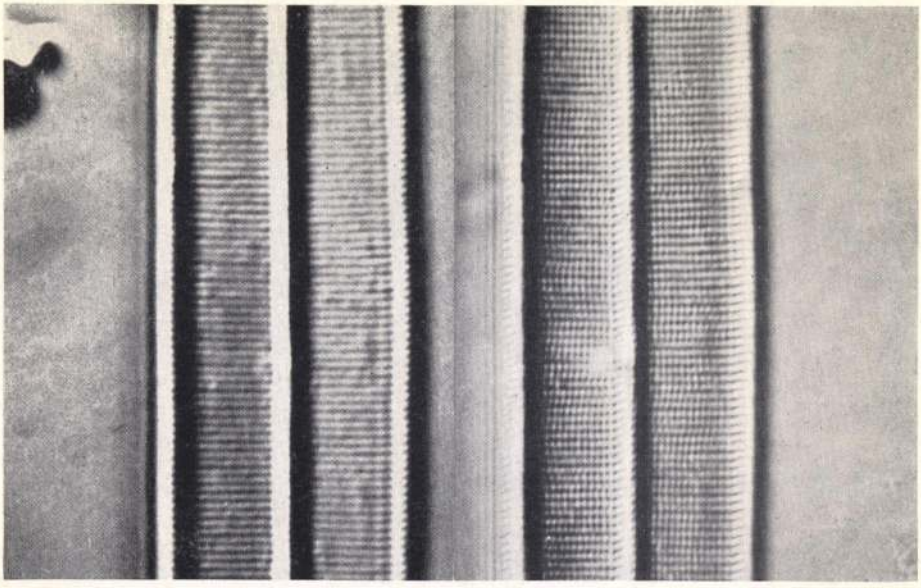
# ULTRA VIOLET
## Photomicrography

AT 3650Å

## Optics and Other Accessories

BAUSCH & LOMB
OPTICAL COMPANY
ROCHESTER NEW YORK

*William A. Sokol*



*Amphipleura Pellucida magnification 4,000X. Left, made with 3 mm 1.40 N. A. apochromatic objective in green light. Right, made with 1.7 mm 1.30 N. A., U V objective in ultra violet light (365 mμ). Both photographs were made with oblique illumination. The angle of obliquity was chosen to show the diatom resolved into beads when photographed in ultra violet light. The same setting of the illuminating apparatus was used to take the photograph in green light. A picture showing more contrast of the lines in the diatom could be taken in green light with a different adjustment of oblique illumination.*

## *Bausch & Lomb Offers*

## *a simple and easy means of making*

# PHOTO-MICROGRAPHS...

## *in the Ultra Violet*

ABOUT sixty years ago, although scientists in general were familiar with the microscope, only a very few had the privilege of using them and fewer still could claim proud ownership. Looking back on the wonderful advances the microscope has made possible, we can value today the tremendous service that has been rendered by this instrument.

Now, Bausch & Lomb announces a development in ultra violet photomicrography. It is an extremely convenient and comparatively inexpensive means of taking advantage of the superior resolution obtained when a shorter wave length of light is used for illumination.

## *RESOLUTION*

Reviewing the capabilities and limits of the microscope, the word "resolution" stands out as most important. Since resolution is the distinct separation of two small details in a structure, it is apparent that all other qualities of the microscope are useless without this prime factor. High magnification is useless if it is not accompanied by a proportionate high resolving power.

The resolving power of a microscope is determined by the numerical aperture of the objective and the wave length of light used for illumination. Stated as a formula this is:

$$\text{Resolution} = \frac{\lambda}{2\ \text{N.A.}}$$

The numerical aperture, or N.A., of an objective is limited by several factors. The most important are focal length and angular aperture. In reducing the focal length of high powered objectives to 1.9 mm, the practical limits have been reached, since some working distance must be allowed. Aperture angle is widened to approximately its greatest degree by the use of immersion oil between the objective lens and the object. Therefore, there is but one element within our power to change . . . the wave length of light. As can be seen from the formula, the shorter the wave length of light the greater the resolving power.

The use of shorter wave lengths, however, presents two serious obstacles: the finding of a material with the necessary physical characteristics that will allow it to be made into a lens, passing ultra violet light in sufficient intensity; and the difficulty of focusing an invisible ray.

## *Photomicrography with the 275 mμ Line*

Some time ago there was developed an apparatus that uses the wave length of 275 mμ, the cadmium line. This gives a greatly superior resolution over the best microscopes using visible light. And this gives the best resolution obtainable today. But there are several drawbacks to its use. In order to pass the λ 275 mμ quartz optics are necessary throughout, and a spectroscopic outfit to sharply cut out all but the cadmium line. Both of these are expensive. Also, in making photomicrographs with this apparatus, several plates must be exposed within the limits of focus, and after they are developed the final plate is made at the exact point of focus by interpolation from the others.

## *Photomicrography with the 365 mμ Line with the Bausch & Lomb Ultra-Violet Accessories.*

Considering these characteristics which have prevented the wide use of ultra violet photomicrography, A. P. H. Trivelli, of the Eastman Kodak Laboratories, and L. V. Foster, of the Bausch & Lomb Scientific Bureau, began the development of an apparatus that would eliminate the difficulties. Bausch & Lomb is now privileged to offer the results to the microscopist.

The features of this new method:

1. Uses the 365 mμ ultra violet wave length for illumination.
2. The resolution is from 12% to 19% better than that obtained with the best microscopes using visible light.

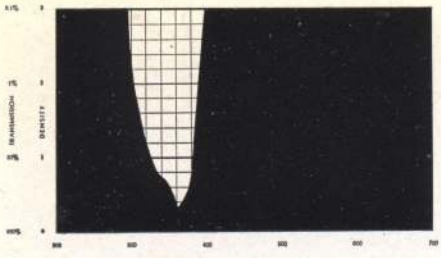


*Fig. 2. Transmission in terms of density of the UV Filter. Note the density at 300 mμ and the 400 mμ is 2.75. This is sufficient density to cut out the lines 302, 312, 404 and 407 mμ.*

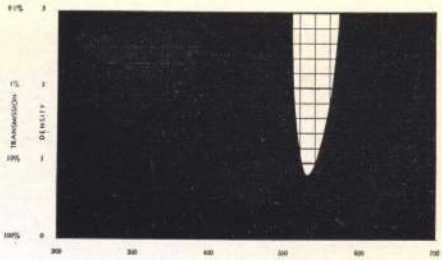


*Fig. 3. Transmission in terms of density of the No. 62 Wratten Filter. Note that the strong 577 mμ line is entirely cut out by the filter which permits the transmission only of the 546 mμ line.*

3. The optical system is of glass.
4. This optical system is corrected to focus both the 365 mμ ultra violet and the visible 546 mμ wave lengths at the same point. Therefore focusing is *visible* and positive.
5. The illuminant is simply a quartz mercury arc lamp of high intensity, with filters.
6. The 365 mμ line is selectively absorbed by many substances, thus bringing out structural detail unobtainable by any other method. This is equally true of transparent and opaque materials.

From these points it can be seen that while this equipment permits of a resolution approaching that of the apparatus using the 275 mμ line, it does not have the difficulties of use and avoids the expense of a quartz optical system and a spectroscopic outfit.

Since the 365 mμ line is well into the ultra violet, it follows that this apparatus will have a resolving power markedly above the best than can be had in microscopes using visible light. By using the formula given for resolving power, it can be seen that with a 1.40 N. A. objective and the 365 mμ wave length the resolving power is 0.13 μ. With an objective of 1.30 N. A. the resolution is 0.14 μ.

In contrast, visible blue light with objectives of the same numerical apertures give resolutions of 0.16 and 0.17 μ respectively. These latter resolutions, however, are practically useless, since blue light has very poor visibility to the human eye, but they serve to show the extremes of resolution under visible light. Likewise, the highest resolution available with quartz optics and the 275 mμ line is 0.11 μ, since quartz objectives are limited for practical use to an N. A. of 1.25.

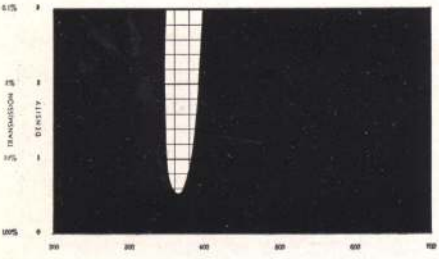


*Fig. 4. Transmission curve in terms of density of the 16 m/m. (42-32-23) UV Objective and the UV Filter. The objective and filter transmit 28.2% of the intensity of the triplet 365 mμ and only 2.5% of the weak line 390 mμ. The intensity of this line is so weak that it will have no effect on a photographic plate which has been only sufficiently exposed for the 365 mμ triplet.*

*Fig. 7. Spectrum of quartz mercury arc lamp. Made with Bausch & Lomb medium quartz spectrograph. Slit with 5 microns—Aperture F:11.0. Shows the strong triplet at 365 mμ and only relatively weak lines at 390, 404 and 407. Lines 302 and 312 in the spectrogram are practically cut out by the UV filter. 334 line is partially passed by this filter.*

For the optics of the Ultra Violet Accessories Bausch & Lomb has developed a special glass which readily transmits in sufficient intensity the 365 mμ wave length. Quartz is unnecessary unless it is preferred for the condenser by the individual microscopist. These glass lenses are corrected to bring into the same plane of focus with λ 365 mμ ultra violet the visible 546 mμ line. λ 546 mμ is green light.

The quartz mercury arc lamp of the Bausch & Lomb Ultra Violet accessories is a special design that gives very high emission in the ultra violet spectrum. The mercury arc being a line spectrum of comparatively few lines lends itself to the formation of a monochromatic source. As can be seen from a study of figures 2, 3 and 7, a single U-V filter can be used to cut out all visible lines and all invisible lines shorter than 300 mμ. This filter transmits the strong triplet at 365 mμ and a small proportion of the other weaker lines 302, 312, 334 and 390. When the absorption of the microscope objectives and the U-V filter is added together, it can be seen from figures 4, 5 and 6, that all of the 302, 312 and 334 mμ lines are absorbed, having a high transmission for the 365 mu triplet and a very low transmission for the weak 390 mμ line.

To focus for photography the green filter provided with the accessories furnishes monochromatic green light. This can be seen by noting in figure 7 that in the middle spectrum the lines are at 404, 407, 436, 546, and 577. All lines except 546 mμ are completely

*Fig. 5. Transmission curve in terms of density of the 6 m/m. (42-32-46) UV Objective and the UV Filter The objective cuts out everything below 350 mμ, with the result that the objective and filter transmit 15.1% of the intensity of the triplet 365 mμ and only 1.6% of the weak line 390 mμ. The intensity of this line is so weak that it will have no effect on a photographic plate which has been exposed for the 365 mμ triplet.*

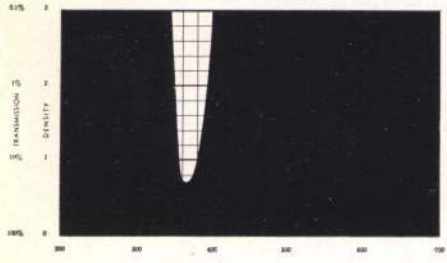
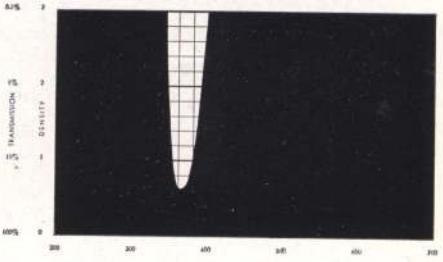


*Fig. 6. Transmission curve in terms of density of the 1.7 m/m. (42-32-45) UV Objective and the UV Filter. The objective cuts out everything below 350 mμ, with the result that the objective and filter transmit 21.9% of the intensity of the triplet 365 mμ and only 2% of the weak line 390 mμ. The intensity of this line is so weak that it will have no effect on a photographic plate which has been exposed for the 365 mμ triplet.*



absorbed by No. 62 Wratten filter as shown in figure 3. When this filter is used between the light source and the microscope, the object can be seen with perfect ease since the eye is exceptionally sensitive to green light. Perfect focus can be made and afterwards the U-V filter substituted, while an exposure is made on a photographic plate. Since the objectives described above are achromatic for these two wave lengths, namely, 546 mμ and 365 mμ, the image formed on the photographic plate is as sharp as that seen by the eye.

Photomicrography with the 365 mμ line has two unique advantages:

1. The selective absorption of this wave length by materials which transmit or reflect light in the visible region frequently brings out detail unobtainable in any other way. Every field of science and industry interested in differentiating between structural differences thus has at hand a new tool for investigation. Such diverse lines of endeavor as botany, entomology, biology, crystology, cytology and metallography obtain new data from the use of ultra violet photomicrography.
2. The increased resolution of the shorter wave lengths allows the investigator to go a step farther in resolving the detail now just beyond the power of his instrument. This advantage applies specifically to the oil immersion objective, which gives the utmost resolution obtainable by this method. In metallography, new structure and detail are rendered. In the biological sciences, visible cell structure and detail are greatly increased.

This is a new field of investigation and it will offer rich returns to all those forward looking investigators, interested in adding to their knowledge through the use of the microscope. For further technical details the reader may refer to the original paper

"Photomicrography with the 365 mu Mercury Arc Line" By R.P.H. Trivilli and L. V. Foster, 2-31, Journal of the Optical Society of America.

For metallography and other work with opaque materials we suggest: U-V objectives 16 mm, 6 mm and 1.7 mm oil immersion, U-V Accessories complete including the Uviarc burner, quartz condenser, filters, etc. When ordering give the type of current, whether A.C. or D.C. and the type of camera that you use.

For transmitted illumination (work with transparent objects) we suggest: U-V objectives 16, 6 and 1.7 mm and the complete U-V accessories with Uviarc burner, quartz condenser, filters, etc. See the price list for details and note the accessory dark field quartz condenser for dark field work.

Canada balsam can be used as a mounting medium, but the usual cedar oil should be replaced by sandlewood oil which has not as high an absorption for λ 365 mμ.

# Specifications and Prices

| Code Word | Catalog No. | Specifications | Price |
|---|---|---|---|
| | | *Achromatic Objectives for Transparent Objects* | |
| *Agujk* | 31-10-23-01 | *Objective* corrected for 365 mμ, 16 mm, 0.25 N.A., dry | $13.50 |
| *Agulm* | 31-10-46-01 | *Objective* corrected for 365 mμ, 6 mm, 0.65 N.A., dry | 27.50 |
| *Agukl* | 31-10-45-04 | *Objectives* corrected for 365 mμ, 1.7 mm, 1.30 N.A., oil. Including bottle of sandalwood oil | 40.00 |
| | | *Stage Accessories* | |
| *Agugh* | 31-51-20 | *Quartz Dark Field Condenser*, Abbe type, with iris diaphragm | 37.50 |
| *Aguhj* | 31-73-81 | *Corex Slide* | 2.50 |
| | | *Lamps and Accessories* | |
| *Ceepg* | 42-44-01-21 | *UV Accessories* for use on type GB Camera, including lamp housing, Uviarc burner, resistance for 110 volt, D.C., quartz condenser, 2 filters (visual and photographic), filter holder, in case | 153.50 |
| *Ceerh* | 42-44-02-22 | *UV Accessories*, the same as above, but with auxiliary for use of 110 volt, A.C | 188.50 |
| *Ceesj* | 42-44-01-31 | *UV Accessories* for use on Type R Camera, with resistance for 110 volt, D.C., the same as for the GB Camera above, but with bracket for fastening filter holder to lamp support | 156.00 |
| *Ceetk* | 42-44-02-32 | *UV Accessories* for Type R Camera, same as above, but for 110 volt, A.C | 191.00 |
| *Ceevl* | 42-44-01-41 | *UV Accessories* for JR type Camera, with resistance for 110 volt, D.C., same as above, but with lamp house support with vertical, adjustable post and inclination joint | 175.00 |
| *Ceexn* | 42-44-02-42 | *UV Accessories* for Type JR Camera, but for 110 volt, A. C | 210.00 |
| *Ceezr* | 42-44-01-51 | *UV Accessories* for Type H, J and K Cameras, for 110 volt, D.C. same as above, but lamp has pedestal base | 178.00 |
| *Cefap* | 42-44-02-52 | *UV Accessories* for Types H, J, and K Cameras, same as above, but for 110 volt, A.C | 213.00 |
| | | *Achromatic Objectives for Opaque Objects* | |
| *Cebal* | 42-32-23 | *Objective* corrected for 365 mμ, 16 mm, 0.25 N. A., dry | 14.50 |
| *Cebem* | 42-32-46 | *Objective* corrected for 365 mμ, 6 mm, 0.65 N. A., dry | 29.00 |
| *Cebin* | 42-32-45-08 | *Objective* corrected for 365 mμ, 1.7 mm, 1.30 N. A. oil. Including bottle of sandalwood oil | 45.00 |
| *Cauvm* | 42-32-92 | *Handle* for objectives used on ILS | 2.00 |
| | | *Lamps and Accessories* | |
| *Cefer* | 42-44-01-01 | *UV Accessories* for use with type ILS Microscope and Camera, or Metallographic Outfit, including lamp housing, Uviarc burner, resistance for 110 volt, D.C., condenser, collective, 2 filters (visual and photographic), filter holder, in case | 162.00 |
| *Cefis* | 42-44-02-02 | Same as above but with transformer for 110 volt, A.C | 197.00 |
| | | *Burners* | |
| *Ceenf* | 42-42-01 | *Uviarc Burner*, for 110 volt, D.C. M.C.P. | 84.00 |
| *Ceemd* | 42-42-02 | *Uviarc Burner*, for 110 volt, A.C. M.C.P. | 90.00 |
| | | *Filters* | |
| *Cegar* | 42-47-73-74 | Two Filters (visual and photographic) | 10.00 |
| | | *Immersion Oil* | |
| *Agufg* | 31-50-08 | 10 cc. bottle of sandalwood oil | 1.00 |

M. C. P.—Manufacturers Current Price.

*The prices herein are subject to change without notice and to increase for taxes, excises or other charges imposed by governmental authorities with respect to articles listed herein or to the sale thereof.*

BAUSCH & LOMB OPTICAL CO.
Rochester, N. Y.

*William A. Sokol*

E-233, 7½ X-33