Application diverses 3D…
…en sciences des matériaux et sciences de la vie

Marco Cantoni

Centre Interdisciplinaire de Microscopie Electronique (EPFL-CIME)

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Since August 2008: NVision 40

e-beam: ZEISS Gemini
1-30kV, 1nm @ 30kV, 2.5nm @1 kV

Ion-beam: 1-30kV, 4nm @ 30kV

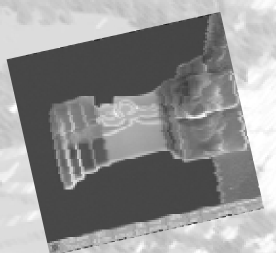
EDS X-MAX (SDD) 80mm² detector

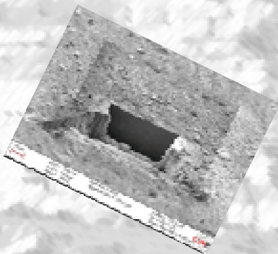
Kleindiek micromanipulator (TEM prep)

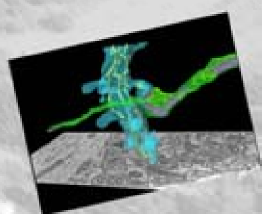
>4000 Ga beam hours (~2 sources) / year

FIB Applications @ CIME

- **Materials Science:**
  TEM Lamellae preparation
  cross-sectioning, 3D reconstruction
  3D EDX (in collaboration with ZEISS)
  3D reconstruction of biocompatible materials
- **Life Science:**
  Serial sectioning of Brain tissue:
  SUPER-STACKS







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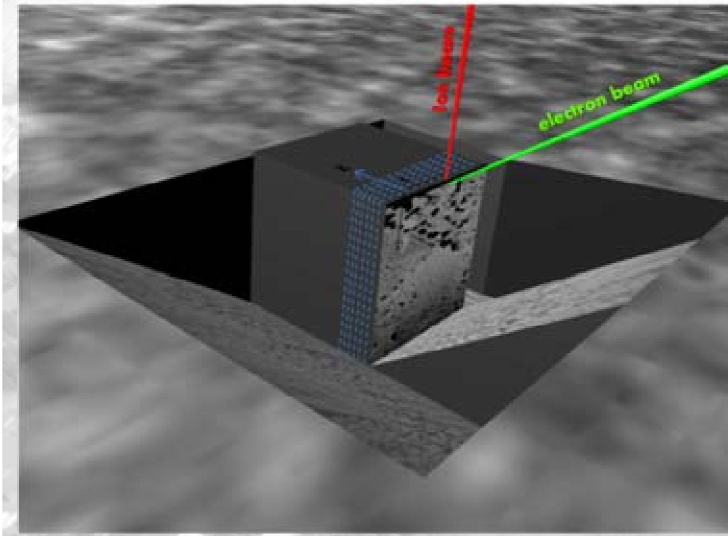
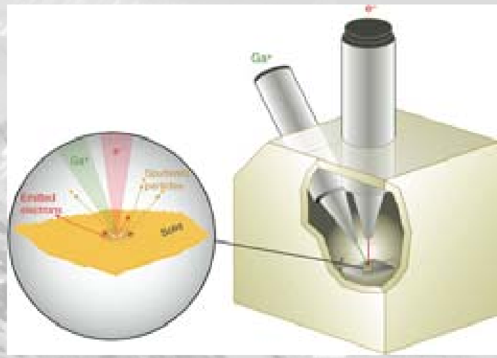
3D FIB/SEM: volume reconstruction

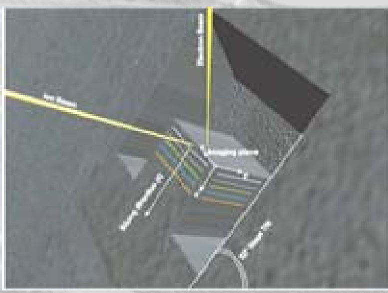


Three-dimensional analysis of porous $BaTiO_3$ ceramics using FIB nanotomography

L. HOLZER, F. INDUTNYI, PH. GASSER, B. MÜNCH & M. WEGMANN

Journal of Microscopy, Vol. 216, Pt 1 October 2004, pp. 84–95

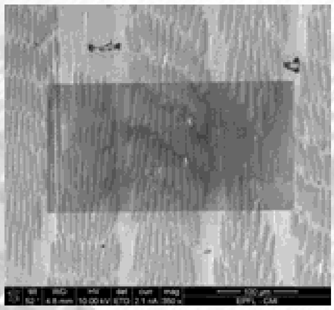


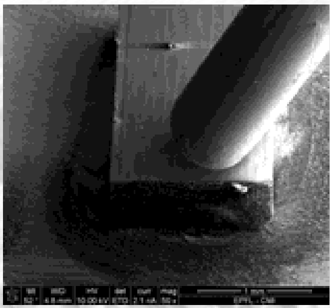


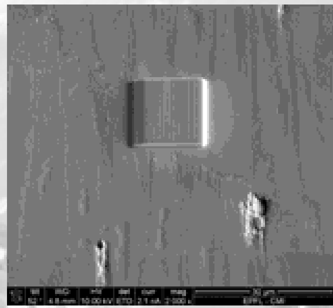
key to success:

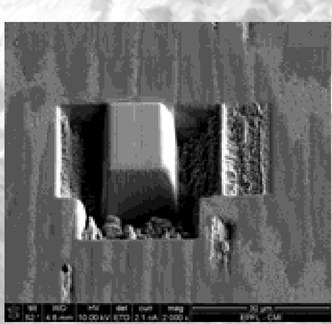
- Choice of parameters
  - What do I need resolution/pixel/voxel
  - Conditions of the microscope: HT, current
  - Detector(s): Which image information do I need for a successful segmentation ?
- Stable environment !
- Segmentation, image processing !
- Specimen preparation !

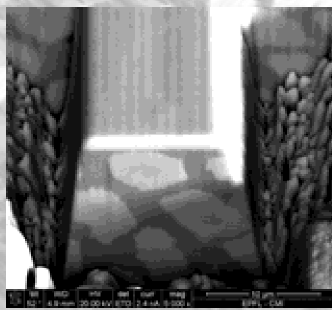
3D slicing of multifilament Nb_3Sn superconductor

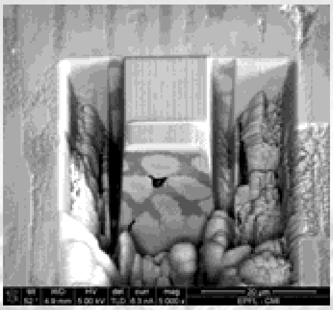




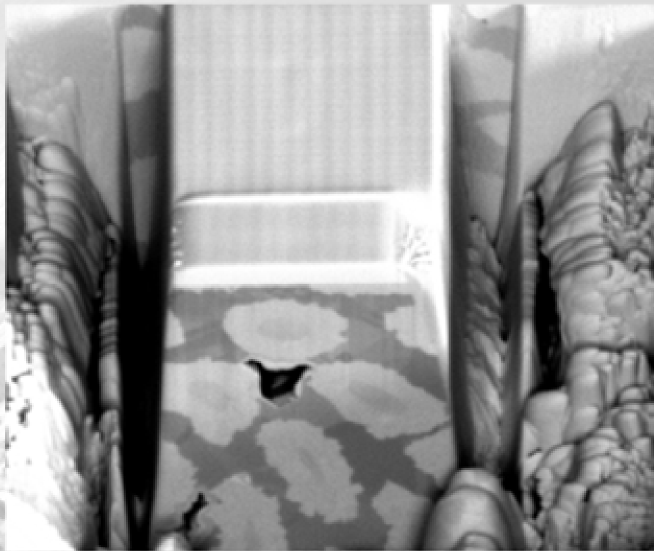






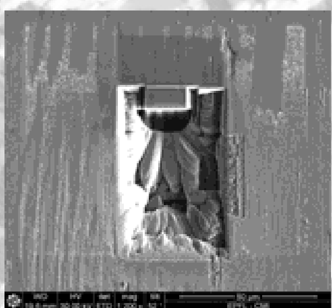


Preparing for slicing



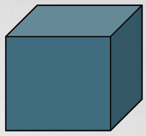
Automated milling and imaging of 170 slices (10h)

the end



3D FIB/SEM: volume reconstruction

"Leitmotiv"
Isometric voxel size x = y = z



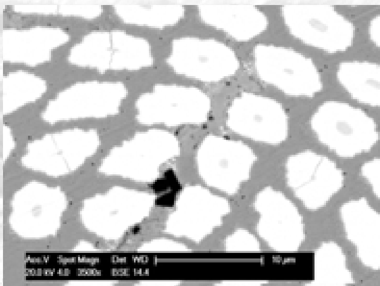
- **Slice thickness (z) = image pixel size (x,y)**
  Z dimension ~ X or Y, typical: 10nm, possible 5nm (3nm)
- **Image dimensions** / data size (8-bit grey level tiff):
  - 1024 x 786: 800 slices -> 640 Mb
  - 2048 x 1572: 1600 slices -> 5 Gb
  - 3096 x 2358: 3000 slices -> 21 Gb
- **Acquisition time ~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 !!!

choice of parameters

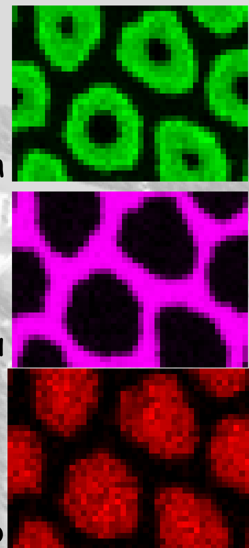
Nb_3Sn multifilament superconducting cable

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

Solid State BSE detector
20kV acceleration voltage



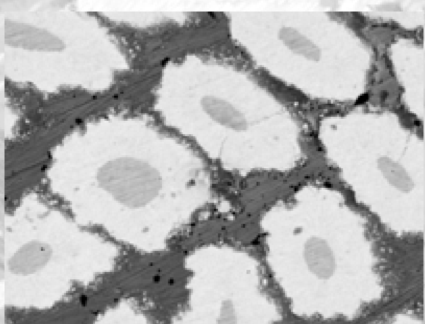
EDX maps

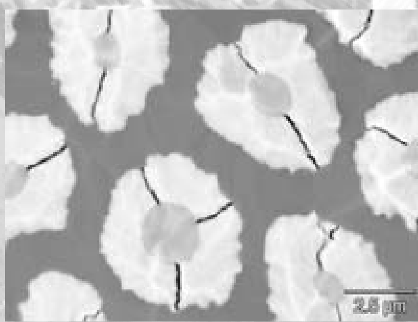


Sn

Cu

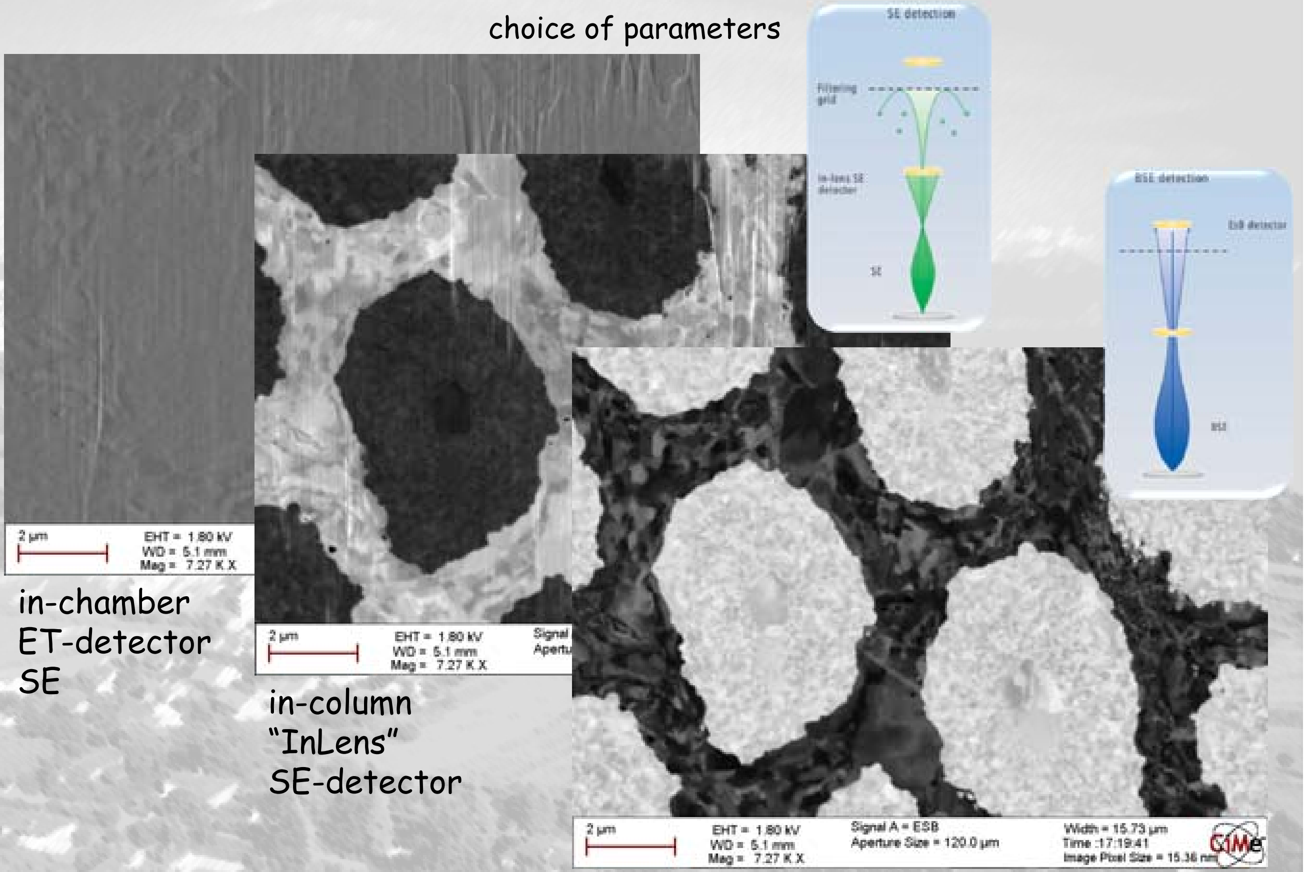
Nb





Mechanical polishing <-> Ar ion beam polished

choice of parameters

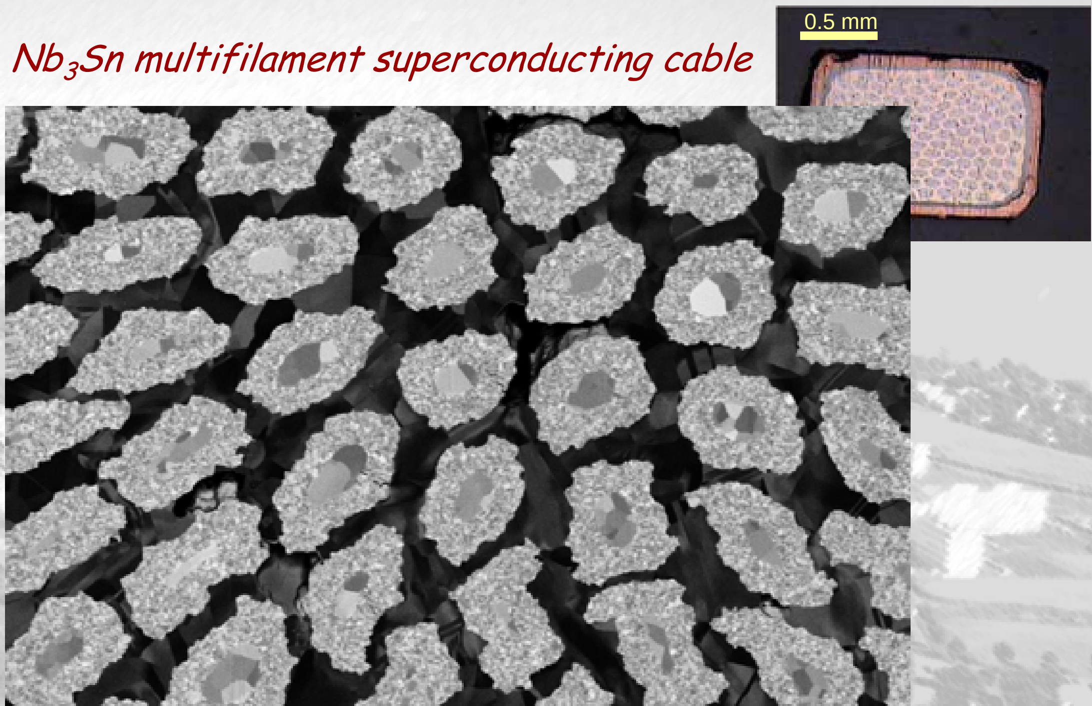


in-chamber
ET-detector
SE

in-column
“InLens”
SE-detector

in-column, “energy-selective” EsB
BSE-detector

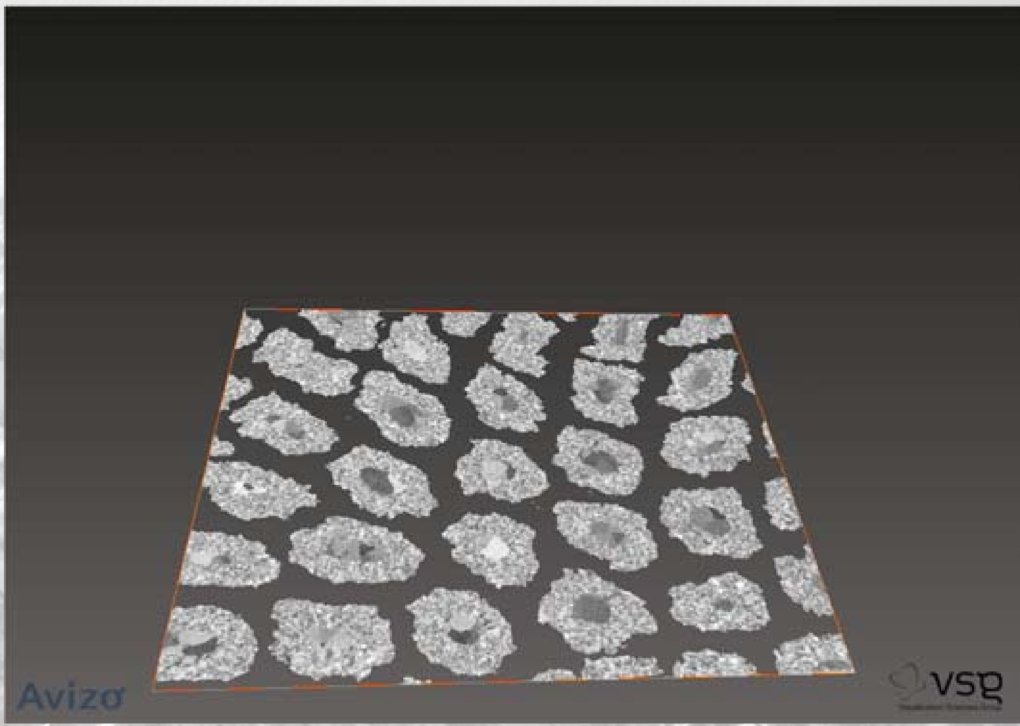
Nb_3Sn multifilament superconducting cable



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

1.8kV EsB detector: Materials & orientation contrast

3D Microanalysis by FIB/SEM volume reconstruction



Materials & grain contrast

2048x1536x1700, (10x10x10nm voxel), 28hours

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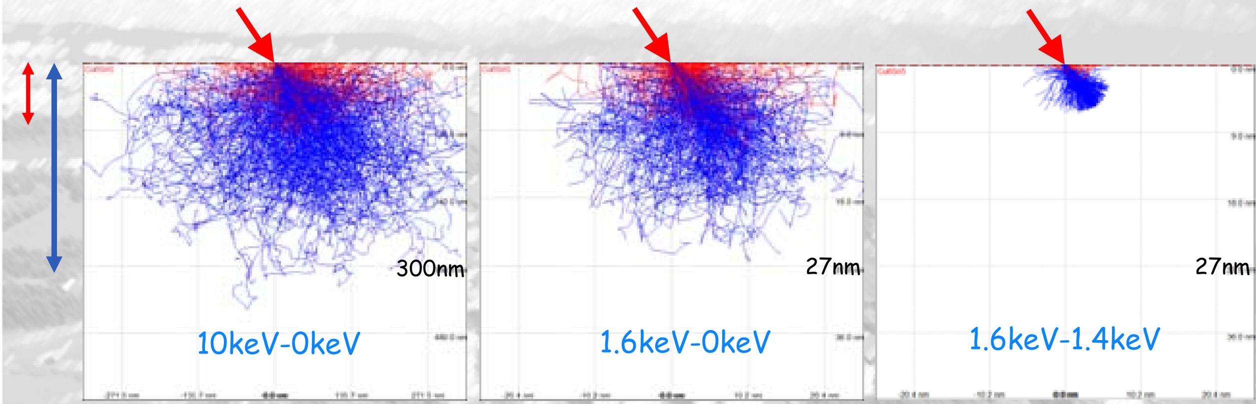
CIME

3D FIB/SEM: volume reconstruction

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

Scatter range in Nb_3Sn:
Monte-Carlo Simulation (CASINO 2.42) of electron trajectories
backscattered electrons

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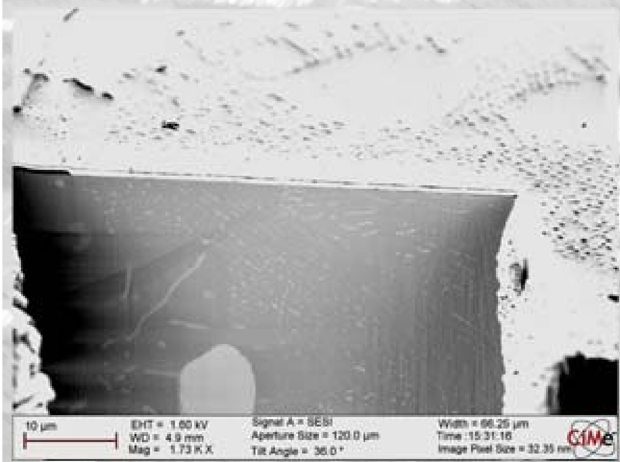
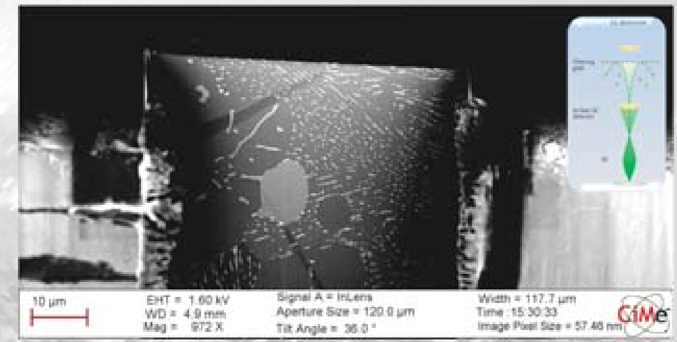
CIME

Pb-free solder:
"one detector is not enough"

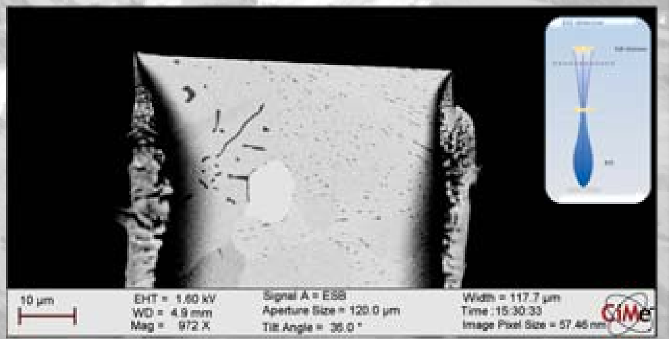
M. Maleki,
EPFL-LMAF



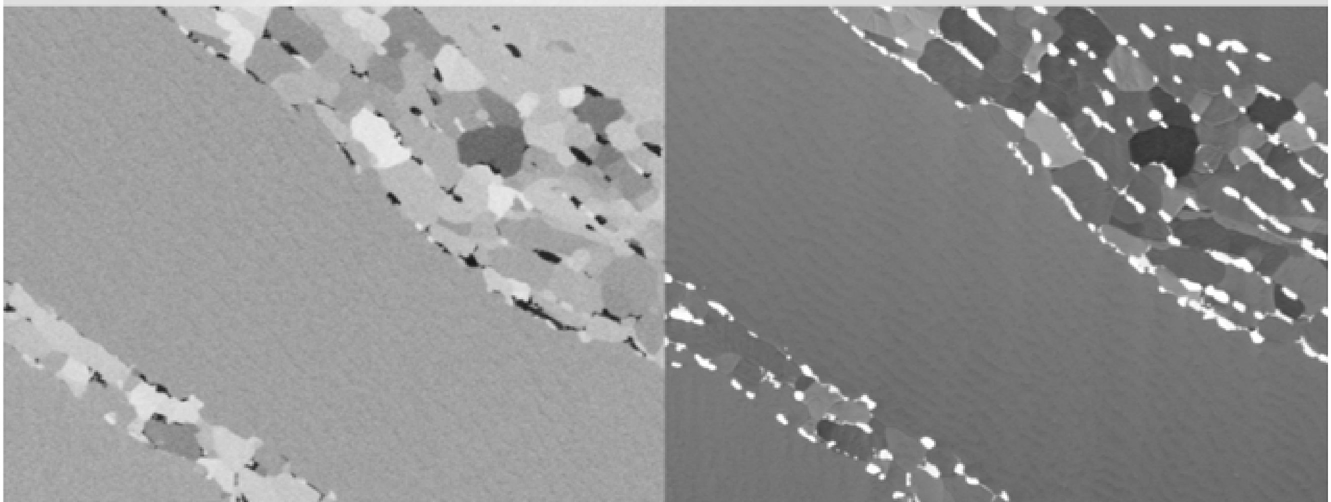
ETD (SE classic)



InLens: SE low energy

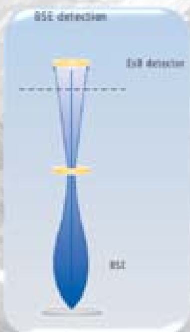


EsB: Energy selective Backscattered

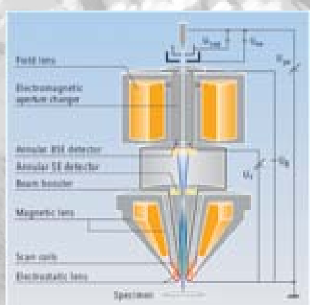


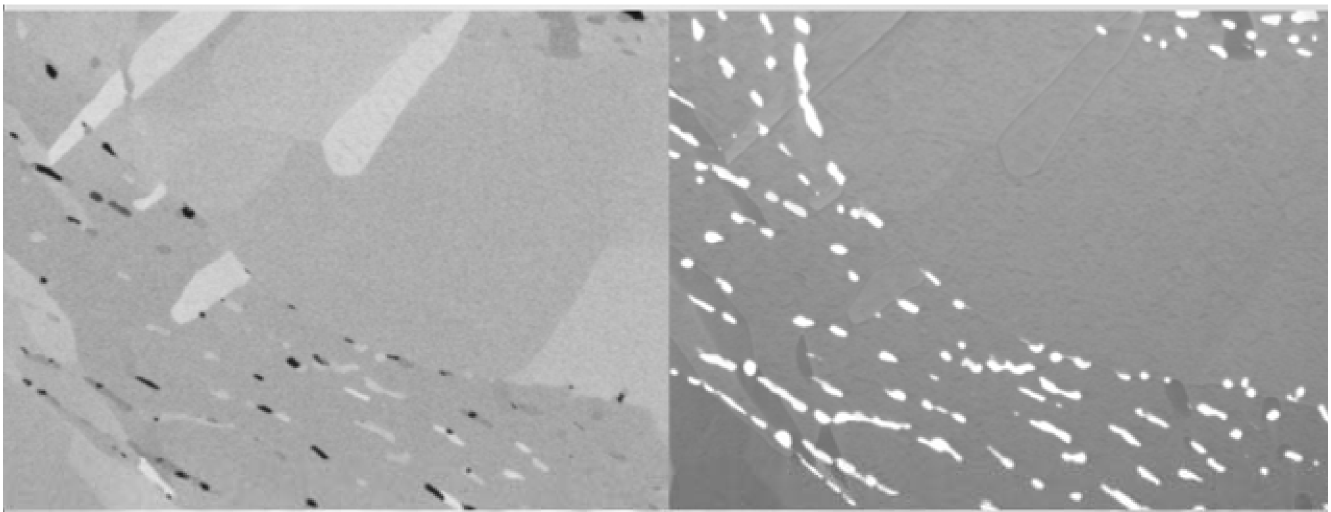
EsB

InLens SE



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





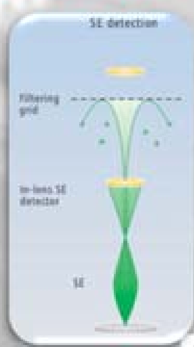
EsB

InLens SE

10x10x10nm voxel size, 2048x1536x2000

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

12Gb data



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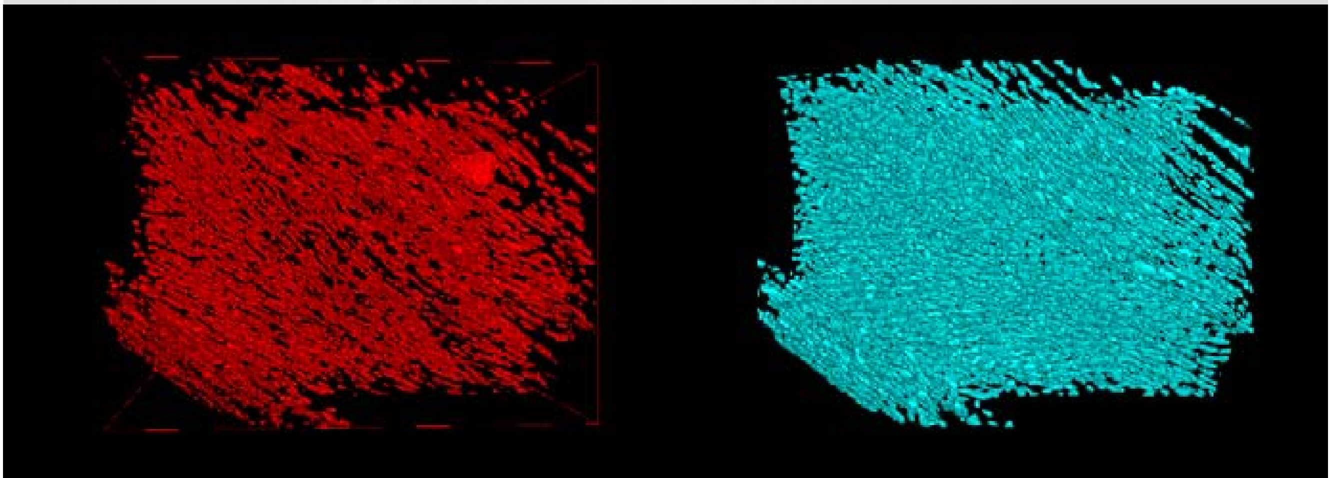

Phase 1

Dark in EsB image

White in SE-InLens

Phase 2

White in SE-InLens - Dark in EsB image

10x10x10nm voxel size, 2048x1536x2000 pixel/slices

2 images (3Mb) / slice ...... 12Gb data

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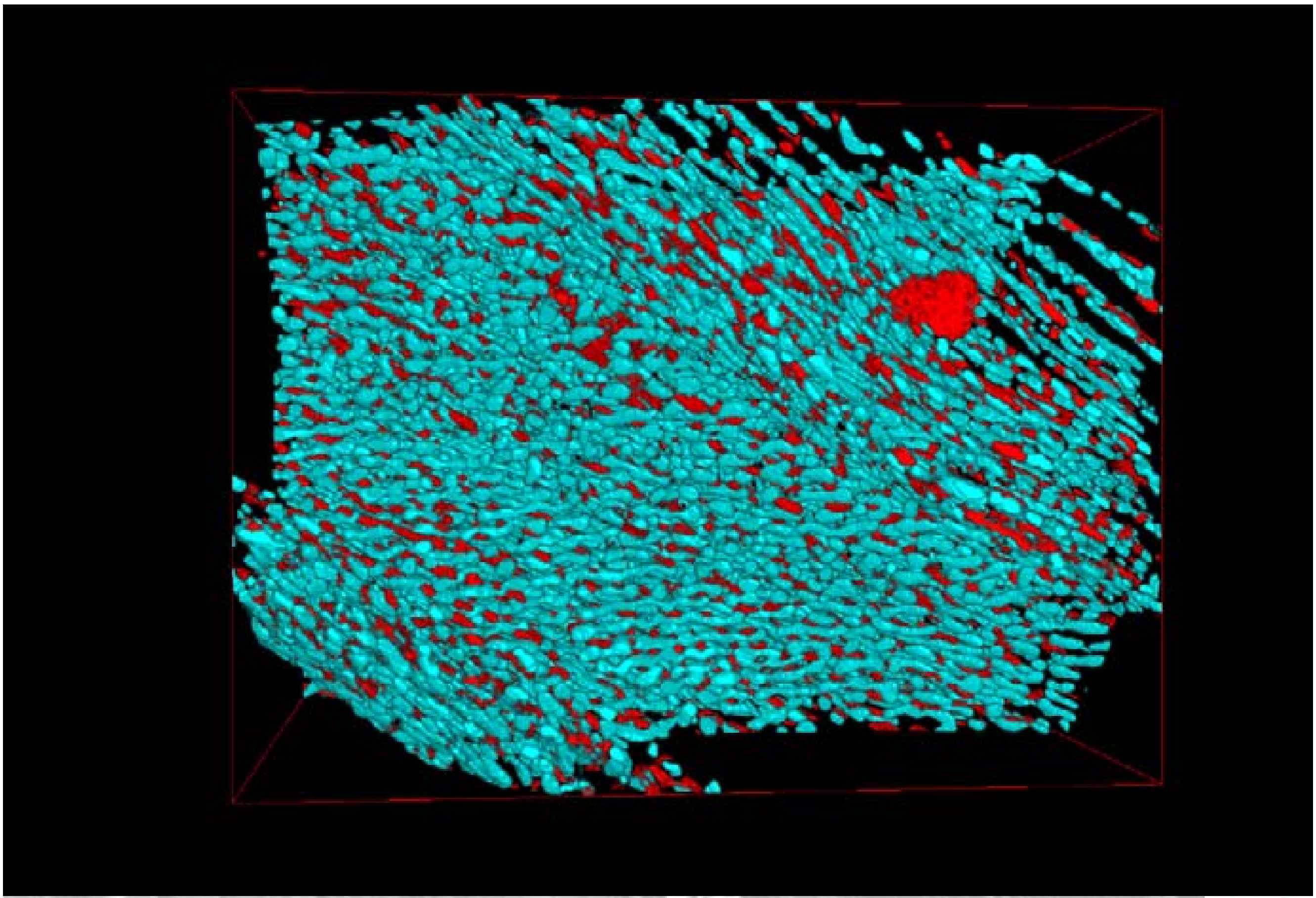

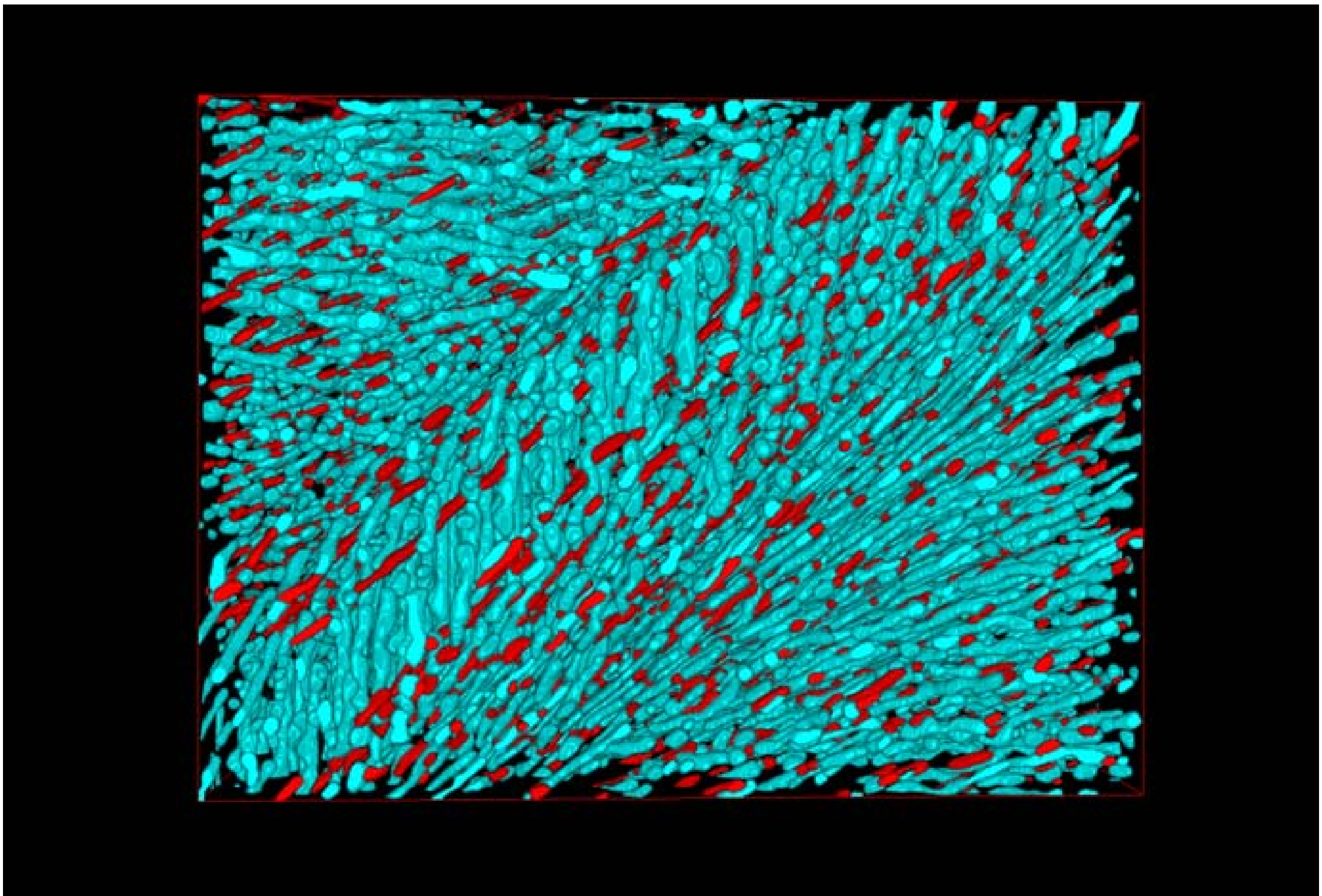

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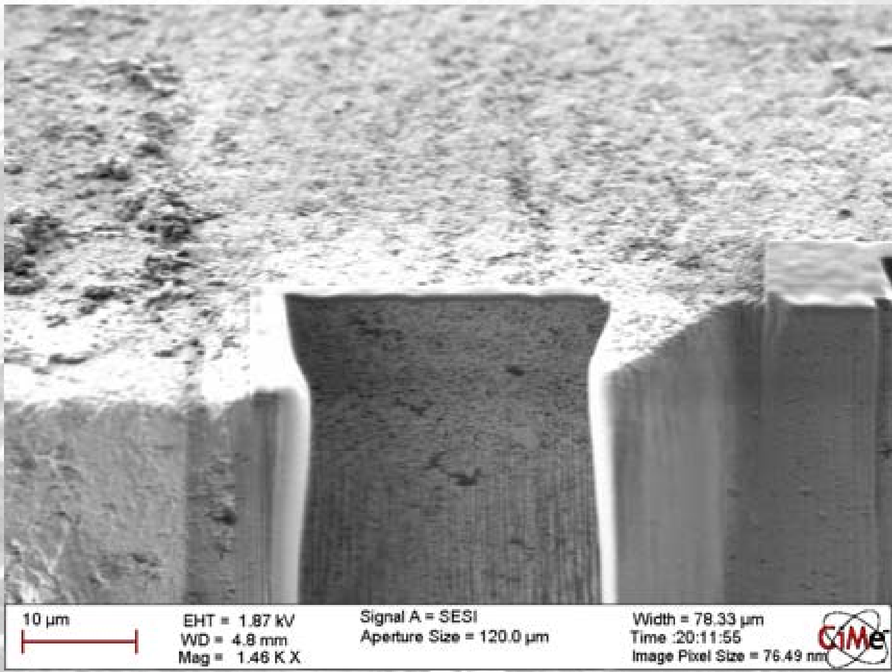
Solid Oxide Fuel Cell cathode

P. Tanasini, LENI



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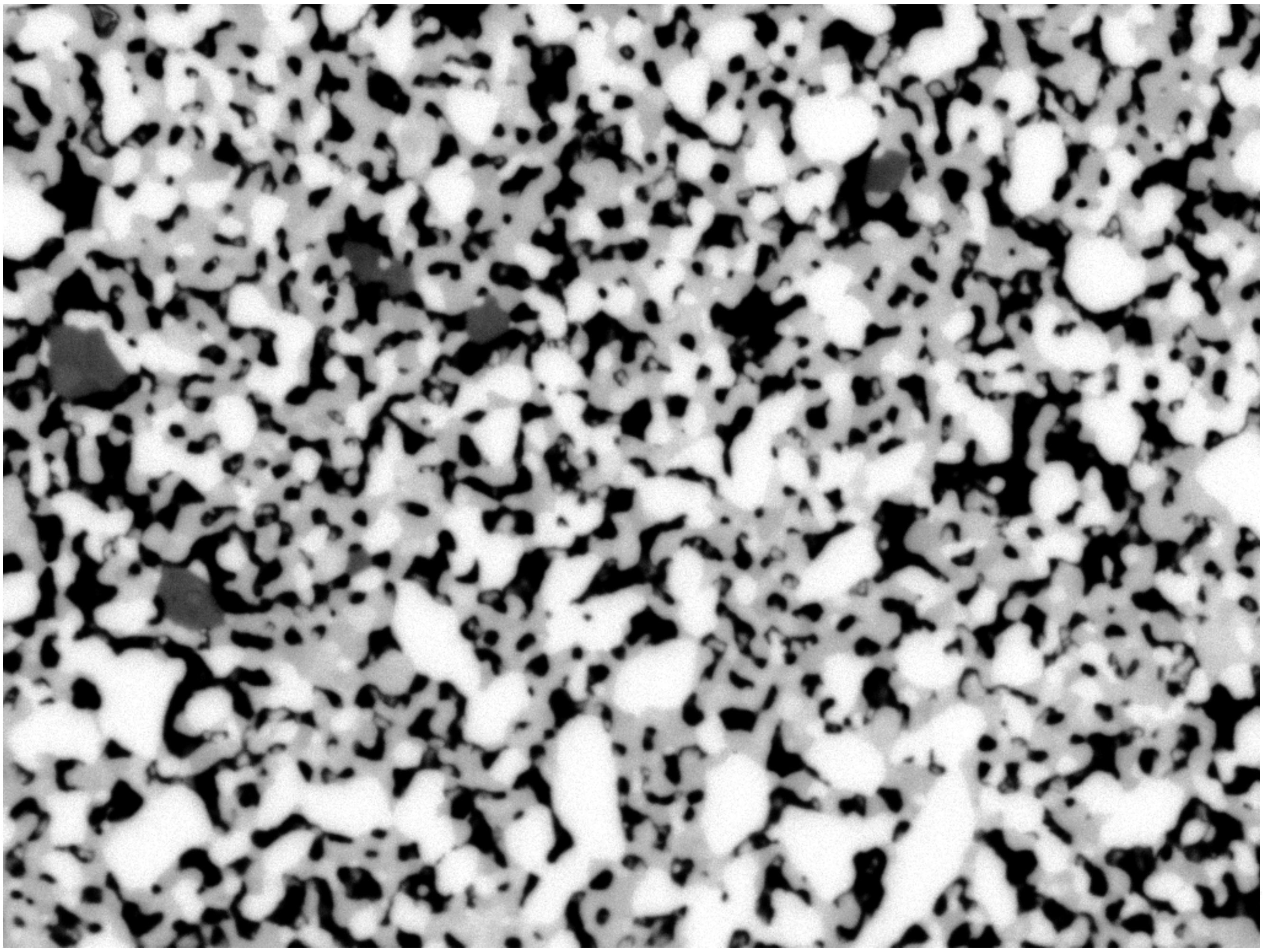

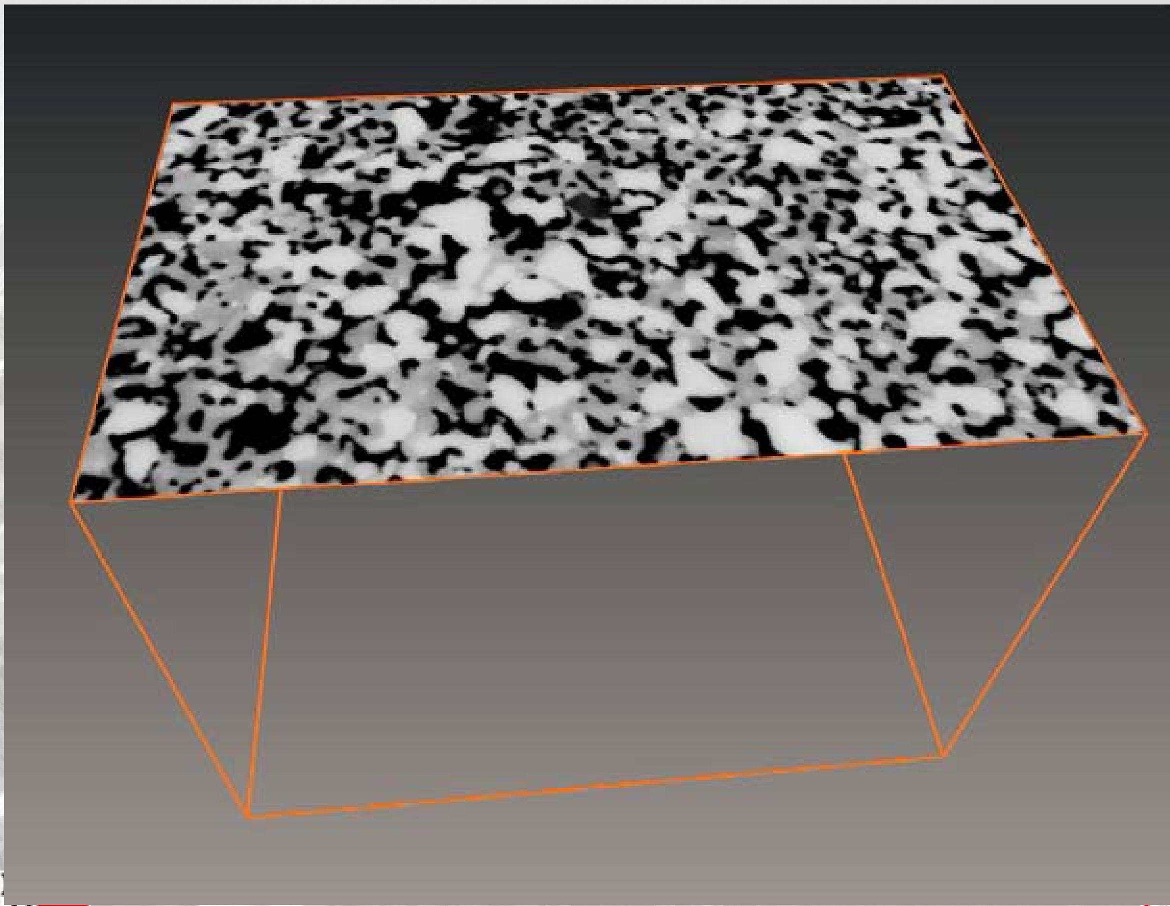

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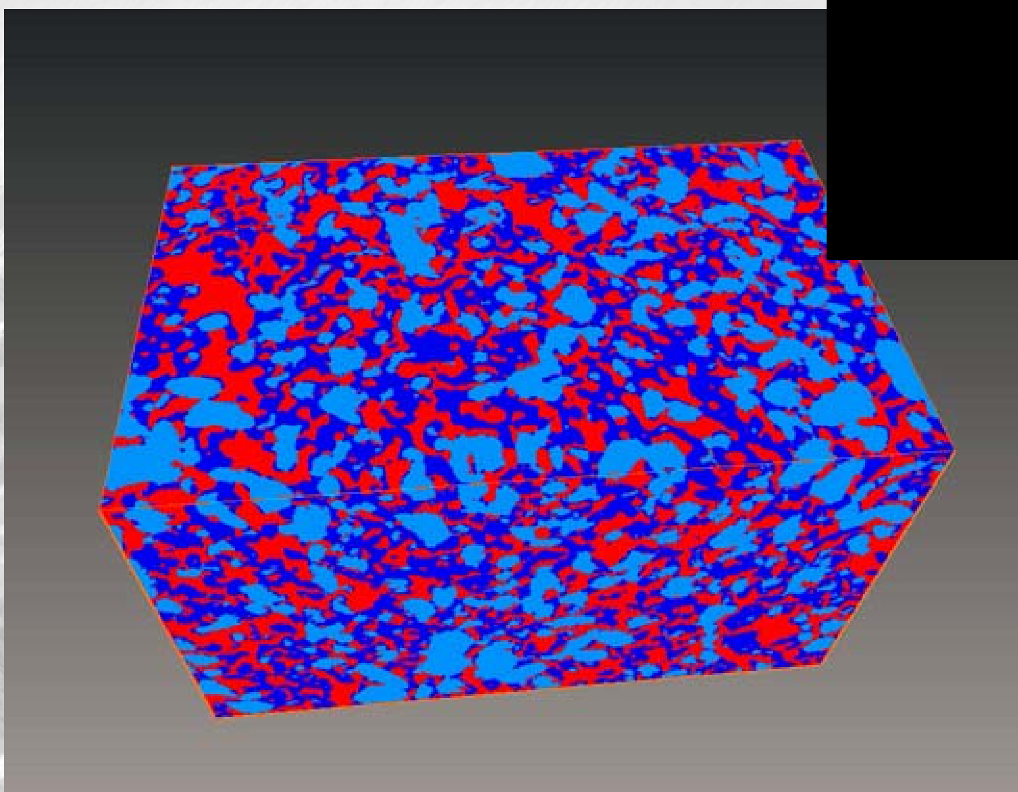
CiMe



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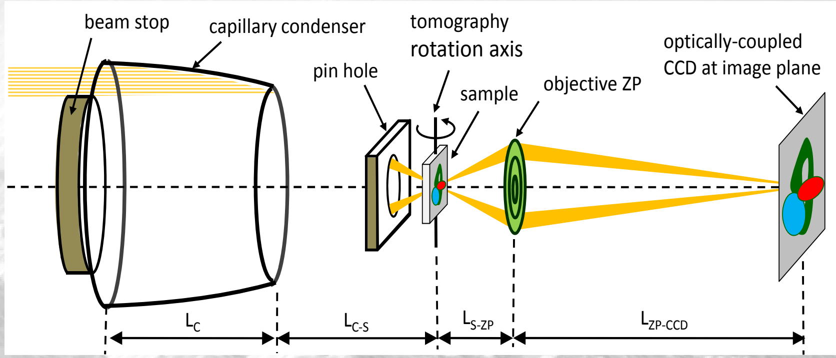
FÉDÉRALE DE LAUSANNE

CiMe

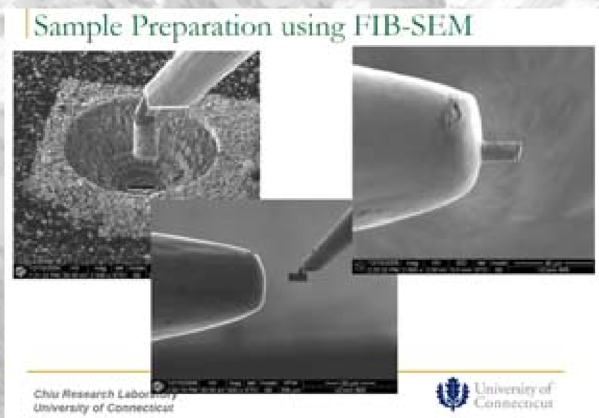
Comparison with Transmission X-ray Microscopy (TXM)



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

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



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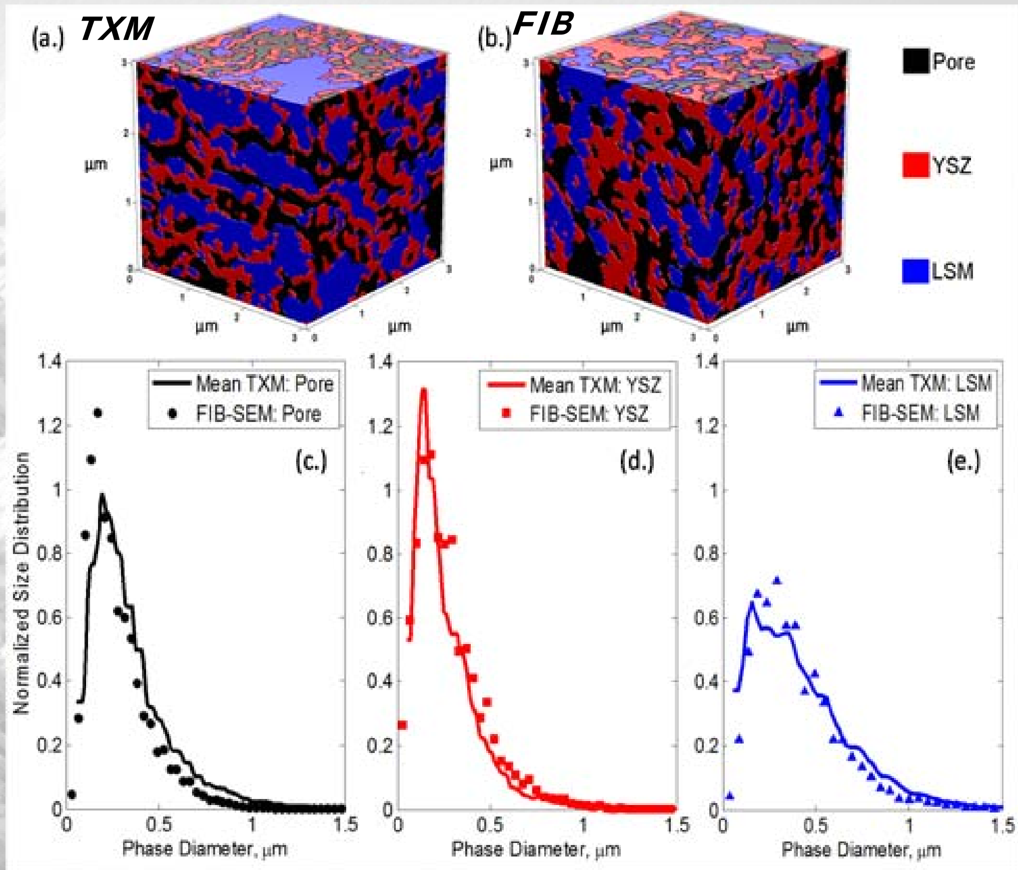

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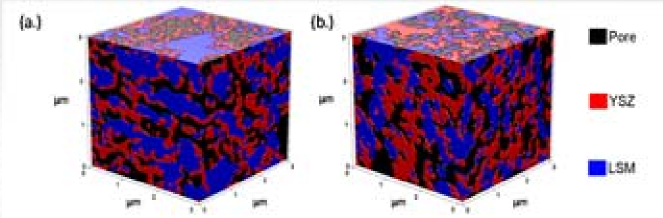


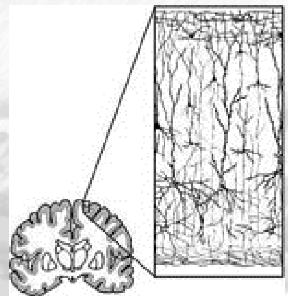
Table 1. Microstructural characterization results for the cathode samples obtained using x-ray nanotomography and FIB-SEM. The FIB-SEM results were down-sampled using 3×3×3 binning to allow comparison at comparable pixel size.

Parameters and Phases	X-ray Nanotomography		FIB-SEM	
Mean Phase Diameter (μm)				
Pore	0.34 (±0.045)		0.27 (±0.030)	
YSZ	0.27 (±0.045)		0.28 (±0.030)	
LSM	0.42 (±0.045)		0.40 (±0.030)	
Volume Fraction				
Pore	0.34		0.34	
YSZ	0.32		0.33	
LSM	0.34		0.33	
Interfacial Characteristics	Total	Effective	Total	Effective
TPB Length (m m^{-3})	6.6E+13	6.0E+13	8.3E+13	7.0E+13
Interfacial Area (m m^{-2})				
Pore-YSZ	7.7E+06	7.6E+06	7.4E+06	7.3E+06
Pore-LSM	2.9E+06	2.7E+06	3.4E+06	3.1E+06
YSZ-LSM	6.4E+06	6.1E+06	5.7E+06	4.9E+06

George J. Nelson, William M. Harris, Jeffrey J. Lombardo, John R. Izzo, Jr., and Wilson K. S. Chiu[*]
Department of Mechanical Engineering, University of Connecticut

A big Challenge in Life Science

brain research

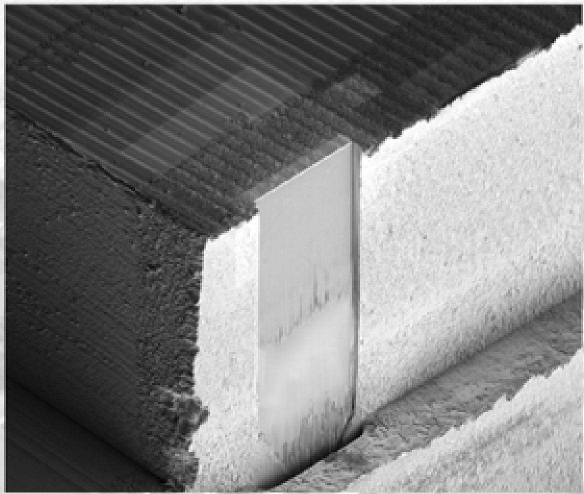


Blue Brain Project

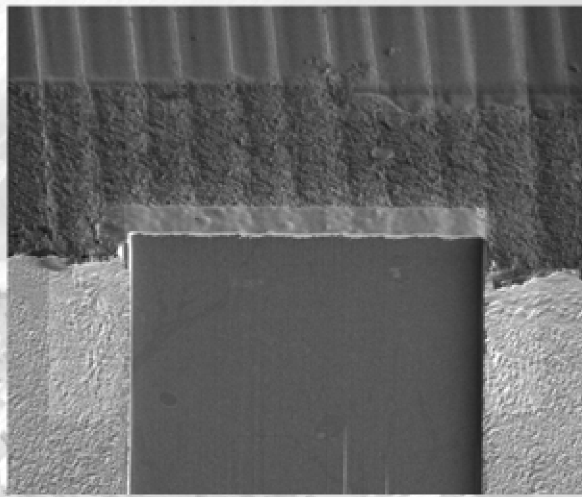
Simulation of brain function with 10'000 neurons

~1'000'000'000 neurons
~1'000'000'000'000 connections

What about FIB…?
Automated acquisition…!



Secondary electron images of mouse brain sample



The imaging window - 100 um width

What do we see….?

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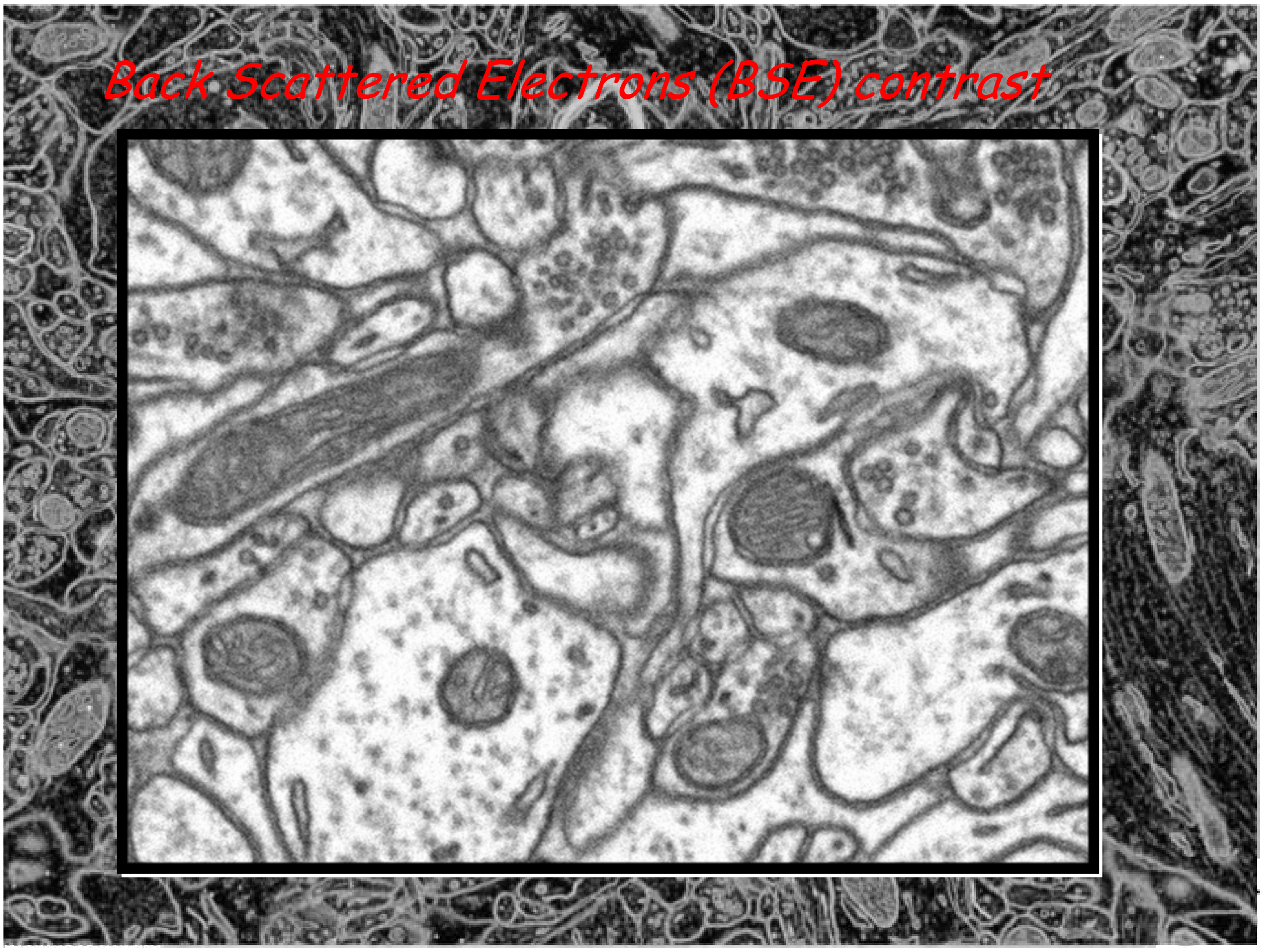
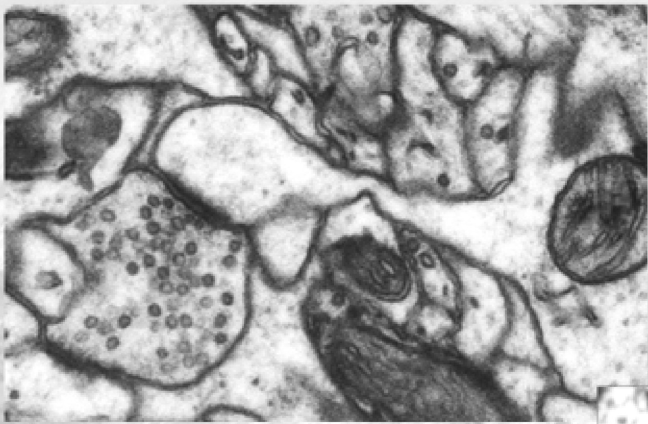
Back Scattered Electrons (BSE) contrast



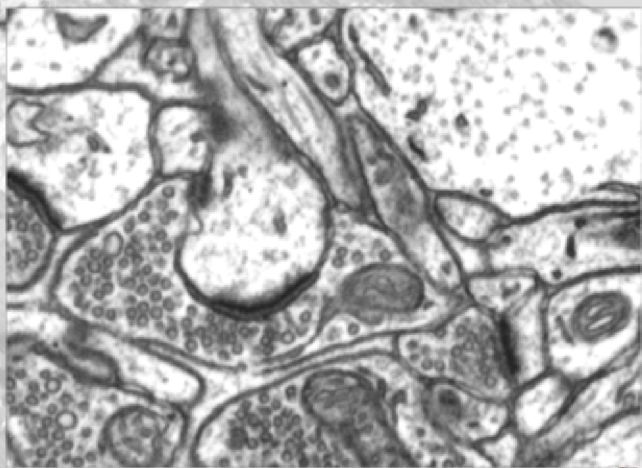
Resolution Bio-FIB…?



TEM , 100kV

thin (50nm) section
prepared by Ultramicrotomy

Brain tissue:
synapse,
vesicles (~50nm)
mitochondria



SEM (FIB) , 1.4kV

"surface", (<5nm escape depth)

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CIME

Which detector…?

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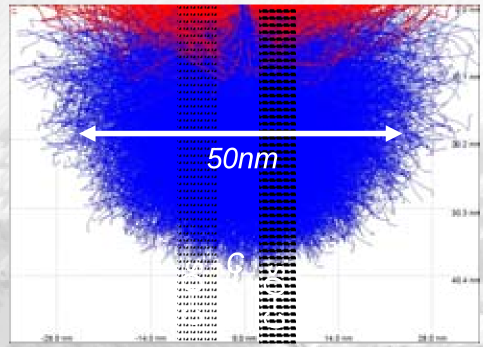
CIME

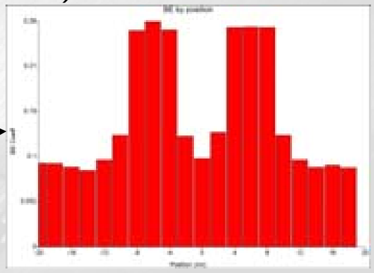
EsB detector: energy filtering, e-beam: 1.5kV

Monte-Carlo Simulations (Casino 2.42)



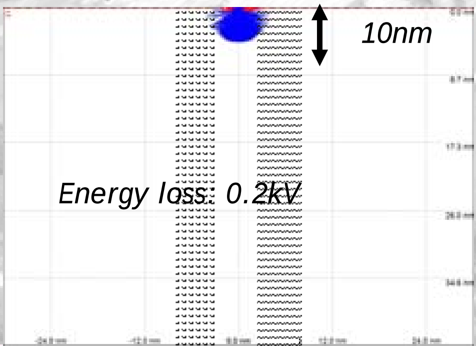
complete energy range

e-beam: 1.5kV

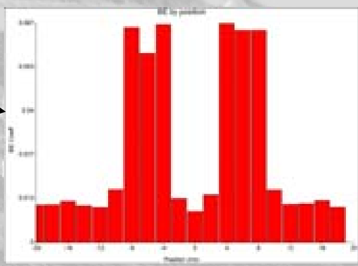


EsB grid 1.3kV

Selection of BSE with Energy loss of 0.2kV



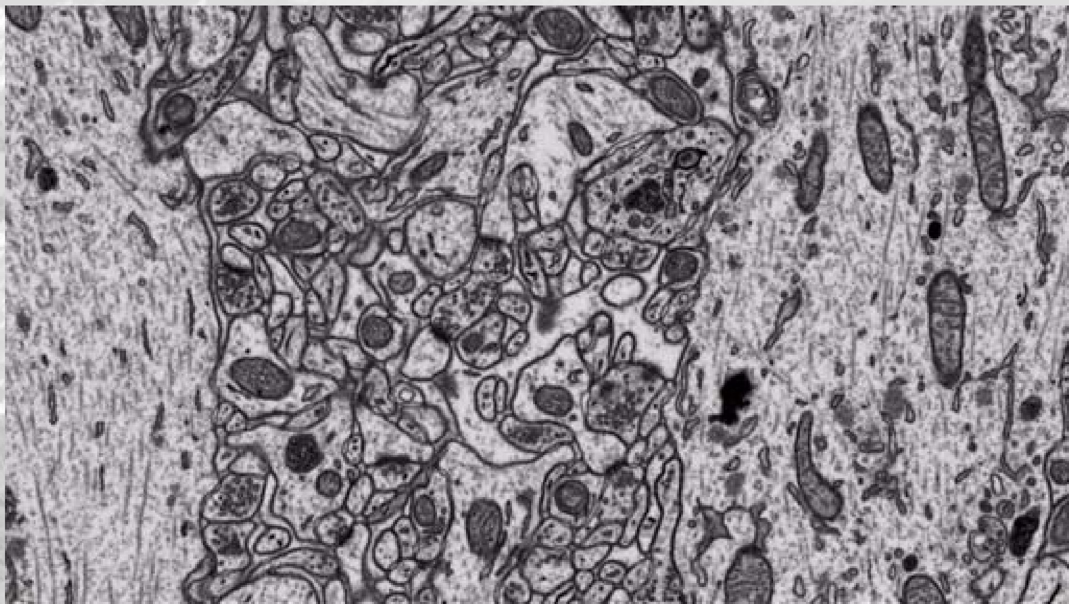
Escape depth of "single" backscattered electrons < 5nm



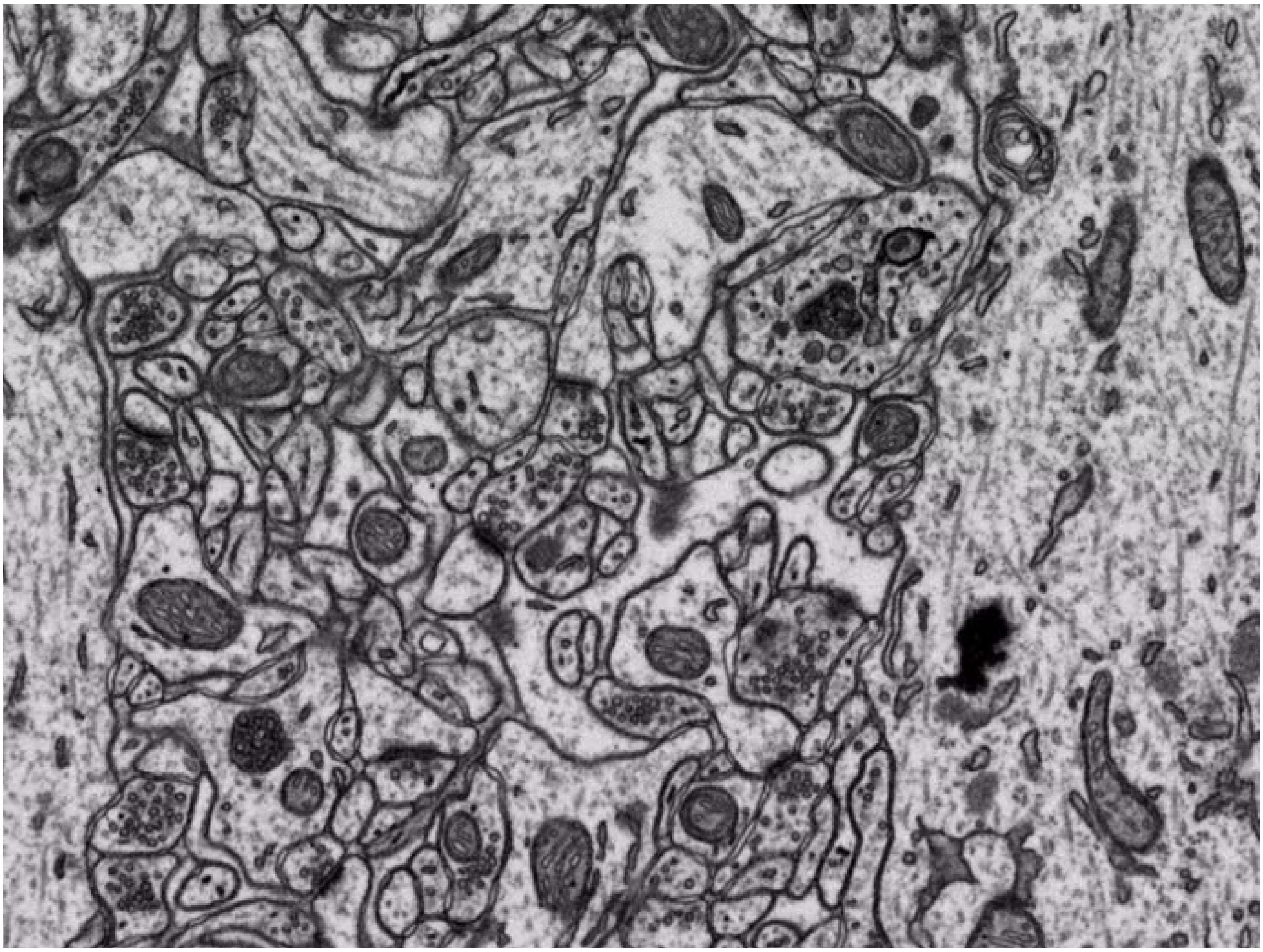
SUPER STACK

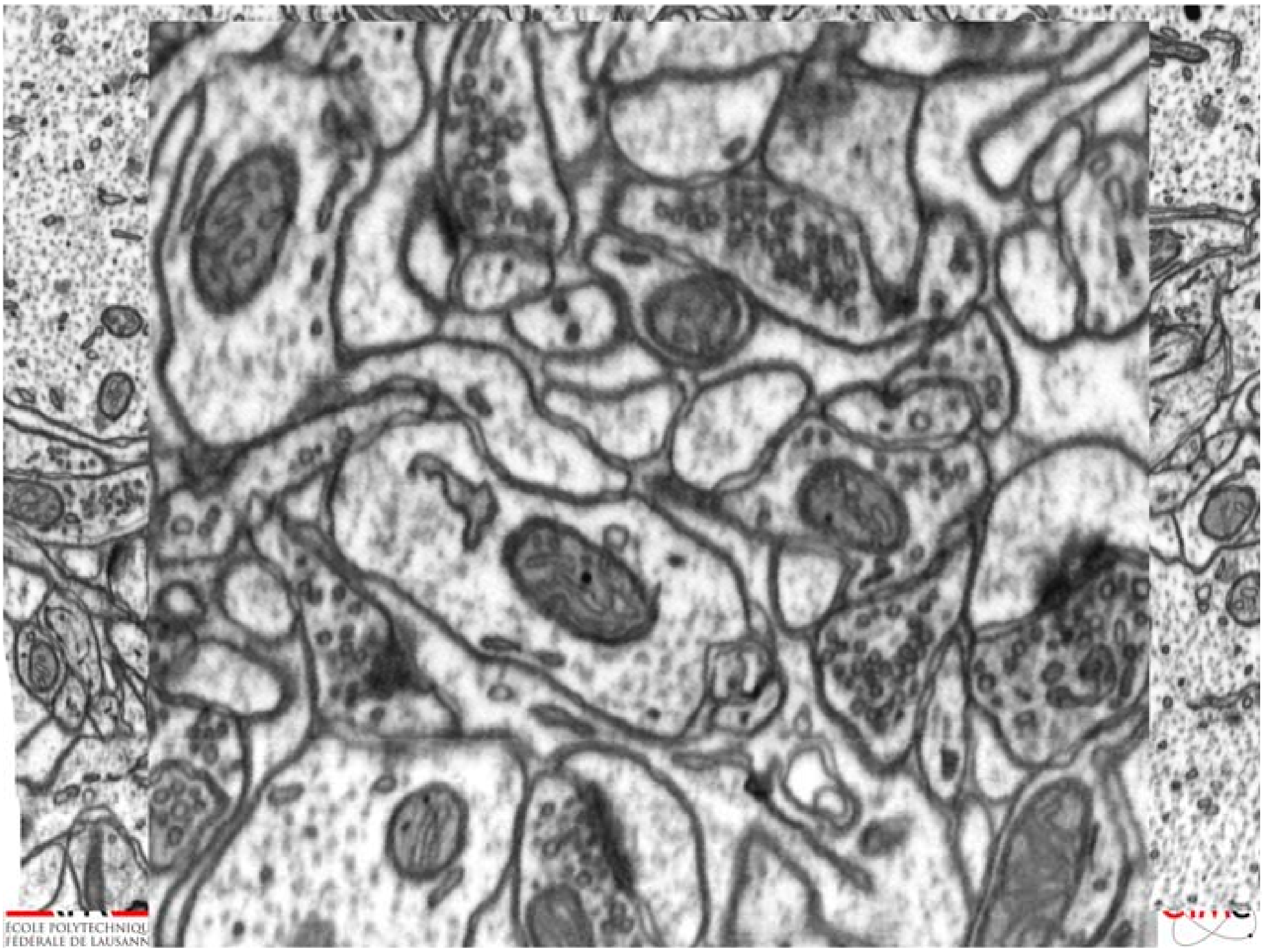
2048 x 1536 x 1600 **Volume: 10 x 8 x 8 um** **voxel: 5x5x5nm**

2 days of fully automated acqusition
5 ~GB of Data



Milling current 700pA,20sec. milling , 1.2min.imaging / slice

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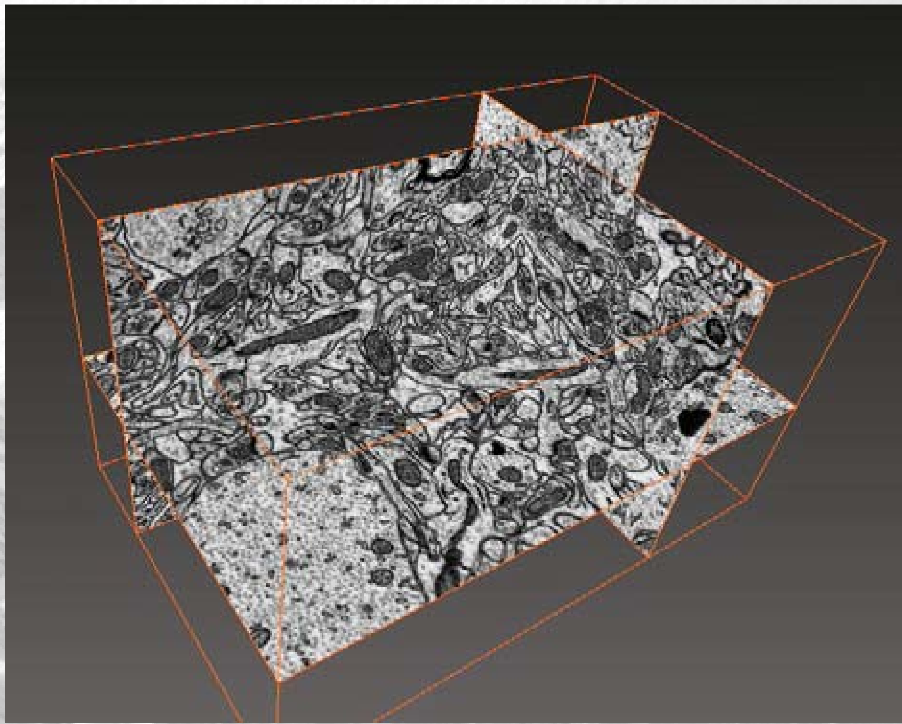
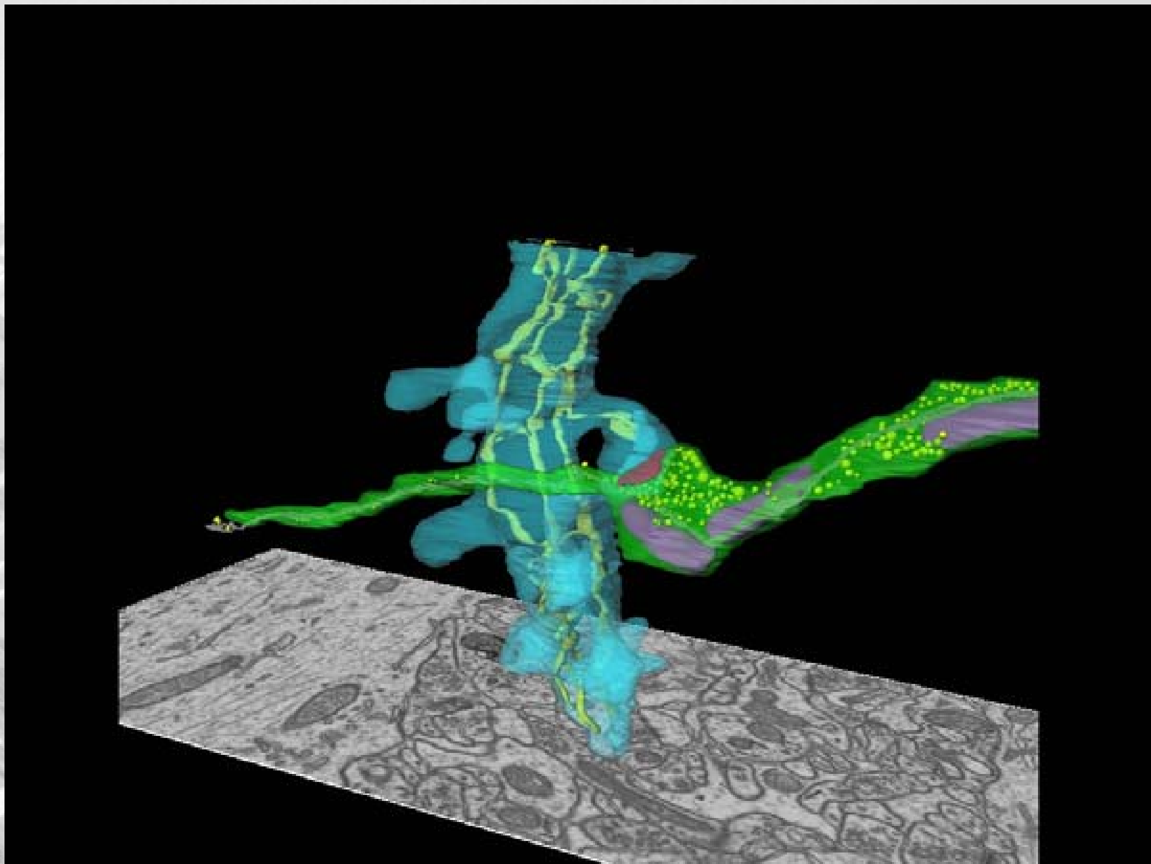
SUPER STACK(S)

2048 x 1536 x 1600 **10 x 8 x 8 um** **voxel: 5x5x5nm**

2 days of fully automated acqusition

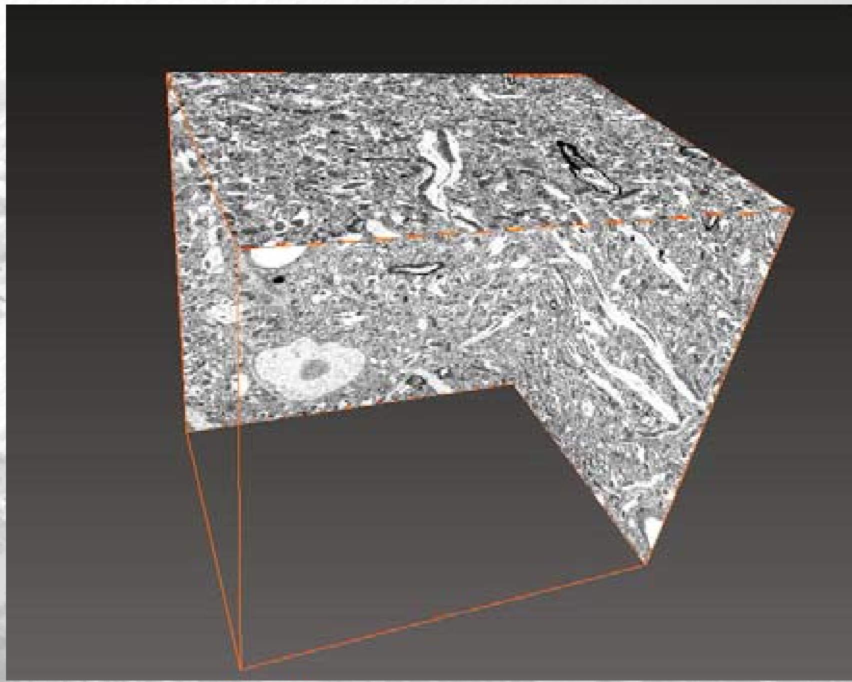




Reconstruction: Christel Genoud

Bigger volumes…!

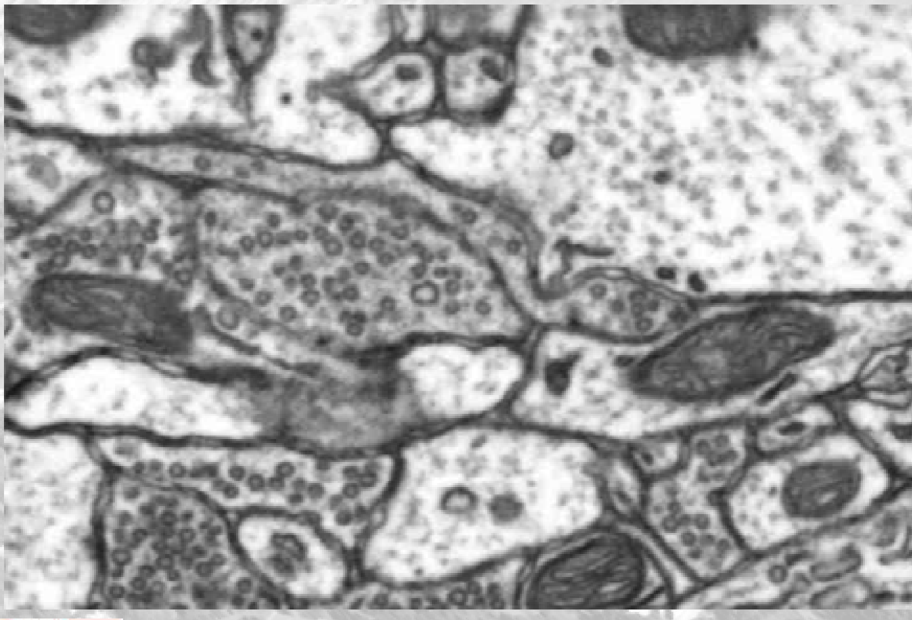
- Voxel: 7.5x7.5x7.5nm
- Image 3096x2304
- 3300 slices (48hours)
- 23x17x24 um
- 9700um^3
- ~7000 synapses
- 23Gb data



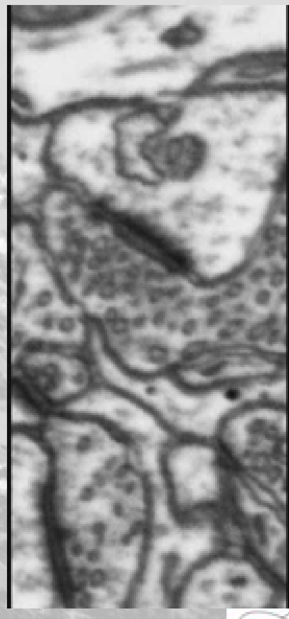
Where is the limit ..?

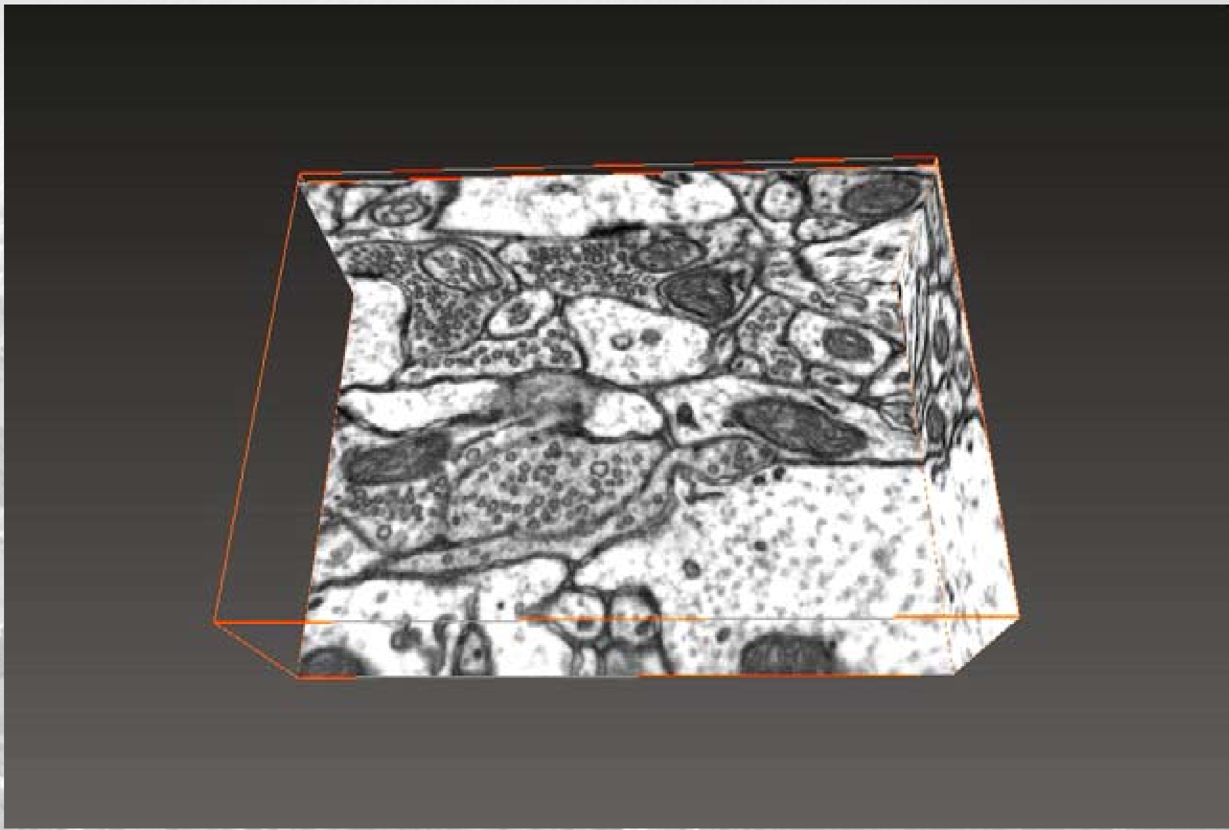
3nm x 3nm x 3nm voxel

X – Y plane
(image)

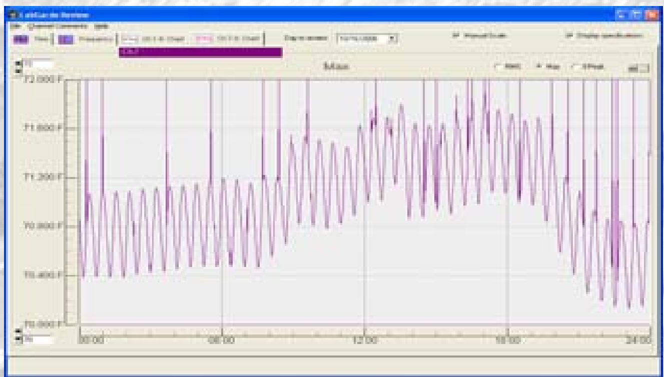


Y -Z plane
(virtual)

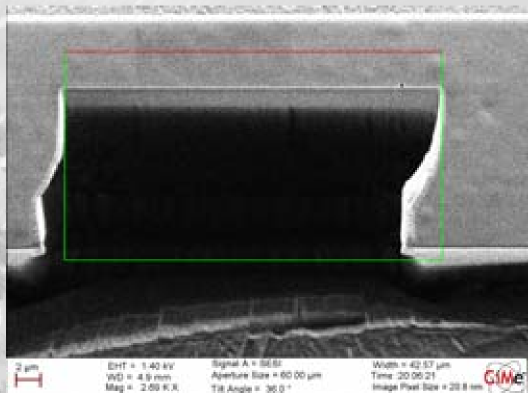




Very important: stable temperature



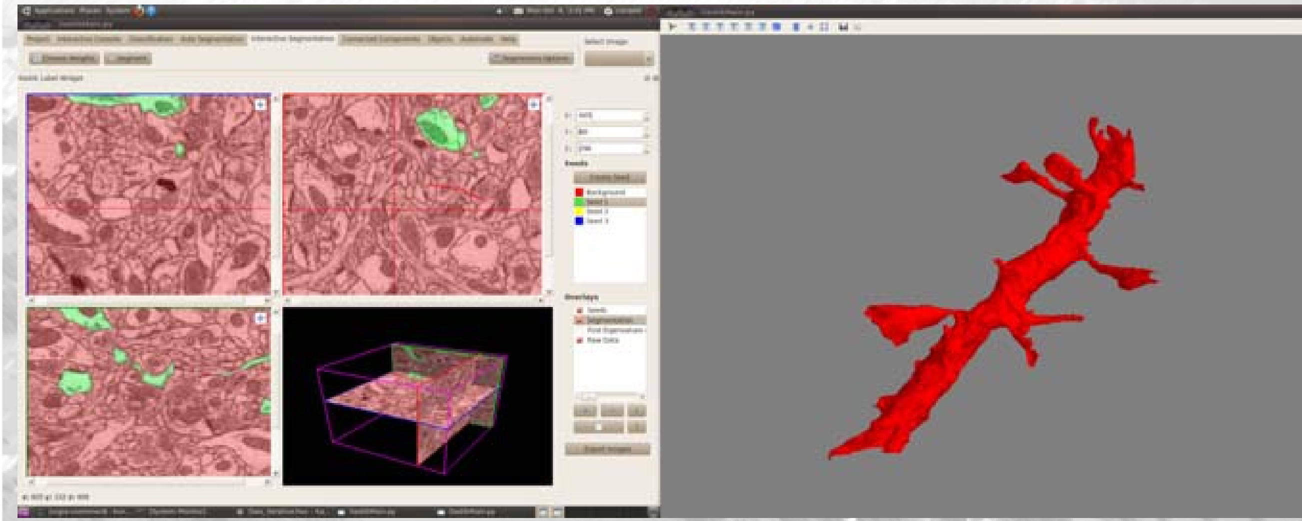
Cooling water (out)



Chamberscope LEDs...!

Automated segmentation of neuronal structures

Ilastik v0.5 - Fred Hamprecht, University of Heidelberg



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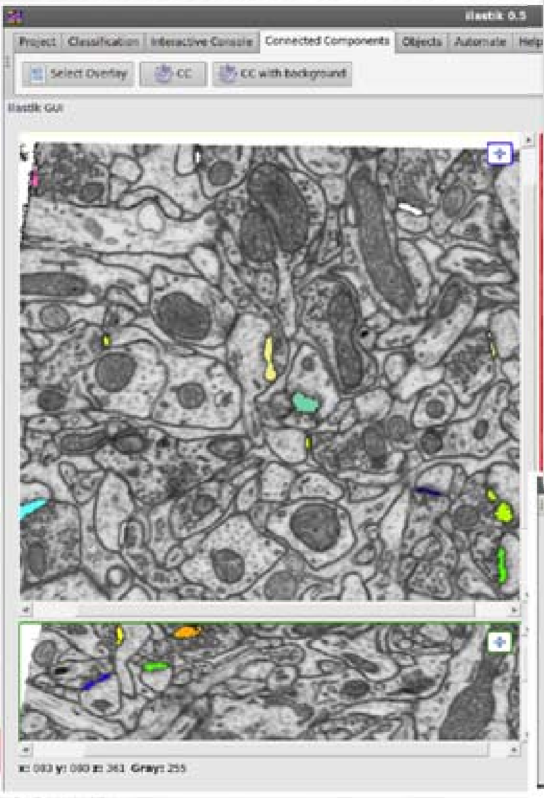
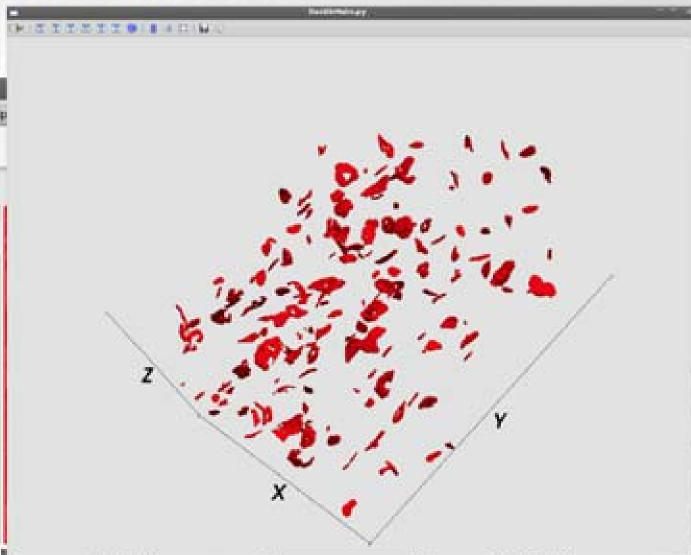
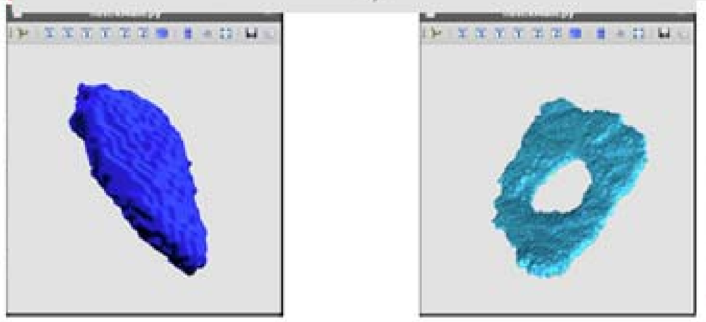
Automated segmentation of neuronal structures

Ilastik v0.5 - Fred Hamprecht, University of Heidelberg

Synapse recognition - Anna Kreshuk







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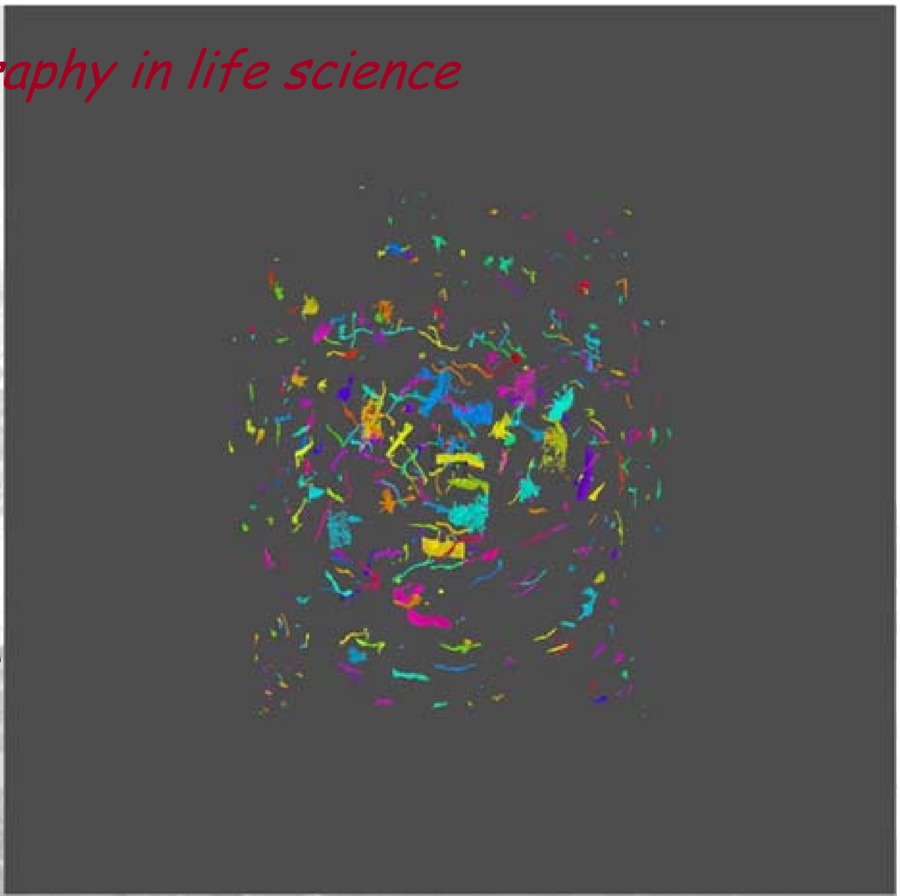
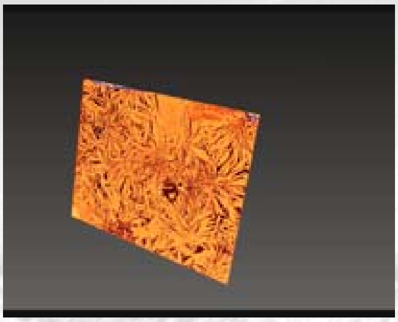
FIB Nanotomography in life science

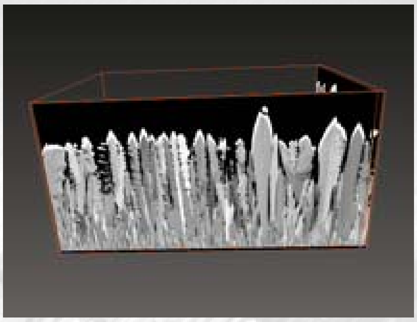
- Specimen preparation (fixation, staining, dehydration, resin infiltration same as for BIO-TEM)
- Image contrast and resolution TEM quality
- Stable and reliable automated acquisition (less artifacts than ultra-microtomy)
- To solve: segmentation and data analysis



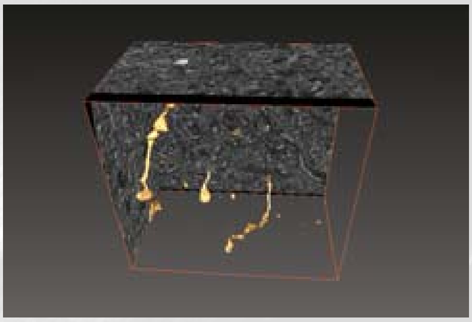
life science and materials science are not as far apart as one might think…



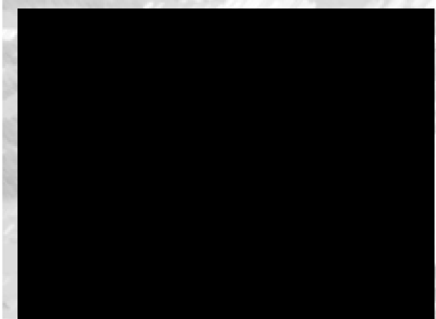
Cement, $(10nm)^3$ voxel



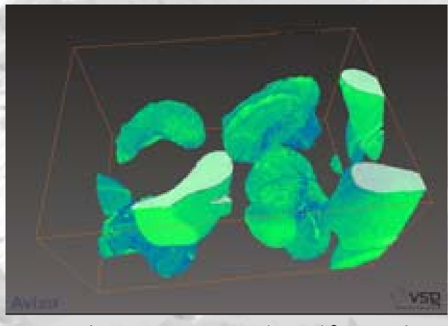
Solar cell: ZnO, $(10nm)^3$ voxel



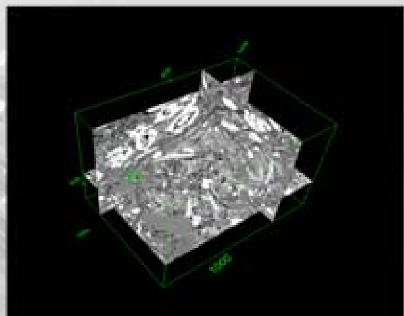
Rat brain, $(5nm)^3$ voxel



SOFC, $(10nm)^3$ voxel



Malaria parasite, $(8nm)^3$ voxel

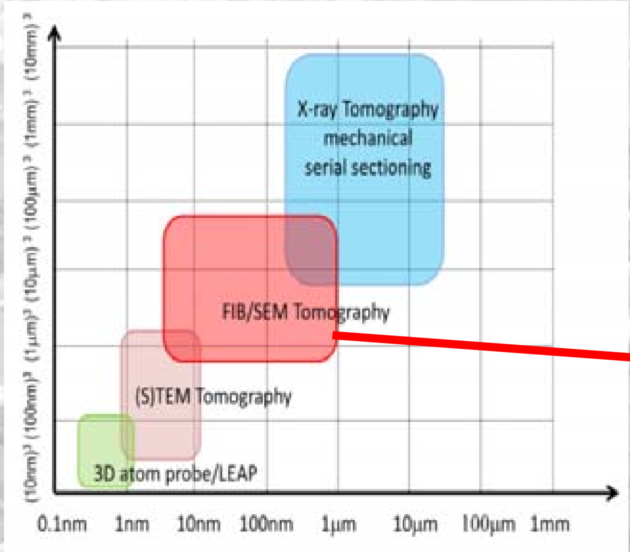


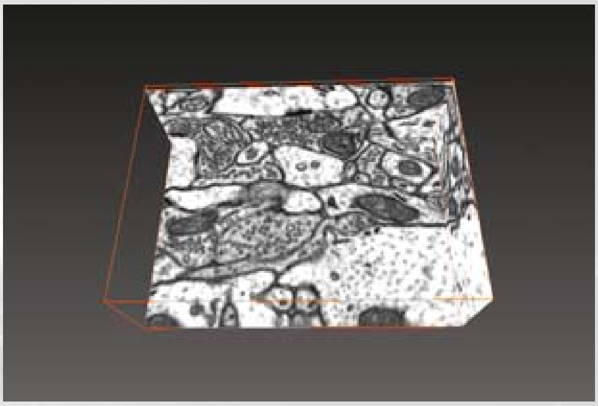
clay, $(10nm)^3$ voxel

FIB/SEM Nanotomography, volume reconstruction
Typical voxel sizes

FIB-NT compared with other 3D-techniques

- Voxel size ~5-10nm
- Dwell time ~10μsec.
- 1 slice, image / min.
- HT: 1-2kV
- Escape depth of signal (BSE) ≤ 5nm





3x3x3 nm voxel brain tissue

New possibilities in 3D-microscopy: Combination with quantitative analytical SEM techniques: EBSD, EDX

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