

Mr. T. W. Barkley

# WATS ON

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## SALES CONFERENCE

The Sales Conference has now been confirmed and will take place on Wednesday and Thursday, July 8th and 9th. The theme of this next Conference will be "Selling Quality" and Mr. Macdonald will be writing separately to each of you with a draft programme of the meeting.

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## FORTHCOMING EXHIBITIONS

The Company is holding one of its University exhibitions in the Department of Anatomy, University of Manchester on the 30th June and the 1st July, where a full range of equipment will be on display. Invitations approaching the 1,000 mark are being sent out early next week and the exhibition will be staffed by Mr. Barkley, Mr. Thompson and Mr. Jackson.

A small exhibition is being held at University College of London on July 14th, in conjunction with a conversazione held by the Institute of Biology and we are demonstrating the Zoom stereo, the Land Camera, the W.I.S.E and low cost phase contrast with Service 3. The last Institute of Biology meeting was held at Imperial College just over 12 months ago and was extremely well attended and we are hopeful that this one will also be a success.

On the same day and also the day after, July 15th, we are staging an exhibition at Imperial College, London, in conjunction with the International Congress of Entomology. Space is again limited, but apart from the products being shown at the above mentioned conversazione, we are also demonstrating the 35mm. Camera Attachment, the Fluorescent Illuminator and a range of stereoscopic instruments. Both of these exhibitions will be staffed by personnel based at Barnet.

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From August 4th to August 8th we are committed to a fairly extensive exhibition in Edinburgh in conjunction with the International Botanical Congress where a comprehensive range of equipment will be demonstrated.

We are anxious to have further Watson exhibitions, particularly in University cities and at the moment we are hopeful of holding these demonstrations in Liverpool and Belfast during September. Other venues proposed for later exhibitions are Cardiff, Leeds, Cambridge, Leicester, Southampton and London.

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## NEW PRODUCTS

The x10 micrometer eyepiece for use with the 5:1 Zoom stereo-microscope has now been designed and approved and placed into production. A costing has been formulated and this will be listed at £15.0.0d. to include any standard 24mm. diameter graticule. The Code No. is 508 and Production is expected to have these available for delivery by mid-October.

The x3 Parachromatic objective, Code No. 1200, has now been approved and placed in Production. This is expected to be available mid-October and will be priced at £3.14.0d. It is important to note that this will only be manufactured in the new 45mm. parfocal length, but it can be used on any existing microscope and it will, of course, be parfocalled with high power objectives when supplied with a Service 3 microscope. This objective is intended to replace the present 50mm, x2.75 Parachromatic objective which after about 12 months will become completely obsolete.

The x5 Parachromatic objective has also been approved and placed in Production. Deliveries are expected mid-October. This objective will be made in both 34 and 45mm. parfocal lengths, Code No's 202 and 1202 respectively. These objectives will, of course, be parfocalled with higher powers of their own parfocal length category and will replace in due course the existing 40mm, x4 Parachromatic objective N.A. 0.13. Both of the objectives (Code No's 202 and 1202) will be priced at £3.14.0d. each.

Polarising accessories for use with the Bactil-60 are in the course of development and further details will be given to you at the Sales Conference in July.

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## PHASE CONTRAST ACCESSORIES FOR SERVICE 3

As promised under J12/5.64 we are now able to give you further information regarding this equipment. The means by which alignment of the annular disc and the phase plate is undertaken will now be by either an auxiliary telescope or a built-in Bertrand lens. The cost of either method is £9.10.0d., but you will appreciate that customers requiring the Bertrand lens must order this at the time of purchasing a new Service 3 microscope. The complete phase contrast outfit will be supplied in a fitted box which will be sufficiently large to accommodate all of the following items: Auxiliary telescope - £9.10.0d., three objectives fitted with phase plates complete in power boxes, phase plates at £7.0.0d. each, annulus centring device complete with lamp condensing lens and annular discs at £7.0.0d., Universal No. 1 condenser in either plain or centring mount at current list prices. The outfit will, of course, include full operating instructions.

Although it is possible to use the phase contrast equipment with a Universal condenser in plain mount, a centring mount will be found more useful and simplify the setting up procedure and this should be recommended. The accuracy with which plain mounts are centred to the optical axis is not consistent. With regard to the phase plates, these can only be fitted at the Works to the 45mm. parfocal length objective and will not be removeable by the customer. Existing objectives, however, can be fitted with suitable phase plates so that a customer can add phase contrast equipment to a Service 3 microscope at any time.

The first advertisement for this equipment will appear in July in conjunction with the announcement of some other improvements of the Service 3 microscope. This advertisement will at the same time be printed as a leaflet to be distributed with all Service 3 catalogues and be backed by complete details of the phase contrast equipment and more detailed information of other improvements.

The above mentioned improvements are:- 360° rotation to all Service 3 monocular and binocular heads; availability of 704 flat top mechanical stage (Unit Construction Development), and Service 3 finished black. All of these improvements will be available for delivery by August/September of this year.

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You will be pleased to learn that we recently received a substantial order for Service 3 microscopes and also for stereoscopic instruments from the University of Bristol. Congratulations to Mr. R. E. Fryer.

Congratulations are due also to Mr. A. Bailie for negotiating the first quantity order for Zoom stereos from Imperial College.

Congratulations seem to be the order of the day this month and last but by no means least of all, our best wishes go to two hard-working members of the Sales staff, Miss V. Dellow and Miss V. Wheeler, both shorthand-typists, who recently married and are now away honeymooning. I am sure you will all join with the Editor in wishing them every happiness in their future lives.

Reference J15/564, the Editor wishes to acknowledge communication received from Mr. J.D. Casartelli, dated the 21st May, giving his thoughts and suggestions on a possible replacement for the Abbe Camera Lucida. The proposals have not yet been discussed with our Technical Department due to pressure of other developments, but his remarks will be valued when discussion eventually arises.

The Editors of both this section and the Technical section are most disappointed that they have not received contributions from representatives and it would seem that you are all leaving it to Mr. Casartelli to set the pace. Quite apart from his letters to the Editor we now understand that he has very recently completed the bulk of the work on his new book.

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## THE MARKET

There is some evidence that business is looking up and although money is still very restricted in University Departments where examinations are in full swing, there is an increase in demand for student microscopes. This increase is almost certainly due to the increase of student numbers and it should be remembered that almost every University is accepting more students this year.

Most Departments have already spent their grants for the current financial year ending in July, but finance is being made available in some way or other as the instruments are required for use during the next session starting in October.

The competition for these orders is very tough and must never be ignored even when you feel that the order is "in the bag". With almost every one of the recent Departmental orders the representative concerned has found the negotiations long and exacting and he has had to do a very real selling job on the features of the instrument being offered. On many occasions he has done an excellent job only to find that the competition is queuing up at the door to offer something different or a little better and the importance of following up of potential business cannot be overstressed. If the order is a substantial one the follow up visits should be frequent and at regular intervals so that the representative can get the "feel" of the way things are going.

Quite naturally the first demonstration and interview with the customer is perhaps the most important of all and an effort should be made at this stage to find out as much as possible. For example:- What do they want? What do they want it for? When do they want it? (This information is vital and a certain knowledge of their requirements in this respect will go a long way to ensure a successful sale in the end). When do they expect to order it? Is the money available yet? How much money is available? Would they really like something different? (Perhaps a minor modification). These are but a few of the obvious questions to ask and above all do not be afraid to ask them, (tactfully, of course), and do not forget to put all the answers on your report.

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## TECHNICAL SALES MEMORANDUM NO. T 12

### Introduction to Fluorescence Microscopy

In conventional microscopy a beam of light is passed through a specimen, or reflected from it, and an image is formed indicating the way in which the specimen has modified the beam. A much simpler form of microscopy is possible with specimens emitting visible light but this has not been important in the past as such specimens are rare. Self luminous specimens being examined microscopically include the 'phosphor' coatings of cathode ray tube screens and also certain semiconducting materials which emit light when an electric current is passed through them. Many materials are also known which emit visible light when irradiated with shorter wavelength light usually in the near ultra-violet - this phenomena is called fluorescence.

The whole field of fluorescence microscopy stems from the fact that some fluorescent materials - called fluorochromes - are not only highly selective stains but also show enough fluorescence to form a usable image at high magnifications when used at extreme dilutions of a few parts per million.

Conventional biological microscopy uses either fixing and staining technique with bright field illumination or alternatively, phase contrast or interference contrast microscopy on fresh unprepared material. The former suffers from the major disadvantage that the specimen preparation often alters or destroys the fine structures to be examined and the latter from the disadvantage that no selective staining is possible and the image indicates only the density distribution of the specimen with no indication of the biochemistry. (Phase Contrast is singularly unsuccessful with stained material because the technique uses an interference phenomena between light refracted by the specimen and the background light. Staining the specimen alters the spectrum of one beam relative to the other and reduces the interference effect dramatically. Even very light staining may reduce the contrast). Fluorescence microscopy is remarkable insofar as it enables selective staining to be used with minimum 'treatment' of the specimen and in some cases with living specimens. The main difficulty is the expensive cumbersome equipment required to produce the primary ultra-violet beam of exciting radiation.

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The notes below indicate the main requirements of the various components of the equipment.

1. Light Source. An intense source of near ultra-violet is required. Mercury Arc lamps are commonly used but they have many disadvantages including high cost and intense radiation at many other wavelengths making elaborate filtration necessary.
2. Primary Filters. To remove heat and visible light from the beam with minimum attenuation of the near ultra-violet. The most useful filter is a glass ultra-violet filter (Chance OX1) but this also passes some red and infra-red. The heat and red-end can be filtered out with a copper sulphate bath or a blue filter - the former attenuates the near U.V. less but requires frequent topping-up. An interference filter can be used to give higher transmission at 3650 Å than glass filters but this does not help with certain fluorochromes which depend on the smaller energies at shorter wavelengths. Acridine orange and some similar fluorochromes are used with blue light fluorescence when blue glass filters only are used as primary filters.
3. Substage Condenser. It must be remembered that the fluorescent radiation from the specimen will be emitted in all directions so that the objective will always be working at full aperture and the numerical aperture of the condenser does not affect image quality. The function of the condenser is merely to concentrate as much primary radiation as possible onto the specimen with fairly even intensity over the field. Achromatic condensers should be avoided as their corrections are meaningless in the U.V. and the thickness of glass absorbs the shorter wavelengths. Also the cements used in their construction are a possible source of stray fluorescence. Best results are obtained with simple uncorrected condensers or with reflecting dark ground condensers for high magnification work. An immersion dark ground condenser is best for a 2mm. objective as it concentrates the beam into a small area and also because most of the primary beam does not enter the objective, less dense primary filters can be used.
4. Immersion Fluids. Cedar wood oil is unsuitable as it fluoresces. Mineral immersion oils (like Watson immersion oil) are satisfactory when fresh and kept in a stoppered bottle but their oxidation products fluoresce and stale oil is unsatisfactory. Commercially available non fluorescent

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immersion oil is similar to the ordinary mineral oil but may be more stable.

Liquid Paraffin or Blycerol are completely non fluorescent but do not have the correct refractive index.

5. Slides. White glass slides are alright but the cheaper green ones tend to fluoresce.
6. Objectives. Standard Watson objectives have not been observed to fluoresce but some manufacturers charge fancy prices for special non fluorescing objectives.
7. Secondary Filters. The clear U.V. stopping filters used as haze filters for outdoor colour photography are not suitable as they do not attenuate the very near U.V. (3650 Au) sufficiently to be safe but they can be used in conjunction with a dark ground condenser. Pale green (OY13) filters are usually used. For blue light fluorescence an orange secondary filter is required.
8. Binocular Heads. For many techniques there is insufficient light available for a binocular head. Increasing the primary excitation does not always increase the fluorescence owing to a phenomena called quenching. In some cases quenching occurs after a period of exposure so that it may not be possible to observe the same area of a specimen for a prolonged period.
9. Glare. All parts of the primary illuminating system have to be completely screened from the operator and an orange glass screen is also provided, in most equipment, to prevent the operator viewing the specimen directly.

Fluorescent microscopy has become an accepted research tool and is used to a small extent for diagnostic work mainly for T.B. The \$ 64,000 question, for microscope manufactures, is whether it will become a routine diagnostic method for a range of diseases especially cancer. The recent decision by the M.O.H. to establish Training Centres for teaching the identification of malignant cells by conventional microscopy indicates that the breakthrough with fluorescence methods has not yet arrived in this field.

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## CHEMICAL MICROSCOPY

by G. G. Cocks Ph.D.

Reproduced from the American Chemical Society 1964 Annual Reviews.

The previous review in this series by Coven and Cox (73) covered the two-year period ending in October 1961. This review covers the two-year period ending in October 1963, but also contains references to a few earlier publications, and to some publications in November and December of 1963.

It is obviously impossible to review all the publications in which chemical microscopy has played an important role. Therefore, no attempt has been made to cover publications in the biological, metallurgical, and geological applications of microscopy, except for those which seemed to be of general interest to chemical microscopists. Electron microscopy is not included as it is the subject of a separate review. An attempt has been made to include references to publications in the fields of optics and crystallography which are of potential interest to chemical microscopists.

## MEETINGS AND SYMPOSIA

The first American meeting of the Royal Microscopical Society was held in April 1963 in celebration of the tercentenary of the microscope in living biology. Although this meeting was primarily concerned with biological applications, a number of papers of general interest were presented. Among these were "Converters and Vidicons" by G. Z. Williams, "Staticons" and "Interference Microscopy and the Living Cell" by R. Barer, "Flying Spot Scanners" by P.O'B. Montgomery, "Fluorescence Microscopy and the Living Cell" by D. Wittekind, "Phase Microscopy and the Living Cell" by G. G. Rose, "The Synthesis of New Methods Incorporating Microscopes for the Analysis of Molecular Behaviour of Cells" by K. R. Porter, and "Photo Materials for Recording Low Light Intensity through the Microscope" by E. H. Land.

The series of symposia sponsored by McCrone Research Institute was continued, with "Micro-62" being held in Chicago and "Micro-63" in Brighton, England. The papers presented in the "Micro-63" meeting are being published in The Microscope and Crystal Front, which recently changed its name from The Microscope and Entomological Monthly.