INTEGRATED 3D CORRELATIVE LIGHT AND ELECTRON MICROSCOPY

Sergey V. Loginov, Alexandra V. Agronskaja, Gerhard A. Blab, Hans C. Gerritsen

Soft Condensed Matter and Biophysics, Debye Institute, University of Utrecht,
Princetonplein 1, 3584 Utrecht, The Netherlands
E-mail: S.Loginov@uu.nl

KEY WORDS: integrated Correlative Light and Electron Microscopy, FIB/SEM, CLSM.

Integration of a Confocal microscope and a 3D Electron Microscope (EM) in one set-up results in efficient Correlative Light and Electron Microscopy (CLEM) in 3 dimensions. The Confocal Laser Scanning Microscope (CLSM) is used to rapidly acquire a 3D image of the specimen which is used to identify regions of interest (ROI) with a limited resolution. Next, the ROI is studied in detail with nm resolution using a Focused Ion Beam – Scanning Electron Microscope (FIB/SEM).

Here, the integration of a CLSM inside a FIB/SEM (Scios, Thermo Fisher) is realized by mounting the optical microscope objective inside and the confocal scan head (Yanus, Thermofisher) outside the vacuum chamber of the FIB/SEM. To switch between the two imaging modalities the specimen is shuttled between two imaging positions. This approach requires the comparatively large objective to be vacuum compatible, no immersion objective can be used and as a result the numerical aperture of the objective is comparatively low (0.9).

In this work the setup is discussed (left panel) and examples of correlative imaging are presented. So far we investigated specimens from catalysis, geosciences and biology. In the biological example in fig. 1, the faith of fluorescent nano particles inside HeLa cells is studied. In the middle panel groups of fluorescent nano particles are visible in a 3x3 μm² image. The right panel shows a section through the nano particles recorded with the FIB/SEM. It reveals that the particles are present in lysosomes. Without the guidance of the CLSM finding the nano particles with the FIB/SEM is a matter of trial and error. To this end 10x10x10 μm³ stacks are recorded which takes about 50 hours per stack (including sample preparation). Recording a 3x3x3 μm³ stack takes less than 7 hours which exemplifies the speed gain of the integrated approach.

Figure 1. Left: The integrated CLSM. Middle: Confocal image of a ROI containing fluorescent nanoparticles. Right: Virtual 3D FIB/SEM slice showing the same groups of nanoparticles.