X-RAY FLUORESCENCE MICROSCOPY AND LENSLESS IMAGING – UNIQUE OPPORTUNITIES WITH AN MBA SOURCE

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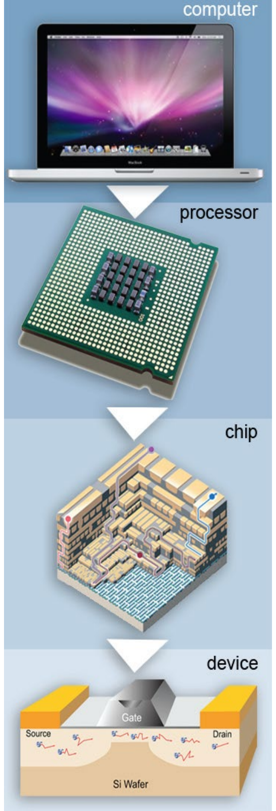
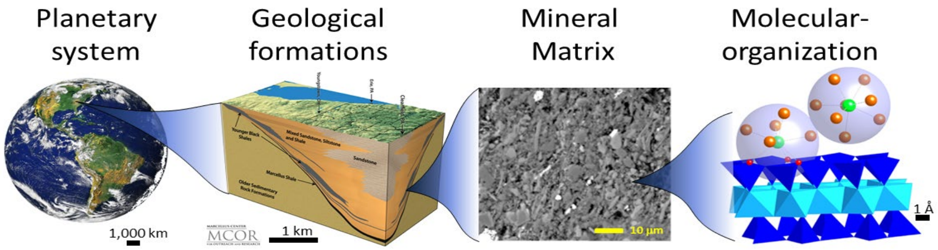
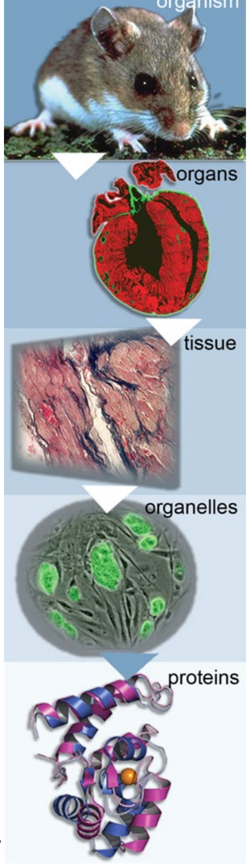
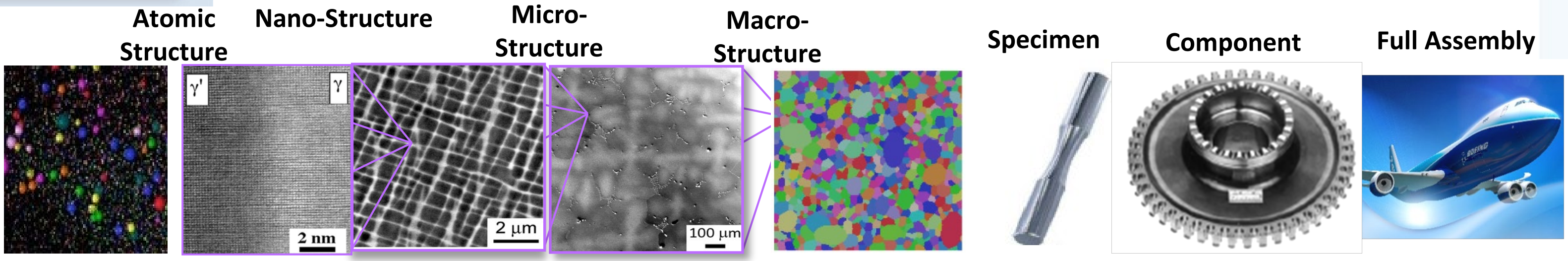






HIERARCHICAL STRUCTURE IN COMPLEX SYSTEMS
=>
NEED TO <u>VISUALIZE</u> ALL RELEVANT LENGTHSCALES

- Heterogeneity
  - MBA sources provide coherent flux to turn basically any conventional technique into a microscopic technique
- Multi-scale (time and spatial)
  - MBA source provides brightness and energy to effectively probe across length and time scales, including rare events
- *in situ / operando*



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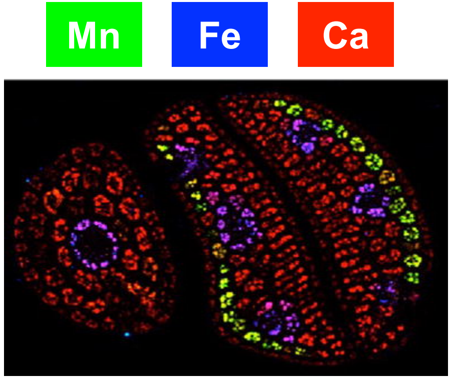
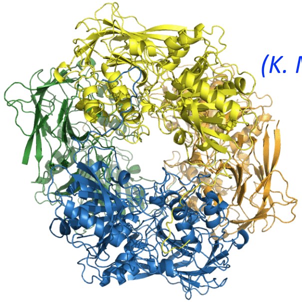
SYNCHROTRON HARD X-RAYS

- **Penetrate matter deeply, including opaque matter**
  - Imaging & tomography
  - Scanning X-ray scattering
  - Coherent diffractive imaging
  - ➢ Enables imaging of thick samples, high resolution
- **Stimulate X-ray fluorescence (XRF)**
  - Quantitative ion distributions at physiological concentrations
  - Low background
  - No labeling needed
  - ➢ Visualization of trace elements and chemical states
- **Diffract**
  - ➢ Structure determination
- **Damage (biological) samples**
  - Radiation damage mitigation:
    - Cryogenic temperatures
    - Reduction of X-ray dose
    - Fast data acquisition to spatially outrun damage

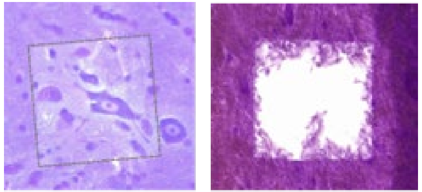
Time resolved x-ray tomography to follow structural reorganization during embryonic development *(Moosmann et al, Nature, 2013)*



Elemental distribution in Arabidopsis seeds collected by synchrotron micro-XRF *(Punshon T et al., 2012)*

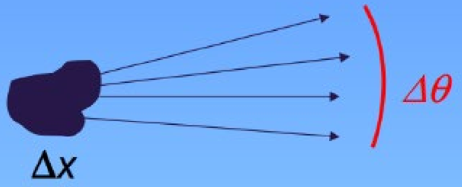


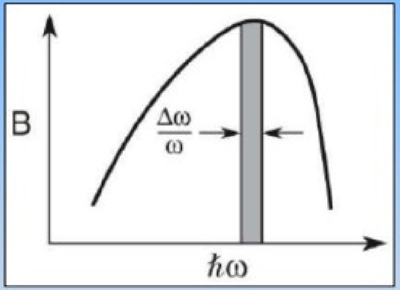
(K. Michalska et al. 2015)



Fixed Unfixed

Brightness





$$\boxed{B = \frac{\text{Photons in unit spectral range in unit time}}{(\text{source size} \times \text{divergence})^2}} \rightarrow \begin{cases} \textbf{Peak} \\ \textbf{Average} \end{cases}$$

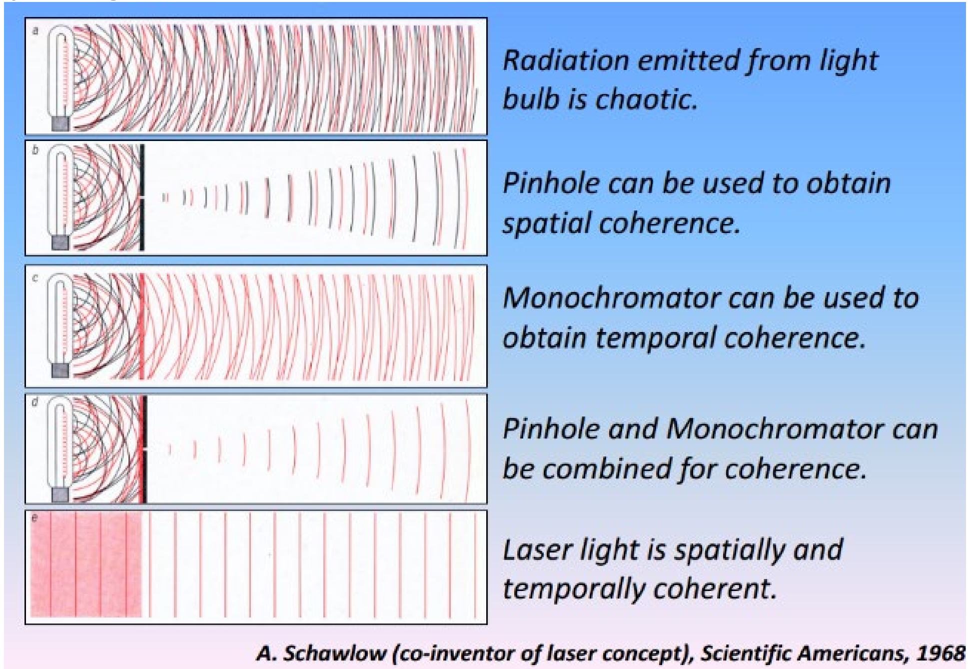
Units: photons/s/mm²/mrad²/0.1%BW

13

=> For microprobes: brightness of sources determines amount of focused flux on sample

BRIGHTNESS (=BRILLIANCE) VS FLUX / INTENSITY, AND WHAT IS COHERENCE ?

100 W incandescent light bulb
(a lot of total flux / intensity, but goes into 4Pi sterradian



5mW laser pointer
Low total intensity, but very bright!!!
All light goes forward.

- Coherent source: cannot distinguish the source from a point source
- Can make any source spatially 'coherent' by putting it at infinity, or putting slits in front of it (but loose a lot of photons)
- Can make any source temporally coherent by using a narrow enough monochromator (but flux)

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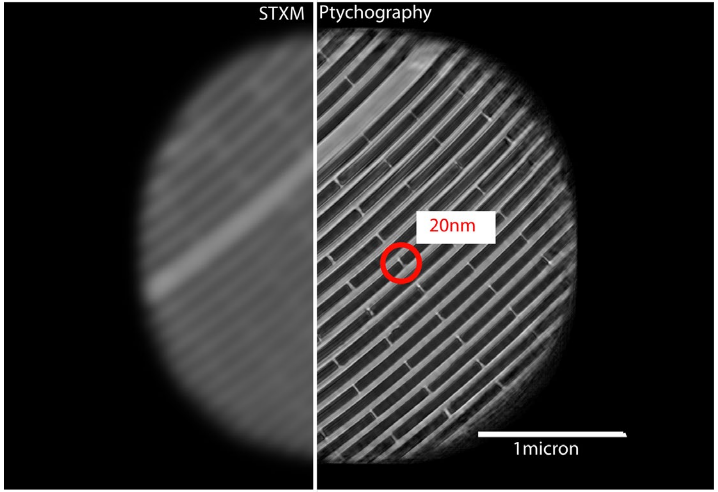
KEY ENABLER: COHERENCE

- Lensless imaging techniques overcome resolution limit of X-ray optics
  - Highest spatial resolution even in non-periodic materials
  - Direct visualization of nanostructures in cells and tissues
  - Complementary information to X-ray fluorescence
  - Spatial resolution limited by the max. scattered angle of signals on the detector

$$\delta_t = \frac{\lambda}{\theta_{max}}$$

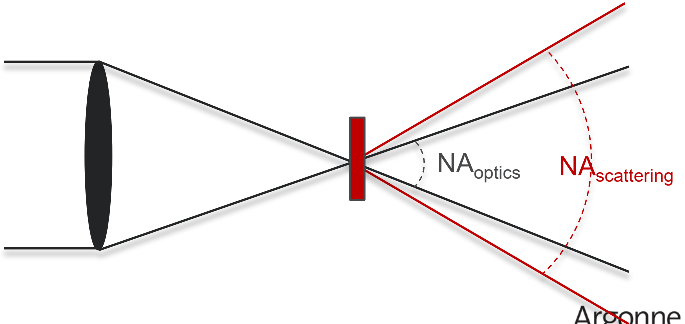
- 'Naturally' uses phase contrast - increased contrast, lower dose
- Key for using Nanoprobes at the diffraction limit of optics.

Platinum nanostructure

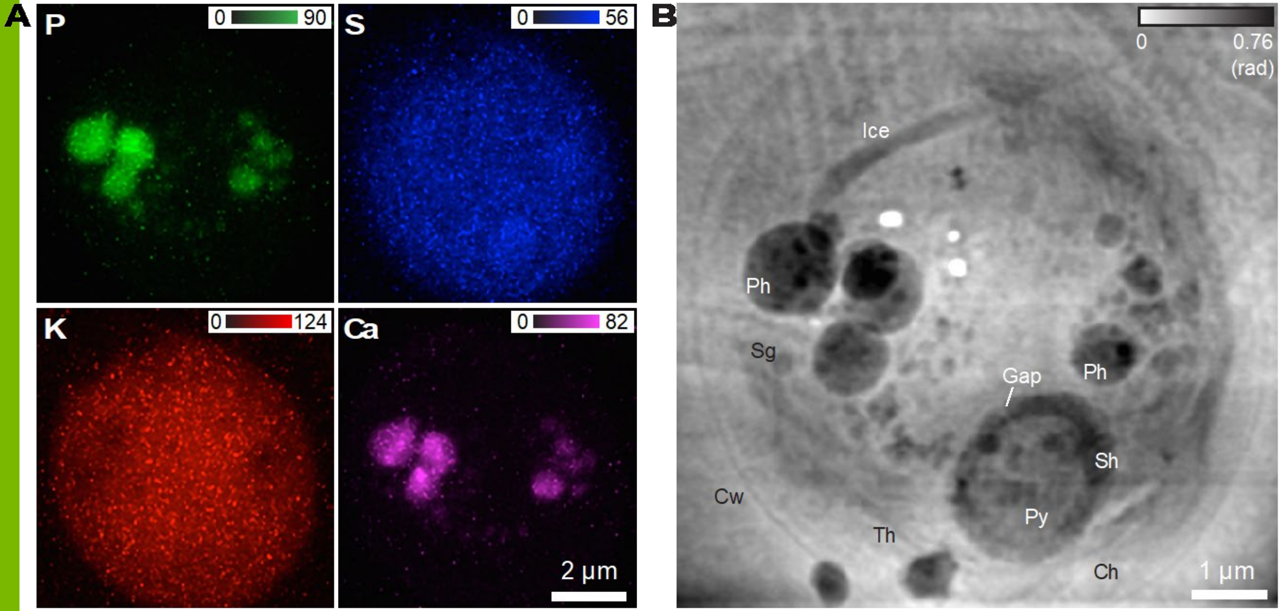


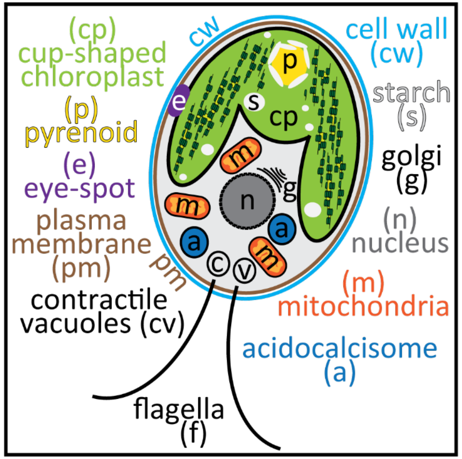
NA$_{optics}$

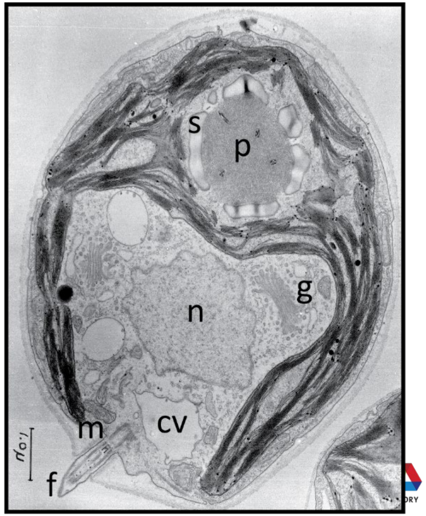
NA$_{scattering}$



CRYO-PTYCHOGRAPHY & XRF OF CHLAMYDOMONAS REINHARDTII





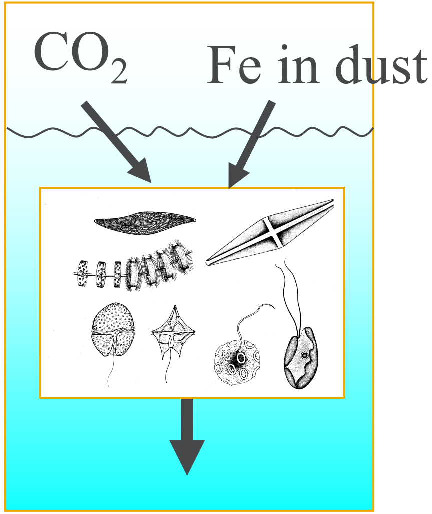


- Can combine XRF mapping for elemental sensitivity with lensless imaging methods (ptychography) to image structure
- Cryo preservation to allow imaging of hydrated samples, without chemical fixation
- Here: white spots beam damage (not careful)
- ~20 nm resolution (ptychography) 50 nm XRF

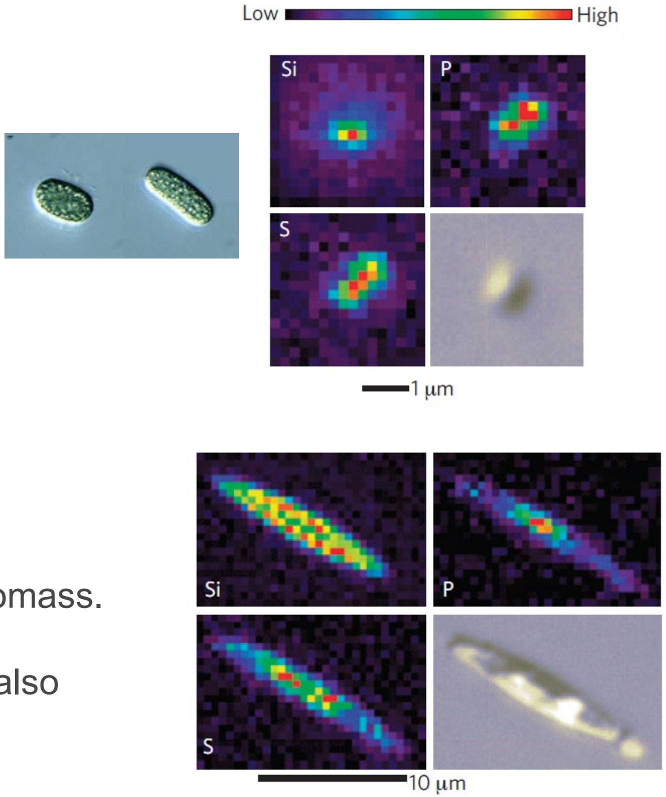
=> Beautiful structural visualization, strong contrast

Junjing Deng et al., PNAS 2015

SURPRISING ROLE FOR PICOCYANOBACTERIA



- Phytoplankton converts dissolved carbon into biomass.
- Key limiting factors: micronutrients (Fe), but also Silicon …



- Picocyanobacteria make up majority of organisms in ocean. 50+% (!) of O_2 generated by ocean.
- *Synechococcus* can show silicon ratios similar to diatoms
- significant, previously not know Si sink
- mechanism of Si accumulation is not yet known, in part because we cannot resolve the form and precise location of the Si associated with the cell.

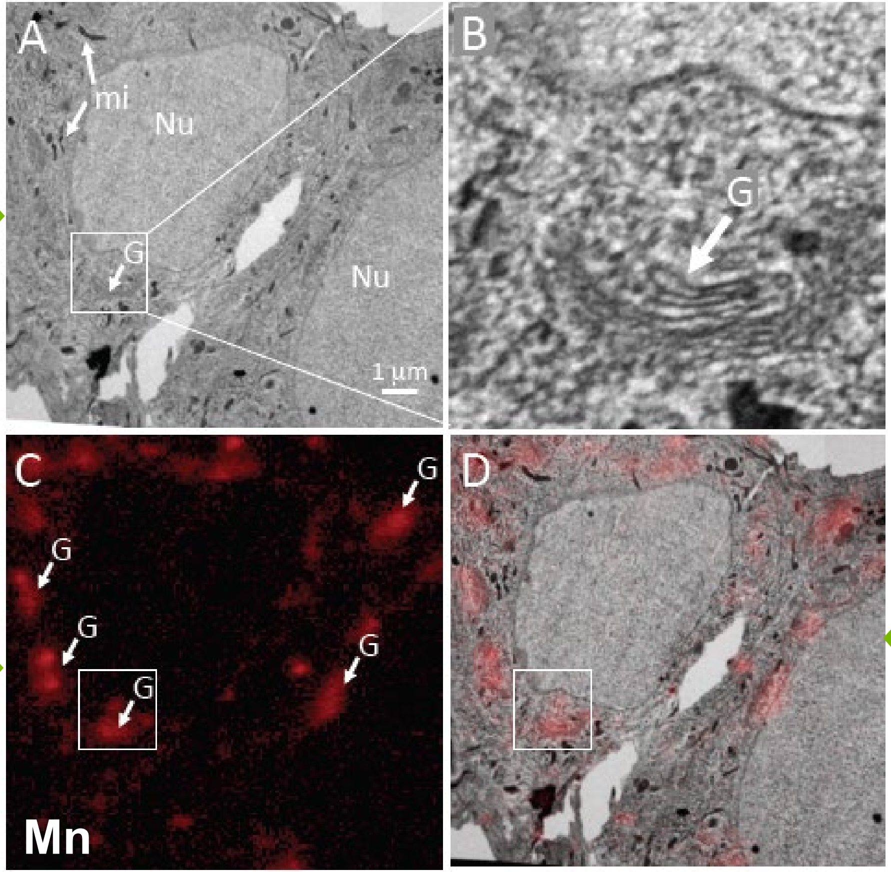
S. Baines et al, Nature Geosciences, 2012

MBA source: spatial resolution to map elements to 10 nm (5 nm w/ chemical fixation and plastic embedding), and possibly in combination with emission spectroscopy to additionally map chemical state

Mn in neurons

TEM reveals ultrastructure: nuclei, mitochondria, Golgi apparatus

XRF map shows high Mn in Golgi: highest Mn concentration: ~15 mM



Golgi membranes

Overlay reveals strong correlation of Mn with Golgi;

Detoxification process: Mn sequestered into Golgi vesicles

[Aronova et al, 2018], Slide after Si Chen, APS

MBA to achieve:
- Improved resolution to localize Mn where in Golgi exactly
- Ptychography to visualize cellular ultrastructure
- Tomography to visualize in 3D

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KEY ENABLER: BRIGHTNESS

With Brightness nearly all techniques will become microscopies

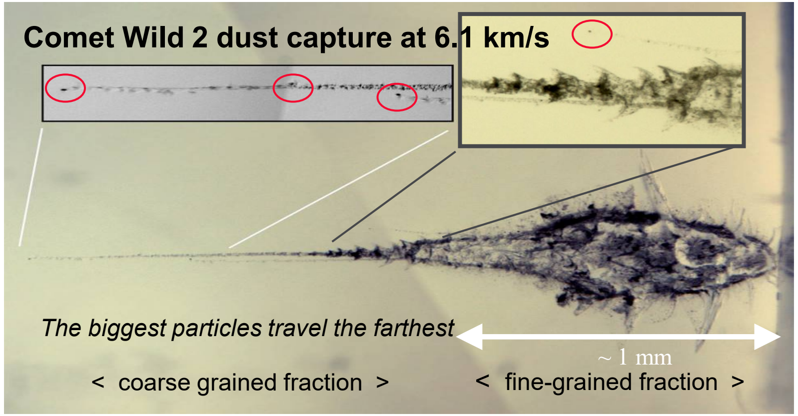
- Unlocks a series of multi-modal approaches (e.g., trace element mapping & visualization of ultrastructure)
- Enables fast data acquisition: speed can be improved by a factor >1000
  - 100-200x gain in brightness
  - 10-100x gain due to improvements in IDs, optics, detectors, methods such as dose fractionation, …
- Large 2D samples at simultaneous high resolution (needle in haystack problems)
- Statistically relevant measurements (eg, single cells)

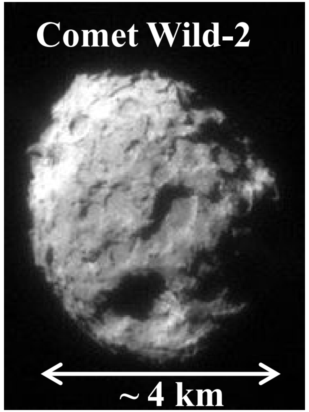
Connecting nanometer features across macroscopic dimensions

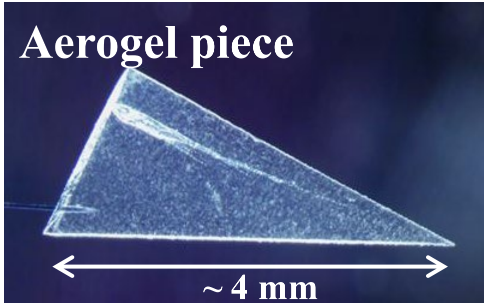
		APS		APS-U	
Scan area	**Resol.**	**Dwell**	**Scan time**	**Dwell**	**Scan time**
10 x 10 mm	100 nm	5 ms	1.5 years	25 μs	3 days
100 x 100 μm	20 nm	0.1 s	29 days	0.5 ms	3.5 h
10 x 10 μm	5 nm	0.1 s	5 days	0.5 ms	0.5 h

STARDUST COMET DUST

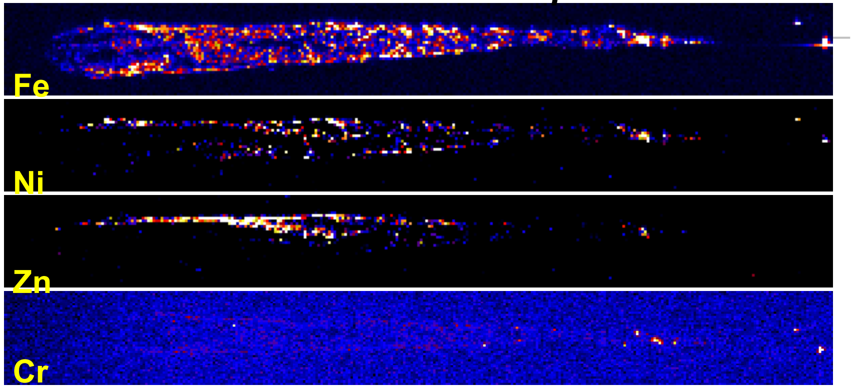
The Stardust Mission brought back samples (several thousand) from the comet Wild-2, embedded in aerogel.







Stardust Track 3 microXRF maps

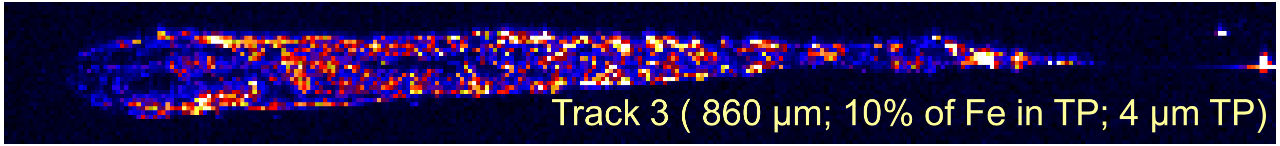


XRF mapping:
Zn predominantly near the entry
Cr near the terminal end.

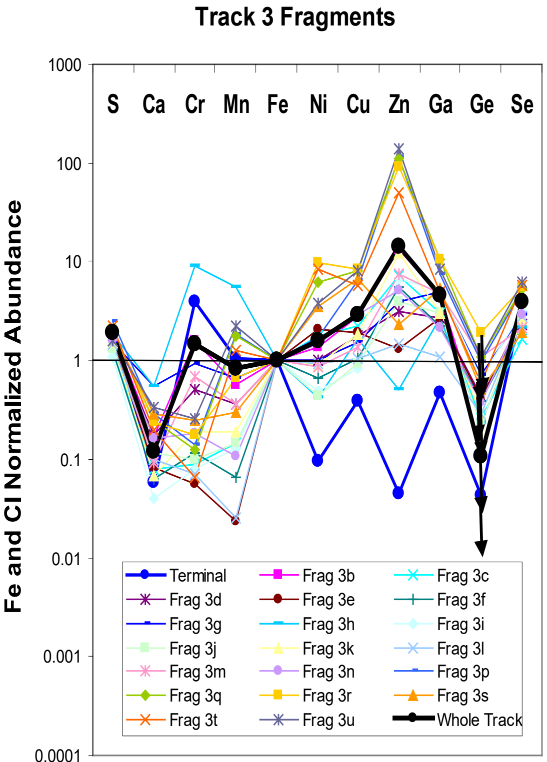
Flynn, G., and 80 others (2006) Elemental Compositions of Comet 81P/Wild 2 Samples Collected by Stardust. Science, 314, 1731-1735.

Slide content courtesy
Steve Sutton, U Chicago

COMPOSITION OF COMETARY FRAGMENTS ALONG TRACK 3



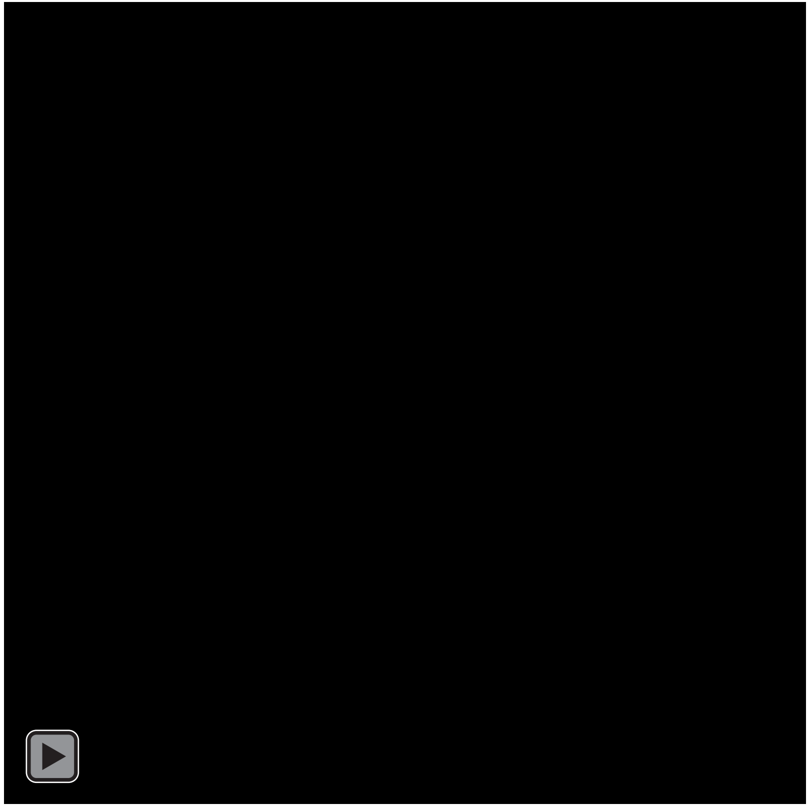
- 20 most intense Fe-hot spots were re-analyzed for a much longer time.
- Grain-to-grain variation is significant -- $>10^3$ for Zn, $>10^2$ for Ni.
- **"Whole track composition" (black) differs significantly from that of the terminal particle (blue) and more volatile rich than carbonaceous meteorites**
- High temperature minerals!

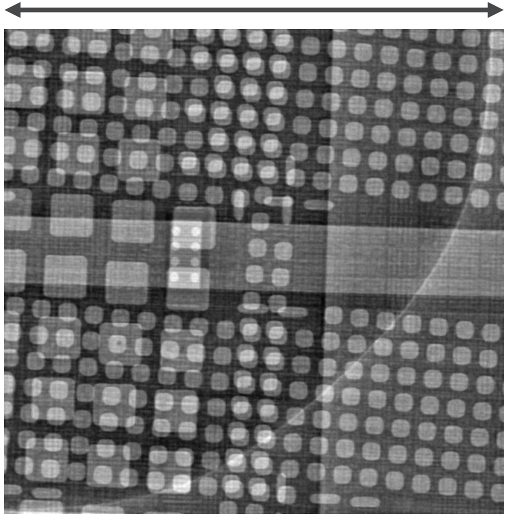


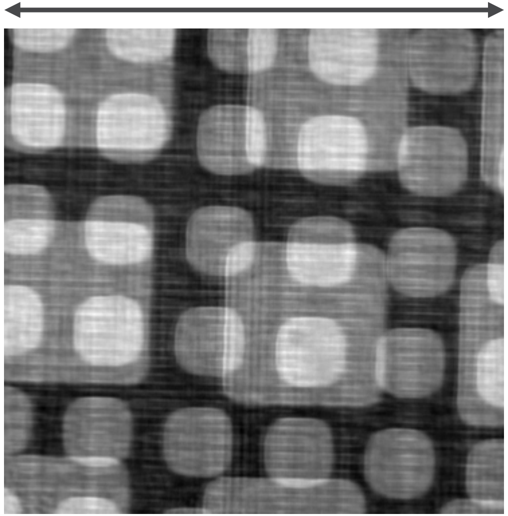
MBA source will advance the capabilities for defining the trace element speciation at nanoscales.

- Faster mapping of large areas at high spatial resolution
- Elemental and spectroscopic analyses at lower concentrations in smaller samples/voxels

High-speed imaging of large samples



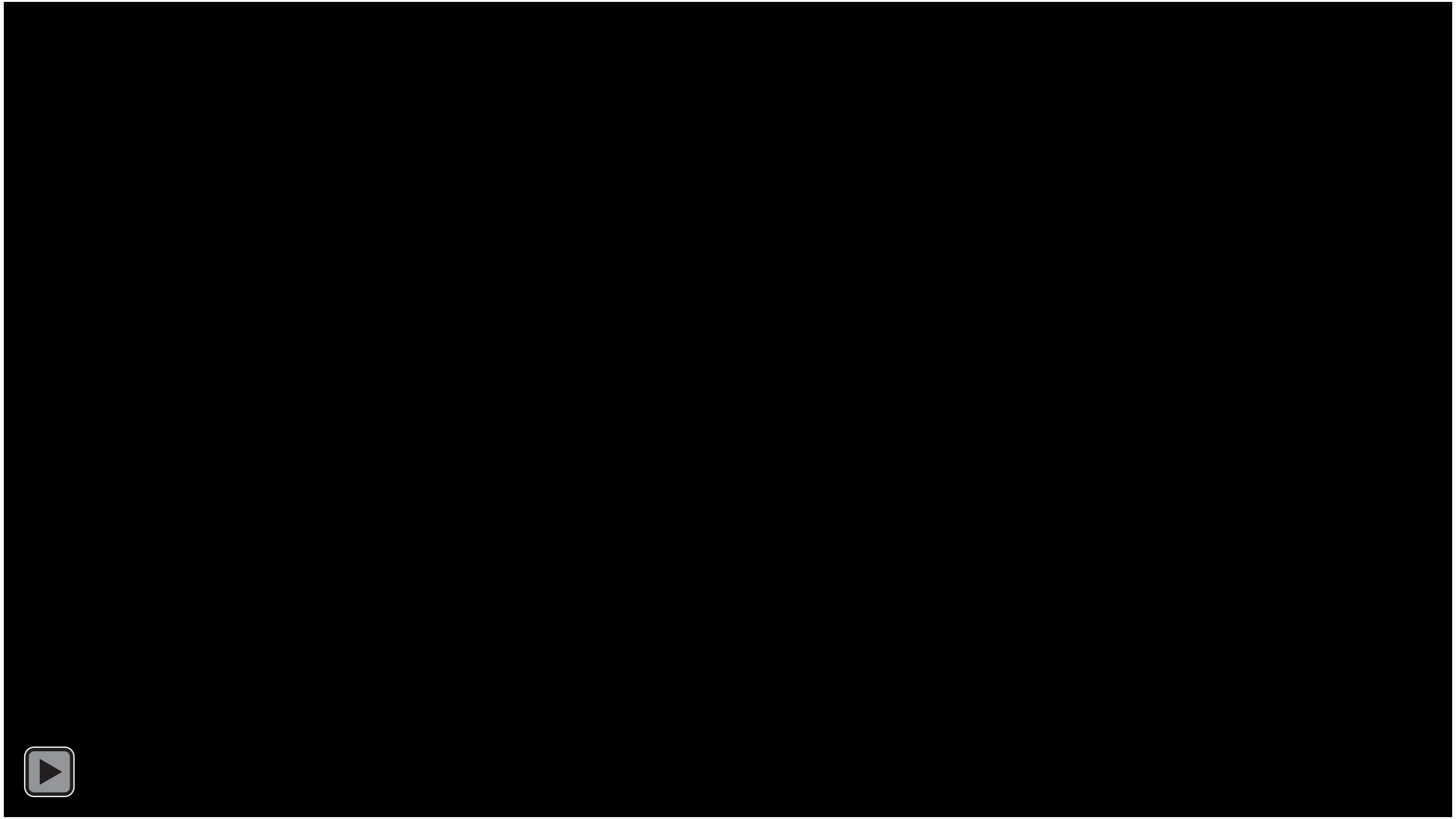




(unpublished)

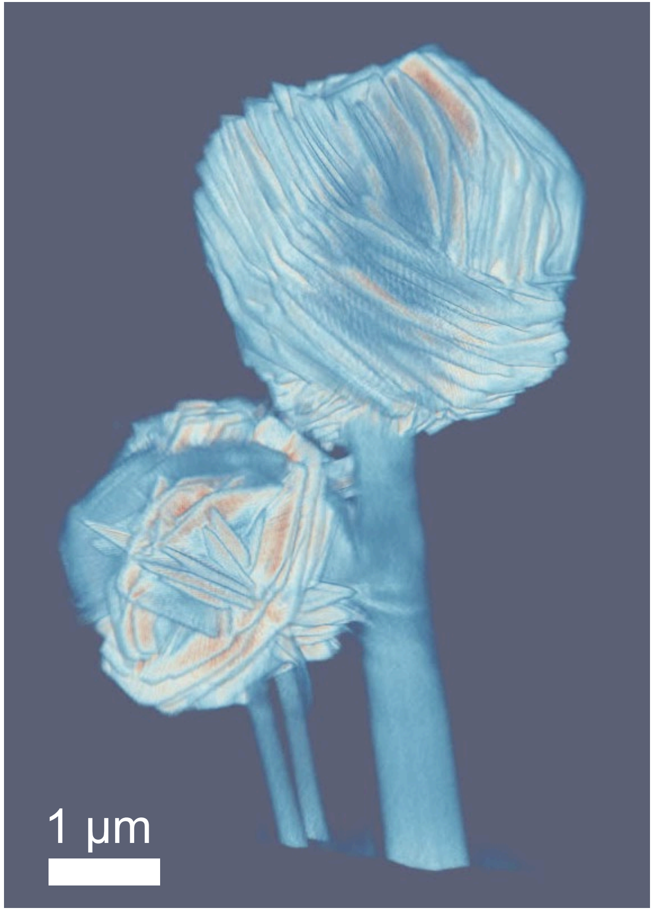
- Energy=8.8 keV
- Field of view: 1 mm^2
- Pixel size: 28 nm
- Frame rate: 2000 Hz
- Data acquisition time: ~3.3 hours
- Total # of diffraction patterns: ~24 million

LAMINOGRAPHY OF 16 NM IC



Scan FOV: 50 µm diameter

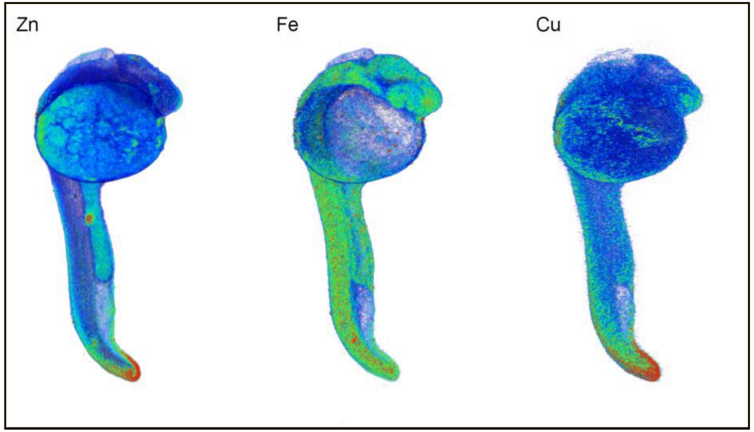
Need for high-speed 3D ptychography for dynamic processes



- CuS secondary particles--An important multifunctional semiconductor with potential applications in gas sensors, lithium ion batteries and solar energy devices.
- 8.8 keV, Detector 500 Hz
- 171 projections, -85° to 85°
- 24 s/proj., ~1.2 hours in total

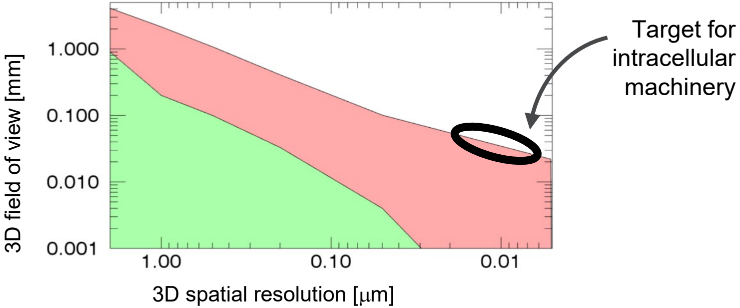
- At the moment limited to ‘static’ samples and snapshots in time.
- MBA source (with detector and methods developments):
  investigated dynamic processes *operando* !

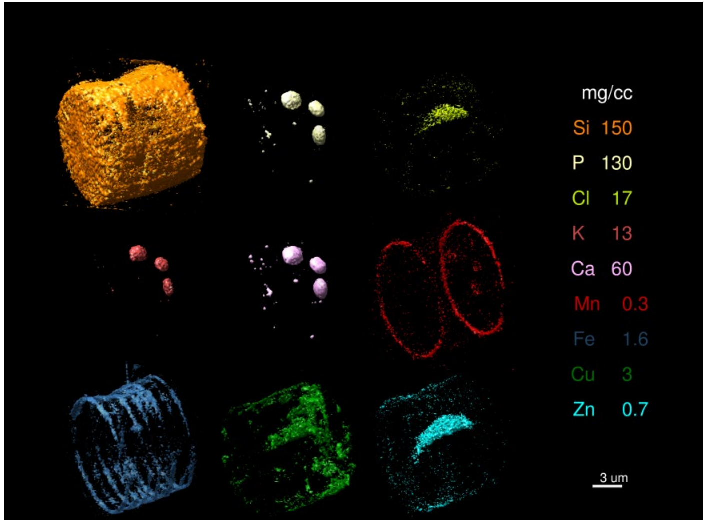
SPEED ENABLES X-RAY (FLUORESCENCE) TOMOGRAPHY



3D imaging of transition metals in the zebrafish embryo by X-ray fluorescence microtomography (metalloprotein cofactor metal distributions)
Bourassa D, Gleber SC, Vogt S, Yi H, Will F, Richter H, Shin CH, Fahrni CJ. Metallomics. 2014; 6(9):1648-55.

- Field of view ~800x1500um, 400x750 pixels, 60 projections, dwell:10 ms/pixel.
- Total data acquisition time: 3-4 days
- Resolution limited only by available flux (scan time).





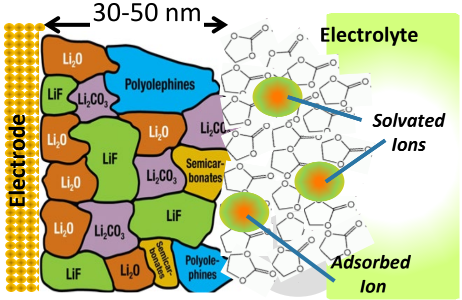
3D rendering of trace elements in a freshwater diatom
M. de Jonge, *et al*, PNAS, 2010

On statistically relevant sample numbers / sizes!!!

PUSHING THE LIMITS, JUST A FEW EXAMPLES

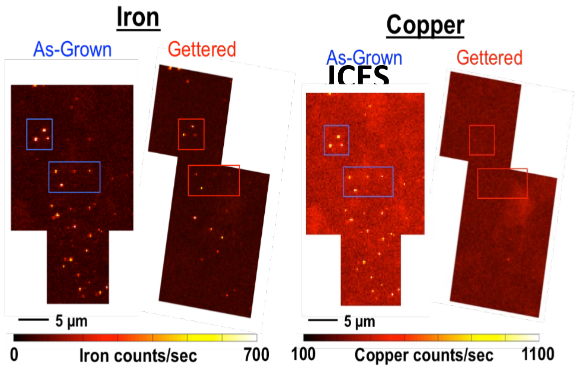
Energy Storage:

- Formation, structure, and function of the solid-electrolyte interface in batteries: relevant length scale 5 nm



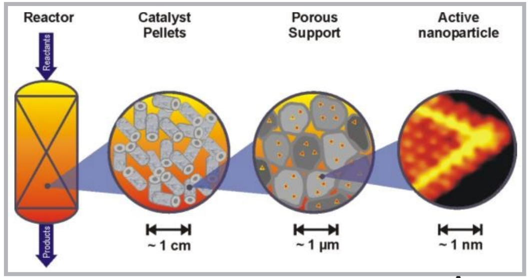
Photovoltaics:

- Nanodefect engineering for better/cheaper mc/Si solar cells
- Novel Materials
- Need sensitivity to detect few atoms of dopants, single dislocations, …



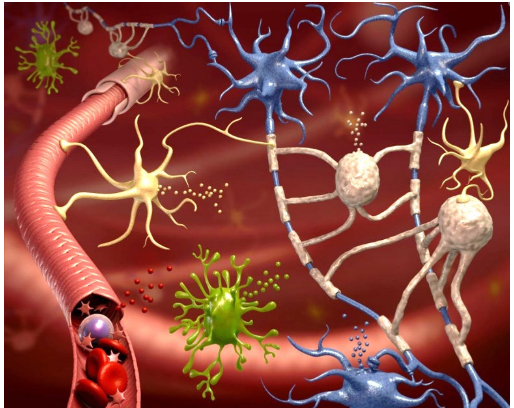
Catalysis:

- Hierarchical 3D structures: **mm**-size pellets, **µm**-size support pores, **nm**-scale catalytic nanoparticles

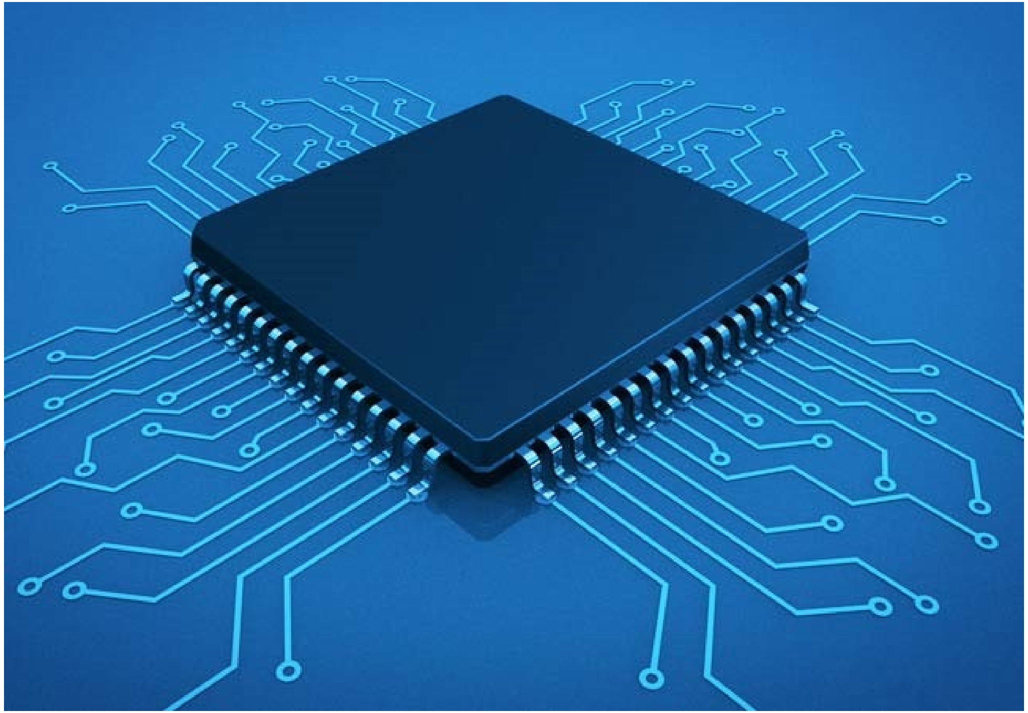


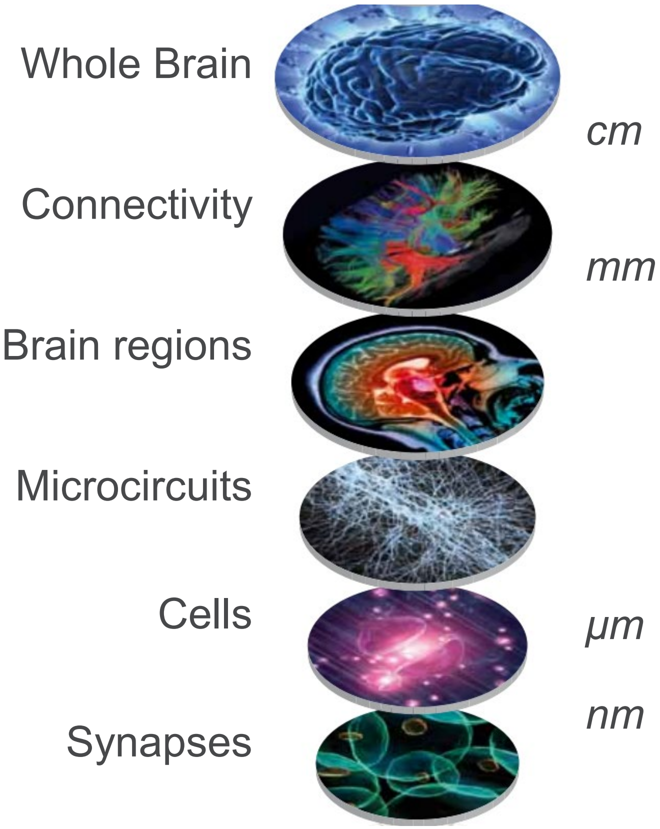
Life Sciences:

- Visualize individual nanoparticles and metal clusters in the context of organelles, cells, tissues in 3D: relevant resolution 10 nm



Stretch goal – image the whole brain (silicon or animal)





With APS-U: coherent flux to image $1 mm^3$ at 10 nm 3D resolution in ~1 day

THE FUTURE IS BRIGHT

Many facilities either recently built, or upgraded or upgrades are underway or planned.

As an example, for APS / APS-U:

⇒ Up to 500x higher brightness

⇒ micro/nanoprobe:

  ⇒ increased flux, smaller spot sizes, higher speed, higher sensititvity

⇒ Lensless imaging:

  ⇒ Higher speed, higher resolution

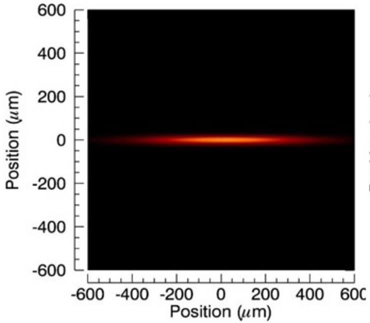
⇒ Experiments become feasible we can only dream about today…

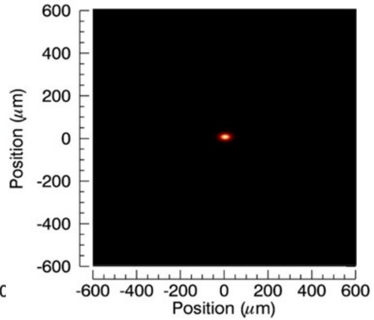
⇒ ~ 1 nm structural information

⇒ 5 nm elemental information

⇒ In situ / operando, 4D/5D

APS Today ⇨ APS Upgrade





Note: Does 500x matter ?
fastest human: 45km/h - Speed to leave earth’s orbit: 7.9km/s => factor 600

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THANK YOU !