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(54) Title: A MICROSCOPY SYSTEM AND A METHOD FOR ANALYZING FLUIDS

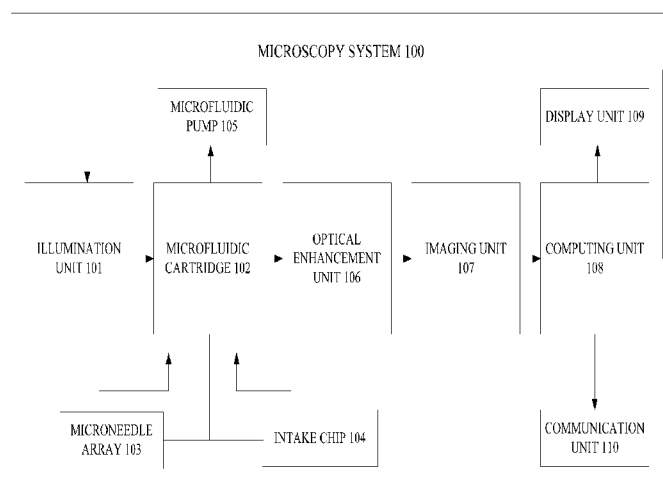


Figure 1

(57) Abstract: Embodiments of the present dis-
closure relate to a microscopy system and a meth-
od to analyze fluids in flow. The microscopy sys-
tem comprises a microfluidic cartridge to receive
input comprising at least one of fluids and re-
agents. The microfluidic cartridge further com-
prises a mixing zone to receive the at least one of
fluids and reagents to form a mixture and an im-
aging zone to receive the mixture from the mixing
zone. An illumination system is configured in the
microscopy system to illuminate the imaging zone
and at least one imaging unit to capture plurality
of images of one or more microscopic elements of
the mixture flowing through the imaging zone.
The plurality of images is processed by a comput-
ing unit associated with the microscopy system for
analyzing fluids.



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5 **“A MICROSCOPY SYSTEM AND A METHOD FOR ANALYZING FLUIDS”**

TECHNICAL FIELD

10 Embodiments of the present disclosure relate to diagnostic test devices. More particularly, embodiments of the disclosure relate to a microscopy system and method for performing rapid analysis of fluids and other flowing entities with low frame rate imaging devices at high throughput.

BACKGROUND

15 Presently, it is well known that a majority of cancer related deaths are due to the late diagnosis of the disease. Early detection of cancer may help in preventing a significantly large percentage of the cancer related deaths, as cancer is curable in its initial stages. However, early diagnosis is limited to the type of cancer where a surface effect may be
20 observed and the required resources needed for testing are available. Also, affordability of the test is another major limitation in the diagnosis. Further, the cancers that do not display surface effects like lung, blood related cancer etc. usually cannot be recognized or may go unnoticed and may be diagnosed only at an advanced stage of the cancer, which is difficult to
25 be cured. In view of this, the effective way of reducing the cancer burden would be to implement mass population screening. Drawbacks for the implementation of cancer screening campaigns are the lack of affordability and automated point-of-care device or instrument capable of performing a quick early diagnosis of various types of cancer.

30 In some of the prior arts, cancer diagnosis includes the use of immunohistochemistry stains, fluorescence in-situ hybridization, PCR-based detection, frozen section and sentinel lymph node biopsy. For the majority of the diagnosis procedures, preparing smear on a slide and its microscopic examination by a cytologist is required. Also, most of these techniques require expensive instrumentation, devices or systems and one or more skilled technicians, which impede its widespread availability.

35 Conventionally, cancer disease is diagnosed by performing a general biopsy or a Fine-Needle Aspiration Biopsy (FNAB). The FNAB comprises obtaining a tissue sample from the suspected tumour by a surgeon and sending the sample to a pathologist or a cytologist for analysis. Most of the times the surgeon and the pathologist are not present together in the

5 same medical facility, in such cases, slides of the biopsy samples are prepared and sent to the pathologist or a cytologist, who studies the slides and prepares a report. This process may take up between a few days to a few weeks, depending on the distance between the sample collection points, the point of analysis and availability of the pathologist/cytologist for analysis. The delay involved impedes cancer diagnosis, as it deters patients from remote areas
10 to get tested at an early stage of the symptom. Another disadvantage is improper slide or smear preparation and handling, which results in generation of false/ wrong diagnosis, which may cause unnecessary panic and trauma in person who is diagnosed.

Currently the flow-cytometers methods are used pre-dominantly for detecting cancer
15 using biomarkers for a certain cancer type. The biomarkers are selectively labelled using fluorescent dyes. The sample cells are passed through a zone of illumination using sheath flow. The cells of interest which is having a biomarker is identified or separated from the other cells based on their fluorescence. The flow cytometers are very expensive, costing over fifty lakh rupees, bulky and not portable. The labelling fluorescent dyes constitute a
20 significant continuous expenditure for flow-cytometer methods. There are image-based flow-cytometers which are being used, which make use of a combination of photometric that is multi-colour fluorescence imaging and morphometric analysis for detecting a disease. These flow cytometers are very expensive which costs over rupees one crore and are not a label-free technique. Also, there are methods which provide the quicker diagnosis and are affordable,
25 but these methods provide only 'Yes or No' type diagnosis, whereas for disease treatment one needs to know the extent of the infection.

SUMMARY

30 The shortcomings of the prior art are overcome and additional advantages are provided through the provision of method of the present disclosure.

Additional features and advantages are realized through the techniques of the present disclosure. Other embodiments and aspects of the disclosure are described in detail herein
35 and are considered as part of the claimed disclosure.

In one embodiment the present disclosure provides a microscopy system for analyzing fluids. The microscopy system comprises a microfluidic cartridge to receive input, an illumination unit and at least one imaging unit. The input to the microfluidic cartridge

5 comprises at least one of fluids and reagents. The microfluidic cartridge comprises, a mixing zone to receive the at least one of fluids and reagents to form a mixture and an imaging zone to receive the mixture from the mixing zone. The illumination unit illuminates the imaging zone and also the at least one imaging unit to capture plurality of images of one or more microscopic elements of the mixture flowing through the imaging zone. The plurality of
10 images is processed by a computing unit associated with the microscopy system for analyzing fluids.

In one embodiment of the present disclosure, a method for analyzing fluids using a microscopy system, the method comprising, receiving, by a cartridge, at least one of, fluids
15 and reagents, where the at least one of the fluids is mixed with the at least one of the reagents in a predefined proportion in a mixing zone. The method further comprises illuminating an imaging zone of the microscopy system by an illumination unit. In one embodiment, capturing plurality of images of one or more microscopic elements of the mixture flowing through the imaging zone by an imaging unit, where the at least one of the plurality of images
20 is processed, by a computing unit for analysis.

It is to be understood that the aspects and embodiments of the invention described above may be used in any combination with each other. Several of the aspects and embodiments may be combined together to form a further embodiment of the invention.

25 The foregoing summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative aspects, embodiments, and features described above, further aspects, embodiments, and features will become apparent by reference to the drawings and the following detailed description.

30 **BRIEF DESCRIPTION OF THE DRAWINGS**

The novel features and characteristic of the disclosure are set forth in the appended claims. The embodiments of the disclosure itself, however, as well as a preferred mode of
35 use, further objectives and advantages thereof, will best be understood by reference to the following detailed description of an illustrative embodiment when read in conjunction with the accompanying drawings. One or more embodiments are now described, by way of example only, with reference to the accompanying drawings.

5 **Fig. 1** illustrates an exemplary block diagram representation of a microscopy system in accordance with an embodiment of the present disclosure;

Fig. 2 shows an exemplary block diagram of an illumination unit in accordance with an embodiment of the present disclosure;

10 **Fig. 3** shows an exemplary block diagram of microfluidic cartridge in accordance with an embodiment of the present disclosure;

Fig. 4 shows an exemplary diagram of exemplary working model of a microscopy system, in accordance with an embodiment of the present disclosure;

Fig. 5A shows a flowchart illustrating a method for analyzing fluids using a microscopy system in accordance with other embodiments of the present invention.

15 **Fig. 5B** shows an example flowchart illustrating a method for detecting foreign bodies in yeast cells in accordance with an embodiment of the present disclosure;

Fig. 6 shows a plot illustrating yeast cell imaging and count, in accordance with an embodiment of the present disclosure;

20 **Fig. 7** shows an exemplary representation of an application to detect infected cells using the image based cytometer system, in accordance with an embodiment of the present disclosure;

Fig. 8 shows an illustration of malaria diagnostic analysis using an image analysis software application, in accordance with an example embodiment of the present disclosure;

25 **Fig. 9A** shows malaria diagnosis test experimental results using microfluidic cartridge showing representative images of healthy red blood cells (RBC); and

Fig. 9B shows malaria diagnosis test experimental results using microfluidic device showing representative images of malaria-infected RBCs, which have absorbed the stain.

30 The figures depict embodiments of the disclosure for purposes of illustration only. One skilled in the art will readily recognize from the following description that alternative embodiments of the structures and methods illustrated herein may be employed without departing from the principles of the disclosure described herein.

DETAILED DESCRIPTION

35

 The foregoing has outlined rather broadly the features and technical advantages of the present disclosure in order that the detailed description of the disclosure that follows may be better understood. Additional features and advantages of the disclosure will be described

5 hereinafter which form the subject of the claims of the disclosure. The novel features which are believed to be characteristic of the disclosure, both as to its organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be expressly understood, however, that each of the figures is provided for the purpose of
10 illustration and description only and is not intended as a definition of the limits of the present disclosure.

While the disclosure is susceptible to various modifications and alternative forms, specific embodiment thereof has been shown by way of example in the drawings and will be
15 described in detail below. It should be understood, however that it is not intended to limit the disclosure to the particular forms disclosed, but on the contrary, the disclosure is to cover all modifications, equivalents, and alternatives falling within the spirit and the scope of the disclosure.

20 The terms “comprises”, “comprising”, or any other variations thereof, are intended to cover a non-exclusive inclusion, such that a setup, device or method that comprises a list of components or steps does not include only those components or steps but may include other components or steps not expressly listed or inherent to such setup or device or method. In other words, one or more elements in a system or apparatus preceded by “comprises... a”
25 does not, without more constraints, preclude the existence of other elements or additional elements in the system or apparatus.

Embodiments of the present disclosure relate to a method for analyzing fluids, using a microscopy system.

30

In one embodiment, the present disclosure provides a microscopy system. The microscopy system comprises an illumination unit and a microfluidic cartridge. The microfluidic cartridge comprises one or more input channels to carry one of, fluids and reagents. Further, the microfluidic cartridge comprises a mixing zone to receive the input
35 from the one or more input channels, to form a mixture. Also, the microfluidic cartridge comprises an imaging zone to receive the mixture from the mixing zone. Also, the microscopy system comprises a microfluidic pump to control the flow rate of the input and the mixture in the cartridge. An illumination unit is configured in the microscopy system to illuminate the imaging zone. In an embodiment, the microscopy system comprises at least

one imaging unit to capture one of, a video and plurality of images of microscopic elements of the mixture, flowing through the imaging zone. Furthermore, the microscopy system may comprise at least one computing unit to process and analyze the plurality of images. In an embodiment, the computing unit may be associated with the microscopy system and connected through communication network.

The one or more channels of the microfluidic cartridge are designed specifically to regulate the flow of the one of, fluids and reagents in the system. Also, the one or more channels assist in proportionately mixing the fluids and reagents in the mixing zone.

Fig. 1 illustrates an exemplary block diagram representation of a microscopy system 100 in accordance with an embodiment of the present disclosure. The microscopy system 100 comprises an illumination unit 101, microfluidic cartridge 102, a micro-needle array 103, an intake chip 104, a microfluidic pump 105, an optical enhancement block 106, imaging unit 107, a computing unit 108, a display unit 109 and a communication unit 110.

As shown in **Fig. 1**, the illumination unit 101 illuminates the microfluidic cartridge 102 uniformly at the high-throughput channel geometry region. The microfluidic channels present in the microfluidic cartridge 102 are magnified with predefined spatial resolution, so that images of microscopic elements of the mixture have high-fidelity. This is achieved by optically enhancing the image of the microscopic elements using optical enhancement unit 106. In one embodiment, this can be achieved by using an objective lens and an eyepiece, in other embodiments this can be achieved by using custom designed lens systems that may have one or more lenses. The lens are one of removable or non-removal type of lenses from the camera. Hence, any digital camera may be used as a compact digital microscope.

In one embodiment of the present disclosure, the imaging unit 107 may include, but is not limited to, an imaging sensor, a camera, a digital camera of a mobile device, Charge Coupled Device (CCD) and Complementary Metal Oxide Semiconductor (CMOS) sensor, which obtains the images of the microscopic elements flowing in the microfluidic cartridge 102.

In an embodiment, a micro-needle array 103, sample intake chip 104, and portable microfluidic pump 105 are configured in the microscopy system 100. The micro-needle array 103 or sample intake chips 104 is used to intake a predefined sample from a user and deliver

5 to the microfluidic cartridge 102. The predefined intake sample is at least one of blood, urine, saliva and any other fluids, which require visualization of microscopic specimen (present within them) like pollen, particles (in aerosols) etc.. The input sample is introduced in to the microfluidic cartridge 102 through at least one of one or more reservoirs, at least one external container, and at least one micro-needle array. In one embodiment, the microfluidic
10 cartridge 102 can be used as a disposable or a replaceable unit, when it is fabricated using inexpensive materials (polymers) like polydimethylsiloxane (PDMS), Poly(methyl methacrylate) (PMMA) etc. using fabrication techniques like soft and photolithography . In another embodiment, the microfluidic cartridge can be reusable for several tests to be performed, in which case, it can fabricated out of robust materials like glass, fused silica etc.,

15

In one embodiment of the present disclosure, the computing unit 108 may comprise at least one of Single Board Computer (SBC)(which is equipped with a single core or a multicore processor), a microcontroller, microprocessor, any portable device and any computing device, which is used to acquire data from the imaging device 107. The imaging
20 device 107 captures one of images or videos of the sample microscopic elements. The videos captured by the imaging device 107 may be split into frames and processed by the computing unit 108. The computing unit 108 may control or trigger the illumination to obtain a temporally coded illumination. The videos captured using this coded illumination may require additional computational processing such as, but not limited to de-convolution and
25 motion de-blurring. Also, the computing unit 108 is used to drive the display unit 109 and the communication unit 110. The communication unit 110 further comprises an on board memory unit for storing the analysis results.

In an exemplary embodiment of the present disclosure, the computing unit 108 may
30 be one of microcontroller, microprocessor and a Charge Coupled Device (CCD) built into the microscopy system 100. An imaging unit 107 can be integrated with the microprocessor that can be attached to portable devices. The portable devices can be, but not limited to a computer web-camera, mobile phone, tablet, Personal Digital Assistant (PDA) or any other mobile computing device. The computing unit 108 is configured to receive at least one of a
35 video and plurality of images of the microscopic elements of the mixture flowing through the imaging zone 302 of the microfluidic cartridge 102. The computing unit 108 performs at least one of de-convolution and motion de-blurring on plurality of images to form a processed images, converting the at least one of video in to plurality of images and analyzing the

5 processed images. The analysis results are displayed by at least one display unit 109 configured with the computing unit 108.

In one embodiment of the present disclosure, the display unit 109 is configured to display the results of the analysis or the test being performed on the sample intake. In one
10 embodiment, the display unit 109 takes one or more inputs required for processing data such as, but not limited to patient details, disease or test to be carried out, and any other data which may be integrated. The microscopy system 100 is connected to remote areas for data transmission through means including, but not limited to, radio communication, internet, Wi-Fi, low-energy Bluetooth and any other communication means for data communication/
15 transmission.

In one embodiment of the present disclosure, the microscopy system 100 comprises a communication unit 110. The communication unit 110 communicates the analysis results to a concerned person or a system including, but not limited to, a clinical pathologist or a
20 system. In one embodiment, the communication unit can also be used to transmit the raw data to a cloud / server based computing unit. The raw data consisting of video or plurality of images is processed to obtain the results of the test. The processed images and results can be received by the communication unit from the server.

25 **Fig. 2** shows an exemplary block diagram of illumination unit 101 in accordance with an embodiment of the present disclosure. The illumination unit 101 is shown in **Fig. 2**, comprises illumination optics, which comprises one of, an LED 201, fluorescent lamp (not shown in the figure) and a ground glass diffuser 202, to illuminate the microfluidic cartridge 102. A LED 201 and a ground-glass diffuser 202 are used to provide the uniform illumination
30 in the object plane of the imaging unit 107. The illumination unit 101 can be controlled by the computing unit 108 according to the test being performed on the input fluids. In an embodiment, the computing unit 108 may control powering of the illumination unit, intensity of illumination, type of illumination and time interval of each type of illumination.

35 **Fig. 3** shows an exemplary block diagram of a microfluidic cartridge 102 in accordance with an embodiment of the present disclosure. As shown in **Fig. 3**, the microfluidic cartridge 102 comprises on-chip sample preparation, which may be carried out on the microfluidic cartridge 102. The microfluidic cartridge 102 comprises an input channel (also referred as first channel and one or more second channels) that are able to handle fluids

5 and image objects flowing through the channels carried by the fluid, such as a gas or liquid. The microfluidic cartridge 102 comprises at least one inlet and at least one outlet. The fluid sample may be introduced from one of reservoir(s), external container(s) that are connected to the microfluidic cartridge 102 and/or a micro-needle array 103 may be attached to the microscopy system 100 through the first or input channel such that a sample can be directly
10 administered into the microfluidic cartridge 102 by layperson or patient without the need of medical training or any person skilled in the respective art. The sample may be blood or any other fluids which includes but not limited to urine, saliva etc., and the amount of fluid required for diagnosing not be large, such that the cartridge is referred to as a microfluidic cartridge.

15 In one embodiment, the reagents may be fed in to the microfluidic cartridge 102 by one of, manually inputting of the one or more reagents in to the microfluidic cartridge 102, inputting the one or more reagents in to the microfluidic cartridge 102 from one or more reservoirs and pre-load the microfluidic cartridge 102 with one or more reagents. The
20 essential reagents are one of, but not limited to, anti-coagulants, staining dyes, nano-particles needed for sample preparation. The reagents may be stored in one or more tanks in accordance with an embodiment, which may be fed through one or more second channels at one or more points, leading to the possibility of simultaneous testing of different properties or diseases present in the sample intake through the first channel or input channel. The one or
25 more reagent chambers are represented as but not limited to A, B, C as shown in **Fig. 3**. The reagents are mixed with the input sample in the mixing zone 301 or also referred as a third channel. The mixture i.e. reagents from the plurality of second channels and the input sample, flowing in the third channel is collected in at least one waste reservoir (not shown in the figure) after the imaging is performed. In the third channel of the microfluidic cartridge 102,
30 a predefined region called imaging zone 302 is imaged by the imaging unit 107. The other end of the microfluidic cartridge 102 comprises a portable microfluidic pump 105, which is a portable pump that does not require electrical energy for its operation, is used to facilitate sample flow across the cartridge. For example, the pumping can be enabled by employing negative pressure at the outlet of the microfluidic cartridge. The negative pressure is
35 employed with the use of a negative pressure VacLok syringe. By pulling the piston of the syringe and locking it, negative pressure is applied. The flow rate is dependent on the negative pressure generated, which in turn depends on the position in which the syringe is locked.

5

The reagents can include, but are not limited to, anti-coagulants, staining dyes, Antibody Conjugated nanoparticles needed for sample preparation.

10 In an exemplary embodiment, the anti-coagulants are used along with other embodiments of the present disclosure for microscopy based diagnostic tests. The present disclosure discloses the use of in-suspension staining techniques. The reagents being used for performing the test can be different for different samples or body fluids being analyzed. Depending upon the cellularity and type of cells present in the sample, different staining and fixing buffers may be used. In case of blood, 10 mM 1X Phosphate Buffered Salines (PBS) is
15 used as the dilution buffer. Ethylene Diamine Tetra Acetic Acid (EDTA) is used as the anti-coagulant and is added to the blood, immediately after extraction. Staining of the parasite or nuclear content within cells is performed using 5 % methylene-blue solution. It is found that methylene-blue staining provided enough contrast, so as to differentiate cells infected with parasites and healthy cells. Similar staining methods can be used to image cells with nuclear
20 content. For examples, while analyzing whole blood suspension, white blood cells (containing nucleus) can be differentiated from Red Blood Cells (not containing nucleus). Further, other in-suspension protocols can also be used in combination with the current disclosure. In case of urine, dilution buffer is not required and staining of the epithelial cells can be performed using methylene blue or Safranin dyes. Also, there is scope for
25 development of in-suspension staining techniques, which can be much more cost effective and robust. Also, the device is compatible with reagents, which are used for conventional flow cytometric analysis. The sample suspension obtained after employing conventional flow cytometric sample preparation techniques, can be analyzed using the present disclosure. The present disclosure can also be used to perform other biological tests like pollen viability.
30 Pollen viability is in general performed using colorimetric tests, where a colorant is added to pollen and microscopic images of the pollen are acquired. Pollen viability is assessed in relation to the level of staining. The colored pollen is in general known to be viable, whereas colorless pollen is non-viable. With the current disclosure, suspension of pollen can also be analyzed. In this case, other staining dyes compatible with pollen are employed. In general, 2,
35 3, 5-triphenyl tetrazolium chloride (TTC) and acetocarmine are used as staining dyes for pollen viability assessment. Further, the disclosure can also be used to check for the presence of micro-scale impurities in water or any other fluids like oil. In one embodiment, the current disclosure can also be used to perform analysis of gases. The ability to assess particle size in

gases would enable particulate estimation and detection of pollen present in air. Particulate size estimation is important in the context of the air quality monitoring and pollution control. Also, the presence of pollen/spores and other micro particles causes allergic reactions, asthma attacks to people breathing the air. In such cases, it is essential to determine pollen/mold counts to assess the number of pollen (total and differential counts) present in the air. By passing the air collected from the location of investigation, through the microfluidic cartridge 102, the required sample preparation can be enabled. Following which, the images of pollen can be acquired and analyzed to assess the sizes and number of pollen present in the air. Similar approach can also be employed to assess the type and quantity of particulates present in the air.

In another embodiment, a suspension of nanoparticles for example but not limited to polymer nanoparticles or metal nanoparticles can be used to enhance the imaging contrast. For example, metal nanoparticles can be used as reagent to improve the imaging contrast. The metal nanoparticles present in the suspension are functionalized with anti-bodies, specific to antigens present on the surface of target cells. When the nanoparticles are mixed with suspension containing the target cells, the nanoparticles functionalized with anti-bodies bind to the antigens present on the surface of target cells/entities. The tagging of cells/entities enables detection (via imaging) of even very small biological entities like bacteria, viruses. Further, nanoparticle tagging enables detection of rare cells like Circulating Tumor cells (CTCs), which are very low in number in a given sample of body fluids. Selective tagging of nanoparticles helps in differentiating CTCs from normal cells. The clusters of metal nanoparticles attached to cells are observed as significantly darker portions (as compared to the background). The target cells are identified based on the presence of clusters of nanoparticles on the surface of cells. A given cell is identified as the target cell, if the nanoparticles are tagged to it. Using image processing algorithms including, but not limited to, contrast adjustment and thresholding, cells tagged with nanoparticles can be easily identified from the cells without nanoparticles.

The microfluidic pump 105 regulates the flow rate at which the sample is flown through the microfluidic cartridge 102. By regulating the flow rate of the sample, the sample is flown at a rate that allows for optimal imaging conditions. This can be controlled based on the properties of sample, microfluidic cartridge 102 design and viscosity. By varying the pumping speed, pumping channel shapes, the sample microscopic elements are deformed to

5 derive more information when compared without deforming, such as information obtained from one of single image, a static image and real-time focus variation to obtain 3D image, for e.g. morphological information about the cell.

Fig. 4 shows an exemplary diagram of a microscopy system 100, in accordance with an embodiment of the present disclosure. **Fig. 4** discloses the working order of the microscopy system 100. An illumination unit 406 as shown in **Fig. 4** illuminates a portion of interest of a microfluidic cartridge 402 which receives input through one or more input channels. A microfluidic pump is used to regulate the flow of input and mixture formed in the microfluidic cartridge 402. An optical enhancement unit 403 is configured in the microscopy system 100 to enhance the image of microscopic elements of the mixture. An imaging unit 404 captures plurality of images of the microscopic elements of the mixture. The plurality of images are processed and analyzed by a computing unit (not shown in the figure) and the analysis results are displayed by the display unit 405. In one embodiment, the imaging unit can include, but is not limited to, a low frame rate digital camera which is integrated in an attachment and the replaceable microfluidic cartridge 102. The attachment consists of an opening slot, to insert the microfluidic cartridge 102 into this slot of the imaging unit 107. The microfluidic cartridge 102 is positioned in the sample plane of the imaging unit 107, which enables the imaging unit 107 to image the biological specimen flowing through the microfluidic channels of the device. There are two openings for the inlet and outlet fluidic connectors to the microfluidic device. The flow of the input sample through the microfluidic cartridge 102 is facilitated with the help of a syringe pump.

Fig. 5A shows a flowchart illustrating a method for analyzing fluids using a microscopy system in accordance with some embodiments of the present disclosure.

30

As illustrated in **Fig. 5**, the method 500 comprises one or more blocks for analyzing fluids using a microscopy system 100. The method 400 may be described in the general context of computer executable instructions. Generally, computer executable instructions can include routines, programs, objects, components, data structures, procedures, modules, and functions, which perform particular functions or implement particular abstract data types.

35

The order in which the method 500 is described is not intended to be construed as a limitation, and any number of the described method blocks can be combined in any order to implement the method. Additionally, individual blocks may be deleted from the methods

5 without departing from the spirit and scope of the subject matter described herein. Furthermore, the method can be implemented in any suitable hardware, software, firmware, or combination thereof.

At **block 501**, receive input by a microfluidic cartridge 102. The input received by
10 the microfluidic cartridge 102, may be one of, fluids and reagents. Further, the fluids may be one of, a blood sample, urine sample, gas and any other fluids. The receiving of the at least one of the fluids is performed through an input channel designed in the cartridge, using the at least one of one or more reservoirs, at least one external container, and at least one micro-needle array. Receiving the at least one reagent is performed through one of, manually, one or
15 more reservoirs and a predefined chip comprising the reagents.

At **block 502**, illuminate, by an illumination unit, the mixture flowing through an imaging zone 302 of the microscopy system 100. The illumination unit 101 as shown in **Fig. 2** comprises illumination optics, which may use one of, an LED 201 and fluorescent lamp
20 (not shown in the figure) and a ground glass diffuser 202, to illuminate the microfluidic cartridge. An LED 201 and a ground-glass diffuser 202 have been used to provide the uniform illumination in the object plane of the imaging unit 107. The illumination unit 101 is controlled by the computing unit 108 according to the test performed on the input fluids.

At **block 503**, capture plurality of images by an imaging unit 107. The imaging unit
25 107 is configured in the microscopy system 100 to capture plurality of images of microscopic elements flowing through the imaging zone 302 of the microfluidic cartridge 102. The imaging unit may be a digital camera which may be integrated in an attachment and the replaceable microfluidic cartridge. The plurality of images is processed to form a processed
30 image. These images of microscopic elements may be recorded or stored in a storage device or memory unit and analyzed on the system. The computing unit 108 performs at least one of de-convolution and motion de-blurring on plurality of images to form a processed images, wherein converting the at least one of video in to plurality of images for further analysis. Further, the processed images are analyzed by the computing unit 108. The analysis of the
35 processed image is as required by the image acquisition to be performed. Further Confirmatory analysis of the processed image is conducted by one of, a computer system, a concerned person and any other unit.

Fig. 5B shows a flowchart illustrating a method for detecting predefined cells and obtaining their quantitative morphological features, in accordance with an embodiment of the

present disclosure. In an example embodiment, the predefined cell is a yeast cell. The yeast cell imaging is performed using the microscopy system 100 or microscopy system 100. The performance of the microscopy system 100 is measured by a morphological analysis based on suspension of yeast cells. The yeast species *saccharomyces cerevisiae* is cultured in yeast extract, peptone and dextrose (YPD) i.e. Himedia G037, liquid medium overnight at a temperature of approximately 37° C in orbital shaking incubator. The cells are harvested after incubation by centrifuging at 3000g for about 20 minutes. The harvested cells are re-suspended in phosphate buffer to a concentration of 106 cells/ ml. The suspension of cells is performed through the microfluidic cartridge 102, at a flow rate of 25 μlh^{-1} .

Using the integrated microscopy system 100, a video of the flow stream containing yeast cells is recorded. The data is analyzed using an image analysis application. The captured video is first split into frames and each frame is individually analyzed to extract the morphometric features of cells. Each frame is first threshold in order to identify the location of cells present in the frame. Using the contour analysis functions present in the image analysis application, the morphological features such as, but not limited to major and minor axis lengths of the cells are extracted. The images of about 569 yeast cells are captured, in an example embodiment.

Fig. 6 shows a plot illustrating yeast cell imaging and count, in accordance with an embodiment of the present disclosure. As shown in **Fig. 6**, a scatter plot of major and minor axis lengths for the recorded yeast cell population. From the scatter plot, it is evident that a wide range of morphologies are displayed by the yeast cell population. The largest entity identified in the scatter plot with label L^* is found to be a cluster of four cells shown in the **Fig. 6**. If the same population of cells had been analyzed using a flow cytometer of conventional method, then due to the non-availability of the image, the cluster of cells L^* would have been mistaken for a large single yeast cell.

In one embodiment, to obtain the distribution of morphological features, the images of cells corresponding to four gated regions of the scatter plot are analyzed. It can be seen from the images of the **Fig. 6** that the cluster gated region- (a) with the largest major and minor dimensions corresponds to cells, which are budding. From the gated regions and their corresponding representative images, it is evident that the size of cells correlates with their location in the scatter plot. Hence, this automated microscopy system 100 is capable of distinguishing cells based on their size and morphological features. In addition to its use in

5 medical diagnostics, this automated microscope has immense applicability for field research in environmental microbiology. The common practice in environmental microbiology studies is to collect field samples of water, bring them to lab to study the microorganisms present in the sample. The sample preparation mechanisms involved may be incorporated into the microfluidic chip and the process of analysis can be carried out on the field.

10 In an exemplary embodiment of the present disclosure, the microscope may be configured to provide rural clinical diagnostic settings, for performing malaria diagnosis test on a given blood sample. An in vitro culture of asexual stage *Plasmodium falciparum* infected RBC culture is diluted to obtain a suspension of cells with a concentration of 5×10^4 cells/ μ l. The staining buffer constitutes PBS of 10 mM phosphate buffer saline, mixed with 15 % methylene blue reagent. The methylene blue concentration of 0.5, 1, 2, and 5 % in PBS is used for staining infected cells in suspension, and the optimum concentration of 1% methylene blue resulted in appropriate staining of parasite as well as maintaining osmolarity for cells. Also, *falciparum* infected red blood cell cultures are used for proof of principle demonstration. However, the actual field trial is carried out on a whole blood sample. Thus, 20 the sample preparation step in the field trial would only involve mixing of three components such as, dilution buffer, blood and stain.

25 The suspension of stained malaria-infected RBC cultures is passed through the microfluidic device at a flow rate of about $50 \mu\text{lh}^{-1}$. The video of the flow stream is recorded for about 10 seconds at frame rate of 30 fps. As video is recorded only for 10 seconds, the effective analyzed is performed only a small portion ($\sim 35\text{nl}$) of the sample and detected the presence of malarial parasites in this small portion of the sample.

30 In one embodiment, the software application is used to analyse the video and automatically count the number of infected and healthy red blood cells. The software application is built to function on mobile operating system like but not limited to Android, iOS, Embedded Windows etc., and non-mobile operating systems like but not limited to Windows, Linux, MAC, etc. A color based thresholding is used to identify and count cells 35 which have absorbed the stain. The RGB frames are first converted into HSV space, following which appropriate thresholding applied to filter out the unstained, which are non-colored, regions of the frame. The contours, which are colored, left in the frame are counted, so as to obtain number of infected cells in a given frame. The flow chart of the algorithm to count the stained cells has been shown in **Fig. 7**.

5 **Fig. 7** shows an exemplary representation of an application to detect infected cells using the microscopy system 100, in accordance with an embodiment of the present disclosure. A comparative analysis of outputs of algorithms at different steps for infected and healthy cells is shown in **Fig. 7**. An application or algorithm used to count the number of yeast cells is used to count the uninfected cells. The analysis is performed by the computing unit 108 and using a custom-developed application, without the need for any other additional hardware. The method of counting the cells comprises recording a video of the flow stream using the pre-built imaging unit 107. After recording the video, the application analyzes the recording. The application receives inputs as the user details such as, but not limited to name, age, gender and other details. Thereafter, the user is directed to the gallery on the imaging unit 107, so as to select the video to be processed. The application processes the video to obtain the quantitative results and store in the on-board memory of the imaging unit 107. Finally, the display unit 109 provides at least one option such as display the results, show video and send results, to process the results to be displayed. The complete work-flow of the application is shown in **Fig. 8**.

Fig. 8 shows an illustration of malaria diagnostic analysis using an application microfluidic device, in accordance with an example embodiment of the present disclosure.

25 As shown in **Fig. 8**, the application takes user input, the input may be but not limited to the test being performed, user details, display reports, test to be performed, etc. **Fig. 8a** shows a user interface of the application. The application asks the user to enter the user details such as but not limited to name, gender, etc. **Fig. 8b** shows the page with the details entered. **Fig. 8c** shows the photographed images of the input sample to be analyzed. **Fig. 8d** shows the page asking the user to select one of, display analysis result, display video of the mixture flowing through the imaging unit 107. **Fig. 8e** shows the page displaying the analysis result for the performed test.

35 The integrated microscopy system 100 provides count stained and unstained cells in flow, without the use of any additional hardware. The integrated microscopy system 100 may be used to diagnose a multitude of diseases, which are conventionally diagnosed based on staining and microscopic examination. As it employs sample on-chip preparation, the microscope may potentially function as a point-of-care diagnostic device, which can be used to perform both qualitative and quantitative analysis of a given diagnostic raw sample.

5 **Fig. 9A** shows malaria diagnosis test simulation results, analyzed by the microscopy system 100 showing representative images of healthy red blood cells (RBC). **Fig. 9B** shows malaria diagnosis test simulation results, using microscopy system 100 showing representative images of malaria-infected RBCs, which have absorbed the stain. The images of cells obtained using the imaging unit 107 of the microscopy system 100 is in **Fig. 9A** and **Fig. 9B**. In an example embodiment, a total of 594 image of red blood cells have been captured, of which 18 were found to be infected i.e. absorbed the stain. From the captured cells, the quantitative level of parasitemia was assessed to be 3.1%. In conventional methods, it would take a skilled technician at-least 1 to 2 hours to perform a malaria diagnostic test, whereas the microscopy system 100 counts and images 3500 cells in a minute, in an example embodiment. Using the high frame rate imaging mode (60 fps) of the camera, a higher imaging throughputs may be obtained of about 465 cells per second or 27900 cells in a minute. Hence, the microscopy system 100 takes about 3.5 minutes to image or 100,000 cells, so as to detect parasitemia levels of 0.001%. The ability to assess quantitative level of parasitemia on such a portable platform potentially enables automated drug dosage level determination and helps in monitoring disease prognosis without the need for a skilled personnel.

Embodiments of the present disclosure provide the advantages such as, but not limited to determining the type of parasite, RDTs, inexpensiveness, automation, speed, high-sensitivity, and quantitative morphometric analysis. Also, the test results demonstrate for the first time, custom designed optics and microfluidics are fully automated and versatile integrated optofluidic devices which may be used for clinical diagnosis as well as biological research.

Embodiments of the present disclosure address the need of a label free detection of cells using just simple bright-field imaging for disease diagnosis. Also, the system facilitates detecting cancer at an early stage where the onset of a disease is characterized by a change on/within the cells of a biological sample. The changes may be detected by the analysis of bright-field images obtained by the custom built imaging flow cytometer. Further, the detection is improved by using a phase imaging coupled with flow cytometry. The phase imaging flow cytometry provides the volumetric information and 3-D shape of cells, which may help in providing a very reliable signature of diseases as compared to 2-D images alone.

5 Finally, the language used in the specification has been principally selected for readability and instructional purposes, and it may not have been selected to delineate or circumscribe the inventive subject matter. It is therefore intended that the scope of the invention be limited not by this detailed description, but rather by any claims that issue on an application based here on. Accordingly, the disclosure of the embodiments of the invention is
10 intended to be illustrative, but not limiting, of the scope of the invention, which is set forth in the following claims.

With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the singular to
15 the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for sake of clarity.

In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby
20 described in terms of any individual member or subgroup of members of the Markush group.

While various aspects and embodiments have been disclosed herein, other aspects and embodiments will be apparent to those skilled in the art. The various aspects and embodiments disclosed herein are for purposes of illustration and are not intended to be
25 limiting, with the true scope and spirit being indicated by the following claims.

REFERENCE NUMERALS

Reference numbers	Description
100	Microscopy System
101	Illumination Unit
102	Microfluidic Cartridge
103	Micro-needle Array
104	Intake chip
105	Microfluidic Pump
106	Optical Enhancement Unit
107	Imaging Unit

108	Computing Unit
109	Display Unit
110	Communication Unit
201	LED
202	Ground Glass Diffuser
301	Mixing Zone
302	Imaging Zone
303	First Channel
304	Second Channel
305	Third Channel
500	Method to analyze fluids using a microscopy system

5 Claims:

1. A microscopy system for analyzing fluids, comprising:
a microfluidic cartridge to receive input comprising at least one of fluids and reagents,
the cartridge comprising:
10 a mixing zone to receive the at least one of fluids and reagents to form a mixture;
and
an imaging zone to receive the mixture from the mixing zone;
an illumination unit to illuminate the imaging zone; and
at least one imaging unit to capture plurality of images of one or more microscopic
15 elements of the mixture flowing through the imaging zone, wherein the plurality of
images is processed by a computing unit associated with the microscopy system for
analyzing fluids.
2. The microscopy system as claimed in claim 1, wherein the microfluidic cartridge is
20 replaceable based on the fluids to be analyzed.
3. The microscopy system as claimed in claim 1, wherein the input is received by the
cartridge from one or more input channels of the cartridge.
- 25 4. The microscopy system as claimed in claim 1, wherein the at least one of fluids and
reagents are mixed in a predefined proportion in the mixing zone to form a mixture.
5. The microscopy system as claimed in claims 3, wherein the predefined proportion of the
at least one of fluids and reagents is regulated by geometry of one or more mixing
30 channels in the mixing zone.
6. The microscopy system as claimed in claim 1, further comprises a pump to control the
flow rate of the input and the mixture in the cartridge.
- 35 7. The system as claimed in claim 1, wherein the illumination provided by the illumination
unit is controlled by the computing unit, as required by the image acquisition to be
performed.

- 5 8. The microscopy system as claimed in claim 1, wherein the plurality of images is processed by the computing unit by receiving the plurality of images of one or more microscopic elements of the mixture;
9. The microscopy system as claimed in claim 1, wherein result of the fluid analysis is
10 displayed on at least one display unit.
10. The microscopy system as claimed in claim 1, comprises a communication module for communicating at least one of the plurality of images and result of the fluid analysis.
- 15 11. A method for analyzing fluids using a microscopy system, the method comprising:
receiving, by a cartridge, at least one of, fluids and reagents, wherein the at least one of the fluids is mixed with the at least one of the reagents in a predefined proportion in a mixing zone;
illuminating, by an illumination unit, an imaging zone of the microscopy system;
20 capturing, by an imaging unit, plurality of images of one or more microscopic elements of the mixture flowing through the imaging zone, wherein the at least one of the plurality of images is processed, by a computing unit for analysis;
12. The method as claimed in claim 11, wherein receiving the at least one of the fluids and
25 reagents is performed through an input channel of the cartridge.
13. The method as claimed in claim 11, wherein the proportion of at least one of the fluids and reagents are regulated by the geometry of one or more mixing channels in the mixing zone.
30
14. The method as claimed in claim 11, wherein the illumination unit illuminates the imaging zone, as required by the image acquisition to be performed.
15. The method as claimed in claim 11, wherein the imaging unit captures plurality of
35 pictures of the one or more microscopic elements of the mixture, the method further comprising, processing the at least one of the plurality of images by the computing unit for analysis.
16. The method as claimed in claim 11, wherein the method comprises displaying the
40 analysis results on a display by the computing unit.

5

17. The method as claimed in claim 11, wherein the method comprises communicating the at least one of the analysis results and the plurality of images by a communication unit.

10

18. The method as claimed in claim 11, wherein the microfluidic cartridge is replaceable based on the fluids to be analyzed.

15

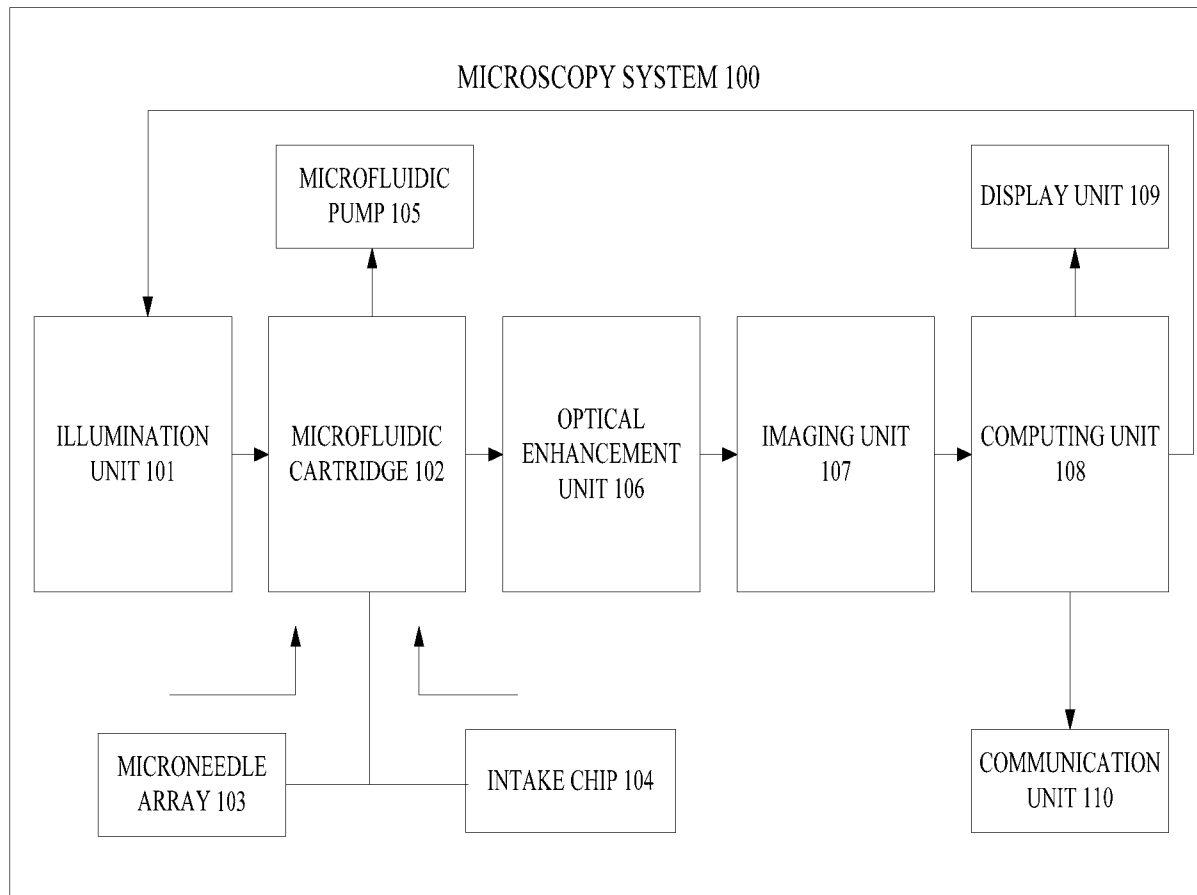


Figure 1

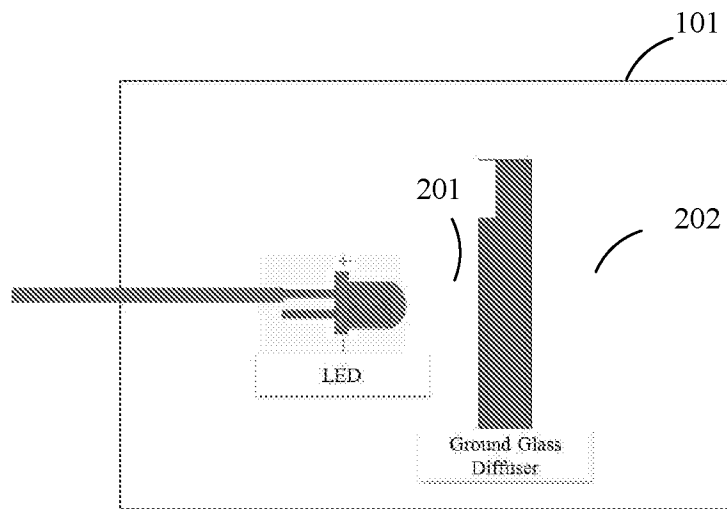


Figure 2

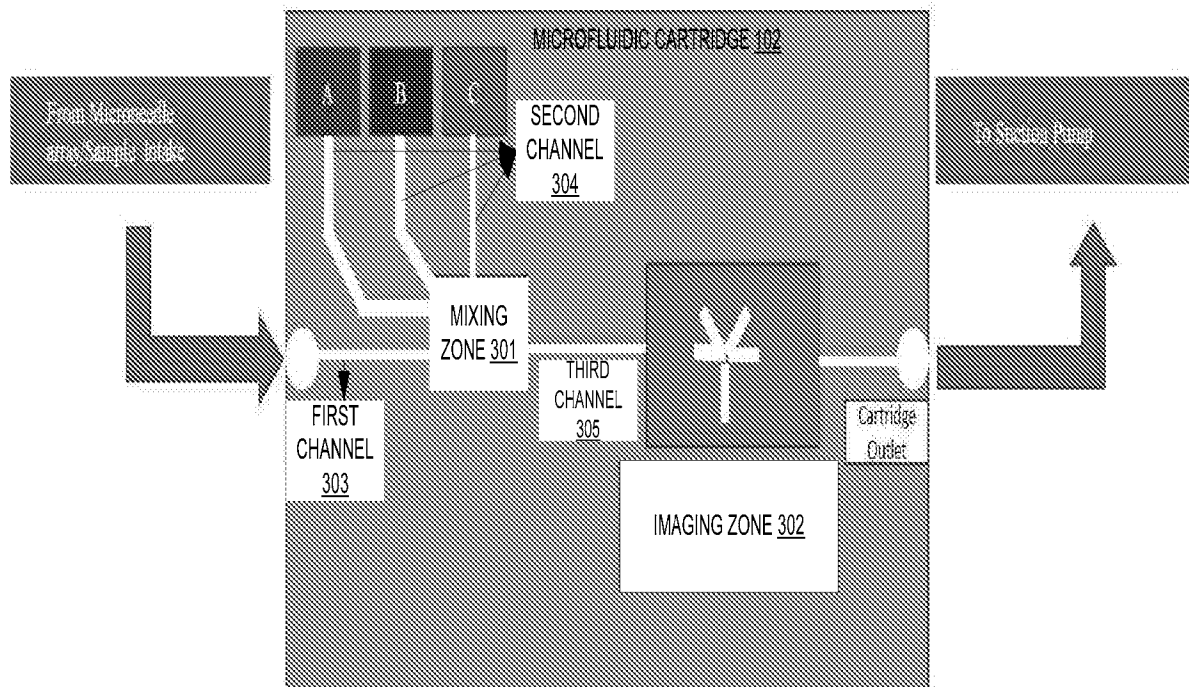


Figure 3

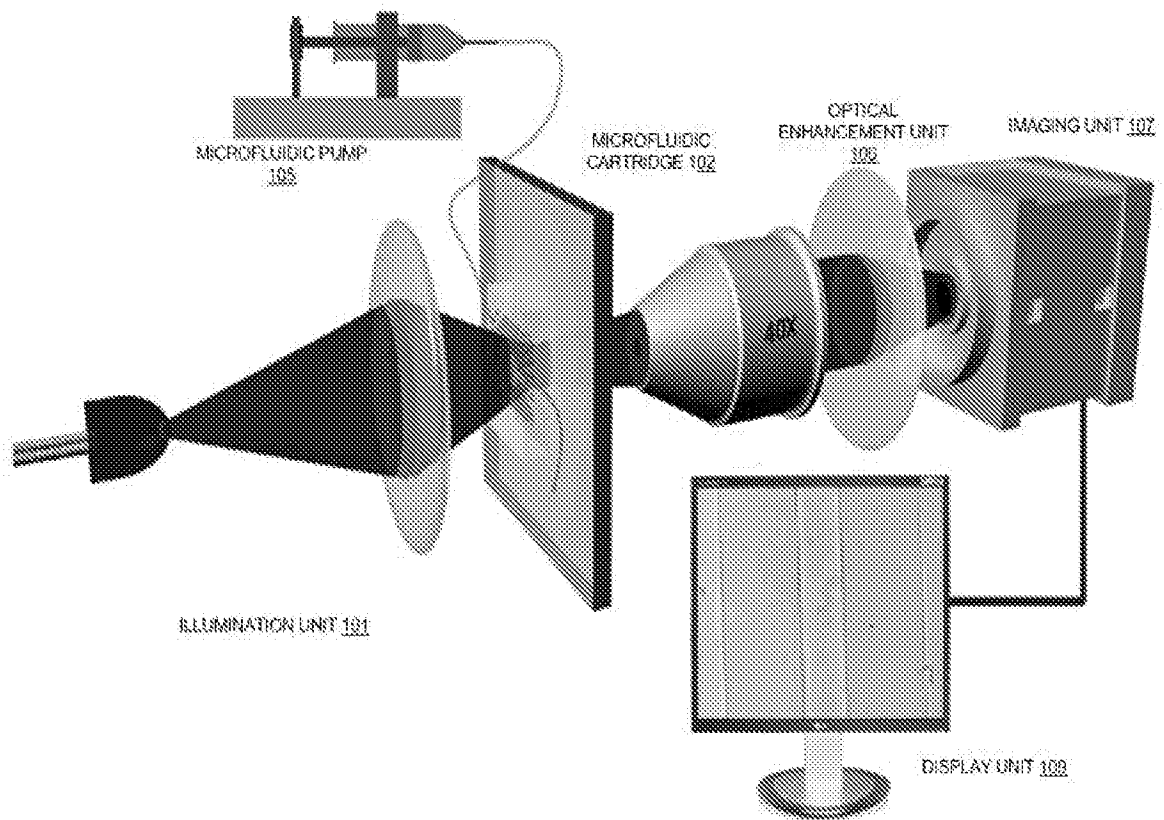


Figure 4

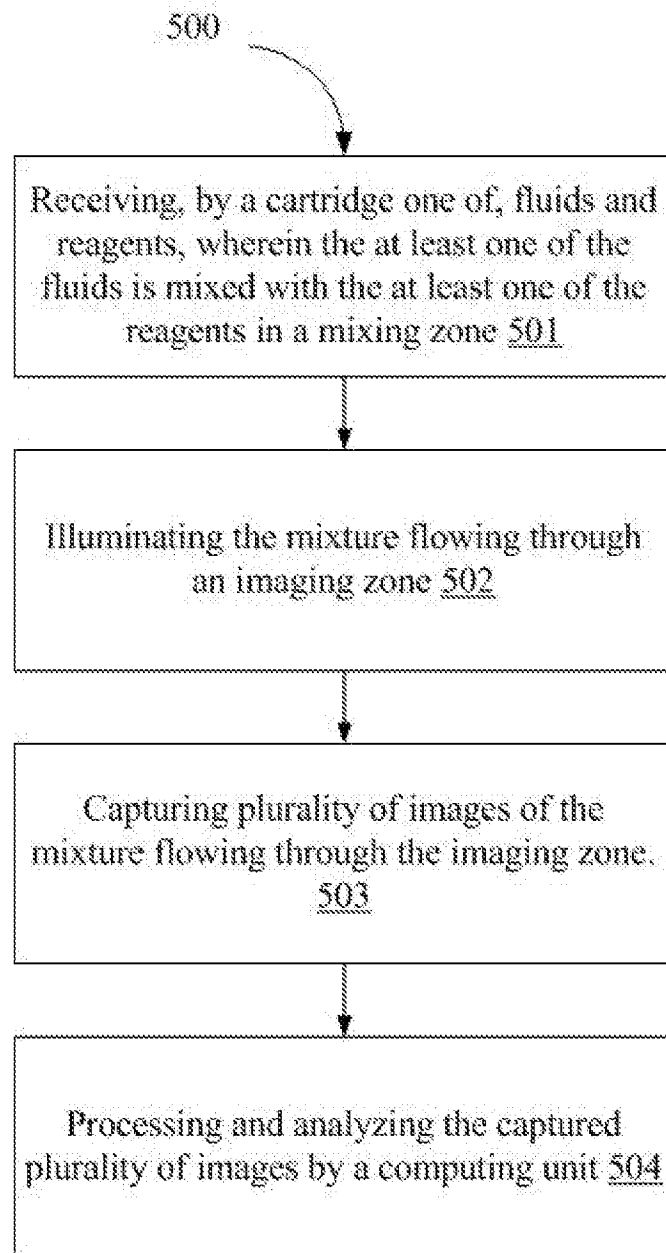


Figure 5A

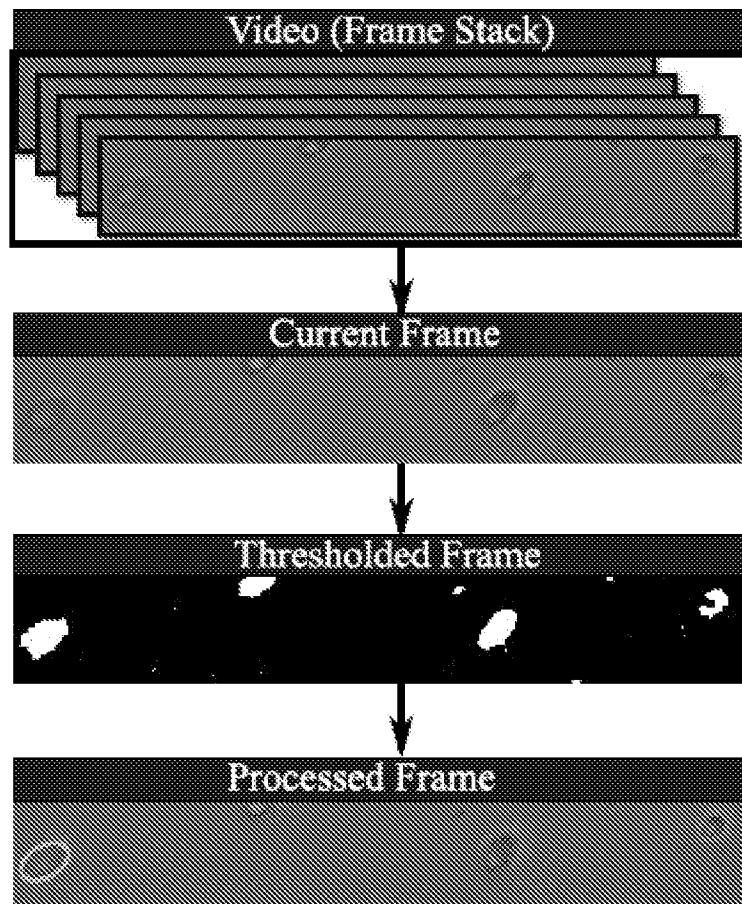


Figure 5B

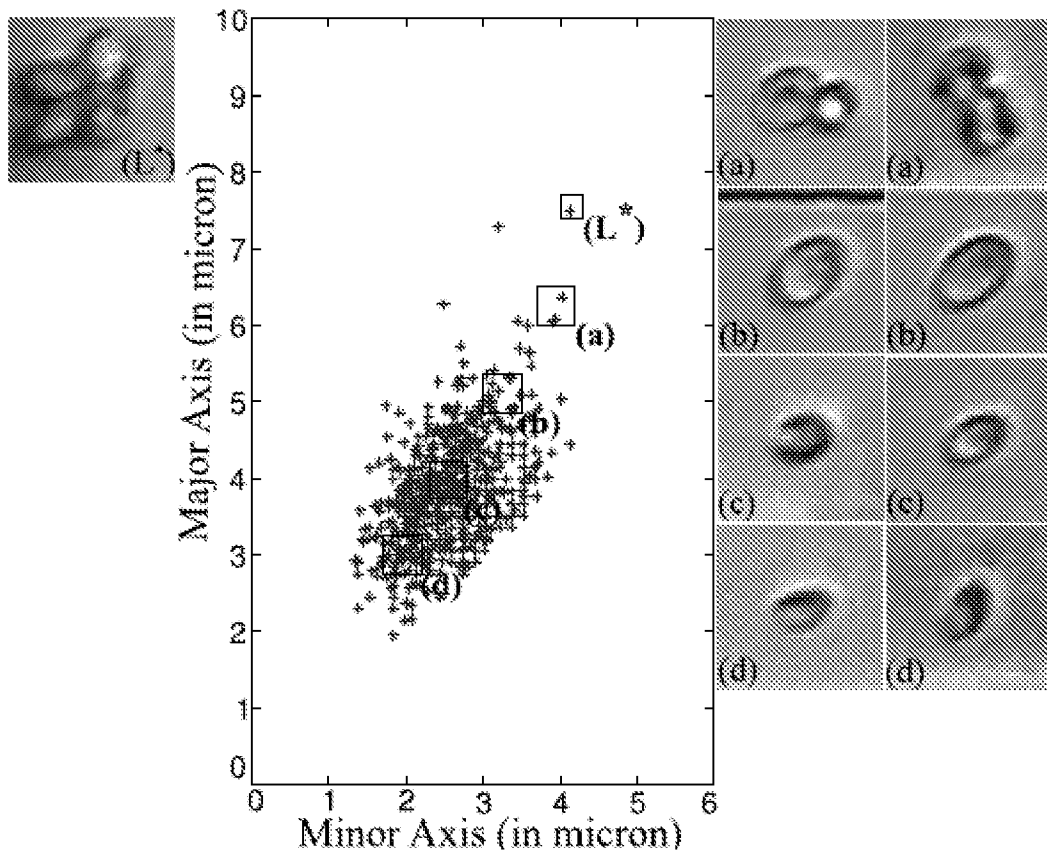


Figure 6

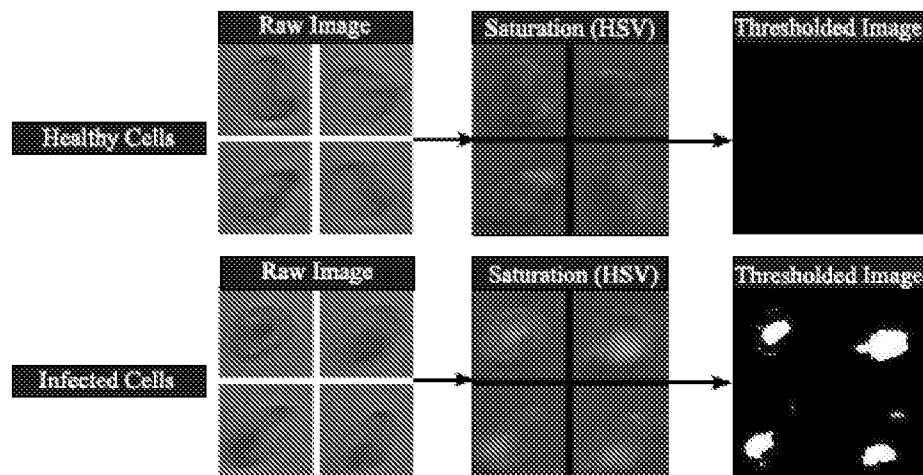


Figure 7

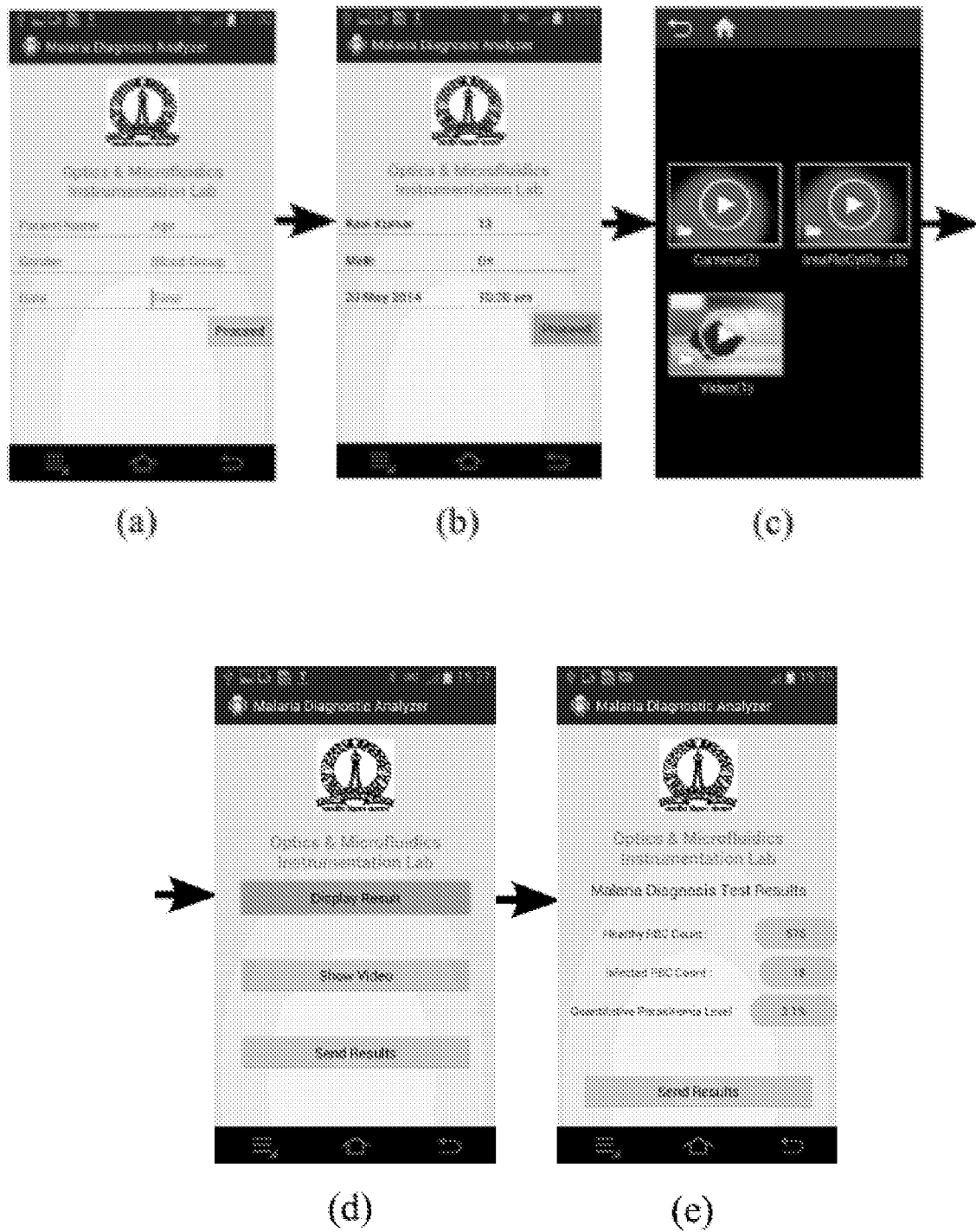


Figure 8

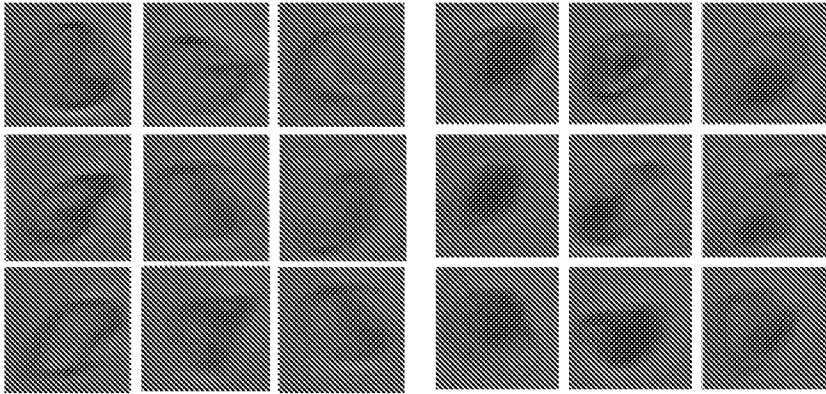


Figure 9A

Figure 9B