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# Correlative Light/Electron Microscopy: a Tool for Investigating Infectious Diseases

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The tremendous advances in the microscopy world are bringing a fundamental contribution to unraveling the complex mechanism of host-pathogen interaction. Different imaging modalities, spacing from light to electron microscopy-based techniques, provide us with different type of information on cellular responses to the infectious agent (pathogenic infection) at different resolutions. At the light microscopy level, the dynamic interaction of a pathogen with its host can be captured with fluorescent-light microscopy (FLM) by highlighting with fluorescent markers the molecular players involved in the infectious process. The spatial resolution of FLM until recently limited by optical diffraction to ~ 200 nm can now reach a few tenth of nanometers thanks to the new super-resolution light microscopy based techniques (like photo-activated localization microscopy (PALM), stimulated emission depletion (STED) microscopy and structured illumination). However, only the tagged molecules of interest can be detected, while the information on their structural organization with respect to the other cellular components is missing. This complementary information can be retrieved by investigating the same with electron microscopy (EM).

The combination of FLM with EM techniques (CLEM) is a powerful method for correlating dynamic functional information from FLM with "static" high resolution structural information from EM. For instance, specific time points or rare events of a dynamic process – like the entry of a pathogen into a host cell – can be captured in 3D by FLM and subsequently relocated and imaged in 2D or in 3D in the electron microscope. The relocation of the labeled structures of interest is a crucial step in a correlative microscopy approach. It involves the transfer of the sample and of the coordinates of the area of interest to the electron microscope. The possibility of growing or depositing the infected cells on supports provided with markers, like cell-locate glass slides or finder grids (see Fig.1), is a fundamental prerequisite for CLEM. The accuracy of the relocation depends on the type of molecular markers used, on the resolution at the FLM level and on the error on the coordinates transfer. The highest accuracy is reached with probes that are both fluorescent and electron dense - like antibodies coupled simultaneously to a fluorescent dye and to a gold nanoparticle (fluoronanogold) or to quantum dots.

We have successfully developed and implemented a CLEM approach for scanning (SEM) and for transmission electron microscopy (TEM). Our method can be applied both at room temperature (see Fig.1 and Fig.2) on classically prepared samples and in cryo-conditions on samples that are rapidly frozen in their native state. In the cryo-CLEM approach, the same event is imaged with cryo-FLM

followed by cryo-EM and cryo-electron tomography [1]. This approach ensures the highest structural preservation of the biological material but, unlike the classic CLEM approach, is generally not compatible with immunocytochemistry.

We are applying both CLEM approaches to the investigation of the clathrin-mediated endocytosis of Listeria Monocytogenes and large beads into host cells. This new process was recently established by Veiga and Cossart [2] with a combination of biochemical methods and fluorescence microscopy observation. We aim at determining how clathrin is delivered and assembled around entering bacteria and large ligand-coated beads. Our room temperature approach combines 3D confocal microscopy both on transfected and on immunolabelled cells with 3D reconstructions from serial sections and tomography acquisitions. Our findings clearly show that clathrin is delivered on membranous compartments of variable sizes, that are connected to cytoskeleton elements like actin and septin, that are known to play a role in the internalisation of the bacteria. We are currently optimising our system for a cryo-CLEM approach, which is emerging as a promising tool for studying isolated pathogens or host-pathogen interactions, especially in combination with cryo-preservation and cryo-sectioning [3].

References

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[2] E. Veiga et al., *Nat. Cell Biol.* 7 (2005) 894.
[3] M. Cyrklaff et al., *Imaging and Microscopy* 9 (2007) 50.

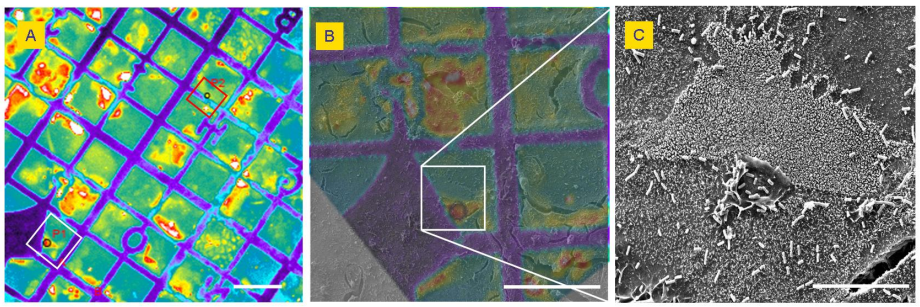


FIG. 1. Polarised CaCo-2 cells, grown on marker electron microscopy grids; the cells invaded by *Shighella Flexneri* are identified by calcium responses (see cells P1 – white box and P2 – red box in **A**. Scale bars: **A** and **B** 100 µm, **C** 20 µm).

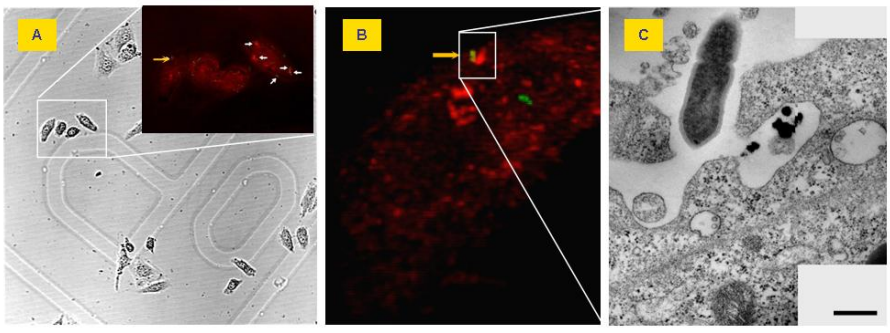


FIG. 2. Clathrin (red) mediated entry of *Listeria Monocytogenes* (green) in HeLa cells grown on cell-locate coverslips. The same entry event is localised with fluorescence microscopy (inset in **A** and **B**) and then (**C**) relocated and imaged in the electron microscope (**C**, scale bar = 500 nm).

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