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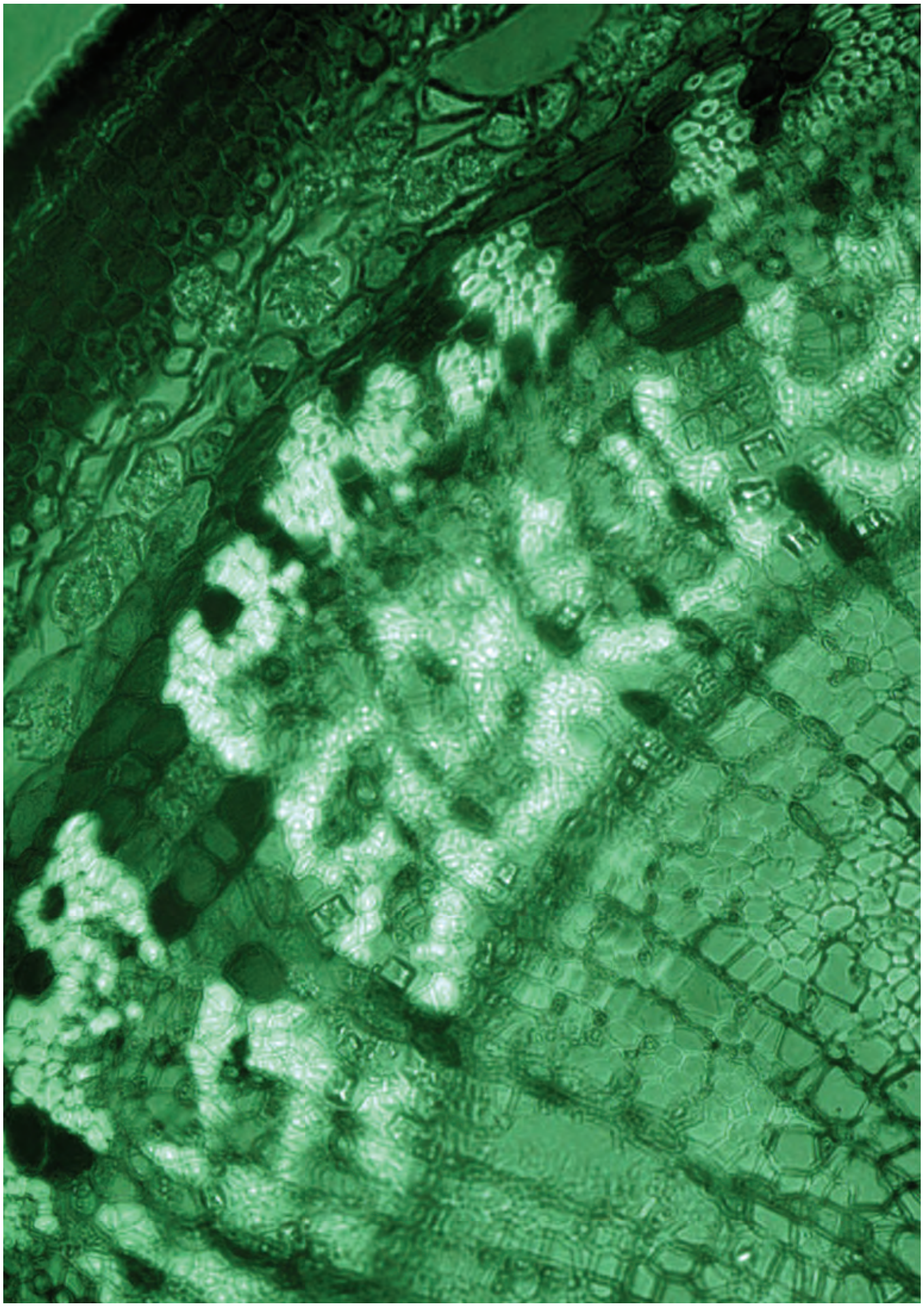

Applications of a Compact, Easy-to-Use Inverted Fluorescence Microscope

by Jennifer Kahle, Robert Levin, Walter Niles, Brian Rasnow, Mel Schehlein, and Chris Shumate

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The scientific and medical applications of fluorescence microscopy have expanded substantially in the past two decades,[1] and the potential to expand further is tremendous. The expense and commitment required to purchase, house, and operate typical research-grade fluorescence microscopes, however, currently limits the accessibility of such microscopes to well-funded laboratories.

The LumaScope™ (**Etaluma Inc.**, Carlsbad, CA) fluorescence microscope utilizes improved and miniaturized universal serial bus (USB) communications, light-emitting diodes (LEDs), and complementary metal-oxide semiconductor (CMOS) sensor technologies. It is small and economical, yet provides laboratory-grade images.[2] The microscope was designed to be sturdy and easy to use, and to withstand frequent use by multiple students and laboratory technicians, without requiring special training.[2] It was also intended as an inverted microscope to allow the observation of many more types of cellular preparations due to its open deck design and ability to accommodate the various focal lengths required for Petri dishes, flasks, microplates, and chambered slides.[3] The small size allows it to fit easily within incubators, hoods, and Faraday cages; under stereoscopes; on desks; and at single laboratory bench stations. It can be stacked for compact, safe storage when not in use.

This article presents examples of recent images of diverse preparations collected from first users in various research laboratories, teaching laboratories, patient examination rooms, and living rooms across the United States.

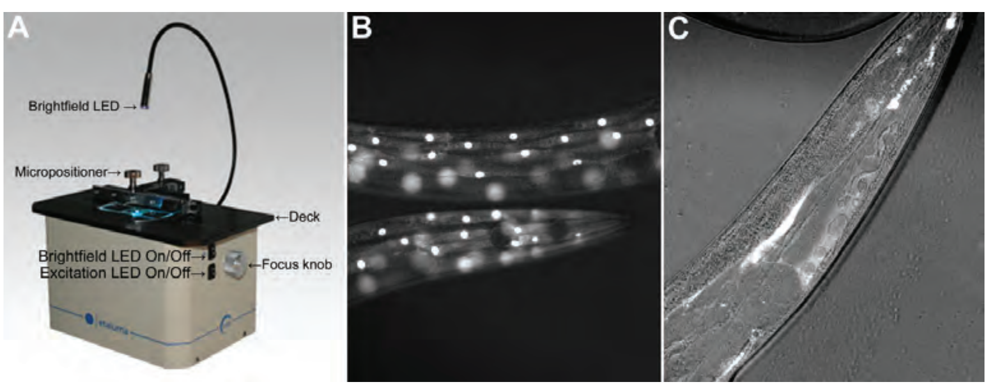

Figure 1 *Live* C. elegans, *imaged by college students in a molecular biology class. a) LumaScope with controls labeled. The micropositioner and brightfield illuminator are easily removed. A standard glass slide is shown clipped into the micropositioner for scale. b) Live* C. elegans *undergoing RNAi against a control gene other than green fluorescent protein (GFP) in muscle cells with a nuclear localization tag. c)* C. elegans *undergoing RNAi against GFP. The entire right image became much lighter with optimization of intensity because of the low levels of fluorescence in these worms; 40× objective, cropped, with standard automatic optimization of intensity, saved as gray-scale jpegs. (Courtesy of Drs. Matthew Jones-Rhoades and Esther Penick, Assistant Professors of Biology, Knox College, Galesburg, IL.)*

Device development

To economize the light source, a conventional arc lamp or laser was replaced with a high-brightness LED.[4] Collimation and filtering of the long-wavelength tail of the light from the LED were critical to achieving high fluorescence performance. The highest-quality filters were used, but cost was saved by miniaturizing the optical path. Directly coupling the objective to a small digital imaging sensor, without an interface to the human eye, allowed smaller-diameter (and thus less expensive) filters while achieving greater intensities and efficiencies.

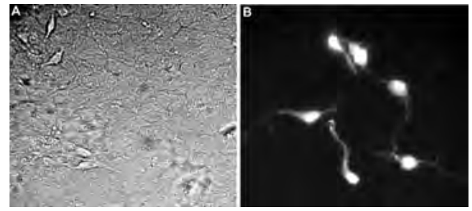

Figure 2 *Live neural stem cells, imaged by a senior researcher from a private research institute. Mouse neural stem cells were followed over time by the author (W.N.) as they developed in culture; these images were collected after seven days. In brightfield (a), the confluence of the cell monolayer was apparent. The fluorescence image (b) shows cells that were developing neuronal attributes. The expression of enhanced green fluorescent protein (EGFP) in these cells was under the control of a neurotrophin-3 promoter, which is only active when the stem cells start to differentiate into neurons; 40× objective, cropped, standard automatic optimization of intensity and contrast, saved as gray-scale jpegs. (Cells courtesy of the Sanford-Burnham Medical Research Institute, La Jolla, CA.)*

Inexpensive and compact CMOS image sensors were used.[5] Their pixel sizes of 3–5 μm provide nearly diffraction-limited imaging with optical magnification of ~20×. The high pixel and frame rates permit continuous, real-time readout necessary for focusing and exploring the sample in fluorescence mode. The sensors' low power consumption enabled the microscope (including fluorescence illumination) to be powered from the host computer's USB port. Thus, the microscope and a laptop computer are a self-contained, portable fluorescence microscopy workstation.

The manual controls were also kept as simple as possible, with a single focus knob and two on/off switches control-

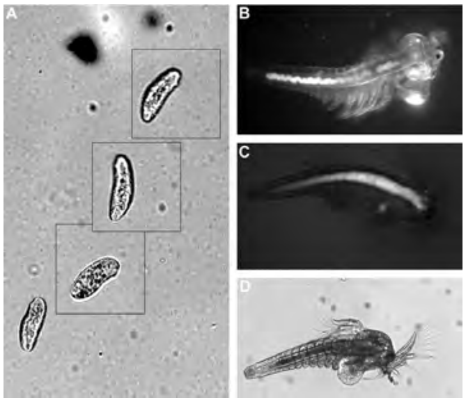

Figure 3 Live paramecium in standing rainwater and brine shrimp, imaged by middle-school (12 years old) and high-school students (14 years old). a) (Montage)—Paramecium moving bottom-to-top in a drop of standing rainwater, imaged using the LumaScope set up by a 12-year-old as a 1.4-frames/sec time series using a 40× objective in brightfield mode. Brine shrimp larva (*A. salina*) images were captured by a 14-year-old using oblique brightfield (b) and fluorescence (c) mode and a 10× objective. Another younger brine shrimp larva imaged in standard brightfield clearly shows swimming setae (d). Note: Crown was not detected on brine shrimp; active digestion and rapid movement of the setae, however, were easily visible. Both types of water samples were imaged at a residence using standard glass slides without coverslips. The brine shrimp were returned to the colony upon completion of the imaging session. Images were cropped, with standard automatic optimization of intensity and contrast, saved as gray-scale jpegs.

ling the fluorescence and brightfield LEDs (*Figure 1a*).

Live samples

The open deck design facilitates imaging many types of live preparations, even at high magnification. Some examples are live C. *elegans* (Figure 1*b* and *c*), neural stem cells developing in culture (*Figure 2*), an unfiltered sample of standing fresh water containing many microorganisms (*Figure 3a*), and brine shrimp larva (Figure 3*b*–*d*).

These images were captured by people of a wide range of ages and scientific experience. The C. *elegans* images were collected by college students; this was their first time using the microscope. The students reported that the LumaScope was very easy to use.

The stem cell images were collected by a highly experienced scientist from a private research institute. The water sample images were collected by middle-school and high-school students at their residence; this was their first experience with making slides with fresh water samples.

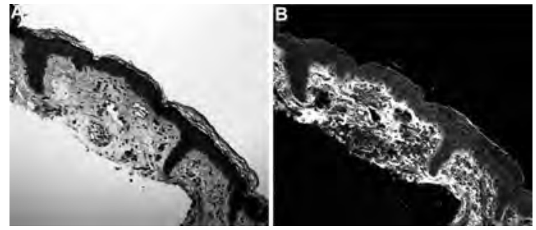

Figure 5 Human skin, imaged by a senior scientist in clinical patient examination room. Human skin samples (mole) stained for H&E, imaged by the author (B.R.) in a dermatology patient examination room with the overhead lights on, conditions traditionally considered unfavorable for imaging. Brightfield (a) and fluorescence (b) images; 20× objective, cropped, standard automatic optimization of contrast and intensity, saved as gray-scale jpegs.

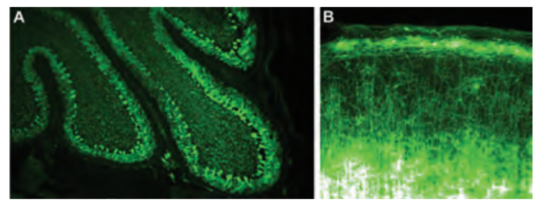

Figure 6 Cerebellum and cortex, imaged by scientists at industrial and academic research laboratories. a) Purkinje cells in rat cerebellum labeled with fluorescent antibody; 10× objective, cropped, standard optimization of intensity and contrast, saved as RGB jpeg. (Courtesy of Dr. Randall Wetzel, Director of Cytometry, Cell Signaling Technology, Danvers, MA.) b) Oligodendrocytes labeled with anti-oligodendrocyte RIP antibody in rat brain cortex; 40× objective, cropped, standard optimization of intensity and contrast, pseudocolored green from gray-scale jpeg. (Courtesy of Andrew Woolley, graduate student in the Departments of Biological Sciences & Biomedical Engineering, Purdue University, West Lafayette, IN.)

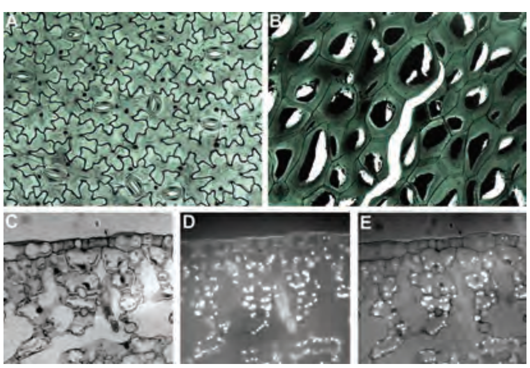

Figure 4 Plant tissues, imaged by a senior scientist at home. Brightfield images of prepared slides of broad bean epidermis (a) and Diospyros endosperm (b) collected by the author (B.R.) at a residence under standard room light conditions. A fern leaf section in brightfield (c), fluorescence (d), and both (e). The position of the fluorescent intracellular organelles inside the cells is easily observed in the image of both brightfield and fluorescence. This is not an overlay, but an image in both modes at the same time; 40× objective, cropped, standard optimization of contrast and intensity, saved as RGB (top) or gray-scale (bottom) jpegs.

Plant and animal cells: Prepared tissue

Many different types of tissue are easily imaged using the LumaScope. *Figures 4* and 5 show prepared slides of plant and human tissue. Many samples that have not been specially prepared with fluorescent labels exhibit fluorescence visible using the LumaScope, for example, the brine shrimp in Figure 3, fern in Figure 4, and conventional hematoxylin and eosin (H&E)-stained human histology tissue in Figure 5. *Figure 6* shows images of animal tissues that were prepared especially for fluorescence imaging.

These images were also collected in diverse places and under varied conditions. The images of plant cells were collected in a residential living room; the cerebellar and cerebral tissue images were collected in industrial and academic research laboratories, respectively, and the skin samples were imaged in a dermatology patient examination room.

Calcium imaging and electrophysiology

The LumaScope fits easily within a Faraday cage and under a stereoscope for positioning of a micropipet near target cells. The inverted open-stage design allowed for complete access to the sample from above with recording micropipets, fiberoptic lights, perfusion tubing, stimulation electrodes, drug application pipets, etc. The sturdy design minimized vibration issues and focal drift, permitting the impaling of single cells under visual control and recording of ongoing cellular events without requiring use of a sophisticated antivibration air table (*Figure 7*). The LumaScope accepts all infinity-

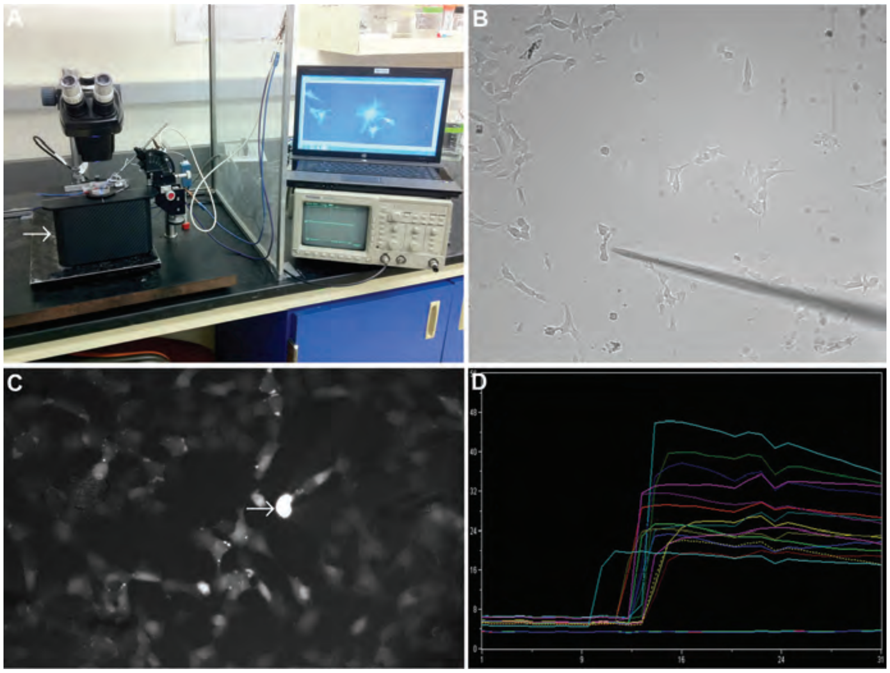

Figure 7 *Calcium signals and electrophysiology, imaged by senior researchers during a university graduate-level electrophysiology laboratory course. a) Modified LumaScope (arrow) embedded with a stereoscope, micromanipulator, and patch-clamp headstage inside a Faraday cage. b) Brightfield image of cultured 5YSY cells with sharp glass electrode for patch-clamping, stimulating with agonist, or loading with calcium indicator dye; 20× standard Meiji objective, no optimization, saved as gray-scale jpeg. c) Field of cells loaded with Fluo-8 calcium indicator dye (**TEFLabs**, Austin, TX). White arrow indicates single cell stimulated via a glass electrode (coming from top right corner); 40× Plan Apo objective, no optimization, saved as gray-scale jpeg. d) Traces represent calcium signals from multiple cells following bath application of agonist, analyzed using Metamorph® (**Molecular Devices Corp.**, Sunnyvale, CA). Each line represents a signal from a single cell. (Courtesy of Dr. Ian Parker, University of California, Irvine, and **Biophotonic Systems** [Irvine, CA].)*

corrected, Royal Microscopical Society (RMS) threaded objectives; a high-numerical-aperture **Olympus** 40× Plan Apo objective (Center Valley, PA) was used in the imaging in Figure 7*c*.

Conclusion

A device was developed at the very simplest level that would produce satisfactory images for routine cell inspection, for example, checking cell confluence in a tissue-culture facility or samples at a point-of-care medical clinic. The applications of the LumaScope microscope are already expanding as future users envision it in their laboratories, develop applications, and suggest modifications that continue to refine the design. The lack of eyepieces offers numerous advantages: ergonomic use, multiple users can share and interact with the real-time image, no subtle controls or adjustments are required, and the smaller optical path enables greater sensitivity and rejection of extraneous room light. The small size and ease of use of the device increases the accessibility of fluorescence microscopy to a wide range of users in diverse situations.

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