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

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The scientific and medical applications of fluorescence microscopy have expanded substantially in the past two decades, 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. 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. 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. 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.

Device development

To economize the light source, a conventional arc lamp or laser was replaced with a high-brightness LED. 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.

Inexpensive and compact CMOS image sensors were used. 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-
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 1b 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 3b–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.

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, fluorescence 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-
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.

References


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