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Correlating Multiscale Measurements of Nanoparticles in Primary Cells

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Nanoparticles are emerging as invaluable tools in disease diagnosis, disease treatment and imaging contrast enhancement agents [1]. The interactions of nanoparticles with host organisms are complex and affect biological systems over length scales that vary from the size of molecules to that of full organisms. In order to understand these interactions between nanoparticles and organisms, a variety of imaging and measurement techniques are required. We present imaging and analysis methods to statistically catalog cellular development on the macroscale, to identify the location of nanoparticles in cellular cultures on the microscale, and to identify the interaction of cellular organelles and nanoparticles on the nanoscale.

Cell cultures can provide a simple model for an organism and they are often used in clinical trials and the complex task of correlating the holistic response of an organism to its exposure to nanoparticles. For example, monocultures of human or animal cells derived from cancerous tumors or immortalized by other means can be used as model systems. However, primary cell cultures with multiple cell types [2], provide an *in vitro* test system closer to that of *in vivo* models. To develop the metrology tools needed for the investigation of nanoparticle-cell interactions in complex cultures, we have studied rat cortical neural progenitor cells at different points in their development and their interaction with citrate stabilized gold nanoparticles of varying size.

The statistical description of cellular cultures is best suited for optical techniques because of contrast mechanisms and relative field of views. A full population of mammalian progenitor cells can be surveyed and the density of cells measured using optical techniques. Unfortunately, limits on contrast and resolution prevent the classification of all cells found in the culture, the measurement of neurites, or the location and characterization of nanoparticles. One solution to enhance contrast is treatment with specific fluorophores and use of fluorescent techniques. This provides the ability to label cellular proteins that identify the cell type (e.g., neurons or astrocytes) in a mixed culture and creates almost binary contrast. However, preparation with these fluorescent tags degenerate the cellular membrane and still does not provide any measurement of nanoparticle size or location. In order to study these, a combination of higher resolution techniques is required. In particular, scanning electron microscopy, focused ion beam cross-sectioning, helium ion microscopy, transmission electron microscopy and transmission electron tomography provide the ability to locate and measure a nanoparticles size inside and outside of a cellular context.

We present correlated phase contrast and fluorescence images demonstrating that differentiated neurons and astrocytes identified by fluorescent tags can also be identified by morphology in cultures allowed to develop for 14 days or 21 days. We then image cellular morphology using scanning electron microscopy, determining the location of representative neurons or astrocytes. Once cells are located, we determine the presence of nanoparticles within the cell membrane using focused ion beam cross-sectioning. To measure the diameter of these nanoparticles more accurately, we image with

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helium ion microscopy on cross-sectioned cells. And finally, we use electron tomography to locate the nanoparticles in relationship to cellular structure.

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Technique	Example	Lateral Field of View	Information
Optical		450 μm	Cellular density
Fluorescence		450 μm	Cell type and Neurite outgrowth
Scanning Electron Microscopy		12 μm	Cell size and cellular morphology
Focused Ion Beam Cross Sectioning		5 μm	Nanoparticle localization
Helium Ion Microscopy		0.8 μm	Nanoparticle size within the cell for thick samples
Transmission Electron Microscopy		2 μm	Nanoparticle size and cellular structure
TEM Tomography		0.8 μm	Cellular structure, nanoparticle location in 3D

Figure 1. Table of Imagining Techniques for Cell-Nanoparticle Interactions. Each technique provides insight into the cell, nanoparticle, or the interaction of the two.

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