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Assembly and Performance of a Double-Beam Microscope Spectrophotometer from Commercial Instruments*

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A microscope spectrophotometer assembled from commercially available instruments has been used to obtain visible and near-infrared spectra on selected specimen areas as small as 1 μ^2. A commercial spectrophotometer is coupled to a research microscope equipped with a photometer tube to which the photodetector of the spectrophotometer is attached. The miscroscope spectrophotometer can be assembled easily, quickly, and in a very compact form with a minimum of machine work. The performance characteristics are discussed, as well as some applications. The instrument was designed for spectral investigations of substances under pressure, but is equally well suited to the study of conventional microscope specimens. The incorporation of cameras and polarizing optics makes the instrument particularly suited to phase studies as well. Applications discussed are the shift of absorption bands with pressure, the determination of the pressure gradient in the diamond high pressure cell, and the determination of the spectra of microsections of stained biological specimens.

INTRODUCTION

AN instrument for the determination of the absorption spectra of selected microsections of biological specimens was first described by Caspersson[1] more than twenty-five years ago. The progress in instrumentation since then has recently been carefully reviewed by Wolken and Strother.[2] They have noted that the microscope spectrophotometers presently in use are laboratory-built instruments and are designed primarily for biological investigations.

The necessary components, viz. monochromator, light chopping device, microscope, photodetector, amplifier, and appropriate recording equipment can now be conveniently purchased. However, the assembly and matching of the components requires a considerable amount of machine work. The problems and delays inherent in such an assembly have made the construction of the microscope spectrophotometer feasible only in connection with a lengthy and comprehensive research program.

The present paper describes a recording double-beam microscope spectrophotometer which can be readily assembled from a commercial spectrophotometer and microscope which have an unusual physical and functional compatibility. The resulting instrument is very compact and is easily assembled. The instrument has the known performance characteristics of a proven spectrophotometer, and the microscope used offers a maximum of flexibility and precision including visual and photographic accessories.

Previously described instruments have been used for the investigation of biological specimens and, therefore, have conventional microscope requirements. The present instrument, however, has been designed for the investigation of substances in a diamond high pressure cell.[3] The physical dimensions of the high pressure cell impose the additional requirement of long working-distance optics. In general, a larger variety of optics for all spectral regions is available with conventional working distances, so that the instrument can be expected to perform as well, or better, in conventional microscopic investigations.

The instrument will be discussed on the basis of microscope spectrophotometry of substances in the diamond high pressure cell in the visible and near-infrared regions of the spectrum. Ultraviolet and conventional nonpressure microscopic applications will then be presented.

INSTRUMENTATION

After some investigation of commercial instruments, it was found that the Perkin-Elmer model 350 spectrophotometer[4] and the Leitz Ortholux microscope[5] were particularly suited to each other in several ways. In normal use, the light enters the Ortholux microscope horizontally and from the rear. This permits the mounting of the microscope in front of the sample compartment of the spectrophotometer so that the specimens can be conveniently mounted on the microscope. A photometer tube, designed for use with the Ortholux microscope in the Leitz single-beam spectrophotometer,[6] is available and simplifies the microsectioning operation as well as the coupling of the microscope to the photodetector described later.

The model 350 spectrophotometer has two matched photodetectors which permit the sample detector to be mounted on the photometer tube of the microscope. In

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[1] T. Caspersson, Skand. Arch. Physiol. **73**, Suppl. 8 (1936); J. Roy. Microscop. Soc. **60**, 8 (1940); Experientia **11**, 45 (1955).

[2] J. J. Wolken and G. K. Strother, Appl. Opt. **2**, 899 (1963).

[3] C. E. Weir, A. Van Valkenberg, and E. R. Lippincott in *Modern Very High Pressure Techniques*, edited by R. A. Wentorf (Butterworths Scientific Publications, Inc., Washington, D. C., 1962), p. 51.

[4] Perkin-Elmer Corporation, Norwalk, Connecticut.

[5] E. Leitz, Inc., 468 Park Avenue South, New York 16, New York, Bulletins 51-40a, 55-20.

[6] E. Leitz, Inc., 468 Park Avenue South, New York 16, New York, Catalog No. 52-D.1.

order to exactly match the two photodetectors, the model 350 is equipped with an I_0 compensator consisting of a bank of 18 trimmer potentiometers corresponding to different wavelengths throughout the spectral range. In the present application, the I_0 compensator is used to correct for the absorbance of the microscope optics. (When a particular diamond high pressure cell is used for an extended period of time, this procedure can be extended to correct for the absorbance of the diamond cell as well as the microscope optics.) An additional factor in the selection of the model 350 spectrophotometer is that it appears to be well suited to fluorescence microscope spectrophototometry. This modification is now being undertaken.

The assembled instrument is shown in Fig. 1 with some of the important visible components labeled. A schematic diagram given in Fig. 2 will aid in the discussion of the modifications and components.

A front surface mirror is placed in the sample compartment of the spectrophotometer so that the chopped monochromatic radiation, normally passing through the sample, is projected into the rear of the microscope and is reflected vertically into the condenser by the front surface mirror in the base of the microscope. A tube at the back of the microscope permits the mounting of a conventional microscope lamp so that the sample may be observed in white as well as monochromatic radiation.

The monochromator slit image is brought to focus at the sample, the size of the image being controlled by the selection of the condenser and the use of an optional monochromator beam lens (approximately 3.5×). The requirement of a long working distance is best satisfied by use of a coniscopic objective as a condenser. Objectives of this type may be obtained in magnifications between 5× and 40×, so that the monochromator slit image may be reduced in size by a factor of from 2 to 40 with a resulting (but not corresponding) increase in monochromator beam intensity. The size of the slit image at the sample is not critical as long as it is large compared with the area being scanned. Hence, the condenser system may be used primarily as a control of the monochromator beam intensity.

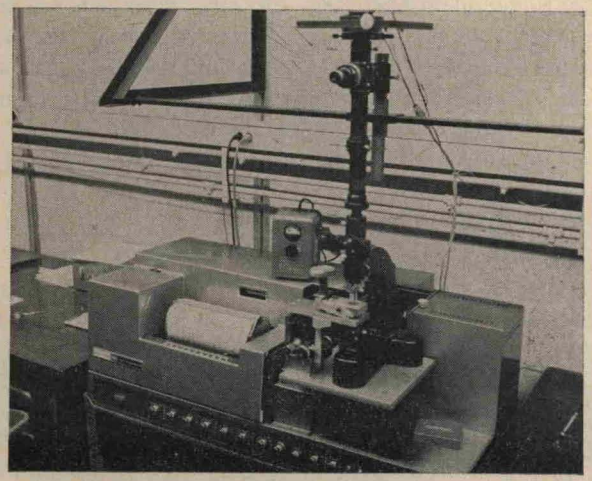

FIG. 1. Photograph of microscope spectrophotometer.

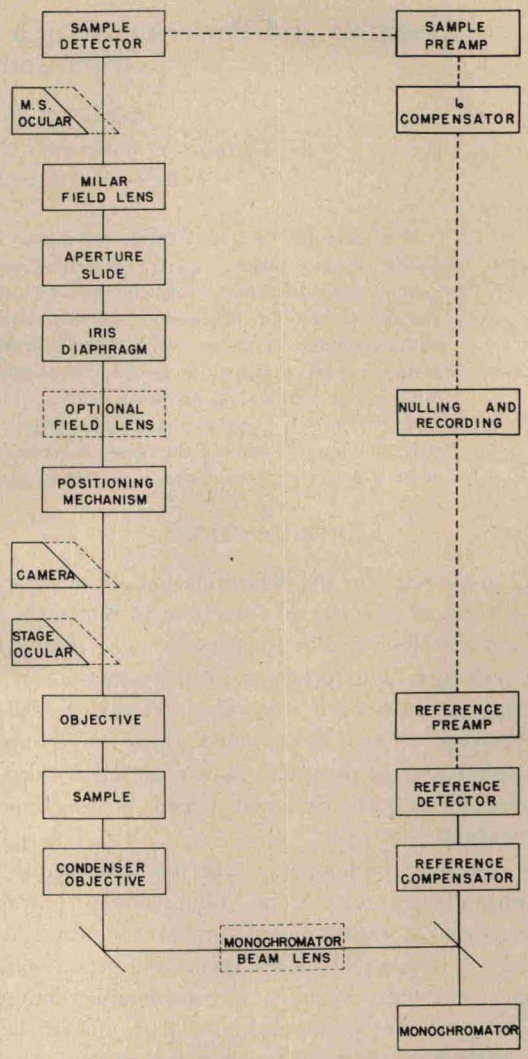

FIG. 2. Schematic diagram of microscope spectrophotometer.

The diamond high pressure cell may be mounted on a conventional microscope stage for observation at a given pressure. However, the force required to alter the pressure makes it desirable to mount the cell more firmly for investigations at different pressures. To satisfy this requirement, a more substantial stage is attached directly to the spectrophotometer.

The objectives used for observations in the high pressure cell are the same as those used in the condenser. The choice of the objective magnification is governed by the area of the specimen to be scanned and will be discussed in connection with the photometer tube.

The stage ocular, a binocular phototube with inclined 6× or 10× periplane eyepieces, is used in the preliminary