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ANR

Structure and Organization of Cell Components Using CEMOVIS and CET

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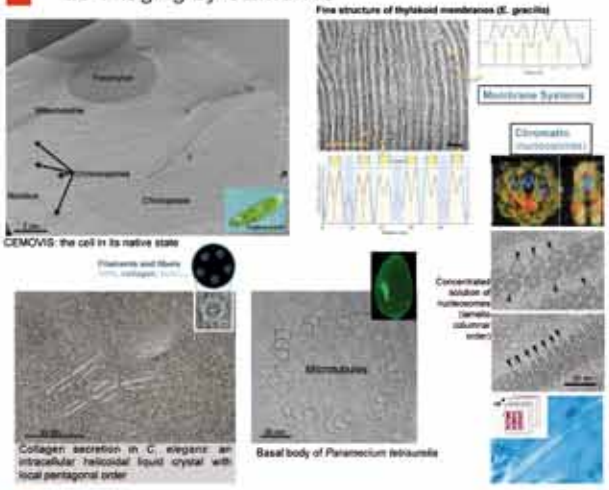
State of the art and objectives

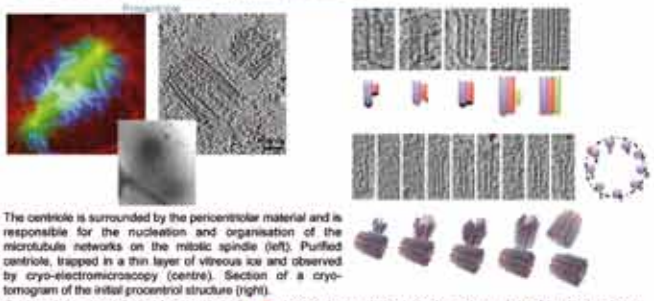
The cell structure and organization has been revisited in the last years with the use of fluorescence imaging and the development of fluorescent tags. In the meantime, **Cryo-Electron Microscopy Of VItreous Sections** (CEMOVIS) and **Cryo-Electron Tomography** (CET) are emerging imaging techniques that 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



3D imaging by CET

Centrosomes from KE37 lymphoblastic cells

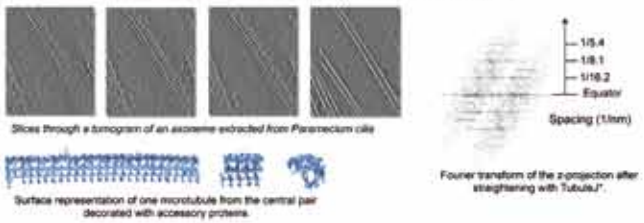


The centriole is surrounded by the pericentriolar material and is responsible for the nucleation and organisation of the microtubule networks on the mitotic spindle (left). Purified centriole, trapped in a thin layer of vitreous ice and observed by cryo-electromicroscopy (centre). Section of a cryo-tomogram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



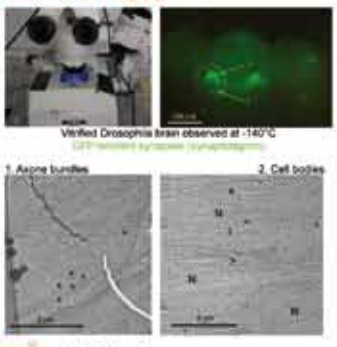
Slices through a tomogram of an axoneme extracted from Paramecium cilia

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

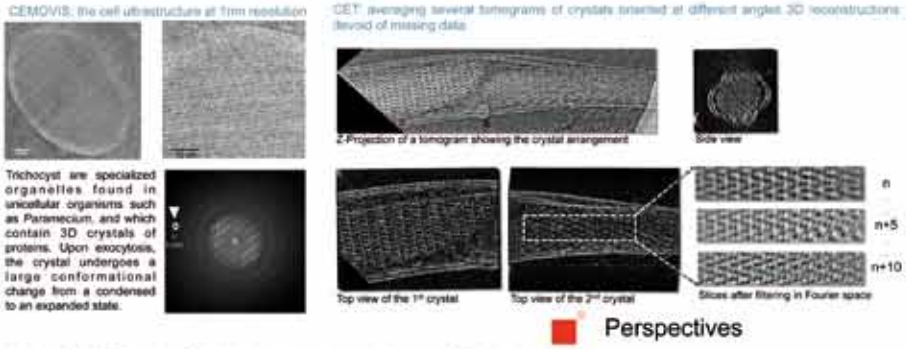
Fourier transform of the z-projection after straightening with TubuleJ*.

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.
**Biesiel et al., 2009. Proceedings of the IEEE International Symposium on Biomedical Imaging.*

Multiscale imaging



Combination of CEMOVIS & CET: Trichocysts from Paramecium



Publications

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- 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., Blesiel, S., Heichette, C., Arnal, I., Kervrann, C. and Chrétien, D. (2011). Cryo-electron tomography of microtubules assembled in vitro from purified components. *Methods in Molecular Biology*. In press.

Perspectives

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

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