Photonic Resonator Outcoupler Microscopy (PROM) for Quantitative Monitoring of Stem Cell Focal Adhesion Area

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Abstract: We developed a novel label-free imaging approach, named Photonic Resonator Outcoupler Microscopy (PROM) utilizing the reduction of the peak-resonance intensity reflected from a photonic crystal surface. PROM can monitor the variation of focal adhesion areas in live cells dynamically and quantitatively for extended time.
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1. Introduction

Focal adhesions (FAs) are large macromolecular assemblies located on plasma membranes which can transmit signals between the extracellular matrix (ECM) and the cytoskeletons of cells. FAs are essential sub-cellular components for dynamic regulation of cell adhesions and migrations. A novel label-free imaging method based on photonic crystal (PC), named Photonic Resonator Outcoupler Microscopy (PROM), has been developed in our lab to monitor the cell adhesion without fluorescent tags. We demonstrate the measured FA area variations caused by the highly localized reduction of peak-resonance intensity value (PIV) on a PC slab surface. PROM can also measure the peak-resonance wavelength shift image which was developed in our lab previously. Therefore, PROM is capable of measuring the FA area variations in live cells by two orthogonal label-free modalities simultaneously (which represent two different physical quantities).

2. Materials and Methods

The PROM instrument is a modified brightfield microscope that utilizes a line-scanning approach to measure the spatiotemporal distribution of optical spectra across a PC surface. The surface-confined electromagnetic standing waves lead to high-reflection efficiency on the PC surface at the resonant wavelength. These standing waves extend into the surrounding medium in the form of an evanescent electromagnetic field, and can cause reflection efficiency variation by interacting with the subject within the field. This variation is sensitive enough to be measured by PROM system and the light-matter interaction only occurs with the surface-attached cell components at the bottom of the cell body within the evanescent field, while being insensitive to the rest of the cell body that is not engaged with the PC surface. Therefore, by utilizing an imaging system based on this principle, we achieved with high-axial resolution (~200 nm) label-free imaging for live cells.

3. Results and Discussion

To highlight how information from PROM images complements that obtained by orthogonal imaging modalities, five stem cells were selected and shown in Fig. 1 imaged by peak-resonance wavelength shift images (PWS), peak-resonance intensity shift images (PIS), confocal images with fluorescence dyes (FL), phase contrast images (PH), and scanning electron microscope images (SEM). Note that PROM images obtained using PWS and PIS information reveal different features of cell attachment, and both show clear details of the cell attachment boundary at high-axial resolution that only highlight the behavior associated with the cell-ECM interfaces, and do not show behavior from the rest of the cell body. Unlike SEM and fluorescent images, the PWS and PIS images yield dynamic and quantitative information of live cells that can be visualized graphically. The dramatic increase of PIS is likely due to the development of FAs along cell boundaries, which is confirmed in FL images in Fig. 1.

4. Conclusions

We developed a novel label-free imaging approach PROM and show that images of the PIS and PWS can be gathered from the same spectral information for the same cells. The two imaging modalities in PROM have distinct

spatiotemporal patterns and thus can provide complementary information about cell-surface activity. We expect PROM to be a highly useful tool that can reveal the mechanisms of biological processes that occur near the cell membrane when the membrane is attached to extracellular matrix materials during cell adhesion, migration, division, apoptosis, cancer cell metastasis, and stem cell differentiation.

4. References

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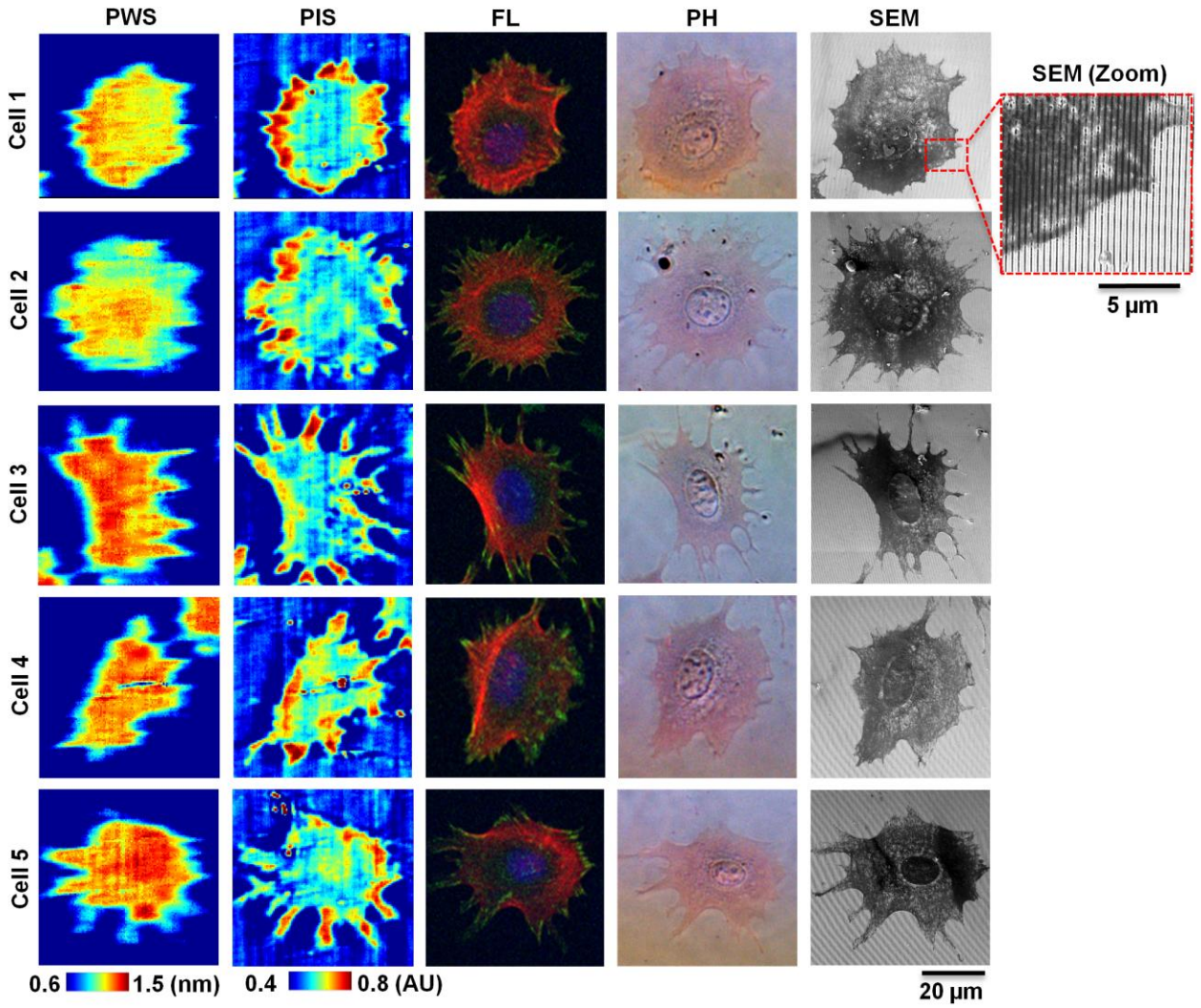

Fig. 1. Image modality comparison and dynamic analysis of PROM images during cell adhesion. Five selected cells imaged by Peak-resonance Wavelength Shift (PWS), Peak-resonance Intensity Shift (PIS), confocal FLuorescence microscopy (FL) (red-Actin, green-Vinculin, blue-Nucleus), PHase contrast microscopy (PH), and Scanning Electron Microscopy (SEM). Scale bar: 20 µm. (Top-right Inset: Zoom-in SEM image for cell 1. Scale bar: 5 µm).