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Localization and Number of Au Nanoparticles in Optically Indexed Cells by FIB Tomography.

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Gold nanoparticles (GNPs) are gaining importance as therapeutic chemical delivery vehicles, medical diagnostic tools, and phototherapeutic and contrast enhancement agents. GNPs are uniquely suited for these biological uses because of their chemical stability, novel optical properties, and broad potential for functionalization. Additionally, each of these beneficial properties is further enhanced by the ability to manufacture GNPs in an almost endless combination of sizes and shapes. This versatility has allowed researchers to access and modify biological processes inside of a large variety of cells [1] and the observation of innocuous uptake of citrate stabilized GNPs [2]. To describe the effect of GNPs, characterization of affected cells and tissues is required from the macroscopic to nanoscopic level. In particular, the location of cells in the tissue or culture of interest and then the mapping of the number and spatial distribution of the GNPs inside of those cells is required, and frequently requires multiple imaging techniques [3]. We achieve the large scale mapping of mammalian stem cells using reflection optical microscopy and then explore the location and number of the nanoparticles inside these cells after exposure to 60 nm GNPs using focused ion beam – scanning electron (FIB-SEM) based tomography.

To map the exterior of the cells at the size scale of a culture, here ~ 60,000 cells grown *in vitro* as a monolayer, to that of a single cell, we employ reflection optical microscopy. To access such different length scales, we stitch many optical fields of view together at low and high magnification. Figure 1 is a mosaic of 8 x 10 images stitched together using a 5x objective. In addition to the lateral mosaic we have also used several images at different objective-sample distances to extend the depth of field. Although reflection optical microscopy is not common in the biological sciences, it provides several distinct advantages for correlative imaging by optical and electron microscopy. First, sample preparation does not involve index matching fluid for optical microscopy, which removes a cleaning step in preparation for electron microscopy or the need for an environmental chamber. Second the sample viewing conditions closely mimic those of the FIB-SEM, allowing for the biological tissue or culture of interest to be viewed on the same specimen holder in the same orientation, making cross-tool registration easier. Finally, using a specialized optical microscope we can image large areas of the tissue or cell culture quickly and then create an index of specific cells of interest to be further investigated in FIB-SEM tomography (See figure1).

Once the specimen has been mapped with optical microscopy, a small amount of conductive metal is deposited and the cells to be investigated are chosen. Individual cells are then milled using the ion beam and imaged using the electron beam creating a series of images of the interior of the cell. In order to enhance contrast and retain the external structure of the cell, cells have been fixed in formaldehyde, and desiccated by sequential rinses in a mixture of ethanol and phosphate-buffered saline, in which the concentration of ethanol increases. This preparation technique, while not highlighting intercellular structure, has been found to preserve the contrast between cells and nanoparticles, allowing for fast tomography that clearly resolves the location and number of nanoparticles inside of cells (see figure 2).

References:
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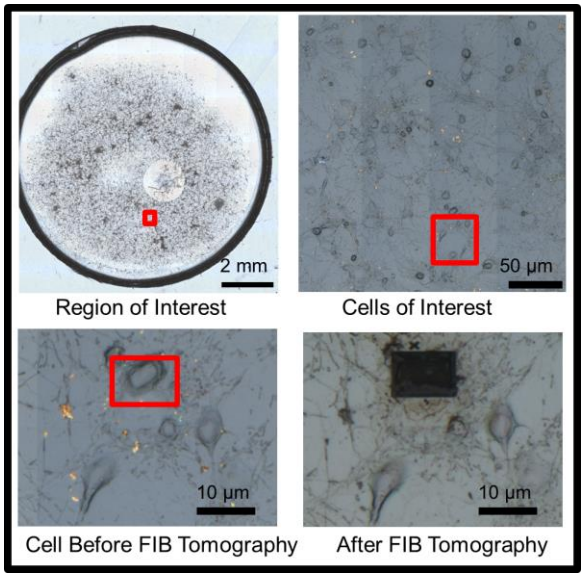

Figure 1. Optical Imaging Based Maps of Cells of Interest. By mosaicking optical micrographs at different magnifications, cells of interest can be located in the *in vitro* culture and then sectioned to give a tomographic representation identifying nanoparticle location inside of the cell.

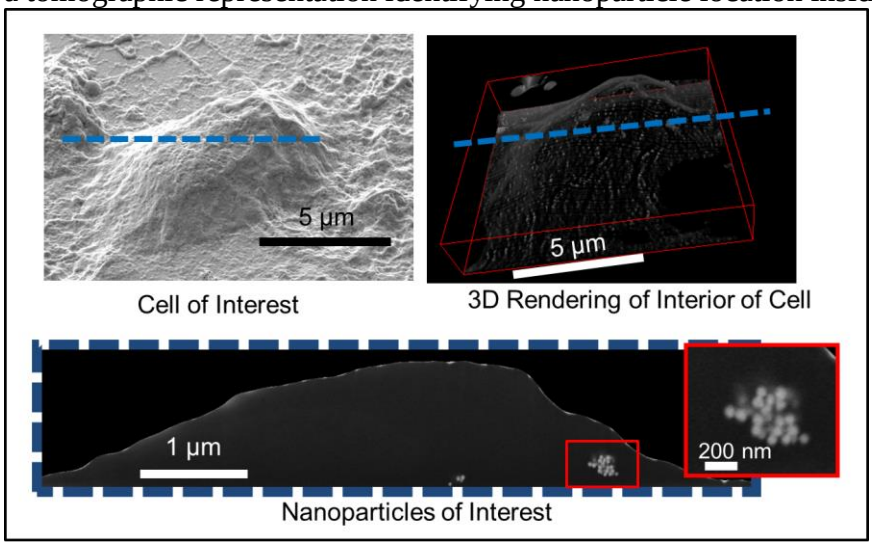

Figure 2. SEM and FIB Tomography to Count Nanoparticles Inside of Cells. The repeated sectioning and imaging of a cell using FIB-SEM techniques creates a 3D representation of the cell, which allows for the determination of nanoparticle location and number. It is observed that nanoparticles inside of stem cells enter and reside in the cells as clusters, a single tomographic slice with a highlighted cluster is shown above (Bottom).