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COOKE PHASE CONTRAST MICROSCOPE

Abbey 3317

SC 20

Reproductions of recent work with the Phase contrast Microscope are included at the end of this booklet for which we are indebted to the Director of the National Institute for Medical Research for Nos. 1, 2, 4, 5, 6, 7, 9, 14, 16, 17 and 18, to Dr. Robert Barer, Department of Human Anatomy, Oxford for Nos. 3, 10, 11 and 12, 13, 15, 19 and 20, and to Dr. A. F. W. Hughes and Dr. A. Martin, Strangeways Research Laboratory, Cambridge, for No. 8.

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Cooke Troughton & Simms

PHASE CONTRAST MICROSCOPY

The phase contrast microscope has already been applied to a number of problems with interesting results and new applications are constantly arising. To assist the biologist we have listed below some of the directions in which useful progress has recently been made.

1. For the study of living cells in tissue cultures when the explanted fragments of tissue grow outwards in a thin sheet on the nutritive medium. Both embryonic and adult dividing cells may be examined and records of the mitotic process obtained.

2. Examination of living cells in accessible material such as insect larva, membranes such as the mesentry, tails of tadpoles, etc.

3. Living macrophages, protozoa, etc., may be studied.

4. The motility and morphology of spermatozoa.

5. The study of living bacteria including the action of drugs thereon. Counting of blood and bacteria in haemacytometer.

6. As an adjunct to the routine methods for the study of ordinary fixed and stained (or unstained) material.

7. In pathology for the study of exudates, urinary deposits, smears from tumour biopsies, etc.

THE PHASE CONTRAST MICROSCOPE

One of the most difficult tasks with which the microscopist is confronted is that of rendering visible the detail in transparent material which is immersed in a medium of almost identical refractive index. This problem may arise in various forms and occurs commonly in the examination of small living organisms formed of transparent tissue immersed in an aqueous medium. Apart from staining techniques (which usually involve fixation and consequent death of the specimen) the problem has been partially solved previously by two methods :—

(a) By the use of dark ground illumination. This method is very satisfactory for some types of object, but tends to reveal the surface layers rather than the internal details of the object. It also necessitates a very intense light source, which may damage delicate specimens.

(b) By the use of bright field illumination with a very narrow cone of rays, i.e. with the condenser iris diaphragm nearly closed. Considerable diffraction effects are produced by this means, so that the extent to which the image is a true representation of the object is doubtful and the full resolving power of the objective is not utilized. Also the depth of focus is very great, which is a disadvantage for many purposes.

The phase-contrast method does not suffer from any of the limitations enumerated above and the internal details of living cells are brought out in a striking manner. Those parts of the object having higher refractive indices than the surrounding medium appear dark against a lighter background. A light source of only low intensity need be used, and the whole aperture of the objective contributes in forming the final image, which is therefore well defined and the depth of focus small.

The principle of the phase-contrast method has been described by Zernike and others (see references on page 19) and may be stated briefly in non-mathematical terms, as follows :—

According to the Abbe theory of illumination * in the microscope, when light from the condenser is incident upon an object consisting of a fine grating, diffraction spectra are formed and may be observed in the back focal plane of the objective. The detail in the image is a result of interference between the direct and diffracted light and is resolvable if at least the zero order and first order spectra can be observed. This is commonly verified by observing the diatom Pleurosigma Angulatum, the regular structure of which gives rise to a striking series of spectra when observed with a high power objective.

* Martin. An Introduction to Applied Optics, pages 100-119. Pitman, 1932.

If now the grating be regarded as composed of alternate strips of transparent material having slightly different refractive indices (representing, say, the detail in transparent cells in an aqueous medium) then rays of light which have passed through alternate strips acquire slight phase differences which, in the case under consideration, are so small that the observed differences in intensity are slight or negligible under ordinary observing conditions and the image of the grating is barely visible. The introduction of a phase plate into the focal plane of the objective converts these slight differences of phase into appreciable changes in intensity.

A diagrammatic scheme of the arrangement adopted in the Cooke Phase Contrast Outfit is shown in Fig. 1.

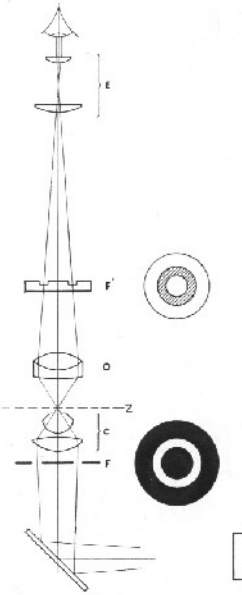


Fig. 1

An annular diaphragm is mounted in the anterior focal plane, F, of the substage condenser so that the object Z is illuminated by a hollow cone of light. The direct image of this bright annulus is formed by the objective O in its back focal plane F' together with other diffraction images (due to structure in the object) which are displaced from the optic axis. In the plane F' is placed the phase retarding plate which consists of another annulus, exactly matching the direct (or zero order) image of the condenser annulus. This introduces a phase difference of $\pi/2$ radians (one quarter of a wavelength) between the beam which is directly transmitted and the light diffracted by the object and passing through the area of the back aperture of the objective not covered by the annulus. A sectional view of the phase plate is shown in Fig. 2 greatly exaggerated in vertical dimensions.

The optical paths AB and ab differ by one quarter wavelength of green light and therefore introduce a phase difference of $\pi/2$ radians.

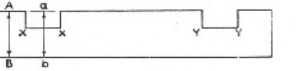


Fig. 2

The effect of this, as has already been stated, is to convert phase differences into intensity variations and causes the image of the grating previously considered to consist of alternate light and dark strips, instead of being almost invisible.

Whilst the method gives the above advantages when used on a transparent object, it does not help in the observation of light absorbing material, such as stained specimens. In fact, it will be found that a less satisfactory image results when phase contrast is used on an object which varies the amplitude and not the phase of the incident light.

The annulus XX, YY, is metallized in order to reduce its transmission, so that the intensities of the two interfering beams may be more nearly equal and result in enhanced contrast.

It is important that the annular diaphragm at F shall be positioned so that its image is entirely covered by the phase annulus XX, YY, and hence accurate centring means are provided.

The Cooke Phase Contrast Microscope is illustrated on page 1. Its most important features are :-

(a) Phase contrast objectives. 2 mm. achromatic, oil immersion
4 mm. ,,
8 mm. ,,
16 mm. ,,

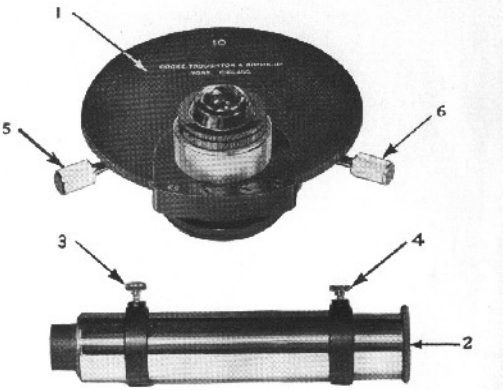
each fitted with a phase retarding annulus in its back focal plane. This can be seen as a grey ring when the objective is held up to the light, but does not interfere with its use by other methods of illumination, except when the requirements are of a most exacting nature.

(b) Rotatable changer for annular condenser diaphragms, mounted beneath special 1.0 N.A. condenser. A separate diaphragm is required for each objective and centring is provided by means of the two screws. The iris diaphragm has independent centring adjustment.

(c) Auxiliary microscope for examining the back focal plane of objectives and ensuring that the image of the condenser annulus is in coincidence with the grey ring of the phase plate. This fits into the microscope tube in place of the eyepiece and has a considerable range of focusing movement.

The condenser is designed to have a working distance adequate for slides of standard thickness.

The phase-contrast outfit is suitable for use with microscopes of the M1000 (except M1005/25/27), M2000, M3000, M4000 and M7000 series, both with monocular and binocular eyepieces.



Adjustment

The microscope is set up in the usual way, except that the special condenser unit is fitted to the instrument in place of the standard condenser and the phase-contrast objectives are screwed into the objective changer.

The condenser annulus changer (1) is rotated until the figure O appears in the circular window and the iris diaphragm is stopped down and brought to a focus. The iris is then centred by means of the adjustment provided (not shown above). The object is brought into focus, using the required objective, the annulus changer being then rotated until the appropriate magnification figure (10, 20, 40 or 95) appears in the window.

The eyepiece is now replaced by the auxiliary microscope (2) which can be adjusted upon unclamping the screws (3 and 4) until the brightly illuminated condenser annulus is sharply focused and can be seen overlapping the grey ring of the phase plate, Fig. 3a.

The condenser annulus is centred by means of the screws (5 and 6) until its image is concentric with and is completely covered by the phase annulus, Fig. 3b. If necessary the condenser may be raised or lowered slightly in order to perfect the registration, and a slight movement of the substage mirror may be desirable to ensure that the annulus is uniformly illuminated.

Fig. 3a

Fig. 3b

The system is then in adjustment, and on replacing the auxiliary microscope by the eyepiece a phase contrast image is observed. The registration of the two rings must be reset as above whenever the objective is changed, but it will be found that with practice this becomes a very simple and rapid operation.

If it is required to examine the object under bright-field conditions, it is only necessary to rotate the annulus changer until the "O" appears in the window and close the iris diaphragm to the desired extent.

M1741 Phase-contrast Unit for use with normal slides with cover glasses, complete with condenser, four annular diaphragms and auxiliary microscope, for use on Cooke microscopes of M1000 (except M1005/25), M2000, M3000, M4000 and M7000 series equipped for use with transmitted light.

M621 Phase-contrast Unit as M1741 but suitable for use on Vickers Projection Microscopes of M500 series equipped for use with transmitted light.

Achromatic Objectives equipped for phase contrast and corrected for 160 mm. tube length.

For Bench Microscopes	For Vickers Projection Microscopes	
M1461	**M661**	16 mm., N.A. 0·28, 10×
M1466	**M666**	8 mm., N.A. 0·45, 20×
M1471	**M671**	4 mm., N.A. 0·65, 40×
M1476	**M676**	1·8 mm., N.A. 1·30, 95×

M575 Corrector Lens, required when using M661, M666, M671 or M676.

PHASE-CONTRAST EQUIPMENT FOR INCIDENT LIGHT

Incorporating positive and negative phase contrast and dark ground illumination.

Phase-contrast equipment is also made for use with incident light for examination of opaque specimens. The illustration on page 10 shows the equipment mounted on a Cooke M3140 stand, and it may be supplied with fittings suitable for attachment to most Cooke microscopes, including the Vickers Projection Microscope.

The illuminator tube carries the annulus which is used for all powers of objectives. The annulus is readily moved in and out of action and is provided with a centring adjustment. The illuminator tube also contains a condensing lens, fitted with a variable power adjustment whereby the annulus may be made to coincide with the phase plates. An auxiliary microscope, which is inserted in place of the normal eyepiece, enables the annulus to be centred on to the phase plates.

Each objective is fitted with quick-change adaptor enabling it to be mounted in the reflector body. The latter carries a slide enabling four systems of illumination to be available, viz., positive and negative phase contrast, dark ground illumination and normal incident illumination.

The optical systems are " bloomed " in accordance with our usual practice for incident light microscopy.

M1753 Incident light phase-contrast equipment for use on Cooke Microscopes of **M1000 series.**

M2753 as M1753 but for use on Cooke Microscopes of **M2000** or **M3000 series.**

M4753 as M1753 but for use on Cooke Microscopes of **M4000 series.**

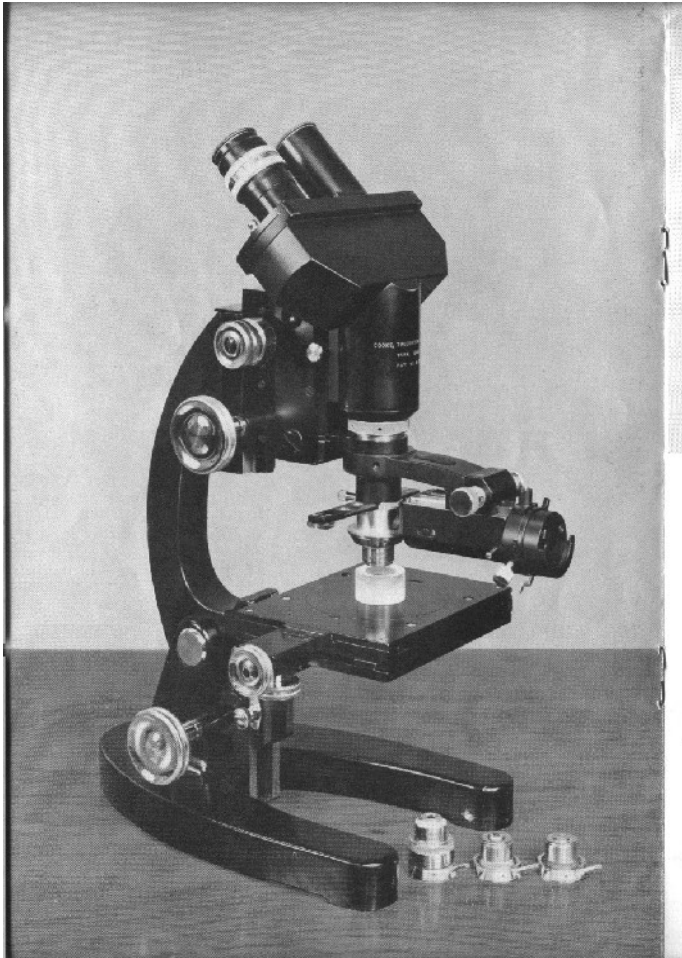
M7853 as M1753 but for use on Cooke Polarizing Microscopes of **M7000 series.**

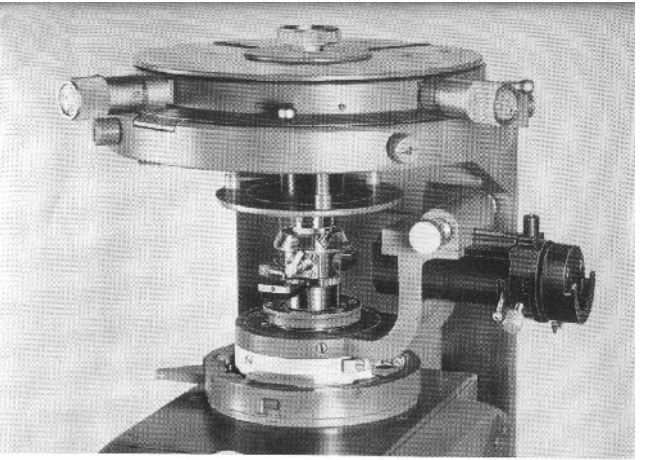
N.B. Polarizing agents cannot be used in conjunction with phase-contrast.

Objectives for use on Bench Microscopes :—

Achromatic, provided with individual objective centring quick-change adaptor. Corrected for use on uncovered specimens and 160 mm. tube length.

M1463 16 mm., N.A. 0·28
M1468 8 mm., N.A. 0·45
M1473 4 mm., N.A. 0·65
M1478 2 mm., N.A. 1·30



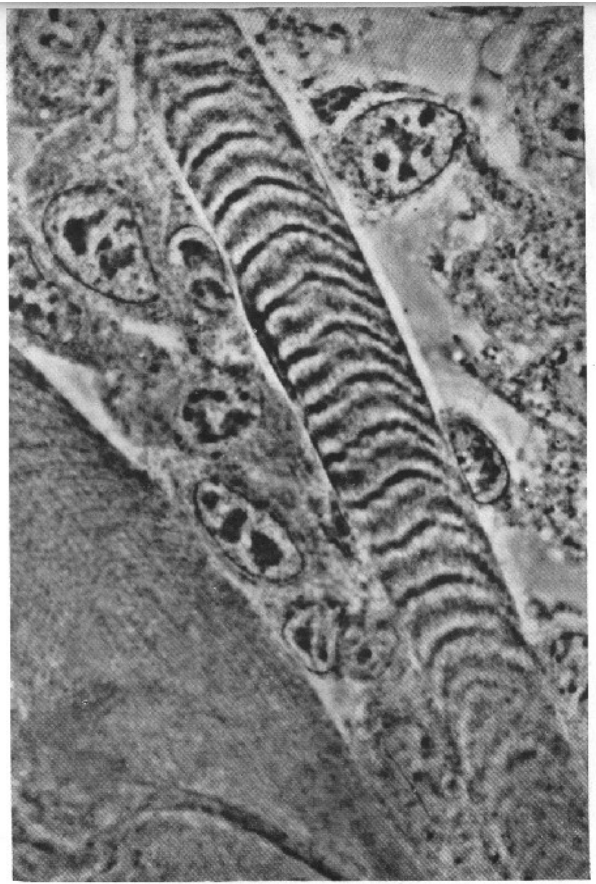


M653 **Incident light phase-contrast equipment** for use with **Vickers Projection Microscope,** as shown above. The unit supporting the objective and illuminator tube is attached to the instrument by a bayonet joint and is interchangeable with the universal illuminator. The specimen is supported on a superstage which is clamped firmly to the main stage of the instrument. The reflector body, which incorporates a slide with four positions, as described on page 9, is provided with a magnetic centring mount and a quick-change system for the objectives.

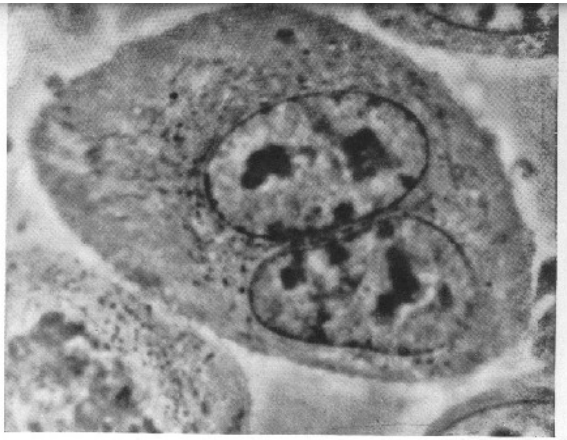
Objectives for Vickers Projection Microscope :—

Achromatic, provided with quick-change adaptor. Corrected for use on uncovered specimens and 250 mm. tube length.

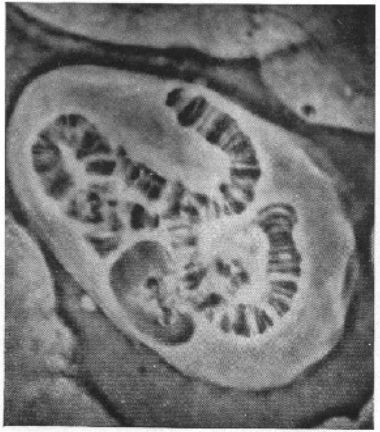
M663 16 mm., N.A. 0·28
M668 8 mm., N.A. 0·45
M673 4 mm., N.A. 0·65
M678 2 mm., N.A. 1·30



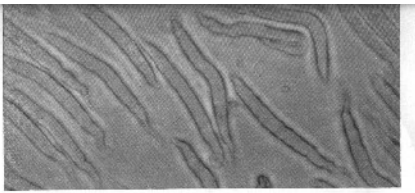
1. Living preparation of sarcoma cells infiltrating muscle fibres. 3250×



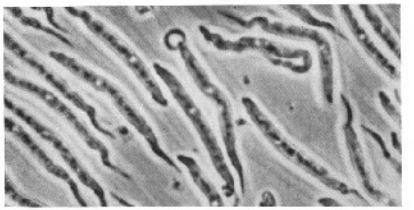
2. Structure of a binucleate malignant cell (sarcoma). 2600×



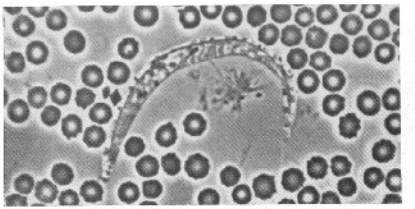
3. Living salivary gland nucleus of Chironomus, showing nucleus, nucleolus and chromosomes. 850×



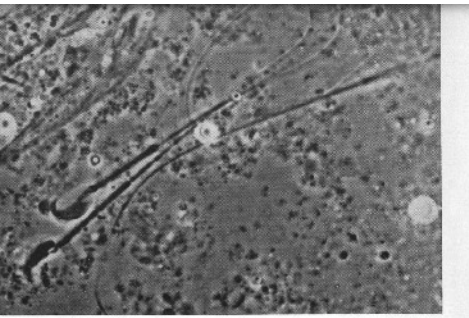
4. Living microfilaria from adult worm by transmitted light. Sheath surrounding organisms not visible. 810×



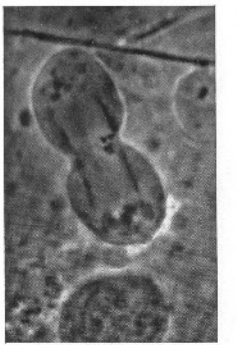
5. The same field as preceding figure by phase-contrast microscopy. Note indications and enveloping sheaths, although flare obliterates their outline in the vicinity of the organisms. 810×



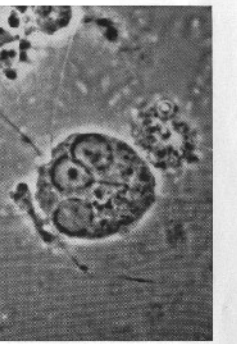
6. Living microfilaria in blood of monkey by phase-contrast microscopy showing enveloping sheath. 810×



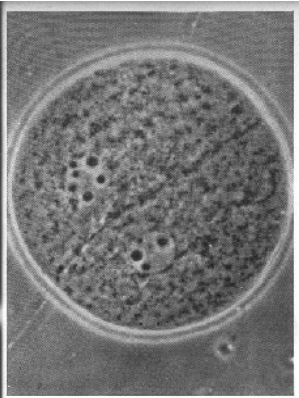
7. Immature spermatozoa from adult mouse, showing spiral formation in mid piece. 1060×

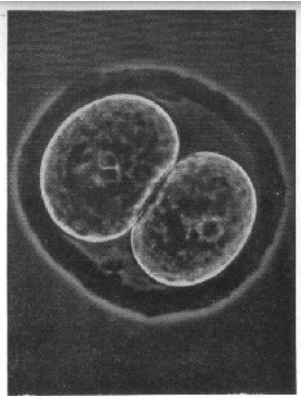


8. Telophase of primary spermatocyte of locust. 1000×



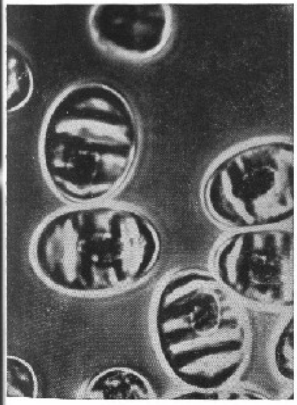
9. Preparation from the testicles of adult mouse. Three spermatids developing within one cell showing developing head, cap and tail. 1750×

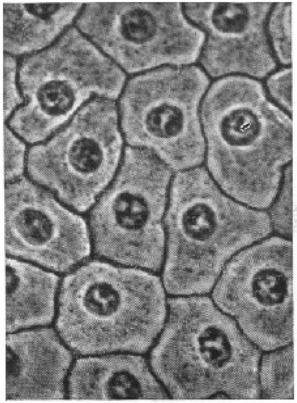




The fertilisation and early development of the living rat egg.
17. Egg in 2-cell stage. Pressed 570 ×

18. Egg in 2-cell stage. Unpressed 570 ×





19. Red blood corpuscles of salamander. 700 ×

20. Squamous epithelium from skin of frog. 750 ×

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	M1708 For 16mm. objectives, M522					7 14
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	M1659	12	M1704	9 4	M1731	29 18
	M1661	2	M1708	7 14	M1732	36 16
	M1662	4 0				
16	M1704	9 4	M1703	14	M1591	6 6
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	E845	3 14	M1695	4	M1376	3 0
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	M1659	12	M1581	1 2	M1379	1 6
	M1660	12	M1586	2 2		
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	M6070	62 12	M6416	6 10	M1524	4 8
	M6100	71 4	M6419	9 10	M6507	6 8
	M6110	62 12	M6421	6 18	M6512	6 8
	M6390	3 8	M1509	3 2	M6517	6 8
	M6406	5 12	M1517	2 12	M6522	6 8
18	M6200	36 0	E125	3 14	M6512	6 8
	M6210	30 10	E230	1	M6517	6 8
	M6391	12	M6502	3 14	M6522	6 8
	M6443	5 6	M6507	6 8		
19	M6131	58 18	M6151	61 6	M6230	34 12
	M6132	58 18	M6152	61 6	M6240	37 0
20	M7021P	91 4*	M7734	15 18	M7659	12
	M7306	5 4	M7831	14 12		

Page		£ s.		£ s.		£ s.
21	M7110Q	114 8*	M7734	15 18	M7316	1 8
	M7306	5 4	M7831	14 12		
22	M7210R	230 4*	M7306	5 4	M7734	15 18
	M7250R2	257 16*	M7316	1 8		
23	M7806	5 2	M7921	9 4	M1535	2 12
	M7811	4 4	M7922	9 4	M7506	4 4
	M7818	6 16	M7923	11 0	M7511	5 12
	M7820	9 4	M7931	16 8	M6516	3 0
	M7821	7 8	M7508	2 8	M6521	3 0
	M7822	7 8	M7509	3 18	M7513	6 16
	M7823	9 4	M7516	3 6	M7585	5 12
	M7831	14 12	M7517	4 16	M7586	2 2
	M7906	6 18	M1521	1 10	M7587	1 12
	M7918	8 12	M1522	3 6	M7590	8 10
	M7920	11 0				
24	M1865	21 16	M1867	35 6	M1900	3 18
	M1866	21 16	M1868	35 6	M1919	3 18
25	M1878	71 6	M1919	3 18	M7701	4 2
	M1879	71 6	M2303	4 2	M7706	10 14
	M1900	3 18	M2304	10 14		
26	M1752	5 6	E845	3 14	M1816	50 0
	M1806	11 16	E850	14	M1820	50 0
	M1808	11 16				
27	M1818	50 0	M1839	40 16	M1759	10
	M1822	50 0	M1841	40 16	M1760	10
	M591	3 2	M1813	2 8	M1761	10
	M592	3 2	M1845	66 4	M1762	10
	M1824	74 12	M1849	66 4	M1763	10
	M1828	81 8	M1847	66 4	M1764	10
	M1826	74 12	M1851	66 4	M1765	4
	M1830	81 8	M1945	45 18	M597	1 8
	M598	12 18 10	M1758	10		

*Prices of microscopes without optical outfits:—

	£ s.		£ s.
M1150	49 12	M1225	48 14
M1170	56 2	M7021	66 4
M1155	87 10	M7110	85 6
M2150	121 12	M7210	190 14
M2190	133 6	M7250	215 6
M1235	55 6		