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THE DIFFERENCE METER FOR MEASURING INTERIOR QUALITY OF FOODS AND
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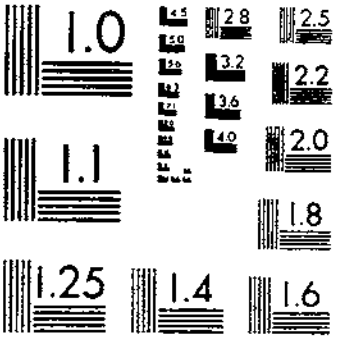

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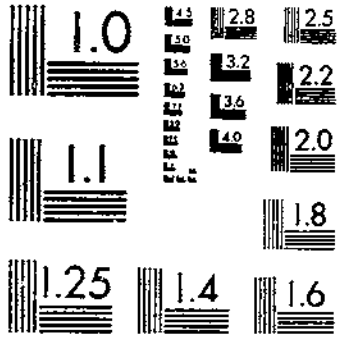

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THE DIFFERENCE METER

for Measuring Interior Quality of Foods *and* Pigments in Biological Tissues

Technical Bulletin No. 1341

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CONTENTS

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SUMMARY

It is generally believed that objects such as whole apples, tomatoes, or potatoes are opaque. Actually these objects, and biological tissue in general, will transmit some of the light falling on them. Although the amount of light that can be transmitted through a 2-inch-diameter apple is small, it is sufficient to permit evaluation of the internal color under controlled conditions without damage to the fruit. Specifically, measurements can be made to determine if the flesh of a red apple is green or white, whether or not there is blood in an egg, and if the interior of potatoes is the normal white flesh or if browning is present. The most satisfactory method of making these evaluations in a practical manner has been to measure the optical density of the product at two wavelengths and compute the optical-density difference. A number of instruments have been developed in the Instrumentation Research Laboratory of the U. S. Department of Agriculture to make such a measurement quickly. This report describes a design that has evolved over a period of years in the use of such instruments.

The instrument described here is compact, portable, easy to use, and has given very reliable service. The instrument employs interference filters to isolate the desired wavelengths and a photometer capable of measuring the extremely low light levels transmitted by intact products.

The instrument is of considerable value in the biological research laboratory because an estimation of the concentration of pigments in fresh tissue can be determined without making an extract. The convenience of use and the nondestructive feature make the instrument useful for the quality control laboratory and for establishing quality grades.

THE DIFFERENCE METER

for Measuring Interior Quality of Foods *and* Pigments in Biological Tissues

By G. S. BIRTH and K. H. NORRIS, Instrumentation Research Laboratory, Market Quality Research Division, Agricultural Research Service

INTRODUCTION

The interior quality of agricultural commodities is as important as the exterior, if not more so. A method of evaluating the interior quality without damaging the sample would benefit marketing, and research has been directed toward this goal. A spectrophotometric method for detecting blood in intact eggs was described in 1953 (*5*).[1] A commercial development of this method for automatic sorting of eggs (*22*) has proved to be more accurate and more economical than hand candling (*12*). Similar equipment has also been developed to detect certain types of bacteriological spoilage in eggs (*16, 17*).

Later, exploratory tests indicated that the technique had a potential application for the evaluation of the interior quality of horticultural products (*18*). This broader application required a more efficient light-measuring system because a sample such as an apple transmits less than 0.1 percent of the light falling on it.

To explore this technique further, two distinct types of instrumentation were developed: (1) The biological spectrophotometer for recording absorption spectra of intact samples (*8, 18, 19, 21*), and (2) abridged instruments for making a specific type of measurement (*1, 2*). The spectrophotometer is a research tool used to develop the criteria for a measurement to solve a specific problem. Mostly, such a measurement has taken the form of an optical-density difference between two wavelengths or Δ O.D. ($\lambda_A - \lambda_B$).

Several abridged instruments, using interference filters to isolate the respective wavelengths for such a difference measurement, have been constructed in USDA's Instrumentation Research Laboratory. The dual-monochromator spectrophotometer (*1*) was designed to make the difference measurement rapidly and yet maintain the advantages of the spectrophotometer by using monochromators to isolate the wavelengths for the difference measurements. Later developments have resulted in more sophisticated spectrophotometers and simpler, more portable, abridged instruments for the difference measurements. A prototype of a portable difference meter was developed in 1960, and later was redesigned, as described here.

[1] Italic numbers in parentheses refer to items in Literature Cited, page 19.

DIFFERENCE METER DESIGN

High reliability, ruggedness, portability, convenience, and speed of operation were considered the primary requirements for the new difference meter. A single-beam optical design, using single-wavelength interference filters to isolate the respective wavelengths, was chosen because of its simplicity. This system requires a method of exchanging filters in the light beam. A reciprocating design was considered but abandoned in favor of a rotating filter wheel. Hecht and others (*13*) showed the advantages of this type of optical unit in the measurement of low concentrations of water in petroleum products.

The difference meter was designed in four parts: Sample compartment, optical unit, electronic unit, and cabinet.

Sample Compartment

A vertical light path at the sample position is used. A window, which passes the monochromatic radiation to illuminate the sample, is surrounded by sponge rubber to hold the sample.

The chief requirement for position-

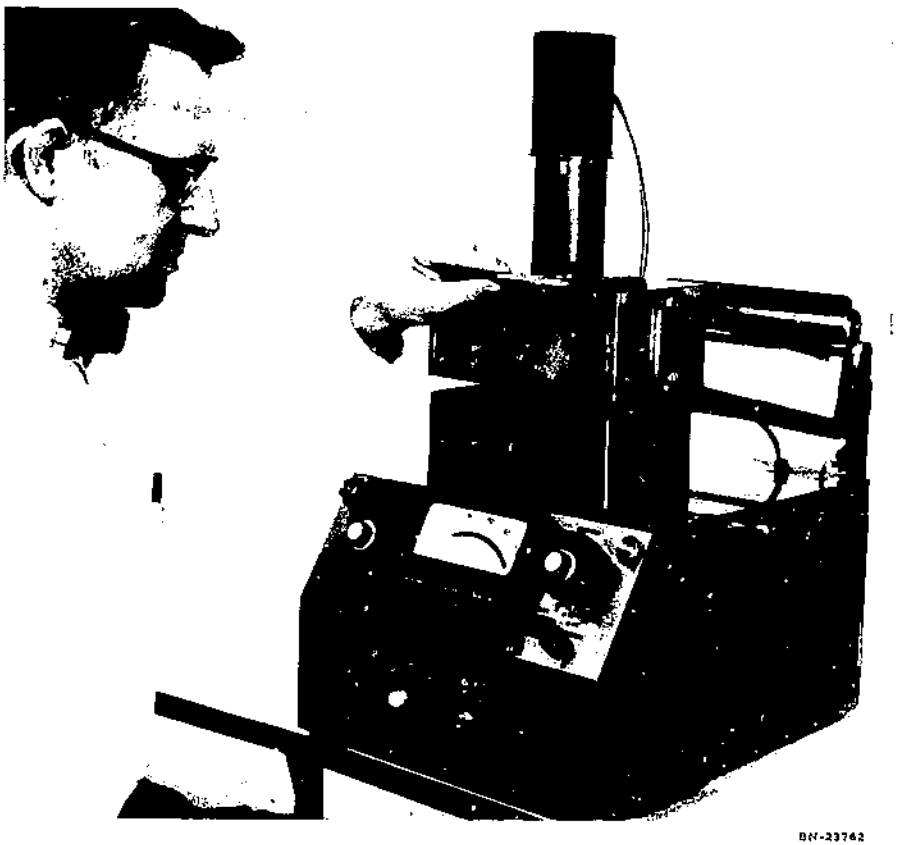

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FIGURE 1.—The horticultural difference meter for measuring optical-density differences of intact agricultural commodities.

FIGURE 2.—The phytochrome difference meter developed for studying phytochrome, a pigment controlling the physiological development of many plants.

ing the phototube is that it be placed as close to the sample as practicable. Since no critical optical alinement is necessary, the phototube is mounted in the movable part of the sample compartment at the top of the instrument. During the measurement, this movable portion of the compartment rests on the sample. This added weight on the sample compresses the sponge rubber to conform to the shape of nonsymmetrical samples, thus providing an effective light seal at both the window and the phototube. When the phototube housing is closed, its height varies with the size of the sample, for samples 2 inches or larger in diameter. Thus, with an appropriate readout device, it is possible to measure size while measuring the Δ O D value. An instrument with a sample compartment of this type is shown in figure 1.

Figure 2 shows a difference meter designed for a specific problem; that is, for measuring the activity of a reversible photoreaction regulating plant growth (9). The instruments (figs. 1 and 2) are essentially identical except for the sample compartment. In the phytochrome instrument, the sample is normally a liquid or a small section of tissue. Thus, a large sample compartment is not necessary, but a convenient means of irradiating the sample with red or far-red light is desired. This is accomplished by mounting a 12-inch-diameter wheel on top of the cabinet. This wheel covers a 4-inch-diameter sample compartment. An access port, a phototube, and lamps

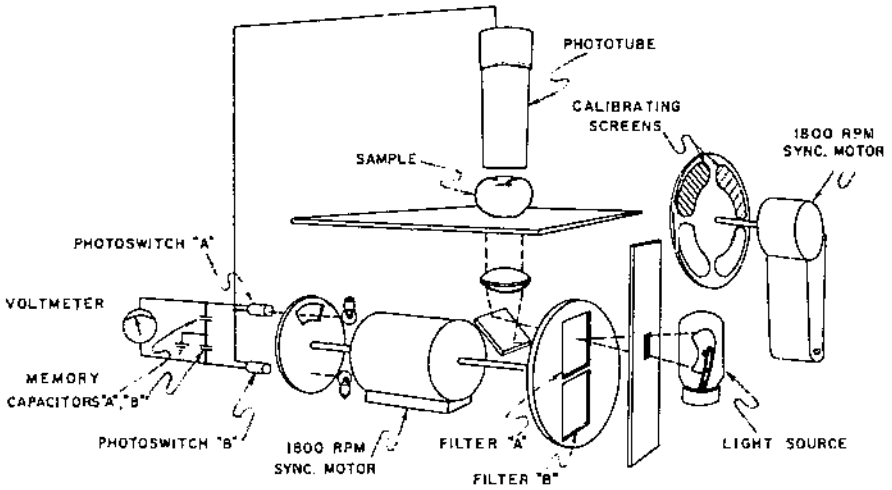

FIGURE 3.—The optical design of the difference meter, including the basic elements of the measuring circuit.

with filters for irradiating with red or far-red light can be positioned over the sample as needed. A timer is included to control the time for the irradiating lamps.

Optical Unit

Figure 3 shows the optical unit schematically. This unit can be removed intact from the instrument (fig. 4). The filter wheel, mirror, and lens are in a rigid housing. When placed in the cabinet, this housing is bolted rigidly to the base and top of the cabinet.

A 150-watt projection lamp (ASA Code DFC) with an internal reflector was selected for the light source. This lamp is about three times as efficient in collecting light as the conventional lamp and lens system. A variable voltage transformer is used to power the lamp. The lamp voltage is normally limited to 70 percent of its rated value or less. At 70 percent of rated voltage, the required heat dissipation is reduced by 50 percent and the lamp life is increased 50 times, with only a 3-percent sacrifice in the overall optical-density range of 10.0.

The lamp illuminates a ¼-inch by ½-inch slit positioned as close as practicable to the filter wheel. Each opening in the filter wheel can hold two 2-inch by 2-inch filters with a total thickness of 0.40 inch. A mirror provides a vertical light beam at the sample position. An f/1 lens used in conjunction with the DFC lamp provides a nearly parallel light beam. To illuminate a large area on the sample, as is normally done with large samples such as apples, no other lenses are necessary. For small samples, supplementary lenses and apertures can be used. The relatively large, highly accessible sample compartment permits considerable latitude in the use of special adapters for specific problems.

An optional filter slot is provided above the lens. Filters in this position are in the beam for both wavelengths. Screens or filters can be placed here to attenuate the light beam for measurements on samples having low optical densities or to decrease the stray light transmitted by the interference filters.

A convenient method of calibrating the difference meter in optical density is included with the optical unit.

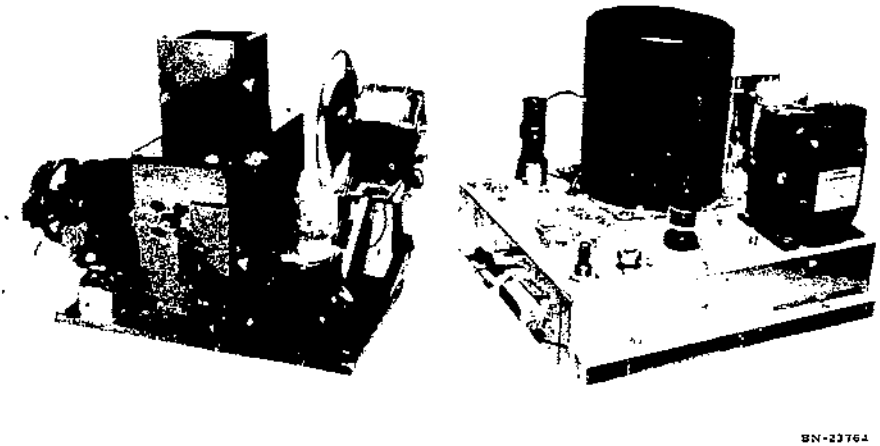

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FIGURE 4. Optical unit (left) and electronic chassis (right) of the difference meter.

An independent 1,800-r.p.m. synchronous motor is used to drive a wheel containing four openings with calibrating screens in two adjacent openings. This unit is pivoted so it can be moved to bring the screens into the light beam for calibration. In operation, the filter wheel and the calibrating wheel rotate at synchronous speed. With proper phasing, a screen will appear in front of one filter and the beam will be unobstructed for the other filter. Thus, while calibrating, one of the following four conditions will occur:

Possible calibrating positions:

$$\Delta\,O\,D = O\,D_{\lambda A} - (O\,D_{\lambda B} + C)$$

$$\Delta\,O\,D = O\,D_{\lambda A} - (O\,D_{\lambda B} + C')$$

$$\Delta\,O\,D = (O\,D_{\lambda A} + C) - O\,D_{\lambda B}$$

$$\Delta\,O\,D = (O\,D_{\lambda A} + C') - O\,D_{\lambda B}$$

Normal operation:

$$\Delta\,O\,D = O\,D_{\lambda A} - O\,D_{\lambda B}$$

C = optical density of screen 1

C' = optical density of screen 2

By momentarily interrupting the power to the calibrating-wheel-drive motor, the operator can shift from one condition to another in an identifiable fashion. The change in the meter reading resulting from introducing a screen into the system is equated to the density of the respective screen. The selection of the calibrating screens depends upon the sensitivity at which the instrument will be operated most of the time, but generally one screen would be five times as dense as the other. A standard, formed of calcium carbonate powder, is used to provide a check on the stability of the zero adjustment.

Electronic Unit

The photometer consists of a multiplier-type phototube operated with a constant-anode-current circuit as described in a previous paper (*20*).

The phototube is energized directly from a 2,500-v. transformer without filtering (fig. 5). The 6842 control tube blocks the positive half of the electrical cycle so that only the negative portion of the cycle appears on the photocathode as shown in figure 6. With this arrangement, the filter wheel must be driven by a synchronous motor with proper phasing so that a filter appears in the light beam when the phototube is

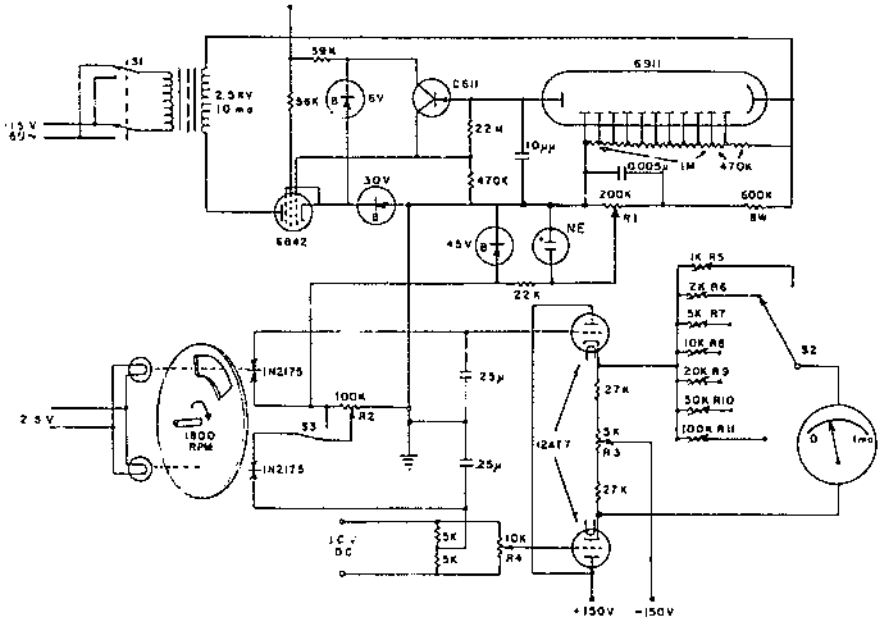

FIGURE 5. Circuit of the difference meter showing the photometer, the photoswitch, memory capacitors and voltmeter with sensitivity and zero controls.

energized. This design has three advantages over the previous direct current design (*21*): (1) Fewer components are required, (2) the noise level is lower, and (3) the phototube is not energized when there is no light on it; that is, during the interval between filters. A C-611 transistor current amplifier is mounted at the phototube socket so that the feedback circuit (anode of the phototube to the grid of the 6842 control tube) has sufficient response speed to follow the 60-cycle voltage.

Figure 7 shows the optical density vs. accelerating-voltage curve for this photometer, using a 6911 phototube. The unit exhibits an optical-density range of 10.

The accelerating voltage is attenuated with the 600 K resistor and potentiometer R 1 for controlling the voltage to the measuring circuit so that the neon indicator light goes on as the phototube approaches its dark-current condition. When the instrument is turned on the filters may appear in the light beam 90 degrees out of phase. This phasing situation is indicated by a neon light and can be corrected with switch S 1.

The 45-v. regulator diode protects the 1N2175 photoduodiodes which have a 50-v. maximum. These photoduodiodes provide low noise, solid-state switching for coupling the voltage from the photometer to the memory capacitors. The photoduodiodes vary from about 10^9 ohms when dark to less than 10,000 ohms when illuminated. Figure 6 shows how the resistance of each photoswitch varies during one revolution of the filter wheel. Mechanical switching has not been satisfactory in this part of the circuit. Commutators and automotive breaker points with stationery contacts were tried, but these devices developed too much noise. A circuit with a mercury-wetted contact relay was designed and has been used in two of the difference meters. This system gave good results, but required several additional compo-

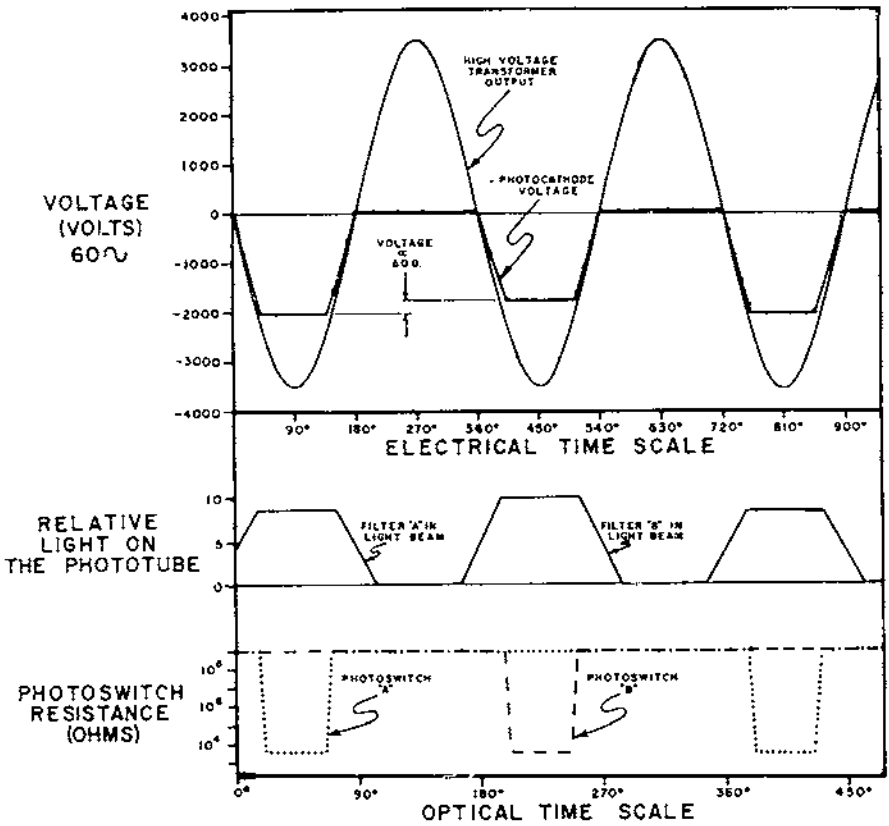

FIGURE 6.—Curves showing the optical, electrical, and time relationships of the photometer and photoswitch. One cycle on the optical time scale is 1/30 sec.

nents, including a separate power supply.

The instrument normally computes an optical-density difference between two wavelengths ($OD_{\lambda A} - OD_{\lambda B}$) or ΔOD ($\lambda_A - \lambda_B$). A special control (R 2) was incorporated in the instrument to permit an additional computation ($OD_{\lambda A} - K \cdot OD_{\lambda B}$) where the value of K is between 1.0 and 0.0. Switch S 3 allows the user to convert back to the normal operation without changing the value of K.

The relationship between accelerating voltage and optical density is nearly linear over the voltage range from 400 v. to 1,000 v. The source-lamp voltage can be adjusted for a given test to operate in this range. Under this condition, the voltages on memory capacitors A and B will vary linearly with the optical density of the sample at wavelengths A and B, and the difference voltage will give ΔOD ($\lambda_A - \lambda_B$). The 12AT7 tube provides a high-impedance voltmeter to measure the voltage difference between the memory capacitors, so that the reading on the meter is proportional to the optical-density difference. A zero control with a maximum adjustment equivalent to an optical-density change of ± 0.7 is provided by the 10-v. isolated d.c. power supply and potentiometer R 4. Larger zero adjustment can be made by attenuating one beam with a screen placed in the filter wheel.

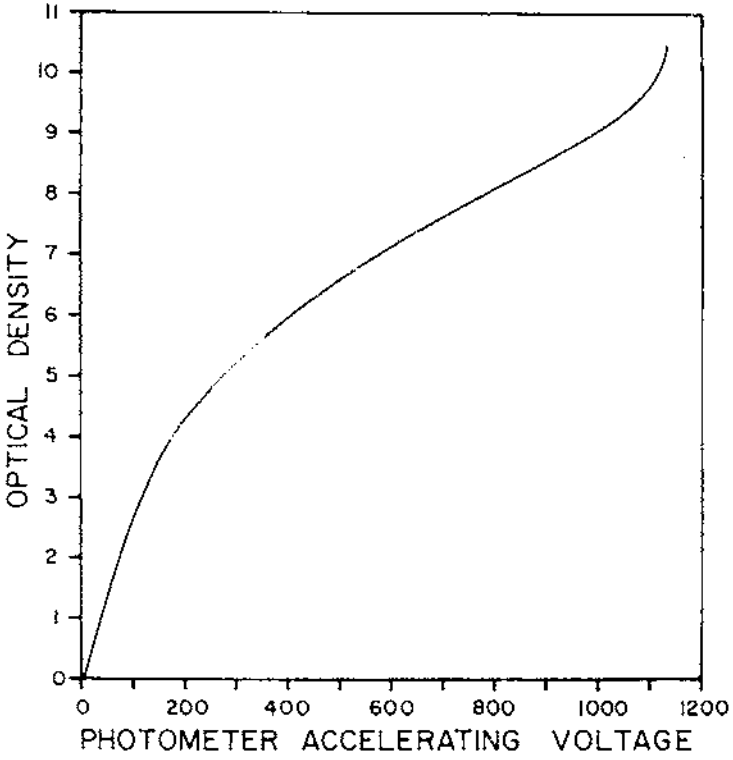

FIGURE 7. Optical density vs. voltage curve of the photometer circuit. At zero optical density, the lamp was at full intensity and no optical attenuation in the system (no filters or sample). When the filters were inserted in the filter wheel the accelerating voltage was about 80 volts. With a sample having an optical density of 4 in the system, the photometer would be at 400 volts and 6 O.D., or at the lower end of the linear portion of the curve.

The sensitivity is controlled by varying the resistance in series with the meter. Six preset sensitivity positions (R 5 to R 10), selected with switch S 2, provide full-scale optical-density ranges from 0.05 to 2.00. One additional continuously adjustable sensitivity control, R 11, is available on the front panel to conveniently obtain any sensitivity desired within the range of the instrument.

Cabinet

The cabinet for the difference meter consists of aluminum sheet bolted to a rigid frame. The front and rear of the cabinet are hinged to provide convenient access to the interior of the instrument. Switches and controls used in the normal operation of the instrument are mounted on the front door and are readily accessible for servicing. The rear vertical members of the cabinet frame on the horticultural difference meter provide the support for the movable part of the sample compartment which moves on a parallel arm linkage so that the phototube remains vertical from the open to closed position.

PERFORMANCE

Figure 8 shows the response speed and noise level of one of the difference meters. With a response speed of about ½ second to 90 percent full scale, the noise level is equivalent to an optical-density change of 0.0005.

Through 3 years of use, the only servicing necessary has been an occasional replacement of a lamp or vacuum tube.

The wavelength range of the instrument is from 400 nm to 1,000 nm. Although phototubes are available that have a response over most of this wavelength region, for any one problem it is important to select a phototube having a high response for the wavelengths being measured, to maintain the necessary wide optical-density range (table 1). The effective spectral characteristic of the system, which is a function of source, filters, and phototube, must be such that the instrument measures the density of the sample at the desired wavelengths for all conditions of the sample.

Interference filters are readily available for the wavelength range of the instrument. For many problems, filters with a spectral bandpass of 10 nm to 15 nm are acceptable.

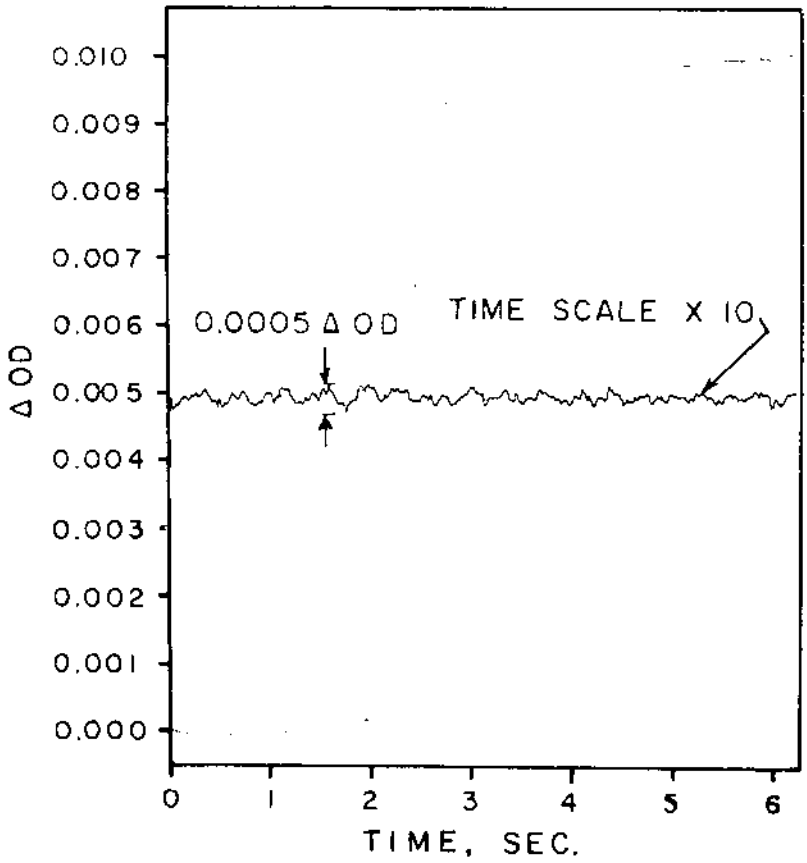

FIGURE 8.—Response speed and noise curves of the difference meter.

TABLE 1.—*Phototubes used with the difference meter*

Photosurface	Phototube [1]	Wavelength region
S 1	6911	650 nm–1000 nm
S 11	6342	370 nm 550 nm
S 20	9558 [2]	370 nm 800 nm
S 10	6217	500 nm 700 nm

[1] Phototubes listed are those used in the Instrumentation Research Laboratory. Other tubes with comparable characteristics can be used.

[2] This tube uses a different type of socket.

Stray light and extra order bandpasses of the filters can be serious limitations, particularly when the user wishes to measure at a wavelength in a region of relatively high optical density.

The instrument is rugged; normal stresses on the cabinet (for example, picking up the instrument while operating) cause less than 0.001 optical-density change in the output. The difference meter, which weighs about 70 pounds, is sufficiently portable to be conveniently moved from one laboratory to another. The instrument is most easily handled by two persons. Two operators, one to record data and the other to handle the fruit, can measure apples, or other products of similar size, at the rate of 600 per hour. An alternative method is to sort the fruit into several categories rather than to record data. In that case, one person can handle the operation at nearly the same rate, provided there is assistance in handling the containers of fruit.

RESULTS

Horticultural Applications

Figures 9, 10, 11, 12, and 13 show several applications of the difference meter. Additional information on twelve applications is listed in table 2. The wavelengths selected for each measurement are the result of a research study on the particular problem, using a biological spectrophotometer. Details of these studies can be secured from the literature cited. The difference meter is used to make the measurement shown. It would not be practical to use the difference meter to develop such a measurement.

Readings on the difference meter are based on an arbitrary scale of 0 to 100. This can be calibrated. The usual procedure is to adjust the instrument so that the range of samples being measured is displayed over the full scale, with one extreme condition of the sample (generally high quality) reading near 100 and the other extreme reading near zero. This relationship can be reversed by exchanging the filters in the filter wheel.

For the peaches, cherries, and tomatoes illustrated in figures 9, 12, and 13, the meter scale was arbitrarily divided into several equal parts and the product sorted into corresponding categories. In detecting hollow heart in potatoes, a preliminary test was made to determine the value on the meter scale to use for separating the potatoes into categories with a minimum number of errors.

Biochemical Applications

A two-wavelength, optical-density difference measurement as made by the difference meter is a valuable tool

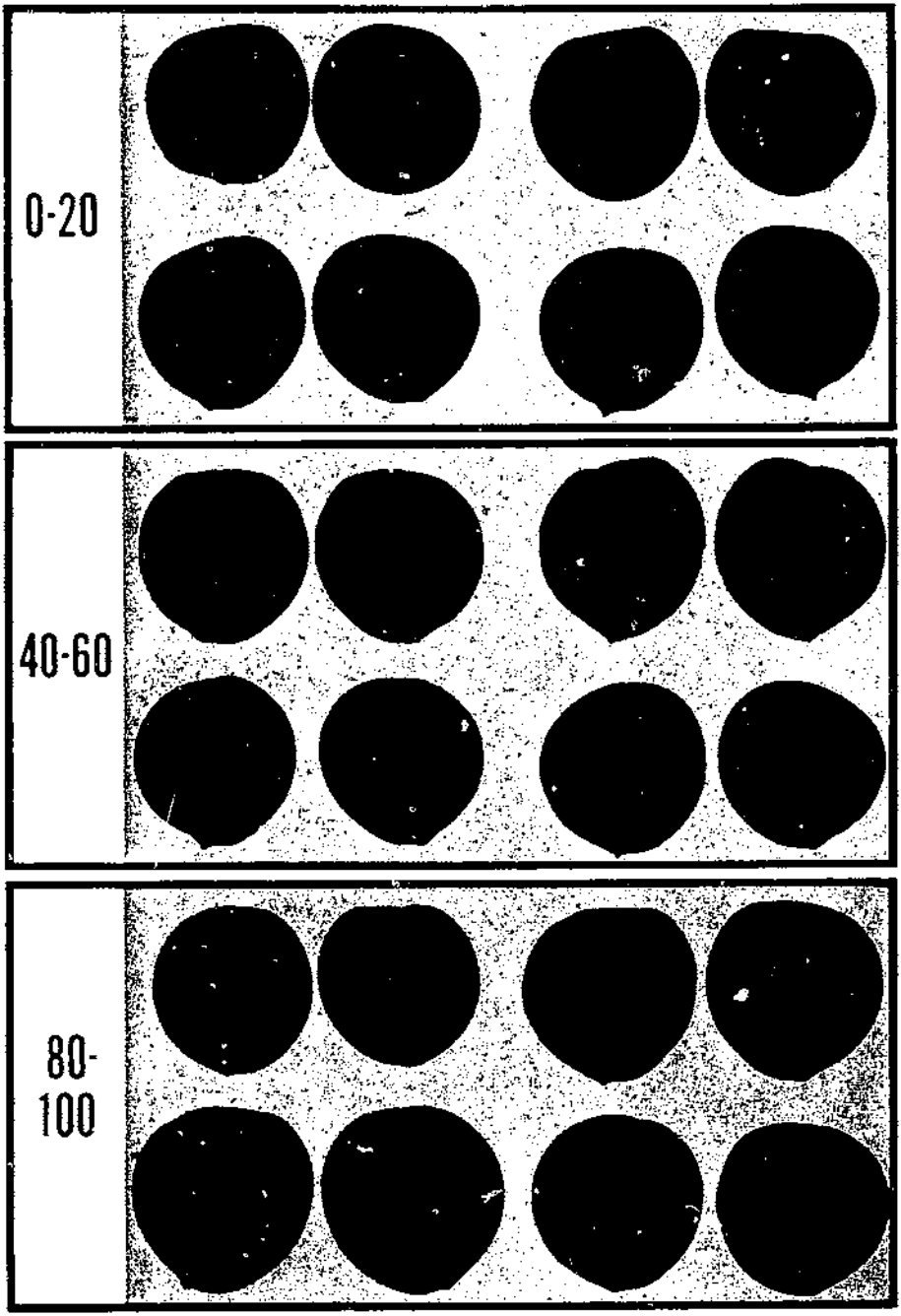

FIGURE 9. Peaches sorted for maturity. Elberta peaches were harvested and sorted into 5 categories with the difference meter. This picture of the cut peaches was taken shortly after the peaches were sorted. Measurement— Δ O D (740nm—700 nm). Full scale Δ O D sensitivity -1.0.

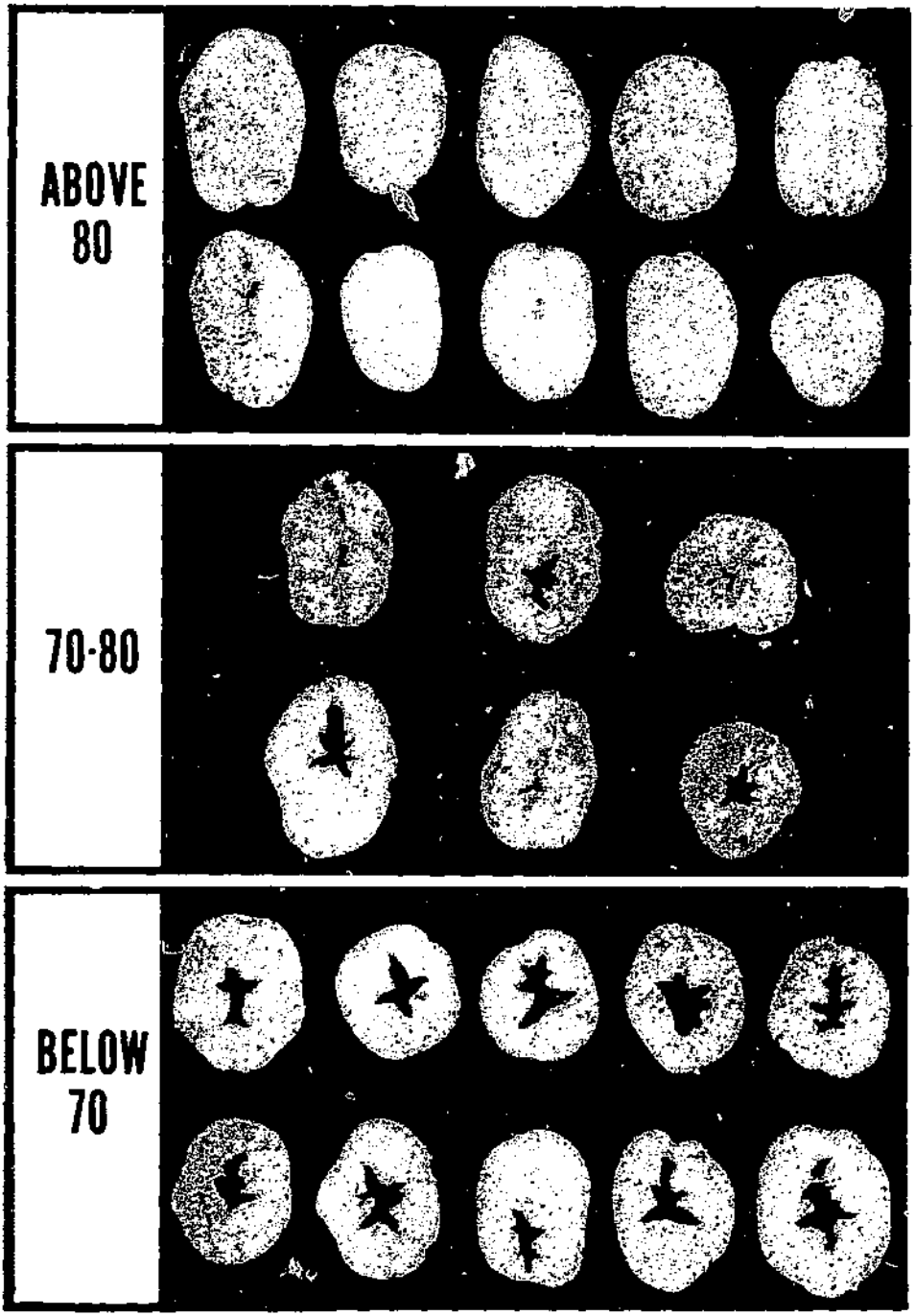

FIGURE 10.—Hollow heart detected nondestructively in potatoes. Potatoes were sorted with the difference meter into 3 categories prior to cutting and photographing. Measurement—Δ O D (800nm—710nm). Full scale Δ O D sensitivity—0.6.

for biochemical research. Measurements of the concentration of specific compounds can be made in the presence of many other substances. Chance has used this type of measurement extensively in the study of enzyme systems for making optical measurements on samples containing light-scattering materials (11). The instrumentation developed by Chance (10) operates in the density range of 0.0 to 2.0. The difference meter was developed to operate over a much wider optical-density range (0.0 to 10.0) with samples having dense light scattering.

An important effect of the light scattering in such samples is an increase in the effective path length of the light passing through the sample (7). This means that very low concentrations of pigments can be detected. In addition to the light-scattering effect, intact biological tissue normally contains several energy-absorbing substances. Considerable attention must be given to the selection of the wavelengths used in

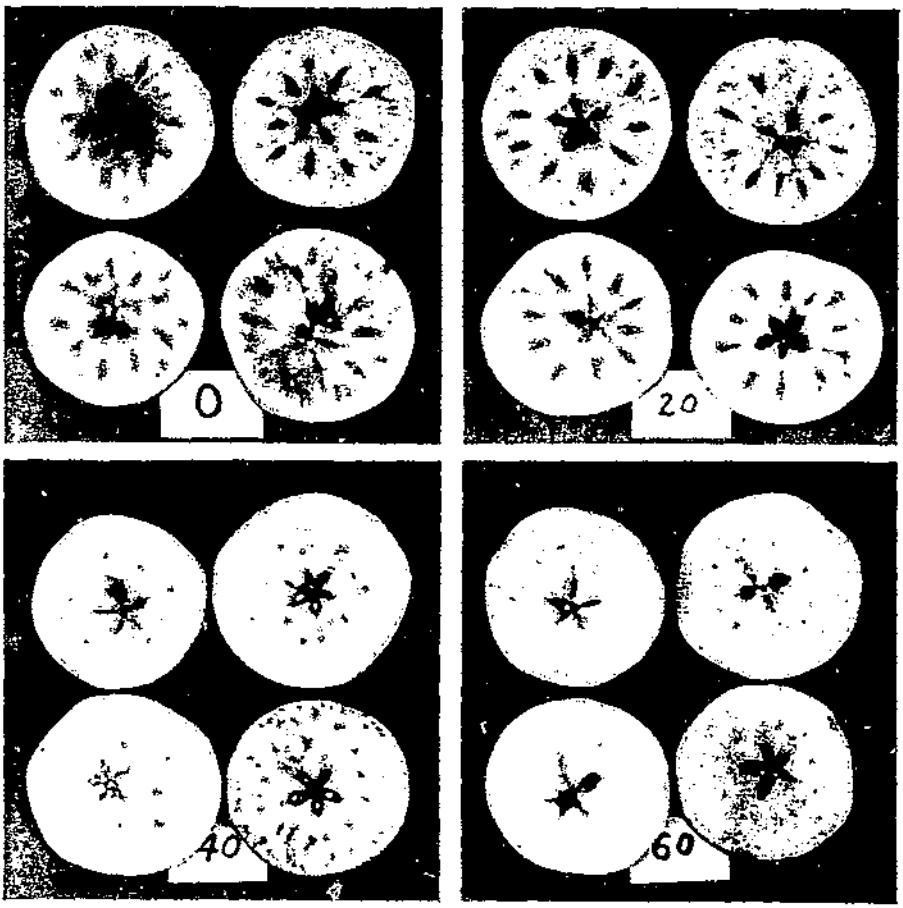

FIGURE 11. Water core detected nondestructively in apples. Apples in each group were selected on the basis of having comparable readings on the difference meter. Measurement: λ OD 760mμ - λ OD 810mμ. Full scale Δ OD sensitivity = 1.0.

FIGURE 12.—Red tart cherries sorted for anthocyanin pigmentation. Cherries of the Montmorency variety were measured and sorted into 5 categories at harvesttime. The cherries were pitted, canned, and stored for about 5 months; then the cans were opened and the cherries were photographed. Measurement—$\triangle$ OD (590nm—620 nm). Full scale $\triangle$ OD sensitivity—1.5.

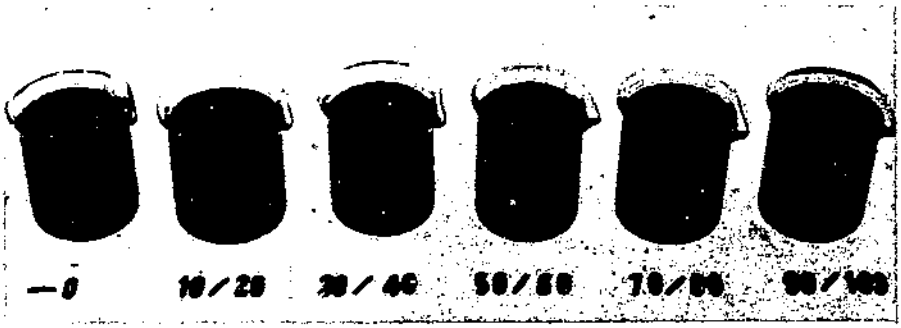

FIGURE 13. Juice of tomatoes that were sorted for internal color. After harvesting, tomatoes were sorted into 10 categories with the difference meter. Each category was juiced and samples of the alternate categories are shown here. Viewed externally, a few of the tomatoes in the category 10-20 could have been classed as culls, but others in that same category appeared the same as some of the tomatoes in the 90-100 category. Subsequent inspection of the cut tomato verified the evaluation made by the difference meter. Measurement—$\triangle$ OD (620nm—670nm). Full scale $\triangle$ OD sensitivity—0.5.

a difference measurement so the concentration of the substance in question can be evaluated in a valid manner.

In the USDA's Plant Physiology Pioneering Research Laboratory, the difference meter has proved to be an extremely useful tool in the study of phytochrome. Phytochrome is a reversible pigment that exists in two forms; it can be changed from one form to the other by illuminating it with red light or far-red light, depending on which form the pigment is in. This pigment controls certain growth characteristics, such as germination, flowering, and elongation, of many plants.

By means of a biological spectrophotometer, it was established that a $\triangle$ OD (660nm—730nm) measurement could be used to assay the reversibility of phytochrome. That is, measurements are made after illuminating the sample with red light and with far-red light; the difference be-

TABLE 2.—*Applications of the difference meter*

Quality evaluation problem	Measurement	Substance measured	Correlation of meter reading vs.	Reference
Mold damage in yellow corn.	Δ O D (800nm-950nm)	Mold spores	% damage 0.90	(*14*)
Degree of milling of rice	Δ O D (660nm-850nm)	Brown substances in the bran layers.	% surface lipids 0.84	(*26*)
Color of red tart cherries	Δ O D (500nm-620nm)	Anthocyanin	% anthocyanin content per gram fresh wt. 0.83	(*27*)
Maturity of peaches	Δ O D (700nm-740nm)	Chlorophyll	% chlorophyll content per gram fresh wt. 0.94	(*24*)
Maturity of apples	Δ O D (690nm-740nm)	Chlorophyll	% chlorophyll content per gram fresh wt. 0.92	(*28*)
Blood in eggs	Δ O D (597nm-577nm)	Blood		(*22*)
Water core in apples	OD 760nm-.8 OD 810nm	Physical changes in the apple tissue.	Visual rating of the cut apple 0.93	(*23*)
Hollow heart in potatoes	Δ O D (710nm-800nm)	Brown substances in the vicinity of the void.		(*3*)
Smut content of wheat	Δ O D (800nm-930nm)	Smut spores	Spore count 0.95	(*2*)
Color of tomatoes	Δ O D (620nm-670nm)	Lycopene and chlorophyll	Color of extracted juice 0.95	(*4*)
Greenness of oranges	Δ O D (690nm-740nm)	Chlorophyll		
Maturity of peanuts	Δ O D (490nm-520nm)	Carotenoid pigments		(*15*)

tween the measurements serves as the index of the activity of phytochrome. This is normally referred to as Δ [Δ (D) (660nm—730nm)]. These measurements can readily be made on the phytochrome difference meter (fig. 2). Since fresh tissue can be used in the instrument, many plant materials were evaluated as potential sources of phytochrome. The best source turned out to be dark-grown seedlings. A typical Δ (Δ (D)) value for a 1.5-cm.-thick sample of dark-grown seedlings is 0.03. The concentration of phytochrome in such a sample is in the order of 5×10^{-8} M (6).

The difference meter was used in the procedure for extracting phytochrome (25) where a measurement to detect phytochrome could be made on the original tissue, a suspension of the ground tissue, the extract, or the residue. Furthermore, the instrument has been used to observe changes in the pigment in live intact tissue as a function of changes in illumination, temperature, time, atmosphere, etc.

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