SURFACE PLASMON BASED SUBSTRATE FOR ENHANCED FLUORESCENCE LIVE CELL IMAGING

Manas Ranjan Gartia[1], Austin Hsiao[1], Mayandi Sivaguru[2], Yi Chen[1], Tae-Jin Kim[3], Yingxiao Wang[3] and Gang Logan Liu[1,*]

[1]Micro and Nanotechnology Laboratory, [2]Institute for Genomic Biology, [3]Beckman Institute for Advanced Science and Technology, University of Illinois, Urbana –Champaign, IL, 61801 USA

ABSTRACT

We have created a randomly distributed nanocone substrate on silicon and coated with silver for surface-plasmon-enhanced fluorescence detection and cell imaging. We observed three dimensional fluorescence enhancement on our substrate evidenced from the confocal fluorescence imaging of Chinese Hamster Ovary (CHO) cells and transfected HeLa cells grown on the substrate. The fluorescence intensity was amplified more than 100 fold as compared to glass substrate. Strong scattering within nanostructured area coupled with random total internal reflection inside the cell resulted in the observed enhancement in fluorescence further away from the substrate surface.

KEYWORDS: Confocal Microscopy, Surface Plasmon, Cell Imaging, Metal Enhanced Fluorescence

INTRODUCTION

Cell imaging is generally done on glass or polymer based substrates. Hence, the substrate itself does not contribute to the sensitivity of fluorescence imaging process and the imaging process is limited by the fluorophore properties (e.g. quantum yield, photostability) as well as the imaging set up (e.g. objective lens numerical aperture, magnification, resolution and depth of field). Partly to overcome these limitations metal-coated slides can be utilized for enhanced cell imaging which rely on the principle of metal-enhanced fluorescence [1]. But it is sensitive up to few tens of nanometers and hence less sensitive for cellular matrix away from the metal surface [2]. We have fabricated a randomly distributed nanocone substrate on silicon and coated with silver for surface-plasmon-enhanced fluorescence detection and cell imaging.

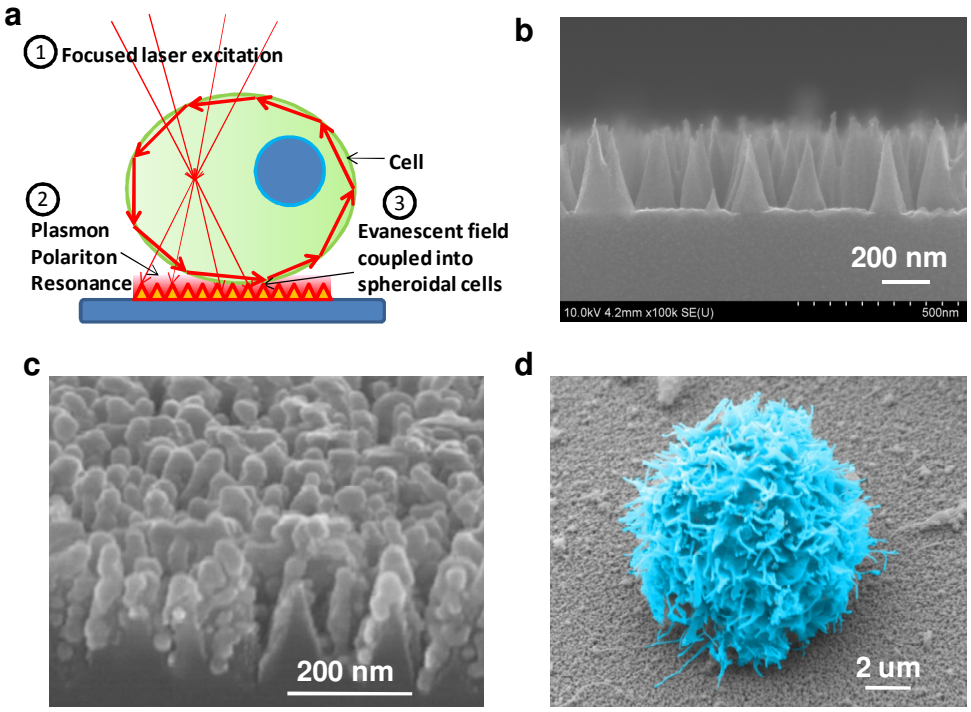

Figure 1: (a) Schematic of nanoplasmon coupled cell imaging. SEM image of nanocone substrate (b) before metal deposition (c) after 80 nm silver deposition (d) SEM image of a CHO cell on the nanoplasmon substrate.

EXPERIMENTAL

The nanocone structures are fabricated by Simultenous Plasma Enhanced Reactive Ion Synthesis and Etching (SPERISE) process. In order to make high-aspect-ratio nanopillar structures, an etching-passivation method is used. First, the native oxide layer is removed from the single crystal silicon wafer (4") using a wet etch process (10:1 buffered oxide etching for 1 min). Then the polysilicon substrate is etched by HBr and oxidized by O_2 simultaneously. PlasmaTherm SLR-770 In-

ductively Coupled Plasma (ICP) Reactive Ion Etcher (RIE) was used for the etching process. The temperature was set to 40 ^{o}C, the throat pressure was 20 mTorr, the RF power was 200 W and the etching time was 6 min during the fabrication process. The HBr flow rate was 20 sccm whereas the O_2 flow rate was set to 8 sccm. During etching, initially a nano-mask made of silicon oxybromide natually forms and then HBr gas etches the unmasked silicon substrate. Since HBr has a high etching selectivity of silicon to oxide (200:1), high-aspect-ratio nanocone structures are formed.

In our experimental configuration (Figure 1a) living cells are situated on top of a nanoplasmonic substrate which is composed of tightly packed silicon nanocone array covered by a thin layer of silver. The silver coated nanocone array as depicted in Figure 1b&c can support both propagating surface plasmon and localized plasmon at visible wavelength range which can then be coupled to a cell. Figure 1d shows the SEM of a spheroid CHO cell on the nanocone plasmonic substrate. For the fluorescence imaging experiments, the cells are grown on the substrate for one day and stained just before the imaging experiments. The cell nucleus is stained with blue fluorescent Hoechst dyes and the calcium ions in the cytoplasm are labeled with green fluorescent dye Fluo-4, AM.

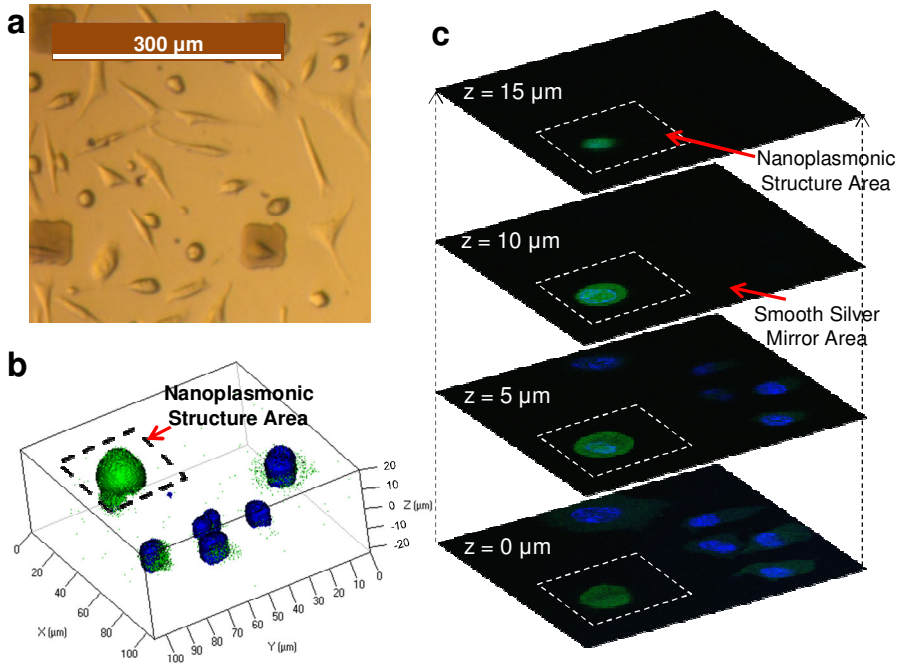

Figure 2: (a) Bright field image of CHO cells on the nanoplasmonic substrate (b) 3-D confocal fluorescence cell imaging of cytoplasm labeled CHO cells (c) Stacked confocal images of cytoplasm labeled cells showing enhancement away from the surface of the substrate .

A typical bright field image of large number of cells grown on the substrate is shown in Figure 2a. The square region is the nanoplasmonic area. Due to the far field fluorescence enhancement facilitated by surface plasmon supporting substrate, only the cytoplasm calcium (label in green fluorescence) in the cell grown on the nanoplasmonic substrate can be observed in 3D fluorescence imaging (Figure 2b) with an extremely low laser power and short imaging time (0.2% of 25 mW laser power for green fluorescence excitation and 1.58 us pixel dwell time) while other cells on smooth silver surface exhibit undetectable to extremely low fluorescence emission only except near the immediate surface of smooth silver film. It should be noted that the fluorescence intensity below and beyond cell span is due to the point spread function of the fluorescence emission.

The vertical cross-sectional cell images (Figure 2c) at various z-positions show that the strong cytoplasm calcium fluorescence enhancement on the nanoplasmonic substrate is extending even up to the top position on the spheroidal cell, around 12 µm above the nanoplasmonic substrate and far beyond the 100 nm domain of metal surface enhanced fluorescence. However the fluorescence enhancement for the nucleus fluorescence is relatively modest due to the mismatch of excitation light and optical resonance wavelengths. Similar experiments has also been performed where cell membrane and nucleus are labeled by red (FM 1-43 FX) and blue (Hoechst) emission fluorophores respectively. The quantitative analysis for fluorescence enhancement factor is performed by comparing the average fluorescence intensity at each depth (z-coordinate) of the z-stack images taken by confocal microscope. The average fluorescence intensities for the cell membrane, cytoplasma and nucleus are shown in Figure 3. Here, increasing z denotes imaging plane away from the substrate surface

i.e. z = 0 is on the substrate. The actual height or thickness of the cell is around 12 μm or z = 12 μm. On the cell membrane we observed a 23 fold fluorescence intensity increase on the nanoplasmonic substrate compared to that on the smooth silver mirror surface at the same imaging plane around z = 6 μm. Given that the smooth mirror surface can provide approximately 5 fold fluorescence enhancement which was confirmed from the fluorescence spectroscopy experiment, the total far field fluorescence enhancement factor for the cell membrane obtained by the surface plasmon substrate is 115 folds with respect to a glass slide surface.

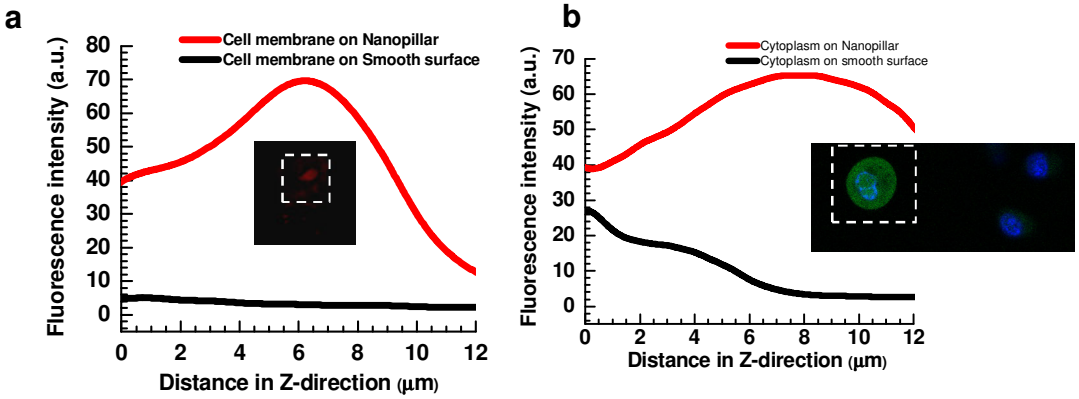

Figure 3: Quantitative analysis of fluorescence enhancement (a) Cell membrane on the nanoplasmonic substrate(b) Cell cytoplasm on the nanoplasmonic substrate.

Finally, we have imaged early stage of transfection using our nanoplasmonic substrate. The typical imaging window post transfection is about 36-54 hrs. The limitation is imposed because of low fluorescence intensity at early stage of transfection. Due to significant fluorescence enhancement on nanoplasmonic substrate, we can possibly image the early stage of transfection. In the present experiment, we transfected a cervical cancer line (HeLa) with a fluorescent protein (mCherry-paxillin) and grew them on nanoplasmonic as well as traditional petridish (glass substrate). The cells were analyzed by confocal microscopy 12-18 hr post transfection. We observed enhanced fluorescence for cells on the nanoplasmonic substrate as shown in Figure 4.

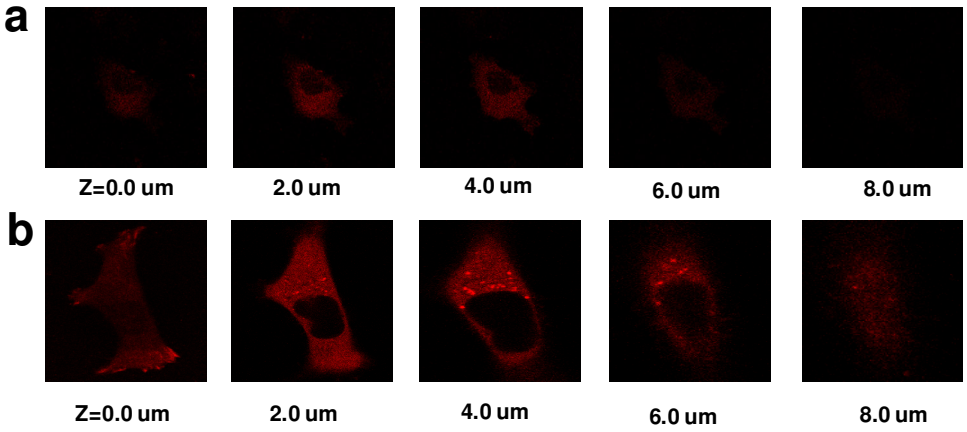

*Figure 4: Comparison of mCherry-paxillin intensity in HeLa cell **(a)** on a glass substrate and that **(b)** on a nanoplasmonic substrate.*

CONCLUSION

In conclusion, we created a nanoplasmonic substrate on which three-dimensional confocal fluorescence cell imaging sensitivity is amplified for more than 100 folds especially for cell membrane and cytoplasm.

REFERENCES

[1] E.L. Moal, E. Fort, S.L. Fort, F.P. Cordelieres, M.P. Fontaine-Aupart and C. Ricolleau, "Enhanced Fluorescence Cell Imaging with Metal-Coated Slides," *Biophysical Journal*, 92, 2150-2161 (2007).

[2] K. Lee , L.D. Hahn , W.W. Yuen , H. Vlamakis, R. Kolter and D.J. Mooney, "Metal-Enhanced Fluorescence to Quantify Bacterial Adhesion," *Adv. Mater.*, 23, H101–H104 (2011).