ELECTRON-BEAM INDUCED FLUORESCENCE SUPERRESOLUTION IN INTEGRATED LIGHT AND ELECTRON MICROSCOPY

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We present a novel superresolution (SR) method using integrated correlative light and electron microscopy. Correlative light and electron microscopy (CLEM) [1] is a powerful technique that combines the nanometric resolution capabilities of electron microscopy (EM) with the high specificity and large field of view that fluorescence microscopy offers. However, for precise correlation, the two order resolution gap between them still has to be mitigated. While correlation of SR data with ultrastructural images obtained with electron microscopy (EM) has been demonstrated [2] , the requirements for SR microscopy are often in conflict with those for EM. Further, the optical localization accuracy in the correlation image may be severely compromised compared to the SR resolution by the additional error introduced by aligning the separate SR and EM images. Here, we present a novel approach for correlative SR-EM using a focused electron beam to locally modify the fluorescence signal of fluorophores, and detecting the instantaneous change in fluorescence intensity with a wide-field epi-fluorescence microscope. We use an integrated light-electron microscope [3] that facilitates the recording of the fluorescence signal while scanning the electron beam through the fluorescence field of view. By correlating changes in the fluorescence decay with the instantaneous electron beam position and the other EM signals, we obtain a SR fluorescence image, that is in perfect registry with the simultaneously acquired EM image.

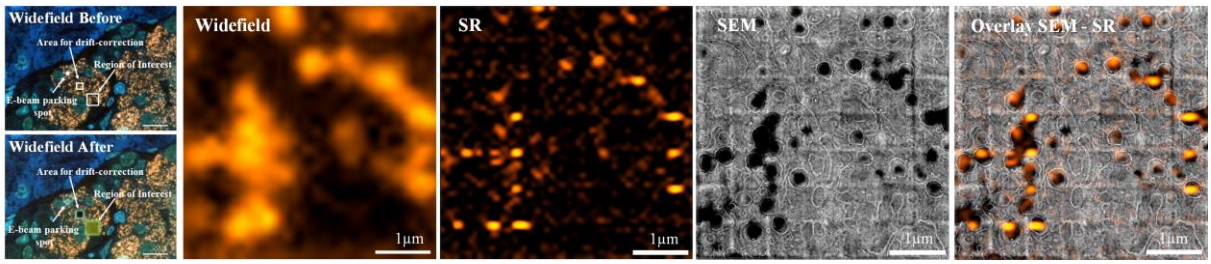

Figure 1: 100nm lateral superresolution on rat pancreatic tissue sections immunolabeled for insulin with Alexa Fluor 594

We have therein achieved a lateral resolution below 100nm in rat pancreatic tissue sections, immunolabeled for insulin using standard Alexa Fluor dyes. We will further discuss the applicability of the technique to multiple dyes and its extension to higher resolution, thereby paving the way towards unambiguous correlation. We will also discuss the photobleaching behaviour of fluorophores under vacuum conditions.

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