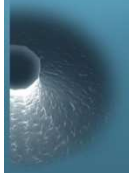
ECOLE D'ETE GN-MEBA

Microscopie Electronique à Balayage et Microanalyses



Analyse d'images, traitement des données 3D

Marco Cantoni

EPFL-CIME

Organisation :

Supports techniques :

Autres supports :

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

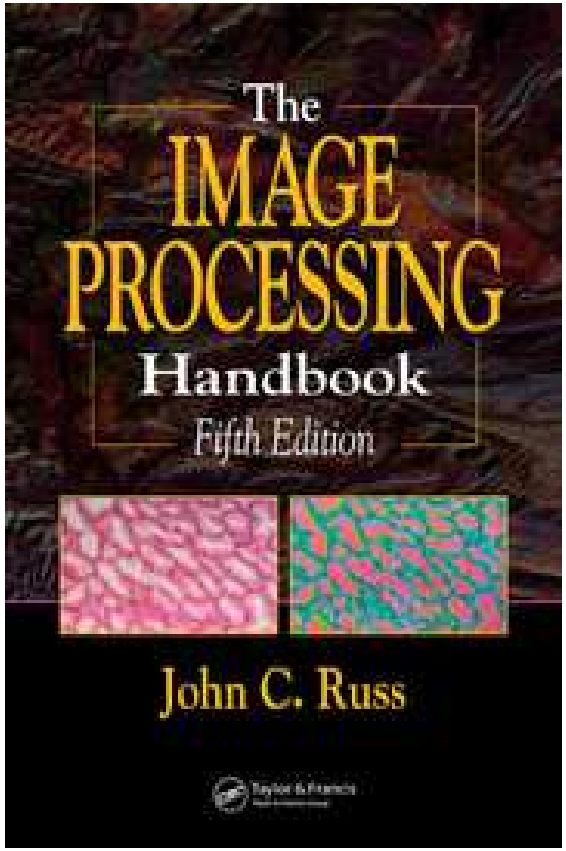
Image processing basics

"the bible"

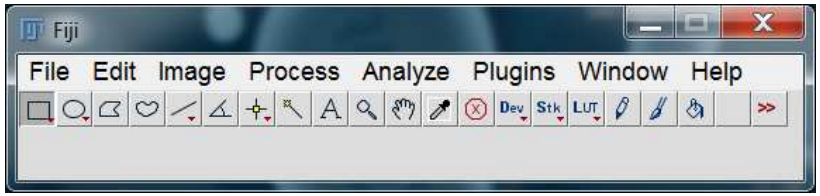
John C. Russ (2002)

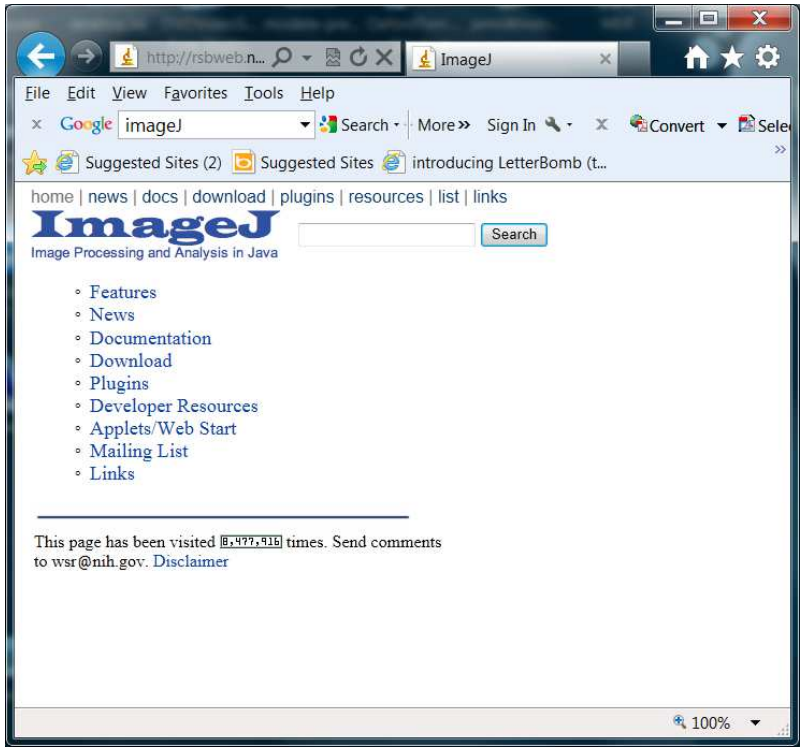
The Image Processing Handbook

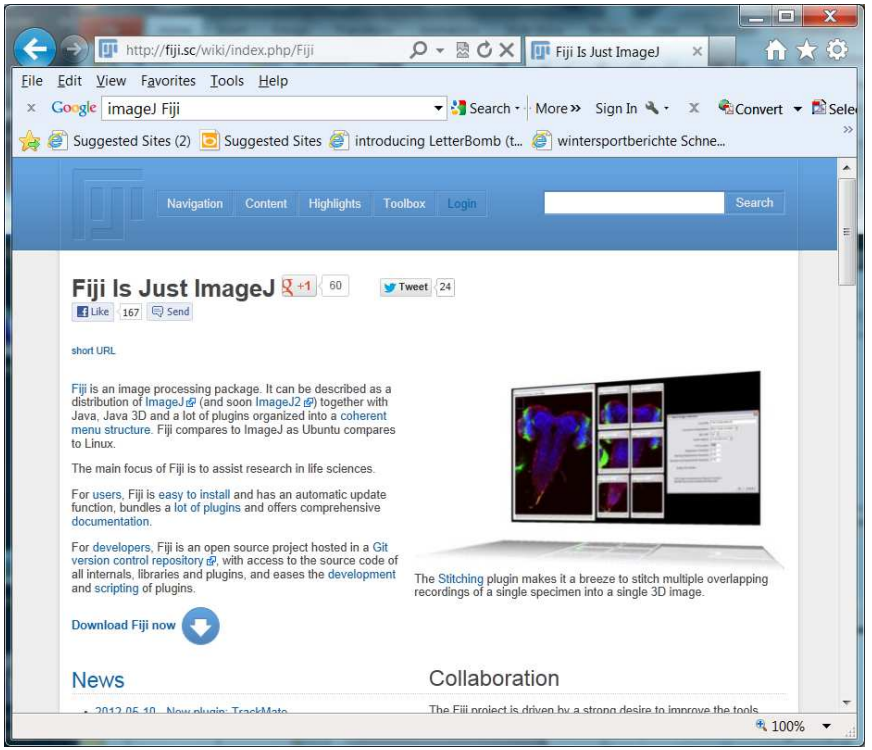
5th edition, CRC Press



The (free) tools



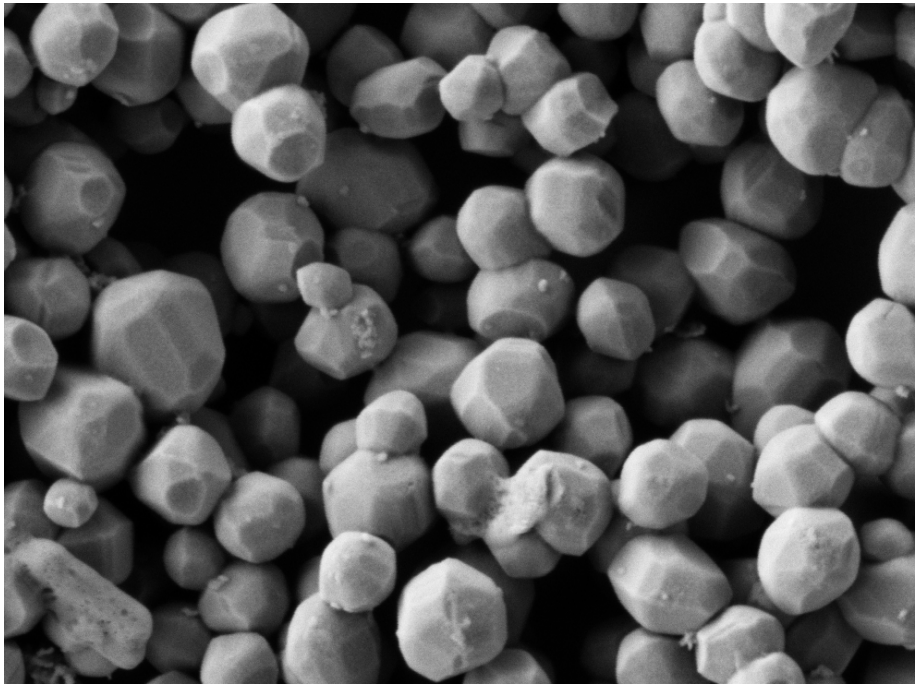


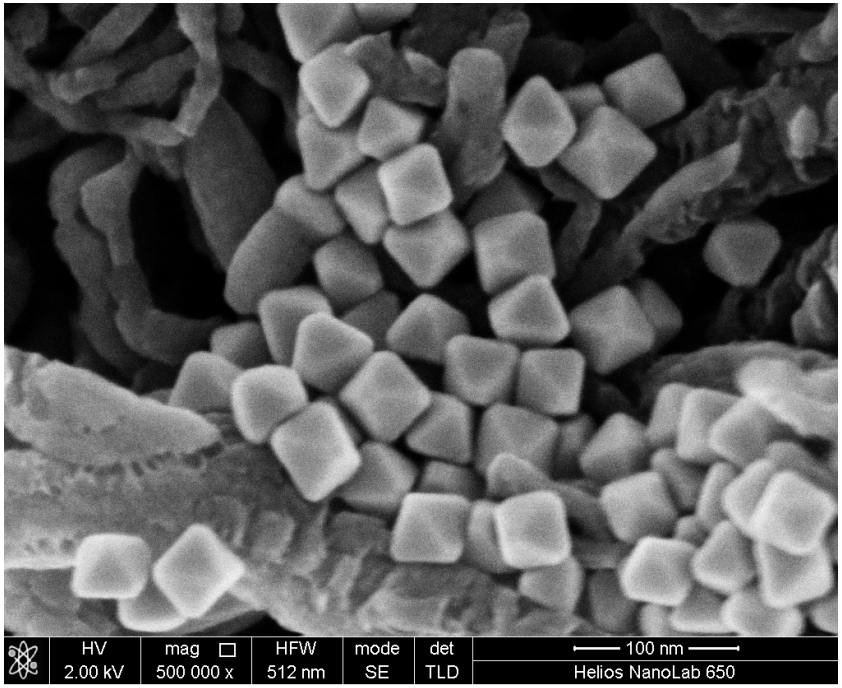


http://rsbweb.nih.gov/ij/

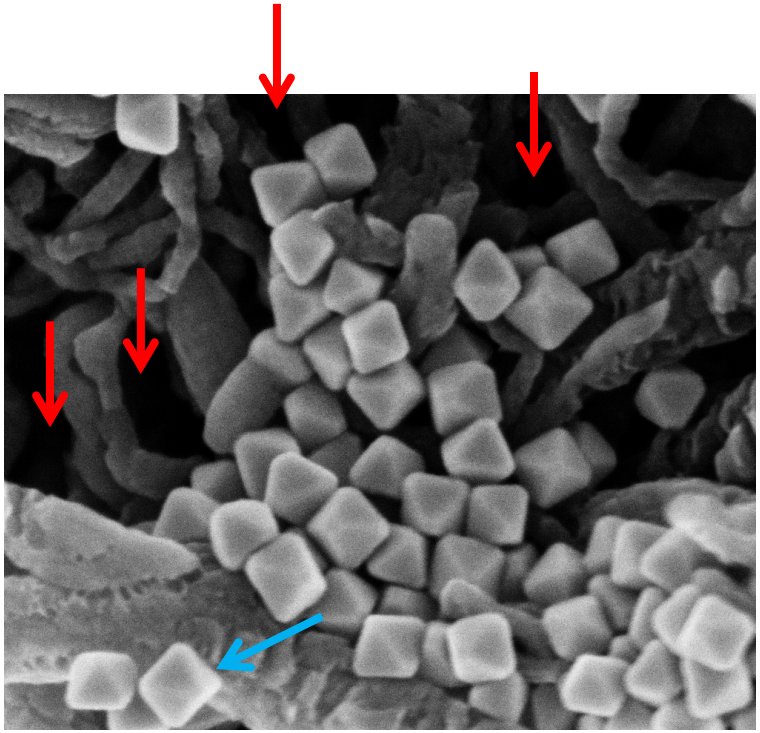
http://fiji.sc/wiki/index.php/Fiji

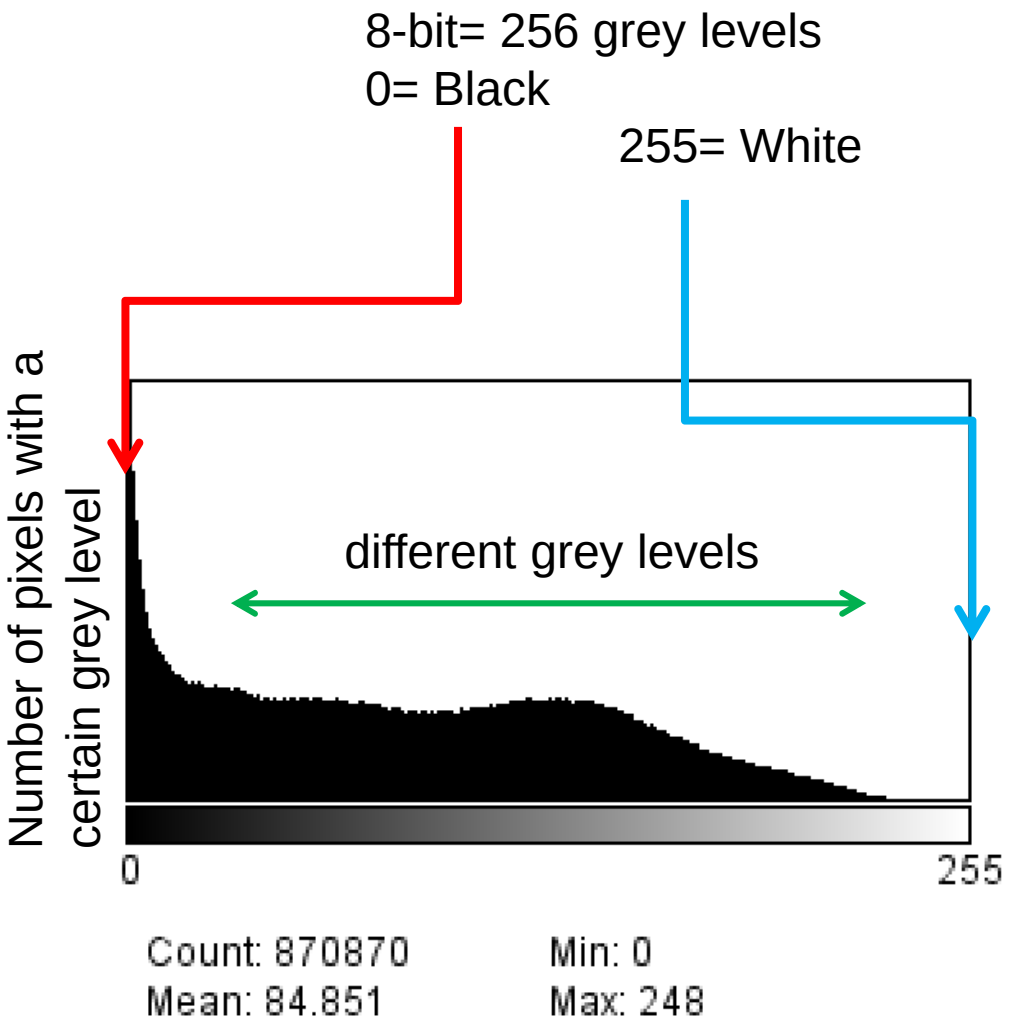
What is a good image..?

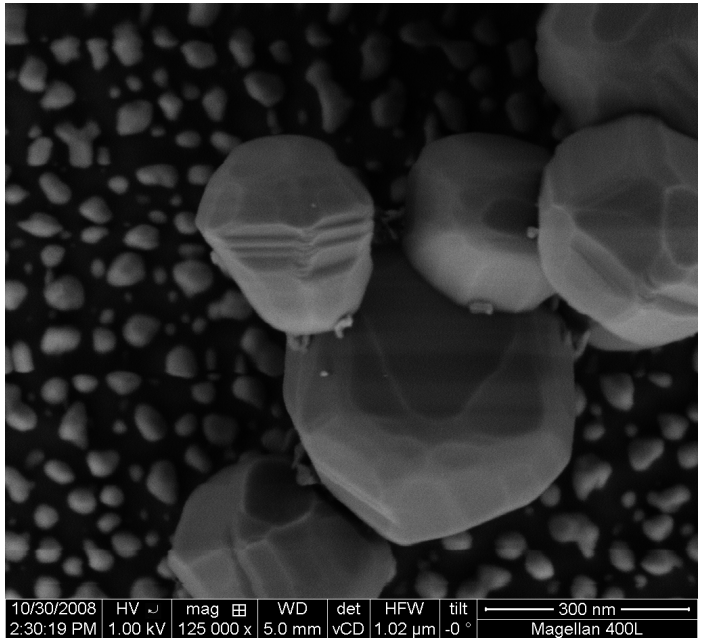




Histogram of 8-bit Tif image



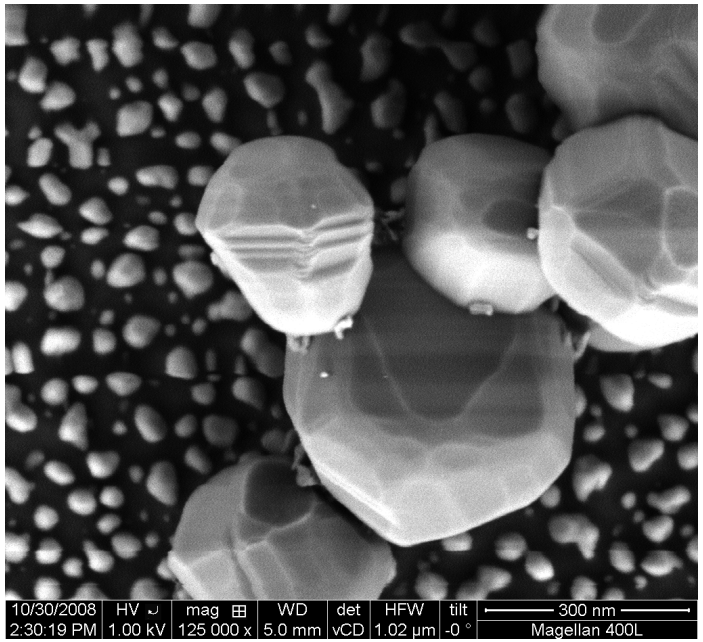


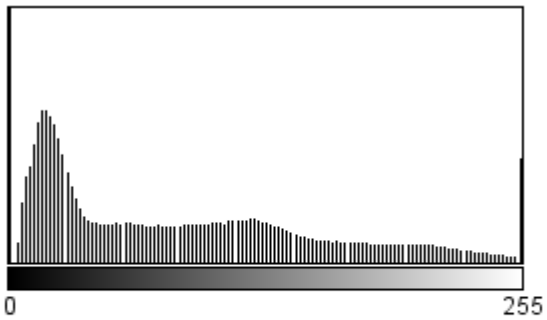


Count: 961536
Mean: 41.789
StdDev: 39.195

Min: 0
Max: 255
Mode: 0 (49648)

A little too dark



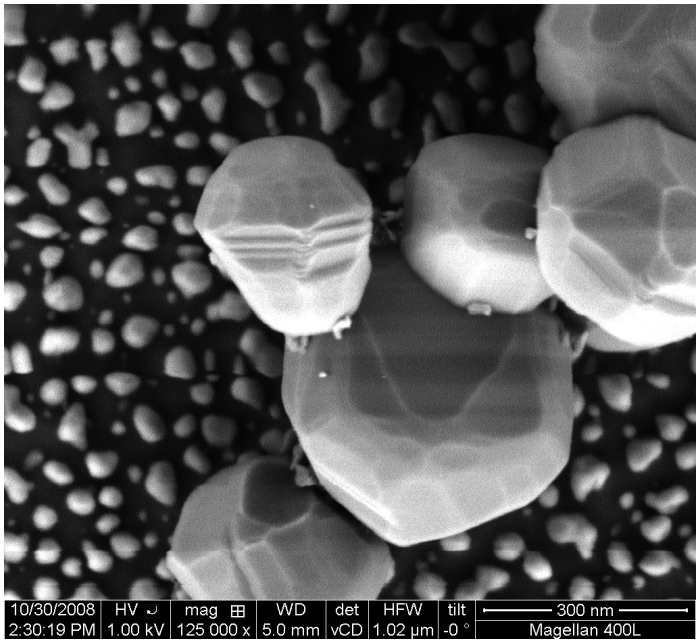


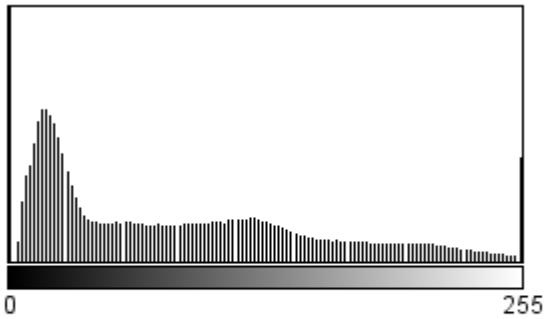
Count: 961536
Mean: 83.493
StdDev: 70.754

Min: 0
Max: 255
Mode: 0 (49648)

Adjusted contrast/brightness (after recording)

missing grey levels !





Count: 961536
Mean: 83.493
StdDev: 70.754

Min: 0
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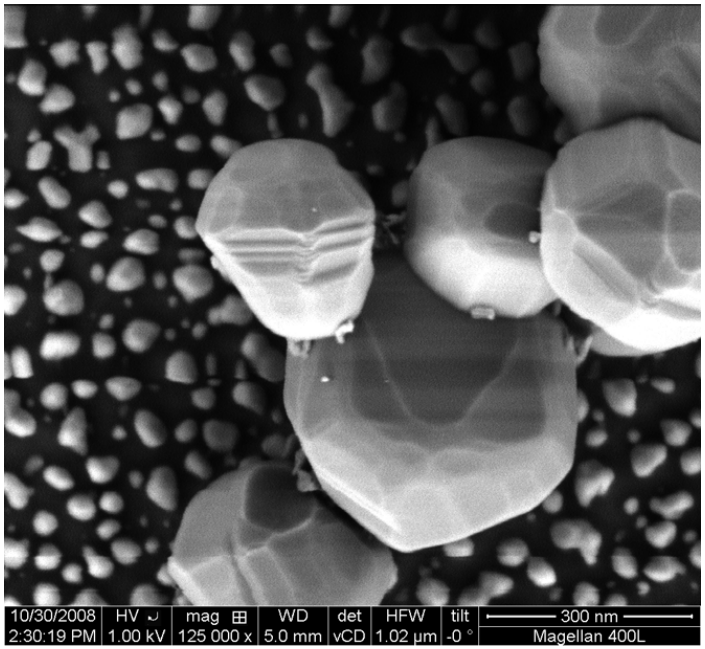
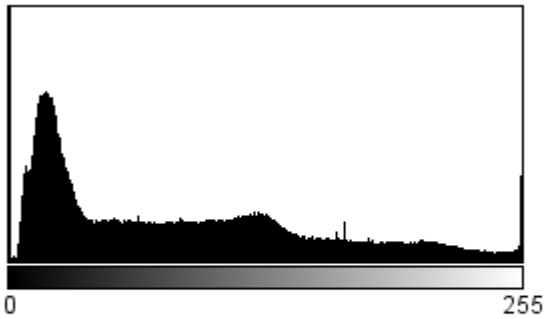
Mode: 0 (49648)

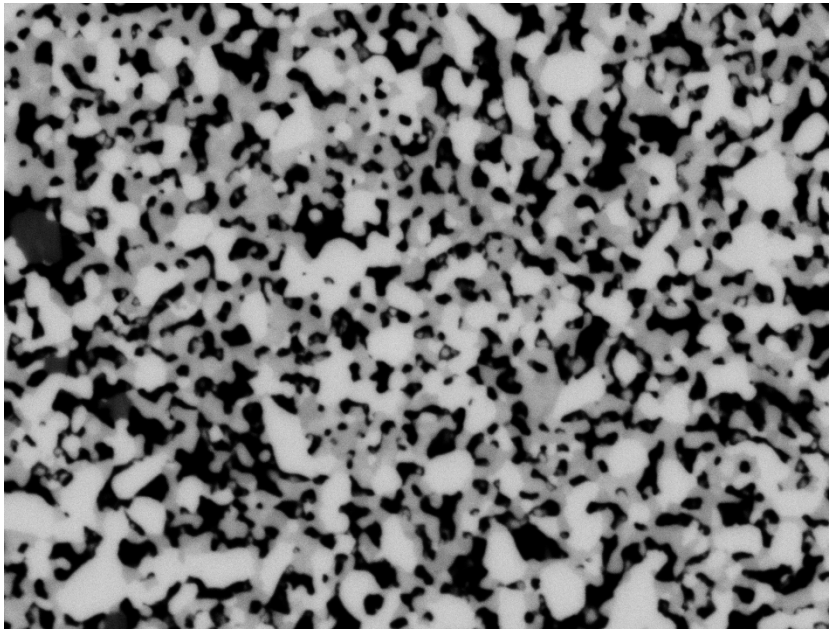


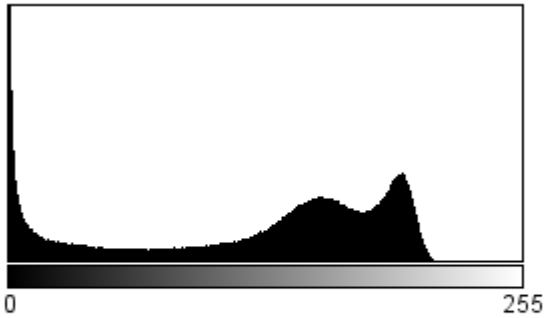


Count: 408258
Mean: 83.562
StdDev: 69.314

Min: 0
Max: 255
Mode: 0 (16913)

After resampling the image (inventing pixels)





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Mean: 116.526
StdDev: 70.839

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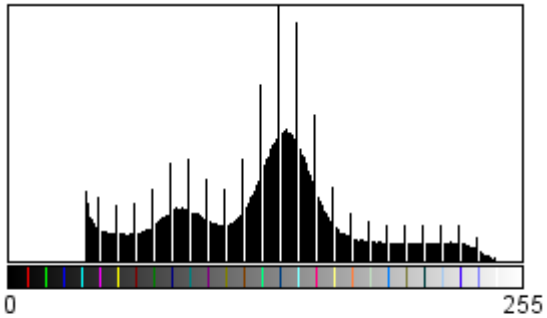
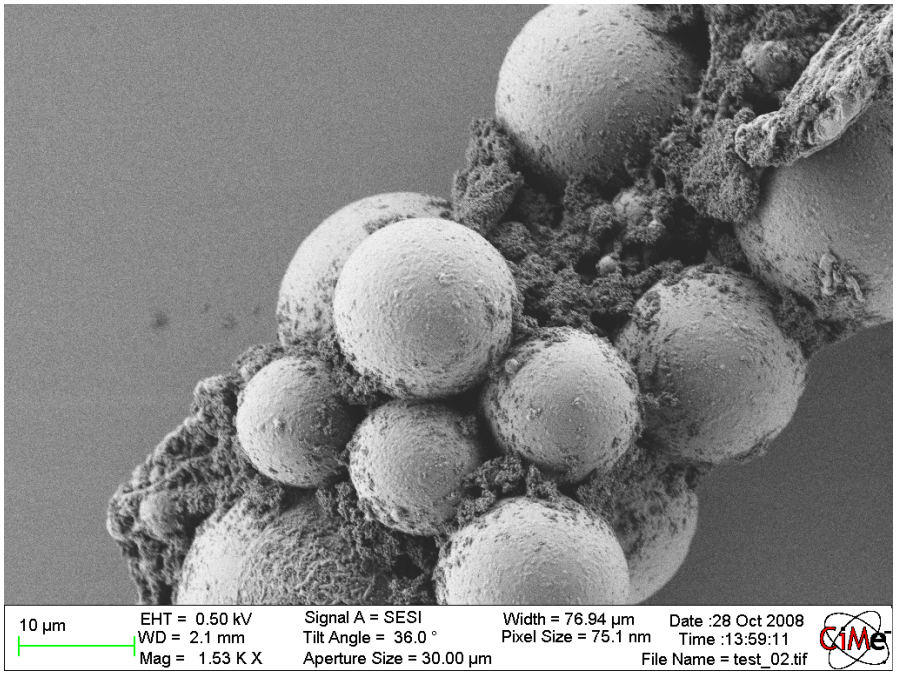
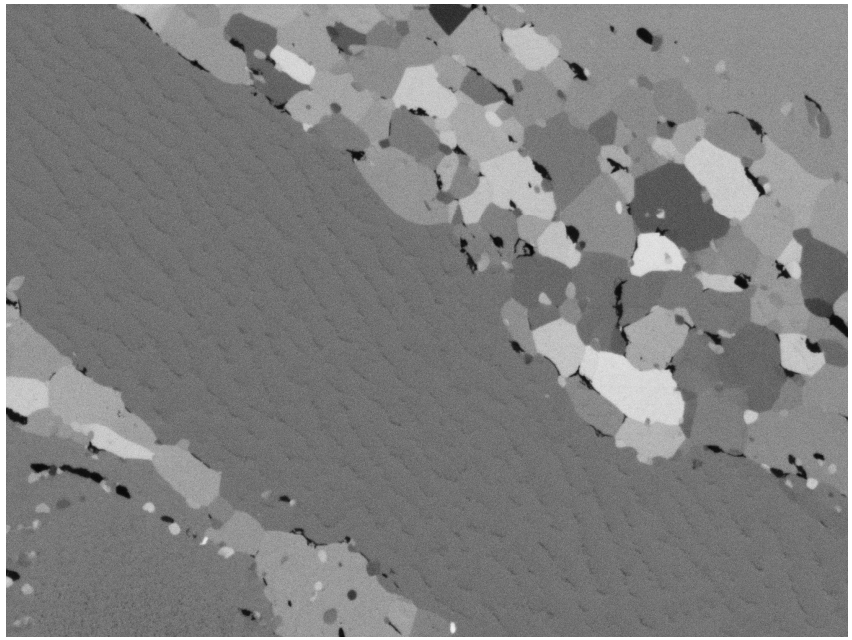
Mode: 0 (61194)

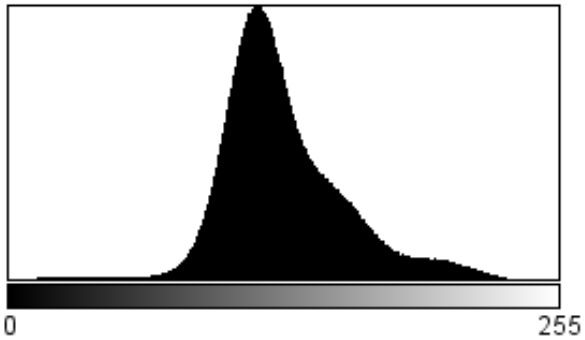


Count: 674674
Mean: 127.019
StdDev: 45.457

Min: 37
Max: 252
Mode: 134 (19589)

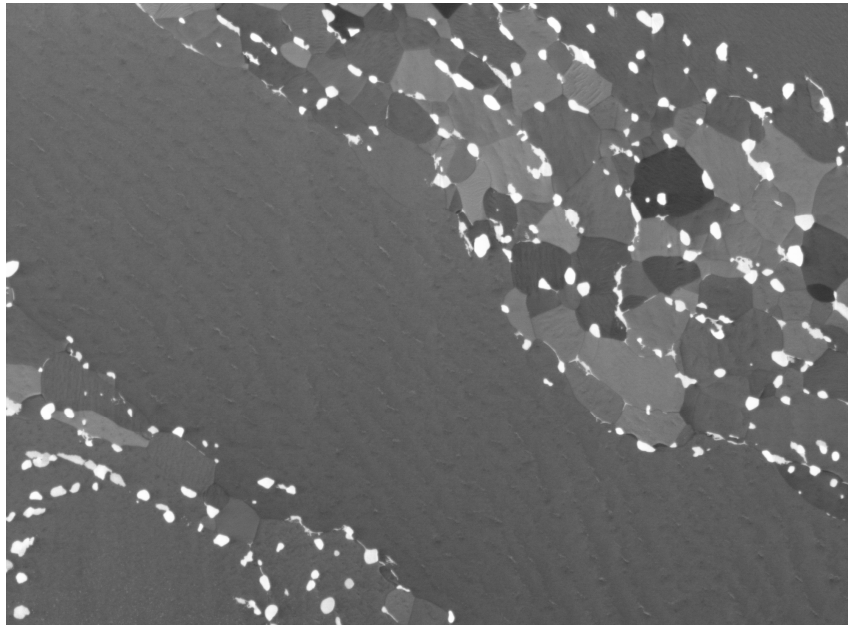


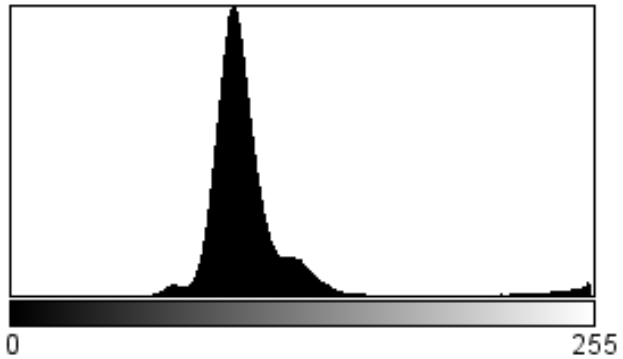




Count: 2946168
Mean: 127.193
StdDev: 29.568

Min: 1
Max: 251
Mode: 115 (57613)

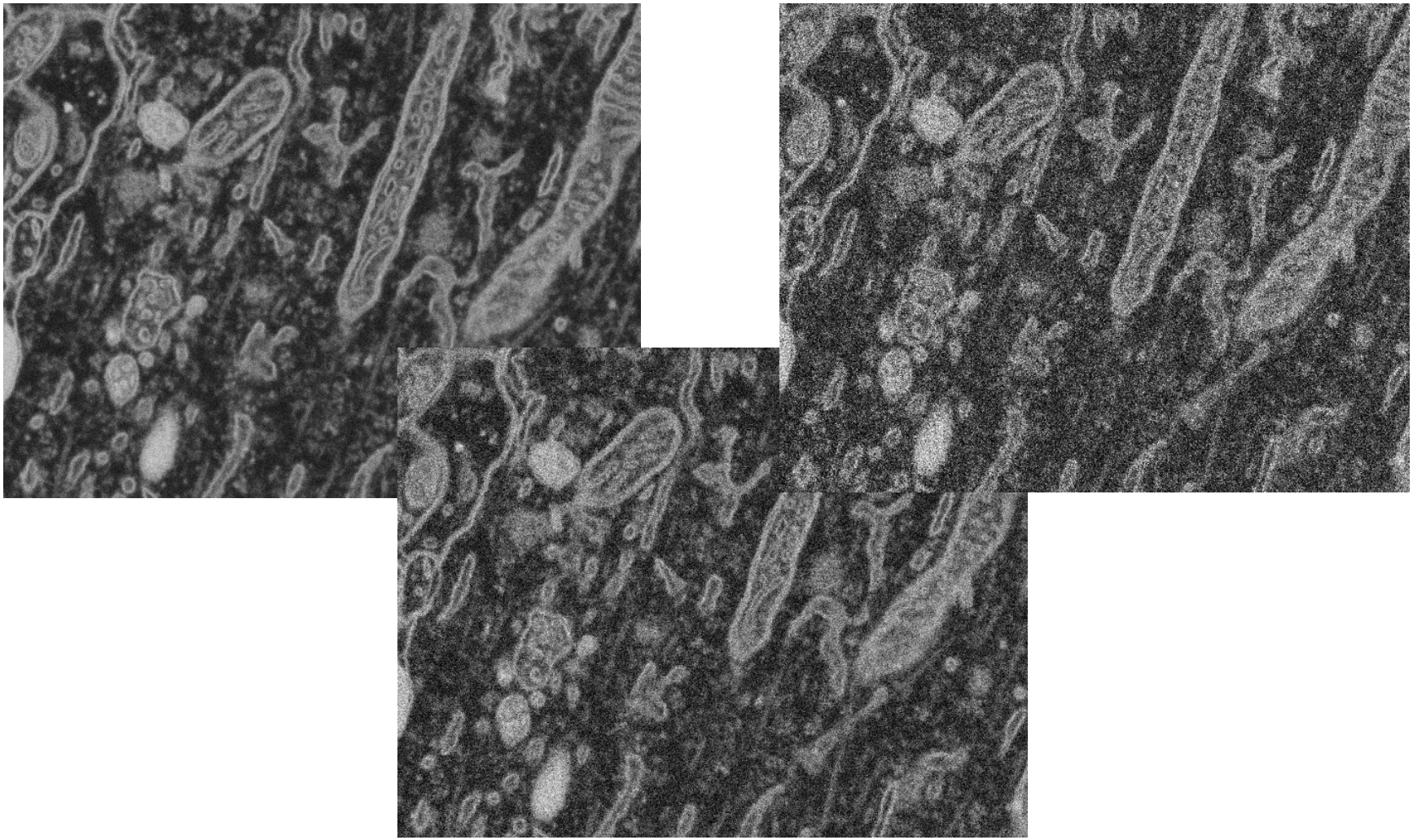


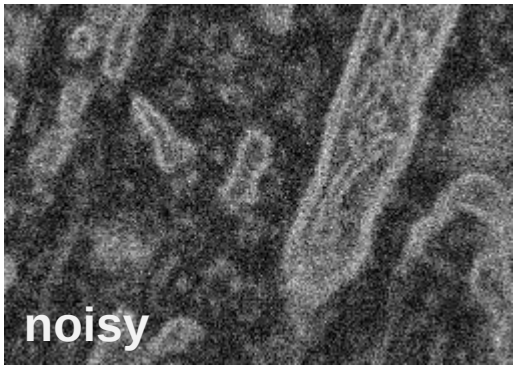


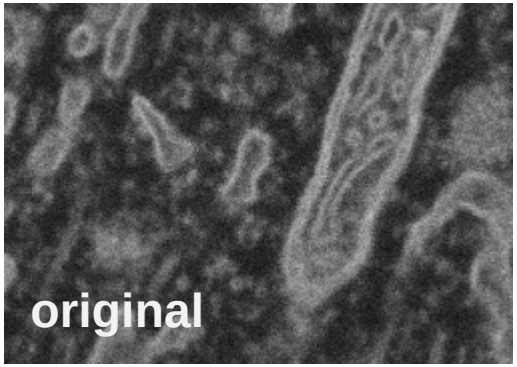
Count: 3145728
Mean: 106.215
StdDev: 28.778

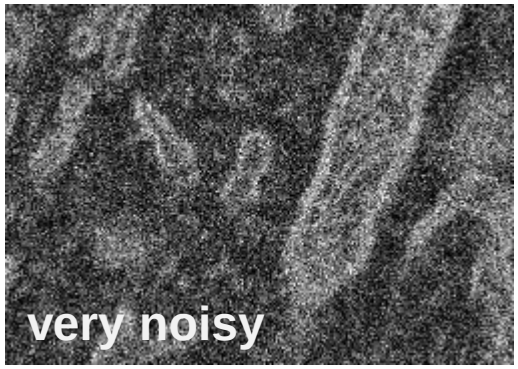
Min: 14
Max: 254
Mode: 97 (133319)

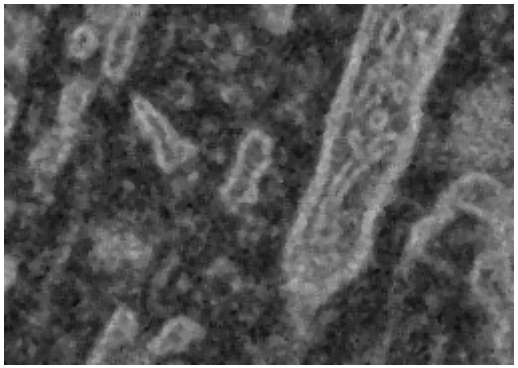
dealing with noise



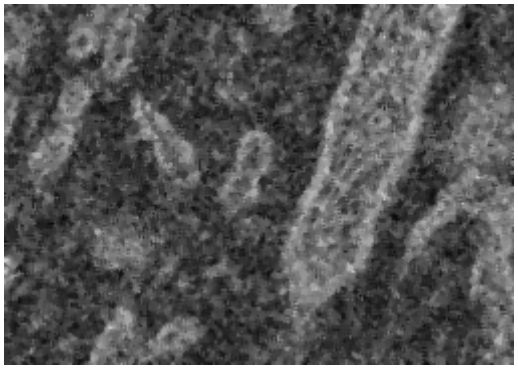


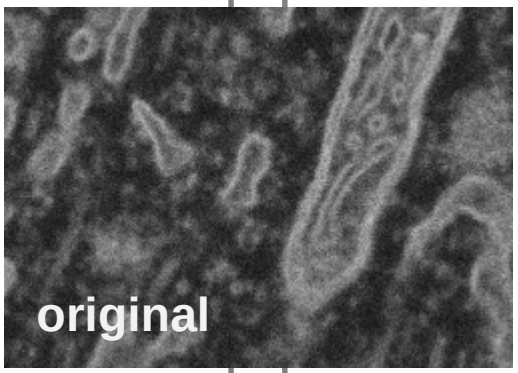


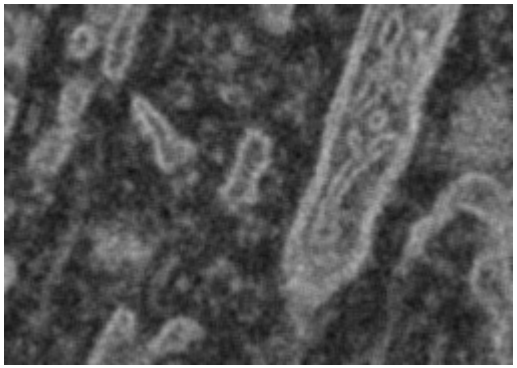




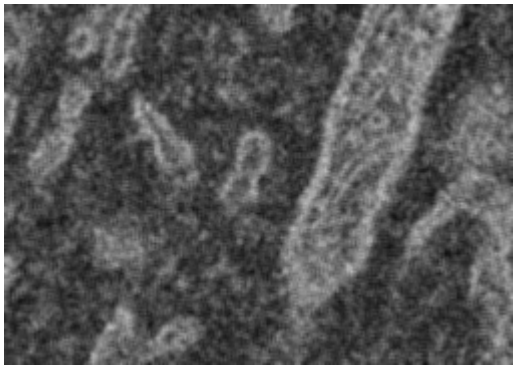
median filter







smooth



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

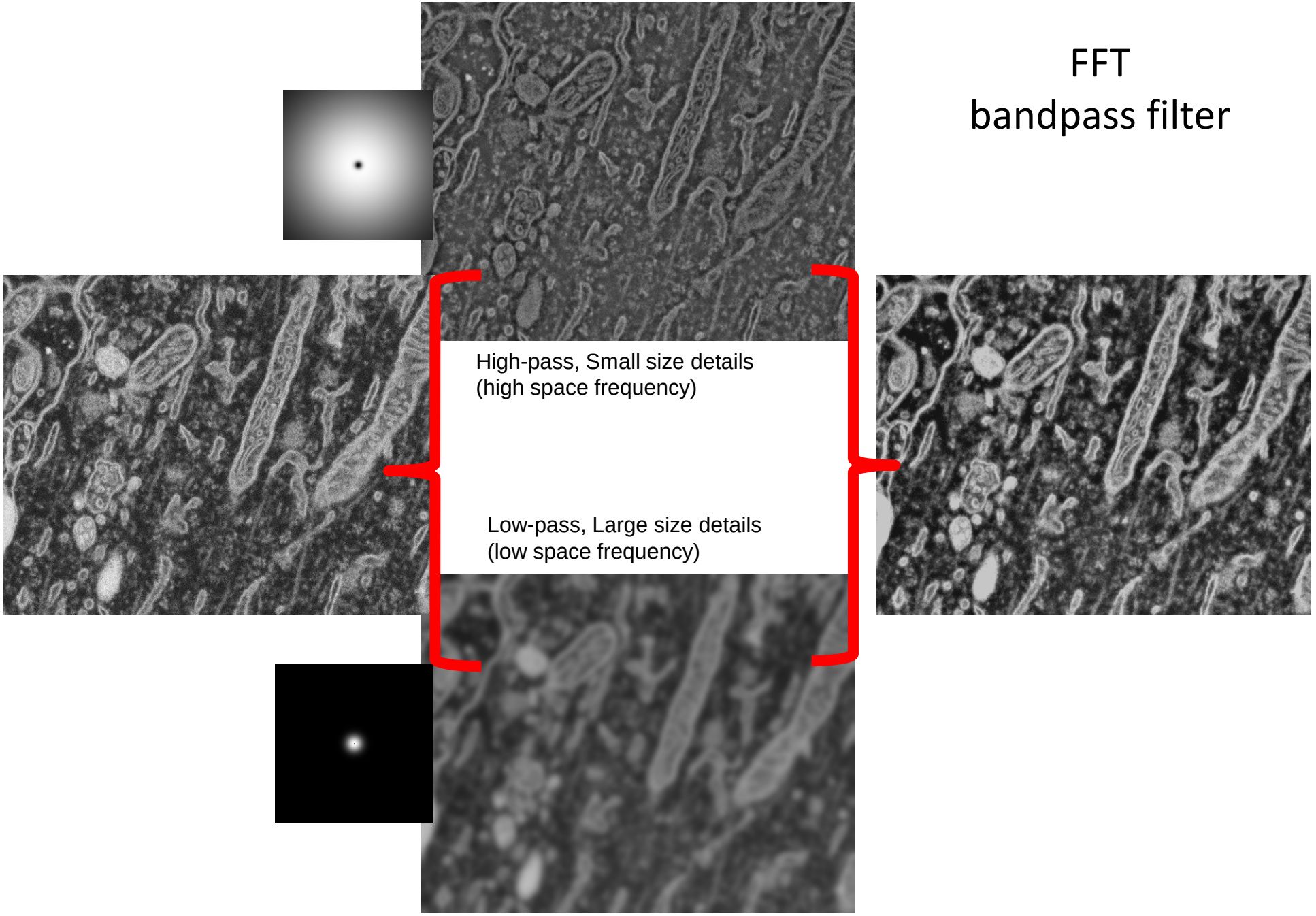
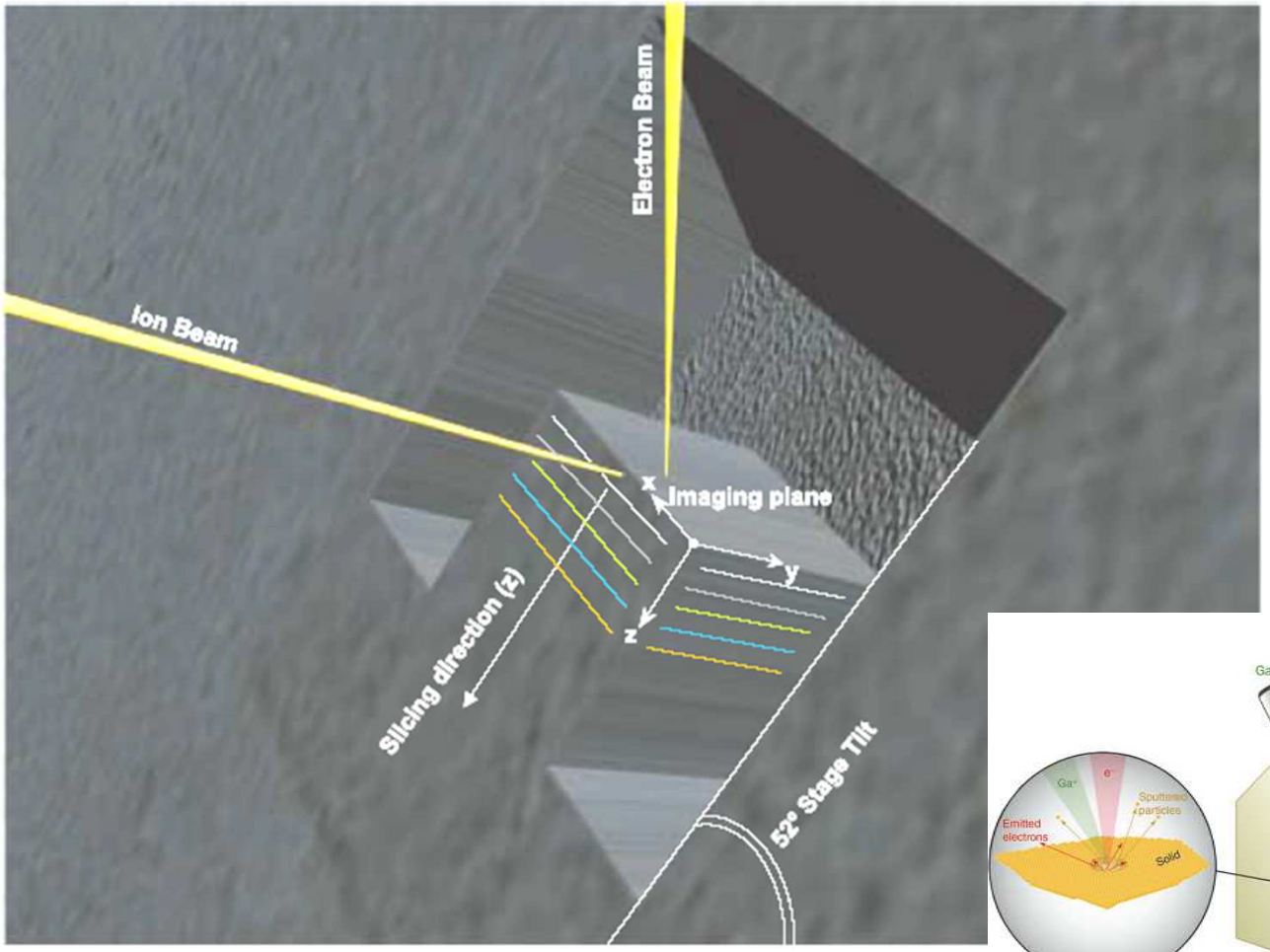
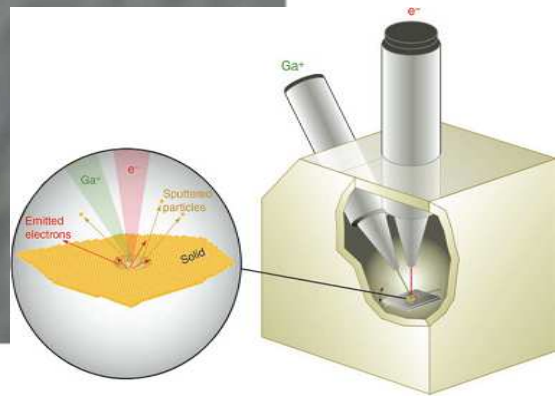


Image processing in 2D

- 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

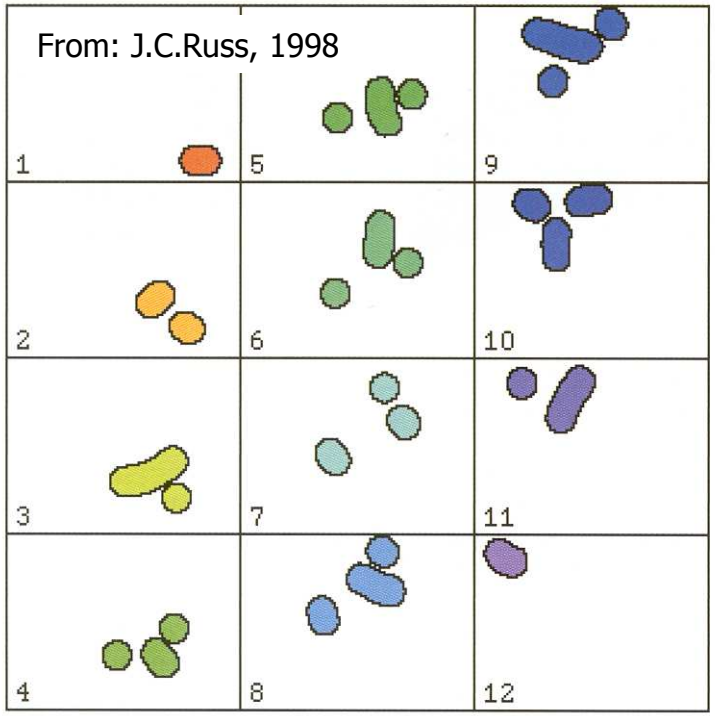
3D imaging with a FIB/SEM
FIB Nanotomography



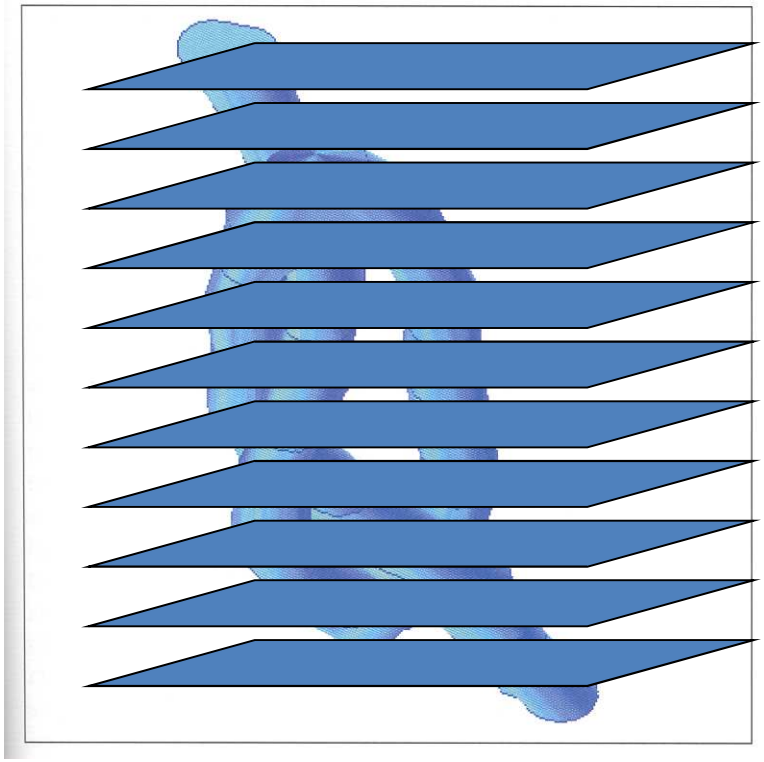


3D Microscopy

Problem of serial sectioning:
3D-reconstruction of disordered microstructures



3D

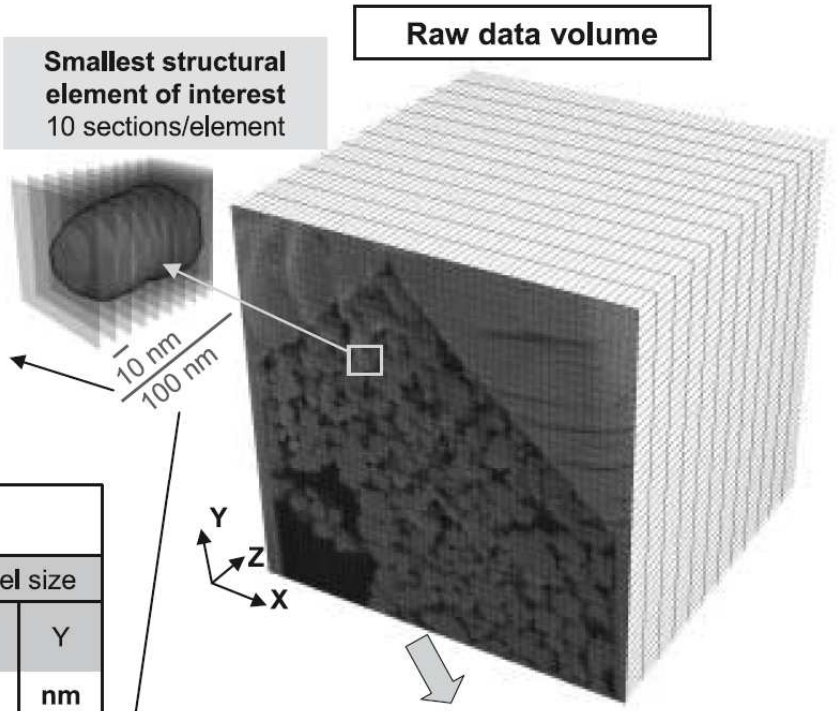


2D Volume fraction

?? Nr of particles ??
?? Shape ??

Voxel, Resolution, Pixel size

Ion-Beam			
Smallest element	Mag	Ideal z-spacing	Beam current
nm	**kX**	**nm**	**pA**
1000	5	100	500
500	10	50	300
300	15	30	100
200	30	20	50
100	50	10	50

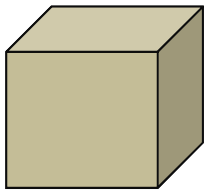


Electron-Beam					
Smallest element	Mag	Image width		Pixel size	
		1024 pxls, X	884 pxls, Y	X	Y
nm	**kX**	**µm**	**µm**	**nm**	**nm**
1000	5	60.0	65.73	58.6	67.9
500	10	30.0	32.86	29.3	33.9
300	15	20.0	21.91	19.5	22.6
200	30	10.0	10.95	9.8	11.3
100	50	6.0	5.18	5.9	6.8

Present example:	x	y	z
Cube size [**µm**]	6.04	6.01	1.74
Voxel matrix	1024 [pixels]	884 [pixels]	105 [sections]
Voxel size [**nm**]	5.9	6.8	16.6

3D FIB/SEM: volume reconstruction

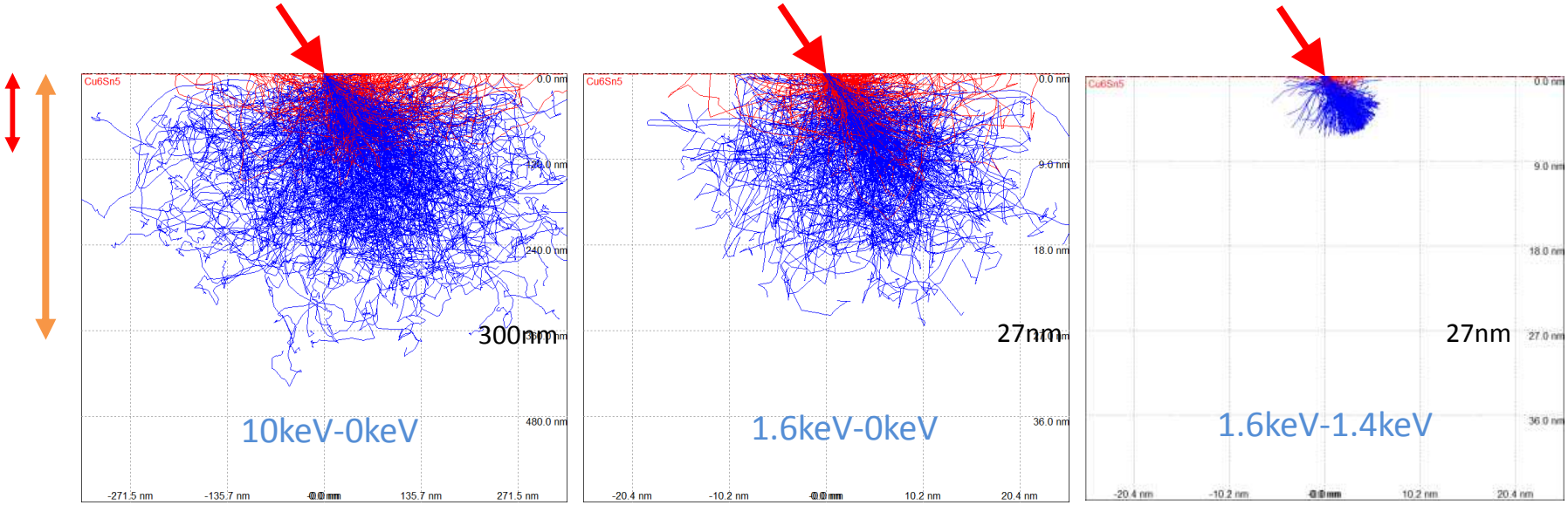
- **Slice thickness (z) = image pixel size (x,y)**
  Z dimension ~ X or Y, typical: 10nm, possible 5nm (3nm)
- **Image dimensions** / data size (8-bit grey level tiff):
  - 1024 x 786: 800 slices -> 640 Mb
  - 2048 x 1572: 1600 slices -> 5 Gb
  - 3096 x 2358: 3000 slices -> 21 Gb
- **Acquisition time ~1min / slice**
  (40-60 slices / hour)
  -> high S/N ratio, beam current (1-1.5nA), detector efficiency
- **Dwell times/pixel 5- 15µsec**. (detector signal -> 256 grey levels)
- **High throughput:** minimise overhead, no tilting, rotating, drift correction
- **Z- Resolution:** low kV !!!



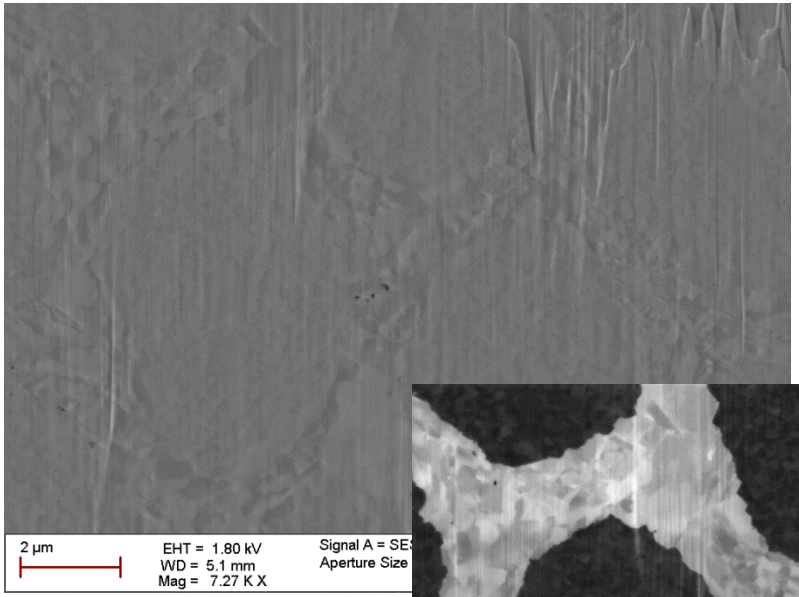
"Leitmotiv"
Isometric voxel size
x = y = z

What is the spatial resolution of BSE electrons ?

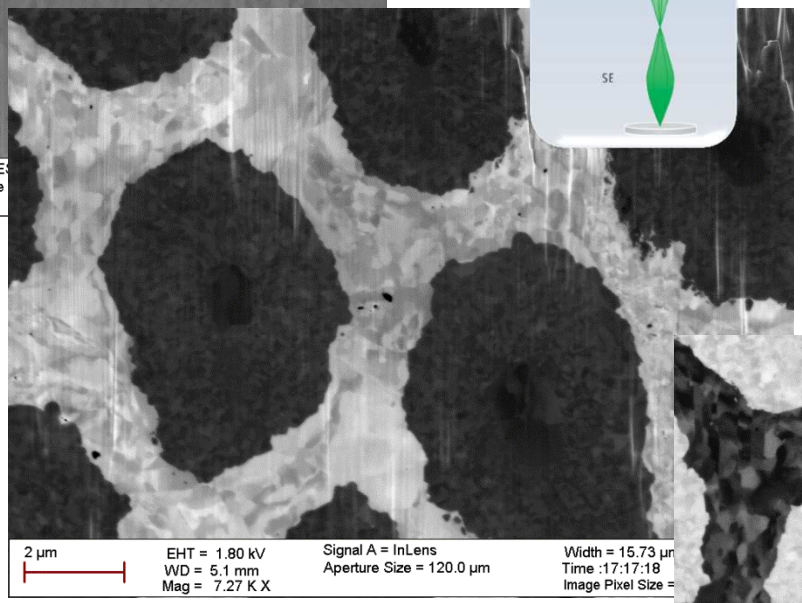
Scatter range in Nb_3Sn:



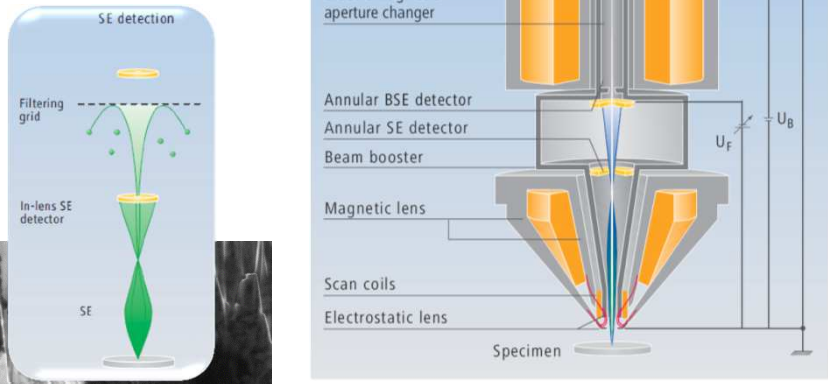
HT	10keV	1.6keV	1.6keV (low loss, EsB grid at 1.4kV)
BSE esc. depth	100nm	10nm	2-3nm
penetration	300nm	20nm	(20nm) Energy selective BS

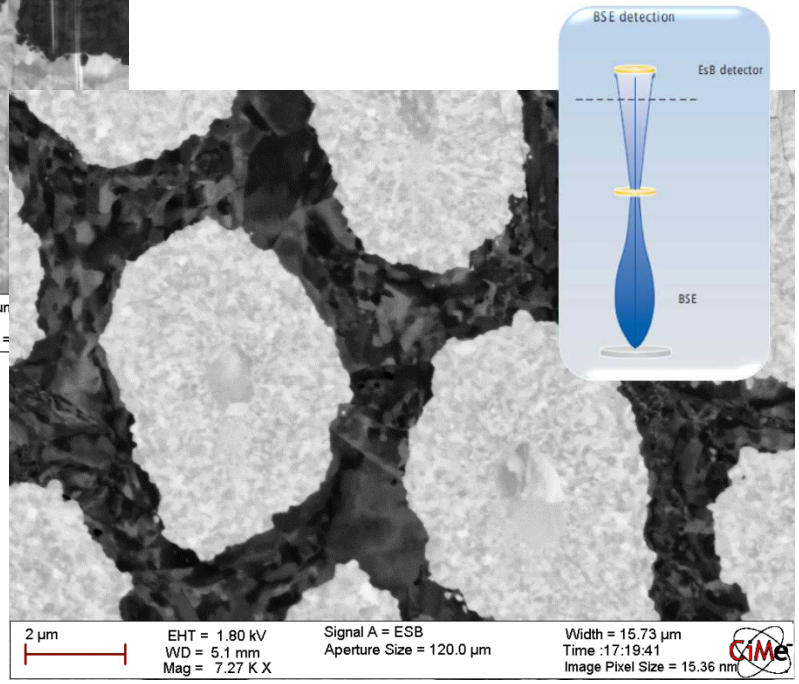


in-chamber ET-detector, SE



in-column "InLens", SE-detector

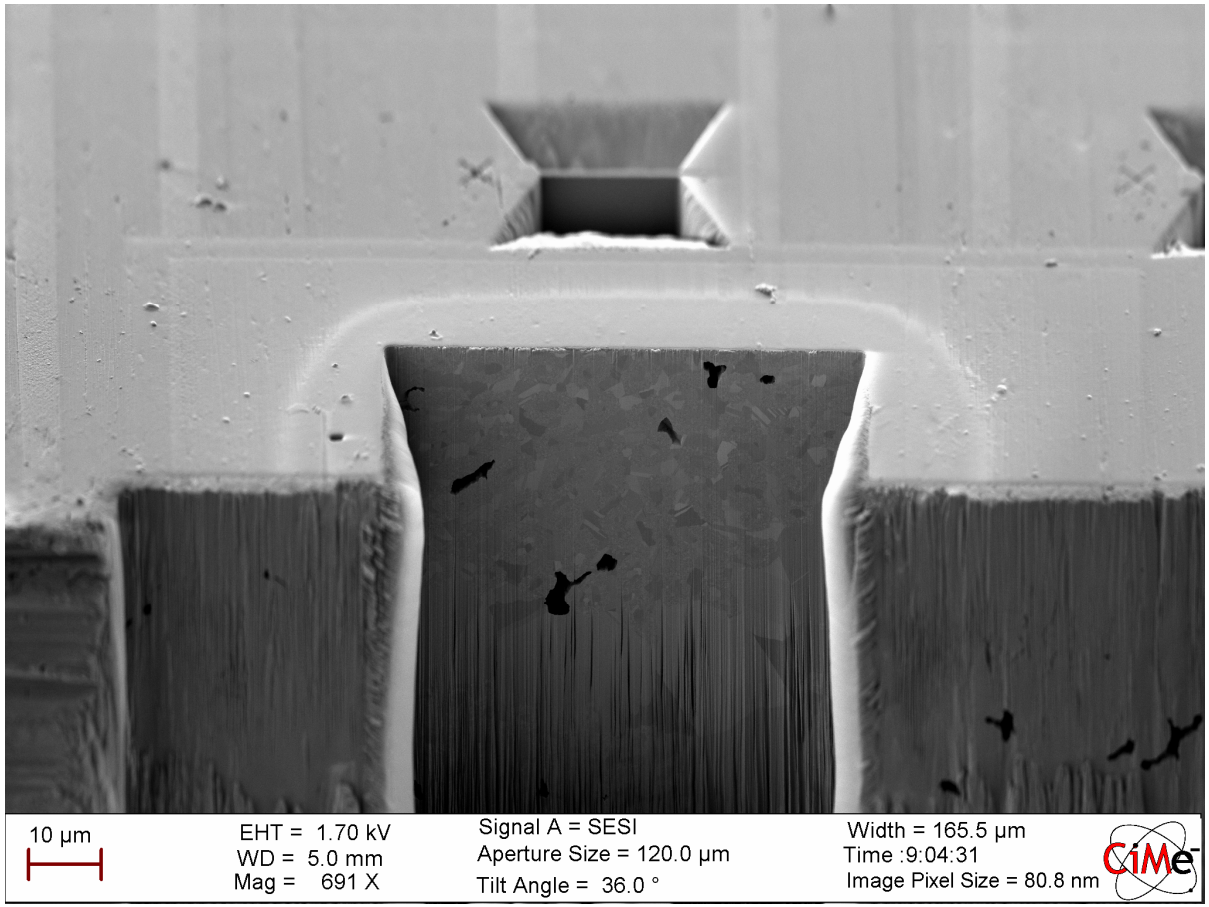


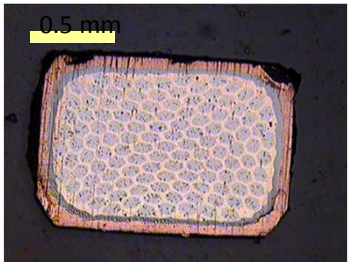


in-column, "energy-selective" EsB, BSE-detector

Low kV imaging:
acceleration voltage: 1.8 kV
In-column detectors

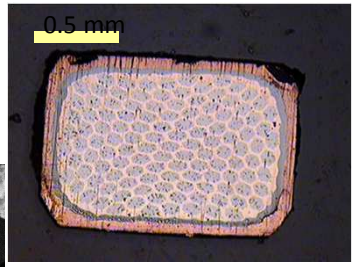
Nb_3Sn multifilament superconducting cable

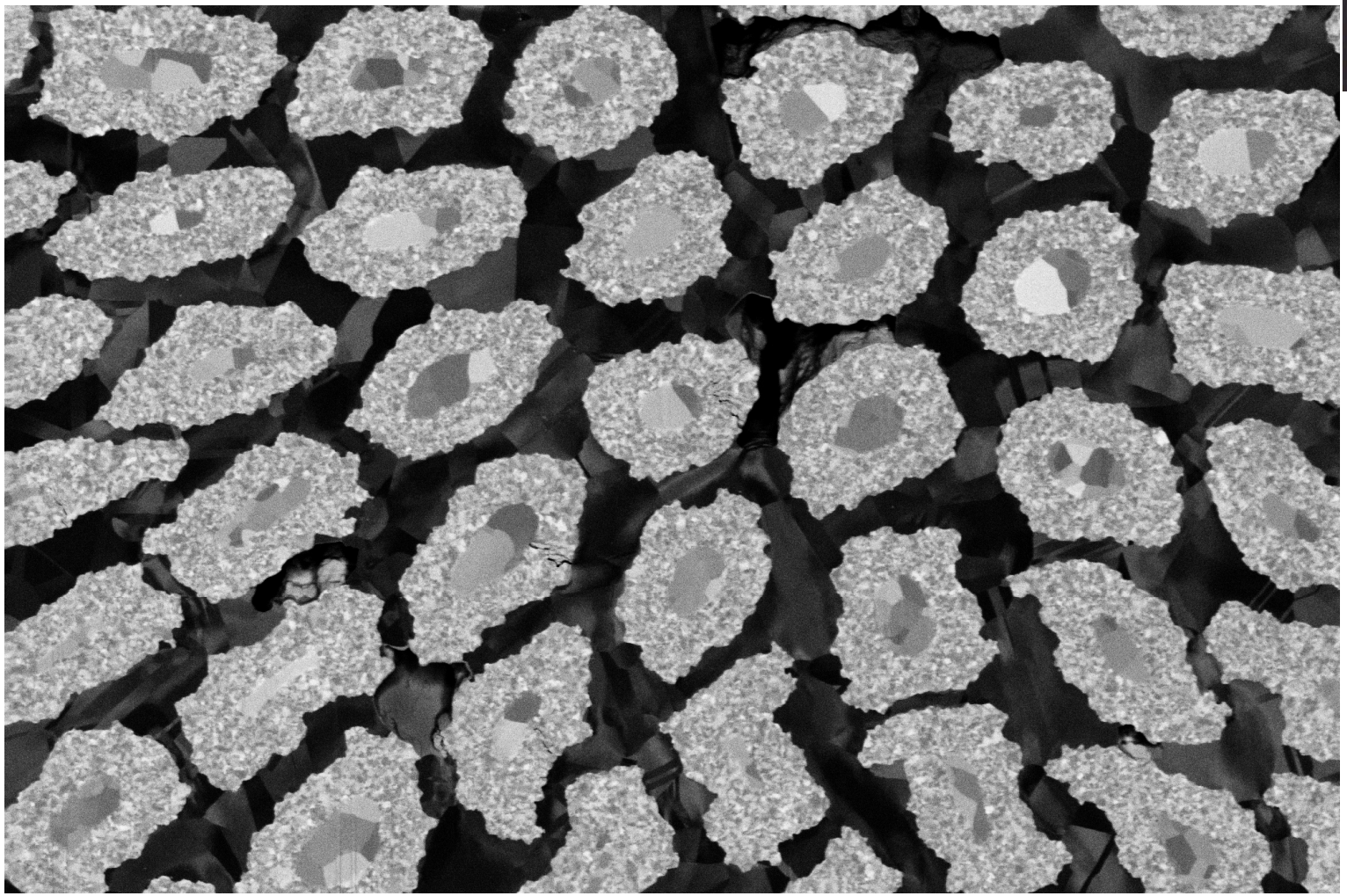




Nb_3Sn superconductor multifilament cable: 14'000 Nb_3Sn filaments (diameter ~5um) in Cu matrix

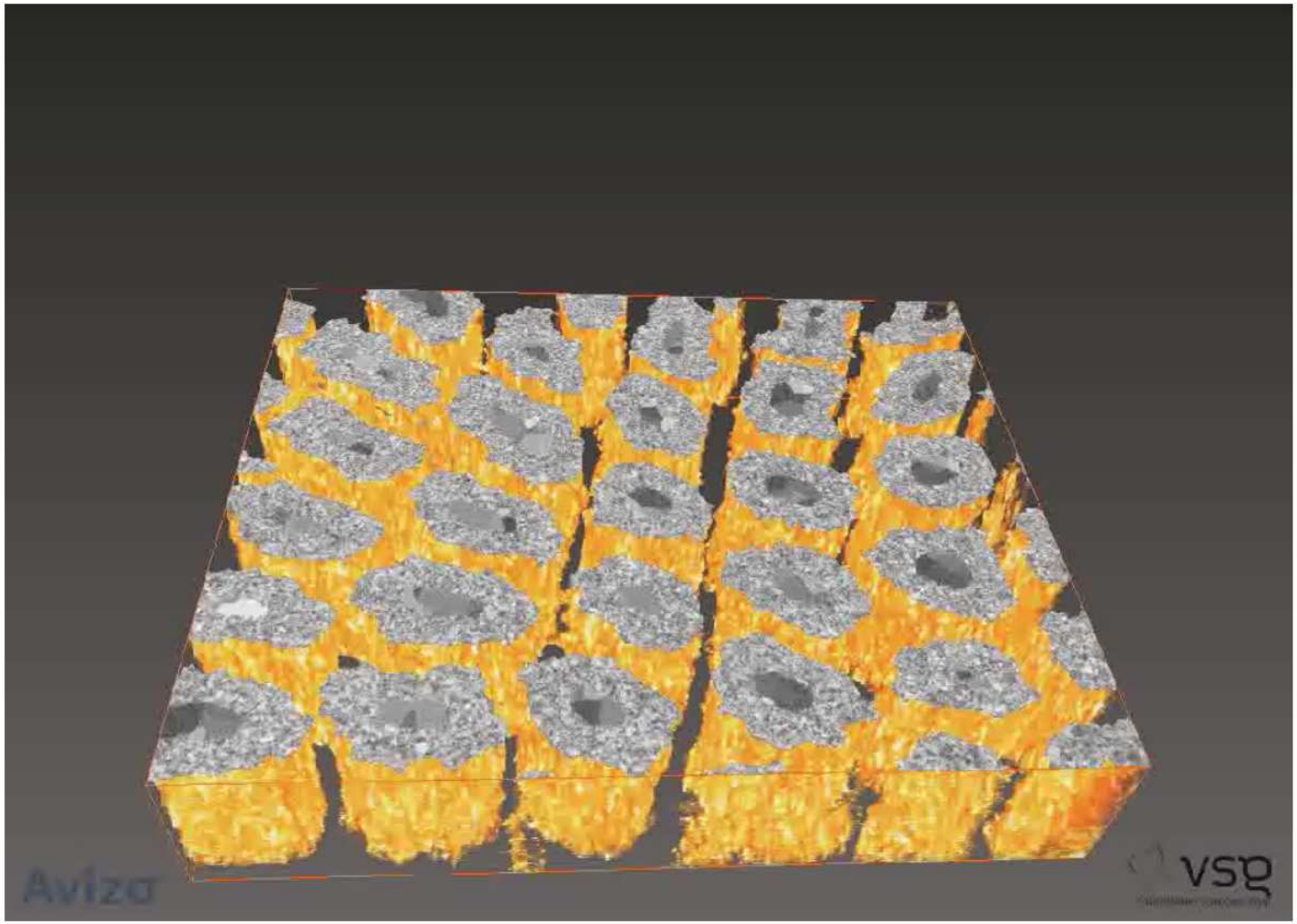
Nb_3Sn multifilament superconducting cable





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

1.8kV EsB detector: Materials & orientation contrast



Materials & grain contrast

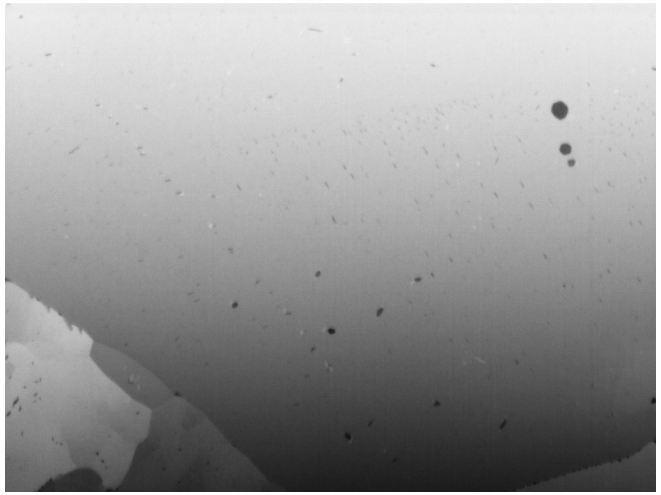
2048x1536x1700, (10x10x10nm voxel),
28hours

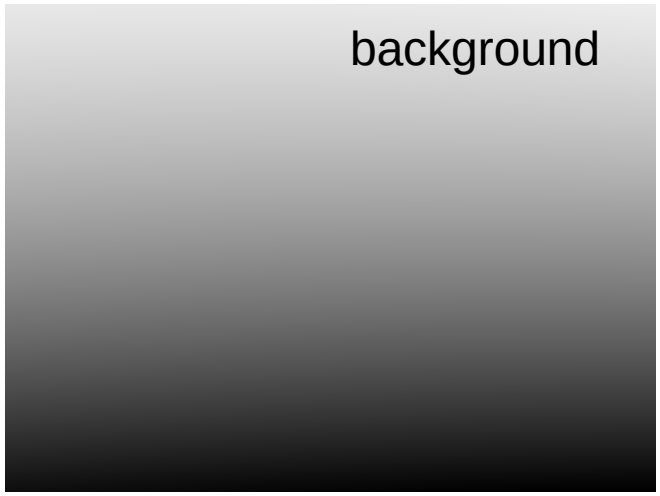
Image processing in 3D

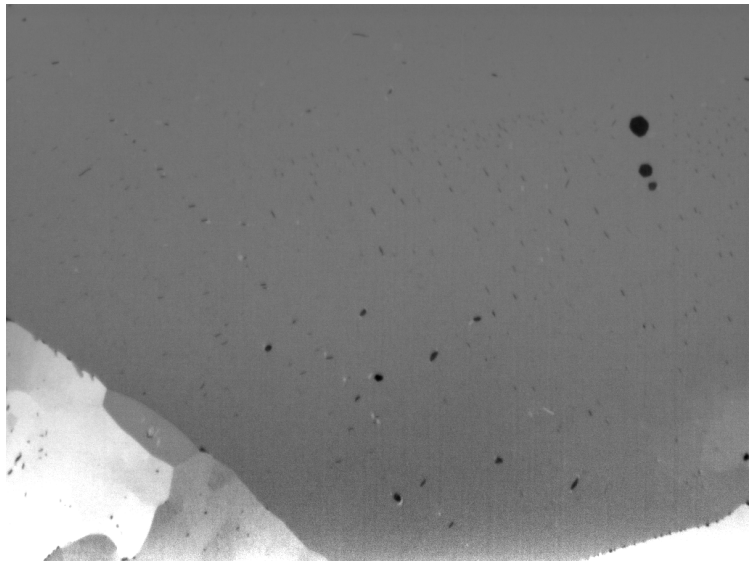
- 2D processing (shading correction)
- Image alignment (registration)
- Cropping (subvolume)
- Noise reduction (2D or 3D)

- Segmentation
- Visualization

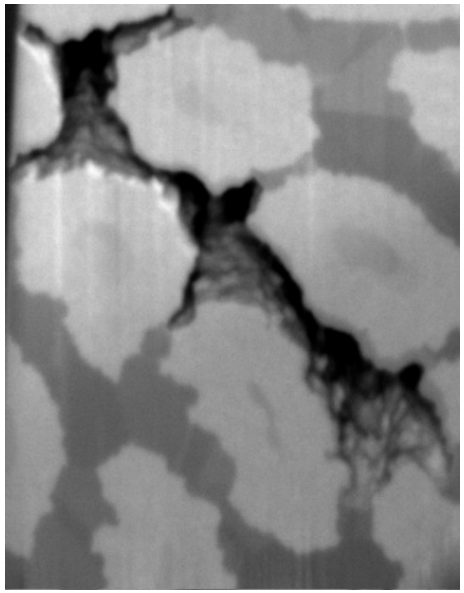
Shading correction

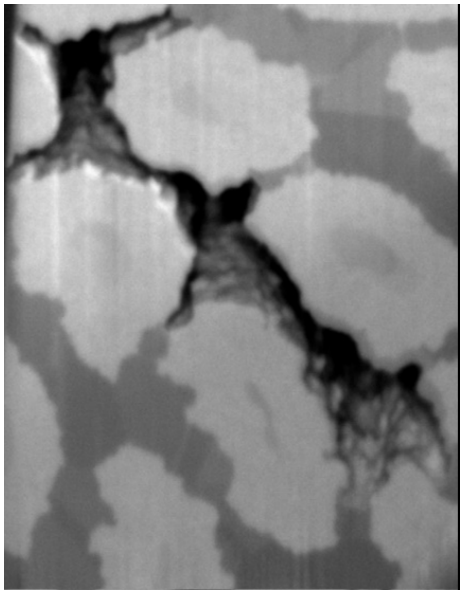
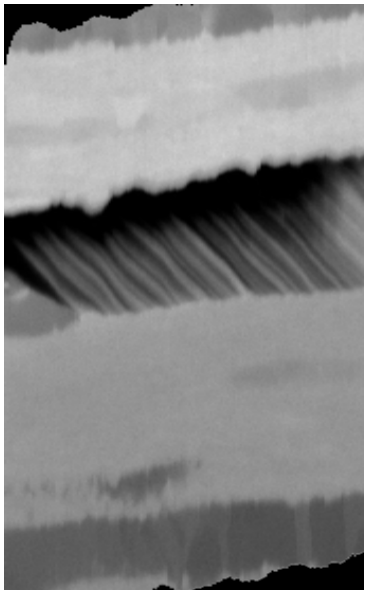
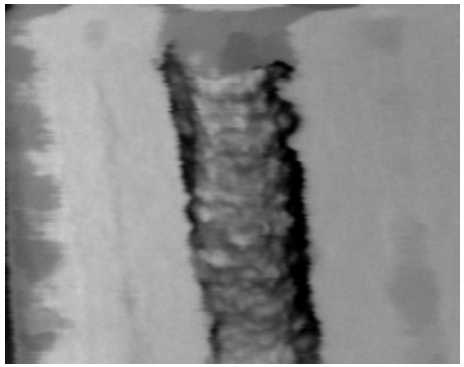




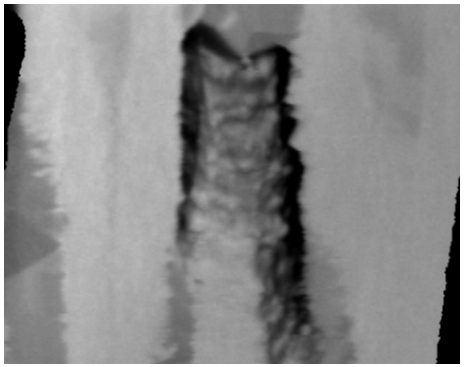


Registration



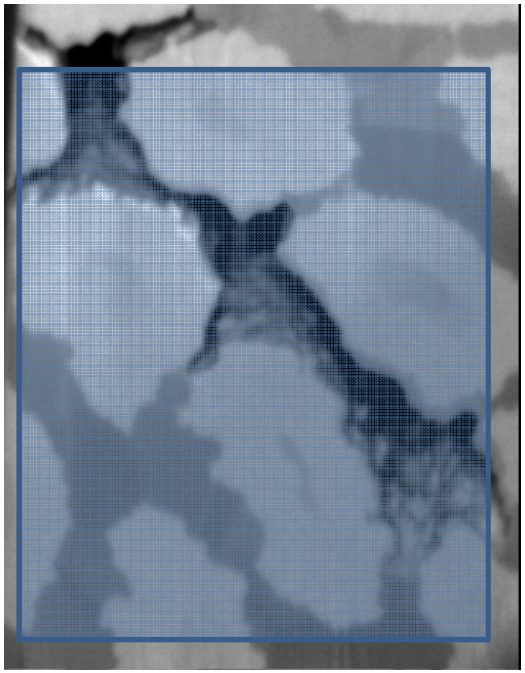




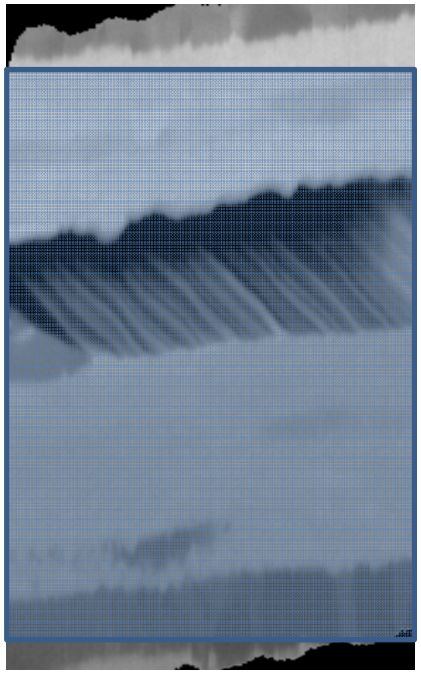
before

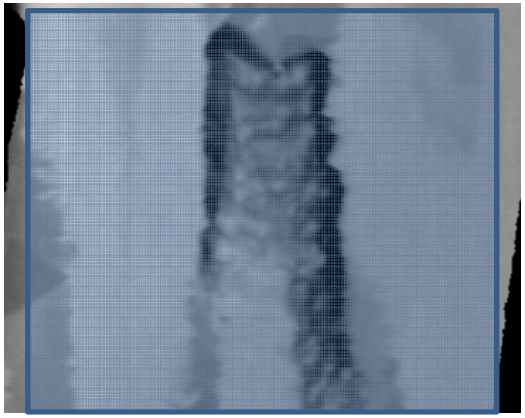


after

cropping







Cutting of the border

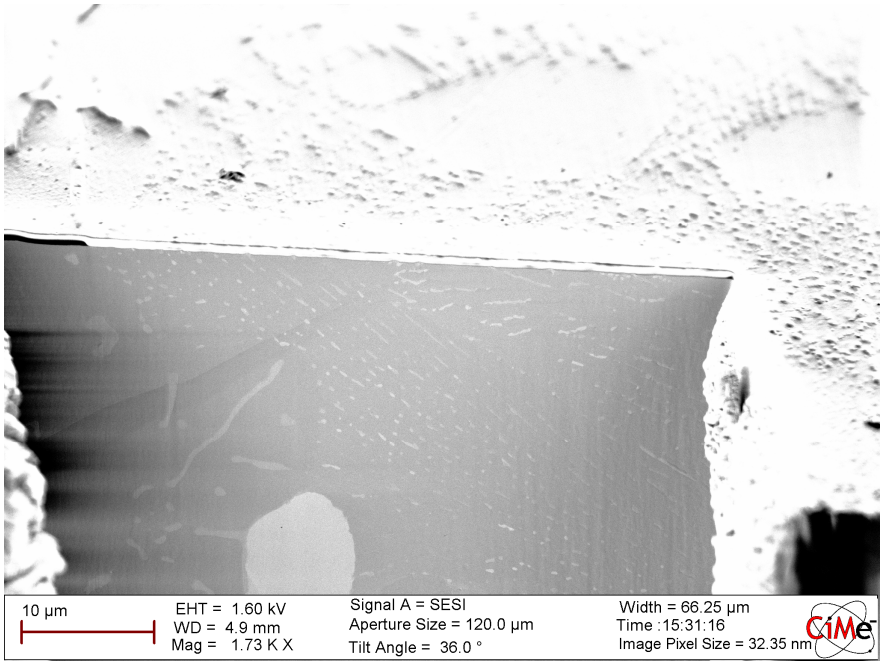
Segmentation

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

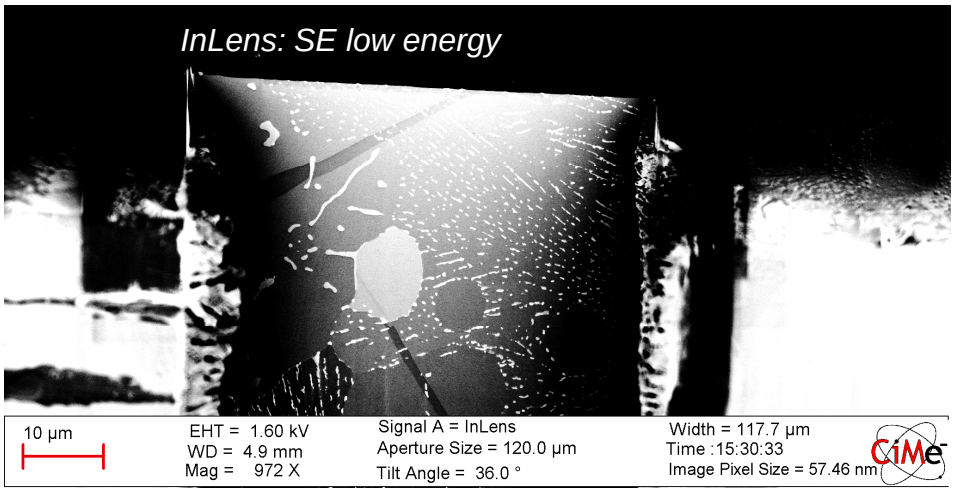
Pb-free solder SnAgCu:

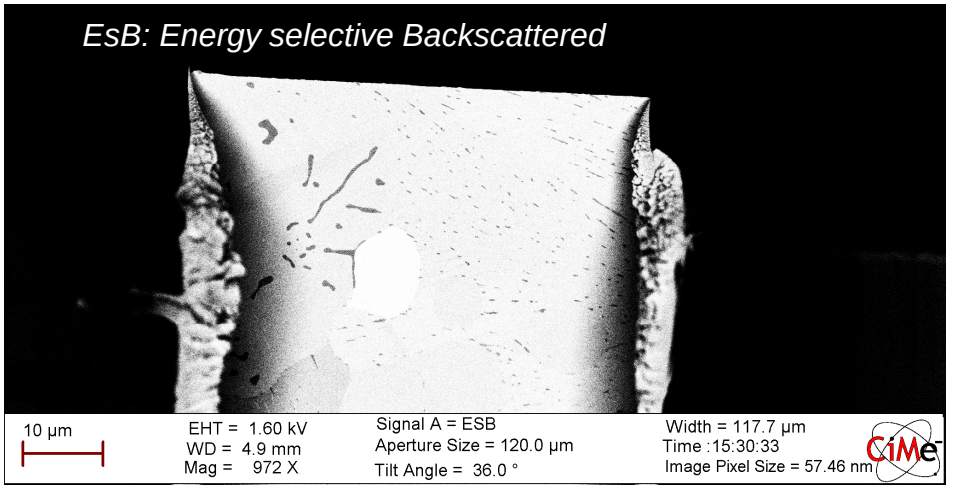
"one detector is not enough"

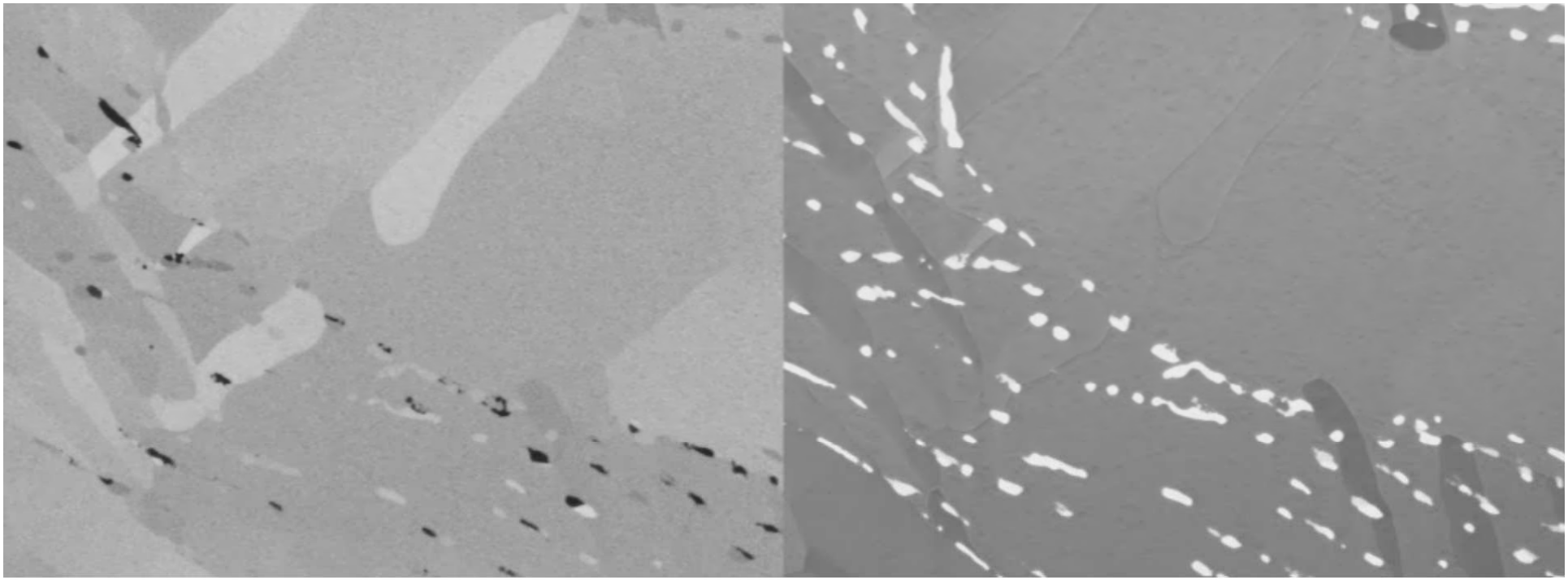
M. Maleki, EPFL-LMAF

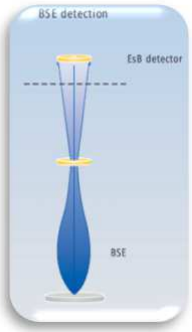


ETD (SE classic)









EsB

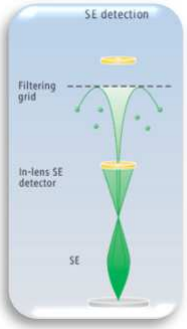
10x10x10nm voxel size, 2048x1536x2000

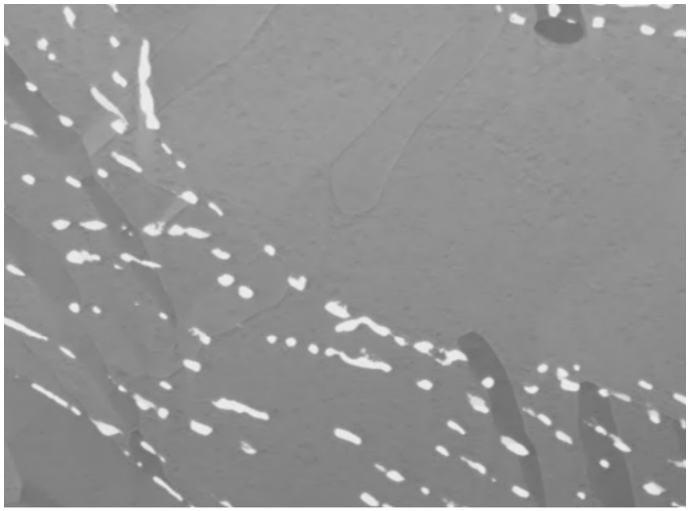
2 images (2x3Mb) / slice …! (DUAL Channel !)

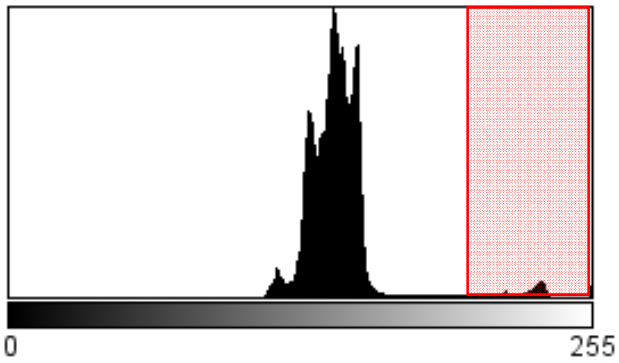
1.6keV, EsB & InLens-SE detector

12Gb data

InLens SE

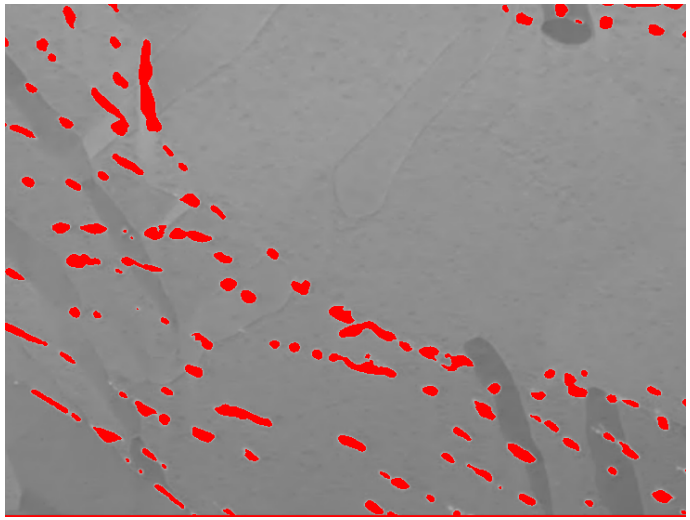


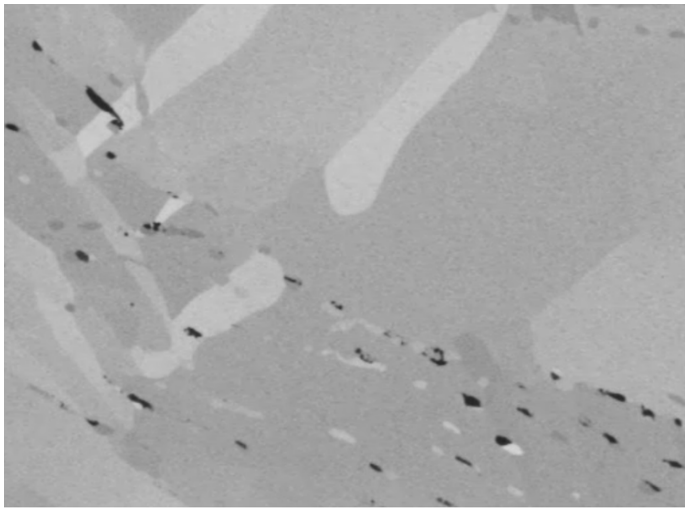


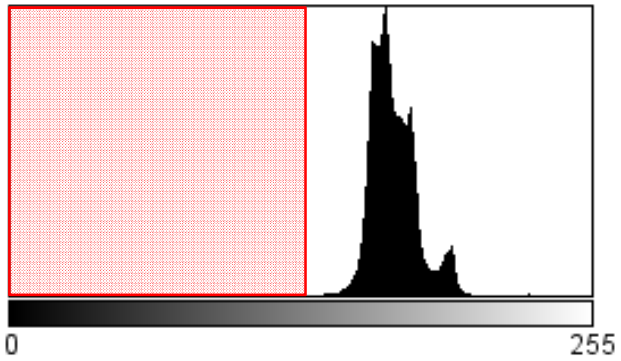


Count: 411792
Mean: 145.445
StdDev: 18.865

Min: 101
Max: 255
Mode: 142 (18748)

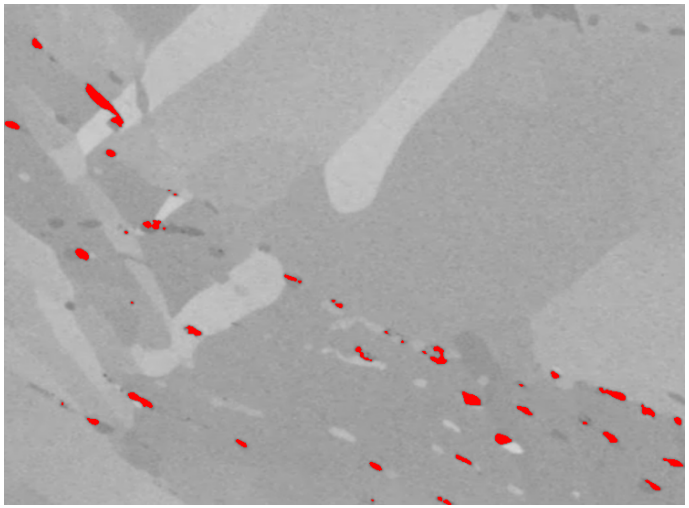


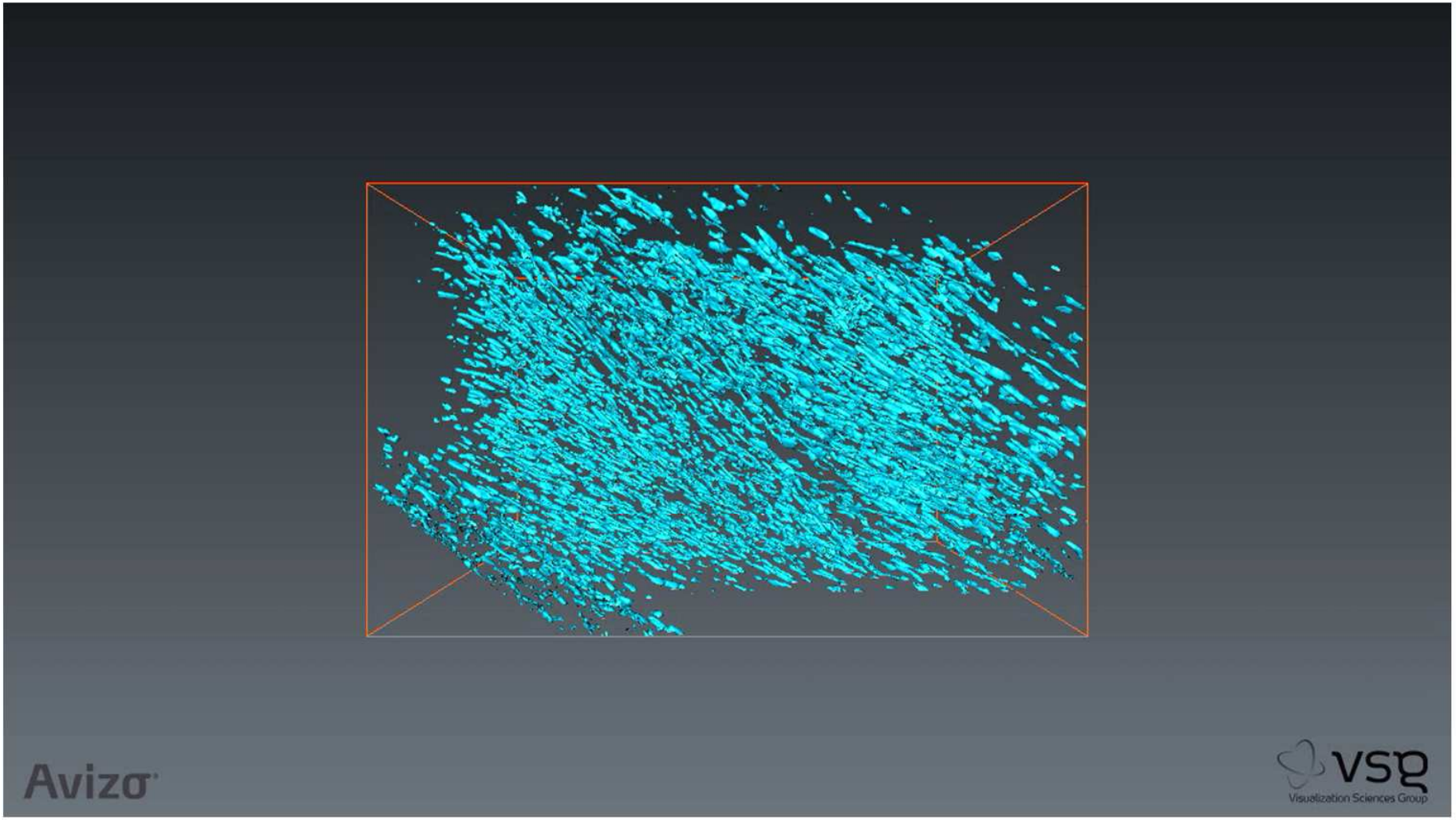




Count: 411792
Mean: 167.731
StdDev: 13.290

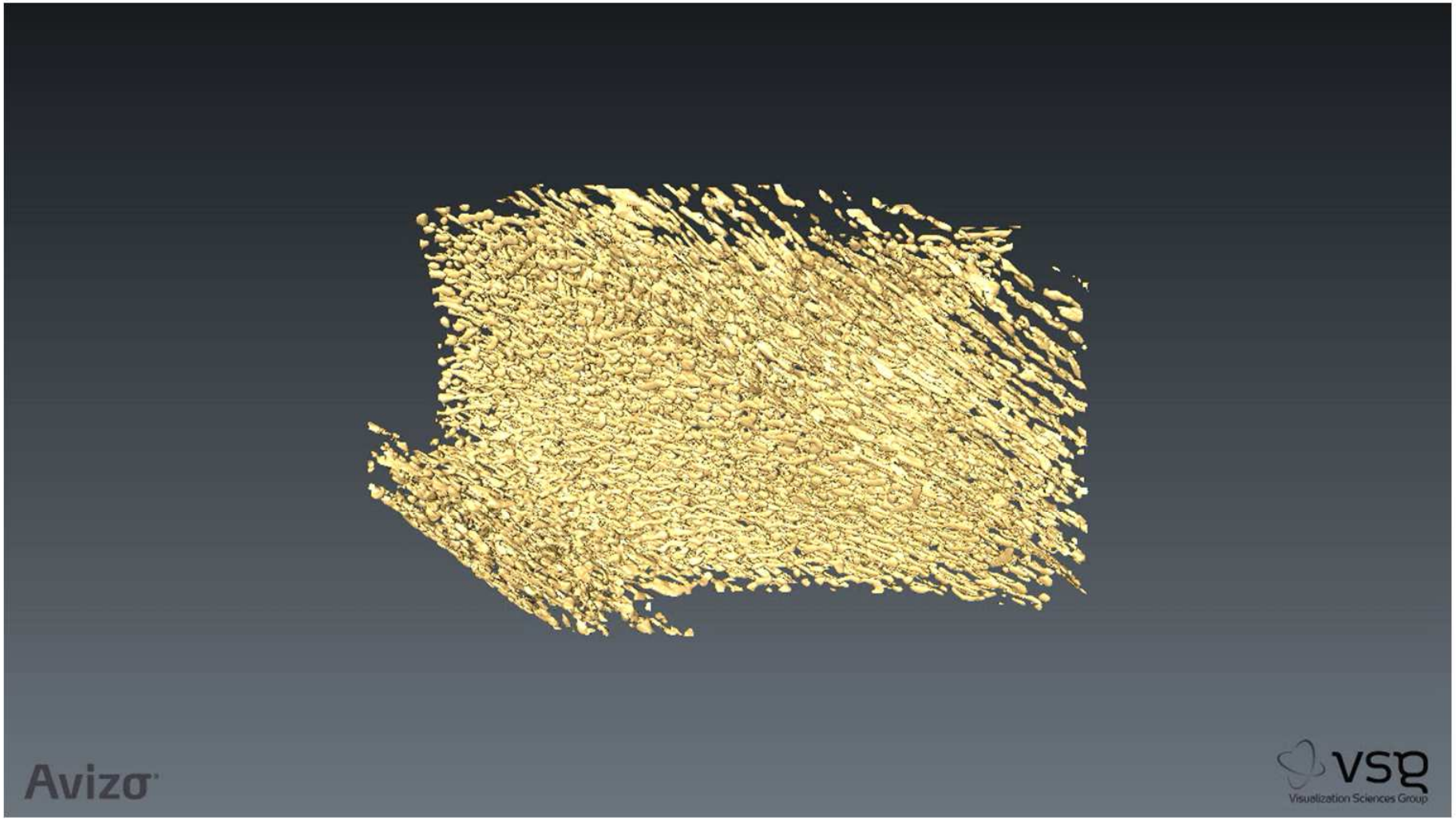
Min: 36
Max: 236
Mode: 165 (20261)





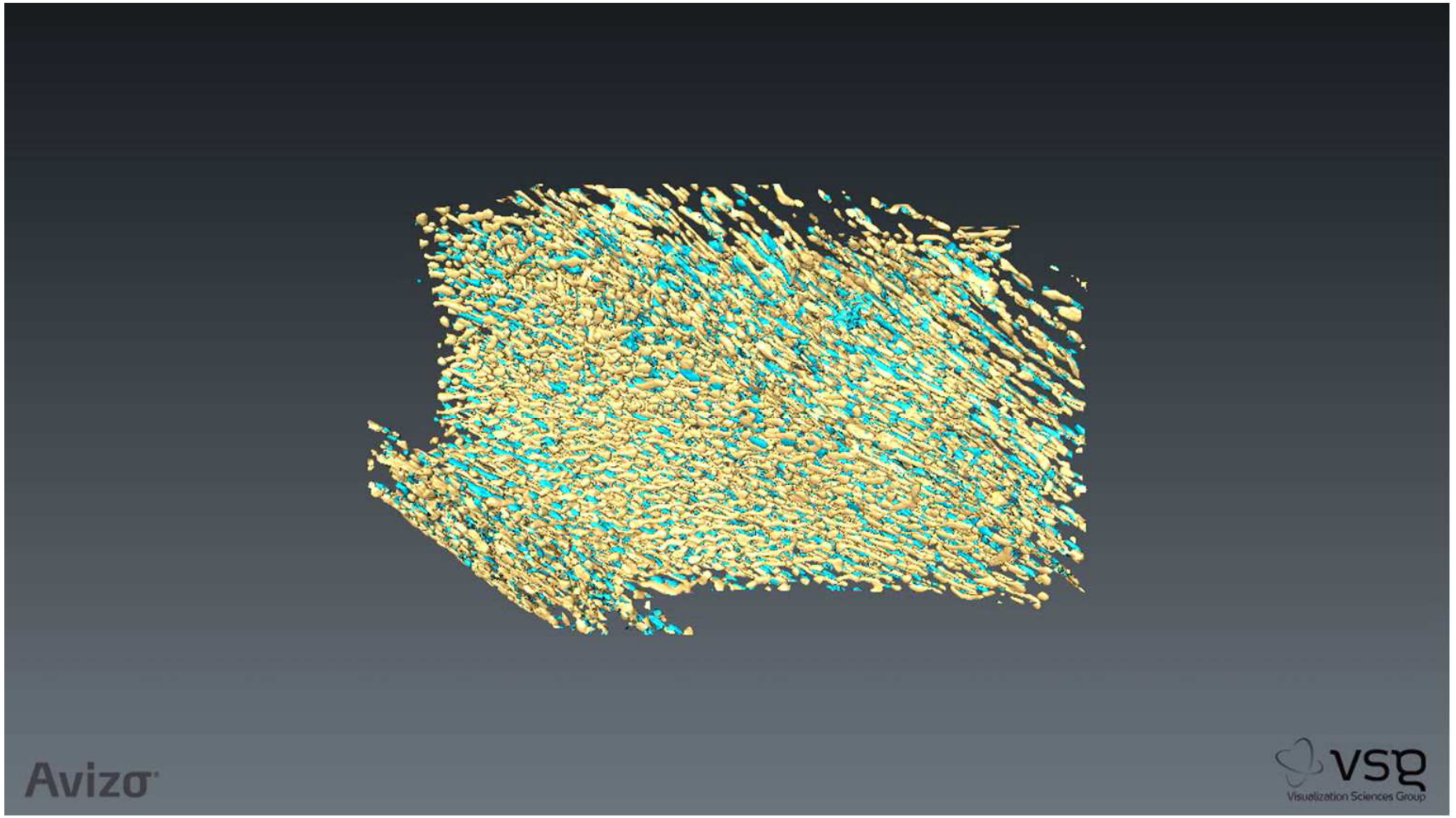
Phase 1. Dark in EsB image, White in SE-InLens

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


Avizo
vsg
Visualization Sciences Group

Segmentation tools for life-science

Problem:

- Structure (shape) determines function of objects

Single cell on substrate

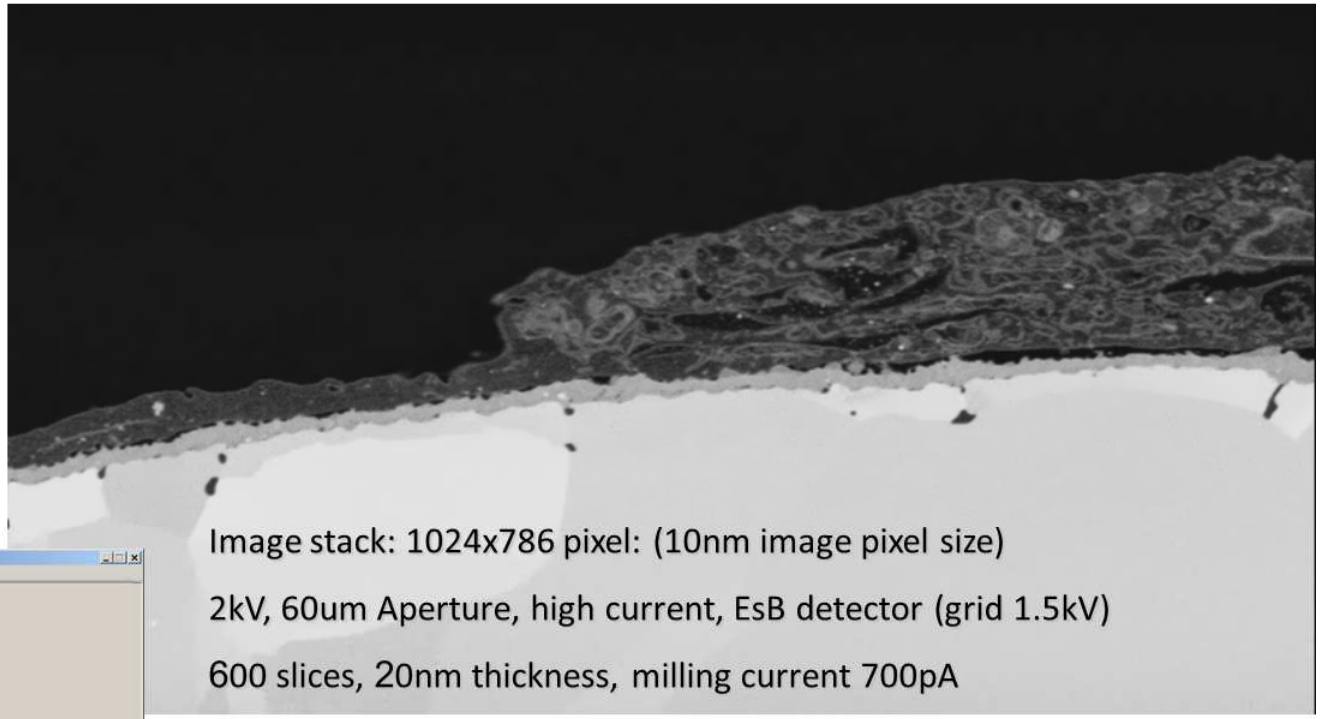
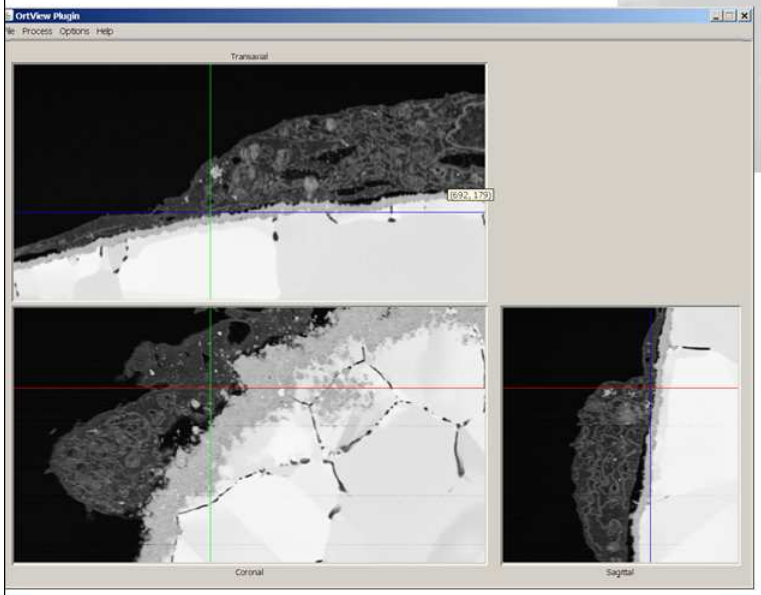


Image stack: 1024x786 pixel: (10nm image pixel size)

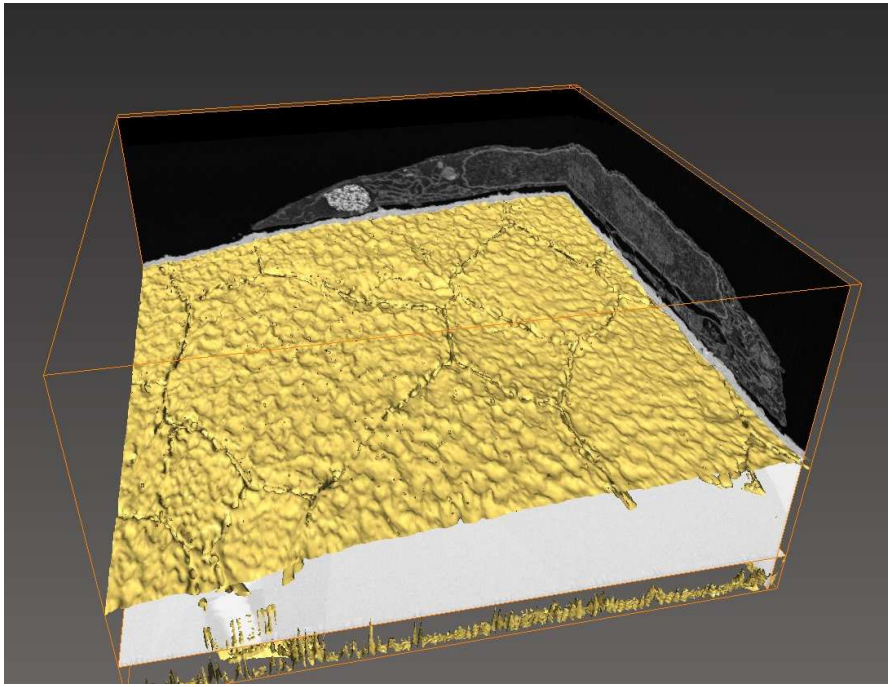
2kV, 60um Aperture, high current, EsB detector (grid 1.5kV)

600 slices, 20nm thickness, milling current 700pA

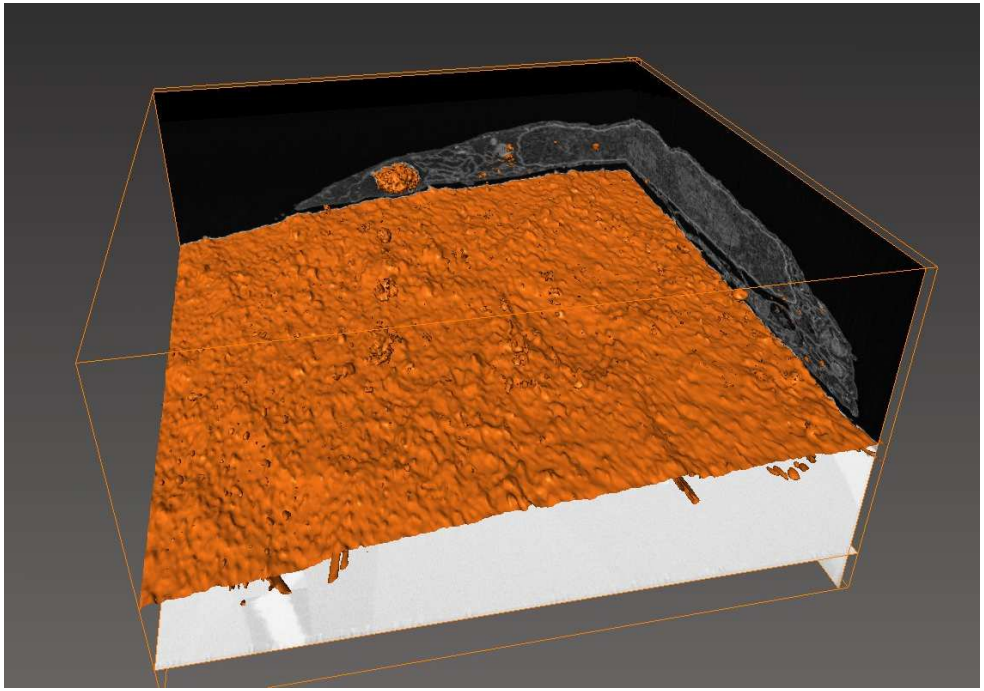


Segmentation based on grey levels

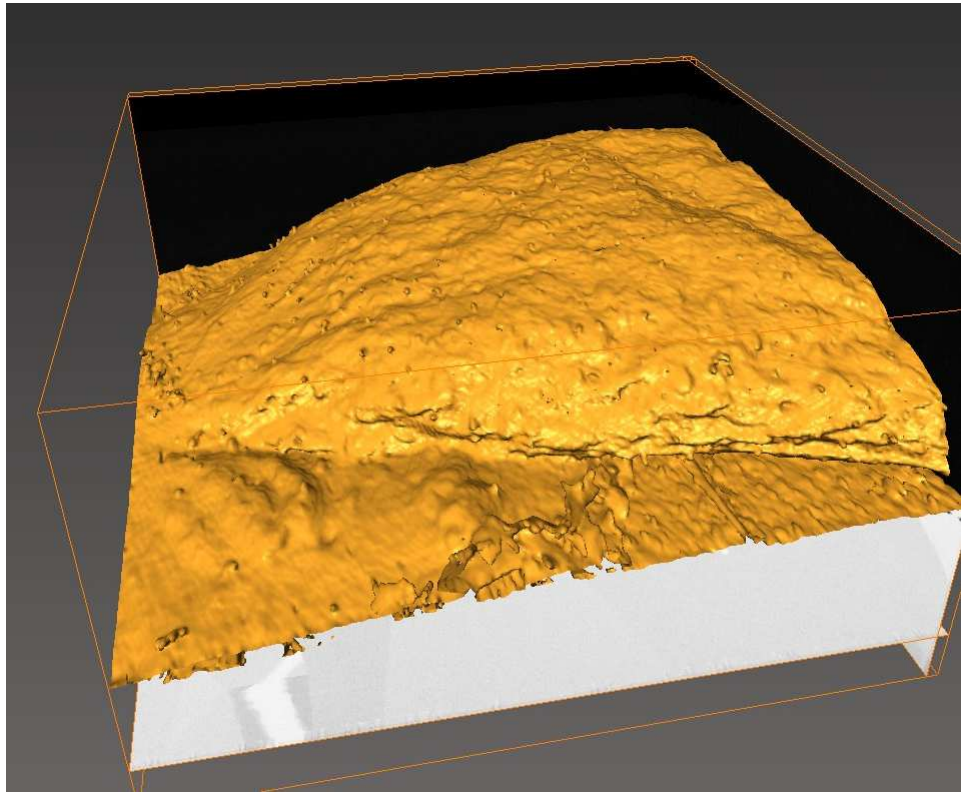
Medical steel



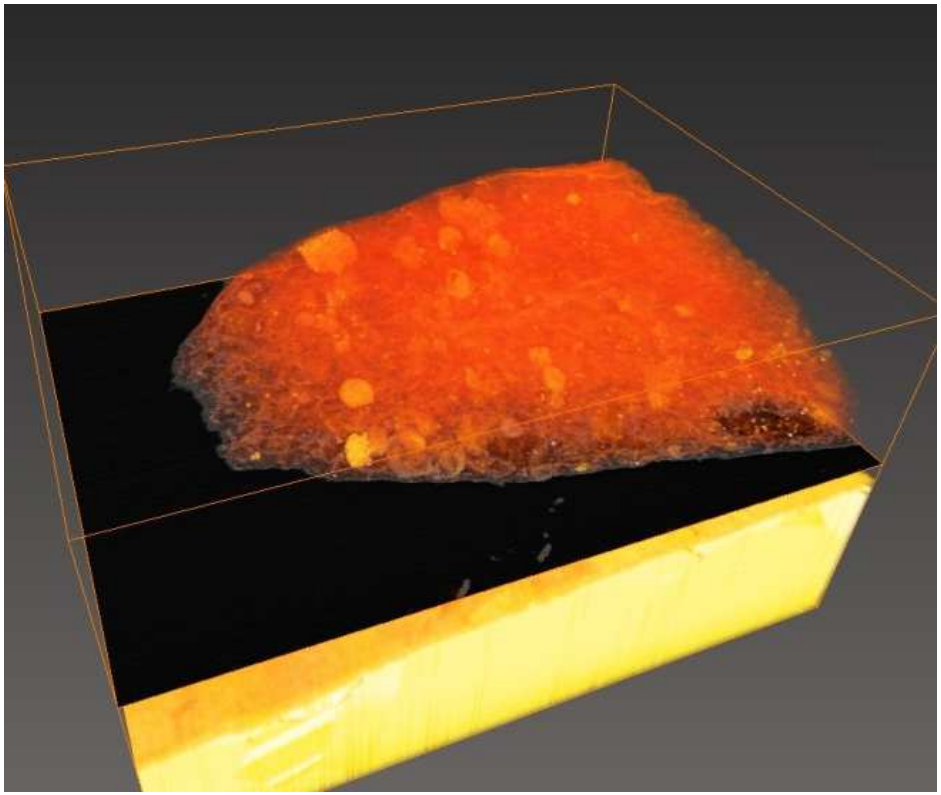
Ceramic coating: TiO_2

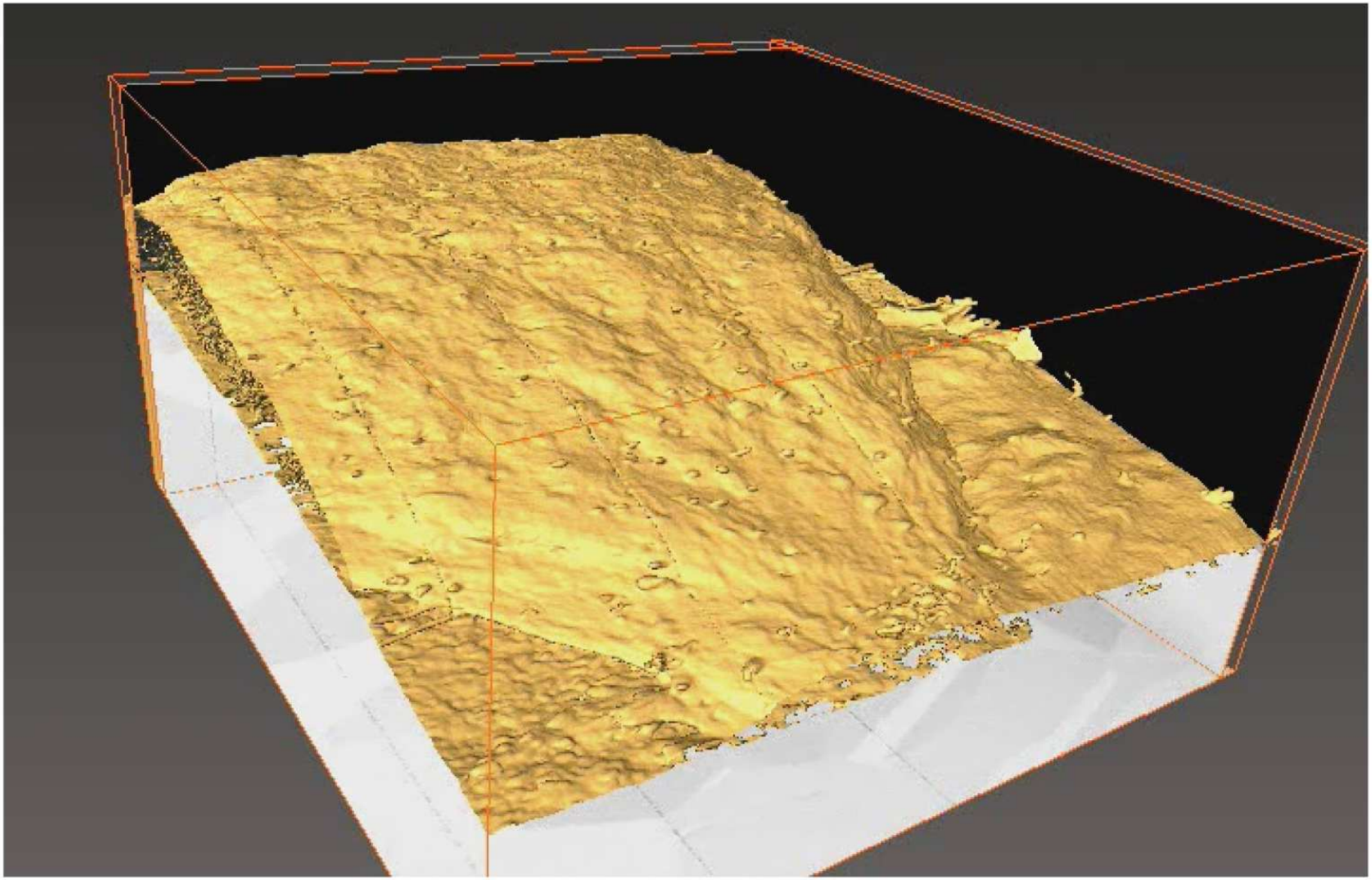


Cell outer membrane



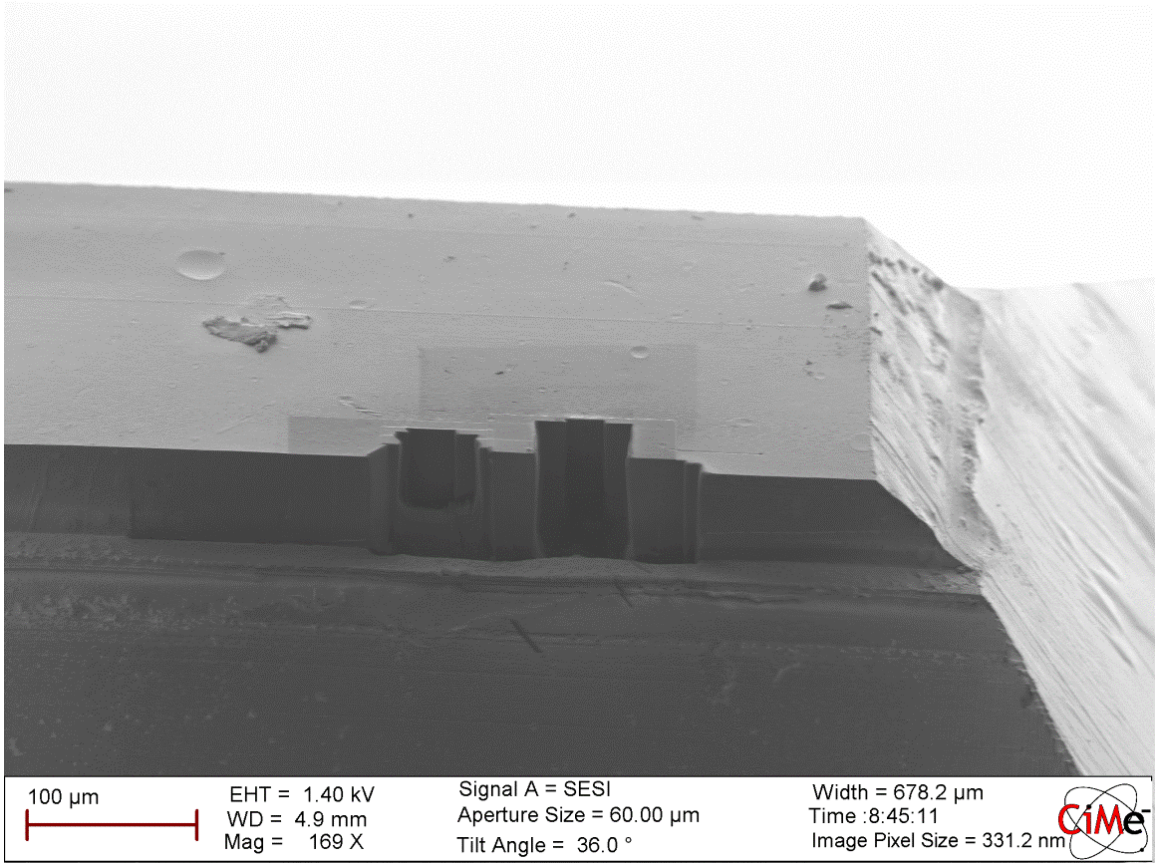
and more…

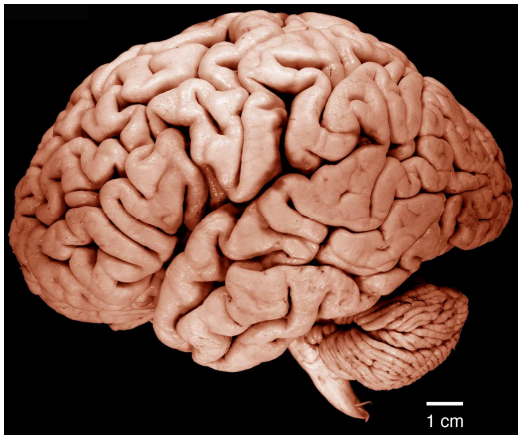


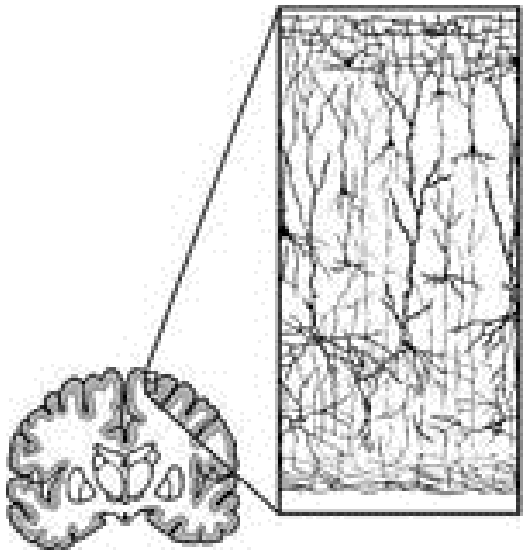


A little bit more complex….

brain tissue, resin embedded





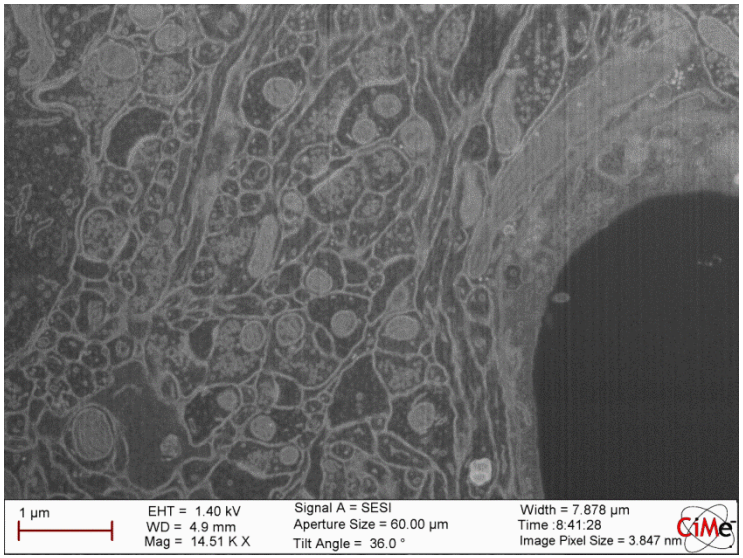


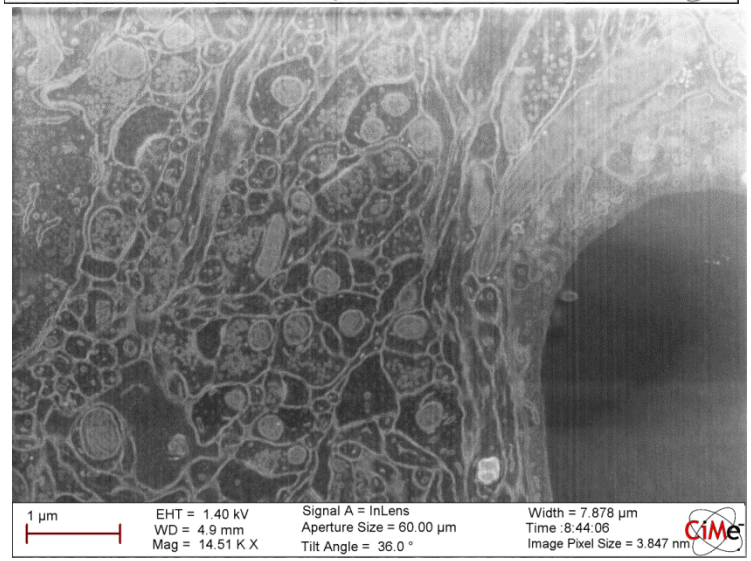
Which detector…?

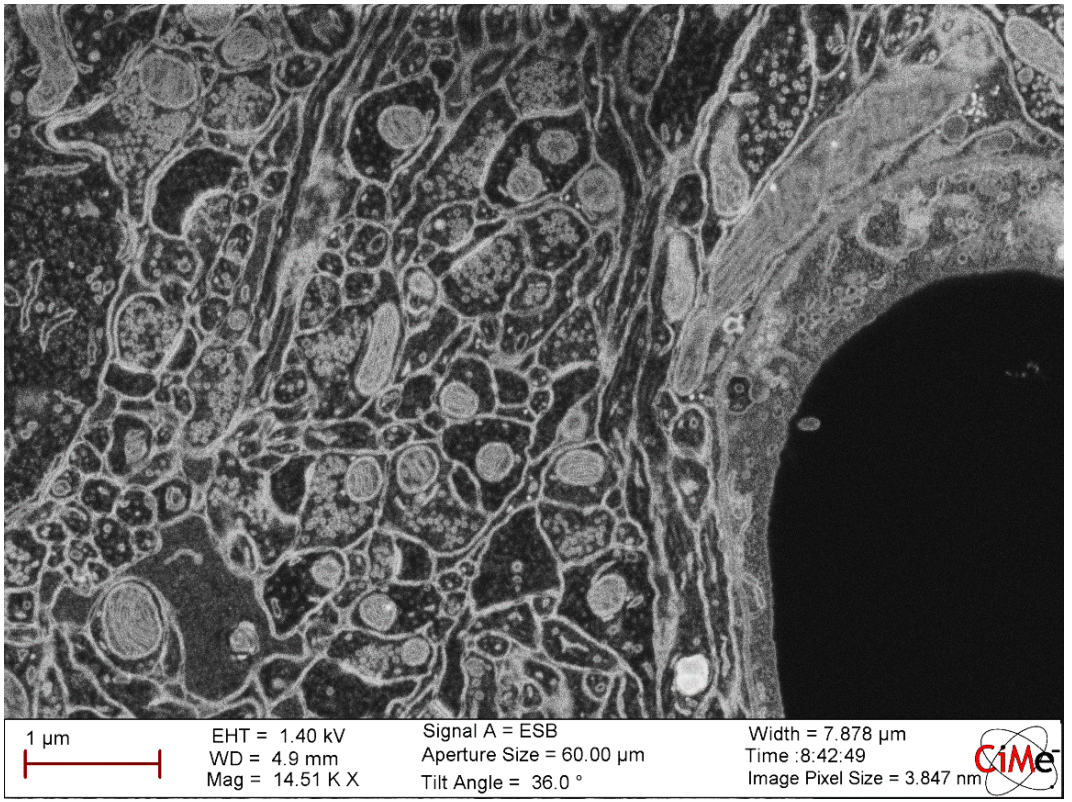
In-chamber SE (Everhard-Thornley)

in-Lens SE

in-Lens BSE (energy selective)

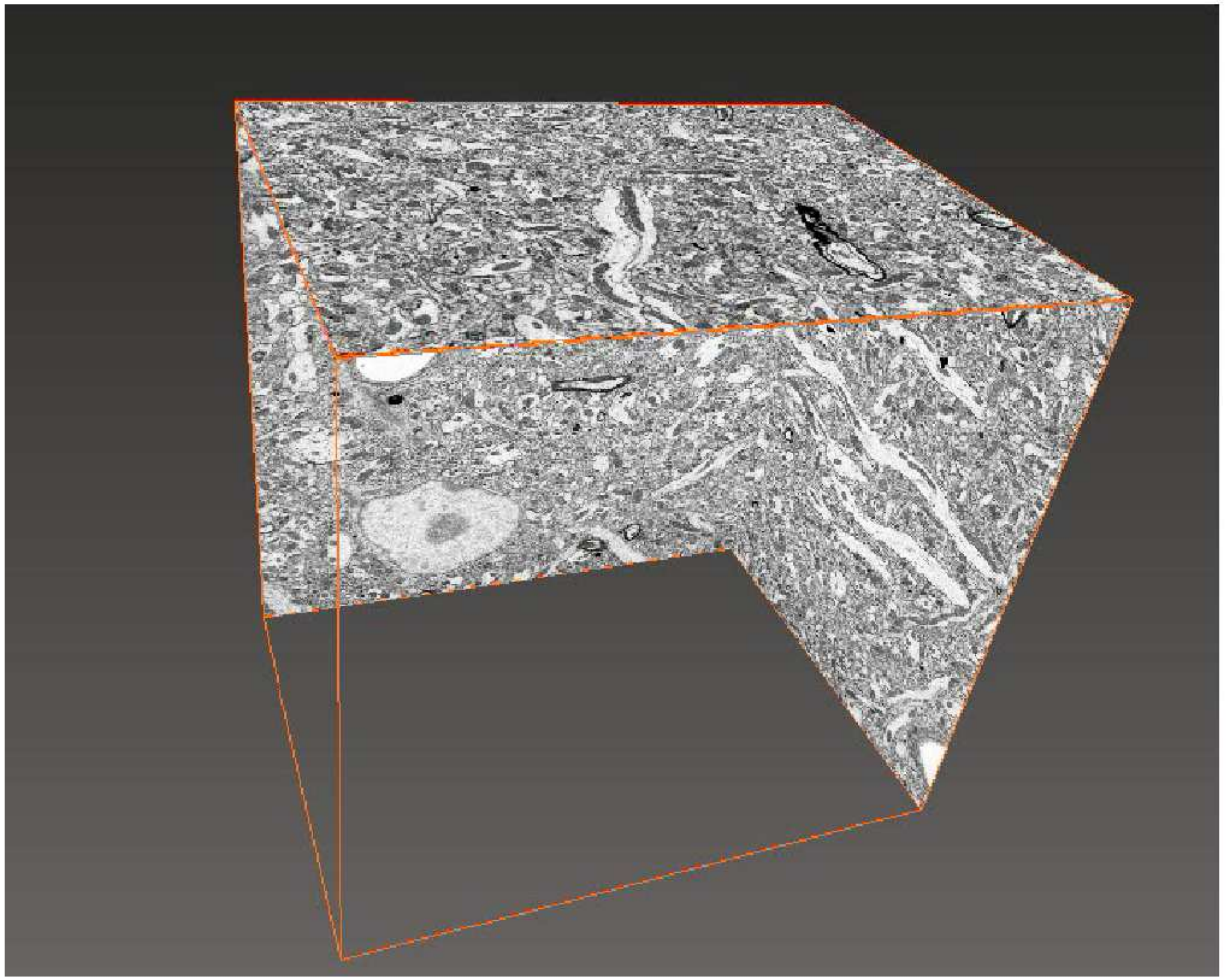


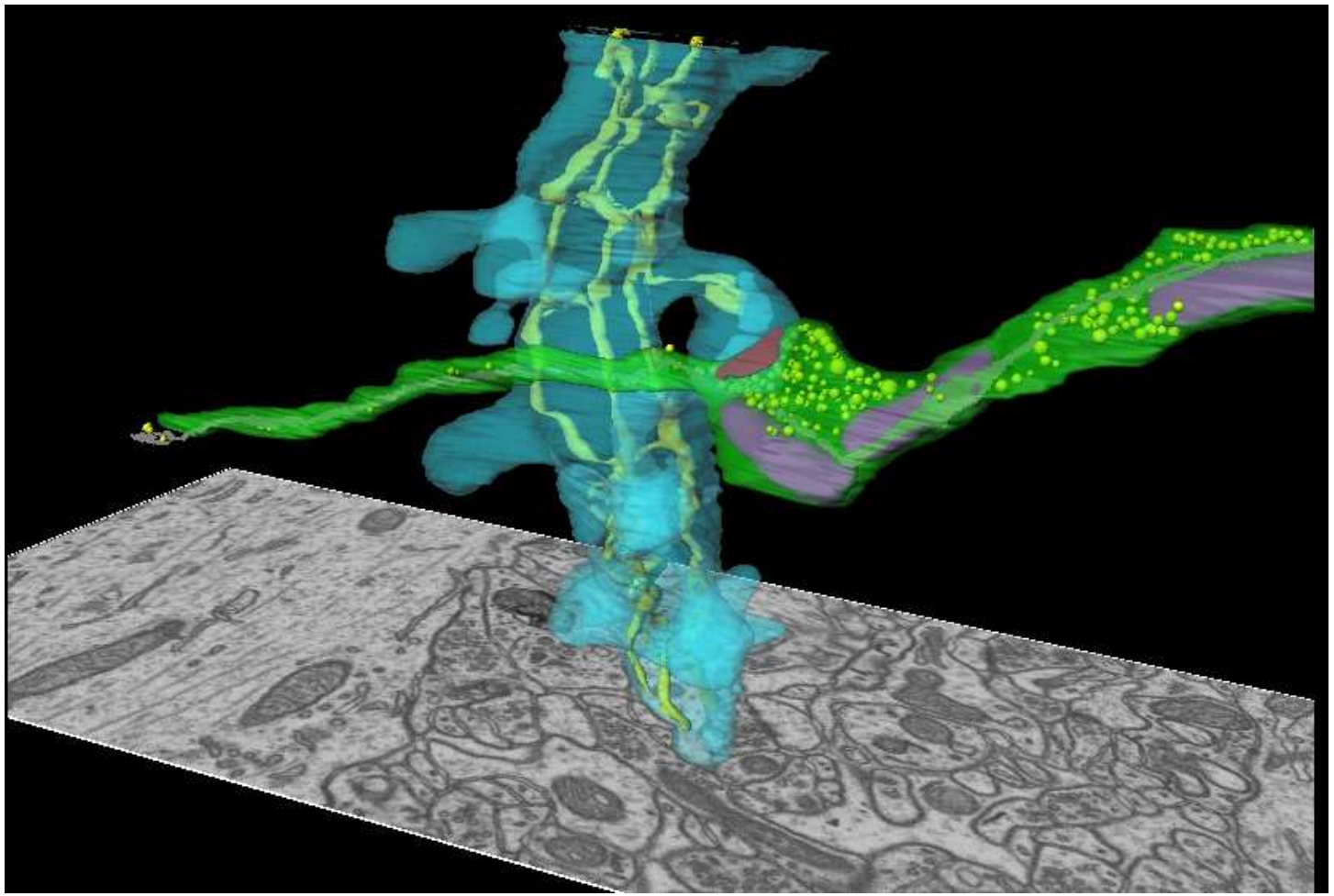




Big volumes

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



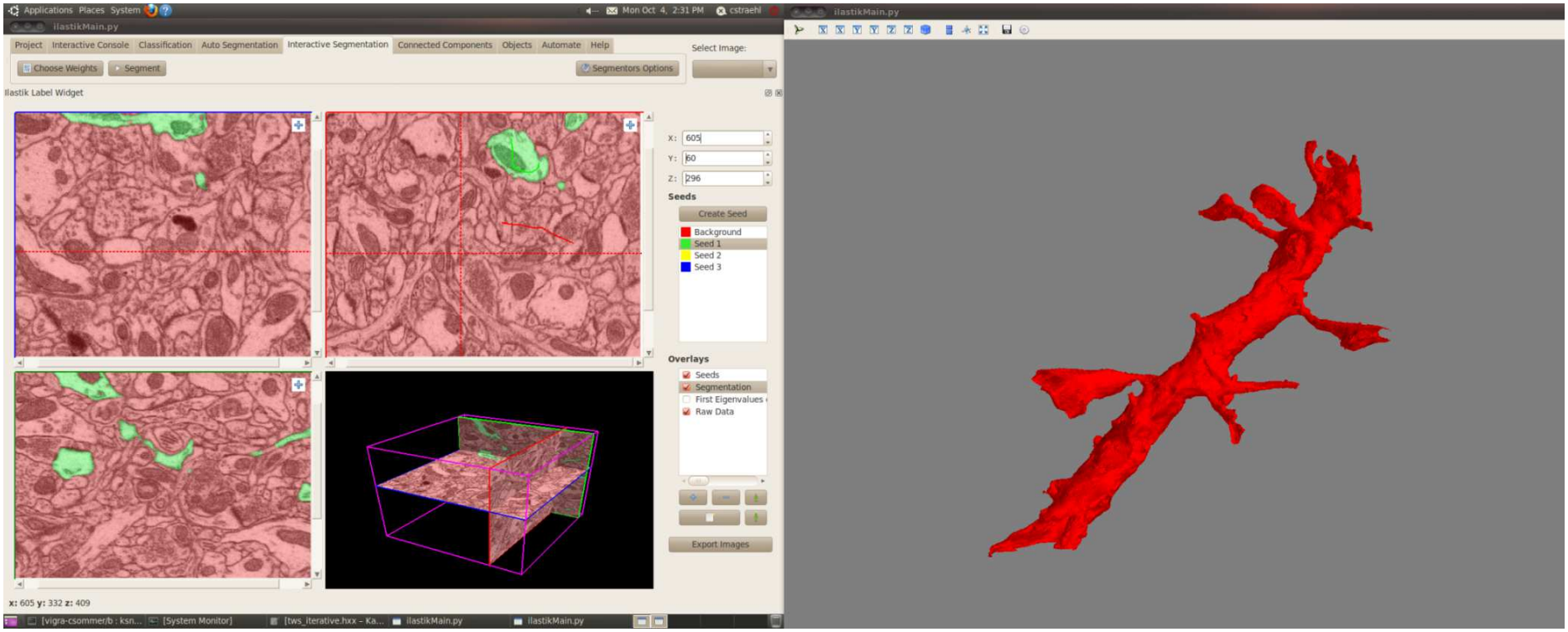


Reconstruction:
Christel Genoud

2weeks of work

Automated segmentation of neuronal structures

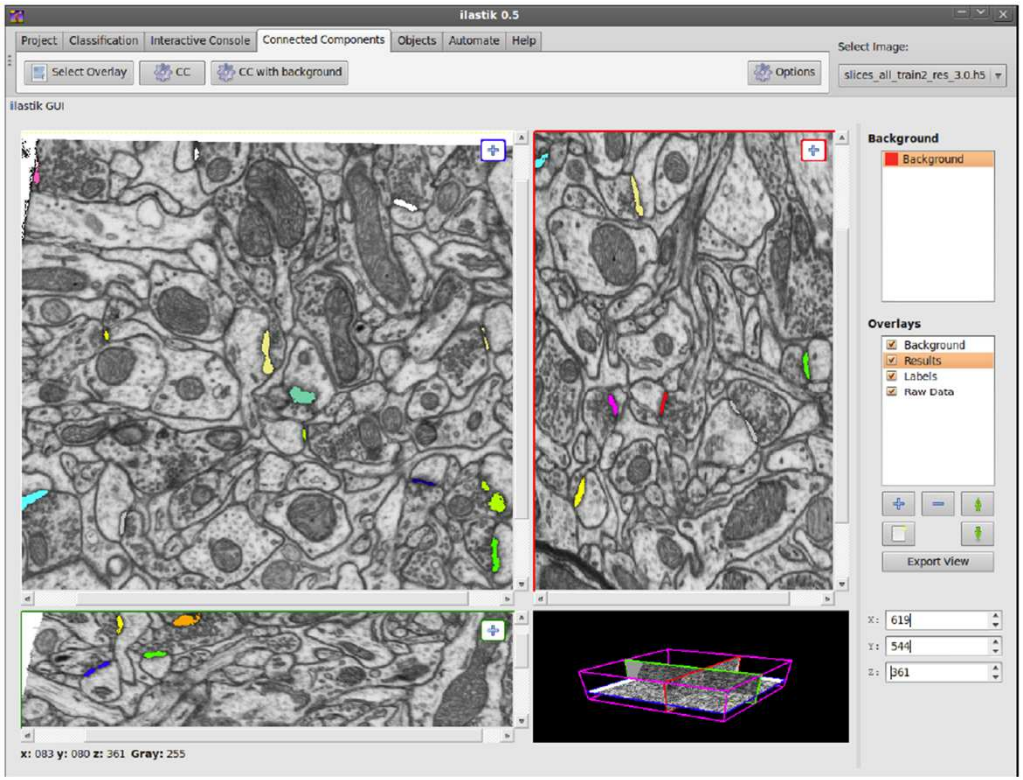
Ilastik v0.5 - Fred Hamprecht, University of Heidelberg

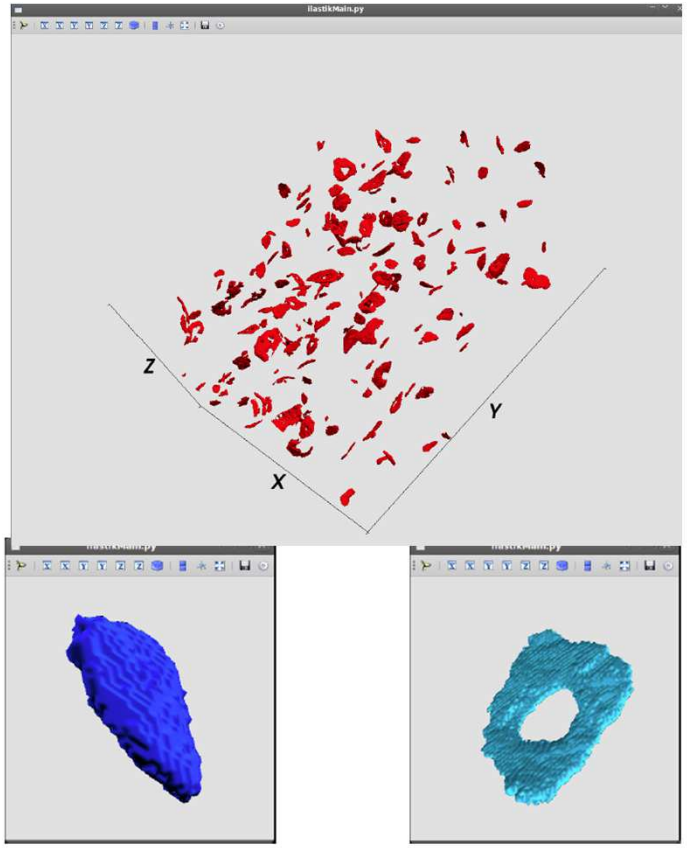


Automated segmentation of neuronal structures

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)

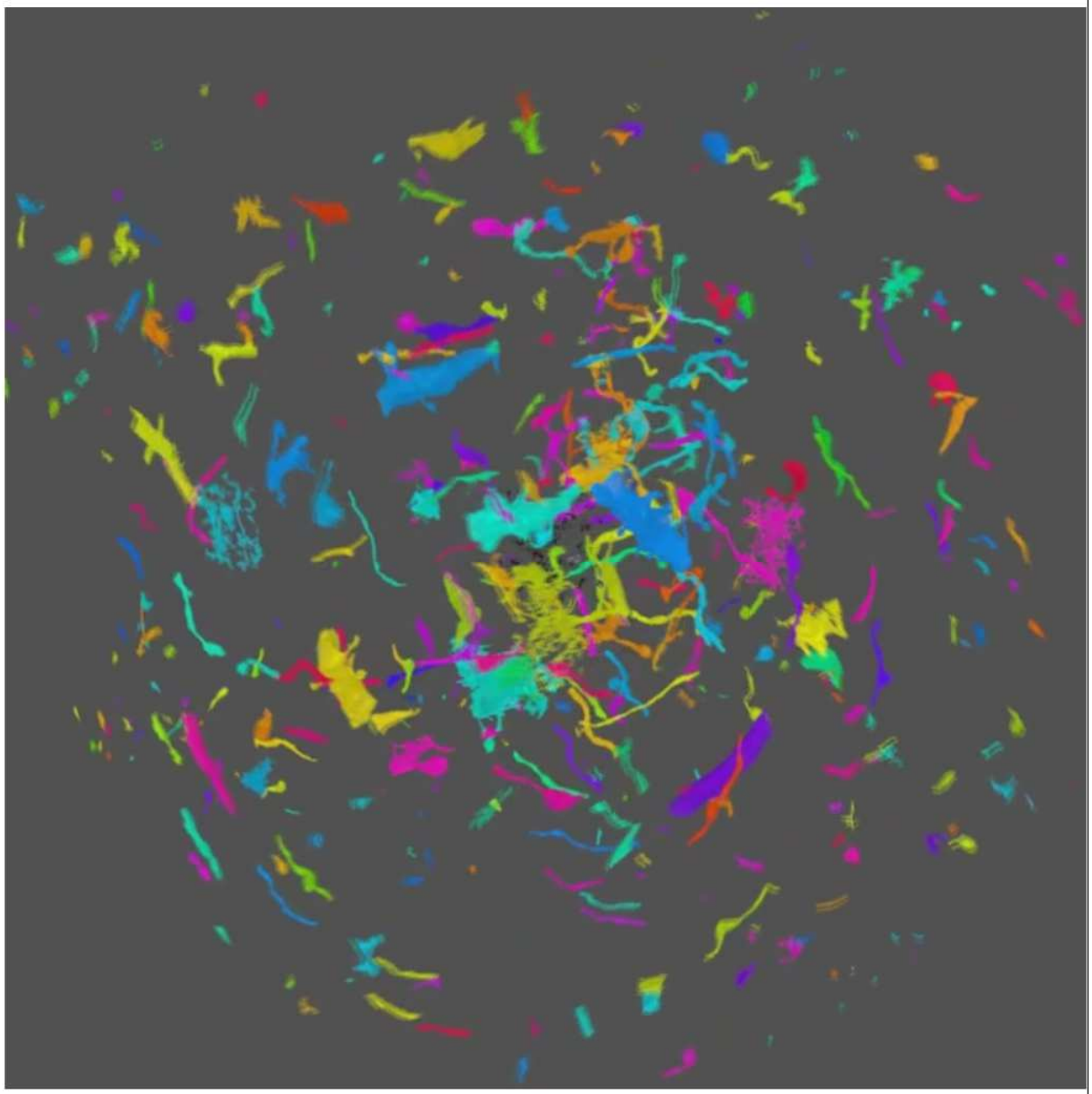


Image processing in 3D

- 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)