Optimizing the intraoperative indocyanine green (ICG) imaging system

Forrest Lin[1], Brandon O'Neill[1], Timothy Markman[2], Brian Salzberg[3], Saman Nazarian[2], Charlie Bleau[4], Chun Bleau[4]

[1]School of Engineering and Applied Sciences (2027), University of Pennsylvania, Philadelphia, PA; [2]Department of Cardiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [3]Department of Neuroscience and Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [4]SciMeasure, North Decatur, GA

INTRODUCTION

- Voltage-sensitive dyes alter their optical properties based on changes in membrane potential, making them excellent fluorophores for recording the firing activity of neurons and myocytes
- Indocyanine green (ICG) dye is the only voltage-sensitive fluorescent dye approved by the FDA for clinical use
- ICG has previously been shown to identify scar and neural tissue outside the heart, crucial for surgical navigation and to identify cardiac arrhythmias
- An imaging device employing ICG has been developed to enhance visualization of anatomical structures, including scarring and conduction circuits, inside the heart

OBJECTIVE

The objective of this project was to fully optimize the ICG imaging system, observe optical properties of ICG in solvents of various polarities, and visualize the electrical activity of neonatal cardiomyocyte rat cultures

METHODS

- When exposed to near-infrared light, ICG becomes excited and emits light (fluorescence) at a slightly longer wavelength
- The overlap of wavelengths in excitation and emission spectra causes artifactual reabsorption of light in the camera, so bandpass (BP) and long-pass (LP) filters are installed in the camera lens and in the LED (Light Emitting Diode) illuminator

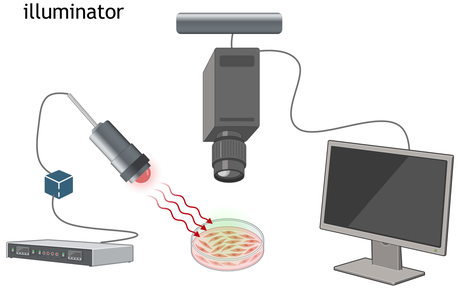

Figure 1. An LED benchtop current controller causes the LED to emit near-infrared light (780 nm) that travels to a filter cube and eventually to the LED head. A 2K-CMOS camera, installed with bandpass filters, is connected to a computer to take recordings.

RESULTS

Imaging Configurations

- A 750/800 nm BP filter was installed in the filter cube; only excitation light between 750 to 800 nm can pass through the LED head
- A 50-mm spacer holding two 830 nm LP filters and one 790 LP filter was installed above the camera lens; only emission light having wavelengths longer than 830 nm can be recorded in the camera and no excitation light should be recorded
- A dark frame is acquired prior to recording to subtract background noise
- The entire imaging system was placed on a piezoelectric vibration isolation table to reduce slight movements in the specimen
- A black plastic sheet was placed between the metal surface and the specimen to eliminate infrared light reflections from the metal
- Recordings were taken in a dark room to optimize signal/noise ratio
- Camera records at 640 Hz frame rate in a 512-by-512 pixel frame, and a single recording takes 5000 images within 2 ms
- The camera, with its lens, exhibited a 13 cm working distance and approximately 3.5 by 3.5 cm field of vision

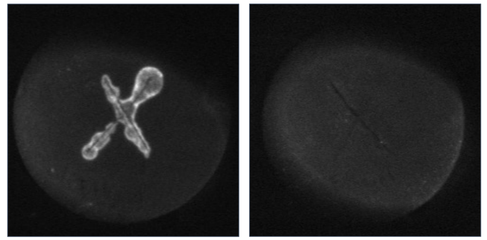

Figure 2. (above) Images taken of ethanol with (left) and without (right) ICG stained on filter papers

Optical Properties of ICG in Various Solvents

- We generated equal dilutions of ICG in ethanol, octanol, Tyrode's*, and distilled water and measured absorbance spectra of each solution with UV-vis spectrophotometer
- We observed absorbance peaks at 779.4, 780.2, 788.2, and 796.0 nm for water, Tyrode's, ethanol, and octanol respectively (see Fig. 3)
- Octanol, being the most non-polar solvent, exhibits an ICG absorbance peak at the longest wavelength, followed by ethanol (polar) and Tyrode's (very polar)

**Tyrode's is an interstitial fluid containing mostly salts (sodium, potassium, calcium, magnesium, etc.) commonly used in physiological experiments; ICG does not dissolve well in Tyrode's*

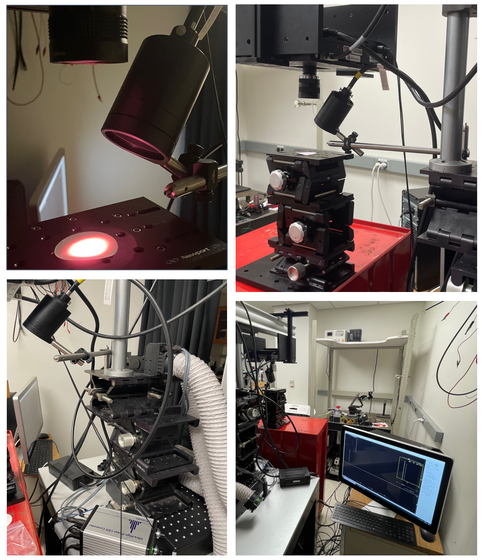

Figure 3. (above) Images taken of the entire system; top left depicts a close-up of the LED head, camera, and stage; top right shows a wider view of the camera mounted directly above the stage; bottom left displays the electrical and optical components of the system, including the LED current controller and the filter cube; bottom right shows a computer attached to the system with software used to take recordings

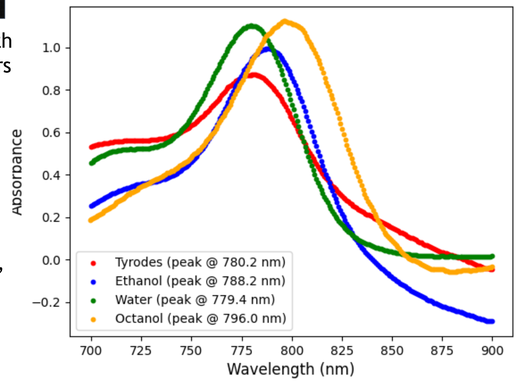

Figure 4. (above) Absorbance Spectra of ICG in Tyrode's Solution (red), Ethanol (blue), Water (green), and Octanol (orange)

Staining and Imaging Cardiomyocyte Cultures

- Staining solution was prepared by dissolving ICG in ~1 ml distilled water and then diluting by ~100x in Tyrode's solution
- Confluent myocyte cultures were stained for 20 minutes before the ICG-Tyrode's solution was replaced with pure Tyrode's
- We added small amounts of calcium, raising extracellular calcium to ~4mM, to detect mechanical contraction in cells and to determine that cells were spontaneously active
- Wire electrodes touching the cultures were attached to opposite ends of the dish and connected to a stimulator to induce contraction in cell cultures
- Recordings were taken in attempt to capture action potentials triggered by single stimulations

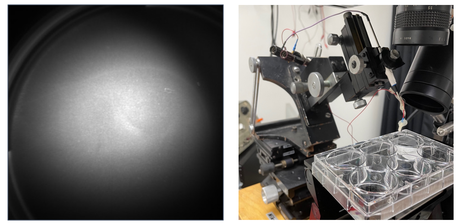

Figure 5. (above) Single frame of a recording taken of a stained, confluent myocytes cultures (left) and an image depicting the configuration for stimulating the cell cultures

Visualizing Action Potentials and Contraction

- Decent fluorescence and bleaching curves are evident in the camera recordings and individual cells are visible under the camera
- No fluorescence changes were detected in the camera because confluent cells were not prepared to contract synchronously
- Synchronous contraction is necessary to have a sufficient number of cells undergo an action potential simultaneously, yielding an optical signal large enough to visualize
- No contraction was detected by the camera, even though cells were visibly contracting individually, but asynchronously, imaged in a microscope
- A larger synchronously active preparation, perhaps a live muscle strip or a frog heart, is recommended to visualize contraction and fluorescence changes in the future

DISCUSSION

- Even though two 830 LP filters should eliminate all excitation bleed-through in the camera, adding a 790 LP filter decreased the camera background, so it was kept into the final configuration
- Solvents of higher polarities (water and Tyrode's) tend to have peaks at shorter wavelengths, whereas ethanol and octanol, which show limited polarity, have longer peak wavelengths
- Ethanol is more polar than octanol but less polar than Tyrode's, so its peak wavelength lies in between the two solvents
- A fluorescence spectrum could be obtained to visualize an overlap between excitation and emission spectra for ICG
- Camera was unable to detect an action potential within myocytes since contraction was asynchronous, however it observed a substantial amount of fluorescence and bleaching from ICG
- The current imaging system is in good shape to perform experiments on a frog heart, the next step of this project
- Frog hearts are inexpensive, easy to prepare, and beat spontaneously in Tyrode's solution containing calcium, making them great candidates for visualizing action potentials
- Frog hearts are easily stained by injecting dye into the aorta, where the heart will circulate dye around the body and stain itself
- In the absence of calcium, frog hearts do not undergo mechanical contraction, so injecting blebbistatin is not necessary to restrict movement

ACKNOWLEDGEMENTS

Research funding in support of Forrest Lin and Brandon O'Neill was provided by University Scholars and Penn Undergraduate Research Mentoring Program. We are indebted to Dr. Timothy Markman and Dr. Brian Salzberg for mentoring us on this project. Special thanks to Charlie Bleau and Chun Bleau at SciMeasure for guidance on imaging software.

REFERENCES

Chon, B., Ghann, W., Uddin, J., Anvari, B., Kundra, V. (2023). Indocyanine Green (ICG) Fluorescence Is Dependent on Monomer with Planar and Twisted Structures and Inhibited by H-Aggregation. *Int. J. Mol. Sci.*, 24(17), 13030. https://doi.org/10.3390/ijms241713030.