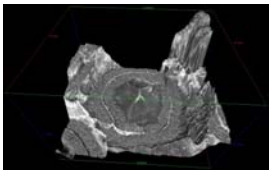
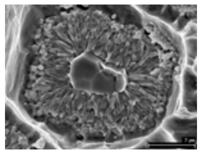
3D Microscopy and FIB Nanotomography

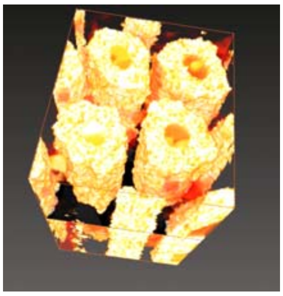


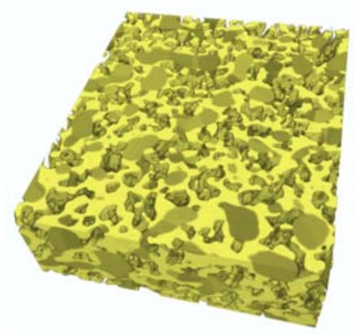


Marco Cantoni

CIME

mxc-133, 34816





Introduction

- Goals:
- Overview of 3D techniques in EM
- FIB Nanotomography: compared to other tomographic techniques (X-ray, TEM tilt-series, atom-probe)
- Introduction to image processing and reconstruction with ImageJ/Fiji

Program 1st day

Topics

1) Introduction: Imaging by SEM:
- Contrast formation of SE and BSE imaging.
- Interaction volume, resolution.
- Principle of 3D surface (2½ D) reconstruction (SEM)

2) Introduction to TOMOGRAPHY
- Tomography: serial sectioning (FIB/SEM, TEM) and tilt-series tomography. Comparison of the different techniques: possibilities and limitations for materials science applications.

3) Introduction to FIB
- Basics, FIB as a tool for machining, Ion beam induced deposition
- Typical applications: Micro-machining, TEM Lamellae preparation, Cross-sectioning

4) FIB-Nano-Tomography
- serial-sectioning, FIB-Nanotomography in Materials and Life Science

5) Introduction to Image Processing

Program 2nd day

6) Image Processing practicals
- **Fiji (ImageJ & plug-ins)**
- Basic functions:
- Stack operations: opening a stack, registration (alignment) of the images, z-scaling
- Simple image procession: noise reduction/filtering (3D mean,…)
- Reconstruction and visualization: ortho-view, banalization (segmentation), generating 3D views of the stack 3D volume viewer….

7) FIB demo
- Setting up a serial sectioning: Preparing the sample, rough milling of the block face, the choice of the right detector, running the acquisition of a stack

Program 3rd day

- **8) advanced FIB Nano-Tomography in Materials and Life Science**
- 3D-EDX
- FIB-nt in Life Science: Segmentation problem

- **9) Exercise and report**

Excercises/Report

- **working with Fiji**
- Processing of selected image stacks

- **For the credit:** short report about 3D volume analysis of a selected stack

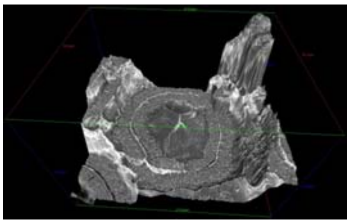
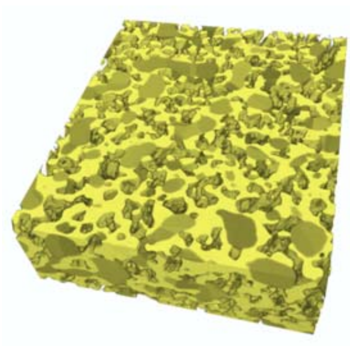
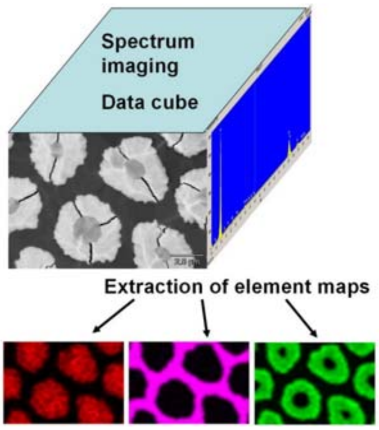
1. Introduction

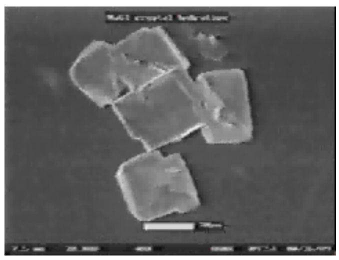
- A single image produced by an Electron Microscope generates a 2D image only.
- Any information about the 3rd dimension requires at least 2 images.

3rd Dimension
- Surface (2½ D)
- Volume
- Time
- Composition

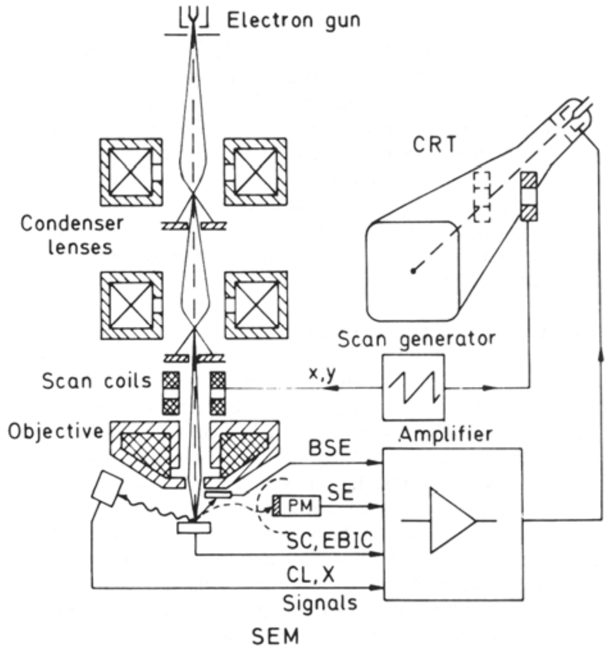
Introduction

3rd Dimension
- Surface
- Volume
- Time
- Composition

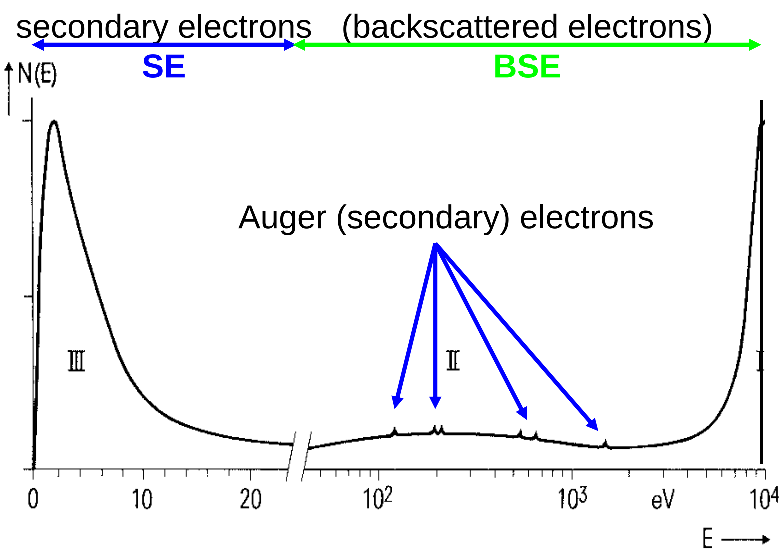


SEM principle



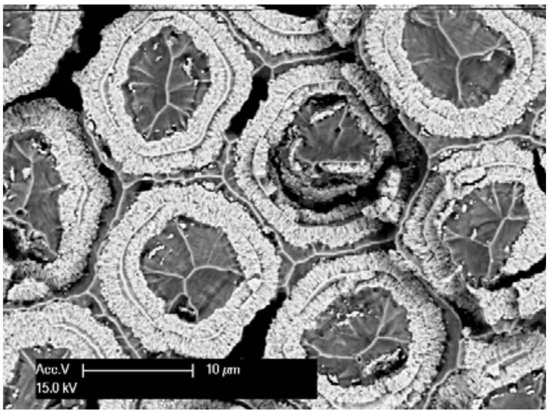
(from L. Reimer, Image formation in Low-Voltage Scanning electron microscopy.)

Energy spectrum of electrons leaving the sample

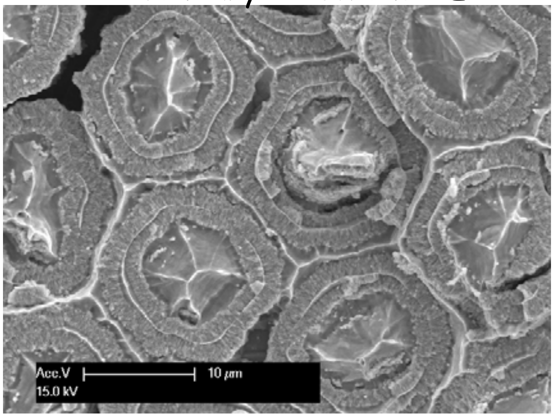


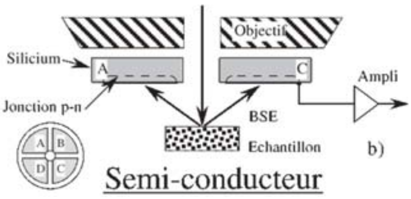
SEM detectors

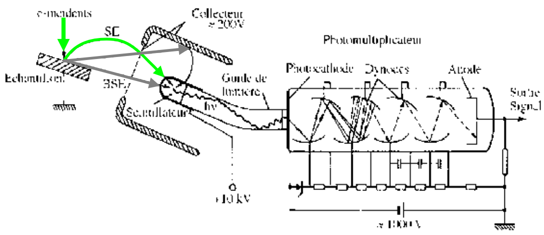
Backscattered electrons BSE



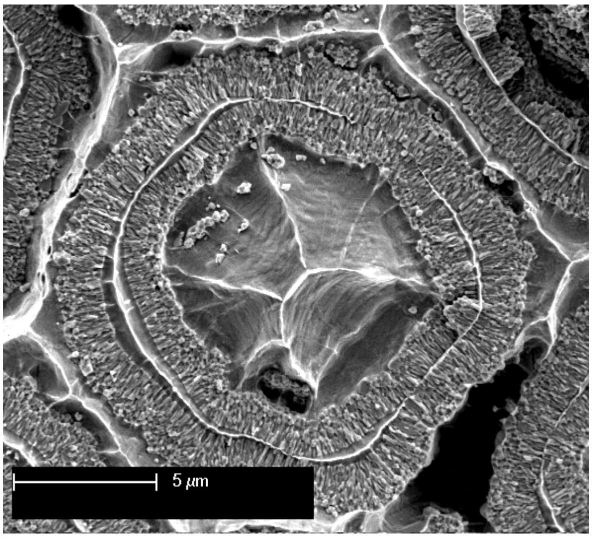
Secondary electrons SE







effect of accelerating voltage



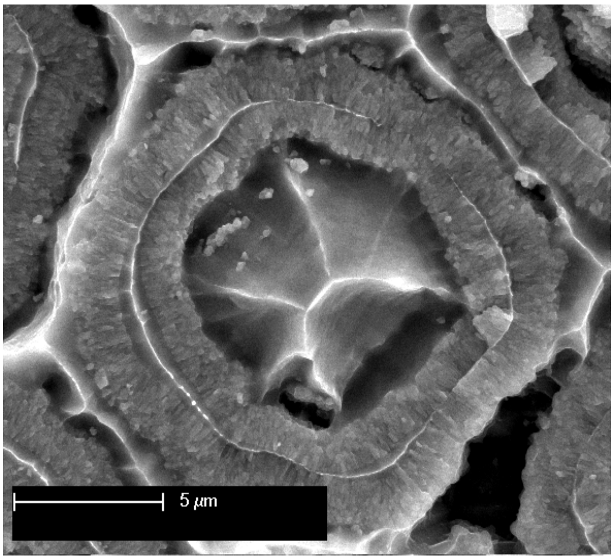
Low kV (5kV)

More surface details

SE detector

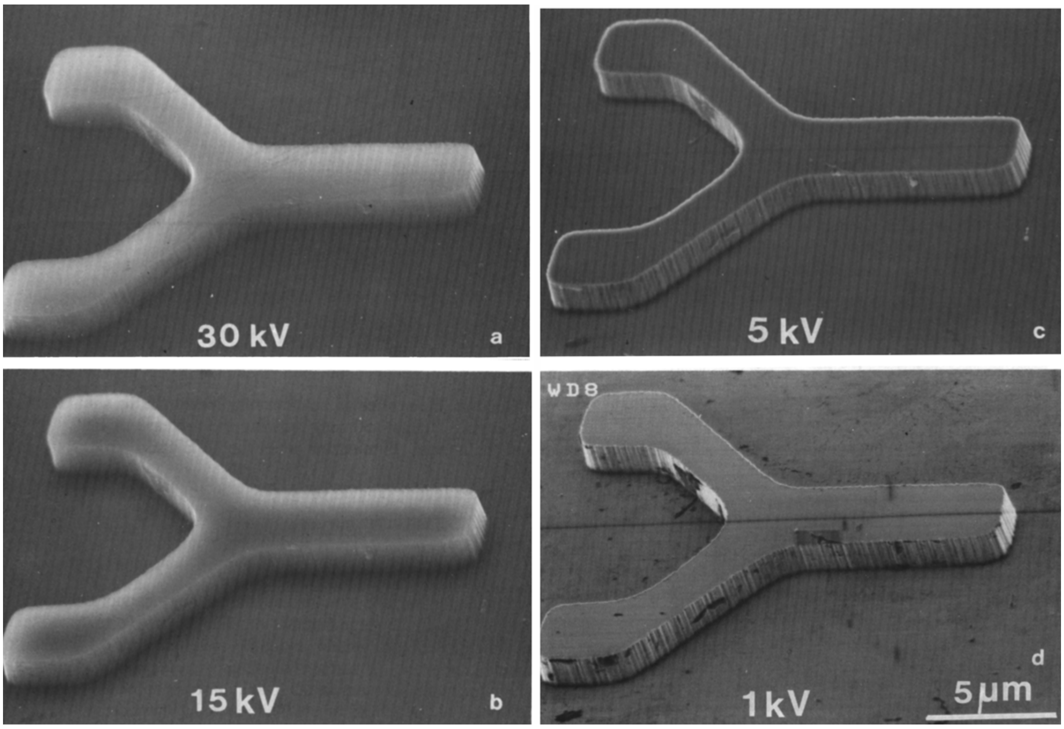
high kV (30kV)

Small grains seem “transparent”

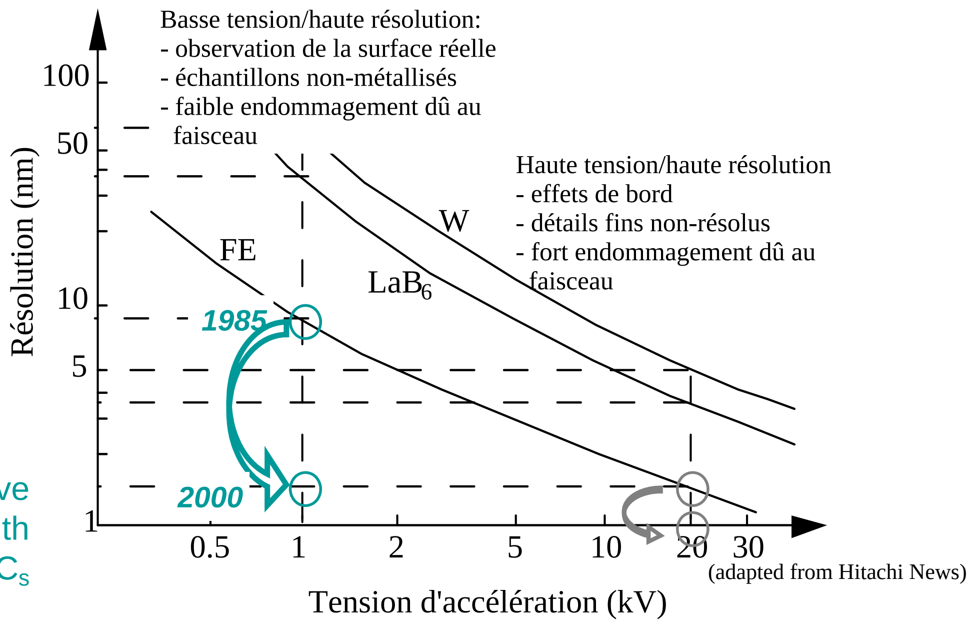


effect of accelerating voltage

SE detector

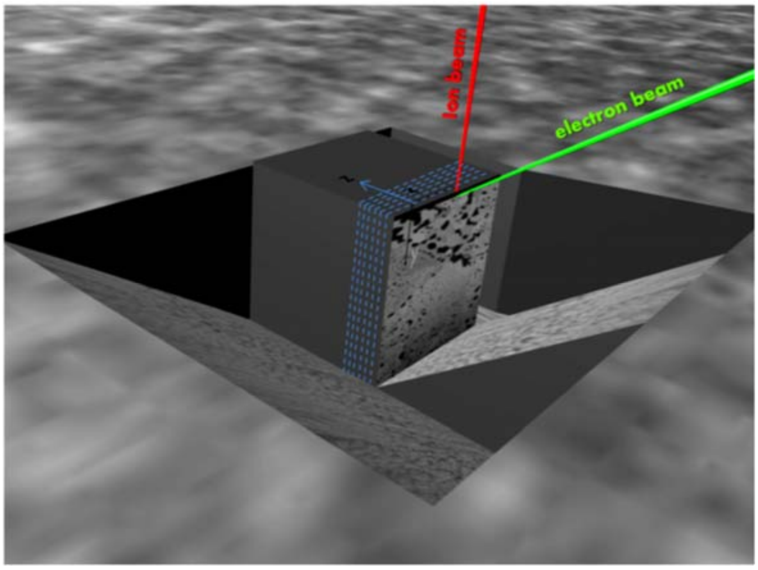


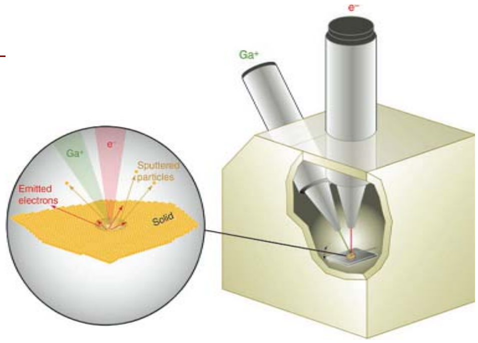
SEM: Loss of resolving power at low accelerating voltage

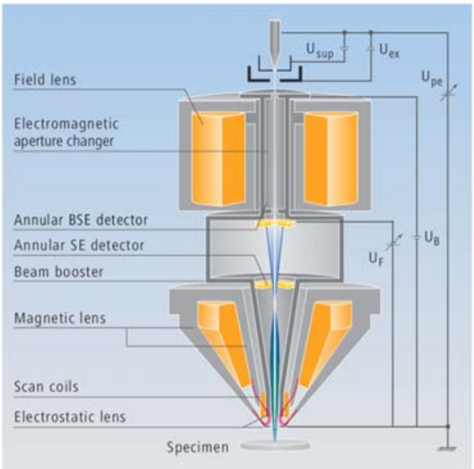


Shorter objective lens focal length and C_s

Modern SEM in column detectors

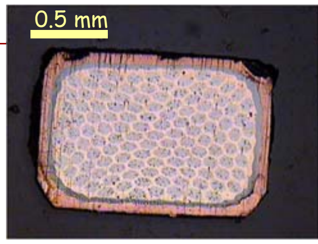






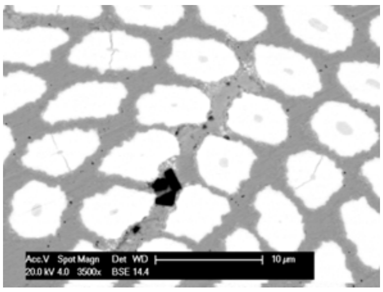
WYSIWYG: What You (detector) See Is What You Get

Classical BSE conditions

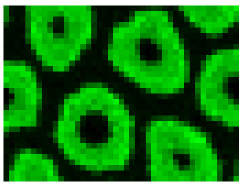


Nb_3Sn superconductor multifilament cable:
14'000 Nb_3Sn filaments (diameter ~5um) in bronze matrix

Solid State BSE detector
20kV acceleration voltage

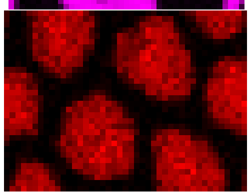


EDX maps

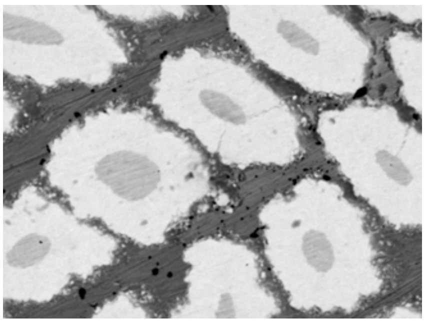


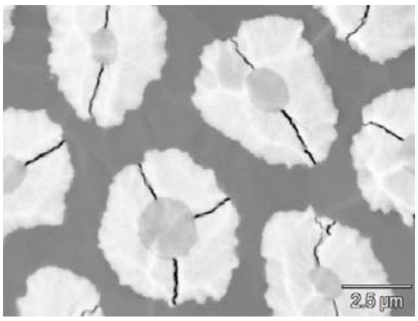
Sn

Cu

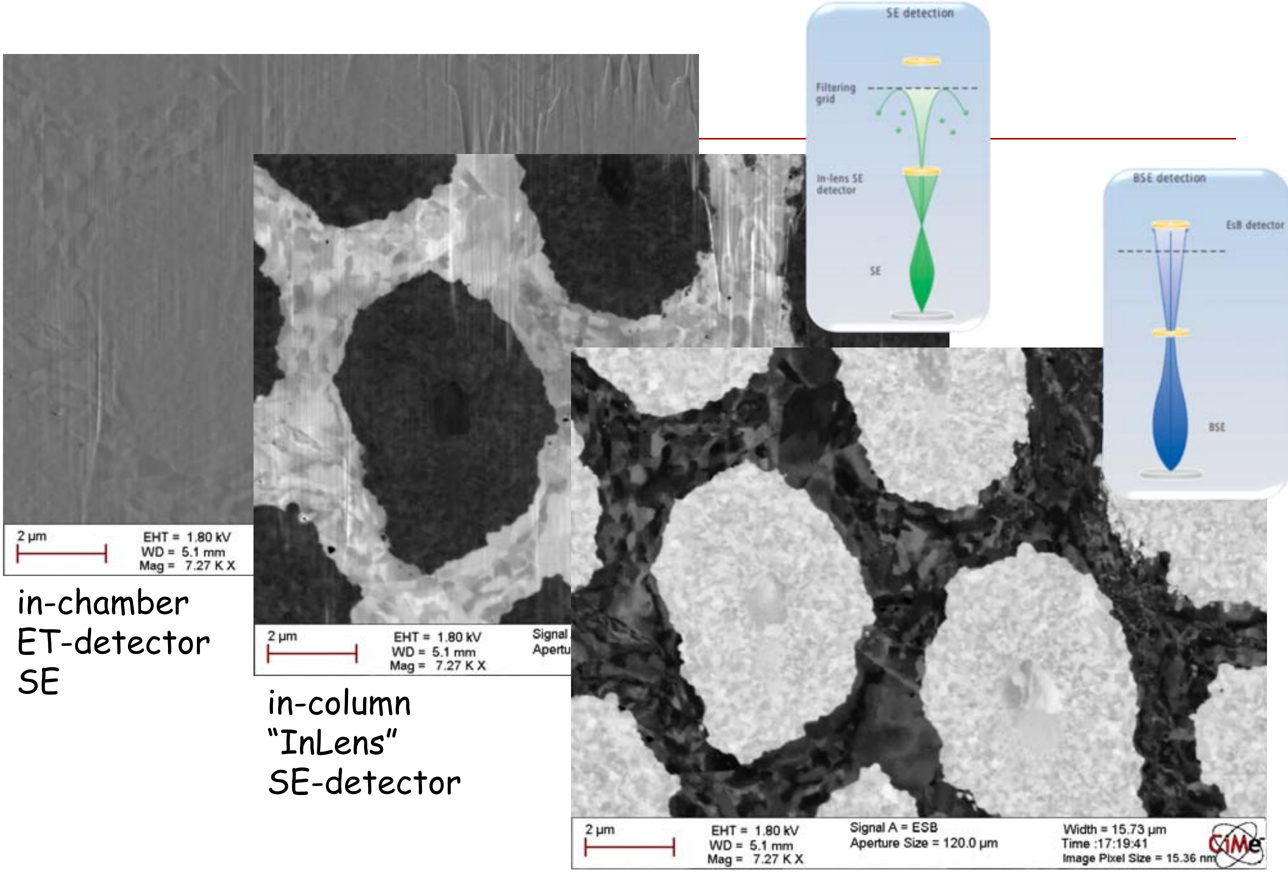


Nb





Mechanical polishing <-> Ar ion beam polished

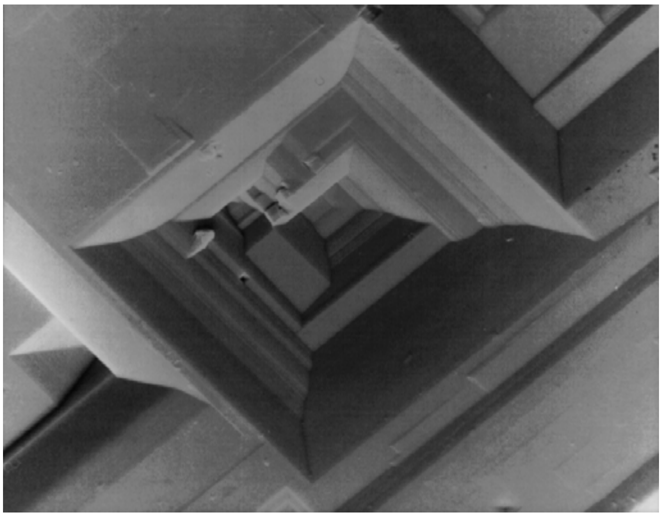


in-chamber
ET-detector
SE

in-column
"InLens"
SE-detector

in-column, "energy-selective" EsB
BSE-detector

Review SEM



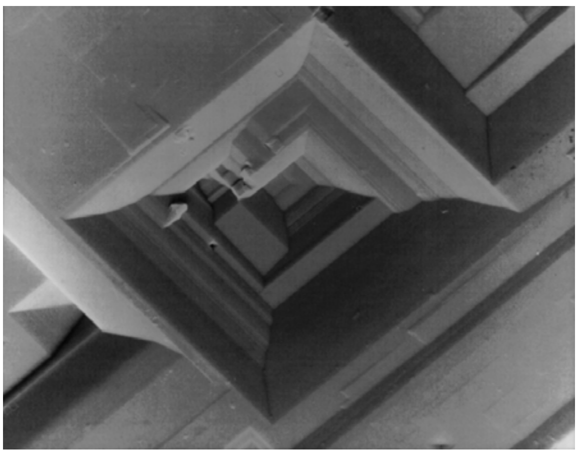
- What does it suggest?
- Which objective information?

Review SEM

- What does it suggest?
- Which objective information?

Detectors !!!

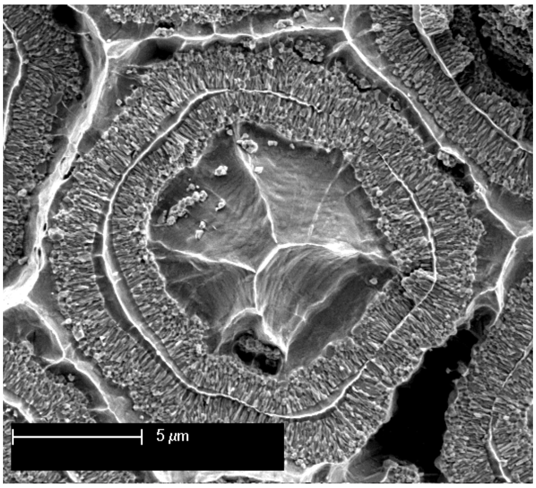
Detector ? **Detector ?**

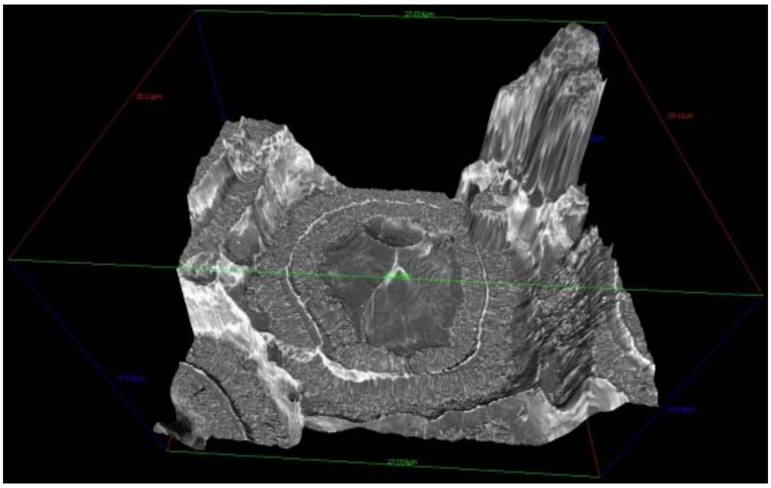


? µm

pyramide? etch-pit?

Stereographic reconstruction





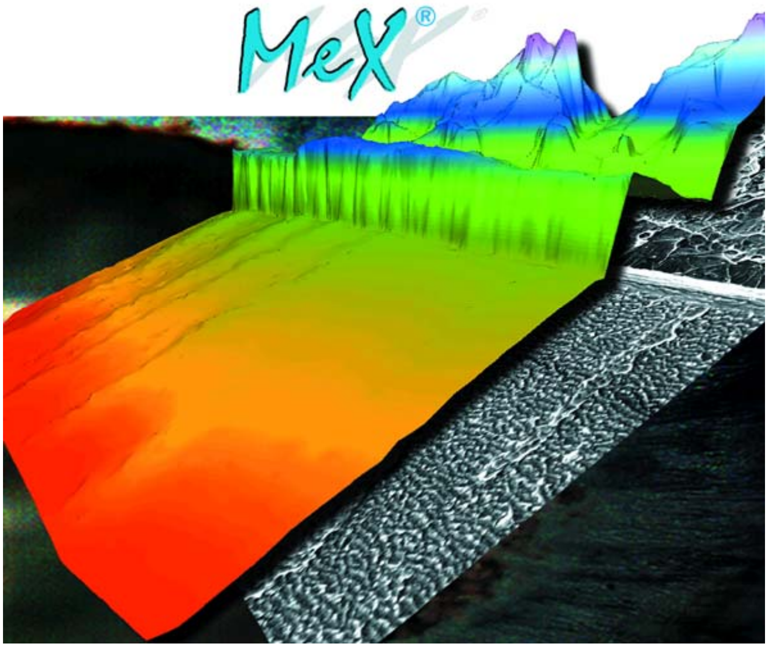
3D surface reconstruction

alicona imaging

MeX –
the real metrology package in SEM

- Alicona Imaging GmbH
- Algorithms

MeX – the real metrology package



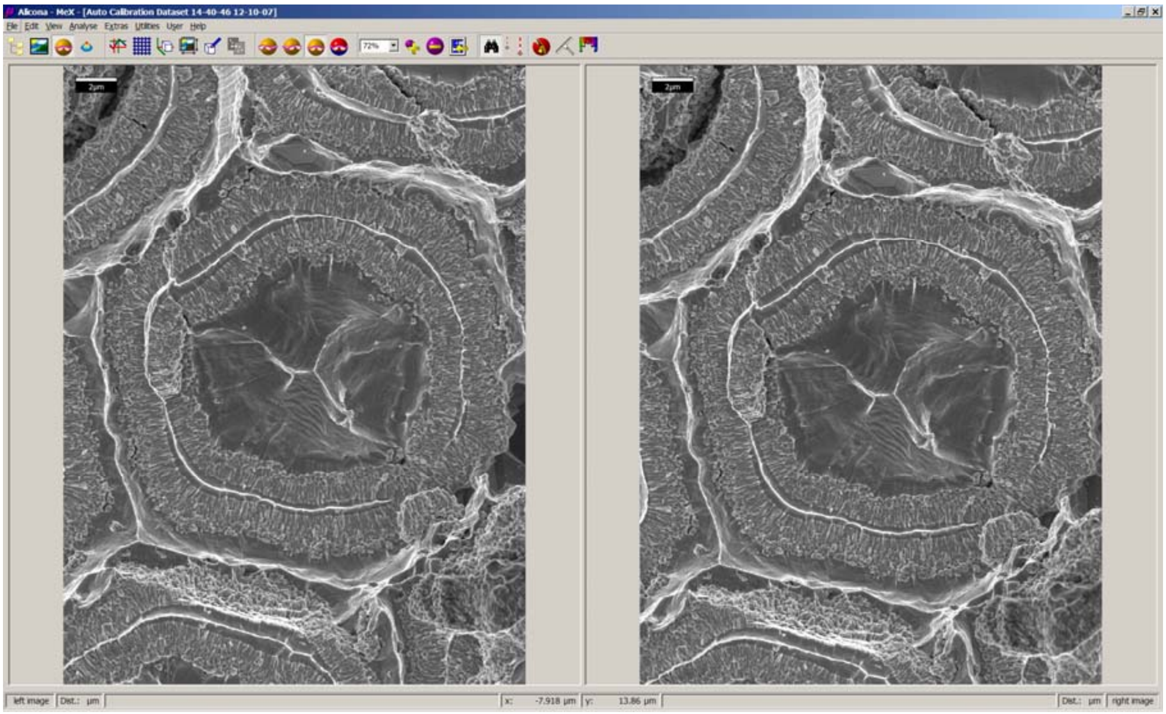
What is MeX

Software for 3D surface reconstruction and analysis in SEM

- Automatic, robust and accurate reconstruction within minutes
- Automatic calibration with traceable results
- Applicable at any microscope at any magnification
- Z-resolution approx. 3 times image resolution
- Comprehensive analysis (z.B. EN/ISO)

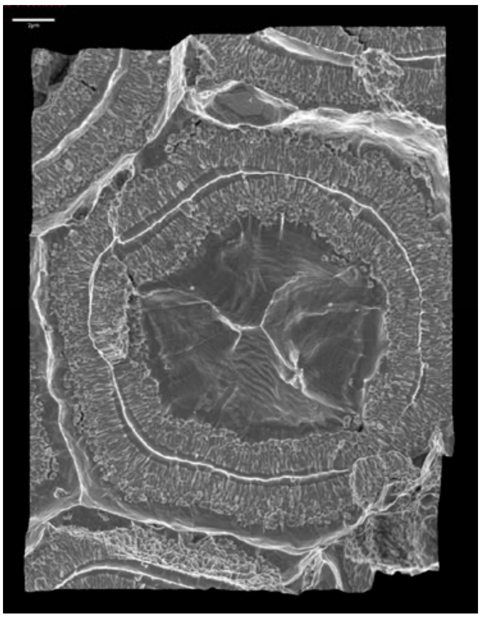
SEM becomes fully operational 3D metrology device

3D dataset from stereopair

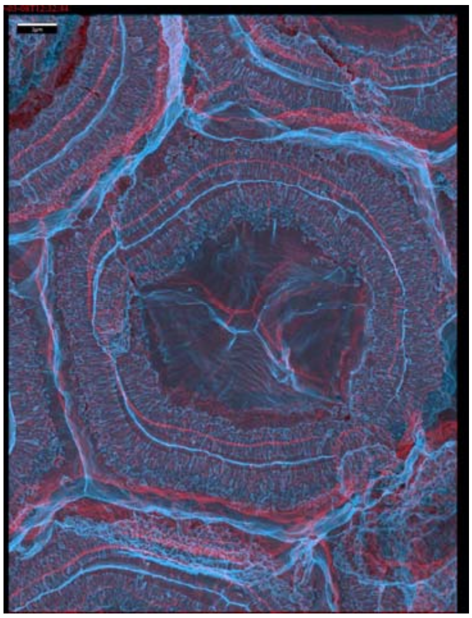


Views from different angle (5 degrees tilt)

Reconstructed surface (anaglyph)



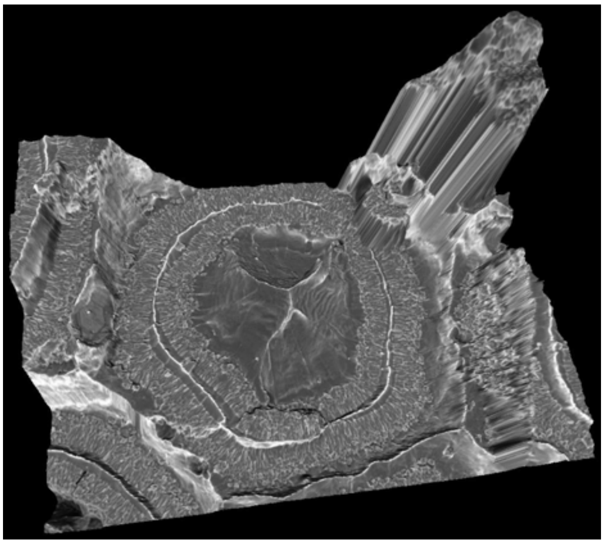
Topview 3D

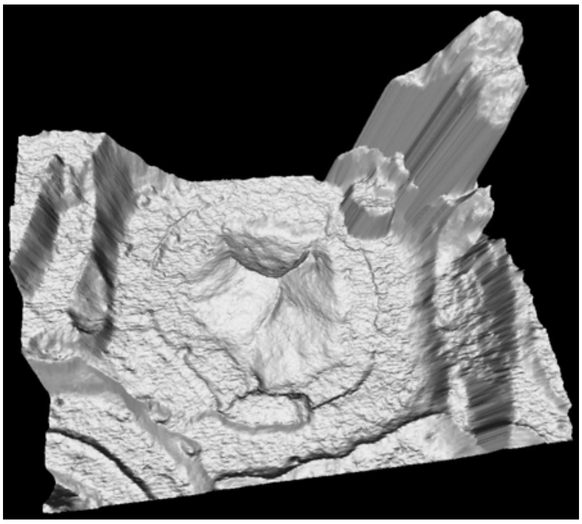


Anaglyph
(to view with red and cyan filter)

3D Dataset

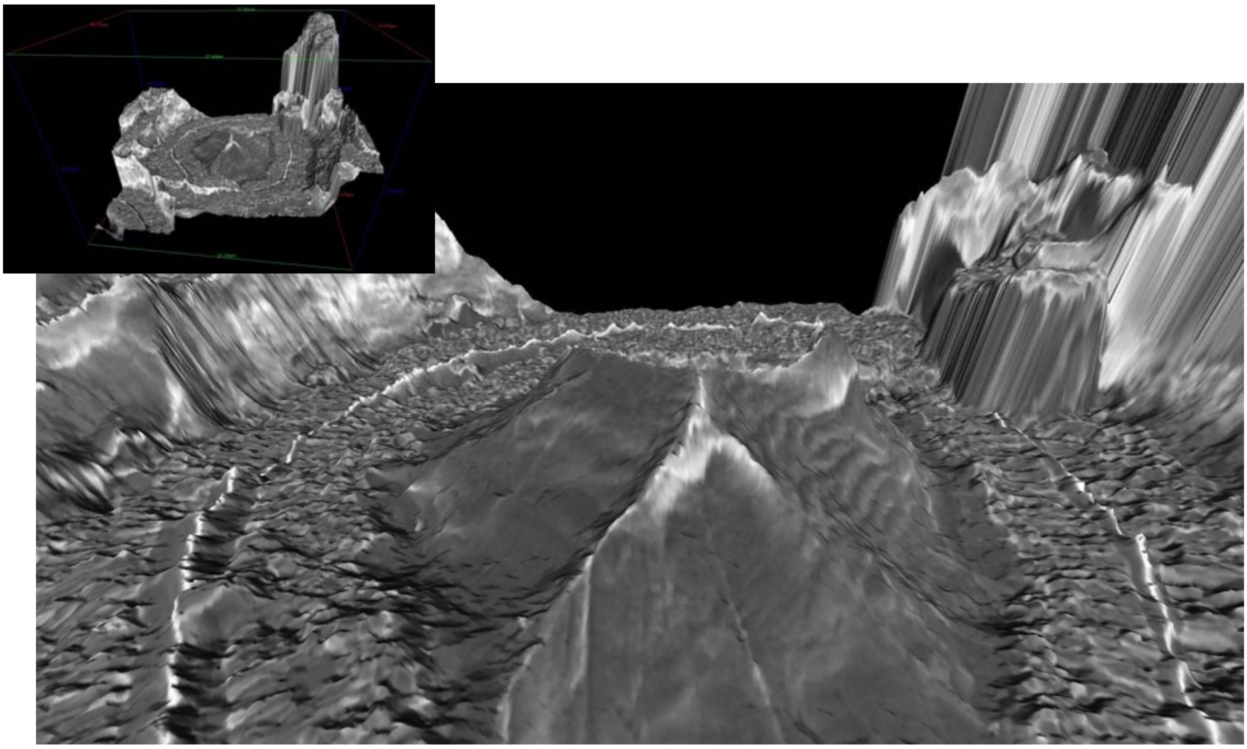
model with image overlay



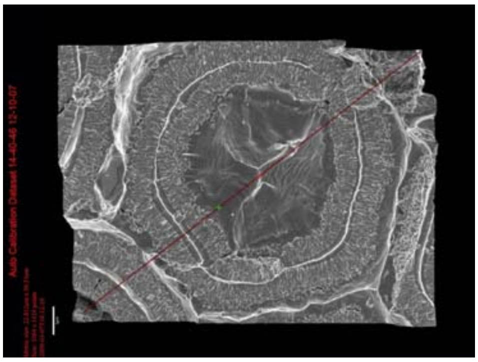


surface model

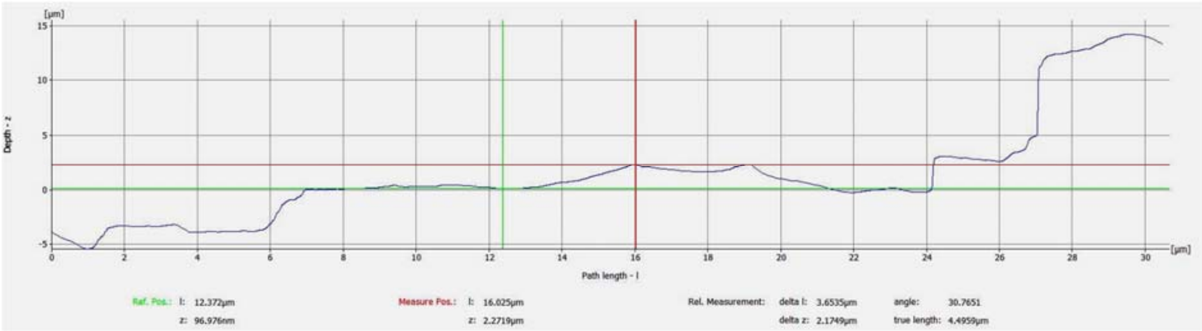
3D model

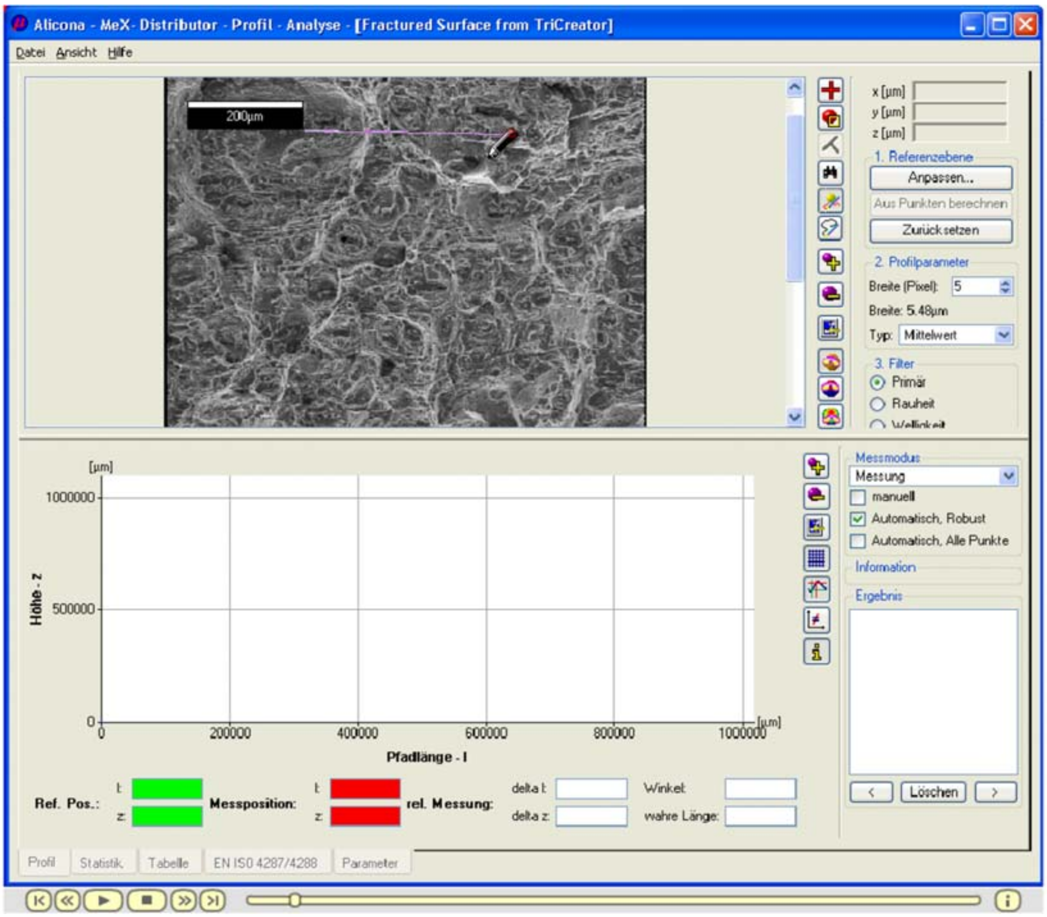


Quantification



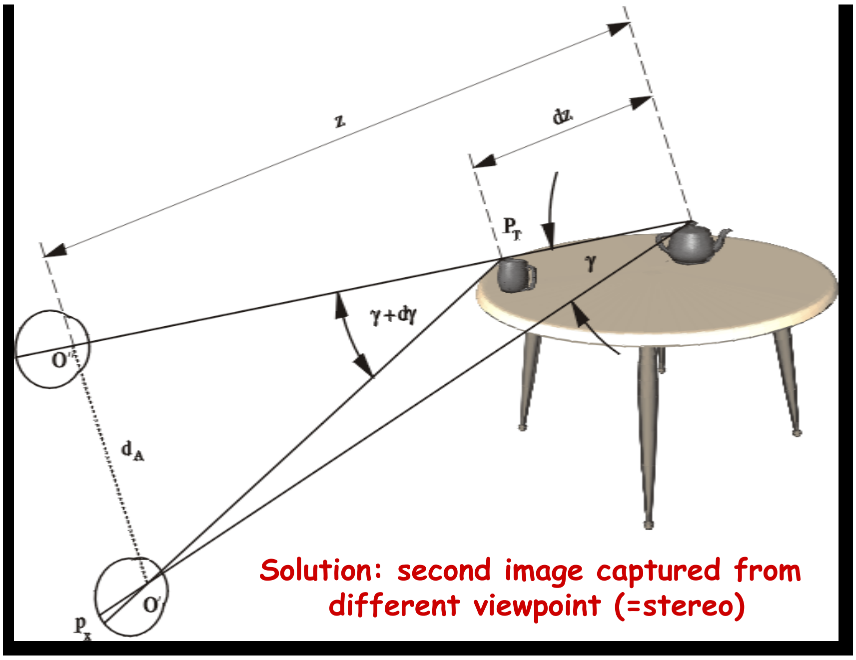
Quantitative:
- height profile
- roughness



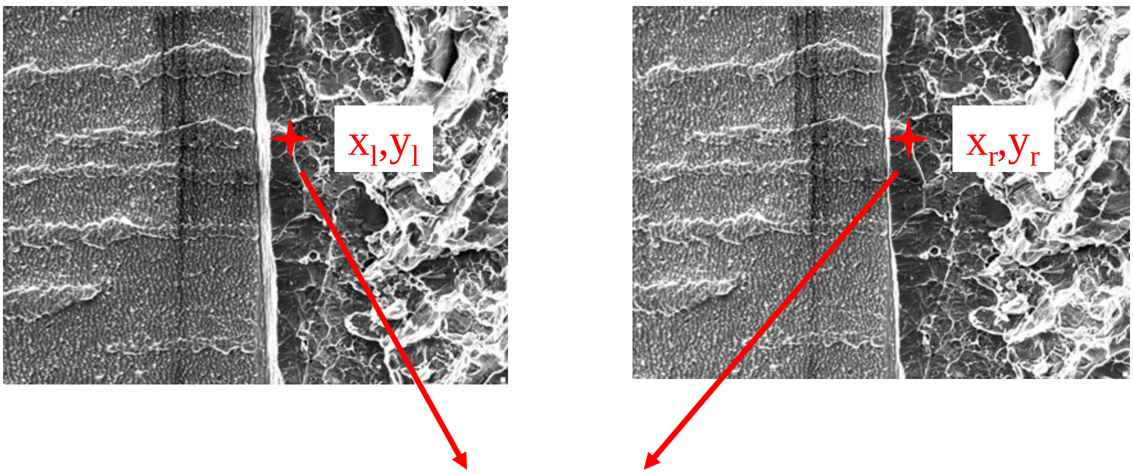


Shape-from-Stereo

Projection 3D object -> 2D image plane
= loss of information



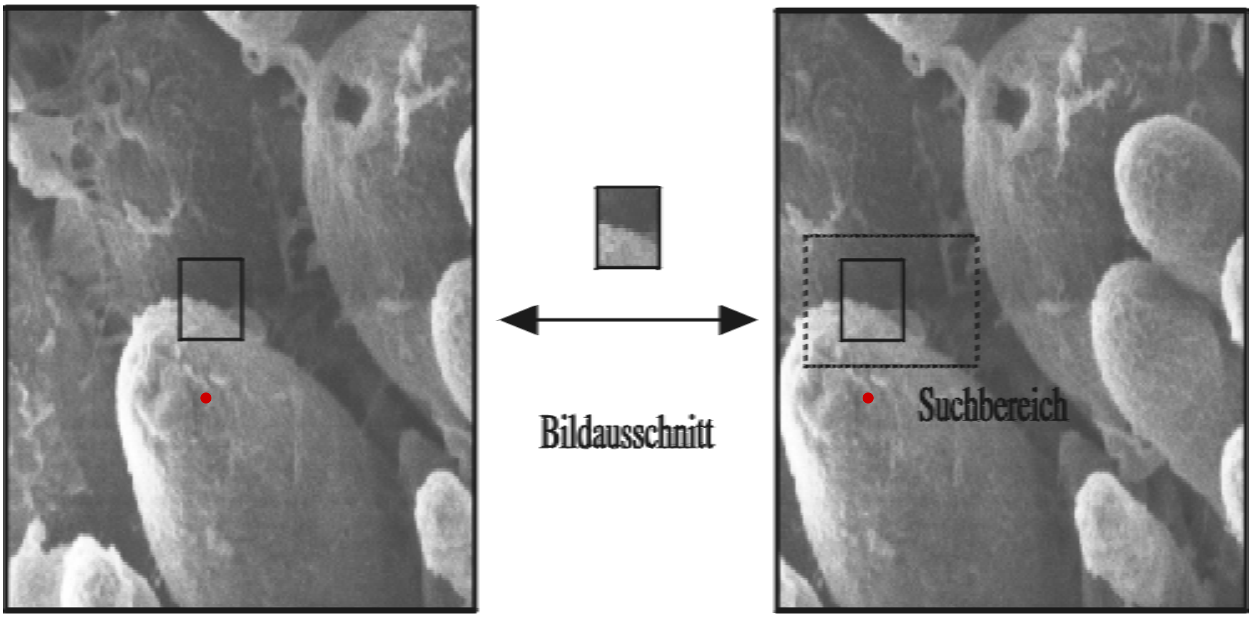
How is the 3D Dataset Created?



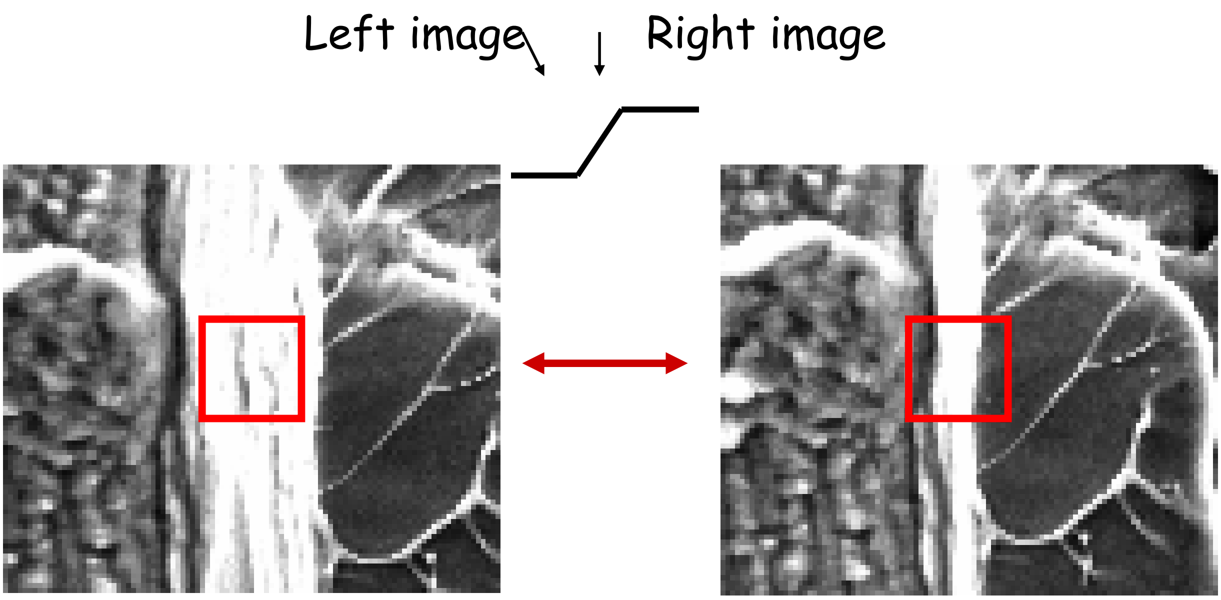
1. Matching
2. Calibration

X,Y,Z

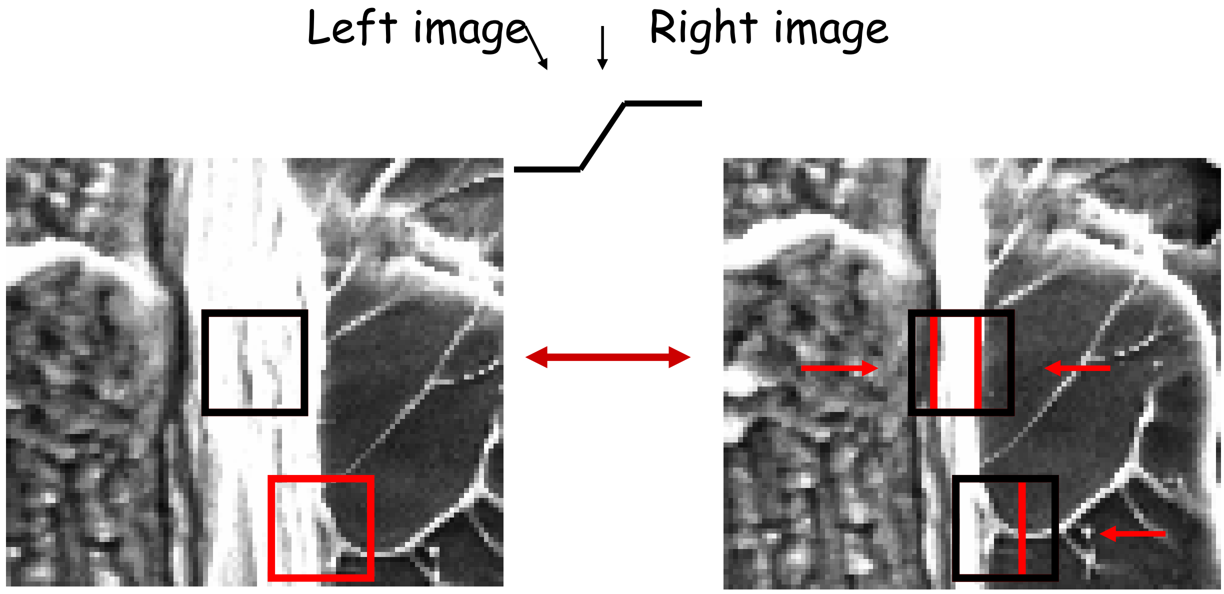
Classical Approach – Area Based Correlation



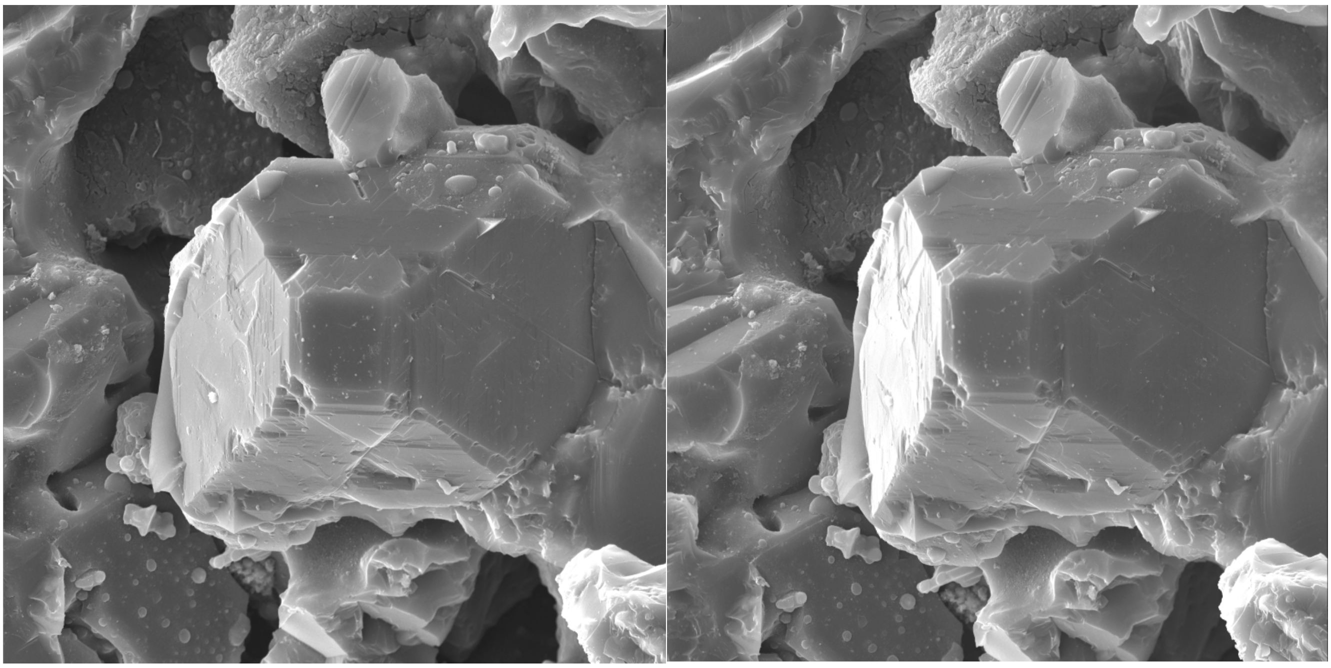
Problem: Distortions

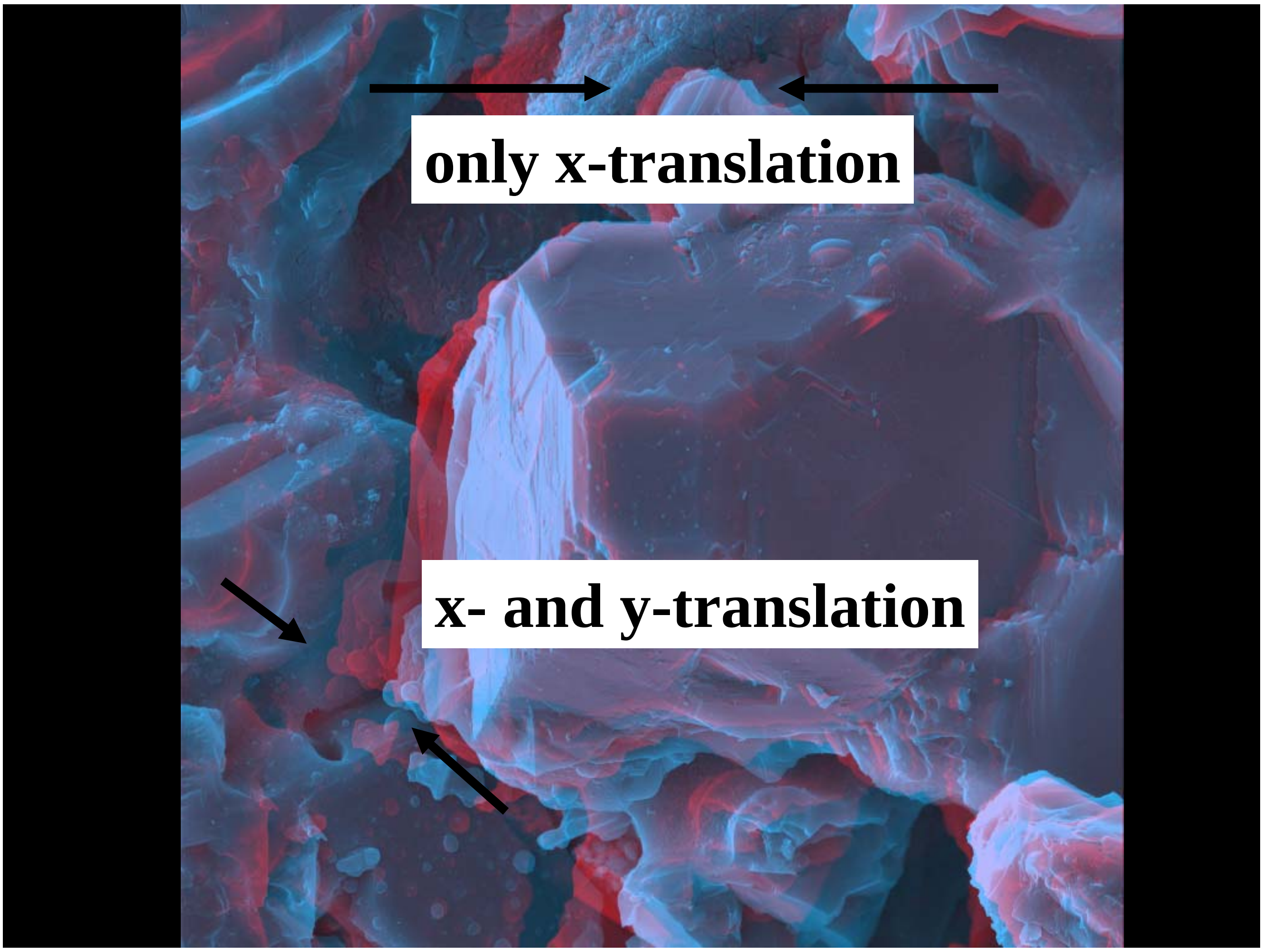


Solution – Adaptive Window

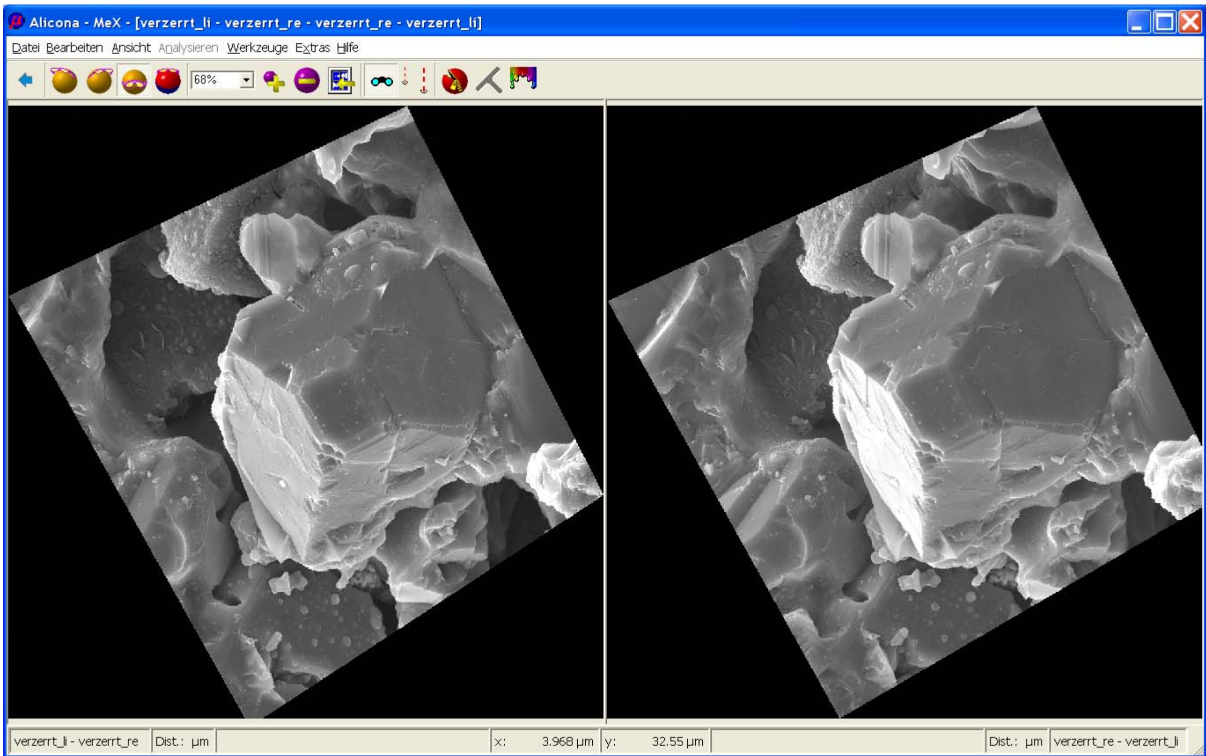


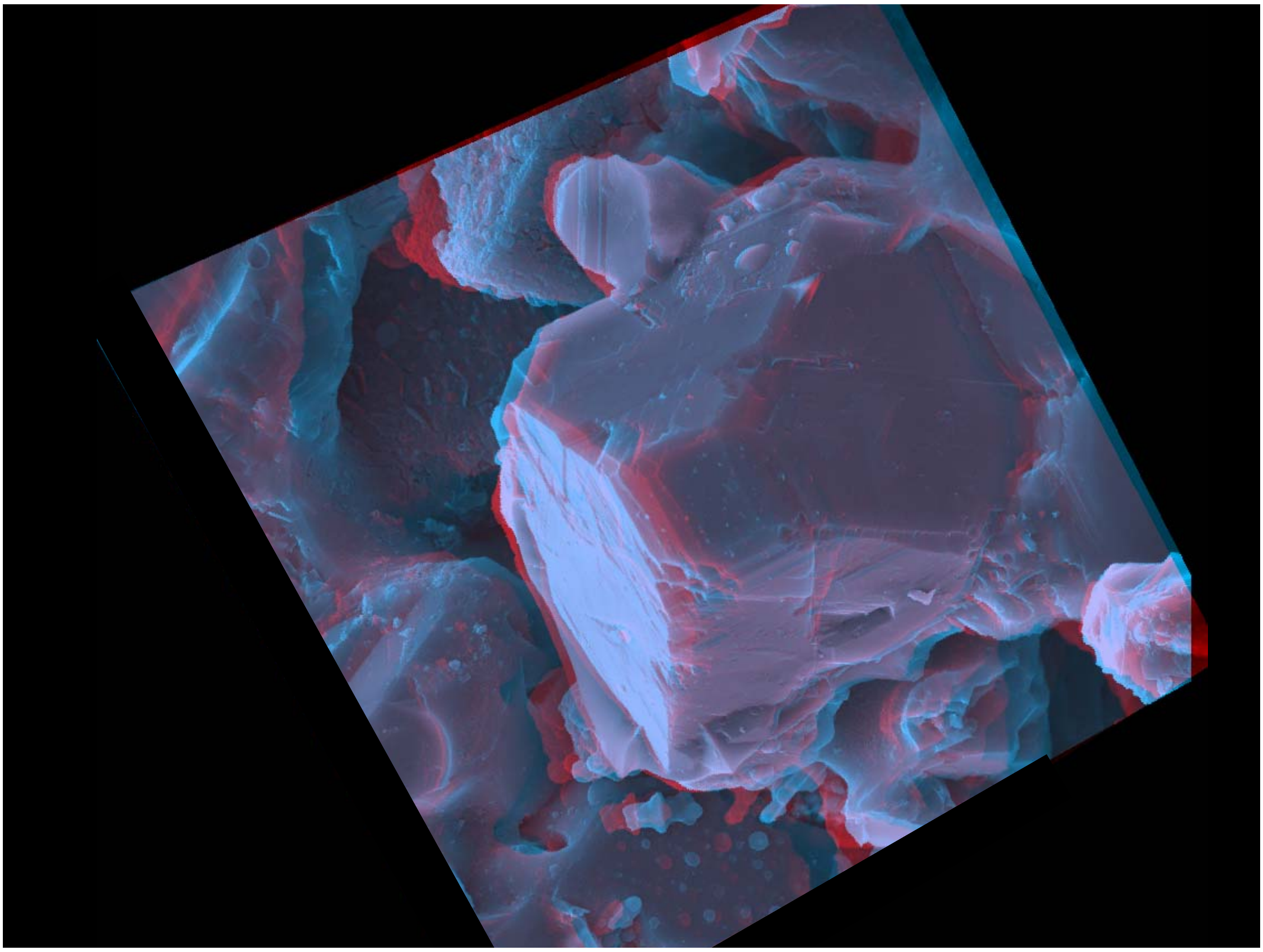
Problem Calibration



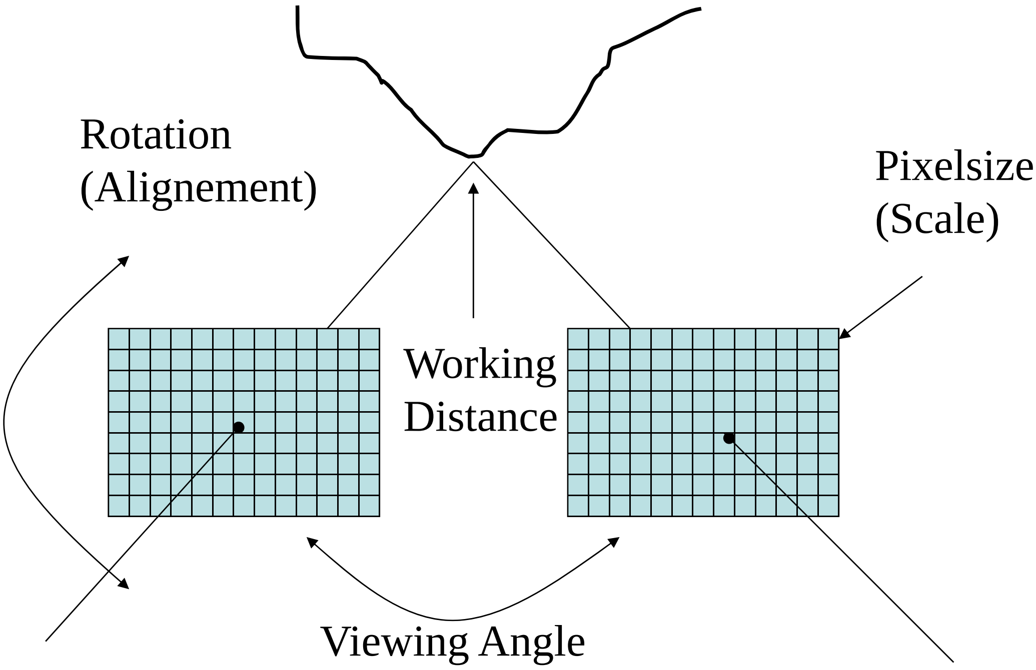


Automatic Rectification





Calibration



Viewing Angle Critical for Accuracy

Automatic Calibration

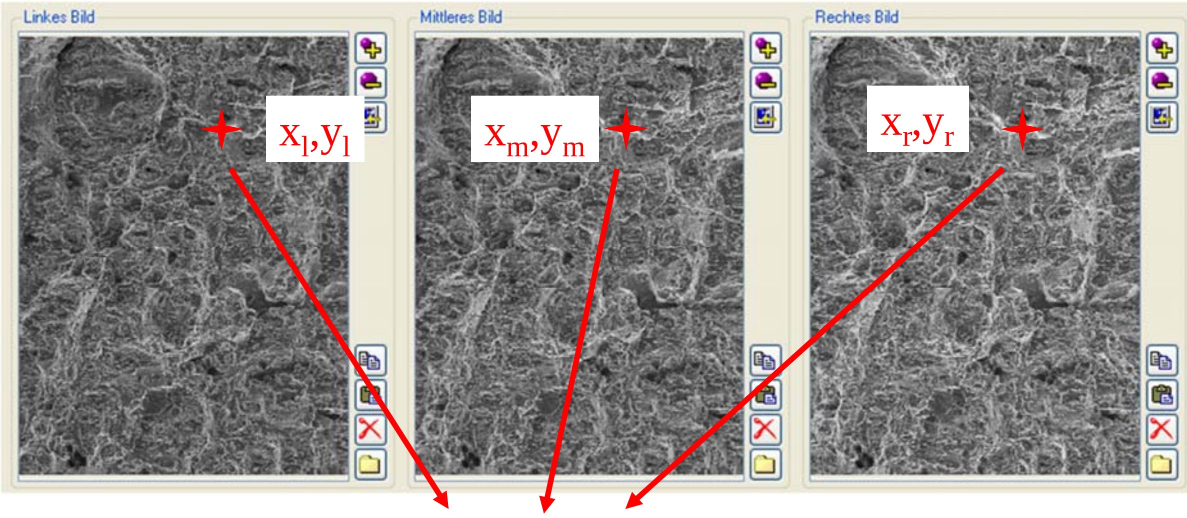
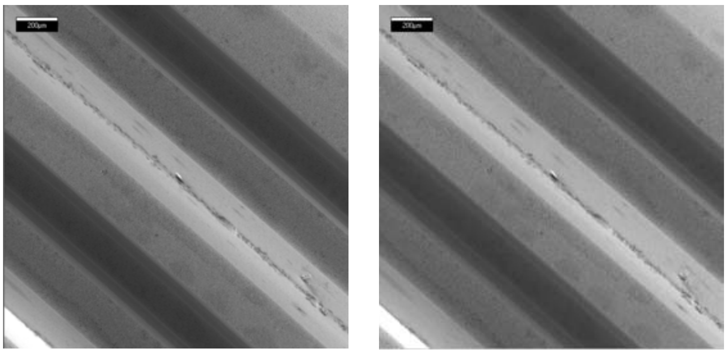


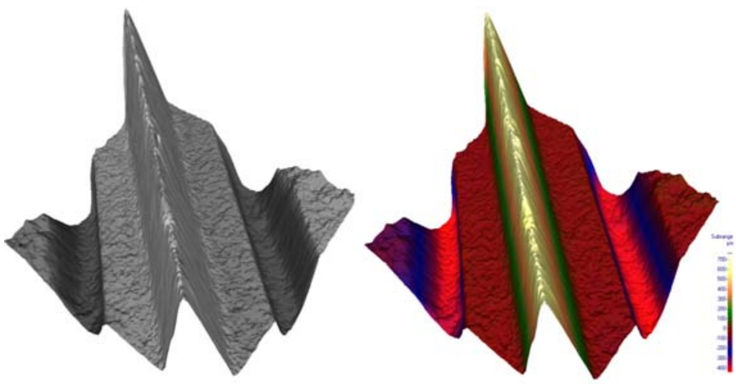
Image Matching + Automatic Calibration

= 3D Dataset

Verification – Steep flanks

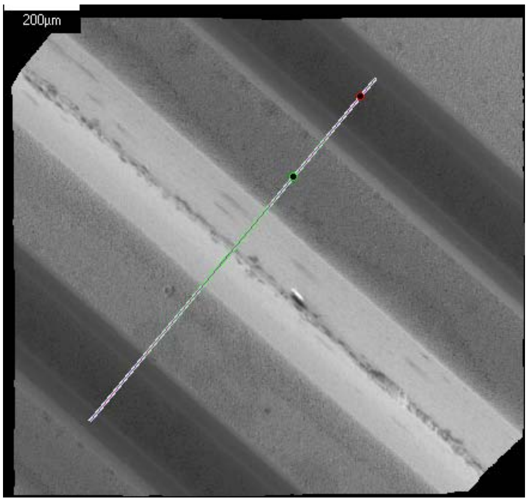


Left and right input image from the SEM (angles -2° and +2°) The center image at 0° is not shown.

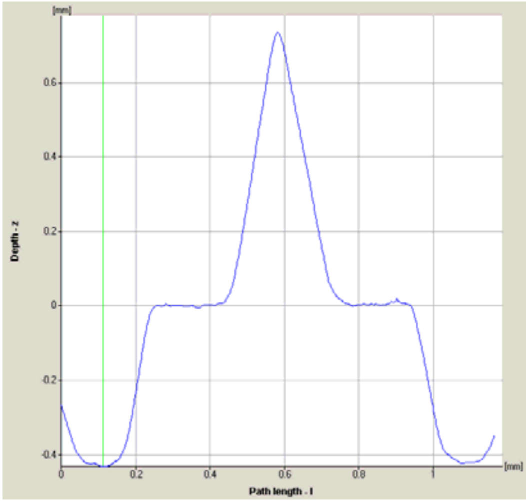


3D reconstruction using the AutoCalibration
Left: with overlaid SEM image
Right: pseudo colour mode

Verification – Steep flanks



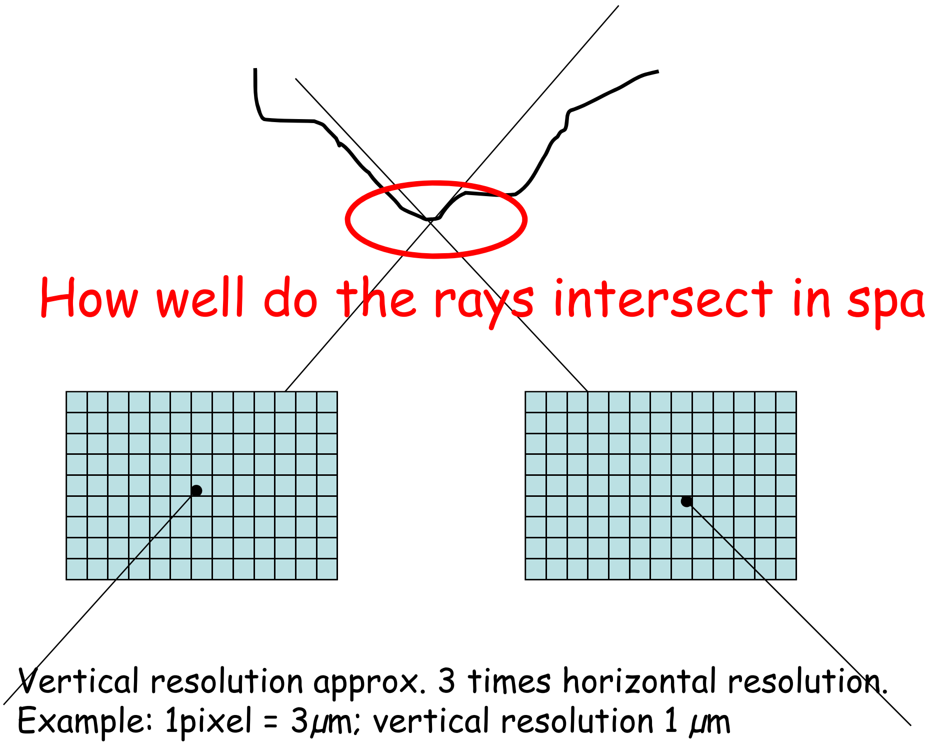
Measurement path



Extracted surface profile

=> MeX can be used to recontruct steep flanks of more than 80°

Accuracy vs. Resolution

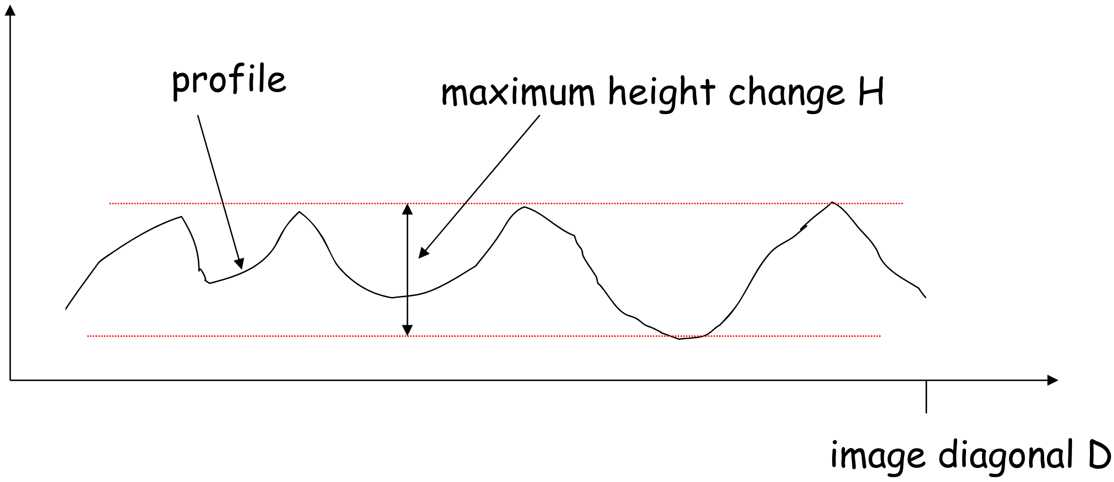


How well do the rays intersect in space?!

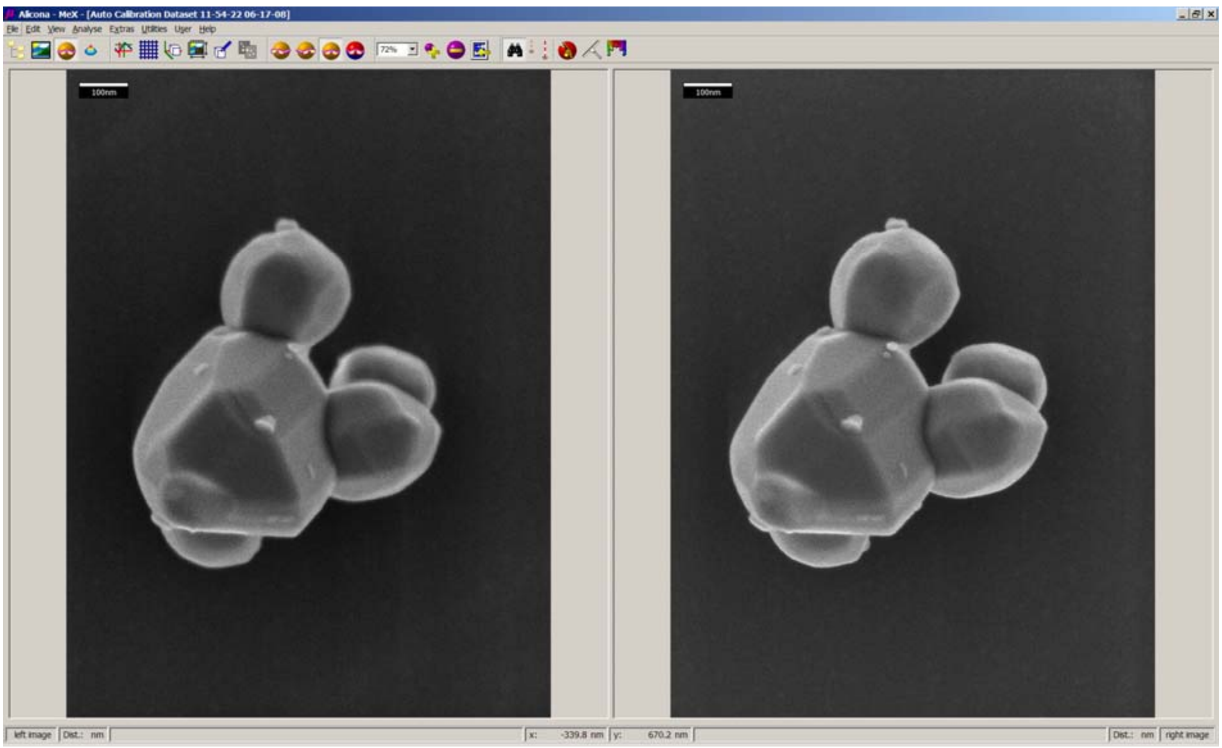
Vertical resolution approx. 3 times horizontal resolution.
Example: 1pixel = 3µm; vertical resolution 1 µm

Field of View vs. Structure

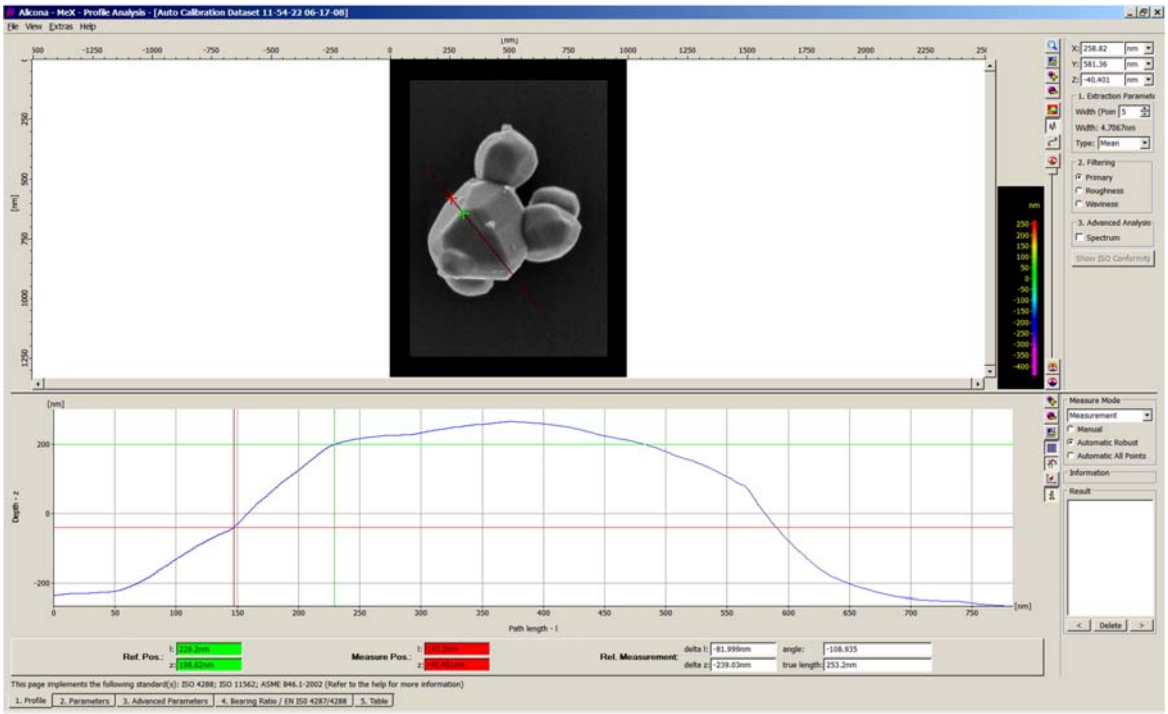
best results can be expected if the ratio D/H ≤ 70



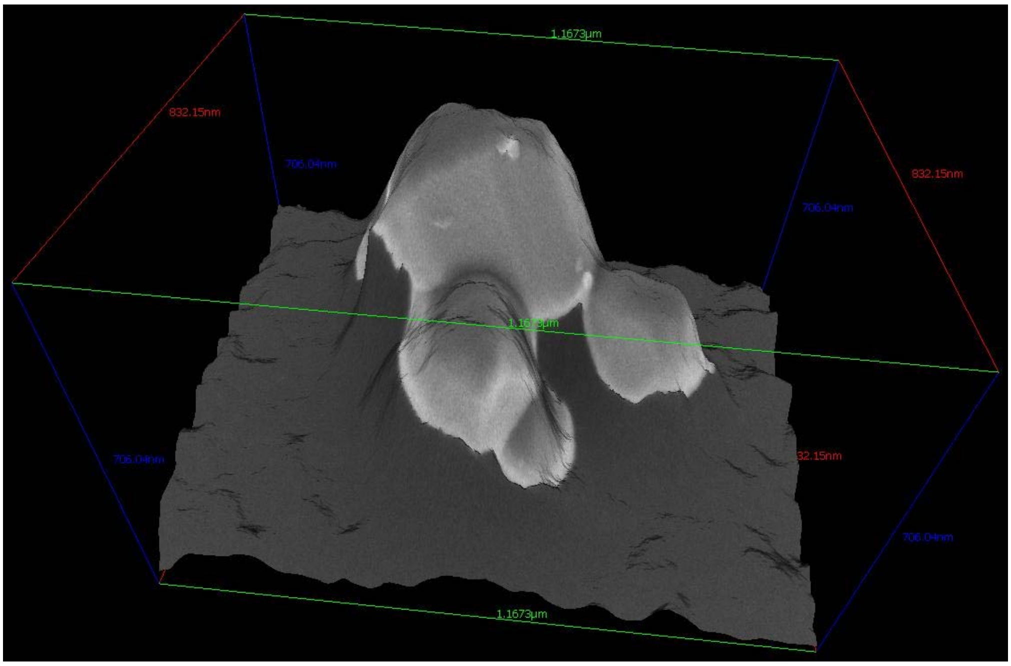
Example Al_2O_3 nano-crystal



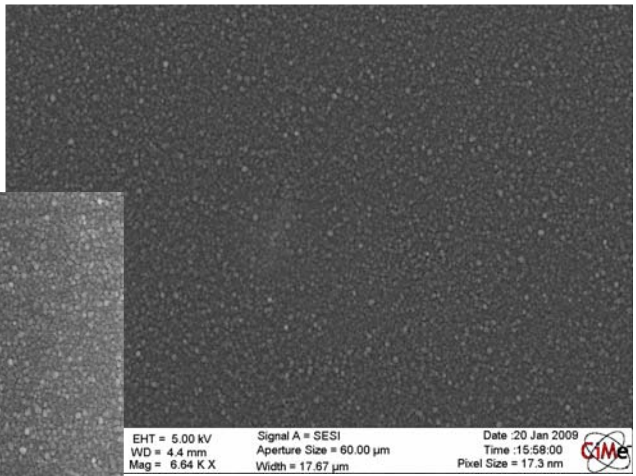
Example Al_2O_3 nano-crystal



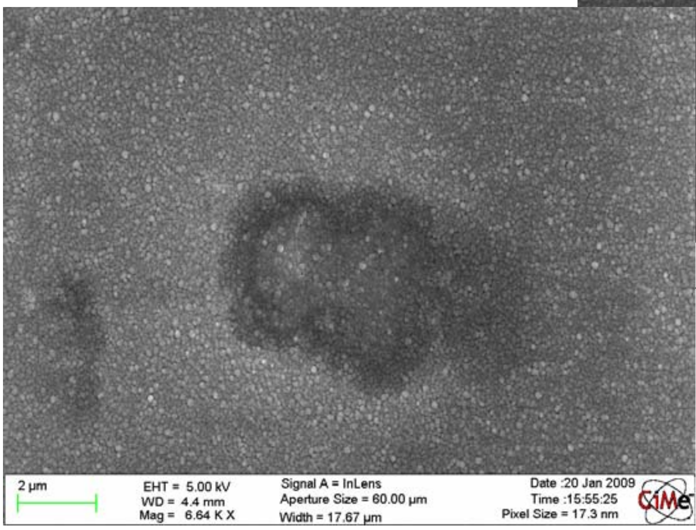
Example Al_2O_3 nano-crystal



Example Alumine

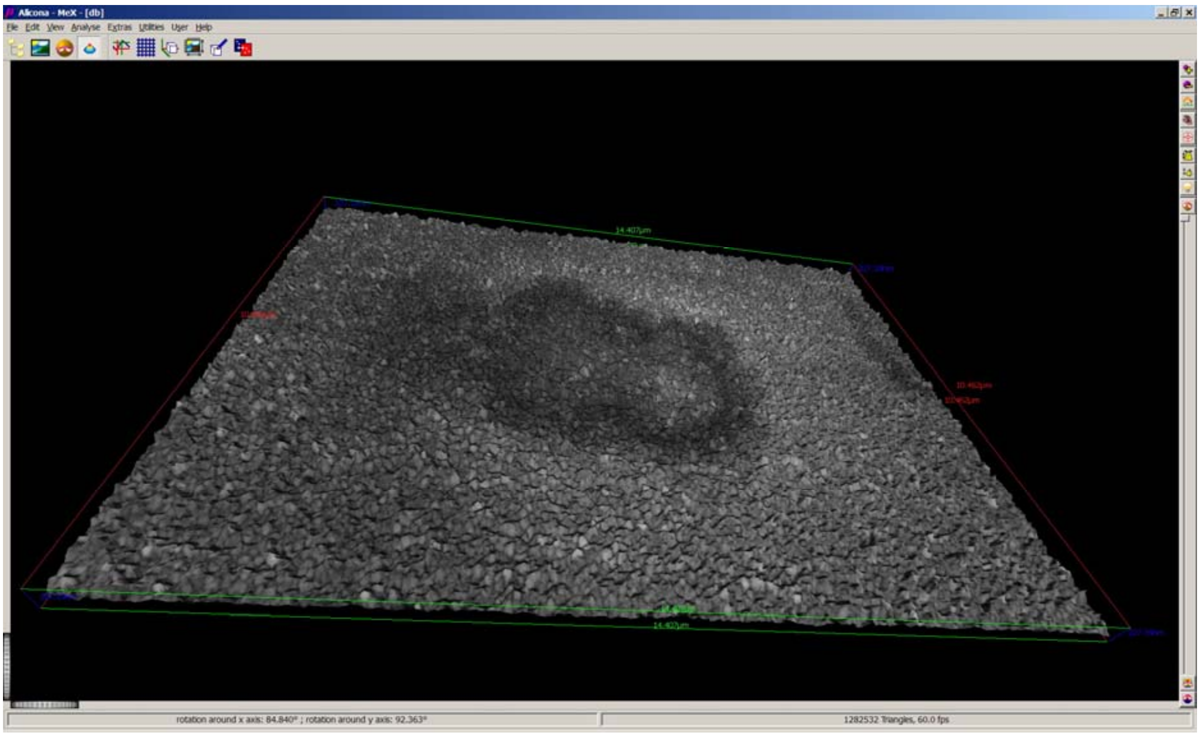


- ETD detector

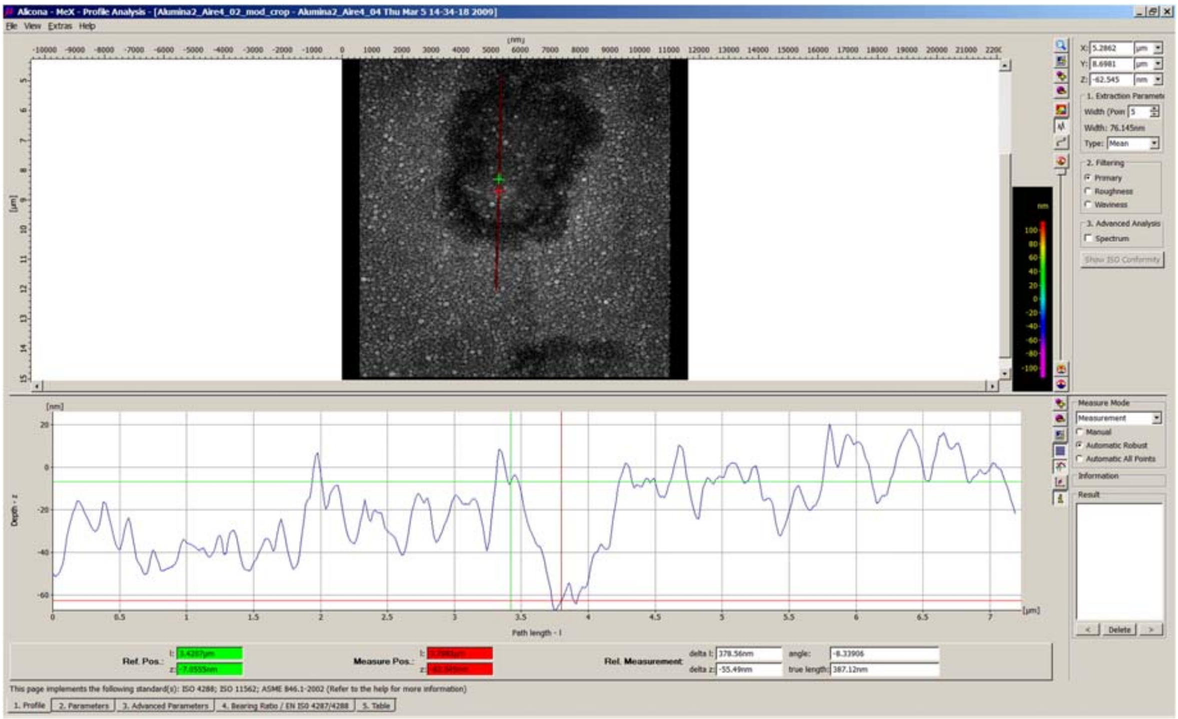


In-lens detector

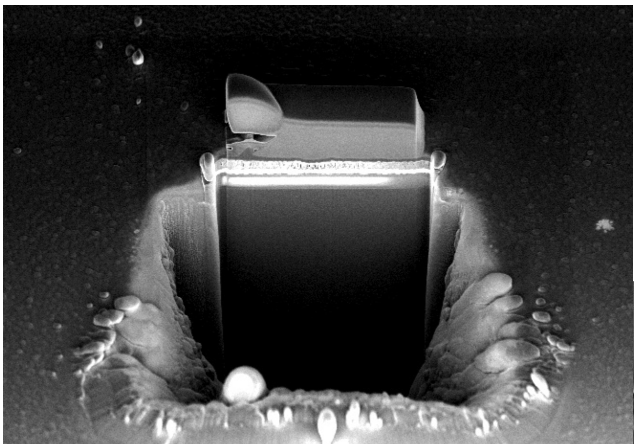
Example Alumine

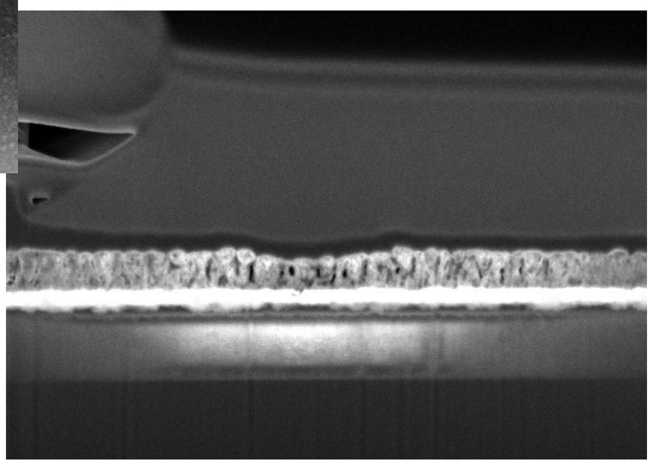


Example Alumine



Example Alumine





Summary

- MeX allows metric accurate, robust and dense 3D measurements directly in SEM images
- MeX automatically refines the calibration data