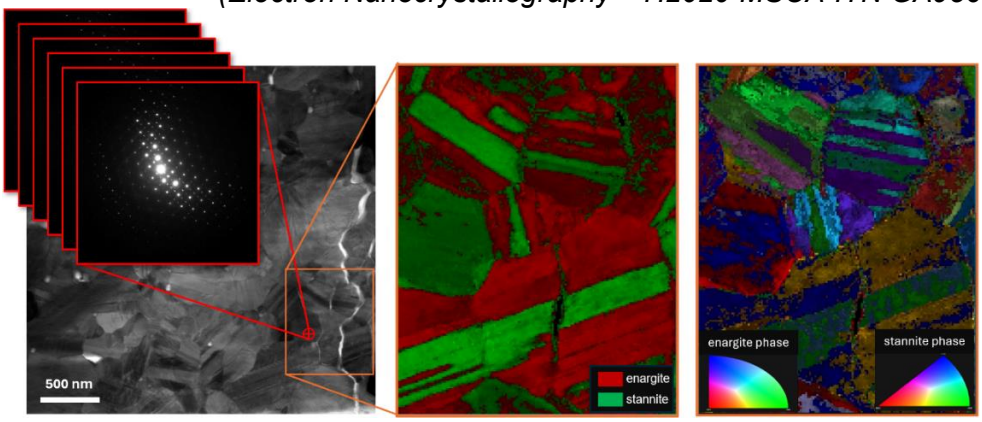

Fig. 1: (Left) STEM image of the $Cu_{2.3}Mn_{0.7}GeS_4$ [2] nanocomposite made of enargite and stannite phases. Precession-assisted 4D-STEM was used to obtain both 3D ED type data for crystallographic structure analysis (in red) and phase (middle) and orientation (right) maps from the same ROI (orange rectangle).

References:
[1] M. Gemmi, et al., ACS Cent. Sci. **5**, 1315-1329 (2019).
[2] V. Pavan Kumar, et al., Angew. Chem. Int. Ed. **61**, e20221060 (2022).
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TOWARDS RELIABLE INVESTIGATIONS OF MATERIALS IN THEIR NATIVE STATE

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The path towards a circular economy requires developing and optimizing technologies that will enable the green transition of society. Energy materials, catalysts, materials for pollutants removal and quantum materials are at the heart of these crucial sectors. Advanced microscopy is of huge importance when developing new materials. However, the afore mentioned materials are often sensitive to the electron beam or influenced by the magnetic field of the TEM, which can severely hamper reliable investigations.
The mission of Thermo Fisher Scientific is to make the world healthier, cleaner, and safer. One important way to achieve this mission is to support the rapidly developing need for atomistic investigations of electron beam and magnetic field sensitive materials. Thermo Fisher Scientific is developing dedicated workflows interlinking both hardware and software innovations.
In this contribution we will highlight and discuss software and hardware innovations as well as workflows, illustrating the power of our solutions with several practical examples.

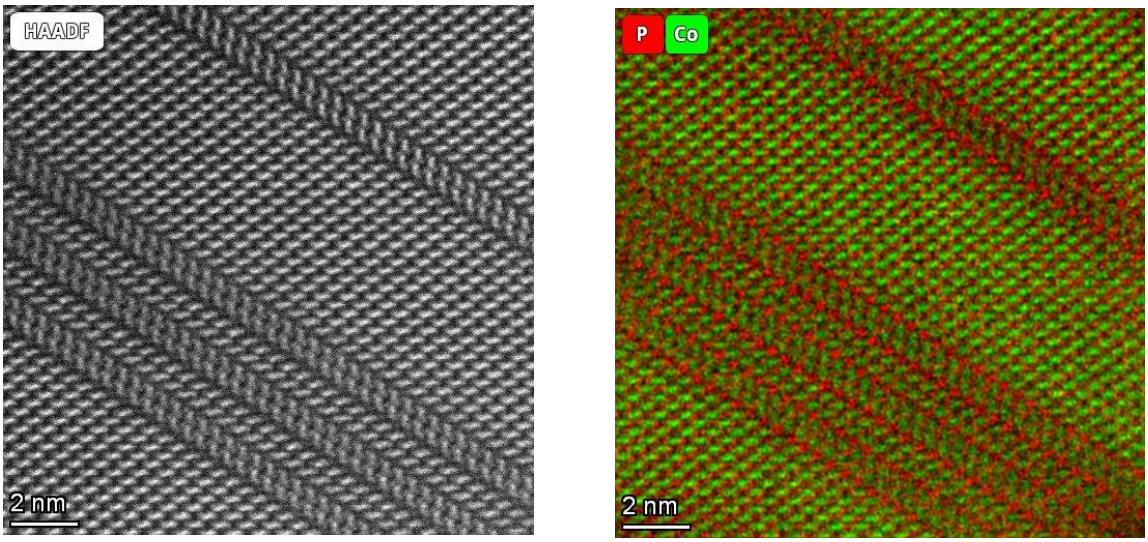

Fig. 1: Atomically resolved HAADF-STEM image of $LiCoPO_4$ cathode material alongside the corresponding Ultra X EDX map collected with a total dose of ~ 4000 ke/Å^2 at 300 kV accelerating voltage. Electron dose mitigation workflow was used prior to the EDX data collection.

STEM EDS IN SEM AND TKD COMBINED FOR ELEMENTAL AND CRYSTALLOGRAPHIC ANALYSES

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We demonstrate the combination of large collection angle energy-dispersive X-ray spectroscopy (EDS) and transmission Kikuchi diffraction (TKD) in SEM for studying the element composition and crystallographic properties of various materials. Examples shown include solar cell, LED, and other semiconductor nanostructures, but applicable as well are partly soft materials and similar, prepared as FIB lamellae. We show that achieving useful quantitative results at nanometer scale in a suitable amount of time in SEM, not STEM, is possible by combining on-axis TKD [1] with an annular EDS detector [2]. EDS data on FinFET structures using windowless large area standard EDS in SEM will also be shown.
The annular arrangement of active SDD quadrants parallel to the specimen surface around a central aperture enables EDS at a particularly high take-off angle, minimizes absorption effects, and allows the analysis of rough topography or bent specimens, all at high signal yield. This geometry is easily combined with diffraction pattern acquisition by an electron detector directly underneath the electron transparent lamellae. Thus, element distribution and specimen thickness, if applying the EDS Zeta-factor method [3], are delivered by quantitative EDS, and crystallographic grain phase, size and orientation are accessible via TKD.
The combination of crystallographic and chemical information is particularly promising for fast correct quantitative analysis on the nanoscale. Small grains can lie in front of each other and produce mixed diffraction patterns. Therefore, to interpret grain size and orientation from TKD correctly, the lamella thickness must be known. Lamella thickness can be determined using sample tilt in FIB, which in turn also enables calibrating the Zeta-factors for absolute quantitative chemical analysis.

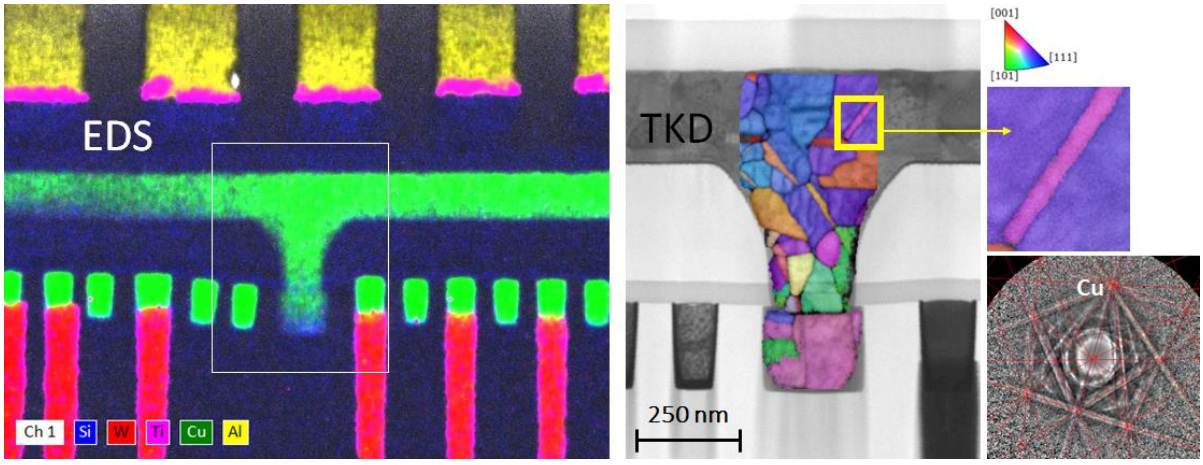

Fig. 1: Composition and crystallographic grain structure of a Cu interconnect studied at nm resolution. Note the intermixing of Si and Cu signals in EDS at the lower end of the connect.

References:
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CRYOCLOUD: A CLOUD-NATIVE CRYOEM DATA ANALYSIS PLATFORM FOR INCREASED ACCESSIBILITY, THROUGHPUT AND COLLABORATION

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CryoCloud is a cloud-native platform for cryoEM data analysis, providing solutions for fast and secure data uploads, data storage and end-to-end data analysis. CryoCloud is accessible via an intuitive, newly designed web-application that adheres to highest security standards and can be easily accessed via a web-browser - thus CryoCloud removes the need for complex IT infastructure setup and maintenance. It provides access to image analysis as well as project and data administration tasks via a user-friendly UI. Image analysis tasks are computed by fast and scalable cloud resources that are launched upon demand and have been optimized for each job, reducing running costs and charging users only for their actual runtime. Next to fast compute resources, CryoCloud provides data management solutions including tools for live data uploads and an archiving feature that moves datasets to cost-efficient cold storage at the click of a button and allows for easy temporary retrieval. CryoCloud's end-to-end workflow for single particle analysis has been successfully used by scientists in industry, academic research groups and at facilities, and has been now expanded with a workflow for cryoET data analysis as well as tools for protein structure prediction and automated model building.

VITROJET: USE CASES SHOW VERSATILITY FOR GRIDS AND SAMPLES

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The VitroJet was used in multiple labs across the world to demonstrate suitability for a variety of samples and workflows. Any grid type can be used that is pre-clipped into an Autogrid ring, accommodating for preferences in sample behavior. A stock volume of 0.5 µL is loaded into the pipette tip, while the pin only consumes 0.5 nL sample per grid. The sample can be exchanged or retained for each grid, and several case studies are presented below (Fig. 1):

I. Membrane protein (2.7 Å): The streamlined workflow enables good results for novices.
II. Nucleosome (3 Å): Less orientation bias on the VitroJet for a 3D reconstruction.
III. Fatty acid synthase (3.3 Å): With the VitroJet, a higher percentage of intact particles was observed in the absence of continuous support.
IV. Tick-borne virus: VitroJet works effectively in combination with a wide range of particle sizes, for this sample up to 18 particles per hole were detected.
V. Lipid nanoparticles: Integrated plasma cleaning on continuous support results in reproducible ice thickness for larger particles.
VI. Tobacco mosaic virus (5.4 Å): Jet-vitrification reduced the areas of non-vitreous ice and achieved the maximum theoretical STEM resolution at the used convergence semi angle.
VII. Bacteriophage: Successful deposition and vitrification of large particles with a 150 nm diameter and 120 nm tail.

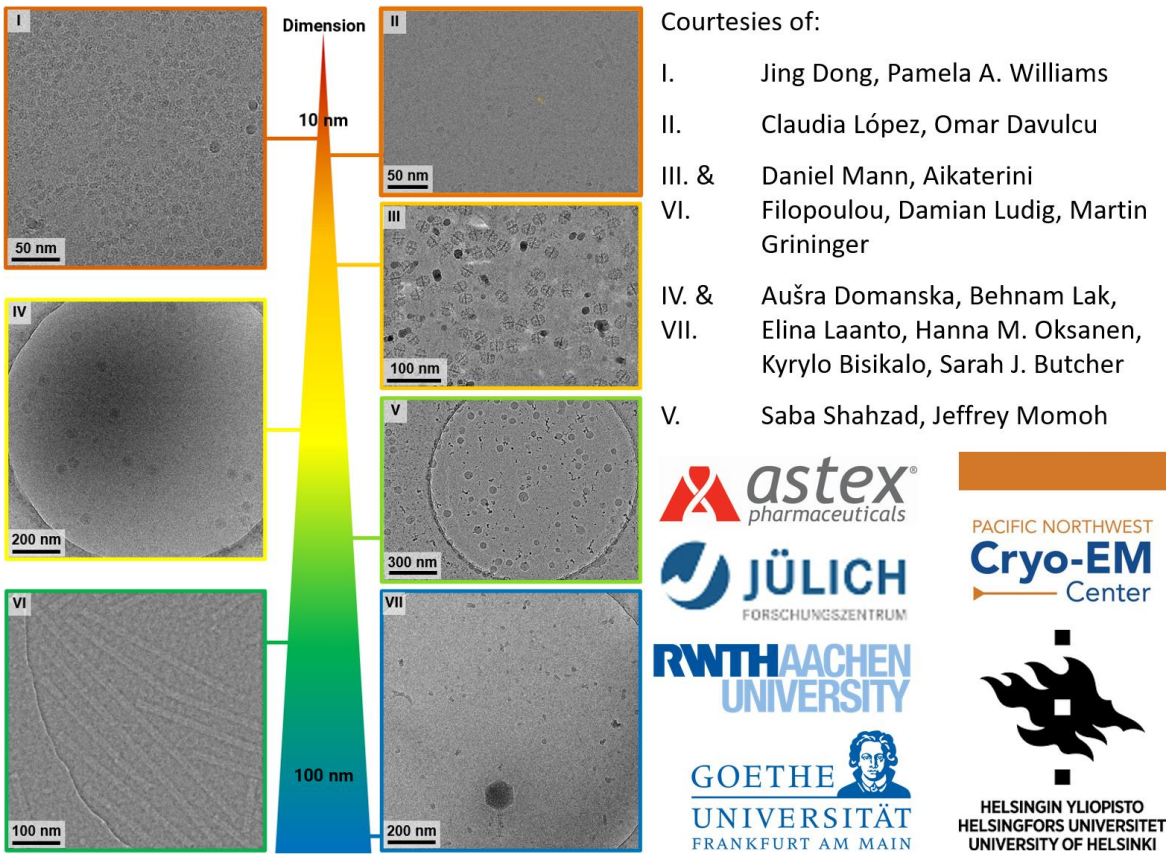

Fig. 1: VitroJet use cases demonstrate applicability with wide variety of samples

STREAMLINING GRAPHENE LIQUID CELL PREPARATION: VITROTEM'S NAIAD SYSTEM

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Graphene Liquid Cells (GLCs) offer unprecedented capabilities for imaging samples in their native hydrated state, providing insights at atomic resolution [1]. However, manual assembly of GLCs presents significant challenges, limiting their widespread adoption. This poster presents VitroTEM's Naiad system, a revolutionary approach to GLC preparation. By employing a layered structure consisting of monolayer graphene sheets on standard TEM grids, the Naiad system rapidly constructs GLCs, encapsulating liquid samples for imaging [2]. Our poster showcases images of ferritin particles in their native environment, demonstrating the system's efficacy in biological materials research. Additionally, we present atomic resolution images of Au nanoparticles, highlighting its utility in nanomaterial science. The Naiad system simplifies GLC assembly, enabling researchers to focus on sample imaging rather than grappling with graphene preparation. This poster emphasizes the Naiad system's potential to accelerate discoveries in diverse fields reliant on high-resolution imaging of liquid-phase samples.

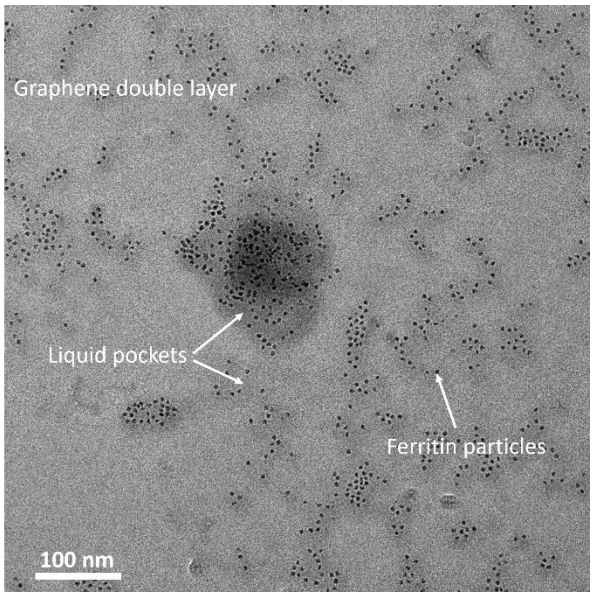

Fig. 1: Ferritin particles encapsulated in GLC pockets.

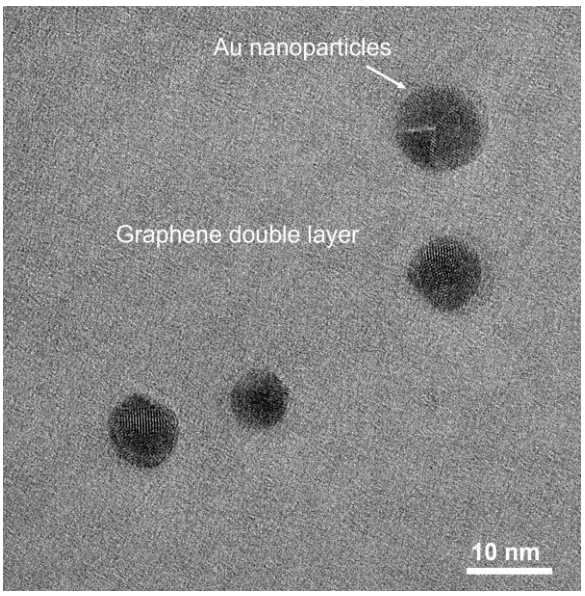

Fig. 2: High resolution TEM image of Au nanoparticles encapsulated in a very thin GLC pocket.

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STABLE CRYOGENIC IN SITU BIASING AND HEATING SYSTEM FOR ATOMIC RESOLUTION (S)TEM

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The application of cryogenic TEM (cryo-TEM) methods to Materials Science and energy-related fields has been mainly limited to beam sensitive materials like lithium-ion batteries, organic semiconductors, pervoskite-based solar cells, polymers, Metal Organic Frameworks (MOFs), etc. Despite their widespread use, grid-based cryogenic systems suffer from poor stability, making it challenging to obtain adequate atomic-resolution imaging conditions and, are time-consuming [2]. Additionally, these systems often have the inability to set intermediate temperatures, which limits the experiments to be performed at either liquid nitrogen temperature or room temperature. Furthermore, such systems are limited in applications when understanding the structure, electronic and transport properties of materials under an applied electrical stimuli at low temperatures is of interest. For applications like quantum materials, magnetic materials and nanostructures, ferroelectrics, a system with low sample drift that combines liquid nitrogen cooling with an external voltage bias is needed.

Here we will present our latest developments with respect of a combined in situ cooling, biasing and heating system. The system includes a novel double-tilt cryogenic holder which is MEMS-chips based, and uses liquid nitrogen for cooling. It has multiple electrical contacts and is compatible with DENSsolutions heating and biasing Nano-Chips, allowing to continuously control the temperature of the sample from -175^{o}C to +800^{o}C while keeping the holder in a cooled state. Without the presence of the cooling agent, it is also possible to perform heating and/or biasing experiments up to 800^{o}C. By exploiting the high stability of this system and its double tilt capability, it is possible to achieve atomic resolution imaging while applying a bias to the sample and/or heating over almost 1000^{o}C temperature range.

A NEW WORKFLOW FOR MANAGING LARGE *IN SITU* DATASETS FROM HIGH FRAME RATE TEM DATA

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The dynamics of materials studied using in situ techniques can evolve quite rapidly, such as the sintering of nanoparticles and some phase transformations. High speed TEM cameras have played an important role in capturing these rapid events, with the latest generation operating at 4K x 4K and 50 fps [1], and much faster as resolution is reduced. Because the exact onset of these events is often unknown, features including Gatan's LookBack™ allow the user to record a buffered history of data to prevent missing the event entirely. If data is captured at full resolution, even sporadic recording at high frame rates can easily result in TBs of saved data. Reviewing this dataset in its entirety after a session is a very challenging task, especially when the exact moments of interest many only be an extremely small subset of the data recorded. This poster will present a new product, AXON High Frame Rate™, which allows a user to open very large datasets in their entirety to quickly locate the most important frames on a large timeline. Furthermore, data that has no value can quickly be identified and deleted, reducing the overall size and increasing the value of saved information. Using this revolutionary new workflow combining Gatan™ IS cameras and Protochips AXON, the poster will show the sintering process of Au nanoparticles isolated for analysis within a dataset ~2TB in size.

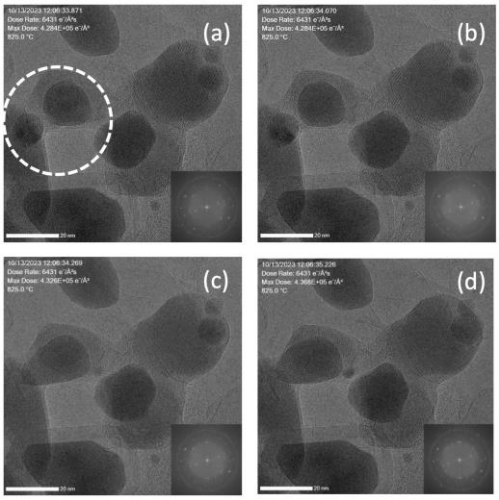

Fig. 1: The evolution of two Au NPs sintering (a-d) highlighted in the dashed circle.

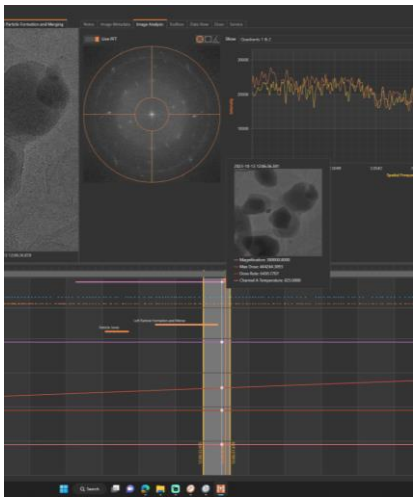

Fig. 2: A section of the AXON Studio™ software displaying the full 2TB dataset opened and the relevant section highlighted

References:

[1] https://www.gatan.com/products/tem-imaging-spectroscopy/clearview-camera

LIQUID HELIUM TEM SAMPLE HOLDER: SWIFT COOL-DOWN AND LONG HOLDING TIME

Sutter, Denys[1*], Lu, Peng-Han[2], Kovács, András[2], Lu, Yan[2], Vas, Joseph V.[2], Faley, Michael[2], Denneulin, Thibaud[2], Estry, Amelia[1], Bucher, Damian[1], Chang, Johan[1], Biscette, Dominik[1], Dunin-Borkowski, Rafal E.[2]

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Quantum materials display a variety of unique electronic and magnetic properties—including superconductivity, charge ordering, and topological states—which are predominantly observed at cryogenic temperatures [1, 2]. Swift progress in cryogenic scanning transmission electron microscopy (S)TEM methodologies has been achieved at liquid nitrogen (LN_2) temperatures. While LN_2-cooled side-entry sample holders and cartridge-integrated microscopes are tailored to suit the demands of the life sciences, the study of phase transitions within materials science typically necessitates adjustable temperatures with a base in the liquid helium (LHe) range [3].

Historically, LHe solutions for electron microscopes were constructed in a cryo-stage setup [4-7], achieving temperatures as low as 1.5 K utilising superfluid helium alongside LN_2-cooled shields, and thus enabling high-resolution imaging over a continuous five-hour span [8]. Despite these achievements, the preference for technically versatile side-entry holders has hindered further advancements in cryo-stage development. Presently, LHe side-entry holders are limited by considerable mechanical and thermal instability, and their base temperature holding times are short due to LHe's low latent heat and the limited cryogen storage capacity of the dewar attached to the holder. Efforts to extend cryogenic holding periods have led to integrating a commercial LHe continuous flow cryostat into a modified 60 mm pole piece gap following the removal of the objective lens [9]. This modification has facilitated temperature control within a range of 6.5 K to 400 K, maintainable over several days. However, the substantial alterations to the microscope structure and the requirement to vent the column for specimen loading present practical challenges for routine operation.

Here, we present recent innovations of a lightweight, ultra-low-temperature LHe TEM sample holder. Starting from room temperature, a base temperature of 5.2 K—measured adjacent to the specimen by a Cernox sensor—can be attained within one minute and sustained for days with a temperature stability of +/- 2.5 mK. Initially designed for X-ray diffraction studies of quantum matter in pulsed magnetic fields, condenZero, a spin-off company from the University of Zurich, has adapted their miniaturisable cryostat design for cryo-TEM usage. Collaborative efforts with the ER-C at the Research Centre Juelich have led to additional enhancements and optimisations. Here, we demonstrate the capabilities of our latest LHe cryo-TEM setup.

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DYNAMICAL STUDIES COMBINING *IN SITU* 4D-SPED MAPPING AND PIXELATED DETECTORS

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Automatic crystal orientation & phase mapping (ASTAR technique) can be applied in a variety of materials, is known also as 4D-STEM technique [1, 2]. We apply a dedicated acquisition process (named as 4D-SPED "scanning Precession Electron Diffraction") where beam precession (to improve ED patterns quality [3,4]) in TEM is used in combination with beam scanning. ASTAR technique is very similar to the traditional EBSD-SEM method, where the major advantage is the significant improved spatial resolution that can be down to 1-3 nm (in case of FEG-TEM).

We have studied many advanced materials using 4D –SPED ASTAR, to investigate local properties and/or study of nanosized samples. In addition, *in situ* methods have been extensively applied towards dynamical studies. The combination of the 4D-SPED ASTAR with the new generation of pixelated detectors, allows the use of extremely low electron dose (up to 0.001 e/Å2/sec) with significantly fast acquisition times (500-1000fps). Combining pixelated detectors (like QD & ASI Medipix,Dectris,PnCCD etc…) with NanoMEGAS 4D-SPED ASTAR system, not only acquisition time is greatly reduced, but more critically, data quality is enhanced, allowing in situ dynamical studies by sequential mapping even for beam sensitive samples.

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APPLICATION OF FRAME-BASED AND EVENT-BASED DIRECT ELECTRON DETECTORS IN ELECTRON MICROSCOPY: MERLINEM, CHEETAH M3 AND CHEETAH T3

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Hybrid pixel direct electron detectors (HPDEDs) are quickly becoming ubiquitous in modern electron microscopy (EM) applications, owing to their high detective quantum efficiencies (DQEs), readout speeds, dynamic ranges and radiation hardnesses. HPDEDs can be distinguished by their readout mode: the MerlinEM and CheeTah M3 are frame-based detectors, and the CheeTah T3 offers event-based detection capability. Here, we explore the advantages and example applications of both readout modes.

HPDEDs have enabled 4D scanning transmission EM (4D STEM), a data collection protocol in which the entire diffraction pattern at each probe position is measured. This generates datasets that are ripe for a host of data processing algorithms and pipelines, facilitating extraction of important sample information: from measurement of electromagnetic fields [1], to ptychographic reconstruction [2], and to measurement of electron energy loss spectroscopy (EELS) data when coupling the detector with an energy filter [3]. Studies report that the MerlinEM and CheeTah T3 are capable of tolerating 10^6 e/pixel/s [4] and 600 e/pixel/s [5], respectively. This suggests that frame-based detectors are best suited for measuring intense features such as the central disc or zero-loss peak in 4D STEM EELS. Moreover, this extends to the measurement of 3D electron diffraction (3D ED) or MicroED data for the elucidation of the sample crystal structure (Fig.1). Conversely, the promise of achieving traditional STEM scan speeds by event-based detection is realised under sparse imaging conditions, implying suitability for ultrafast low-dose experiments, including cryo-EM [6] and Lorentz imaging of optical fields [7] (Fig. 2).

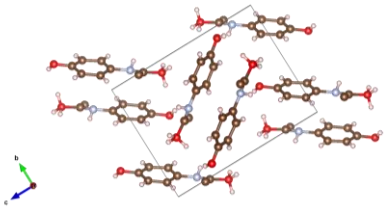

Fig. 1: Structure of paracetamol solved using data collected with MerlinEM

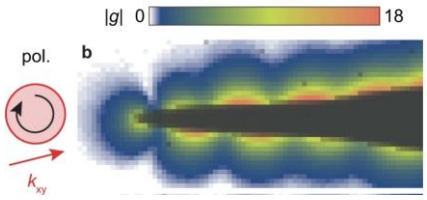

Fig. 2: Map of the magnitude of the near-field coupling coefficient for a specific polarisation [7].

References:
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ROAD MAPS AND FLOOR PLANS

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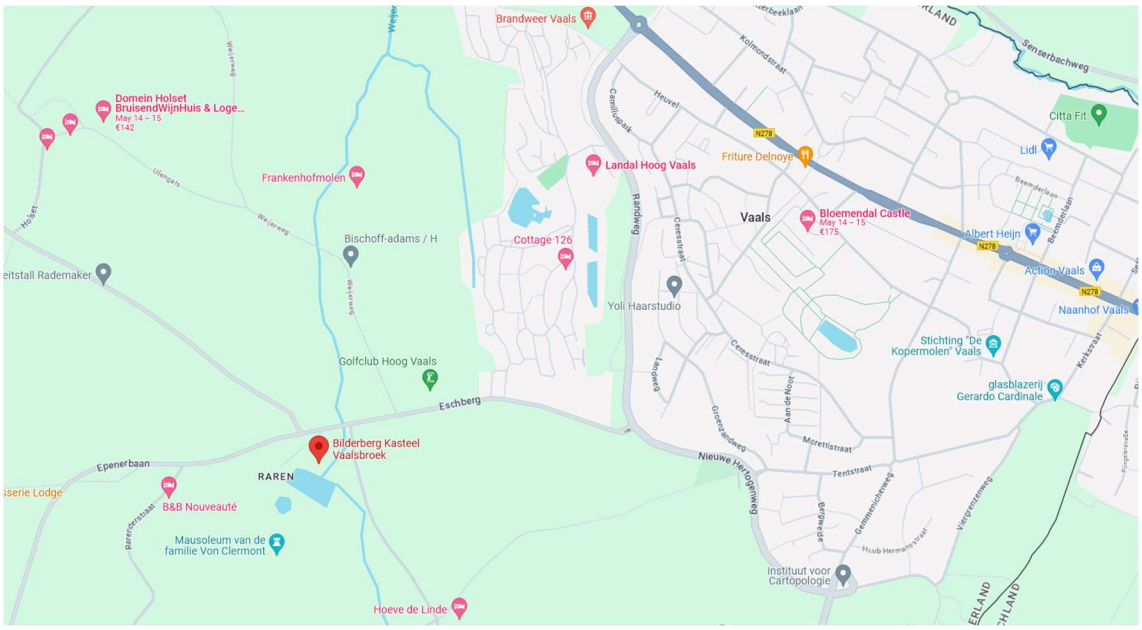
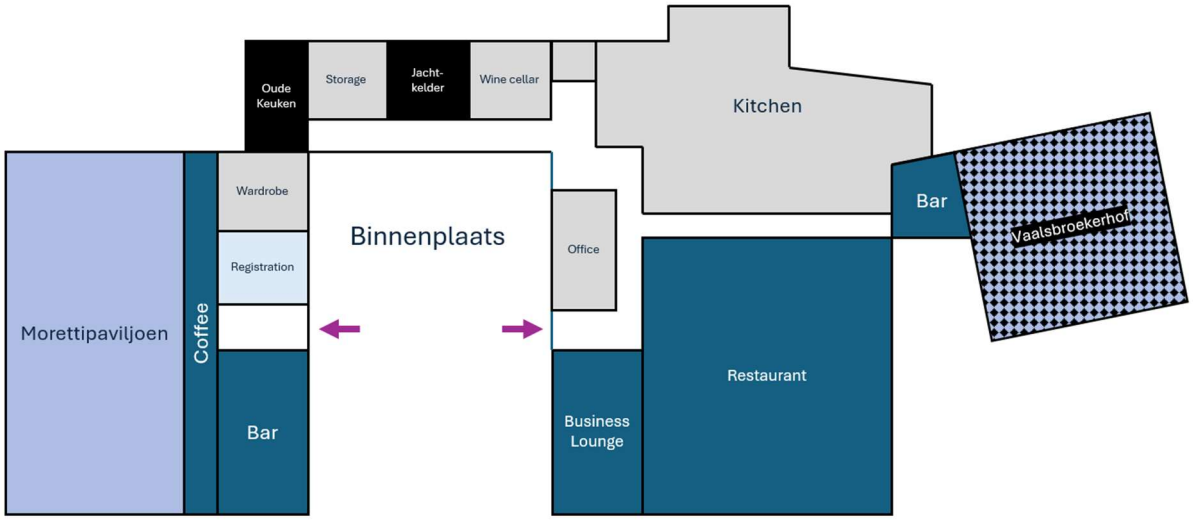
ROAD MAP

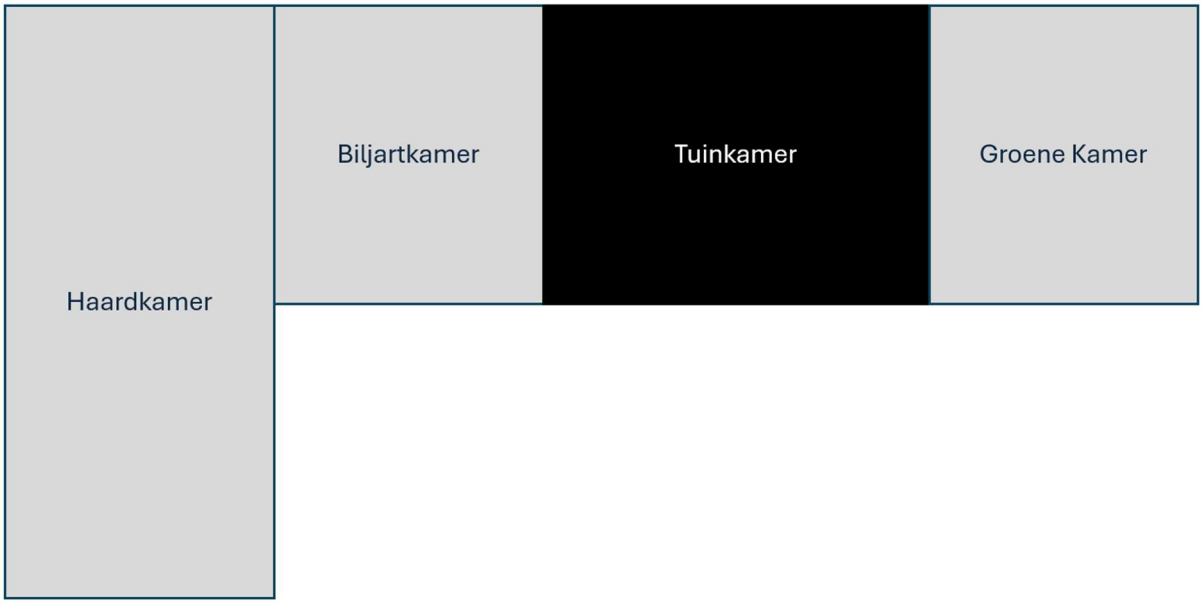


FLOOR PLAN

GROUND FLOOR



FIRST FLOOR



SECOND FLOOR