Electron-Beam Induced Fluorescence Superresolution in Integrated Correlative Light and Electron Microscopy

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We present a novel optical superresolution (SR) technique in the context of integrated correlative light and electron microscopy. Correlative light and electron microscopy (CLEM) [1] represents a powerful set of tools that combine the nanometric resolution potential of electron microscopy (EM) with the excellent molecular specificity of fluorescence microscopy (FM). Thereby enabling fluorescent regions of interest to be mapped to their ultrastructural backdrop.

However, for precise correlation, the two order resolution gap between EM and FM still has to be mitigated. Optical superresolution (SR) techniques have witnessed rapid developments in recent years by achieving image resolution well below the diffraction limit. While correlation of such superresolution data with ultrastructural images obtained with EM has been demonstrated [2] [3] , the requirements for SR microscopy are often in conflict with those for EM and their practical implementation still remains difficult. 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/registering 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 emitters and detecting the instantaneous change in fluorescence intensity with a high numerical aperture (N.A.) wide-field epi-fluorescence microscope. We use an integrated light-electron microscope [4] that facilitates the recording of the fluorescence signal while scanning the electron-beam through the optical field of view. By correlating changes in the fluorescence decay with the instantaneous electron-beam position and the other EM signals, we reconstruct a SR fluorescence image, that is in perfect registry with the simultaneously acquired EM image.

We have therein achieved a lateral resolution below 100nm in rat pancreas tissue sections, immunolabeled for insulin using standard Alexa Fluor dyes. We will demonstrate the applicability of the technique to multiple dyes and its extension to higher resolution, thereby paving the way towards simultaneous and unambiguous correlation. Further, we will present theoretical analysis of the underlying resolution limit that can be achieved.

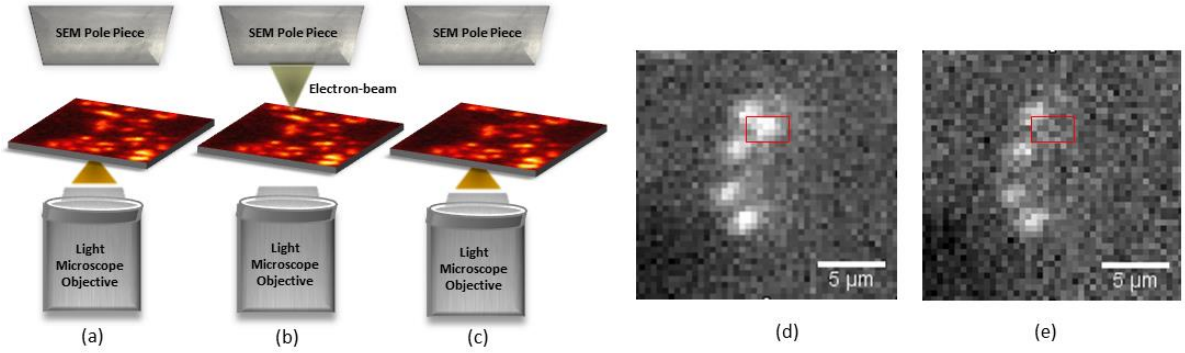

Figure 1: Schematic illustrating the technique. (a) Firstly a wide-field fluorescence image is taken, (b) thereafter the electron-beam impinges on the sample causing a local decay in fluorescence if an emitter is present. (c) Following which, a fluorescence image is taken again and the intensity of the irradiated area is monitored. (d) and (e) show actual images of fluorescent microspheres (*FSDG001-Dragon Green, Bangs Laboratories,Inc.*) before and after electron-beam impingement respectively (red inset).

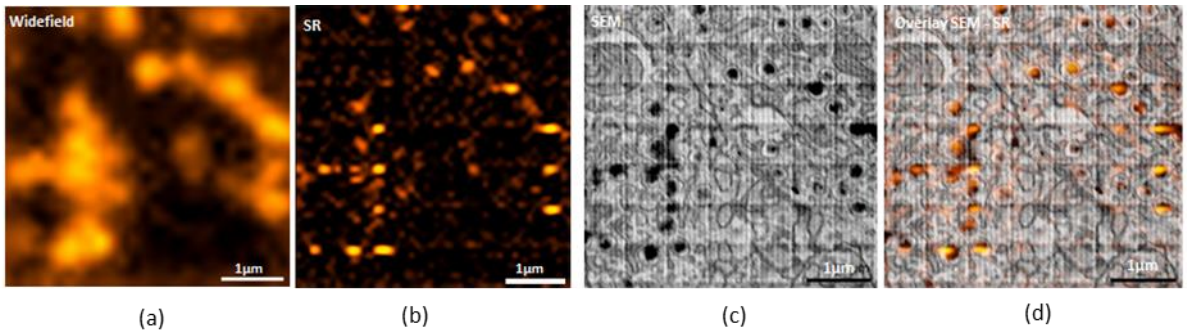

Figure 2: ~100nm lateral superresolution on rat pancreas tissue sections immunolabeled for insulin with Alexa Fluor 594. (a) Widefield fluorescence image, (b) SR image , (c) Scanning electron microscope (SEM) image of the same region and (d) Overlay of SEM with SR.

[1] P. de Boer *et al.*, *Nature Methods,* **12(6)**, 503-513 (2015)
[2] D. Kim *et al., PLoS ONE,* **10(4)**, 1-20 (2015)
[3] C.J. Peddie *et al, Journal of Structural Biology*, **199**,120-131 (2017)
[4] A.C. Zonnevylle *et al.*, *Journal of Microscopy*, **252**, 58-70 (2013)