Towards Two-Color Fluorescence Imaging for Diagnosis of Microbial Keratitis

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Abstract: Novel methods are needed for diagnosis of microbial keratitis. Two-color fluorescence imaging is proposed and tested on *ex vivo* porcine eyes. Results show the technique may be feasible but further quantitative characterization is needed.

1. Introduction

Microbial Keratitis (MK) is a leading cause of preventable blindness worldwide [1], and is responsible for ∼1.5 million cases of blindness each year [2]. MK is difficult to diagnose, with standard laboratory techniques only positive in ∼50% of cases [1]. We propose two-color line-of-sight fluorescence imaging as a possible strategy to aid in diagnosis of MK. Microbial fluorescence spectroscopy is known to have utility in distinguishing between species [3,4] *in vitro*, but significant effort is needed to develop rapid, low-cost imaging tools for diagnosis of MK. In this paper, we describe preliminary two-color fluorescence imaging results from a simple *ex vivo* porcine eye.

2. Methods

A fluorescence imaging device was constructed from two nominally-identical cameras (ThorLabs CS165MU1), a visible beamsplitter with 458-nm cutoff (Semrock FF458-Di02), and an $f/2$ achromatic imaging lens (Edmund Optics #65-976). The imaging bands are further restricted using a UV-cutoff filter (ThorLabs FELH0400), a 450-nm longpass filter (Red band; ThorLabs FELH0450) and a 450-nm shortpass filter (Blue band; ThorLabs FESH0450). 365-nm LED emission is directed along the imaging axis using a dichroic beamsplitter (Semrock FF389-Di01) which provides a peak fluence of 30 mW/cm^2 and is uniform over a 1-cm square in the object plane. The LED is synchronized with the cameras and gated using a LED driver (ThorLabs LEDD1B); the cameras and LED are gated for 50 ms (total fluence 1.5 mJ/cm^2), well below established safety thresholds for human use.

Seven *ex vivo* porcine eyes (Animal Technologies Inc., Porcine eye-globe only) were acquired to demonstrate the technique. First, a fluorescence emission spectrum was collected for each eye and for three bacteria smears for comparison (*B. cereus*, *B. subtilis*, and *E. coli*) using a spectrometer (Ocean Insight USB2000) with fiber-coupled lens (ThorLabs F810SMA-635) and UV-cutoff filter (ThorLabs FELH0400). Spectra were collected using a 1 to 3 s exposure and 30 to 40 samples were averaged; spectra were corrected for background and relative spectral response using a quartz-tungsten halogen lamp (World Precision Instruments D2H), and normalized by integrated emission intensity. The eyes were then placed on a rotating platform at the object plane of the imaging system, and imaged at five different incidence angles (-90, -45, 0, 45, and 80 degrees relative to the optical axis). A series of 10 images were acquired at each orientation. Images were averaged and background subtracted before analysis. Imaging measurements were repeated for each eye after inoculating with ∼2 µL of *B. cereus*.

3. Results

Measured fluorescence spectra are shown in Figure 1 with the spectra of *B. cereus*, *B. subtilis*, and *E. coli* (measured from ∼ 0.1 mm thick smears) overlaid for comparison. The intrinsic eye and bacteria fluorescence spectra are similar; both measurements are likely dominated by pyridine nucleotide fluorescence. The porcine eye fluorescence spectra are very consistent across the seven eyes, but there are significant differences in spectral content across the three bacteria samples that could be exploited for detection of bacteria on the eye.

Red-band fluorescence and fluorescence intensity ratio (FIR) images are shown in Figure 2 for three selected eyes, both healthy and infected, at each orientation and summary statistics are shown in Table 1. The healthy eye images show a bright pupil with a dark iris, indicating that the majority of fluorescence originates in the lens. While there is variation in intensity within the pupil, the FIR measurement is uniform which illustrates the utility of the two-color approach. Comparing with the inoculated eye images, the microbe smears are visible as changes in relative intensity. Thin bacteria layers appear to exhibit a combination of extinction of intrinsic eye

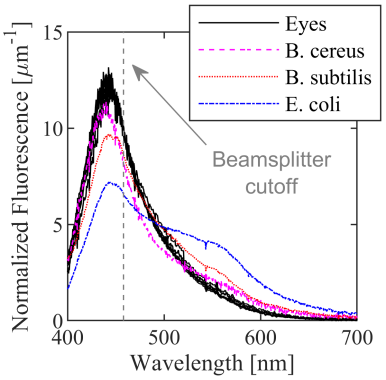

Fig. 1: Normalized fluorescence spectra of porcine eyes and three bacteria smears (*B. cereus*, *B. subtilis*, and *E. coli*) with 365nm excitation.

Table 1: Normalized intensity and FIR for each eye averaged over the pupil, and estimated effective fluorescence quantum yield (FQY).

		Intensity [counts]		
Eye	**FIR**	**Red**	**Blue**	**FQY [%]**
1	1.75	421	238	3.5
2	1.64	416	253	3.6
3	1.58	405	257	3.6
4	1.60	361	223	3.1
5	1.59	464	289	4.0
6	1.61	469	291	4.1
7	1.73	371	210	3.1

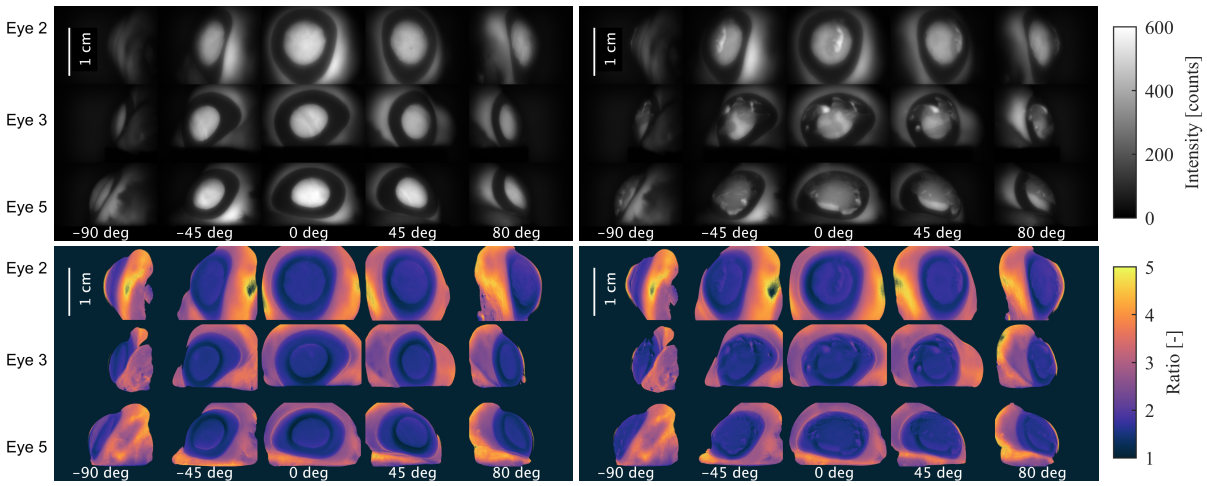

Fig. 2: Red-band fluorescence intensity (top) and FIR (bottom) images for three healthy (left) and *B. cereus*-inoculated (right) porcine eyes.

fluorescence with a modest amount of microbial fluorescence resulting in reduced image intensity. Conversely, thicker bacteria layers appear to be dominated by microbial fluorescence and exhibit significantly increased image intensity. Further analysis will need to consider radiative transfer effects within microbe layers in addition to fluorescence emission. Changes in FIR are apparent with the addition of the bacteria smear, but the FIR value is inconsistent likely due to radiative transfer effects in the microbe layer.

4. Conclusion

Two-color line-of-sight fluorescence imaging is proposed as a new tool to aid in the diagnosis of microbial keratitis because it is simple, low-cost, and has the potential to provide species-specific information while avoiding reflections and ambient light. Preliminary imaging results of porcine eyes (both healthy, and streaked with *B. cereus*) demonstrate the features of the method. Streaked regions are identifiable in the data as a change in fluorescence intensity and FIR. Radiative transfer processes inside the bacteria film appear to be significant in some cases, and further investigation of these effects is warranted. Future work will additionally require spectroscopic characterization of eyes and species implicated in MK.

References

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