Photonic crystal enhanced microscopy

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Abstract: By modifying a microscope to perform hyperspectral imaging of reflectance from a photonic crystal, we describe a new microscopy approach that enables quantitative, spatially resolved imaging of the interaction of cells and nanoparticles with surfaces.
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1. Introduction

Cell membrane interactions with surfaces are fundamental aspects of many *in vivo* biological phenomena including differentiation, growth, apoptosis, tumor metastasis and injury response. Characterizing these processes in the laboratory traditionally involves fluorescent dyes, fluorescent proteins, histological stains, or fixation. Such approaches are either cytotoxic, or temporally constrained by the effects of fluorescence photobleaching. While these techniques elucidate the mechanics and outcomes of cellular processes, the lack of long-term, time-course data collection poses a serious compromise to the study of natural cell behavior during processes that occur over extended time scales like cell invasion and chemotaxis.

In a recent publication [1], we demonstrated photonic crystal enhanced microscopy (PCEM) as a label-free biosensor-based cell attachment imaging approach that quantifies cell-surface interactions with spatial resolution sufficient for monitoring intra-cell attachment distribution, and temporal resolution sufficient for generating time-lapse movies during processes that include chemotaxis, apoptosis, differentiation, and division. Critically, these studies can be performed on extracellular matrix (ECM) protein functionalized substrates, retaining the capacity to define the chemistry of cell-matrix interactions. Seeking to explore the limits of spatial resolution for the new imaging modality, and to determine the ultimate limits of detection for nano-objects, we next explored detection of metal and dielectric nanoparticles via PCEM [2]. Our work showed the ability to sense the presence of surface-adsorbed single nanoparticles of TiO_2 or gold through mechanisms of resonance wavelength shifting, resonant absorption quenching, and outcoupled light scattering. Further, we demonstrated substantially enhanced optical absorption for metal nanorods that exhibit surface plasmon resonances that couple with the photonic crystal resonances. The resonant electric fields at the photonic crystal surface may not only be used for label-free detection of surface-adsorbed materials, but may also be used to excite surface-adsorbed fluorescent dyes with intensities that scale with the distance of the fluorescent emitter from the photonic crystal surface. We recently described how photonic crystal enhance fluorescence (PCEF) can be combined with PCEM to estimate the distance between the cell membrane or internal cell bodies through derivation of spatial images of the fluorescence enhancement factor [3].

This invited talk with review recent progress in the Cunningham Group at Illinois in the design of photonic crystal surfaces and a detection system for performing PCEM imaging for cells and nanoparticles. Having demonstrated the ability to perform high resolution, time resolved imaging of cell-surface attachment and nanoparticle capture, our future plans involve demonstrating new applications for PCEM in drug discovery, as a new tool for understanding the interaction of a variety of cell types with surfaces (including stem cells, cancer cells, and bacterial biofilm), and digital-resolution biomolecule detection for disease diagnostics.

2. PCEM for Label-Free Cell-Surface Interaction Analysis

A form of microscopy that utilizes a photonic crystal biosensor surface as a substrate for cell attachment enables label-free, quantitative, submicron resolution, time-resolved imaging of cell-surface interactions without cytotoxic staining agents or temporally-unstable fluorophores. Other forms of microscopy do not provide this direct measurement of live cell-surface attachment localization and strength that includes unique, dynamic morphological signatures critical to the investigation of important biological phenomena such as stem cell differentiation, chemotaxis, apoptosis, and metastasis. We initially applied PCEM to the study of murine dental stem cells to image the evolution of cell attachment and morphology during chemotaxis and drug-induced apoptosis. PCEM provides rich, dynamic information about the evolution of cell-surface attachment profiles over biologically relevant time-

scales. Critically, this method retains the ability to monitor cell behavior with spatial resolution sufficient for observing both attachment footprints of filopodial extensions and intracellular attachment strength gradients.

3. PCEM for Imaging of Individual Metal and Dielectric Nanoparticles

We next demonstrated a label-free biosensor imaging approach that utilizes a photonic crystal surface to detect surface attachment of individual dielectric and metal nanoparticles through measurement of localized shifts in the resonant wavelength and resonant reflection magnitude from the photonic crystal. Using a microscopy-based approach to scan the photonic crystal resonant reflection properties with 0.6 μm spatial resolution, we show that metal nanoparticles attached to the biosensor surface with strong absorption at the resonant wavelength induce a highly localized reduction in reflection efficiency and are able to be detected by modulation of the resonant wavelength. Experimental demonstrations of single-nanoparticle imaging are supported by Finite Difference Time Domain computer simulations. The ability to image surface-adsorption of individual nanoparticles offers a route to single molecule biosensing, in which the particles can be functionalized with specific recognition molecules and utilized as tags.

4. Enhanced Fluorescence Imaging of Live Cells using PCEM

Finally, we demonstrated photonic crystal enhanced fluorescence (PCEF) microscopy as a surface-specific fluorescence imaging technique to study the adhesion of live cells by visualizing variations in cell-substrate gap distance. This approach utilizes a photonic crystal surface incorporated into a standard microscope slide as the substrate for cell adhesion, and a microscope integrated with a custom illumination source as the detection instrument. When illuminated with a monochromatic light source, angle-specific optical resonances supported by the photonic crystal enable efficient excitation of surface-confined and amplified electromagnetic fields when excited at an on-resonance condition, while no field enhancement occurs when the same photonic crystal is illuminated in an off-resonance state. By mapping the fluorescence enhancement factor for fluorophore-tagged cellular components between on- and off-resonance states and comparing the results to numerical calculations, the vertical distance of labeled cellular components from the photonic crystal substrate can be estimated, providing critical and quantitative information regarding the spatial distribution of the specific components of cells attaching to a surface. As an initial demonstration of the concept, 3T3 fibroblast cells were grown on fibronectin-coated photonic crystals with fluorophore-labeled plasma membrane or nucleus. We demonstrate that PCEF microscopy is capable of providing information about the spatial distribution of cell-surface interactions at the single-cell level that is not available from other existing forms of microscopy, and that the approach is amenable to large fields of view, without the need for coupling prisms, coupling fluids, or special microscope objectives.

4. References

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