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An interference-filter monochromator system for the irradiation of microscopic objects

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INTRODUCTION

In photobiological experiments interference filters are widely used for the effective isolation of narrow spectral regions. Withrow (1957) and Mohr and Schoser (1959, 1960) have recently described equipments allowing the irradiation of experimental objects with monochromatic light of relatively high intensity, i.e. of the order of some thousand ergs per cm^2 sec., obtained from interference filters in the visible and near-visible range. Cyto-physiological researches undertaken in this laboratory have necessitate the construction of a similar equipment allowing the irradiation of microscopic objects with monochromatic light of very high intensity, of the order of some tens of thousands ergs per cm^2 sec. The present report describes this equipment and the method of measuring light intensity.

DESCRIPTION OF THE APPARATUS

The apparatus consists of ten identical units each composed of a projection lamp, a collector lens, an aqueous filter, an interference filter, a microscope mirror, and a condenser. The units are arranged side by side on a common bench. The lamps have one common housing and the condensers are mounted side by side on a common stage more than 100 cm. long. The microscope tube travels on a raul above the stage and is provided with a spring catch which allows to set it in line with the optical axis of each unit. This equipment makes possible the simultaneous irradiation of 10 microscopic objects with monochromatic light of different wave lengths and the successive microscopic observation of the objects (Fig. 1 and 2).

The sources of light are 100 W or 750 W — according to the desired light intensity — incandescent lamps with tungsten biplane filaments and a nominal voltage 110 V. The lamps are operated with 100 V current from three variable transformers, each transformer feeding three or four units.

A voltmeter connected to each set of units allows the control of the voltage. The socket of each bulb is so constructed that its position can be adjusted in three directions. All the lamps are housed under a common cover of aluminium sheet lined inside with asbestos fabric. When 100 W lamps are used no cooling is necessary, but with the 750 W ones 10 centrifugal air blowers must be started to cool the bulbs.

The optical system of each unit is fixed on a common rail and consists of a double lens, each f = 6,7 cm (+ 15 D), a diafragm placed between the lenses for limiting the light field, a plane glass cell for the aqueous filter of path length 5 cm., a lens of focal length 10 cm, and a microscope

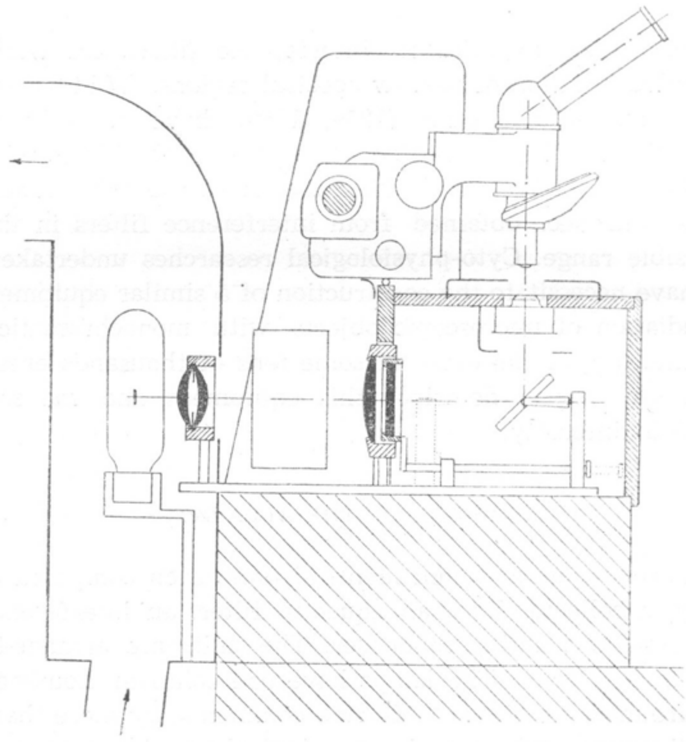

Fig. 1. Cross-section view of the apparatus for the irradiation of microscopic objects (for details see text)

mirror. Directly behind the second lens there is the interference filter mounted in a way allowing its easy and rapid removal from the path of the light. The mirror directs the light beam upwards onto the microscope condenser (N. A. 1,2) composed of two lenses and mounted in the microscope stage. Below the stage there is a sliding frame for the neutral filters used to reduce the light intensity. Fig. 3 shows the dimensions of the optical system and the path of light rays. As is to be seen the lenses

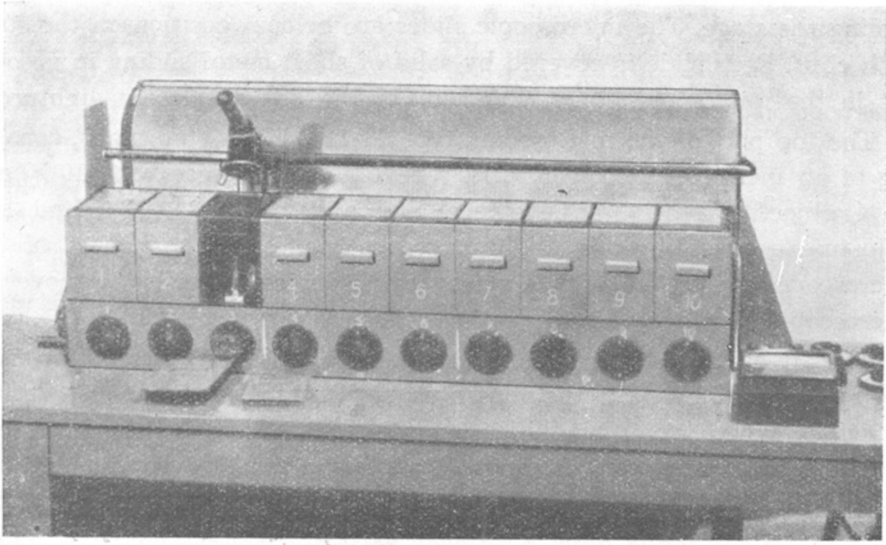

Fig. 2. General view of the apparatus

are arranged according to the Kohler's system which gives a uniform distribution of light intensity in the light spot when the system is properly regulated. Measurements with a photomultiplier of the light intensity distribution in the image of the field of vision of the microscope have shown that the local differences do not exceed ± 10 per cent. The diameter of the lighted field in this equipment is 2 mm. when the diafragm aperture diameter is 16 mm. and can be increased by widening the diafragm aperture.

The microscope stage is of duralumin and its under side is provided with a copper tube spiral connected to an ultrathermostat for heating or

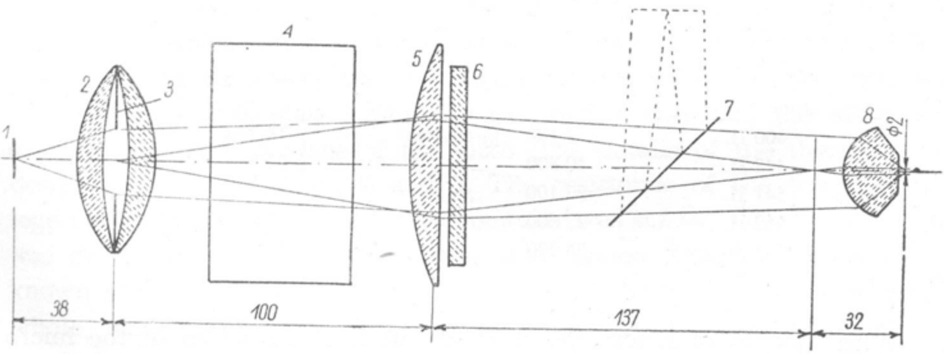

Fig. 3. The optical system: *1* — filament of bulb; *2* — lens f = 6,7 cm. diameter 45 mm.; *3* — diafragm diameter 16 mm.; *4* — water filter; *5* — lens f = 10 cm. diameter 58 mm.; *6* — interference filter diameter 50 mm.; *7* — mirror; *8* condenser

cooling the stage. The microscopic slides are held in position on the stage with clips. Each slide is covered by a lid of sheet metal sliding in grooves cut in the microscope stage which make the lids absolutely lightproof.

The top part of the microscope (type M-110, PZO, Warszawa), consisting of an inclined monocular tube, a revolving nosepiece with 10 × and 40 × objective lenses, an eyepiece, and a device for coarse and fine adjustment, travels along the microscope stage. The top part of the microscope is provided with a spring catch device allowing to move the microscope from one unit to another within a few second.

The air-cooling of strong lamps may cause vibrations transferred from the rotors and blowers to the apparatus, and this may impede observations especially when larger magnification are used. However, the vibrations can be almost completely eliminated by placing the blowers on a separate base and connecting them to the lamp housing through wide rubber tubes.

THE EFFICIENCY OF THE OPTICAL SYSTEM

Single line interference Zeiss (type JF) and Schott (type IL) filters ranging from 400 to 750 mμ are used. Their optical characteristics have been described by various workers, e.g. Günzler (1956), and Mohr and Schoser (1959). The heat filters consist of the following water solusions: $CuSO_4$ 100 g/l with 2 per cent addition of sulfuric acid for the wave length range 400—480 mμ, $Fe(NH_4)_2(SO_4)_2$ 70 g/l with 1 per cent addition of sulphuric acid for the wave length range 490—670 mμ, and pure water for the wave length range 680—750 mμ (Withrow and Price 1956).

Table 1

Filter	Maximum light intensity with bulb of	
	100 W	750 W
400 IL	3 100 ergs/cm²sec	14 700ergs/cm²sec
442 IL	10 800 ,,	63 500 ,,
541 IL	23 100 ,,	86 400 ,,
662 IL	22 600 ,,	92 000 ,,
713 JF	28 200 ,,	108 000 ,,

With this set of filters the intensity of the irradiation of the microscope objects ranges from several thousand to several tens of thousands ergs per cm² per sec. according to the wave length and the power of the lamps. Some examples of maximum light intensity are shown in table 1.

Table 2

	Diameter of illuminated surface	Maximum light intensity in ergs/cm²sec.	
		about 410 mμ	about 620 mμ
Withrow (1957) lamp of 750 W	20 cm	200	1 000
Withrow (1957) carbon arc	20 cm	—	20 000
Mohr and Schoser (1959) lamp of 750 W	5 cm	1 500	14 000
Mohr and Schoser (1960) xenon arc	9 cm	4 700	6 300
Apparatus described in this paper			
lamp of 100 W	2 mm	4 000	22 000
lamp of 750 W	2 mm	15 000	90 000

Since the light intensity changes as the lamps are used up measurements must be carried out after keeping a lamp alight for 6—10 hours.

The direct comparison of the apparatus here described with similar equipment constructed by Withrow and by Mohr and Schoser is difficult because those workers designed their equipment for irradiating larger surfaces. The approximate maximum intensities of blue and red light as well as the diameters of the illuminated surfaces in different types of monochromators are compared in table 2.

MEASUREMENTS OF LIGH INTENSITY

For measurements of absolute light intensity on the small lighted area of the microscopic slide a specially designed thermocouple was used. It consisted of a manganine-constantan strip 7 μ thick, 0,16 mm wide and 7 mm. long streched between two copper wires 0,5 mm. in diameter. The attachments of the strip to the wires were carefully soldered with Wood metal and the strip was coated with lamp black. The thermocouple was mounted on a slide glass 1 mm. thick in such a way that the strip was 0,3 mm. above the surface of the glass. The details of the thermocouple design are shown in figs. 4 and 5. The resistance of the thermocouple together with about one meter of conduits was 5,2 Ω. The thermocouple was calibrated according to a Kipp and Zonen type El thermopile of known absolute sensitivity. The sensitivity of the thermocouple when its whole length was irradiated was 9,28 μV/mW. However, during the measurements of the intensity of the light spot only a part of the strip was illuminated and thus it was necessary to examine the sensitivity of the various segments of the thermocouple. For this purpose the thermo-

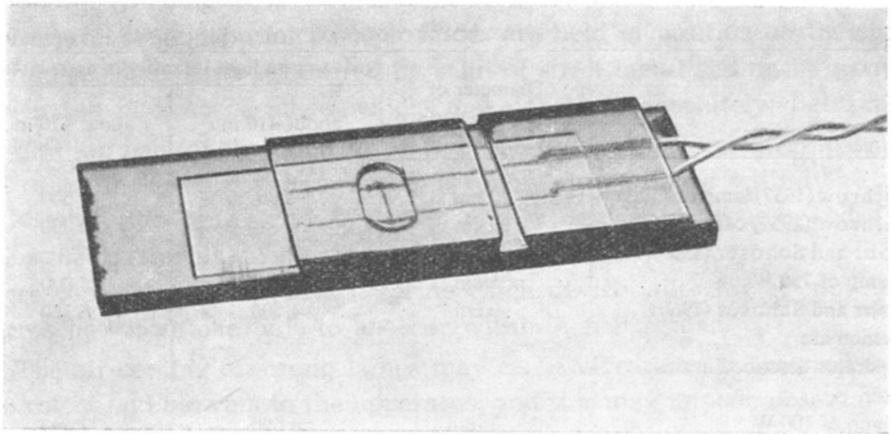

Fig. 4. Thermocouple for measurements of absolute light intensity

couple was moved over a narrow slit of light (obtained in a microscope by applying a slit diafragm in the Köhler system) and from the deflections of the galvanometer the point of the greatest sensitivity of the thermocouple was found (Fig. 6 a). Then the thermocouple was placed with the point of the greatest sensitivity over the centre of the light slit and by altering the width of the slit the length of the illuminated thermocouple segment was changed at constant intensity of the incident light; the

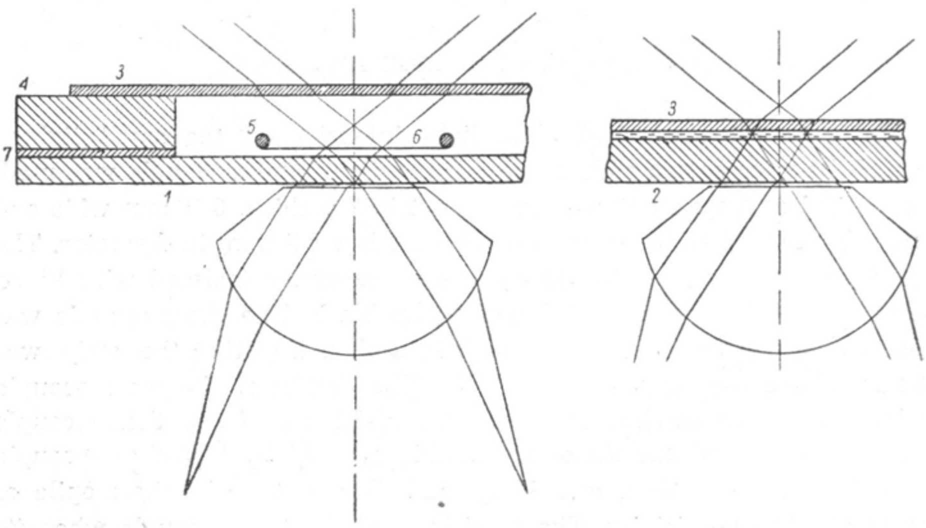

Fig. 5. Cross-section of thermocouple (left) and cross section of microscopic object (right) with marked paths of light rays. *1* — glass slide 1 mm. thick; *2* — glass slide 1,5 mm. thick; *3* — cover glass; *4* — plexiglass plate 2 mm. thick with imbedded copper wires; *5* — copper wires diameter 0,5 mm.; *6* — thermostrip; *7* — plate 0,3 mm. thick

galvanometer recordings showed the dependence of the thermocouple potential on the size of the light spot (Fig. 6 b). The standard curve plotted from a series of such recordings was used for establishing the corrections to be used in calculations of the absolute light intensity. E.g. in the case of a light spot 2 mm. in diameter applied in the apparatus here described the thermoelectric potential was 68 per cent of the potential which would have been obtained if the strip was irradiated over its whole length with the light of the same intensity.

The wiring system of the thermocouple and the galvanometer was for every recording the same as the circuit described by K o k (1948) for the calibration of galvanometers. The magnitude of the resistance applied

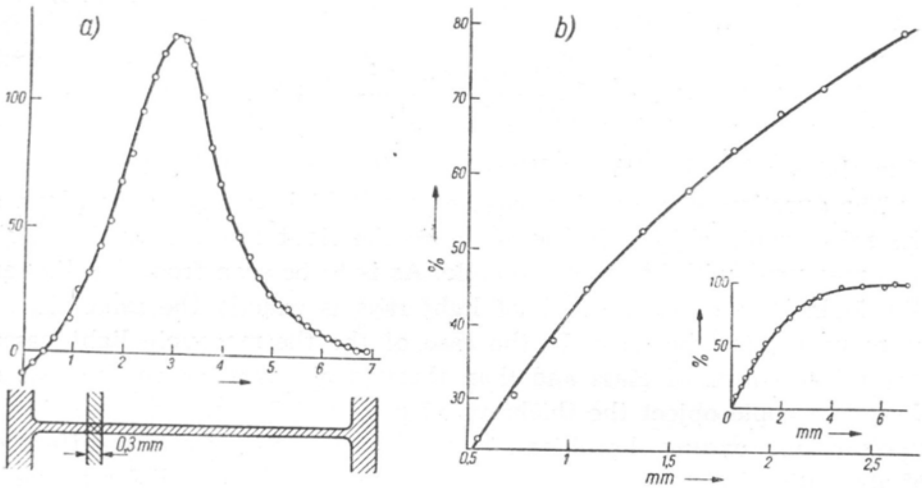

Fig. 6. The local sensitivity of the thermocouple: *a* — galvanometer deflection according to the position of light beam 0,3 mm. wide, *b* — dependence of the thermoelectric potential on the width of the light spot when the centre of the light spot coincides with the point of maximum sensitivity of the thermocouple strip

in the voltage regulator was known to within 0,01 per cent. The following galvanometers were used: a Kipp and Zonen portable galvanometer type A 70 of internal resistance 80 Ω, and a Zeiss string galvanometer of internal resistance 5,5 Ω.

The absolute light intensity was calculated from the formula:

$$W = x \frac{V \cdot r}{z \cdot y \cdot s}$$

where W is the absolute light intensity in mW/cm^2.,

x is the mean galvanometer deflection after switching on the light, z is the mean galvanometer deflection after switching on the microvolt potentiometer,

V is the voltage of the element of the voltage regulator in μV.,

r is the ratio of the voltage distribution R_0/R_x

y is the absolute sensitivity of the thermocouple in μV/mV,

s is the lit area coefficient of the thermocouple.

Here is an example of actual measurements:

Filter 622 mμ, 100 W lamp, A 70 galvanometer, mean deflection after switching on the light $x = 19{,}42$, mean deflection of the galvanometer $z = 44{,}04$ when the voltage element was $V = 1{,}58$ V and the arrangement of the resistance was such that the ratio of the voltage distribution was $r = 1{,}77 \times 10^{-5}$. The absolute sensitivity of the thermocouple was 9,28 μV/mW and the surface coefficient for the light spot 2 mm. in diameter was $s = 0{,}68$. Therefore,

$$W = 19{,}42 \frac{1{,}58 \cdot 10^{6} \cdot 1{,}77 \cdot 10^{-5}}{44{,}04 \cdot 9{,}28 \cdot 0{,}68} = 2{,}074 \text{ mW/cm}^2.$$

Thus the absolute light intensity was 20 740 ergs per cm^2. sec.

The question still remains whether the light intensity in the plane of the microscopic objects is the same as the light intensity of the light spot measured with the thermocouple. As is to be seen from the diagram (fig. 5) in both cases the path of light rays is exactly the same till the beam penetrates the slide. In the case of the thermocouple light passes through one mm. of glass and than through air, whereas in the case of the microscopic object the thickness of glass is 1,5 mm. and water is the medium surrounding the object. Thus in each case there is a difference of absorption in the glass and of reflection caused by the different media (glass-air and glass-water). In view of the very small absorption of light in glass in the 400—2000 mμ wave lenght range the difference of 0,5 mm. in the thickness of slides may be disregarded. However, in the two cases the losses of light intensity caused by reflection differ significantly. When the incident light rays are normal to the boundary between the two media the theoretically calculated losses caused by reflection are 0,425 per cent when light passes from glass ($n = 1{,}52$) to water ($n = 1{,}33$) and 4,25 per cent when light passes from glass to air ($n = 1$). In a beam of light passing through a condenser of given aperture not all the light rays are normal to the boundary, but on the contrary most rays strike the boundary at different angles. The reflection of rays striking the boundary obliquely may be much greater, especially if the angle of incidence approaches the critical angle. The drop of light intensity according to the angle of incidence of light rays calculated theoretically from Fresnel's laws of reflection (J e n k i n s and W h i t e 1957) is illustrated by the curve in fig. 7. As is to be seen from the graph the difference of reflection between the glass-water boundary and the glass-air boundary increases markedly only when the angle of incidence of the beam is greater than 30—35°. In the beam of light here considered rays striking the boundary at this angle occur only when the aperture is more than 0,8, and even than their proportion is small. In order to measure experimentally how the difference of reflection depends on the aperture number a series of observation was carried out on two different microscopic objects: 1) glass slide 1,5 mm. thick, water and cover glass, and 2) glass slide 1 mm. thick, air and cover glass. The intensity of transmitted light was measured in a beam of parallel rays and for different apertures. The light was monochromatic of the wave length 546 mμ. The objectives used for these experiment always had a larger aperture than the apertures used in the illuminating system and were always set to the sharpness plane of the light spot (in the object consisting of glass and water the light spot is situated about 0,2 mm. higher than in the object consisting

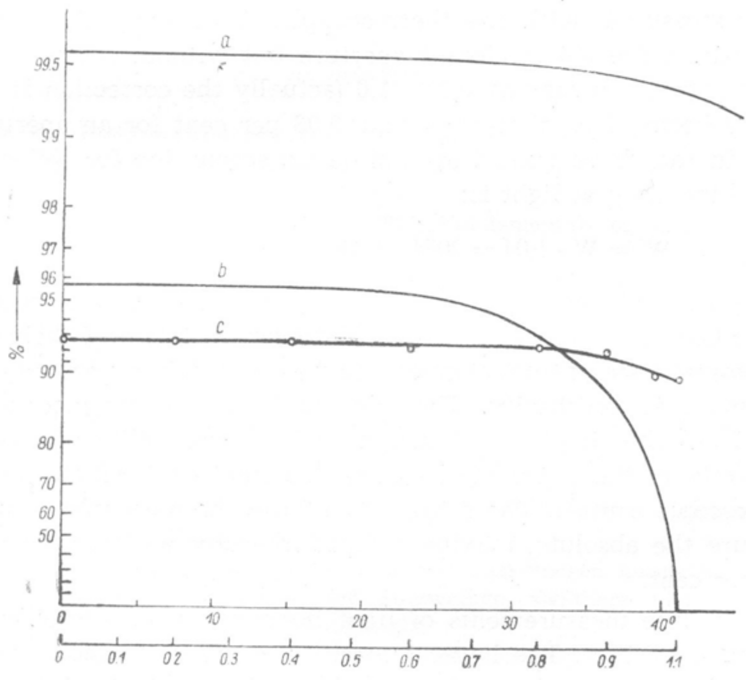

Fig. 7. Reduction of light intensity caused by reflection: *a* — theoretical for glass--water boundary plotted against incidence angle, *b* — theoretical for glass-air boundary plotted against incidence angle, c — experimental for two a glass-air boundaries as compared with two glass-water boundaries (assumed as 100 per cent) plotted against aperture

of glass and air). The light detector was a photo-element placed over the objective in the plane in which the image is formed and connected to a Zeiss scale galvanometer. The loss of light intensity caused by reflection was in this case twice as large as in measurements of absolute light intensity becouse the light rays passed twice through the boundaries between different media (glass-water and glass-air). The results of the measurements are shown on the graph in fig 7. The drop of intensity in a normal beam of parallel rays was in accordance with the theoretical calculations. The intensity of a beam of light passed through a dry object was 92,76 per cent as compared to the intensity of a beam passed through the other object taken as 100 per cent. When the beam was composed of convergent rays the light intensity decreased only slightly, dropping to 89,5 per cent when the aperture was 1,0. The reason for such a small decrease was that even in the case of aperture approaching 1,0 the rays striking the boundary at angles close to the critical angle amounted to only a small fraction of the whole beam.

From the above results it has been accepted that the intensity of light for a microscopic object (water medium) is 4 per cent higher than the intensity measured with the thermocouple. This correction has been established for the 0,8 condenser aperture but without major error it is valid for appertures ranging 0,5 to 1,0 (actually the correction is 3,35 per cent for a beam of parallel rays and 5,03 per cent for an aperture 1,0).

Thus in the above quoted example after accounting for reflection the measured intensity of light is:

$$W' = W \cdot 1{,}04 = 2074 \cdot 1{,}04 = 21\,560 \text{ ergs/cm}^2 \text{ sec.}$$

A thermocouple similar to the one described here was also constructed by Manten and Milatz (1953). However, the strip of their thermocouple was of lesser width, thinner and was placed in a vacuum chamber which improved sensitivity. The advantages of the thermocouple here described are the simple construction and the possibility of placing the strip exactly in the plane of the measured light spot which is obtained by microscopic control. The sensitivity of the thermocouple is sufficient to measure the absolute, maximum light intensity with an error of less than 5 per cent.

The absolute measurements of light intensity are made only for the maximum intensities. The lower intensities obtained by the interposition of neutral filters are measured relatively to the maximum intensity. For this purpose a photomultiplier with a RCA 931A cell is used. The cell is placed over the microscope eyepiece in the plane of the field of vision. The set of neutral filters makes possible the reduction of light intensity to any desired level.

SUMMARY

The monochromator apparatus with interference filters is used for irradiation of microscopic objects. The apparatus allows the simultaneous irradiation of 10 slides with monochromatic light within the range 400 to 750 mμ. The diameter of the irradiated field is 2 mm. The maximum intensity when single line filters are used ranges from 15 000 ergs/cm²sec. for 400 mμ to 100 000 ergs/cm²sec. for 700 mμ.

A method is described for measuring the absolute light intensity when the illuminated surface has a small area. The method consists in the application of a specially constructed thermocouple allowing measurements of light intensity of a beam of light 0,3 to 6 mm. in diameter. The sources of errors arising in the measurements are analysed.

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