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(54) **SYSTEMS AND METHODS FOR IDENTIFYING AND RECOVERING RARE BIOLOGICAL CELLS FROM A SAMPLE**

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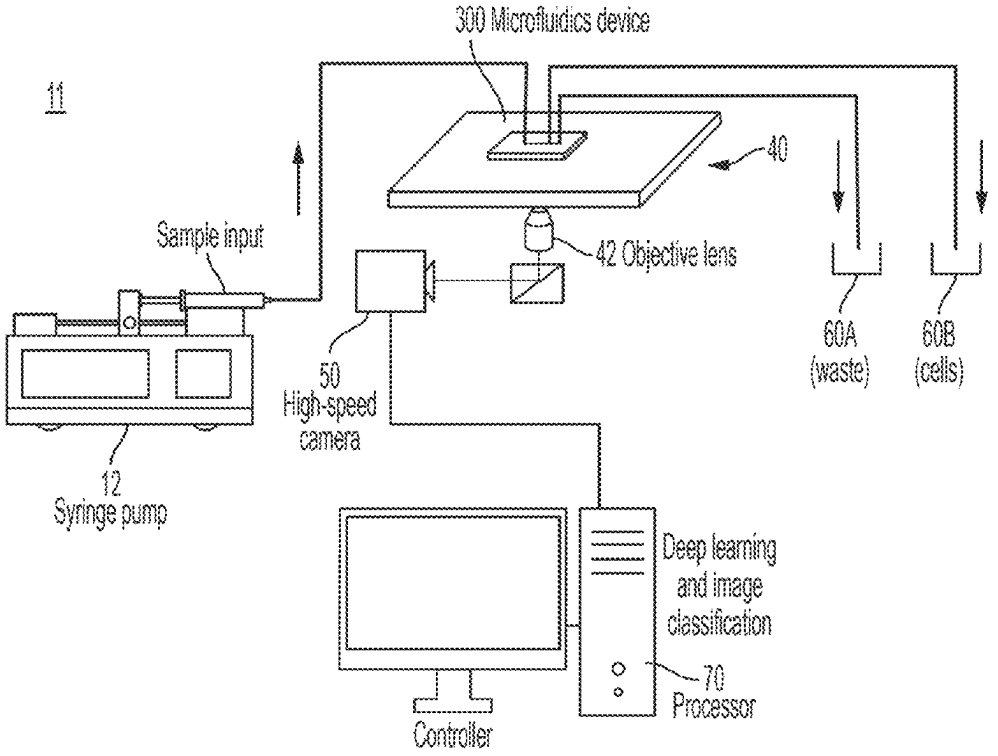
(57) **ABSTRACT**

Biological cells can be identified and recovered from a sample by pumping the sample through a flow channel so that the sample flows through the flow channel with a non-turbulent flow (e.g., a laminar flow). A plurality of images of the sample are captured as the sample passes through the flow channel. These images are analyzed to determine which portions of the sample include a target biological cell. Portions of the sample that were determined to include a target biological cell are routed into at least one first container, and portions of the sample that were not determined to include a target biological cell are routed to at least one second destination (e.g., one or more second containers).



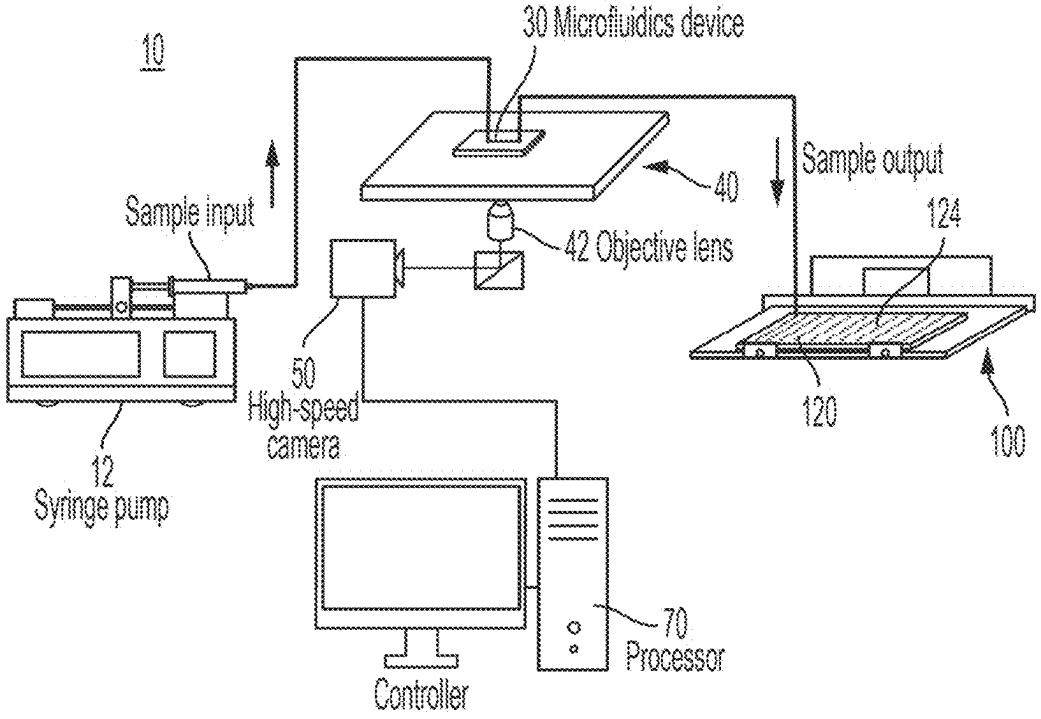


FIG. 1

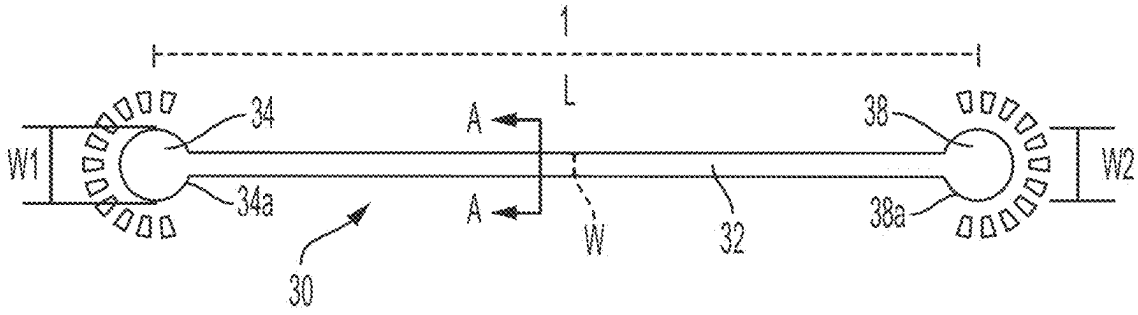


FIG. 2

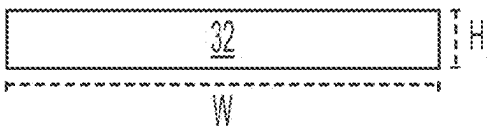


FIG. 3

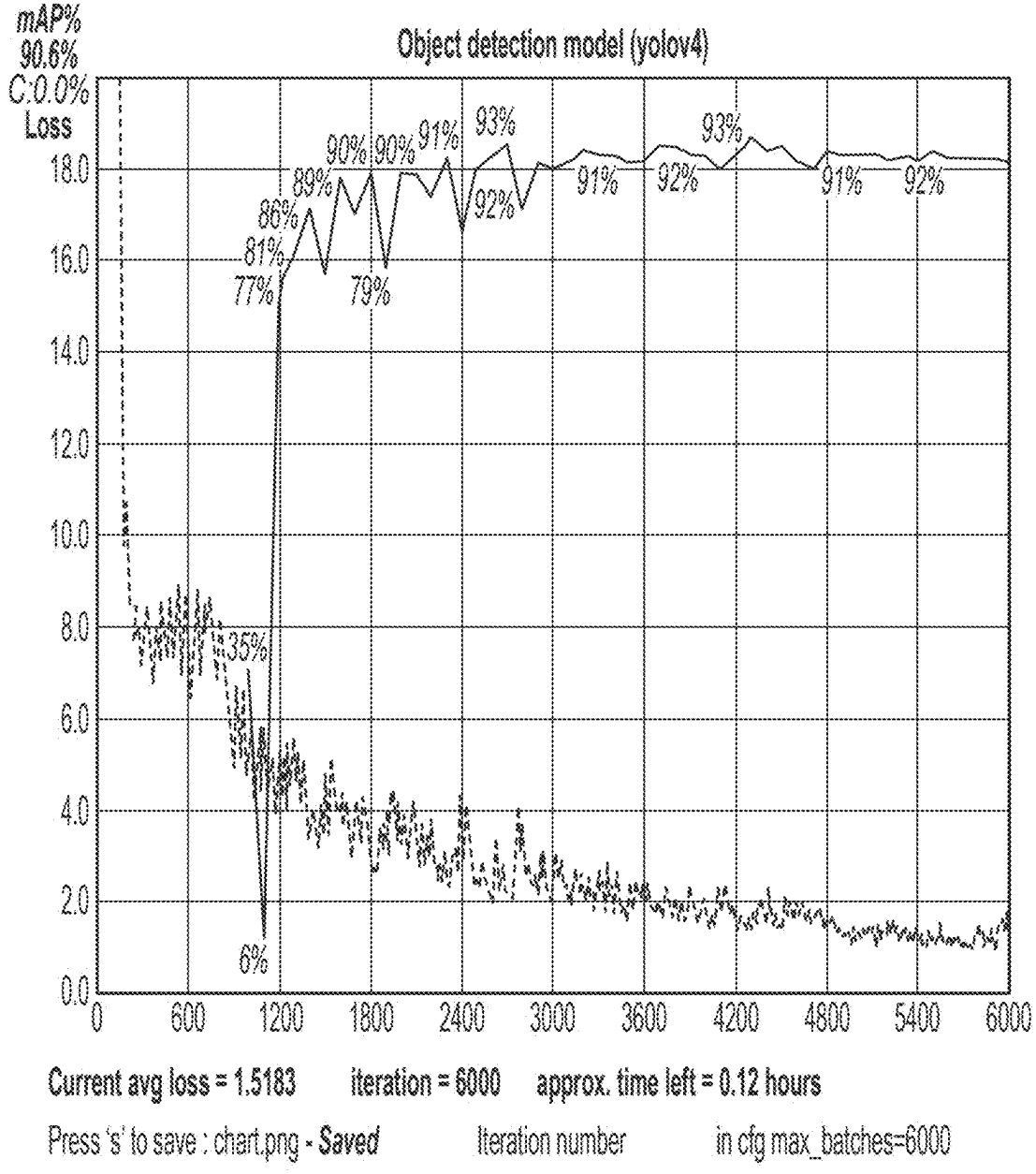

mAP%
90.6%
C:0.0%
Loss
Object detection model (yolov4)
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0
600
1200
1800
2400
3000
3600
4200
4800
5400
6000
77%
81%
86%
89%
90%
90%
91%
92%
79%
91%
92%
93%
91%
92%
93%
91%
92%
35%
6%
Current avg loss = 1.5183 iteration = 6000 approx. time left = 0.12 hours
Press 's' to save : chart.png - Saved Iteration number in cfg max_batches=6000

FIG. 4

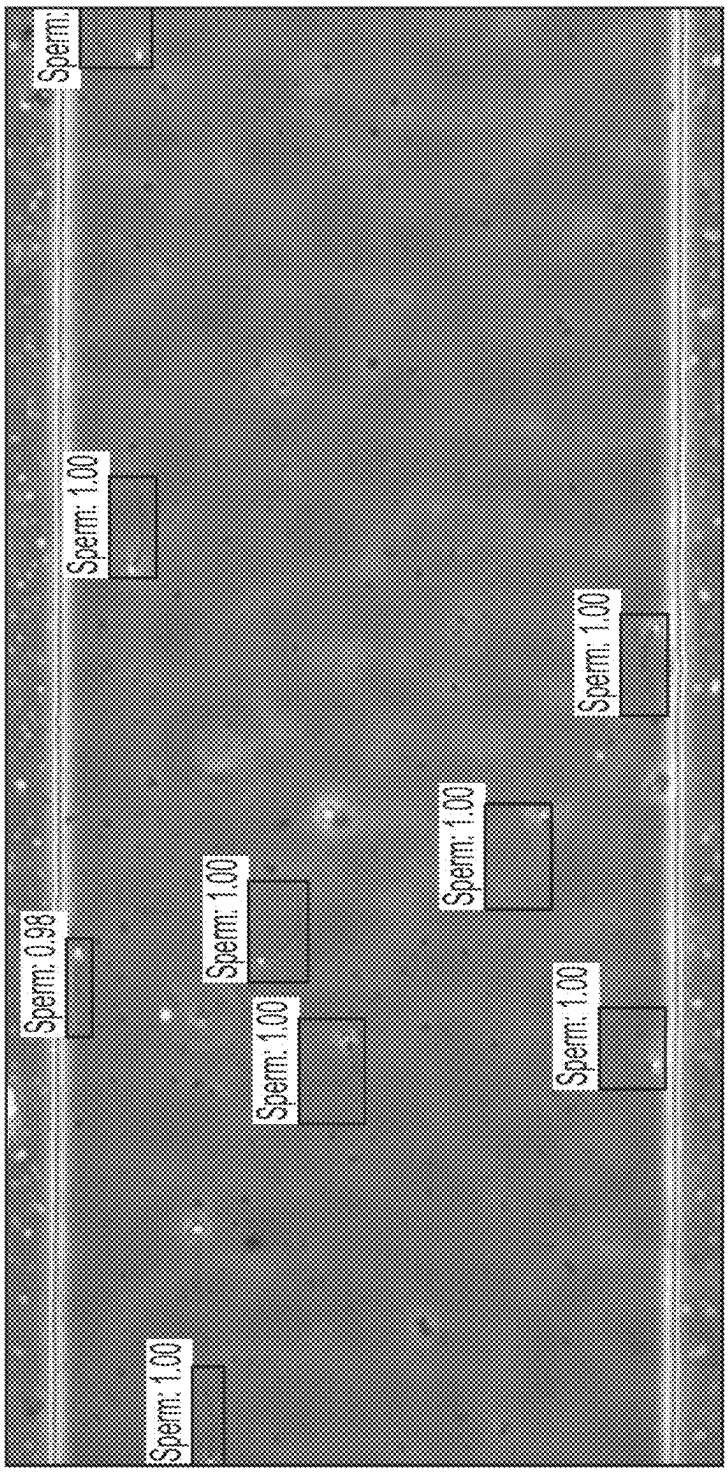

Sperm:
Sperm: 1.00
Sperm: 0.99
Sperm: 1.00
Sperm: 1.00
Sperm: 1.00
Sperm: 1.00
Sperm: 1.00
Sperm: 1.00

FIG. 5

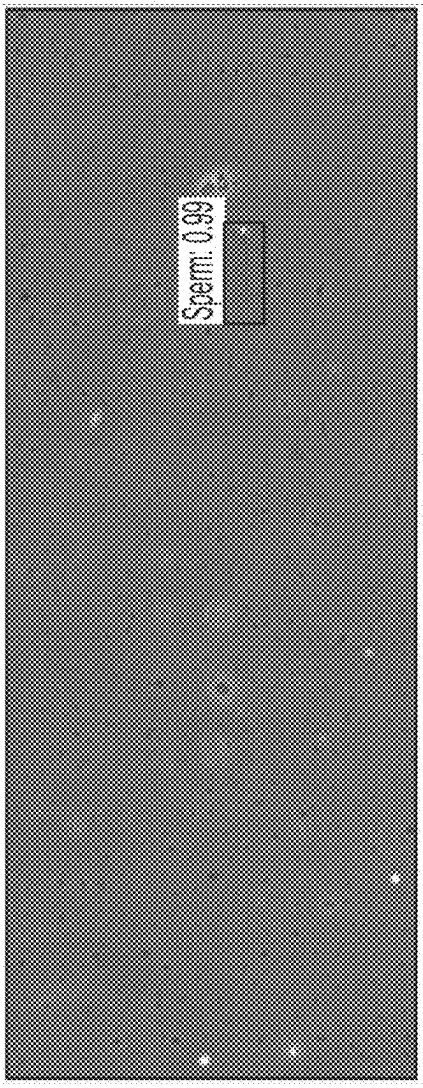

Sperm: 0.99
Sperm probability: 99%

FIG. 6

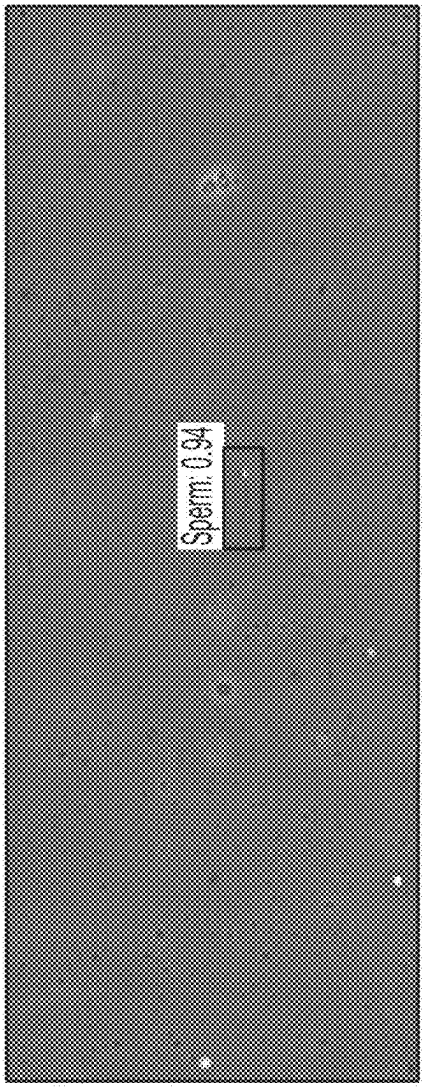

Sperm: 0.94
Sperm probability: 94%

FIG. 7

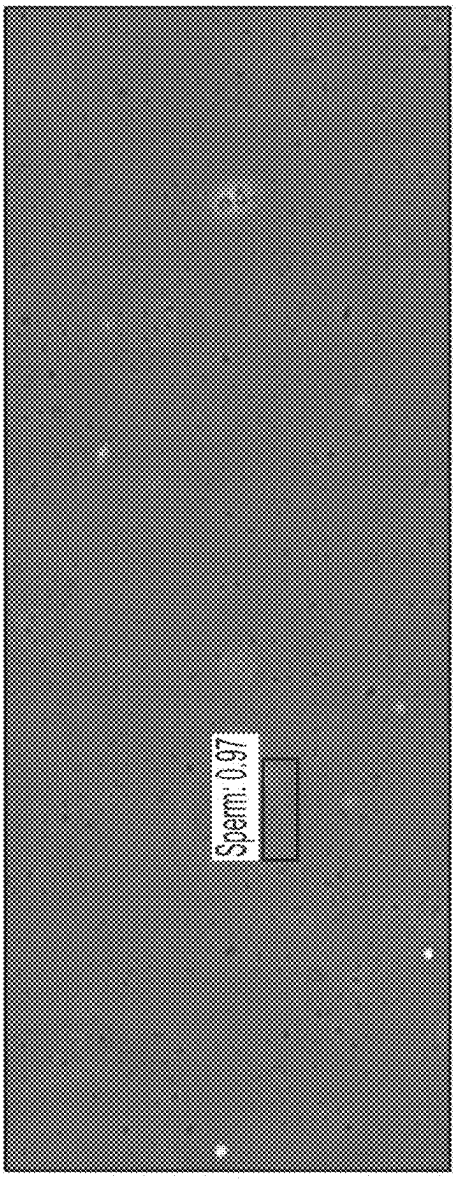

Sperm: 0.97
Sperm probability: 97%

FIG. 8

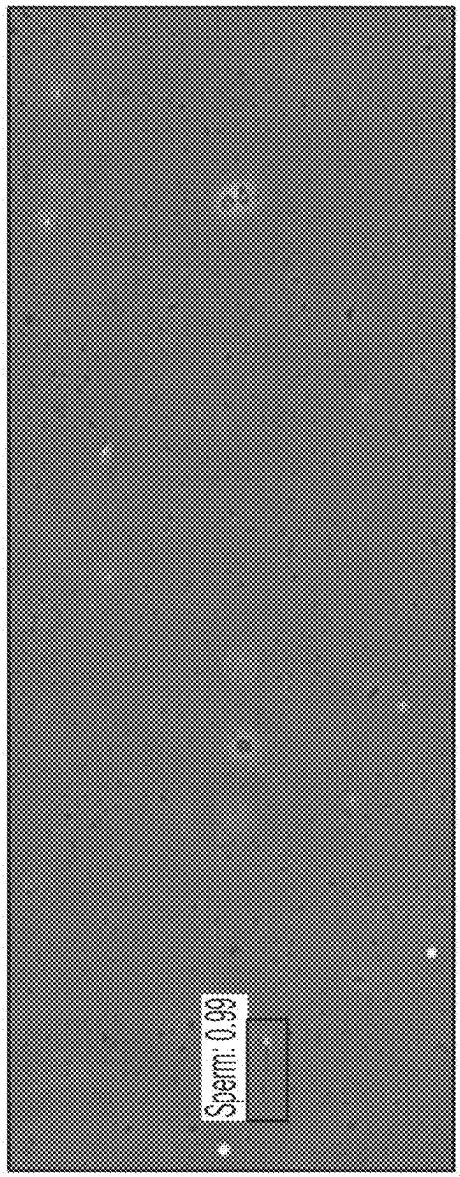

Sperm: 0.99
Sperm probability: 99%

FIG. 9

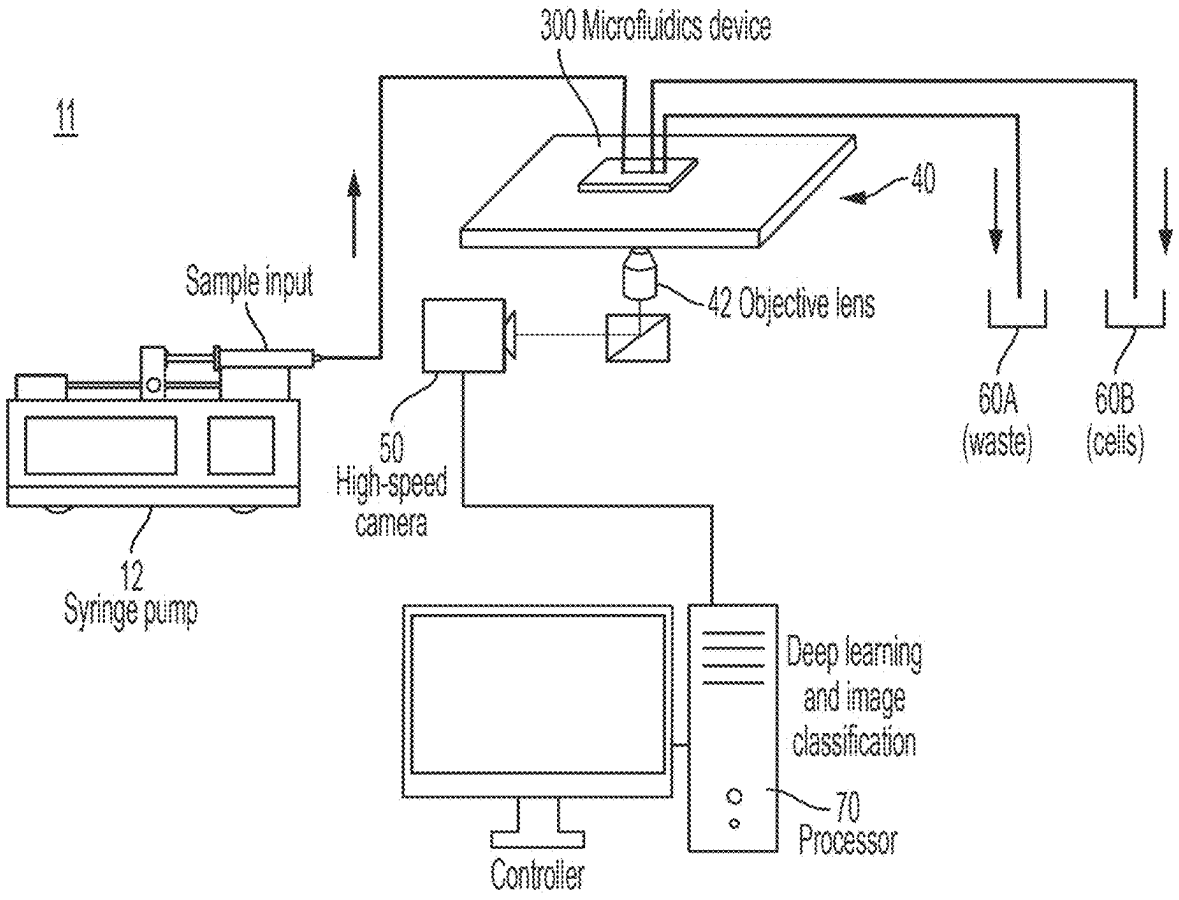

300 Microfluidics device
11
40
Sample input
42 Objective lens
60A
(waste)
60B
(cells)
50
High-speed
camera
12
Syringe pump
Deep learning
and image
classification
70
Processor
Controller

FIG. 10

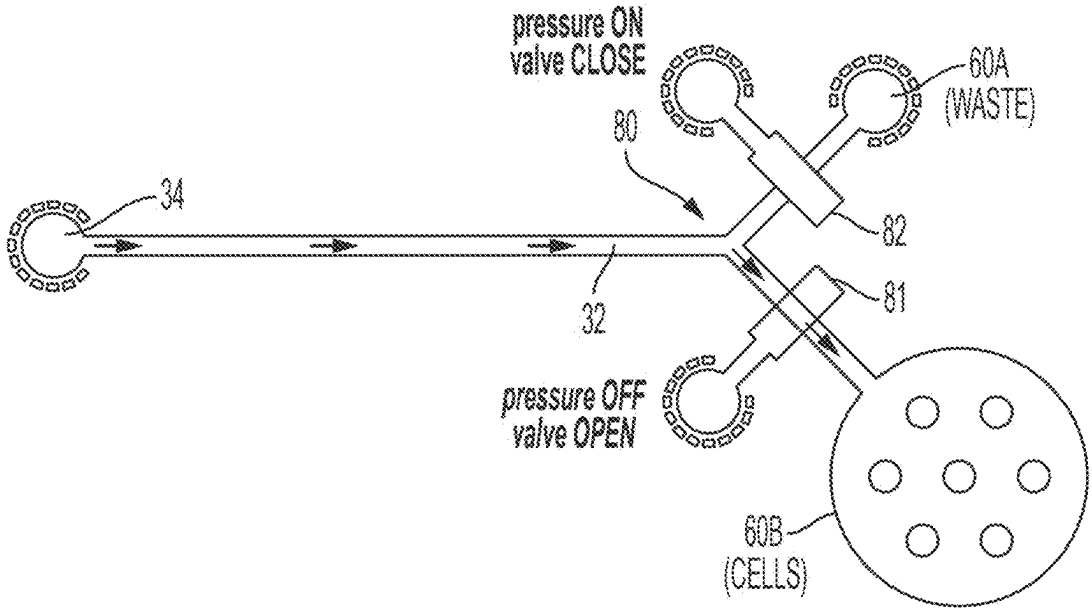

pressure ON
valve CLOSE
60A
(WASTE)
80
34
82
32
81
pressure OFF
valve OPEN
60B
(CELLS)

FIG. 11

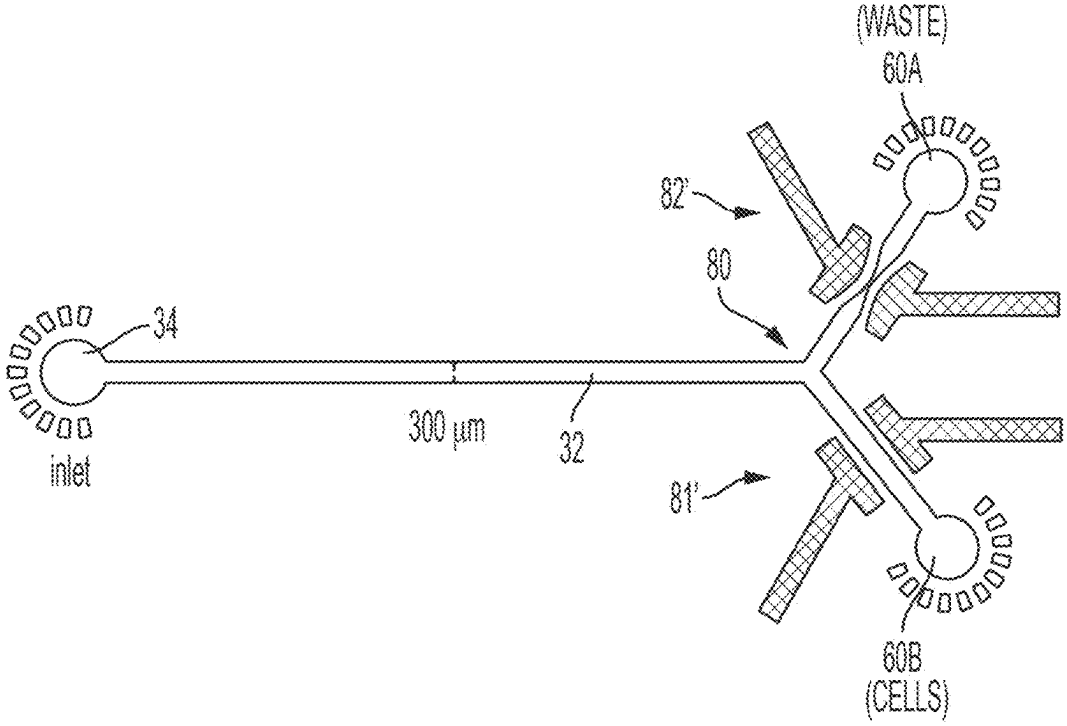

(WASTE)
60A
82'
80
34
300 µm
32
inlet
81'
60B
(CELLS)

FIG. 12

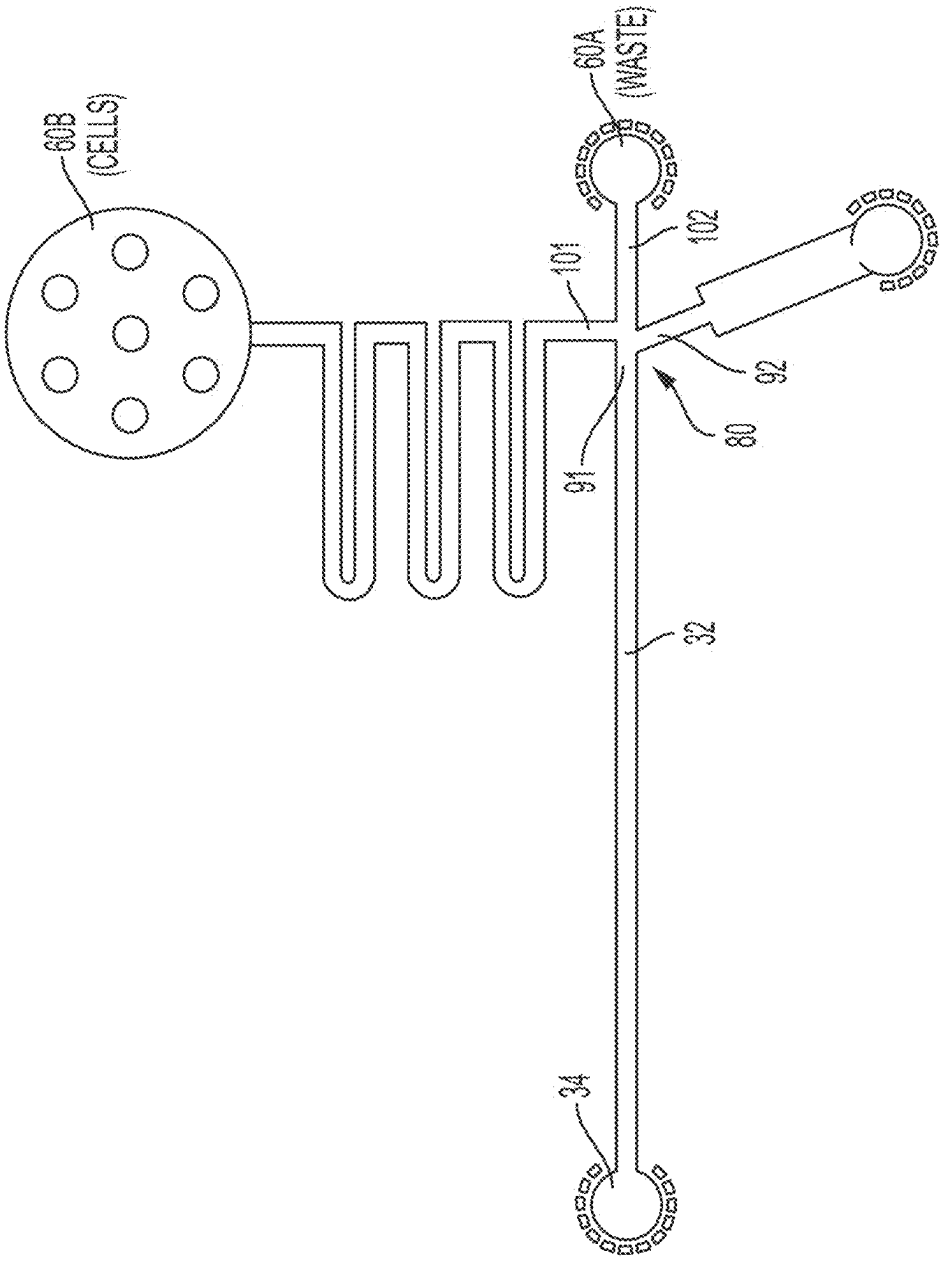


FIG. 13

SYSTEMS AND METHODS FOR IDENTIFYING AND RECOVERING RARE BIOLOGICAL CELLS FROM A SAMPLE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 19/036,372, filed Jan. 24, 2025, which is a continuation-in-part of International Application No. PCT/US2023/028843, filed Jul. 27, 2023, which claims the benefit of U.S. Provisional Application 63/392,628, filed Jul. 27, 2022, each of which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] A normal sperm count is between 15 million to more than 200 million sperm per ml of semen. Below those levels, as long as the semen contains a significant amount of sperm (e.g., around 1 million sperm per ml), many techniques exist for extracting sperm for in vitro fertilization. But some men have extremely low sperm counts, e.g., less than 10 sperm cells per ml. Identification and recovery of sperm cells in semen samples from these men and in testicular biopsy samples has heretofore been challenging because the semen sample includes very few sperm cells but a high number of other cells and debris. Identification and recovery of sperm cells for use in fertilization is also difficult because, in order to use the sperm cells in subsequent procedures, the identification and recovery processes must not damage the sperm cells. The aforementioned challenges also apply to identifying and recovering other types of rare biological cells such as cancer cells, fetal cells, and placenta cells.

[0003] Existing cell identification and sorting technologies do not provide any good solutions for the problems described above. For example, a fluorescence-activated cell sorting (FACS) sorting approach is impractical for sorting sperm cells and other rare cells due to lack of specific monoclonal antibodies against the cells. Further, the high-pressure system used in a FACS machine could potentially damage the cells, and would therefore be detrimental to the use of the cells for any sensitive biological applications. In addition, FACS requires at least 10,000 cells. Other sorting approaches, such as magnetic-activated cell sorting (MACS) based separation, are unsuitable for use with sperm cells that are present in very low numbers or other rare cells because these approaches lack sensitivity and work only at high cell concentration ranges.

SUMMARY OF THE INVENTION

[0004] One aspect of this application is directed to a first apparatus for identifying and recovering biological cells from a sample. The first apparatus comprises a fluid flow conduit, a pump, a camera, a stage, and a processor. The fluid-flow conduit has an inlet and an outlet, with a flow channel disposed between the inlet and the outlet. The inlet and the flow channel are configured so that the sample flows through the flow channel with a non-turbulent flow. The pump is configured to pump the sample into the inlet so that the sample flows through the flow channel with a non-turbulent flow and exits the outlet, and so that respective portions of the sample are dispensed from the outlet at respective times. The camera is positioned to capture a plurality of images of the sample as the sample passes through the flow channel. The stage is configured to move a plate that includes a plurality of microwells to respective positions at which respective portions of the sample flow out of the outlet and into respective ones of the plurality of microwells. And the processor is configured to analyze the plurality of images to detect which of the plurality of images include a target biological cell, and keep track of which microwells have been filled with a target biological cell.

[0005] In some embodiments of the first apparatus, the inlet and the flow channel are configured so that the sample flows through the flow channel with a laminar flow, and the pump is configured to pump the sample into the inlet so that the sample flows through the flow channel with a laminar flow.

[0006] In some embodiments of the first apparatus, the flow channel has a height between 10 and 50 μm, and a width between 100 and 400 μm. In some embodiments of the first apparatus, the flow channel has a height between 15 and 25 μm. In some embodiments of the first apparatus, a flow rate of the sample is between 800 and 2000 μL/hr. In some embodiments of the first apparatus, a width of the inlet is greater than a width of the flow channel, and the inlet includes a conical transition to the flow channel.

[0007] In some embodiments of the first apparatus, the processor keeps track of which microwells have been filled with the target biological cell by recording data corresponding to the plurality of images.

[0008] In some embodiments of the first apparatus, the camera is positioned and focused to capture images of a vertically central portion of the flow channel having a height between 2 and 8 μm. In some embodiments of the first apparatus, the camera is positioned and focused to capture images of a vertically central portion of the flow channel having a height between 4 and 6 μm.

[0009] In some embodiments of the first apparatus, the processor is further configured to analyze the plurality of images using an object detection model. In some embodiments of the first apparatus, the processor is further configured to analyze the plurality of images using a YOLO object detection model. In some embodiments of the first apparatus, the target biological cell is any one of a sperm cell, a cancer cell, a fetal cell, and a placenta cell. In some embodiments of the first apparatus, the camera is further configured to capture the plurality of images at a rate of at least 100 frames per second.

[0010] Some embodiments of the first apparatus further comprise a controller configured to issue commands to the stage to cause the stage to move the plate to the respective positions.

[0011] Another aspect of this application is directed to a first method of identifying and recovering biological cells from a sample. The first method comprises pumping a sample through a flow channel so that the sample flows through the flow channel with a non-turbulent flow; capturing a plurality of images of the sample as the sample passes through the flow channel; analyzing the plurality of images to detect which of the plurality of images include a target biological cell; moving a plate including a plurality of microwells to respective positions at which respective portions of the sample flow out of an outlet of the flow channel and into respective ones of the plurality of microwells; and keeping track of which microwells have been filled with a target biological cell.

[0012] In some instances of the first method, the sample flows through the flow channel with a laminar flow. In some instances of the first method, the sample flows through the flow channel at a flow rate between 800 and 2000 μL/hr. In some instances of the first method, the keeping track of which microwells have been filled with the target biological cell includes recording data corresponding to the plurality of images.

[0013] In some instances of the first method, the capturing of the plurality of images includes capturing the plurality of images using a camera focused on a vertically central portion of the flow channel having a height between 2 and 8 μm. In some instances of the first method, the capturing of the plurality of images includes capturing the plurality of images using a camera focused on a vertically central portion of the flow channel having a height between 4 and 6 μm.

[0014] In some instances of the first method, the analyzing of the plurality of images is implemented using an object detection model. In some instances of the first method, the analyzing of the plurality of images is implemented using a YOLO object detection model. In some instances of the first method, the target biological cell detected in the image is any one of a sperm cell, a cancer cell, a fetal cell, and a placenta cell. In some instances of the first method, the capturing of the plurality of images includes capturing the plurality of images at a rate of at least 100 frames per second.

[0015] In some instances of the first method, portions of the sample that correspond to images that include a target biological cell are deposited into one well and portions of the sample that correspond to images that do not include a target biological cell are deposited into a different well.

[0016] Another aspect of this application is directed to a second method of identifying and recovering biological cells from a sample. The second method comprises pumping the sample through a flow channel so that the sample flows through the flow channel with a non-turbulent flow; capturing a plurality of images of the sample as the sample passes through the flow channel; and analyzing the plurality of images to determine which portions of the sample include a target biological cell. Portions of the sample that were determined to include a target biological cell are routed into at least one first container, and portions of the sample that were not determined to include a target biological cell are routed to at least one second destination.

[0017] In some instances of the second method, the sample flows through the flow channel with a laminar flow. In some instances of the second method, the sample flows through the flow channel at a flow rate between 800 and 2000 μL/hr. In some instances of the second method, the capturing of the plurality of images includes capturing the plurality of images using a camera focused on a vertically central portion of the flow channel having a height between 2 and 8 μm. In some instances of the second method, the capturing of the plurality of images includes capturing the plurality of images using a camera focused on a vertically central portion of the flow channel having a height between 4 and 6 μm.

[0018] In some instances of the second method, the analyzing of the plurality of images is implemented using an object detection model. In some instances of the second method, the analyzing of the plurality of images is implemented using a YOLO object detection model. In some instances of the second method, the target biological cell is any one of a sperm cell, a cancer cell, a fetal cell, and a placenta cell. In some instances of the second method, the capturing of the plurality of images includes capturing the plurality of images at a rate of at least 100 frames per second.

[0019] In some instances of the second method, portions of the sample that were determined to include a target biological cell are deposited into a single first container, and portions of the sample that were not determined to include a target biological cell are deposited into a single second container.

[0020] Another aspect of this application is directed to a second apparatus for identifying and recovering biological cells from a sample. The second apparatus comprises a flow channel, a pump, a camera, a processor, and routing means. The pump is configured to pump the sample through the flow channel, and the flow channel and the pump are configured so that the sample flows through the flow channel with a non-turbulent flow. The camera is configured to capture a plurality of images of the sample as the sample passes through the flow channel. The processor is configured to analyze the plurality of images to determine which portions of the sample include a target biological cell. And the routing means is configured for (a) routing portions of the sample that were determined to include a target biological cell into at least one first container, and (b) routing portions of the sample that were not determined to include a target biological cell to at least one second destination.

[0021] In some embodiments of the second apparatus, the flow channel and the pump are configured so that the sample flows through the flow channel with a laminar flow. In some embodiments of the second apparatus, the flow channel and the pump are configured so that the sample flows through the flow channel at a flow rate between 800 and 2000 μL/hr. In some embodiments of the second apparatus, the camera is focused on a vertically central portion of the flow channel having a height between 2 and 8 μm. In some embodiments of the second apparatus, the camera is focused on a vertically central portion of the flow channel having a height between 4 and 6 μm.

[0022] In some embodiments of the second apparatus, the processor analyzes the plurality of images using an object detection model. In some embodiments of the second apparatus, the processor analyzes the plurality of images using a YOLO object detection model. In some embodiments of the second apparatus, the target biological cell is any one of a sperm cell, a cancer cell, a fetal cell, and a placenta cell. In some embodiments of the second apparatus, the camera captures the plurality of images at a rate of at least 100 frames per second.

[0023] In some embodiments of the second apparatus, the routing means is configured so that portions of the sample that were determined to include a target biological cell are deposited into a single first container, and portions of the sample that were not determined to include a target biological cell are deposited into a single second container.

[0024] Another aspect of this application is directed to a third apparatus for identifying and recovering biological cells from a sample. The third apparatus comprises a fluid flow conduit, a pump, a camera, at least one valve, and a processor. The fluid-flow conduit has an inlet and an outlet, with a flow channel disposed between the inlet and the outlet. The inlet and the flow channel are configured so that

the sample flows through the flow channel with a non-turbulent flow. The pump is configured to pump the sample into the inlet so that the sample flows through the flow channel with a non-turbulent flow and exits the outlet, and so that respective portions of the sample exit the outlet at respective times. The camera is positioned to capture a plurality of images of the sample as the sample passes through the flow channel. The at least one valve is configured to route a plurality of first portions of the sample that exit the outlet into at least one first container and to route a plurality of second portions of the sample that exit the outlet to at least one second destination. The processor is configured to analyze the plurality of images to determine which portions of the sample include a target biological cell. And the processor is also configured to, based on the analyzing, control the at least one valve so that (a) portions of the sample that exit the outlet and were determined to include a target biological cell are routed into the at least one first container and (b) portions of the sample that exit the outlet and were not determined to include a target biological cell are routed to the at least one second destination.

[0025] In some embodiments of the third apparatus, the inlet and the flow channel are configured so that the sample flows through the flow channel with a laminar flow, and the pump is configured to pump the sample into the inlet so that the sample flows through the flow channel with a laminar flow. In some embodiments of the third apparatus, the flow channel has a height between 10 and 50 μm, and a width between 100 and 400 μm. In some embodiments of the third apparatus, the flow channel has a height between 15 and 25 μm. In some embodiments of the third apparatus, a flow rate of the sample is between 800 and 2000 μL/hr.

[0026] In some embodiments of the third apparatus, a width of the inlet is greater than a width of the flow channel, and the inlet includes a conical transition to the flow channel. In some embodiments of the third apparatus, the camera is positioned and focused to capture images of a vertically central portion of the flow channel having a height between 2 and 8 μm. In some embodiments of the third apparatus, the camera is positioned and focused to capture images of a vertically central portion of the flow channel having a height between 4 and 6 μm.

[0027] In some embodiments of the third apparatus, the processor is further configured to analyze the plurality of images using an object detection model. In some embodiments of the third apparatus, the processor is further configured to analyze the plurality of images using a YOLO object detection model.

[0028] In some embodiments of the third apparatus, the target biological cell is any one of a sperm cell, a cancer cell, a fetal cell, and a placenta cell. In some embodiments of the third apparatus, the camera is further configured to capture the plurality of images at a rate of at least 100 frames per second.

[0029] In some embodiments of the third apparatus, the valve is configured to route a plurality of first portions of the sample that exit the outlet into a single first container and to route a plurality of second portions of the sample that exit the outlet to a single second destination. Optionally, in these embodiments, the single second destination can be a single second container.

[0030] In some embodiments of the third apparatus, the at least one valve comprises a plurality of PDMS quake valves. In some embodiments of the third apparatus, the at least one valve comprises a plurality of lateral deflection valves, and each of the lateral deflection valves is implemented using a single PDMS layer.

[0031] In some embodiments of the third apparatus, the at least one valve comprises a fluid-flow circuit having a main flow inlet, a first outlet, a second outlet, and a control flow inlet, and the fluid-flow circuit is configured so that (a) applying a control flow to the control flow inlet at a first flow rate causes fluid from the main flow inlet to flow out of the first outlet and (b) applying a control flow to the control flow inlet at a second flow rate causes fluid from the main flow inlet to flow out of the second outlet. Optionally, in these embodiments, the first flow rate is larger than the flow rate at the main flow inlet, and the second flow rate is smaller than the flow rate at the main flow inlet.

[0032] Other features and aspects will be apparent from the following detailed description, the drawings, and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] FIG. **1** is a diagram of a system for identifying and recovering biological cells that are present in a sample.

[0034] FIG. **2** is a plan view of a fluid-flow conduit of the system of FIG. **1**.

[0035] FIG. **3** is a cross-sectional view taken along line A-A of FIG. **2**.

[0036] FIG. **4** is a graph illustrating accuracy of the system of FIG. **1** in identifying sperm in a human semen sample using a YOLOv4 object detection model, according to an experimental embodiment.

[0037] FIG. **5** is an image processed by the system of FIG. **1** that includes sperm identified using the YOLOv4 object detection model, according to an experimental embodiment.

[0038] FIGS. **6-9** depict successive images of a test sample that were captured while a single sperm cell moved through a flow channel in an experiment.

[0039] FIG. **10** depicts another system for identifying and recovering target cells that are present in a sample.

[0040] FIGS. **11-13** depict three alternative approaches for routing different portions of the sample to different destinations in the FIG. **10** embodiment.

[0041] Throughout the drawings and the detailed description, the same reference numerals refer to the same elements. The drawings may not be to scale, and the relative size, proportions, and depiction of elements in the drawings may be exaggerated for clarity, illustration, and convenience.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0042] In view of the shortcomings of the prior art identified above, it would be desirable to provide a system and method that are capable of accurately and efficiently identifying and capturing sperm cells from samples that contain an extremely low number of sperm cells, without damaging those sperm cells, so that they can subsequently be used (e.g., for in vitro fertilization).

[0043] Ordinarily, if a semen sample that contains very few sperm cells (e.g., less than 100 sperm cells per ml) is pumped through a channel that is large enough for semen to flow through without clogging, conventional techniques will not be able to identify and capture the sperm cells. Two factors contribute to this difficulty: first, other cells and

debris in the semen can outnumber the target sperm cells by over 100,000:1. And second, the cross-section of the channel is so large that it is impossible to keep the entire cross-section of the channel in focus to identify the extremely rare target sperm cells for subsequent capture.

[0044] The embodiments described herein overcome this problem by, inter alia, making sure that non-turbulent flow (e.g., laminar flow) is maintained through the channel. When laminar flow is a maintained, the flow is slower near the walls of the channel and faster near the center of the channel. As a result, the majority of the sample ends up flowing through the center of the channel. Because the majority of the sample flows through the center of the channel, it becomes possible for a microscope to maintain focus on the entire cross-section of the center of the channel through which the majority of the sample flows. And when the entire cross-section of flow can be kept in focus, the system can spot the rare sperm cells in images captured using the microscope and a camera as they traverse the channel.

[0045] FIG. **1** illustrates an embodiment of a system **10** for identifying and recovering target cells present in a sample. The system **10** will be described in the context of identifying and recovering sperm cells present in a semen sample, but can also be used in a variety of other contexts.

[0046] The FIG. **1** embodiment includes a syringe pump **12**, a fluid-flow conduit **30** including a flow channel **32**, a camera **50**, a processor **70**, and a stage **100** supporting a microplate (or plate) **120**. As will be described later in more detail, the syringe pump **12** pumps a semen sample containing sperm cells through the fluid-flow conduit **30**, the camera **50** captures images of the semen sample and the sperm cells therein as the semen sample flows through a fluid channel **32** of the fluid-flow conduit **30**, and the processor **70** detects the sperm cells in the semen sample. The microplate **120** is positioned using the stage **100** to collect respective portions of the semen sample as the semen sample flows out of the fluid-flow conduit **30**.

[0047] To ensure that that the camera **50** is able to capture sharply focused images and the sample collection station properly captures portions of the semen sample, it is important for the semen sample to have a consistent, precise flow rate through the flow channel **32** of the fluid-flow conduit **30**. A semen sample will typically have a volume of 1 to 3 ml. In the illustrated embodiment, a syringe pump **12** is configured to pump the semen sample through the flow channel **32** of the fluid-flow conduit **30**. But in alternative embodiments, different types of pumps may be used. In some embodiments, the pump **12** pumps the semen sample through the flow channel **32** at a flow rate of 800 to 2000 μL/hr (e.g., 1000 μL/hr). The inventors have experimentally determined that flow rates in these ranges can advantageously process human semen samples quickly without causing image blurring. But in alternative embodiments, flow rates outside these ranges can also be used.

[0048] Referring to FIGS. **1-3**, the fluid-flow conduit **30** includes the flow channel **32**, an inlet **34** disposed at a first end of the flow channel **32**, and configured to receive the semen sample from the pump **12** and introduce the semen sample to the fluid flow channel **32**, and an outlet **38** disposed at a second end of the flow channel **32** and configured dispense the semen sample to the microplate **120**. The camera **50** is configured to capture images of the semen sample and the sperm cells therein as the semen sample flows through the flow channel **32**. Thus, the fluid-flow conduit **30** or at least the flow channel **32** should be made of a transparent material.

[0049] If the sperm cells in the semen sample are stacked or widely distributed through the cross-section of the flow channel **32** in the direction of the viewing axis of the camera **50**, the camera **50** will fail to image the majority of the sperm cells as they flow through the flow channel **32** and/or will capture unfocused images of the majority of the sperm cells. That is, such stacking or distribution of sperm cells will cause the majority of the sperm cells to be disposed outside of the depth of field (DOF) of the camera **50**. Further, if the semen sample forms clumps of semen that do not flow consistently in the flow channel **32**, the camera **50** cannot accurately image the sperm cells throughout the length of the flow channel **32**, thereby preventing the system **10** from tracking and recovering at least some of the sperm cells.

[0050] For example, referring to FIGS. **2** and **3**, if a height H (in the direction of the viewing axis of the camera **50**) of the flow channel **32** is too large, the aforementioned problem of stacking and wide distribution of the sperm cells will occur along the height H. If the height H is too small, the aforementioned problem of clumping of the semen sample will occur. Additionally, stacking of sperm cells along the height H or clumping of the semen may occur if a width W of the flow channel **32** is not sized in appropriate proportion to the height H.

[0051] Further, unless care is taken to ensure that the flow of the sample through the flow channel **32** is non-turbulent, turbulence in the sample will cause blurring in images captured by the camera **50** due to erratic motion of the semen sample, and will prevent the flow of the semen sample from being concentrated at a vertically central portion of the flow channel **32**, thereby causing the problem of stacking and wide distribution of the sperm cells along the height H.

[0052] To avoid the problems described above and enable the camera **50** to capture well-focused images of all of the sperm cells in the semen sample, the flow channel **32**, the inlet **34**, and the outlet **38** are shaped and dimensioned to provide a non-turbulent flow of the semen sample from the inlet **34**, through the flow channel **32** and to the outlet **38**, and to maintain the flow of the semen sample at a vertically central portion of the flow channel **32**. For example, the flow channel **32**, the inlet **34**, and the outlet **38** can be shaped and dimensioned to provide a laminar flow of the semen sample throughout the entire flow channel **32**.

[0053] FIGS. **2** and **3** depict a suitable set of dimensions for an inlet **34**, flow channel **32** and an outlet **38** that results in a non-turbulent, laminar, flow through the flow channel **32**. In this embodiment, the flow channel **32** has a substantially rectangular cross-sectional shape, the inlet **34** has a shape including a conical transition **34***a* to the flow channel **32**, and the outlet **38** has a shape including a conical transition **38***a* from the flow channel **32**. The inlet **34** and the outlet **38** have respective widths W **1** and W **2** that are greater than a width W of the flow channel **32**. One example of suitable dimensions for the flow channel **32** that facilitates non-turbulent flow is a height H of 40 μm, a width of 300 μm, and a length of 1 cm. But in alternative embodiments, the height of the flow channel **32** could range from 10 to 50 μm, and the width of the flow channel **32** could range from 100 to 400 μm. The length of the flow channel **32** is less critical, and in alternative embodiments the length could range from 0.5 to 5 cm.

[0054] These dimensions for the flow channel **32**, the inlet **34**, and the outlet **38** were found to provide excellent sample flow characteristics when the system **10** was used to identify sperm cells in human semen samples. That is, the foregoing configurations of the flow channel **32**, the inlet **34**, and the outlet **38** exhibited laminar flow of the semen samples through the flow channel **32** without clumping, and the flow of the semen samples was located within a vertically central portion of the flow channel **32**.

[0055] Notably, with the foregoing configurations of the fluid-flow conduit **30**, most of the sample will flow with a laminar flow through the central-most 8 μm portion of the flow channel **32**. In some embodiments, most of the sample will flow through the central-most 6 μm portion, the central-most 5 μm portion, the central-most 4 μm portion, the central-most 3 μm portion, or even the central-most 2 μm portion of the flow channel **32**. Thus, the flow of the sample is confined within a vertically central region of the flow channel **32**, such that the target cells are not vertically stacked in the flow channel **32** and the flow of the sample falls within a DOF of the camera **50**, which can be small (e.g., 2, 3, 4, 5, 6, or 8 μm).

[0056] Additionally, the disclosed values for the width W of the flow channel **32** promote the desired flow characteristics and provide an optimal region of interest (ROI) in which images of the sample are captured by the camera **50**. Further, the disclosed values for the length L promotes the desired sample flow characteristics and enables the camera **50** to acquire an adequate number of images of sperm cells as the sperm cells travel through the flow channel **32** as described below. Note that in alternative embodiments, the height and width of the flow channel **32** could be outside the numeric ranges indicated above.

[0057] Maintaining non-turbulent (e.g., laminar) flow is important because turbulent flow would cause the sperm cells and debris to be distributed across the full z-axis, which would make it difficult if not impossible to keep the relevant cells in focus and to keep track of the sperm cells as they traverse the flow channel. For if the sperm cells were distributed across the full z-axis, (a) only a small fraction of sperm would be visible in any given image and (b) those sperm would likely NOT remain seen across multiple images and thus would be much more difficult to positively identify. In contrast, when non-turbulent (e.g., laminar) flow is maintained, the majority of the sample (including the sperm cells) will flow through a vertically central region of the flow channel **32** that is short enough in height (e.g., a few μm) so that the majority of the sample (including the sperm cells) will remain in focus as they traverse the flow channel **32**. This is important because it allows the sperm cells to be visualized by the camera **50** as they pass under the microscope. Notably, in experiments where 30 sperm cells were inserted into a sample and pumped through a flow channel **32** with a height H of 40 μm, a width of 300 μm, and a length of 1 cm, 8-12 sperm cells were consistently detected, and sometimes more. This means that at least those sperm cells flowed through a portion of the flow channel **32** that was short enough such they were sufficiently in focus to be visualized.

[0058] The fluid-flow conduit **30** can be included in a microfluidics device and can be positioned on a microscope **40** such that the fluid channel **32** is optically aligned with an objective lens **42** of the microscope **40**. The microscope **40** can be a phase contrast microscope, but is not limited thereto.

[0059] The camera **50** includes one or more lenses configured to be aligned with the objective lens **42** to capture images of the semen sample as the semen sample flows through the flow channel **32**. Since the flow of a semen sample through the flow channel **32** is confined to the vertically central portion of the flow channel **32**, as described above, the camera **50** can be precisely focused on the entire vertically central portion of the flow channel **32** and can therefore capture clear, properly focused images of most of the sperm cells in the semen sample. For example, the camera **50** can be focused on a vertically central portion of the flow channel **32** having a height in a range of 2 to 8 μm.

[0060] The camera **50** is a high-speed camera. The resolution, shutter speed, frame rate, trigger time, and other parameters of the camera **50** should be such that the camera acquires multiple images of sperm cells in the semen sample as the sperm cells pass through the flow channel **32**. The parameters of the camera **50** are optimized to make sure captured images are not blurry and the same sperm cell is captured in multiple images, at various locations along the flow channel **32**. The camera **50** can be configured to capture images at a frame rate of at least 100 frames per second. For example, the camera **50** can capture images at a frame rate of 500 frames per second. The camera **50** can capture images of the same sperm cell at least 5 times as the sperm cell passes through the flow channel **32**, and can capture more than two million images of the sperm sample. Capturing multiple images of the same sperm cell as the sperm cell travels along the flow channel **32** can advantageously improve the system's confidence that what appears to be a sperm cell in one image is indeed a sperm cell.

[0061] Operation of the camera **50** can be controlled by the processor **70**, or by a dedicated controller of the camera **50**. Images captured by the camera **50** can be stored in a memory, which can include one or more high-capacity solid state drives (SSDs). The processor **70** analyzes the images captured by the camera **50** to identify target sperm cells in the semen sample and keeps track of which images contain the target sperm cells (e.g., in a memory, not shown). The processor **70** can include one or more GPU-based computational systems that apply a pre-trained neural network to the images captured by the camera **50** to identify the target sperm cells in the images.

[0062] In a semen sample from a person who has an extremely low sperm count (e.g., less than 10 sperm cells per ml), sperm cells are present in very small numbers and are therefore rare cells. Accurate identification of the sperm cells is challenging because other cells and debris are present in large proportion in the sample, relative to the sperm cells. For example, semen samples may also include the following components that are visible through microscopy: epithelial cells from the urogenital tract; myeloid cells; leukocytes (white blood cells); red blood cells; immature germ cells; and fragments of cells. Due to the large proportion of other cells and debris in the semen sample, the techniques that are used to identify the sperm cells must have extremely low error rates in order to provide meaningful identification results. That is, due to the large number of other cells and debris in the semen sample, even a modest error (false

positive) rate in a sperm cell identification technique will produce a very large number of false positive sperm cell identifications.

[0063] Existing neural-network based image classification techniques have proved to be insufficiently accurate for identifying rare sperm cells. But when the processor **70** was programmed to implement a trained object detection model to identify sperm cells in a semen sample, successful results were obtained. M ore specifically, successful results were obtained when the processor **70** was programmed to implement a trained "you only look once" (YOLO) object detection model. For example, the object detection model can be a YOLOv4 object detection model. The trained object detection model is trained based on millions of training images containing sperm cells being input to an object detection model in a training phase.

[0064] The processor **70** inputs each of the captured images of the semen sample flowing through the flow channel **32** to the object detection model, and identifies sperm cells in the captured images based on outputs generated by the object detection model. The processor **70** records data regarding each of the images input to the object detection model. The recorded data can include, for example, time information indicating a time at which the image was captured, position information indicating a position of the sperm cell in the flow channel **32** when the image was captured, target cell information indicating whether the image contains a sperm cell, and an indication of which images correspond to which microwells.

[0065] FIG. **4** is a graph illustrating accuracy of the system **10** in identifying sperm cells in a human semen sample using a trained YOLOv4 object detection model, according to an experimental embodiment. As illustrated in FIG. **4**, the system **10** exhibited a high mean average precision (mAP) and low loss.

[0066] FIG. **5** is an image processed by the system **10** that includes sperm cells identified using the YOLOv4 object detection model, according to an experimental embodiment. Sperm cells were accurately identified with high probability scores.

[0067] FIGS. **6-9** depict successive images of a test sample that were captured while a single sperm cell moved through a flow channel **32** from right to left in an experiment. Notably, the same sperm cell appears at different locations in each of the successive images, which indicates that the sperm was moving from right to left (which was consistent with the direction of flow of the sample through the flow channel **32**). Because each sperm cell appears in multiple images as the sperm cell moves through the flow channel **32**, the probability of identifying any given sperm cell increases dramatically, and the probability of a false positive identification decreases dramatically. Assume, for example, that a given sperm cell was identified in four successive images with respective probabilities of 99%, 94%, 97%, and 99%. This means that the respective probability of a false positive in those images would be 1%, 6%, 3%, and 1%. While the risk of a false positive may not be sufficiently low in any single given image, combining the information from multiple images (e.g., 2, 3, 4, 5, 6, or >6 images) reduces the overall probability of a false positive to the point where the false positive rate becomes negligible.

[0068] Referring back to FIG. **1**, the microplate **120** is disposed on or supported by the stage **100**, and arranged to collect portions of the sperm sample as they exit the outlet **38** of the fluid-flow conduit **30**. M ore specifically, the microplate **120** includes a plurality of microwells **124** configured to contain respective portions of the semen sample. For example, the microplate **120** can include between 50 and 500 microwells (e.g., 96 or 384 microwells).

[0069] In some embodiments, the microwells **124** are arranged in a two-dimensional array and the stage **100** can move in the two dimensions (e.g., X and Y) of the array, and thereby move the microplate **120** in the two dimensions so that respective microwells receive respective portions of the semen sample. In less preferred embodiments, the microwells can be arranged in a one-dimensional array, in which case the stage only has to move in a single dimension to receive the respective portions of the semen sample. In other embodiments, the stage **100** can move in three dimensions (e.g., X, Y, and Z), and thereby move the microplate **120** in three dimensions to receive portions of the semen sample.

[0070] The stage **100** is configured to move precisely in one or more dimensions, to thereby move the microplate **120** in the one or more dimensions to respective positions where the respective portions of the semen sample flow out of the outlet **38** and into the respective ones of the plurality of microwells **124**. Each microwell **124** can receive a prescribed volume of the semen sample, and the entire semen sample can be collected by the microwells **124**.

[0071] The above-described movements of the stage **100** can be controlled by the processor **70** or by an independent controller (not shown). More specifically, the processor **70** or the controller can issue commands to the stage **100** to cause the stage **100** to move in one or more directions as described above.

[0072] The processor **70** keeps track of which microwells contain sperm cells. More specifically, the processor **70** determines which of the microwells **124** include sperm cells based on the recorded data of images in which the sperm cells are identified. For example, the recorded data of images in which a sperm cell is identified can include the time information indicating times at which the images were captured, position information indicating positions of the sperm cell in the flow channel **32** when the images were captured, and the and target cell information indicating that the images contain the sperm cell. Additionally, the processor **70** is informed of the flow rate of the semen sample and a time at which each of the microwells **124** is positioned to receive a respective portion of the semen sample from the outlet **38**. Thus, the processor **70** can correlate the recorded data of the images corresponding to the sperm cell to a microwell **124** that was positioned to receive a portion of the semen sample including the sperm cell to determine which microwell **124** includes a sperm cell.

[0073] Since a microwell **124** including the sperm cell can be identified by the processor **70**, the sperm cell can be recovered from the identified microwell **124**. Further, since the sperm cell is identified and recovered without the sperm cell being damaged, the recovered sperm cell can be used for a subsequent procedure. For example, the recovered sperm cell can be used for an IVF treatment.

[0074] The system described above in connection with FIGS. **1-3** was tested using a microTESE (microscopic testicular sperm extraction) sample. MicroTESE is a surgical procedure used to retrieve sperm from the seminiferous tubules of a male's testes. Extensive search for sperm cells in the sample by lab experts using conventional approaches failed. In contrast, the system of FIGS. **1-3** detected **55**

sperm cells, even though the sample contained significantly more background cells (RBCs and other blood cells) and debris than ordinary semen samples.

[0075] The system described above in connection with FIGS. **1-3** was also tested using a post-vasectomy semen sample, where no sperms were found using conventional approaches. In contrast, the system of FIGS. **1-3** detected **22** sperm cells.

[0076] Many factors can influence the overall functioning of the system, and the inventors have come up with the following equation that takes into account these parameters. Other versions of the system, for example, with different height of microfluidics channel, flow rate, or frames per second capture rate etc. will follow this equation (1):

$$NTF \approx \frac{VROI \times FPS \times 3.6}{FR} \text{ where, } VROI = \frac{L \times W \times H}{10^6}$$

ROI—Region of interest. Region in the channel that is captured by the camera

NTF—Number of times the target cell is captured in ROI

VROI—Volume in region of interest (in nanoliters)

FPS—Frames per second capture by camera

FR—Flow rate (microliters per hour)

L—Length of ROI (in micrometers)

W—Width of ROI (in micrometers)

H—Height of ROI (in micrometers)

[0077] The embodiments described above in connection with FIGS. **1-3** sequentially aliquots the sample into respective ones of a relatively large number of microwells (e.g., >100). And based on the time stamp of sperm image capture, its destination microwell can be determined. In the multi-microwell embodiment, an ODS of ~90 images per second and a camera FPS 500 (ODS<FPS) may be used.

[0078] In alternative embodiments, a two-microwell system may be used in which most of the fluid from outlet will flow into microwell 'A' (discard). But every time a target cell is detected, the output is switched to microwell 'B' (concentrator). Note, however, that this two-microwell embodiment requires a significantly faster object detection speed (ODS) that is faster than FPS (frames per second) i.e., ODS>FPS. Using equation (1) set forth above, we can bring the FPS down to 100 by playing with Flow rate and NTF. With a sufficiently powerful workstation, the ODS can be increased to 200 thereby achieving ODS>FPS, thereby making this two-microwell system feasible.

[0079] FIG. **10** illustrates an embodiment of another system **11** for identifying and recovering target cells present in a sample. The system **11** is similar to the system **10** described above in connection with FIG. **1**, with similarly-numbered components operating as described above in connection with FIGS. **1-9**. Except that instead of capturing respective portions of the sample in respective microwells and keeping track of which microwells contain target cells (as described above in connection with FIGS. **1-9**), the microfluidics device **300** of the system **11** routes portions of the sample that were determined to include a target biological cell into a single first container **60**B, and routes portions of the sample that were not determined to include a target biological cell into a single second destination (i.e., a single second container **60**A).

[0080] This can be accomplished by using a microfluidic device **300** to implement at least one valve that is configured to route a plurality of first portions of the sample that exit the outlet into the single first container **60**B and to route a plurality of second portions of the sample that exit the outlet to the single second container **60**A.

[0081] FIG. **11** depicts one approach for using the microfluidic device **300** to implement the at least one valve in the FIG. **10** embodiment. This approach uses two PDMS (polydimethylsiloxane) quake valves **81** and **82**, and the sample flows through the flow channel **32** from left to right in FIG. **11**. When the processor **70** determines that a portion of the sample that includes a target biological cell is approaching the branching point **80**, the processor **70** issues commands that cause the first valve **81** to open and the second valve **82** to close. This will route the portion of the sample that includes the target biological cell to the first container **60**B. On the other hand, when the processor **70** has not determined that the portion of the sample that is approaching the branching point **80** includes a target biological cell, the processor **70** issues commands that cause the first valve **81** to close and the second valve **82** to open. This will route this portion of the sample to the second container **60**A.

[0082] FIG. **12** depicts another approach for using the microfluidic device **300** to implement the at least one valve in the FIG. **10** embodiment. This approach uses two single-layer PDMS sidewall valves **81'** and **82'**, and the sample flows through the flow channel **32** from left to right in FIG. **12**. When the processor **70** determines that a portion of the sample that includes a target biological cell is approaching the branching point **80**, the processor **70** issues commands that cause the first valve **81'** to open and the second valve **82'** to close. This will route the portion of the sample that includes the target biological cell to the first container **60**B. On the other hand, when the processor **70** has not determined that the portion of the sample that is approaching the branching point **80** includes a target biological cell, the processor **70** issues commands that cause the first valve **81'** to close and the second valve **82'** to open. This will route this portion of the sample to the second container **60**A.

[0083] FIG. **13** depicts yet another approach for using the microfluidic device **300** to implement the at least one valve in the FIG. **10** embodiment. This approach uses a fluid-flow circuit having a main flow inlet **91**, a first outlet **101**, a second outlet **102**, and a control flow inlet **92** arranged as depicted in FIG. **13**. The fluid-flow circuit is configured so that (a) applying a control flow to the control flow inlet **92** at a first flow rate causes fluid from the main flow inlet **91** to flow out of the first outlet **101** and (b) applying a control flow to the control flow inlet **92** at a second flow rate causes fluid from the main flow inlet **91** to flow out of the second outlet **102**. The sample flows through the flow channel **32** from left to right in FIG. **13**.

[0084] Computational fluid dynamics (CFD) simulations show that when the sample flows through the flow channel **32** at a rate of 600 μl per hour and the control flow that is applied to the control flow inlet **92** is 10 μl per hour (which is much smaller than the flow rate at the main flow inlet **91**), the sample will flow through the flow channel **32** and into the main flow inlet **91**, and will then continue to the right and exit via the second outlet **102**. On the other hand, when the control flow that is applied to the control flow inlet **92** is 4 ml per hour (which is much larger than the flow rate at the main flow inlet **91**), the control flow will exit via the second outlet **102**, which will cause the sample arriving via the main

flow inlet **91** to be diverted into the first outlet **101**. In view of this configuration, when the processor **70** determines that a portion of the sample that includes a target biological cell is approaching the branching point **80**, the processor **70** issues commands that cause the control flow to operate at 4 ml per hour. This will route the portion of the sample that includes the target biological cell out of the first outlet **101** and into the first container **60**B. On the other hand, when the processor **70** has not determined that the portion of the sample that is approaching the branching point **80** includes a target biological cell, the processor **70** issues commands that cause the control flow to operate at 10 μl per hour. This will route this portion of the sample (which does not include a target biological cell) out of the second outlet **102** and into the second container **60**A.

[0085] According to embodiments disclosed herein, a system and method are provided to identify and recover sperm cells from a semen sample. The disclosed system and method accurately and efficiently identify and recover rare sperm cells in a semen sample having an extremely low sperm count. Moreover, the disclosed system and method do not need to employ potentially harmful markers such as stains, lasers, antibodies, or other markers to identify the sperm cells, and do not require the application mechanical or electrical force to capture the sperm cells. Therefore, the sperm cells can be recovered without being damaged, and thus can be used in subsequent procedures after recovery.

[0086] Another application for the embodiments described above in connection with FIGS. **1-13** is to process semen samples for use in In vitro fertilization (IVF), Intrauterine Insemination (IUI), and/or freezing (Cryo) in samples where the sperm count is normal or only a little low (as opposed to extremely low). Currently, semen samples for use in IVF, IUI or Cryo are typically processed using time consuming and labor intensive steps that can often be damaging to sperm. This includes e.g., multiple rounds of centrifugation, sometime in a toxic gradient solution. In contrast, a small aliquot of the semen sample can be diluted in a gentle buffer and then run through the embodiments described above in connection with FIGS. **1-13**. The system will then select out the sperm and concentrate them for future use in a much more gentle manner.

[0087] The foregoing description describes systems and methods in a context in which a semen sample is the sample to be processed and sperm cells are the target cells to be identified and recovered. However, identifying and recovering other types of rare biological cells in other contexts present similar concerns. Therefore, the methods and system disclosed herein are not limited to identifying and recovering sperm cells. To the contrary—the disclosed systems and methods can identify and recover other types of target cells from other types of samples. For example, the disclosed systems and methods can identify and recover rare biological cells such as, but not limited to, cancer cells, fetal cells, or placenta cells present in a blood sample, a placental fluid sample, or another fluid sample. Additionally, the disclosed systems and methods can be used to perform a complete blood count (CBC), which is routinely used to detect leukemia, anemia, infections, and other disorders.

[0088] While the present invention has been disclosed with reference to certain embodiments, numerous modifications, alterations, and changes to the described embodiments are possible without departing from the sphere and scope of the present invention, as defined in the appended claims. Accordingly, it is intended that the present invention not be limited to the described embodiments, but that it has the full scope defined by the language of the following claims, and equivalents thereof.

What is claimed is:

1. A method of identifying and recovering biological cells from a sample, the method comprising:

diluting the sample in a buffer;

pumping the diluted sample through a flow channel, wherein the diluted sample flows through the flow channel with a non-turbulent flow;

sequentially capturing a plurality of images of the diluted sample as the diluted sample passes through the flow channel;

analyzing the captured images to determine which portions of the diluted sample include a target biological cell;

routing portions of the diluted sample that were determined to include a target biological cell into at least one first container; and

routing portions of the diluted sample that were not determined to include a target biological cell to at least one second destination.

2. The method of claim **1**, wherein the diluted sample flows through the flow channel with a laminar flow.

3. The method of claim **1**, wherein the diluted sample flows through the flow channel at a flow rate between 800 and 2000 μL/hr.

4. The method of claim **1**, wherein the capturing of the plurality of images includes capturing the plurality of images using a camera focused on a vertically central portion of the flow channel having a height between 2 and 8 μm.

5. The method of claim **1**, wherein the capturing of the plurality of images includes capturing the plurality of images using a camera focused on a vertically central portion of the flow channel having a height between 4 and 6 μm.

6. The method of claim **1**, wherein the analyzing of the captured images is implemented using an object detection model.

7. The method of claim **1**, wherein the analyzing of the captured images is implemented using a YOLO object detection model.

8. The method of claim **1**, wherein the target biological cell is any one of a sperm cell, a cancer cell, a fetal cell, and a placenta cell.

9. The method of claim **1**, wherein the capturing of the plurality of images includes capturing the plurality of images at a rate of at least 100 frames per second.

10. The method of claim **1**, wherein portions of the diluted sample that were determined to include a target biological cell are deposited into a single first container, and portions of the diluted sample that were not determined to include a target biological cell are deposited into a single second container.

11. The method of claim **1**, wherein a determination that a given portion of the diluted sample includes a target biological cell is based on identifying a given target biological cell at respective different locations in a plurality of the captured images.

12. A method of identifying and recovering sperm cells from a semen sample that includes at least 1 million sperm per ml, the method comprising:

diluting the semen sample in a buffer; and

processing the diluted sample using an apparatus that is capable of extracting sperm cells from fluids that include fewer than 10 sperm cells per ml,

wherein the apparatus extracts the sperm cells from the diluted sample.

13. The method of claim **12**, wherein the apparatus causes the diluted sample to flow through a flow channel with a non-turbulent flow, sequentially captures a plurality of images of the diluted sample as the diluted sample passes through the flow channel, analyzes the captured images to determine which portions of the diluted sample include a sperm cell, routes portions of the diluted sample that were determined to include a sperm cell into at least one first container, and routes portions of the diluted sample that were not determined to include a sperm cell to at least one second destination.

14. The method of claim **13**, wherein a determination that a given portion of the diluted sample includes a sperm cell is based on identifying a given sperm cell at respective different locations in a plurality of the captured images.

15. The method of claim **13**, wherein the diluted sample flows through the flow channel with a laminar flow.

16. The method of claim **13**, wherein the diluted sample flows through the flow channel at a flow rate between 800 and 2000 μL/hr.

17. The method of claim **13**, wherein the capturing of the plurality of images includes capturing the plurality of images using a camera focused on a vertically central portion of the flow channel having a height between 2 and 8 μm.

18. The method of claim **13**, wherein the analyzing of the captured images is implemented using a YOLO object detection model.

19. The method of claim **13**, wherein the capturing of the plurality of images includes capturing the plurality of images at a rate of at least 100 frames per second.

20. The method of claim **13**, wherein portions of the diluted sample that were determined to include a sperm cell are deposited into a single first container, and portions of the diluted sample that were not determined to include a sperm cell are deposited into a single second container.

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