AD NUMBER

AD837022

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; JUN 1963. Other requests shall be referred to Department of the Army, Fort Detrick, MD.

AUTHORITY

SMUFD D/A ltr, 8 Feb 1972

TRANSLATION NO. 241

DATE: June 1963

INSTRUMENTATION FOR QUANTITATIVE CYTOCHEMISTRY

[Following is the translation of a German-language article by T. Caspersson, G. Lomakka and L. Carlson, Institute for Cytology and Genetics, Karolinska Institutet, Stockholm, published in Acta Histochemica, No. 9, 1960, pages 139-156.]

(Note: The work herein was carried out with the support of the Swedish cancer society, of the Wallenberg Foundation and under Grants C-2740 C-3082 of the United States Public Health Service.)

The combination of microspectrographic methods in the visible and in the ultraviolet range of the spectrum through microinterferometric and microradiographic methods suitable primarily for volumetric analysis but under certain circumstances also for ultimate analysis, affords a satisfactory basis for general quantitative cytochemical work within the dimensional range of cell structures. Actual techniques are often rather complicated in some of these methods, especially when several methods are being applied to one and the same biological object. We therefore developed in the Stockholm Institute in recent years a group of inter-coordinated methods with the intention of being able to utilize their investigative properties for practical biological routine tests. The following is intended, with the aid of a series of pictures and diagrams, to give a review on such instrumentation (cf. references 1-10 for details).

Figure 1 shows the most important areas of application for techniques of this type. The most highly developed area is ultramicro-investigation by direct methods. However, indirect methods will gain increasingly in importance in the future. Among such indirect methods, the Feulgen reaction for determination of DNS has been employed especially frequently at the present time. This histochemical reaction is unique in regard to reproducibility and stability due to the peculiar colloid-chemical properties of DNS. The difficulty of

using histo-chemical methods, based on staining and/or chemical reactions, more generally in the quantitative sense lies in producing a suitable calibration or reference system definitely necessary for control of staining and/or the reaction sequence in the specific reaction environments offered by the biological material. Without further discussing this field, I would like to stress that the microspectrographic methods here indicated and complemented with the "methods for measuring on r[illegible]t systems" given further below offer a possibility for developing such calibration systems for wide groups of histochemical processes. So far this possibility has been exploited only to a very limited extent.

Figure 2 shows the most important operating methods with the optical ultra-microspectrograph in the visible and in the ultra-violet range. Figures 2, 1-a and 1-b show the determination of the absorption sequence within individual object sections or "point measurements." The individual "measuring point" as a rule must amount to 0.5 μ by reason of the optical non-homogeneity in general biological material. Especially in the problem of nucleotide and protein metabolism, microspectrographic total volumetric analysis in individual cells or cell sections predominate in practical biological work. Due to the high optical non-homogenity in nearly all of the cell material encountered, such total determinations must be carried out in the following manner. It is initially necessary to measure a very large number of points of the indicated magnitude; subsequently the transmission values must be transformed into extinction values and the extinction values must be summated over the surface. This means alone for a cell of general magnitude in mammalian material as a rule several 100-1,000 measurement points of which the data must be processed and summated. It is generally further necessary to repeat the measurements in several wave lengths. By reason of the instability of the biological material and other reasons, it was definitely necessary to automate the measuring methods. This was done by electronic transformation of function, in the simplest case from transmission to extinction, as well as through electronic integration of the extinction values over the surface. Figure 2, 2-b demonstrates the meterologically simplest case in which the measurement object has a free background and the entire examination process can be automated. Usually, however, the situation is the one shown in 2-a where the measurement object is surrounded by material which is not intended to enter into calculation. For this case, the spectrographs are so arranged that the scanning areas are continuously registered and each individual point on the diagram can be correlated accurately with a corresponding measuring point. On the other hand, we can then limit the measurement to the desired measurement objective from the registered data, eventually with the aid of a special function transformer and integrator, by manual operation of a curve-trace

indicator (Figure 12). Meteorologically, this case is obviously the best. If we select a cell population in which the cells lie free of each other, the saving of time in a specific biological problem is often considerable. Where selective staining of nuclei is concerned as, for instance, in the Feulgen reaction, there exists also for connected tissues a measurement situation corresponding to Figure 2, 2-b.

Figure 3 shows the large universal microspectograph of the Institute which is specially designed within the ultraviolet and the visible spectrum range in order to carry out measurements in the was indicated. It can be utilized with different types of microscope optics. Especially in the ultraviolet range, refraction optics are preferable due to certain optic relations of reflection optics. (The Carl Zeiss Company in Oberkochen has designed, in collaboration with us, a commerical model of this instrument and has also designed special achromatic ultraviolet optics corresponding to the special requirements of microspectrographic work). Both of the recording instruments of the spectrograph register the functions indicated in Figure 2.

Figure 4 shows the course of a measurement in principle. After appropriate preparation, the measurement is carried out in the ultra-microspectograph (UMSP). Eventually control of optic properties, primarily the dispersion of light in the preparation, by special equipment precedes the measurement.

For the analysis of the measurement data obtained, knowledge of the optic constants for the substances is required under the conditions obtaining in the object during the measurements. Especially in stabilized preparations but also in objects which may be assimilated to a highly concentrated protein solution, the optical constants often deviate essentially from the values in diluted solution. For the determination of these constants, equipment (cf. below) has been developed which is identified in the picture as "model substance line."

If the non-homogeneity degree of the cell material is very low, the mass in cells and cell sections can be determined directly with the aid of the interference microscope although the accuracy of this method is generally very low. If significant differences exist in the refraction indices which is generally the case for biological material, it is absolutely necessary to employ scanning methods with very small measurement points here also as in absorption measurements.

By utilizing Baker-Barer interference-microscope optics, Svensson (10) developed a scanning micro-interferometer. In principle, it is also possible to install interference optics suitable for absorption

measurement in our ultra-microspectrograph and carry out scanning micro-interferometric measurements. Even large objects such as protozoae with large differences in the density of different cell parts can be measured with great reproducibility and accuracy.

Figure 5 demonstrates the manner of operation of a new rapid-scanning micro-interferometer which is an improvement of an instrument described earlier. Through the introduction of an electronic integration system, it is possible to handle even relatively large cell populations with this instrument. Figure 6 shows the instrument with the two recorders for increment and the integral of the increment.

Micro-radiographic mass determination furnishes in principle the same indications as micro-interferometrics. We use a high-resolution roentgenospectrograph built by Lindstroem and shown in Figure 7.

Figure 8 describes the principal method of operation, the exponentiation of a micro-radiogram with subsequent scanning measurements in the microspectrograph. The great advances of micro-interferometrics lie in the circumstance that the object can be investigated in a liquid embedding medium and also under high optical resolution. In contrast, microradiography requires working in a vacuum but has the very great advantage, as compared to micro-interferometrics, that a "model substance line" is not required because roentgen absorption is independent from the physical state of the material. For practical biological work, microradiography can be utilized primarily for calibration of the interference methods. Recently, however, Carlson has developed a procedure suitable also for routine examination which allows us to work in low absorbing biological objects with a higher resolubility than 1/u within an area of the density curve of the plate so that it is possible to carry out surface measurements without scanning. This results in such an increase of working speed that the x-ray method is as quick, at least for the examination of large cell populations, as the micro-interferometrics described above. Figures 9 and 10 represent the principles of photometric plate measurements and/or the appearance of the measuring equipment.

The measurement of element-specific roentgen absorption can also be carried out in the roentgen microspectrograph developed by Engstroem-Lindstroem. The photo plates are analyzed in this case in analagous manner for the determination of certain elements.

In all of these measurements, electronic evaluation of the data plays a central role because large biological material can be processed only after a high measuring speed has been achieved. Figure 11 shows the basic diagram of the four most important

measurement methods and also the general arrangement of the instrumentation. The upper branch of the diagram corresponds to instruments described above. The lower branch represents a special function transformer (Figure 12) with the aid of which the measurement curves of the spectrograph or interferometer are evaluated through manual tracing of the curve which is an advantage for certain types of objects. This method of operation is of great advantage for more complicated objects, especially such as listed in Figure 2, 2-a.

If the diagrams mentioned are grouped in a general review of the coordination of the different equipment, this produces an instrumentation diagram as shown in Figure 13 (each square in the diagram represents a specific instrument).

The model substance measurements (Figure 14 and also Figure 13, bottom) attempt to obtain exact values for the optic constants of the substances concerned which are necessary for the analysis of the microspectrographic and micro-interferometric measurements. Figure 14 represents the three most important cases. The top case offers no technical difficulties but occurs practically never in standard biological work. The data can here be acquired with conventional measuring instruments in the macro-range. For the simple case where the object corresponds to a concentrated solution, the refraction index in the visible range can be easily determined with conventional equipment. However, for the determination of the extinction coefficient, special equipment will be necessary as well as for routine refraction-index determination in the ultraviolet wave range. Svensson has constructed a "lens cuvette" (Figures 15 and 16) for this purpose.

At the bottom of Figure 14, the most general situation for the measurement of biological material is represented, i.e. where the object is free of water.

Figure 17 shows schematically how the determination of the optic constants can be carried out in this case with the aid of the "drop method". In the illustration, the object is intended to represent a very small drop of highly concentrated protein solution. The drop is either dried or stabilized on a suitable base. The material is then generally distributed very irregularly over the measuring surface. Total volumetric analysis is made by scanning microradiography and, when the material volume is known, the necessary determination of constants can be carried out in the object with scanning interferometrics or micro-spectrophotometrics.

Rapid measuring methods for specific problems: The instrumentation groups described above are intended for more general work on biological material of different nature. If the problematics are

restricted to special substances or special types of biological material, significant advantages can be obtained when time-consuming labor is shortened by the construction of specialized measuring apparatus.

As an example, I should like here to refer to two designs recently developed for an extremely actual field within experimental cancer research, i.e. the cytochemical differences in the nucleotide and protein components of the different cells in large populations of tumor cells. These working possibilities of special interest for the investigation of the manner of action of various anti-cancer substances as well as for the study of early stages in cancerogenesis. For tumor problems of this kind, we can frequently select as investigative material cells lying free of each other (cf. Figure 2, 2-b) which make possible extensive mechanization of measurements as described above. A particularly rapid-ultramicrospectrograph for the ultraviolet and the visible range has been constructed for material of this type. Its manner of operation can be seen in Figure 18. By foregoing registration, the measuring time for a customary mammalian cell could be reduced to a few seconds through two-dimensional scanning of the objects with direct reading of the terminal values. A further increase of measuring speed is pointless because the adjustment of the new measuring objects generally requires at least 10 times the measuring time. The instrument, shown in Figure 19, is primarily suitable for the investigation of large cell populations in ultraviolet and in visible light. It is also very appropriate for the determination of DNS by means of the Feulgen reaction and, in this case, tissue sections can be employed also because absorption in the cytoplasma can be neglected in the measurement of the Feulgen-absorption maximum.

The instrument for microinterferometrics shown in Figures 5 and 6 was developed especially for work with large cell series. However, it is appreciably slower than the instrument for absorption measurement described above.

For the greater part of cytochemical investigation of cell populations, the determination of optical cross section of both nucleoli and nuclei as well as of cytoplasma plays an essential role. An instrument for this purpose can also be mentioned. The author earlier described a microplanimeter which works on the principle indicated on the left side of Figure 20. An adjustable weak lens is moved by the observer above a microscope objective in a plane at a right angle to the axis of the microscope. Subjectively, this gives the impression that cross-hairs provided in the eyepiece move around the measurement object. The motion of the lens is transmitted to a planimeter system. For series measurement, this arrangement was provided with an electrical registration system working in 3

channels. Practically, the work procedes in such manner that initially a nucleolus is measured. The observer presses a button and a three-point recorder prints a point on the paper of the recording instruments in column 1. Subsequent to this, the cell nucleus is measured and activation of button 2 gives a recording in column 2. Channel 3 is intended primarily for cytoplasma measurements. Simple summation of the points in the columns directly produces the desired histogram. The equipment is particularly suitable for measurement of very small objects because the accuracy of measurement is limited only by the resolubility of the optics employed.

Summary: With the aid of diagrams and illustrations, a number of instruments are shown which produce the possibility, by their coordination, for simultaneously exploiting the quantitative cytochemical working possibilities (for general biological problematics) afforded by ultramicrospectography in the visible and ultraviolet range as well as in the radiographic field and in microinterferometrics. In addition, special instruments for work with large cell populations are described.

Literature References

Caspersson, T: Cell Growth and Cell Function. W. W. Norton New York (1950).

Caspersson, T: Quantitative Cytochemical Methods for the Study of Cell Metabolism. Experientia, XI: 2, 45-60 (1955).

Caspersson, T: Quantitative Ultramicrospectographic Method. Vienna Conference. Mikrochimica Acta. Heft 1-6 (1956).

Svensson, G: Determination of Absorption and Refraction Index of Extreme High-absorbent Solutions in Microscale. Vienna Conference. Mikrochimica Acta, Heft 1-6 (1956).

Caspersson, T: Quantitative Cytochemical Determinations on Endonuclear Structures. Coll Spring Harbor Sympos. Quant. Biol. (1956).

Carlson, L: Evaluation of Microradiograms for Dry Weight Determination. Exptl Cell Research, Suppl. 4, 193-196 (1957).

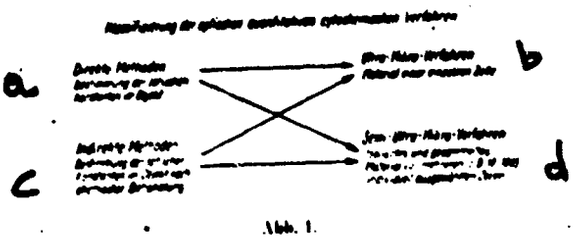

Abb. 1

Figure 1 - Classification of Optical Quantitative Cytochemical Methods. a - direct methods: determination of optical constants in objects; b - ultramicro method: material in an individual cell; c - indirect methods: determination of the optical constants in the object after chemical treatment; d - semi-ultramicro methods: isolated and collected material of several (e.g. 10-100) individually selected cells.

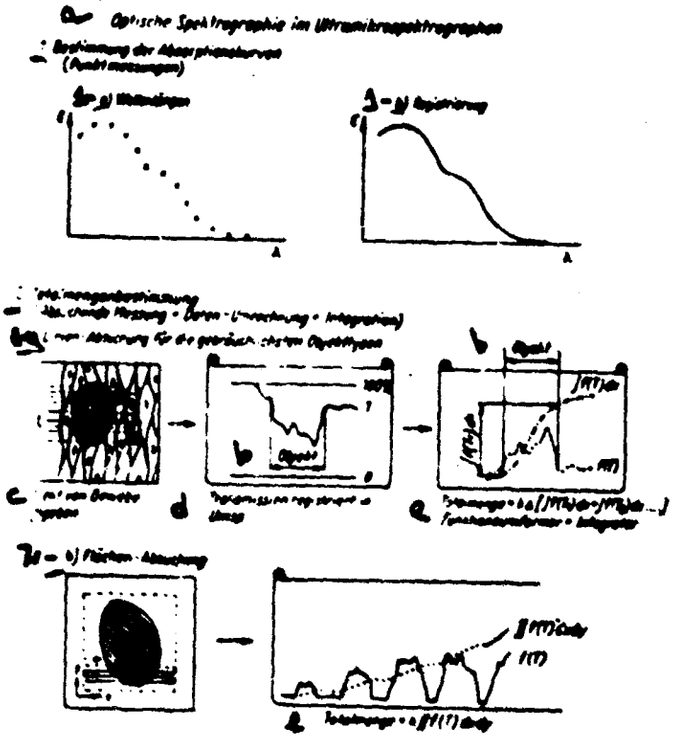

Figure 2 - The Most Important Working Methods in Optical Miespo-spectography.
a - optical spectography in ultramicrospectrograph;
1 - determination of absorption curves (point measurements);
1-a - wave lengths; b-b - recording; 2 - total volumetric determination; scanning measurement/data - recalculation / integration); 2-a - line scanning for the most usual types of objects; b - object; c - directly surrounded by tissue; d - transmission recorded in ultra-microspectrograph;
e - total volume = $k\Delta[\int f(T_1)d\,x \,/\, \int f(T_2)d\,x \ldots]$, function transformer / integrator; 2-b - surface scanning.

GRAPHIC NOT REPRODUCIBLE

Abb. 3. Der Universal-Ultramikrospektrograph.

Figure 3 - Universal Ultra-microspectrograph.

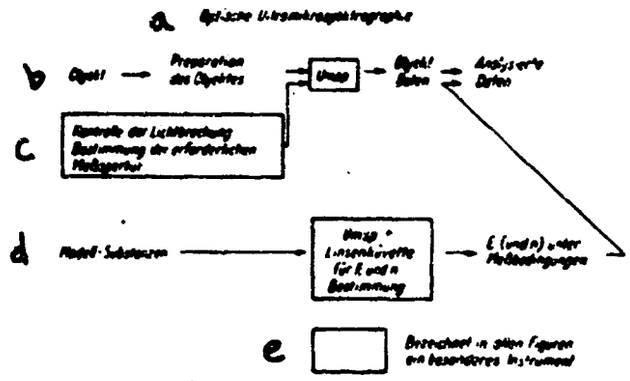

Abb. 4.

Figure 4

a - Optical Ultramicrospectrography; b - object - preparation of object - ultra-microspectrograph - object data - analyzed; c - control of light refraction. Determination of required measuring aperture. d - model substances - ultra-microspectrograph / lens cuvette for determination of $\mathcal{E}$ and n - $\mathcal{E}$ (and n) under measurement conditions; e - signifies a special instrument in all illustrations.

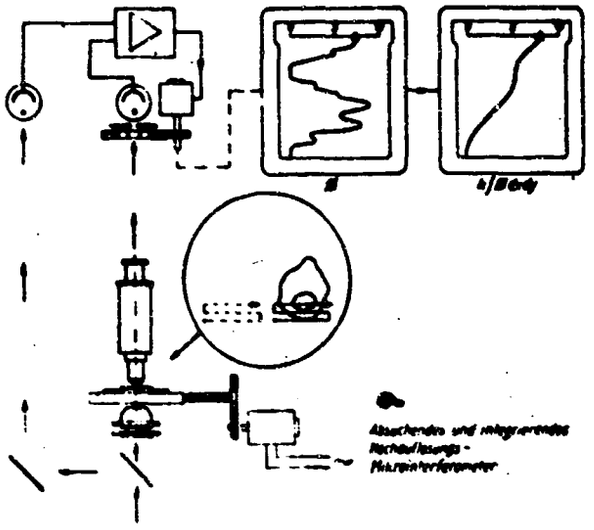

Abb. 5. Diagramm eines Mikrointerferometers.

Figure 5 - Diagram of Microinterferometer.
a - scanning and integrating high-resolution microinterferometer.

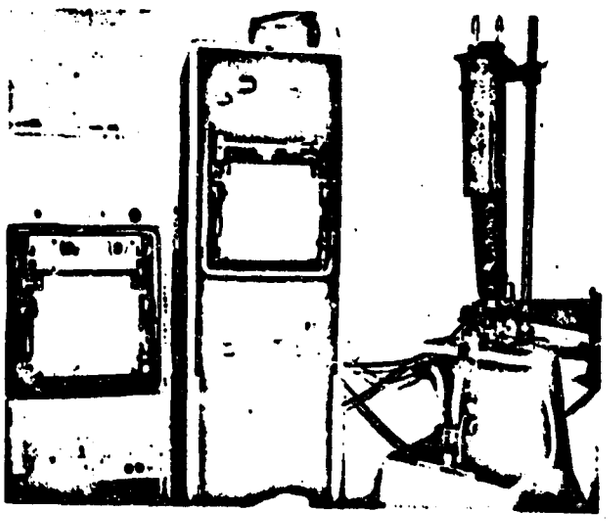

Abb. 6. Das Mikrointerferometer.

Figure 6 - Microinterferometer.

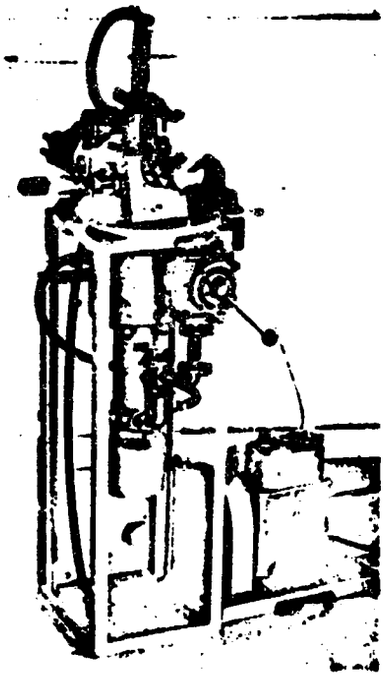

Abb. 7. Hochauflösungs-Röntgenspektrograph für biologische Objekte nach Lindström

Figure 7 - High-Resolution X-ray Spectrograph for Biological Objects According to Lindstroem.

GRAPHIC NOT REPRODUCIBLE

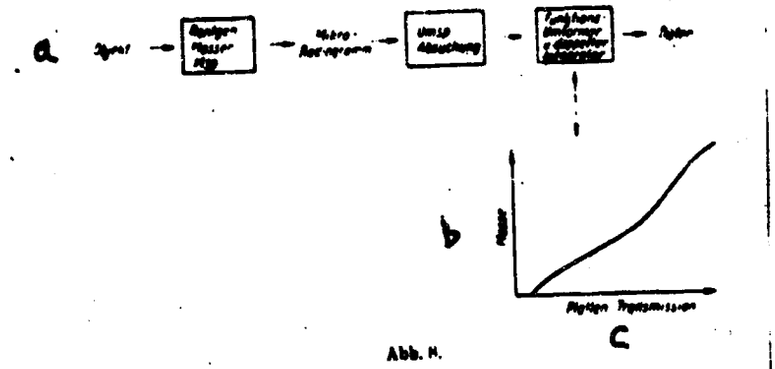

Abb. 8.

Figure 8
a - object - x-ray mass microspectrograph - microradiogram - ultramicrospectrographic scanning - function transformer and double integrator - data; b - mass; c - plate transmission.

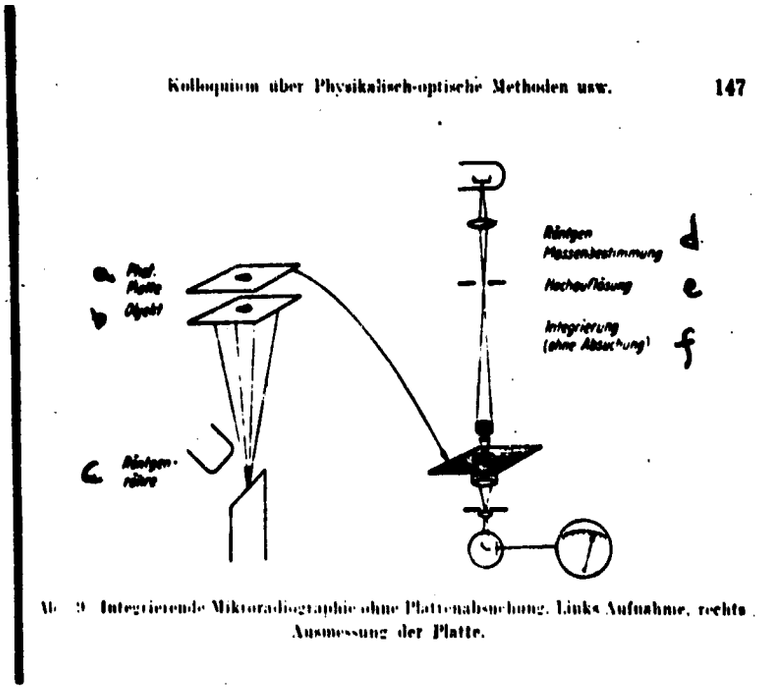

Abb. 9. Integrierende Mikroradiographie ohne Plattenabtastung. Links Aufnahme, rechts Ausmessung der Platte.

Figure 9 - Integrating Microradiography Without Plate Scanning. Left - photograph; right - measuring of plate. a - photographic plate; b - object; c - x-ray tube; d - x-ray mass determination; e - high resolution; f - integration (without scanning).

GRAPHIC NOT REPRODUCIBLE

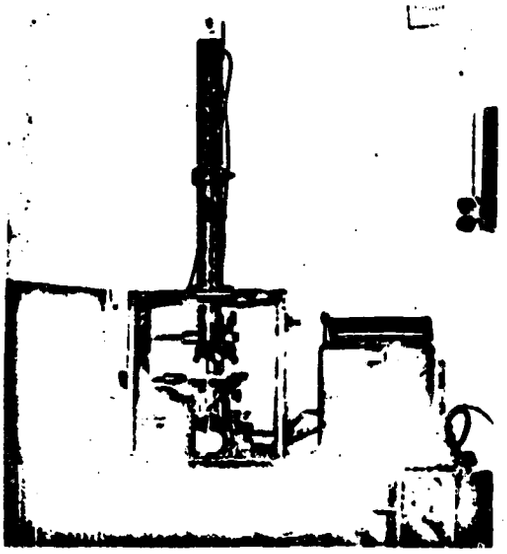

Abb. 10. Plattenausmeßgerät für Mikroradiogramme.

10*

Figure 10 - Plate Measuring Instrument for Microradiograms.

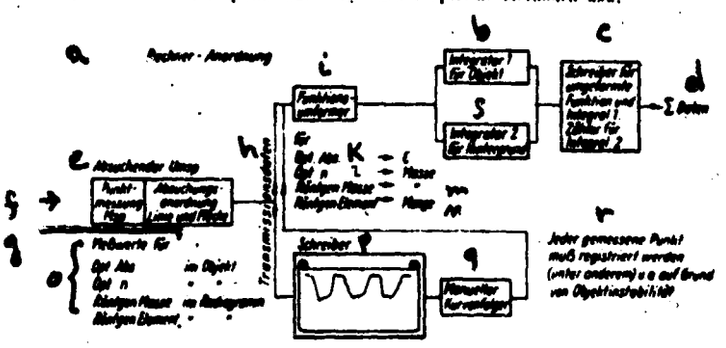

Abb. 11. Allgemeine Übersicht über die Verwendung von elektronischen Umrechenanordnungen und Integratoren in den Arbeitslinien. Die Abbildung zeigt, wie diese Geräte für verschiedene Arbeitsarten verwendet werden können.

Figure 11 - General Diagram of the Utilization of Electronic Computer Arrangements and Integrators in Work Flow. The illustration shows how the instruments can be utilized for different methods of procedure.
a - computer arrangement; b - integrator 1 for object; c - recorder for transformed function and integral 1. Counter for integral tube; d - data; e - scanning ultramicrospectrograph; f - point measurement microspectrograph; g - scanning arrangement, line and surface; h - transmitted data; i - function transformer; k - for optical scanning - £ ; l - optical n - mass; m - x-ray mass - mass; n - x-ray element - quantity; o - measurement values for optical scanning in object, optical n in object, x-ray mass in radiogram, x-ray element in radiogram; p - recorder; q - manual curve tracer of object instability; s - integrator 2 for background.

GRAPHIC NOT REPRODUCIBLE

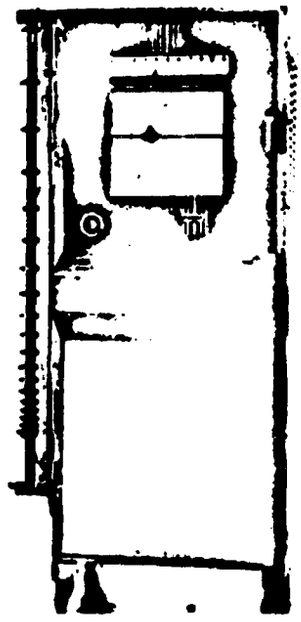

Abb. 12. Funktionsgenerator mit Integrator und mit Anordnung zur manuellen Verfolgung der zu analysierenden Kurve.

Figure 12 - Function Generator with Integrator and with Arrangement for Manually Tracing the Curve to be Analyzed.

150 Kolloquium über Physikalisch-optische Methoden usw.

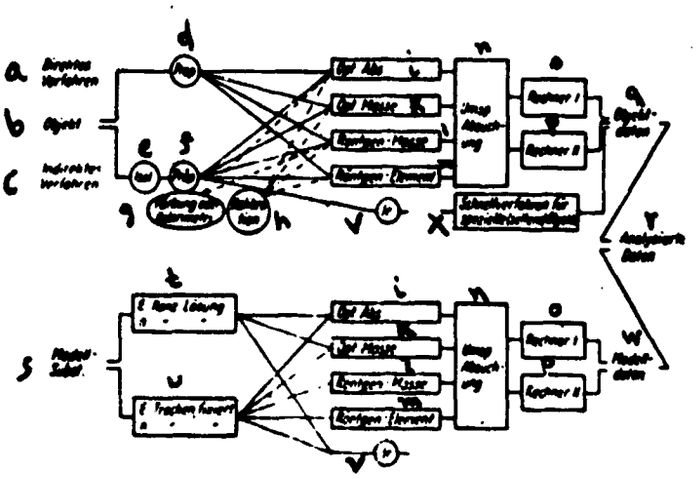

Abb. 18. Allgemeine schematisierte Übersicht der verschiedenen Arbeitslinien [illegible] Instrumente. Opt. abs., Mikrospektrographie, Opt. Masse, Mikrointerferometrie, Rön[illegible] Masse, mikroradiographische Massen-Bestimmung, Röntgen-Element, Elementbestimmung. Rechtecke bedeuten besondere Instrumente, Ringe deuten Arbeitsverfahren an.

Die Abbildung demonstriert, wie sowohl für die Messungen am Objekt, als auch f[illegible] Modellsubstanzmessungen weitgehend dieselben Anordnungen für Absuchung u[illegible] elektronische Datenbearbeitung benutzt werden können.

Figure 18 - General Diagram of Different Types of Work Flow and Instruments, Optical Scanning, Microspectrography, Optical Mass, Microinterferometrics, x-ray mass, microradiographic mass determination, X-ray Element, Element Determination. Squares signify special instruments, circles signify working methods. The illustration demonstrates how the same arrangements can be used extensively for scanning and electronic data-processing both for measurements on the object as well as for measurements of model substances.

a - direct process; b - object; c - indirect process; d - preparation; e - isolation; f - preparation; g - staining or color-metering; h - calibration; i - optical scanning; k - optical mass; l - x-ray mass; m - x-ray elements; n - ultra-microspectrographic scanning; O - computer I; p - computer II; q - object data; r - analysed data; s - model substances; t - & concentrated solution, n concentrated solution; u - dry-fixation, n dry fixation; v - radiation; w - model data; x - rapid method for inhabitual objects.

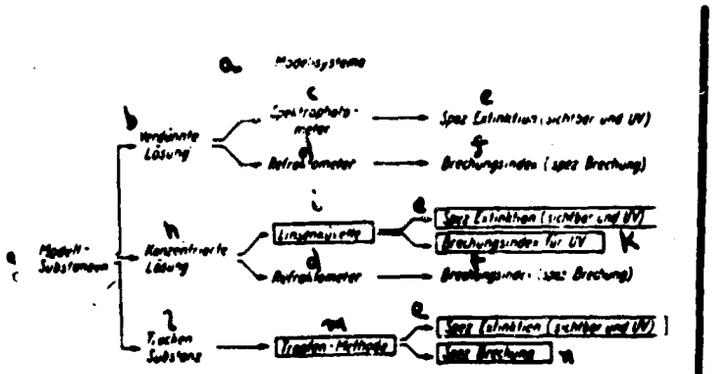

Abb. 14. Die verschiedenen Typen von Modellsubstanzmessungen, die de[n] v[er]schiedenen Typen von Verhältnissen in den biologischen Meßpräparaten entsp[re]chen. Rechtecke bedeuten, daß besondere Geräte für diesen Abschnitt der Arbeit ent[wi]ckelt worden sind.

Figure 14 - Different Types of Model Substance Measurement which Correspond to the Different Types of Conditions in Biological Preparations in which Squares indicate that Special Instruments have been Developed for the Particular Section of the Method.

a - model substance; b - diluted solution; c - spectrophotometer; d - refractrometer; e - specific extinction (visible and ultra-violet); f - refraction index (specific refraction); g - model substances; h - concentrated solution; i - lens cuvette; k - refraction index for ultra-violet; l - dry substance; m - drop method; n - specific refraction.

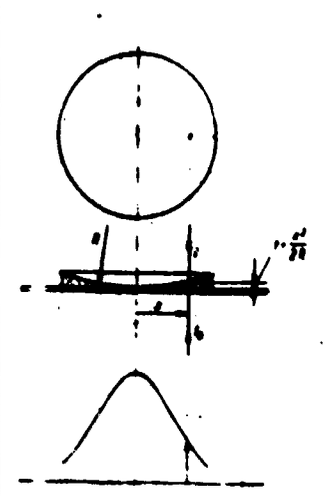

Abb. 15. Die „Linsenküvette", ein [illegible] für Messung mit Hilfe des registrierenden Ultramikrospektrographen der Absorptionskonstanten im optischen Spektrum von sehr hochkonzentrierten Lösungen in sehr geringen Schichtdicken.

TEXT NOT REPRODUCIBLE

Figure 15 - The "Lens Cuvette" is an Instrument for Measurement (by means of the recording ultramicrospectrograph) of the Absorption Constants in the Optical Spectrum of very High-Concentrated Solutions in Very Low Layer-Thickness.

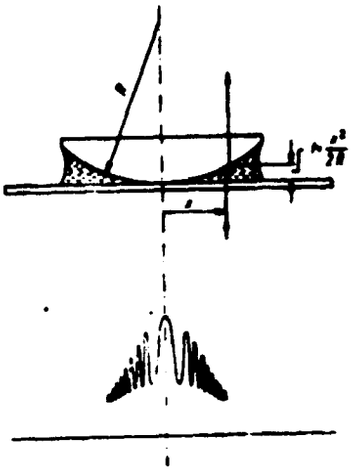

Abb. 16. Eine Linsenküvette mit halbverspiegelten Zwischenflächen für Brechungsindexmessungen in hochabsorbierenden Lösungen im sichtbaren und im Ultravioletten. Die Messung erfolgt durch Registrierung der Newtonschen Ringe bei parallelem Lichteinfall mit Hilfe des Mikrospektrographen.

Figure 16 - A Lens Cuvette with Semi-Mirrored Interfaces for Refraction Index Measurement in High-Absorbent Solutions in the Visible and in the Ultraviolet Range. Measurement is carried out by recording the Newton rings under parallel light incidence by means of the microspectrograph.

GRAPHIC NOT REPRODUCIBLE

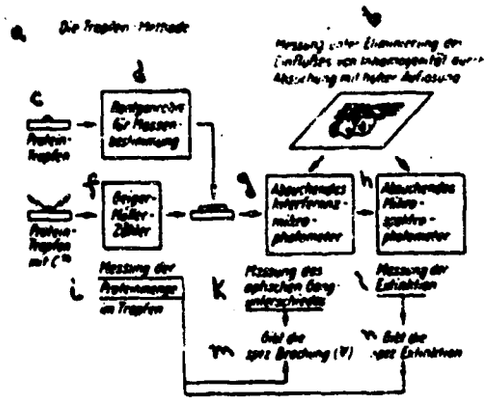

Abb. 17. Die „Tropfenmethode" (vgl. Abb. 14) zur Bestimmung der optischen Konstanten von Trockensubstanzen der Art, wie man sie in verschieden fixierten oder getrockneten biologischen Präparaten findet. Als Modellobjekt ist in der Abbildung ein Tropfchen konzentrierter Eiweißlösung angenommen. Zwei alternative Wege zur Mengenbestimmung sind angegeben.

Figure 17 - The Drop Method (cf. Figure 14) for Determination of the Optical Constants of the Dry Substances of the Type Found in Various Stabilized or Dessicated Biological Preparations. A droplet of concentrated protein solution is assumed as model object in the illustration. Two alternate ways for volume determination are indicated a - the drop method; b - measurements by elimination of the influence of non-homogeneity through scanning with high resolution; c - protein droplet; d - x-ray tube for mass determination; e - protein droplet with C^{14}; f - Geiger-Muller counter; g - scanning interference microphotometer; h - scanning microspectrophotometer; i - measurement of protein amount in drop; k - measurement of optical phase difference; l - measurement of extinction; m - gives specific refraction; n - gives specific extinction.

GRAPHIC NOT REPRODUCIBLE

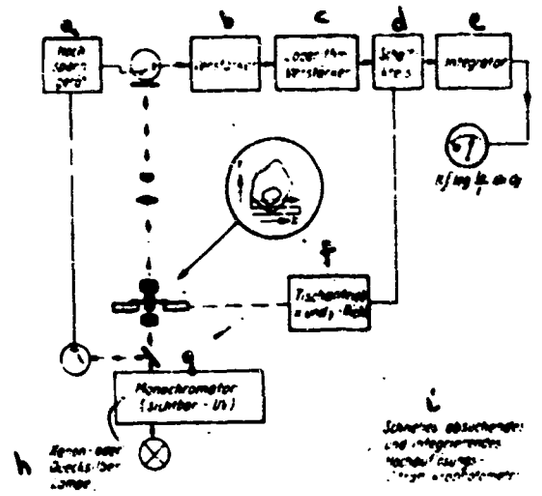

Abb. 18. Schnelles Ultramikrophotometer für Arbeit mit z. B. großen Zellpopulationen. Arbeitsdiagramm.

Figure 18 - Rapid Ultramicrophotometer for Working with Large Cell Populations. Working diagram. a - high-tension instruments; b - amplifier; c - logarithmic amplifier; d - circuit; e - integrator; f - table drive, and direction; g - monochromator (visible-ultraviolet); h - xenon or mercury lamp; i - rapid scanning and integrating high-resolution ultramicrophotometer.

GRAPHIC NOT REPRODUCIBLE

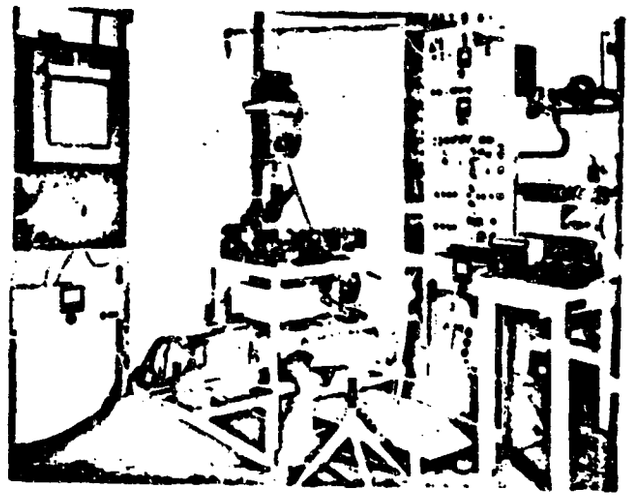

Abb. 19. Das schnelle Ultramikrophotometer.

Figure 19 - Rapid Ultramicrophotometer.

GRAPHIC NOT REPRODUCIBLE

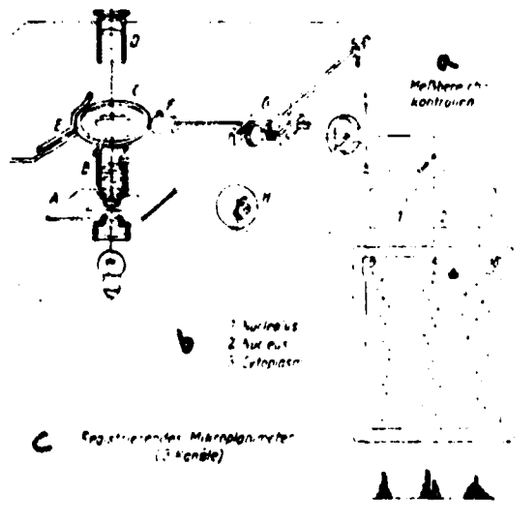

Abb. 20. Arbeitsdiagramm des registrierenden Mikroplanimeters.

Figure 20 - Operating Diagram of Recording Microplanimeter. a - measuring range control; b - 1. nucleolus, 2. nucleus, 3. cytoplasma; c - recording microplanimeter (3 channels).

GRAPHIC NOT REPRODUCIBLE

Abb. 21. Das Mikroplanimeter.

Figure 21 - Microplanimeter.

6198
CSO: R-3126-D

- END -