

April 28, 1970

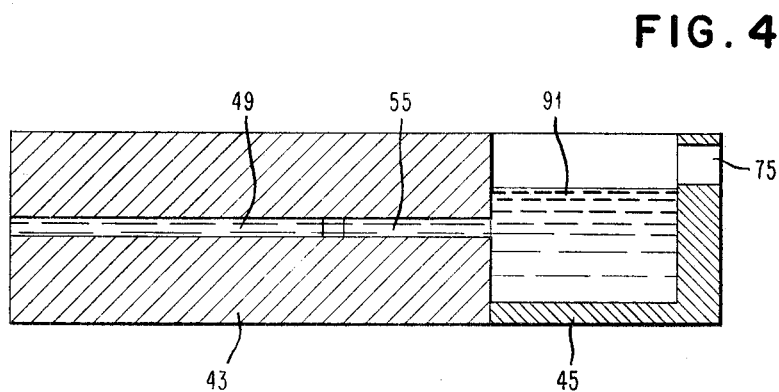
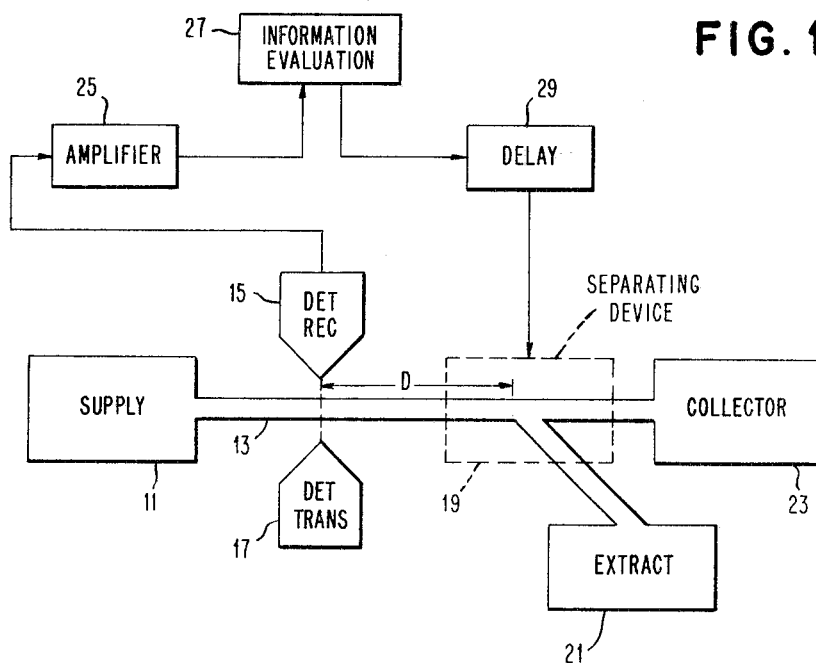
H. H. GLAETTLI

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PARTICLE SEPARATION APPARATUS

Filed Feb. 1, 1967

3 Sheets-Sheet 1



INVENTOR.

HANS H. GLAETTLI

BY

Thomas J. Kilgannon Jr.
ATTORNEY

April 28, 1970

H. H. GLAETTLI

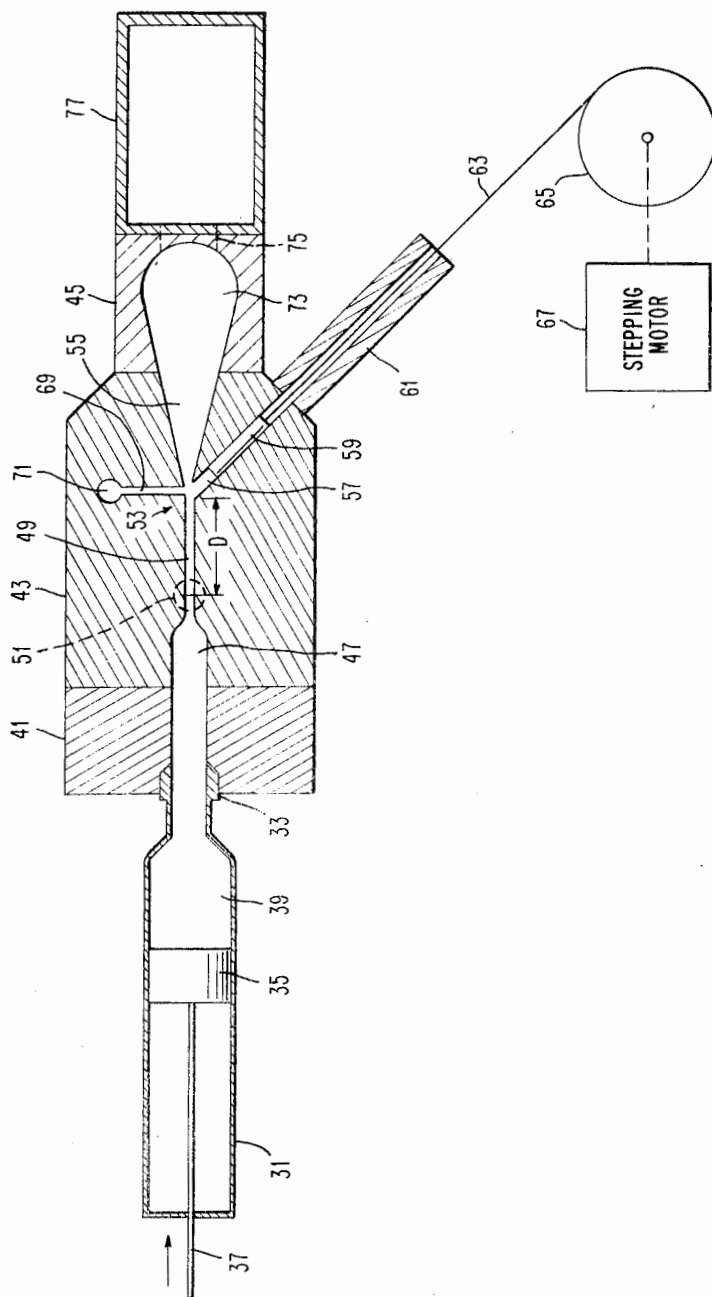
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PARTICLE SEPARATION APPARATUS

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FIG. 2



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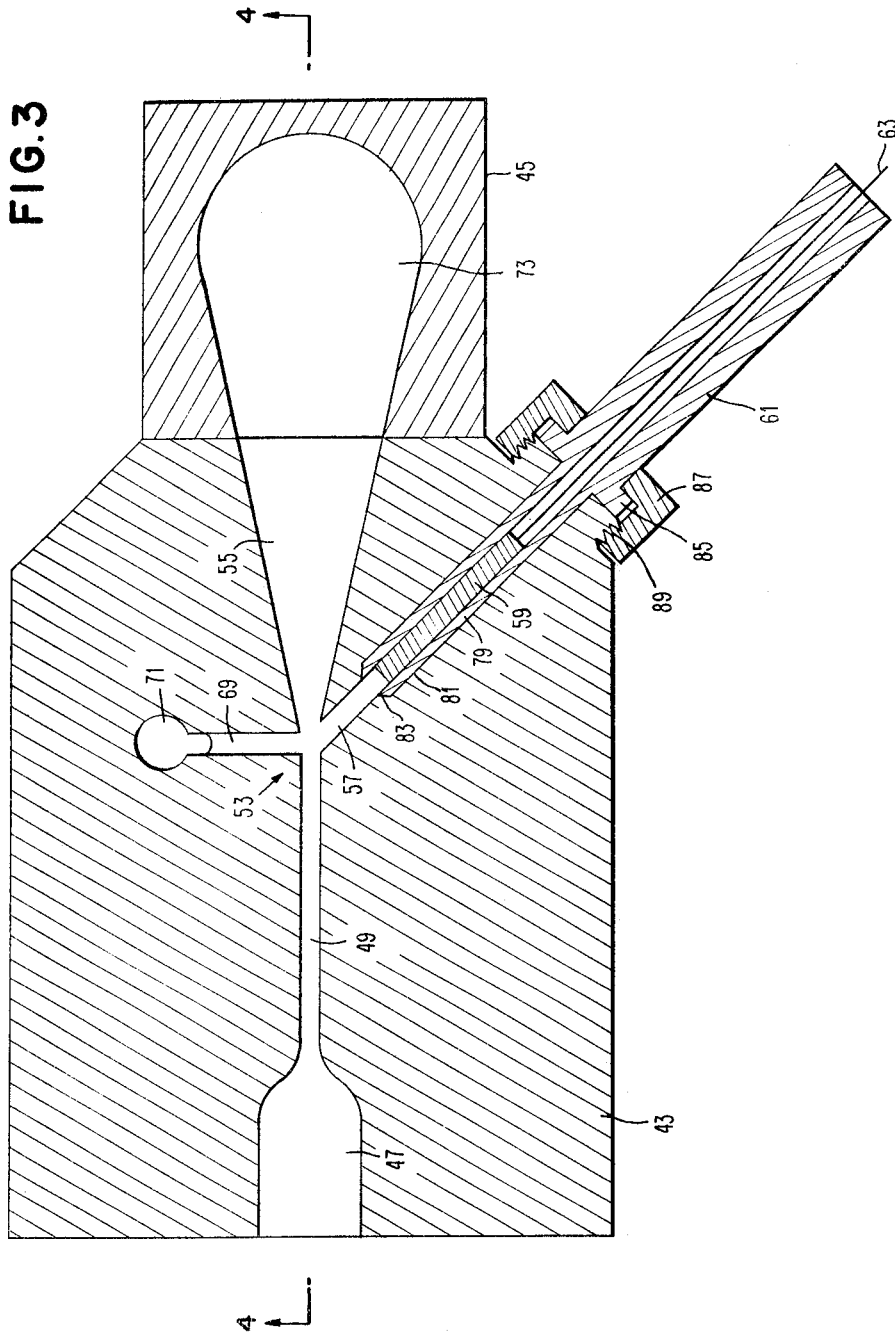
H. H. GLAETTLI

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PARTICLE SEPARATION APPARATUS

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PARTICLE SEPARATION APPARATUS

Hans H. Glaetli, Kunsnacht, Zurich, Switzerland, assignor to International Business Machines Corporation, Armonk, N.Y., a corporation of New York

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12 Claims

ABSTRACT OF THE DISCLOSURE

A particle separating apparatus for separating suspended biological bodies having specified identification criteria which has a main channel upstream of a junction from which three branches emanate. A closed channel containing an air bubble at its closed end extends substantially perpendicularly from the main channel, while a diffuser channel of gradually widening size extends from the junction along the same axis as the main channel. An extraction channel extends from the junction preferably at an included angle of 135° from the axis of the main channel and contains a piston within the channel which, when actuated at a desired time, removes a biological body appearing at the junction from the junction and causes it to enter the extractor channel. The separated particles are subsequently removed from the extraction channel for examination.

BACKGROUND OF THE INVENTION

This invention relates generally to apparatus for separating selected particles from a large group of particles according to specified criteria. More specifically, it relates to apparatus for separating selected biological particles from a large number of similar particles suspended in a fluid.

Field of the invention

In medical practice it is often necessary to investigate tissue samples or other particles which are contained in a fluid, in order to identify diseases and to establish diagnoses, or to study physiological processes in detail. In many cases, only a minor fraction of a given sample constitutes significant material. This fact requires separation of the particles of actual interest, e.g., biological cells, from a large quantity of other particles before a detailed analysis can be made, e.g., by a pathologist using a microscope.

The prior art indicated below has already suggested the use of an apparatus for the detection of cells of special interest if they exhibit automatically measurable properties distinguishing them from other cells with which they are mixed. Such an automatic separation is especially desirable for large scale series of laboratory tests. For example, there is a fair chance of curing certain cancers if their symptoms are recognized at an early stage. This could be achieved by regular tests.

Description of the prior art

Cancer cells usually exhibit optical properties different from normal healthy ones since their nuclei contain a greater amount of nucleic acids. Due to this, the absorption of light of a certain wavelength is altered so that suspicious cells can be detected by spectrophotometric measurements.

The following articles, among others, were published on this phenomenon:

(a) R. C. Mellors and R. Silver: A microfluorometric scanner for the differential detection of cells: Application

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to exfoliative cytology. Science, vol. 114, Oct. 5, 1951, pp. 356-360.

(b) R. C. Mellors, J. F. Keane, Jr., and G. N. Papanicolaou: Nucleic acid content of the squamous cancer cell. Science, vol. 116, Sept. 12, 1952, pp. 265-269.

Based on principles defined in the above articles, one can, therefore, detect in a device for the automatic testing of cells whether a given sample of cells contains suspicious cells. If such cells are detected, they should be separated at once in order that a detailed analysis can be effected on a concentrated sample by a pathologist. The detection and separation of such suspicious cells, of course, represents a considerable saving in time and money particularly where mass screening of the population is contemplated. Other relevant prior art relating specifically to cell separators may be found in a patent application entitled, "Particle Separator," filed in the name of L. A. Kamensky, on Nov. 17, 1965, S.N. 508,307, and assigned to the same assignee as the present invention.

SUMMARY OF THE INVENTION

In accordance with the present invention, apparatus for separating selected individual particles from a larger number of similar particles which are suspended in a fluid is provided. This apparatus broadly comprises a source of suspended particles from which certain particles are to be separated in accordance with given criteria. It further comprises detection apparatus for sensing the particles which meet the criteria. The criteria are evaluated in evaluation apparatus which may consist of a biased amplifier which, if it passes signals in excess of the bias level, indicates the presence of a cancer cell. The resulting signal is then coupled to delay apparatus which takes into account the various system delays including time of passage of the cell from the detection apparatus and actuation time of system components. The cell after detection is passed to separation apparatus which consists of a closed channel containing an air-bubble at its closed end, extending perpendicularly from a main flow channel at a junction; a diffuser channel of gradually widening width which maintains substantially atmospheric pressure at the junction from which the diffuser channel also extends; and an extractor channel also extending from the junction containing an actuable member which upon retraction applies suction to a particle appearing at that instant at the junction. The actuable member may be a piston which is attached to a stepping motor which is actuated from the above mentioned delay apparatus. The stepping motor and piston are arranged so that for a given size tubing of the extractor channel, a sufficiently large volume of fluid is extracted from the junction to include the selected cell and withdraw it sufficiently far from the junction so that it cannot be affected by the flow of fluid which passes directly from the main channel through the junction to the diffuser channel. The piston may be withdrawn in extractor channel sufficiently far that it travels beyond a removable connector and into an extractor channel extension. By removing the connector and extractor channel extension, fluid containing the selected, separated cells may be recovered.

It is an object of the present invention to provide a particle testing apparatus, e.g., an apparatus for analyzing cells and a separator which provides for fast and reliable separation of particles being selected according to certain criteria from other particles not exhibiting these criteria.

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of a preferred embodiment of the invention as illustrated in the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a block diagram of cell testing apparatus in which the separator of the present invention may be used,

FIG. 2 is a cross-sectional view of an embodiment of a particle separator showing the juxtaposition of the channels which branch from a junction at which a cell-to-be-separated appears,

FIG. 3 is an enlarged representation of the particle separator according to FIG. 2 showing additional details,

FIG. 4 is a cross-section of the separator taken along line 4—4 of FIG. 3.

DESCRIPTION OF THE PREFERRED EMBODIMENT

FIG. 1 shows a block diagram of an apparatus which may be used for separating cells with specific properties from a solution containing biological cells. The task may be, for example, to eliminate cancer suspicious cells from a tissue sample suspended in a fluid. A similar task would be the separation of certain blood cells from a blood sample.

Generally, such an apparatus might be employed not only for separating biological cells but also for separating any particles from similar particles with which they are initially mixed. It would be advantageous to have all particles the same size; however, it is not a necessary requirement for the workability of the separator.

In the following description of an embodiment an apparatus for separating cancer suspicious cells is assumed.

The cells to be tested pass a detector device in an examination channel and thereafter a separator, and normally flow subsequently together with the fluid into a collector receptacle. If, however, a desired cell is detected, by the detector a signal is generated which causes the extraction of this cell by the separator, the latter being the object of the present invention.

A large number of cells to be tested is inserted with a solution into supply receptacle 11. A pressure exerted on the supply causes the solution with the cells to flow through examination channel 13 at constant speed. The detector consisting of transmitter 17, e.g., a source of radiation, and receiver 15, detects certain properties of the cells when these pass between transmitter and receiver. Choosing a suitable cross-sectional area for examination channel 13 provides for a good probability that the cells pass in sequentially between the two detector parts.

A measurable property by which cancer suspicious cells differ from healthy cells is the ability to absorb optical waves of certain wavelength, a fact that is already briefly described in the early part of this specification.

Signals generated in detector receiver 15, due to the scanning of cells, are amplified for further processing by amplifier 25 and then transferred to information evaluation means 27. The distinction between signals caused by healthy cells and those caused by suspicious cells might possibly not be sufficient for clearly controlling a separator. For example, large cells with a normal core, and small cells with an increased amount of nucleic acid and, therefore, being cancer suspicious, will yield almost equal signals in an absorption measurement. Therefore, to enable a clear separation the ratio of nucleic acid to the size of the entire cell must be taken into account. This is possible by a combined absorption and scattering measurement with subsequent evaluation.

Such an evaluation is accomplished in apparatus indicated schematically by block 27. A signal appears at its output only if a cell to be extracted (a suspicious cell) has just passed the detector. After passing a delaying unit 29, this signal is used to control separator 19.

The delay is necessary because the cells need a finite time interval to flow with the solution from the detector

to the separator, i.e., to travel through distance D. The actual time delay is established by subtracting the response time of the separator from the time interval required for traveling through distance D.

If operation with different flow rates is desired, a delaying unit adjustable to different delay times must be provided.

In the actual separating device 19 discussed in more detail in the following, examination channel 13 branches into two separate channels. As long as there is no extraction of cells the solution flows into collector receptacle 23. However, as soon as a cell to be extracted reaches the junction point in the separator the signal arriving simultaneously from the delaying unit causes suction of this cell into the side channel so that finally the separated cells are collected as an extract from the initial supply of suspension, as is indicated schematically by block 21.

In principle, it is possible to provide a plurality of such separating devices in a single testing apparatus, and to control these separators by different detector signals, so that cells with different properties can be extracted at different points in one operation. In the present embodiment, however, only one separating device is provided. A still more detailed description of the device, which is a preferred embodiment of the invention, is now given in connection with FIGS. 2 to 4.

FIG. 2 shows on the left side a supply receptacle 31 similar to a syringe and ending in a pointed head 33. A fixed connection, e.g., a thread, can be made between part 33 and block 41 through which a channel passes, so that a leak-proof connection is established between chamber 39 of the receptacle and the channel passing through block 41.

A piston 35 and piston rod 37 are arranged movably in receptacle 31. If part 33 of the receptacle is dipped into the sample to be examined, i.e. the cell suspension, chamber 39 can be filled with the solution by retracting piston 35. Thereafter, part 33 is connected to block 41. Now piston rod 37 is displaced at constant speed in the direction of the arrow by driving means not shown in the drawing, causing the cell suspension contained in chamber 39 to flow through block 41 into channel 47 of block 43. Examination channel 49 of reduced cross-sectional area leads from channel 47. The cross-sectional area of this examination channel should not be much larger than the cross-sectional area of the cells to be examined. An inner diameter of about 0.1 mm. may be chosen. This forces the cells to pass in sequential order through the examination channel 49. The spot where measurement of cell properties is effected by the detector is indicated by dotted circle 51. The junction of the examination channel where extraction of the desired cells is effected is designated by 53.

At junction 53, examination channel 49 connects into diffusor 55, i.e., a channel with continuously increasing cross-sectional area. Due to diffusor 55, the pressure at junction 53 is almost equal to atmospheric pressure.

At junction 53 where examination channel 49 connects into diffusor 55 a side-channel 57 branches off at an included angle of about 135° with examination channel 49. The cells to be separated, which were detected at 51 by the detector, are extracted from the suspension flowing through junction 53 by a suction pumping device connected to channel 57, referred to hereafter as extraction channel 57.

The operation is as follows: When a cell to be separated has traveled through distance D, an impulse is transmitted to a stepping motor 67. A movement of the latter is transferred by means of a pulley 65 to wire 63 which retracts over a certain distance a valve piston 59 having the same cross-sectional area as extraction channel 57. Due to the retraction, a low pressure or pressure differential is established at the extraction channel's entrance, forcing the cell then located at junction 53 into extraction channel 57. Cavitation, due to the acceleration of a

fluid from examination channel 49 is prevented at junction 53 by the presence of channel 69 which extends from junction 3 and terminates in a dead end 71 which contains an air bubble. Fluid in channel 69 enters junction 53 upon the retraction of piston 59. Thus, a pressure differential is established at the entrance of extraction channel 57 which is sufficient to remove a selected particle but of insufficient magnitude to cause cavitation at the entrance of distraction channel 57. The orifice of the extraction channel should have the same or a slightly larger cross-sectional area as the examination channel.

The part of the extraction channel guiding valve piston 59 may have, in principle, a larger cross-sectional area than its orifice situated directly at junction 53. This will permit the extraction of a larger volume of suspension in the same length.

However, the cross-sectional area of (valve piston 59) should not be larger than three times the cross-sectional area of the orifice at junction 53. In any case, variations in the cross-sectional area must be steady and not show discontinuities. It is assumed, however, in the embodiment described, that extraction channel 57 has a constant cross-sectional area over its entire length. This is an optimum solution with regard to fluid mechanics.

In order to obtain an optimum pressure distribution for the operation of this device, a Reynolds number of about 100 should be maintained in examination channel 49.

A possible response time of the device, i.e., a time interval between the transfer of an impulse to the stepping motor and extraction of a cell, must be considered as indicated above.

The step length of the piston should be large enough to ensure movement of the fluid in the orifice of the extraction channel over at least two to three channel-widths, so that a cell after having been sucked into the extraction channel is not removed subsequently by secondary flows.

In the event non-desired cells enter extraction channel 57 due to a heavy concentration of cells in examination channel 49, they may be eliminated by passing the resulting extract together with additional fluid through the apparatus a second time.

Another channel 69, having a dead-end 71 which contains a gas bubble, e.g., air, leads into junction 53 opposite extraction channel 57. This considerably reduces the length of the column of fluid material which must be accelerated during the extraction process. As a result, cavitation effects are inhibited which tend to increase the response time and could possibly destroy the cells to be extracted. The inner width of this additional channel should be in the same order of magnitude as the inner widths of the inspection channel and the extraction channel.

In order to enable the separated cells to be removed from the device, suction pump 61 is detachable from block 43. Suction pump 61 has a central bore which is a continuation of and is of the same cross-sectional area as extraction channel 57. However, as indicated above they may have a cross-sectional area two to three times as large but this requires a carefully machined transition between the different cross sections. Detachable suction pump 61 is shown in more detail in FIG. 3.

In FIG. 3, the front end of pump 61 is fitted into a bore 81 of block 43. A conically ground surface 83 on both parts provides for a continuation of extraction channel 57 at the joint without surface irregularities or alterations of the cross-sectional area. Pump 61 can be fixed to block 43 by a thread 89 and a clamping nut 87 which engages a flange 85 of the pump.

An optical or mechanical sensing device can be provided for automatically detecting the fact that valve piston 59 has been retracted up to the end of pump 61 after a number of cell extractions thus indicating that extraction channel 57 is completely filled. Such sensing devices are

well-known in the art and, therefore, are not shown in connection with the embodiment described here.

Upon detecting that the piston has reached its final position, a signal must be transmitted to stop the driving of piston 35 in the supply receptacle and, thereby, to interrupt the examination process. Now, suction pump 61 can be removed from the separating device. If a suitable joint is provided, also wire 63 and pulley 65 can be disconnected. By pushing forward wire 63 and piston 59 the collected extracted cells can be ejected out of the extraction channel and applied to a glass slide for further analysis.

It is evident that any other suitable device could be provided for removing the extracted cells from the device by the separator. It is a condition, however, that gas bubbles be strictly avoided. This is achieved most easily if the extraction channel has an almost constant cross-sectional area over its entire length.

In order to ensure the desired effect of diffusor 55 beginning at the end of examination channel 49, i.e., at junction 53, it must always be completely filled with fluid. FIG. 4 representing a cross-section through the separating device indicated by line 4—4 in FIG. 3 shows how this may be achieved.

The top and bottom positions of the diffusor 55 have a triangular form and are parallel to each other, whereas the perpendicular side planes are small strips extending from junction 53 at a desired angle (e.g., 24°) with the axis of symmetry of diffusor 55. A useful range of angles is 20°–30°. Such a diffusor has a wedge-like shape and can easily be obtained by milling if block 43 comprising the separator is built-up by parallel layers of plastic.

An overflow reservoir 45 including a chamber 73 is attached to block 43. The chamber is connected with the diffusor and must always be filled with a fluid 91 to a height sufficient to keep the diffusor outlet completely below the surface of fluid 91. An overflow opening 75 is provided in the wall of overflow reservoir 45 so that the suspension entering from the separating device may again leave chamber 73. In FIG. 2 a collector receptacle 77 is shown attached to reservoir 45, into which the suspension can flow through overflow opening 75. Collector receptacle 77 may have any shape and size and should be removable for emptying.

What is claimed is:

1. In a system for detecting particles in accordance with given criteria, apparatus for separating particles suspended in a fluid comprising:

a source of particles disposed in a carrier fluid, means for detecting said particles in accordance with said given criteria,

a main fluid channel and an extracting channel meeting at a junction said extracting channel being disposed at an angle with said main channel and of given width,

means for delivering said particles through said main channel at a given pressure to said junction,

means connected to said junction for maintaining a pressure lower than said given pressure at said junction,

means connected to said junction for inhibiting cavitation effects at said junction, and

means responsive to said detecting means for extracting portions of said fluid containing selected of said particles through said extracting channel from said junction.

2. In a system for detecting particles in accordance with given criteria, particle separating apparatus according to claim 1 further including means connected to said extracting means for removing said selected particles from said system.

3. In a system according to claim 1 wherein said means for detecting said particles includes means for evaluating information relating to said criteria to provide an output signal when said criteria are met, and delay means con-

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nected to said evaluating means and said extracting means to provide a delayed signal to actuate said extracting means.

4. In a system according to claim 1 wherein said means for maintaining a pressure lower than said given pressure includes a diffuser channel the beginning of which is contiguous with said extraction channel and of gradually increasing cross-sectional area as said diffuser channel extends from said junction.

5. In a system according to claim 4 wherein said diffuser channel comprises top and bottom walls disposed in parallel spaced relationship and having tapering side walls the angle of the taper being in a range of 20°-30° relative to the axis of symmetry of said diffuser channel.

6. In a system according to claim 5 wherein the angle of said taper is preferably 24° relative to the axis of symmetry of said diffuser channel.

7. In a system according to claim 4 wherein said pressure lower than said given pressure is approximately atmospheric pressure.

8. In a system according to claim 1 wherein said means for inhibiting cavitation effects includes a closed channel containing a gas bubble at the closed end thereof the open end of which is disposed opposite said extraction channel.

9. In a system according to claim 1 wherein said means for extracting includes a piston disposed in said extrac-

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tion channel and retraction means connected to said piston to displace said piston in said extraction channel to apply a pressure differential at said junction.

10. In a system according to claim 9 wherein said retraction means includes a stepping motor responsive to said detecting means which retracts said piston away from said main fluid channel in steps of fixed length.

11. In a system according to claim 1 wherein said given angle is 135°.

12. A system according to claim 4 further including an opening from said diffuser channel, reservoir means for collecting said fluid from said opening and an overflow outlet disposed above the level of said opening to maintain said opening beneath the level of said fluid.

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REUBEN FRIEDMAN, Primary Examiner

T. A. GRANGER, Assistant Examiner