Further improvements on the integrated laser and electron microscope

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Combining light and electron microscopy has several advantageous: light (fluorescence) microscopy can be used to rapidly locate specific labels/events in large fields of view but has limited space resolution. Electron microscopy on the other hand has superior resolution over a limited field of view and therefore a poor method to locate events in large fields of view.

Combining light and electron microscopy (Correlative Light Electron Microscopy, CLEM) has been used for a long time and is normally performed by employing separate light (fluorescence) and electron (transmission) microscopes. This approach to CLEM is time and labor consuming: it requires additional sample preparation steps, the setting up of two imaging systems and overcoming difficulties in retrieval of coordinates of region of interest in both microscopes. Furthermore specimens may get damaged or lost in the transport between the two microscopes. An elegant way to simplify and speed up CLEM is to integrate both microscopes in one setup. Previously we demonstrated that integration of fluorescence microscopy in a transmission electron microscope (TEM) is not only feasible [1], but also very useful [2].

Our integrated approach is based on a specially designed scanning fluorescence microscope that is mounted on one of the side ports of a standard TEM (Technai 12, FEI Company). The fluorescence microscope has a lateral resolution of 0.55 μm, maximum field of view of 500x500 μm^2, and detection rate of up to 1 frame per second.

The fluorescence microscope shares the sample stage with the TEM and coordinates of regions of interest can be correlated with an accuracy of ±0.5 μm.

The essence of the CLEM is to identify regions of interest with the help of fluorescence markers. Therefore in the first version of Integrated Laser and Electron Microscope (ILEM) we employed only a single detection channel. Recently, we added a second detection channel that can be used for two color fluorescence imaging, but also for detection of back reflected laser light. Detection of back reflected laser light is particularly useful for finding coupes on standard grids. Interestingly, in the case of Tokuyasu cryo sections of cells, the back reflected laser light images strongly resemble low magnification TEM images. We note that the back reflected laser light is much stronger then the fluorescence signal, therefore the recording of reflection images requires only very low laser powers. Consequently virtually no photo degradation (bleaching) of the sample is introduced by reflection imaging of coupes on the grid.

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A major advantage of the integrated correlative microscopy approach is the gain in acquisition speed of correlative images. The acquisition rate of the CLEM can be further increased by automated acquisition of the fluorescence images. To streamline automated acquisition an auto-focusing unit was added to the fluorescence microscope. This unit consists of an infra red diode laser and a CCD camera. Details will be given during the presentation.

The integrated approach for CLEM requires new sample preparation techniques that provide contrast in both fluorescence and electron microscopy. At this moment there are only few protocols that are compatible with the integrated approach [3,4]. Therefore new, standardized protocols need to be developed. Possible routes to solve the difficulties related to integrated protocols will be discussed.

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