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Correlated 3D Light Microscopy and 3D Electron Microscopy: Applications of an Integrated Setup of a CLSM and a FIB/SEM

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Correlative Light and Electron Microscopy (CLEM) becomes much simpler when performed in an integrated setup, as it allows for a much simplified image registration of the Light and Electron Microscopy modalities. Several integrated CLEM systems with different geometries were realized recently [1–4]. Some of these systems are even commercially available now (iCorr+ from ThermoFisher, SECOM from Delmic). However, so far all integrated CLEMs were limited to 2D imaging since at least one of the modalities used was unsuitable for 3D imaging.

Our group has developed a novel set-up which possesses full 3D CLEM capabilities in both modalities. For this goal, we have successfully integrated a Confocal Laser Scanning Microscope (CLSM) into a Focused Ion Beam / Scanning Electron Microscope (FIB/SEM). The added benefit is the relatively high-speed of 3D imaging of a CLSM (~10^4 μm^3/min) when compared to the speed of a FIB/SEM (~10^{-2} μm^3/min).

In order to preserve the 3D functioning of our FIB/SEM (Scios, ThermoFisher), the ability to tilt the stage towards the FIB column must be retained. This requirement severely limited the possible placements of the CLSM inside the vacuum chamber. Consequently, the CLSM is put very snugly in between the other instruments already present in the FIB/SEM chamber. The objective lens has to be vacuum compatible, so no immersion liquid can be used and as a result the numerical aperture of the objective is comparatively low (we use a Nikon objective with NA 0.9 and W.D. 2 mm). The discrepancy between the working distances of CLSM and FIB/SEM (2 mm vs 7 mm) forces to shuttle the specimen between two imaging positions.

Because of the difficult access into the crowded vacuum chamber it is very important to be able to pre-align the CLSM while it is not yet integrated into the FIB/SEM. We have reached this goal by using a special mount for the optical microscope (figure 1, upper left panel). The CLSM can be assembled in this mount from numerous optical parts and it can be aligned with an ease of an optical bench. The mount with the CLSM aligned can then be split into three pieces which are easy to install inside the setup. With this approach, we have been able to achieve the Point Spread Function of our integrated CLSM close to the diffraction limit (FWHMs across dimensions are ~0.4 μm×0.4 μm×1 μm at wavelengths of 532 nm for excitation and ~560 nm for emission).

We have investigated several samples in our integrated 3D CLEM set-up, such as biological (figure 1, upper right panel), catalytical (figure 1, lower right panel), and geological (not shown) specimens. For every case we have observed the added value of the integrated 3D CLEM. For example, in the biological sample in (figure 1, upper right panel), we have shown increase the throughput of 3D imaging of rear events with the FIB/SEM. By using the fluorescent signal to navigate the FIB/SEM towards the ROI we have been able to decrease the acquisition volume from typical ~10x10x10 μm^3 to ~3x3x3 μm^3. This led to consequent time decrease from ~50 hours per stack to just above 3 hours in this case.

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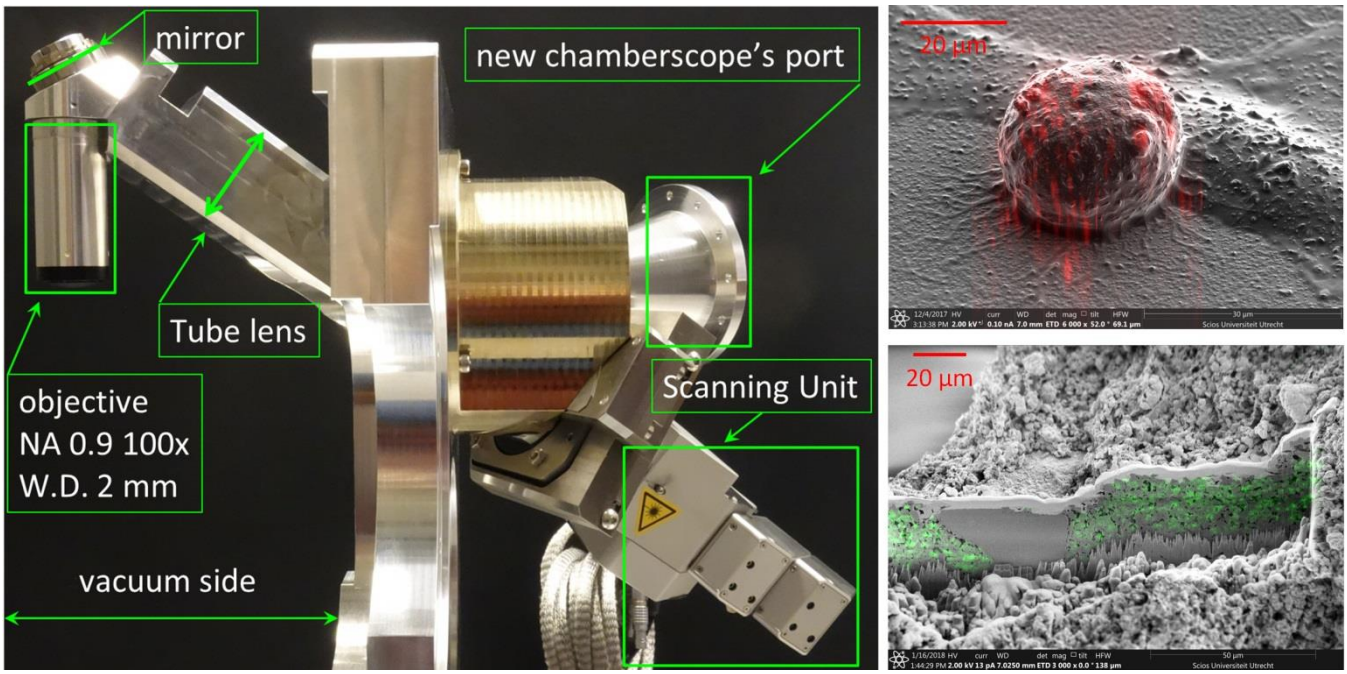

Figure 1. Left: The pre-integrated CLSM mounted on the model of the chamber wall. The numerous optical components are assembled and aligned on this mount. Upper right: an overlay of the 3D CLSM image with a SEM overview of a resin embedded HeLA cell. The fluorescent signal (red) is caused by the nanoparticles introduced to the live cell culture. Lower right: fluorescent signal (green) from catalytical reaction products within the catalyst. The trench is FIB-prepared in the same system.

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