SMITHSONIAN MISCELLANEOUS COLLECTIONS
VOLUME 87, NUMBER 12

A SPECTROPHOTOMETRIC DEVELOPMENT FOR BIOLOGICAL AND PHOTOCHEMICAL INVESTIGATIONS

(WITH THREE PLATES)

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Division of Radiation and Organisms, Smithsonian Institution

(PUBLICATION 3176)

CITY OF WASHINGTON
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A SPECTROPHOTOMETRIC DEVELOPMENT FOR BIOLOGICAL AND PHOTOCHEMICAL INVESTIGATIONS

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Investigations of the effect of radiation upon biological material can be carried out advantageously with microscopic organisms such as unicellular algae, bacteria, yeast, and fungi, along lines closely analogous to customary spectroscopic practice. The great advantage of this general method of approach is that one is able to obtain numerical evaluations which depend statistically on large numbers of organisms without going to equipment of cumbersome dimensions. The needs are, however, sufficiently different as to make desirable the development of special equipment and methods. It is our purpose to describe the development along these lines which has been undertaken in the Division of Radiation and Organisms, with a view to carrying out cooperative investigations with biologists who have specialized in the study of particular types of small organisms. It has been our idea to make the special equipment developed available not only to members of the Division but more generally to biologists who may arrange to carry on investigations at the Smithsonian Institution in cooperation with the Division.

The first of these cooperative experiments has been carried out with Dr. Florence E. Meier, National Research Fellow, working with the alga *Chlorella vulgaris* of her collection. The results of her experiments have been presented in a previous publication, Smithsonian Miscellaneous Collections, Vol. 87, No. 10, 1932. Some of the problems of a physical nature which have arisen in the course of these experiments will be taken up in connection with this discussion.

In order that advantage may be gained from comparative observations of a differential type it is desirable that the organisms be exposed to several wave lengths at the same time. Where slides can be prepared coated with a layer of microscopic organisms, they may be exposed in an instrument of the spectrograph type. Since, however, there exist essential difficulties in securing either as great uniformity or as fine texture or structure as is presented by the photographic plate, it is desirable to secure as large an area exposed to a given wave length as possible. In other words, one wishes to work with a wide and long slit and as large monochromatic images of the slit as is compatible with essential spectral purity. A second demand is that provision be made for a nonselective determination of the relative intensities of the different wave lengths incident upon the organisms. A third demand is that provision be made for the exposure of the organisms without great hazard of contamination.

These three requirements determine the general character of the combined recording monochromator and biological spectrograph which has been constructed. Since one of the most interesting regions from a biological standpoint lies between 2,500 A. and 3,000 A., fused quartz serves very well as the material for the construction of the optical parts. The difficulty which such material presents due to its slight inhomogeneity is not of great moment in this connection. The resulting loss of definition is of no consequence because of the wide slits demanded by the coarse-structured biological plates. In order that a comparatively high degree of spectral purity might be maintained despite the use of large slit-widths, a relatively great dispersion is required. This is obtained by the use of two 60° prisms, together with focal lengths of collimator and telescope of 50-60 cm. In order to maintain a high light intensity, unusually large prisms have been employed, yielding a numerical aperture of f. 4 to f. 5 depending on the wave length. The second prism is slightly larger than the first, the first being 13 cm high x 13 cm diagonal face, the second 14 cm high x 16.5 cm diagonal face. The use of a larger second prism minimizes the reduction in aperture for the beams of least and greatest deviation. The optical arrangement is shown diagrammatically in Figure 1. With the high numerical aperture, it was necessary to have the lenses ground aspherically in order to minimize spherical aberration. Since it was desirable that the instrument should be used efficiently over a wide range of wave lengths either as a spectrograph or monochromator, provision was made to swing both collimator and camera. The first slit " s " is curved, compensating for the change of prismatic deviation off the axis and thus yielding straight line images of the slit. A slit-length as great as 5 cm proves to give satisfactory images. Provision is made for the independent focusing of both collimator and camera. The lenses are 16.5 cm in diameter. The camera has been specially constructed to serve first, with a conventional plate holder, for photographic purposes, and second, with a special plate holder which can be sterilized in an autoclave, for biological purposes. This plate holder is constructed of metal and provided with both shutter and quartz window so as to isolate the biological material under sterile conditions. Furthermore, provision is made to traverse the spectrum with a thermocouple. This is supported by a worm-driven dovetail slide. Last, a second slit may be supported in place of the thermocouple and the instrument used strictly as a monochromator. Provision has been made for automatically recording the galvanometer deflections when the spectrum is traversed by the thermo-

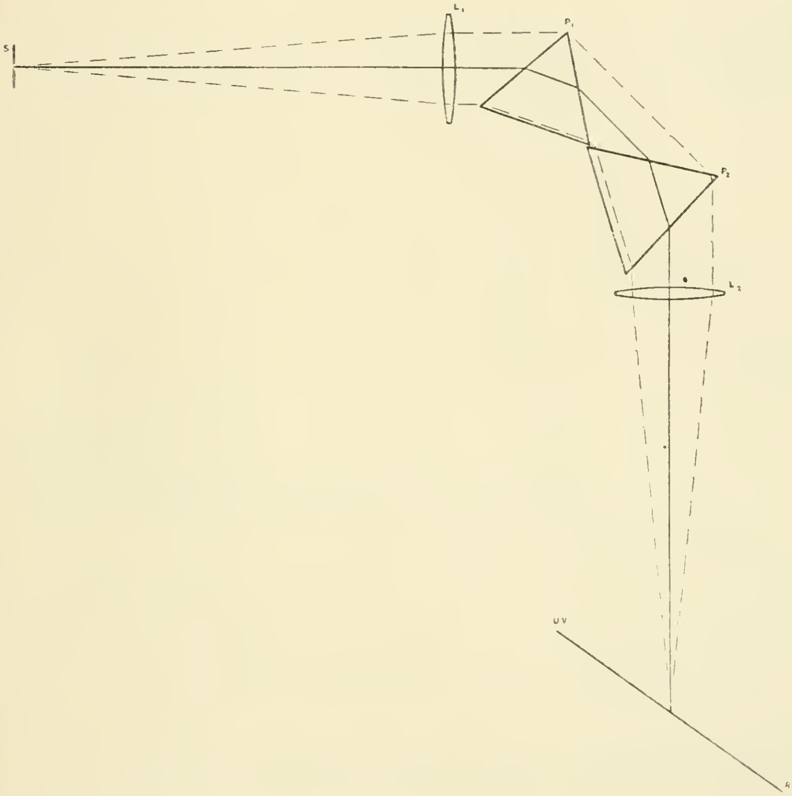


FIG. 1.—Diagram of optical arrangement.

couple. The instrument is provided with scales and is of such rigid construction as to make possible a high reproducibility of setting.

Plate 1 shows the spectrograph with the prisms exposed. A number of unique features will be noted; the extra long slit is supported by parallel rods which extend so as to carry the source and the condenser lenses when used. This permits the swinging of the collimator without realignment of the preliminary system. As will be noted, the instrument extends from one room to another. Provision is made for closing the window, thereby isolating the working room from the source of ultra-violet light. The standard plate holder mounting is shown at the left while the special biological plate holder lies at the front of the concrete table. It is shown with the back removed. Slide and quartz window are placed at 45° to the plate support in order to minimize reflection from the window.

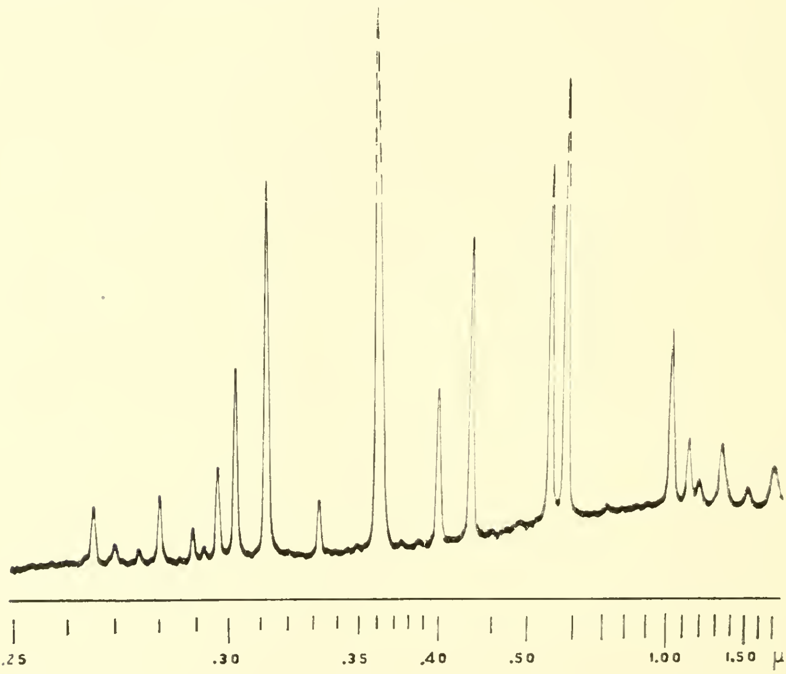


Fig. 2.—Record of mercury spectrum taken with open thermocouple.

Plate 2 shows the camera, thermocouple, and recording system in the adjoining room. The biological plate holder is in place with the back and slide removed. The thermocouple can be moved across the spectrum under these conditions, the traverse thus being made under the identical conditions to which the biological material is exposed. At the extreme right the box is shown which houses a cylinder upon which the galvanometer record is made through a cylindrical lens. Gear mechanisms accomplish convenient reduction of motion so as to secure the desired spread of spectrum upon the record. An interchange of standard reduction gears allows a modification of ratio.

Figure 2 is a direct record of the mercury spectrum from 2,500 A. to 1.7μ. This record was made with an open thermocouple, thus avoiding the difficulty of correcting for window transmission. Only two settings of collimator and camera have been required for the recording of the entire range of the spectrum, the region from 2,500 A. to 3,900 A. being traversed at one setting and the region from 3,900 A. to 1.7μ at the other. In this case a small spread or high gear ratio has been used in recording to show conveniently the entire spectrum.

Figure 3 is another automatic record of the mercury arc taken with a vacuum thermocouple of the type described by Brackett and McAlister.[1] As will be seen this record has been taken with a finer slit-width, the spectral range subtended by the slit being of the order of

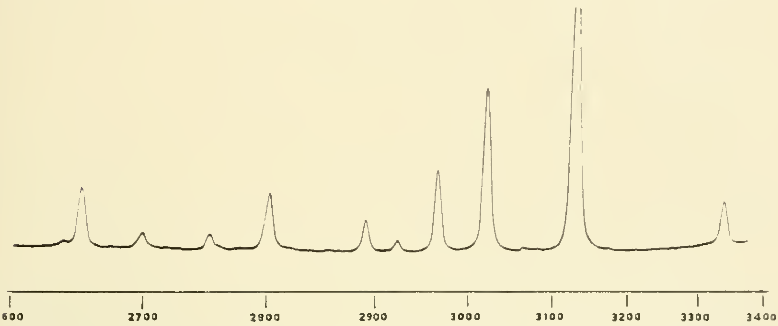


FIG. 3.—Record of mercury spectrum taken with vacuum thermocouple showing higher resolution and greater stability.

8 A. Here a lower gear ratio has been used in order to secure a larger spread. This permits the ready integration of areas for quantitative comparisons. One will note that the same order of deflection has been achieved despite the great increase in resolution and improvement in stability of zero.

Plate 3, Figure 1, shows a photographic spectrogram of the quartz mercury arc. Although rather a wide slit has been used it will be seen that the finer lines are sharply defined. Furthermore, although over 4 cm of slit-length has been shown in the illustration excellent straightness of line has been obtained.

Plate 3, Figure 2, shows a typical spectrogram obtained with biological material where the organisms are destroyed by the wave lengths of a given spectral region. This was obtained by Doctor Meier, using unicellular algae, and was discussed in her paper " The Lethal Action

[1] The automatic recording of the infra-red at high resolution. Rev. Sci. Instruments, vol. 1, no. 3, pp. 181-193, 1930.

of Ultra-Violet Light on a Unicellular Green Alga."[1] As will be noted in comparing Plate 3, Figures 1 and 2, lines in the region from 2,500 A. to 3,000 A. appear in much their usual character, while the very heavy lines shown between 3,000 A. and 3,200 A. are wholly lacking. In order that such a biological spectrogram may be made to yield a quantitative measure of lethal effect, one may resort to the microphotometer methods which are in use for photographic purposes. Again, the only essential difference is that one is dealing with a plate of less uniformity and greater coarseness of structure in the biological work. Consequently, a recording microphotometer of the

FIG. 4.—Photometric record of algal spectrogram of mercury arc.

Moll type may be used, provided it is equipped with unusually long and wide slits, thus providing an integration over a considerable area.

Figure 4 shows such a record by the microphotometer of the algal plate. The lines are readily recognized although it will be observed that the heavier ones have been so overexposed as to present a flat-topped or truncated appearance. Such a thin plate effect may be partially corrected by extrapolating the curves to a normal pyramidal shape. This is, of course, only a first approximation, accurate work requiring varied exposures so that one stays within a narrow range of effective lethal action.

Figure 5 shows a similar record by the microphotometer of the photographic spectrogram, Plate 3, Figure 2. Although there is in this case no evidence of a thin plate effect, the fact that the sensitivity of the plate varies widely, not only for different wave lengths but also for different intensities, is readily observed by a comparison of this record with Figure 3. For convenience, the microphotometer used has been equipped with extra large slits, special lenses being substituted for the microscopic type in standard use. Thermocouple and second lens have been eliminated, a Weston photronic cell being sub-

[1] Smithsonian Misc. Coll., vol. 87, no. 10, 1932.

stituted for this work. Because of its large area and uniform response this arrangement proves quite satisfactory. It is, of course, particularly suitable to the algal plate. As the same set-up of the photometer was used with the photographic plate, considerable of the detail is not brought out.

This combination of equipment, spectrograph and microphotometer, makes possible a type of biological investigation which is capable of yielding quantitative results, approaching those of photographic spectrophotometry, the limit being set simply by the uniformity of texture of the biological plates which can be prepared.

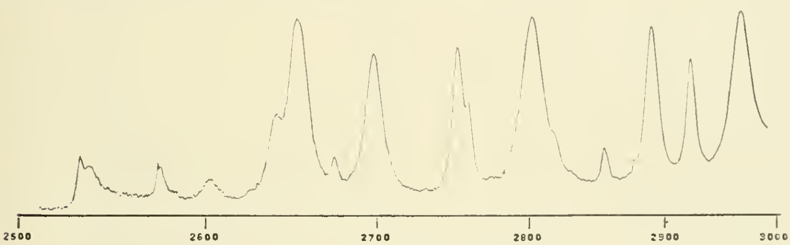
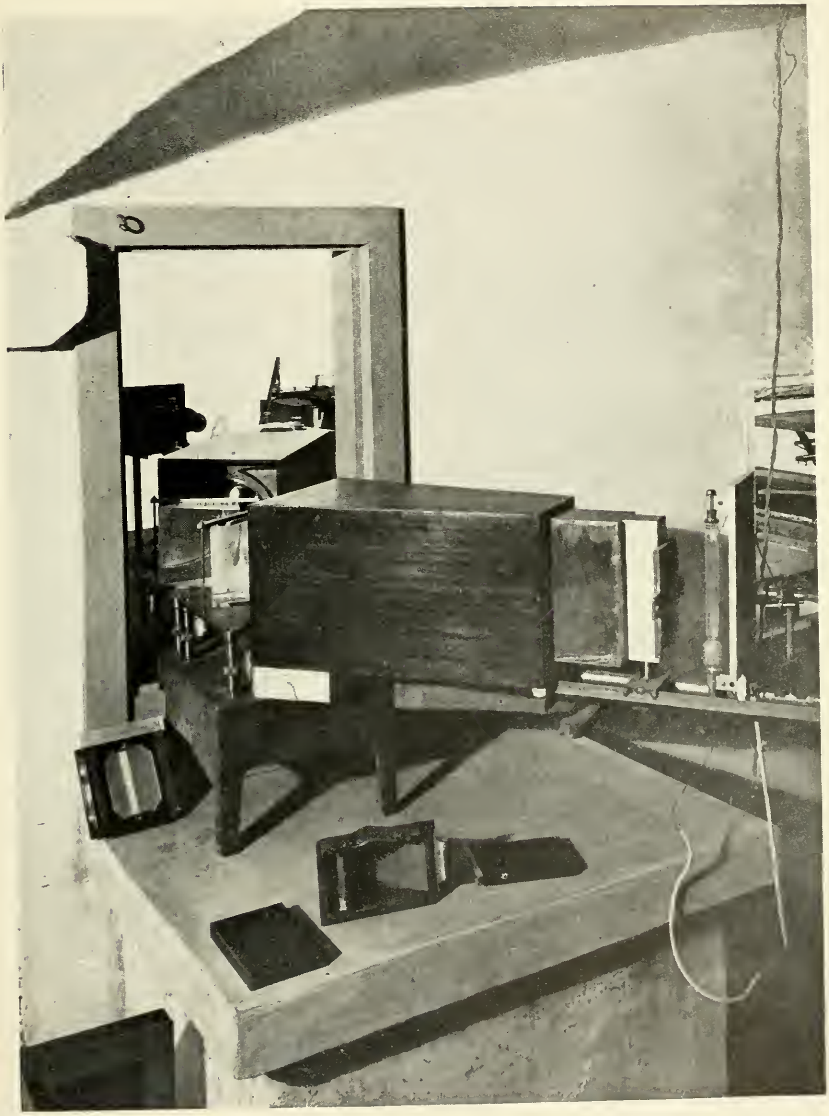
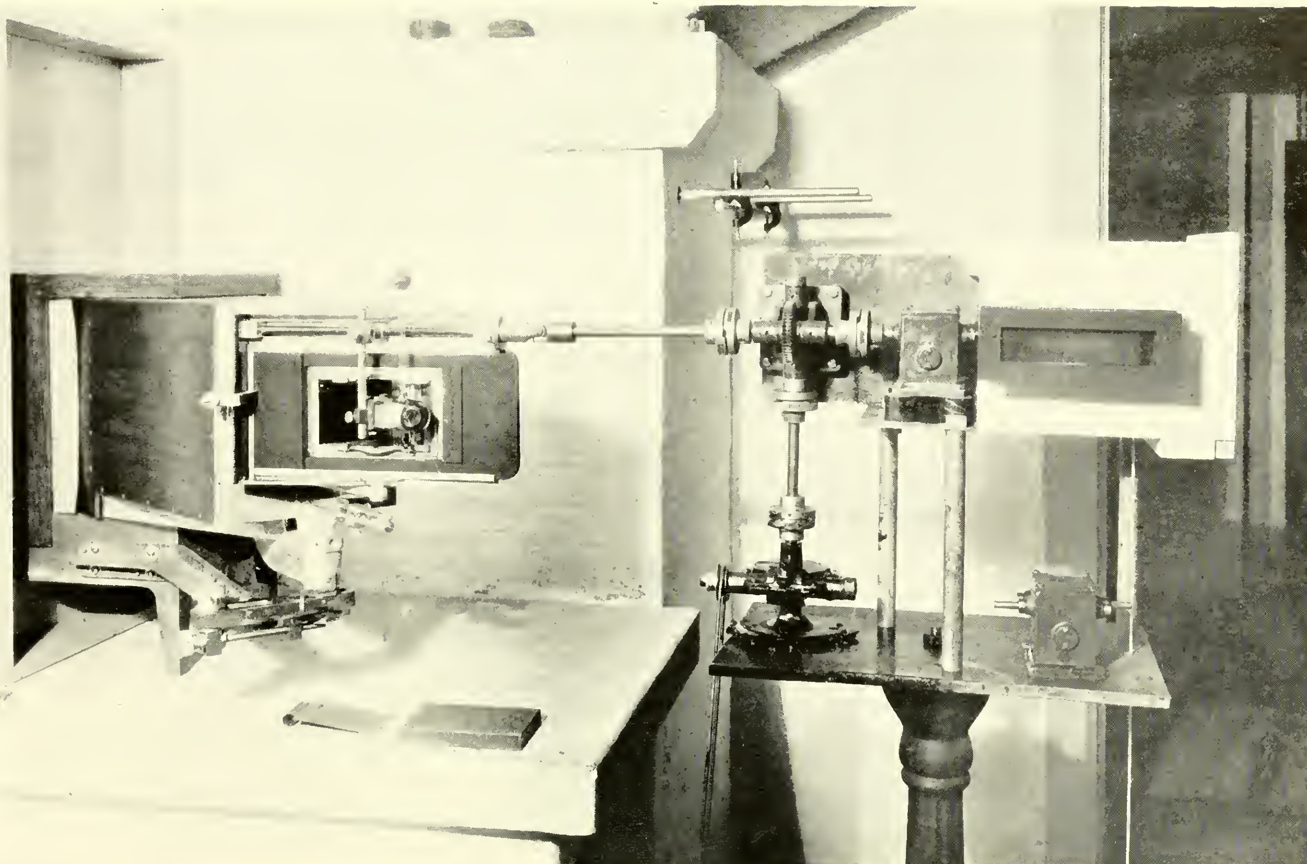


Fig. 5.—Photometric record of photographic spectrogram of mercury arc.

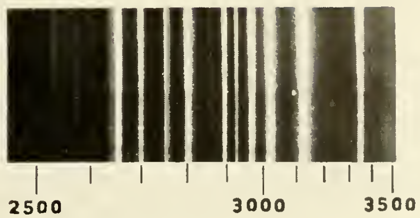
Obviously the equipment developed presents unusual advantages for pure photochemical investigation. Because of the large aperture and long slit it is possible to secure a large amount of monochromatic energy, thus enabling one to work with large enough quantities of material for convenient analysis. A mounting has been developed which permits the simultaneous exposure of 6 small test tubes to different lines of the spectrum. As in many cases slit-widths as great at 2 mm are possible without overlapping of lines, amounts of energy become available of the order commonly secured in photochemical practice by technique which yields considerably less resolution. Thus, with lines varying in intensity from 4.8/ergs/sec./mm ^{2} to 125.6 ergs/sec./mm ^{2}, and slit 2 mm wide by 50 mm long, one obtains corresponding rates of energy supply 480 ergs per sec. to 12,560 ergs per sec. Each test tube is surrounded by its own cylindrical cover in which a slit of the desired width has been cut. This second use of the instrument has already proved of considerable interest, cooperative work having been carried out with the United States Department of Agriculture upon photochemical changes in plant products.



Spectrograph for biological and photochemical investigations.

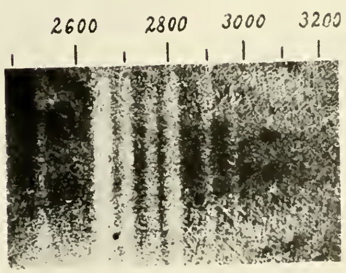


Camera of spectrograph showing mechanism for moving thermocouple and directly recording the spectrum.



1

Photographic spectrogram of mercury arc.



2

Spectrogram of mercury arc shown by lethal effect on algae.