Building a fluorescent microscope to visualize C. elegans

Abstract

Fluorescent microscopy is a mainstay biological tool used to image selectively stained tissues, bacteria and other pathogens. Fluorescent detection and microscope components are costly, and can be substituted in creative ways to reduce cost and develop understanding of the underlying optics and physics. Here, green fluorescent microspheres and C. elegans are imaged at 50-70X magnification at a spectral range of about 490 - 555nm. Not only is this range conducive to visualizing the fluorescent microspheres, but it allows visualization of some autofluorescent emissions of *C. elegans* near senescence.

Introduction

Sir George Stokes published on the phenomenon of specific organic and inorganic specimens emitting light when excited by ultraviolet light in 1852. This began the discovery of the fluorescent process. Fluorescence entails the nearly simultaneous (within nanoseconds[1]) events of an atom or nuclei's absorption and subsequent emission of a photon. Fluorescent emission occurs at a longer wavelength and lower energy than that of the excitation light, this is the Stokes shift. In the 1930s, fluorescent techniques permeated the biology community to investigate stained tissue, bacteria and other pathogens. Since this introduction, organic chemists have worked to provide a means of labeling fluorescent probes for a wide variety of biological systems. ThermoFisher Scientific's Molecular Probes Handbook is the most complete compendia of fluorescence information, containing information on over 3,000 reagents.

Due to the Stokes shift, by completely filtering out the excited light, but not blocking the emitted fluorescence, it's possible to selectively focus on the objective, as it's the only thing illuminated. Fluorescent detection components are costly, and when working with particularly bright fluorescent markers, their fidelity can be superfluous. The Zhong Lab at RICE University proposed a method to convert a standard microscope into one that can detect fluorescence. We propose a similar method, yet further reducing the cost by building the microscope component out of a webcam mounted with spare lab materials. The fluorescent microspheres imaged in the following study are synthesized to be bright and easy to image. The C. elegans are about 1 mm long and are conducive to imaging at magnification levels under 100x. The continuous video capture supplied by the webcam-driven microscope is conducive to watching the undulations and behaviors of the nematodes.

Methods

Scope Build

Webcam

Reversing the orientation of a webcam's (Stopmotion Explosion's Pro HD 1080P Webcam) lens with respect to the CMOS sensor drastically effects its magnification. To do so, open the webcam, making sure

not to damage the internal components. Reverse the lens and secure it 2-3 mm away from the CMOS sensor. Glue components into place and replace the webcam's cover.

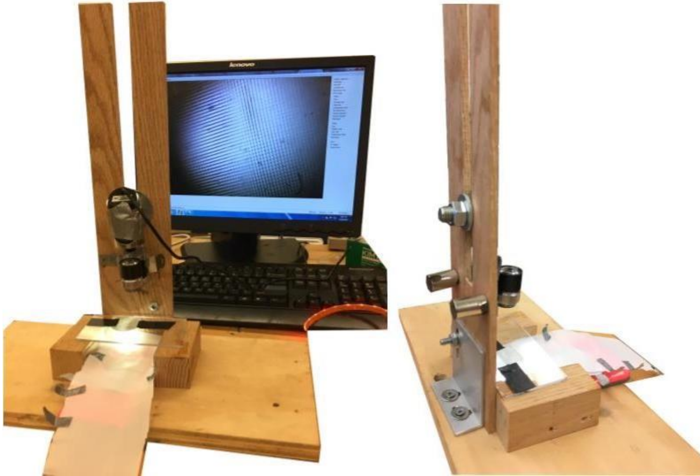

Figure 1. *DIY Microscope build with multi lens system including adapted ~200X reversed-webcam secured by nut and bold, and 10X lens in lens holder secured by rare earth magnets. Subject is 200-micron mesh light from beneath with diffused broadband light source.*

Stand

As in Figure 1, A linear slot was machined along the midline length of a spare 2x4, open at the top, and ending a few inches before the bottom. This plank will act as a vertical stand when attached to a base via L-bracket. The slot was wide enough for a nut to pass through, and secured with washers and a bolt on the back side. The webcam is attached to the head of this nut with epoxy and left overnight to set. Care was taken to attach the webcam and the nut in such a way that the camera looks straight down. An extra lens is necessary to increase the working distance of the system. This lens was glued into a plastic holder, which was taped to metal angle brackets to both attach to the vertical stand and aline the auxiliary lens with the optical axis of the webcam. This extra lens was secured to the vertical stand with rare earth magnets. In this way, both the webcam and auxiliary lens exhibited freedom to move along the vertical axis.

Optics

Working distance & Magnification

The inverted webcam resulted in a magnification of ~200x, calibrated experimentally with 200 micron mesh. The DIY scope has a working distance of 1.5 cm, and is unfit to be used independently in our fluorescent microscope system. The addition of a 10x magnification lens into our multiple lens system increased our working distance to ~1.5". This provided enough room to shine the targeted excitation photons at the fluorescent sample.

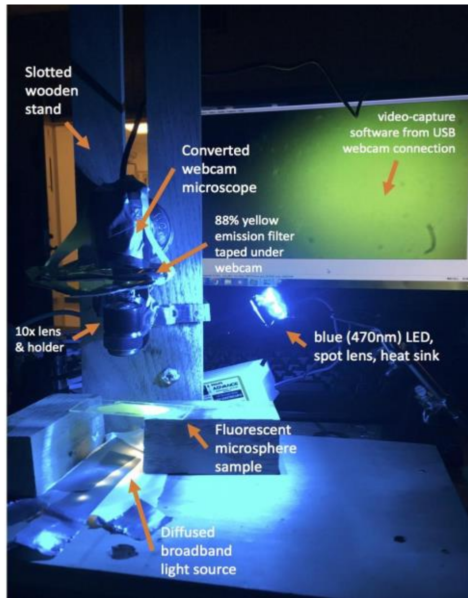

Figure 2. *Labeled DIY fluorescent microscope build. DIY microscope made of multiple lens system including reversed webcam and auxiliary 10X magnification lens. Sample (green fluorescent microspheres) is lit from beneath with diffused broadband light source, and excited by 470 nm blue LED and spot lens. Fluorescent emission is filtered with Roscolux 12: Straw gel, attached under webcam.*

Excitation / Emission Spectra

An emission filter (Roscolux 12: Straw) was placed under the webcam. Blue LED (470nm) was passed through a spot lens by way of nonconductive adhesive to an optic holder. The targeted visualization spectrum falls between 490nm-555nm, including cyan to green fluorescence [Figure 3]. The backside of the LED was attached to a heat sink, and the assembly was supported by Xacto helping hands. The LED was soldered to a 700mA constant current titanium LED driver, which was soldered to a 18AWG lamp cord with switch (LED gets very hot and easy switch access is desirable). We aimed to image green fluorescent microspheres (~=525 nm) and their interaction with C. elegans. The sample was also lit from below with a diffused broadband light source [Fig 2].

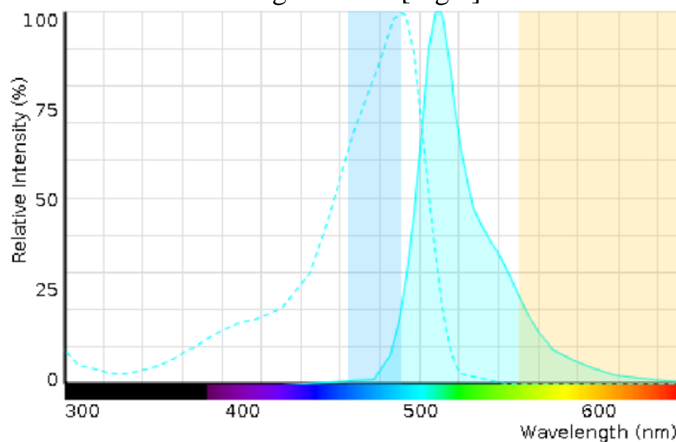

Figure 3. *Fluorescence SpectraViewer (ThermoFisher Scientific) graph of experimental conditions. Excitation and emission bands are represented by blue 470 nm LED with 30nm bandwidth, and Roscolux 12: Straw gel at 555+ nm, respectively. Projected visualization parabola (shown in cyan) is GFP emission curve.*

Results

Optical

The DIY fluorescent microscope was able to visualize and capture real-time video of fluorescent microspheres and C. elegans in a gel substrate. Calculated experimentally, the multi lens system had an overall magnification of 50-70X. This was calculated on various still frames from equation (1), the known pixel density of a 1080p webcam, and the average length of C. elegans = 1 mm. When the blue (=470 nm) excitation LED is on, the beads appear green and the worm tinged cyan. When the excitation LED is off, the c. elegans and fluorescent microspheres are indistinguishable, and contrasted against the broad spectrum white LEDs under the sample [Fig 4].

Equation 1. *Magnification = image height / object height.*

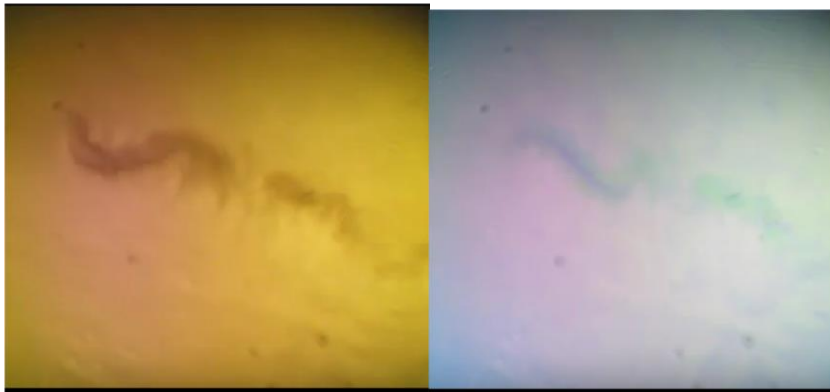

Figure 4. *Undulating C. Elegans visualized without and with blue LED excitation light.*

Biological

The materials gathered to image under this scope came from various labs, and compatibility was overlooked. This resulted in the exposure of *C. elegans* to fluorescent microspheres containing inorganic fluorescent compounds that were toxic to the nematodes. When c. elegans interacted with the microspheres, they struggled to swim, changed swimming patterns, and/or swam away. This is visualized in Figure 5, as a *C. elegans* is swimming out of a concentrated area of green fluorescent microspheres.

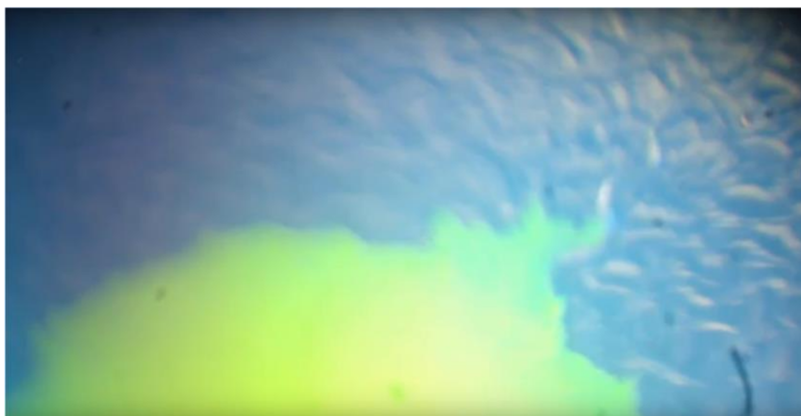

Figure 5. *C. elegans swimming out of concentrated area with green fluorescent microspheres.*

Another unexpected outcome of this experimental set up was the intestinal autofluorescence of C. elegans undergoing senescence [Fig 6]. Lipofuscin (also known as the "age pigment") is an autofluorescent material

that builds up over time in cells and tissues with low turnover[3]. Lipofuscin is composed of highly-oxidized, insoluble cross-linked proteins and lipids. This build up is usually found in the intestinal cells[2]. The precise relationship between autofluorescence, aging and lifespan in C. elegans remains a topic of debate. However, blue fluorescence has been found to provide a measurement of the fraction of dead or near-dead individuals, as it is an indicator of incipient or recent demise[3].

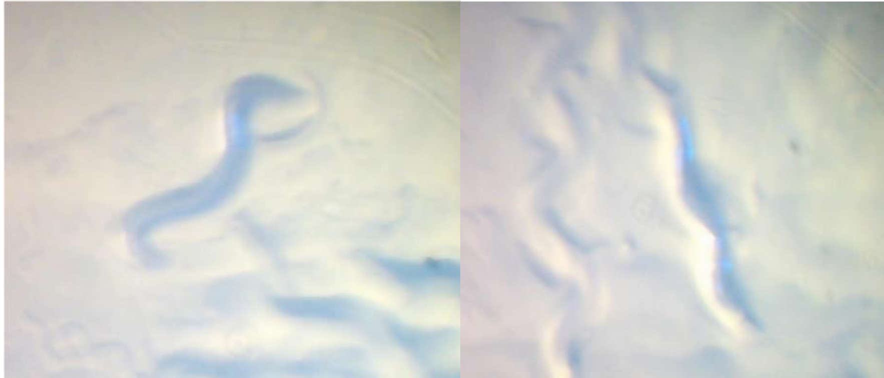

Figure 6. *C. elegans in gel emitting cyan/blue fluorescence after being exposed to green fluorescent microspheres.*

Discussion

Fluorescence microscopy can be executed at a lower price point than that of standard issue scopes. If the desired visualization material has strong enough fluorescent properties, the fidelity of our DIY scope is enough to detect the excited spectra. Excitation at 470 nm and subsequent filtering of emissions above 555 nm provide a range of visible fluorescence incorporating cyan and green hues. This range is sufficient to view both green fluorescent microspheres and some autofluorescence from *C. elegans.* Using a higher energy light (e.g. purple) to excite the subject could have shown more blue autofluorescence from the *C. elegans.* The cyan components of this biomarker for senescence is imaged, however it is likely there are darker blues that could have been imaged at this event.

Further, blue spectra are indicative of dying or near-dead nematodes. If another wavelength-range had been targeted, e.g. red, the experimental procedure could have had longitudinal implications to study *C. elegans* life cycle and health. Red autofluorescence is known to correlate with an individual's remaining days of life, and is thus a marker of health (instead of senescence)[3]. Another potential visualization objective could have been C. elegans with fluorescent material that is not toxic to them. Once the C. elegans were exposed to our green microspheres, their behavior and health changed drastically. A biocompatible compromise of these two could provide a prolonged ability to image healthy worms, as well as more humane experimental conditions.

References

1. Lichtman, Jeff, J.-A. C. (2005). Fluorescence microscopy. *Nature Methods, 2*(12), 910-919.

2. Clokey GV, J. L. (1986). The autofluorescent "lipofuscin granules" in the intestinal cells of Caenorhabditis elegans are secondary lysosomes. *Mechanisms of Ageing and Development, 35*(1), 79-94.

3. Pincus, Zachary, T. C. M., and Frank J. Slack. (2016). Autofluorescence as a measure of senescence in C. elegans: look to red, not blue or green. *Aging, 8*(5), 889-898.

Special thanks to: Curtis Lab for fluorescent microspheres, McGrath lab for C. elegans, BME Machine Shop and CRAB Lab.