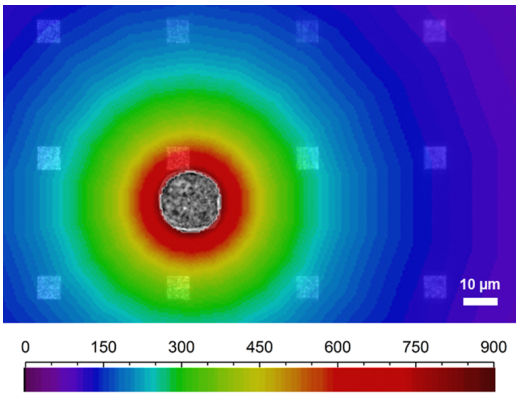
Naval Research Laboratory

The Corporate Laboratory for the Navy and Marine Corps

Imaging Protein Secretions from Single Cells in Real Time

The current-state-of-the-art measurements of cellular secretions of proteins produce just one data point every two hours with a spatial resolution of hundreds of microns: orders of magnitude coarser than what is needed to precisely map how cells organize protein secretions in space and time for multi-cellular functionality. To address this roadblock, the Naval Research Laboratory has invented a label-free technique based upon nanoplasmonic imaging which enables the measurement of individual cell secretions with time resolutions below one second and spatial resolutions below 10 µm. This is accomplished by lithographically patterning gold plasmonic nanostructures into arrays atop standard glass coverslips. The nanostructures are functionalized for biomolecular detection using standard thiol chemistries and the detection of analyte binding is imaged by a CCD camera. The technique integrates seamlessly on to commercially available wide-field and confocal microscopes, allowing real-time transmitted light and fluorescence imaging of the cells, as well as the plasmonic imaging of secreted proteins.



Overlay of the two images (LSPR, transmitted light) with a map of secreted antibody concenttrations as generated by Finite Element Analyses. The colored concentration scale has units of pM and the distance scale bar is 10µm

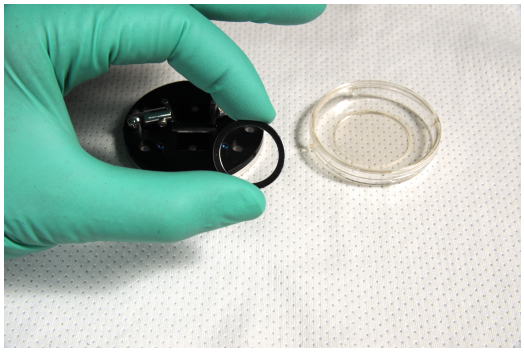
Advantages

Protein secretions measured in real-time with the frequency of time points limited only by the exposure time of the camera, typically 250-400 ms; Gold plasmonic nanostructures lithographically patterned on standard glass coverslips enabling fluorescence and bright field imagery to be readily integrated into the experiments; Nanostructures are calibrated for the quantitative determination of secreted protein concentration as a function of time and space; Platform is designed to accommodate a wide variety of cell types relevant to wound healing, including neurons, blood cells and epithelial cells

The combination of these advantages enabled us to measure secreted antibodies at sub-nanomolar concentrations with unparalleled spatial and temporal resolutions, while also monitoring cell health using fluorescence and transmitted light microscopy.

Opportunities

Because the cellular secretion of proteins plays a central role in cellular communication, the applications for this technique span biology. Protein secretions are utilized by cells in a diverse range of fields such as immunology, wound healing, developmental biology and diseases such as cancer. The plasmonic imaging technique is designed to integrate seamlessly with existing commercially available wide field and confocal microscopes. It is meant to enable investigators to extend their current light microscopy techniques to incorporate a measurement that was previously unattainable: real time spatio-temporal mapping of protein secretions from single cells.



Licensing and Collaboration Opportunities

US Filed Patent Application No. 14/0039632 is available for License to companies with commercial interest. Collaborative research and development is available under a Cooperative Research and Development Agreement (CRADA).

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