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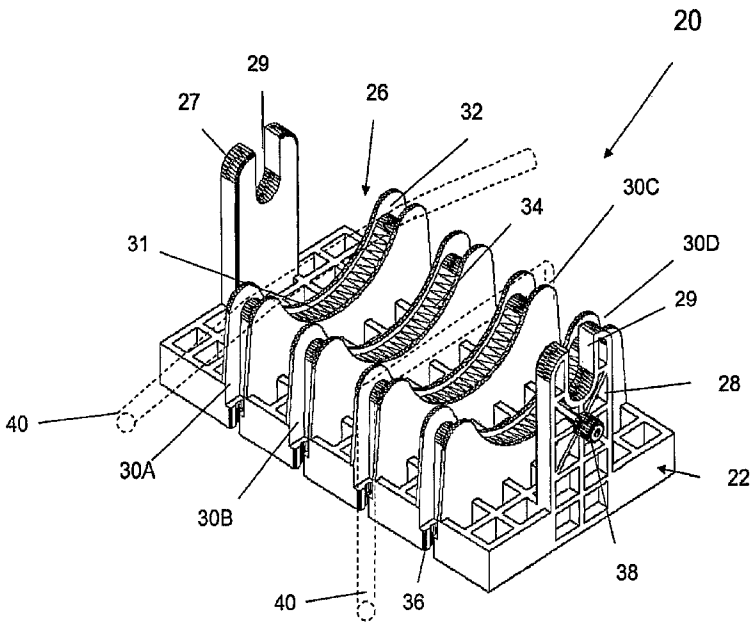
(54) Title: MEDIA CIRCULATION SYSTEM FOR A CELL CULTUREWARE MODULE



(57) Abstract: A media circulation system for a self-contained cell culture environment includes a multi-channel, variable output pump located in a reusable instrumentation base device having hardware to support cell culture growth. The pump circulates cell culture medium through a disposable cultureware module removably located on the instrumentation device. A pump cassette (20) having attached tubing is removably located on the disposable cultureware module, the pump cassette and tubing being insertable into the multi-channel pump.

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MEDIA CIRCULATION SYSTEM FOR A CELL CULTUREWARE MODULE

BACKGROUND OF THE INVENTION

1. Field of the Invention:

[0001] The present invention relates to a media circulation system for a self-contained culture environment, and more particularly to a multi-channel, cassette loading peristaltic pump having discrete variable output control for each channel for circulating cell culture medium in a cell culture system incorporating a disposable cultureware module and a reusable compact instrumentation base device that is capable of expanding patient- specific cell lines in a highly controlled, contaminant-free manner.

2. Description of the Related Art:

[0002] The anticipated growth of personalized medicine will require new paradigms for the manufacture of therapies tailored to the needs of individual patients. The greatest challenge is expected to come in the area of cell based therapies, especially when such therapies are autologous in nature. In such cases each cell or cell based product will need to be manufactured from scratch for each patient. Manual methods for mammalian cell culture, by their nature, are prone to technician error or inconsistency leading to differences between supposed identical cultures. This becomes especially evident as more and more autologous cells are expanded for personalized therapies. Patient-specific cells, or proteins, are subject to variation, especially when scaled beyond levels that can be managed efficiently with manual methods.

[0003] In addition to being labor intensive, the stringent requirements for segregation of each patient's materials from that of every other patient will mean that manufacturing facilities will be large and complex, containing a multitude of isolation suites each with its own equipment (incubators, tissue culture hoods, centrifuges) that can be used for only one patient at a time. Because each patient's therapy is a new and unique product, patient specific manufacturing will also be labor intensive, requiring not just direct manufacturing personnel but also disproportionately increased manpower for quality assurance and quality control functions.

[0004] Moreover, conventional approaches and tools for manufacturing cells or cell based products typically involve numerous manual manipulations that are subject to variations even when conducted by skilled technicians. When used at the scale needed to

manufacture hundreds or thousands of patient specific cell based therapies, the variability, error or contamination rate may become unacceptable for commercial processes.

[0005] Small quantities of secreted product are produced in a number of different ways. T-flasks, roller bottles, stirred bottles or cell bags are manual methods using incubators or warm-rooms to provide environments for cell growth and production. These methods are very labor intensive, subject to mistakes and difficult for large scale production. Another method, ascites production, uses a host animal (usually a mouse) where the peritoneum is injected with the cells that express the product and are parasitically grown and maintained. The animals are sacrificed and the peritoneal fluid with the product is collected. This method is also very labor intensive, difficult for large scale production and objectionable because of the use of animals. Another method is to inoculate and grow the cells in a small stirred tank or bioreactor or bag-type chamber. The tank provides the environmental and metabolic needs and the cell secretions are allowed to accumulate. This method is costly in terms of facility support in order to do a large number of unique cells and produces product at low concentration.

[0006] Another method is to use a bioreactor (hollow fiber, ceramic matrix, fluidizer bed, etc) in lieu of the stirred tank. This can bring facilities costs down and increases product concentration. Biovest International of Coon Rapids, MN, has or had instruments using these technologies - hollow fiber, ceramic matrix, fluidized bed and stirred tanks.

[0007] Cell culturing devices or cultureware for culturing cells in vitro are known. As disclosed in U.S. Patent No. 4,804,628, the entirety of which is hereby incorporated by reference, a hollow fiber culture device includes a plurality of hollow fiber membranes. Medium containing oxygen, nutrients, and other chemical stimuli is transported through the lumen of the hollow fiber membranes or capillaries and diffuses through the walls thereof into an extracapillary (EC) space between the membranes and the shell of the cartridge containing the hollow fibers. The cells that are to be maintained collect in the extracapillary space. Metabolic wastes are removed from the cultureware. The cells or cell products can be harvested from the device.

[0008] Preparing the system to start the cell culture is a very labor intensive process. The prior art cultureware must be assembled and sterilized or probes must be prepared, sterilized and aseptically inserted into the pre-sterilized portion of the cultureware. The cultureware assembly is then loaded onto the instrument. A series of manual operations are needed to check the integrity of the assembly, introduce fluid into the cultureware flow path, flush the toxic residuals from the cultureware, start the cultureware in a pre-inoculation

mode, introduce factors into the flow path getting it ready for the cells, inoculating the cells into the bioreactor and starting the run (growth of the cell mass and eventual harvest of product).

[0009] Each unique cell line must be cultured, cell secretions harvested and purified separately. In order to do a large number of unique cell lines, a considerable number of instruments would be needed. Compactness of the design and the amount of ancillary support resources needed have become an important facilities issue. The systems currently available are general purpose in nature and require considerable time from trained operators to setup, load, flush, inoculate, run, harvest and unload. Each step usually requires manual documentation.

[0010] The previous methodologies that utilize off-line sampling are subject to contamination problems and depend on the skill of the operator in predicting future lactate levels and influence of media dilution rate. Sampling equipment need interfacing to the culture fluidic circuit and an interface for the feedback signal. The lactate probe requires interface with the fluid circuit, a method for sterilization or a sterile barrier, and interface electronics to convert the probe signal to a useful feedback.

[0011] Another problem with the known cultureware is the process for circulating the growth medium therethrough. U.S. Patent No. 5,882,918 discloses a cell culture incubator having a continuous batch circulation system that uses a peristaltic pump to circulate the medium between a cell culture receptacle and a control portion.

[0012] Typical multi-channel peristaltic pump applications operate using a rotational drive shaft that is common to all rotors. This causes all of the rotors to turn at the same RPM yielding the same fluid output. Different diameter flexible tubing is used to give a fixed ratio delta output from one rotor to the next. To obtain a variable output of the peristaltic pump segments individual pump heads and drives are used. This requires individual tubing cassettes that must be loaded individually and does not allow for close center to center distance between pump heads. Examples of such existing pumps are the Masterflex L/S series pump manufactured by Barnant Inc. and the multi-channel pump manufactured by Ismatec SA, both being distributed by Cole-Parmer of Vernon Hills, Illinois. As disclosed in U.S. Patent No. 4,886,431, these pumps offer up to eight channels that all rotate at the same RPM. Different types of cassettes are available that hold different sizes of tubing yielding different outputs per revolution.

[0013] One limitation with such pumps is that as the drive servo increases or decreases each of the channels has a corresponding increase or decrease in flow rate. The pump system does not allow for one channel to be increased and another to be decreased.

[0014] Another problem is the difficulty in loading the various tubing lines in the respective pump cassettes. This is a labor intensive procedure that requires a skilled technician to connect the appropriate cassette to the cultureware system.

[0015] Accordingly, there is a need for a system and method having a multi-channel pump with variable control from channel-to-channel to enable different outputs per channel in a fully automated, rapid and sterile manner.

[0016] Moreover, an easy loading and locking mechanism to reduce tubing segment loading errors and installation time is also necessary.

SUMMARY OF THE INVENTION

[0017] One aspect of the present invention is to provide a multi-channel pump having discrete variable control of each channel so that the output of one channel can be increased while another decreased to pump different fluid components in a cell culture system at different rates in a fully automated, rapid and sterile manner.

[0018] Another aspect of the present invention is to provide an easy loading cassette and locking mechanism to reduce tubing loading errors and installation time.

[0019] Yet another aspect of the present invention is the ability to move fresh basal media into the cultureware, remove spent media, add high molecular weight factor and remove product harvest at different rates.

[0020] The system of the present invention incorporates features that greatly reduce the operator's time needed to support the operations (e.g. integrated pump cassette) and designed automated procedures and apparatuses which allow the system to sequence through the operations.

[0021] According to these and other aspects of the present invention, there is provided a media circulation system for a self-contained cell culture environment including a multi-channel pump located in a reusable instrumentation base device having hardware to support cell culture growth. The pump circulates cell culture medium through a disposable cultureware module removably located on the instrumentation device. A pump cassette having attached tubing is removably located on the disposable cultureware module, the pump cassette and tubing being insertable into the multi-channel pump.

[0022] According to these and other aspects of the present invention, there is also provided a method of circulating cell culture medium in a cell culture system including the steps of providing a disposable cultureware module including a cell growth chamber, and a reusable instrumentation base device incorporating hardware to support cell culture growth. The base device includes a multi-channel pump for circulating media through the cell growth chamber. A pump cassette for supporting a plurality of lengths of tubing is provided. The tubing is loaded in the cassette. The cassette is removably attached to the cultureware module. The cultureware module in turn is removably attached to the instrumentation base device. The cassette and tubing are inserted into the multi-channel pump. At least one source of media is fluidly attached to the cultureware module. The at least one source of media is pumped through the cell growth chamber to grow cells or cell products therein.

[0023] These and other features, aspects, and advantages of the present invention will become more apparent from the following detailed description of the preferred embodiment relative to the accompanied drawings, in which:

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] Fig. 1 is a perspective view of the system for producing cells and/or cell derived products according to the present invention.

[0025] Fig. 2 is another perspective view of the system of the present invention.

[0026] Fig. 3 is a perspective view of the disposable cultureware module and pump cassette of the present invention.

[0027] Fig. 4 is a perspective view of the pump cassette of the present invention.

[0028] Fig. 5 is a perspective view of the variable output, multi-channel pump of the present invention.

[0029] Fig. 6 is a perspective view of the variable output, multi-channel pump of Fig. 5 in an unassembled state.

[0030] Fig. 7 is a front view of the variable output, multi-channel pump of Fig. 5.

[0031] Fig. 8 is a side view of the variable output, multi-channel pump of Fig. 5.

[0032] Fig. 9 is a view of a technician loading an assembled cassette on the module.

[0033] Fig. 10(a) – (g) are views of the various steps in set-up and loading of the system of the present invention.

[0034] Fig. 11 is a flow diagram of the system of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0035] Referring to Fig. 1, the present invention provides a fully integrated system 10 for producing cells and cell derived products in a closed, self-sufficient environment. More specifically, the system allows for cell expansion and harvest of cells and their products with minimal need for technician interaction. As will be described further herein, the device incorporates cell culture technology, for example, hollow fiber bioreactor perfusion technology, with all tubing components encased in a single-use, disposable cultureware module 12. Following bioreactor inoculation with cells, the system follows pre-programmed processes to deliver media, maintain pH, maintain lactate levels, control temperature and harvest cells or cell-secreted protein. Standard or unique cell culture methods can be programmed prior to bioreactor inoculation, such that, various cell types or proteins can be expanded and harvested in an efficient, reproducible manner that is free of human error.

[0036] The system is based on cell growth chamber technology. For example, bioreactors that have a plurality of semi-permeable hollow fibers potted in a housing to create a space inside the fiber (intracapillary or IC space) separate from that outside the fibers (extracapillary or EC space). Fluid distribution between the IC and EC space occurs through the fiber pores which can range in size from 10Kd to 0.2μm. Cells are placed on one side of the fiber, usually in the EC space, in a complete cell culture medium, which is usually the same medium used to expand cells prior to bioreactor inoculation (serum containing, serum-free, or protein-free medium). Cells are usually placed in the EC space when secreted protein is the desired product. In some instances, when cells are the desired product, it may be beneficial to place cells in the IC space.

[0037] Medium is perfused through the bioreactor by circulating through the IC space at a fast rate. This serves to deliver nutrients to the cell space and conversely, removes or prevents a toxic build-up of metabolic waste. It should be appreciated that other culture vessels are contemplated by the present invention.

[0038] The system 10 provides significant efficiencies and cost reduction through its disposable component and enclosed operation. As such, cell lines are contained in a closed system and continuously cultured without the need for specialized, segregated clean rooms. This fully integrated apparatus eliminates the need for cleaning and sterilization validations, as well as the need for hard plumbing associated with conventional cell culture facilities.

[0039] Referring again to Fig. 1, the system consists of two individual parts: an instrumentation base device 14 that is reusable and an enclosed cultureware 12 that is used

for a single production run and is disposable. Disposable cell culture module 12 is removably attachable to device 14. The module requires multiple mechanical and electrical interfaces to the control instrumentation of device 14. Module 12 has interface features integrated into the module that mate with instrument interface features in the device to allow for a single motion installation.

[0040] The instrument provides the hardware to support cell culture growth and production in a compact package. As shown in Fig. 2, and as will be described in further detail herein, an easy-load multiple channel peristaltic pump 16 is located within device 14 and moves fresh basal media into the cultureware, removes spent media, adds high molecular weight factor and removes product harvest. An integrated cool storage area 80 maintains the factor 82 and harvest 84 at a low temperature (approximately 4°C).

[0041] Module 12 is heated to maintain cell fluid temperature and the cell environment to promote growth and production. The cell culture, disposable modules 12 requiring elevated temperatures are warmed by fully encapsulating the module and attaching the module to the controlling instrument 14, such that air ports are aligned and warmed air is forced into the module from the instrument at one location and allowed to escaped at another.

[0042] The one-time use cultureware module 12 is provided pre-sterilized. It is designed for quick loading onto the instrument ("quick-load"). The loading of the cultureware body makes connections to the instrument. A pump cassette 20, the front of which is shown in Fig.1 and the back of which being shown in Figs. 2-4, accommodates a plurality of lengths of tubing 40 (Fig. 4) and allows the user to quickly load the pump segments. The design and layout minimizes loading errors.

[0043] As will be explained further herein and as shown in Figs. 9 and 10, the tubing lengths 40 are attached to the disposable containers for harvest collection. The operator also attaches a media source, factor bag and spent media container to the cultureware via tubing 40 before running. The media and spent media container is disconnected, the pump cassette is unloaded, the cultureware body is unloaded and the used cultureware is placed in a biohazard container for disposal.

[0044] The system of the present invention has application in a regulated cell culture environment. It is anticipated that autologous whole cell therapies or patient-specific proteins (vaccines) therapies, would by their nature, require the simultaneous culture of numerous cell lines in a single facility. In addition to the segregation created through this closed culture approach, the apparatus is designed to support a standard information management system (such as a LIMS or MIS) protocol. This capability contributes to the creation of thorough

batch records and verification of culture conditions to ensure standardization, tracking and safety of each product. This capability facilitates the multi-product concept that is pivotal to facilities involved with autologous or patient-specific products.

[0045] Referring again to Figs 1-4, cassette 20 is positionable between an assembly position where it is mounted on the cell culture module 12 (Figs. 2 and 3) and an installation position (Fig. 1) where the cassette is connected to pump 16. Module 12 includes a recess 18 for receiving a handle 25 mounted on front 24 of cassette 20. Cassette 20 is mounted on module 12 during the positioning of module 12 on device 14. As described above, cassette 20 can be pre-loaded with lengths of peristaltic tubing 40 to reduce loading errors and to reduce installation time. See Fig. 10. As will be described further herein, when cassette 20 is installed into pump 16 the tubing interfaces with each channel of the pump.

[0046] As shown in Fig. 4, cassette 20 includes a plurality of sections 30A-30D. Each section 30 includes a canal 32 for receiving a length of tubing 40. The sections 30 have a curvature 34 that corresponds with a shape of the rotors of pump 16, which will be described further herein.

[0047] The cassette configuration is structured to hold multiple peristaltic tubing segments. A gripping feature 36 on the top and the bottom of each section prevents the tubing from creeping during operation. The design allows for all tubing segments to be loaded into the pump drive mechanism at the same time.

[0048] Cassette 20 is pre-loaded with peristaltic tubing (Figs. 9 and 10) and positioned in groove 18 on module 12. Referring to Figs. 4-5, after module 12 is positioned on device 14, cassette 20 is removed and inserted into interface or plate 42 of pump 16. As shown in Figs. 5 and 7, a plurality of pump recesses 44 are located in device 14. When positioned into the pump, each cassette section 30A -D supporting a length of tubing 40 is inserted simultaneously into a respective recess 44A-44D formed in plate 42. This configuration reduces tubing segment loading errors with pre-loaded multi-position cassettes, and reduces installation time.

[0049] Referring to Figs. 6-8, the present invention incorporates a multi-channel, cassette loading, peristaltic pump 16 with discrete, variable output control for each channel. Although four channels are shown, it should be appreciated that pump 16 can have more or less channels. As shown in Fig. 6, the pump has a plurality of channels 50 with individual, variable control of the output of each channel. Each channel includes a pump rotor 52 and an occlusion roller 56. Pump rotors 52A-52D are mounted on a common fixed axial shaft 54. A plurality of occlusion rotors 56A to 56D are driven by respective pump rotors 52A-52D, and

in turn are mounted on the single shaft 54. Pump rotors 52A-52D have internal bearings that allow for independent functional control by a respective reacting servo drive 58A-D. The single shaft minimizes tolerance accumulations typically caused by misalignment of individual rotors and shafts mating with a multi-channel cassette. Feedback sensors are included to verify rotation of the pump rotors. Servo drives 58A-D are controlled by control board 48. Each pump rotor 52 is connected to and driven by the respective servo 58 via a drive belt 53.

[0050] The cassette mechanism includes side walls 27 and 28 that extend through apertures 45 (Fig. 7) of plate 42 when inserted into pump 16. Each of the side walls 27, 28 have a recess 29 that engages shaft 54 when the cassette is inserted. Side wall 28 includes a cam operated cassette insertion feature 38 that interfaces with a cam operated latch 57 (Fig. 6) on pump 16 to lock the cassette in the pump. Cam feature 38 is also included to provide a bearing surface for the cam-operated latch 57 to react upon. As shown in Fig. 6, a knob 48 is rotated to move cam feature 38 into position to aid initial tubing occlusion during loading.

[0051] The occlusion rotors 56A-D each include a plurality of rollers 55 (Fig. 6) equally spaced in a circular path that corresponds with the curvature 34 of the cassette channels 32. The rollers 55 engage the tubing so that at least one roller is compressing the tubing against a pressure surface 31 of each section 30. As is known, the liquid components are pumped as rollers 55 engage and disengage the portion of the tubing contacting surface 31.

[0052] As shown in Fig. 7, occlusion rotors 56A-D are in line with recesses 44A-D so that the tubing lengths supported by cassette 20 mate with a respective occlusion rotor of the pump when the cassette is inserted into the pump. Unlike typical multi-channel peristaltic pumps that operate using a rotating drive shaft common to all rotors, the pump of the present invention has individually driven channels that need not turn at the same RPM, yielding different fluid output. Thus, there is not the need for different inside diameter tubing from one rotor to another. Thus, to obtain a variable output of the peristaltic pump segments, individual pump heads and drives are used..

[0053] Referring again to Figs. 9 and 10, the technician loads tubing onto the cassette 20 (Fig. 10(b)). Cassette 20 is then located on module 12 and module 12 is connected to device 14 (Fig. 10(c)). The loaded cassette is then inserted into pump 16, the locking mechanism operated and the various connections are made (Fig. 10(d)). Thereafter, the system is run to cultivate the cells.

[0054] Referring to the flow diagram of Fig. 11, pump 16 moves fresh basal media into the cultureware at media line 210. Media line 210 is connected to a user provided container of fresh media to provide the growth nutrients to the cell culture that are pumped into the disposable. Outflow line 214 is connected to a user provided container to collect the waste or spent media being pumped out of the disposable. Factor line 212 is connected to a user provided container of growth factors that are pumped into the disposable. Inoculate can be added at 220 and IC sample at 216. Product harvest is removed at 114. The cells are harvested at 218. Harvest line 218 is a pre-attached container that is part of the disposable that is used to collect the product that is pumped out of the disposable. Pump 16 has multiple lines 210, 214, 212 and 114. Because the pump of the present invention has a common fixed axial shaft and individual servo driven rotors, the control of the flow of each can be independent, allowing one channel or flow to be increased while another decreased.

[0055] At present, the present invention fully integrates the concept of disposable cultureware into automated process control for maintaining and expanding specialized (autologous or other) cell lines for a duration of any time needed . To accomplish this, the system of the present invention was designed for EC space fluid flow that enhances cell growth in high density perfusion culture, yet remains completely closed and disposable. The integrated pre-assembled cultureware, which consists of all tubing, bioreactor, oxygenator, pH probe, is enclosed in a single unit that easily snaps into the apparatus. In addition to this error-proof, quick-load design, the entire cultureware unit enclosed by the casing becomes the cell culture incubator with temperature control regulated through automated process control of the instrument. Pumps and fluid control valves facilitate disposability and error-proof installation, eliminating the possibility of technician mistakes. Finally, during the course of any culture, the closed system has restricted access except for trained and authorized personnel. Manipulations or sampling, outside of program parameters, require password and bar code access before they can be implemented.

[0056] Each unique cell line must be cultured, cell secretions harvested and purified separately. In order to manage a large number of unique cell lines, as for example might be required for the production of large numbers of autologous cell therapeutic products or large numbers of unique monoclonal antibodies, a considerable number of instruments would be needed. Compactness of the design and the amount of ancillary support resources needed become an important facilities issue. Small stirred tank systems require a means of steam generation and distribution (for steam-in-place sterilization) or autoclaves to sterilize the vessels and supporting plumbing. To support a large number of units becomes a logistics

problem for the facility. The system of the present invention has no such requirement. Larger scale cell culture is historically done in segregated steps that often require separate types of equipment. Manual handling, storage and tracking is needed for all these steps as the culture expands and product is harvested. The method of the present invention integrates these steps into a continuous, fully integrated sequential process. This eliminates the handling risk and facilitates the data gathering required for thorough documentation of the entire process.

[0057] Although the present invention has been described in relation to particular embodiments thereof, many other variations and modifications and other uses will become apparent to those skilled in the art. It is preferred therefore, that the present invention be limited not by the specific disclosure herein, but only by the appended claims.

WHAT IS CLAIMED IS:

1. A media circulation system for a self-contained cell culture environment comprising:

a multi-channel, variable output pump located in a reusable instrumentation base device, the device incorporating hardware to support cell culture growth, wherein the pump circulates cell culture medium through a disposable cultureware module removably located on the instrumentation device; and

a pump cassette having attached tubing removably located on the disposable cultureware module, the pump cassette and tubing being insertable into the multi-channel pump.

2. The media circulation system of claim 2, wherein the pump moves high molecular weight factor into a cell growth chamber of the module and removes product harvest from the cell growth chamber.

3. The media circulation system of claim 1, wherein the pump is a variable output, multi-channel peristaltic pump.

4. The media circulation system of claim 3, wherein the pump is comprised of a plurality of pump rotors disposed on a common axial shaft.

5. The media circulation system of claim 4, wherein each of the plurality of pump rotors is driven by a separate servo drive to enable individual variable control of each channel.

6. The media circulation system of claim 5, wherein the pump includes a plurality of occlusion rotors disposed on the axial shaft, each of the plurality of occlusion rotors being driven by a respective pump rotor.

7. The media circulation system of claim 3, wherein said pump cassette has a plurality of sections, each of said plurality of sections communicating with a respective pump channel.

8. The media circulation system of claim 7, wherein each cassette section receives a length of tubing and includes a gripping feature to retain the tubing therein.

9. The media circulation system of claim 8, wherein each cassette section include a pressure surface that contacts the length of tubing when the cassette is inserted into the pump.

10. The media circulation system of claim of claim 9, wherein the instrumentation device includes a plurality of recesses.

11. The media circulation system of claim 10, wherein each section of the cassette is received in one of the plurality of recesses when said cassette in inserted into the pump.

12. The media circulation system of claim 11, wherein each of the pump channels communicates with one of the plurality of recesses so that the occlusion rotors of each channel correspond with a respective recess.

13. The media circulation system of claim 12, wherein one of the plurality of cassette sections and a length of tubing are received in one of the recesses to locate the tubing length between the pressure surface of the cassette and the occlusion rotors when the pump cassette is inserted into the pump.

14. A method of circulating cell culture medium in a cell culture system comprising the steps of:

providing a disposable cultureware module, said module including a cell growth chamber;

providing a reusable instrumentation base device incorporating hardware to support cell culture growth, said base device including a multi-channel, variable output pump for circulating media through said cell growth chamber;

providing a pump cassette for supporting a plurality of lengths of tubing;

loading the tubing in said cassette;

removably attaching said cassette to the cultureware module;

removably attaching said cultureware module to said instrumentation base device;

inserting said cassette and tubing into said multi-channel pump;

fluidly attaching at least one source of media to said cultureware module; and

pumping the at least one source of media through said cell growth chamber to grow cells or cell products therein.

15. The method of claim 14, wherein the pump cassette has a plurality of sections and the step of loading the tubing comprises loading a length of tubing in each of the sections.

16. The method of claim 15, wherein the pump has a plurality of channels, each channel including a pump rotor and occlusion rotors, wherein the step of inserting the

cassette into the pump comprises inserting each section of the cassette and tubing into a respective channel simultaneously.

17. The method of claim 16, wherein each of the pump rotors are driven by an independently controlled servo motor and the step of pumping comprises individually and variably controlling the output of each channel.

18. The method of claim 17, further comprising the steps of fluidly attaching a source of high molecular weight factor to said cultureware module, and pumping the high molecular weight factor through said cultureware module.

19. The method of claim 14, further comprising the step of pumping spent media from the cultureware module.

20. The method of claim 14, further comprising the step of pumping harvested cells or cell products from the cultureware module.

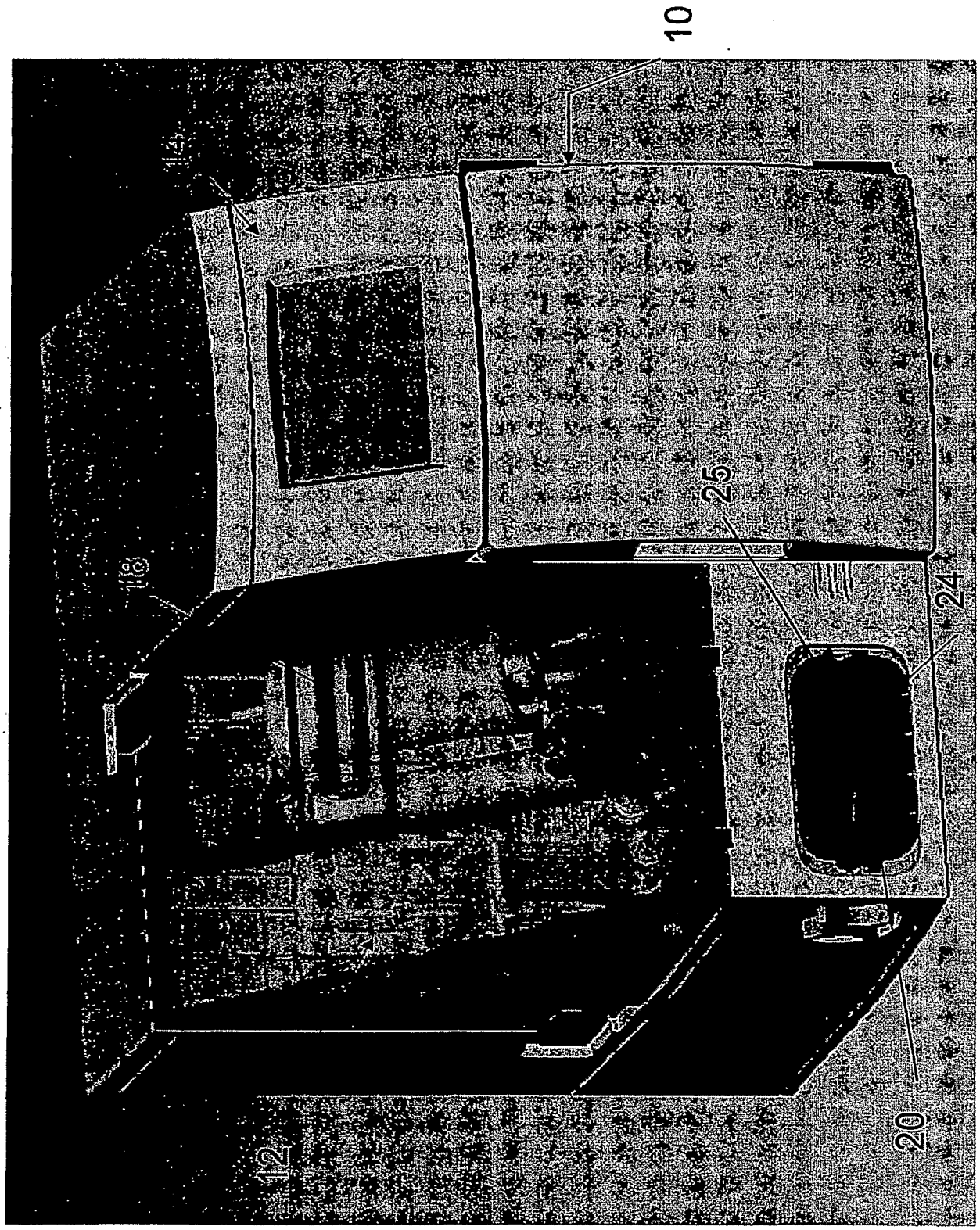


Fig. 1

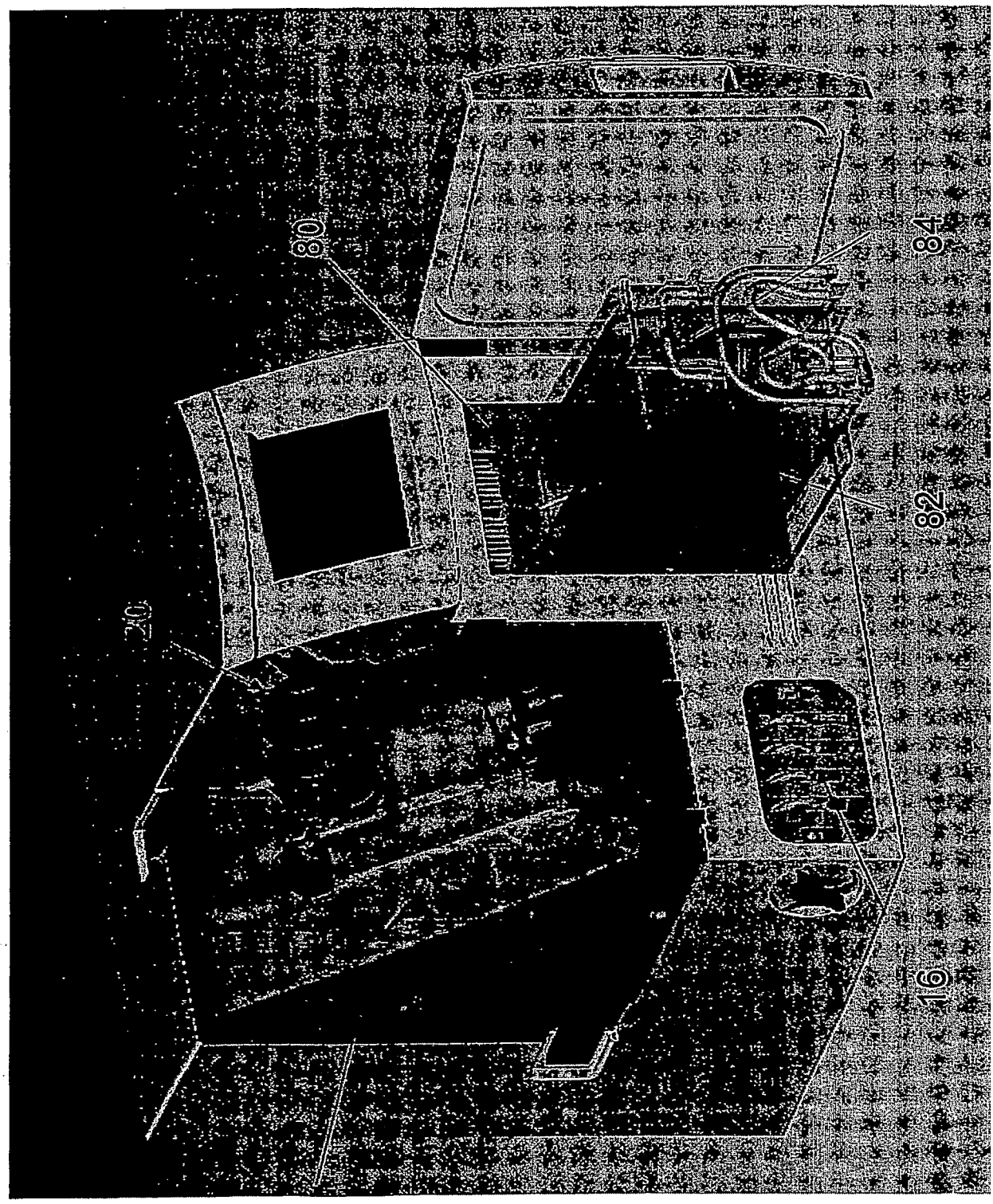
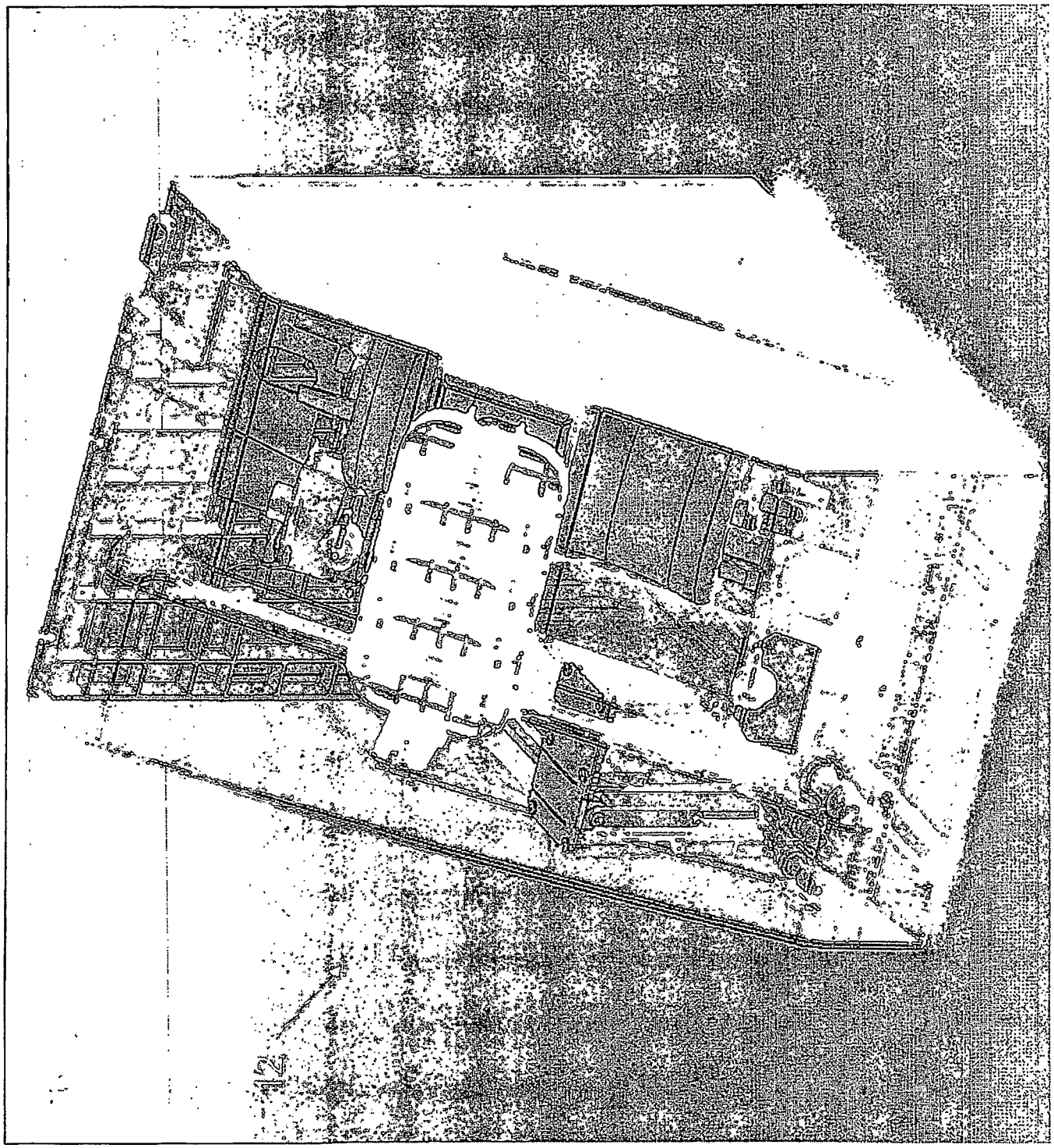


Fig. 2



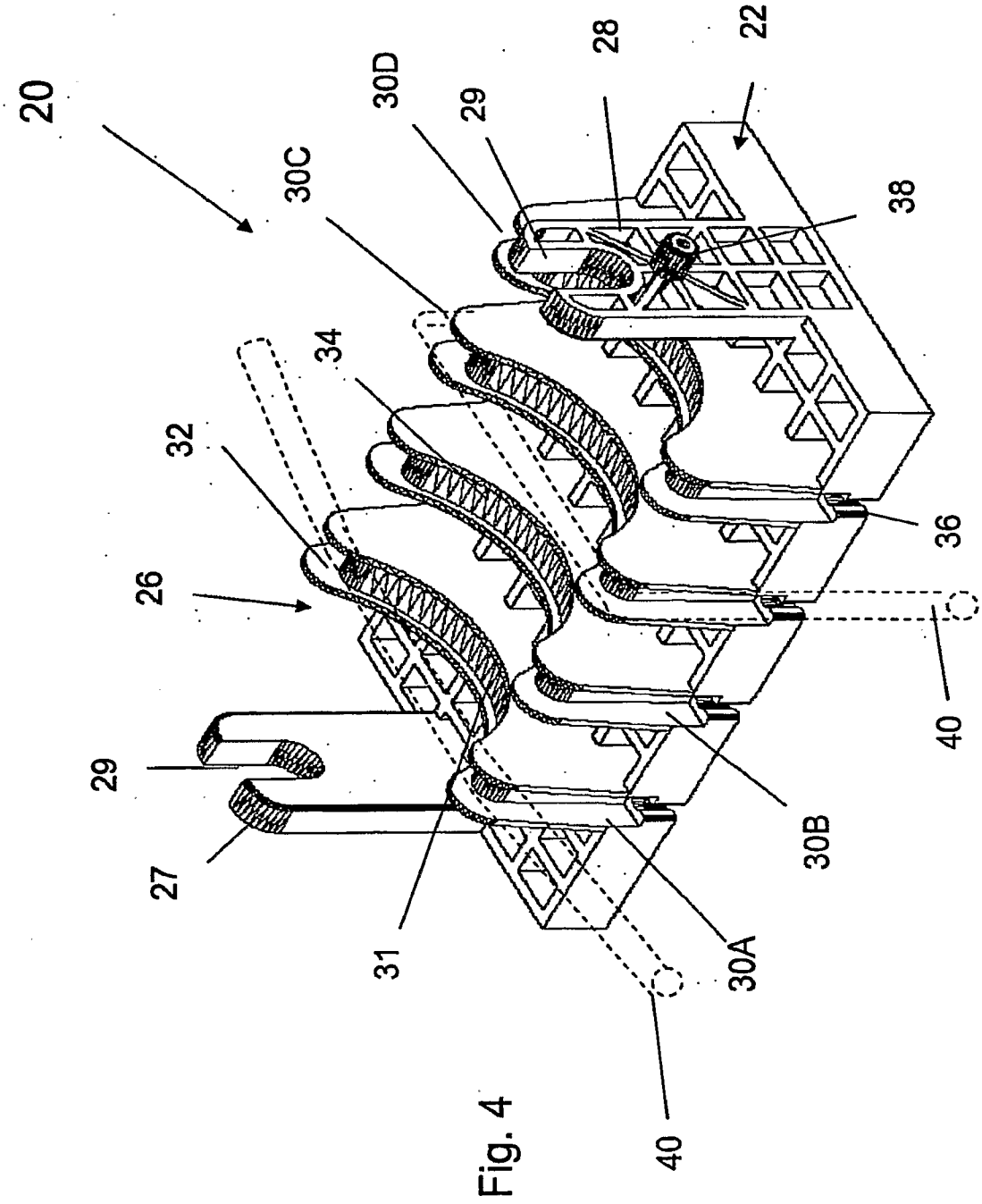


Fig. 4

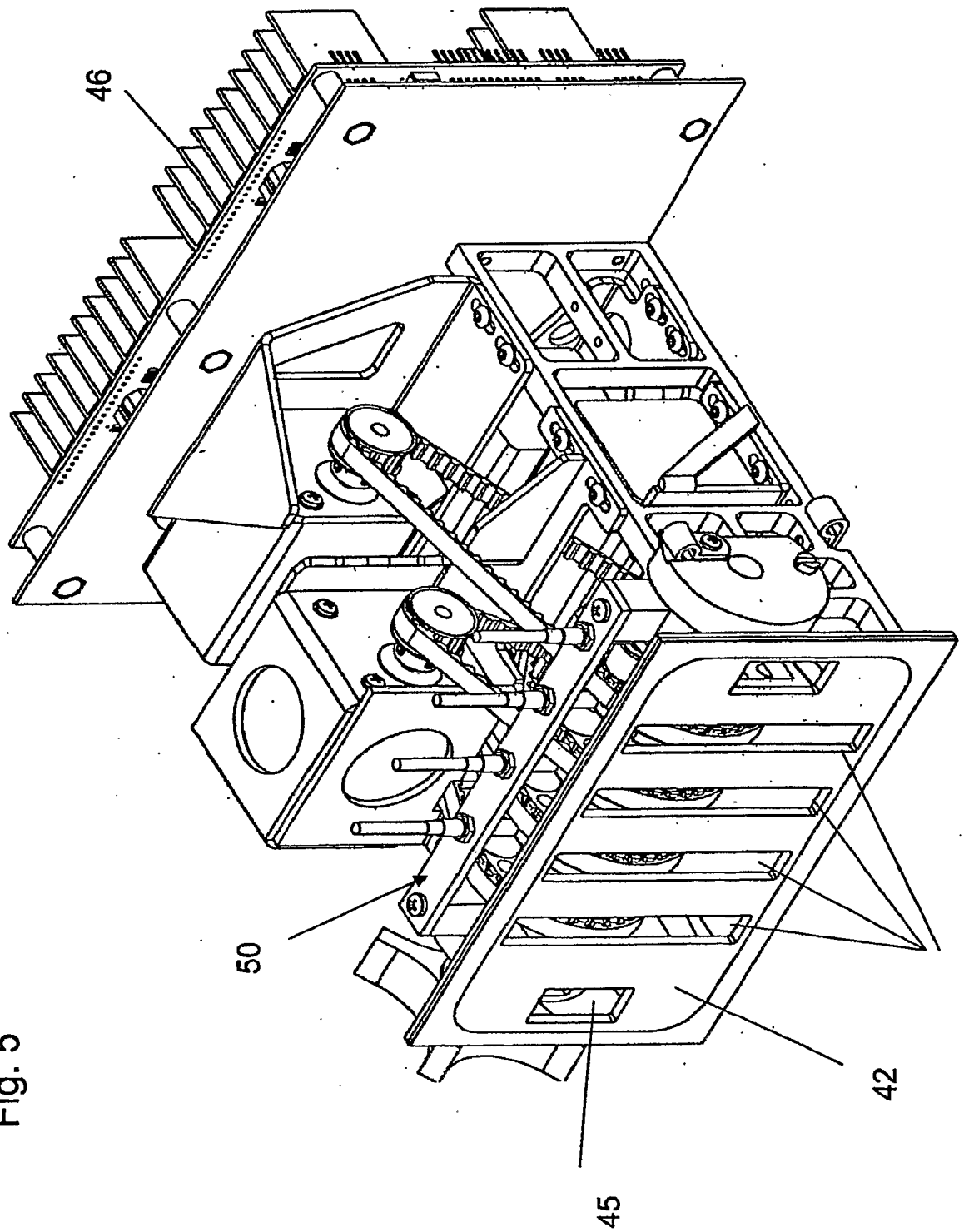
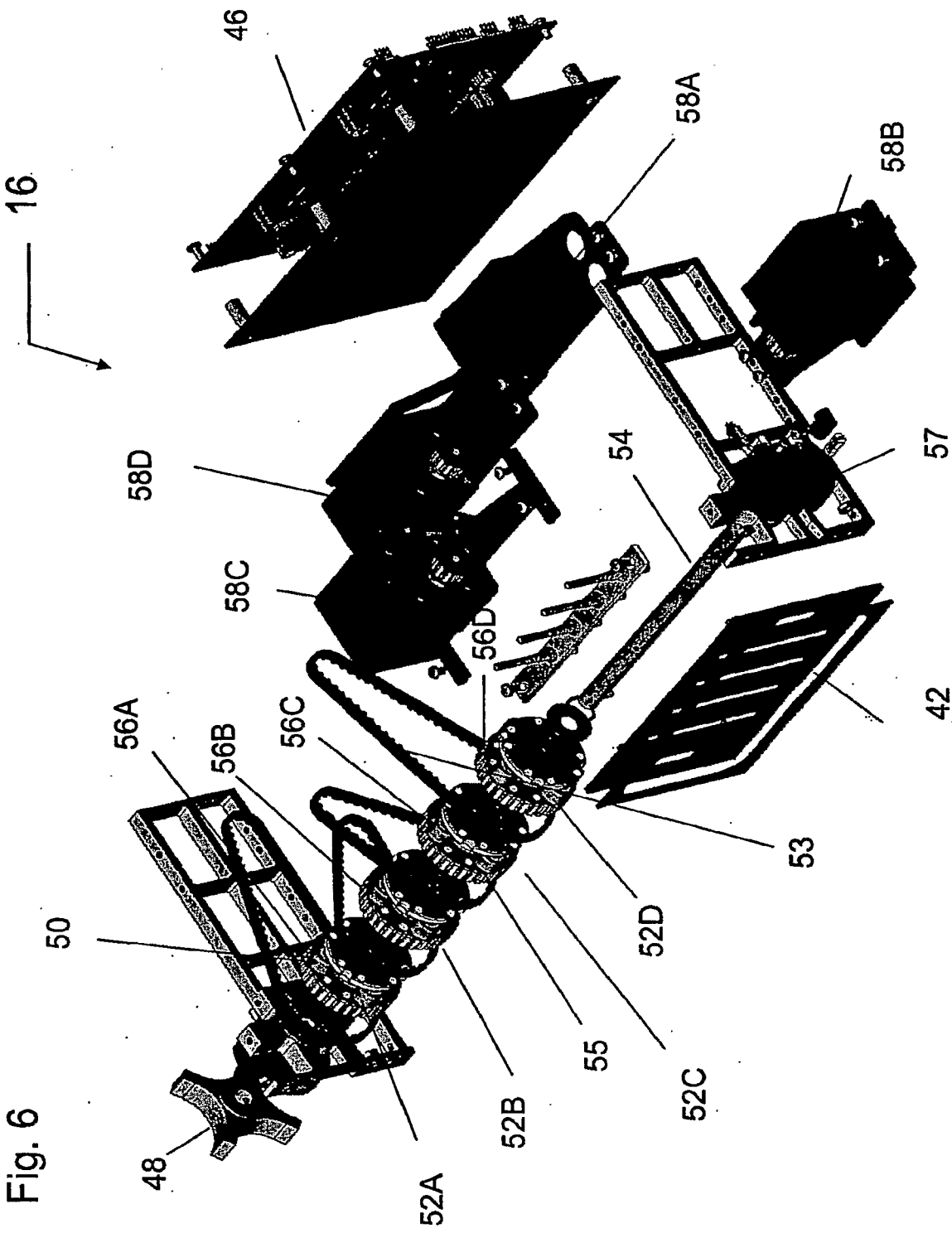


Fig. 5


16
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58C
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52C
52B
48
52A

Fig. 6

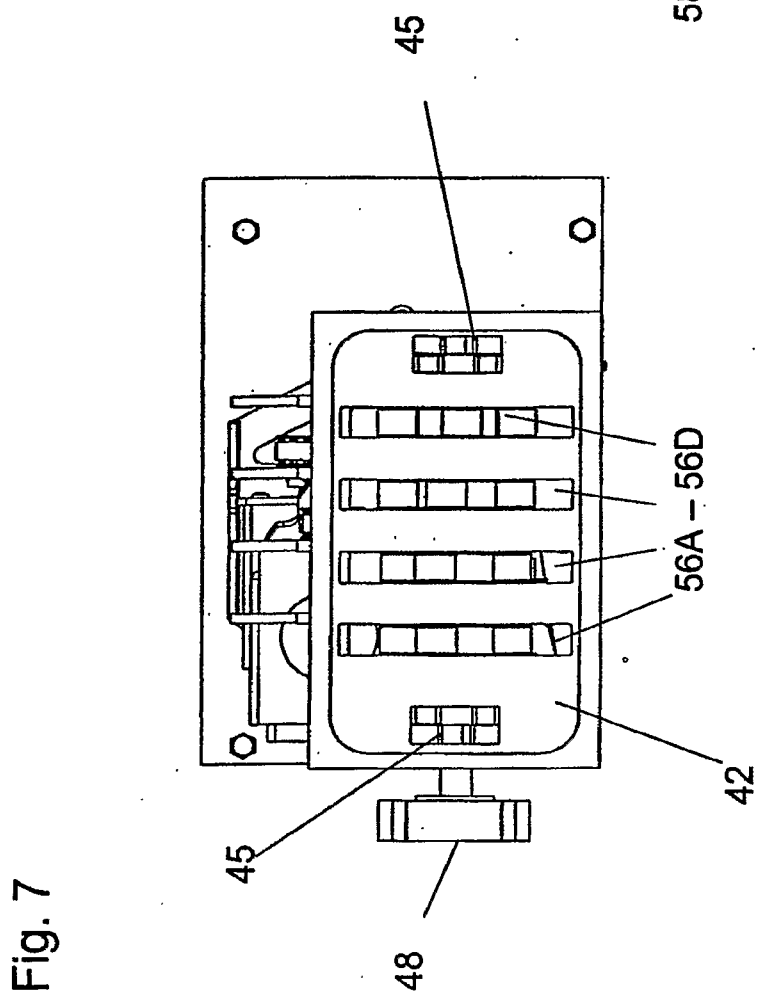


Fig. 7

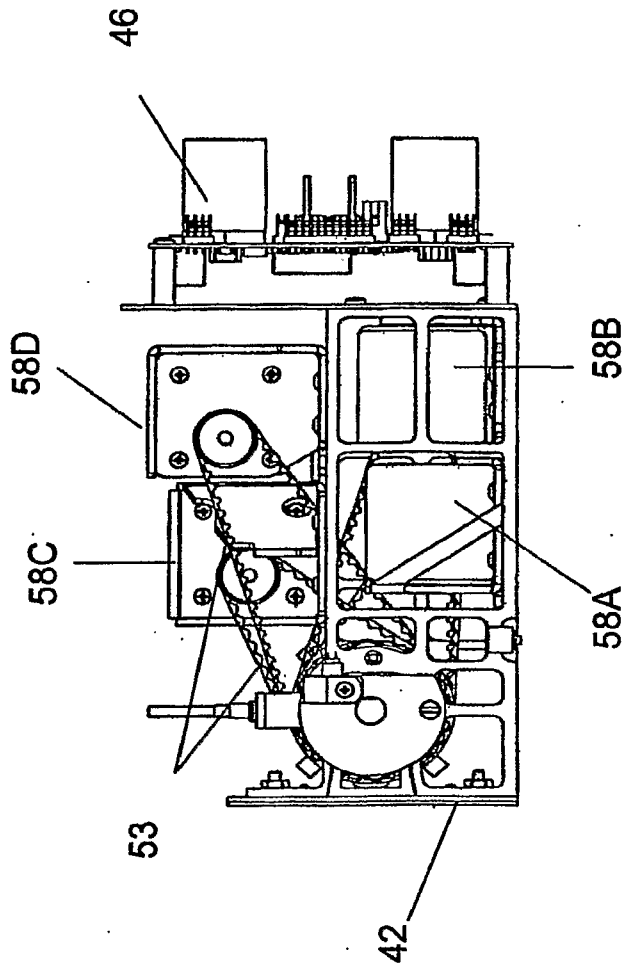


Fig. 8

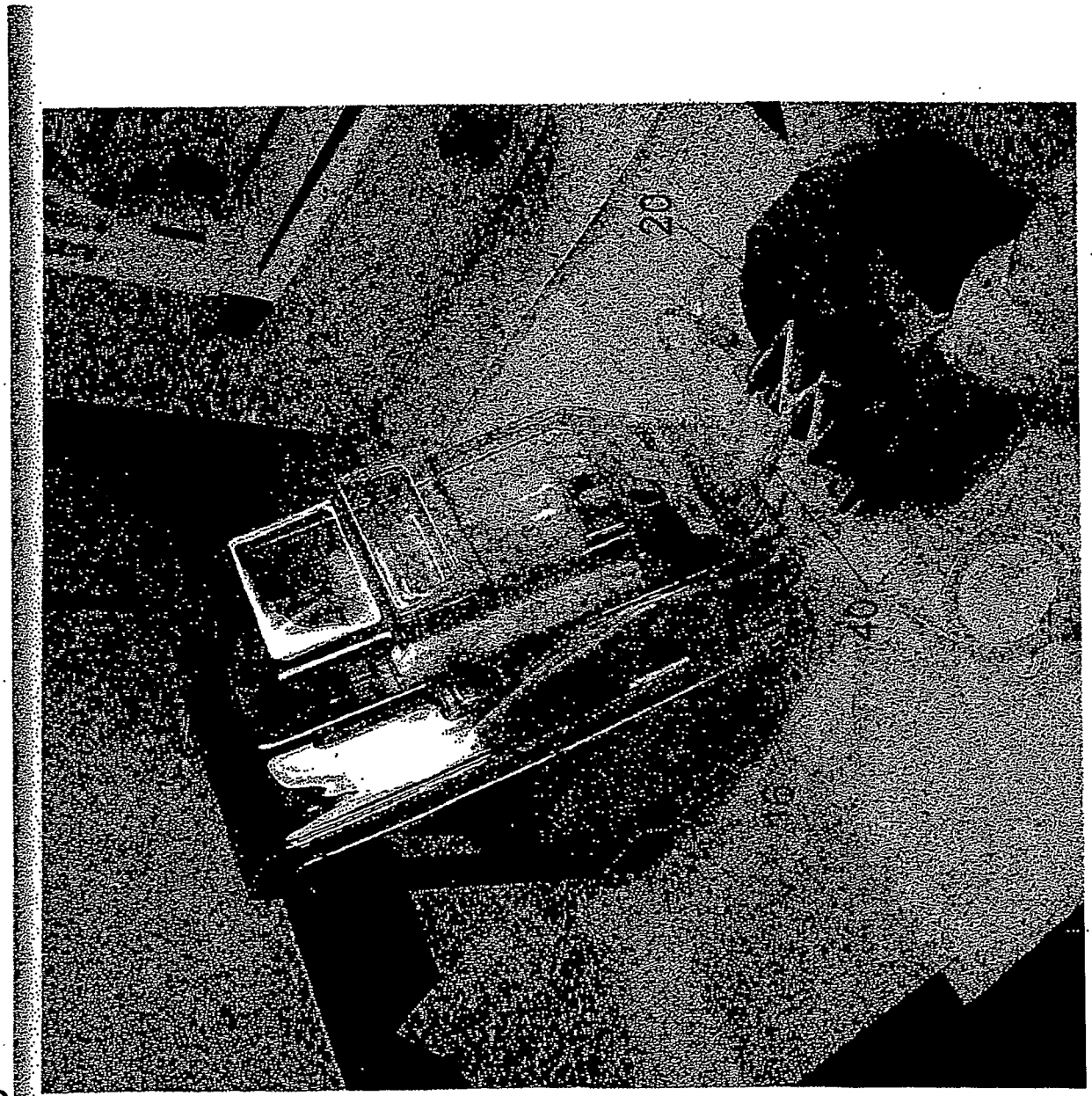
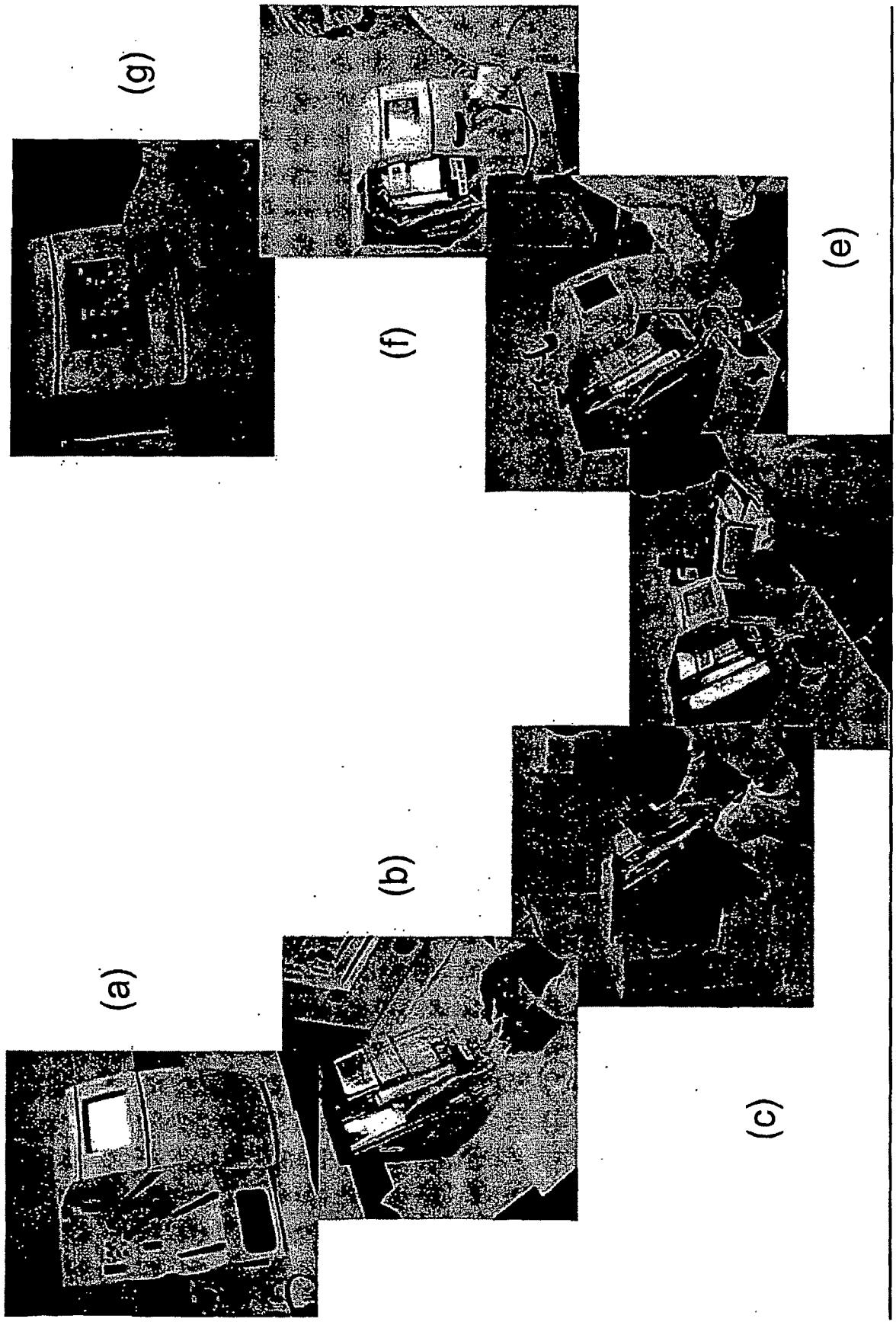


FIG. 9

Fig. 10



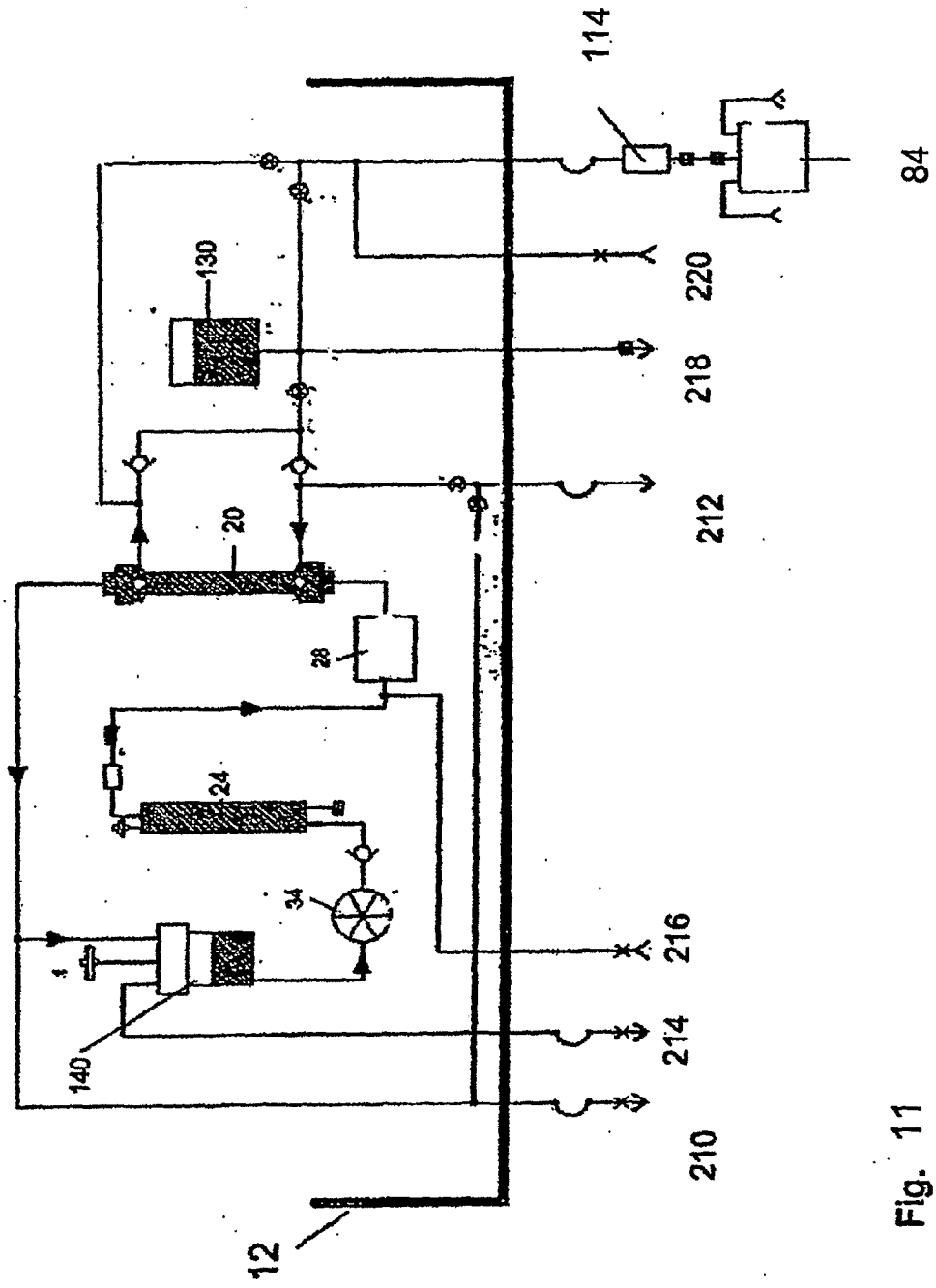


Fig. 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/012054

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12M3/00 F04B43/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12M F04B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 02/087662 A (NEXELL THERAPEUTICS INC [US]; JOHNSON CRAIG [US]; JULIAR RENA [US]; NO) 7 November 2002 (2002-11-07) page 1, line 15 - line 20 page 4, line 9 - line 22 page 6, line 21 - line 30 figures 1,2A	1-20
Y	EP 1 400 691 A2 (ISMATEC SA LABORATORIUMSTECHNI [CH]) 24 March 2004 (2004-03-24) claims 1-9 figures 1-4	1-20
A	EP 0 164 020 A1 (ISCO INC [US]) 11 December 1985 (1985-12-11) claims 1-7 figures 1-3	1-20

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 September 2007	25/09/2007

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cubas Alcaraz, Jose

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/012054

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	US 4 604 038 A (BELEW ROBERT R [US]) 5 August 1986 (1986-08-05) column 2, line 60 - column 3, line 34 column 4, line 24 - line 55 figures 1,5,6	1-20
A	WO 2005/031167 A (ISMATEC SA LABORATORIUMSTECHNI [CH]; MICHELS STEPHAN [CH]; FAESSLER RE) 7 April 2005 (2005-04-07) claim 1; figure 1	1-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2007/012054

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