AN INTERFERENCE-FILTER MONOCHROMATOR SYSTEM FOR THE IRRADIATION OF BIOLOGICAL MATERIAL

R. B. WITHROW

Smithsonian Institution, Washington, D. C.

The determination of action spectra for biological photoreactions requires that large areas be irradiated uniformly at high intensities and with narrow band widths for the resolution of spectral details. When the material is changing irreversibly with time or when long irradiation periods are required, it may be necessary to irradiate samples at ten or more different points in the spectrum simultaneously. This excludes use of the conventional single-exit-slit monochromator and requires a spectrograph with a series of wide slits arranged in the focal plane of the spectrum. The necessity of both high radiant power and narrow band width are difficult to meet with the prism or grating spectrograph. These instruments are capable of producing band widths of a small fraction of a millimicron but the intensity of the beam falls rapidly as the wavelength is decreased. Large prism (8) and grating (7) spectrograph systems using 5 to 15 kw carbon-arc sources have been described for the simultaneous irradiation of relatively large objects with monochromatic energy at many wavelengths. The band width of such systems is usually from 5 to 20 mμ when the radiant power is sufficiently high. The properties and application of these systems have been discussed elsewhere (14). The principal practical limitations of the spectrograph system are: 1) the large space required, 2) the unequal irradiance distribution over the spectrum, and 3) the cross-scattering of energy between wavelength stations. Because of the magnification required of the optical system, it is necessary to project the beam over relatively large distances of 5 to 15 meters. The relative intensity distribution between the various wavelength stations is arbitrarily determined by the spectral energy distribution of the source and transmission of the spectrograph. Therefore, it is not possible to obtain an equal irradiance or equal quantum-intensity spectrum. While this is not always essential for action spectrum studies, it is certainly desirable for convenience of analysis and interpretation of results. It is especially desirable for biological systems for which reciprocity does not hold.

The interference-filter monochromator presents several important advantages over the prism and grating instruments. A system of 10 or 20 wavelength stations can be placed in a relatively small constant-condition room. The spectral bands can be completely isolated in small cabinets and the intensity of each wavelength station adjusted independently by electrical control of the source or by neutral density filters so as to produce an equal irradiance or equal quantum-intensity spectrum. The system is simple optically, does not require high quality optical components, and is much easier to adjust than grating and prism instruments.

The interference-filter monochromator system to be described has been developed for the determination of action spectra in plants and for kinetical studies where it is desired to irradiate the material with high intensities of monochromatic energy of high spectral purity.

**Characteristics of Interference Filters**

The monochromator system uses 50 mm x 50 mm second-order transmission interference filters of the Fabry-Pérot type. These filters have been discussed in several papers (4, 11) and manufacturers' brochures (1, 10). Therefore, only those properties of the filters which are of special importance in the design and use of the monochromator will be discussed here. Interference filters are now available from 340 to 1000 mμ in at least 10 mμ steps.

The transmission interference filter rejects the untransmitted energy by reflection; consequently the rejected energy is not absorbed and the filters with cemented cover glasses may be used at temperatures up to approximately 80° C. The Fabry-Pérot interference filter consists of two semi-transparent layers of silver separated by a very thin layer of a transparent dielectric material. The filter has transmission bands where the equivalent thickness of the dielectric layer is an integral multiple of a half-wavelength. Therefore, the filter has a first-order transmission band at the wavelength at which the equivalent thickness is one half-wavelength, a second-order band at two half-wavelengths, a third-order band at three half-wavelengths, and so on. The various orders of the transmission bands occur in approximate wavelength ratios of 1:1, 1:2, 1:3, etc. In general, the peak transmittance and the band width decrease with higher orders and, as a general compromise, most of the interference filters now available are of the second-order type which have a peak transmittance of from 20 to 45% and a wavelength band width at half the peak transmittance (waveband half-width) of from 8 to 12 mμ in the visible spectrum, which increases to 20 mμ in the ultraviolet.

When using the second-order band of a transmission-type interference filter, it is necessary to remove the first and third orders with supplementary absorption filters. The supplementary filters must absorb transmission bands at twice the second-order wavelength on the long wavelength side and at two-thirds of the wavelength on the short wavelength side. It is desirable to select the supplementary filters with as narrow a band pass as possible because all interference filters have low transmittance of from a few

---

1 Received February 12, 1957.
2 Published with the approval of the Secretary of the Smithsonian Institution.
3 This work was in part supported by a research contract with the Atomic Energy Commission.
The slame the cipal transmission bands. be obtained with tandlem. in of several The which in(lividual were not is paired transmittance a and of of vantage of 2 of of vantage of closer than than three. The data of figure 1 were obtained for single and paired filters in a Beckman spectrophotometer. The peak transmittance varies partly because the filters were not precisely matched. Matching in dominant wavelengths is important since the transmittance curve of an interference filter is sharply peaked (fig 1) and a divergence of several mΦ between filters in tandem often decreases the peak transmittance considerably below that which normally would be obtained with perfect matching. It is seldom possible to obtain filters with dominant wavelengths matched to closer than 0.3% of the dominant wavelength, or 1 to 2 mΦ. Even a 2-mΦ mismatch will cause the half-width of paired filters to be equal to or larger than that of a single filter. Therefore, the principal advantage of using filters in tandem is the very great reduction in leakage of energy outside of the band pass.

LAMP SOURCES

Suitable sources include the projection-type incandescent lamps, the medium and high-pressure mercury arcs, and the zirconium, xenon, and carbon arcs (12, 14).

The most useful incandescent sources are an interchangeable group of projection lamps having medium pre-focusing bases and a distance from the base to the center of the filament structure of 2 3/16 inches. The wattage and code numbers of a series of seven 120 volt American-manufactured lamps are: 100w, 100T 8½/8; 200w, PH/200T 10P; 300w, PH/300T 10P/61; 400w, PH/400T 10P; 500w, PH/500T 10P; 750w, PH/750T 12P; and 1000w, PH/100T 12P. The five lamps of 300w and above have biplane filaments which yield more uniform flux distribution than those with monoplane filaments. Small changes in intensity can be obtained by varying the applied voltage with a variable transformer.

As the wattage of an incandescent lamp is increased, the size of the filament structure increases correspondingly because the lamps are operated at the maximum permissible temperature for the tungsten filament. Therefore, as the wattage of the source is increased, the focal length of the condenser must be made greater in order to reduce the magnification and keep the image within the effective aperture of the filter. The increase in focal length of the condenser results in a corresponding decrease in angular aperture and the proportion of the flux that is collected by the optical system. With a specified condenser system and size of interference filter, incandescent sources larger than a certain limit produce no increase in transmitted radiant power. The limit is determined by the effective aperture of the filter and the focal length of the condenser.

In the blue and near ultraviolet, it is often advantageous to use the mercury lines. At 365, 405 and 435 mμ, a 100w mercury arc is more effective than a 500w incandescent lamp. By slightly modifying the admixture of mercury are lampholders for the 100w H-4 lamp, it is possible to use it in the same mounting as for the projection lamp. Since arcs have a negative resistance characteristic and require a minimum voltage for firing on each half of the alternating current cycle, it is necessary to introduce series resistance between the arc and the ballast. The 100-ohm rheostat shown in figure 3 makes it possible to reduce the intensity of a 100w lamp about 80%. Further reduction in intensity produces instability of the arc and excessive damage to the cathodes.

A voltage-regulated power supply is especially important when incandescent lamps are used for the blue or ultraviolet (14). The peak of the spectral energy distribution curve of incandescent sources shifts to longer wavelengths as the temperature is reduced. A one percent reduction in voltage causes a decrease of about one percent in the near infrared, three percent in the visible and six percent in the middle ultraviolet. This radiation magnification of voltage changes can make it very difficult to balance intensities and determine incident energy values without a voltage-regulated supply.

OPTICAL SYSTEM

Unlike the prism or grating as a dispersing element, the interference filter does not require precise collimation of the incident flux. Within the limits of a 10-mμ resolution, the rays incident to the filter may diverge from the normal by as much as 15 degrees. The peak transmission shifts to the shorter wavelengths for off-axis rays, although up to 12 degrees, the shift does not exceed 6 to 8 mμ. This makes it possible to use a simple condenser without slits or
collimator optics. Short-focus, large angular-aperture condensing lenses may be used with concentrated sources in which the object distance is not less than 2.5 times the effective diameter of the lens. This gives a divergence from normal of about 13 degrees for a small concentrated source. The maximum energy is transmitted when the focal length of the condenser is such that a magnified image of the source is formed at the interference filter and the image is just large enough to include the full aperture of the filter.

The general principles of the optical system used in the interference-filter monochromator are presented diagrammatically in figure 2. This system is very similar to that of a slide projector. The condenser system, L₁ and L₂, focuses an image of the source on the projection lens, L₃. The lens, L₃, then projects an image of the condenser lens aperture in the objective plane, A'. The object to be irradiated is placed at A' where the flux distribution is very uniform.

It is possible to dispense with L₃ and to irradiate the material with a diffused, out-of-focus image of the source, S. However, no matter how far out-of-focus the specimen is placed, some of the irregular brightness details of the source will be reproduced and the intensity distribution is not as uniform as when using a projection lens to produce an image of the condenser-lens aperture. The condenser aperture is irradiated with relative uniformity by the source. However, the center is always more intense than the edges by approximately 20%, depending in part upon the source distance, f₁. If extremely uniform flux distribution is desired, it is necessary to use a long focal-length condenser lens. The source is relatively far removed from the condenser and the condenser aperture more uniformly irradiated. A larger wattage lamp is then required to produce the same transmitted flux.

The interference filters are placed as close to the objective lens, S', as possible. In the case of the Bausch and Lomb interference filters, which are approximately 50 mm square, the useful aperture is only 38 mm. The filters are not aluminized over the whole face of the glass plate, in order to leave a small margin of unsilvered glass for bonding the cover glass. The filter should be blackened on the edges and on the margins of both faces for about 6 mm to prevent scattering of unfiltered energy around the edges of the silver-dielectric film (fig 2).

A primary aqueous filter, F₁, is used between the

![Fig. 2. Schematic diagram of the optical system of a transmission interference-filter monochromator unit.](image)

Legend:
- **S**—Source
- **S'**—Image of source produced by condenser system L₁ and L₂ of focal lengths f₁ and f₂ respectively
- **F₁**—Aqueous filter of refractive index, n, and path length, d. This filter increases the actual object distance by d(1−1/n).
- **F₂**—Assembly of two interference and one dyed-gelatin filters
- **L₁**—Projection lens of focal length, f₁
- **M**—Mirror
- **A'**—Magnified image of uniformly irradiated aperture, A, at position of condenser lens, L₂
- **u**—Object distance of L₂
- **v**—Image distance of L₂

Copyright © 1957 American Society of Plant Biologists. All rights reserved.
condenser and the interference filter, \( F_2 \) (13). Because of the relatively great depth, \( d \), of this filter (10 cm in fig 3), the effective image distance is appreciably increased (6). Formulas used for calculating the image distances are given in figure 2. It will be noted that the increase in effective distance between the condenser lens and the source image is a function of the refractive index of the filter solution which is approximately 1.3 for aqueous filters. The upper formula gives the increase in apparent focal length of \( L_g \) in a two-lens condenser. The lower formula gives the increase in objective distance which would be obtained if a single condenser lens is used.

**Monochromator Unit**

The complete details of a single monochromator unit with associated electrical circuitry and cabinet are given in figure 3. In our laboratories we have ten of these units mounted on a long table in a constant-condition darkroom. They are powered from a single voltage regulator and the whole system is controlled by a time switch. The ten-station system is designed for the irradiation of small leaves and stems. These are arranged in a circle in a 150-mm-diameter dish, \( D \), centered on the emergent beam.

The filter system consists of four or five components, depending upon the region to be investigated. The first supplemental filter, \( F_1 \), is water or an aqueous solution of ferrous ammonium sulfate or copper sulfate, in an all-glass filter cell of 10 cm internal length. The properties of these filters have been discussed (2, 5, 9, 13, 14). This filter is cooled by a small hairpin loop of \( \frac{1}{4} \) inch silver tubing. Fine silver is used because it is very ductile and not reacted upon by salts of copper and iron in the presence of free acid. The interference filters, \( F_2 \) and \( F_4 \), and the gelatin filter, \( F_b \), (3, 13) are mounted in a half-inch aluminum plate. The neutral density filter, \( N \), is useful for obtaining very low irradiances.

The filter, \( F_5 \), absorbs stray flux and is only of value under certain conditions. For determination of a red-sensitive response in the green, blue and ultraviolet, the filter, \( F_b \), is a solution of copper sulfate. For a blue-sensitive biological system, a yellow filter is required. The window, \( F_b \), is essentially a far-infrared-absorbing filter of lucite and prevents air, circulating from the blower, \( B \), from entering the irradiation cabinet. Warm components produce long-wavelength infrared which can cause appreciable deflection of a thermopile and often is confused with the energy transmitted by the filters. This annoying property is practically eliminated by a \( \frac{1}{4} \) inch plate of methacrylate plastic such as plexiglas or lucite (14).

A listing of the supplemental filters for use with tandem interference filters is given in table 1. For wavelengths longer than 520 \( \mu \)m, long-wave-pass gelatin filters with sharp cut-off are indicated, with band pass filters specified for the shorter wavelengths. In general, the selection of supplemental filter combinations was made so as to produce as narrow a band pass as possible, consistent with reasonably high transmittance.

With two interference filters and a 750w lamp, the monochromator of figure 3 will irradiate an object 20 cm in diameter with 5 to 20 \( \mu \)W/cm\(^2\) at any point in the spectrum from 365 to 800 \( \mu \)m and with a single filter, 20 to 100 \( \mu \)W/cm\(^2\). A larger monochromator unit has been designed for a 7-kw condenser-type rotating carbon arc. It employs 6-inch diameter condenser lenses. This system produces irradiances in the red of 300 \( \mu \)W/cm\(^2\) with double filters and up to 2000 \( \mu \)W/cm\(^2\) with a single filter. When properly adjusted, both the small and large units produce a variation of about \( \pm 10\% \) in intensity over the 20-cm diameter aperture. By arranging the material to be irradiated in a circular pattern, the variation in irradiance can be kept very small. The spectral resolution of the system is adequate for resolving spectral details separated by 10 \( \mu \)m. With careful selection

**Table I**

**Monochromator Filter Systems**

<table>
<thead>
<tr>
<th>Interference, ( \mu )m **</th>
<th>Gelatin or Glass No. †</th>
<th>Aqueous, 10 cm Pathlength ††</th>
<th>Salt Conc. gm/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>365 (Hg)</td>
<td>18A</td>
<td>Cu</td>
<td>200</td>
</tr>
<tr>
<td>365 (Hg)</td>
<td>3A</td>
<td>Cu</td>
<td>200</td>
</tr>
<tr>
<td>385 (Hg)</td>
<td>3A</td>
<td>Cu</td>
<td>200</td>
</tr>
<tr>
<td>400 (Hg)</td>
<td>3A</td>
<td>Cu</td>
<td>200</td>
</tr>
<tr>
<td>450 (Hg)</td>
<td>4A</td>
<td>Cu</td>
<td>200</td>
</tr>
<tr>
<td>520 (Hg)</td>
<td>5A</td>
<td>Cu</td>
<td>100</td>
</tr>
<tr>
<td>550 (Hg)</td>
<td>6A</td>
<td>Cu</td>
<td>100</td>
</tr>
<tr>
<td>600 (Hg)</td>
<td>7A</td>
<td>Cu</td>
<td>100</td>
</tr>
<tr>
<td>650 (Hg)</td>
<td>8A</td>
<td>Cu</td>
<td>100</td>
</tr>
<tr>
<td>700 (Hg)</td>
<td>9A</td>
<td>Cu</td>
<td>100</td>
</tr>
<tr>
<td>750 (Hg)</td>
<td>10A</td>
<td>Cu</td>
<td>100</td>
</tr>
<tr>
<td>800 (Hg)</td>
<td>11A</td>
<td>Cu</td>
<td>100</td>
</tr>
<tr>
<td>850 (Hg)</td>
<td>12A</td>
<td>Cu</td>
<td>100</td>
</tr>
<tr>
<td>900 (Hg)</td>
<td>13A</td>
<td>Cu</td>
<td>100</td>
</tr>
</tbody>
</table>

**Incandescent lamps used as sources except as noted for mercury arc, Hg.
† Kodak Wratten No. 3 except for those designated * see (13).
†† Cu = CuSO\(_4\) · 5 H\(_2\)O. Fe = Fe(NH\(_4\))\(_2\)(SO\(_4\))\(_2\) · 6 H\(_2\)O. Salts prepared in 1% sulfuric acid.**

358 PLANT PHYSIOLOGY

Copyright © 1957 American Society of Plant Biologists. All rights reserved.
Fig. 3. Diagram of a single interference-filter monochromator mounted on exposure cabinet. Control circuits are shown for both incandescent and mercury arc lamps.

Legend:

IS—Incandescent source, 100-1000-watt projection lamp
AS—Arc source, 100-watt, H100-A4 type mercury arc
BL—Blower
Ls—Condenser lens, 65 mm diam, 50 mm focus
Lp—Projection lens, 65 mm diam, 80 mm focus
F1—Aqueous filter, 10 cm path length
F2, F4—Second-order, interference filters, 50 mm x 50 mm
F5—Dyed-gelatin filter
F6—Secondary aqueous filter for removing scattered flux
F7—Secondary infrared filter of ¼ in. methacrylate plastic
NF—Neutral filter
FC—Filter cell inner cover
SC—Silver cooling coil
M—Plane mirror
H—Removable housing
D—Petri dish, 150 mm diam
CB—Centering board
CP—Cover plate
V—Voltmeter
VT—Variable transformer
EVR—Electronic voltage regulator
Ba—Mercury arc lamp ballast
R—Rheostat, 150 ohms, 150 watts
of filters, it might be possible to obtain 5 mμ resolution.

SUMMARY

The design of a small compact interference-filter monochromator system for the spectral range from 365 to 800 mμ is presented, together with a theoretical discussion of optical principles. The characteristics of interference filters and supplemental gelatin and aqueous filters are given. The monochromator has a resolution of about 10 mμ throughout the visible spectrum and can produce relatively high irradiances with an incandescent lamp. Irradiances as high as 2000 μw/cm² can be secured with a larger unit employing a 7-kw carbon arc.

The author wishes to express appreciation to Dr. W. H. Klein and Mr. V. Elstad who made many valuable suggestions for modifications while using the monochromator system, and to Mr. D. G. Talbert who constructed the instrument and Mr. J. H. Harrison who assisted in the design of the optical system.

LITERATURE CITED


THE RELATION OF OPTICAL FORM TO THE UTILIZATION OF AMINO ACIDS.
I. UTILIZATION OF STEROISOISOMERIC FORMS OF GLUTAMIC ACID BY CARROT ROOT DISKS

F. D. H. EL-SHISHINY AND M. A. NOSSEIR

Botany Department, Faculty of Science, Alexandria University, Egypt

According to the classical idea of Liebig the plants are able only of assimilating inorganic nitrogen. But now there is no longer reason to doubt the ability of plants to absorb and utilize organic nitrogenous compounds, since in sterile culture plants can utilize various organic nitrogenous compounds, amino acids and amides. The extensive early literature describing the utilization of organic nitrogenous compounds by plants has been reviewed by Hutchinson and Miller (7) and by Brigham (2). Several other reports concerning the availability of amino acids for plant growth, that followed these papers, were reviewed by Ghosh and Burris (6).

Very little experimental work was carried out on the relation of optical form to the utilization of amino acids in higher plants, and our knowledge in this field is very scanty. The utilization of both optical forms of aspartic and glutamic acids by pea and clover was reported by Virtanen and Linkola (15). The synthesis of both isomers of glutamine from their respective isomers of glutamic acid catalysed by a purified enzyme from pea was shown by Levintow and Meister (9, 10).

The purpose of this investigation is to compare the ability of carrot root cells to absorb and assimilate the various stereoisomeric forms of glutamic acid and to attempt some explanations of the results obtained.

MATERIAL, METHODS, AND EXPERIMENTS

The disks for this investigation were prepared from carrot roots var. Chantenay. The general procedure of preparation and pre-treatment of the disks as well as the determination of the nitrogenous fractions were as described by El-Shishiny (4). Twenty grams of disks, taken at random from a stock of disks prepared for each experiment, were used for each sample. The samples were washed for four days in aerated distilled water. The samples were then washed several times with sterilized distilled water and transferred into the sterile culture solutions, kept at 25°C (± 0.1°C). The samples were aerated for

1 Revised manuscript received April 18, 1957.