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(54) CELL CHARACTERISATION APPARATUS

(71) I, DR. WOLFGANG GOHDE, a German citizen of von Stauffenberg-Strasse 40, 4400 Münster, Germany, do hereby declare the invention, for which I pray that a patent may be granted to me and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a system for detecting characteristics of cells suspended in a liquid medium and to a flow chamber for use in the system.

Biological cells, such as cells taken from the human body, can be investigated by treating the cells with various chemical substances and observing and measuring luminous phenomena (e.g. fluorescence of the cells) occurring after this treatment and irradiation of the cells by light. By way for example, cells are stained with two different fluorescent dyes, one of which causes phenomena of fluorescence characteristic of the DNA and the other one characteristic of the protein of the cell.

To accomplish this, dyes are employed which show peaks, or maxima, of absorption at certain wavelengths during the fluorescence excitation. When using devices available prior to the present invention, errors occur in the case of simultaneous measurement, because the fluorescence spectra of the dyes utilized are comparatively broad and overlap one another. In these instruments part of the light of one dye invariably reaches the photomultiplier intended for the other dye. A device built for this purpose is described by M. Stohr in "Double Beam Application in Flow Technic and Recent Results", *Pulse-Cytophotometry*, 1976, pp. 39—45. The apparatus is equipped with an argon-ion laser and a helium-cadium laser emitting at wavelengths of 448 nm. and 441 nm., respectively. It also has special mirrors for 325 nm. According to Stohr, difficulties can be avoided if the points of interaction between a particle and the illuminating laser beams are physically separated. However, no solution is offered to the problem in that paper.

Other examples of prior art devices in this general field are U.S. Patents 3,513,319; 3,541, 336 and 3,609,048.

According to one aspect of the invention, there is provided a flow chamber for use in an optical system for detecting characteristics of cells suspended in a liquid, the chamber comprising a body having a transparent wall portion and a flow passage within the body through which the liquid and cells can pass, the flow passage having two passage portions which intersect adjacent to the wall portion and which form an angle therebetween less than 180°, the passage portions defining therebetween at the point of intersection an edge which lies in a plane spaced from and parallel to the wall portion, whereby cells in the liquid pass from one passage portion through said plane toward the wall portion and to the other passage portion away from the wall portion.

According to another aspect of the invention an apparatus for determining optical characteristics of cells comprises a flow chamber as defined above and an optical system including lens means for establishing an observation plane through said passage portions, adjacent said edge and substantially parallel with said transparent wall portion, light source means for providing light at two different wavelengths and for illuminating, through said lens means, the cells passing through said observation plane so that the cells in the inlet portion of said plane are illuminated at one wavelength and the cells in the outlet portion are illuminated at the other wavelength, and means responsive to light emanating from said cells to provide an indication of a characteristic thereof.

In the device according to the invention, both observation points have substantially identical flow conditions. They are physically separated, yet are so close to one another that the two separate light sources required for illumination need not be expensive lasers. Instead, according to the invention, they

may be two adjacent filters having different spectral or wavelength transmission characteristics and arranged between a primary light source and the observation point. By means of a conventional dichromatic beam splitter, the light from the primary light source is emitted by the two filters in accordance with the Kohler illumination principle to the two observation points, one of which receives the light of one filter and the other that of the other filter. Two photomultipliers are arranged behind the beam splitter, using a second beam splitter. Either of these photomultipliers receives the fluorescence from the particular observation point properly divided via half-side diaphragms.

Accordingly, the whole assembly is of a substantially simpler design and is less expensive than the devices now in use. Nevertheless, with the apparatus built in accordance with the principles of the invention, it is possible to excite the cells separately and properly with respect to the excitation wavelengths of light during their passage through the two observation points, and to measure them separately and correctly with respect to the fluorescence wavelengths of light. Special adjusting hitherto intended to minimize any overlapping is no longer necessary. The cells may be fed to the observation points with envelope flow. Thus, simplification is achieved when compared with flow chambers operating with a cross flow. The two observation points are symmetrical to one another, either of them forming a section of the focusing plane of the objective that can be fixed properly. Further, it is possible to provide an objective with a large aperture. By virtue of the envelope flow arrangement and proper adjustment of the flow conditions, the cells can be passed through the two observation points practically one at a time.

These and other advantages of the present invention will be readily apparent upon a consideration of the following description taken in conjunction with the accompanying drawings wherein:

Fig. 1 is a simplified side elevational view, schematic and in partial section, of a flow chamber according to the present invention; and

Fig. 2 is a simplified schematic view of the overall apparatus in accordance with the present invention.

The device according to the invention shown in the drawings has a flow chamber 10 provided with a flow channel 12 which is bent like a knee about an inner separating corner member 16. The vertex of the knee angle is cut off and replaced by a transparent glass cover 14 extending substantially at right angles to the bisectrix of the corner 16.

Parallel with the surface of the plane glass cover 14 lies the cross section of the flow to be observed via an objective lens 24, the observation plane being marked by the dotted line A—B. This cross section lies partly in the inflow leg of channel 12 and partly in the outflow leg thereof, these passage portions being separated from each other by edge 18 of corner 16, thus giving rise to the two observation zones or points 20 and 22, which are separated from one another and through which flow the particles or cells to be observed under substantially identical conditions. Edge 18 constitutes a line which is perpendicular to the plane of the paper and parallel with window plate 14.

The diameters of the flow passages in the zone of observation points 20 and 22 are determined by the nature of the particles under study. Depending on their size, the diameters vary between 0·1 and 0·5 mm. Diameters of 0·2 mm. are suitable for mammal cells.

Either or both of the observation zones may have slight differences in illumination level across the zone. To avoid measuring errors resulting therefrom, an envelope flow arrangement is employed with the object of passing all the cells through a predetermined point of the focusing plane, or observation point, so that all the cells are exposed to the same light energy. A cell suspension is fed to a feed channel 26 from a supply marked by arrow 28. This feed channel 26 ends in the inflow leg 12. An envelope of liquid is introduced to inflow leg 12 by a feeding mechanism comprising an annular inlet marked by arrow 30. The envelope liquid may consist of the same medium as the liquid in which are suspended the cells under study.

The outflow leg of channel 12 leads at 32 to a discharge tank. However, at 32 a sorting system for the cells may alternatively be connected to the outflow leg, the sorting being controlled by the readings taken at observation points 20 and 22.

The proper separation of observation points 20 and 22 by edge 18 of corner 16 permits a very simple design of the illumination system. A primary light source 40 is provided with a collector system 42 which makes parallel the rays of the beam of light emitted by a source 40. Using lens 44 the light source is imaged into the "inner pupil" of the microscope objective. This corresponds to the Kohler illumination of the observation point. Two filters 46 and 48 are arranged side-by-side in the path of rays such that the beam of light of the primary light source 40 is divided into two different parallel beams arranged side-by-side. Depending on the filter characteristics, this gives rise to two different substantially monochromatic beams of light I and II, so that filters 46 and 48 may be considered secondary light sources. A dichromatic beam splitter 50 is arranged at a 45° angle to the optical axis of the objective 24. This optical axis of objec-

tive 24 corresponds to the bisectrix of corner 16 and is thus normal to the surface of observation points 20 and 22. Observation point 20 is illuminated through the use of the beam splitter by beam I which has traversed filter 46, and observation point 22 by beam II which has traversed filter plate 48.

With the aid of a second beam splitter 54, which is likewise arranged on the optical axis of objective 24 at a 45° angle behind beam splitter 50, the phenomena of fluorescence at observation points 20 and 22 are received by means of photomultipliers 60 and 62. There is arranged ahead of either multiplier a half-side closing diaphragm 56 or 58 in the eyepiece focal plane of the reflected light microscope such that the light reaches photomultiplier 60 from observation point 20 through diaphragm 56 while the latter is being shielded against light from observation point 22. Conversely, light from observation point 22 traverses beam splitter 54 and diaphragm 58 and reaches photomultiplier 62, while the latter is being shielded against light from observation point 20 by the half-side closing of diaphragm 58.

With the arrangement constructed in accordance with the teachings of the present invention, it is possible to work with a comparatively large inner pupil 52 and a correspondingly large aperture of objective 24.

WHAT I CLAIM IS:—

1. A flow chamber for use in an optical system for detecting characteristics of cells suspended in a liquid, the chamber comprising a body having a transparent wall portion and a flow passage within the body through which the liquid and cells can pass, the flow passage having two passage portions which intersect adjacent to the wall portion and which form an angle therebetween less than 180°, the passage portions defining therebetween at the point of intersection an edge which lies in a plane spaced from and parallel to the wall portion, whereby cells in the liquid pass from one passage portion through said plane toward the wall portion and to the other passage portion away from the wall portion.

2. A flow chamber according to Claim 1 wherein said transparent wall portion is substantially planar and said edge parallel to said wall portion.

3. A flow chamber according to Claim 1 or 2 wherein said angle is an acute angle between about 50° and about 90°.

4. A flow chamber according to Claim 3 wherein said acute angle is about 60°.

5. A flow chamber according to any of Claims 1 to 4 wherein one of said first and second passage portions constitutes an inlet passage, said inlet passage having a central conduit for delivery of cells suspended in liquid and an annular conduit around said central conduit for delivery of liquid cells, whereby envelope flow is established in said inlet passage to maintain said cells substantially centrally located in said flow passage.

6. A flow chamber substantially as herein described with reference to and as shown in the accompanying drawings.

7. An apparatus for determining optical characteristics of cells comprising a flow chamber as claimed in any of claims 1 to 6 and an optical system including lens means for establishing an observaton plane through said passage portions, adjacent said edge and substantially parallel with said transparent wall portion; light source means for providing light at two different wavelengths and for illuminating, through said lens means, the cells passing through said observation plane so that the cells in the inlet portion of said plane are illuminated at one wavelength and the cells in the outlet portion are illuminated at the other wavelength; and means responsive to light emanating from said cells to provide an indication of a characteristic thereof.

8. Apparatus according to Claim 7 wherein said transparent wall portion and said observation plane are substantially perpendicular to a line bisecting the angle between said inlet and outlet portions.

9. An apparatus according to Claim 7 or 8 wherein said inlet portion includes means for delivering said cell-liquid suspension to said observation plane in envelope flow.

10. Apparatus according to any of claims 7 to 9 wherein said light source means includes two filters arranged side-by-side and having different light wavelength transmission properties, and a primary light source illuminating said filters, said filters being disposed between said primary source and said observation plane.

11. Apparatus according to any of claims 7 to 10 wherein said means responsive to light emanating from said cells includes a photomultiplier arranged to receive light from cells in one side of said observation plane.

12. Apparatus according to any of claims 7 to 11 wherein said optical system includes beam splitters for coupling said light source means and said means responsive through said transparent wall portion from the same side.

13. Apparatus for determining optical characteristics of cells substantially as herein described with reference to and as shown in the accompanying drawings.

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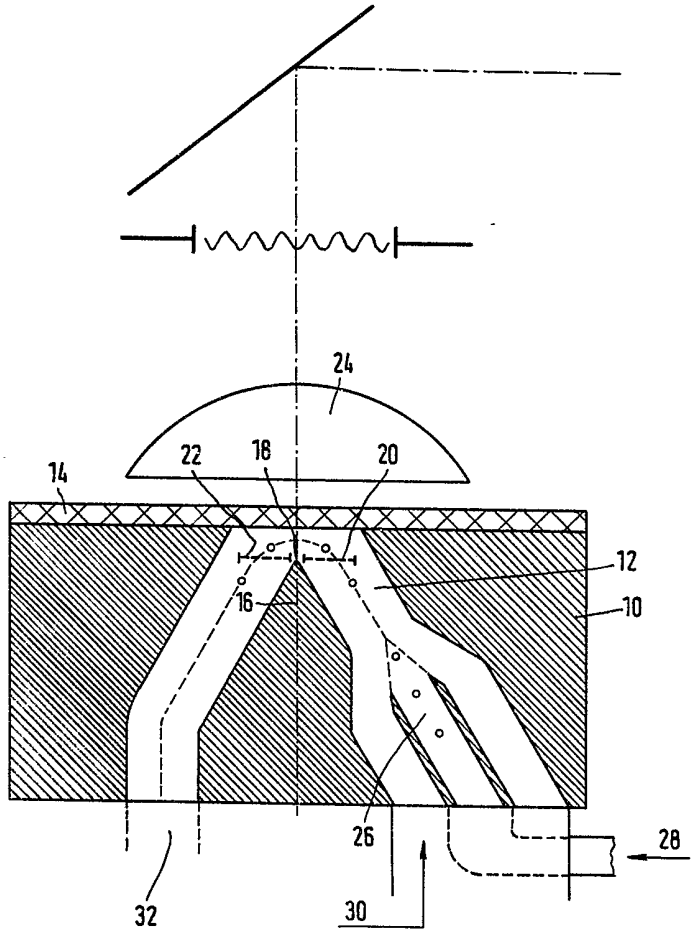
2 SHEETS *This drawing is a reproduction of the Original on a reduced scale*
Sheet 1

Fig.1



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Sheet 2

Fig.2