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Design and construction of a modular low-cost epifluorescence upright microscope for neuron visualized recording and fluorescence detection

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HIGHLIGHTS

- We provide instructions for building a low-cost epifluorescence upright microscope.
- The design of a microscope using catalog-available parts reduces the time and price of assembling.
- This microscope is suitable for visualized recording and fluorescence detection.
- This open-box microscope allows researcher to modify it for a wide variety of experimental setups.

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ABSTRACT

Background: One of the limitations when establishing an electrophysiology setup, particularly in low resource settings, is the high cost of microscopes. The average cost for a microscope equipped with the optics for infrared (IR) contrast or microfluorometry is $40,000. We hypothesized that optical elements and features included in commercial microscopes are not necessary to IR video-visualize neurons or for microfluorometry.
New method: We present instructions for building a low-cost epifluorescence upright microscope suitable for visualized patch-clamp recording and fluorescence detection using mostly catalog-available parts.
Results: This microscope supports applications such as visualized whole-cell recording using IR oblique illumination (IR-OI), or more complex applications such as microfluorometry using a photodiode. In both IR-OI and fluorescence, actual resolution measured with 2-μm latex beads is close to theoretical resolution. The lack of movable parts to switch configurations ensures stability when doing intracellular recording.
Comparison with existing methods: The low cost is a significant advantage of this microscope compared to existent custom-built microscopes. The cost of the simplest configuration with IR-OI is ~$2000, whereas the cost of the configuration with epifluorescence is ~$5000. Since this design does not use pieces discarded from commercial microscopes, it is completely reproducible.
Conclusions: We suggest that this microscope is a viable alternative for doing in vitro electrophysiology and microfluorometry in low-resource settings. Characteristics such as an open box design, easy assembly, and low-cost make this microscope a useful instrument for science education and teaching for topics such as optics, biology, neuroscience, and for scientific "hands-on" workshops.

Abbreviations: IR, infrared; OI, oblique illumination; DIC, differential interference contrast; CCD, charge coupled device; LED, light-emitting diode; CMOS, complementary metal oxide semiconductor.

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1. Introduction

Cell imaging, fluorescence microscopy and IR differential interference contrast (IR-DIC), in combination with patch-clamp recording are powerful techniques used in physiology (Foskett, 1988; Sokabe and Sachs, 1990; Dodt and Zieglgänsberger, 1990). A microscope, an imaging/detection device and a light source are all necessary for visualization of fluorescent staining, recording of fluorescent ion probes or to perform visualized electrophysiological recording (Hamaguchi and Hamaguchi, 1990; O'Rourke et al., 1990).

One of the main limitations when establishing an electrophysiology setup, particularly in low resource settings, is the high cost of research microscopes. In order to perform visualized electrophysiological recording of neurons, commercially available microscopes require expensive and time-consuming adaptations. First, the microscope must include the proper optics for DIC or IR-DIC. Depending on the research needs, one must choose a fixed stage with a movable microscope or vice versa. Adaptations to either option are not included in the retailer's price and range from $4000 to $6000. When using a high-speed camera for Ca^{2+} imaging, a device to split the signal or an additional port is needed for IR-DIC image visualization, which is often performed with a video-rate CCD (charge coupled device) camera.

Researchers are continuously searching for new technology or modifying existing tools to improve imaging in neuroscience (Foskett, 1988; Dodt and Zieglgänsberger, 1990; Filler and Peuker, 2000; Demontis et al., 2005; Safronov et al., 2007; Hayar et al., 2008). Low-cost microscopes for diverse purposes have been developed (Szücs et al., 2009; Zimic et al., 2010; Shin et al., 2010; Miller et al., 2010). Reproducibility of some designs is limited because they involve the recycling of pieces discarded from obsolete microscopes (Peidle et al., 2009).

In low-resource settings, acquisition of a well-equipped microscope may take years due to funding limitations. Here, we report a design for a low cost, modular, IR-OI and epifluorescence upright microscope using light-emitting diodes (LEDs), a webcam, and ready-to-assemble commercially available parts. We suggest that this microscope is a low-cost alternative for visualized recording for in vitro electrophysiology, fluorescence detection and microfluorometry in low-resource settings. This microscope may reduce the cost of hands-on workshops and may be useful for scientific educators and teachers in topics such as optics, biology and neuroscience.

2. Materials and methods

We designed a modular microscope to satisfy several levels of complexity. Module 1 forms the core of the microscope with a turret, simple optics, IR OI and a single port, with a webcam as the imaging device. Module 1 is suitable for visualized electrophysiology recording. Module 2 upgrades the design to an epifluorescence microscope, incorporating a blue (dominant wavelength 470 nm) high power LED, optics for detection of green fluorescence (516–556 nm) and an additional port for a second imaging/detection device such as a camera or a photodiode. Fluorescence from other wavelengths can be detected by substitution of the light source and filters (data not shown).

We used parts from Thorlabs (Newton, NJ, USA), 80/20 Inc. (Columbia City, IN, USA), Solino c/o Opto GmbH (Germany) and filters from Semrock (Rochester, NY, USA) (see Table A1 in appendix). We used imperial units; however, compatibility with user's existing hardware (e.g., optic table) should be considered. Thorlabs catalog provides same parts in metric units.

Accurate drawings for each module preserving the scale were made in SolidWorks Premium 2012 with imported files from the Thorlabs website.

2.1. Fixed stage

The fixed stage was designed to accommodate an open diamond bath-imaging chamber with a platform (RC-26 and P1 from Warner Instruments). The minimal design required a solid stainless steel Ø 1.5″, 10″ tall post, a Ø 1.5″ mounting post bracket, a Ø 1.5″ post pedestal base adapter, and a clamping fork. A more expensive alternative to the minimal design is the chamber column for Warner Instruments Series 20 recording chamber (Sutter MT-150/W20R, $790.00).

2.2. Module 1. IR OI for visualized recording

Module 1 is a basic upright microscope. An *XYZ* translation stage (PT3/M, Thorlabs, NJ, USA) was used to provide movement over the specimen. The *XYZ* translation stage was mounted on a mounting post bracket, which was fixed to a solid stainless steel Ø 1.5″, 10″ tall post. The PT3/M *XYZ* translation stage provided precise motion over 25 mm (1″) travel range in each axis. The Module 1 microscope was assembled using an objective lens turret (OT1, Thorlabs), a plano-convex lens with a positive focal length (f) of 150 mm (LA1433, Thorlabs), two lens tubes (SM1L30 and SM1L40, Thorlabs) assembled to achieve a distance of 150 mm between the plano-convex lens and the sensor of the imaging device. A webcam (LifeCam Cinema, True 720p, Microsoft) was used as the imaging device (see Fig. A1 and Table A1 in the appendix). All the optical components of the webcam were removed, and the CMOS (complementary metal oxide semiconductor) chip was exposed. The webcam contained a ¼-in. OV9712 imaging chip that offered 720p HD video performance at 30 frames per second with 3 μm × 3 μm pixel size and low-light sensitivity (3.3 V/(lx-s); OmniVision Technologies, Inc., CA, USA). The array size was 3888 μm × 2430 μm with a resolution of 1 megapixel (MP). The camera was inserted into the upper SM1 lens tube at the proper distance for image formation (see Fig. 1). The camera with no optical components fit the SM1 lens tube. A retaining ring was positioned to maintain the CMOS chip in a distance of 150 mm. The port where the webcam was placed is referred to as the upper port.

The following objectives were used: Olympus: UMPLFLN ∞ 20× numerical aperture (NA) 0.5 W; LUMPlanFl ∞ 60× NA 0.90 W. Leica: HCX APO ∞ 40× NA 0.80 W U-V-I; EF 160/– 10× NA 0.25; NPlan ∞ 5× NA 0.12. Nikon: Plan UW ∞ 2× NA 0.06.

IR OI was achieved using an infrared (940 nm) high power LED (M940L2, Thorlabs) pointed toward the specimen from below in an oblique 30° angle with respect to the specimen (modified from Safronov et al., 2007). The LED was enclosed within a lens tube (SM1L20, Thorlabs). The IR light was collimated by an uncoated plano-convex lens with f = 30 mm (LA1805, Thorlabs) (Fig. 1). The distance between the lens and the specimen was ~0.5–1.0 cm. Two-μm latex beads (Life Technologies, NY, USA) were visualized for calibration.

2.3. Module 2. Epifluorescence

A blue LED (peak wavelength 470 nm; M470L2, Thorlabs) was used as the light source for fluorescence visualization (see Figs. 2 and 3). The blue light was collimated by a plano-convex lens with f = 30 mm (LA1805, Thorlabs) and followed by an iris diaphragm (SM1D12D, Thorlabs), an excitation filter (FF01-482/35-25, Semrock) and, a dichroic mirror