Basics of Image and data analysis in 3D

outline

- Why image processing, and how…?
- Image processing in 2D
  - What is an ideal image …? Histogram tells stories…!
  - Before taking the image: the right imaging conditions !
- Tools for 2D image analysis/processing
- 3D volume reconstruction by FIB/SEM
- Processing steps for 3D reconstruction
- Registration, Segmentation, Quantification
- Visualisation

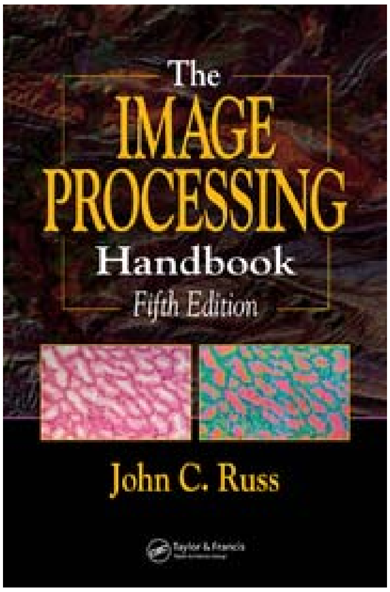
Image processing basics

"the bible"

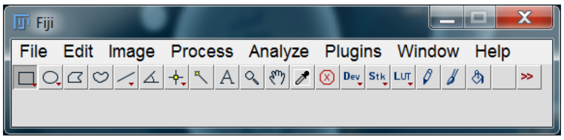
John C. Russ (2002)

The Image Processing Handbook

5th edition, CRC Press



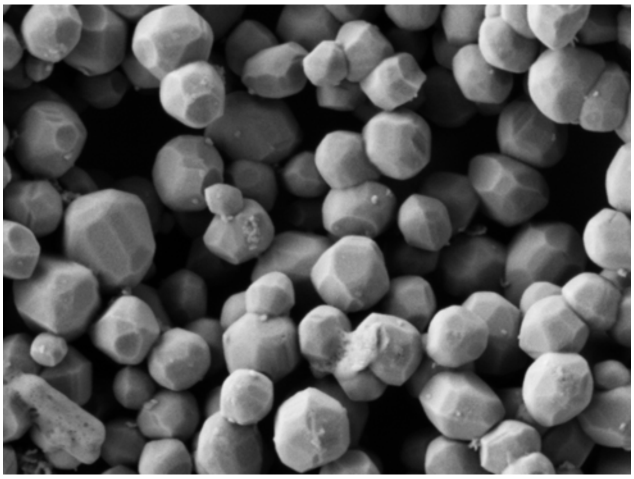
The (free) tools

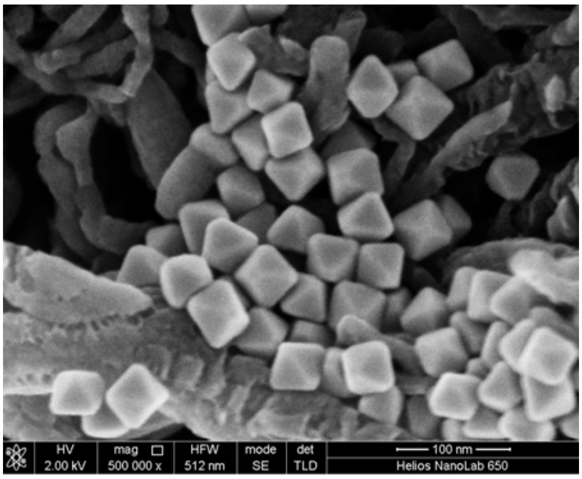


http://rsbweb.nih.gov/ij/

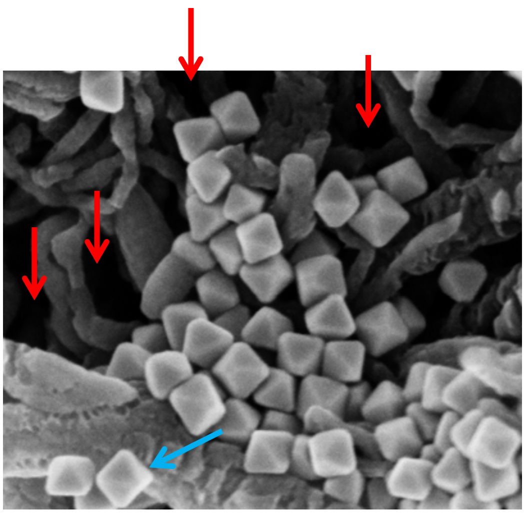
http://fiji.sc/wiki/index.php/Fiji

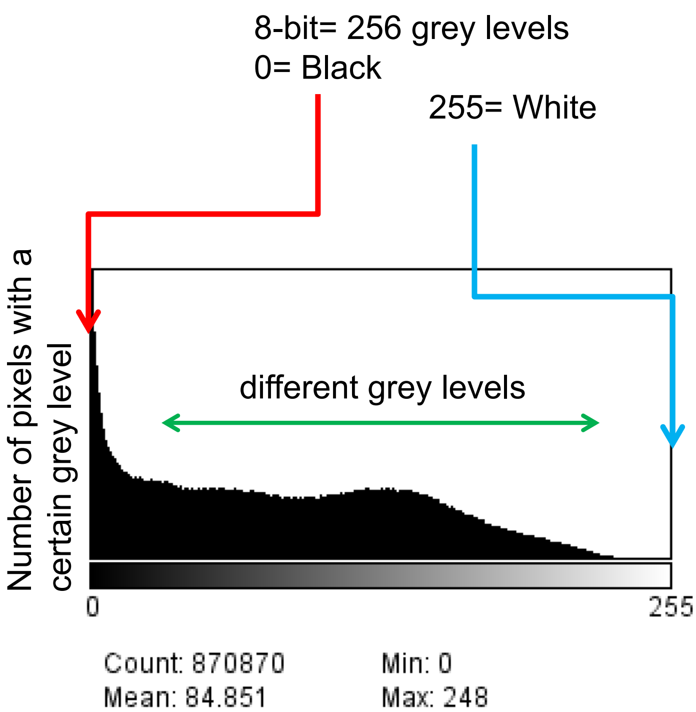
What is a good image..?

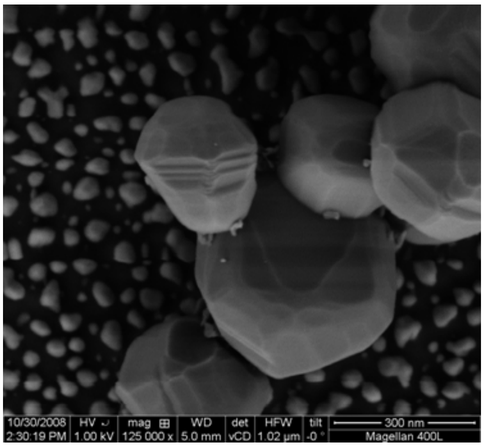


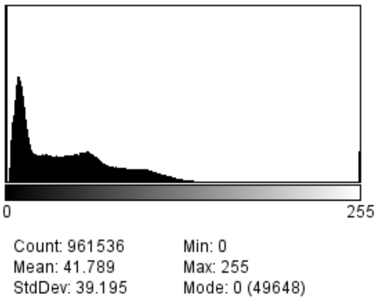


Histogram of 8-bit Tif image

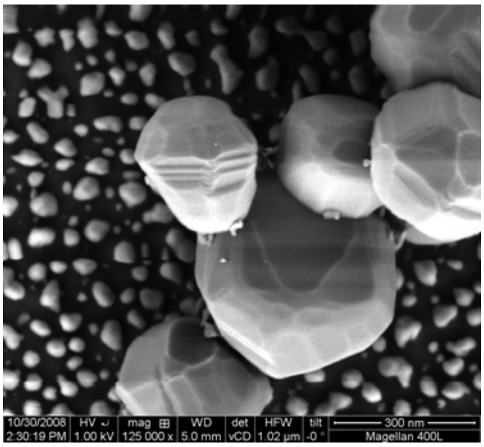


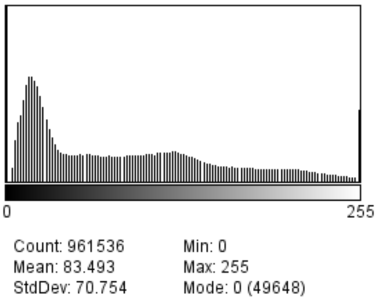






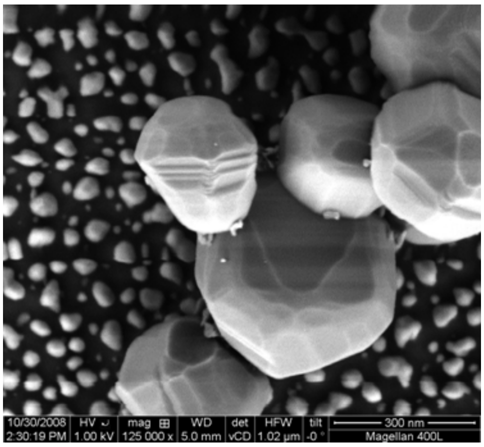
A little too dark

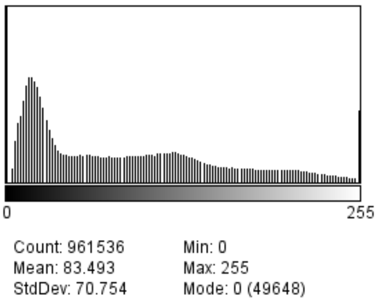


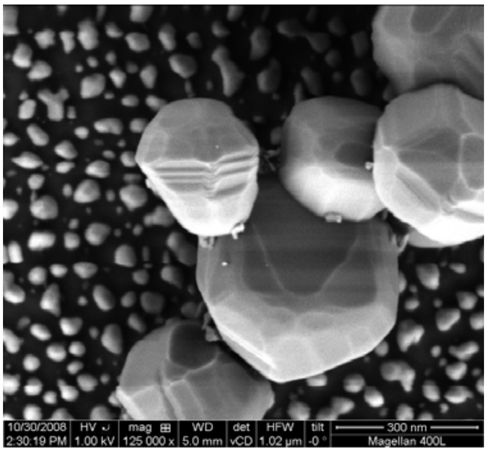


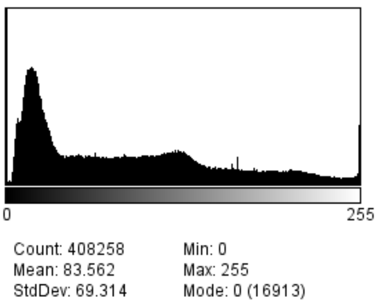
Adjusted contrast/brightness (after recording)

missing grey levels !

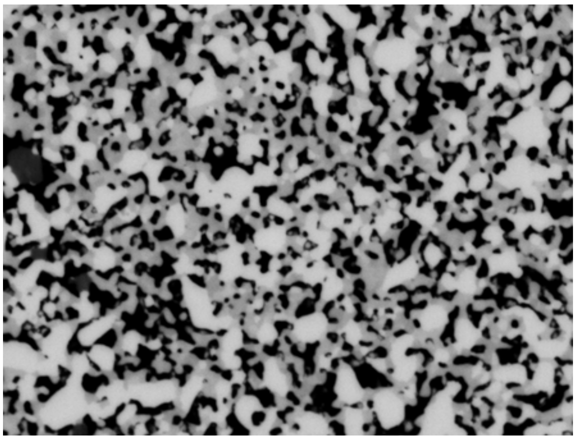


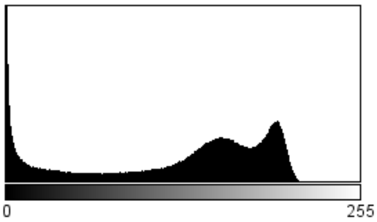




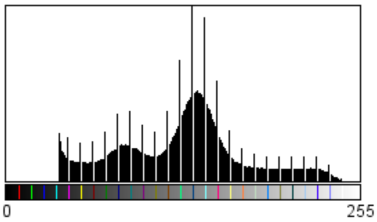


After resampling the image (inventing pixels)

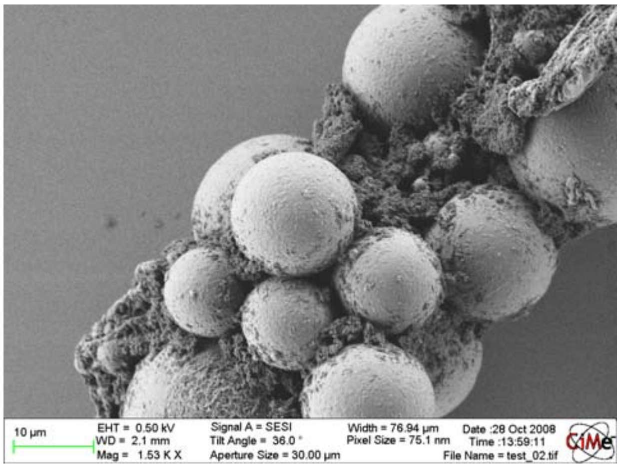


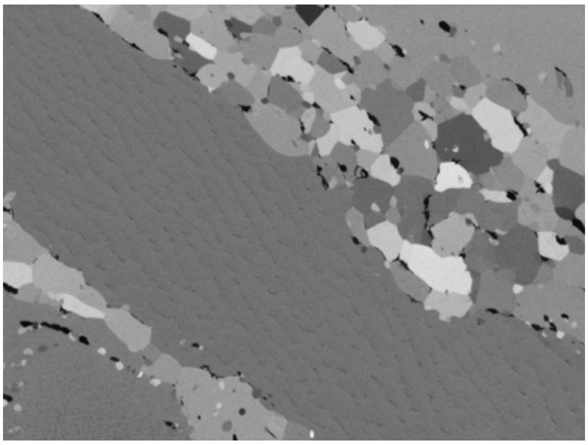


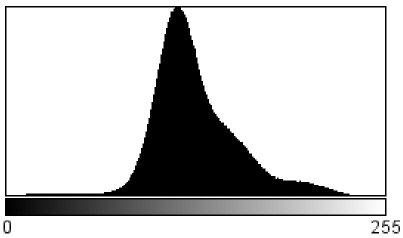
Count: 786432 Min: 0
Mean: 116.526 Max: 220
StdDev: 70.839 Mode: 0 (61194)



Count: 674674 Min: 37
Mean: 127.019 Max: 252
StdDev: 45.457 Mode: 134 (19589)



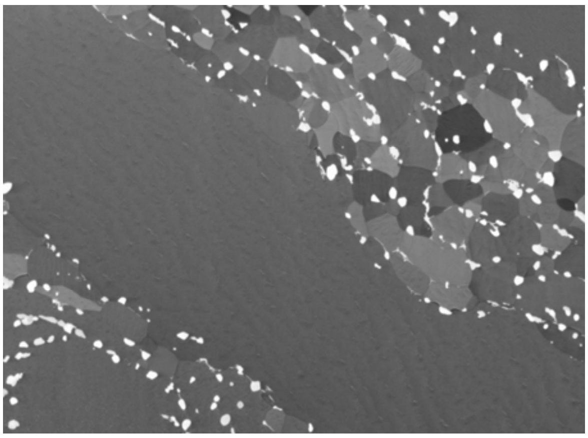


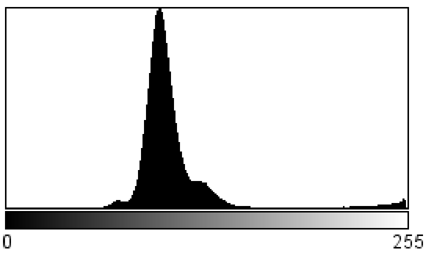


Count: 2946168 Min: 1
Mean: 127.193 Max: 251
StdDev: 29.568 Mode: 115 (57613)

BSE

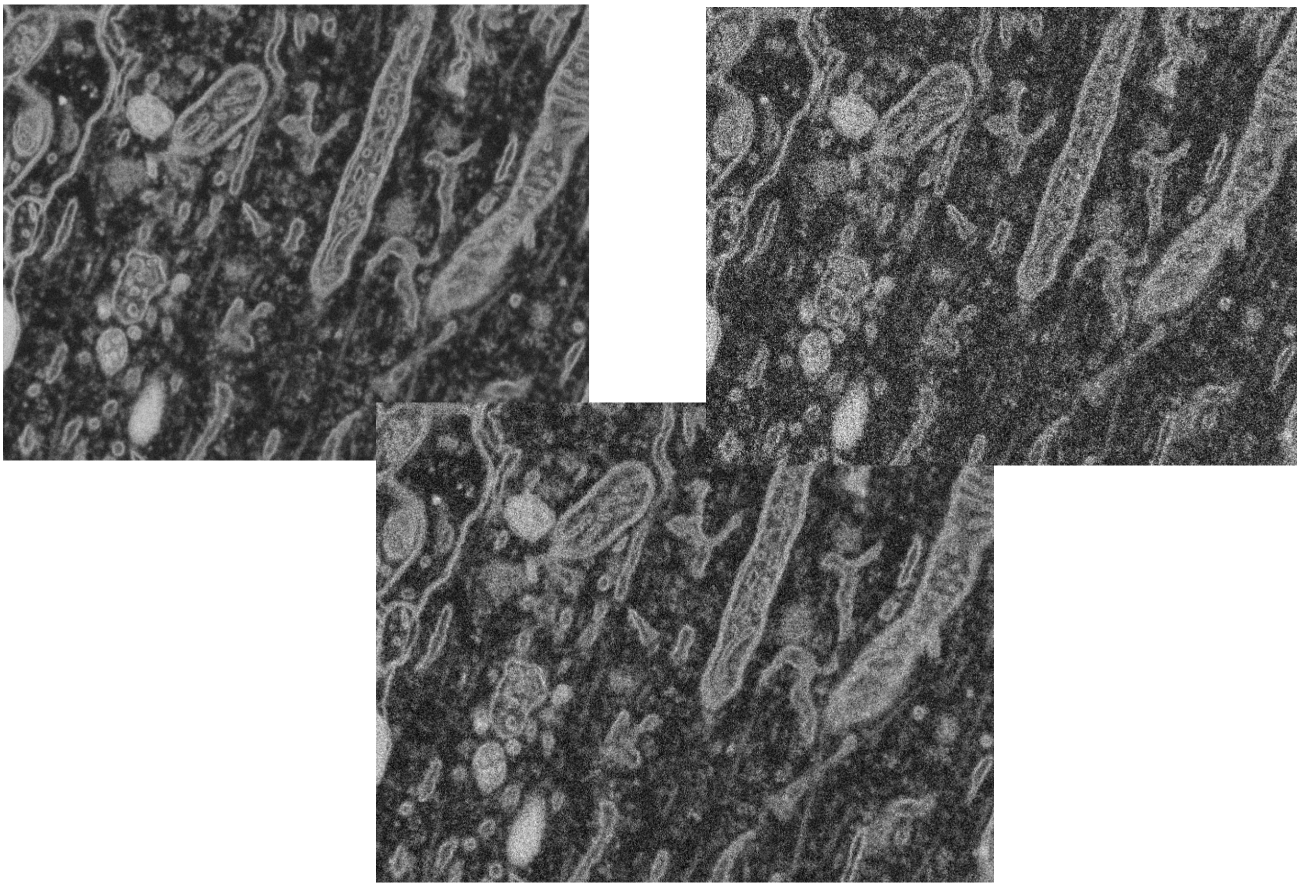
SE

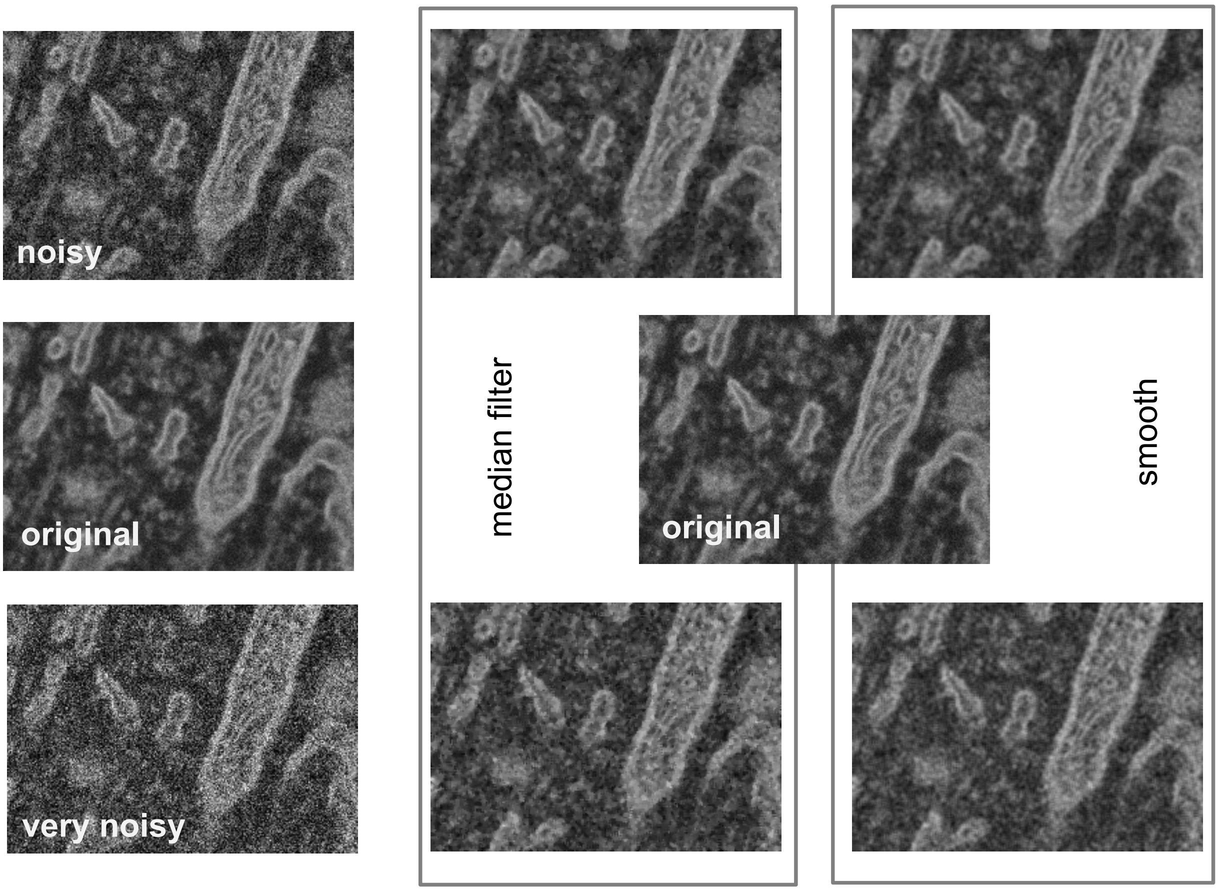




Count: 3145728 Min: 14
Mean: 106.215 Max: 254
StdDev: 28.778 Mode: 97 (133319)

dealing with noise





Filters….

- Median
- Gaussian blur
- Mean

…reduce in general the resolution

They take into account the neighbourhood of a pixel

- FFT (fast fourier transform) filter (bandpass)

FFT
bandpass filter

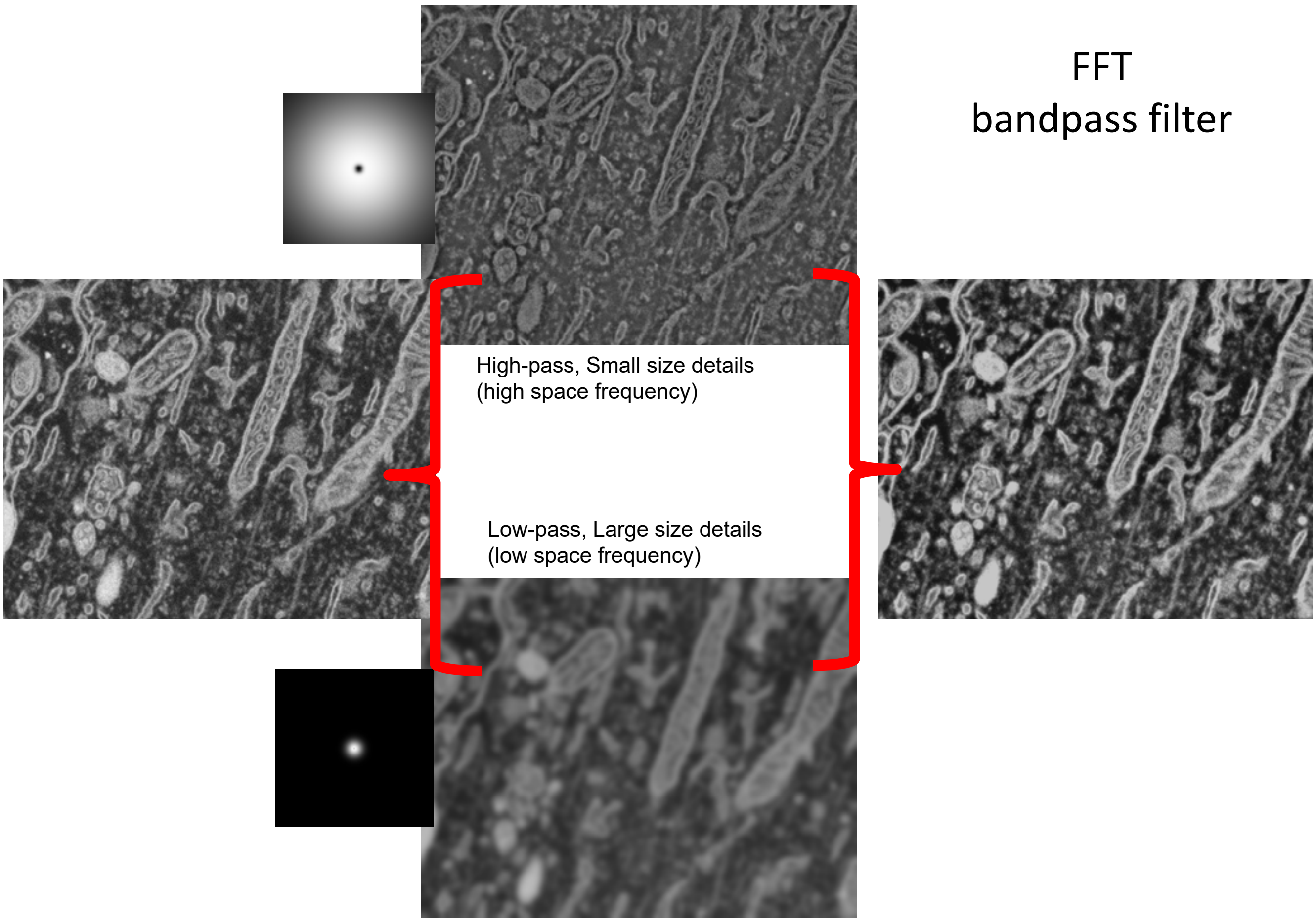
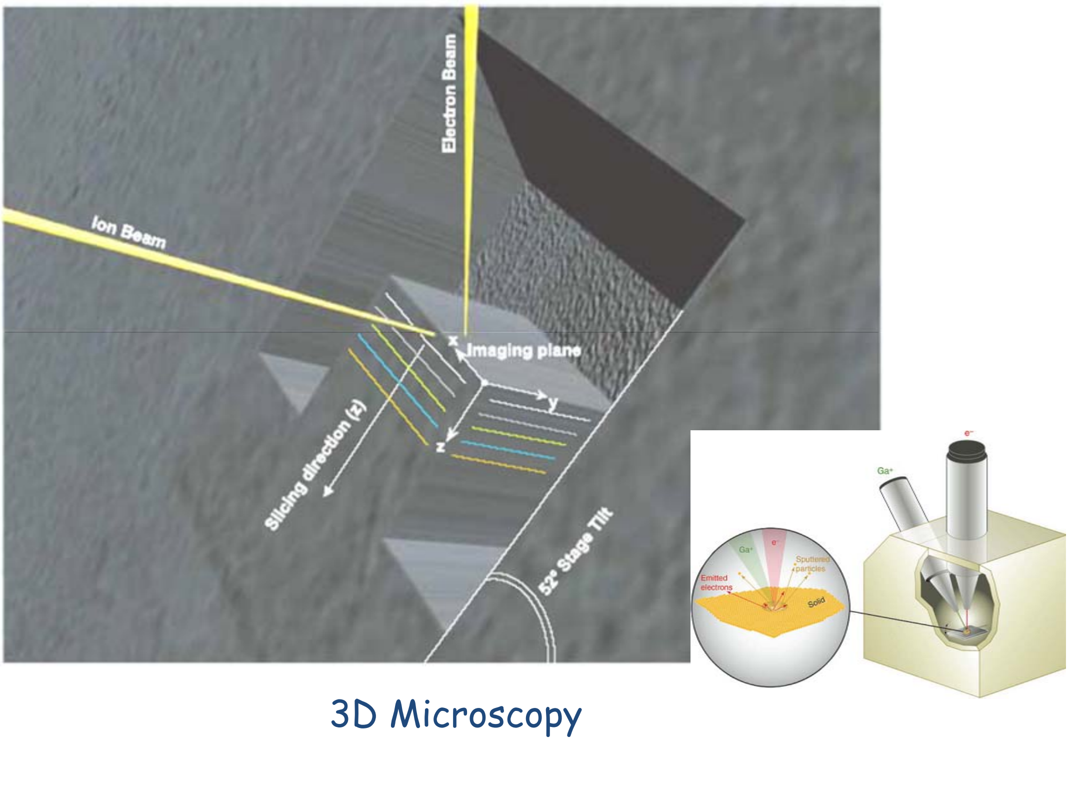


Image processing in 2D

- Best: start with a “good” image:
  - equalized histogram, use all 256 grey levels
  - Reduce noise during acquisition
- Carefully use filters
  - Filtering means “mixing-in” of information from neighbouring pixels: loss of resolution
  - try several to see which one works best
  - do resampling only in the very end

3D imaging with a FIB/SEM
FIB Nanotomography



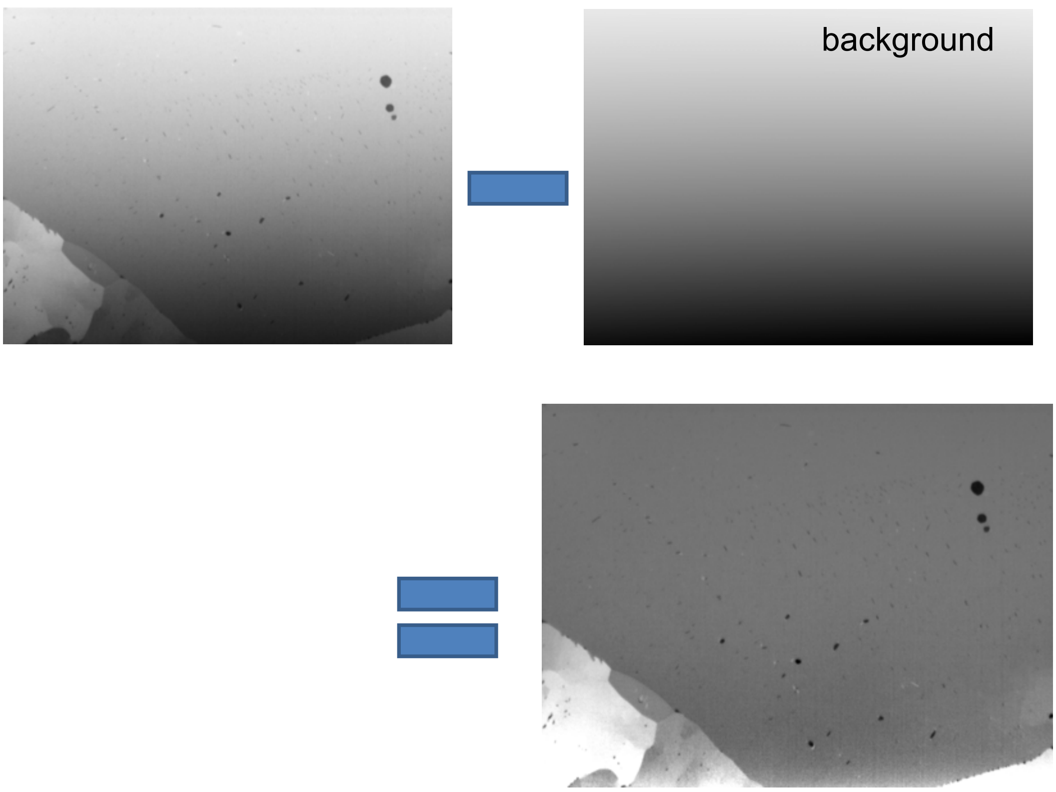
3D Microscopy

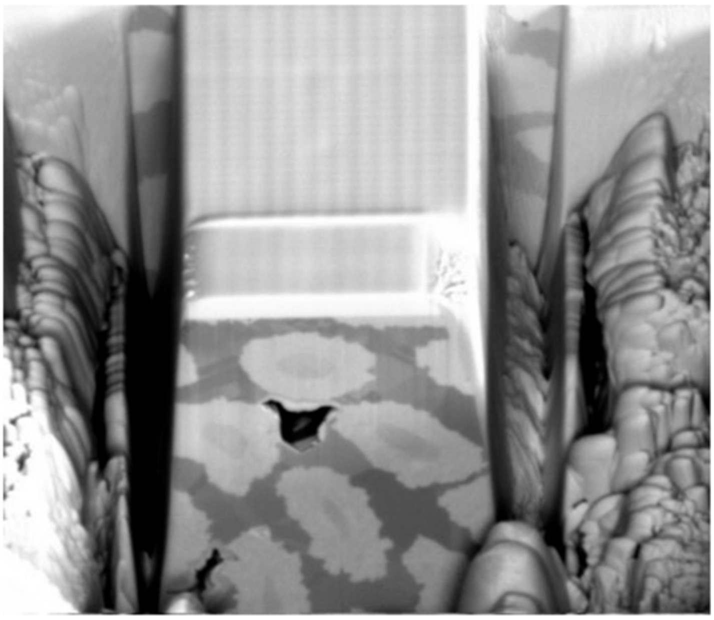
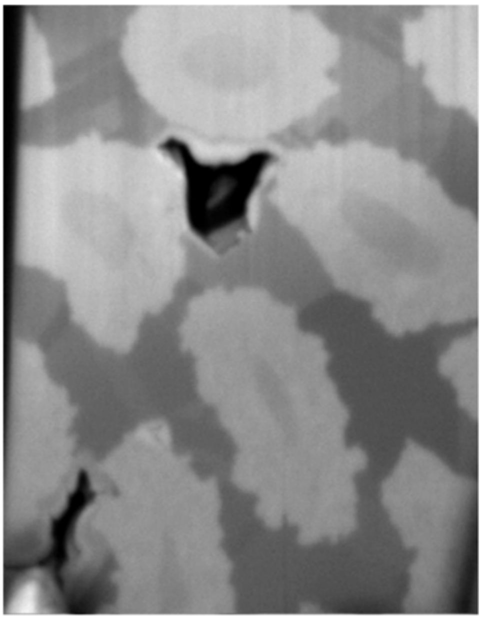
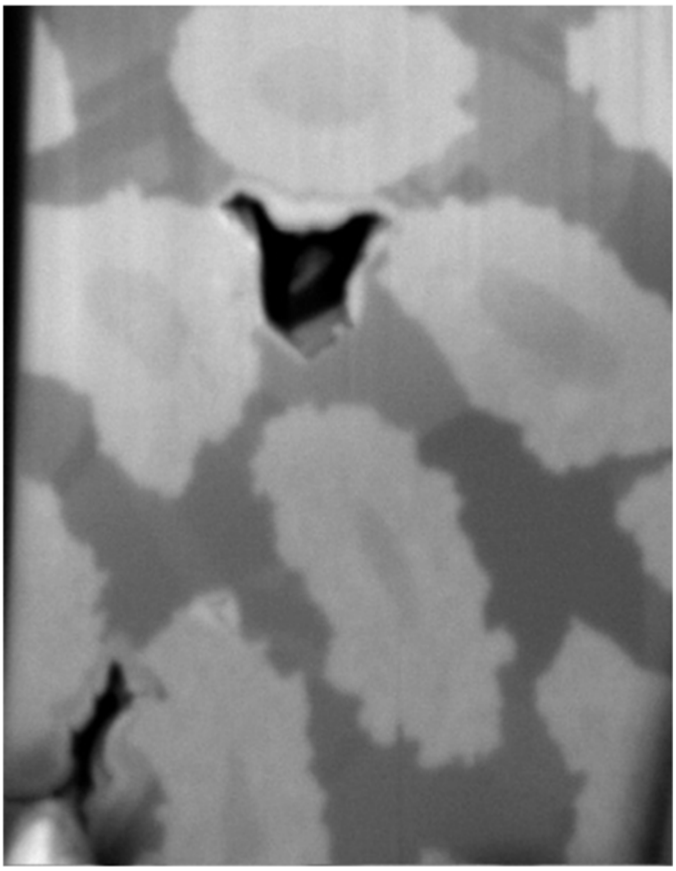
Image processing in 3D

- 2D processing (shading correction)
- Image alignment (registration)
- Cropping (subvolume)
- Noise reduction (2D or 3D)

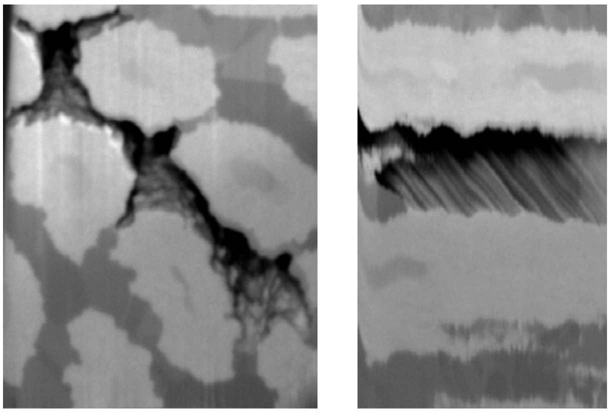
- Segmentation
- Visualization

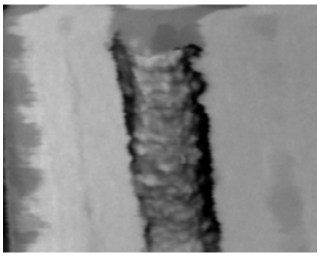
Shading correction



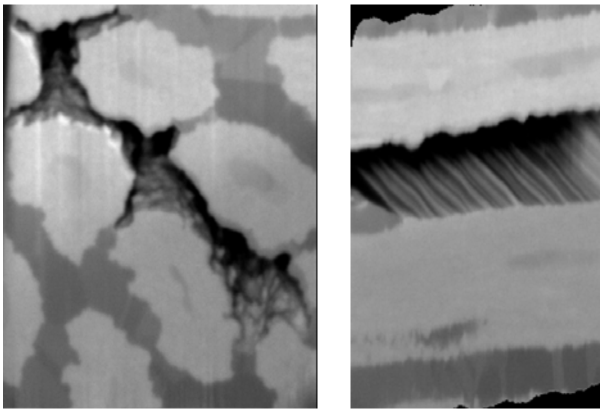




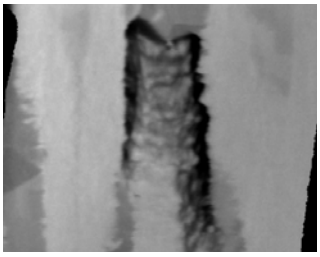
Registration





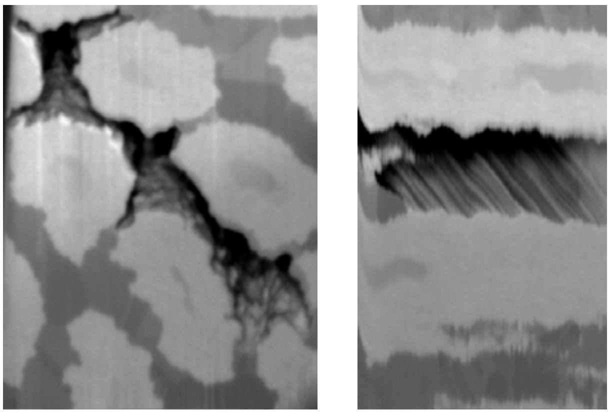
before

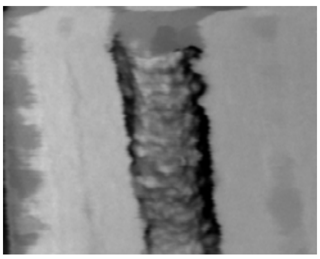




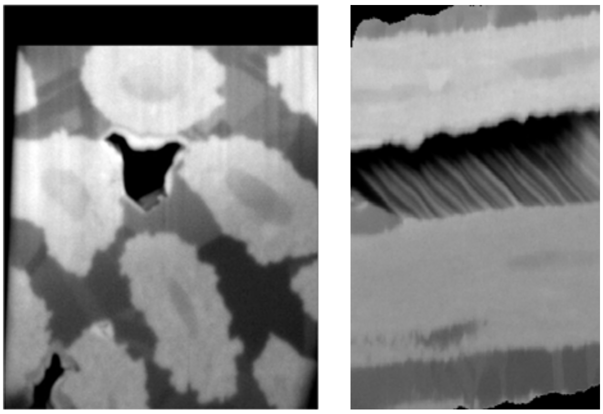
after

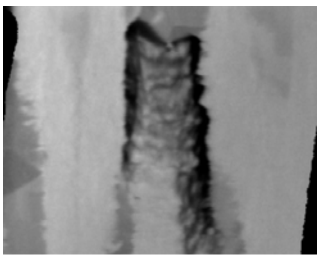
Registration





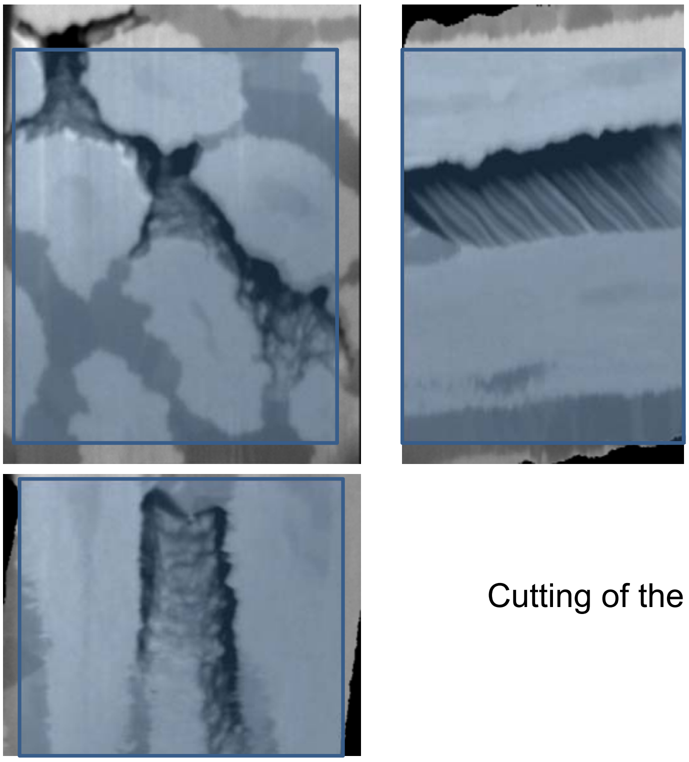
before





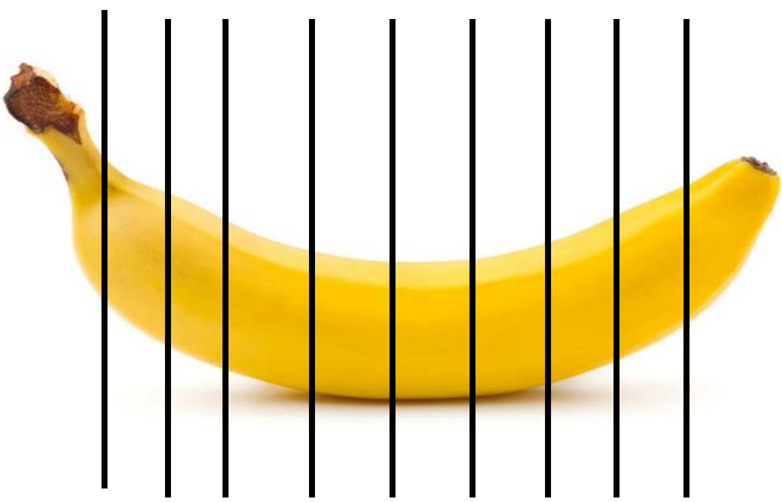
after

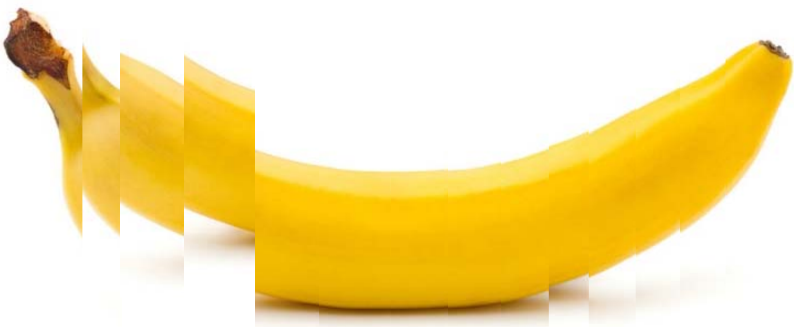
cropping

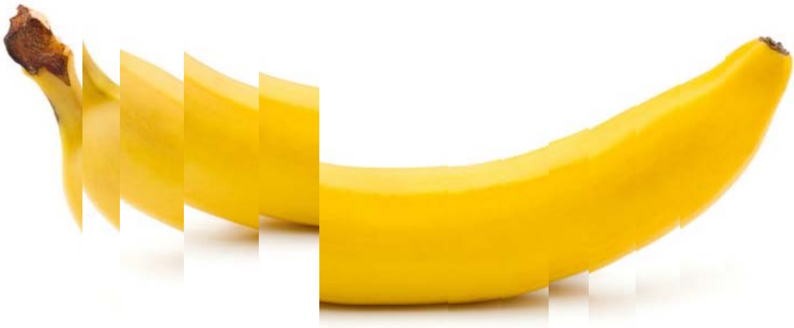


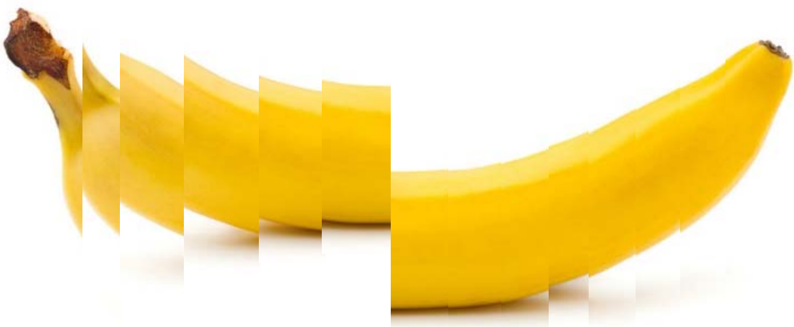
Cutting of the border

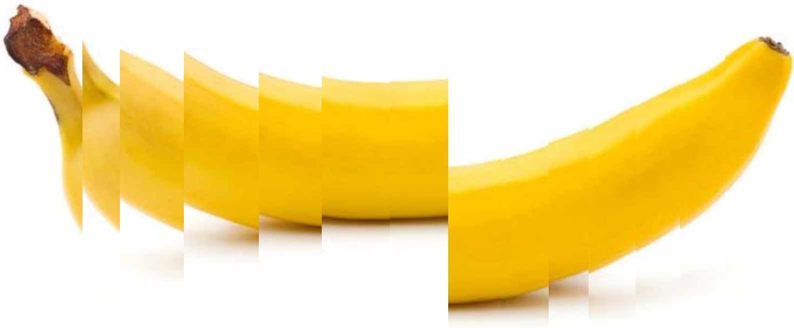
Post-acquisition alignment (registration) “banana <-> cucumber problem”

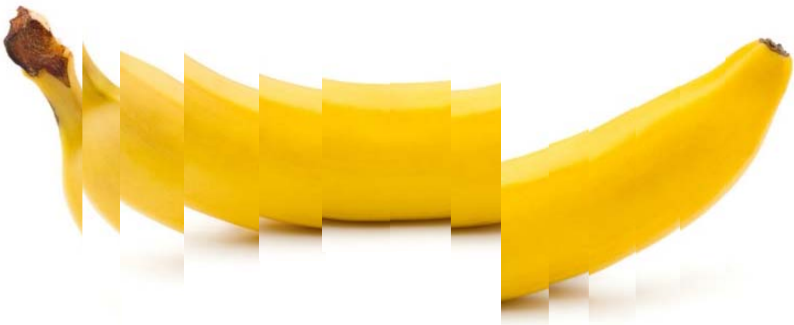


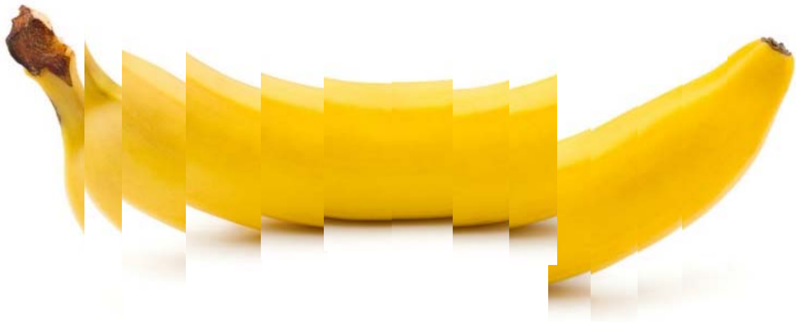


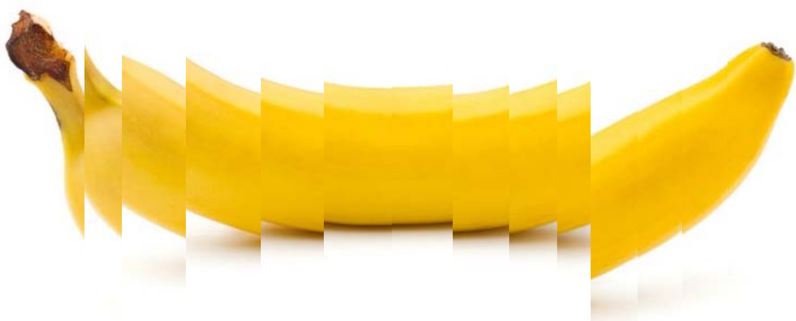


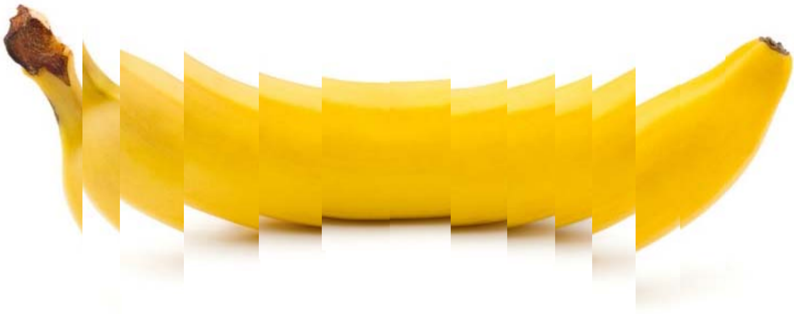


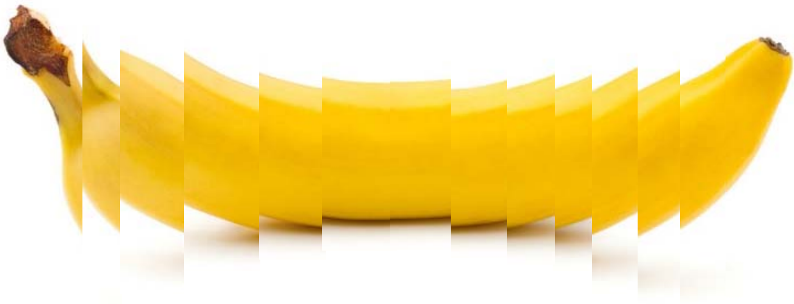


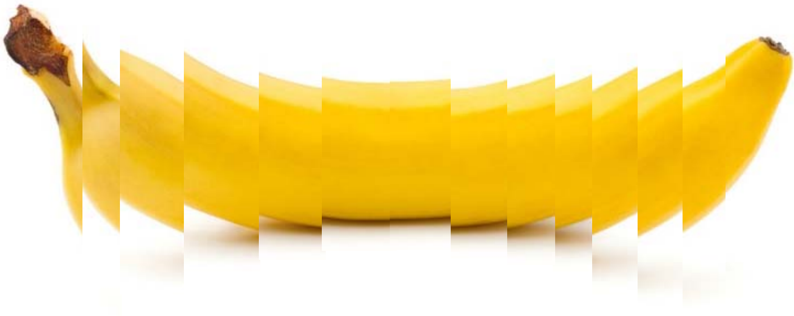


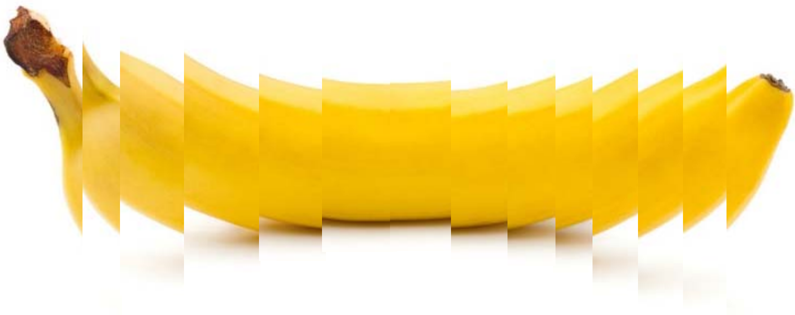




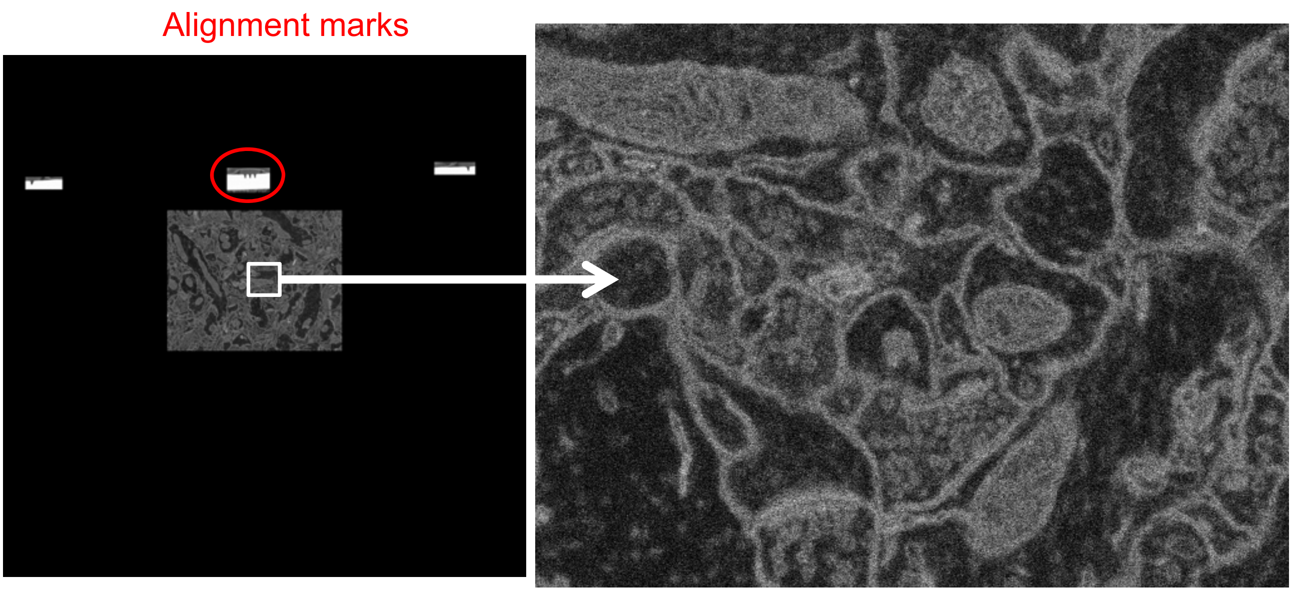








4800 images (5nm pixel size) at 1.7nm slice thickness

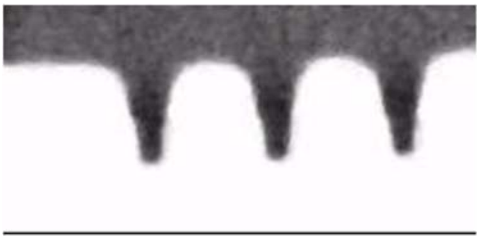


8kx8k image

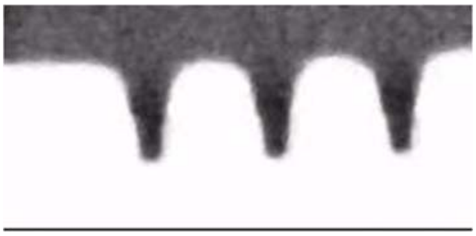
20sec. For 2kx2k ROI

5nm pixel

Alignment on notches

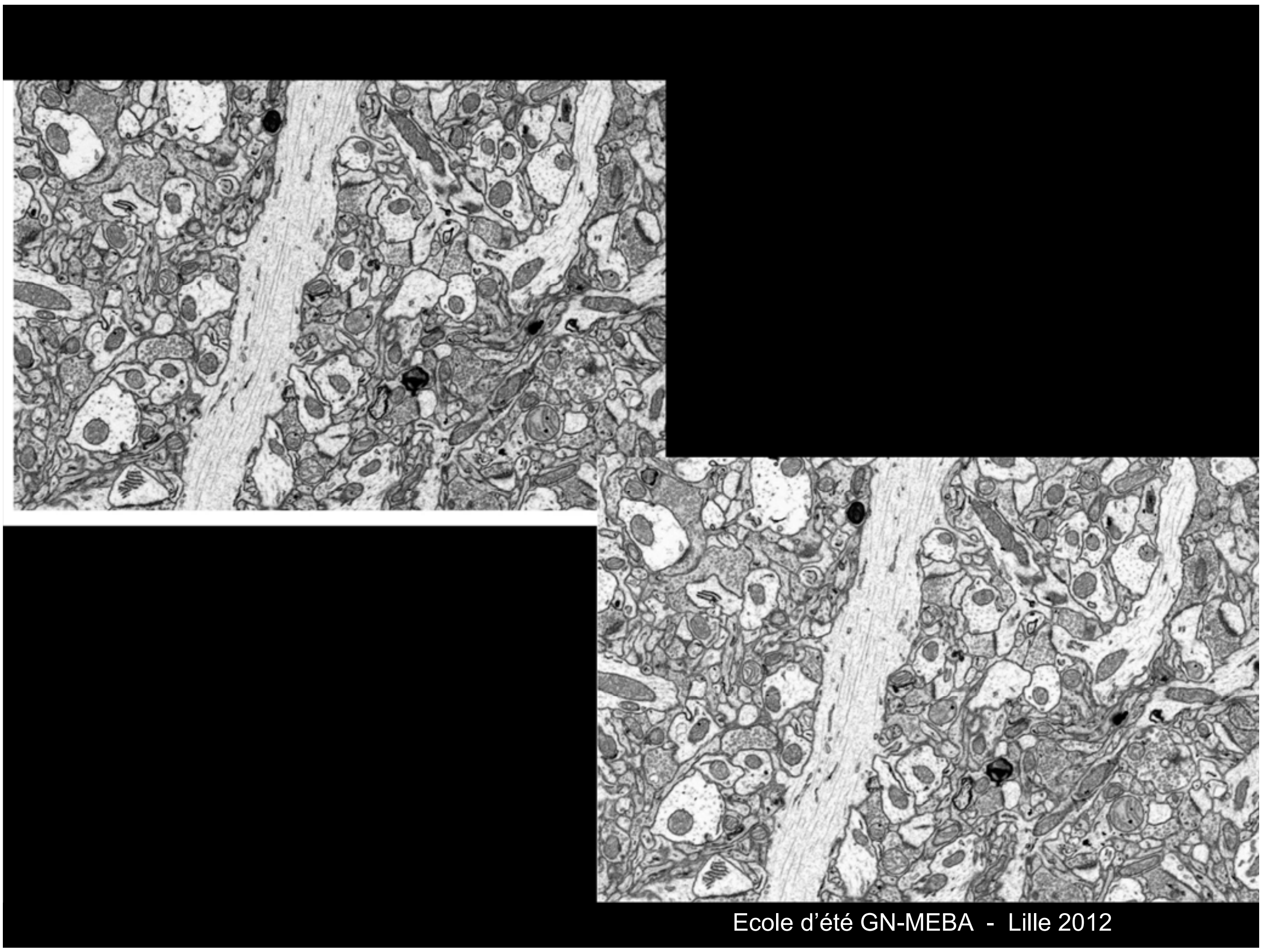


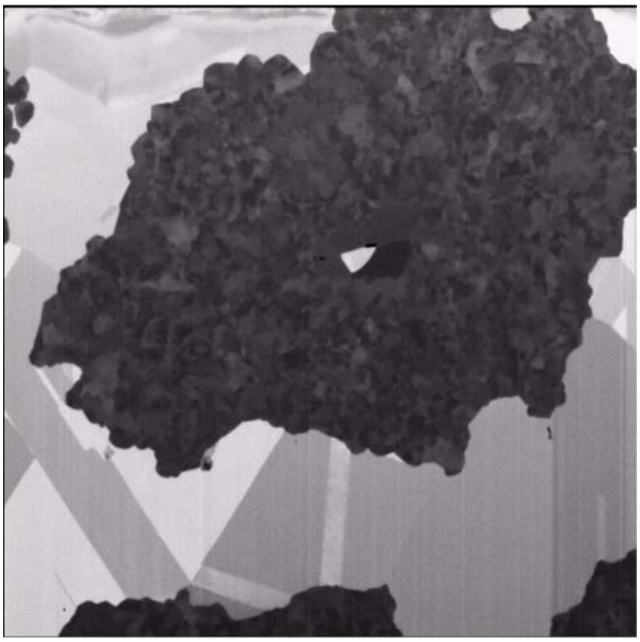
raw



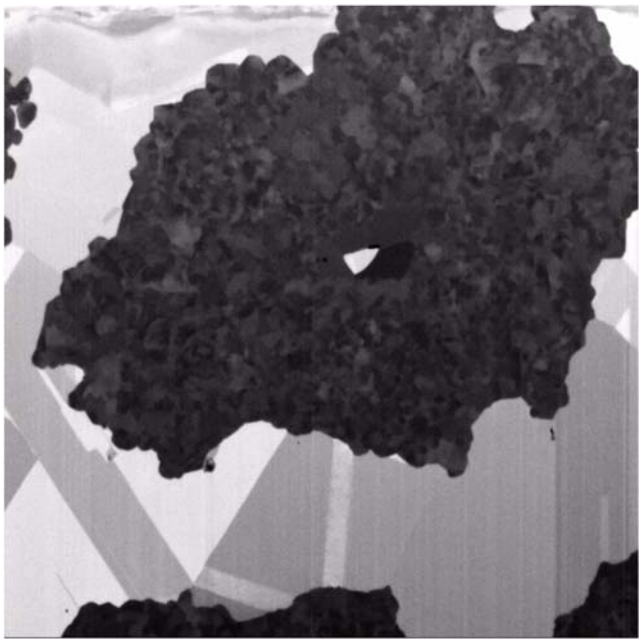
StackReg on unprocessed images

StackReg on “binary” enhanced images



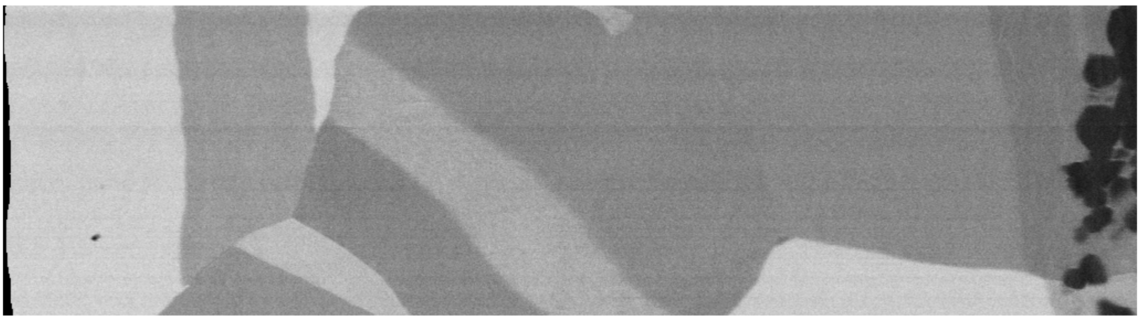


StackReg



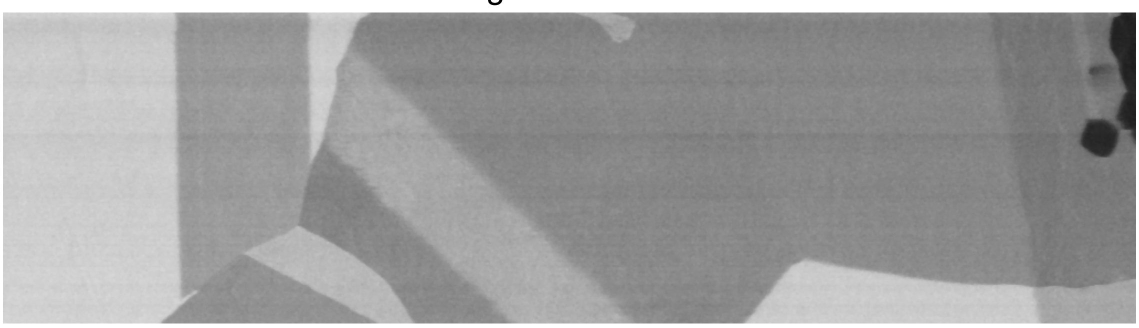
Aligned on Notches

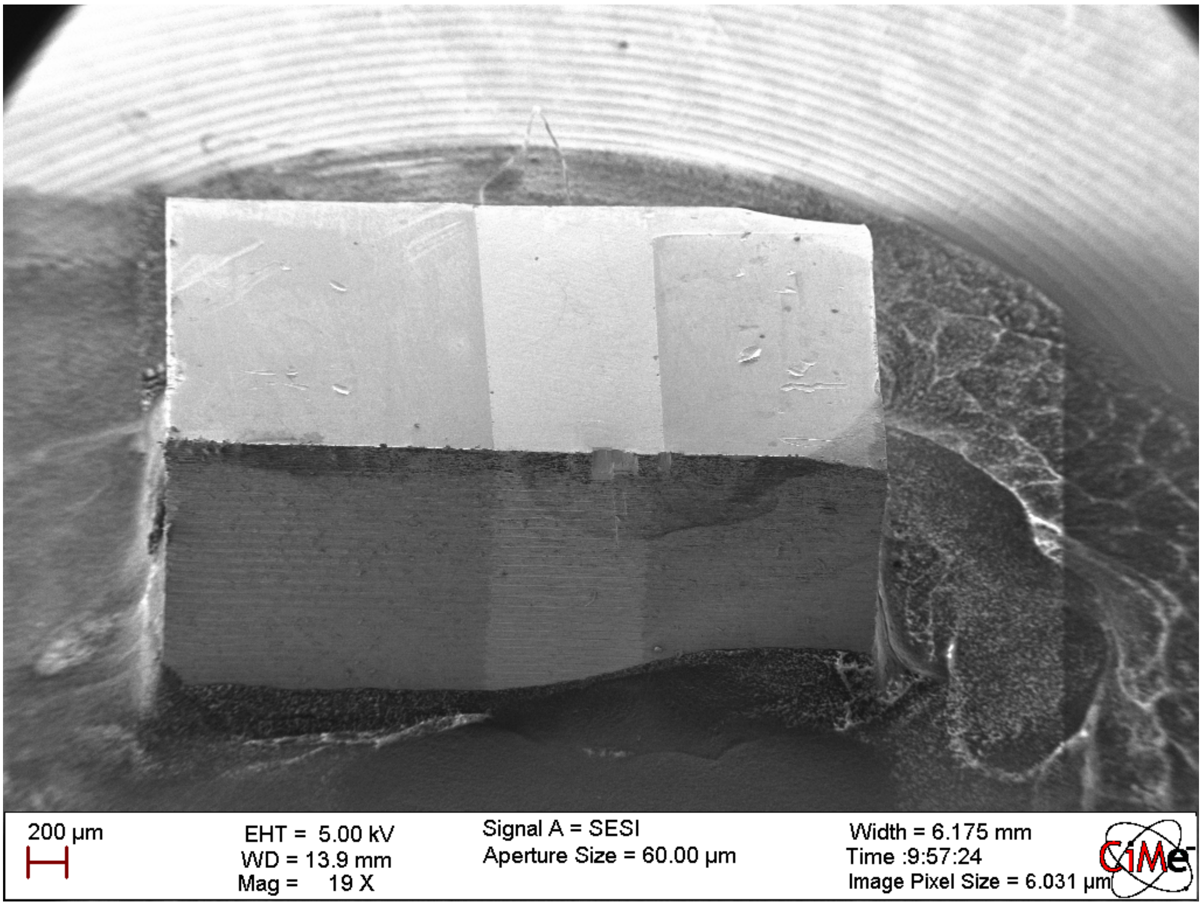
X-Z plane

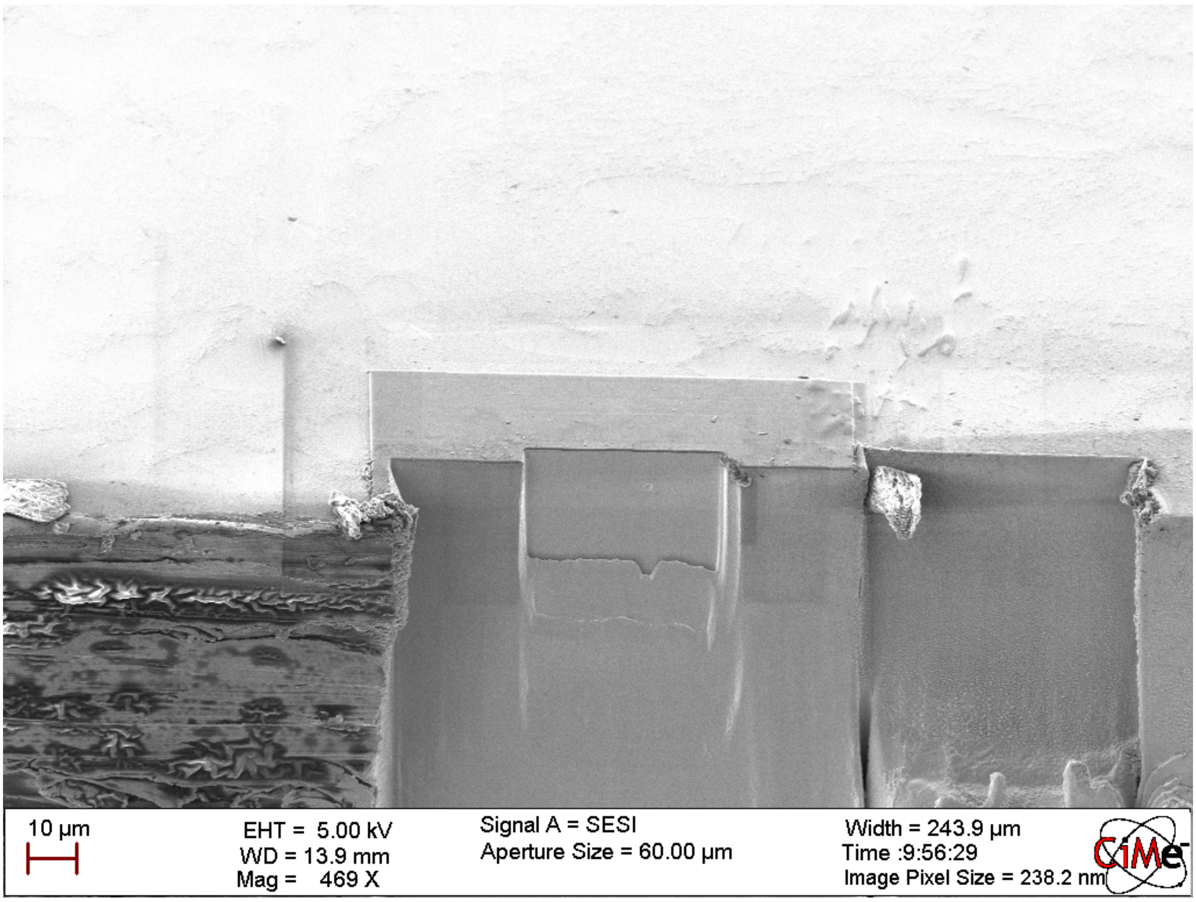


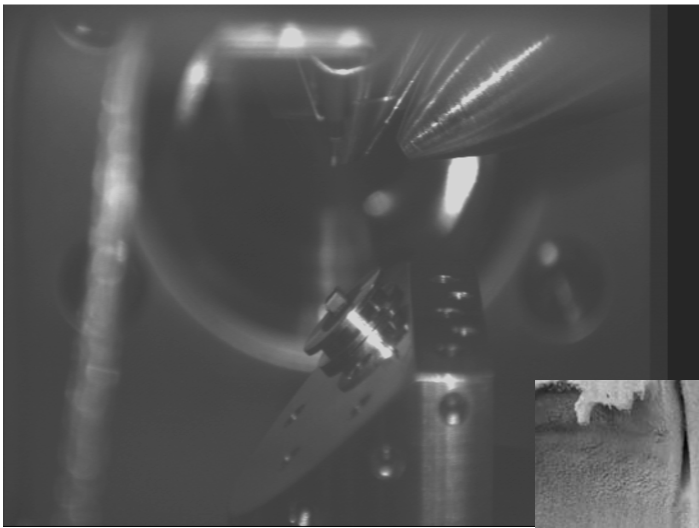
StackReg

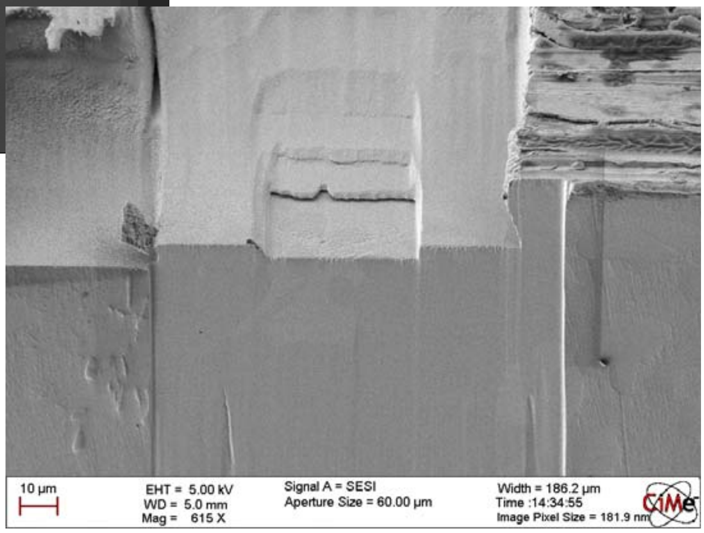
Aligned on Notches

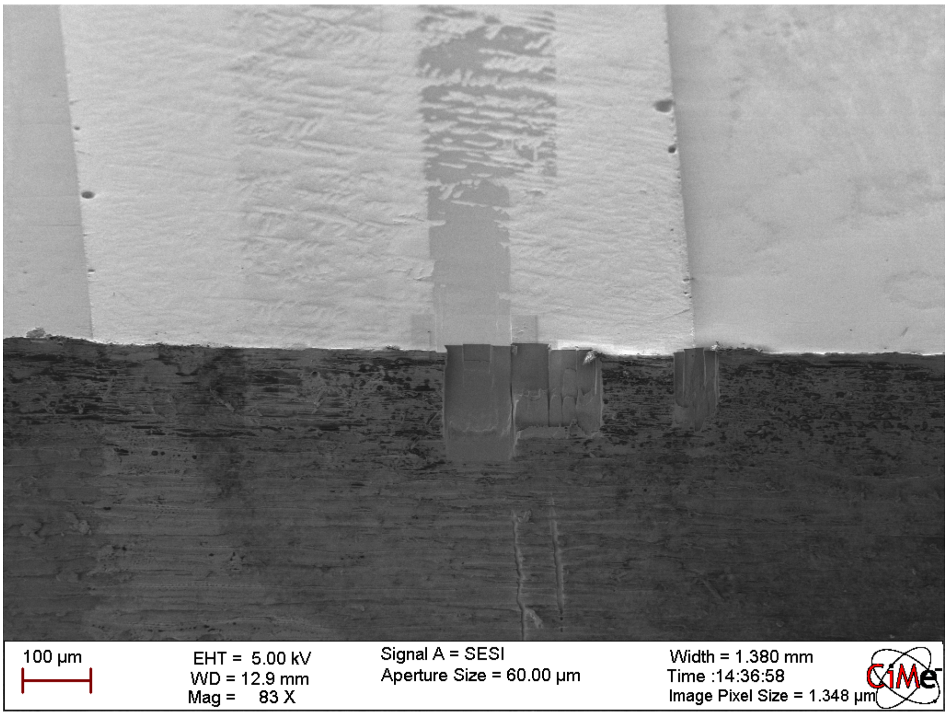


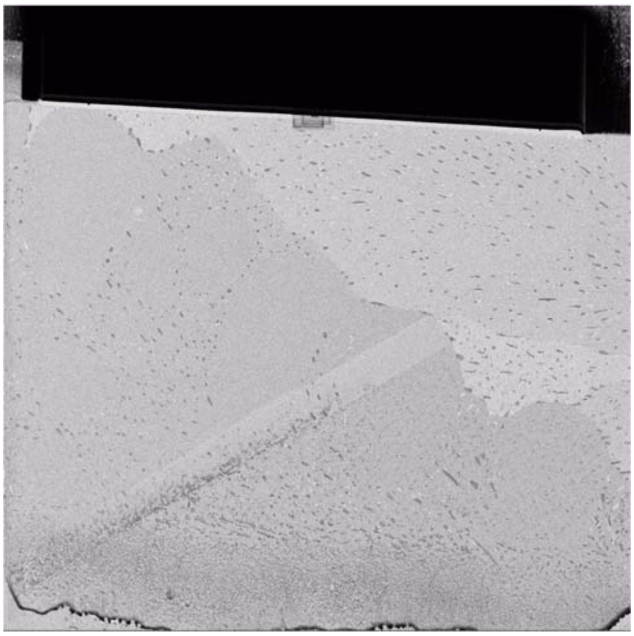

200 µm
EHT = 5.00 kV
WD = 13.9 mm
Mag = 19 X
Signal A = SESI
Aperture Size = 60.00 µm
Width = 6.175 mm
Time :9:57:24
Image Pixel Size = 6.031 µm
CiMe

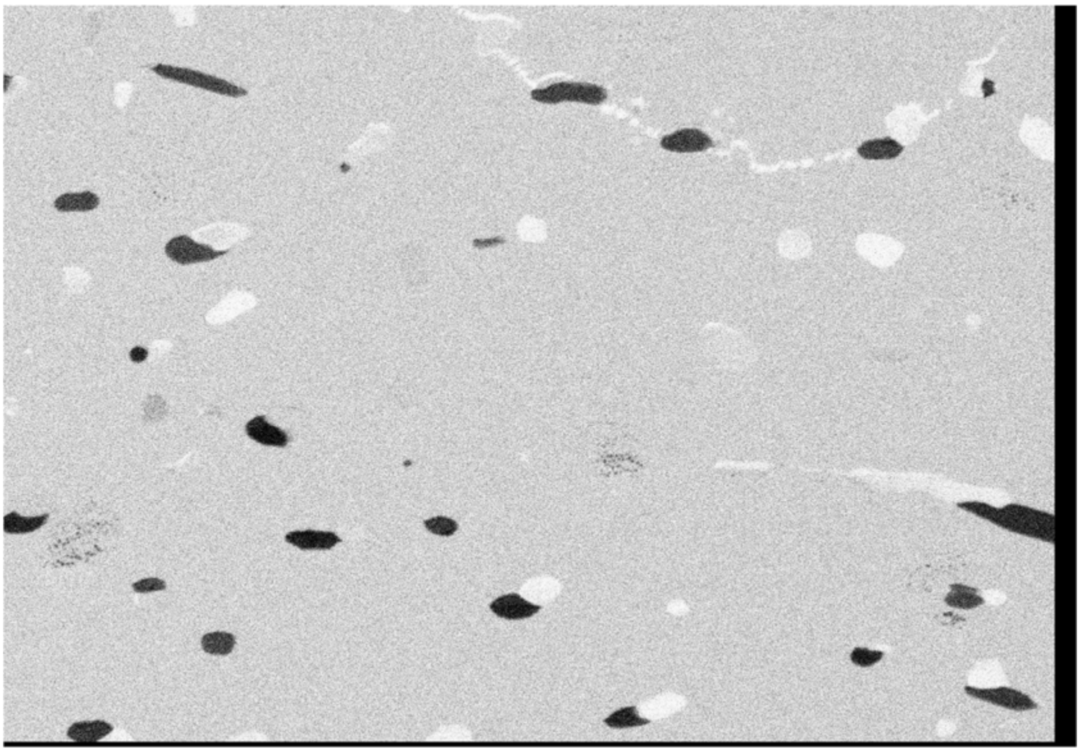

10 µm
EHT = 5.00 kV
WD = 13.9 mm
Mag = 469 X
Signal A = SESI
Aperture Size = 60.00 µm
Width = 243.9 µm
Time :9:56:29
Image Pixel Size = 238.2 nm
CiMe



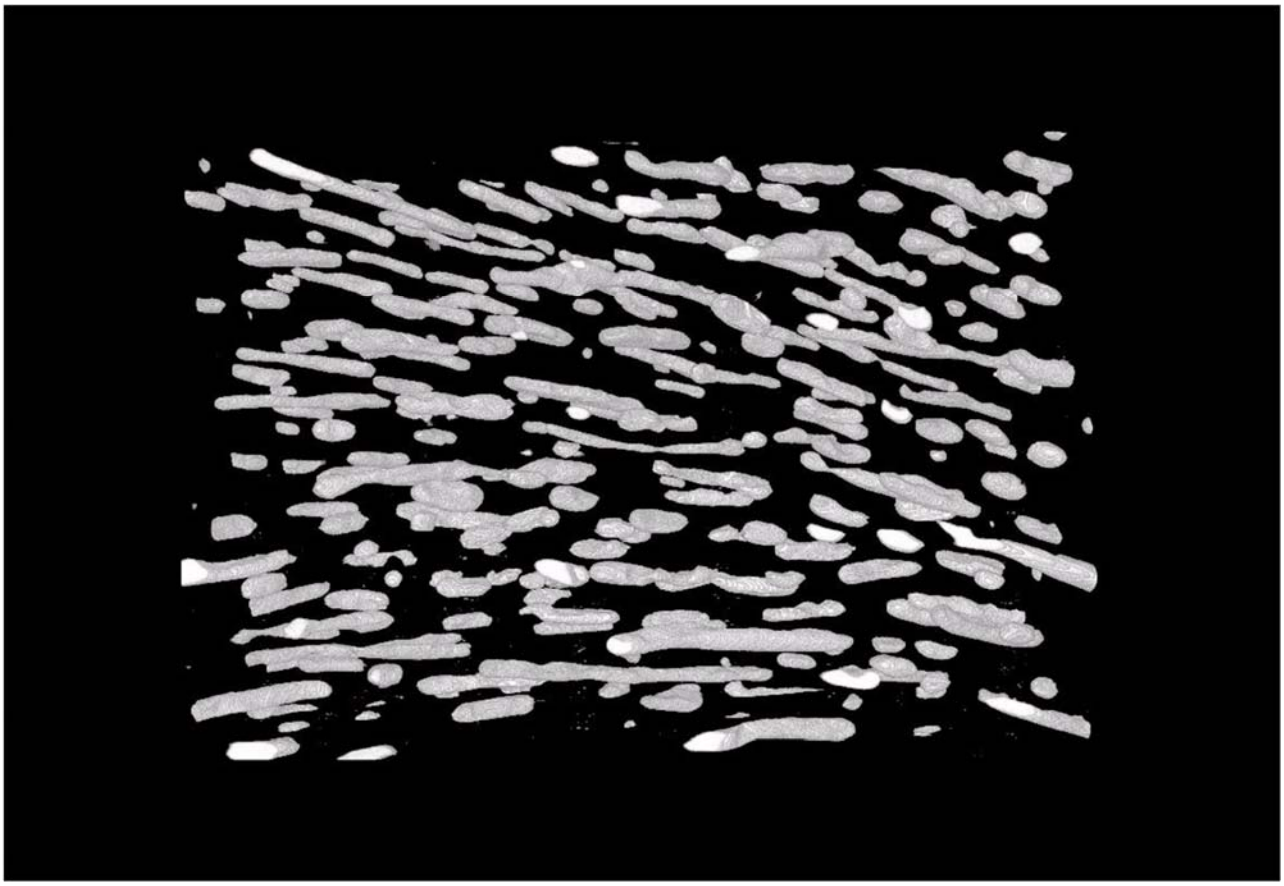

10 µm
EHT = 5.00 kV
WD = 5.0 mm
Mag = 615 X
Signal A = SESI
Aperture Size = 60.00 µm
Width = 186.2 µm
Time :14:34:55
Image Pixel Size = 181.9 nm
CiMe


100 µm
EHT = 5.00 kV
WD = 12.9 mm
Mag = 83 X
Signal A = SESI
Aperture Size = 60.00 µm
Width = 1.380 mm
Time :14:36:58
Image Pixel Size = 1.348 µm
CiMe





Aligned with StackReg



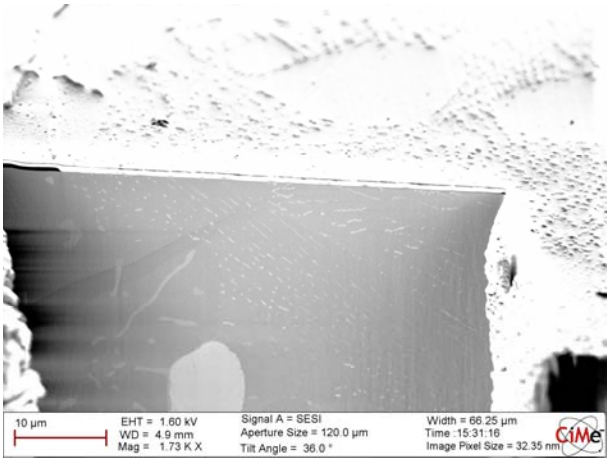
Segmentation

- Identifying the different phases/volumes/objects
- Creating binary images/stacks of the different objects

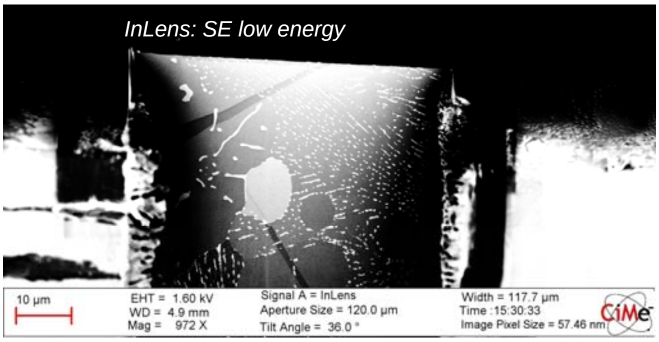
Pb-free solder SnAgCu:

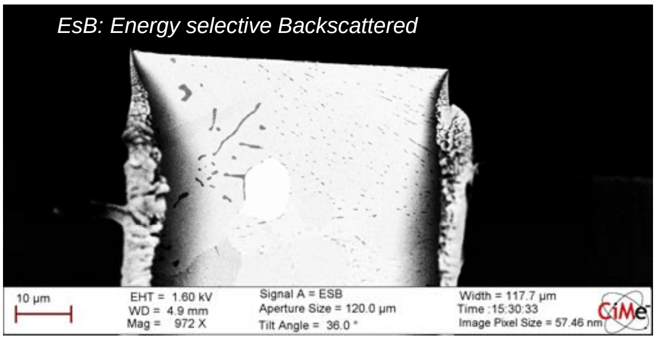
"one detector is not enough"

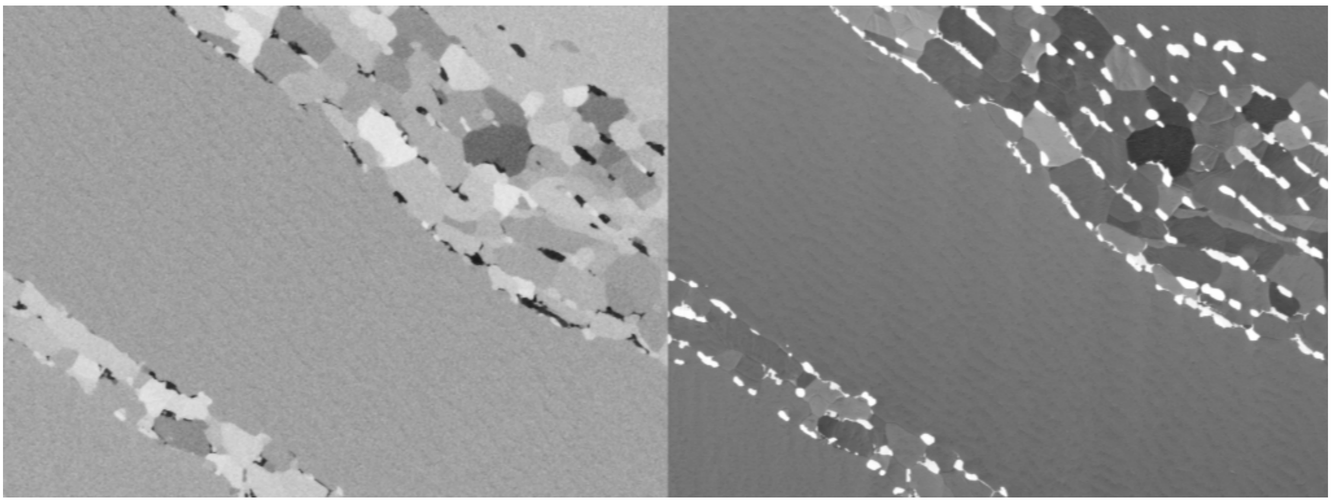
M. Maleki, EPFL-LMAF

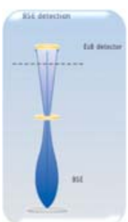


ETD (SE classic)









EsB

10µm

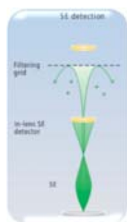
10x10x10nm voxel size, 2048x1536x2000

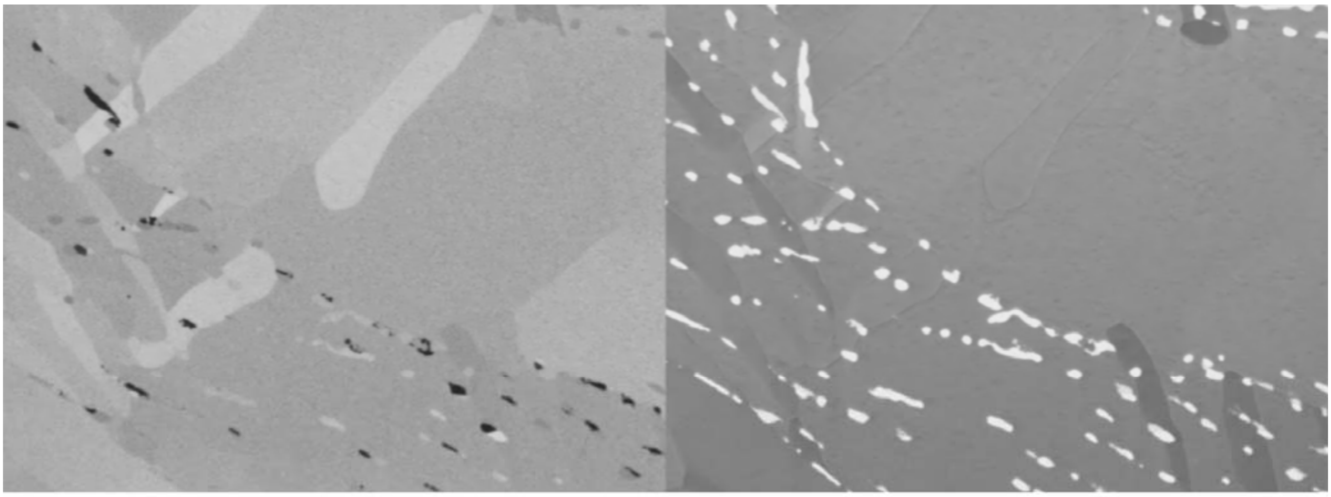
2 images (2x3Mb) / slice ...! (DUAL Channel !)

1.6keV, EsB & InLens-SE detector

12Gb data

InLens SE

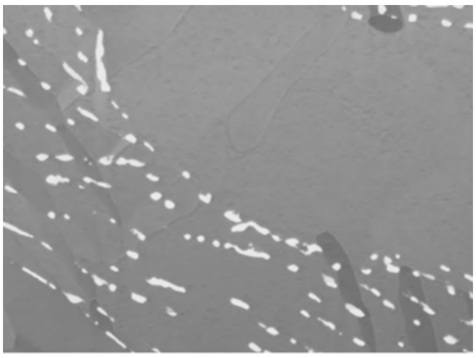


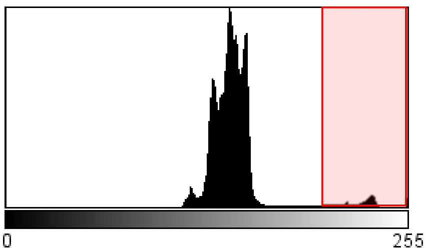


EsB

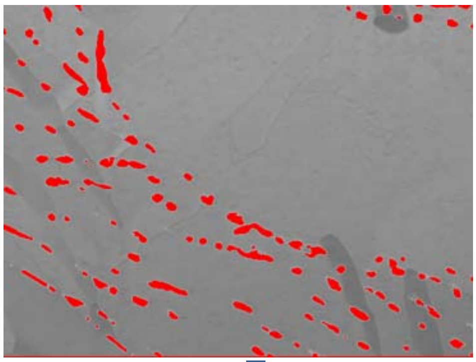
10x10x10nm voxel size, 2048x1536x2000
2 images (2x3Mb) / slice ...! (DUAL Channel !)
1.6keV, EsB & InLens-SE detector
12Gb data

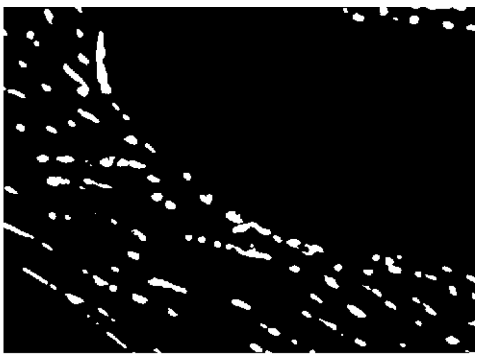
InLens SE

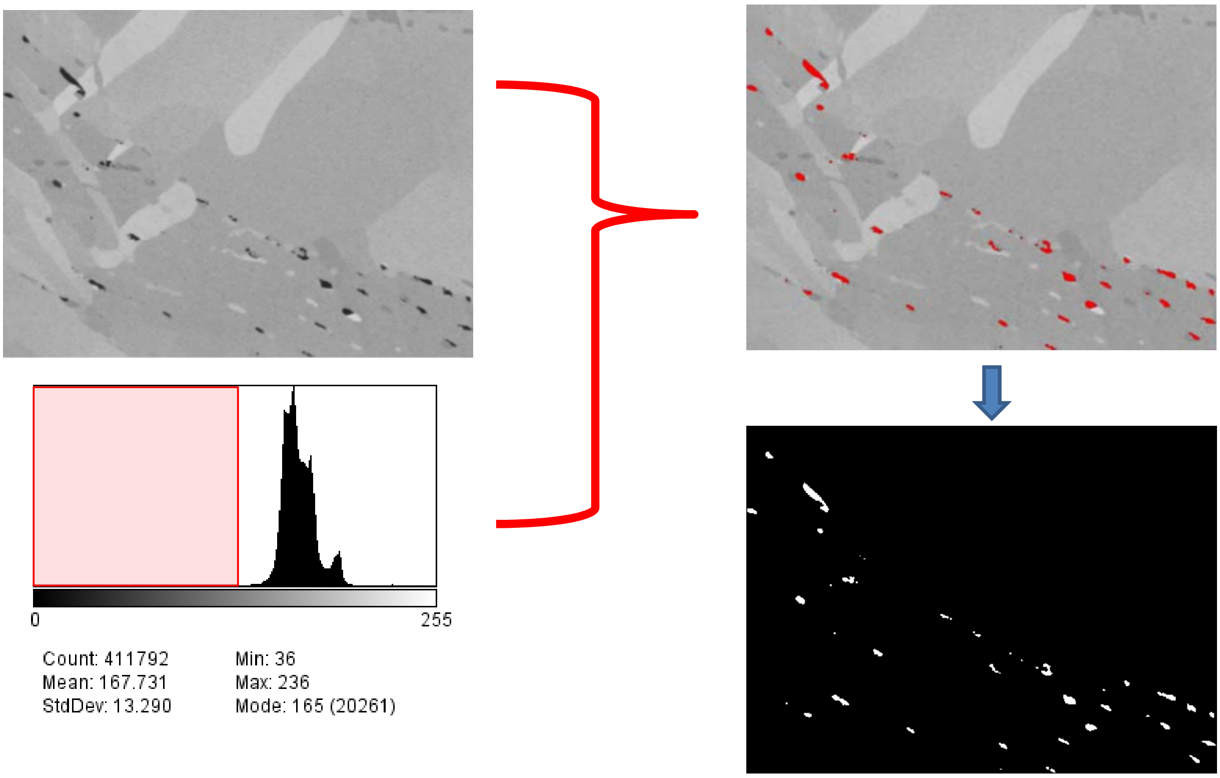


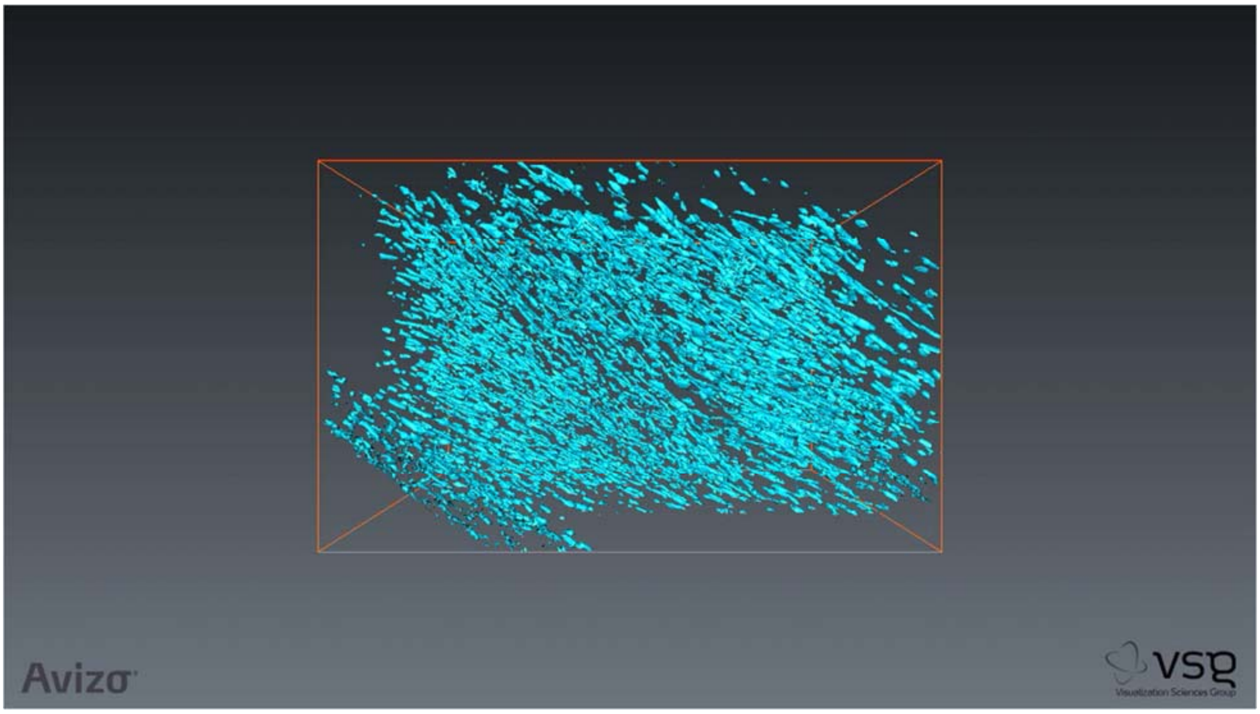


Count: 411792
Mean: 145.445
StdDev: 18.865
Min: 101
Max: 255
Mode: 142 (18748)







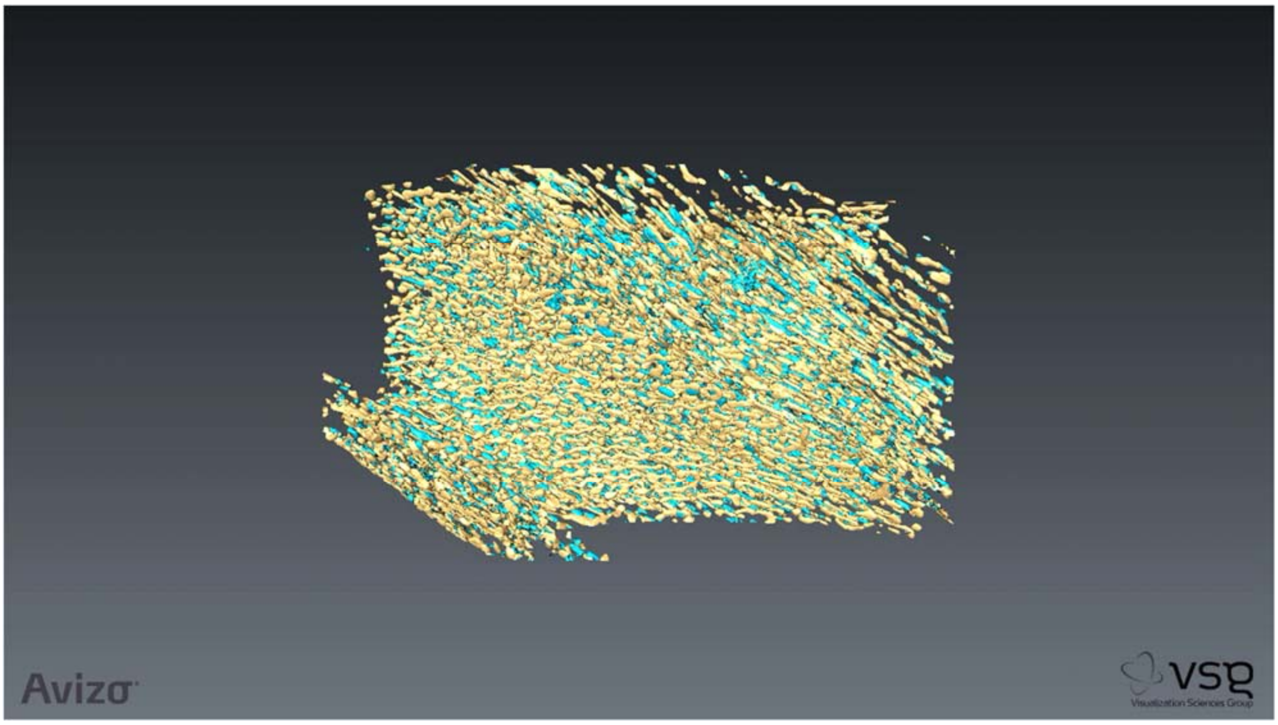


Phase 1. Dark in EsB image, White in SE-InLens

10x10x10nm voxel size, 2048x1536x2000 pixel/slices
2 images (3Mb) / slice …… 12Gb data

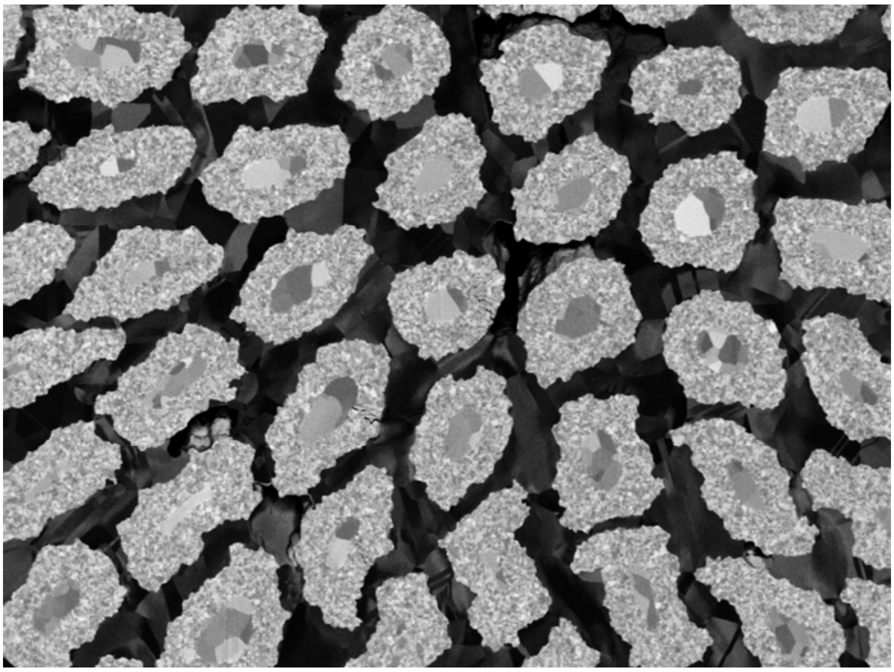
Phase 2: White in SE-InLens - Dark in EsB image

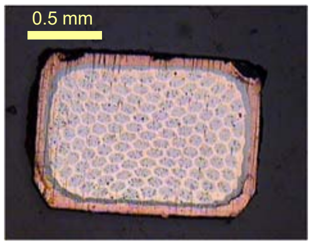
10x10x10nm voxel size, 2048x1536x2000 pixel/slices
2 images (3Mb) / slice …… 12Gb data



3D FIB/SEM: volume reconstruction

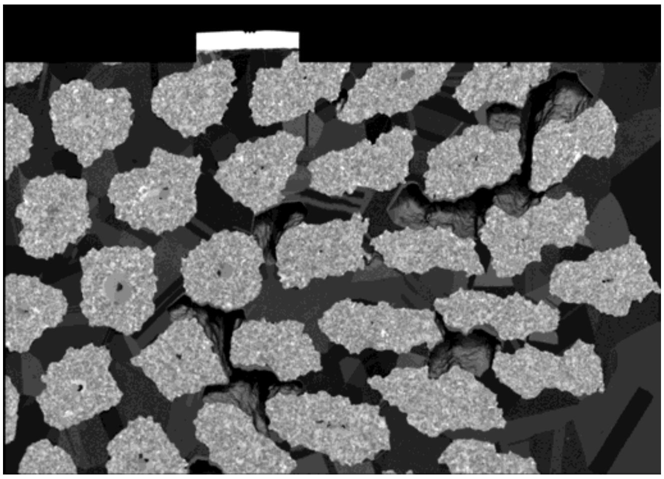
Nb_3Sn multifilament superconducting cable

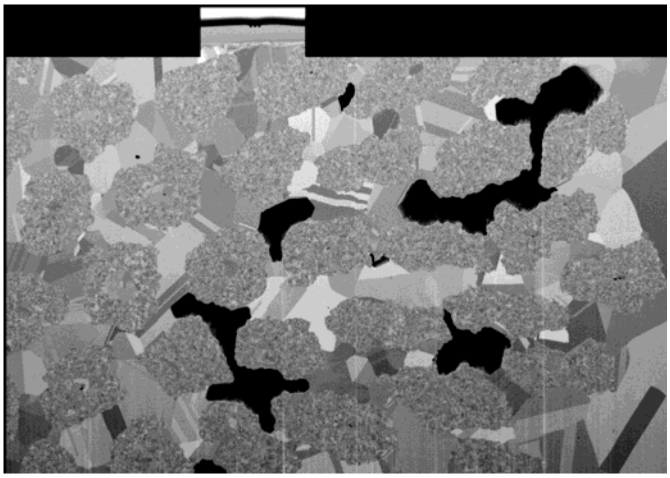




Nb_3Sn superconductor multifilament cable: 14'000 Nb_3Sn filaments (diameter ~5um) in Cu matrix

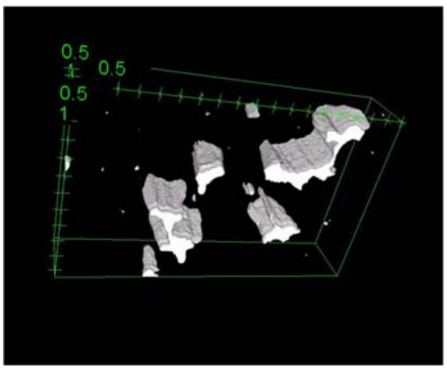
1.8kV EsB detector: Materials & orientation contrast

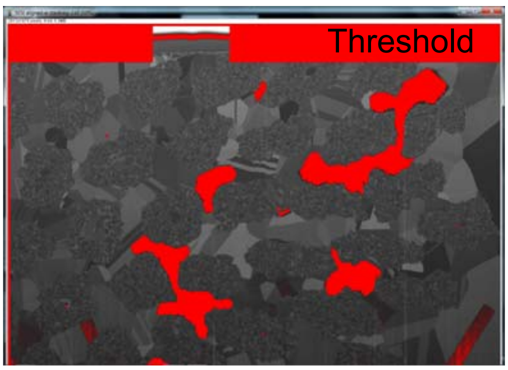




Es
B

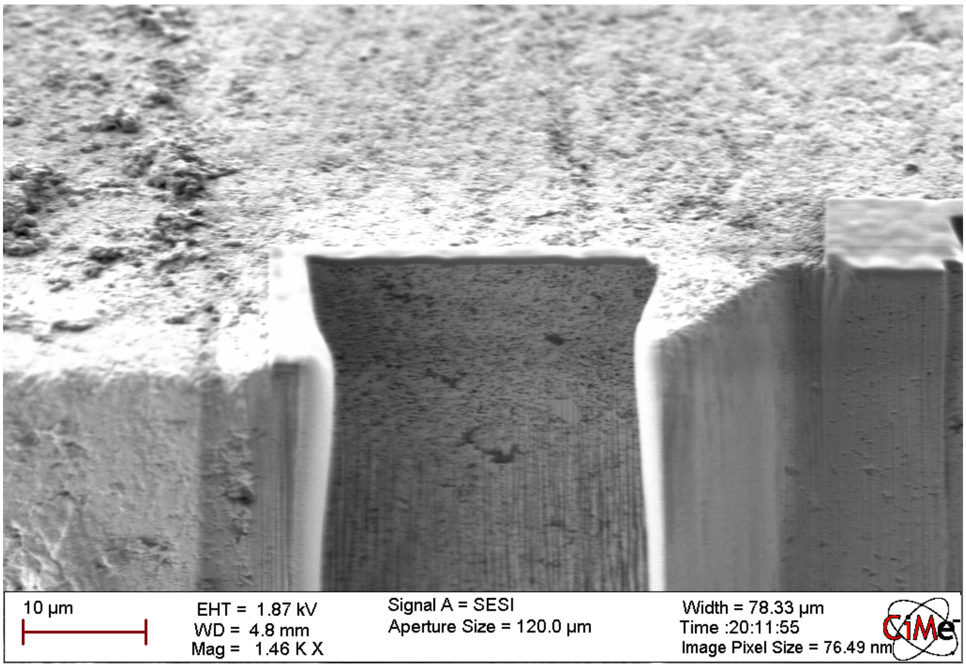
SE





Solid Oxide Fuel Cell cathode

P. Tanasini, LENI



The right conditions

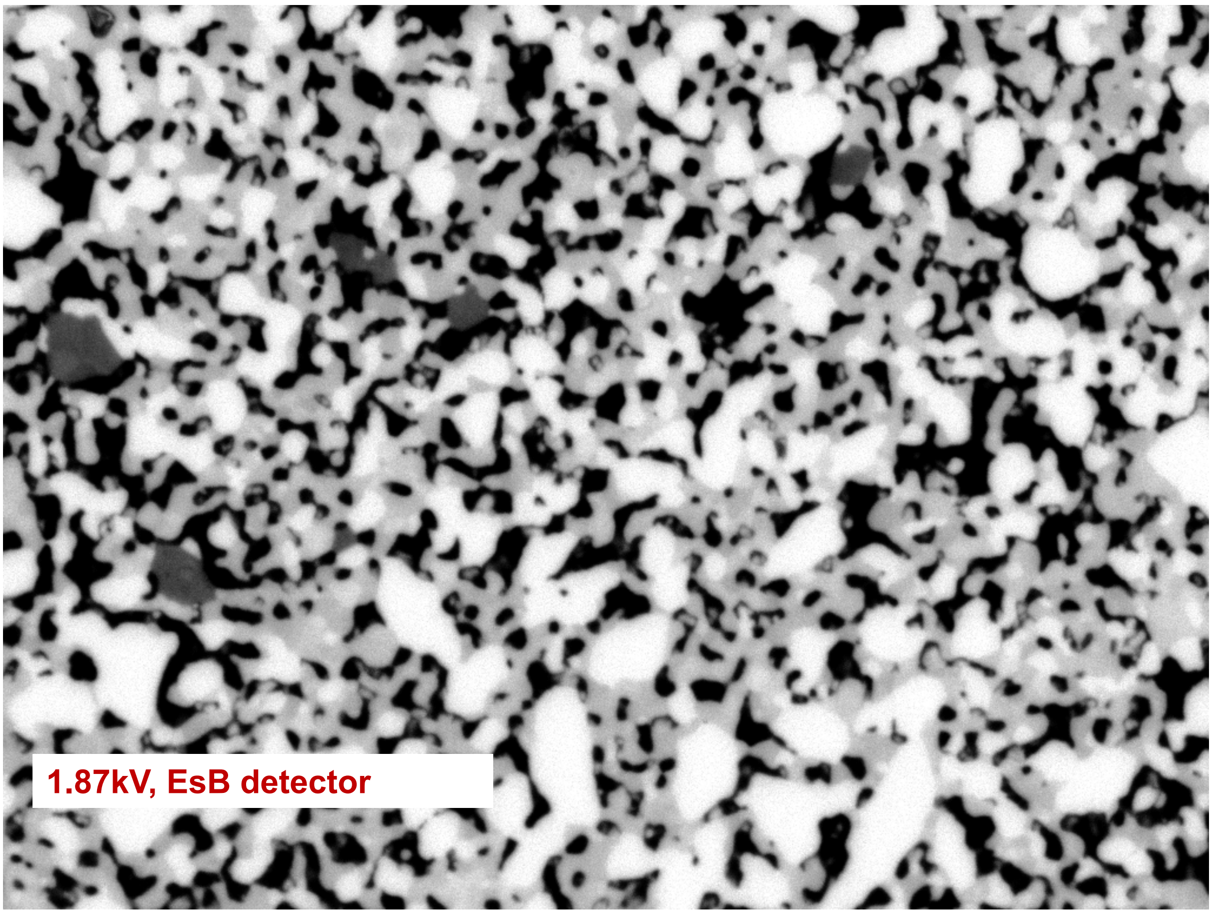
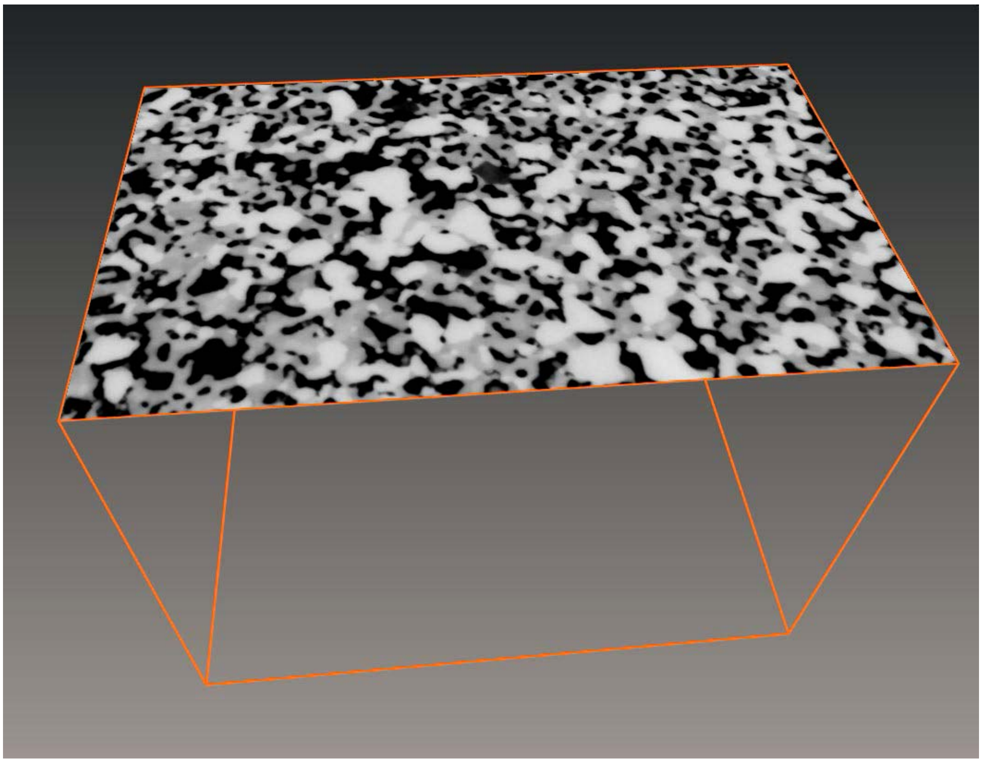
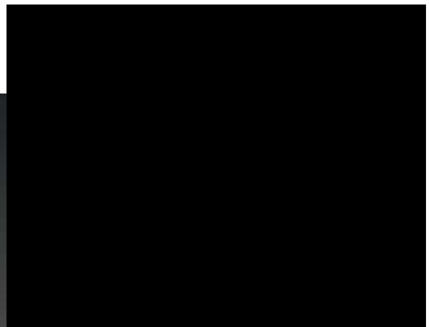
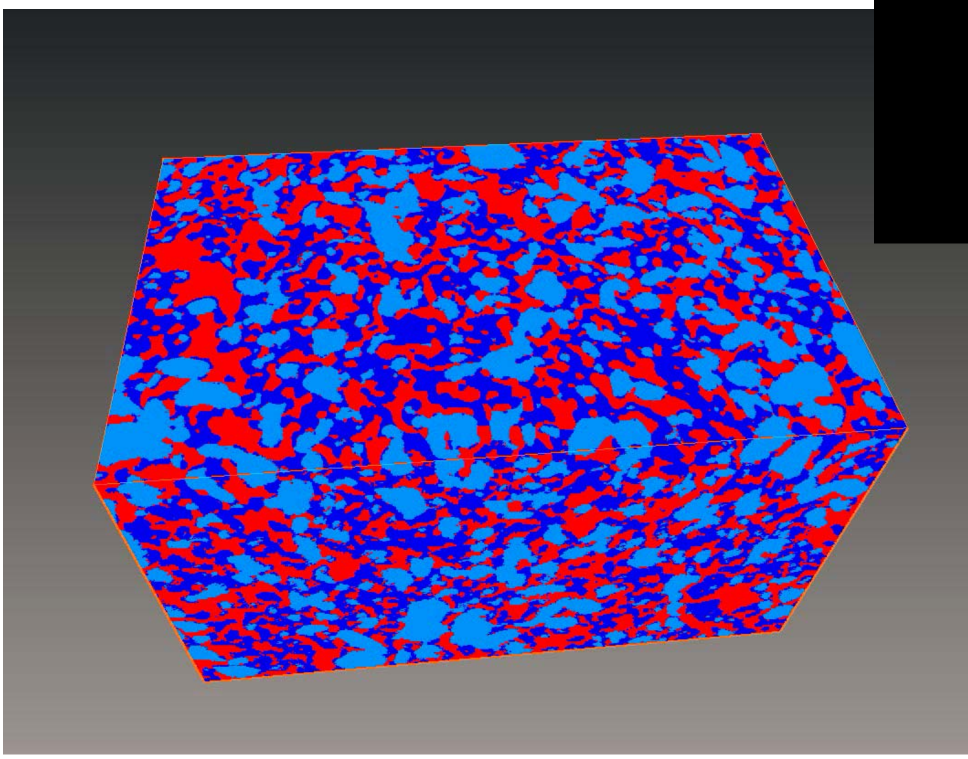


Image:
2048x1536
10nm pixel size

2200 images
36hours



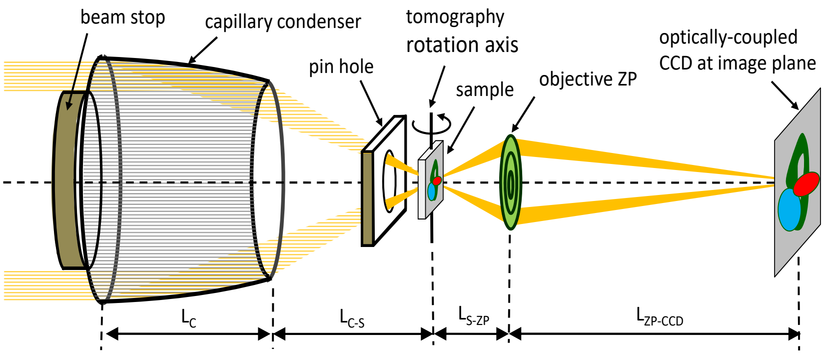




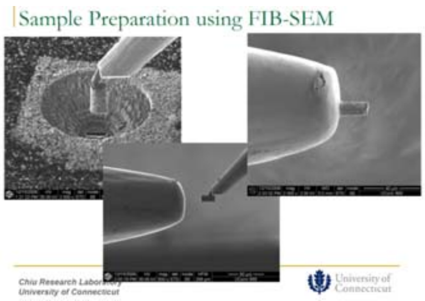
Segmentation and analysis

Comparison with Transmission X-ray Microscopy (TXM)

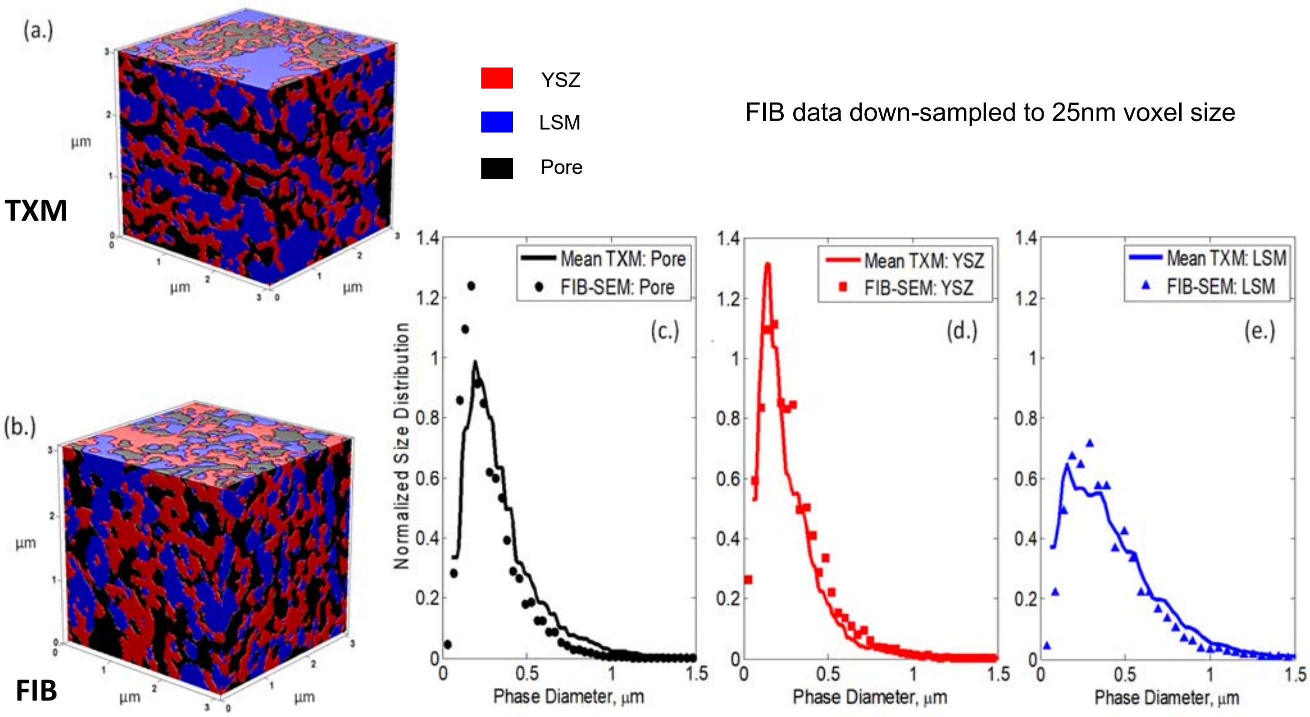
Joy C. Andrews, Yijin Liu, and Piero Pianetta
Stanford Synchrotron Radiation Lightsource
Stanford Linear Accelerator Center
Yong S. Chu
National Synchrotron Light Source II
Brookhaven National Laboratory



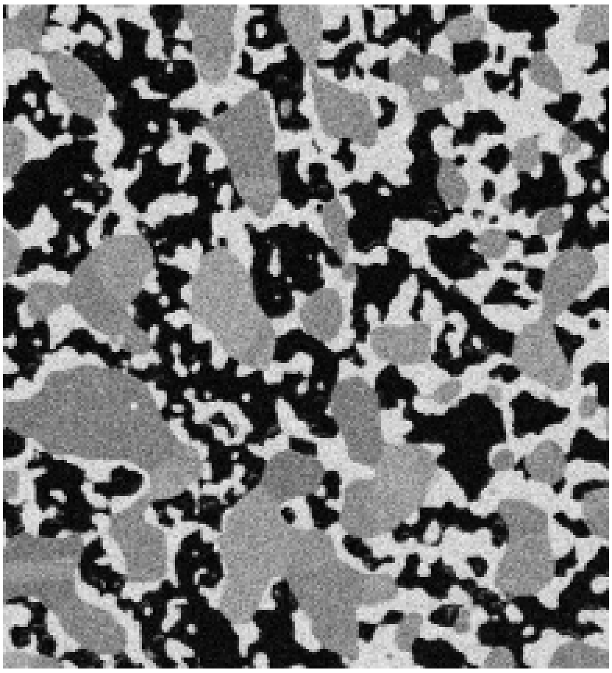
George J. Nelson, William M. Harris, Jeffrey J. Lombardo, John R. Izzo, Jr., and Wilson K. S. Chiu*

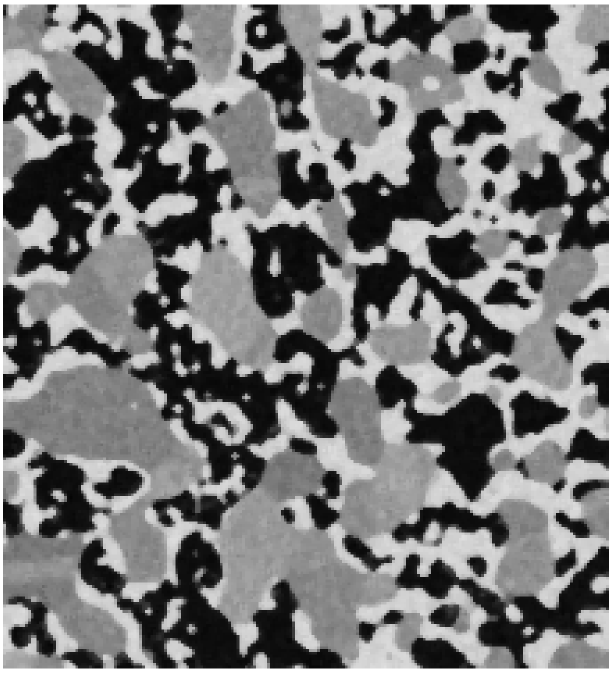


FIB data down-sampled to 25nm voxel size

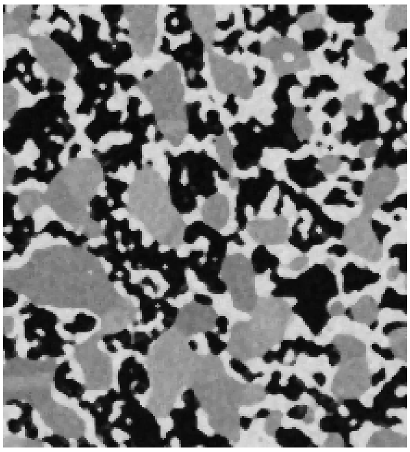


The segmentation dilemma

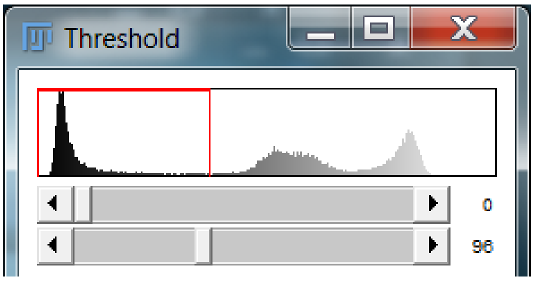


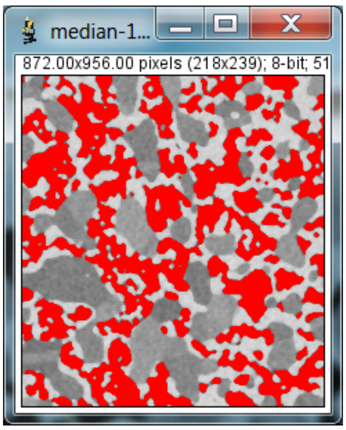


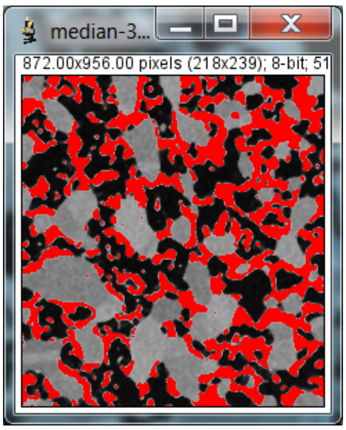
Median filter

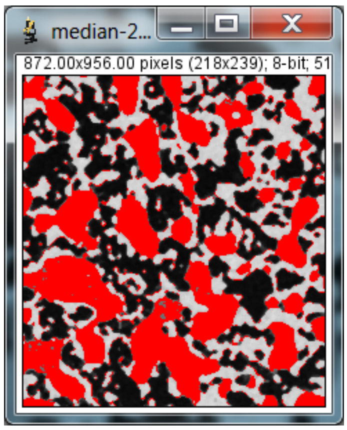


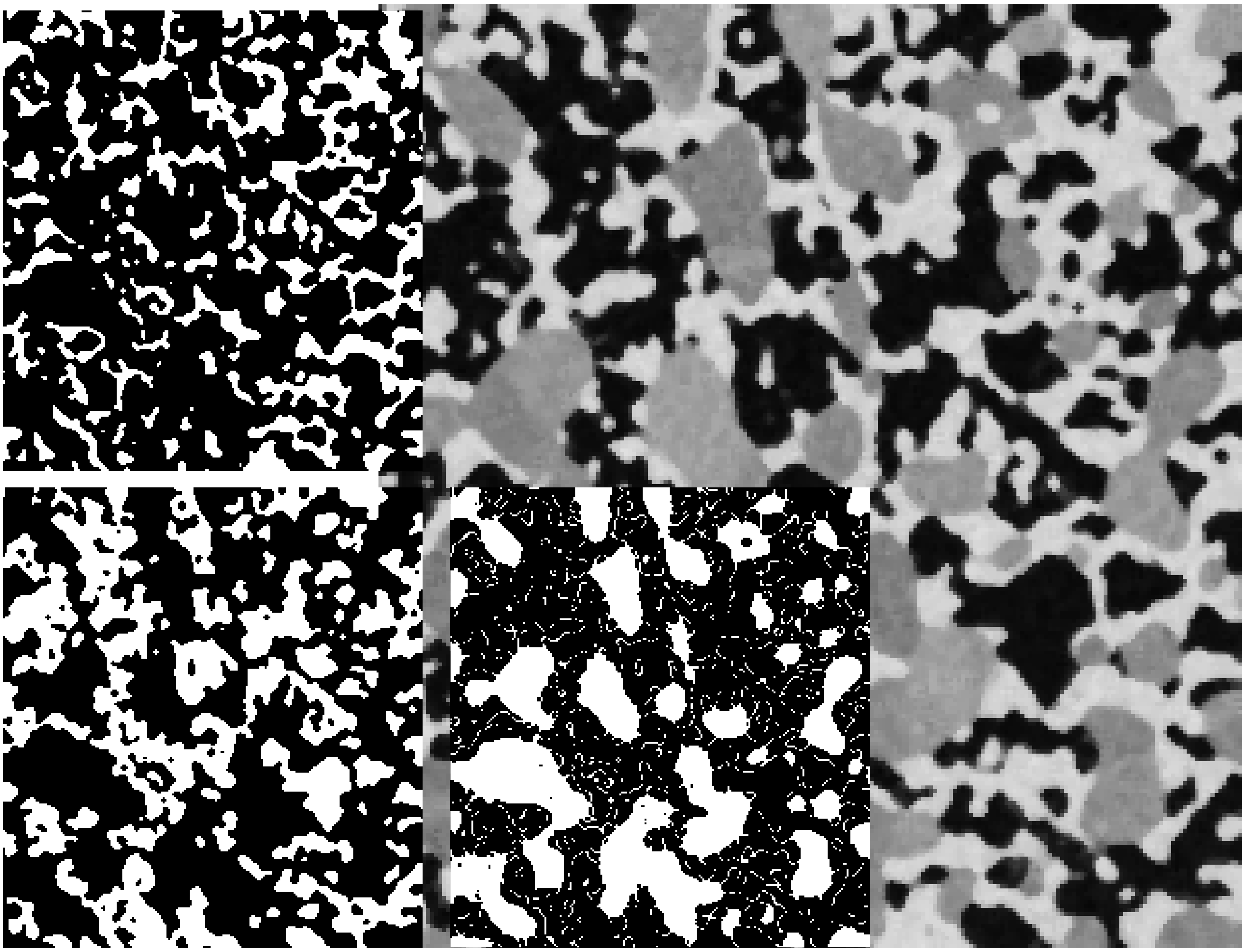
thresholding



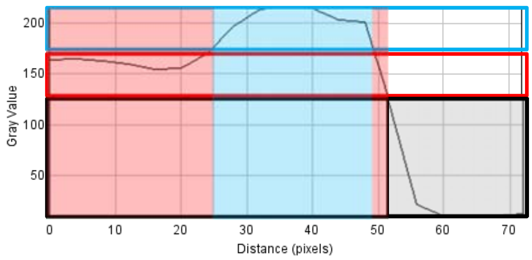


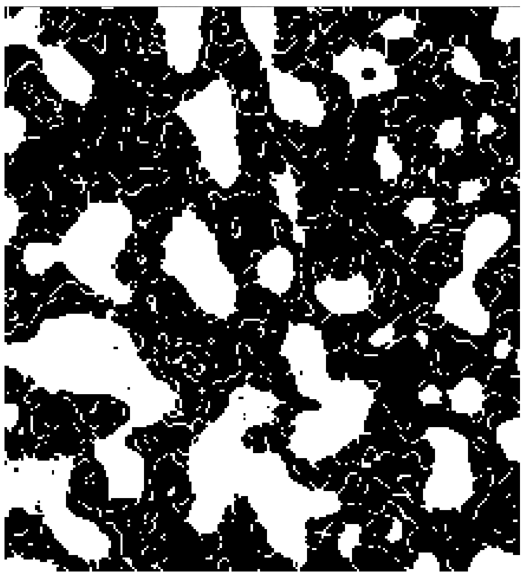


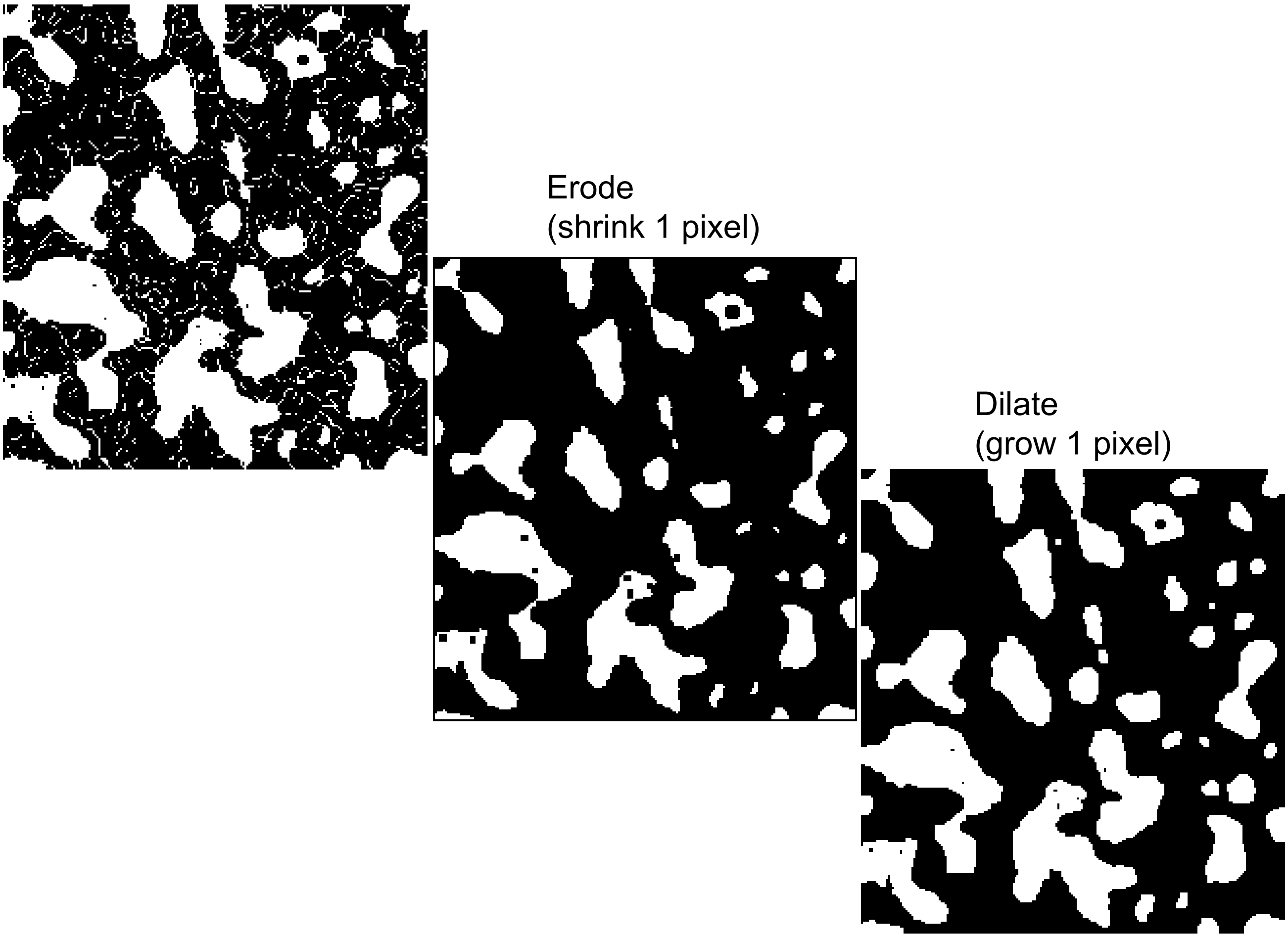




The gradient problem…







Segmentation tools for life-science

Problem:

- Structure (shape) determines function of objects

Single cell on substrate

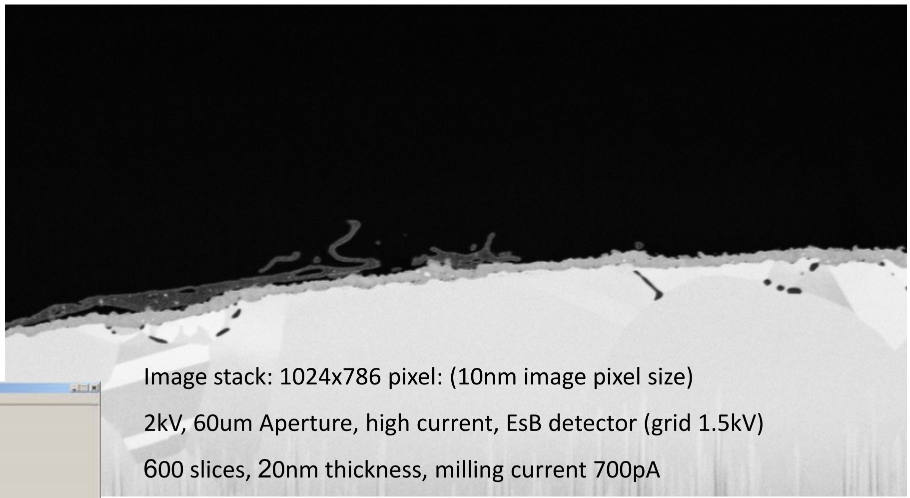
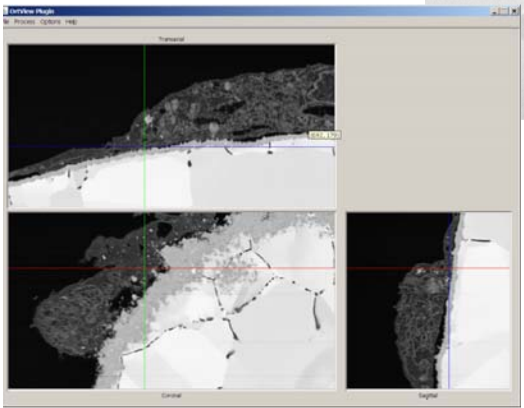


Image stack: 1024x786 pixel: (10nm image pixel size)

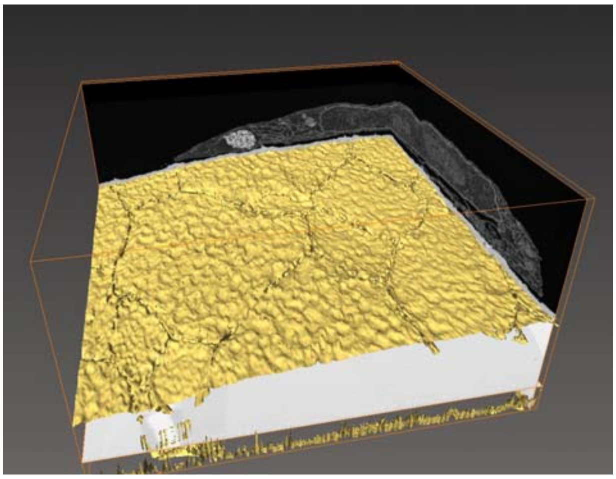
2kV, 60um Aperture, high current, EsB detector (grid 1.5kV)

600 slices, 20nm thickness, milling current 700pA

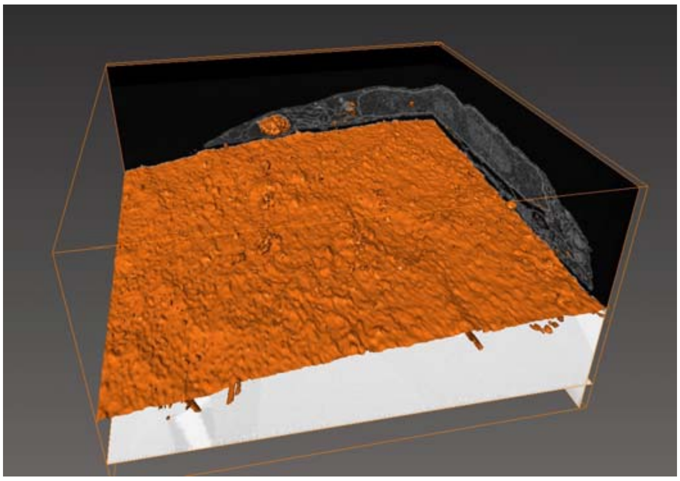


Segmentation based on grey levels

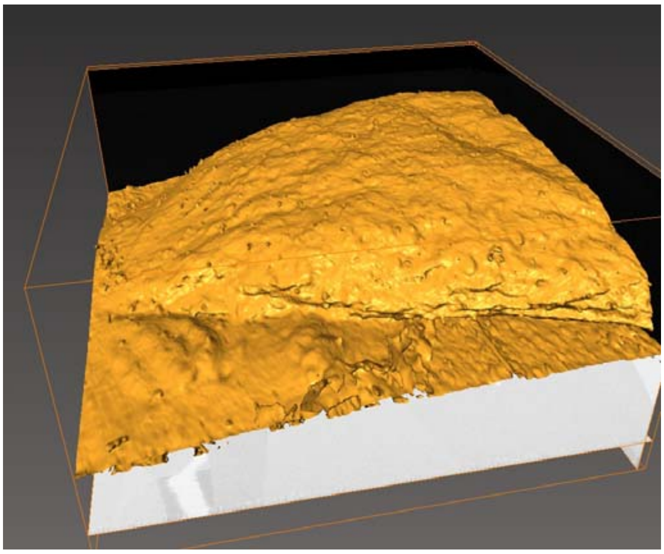
Medical steel



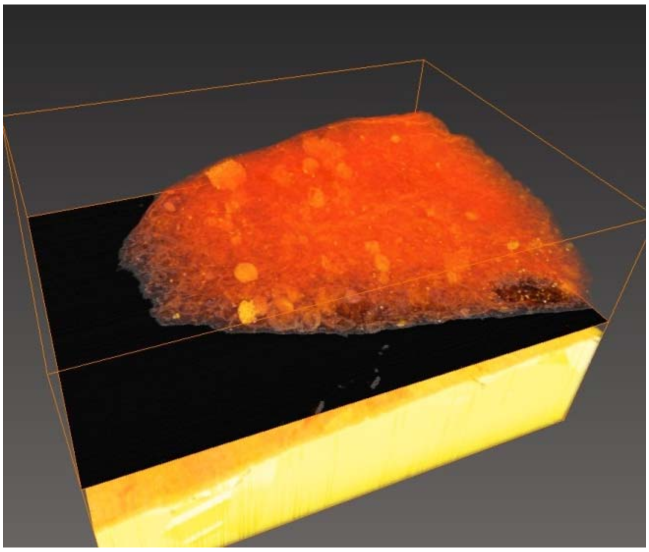
Ceramic coating: TiO_2

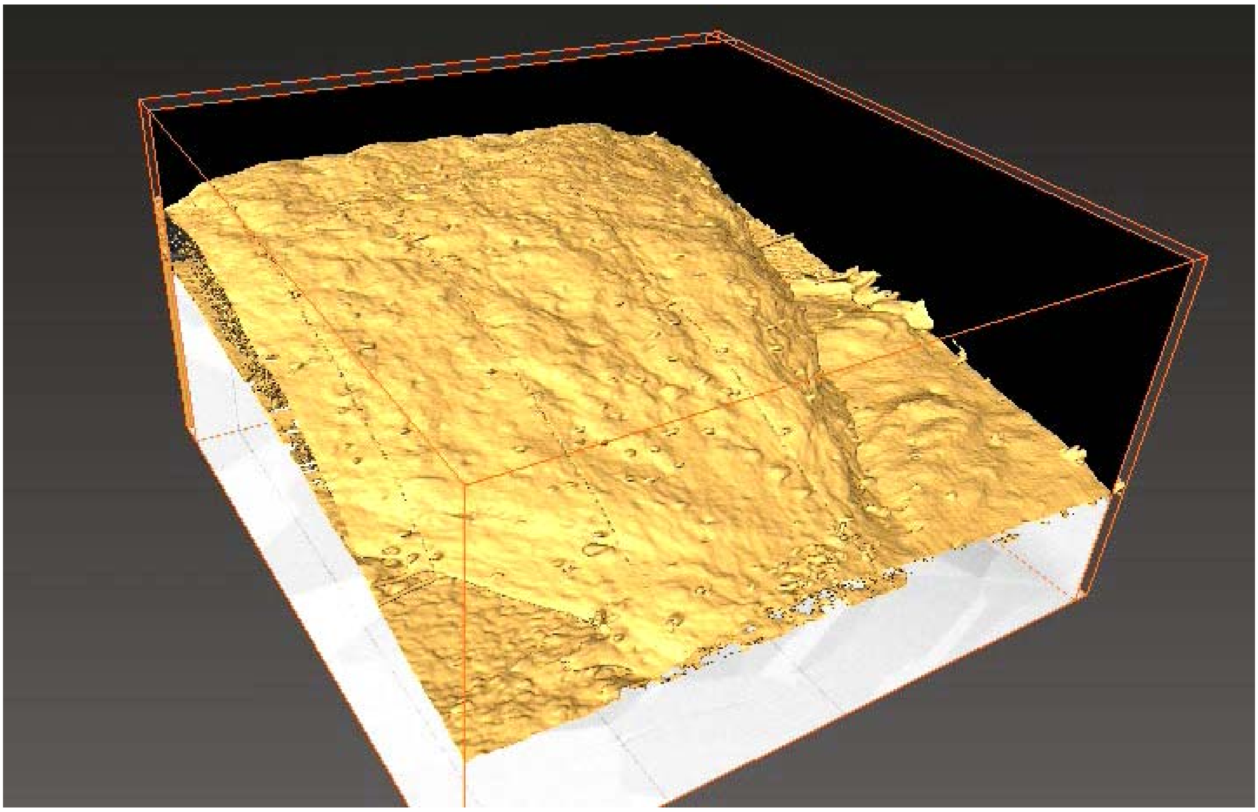


Cell outer membrane



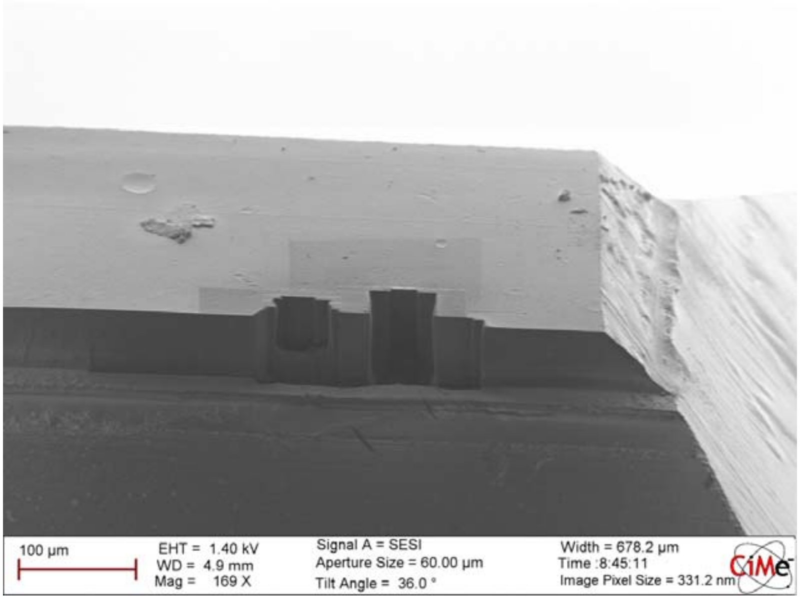
and more…

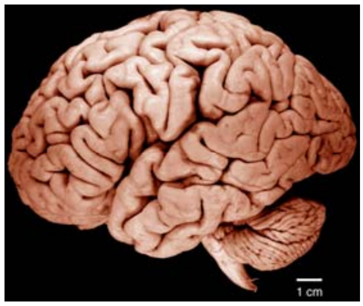


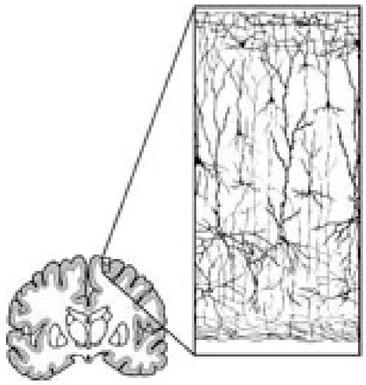


A little bit more complex….

brain tissue, resin embedded

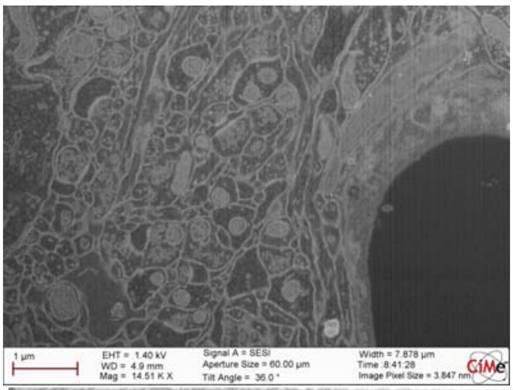


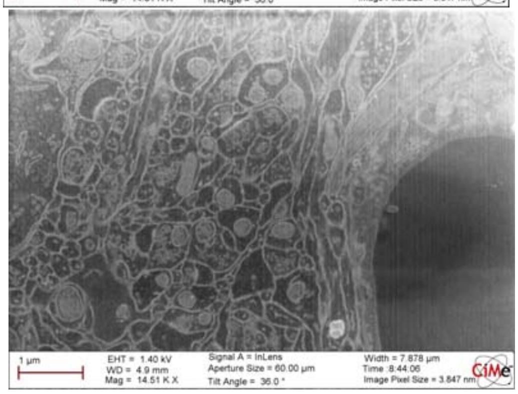


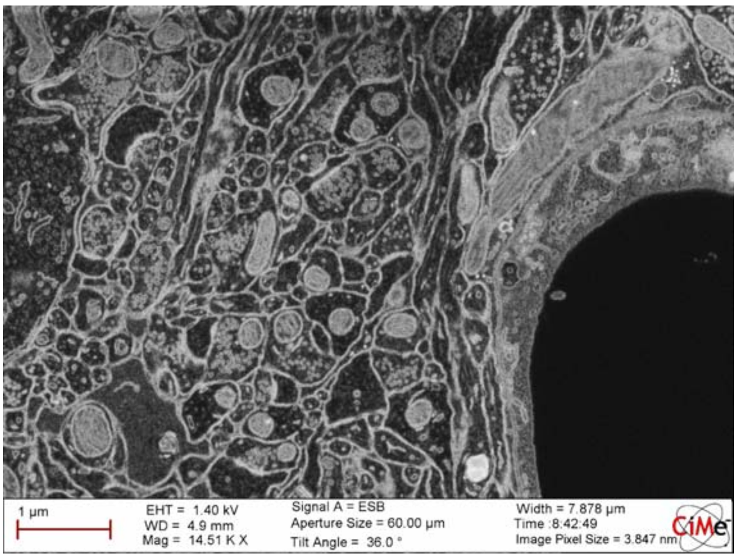


Which detector…?

In-chamber SE (Everhard-Thornley)
in-Lens SE
in-Lens BSE (energy selective)

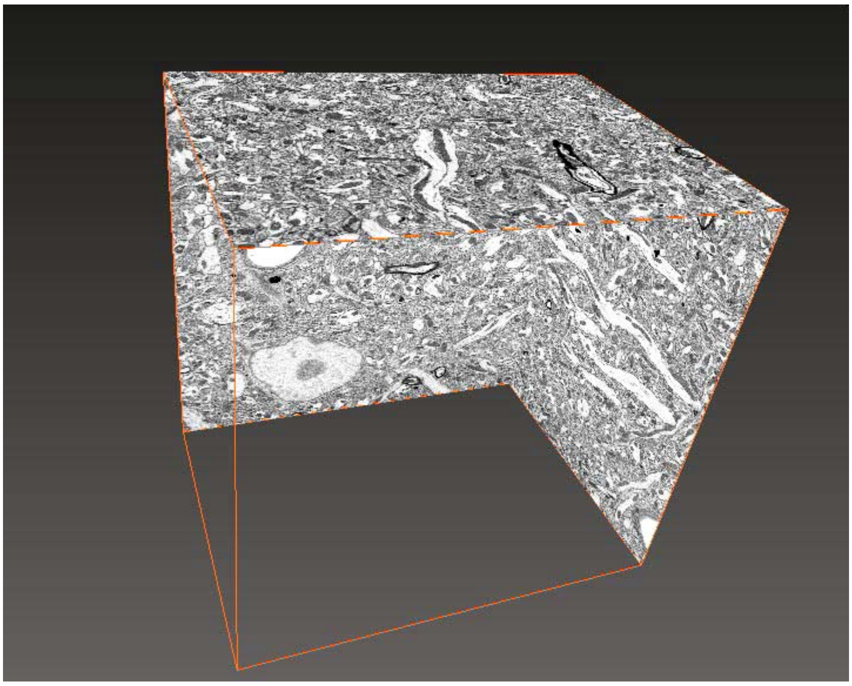


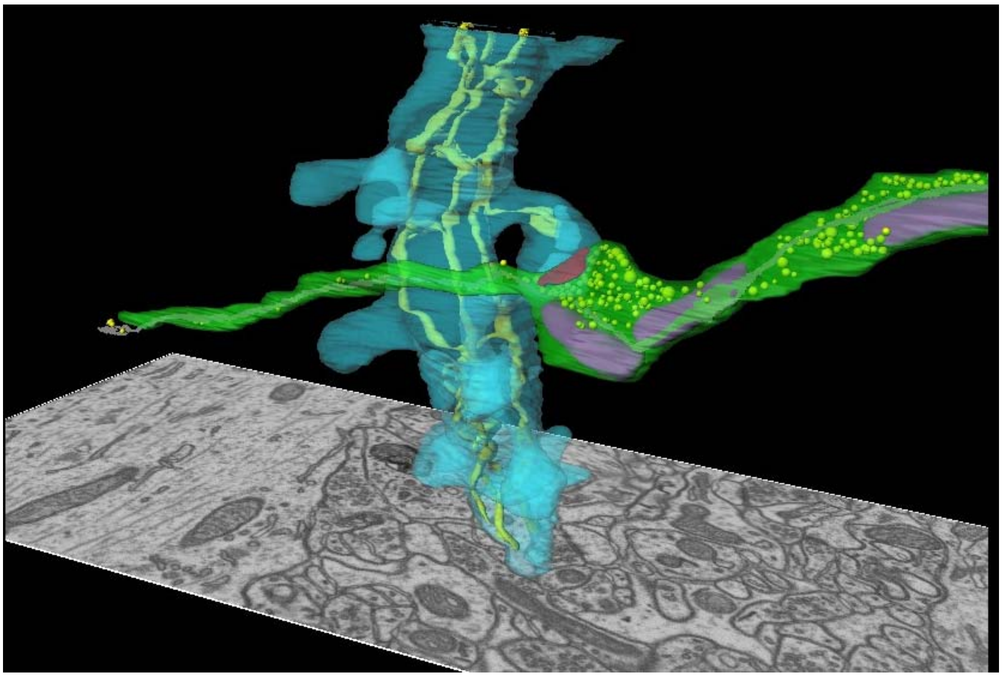




Big volumes

- Voxel: 7.5x7.5x7.5nm
- Image 3096x2304
- 3300 slices (48hours)
- 23x17x24 um
- 9700um^3
- ~7000 synapses
- 23Gb data



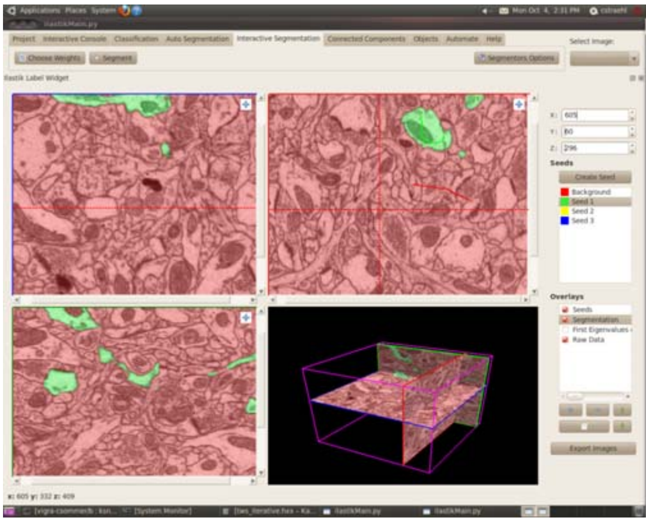


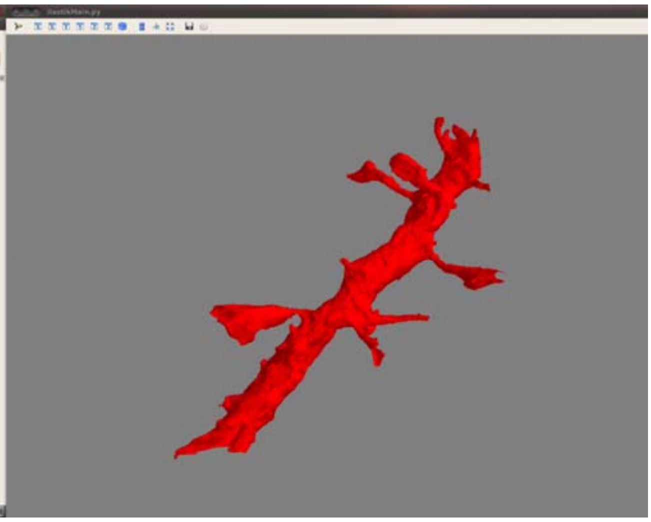
Reconstruction: Christel Genoud

2weeks of work

Automated segmentation of neuronal structures

Ilastik v0.5 - Fred Hamprecht, University of Heidelberg

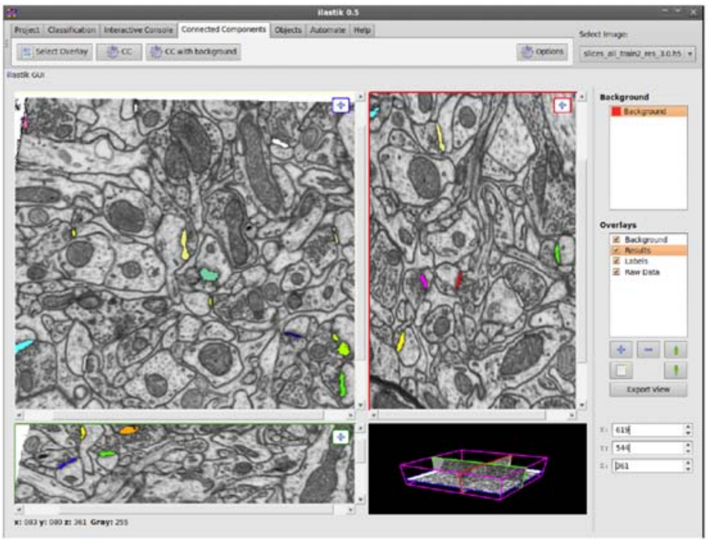


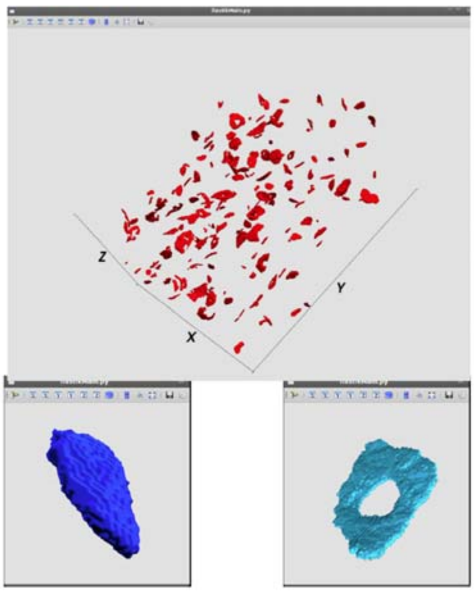


Automated segmentation of neuronal structures

Ilastik v0.5 - Fred Hamprecht, University of Heidelberg

Synapse recognition - Anna Kreshuk





FIB Nanotomography

in life science

- Specimen preparation (fixation, staining, dehydration, resin infiltration same as for BIO-TEM)
- Image contrast and resolution TEM quality
- Stable and reliable automated acquisition (less artifacts than ultra-microtomy)

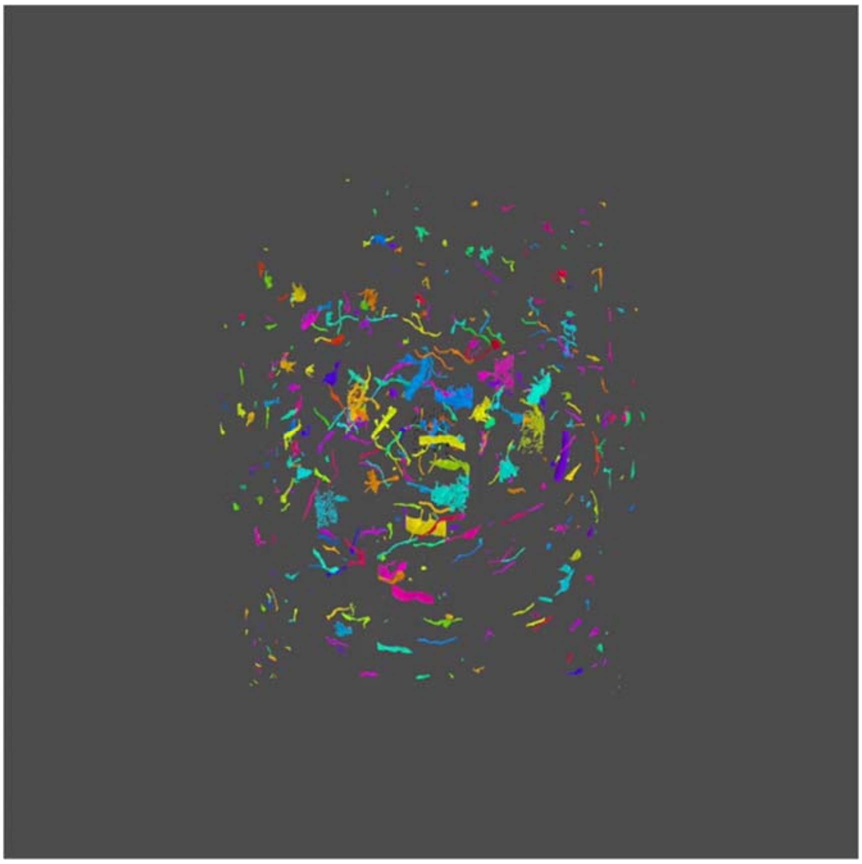


Image processing in 3D

- Start with a “good” image (low noise, equilibrated histogram, right detector)
- 2D processing (shading correction)
- Image alignment (registration)
- Cropping (subvolume)
- Noise reduction (2D or 3D)

- Segmentation (most important)
- Visualization (strong area for commercial software)