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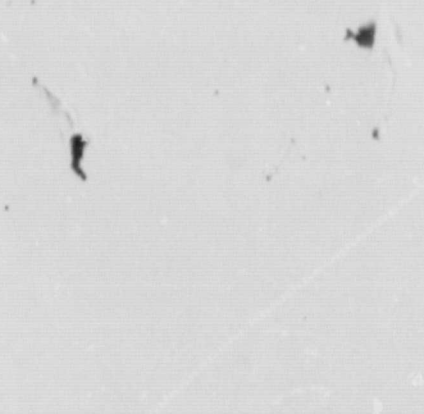
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Summary Report
to
National Aeronautics and Space Administration

Grant No. NGR-39-002-011-4D

DEVELOPMENT OF NEW MICROSPECTROPHOTOMETRIC INSTRUMENTATION

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8/27/71

15 August 1971

FACILITY FORM 602
N71-35515 (ACCESSION NUMBER)
24 (PAGES)
CR-122066 (NASA CR OR TMX OR AD NUMBER)
(THRU) 1
(CODE) 14
(CATEGORY)

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I. INTRODUCTION

For the past decade, we have been developing a new instrument to study the chemistry of the cell in its living state. To do so, we have combined microscopy with spectroscopy in constructing a rapid recording microspectrophotometer M-5 (20, 34-36).

In the year 1970-1971, we have carried on further developmental experimental studies related to this instrumentation. These are:

1. Improvements in the recording of spectral data
2. Single crystal spectroscopy
3. Redesigning the instrument so that fluorescence microspectrophotometry can be accomplished

II. MICROSPECTROPHOTOMETRY: BRIEF HISTORY OF INSTRUMENT DEVELOPMENTS

In the late 1930's, Caspersson initiated the development of microspectrophotometry, by combining a microscope with a monochromator (4). By this method, he was able to measure the ultraviolet absorption spectrum of the cell nucleus for the identification of nucleic acids.

The essential components of all microspectrophotometers are a microscope, a monochromatic light source, a light chopper, a sensitive photocell (usually a photomultiplier tube), an amplifier and an output device for recording the absorption.

A rapid advance in microspectrophotometer instrumentation was made in the 1960's for obtaining absorption spectra of living cells. These instruments are of various types, from cytophotometers and cytodensitomitors to very complex microspectrophotometers in which spectra are rapidly recorded and the data stored by computer techniques (1-3, 5, 6, 10, 11,14, 15, 17-19).

The microspectrophotometers presently in use are primarily laboratory-built instruments. There are, though, several commercial recording microspectrophotometers that have recently become available (Carl Zeiss, Incorporated; Canal Industrial Corporation; Olympus Corporation of America). However, these still lack features which have been designed into laboratory-built instruments.

For the study of photosensitive pigments in living cells, better resolution, faster time constants, low light-levels and rapidly recorded absorption spectral data are necessary.

To meet these requirements, a recording spectrophotometer can be adapted for microspectrophotometry. Brown (3) converted a Cary Model 14 dual-beam recording spectrophotometer into a microspectrophotometer by replacing the sample cell compartment with a microscope for the sample light beam and equivalent optics for the reference beam. This instrument can be used for solution spectroscopy or for microspectrophotometry by simply interchanging components. For sample areas of 0.1 to 1 mm in diameter, the usable wavelength range is 300-700 nm. Additional provisions include increasing the sensitivity of the recorder from 0-1 optical density units to 0.01 optical density units full scale and the addition of a monochromator light source for locating the specimen. This modified Cary 14 microspectrophotometer can sweep the visible spectrum in less than 2 minutes and has been successful in obtaining recorded absorption spectra with a peak-to-peak noise level of about 10^{-2} optical density units. A disadvantage of this system is that the reference beam does not pass through the microscope slide chamber containing the experimental specimen, and unless a pure solution is used for the suspending medium, the absorption of other cellular constituents surrounding the sample area under investigation must be corrected for.

Recording microspectrophotometers have been constructed in several laboratories (1, 2, 6). One such instrument is that of a single-beam recording microspectrophotometer, designed and constructed by Chance et al (15). This instrument covers the spectral range from 240 to 600 nm, with a noise level of the order of 10^{-4} optical density units to detect concentrations of 10^{-18} mole at a signal-to-noise ratio of 50 to 1. A modified design of this microspectrophotometer was made by Liebman (8-10) to investigate absorption and dichroism in isolated single retinal rods and cones. In these studies, the microscope was fitted with Zeiss ultrafluar optics (320 X) and was illuminated by a Bausch and Lomb 250 mm grating monochromator with 1200 lines per mm. To fill the condenser back aperture (N.A. = 0.4), the monochromator exit slit-width was set at 0.8 mm for a spectral bandwidth of 5 μ. A beam splitter above the ocular permits viewing of the specimen and provides a light path for the signal, which is imaged in the plane of a pair of circular apertures (in the object plane). A mechanical shutter alternately selects the object or the reference area at 60 cycles per second for illumination of the photomultiplier. The output of

the photomultiplier is controlled by a dynode feedback circuit. Integration of the measure-reference signal is performed by an RC network, and the output is recorded on a dc recorder in percent absorption, proportional within 10% to an optical density of 0.1 or less. A time constant of 2 sec and a noise level of about 4×10^{-4} optical density units peak-to-peak are possible with this instrument.

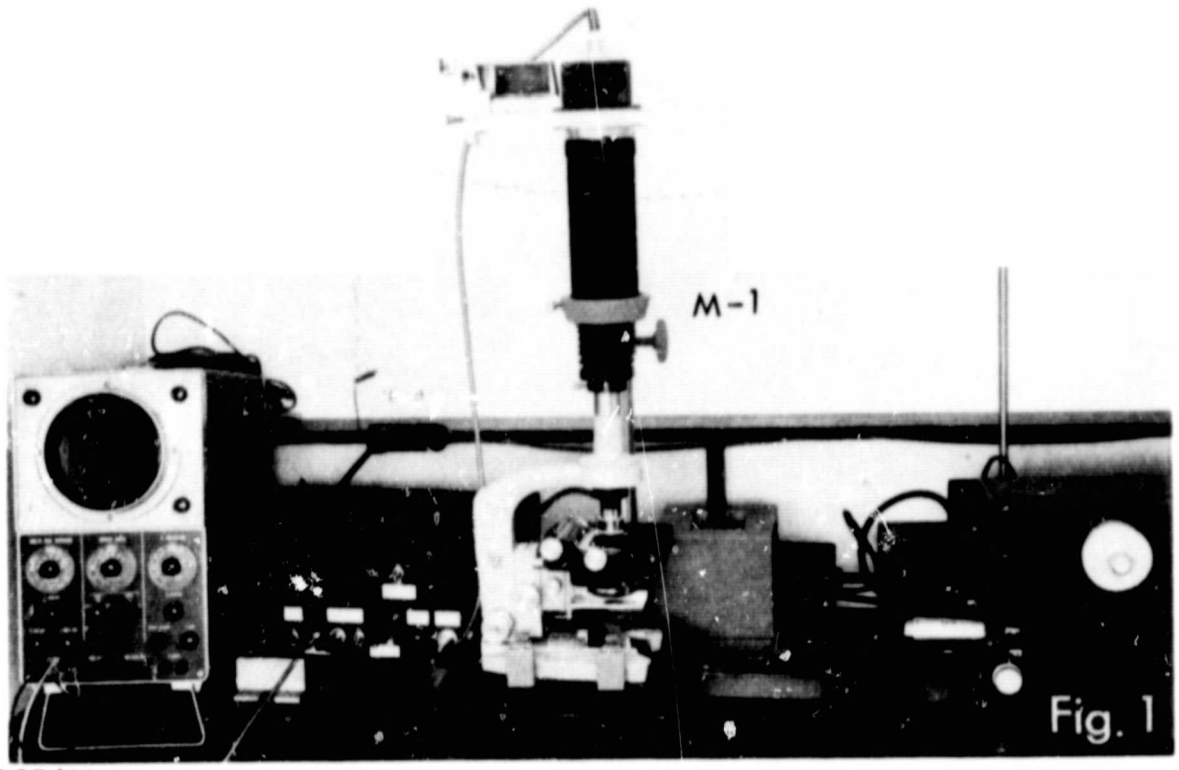
III. DEVELOPMENTS IN MICROSPECTROPHOTOMETRY IN THE BIOPHYSICAL RESEARCH LABORATORY

Over the past decade, we have designed and constructed five instruments for microspectrophotometry, from the simplified M-1 to the rapidly recording M-5 (Figures 1-4, and References 16, 35, 36).

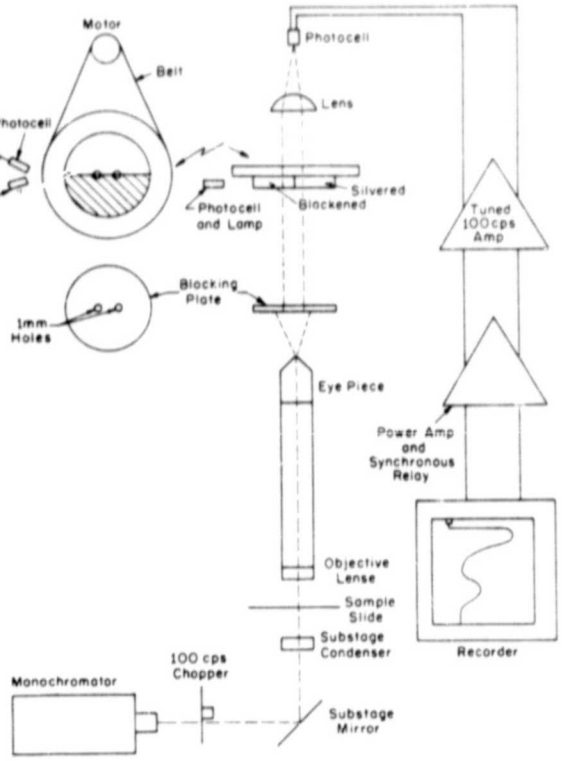
Microspectrophotometer M-1

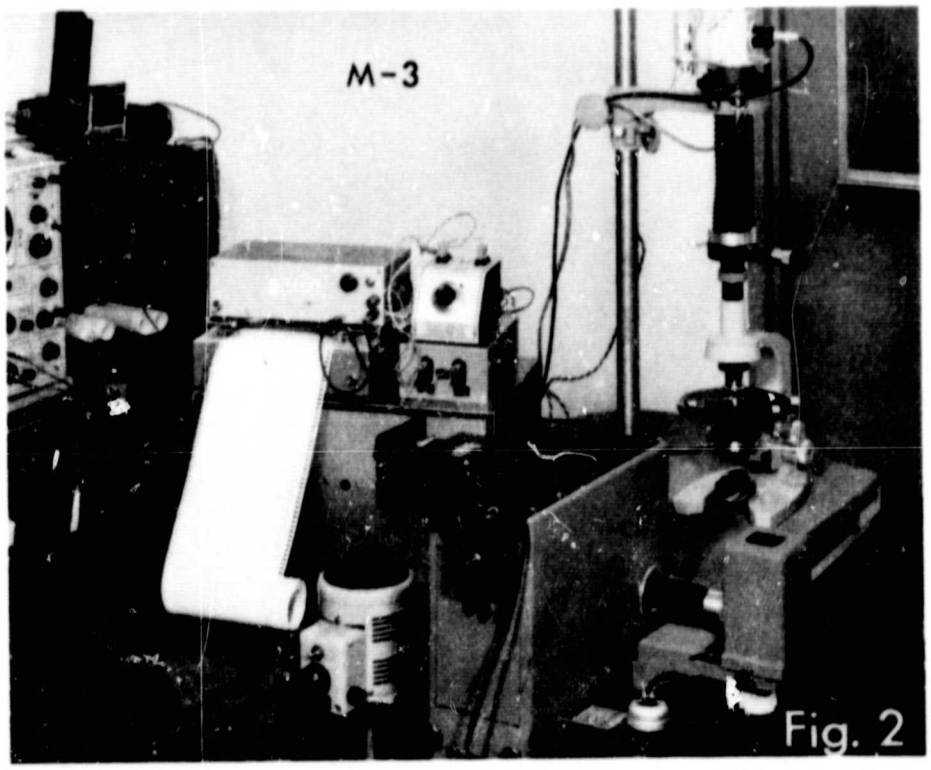
The simplified microspectrophotometer (M-1) uses a cadmium selenide (CdSe) photoconductive cell as the light sensitive element, which covers the ultraviolet and the visible spectrum and can be applied to spectral measurements in the infrared and to x-rays. The microspectrophotometer M-1 uses either a xenon arc or a tungsten ribbon filament light source and a Bausch and Lomb 250 mm grating monochromator. The microscope is equipped with reflecting objectives (50 X with numerical aperture of 0.5). The instrument is operable over the wavelength range from 200 to 900 nm in a single sweep (16, 35).

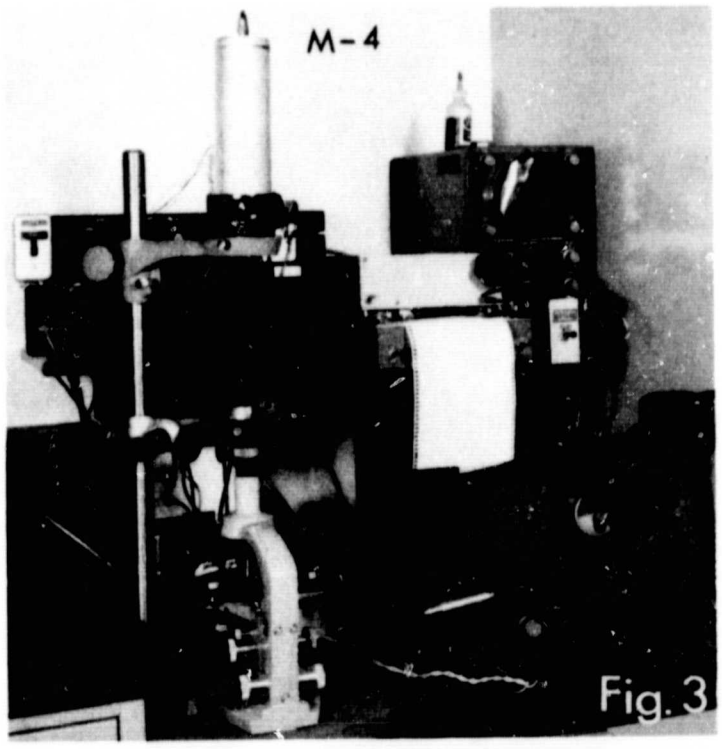
The ground glass viewer and the photocell are operated on a sliding track. The entire assembly is swung into position by a vertical bar arrangement mounted on a base plate (Figure 1). The specimen can be viewed at all times through the telescope. The maximum magnification employed is 500 X. At this magnification, spectra from areas of 2 μ^2 (1 x 2 μ in the object plane) are easily obtained. No limiting apertures are used for measurements. Optical alignment is not critical, and no light chopping is necessary. A 67.5-V bias supply is used, and at each wavelength setting the dc photocell current is measured by means of the dc voltage drop across a 2 megohm load resistor. The noise level as determined by photocell current fluctuations is about 2×10^{-3} optical density units. Since the entire photosurface of the cell is exposed to the light beam, no effects due to variation in the sensitivity of the photosurface are observed.

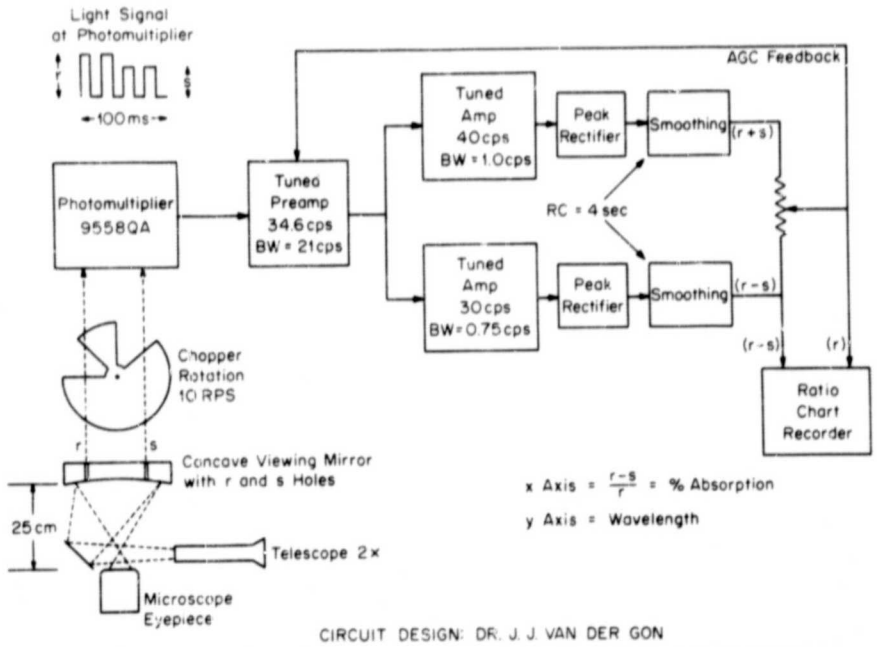


M-3 BLOCK DIAGRAM









CIRCUIT DESIGN: DR. J. J. VAN DER GON

M-4 BLOCK DIAGRAM

Microspectrophotometers M-2 Through M-4

Recorded data and faster time responses than those available with the M-1 were necessary for the spectroscopy of photosensitive pigments. As a result, a single-beam recording microspectrophotometer, a modification of the simplified M-1, which also uses a photoconductive photocell, was constructed and designed as M-2.

Recording Instruments. To achieve further improvements, a recording microspectrophotometer (M-3) was then developed (Figure 2). In this instrument, light from the monochromator is passed through a 100 cps chopper using an ac-tuned amplifier, to the substage mirror (front surface) of a microscope equipped with reflecting substage condenser and objective lenses. The sample image is focused on a plate having two holes of the same size (holes of approximately 1 mm diameter and a magnification of 500 X are used, but these can be varied depending on the sample size). The image of the specimen is adjusted over one hole by moving the slide; the other hole is used for the reference area. Above the blocking plate, a second chopper in the form of a half disc, rotating at 10 cps, alternately exposes the sample and reference holes and allows the light to fall on a converging lens which is focused on the detecting photocell. A mechanical adjustment for the phasing network is necessary to allow the electronic switching circuit to be synchronized with the above 10 cps chopper. The outside edge of this chopping disc is black for 180° and silvered for the other 180°. A Clairex CL-3 photocell and lamp are used as a phasing network and mounted so that light reflecting from the silvered edge of the chopping disc will operate the synchronous relay.

The output of the detecting photocell supplied by a 135-V battery and developed across a 2 megohm resistor is coupled to the ac tuned amplifier. A 6V6 tube and plate transformer are used in a conventional manner as a power amplifier. The output of this stage is separated by the synchronous relay into sample and reference signals. After filtering, the signals are compared by a conventional null balance servo amplifier. To obtain a recording, a second 1000-ohm transmitting potentiometer supplied by an adjustable voltage source is operated with the balancing potentiometer, and its output is measured in a dc recorder.

The photoconductive cells used in the M-1, M-2 and M-3 instruments are special purpose cells (manufactured by the Clairex Corporation of New York and

RCA) with a photosensitive surface of 0.5 x 1 mm and a radiation sensitivity per unit area roughly equivalent to a photomultiplier tube. They do not require high voltages. The spectral response of the CdSe cells extends from the far ultraviolet to the near infrared, with a rather sharp cutoff at about 1 μ. The peak response is in the visible region of the spectrum at 720 nm. At high light intensity the time constant of the cell is of the order of milliseconds. At extremely low light levels, the time constant is of the order of seconds.

To secure a more rapid and reliable sensing device, an EMI photomultiplier tube (S-20) with a diameter of 4.5 cm was chosen and integrated into the M-4 instrument (Figure 3). The cathode material is SbNaKCS on fused silica. The photocathode sensitivity is about 140 amps/lumen; the overall sensitivity of the photomultiplier tube is 200 amps/lumen. The photomultiplier tube with a pyrex glass window has a spectral response between 300 and 840 nm. A quartz window extends this range to 165 nm in the u.v.

The dark current is less than 10^{-9} amps. The ratio of the high sensitivity to low dark current means an extremely high signal to noise ratio. The photomultiplier tube may be cooled to -180°C which increases the signal to noise ratio more than 100 times. Another important characteristic of the photomultiplier tube is the very fast rise time (7×10^{-9} sec), which improves the time constant in obtaining spectral data.

The photomultiplier tube (S-20) in its housing can be mounted directly above the rotating light chopper, replacing the CdSe cell. The two light beams (the reference and the sample) emerge from behond the viewing mirror and come alternately through the light chopper, falling on the photomultiplier cathode about 2 cm apart. Sensitivity over the cathode surface varies, but equal sensitivity for each light beam was achieved, within the limitations of the amplifier and recording systems, by rotating the photomultiplier tube. Although the photomultiplier cathode is large in comparison to the CdSe cell, the problem of alignment of the two light beams is not critical. Therefore, the only change required in adapting the photomultiplier tube is the disconnection of the dc potential used for the CdSe diode. The 2M7 grid resistor at the input tube of the preamplifier serves as the load resistance for the photomultiplier tube.

When the photoconductive CdSe cell requires a dc potential of approximately 100 volts from the amplifier, the photomultiplier tube requires a high voltage

(1600 volts dc), and the voltage regulation is critical. A Hamner B-401 power supply, regulated to 0.002%, makes it possible to take full advantage of the sensitivity and signal to noise ratio of the photomultiplier tube. The sensitivity of the photomultiplier tube with the simple power supply is of the order of 100 times that of the cadmium selenide (CdSe) cell.

IV. MICROSPECTROPHOTOMETER M-5

Because of limitations in the microspectrophotometers M-1 to M-4, a completely new instrument, the M-5, was developed (Figure 4).

The objective was to extend the usefulness of the M-4 recording microspectrophotometers by: 1. increasing the degree of optical resolution; 2. increasing the sensitivity throughout the wavelength scale from the ultraviolet through the visible and to the infrared; 3. reducing the time constants so that spectral data can be obtained in the order of seconds; and 4. to automate and miniaturize the instrument so that it also could be adapted for oceanic, astronomical and space flight research.

Component Parts of the M-5

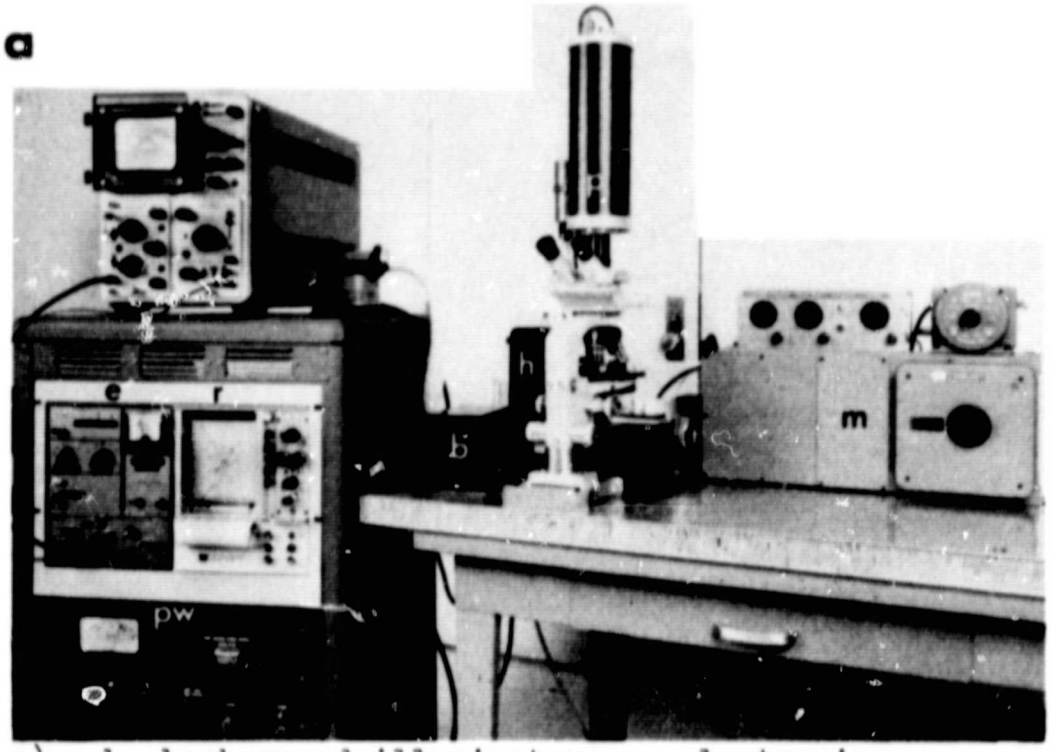
Microscope. The instrument at present uses an American Optical microscope base. The optics are Zeiss ultrafluar objectives, which require special optical eyepieces, and a Unitron BTMS microscope stage to reduce vibration.

To view specimens which are photosensitive, additional equipment was found necessary; a cold stage to reduce thermal "bleaching" effects, and an infrared image converter which can be adapted to the viewing eyepiece (Figure 5a and b). To better observe the specimen, an image intensifier tube is being investigated which allows us to view specimens under very low light levels of illumination and increases the optical resolution. A schematic of the microspectrophotometer M-5, a photograph of the instrument in present operation, is shown in Figure 4a and b.

Optimal resolution in viewing the specimen is important. Two types of optics are used. One is reflecting optics, which has practically no dependency on wavelength and consequently the focal point does not change with respect to wavelength. They do lack resolving power. The other is Zeiss ultrafluar optics which give better resolving power than the reflecting optics but are limited in their spectral range.

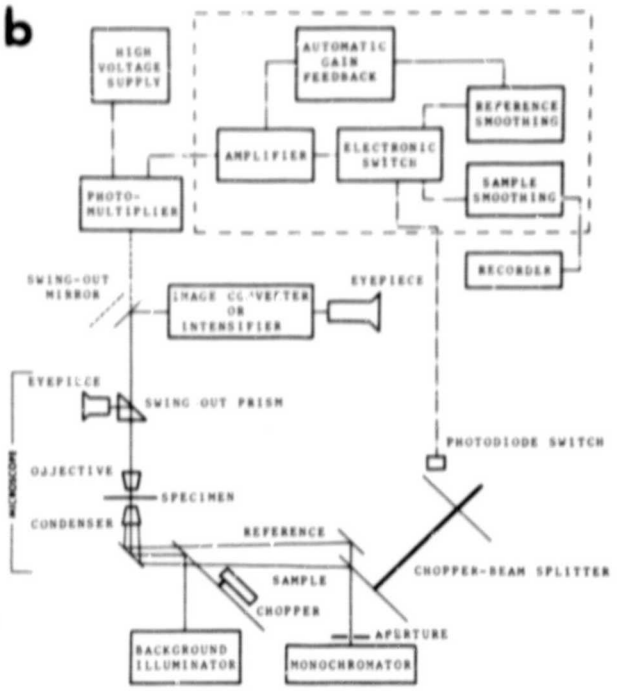
Microspectrophotometer M-5

a

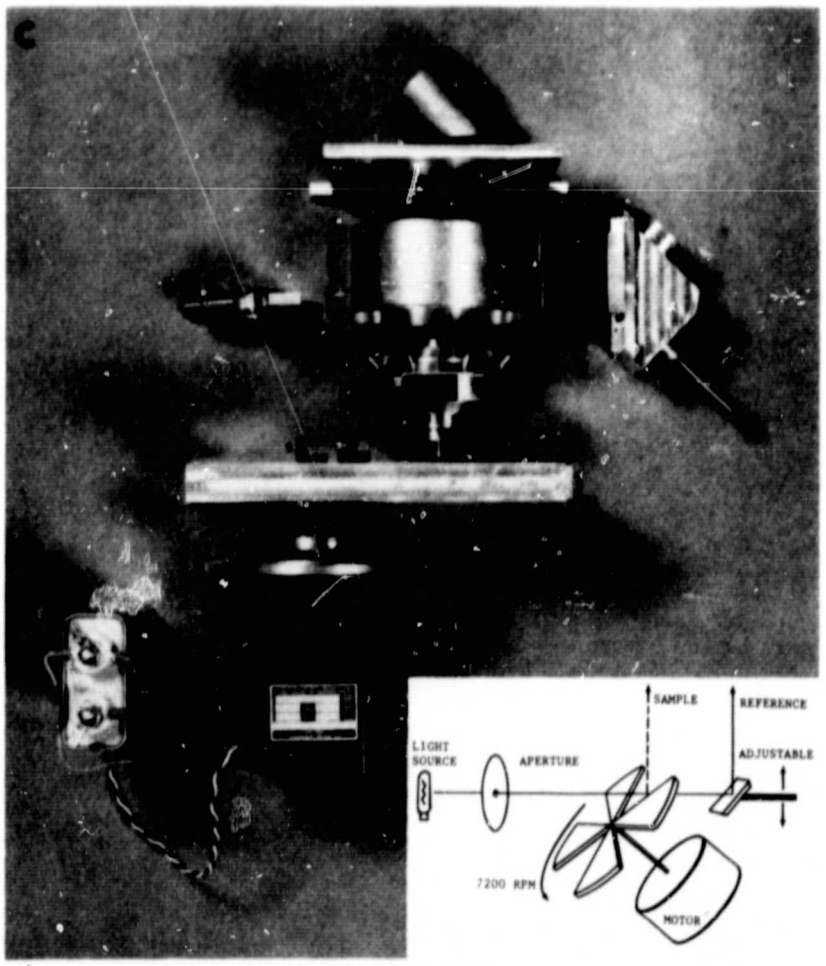


a). b. background illuminator; e. electronics; h. light source; m. monochromator; p. photomultiplier tube housing; pw. power supply; r. chart recorder.

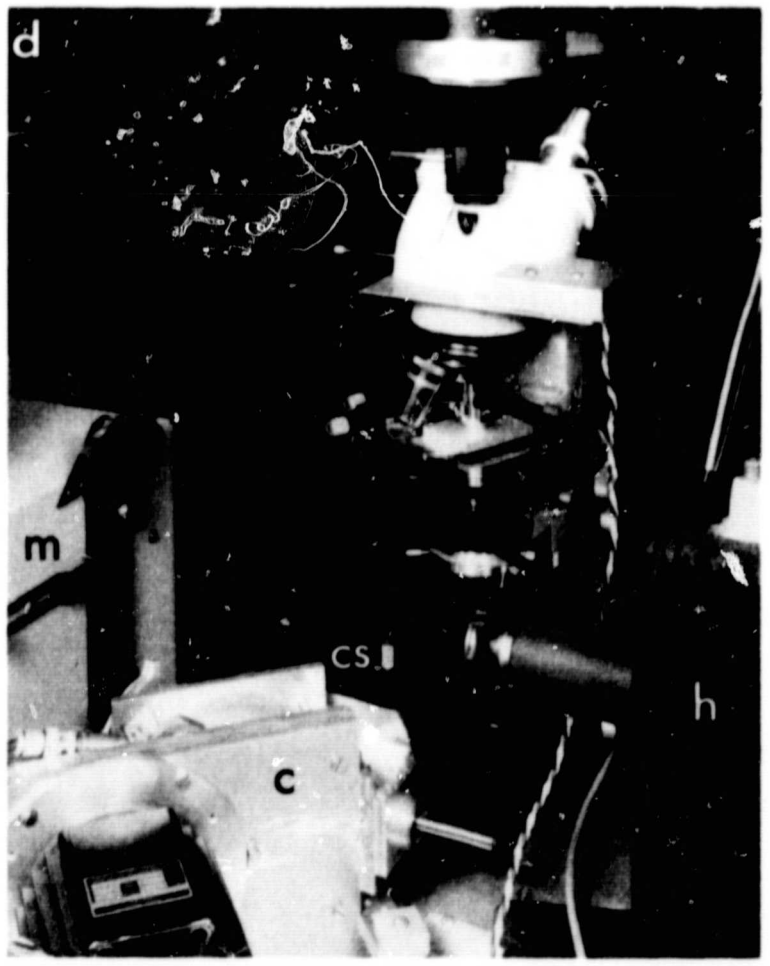
b



M-5 BLOCK DIAGRAM



c). chopper and schematic.



d). c. chopper; cs. cuvette chamber for solution spectroscopy; h. light source; m. monochromator.

Fig. 4

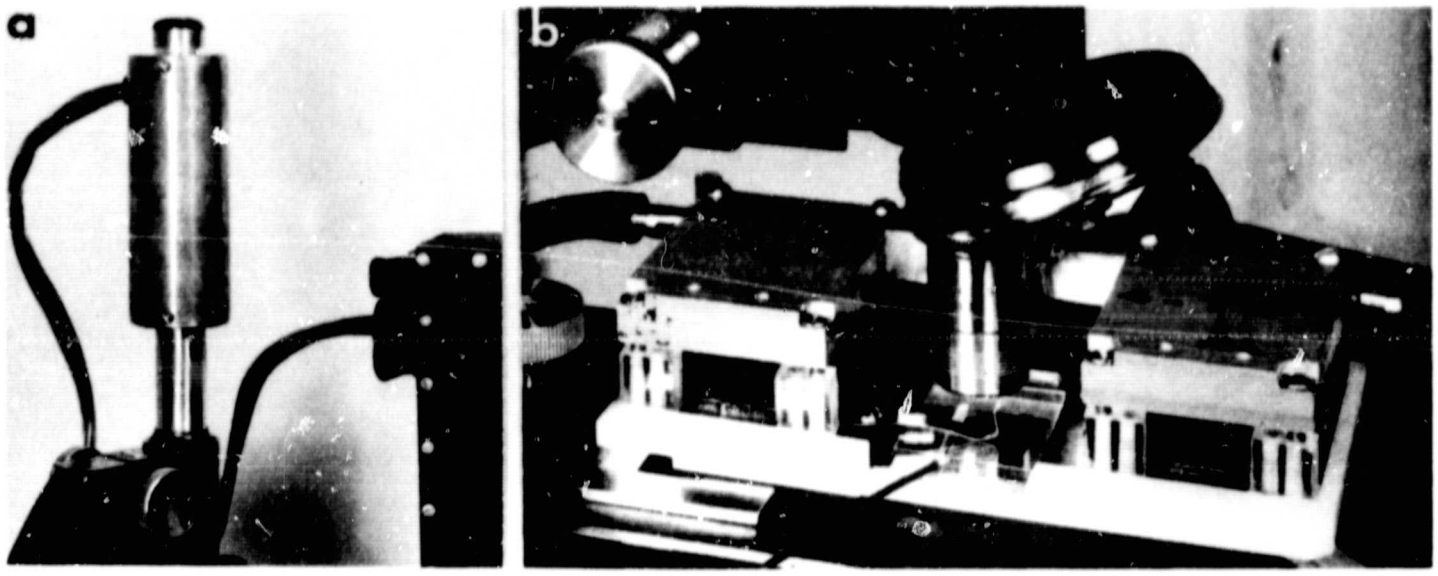


Fig. 5 a). Infrared image viewer. b). Temperature control microscope stage chamber (-20° to +100°C).

Detector. The detector is an EMI 9558Q Photomultiplier with a housing to cool the photomultiplier. The photomultiplier enables the instrument to make measurements with lower levels of light and improves the instrument sensitivity. The EMI 9558Q Photomultiplier Tube has a spectral response from 162 nm to 840 nm, with a quantum efficiency of about 20 percent from 200 to 420 nm, 10 percent efficiency at 480 nm, and 1 percent efficiency at 785 nm. The dark current noise is extremely low, and with cooling (-12°C) is almost negligible.

Monochromator and Light Source. The monochromator used is a Canalco rapid-scanning one, which has 600 line/mm grating and has ten different speeds, from three seconds (0.2 to 200 nm per second) over a 200 to 800 nm range. Its dispersion is 4 nm/mm and peaks at 500 nm. A disadvantage of all grating monochromators is that the light is about 10 to 20 percent polarized (a part of this polarization can be removed by using a quartz diffusing plate).

A quartz lens of about a 10 cm focal length was inserted into the light path in front of the aperture and adjusted so that the filament in the source and the slit was out of focus. This was found to concentrate more of the light on the aperture.

Chopper. The accuracy in measuring the relative percentage of absorption (as far as electronics are concerned) depends on the relative heights of the two pulses; the sample and reference areas are not measured simultaneously but

alternately in time. Therefore, if the overall accuracy is to stay within one percent, any change in the relative height of the pulses due to relative spectral quantum efficiency of the photocathode, the relative spectral distribution of the light source, or the gain of the amplification should be within one percent during the time for one pair of pulses. Consequently, a short-scanning time requires a correspondingly short pulse pair-time. The desired short pulse pair-time required the development of a precision, high-speed chopper and special electronic circuits in the amplifier. The chopper in the M-5 has been the main design improvement over previous instruments (Figure 4c and d).

Amplifier. The electronics consist of a preamplifier mounted on the case of the photomultiplier tube, a main amplifier, an electronic switch, an automatic gain amplifier, a feedback circuit, an integrating circuit for each sample and reference pulse, and a panel for necessary controls.

The lead from the photomultiplier to the preamplifier is as short as possible in order to minimize lead-in capacity. The preamplifier then has a current gain of about 150 X. The main amplifier gives the final amplification to the signal, which at this point consists of both sample and reference pulses alternately in time. The dc level is restored by a clampling circuit. The electronic switch, synchronized to the chopper, then separates the signals. The reference pulse goes to the automatic gain amplifier, where it is further amplified, and then to the feedback circuit.

The feedback circuit consists of a field effect transistor which acts as the load resistance for the photomultiplier. The output from the automatic gain amplifier is connected to the gate of the field effect transistor. The resistance of the field effect transistor depends on the potential on its gate in such a way that a high voltage input to the amplifier causes a low voltage output which results in a high resistance. A low voltage input to the amplifier causes a high voltage output, which results in a low resistance. As the signal from the photomultiplier increases, the output of the automatic gain amplifier, which goes to the gate of the field effect transistor, decreases. There is also a corresponding decrease in load resistance which decreases the signal. This operates over a range of 5000 to 1.

The circuit holds the reference pulse constant over a range of 5000 times within two percent. Now, the sample pulse is simply integrated, and sent to the recorder where the data is plotted.

Recorder. The recorder for the M-5 is a D'Arsonval-type pen drive, rather than the servomechanism-type recorder used for the M-3 and M-4 instruments. The electronics are much more versatile and enhance the response speeds. The instrument will scan the visible spectrum, 400 to 700 nm in the order of seconds, or 100 wave numbers in 0.33 seconds.

Operation of the M-5 Instrument

In operation, light from a tungsten or a xenon lamp passes through a Canalco grating monochromator with variable slits to a specially designed chopper. The chopper divides the beam of light into two beams which pulse alternately in time. Each beam is on 40% of the time and is pulsed 480 times/sec. The two beams of light are directed into a condenser of a microscope, which is an ultrafluar objective lens of 100 X or less. Its purpose is to focus the two light beams as two spots of light on the specimen slide. The slide is moved to locate the specimen area to be investigated over one of the spots while the other spot is used as a reference. The light is then collected and focused by the objective, and a simple quartz lens (focused on the back diaphragm of the objective) directs all the light on the photomultiplier tube.

For viewing the specimen and locating it with respect to the spots of light, a swingout prism directs the light to the binocular eyepieces. A rotating reflecting blade serves to direct light from the microscope illuminator into the light path to illuminate the background which can then be viewed simultaneously with the monochromatic spots of light. When the swingout prism is in position for a measurement, the rotating reflecting blade is automatically stopped so as not to interrupt the monochromatic light path, and only the monochromatic light goes to the photomultiplier.

The photomultiplier output is fed into the ac coupled amplifiers. The dc level is restored by a combination of a clampling circuit and a pulse shaping circuit. The signal is then synchronously switched into separate RC smoothing circuits. The synchronization signals are supplied by a light source and phototransistor arrangement at the chopper. Now that the signal has been separated the sample signal is fed into a recorder. The reference signal or AGC is then

amplified and the output is applied to the gate of a load resistance field effect transistor located at the photomultiplier output. This keeps the average level of the reference signal at the amplifier output a constant. The average level of the sample signal is then directly proportional to the absorption of the specimen.

For kinetic studies, a temperature-controlled specimen chamber with thermoelectric elements was constructed and adapted to the microscope stage; temperatures from -20° to 100°C are easily obtainable in the specimen chamber (Figure 5b). The cells can be grown or maintained in this chamber; chemical reactions can also be carried out in this chamber.

Advantages of the M-5

The Microspectrophotometer M-5 has an advantage over other laboratory and commercial spectrophotometers in being able to scan with increased spectral sensitivity from the ultraviolet through the visible to the infrared, 225-800 nm, in less than two seconds, at very low light levels (10^4 photons per second). Using other photodetectors or photomultipliers, spectra into the infrared beyond one micron are obtainable; however, with longer time constants. Spectra can be obtained from single cells, from cell organelles and from particles of about 0.5 micron in diameter. Solution spectroscopy are also obtainable by incorporating in the design a chamber to hold spectroscopic cuvettes (Figure 4d).

To really make this instrument adaptable and most useful, we are exploring the possibilities for not only measuring the absorption spectrum but the fluorescence emission from cell organelles and crystals under study. This is not an easy task, even though several recording microspectrofluorometers have already been built (12, 13, 17).

V. APPLICATIONS AND SOME EXAMPLES OF SPECTRA OBTAINED

Photosynthesis

In research related to photosynthesis, the absorption spectrum of chlorophyll and the pigments of the chloroplast are of considerable importance in elucidating the process (24, 28, 30-33). In Figure 6, the absorption spectrum of the Euglena chloroplast from the ultraviolet through the visible was obtained (within 13 sec) with a single sweep. The spectrum is similar in peak position

to the spectrum of chlorophyll a (Figure 7), which is known to account for 80-90% of the Euglena chloroplast chlorophylls. The peak near 480 nm is that of carotenoid which is associated with the chlorophylls in the chloroplast. The instrument also permits us to follow the synthesis of chlorophyll in the cell during photosynthesis (Figure 8).

Vision

Microspectrophotometry has immediate application to photochemistry and to the understanding of how the retinal photoreceptors function from eyespots (Figure 9) to retinal rods and cones of the eye in vision. Studies of the retinal photoreceptors, the rods and cones of the eye which are known to be sensitive to visible light, indicate that microspectrophotometry is most useful in studying the visual pigments (i.e., the photochemistry of the visual process during light ↔ dark reactions). This is not only important to how we see, but to understanding how we perceive colors. The principal component of the retinal rods is the visual pigment rhodopsin (21). It accounts for about 40% of the dry weight of the frog retinal rod outer segment. Figure 10 is a typical absorption spectrum from 250 to 650 nm for a frog retinal rod outer segment. Curve 1 of the retinal rod shows the spectrum of rhodopsin, and that the major absorption is near 500 nm; in addition, there are peaks near 280 and 350 nm, which are associated with opsin and retinal. The light-bleached spectra, curves 2, 3 and 4, show the release of retinal from the rhodopsin complex with a shift in the spectral peak towards 380 nm, that of retinal$_1$. The protein opsin absorption peak near 280 nm does not change with light bleaching. The search for the identification of the pigments in the retinal cones responsible for color vision have now been determined (9, 10).

Concentration of Pigment. The number of chlorophyll molecules in a chloroplast or in a retinal rod outer segment can be calculated from such spectra (Figures 6-8 and 10), if the thickness of the specimen and the pigment extinction coefficients are known. For example, calculations from the absorption spectrum of the Euglena chloroplast indicate that there would be 1.3×10^9 molecules of chlorophyll in the chloroplast, and from the absorption spectrum of the frog retinal rod outer segment there would be 3×10^9 rhodopsin molecules in the retinal rod.

The Red Blood Cell and Respiration

Another example is that of the identification of the hemoglobins, the oxygen-carrying pigments of the red blood cell. Hemoglobin is present in large quantities in the cytoplasm of the red blood cell. The hemoglobin molecule contains four hemes which are bound to the protein globin. The heme pigments can be identified by their absorption peaks. The characteristic spectrum of hemoglobin is very useful for the rapid detection by microspectrophotometry of hemoglobins in a single blood cell to identify specific blood abnormalities (22). Using the microspectrophotometer, the absorption spectrum of a single human red blood cell is shown in Figure 11, which shows the absorption peaks near 415, 541 and 577 nm. Other red blood cell spectra are illustrated in Figures 12 and 13.

Single Cells, Phototropism and Crystal Spectroscopy

In an attempt to comprehend at the molecular level how visible light initiates phototropism and how this is related to vision and nervous excitation, two model living single cells were chosen for study (23-27, 29, 33). These are the algal flagellate Euglena (Figures 6-9) and the fungus Phycomyces (Figures 14-17).

The problem undertaken here was to see if it was possible to identify the photopigment responsible for the organisms phototropism by microspectrophotometry. For Phycomyces, Figure 14 shows two spectral scans in the growth zone. Spectrum (a) is typical of that of β-carotene, while spectrum (b) is more typical of a photoreduced flavin or a flavin semiquinone. The Phycomyces mutant car-10 (Figure 15) which is deficient in β-carotene, shows only that of Figure 14b, a flavin-like molecule. In Figure 16, the spectra of the crystal zone of Phycomyces is compared to riboflavin crystals.

The uniqueness of the instrument is illustrated in Figure 17, which shows the spectrum of a single cyrstal. Thus, it provides the basis for study of single crystal spectroscopy.

Model for Origin of Life Studies

In collaboration with Professor Sidney Fox, Institute of Molecular Evolution, University of Miami, Miami, Florida, information regarding the chemistry of laboratory synthesized microspheres, believed to be a precursor structural model in the evolution of life, is being explored. Microspectrophotometry also provides information on cellular DNA, RNA and protein synthesis.

Paleontology, Geology and Oceanography

The application to the study for the identification of particular organic compounds in fossils would be of considerable help to paleontologists.

In collaboration with Professor G. Mueller, Institute of Molecular Evolution, University of Miami, Miami, Florida, samples of foxite (from a mineral vein, Derbyshire, England); oxocerite, a film extracted from quartz found in S.W. Africa which contains microsphere-like inclusions, were studied.

Professor Mueller also brought a small sample of the lunar breccia from the Apollo 11 Mission to this Laboratory in order to obtain spectra of the very small "glass" spheroids. These range in size from 0.1 to 500 microns. A selection was made of those ranging from 0.5 to about 40 microns, with most being 10 to 15 microns in diameter. Gross spectra were made of the whole spheroids as well as spectra of specific parts that appeared to differ from the whole. The microspectrophotometry of these particles indicated differences from particle to particle, but in most cases, a uniformly high ultraviolet absorption and low red absorption which is in line with their salmon pink coloration. This does, however, differ from volcanic, meteorite and in part, glasses, which exhibit a relatively greater uniformity in comparison (7).

Dr. R.V. Gentry, Institute of Planetary Science, Columbia Union College, Takoma Park, Maryland, applied this instrument to particular minerals and mica samples in search of identifying inclusions by spectra in these structures.

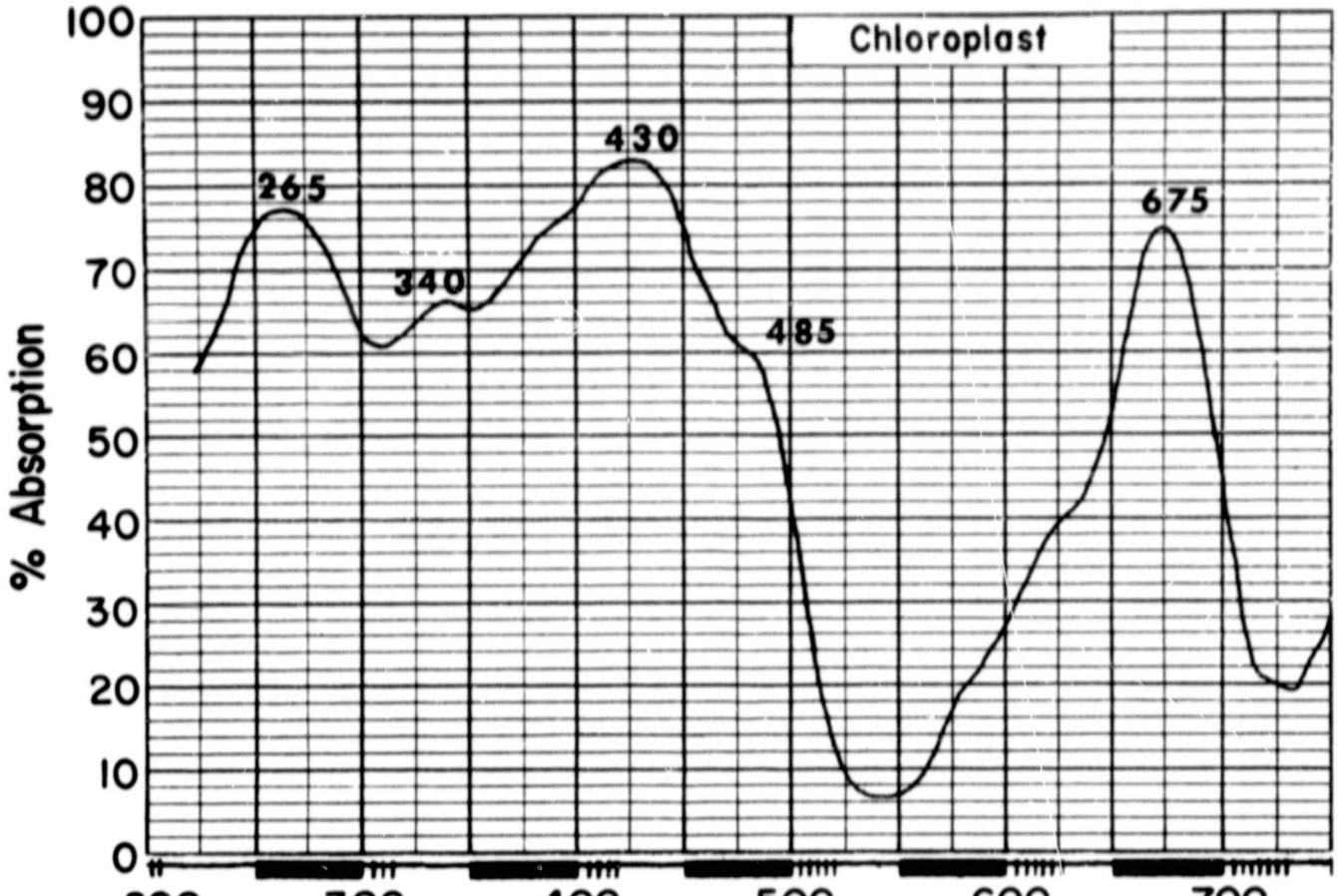
Related Studies

Other related problems under investigation are: What chemical changes control the growth of cells; what pigments, if any, are involved in abnormal cell growth; and what are the aging pigments?

In addition, the instrument has application to the study of the spectra of single crystals, especially those of biological origin (Figure 17). It also can be useful for studies in the identification of organic molecules in oceanic sediments, fossils, meteorites and extraterrestrial matter.

Therefore, microspectrophotometry is a versatile, sensitive and quantitative microanalytical tool for performing a variety of spectroscopic analyses of living cells, cell organelles, crystals, particles and dilute solutions which would be difficult if not impossible to obtain by other methods.

Chloroplast and Chlorophyll
absorption spectra



Time of scan 13 secs.

Fig. 6

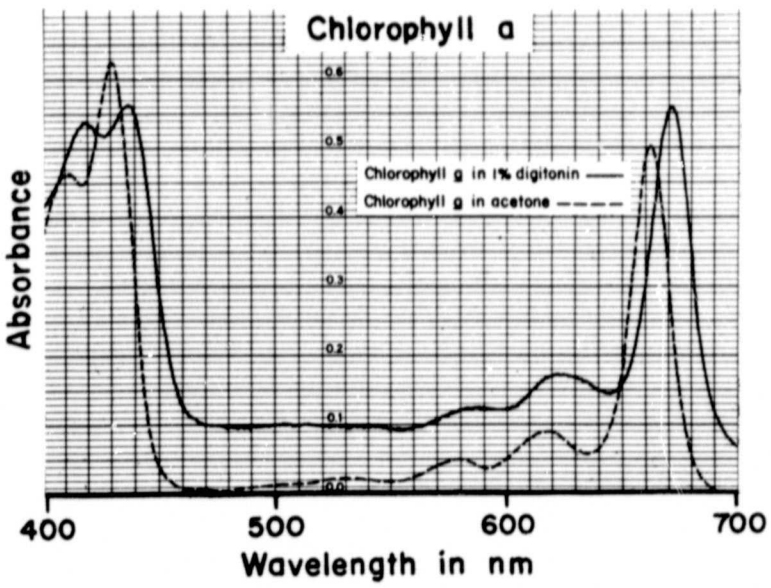


Fig. 7

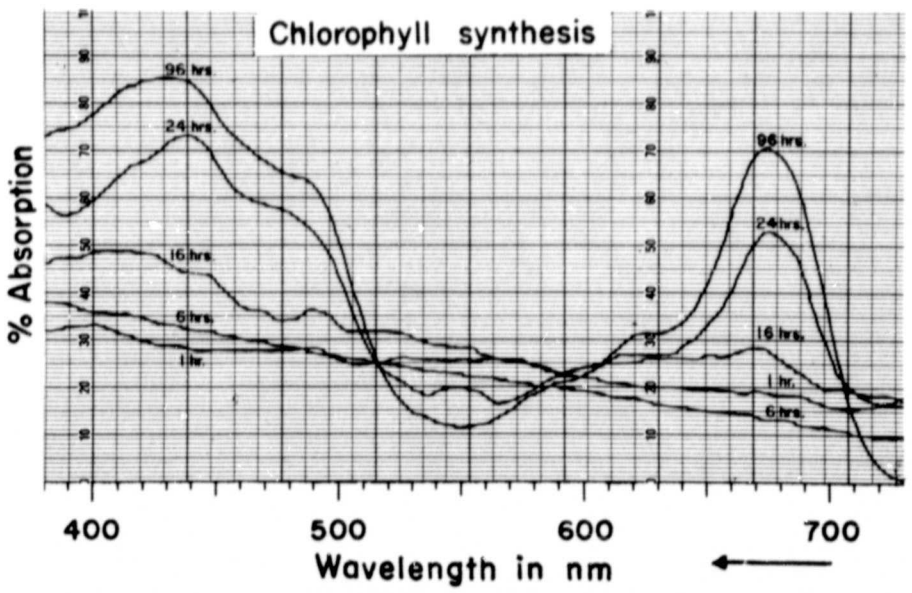


Fig. 8

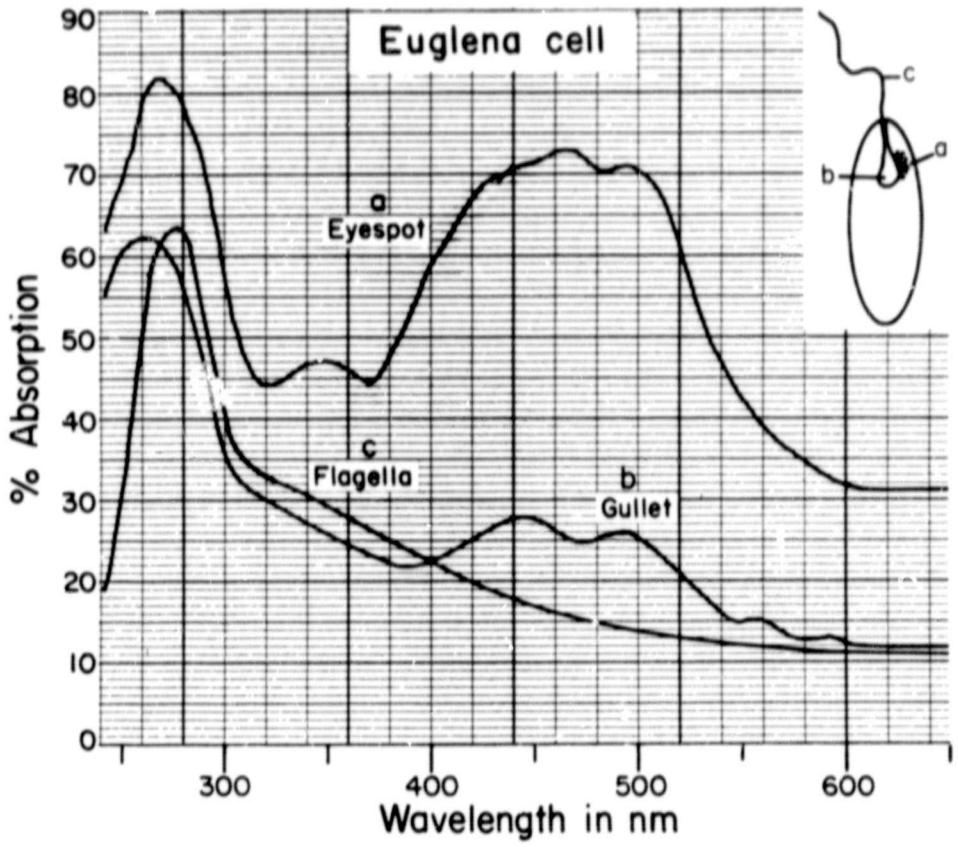

Euglena cell
a
Eyespot
c
Flagella
b
Gullet
c
a
b
% Absorption
90
80
70
60
50
40
30
20
10
0
300
400
500
600
Wavelength in nm

Fig. 9

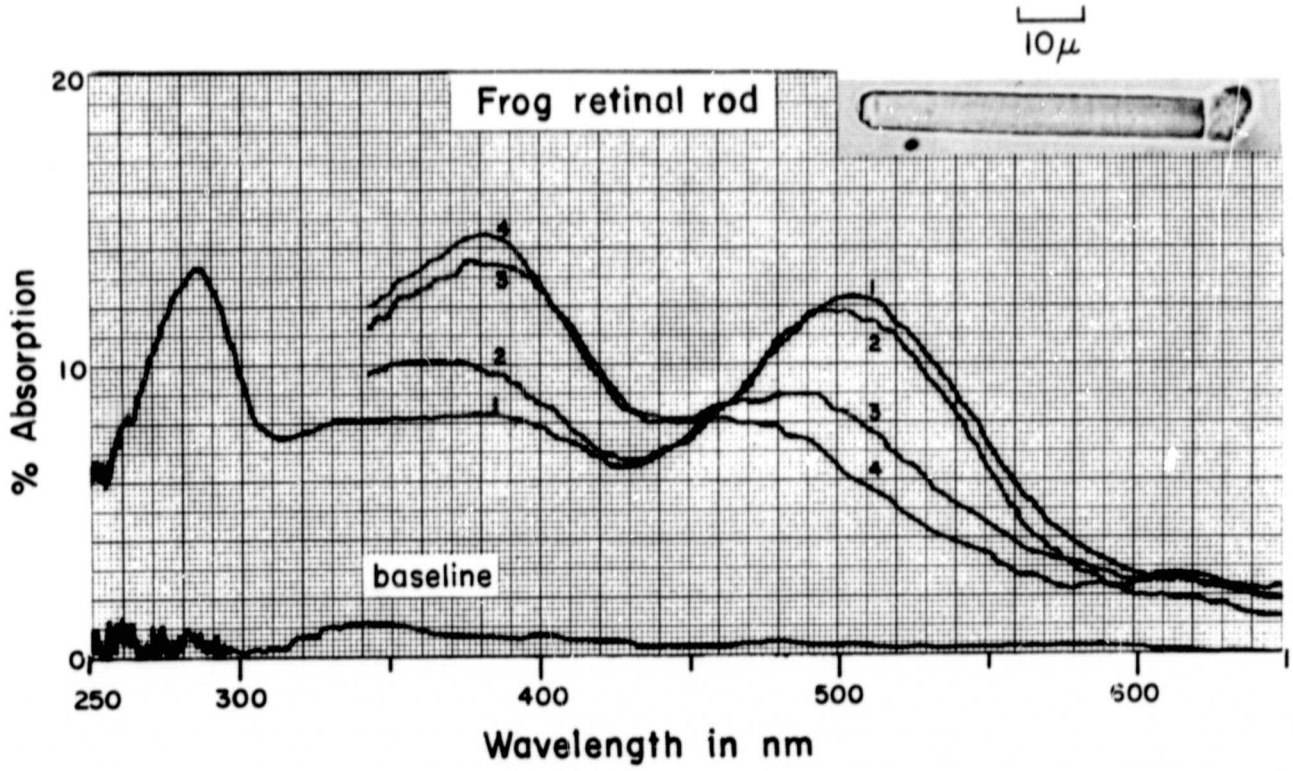

10μ
Frog retinal rod
baseline
1
2
3
4
% Absorption
20
10
0
250
300
400
500
600
Wavelength in nm

Fig. 10

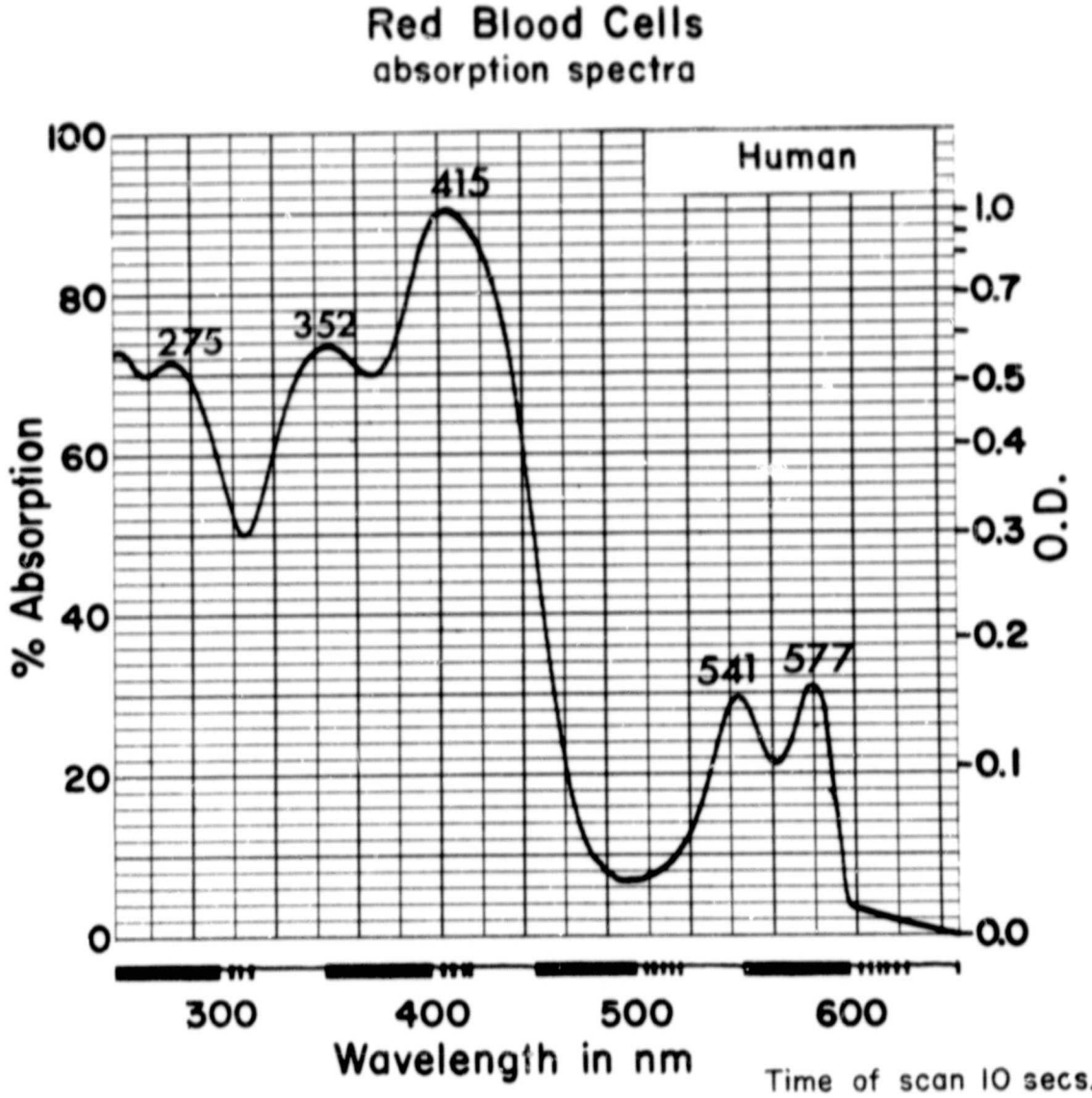


Fig. 11

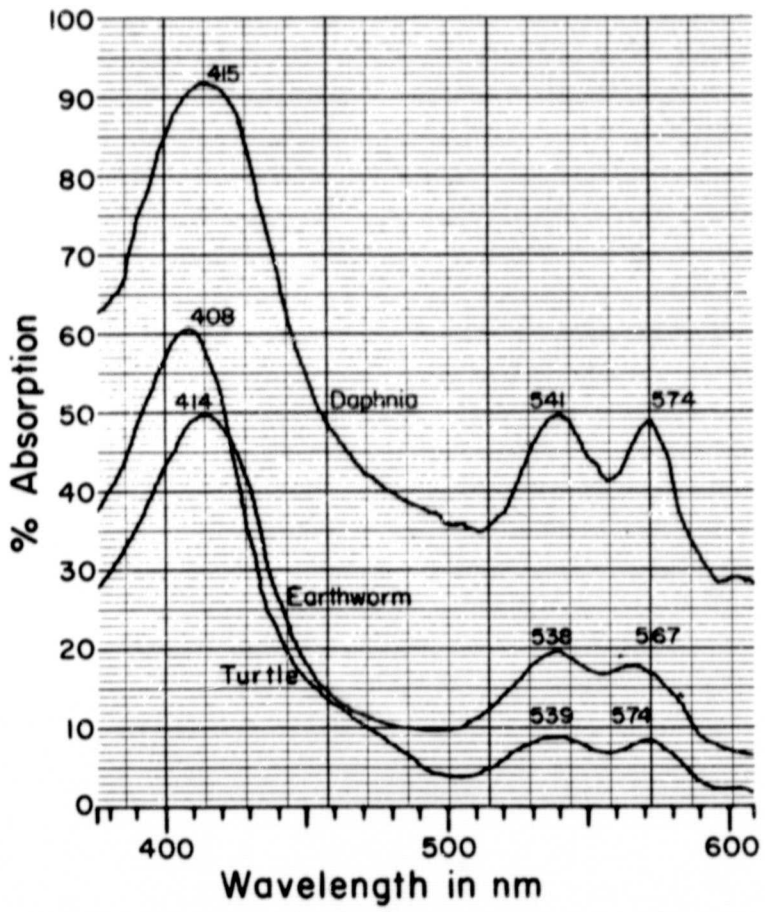


Fig. 12

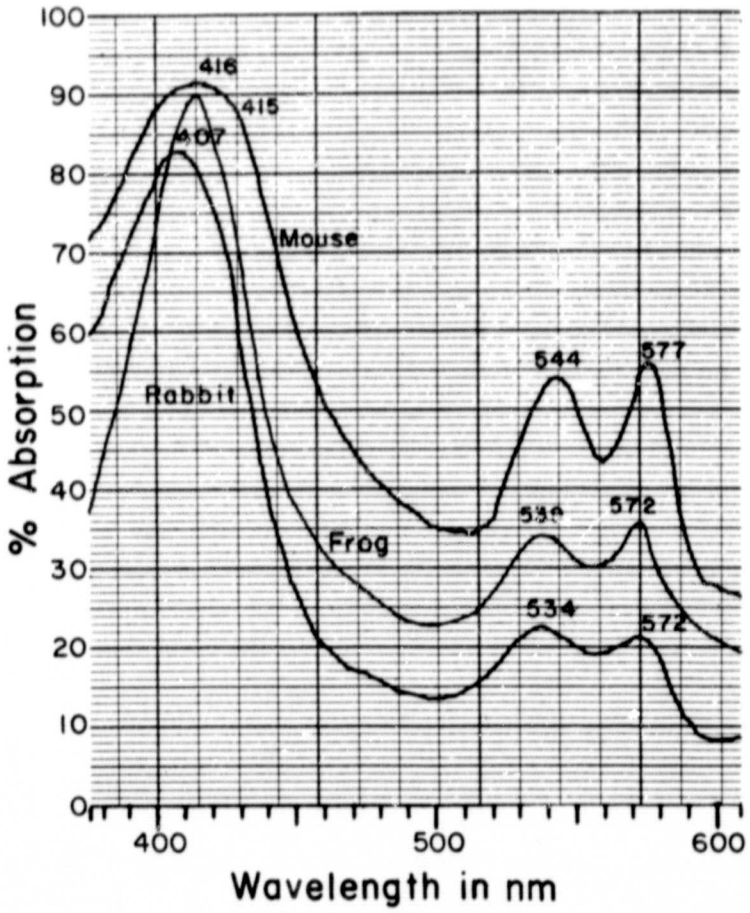


Fig. 13

Phycomyces Cell
absorption spectra

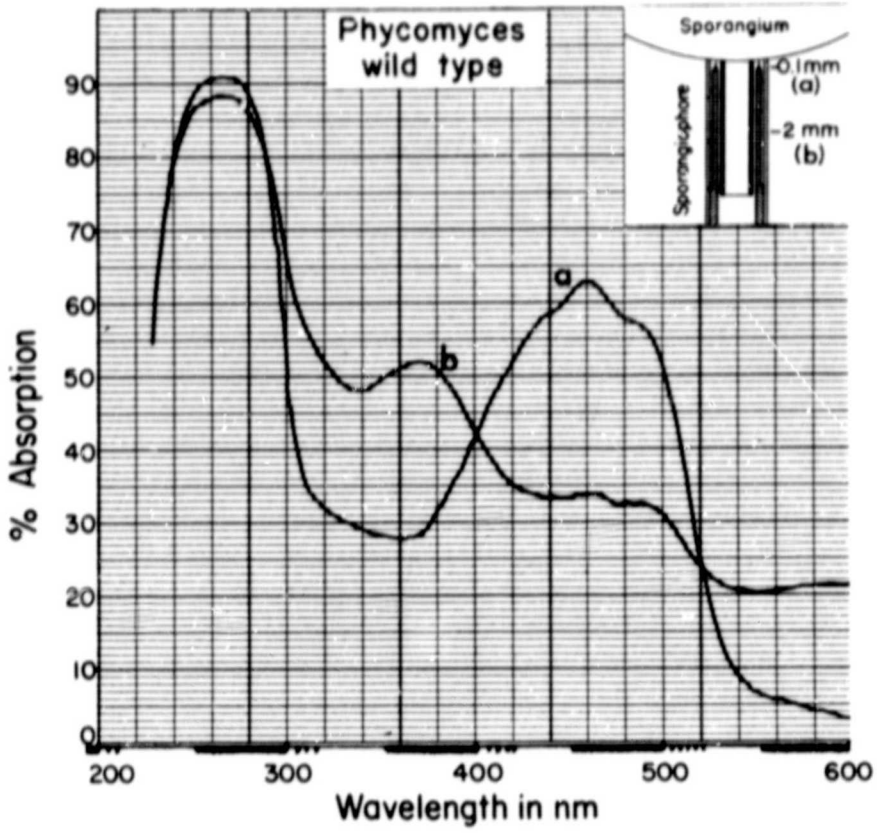


Fig. 14

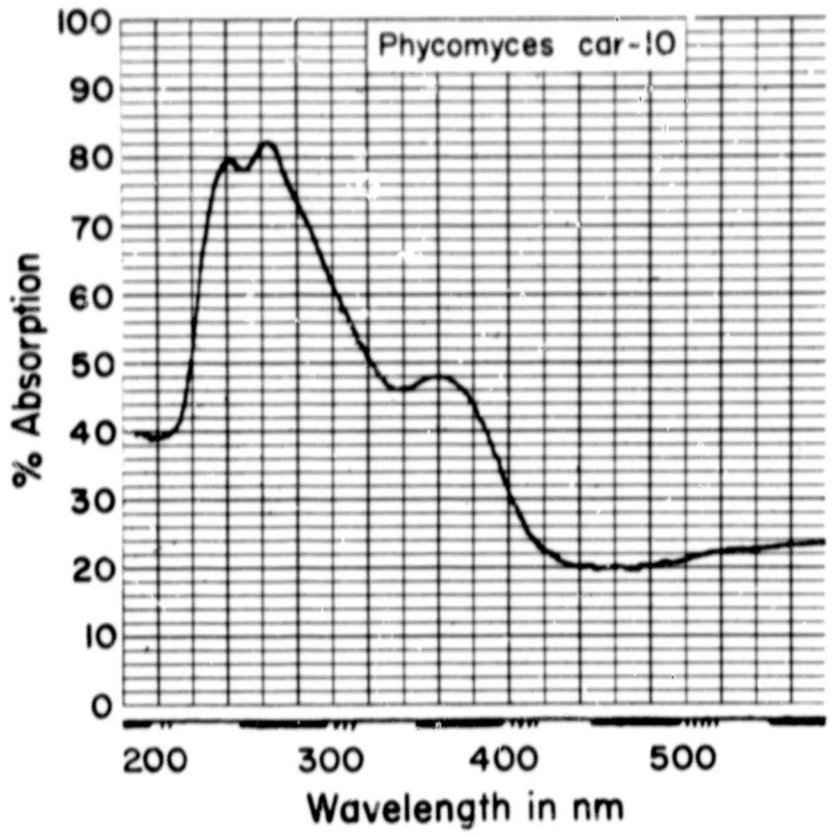


Fig. 15

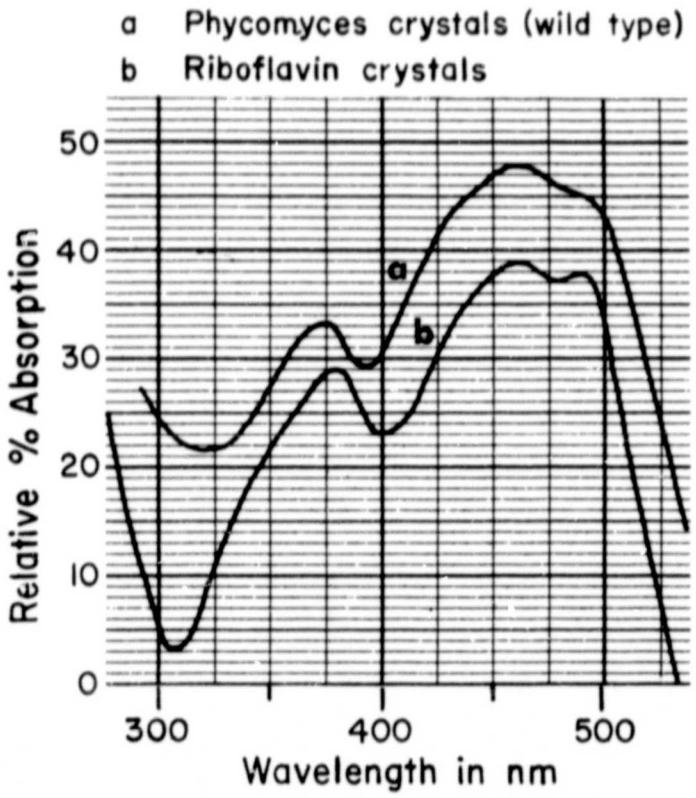


Fig. 16

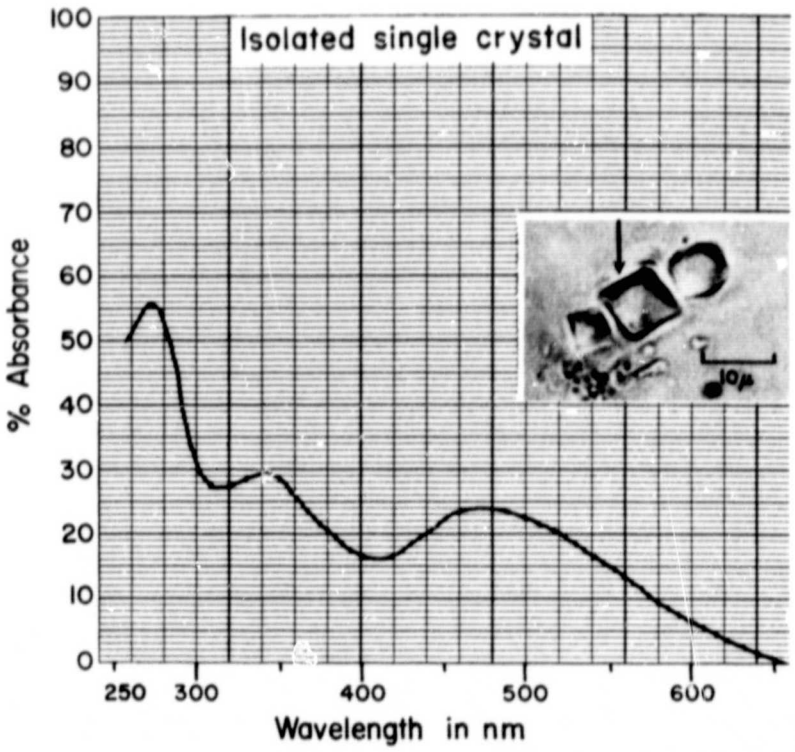


Fig. 17

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