existed per genome, 1800 count/min would have been observed in the DNA density region of the gradient and none were found. Other variations included raising the initial temperature to 65°C in the annealing process, increasing viral RNA to amounts five times above saturation for ribosomal RNA, a preliminary heat denaturation of viral RNA, and the use of DNA preparations from a variety of strains of *E. coli*. Finally, DNA was isolated from host cells after infection at various intervals from 20 to 45 minutes. No hybrid formation was detected with any of these preparations. The absence of hybrid formation could not be ascribed to the fact that only one of the two possible complementary RNA strands was being tested. The DNA which was being challenged contained both strands and one of the two would have satisfied the condition of complementarity, if it existed.

It is evident that neither before nor after infection can one find sequences in the DNA which are complementary to the viral RNA. In evaluating these negative findings several features of these experiments must be recalled. First, the hybridization process was monitored internally. Second, the relative specific activities in counts per minute was such that the sensitivity per strand of detecting hybrids with viral RNA was ten times that possible with the ribosomal RNA. Finally, the procedure used was adequate to detect, with ease, complementary stretches in the DNA corresponding in length to 10 percent of the length of viral RNA.

These results have a number of interesting implications. They obviously lend no support to the rather attractive speculation that the RNA viruses might represent "escaped" genetic messages of the host. The data do suggest that RNA viruses do not employ the "DNA to RNA to protein" path of information transfer. This in turn implies that the viruses have evolved a mechanism of transcription and replication at the level of RNA. We would then predict the existence of an enzymatic mechanism involving an RNA-dependent RNA polymerase. It seems highly unlikely that an enzyme of this sort pre-exists in the cell. All recognized nucleic acid components, including "informational" RNA (*9*), ribosomal RNA (*11, 12*), and RNA which transfers amino acid (*15*) have been shown to be complementary to some sequences in homologus DNA. Further, actinomycin-*D*, which inhibits the DNA-dependent RNA polymerase (*16*), prevents synthesis of RNA in both bacterial (*17*) and animal cells (*18*) but does not inhibit production of RNA virus (*18*). One must conclude, therefore, that the incoming viral RNA contains the structural program for this new polymerase. Since this enzyme must be synthesized before replication, the viral RNA must be conserved during its translation into protein (*19*). Results of experiments confirming some of these predictions are in preparation for publication.

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Photographic Cytophotometry with a Dual Microscope

Abstract. A modified comparison microscope assembly was developed for quantitative cytochemical analysis of stained or naturally pigmented objects. Test and control objects were photographed simultaneously on the same frame of color film. Pigments in the film image were then extracted and analyzed in a recording spectrophotometer. The total procedure is an extension and simplification of existing methods.

Cellular or tissue inhomogeneity was first recognized by Caspersson as a major obstacle in microscopic photometry (*1*). In succeeding early work, the problem demanded tedious photographic densitometry, now replaced by two modern techniques involving use of photoelectric receivers. Complicated scanning-recording instrumentation permits area measurements and yields total extinctions (*2*). Less complicated apparatus is satisfactory for spot measurements (*3*) which meet certain inhomogeneity situations if the "two-wavelength method" is applied (*4*). As a simple solution to distributional error, Ornstein also suggested a novel photographic method in which absorption is measured in terms of reduced silver in a specially prepared positive image. It is the purpose of this report to describe an assembly which exploits the photographic method, uses color film, and greatly simplifies the technique.

There are two fundamental operations in the entire procedure (Fig. 1). The details are described in terms of the instrumental components as they now exist.

The light source is a Mikrark 100-watt zirconium arc (Sylvania C-100/DC) whose power supply is operated from a Sola constant-voltage transformer through a variable transformer that is usually set for 115 volts. Köhler illumination is strictly maintained and is attenuated only by neutral density filters, with or without a Corning blue filter (5800, melt 528).

The beam-splitter is a complex of one half-aluminized optical flat (Bausch and Lomb No. 45-10) and three first-surface mirrors. The mirrors are mounted on double ball-and-socket clamping joints.

The triocular microscopes (Bausch and Lomb Dynazoom) have apochromatic objectives, compensating eyepieces, achromatic centering condensers (1.40 numerical aperture), and first-surface mirrors. Visual and photographic coupling is effected by a Bausch and Lomb comparison eyepiece between the vertical microscope tubes. Specific design of the microscopes is not critical, although focusing by movement of the stage eliminates the eyepiece binding that occurs with movements of the body tube.

Photomicrographs were made on

standard daylight or type II daylight Kodachrome 35-mm film. Larger films or plates would automatically provide greater sensitivity, especially useful for small objects or objects of low density. For most cells photographed with 12.5×-ocular and 90×-objective, disks were conveniently punched at 6-mm diameter. These disks weighed 5.9 to 6.5 mg among all films, varying only 0.08 to 0.13 percent within a single frame. Pigments (10 to 50 μg per disk) were extracted by overnight digestion of emulsions in 0.1 percent trypsin at 37° to 50°C or, better, by 1-hour treatment with 70 percent ethanol at 40°C. The supernatant liquids of the centrifuged extracts were then analyzed from spectra (350 to 750 mμ) obtained in a recording spectrophotometer (Bausch and Lomb 505). Standard 1.0-cm light path cuvettes were used for volumes of 3 to 5 ml; sensitivity was sometimes increased by extracting disks in only 1.0 ml and by using modified cuvettes. Full spectra were necessary to monitor curve shapes (envelopes of three emulsion pigments) and to obtain absorption integrals by planimetry or by dissection and weighing of curve tracings.

Preliminary applications of the dual-microscope technique to biological materials have revealed no serious defects. Duplicate measurements of total absorption for a given object varied less than 1 percent when made on the same film strip and less than 3 percent when made on different film strips. The total absorption values obtained for an object when it was interchanged with its control for a pair of photomicrographs agreed within 1 percent. To study representative objects in free surroundings, preparations made from human blood and bone marrow cells, peritoneal mast cells and lymphocytes from a rat, and ascites tumor cells from a mouse were treated with Feulgen reagents to reveal DNA, or with Astrablau at *p*H 0.2 (*5*) to show acid mucopolysaccharides. To illustrate the method for sectioned tissue, preparations of fetal cartilage from a dog were stained with Astrablau, rat kidney was treated to show alkaline phosphatase, and human liver obtained from biopsy was stained for glycogen by the periodic acid-Schiff method with and without digestion by amylase.

Figure 2 illustrates the distinction between "control" and "standard" objects. Control objects were arbitrary (usually one cell stained in the same manner as similar cells to be measured), since their main function was the evaluation of variation from frame to frame in the photomicrographs. Control objects might eventually be model droplets, such as proteins or other constituents of cells; they could then be weighed by interference microscopy, and stained or reacted and compared to similar constituents still contained within the cell (*6*). Standards, such as mixed preparations of crushed optical filters, were used partially to calibrate the microscopes and also to check instability in the control object such as fading. It will be evident that each half-frame photographed in the dual microscope is analogous, with its "blank" area, to the usual photoelectric spot measurement requiring two successive readings.

The working time for measuring absorption of 18 to 20 units was about 2 hours, superimposed on 24-hour film

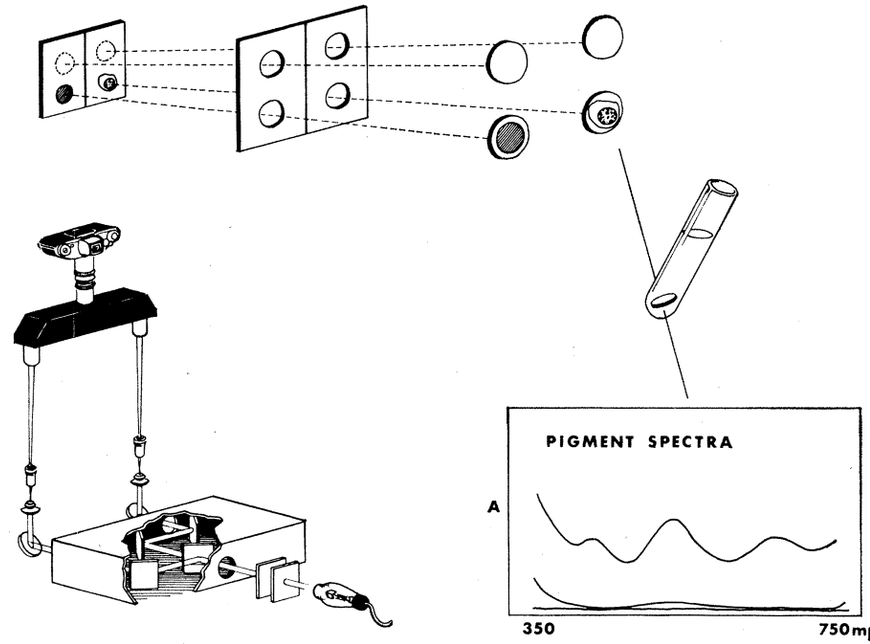

Fig. 1. Summary of the dual microscope procedure. Microscopes with beam-splitter and comparison eyepiece are at the lower left. Film cutouts for the object of interest, the comparison object, and two corresponding blank areas are shown at the top of the figure. From solution extracts made of each disk, pigment and background spectra (lower right) yield a corrected value for "amount of chromophore" in the object of interest.

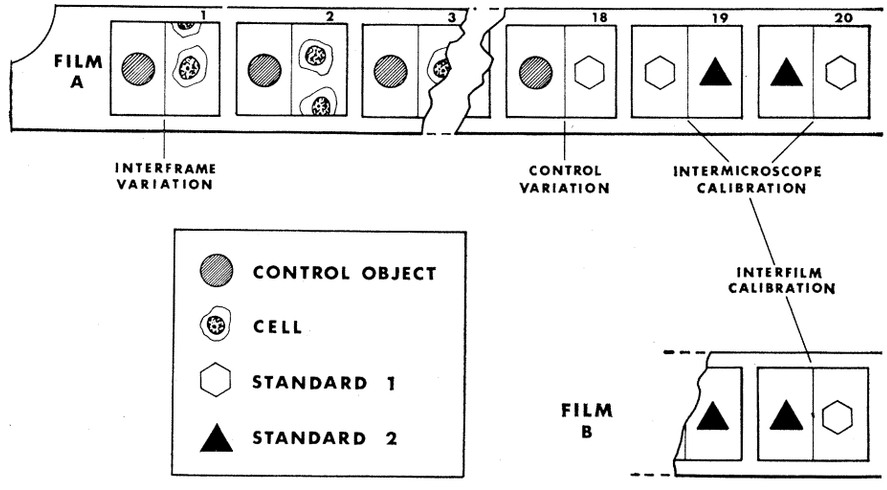

Fig. 2. Management of controls and standards. Most of film A is used to record a cell series against a single control object. The control object, if unstable (for example, fading), is then photographed in frame 18 against standard 1 (colored glass fragment). The comparison of standard 1 with a similar standard 2, including exchange between the microscopes (frames 19 and 20), serves the dual purpose of partially calibrating the microscopes and linking one film strip with another. (Blank areas ordinarily cut from each half-frame are not shown in this figure.)

processing. The procedure is greatly accelerated by photographing more than one unit per frame, eliminating disk-weighing, and substituting readings of one wavelength for spectral curve planimetry, all possible under certain conditions.

The inherent superiority of photographic over photoelectric recording of absorbance in heterogeneous microscopic objects was eloquently outlined by Ornstein (*4*) and later extended to include color transparencies (*3*). Mendelsohn (*7*) also described a method for converting black-and-white films to monochromes, with special application to the "two-wavelength method." Only Niemi (*8*) has applied the photographic method (standard microscope, silver analysis) to a quantitative study of erythrocytes in types of human anemia. The use of two microscopes and one light source in the photographic method eliminates concern for light source fluctuations, that otherwise demand rigorous control. The simplifying substitution of white light and color film for monochromatic light and specially prepared monochrome transparencies shifts a measure of responsibility to the film manufacturer and processor, although monitoring of variations in film emulsions and processing is a function intrinsic to the dual microscope procedure.

The successful performance of these two light microscopes suggests similar arrangements for appropriate quantitative applications of ultraviolet, interference polarizing, and fluorescence microscopes. The photographic basis would be essentially the same: formation of a "chemical model" to a scale suitable for an object otherwise too small for dissection and macroanalysis (*9; 10*).

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Correspondence between Stevens' Terminal Brightness Function and the Discriminability Law

Abstract. Stevens' terminal brightness function, an adaptation curve derived from power law data, closely agrees with Troland's just-noticeable-difference (jnd) summation for brightness. The power law itself describes sensation magnitude *before* adaptation to a test stimulus, whereas the Fechnerian discriminability law describes sensation magnitude *after* adaptation. This suggests their synthesis in a more general psychophysical equation.

Stevens, Garner, Helson, and others (*1*) have done considerable work since the 1930's in developing scales to describe the relation between the physical intensity of a stimulus and the resulting subjective sensation. In the main, Stevens' work has been the most influential and his proposed power function law of sensation has found considerable acceptance, even though such acceptance has seemed to necessitate the rejection of Fechner's law, which has been in use for the last 100 years.

The sensory scaling system developed by Fechner was based on Weber's law, $\Delta I/I = C$, in which the minimum detectable change in a physical stimulus, ΔI, is related to the associated intensity of the stimulus, I, by a constant, C. The idea of Fechner was that the C in Weber's law corresponded to a basic sensation unit, $\Delta\Psi$, the "just-noticeable" sensation change which was associated with an incremental change in physical stimulus. He hypothesized further that the just-noticeable difference (jnd) had the same sensation magnitude at all intensity levels of the physical stimulus. Fechner's hypothesis was, then, that, $\Delta I/I = K_1\Delta\Psi$. If the equation is integrated after suitable mathematical assumptions, a form of Fechner's law is found that gives the magnitude of sensation as a function of physical stimulus intensity, $\Psi = K_{1\log}I + C$. Fechner's law is a theoretical discriminability law, since it is based on a mathematical integration of jnd's, units of discrimination. An empirically based discriminability law can be obtained, however, by the summation of empirically determined jnd's. This was done, for instance, by Troland (*2*). In both scales the basic assumption is that the sum of jnd's (sensation units) equals a sensation magnitude.

Stevens, however, has found by direct estimation of the magnitude of sensation associated with a physical stimulus, that the psychophysical law is not a log function but is a power function, $\Psi = KI^n$ (sensation magnitude is proportional to a power of the physical stimulus intensity). He has, therefore, suggested that Fechner's law be "repealed" (*1*).

It is important to note, however, that a significant operational difference exists between the methods of Stevens and those of classical psychophysics, a difference which accounts for the discrepancy between the two formulations. In power law experiments, Stevens' experimental subject is first adapted to a given stimulus level and then is asked to estimate the sensation magnitude of a test-stimulus to which he is *not* adapted. But in classical psychophysics, the jnd's on which the discriminability law for brightness is based were determined only at stimulus levels to which the experimental subject was essentially adapted. Troland's discriminability scale, it should be noted, is based on Hecht's averages (*3*) of the work of Aubert (1865), Konig and Brodhun (1889), and Blanchard (1918), all of whom determined jnd's at levels to which their subjects were essentially adapted. It can be inferred, then, that Troland's discriminability scale can be valid only *after* adaptation to a stimulus level, whereas power law scales can be valid only *before* adaptation.

With this methodological difference in the construction of brightness scales in mind, it is of interest to consider the following: In a recent paper, Stevens (*4*) describes a brightness-luminance (psychophysical) relationship derived from power law data which he calls the "terminal brightness function" and of which he says, it "does *not* follow a power law." Of significance is the fact that the terminal brightness function represents the brightness (sensation magnitude) which would be experienced by an observer *after* adaptation to the test stimulus. In other words, the function seems to represent