Preparation and observation of cells at ID16A nanoprobe

Sylvain Bohic
Inserm U1264, UGA, ESRF, CHU
STROBE – Synchrotron Radiation for Biomedecine
ESRF scientific collaborator – ID16A
bohic@esrf.fr

Acknowledgements

ID16A staff


P. Cloetens BLR


JC Da Silva


A.Pacureanu


Y Yang


M Salomé (ID16A /ID21)


L André


L. Bloch


M Hubert


F. Fus

Gérard JAOUEN
Institut Universitaire de France
Ecole Nationale Supérieure de Chimie de Paris
ChemBio team: organometallic complexes


Bissardon : Post-doc INCA/Inserm (2016-2018)

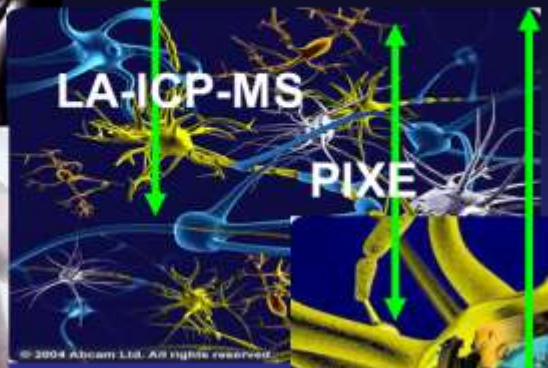

M. Carriere

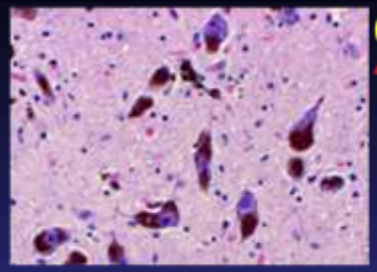
Nucleic Acid Lesions Laboratory
CEA/DRF/INAC/SyMMES


A. Bouron

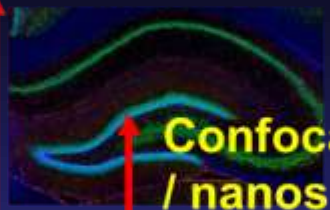
Team BioMet
Laboratory of Chemistry and Biology of Metals (LCBM) - CEA/BIG

MRI

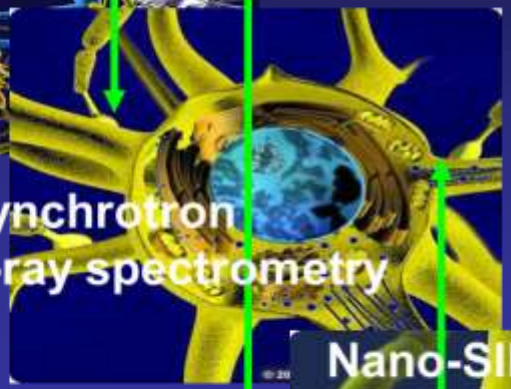


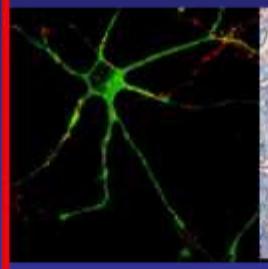


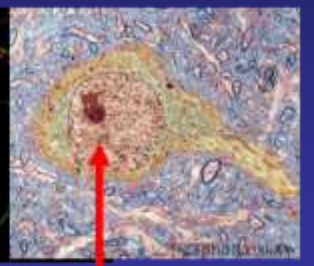
Optical microscopy

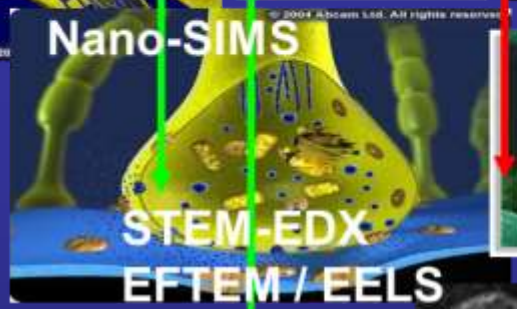


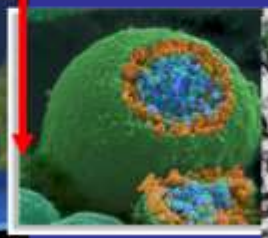
Confocal microscopy / nanoscopy

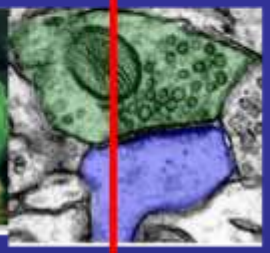




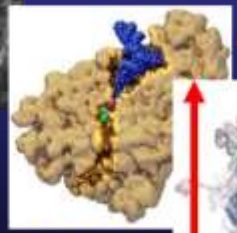


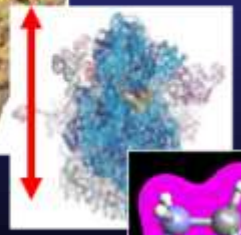




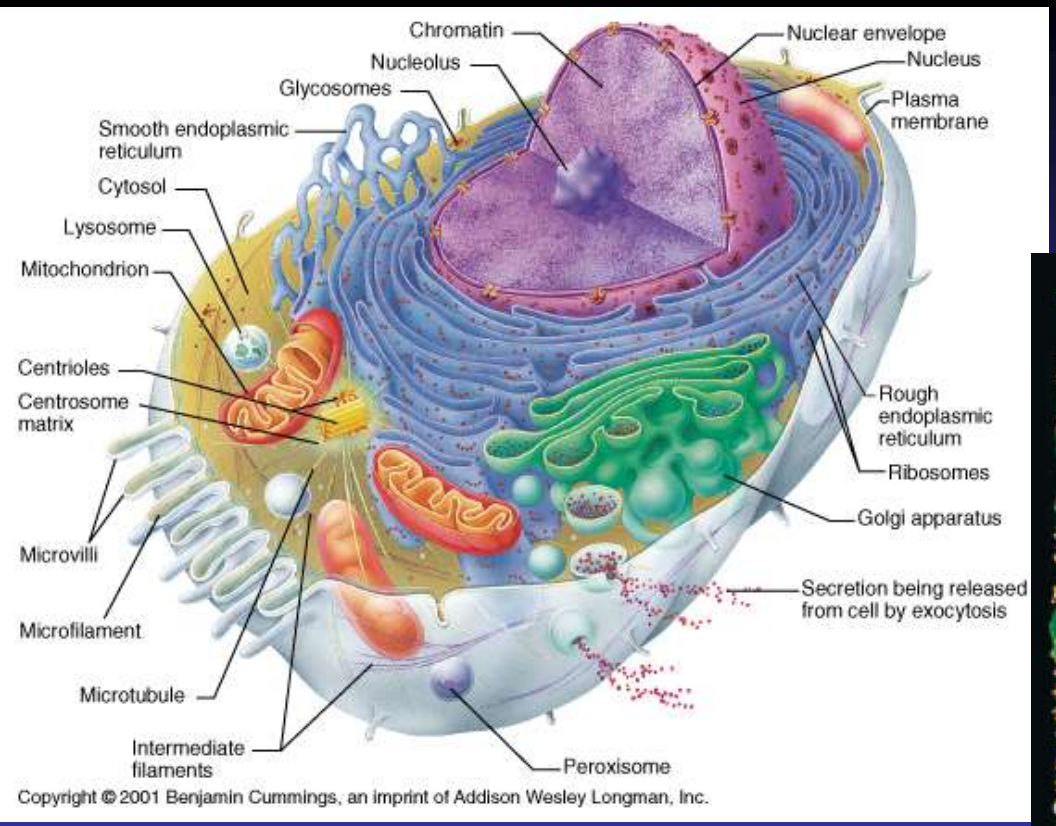


TEM





NMR
X-ray



Slab of cytoplasm, 3.1 × 3.2 × 1.2 µm3

ER, yellow; membrane-bound ribosomes, blue; free ribosomes, orange; MTs, bright green; dense core vesicles, bright blue; clathrin-negative vesicles, white; clathrin-positive compartments and vesicles, bright red; clathrin-negative compartments and vesicles, purple; mitochondria, dark green.

B.J. Marsh, et al, (2001) PNAS. 98:2399–2406

Nanochemical analysis : How to preserve of cellular morphology and chemical element distribution integrity ?

Air dried

Freeze-dried

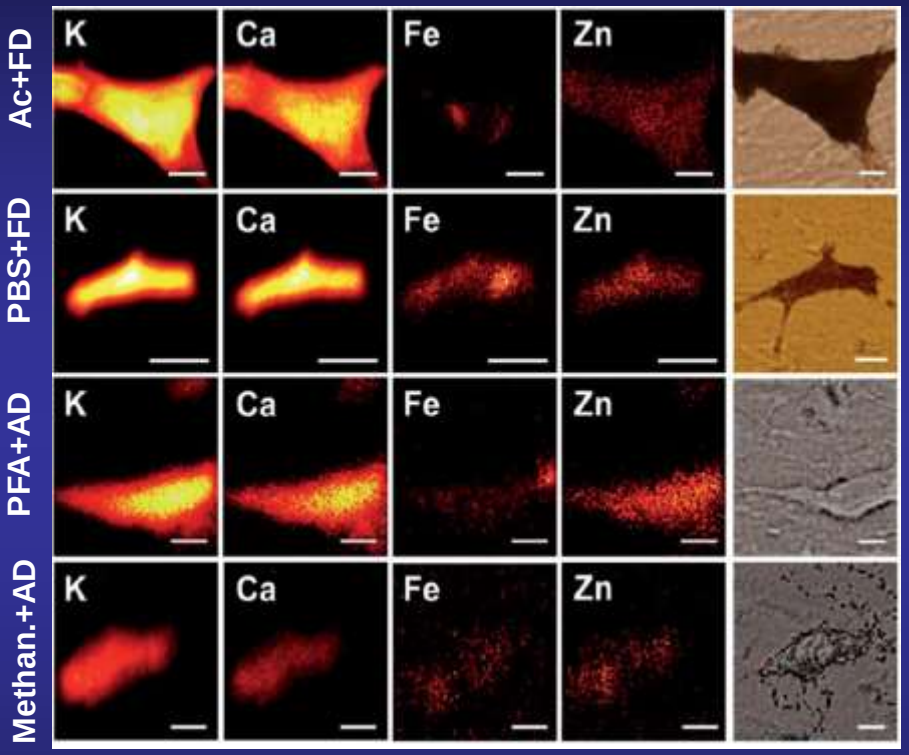
Native

Chemically fixed

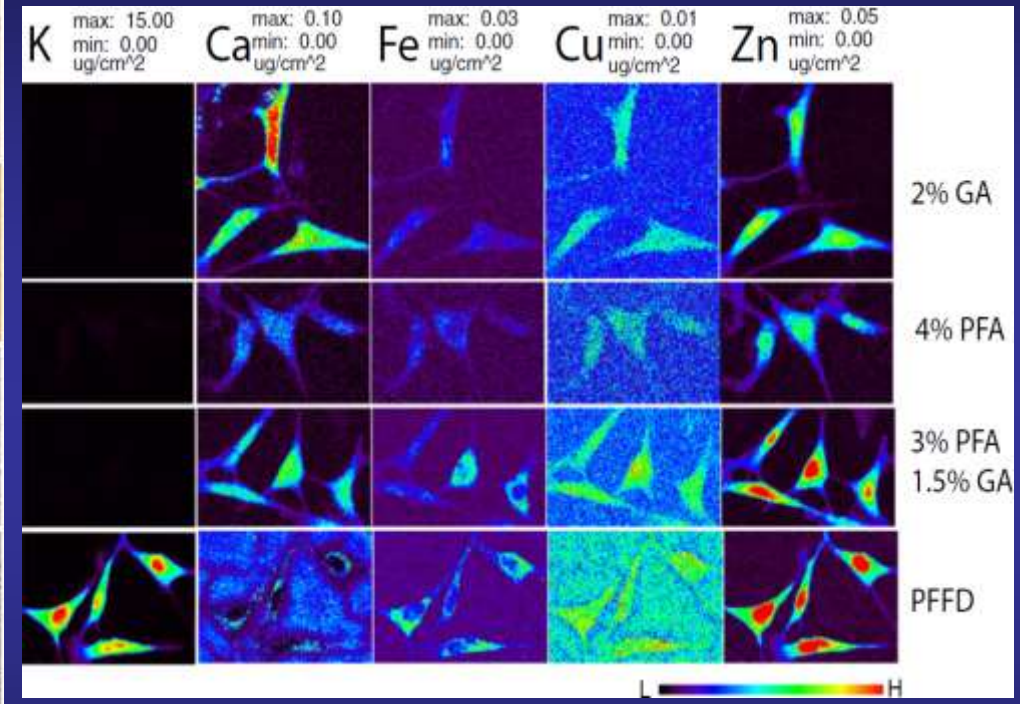
Frozen hydrated

Nanochemical analysis : How to preserve of cellular morphology and chemical element distribution integrity ?

- *Perrin, L., et al. (2014) JAAS 30, 2525. PC-12 rat cells*



- *Jin, Q., et al. (2017). J. Microsc,, 265, 81. NIH/3T3 mouse embryonic fibro.*



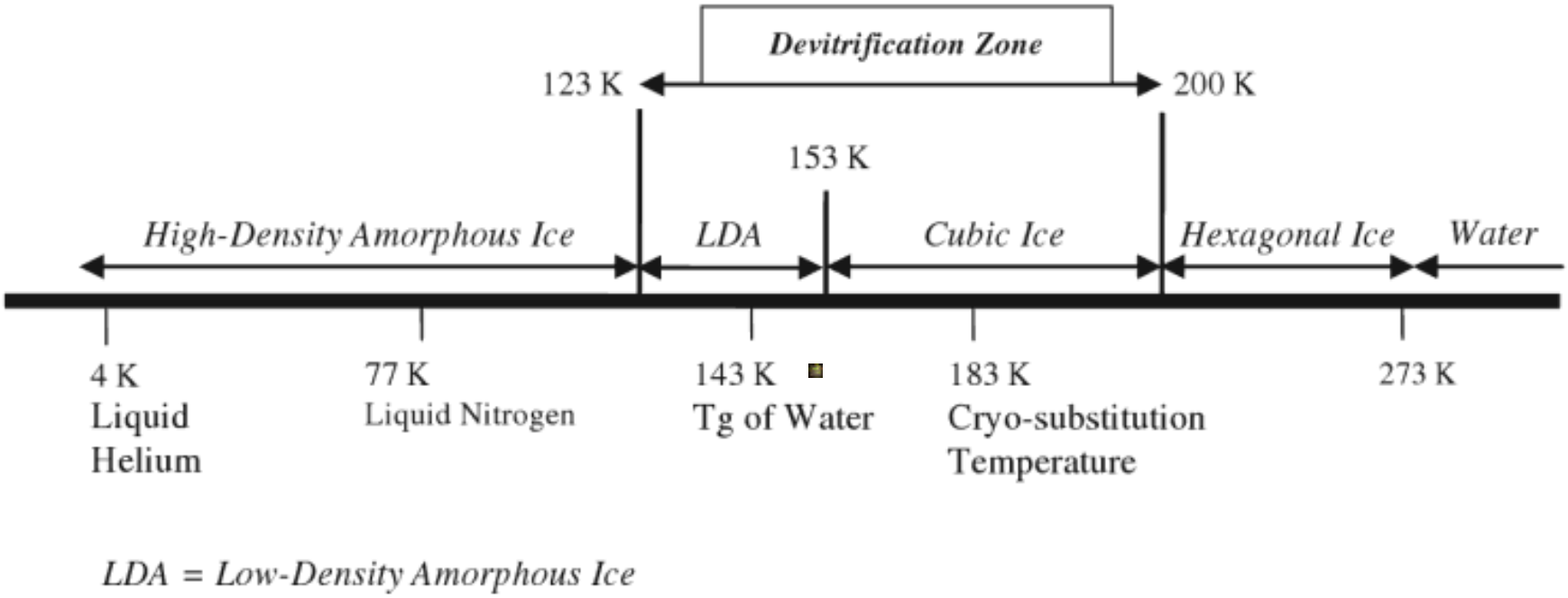
Cryopreparation : complexity and sometimes cost, limit its use.

Still, it is the best way to be as close as possible to cell native state and preserve morphology + chemical integrity

I will not talk about the possibility of combined High-pressure freezing and freeze substitution techniques : ***see Veronesi G., talk;*** ***neither*** *chemical fixation.*

Freezing diagram for organic solution in water

(biologic sample osmolarity ~ 270 - 300 mOsm/kg e.g. D-PBS -Ca^{2+}/-Mg^{2+})

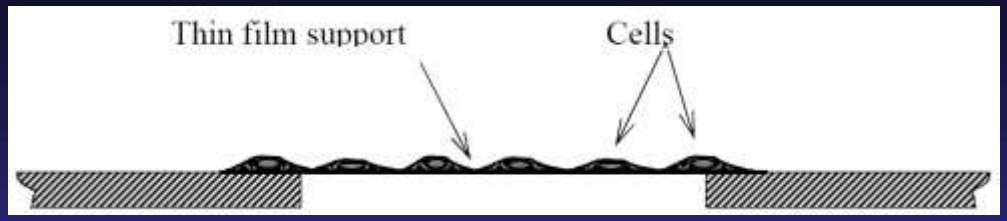


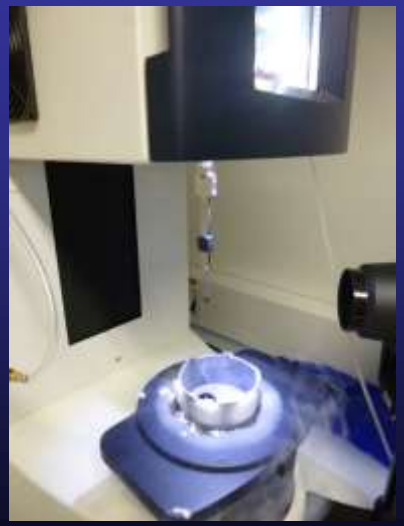
In a biological-type heterogeneous sample – devitrification occurs ~ 153K ~ -120 °C

Ideally: safest to keep sample < 130-120 K ~ -130/-150 °C

Ayache J., Boumendil J. et al.: Sample Preparation Handbook for Transmission Electron Microscopy (2010)

Cryopreparation





Plunge freezing

- Rinsing: good start 120-150 mM ammonium acetate, pH 7.4

- Blotting filter paper

5 sec.

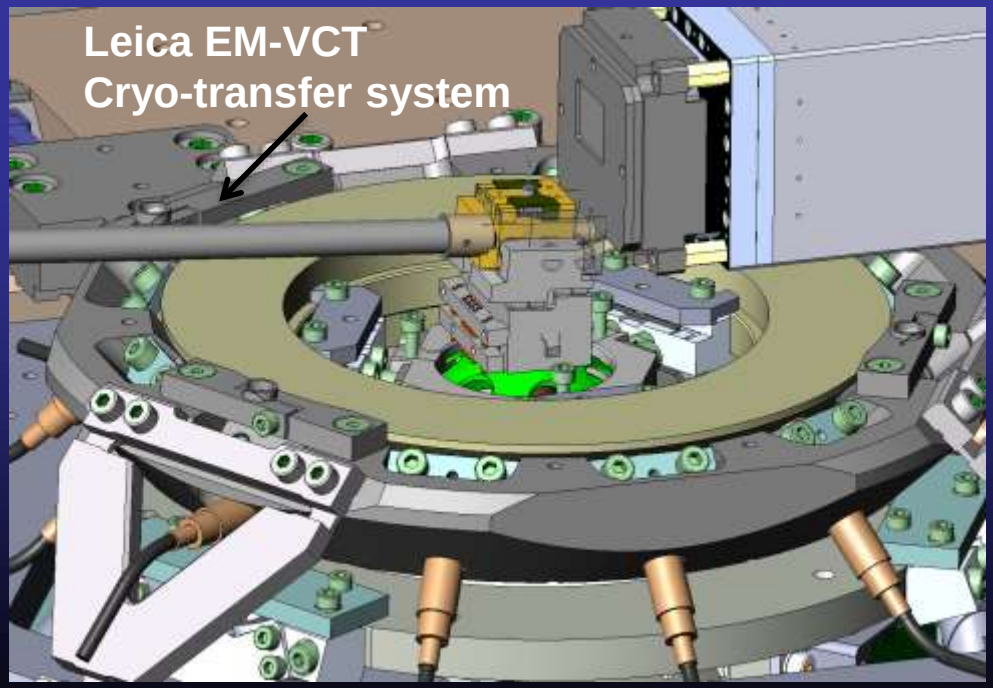
3-5 sec.

> ~ 5 sec.

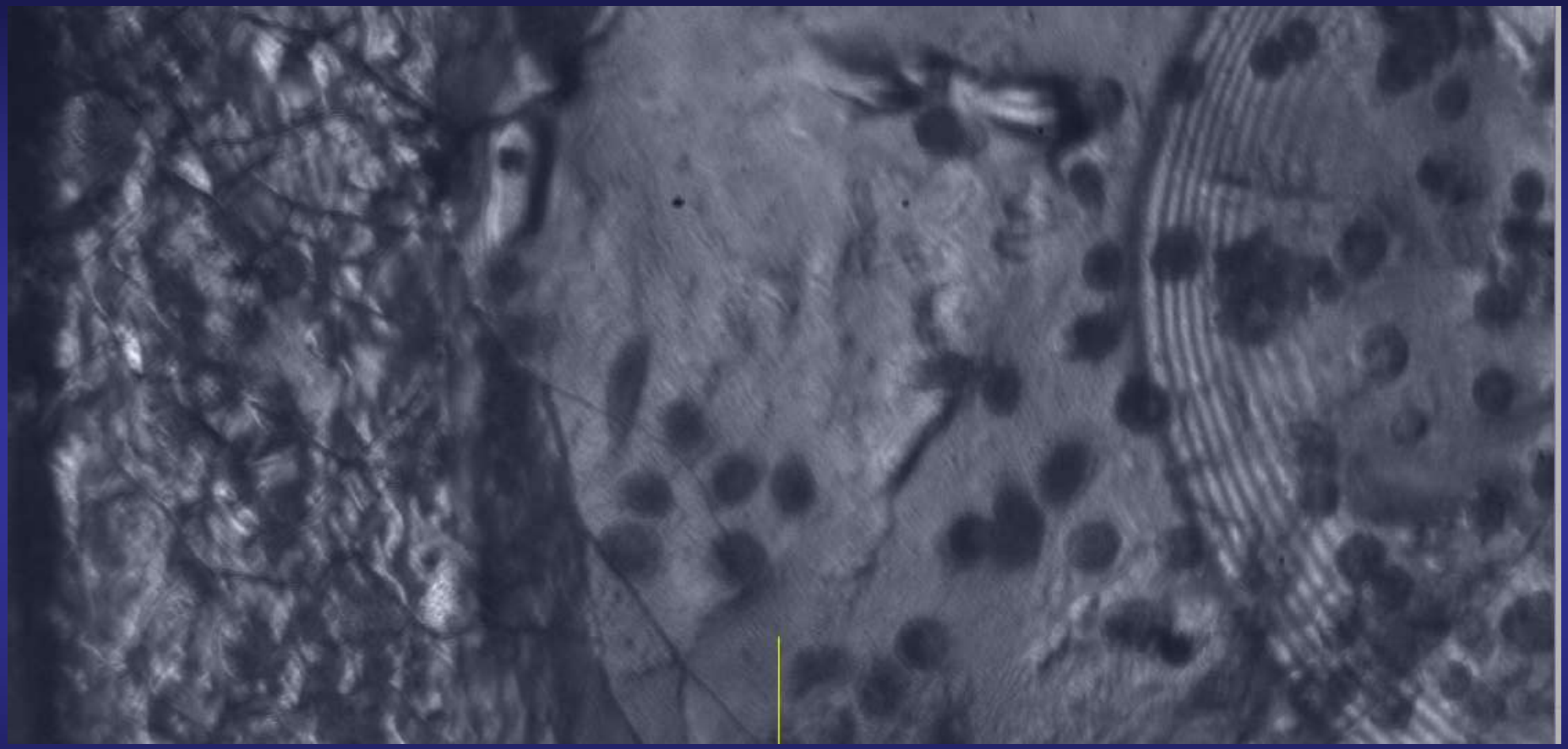
Plunge freezing

Movie

CRYOGENIC WORKFLOW at ID16A



Vitrified sample – VLM online view on ID16A

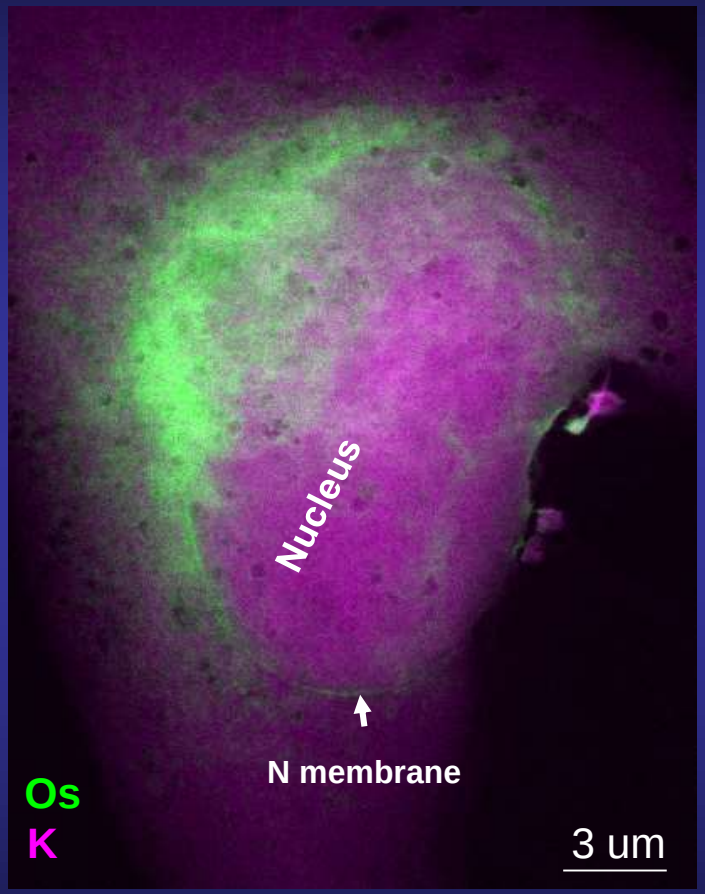


15-20 µm ice

Importance of having as far as possible vitrified rather than frozen specimens: EM beam radiation damages more severe at interfaces organic material/crystalline ice than with vitreous ice: Talmont Y, Adrian M, Dubochet J (1986) J, Microscopy 14:375,

XRF on chemically fixed cells v.s. Cryo-XRF

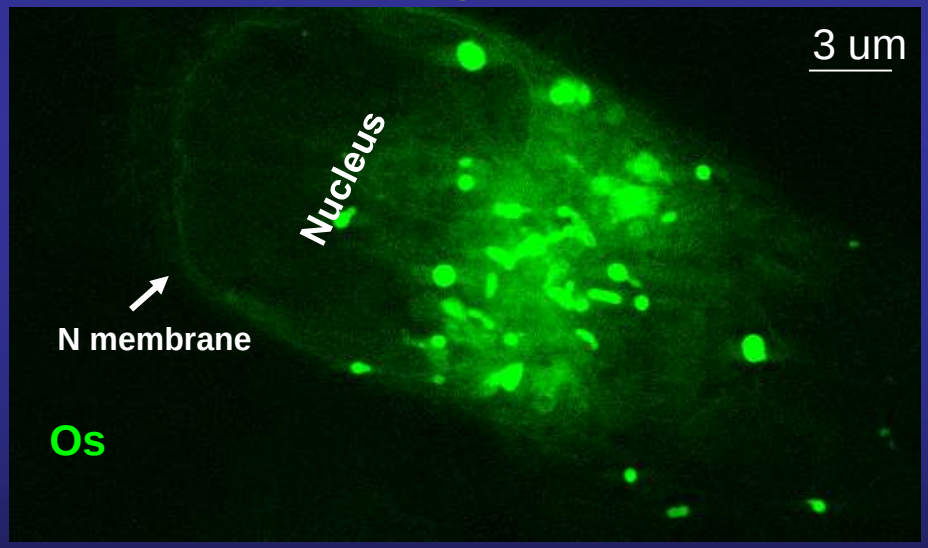
Room Temperature – light chemical fixation



MDA-MB-231 human breast cancer cell line
1h. incubation Osmocifen compound, 2 μM

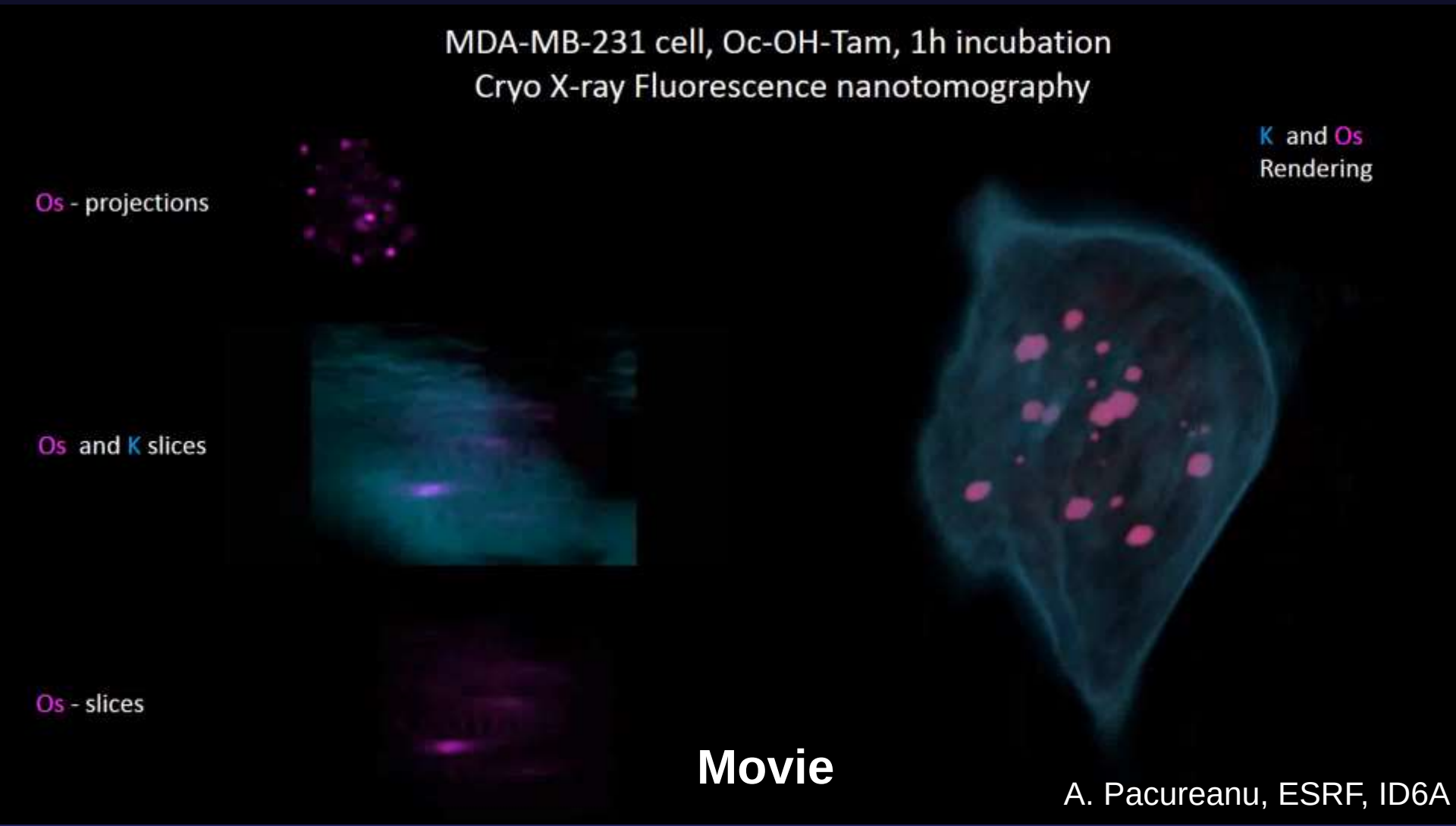
50 nm pixel size
E_0 = 17 keV, 50 ms dwell-time

Cryo



Fus, F. , Yang, Y. , Lee, S. , Top, S. , Carriere, M. , Bouron, A. , Pacureanu, A. , Da Silva, J. , Salmain, M. , vessieres, A. , Cloetens, P. , Jaouen, G. and Bohic, S. (2019), Intracellular localization of an osmocenyl-tamoxifen derivative in breast cancer cells revealed by synchrotron radiation X-ray fluorescence nanoimaging. Angew. Chem. doi:10.1002/ange.201812336

Subcellular imaging of organelles targeted by cancer drugs



Movie

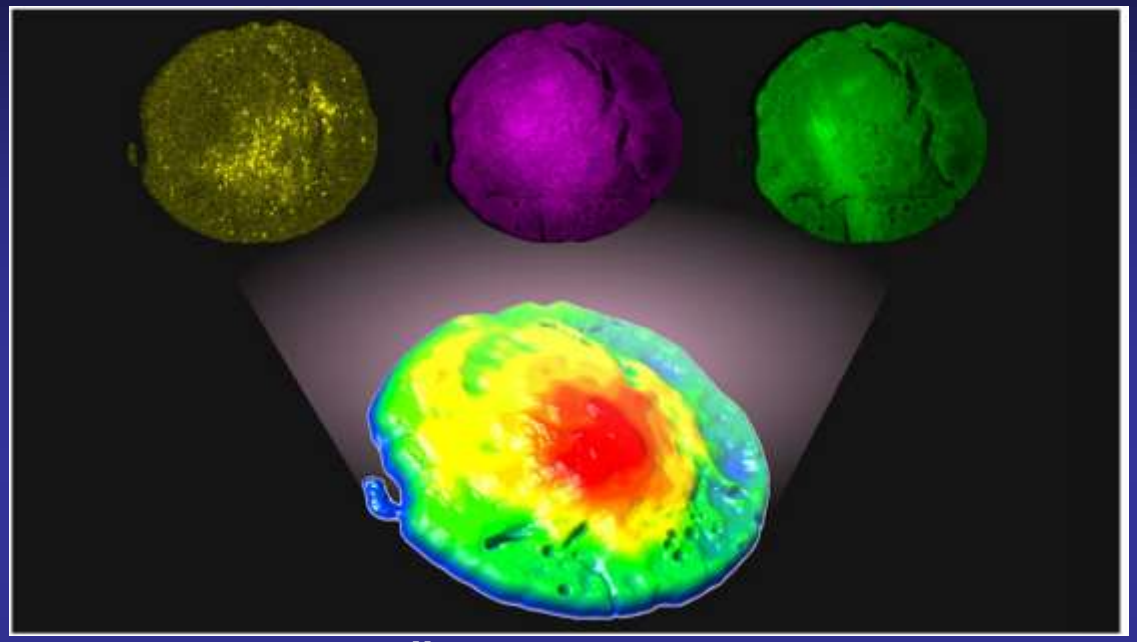
A. Pacureanu, ESRF, ID6A

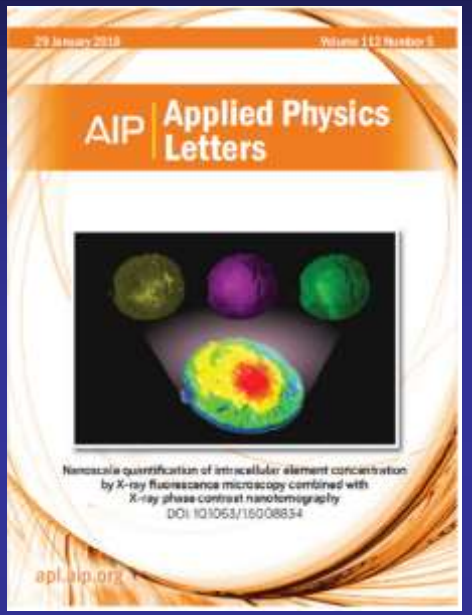
Confirm a distribution endomembrane system that encompass : nuclear membrane, perinuclear space (endoplasmic reticulum; vacuolar regions)

Fus, F. , et al. (2019) Angew. Chem. doi:10.1002/ange.201812336

See also poster @ USM – 05/02/19

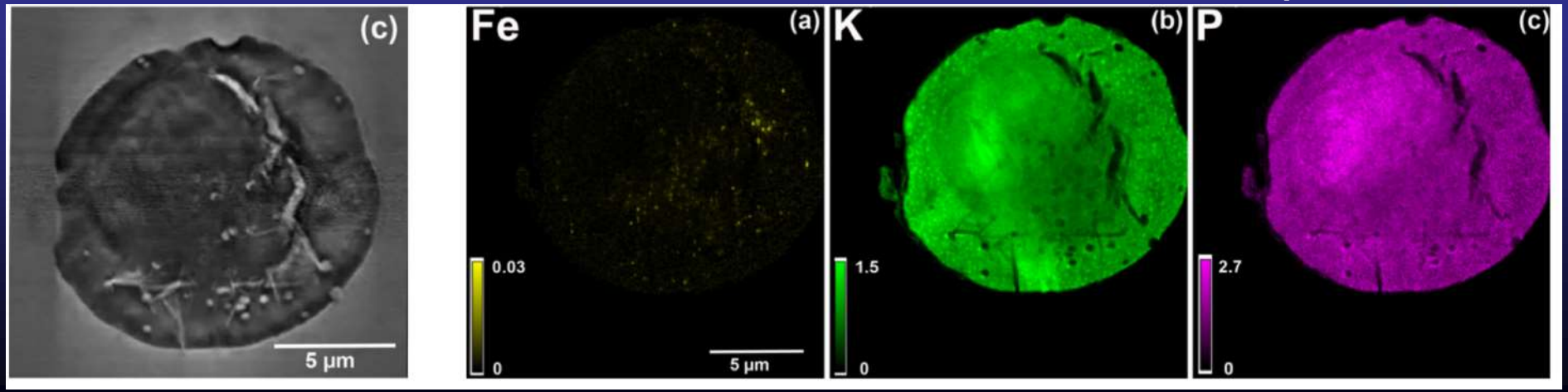
Gramaccioni et al.: Cryo-nanoimaging of single human macrophage cells: 3D structural and chemical quantification



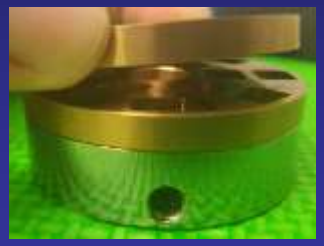


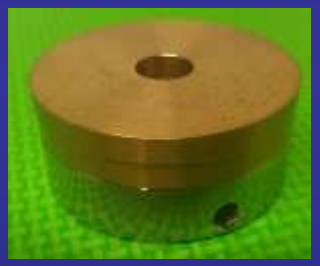
Holo-tomo, transverse slice

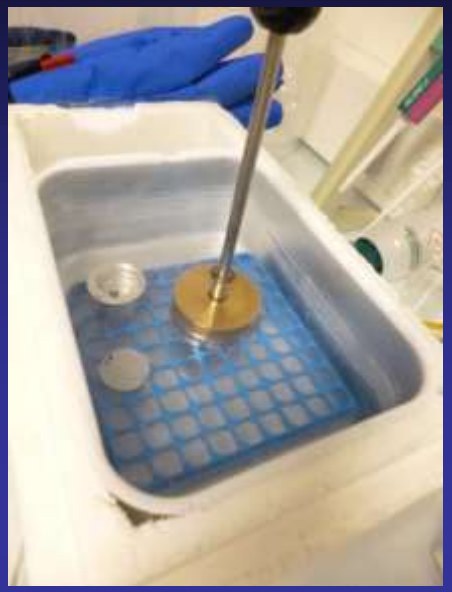
XRF molar concentration maps



Freeze drying protocol @ ID16







Protocol we recommend for smooth freeze-drying of cells. (manual LN2 refilling)

- 2h @ 153 K
- 1h: increase 153 K to 193 K
- 2h @ 193 K
- 1h : increase 193 K to 223K
- 2h @ 223 K
- 6h: increase 223 K to 303 K

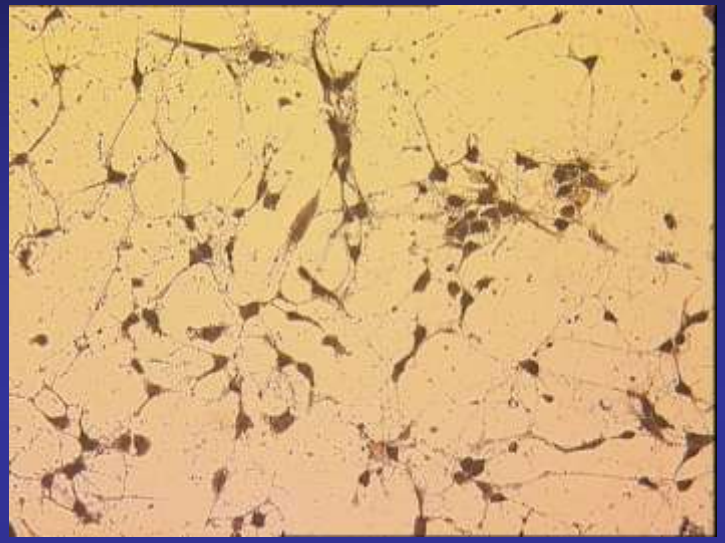
Turbo pump

7. 10^{-5} mbar

Cryopreparation – freeze-drying

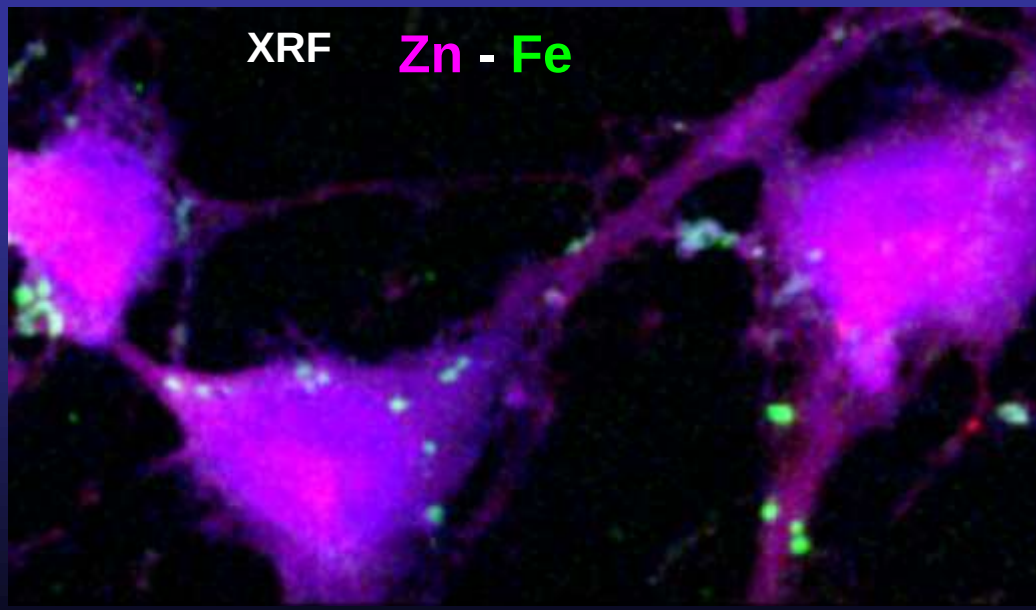
Freeze-dried neurons cultured on Si_3N_4 membranes

[Poly-L-lysine (0.0025% in H2O, 90 min @ 37 °C), followed by poly-L-ornithine(0.0033%inH2O,90min. @ 37 °C)]



Room temperature, in-air analysis
optical microscopy view in transmission

In-vacuum analysis requires
30-50 nm carbon coating on the FD sample



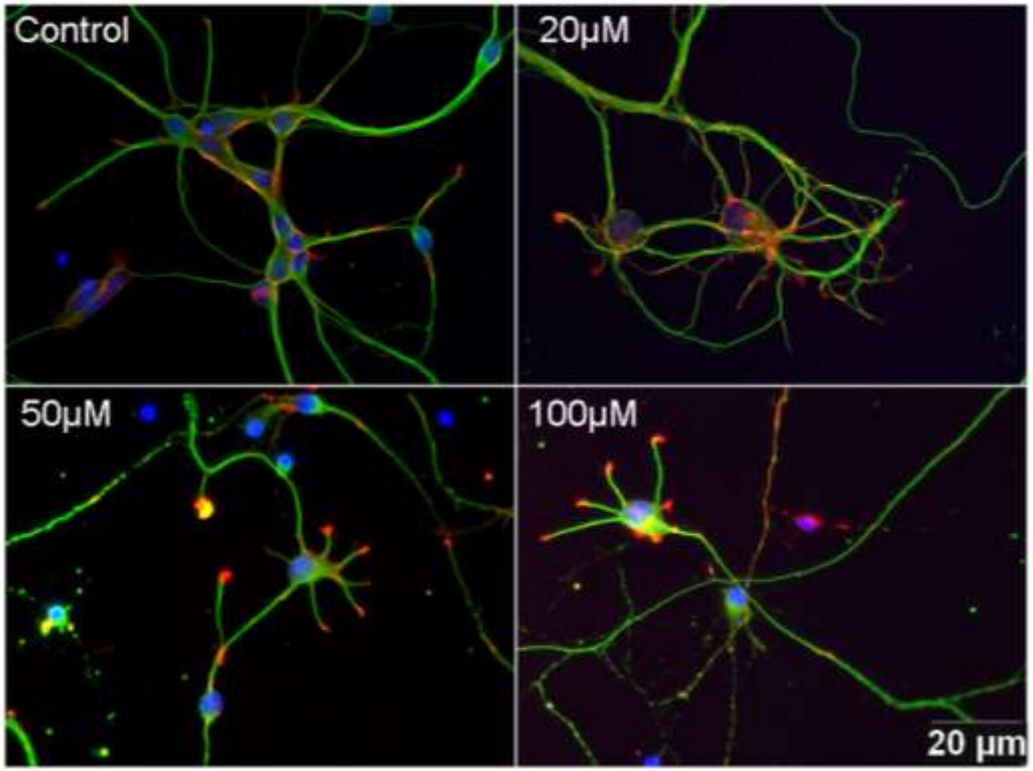
Daoust, A., Saoudi, Y., Brocard, J., Collomb, N., Batandier, C., Bisbal, M., salome S, Andrieux A., Bohic S.& Barbier, E. L. (2014) Hippocampus, 24(5), 598-610.

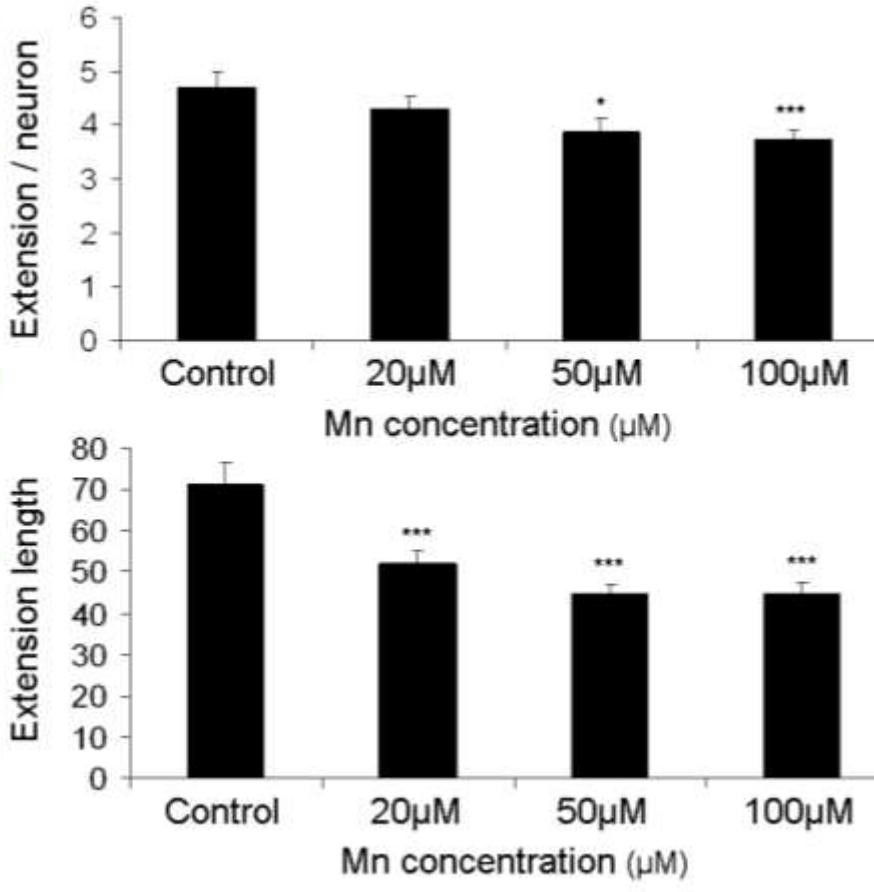
Gibon, J., Tu, P., Bohic, S., Richaud, P., Arnaud, J., Zhu, M., ... & Bouron, A. (2011). *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1808*(12), 2807-2818.

Kosior, E., Bohic, S., Suhonen, H., Ortega, R., Devès, G., Carmona, A., ... & Cloetens, P. (2012). *Journal of structural biology*, *177*(2), 239-247.

Morphologic neuronal characteristics

Tubulin
Actin
Nucleus





n=60; *p<0.05; ***p<0.001; mean ± SEM

► **Decrease of neuritic extension number and extension length upon MnCl2 exposure**

Daoust A., et al. (2014) Hippocampus.

CRYOGENIC WORKFLOW at ID16A

IBS – EM platform / onsite

High Pressure Freezing,
Cryo-Ultramicrotomy

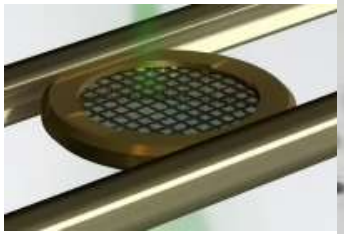
or

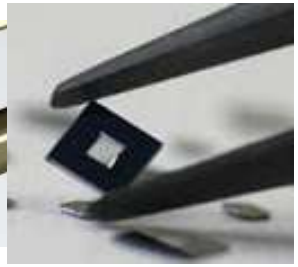
Grid Plunging

Cryo-Transfer System

Leica Cryo Light Microscopy

Cryo CLEM



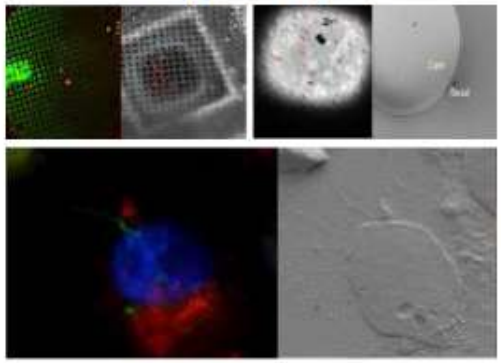


Leica LAS X Widefield Images used for correlation of LM marked structures in EM

Transfer to cryoTEM

Cryo-TEM

Image Analysis



X-ray nanoprobe ID16A

Selected references – good start

- *Quintana, C. Cryofixation, cryosubstitution, cryoembedding for ultrastructural, immunocytochemical and microanalytical studies. Micron 25, 63-99 (1994)*
- *Nagata, T. X-ray microanalysis of biological specimens by high voltage electron microscopy. Progress in Histochemistry and Cytochemistry 39, 185-319 (2004)*
- *Malucelli, E., Fratini, M., Notargiacomo, A., Gianoncelli, A., Merolle, L., Sargenti, A., ... & Iotti, S. (2016). Where is it and how much? Mapping and quantifying elements in single cells. Analyst, 141(18), 5221-5235*
- *Perrin, L., Carmona, A., Roudeau, S., & Ortega, R. (2015). Evaluation of sample preparation methods for single cell quantitative elemental imaging using proton or synchrotron radiation focused beams. Journal of Analytical Atomic Spectrometry, 30(12), 2525-2532.*
- *Jin, Q., Paunesku, T., Lai, B., Gleber, S. C., Chen, S. I., Finney, L., ... & Jacobsen, C. (2017). Preserving elemental content in adherent mammalian cells for analysis by synchrotron-based x-ray fluorescence microscopy. Journal of microscopy, 265(1), 81-93*
- *Pascolo, L., Venturin, I., Gianoncelli, A., Bortul, R., Zito, G., Giolo, E., ... & Ricci, G. (2018). Light element distribution in fresh and frozen-thawed human ovarian tissues: a preliminary study. Reproductive biomedicine online*
- *Zierold, K. (1991) Cryofixation methods for ion localization in cells by electron-probe microanalysis: a review. J. Microsc. 161(2), 357-366*