

Technical methods

Improved immunofluorescence obtained with a tungsten halogen lamp in a modified inverted microscope

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The potential value of an iodine quartz lamp for use in a fluorescence microscope intended for immunofluorescence studies has been known for several years but development for this purpose has been restricted, mainly because suitable optical filters required for use with it have not been available. The fluorescence microscope described here incorporates an iodine quartz lamp with a larger filament than usual. Moreover, a high proportion of the available

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light is used. This results in brighter fluorescent images being observed.

The fluorochrome most commonly used for immunofluorescence studies is fluorescein isothiocyanate (FITC) and is therefore the one considered here.

Young and Armstrong (1967) reported on the usefulness and convenience of the 100 watt iodine quartz lamp for fluorescence microscopy. Tomlinson (1970a, 1970b) discussed this lamp and filters required to be used with it for the excitation of fluorescein isothiocyanate. A satisfactory all-dielectric interference filter for the excitation of FITC has been developed by Rygaard and Olsen (1969, 1971), and is now commercially reproducible. Improved fluorescence emission is obtainable with the 100 watt iodine quartz lamp and such a filter. Further improvements in the image are obtained with a Union MiC Bi inverted microscope which has been adapted for fluorescence microscopy. Figure 1

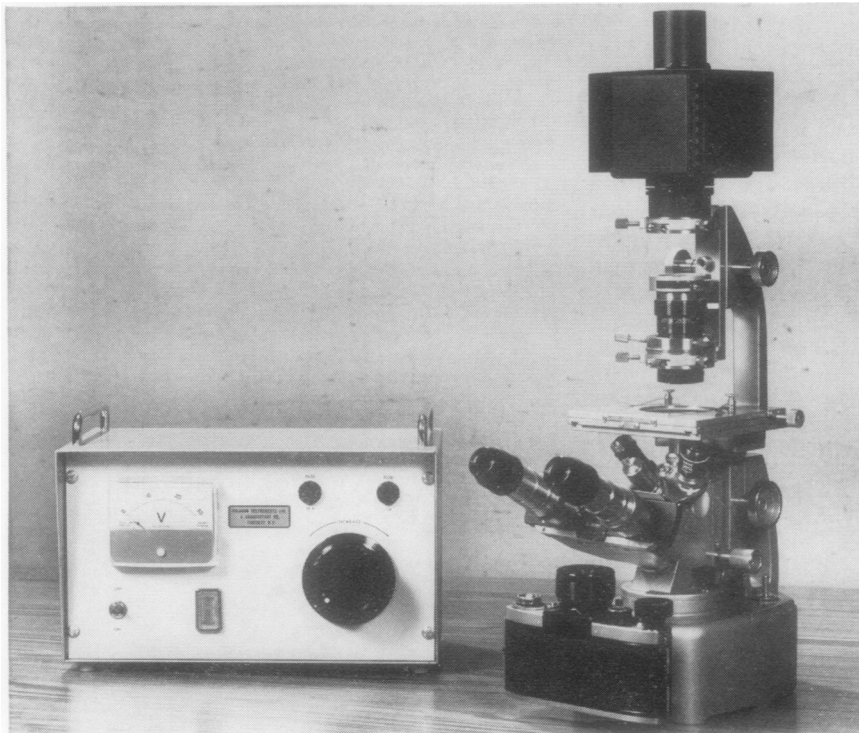


Fig. 1 *The apparatus.*

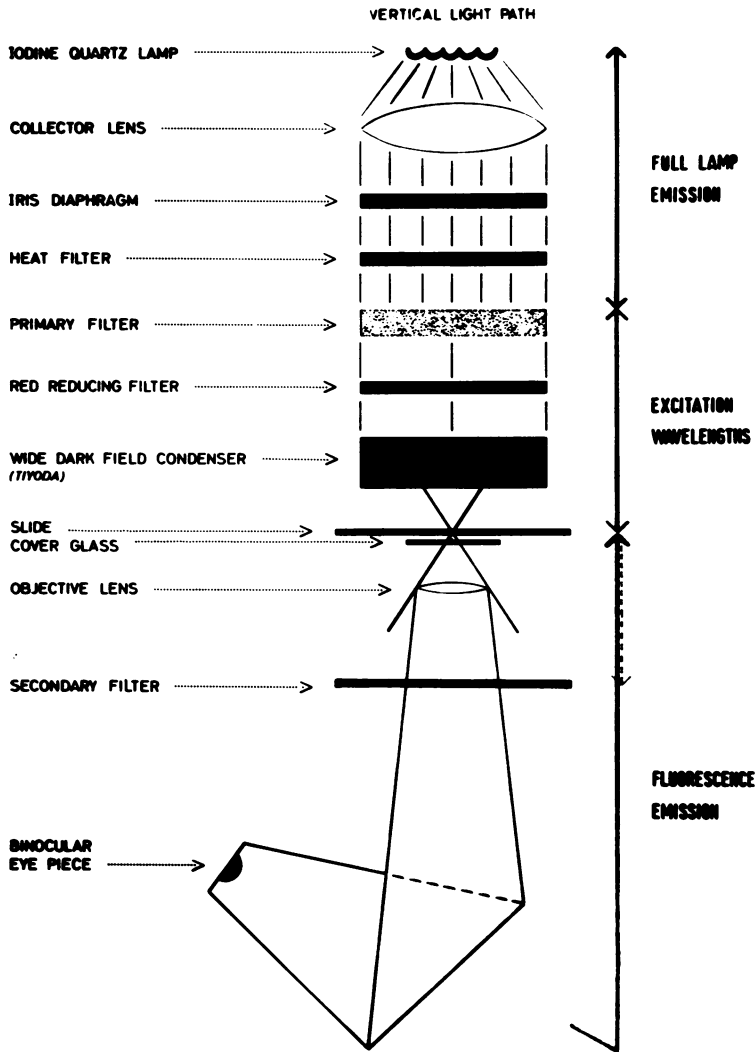


Fig. 2 Light path of a Union inverted microscope adapted for immunofluorescence studies.

shows the microscope, the power supply unit, and camera. The light shield has been removed from the front of the lamp housing to reveal adjustable components.

Figure 2 shows a diagram of the light path through the components of the microscope which does not require a mirror in the light path. Controls for obtaining precise vertical optical alignment are conveniently to hand. Goldman (1968) emphasized the importance of correct optical alignment and the precautions to be taken with the mirror if this forms part of the microscope.

This microscope has been used for experimental and routine immunofluorescence work for the past two years. Optical efficiency and cost (initial,

upkeep, and maintenance) compare favourably with the equipment described by Lidwell, Taylor, Clark, and Heimer (1967).

Photomicrography is simple, and bright coloured transparencies of immunofluorescing objects are obtainable with only a few seconds' exposure.

The essential components introduced into the inverted microscope, and its operation for the purpose of immunofluorescence studies, are described and briefly discussed under the following headings: (1) illuminator, (2) light filters, (3) dark-field condenser, (4) optical alignment, (5) fluorescence photomicrography.

Procedures 4 and 5 are supplementary to those found in the manufacturers' instruction manual

accompanying the basic microscope. This microscope and the components required for fluorescence microscopy are available from Polaron Equipment Ltd, 4 Shakespeare Road, London, N3 1XH.

1 Illuminator

Characteristics of the lamp, which was supplied by Thorn Lighting Limited, Thorn House, London, WC2, include:

Type	Tungsten halogen A1/216
Watts	150
Volts	24
Light output at 24 volts	5,000 lumens
Life	50 hours
Colour temperature	3,400°K
Lighted length of the filament	5.3 mm

Unlike the high pressure mercury vapour lamp, a warming-up period is not required, so that maximum illumination is immediately available. The lamp may be repeatedly switched on and off without having to wait for it to cool. Variable intensity of illumination is controlled from the power supply unit seen in Figure 1. The life of the lamp can be extended by reducing the voltage supply to an acceptable limit. Twenty-four volts is the designed operating voltage. Although the lamp is commonly referred to as iodine quartz, bromine has now replaced iodine as the halogen of choice. All lamps of this type are now usually grouped under the heading 'tungsten halogen lamps'. Figure 3 shows its spectral energy distribution curve. The lamp is fixed horizontally in its housing. A comparison between a new 100 watt 12 volt and a new 150 watt 24 volt iodine quartz lamp has been made at 12 and 24 volts respectively by measuring immunofluorescence emission from single bacilli by

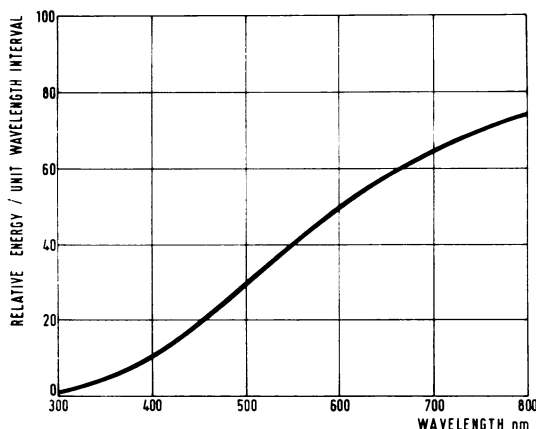


Fig. 3 Spectral energy distribution curve for tungsten halogen lamps 12 v/100 w and 24 v/150 w.

means of the photometric equipment described by Taylor, Heimer, and Lidwell (1971). With the 150 watt lamp, emission of 7.4 arbitrary units was obtained compared with 3.6 arbitrary units with the 100 watt lamp. These measurements are the mean of 10 readings, each of which was made from a fresh microscopical field.

The lamp housing, which is conveniently sited above the level of the microscopist's head is seen in Figure 4. Heat generated by the lamp is convected upwards through the chimney without the aid of a fan.

2 Light Filters

The following filter system is used: (a) Schott KG1 toughened glass heat filter 3 mm thick and 30 mm in diameter fitted in the lamp housing; (b) primary FITC all-dielectric interference filter Olsen no. 216¹. As this excitation filter also reflects heat it reinforces (a). For the purpose of cleaning, the interference filter, which has glass surfaces, should be treated with the same care as an ordinary lens.

(c) Schott BG38, 2, 3, or 4 mm thick, 30 mm in diameter. A choice of one or more of these is used to reduce the amount of red transmission which occurs in the region of 630 nm (Figure 5). Different intensities of contrasting red light are therefore available in the image plane to illuminate the background if desired.

(d) The secondary filter obtained with its transmission characteristics from H. V. Skan Ltd, Solihull, Warwickshire, England, is made from a specially selected piece of Schott coloured glass OG530 3 mm thick (525-530 nm). With a standard Schott OG530 filter, the blocking point at 50% transmission may be anywhere between 523 and 537 nm. For more precise filtering and better results, a filter transmitting to within 2 nm of the required wavelength may be obtained on request. Rygaard and Olsen (1971) emphasize the importance of properly matching the secondary filter to their primary FITC interference filter so as totally to exclude light in the excitation waveband, thus allowing maximum contrast for the fluorescent image. A suitable secondary filter is recommended for use with each primary filter as some variation in characteristics occurs between individual primary interference filters.

Figure 5 shows transmission curves of a pair of primary and secondary filters as well as the positions of maximum absorption of FITC (495 nm) and of fluorescence emission (525 nm).

¹Improved primary FITC all-dielectric interference filters with and without red transmission are manufactured by Optisk Laboratorium, Lundtoftevej 100, 2800 Lyngby, Denmark.

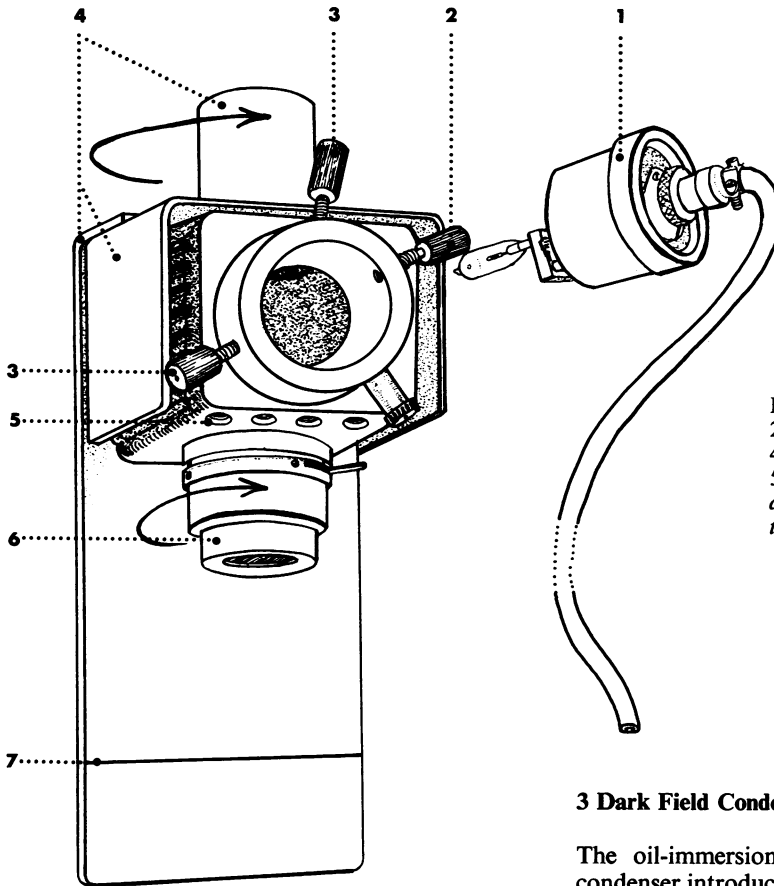


Fig. 4 1 adjustable lamp holder, 2 screw clamp, 3 aligning screws, 4 light shield with chimney vent, 5 air intake holes, 6 collector lens and iris diaphragm, 7 position of toric lens on Tiyoda condenser.

3 Dark Field Condenser

The oil-immersion Tiyoda super-wide dark field condenser introduced into the light path (Figs 1 and 2) has been described and recommended for immunofluorescence microscopy by Nairn (1969) and Taylor (1970). This component, which incorporates a toric lens, is more efficient than the conventional cardioid dark field condenser as it accepts and utilizes most of the available light. Another feature is the wide field illumination observed with a range of low-to-high-power objectives. Adjusting screws in the condenser mount are available for manoeuvring it in the light path.

4 Optical Alignment

PROCEDURE FOR ADJUSTING THE LAMP FILAMENT TO THE OPTICAL AXIS

This is shown in Figure 4.

(a) Switch on power unit and adjust to approximately 10 volts to illuminate the lamp filament.

(b) Close iris diaphragm (6).

(c) Place a suitable piece of white card in position of toric lens of Tiyoda condenser (7).

(d) Place lamp and lampholder (1) into the lamp house so that the filament lies approximately across

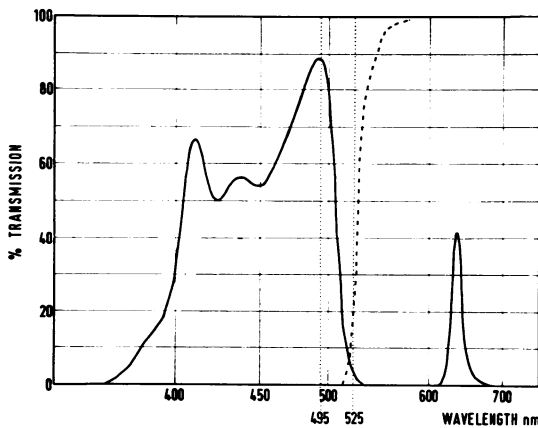


Fig. 5 Transmission curves of primary and secondary filters

the centre of the collector lens. Adjust (1) so that a flat image of the filament falls on to the white card.

(e) Tighten (2).

(f) Focus image of lamp filament by rotating (6).

(g) Fix lamp house on the microscope by means of the adjustable ring mount.

(h) Place pin hole component in adjustable ring mount in place of the dark field condenser.

(i) Increase the voltage.

(j) Adjust position of the lamp until image of filament falls approximately across the centre of the pin hole component.

(k) Place suitable oculars in the microscope.

(l) Focus the $\times 10$ objective onto the outline of the pinhole.

(m) Adjust pinhole component to the centre of the field. The central position is readily found with the aid of a 35 mm photofinder in an ocular.

(n) Rack up the lamp house and adjust its ring mount to centre the illuminated area.

(o) Open the iris diaphragm (6) and replace the pin hole component with the dark field condenser.

(p) With a low-power objective adjust the condenser to bring it centrally in the light path while viewing a preparation.

(q) Increase the voltage up to 24 volts for the required intensity of light.

5 Fluorescence Photomicrography and Colour Transparencies

In this system light from a fluorescent object is not split for the purpose of focusing and photographic exposure. The fluorescent image is first viewed in the mirror of the 35 mm Mirax Laborec reflex camera (Fig. 1) and then the film is exposed to all the light emitted from the object.

The iodine quartz lamp provides a stable source of excitation and the fading rate of microscopical images is considerably less than with a mercury vapour lamp (Taylor *et al.*, 1971). Estimated exposure times based on records of photographic data may be used. For example, the following results have been obtained without an exposure meter and may be used as a guide.

SERIES 1

Preparation

Escherichia coli type 026 in faeces stained by the direct method with conjugate diluted as for routine use.

Film

Kodak high speed ektachrome daylight type ASA 160.

Developing time

Standard.

BG38 Filter (mm thick)	Objective	Satisfactory Exposure (sec)
4	$\times 40$	10
9	$\times 40$	20
4	$\times 100$	10
9	$\times 100$	20

SERIES 2

Preparation

Epstein-Barr virus infected cells stained by the indirect method with anti-IgG conjugate diluted as for routine use.

Film

Ansochrome colour slide daylight type ASA 500.

Developing time

Standard.

BG38 Filter (mm thick)	Objective	Satisfactory Exposure (sec)
4	$\times 40$	2
9	$\times 40$	4
4	$\times 60$	2
9	$\times 60$	4
9	$\times 10$	2

The exposure times may be halved or doubled so as to provide a suitable range of exposure for photographing a selected field.

Remarks

A prominent red contrasting background is obtained with the 4 mm BG38 filter and a much darker background with the 9 mm filter. The $\times 100$ objective is an oil immersion type with an iris diaphragm for adjusting the numerical aperture.

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