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SHORT COMMUNICATION

A SIMPLE AND LOW-COST ILLUMINATION SYSTEM FOR *IN VIVO* FLUORESCENT VISUALIZATION OF NEURONES

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The use of fluorescent dyes for staining neurones has become very common since the introduction of Lucifer Yellow (Stewart, 1978) because it permits high-luminance stainings, for detailed structural analysis, with a minimum of filling time. Lucifer Yellow has been used in a variety of techniques in neurophysiology: *in vitro* visualization (Stewart, 1978); killing of neurones or parts of neurones (e.g. Miller & Selverston, 1979; Miller & Jacobs, 1984); and *in vivo* visualization (e.g. Reichert & Krenz, 1986; Kater & Hadley, 1982). We describe a new system for *in vivo* and *in vitro* visualization. It can be used with a standard dissecting microscope, features a very simple optical design and costs much less than other systems used previously.

The system is constructed around a new medium-pressure mercury arc lamp, type HQI-T150. This lamp was developed by Osram (Osram GmbH, Postfach 900620, D-8000 München 90) for high-intensity spotlight illumination in showrooms. It operates at 220 V a.c. and only requires a choke and a high-voltage starter (parts can be purchased at May & Christe GmbH, Postfach 1120, D-6370 Oberursel/Taunus). The total power consumption of the lamp is 150 W. The size of the luminous spot is 4 mm × 2 mm, and the luminous density is 8000 cd cm^{-2}. The lamp has a lifetime of 6000 h; in comparison, the standard high-pressure mercury lamp (i.e. HBO 100/2) has a maximal lifetime of 1000 h. The HQI-T150 lamp and its accessories are commercially available: a replacement lamp costs about 130 DM; the socket, ignition system and choke assembly are available for approximately 150 DM.

The principle of the optical setup is illustrated in Fig. 1. The light emerging from lamp L is first transformed into a parallel beam using the condensor lens L_1 (planoconvex f = 50 mm, diameter 22 mm). After passing through a filter set and an iris diaphragm, the light is focused by lens L_2 (same type as L_1, f = 40 mm) into a spot L′, which represents an image of the lamp's arc of slightly reduced size. To obtain optimal illumination of a small target neurone, precise positioning of the spot L′ is essential. To achieve this, the light source is first adjusted so that the spot L′ comes within a few millimetres of the target. Then, fine adjustment can be

Key words: Lucifer Yellow, *in vivo* fluorescence, illumination system.

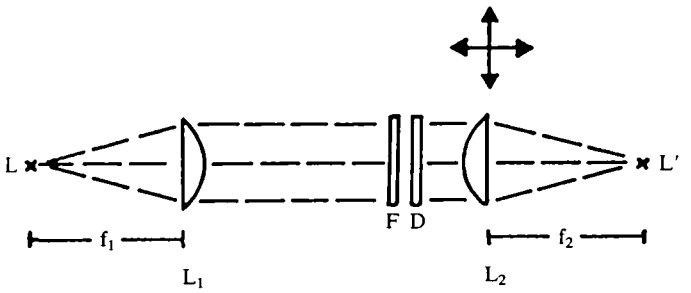


Fig. 1. The principle of the optical setup. A drawing of the centre beam and parallel rim beams. L, lamp; L_1, L_2, lenses; F, filter; D, iris diaphragm; f_1, f_2, focal distances of L_1, L_2, respectively; L', image of lamp L.

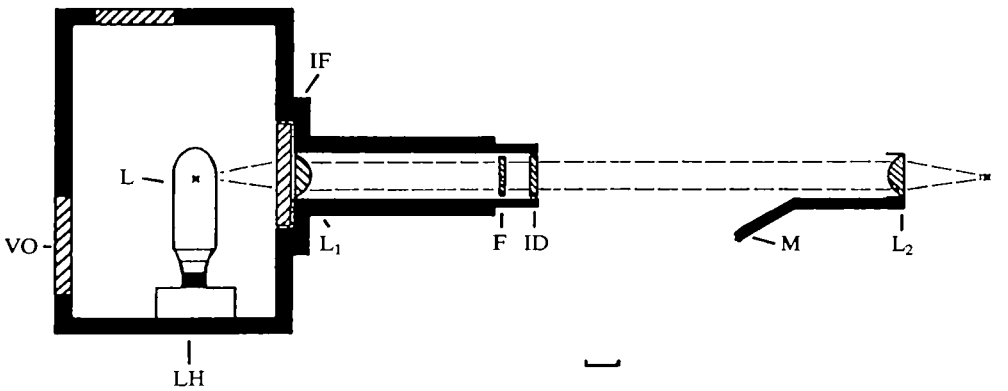
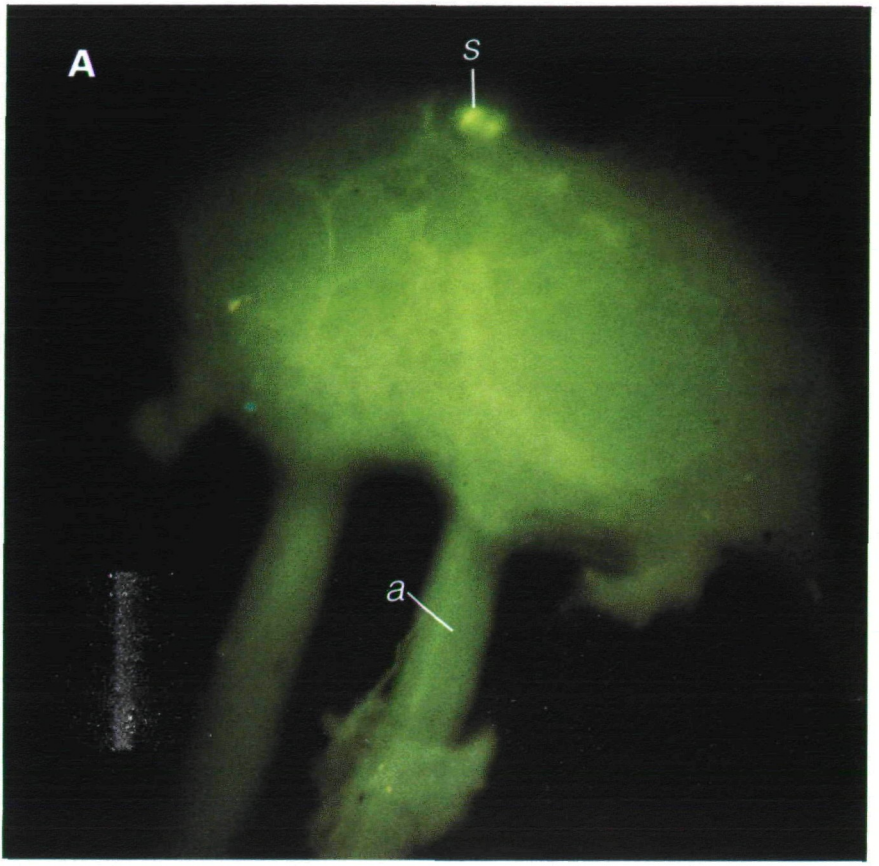


Fig. 2. Scale drawing of the illumination system. LH, lamp housing; VO, ventilation opening; IF, infrared blocking filter; L_1, condensor lens; F, filter; ID, iris diaphragm; M, connection point to xyz manipulator; L_2, focusing lens; scale bar, 2 cm.

achieved by moving L_2, since the spot L' moves with L_2. As long as L_2 is not moved too far out of the axis of the parallel beam, there will only be a small loss of intensity. The advantage of this positioning system is that only a small lightweight xyz-manipulator is necessary to position L_2, whereas the lamp with its housing can be mounted on an ordinary laboratory stand.

A scale drawing of the lamp housing and the focusing and positioning setup is shown in Fig. 2. The lamp housing is constructed from 8 mm thick aluminium plates which are connected with 4 mm screws. It has two ventilation openings, one at the back and one at the top. The openings are provided with blackened light baffles. The lamp HQI-T150 is fixed in a special china socket (supplied by Bender & Wirth, Elektrotechnische Fabrik, Postfach 1127, 5883 Kierste 1). The lenses and the iris diaphragm (all 25 mm outer diameter) are components of an optical kit (Spindler & Hoyer GmbH & Co., Königsallee 23, Postfach 3353, D-3400 Göttingen). The optical filter (BG 1, d = 2 mm) was bought in the 50 mm × 50 mm



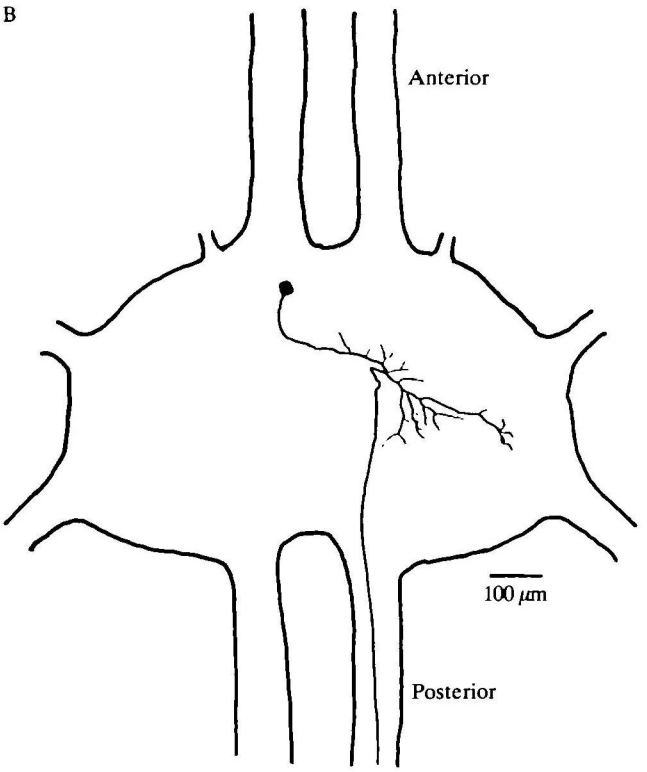


Fig. 3

standard size (Schott Glaswerke, Geschäftsbereich Optik, Postfach 2480, D-6500 Mainz) and then cut and ground to a circular disc 22 mm in diameter in an optician's shop. The filter was mounted in a component holder of the Spindler & Hoyer kit. The filter BG 1 also works as a blocking filter against ultraviolet rays. Between the lamp and lens L_1 an infrared blocking filter is fixed to avoid thermal damage of the lens and the following filter.

To mount L_1, the filters and the diaphragm, a brass tube of 25 mm inner diameter is used. It is provided with a longitudinal slit permitting adjustment of the lens and fixation by slight compression with a hose clamp. Lens L_2 is epoxy-glued to a 5 mm diameter brass rod which is clamped into the micromanipulator. The lamp housing is mounted in the electrophysiological recording setup such that it illuminates the object (e.g. an invertebrate ganglion) obliquely from the side or behind at an angle of 40–70°. The object is then viewed as usual through a dissecting microscope mounted above the preparation. Thus, the normal recording technique is not affected.

The spot size of the light source was 5 mm × 2 mm; the beam intensity was measured using a Coherent radiation power meter 210. With the BG 1 filter in place, the transmitted power density was 330 mW cm^{-2}. To investigate *in vivo* fluorescence, a Carl Zeiss dissecting microscope type IV (or type DR) was used. The appropriate blocking filters (Schott GG495 or Zeiss green interference-filter) were mounted directly in front of the objective of the dissecting microscope. Interneurones in the mesothoracic ganglion of *Carausius morosus* were impaled with 70 MΩ microelectrodes filled with 4% Lucifer Yellow. The neurones were stained for 15 min at −6 nA. Although the ganglia of *Carausius* are rather turbid, the soma and primary neurites of the neurones could be seen even with a reduced opening of the diaphragm (see Fig. 3).

When the ganglion had been excised, fixed and cleared using methylsalicylate, all smaller stained ramifications could be seen using the Zeiss DR dissecting microscope with a 6·3× objective.

The performance of our light source is certainly lower than that of a high-pressure mercury arc lamp such as HBO 100/2 or a laser, especially in power density. However, if it is intended not to kill the cell, the performance is adequate. The reduced costs in setting up and operating the system may help to make the benefits of fluorescence staining available to a large number of investigators.

We are grateful to Mr H. Birkhofer, Osram AG München, for providing us with a prototype of the HQI-T150 lamp. We are also indebted to the companies Bender

Fig. 3. (A) A photograph of a mesothoracic ganglion of *Carausius morosus in vivo*. One can see the somata (*s*) and the axons (*a*) of two descending interneurones. Since the axons run close together they are not separated in this overview photograph. These interneurones receive inhibitory input from the femoral chordotonal organ (for details, see Büschges, 1989). The white structures in the ganglion are caused by autofluorescing tracheae. This photograph was made using a Zeiss DR dissecting microscope and the lamp system described in the text. Magnification ×100. (B) *Camera lucida* drawing of one of the two interneurones.

& Wirth (Kierste) and May & Christe (Oberursel/Taunus) for the donation of a socket, ignition system and choke assembly.

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