Fluorescence Detection System with Miniaturized Integrating Sphere

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Abstract—**We report on the use of non-imaging reflectors to improve the collection efficiency of fluorescence detection systems. Half-sphere mirrors redirect the isotropic fluorescent emission toward the photodetector, and significantly enhance the fluorescence signal.**

Biosensors; Fluorescence detection; Non-imaging optics

I. INTRODUCTION

Because of its sensitivity, selectivity, and efficiency, fluorescence detection systems for biochemical assays are being widely used in a number of bioanalytical applications including microscopy and diagnostics [1]. Typical fluorescence detection systems excite fluorescent molecules with a focused light beam, and collect the emitted fluorescence from labels by refractive optical lenses [2]. Although optical lenses play crucial roles in microscopy and bioimaging applications, there exist some biomedical applications, such as point-of-care diagnosis, that do not require the detailed two-dimensional fluorescent image information. For example, portable fluorescence-based point-of-care systems might need to only detect the presence (and absence) of certain target fluorescence for rapid on-site diagnostic evaluations [1].

In this paper, we suggest that non-imaging reflective optics can significantly improve the collection efficiency of the fluorescence detection systems and reduce the overall system volume and complexity, when the imaging capabilities are not necessary.

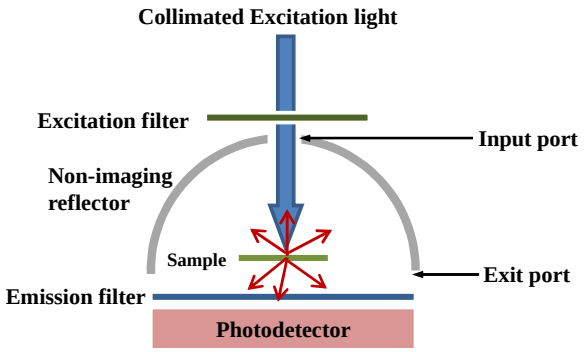

Figure 1. Schematic diagram of the proposed fluorescence emission detection system with a non-imaging reflector.

II. OPTICAL SYSTEM DESIGN

Light emission from the fluorescent molecules is nearly isotropic in space, and covers whole 4π steradians [3]. When an optical lens is used to capture the fluorescent signals such as in typical fluorescence microscopes, the collection efficiency is directly related with the lens performance, and given by $(1-\sqrt{1-NA^2})/2$, where *NA* is the lens numerical aperture in air. Since the numerical aperture of typical lenses ranges from 0.3 to 0.8, the theoretical fluorescence collection efficiency is at most 20 %, which means that most fluorescence is not captured and wasted. Moreover, optical lenses typically become bulkier and more expensive as their numerical apertures increase, and the alignment tolerance gets tighter as well.

Figure 1 shows the schematic diagram of the proposed fluorescence detection optical system with a non-imaging reflector for improved fluorescence collection efficiency. The collimated light through the entrance port excites the sample, and the curved reflector surface reshapes and redirects the isotropic fluorescent emission toward the photodetector at the exit port to enhance the overall fluorescence collection efficiency. This type of non-imaging optical systems, such as integrating spheres, are being popularly used to measure the total radiation from a light source, but have not been used for fluorescent detection [3]. Unlike the conventional detection systems with refractive optical lenses, the fluorescence collection efficiency of the non-imaging reflectors can ideally reach up to 100%.

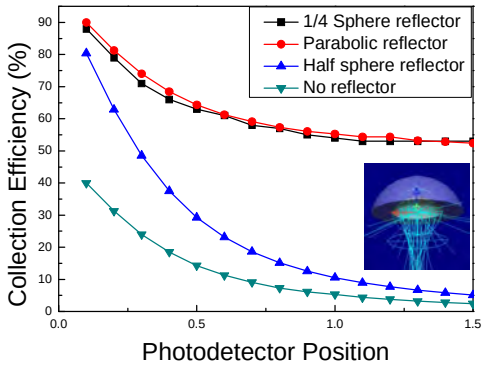

Figure 2. Simulated fluorescence collection efficiency for various reflector types as a function of the photodetector position from the exit port. The inset shows an example of the ray tracing results.

We used a commercial ray tracing software to estimate the collection efficiency of various reflector surfaces as a function of the photodetector position from the exit port, and the simulation results are shown in Fig. 2. We assume that the photodetector size is the same with the exit port size (1 inch diameter in our simulation), and the photodetector position is normalized by the photodetector size. When the photodetector is in direct contact with the exit port, the collection efficiency approaches 100%.

III. Results and Discussion

In our prototype optical system, a sample mount is placed between a silicon photodetector and a miniaturized integrating half sphere with an entrance pupil (6 mm diameter) for collimated excitation light. Fluorescence signals emitted downward is directly captured by the photodetector, while the upward emission is reflected by the half sphere mirror surface toward the photodetector.

A light emitting diode (LED) with a peak wavelength of 405 nm and a spectral width of 15 nm is used as an excitation light source. An LED is chosen because of its low cost and long lifetime [4]. An optional excitation filter (bandpass optical filter) at the entrance pupil transmits a well-defined excitation wavelength range that does not overlap with the fluorescence emission spectrum. Our target fluorescent molecule is 7-diethylaminocoumarin-3-carboxylic acid, and has a maximum absorption and emission at 409 and 473 nm, respectively [5]. When the emission spectrum does not overlap with the source spectrum, the excitation filter is not necessary. Finally, an emission filter (long pass optical filter with the transition edge at 442 nm) rejects the excitation LED light, and passes only the fluorescence emission signals to the photodetector with a large contrast.

The integrating half-sphere mirror was fabricated by evaporating metals (500 nm Ag and 10 nm Cr) on an injection-molded plastic half sphere (20 mm inner diameter). Ag surface has high reflectivity (>0.9) in the visible spectrum range [6], and reflects the fluorescent emission back to the photodetector plane. Additional slits and holes can be used to prevent back-scattering of excitation light between the mirror and emission filter. The suggested optical system has high fluorescence collection efficiency, and does not require bulky optical components such as high NA objectives and beam splitters.

Figure 3 shows the measured fluorescence signals from the silicon photodetector as a function of the excitation light intensity. When the reflectors are not used, the fluorescent intensity levels do not change much regardless of the photodetector diameter. This means that downward fluorescence emission is being mostly captured by the photodetector. Considering the distance between the sample and photodetector, the estimated collection efficiency for this downward fluorescence emission is 45 %. When the integrating half-sphere mirror is used, the detected fluorescence signal from the 10 mm photodetector is increased by ~70 %, and this corresponds to the overall collection efficiency of 76 %.

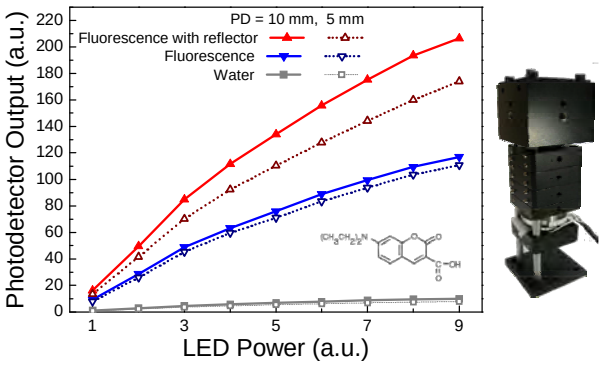

Figure 3. Measured fluorescence intensity with and without the integrating half-sphere reflector. Two photodetector dimensions (5 mm and 10 mm diameter) are used for comparison. The inset shows the picture of the prototype fluorescence detection system, and the chemical structure of the target fluorescent molecule.

IV. Conclusion

We demonstrate that non-imaging reflective optics can significantly improve the fluorescence collection efficiency by sacrificing the detailed imaging capability and integrating the isotropic fluorescent emission. For rapid on-site diagnostic applications, the proposed systems can be more efficient, compact, and less expensive than the conventional lens-based fluorescence detection systems. We believe that this idea can also be implemented in various other platforms, such as microfluidic channels and flow cytometry, to enhance the light detection efficiency.

Acknowledgment

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