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# Site-specific, Automated 3D Imaging of Cells and Tissues Using a Dual Beam Microscope

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Current approaches to 3D imaging at subcellular resolution using confocal microscopy and electron tomography, while powerful, are limited to relatively thin and transparent specimens. To facilitate rapid and automated imaging of tissue specimens at the highest resolution, we are developing an alternative novel microscope technology that is capable of site-specific imaging of cellular and tissue specimens at spatial resolutions better than 100 nm. The principle of imaging is based on using a focused ion beam (FIB) to create a cut at a designated site in the specimen, followed by viewing the milled surface with a scanning electron beam. Iteration of these two steps several times thus results in the generation of a series of surface maps of the specimen at regularly spaced intervals. The 3D maps obtained with this sequential "slice-and-view strategy" has the potential to generate 3D maps of biological specimens at resolutions as high as ~ 50 nm in-plane and ~ 100 nm in the vertical direction. Besides the substantially higher resolution as compared to optical microscopy, the new technology provides a new tool for molecular imaging by simultaneously imaging cellular architecture and the spatial location of specifically targeted nanoparticle reagents such as those used in cancer diagnosis and treatment.

The principle of recording images using dual beam microscopy is shown in Fig.1. In the experiments reported here, we have used a Nova series of FEI DualBeam instruments equipped with a field-emission gun electron source for imaging and a gallium ion source for milling. Experiments that were conducted at room temperature were carried out using specimens that were preserved by critical point drying. Figure 2a depicts the result of FIB-milling into a pellet of baker's yeast cells that were subjected to critical point drying. A higher magnification view of the milled region is shown in Fig. 2b, and illustrates the level of contrast and resolution that can be typically obtained with such specimens. By iteration of the milling and imaging steps, a 3D map of a single yeast cell that is in the process of cell division was generated (Fig. 2c).

We have extended these studies to analyze 3D structures of frozen cells and tissues. Direct, site-specific imaging of frozen specimens using this dual-beam approach bypasses the need for chemical fixation and allows for the rapid evaluation and characterization of morphological features in 3D. For work at cryogenic temperatures, the Nova instrument was equipped with a Polaron cryo-transfer station, which allowed the specimen to be maintained at –150 C. The quality of the images obtained at cryogenic temperatures is beginning to be superior to that shown for room temperature imaging, and is likely to be a useful complement to optical imaging methods. We envisage a broad range of future applications for these methods in areas ranging from imaging of brain and tumor tissues to the imaging of subcellular architecture.

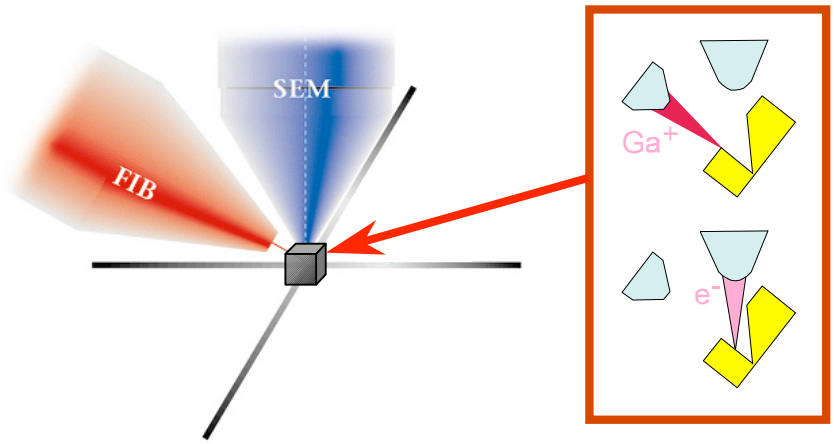


Fig. 1: Principle of imaging in a dual beam electron microscope. The sample is located at the intersection of the focused gallium ion beam (red) and the scanning electron beam (blue). Irradiation with the gallium ion beam results in excavation and milling of the sample in proportion to the strength of the beam current. A weak electron beam is used to record an image of the milled surface. A 3D image of the specimen is constructed by iteratively exposing the specimen to the gallium and electron beams. The specimen can be imaged either at room temperature, or at temperatures as low as –150 C using a customized specimen holder.

a)

b)

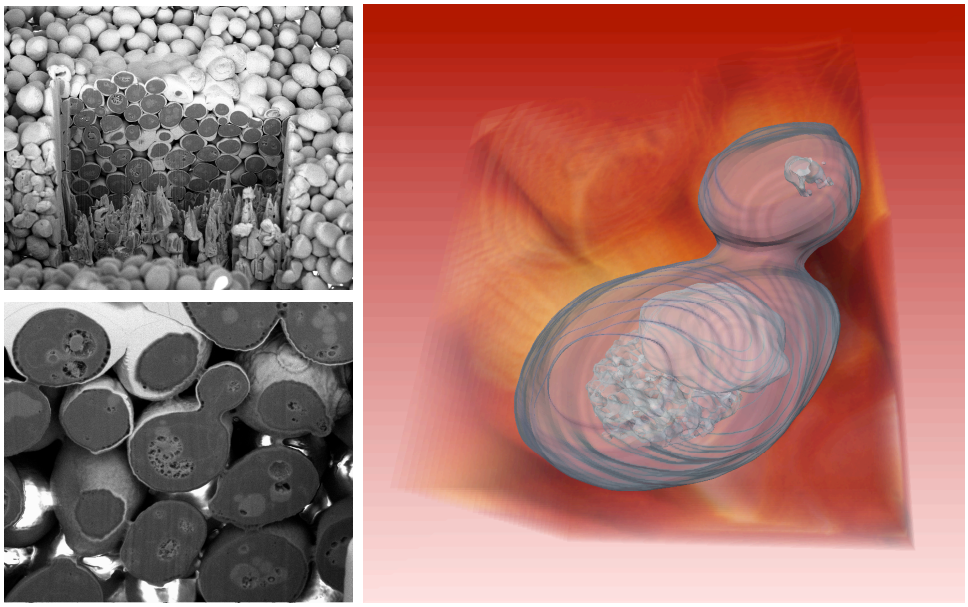

Fig. 2: Three stages in the structural analysis of a critical point-dried yeast sample. Panel (a) shows a pellet of yeast cells that has been milled with the ion beam. Panel (b) shows one of many successive slices through the region following the initial excavation shown in (a). Panel (c) shows a segmented surface rendering of a dividing yeast cell that can be seen in the central region of the slice in panel (b). The roughly contoured internal region represents a large vacuole, while the smoother contour corresponds to the surface of the nucleus.

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