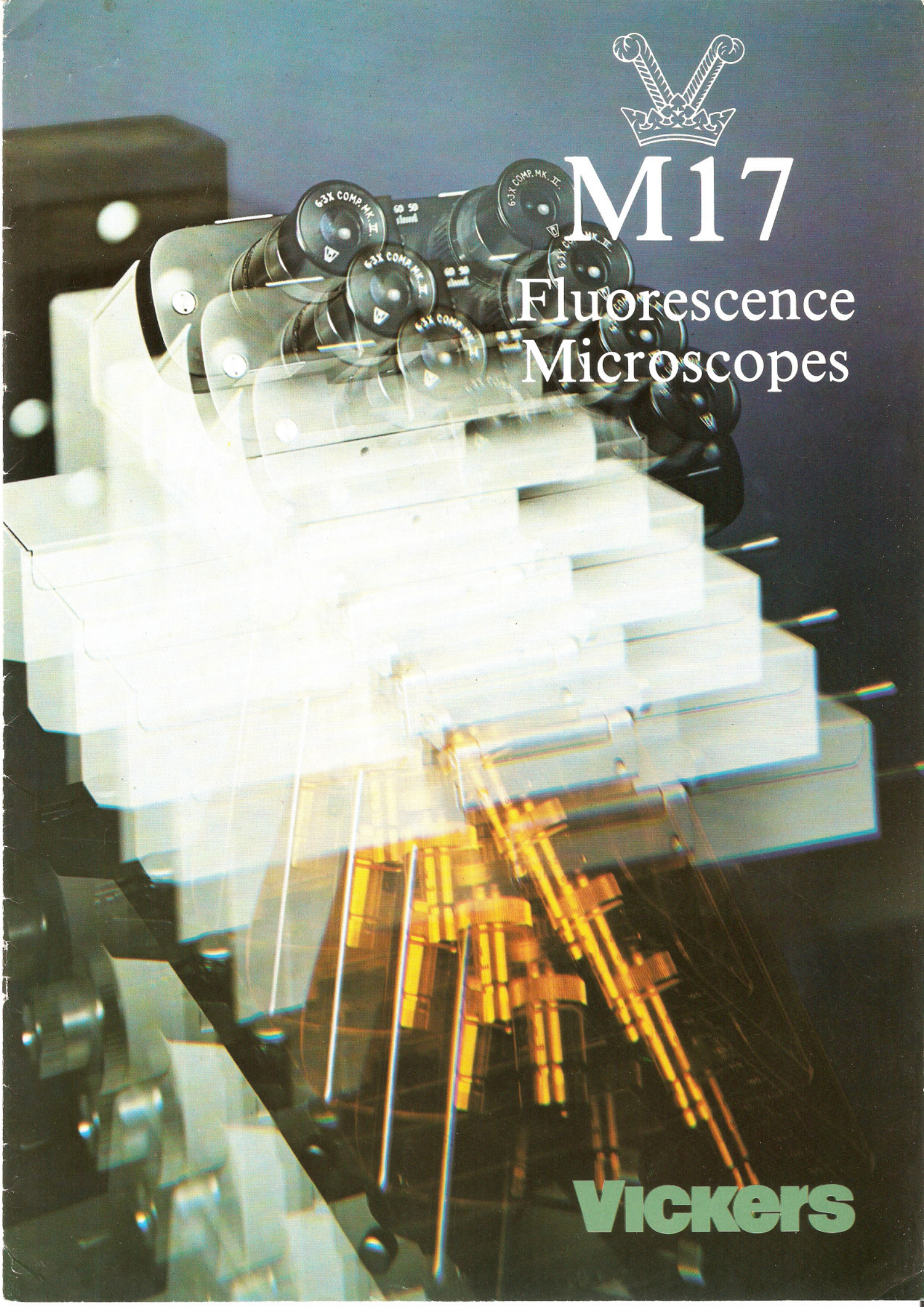


M17

Fluorescence Microscopes

Vickers

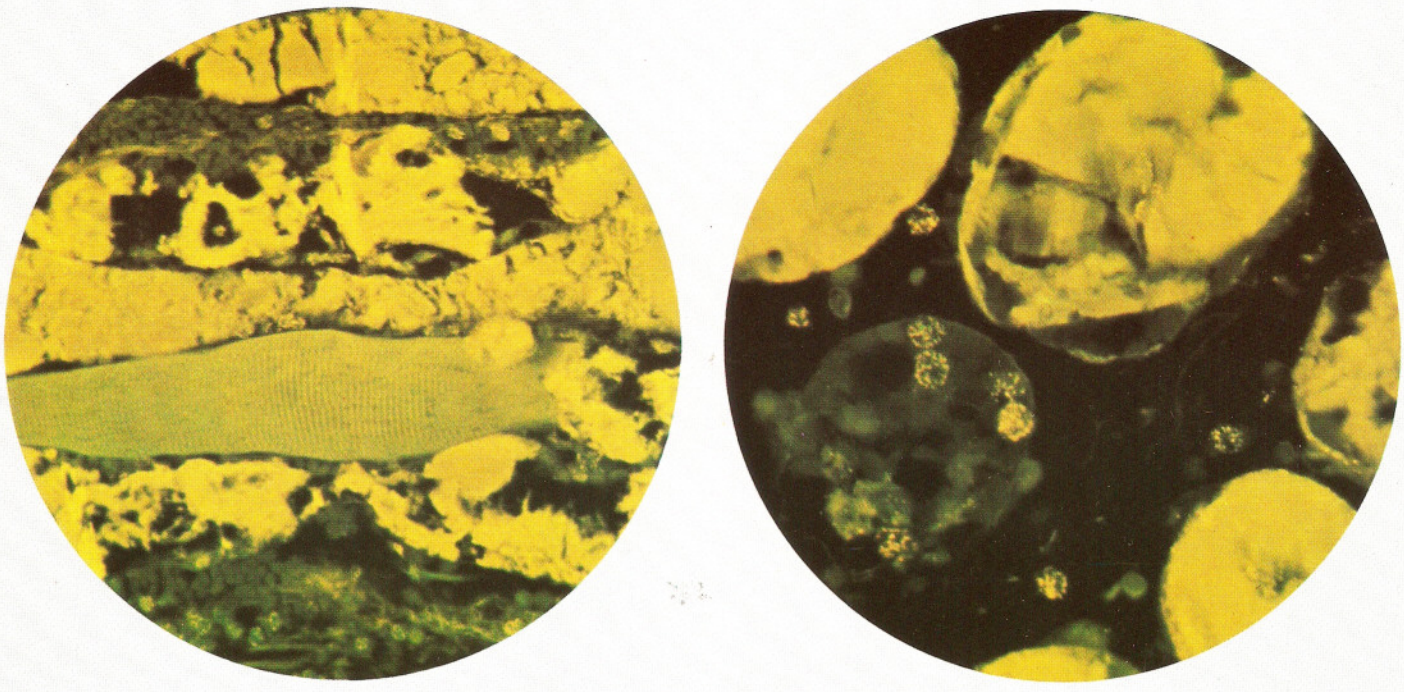
Vickers

M17
Fluorescence Microscopes

Incident light fluorescence microscopy has largely been developed on the basis of work by Dr. J. S. Ploem of the University of Leiden. It offers great advantages over traditional transmitted light methods and the Vickers M17 microscope now incorporates facilities for incident fluorescence work at four different wavelengths.

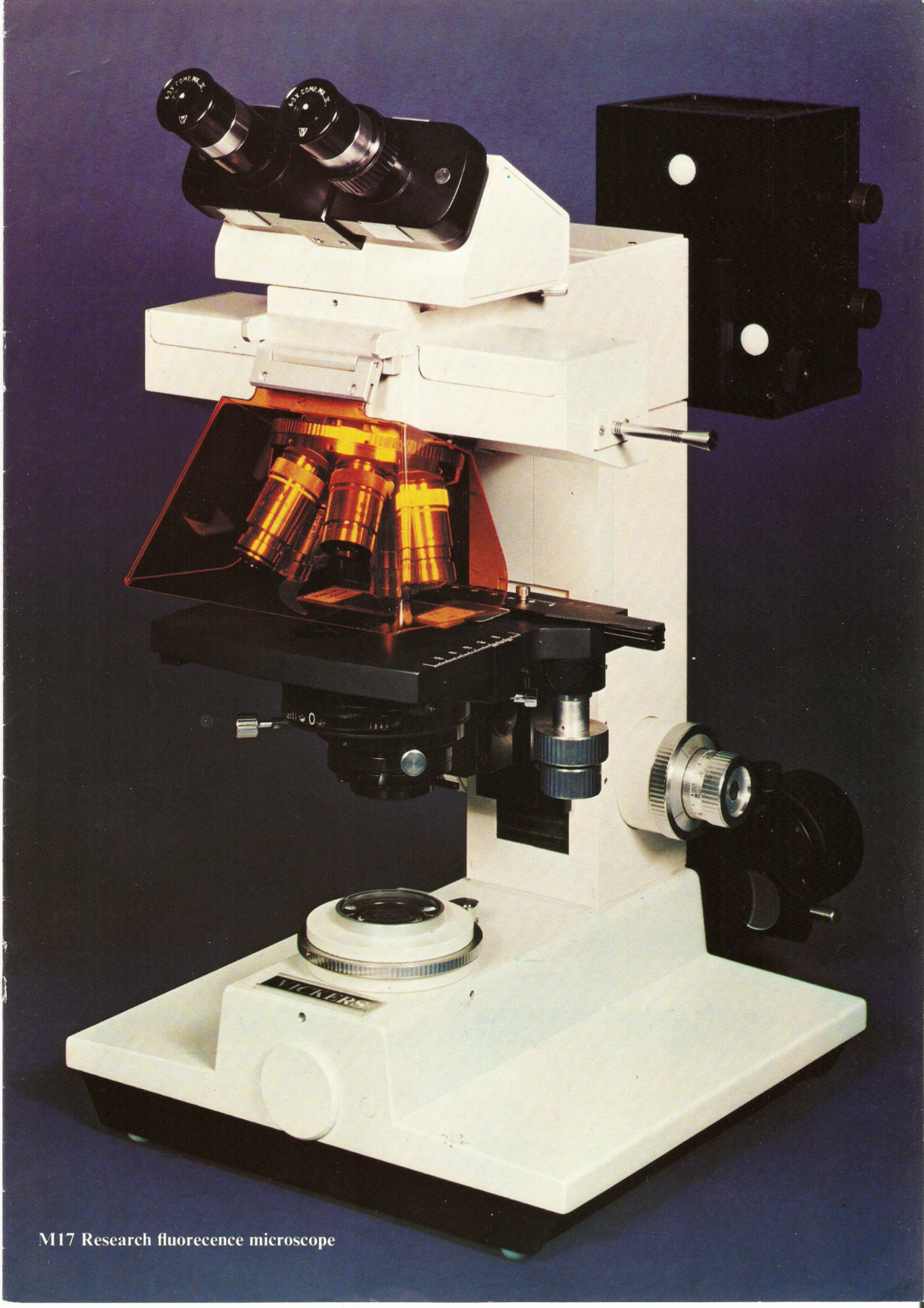
We believe that a high proportion of work in the fluorescence field can be done with just four standard sets, each comprising exciter filter, dichroic reflector and barrier filter. The sets provided cover U.V., violet, blue and green excitation. Moreover, we think that the great majority of users will welcome this restriction on the variety of filters and dichroic reflectors. The user whose work demands the use of other filters is still able to change them with ease. The dichroic reflectors are factory adjusted and are not interchangeable.

Despite the advantages of incident methods in fluorescence microscopy, there is still a place for transmitted light excitation. Vickers have a near ideal instrument for this purpose in the M17 with 0.8X corrector lens. Images are around 50% brighter than with a unit power microscope.



Necrotic muscular fibres stained with procion yellow

(Mr. J. J. Fulthorpe, Muscular Dystrophy Group Research Laboratories, Newcastle General Hospital)



M17 Research fluorecence microscope

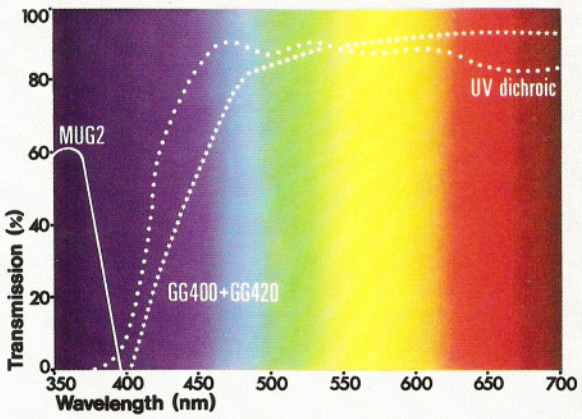
Microscope stands

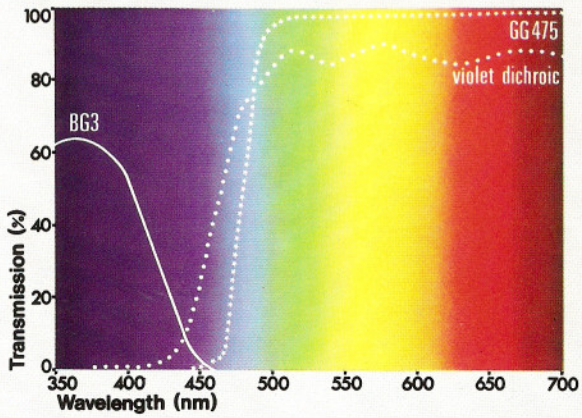
Incident light—research

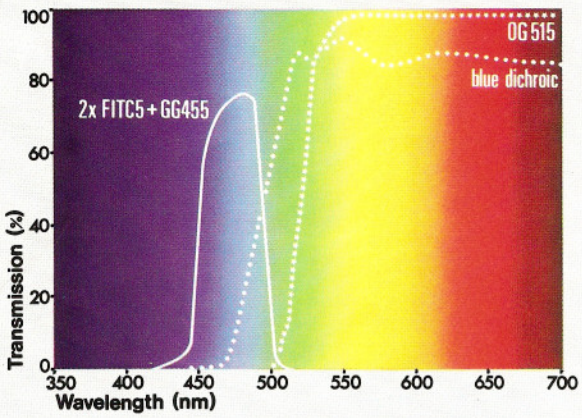
Four sets of filters, each comprising exciter filter, dichroic reflector and barrier filter are mounted on a slide in the microscope head. Thus, in general, ultra-violet, violet, blue (including F.I.T.C.) and green (including T.R.I.T.C.) excitation can be employed merely by use of a push-bar control.

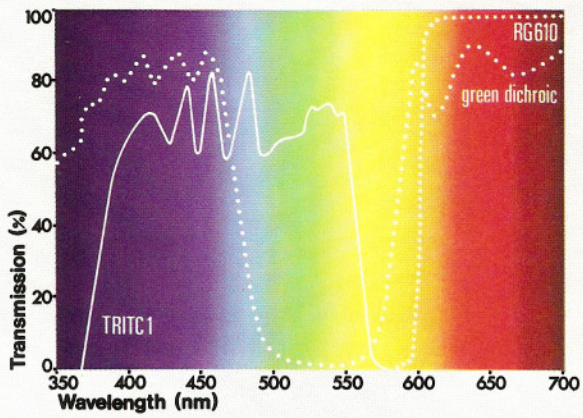
Two models are provided. Model 1 is for general use with a variety of fluorochromes; model 2 has filter sets modified specially for the F.I.T.C.—rhodamine double staining technique. Since the F.I.T.C. and T.R.I.T.C. filter sets occupy neighbouring positions on the slide, it is possible to flick rapidly between the positions, retaining a visual memory of the fluorescence visible in each.

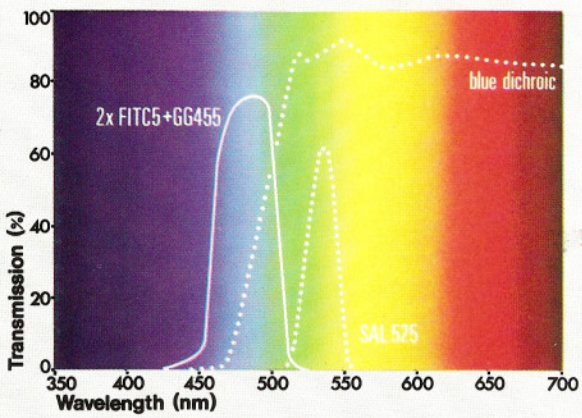
With these two models, the research incident light instruments are ideal for the laboratory where a wide range of specimens is encountered and/or where double-staining techniques are in use. If desired, the user can quickly replace any of the exciter or barrier filters of the standard sets by different filters appropriate to his particular work.

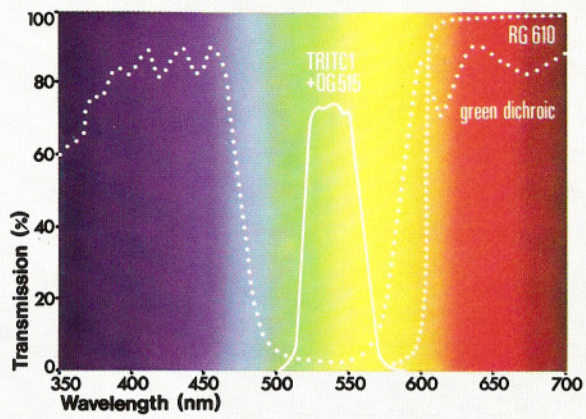


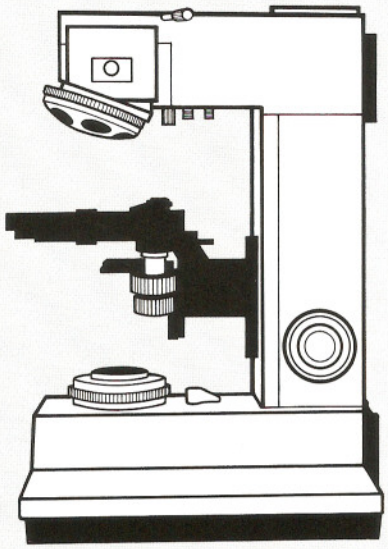




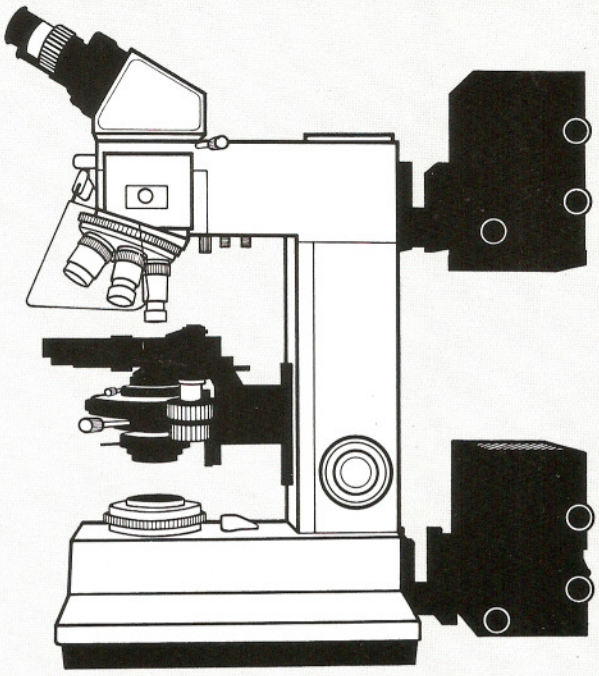








M171070 or M171075 basic stand for research fluorescence microscope

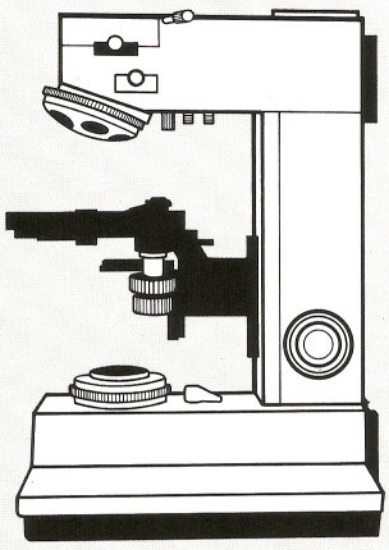


Complete research fluorescence microscope

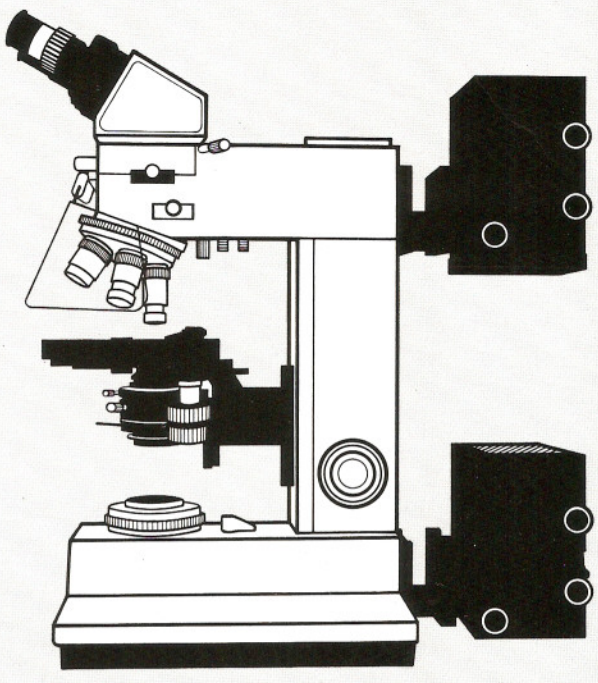
Incident light—routine

This microscope is intended primarily for routine F.I.T.C. investigations. The single dichroic reflector designed for F.I.T.C. work and the exciter filter combination are carried in separate sliding mounts, in which they can be removed from the light path. Slide mounted OG515 and OG530 barrier filters are carried in a slot above the dichroic reflector. Alternatively, the OG515 filter can be supplied in a four position slide with other filters for "white light" use in transmitted light.

The 12V 100W tungsten halogen lamp is the usual choice of light source for this instrument although a mercury vapour lamp can be supplied if required.



M171080 basic stand for routine fluorescence microscope

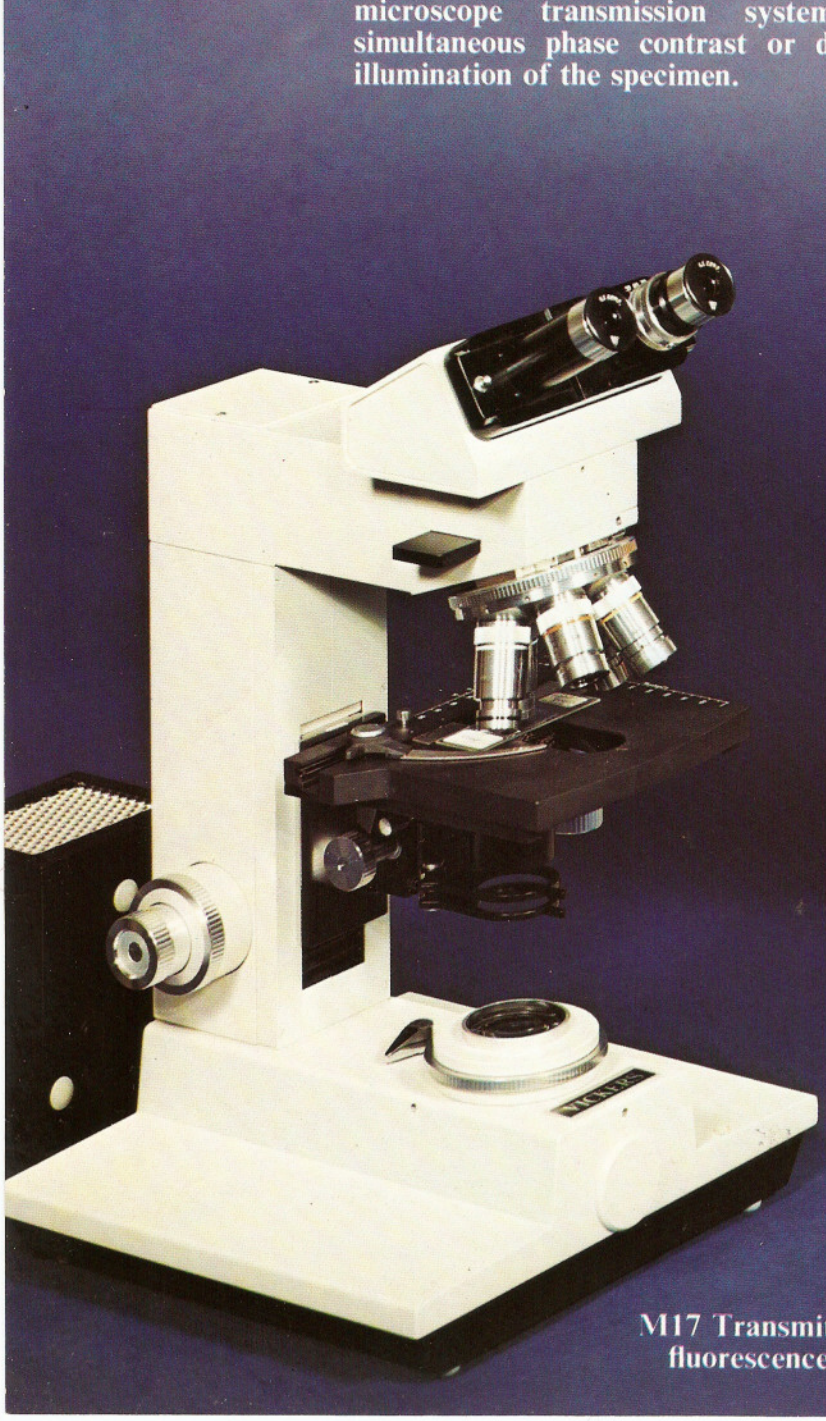


Complete routine fluorescence microscope

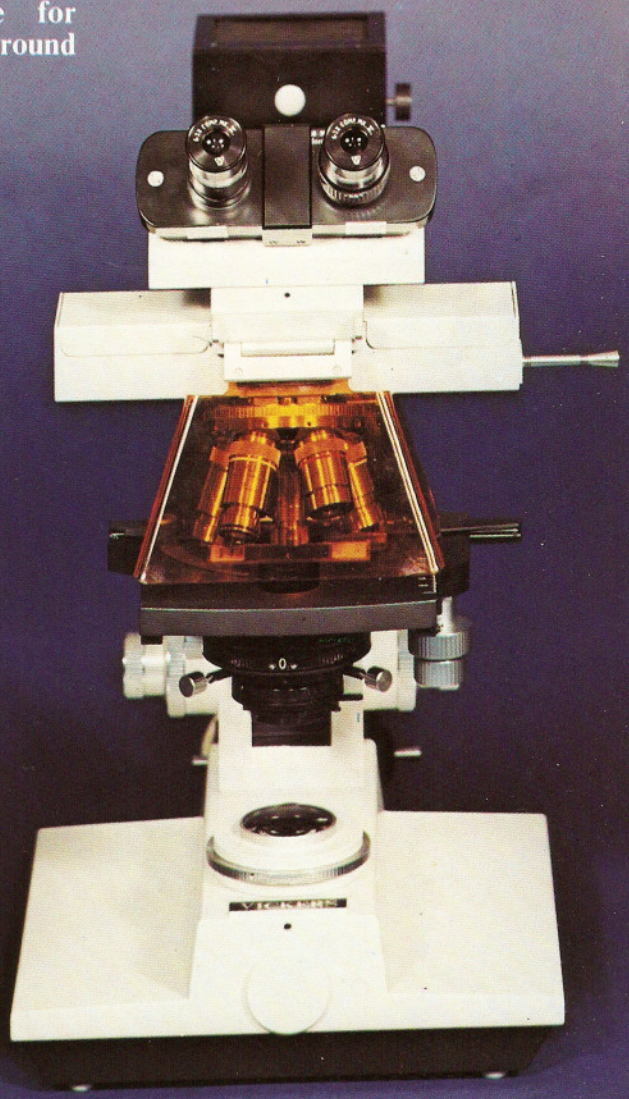
M17 Fluorescence

Incident light fluorescence illumination offers many advantages.

- Incident light excites fluorescence in or near the surface of the specimen —fluorescence not reduced by passage through the specimen.
- The objective acts as its own condenser making high N.A. relative to magnification very advantageous.
- Stray exciting light travels away from the observer's eye, giving a darker background with less secondary filtration.
- The dichroic reflector, throwing light down on to the specimen, serves as an exciter filter and supplements ordinary transmission filters.
- Exciter filters, dichroic reflectors and barrier filters for four different wavelengths of excitation and emission can be combined in one slide, allowing instant change from one wavelength to another.
- Incident fluorescence excitation leaves the microscope transmission system free for simultaneous phase contrast or dark ground illumination of the specimen.



M17 Transmitted fluorescence microscope

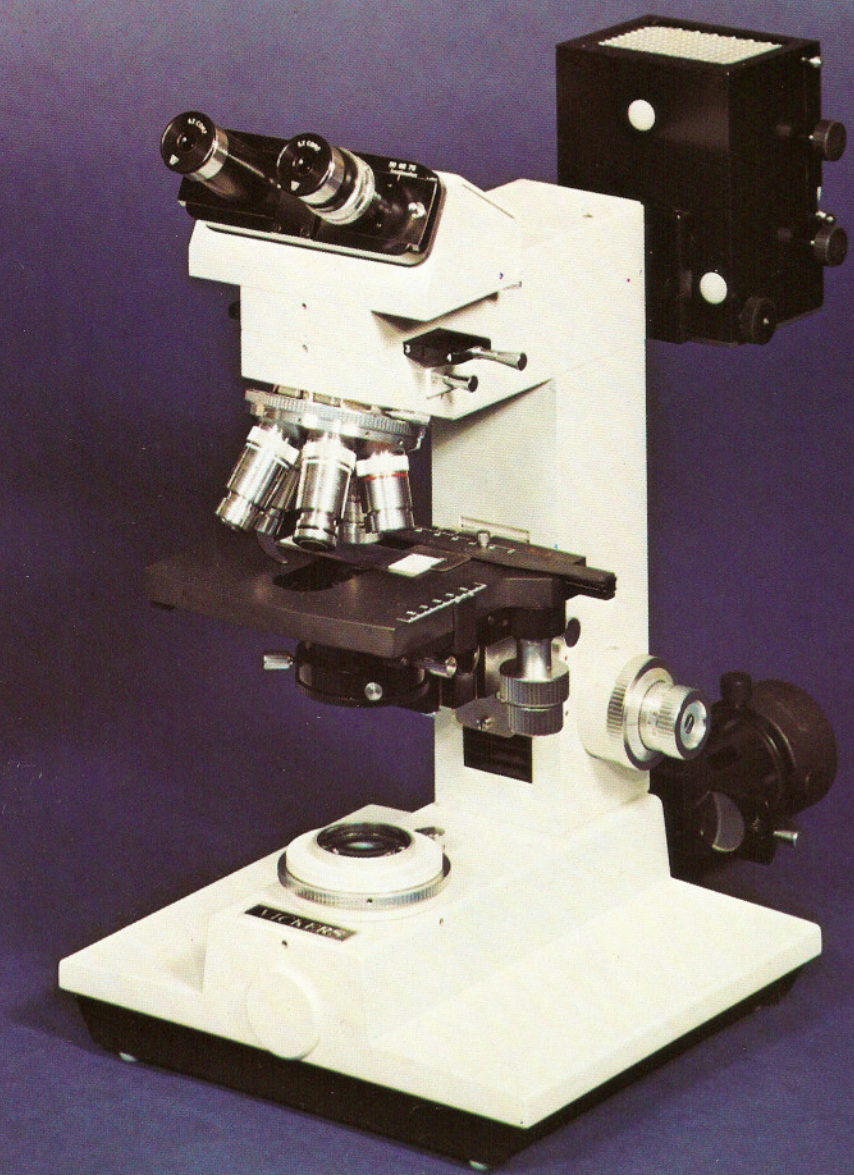


M17 Research fluorescence microscope

Microscopes

Transmitted light fluorescence illumination still has its uses and the M17 system is outstandingly efficient.

- The primary magnification of this model of the M17 is 0.8X, and it gives fluorescence over 50% brighter than with unit power instruments.
- A variety of exciter or barrier filters can quickly be interchanged, providing a very flexible instrument.
- Use of the dark ground condenser prevents direct exciting light reaching the objective and avoids loss of brightness by heavy barrier filtration.
- The system is well suited for use where a proportion of time on the instrument is devoted to other than fluorescence work.



M17 Routine
fluorescence microscope

Vickers

Microscope stands

Transmitted light — extra wide field

This is the fluorescence version of the M17 extra wide field biological microscope. By use of a suitable corrector lens system in the upper limb, the effective magnification of each objective is reduced by a factor of 0.8X, the field of view it covers increased by 1.25X. Thus, using eyepieces with field of view number 20, the microscope gives a 25 mm field of view.

Such a microscope is particularly suitable for fluorescence work as the image brightness varies inversely as the square of the magnification, i.e. it is $1/0.8^2$, or more than 50% greater than with a unit power instrument.

The light sources are the 100W tungsten halogen lamp or the 200W mercury vapour lamp which necessitates use of the riser plate for the microscope.

Exciter filters

Both coloured glass filters and interference filters are supplied 32 mm diameter for use in a substage unit having two swing-out filter trays.

Suppressor filters

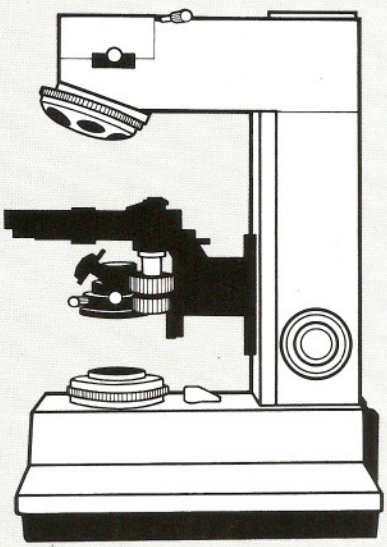
These are supplied 40 mm diameter and held with spring rings in metal mounts provided with a handle. They are carried either in a filter holder between the lamp and the back of the microscope limb, or in a substage filter holder on the miscroscope base. The former holder takes three and the latter takes two, mounted filters.

Colour contrast filters

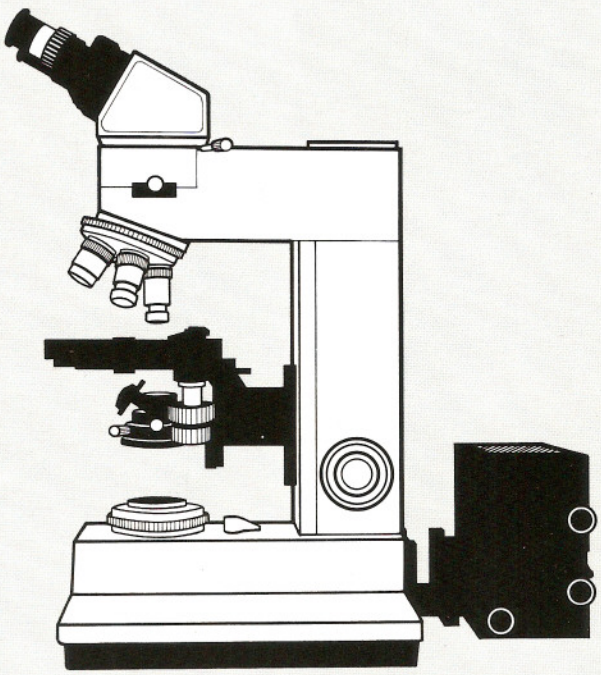
Colour contrast filters for ordinary microscopy are supplied mounted similarly to the suppression filters.

Barrier filters

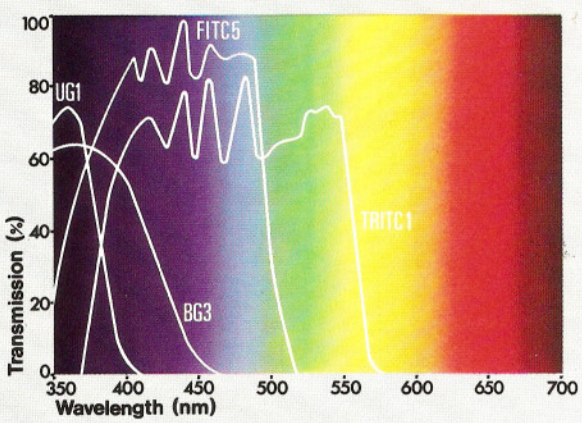
Up to four 19mm diameter barrier filters can be supplied in one slide. The slide fits in a slot in the microscope head and indexes in four positions.



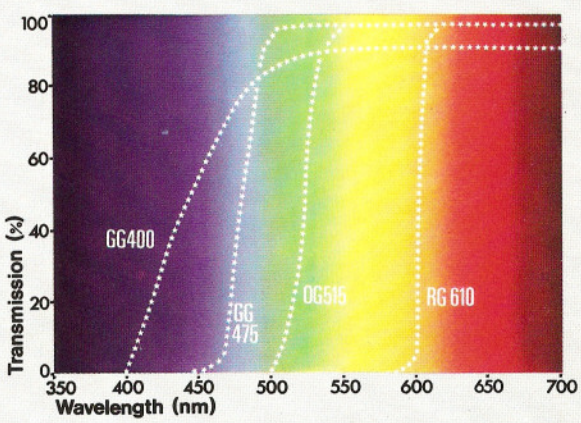
M171010 basic stand for transmitted fluorescence microscope



Complete transmitted fluorescence microscope



Exciter filters



Barrier filters

Interchangeable components

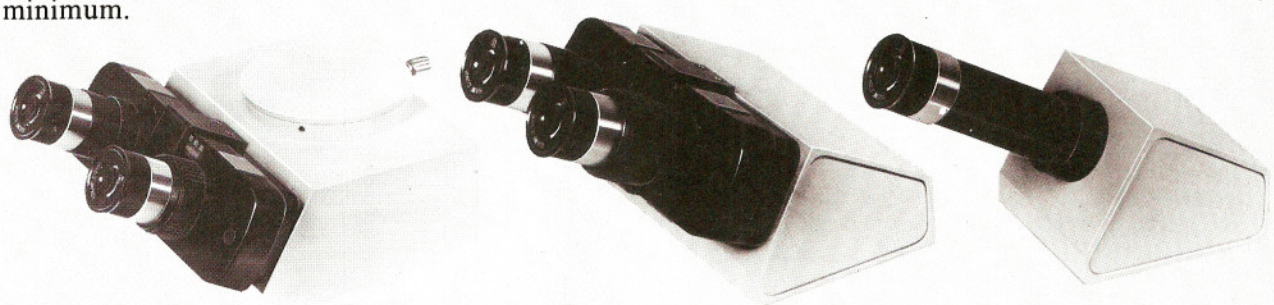
Viewing heads

Inclined eyetubes are at 30° to the horizontal, giving greatly increased comfort in use as compared with the traditional 45°.

Interocular separation is variable from 52 to 74 mm.

The reflector which splits the light between the two eyetubes has a high-efficiency interference coating so that light loss is kept down to the minimum.

The M171900 photovisual head and the M172120 binocular head have a compensating mechanism which maintains tube length and magnification constant, when interocular separation is altered. If the brightest possible image is more important than comfort in use, then use of the M172000 inclined monocular head might be considered.



M171900 Photovisual binocular head

M172120 Compensating binocular head

M172000 Monocular head

Objectives and Eyepieces

In choosing objectives for fluorescence work, where image brightness may be low, the aim should be a relatively high numerical aperture relative to magnification. This is especially valuable when using incident fluorescence illumination.

Similarly, to obtain as bright images as possible, low eyepiece magnification helps and a pair of M071121 6.3X compensating eyepieces should be used.

Condensers (for transmitted light background to incident light fluorescence)

The Vickers range of condensers is available for use in the transmitted light path simultaneously with fluorescence illumination in the incident path. The M410975 phase condenser and M150970 dark ground condenser are particularly useful for background illumination of the specimen and identification of a fluorescing structure.

For non-fluorescence transmitted light use of the research incident fluorescence microscope, the M152970 1.25 N.A. achromatic condenser in centring mount is very suitable.

M150970 Tiyoda condenser

M410975 Research phase condenser

M152970 Achromatic condenser

Condensers (transmitted light fluorescence)

The M17 transmitted light fluorescence microscope takes the normal Vickers range of condensers. If a bright field condenser is needed, the best choice is the M174900 achromatic condenser with swing-out top lens, which fills the 25 mm field at the lowest objective power ($2\frac{1}{2}$X).

There are strong reasons, however, for using a dark ground condenser in transmitted light fluorescence work, and the M150970 Tiyoda condenser is offered. In such condensers, a hollow cone of illuminating light is arranged to have an angle greater than can be accepted by the objective. A less dense barrier filter is then adequate to remove scattered light of the excitation wavelength than would be necessary to suppress light from the whole exciting cone if that could enter the objective. A dark background therefore, can be obtained with much brighter fluorescence images than would otherwise be the case.

Light sources

For general fluorescence microscopy a mercury vapour source is the most suitable. The 200W lamp provides rather brighter fluorescence than does the 50W lamp. The latter, though, is adequate for most incident light purposes and is less bulky and a good deal cheaper. The 50W lamp is unsuitable for transmitted light work. A mercury vapour source is essential for ultra-violet excitation and has a very high output of light at 365nm.

The 12V 100 W tungsten halogen lamp provides a convenient source, which is often adequate for longer wavelength excitation and can be recommended for routine F.I.T.C. work in incident or transmitted light. It is excellent as a white light source to provide phase contrast or dark ground background to incident fluorescence illumination.

The 6V 30W tungsten source provides an efficient and cheaper alternative as a transmitted white light source only.

Photomicrography

Photographs are taken on the M17 in the most convenient way possible. The focusing of the image through the visual eyepieces of the M171900 photo-visual head automatically assures that it is focused in the camera.

One of the pair of 10X eyepieces used contains a graticule showing the field of view of the camera in use. It is only necessary to bring the area to be photographed within the graticule.

As a refinement, eyepiece and camera body are provided with rotational scales. An elongated object can be orientated to lie along the length of the camera frame by rotating the eyepiece and then the camera through the same angle. Need for an expensive "photographic" stage, with a rotating movement is thus eliminated.

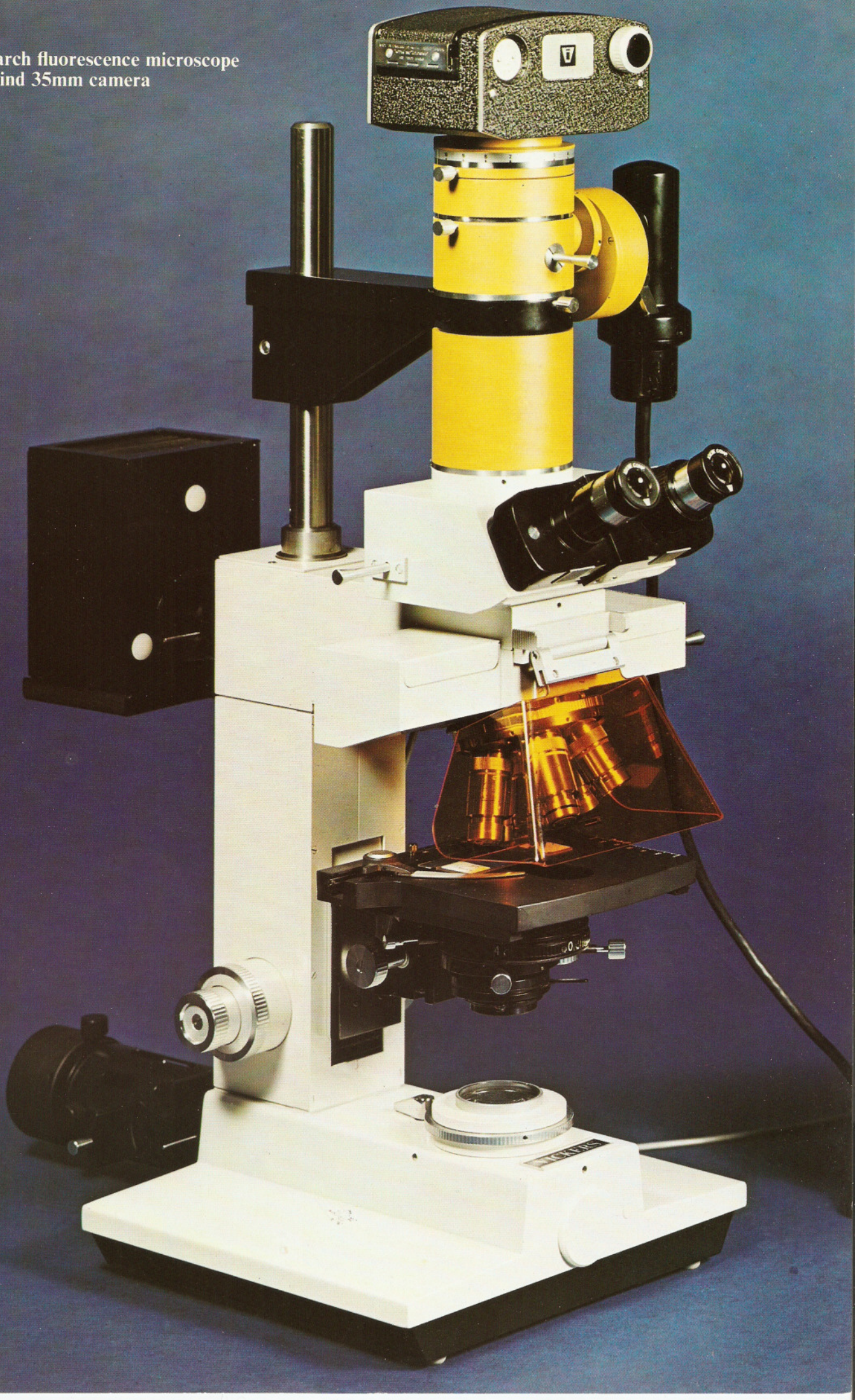
The photovisual head has a sliding beam-splitter which directs all the light to the visual eyepieces or 80% to the camera and 20% to the eyepieces. Camera magnifications are varied by using different eyepieces from 6.3X to 20X in the photo-tube.

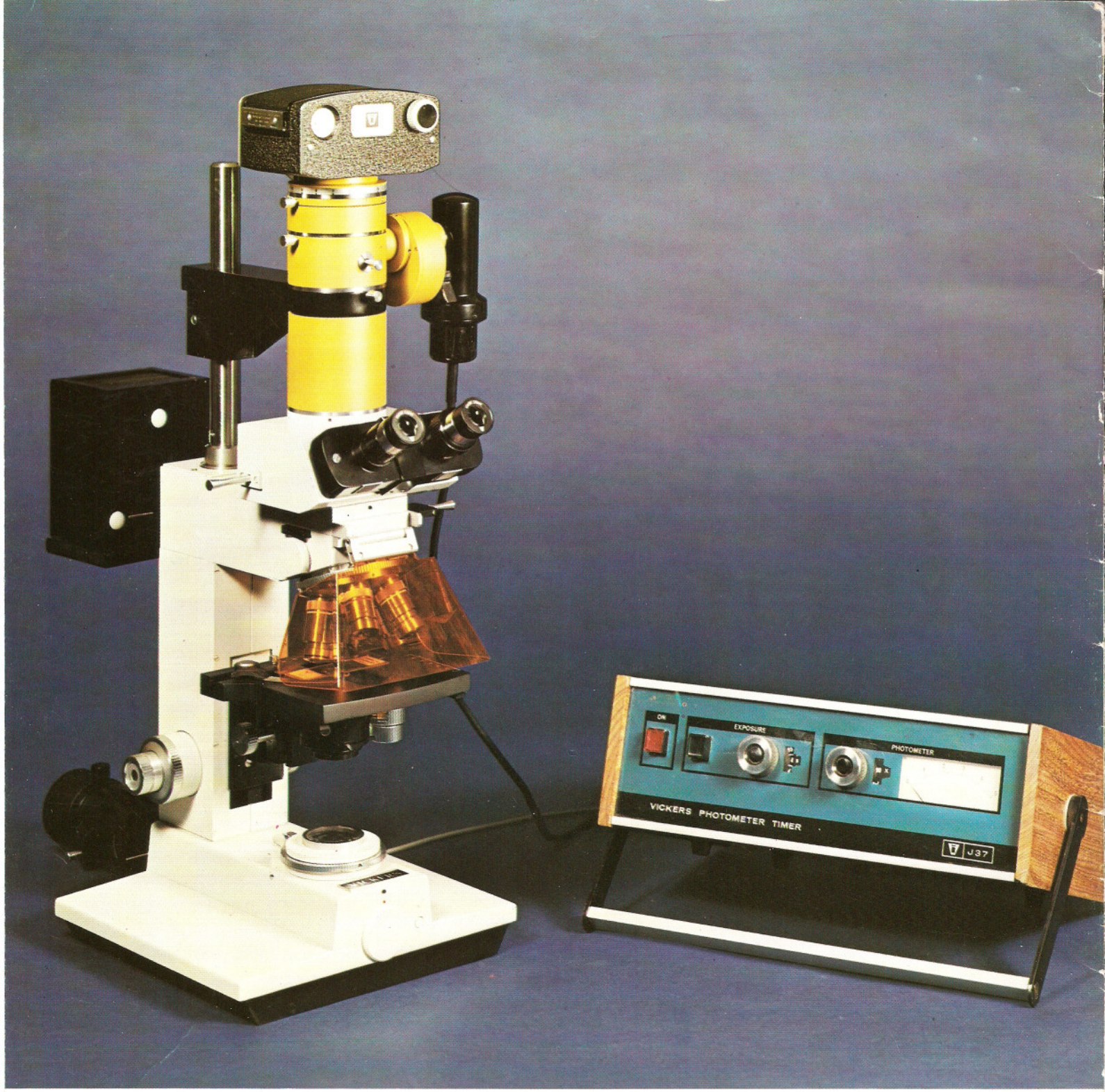
For fluorescence work the 6.3X eyepiece is usually to be preferred as it gives the maximum brightness in the film plane and minimum exposure time—often important to counter specimen fading. M17 camera equipment is carried rigidly from a pillar screwed into the top of the microscope stand. 35 mm, Polaroid CB101 and 5″ × 4″ camera bodies are interchangeable, but the 35 mm format is most often used in fluorescence work.

A mechanical shutter speeded from 1/125—1 sec. B.T. is available for use when exposures are to be made on the basis of previous experience or with simple means of estimating brightness.

The J37 high sensitivity partial field photometer-timer measures the available light and then is set to operate an electromagnetic shutter so as to give the required exposure. The light measurement can be based on the whole field of view, or on the central 1/100 of the field. Accurate exposure therefore, can be based on the brightness of a local area of fluorescence, unbiased by a much larger area of dark background.

M17 Research fluorescence microscope
with autowind 35mm camera





M17 Routine incident fluorescence microscope with autowind 35 mm camera and J37 partial field photometer

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