Large area automated image acquisition for integrated CLEM

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Imaging techniques in the life sciences have seen great advances in recent times. Super-resolution optical microscopy techniques like STORM, STED and PALM enable biologists to overcome the diffraction limit in optical imaging. At the same time, advances in electron microscopy have made it possible to achieve both sub-nanometre resolution and volume imaging by means of advances in sample preparation such as cryogenic techniques and quick freeze substitution, and techniques like array tomography, FIB-SEM and SBFSEM. With these developments, correlative light and electron microscopy (CLEM) has also grown in popularity. The ability to combine functional information in the context of high resolution structural information using CLEM has helped in increasing the understanding of biological systems.

One of the main challenges in the field of CLEM is still the amount of labour needed to obtain the final image. This is partly due to the time consuming workflow for conventional CLEM, involving imaging the sample in the optical microscope, removing it from the microscope, staining it for electron imaging, transferring it to the electron microscope and imaging it there. This brings about the risk of sample contamination, often requires time and effort to locate the region of interest again in the electron microscope, and requires considerable time to perform the overlay manually. A fundamental problem is also that as a result of removing the sample and staining it for EM, it is not actually the same sample that is being imaged in the two systems. And finally, since there is no ground truth available for overlaying the two images, the manual overlay performed by inspection of the images themselves, is biased. Fig. 1 shows an imaging platform developed for performing integrated CLEM (iCLEM). It is designed as an optical microscope that is retro-fitted onto any SEM, and overcomes these challenges by enabling simultaneous imaging of the sample with light and electrons, that is, without any sample transfer between the two imaging modes. The overlay is based on markers generated by cathodoluminescence (Fig. 2) when the electron beam is incident on the sample. It is highly accurate, completely automated and is performed at the end of each acquisition, saving the user valuable time.

In this work, we address the next challenge in CLEM. In order to go from imaging to understanding, it is essential that the imaging is performed over a large area of the sample. An automated tiling software has been developed which acquires a series of images by moving the sample stage, detects and corrects for the image shift using cross-correlation, and stitches them to create a high-resolution large area image. Fig. 3 shows an example of a tile of 5 x 5 images of a thin section of HeLa cells on a transparent conducting substrate, acquired using automated tiling, in a Thermo Fisher Verios 460 SEM using the in-lens secondary electron detector. Multiple fluorescence streams acquired simultaneously with the electron image, resulting in successful automated large area correlative imaging, will be presented at the meeting.

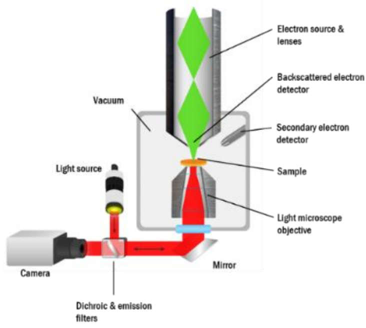

Fig. 1 Schematic of the optical imaging platform fitted onto a Scanning Electron Microscope for performing iCLEM.

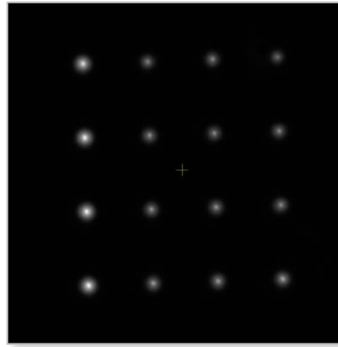

Fig. 2 Optical image showing an array of cathodoluminescence-based markers generated by the impingement of the electron beam on the sample, used for automated CLEM overlay.

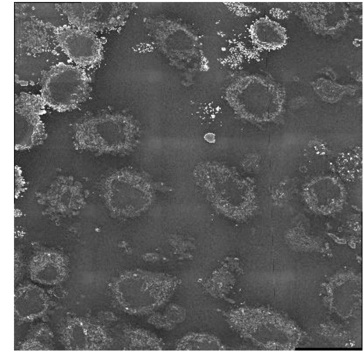

Fig. 3 A tile of 5 x 5 images of a thin section of HeLa cells on a transparent conducting substrate, acquired using automated tiling, in a Thermo Fisher Verios 460 SEM using the in-lens secondary electron detector.