

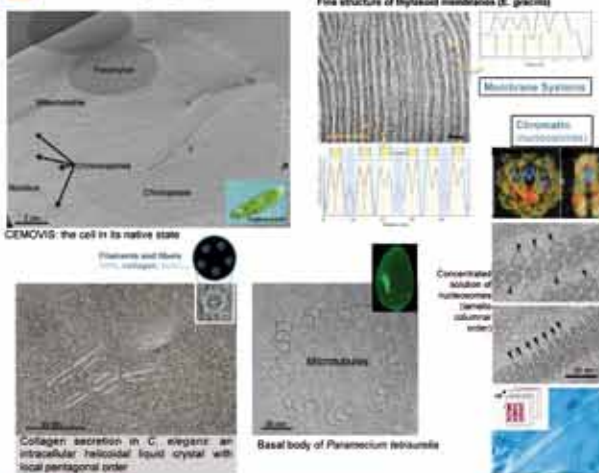
Structure and Organization of Cell Components Using CEMOVIS and CET

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State of the art and objectives

The cell structure and organization has been revisited in the last years with the use of fluorescence imaging and the development of fluorescent tags. In the meantime, **Cryo-Electron Microscopy Of Vitreous Sections (CEMOVIS)** and **Cryo-Electron Tomography (CET)** are emerging imaging techniques that access the visualisation of the cell components in their native environment and at the molecular level of resolution (1 to 5 nm). These promising methods are developed in a limited number of laboratories over the world. They should allow us to get a 3D understanding of cell snapshots under conditions as close as possible to the initial native state. Our goal is to develop these two approaches to image cell components both *in situ* inside the vitrified cell or *in vitro* after purification.

2D imaging by CEMOVIS



CEMOVIS: the cell in its native state

Fluorescence and TEM
 (TEM, cryo-EM, X-ray)

Collagen secretion in *C. elegans*: an intracellular vesicular liquid crystal with local pentagonal order

Basal body of *Paramecium tetraurelia*

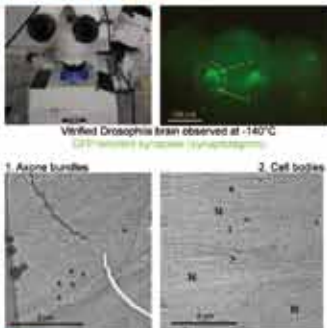
Microtubules

Concentrated solution of microtubules (dense liquid crystal order)

Membrane Systems

Cytoskeletal (microtubules)

Multiscale imaging

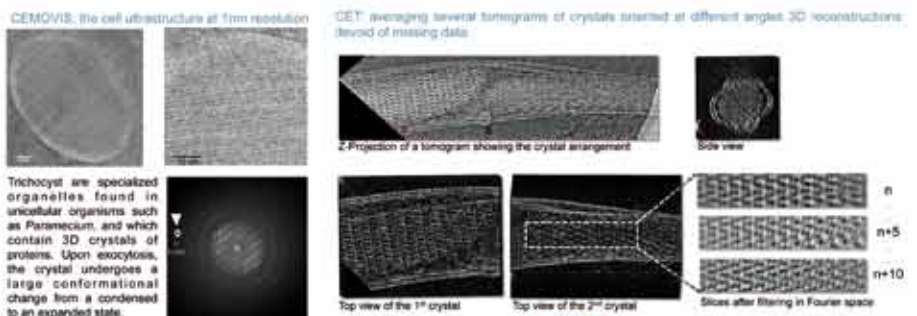


Vitrified *Drosophila* brain observed at -140°C
 GFP revealed by TEM (cryo-EM)

1. Axone bundles

2. Cell bodies

Combination of CEMOVIS & CET: Trichocysts from Paramecium



CEMOVIS: the cell ultrastructure at 11nm resolution

CET: averaging several tomograms of crystals oriented at different angles. 3D reconstructions (avoid of missing data)

Trichocyst are specialized organelles found in unicellular organisms such as *Paramecium*, and which contain 3D crystals of proteins. Upon exocytosis, the crystal undergoes a large conformational change from a condensed to an expanded state.

Z-Projection of a tomogram showing the crystal arrangement

Side view

Top view of the 1st crystal

Top view of the 2nd crystal

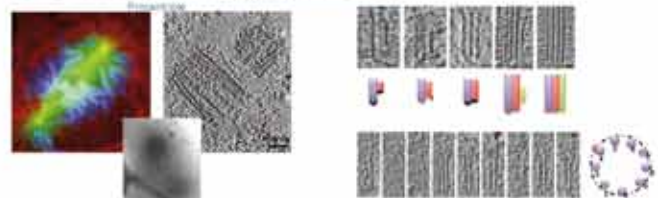
Slices after blurring in Fourier space

Publications

- Leforestier A, Berlin A, Dubochet J, Richter K., Sartori Blanc N., Livolant F (2008) Expression of chirality in columnar hexagonal phases of DNA and nucleosomes. *C. R. A. S. Chimie*, 229, 1815-2833.
- Odin, C., Heichette, C., Chrétien, D., and Y. Le Grand. (2009). Second harmonic microscopy of axonemes. *Optics Express*, 17:9235-40
- Guichard, P., Chrétien, D., Marco, S., and A.-M. Tassin (2010). Procentriole assembly revealed by cryo-electron tomography. *EMBO J.*, 29:1565-72.
- Coquelle, F. M., Blest, S., Heichette, C., Arnal, I., Karvann, C. and Chrétien, D. (2011). Cryo-electron tomography of microtubules assembled *in vitro* from purified components. *Methods in Molecular Biology*. In press.

3D imaging by CET

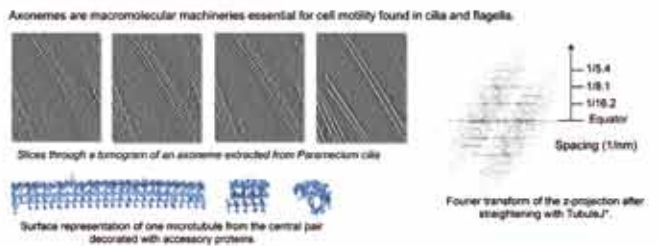
Centrosomes from KE37 lymphoblastic cells



The centriole is surrounded by the pericentriolar material and is responsible for the nucleation and organization of the microtubule networks on the mitotic spindle (left). Purified centriole, trapped in a thin layer of vitreous ice and observed by cryo-electron microscopy (centre). Section of a cryo-tomogram of the initial procentriole structure (right).

Procentriole microtubule triplet growth. The B-microtubules assemble using the outer surface of A-microtubules as a template and polymerises bidirectionally (Similarly for the C-microtubules). The distal end of the triplet continues to grow while the proximal end stops, leading to blunt extremities just above the γ -TuRC. In the mature centriole, the A-microtubules are no longer capped. The nine microtubule triplets grows independently. Scale bar, 20 nm.

Axonemes from Paramecium



Axonemes are macromolecular machineries essential for cell motility found in cilia and flagella.

Slices through a tomogram of an axoneme extracted from *Paramecium* cilia

Surface representation of one microtubule from the central pair decorated with accessory proteins.

Fourier transform of the z-projection after straightening with TubuleT.

Spacing (1/nm): 1/15.4, 1/18.1, 1/19.2, Equator

We have started to analyze the structure of axonemes from *Paramecium* in wild type and mutated cells. Cryo-ET reveals nicely the supramolecular architecture of the axonemal microtubules. *Blest et al., 2009. Proceedings of the IEEE International Symposium on Biomedical Imaging.

Perspectives

- Double axis tomography
- CETOVIS
- Electron dense labelling *in situ*
- Correlative light and electron microscopies

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