402-21-Excellence-mini_microscope-Executive Summary

Affordable FRET Microscope for Education

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Advisor: Professor Jeremy Rogers Client: Professor Matthew Merrins

Our team is designing an affordable FRET-capable microscope that will be used in a classroom setting. Fluorescence Resonance Energy Transfer (FRET) is an imaging technique where a single wavelength of light is used to excite an acceptor fluorophore, at which point the acceptor fluoresces at a specified wavelength. The acceptor fluorescence excites a donor fluorophore, and a fluorescence ratio of the two fluorophores can be used as an intracellular sensor. Our client, Professor Matthew Merrins, teaches a human biochemistry lab at the University of Wisconsin-Madison, in which his students utilize a FRET-based biosensor to study lactate levels in pancreatic islet beta cells. His current microscope costs over $100,000, which far exceeds his course budget, since he would like multiple devices for his students. The goal of this project is to design a cheaper FRET microscope that Professor Merrins can fabricate himself. With the opportunity to collect data through hands-on experiments, students will learn to establish their own experimental procedures and acquire analytical skills necessary for academic research.

Research microscopes on the market are expensive partially due to their broad, built-in functionality. However, these functionalities are not always required in an educational setting where the client requires specific experiences for his lab. Currently, the Lumascope 620 is a common FRET microscope used to image living cells; however, the variety of configurations of the objective lens, laser options, filters, and detectors make the device too expensive to obtain multiple devices for student use. On the other hand, the Dino-Lite is an affordable fluorescence microscope, but the Dino-Lite only detects a single wavelength of light. This is not ideal for FRET since FRET requires the use and detection of two fluorophores, and therefore two wavelengths of light.

The current design involves a simplified microscope with a 3D printed sample stage, 430 nm LED excitation source, platform with filter-switching interface, objective and tube lenses, one triple bandpass filter, fold mirror, emission filters, and camera. The device will image the two chromophores of interest at 470 and 535 nm. Through the development of a software package, the microscope's electromechanical components, including the camera, can be easily controlled by the student, and the FRET ratios can be directly computed from the compiled images. These features make this microscope design easily accessible and intuitive for students new to FRET microscopy, and each device is estimated to cost around $7,500, within our client's $10,000 budget.

Testing began with camera validation to determine if our cheaper option was functional for the design requirements, and it was able to resolve the islets. Then the excitation source was designed iteratively, starting with a basic LED array that evolved into a system that excites through the objective lens. Current testing on the complete prototype has suggested that the microscope can effectively detect these chromophores, and the software produces relatively accurate quantitative results along with great qualitative results in terms of FRET ratio patterns, which is the goal of the client. Furthermore, the smallest bar on a 1951 bar target, a common standard used to characterize optical systems, showed that our design can resolve at least 5.5 μm objects. In comparison to a professional FRET microscope, the Nikon TI Eclipse, our system is able to image islets at a 250 ms exposure time, whereas the Nikon scope is able to do so at an exposure time of 100 ms. Additionally, our design features a 2.26 signal-to-noise ratio (SNR), while the Nikon scope produced an SNR of 3.5. At under $10,000, this device adequately addresses the client's need for a less expensive microscope for educational use.