Biological investigation of samples, both in their native state as well as under dried, fixated conditions, is predominantly achieved by light microscopy (LM). LM allows biomolecule identification, but has a diffraction-limited resolution and is incapable of revealing the ultrastructure. On the other hand, electron microscopy (EM) offers sub-nm resolution, but only allows grey scale images of the cellular ultrastructure of dehydrated and/or frozen samples without providing the functional biomolecular information. By combining both techniques in CLEM, biomolecule identification within the ultrastructure is possible at high resolution and sensitivity.

Nowadays, the number of researchers using CLEM is still relatively limited, compared to the amount of LM and/or EM users. This can be related to transfer steps between separate EM and FM setups, crucial differences in sample preparation protocols, the need for fiducial markers visible in both LM and EM to retrieve regions of interest, and limitations in correlation accuracy. Additional challenges are to increase spatial resolution in LM and incorporate temporal resolution in EM.

We have recently developed a setup that enables LM and EM imaging simultaneously on the same area of a sample [1]. This eliminates the transfer and ROI retrieval steps [2]. Thus allowing for easy, quantitative investigation of large areas and datasets with a drastic reduction of inspection time and reduced risk of sample contamination2. With this microscope, high registration accuracy down to five nanometer can be obtained without the need for fiducial markers.

However, comprehensive investigation of biological activities of cells requires high resolution imaging in the native state of the cell. Therefore, a microchip was designed as part of this setup, with a thin electron transparent window as well as a light transparent window [3]. This facilitates correlative microscopy of cells in liquid. We will present the microscope design, show application examples and discuss the possibilities for near-native state correlative imaging. We expect that with new developments like our setup, CLEM can become a powerful tool for fundamental biological research, applied industrial research and medical diagnostics.


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Fig. 1: Optical components on the outside of the vacuum door (A), integrated microscope in SEM with final electron lens above and light objective lens below the sample (B) and vacuum parts of the CLEM platform [1].

Fig. 2: Schematic illustration of the simultaneous observation with fluorescence (from below) and scanning electron microscopy (from above) of a sample in liquid, shielded from the vacuum by a thin, electron-transparent membrane [3].

Fig. 3: (A) Fluorescence image of CV1 cells, fixed during uptake of EGF-conjugated QDs (B), the enlarged image of the boxed area in a), (C) SEM image of the same area (reversed contrast) and (D) Similar high-magnification SEM image of part of a CV1 cell taken in liquid. Arrows point to the internalized EGF-conjugated QDs.