Application Note

Poseidon™

Correlative Light and Electron Microscopy (CLEM) of Eukaryotic Cells in Liquid

Reference: Dr. Niels de Jonge, Vanderbilt University School of Medicine, Nashville TN. M.J. Dukes, D.B. Peckys, & N. de Jonge; ACS Nano 4, 4110-4116, 2010

Introduction

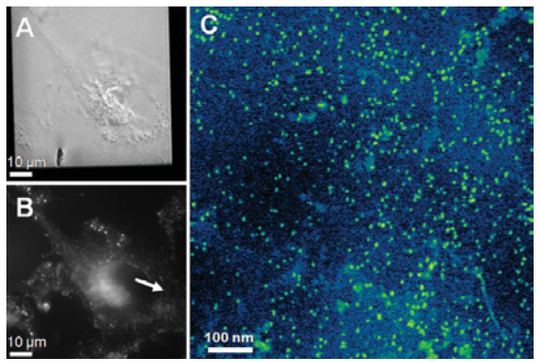
Correlative Light and Electron Microscopy (CLEM) is an imaging strategy that combines the functionality of light microscopy with the high resolution of electron microscopy. A key limitation in biological applications of CLEM is the need to dry or cryogenically freeze samples, which complicates experimental procedures and are prone to introducing artifacts. The Poseidon liquid holder enables electron microscopy imaging of wet samples. Thus, cellular receptors tagged with nanoparticles such as quantum dots (QDs) can be imaged with nanometer resolution under physiologically relevant conditions.

Experiment

COS-7 fibroblast cells were cultured directly on Poseidon E-chips™ sample supports. The epidermal growth factor (EGF) receptors were labeled bynincubating the cells for 5 minutes in a solution of 5 nM CdSe QDs coupled to EGF. After labeling, the cells were washed, fixed with glutaraldehyde, and stored in phosphate buffered saline. Fluorescence images were obtained by inverting the E-chips containing cells and placing them in a glass bottom culture dish.

Images were recorded using a wide field fluorescence microscope equipped with an oil immersion objective. Next, the E-chip was positioned in the Poseidon holder with a liquid thickness of 5 µm and inserted into an FEI CM200 electron microscope. The sample was imaged in scanning TEM and a liquid flow rate of 2 µL/min was maintained throughout the duration of the imaging session.

Discussion



The electron microscopy images were correlated with their corresponding location in the light microscopy image. The rectangular design of the E-chip window served as a reference system for matching position coordinates between images.

Figure A shows the direct interference contrast image of a cell on the E-chip window. The corresponding quantum dot fluorescence is shown in Figure B. The region indicated with an arrow was using scanning TEM (Figure C). The individual QDs are visible as yellow-green spots distributed throughout the cell (false colorized to enhance contrast). Cellular contours appear as regions of blue shading. Thespatial resolution was determined to be 3 nm using the 25-75% edge width of line scans taken over 10 QDs.

Application

These results demonstrate the utility of the Poseidon for CLEM imaging of protein distribution on whole cells. The resolution obtained is sufficient to discriminate among nanoparticles of different size, shape, and electron density to facilitate multiplex imaging studies. Contact us to discuss the Poseidon's full range of capabilities. We can be reached at (919) 377-0800 or contact@protochips.com.

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