Photonic Crystal Enhanced Microscopy (PCEM) for Multimode Dynamic Quantitative Imaging of Cell Adhesion

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***Abstract*—We describe a novel label-free biosensor imaging modality within Photonic Crystal Enhanced Microscopy in which cell membrane components locally reduce resonant reflection efficiency through photon out-scattering caused by protein clusters within focal adhesion sites.**

I. Introduction

Focal adhesions (FAs) are critical cellular membrane components that regulate cell adhesion and migration. We have developed a novel label-free biosensor imaging approach, Photonic Crystal Enhanced Microscopy (PCEM), for dynamic, long-term, quantitative imaging FAs during cell-surface interactions. In PCEM measurements, cellular membrane-associated protein aggregates serve to outcouple photons from the resonant evanescent field of a photonic crystal (PC) biosensor, resulting in highly localized shift of peak wavelength and highly localized reduction of the resonantly reflected light intensity. We demonstrate that cell-surface contact and FA formation can be imaged by two orthogonal label-free modalities with PCEM, providing a general-purpose tool for kinetic, high-resolution monitoring of cell interactions with substrates during processes that include apoptosis, stem cell differentiation, and chemotaxis.

II. Methods

In our previous reports of cell imaging by PCEM, we utilized an imaging modality in which the resonant reflected peak was measured over the imaging field of view to derive images of Peak Wavelength Shift (PWS) that occur when cells attach to the PC surface. In this work, we report a novel and orthogonal imaging modality within PCEM in which we measure the resonant reflected Peak Intensity Shift (PIS) from the PC during live cell attachment. Images of the PIS reveal highly localized and easily observed loci of protein clusters that correlate with the spatial distribution of FAs. We describe our hypothesis that the observed reduction in reflected intensity from the PC is caused by outcoupling of resonant standing wave photons via scattering. In this work, we show that PCEM is a quantitative, dynamic, and label-free approach for observation of the formation and evolution of FA clusters that provides information that is not available by another imaging modality, particularly for continuous observation of a single cell or monitoring of cell populations for extended time periods.

III. Results and Conclusion

Using dental epithelial stem cells (mHAT9a) attaching to a fibronectin coated PC surface as a representative example, we show that PWS and PIS images of the same cells display distinct and complementary information. Figure 1 demonstrates that PIS (2nd row) and PWS (3rd row) images show distinct distribution patterns, which suggests that they likely represent two different physical measurements. While regions of greatest PWS represent the locations at the cell-surface interface in which uniformly distributed regions with the greatest surface engagement occur, regions with the greatest PIS represent the formation of highly concentrated protein clusters at the cell-surface interface that are capable of scattering photons.

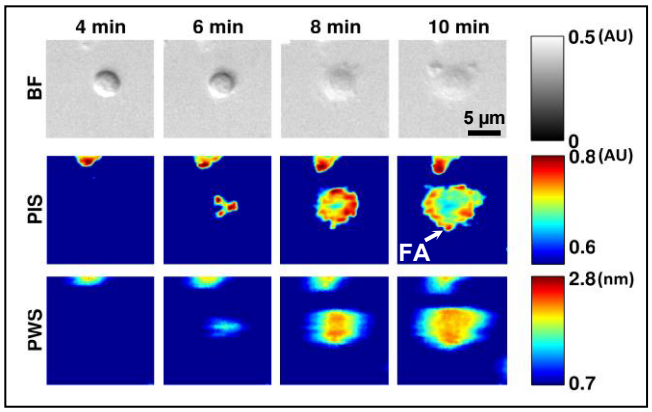

Figure 1. PCEM Brightfield (BF), PIS and PWS images of mHAT9a cells.

To conclude, this work describes a novel imaging modality for PCEM. The PIS images can be quantitatively and dynamically measured with PCEM for surface-attached live cells. PCEM enables long-term, label-free dynamic measurement of live cell interaction with surfaces, and quantitative study of FA formation and evolution.

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