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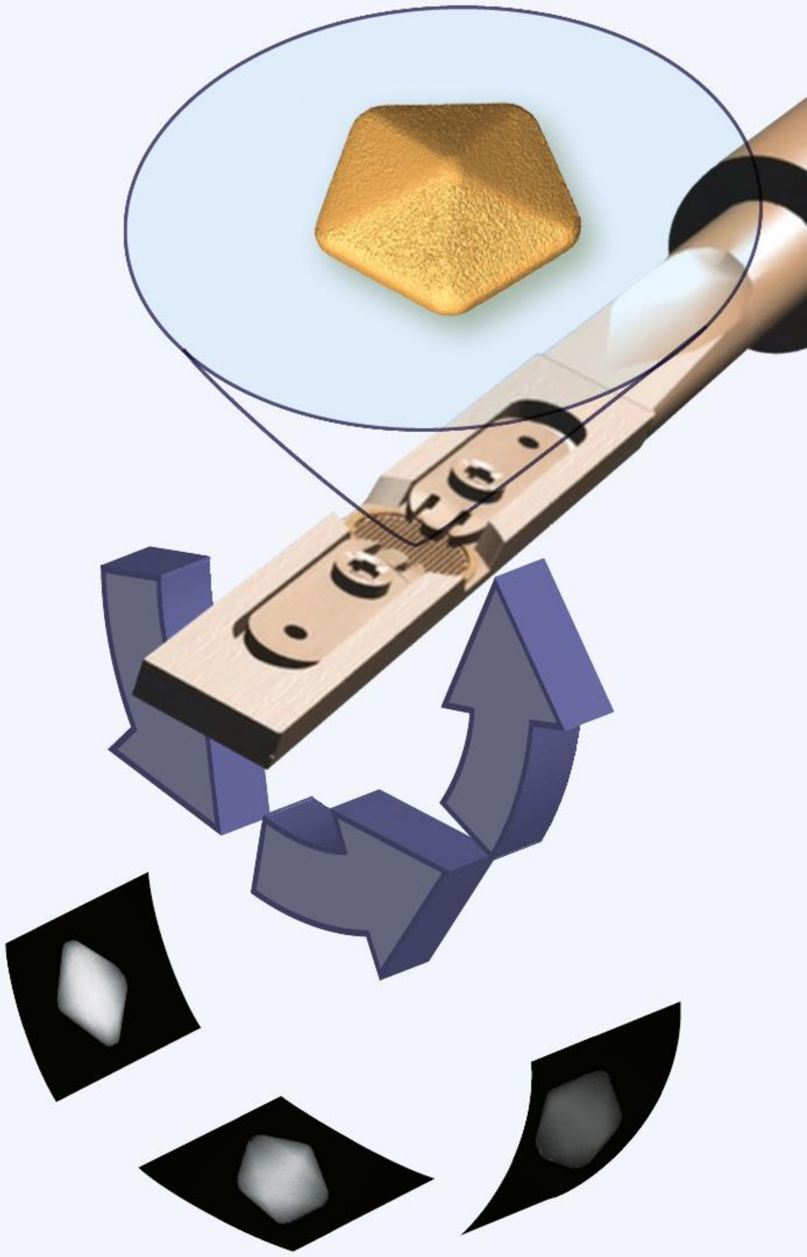
ELECTRON TOMOGRAPHY

From 2D to 3D

Robin Girod, Sara Bals

16.05.2025 – QEM2025



Electron tomography: what you'll learn

- Fundamental principle of tomographic reconstruction

Why it works and why we need it

- The basic electron tomography workflow (with practical insights)

How to go from a sample to a reconstruction

- A (brief) review of advanced methods

Insights into the range of analyses enabled by tomography (for the physical sciences)

→ Extra practical at 2pm: make your own reconstruction with Tomviz!

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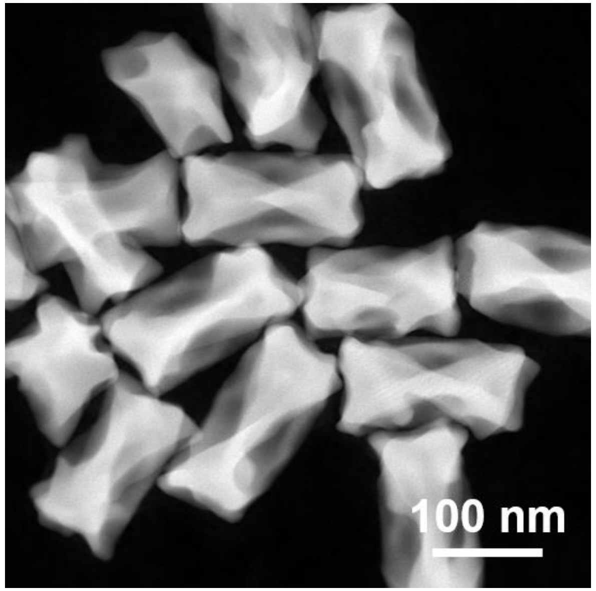
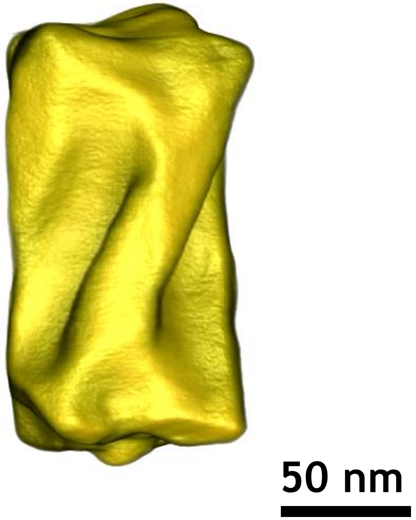
Why 3D?

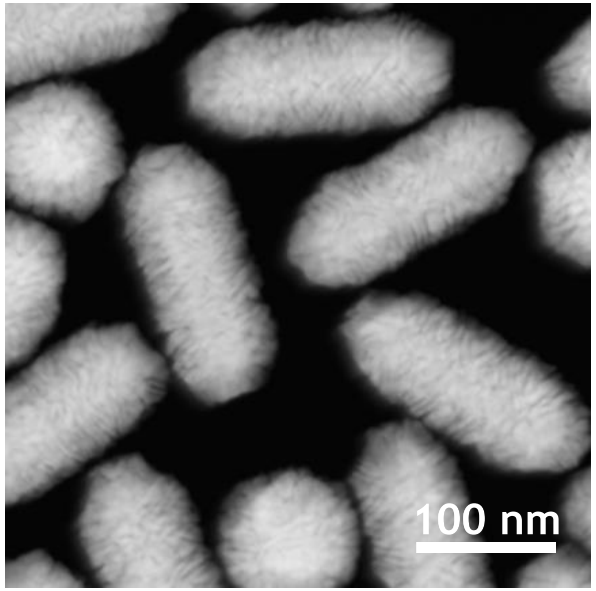
A 2-dimensional projection of a 3-dimensional object can be very misleading

Why 3D?

2D images are not always enough to understand complex shapes, interactions, composition at the nanoscale





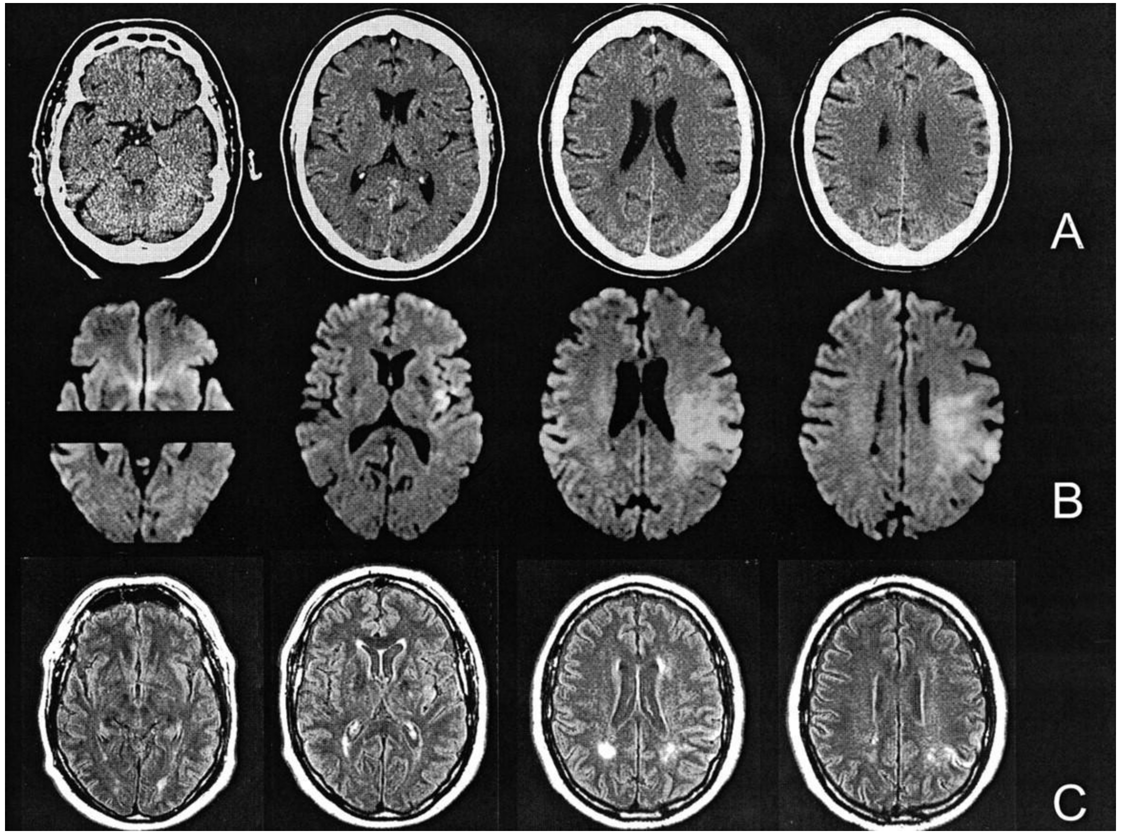


Van Gordon, Angew. Chem. (2024)

Tomography in (almost) everyday life

Tomos (Greek): slice, section

Tomography is a technique for imaging by **sections**



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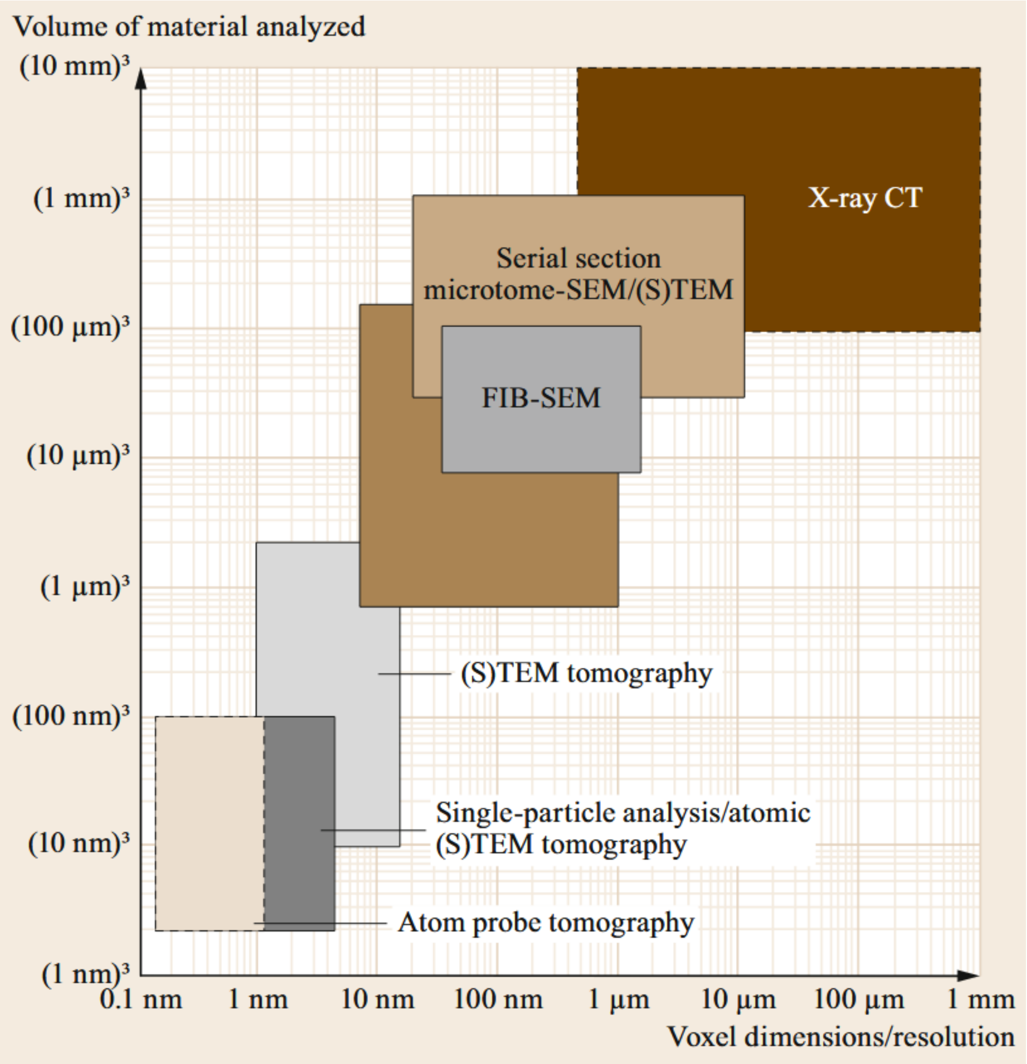
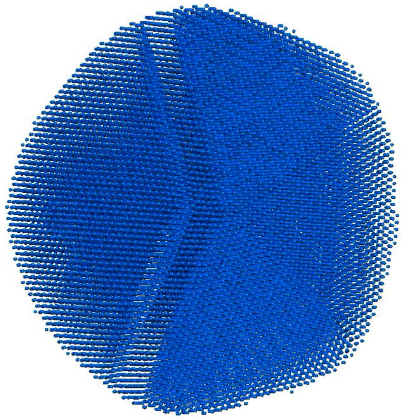
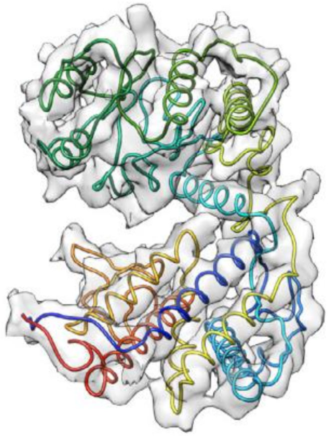
Why (S)TEM?



Down to atomic resolution

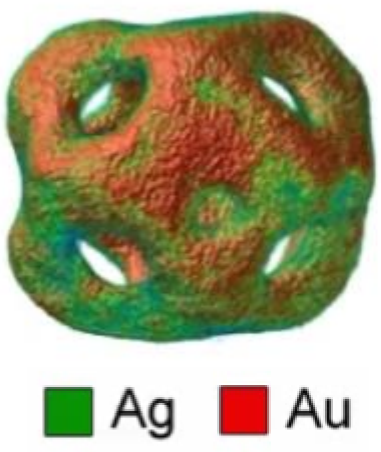


Goris, Nano Lett. 15 (2015)

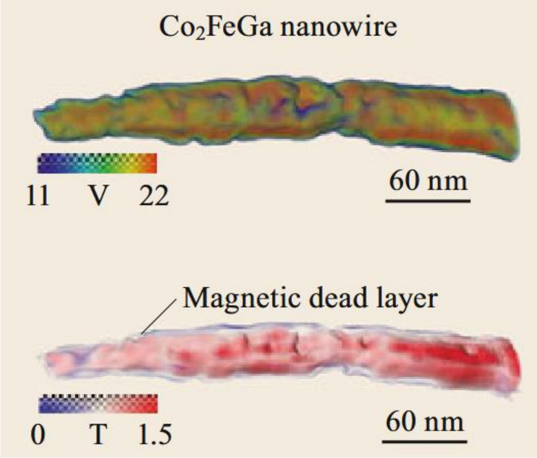


Chen, J. Struct. Bio. 196 (2016)

Composition & more



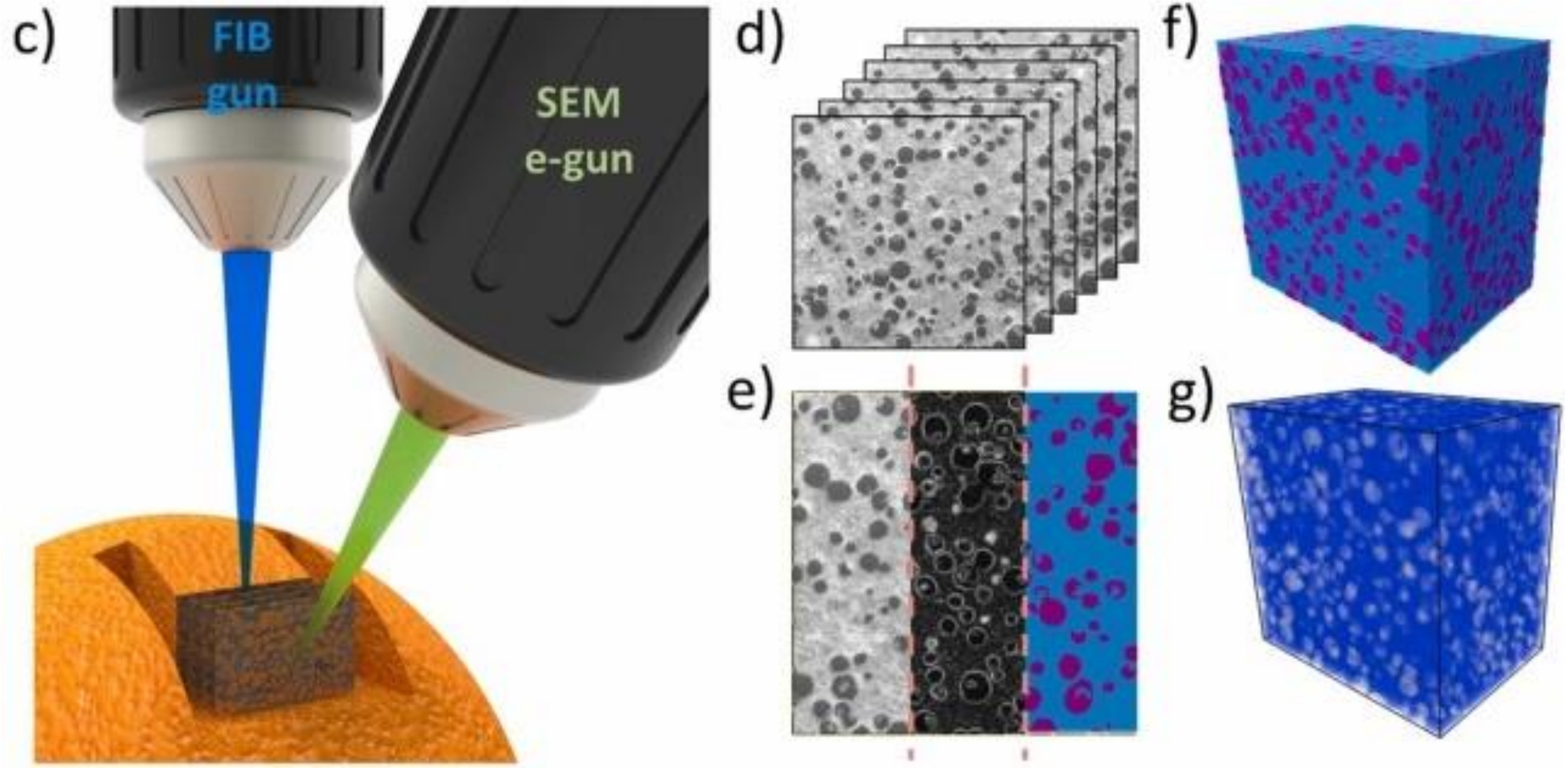
Goris, Nano Lett. 14 (2014)



Simon, Nano Lett. 16 (2016)

Electron tomography: two ways

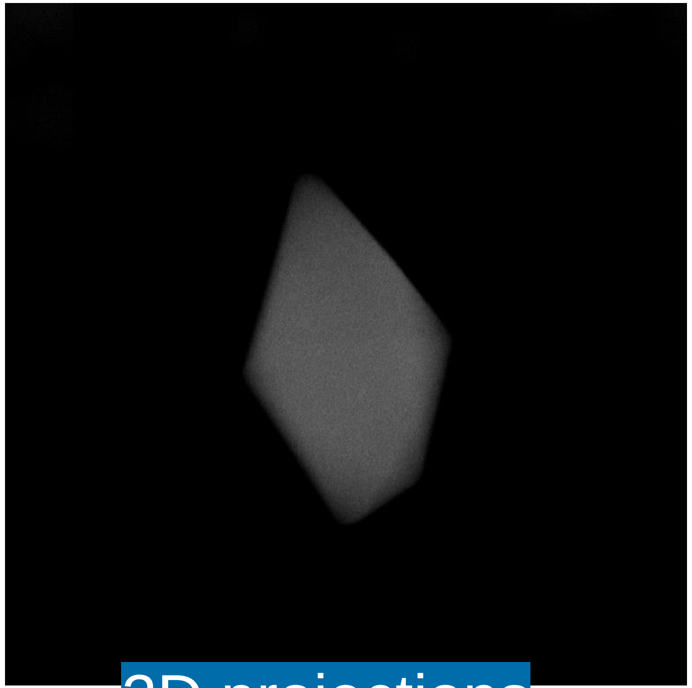
Using Focused Ion Beam – Scanning Electron Microscopy



Rodenas, T. & Prieto, G. *Catalysis Today* **405–406**, 2–13 (2022)

Electron tomography: two ways

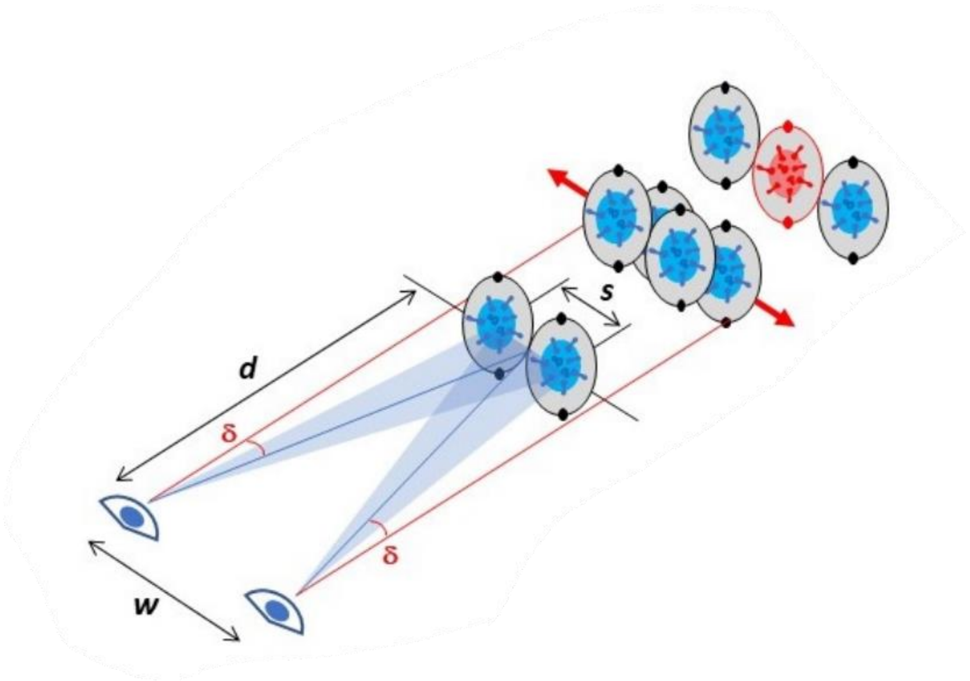
Using TEM images

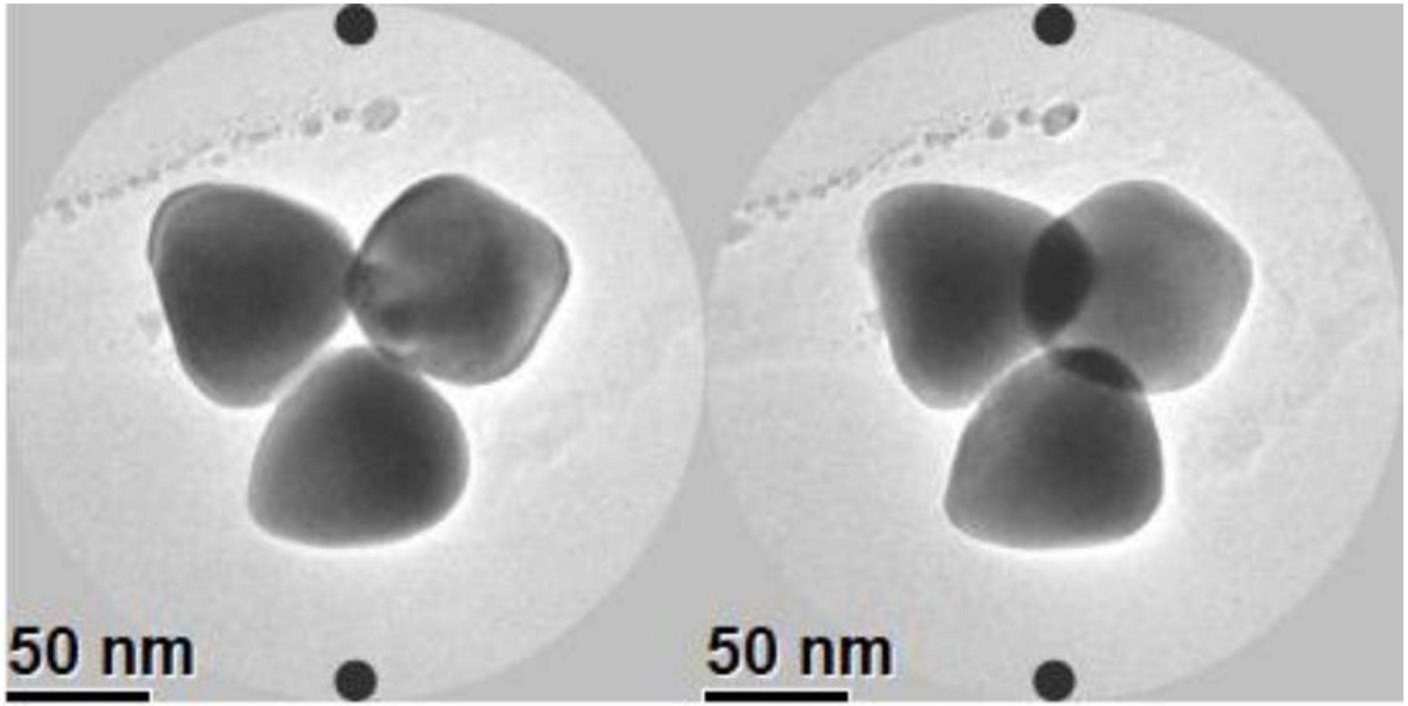


2D projections

Electron tomography: two ways

What about stereo imaging?

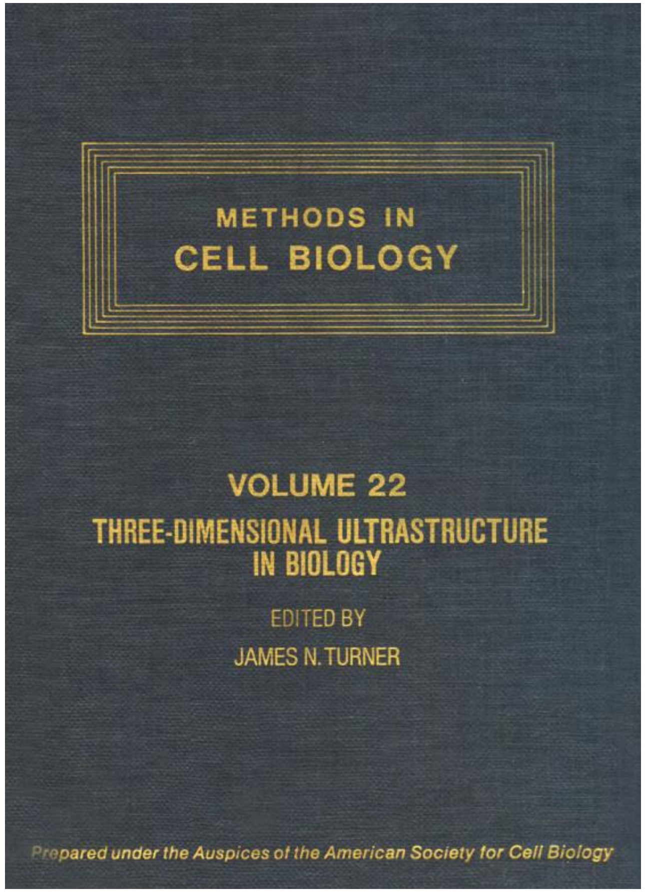




Potapov, P. & Sturm, S., *Simplest stereo view of TEM images*, 2023

Electron tomography: two ways

What about stereo imaging? (in 1981)



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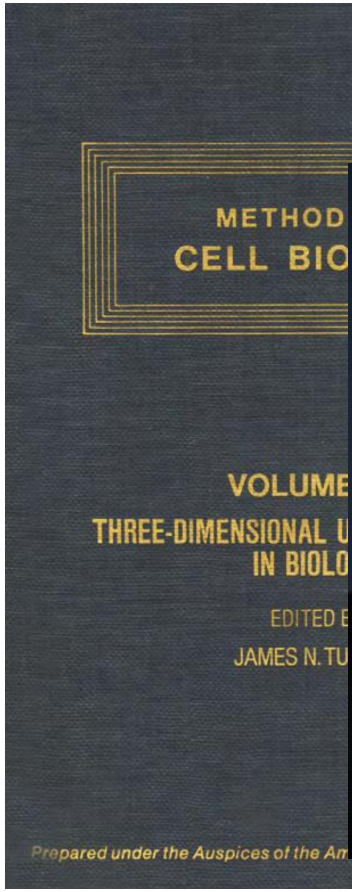
Electron tomography: two ways

What about stereo imaging? (in 1981)



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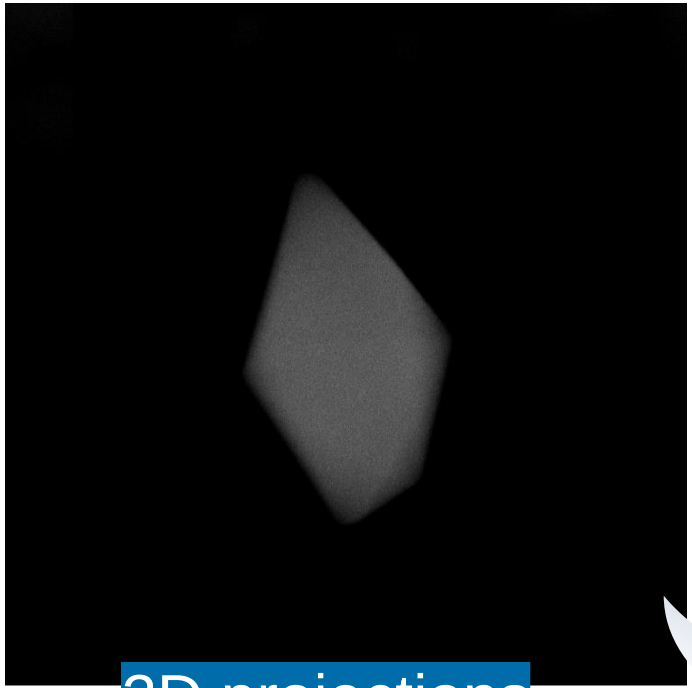
"Joachim Frank developed a method for analyzing and merging blurry two-dimensional images of the electron microscope into a sharp three-dimensional image"

- Nobel Prize in Chemistry 2017

Introduction

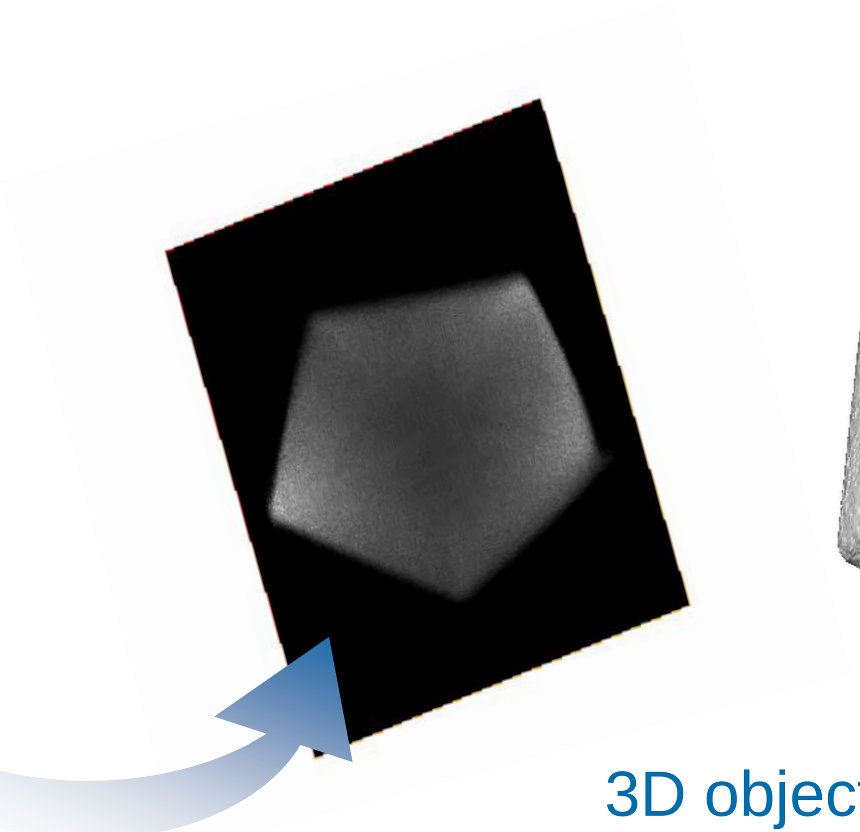
Electron tomography: two ways

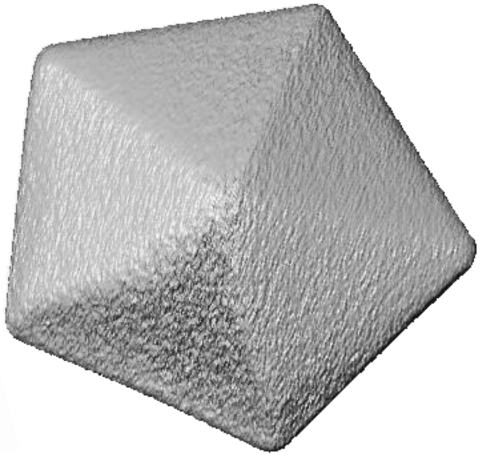
Using TEM images



2D projections

Mathematical algorithm





3D object

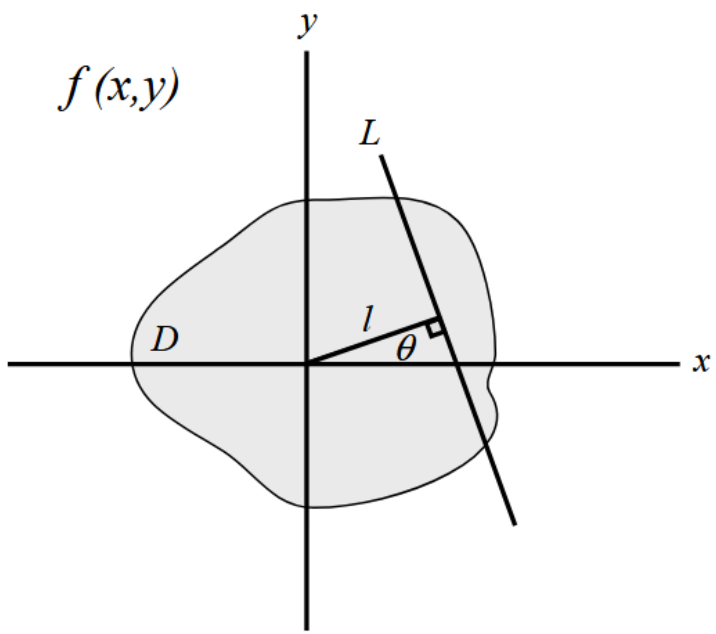
Foundations of tomography

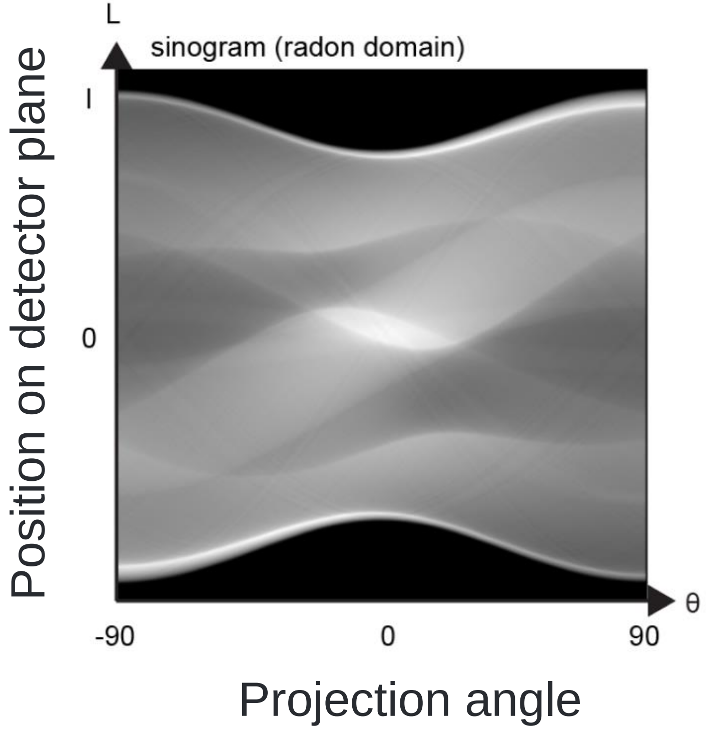
The **Radon transform**

$$Rf = \int_l f(x,y)\,\mathrm{d}s.$$

maps an object $f(x,y)$ to the **Radon space** (l, θ)

➡ **An object and its projections are equivalent under a mathematical transform**





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J. Radon. Math.Phys. Kl. 69 (1917) 262.

Early applications of the Radon principle

TWO–DIMENSIONAL AERIAL SMOOTHING IN RADIO ASTRONOMY

By R. N. Bracewell*

[*Manuscript received January* 27, 1956]

Radio telescope

CT scanner

JOURNAL OF APPLIED PHYSICS VOLUME 34, NUMBER 9 SEPTEMBER 1963

Representation of a Function by Its Line Integrals, with Some Radiological Applications

A. M. Cormack
Physics Department, Tufts University, Medford, Massachusetts
(Received 28 January 1963; in final form 26 April 1963)

[54] METHOD OF AND APPARATUS FOR EXAMINING A BODY BY RADIATION SUCH AS X OR GAMMA RADIATION

[75] Inventor: **Godfrey Newbold Hounsfield,** Newark, England

[73] Assignee: **EMI Limited,** Hayes, England

[22] Filed: **May 9, 1974**

Introduction

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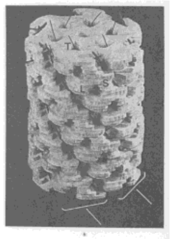
The beginning of electron tomography

Reconstruction of Three Dimensional Structures from Electron Micrographs



D. J. DE ROSIER & A. KLUG

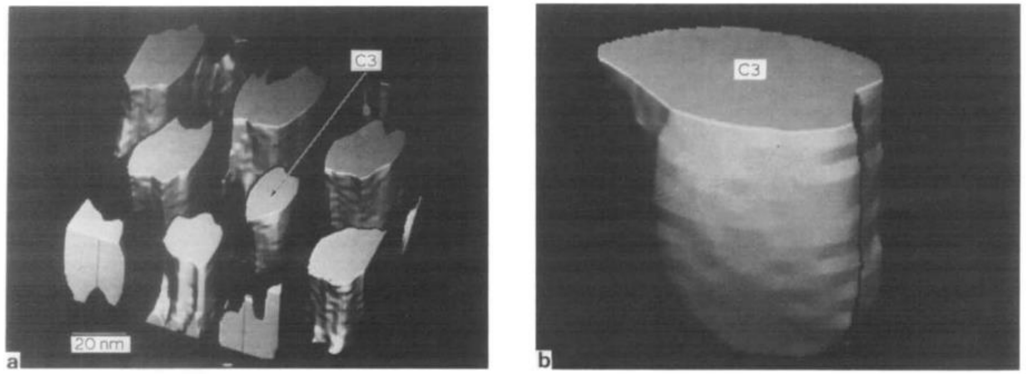
Nature **217**, 130–134 (1968) | Cite this article

1988

Polymer paper

Three-dimensional study of cylindrical morphology in a styrene-butadiene-styrene block copolymer

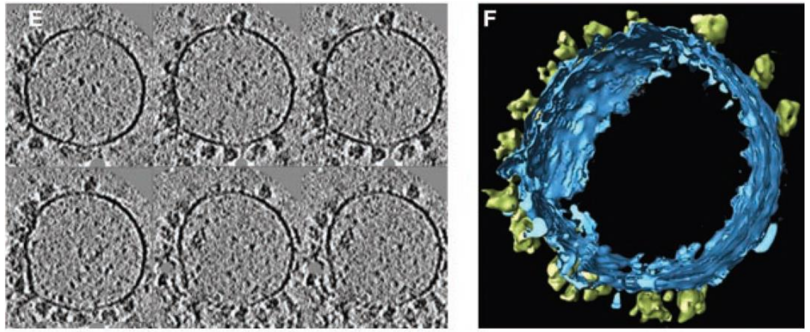
Richard J. Spontak, Michael C. Williams, David A. Agard



Macromolecular Architecture in Eukaryotic Cells Visualized by Cryoelectron Tomography

OHAD MEDALIA, IGOR WEBER, ACHILLEAS S. FRANGAKIS, DANIELA NICASTRO, GÜNTHER GERISCH, AND WOLFGANG BAUMEISTER Authors Info & Affiliations

SCIENCE • 8 Nov 2002 • Vol 298, Issue 5596 • pp. 1209-1213 • DOI: 10.1126/science.1076184



ultramicroscopy

3D electron microscopy in the physical sciences: the development of Z-contrast and EFTEM tomography

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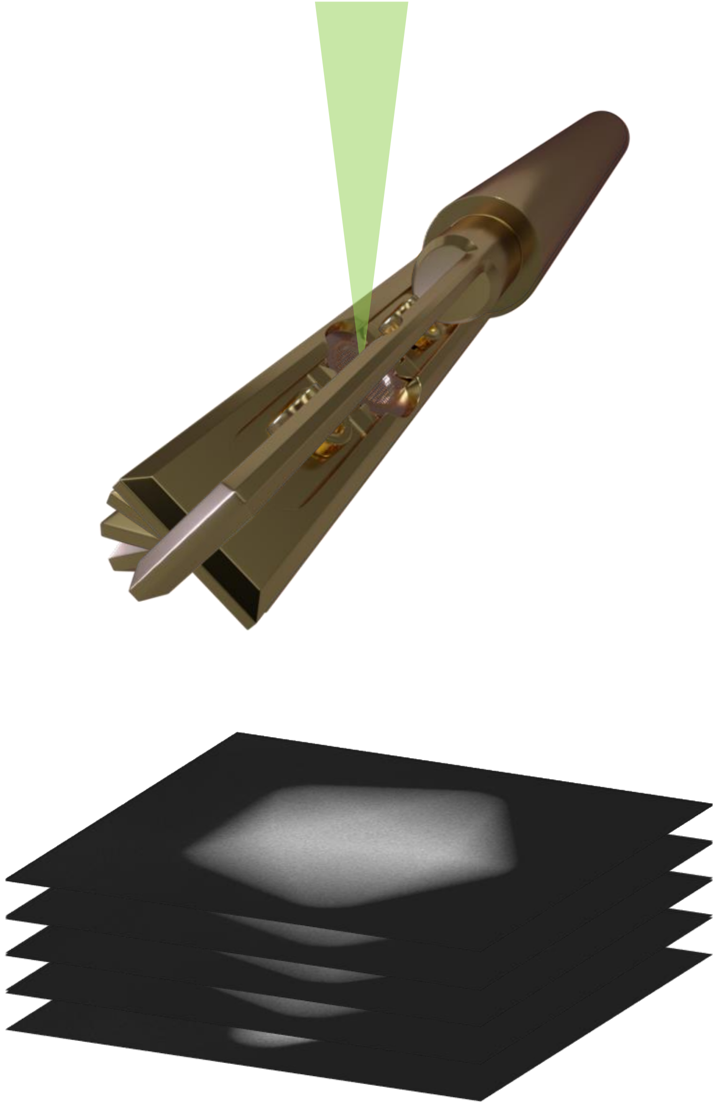
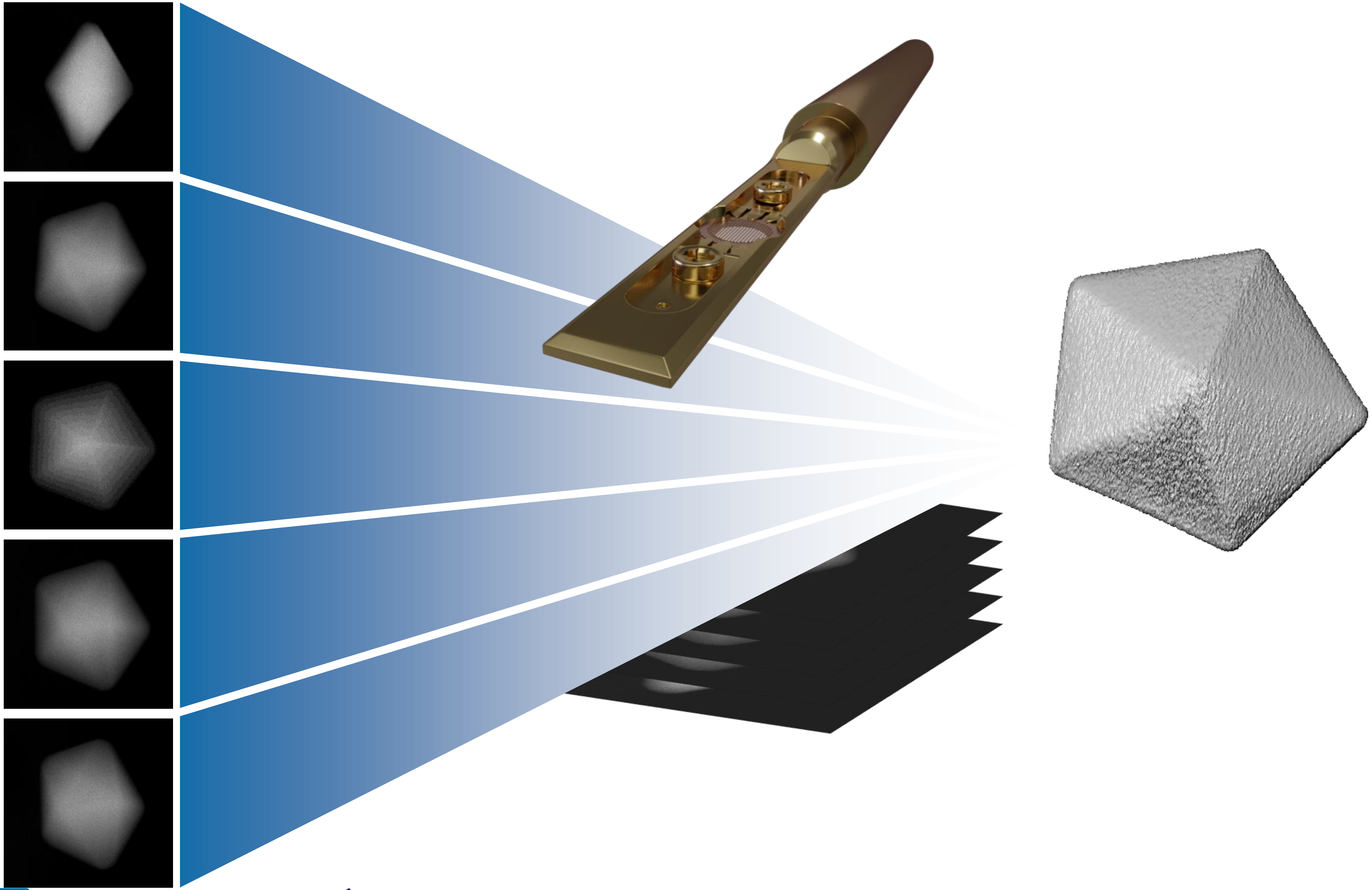
Department of Materials Science and Metallurgy, University of Cambridge, Pembroke Street, Cambridge CB2 3QZ, UK

Received 6 August 2002; accepted 27 October 2002

Basic principle

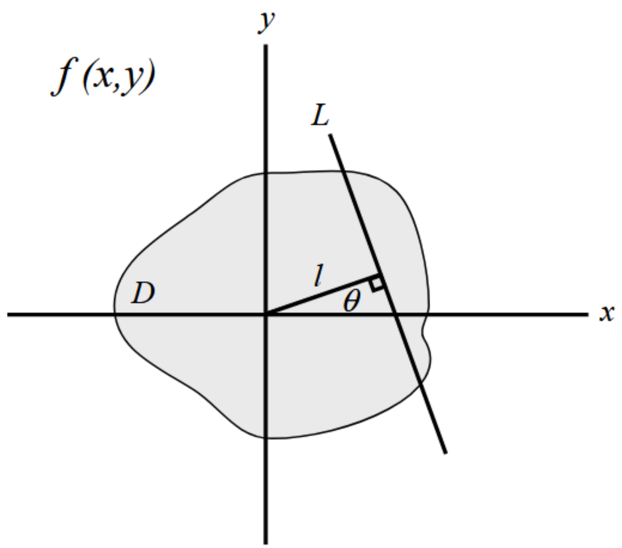


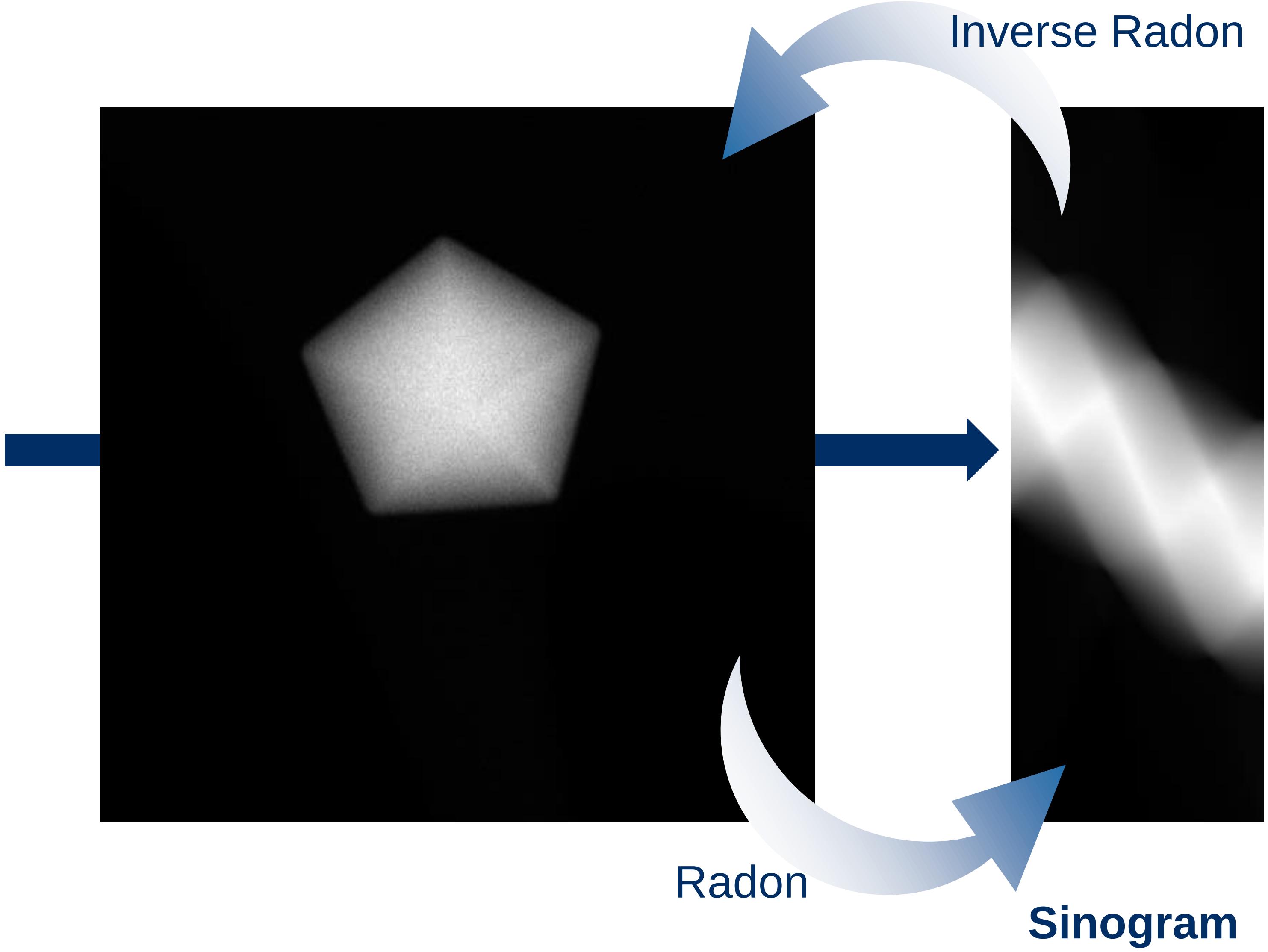


Reconstruction: intuitions from the Radon transform

Radon transform

$$Rf = \int_L f(x, y)\, \mathrm{d}s.$$

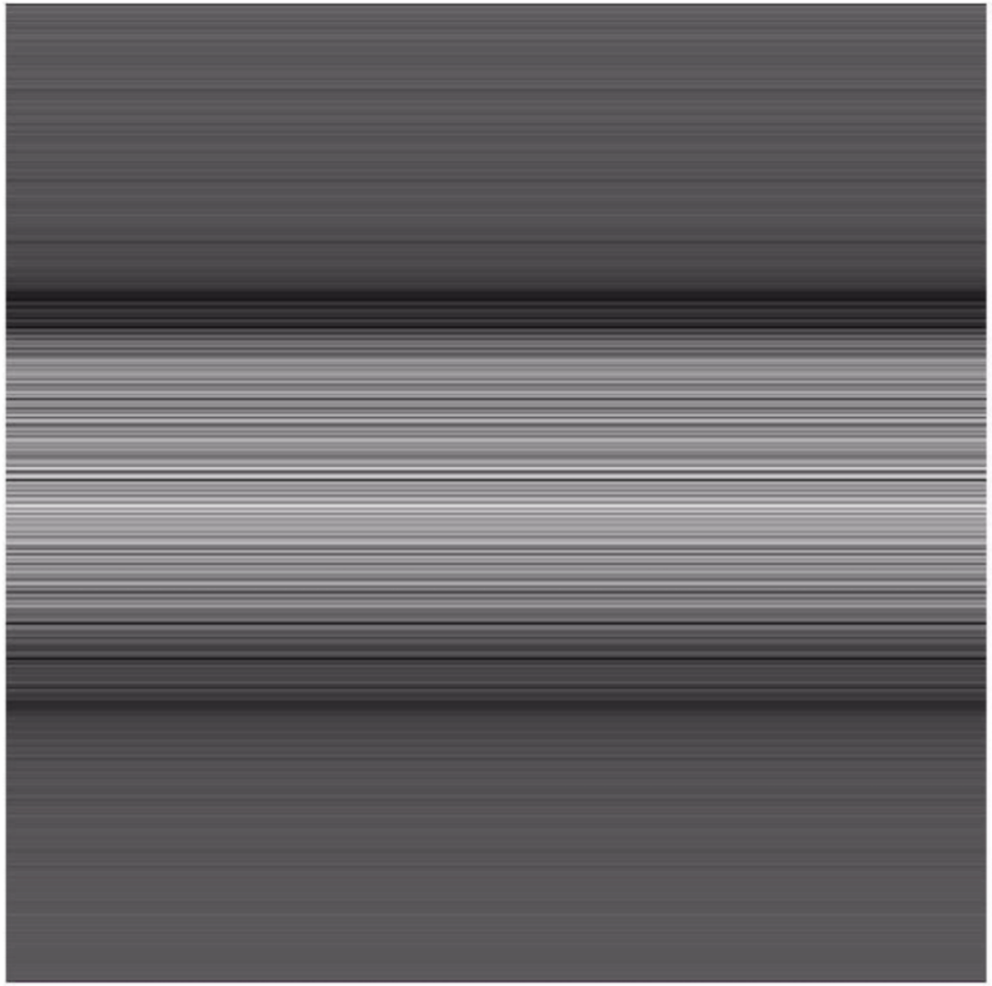




Inverting the Radon transform: the backprojection

In **real space** a direct way to inverse the Radon transform is the **backprojection** algorithm

Projections are *smeared* back into the space at the angle at which they were acquired. The summation of intensities generate the original object



angles

1

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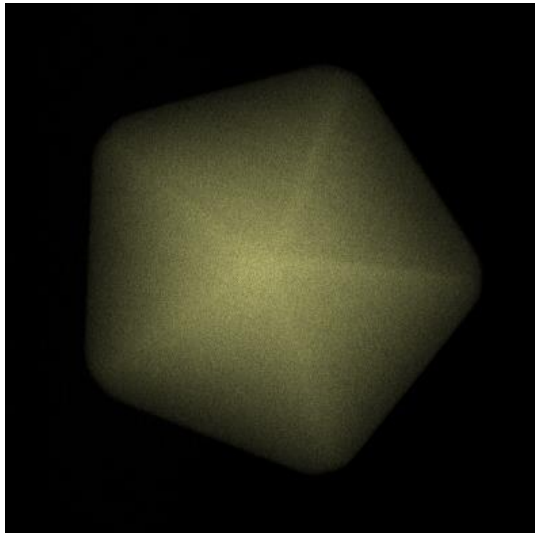
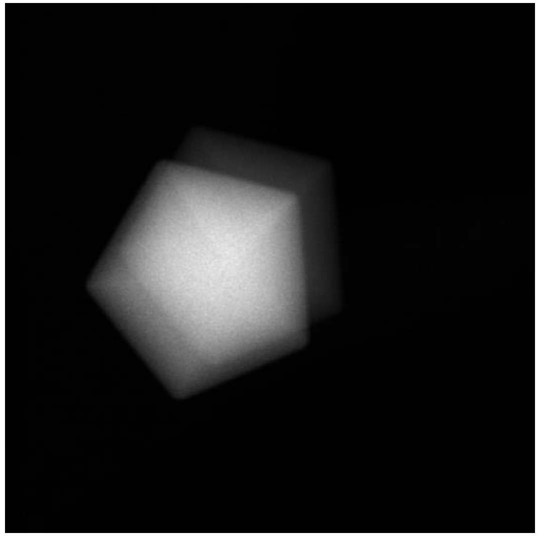
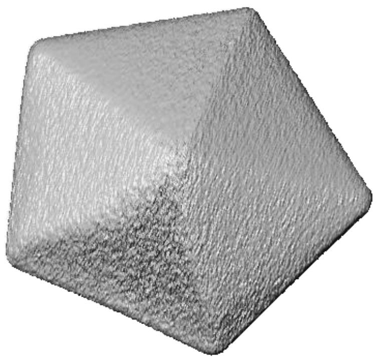
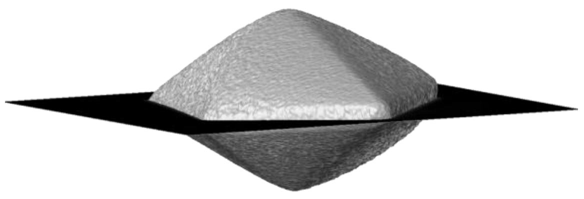
Tomography experiment

Acquisition	Alignment	Reconstruction	Quantification









Electron microscopy and projections

Radon transform

An object and its projections are equivalent under a mathematical transform

➡

The projection requirement

For tomographic reconstruction, the signal detected must be a projection of the object. That is, the image contrast should vary **monotonically** with some properties of the object: thickness, mass, density, concentration, etc.

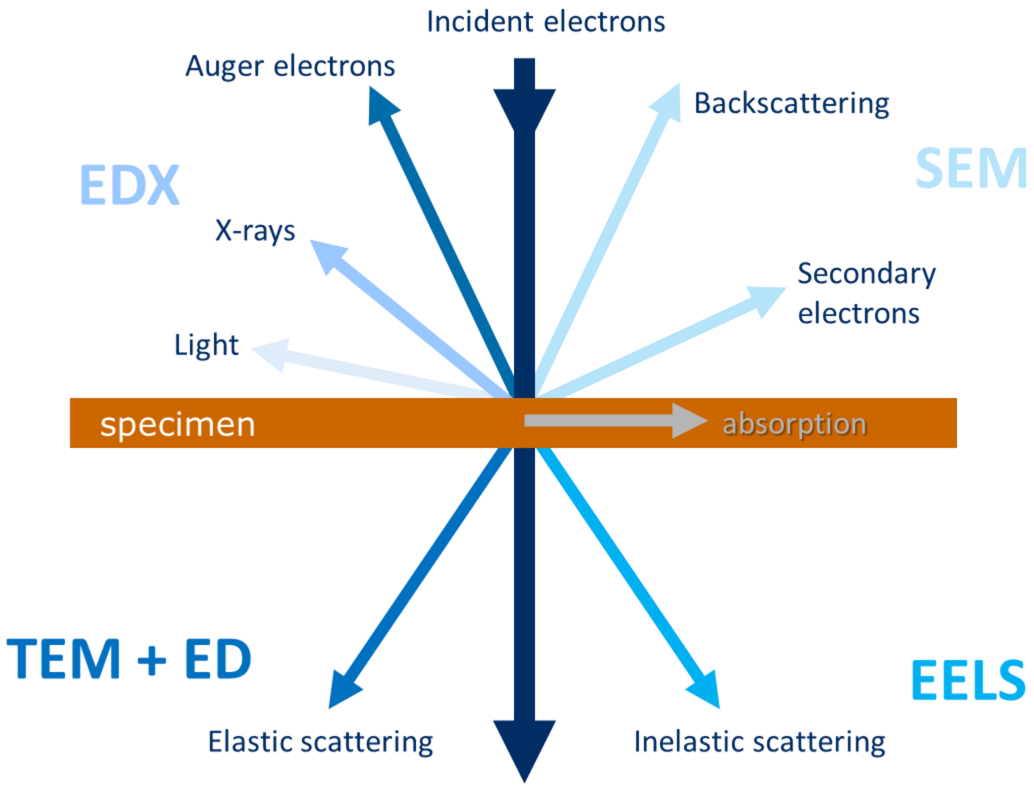
Electron microscopy and projections

Are (S)TEM images projections of the sample?

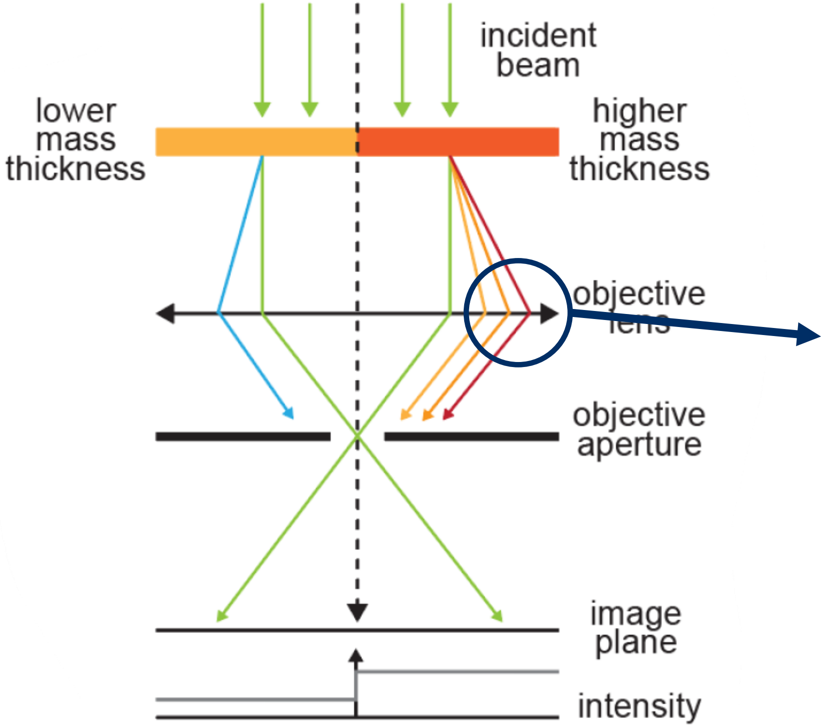
A. Yes
B. No
C. It depends ✓

The contrast mechanism in TEM images depends on …

- The electron-matter interactions that participate in the image
- The acquisition mode and parameters



Images in bright-field TEM mode (amplitude contrast)



Bragg (diffraction) scattering

- Elastic
- Coherent
- Occurs at specific tilt angles in crystalline specimen

Rutherford scattering

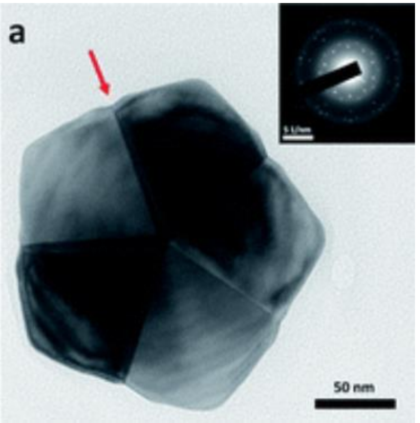
- Elastic
- Incoherent
- Scattering cross section ~$tZ^{1.7}$

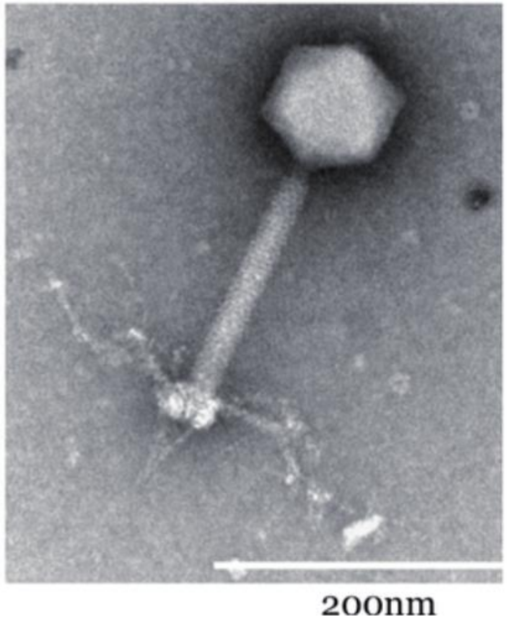
Also, diffraction contrast changes with defocus, delocalization of information, etc.

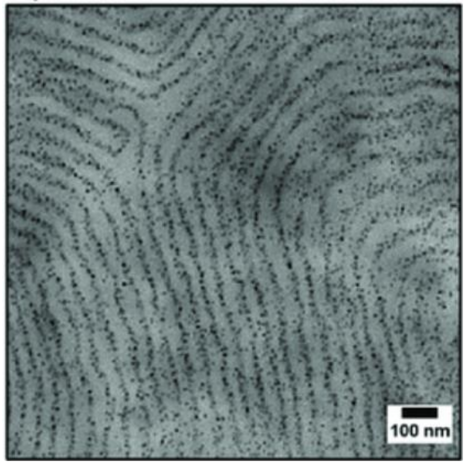
Images in bright-field TEM mode

For which object(s) will the projection requirement be fulfilled in BF-TEM?

A. A virus ✓
B. A gold nanoparticle
C. A polymer network ✓



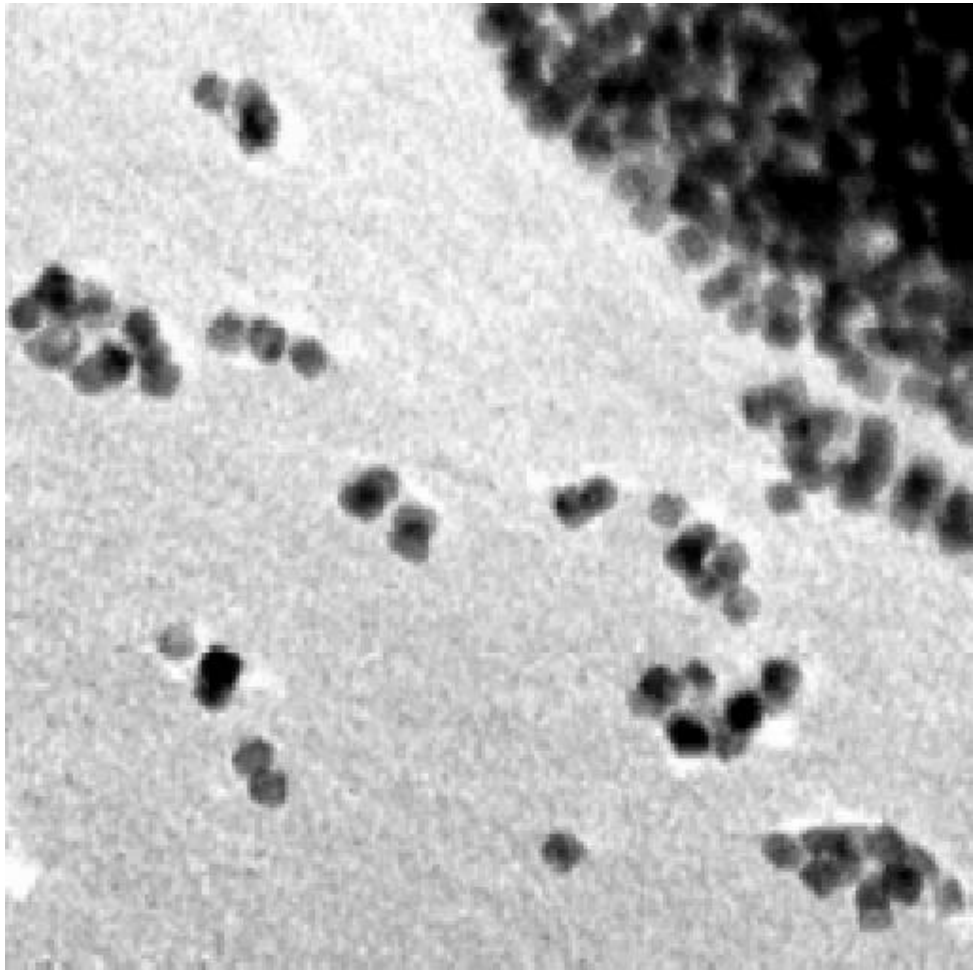


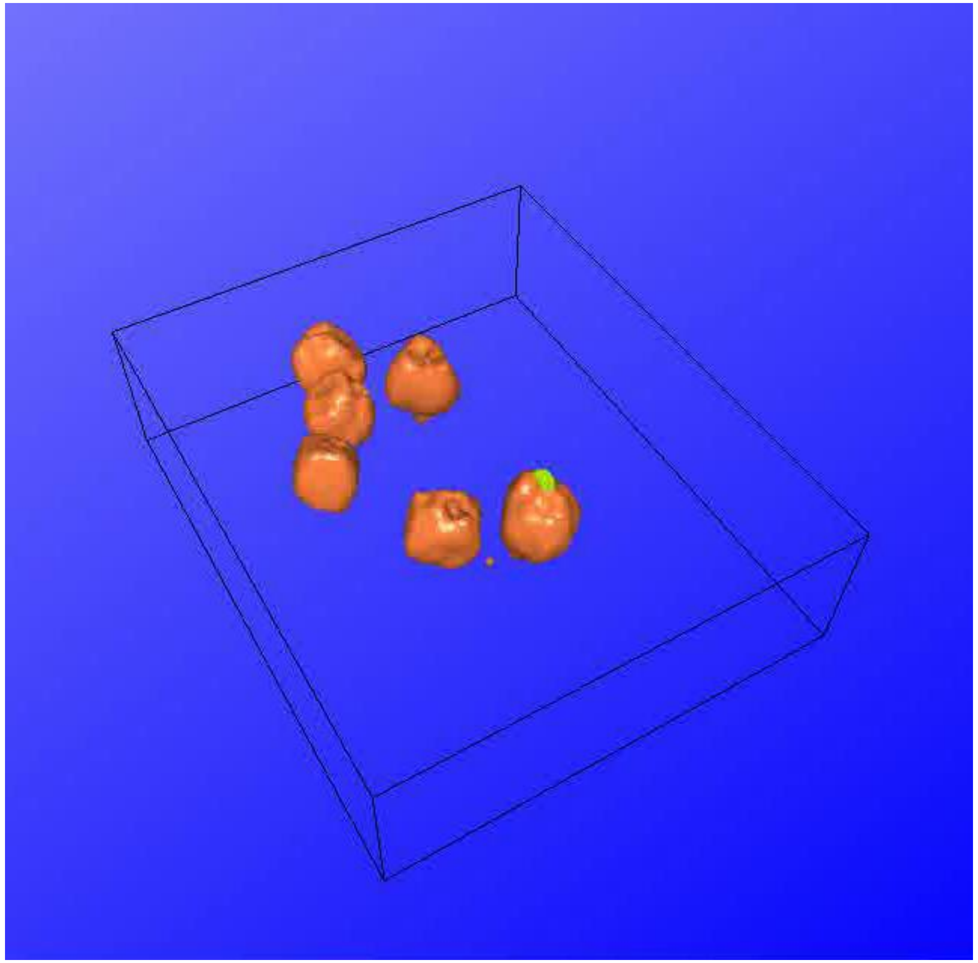


➡ BF-TEM is generally adequate for soft-matter

At high magnification, the contrast in BF-TEM is mostly *phase* contrast created by the interference of diffracted and direct beams. Phase contrast *can* meet the projection requirement under specific conditions: weak phase object, thin & light

Effects of diffraction contrast in BF-TEM





Images in HRTEM mode (phase contrast)

At high magnification, the contrast in HRTEM is mostly *phase* contrast created by the interference of diffracted and direct beams. Phase contrast *can* meet the projection requirement under specific conditions: weak phase object, thin & light

Recall the objective lens transfer function

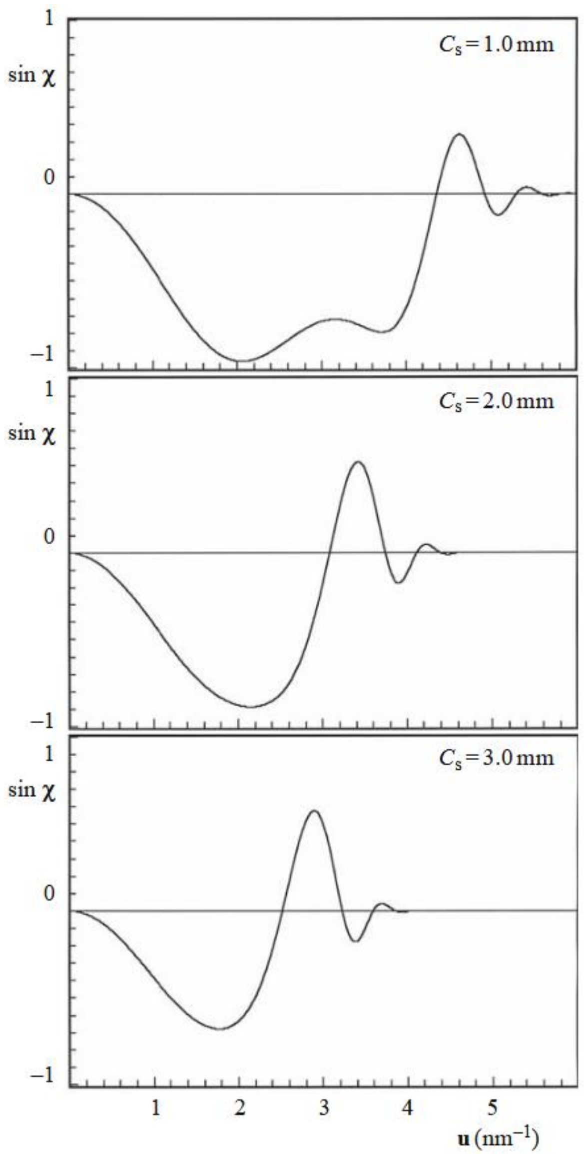
$$T(\mathbf{u}) = A(\mathbf{u})E(\mathbf{u})2\sin\chi(\mathbf{u})$$

A is the aperture function, ***E*** the envelop function, and,

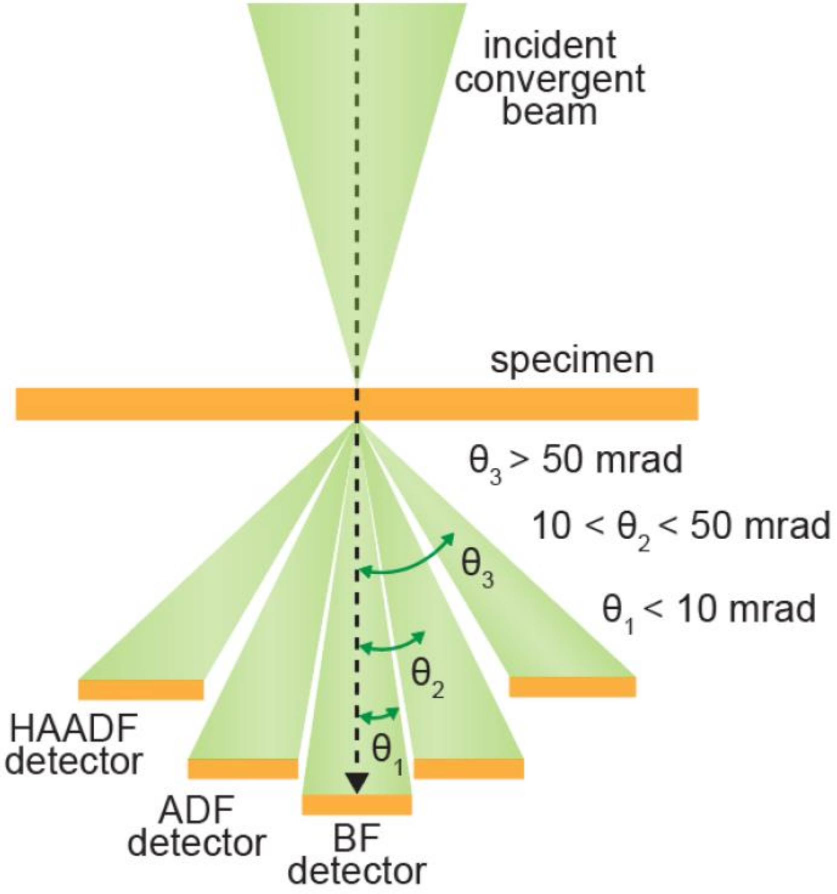
$$\chi = \pi\Delta f\,\lambda u^2 + \frac{1}{2}\pi C_s\lambda^3 u^4$$

Under the weak phase object approximation

→ The sign of the intensity varies with the spatial frequency, the defocus *Δf* and the aberrations C_s
→ Only OK for tomography within the first passband



Images in dark-field scanning TEM mode



Electron tomography in materials science mostly relies on high angle annular dark field (HAADF) to collect only Rutherford-scattered electrons

➡ Scattering cross section $\sim tZ^{1.7}$

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Ultramicroscopy 96 (2003) 413–431
ultramicroscopy
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3D electron microscopy in the physical sciences: the development of Z-contrast and EFTEM tomography

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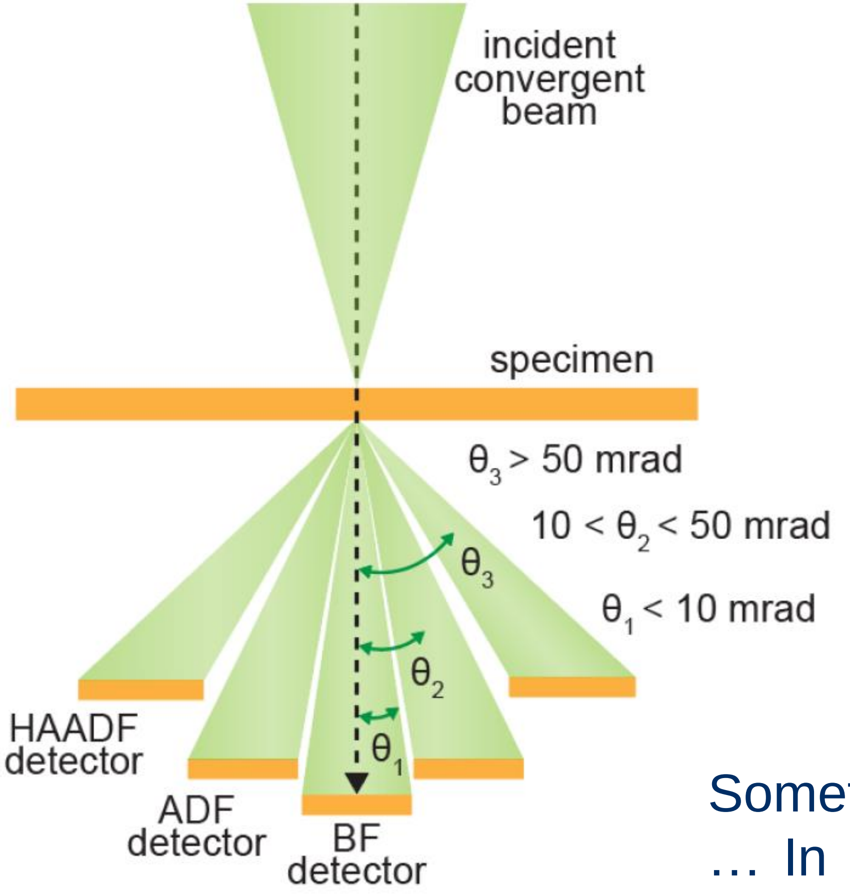

In which cases is HAADF not ideal?

A. Heavily twinned particles
B. Soft matter ✓
C. Mixed light and heavy materials ✓

Sometimes ~$tZ^{1.7}$ is too much, or materials are beam sensitive … In this case phase imaging (~tZ for weak phase objects) is better but these are less straightforward experiments!

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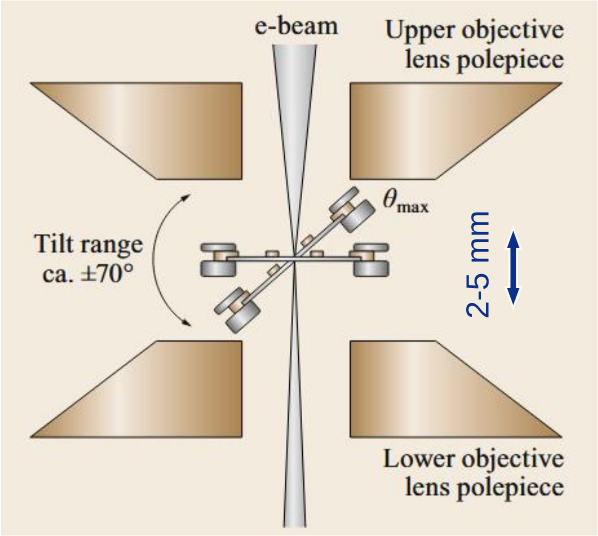
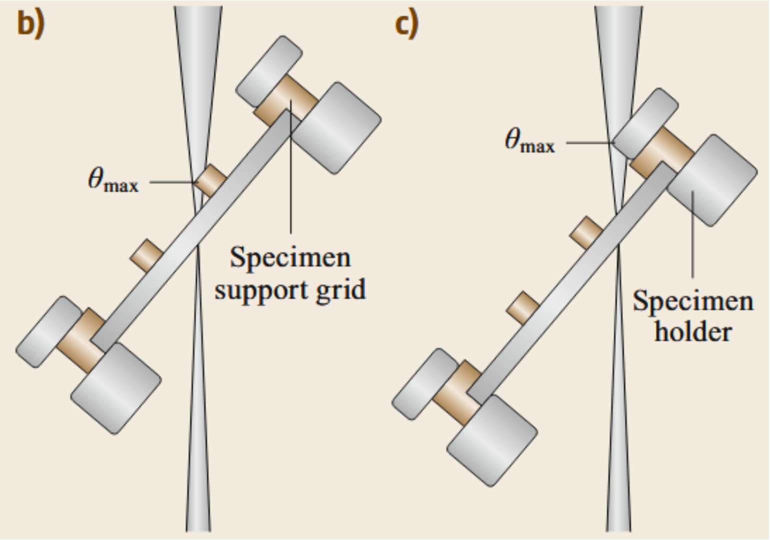
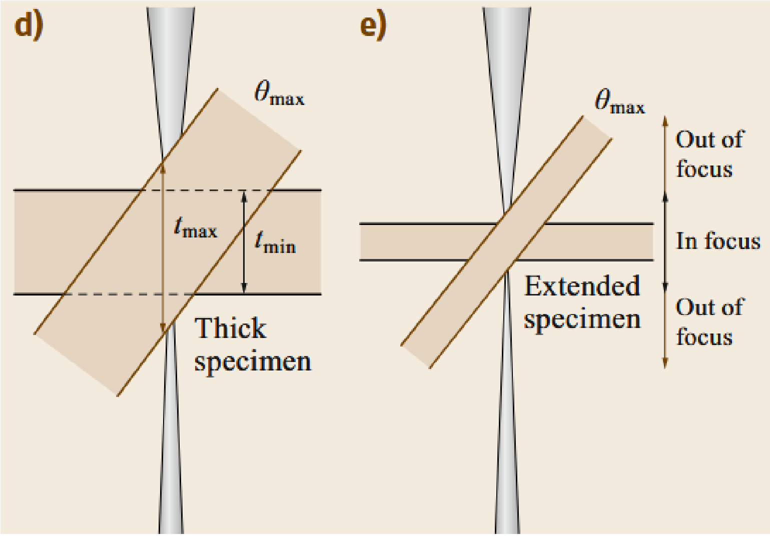
Acquisition at the microscope: tilting the sample

An object and its projections are equivalent under a mathematical transform

Ideally: tilt fully at 180° (± 90°), very fine (infinite) angular sampling
Reality: limited tilting ~ 150° (± 75°), coarse sampling (2-5°) for the sake of time







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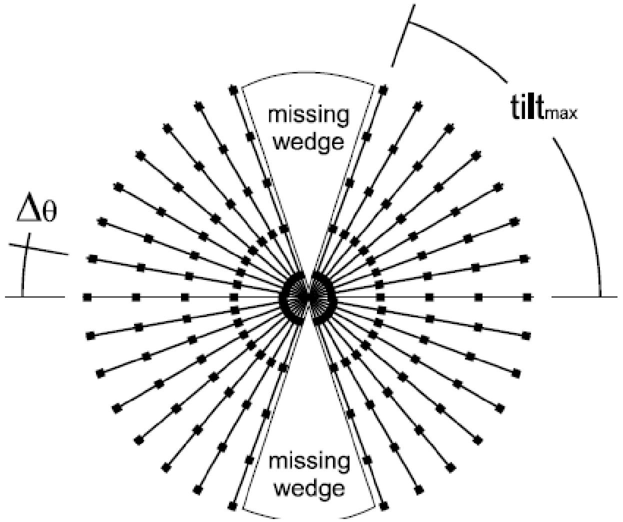
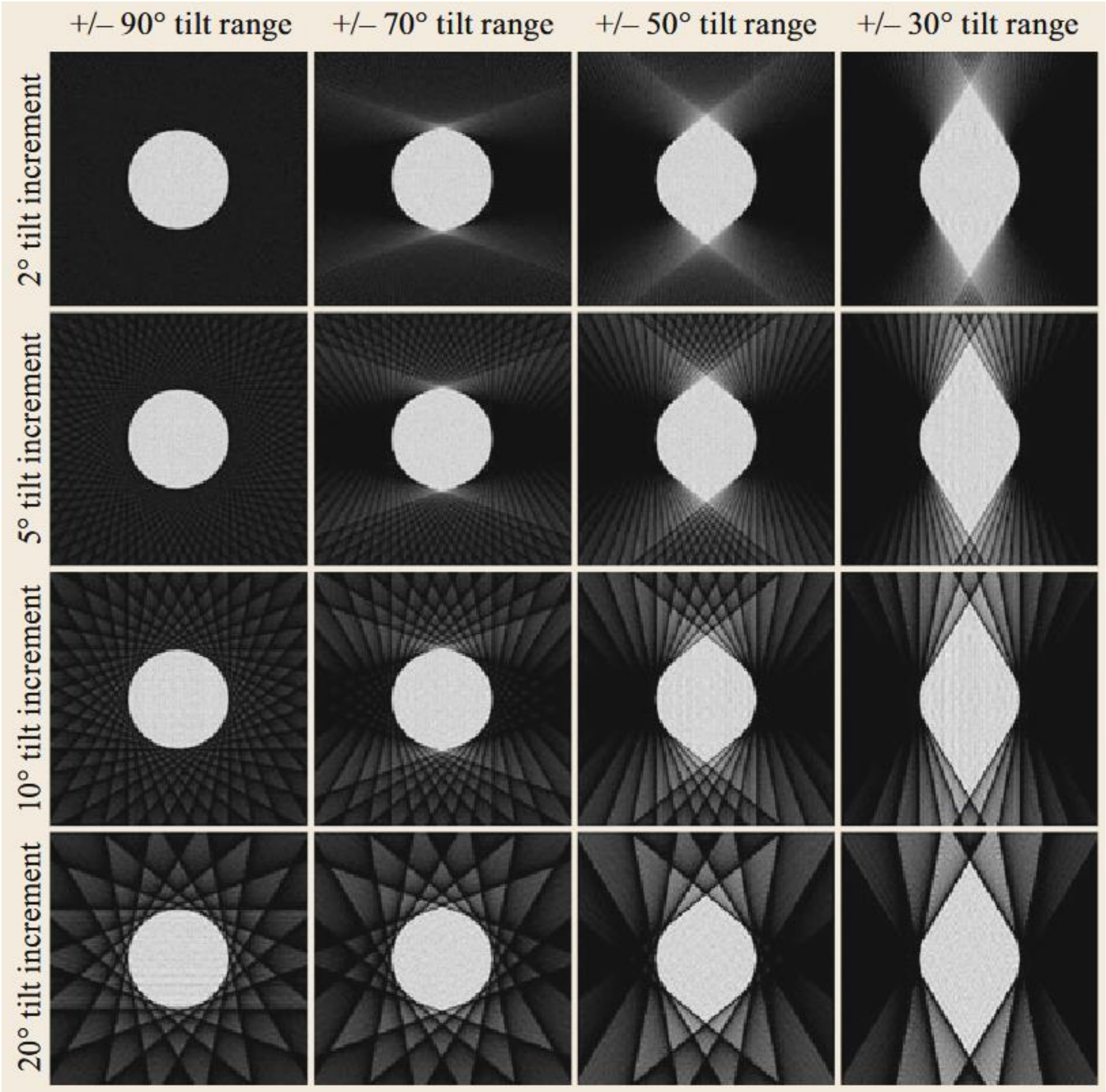
Effect of the missing wedge and of the tilt increment

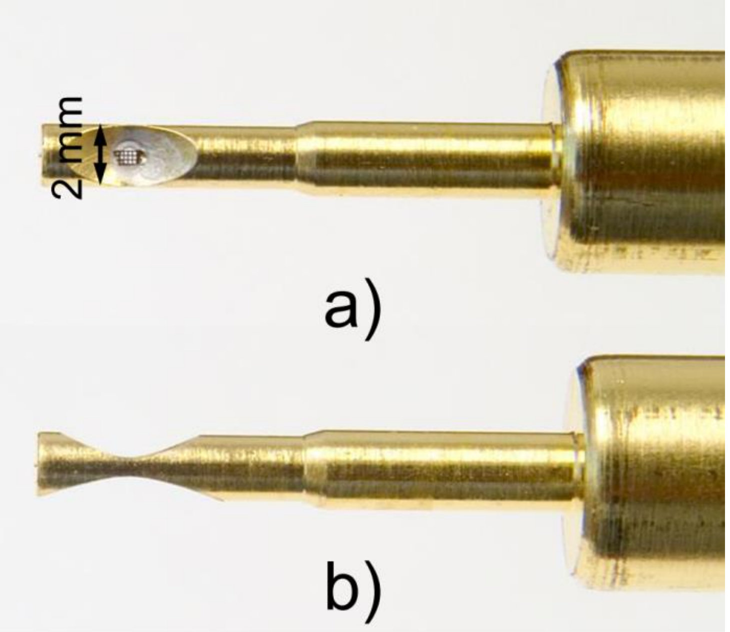


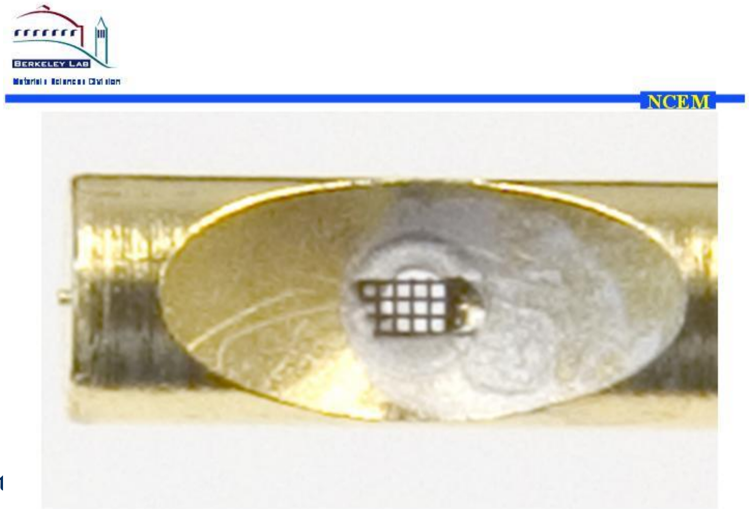


The missing wedge elongates the reconstructed features in the direction parallel to the beam

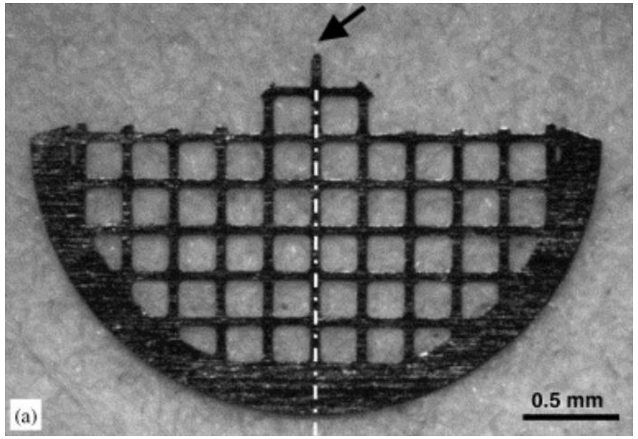
A large tilt increment decreases the overall resolution

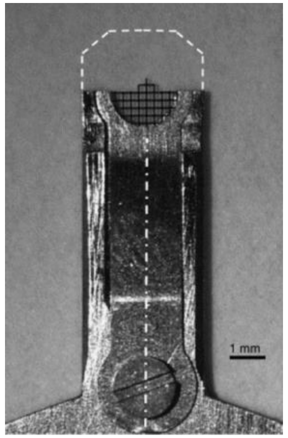
Development of tomography holders





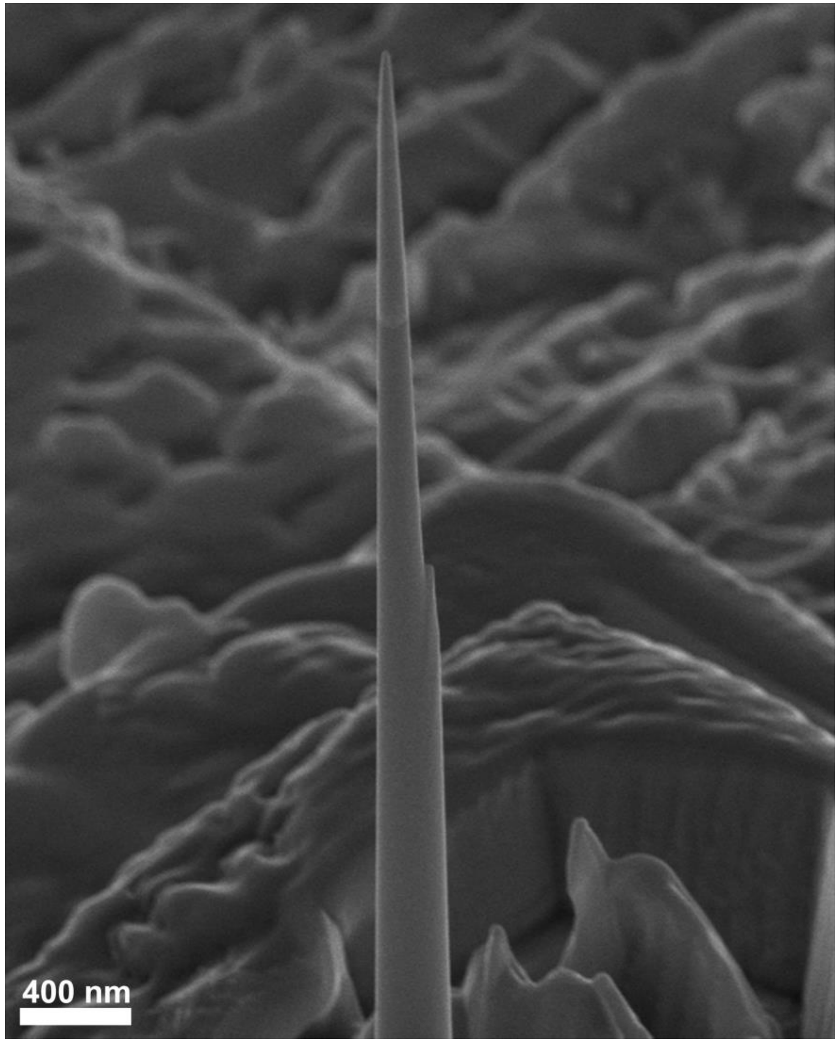
Development of tomography holders





Kawase, N., Kato, M., Nishioka, H. & Jinnai, H. Ultramicroscopy 107 (2007) 8

On axis rotation tomography holder (Fischione instruments, Model 2050)



Development of tomography holders

Ultra-Narrow Gap tomography holder
(Fischione instruments, Model 2030)

Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Structural Biology 153 (2006) 55–63
ELSEVIER
Journal of Structural Biology
www.elsevier.com/locate/yjsbi

A novel dual-axis iterative algorithm for electron tomography

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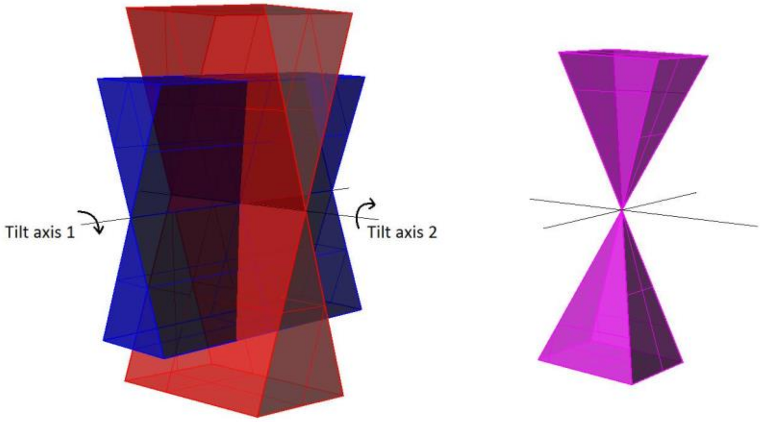
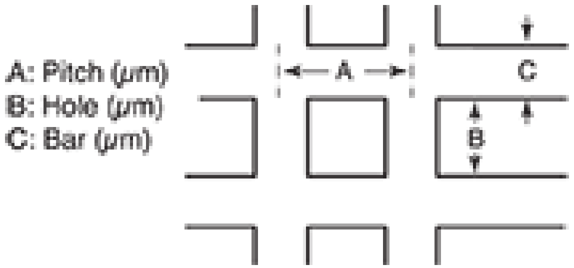
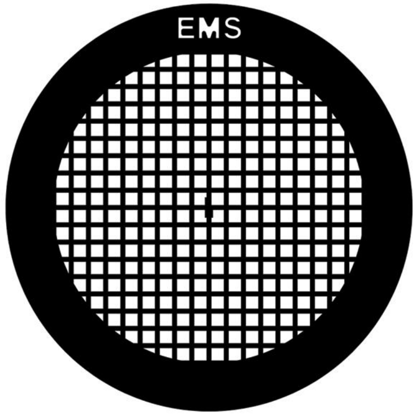


“Regular” tomography: what to consider for sample preparation?

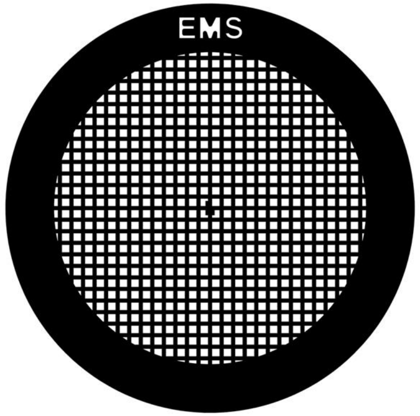
The choice of grid is important (≥ 200 mesh), the region of interest should be close to center within a grid square, watch out for shadowing from neighboring particles, position the grid properly

→ Maximizes tilt range (see the **demo**)

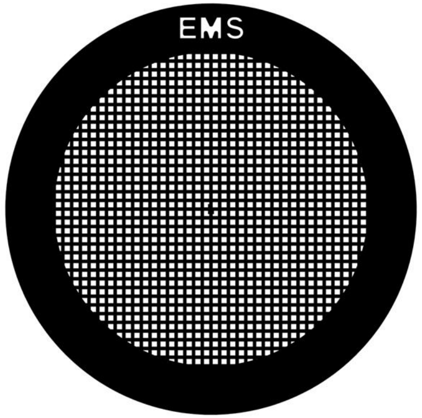




200 mesh

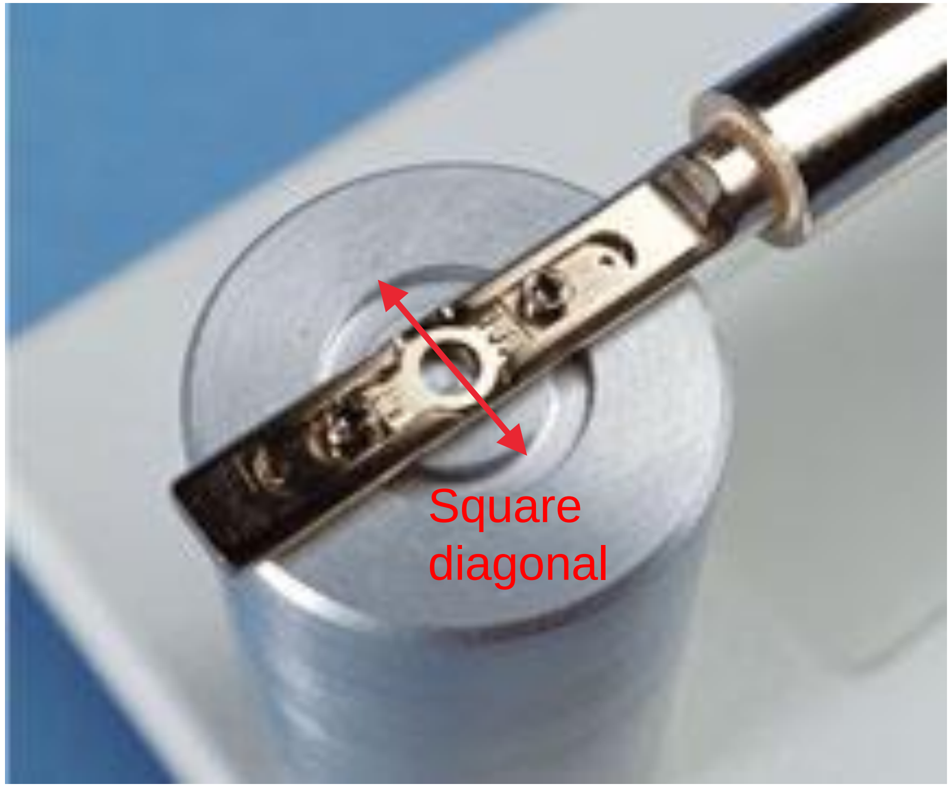


300 mesh

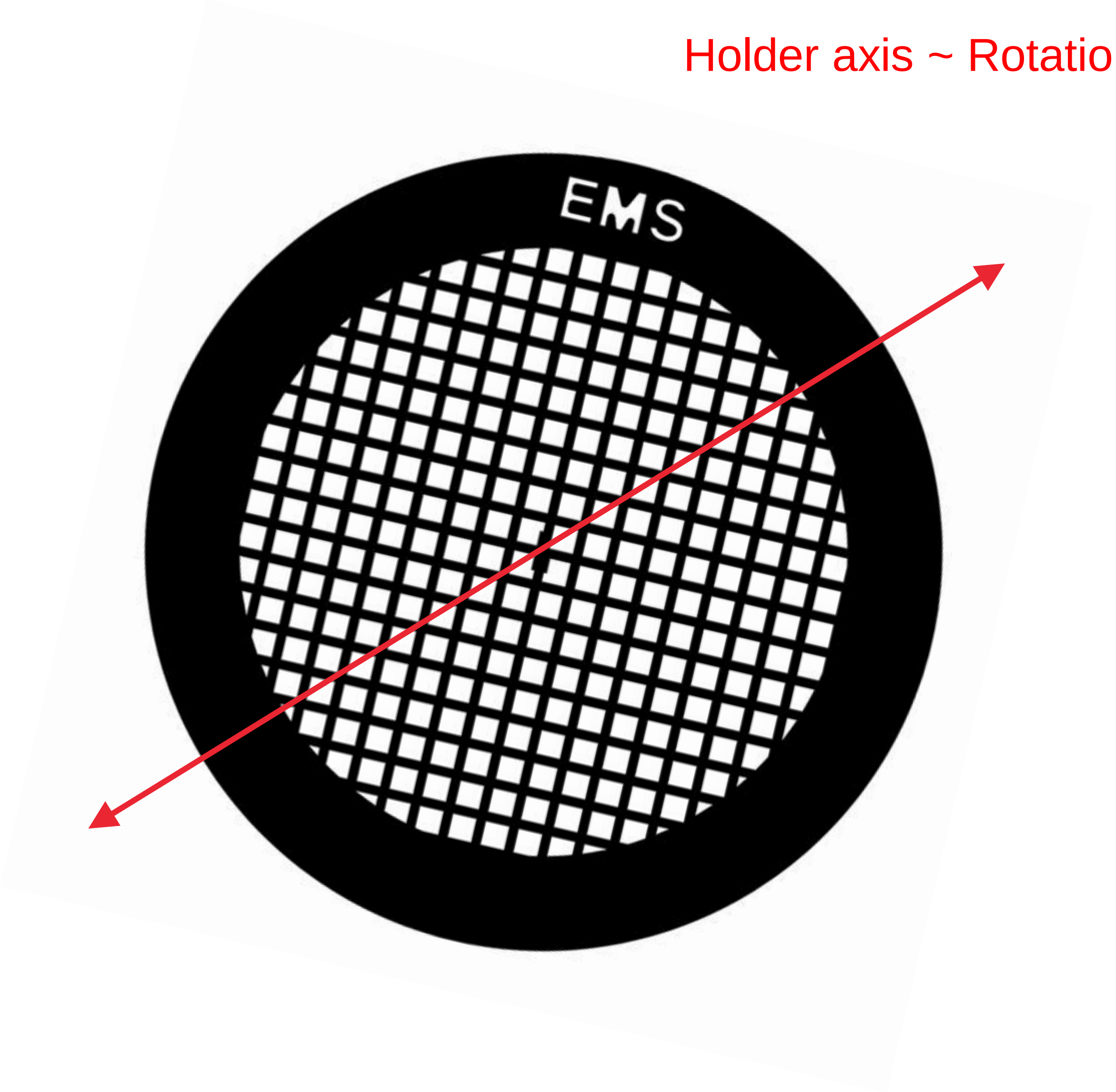


400 mesh

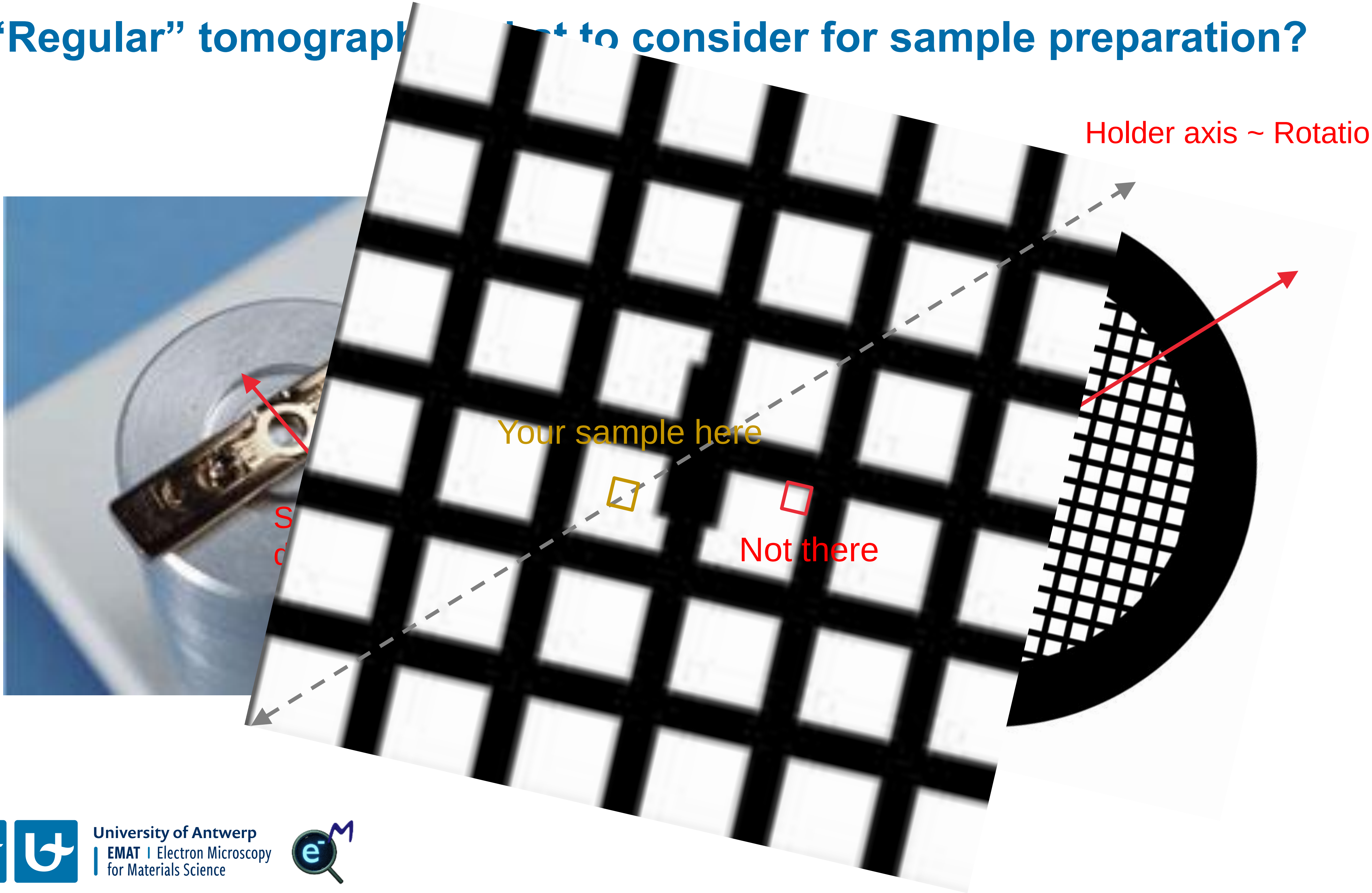
“Regular” tomography: what to consider for sample preparation?



Holder axis ~ Rotation axis



“Regular” tomograph[...] to consider for sample preparation?



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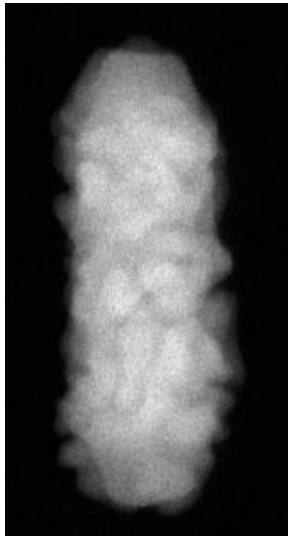
→ Maximizes tilt range (see the **demo**)

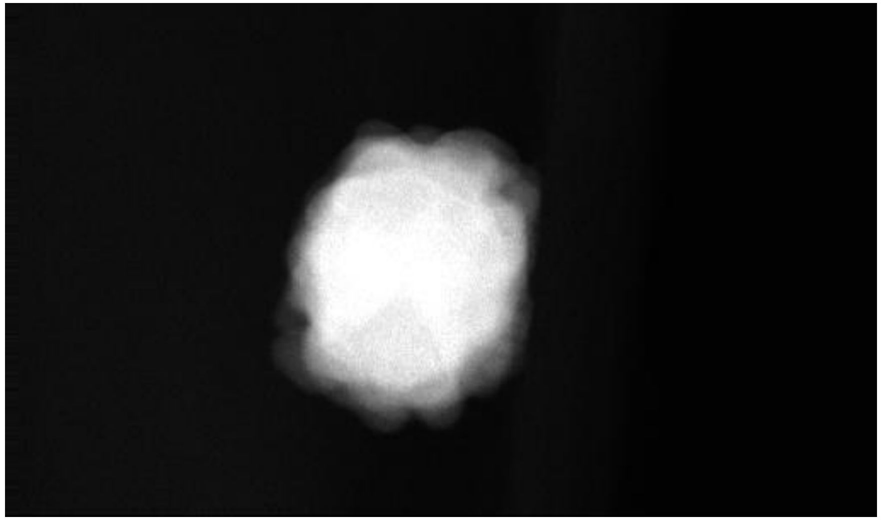
Anisotropic objects should have their long axis as close as possible to the rotation axis

→ Minimizes the thickness and the reconstruction size

Aligned



Not aligned



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“Regular” tomography: what to consider for sample preparation?

The choice of grid is important (≥ 200 mesh), the region of interest should be close to center within a grid square, watch out for shadowing from neighboring particles, position the grid properly

→ Maximizes tilt range (see the **demo**)

Anisotropic objects should have their long axis as close as possible to the rotation axis

→ Minimizes the thickness

In BF-TEM or HRTEM, lacey carbon is better than C-flat and will have more particle on the edges

→ Minimizes background

For nanoparticles, dispersion is critical!!

And also, lamellas, microtomed sections, cryo-transfer for frozen liquid / polymer assemblies, etc.

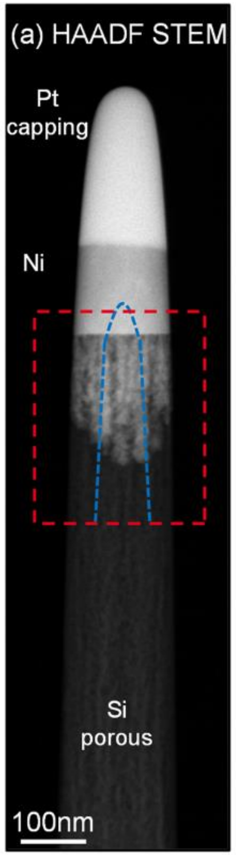
“~~Regular~~” tomography: what to consider for sample preparation?

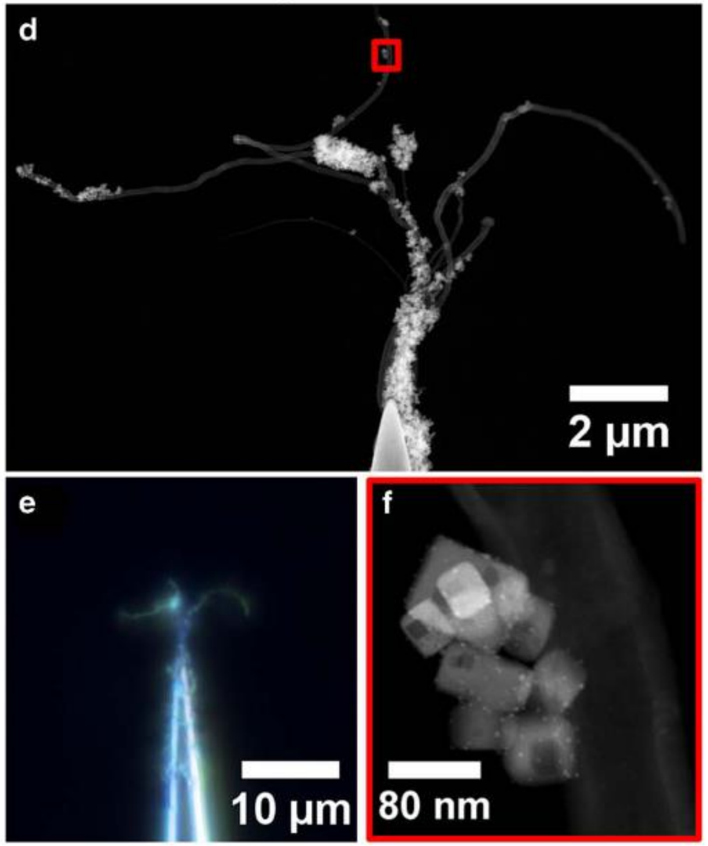
Making probes / pillar

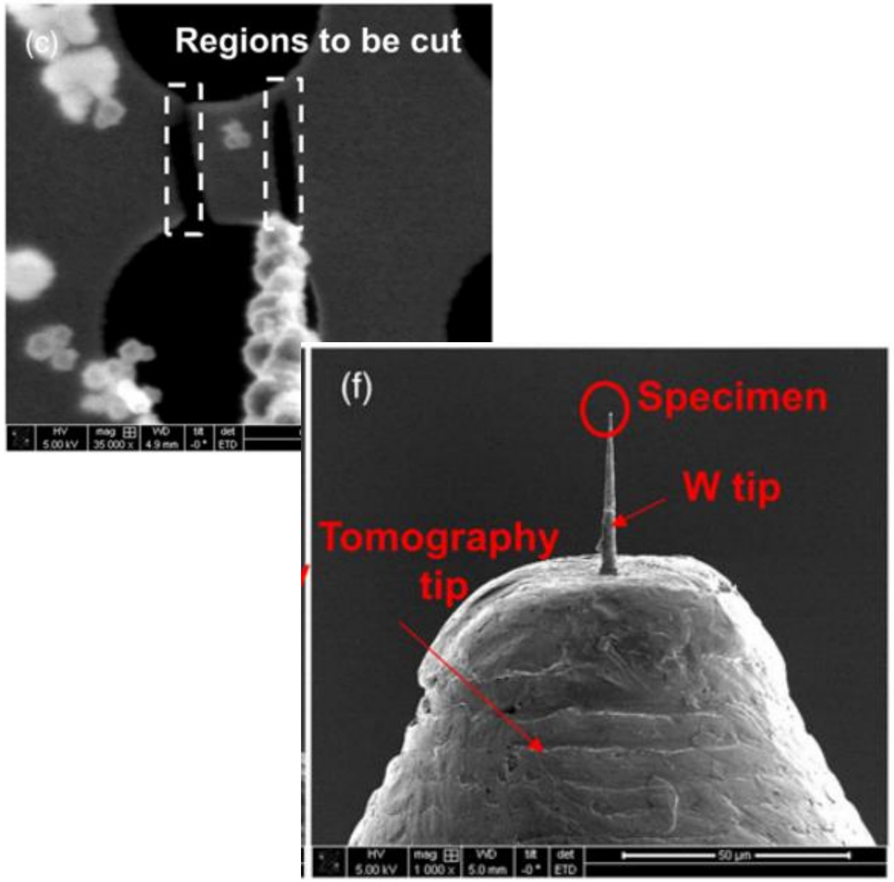
FIB

Deposition

Transfer / picking







Mouton, I., *et al.* *Ultramicroscopy* **182**, 112-117 (2017)

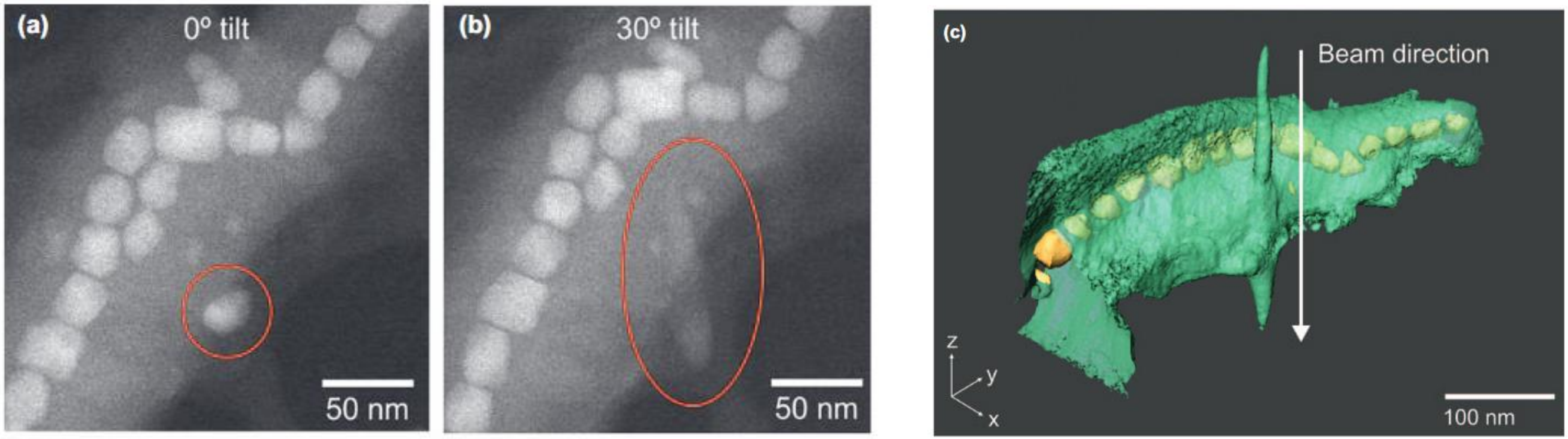
Padgett, E., *et al.* *Microsc Microanal* **23**, 1150-1158 (2017)

Huang, X., *et al.* *Microsc Microanal* **28**, 1981-1988 (2022)

“Regular” tomography: what to consider for sample preparation?

Tomography typically involves > 60 images for acquisition + tracking and focusing (“overhead”)

→ Watch out for contamination, consider plasma cleaning, beam shower, ligands removal
→ Most samples are at least a bit beam sensitive – don’t spend more time than necessary, consider automated software

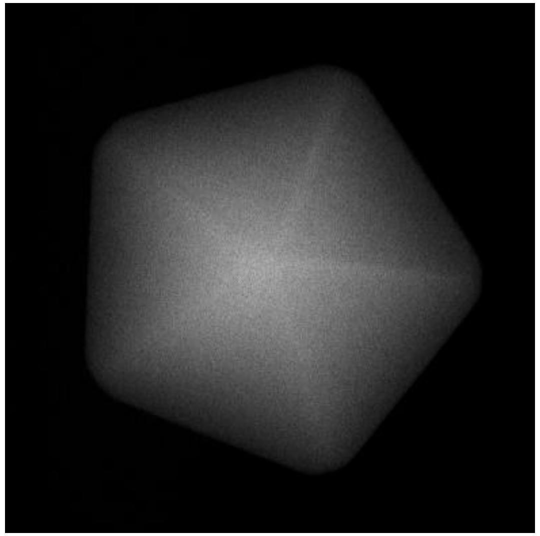
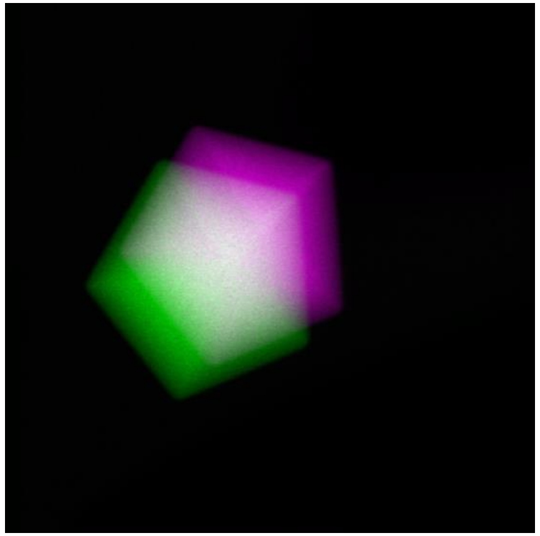
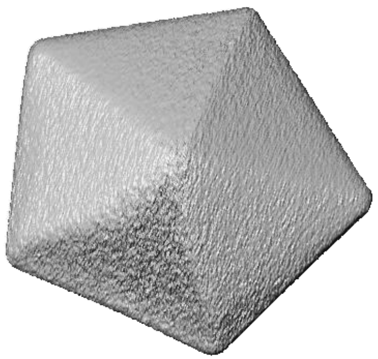
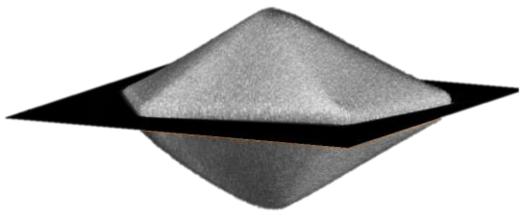


Weyland, M. & Midgley, P. A. Electron Tomography in *Nanoscience & Nanotechnology Series,* 2015

Li, C., *et al. Ultramicroscopy* **221**, 113195 (2021)

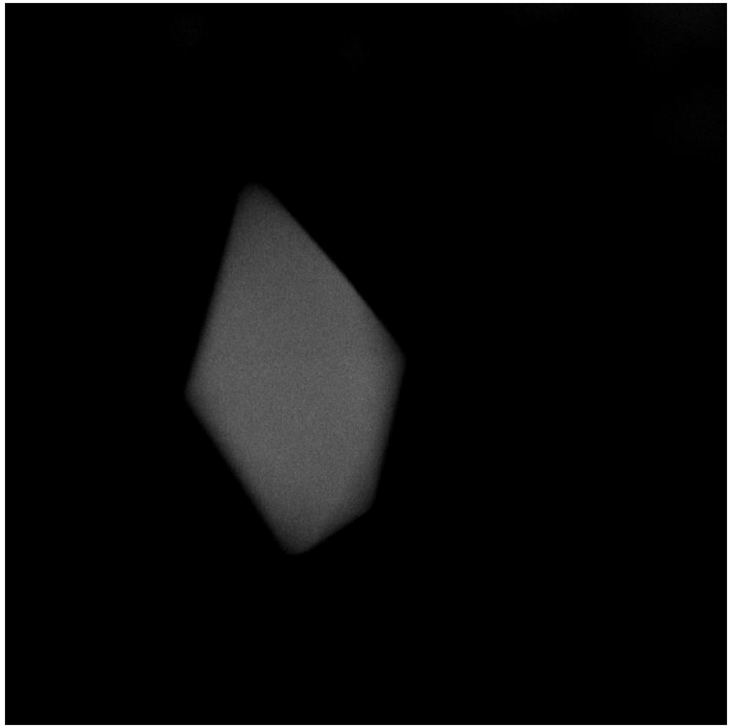
Tomography experiment

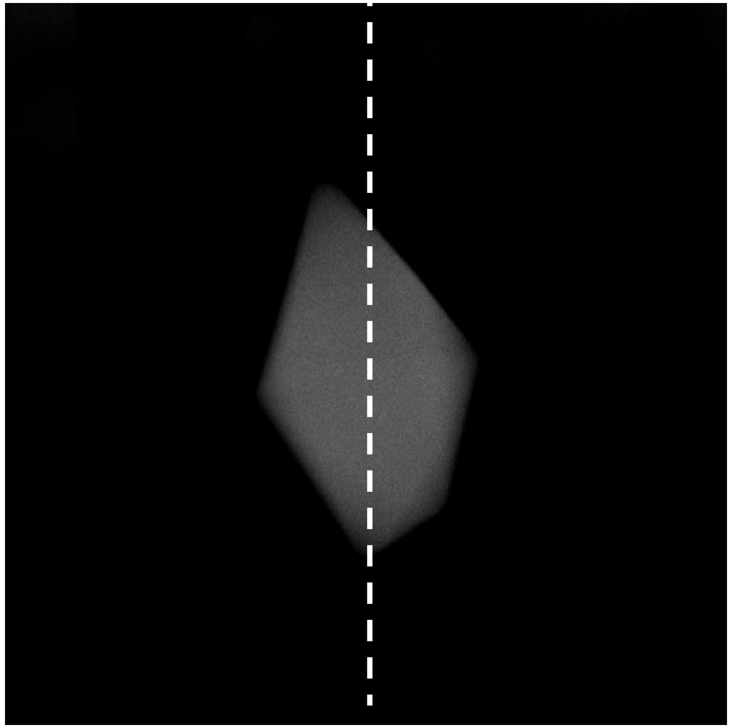
Acquisition	Alignment	Reconstruction	Quantification

Why is alignment required?

Reconstruction algorithms assume a **common**, **central**, **vertical** rotation axis in the tilt-series.
Alignment makes or breaks your reconstructions!!





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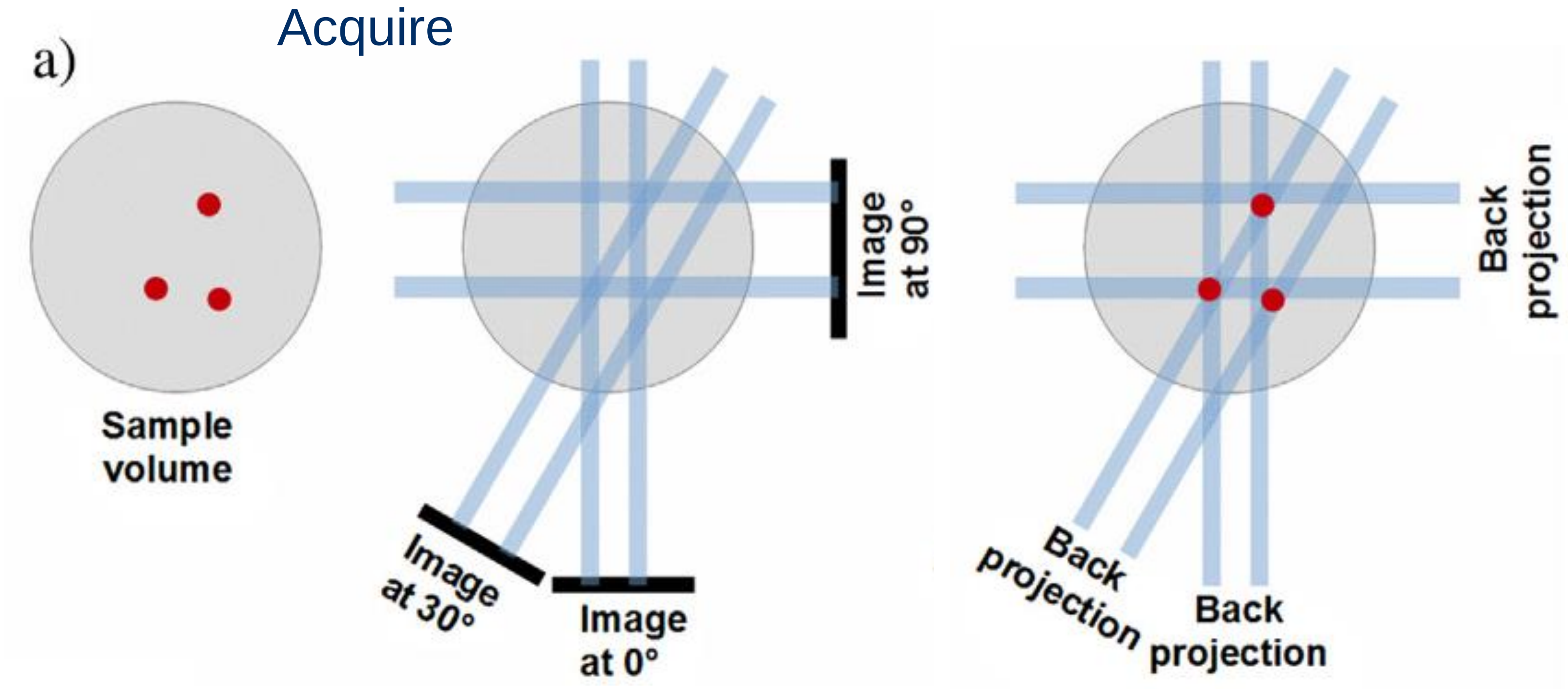
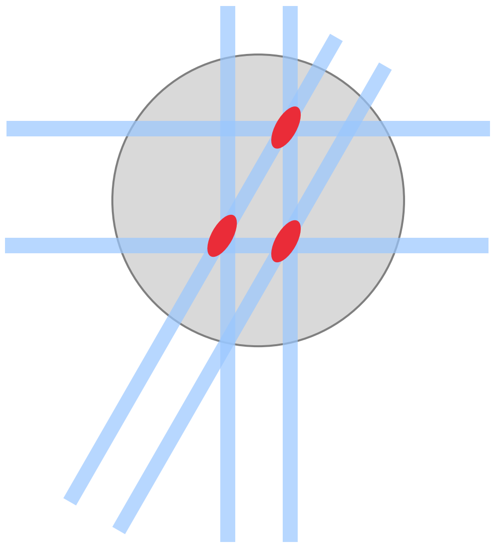
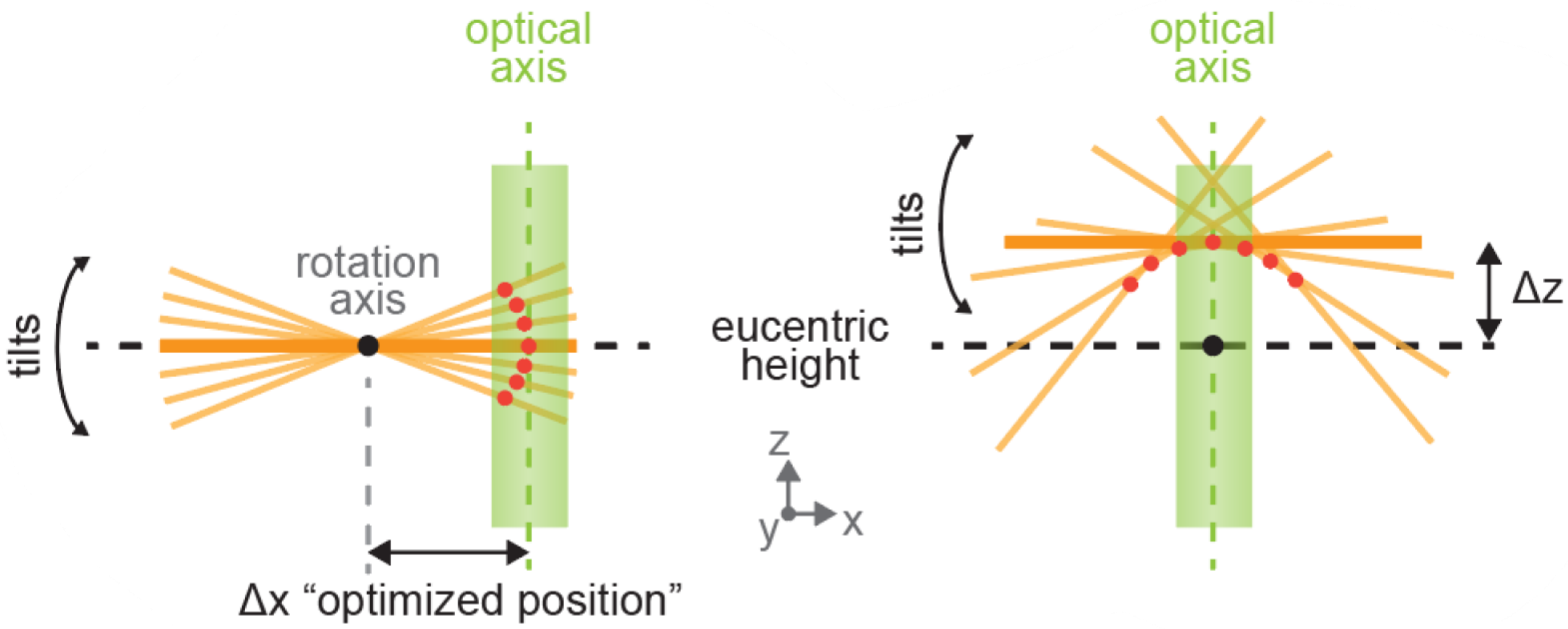




Backprojection - misaligned

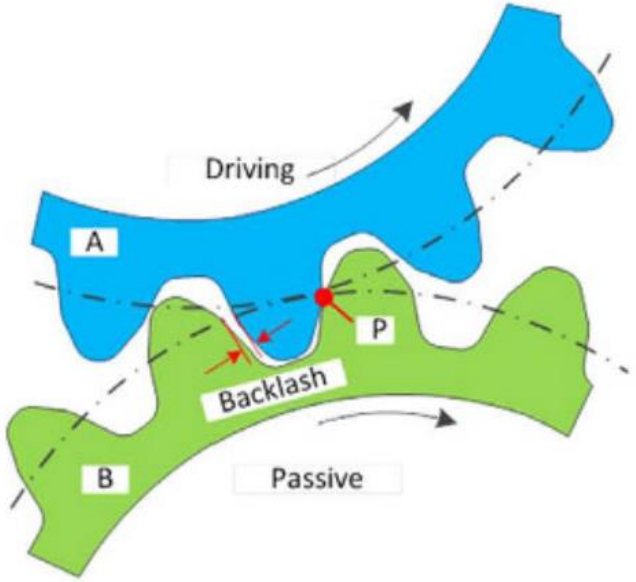
Why is alignment required?

- Mechanical precision of the tilting stage is on par with the dimensions of the objects
- Sample drift over time
- In TEM, the **stage** tilts, and the holder moves within the stage, so if the grid or the optical axis are not aligned with the rotation axis, systematic shifts will be experienced



In practice: optimizing alignment at the microscope

- Watch out for your eucentric height! (see **demo**)
- The optical axis can be tuned with some software (TFS) with beam shifts (+ image shifts in TEM mode)
- As much as possible, always approach the target angle from the same direction to avoid backlash (e.g., if your series starts at -70°, go to -72° and back to 70°; this is often automated by the software!)



Liu, J., *et al. Sci Rep* **6**, 29231 (2016)

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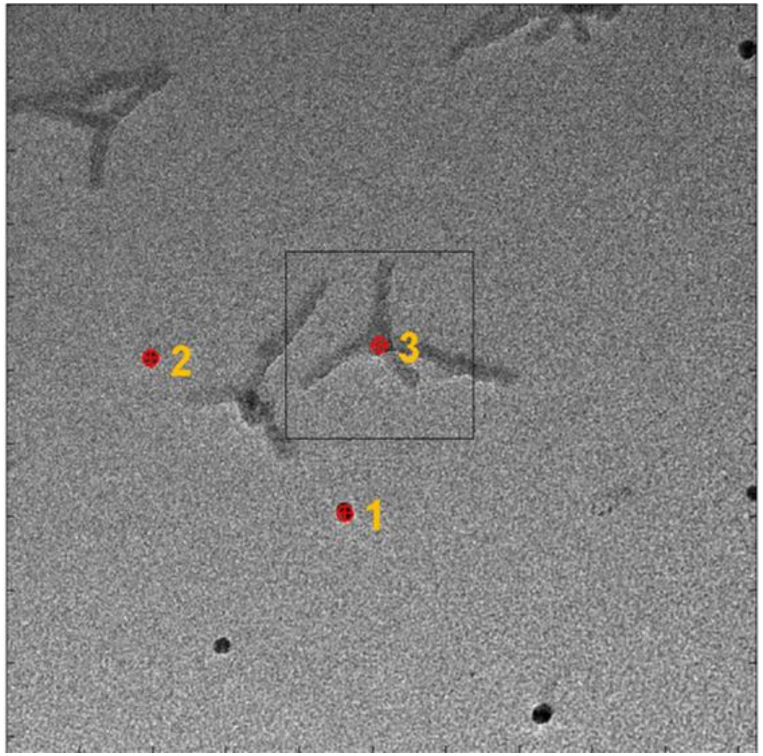
Aligning to a common tilt axis: three strategies (of many)

Fiducial markers



- Fiducial markers are small (5 nm), easily trackable nanoparticles that are added onto the grid
- Each marker should follow a parabolic space curve during rotation. Therefore, the images can be iteratively shifted until the position of the tracked markers are consistent with their ideal projected 3D trajectories

\+ Very accurate, solves displacement, distortions, rotation
\- Adds bright markers to the sample, user involvement

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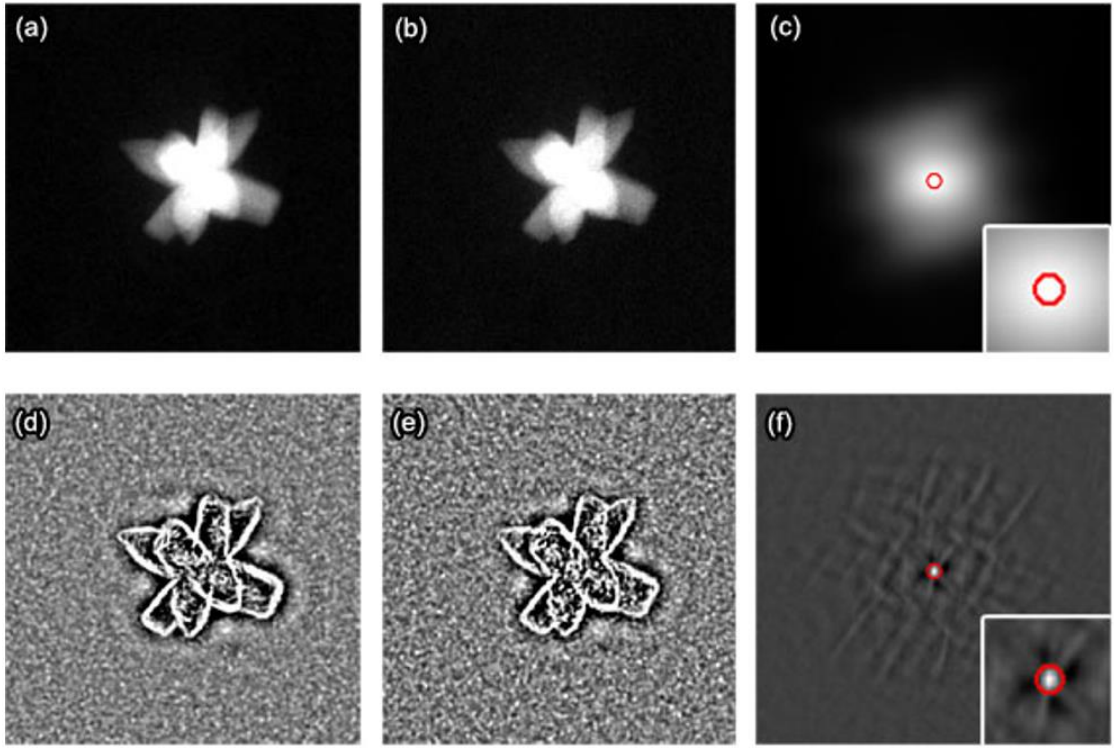
Aligning to a common tilt axis: three strategies (of many)

Cross-correlation



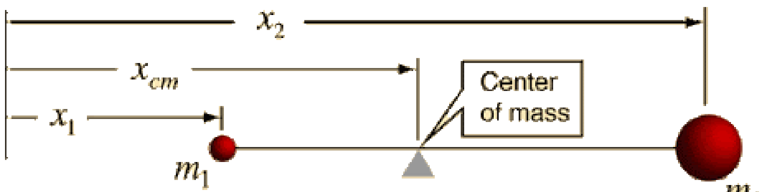
The cross-correlation or phase-correlation is obtained by multiplying the Fourier transform of one image by the complex conjugate of the other, then using peak detection to find the optimal shifts

Numerical edge filters can enhance the precision of the x-corr maxima

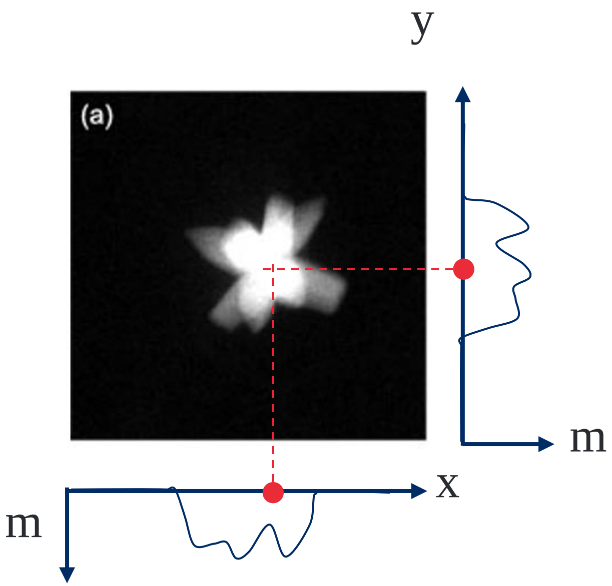
+ Fast, accurate if there are no artefacts
- Typically done on two consecutive images so small errors can accumulate. Requires bright features

Aligning to a common tilt axis: three strategies (of many)

Center of mass



For two masses: $x_{cm} = \dfrac{m_1 x_1 + m_2 x_2}{m_1 + m_2}$



For n pixels:

$$x_{cm} = \frac{\sum_i x_i m_i}{\sum_i m_i}$$

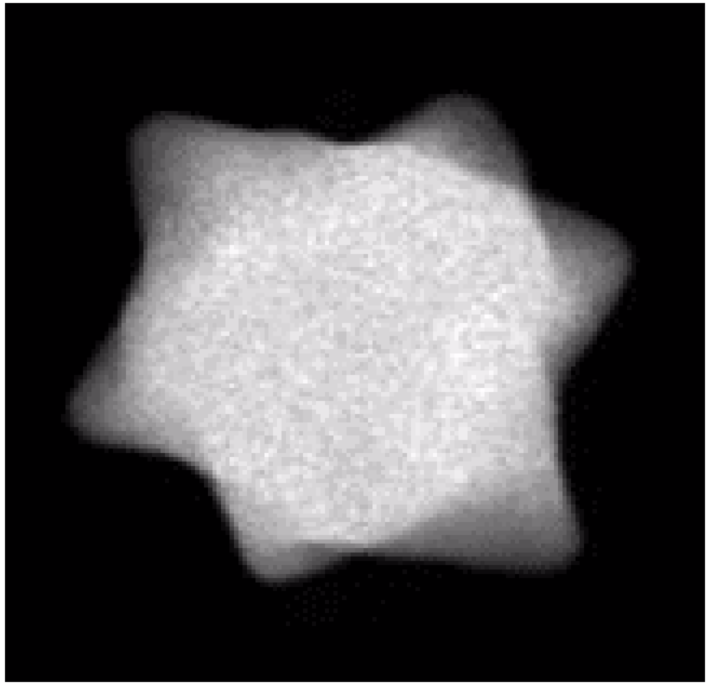
The "mass" is the intensity

The centers of mass in the x and y axis should coincide for all angles

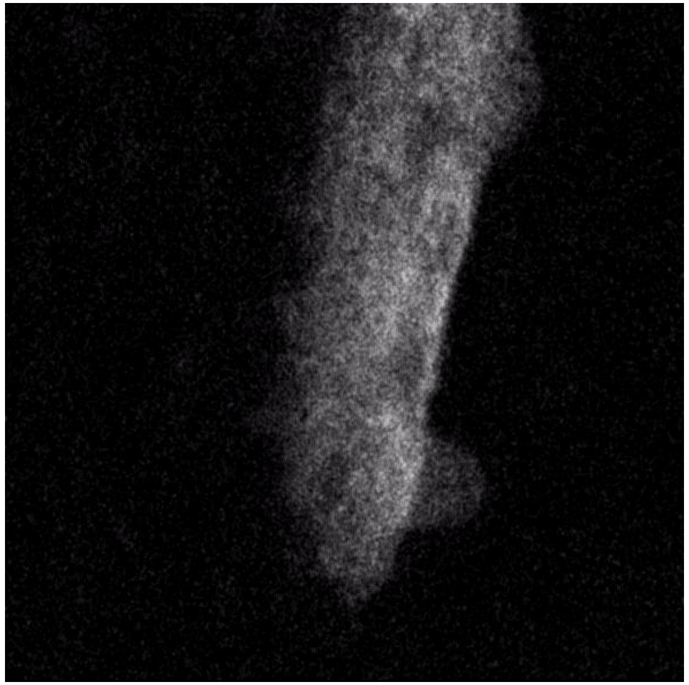
\+ Fast, accurate if there are no artefacts

\- Requires a perfectly isolated object and nothing else in the image. Sensitive to noise

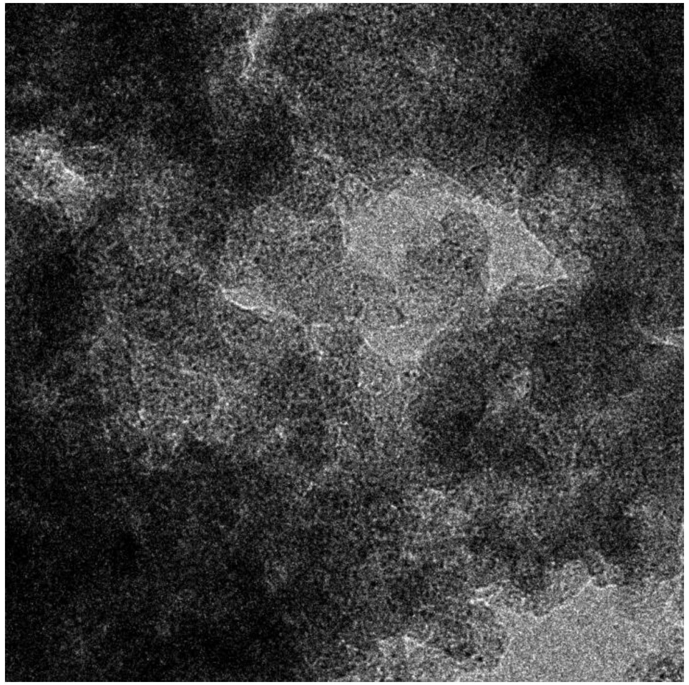
What would you use?



- Cross-correlation
- Center of mass



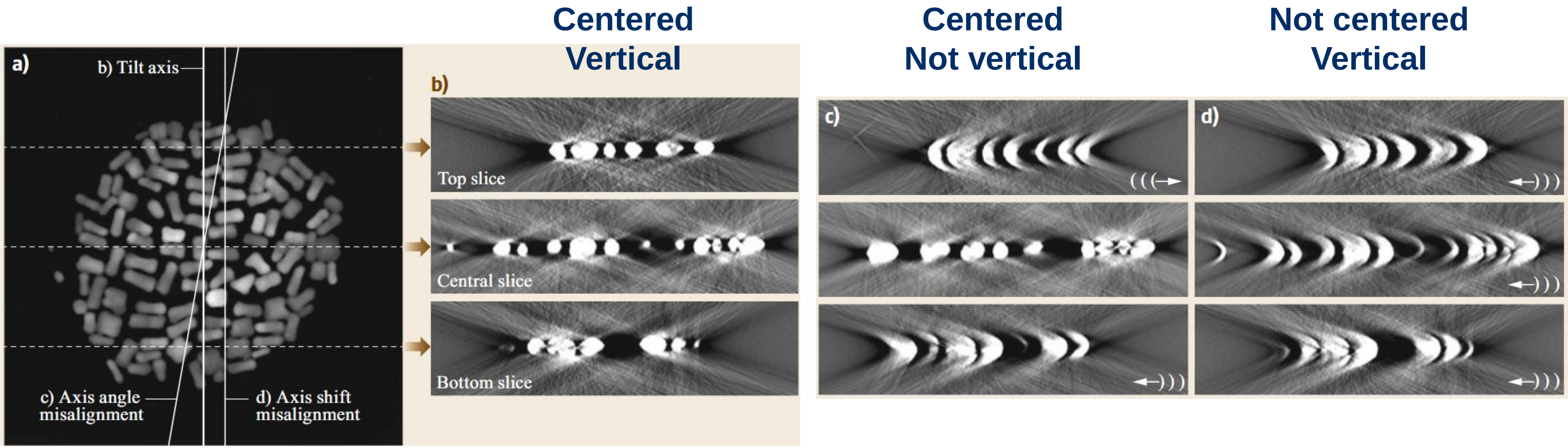
- Cross-correlation
- Markers if ok for the sample



- Cross-correlation
- Markers if ok for the sample

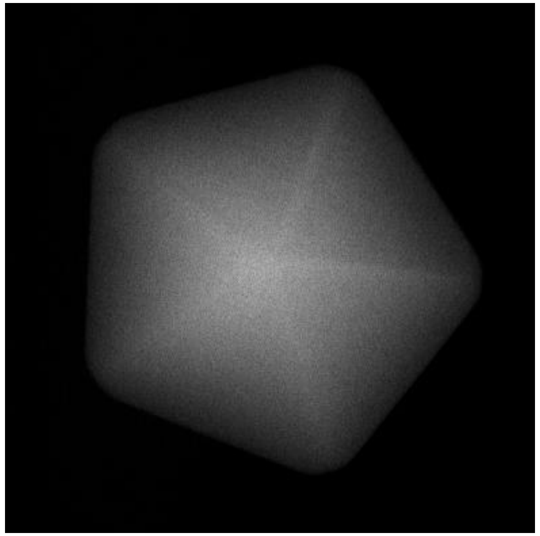
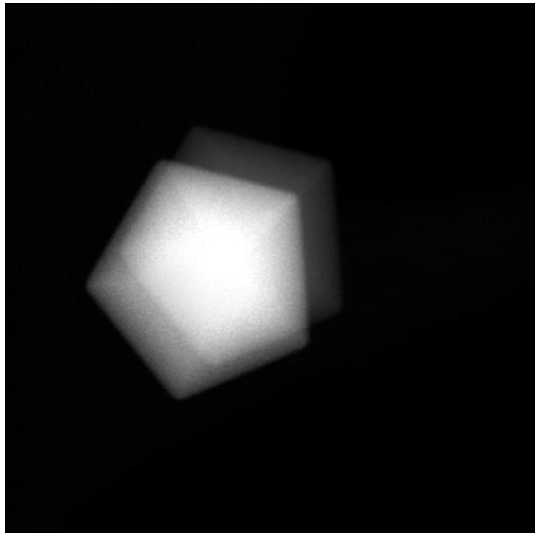
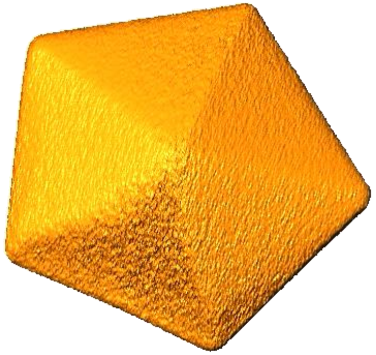
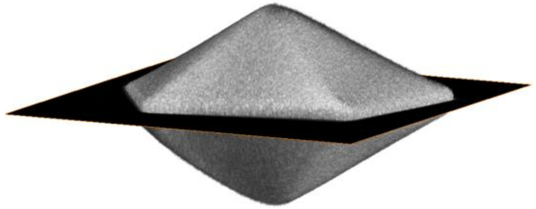
If nothing works you can always do it fully manually …

Aligning to a vertical, center tilt axis



- A misaligned tilt axis results in **arc artefacts** and make interpretation and data processing very challenging!
- Can be automatized but is typically fast to do manually (see practical)
- Before acquisition, ensure that the scan direction is orthogonal (or aligned) with the tilt axis! If you need to rotate the image by 45° in post-processing you will lose information ….

Tomography experiment

Acquisition	Alignment	Reconstruction	Quantification
			

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Algorithms

Direct transforms

Practical:

Filtered Backprojection (FBP)

Direct Fourier Inversion

Iterative transforms

Expectation Maximization (EM)

Simultaneous Iterative Reconstruction Technique (SIRT)

Advanced:

L1 regularization (LASSO)

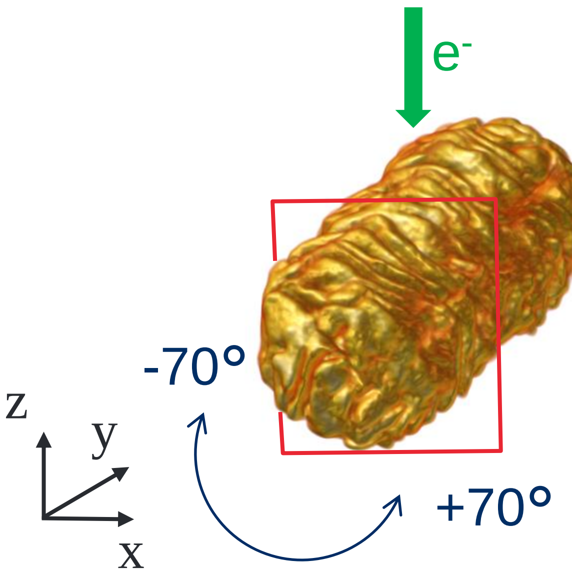
Total-Variation Minimization (TVM)

Discrete Algebraic Reconstruction Technique (DART)

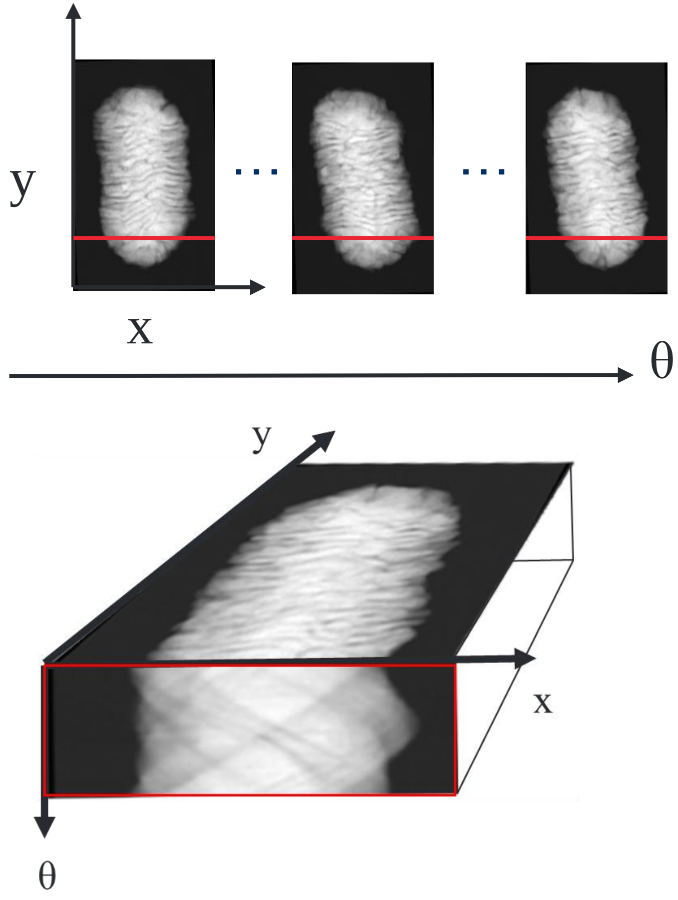
And more …

Reconstruction: 2D series or 3D?

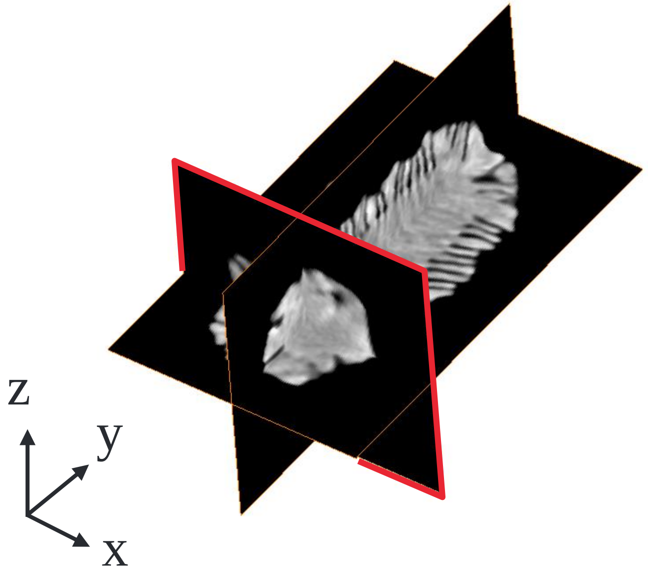
Acquisition geometry



Tilt-series geometry



Reconstruction geometry

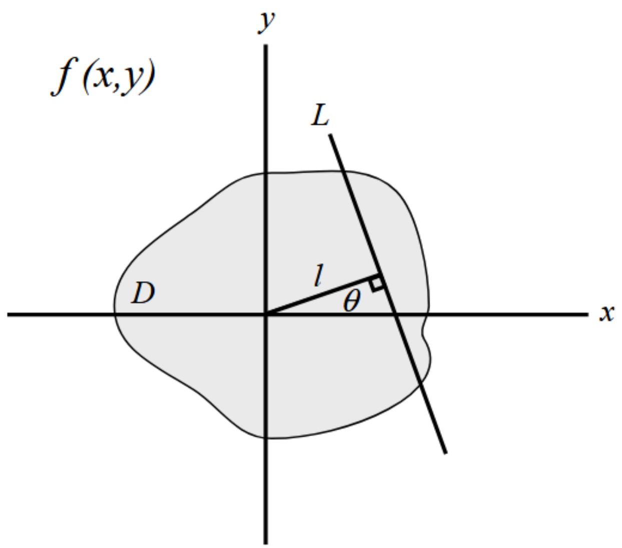


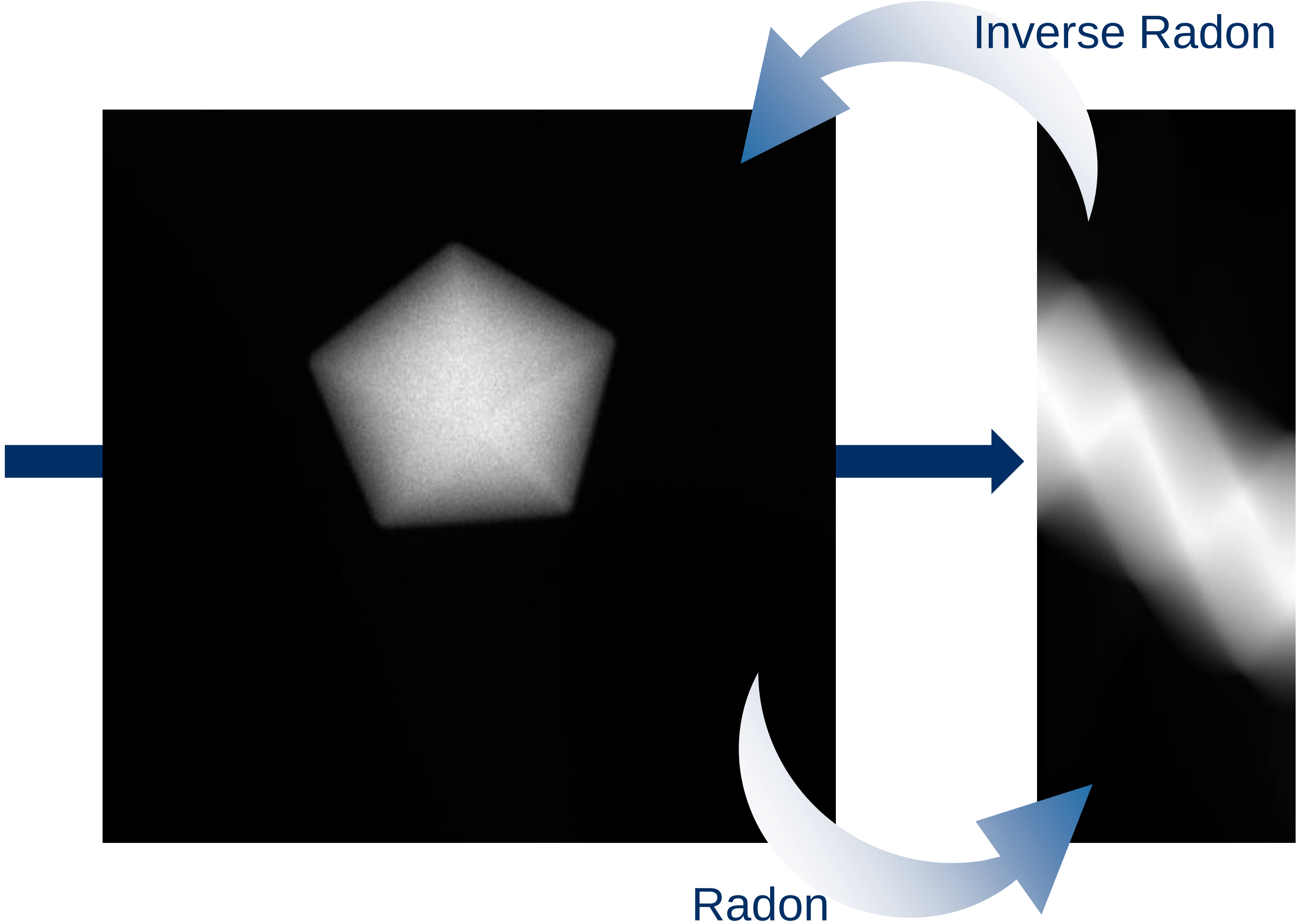
Most algorithms are applicable on the entire 3D geometry, or to reconstruct a series of 2D (xz) sections slice-by-slice. 2D series are typically easier on the (V)RAM

Reconstruction: intuitions from the Radon transform

Radon transform

$$Rf = \int_L f(x,y)\,\mathrm{d}s.$$

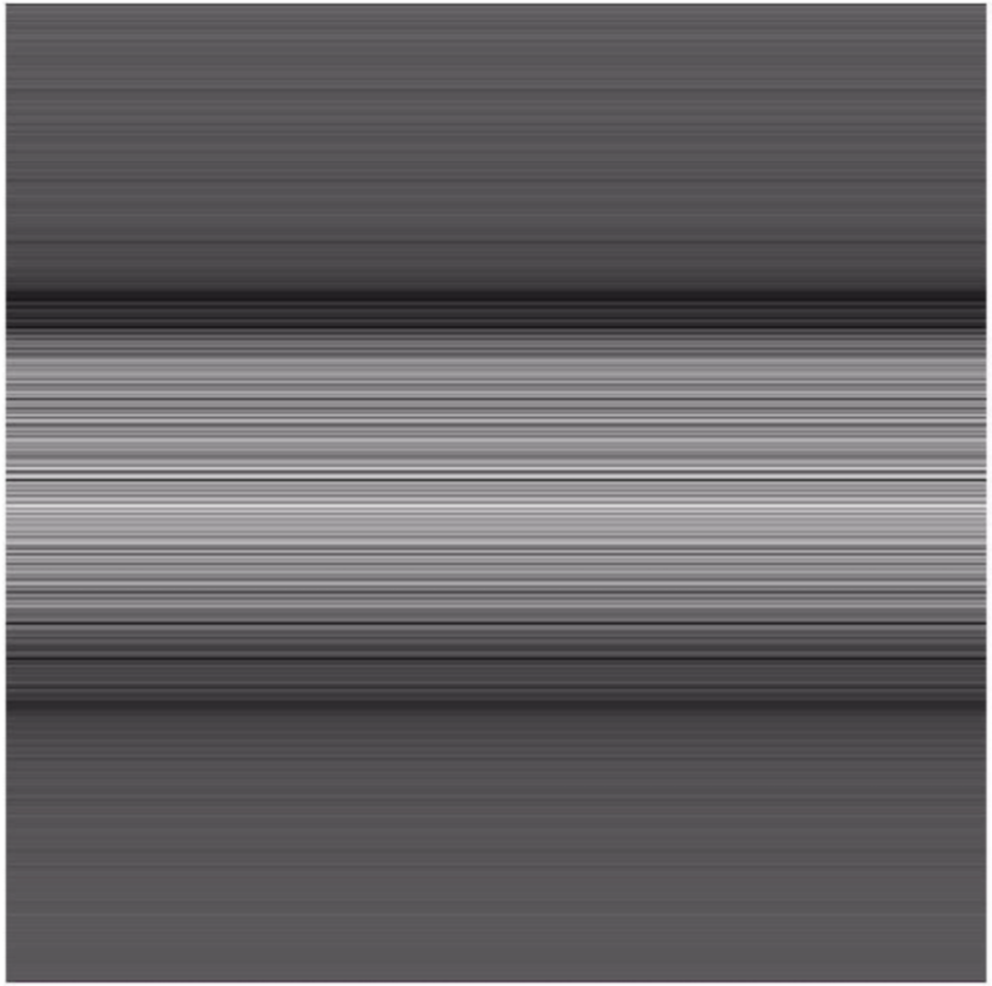




Inverting the Radon transform: in real space

In **real space** a direct way to inverse the Radon transform is the **backprojection** algorithm

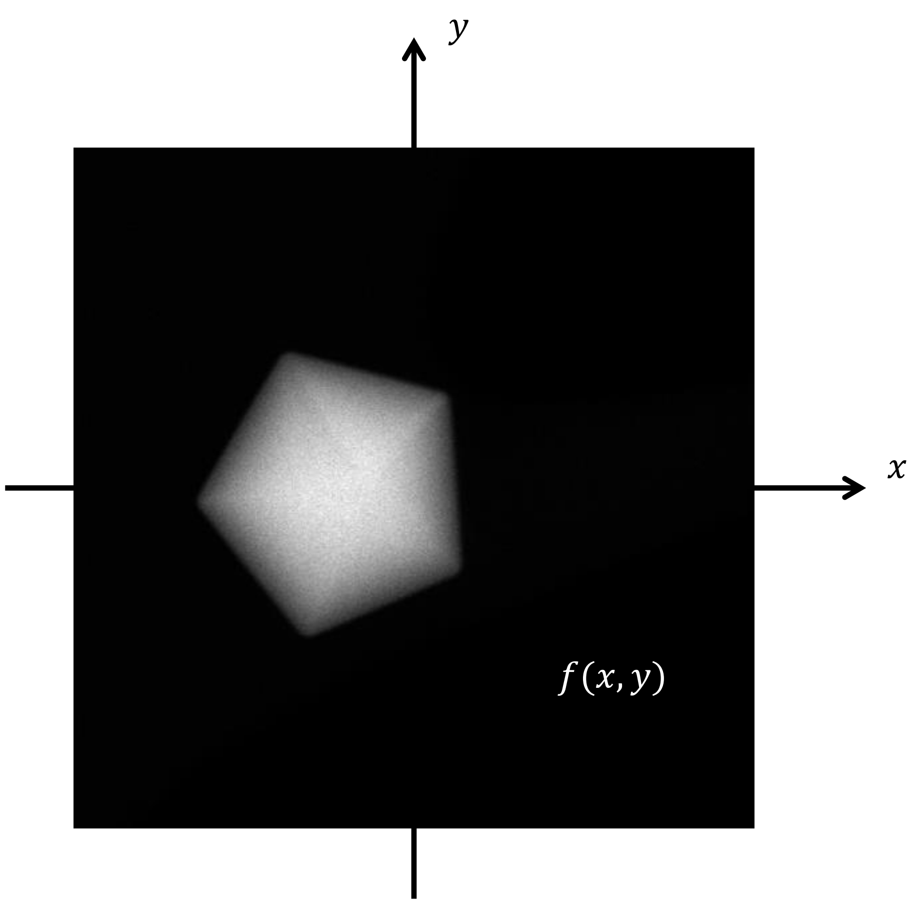
Projections are *smeared* back into the space at the angle at which they were acquired. The summation of intensities generate the original object

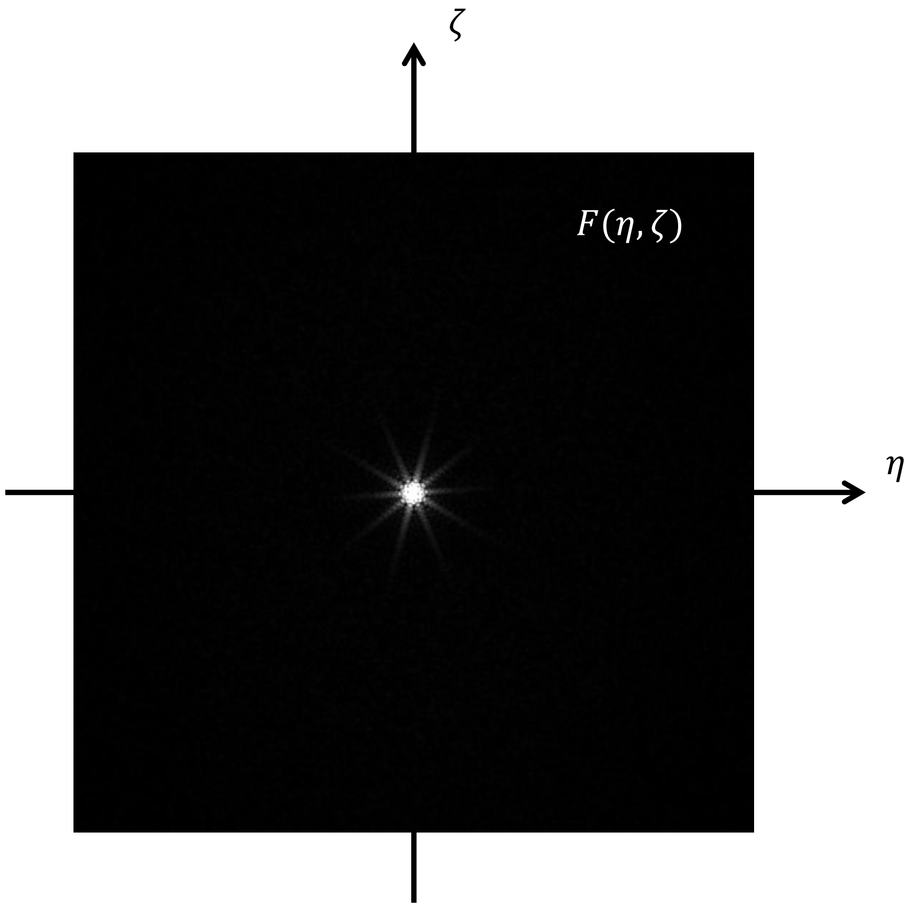


angles

1

Inverting the Radon transform: in Fourier space



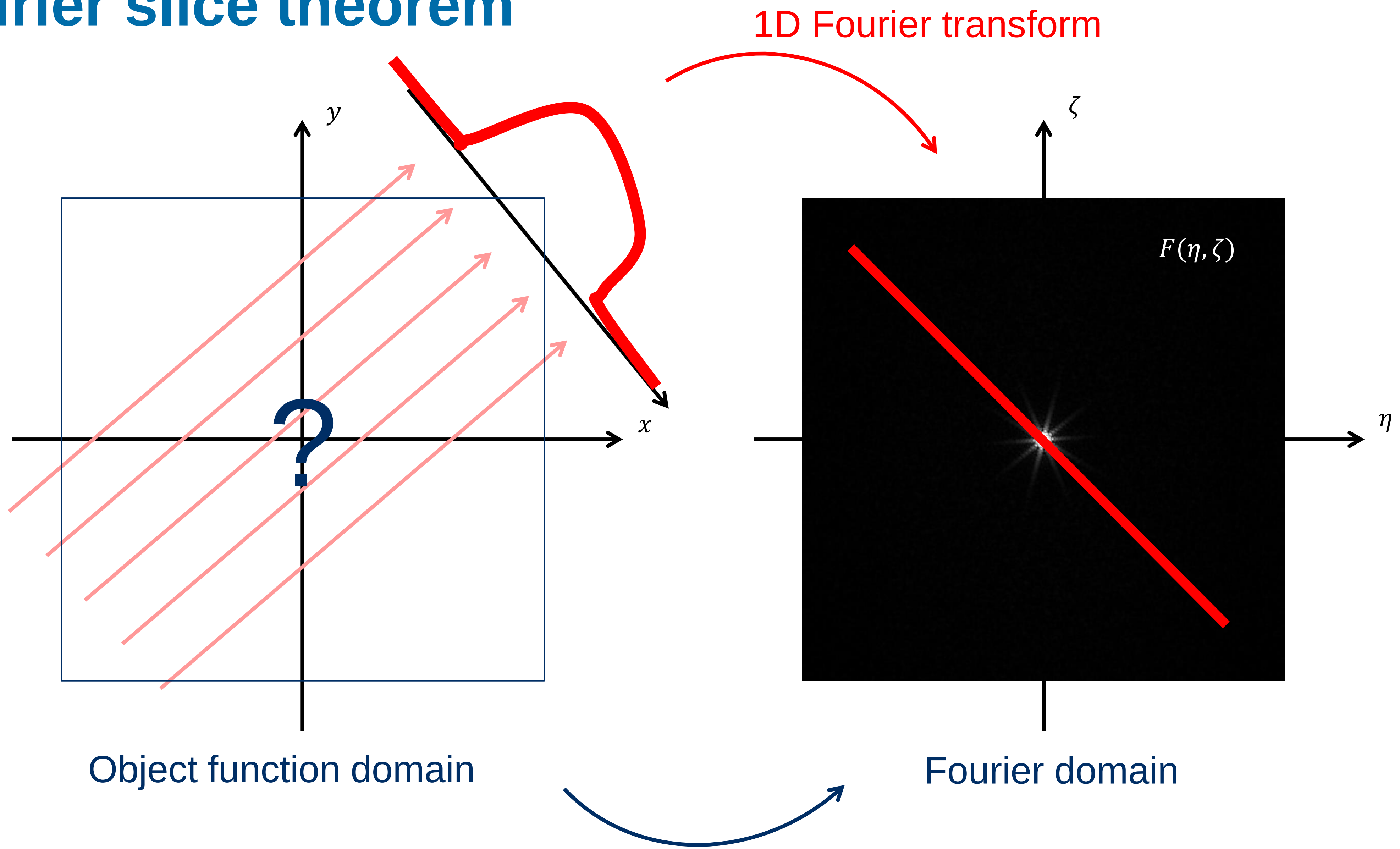


Object function domain

Fourier domain

2D Fourier transform

Fourier slice theorem



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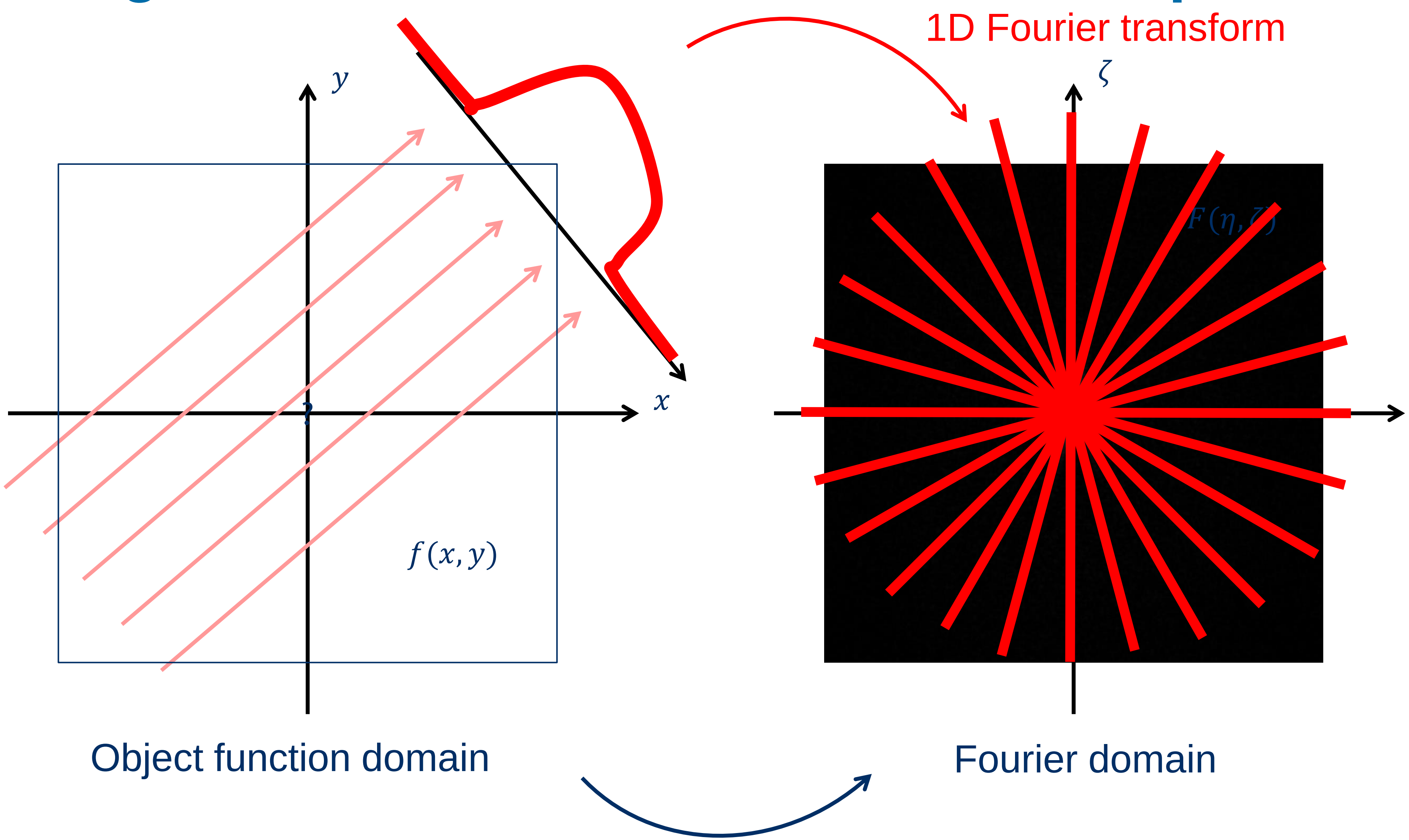
Acquisition
Alignment
Reconstruction
Quantification

Inverting the Radon transform: in Fourier space

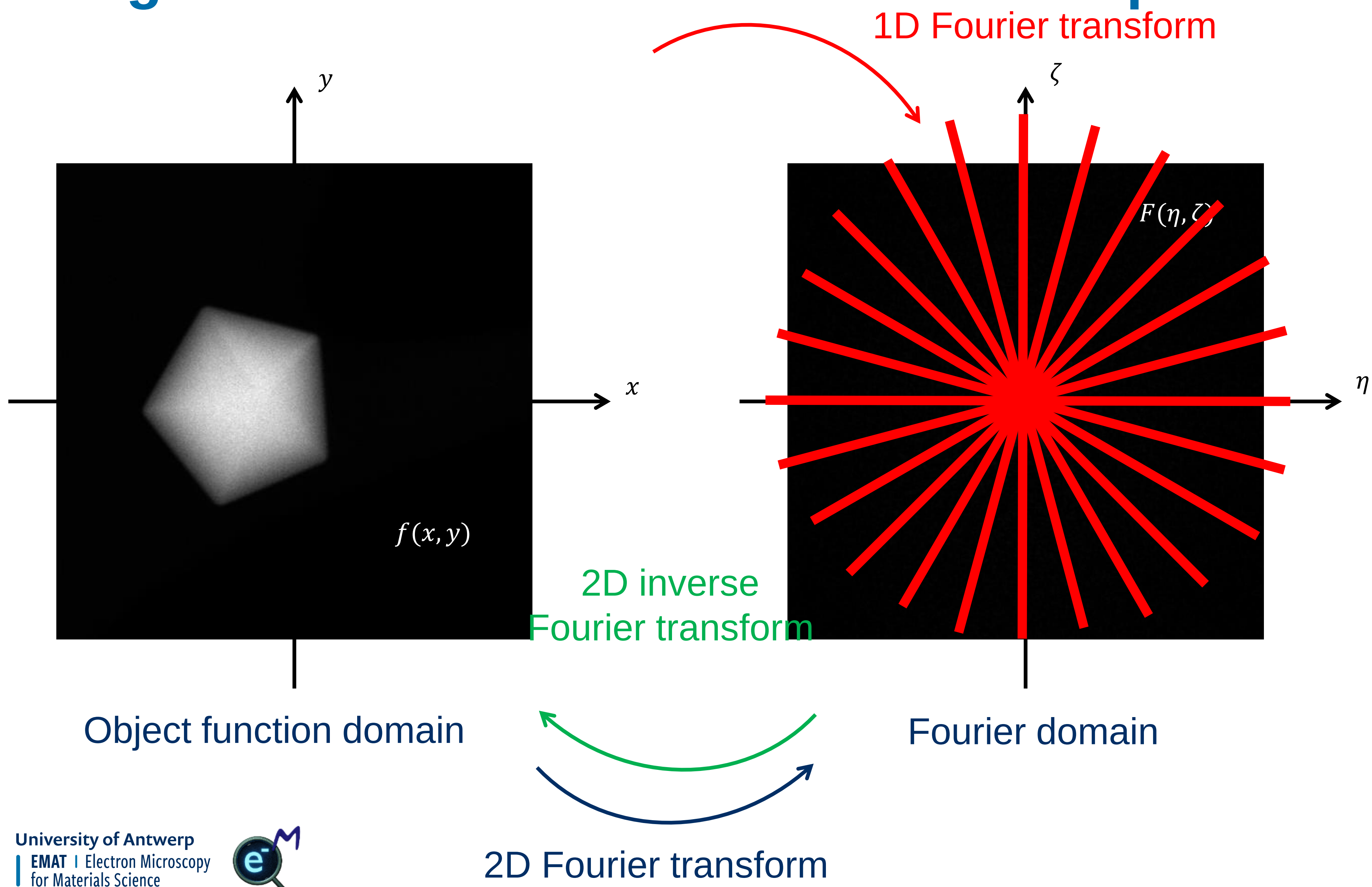


Object function domain

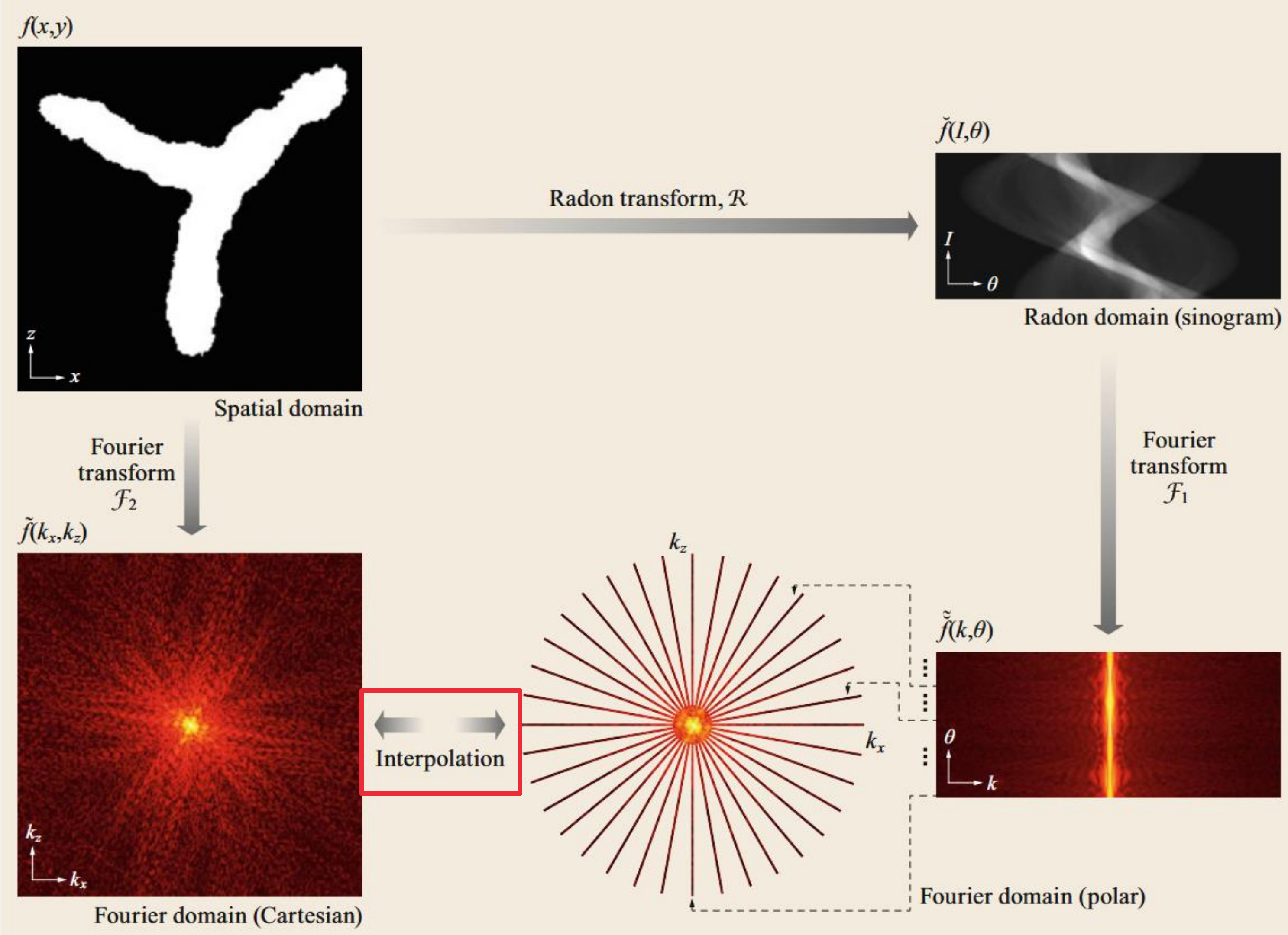
Fourier domain

2D Fourier transform

Inversing the Radon transform: in Fourier space



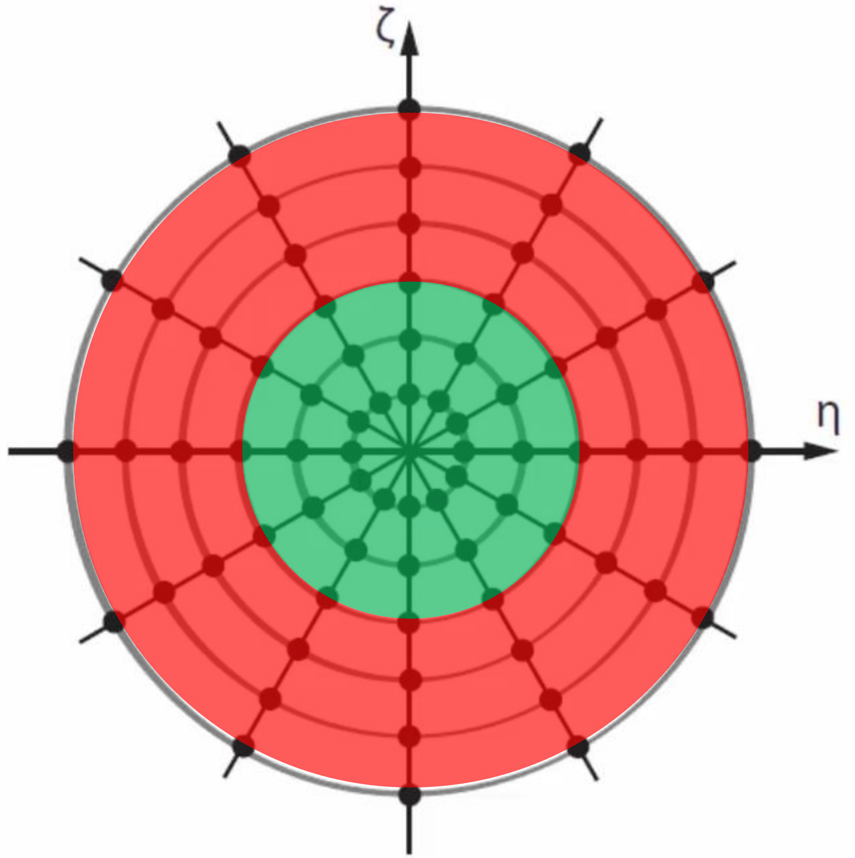
Inverting the Radon transform: in Fourier space



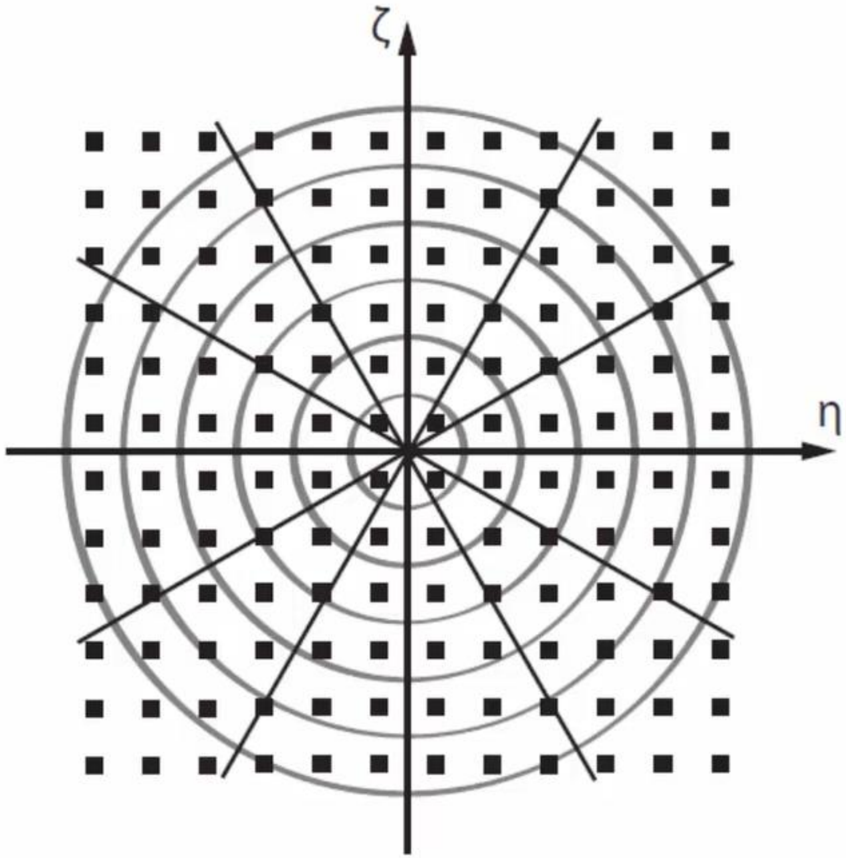
Inversion in Fourier space builds on the **Fourier slice theorem**:

The Fourier transform of a projection is a slice through the Fourier transform of the original object

Inverting the Radon transform: in Fourier space



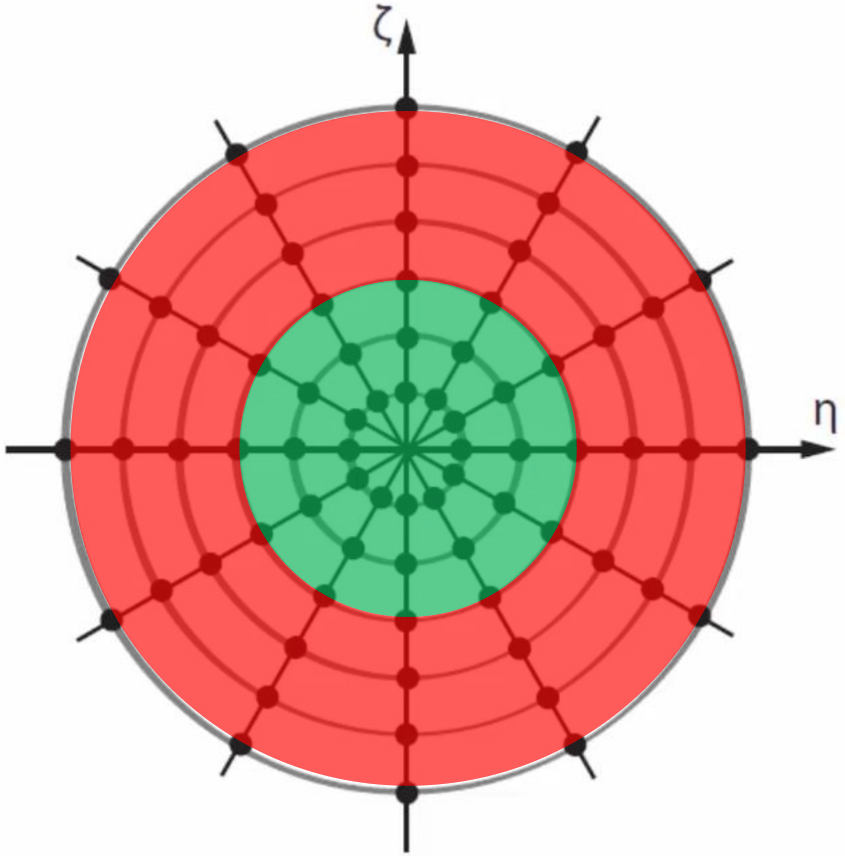
Fourier sampling with
Fourier slice theorem



Sampling required by Fast
Fourier Transform

2 problems: 1) **interpolation** is needed 2) sampling in Fourier space is **uneven**

Inverting the Radon transform: in Fourier space



Fourier sampling with
Fourier slice theorem

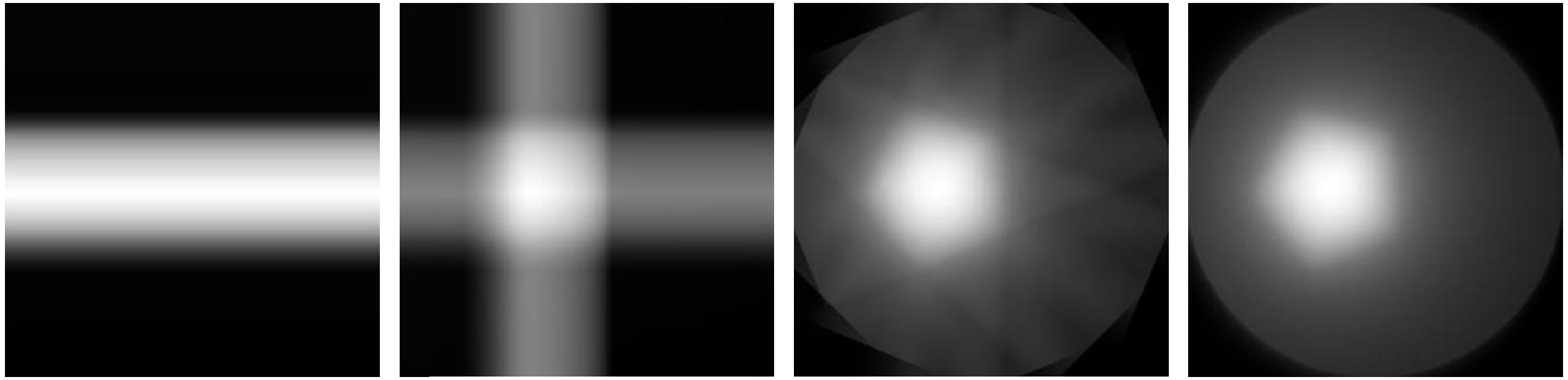
Why is an uneven sampling an issue? How will the reconstruction look like?

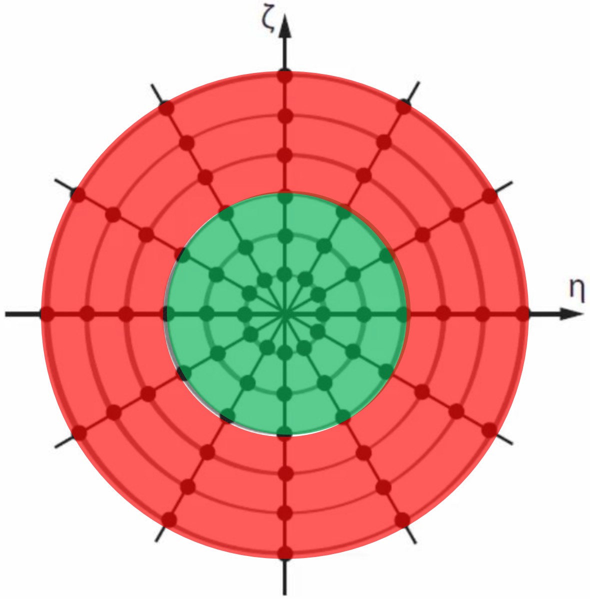
A. The intensity will be low
B. Sharp features will be blurred ✓
C. The contrast will be low

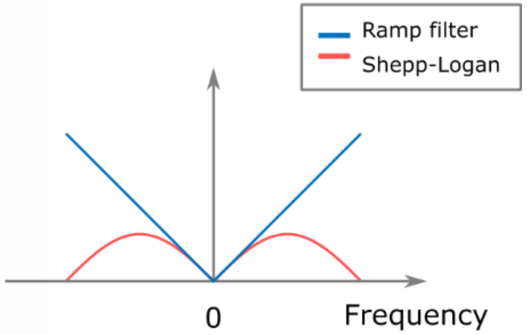
Effect of the uneven sampling

	1 angle	2 angles	8 angles	180 angles

Backprojection







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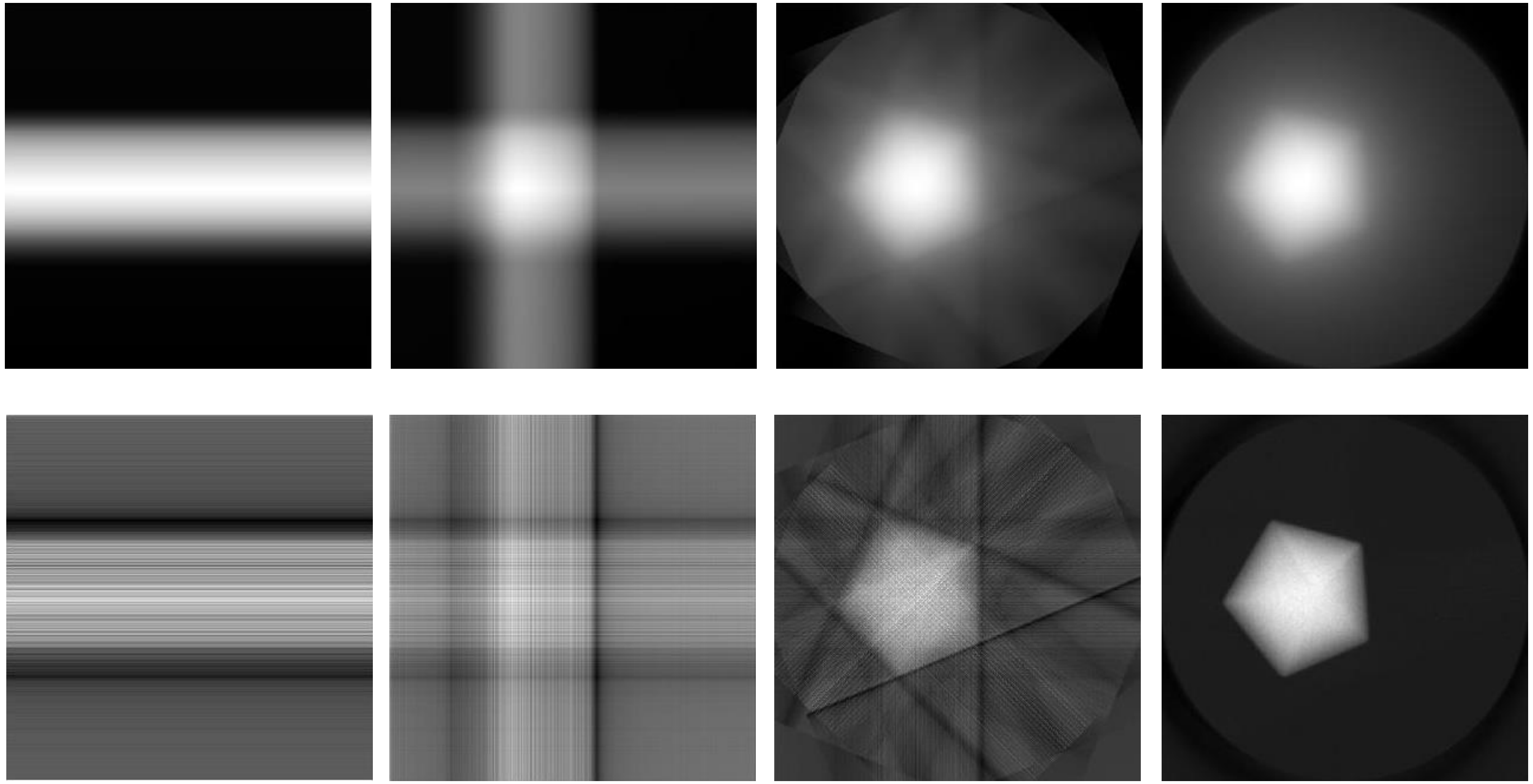
Filtered backprojection

1 angle | 2 angles | 8 angles | 180 angles

Backprojection

Filtered Backprojection



Advantages: computationally efficient, resolution independent.

Shortcomings: sensitive to noise (high-pass filter, streaky artefacts due to reduced number of measurements

Iterative and advanced reconstruction

General principle: the reprojections of a computed reconstruction should be equal (as much as possible) to the experimental data

Example: the simultaneous iterative reconstruction (SIRT) *iteratively* updates the reconstruction with the difference to the acquired projections

Simultaneous Iterative Reconstruction Technique (SIRT)

Backprojection operator

Forward projection operator

$$v^{(k+1)} = v^{(k)} + CW^T R\left(p - W v^{(k)}\right)$$

Experimental projections

Reconstruction at step k

Weighted projection difference

Weighted backprojection

Update

$$R \in \mathbb{R}^{m\times m} \qquad r_{ii} = 1 \,/ \sum_{j=0}^{n-1} w_{ij}$$

$$C \in \mathbb{R}^{n\times n} \qquad c_{jj} = 1 \,/ \sum_{i=0}^{m-1} w_{ij}$$

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Simultaneous Iterative Reconstruction Technique (SIRT)

$$v^{(k+1)} = v^{(k)} + CW^T R\left(p - Wv^{(k)}\right)$$

Weighted projection difference

Weighted backprojection

Update

$$R \in \mathbb{R}^{m\times m} \qquad r_{ii} = 1 \,/ \sum_{j=0}^{n-1} w_{ij}$$

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Weighted backprojection

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$$C \in \mathbb{R}^{n \times n} \qquad c_{jj} = 1 \,/\, \sum_{i=0}^{m-1} w_{ij}$$

$v^* = argmin_v \|p - Wv\|_R$ with $\|x\|_R = x^T R x$

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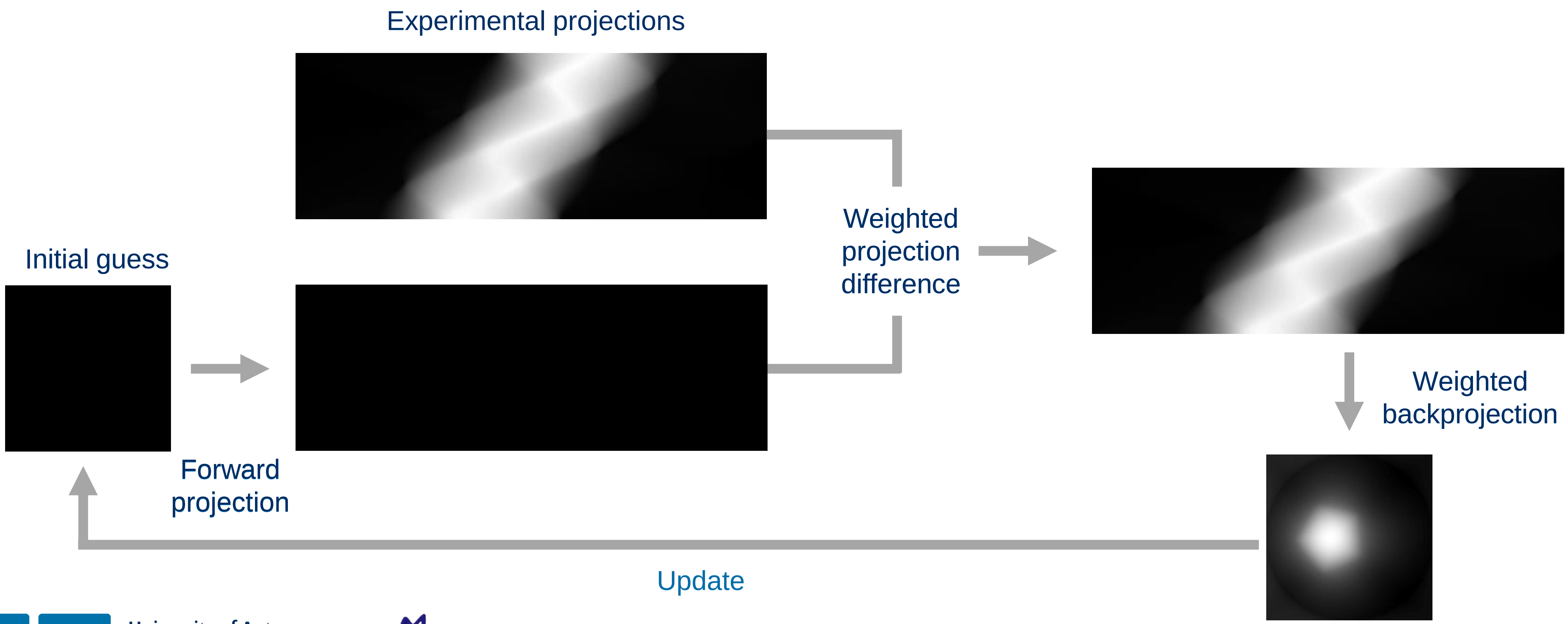
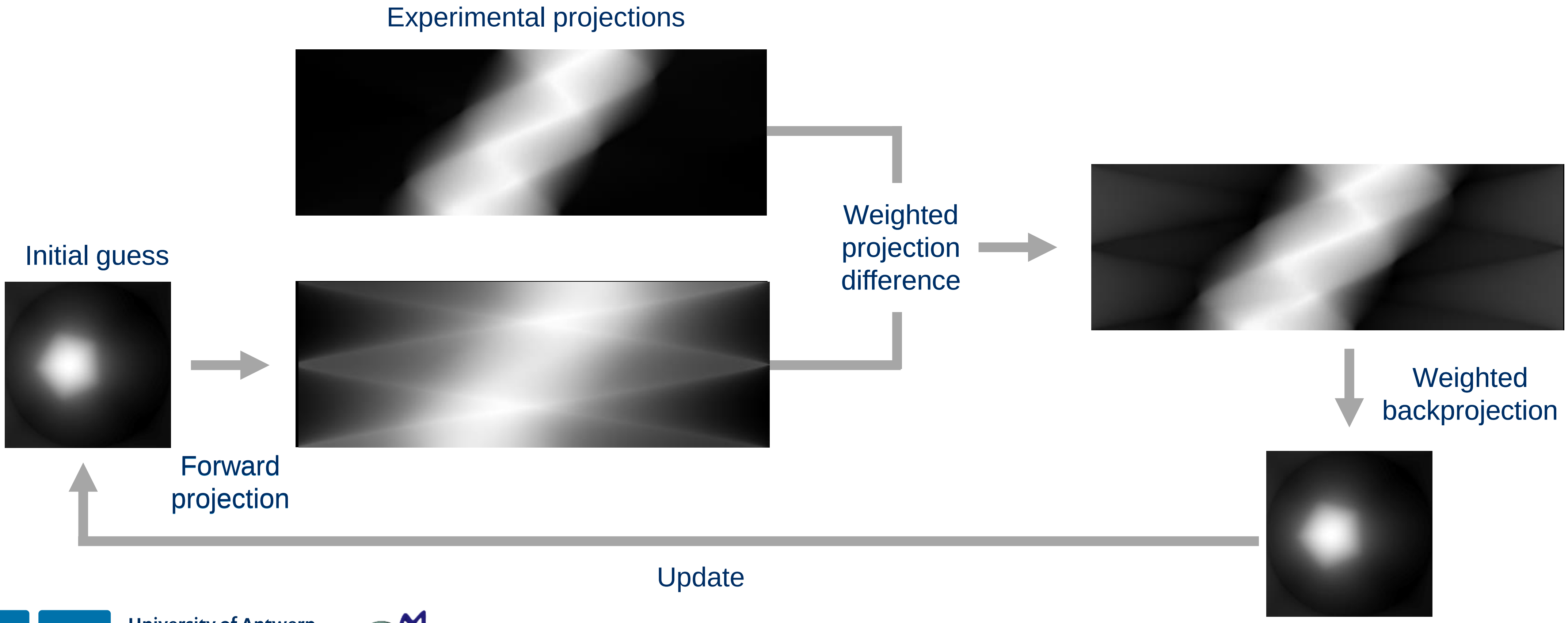
Simultaneous Iterative Reconstruction Technique (SIRT)

$$v^{(1)} = v^{(0)} + CW^{T}R\left(p - Wv^{(0)}\right)$$



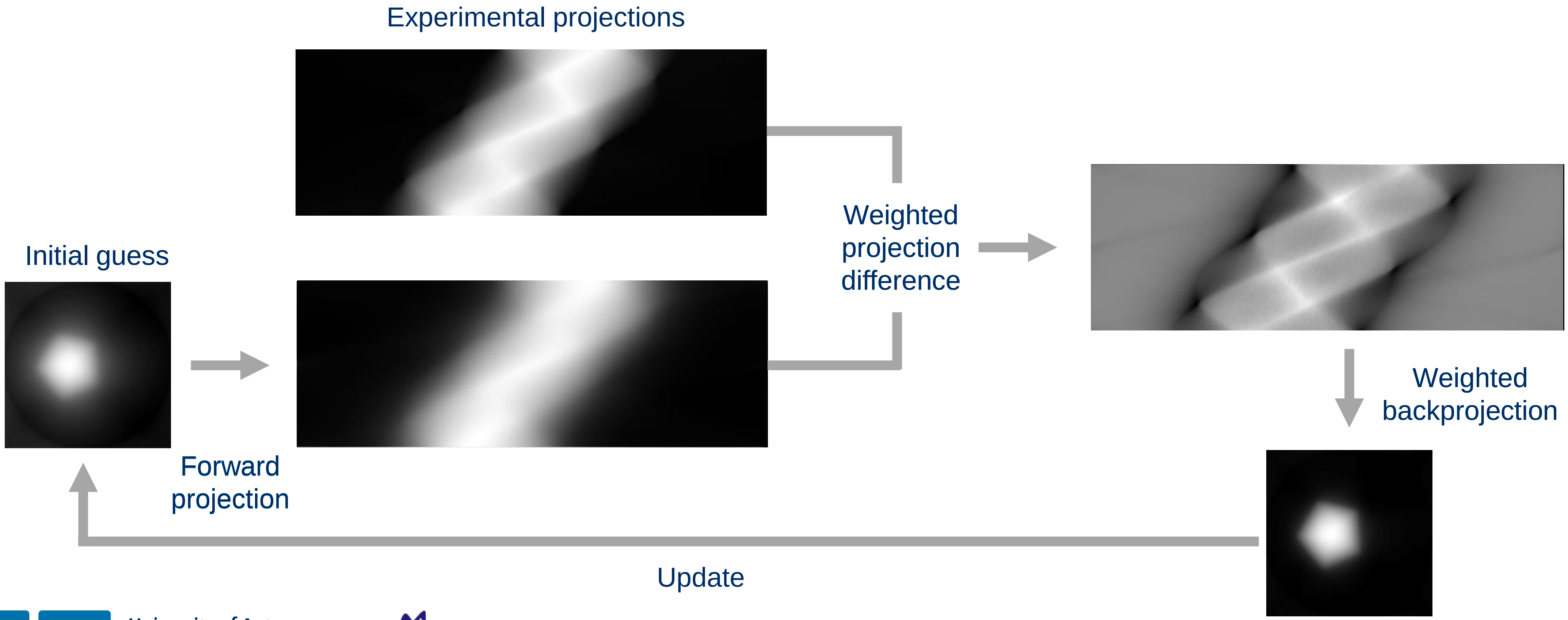
Simultaneous Iterative Reconstruction Technique (SIRT)

$$v^{(1)} = v^{(0)} + CW^{T}R\left(p - Wv^{(0)}\right)$$

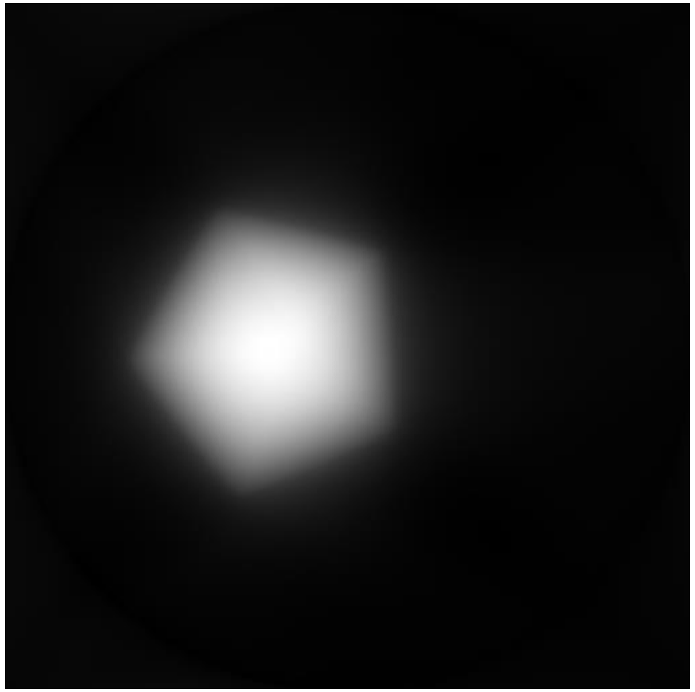


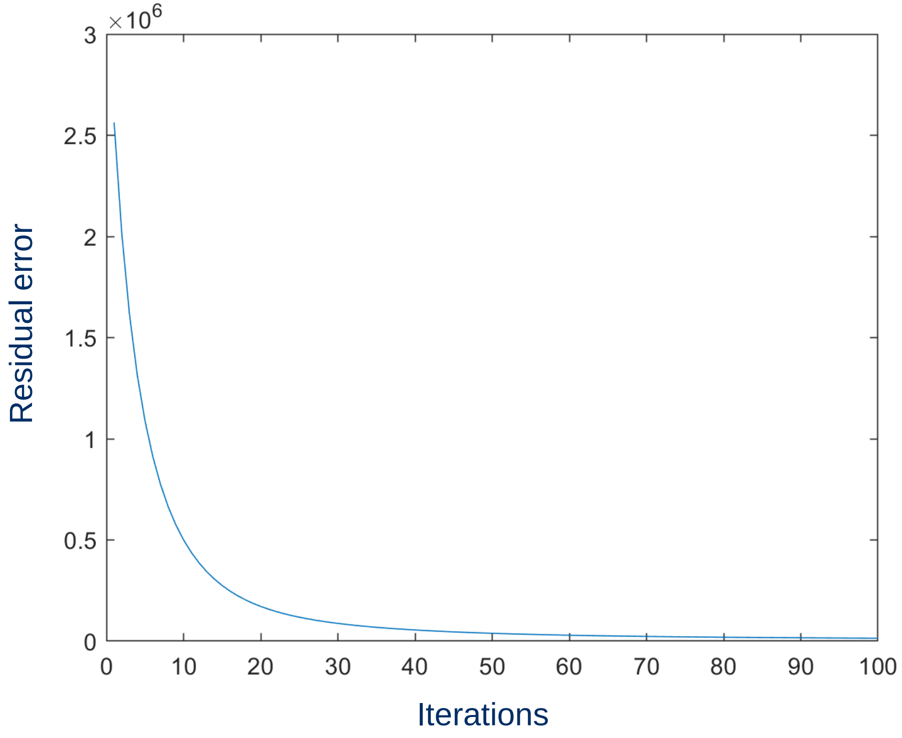
Simultaneous Iterative Reconstruction Technique (SIRT)

$$v^{(1)} = v^{(0)} + CW^T R\left(p - Wv^{(0)}\right)$$



Simultaneous Iterative Reconstruction Technique (SIRT)





Number of iterations: 20-100

Advantages: fast computation, stable solution, fairly robust to noise

Shortcomings: streaky artefacts, boundary effects

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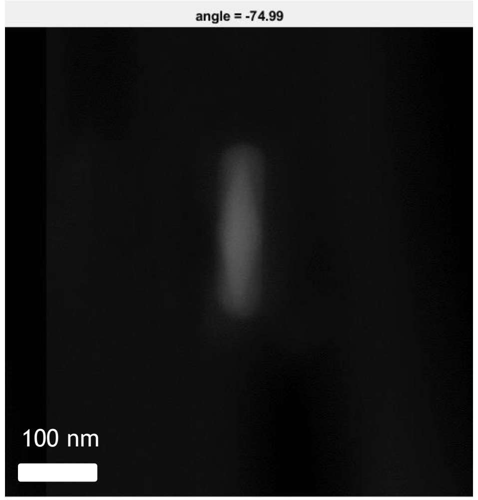
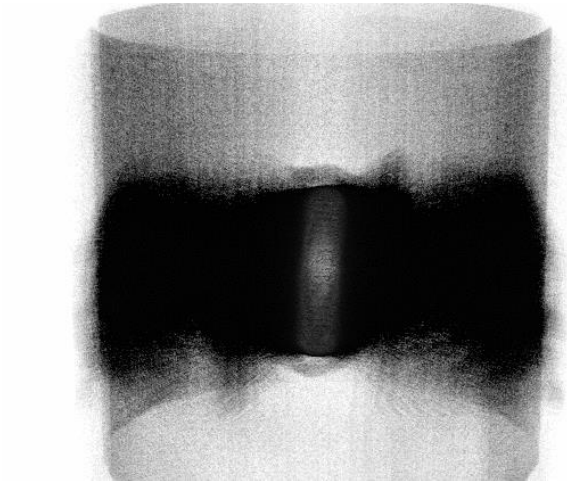
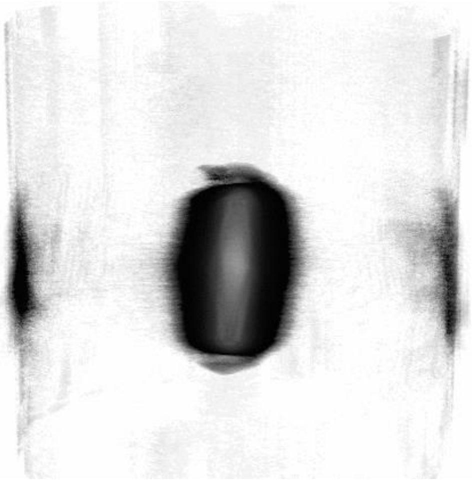
Comparison

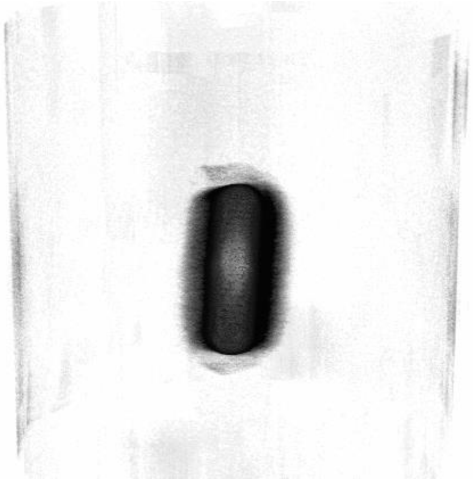
Gold+Silver Nanorod: 53 angles between -75 to 75 degrees

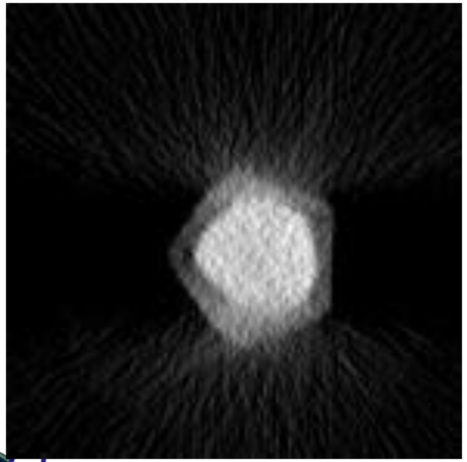
Tilt-series	FBP	SIRT	EM

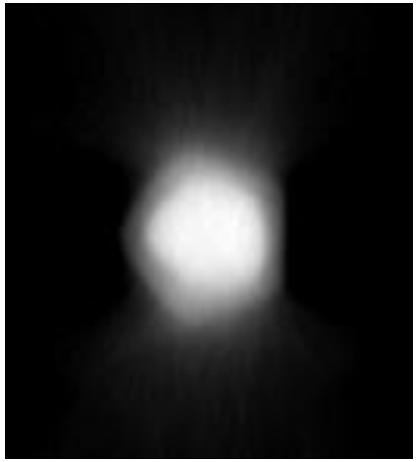


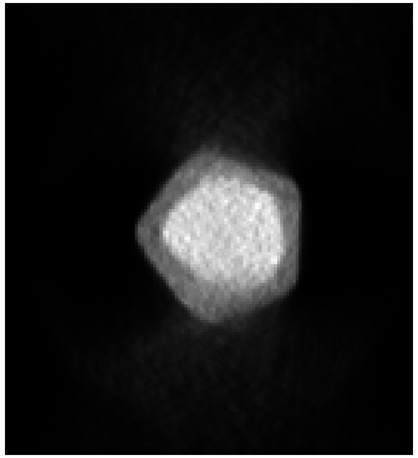












Algorithms

Practical:

Filtered Backprojection (FBP)

Expectation Maximization (EM)

Simultaneous Iterative Reconstruction Technique (SIRT)

Advanced:

L1 regularization (LASSO)

Total-Variation Minimization (TVM)

Discrete Algebraic Reconstruction Technique (DART)

Iterative and advanced reconstructions

General principle I: the reprojections of a computed reconstruction should be equal (as much as possible) to the experimental data

And the data can be forced to be consistent with constraints

Typical example: TV minimization solves the problem

$$\hat{\mathbf{x}}_\lambda = \arg\min_{\hat{\mathbf{x}}} \left\{ \|\Phi\hat{\mathbf{x}} - \mathbf{b}\|_{\ell_2}^2 + \lambda \underline{\|\Psi\hat{\mathbf{x}}\|}_{\ell_1} \right\}$$

reconstruction

(re)projection operator

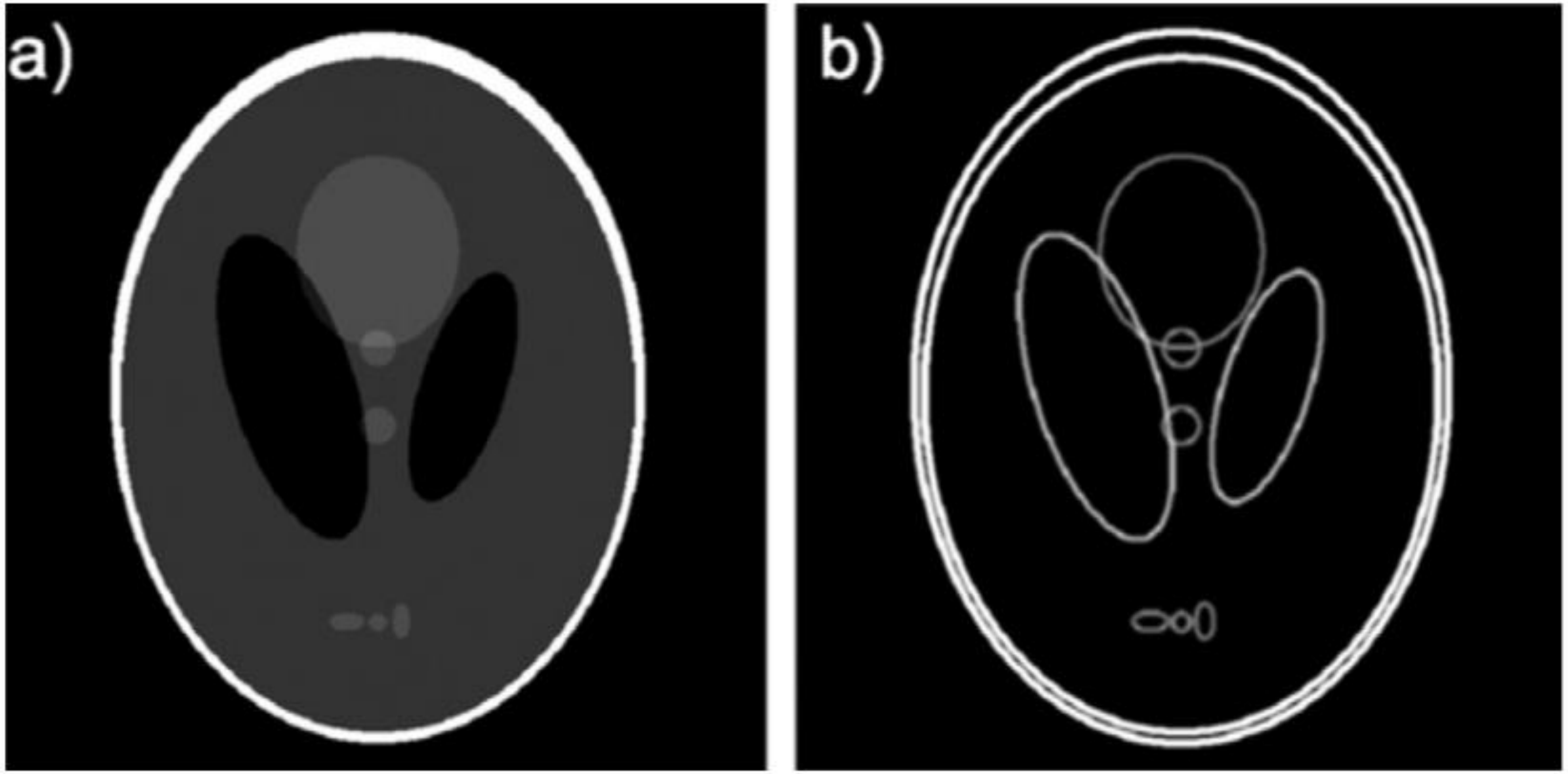
experimental acquisition

TV constraint (minimizes the "number" of edges) when ψ is the image gradient operator

(Image gradients?)

Image

Image gradient



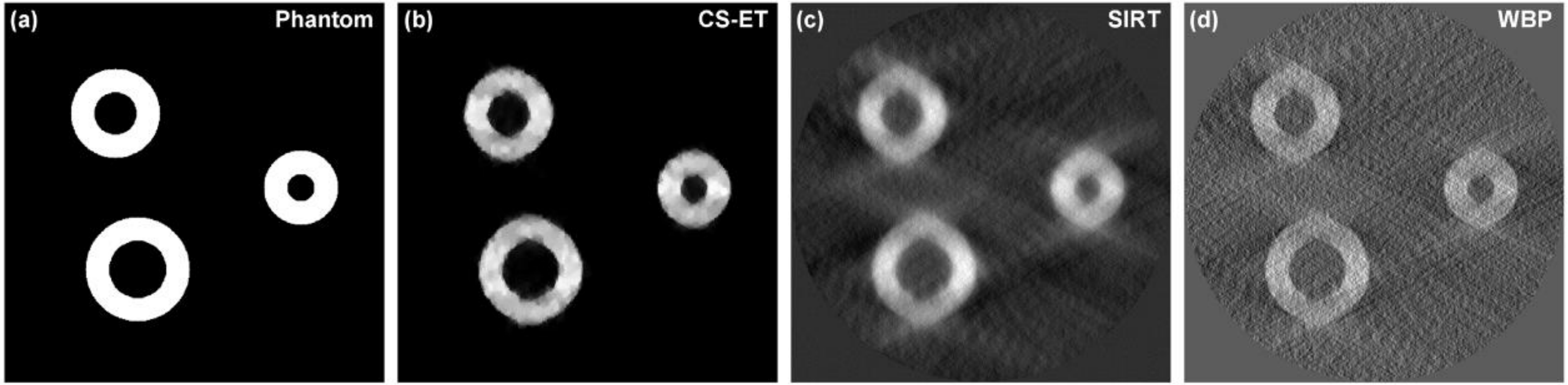
TV operator

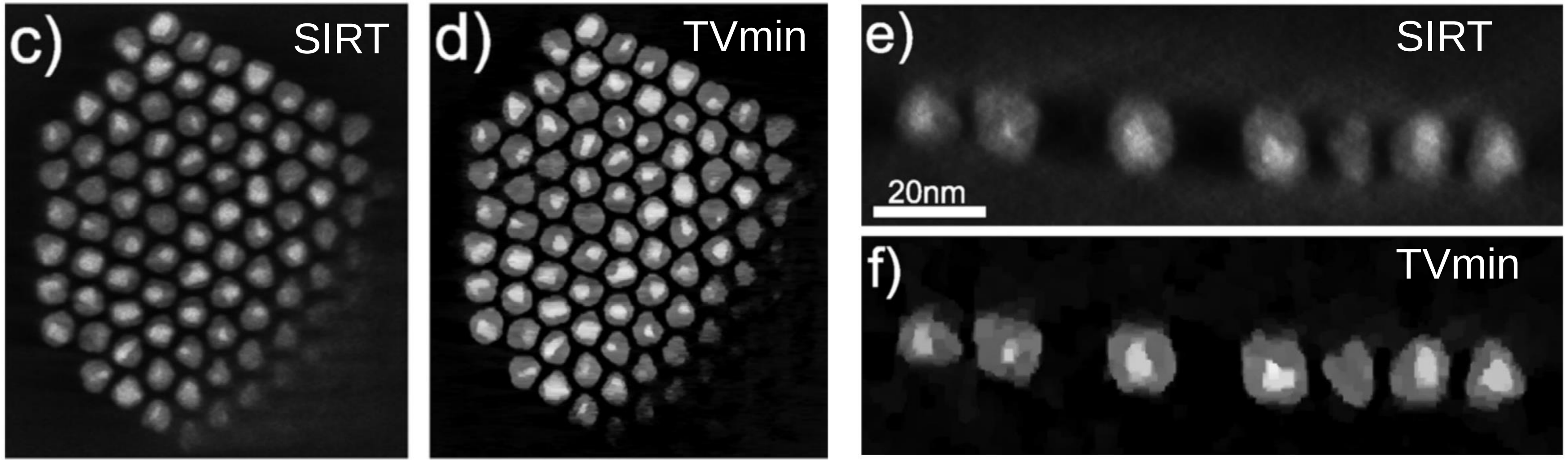
(L1 norm of the gradients)

$$\|\Psi\hat{\mathbf{x}}\|_{\ell_1}$$

Leary, R., *et al.* *Ultramicroscopy* **131**, 70–91 (2013)
Goris, B., *et al.* *Ultramicroscopy* **113**, 120-130 (2012)

TV minimization / compressed sensing ET





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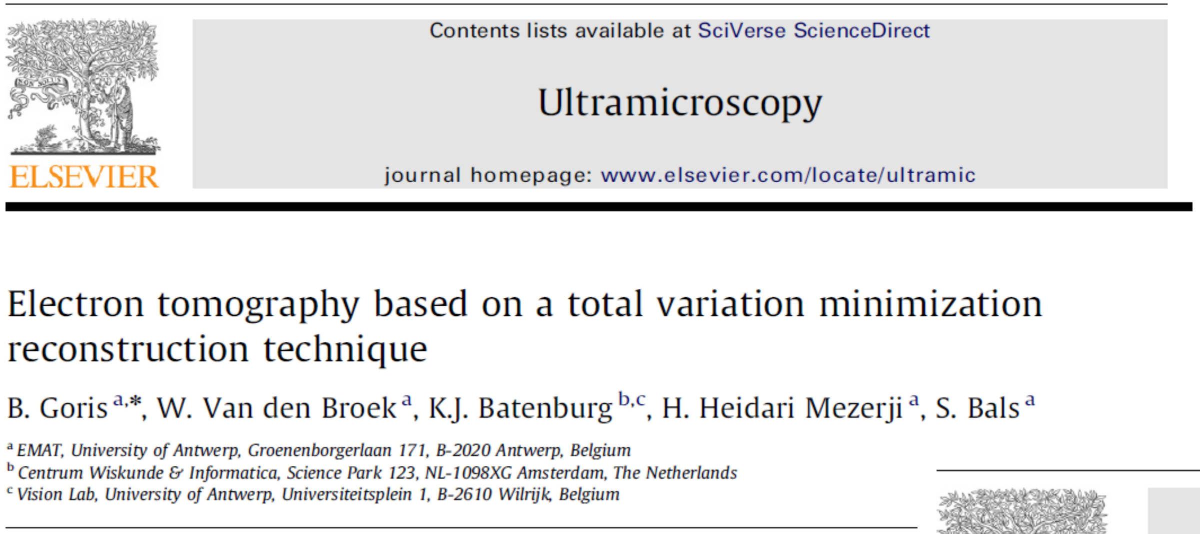
Leary, R., *et al.* *Ultramicroscopy* **131**, 70–91 (2013)
Goris, B., *et al.* *Ultramicroscopy* **113**, 120-130 (2012)

TV minimization / compressed sensing ET



Contents lists available at SciVerse ScienceDirect

Ultramicroscopy

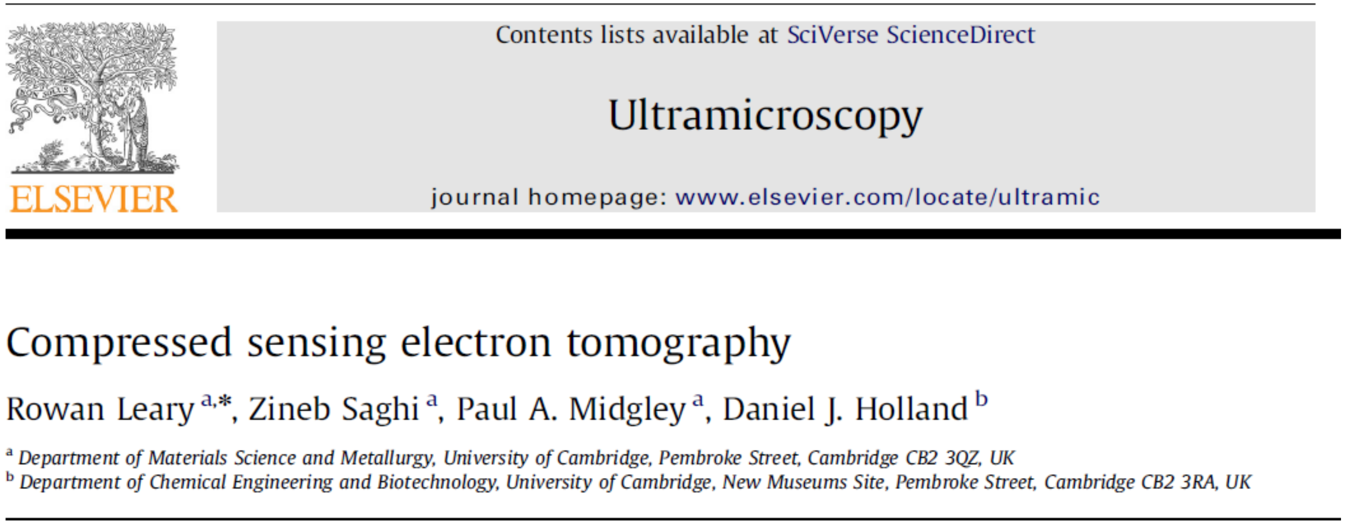
journal homepage: www.elsevier.com/locate/ultramic

ELSEVIER

Electron tomography based on a total variation minimization reconstruction technique

B. Goris [a,*], W. Van den Broek [a], K.J. Batenburg [b,c], H. Heidari Mezerji [a], S. Bals [a]

[a] EMAT, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium
[b] Centrum Wiskunde & Informatica, Science Park 123, NL-1098XG Amsterdam, The Netherlands
[c] Vision Lab, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium



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journal homepage: www.elsevier.com/locate/ultramic

ELSEVIER

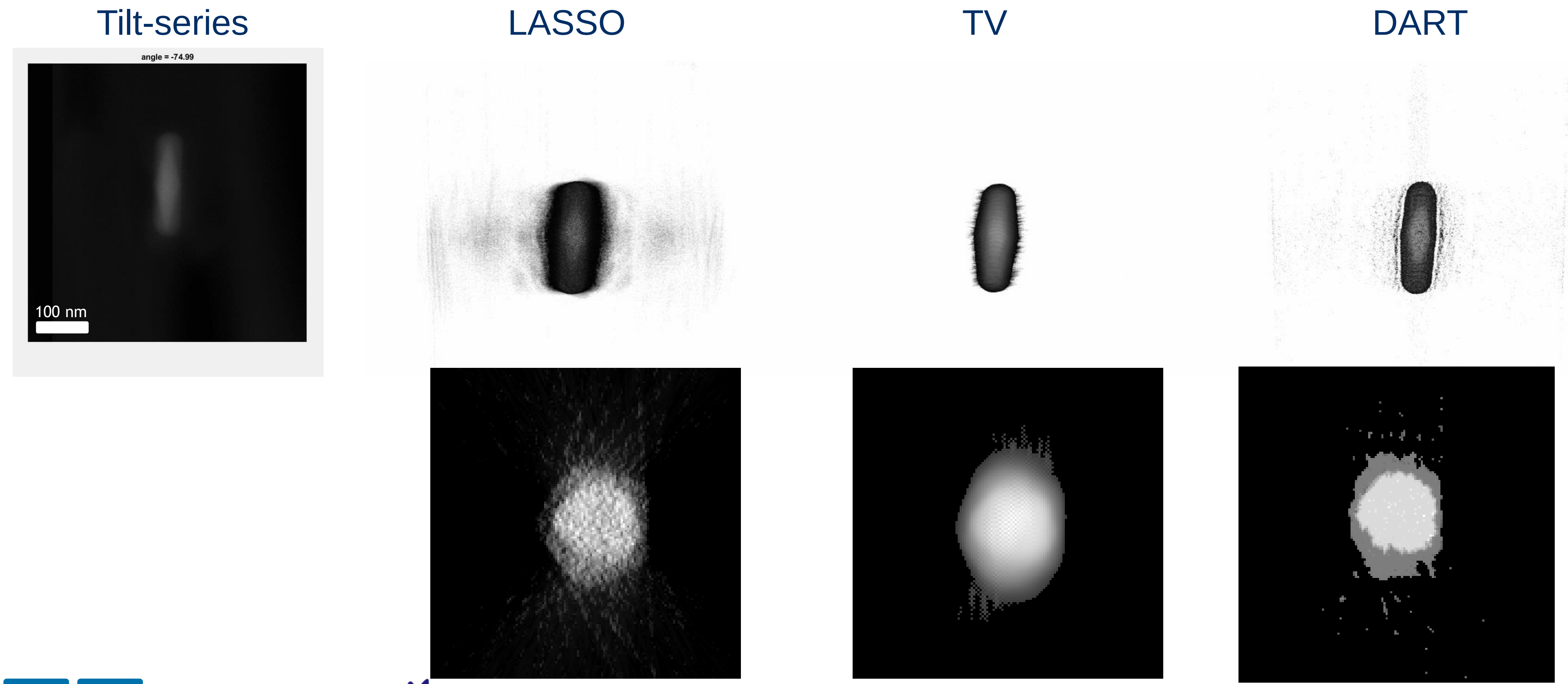
Compressed sensing electron tomography

Rowan Leary [a,*], Zineb Saghi [a], Paul A. Midgley [a], Daniel J. Holland [b]

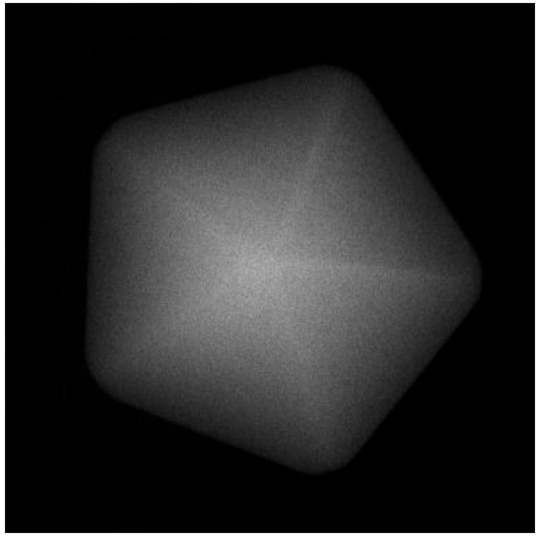
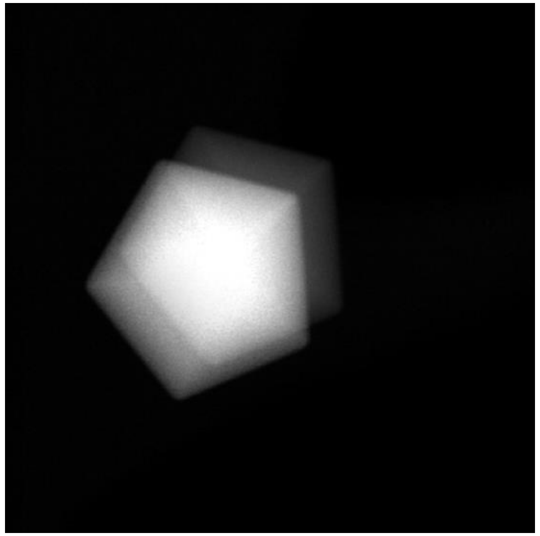
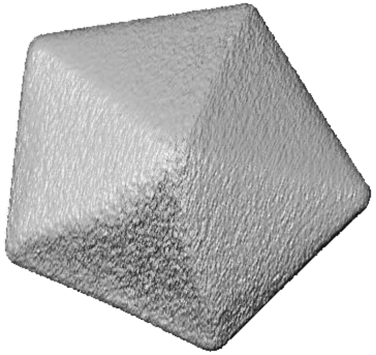
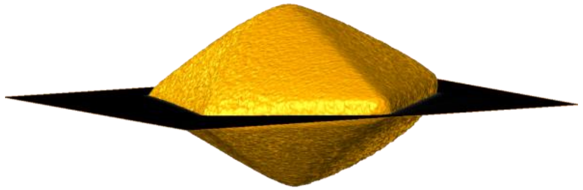
[a] Department of Materials Science and Metallurgy, University of Cambridge, Pembroke Street, Cambridge CB2 3QZ, UK
[b] Department of Chemical Engineering and Biotechnology, University of Cambridge, New Museums Site, Pembroke Street, Cambridge CB2 3RA, UK

Comparison

Gold+Silver Nanorod: 14 angles between -75 to 75 degrees

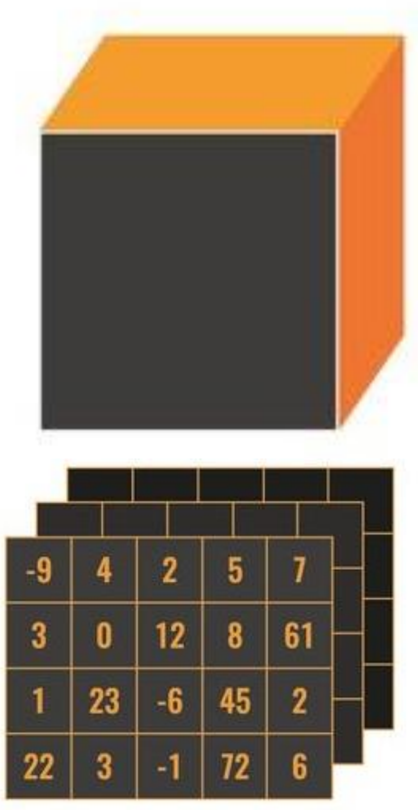


Tomography experiment

Acquisition	Alignment	Reconstruction	Quantification
			

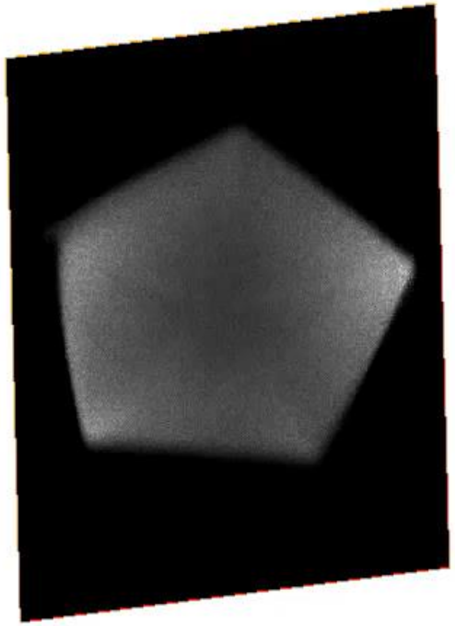
Visualization

Reconstruction output

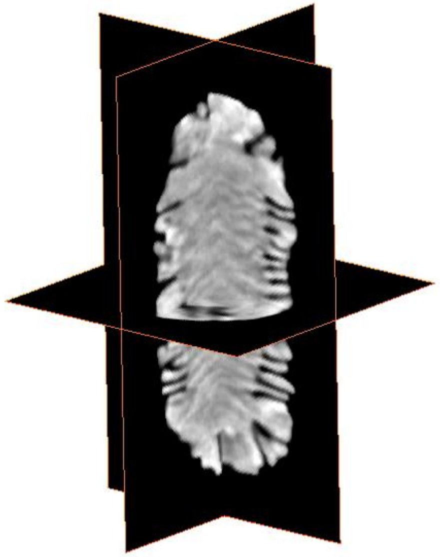


Data cube, volume, 3D tensor, …

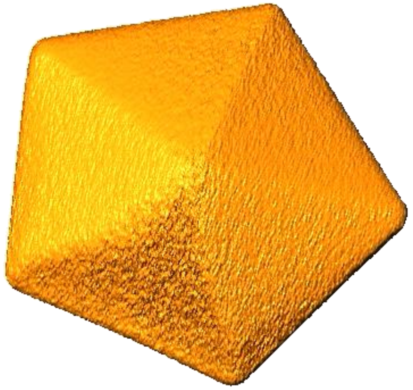
Orthoslices



Multiorthoslices



3D surface rendering



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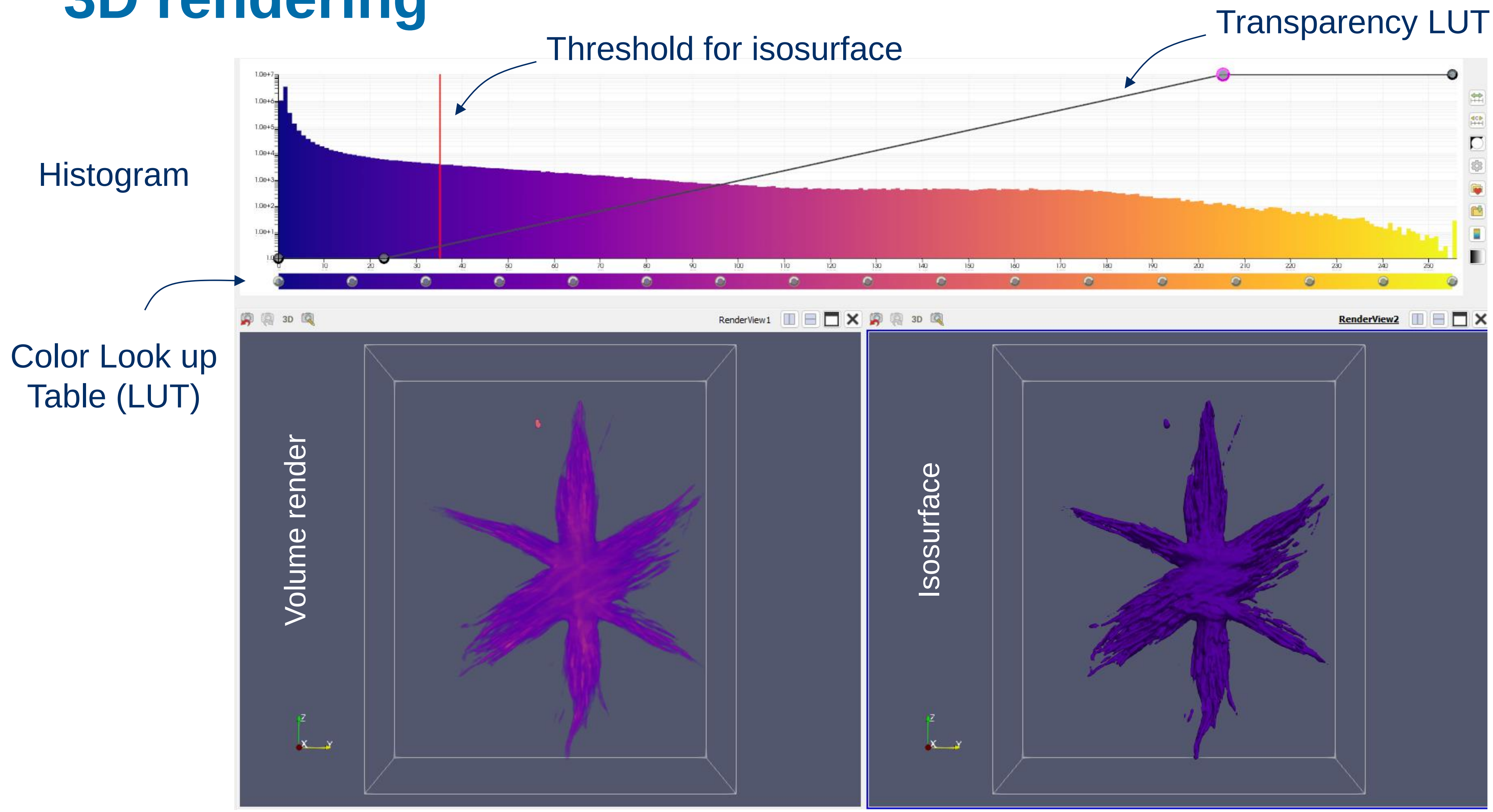
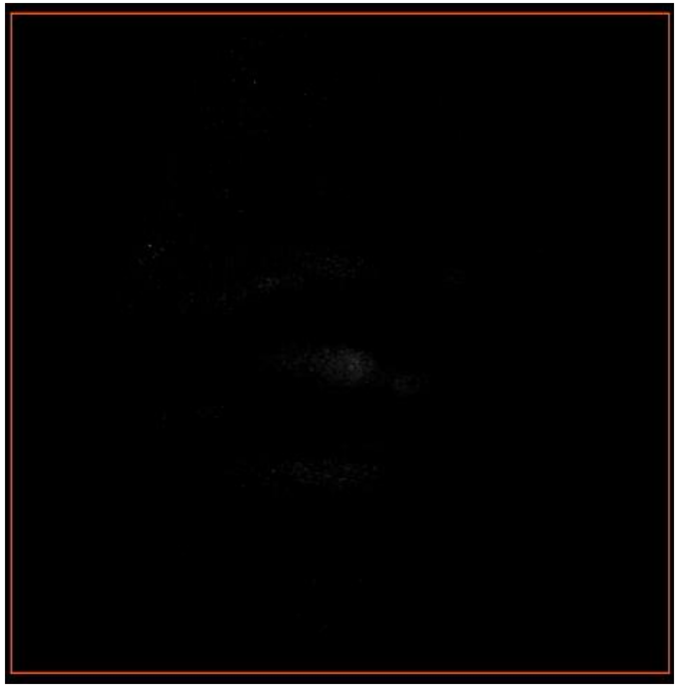
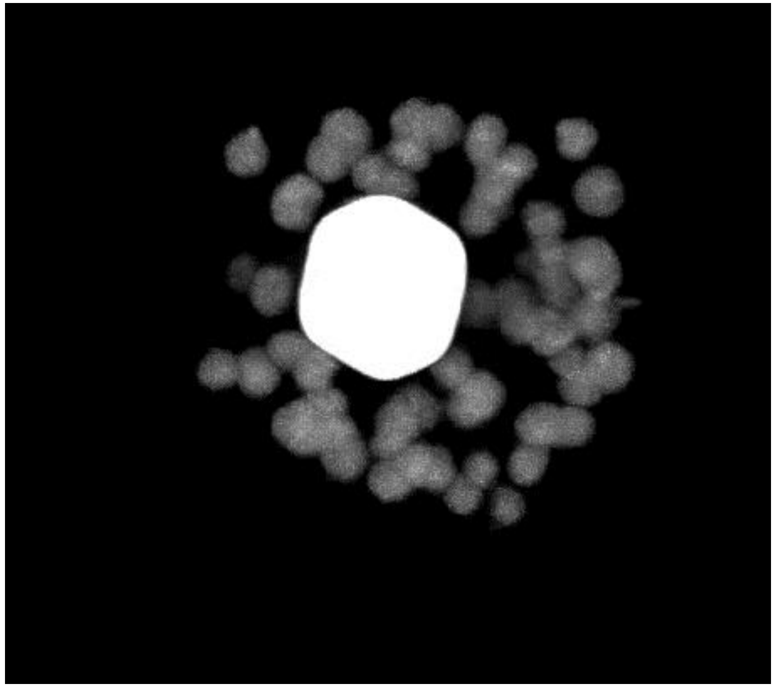
3D rendering



The analysis workflow





Reconstruction → Post-processing (denoising) → Segmentation → Quantification Analysis Statistics

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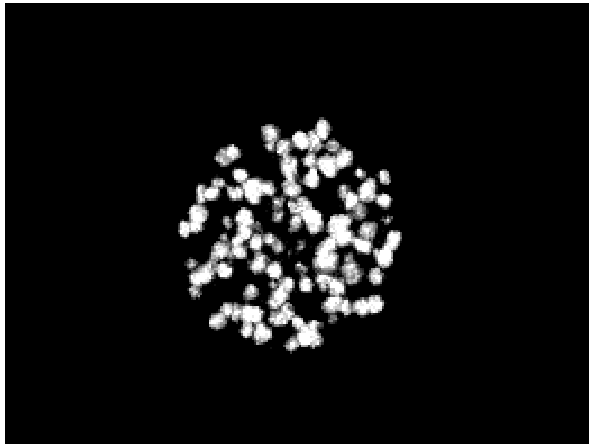
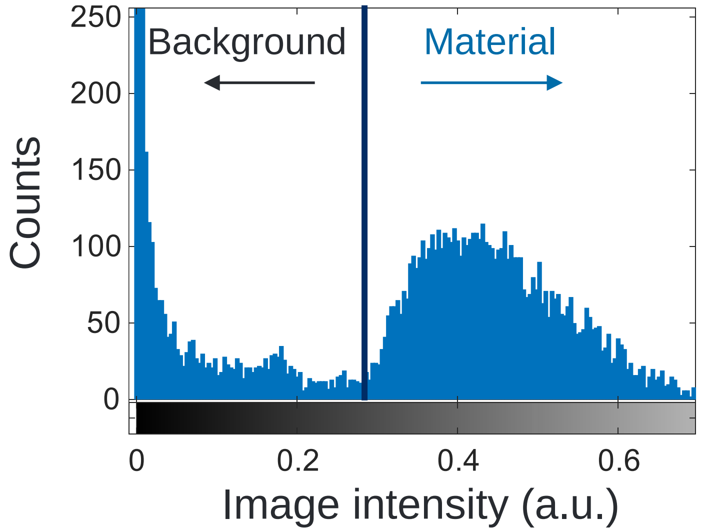
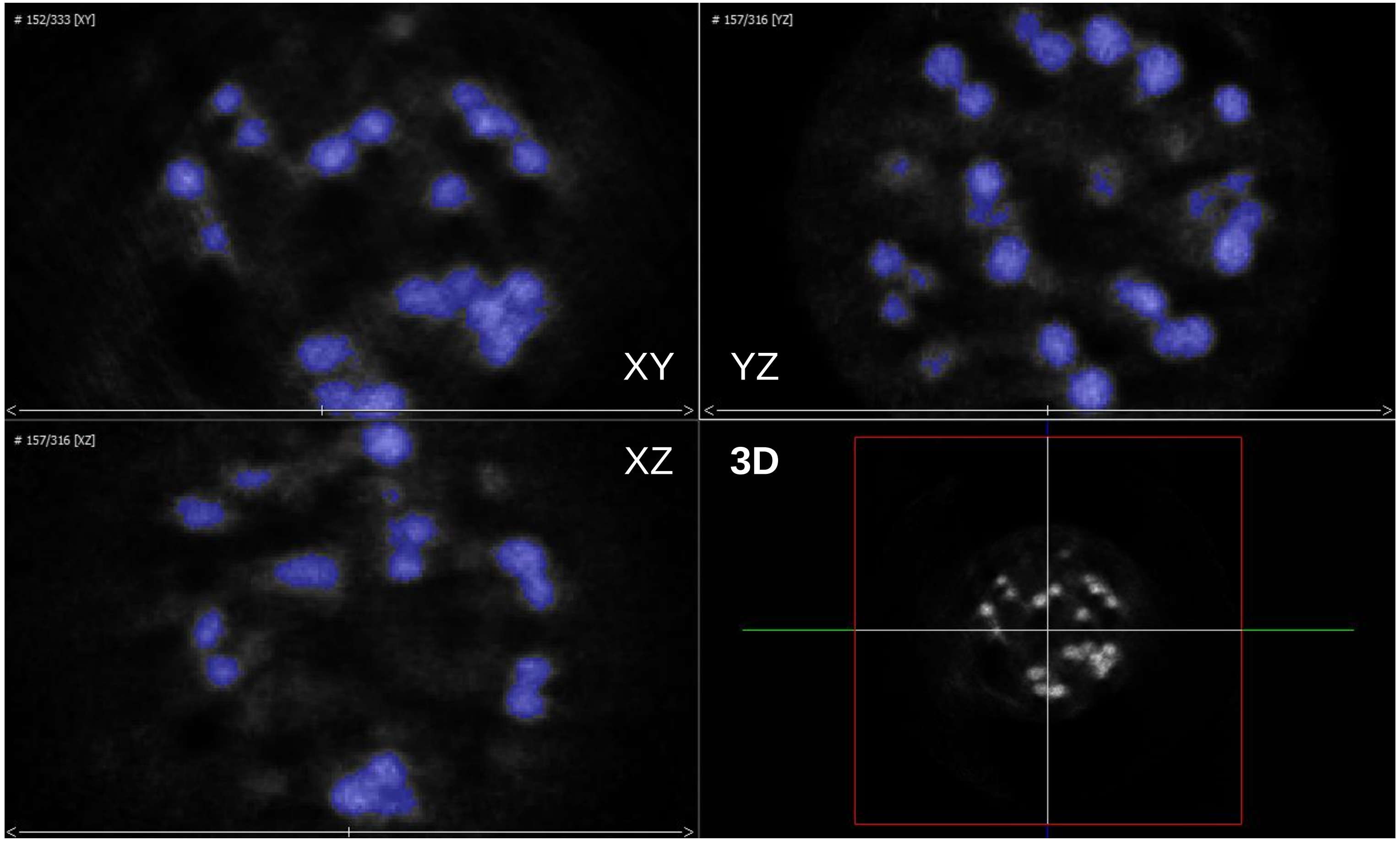
S. Kavak, A. Anil Kadu, N. Claes, A. Sánchez-Iglesias, L. M. Liz-Marzán, K. J. Batenburg, S. Bals, J. Phys. Chem. C (2023)

Segmentation: manual threshold







Segmentation aims at attributing a **class** to each pixel (voxel)

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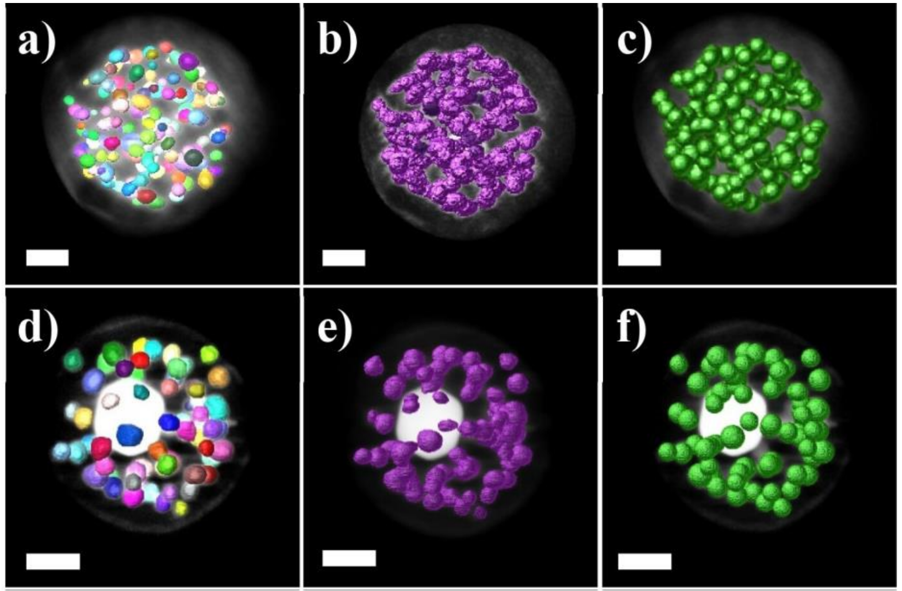
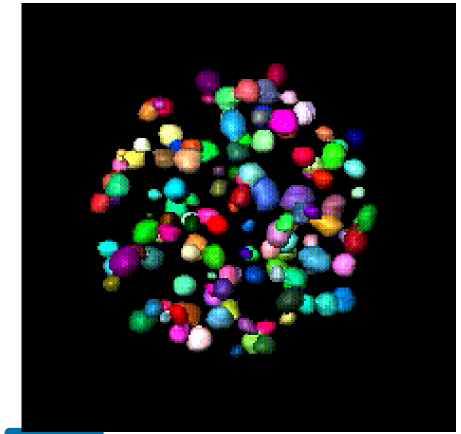
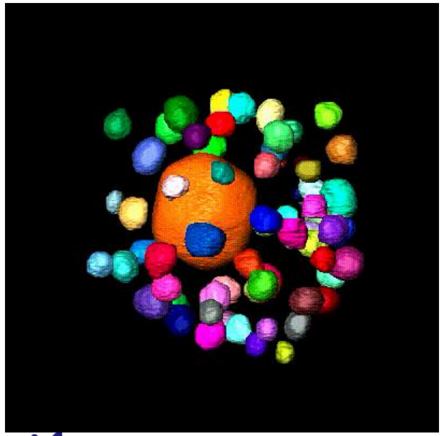
Segmentation: automated

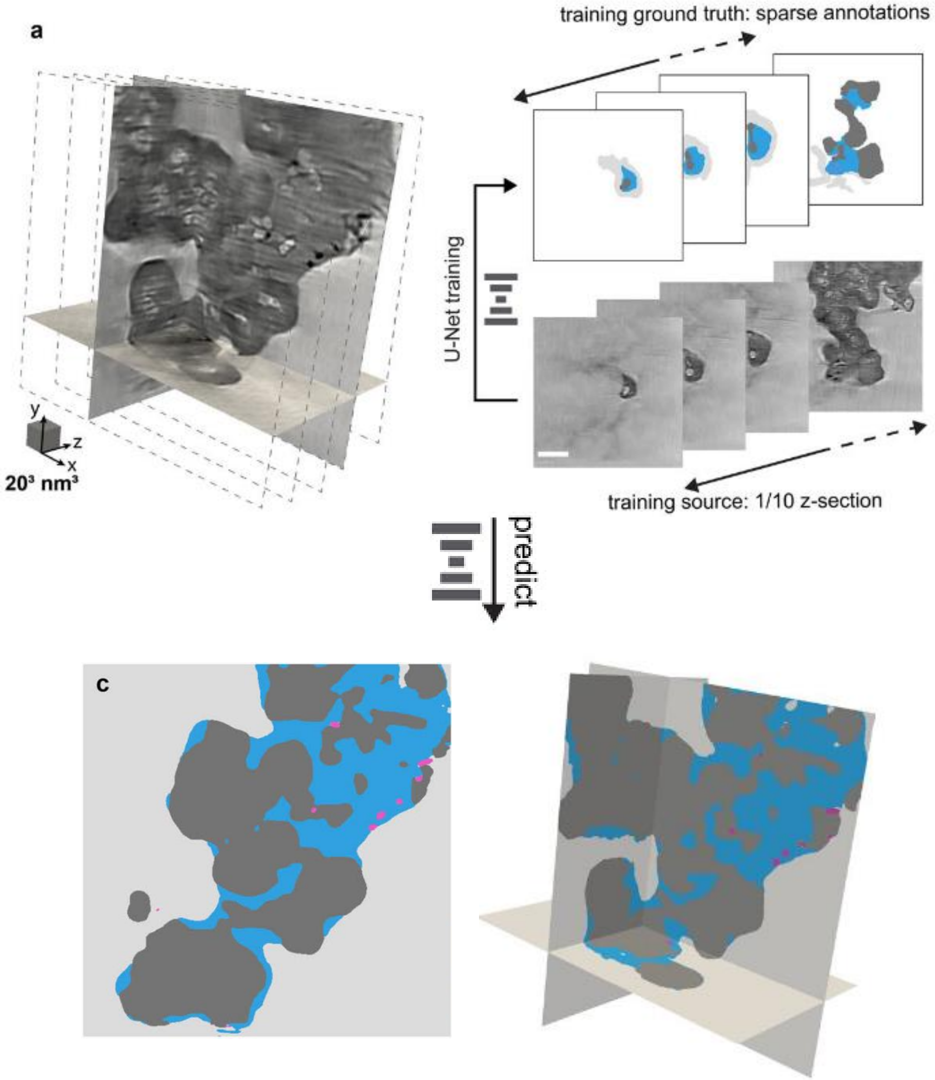
Manual | Watershed | Spherical Hough transform







Neural networks

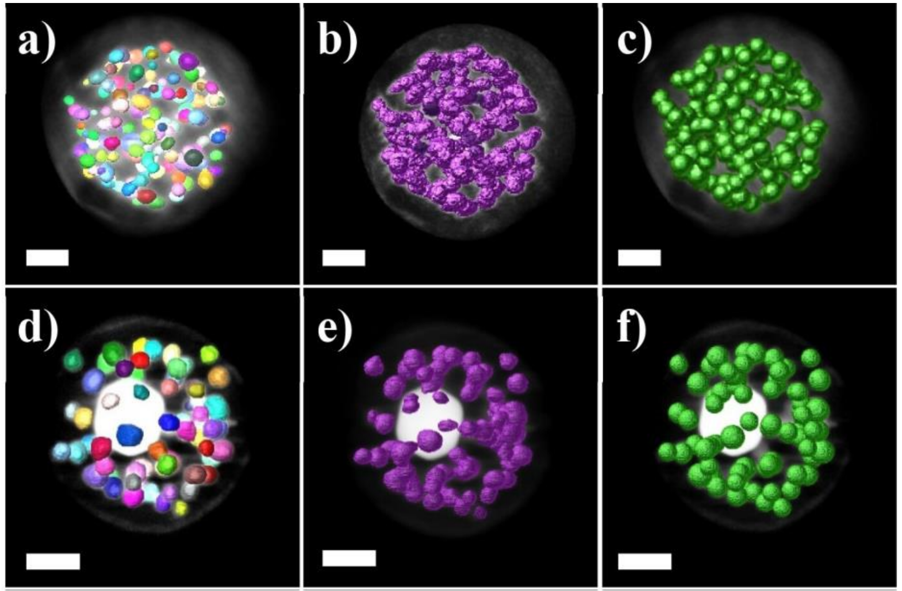


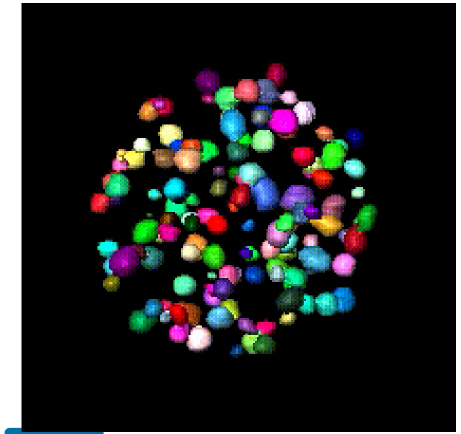
S. Kavak, et al., J. Phys. Chem. C (2023)

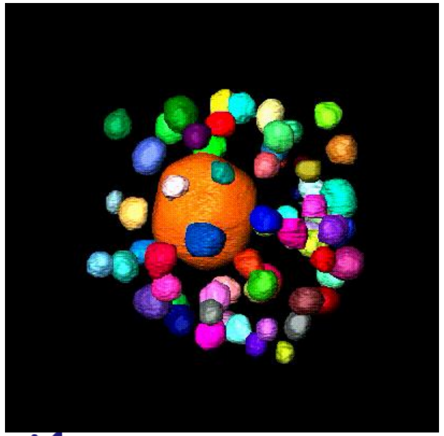
R. Girod, et al., Nat Catal (2023)

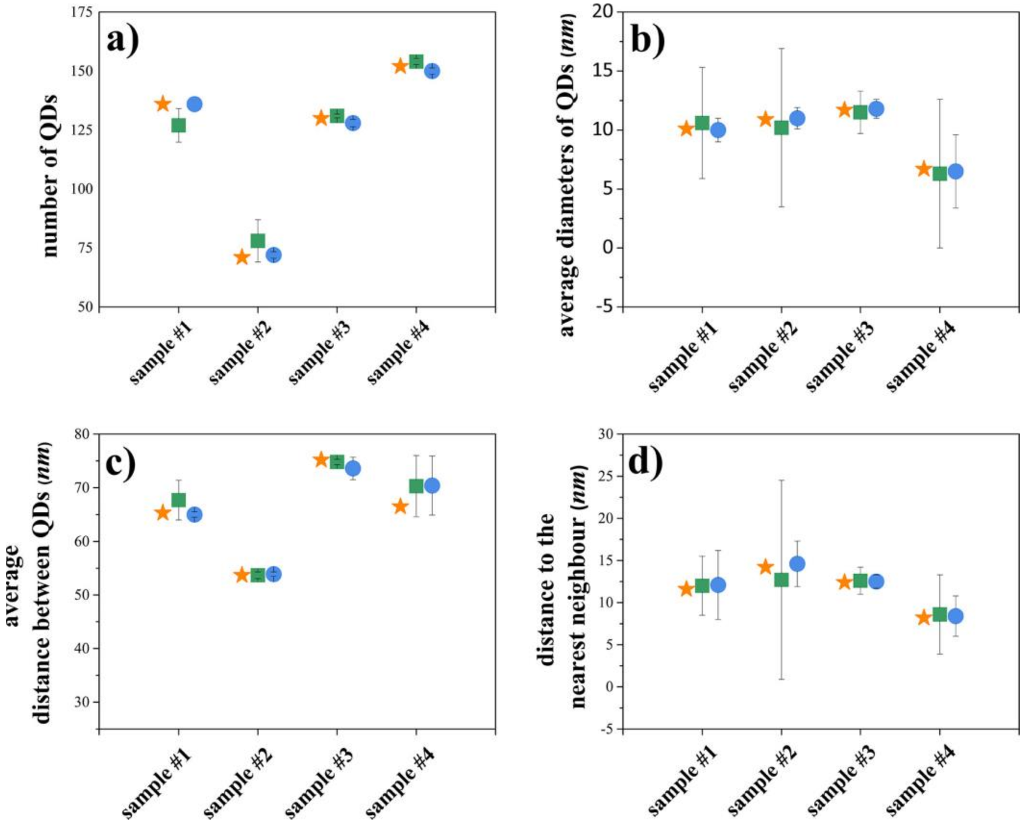
Quantification of a quantum dots assembly

Manual | Watershed | Spherical Hough transform





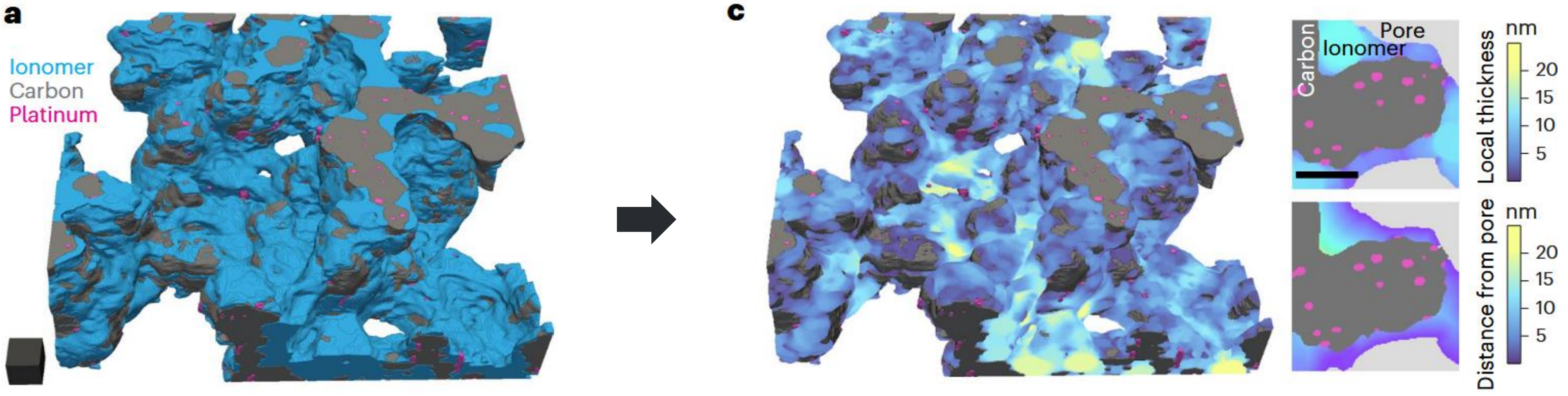




★ Manual segmentation
■ Watershed
● Spherical Hough

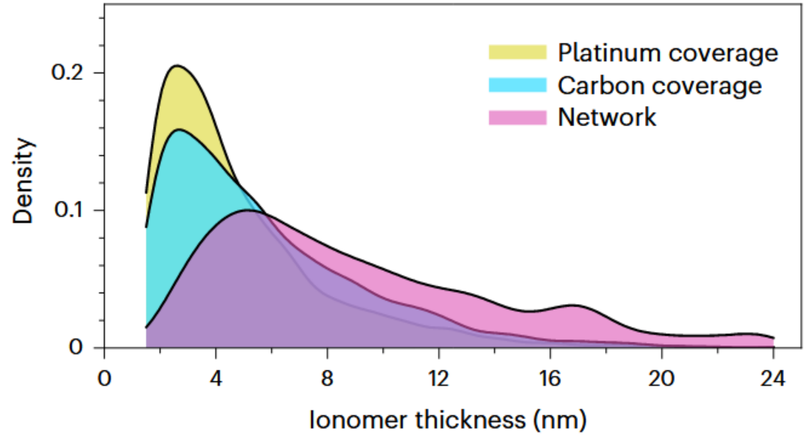
S. Kavak, A. Anil Kadu, N. Claes, A. Sánchez-Iglesias, L. M. Liz-Marzán, K. J. Batenburg, S. Bals, J. Phys. Chem. C (2023)

Quantification of a proton-conducting network for fuel cells



In fuel cells, proton-conducting polymers are added to maximize the utilization of cost-bearing Pt catalysts, but little is known about their network properties …

Challenges: soft-matter, low contrast, **very** beam sensitive



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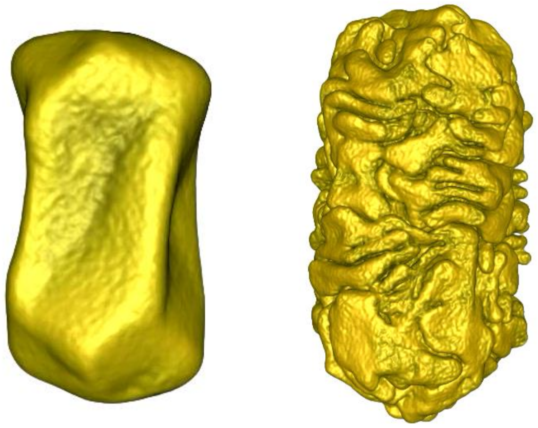
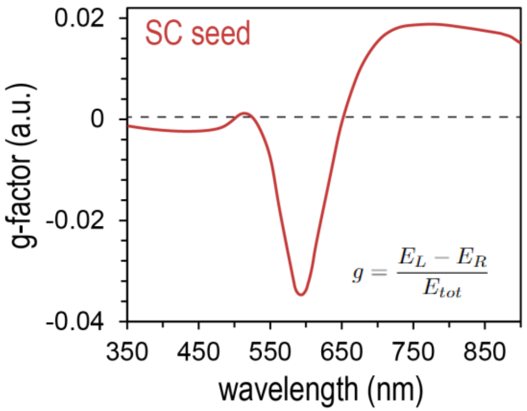
Girod, R., Lazaridis, T., Gasteiger, H. A. & Tileli, V. *Nat Catal* **6**, 383–391 (2023)

Quantification of chirality in plasmonic nanoparticles

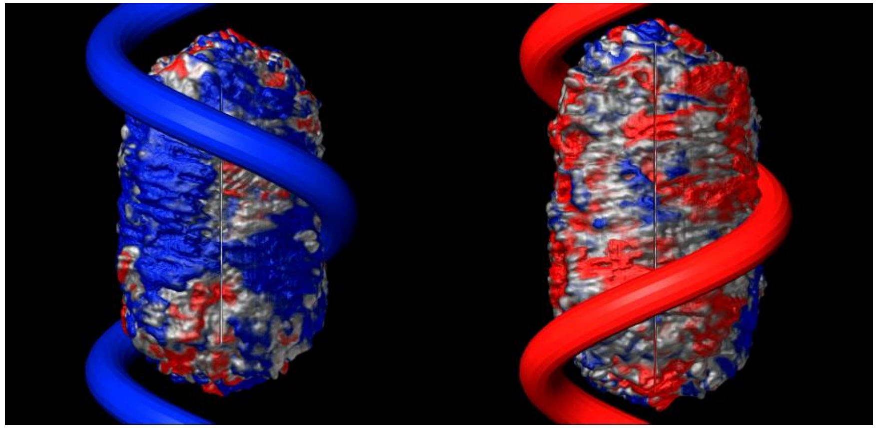
Helical gold nanorods



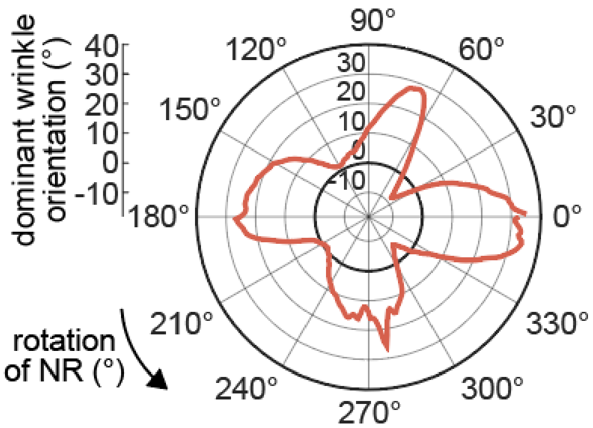


Some particles exhibit very strong optical activity, can we relate this to their shape? Understand their growth pathway? Can we establish quantitative shape-optical relationship? Can we guide the design of nanomaterials with desirable properties?

Quantification of helicity



Quantification of wrinkle orientation



K. Van Gordon, Angewandte Chemie (2024) e202403116
W. Heyvaert, ACS Materials Letters 4 (2022) 642

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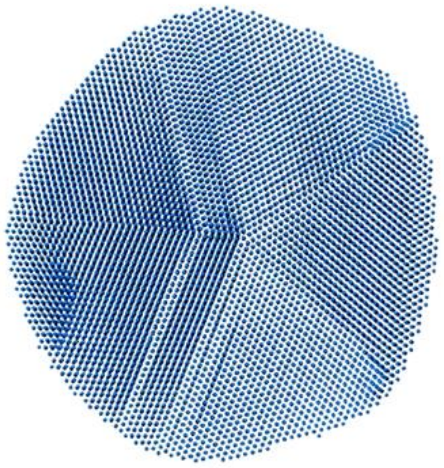
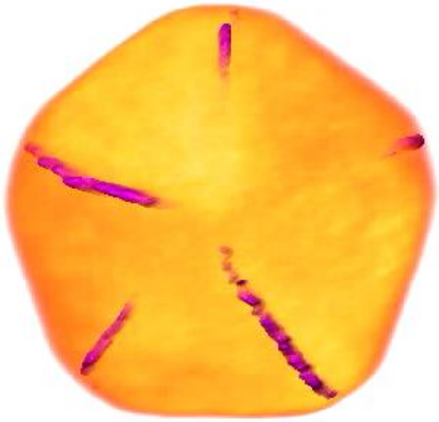
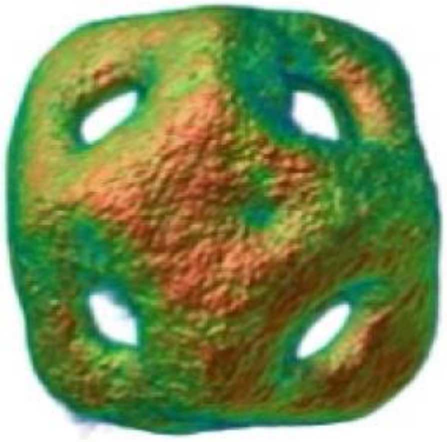
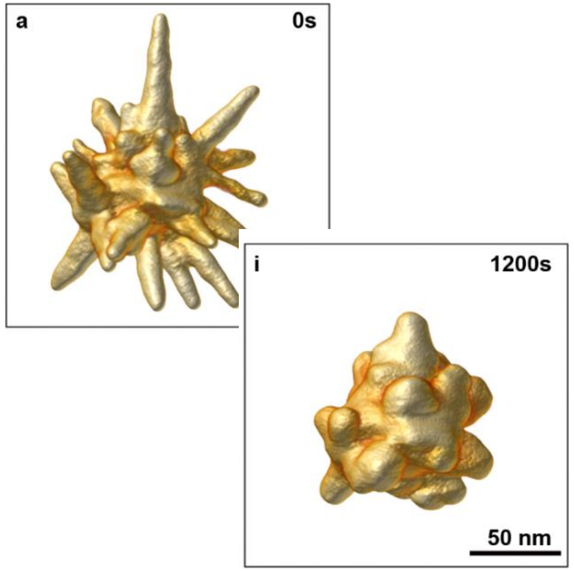
Advanced methods

Atomic ET	Multimode	Composition	Fast ET

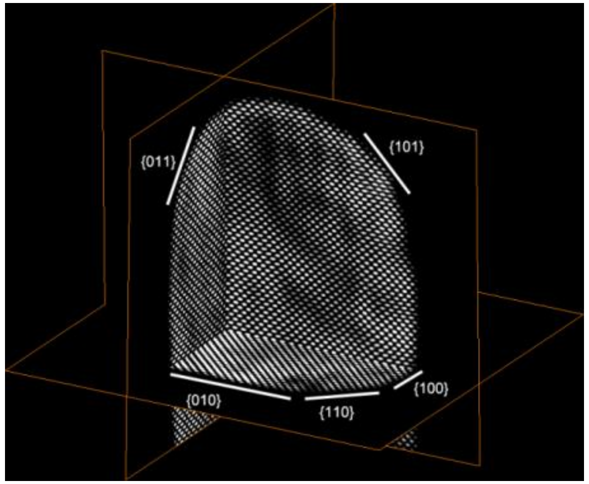


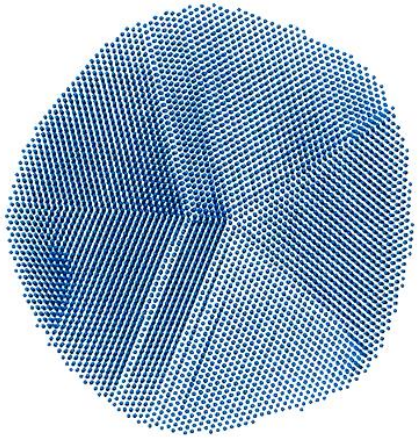


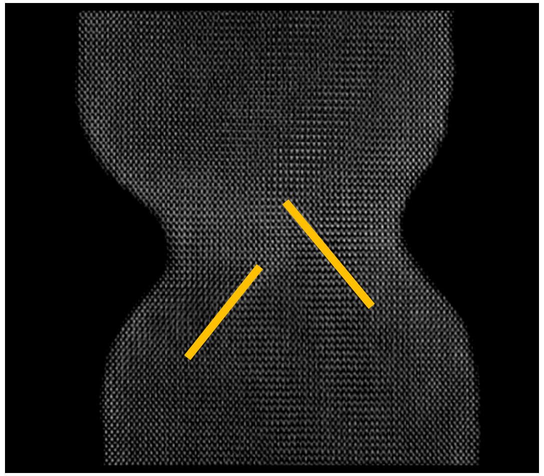




High resolution electron tomography







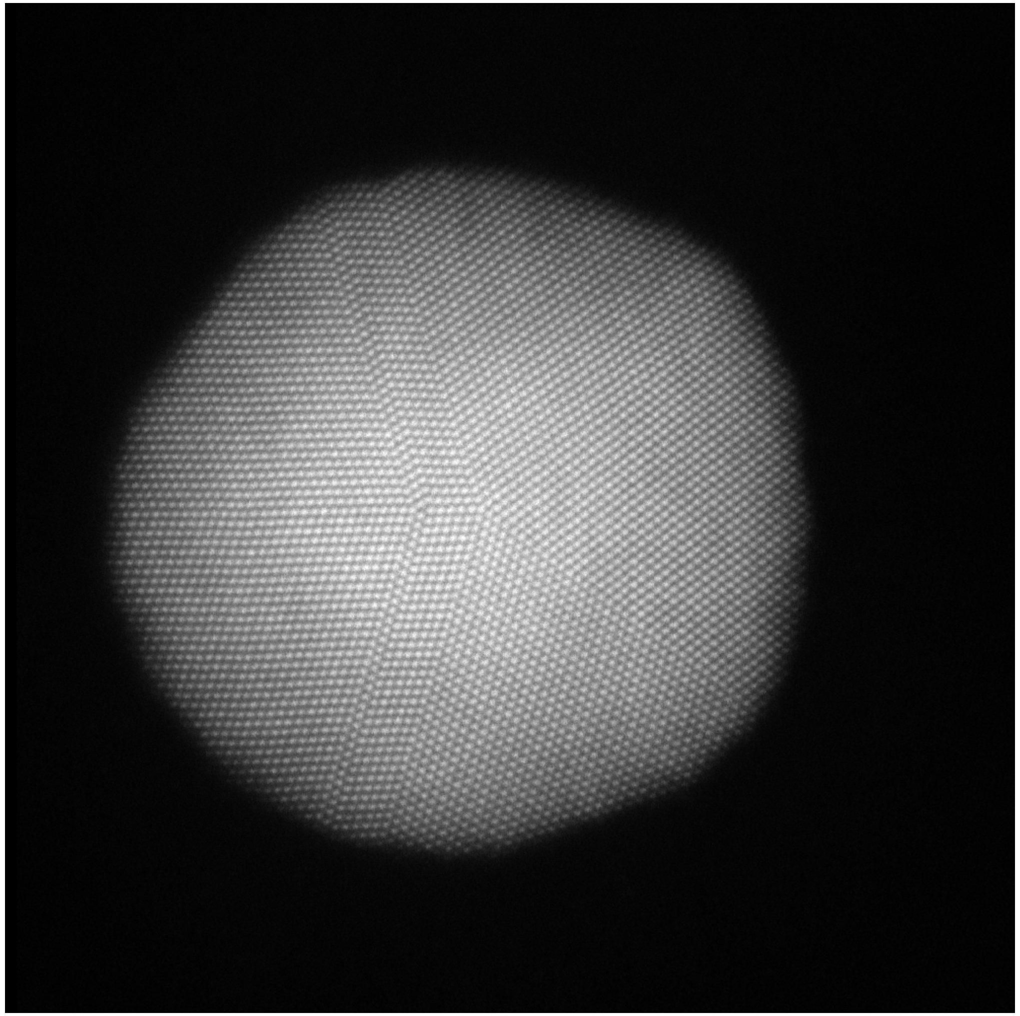
B. Goris, S. Bals, W. Van den Broek, E. Carbo-Argibay, S. Gomez-Grana, L.M. Marzan, G. Van Tendeloo Nature Materials 11 (2012) 930

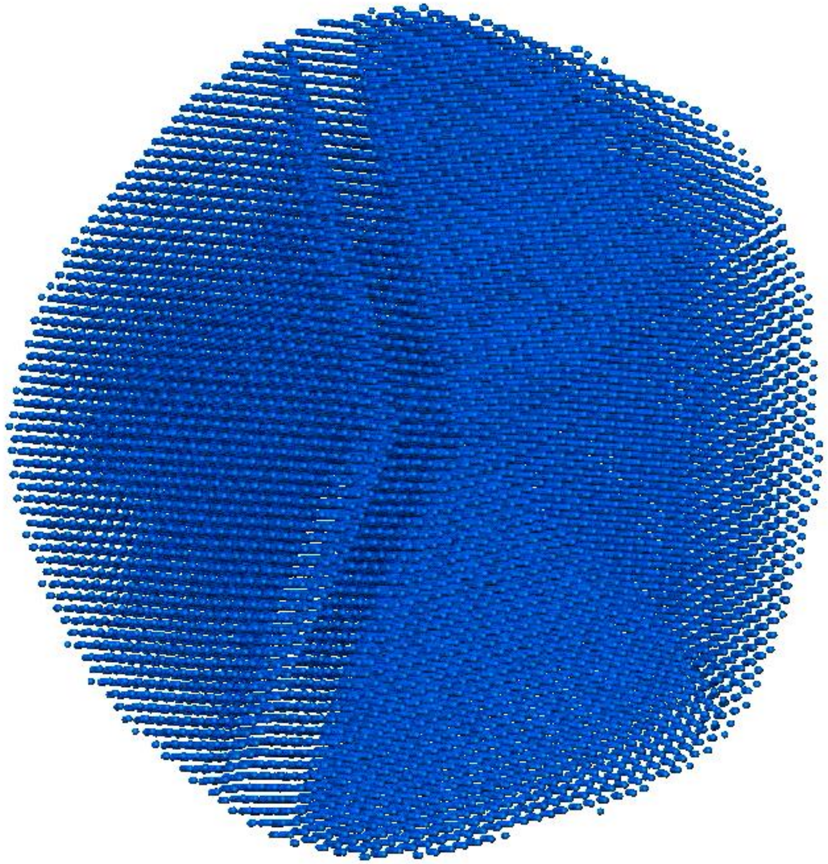
B. Goris, S. Bals, G. Van Tendeloo, et al. Nano Letters 15 (2015) 6996

T. Milagres de Oliveira, W. Albrecht, G. González-Rubio, T. Altantzis, I. Pedro Lobato Hoyos, A. Béché, S. Van Aert, A. Guerrero-Martínez, L.M. Liz-Marzán, S. Bals
ACS Nano 14 (2020) 12558

Only 4 projection in zone axis + TVmin

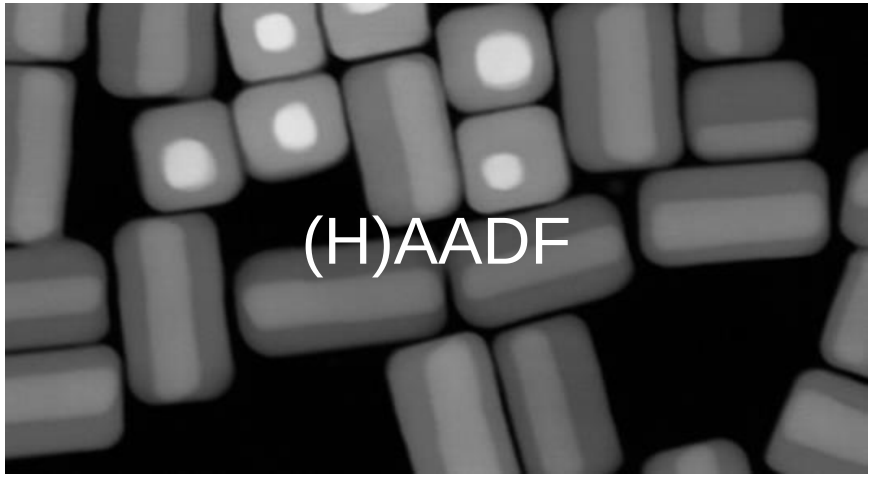
Seeing atomic scale defects in 3D

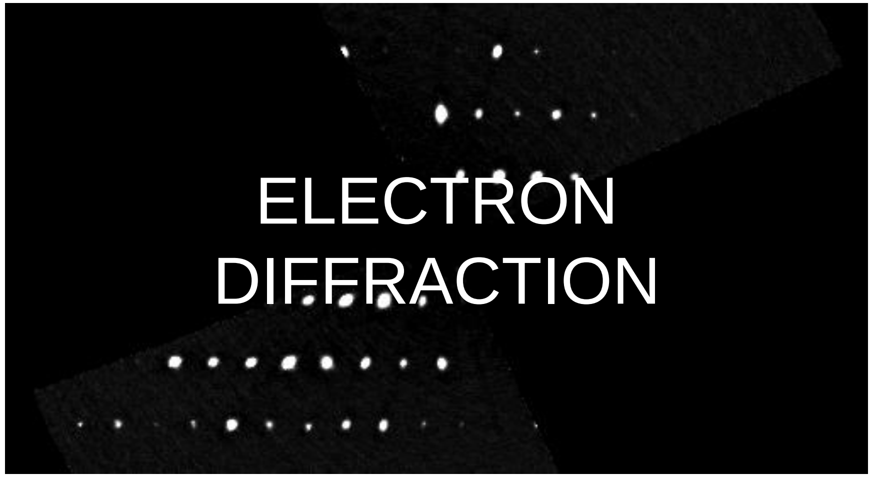


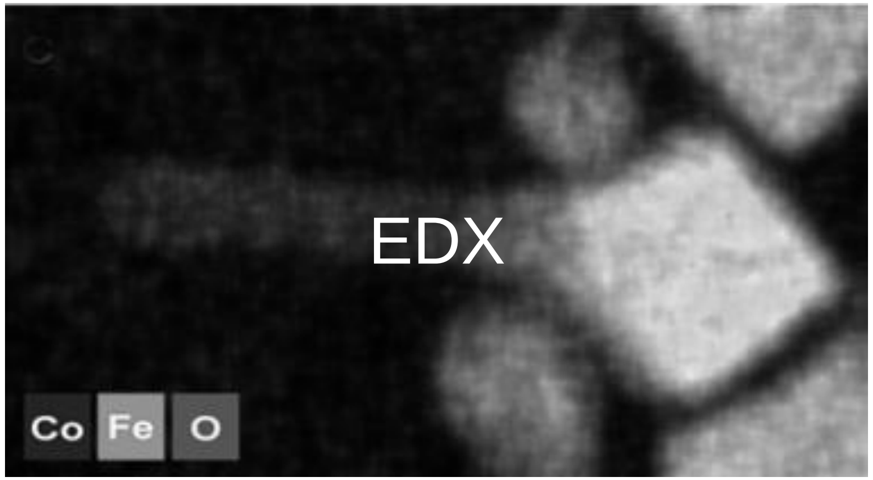


B. Goris, J. De Beenhouwer, A. De Backer, D. Zanaga, K.J. Batenburg, A. Sánchez-Iglesias, L. M. Liz-Marzán, S. Van Aert, S. Bals, J. Sijbers, G. Van Tendeloo, Nano Letters 15 (2015) 6996

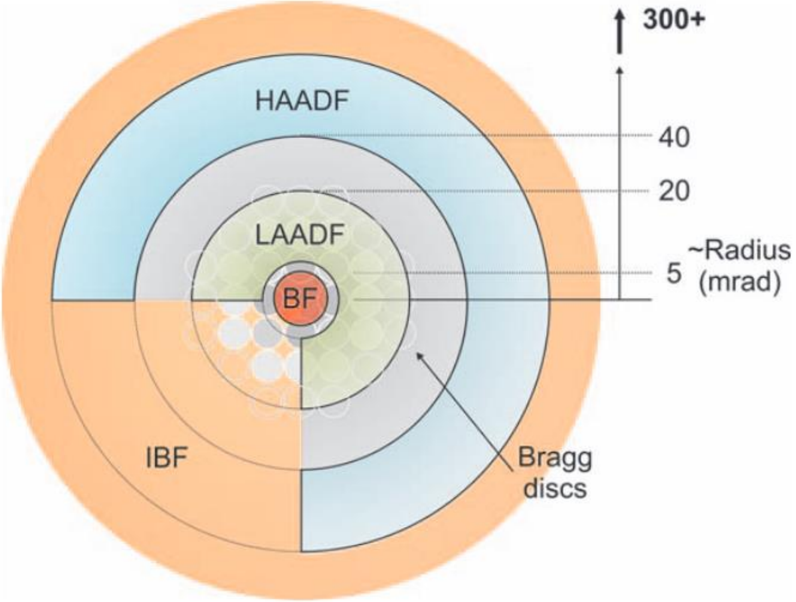
Multimode electron tomography

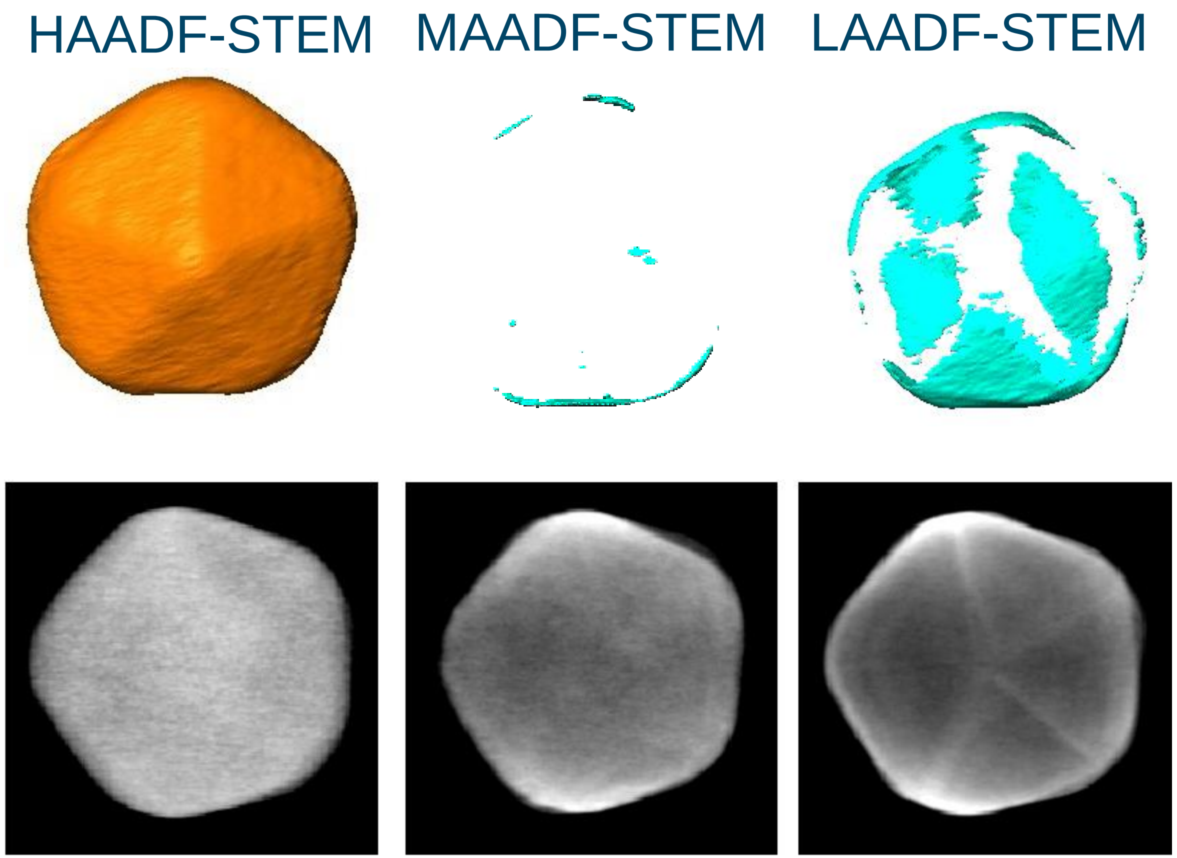






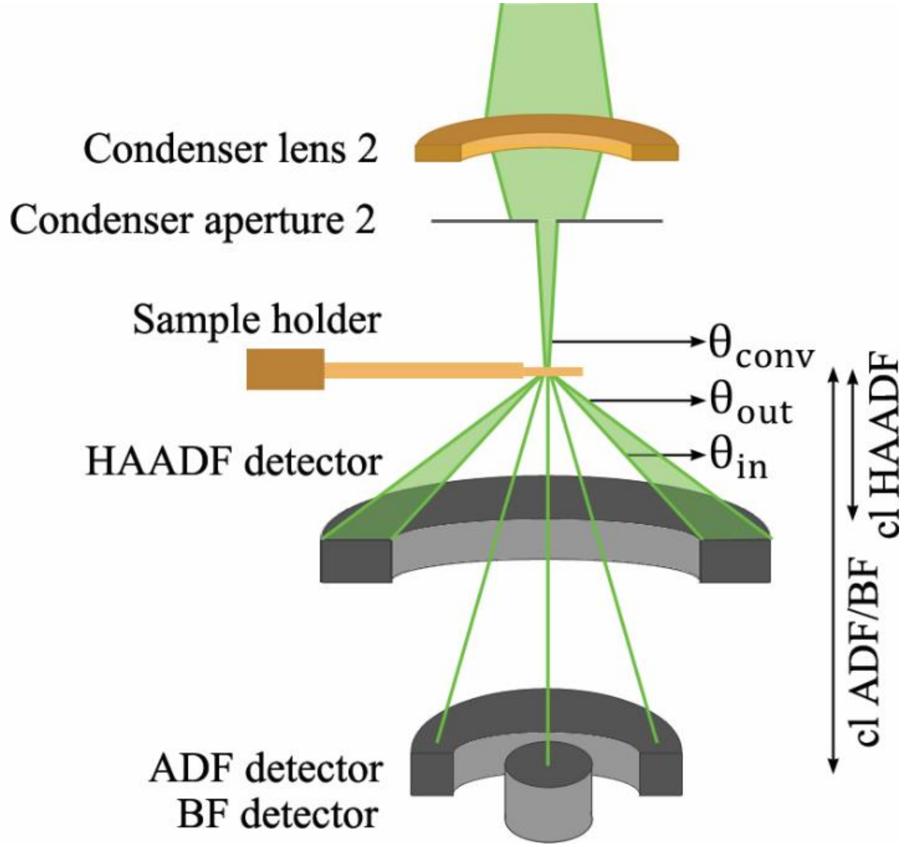
Multimode electron tomography: defects

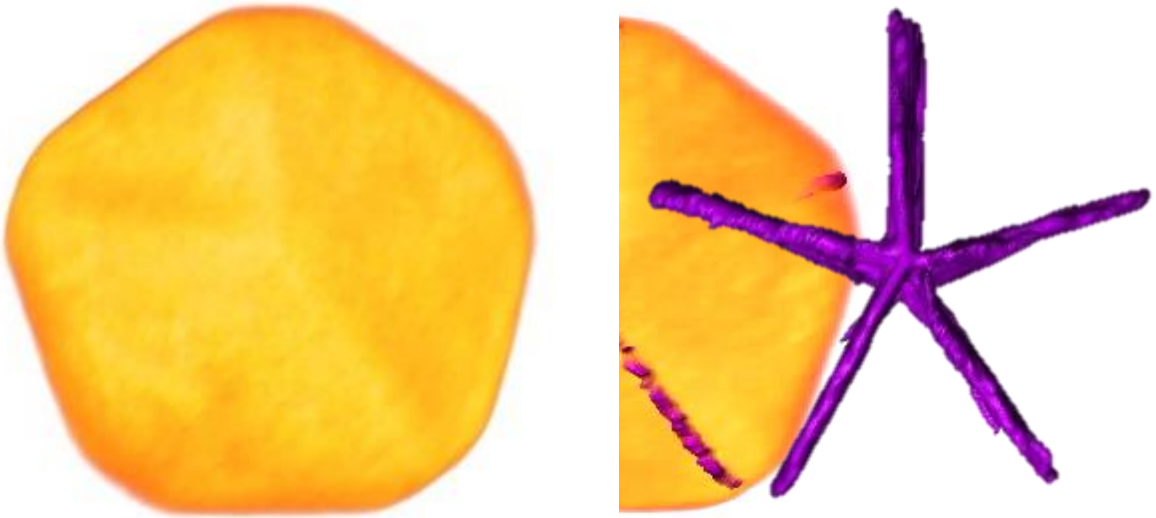




Annular Dark Field

Multimode electron tomography: defects



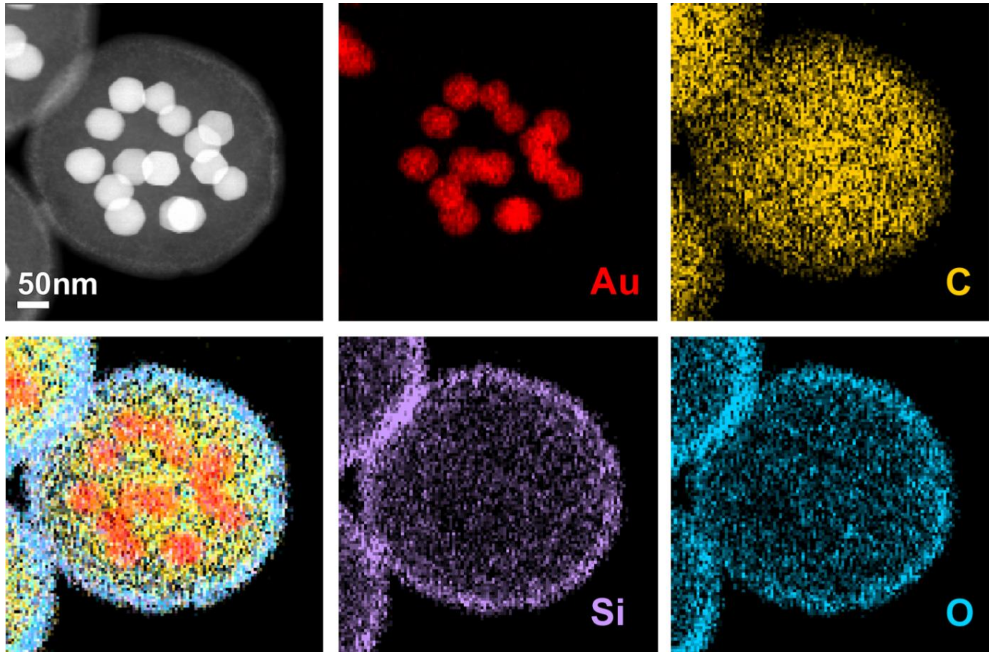


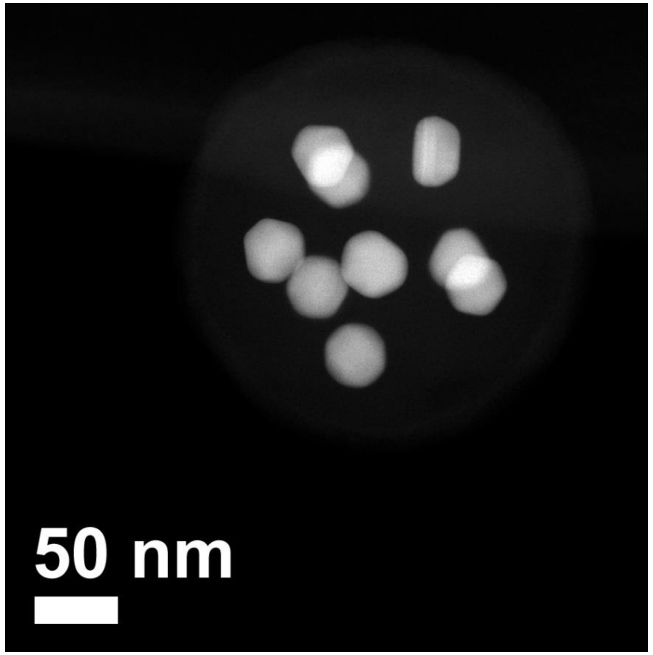
AET
Multimode
Composition
Fast ET

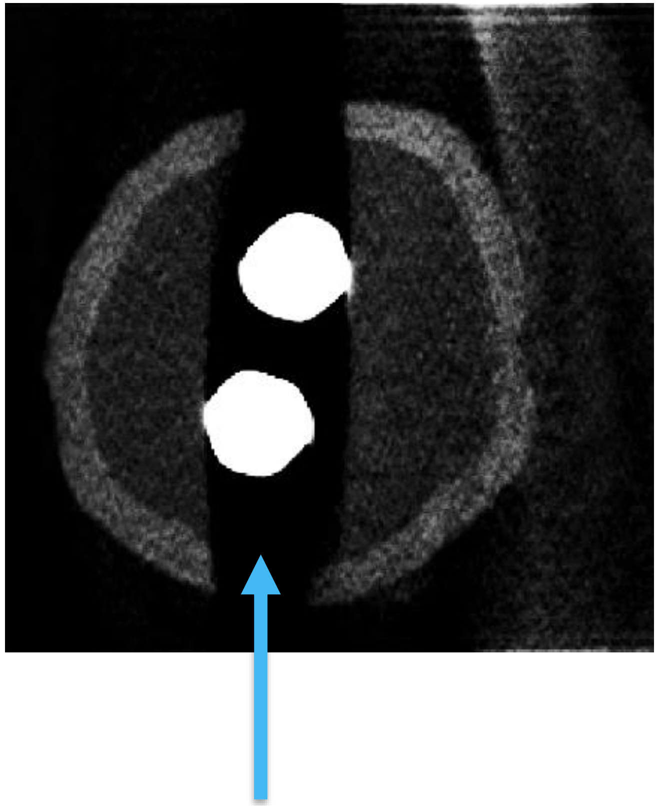
N. Winckelmans, T. Altantzis, M. Grzelczak, A. Sánchez-Iglesias, L. Liz-Marzán, S. Bals The Journal of Physical Chemistry C J. Phys. Chem. C 25 (2018)) 13522

Multimode electron tomography: soft-hard materials

Stimuli responsive particles for colorimetric sensors







K. Sentosun, M.N. Sanz Ortiz, K.J. Batenburg, L.M. Liz-Marzán and S. Bals, Particle & Particle Systems Characterization, 32, 12 (2015) 1063-1067.

A. Sanchez-Iglesias, N. Claes, D.M. Solis, J.M. Taboada, S. Bals, M. Grelczak and L.M. Liz-Marzán, Angewandte Chemie International Edition, 57, 12 (2018) 3183-3186.

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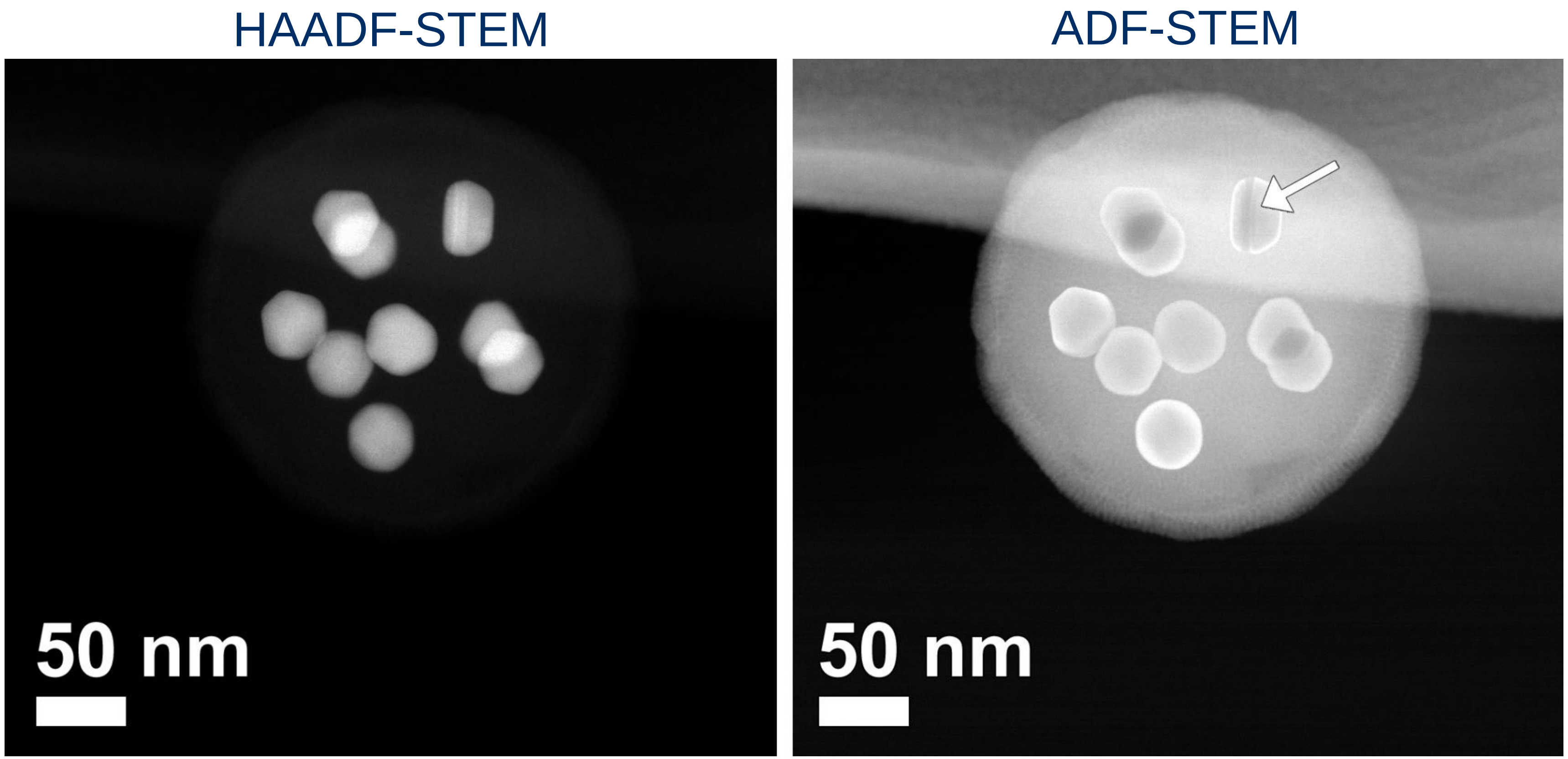
EMAT | Electron Microscopy for Materials Science

Multimode electron tomography: soft-hard materials



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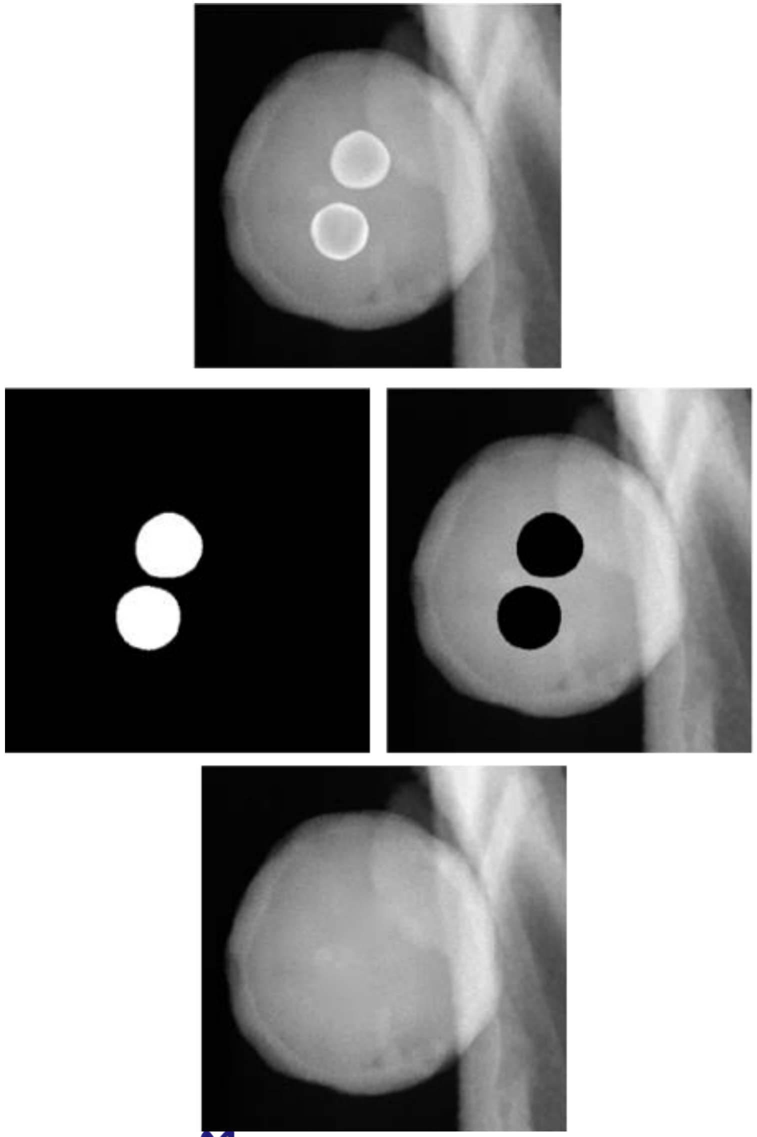
A. Sanchez-Iglesias, N. Claes, D.M. Solis, J.M. Taboada, S. Bals, M. Grelczak and L.M. Liz-Marzán, Angewandte Chemie International Edition, 57, 12 (2018) 3183-3186.

Multimode electron tomography: soft-hard materials



ADF STEM image

↓

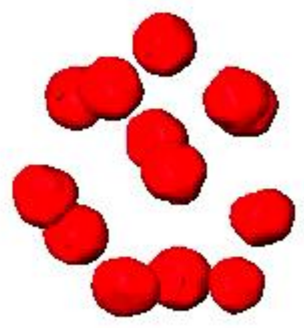
Mask

↓

Inpainting

K. Sentosun , M. N. Sanz Ortiz , K.J. Batenburg , L.M. Liz-Marzán, S. Bals, Part. Part. Syst. Charact. 2015, 32, 1063–1067

Multimode electron tomography: soft-hard materials



Nanoparticle coordinates

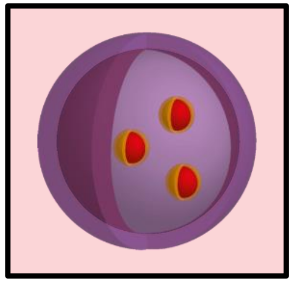
↓

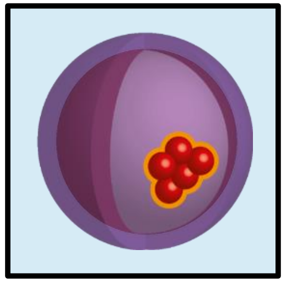
Theoretical calculations optical properties

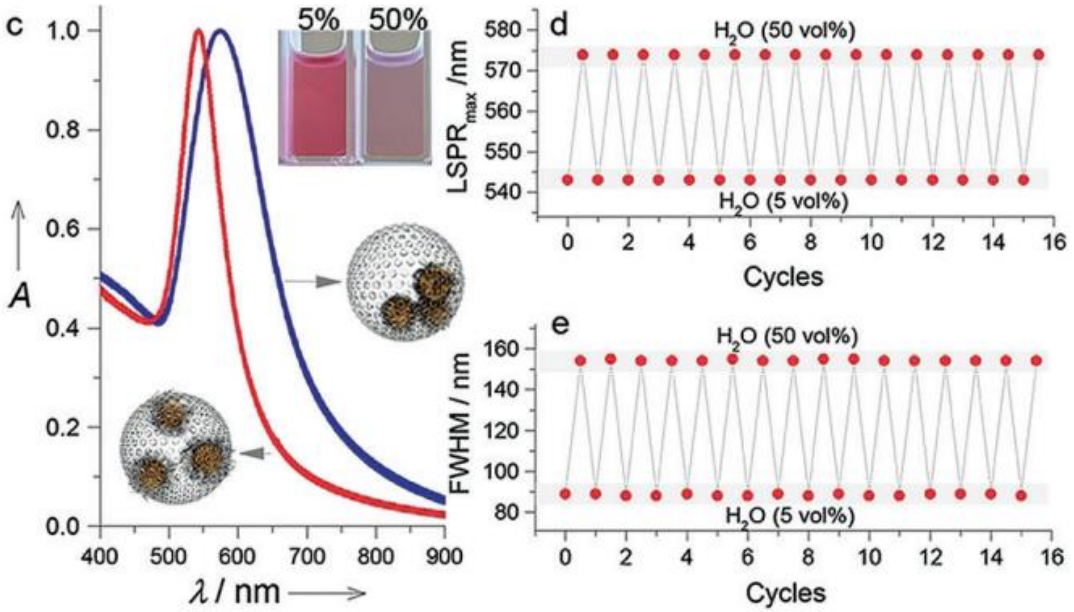
A. Sanchez-Iglesias, N. Claes, D.M. Solis, J.M. Taboada, S. Bals, M. Grelczak and L.M. Liz-Marzán, Angewandte Chemie International Edition, 57, 12 (2018) 3183-3186.

Applications

Colorimetric sensor – stimuli responsive systems







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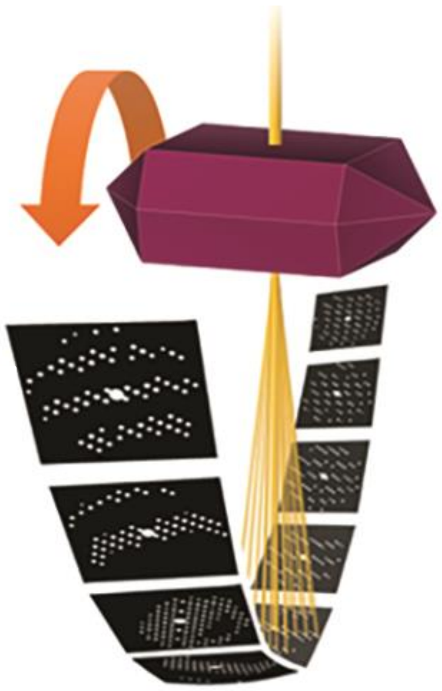
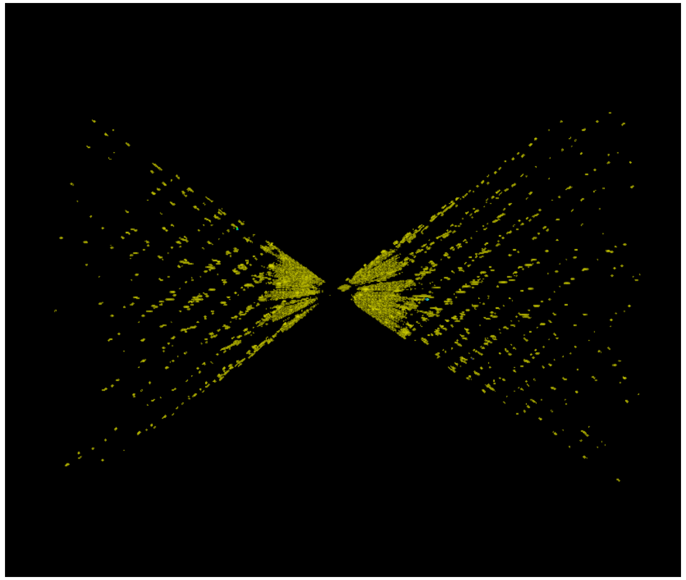
A. Sanchez-Iglesias, N. Claes, D.M. Solis, J.M. Taboada, S. Bals, M. Grelczak and L.M. Liz-Marzán, Angewandte Chemie International Edition, 57, 12 (2018) 3183-3186.

3D electron diffraction (3DED)

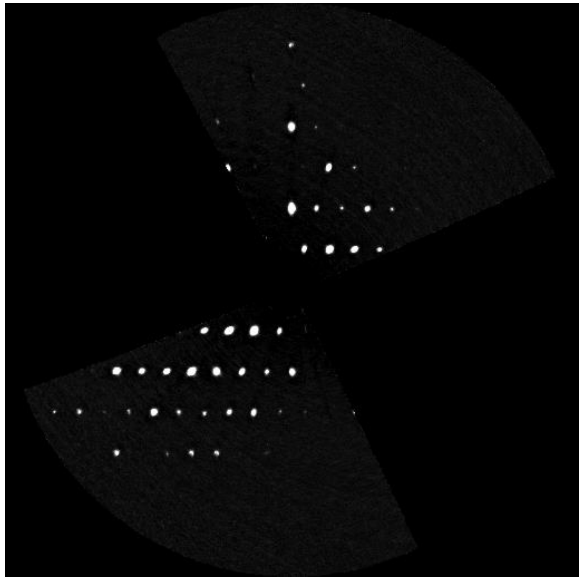
ED tomography



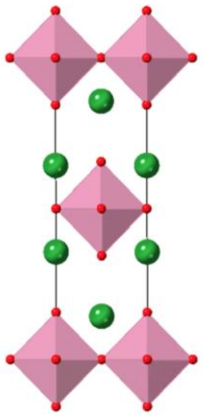
3D reciprocal space



2D zone reconstructions
indexing & integration



Structure solution
& refinement

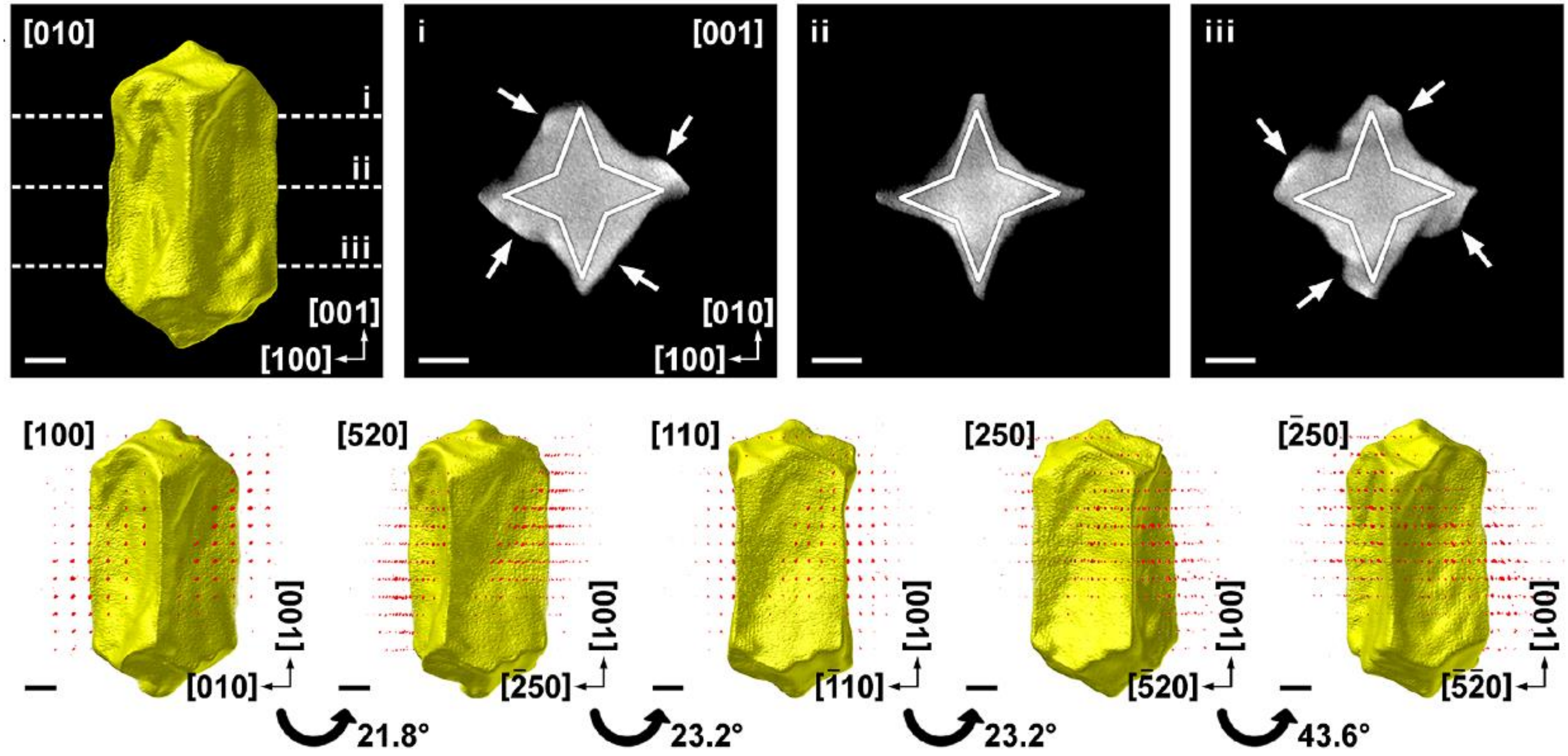


Advantage: no orientation to crystallographic zone required

O. Karakulina, Microsc. Microanal. (2019) 25
Kolb U. et al., Ultramicroscopy 2007, 107, 507

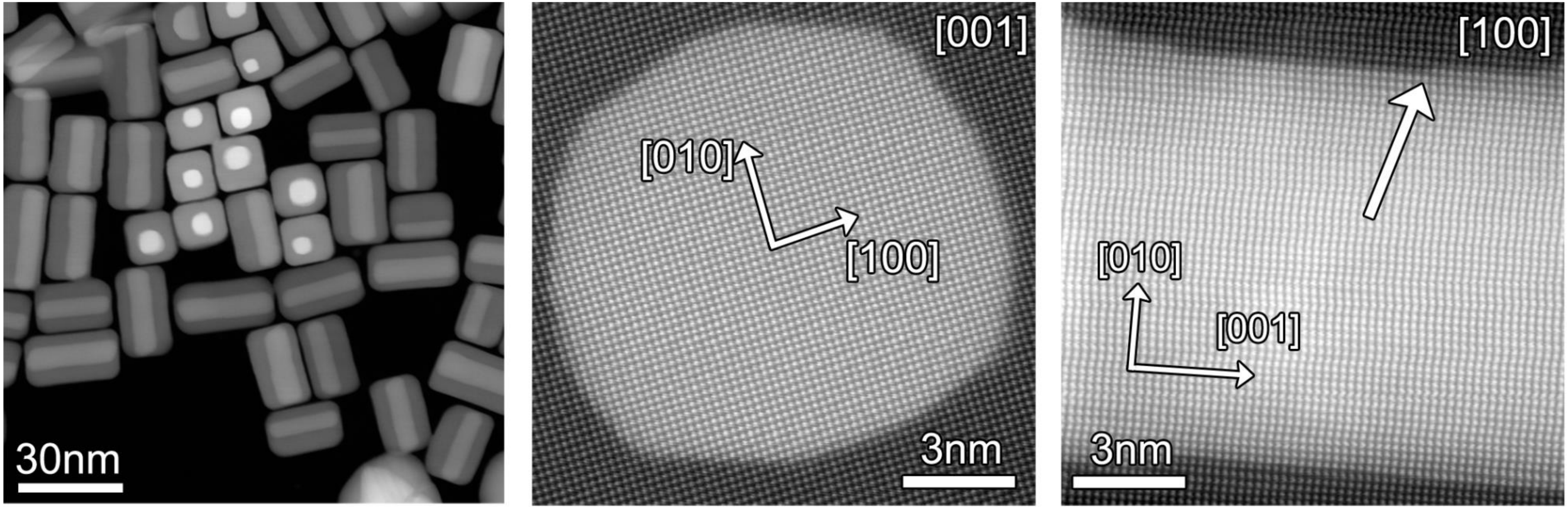
Courtesy of Daphne Vandemeulebroucke
Palatinus, L., PETS software, pets.fzu.cz. (2018)

3D electron diffraction (3DED)



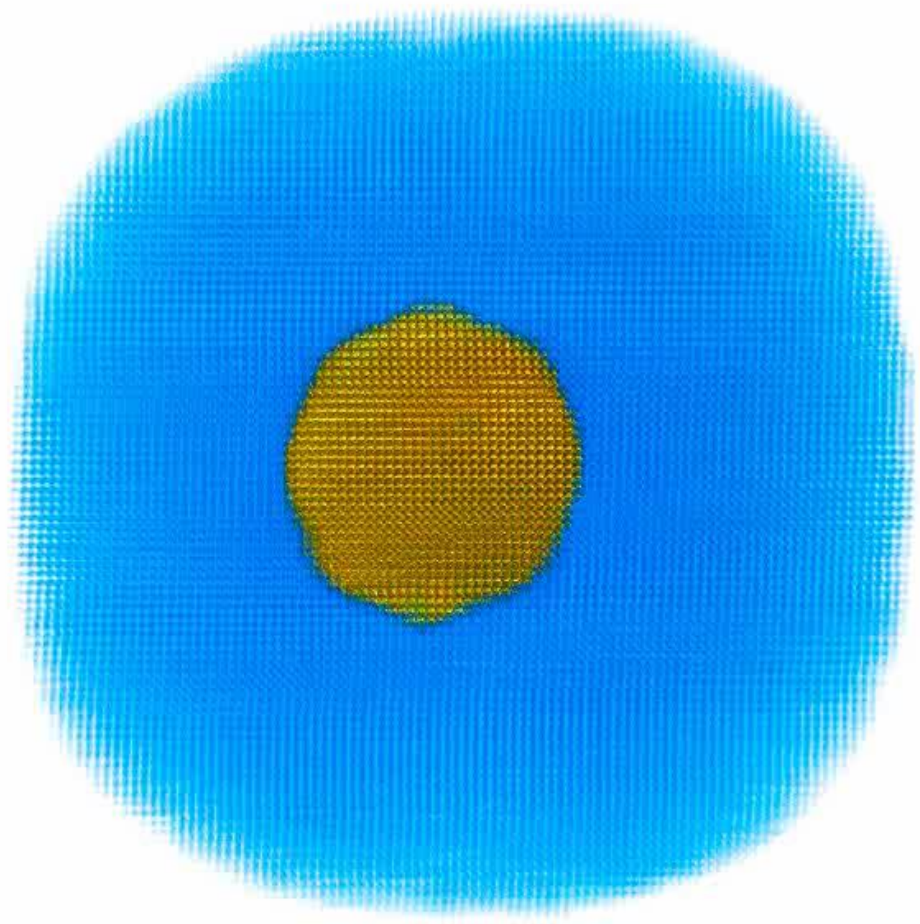
Van Gordon, K., *et al.* *Nano Lett.* **23**, 9880–9886 (2023)

Analytical ET



B. Goris, A. De Backer, S. Van Aert, S. Gomez-Grana, L.M. Liz-Marzan, G. Van Tendeloo, S. Bals, Nano Letters 13 (2013) 4236

Analytical ET



B. Goris, A. De Backer, S. Van Aert, S. Gomez-Grana, L.M. Liz-Marzan, G. Van Tendeloo, S. Bals, Nano Letters 13 (2013) 4236

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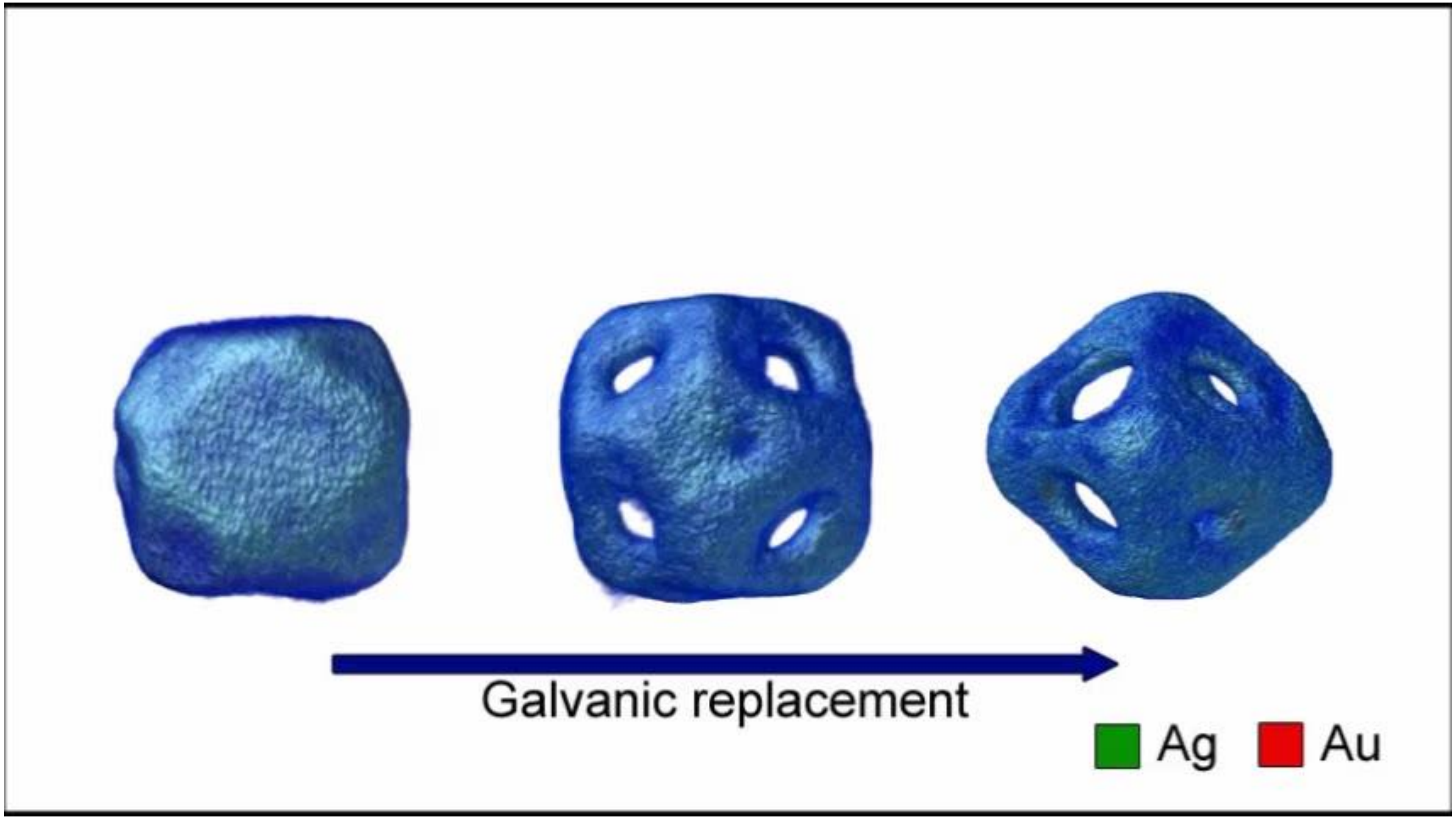
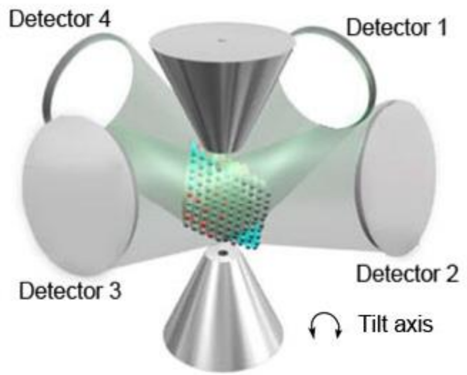
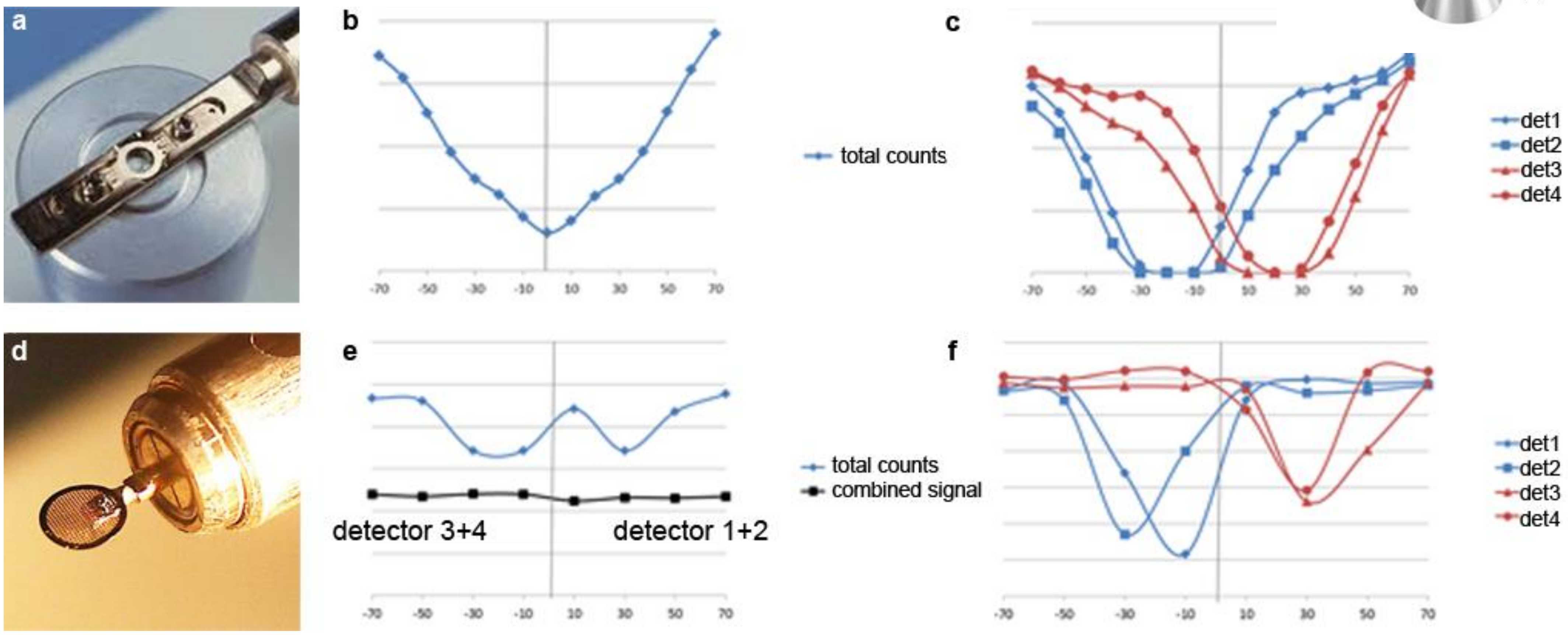
Analytical ET: EDS



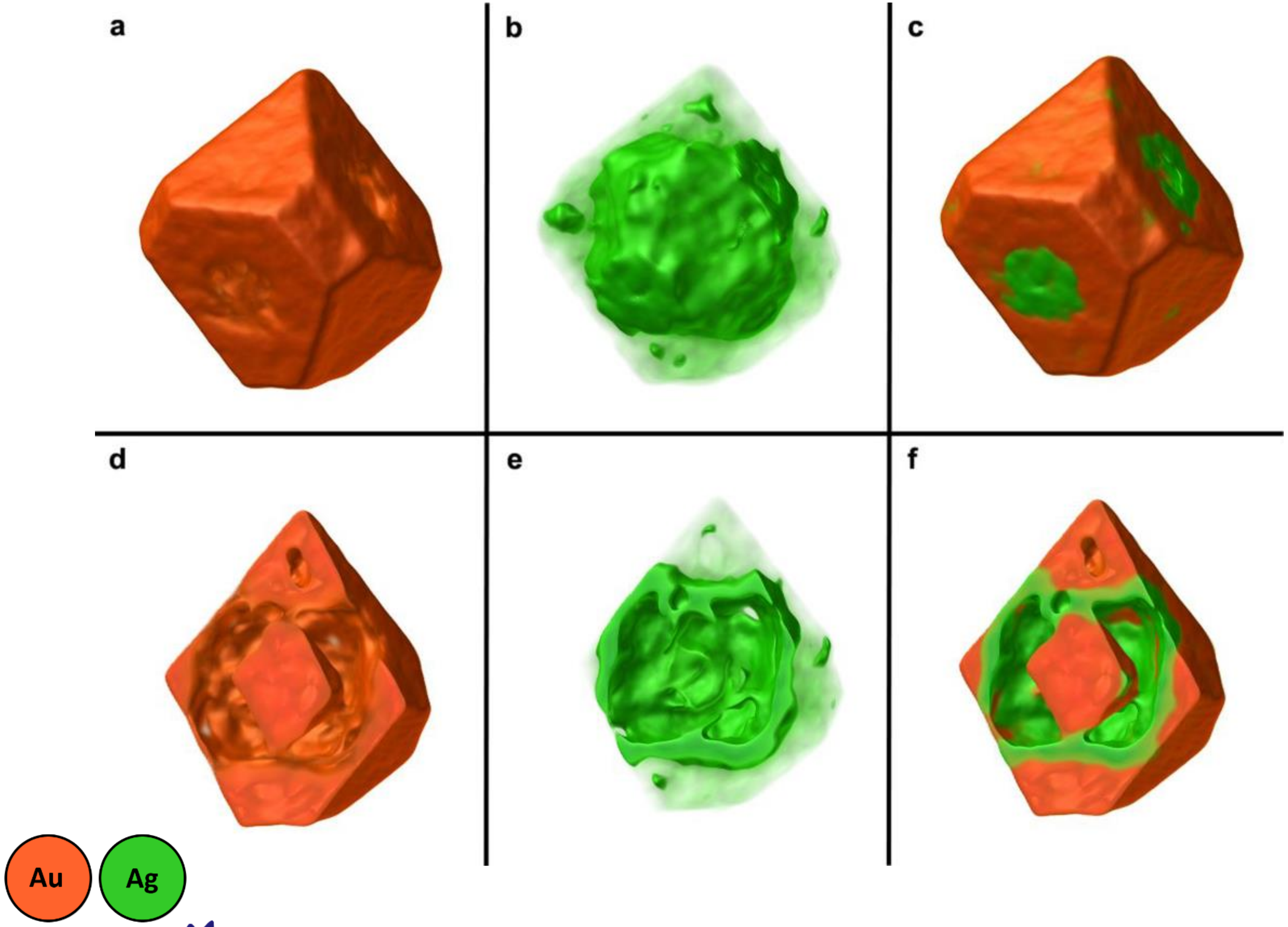
B. Goris, L. Polavarapu, S. Bals, G. Van Tendeloo, L. M. Liz-Marzán, Nano Letters 4 (2014) 3220-3226

Analytical ET: EDS



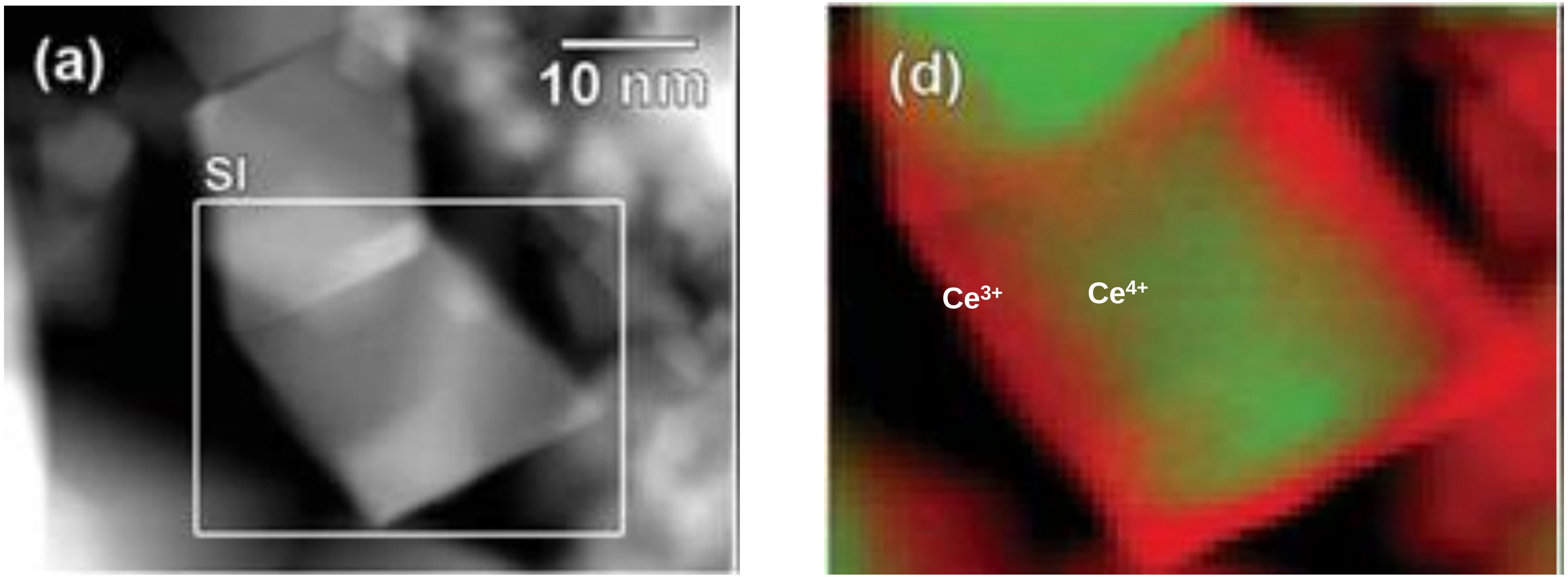


Analytical ET: EDS



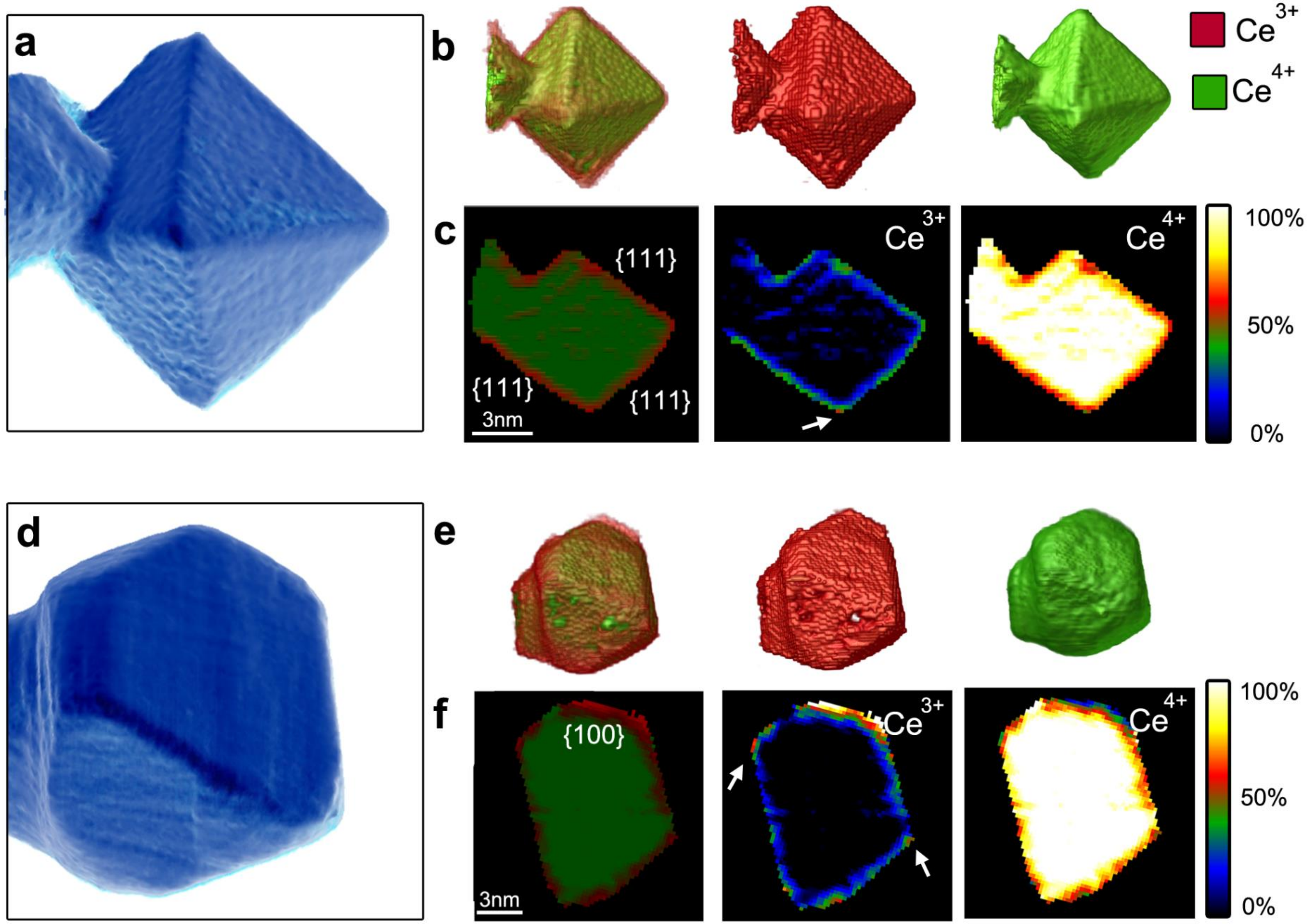
D. Zanaga, T. Altantzis, L. Polavarapu, L.M. Liz-Marzán, B. Freitag, S. Bals, Particle & Particle Systems Characterization, 33 (2016)
D. Zanaga, T. Altantzis, J. Sanctorum, B. Freitag, S. Bals, Ultramicroscopy 164 (2016) 11

Multimode electron tomography: quantitative EELS



S. Turner, S. Lazar, B. Freitag, R. Egoavil, J. Verbeeck, S. Put, Y. Strauven, G. Van Tendeloo, Nanoscale, 3 (2011) 3385

Multimode electron tomography: quantitative EELS



B. Goris, S. Turner, S. Bals, G. Van Tendeloo, ACS Nano, 8 (2014) 10878

Fast HAADF-STEM tomography: acquisition

Conventional tomography

60 minutes

Fast tomography

4-6 minutes

H. Vanrompay, E. Bladt, W. Albrecht, A. Béché, M. Zakhozheva, A. Sánchez-Iglesias, L.M. Liz-Marzán, S. Bals, Nanoscale 10 (2018) 22792

V. Migunov, H. Ryll, X. Zhuge, M. Simson, L. Struder, K.J. Batenburg, L. Houben, R. Dunin-Borkowski, Scientific Reports 5 (2015) 14516
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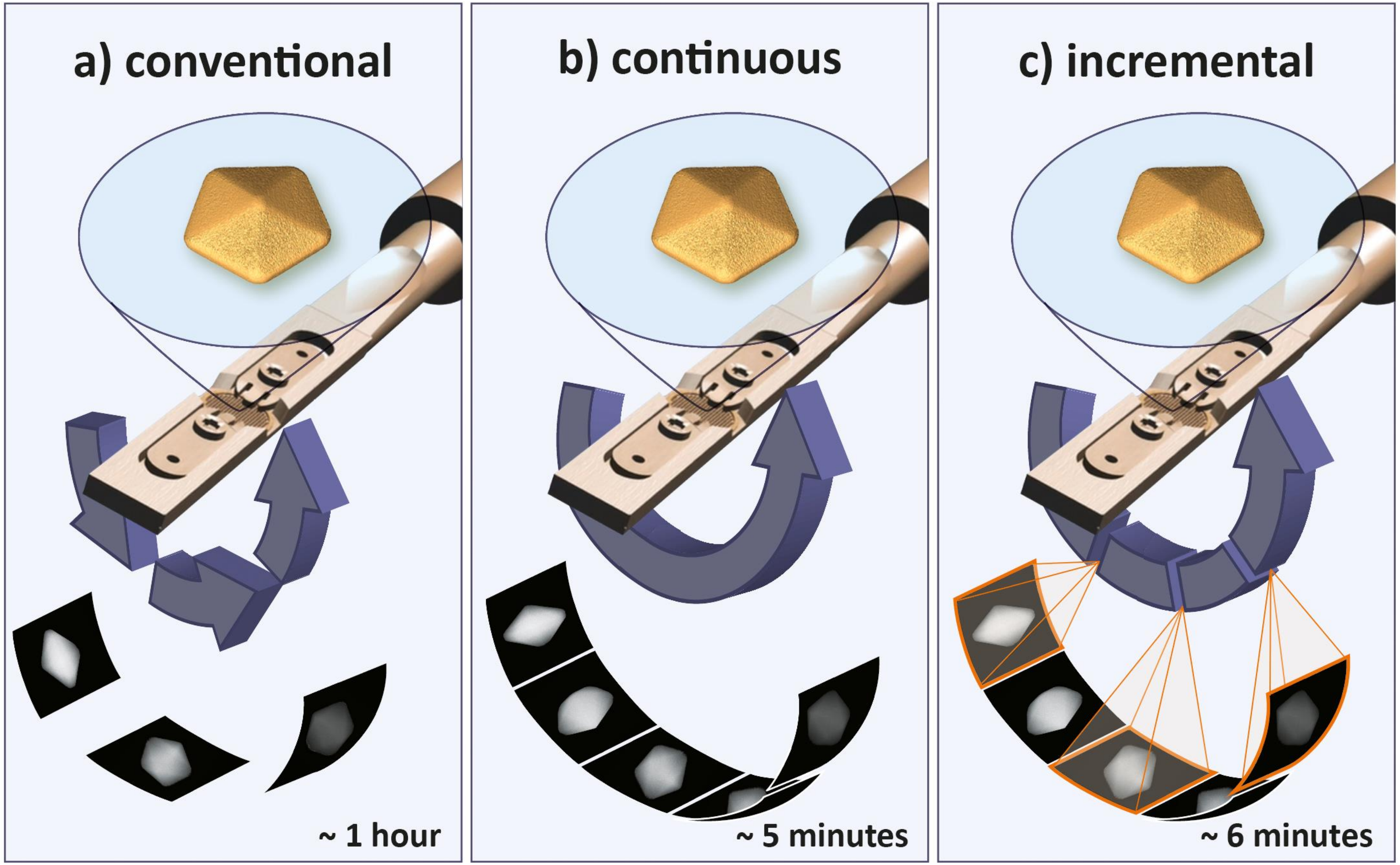
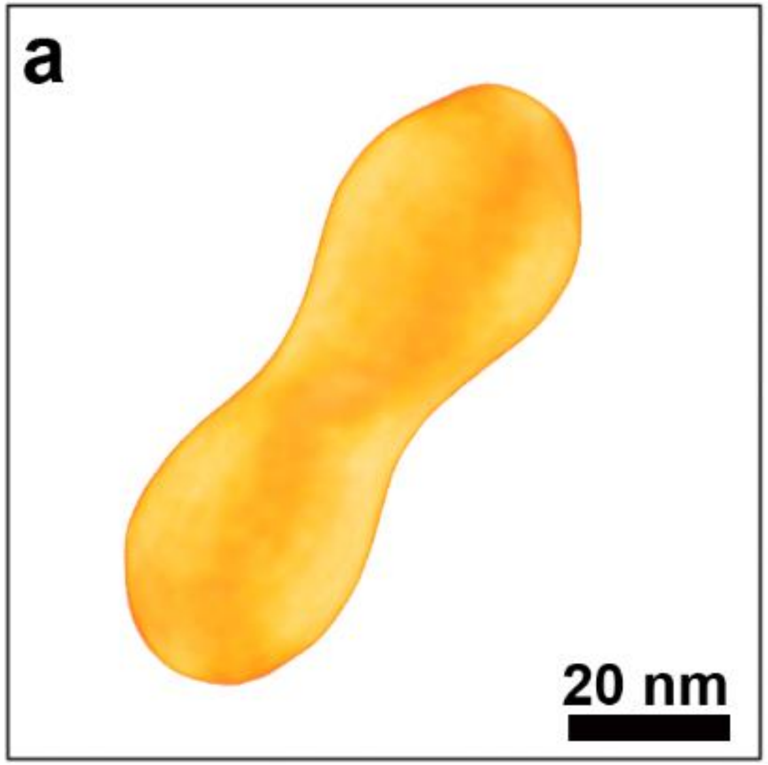
Fast HAADF-STEM tomography

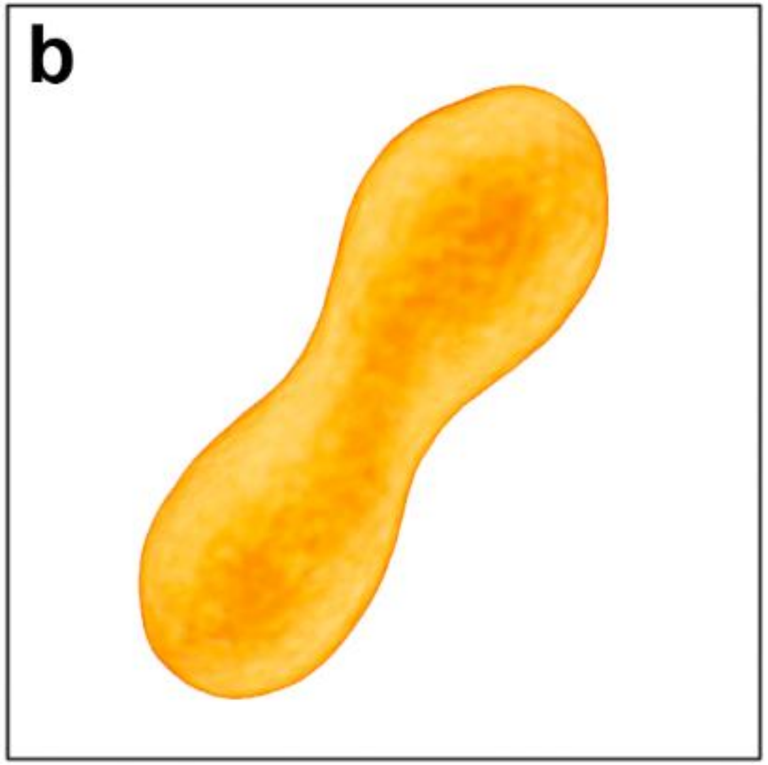


H. Vanrompay, A. Skorikov, E. Bladt, A. Béché, B. Freitag, J. Verbeeck, S. Bals, Ultramicroscopy 221, 113191 (2021).

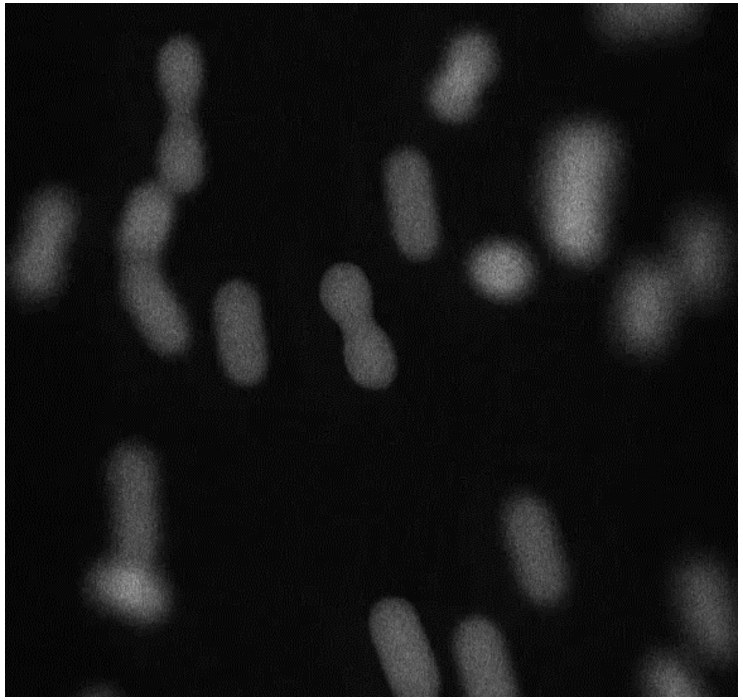
Fast HAADF-STEM tomography



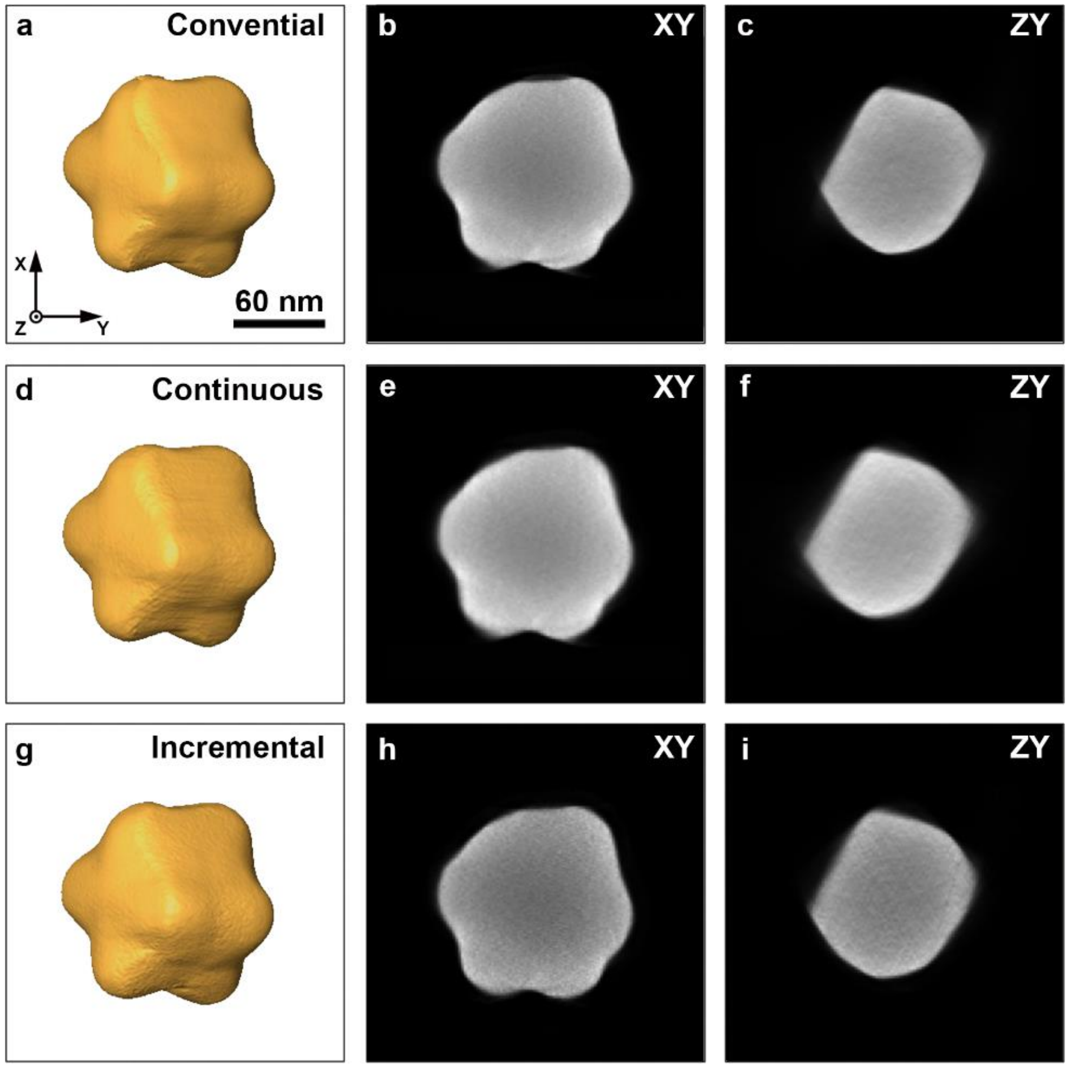
49 images, 1 hour

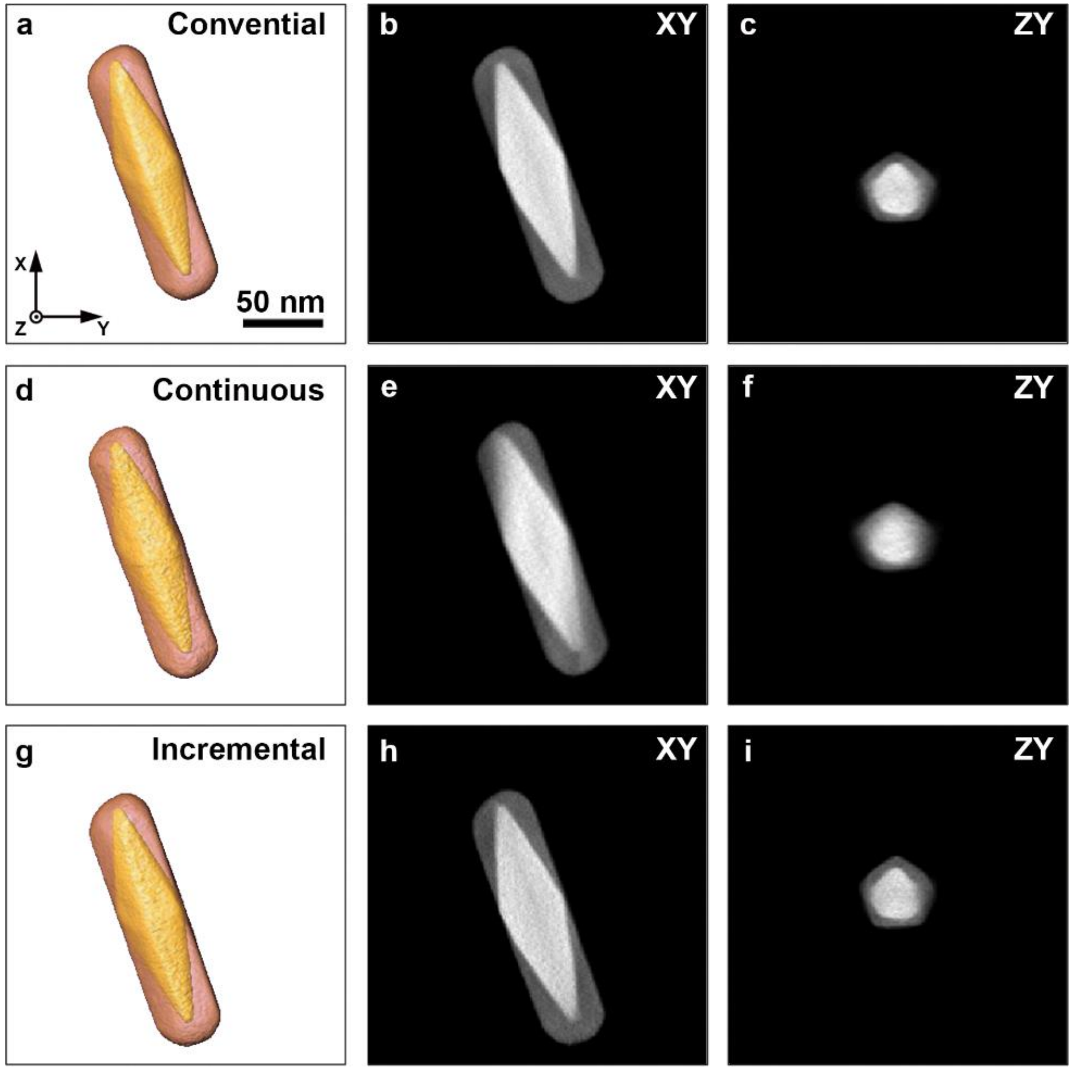


350 images, 6 minutes



Fast HAADF-STEM tomography



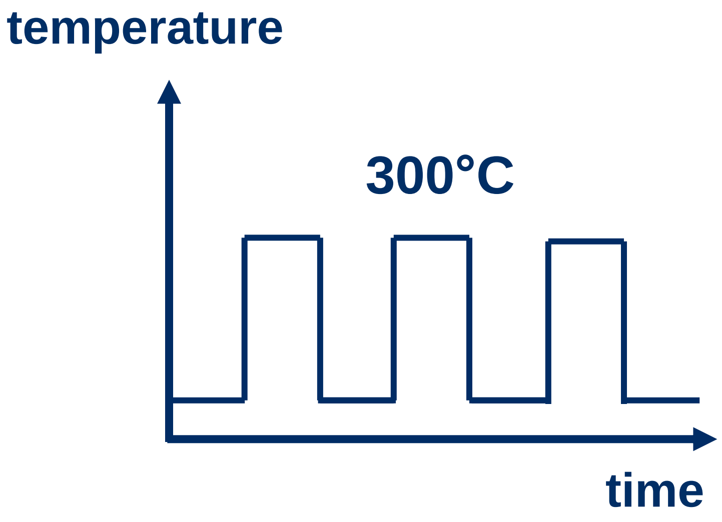


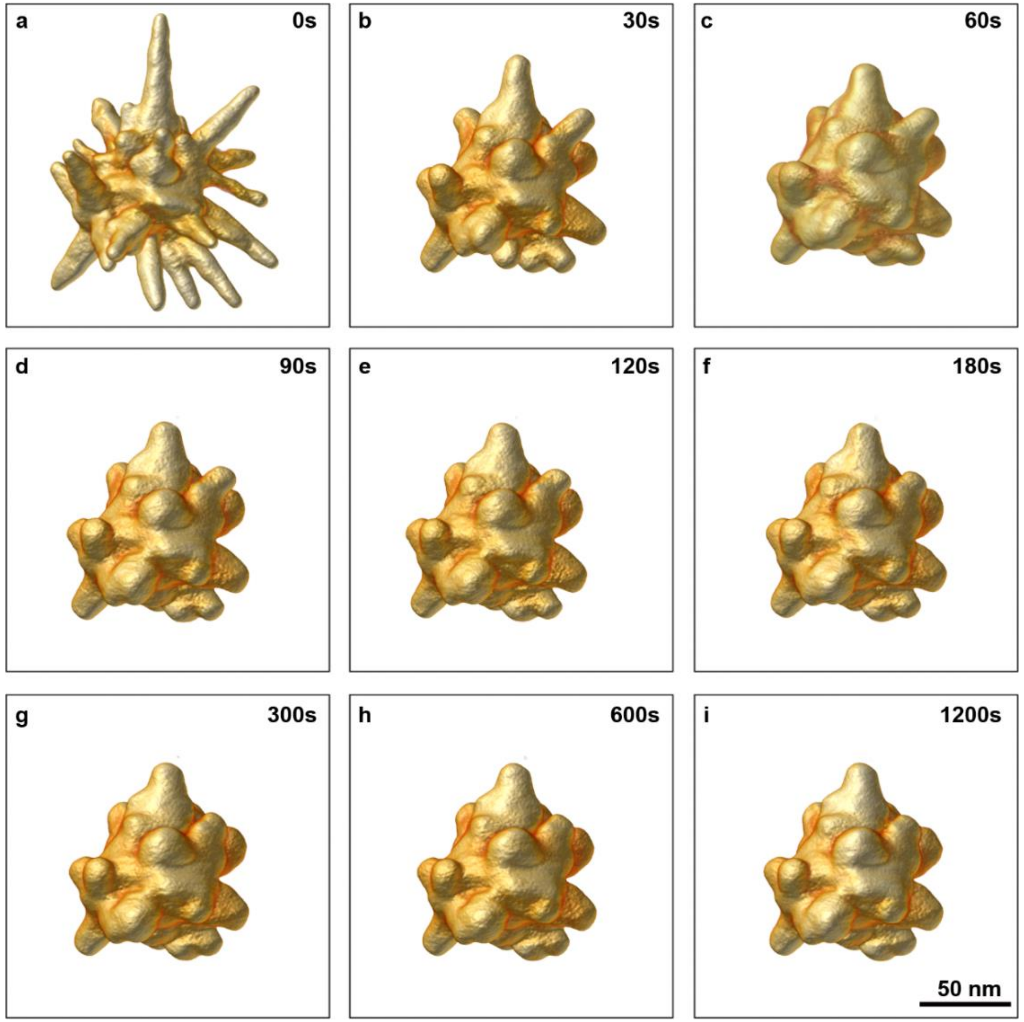
H. Vanrompay, A. Skorikov, E. Bladt, A. Béché, B. Freitag, J. Verbeeck, S. Bals, Ultramicroscopy 221, 113191 (2021).

Fast HAADF-STEM tomography

What happens at high temperatures?

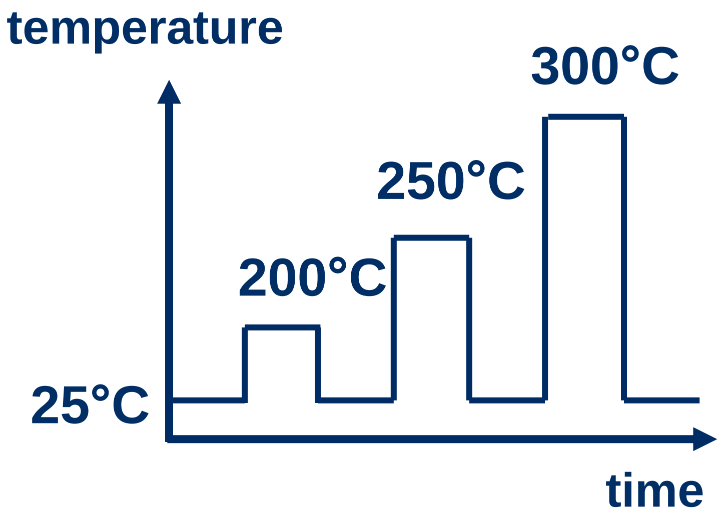
Morphological changes while heating: Au nanostars





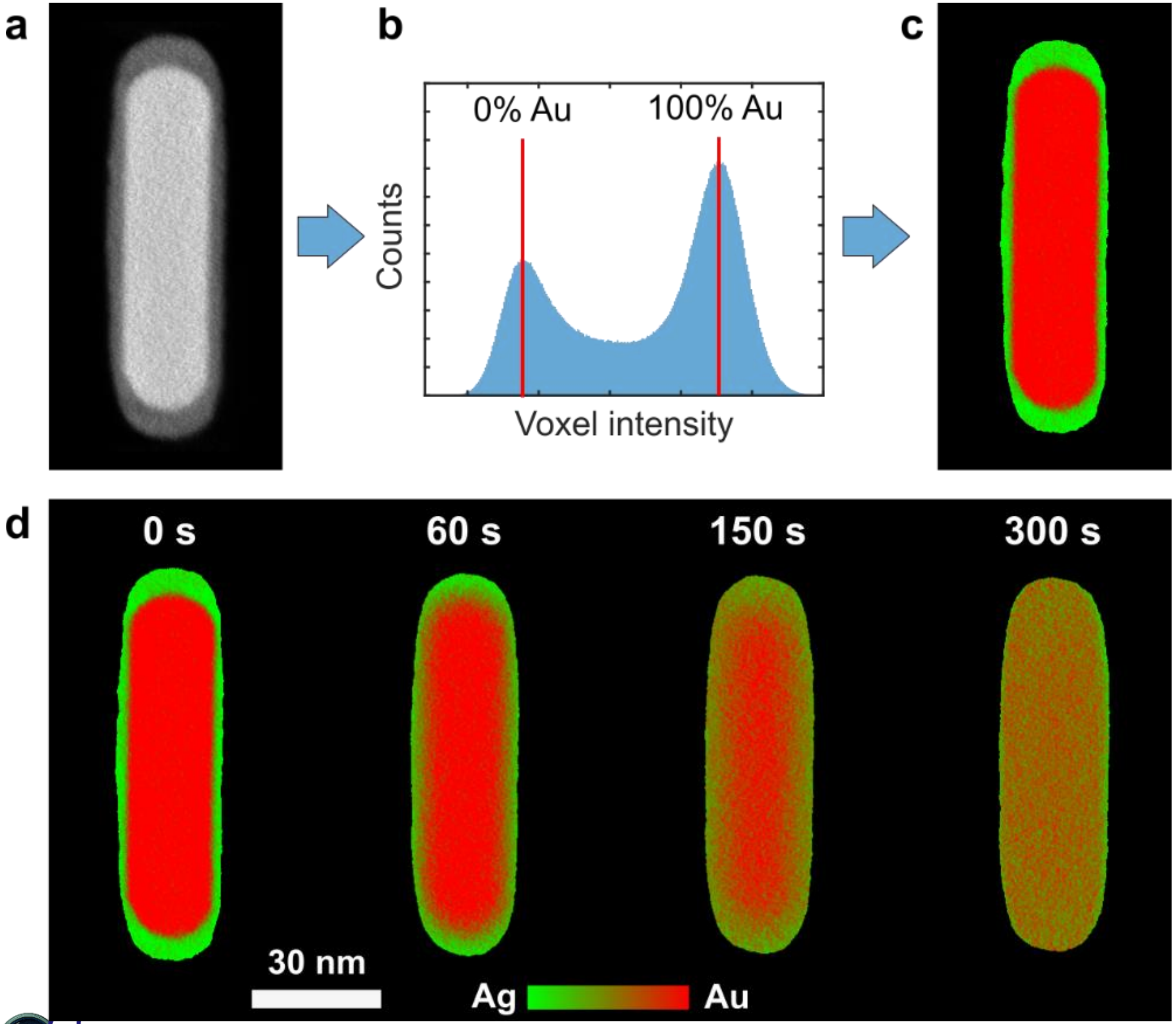
H. Vanrompay, E. Bladt, W. Albrecht, A. Béché, M. Zakhozheva, M., A. Sánchez-Iglesias, L.M. Liz-Marzán, S. Bals, Nanoscale 10 (2018) 22792

Morphological changes while heating: AuPd octapods



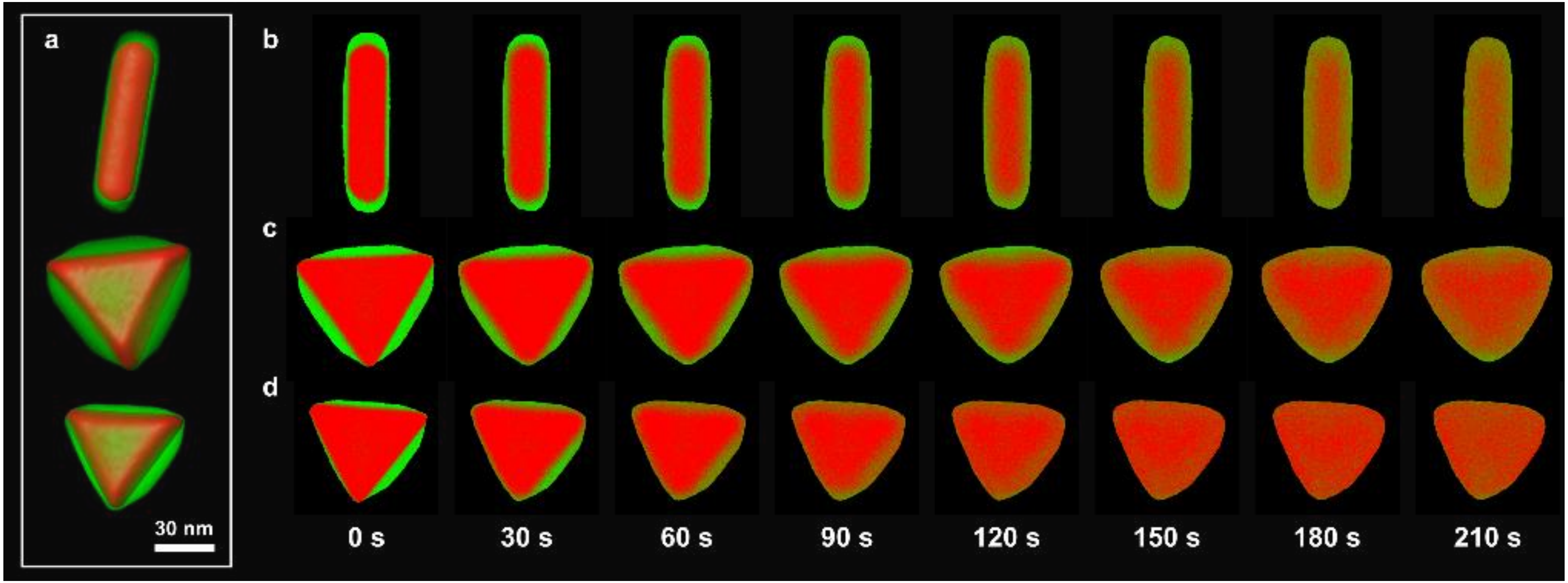
W. Albrecht, E. Bladt, H. Vanrompay, J. Smith, S. Skrabalak, S. Bals, ACS Nano 13 (2019) 6522-6530

Compositional changes while heating: AuAg nanorods



A. Skorikov, W. Albrecht, E. Bladt, X. Xie, J.E.S. van der Hoeven, A. van Blaaderen, S. Van Aert , S. Bals, ACS Nano, 13 (2019) 13421

Compositional changes while heating: AuAg nanorods

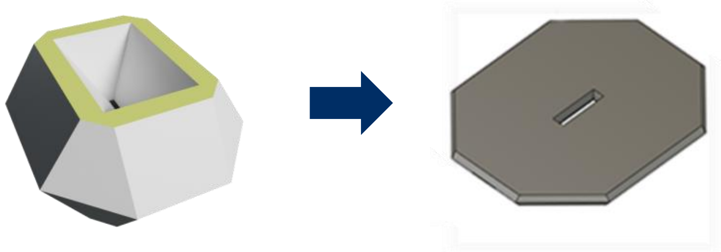


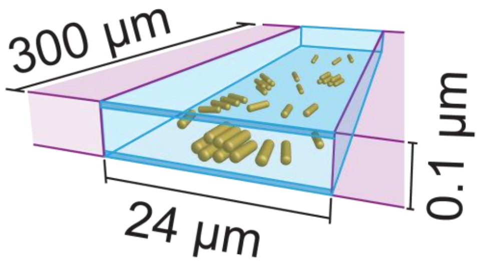
A. Skorikov, W. Albrecht, E. Bladt, X. Xie, J.E.S. van der Hoeven, A. van Blaaderen, S. Van Aert , S. Bals, ACS Nano, 13 (2019) 13421

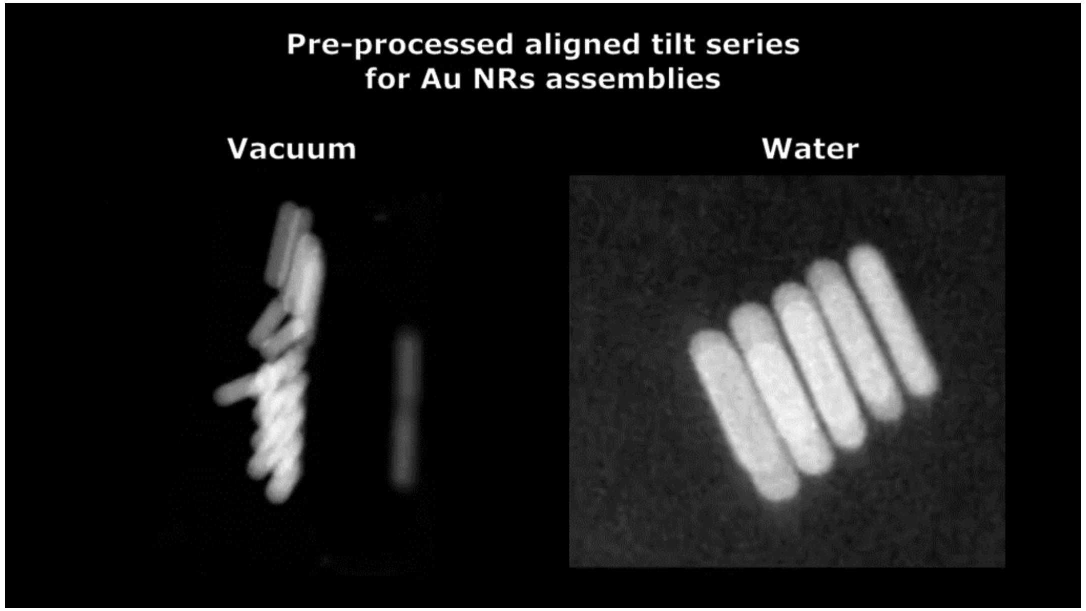
Morphological changes in liquid

K-Kit

Tomochip



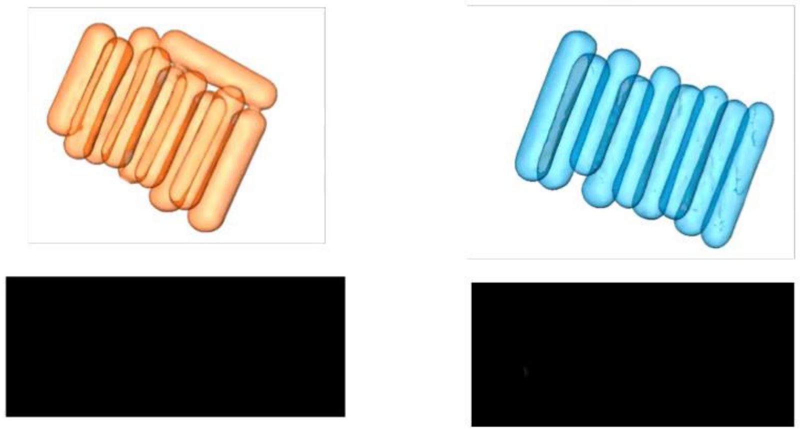




3D reconstructions of Au NRs bilayer assemblies

FET-vacuum

LP-FET



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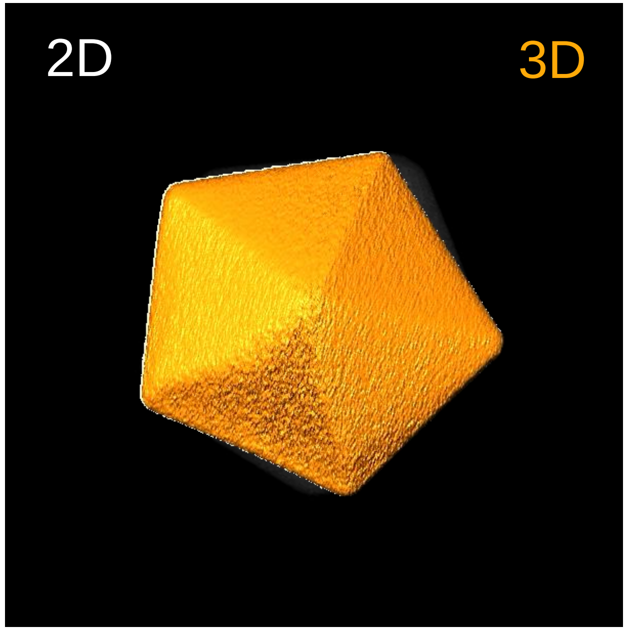
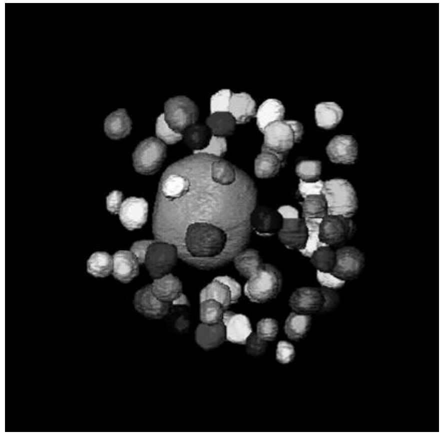
D.A. Arenas Esteban, D. Wang, A. Kadu, N. Olluyn, A. Sanchez-Iglesias, A. Gomez-Perez, J. Gonzalez-Casablanca, S. Nicolopoulos, L. Z. Liz-Marzan, S. Bals. Nature Communications 15 (2024) 15:6399

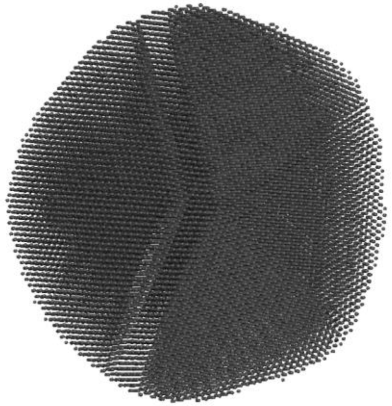
Conclusions



Morphology

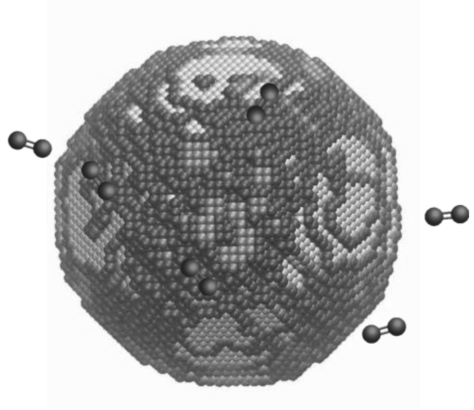


Defects



Composition

Realistic conditions



To go further …

Some practical, easy to follow reviews

- Midgley, P. A. & Weyland, M. 3D electron microscopy in the physical sciences: the development of Z-contrast and EFTEM tomography. *Ultramicroscopy* **96**, 413–431 (2003).
- Leary, R. K. & Midgley, P. A. Electron Tomography in Materials Science. in *Springer Handbook of Microscopy* (eds. Hawkes, P. W. & Spence, J. C. H.) (Springer International Publishing, Cham, 2019).

Advanced and theoretical resources

- *Electron Tomography: Methods for Three-Dimensional Visualization of Structures in the Cell*. (ed. Frank, J.) (Springer, New York ; London, 2006).