Characterization of a Four-Camera Ratiometric Optical Mapping System

Zachary Collins

Zachary J. Collins and Steven Poelzing Ph.D.

Department of Bioengineering

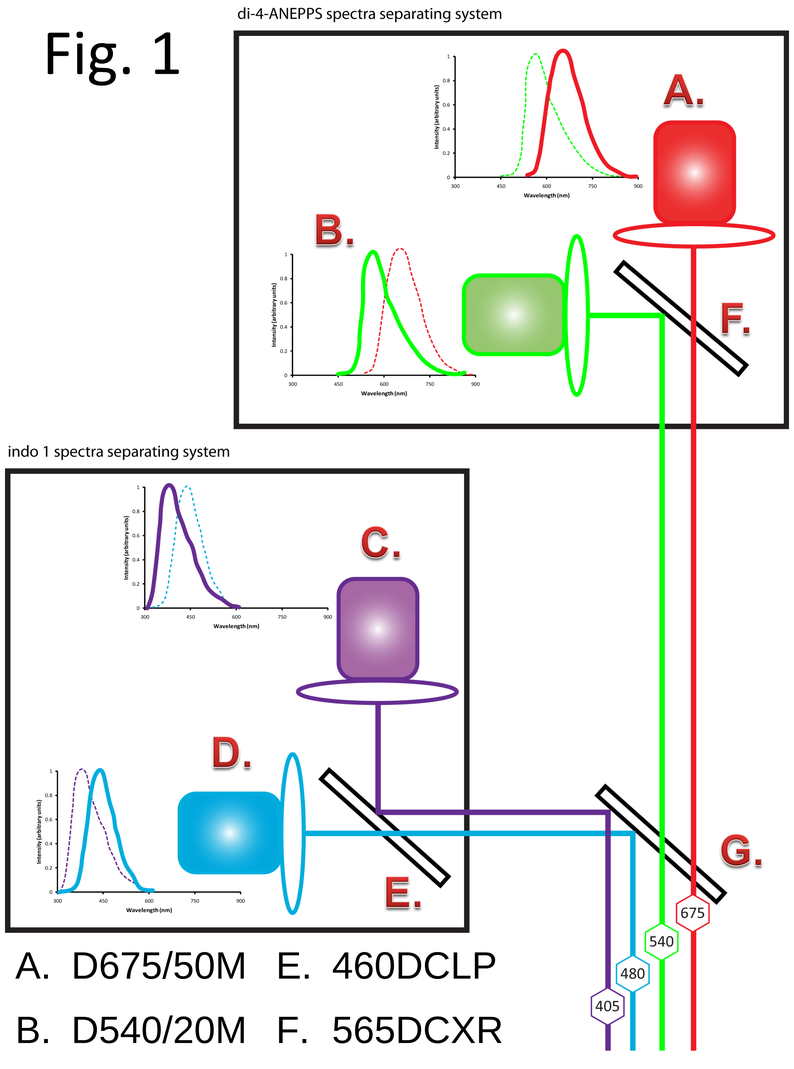
THE UNIVERSITY OF UTAH

Steven Poelzing Ph.D.

INTRODUCTION

- Electrical activity precedes calcium mediated heart contractions.
- Optical mapping takes advantage of minimally invasive fluorescent molecular probes to image both electrical and calcium activity. These molecular probes allow for ratiometric imaging.
- We characterize a four camera system capable of simultaneously capturing ratiometric images of voltage and calcium

Fig. 1



METHODS

- Optical mapping system as shown in Fig. 1
- Langendorf perfused guinea pig hearts.
- Di-4-ANEPPS or Indo1 perfused into Tyrode's Solution.
- Light intensity recordings performed with and without fluorescent dyes.

RESULTS

- Results are discussed below accompanied by graphical representations of the data.

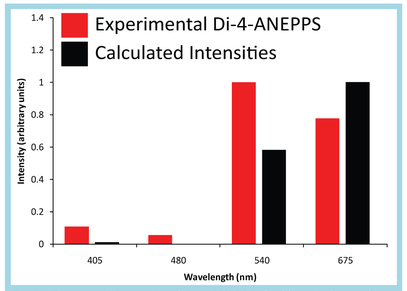


Fig. 2 - Fluoresced Intensity of Di-4-ANEPPS: Red bars indicate the relative intensity of light incident upon each of the cameras when di-4-ANEPPS was loaded in the preparation and excited. The black bars are the calculated intensity of light incident upon each camera due to fluorescence of di-4-ANEPPS. Camera 540 demonstrated experimentally the greatest intensity of fluorescence and 675 fluoresced less than calculated. Fluoresced intensity bleedover into camera 405 & 480 is not considered significant.

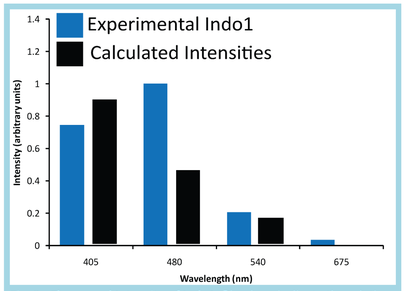


Fig. 3 - Fluoresced Intensity of Indo1: Blue bars indicate the relative intensity of light incident upon each of the cameras when indo1 was loaded in the preparation and excited. The black bars the intensity of light expected to be incident upon each camera due to fluorescence of indo1. Camera 480 fluoresced significantly more than calculated. Note that bleedover into camera 540 was significantly less than calculated.

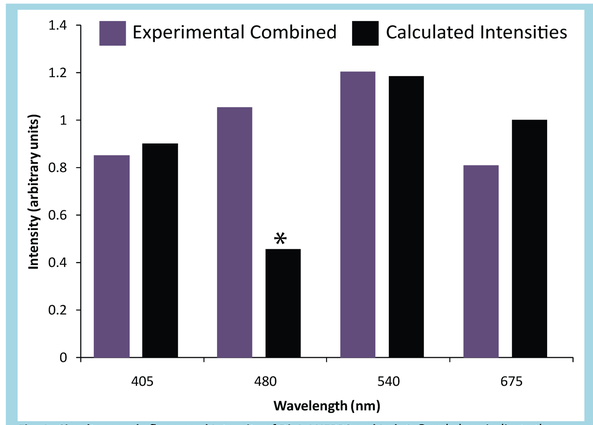
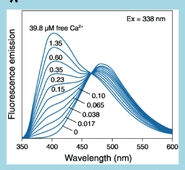


Fig. 4 - Simultaneously fluoresced Intensity of Di-4-ANEPPS and Indo1: Purple bars indicate the relative intensity of light incident upon each of the cameras when both di-4-ANEPPS and indo1 were loaded in the preparation and excited. The black bars indicate the calculated intensity of light incident upon each camera due to fluorescence of both di-4-ANEPPS and indo1. Here the fluoresced intensities very closely match the calculations made in cameras 405, 540, and 675. Camera 480 experienced a significant difference (see the * side panel). Overall the results from this experiment show that calculated intensities closely match experimental conditions.

*



The wavelength shift graph above is representative of Indo-1. This figure demonstrates how the intensity in each wavelength changes as calcium ion concentration changes from the bound to the unbound state. Note that the maximum states for unbound and bound correspond to the bandwidths of camera 405 and 480, respectively. A similar graph (not shown) can be constructed for the wavelength shift behavior of Di-4-ANEPPS. The green peak of Di-4-ANEPPS correlating with camera 540 and the red peak with camera 675.

SUMMARY

- **Di-4-ANEPPS**
  - Light intensity occurred principally in cameras 540 (C) and 675 (D).
  - Theoretical light intensity in camera 540 (C) is less than expected.
- **Indo-1**
  - Light intensity contributes to cameras 405 (A), 480 (B), and 540 (C).
  - Theoretical light intensity in camera 480 (B) is significantly higher than expected.
- **Combined**
  - Observed light intensity is similar to theoretical light intensity for cameras 405 (A), 540 (C), and 675(D).
  - Theoretical light intensity in 480 (B) is less than the observed experimental light intensity.

CONCLUSIONS

Di-4-ANEPPS

- The di-4-ANEPPS signal is restricted to cameras 540 (C) and 675 (D) with minimal signal overlap in cameras 405 (A) and 480 (B).
- Differences between experimental and theoretical di-4-ANEPPS are likely due to idealized fluorescent spectra of di-4-ANEPPS that does not accurately reflect dye activity while under physiological conditions.

Indo-1

- The signal from Indo-1 is present in cameras 405 (A), 480 (B), and 540 (C). The overlap is significant in 540 (C).
- Differences between experimental and theoretical Indo-1 data are likely due to idealized experimental fluorescent spectra of Indo-1 that does not accurately reflect dye activity while under physiological conditions.

Future Directions

- Carry out additional experiments to form a strong statistical basis upon which corrective mathematical formulas can be developed and subsequently integrated into the current data analysis software.
- Consider current filter selection and possible changes to system.

This work was also supported by a grant from the Nora Eccles Treadwell foundation awarded to Dr. Poelzing.