Stem Cell Adhesion Imaging with Photonic Resonator Outcoupler Microscopy (PROM)

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Introduction: Focal adhesions (FAs) are critical cellular membrane components that regulate cell adhesion and migration [1]. We developed a label-free imaging biosensor approach, named Photonic Resonator Outcoupler Microscopy (PROM), for dynamic, long-term, quantitative imaging of cell-surface interactions [2-7]. Our measurements show that membrane-associated adhesion protein aggregates scatter photons from the resonant electromagnetic standing wave on a photonic crystal surface, resulting in a highly localized reduction of the reflected light intensity.

Materials and Methods: By mapping the changes in the resonant reflected peak intensity from the photonic crystal biosensor surface, we demonstrate the ability of PROM to detect cellular focal adhesion dimensions and their dynamic evolution as a function of time. We show a high degree of correlation for the location of PROM-measured focal adhesions of live dental epithelial stem cells (mHAT) with those observed by conventional fluorescent staining in fixed cells. We constructed a hyperspectral imaging detection instrument that utilizes low intensity illumination from an LED that gathers and processes PROM images at 10 sec increments using a 10X microscope objective lens. Due to utilization of low illumination intensity, PROM does not introduce phototoxicity and as a label-free sensing approach, is not subject to the limitations of photobleaching.

Results and Discussion: In Fig. 1, the top row shows brightfield (BF), peak wavelength shift (PWS), and peak intensity shift (PIS) images and the bottom row displays fluorescence images of three different cellular components (nucleus, actin, and vinculin), respectively. The fluorescent image of vinculin indicates that filopodia reside in the FA area along the cell boundary. As shown in Fig. 1 (right column), the PIS image shows a nearly identical distribution pattern along the cell peripheral region to that fluorescence labeled vinculin where the FA areas are concentrated along the cell boundary.

Conclusions: PROM provides contrast in the reflected resonant intensity that is induced by the refractive index contrast of the localized protein clusters. These protein clusters typically occur at the cell-surface interface, which comprise focal adhesion sites. PROM is a general-purpose label-free imaging tool for kinetic, high axial-resolution monitoring of cell interactions with cellular basement membranes.

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References: **[1]** Y. Zhuo, et. al, Sci. Rep, 2015, 5 (12963). **[2]** B. T. Cunningham, et. al, Sensor Actuat B. Chem, 2002, 85(3), pp.219-226. **[3]** Y. Zhuo, et. al, 2014, Analyst, 139, pp.1007-1015. **[4]** Y. Zhuo, B. T. Cunningham, Sensors (Basel). 2015 Sep; 15(9), pp.21613–21635. **[5]** B. T. Cunningham, et. al, 2016, IEEE Sens. J., 16 (10), pp. 3349-3366. **[6]** Y. Zhuo, et. al, Prog Quantum Electron, 2016, 50, pp.1-18. **[7]** Y. Zhuo, et. al, Light Sci. &. Appl, 2018, in press.

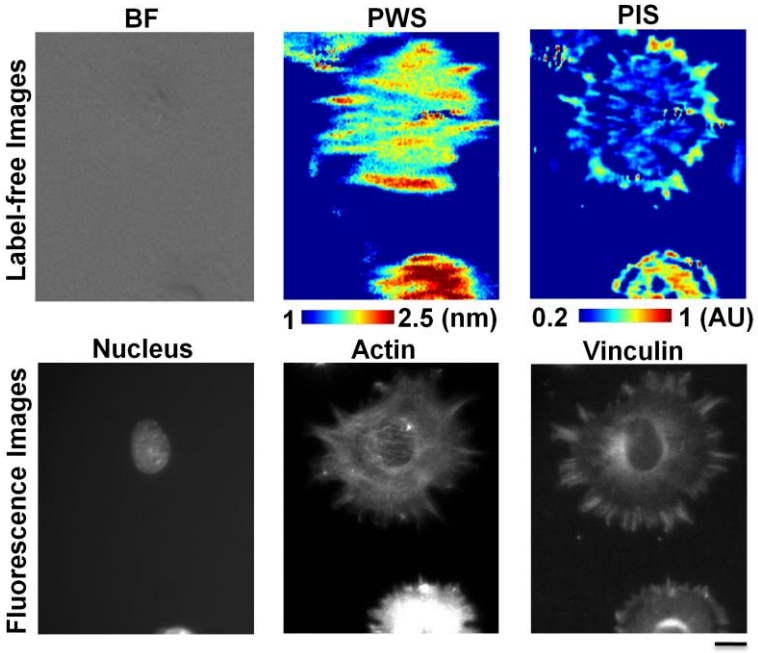

Fig. 1. Comparison of label-free PROM images and fluorescence images. Top row: brightfield image (BF), PWS image and PIS image. Bottom row: fluorescence images, including dyes that selectively stain nucleus, actin, and vinculin. Scale bar: 20 μm.