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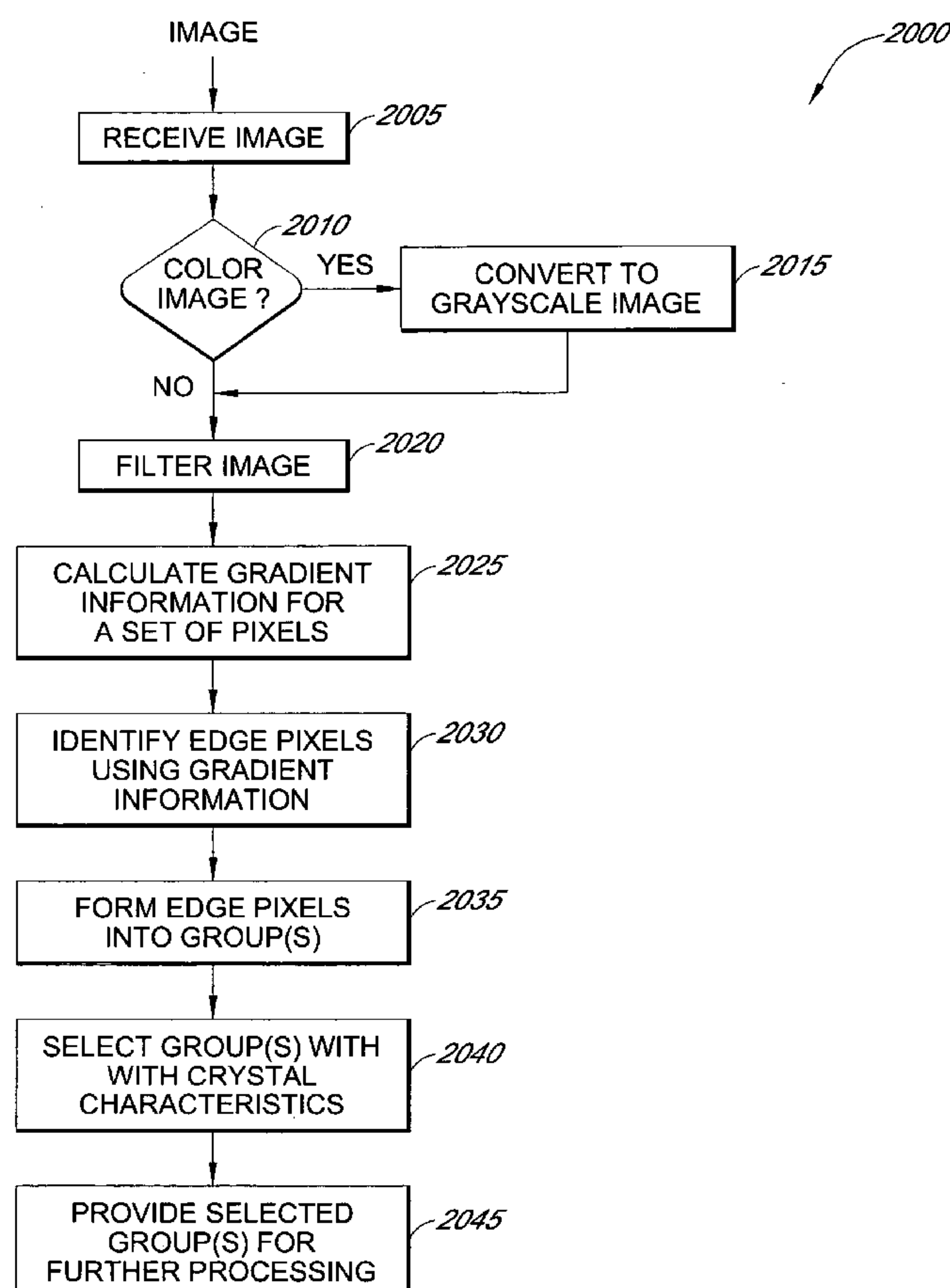
(19) **United States**(12) **Patent Application Publication**  
**Affleck et al.**(10) **Pub. No.: US 2004/0218804 A1**(43) **Pub. Date: Nov. 4, 2004**(54) **IMAGE ANALYSIS SYSTEM AND METHOD****Publication Classification**(76) Inventors: **Rhett L. Affleck**, Poway, CA (US);  
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(US)(51) **Int. Cl.<sup>7</sup>** ..... **G06K 9/00**(52) **U.S. Cl.** ..... **382/141**(57) **ABSTRACT**

An image analysis system and related methods for automation of the monitoring of samples to determine crystal growth. Samples are imaged from time to time using a set of imaging parameters. The resulting images are evaluated to determine the contents of the samples. If the evaluation of the image indicates the presence of crystals, the sample may be re-imaged using a different set of imaging parameters, and the resulting image analyzed to determine its contents. The sample may also be evaluated by generating multiple images of a sample using various sets of imaging parameters, identifying pixels that depict regions of interest in a plurality of images, merging the pixels from each region of interest into a composite image and analyzing the composite image. An image of a sample can also be evaluated by classifying the pixels of the image based on the contents of the sample that they depict, color-coding the pixels using the classifications, and displaying the colored image for analysis.

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**IRVINE, CA 92614 (US)**(21) Appl. No.: **10/769,150**(22) Filed: **Jan. 30, 2004****Related U.S. Application Data**

(60) Provisional application No. 60/474,989, filed on May 30, 2003. Provisional application No. 60/444,586, filed on Jan. 31, 2003. Provisional application No. 60/444,519, filed on Jan. 31, 2003. Provisional application No. 60/444,585, filed on Jan. 31, 2003.



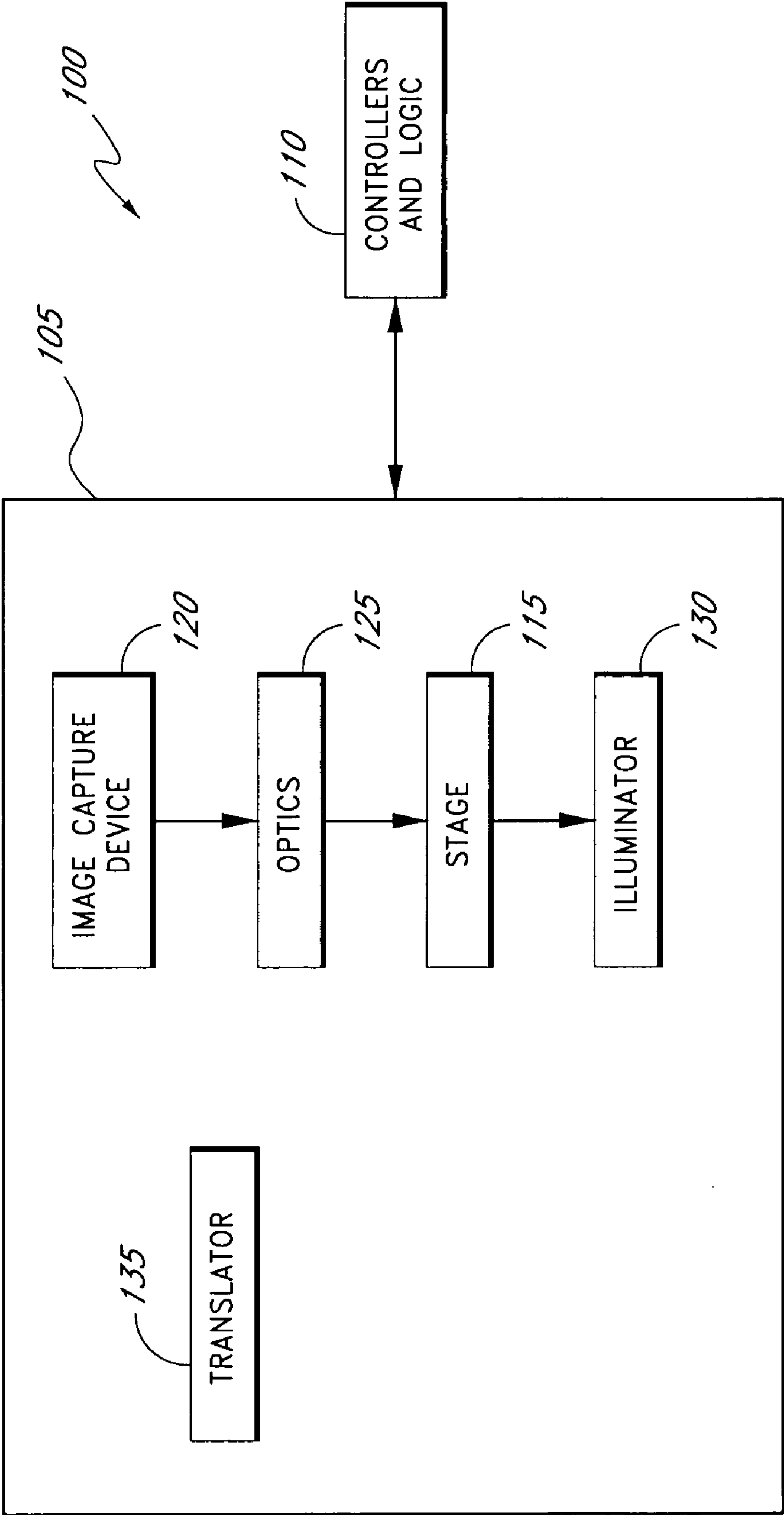


FIG. 1A

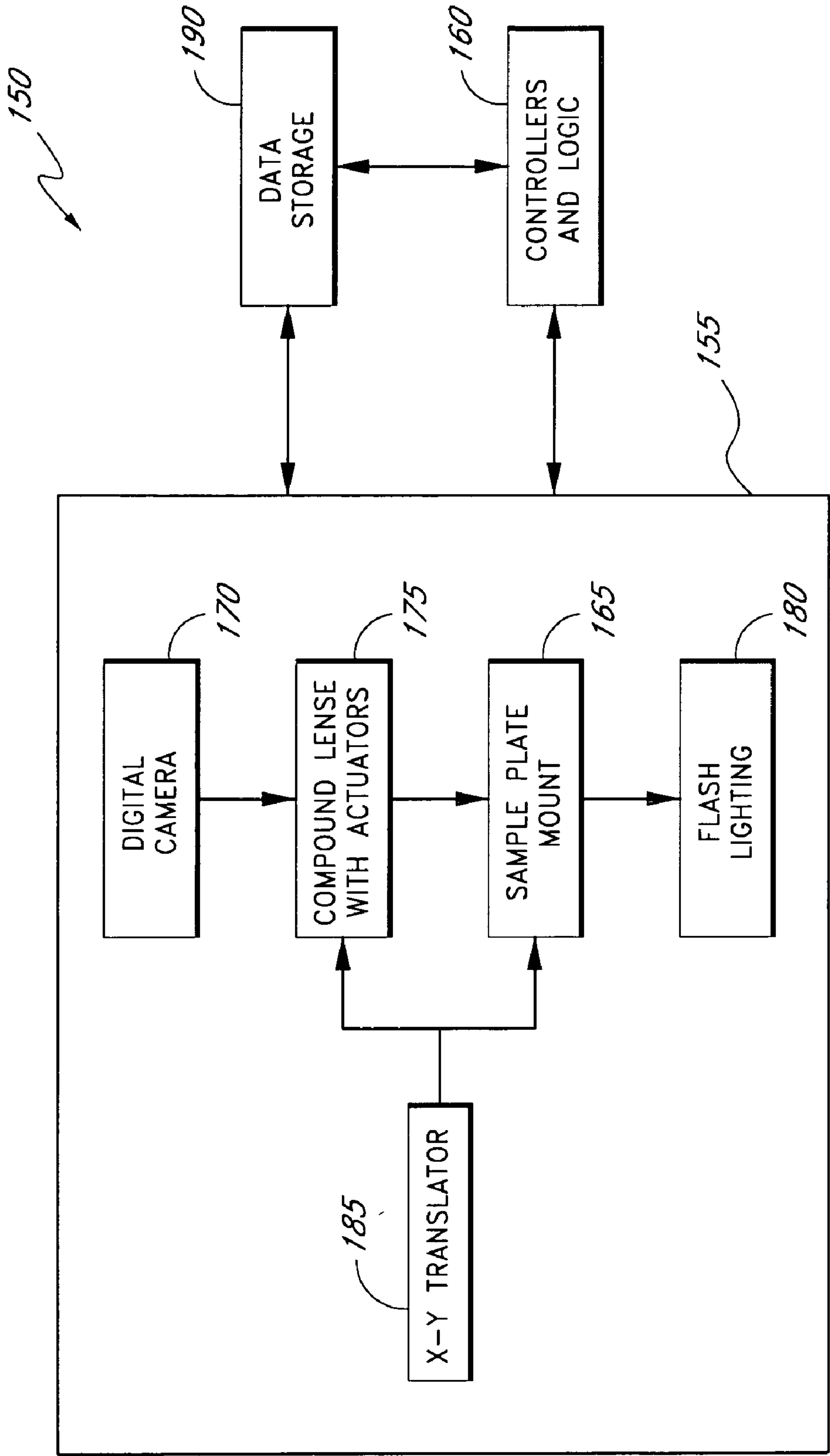


FIG.1B

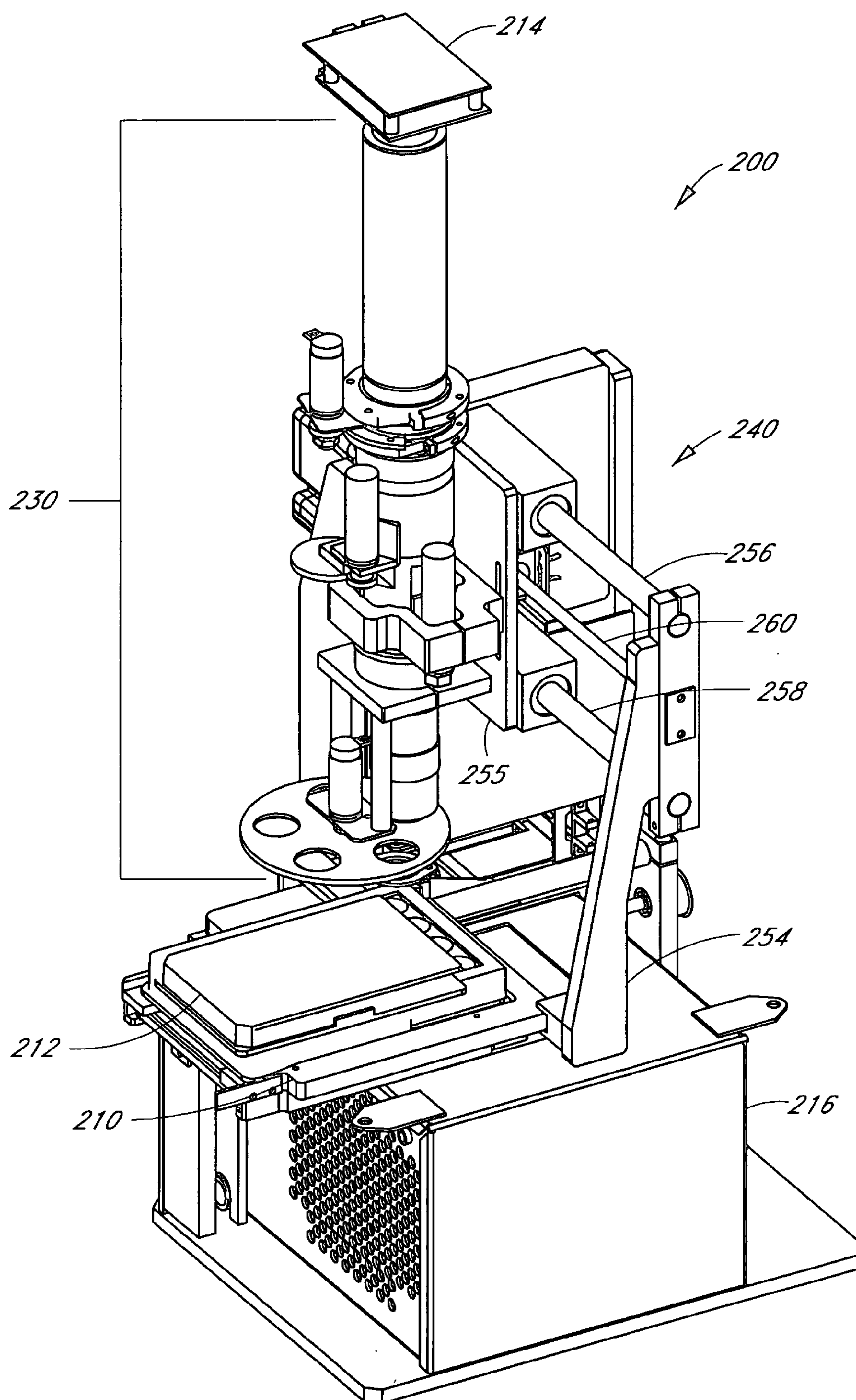


FIG. 2



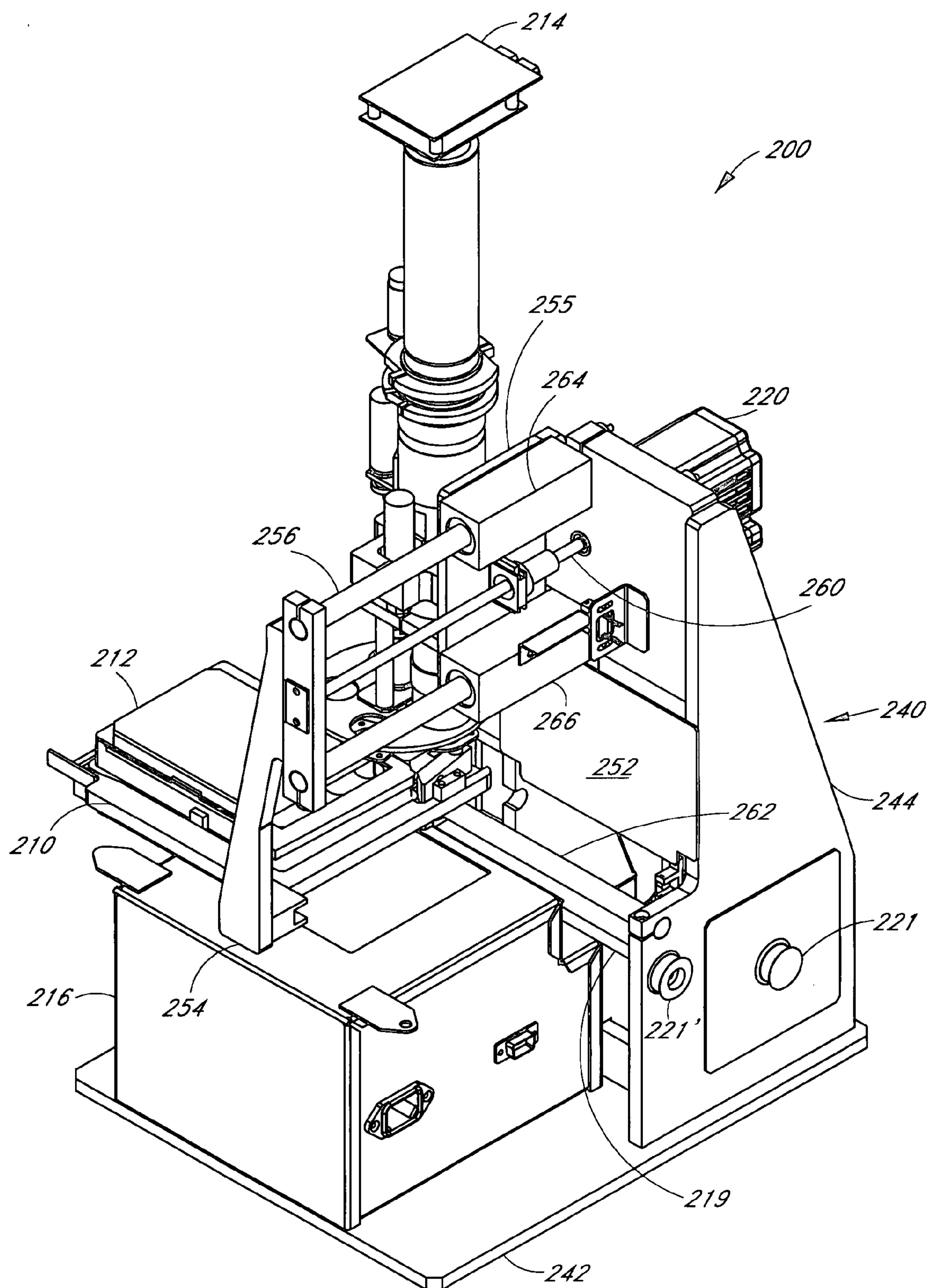


FIG. 3

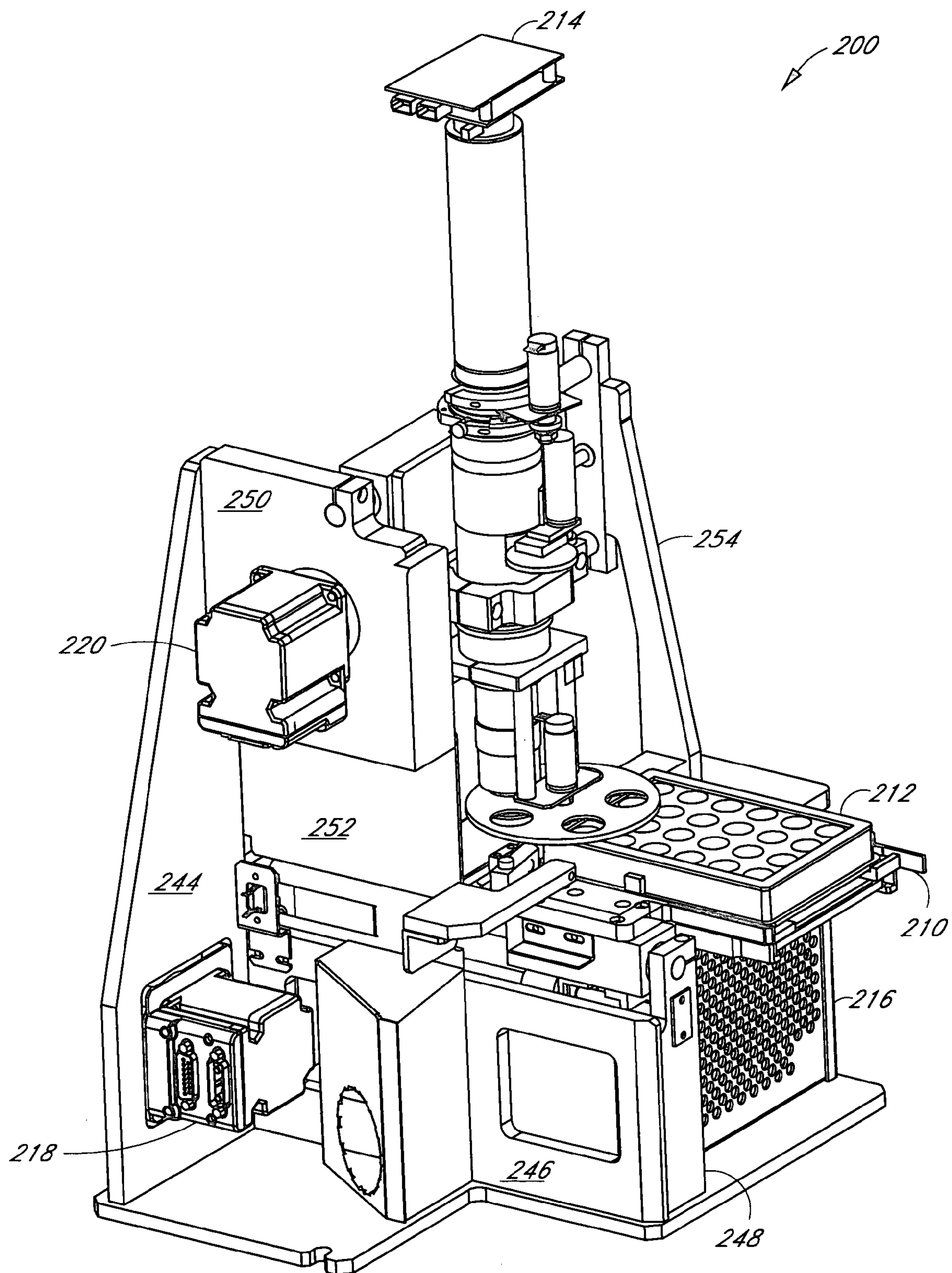


FIG. 4

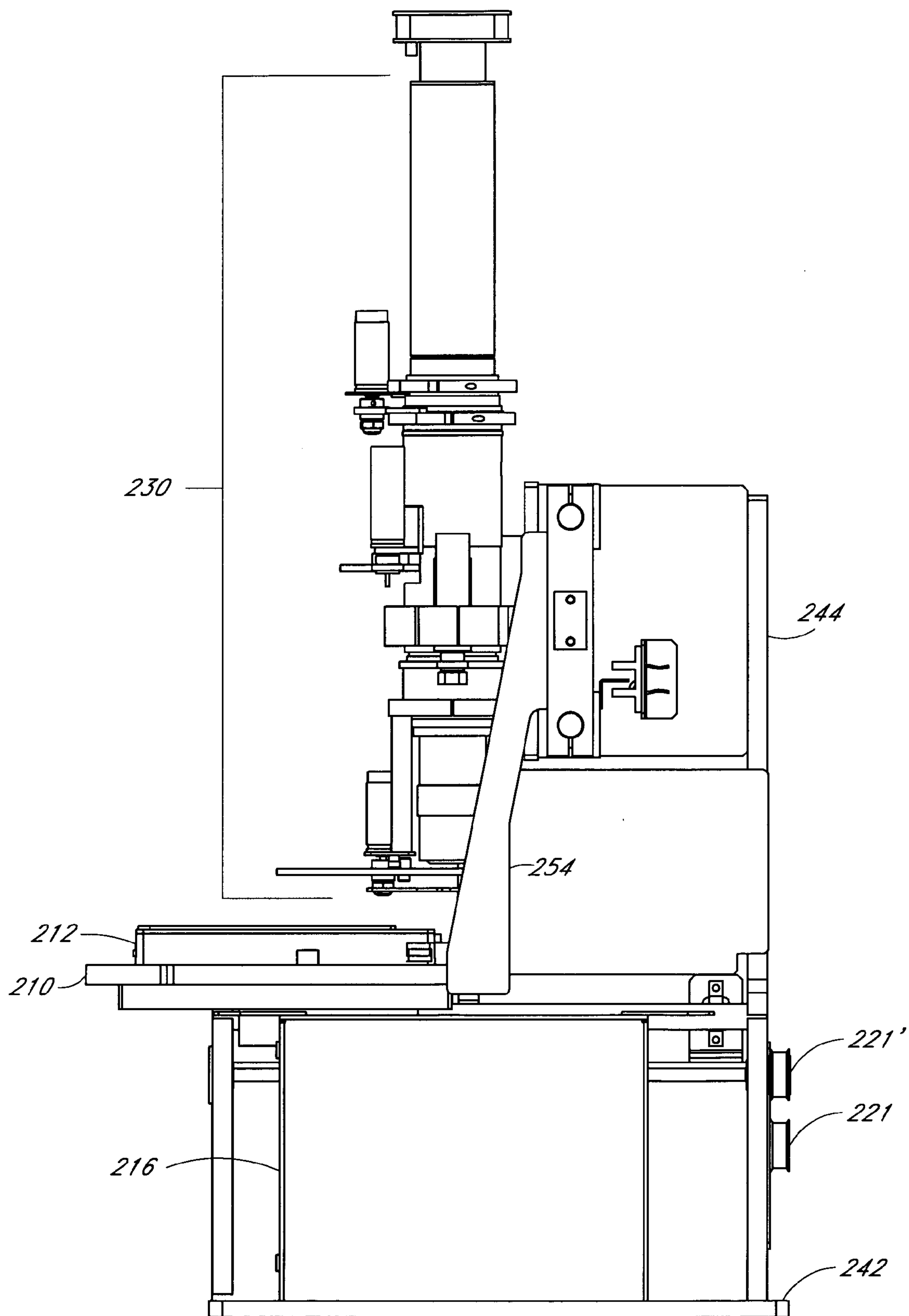


FIG. 5

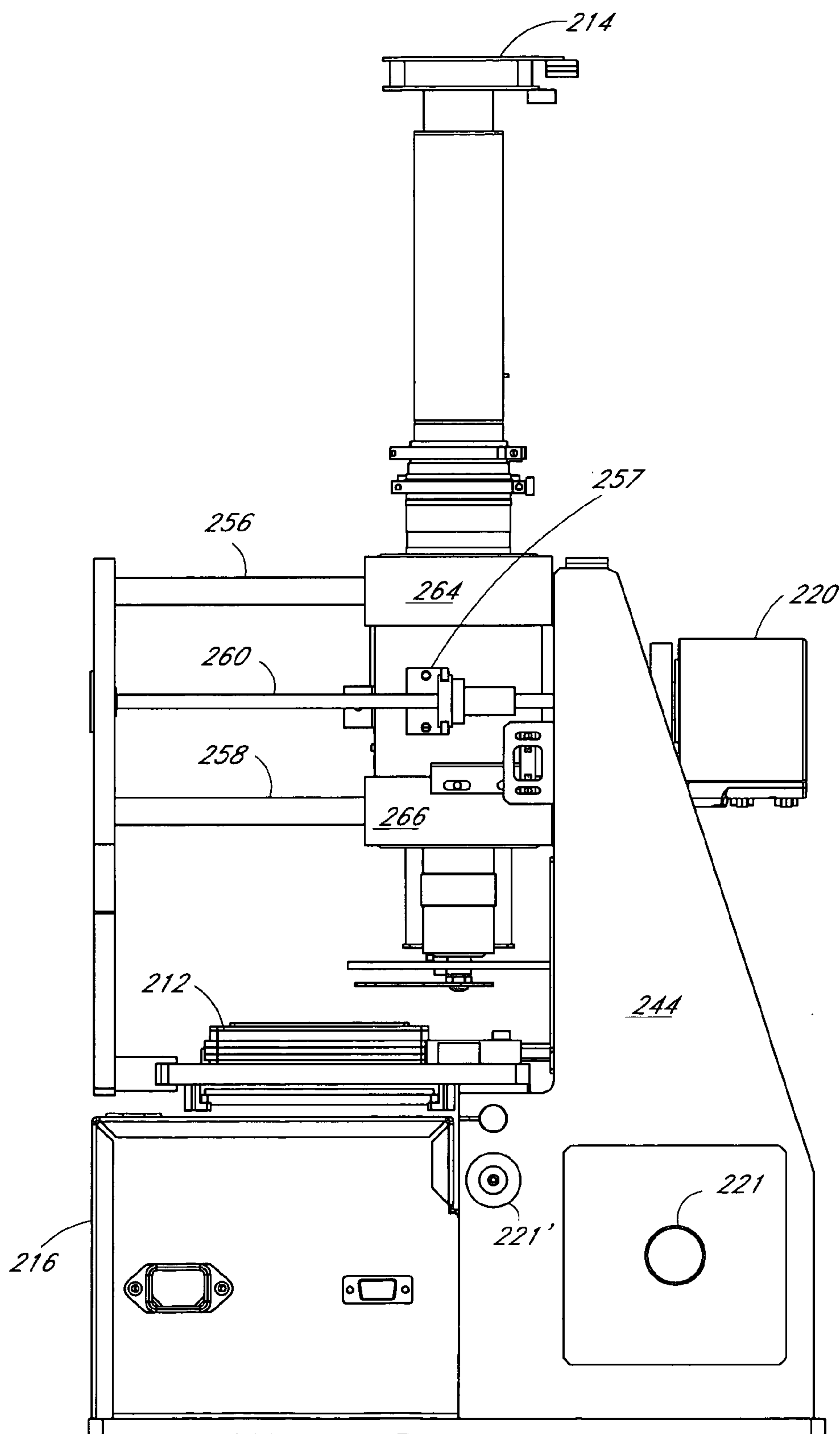


FIG. 6



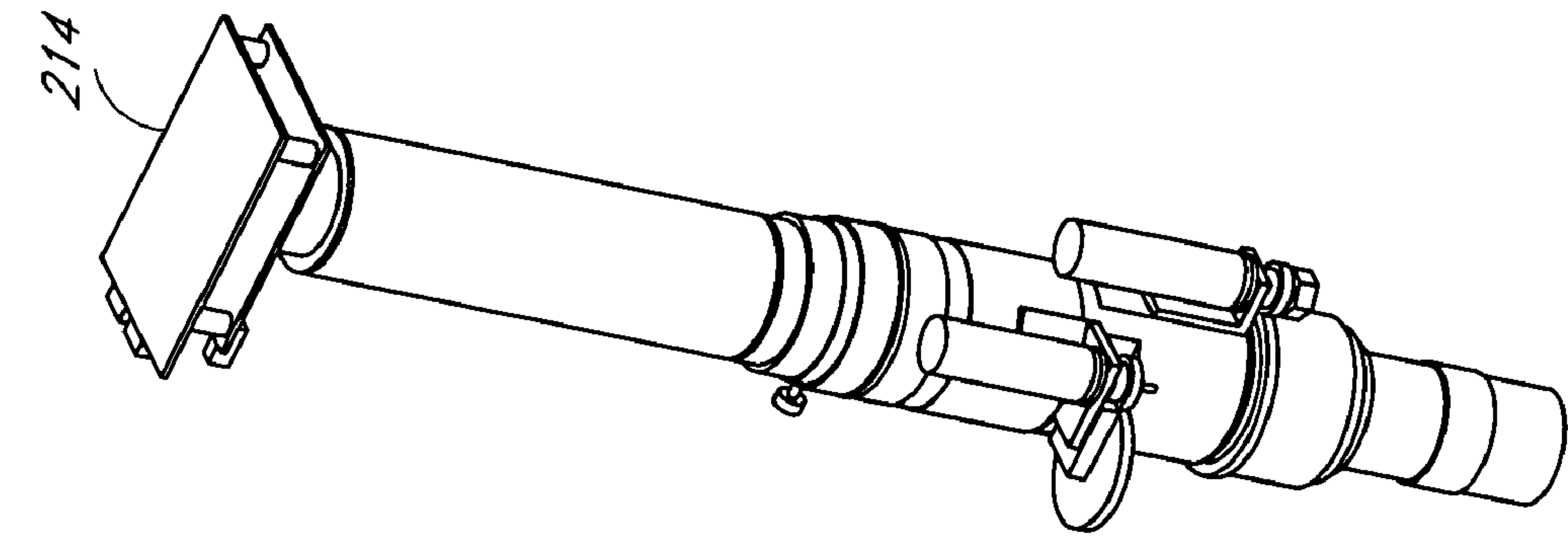


FIG. 7A

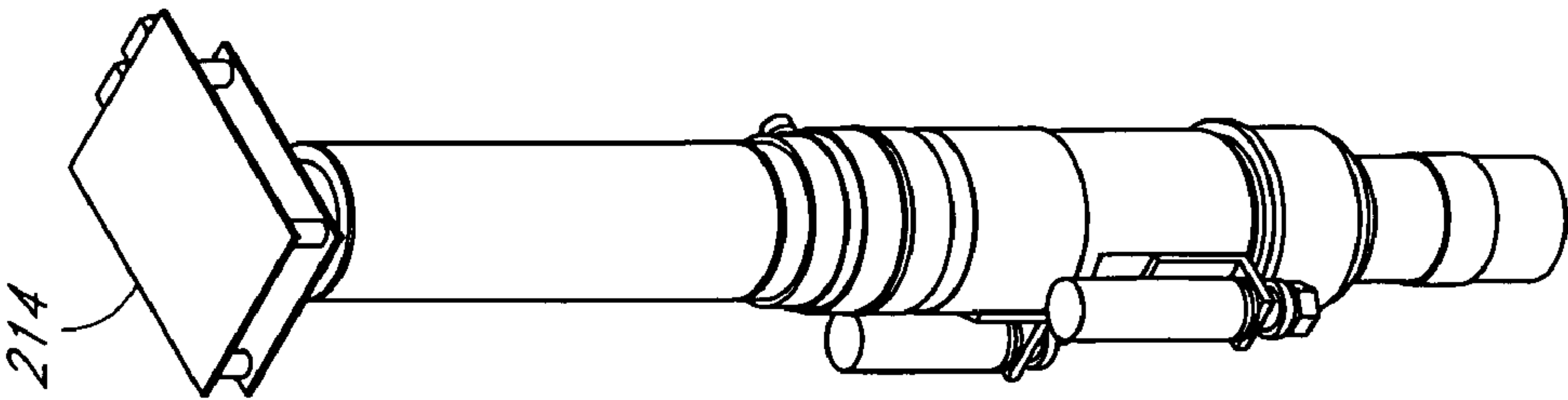
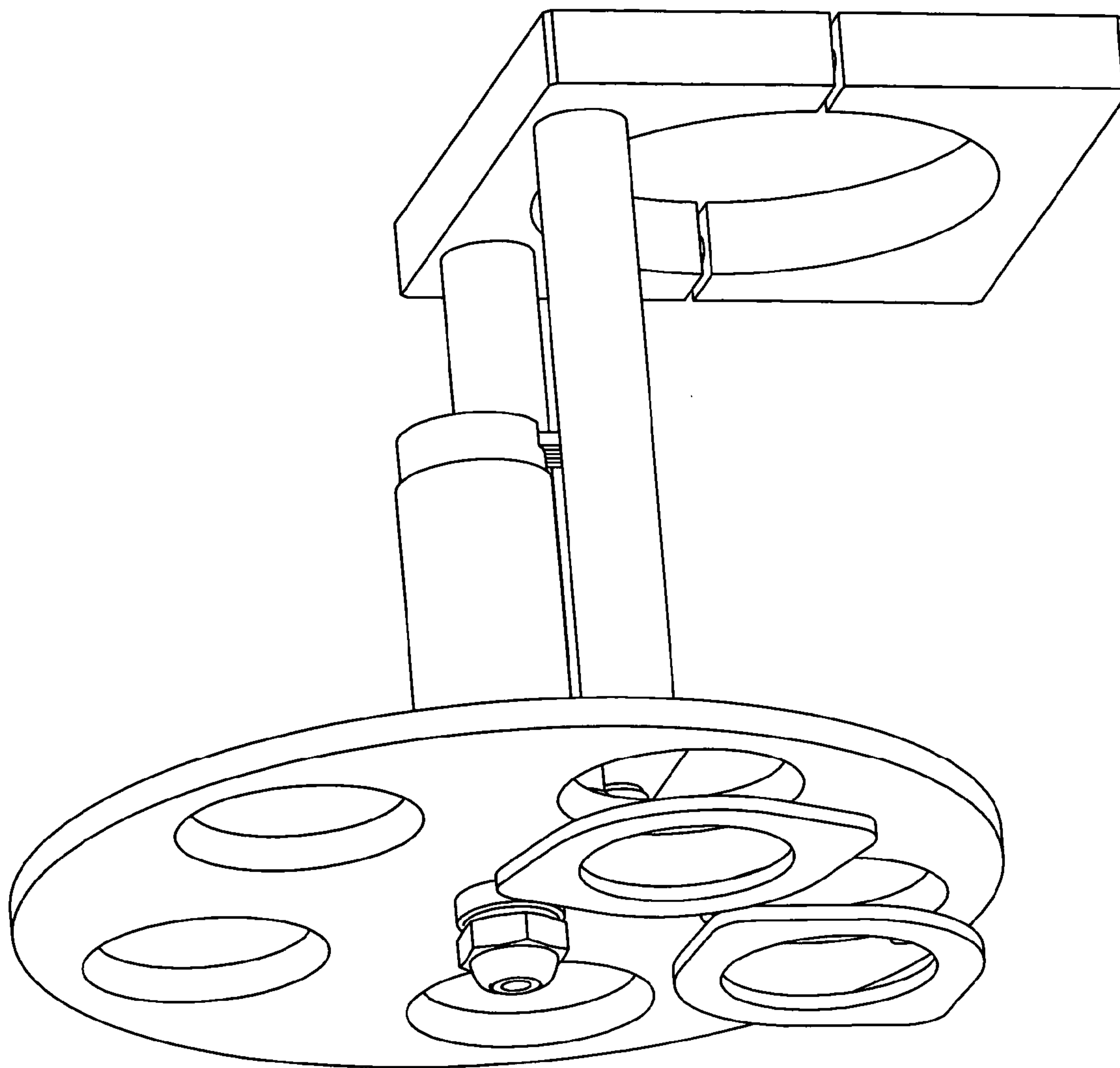


FIG. 7B



*FIG. 8*

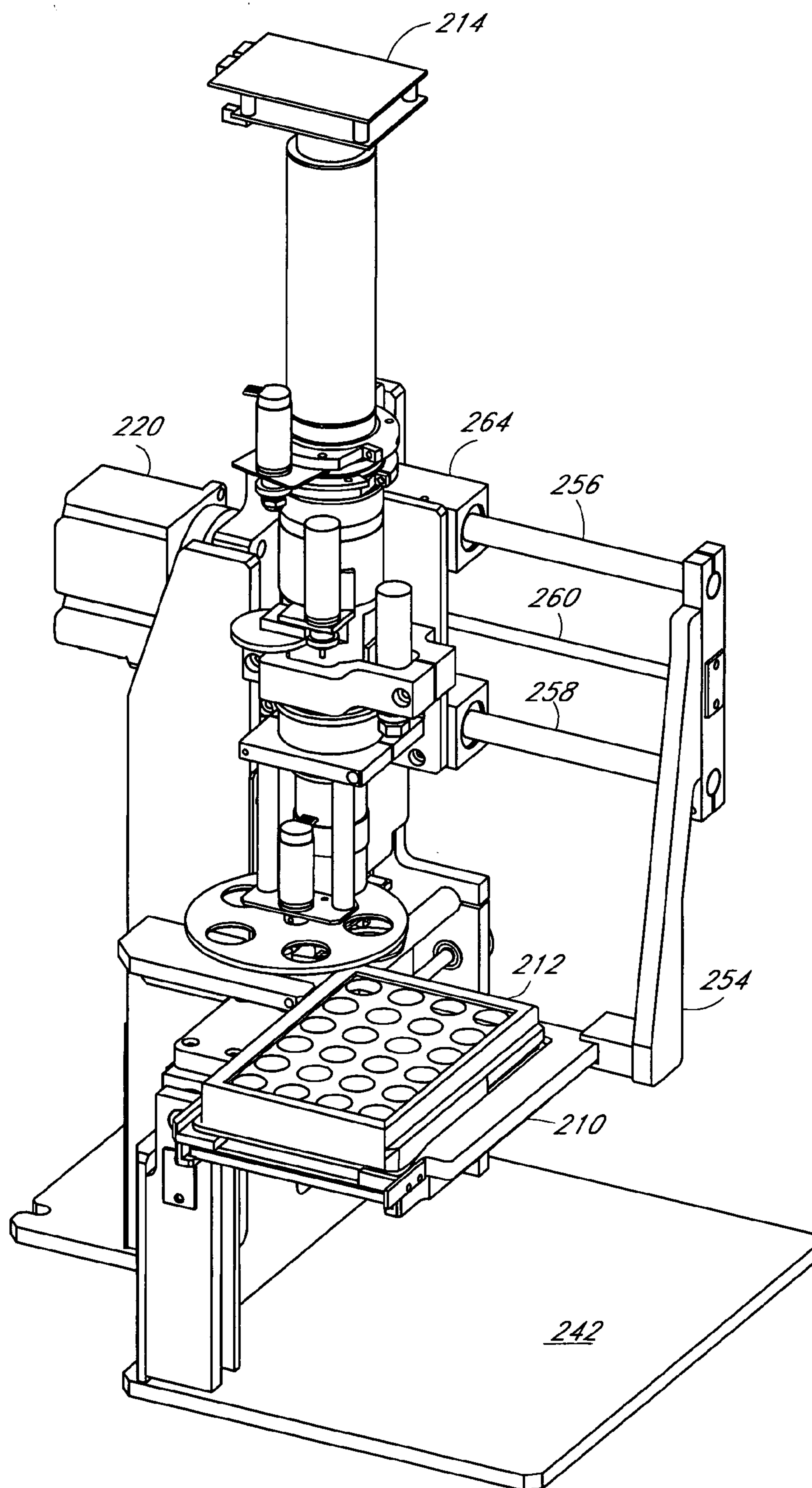


FIG. 9

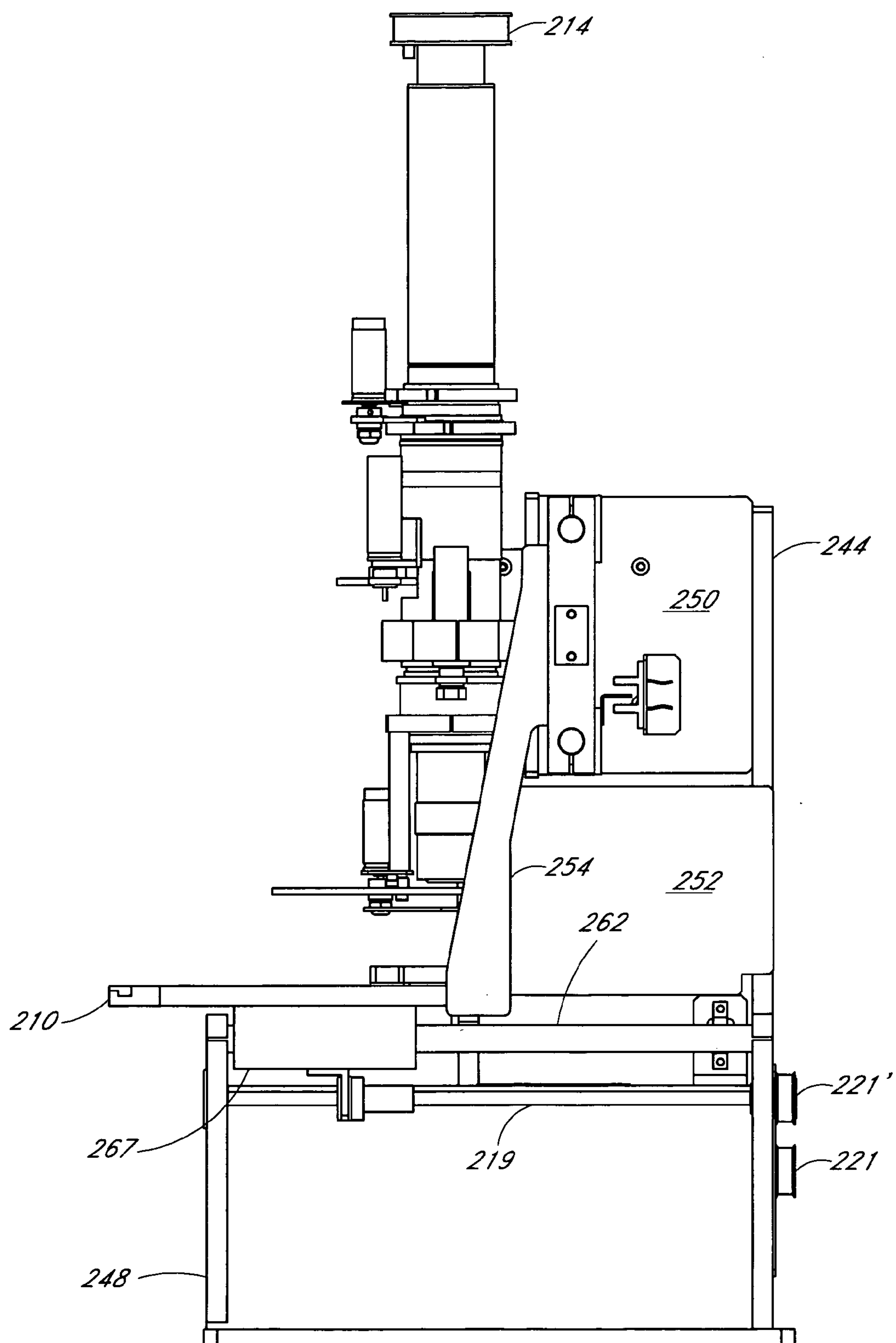


FIG. 10

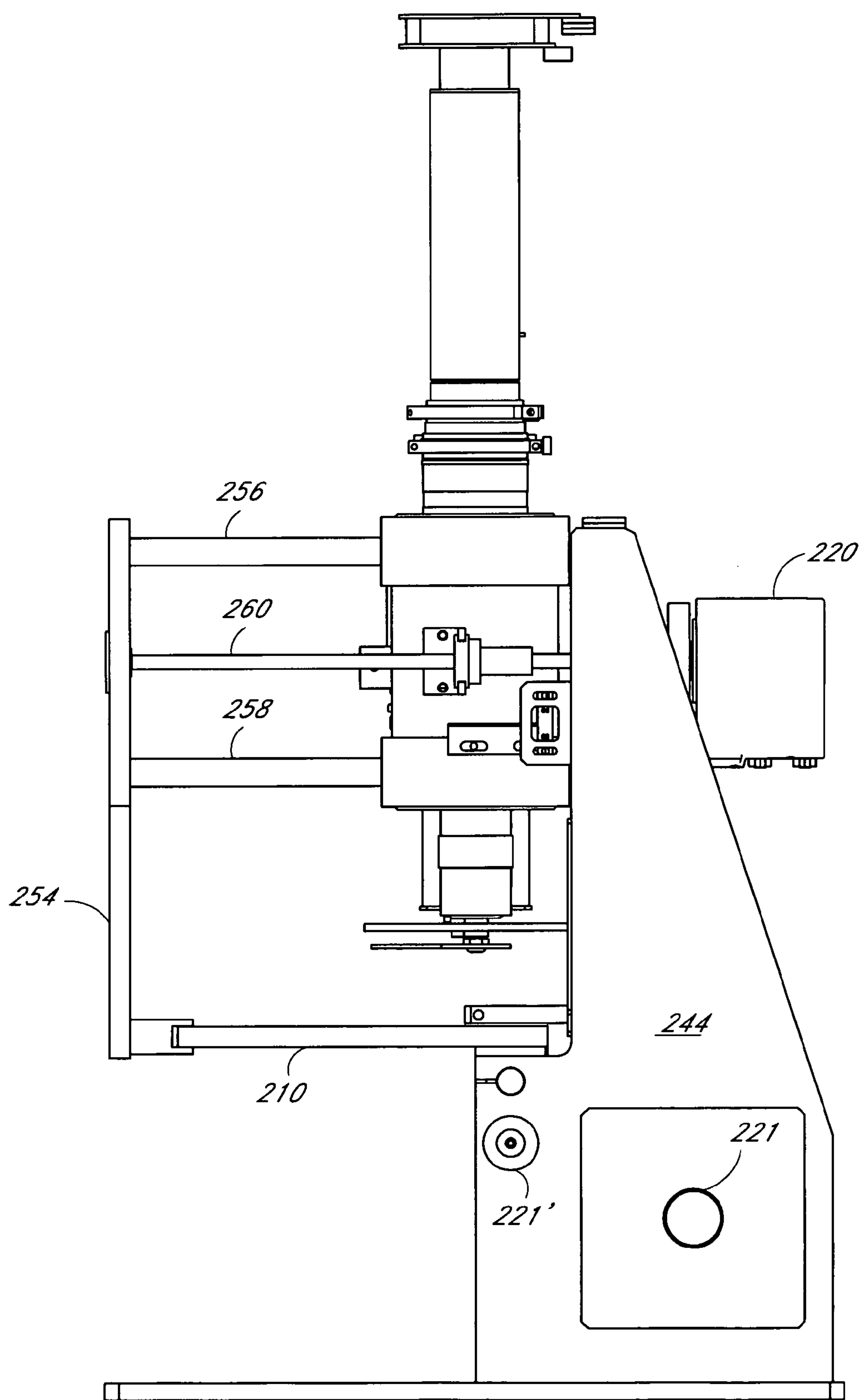


FIG. 11



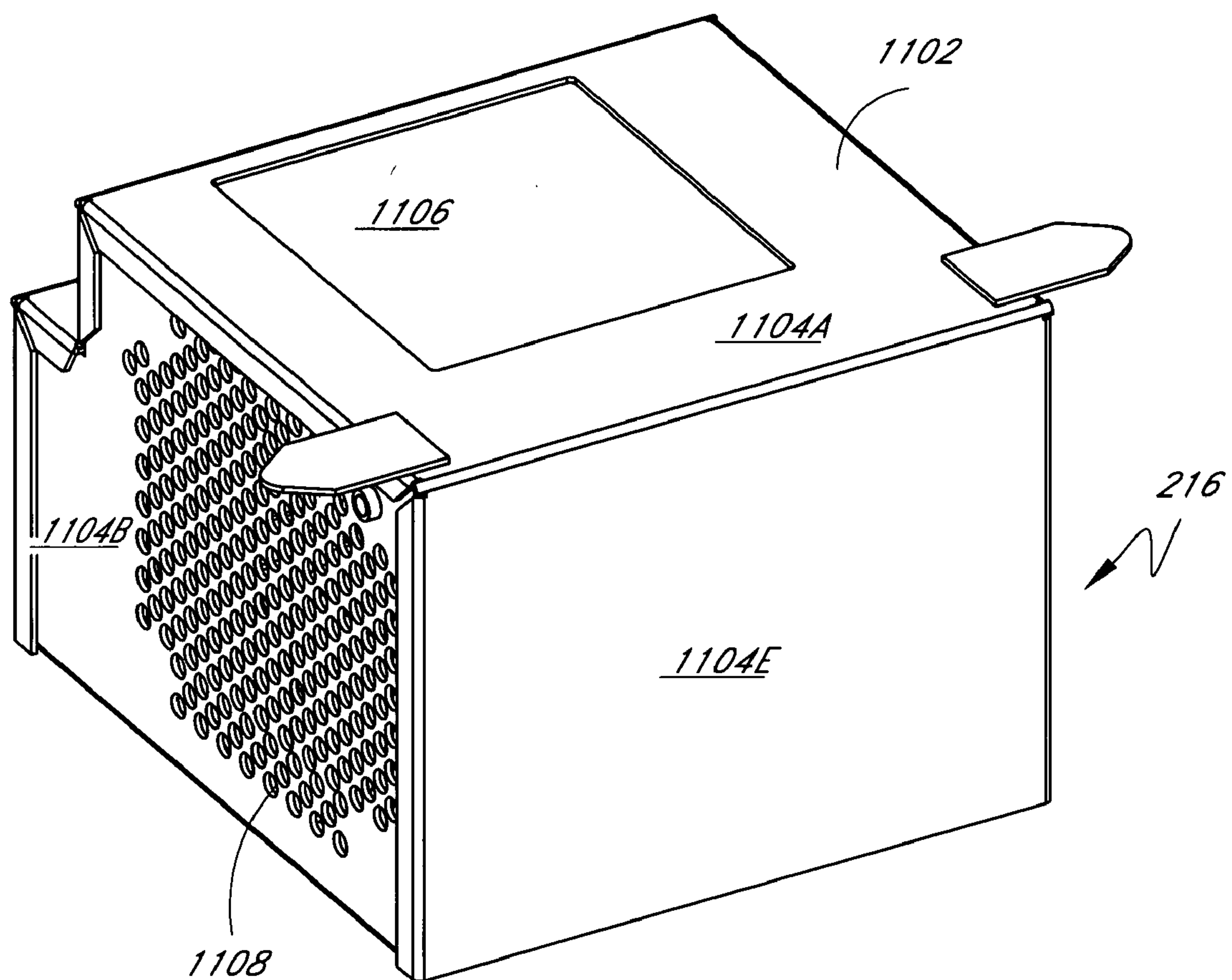


FIG. 12

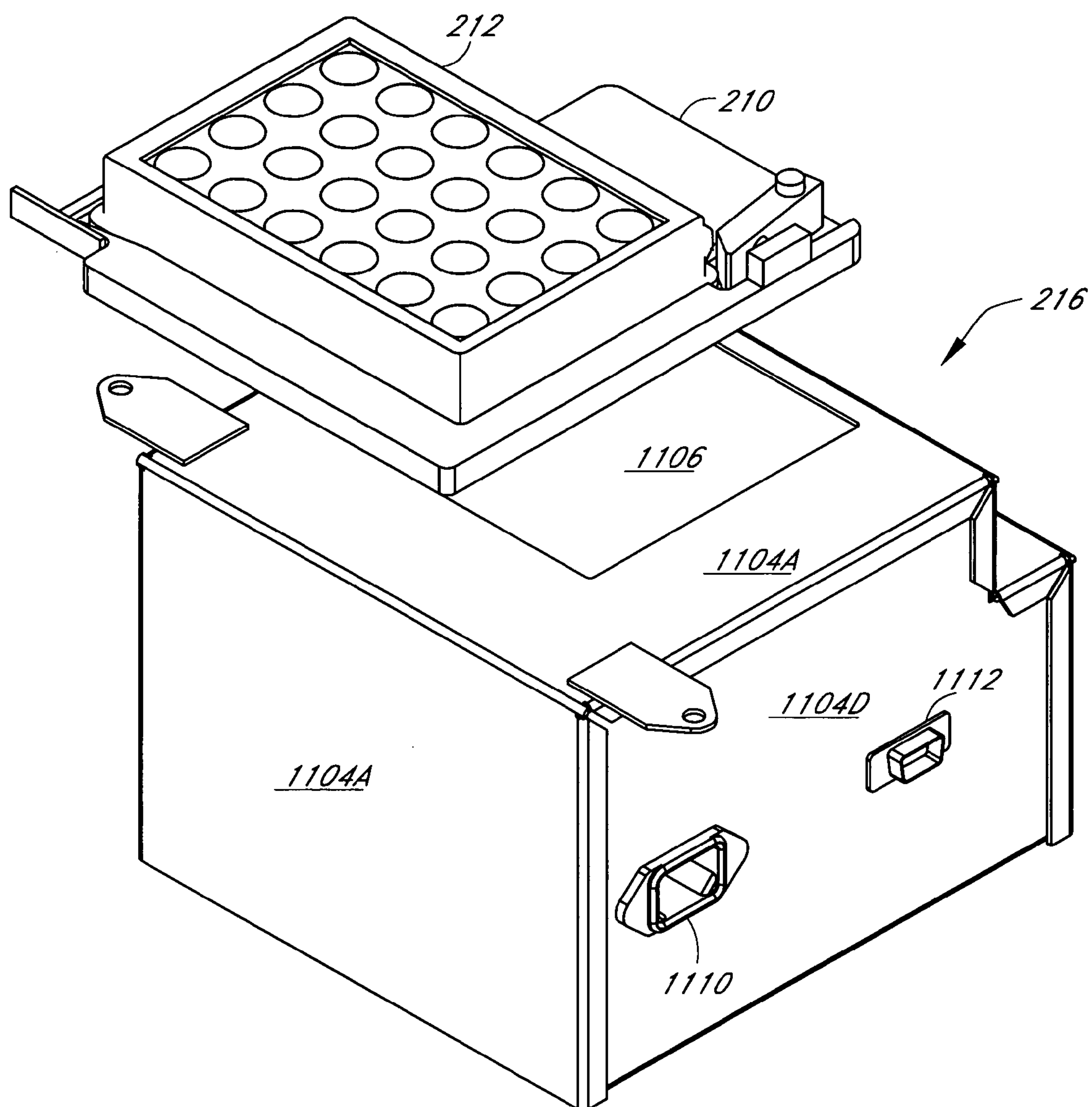


FIG. 13

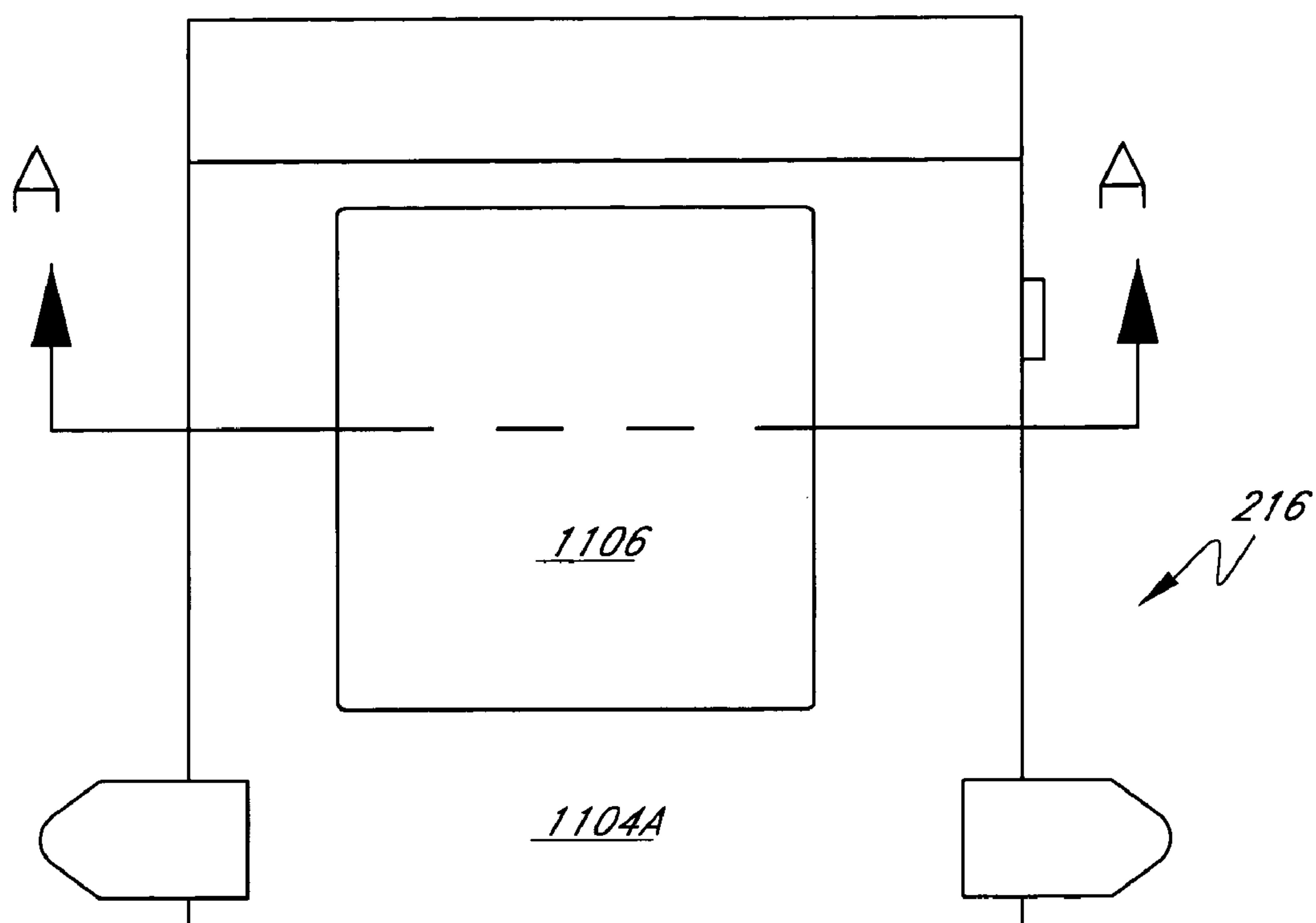


FIG. 14A

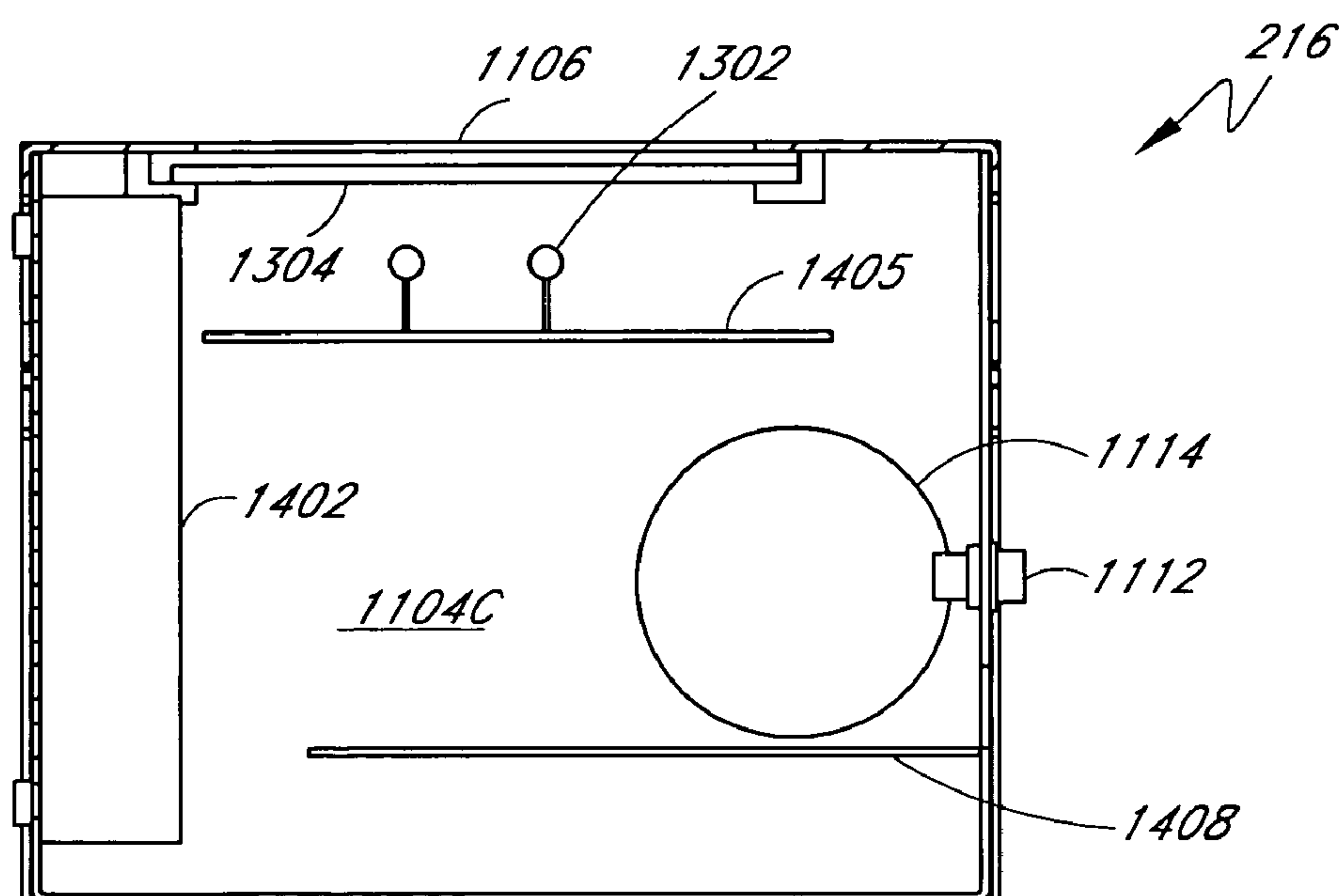


FIG. 14B

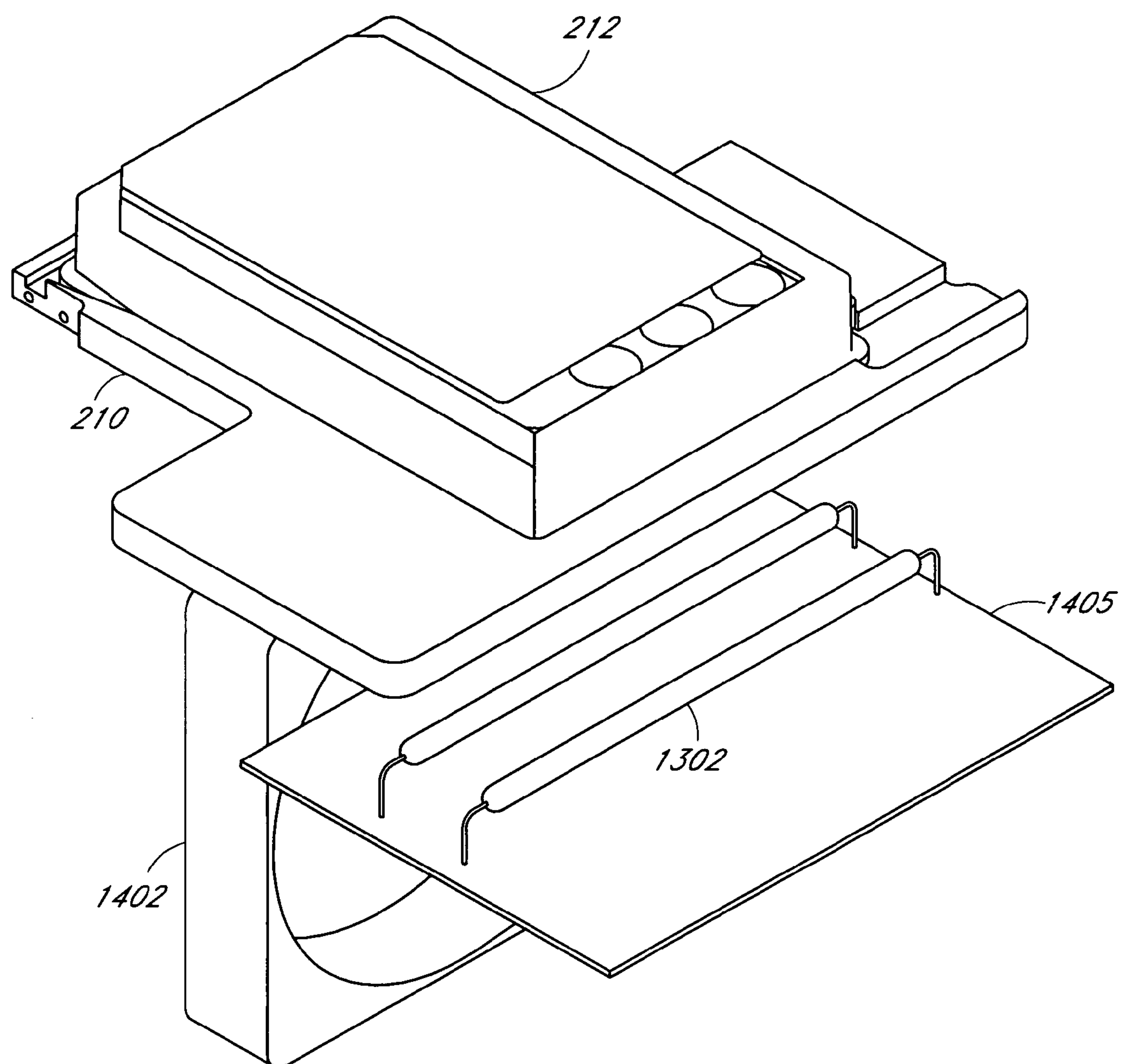
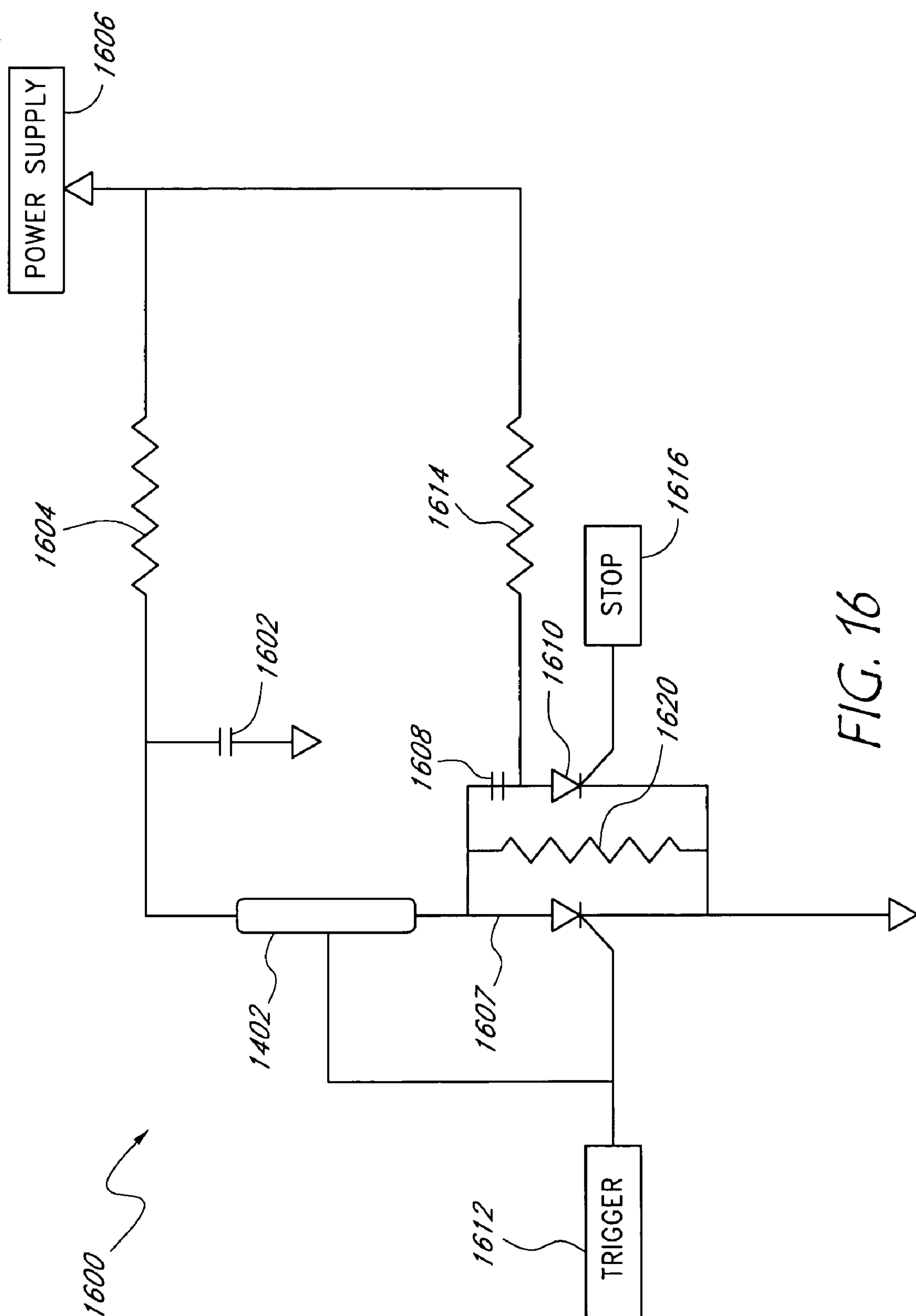
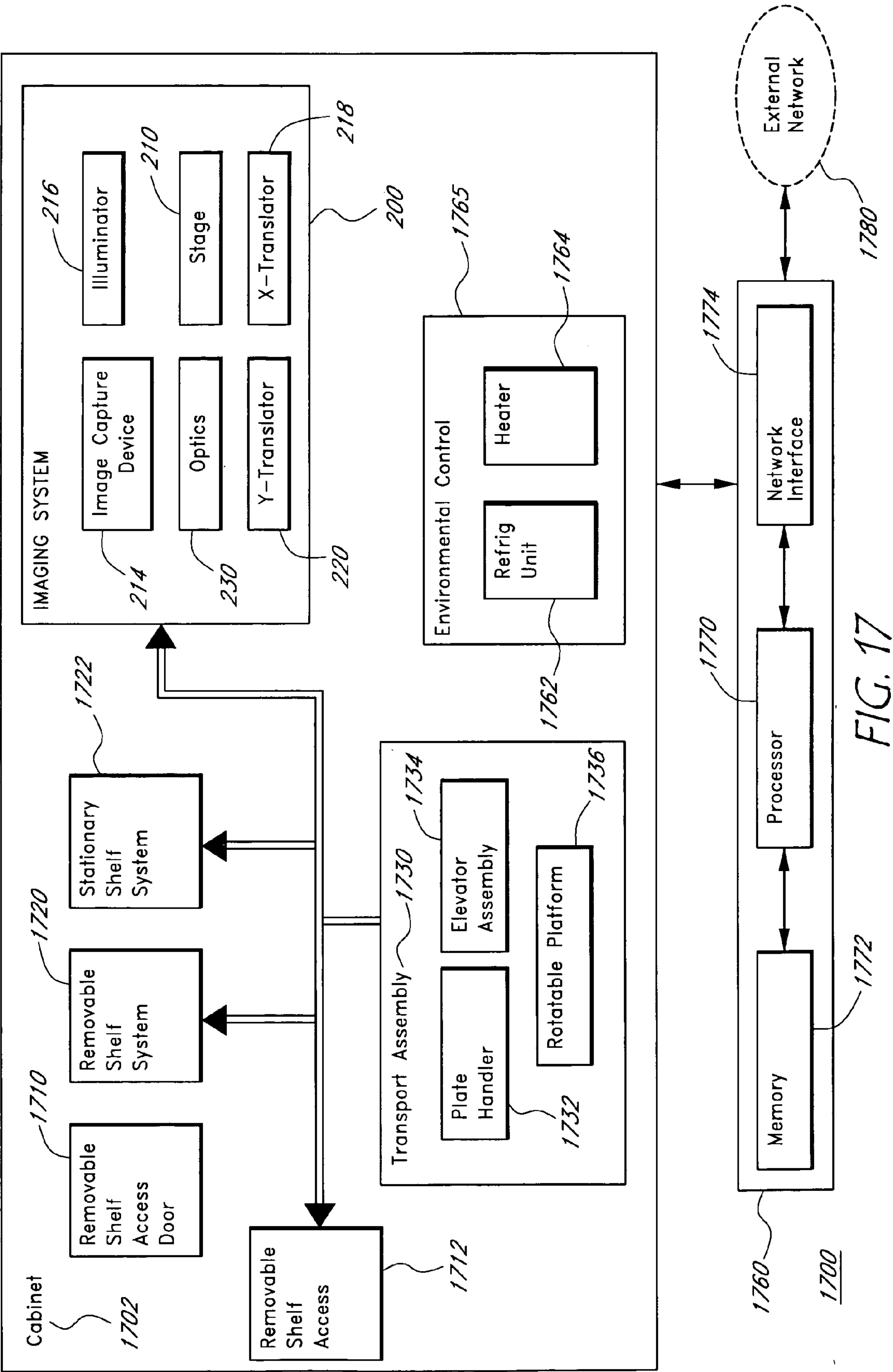


FIG. 15







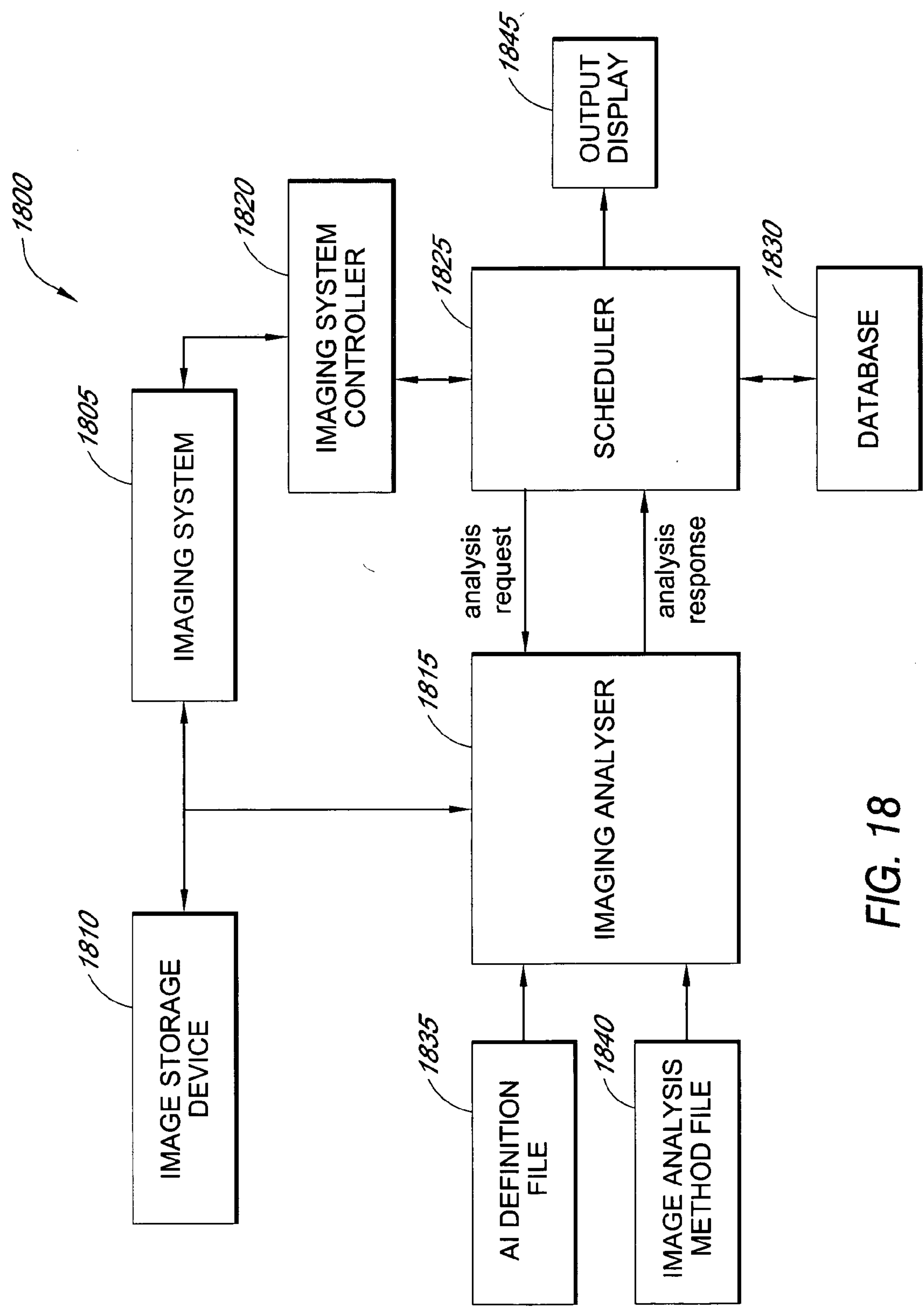


FIG. 18

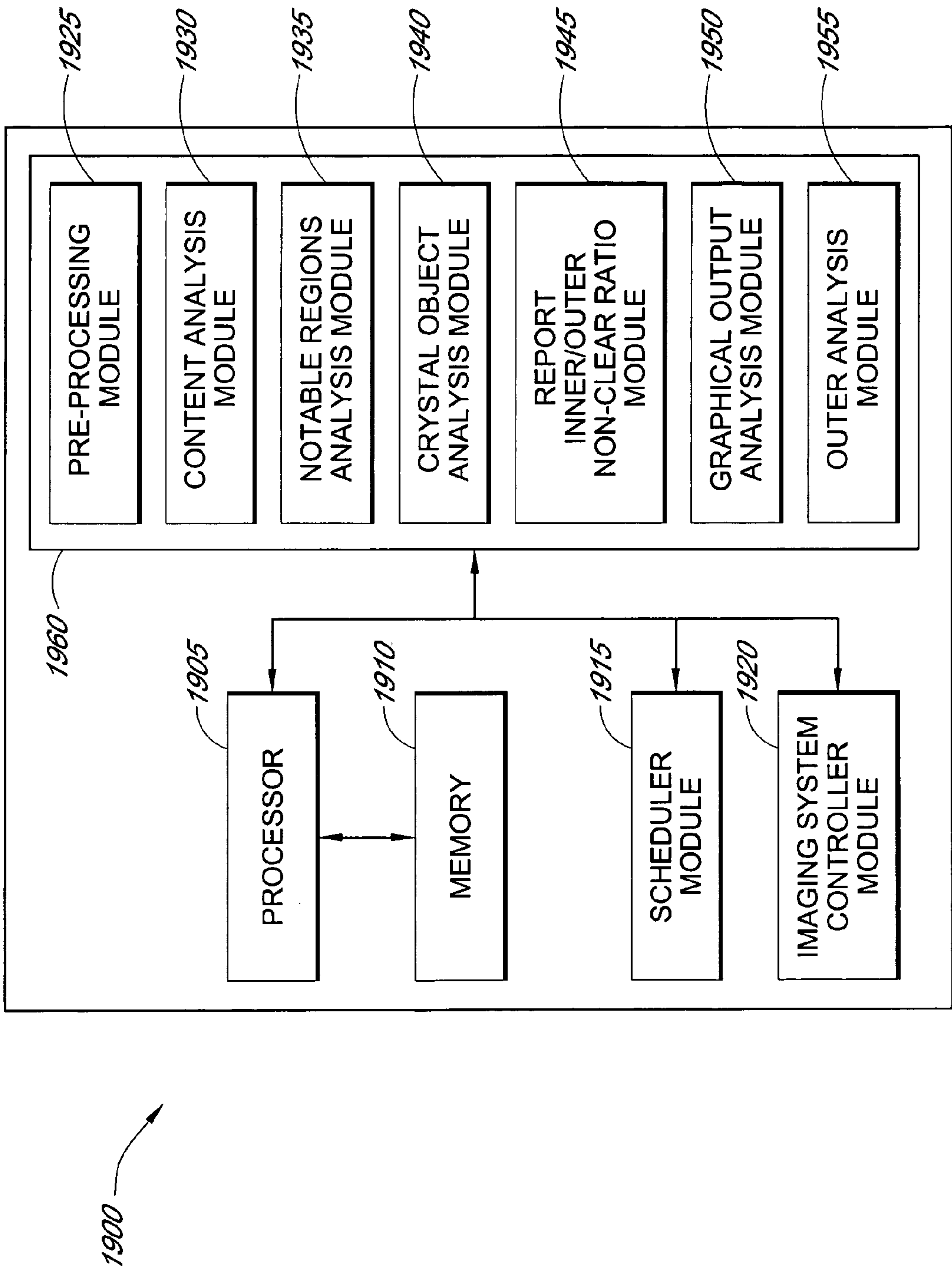


FIG. 19

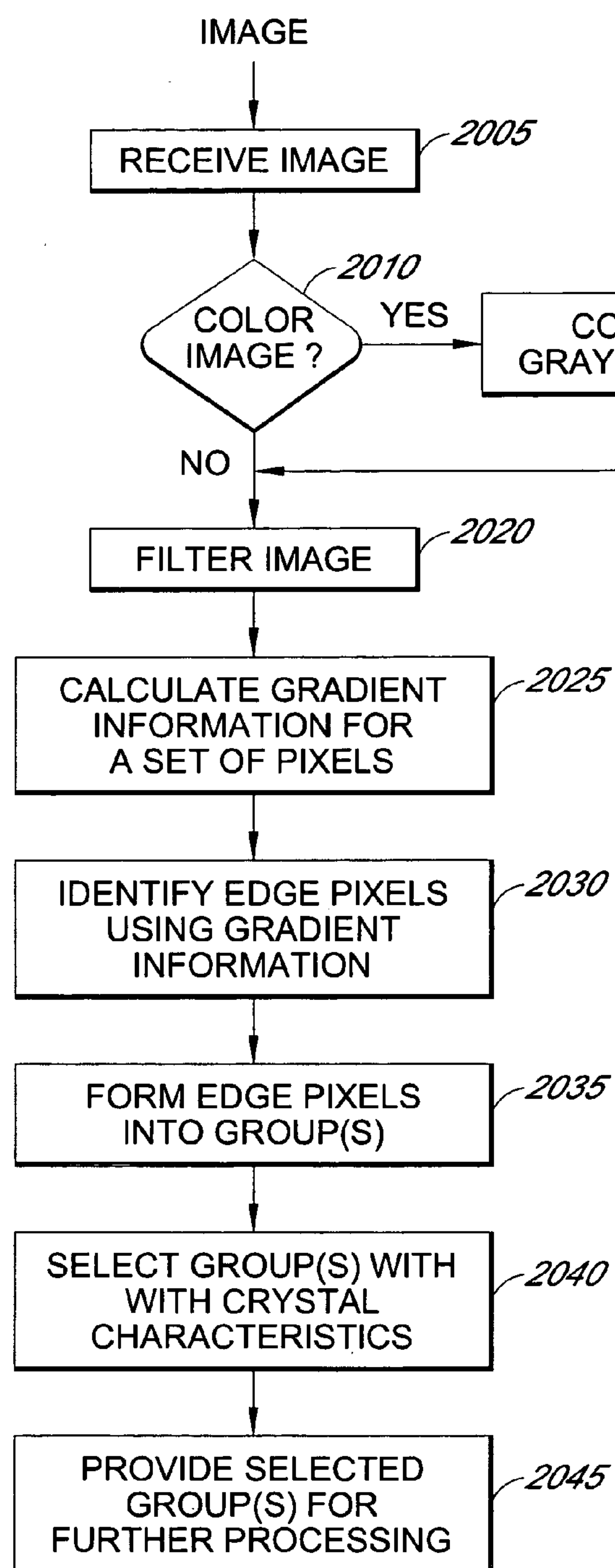


FIG. 20A

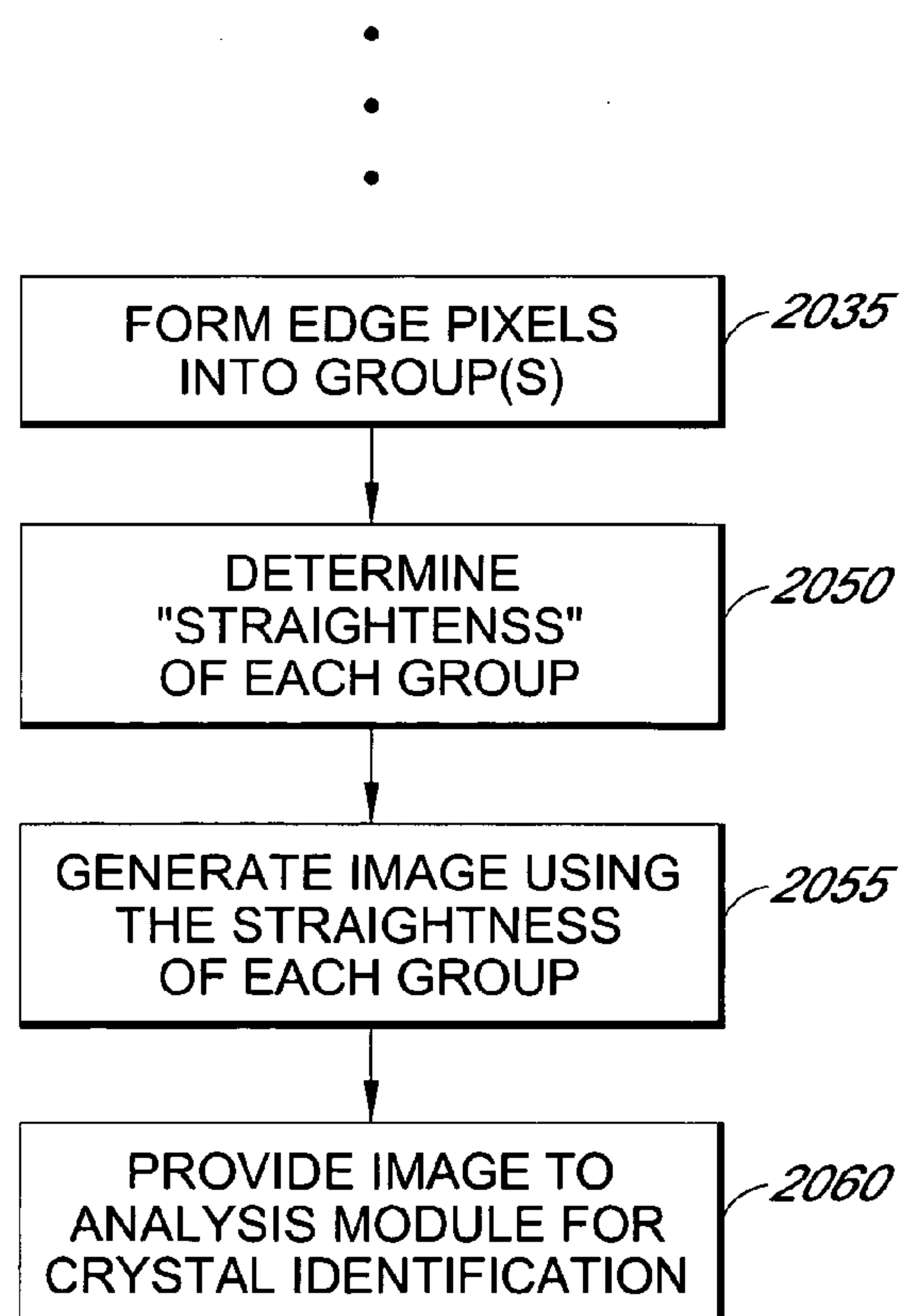


FIG. 20B

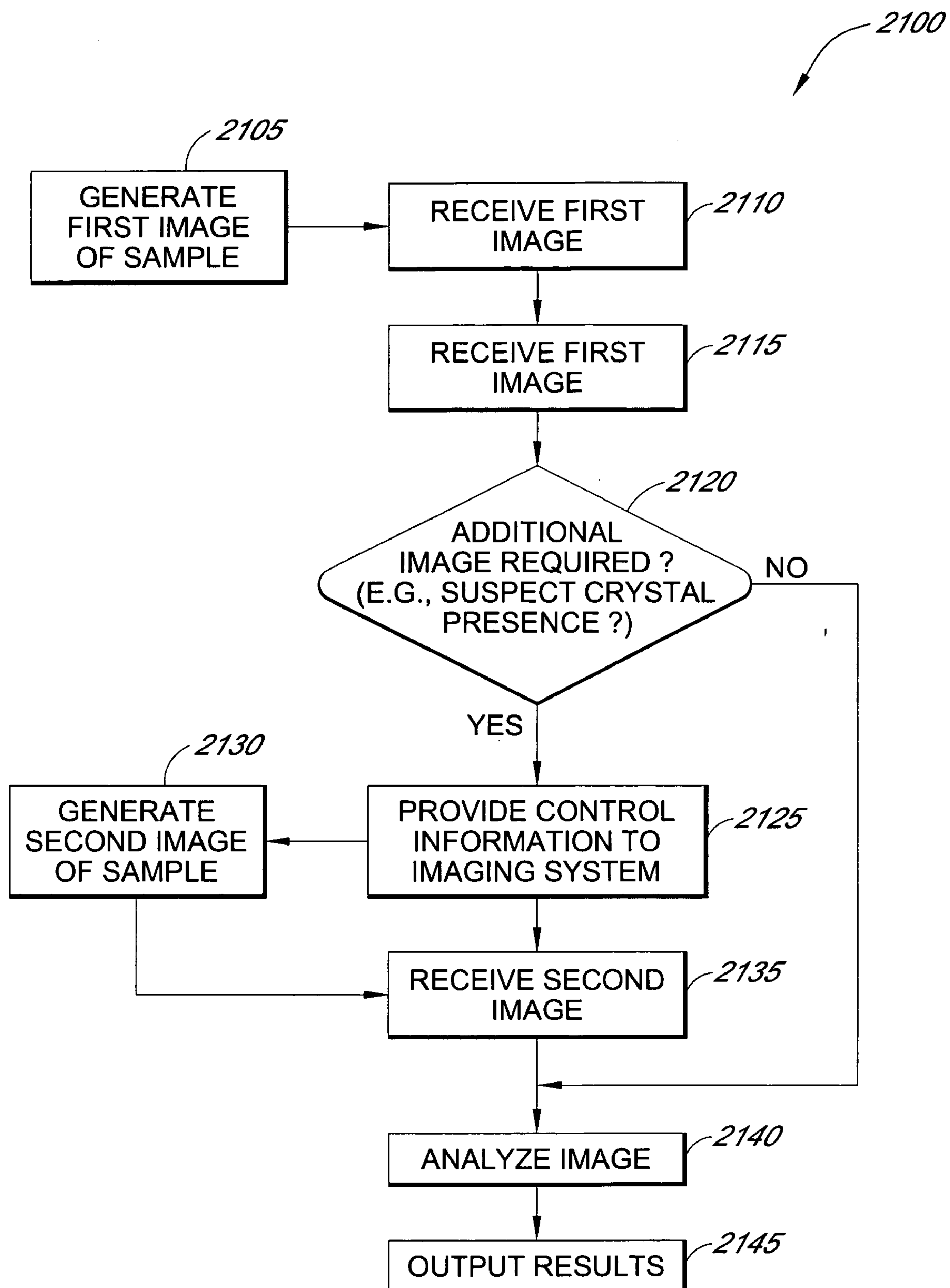


FIG. 21



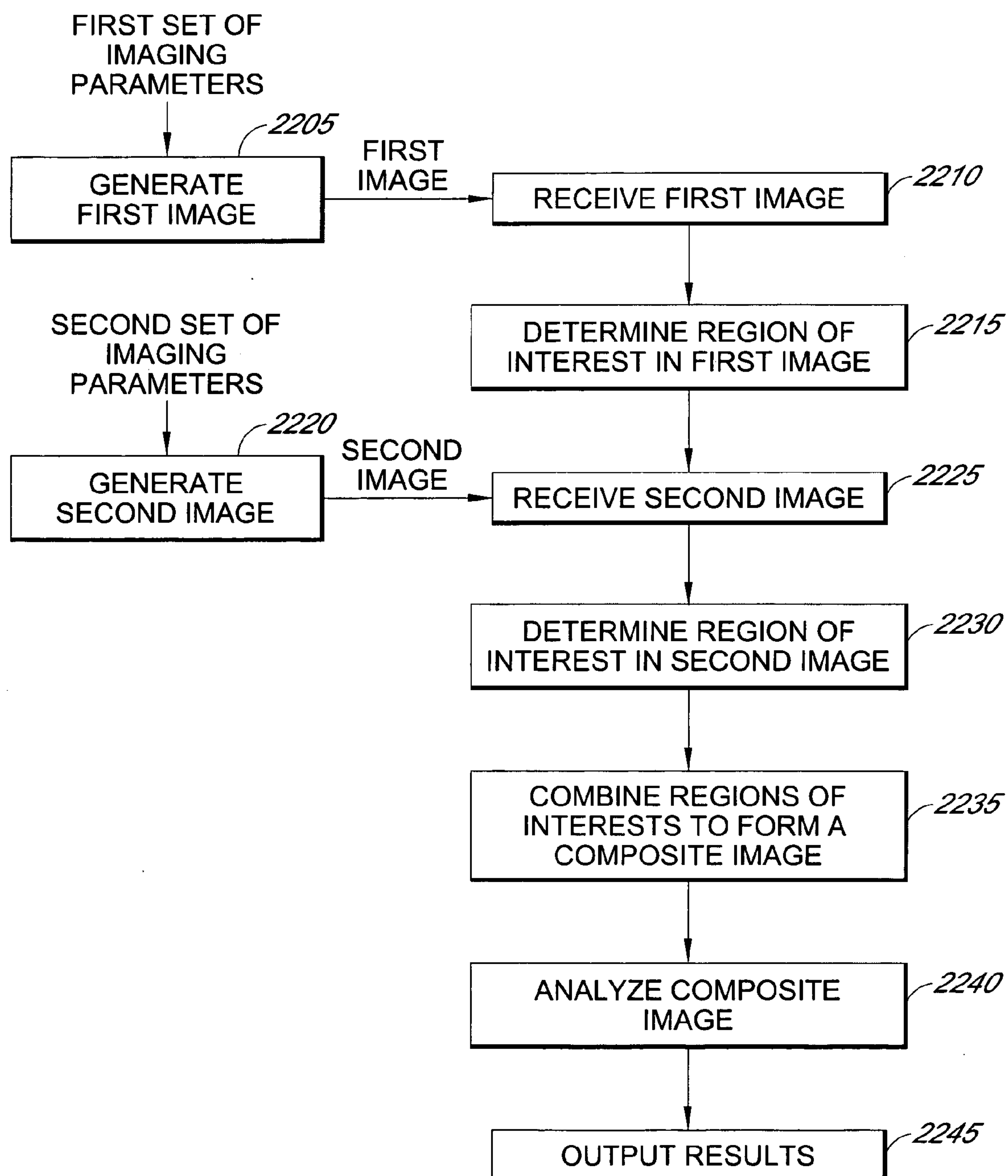
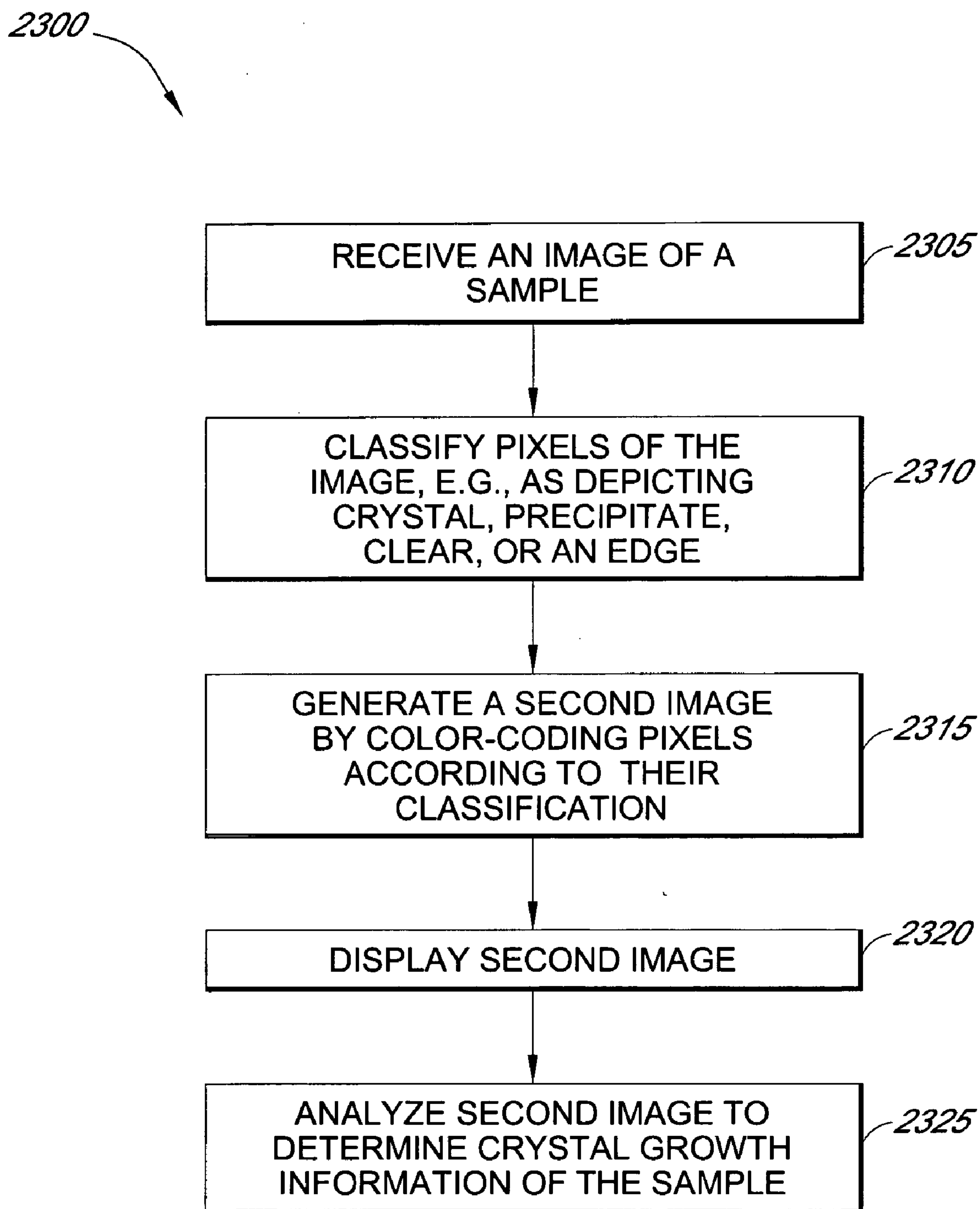


FIG. 22

**FIG. 23**



## IMAGE ANALYSIS SYSTEM AND METHOD

### RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. §119(e) to U.S. Provisional Application No. 60/474,989 filed on May 30, 2003, and entitled IMAGE ANALYSIS SYSTEM AND METHOD, U.S. Provisional Application No. 60/444,586 filed on Jan. 31, 2003, and entitled AUTOMATED IMAGING SYSTEM AND METHOD, U.S. Provisional Application No. 60/444,519 filed on Jan. 31, 2003, and entitled AUTOMATED SAMPLE ANALYSIS SYSTEM AND METHOD, and U.S. Provisional Patent Application No. 60/444,585 filed on Jan. 31, 2003, and entitled REMOTE CONTROL OF AUTOMATED LABS, the entirety of which are incorporated herein by reference.

### BACKGROUND OF THE INVENTION

#### [0002] 1. Field of the Invention

[0003] This invention generally relates to systems and methods for analyzing and exploiting images. More particularly, the invention relates to systems and methods for identifying and analyzing images of substances in samples.

#### [0004] 2. Description of the Related Technology

[0005] X-ray crystallography is used to determine the three-dimensional structure of macromolecules, e.g., proteins, nucleic acids, etc. This technique requires the growth of crystals of the target macromolecule. Typically, crystal growth of macromolecules is dependent on several environmental conditions, e.g., temperature, pH, salt, and ionic strength. Hence, growing crystals of macromolecules requires identifying the specific environmental conditions that will promote crystallization for any given macromolecule. Moreover, it is insufficient to find conditions that result in any type of crystal growth; rather, the objective is to determine those conditions that yield well-diffracting crystals, i.e., crystal configurations that provide the resolution desired to make the data useful.

[0006] Modern chemistry and biology laboratories produce and analyze multiple samples concurrently in order to accelerate the crystal growth development cycle. The samples are often produced and stored in a sample storage container, such as the individual wells in a well plate. Alternatively, drops of multiple samples are placed at discrete locations on a plate, without the need for wells to contain the sample. In either case, hundreds, thousands, or more, different sample drops may be placed on a single analysis plate. Similarly, a single laboratory may house thousands, millions, or more, samples on plates for analysis. Thus, the number of drops to monitor and analyze may be extremely large.

[0007] In the screening experiments, samples under investigation are periodically evaluated to determine if suitable crystallization of the sample has taken place. In a conventional laboratory, a technician manually locates and removes each plate or sample storage receptacle from a storage location and views each sample well under a microscope to determine if the desired biological changes have occurred. In most cases, the plates are stored in laboratories within a controlled environment. For example, in protein crystallization analysis, samples are often incubated for long periods of time at controlled temperatures to induce production of

crystals. Thus, the technician must locate, remove, and view the samples under a microscope in a refrigerated room. Further increasing the demand for technician labor, hundreds or thousands of samples in sample wells may need to be periodically viewed or otherwise analyzed to determine the existence of crystals in a sample well.

[0008] As an alternative, an image may be periodically generated for each sample and provided to a technician, who need not be geographically co-located with the sample, to analyze the image to evaluate crystal growth. Automated image evaluation techniques can also be used to analyze the image and evaluate the presence of crystal growth and increase system throughput. However, current image analysis techniques do not always receive sufficient information from the sample image to accurately evaluate crystal growth. Important information learned as a result of analyzing the image is not automatically exploited, or used for further analysis to facilitate a user's evaluation of the image. Additionally, in current systems, the results of analyzing the image are not adequately provided to facilitate easy interpretation and efficient decision making.

[0009] Accordingly, there is a need in the industry for systems and methods that overcome the aforementioned problems in the current art.

### SUMMARY OF CERTAIN INVENTIVE ASPECTS

[0010] This invention relates to systems and methods for automation of the monitoring of samples to determine crystal growth. According to one embodiment, the invention comprises a method of evaluating crystal growth in a crystal growth system, comprising receiving a first image of a sample, said first image generated by an imaging system using a first set of imaging parameters, analyzing information depicted in said first image to determine the contents of said sample, determining whether to generate another image of said sample based on the contents of said sample, providing information to said imaging system to generate a second image of the sample using a second set of imaging parameters, wherein said second set of imaging parameters comprises at least one imaging parameter that is different from an imaging parameter in said first set of imaging parameters, receiving said second image of said sample, and analyzing information depicted in said second image to determine the contents of said sample. According to other embodiments, the different imaging parameter included in the method can be depth-of-field, illumination brightness level, focus, the area imaged, the center location of the area imaged, illumination source type, magnification, polarization, and/or illumination source position. According to other embodiments of the method of evaluating crystal growth, analyzing said first image comprises determining a region of interest in said first image and wherein said information comprises is used to adjust said second set of imaging parameters so that the imaging system generates a zoomed-in second image of said region of interest.

[0011] According to another embodiment, analyzing information in the method of evaluating crystal growth in a crystal growth system comprises determining whether said first image depicts the presence of crystals, and can further comprise, wherein said first image comprises pixels, and said determining comprises classifying said pixels and comparing the number of pixels classified as crystals to a threshold value.



[0012] According to another embodiment, a method of evaluating crystal growth in a crystal growth system comprises counting the number of said pixels depicting objects in the sample and evaluating said number using a threshold value.

[0013] According to another embodiment of the invention, the method of analyzing crystal growth comprises receiving a first image having pixels depicting crystal growth information of a sample, identifying a first set of pixels in said first image comprising a first region of interest, receiving a second image having pixels depicting crystal growth information of said sample, identifying a second set of pixels in said second image comprising a second region of interest, merging said first set of pixels and said second set of pixels to form a composite image, and analyzing said composite image to identify crystal growth information of said sample. According to another embodiment said first image is generated by an imaging system using a first set of imaging parameters, said second image is generated by said imaging system using a second set of imaging parameters, and wherein said second set of imaging parameters comprises at least one imaging parameter that is different from the imaging parameters in said first set of imaging parameters.

[0014] According to another embodiment of the invention, a method of analyzing crystal growth information comprises receiving a first image comprising a set of pixels that depict the contents of a sample, determining information for each pixel in said set of pixels, wherein said information comprises a classification describing the type of sample content depicted by said each pixel, and a color code associated with each classification, generating a second image based on said information and said set of pixels, displaying said second image, and visually analyzing said second image to determine crystal growth information of the sample.

[0015] According to another embodiment, the invention comprises a system for detecting crystal growth information comprising an imaging subsystem with means for generating an image of a sample, wherein said image comprises pixels that depict the content of said sample, an image analyzer subsystem coupled to said imaging system with means for receiving said image, means for classifying the content of said sample using said pixels and means for determining whether said sample should be re-imaged based on said classifying; and a scheduler subsystem coupled to said imaging analyzer system with means for causing said imaging subsystem to re-image said sample.

[0016] According to another embodiment, the invention comprises a computer-readable medium containing instructions for analyzing samples in a crystal growth system, by receiving a first image of a sample, said first image generated by an imaging system using a first set of imaging parameters, analyzing information depicted in said first image to determine the contents of said sample, determining whether to generate another image of said sample based on the contents of said sample, providing information to said imaging system to generate a second image of the sample using a second set of imaging parameters, wherein said second set of imaging parameters comprises at least one imaging parameter that is different from an imaging parameter in said first set of imaging parameters, receiving said second image of said sample, analyzing information depicted in said second image to determine the contents of said sample.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0017] The above and other aspects, features, and advantages of the invention will be better understood by referring to the following detailed description, which should be read in conjunction with the accompanying drawings, in which:

[0018] FIG. 1A is a high-level block diagram of an imaging system according to the invention.

[0019] FIG. 1B is high-level block diagram of another imaging system according to the invention.

[0020] FIG. 2 is a perspective view of an imaging system according to the invention.

[0021] FIG. 3 is a perspective view of the imaging system shown in FIG. 2, viewed from a different angle.

[0022] FIG. 4 is a perspective view of the imaging system shown in FIG. 2, viewed from yet a different angle.

[0023] FIG. 5 is a plan front view of the imaging system shown in FIG. 2.

[0024] FIG. 6 is a plan, right side view of the imaging system shown in FIG. 2.

[0025] FIGS. 7A and 7B are perspective views from different angles of a lens system as can be used with the imaging system shown in FIG. 2.

[0026] FIG. 8 is a perspective view from below of a photo-filter carriage that can be used with the imaging system shown in FIG. 2.

[0027] FIG. 9 is a perspective view of certain components as assembled in the imaging system shown in FIG. 2.

[0028] FIG. 10 is a plan front view of certain components as assembled in the imaging system shown in FIG. 2.

[0029] FIG. 11 is a plan, right side view of the components shown in FIG. 10.

[0030] FIG. 12 is a perspective view of a light source as can be used with the imaging system shown in FIG. 2.

[0031] FIG. 13 is a perspective view of a sample mount with the light source shown in FIG. 12, viewed from a different angle.

[0032] FIG. 14A is a plant top view of the light source shown in FIG. 12.

[0033] FIG. 14B is a cross-sectional view along the plane A-A of the light source shown in FIG. 14A.

[0034] FIG. 15 is a exploded, perspective view of certain components of the sample mount and the light source shown in FIG. 13.

[0035] FIG. 16 is a functional block diagram of an illumination duration control circuit as can be used with the light source shown in FIG. 12.

[0036] FIG. 17 is a functional block diagram of an automated sample analysis system in which the imaging system according to the invention can be used.

[0037] FIG. 18 is a block diagram of an imaging and analysis system.



[0038] FIG. 19 is a block diagram of a computer that includes a Crystal Resolve analysis module, according to one aspect of the invention.

[0039] FIG. 20A is a block diagram of an analysis system process, according to one embodiment of the invention.

[0040] FIG. 20B is a block diagram of an analysis system process, according to one embodiment of the invention.

[0041] FIG. 21 is a flow diagram of an imaging analysis process, according to one embodiment of the invention.

[0042] FIG. 22 is a flow diagram of an imaging analysis and control process, according to one embodiment of the invention.

[0043] FIG. 23 is a flow diagram of an analysis process, according to one embodiment of the invention.

#### DETAILED DESCRIPTION OF CERTAIN INVENTIVE EMBODIMENTS

[0044] Embodiments of the invention will now be described with reference to the accompanying figures, wherein like numerals refer to like elements throughout. The terminology used in the description presented herein is not intended to be interpreted in any limited or restrictive manner, simply because it is being utilized in conjunction with a detailed description of certain specific embodiments of the invention. Furthermore, embodiments of the invention may include several novel features, no single one of which is solely responsible for its desirable attributes or which is essential to practicing the inventions herein described.

[0045] The imaging and analysis system and methods disclosed here are related to embodiments of an automated sample analysis system having an imaging system that is described in the related U.S. provisional patent application No. 60/444,519, entitled "AUTOMATED SAMPLE ANALYSIS SYSTEM AND METHOD." An imaging system that can provide images of samples for analysis, in response to control information, is described hereinbelow, followed by a description of a system and processes for analyzing the images. It should be noted here that the terms "image", "subimage" or "pixels" as used herein at various locations do not necessarily mean an optical image, subimage or pixels which are either usually displayed or printed, but rather include digital representations or other representations of such image, subimage or pixels. It should also be noted that the term "sample" as used herein refers to any type of suitable sample, for example, drops, droplets, the contents of a well, the contents of a capillary, a sample in gel or any other embodiment of containing a sample or material.

[0046] FIG. 1A is a high-level block diagram of an imaging system 100. In this embodiment, the imaging system 100 has an assembly 105 that is controlled by controllers and logic 110. The assembly 105 includes a stage 115 that holds and transports target samples to be imaged by an image capture device 120. The imaging system 100 employs an optics assembly 125 to enhance the view of the target samples before the image capture device 120 obtains the images of the samples. An illuminator 130 is configured as part of the assembly 105 to direct light at the samples held in the stage 115.

[0047] The assembly 105 also includes a translator 135 that provides the structural support members and actuators

to move any one combination of the stage 115, image capture device 120, optics 125, or illuminator 130. The translator 135 may be configured to move the combination of components in one, two, or three dimensions. As will be discussed in detail below, in some embodiments the stage 115 remains stationary while the translator 135 moves the image capture device 120 and optics 125 to a desired well position in a sample plate held by the stage 115. In other embodiments of the imaging system 100, the translator 135 moves the stage 115 in a first axis and the image capture device 120 and optics 125 in a second axis which is substantially perpendicular to the first axis.

[0048] The controllers and logic 110 of the imaging system 100 provide instructions to and coordinate the activities of the components of the assembly 105. The controllers may include a microprocessor, controller, microcontroller, or any other computing device. The logic includes the instructions to cause the controller to perform the tasks or processing described here.

[0049] FIG. 1B is high-level block diagram of an imaging system 150. The imaging system 150 includes an assembly 155 in communication with controllers and logic 160. The assembly 155 may also be in communication with a data storage device 190, which itself may be configured for communication with the controllers and logic 160. The controllers and logic 160 control and coordinate the activities of the components of the assembly 155.

[0050] In this embodiment, the assembly 155 includes a sample plate mount 165 suitably configured to receive micro-titer plates of various configurations and sizes. Alternatively, the sample plate mount 165 can be configured to receive any sample matrix that carries samples, regardless of whether the samples are stored in individuals sample wells, rest on the surface of the sample matrix (e.g., as droplets), or are embedded in the sample matrix. A source of flash lighting 180 is arranged to direct light bursts to the samples stored in the micro-titer plate carried by the sample plate mount 165. An inventive system and method of providing the flash lighting 180 will be discussed with reference to FIG. 16.

[0051] The assembly 155 includes a compound lens 175 that cooperates with a digital camera 170 to acquire images of the samples in the sample plate. The compound lens 175 may consist, for example, of an objective lens, a zoom lens, and additional optics chosen to provide the digital camera 170 with the desired image from the light from the samples. In one embodiment, as will be discussed further below, the compound lens 175 may be motorized (i.e., provided with one or more actuators) so that the controllers and logic 160 can automatically focus the scene, zoom on the scene, and set the aperture.

[0052] In this embodiment, the assembly 155 includes an x-y translator that moves either the sample plate mount 165 or the compound lens 175, or both. Of course, if the digital camera 170 is coupled to the compound lens 175, the x-y translator moves both the digital camera 170 and the compound lens 175. In some embodiments, the x-y translator 185 is configured to move the sample plate mount 165 in two axis, e.g., x and y coordinates. Alternatively, the x-y translator 185 moves the compound lens 175 in two axis, while the sample plate mount 165 remains stationary. In yet another embodiment, the x-y translator consists of multiple



and separate actuators that move independently from one another the sample mount **165** or the compound lens **175**.

[0053] It should be noted that the assembly **155**, the controllers and logic **160**, and the data storage **190** are depicted as separate components for schematic purposes only. That is, in some embodiments of the imaging system **150** it is advantageous to, for example, integrate the data storage device **190** into the assembly **155** and to include the controllers and logic **160** as part of one or more of the components shown as being part of the assembly **155**. Similarly, the sample mount **165**, digital camera **170**, compound lens **175**, flash lighting **180**, and x-y translator **185** need not all be configured as part of a single assembly **155** as shown.

[0054] Exemplary ways of using and constructing embodiments of the imaging system **100** or **150** will be described in detail below with reference to **FIGS. 2-16**, which depict a specific embodiment of the imaging system. Of course, since there are multiple ways to implement the imaging system, the following description of the specific embodiment should not be taken to limit the full scope of the inventive imaging system.

#### [0055] Illustrative Embodiment

[0056] With reference to **FIGS. 2-6** and **9-11**, perspective and plan views of an imaging system **200** according to the invention are illustrated. The imaging system **200** includes a sample plate mount **210** that receives a sample plate **212**. An x-translator having an actuator **218** (see **FIG. 4**) is coupled to the sample plate mount **210** to move the sample plate mount **210** into position above a light source **216** and below a lens assembly **230**. A digital camera **214** is coupled to the lens assembly **230** to capture images of the wells in the sample plate **212**. A y-translator having an actuator **220** (see **FIG. 3**) is coupled to the lens assembly **230** to move the lens assembly **230** into position over a desired well of the sample plate **212**.

#### [0057] Support Platform

[0058] The digital camera **214**, lens assembly **230**, sample plate mount **210**, light source **216**, x-translator **218**, and y-translator **220** are mounted on a platform **240** (see **FIG. 2**). The platform **240** generally consists of several structural members, brackets, or walls, e.g., base **242**, side wall **244**, front wall **250**, bracket **252**, bracket **246**, post **248**, and support member **254**. The light source **216** can be fastened to the base **242**. Rails **256** and **258**, which support the lens assembly **230** are fastened to the wall **250** of the platform **240** and to the support member **254**. The sample plate mount **210** is supported by a rail **262** and an outport guide **253** of the support member **254**. The rail **262** is supported through attachment to the side wall **244** and the post **248**. Of course, there are multiple, equivalent alternatives to providing support for and configuring the lens assembly **230**, sample plate mount **210**, light source **216**, and x-, y-translators **218** and **220** on the platform **240**.

[0059] The platform **240** may be constructed of any of several suitable materials, including but not limited to, aluminum, steel, or plastics. Because in some applications it is critical to keep vibration of the platform **240** to a minimum, materials that provide rigidity to the platform **240** are preferred in such applications. With regard to the rails **256**, **258**, and **262**, these are preferably manufactured with very

smooth surfaces to carry the lens assembly **230** or the sample plate mount **210** in a smooth fashion, thereby avoiding vibrations. As illustrated in **FIGS. 3** and **9**, supporting the lens assembly **230** may be done by coupling the linear plain bearings **264** and **266** to the rails **256** and **258**. A similar coupling using a “bushing” **267** (see **FIG. 10**) may be employed to fasten the sample plate mount **210** to the rail **262**. Bearings **264**, **266**, and **267** are chosen to provide smooth bearing surfaces for smooth translation of the load, e.g., the lens assembly **230** or the sample plate mount **210**.

#### [0060] Sample Plate Mount

[0061] The sample plate mount **210** may be constructed from any rigid material, e.g., steel, aluminum, or plastics. Preferably the sample plate mount **210** is configured to accommodate, either directly or through the use of adapters, various standard sizes of micro-titer plates. Micro-titer plates that may be used with the sample plate mount **210** include, but are not limited to, crystallography plates manufactured by Linbro, Douglas, Greiner, and Corning. As will be described further below, the sample plate mount **210** is coupled to an actuator **218** for moving the sample plate mount **210** in one axis.

#### [0062] Translators

[0063] The imaging system **200** includes two independent translators. Typically, the sample plate mount **210** and the lens assembly **230** move on a plane that is substantially parallel to a plane defined by the sample plate **212** carried by the sample plate mount **210**. In one embodiment, the controllers and logic **110** or **160** can control x-, y-translators to position the sample plate mount **210** and the lens assembly **230** at the coordinates of a specific well of the sample plate **212**.

[0064] An x-axis translator for moving the sample plate mount **210** consists of an actuator **218** (see **FIG. 4**) that rotates a threaded rod **219** (or “lead screw”) about its axis in clockwise or counter-clockwise directions. In the embodiment shown in **FIGS. 3, 4**, and **10**, the actuator **218** is coupled to the rod **219** via a belt (not shown) and pulleys **221** and **221'**. The sample plate **210** mount is fastened to a “bushing” **267** (see **FIG. 10**) that rides on the rail **262**. The sample plate mount **210** is also supported by the outport guide **253** (see **FIGS. 6** and **11**) of the support member **254**. The “bushing” **267** is additionally coupled in a known manner to the rod **219**. When the actuator **218** turns in one direction, its power is transmitted via the belt and pulleys **221** and **221'** to the rod **219**, which then moves the “bushing” **267** and, thereby, moves the sample plate mount **210** in a linear direction.

[0065] A y-axis translator for moving the lens assembly **230** consists of an actuator **220** (see **FIG. 3**) that rotates a threaded rod **260** about its axis in clockwise or counter-clockwise directions. In the embodiment shown in **FIGS. 3, 6**, and **9**, for example, the actuator **220** is coupled to the rod **260** through a slotted disc coupling (not shown). The lens assembly **230** is coupled to bearings **264** and **266** that respectively ride on rails **256** and **258**. The bearings **264** and **266** are coupled to the rod **260** through plate **255** and the bracket **257** (see **FIG. 6**) in a known manner. When the actuator **220** turns in one direction, its power is transmitted via the slotted disc coupling to the rod **260**, which then moves the bearings **264** and **266** and, thereby, moves the lens assembly in a linear direction.



[0066] The actuators **218** and **220** may be direct current gear motors or 3-phase servo motors, for example. Of course, the type of motors employed as the actuators **218** or **220** will depend on, among other things, the weight of the sample plate mount **210** plus sample plate **212** or the lens assembly **230** and the digital camera **214**. Another factor in determining the type of motor is the desired speed. In one embodiment, actuators **218** and **220** having a positioning precision of 10-microns are used. Suitable motors may be obtained from PITMANN® of Harleysville, Pa.

[0067] In the embodiment of the x-, y-translators described above, each translator mechanism independently translates along an axis of motion each of the sample plate mount **210** and the lens assembly **230**. However, it should be noted that in other embodiments of the imaging system **200**, it may be desirable to maintain the lens assembly stationary and only move the sample plate mount **210**, which would then have one or more translators to position the sample plate mount **210** anywhere in an x-y coordinate area. Similarly, the imaging system **200** may be configured so that an x-y translator (or set of x-, y-translators) moves the lens assembly in the x-y coordinate area, while the sample plate mount **210** remains stationary over the light source **216**. In one embodiment, the x-, y-translators employ optical sensors **285** and **287** (see FIG. 5) as to sense the start or end positions (“home positions”) of the lens assembly **230** or the sample plate mount **210**.

[0068] In yet another embodiment, the imaging system **200** may also include a z-axis translator (not shown) to lift or lower the sample plate mount **210**, lens assembly **230**, or light source **216**. The z-axis translator may consist of, for example, an actuator, a lead screw, one or more rails, and appropriate bearings and fasteners.

[0069] The actuators **218** and **220** may be governed by a controller (not shown). Suitable controllers may be obtained from J R Kerr Automation Engineering of Flagstaff, Arizona. The controller may be configured to interpret high level commands from a computing device. In one embodiment, when a specific axis is addressed, the controller causes the actuator **220**, for example, to move and keeps count of the travel distance and final location. The controller can be programmed to move the actuator **220** at varying speed, torque, and acceleration.

#### [0070] Image Capture Device

[0071] In some embodiments of the imaging system **200**, the image capture device can be a film camera, a digital camera, a CMOS camera, a charge coupled device (CCD), and the like, or some other apparatus for capturing an image of an object. The embodiments of the imaging system **200** described here employ a digital camera **214**. A suitable digital camera **214** is, for example, a CMOS digital camera. However, it should be apparent that several digital photography devices could also be employed. The CMOS camera **214** is preferred because it provides random access to the image data and is relatively low cost. In conventional imaging systems for crystallography, a CMOS camera is typically not used because in those systems the level of light is insufficient for this type of camera. In contrast, the imaging system **200** is configured to provide the level of light necessary to allow use of a CMOS camera.

[0072] The digital camera **214** can be a CMOS camera having a pixel resolution of 1280×1024 pixels, Bayer color

filter, a pixel size of 7.5×7.5 microns, and a data interface governed by the IEEE 1394 standard (commonly known as “Firewire”). The digital camera **124** may be fully digital and not require a frame grabber. The digital camera **124** may also have a centered pixel area, e.g. a 1024×1024 or 800×600 pixel subset of the array, which enhances the image quality since the edges of the array where optical distortions increase are avoided. In one embodiment, the digital camera **214** is connected separately to a host computer (not shown) via a Firewire data interface. This allows for rapid transfer of large amounts of image data, e.g., five images per second.

#### [0073] Lens Assembly

[0074] One embodiment of the lens assembly **230** includes an objective lens **231**, a zoom lens **233**, and an adapter **235**. These optical components are chosen to provide suitable field of view, magnification, and image quality. The objective lens **231**, zoom lens **233**, and adapter **235** may be purchased from, for example, Navitar Inc. of Rochester, N.Y.

[0075] In one embodiment, the zoom lens **233** may be the “12×UltraZoom” zoom lens manufactured by Navitar. The zoom lens **233** may provide a 12:1 zoom factor, a focus range of about 12-mm, and an aperture of about 0.14. The zoom lens **233** preferably includes adapters for mounting the objective lens **231**. The zoom lens **233** may have actuators **233A**, **233B**, and **233C** for providing, respectively, automatic aperture adjustment, autozoom, and autofocus functionality. In one embodiment, actuators **233B** and **233C** have gear reductions of 262:1. Of course, the gear reduction ratio is chosen to suit the particular application. For example, a 5752:1 gear ratio for the focus actuator **233C** may be too slow for some applications of the imaging system **200**. The actuators **233A**, **233B**, and **233C** may be obtained from Navitar or from MicroMo Electronics, Inc. of Clearwater, Florida.

[0076] The objective lens **231** may be, for example, a 5× Mitutoyo Infinity Corrected Long Working Distance Microscope Objective (model M Plan Apo 5) microscope accessory. The objective lens **231** is coupled to the zoom lens **233**. Since the light source **216** delivers sufficient light to the sample plate **212**, the lens assembly **230** is configured to allow for setting a small aperture in order to increase the depth of field. The objective lens **231** preferably provides a working distance that allows adequate room beneath the lens assembly **230** to manipulate a sample plate **212** and provide a photo-filter carriage **237** in the image path. In one embodiment, the working distance of the objective lens **231** is about 34-mm.

[0077] The adapter **235** serves to allow use of the digital camera **214**. The adapter **235** may be, for example, a 1× Adapter model number 1-6015 sold by Navitar. Of course, different combinations of objective lenses **231** and adapters **235** may be used, e.g., a 2× Adapter and 2× Objective combination. The combination of 1× Adapter and 5× Objective provides a suitable image for most applications of the imaging system **200**. In some embodiments, it is desirable to use a 0.67× Adapter **235** with a 10× Objective **231**, for example, to provide a higher image resolution.

[0078] The optical components of the lens assembly **230** can be provided with actuators for remote and automatic control. To allow software control of the optical compo-



nents, controllers and control logic (not shown) can control the actuators **233A**, **233B**, **233C**, and **233D**. The actuators (e.g., dc motors) may be coupled to the aperture of the magnification and focus of the zoom lens **233**, as well as the photo-filter carriage **237**. In some embodiments, the actuators **233A**, **233B**, **233C**, and **233D** are preferably provided with encoders to provide position information to the controllers. In one embodiment, the actuators on the lens assembly **230** are 17-mm direct current motors with 100:1 gear reducers. These motors may be obtained from PIT-MANN® of Harleysville, Pennsylvania.

[0079] The lens assembly **230** may also include a photo-filter carriage **237** that is configured to hold optical filters (not shown). For example, the photo-filter carriage **237** can hold polarization plates or color light filtering plates. **FIG. 8** illustrates one embodiment of a photo-filter carriage **237** that may be used with the imaging system **200**. The photo-filter carriage **237** includes a filter wheel **237A** for receiving one or more photo-filters (not shown) in openings **237B**. The photo-filters may be held in place in the filter wheel **237A** in a variety of ways. For example, in the embodiment illustrated in **FIG. 8**, caps **237C** in cooperation with suitable fasteners hold the photo-filters in place. The filter wheel **237A** may be coupled to an actuator **233D** for remote and automatic control of the filter wheel **237A**. The actuator **233D** and the filter wheel **237A** may be fastened, in a conventional manner, to a clamp **237D** that is coupled to, for example, the objective lens **231** or the zoom lens **233** (see **FIGS. 1 and 9**). In one embodiment, a polarization filter is coupled to a filter wheel so that the polarization filter covers about 90 degrees of the wheel. In this embodiment, the polarization filter can be rotated so that the applied polarization varies between zero and ninety degrees. Thus, the use of the polarization filter with a polarized light source can provide analysis of the effect of samples on polarized light. For example, when a polarized light source and the polarization filter are cross-polarized then minimal light should get to the objective lens **231**, unless the sample re-orientes the polarized light, such as can happen when the light passes through crystals.

[0080] The digital camera **214** in combination with the lens assembly **230** provides a broad depth of field to allow imaging of objects such as protein crystals at varying depths within a sample droplet stored in a sample well of a sample plate **212**. In one embodiment, the lens assembly **230** has a 12:1 zoom lens and, in cooperation with the digital camera **214**, can provide a 1 micron optical resolution. In some embodiments, the lens assembly **230** and the digital camera **214** may be integrated as a single assembly.

#### [0081] Light Source

[0082] The light source **216** will now be described with reference to **FIGS. 12-15**. **FIG. 12** shows a perspective view of the light source **216**. Since the crystallization of substances is often highly sensitive to temperature changes, the light source **216** is preferably configured to minimize the amount of heat transferred to the sample plate **212**, e.g., by isolating and removing heat generated by the electronics **1408** and illuminators **1402** (see **FIG. 14B**).

#### [0083] Housing

[0084] With reference to **FIGS. 12, 14B and 15**, the light source **216** includes a housing **1202** adapted to store one or

more illuminators **1402** (see **FIGS. 14B and 15**), cooling elements **1404**, heat reflecting glass **1406**, light diffuser plate **1206**, and corresponding electronics **1405** and **1408**. In one embodiment, the housing **1202** consists of a plurality of walls that serve as structural support for the internal components and that substantially isolate the internal components from the external environment. The housing **1202** can be constructed of a variety of materials including, but not limited to, stainless steel, aluminum, and hard plastics. A material with a low coefficient of heat transfer is preferred so as to substantially keep heat generated within the housing **1202** from reaching the outside through the walls of the housing **1202**. However, depending on the application, use of metals is appropriate when cooling elements **1404** are provided. In some embodiments, one or more of the internal surfaces of the walls of the housing **1202** may be coated with a suitable material that absorbs or reflects various types of radiation and prevents them from reaching the outside of the housing **1202**.

[0085] In the embodiment of the light source **216** shown in **FIGS. 12-14B**, the top wall **1204A** of the housing **1202** has an opening to receive and support a light diffuser plate **1206**. The plate **1206** serves to diffuse light from the illuminators **1402** onto the sample plate **212**. The plate **1206** may be, for example, a sheet of translucent plastic. In one embodiment, inside the housing **1202** and adjacent and below the plate **1206**, a heat reflecting glass ("hot mirror") **1406** (see **FIG. 14B**) is provided. The heat reflecting glass **1406** prevents most infra-red energy from exiting the housing **1202**.

[0086] The wall **1204B** of the housing **1202** may be provided with a plurality of orifices **1208** that allows a cooling element **1404**, such as fan, to draw air into the housing **1202** for cooling the internal components. A wall **1204C** (see **FIG. 14B**) of the housing **1202** can be fitted with an opening **1410** for receiving a duct that guides forced air out of the housing **1202**. A wall **1204D** (see **FIG. 13**) of the housing **1202** can be fitted with a power plug **1208** and a communications port **1302**. The housing **1202** is preferably adapted to isolate an operator of the imaging system **200** from high voltages that may be used to fire the illuminators **1402**.

[0087] Of course, the housing **1202** may be configured in a variety of ways not limited to that detailed above. For example, the ventilation openings **1208** on wall **1204B** may be replaced by one or more fans built into the wall **1204B** or the wall **1204E**. Moreover, depending on the specific location of the light source **216** in any given application of the imaging system **200**, the ventilation openings **1208** may be located on the bottom wall (not shown) of the housing **1202**, for example.

#### [0088] Illuminators

[0089] With reference to **FIGS. 14B and 15**, the light source **216** includes one or more illuminators **1402** that generate light rays. The illuminators **1402** may be various types, for example, incandescent bulbs, light emitting diodes, or fluorescent tubes of various types including, but not limited to, mercury- or neon-based fluorescent tubes. In one embodiment, the illuminators **1402** are two xenon tubes. Xenon tubes are well known in the relevant technology and are readily available. The xenon tubes **1402** can include borosilicate glass that absorbs ultra-violet radiation. Xenon tubes are preferred because they produce sufficient light to



allow use of a CMOS camera **214** in the imaging system **200**. Xenon tubes are also preferred since they provide a broad spectrum of light rays, which enables use of color to enhance detection of crystal growth in the wells of the sample plate **212**.

[0090] The actual dimensions of the illuminators **1402** are chosen to suit the specific application. For example, in the imaging system **200** the xenon tubes **1402** are long enough to cover one dimension of the sample plate **212** so that it is not necessary to move the light source **216** when the lens assembly **230** or sample plate mount **210** are repositioned. As shown in **FIG. 14B**, the illuminators **1402** may be supported on a board **1405**, which may also support electronics for control of the illuminators **1402**.

#### [0091] Off-Axis Lighting

[0092] In one embodiment, two illuminators **1402** are positioned to provide different locations of the illumination source, e.g., both on-axis and off-axis lighting of the wells in the sample plate **212**. As used here, the imaging axis of the lens assembly means the principal axes of the lens assembly. For example, first and second xenon tubes **1402** can be positioned, respectively, a first and second distance from the imaging axis of the lens assembly **230**. Typically, the first and second distances are substantially equal in length, and the first xenon tube is positioned opposite the imaging axis from the second xenon tube.

[0093] In one embodiment, the xenon tubes **1402** are mounted about an inch on either side of the area directly under the lens assembly **230**. This configuration allows the use of an indirect lighting effect when only one xenon tube is fired. That is, when two xenon tubes are positioned off the imaging axis, the controllers and logic **110** or **160** can control the tubes to provide on-axis or off-axis illumination of the sample plate **212**. One xenon tube can be fired to provide off-axis illumination of the sample plate **212**. When the two xenon tubes are fired simultaneously a more conventional backlit scene is obtained. In some applications, off-axis illumination is preferred because it produces shadows on small objects in a sample droplet stored in a well of the sample plate **212**. The shadows caused by off-axis lighting enhance the ability of the controllers and logic **110** or **160**, or an operator, to detect objects in the sample.

[0094] In one embodiment, for example the imaging system **150** shown in **FIG. 1B**, the controllers and logic **160** control the assembly **155** to capture two images of a droplet in a well plate of the sample plate **212**. The imaging system **150** captures one image with the light source **216** lighting the sample with a first xenon tube. The imaging system **150** captures a second image with the light source **216** lighting the sample with the second xenon tube. The controllers and logic **160** can then combine the data from both images and perform an analysis based on the combined data. This results in enhanced characterization of the sample since the combination of the images typically provides more information about crystallization of the sample than a single image acquired with standard back lighting of the scene.

#### [0095] Filters

[0096] In one embodiment, a source filter **270** (**FIG. 2**) may be inserted in a filter slot **272** so that the filter **270** is interposed between the light source **216** and the sample plate **212**. The various filters **270** may be inserted and removed

from the filter slot **272** by a plate handler. Thus, the filter **270** may be automatically removed, or exchanged with another filter, by the imaging system **200**. The source filter **270** may be any type of filter, such as a wavelength specific filter (e.g. red, blue, yellow, etc.) or a polarization filter.

#### [0097] Flash Mode

[0098] In one embodiment of the imaging system **200**, the light source **216** includes one or more illuminators **1402** (e.g., fluorescent tubes) adapted to provide flash lighting. That is the illuminators **1402** are controlled to illuminate only momentarily the sample plate **212** as the digital camera **214** captures an image of a well in the sample plate **212**. This arrangement provides benefits over known devices in which illuminators remain in the on-position throughout the entire time that the sample plate **212** is handled by an imaging system. In the imaging system **200**, since the illuminators **1402** are turned on for only a fraction of a second per image, very little heat radiation is transferred to the wells of the sample plate **212**. Hence, one benefit of this configuration is that the imaging system **200** can provide high illumination levels for the camera **214** while minimizing energy or radiation transfer to the samples in the sample plate **210**. An exemplary control circuit **1600** that provides controlled flash lighting is described below with reference to **FIG. 16**.

#### [0099] Flash Lighting Circuitry

[0100] **FIG. 16** is a functional block diagram of an illumination duration ("flash") control circuit **1600** for an illuminator **1402**. Although only one illuminator **1402** and control circuit **1600** is shown, multiple illuminators **1402** can be used and independently controlled using additional control circuits **1600**. The illuminator **1402** can be, for example, a xenon tube having a length greater than the maximum width of the sample plate **212** to be used in the imaging system **100**, **150**, or **200**. By having such a dimension, the illuminator **1402** can be located underneath and along one axis of the sample plate **212** to illuminate all the wells in one row or column of the sample plate **212** without repositioning the illuminator **1402**.

[0101] A first end of the illuminator **1402** is connected to a first capacitor **1602** and a first resistor **1604**. The opposite end of the first resistor **1604** is connected to a power supply **1606**. The power supply **1606** may be controlled by a dedicated RS232 line, for example. The opposite or second end of the first capacitor **1602** that is not connected to the illuminator **1402** is connected to ground or a voltage common.

[0102] The second end of the illuminator **1402** is connected to the anode of a first silicon controlled rectifier ("SCR") **1607** and a first terminal of a second capacitor **1608**, respectively. An SCR is a solid state switching device that can provide fast, variable proportional control of electric power. A resistor **1620** is connected between the first terminal of the second capacitor and the cathode of a second SCR **1610**. The second terminal of the second capacitor **1608** is connected to an anode of the second SCR **1610**. The cathode of the first SCR **1607** is connected to the ground or voltage common potential. The cathode of the second SCR **1610** is connected to the cathode of the first SCR **1607** and is similarly connected to ground or the voltage common potential. The anode of the second SCR **1610** is also connected to a second resistor **1614** that connects the anode of the second SCR **1610** to the power supply **1606**.



[0103] A trigger **1612** of the illuminator **1402** is connected to the gate of the first SCR **1607** so that both can be triggered simultaneously. This common connection controls the trigger **1612** of the illuminator **1402** and the start of illumination. The gate of the second SCR **1610** controls a stop or end of illumination.

[0104] The duration of illumination provided by the illuminator **1402** can be controlled as follows. Initially, the first and second SCRs **1607** and **1610**, respectively, are not conducting. The first capacitor **1602** is charged up to the level of the voltage of the power supply **1606** using the first resistor **1604**. The power supply **1606** can, for example, charge the first capacitor to 300 volts or more.

[0105] The size of the first capacitor **1602** relates to the amount of energy that can be transferred to the illuminator **1402**. The illuminator **1402** provides an illumination based in part on the amount of energy provided by the first capacitor **1602**. The first capacitor **1602** can be one capacitor or a bank of capacitors. The first capacitor **1602** can be, for example, a 600° F. capacitor.

[0106] The size of the resistors **1620** and **1614** are determined in part by the desired voltage rise time on the second capacitor **1608**. Smaller resistors **1620** and **1614** allow the second capacitor **1608** to charge quickly. However, the second SCR **1610** can inadvertently trigger if the voltage impulse at its anode is too great. Thus, the value of the resistors **1620** and **1614** are typically chosen to allow the second capacitor **1608** to recharge before the next image flash trigger, but not to recharge so quickly as to inadvertently trigger conduction in the second SCR **1610**.

[0107] The resistor **1620** provides an electrical path from the anode of the first SCR **1607** to ground or voltage common to allow the second capacitor **1608** to charge.

[0108] The illuminator **1402** is ready to trigger once the first capacitor **1602** is charged. The second capacitor **1608** is charged by the power supply **1606** through the second resistor **1614** concurrent with the charging of the first capacitor **1602**. The second capacitor **1608** is chosen to be large enough to generate a current potential that shuts off the first SCR **1607** and, thus, to terminate illumination by the illuminator **1402**. The second capacitor **1608** can be a single capacitor or can be a bank of capacitors. The second capacitor **1608** can be, for example, a 20  $\mu$ F capacitor.

[0109] After the first and second capacitors **1602** and **1608** have been charged, the duration of illumination can be controlled. The illuminator **1402** initially illuminates when the trigger signal is provided to the control of the illuminator **1402** and the gate of the first SCR **1607**. The illuminator **1402** can include a triggering circuit that triggers the illuminator **1402** in response to a logic signal. If the illuminator **1402** does not include this circuit, an external triggering circuit can be included.

[0110] The first SCR **1607** conducts in response to the trigger signal. The first SCR **1607** then continues to conduct even in the absence of a gate signal. The first SCR **1607** can be shut off by interrupting the current through the SCR or by reducing the voltage drop across the first SCR **1607** to below the forward voltage of the device.

[0111] The second SCR **1610** is controlled by a stop signal generator **1616** to connect the second capacitor **1608** in

parallel with the first SCR **1607**. However, the second capacitor **1608** is charged in opposite polarity to the voltage drop across the first SCR **1607**. Thus, when the second SCR **1610** initially conducts, the voltage from the second capacitor **1608** is placed in opposite polarity across the first SCR **1607** thereby shutting off the first SCR **1607**.

[0112] After the first SCR **1607** is triggered by a gate signal and begins to conduct, the second end of the illuminator **1402** and the first terminal of the second capacitor **1608** are pulled to ground via the first SCR **1607**. The illuminator **1402** then illuminates in response to the current flowing through the illuminator **1402**. The second SCR **1610** controls turn-off of the illuminator **1402**. The second SCR **1610** begins to conduct when a stop signal is applied to the gate of the second SCR **1610**. This pulls the second terminal of the second capacitor **1608** to ground. Because a capacitor resists instantaneous voltage changes, the voltage across the second capacitor **1608** momentarily causes the voltage at the anode of the first SCR **1607** to be pushed below the ground or voltage common potential. A negative voltage at the anode of the first SCR **1607** results in a loss of current flowing through the first SCR **1607**, which results in shut down of the first SCR **1607**. The second capacitor **1608** discharges almost immediately. The illuminator **1402** shuts off when the first SCR **1607** turns off because there is no longer a current path through the illuminator **1402**.

[0113] Thus, a microprocessor, controller, or microcontroller can be programmed to control the trigger **1612** and stop signal generator **1616**. The processor controls the trigger signal to initiate illumination with the illuminator **1402**. The processor then controls the stop signal to control termination of the illuminator **1402**. The processor can thus control the trigger and stop signals to control the duration of the illumination. The processor can control the duration of the illumination (a "flash") in predetermined intervals or can control the duration of the illumination over a range of time. For example, the processor can control the duration of the flash in microsecond steps across an interval of approximately 20  $\mu$ S-600  $\mu$ S. Alternatively, the processor can control the lower range of the duration of the flash to be 0, 20, 40, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, or 550  $\mu$ S. In another alternative, the processor can control the upper range of the duration of the flash to be 40, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550 or 600  $\mu$ S. In one embodiment, the digital camera **214** issues the signal to turn on the illuminator **1402** so that the "flash" will be in synchronization with the electronic shutter of the digital camera **214**.

[0114] The power supply **1606** can be a controllable high voltage power supply. The microprocessor, controller, or microcontroller can also control the output voltage of the power supply **1606** to further control the illumination provided by the illuminator **1402**. For example, the microprocessor can control the output voltage of the power supply **1606** to vary the illumination provided by the illuminator **1402** for the same illumination duration. Thus, for a given illumination duration, the microprocessor can control the power supply **1606** to a lower output voltage to minimize the illumination. Similarly, for the same illumination duration, the microprocessor can control the power supply **1606** to a higher output voltage, thereby increasing the illumination.

[0115] The microprocessor can control the output voltage of the power supply **1606** over a range of, for example,



180-300 volts. The illuminator **1402** may not consistently illuminate for voltages below 180 volts when the illuminator **1402** is a xenon flash tube. The microprocessor can control the output voltage of the power supply **1606** using a digital control word. Thus, the microcontroller can control the output voltage of the power supply **1606** in steps determined in part by the number of bits in the control word and the tunable range of the power supply **1606**. The microcontroller can, for example provide a 10-bit control word, an 8-bit control word, a 6-bit control word, a 4-bit control word, or a 2-bit control word. Alternatively, the power supply **1606** output voltage can be continuously variable over a predetermined range.

[0116] Thus, the microcontroller can control a level of illumination by controlling the illumination duration, the power supply **1606** output voltage, or a combination of the two. The microprocessor's ability to control the combination of the two permits a wider range of brightness outputs than if only one parameter were controllable. The microprocessors ability to control both illumination duration and power supply **1606** output voltage is advantageous for different lens zoom conditions. When magnification is low, such as when the lens is zoomed out, a relatively small amount of light is required. When magnification is high, a relatively large amount of light is required to capture an image. Use of filters and varying apertures may also be used to adjust the amount of light from the light source.

[0117] Operation

[0118] The imaging system **200** includes software modules that control and direct the lens assembly **230** to perform the following functions. In one embodiment, the imaging system **200** is configured to automatically control the brightness of the image. For example, after the camera **214** captures an image of a well of the sample plate **212**, the software determines whether the brightness is within predetermined thresholds. If the brightness does not fall within the thresholds, the controllers and logic of the imaging system **200** iteratively adjust the illumination intensity of the illuminators **1402** to adjust the brightness of the images until the brightness falls within the thresholds. In some embodiments, the brightness of the image may be evaluated based on a predetermined region (or set of pixels) of the image captured.

[0119] The brightness of the illuminators **1402** may be adjusted when capturing a plurality of images of the same sample droplet. In one embodiment, for example, the imaging system **150** shown in FIG. 1B, the controllers and logic **160** control the assembly **155** to capture two images of a droplet in a well plate of the sample plate **212**. The imaging system **150** captures one image with the light source **216** lighting the sample with a first brightness level. The imaging system **150** captures a second image with the light source **216** lighting the sample with a second brightness level. In one embodiment the controllers and logic **160** can then combine the data from both images and perform an analysis based on the combined data, which may result in enhanced characterization of the sample. In some embodiments, the brightness used for the second image may be logically controlled based on analyzing the brightness of the first image, determining if a lighter or darker second image may result in enhanced characterization of the sample, and adjusting the light source **216** to light the sample accordingly.

[0120] The imaging system **200** can also be configured with software to automatically focus the image. An exemplary autofocus routine is as follows. Once the lens assembly **230** is positioned over a sample of the sample plate **210**, the objective lens **231** is moved along its imaging axis to a predetermined starting position. The camera **214** then acquires an image of the sample and/or well at that focus position. In one embodiment, the software obtains a "focus score." This may be done, for example, by examining the brightness values of a set of pixels (e.g., a 500x3 pixel area) in the captured image, applying a low pass filter, and computing the sum of the squares of the differences in brightness of adjacent pixels for the set of pixels. The position and focus score data points are stored in an array. The objective lens **231** is moved to the next predetermined incremental position on its imaging axis, and the process of acquiring an image, computing the focus score, and storing the position and focus score values is repeated. This process continues until the objective lens **231** has been moved to all the predetermined or desired positions, e.g., until it reaches a predetermined end position by incrementally moving in a predetermined step size from the starting position. In one embodiment, the step size depends at least in part upon a predetermined maximum number of images to be acquired during the autofocus routine.

[0121] Next, the software searches the lens position/focus score array to identify the lens position with the best focus score. In one embodiment, the software then proceeds to compute the lens positions that are midway from the best focus score position to positions adjacent to it in the array. That is, the software examines the array of positions already imaged, finds the nearest position greater than the lens position associated with the best focus score, and calculates a "midpoint" position between them. A similar process is performed with regard to the nearest lens position that is less than the best focus score position. The software then acquires images at the midpoint positions and obtains corresponding focus scores. The software once again evaluates the array to identify the image with the best focus score, using a step size that is, say, one-half of the initial step size. These tasks are repeated until, for example, a maximum number of images acquired during autofocus, or a minimum step size, has been reached.

[0122] In some embodiments, the imaging system **200** performs the processes of autofocusing and automatically adjusting the brightness, as described above, for each well sample of a sample plate **212** received by the imaging system **200**. After the desired brightness and focus are set, the imaging system **200** then captures an image and stores it in, for example, the data storage **190**. In one embodiment, the automatically determined brightness and focus are also stored for each sample. In another embodiment, the software of the imaging system **200** calculates and stores a value associated with the mean of the brightness and focus positions for the aggregate of wells samples of the first plate. This value is then associated with each of the position/focus score data points in the array. Subsequent plates are examined using the mean brightness and focus as initial imaging values.

[0123] The imaging system **200** may also include additional functionality related to automatically finding the edges of a droplet in a well of a sample plate **212**. In one embodiment, after the edges of the drop have been found,



the imaging system **200** finds the centroid of the droplet and moves the lens assembly **230** to the centroid. The imaging system **200** then determines the magnification required to image substantially only that area corresponding to the droplet, adjusts the zoom, and acquires the image.

[0124] In another embodiment, the imaging system **200** may be configured to perform automatic adjustment of aperture. In this embodiment, the imaging system **200** receives settings for either maximum image resolution or maximum depth of field. The imaging system **200** then determines the corresponding aperture by, for example, looking one or more tables having values correlating aperture with maximum resolution and/or maximum depth of field. Of course, magnification data may be part of these tables.

[0125] In yet another embodiment, the imaging system **200** may be configured to perform automatic zoom of a substance in a sample stored in a well of the sample plate **212**. In one embodiment, for example, the imaging system identifies a “crystal-like object” in the sample, calculates its centroid, moves the lens assembly **230** and digital camera **214** to the centroid, adjusts the zoom level, and captures an image of the “crystal-like object.” In another embodiment, the imaging system **200** can be configured to capture an image of a sample or a crystal-like object, perform image analysis of the image, adjust imaging parameters (e.g., focus, depth of field, aperture, zoom, illumination filtering, image filtering, brightness, etc.) and retake an image of the sample or crystal-like object. The imaging system **200** can perform this process iteratively until predetermined thresholds (e.g., contrast, edge detection, etc.) are met. In some embodiments, the images captured in an iterative process can be either analyzed individually, or can be combined with other images and the resulting image analyzed.

[0126] Thus, in one embodiment of the imaging system **200**, the imaging system receives a sample plate **212** and for each well sample performs the following functions including, automatic adjustment of brightness and aperture, auto-focus, automatic detection of the sample droplet, and acquisition and storage of images. The imaging system **200** stores the aperture, brightness, focus position, drop position and/or size. The imaging system **200** may then use mean values of these factors as initial imaging settings for subsequent plates.

[0127] To increase the amount of data available for analysis of the sample, or crystal detection, in some embodiments an illumination source filter **270** (FIG. 2) may be inserted in the filter slot **272** so that the filter **270** is (not shown) may be interposed between the light source **216** and the sample plate **212**. In one embodiment the various filters **270** may be inserted and removed from the filter slot **272** by a plate handler. Thus, the filter **270** may be automatically removed or exchanged by the imaging system **200**. Alternatively, or additionally, an image filter (such as those that may be placed in the photo-filter carriage **237**) may be interposed between the sample droplet in the sample plate **212** and the objective lens **231**. In one embodiment, the image filter includes a polarization filter that provides a variable amount of polarization on the light incident on the objective lens **231**. The use of these filters can be automatically controlled by imaging software routines and/or determined by operator defined variables.

[0128] The motorized control of aperture, focus, and zoom of the lens assembly **230** in conjunction with remote control of the light source **216** (e.g., brightness and direction of illumination) allows dynamic optimization of contrast, field of view, depth of field, and resolution.

[0129] Imaging System Integrated with Automated Sample Analysis System

[0130] FIG. 17 depicts a functional block diagram of an automated sample analysis system **1700** having an imaging system **100**, **150**, or **200**. The system **1700** includes controllers and logic **1760** for controlling various subsystems housed in a cabinet **1702**. The system **1700** can further include a shelf access door **1712** for allowing access to a removable shelf system **1720** and/or a stationary shelf system **1722**. In one embodiment, a removable shelf access door **1710** can be provided. The system **1700** can include a transport assembly **1730** that can consist of a plate handler **1732**, an elevator assembly **1734**, and a rotatable platform **1736**. The system **1700** can further include an environmental control subsystem **1765** that employs a refrigeration unit **1762** and/or a heater **1764**.

[0131] In one embodiment, the system **1700** also includes an imaging system **200** as has been described above. The imaging system **200**, having subcomponents **210**, **214**, **216**, **218**, **220**, and **230**, which are fully detailed above with reference to FIGS. 2-16, can be housed in the cabinet **1702**. This arrangement ensures that the samples in the sample plates remain at all times within the confines of a controlled environment. That is, once a sample plate is stored in the cabinet **1702**, it is unnecessary to expose the sample plate to the environment external to the cabinet since the system **1700** is capable of automatically (i.e., without operator intervention) carry out the imaging of the sample within the cabinet **1702**.

[0132] Embodiments of an automated sample analysis system **1700** having an imaging system in accordance with the invention are described in the related United States Provisional Patent Application entitled “AUTOMATED SAMPLE ANALYSIS SYSTEM AND METHOD,” having U.S. Patent Application No. 60/444,519, which is referenced above.

[0133] Sample Analysis System

[0134] FIG. 18 depicts a block diagram of an imaging and analysis system **1800**, according to one embodiment of the invention. The imaging system **1805** can be an imaging system **100**, **150**, or **200** as described above, or another suitable imaging system that provides similar functionality to the imaging systems described herein. The system **1800** includes an imaging system controller **1820** that provides logical control of the imaging system **1805** to, for example, direct the imaging system **1805** to image a particular sample on a particular sample plate **212**, all the samples on the sample plate **212**, or image a subset of the samples. The imaging controller **1820** may also control the imaging parameters used by the imaging system **1805**. Such imaging parameters can include, for example, focus, depth of field, aperture, zoom, illumination filtering, image filtering and brightness.

[0135] The system **1800** also includes an image storage device **1810** that stores images of samples captured by the imaging system **1805**. The image storage device **1810** can be



any suitable computer accessible storage medium capable of storing digital images, e.g., a random access memory (RAM), hard disk floppy disk, optical disk, compact disks, or magnetic tape. The system **1800** shows the image storage device **1810** separate from the imaging system **1805**. In some embodiments, the image storage device **1810** can be included in the imaging system **1805**, or it may be included in a system that may also include an image analyzer **1815**, the imaging system controller **1820**, or a scheduler **1825**. In one embodiment, a computer includes all the control, scheduling, analysis and imaging software for the system **1800**. Alternatively, the software for the system **1800** may reside and run on a plurality of computers that are in communication with each other. In some embodiments, the imaging system **1805** may be configured to provide captured images directly to the image analyzer **1815**, or it may be configured to typically store images on the image storage device **1810** and provide images to the image analyzer **1815** as directed by the imaging system controller **1820**.

[0136] The scheduler **1825** communicates with the image analyzer **1815** and the imaging system controller **1820** to control the analysis and imaging of samples based on user provided input. For example, the scheduler can schedule the imaging of a particular droplet or a plurality of droplets on a sample plate, and coordinate the imaging of said droplet or plurality of droplets with its subsequent analysis. The scheduler **1825** can use a database **1830** to store information relating to scheduling the images and image specific information, for example, the size of pixels in each of the stored images, in a suitable format for quick retrieval. Knowing the pixel size can allow the analyzer **1815** to reduce sampling to an appropriate density and size for particular objects in the image. The information in the database **1830** can be available with each request to process an image. The database **1830** can reside on the same computer as the scheduler **1825** or on a separate computing device.

[0137] The scheduler **1825** provides an analysis request to the image analyzer **1815**. According to one embodiment, the analysis request includes an image list, including the resolution of each image and the absolute X,Y location of its center. The image list typically contains only one image but may contain a plurality of images. The analysis request can also contain an analysis method including a list of parameters that specify options controlling how to analyze the image(s) and what to report. Additionally, the analysis request can include the Uniform Resource Locator ("URL") of a definition file **1835**, i.e., an electronic address that may be on the Internet, such as an ftp site, gopher server, or Web page. The definition file **1835** defines parameters used by the image analyzer **1815**, e.g., neural network dimensions, weights and training resolution (e.g., pixel granularity, or the spacing between pixels, of images used to train the neural network). The definition file **1835** may be a single file or a plurality of files, but will be referred to hereinafter in the singular.

[0138] The image analyzer **1815** also receives an analysis method file(s) **1840**. The analysis method file may be a single file or a plurality of files, but will be referred to hereinafter in the singular. The analysis method file **1840** includes parameters that can be used by the various image analysis modules contained in the image analyzer **1815**, e.g., a content analysis module **1930**, a notable regions module **1935**, and a crystal object analysis module **1940** (FIG. 19),

described below, according to one embodiment. The image analyzer **1815** can also include functionality that determines the content of an image in terms of objects and/or regions of, for example, crystals or precipitate, or clear regions, that is, regions that do not show any features. The image analyzer **1835** includes a neural network to identify features, e.g., crystals, precipitate, and edges, that are depicted in the image, according to one embodiment. Preferably, the image analyzer **1815** is configured to identify objects and regions of interest in an image quickly enough to allow the system **1800** to re-image specific objects or regions, if desired, while the corresponding sample plate is still in the imaging system **1805**.

[0139] The image analyzer **1815** provides an analysis response to the scheduler **1825**. The analysis response, described in further detail below, typically includes the parameters used to for the analysis and the results of the particular analysis performed, e.g., the count of crystal, precipitate, clear and edge samples, regions of crystals, and/or a list and description of objects found in the image.

[0140] The analysis results can be reviewed using an output display **1845** that can be co-located with the scheduler or at a remote location. The output displays may be coupled to the system **1800** via a web server, or via a LAN or other small network topology. Embodiments of a remote output display in accordance with the invention are described in related United States Provisional Patent Application entitled "REMOTE CONTROL OF AUTOMATED LABS," having Application No. 60/444,585.

#### [0141] Illustrative Embodiment

[0142] A computer containing analysis and control modules, and methods related to controlling an imaging and analysis system are illustrated and described with reference to FIGS. 19-22, according to one embodiment of the invention. FIG. 19 depicts a computer **1900** that includes a processor **1905** in communication with memory **1910**, e.g., a hard disk and/or random access memory (RAM). The processor **1905** is also in communication with an image analysis module **1960** that can include various modules configured to perform the functionality of the image analyzer **1815** (FIG. 18) described herein.

[0143] The computer **1900** may contain conventional computer electronics that are not shown, including a communications bus, a power supply, data storage devices, and various interfaces and drive electronics. Although not shown in FIG. 19, it is contemplated that in some embodiments, the computer **1900** may include a video display (e.g., monitor), a keyboard, a mouse, loudspeakers or a microphone, a printer, devices allowing the use of removable media including, but not limited to, magnetic tapes and magnetic and optical disks, and interface devices that allow the computer **1900** to communicate with another computer, including but not limited to a computer network, a LAN, an intranet, or a WAN, e.g., the Internet.

[0144] The computer **1900** is in communication with an imaging storage device, for example, image storage device **1810** (FIG. 18), and is configured to receive an image of a sample from the storage device and determine the contents of the sample, using one or more analysis processes. The computer **1900** can be co-located with the image storage device, located near the image storage device, e.g., in the



same building, or geographically separated from the image storage device. The computer **1900** can receive the image from the image storage device via, e.g., a direct electronic connection or through a network connection, including a local area network, or a wide area network, including the Internet. It is also contemplated the computer **1900** can receive the image via a suitable type of removable media, e.g., a 3.5" floppy disk, compact disc, ZIP drive, magnetic tape, etc.

[0145] It is contemplated the computer **1900** can be implemented with a wide range of computer platforms using conventional general purpose single chip or multichip microprocessors, digital signal processors, embedded microprocessors, microcontrollers and the like. The computer **1900** can operate independently, or as part of a computing system. The computer **1900** may include stand-alone computers as well personal computers, workstations, servers, clients, mini-computers, main-frame computers, laptop computers, or a network of individual computers. The configuration of the computer **1900** may be based, for example, on Intel Corporation's family of microprocessors, such as the PENTIUM family and Microsoft Corporation's WINDOWS operating systems such as WINDOWS NT, WINDOWS 2000, or WINDOWS XP.

[0146] The computer **1900** includes one or more modules or subsystems that incorporate the analysis processes described herein. As can be appreciated by a skilled technologist, each module can be implemented in hardware or software, or a combination thereof, and comprise various subroutines, procedures, definitional statements, and macros that perform certain tasks. For example, in a software implementation, all the modules are typically separately compiled and linked into a single executable program. The processes performed by each module may be arbitrarily redistributed to one of the other modules, combined together with other processes in a single module, or made available in, for example, a shareable dynamic link library. A module may be configured to reside on the addressable storage medium and configured to execute on one or more processors. Thus, a module may include, by way of example, other subsystems, components, such as software components, object-oriented software components, class components and task components, processes, functions, attributes, procedures, subroutines, segments of program code, drivers, firmware, microcode, circuitry, data, databases, data structures, tables, arrays, and variables. It is also contemplated that the computer **1900** may be implemented with a wide range of operating systems such as Unix, Linux, Microsoft DOS, Macintosh OS, OS/2 and the like.

[0147] The analysis module **1960** can include a pre-processing module **1925** that can filter the received image prior to further processing. The image may be filtered to remove "noise" such as speckles, high frequency noise or low frequency noise that may have been introduced by any of the preceding steps including the imaging step. Filtering methods to remove high frequency or low frequency noise are well known in image processing, and many different methods may be used to achieve suitable results. For example, according to one embodiment in a filtering procedure that removes speckle, for each pixel, the mean and standard deviation of every other pixel along the perimeter of a 5x5 pixel area centered on a pixel are computed. If the center pixel varies by more than a threshold multiplied by

the standard deviation, then it is replaced by the mean value. Then the slope of the 5x5 image pixel intensities is calculated and the center pixel is replaced by the mean value of pixels interpolated on a line across the calculated slope.

[0148] The analysis module **1960** also includes one or more modules that perform image analysis to determine information about the sample contents, including content analysis module **1930**, notable regions analysis module **1935**, and crystal object analysis module **1940**. The content analysis module **1930** determines the count of crystal, precipitate, clear and edge pixels in the image, and can be optionally enabled to operate only inside a specific region of the sample. The notable regions analysis module **1935** determines a list of regions of a specified pixel type, e.g., crystal, precipitate, clear and edge pixels. The crystal object analysis module **1940** determines objects containing crystal pixels that meet certain criteria, for example, size, area, or density.

[0149] FIG. 19 also shows analysis module **1960** includes a report inner/outer non-clear ratio module **1945** that determines the ratio of non-clear pixel density inside a sample region over non-clear pixel density outside a sample region. The analysis module **1960** also includes a graphical output analysis module **1950** that generate a color-coded image depicting each of the various features found in a sample image in a specified color. These modules are further described hereinbelow. Other analysis modules **1955** that incorporate different image analysis processes may also be included in the analysis module **1960**. In one example, an analysis module **1955** can analyze the change in two or more images of the same sample taken at two different times. The analysis module **1955** can receive the count of pixels that are classified as crystal, precipitate, clear or edge pixels in an image of a particular region of a sample at a time T1 and save the count information with a reference to the region of a sample imaged. When the same region of a sample is re-imaged at a later time T2, the analysis module **1955** receives the count of pixels that are classified as crystal, precipitate, clear and edge pixels in the image of the sample region at time T2. The analysis module **1955** can compare the count information from time T1 and T2 to determine if the droplet contains a crystal(s). One analysis method compares the total number of pixels classified as crystal pixels at time T1 and T2 to determine if the sample contains crystal. Another comparison method compares the percentage of crystal pixels at time T1 to the percentage of crystal pixels at time T2. If the count or the percentage of crystal pixels increases beyond a threshold value, the sample will be deemed to contain crystals. The other pixel classifications (e.g., precipitate, clear and edge) can also be compared and evaluated to facilitate the crystal analysis. A time-based comparison method, where the count information is saved for one image and compared to a second subsequent image, can be used with any sample processing algorithm.

[0150] In another example, the analysis module **1955** may analyze a series of two or more images crystal growth using a grid approach. In this analysis method, two images **11** and **12** are divided up into grids, and the corresponding grids in each image are compared for change in the number of crystal, using, for example, the actual number of pixels or the percentage of crystal pixels. The pixel count information can be kept for each image and used to compare to other images taken at a different time. In any of the analysis



methods described herein, the method can include analyzing every pixel, or skipping one or more pixels between the pixels analyzed.

[0151] A scheduler module **1915** and an imaging system controller module **1920** are also included in computer **1900**, according to one embodiment. These modules are configured to include functionality that schedules the imaging of sample plates/droplet samples and subsequent analysis of the images, and controls the imaging system **100**, **150**, **200**, **1805**, as described herein, e.g., for scheduler **1825** and imaging system controller **1820**, respectively.

[0152] The image analysis software package may include support software that performs training and configuring of perception and analysis functionality, e.g., for a neural network. Some of the algorithms included in the image analysis software modules may use stochastic processing and may include the use of a pseudo-random number generation to find answers. All such functions can be provided a random number generator seed in request parameters received by the software module. When the analysis modules are properly configured, the same results should be obtained for a given image given the same parameters that affect its algorithms and any pre-processing of the image. The image analysis modules can be configured so that an analysis method using a pseudo-random number does not affect the results of a different analysis method or software module.

[0153] In one embodiment, the image analysis software works with an image size of, for example, 800 by 600 pixels, a zoomed-in resolution of 2,046 pixels/mm (0.5  $\mu\text{m}/\text{pixel}$ ), and a zoomed-out resolution of 186 pixels/mm, (5.4  $\mu\text{m}/\text{pixel}$ ), or 1,024 by 1,024 pixels, a zoomed-in resolution of 2,460 pixels/mm (0.41  $\mu\text{m}/\text{pixel}$ ), and a zoomed-out resolution of 220 pixels/mm, (4.5  $\mu\text{m}/\text{pixel}$ ). The image analysis modules may optionally use the same neural network for both zoomed-in and zoomed-out images, however, quality of the results may suffer if only one neural network is used and it may be advantageous to train multiple neural networks, e.g., one for zoomed-in images and one for zoomed-out images. The image analysis software can also be adaptable to other image sizes and pixel resolutions, however, the training of new neural networks may be necessary in order to suitably process these images. If the resolution of the images vary, each definition file may include its training resolution, that is, the spacing between sampled pixels that was used to train the neural network. This information allows the algorithms to consider how to adapt images of varying resolution for use with the neural networks.

[0154] The analysis module receives an analysis request (**FIG. 18**) containing an image list that includes the images to be analyzed. The analysis request also includes, for each image, its resolution in pixels/mm and the absolute X-Y location of the center of the image. Typically, there is only one image in the image list, however, multi-image methods may also be used. The analysis request also includes an analysis method, which is a collection of parameters that specify options controlling how to analyze the images and what to report. In specifying the analysis method, a URL of the definition file is included. The definition file defines the neural network's dimensions, weights and training resolution, i.e., a pixel granularity of the images that were used to

train the neural network. Examples of the parameters are first described generally below, and then specifically as they relate to the content analysis module **1930**, notable regions analysis module **1935**, and the crystal object analysis module **1940**, according to one embodiment.

[0155] The analysis request may include parameters that specify how a working copy of the image is prepared for all subsequent processing. For example, parameters can include options for a color to grayscale conversion of the image, and resizing of the image using pixel interpolation methods. Also, the parameters may specify the output of an image, for example, they may specify whether and how an image file representing the pixel interpretation should be generated. This generated image file may be visually displayed and further evaluated by a user. The parameters are also used by the analysis modules, e.g., in the content analysis module, the parameters specify whether an image is scanned and analyzed to determine statistics of its contents in terms of crystal, precipitate, clear and edge features. These parameters specify whether crystal-like objects should be searched for and reported. Options may include a scan grid, an ID criteria and the maximum number of objects to find.

[0156] The parameters may also be used by the notable region analysis module **1935** to specify whether notable regions in an image should be reported and, if so, the scan grid in micrometers, the size that is the width times the height in micrometers, the ID criteria, and the quantity of regions to report. The crystal object analysis module **1940** can use the parameters to specify whether effective contiguous subregions of crystals are identified and reported as crystal objects, how this identification should be performed, and the quantity of crystal objects to identify.

[0157] The parameters can also specify whether to report the inner/outer non-clear ratio. If this ratio is to be specified, the output includes a ratio of the non-clear pixel density inside a sample region over the non-clear pixel density outside of the sample region. For example, the ratio would be 3.0 if every 100th pixel inside of a sample region is non-clear and every 300th pixel outside of a sample region is non-clear. According to one embodiment, ratios above 1 billion are truncated to that value.

[0158] Image sampling parameters may include, for example, a color processing parameter which specifies how each pixel is converted to a floating point intensity value, or it may specify the linear grayscale for image conversion. If the image is already grayscale, pixels are converted to black, e.g., 0.0, or to white, e.g., 1.0. If color is selected, the pixels are linearly converted to 0.0 to 1.0 with equal channel weighting for each color. Pixel interpolation parameters may include, for example, no pixel interpolation, that is, only a closest pixel method will be used for pixel interpolation. This is generally the fastest interpolation method but typically results in reduced image quality. Interpolation methods that may be selected include bilinear and cubic spline interpolation, which yield higher quality images but they are more computationally complex and take more time or resources to generate. The re-size parameter includes options of 1:1, that is, the image is not resized, automatic, where the image is resized to match the training resolution using the specified interpolation method, and scale factor, where the image is re-sized using this factor and specified interpolation method.



[0159] The analysis modules **1930**, **1035**, **1940** are configured to receive an analysis request from a scheduler module **1915** and generate a response, as described below. The content analysis module **1930** determines counts of types of pixels in the sample images, e.g., crystal, precipitate, clear and edge pixels, as depicted in the image. In the illustrative embodiment described herein, the content analysis module **1930** is implemented as a neural network.

[0160] The content analysis module **1930** receives a set of parameters that include parameters that indicate whether this module should be enabled, whether the content analysis should take place inside the sample region only or inside and outside the sample region, and the number of pixels to be skipped during the image analysis. If enable is set to NO, no analysis by the content analysis module **1930** is done and nothing is reported. If enable is set to YES, then the content analysis module analyzes the sample image. If the inside-sample-region-only option is set to YES, the edge of the sample region is found first, and the analysis is done only within the sample region edge. If inside-sample-region-only is set to NO, then checking is done inside and outside the sample region. A process for identifying the edge of a sample region is described hereinbelow in reference to **FIG. 20**, according to one embodiment. If the number of pixels to be skipped is set to 0, all the pixels in the image will be used. If the number of pixels to be skipped is set to 1, every other pixel in the image will be used for the content analysis, if 2, every third pixel will be used, etc. The default parameter for skipped pixels is typically set to 0.

[0161] The response of the content analysis module **1930** includes an “echo” of the parameters used during the content analysis processing, and the counts of each pixel type pixels of crystal, precipitate, clear and edge samples found in the image. If the inside-sample-region-only option is enabled, the edge count can be used to assess how well the edge of the sample region was found. If it is not enabled, the edge count may be ignored.

[0162] The notable region analysis module **1935** processes an image and determines regions of a specified size that include the minimum levels of crystal, precipitate or non-clear pixels. The request parameters for the notable region analysis module **1935** can include an enable parameter which is set to either “YES” or “NO” that determines if notable region analysis should be performed and reported. The request parameters can also include a region size or area that is used to determine the size of the smallest region the notable region analysis module will identify. A skip-pixel parameter can be included to control the number of pixels that will be skipped during processing, where “0” means to check all of the pixels, “1” means to sample every other pixel, that is, sample the pixels with one unsampled pixel between them, etc. Typically, the default value for skip-pixel is “0.”

[0163] The request parameters can also include the maximum number of regions to report and the minimum percentages of crystal pixels, precipitate pixels and non-clear pixels to report. Typically, pixels determined to be edge-type pixels are ignored. The notable region analysis module **1935** can be configured to identify regions with the highest percentage of each specified pixel type. If the regions contain less than the minimum percentage of pixels, it is not saved and the search for regions ends. Regions typically do

not go outside of the input image. Newly found regions generally do not overlap existing regions. The report of results from the notable regions analysis module includes all the request parameters and a list of the regions identified. The results for each region can include its absolute position, size, the number of crystal pixels and the total pixels sampled, not including edge pixels.

[0164] The crystal object analysis module **1940** identifies small regions in the image that are rich in crystal pixels. The small regions, or objects, comprise one or more “cells.” The request parameters for the crystal object analysis module can include an enablement parameter which determines if this analysis should be performed and reported. The request parameters also include a skip-pixels parameter that operates as previously described above, parameters that control the size of the cells identified, for example, a cell-minimum-size parameter to control the smallest width or height of a cell, a cell minimum area which indicates the smallest overall area of a cell, a cell minimum density parameter which indicates the proportion from 0 to 1 of crystal pixels the cell must contain in order to be reported, and an object-minimum-size parameter which indicates one or more dimensions that the overall object must achieve in order to be reported. The request parameters can also include a pseudo-random generator seed which is used for the crystal object analysis stochastic processing. The crystal object analysis module **1940** typically includes the limitation that the center of a cell cannot be inside another cell. Identified cells that touch are grouped and identified as a single crystal object, and the largest overall dimension of the crystal object is computed. If the largest overall dimension is less than the minimum size, the object is discarded. The crystal object analysis processing can also compute an object area as the sum of the cell density times the cell area, and further compute the object centroid. The results from the crystal object analysis module **1940** can include all the request parameters provided to the module, a list of objects identified and their description. The list is sorted in descending order by an object’s area. Each object description includes the object area ( $\mu\text{m}^2$ ), the centroid (X, Y in  $\mu\text{m}$ ) and a list of cells that make up each object. Each cell is described with its absolute position in size ( $\mu\text{m}$ ), crystal pixel count and total pixel count.

[0165] The graphical output module **1950** generates a representation of the analyzed image which can be displayed and further analyzed. For example, grayscale and/or color coding pixel characteristics may be adjusted by the graphical output module **1950**. The analysis request for the graphical output module **1950** includes an image path parameter that defines where the image to be analyzed is found. If the image path parameter is empty, no further processing is done. A base value parameter indicates whether a “base image,” i.e., an image used to generate the representation of the analyzed image, is either black, gray or white. If the base value is gray, the base image begins as a grayscale rendition of the resampled image. Otherwise, the base image begins as a white or black image, as indicated by the base value. The parameters include a gray “min” value and a gray “max” value, which are typically from 0 to 1, and specify the linear grayscale compression. For example, adjusting the gray min or max values can control the color coding contrast or flatten the image, and they are typically set to defaults of 0 for the gray min and 0.75 for the gray max.



[0166] An opaque parameter indicates whether a pixel in the base image should be replaced with the color coding associated with the particular type of corresponding pixel in the analyzed image. For example, if the opaque parameter is set to YES or the base parameter equals black or white, the appropriate color coding replaces the pixel. If the opaque parameter is set to no, the color for a base image pixel is generated by OR'ing the color with the corresponding pixel in the analyzed image. A crystal color parameter provided in the analysis request sets the color coding value for pixels identified as crystals, a precipitate color parameter sets the color coding for precipitate pixels, and an edge color sets the color coding for pixels identified as edges. For example, the default values for the crystal color parameter may be blue, the precipitate color parameter may be green and the edge color parameter may be red. The graphical output module 1950 writes the color coded image file to the image path specified in the request parameters, unless the path parameter is empty or invalid. The generated color-coded image file typically does not contain region annotations, but annotations can be superimposed on the image file by another process, if desired. The graphical output module 1950 provides an analysis report to the scheduler module 1915 that includes the request parameters that were used to produce the color coded image file.

[0167] In one embodiment, the analysis modules 1930, 1935, 1940 can function as service functions that are capable of quickly identifying objects and/or regions within an image, so that a scheduler module 1915 can dispatch control information to the imaging controller module 1920, which in turn directs the imaging system to re-image specific areas of a droplet using at least one different imaging parameter, (e.g., the magnification or zoom level may be different, a different configuration of lighting, such as, off-axis lighting may be used, etc.), while the sample plate containing the sample just analyzed is in the imaging device. In one embodiment, an analysis module 1960 can analyze at least 10,000 images per day under typical conditions, where the images are less than or equal to 1.0 mega pixels, i.e., the equivalent of processing each image in 8.64 seconds, and where one instance of the image analysis software is running on one PC. The analysis module may be packaged and distributed in a Java 2 file. Java message service may be used to receive requests and send the responses from the analysis module(s). Extensible markup language (XML) may also be used for the analysis requests and responses.

[0168] Test images are used with training software to train the neural networks to analyze crystal growth in sample droplets. As general software implementation of a neural network is well known in the art, only the training of a neural network is described, according to one embodiment of the invention. Training software allows the user to create, open, display, edit and save lists of images in training/test set files, and is described herein according to one embodiment of the invention. The test images include identified subimages containing edge, crystal, precipitate and clear pixels within a wide variety of images. For each image, the user can designate "training subimages" as crystal, precipitate, edge or clear. The resolution of the subimages can be user-adjustable. To minimize user fatigue during image designation, the software can include a single-click designation action that efficiently designates the subimages as crystal, precipitate, edge or clear. The images containing the designated training subimages can be saved as a set of training

files. The training software can display training subimages in table form and/or as color-coded markers on an image. Subimages may be moved by either dragging the marker or editing the table. Subimages may also be deleted either from the image or from the table. The training software can be configured to allow a user to define the neural network dimensionality, select a training set file and another file for testing, and perform iterative training and testing using the selected sets of files. Training data, e.g., neural network weights, training and test error, and the number of iterations is saved in a definition file.

[0169] To train the neural network, the intensity levels of pixels in a selected image area, e.g., a subimage, are provided as an input to the neural network. The neural network identifies each pixel as a particular type of pixel, e.g., edge, clear, crystal or clear. The results are compared to what is actually correct, and corresponding error values are calculated. Small adjustments are made to the weights within the neural network based on the error values, and then another test image containing a designated subimage is provided as an input to the neural network. This process is performed for other test images and can be repeated for many thousands of iterations, where each time the weights may be slightly adjusted to provide a more accurate output.

[0170] When the neural network is used for content analysis, an image of a sample droplet is provided as an input to the neural network. The output of the neural network includes a rating for each pixel that indicates a degree of confidence that the pixel depicts each of the different pixel classifications, for example, edge, crystal, precipitate, and clear. The rating is typically between zero and one, where zero indicates the lowest degree of confidence and a 1 indicates the highest degree of confidence. The overall content of an image can be determined counting the number of pixels of each classification by computed as a percentage of the crystal, precipitate, edge and clear pixels contained in the image.

[0171] When considering the content analysis strategy, accuracy of the results is important, but so is the speed of the analysis. Analysis algorithms can allow the user to balance and prioritize the characteristics of speed and quality. For example, one analysis option identifies edges of a drop within the image, and may be used with quick and coarse resolution search parameters to first identify the edge of the drop, and then the interior of the drop may be analyzed with a higher resolution search.

[0172] According to one embodiment of the invention, a supervised learning type of neural network is used to classify the subimages as crystal, precipitate, edge of drop or clear, using the pixel intensity, not the pixel hue. In one embodiment, the entire image is scanned, sampling subimages on a host-specified grid, where the spacing of the grid is in millimeters, not pixels. The resolution of the images is provided as a parameter received from the host. Pie charts can be generated graphically showing the results of the neural network analysis. According to one embodiment, the outputs of a neural network can be summed for each type of object identified and divided by the sum of all the outputs, for example, the results can be A% crystal, B% precipitate, C% clear, where  $A+B+C=100\%$ .

[0173] Each image analysis method file contains neural network definitions, e.g., "dimensions" and "weights." The



method file also includes parameters that specify the analysis options including whether to perform drop edge detection, and if drop edge detection is selected, the sample grid spacing used to find the edges of the drop, and the sample grid spacing to find crystals within the drop. For example, drop edge detection finds the edge of a drop quickly with a relatively coarse grid spacing scan and then use a relatively fine grid spacing scan inside the drop, according to one embodiment. A database can be used to associate the image analysis file with the image analysis results, so that if a better image analysis method is available at a later time, an image may be re-analyzed using the later analysis method.

[0174] The analysis modules can use a neural network to classify the contents of an image. To aid the neural network in the classification process, a fast operator can be used to identify if a pixel has a particular crystal characteristic. One embodiment of an edge detection process is described below and illustrated in FIG. 20A. Color or black and white images of a sample droplet can be generated and used for identifying crystals. At step 2005, the edge detection process 2000 receives the image of a sample that may contain crystals. At step 2010 the process 2000 determines if the image received is a color image. If the image is a color image, it is converted to a grayscale image at step 2015. The image may be filtered at step 2020 to remove minimize undesirable characteristics such as speckle or other types of image “noise” during subsequent processing.

[0175] The edge detection process 2000 uses the gradient of the intensity of the pixels in the image to identify edges. At step 2025, for a plurality of pixels in the image, gradient information is calculated from a 3×3 set of pixels using a calculation based on the best fit of a plane through the image points. The gradient of intensity of the pixel in the center of the 3×3 set of pixels is the direction and magnitude of the maximum slope of the plane. The use of a 3×3 set of pixels helps to eliminate some of the effects of image noise on the process. Gradient information is calculated for selected pixels in the image. All the pixels in the image may be selected, or a subset of the pixels, e.g., an area of interest in the image which may be smaller than the whole image, may be selected. Gradient information is calculated for each selected pixel and stored in three arrays of the same dimensions as the received image. The first array contains the cosine of the angle of the gradient direction. The second array contains the sine of the angle of the gradient direction. The third array contains the magnitude, or steepness, of the gradient. Pixels with a calculated magnitude less than a given threshold have their gradient information set to zero so they are eliminated from further processing.

[0176] At step 2030, edge pixels are identified using the gradient information. An edge pixel can be defined as a pixel for which the magnitude of the gradient of the image is a local maximum in the direction of the gradient. These pixels represent the points at which the rate of change in intensity is the greatest. A separate array of pixels is used (of the same dimensions as the original image) to store this information for further processing.

[0177] At step 2035, edge pixels are formed into groups based on the direction of their gradient. A threshold on the difference in direction is used to include or exclude pixels from a group. Each pixel in a group should be adjacent to another pixel in the group. The edge pixels are labeled

identifying the group to which they belong. At step 2040, the group(s) with crystal characteristics are selected and at step 2045 the selected groups are provided to another analysis process for aid in further analysis of the image.

[0178] One characteristic that separates a crystal from other objects in an image is the straightness of the edge of the crystal. FIG. 20B includes the same steps 2005-2035 as in FIG. 20A, and then uses the crystal characteristic “straightness” to determine whether a group of pixels depict a crystal. At step 2035 in FIG. 20B, edge pixels are formed into a group(s), as described above for FIG. 20A. At step 2040, the edge detection process 2000 determines the “straightness” of each labeled group of pixels using linear regression, according to one embodiment. The correlation from the linear regression and the number of pixels in the group is used to determine the “straightness” of the group. The straightness can be defined as the product of the count of pixels in the group and the reciprocal of 1.0 minus the fourth power of the correlation coefficient for the group, according to one embodiment. If the count of pixels is below a given threshold, the count is set to zero.

[0179] At step 2055, the edge detection process 2000 generates an image, hereinafter referred to as a “lines image,” using the previously calculated straightness information. The lines image is the same shape and size as the subset of pixels selected for edge detection. The intensity value for a pixel in the lines image is set to the straightness value of the group that its corresponding pixel belongs to. At step 2060, the lines image, containing information indicating where “straight” pixels may be found, is provided to an analysis module to aid in crystal identification.

[0180] Referring now to FIGS. 18 and 21, in a typical imaging and analysis process, the scheduler 1825 controls the imaging of samples by communicating to the imaging system controller 1815 the necessary information for imaging a particular plate and the droplet samples on that plate. The imaging system controller 1815 directs the imaging system 1805 to generate the images of the particular plate and droplet sample at a specified time or in a specified sequence, and the images are stored on the image storage device 1810. After an image is generated for a particular sample, the scheduler 1825 sends an analysis request to the image analyzer 1815, and the corresponding image for that sample is provided to the image analyzer 1815. The image analyzer 1815 determines the contents of the image using one or more of the various analysis modules, and provides results to the scheduler 1825 in an analysis response.

[0181] FIG. 21 shows a process 2100 that uses the results of analyzing an image for subsequent imaging of the same sample, according to one embodiment of the invention. At step 2105, a first image of a sample is generated using a first set of imaging parameters, which may include for example, focus, depth of field, aperture, zoom, illumination filtering, image filtering, and/or brightness. An analysis process receives the first image at step 2110 and analyzes the first image in accordance with the analysis request at step 2115. At step 2120, the process 2100 determines whether crystal formation in the first image is suspected, the presence of which can make an additional image of the sample desirable. For example, to determine if an additional image is desired, a score can be computed for the image. The score can be based upon user-adjustable thresholds and weighting fac-



tors, allowing the user to tailor preferences with experienced personal judgment. If the overall score exceeds a specific threshold, reimaging is warranted and an appropriate reimaging request is dispatched. Scoring and threshold may be a function of apparent image content and/or also a function of system bandwidth and scheduling issues. The more available system resources are, e.g., the imaging subsystem, the more likely zoomed-in reimaging occurs.

[0182] The analysis of the first image at step 2120 can be done using a relatively fast running process, e.g., determining the inner/outer non-clear ratio for the droplet sample, and a further, more thorough analysis can be done at step 2140, according to one embodiment. At step 2125, information is provided to the imaging system that allows the same sample to be re-imaged to create a second image of the sample. Subsequent images generated of the same sample can use imaging parameters that are different than those used to generate the first image, that is, at least one value of a imaging parameter used to generate the second image is different than the values of the imaging parameters used to create the first image. At step 2135, the process 2100 receives the second image of the sample and analyzes the second image at step 2140 using, for example, the analysis methods described herein. Analysis results are output for evaluation or display at step 2145.

[0183] Using the analysis data as feedback to the imaging process and adjusting the imaging parameters accordingly, subsequently generated images can more clearly show the presence of crystal formation. For example, if the formation of crystal in the sample droplet is suspected as a result of analyzing the first image, information can be communicated to the imaging system to zoom-in on the area where the crystal formation is suspected and re-image the droplet using a higher magnification. Other imaging parameters, e.g., focus, depth of field, aperture, zoom, illumination filtering, image filtering, and brightness, can also be changed to obtain an image that may better depict the contents of the sample.

[0184] Timely analysis of the first image can result in a relatively large time savings if a subsequent image of a particular sample is desired. The process for handling a sample plate containing the sample, e.g., fetching the correct plate from a storage location, placing the plate in the imaging device, and returning the plate to its storage location, is very time consuming. When thousands of images are scheduled to be generated in one day, minimizing the amount of plate handling during image generation increases image generation and analysis throughput. According to one embodiment, the images generated from the samples on a sample plate are completely analyzed before the plate is removed from the imaging device. If desired, additional subsequent images of a sample contained on that plate can then be generated without incurring the time required to re-fetch the plate. In another embodiment, a certain percentage of the images are analyzed before the plate is removed. While this may not allow every sample to be re-imaged without re-fetching the plate, e.g., the analysis of the last sample imaged may not be completed before the plate is removed, it may still result in an overall time savings as it may allow quick re-imaging of most of the samples, if desired, while not unduly delaying the removal of the plate from the imaging device.

[0185] FIG. 22 illustrates a process 2200 that includes generating two images of a sample, where each image is generated using a set of imaging parameters that has at least one different imaging parameter than those used for the other image, according to one embodiment of the invention. At step 2205 a first image is generated using a first set of imaging parameters. At step 2210, the first image is received by an analysis process which determines one or more regions of interest in the first image at step 2215. The analysis process may be, for example, an edge detection process or a process implemented in one of the analysis modules, both or which are described hereinabove.

[0186] At step 2220, a second image is generated using a second set of imaging parameters where the second set of imaging parameters includes at least one imaging parameter that is different than the first set of imaging parameters. One or more imaging parameter may be changed to generate the second image. For example, the focal plane may be set to a different height relative to the droplet sample, the illumination of the sample may be changed, including using a different direction of illumination (e.g., lighting the sample from alternate sides and off-axis lighting) or a different illumination brightness level, the magnification or zoom level used may be changed, and different filtering may be used for each image (e.g., polarizing filters). At step 2225 the second image is received by an analysis process, and analyzed to determine a region or regions of interest at step 2230.

[0187] At step 2235, the regions of interest from the first and second images are combined to form a composite image. Typically, the composite image is the same size as the first and second images. The first and second of images are analyzed to determine the portion or portions of each image that will be used to form the composite image. The composite images is generated by copying the values of the pixels from each region of interest in the first and second images into one composite image. At step 2240, the composite image is analyzed for the presence of crystal formation by a user, or automatically by an automatic or interactive analysis method, e.g., using the content analysis module, the notable regions analysis module, the crystal object analysis module, or a report inner/outer non-clear ratio module, as previously described, and the results are output at step 2245.

[0188] Although process 2200 shows a process to form a composite image using two images generated with different imaging parameters, more than two images may also be generated and used to form composite images, where each image is generated using at least one different imaging parameter, according to another embodiment. For example, according to one embodiment, a plurality of images are generated for a sample where the focal plane for each image is set at a different "height" relative to the sample. The resulting images may show varying sharpness in corresponding locations. The sharpness of the corresponding portions of the images are compared to determine which portion of each image should form the composite image. The portion of each image that best satisfies specified sharpness criteria, e.g., where a selected set of pixels exhibits the greatest contrast, may be selected from the plurality of images to form the composite image. The size of a portion of an image compared to the other images may be as small



as a single pixel or several pixels, and may be as large as tens of pixels or hundreds of pixels, or even larger.

[0189] FIG. 23 illustrates a process 2300 for visual evaluation of crystal growth by a user, according to another embodiment of the invention. At step 2305, process 2300 receives an image of a sample. At step 2310, the process 2300 classifies the pixels of the image according to their depiction of the contents of the sample, e.g., the pixels are classified as depicting crystal, precipitate, clear or an edge. The pixels of the image may be classified by processes incorporated into the content analysis module 1930, the notable regions analysis module 1935, the crystal object analysis module 1940, as described above, or another suitable analysis process. At step 2315, process 2300 generates a second image that is color-coded using the pixel classification information from step 2310. Step 2315 may be performed by the above-described graphical output analysis module 1950. To generate the second image, pixels that were classified as edge, precipitate or crystal pixels are depicted as a particular color, e.g., red for crystal pixels, green for precipitate pixels, and blue for edge pixels. One or all the classified pixels may be depicted according to a color-code scheme. The second image can have opaque color-coded information, or translucent color-coded information that also shows the original image through the color. The second image is typically the same size and shape as the image received at step 2305.

[0190] At step 2320, the color-coded second image is visually displayed, for example, on a computer monitor or on a printout. At step 2325, the second image is visually analyzed to determine crystal growth information of the droplet sample. Displaying the color-coded image to a user facilitates efficient interpretation of the contents of the image and allows the presence of crystals in the image to be easily and visualized.

[0191] The foregoing description details certain embodiments of the invention. It will be appreciated, however, that no matter how detailed the foregoing appears in text, the invention can be practiced in many ways. As is also stated above, it should be noted that the use of particular terminology when describing certain features or aspects of the invention should not be taken to imply that the terminology is being re-defined herein to be restricted to including any specific characteristics of the features or aspects of the invention with which that terminology is associated. The scope of the invention should therefore be construed in accordance with the appended claims and any equivalents thereof.

What is claimed is:

1. A method of evaluating crystal growth in a crystal growth system, comprising:

receiving a first image of a sample, said first image generated by an imaging system using a first set of imaging parameters;

analyzing information depicted in said first image to determine the contents of said sample;

determining whether to generate another image of said sample based on the contents of said sample; and

providing information to said imaging system to generate a second image of the sample using a second set of imaging parameters, wherein said second set of imag-

ing parameters comprises at least one imaging parameter that is different from an imaging parameter in said first set of imaging parameters.

2. The method of claim 1, further comprising receiving said second image of said sample.

3. The method of claim 2, further comprising analyzing information depicted in said second image to determine the contents of said sample.

4. The method of claim 1, wherein said different imaging parameter is depth-of-field.

5. The method of claim 1, wherein said different imaging parameter is illumination brightness level.

6. The method of claim 1, wherein said different imaging parameter is illumination source type.

7. The method of claim 1, wherein said different imaging parameter is magnification.

8. The method of claim 1, wherein said different imaging parameter is polarization.

9. The method of claim 1, wherein said different imaging parameter is illumination source position.

10. The method of claim 1, wherein said different imaging parameter is the location of the area imaged.

11. The method of claim 1, wherein said different imaging parameter is the focus.

12. The method of claim 1, wherein said analyzing information comprises determining whether said first image depicts the presence of crystals.

13. The method of claim 12, wherein said first image comprises pixels, and said determining comprises classifying said pixels and comparing the number of pixels classified as crystals to a threshold value.

14. The method of claim 13, wherein said classifying comprises using a neural network.

15. The method of claim 12, wherein said first image comprises pixels, and wherein said determining comprises counting the number of said pixels depicting objects in the sample and evaluating said number using a threshold value.

16. The method of claim 12, wherein said first image comprises pixels, and wherein said determining comprises classifying said pixels as either clear or non-clear and evaluating said classified pixels using a threshold value.

17. The method of claim 1, wherein said analyzing information depicted in said first image comprises determining a region of interest in said first image and wherein said information is used to adjust said second set of imaging parameters so that the imaging system generates a zoomed-in second image of said region of interest.

18. The method of claim 1, wherein said analyzing information depicted in said first image comprises displaying said first image and receiving user input based on said displayed image.

19. A method of analyzing crystal growth, comprising:

receiving a first image having pixels depicting crystal growth information of a sample;

identifying a first set of pixels in said first image comprising a first region of interest;

receiving a second image having pixels depicting crystal growth information of said sample;

identifying a second set of pixels in said second image comprising a second region of interest;

merging said first set of pixels and said second set of pixels to form a composite image; and

analyzing said composite image to identify crystal growth information of said sample.



**20.** The method of claim 19, wherein said first image is generated by an imaging system using a first set of imaging parameters, said second image is generated by said imaging system using a second set of imaging parameters, and wherein said second set of imaging parameters comprises at least one imaging parameter that is different from the imaging parameters in said first set of imaging parameters.

**21.** The method of claim 20, wherein different imaging parameter is depth-of-field.

**22.** The method of claim 20, wherein said different imaging parameter is illumination brightness level.

**23.** The method of claim 20, wherein said different imaging parameter is illumination source type.

**24.** The method of claim 20, wherein said different imaging parameter is magnification.

**25.** The method of claim 20, wherein said different imaging parameter is polarization.

**26.** The method of claim 20, wherein said imaging parameter is illumination source position.

**27.** The method of claim 20, wherein said different imaging parameter is the location of the area imaged.

**28.** The method of claim 20, wherein said different imaging parameter is the focus.

**29.** A method of analyzing crystal growth information, comprising:

receiving a first image comprising a set of pixels that depict the contents of a sample; determining information for each pixel in said set of pixels, wherein said information comprises a classification describing the type of sample content depicted by said each pixel, and a color code associated with each classification;

generating a second image based on said information and said set of pixels; and

displaying said second image.

**30.** The method of claim 29, further comprising determining crystal growth information of the sample.

**31.** The method of claim 29, wherein said classification comprises crystals.

**32.** The method of claim 31, wherein said classification further comprises precipitate, edges, or clear.

**33.** A system for detecting crystal growth information, comprising:

an imaging subsystem with means for generating an image of a sample, wherein said image comprises pixels that depict the content of said sample;

an image analyzer subsystem coupled to said imaging system with means for receiving said image, means for classifying the content of said sample using said pixels and means for determining whether said sample should be re-imaged based on said classifying; and

a scheduler subsystem coupled to said imaging analyzer system with means for causing said imaging subsystem to re-image said sample.

**34.** A computer-readable medium containing instructions for analyzing samples in a crystal growth system, by:

receiving a first image of a sample, said first image generated by an imaging system using a first set of imaging parameters;

analyzing information depicted in said first image to determine the contents of said sample;

determining whether to generate another image of said sample based on the contents of said sample;

providing information to said imaging system to generate a second image of the sample using a second set of imaging parameters, wherein said second set of imaging parameters comprises at least one imaging parameter that is different from an imaging parameter in said first set of imaging parameters;

receiving said second image of said sample; and

analyzing information depicted in said second image to determine the contents of said sample.

**35.** A computer-readable medium containing instructions for analyzing crystals, by:

receiving a first image having pixels depicting crystal growth information of a sample;

identifying a first set of pixels in said first image comprising a first region of interest;

receiving a second image having pixels depicting crystal growth information of said sample;

identifying a second set of pixels in said second image comprising a second region of interest;

merging said first set of pixels and said second set of pixels to form a composite image; and

analyzing said composite image to identify crystal growth information of said sample.

**36.** A computer-readable medium containing instructions for analyzing crystal growth information, by:

receiving a first image comprising a set of pixels that depict the contents of a sample;

determining information for each pixel in said set of pixels, wherein said information comprises a classification describing the type of sample content depicted by said each pixel, and a color code associated with each classification;

generating a second image based on said information and said set of pixels;

displaying said second image; and

visually analyzing said second image to determine crystal growth information of the sample.

**37.** A method of analyzing crystal growth, comprising:

generating a first image having pixels depicting crystal growth information of a sample at a first time;

generating a second image having pixels depicting crystal growth information of said sample at a second time;

analyzing said first image and said second image to identify crystal growth information of said sample.

**38.** The method of claim 37, wherein analyzing comprises comparing the number of pixels depicting crystal growth in said first image with the number of pixels depicting crystal growth in said second image.

**39.** The method of claim 37, wherein analyzing comprises comparing the number of pixels within grid elements of said first image with the number of pixels within respective grid elements of said second image.

**40.** The method of claim 39, wherein the size of the grid elements defined by dividing up the said first image and said second image can vary from 1 pixel up to the total number of pixels in each image.