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A SPECTROPHOTOMETER FOR BIOLOGICAL APPLICATIONS

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SUMMARY:
Instrumentation incorporating digital data recording was developed for measuring the optical properties of intact biological materials. Unique attributes of the instrument are high monochromatic power and provision for handling a wide variety of samples. Results that illustrate the use of the instrument in different geometrical arrangements are included.

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A Spectrophotometer for Biological Applications *

American agriculture is becoming increasingly dependent upon the application of automation technology. Equipment for handling and processing agricultural produce does not readily differentiate between quality classifications unless the engineers designing the equipment incorporate specific devices into their design to recognize quality. Foreign objects and inferior produce can be readily removed from the desired product by properly designed automatic equipment (3,7).

To accomplish this goal, the engineer should have at his disposal a complete description of the physical properties for the product to which his design is applicable.

Radiant energy of the optical frequency range is widely used in assaying product quality for automatic sorting, as well as for laboratory measurements and remote sensing. In order to adequately describe the interaction between radiation and biological materials for the purpose of obtaining information about a specimen, many special purpose research devices have been used (4,5,6 & 8).

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Instrument Design

A new spectrophotometer, Figure 1, designed expressly to obtain data on intact biological samples was recently developed (1).

The radiation, which interacts with the specimen and is subsequently measured, is influenced by the absorption, distribution of the absorbing substances, light scattering, and geometry of the specimen. In order to quantify these factors, the primary design criterion was to develop a monochromator having high power output consistent with good quality spectra. High incident power contributes to improved spatial resolution in geometrical measurements of the radiation reflected or transmitted by the specimen as well as permitting greater freedom in selection of detectors.

Optical Design

The instrument source is a quartz iodide tungsten lamp. The lamp filament is at the focal point of the first or collimating mirror and replaces the entrance slit of conventional monochromator design as shown in Figure 2. Since the lamp radiates in all directions, numerous baffles are used so that the light that enters the monochromator is restricted to that which is actually used by the optics of the system.

The primary dispersion element is a 5" x 6" diffraction grating blazed at 600 nm. An image of the filament is focused on the exit slit. For optimum operation the exit slit must correspond to the cross section of the lamp filament. Thus the filament determines the bandpass of the instrument. A linear variable-wavelength filter for reducing stray light is located at the exit slit and the movement of the filter is synchronized with the grating rotation.

A reference cell is used to monitor the output of the monochromator.

Structural Design

A console arrangement is used for the electronic racks. The top of the console constitutes an optical table made of 3.4" aluminum plate with tapped holes located on a 3" grid pattern. The monochromator is mounted on a bearing so that it can be oriented in position to operate with appropriate sample geometry.

Assemblies for measuring transmittance (Figure 3), large area reflectance (Figure 5), and bi-directional reflectance (Figure 7), are available. Other geometrical arrangements can be readily set up with fiber optics and standard optical bench equipment.

Measurement

D.C: measurement system is available, where the output of the detector is fed directly to an operational amplifier (9). Either linear or logarithmic output can be used. This system is very easy to operate and provides a means of radiation measurement for any source such as fluorescence where light chopping cannot be accomplished easily.

A chopped light system is also incorporated in the instrument. This is a commercial lock-in amplifier and provides lower noise equivalent power than the D.C. system.

Recording

An X-Y recorder is available for direct recording of the output. This provides a means for quickly verifying the validity of the data and facilitates exploratory tests.

Digital recording is available in the instrument and has been set up to record the radiometric signal, a reference signal, and a wavelength signal upon receiving a record command. The record command can be generated at a suitable wavelength interval by the appropriate design of a programming wheel. The radiometric signal is recorded with four significant figures, the other signals with three figures. All signals are acquired at the same time regardless of scanning speed.

For normal use, the data requires corrections for spectral response and wavelength. The system is set up so that these corrections are subsequently made by a computer.

Performance

Monochromator

Output Power (1000 watt lamp) 700nm

10^{-2} watts

Bandpass

10 nm

Wavelength Range

350 - 1200 nm

Scanning Speed

0.03 nm/second to 30 nm/second

Detection

Noise Equivalent Power (530 nm) Photomultiplier

10^{-15} watts

Max Sensitivity

0.001 OD.

Recording

Analog

X-Y Recorder 8 1/2" x 11"

Digital

Punched paper tape

Interfaced with Procsy Computer

Record Interval 10 nm

(determined by design of a programming wheel)

Applications

The transmittance assembly (Figure 3) is similar to that described in earlier publications (2). Transmittance spectra of two grapefruit (Figure 4) illustrate the application of this unit. The optical density difference between the pink flesh fruit and the white flesh fruit is greater than 1.0 at 575 nm and there is no difference at 660 nm. This information can readily be used to develop equipment for sorting grapefruit for internal flesh color.

The large area reflectance assembly (Figure 5) was designed to provide a means of obtaining an area-integrated reflectance measurement. Detector geometry can be modified to suit the experimental requirements. This assembly was used to measure the reflectance spectra of intact potato chips. Two packages of potato chips were obtained having a small but perceptible color difference. The ratio between the reflectances for the two sample was computed and is shown in Figure 6. These results indicate an optimum criterion for distinguishing between the two samples to be the reflectance at 520 nm.

The bi-directional assembly, shown in Figure 7, is used to obtain reflectance data as a function of angle of incidence and angle of detection. Figure 8 illustrates the use of this unit in obtaining directional data on healthy corn leaves for a single angle of incidence.

Summary

The successful utilization of the spectrophotometer depends upon supporting activities, such as a literature file, radiometric standards, machine shop facilities and the availability of a computer.

There are numerous areas where the applications of the instrument can be extended beyond those already described; however, this depends largely upon future research requirements.

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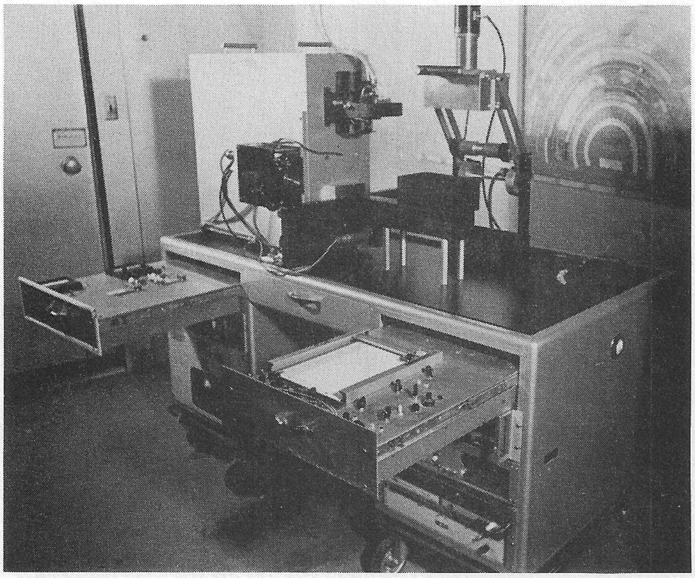

Figure 1. The Spectrophotometer With an Assembly for Measuring Transmittance.

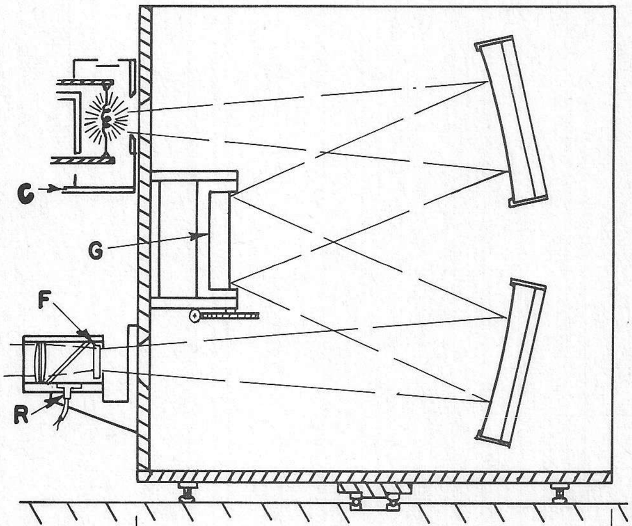

Figure 2. Crossection of the Monochromotor. The labeled components are a lamp cooling system (C); diffraction grating (G); linear-variable-wavelength filter (F); and reference detector (R).

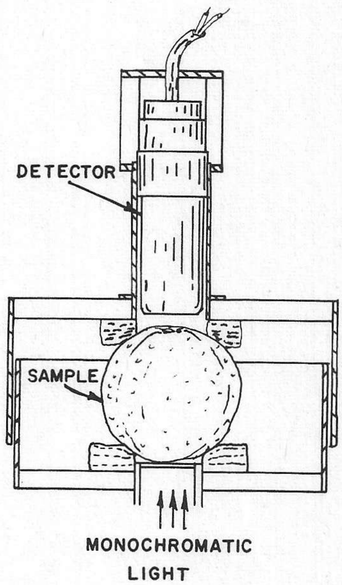

Figure 3. Sample Geometry for the Transmittance Measurement.

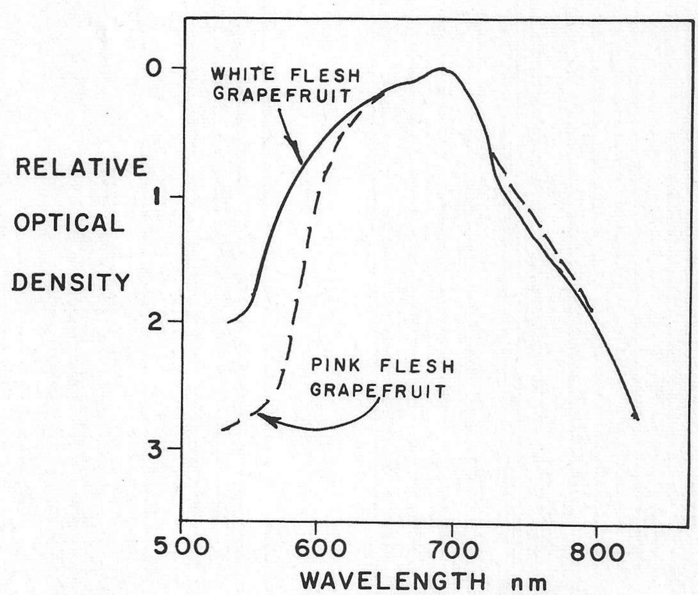

Figure 4. Transmittance Characteristics of Two Intact Grapefruit.

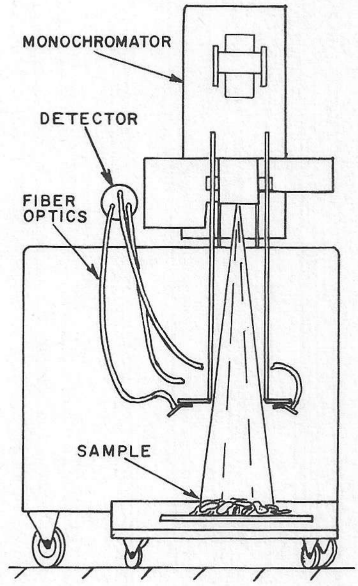

Figure 5. Large Area Reflectance Assembly. The reflected radiation is obtained from three points symmetrically located around the sample and conveyed to a single detector via fiber optics. Illuminated area on the sample can be varied from 1" to 7 3/4" diameter.

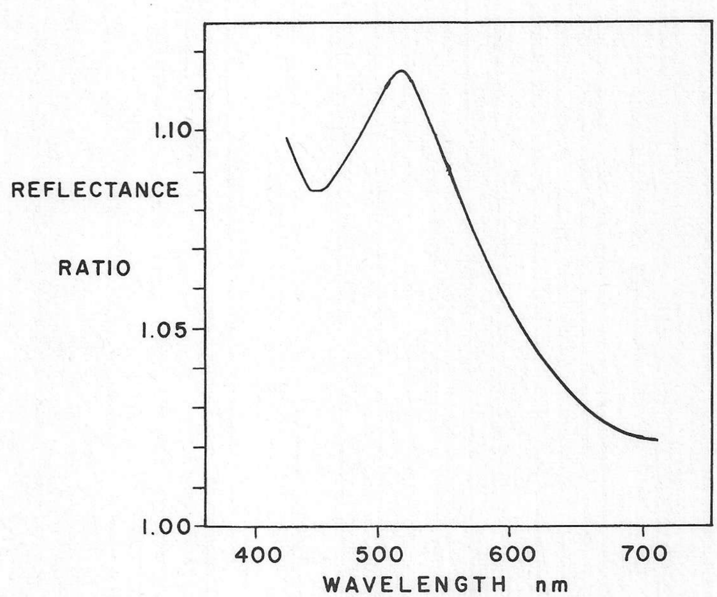

Figure 6. Spectral Reflectance Ratio R_A/R_B of Two Samples of Potato Chips (A and B). The maximum at 520 nm identifies an optimum criterion for separating the groups of potato chips into classes which agree with a visual rating of the two samples.

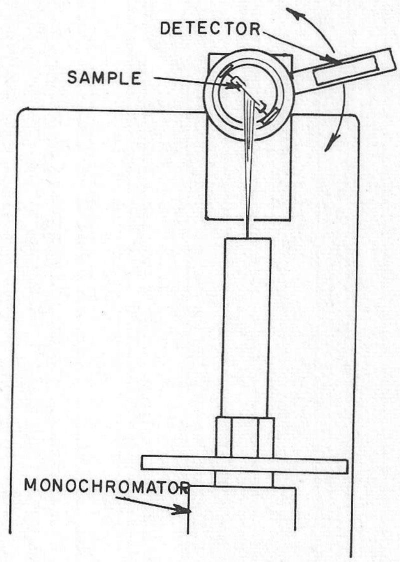

Figure 7. Bidirectional Reflection and Transmission Assembly. The light beam is stationary and the sample is rotated to change the incident angle.

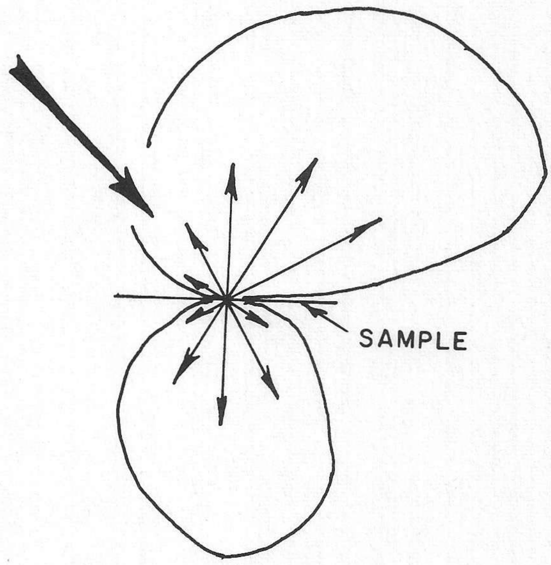

Figure 8. The Geometrical Reflectance and Transmittance of a Corn Leaf for 550 nm Radiation Incident at 45°.