Observing Fluorescent Probes in Living Cells using a Low-Cost LED Flashlight Retrofitted to a Common Vintage Light Microscope

G. A. Babbitt1*, C. A. Hanzlik1,2, and K. N. Busse3

1T.H. Gosnell School of Life Sciences, Rochester Institute of Technology, Rochester, NY 14623,
2Confocal Microscopy Lab, College of Science, Rochester Institute of Technology, Rochester, NY 14623,
3Biomedical Engineering, Kate Gleason College of Engineering, Rochester Institute of Technology, Rochester, NY 14623

INTRODUCTION

Fluorescent microscopy techniques are currently employed in many areas of modern cell biology, in particular with respect to the targeted probing of localized protein structure or gene activity in the cell. There is increasing need to expose high school and college students to hands-on laboratory experiences involving these techniques. Many kit-based biotechnology products utilize fluorescent probes and are designed to be implemented in teaching laboratories (e.g. BioRad’s Biotechnology Explorer product series for manipulating green fluorescent protein or GFP). However, because of the high cost of the equipment, the teaching of fluorescent microscopy techniques often remains financially out of reach for most high school and undergraduate college courses. In the past, fluorescent microscopy illumination required expensive high-energy short arc-discharge mercury or xenon lamps requiring heavy armoring; or alternatively, argon-ion or argon-krypton lasers, making even the simplest of optical systems cost-prohibitive outside of a research lab. However, the recent development of very bright low-voltage light emitting diodes (LEDs) at precise and specific wavelengths may serve as very low-cost and long-life alternatives for fluorescent illumination that can be retrofitted to a variety of modern microscopes (1–4).

Here, we describe in detail a simple and low-cost (< $100) adaptation of a vintage Nikon Optiphot brightfield vertical EPI illuminator, a pair of needle-nose pliers, a drill, a set of small screwdrivers, superglue, a 3W Ultrafire WF-501b green LED tactical flashlight, a rectangular 450DRLP dichroic mirror for 45 degree AOI (25.7 mm x 36 mm) and a small green optical bandpass filter (520 nm for GFP). Alternatively, for viewing orange/red live cell stains, you will need a 3W Ultrafire WF-501b green LED tactical flashlight, a rectangular 575DRLP dichroic mirror for 45 degree AOI (25.7 mm x 36 mm) and a suitable orange-red optical bandpass filter (610 nm for most live cell membrane or mitochondrial stains). Colored LEDs emit at very specific wavelengths thereby eliminating the need for filtering the excitation wavelength. All the optical parts are readily available inexpensively through secondhand instrumentation dealers. The Nikon model S is one of the most common microscopes resold online and older vertical illuminators with broken mirrors are often sold very cheaply for parts. The basic idea is to remove most of the illuminator optics and place the LED flashlight in close proximity to the dichroic mirror. In theory, this sort of procedure can be performed with any older microscope equipped with epi-illumination such as is suitable for metallurgical applications. Prior to the 1980s, many microscope parts were interchangeable between brands (with the exception of Zeiss). Often the older Nikon and Olympus microscopes are sold stripped...
of their original low-voltage halogen light sources. In these cases, a bright white LED flashlight can be fitted to the back of the microscope base. This method of base illumination is advantageous in that it eliminates the problem of stage heating by traditional halogen lighting, which has a tendency to stress or kill live cells and protozoans. Brightness can be controlled at diaphragms in both the condenser and Nikon S-Ke microscope base.

**Vertical illuminator disassembly**

1. Remove all parts of the illuminator to the rear of the field diaphragm ring (marked “F”); this includes the center sleeve, aperture diaphragm (marked “A”), excitation filter optics, filter holders, and lamphouse (if present). Figure 1(A) shows the intact vertical illuminator and its parts. The field diaphragm is marked with an arrow.

2. Remove the plastic field diaphragm ring exposing the brass field diaphragm (Fig. 1(B)). Loosen the two small brass slotted screws that secure the diaphragm in place (Fig. 1(C)). The field diaphragm is also held in place by an internally threaded aluminum ring. This ring can be removed using a pin spanner wrench. If this type of wrench is not available, simply enlarge the holes for the spanner slightly with a small drill bit; then, using needle-nose pliers, turn counterclockwise (Fig. 1(D)). This may require considerable force if the ring is glued in place. The brass field diaphragm will fall out when this ring is removed and the two small brass slotted screws on the sides are loosened (Fig. 1(E)).

**Flashlight attachment to vertical illuminator**

3. Unscrew the lens from the top of the tactical flashlight, allowing the LED (bulb) assembly to come free (Fig. 1(F)). Insert this LED assembly into the position where the field diaphragm was and lock in place by tightening the small brass field diaphragm screws (Fig. 1(G)). Put the body of the flashlight into place against the LED assembly and tighten it using three of the larger screws that originally held the center sleeve against the field diaphragm assembly (Fig. 1(H)). The flashlight should now illuminate the dichroic mirror and reflect downward from the illuminator (Fig. 1(I)). The author often solders 9V DC power cords (salvaged from LED desk lamps) to the flashlights to power the illuminator from the wall instead of using batteries.

**Assembly of internal optics**

4. Remove the internal mirror holder from the main housing by first removing the optical path change-over knob and loosening internal screws. Carefully break away any existing beamsplitters or dichroic mirrors from the internal mirror holder using a paper towel to catch broken glass (appropriate gloves should be used to protect your hands from being cut by the mirror). Any small amounts of glass or glue left on the mirror holder can be chiseled off with a small flathead screwdriver. Using small drops of superglue, place the new mirror and optical filter as shown (Fig. 2(A)). When dry, the mirror and filter assembly should be reinstalled to the illuminator (Fig. 2(B)). The optics on the mirror and filter can be fine adjusted and aligned to the optical train of the microscope using the three screws surrounding the optical path change-over knob (indicated by the black arrow in Fig. 2(C)). Note: the optical filters are critical for protecting the eye, especially when UV excitation is used. These optical filters should transmit only wavelengths observed in the peak emission spectrum of the fluorophor used (e.g. around 520 nm for GFP or 610 nm for red live cell stains). Moderately wide bandpass filters can be used instead of narrow bandpass filters to compensate for the lower brightness of LEDs when compared to modern microscope systems; however, narrow bandpass filters generally produce a darker image background. The optical characteristics of a system designed for GFP excitation at 400 nm using an UV LED light source is shown in Figure 3. The author often orders filters and mirrors from the many used part dealers on eBay. One seller, “Omegabob,” has quite a large selection of
optical filter glass and is, in fact, representing the surplus inventory of Omega Optical Inc., a large producer of precision scientific filters in Brattleboro, Vermont (http://www.omegafilters.com/).

Imaging

We show images of various fluorescent materials and live cell specimens using our retrofitted fluorescent illumination mounted on an inexpensive 1960s era Nikon model S light microscope (Figs. 4(A), 4(C), 4(D), 4(E)). For comparison, we also include comparable images taken on a modern Zeiss Axiovert 200M inverted fluorescent microscope system (Figs. 4(B), 4(F)).

CONCLUSION

An entire fluorescent microscopy system complete with the option of phase contrast can be built for a few hundred dollars when based on Nikon model S components. It is
remarkably well-suited for classroom use in observing a variety of fluorescent probes in living cells. Unlike more expensive modern fluorescent microscopes, the optical paths in this simple retrofitted light microscope are quite obvious and easily accessible for instructors during classroom demonstrations of the optical principles behind fluorescent techniques. For further detailed information regarding fluorescent microscopy, we refer students and instructors to the appropriate article at Nikon’s Microscopy U website that may be sourced via the following link: http://www.microscopyu.com/articles/fluorescence/index.html.

The author welcomes any and all questions from classroom instructors building their own retrofitted fluorescent scopes.

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