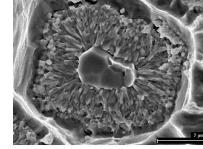
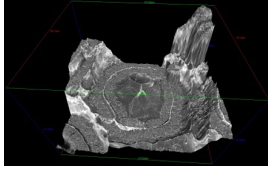


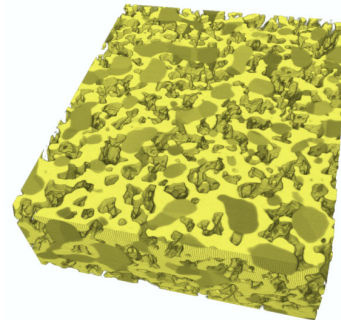
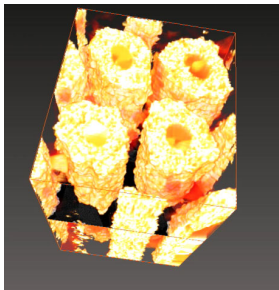
3D Microscopy and FIB Nanotomography



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

MSE-704	3D Microscopy																																																																																									
Tuesday 8.7.	Wednesday 9.7.	Thursday 10.7.																																																																																								
<table border="1"> <tr><td>10:15</td><td>MXG-110</td></tr> <tr><td></td><td>1. Intro</td></tr> <tr><td></td><td>SEM imaging</td></tr> <tr><td>11:00</td><td></td></tr> <tr><td>11:15</td><td>2. Tomography</td></tr> <tr><td></td><td>basics</td></tr> <tr><td>12:00</td><td></td></tr> <tr><td>13:15</td><td>MXG-110</td></tr> <tr><td></td><td>3. FIB</td></tr> <tr><td></td><td>basics</td></tr> <tr><td></td><td>advanced</td></tr> <tr><td></td><td>Nanotomography</td></tr> <tr><td>14:45</td><td></td></tr> <tr><td>15:15</td><td>4. Image Processing</td></tr> <tr><td></td><td>basics</td></tr> <tr><td></td><td>3D</td></tr> <tr><td>17:00</td><td></td></tr> </table>	10:15	MXG-110		1. Intro		SEM imaging	11:00		11:15	2. Tomography		basics	12:00		13:15	MXG-110		3. FIB		basics		advanced		Nanotomography	14:45		15:15	4. Image Processing		basics		3D	17:00		<table border="1"> <tr><td>09:15</td><td>5. PFIB</td></tr> <tr><td></td><td>Carsten Putzke</td></tr> <tr><td></td><td>recorded lecture</td></tr> <tr><td>09:15</td><td>MXG-110</td></tr> <tr><td></td><td>6. ImageJ 1</td></tr> <tr><td></td><td>basics</td></tr> <tr><td>10:00</td><td></td></tr> <tr><td></td><td>working with stacks</td></tr> <tr><td>10:15</td><td></td></tr> <tr><td></td><td>6. ImageJ 2</td></tr> <tr><td></td><td>exercise</td></tr> <tr><td></td><td>Registration</td></tr> <tr><td>12:00</td><td></td></tr> <tr><td></td><td>Segmentation</td></tr> <tr><td>13:15</td><td></td></tr> <tr><td></td><td>demo</td></tr> <tr><td></td><td>XB540/XB550</td></tr> <tr><td></td><td>Lucie Navratilova</td></tr> <tr><td></td><td>Gulnaz Ganeeva</td></tr> </table>	09:15	5. PFIB		Carsten Putzke		recorded lecture	09:15	MXG-110		6. ImageJ 1		basics	10:00			working with stacks	10:15			6. ImageJ 2		exercise		Registration	12:00			Segmentation	13:15			demo		XB540/XB550		Lucie Navratilova		Gulnaz Ganeeva	<table border="1"> <tr><td>08:15</td><td>MXG-110</td></tr> <tr><td></td><td>7. advanced FIB-NT</td></tr> <tr><td></td><td>3D-edx</td></tr> <tr><td>09:00</td><td></td></tr> <tr><td>09:15</td><td>8. Exercises</td></tr> <tr><td></td><td>Report</td></tr> <tr><td></td><td>Questions</td></tr> <tr><td>11:00</td><td></td></tr> </table>	08:15	MXG-110		7. advanced FIB-NT		3D-edx	09:00		09:15	8. Exercises		Report		Questions	11:00	
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Program 1st day

Topics

1) Introduction: Imaging by SEM:

- Contrast formation of SE and BSE imaging.
- Interaction volume, resolution.
- Principle of 3D surface ($2\frac{1}{2}$ 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.

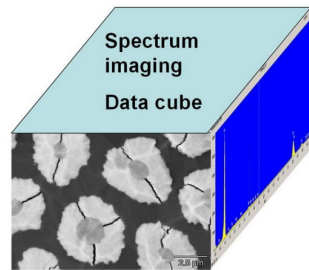
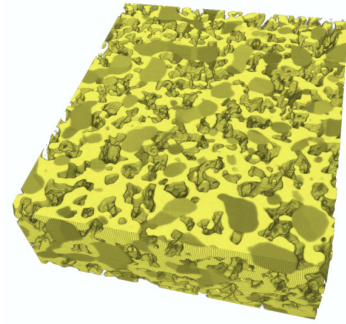
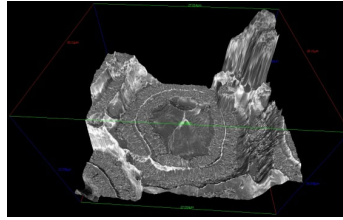
3rd Dimension

- Surface ($2\frac{1}{2}$ D)
- Volume
- Time
- Composition

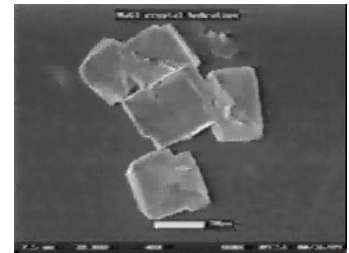
Introduction

3rd Dimension

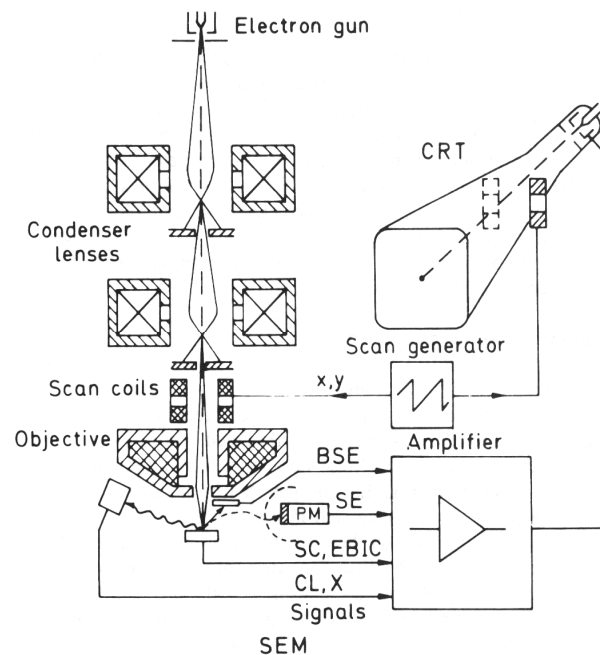
- Surface
- Volume
- Time
- Composition



Extraction of element maps

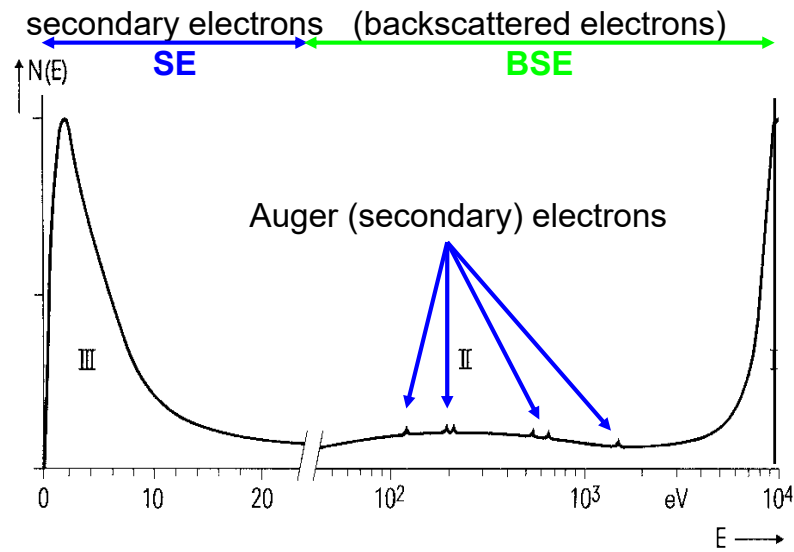


SEM principle



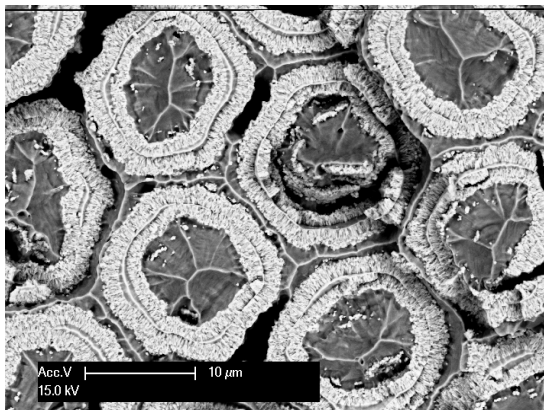
(from L. Reimer, Image formation in Low-Voltage Scanning electron microscopy.)

Energy spectrum of electrons leaving the sample

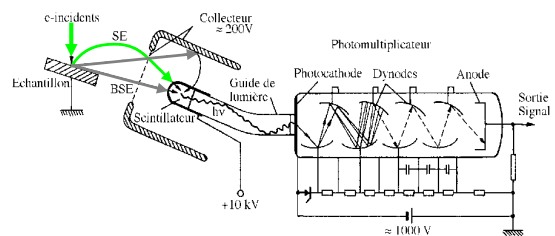
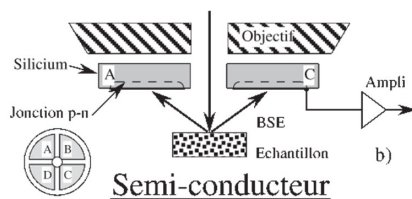
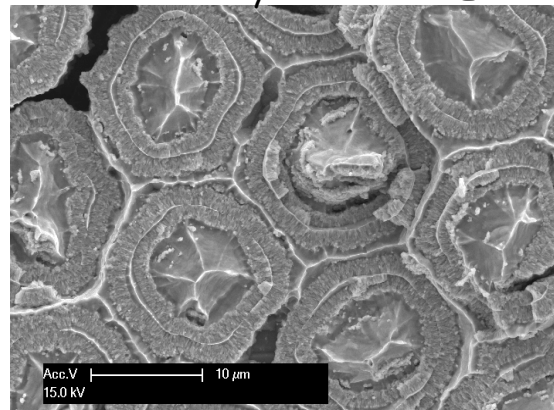


SEM detectors

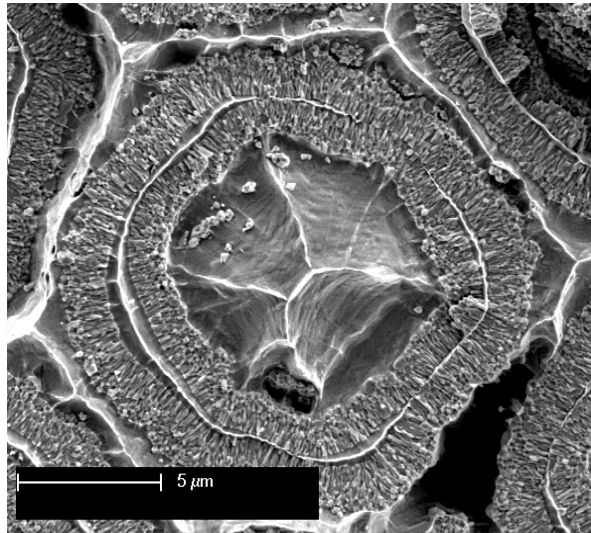
Backscattered electrons BSE



Secondary electrons SE



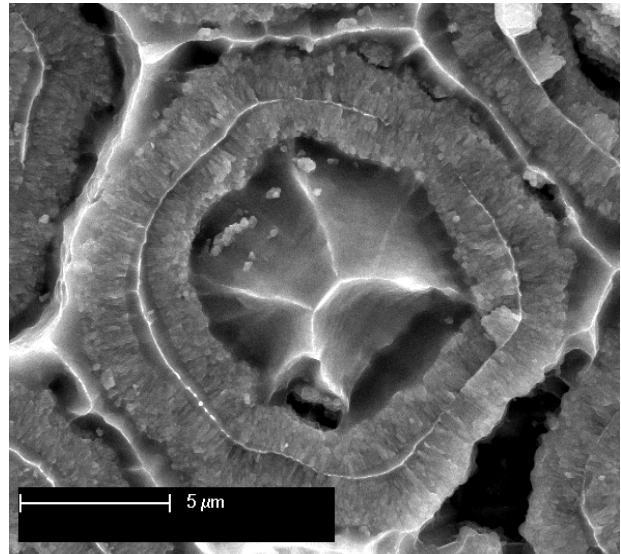
effect of accelerating voltage



high kV (30kV)

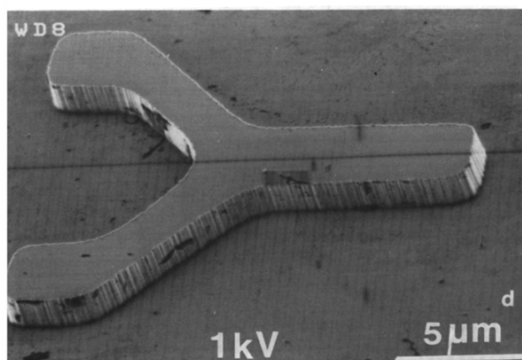
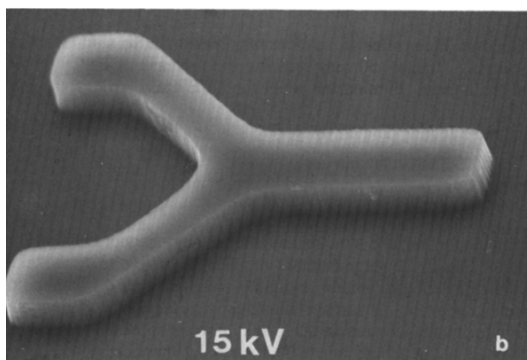
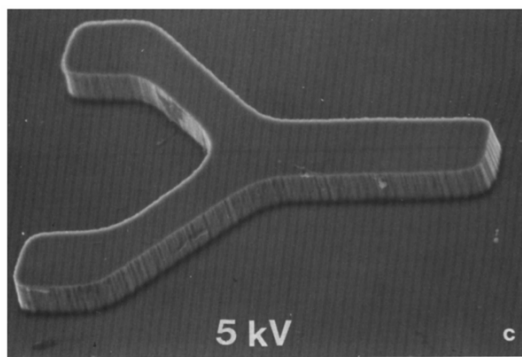
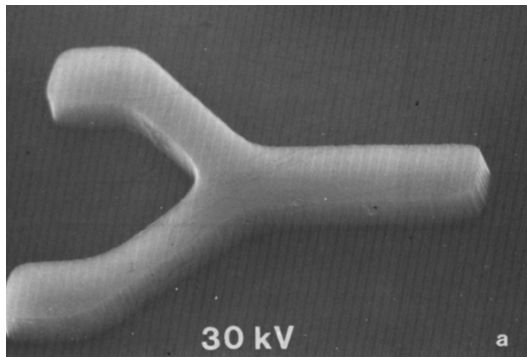
Small grains seem "transparent"

SE detector

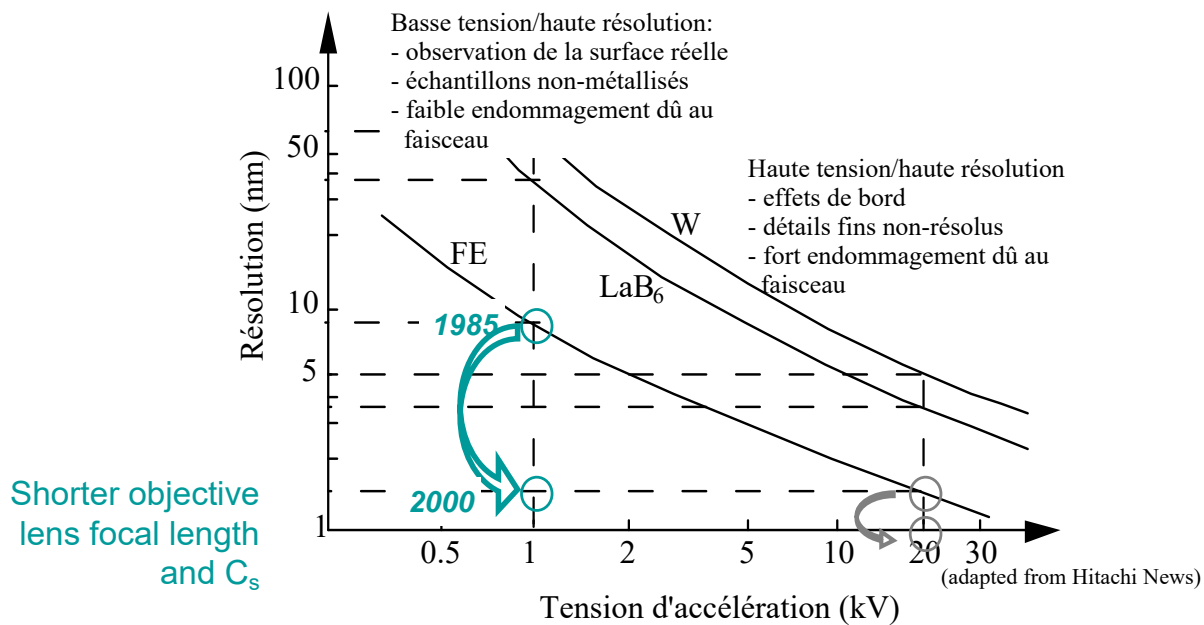


effect of accelerating voltage

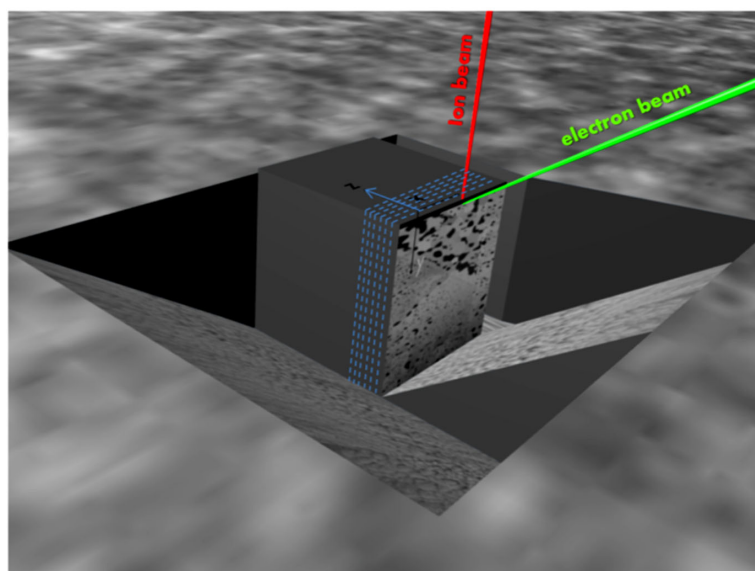
SE detector



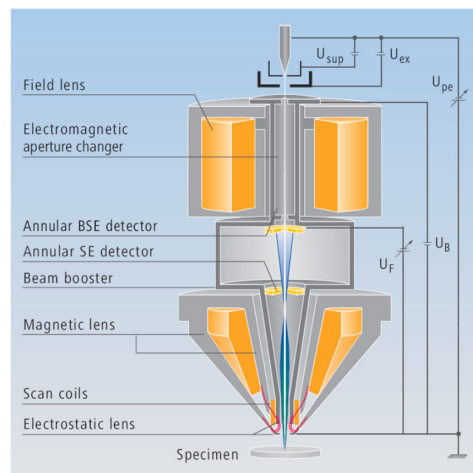
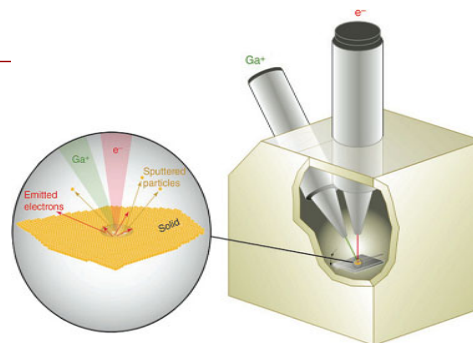
SEM: Loss of resolving power at low accelerating voltage



Modern SEM in column detectors

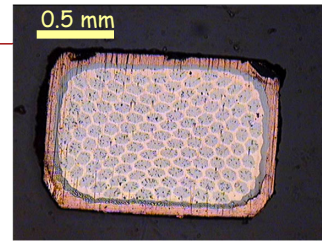


WYSIWYG: What You (detector) See Is What You Get

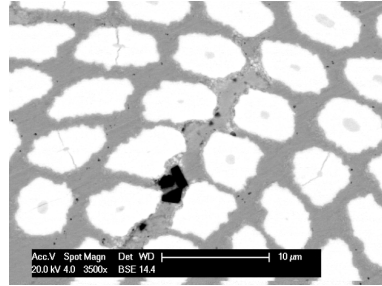


Classical BSE conditions

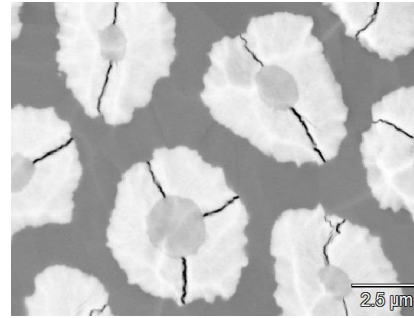
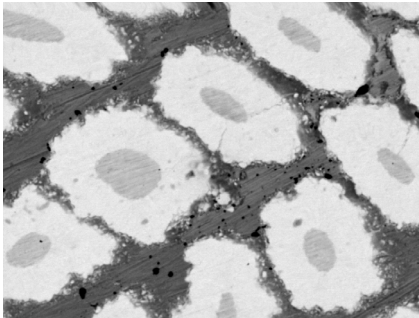
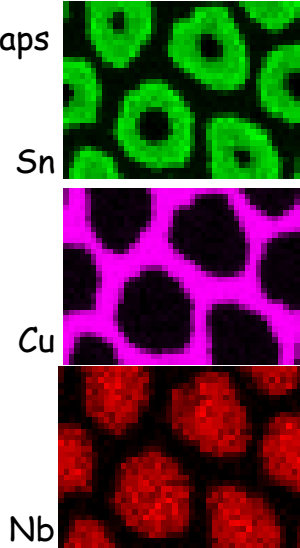
Nb₃Sn superconductor multifilament cable:
14'000 Nb₃Sn filaments (diameter ~5µm) in bronze matrix



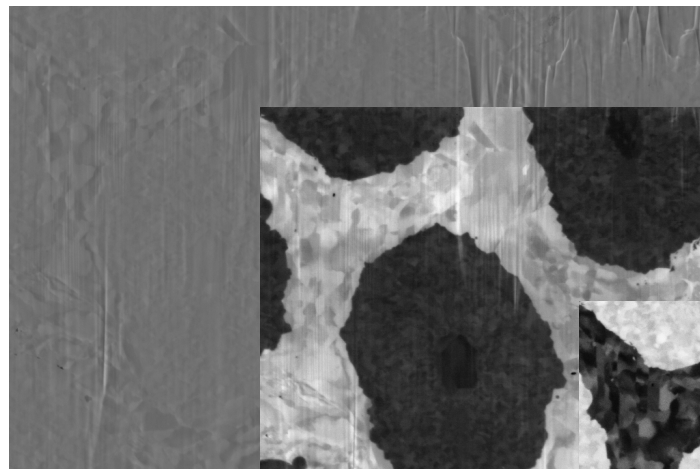
Solid State BSE detector
20KV acceleration voltage



EDX maps

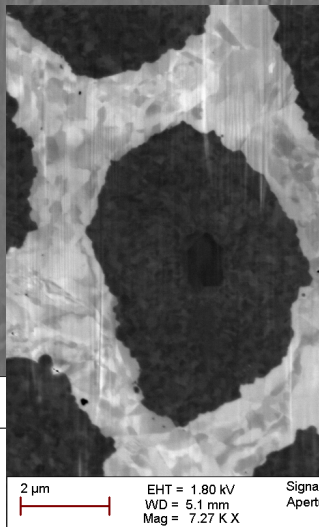


Mechanical polishing <-> Ar ion beam polished



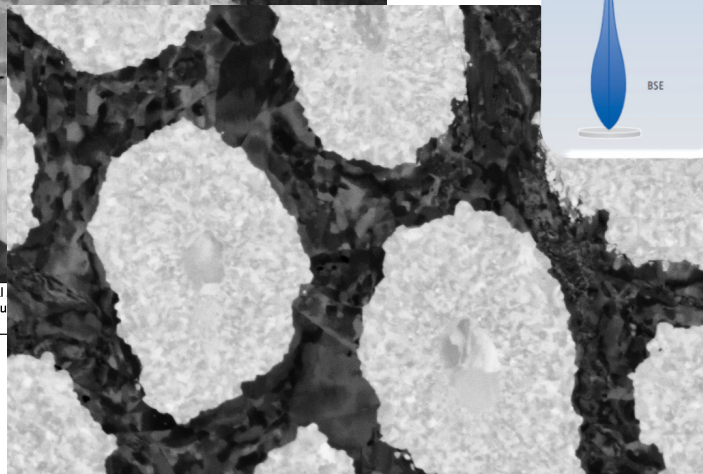
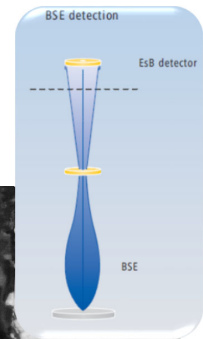
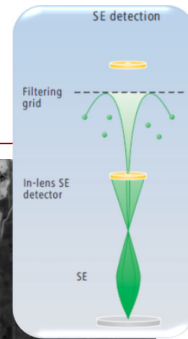
2 µm EHT = 1.80 kV
WD = 5.1 mm
Mag = 7.27 K X

in-chamber
ET-detector
SE



2 µm EHT = 1.80 kV
WD = 5.1 mm
Mag = 7.27 K X
Signal Apertu

in-column
"InLens"
SE-detector



2 µm EHT = 1.80 kV
WD = 5.1 mm
Mag = 7.27 K X
Signal A = EsB
Aperture Size = 120.0 µm
Width = 15.73 µm
Time : 17:19:41
Image Pixel Size = 15.36 nm

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 ?



? μm

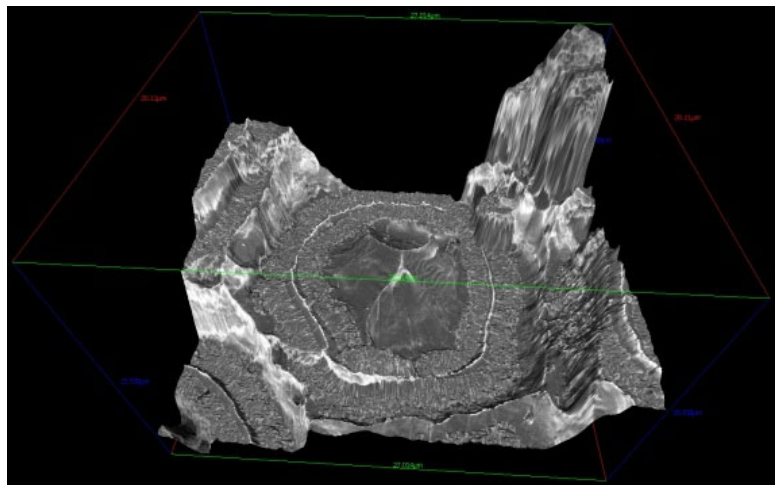
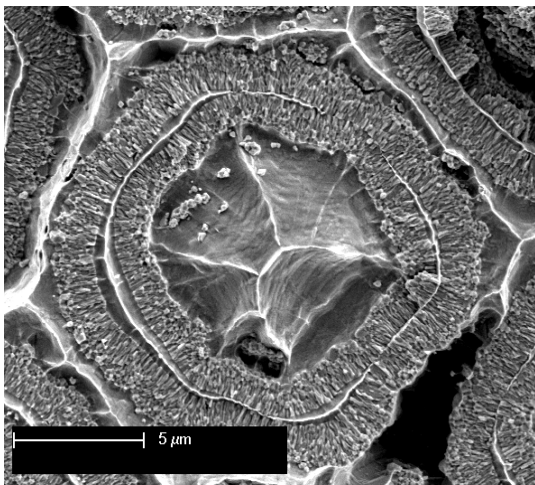
pyramide?

Detector ?



etch-pit?

Stereographic reconstruction



3D surface reconstruction

alicon
imaging

MeX - the real metrology package in SEM

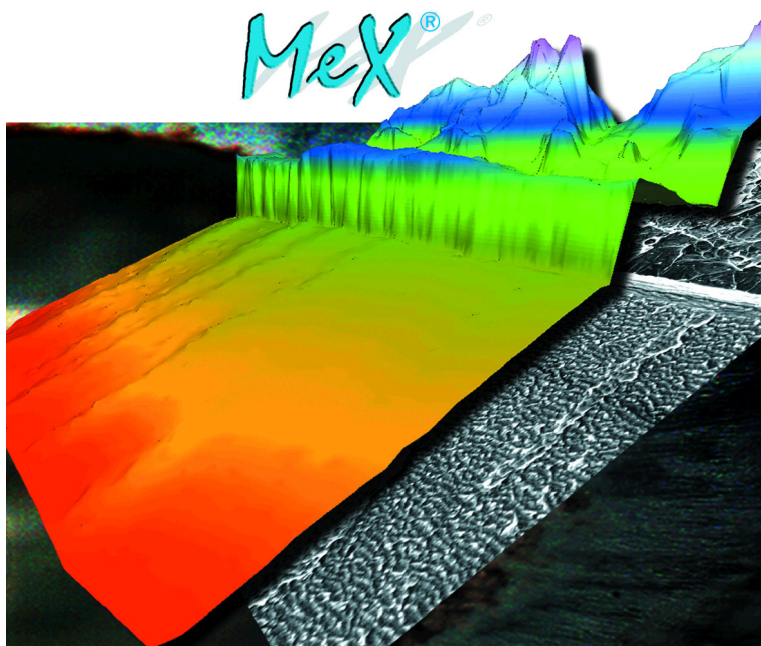
- Alicona Imaging GmbH
- Algorithms



MSE-704 3D Microscopy and FIB Nanotomography



MeX - the real metrology package



MSE-704 3D Microscopy and FIB Nanotomography



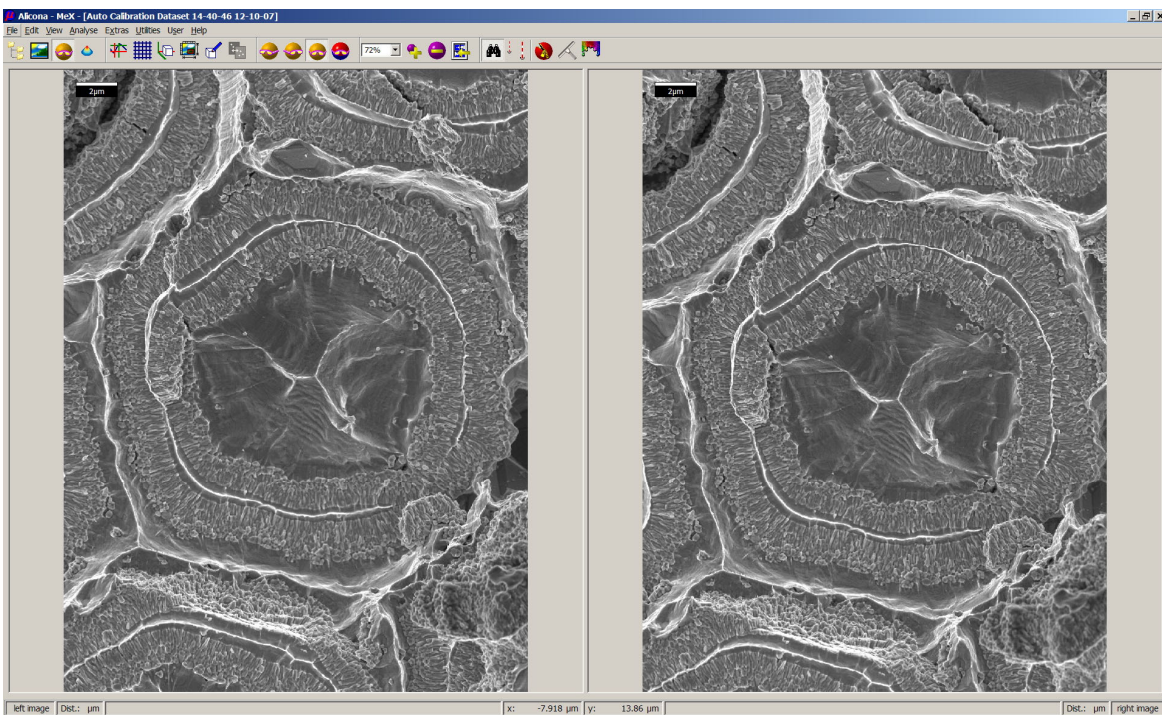
What is MeX

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)

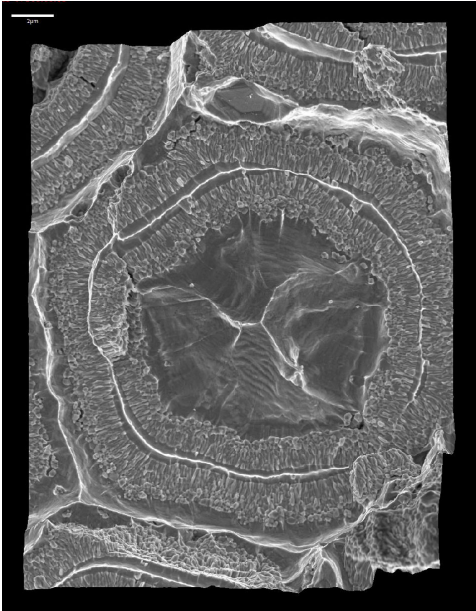
SEM becomes fully operational 3D metrology device

3D dataset from stereopair

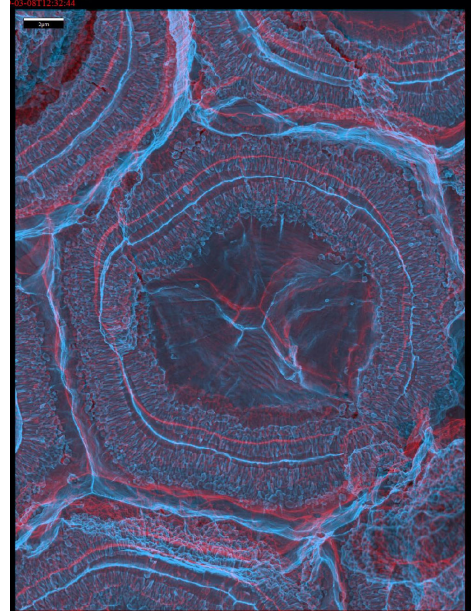


Views from different angle (5 degrees tilt)

Reconstructed surface (anaglyph)



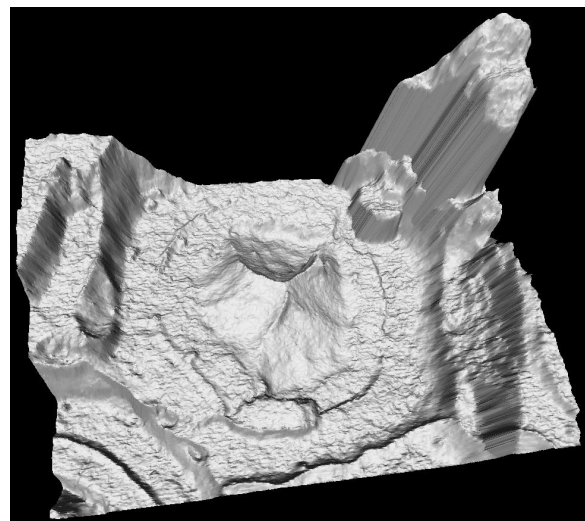
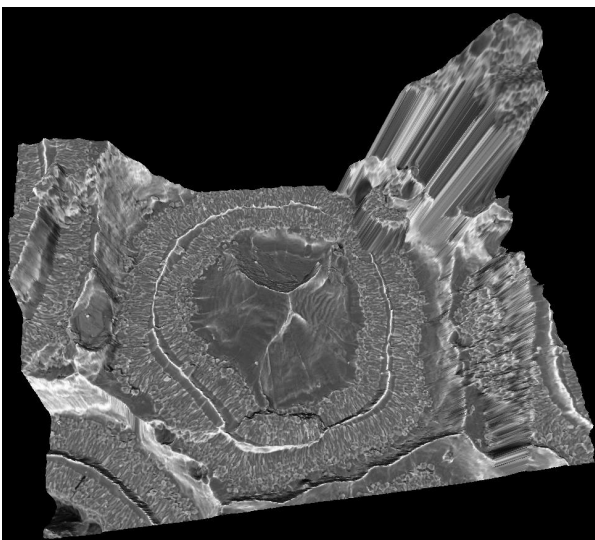
Topview 3D



Anaglyph
(to view with red and cyan filter)

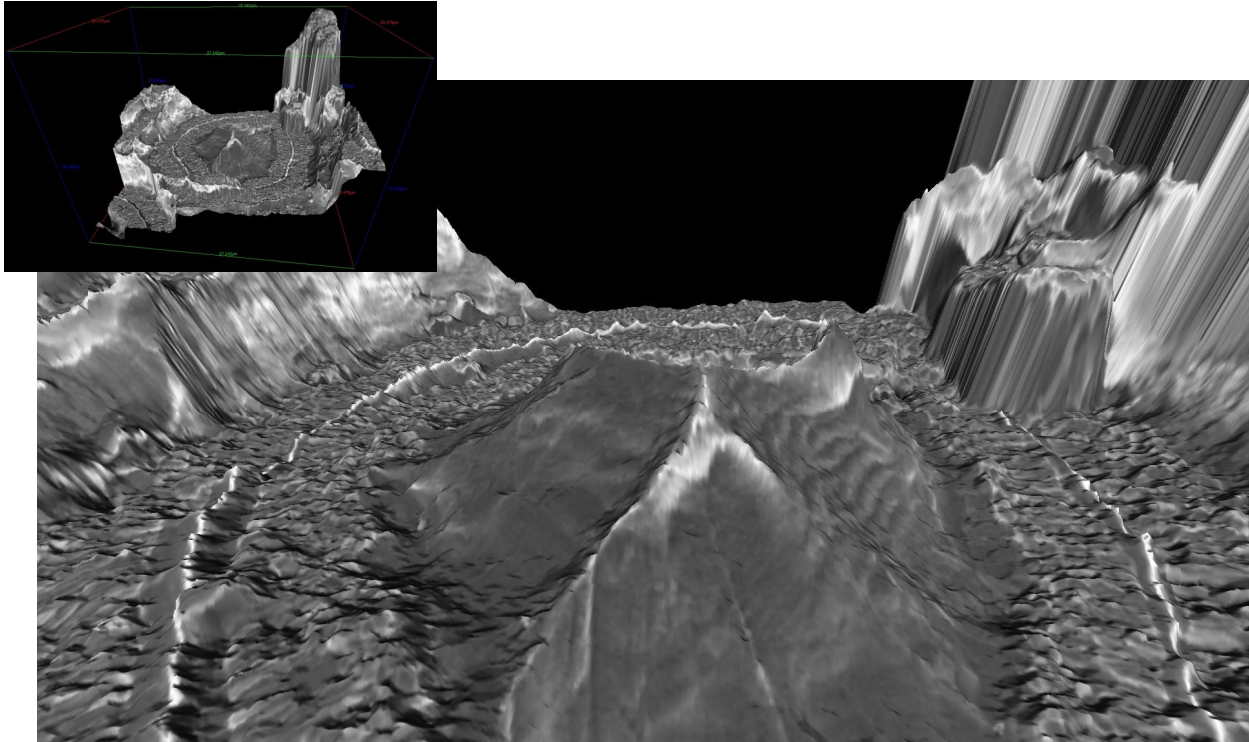
3D Dataset

model with image overlay

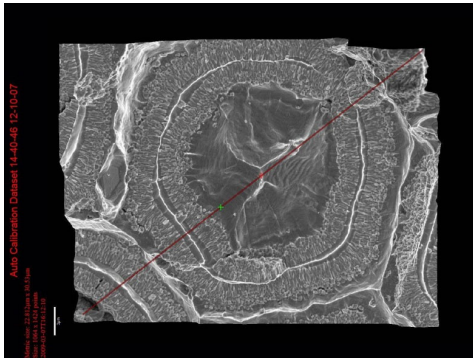


surface model

3D model

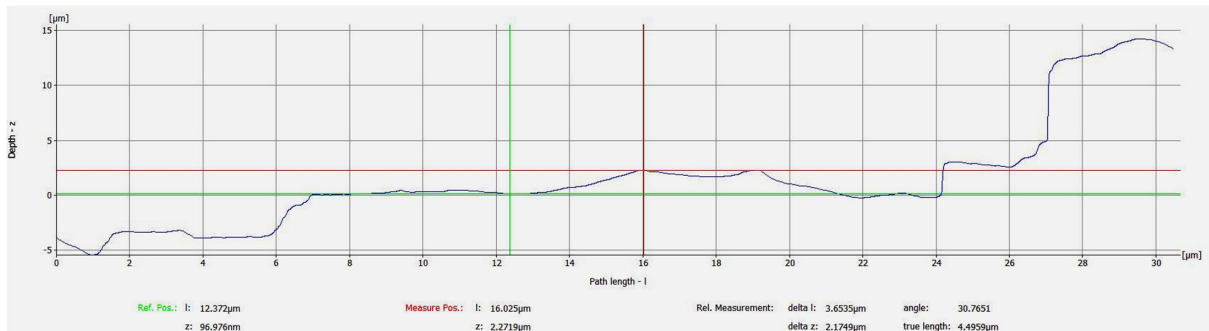


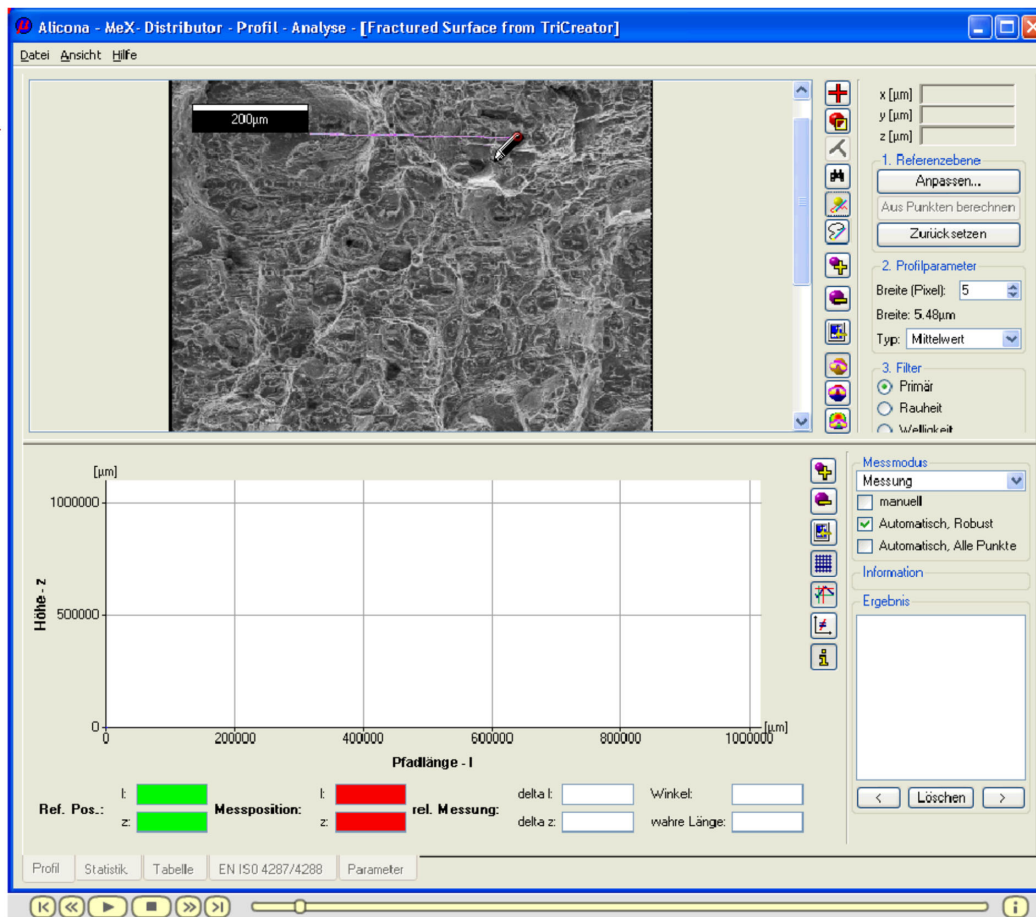
Quantification



Quantitative:

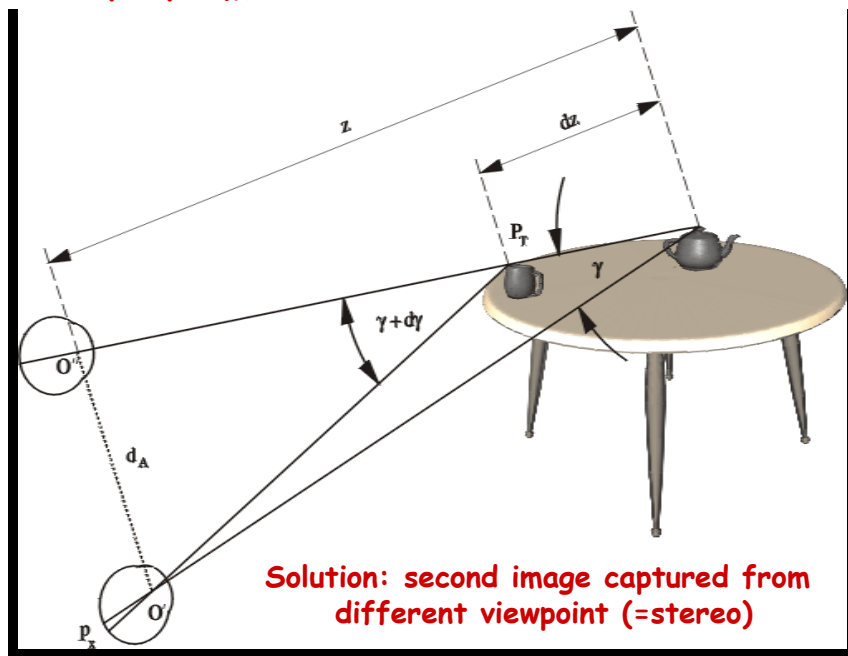
- height profile
- roughness



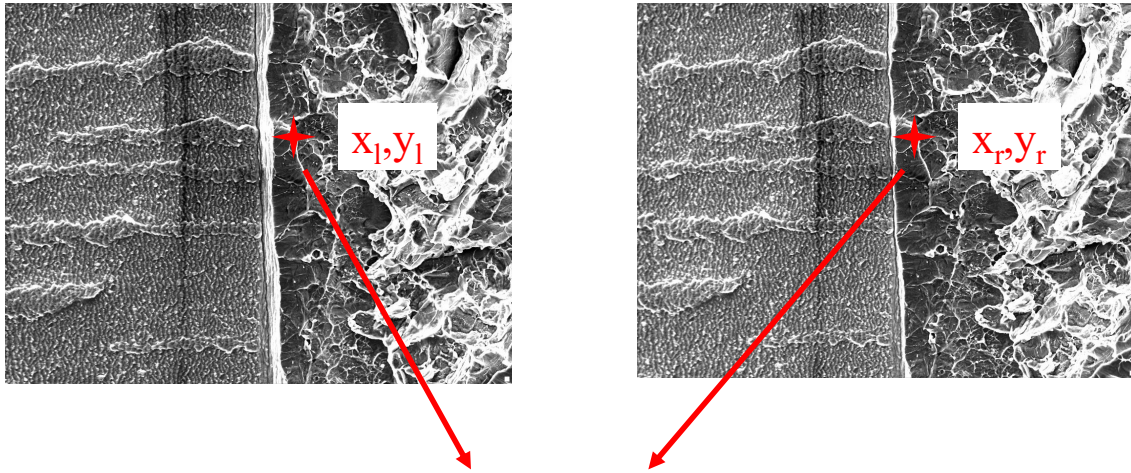


Shape-from-Stereo

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



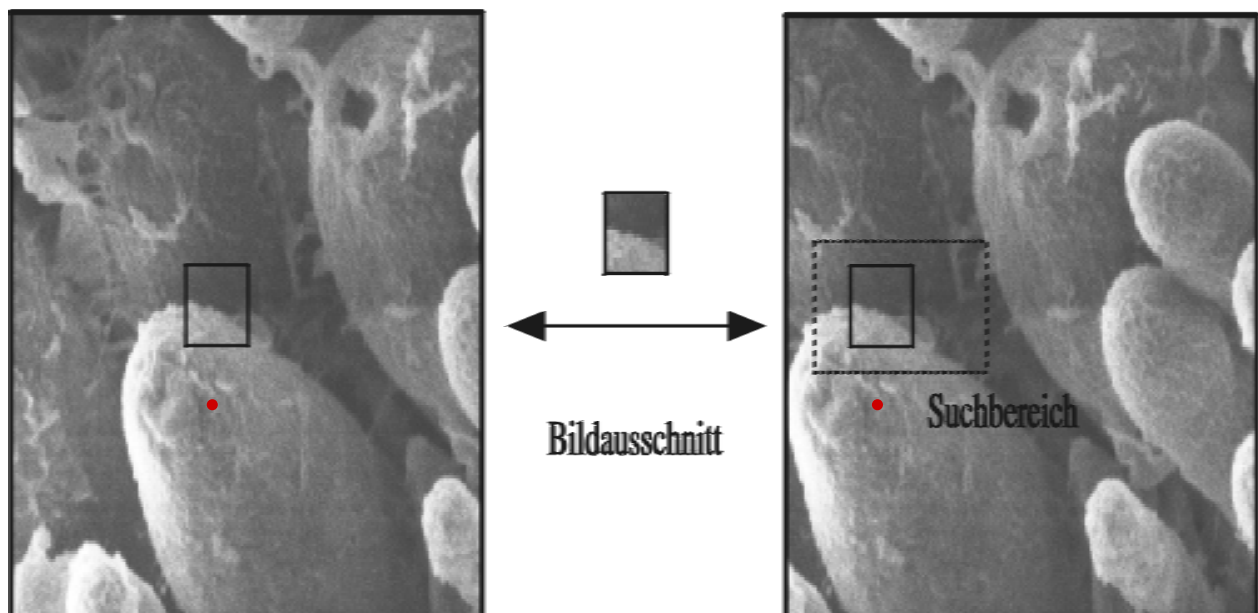
How is the 3D Dataset Created?



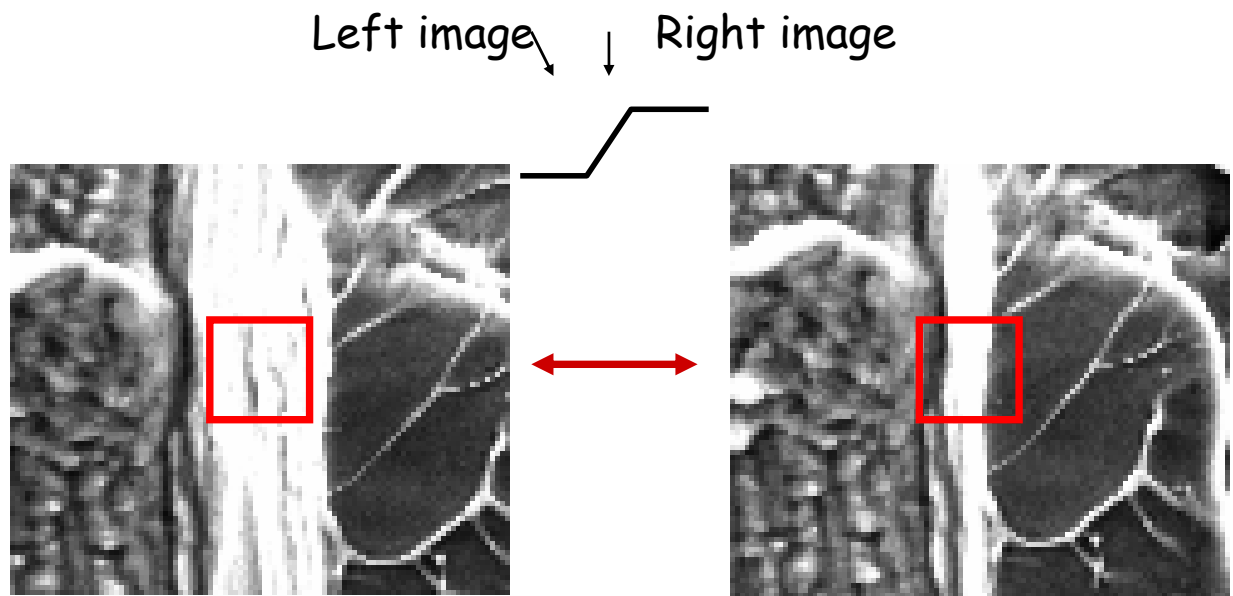
1. Matching
2. Calibration

x, y, z

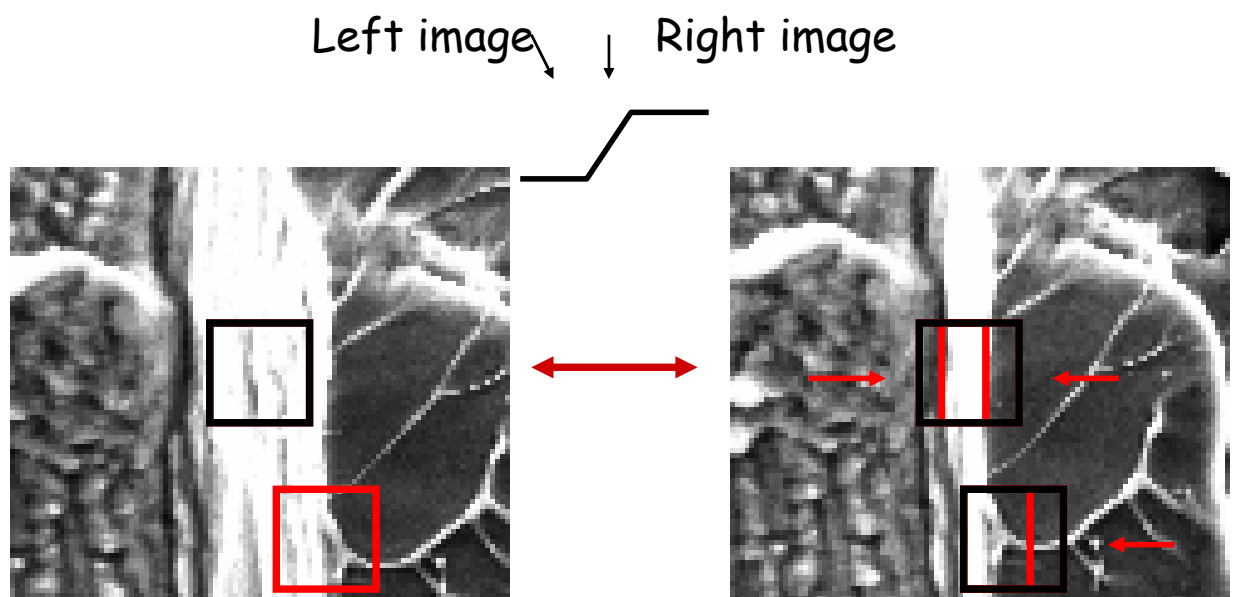
Classical Approach - Area Based Correlation



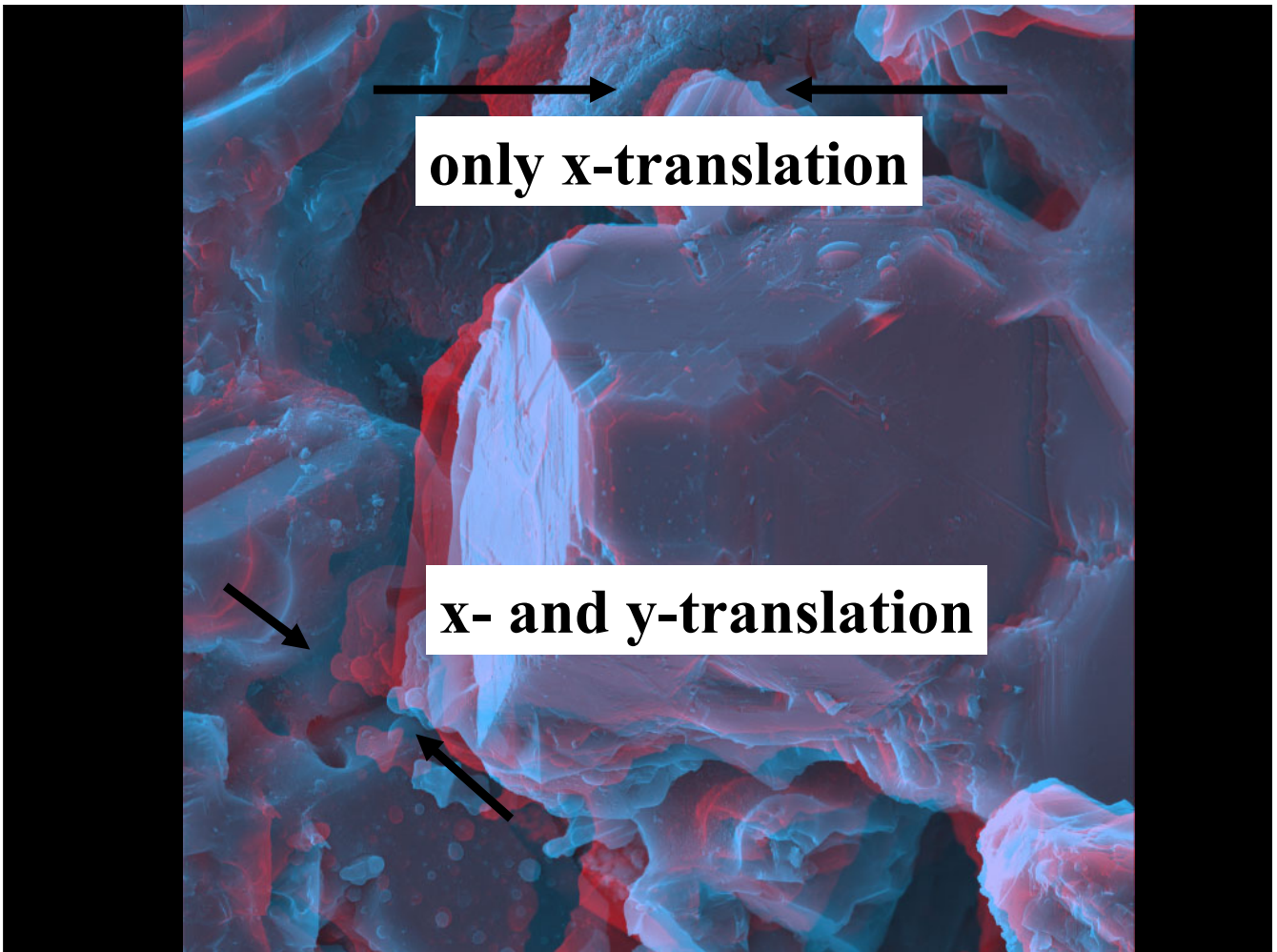
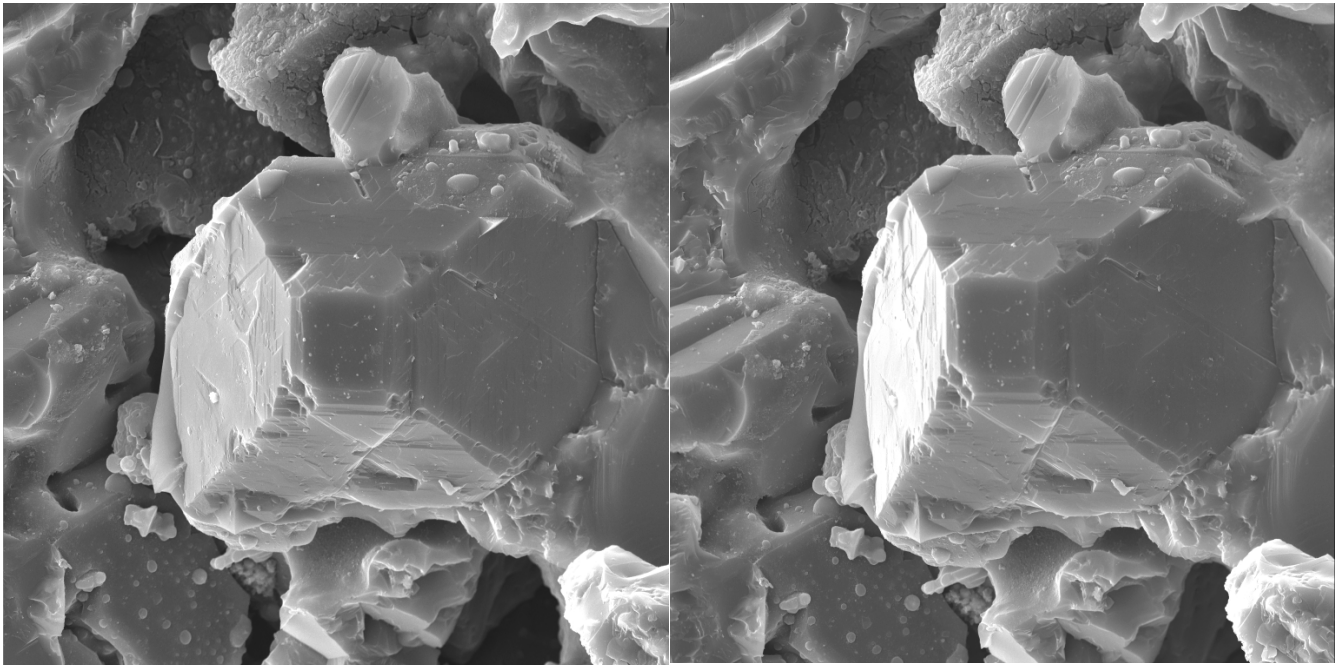
Problem: Distortions



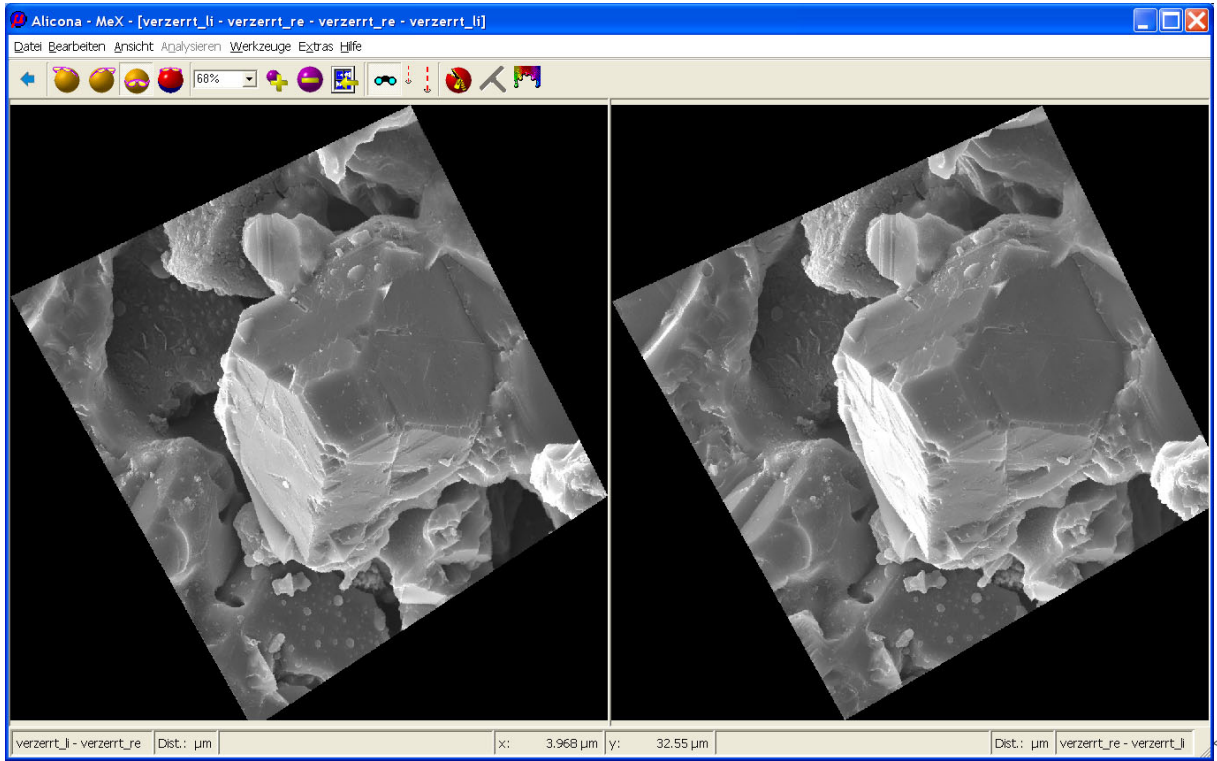
Solution - Adaptive Window



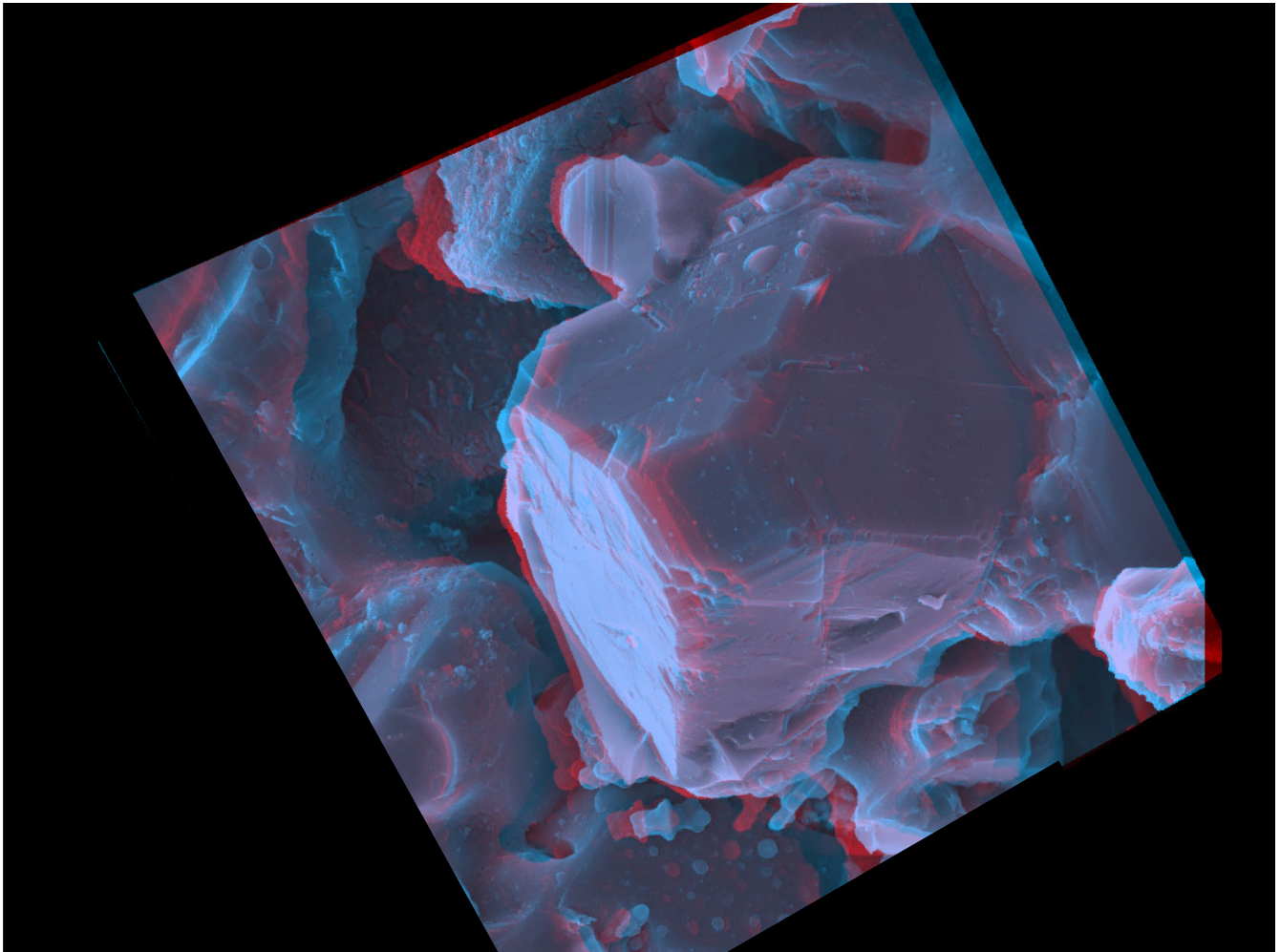
Problem Calibration



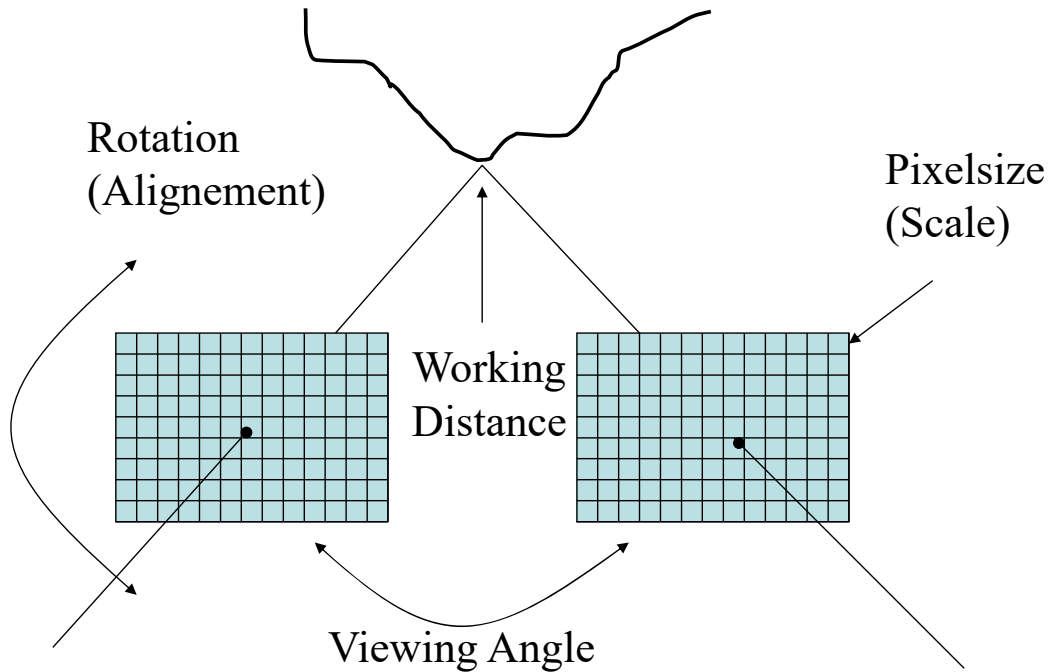
Automatic Rectification



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Calibration



Viewing Angle Critical for Accuracy

Automatic Calibration

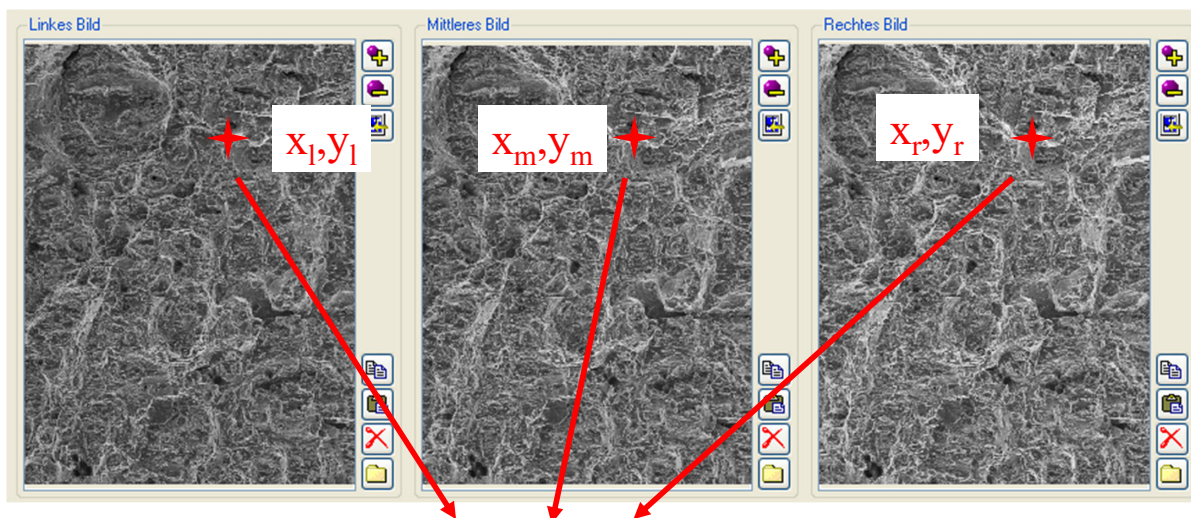
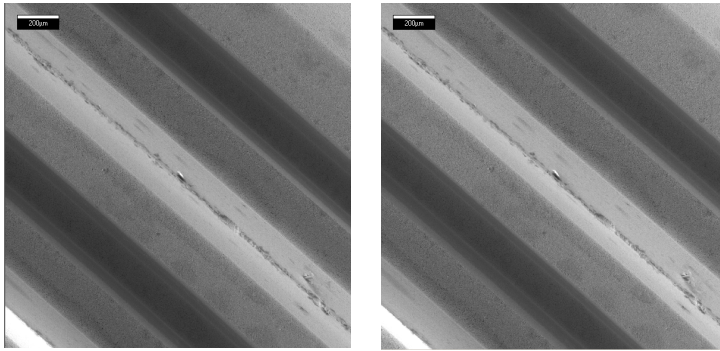
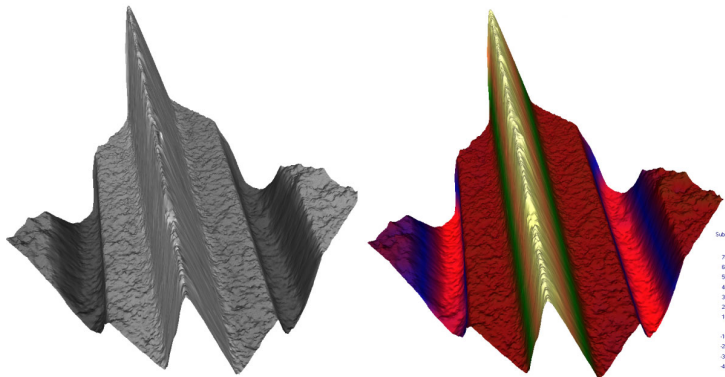


Image Matching + Automatic Calibration
= 3D Dataset

Verification - Steep flanks

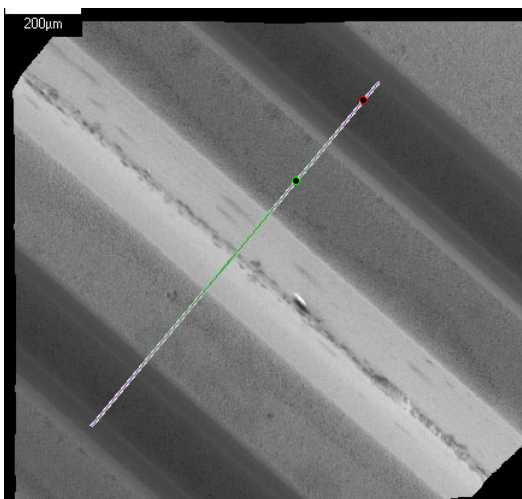


Left and right input image from the SEM (angles -2° and $+2^\circ$)
The center image at 0° is not shown.

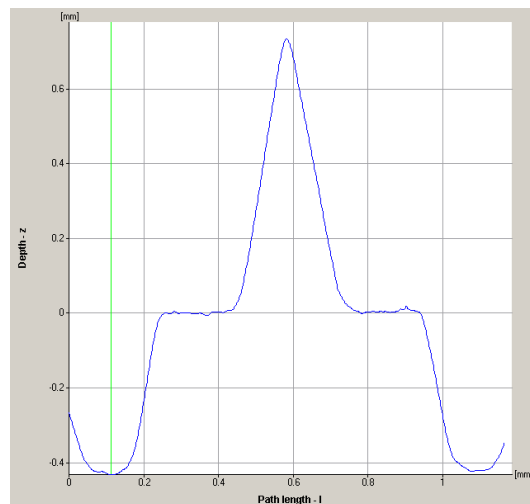


3D reconstruction using the AutoCalibration
Left: with overlaid SEM image
Right: pseudo colour mode

Verification - Steep flanks



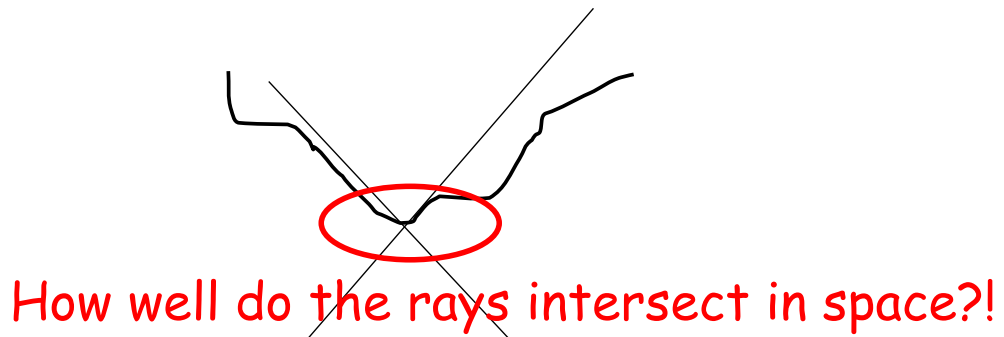
Measurement path



Extracted surface profile

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

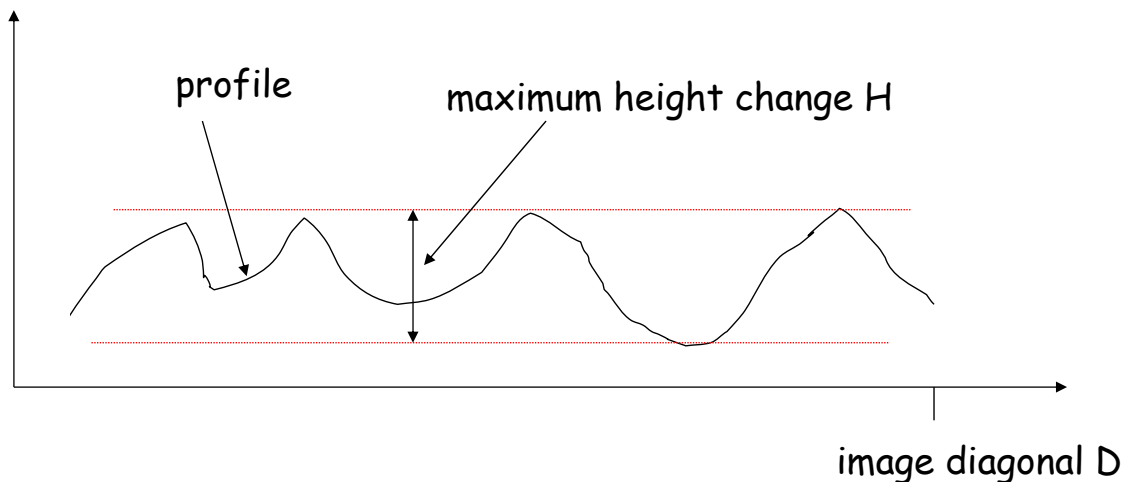
Accuracy vs. Resolution



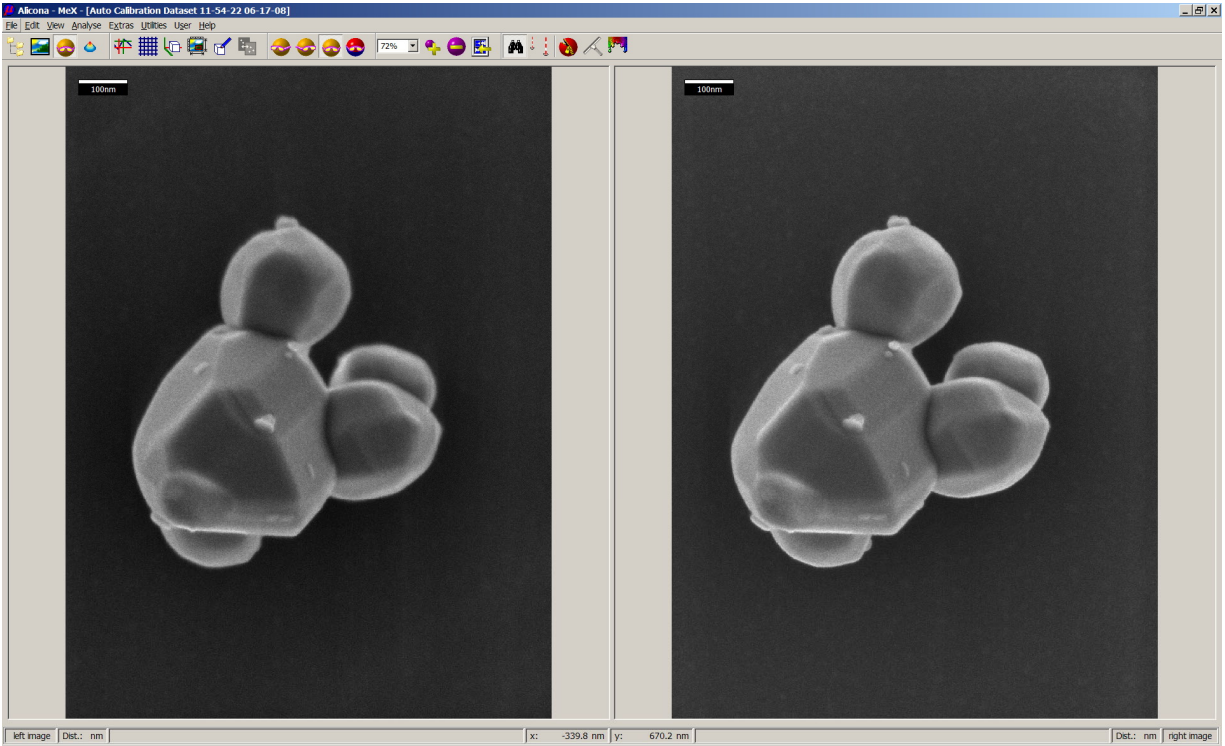
Vertical resolution approx. 3 times horizontal resolution.
Example: 1pixel = $3\mu\text{m}$; vertical resolution $1\mu\text{m}$

Field of View vs. Structure

best results can be expected if the ratio $D/H \leq 70$



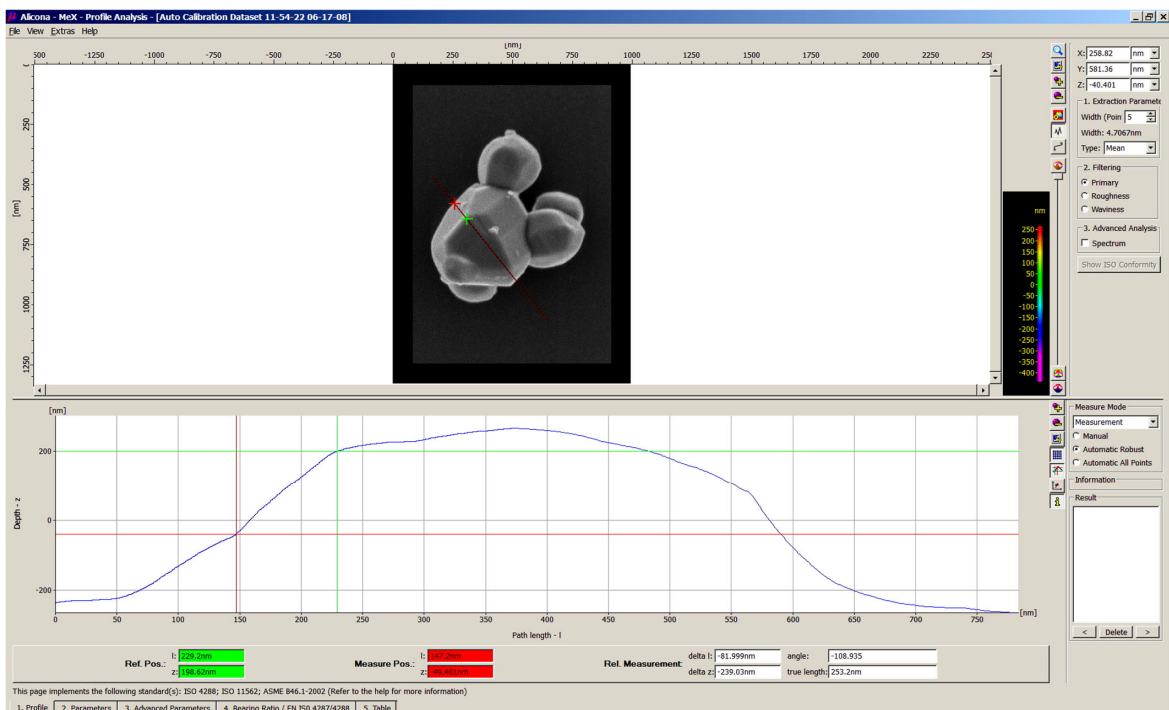
Example Al_2O_3 nano-crystal



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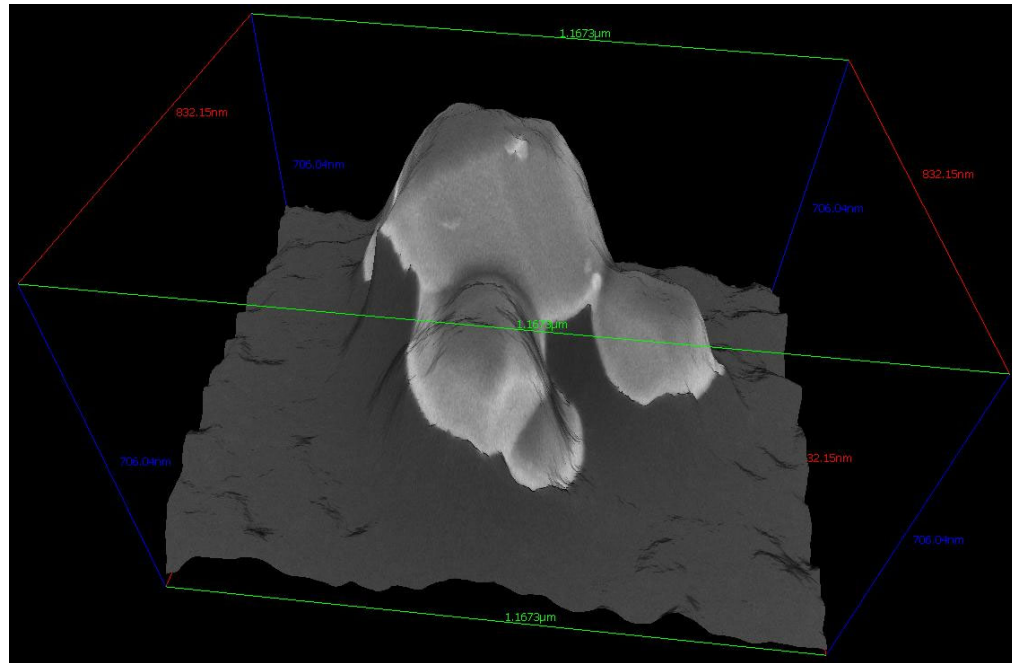
Example Al_2O_3 nano-crystal



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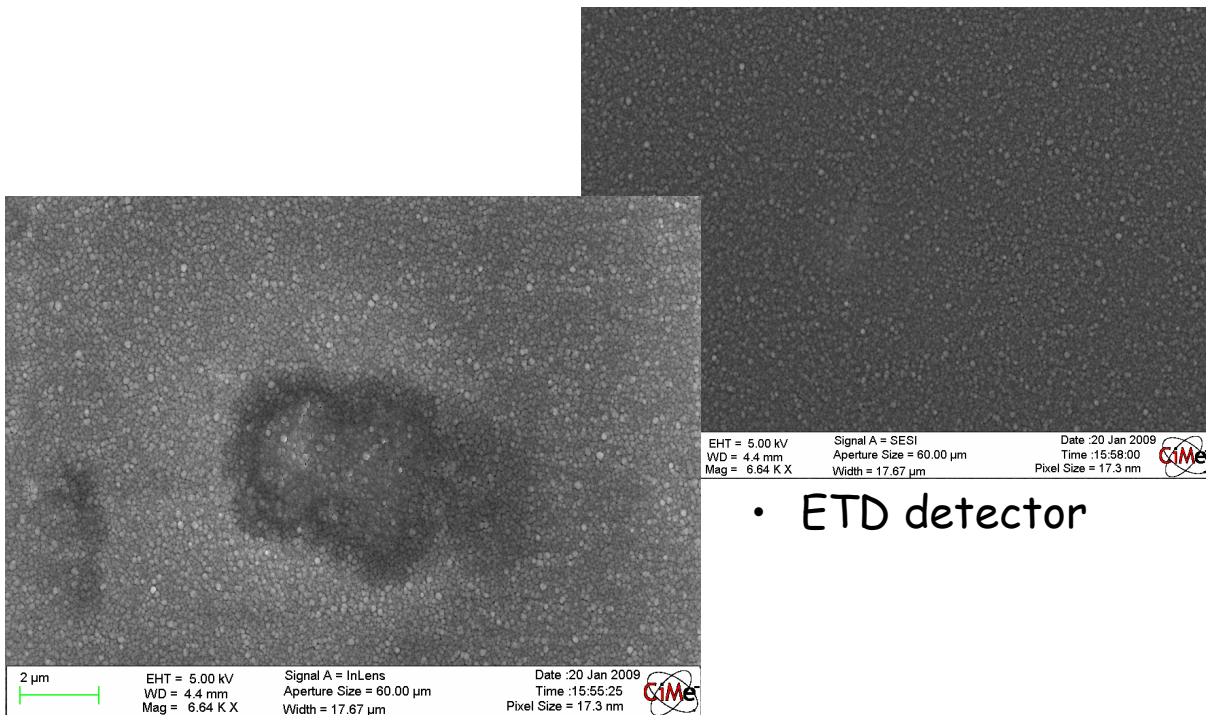
Example Al_2O_3 nano-crystal



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Example Alumine



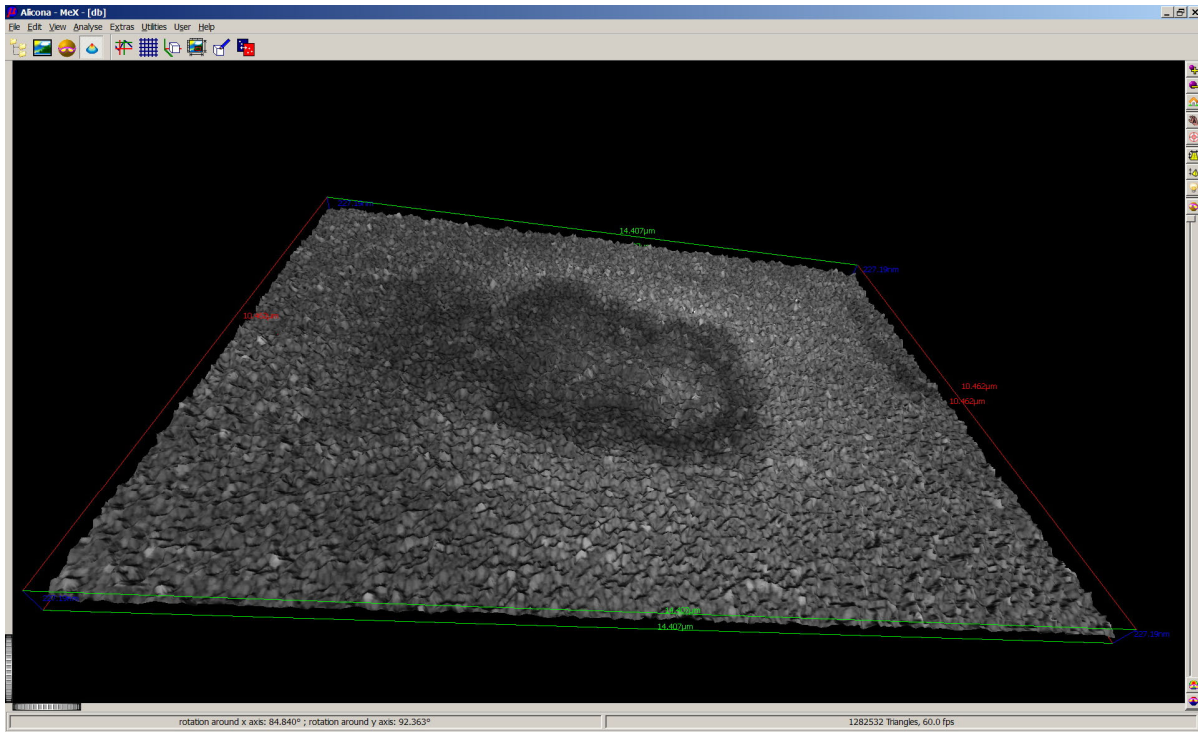
- ETD detector

In-lens detector

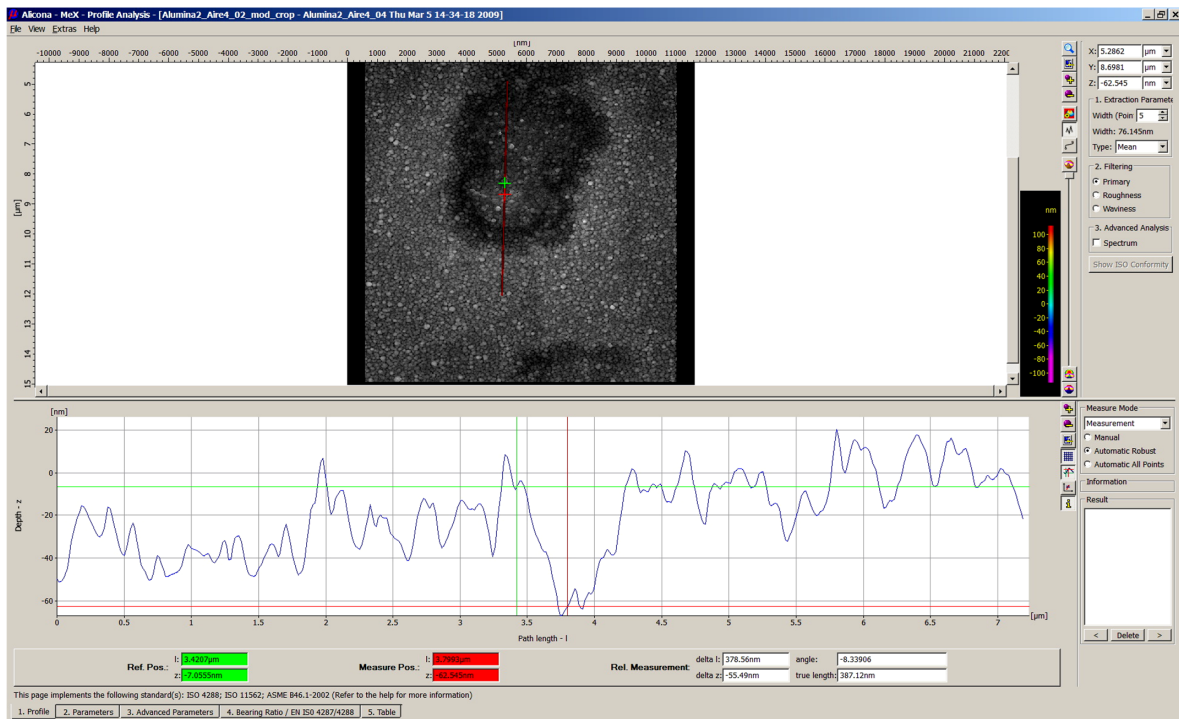
MSE-704 3D Microscopy and FIB Nanotomography



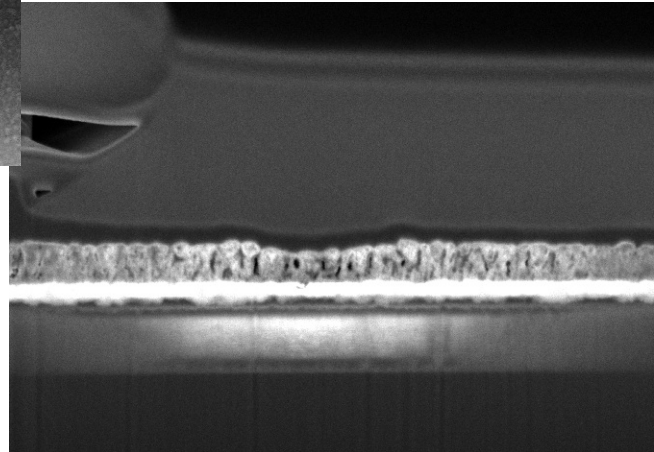
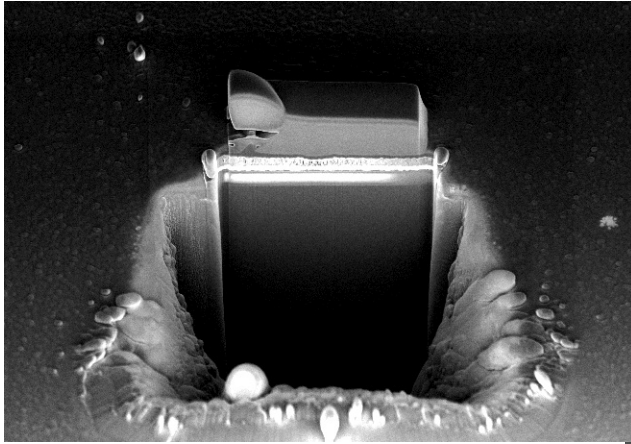
Example Alumine



Example Alumine



Example Alumine



MSE-704 3D Microscopy and FIB Nanotomography



Summary

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

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