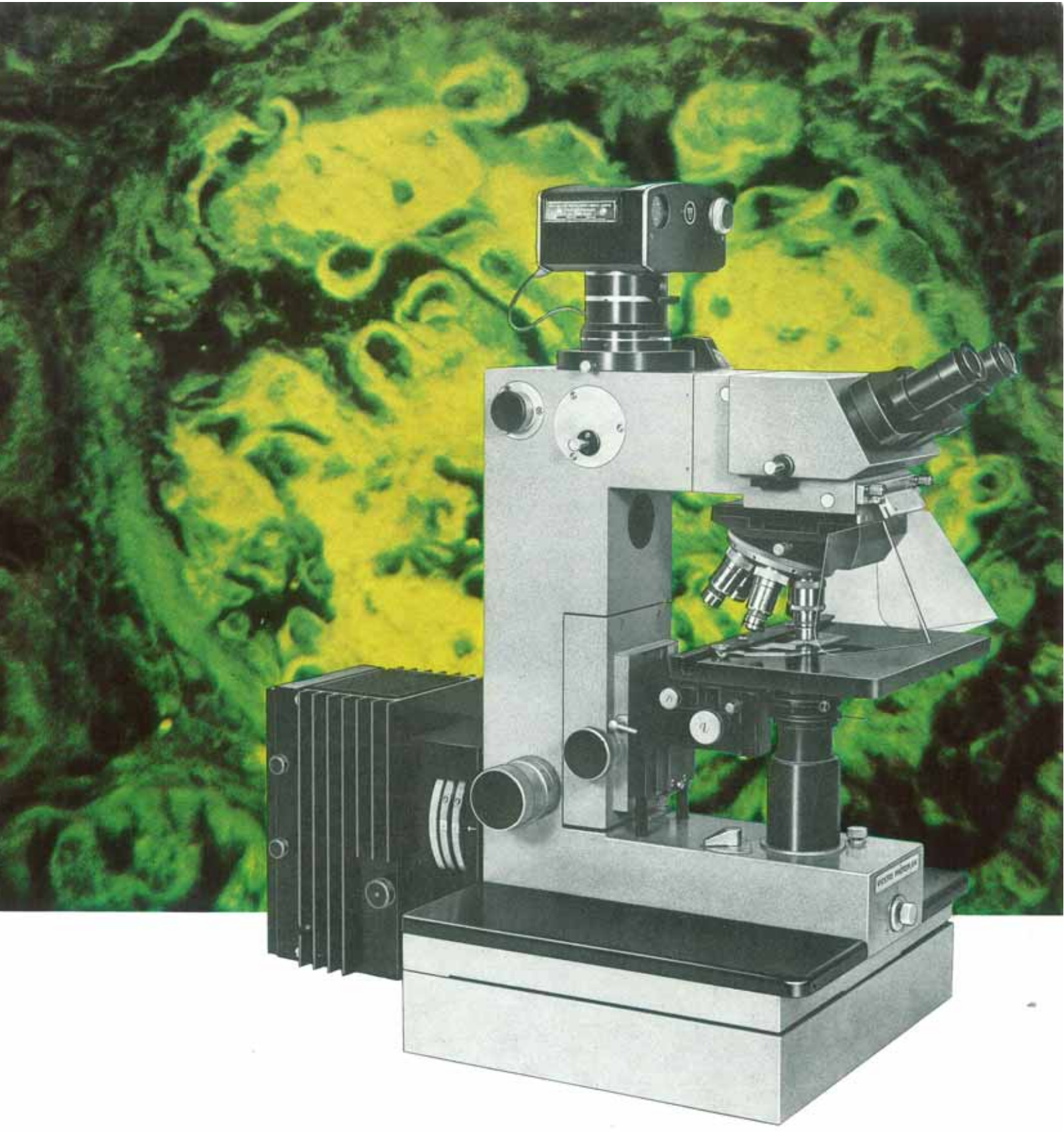


VICKERS M41 PHOTOPLAN
FLUORESCENCE EQUIPMENT

VICKERS M41 FLUORESCENCE MICROSCOPE

It is only during the last decade or so that the fluorescence microscope has assumed the importance which it undoubtedly holds today. The work of A. H. Coons on the fluorescent antibody technique in the early 1940's did much to popularize the use of the fluorescence microscope in the immunological, serological and bacteriological laboratory. The fluorescence microscope has in a very short space of time become accepted as an invaluable tool not only in the field of routine diagnosis but in a host of other disciplines from mineralogy to preventive dentistry. An instrument has long been needed which, besides being simple to operate, facilitated the precise control of the photographic recording of even the faintest fluorescence images.

The Vickers M41 Photoplan fluorescence microscope equipped with the J37 high sensitivity photometer timer and associated partial field device completely fulfils these requirements.

★ **Transmitted ultra-violet and blue light excitation in dark ground and bright field.**

★ **Incident ultra-violet and blue light excitation. Turret mounted dichroic mirrors ensure efficient selection of excitation wavelength.**

★ **Transmitted excitation combined with transmitted phase contrast.**

★ **Incident excitation combined with transmitted phase contrast or dark ground.**

★ **Partial field photographic equipment for perfectly controlled photography of even the weakest fluorescent specimens.**

★ **Integral mounting of the camera unit with the microscope stand for extreme camera stability.**

★ **Direct camera framing and focusing with the standard viewing head.**

★ **Combined transmitted and incident light excitation for observation of very weakly emitting preparations.**

Excitation Modes with the M41 Photoplan Fluorescence Microscope

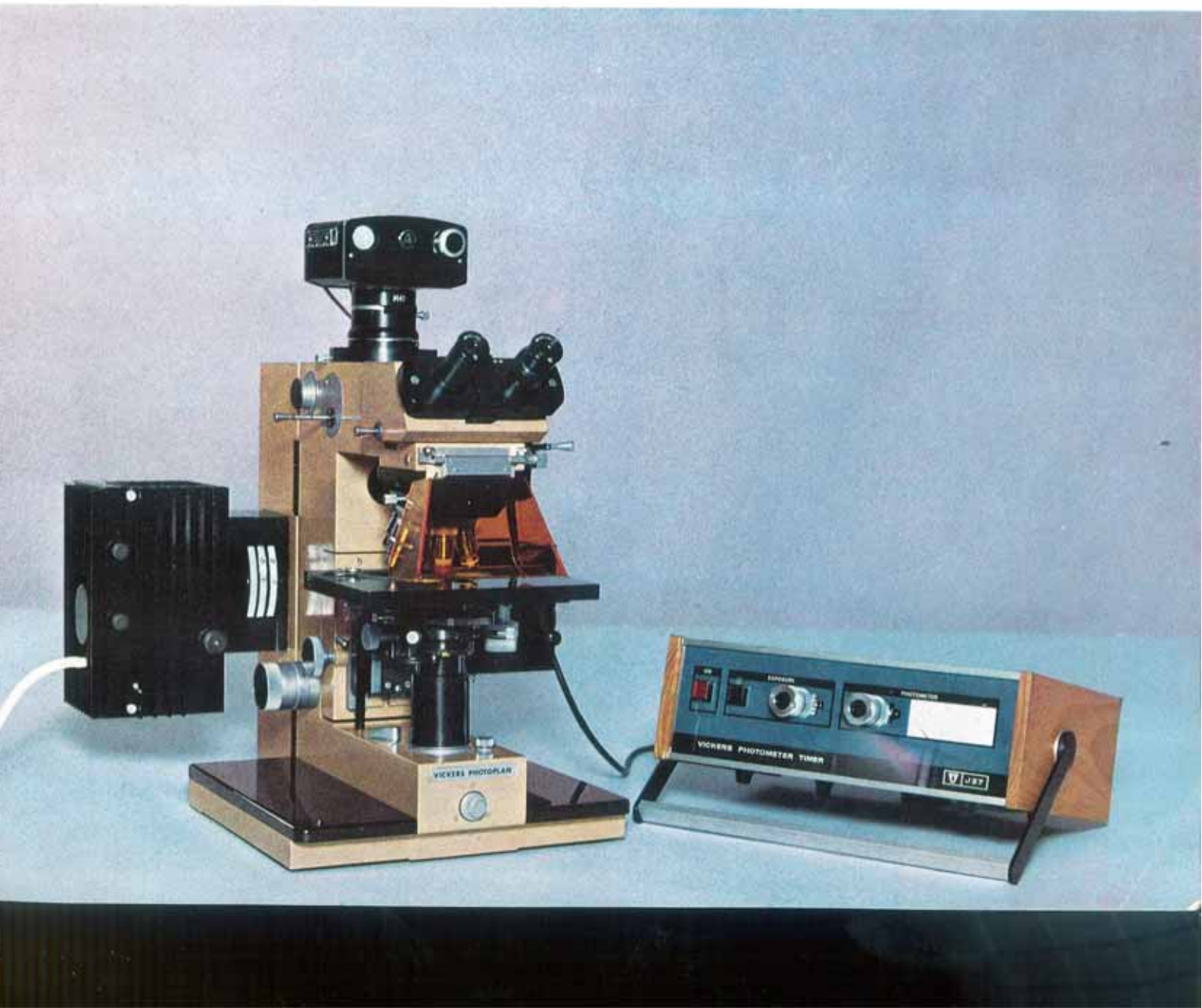
	Tungsten Halogen	Mercury HBO.200	Mercury 50 Watt	Reflector Housing HBO.200 and T.Hal.
1. Transmitted Blue				
2. Transmitted U.V.				
3. Incident U.V.		with incident illuminator	with incident illuminator	with incident illuminator
4. Incident Blue		with incident illuminator	with incident illuminator	with incident illuminator
5. Incident Green		with incident illuminator		with incident illuminator
6. Transmitted Phase Fluorescence				with phase fluorescence condenser
7. Incident Fluorescence with transmitted phase		with incident illuminator and standard phase		with incident illuminator and standard phase
		The lamp unit may be positioned at the top or bottom stand aperture	Generally suitable as low cost system for strongly fluorescing material	The reflector housing will accommodate the HBO.200 and tungsten halogen lamps. The exciter beam suffers a small amount of attenuation

Cover Illustration:

**Amyloid Kidney stained with Thioflavin T.
Blue Light. Transmitted Light. Dark Ground. Apo. 20×.**

*All photomicrographs by courtesy of
Dr. T. H. Flewett, Regional Virus Laboratory, Birmingham.*

M41 Photoplan Fluorescence Microscope equipped with the universal reflector housing and the J37 high sensitivity partial field photographic photometer.



TECHNIQUES IN FLUORESCENCE MICROSCOPY

In fluorescence microscopy the specimen is excited to the emission of visible radiation, primary or secondary fluorescence, by short wave ultra-violet or blue light isolated from a suitable light source by so called exciter filters. The excess short wave radiation remaining together with the emitted light is eliminated by barrier filters mounted above the microscope objectives, so presenting the fluorescence as bright or relatively bright areas on a dark ground.

Bright field excitation

Bright field excitation is employed when the non-fluorescing background does not interfere with the fluorescent image or when it is not required to observe the relationship between the background and the fluorescent image.

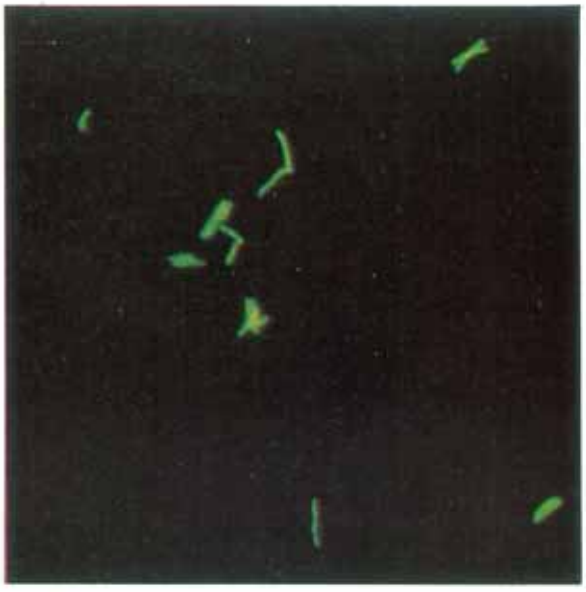
Bright field illumination provides the greatest excitation intensity as full use is made of the condenser aperture.

Dark ground excitation

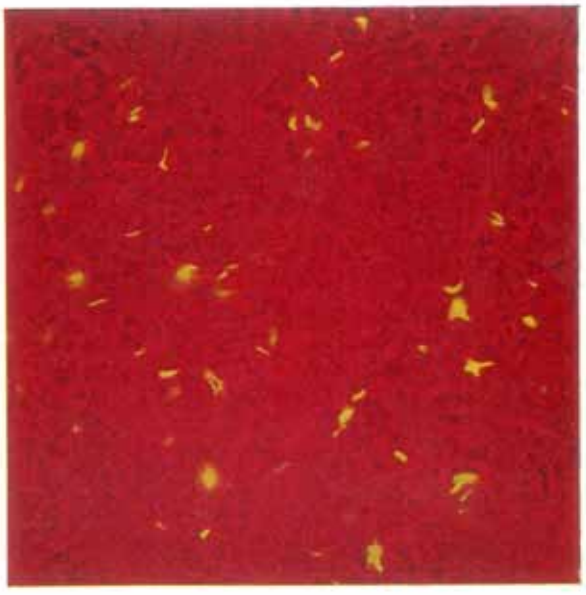
Dark ground excitation finds its widest application in the fields of bacteriology and virology and in similar instances where very small fluorescing bodies are to be observed. The main advantage of this technique is the very black specimen background and the extremely low glare. The general image brightness is however lower than that obtained with bright field excitation.

Fluorescence phase contrast

In fluorescence microscopy it is sometimes desirable to superimpose a white light phase contrast image of the non-fluorescent parts of the specimen upon the fluorescent image so that the general relationship of one to the other may be studied. This is especially true of secondary fluorescence where the specimen is essentially unstained. Both the excitation radiation and the phase contrast illumination are transmitted by a special phase contrast condenser.



Tuberculous Lung. Auramine. Incident light. U.V. Excitation. Microplan 100× oil.



Tuberculous Bacilli in Incident light. U.V. Excitation. Section highlighted by transmitted phase contrast. Apo. 20× Ph.+.

Incident light excitation

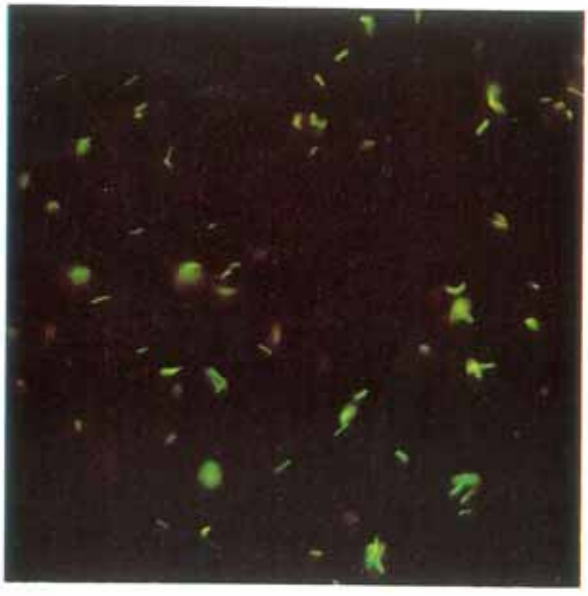
With incident light excitation the objective serves both as a viewing system and as its own condenser ensuring that the illumination centration and illumination focusing are coincident with the specimen image. No excitation radiation is transmitted to the viewing system as occurs with transmitted light excitation. The fluorescent image thus appears similar to that obtained with transmitted light dark ground illumination. The system is however far more efficient than dark ground illumination enabling brighter images to be produced with an equally black background and a minimum of glare.

There are also many circumstances where transmitted light excitation is neither convenient nor applicable. This applies for example where opaque materials such as bones, teeth or minerals are to be observed, and also where much of the transmitted light exciting radiation is absorbed by the lower parts of the specimen before being able to excite the observable top surface.

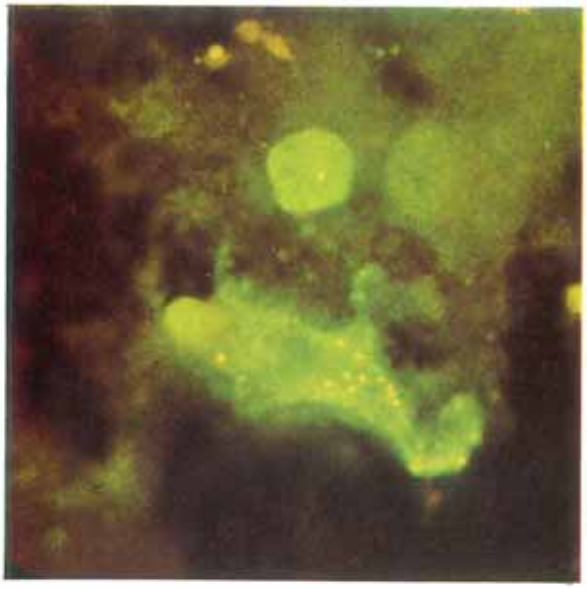
Incident light excitation with transmitted light

The combination of incident light excitation with transmitted light phase contrast or dark ground illumination allows full use to be made of the exciting radiation when observing the non-fluorescent background of the specimen in relation to the fluorescence emission. A standard phase contrast or dark ground condenser may be employed as may other transmitted light accessories. Under suitable conditions it would be possible to combine transmitted light polarizing with incident light fluorescence.

The choice between transmitted phase/transmitted excitation and transmitted phase/incident excitation lies entirely with the general suitability of the specimen.



Section of Tuberculous Lung. Auramine. Incident light. U.V. Excitation. Apo. 20×.



Herpes Encephalitis. Brain Smear. Herpes Antiserum. Incident light. Blue light Excitation. 50× Fluorite oil.

LIGHT SOURCES FOR FLUORESCENCE MICROSCOPY

Generally primary and secondary fluorescence is excited by short wave radiation, blue light or ultra-violet light. These two groupings may be further sub-divided into strong and weak emission. The light source selected will depend upon the excitation properties of the specimen to be examined.

Blue light excitation

The 12 volt 100 watt tungsten halogen lamp provides ample illumination for blue light excitation, a technique finding greatest use in routine applications such as F.I.T.C. staining and those methods developed by Nairn and other research workers for cancer investigations.

Ultra-violet and blue light excitation

Where ultra-violet excitation is to be employed with relatively strongly fluorescent materials then the mercury vapour lamp 50 watt is a suitable source, being very compact and having abundant ultra-violet and blue light in its emission spectrum.

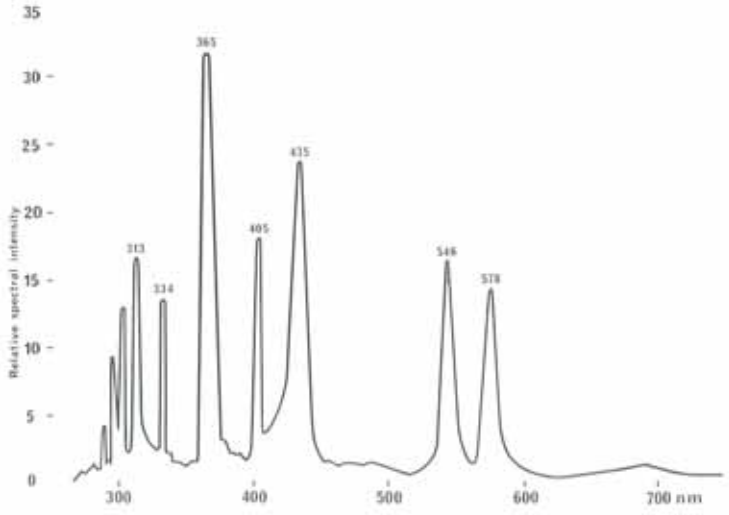
Both the tungsten halogen lamp and the mercury vapour lamp 50 watt are contained in similar small lamphousings. The tungsten halogen lamp carrier is fitted with very convenient centring movements and a swing-in diffuser disc.

The mercury vapour 50 watt lamp carrier is fitted with centring movements and a concave back reflector enabling two images of the light source to be laid alongside one another for maximum field coverage at the lower powers.

The HBO.200 is the ideal high intensity light source for ultra-violet and blue light excitation of all fluorescent materials with every illumination technique. The large lamphousing for the HBO.200 mercury vapour lamp is fitted with centring movements and a concave back reflector enabling two images of the light source to be laid alongside one another for maximum field coverage at the lower powers.

A riser base is required if the large lamp unit is used for transmitted light excitation without the special reflector housing.

The lamphousings together with the filter units may be fitted directly to the rear of the M41 Photoplan stand on dovetail mounts. The upper stand mount serves for incident illumination. The lower stand mount serves for transmitted illumination.



Spectral distribution of the high pressure mercury vapour lamp HBO.200.

Filters for ultra-violet and blue light excitation

The Exciter filters placed between the light source and the condenser are required to transmit only that part of the blue or ultra-violet radiation necessary to excite a particular specimen to full fluorescence.

Filters are required, having differing spectral transmission and density characteristics to cope with the varying needs of ultra-violet and blue light fluorescence.

The exciter filters are contained in two revolvable quadruple discs in a special unit attached on heat resisting fastenings to the lamphousing. The six filters, three to each disc, may be used either singly or in combination covering a wide range of excitation conditions.

The Barrier filters are required to exclude any of the exciting radiation which has passed the specimen after producing fluorescence. The barrier filter combinations are contained in a filter slide inserted in the accessory slot on either the transmitted light carrier or the standard head carrier bracket.

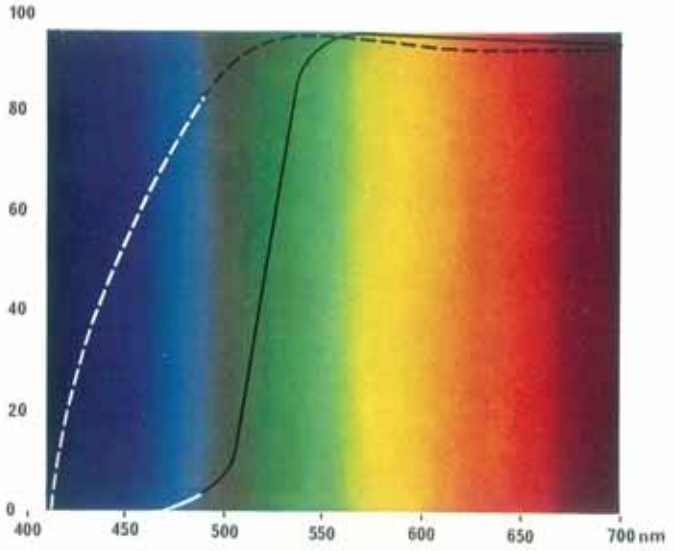
Maximum excitation together with a minimum background colouration can be achieved with a suitable combination of barrier and exciter filters for all excitation techniques.

Disc 1. Nearest Lamp	0. Clear 1. UG.2 2. BG.12 3. BG.12	 1 mm thick 1 mm thick 3 mm thick	A red minus BG.38 filter is mounted permanently in place.
Disc. 2	0. Clear 1. UG.2 2. BG.12 3. BG.12	 2 mm thick 2 mm thick 3 mm thick	

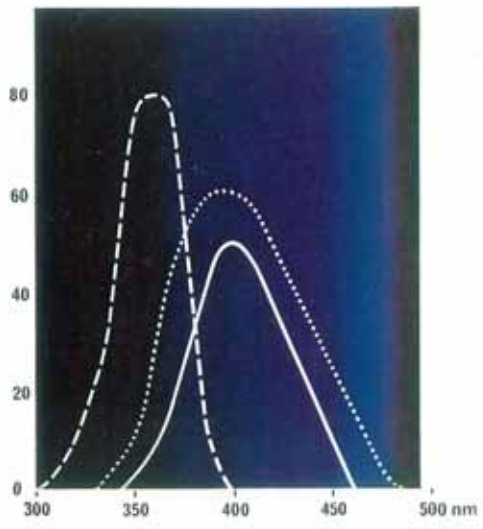
Exciter filters for the tungsten halogen lamp, and mercury vapour lamps 50 watt, HBO.200.

OG.4 1.5 mm. +GG.9 1.5 mm. 1	OG.1 1.5 mm. +GG.9 2.5 mm. 2	GG.4 1.5 mm. +Wratten 2E 3

Barrier Filter combinations in slider.



1. **Transmission characteristics of barrier filters.**
   — — — **GG.4 + 2E**
   ——— **GG.9 + OG.4**



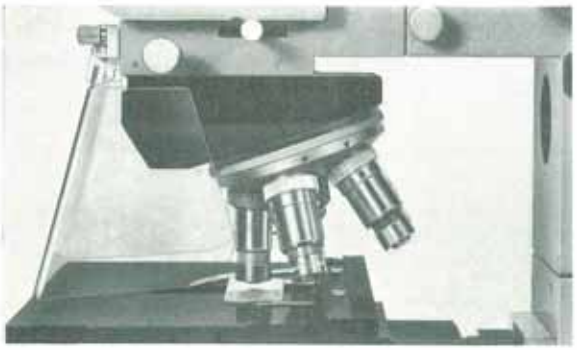
2. **Transmission characteristics of exciter filters.**
   — — — **2 mm. UG.2**
   · · · · · · **3 mm. BG.12**
   ——— **5 mm. BG.12**

Accessory units for transmitted light excitation

One of the prime requirements of fluorescence excitation is that the maximum amount of exciting radiation should reach the specimen without attenuation.

The achromatic bright field condenser has adequate light transmission for fluorescence excitation.

If a simpler condenser with a slightly higher transmission is required then a two lens Abbe type condenser is to be recommended. The bright field condenser has a numerical aperture of 1.2 when oil immersed.



Transmitted light objective changer carrier.

The dark ground condenser for use with medium (20×) and higher power objectives should be used oil immersed. The image definition with a dark ground condenser is greater than with bright field although the image intensity is reduced. The dark ground condenser is to be recommended for bacteriological or virological work. An adjustment collar allows the condenser to be precisely corrected for use with object slides from 0.75 to 1.5 mm thick. Oil immersion objectives chosen for use with dark ground should either be of the type provided with an adjustable iris or be supplied with suitable funnel stops.

All Vickers objectives may be used for fluorescence observation. It is however recommended that objectives be chosen having a high numerical aperture in relation to their magnification. The light gathering power of an objective is inversely proportional to the square of the magnification and directly proportional to the square of the numerical aperture.

The following objectives are therefore of particular value in fluorescence work:—

M023911	Apochromatic	20× N.A. 0.65.
M023611	Fluorite	50× N.A. 0.95 oil.

The oil immersion objectives for dark ground work should be fitted with an adjustable iris diaphragm so that the aperture of the condenser may remain larger than the objective aperture. Even in bright field work the adjustable diaphragm can be of value in reducing general glare.

M022643	Achromatic	100× N.A. 1.3 with iris.
M023541	Fluorite	100× N.A. 1.3 with iris.

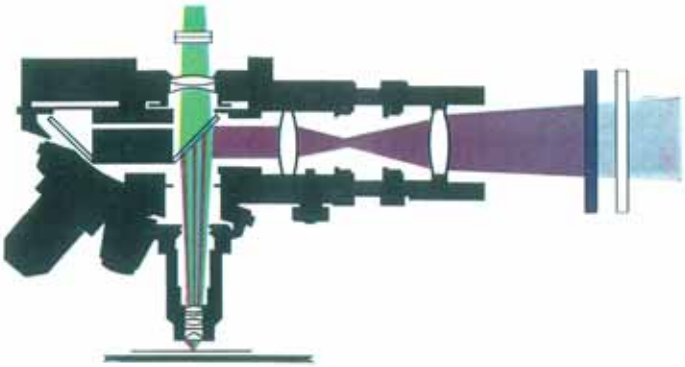
Accessory units for incident light excitation

The objectives for incident light observation and excitation are fitted precentred on an interchangeable quadruple objective changer. The incident illuminator is readily exchanged on a dovetail slide against the transmitted light carrier.

A revolver in the incident illuminator contains four interchangeable illuminating reflectors, each designed to reflect maximum exciting radiation of a given wave band to the specimen and to transmit maximum emitted light to the viewing head. The revolver positions are clearly marked and click stops ensure accurate location.

Incident light illuminator.

Schematic ray path of incident illuminator.



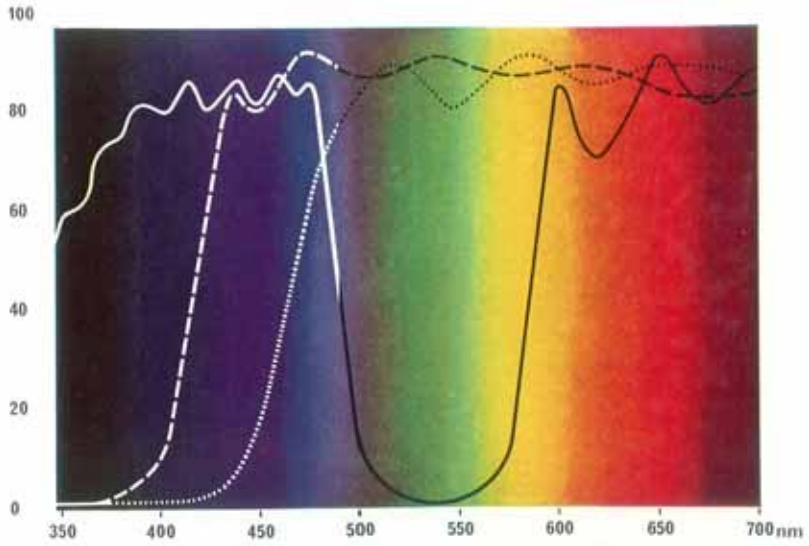
The slide mounted barrier filter must be fitted into the head carrier bracket slot (bracket without Bertrand lens) as the incident illuminator has no filter slot.

A minimum degree of filtration is required with the incident illuminator as excellent discrimination is achieved with the dichroic reflector. An extra 32 mm diameter filter of up to 3 mm thickness may be accommodated in an auxiliary carrier supplied with the unit.

Standard Vickers transmitted light objectives may be employed with the incident illuminator provided that cover slips are retained where necessary (20× objectives and higher). The objective light gathering power assumes double significance with incident excitation as the objective operates both as a viewing system and as its own condenser.

All standard transmitted light techniques may be used in combination with incident light excitation. Conventional bright field, dark ground or phase contrast with positive or negative phase objectives in transmitted light may be employed for the study of the non-fluorescent background without interfering greatly with the intensity of the exciting radiation.

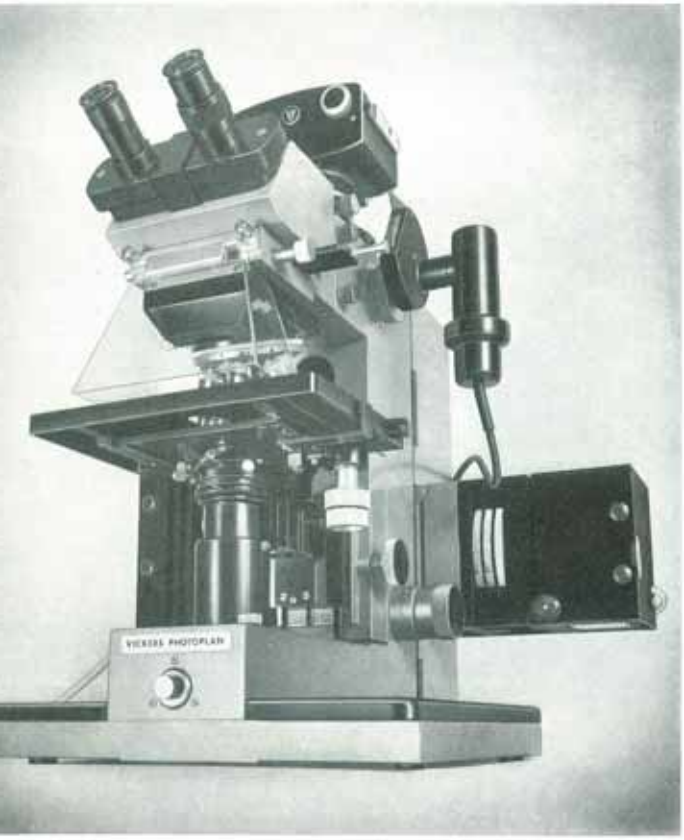
The tungsten halogen housing, provided with white light filters, is fitted to the lower stand aperture and the excitation source is attached to the upper aperture.



Transmission/reflectance characteristics of dichroic reflectors.

— — — U.V. reflector.
- - - - - - Blue light reflector.
——— Green light reflector.

M41 Photoplan fitted with reflector housing.



Universal reflector housing

It is very often necessary to employ a number of fluorescence techniques with the minimum of inconvenience and change-over time from one to the next.

The reflector housing is designed to satisfy this requirement.

The universal reflector housing is slid onto the rear dovetail slides of the M41 Photoplan stand in place of the standard lamp units. The total amount of bench space required for the M41 Photoplan fitted with the reflector housing is very little more than that required for the basic instrument.

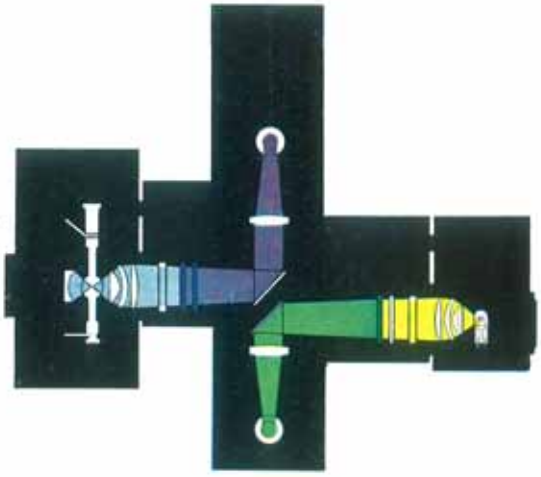
A mercury vapour HBO.200 lamphousing together with a filter unit is fitted on a dovetail mount to the left hand side of the reflector housing. A readily accessible lever operates a swivel mirror directing the excitation radiation either upwards towards the top stand aperture for incident excitation, or downwards to the base aperture for transmitted light excitation.

An aperture is provided at the right hand side for the attachment of a 12 volt 100 watt tungsten halogen lamp.

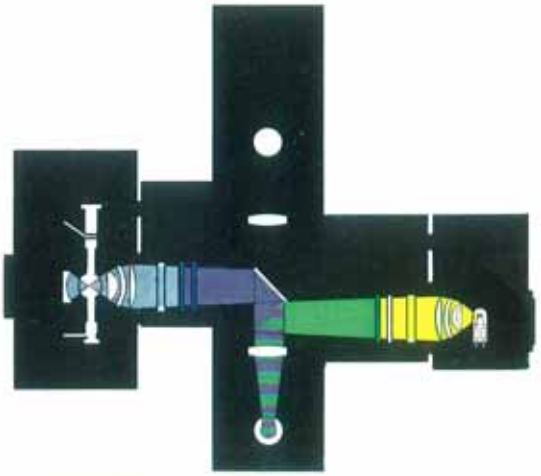
An easily removable dichroic mirror in a plug-in mount allows the visible portion of the tungsten halogen light to be passed to the transmitted light aperture for transmitted light phase contrast with a special condenser. The tungsten halogen lamphousing is fitted with a filter unit containing white light filters for obtaining suitable contrast with the emitted radiation.

The ultra violet or blue-light radiation selected from the mercury vapour spectrum by suitable filtration may be used for incident light excitation alone or in combination with transmitted light phase contrast or dark ground with standard condensers.

Alternatively the exciting radiation may be sent through the dichroic mirror, which passes short wave light, for transmitted excitation. The tungsten halogen light is reflected by the dichroic to a special transmitted light phase contrast condenser fitted with colour discriminating phase annuli.



Schematic ray path of incident fluorescence with transmitted white light.



Schematic ray path of combined transmitted phase contrast fluorescence.

Phase contrast condenser.

The Fluorescence phase condenser allows observation of the specimen by phase contrast, fluorescence or a combination of the two. The condenser is highly suitable for both ultra-violet and blue light fluorescence. Phase annuli in combination with the tungsten halogen lamp filters and lamp rheostat allow colour discriminated phase images to be faded in and out of the general fluorescence foreground. A position is reserved on the condenser for bright field work. A series of both negative and positive phase objectives permit the attainment of a wide range of contrast conditions.

General requirements

Viewing Head

A special viewing head fitted with high efficiency beam splitting prisms is available. This binocular viewing head ensures maximum transmission to the eyepieces and is of particular value with low emission preparations. The standard viewing head does however have sufficient transmission for most purposes.

Immersion Oil

Immersion oil which is in itself non-fluorescing should be employed.

M322245 is a low viscosity oil for use with both objectives and condensers.

M322247 is a high viscosity oil for use with condensers only: e.g. with the dark ground condenser where a slightly longer working distance is encountered.

Dark Adaptation

A special clip-on transparent plastic U.V. light shield is provided to prevent the distracting effect of light scatter from around the specimen. In addition a telescopic tube is fitted between the microscope substage and the transmitted light illumination aperture. A light exclusion sleeve may also be fitted between the incident illuminator and the upper stand aperture.

J37 High sensitivity photometer.

J37 High sensitivity exposure timer and partial field device

The M41 Photoplan photographic microscope is ideally suited to the photomicrography of fluorescence preparations. The stability problems often associated with fluorescence photography are eliminated by the rigid integral mounting of the camera body to the microscope stand.

The J.37 exposure timer enables fully predictable exposures to be taken of specimens consisting of isolated illuminated particles on a very dark background or dark on a light background. The J.37 thus allows the photographic recording of dark ground fluorescence images which have not in the past been amenable to controlled photography.

When the camera prism set is pulled right out, all the light is directed to the J.37 timer photomultiplier. A revolver disc enables the J.37 to sample the light from the whole field, 1/10 by area, 1/100 by area or 1/500 by area. The diaphragm sizes are directly related to a graticule in a focusing eyepiece. It is therefore possible to select an area in the specimen for measurement, occupying a space as little as 1/500 of the field by area, determine the required exposure time and set the timer to open and close the shutter. The system is extremely sensitive such that it is possible to measure the amount of light present with the 1/500 stop in place when the image can only just be seen with the well accommodated eye. The timer can be set for a photometric series from 0.05 seconds to 1 hour. Having timed the exposure all the image light may be sent to the camera. With the J.37 it is possible to time even the faintest fluorescence dark ground image regardless of the light distribution and to accurately record this image on film.

The necessary optics for use of the various configurations must be ordered along with the exposure unit.

SPECIFICATION FOR M41 PHOTOPLAN FLUORESCENCE ACCESSORIES

LAMP UNITS FOR FLUORESCENCE EXCITATION
(Not including excitation filters)

M411320 Tungsten halogen 12 volt 100 watt lamp housing with iris and filter unit but without filter set

M415100 Power supply unit for tungsten halogen lamp 230 volt.

M411420 Mercury vapour 50 watt lamp housing with iris and filter unit but without filter set.

M415050 Power supply unit for mercury vapour 50 watt, 240 volts.

M411520 Mercury vapour HBO.200 lamp housing with iris and filter unit but without filter set.

M415205 Power supply unit for mercury vapour HBO.200, 220 volt.

M413075 Riser plate, essential when large lamp housing is used directly for transmitted light. (HBO.200).

FILTERS FOR FLUORESCENCE MICROSCOPY

M411790 Set of six excitation filters (one set for each lamp unit).

M410665 *Barrier filter slide containing 3 filters.

** The barrier filter slide is inserted in the filter slot on the transmitted light objective changer carrier or the head carrier bracket without Bertrand lens M410650. When incident light accessories are purchased the head carrier bracket M410650 must be available.*

CONDENSERS FOR FLUORESCENCE EXCITATION

M151970 Oil immersion dark ground condenser.

M252779 Akehurst slide for condenser attachment.

M151975 Two lens high transmission Abbe condenser.

M252779 Akehurst slide for condenser attachment.

SPECIAL ACCESSORY

M410850 Binocular head with high efficiency prisms.

INCIDENT ILLUMINATOR

M413500 Incident fluorescence illuminator fitted with dichroic revolver, for blue light, ultra-violet, green and white light excitation, including quadruple revolving incident bright field objective changer.

(The head carrier bracket without Bertrand lens **M410650** is required).

LIGHT SHIELDS

M413630 Plastic U.V. protection shield and telescopic substage light tube.

M413530 Incident light protection tube.

UNIVERSAL REFLECTOR HOUSING AND LAMP UNITS

M412300 Reflector housing for incident and transmitted light with built-in dichroic reflector.

M411320 Tungsten halogen 12 volt, 100 watt, lamp housing with iris and filter unit but without filter set.

M415100 Power supply unit for tungsten halogen lamp 230 volt.

M411780 Set of six filters for white light work.

M411520 Mercury vapour HBO.200 lamp housing with iris and filter unit but without filter set.

M415205 Power supply unit for mercury vapour HBO.200, 220 volt.

M411790 Set of six filters for fluorescence excitation.

FLUORESCENCE PHASE CONTRAST CONDENSER
(for use with reflector housing and standard phase objectives)

M410990 Fluorescence phase contrast unit with individual centrable annuli.

M252779 Akehurst slide for condenser attachment.

SPARE LAMPS

M551649 Spare lamp HBO.200 mercury vapour.

M320545 Spare lamp, 12 volt, 100 watt tungsten halogen.

M411440 Spare lamp, 50 watt mercury.

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