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(54) Title: FACILITATING CONTROLLED PARTICLE DEPOSITION FROM A DROPLET DISPENSER

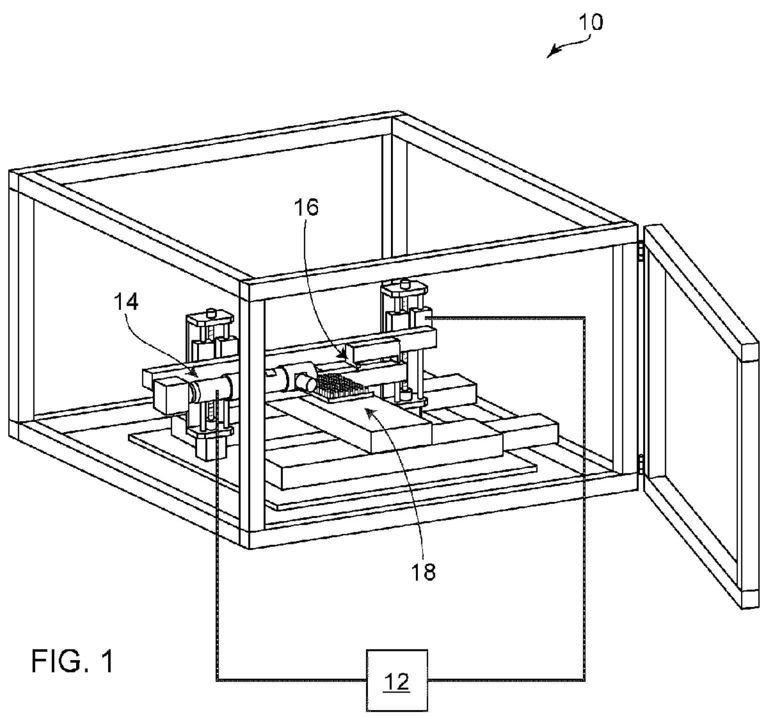

FIG. 1

(57) Abrégé/Abstract:
A method of facilitating controlled particle deposition from a droplet dispenser involves receiving at least one pre-dispensed image of a dispensing portion of the droplet dispenser, the dispensing portion including fluid to be dispensed in a subject droplet to a subject target region, receiving at least one post-dispensed image of the dispensing portion of the droplet dispenser after the subject droplet has been dispensed, comparing the at least one pre-dispensed image and the at least one post-dispensed image to determine a subject droplet particle count representing a count of particles included in the subject droplet, and producing signals for associating a representation of the subject droplet particle count with the subject target region. Other methods, apparatuses, systems, and non-transitory computer readable media are disclosed.

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(54) Title: FACILITATING CONTROLLED PARTICLE DEPOSITION FROM A DROPLET DISPENSER

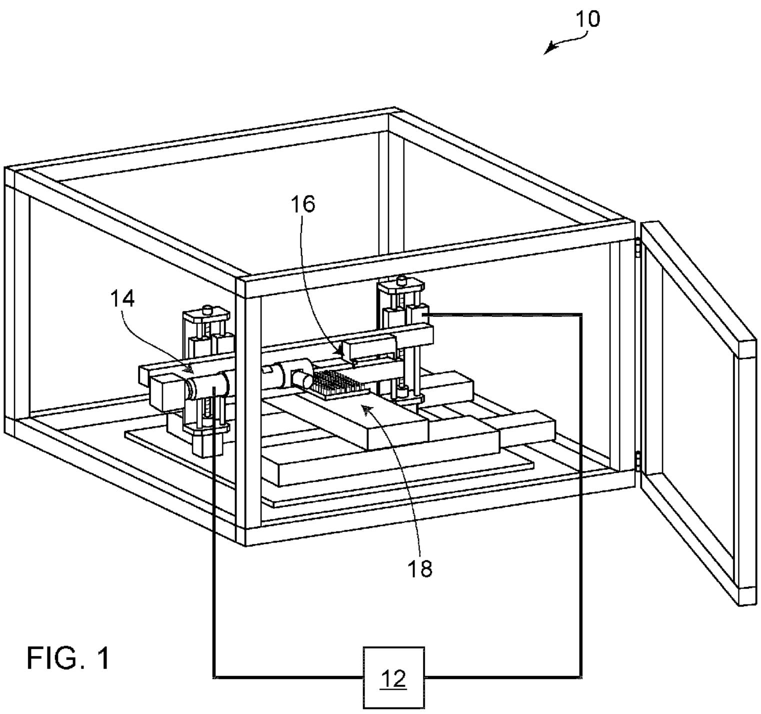

FIG. 1

(57) Abstract: A method of facilitating controlled particle deposition from a droplet dispenser involves receiving at least one pre-dispensed image of a dispensing portion of the droplet dispenser, the dispensing portion including fluid to be dispensed in a subject droplet to a subject target region, receiving at least one post-dispensed image of the dispensing portion of the droplet dispenser after the subject droplet has been dispensed, comparing the at least one pre-dispensed image and the at least one post-dispensed image to determine a subject droplet particle count representing a count of particles included in the subject droplet, and producing signals for associating a representation of the subject droplet particle count with the subject target region. Other methods, apparatuses, systems, and non-transitory computer readable media are disclosed.

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WO 2021/022374 A1

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FACILITATING CONTROLLED PARTICLE DEPOSITION FROM A DROPLET DISPENSER

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/884,007 entitled "FACILITATING CONTROLLED PARTICLE DEPOSITION FROM A DROPLET DISPENSER", filed on August 7, 2019, which is hereby incorporated by reference herein in its entirety.

BACKGROUND

1. Field

Embodiments of this invention relate to controlled particle deposition and more particularly to facilitating controlled particle deposition from a droplet dispenser.

2. Description of Related Art

Controlled particle deposition may be used to isolate particles or cells for various applications including for next generation sequencing workflows. Some known particle deposition systems may use microfluidic chips that can manipulate cell-sized particles, nano-liter sized jetted droplets wherein the droplets encapsulate the particles or cells, microfluidic droplet generators and/or fluorescence-activated cell sorters (FACS), for example. Some systems rely on random partitioning of the particles or cells into nanowells or droplets, following the Poisson distribution. This may result in a low fill factor with a large number of empty wells or droplets with a persistent component of multiple-cell events. Some known systems may lack the ability to validate single-cell events, especially within small timescales, which may be required for some applications, such as, for example, RNA sequencing applications.

SUMMARY

In accordance with various embodiments, there is provided a method of facilitating controlled particle deposition from a droplet dispenser, the method involving receiving at least one pre-dispensed image of a dispensing portion of the droplet dispenser, the dispensing portion including fluid to be dispensed in a subject droplet to a subject target region, receiving at least one post-dispensed image of the dispensing portion of the droplet dispenser after the subject droplet has been dispensed, comparing the at least one pre-dispensed image and the at least one post-dispensed image to determine a subject droplet particle count representing a count of particles included in the subject droplet, and producing signals for associating a representation of the subject droplet particle count with the subject target region.

Comparing the at least one pre-dispensed image and the at least one post-dispensed image may involve causing a representation of the at least one pre-dispensed image and the at least one post-dispensed image to be input into one or more functions.

Causing the representation of the at least one pre-dispensed image and the at least one post-dispensed image to be input into the one or more functions may involve causing the one or more functions to generate a plurality of count confidences, each associated with a respective prospective particle count.

Comparing the at least one pre-dispensed image and the at least one post-dispensed image to determine the subject droplet particle count may involve determining the subject droplet particle count to be the particle count associated with the largest of the plurality of count confidences.

The method may involve comparing the largest of the plurality of count confidences to a threshold confidence to determine whether the largest of the plurality of confidences is less than the threshold confidence.

Comparing the at least one pre-dispensed image and the at least one post-dispensed image may involve generating a first image difference representing a difference between the first pre-dispensed image and the at least one post-dispensed image, and causing a representation of the first image difference to be input into the one or more functions.

The first pre-dispensed image may represent the droplet dispenser at a first pre-dispensed time and the at least one pre-dispensed image may include a second pre-dispensed image representing the droplet dispenser at a second pre-dispensed time prior to the first pre-dispensed time. Comparing the at least one pre-dispensed image and the at least one post-dispensed image may involve generating a second image difference representing a difference between the second pre-dispensed image and the first pre-dispensed image, and causing a representation of the second image difference to be input into the one or more functions.

The method may involve causing a first preceding droplet to be dispensed by the droplet dispenser between the second pre-dispensed time and the first pre-dispensed time.

The at least one pre-dispensed image may include a third pre-dispensed image representing the droplet dispenser at a third pre-dispensed time prior to the second pre-dispensed time, and comparing the at least one pre-dispensed image and the at least one post-dispensed image may involve generating a third image difference representing a difference between the third pre-dispensed image and the second pre-dispensed image, and causing a representation of the third image difference to be input into the one or more functions.

The method may involve causing a second preceding droplet to be dispensed by the droplet dispenser between the third pre-dispensed time and the second pre-dispensed time.

The one or more functions may include one or more neural network functions.

The method may involve training the one or more neural network functions.

Producing signals for associating the representation of the subject droplet particle count with the subject target region may involve determining whether the subject droplet particle count matches a desired particle count and, if the subject droplet particle count matches the desired particle count, producing signals for identifying the subject target region as containing the desired particle count.

The method may involve determining whether the subject droplet particle count is less than a desired particle count and, if the subject droplet particle count is not less than the desired particle count, producing signals for causing the droplet dispenser to be configured to dispense a subsequent droplet to a subsequent target region different from the subject target region.

The method may involve determining whether the subject droplet particle count is less than a desired particle count and, if the subject droplet particle count is less than the desired particle count, producing signals for causing the droplet dispenser to dispense a further droplet to the subject target region.

The method may involve identifying one or more pre-dispensed image particles depicted in the at least one pre-dispensed image, and identifying one or more post-dispensed image particles depicted in the at least one post-dispensed image. Comparing the at least one pre-dispensed image and the at least one post-dispensed image may involve identifying at least one unmatched pre-dispensed image particle of the one or more pre-dispensed image particles as not matching any of the post-dispensed image particles and therefore representing a particle included in the subject droplet, and determining the subject droplet particle count as a count of the at least one unmatched pre-dispensed image particle.

In accordance with various embodiments, there is provided a method of training at least one neural network function for facilitating controlled particle deposition, the method involving receiving a plurality of sets of training images, each of the sets of training images including at least one pre-dispensed image of a dispensing portion of a droplet dispenser, the dispensing portion including fluid to be dispensed in a droplet to a target region, and at least one post-dispensed image of the dispensing portion of the droplet dispenser after the droplet has been dispensed. The method involves receiving a plurality of droplet particle counts, each of the droplet particle counts associated with one of the sets of training images and representing a count of particles dispensed in the droplet that is the subject of the associated set of training images, and causing the at least one neural network function to be trained using representations of the sets of training images as respective inputs and the associated droplet counts as desired outputs.

For each of the sets of training images, the at least one pre-dispensed image included in the set of training images may include a first pre-dispensed image, and causing the at least one neural network function to be trained may involve generating a first image difference representing a difference between the first pre-dispensed image and the at least one post-dispensed image, and causing a representation of the first image difference to be input into the at least one neural network function.

For each of the sets of training images, the first pre-dispensed image included in the set of training images may represent the droplet dispenser at a first pre-dispensed time. The at least one pre-dispensed image included in the set of training images may include a second pre-dispensed image representing the droplet dispenser at a second pre-dispensed time prior to the first pre-dispensed time. Causing the at least one neural network function to be trained may involve generating a second image difference representing a difference between the second pre-dispensed image and the first pre-dispensed image, and causing a representation of the second image difference to be input into the at least one neural network function.

The second pre-dispensed image may represent the droplet dispenser at the second pre-dispensed time prior to the first pre-dispensed time, a first preceding droplet having been dispensed by the droplet dispenser between the second pre-dispensed time and the first pre-dispensed time.

For each of the sets of training images, the at least one pre-dispensed image included in the set of training images may include a third pre-dispensed image representing the droplet dispenser at a third pre-dispensed time prior to the second pre-dispensed time. Causing the at least one neural network function to be trained may involve generating a third image difference representing a difference between the third pre-dispensed image and the second pre-dispensed image, and causing a representation of the third image difference to be input into at least one neural network function.

The third pre-dispensed image may represent the droplet dispenser at the third pre-dispensed time prior to the second pre-dispensed time, a second preceding droplet having been dispensed by the droplet dispenser between the third pre-dispensed time and the second pre-dispensed time.

In accordance with various embodiments, there is provided a system for facilitating controlled particle deposition from a droplet dispenser, the system comprising at least one processor configured to perform any of the above methods.

In accordance with various embodiments, there is provided a non-transitory computer readable medium having stored thereon codes that, when executed by at least one processor, cause the at least one processor to perform any of the above methods.

In accordance with various embodiments, there is provided a system for facilitating controlled particle deposition from a droplet dispenser, the system including means for receiving at least one pre-dispensed image of a dispensing portion of the droplet dispenser, the dispensing portion including fluid to be dispensed in a subject droplet to a subject target region, means for receiving at least one post-dispensed image of the dispensing portion of the droplet dispenser after the subject droplet has been dispensed, means for comparing the at least one pre-dispensed image and the at least one post-dispensed image to determine a subject droplet particle count representing a count of particles included in the subject droplet, and means for producing signals for associating a representation of the subject droplet particle count with the subject target region.

In accordance with various embodiments, there is provided a system for training at least one neural network function for facilitating controlled particle deposition, the system including means for receiving a plurality of sets of training images, each of the sets of training images including at least one pre-dispensed image of a dispensing portion of a droplet dispenser, the dispensing portion including fluid to be dispensed in a subject droplet to a subject target region, and at least one post-dispensed image of the dispensing portion of the droplet dispenser after the subject droplet has been dispensed. The system includes means for receiving a plurality of subject droplet particle counts, each of the subject droplet particle counts associated with one of the sets of training images and representing a count of particles dispensed in the subject droplet for the set of training images, and means for causing the at least one neural network function to be trained using representations of the sets of training images as respective inputs and the associated subject droplet counts as desired outputs.

Other aspects and features of embodiments of the invention will become apparent to those ordinarily skilled in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

In drawings which illustrate embodiments of the invention,

Figure **1** is a schematic perspective view of a system for facilitating controlled particle deposition according to various embodiments;

Figure **2** is a front perspective view of a portion of the system shown in Figure **1** according to various embodiments;

Figure **3** is a schematic view of a controller of the system shown in Figure **1** including a processor circuit according to various embodiments;

Figure **4** is a flowchart depicting blocks of code for directing the controller shown in Figure **3** to perform particle deposition control functions in accordance with various embodiments;

Figure **5** is a representation of a first pre-dispensed image that may be used in the system shown in Figure **1** in accordance with various embodiments;

Figure **6** is a representation of a second pre-dispensed image that may be used in the system shown in Figure **1** in accordance with various embodiments;

Figure **7** is a representation of a third pre-dispensed image that may be used in the system shown in Figure **1** in accordance with various embodiments;

Figure **8** is a flowchart depicting blocks of code that may be included in the flowchart shown in Figure **4** in accordance with various embodiments;

Figure **9** is a representation of a post-dispensed image that may be used in the system shown in Figure **1** in accordance with various embodiments;

Figure **10** is a representation of a neural network that may be used in the system shown in Figure **1** in accordance with various embodiments;

Figure **11** is a flowchart depicting blocks of code that may be included in the flowchart shown in Figure **4** in accordance with various embodiments;

Figure **12** is a representation of a target count record that may be used in the system shown in Figure **1** in accordance with various embodiments;

Figure **13** is a representation of a target count confidence record that may be used in the system shown in Figure **1** in accordance with various embodiments;

Figure **14** is a flowchart depicting blocks of code that may be included in the flowchart shown in Figure **4** in accordance with various embodiments;

Figure **15** is a representation of a target count validity record that may be used in the system shown in Figure **1** in accordance with various embodiments;

Figure **16** is a schematic view of a system for facilitating controlled particle deposition including neural network training in accordance with various embodiments;

Figure **17** is a schematic view of a controlled particle deposition trainer of the system shown in Figure **16** including a processor circuit according to various embodiments;

Figure **18** is a flowchart depicting blocks of code for directing the trainer shown in Figure **17** to perform particle deposition control functions in accordance with various embodiments;

Figure **19** is a schematic view of a controller that may be included in the system shown in Figure **1** including a processor circuit according to various embodiments;

Figure **20** is a flowchart depicting blocks of code for directing the controller shown in Figure **19** to perform particle deposition control functions in accordance with various embodiments;

Figure **21** is a representation of a pre-dispensed image that may be used by the controller shown in Figure **19** in accordance with various embodiments;

Figure **22** is a flowchart depicting blocks of code that may be included in the flowchart shown in Figure **20** in accordance with various embodiments;

Figure **23** is a representation of a filtered image that may be used by the controller shown in Figure **19** in accordance with various embodiments;

Figure **24** is a representation of a particle positions record that may be used by the controller shown in Figure **19** in accordance with various embodiments;

Figure **25** is a representation of a post-dispensed image that may be used by the controller shown in Figure **19** in accordance with various embodiments;

Figure **26** is a representation of a particle positions record that may be used by the controller shown in Figure **19** in accordance with various embodiments;

Figure **27** is a flowchart depicting blocks of code that may be included in the flowchart shown in Figure **20** in accordance with various embodiments;

Figure **28** is a flowchart depicting blocks of code that may be included in the flowchart shown in Figure **20** in accordance with various embodiments;

Figure **29** is a representation of a neural network that may be used in a system generally similar to the system shown in Figure **1** in accordance with various embodiments;

Figure **30** is a schematic view of an implementation of the system shown in Figure **1** in accordance with various embodiments; and

Figure **31** is a representation of an exemplary graphical user interface (“GUI”) that may be displayed by a display included in the system shown in Figure **1** in accordance with various embodiments.

DETAILED DESCRIPTION

Controlled particle deposition may be desirable for various applications, such as, for example, to facilitate single-cell next generation sequencing workflows, such as biological cell genomic sequencing workflows. In some embodiments, by probing at the single-cell level, insights may be gained at much higher phenotypic resolution than was previously attainable at the bulk tissue analysis level. Some single-cell next generation sequencing workflows may be described in four major steps: **1**) single-cell isolation, **2**) content amplification, **3**) sequencing and **4**) data processing. While sequencing technology has matured at a rate outpacing Moore’s Law due to a reduction in cost, an increase in read accuracy, and more parallelized throughput, some cell isolation, such as single-cell isolation, methodologies have lagged behind, limited by low throughput and a lack in data confidence – that is, whether the data was generated from a single cell or from multiple cells. In accordance with various embodiments, there is described herein a controlled particle deposition system that may be used for cell isolation, such as single-cell isolation.

Referring to Figure **1**, a system for facilitating controlled particle deposition in accordance with various embodiments is shown at **10**. In accordance with various embodiments, the system **10** may facilitate a high-throughput particle dispenser, such as a single-cell dispenser, for example, that may be used in various applications, including, for example, next generation sequencing systems.

Referring to Figure **1**, the system **10** includes a controller **12** in communication with an imager **14** and a substrate stage **18**. The system **10** also includes a droplet dispenser **16**. In some embodiments, the droplet dispenser **16** may be controllable via signals produced by the imager **14** and so the droplet dispenser **16** may be indirectly controllable by the controller **12** via the imager **14**.

In various embodiments, the controller **12** may be configured to control particle deposition by the droplet dispenser **16** onto the substrate stage **18**. For example, in some embodiments, the droplet dispenser **16** may be in communication with a fluid source having a source fluid or suspension including a plurality of particles or cells and the controller **12** may be configured to cause the droplet dispenser **16** to dispense, from the source fluid, fluid including a desired number of particles or cells (such as, for example, a single particle or cell) into target regions of the substrate stage **18**. In various embodiments, the controller **12** may be configured to use information received from the imager **14** and/or control of the droplet dispenser **16** and/or the substrate stage **18** to facilitate this control.

Referring to Figure **2**, the imager **14**, the droplet dispenser **16** and the substrate stage **18** are shown from a front perspective view with a zoomed in partial view **40** included.

In various embodiments, the imager **14** may include a camera and lens system fixed to the droplet dispenser **16**. In some embodiments, the imager **14** may include, for example, a FLIR BlackflyS BFS-U3-32S4 camera configured to capture images with exposure time of about 3-5 ms and a Thor Labs MVL12X3Z zoom lens with attachments. In some embodiments, referring to Figure **2**, the imager **14** may be fixed to the droplet dispenser via mount **50**. In various embodiments, by fixing the imager **14** in place relative to the droplet dispenser **16**, continuous imaging feedback of the nozzle may be used and/or images may be acquired and saved at small time scales which may validate the deposition of a desired number of particles, such as a single cell or particle with high confidence.

In various embodiments, the droplet dispenser **16** may include a controllable inkjet dispenser having a dispensing portion **52**, which may for example include an inkjet nozzle, in communication with the fluid source. In various embodiments, an inkjet dispenser may be configured to dispense low volumes of fluid (e.g., in the picoliter range). In some embodiments, for example, the droplet dispenser **16** may include a Microfab ABP-01, which may be configured to dispense 300-600 picoliters at a time. In various embodiments dispensing low volumes of fluid compared to other dispensers may provide various advantages, such as, for example, a decrease in the probability of capturing ambient DNA/RNA within a cell suspension, which may be desirable for single-cell isolation in genomics applications, for example.

In some embodiments, the system **10** may be used to dispense fluid having a high viscosity, a neutrally buoyant solution, and/or the droplet dispenser **16** may include an inkjet nozzle with tuned physical parameters configured to allow for optimized particle deposition. For example, in some embodiments, the inkjet nozzle may have geometries or taper angles that help to ensure that particles are moving forward or downstream towards a nozzle orifice when dispensing takes place. For example, in some embodiments, the taper angles may be less than about 15º (See, for example, B. Enright, E. Cheng, H. Yu, K. C. Cheung, and A. Ahmadi, "Inkjet

System Design Optimization For Reliable Cell Printing," *21st Int. Conf. Miniaturized Syst. Chem. Life Sci.*, 2017). In some embodiments, for example, the solution may be 10% w/v Ficoll PM 400 in Phosphate Buffered Saline (PBS). In various embodiments, by carefully controlling the input cell concentration into the inkjet nozzle, the system **10** may be capable of dispensing cells at a rapid rate with a large population of single-cells. In various embodiments, under controlled conditions, a Poisson distribution may be achieved from dispensing cells within inkjet nozzles as described herein. For example, by controlling the cell concentration, in some embodiments, the system **10** may be able to achieve higher single cell component when compared to the multiple cell component; however, there may be a large component with no cells. Or if the cell concentration is increased, in some embodiments, the system **10** may be able to have a maximum single cell component of about 37% with an equal probability of zero cells. In various embodiments, either of these conditions may be appropriate for use under certain experiments; however, the random seeding of cells and persistent non-one cell component may not be ideal in many cases.

In some embodiments, the droplet dispenser **16** and the imager **14** may be held in a fixed position over the substrate stage **18**, which may be controllable to move relative to the droplet dispenser **16** and the imager **14** to change/update a target region for the droplet dispenser **16** in the substrate stage **18**. In some embodiments, the substrate stage **18** may include an x-y-z controllable stage having thereon a microplate or microtiter plate **30** including a plurality of target regions or wells. In some embodiments, the substrate stage **18** may be configured to control a yaw axis of rotation of the microplate **30**. In various embodiments, the microplate **30** may include a machined aluminum microwell chip including an array of 72 by 72 wells or targets, for example.

Referring still to Figure **2**, the imager **14** may be configured to capture images of the dispensing portion **52** of the droplet dispenser **16** and to transmit signals representing the images of the dispensing portion **52** to the controller **12**. In various embodiments, the controller **12** may be configured to receive at least one pre-dispensed image of the dispensing portion **52** of the droplet dispenser **16**, the dispensing portion including fluid to be dispensed in a droplet to a first target region **54** of the substrate stage **18**. The droplet dispenser **16** may then dispense the droplet into the first target region **54**. For example, in some embodiments, after receiving the pre-dispensed image, the controller **12** may produce signals for causing the imager **14** to cause the droplet dispenser **16** to dispense the droplet into the first target region **54**.

In some embodiments, the received signals by the imager **14** from the controller **12** may also trigger the imager **14** capturing and sending at least one post-dispensed image of the dispensing portion **52** of the droplet dispenser **16** to the controller **12**, the post-dispensed image depicting the dispensing portion **52** after the droplet has been dispensed.

In various embodiments, the controller **12** may be configured to receive the at least one post-dispensed image of the dispensing portion **52** of the droplet dispenser **16** after the droplet has been dispensed from the imager **14**.

In various embodiments, the controller **12** may then compare the at least one pre-dispensed image and the at least one post-dispensed image to determine a droplet particle count representing a count of particles included in the dispensed droplet. For example, in some embodiments, the controller **12** may be configured to cause a representation of the at least one pre-dispensed image and the at least one post-dispensed image to be input into one or more functions, such as a neural network function. In some embodiments, the controller **12** may be configured to generate a plurality of count confidences, each associated with a respective prospective particle count or class and to determine the droplet particle count to be the particle count associated with the largest of the plurality of count confidences.

In various embodiments, the controller **12** may then produce signals for associating a representation of the droplet particle count with the target region. In some embodiments, the controller **12** may produce signals for including the droplet particle count in a target count record in association with the target region. In some embodiments, the controller **12** may generate and store a target count record associating each target region in the substrate stage **18** with a number representing a particle count for the target region.

In various embodiments, the generated target count record may be used to identify which of the target regions included in the microplate **30** of the substrate stage **18** hold a desired particle count and therefore may be useable for next generation genomic sequencing systems. For example, in some embodiments, the system **10** shown in Figure **1** may include a display in communication with the controller **12** and the controller **12** may be configured to cause a representation of the target count record to be displayed by the display to a user. In some embodiments, the user may view the display and use the information as required for their application. In some embodiments, the controller **12** may be configured to save the target count record as a file that may be used later on and/or sent to another device/system for use.

In some embodiments, the system **10** described herein may be configured to skew particle or cell encapsulation towards a single dispensed cell per target by skewing away from the Poisson distribution, in terms of having a more controlled partitioning of cells into targets or nanowells. In some embodiments, simulated results have shown the system **10** to be capable of achieving high single-cell rates (>**80%**) using low cell concentrations which may be advantageous as it may reduce the risk of clogging within the inkjet nozzle.

In some embodiments, because the pre-dispensed and post-dispensed images are being compared to determine a dispensed particle count, no a priori information of the inkjet nozzle's encapsulation properties may be required for the system **10** and so there may be no requirements to identify regions within the inkjet nozzle, which may add to the reliability of the system **10** as no estimations may be necessary. In some embodiments, the encapsulation properties may determine whether or not a particle will be encapsulated into the dispensed droplet, given the present working conditions. In some embodiments, with the system **10** the encapsulation properties may not need to be determined for each nozzle geometry, actuation waveform, cell type, suspending media composition, as may be required by other systems.

In some embodiments, by validating single-cell events while the cells are within the nozzle, the time which a cell may be exposed to unfavorable environments (such as high shear or low CO_2 environments) may be reduced before the cell is lysed which may lead to reliability in various applications, such as, for example RNA applications where gene expression profiles may be time sensitive and can change in response to stress on the cells.

<u>Controller - Processor Circuit</u>

Referring now to Figure **3**, a schematic view of the controller **12** of the system **10** shown in Figure **1** according to various embodiments is shown. Referring to Figure **3**, the controller **12** includes a processor circuit including a controller processor **100** and a program memory **102**, a storage memory **104**, and an input/output (I/O) interface **112**, all of which are in communication with the controller processor **100**. In various embodiments, the controller processor **100** may include one or more processing units, such as for example, a central processing unit (CPU), a graphical processing unit (GPU), and/or a field programmable gate array (FPGA). In some embodiments, any or all of the functionality of the controller **12** described herein may be implemented using one or more FPGAs.

The I/O interface **112** includes an interface **120** for communicating with the imager **14** and an interface **124** for communicating with the substrate stage **18**. In some embodiments, the I/O interface **112** may also include an additional interface for facilitating networked communication through a network such as the Internet. In some embodiments, any or all of the interfaces may facilitate a wireless or wired communication. In some embodiments, for example, interfaces **120** and **124** may be implemented using a USB connection. In some embodiments, each of the interfaces shown in Figure **3** may include one or more interfaces and/or some or all of the interfaces included in the I/O interface **112** may be implemented as combined interfaces or a single interface.

In some embodiments, where a device is described herein as receiving or sending information, it may be understood that the device receives signals representing the information via an interface of the device or produces signals representing the information and transmits the signals to the other device via an interface of the device.

Processor-executable program codes for directing the controller processor **100** to carry out various functions are stored in the program memory **102**. Referring to Figure **3**, the program memory **102** includes a block of codes **190** for directing the controller **12** to perform particle deposition control functions. In this specification, it may be stated that certain encoded entities such as applications or modules perform certain functions. Herein, when an application, module or encoded entity is described as taking an action, as part of, for example, a function or a method, it will be understood that at least one processor (e.g., the controller processor **100**) is directed to take the action by way of programmable codes or processor-executable codes or instructions defining or forming part of the application.

The storage memory **104** includes a plurality of storage locations including location **140** for storing first pre-dispensed image data, location **142** for storing second pre-dispensed image data, location **144** for storing third pre-dispensed image data, location **150** for storing post-dispensed image data, location **152** for storing first image difference data, location **154** for storing second image difference data, location **156** for storing third image difference data, location **158** for storing neural network data, location **160** for storing droplet particle count data, location **161** for storing count confidence data, location **162** for storing dispensed particle count data, location **164** for storing desired particle count data, location **166** for storing target particle count data, location **168** for storing target count confidence data, location **170** for storing count confidence threshold data, location **172** for storing target count validity data, and location **174** for storing target droplet count data. In various embodiments, the plurality of storage locations may be stored in a database in the storage memory **104**.

In various embodiments, the block of codes **190** may be integrated into a single block of codes or portions of the block of code **190** may include one or more blocks of code stored in one or more separate locations in the program memory **102**. In various embodiments, any or all of the locations **140**-**172** may be integrated and/or each may include one or more separate locations in the storage memory **104**.

Each of the program memory **102** and storage memory **104** may be implemented as one or more storage devices including random access memory (RAM), a hard disk drive (HDD), a solid-state drive (SSD), a network drive, flash memory, a memory stick or card, any other form of non-transitory computer-readable memory or storage medium, and/or a combination thereof. In some embodiments, the program memory **102**, the storage memory **104**, and/or any portion thereof may be included in a device separate from the controller **12** and in communication with the controller **12** via the I/O interface **112**, for example.

<u>Controller operation</u>

As discussed above, in various embodiments, the controller **12** shown in Figures **1** and **3** may facilitate controlled particle deposition from the droplet dispenser **16** using the imager **14** and/or the substrate stage **18**.

Referring now to Figure **4**, a flowchart depicting blocks of code for directing the controller processor **100** shown in Figure **3** to perform particle deposition control functions in accordance with various embodiments is shown generally at **200**. The blocks of code included in the flowchart **200** may be encoded in the block of codes **190** of the program memory **102** shown in Figure **3**, for example.

Referring to Figure **4**, the flowchart **200** begins with block **202** which directs the controller processor **100** shown in Figure **3** to receive at least one pre-dispensed image of a dispensing portion of the droplet dispenser, the dispensing portion including fluid to be dispensed in a droplet to a target region. In various embodiments, block **202** may direct the controller processor **100** to communicate with the imager **14** via the interface **120** of the I/O interface **112** to cause the imager **14** to capture a first pre-dispensed image of the inkjet nozzle of the droplet dispenser **16**, as shown at **240** in Figure **5**, for example, representing the droplet dispenser **16** at a first time with the droplet dispenser **16** configured to dispense a first droplet into the first target region **54** as shown in Figure **2**.

In some embodiments, the imager **14** may include a camera and a lens which is configured to focus on the contents or fluid in the dispensing portion **52** of the droplet dispenser **16** shown in Figure **2**. In some embodiments, to avoid interference between the lens and the substrate stage **18** while still allowing the imager **14** to capture focused images above all of the target regions of the microplate **30**, the lens of the imager **14** may need to have a working distance that is larger than a threshold working distance.

In some embodiments, for example, if the substrate stage **18** is not configured to rotate the microplate to allow the imager **14** to image from both sides of the microplate **30**, the threshold working distance may be a shortest side or the width of the microplate **30**. In some embodiments, the largest microplate **30** that may be used in the system **10** may be about 127 mm by 85 mm and so the lens may need a minimum working distance of 85 mm in order to accommodate for this. In some embodiments, the imager **14** may include a high resolution monochrome camera and a long working lens to achieve a resolution of 0.22 μm with a 456x342 μm field-of-view and a working distance of 86 mm. In some embodiments, with a resolving power of 3.34 μm this setup may be appropriate for identifying cell-sized particles. In some embodiments, the imager **14** may include a color camera and/or a camera that is configured to sense fluorescence.

In some embodiments, by using a long working distance lens in the imager **14**, the nozzle may be positioned right above the target region of the microplate **30** which may allow for accurate droplet placement by minimizing the effects of droplet deflection by minimizing the distance which the droplet travels through the air.

In some embodiments, for compatibility with smaller particles such as cell nuclei or certain bacterial cells, a higher resolving power lens may be used. In some embodiments, because long working distance and high resolving power lens may be difficult to source as the resolving power is inversely proportional to the numerical aperture, the substrate stage **18** may be configured to allow rotation of the microplate **30** such that the lens needs a working distance of only about half of the width of the microplate **30**. In such embodiments, the controller **12** may be configured to communicate with the substrate stage **18** to cause the substrate stage to, once half of a microplate **30** is filled, **1**) drop down clearing the lens, **2**) rotate 180° and **3**) raise back up to be printed onto the other side of the microplate. In some embodiments, such rotation may allow a 10x objective lens with a 51 mm working distance and a 1.31 μm resolving power be used in place of a zoom lens, thereby allowing for resolving smaller particles.

Referring to Figure **4**, in various embodiments, block **202** may direct the controller processor **100** to receive the first pre-dispensed image **240** shown in Figure **5** and to store the received first pre-dispensed image in the location **140** of the storage memory **104** shown in Figure **3**.

In some embodiments, the controller **12** may be configured to receive more than one pre-dispensed image. In some embodiments, the at least one pre-dispensed image may include a second pre-dispensed image **248** as shown in Figure **6** representing the droplet dispenser **16** at a second pre-dispensed time prior to the first pre-dispensed time. In some embodiments, the second pre-dispensed image may have been previously received by the controller **12**, generally as described above for the first pre-dispensed image and stored in the location **142** of the storage memory **104**. In some embodiments, the controller **12** may have caused a first preceding droplet to be dispensed by the droplet dispenser **16** between the second pre-dispensed time and the first pre-dispensed time.

In some embodiments, the at least one pre-dispensed image may include a third pre-dispensed image **254** as shown in Figure **7** representing the droplet dispenser **16** at a third pre-dispensed time prior to the second pre-dispensed time. In some embodiments, the third pre-dispensed image may have been previously received by the controller generally as described above for the first pre-dispensed image and stored in the location **144** of the storage memory **104**. In some embodiments, the controller **12** may have caused a second preceding droplet to be dispensed by the droplet dispenser **16** between the third pre-dispensed time and the second pre-dispensed time. In some embodiments, without the requirement for machine movement to a next target (e.g. when printing into the same target) average time between dispensing droplets may be about 14.58 ms. In some embodiments, including machine movement to a next target, average time between dispensing droplets may be about 178.22 ms.

In various embodiments, including more than one pre-dispensed image, such as the second and/or third pre-dispensed images 248 and 254 shown in Figures 6 and 7 in the at least one pre-dispensed images may facilitate improved accuracy in determining particle counts for dispensed droplets using the pre-dispensed images. For example, in some embodiments, using more than one pre-dispensed image may allow for the visualization of the particles or cells as they move across the field of view of the imager **14** within a nozzle or dispenser, giving more information that may be later used by a neural network, for example.

In some embodiments, on average a particle or cell may enter and exit the field of view of the imager **14** within 3 droplets. Accordingly, in some embodiments using 3 pre-dispensed images may facilitate all images of a particle or cell that is dispensed being captured in the 3 pre-dispensed images.

In some embodiments, because a classification is being made using 2D images of a 3D volume, multiple particles or cells may temporarily overlap within the 2D images, appearing very similar to only 1 particle or cell, for example. In various embodiments, by incorporating more than one pre-dispensed image, functions such as a neural network may be trained to identify, using the pre-dispensed images, cases such as where two particles or cells that appear upstream in the nozzle appear to be overlapping in the next pre-dispensed image before being dispensed as an event where 2 particles or cells were dispensed. In various embodiments, if one were only to use a single pre-dispensed image there may be few or no indicators that suggest the pre-dispensed image contained two overlapping particles or cells.

In some embodiments, having a droplet dispensed between capturing the pre-dispensed images may facilitate improved accuracy in determining the particle counts using the pre-dispensed images. For example, in some embodiments, interleaving dispensing of droplets with capturing the pre-dispensed images may facilitate providing further information regarding the particles or cells shown in the pre-dispensed images.

In some embodiments, the controller **12** may be configured to, before dispensing to the first target region, have the third pre-dispensed image captured, dispense a droplet to a discard region, have the second pre-dispensed image captured, and then dispense another droplet to a discard area, before having the first pre-dispensed image captured and targeting the first target region, such that the first, second, and third pre-dispensed images may be provided before the first droplet is dispensed to the first target region. As described in further detail below, after a droplet is dispensed, the controller **12** may be configured to treat the former first pre-dispensed image as a second pre-dispensed image and to treat the former second pre-dispensed image as a third pre-dispensed image for the next droplet to be dispensed.

In various embodiments, block **202** of the flowchart **200** shown in Figure **4** may include the blocks of code shown at **900** in Figure **8**. Referring to Figure **8**, block **902** directs the controller processor **100** to generate an image difference representing a difference between the second pre-dispensed image and the first pre-dispensed image. In some embodiments, block **902** may direct the controller processor **100** to subtract the first pre-dispensed image from the second pre-dispensed image and to store the result as a second image difference in the location **154** of the storage memory **104**.

Referring still to Figure **8**, block **904** directs the controller processor **100** to generate an image difference representing a difference between the third pre-dispensed image and the second pre-dispensed image. In some embodiments, block **902** may direct the controller processor **100** to subtract the second pre-dispensed image from the third pre-dispensed image and to store the result as a second image difference in the location **156** of the storage memory **104**.

In various embodiments, the difference of the images may be taken in order to remove static features within the images leaving only the cells suspended within the nozzle's channel. In some embodiments, this may facilitate improved performance in determining dispensed particle counts, since extraneous information may be removed. In various embodiments, the subtracted images may be calculated according to the following formula.

$$\left\lfloor \frac{(IM_{t=\mathbf{0}} - IM_{t=-\mathbf{1}}) + \mathbf{255}}{\mathbf{2}} \right\rfloor$$

where the subtraction is a pixelwise subtraction of the two images and the pixelwise addition of **255** and division by 2 is to normalize the range of pixel values to within **0**-**255** as used for **8**-bit images. In various embodiments, the floor function may ensure that all pixel vales are whole numbers. In various embodiments, an image pixel resolution of **192**x**313** pixels may be used.

Referring back to Figure **4**, block **204** then directs the controller processor **100** to receive at least one post-dispensed image of the dispensing portion of the droplet dispenser **16** after the droplet has been dispensed. In various embodiments, block **204** may direct the controller processor **100** to produce signals for causing the droplet dispenser **16** to dispense the first droplet of fluid into the first target region of the microplate **30** of the substrate stage **108**. In some embodiments, for example, the block may direct the controller processor **100** to transmit instructions to the imager **14** for causing the imager **14** to cause the droplet dispenser **16** to dispense a droplet. In some embodiments, the signals may also trigger the imager **14** to capture and send to the controller **12** at least one post-dispensed image of the dispensing portion of the droplet dispenser **16** after the droplet has been dispensed.

In some embodiments, the droplet dispenser **16** may be configured to dispense low volumes of fluid, such as, for example, volumes of about 250 pL per droplet.

In some embodiments, for example, the signals transmitted to the imager **14** for causing the imager to cause the droplet dispenser **16** to dispense the droplet may have triggered the imager **14** capturing and sending to the controller **12**, a post-dispensed image **320** as shown in Figure **9** of the inkjet nozzle shown in Figure **2**, the post-dispensed image taken after the first droplet was dispensed by the droplet dispenser **16** to the first target region of the microplate **30**. Block **204** may direct the controller processor **100** to receive the post-dispensed image **320** via the interface **120** of the I/O interface **112** shown in Figure **3**. In some embodiments, block **204** may direct the controller processor **100** to store the post-dispensed image **320** in the location **150** of the storage memory **104** shown in Figure **3**.

In some embodiments, block **204** may include a block generally similar to the blocks **902** or **904** included in the flowchart **900** shown in Figure **8**, such that execution of block **204** directs the controller processor **100** to generate a first image difference representing a difference between the first pre-dispensed image and the post-dispensed image. In various embodiments, block **204** may direct the controller processor **100** to store the first image difference in the location **152** of the storage memory **104**.

Accordingly, in various embodiments, after blocks **202** and **204** have been executed, image differences acting as representations of the first, second, and third pre-dispensed images **240**, **248**, and **254** as shown in Figures **5**, **6**, and **7** and the post-dispensed image **320** as shown in Figure **9** may be stored in the locations **152**, **154**, and **156** of the storage memory **104**. In some embodiments, the image differences stored in the locations **152**, **154**, and **156** may be stored as a single RGB color image in the storage memory **104** with the first, second, and third image differences acting as the R, G, and B values respectively of the RGB color image. In some embodiments, storing the images as a single image or 3D array may make it easier to perform operations on the images. For example, a roll function may be used when adding a new image to the red channel. In some embodiments, the input shape to the neural network may be a 3D array, and so storing the image differences as a 3D array or a single color image may facilitate no further operations on the images being required when running a prediction. In some embodiments, if the image differences are stored as three separate images then a concatenation step may be required first to create a 3D array before being input into the neural network. In some embodiments, saving the image differences as an RGB image may facilitate human inspection as such RGB images may be easy to visually inspect.

Referring back to Figure **4**, the flowchart **200** continues at block **206**, which directs the controller processor **100** to compare the at least one pre-dispensed image and the at least one post-dispensed image to determine a particle count representing a count of particles included in the dispensed droplet. In some embodiments, block **206** may direct the controller processor **100** to cause a representation of the at least one pre-dispensed image and the at least one post-dispensed image to be input into one or more functions. For example, in some embodiments, block **206** may direct the controller processor **100** to input the image differences stored in the locations **152**, **154**, and **156** of the storage memory **104** into a neural network, such as the convolutional neural network (CNN) **1000** as shown in Figure **10**.

In some embodiments, the CNN **1000** may have been previously trained such that the CNN is configured to receive as input, the first second, and third image differences and to output first, second, and third count confidences **1020**, **1022**, and **1024** each associated with a respective prospective particle count or class. In various embodiments, the first count confidence **1020** may represent a confidence that the first droplet includes 0 particles, the second count confidence **1022** may represent a confidence that the first droplet includes 1 particle, and the third count confidence **1022** may represent a confidence that the first droplet includes 2 or more particles.

Parameters defining the CNN **1000**, which may have been obtained during training, may be stored in the location **158** of the storage memory **104**.

In some embodiments, for example, block **206** may direct the controller processor **100** to retrieve the first, second, and third image differences from the locations **152**, **154**, and **156** of the storage memory **104** and to input the first, second, and third image differences into the CNN **1000**. Block **206** may direct the controller processor **100** to retrieve the parameters defining the CNN **1000** from the location **158** of the storage memory **104** and to process the input to generate first, second, and third count confidences. For example, in some embodiments, the first, second and third count confidences may be generated or determined to be 0.02, 0.96, and 0.02 respectively, when the first, second, and third image differences are input.

In various embodiments, the size and hyperparameters used in the CNN **1000** used in block **206** may be as follows:

Layers:	**Parameters:**
2D Convolutional Layer 1	Kernel Size = 5x5, # Filters = 16, Activation = ReLu
2D Convolutional Layer 2	Kernel Size = 3x3, # Filters = 32, Activation = ReLu
2D Convolutional Layer 3	Kernel Size = 3x3, # Filters = 64, Activation = ReLu
2D Convolutional Layer 4	Kernel Size = 3x3, # Filters = 64, Activation = ReLu
Fully Connected Layer 1	# Nodes = 256, Activation = ReLu
Fully Connected Layer 2	# Nodes = 512, Activation = ReLu
Output Layer	# Nodes = 3, Activation = Softmax

In some embodiments, block **206** may direct the controller processor **100** to determine a subject droplet particle count based on the determined count confidences, the subject droplet particle count being a count of particles expected to be in the droplet that was most recently dispensed. In some embodiments, block **206** may direct the controller processor **100** to determine the subject droplet particle count to be the particle count associated with the largest of the plurality of count confidences. In various embodiments, block **206** may direct the controller processor **100** to store the determined subject droplet particle count in the location **160** of the storage memory **104**.

Accordingly, in various embodiments, where the largest count confidence of 0.96 is the second count confidence associated with a particle count of 1, block **206** may direct the controller processor **100** to determine the subject droplet particle count to be 1 and to store the subject droplet particle count of 1 in the location **160** of the storage memory **104**.

In various embodiments, where the largest count confidence is the first count confidence associated with a particle count of 0, block **206** may direct the controller processor **100** to determine the subject droplet particle count to be 0 and to store the subject droplet particle count of 0 in the location **160** of the storage memory **104**.

In various embodiments, where the largest count confidence is the third count confidence associated with a particle count of 2 or more, block **206** may direct the controller processor **100** to determine the subject droplet particle count to be 2 or more and to store the subject droplet particle count of 2 in the location **160** of the storage memory **104**.

In various embodiments, block **206** may direct the controller processor **100** to store the largest count confidence in the location **161** of the storage memory **104**. In various embodiments, the count confidence may be later used to assess validity of the determined particle count and/or any other resulting count.

In various embodiments, after the subject droplet particle count is determined and stored in the location **160** of the storage memory **104**, the controller processor **100** may proceed to block **208**.

Referring back to Figure **4**, block **208** may direct the controller processor **100** to produce signals for associating a representation of the droplet particle count with the target region. In some embodiments, for example, block **208** may direct the controller processor **100** to produce signals for causing a dispensed particle count to be stored, which may represent a total number of particles dispensed in droplets to a target region. In various embodiments, the dispensed particle count may represent or include the subject droplet particle count. Block **208** may direct the controller processor **100** to store the dispensed particle count in a target count record in association with the target region.

In various embodiments, the dispensed particle count may be stored in the location **162** of the storage memory **104** and may represent a total running count of a number of particles dispensed to a target region. In some embodiments, the dispensed particle count may be incremented by the subject droplet particle count for each droplet dispensed to a target region and reset to 0 whenever a new target region is to be dispensed into.

In some embodiments, block **208** may direct the controller processor **100** to first determine whether one or more further droplets should be dispensed to the target region before associating the dispensed particle count with the target region. In some embodiments, if further droplets are to be dispensed to the target region, block **208** may direct the controller processor **100** to treat the present post-dispensed image as a pre-dispensed image for a further droplet to be dispensed. Block **208** may direct the controller processor **100** to execute blocks generally similar to blocks **204**, **206**, and **208** of the flowchart **200** shown in Figure **4**, but dispensing a further droplet to the target region. In such embodiments, a block generally similar to block **206** may direct the controller processor **100** to update the dispensed particle count stored in the location **162** to include a count of particles included in a second droplet summed with the count of particles included in the first droplet in a total dispensed particles count and a block generally similar to the block **208** may treat the total dispensed particle count generally as described above.

Referring to Figure **11**, there is shown a flowchart **500** including blocks of code that may be included in block **208** in various embodiments. The flowchart **500** begins with block **501**, which directs the controller processor **100** to increment the dispensed particle count by the subject droplet particle count. In some embodiments, block **501** may direct the controller processor **100** to read the subject droplet particle count from the location **160** of the storage memory **104**, to add it to the dispensed particle count stored in the location **162** of the storage memory and to store the result in the location **162** of the storage memory as the dispensed particle count.

Block **502** then directs the controller processor **100** to determine whether the dispensed particle count is less than a desired particle count. In some embodiments, for example, the desired particle count may have been previously set by a user, for example, and stored in the location **164** of the storage memory **104**. In some embodiments, the desired particle count may be 1, such as, for example, where single cells or particles are to be dispensed. Block **502** may direct the controller processor **100** to compare the dispensed particle count stored in the location **162** of the storage memory **104** to the desired particle count stored in the location **164** of the storage memory **104**.

If the dispensed particle count is not less than the desired particle count, (i.e., the dispensed particle count is equal to or greater than the desired particle count) then this may indicate that no more particles should be dispensed to the current target region, either because the desired number of particles have been dispensed or too many have already been dispensed. In either case, dispensing to the current target region may be complete and a new target should be used and so block **502** may direct the controller processor **100** to proceed to block **504**.

Block **504** directs the controller processor **100** to record the dispensed particle count in association with the target region. In various embodiments, block **504** may direct the controller processor **100** to copy the dispensed particle count or class from the location **162** of the storage memory **104** shown in Figure **3** to a first target total count field **1062** of a target count record **1060** as shown in Figure **12**, which may be stored in the location **166** of the storage memory **104**. In various embodiments, the target count record **1060** may include target total count fields, some of which are shown at **1062**-**1070**, for storing particle counts associated with each target region of the microplate **30**. In some embodiments, the target count fields may be initialized to 0 and updated as block **504** is executed for each target region. In various embodiments, where the microplate includes a 72x72 grid of target regions, for example, the target count record **1060** may include 5184 target count fields. In some embodiments, a subset of the grid may be used and so fewer than 5184 target count fields may be included. In some embodiments, the target count record **1060** may be stored as an array of numbers representing particle counts, with each position in the array corresponding to a target region of the microplate **30**.

Referring to Figure **11**, in various embodiments, the flowchart **500** may include block **507** which directs the controller processor **100** to record a count or class confidence in association with each count, for example zero, single, or multiple particles, and in association with the target region. In various embodiments, block **507** may direct the controller processor **100** to copy the stored count confidence(s), which may in some embodiments include a respective maximum count confidence and the corresponding count, associated with each droplet dispensed to the target region, from the location **161** of the storage memory **104** shown in Figure **3** to a count confidence field **1102** of a target count confidence record **1100** as shown in Figure **13**, which may be stored in the location **168** of the storage memory **104**. In various embodiments, the target count record **1100** may include target count confidence fields, some of which are shown at **1102**-**1108**, for storing the largest of the three predicted count confidences and its corresponding count associated with each target region of the microplate **30**. In some embodiments, the target count fields may be initialized to 0 and updated as block **507** is executed for each target region. In some embodiments, more than one count confidence may be stored in the location **161** of the storage memory **104** and so block **507** may direct the controller processor **100** to store more than one count confidence in the target count confidence record **1100** in association with a particular target region.

Referring to Figure **11**, in various embodiments, the flowchart **500** may include block **505** which may direct the controller processor **100** to produce signals for causing the droplet dispenser **16** to be configured to dispense a second or subsequent droplet to a next or second target region different from the initial subject or first target region. In some embodiments, block **505** may direct the controller processor **100** to communicate with the substrate stage **18** via the interface **124** of the I/O interface **112** to cause the substrate stage **18** to move the microplate **30** such that the dispensing portion **52** shown in Figure **2** is above a new target region different from the first target region and any target region to which a droplet was previously dispensed. In some embodiments, if at block **505** the controller processor **100** determines that there are no further target regions, the flowchart **500** may end.

Referring still to Figure **11**, after block **505** is executed, block **506** may direct the controller processor **100** to reset the dispensed particle count stored in the location **162** to zero to reflect that a new target is being dispensed into.

In various embodiments, the flowchart **500** may include block **509**, which may direct the controller processor **100** to reset the count confidence by deleting the count confidences stored in the location **161**.

After block **509** or if at block **502** it is determined that the particle count is less than the desired particle count, the controller processor **100** may proceed to block **508** which directs the controller processor **100** to store or use the current post-dispensed image as a pre-dispensed image when considering the next droplet to be dispensed. In some embodiments, block **508** may direct the controller processor **100** to also update how the pre-dispensed images are considered for the next droplet, for example, by treating the current first pre-dispensed image as the second pre-dispensed image when considering the next droplet to be dispensed, and by treating the current second pre-dispensed image as the third pre-dispensed image when considering the next droplet to be dispensed.

For example, in some embodiments, block **508** may direct the controller processor **100** to cause the first image difference data stored in the location **152** to become second image difference data stored in the location **154** and to cause the second image difference data to become third image difference data stored in the location **156**. In some embodiments, block **508** may direct the controller processor **100** to move the image stored in the location **150** to the location **140**, to cause the current post-dispensed image to become the first pre-dispensed image when considering the next droplet to be dispensed. Block **508** may similarly direct the controller processor **100** to move the image stored in the location **140** to the location **142**, to cause the current first pre-dispensed image to become the second pre-dispensed image when considering the next droplet to be dispensed and to move the image stored in the location **142** to the location **144**, to cause the current second pre-dispensed image to become the third pre-dispensed image when considering the next droplet to be dispensed.

Referring to Figure **11**, block **510** then directs the controller processor **100** to perform further controlled droplet dispensing functions. In some embodiments, block **510** may direct the controller processor **100** to return to execute blocks generally similar to blocks **204**, **206**, and **208** of the flowchart **200** shown in Figure **4**, but either dispensing a further droplet to the target region (if block **502** directed the controller processor **100** to proceed to block **508**, for example) or dispensing a droplet to a new target region (if blocks **504**, **507**, **505**, **506**, and **509** have been executed, for example).

In such embodiments, where block **502** directed the controller processor **100** to proceed to block **508**, a block generally similar to block **206** may direct the controller processor **100** to include a count of particles included in a second droplet with the count of particles included in the first droplet in the total dispensed particles count and a block generally similar to the block **208**. In various embodiments, block **206** may direct the controller processor **100** to include the largest count confidence in a set or array of count confidences stored in the location **161** of the storage memory **104**.

In various embodiments, once the flowchart **200** has generally been executed for each target region, the target count record **1060** shown in Figure **12** may include a value stored for each target count field, the value representing a number of particles dispensed into that target region.

In various embodiments, the target count confidence record **1100** shown in Figure **13** may include at least one value stored in each target count confidence field, the values representing respective confidences that droplets dispensed into the associated target region had particular counts.

In some embodiments, the block of codes **190** of the program memory **102** may include blocks of code as shown in flowchart **1140** shown in Figure **14**. In various embodiments, the flowchart **1140** may be executed when a user wishes to view counts associated with the target regions. In some embodiments, the flowchart **1140** may be executed for each target region. The flowchart **1140** begins with block **1142**, which directs the controller processor **100** to consider a target region. In some embodiments, upon a first execution of the flowchart **1140**, block **1142** may direct the controller processor **100** to consider the first target region. In various embodiments, the flowchart **1140** may be executed repeatedly, but considering a different target region each time, until all of the target regions have been considered. In some embodiments, only target regions associated with a desired particle count may be considered. For example, in some embodiments, where single particle or single cell targets are desired, block **1142** may direct the controller processor **100** to consider only target regions associated with a particle count equal to 1.

Block **1144** then directs the controller processor **100** to determine whether the count confidence associated with the target region is greater than a threshold count confidence. In some embodiments, block **1144** may direct the controller processor **100** to determine whether the count confidence for a desired particle count, such as a count of 1, for example, associated with the target regions is greater than a threshold count confidence. In some embodiments, where more than one count confidence is associated with a target region, block **1144** may direct the controller processor **100** to determine whether all of the count confidences are greater than a threshold count confidence. In some embodiments, the threshold count confidence may have been set previously and may be stored in the location **170** of the storage memory **104**. For example, in some embodiments, the threshold count confidence may have been set to 0.95.

In some embodiments, block **1144** may direct the controller processor **100** to read a target count confidence associated with the considered target region from the target count confidence record **1100** stored in the location **168** of the storage memory and the threshold count confidence from the location **170** of the storage memory **104** and to compare the values to determine whether the target count confidence is greater than the threshold count confidence. If at block **1144**, it is determined that the target count confidence is not greater than the threshold count confidence, block **1144** may direct the controller processor **100** to proceed to block **1146**. Alternatively, if it is determined that the target count confidence is greater than the threshold count confidence, block **1144** may direct the controller processor **100** to proceed to block **1148**.

Block **1146** directs the controller processor **100** to associate the target region with a low confidence indicator and block **1148** directs the controller processor **100** to associate the target region with a high confidence indicator. In various embodiments, blocks **1146** and **1148** may direct the controller processor **100** to populate a target count validity record **1180** as shown in Figure **15** with a value of “INVALID” or “VALID” respectively. In various embodiments, the target count validity record **1180** may be stored in the location **172** of the storage memory **104** and may be used to indicate which of the counts included in the target particle count record represent valid or reliable data regarding the count of particles in the associated target region.

In some embodiments, the system **10** shown in Figure **1** may include a display in communication with the controller **12** and the block of codes **190** of the program memory **102** may include code for directing the controller processor **100** shown in Figure **3** to communicate with the display to cause a representation of the target count record **1060**, the target count confidence record **1100**, and/or the target count validity record **1180** to be displayed by the display to a user. In some embodiments, the user may view the display and use the information as required for their application.

In some embodiments, for example, the user may view the display and identify target regions or positions which contain only a single cell for downstream processing (e.g. adding reagents for Polymerase chain reaction (“PCR”) and library preparation) while omitting the other target regions from downstream processing. In some embodiments, for example in monoclonal antibody development the target particle count record may be used to prove clonality of cultured cells.

In some embodiments, the target count record **1060,** the target count confidence record **1100**, and/or the target count validity record **1180** may be saved in one or more files that may be used later on and/or sent to another device/system for use.

Neural Network Training

As discussed above, in various embodiments, parameters defining the CNN **1000** shown in Figure **10** may be stored in the location **158** of the storage memory **104** of the controller **12** shown in Figure **3**. In some embodiments, the parameters may have been generated during neural network training. Referring now to Figure **16** there is shown a system **1240** for controlled particle deposition including neural network training, in accordance with various embodiments.

Referring to Figure **16**, the system **1240** includes a system **1242** for facilitating controlled particle deposition, which may be generally similar to the system **10** shown in Figure **1** and may include a controller generally similar to the controller **12** shown in Figure **3**. In various embodiments, the system **1240** may also include a controlled particle deposition trainer **1244** in communication with a particle deposition training data source **1246**. In various embodiments, the system **1242** may be in communication with the trainer **1244** via a network **1248**, which may in some embodiments, include the Internet, and/or remote mass storage for example.

In operation, the trainer **1244** may be configured to use controlled particle deposition training data received from the training data source **1246** to train the CNN **1000** shown in Figure **10**. In some embodiments, the trainer **1244** may be configured to provide data defining the trained CNN to the controller of the system **1242** shown in Figure **16**. In some embodiments, the CNN **1000** shown in Figure **10** may be trained from a complete data set providing known target particle count records for sets of pre-dispensed and post-dispensed images. For example, in some embodiments, the training data may have been obtained through experiment and analysis of droplets dispensed using the system **1242** or another similar system.

Referring to Figure **17**, a schematic view of the the trainer **1244** of the system **1240** shown in Figure **16** according to various embodiments is shown. In various embodiments, elements of the trainer **1244** that are similar to elements of the controller **12** shown in Figure **3** may function generally as described above having regard to the controller **12** shown in Figure **3**.

Referring to Figure **17**, the trainer **1244** includes a processor circuit including a trainer processor **1300** and a program memory **1302**, a storage memory **1304**, and an input/output (I/O) interface **1312**, all of which are in communication with the trainer processor **1300**.

The I/O interface **1312** includes an interface **1320** for communicating with the training data source **1246** shown in Figure **16** and an interface **1322** for communicating with the controller of the system **1242** via the network **1248** shown in Figure **16**.

Processor-executable program codes for directing the trainer processor **1300** to carry out various functions are stored in the program memory **1302**. Referring to Figure **17**, the program memory **1302** includes a block of codes **1370** for directing the trainer **1244** to perform facilitating controlled particle deposition neural network training functions.

The storage memory **1304** includes a plurality of storage locations including location **1340** for storing training image data, location **1342** for storing training count data, location **1344** for storing training image difference data, and location **1346** for storing neural network data.

In some embodiments, the program memory **1302**, the storage memory **1304**, and/or any portion thereof may be included in a device separate from the trainer **1244** and in communication with the trainer **1244** via the I/O interface **1312**, for example. In some embodiments, the functionality of the trainer processor **1300** and/or the trainer **1244** as described herein may be implemented using a plurality of processors and/or a plurality of devices, which may be distinct devices which are in communication via respective interfaces and/or a network, such as the Internet, for example.

In various embodiments, the trainer **1244** shown in Figures **16** and **17** may be configured to facilitate controlled particle deposition neural network training. Referring to Figure **18**, a flowchart depicting blocks of code for directing the trainer processor **1300** shown in Figure **17** to perform controlled particle deposition neural network training functions in accordance with various embodiments is shown generally at **1400**. The blocks of code included in the flowchart **1400** may be encoded in the block of codes **1370** of the program memory **1302** shown in Figure **17**, for example.

Referring to Figure **18**, the flowchart **1400** begins with block **1402** which directs the trainer processor **1300** to receive a plurality of sets of training images, each of the sets of training images including at least one pre-dispensed image of a dispensing portion of a droplet dispenser, the dispensing portion including fluid to be dispensed in a droplet to a target region, and at least one post-dispensed image of the dispensing portion of the droplet dispenser after the droplet has been dispensed.

Block **1404** then directs the trainer processor **1300** to receive a plurality of droplet particle counts, each of the droplet particle counts associated with one of the sets of training images and representing a count of particles dispensed in a droplet that is the subject of the associated set of training images.

In some embodiments, for example, the training data source **1246** may have previously been provided with training data including sets of training images and associated droplet counts. In some embodiments, for example, the training data source **1246** may have stored thereon training data many training data sets, each including a first, a second, and a third pre-dispensed image and a post-dispensed image, all associated with a droplet particle count. For example, in some embodiments, the training data may include many images having subsets therein associated with droplet particle counts, which may have been determined through experiment and inspection. Each subset of images may include a first, a second, and a third pre-dispensed image and a post-dispensed image which would be associated with the droplet particle count generally as described above.

Referring back to Figure **18**, block **1402** may direct the trainer processor **800** to receive a message including a representation of the training images stored in the training data source **1246** via the interface **1320**, for example. In some embodiments, block **1402** may direct the trainer processor **1300** to store the training images in the location **1340** of the storage memory **1304** shown in Figure **17**.

Block **1404** may direct the trainer processor **1300** to receive a message including a representation of the droplet particle counts stored in the training data source **1246** via the interface **1320**, for example, each droplet particle count associated with a set of training images. In some embodiments, block **1404** may direct the trainer processor **1300** to store the droplet particle counts in the location **1342** of the storage memory **1304** shown in Figure **17**.

In some embodiments, blocks **1402** and **1404** may be executed concurrently and the sets of training images and associated droplet particle counts may be received contemporaneously.

Referring to Figure **18**, block **1406** directs the trainer processor **1300** to cause at least one neural network function to be trained using representations of the sets of training images as respective inputs and the associated droplet counts as desired outputs. In some embodiments, block **1406** may direct the trainer processor **1300** to train the CNN **1000** shown in Figure **10** using the training images and droplet particle counts stored in the locations **1340** and **1342**.

In some embodiments, initial neural network data defining the architecture of the CNN **1000** may be stored in the location **1346** of the storage memory **1304**. In various embodiments, the initial neural network data may have been previously provided when setting up the trainer **1244**, for example.

In some embodiments, block **1406** may direct the trainer processor **1300** to, for each set of training images, generate first, second, and third image differences generally as described above having regard to block **206** of the flowchart **200** shown in Figure **4**. In various embodiments, block **1406** may direct the trainer processor **1300** to use the generated first, second, and third image differences as the inputs for the CNN **1000** as shown in Figure **10**. In some embodiments, block **1406** may direct the trainer processor **1300** to set the desired output based on the droplet particle count by setting the desired outputs for the count confidences according to the training droplet particle count associated with the input training images. For example, in some embodiments, the desired output for a count confidence that is associated with a droplet particle count that matches the training droplet particle count may be set to 1.00 and the desired outputs for the other count confidence outputs may be set to 0.

In some embodiments, block **1406** may direct the trainer processor **1300** to use categorical cross entropy as a loss function for training. In various embodiments, block **1406** may direct the trainer processor **1300** to determine parameters systematically by running Hyperband tuning to determine the hyperparameters such as the # of layers to use. During training an Adam optimizer may be used with a learning rate of 0.0001, for example. In some embodiments, Hyperband tuning may be used to determine an optimal learning rate. In various embodiments, properties of the CNN **1000** including, for example, hyperparameters, may be subject to change given new data.

In various embodiments, after block **1406** has been executed, data defining a trained controlled particle deposition neural network may be stored in the location **1346** of the storage memory **1304** of the trainer **1244** shown in Figure **17**.

In some embodiments, the flowchart **1400** may include a block for directing the trainer processor **1300** to produce signals representing the trained neural network for causing a representation of the trained neural network to be transmitted to the controller of the system **1242** shown in Figure **16**. In some embodiments, the controller of the system **1242** may include a processor circuit generally as shown in Figure **3** and may direct a controller processor of the controller to store the representation of the trained neural network in a location similar to the location **158** of the controller **12** shown in Figure **3**. In various embodiments, the controller may be configured to execute the flowchart **200** shown in Figure **4**, generally as described above, using the trained neural network stored in memory.

<u>Controller alternative embodiments</u>

In various embodiments, controllers generally similar to the controller **12** may be used which use alternative implementations of the flowchart **200** shown in Figure **4**. For example, in some embodiments, a controller **1500** as shown in Figure **19** may be used in in place of the controller **12** shown in Figure **3** in the system **10** shown in Figure **1**.

In some embodiments, the controller **1500** shown in Figure **19** may be configured to identify one or more pre-dispensed image particles depicted in at least one pre-dispensed image and to identify one or more post-dispensed image particles depicted in the at least one post-dispensed image. The controller **12** may be configured to identify at least one unmatched pre-dispensed image particle of the one or more pre-dispensed image particles as not matching any of the post-dispensed image particles and therefore as representing a particle included in the dispensed droplet. The controller may be configured to determine a droplet particle count as a count of the at least one unmatched pre-dispensed image particle.

In various embodiments, the controller **1500** may include elements that function generally similar to those of the controller **12**. Referring to Figure **19**, the controller **1500** includes a controller processor **1501** and a program memory **1502**, a storage memory **1504**, and an input/output (I/O) interface **1512**, all of which are in communication with the controller processor **1501**. The I/O interface **1512** includes an interface **1520** for communicating with the imager **14** and an interface **1524** for communicating with the substrate stage **18**. Processor-executable program codes for directing the controller processor **1501** to carry out various functions are stored in the program memory **1502**.

Referring to Figure **19**, the program memory **1502** includes a block of codes **1570** for directing the controller **1500** to perform particle deposition control functions. The storage memory **1504** includes a plurality of storage locations including location **1540** for storing received image data, location **1542** for storing pre-dispensed image representation data, location **1544** for storing post-dispensed image representation data, location **1550** for storing droplet particle count data, location **1551** for storing target dispensed particle count data, location **1552** for storing desired particle count data, and location **1554** for storing target particle count data.

In various embodiments, blocks of code that function generally similar to those included in the flowchart **200** shown in Figure **4** may be encoded in the block of codes **1570** of the program memory **1502** of the controller **1500** shown in Figure **19**. For example, referring now to Figure **20**, a flowchart depicting blocks of code for directing the controller processor **1501** shown in Figure **19** to perform particle deposition control functions in accordance with various embodiments is shown generally at **1580**. The blocks of code included in the flowchart **1580** may be encoded in the block of codes **1570** of the program memory **1502** shown in Figure **19**, for example.

In various embodiments, the flowchart **1580** begins with block **1582** which directs the controller processor **1501** of the controller **1500** to receive at least one pre-dispensed image of a dispensing portion of the droplet dispenser, the dispensing portion including fluid to be dispensed in a droplet to a target region. In various embodiments, block **1582** may direct the controller processor **1501** to receive a pre-dispensed image **1600** as shown in Figure **21** from the imager **14** and to store the pre-dispensed image **1600** in the location **1540** of the storage memory **1504**.

Referring to Figure **20**, in some embodiments, block **1582** may direct the controller processor **1501** to generate a representation of the pre-dispensed image **1600** shown in Figure **21** and to store the representation of the pre-dispensed image **1600** in the location **1542** of the storage memory **1504**. For example, in some embodiments, the block may direct the controller processor **1501** to generate a representation of the particles included in the pre-dispensed image **1600** and to store the representation of the particles in the location **1542** of the storage memory **1504**.

Referring to Figure **22**, there is shown a flowchart **1640** depicting blocks of code for directing the controller processor **1501** to generate a representation of a received image that may be included in the block **1582** of the flowchart **1580** shown in Figure **20** in accordance with various embodiments. The flowchart **1640** begins with block **1642** which directs the controller processor **1501** to apply segmentation to the received pre-dispensed image to isolate particles. In various embodiments, block **1642** may direct the controller processor **1501** to generate a filtered image depicting only particles included in the pre-dispensed image. In some embodiments, the particles depicted may represent cells, for example. Referring to Figure **23**, there is shown at **1660** a representation of a filtered image that may be generated at block **1642** based on the pre-dispensed image **1600** shown in Figure **21**, in accordance with various embodiments.

Block **1644** then directs the controller processor **1501** to identify each particle included in the pre-dispensed image **1600** and to associate a position with each identified particle. In some embodiments, block **1644** may direct the controller processor **1501** to use the filtered image **1660** shown in Figure **23**, which was generated at block **1642**, to determine a center of mass for each of the particles and to store the x and y pixel coordinates or positions in the pre-dispensed image **1600** for each center of mass for each particle. In some embodiments, for example, upon execution of block **1644** using the filtered image **1660** shown in Figure **23**, block **1644** may generate a first particle positions record **1680** as shown in Figure **24**.

Referring to Figure **24**, the first particle positions record **1680** includes a first particle x field and a first particle y field for storing x and y pixel coordinates for a determined center of mass of a first particle **1602** shown in the pre-dispensed image **1600** of Figure **21**. The first particle positions record **1680** also includes x and y fields **1687** and **1688** for a second particle **1604** shown in the pre-dispensed image **1600** of Figure **21** and x and y fields **1690** and **1692** for a third particle **1606** shown in the pre-dispensed image **1600** of Figure **21**. Block **1644** may direct the controller processor **1501** to store the first particle positions record **1680** as a pre-dispensed particle positions record in the location **1542** of the storage memory **1504** shown in Figure **19**. In some embodiments, block **1644** may direct the controller processor **1501** to order the x and y fields such that x and y field pairs representing particles having the highest y field values representing particles that are the most downstream are listed first.

In various embodiments, the first particle positions record **1680** shown in Figure **24** may be stored as an array, with a position of each value in the array implying the field label for each value. For example, in some embodiments, the first particle positions record **1680** may be stored as an array having values 101, 361, 87, 232, 130, and 133.

In some embodiments, block **1644** may direct the controller processor **1501** to identify shapes of each of the initially identified particles to facilitate identifying each particle and determining a position associated with each particle. In some embodiments, block **1644** may direct the controller processor **1501** to determine whether a potential particle has an irregular or peanut shape (e.g., depicting two overlapping circles), for example. In some embodiments, if a potential particle has a peanut shape, block **1644** may direct the controller processor **1501** to determine that the potential particle is two particles and to include two entries in the generated particle positions record. In some embodiments, block **1644** may direct the controller processor **1501** to best fit an irregular shape with as many circles as possible and to determine that the potential particle is as many particles as can be fit within the irregular shape and to include as many entries as necessary in the generated particle positions record, based on the determined number of circles that best fit the irregular shape.

Referring back to Figure **20**, block **1584** may direct the controller processor **1501** to receive at least one post-dispensed image of the dispensing portion of the droplet dispenser **16** after the droplet has been dispensed. In various embodiments, block **1584** may direct the controller processor **1501** to produce signals for causing the droplet dispenser **16** to dispense the first droplet generally as described above having regard to block **204** of the flowchart **200** shown in Figure **4**. Block **1584** may direct the controller processor **1501** to receive a post-dispensed image **1720** as shown in Figure **25** via the interface **1520** of the I/O interface **1512** shown in Figure **19**. In some embodiments, block **1584** may direct the controller processor **1501** to store the post-dispensed image **1720** in the location **1540** of the storage memory **1504** shown in Figure **19**.

In various embodiments, block **1584** may include blocks generally similar to the blocks included in the flowchart **1640** shown in Figure **22**, such that execution of block **1584** directs the controller processor **1501** to generate a second particle positions record **1740** as shown in Figure **26** and to store the second particle positions record **1740** as a post-dispensed particle positions record in the location **1544** of the storage memory **1504**. Referring to Figure **26**, the second particle positions record **1740** includes a first particle x field **1742** and a first particle y field **1744** for storing x and y pixel coordinates for a determined center of mass of a first particle **1722** shown in the post-dispensed image **1720** of Figure **25**. The second particle positions record **1740** also includes a second particle x field **1746** and a second particle y field **1748** for storing x and y pixel coordinates for a determined center of mass of a second particle **1724** shown in the post-dispensed image **1720** of Figure **25**.

Referring back to Figure **20**, block **1586** may direct the controller processor **1501** to compare the at least one pre-dispensed image and the at least one post-dispensed image to determine a particle count representing a count of particles included in the dispensed droplet. In some embodiments, block **1586** may direct the controller processor **1501** to compare the representation of the pre-dispensed image stored in the location **1542** with the representation of the post-dispensed image stored in the location **1544** of the storage memory **1504** to determine the particle count.

For example, in some embodiments, block **1586** may direct the controller processor **1501** to retrieve the first particle positions record **1680** shown in Figure **24** from the location **1542** of the storage memory **1504** shown in Figure **19** and to retrieve the second particle positions record **1740** shown in Figure **26** from the location **1544** of the storage memory **1504** and to compare the records to determine how many, if any, particles appearing in the pre-dispensed image were dispensed in the dispensed droplet.

In some embodiments, block **1586** may direct the controller processor **1501** to determine whether no particles were identified in the first particle positions record **1680**. If the controller processor **1501** determines that no particles were identified in the first particle positions record **1680**, then block **1586** may direct the controller processor **1501** to determine that no particles were dispensed and so the block may direct the controller processor **1501** to set a droplet particle count stored in the location **1550** of the storage memory **1504** to 0 and to proceed to block **1588** of the flowchart **1580** shown in Figure **20**.

In some embodiments, block **1586** may direct the controller processor **1501** to determine whether no particles were identified in the second particle positions record **1740**. If the controller processor **1501** determines that no particles were identified in the second particle positions record **1740**, then block **1586** may direct the controller processor **1501** to determine that all of the particles included in the first particle positions record **1680** were dispensed and so block **1586** may direct the controller processor **1501** to set the droplet particle count stored in the location **1550** of the storage memory **1504** to a count of the number of particles included in the first particle positions record **1680** and to proceed to block **1588** of the flowchart **1580** shown in Figure **20**.

In various embodiments, block **1586** directing the controller processor **1501** to first check whether the first and/or the second particle positions records **1680** and/or **1740** include no particles as described above may facilitate fast execution of block **1586**.

In various embodiments, block **1586** may include blocks of code for directing the controller processor **1501** to compare the first and second particle positions records **1680** and **1740** shown in Figures **24** and **26** when both the first and second particle positions records **1680** and **1740** include at least one particle. Referring to Figure **27**, there is shown a flowchart **1800** depicting blocks of code that may be included in the block generally similar to the block **206** in accordance with various embodiments.

In various embodiments, the flowchart **1800** begins with block **1802** which directs the controller processor **1501** to identify at least one unmatched pre-dispensed image particle of the pre-dispensed image particles as not matching any of the post-dispensed image particles and therefore representing a particle included in the dispensed droplet. For example, in some embodiments, block **1802** may direct the controller processor **1501** to identify at least one particle represented by the first particle positions record **1680** as not matching any of the particles represented by the second particle positions record **1740** and therefore representing a particle included in a dispensed droplet.

Block **1804** directs the controller processor **1501** to determine the particle count as a count of the at least one unmatched pre-dispensed image particle. In various embodiments, the unmatched particles may be counted via the droplet particle count stored in the location **1550** of the storage memory **1504** and so block **1804** may direct the controller processor **1501** to use the droplet particle count from the location **1550** of the storage memory **1504** to count the number of unmatched particles in the pre-dispensed image. For example, in some embodiments, the number of unmatched particles in the pre-dispensed image may be equal to 1 and so block **1804** may direct the controller processor **1501** to set the dispensed particle count to 1.

Referring to Figure **28**, there is shown a flowchart **1820** depicting blocks of code that may be used to implement the blocks **1802** and **1804** shown in Figure **27** in accordance with various embodiments. The flowchart **1820** begins with block **1822** which directs the controller processor **1501** to consider the most downstream particle from the pre-dispensed image. In some embodiments, block **1822** may direct the controller processor **1501** to consider the most downstream particle represented by the first particle positions record **1680** stored in the location **1542** of the storage memory **1504** shown in Figure **19**. In some embodiments, the most downstream particle may be the particle associated with the largest y pixel coordinate value and so in various embodiments, block **1822** may direct the controller processor **1501** to consider the particle associated with the x and y fields **1682** and **1684** with values of **101** and **361** respectively, as shown in Figure **24**.

Block **1824** may then direct the controller processor **1501** to determine whether a most downstream particle from the post-dispensed image **1720** is more downstream than the considered particle from the pre-dispensed image **1600**. Block **1824** may direct the controller processor **1501** to compare the y field of the most downstream particle from the second particle positions record **1740** with the y field **1684** of the first particle positions record **1680** having a value of **361** to determine whether the y field of the most downstream particle from the second particle positions record **1740** (having a value of **286**) is greater than the y field **1684** having a value of **361**.

In various embodiments, the controller processor **1501** may determine at block **1824** that the y field of the most downstream particle from the second particle positions record **1740** is not greater than the y field **1684** value and so block **1824** may direct the controller processor **1501** to determine that the most downstream particle from the post-dispensed image **1720** is not more downstream than the considered particle from the pre-dispensed image **1600** and so block **1824** may direct the controller processor **1501** to proceed to block **1826**.

In various embodiments, if the most downstream particle from the post-dispensed image is not more downstream than the considered particle, it may be concluded that the considered particle cannot be matched to any particle in the post-dispensed image, since any particle that could be matched to the considered particle should be downstream from it. In some embodiments, the inkjet nozzle having geometries or taper angles that help to ensure that particles are moving forward or downstream towards a nozzle orifice when dispensing takes place (For example, in some embodiments, the taper angles may be less than about 15º) may help to ensure this assumption is true.

Accordingly, block **1826** then directs the controller processor **1501** to increment a droplet particle count to count the unmatched considered particle. For example, in some embodiments, block **1826** may direct the controller processor **1501** to increment the droplet particle count stored in the location **1550** of the storage memory **1504** shown in Figure **19** from an initial value of 0 to a value of 1.

Referring to Figure **28**, in various embodiments, block **1828** then directs the controller processor **1501** to determine whether there is a next most downstream particle included in the pre-dispensed image **1600**. Block **1828** may direct the controller processor **1501** to read the first particle positions record **1680** and determine whether another particle that has a lower y field value than the considered particle is represented in the first particle positions record **1680**. If at block **1828** the controller processor **1501** determines that a next most downstream particle is included in the pre-dispensed image **1600**, block **1828** directs the controller processor **1501** to proceed to block **1830**.

Block **1830** directs the controller processor **1501** to consider the next most downstream particle form the pre-dispensed image. In some embodiments, where the particle associated with fields **1682** and **1684** of the first particle positions record **1680** shown in Figure **24** was considered at block **1822**, block **1830** may direct the controller processor **1501** to consider the particle associated with fields **1687** and **1688** of the first particle positions record **1680**.

Referring to Figure **28**, in various embodiments, block **1830** then directs the controller processor **1501** to return to block **1824** and determine whether a most downstream particle from the post-dispensed image **1720** is more downstream than the newly considered particle from the pre-dispensed image. In some embodiments, for example, where the considered particle is associated with the y field **1688**, block **1824** may direct the controller processor **1501** to determine that the value of 286 stored in the y field **1744** of the second particle positions record **1740** is greater than the value of 232 stored in the y field **1688** of the first particle positions record **1680**. Block **1824** may direct the controller processor **1501** to determine that since the particle associated with the fields **1742** and **1744** is more downstream than the particle associated with the fields **1687** and **1688**, there is a high likelihood that these particles are the same particles and so these particles are matched.

In various embodiments, the most downstream particle in the post-dispensed image may be assumed to be present in the pre-dispensed image, if there is one. In some embodiments, if the most downstream particle in the post-dispensed image is able to be matched to a pre-dispensed particle then all the remaining pre-dispensed particles upstream from the matched pre-dispensed particle in the pre-dispensed image may be assumed to have not been dispensed.

In various embodiments, once a considered particle from the first particle position record **1680** is matched and therefore determined not to have been dispensed, it may be assumed that any particles upstream of the matched particle may also be matchable and not dispensed. Accordingly, block **1824** may direct the controller processor **1501** to, upon determining that the most downstream particle from the post-dispensed image is more downstream than the considered particle from the pre-dispensed image, proceed to block **1832** and end the flowchart **1820**.

Referring to Figure **28**, in various embodiments, if at block **1828**, it is determined that there are no further upstream particles from the considered particle, the controller processor **1501** may also be directed to proceed to block **1832** and the flowchart **1820** may end. After the flowchart **1820** has been executed, there may be stored in the location **1550** of the storage memory **1504** shown in Figure **19**, the droplet particle count representing a count of particles included in the first droplet dispensed into the first target region of the microplate **30**. In various embodiments, after the flowchart **1820** has been executed, execution of block **1586** may be complete.

In various embodiments block **1588** of the flowchart **1580** shown in Figure **20** may then be executed by the controller processor **1501**. Block **1588** may direct the controller processor **1501** to produce signals for associating a representation of the droplet particle count with the target region. In various embodiments, block **1588** may be generally similar to block **208** of the flowchart **200** shown in Figure **4** and may include blocks generally similar to those included in the flowchart **500** shown in Figure **11**, except that no confidences may be used and so blocks **507**, and **509** may be omitted. Further, a block generally similar to block **508** may direct the controller processor **1501** to move the second particle positions record **1740** shown in Figure **26** from the location **1544** to the location **1542** of the storage memory **1504** shown in Figure **19**, such that the second particle positions record **1740** is treated as a pre-dispensed particle positions record.

Accordingly, in various embodiments, after execution of a block generally similar to block **1588** and further executions of the blocks **1584**, **1586** and **1588**, there may be stored in the location **1554** of the storage memory **1504**, a target particle count record including target particle counts for each target, which may have a format generally similar to the target particle record **1060** shown in Figure **12**.

<u>Various embodiments</u>

In some embodiments, block **208** may include further or alternative code for directing the controller processor **100** to produce signals for associating a representation of the droplet particle count with the target region. For example, in some embodiments block **208** may include code for directing the controller processor **100** to produce signals representing the target count record **1060**, for causing the target count record **1060** to be exported to another system and/or used in later testing/processing of the particles dispensed into the microplate **30**. In some embodiments, block **1588** of the flowchart **1580** shown in Figure **20** may include generally similar code for directing the controller processor **1501** to produce signals associating a representation of the droplet particle count with the target region.

In various embodiments, blocks **202** and **204** may direct the controller processor **100** to receive and store alternative or additional representations of pre-dispensed and post-dispensed images. For example, in some embodiments, the complete images may be stored in the locations **140, 142, 144,** and **150** and block **206** may direct the controller processor **100** to compare the pre-dispensed images **240**, **248**, and **254** and the post-dispensed image **320** directly, for example by inputting these images into a neural network. In some embodiments, blocks **1582** and **1584** of the flowchart **1580** shown in Figure **20** may include code for directing the controller processor **1501** to receive and store alternative or additional representations of pre-dispensed and post-dispensed images, such as the complete image, for example.

In some embodiments, block **1586** of the flowchart **1580** shown in Figure **20** may include code for directing the controller processor **1501** to apply one or more neural networks to the pre-dispensed image **1640** and the post-dispensed image **1720** to determine the particle count representing the count of particles included in a droplet. For example, in some embodiments, block **206** may direct the controller processor **1501** to apply a neural network function **600** as shown in Figure **29** to a pre-dispensed image **602** and post-dispensed image **604** shown in Figure **29** to determine a particle count representing the count of particles included in the droplet. In various embodiments, the neural network function **600** may have been previously trained using pre-dispensed and post-dispensed images associated with verified particle counts.

Referring to Figure **29**, the post-dispensed image **604** may be subtracted from the pre-dispensed image **602** and the result may be normalized to generate an image subtraction and normalization image **606**, which may be flattened and input into an input layer **608**. The output of the input layer **608** may be processed by hidden layers **610** to an output layer **612**, with output **614** representing a count confidence associated with no cells dispensed, output **616** representing a count confidence associated with one cell dispensed, and output **618** representing a count confidence associated with multiple cells dispensed.

In some embodiments, use of deep neural networks may outperform traditional algorithms in both accuracy and processing speed by a wide margin. Additionally, in some embodiments, use of a deep learning algorithm may facilitate providing a confidence level for predictions. In some embodiments, this may help decrease the impact of a false positive or negative by providing another metric, as false predictions may have low confidence levels while correct classifications may have very high confidence levels. In various embodiments, this may be difficult or impossible in traditional algorithms as logic systems tend to produce more or less binary results.

In some embodiments, any or all of the at least one pre-dispensed images and/or any or all of the at least one post-dispensed images described herein may each include a plurality of images represented by a video, for example. In such embodiments, the plurality of images may be treated generally as described above regarding the single pre-dispensed images and post-dispensed images.

In some embodiments, block **502** of the flowchart **500** shown in Figure **11** may include a break condition such that block **502** may direct the controller processor **100** to proceed to block **504** and move on to a next target region if a threshold maximum number of additional droplets have been dispensed to a single target region. In some embodiments, block **502** may direct the controller processor **100** to store in location **174** of storage memory **104** a count of the number of times that block **502** has directed the controller processor **100** to proceed to block **508** for a particular target region and block **502** may direct the controller processor **100** to determine whether the count stored in the location **174** of the storage memory **104** is greater than the threshold maximum number of additional droplets. If the count is greater than the threshold maximum number of additional droplets, block **502** may direct the controller processor **100** to proceed to block **504** regardless of whether the dispensed particle count is less than the desired particle count. In some embodiments, the threshold number of additional droplets may be about 9, for example, such that a maximum of 10 droplets may be dispensed for each target region. In various embodiments, this may help the system **10** work with some applications such as genome sequencing where the reagent composition must be closely controlled. For example, in some embodiments, this may help the system **10** work with applications where downstream applications may be volumetrically sensitive (e.g. downstream chemical reactions), because, for example, if too many droplets are dispensed this would dilute reagents that may already be in the target regions or nanowells. For example, if the cells are dispensed directly into a lysis buffer that has previously been aliquoted into the nanowells, dispensing too many empty droplets will dilute the lysis buffer. In various embodiments, the above feature may be implemented in the controller **1500** with the additional droplet count stored in the location **1556** of the storage memory **1504** shown in Figure **19**.

Referring now to Figure **30**, there is shown a schematic representation of elements of the system **10** shown in Figure **1**, in accordance with various embodiments. Referring to Figure **30**, the system **10** includes the controller **12**, which may include a computer running main controlling software, for example, in communication with the imager **14**, which may include a camera, for example, and the substrate stage **18**, which may include an X-Y positioning stage, for example. The system **10** also includes a microcontroller **20** in communication with the imager **14** and a function generator and amplifier **22** and a light emitting diode (LED) **24** both in communication with the microcontroller. In various embodiments, the function generator and amplifier **22** may be in communication with the dispenser **16**, which may include an inkjet nozzle, for example.

In some embodiments, block **204** of the flowchart **200** shown in Figure **4** may direct the controller processor **100** to send a signal via USB to the imager **14** for causing the imager **14** to cause a droplet to be dispensed by the droplet dispenser **16** and for triggering the capture of a post-dispensed image with an exposure time of about 3-5ms for example. Accordingly, in some embodiments, the controller processor **100** may cause the capture of a post-dispensed image to begin prior to dispensing the droplet and so, in some embodiments, the post-dispensed image may be captured during and after the droplet has been ejected. In various embodiments, scientific cameras may typically require exposures on the order of milliseconds while inkjet nozzles may operate at frequencies of 100-1000 Hz and common microcontrollers may operate in the MHz ranges. Therefore, in some embodiments, triggering the camera capture first may save on latency with the propagation of signals between the devices as it may be the rate limiting step and noticeable effects may be small. Accordingly, in some embodiments, capturing the post-dispensed image both during and after the droplet has been ejected may be a fast way to synchronize all necessary components to acquire an image of the nozzle after dispensing.

In some embodiments, upon receiving the signal from the controller **12**, the imager **14** may send a transistor-transistor logic ("TTL") signal via a wire to the microcontroller **20** and the microcontroller **20** may receive the signal as a hardware interrupt. The microcontroller **20** may then send a TTL signal via a wire to the function generator and amplifier **22** which may respond by outputting an actuation signal to the droplet dispenser **16** to cause a droplet to be dispensed. The droplet dispenser **16** may receive the actuation signal and dispense a droplet. The microcontroller **20** may wait for a user defined delay (e.g., between about 100 and about 1000 μs) and then produce signals for causing the LED **24** to turn on. In various embodiments, this delay may be long enough to allow the droplet dispenser **16** to dispense the droplet before turning the LED **24** on. In various embodiments, the LED **24** may remain on until the post-dispensed image exposure time of 3-5ms has elapsed. Once the post-dispensed image has been captured, the imager **14** may transmit a representation of the post-dispensed image to the controller **12** and block **204** may be executed as described herein.

In various embodiments, if at block **208**, a "move" command is necessary, then block **208** may direct the controller processor **100** to send a serial command to the substrate stage **18** via USB, for example, to tell the substrate stage how much to move by on each axis. Once the move is completed the substrate stage **18** may send a serial command back to the controller **12** signaling completion of the move command and the controller **12** may proceed with further processing. In various embodiments, the controller **1500** may be included in the system **10** in place of the controller **12** shown in Figure **30**.

In some embodiments, a system generally similar to the system **10** shown in Figure **1** may include a controller configured to communicate directly with the droplet dispenser **16** and configured to control the droplet dispenser **16**.

In some embodiments, block **208** of the flowchart **200** shown in Figure **4** may direct the controller processor **100** to determine whether a dispensed particle count matches a desired particle count and, if the particle count matches the desired particle count, to produce signals identifying the target region as containing the desired particle count. For example, in some embodiments, block **208** may direct the controller processor **100** to include in the target count record **1060**, in addition to or alternatively to the particle count fields, a target status field associated with each target region. In some embodiments, the target status field may include a status indicator, which in some embodiments may be set to TRUE if the dispensed particle count for the associated target region matches the desired particle count and set to FALSE if the dispensed particle count for the associated target region does not match the desired particle count. In various embodiments, the status indicator may act as a representation of the dispensed particle count. Accordingly, in various embodiments, block **208** may direct the controller processor **100** to determine whether a dispensed particle count matches the desired particle count and to set the target status field accordingly. In various embodiments, block **1588** of the flowchart **1580** shown in Figure **20** may include similar code.

In some embodiments, there may be a range of desired particle counts and block **208** or block **1588** may direct the controller processor **100** or **1501** to determine whether the dispensed particle count matches a desired particle count range.

In some embodiments, the dispensed particle count may be a non-integer, such as, for example, where a neural network is used to determine the dispensed particle count.

In various embodiments, the target status field may include additional or alternative information such as, for example, the number of droplets dispensed into the target before dispensing the particle, information about cell morphology or other features such as from fluorescence image acquisition, phenotypic scores based on multidimensional classification and/or other information.

In some embodiments block **208** or block **1588** may direct the controller processor **100** or **1501** to simply record the droplet particle count as the dispensed particle count and/or record a status indicator in association with each target region, without checking whether further droplets should be dispensed to the target. In some embodiments, a new target may be targeted after each droplet is dispensed.

In some embodiments, all of the images or a subset of the images (e.g., post-dispensed and pre-dispensed images) received may be kept for record keeping purposes.

In various embodiments, the neural network defined by data stored in the location **158** of the storage memory **104** shown in Figure **3** (such as one generally similar to the CNN **1000** shown in Figure **10**) may be configured to output count confidences associated with additional or alternative counts, such as, for example, 2 particles, 3 particles, 4 particles, or any other number of particles and those count confidences may be treated generally as described herein.

In some embodiments, the neural network definition data stored in the location **158** of the storage memory **104** shown in Figure **3** may be stored on a device separate from the controller **12** and in communication with the controller **12**. In some embodiments, the processing required to generate the outputs for the CNN **1000** shown in Figure **10** may be performed by one or more separate devices from the controller **12** and the results may be transmitted to the controller **12**.

In some embodiments, devices or systems described as separate herein may be implemented as a single device. For example, in some embodiments, the trainer **1244** and the controller of the system **1242** shown in Figure **16** may be implemented as a single device.

In various embodiments, block **206** of the flowchart **200** shown in Figure **4** may direct the controller processor **100** shown in Figure **3** to store all of the determined count confidences, and not just the largest count confidence, in the location **161** of the storage memory **104**. In some embodiments, the target count confidence record **1100** shown in Figure **13** may store all of the count confidences and the associated counts for all droplets dispensed to a particular target.

As disclosed herein, in some embodiments, the system **10** shown in Figure **1** may include a display in communication with the controller **12** and the block of codes **190** of the program memory **102** may include code for directing the controller processor **100** shown in Figure **3** to communicate with the display to cause a representation of the target count record **1060** shown in Figure **12**, the target count confidence record **1100** shown in Figure **13**, and/or the target count validity record **1180** shown in Figure **15** to be displayed by the display to a user. Referring to Figure **31**, there is shown an exemplary graphical user interface ("GUI") **1900** which may be displayed to a user using the system **10**. The GUI **1900** may include a representation **1902** of the microplate **30** shown in Figure **2**, wherein the representation **1902** depicts information from the target count record **1060** shown in Figure **12**, showing particle counts for each target region of the microplate **30**. In some embodiments, the representation **1902** may include, for each target region, a visual indicator representing the particle count for that target region, as set out in the target count record **1060**.

In some embodiments, a target region associated with a particle count of 1 may be shown as green (represented by a white box, such as white box **1904**, shown in Figure **31**), a target region associated with a particle count of 2 or more may be shown as red (represented by a box including an X, such as box **1906**, shown in Figure **31**), and a target region associated with a count of 0 may be shown as a black box, such as box **1908** shown in Figure **31**.

In some embodiments, a user may select any of the boxes included in the representation **1902** to view additional information for the target region associated with the selected box. In some embodiments, for example, box **1910** may be selected as shown in Figure **31** and codes included in the block of codes **190** shown in Figure **3** may direct the controller processor **100** to cause a representation **1920** of one or more count confidences for the selected block **1910** to be displayed in the display **1900**. In some embodiments, the box **1910** may be selected as shown in Figure **31** and codes included in the block of codes **190** shown in Figure **3** may direct the controller processor **100** to cause a representation **1930** of a first pre-dispensed image **1932** and a post-dispensed image **1934** used as inputs for the selected box **1910**. In some embodiments, the count confidences, first pre-dispensed image **1932** and post dispensed image **1934** displayed may be those associated with a droplet for which a desired particle count of 1 was determined.

While specific embodiments of the invention have been described and illustrated, such embodiments should be considered illustrative of the invention only and not as limiting the invention as construed in accordance with the accompanying claims.

CLAIMS:

1. A method of facilitating controlled particle deposition from a droplet dispenser, the method comprising:

receiving at least one pre-dispensed image of a dispensing portion of the droplet dispenser, the dispensing portion including fluid to be dispensed in a subject droplet to a subject target region;

receiving at least one post-dispensed image of the dispensing portion of the droplet dispenser after the subject droplet has been dispensed;

comparing the at least one pre-dispensed image and the at least one post-dispensed image to determine a subject droplet particle count representing a count of particles included in the subject droplet; and

producing signals for associating a representation of the subject droplet particle count with the subject target region.

2. The method of claim **1** wherein comparing the at least one pre-dispensed image and the at least one post-dispensed image comprises causing a representation of the at least one pre-dispensed image and the at least one post-dispensed image to be input into one or more functions.

3. The method of claim **2** wherein causing the representation of the at least one pre-dispensed image and the at least one post-dispensed image to be input into the one or more functions comprises causing the one or more functions to generate a plurality of count confidences, each associated with a respective prospective particle count.

4. The method of claim **3** wherein comparing the at least one pre-dispensed image and the at least one post-dispensed image to determine the subject droplet particle count comprises determining the subject droplet particle count to be the particle count associated with the largest of the plurality of count confidences.

5. The method of claim **3** or **4** comprising comparing the largest of the plurality of count confidences to a threshold confidence to determine whether the largest of the plurality of confidences is less than the threshold confidence.

6. The method of any one of claims **2** to **5** wherein comparing the at least one pre-dispensed image and the at least one post-dispensed image comprises:

generating a first image difference representing a difference between the first pre-dispensed image and the at least one post-dispensed image; and

causing a representation of the first image difference to be input into the one or more functions.

7. The method of claim **6** wherein:

the first pre-dispensed image represents the droplet dispenser at a first pre-dispensed time;

the at least one pre-dispensed image includes a second pre-dispensed image representing the droplet dispenser at a second pre-dispensed time prior to the first pre-dispensed time; and

comparing the at least one pre-dispensed image and the at least one post-dispensed image comprises:

generating a second image difference representing a difference between the second pre-dispensed image and the first pre-dispensed image; and causing a representation of the second image difference to be input into the one or more functions.

8. The method of claim **7** comprising causing a first preceding droplet to be dispensed by the droplet dispenser between the second pre-dispensed time and the first pre-dispensed time.

9. The method of claim **7** or **8** wherein:

the at least one pre-dispensed image includes a third pre-dispensed image representing the droplet dispenser at a third pre-dispensed time prior to the second pre-dispensed time; and

comparing the at least one pre-dispensed image and the at least one post-dispensed image comprises:

generating a third image difference representing a difference between the third pre-dispensed image and the second pre-dispensed image;

causing a representation of the third image difference to be input into the one or more functions.

10. The method of claim **9** comprising causing a second preceding droplet to be dispensed by the droplet dispenser between the third pre-dispensed time and the second pre-dispensed time.

11. The method of any one of claims **2** to **10** wherein the one or more functions include one or more neural network functions.

12 The method of claim **11** comprising training the one or more neural network functions.

13. The method of any one of claims **1** to **12** wherein producing signals for associating the representation of the subject droplet particle count with the subject target region comprises determining whether the subject droplet particle count matches a desired particle count and, if the subject droplet particle count matches the desired particle count, producing signals for identifying the subject target region as containing the desired particle count.

14. The method of any one of claims **1** to **13** comprising determining whether the subject droplet particle count is less than a desired particle count and, if the subject droplet particle count is not less than the desired particle count, producing signals for causing the droplet dispenser to be configured to dispense a subsequent droplet to a subsequent target region different from the subject target region.

15. The method of any one of claims **1** to **14** comprising determining whether the subject droplet particle count is less than a desired particle count and, if the subject droplet particle count is less than the desired particle count, producing signals for causing the droplet dispenser to dispense a further droplet to the subject target region.

16. The method of any one of claims **1** to **15** comprising:

identifying one or more pre-dispensed image particles depicted in the at least one pre-dispensed image; and

identifying one or more post-dispensed image particles depicted in the at least one post-dispensed image;

wherein comparing the at least one pre-dispensed image and the at least one post-dispensed image comprises:

identifying at least one unmatched pre-dispensed image particle of the one or more pre-dispensed image particles as not matching any of the post-dispensed image particles and therefore representing a particle included in the subject droplet; and

determining the subject droplet particle count as a count of the at least one unmatched pre-dispensed image particle.

17. A method of training at least one neural network function for facilitating controlled particle deposition, the method comprising:

receiving a plurality of sets of training images, each of the sets of training images including:

at least one pre-dispensed image of a dispensing portion of a droplet dispenser, the dispensing portion including fluid to be dispensed in a droplet to a target region; and

at least one post-dispensed image of the dispensing portion of the droplet dispenser after the droplet has been dispensed;

receiving a plurality of droplet particle counts, each of the droplet particle counts associated with one of the sets of training images and representing a count of particles dispensed in the droplet that is the subject of the associated set of training images; and

causing the at least one neural network function to be trained using representations of the sets of training images as respective inputs and the associated droplet counts as desired outputs.

18. The method of claim **17** wherein, for each of the sets of training images:

the at least one pre-dispensed image included in the set of training images includes a first pre-dispensed image; and causing the at least one neural network function to be trained comprises:

generating a first image difference representing a difference between the first pre-dispensed image and the at least one post-dispensed image; and

causing a representation of the first image difference to be input into the at least one neural network function.

19. The method of claim **18** wherein, for each of the sets of training images:

the first pre-dispensed image included in the set of training images represents the droplet dispenser at a first pre-dispensed time;

the at least one pre-dispensed image included in the set of training images includes a second pre-dispensed image representing the droplet dispenser at a second pre-dispensed time prior to the first pre-dispensed time; and

causing the at least one neural network function to be trained comprises:

generating a second image difference representing a difference between the second pre-dispensed image and the first pre-dispensed image; and

causing a representation of the second image difference to be input into the at least one neural network function.

20. The method of claim **19** wherein the second pre-dispensed image represents the droplet dispenser at the second pre-dispensed time prior to the first pre-dispensed time, a first preceding droplet having been dispensed by the droplet dispenser between the second pre-dispensed time and the first pre-dispensed time.

21. The method of claim **19** or **20** wherein, for each of the sets of training images:

the at least one pre-dispensed image included in the set of training images includes a third pre-dispensed image representing the droplet dispenser at a third pre-dispensed time prior to the second pre-dispensed time; and

causing the at least one neural network function to be trained comprises:

generating a third image difference representing a difference between the third pre-dispensed image and the second pre-dispensed image; and

causing a representation of the third image difference to be input into at least one neural network function.

22. The method of claim **21** wherein the third pre-dispensed image represents the droplet dispenser at the third pre-dispensed time prior to the second pre-dispensed time, a second preceding droplet having been dispensed by the droplet dispenser between the third pre-dispensed time and the second pre-dispensed time.

23. A system for facilitating controlled particle deposition from a droplet dispenser, the system comprising at least one processor configured to perform the method of any one of claims **1** to **22**.

24. A non-transitory computer readable medium having stored thereon codes that, when executed by at least one processor, cause the at least one processor to perform the method of any one of claims **1** to **22**.

25. A system for facilitating controlled particle deposition from a droplet dispenser, the system comprising:

means for receiving at least one pre-dispensed image of a dispensing portion of the droplet dispenser, the dispensing portion including fluid to be dispensed in a subject droplet to a subject target region;

means for receiving at least one post-dispensed image of the dispensing portion of the droplet dispenser after the subject droplet has been dispensed;

means for comparing the at least one pre-dispensed image and the at least one post-dispensed image to determine a subject droplet particle count representing a count of particles included in the subject droplet; and

means for producing signals for associating a representation of the subject droplet particle count with the subject target region.

26. A system for training at least one neural network function for facilitating controlled particle deposition, the system comprising:

means for receiving a plurality of sets of training images, each of the sets of training images including:

at least one pre-dispensed image of a dispensing portion of a droplet dispenser, the dispensing portion including fluid to be dispensed in a subject droplet to a subject target region; and at least one post-dispensed image of the dispensing portion of the droplet dispenser after the subject droplet has been dispensed;

means for receiving a plurality of subject droplet particle counts, each of the subject droplet particle counts associated with one of the sets of training images and representing a count of particles dispensed in the subject droplet for the set of training images; and

means for causing the at least one neural network function to be trained using representations of the sets of training images as respective inputs and the associated subject droplet counts as desired outputs.

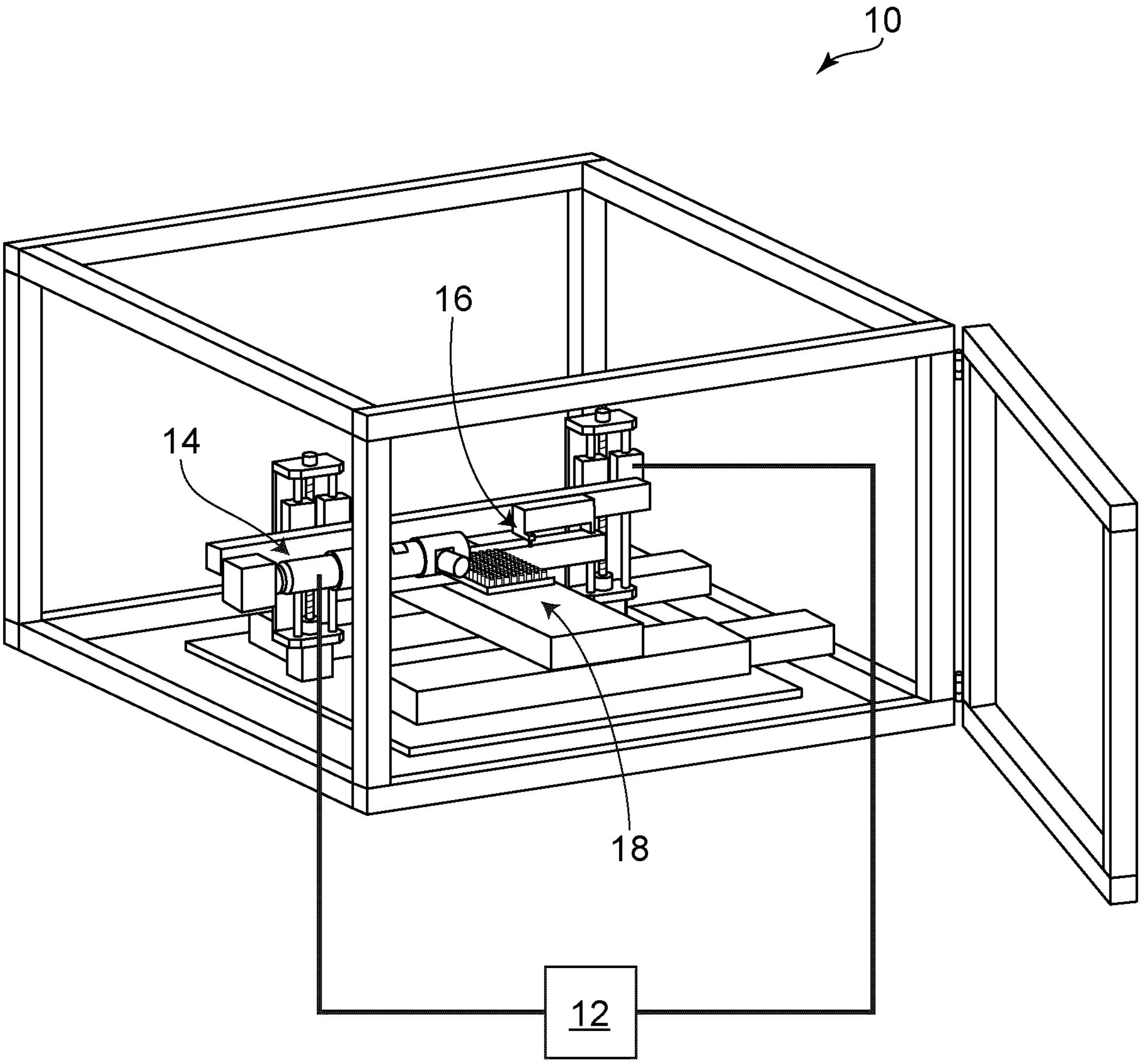

FIG. 1

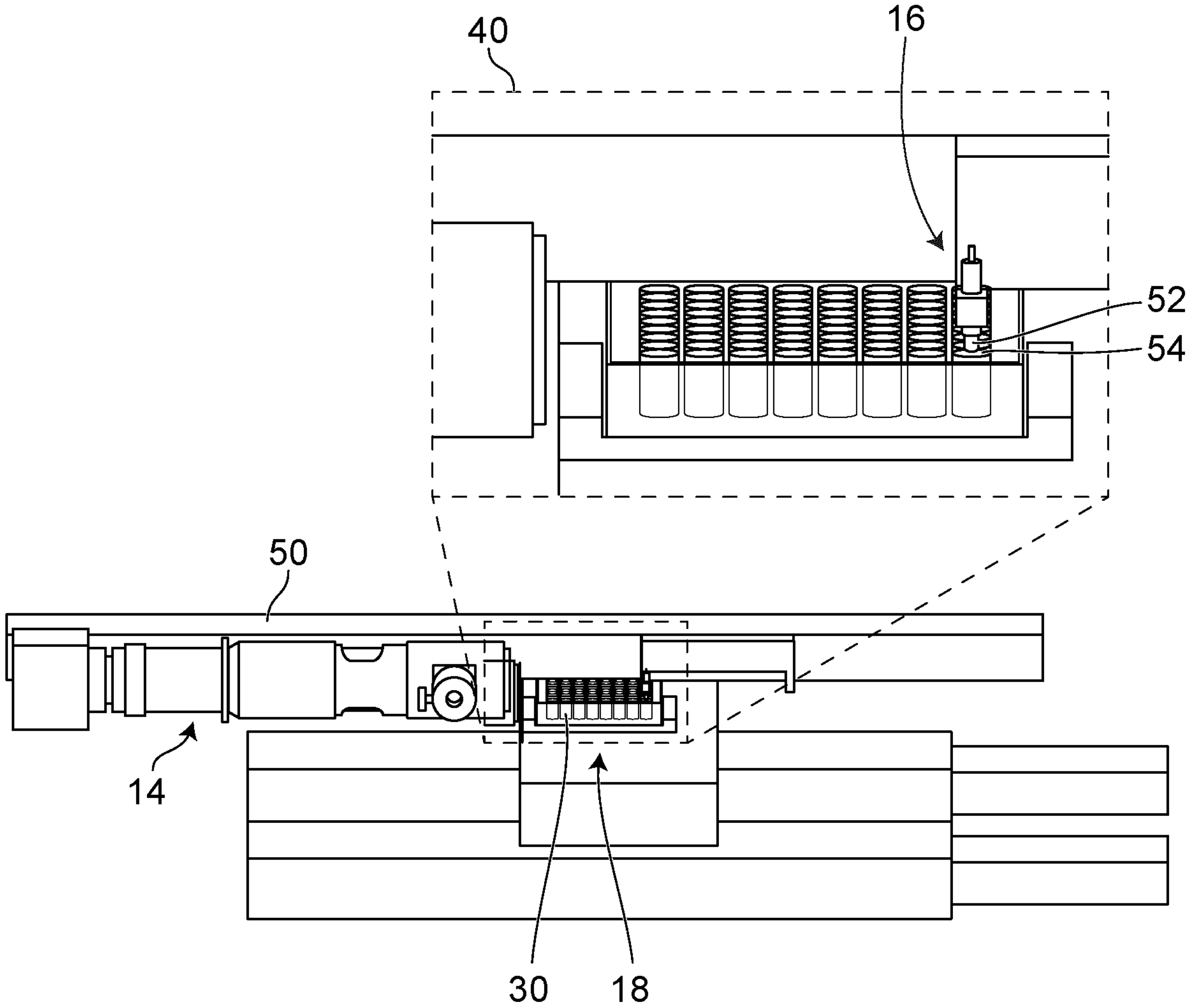

FIG. 2

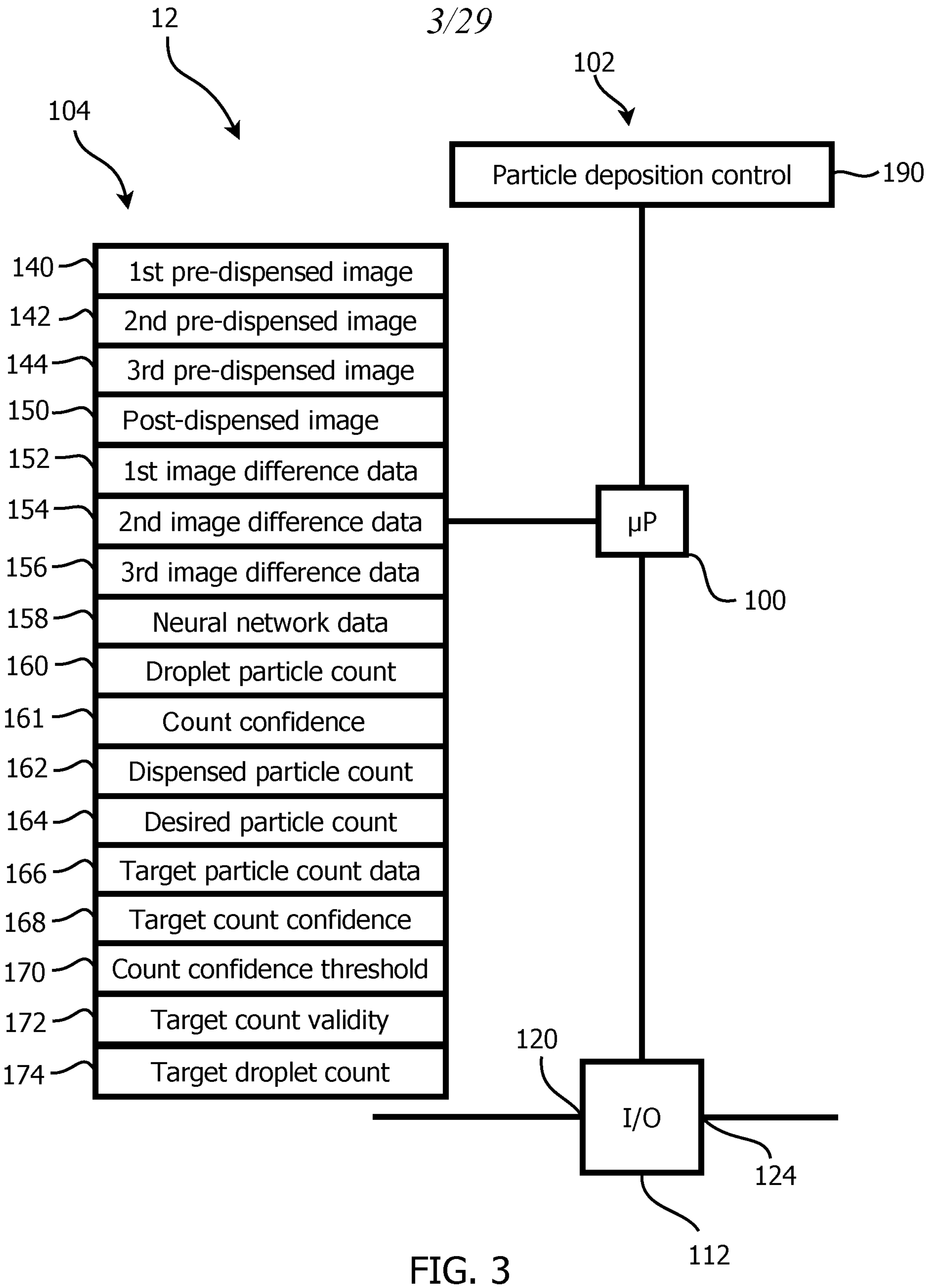
12
102
104
Particle deposition control
190
140
1st pre-dispensed image
142
2nd pre-dispensed image
144
3rd pre-dispensed image
150
Post-dispensed image
152
1st image difference data
154
2nd image difference data
156
3rd image difference data
158
Neural network data
160
Droplet particle count
161
Count confidence
162
Dispensed particle count
164
Desired particle count
166
Target particle count data
168
Target count confidence
170
Count confidence threshold
172
Target count validity
174
Target droplet count
µP
100
120
I/O
124
112

FIG. 3

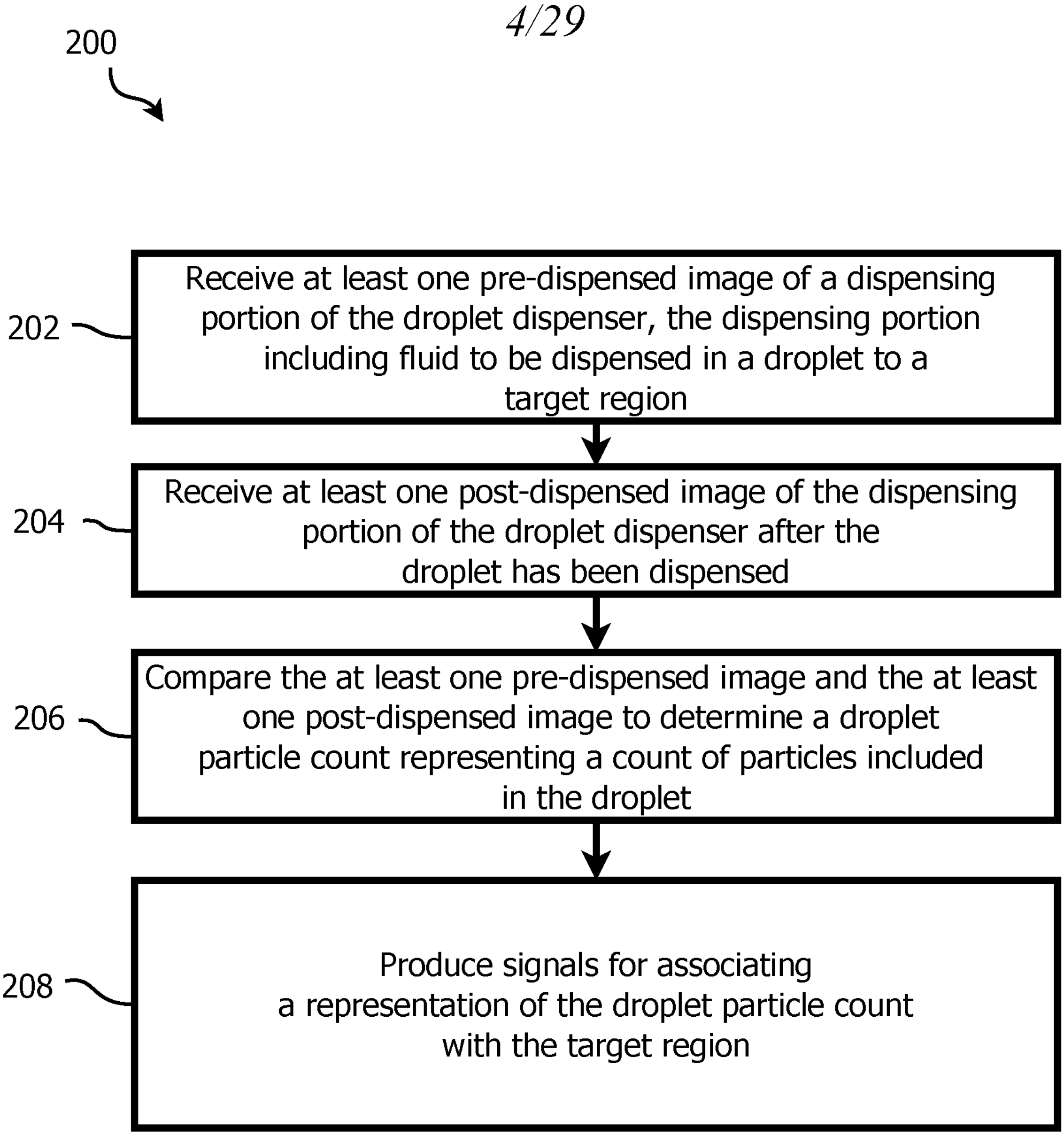
200
202
Receive at least one pre-dispensed image of a dispensing portion of the droplet dispenser, the dispensing portion including fluid to be dispensed in a droplet to a target region
204
Receive at least one post-dispensed image of the dispensing portion of the droplet dispenser after the droplet has been dispensed
206
Compare the at least one pre-dispensed image and the at least one post-dispensed image to determine a droplet particle count representing a count of particles included in the droplet
208
Produce signals for associating a representation of the droplet particle count with the target region

FIG. 4

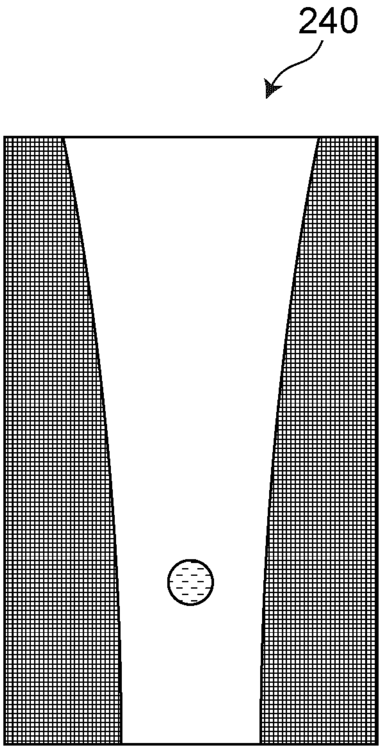

FIG. 5

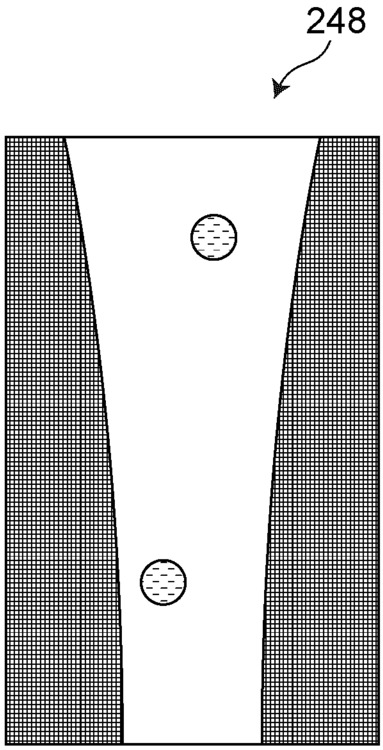

FIG. 6

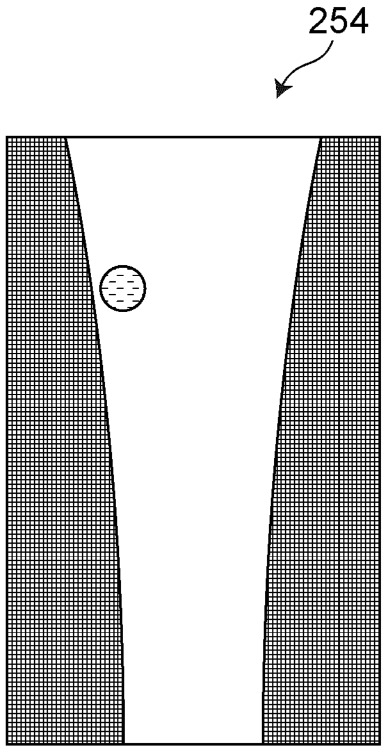

FIG. 7

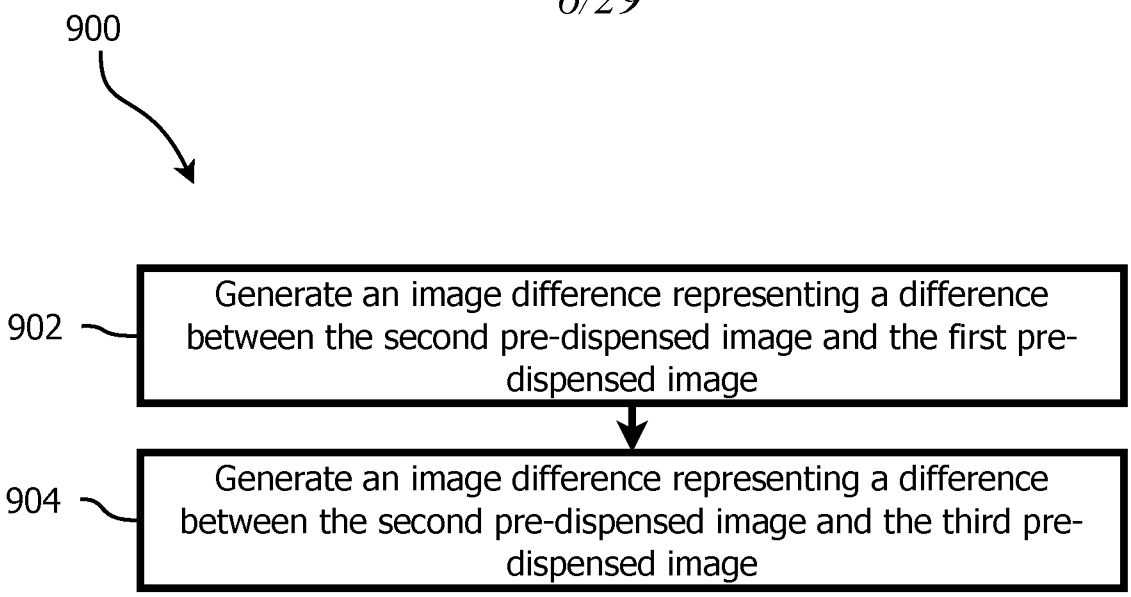
900
902
Generate an image difference representing a difference between the second pre-dispensed image and the first pre-dispensed image
904
Generate an image difference representing a difference between the second pre-dispensed image and the third pre-dispensed image

FIG. 8

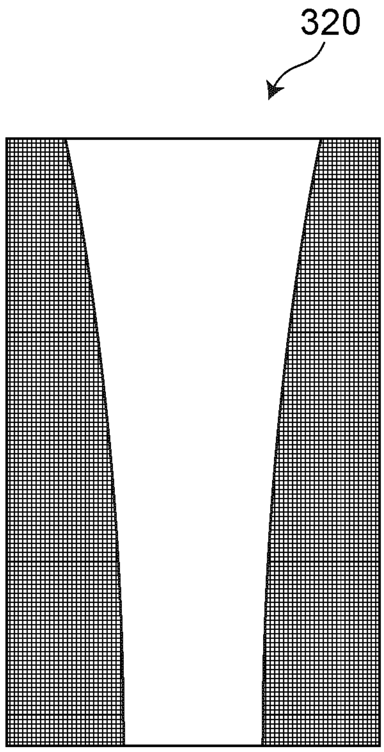

FIG. 9

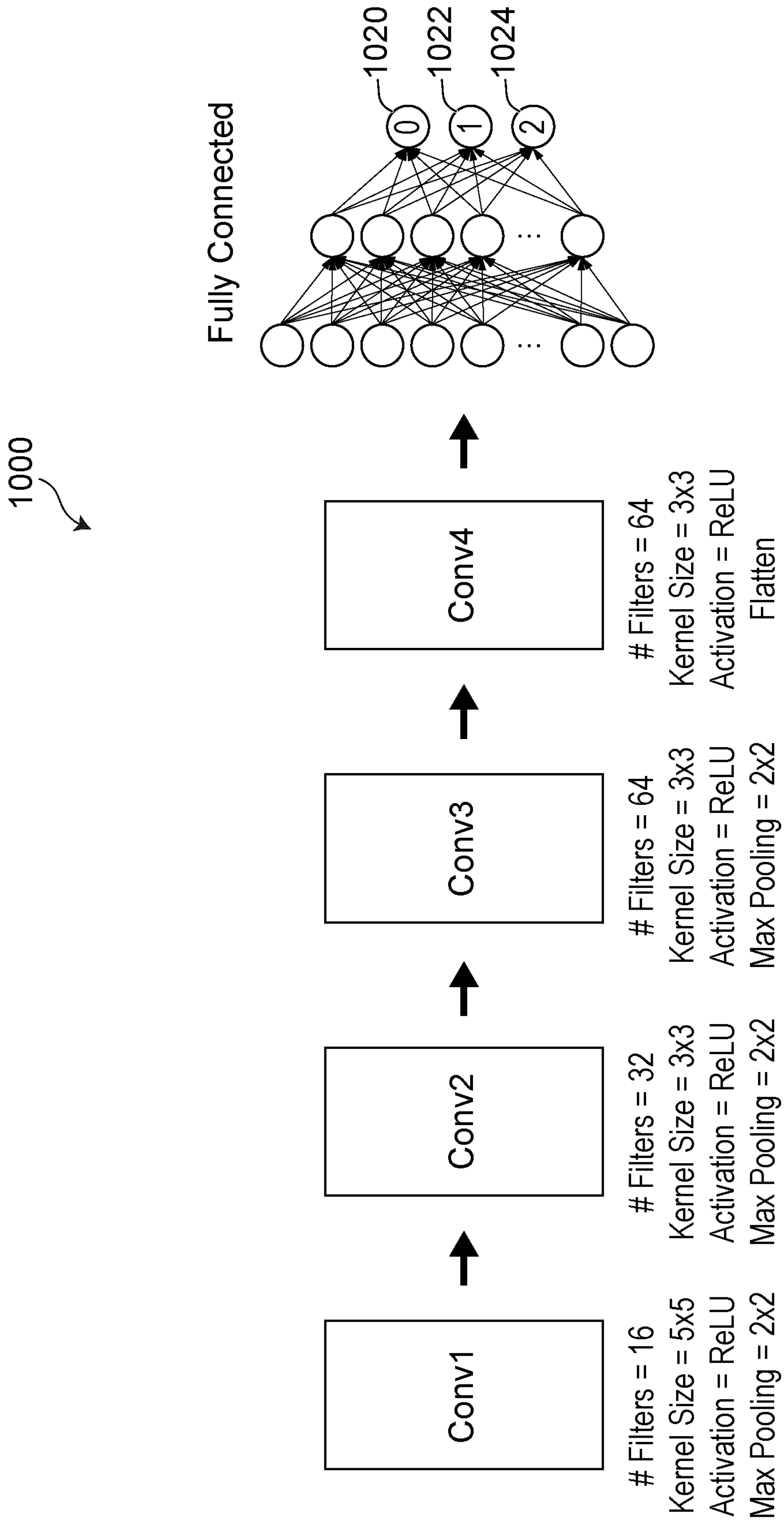
1000
Conv1
Filters = 16
Kernel Size = 5x5
Activation = ReLU
Max Pooling = 2x2
Conv2
Filters = 32
Kernel Size = 3x3
Activation = ReLU
Max Pooling = 2x2
Conv3
Filters = 64
Kernel Size = 3x3
Activation = ReLU
Max Pooling = 2x2
Conv4
Filters = 64
Kernel Size = 3x3
Activation = ReLU
Flatten
Fully Connected
0
1
2
1020
1022
1024

FIG. 10

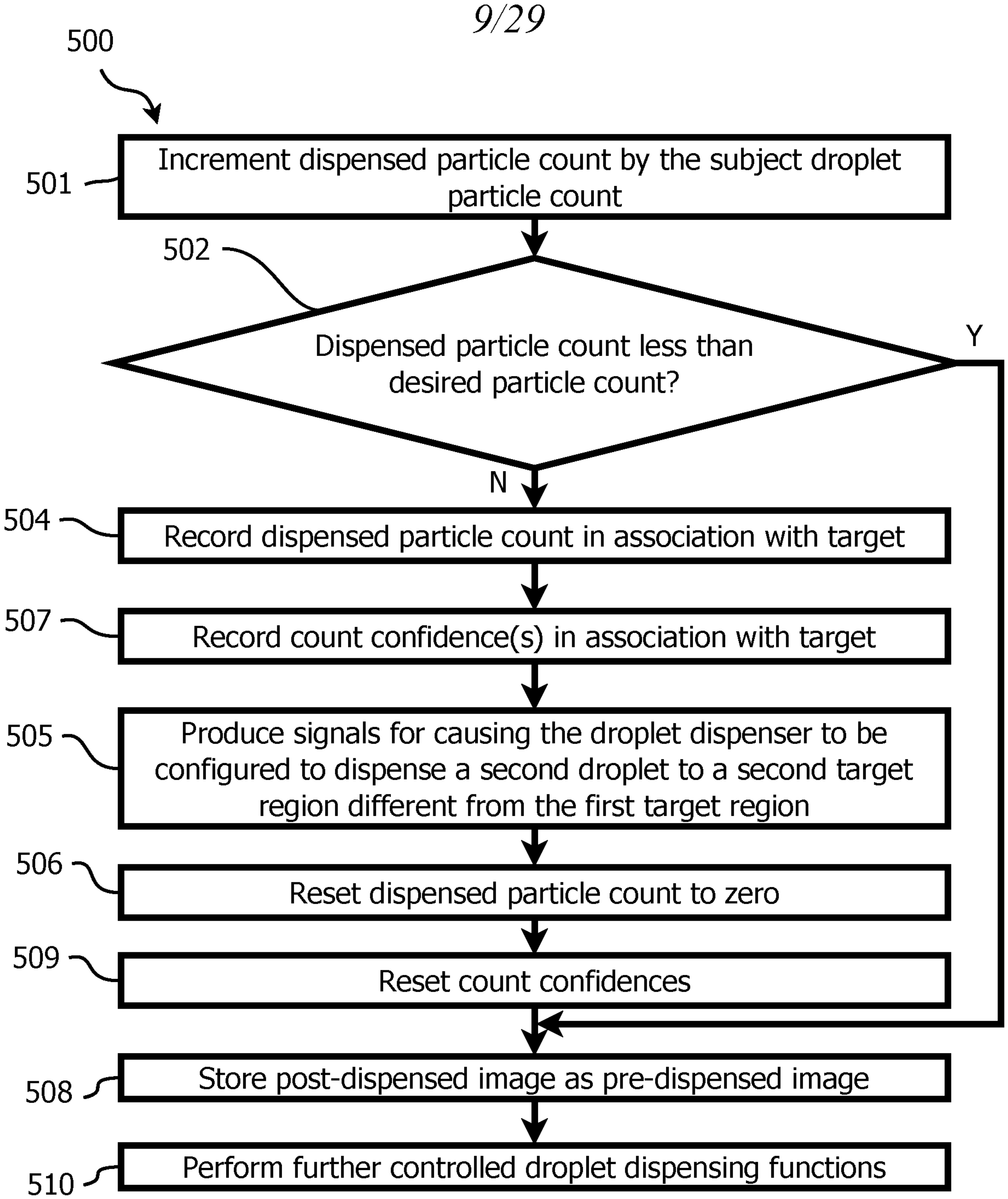
500
501
Increment dispensed particle count by the subject droplet particle count
502
Dispensed particle count less than desired particle count?
Y
N
504
Record dispensed particle count in association with target
507
Record count confidence(s) in association with target
505
Produce signals for causing the droplet dispenser to be configured to dispense a second droplet to a second target region different from the first target region
506
Reset dispensed particle count to zero
509
Reset count confidences
508
Store post-dispensed image as pre-dispensed image
510
Perform further controlled droplet dispensing functions

FIG. 11

1060

Target count record

1062	Target 1 total count	1
1064	Target 2 total count	0
1066	Target 3 total count	0
1068	Target 4 total count	0
	...	
1070	Target 5184 total count	0

FIG. 12

1100

Target count confidence record

1102	Target 1 confidence	0.95
1104	Target 2 confidence	0
1106	Target 3 confidence	0
	...	
1108	Target 5184 confidence	0

FIG. 13

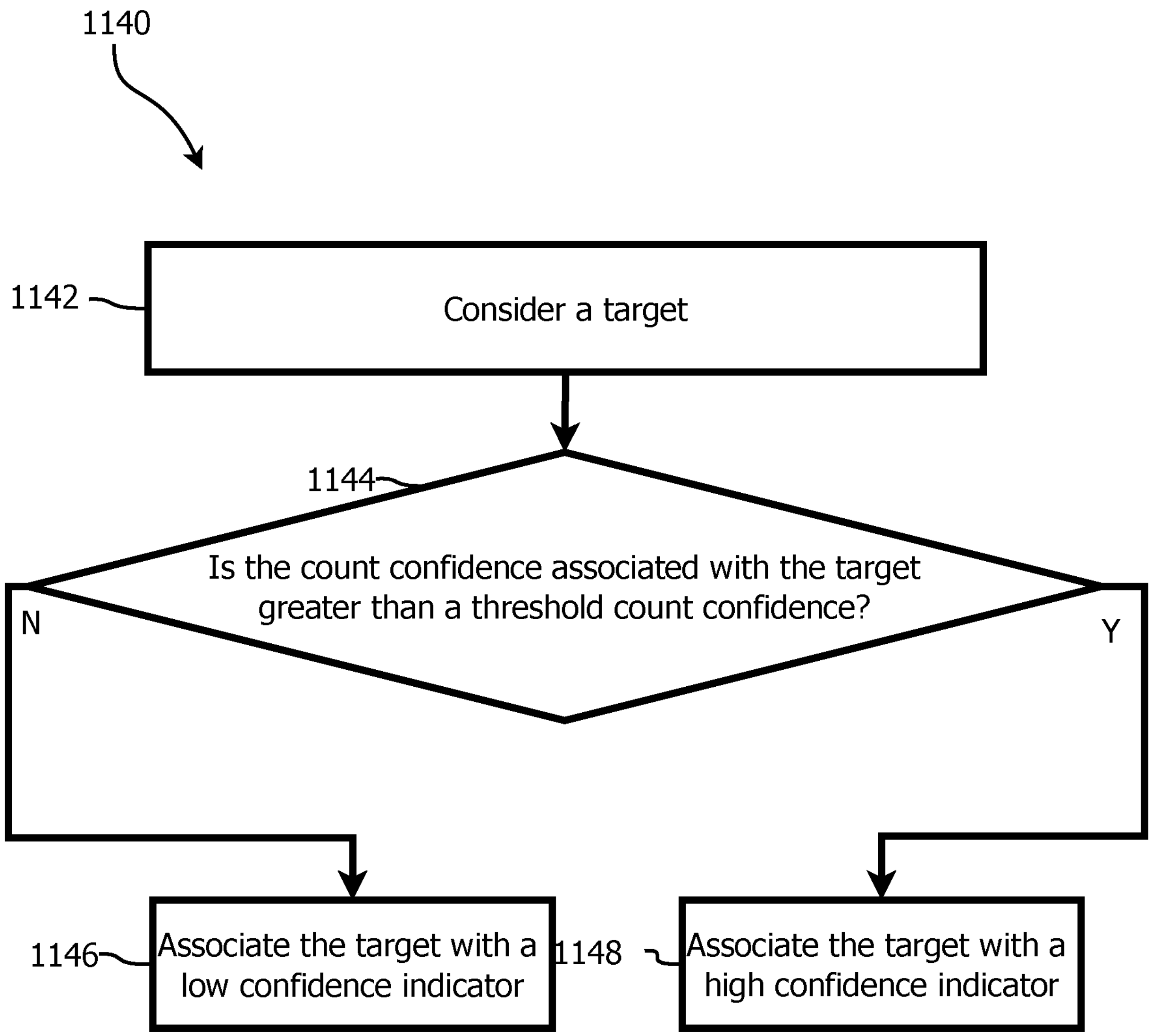
1140
1142
Consider a target
1144
Is the count confidence associated with the target greater than a threshold count confidence?
N
Y
1146
Associate the target with a low confidence indicator
1148
Associate the target with a high confidence indicator

FIG. 14

1180

Target count validity record

Target 1 validity	VALID
Target 2 validity	VALID
Target 3 validity	VALID
Target 4 validity	INVALID
...	
Target 5184 validity	VALID

FIG. 15

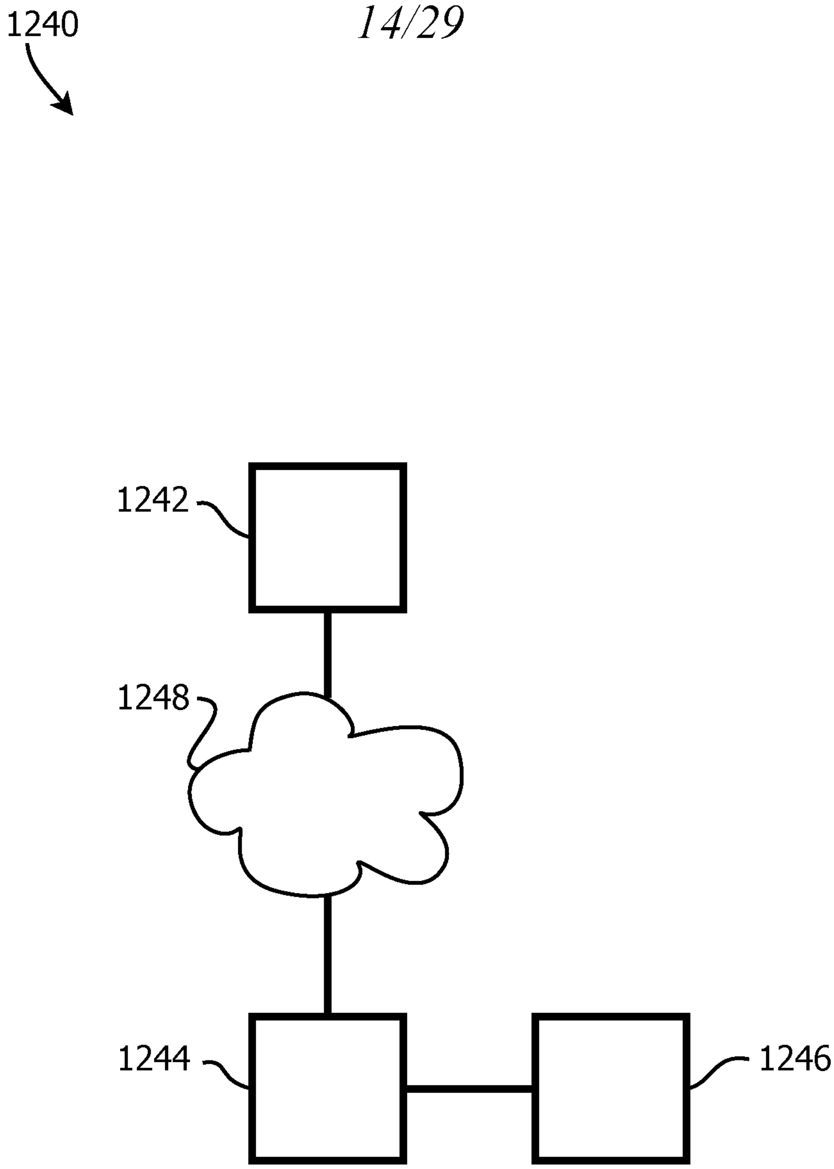

FIG. 16

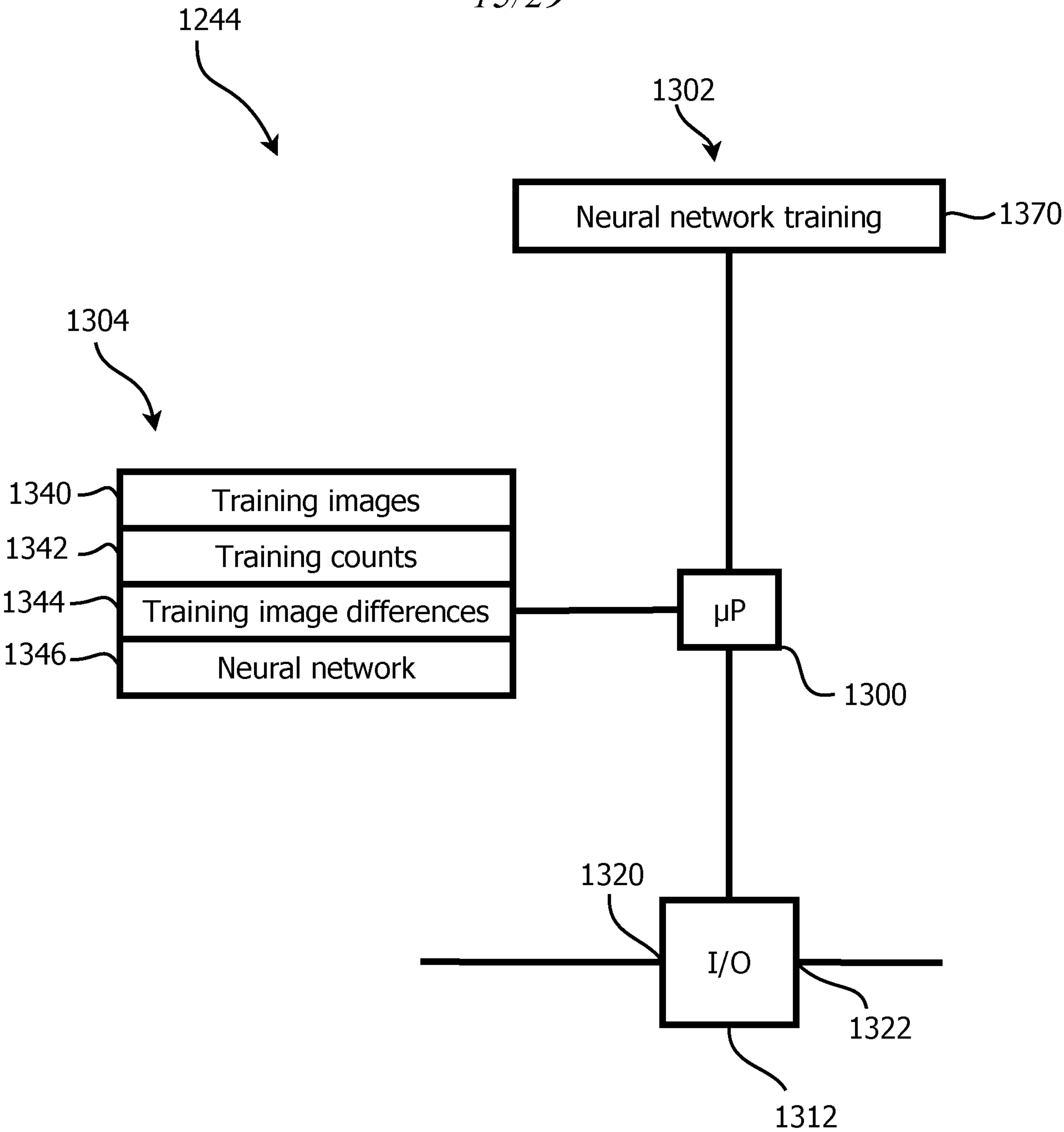
1244
1302
Neural network training
1370
1304
1340
Training images
1342
Training counts
1344
Training image differences
1346
Neural network
μP
1300
1320
I/O
1322
1312

FIG. 17

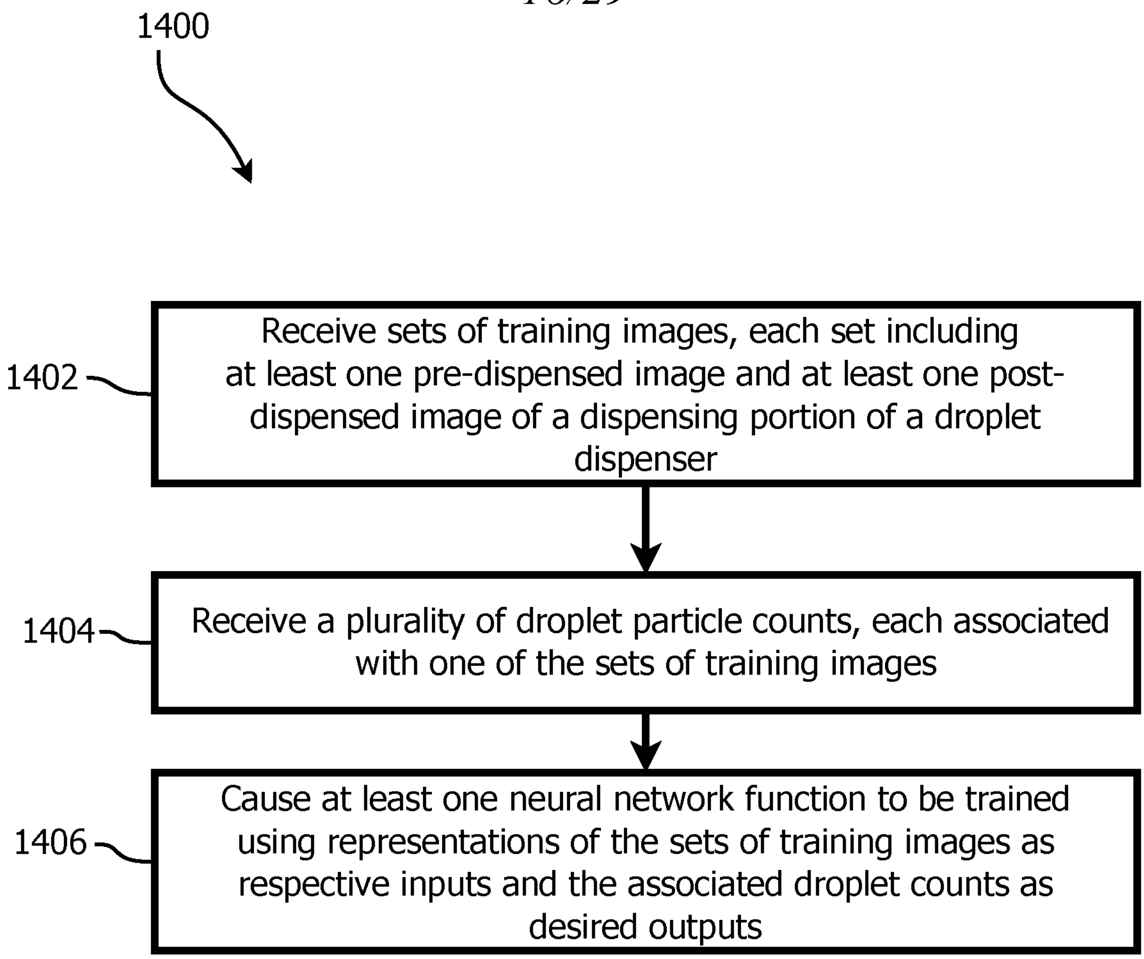
1400
1402
Receive sets of training images, each set including at least one pre-dispensed image and at least one post-dispensed image of a dispensing portion of a droplet dispenser
1404
Receive a plurality of droplet particle counts, each associated with one of the sets of training images
1406
Cause at least one neural network function to be trained using representations of the sets of training images as respective inputs and the associated droplet counts as desired outputs

FIG. 18

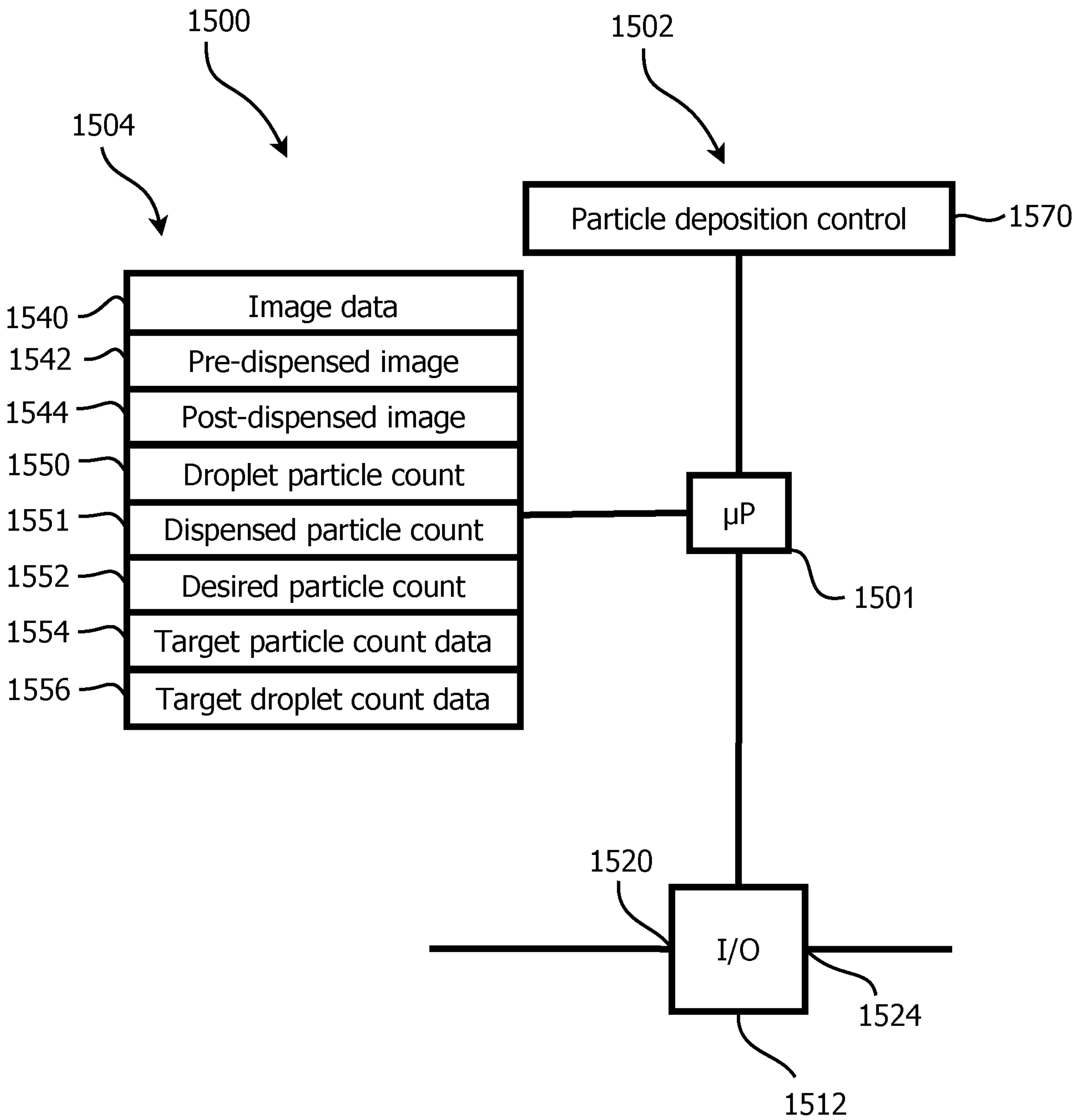
1500
1502
1504
Particle deposition control
1570
1540
Image data
1542
Pre-dispensed image
1544
Post-dispensed image
1550
Droplet particle count
1551
Dispensed particle count
1552
Desired particle count
1554
Target particle count data
1556
Target droplet count data
μP
1501
1520
I/O
1524
1512

FIG. 19

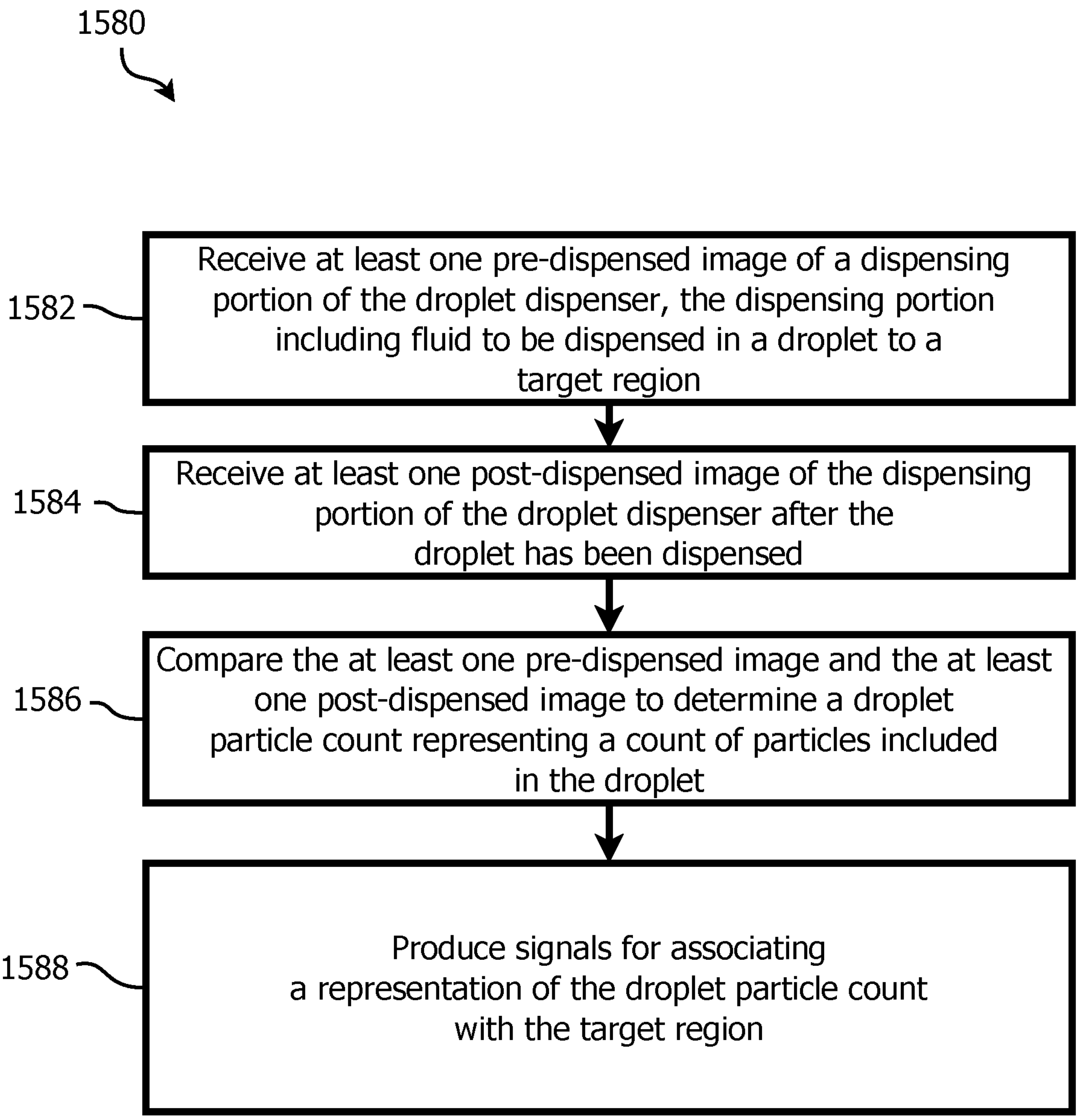
1580
1582
Receive at least one pre-dispensed image of a dispensing portion of the droplet dispenser, the dispensing portion including fluid to be dispensed in a droplet to a target region
1584
Receive at least one post-dispensed image of the dispensing portion of the droplet dispenser after the droplet has been dispensed
1586
Compare the at least one pre-dispensed image and the at least one post-dispensed image to determine a droplet particle count representing a count of particles included in the droplet
1588
Produce signals for associating a representation of the droplet particle count with the target region

FIG. 20

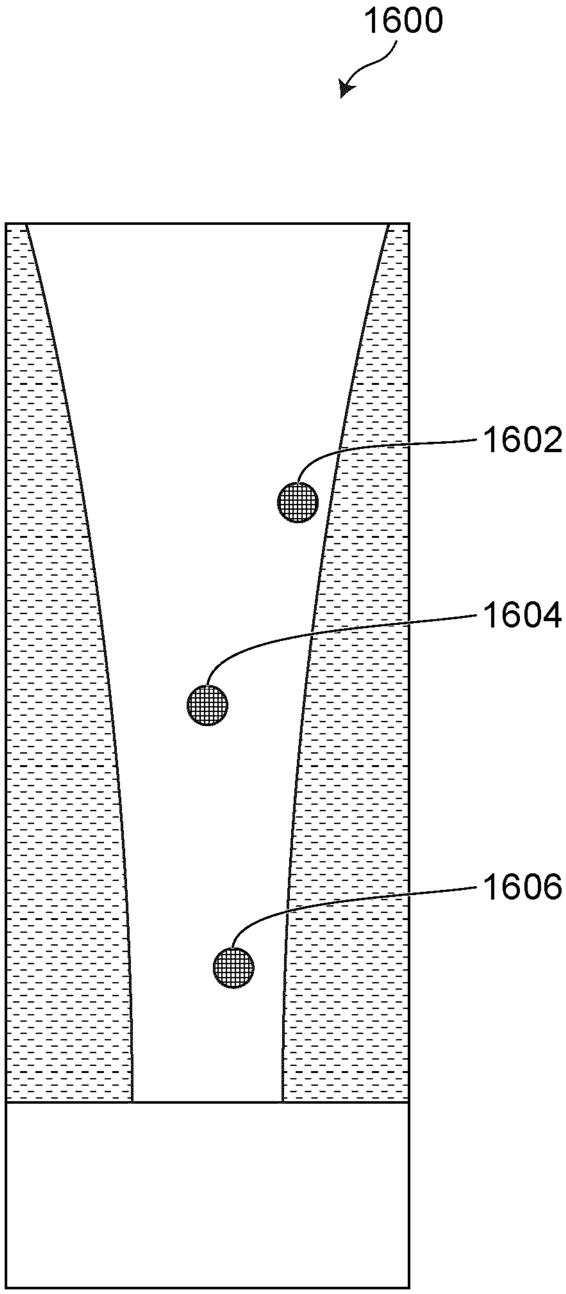

FIG. 21

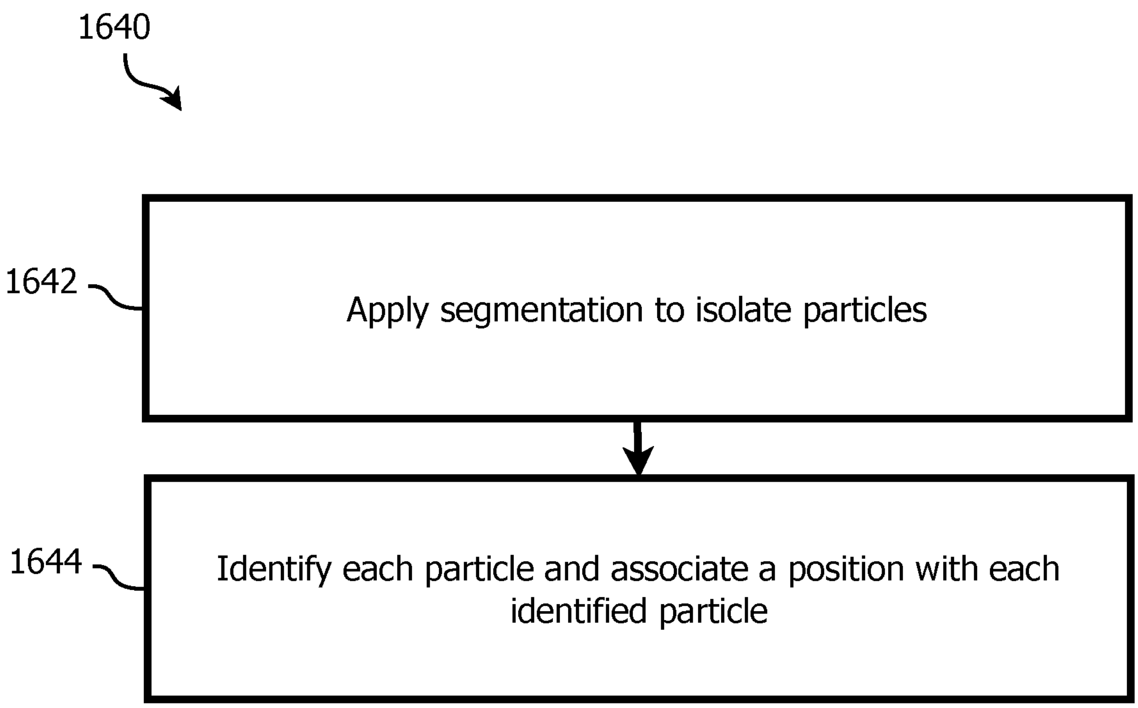
1640
1642
Apply segmentation to isolate particles
1644
Identify each particle and associate a position with each identified particle

FIG. 22

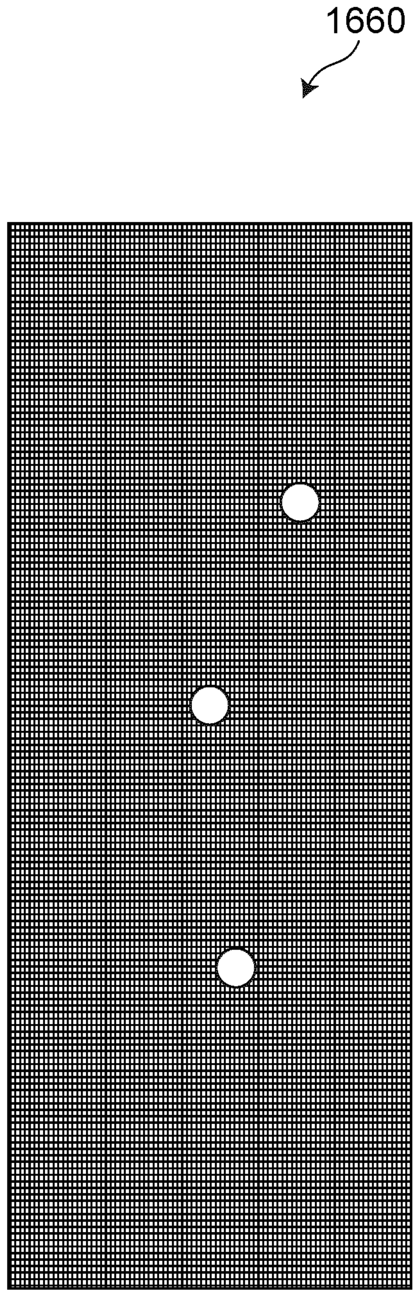

FIG. 23

1680

Particle positions record

1682	First particle x	101
1684	First particle y	361
1687	Second particle x	87
1688	Second particle y	232
1690	Third particle x	130
1692	Third particle y	133

FIG. 24

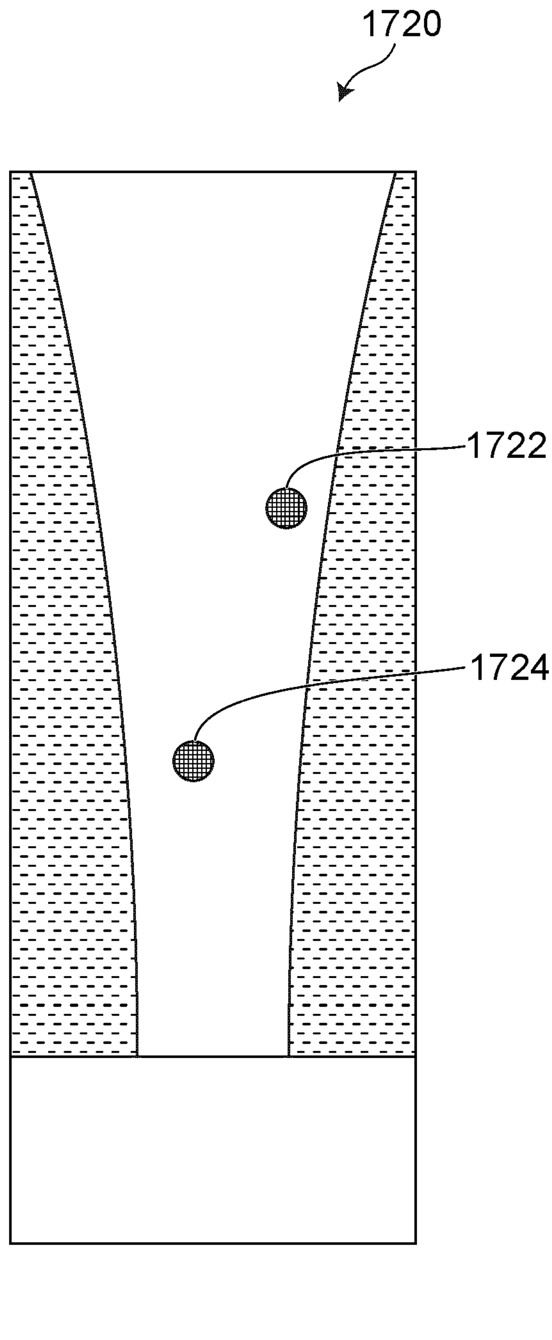

FIG. 25

1740

Particle positions record

1742	First particle x	84
1744	First particle y	286
1746	Second particle x	127
1748	Second particle y	169

FIG. 26

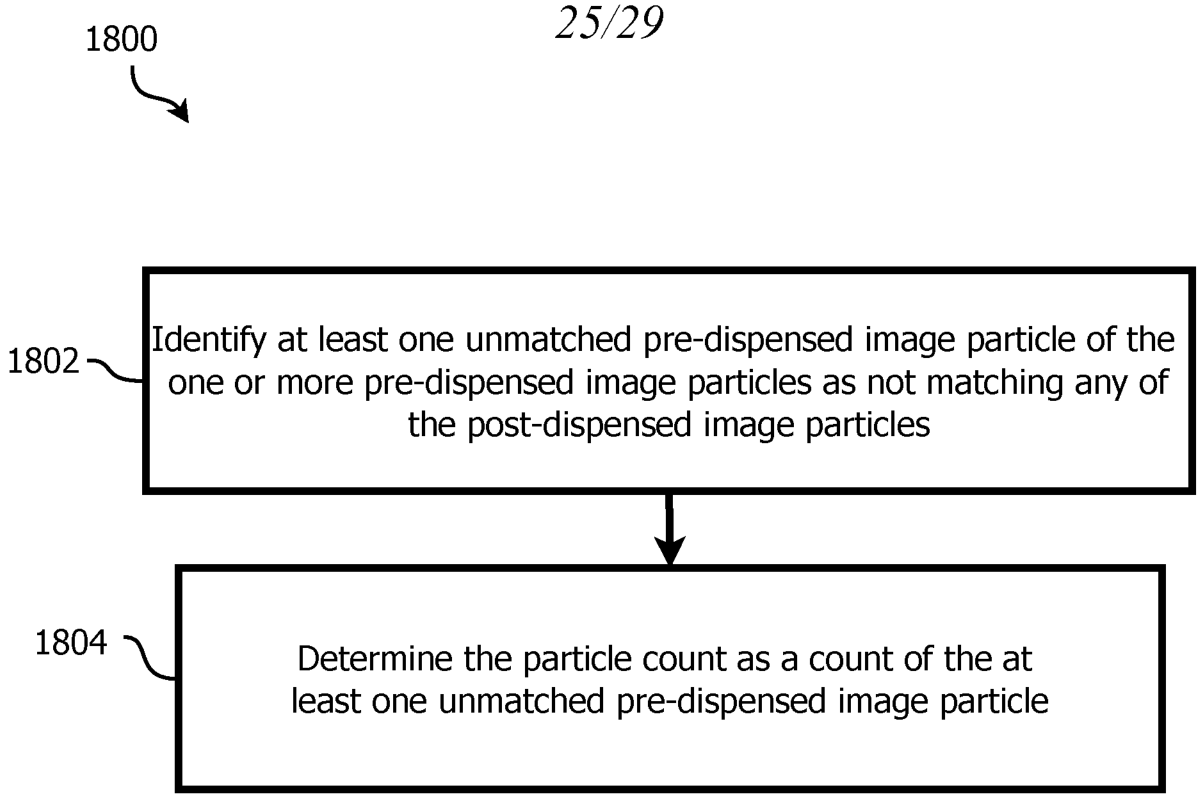
1800
1802
Identify at least one unmatched pre-dispensed image particle of the one or more pre-dispensed image particles as not matching any of the post-dispensed image particles
1804
Determine the particle count as a count of the at least one unmatched pre-dispensed image particle

FIG. 27

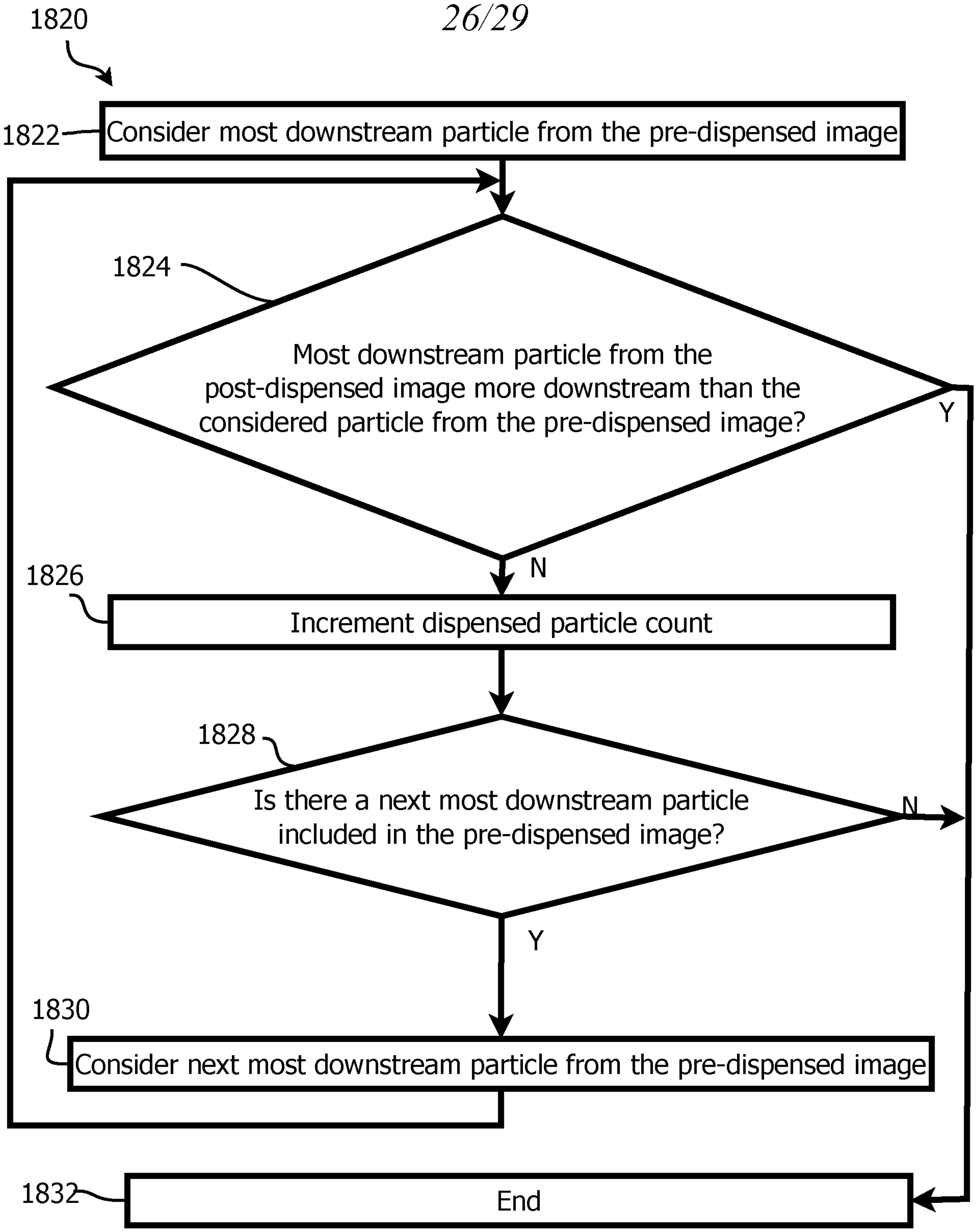
1820
1822
Consider most downstream particle from the pre-dispensed image
1824
Most downstream particle from the post-dispensed image more downstream than the considered particle from the pre-dispensed image?
Y
N
1826
Increment dispensed particle count
1828
Is there a next most downstream particle included in the pre-dispensed image?
N
Y
1830
Consider next most downstream particle from the pre-dispensed image
1832
End

FIG. 28

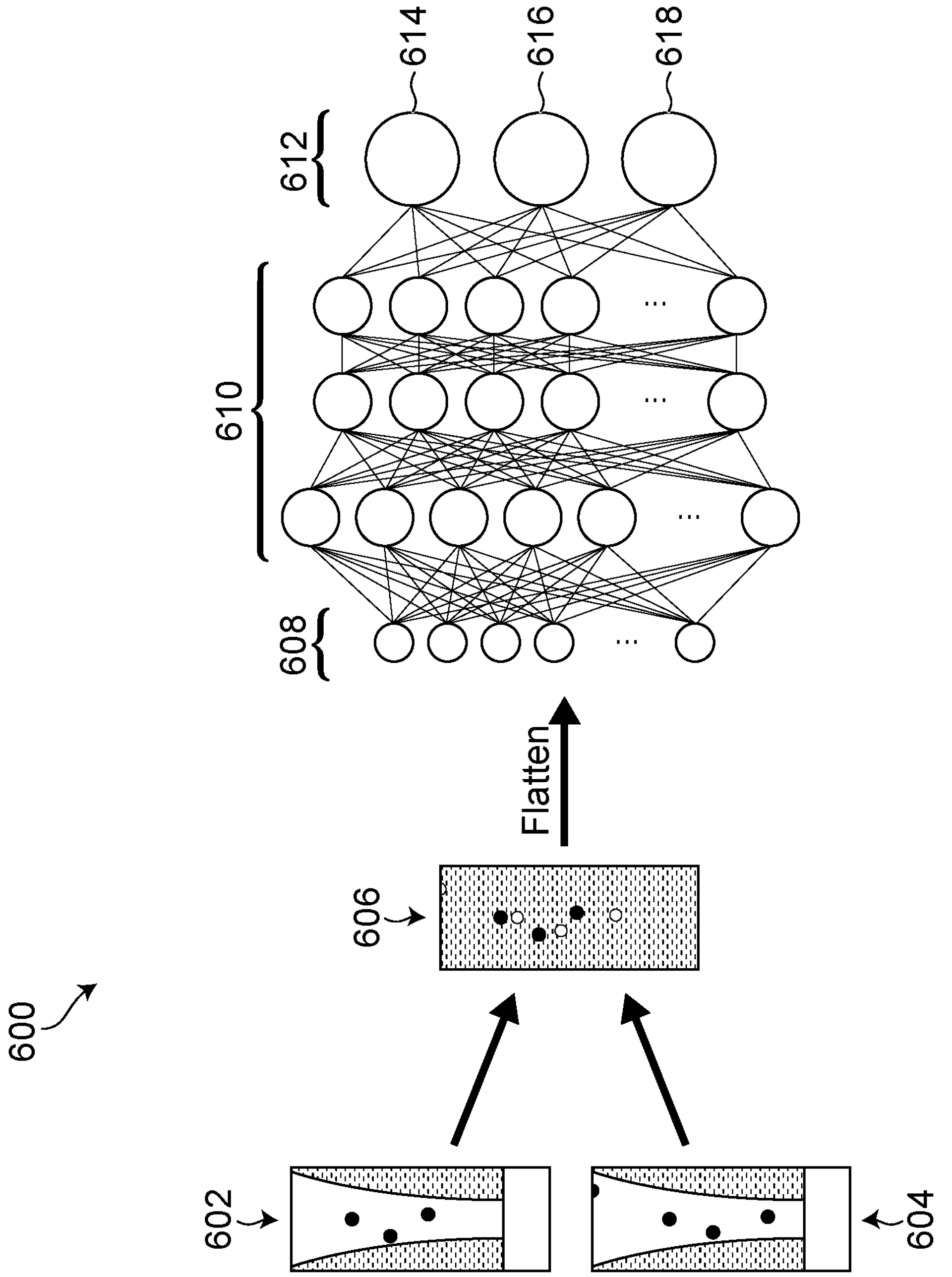

FIG. 29

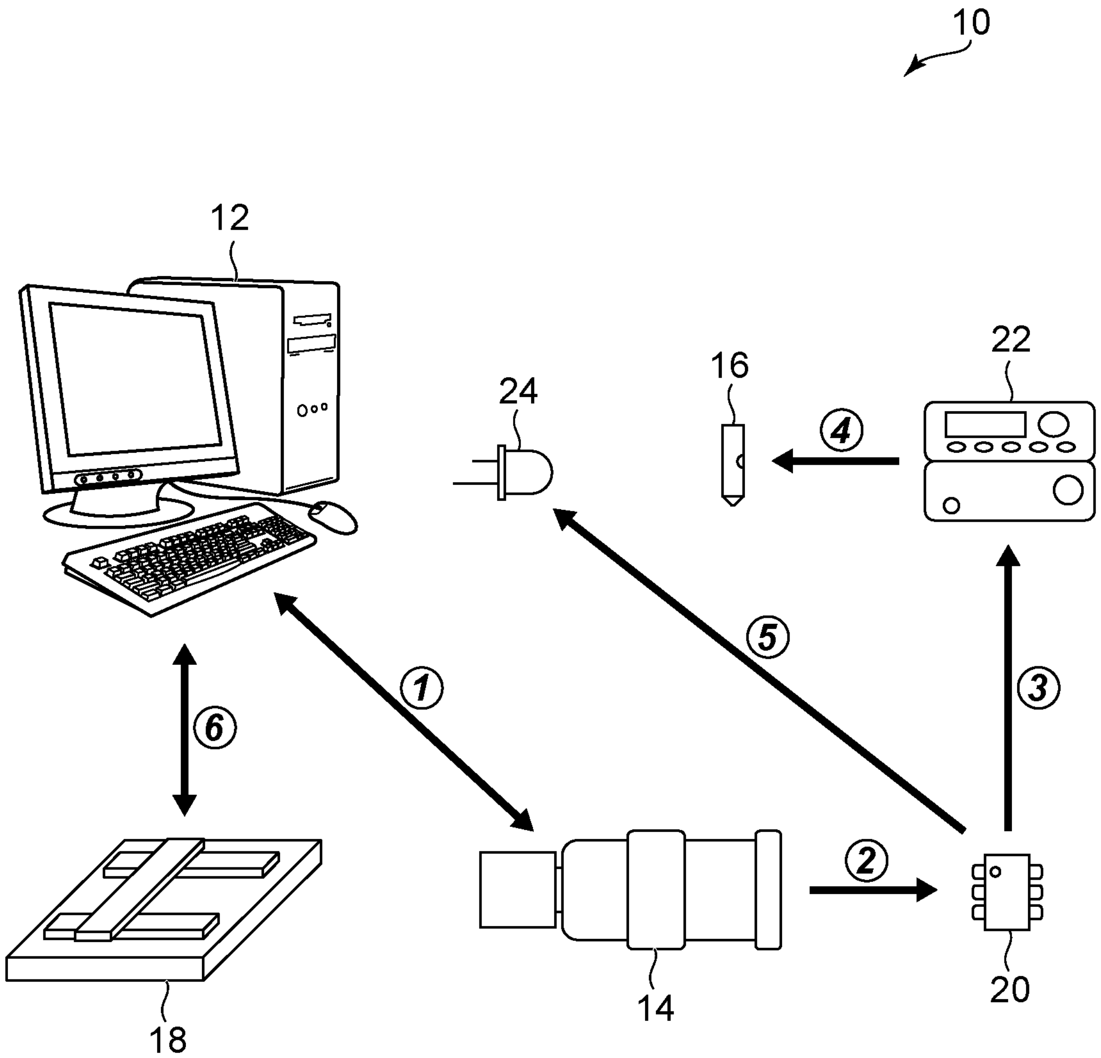

FIG. 30

29/29

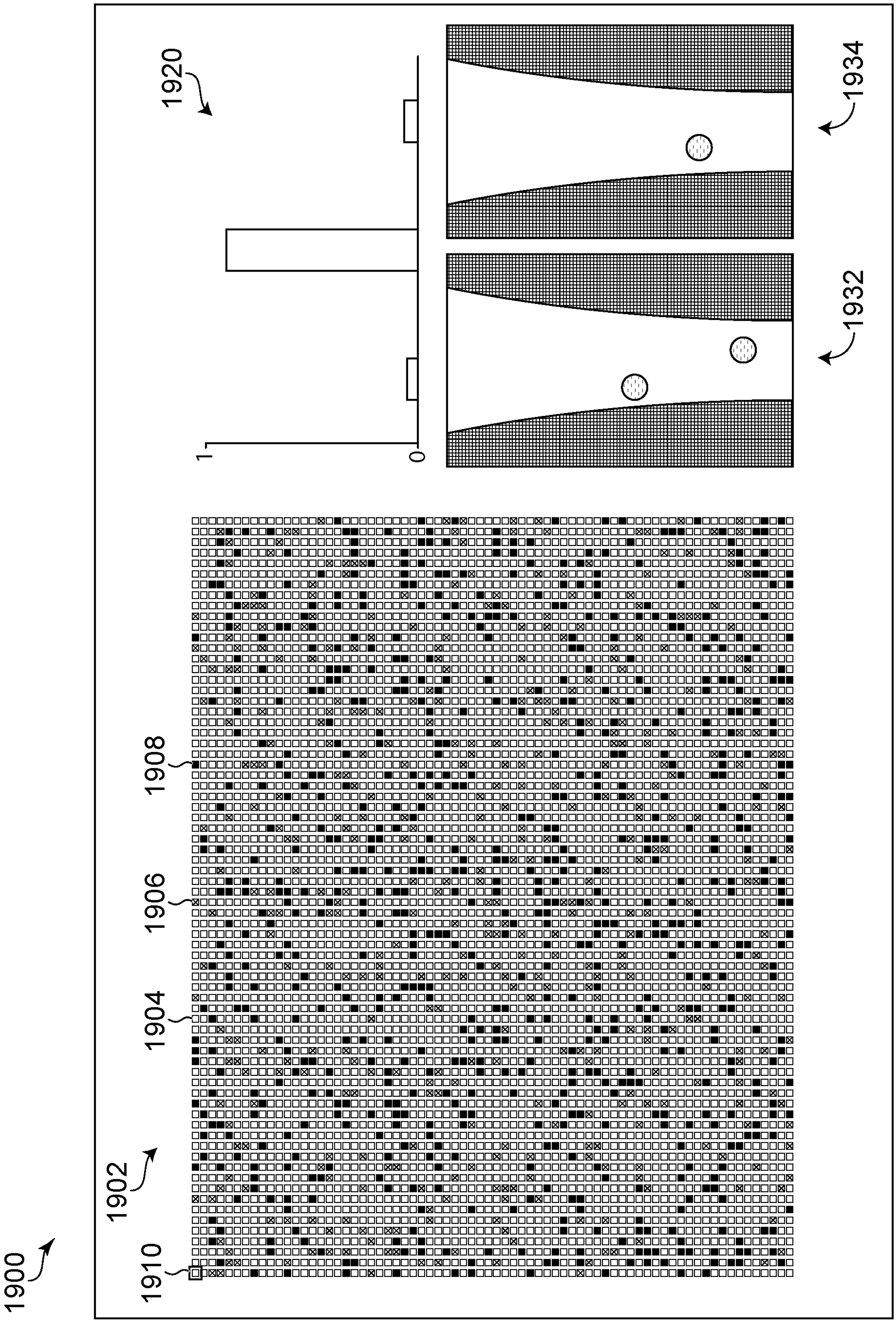

FIG. 31

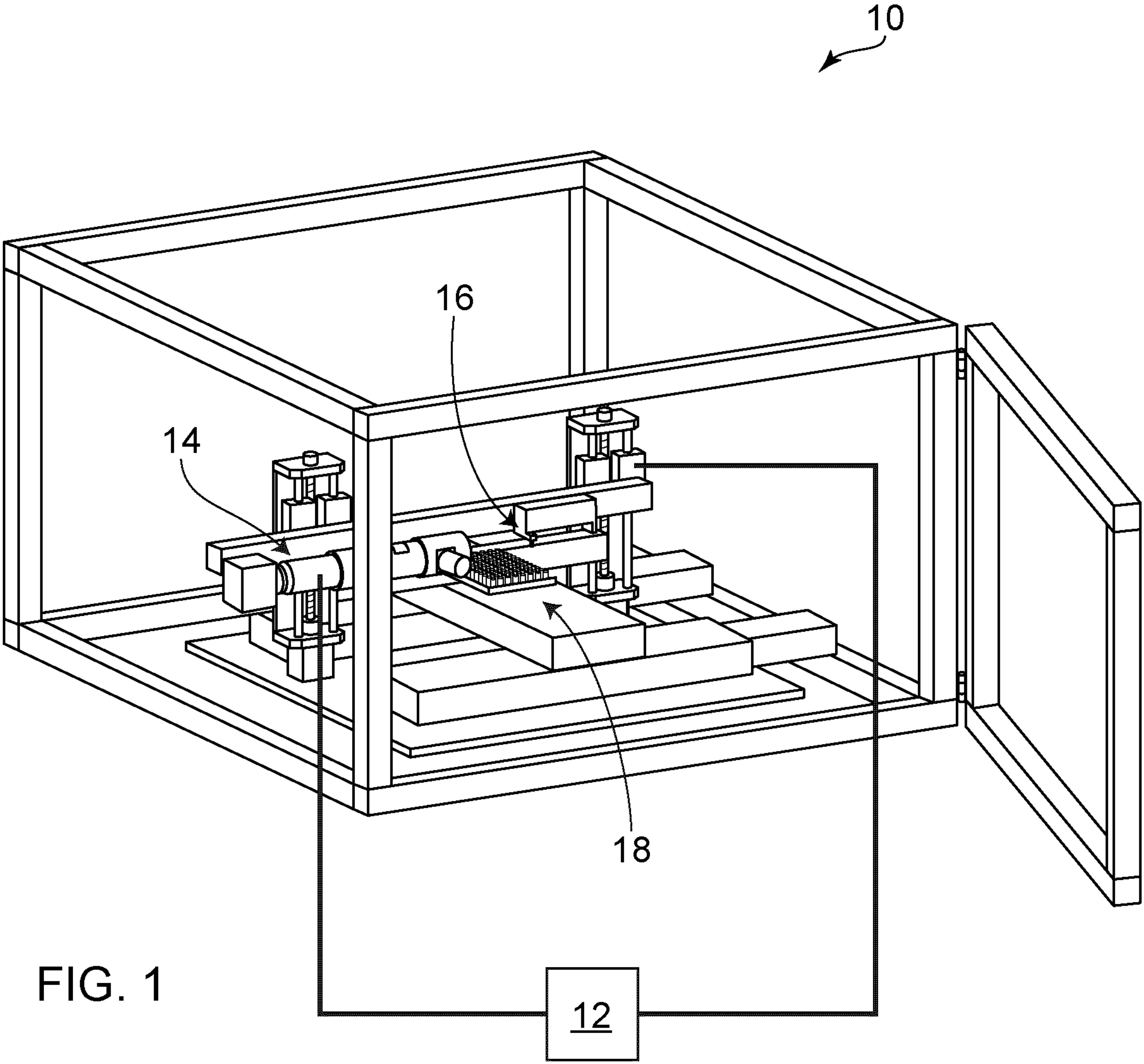

FIG. 1