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Comparison of cone and cone shell configuration for depth sensitive fluorescence measurements in turbid media

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ABSTRACT

We have developed a novel cone shell illumination and detection configurations using combination of axicon lenses for depth sensitive fluorescence spectroscopy. The probe was demonstrated experimentally to be able to selectively detecting fluorescence from different depths from a two-layered turbid agar phantom. In addition to enhanced contrast of subsurface fluorescence measurement as compared to a conventional cone configuration implemented by a microscope objective lens, the axicon lenses based setup eliminated the need of moving the objective lens up or down to achieve depth sensitive measurements, which effectively improves the consistency of optical coupling thus would be preferred in a clinical setting.

Keywords: depth sensitive, fluorescence, spectroscopy, axicon lens based setup, cone shell configuration

1. INTRODUCTION

Human epithelial tissue contains numerous endogenous fluorophore molecules that carry vital diagnostic information for the detection of various diseases.[1] Ultraviolet-visible fluorescence spectroscopy has been widely explored in the detection of human epithelial precancers and cancers due to its light penetration depth of several hundred micrometers, similar to the depths of tissue of interest.[2] Factors such as the progression of disease state, anatomical site, age and menopausal status can affect the depth distribution of these endogenous fluorophores. [3] Hence, depth sensitive fiber probes with excellent layer specific fluorescence contrast will enhance the diagnostic capability of this technique. Currently, depth sensitive fluorescence probe has been achieved in two approaches, fiber-optic based and lens based. Fiber optic probe achieves depth sensitive measurement by varying the spatial offset, aperture diameter [4] and (or) angle of illumination and collection fiber. [3] Interpretation of the fluorescence measured is complicated in the case of stratified diffuse scattering sample as the laser is less focused and the resulting signals may reflect the average contribution of large volume. Lens based setups use a single lens or a combination of lenses to achieve a cone configuration, in which both the excitation and emission volumes in an optically transparent medium would form light cones. One weakness of this setup is the limited sensitivity to deep subsurface layers due to the dominance of fluorescence from shallower layers. Furthermore, it is required to vary the distance between the imaging lens and the tissue surface in order to achieve depth sensitive measurements, which induces uncertainty and great inconvenience in clinical measurements.

In this study, we introduce a novel cone shell illumination and collection configuration with combination of axicon lenses to overcome the weaknesses of the cone configuration in limited depth sensitivity as well as to eliminate the need of altering the lens-sample distance in achieving depth sensitive fluorescence measurements. The advantage of the cone shell configuration in depth sensitivity compared to the cone configuration will be demonstrated experimentally.

2. OPTICAL SYSTEM SETUP AND SAMPLE PREPARATION

Fig. 1 Schematic diagram of (a) cone illumination and collection configuration using a microscope objective lens, and (b) cone shell configuration with combination of axicon lenses.

We constructed a fluorescence probe with cone illumination and collection configuration as shown in Fig. 1(a). The probe was coupled to a diode laser (iFlex-2000, Point Source Ltd., Hamble, UK) through a singlemode polarization maintaining fiber, and has a maximum output power of 30mW at 405 nm. The output laser was collimated using a convex lens ($f = 50.0\text{mm}$), before passing through a 405 nm bandpass filter and reflected by a dichroic mirror towards a microscope objective lens (5X, NA = 0.1). The laser was focused onto the sample and fluorescence signals was collected through the same objective lens and pass through a longpass filter before being focused onto the tip of a collection fiber (400 μm , NA = 0.22). In another setup, a collimated laser of 3 mm in diameter was shined through a pair of axicon lenses with identical apex angle (110°) to create a donut-shape laser ring as shown in Fig. 1(b). The laser ring was then focused onto the sample by axicon 3 with apex angle of 140° , forming cone shell geometry. The laser power at the sample was measured to be 10 mW and 7 mW for cone and cone shell configuration respectively. The integration time for this experiment was one second. The collected signals in both setup will be coupled to a Czerny-Turner type spectrograph ($f = 303\text{mm}$) equipped with a holographic grating (1200 grove/mm) and a Newton spectroscopic CCD (Shamrock, Andor Technology, UK), which yields a spectral resolution of 0.1 nm.

Two layered agar phantom was fabricated to mimic the optical properties of human skin according to the recipe published in earlier work [5]. The thickness of top layer was 1 mm and the thickness of bottom layer was 10 mm whereas the lateral dimension of both layer was made at $>10\text{ mm}$ to represent a semi-infinite medium. Two different endogenous fluorophores, FAD and PpIX, were added to the top and bottom layer of phantom because the non-overlapping peak emission wavelengths of these fluorophores at 540 nm and 630 nm, respectively can facilitate the fluorescence discrimination from each layer. FAD concentration of 38.2 μM and PpIX concentration of 17.8 μM were used so that the intensities of both emission peaks fall within the same order of magnitude to the ease of data analyzing. A piece of plastic wrap was placed at the centre to prevent cross diffusion of fluorophores between layers. Intralipid 20% was added into each layer at different concentration to mimic the light scattering property of epithelium and stroma at 540 nm. Optical properties of epithelium and stroma (μ'_s and g) at excitation wavelength and peak emission wavelengths of fluorophores are shown in Table 1.

Table 1. Optical Properties ($\mu'_s [\text{cm}^{-1}]$ and g) for Top (Epithelial) and Bottom (Stroma) Layers for Tissue Phantom at Excitation Wavelength and at Peak Emission Wavelength of FAD and PpIX [6]

Layer	Wavelength (nm)		
	405 (Excitation)	540 (FAD)	630 (PpIX)
Top	42.4, 0.95	33.4, 0.95	29.0, 0.95
Bottom	266.3, 0.88	209.9, 0.88	182.3, 0.88

Different apparent focal depths were achieved by changing the lens-sample distance in configuration (a) and by changing the distance between axicon lens 1 and 2 in configuration (b). The actual depths of measurement were obtained by correcting the refractive index mismatch between sample and air as described by Everall[7] based on the refractive index of intralipid 20% at 405 nm which is 1.364 [8].

3. RESULTS AND DISCUSSIONS

Fig. 2 Fluorescence spectra measured from two layered turbid media at different focal depths corrected for refractive index mismatch with cone shell configuration.

Figure 2 shows the fluorescence spectra measured at different depth from the two layered phantom using configuration (b). FAD fluorescence intensities detected at the surface (0 mm) and at 1.3 mm depth were highest compare to those detected at greater depths. It is obvious that the FAD peak intensity decreases while PpIX peak intensity increases as the focal depth increases from 1.3 mm to 6.5 mm. This has demonstrated that cone shell configuration using axicon lenses was capable of performing depth sensitive fluorescence spectroscopy without the need of altering lens-sample distance. To compare the contrast improvement achieved by this approach, we have computed the normalized fluorescence peak ratio of top and bottom layer at all depths for both cone and cone shell configuration. First, the intensities of FAD and PpIX were normalized to their maximums (of all depths), respectively. Then, the contrast ratio of top layer was obtained by dividing the normalized FAD peak intensity at one depth to the sum of the normalized peak intensities of both fluorophores at that particular depth. Similarly, the contrast ratio of bottom layer was obtained by dividing the normalized PpIX intensity at one depth to the sum of the normalized intensities of both fluorophores at the same depth.

Fig. 3 The normalized fluorescence intensity contrast ratio of (a) top layer and (b) bottom layer as a function of refractive index mismatch corrected focal depth.

Figure 3 shows the comparison between depth sensitivity of cone and cone shell configuration in probing fluorescence signals from a turbid two layered tissue phantom. In Fig. 3 (a), we can see that cone configuration was more sensitive to top layer fluorescence as the FAD contrast ratio raised from 0.5 at the surface to 0.52 at a depth of 1.3 mm, whereas the FAD contrast ratios for cone shell configuration were 0.45 and 0.5 for the same focal depths. The depth sensitivity of cone configuration was superior at near surface as the microscope objective lens formed a tiny spot of 32.2 μm in diameter which is relatively small compared to the top layer thickness of 1 mm, whereas the axicon lens formed an elongated focal line of around 1.7 mm which is larger than the thickness of top layer. Decreasing the length of focal line formed by axicon 3 would improve the depth sensitivity of cone shell configuration at shallower layer. However, the depth selectivity of cone shell configuration was superior at greater depth as the FAD contrast ratio decreases more rapidly than cone configuration after depth of 1.3 mm, that is from 0.5 to 3.9 at depth of 6.5 mm whereas contrast ratio of the latter decreases from 0.52 to 0.47. This is because when measuring from greater depth, smaller volume of the top layer was illuminated by the excitation light cone shell as compared to cone configuration in a conventional microscope objective lens setup. Since fluorescence begins with absorption of excitation light, smaller illuminated volume of top layer reduces fluorescence originated from the top layer. Thus, cone shell configuration can effectively reduce the excitation and detection of fluorescence from shallower layers when performing subsurface measurement.

Fig. 3(b) shows the comparison of sensitivity to PpIX (bottom layer) between cone and cone shell configuration. The PpIX contrast ratio of cone configuration was smaller than 0.5 for surface to the depth of 1.3 mm but the contrast ratio for cone shell configuration was higher than 0.5 for the same depths. This observation can be due to the elongated focal line in axicon setup that has elevated the contribution of fluorescence from bottom layer when measuring from a shallower layer. However, when the focal depth was increased from 1.3 mm onwards, PpIX contrast ratio of cone configuration increases at a much slower rate than cone shell configuration. The PpIX contrast ratio at 6.5 mm depth was 0.53 for cone configuration and 0.61 for cone shell configuration. The bottom layer fluorescence contrast of cone configuration was low due to the dominance of top layer fluorescence even though the laser intensity of the illumination cone base is much lower at the top layer than that of the illumination cone tip at greater depth. Because cone shell configuration reduces the illumination volume of near surface region when performing deep measurement, contribution from the top layer was effectively weakened thus enhancing the contrast of fluorescence originated from deep. It is noted that conventional microscope objective lens has better focusing power and light collecting efficiency than axicon lens as the magnitude of fluorescence detected by the CCD using microscope objective lens setup was stronger than the latter using similar excitation laser power. However, due to the ability of axicon lens setup in enhancing the contrast ratio of deep fluorescence and in eliminating the need of altering lens-sample distance when performing deep measurement, our axicon lens based cone shell light illumination and collection configuration would improve the performance and consistency of subsurface spectral acquisition in various clinical setting.

4. CONCLUSION

In this study, we have experimentally demonstrated that a cone shell configuration that involves a combination of axicon lenses detects fluorescence with a higher sensitivity to the bottom layer and a larger sensitivity range to either the top or the bottom layer in a two-layered tissue phantom mimicking human skin. Furthermore, these can be achieved without altering the distance between the imaging lens and the sample in the cone shell configuration, which will minimize inconsistency in optical coupling and bring great convenience in clinical measurements.

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