Photonic crystal enhanced fluorescence using a quartz substrate to reduce limits of detection

Anusha Pokhriyal[1], Meng Lu[2], Vikram Chaudhery[3], Cheng-Sheng Huang[3], Stephen Schulz[2] and Brian T. Cunningham[3,4*]

[3]Dept. of Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[2]SRU Biosystems, 14-A Gill St., Woburn, Massachusetts, 01810, USA
[3]Dept. of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[4]Dept. of Bioengineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[*]bcunning@illinois.edu

Abstract: A Photonic Crystal surface fabricated upon a quartz substrate using nanoimprint lithography has been demonstrated with a signal enhancement factor as high as 7500× for the detection of LD-700 dye spin-coated upon the PC. The detection of spotted Alexa-647 labeled polypeptide on the PC exhibits a 330× SNR improvement. Using dose-response characterization of deposited fluorophore-tagged protein spots, the PCEF surface demonstrated a 140× lower limit of detection compared to a conventional glass substrate.

OCIS codes: (050.5298) Photonic crystal; (180.2520) Fluorescence microscopy; (110.3960) Nanolithography.

1. PCEF surface design

The PCEF structure supports resonant modes at a specific combination of wavelength and angle of incidence. The near field intensity associated with the resonant modes is strongly enhanced with regard to the field intensity of excitation light and can amplify the emission intensity from target fluorescent molecules. In this study, the PCEF surface was designed and fabricated to enhance the emission from fluorescent dyes which have an absorption band at λ_{ex}=632.8 nm and an emission band near λ_{em}=690 nm [1]. Quartz was selected for its low autofluorescence characteristics compared to polymer-based PCs, improving the detection sensitivity and signal-to-noise ratio (SNR) of PC Enhanced Fluorescence (PCEF). The PCEF surface was designed to have one TM mode at λ_{ex}=632.8 nm which spectrally matches with the excitation laser wavelength for near field enhancement, resulting in "enhanced excitation." Meanwhile, another TM mode at λ_{em}=690 nm, which spectrally overlaps with the pass band of the emission filter was used to direct the emitted photons towards the detection optics to obtain an "enhanced extraction" effect. Fig. 1(a) shows the spatial distribution of the simulated near-field electric field intensity (normalized to the intensity of incident field) within one period of the PC structure for resonant wavelength λ_{ex}=632.8 nm and resonant angle of θ_{ex}=10.8°. Nanoimprint lithography was used to demonstrate economical fabrication of the subwavelength PCEF surface structure over entire 1x3 in^2 quartz slides. The measured angle spectrum of the fabricated PCEF surface is shown in Fig. 1(b) with FWHM$_\theta$=0.3°.

2. Enhanced excitation and extraction

In order to characterize the signal enhancement capability of the PCEF surface, a fluorescent dye (LD-700) with a peak absorption wavelength of 647 nm and a peak emission wavelength of 673 nm was doped in SU-8 and spin-coated onto the PC surface. The coated film thickness was measured as ~50 nm by ellipsometer. The PCEF surface was then tested using the PCEF microscope instrument [2], which illuminates the surface from beneath the structure with collimated light that can be swept rapidly through a range of incident angles. The system incorporates a TM-polarized HeNe laser, while the emission is collected by a 0.1 NA objective. By varying the angle of incidence from 10° to 20° in increments of 0.02°, the emission intensity was recorded as shown in Fig. 2(a). The enhancement factor of the PCEF surface was calculated by subtracting the background from the signals and dividing the on-resonance net signal by the off-resonance net signal. An enhancement factor of 1500× was obtained, representing the effects of enhanced excitation. Using the same sample, the enhanced extraction effect was also studied with PCEF microscope. The LD-700 coated PC and glass control were illuminated at an off-resonance angle (θ=20°) with the TM polarized HeNe laser. The ratio of background subtracted off-resonance signal and the background subtracted glass control signal gives the extraction enhancement factor of the device. From the above measurement, the PCEF surface gave a factor of 5× for enhanced extraction.

CThQ1.pdf

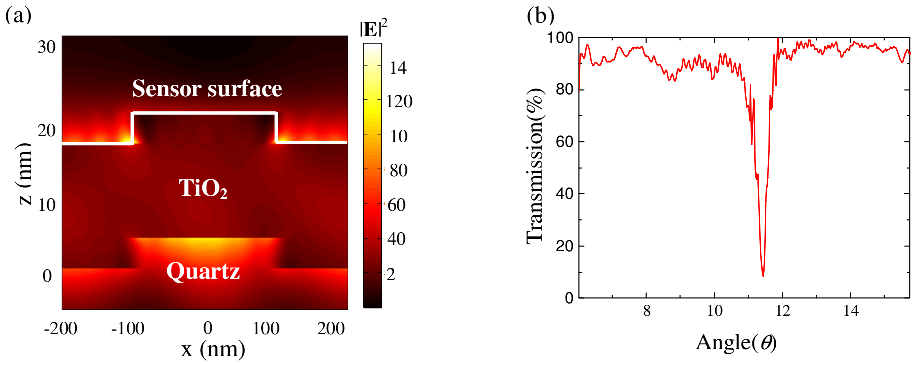

Fig. 1 (a) Simulated near field distribution at λ=632.8 nm (normalized to the intensity of the incident field); (b) Measured angle transmission spectrum at the excitation wavelength λ=632.8nm.

A combined enhancement factor, (defined as the product of the enhanced excitation and the enhanced extraction factors) of 7500× was calculated for the device at the given concentration of LD-700. This enhancement factor represents the maximum achievable enhancement that can be obtained when fluorescent material is allowed to fill a ~50 nm thick volume that conforms to the corrugated PC surface, with no spacer materials between the fluorescent layer and the PC surface for the demonstrated design of the PCEF surface. The addition of the ~50 nm SU8 layer results in a 10% increase of the Q-factor of the PC and thus increases the enhancement factor by ~10%. Addition of biomolecular layers to the bare PC results in approximately the same effect.

3. Detection of dye labeled polypeptide

In order to demonstrate the enhancement in the signal to noise ratio (SNR) and lowering of the limit of detection (LOD) of the analytes on the PCEF surface in the context of a multispot microarray assay, a detection experiment using a dye-labeled protein was performed. Spots of dye-labeled polypeptide with a range of dye concentrations were applied directly to the PCEF surface and a glass surface, and the fluorescence output of spots on each surface were compared in a dose-dependent manner. A SNR improvement of 330× on the PC was demonstrated for the concentration corresponding to the LOD on an unpatterned glass surface. The LOD on the PC slide was lowered by 140× compared to the LOD of PPL-Alexa on the glass control. Fig. 2(b) gives the SNRs calculated from the optimized images for each PPL-Alexa 647 concentration spotted onto the PC and glass slide.

This PCEF surface can be used to provide lower detection limits for broad classes of surface-based fluorescent assays for applications that include DNA microarrays for quantification of gene expression, and next-generation DNA sequencing applications that utilize fluorescent tags.

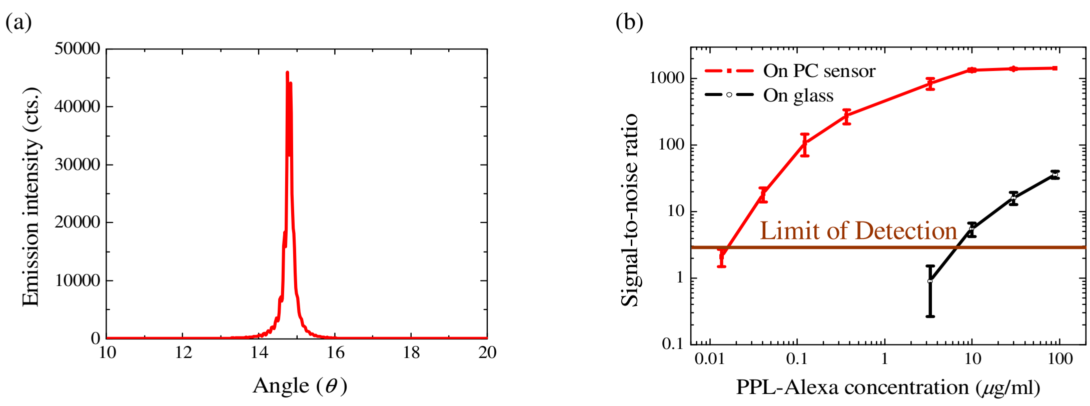

Fig. 2 (a) Fluorescence output as a function of angle of incidence for a ~50 nm film of dye-doped polymer applied directly to the PCEF surface; (b) Signal-to-noise vs PPL-Alexa 647 concentration showing an improvement in limit of detection (LOD) on a PCEF surface by a factor of 140.

4. References

[1] A. Pokhriyal, M. Lu, V. Chaudhery, C. S. Huang, S. Schulz, and B. T. Cunningham, "Photonic crystal enhanced fluorescence using a quartz substrate to reduce limits of detection," Optics Express **18**, 16 (2010).

[2] I. D. Block, P. C. Mathias, N. Ganesh, S. Jones, B. R. Dorvel, V. Chaudhery, L. Vodkin, R. Bashir, and B. T. Cunningham, "A detection instrument for enhanced-fluorescence and label-free imaging on photonic crystal surfaces," Optics Express **17**, 14 (2009).