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DESCRIPTION

CROSS-REFERENCE TO RELATED APPLICATION

[0001] The application claims the benefit of the earlier filing date of co-pending U.S. Provisional Patent Application No. 61/375,703, filed August 20, 2010.

FIELD

[0002] Digital microscope.

BACKGROUND

[0003] In various settings, examination of biological specimens is required for diagnostic purposes. Generally speaking, pathologists and other diagnosticians collect and study samples from patients, utilize microscopic examination, and other devices to assess the samples at cellular levels. Numerous steps typically are involved in pathology and other diagnostic processes, including the collection of biological samples such as blood and tissue, processing the samples, preparation of microscope slides, staining, examination, re-testing or re-staining, collecting additional samples, re-examination of samples, and ultimately the offering of diagnostic findings.

[0004] The examination of a biological sample generally involves magnification of the sample or region of interest of the sample and an assessment by a pathologist or diagnostician. Traditionally, this is done by placing a slide containing a sample on a microscope and examining a magnified view of the tissue sample or region of interest of the tissue sample through a microscope. Recently, digital microscopes have been developed wherein a sample, particularly a sample on a microscope slide is placed in an instrument and a magnified digital image of the sample or region of interest of the sample is captured and displayed on a monitor such as thin film liquid crystal display monitor. While being able to view a sample or origin of interest of a sample on a display rather than through a lens of a microscope may be beneficial to the pathologist or other diagnosticians, often the time it takes to scan a magnified image and display that image presents an inconvenient delay or a significant delay when multiple samples need to be processed (magnified).

[0005] US 4,760,385 A discloses a method to create a mosaic of multiple images and requires that images are stored.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006]

Figure 1 is a block diagram of a system including a digital microscope for examining a sample.

Figure 2 is a top perspective view of an embodiment of a slide carrier for use in a digital microscope system.

Figure 3 is a cross-sectional side view through line 3-3' of **Figure 2**.

Figure 4 shows a top view of an embodiment of a slide having a sample and a label thereon and indicating a field of view of a sensor of an embodiment of a digital microscope.

Figure 5 shows a representation of three adjacent images of a sample each captured by a sensor of a digital microscope.

Figure 6 shows the three adjacent images of **Figure 5** following their stitching together.

Figure 7 shows an image of a slide including the three adjacent images of **Figure 5** stitched together and a slide label.

Figure 8 describes a flow chart of an embodiment of image capture by a system including a digital microscope.

Figure 9 is an embodiment of a screen shot of a display showing an embodiment of different modes of use of a system including a digital microscope.

Figure 10 is an embodiment of a screen shot of a display showing low-resolution images of four slides and their labels.

Figure 11 is an embodiment of a screen shot of a display showing a portion of a sample in a live image mode.

Figure 12 is an embodiment of a screen shot of a display showing a portion of three different samples in a live image mode.

DETAILED DESCRIPTION

[0007] The present invention is directed to a method according to claim 1 and an apparatus according to claim 8. **Figure 1** shows a block diagram of a system for examining a sample such as a tissue sample. Referring to **Figure 1**, system 100 includes computer 110. Computer 110 includes, for example, a central processing unit that in one embodiment is an Intel Core 2 Quad or better having at least 4 gigabytes of random access memory and at least one terabyte hard drive memory. Computer 110 also includes a DVD writer and an operating system such as Windows 7.

[0008] Connected to computer 110 is display 120 configured to display information transmitted from computer 110. Display 120 is, for example, a thin film liquid crystal display monitor that is S-IPS or PVA technology based. A 24-inch or greater color monitor is a representative example. Alternatively, two, three or more displays can be connected to computer 110 to provide a user with more information in a more structured way. For example, one display may provide the image of a sample having a hematoxylin and eosin (H&E) stain, while another one shows images of the same case using a different type of staining method, and a third may show clinical data from other disciplines such as Clinical Chemistry, Hematology, Radiology. Also connected to computer 110 is keyboard 130, mouse 1400A and mouse 1400B. In one embodiment, mouse 1400A is a two-dimensional conventional mouse and mouse 1400B is a three-dimensional mouse such as the 3DConnexion Space Navigator™. Three-dimensional mouse 1400B may, for example, be used to position or navigate the environment and mouse 1400A may, for example, be used to select, create or edit.

[0009] Computer 110 may have internet and/or intranet connection 145 to allow remote transmission operation of system 100 and/or to connect to a network operating system.

[0010] Connected to computer 110 in this embodiment of system 100 is digital microscope 150. Digital microscope 150 may include one or several imaging systems including sensor 160 and sensor 165, optical imaging sub-system 168 and optical imaging sub-system 170, autofocus optics and illuminations. Each imaging system may have a different optical resolution or range of resolution. At least one optical system may reach magnification, $m \leq 1$. The system may also provide high resolution with magnifications, $m > 1$. The digital microscope also includes stage 180 translatable in x-, y- and z-directions and control electronics.

[0011] Digital microscope 150 may be operated as a bright field and/or fluorescence microscope. In the case of a bright field operation, a sensor or sensors sense(s) the absorption of the sample and captures an image of the sample on the stage 180 with a light source on the opposite side of sensor(s) with respect to the sample. As shown in **Figure 1**, light source 195 and light source 196, each, for example, a light emitting diode (LED) light source, are positioned below stage 180. An opening in stage 180 allows light to be emitted through stage 180 to illuminate a sample, such as a slide on stage 180. In the case of a fluorescence microscope operation, the imaging system images fluorescence markers which have been excited by a fluorescence illumination light source. The fluorescence light is typically coupled into the optical system via a dichroic mirror between the infinity corrected microscope objective and a tube lens. In such case, both the sensor and the illumination light source are on the same side of the sample. Referring to optical imaging sub-system 168 and sensor 160, in one embodiment, sensor 160 comprises a commercially available digital camera with an area sensor, for example, a charge-coupled device (CCD). CCD sensors are subdivided in several million photo-sensitive square units (pixels) which describe the resolution of the sensor. A typical pixel size (sensor resolution) of such a sensor is about 5 microns (μm) x 5 microns. The size which a pixel represents using the magnification of the optical system on the sample is commonly referred to as the pixel resolution. Using an optical system with a magnification

$0.1 < m < 40$, results in a pixel resolution of approximately 50 microns to 125 nanometers.

[0012] In one embodiment of an imaging system, sensor 160 is configured to sense and capture an image of a sample, such as an image of a slide or a portion of a slide on stage 180. Optical imaging sub-system 168 in digital microscope 150 includes lens or objective 1680 that focuses light from light source 195 of an illumination sub-system on sensor 60. Light from light source 195 is emitted through an opening in stage 180, through a slide on stage 180. Mirror 1682 of optical imaging sub-system 168 directs the light to lens or objective 1680. Sensor 160 may capture such image by sensing the image, without magnification ($m=1$) or a magnification less than one ($m<1$) through optical imaging sub-system 168. In one embodiment, a location of optical imaging sub-system 168 and sensor are fixed. Mirror 1682 may be moved in an x- and a y-direction by xy-stepper motor 172 and in a z-direction by z-stepper motor 174.

[0013] Computer 110 receives signals representative of a sensed image from sensor 160 and generates a sensed live image for display and displays such generated image on display 120.

[0014] In one embodiment, sensor 165 is similar to sensor 160. Sensor 165 is configured to capture an image of a sample, such as an image of a slide or a portion of a slide on stage 180. Sensor 165 captures such image through optical imaging system 170 ($m>1$). Optical imaging sub-system 170 in digital microscope 150 may include multiple objectives. Objective 1700A, objective 1700B, and objective 1700C are shown. Objective 1700A is, for example, an infinity corrected type from Carl Zeiss having a magnification of 2.5x. Objective 1700B is, for example, an infinity corrected type from Carl Zeiss, having a 20 times (20X) magnification. Objective 1700C is, for example, a Carl Zeiss A-plan objective, having a 40 times (40X) magnification. Interchanging these objectives yield different optical systems, where each system results in a different pixel resolution (e.g., two microns for magnification 2.5 and 250 nanometers for magnification 20). As needed, other objectives can be substituted or more objectives can be added. The individual objectives are movable in an x- and a y-direction by xy-stepper motor 172 allowing a particular objective to be associated with sensor 165 and a slide on stage 180 when desired. Representatively, the objectives and mirror 1682 may individually be connected to a track and are motorized to move along the track and actuated into position when desired.

[0015] Disposed between sensor 165 and optical imaging sub-system 170, in one embodiment, may be automatic focusing system 167 including a beam splitter, an autofocus detector and autofocus illumination. An infrared filter and tube lens may also be disposed between sensor 165 and optical imaging sub-system 170, such as between automatic focusing system 167 and sensor 165.

[0016] In one embodiment, when capturing images through optical imaging sub-system 170, microscope 150 uses light source 196 of illumination sub-system positioned beneath stage 180. Light source 196 may be similar to light source 195. Associated with light source 196 (i.e., disposed between light source 196 and stage 180) and included in the illumination sub-system are motorized apertures or diaphragms 197 providing Kohler illumination that improves the specimen illumination.

[0017] Computer 110 receives signals representative of a sensed live image from sensor 165 and generates an image for display. The generated image is displayed on display 120.

[0018] In the above-described embodiment, multiple sensors (sensor 160, sensor 165) are described to capture an image of a sample on a slide. In another embodiment of an imaging system, system 100 includes a single sensor configured to capture an image of a slide or a portion of a slide without magnification ($m=1$) or with a magnification less than one ($m<1$) and to capture an image or portion of an image through magnifying optical imaging sub-system 170 ($m>1$). In this embodiment, a single sensor may be utilized in connection with interchangeable optics (e.g., optical imaging sub-systems 168 and 170). Similarly, in the above embodiment, instead of light source 195 for optical imaging system 168 and light source 196 for optical imaging system 170, a single light source may be used for each imaging system.

[0019] In one embodiment, digital microscope 150 includes control unit 175. Control unit 175 is connected to computer 110. Control unit 175 is connected to computer 110. Control unit 175 is also connected to the various components of digital microscope 150 to control an operation of digital microscope based on signals received from computer 110. Control unit representatively controls xy-stepper motor, z-stepper motor, light source 185, light source 196, motorized apertures or diaphragms 187, optical imaging sub-system 168, optical imaging sub-system 170, sensor 160 and sensor 165.

[0020] Referring to digital microscope 150 operated as a bright field microscope, in one embodiment, where a tissue sample is a slide having a label with patient identification information and/or other information, including, for example, type of stain or process to which the sample was subjected, printed on the label, digital microscope 150 can sense and capture such information. Using a light source beneath a label to illuminate the label, however, may not make the label information visible. Accordingly, in one embodiment, second light source 198 is utilized to illuminate the slide or a label portion of the slide so that the data on a slide label may be sensed via reflectance. An image of the label may be captured with, for example, sensor 160 and optical imaging sub-system 168.

[0021] Referring again to **Figure 1**, stage 180 is manipulated in three directions by control unit 175: x-direction (side to side as viewed), z-direction (up and down as viewed) and y-direction (into and out of page as viewed). The z-direction may also be accomplished by moving the optics with respect to the sample. Stage 180 is manipulated in an x-direction and a y-direction by xy-stepper motor 172 and in a z-direction by z-stepper motor controlled by control unit 175.

[0022] Referring again to digital microscope 150 of system 100, the microscope includes stage 180. In one embodiment, stage 180 is sized to handle one or more slides. In one embodiment, a slide tray including four slides may be contained on stage 180. **Figure 1** shows slide carrier 210. In another embodiment, a slide loader can be attached to system 100, which allows for the automatic loading and unloading of up to about 240 slides. The slide loader allows system 100 to perform slide imaging automatically, whether a user is present or not in a reflex imaging

mode. Users can select to either use the automatic slide loader or the slide tray.

[0023] Figure 2 shows a representative example of slide carrier 210 on stage 180 within microscope 150. Slide carrier 210 is, for example, a molded polymer material that includes four slide cavities 220A, 220B, 220C and 220D each for retaining an individual slide (e.g., a 25 millimeter x 76 millimeter slide). Three slide cavities (cavity 220A, 220B and 220C), in the illustration of **Figure 2**, contain a slide while fourth cavity (cavity 220D) is empty. Slides may be placed in respective slide cavities by a user or automatically by, for example, a mechanism associated with a slide loader (e.g., a pick and place robotic instrument). In one embodiment, a slide is not mechanically restrained by slide carrier 210 but instead rest partially within a slide cavity.

[0024] Figure 3 shows a side view of slide carrier 210 on stage 180 through line 3-3' of **Figure 2**. In this embodiment, slides 320A, 320B and 320C are shown in slide cavities 220A, 220B and 220C, respectively. No slide is shown in slide cavity 220D. Each slide cavity includes a cavity (cut out) portion and a plateau portion (330A, 330B, 330C and 330D). A slide rests horizontally on a plateau portion. Each plateau portion has a height dimension such that when a slide rests on an upper surface of the plateau portion (as viewed), a portion of a thickness of the slide extends a distance 315 above a height dimension of slide carrier 210 (as viewed).

[0025] Referring to **Figure 2** and **Figure 3**, slide carrier 210 may be seen to be within a pocket or holder of stage 180. Stage 180, in one embodiment, is a molded plastic piece having a size to support slide carrier 210 within digital microscope 150. The pocket or holder of stage 180 is formed by opposing L-shaped brackets protruding from surface 181 (a top surface as viewed) of stage 180. **Figure 2** shows bracket 183 and bracket 184, each having an inverted L shape and opposing one another (with a base of the inverted L or cantilevered portion facing toward the opposing bracket). Bracket 183 and bracket 184 are separated by a distance at least greater than a width dimension of slide holder 210. (For example, if slide holder 210 has a width dimension on the order of 10 centimeters (cm) to 12 cm, bracket 183 and bracket 184 are separated by that distance plus 0.25 cm - 0.5 cm greater.) Each bracket extends a height dimension from surface 181 that is greater than a thickness of slide holder plus a distance 315 to which a slide in a slide cavity of slide holder 210 protrudes beyond surface 181 of slide holder 210. For example, if slide holder has a thickness on the order of 1 cm, a base of the cantilevered or inverted L portion of each of bracket 183 and bracket 184 is at a distance from surface 181 of stage 180 of 1 cm plus more than a distance 315. For example, if distance 315 is 1 mm, the cantilevered portion of each of bracket 183 and bracket 184 is at a distance from surface 181 of stage 180 of 1.2 cm or more. An orientation and configuration of bracket 183 and bracket 184 of stage 180 allow slide holder 210 to be guided in and out of the pocket formed by the brackets.

[0026] Referring to **Figure 3**, in one embodiment, stage 180 includes hump 188 on surface 181 at a distal end of the pocket formed by bracket 183 and bracket 184 (distal from a point where slide holder 210 enters the pocket). Hump 188 has a dimension sufficient to elevate stage holder 210 from surface 181 of stage 180 and bring any slides in slide cavities of slide

holder 210 into contact with cantilevered portions of bracket 183 and bracket 184. In this manner, cantilevered portions of bracket 183 and bracket 184 act to secure or support a position of a slide when slide holder 210 is on stage 180. Representatively, hump has a thickness or height dimension on the order of a few millimeters and a length dimension of 0.5 cm, and a width that extends between bracket 183 and bracket 184. Alternatively, two or more smaller width dimensioned bumps may be used.

[0027] In operation, digital microscope 150 uses one of sensors 160 and 165 to sense and capture images of a sample or a region of interest of a sample on the slide. The sensor captures slide images of the sample and transmits those images in digital signals to computer 110 and such signals are displayed on display 120. In one embodiment, when capturing an image and displaying that image on display 120, it may not be desirable to store the image in the sense that it could be retrieved in the future. Instead, the image is transmitted from sensor 160 or sensor 165 to computer 110 and absent some instruction from a user or from the system to take another action, the images are refreshed representatively at a refresh rate on the order of several images per second. The refreshing rate may vary. If there is no action of the microscope, for example, there is no need to refresh the image.

[0028] In one embodiment, sensor 160 captures an image of a sample on a slide with a magnification of one or less ($m \leq 1$). In other words, where a magnification is less than one ($m < 1$), optical imaging sub-system 168 project an unmagnified or a demagnified image of the sample on the sensor. Representatively, sensor 160 is smaller than a slide (e.g., a sensor is approximately 3 to 4 millimeters in diameter while a slide is approximately 25 millimeters by 76 millimeters). Optical imaging sub-system 168 includes an objective that projects a larger field of view on sensor 160.

[0029] In one embodiment, system 100 creates an overview image of an entire sample on a slide or a portion of an entire sample. The overview image is an image captured without magnification as described above (i.e., a magnification of one or less than one). An advantage to capturing a overview image is the speed at which it can be captured. For example, an image of a full slide can be captured on the order of one to two seconds while capturing a magnified image may take on the order of 20 seconds or more.

[0030] As noted above, a sensor is smaller than a slide and typically smaller than a sample or a portion of a sample on the slide. In order to obtain an acceptable resolution of an image, such as an overview image, an area that individual pixels of the sensor represent is reduced. In one embodiment, to obtain an acceptable overview image of a sample on the slide, sensor 160 will take multiple images and stitch those images together. For example, in one embodiment, a slide or an image sample such as an entire sample on a slide is divided into thirds with a sensor capturing light through a third of the desired area for the overview image (e.g., a third of the usable area of a slide). To coordinate the capture of light representative of a third of a sample, stage 180, in one embodiment, is moved to a desired portion within the field of view of sensor 160.

[0031] Referring to **Figure 2**, stage 180 is translatable in an x- and y-direction. Sensor 160 remains stationary. In one embodiment, stage 180 is translated in response to signals from control unit 175 to xy-stepper motor 172.

[0032] In one embodiment, microscope 150 and system 100 is calibrated using a reference slide carrier such that a nominal position of slides are known within a defined tolerance. The defined tolerance is a result of the xz coordinate system of stage 180 ($\pm p$); the mechanical tolerances of slide carrier 210 and its position when inserted into microscope 150 ($\pm q$); and the mechanical tolerances of slide cavities in slide carrier 210 that accept slides ($\pm r$). The defined tolerance considering these factors is $p + q + r$. An overview image of a slide, in one embodiment, consists of three overlapping images with the field of view of each image and overlap selected to accommodate the defined tolerance and to collectively capture an image of the entire slide. In other words, since the images obtained by sensor 160 will be stitched, in one embodiment, stage 180 is translated from one field of view of sensor 160 to a different field of view such that, at the different field of view, there is an overlap of another field of view (e.g., a previously imaged field of view). In one embodiment, the overlap is at least 20 pixels more than a maximum tolerance of the stage, slide carrier and cavities within the slide carrier (e.g., 20 to 50 pixels).

[0033] **Figure 4** demonstrates an image capture of an image or a portion of an image. Referring to **Figure 4**, stage 180 is positioned so that slide 220A is within the field of view of sensor 160. Slide 220A is positioned so that a first image captured by sensor 160 will be at one edge of a slide. For example, assuming a slide is approximately 76 millimeters long with a slide label occupying approximately 16 millimeters of that length at one end, the remaining approximately 60 millimeters of the slide will constitute the usable area of the slide (i.e., an area where a sample could be located). Sensor 160 will be positioned at an opposite end such that it captures the first 20 to 25 millimeters length of the slide from that end. An edge of a slide or an edge of a slide label on a slide may provide an x-coordinate and a y-coordinate. Stage 180 can use these coordinates to establish a position to capture the first 20 to 25 millimeters of the slide from the slide label. In stitching images together, the first image may be considered a fixed reference to which other images are stitched to form the overview image. At a first portion of an image, **Figure 4** shows sensor 160 capturing a portion of slide 220A designated in area 310 as a first or Time1 image. Stage 180 then moves in an x-direction approximately 20 millimeters and sensor 160 captures a second image at Time2 represented by area 320. Finally, stage 180 moves to a third position in area 330 within the field of view of sensor 160 and sensor captures an image of area 330 at Time3.

[0034] In the description of image capture of adjacent images, in one embodiment, the system requires overlap of the captured images. Overlap is shown in **Figure 5** where area 420 overlaps a portion of area 410 and area 430 overlaps a portion of area 420. The overlap is put to beneficial use when the images are stitched together. In one embodiment, the system strives for approximately 20 to 50 pixels overlap between adjacent images. Following the capture of adjacent images, the adjacent images are assembled or stitched together.

[0035] **Figures 5** and **6** illustrate an embodiment of stitching. In one embodiment, the system uses landmarks of the sample and seeks to align common landmarks to form an overall image. **Figures 5** and **6** show simple geometric landmarks or features to represent this concept. **Figure 5** shows image represented by area 410 and image represented by area 420 are put together using the geometric symbols as landmarks or features common to adjacent areas. In this example, the image represented by area 410 and the image represented by area 420 need to be moved along a single axis for alignment. System 100, however, also allows more alignment possibilities, such as along two axes and rotation. For example, the image represented by area 430 is shown offset in the y-direction relative to the image represented by area 420. Thus, the image represented by area 480 may be moved in two directions (x-direction, y-direction) to align identified common landmarks or features between the images.

[0036] As noted above, in one embodiment, the stitching of portions of an image to gather the overview image is performed as the images are captured. Although **Figure 5** shows three separate images, in one embodiment, in the example with the respect to, the image represented by area 410 and the image represented by area 420 are stitched together immediately upon capturing of each image. As the image represented by area 430 is captured, that image is aligned and stitched to the combined image represented by area 410 and area 420. Images of the individual areas are not saved. Instead, once a sufficiently large area of an image of the sample is stitched and assembled, the assembled area is broken into similarly sized fractions and saved in a file structure or may be compressed using a compression format (e.g., JPEG). The saved image area is deleted from random access memory (RAM) of computer 110.

[0037] System 100 can establish approximately where a sample is on a slide as well as a location of significant features based on the field of view images used to assemble an overview image. For example, each pixel of an overview image represents a specific area, e.g., $5.4\ \mu\text{m} \times 5.4\ \mu\text{m}$. Further, the assembled image is represented by the pixel number of sensor 160 which can be described in terms of x- and y-coordinates, e.g., 2504 x-direction pixels by 3324 y-direction pixels. With this information, a selection by mouse 1400A or mouse 1400B of a position in the overview image is a selection of a pixel or pixels in that image. Since a size of each pixel is known, system 100 can determine the number of pixels in an x-direction and a y-direction that a selected position (e.g., a position of a feature of the image) is located relative to a starting position, e.g., an edge of the image. An approximate coordinate system for the image may thus be established such that system 100 can identify a location of an area including a feature represented by a particular pixel or pixels.

[0038] As noted above, generally a slide has a label fixed to a surface. In one embodiment, it is desirable to have an overview image including not only of the sample on the slide but also the slide label. Because a slide label will obstruct light introduced in digital microscope 150 from below the slide, digital microscope 150 includes sensor 198 that captures an image of the slide label in reflectance. **Figure 7** shows label 450 that may be sized and manipulated (e.g., rotated) so that when assembled with the overview image taken from the slide field sensor (sensor 160), label 450 may be adjacent to the image. **Figure 7** shows an overview image 440

of stitched together portions of the image (a portion represented by area 410, a portion represented by area 420 and a portion represented by area 430) to create a full stitched together image of the active portion of the slide. **Figure 7** also shows an image of label 450 adjacent the overview image of the sample. As noted above, individual portions of the sample are not saved, only the composite stitched slide image (e.g., an overview image). In one embodiment, the stitched slide image is saved separate from the label image. In another embodiment, the stitched slide image is saved with the label image stitched adjacent to it. In either situation, the composite image may be saved with conventional compression software (e.g., JPEG).

[0039] Where sensor 160 and optical imaging sub-system 168 are positioned over a sample on a slide to capture an image of that slide, a user of system 100 can electronically "zoom" in to increase the resolution. In one embodiment, system 100 initially captures an overview image of a sample on a slide (e.g., an overview image of the entire sample) with sensor 160 and an image of the slide label. The initial overview image may be stitched together as described above. In one embodiment, the initial image is displayed at a relatively larger sensor to pixel ratio (multiple pixels of sensor 160 mapped to a pixel of display 120). It is appreciated that pixels on display 120 are generally larger than on sensor 160. For example, pixels on sensor 160 have a size on the order of five microns while a pixel size of display 120 are on the order of 0.5 millimeters.

[0040] In one example, the initial overview image is displayed at a sensor to display pixel ratio of four to one or greater. A user then uses mouse 1400A to select a region of interest in a sample. The user can then zoom in to increase the image resolution at that particular point or that particular region of interest and/or increase the magnification. Representatively, to zoom in electronically at a selected region of interest (selected by mouse 1400A and located by system 100 as described above), a user directs mouse 1400B to zoom in and, in response, system 100 will modify a sensor to display pixel ratio from, for example, four to one toward one to one or more (i.e., map fewer sensor pixels to individual display pixels). It is appreciated that as the individual sensor pixels are mapped to more display pixels, the image will appear to the user as being magnified as the display area of the region of interest on display 120 increases. A user can accept and save the image at any desired ratio of sensor pixel to display pixel.

[0041] At some point, a threshold resolution will be reached (e.g., a one-to-one sensor to display pixel ratio). If the user wishes to continue to zoom in on the region of interest, in response, system 100 will automatically switch from an optical magnification of one or less to the next higher magnification in microscope 150. In an embodiment where a separate sensor is associated with magnifying optics, system 100 will automatically switch to sensor 165 and position magnifying optical imaging sub-system 170 over the region of interest. Representatively, when the threshold resolution of the image is reached with sensor 160 and optical imaging sub-system 168, system 100 will switch to magnifying the image through objective lens 1700A. Objective lens 1700A is, for example, a 2.5X magnification.

[0042] Upon switching to capturing an image through magnifying optical imaging sub-system

170, the sensor to display pixel ratio will once again begin at a pixel ratio greater than one to one (e.g., four to one). A user can accept that captured image and save that captured image or continue to zoom in and accept and save the image at any desired ratio. Continuing to zoom in again initially involves modifying a sensor to display pixel ratio from a sensor to display pixel ratio greater than one to one towards a ratio of one to one or more. Once the threshold resolution is reached, system 100 will change objectives from objective 1700A to objective 1700B with the next higher optical magnification. In one embodiment, objective 1700B is a 20X magnification. Continued zooming follows the same action.

[0043] In one embodiment, when a slide is placed in digital microscope 150, system 100 immediately creates an overview image with a magnification of one or less than one. A user can zoom in where sensor 160 is capturing an image from a sample in the slide as described above, or a user can alternatively capture a greater magnification of a region of interest on the sample by telling the system to increase magnification. One way a user does this is by using mouse 1400A and selecting a region of interest on the overview image and indicating the desired magnification. In the latter case, it is appreciated that a user may make the selection on an overview image whether or not a particular sample/slide is currently the slide to which sensor 160 may be capturing an image. For example, where sensor 160 is currently capturing an image of slide 320A (see **Figure 3**) and a user wants a magnified image of a region of interest of slide 320B, a user will navigate with mouse 1400A to a thumbnail image of slide 320B present, for example, with thumbnail images of other slides along a side of display 120. The thumbnail image may be a smaller presentation of the image or a portion of the image, for example, a representation of a portion of the image using a reduced number of pixels. A user may then select the thumbnail overview image of slide 320B with mouse 1400A (e.g., by clicking on it). System 100 will then display a larger image of the overview image of slide 320B on screen 120 and stage 180 may move slide 320B into position for image capture by sensor 160. If a magnified image is stored in a memory of computer 110, system 100 will retrieve it and display it on display 120. If, however, a magnified image does not exist, system 100 will generate one. It is appreciated that slide 320B must be in slide carrier 210 in digital microscope 150.

[0044] Using the example of a user wanting a magnified view of a portion of a sample on slide 320B, initially a saved overview image of slide 320B will be displayed on display 120. If, for example, a user wants a magnified image of a portion of the image (e.g., a region of interest), the user can drag mouse 1400A to a desired position of display 120 showing a sample on slide 320B and then indicate to system 100 the desired area of magnification by clicking on mouse 1400A. As noted before, a specific coordinate system of the overview image may not be saved. However, system 100 knows the approximate location selected by a user, because it knows where on display 120 a user indicated (e.g., clicked), it knows the size of individual pixels in the overview image (e.g., 50 μm x. 50 μm) and it knows the pixel size of the image (e.g., 3324 x. 2504 pixels). Since the system previously identified slide 320B in slide carrier 210 and the approximate location of the sample on the slide, system 100 will approximately know the region of interest to capture a magnified view of the region of interest. Similarly, if a magnified image including a region of interest had previously been saved, system 100 can retrieve that image

based on a user indicating the region of interest on the overview image. In other words, an ability to identify a region of interest by a pixel position in an overview image applies not only to the overview image of a sample but to any other image of that sample.

[0045] With regard to saving images (e.g., an overview image, a magnified image), in one embodiment, a single assembled image of a sample is saved. In another embodiment, a hierarchy of images of the sample is saved. In one embodiment, a hierarchy of images of a sample is created based on a sensor to display pixel ratio. In this embodiment, a highest ranked image in the hierarchy is an image having a one-to-one pixel ratio (full resolution sample mapping each sensor pixel with each display pixel). One or more lower ranked images of increasing sensor to display pixel ratio (a sensed image is displayed on display 120 such that one sensor pixel is mapped to more than one display pixel, e.g., 2:1, 4:1, etc.) make up the remainder of the hierarchy of images. Each of the full resolution sample and the one or more lower ranked images may be stored together in a data set.

[0046] For objectives with high magnification, a depth of field (i.e., objects within the depth of field (z-direction range)) is relatively small. The z-direction range is so small (e.g., 1 μm) that the image captured may not capture all objects in a sample having a thickness, for example, on the order of 10 μm , in one image capture. To capture as many objects as possible, in one embodiment, system 100 may capture several images at different focal planes by a depth of field. In the example, system 100 may capture 10 images moving stage 180 one micron in a z-direction between each capture. Such an operation will result in 10 image planes representing a z-direction stack or z-stack of the sample.

[0047] In another embodiment, a coordinate system for each slide may be established using a label image. In one embodiment, a slide label may be printed with more than one perceivable dot or point. **Figure 7** shows an embodiment of a slide label having three points (point 4500A, point 4500B, point 4500C). Sensor 160 or sensor 165 can perceive these points and, having perceived them, system 100 can determine a pixel location of each point and the number of pixels between each point. By associating image 440 adjacent label 450, system 100 can locate any position on image 440 based on its x- and y-distance from one or more points.

[0048] In one embodiment, a data set including a saved image or hierarchy of images of a sample, a z-stack, a coordinate system for that image, if any, and separately saved label image is assembled in a memory of computer 110. Such a data set may also contain comments or annotations (including markings on an image made by a user) and content of label (e.g., interpretation of label).

[0049] Having described certain components of system 100, a brief description of operation is now presented. In one embodiment, use of system 100 is software driven. In other words, a machine or computer readable medium is provided in computer 110 containing program instructions that, when executed, will carry out the various methods of operation described.

[0050] In one embodiment, a method of operation is illustrated in **Figure 8**. Method 500 will be

described with reference to components of system 100 and various screen shots that, in one embodiment, are displayed on display 120.

[0051] As a starting point, slide carrier 210 may be loaded into digital microscope 150 and placed on stage 180. Sensors may be located on stage 180 to sense a slide carrier. Computer 110 is responsive to such sensors. When computer 110 senses slide carrier 210 on stage 180, in one embodiment, system 100 has three modes: a live image mode; a scanning mode; and a viewing mode. The live image mode and viewing mode are interactive modes in that they include user interaction. The scanning mode may be operated interactively or be fully automated with specific predefined parameters or configurations for scanning (saving) images. For example, in the scanning mode, slide(s) may be loaded onto a slide carrier and inserted into a digital microscope and the system will sense and save one or more images of samples on a slide.

[0052] **Figure 9** shows an example of a screen shot of display 120 of system 100. This is an example of an entry screen where a user can select a live image mode, a scanning mode or a viewing mode. An entry screen may also include an opportunity to enter or modify the basic settings of the instrument by selecting "Settings" 640 as well as an opportunity to exit the system by selecting "Exit" 650.

[0053] In the example where a user chooses a live image mode by selecting "Live" 610, computer 110 will direct digital microscope 150 to move slide carrier 210 to a loading position, such as by extending slide carrier 210 out of the instrument so it is accessible by a user. At this time, when slide carrier 210 is accessible, computer 110 may indicate that the slide carrier may be removed from digital microscope 150 and loaded with one or more slides. One way to indicate this is by an announcement on monitor 120 (block 505, **Figure 8**).

[0054] In the embodiment where slide carrier 210 has four slide cavities (slide cavities 220A, 220B, 220C and 220D), a user may place up to four slides onto slide carrier 210. After placing one or more slides on slide carrier 210, the carrier is loaded into the instrument and the instrument pulls the carrier inside and senses its presence and location (block 510, **Figure 8**).

[0055] Once slide carrier 210 is placed within digital microscope 150, system 100 determines the number and position of slides inserted into slide cavities 220A, 220B, 220C and/or 220D (block 515, **Figure 8**). System 100 selects each slide for imaging (block 520, **Figure 8**). System 100 then aligns a selected slide and sensor 160/optical imaging sub-system 168 (block 520, **Figure 8**). If a selected slide has been reinserted into the instrument and information of a previous session needs to be correlated, system 100 determines the rotation and shift of the selected slide with respect to a previous session. In one embodiment, sensor 160 initially senses an image or images of a sample on each slide without magnification or with a magnification of less than one (block 530, **Figure 8**). The sensed live image along with a separately sensed image of a label on the slide may then be displayed (block 535, **Figure 8**). A low-resolution overview image of all inserted slides and of their labels is then taken.

[0056] The screen shot in **Figure 10** is an embodiment of a slide selection graphical user interface (GUI). In GUI title bar 710, the steps for the workflow are illustrated and the current step ("slide selection") highlighted. To the right of these tabs (as viewed), the title bar includes controls to move within of the workflow (buttons 715), to eject the slide carrier (button 720) or to get assistance (button 725).

[0057] Main part 730 of screen 700 is divided into slide selection section 740, profile selection section 750 and slide information section 760. Slide selection section 740 shows a sketch of slide carrier 180 (see **Figure 2**). In the occupied cavities of slide carrier 180, overview images 770A, 770B, 770C and 770D of slides in slide cavities 220A, 220B, 220C and 220D, respectively (see **Figure 2**), as well as the labels of the respective slides can be seen. The GUI allows a user to select and group correlated slides. In one embodiment, grouped slides are scanned and simultaneously displayed. This feature allows the user to, for example, compare structures of different slides of the same group. A group of slides may be referred to as a case. In the "live image mode," a user is able to add pre-scanned, stored slides to a case. For example, in addition to any slides present on slide carrier 180 within digital microscope 150, computer 110 of system 100 may store previously captured (saved) images (e.g., overview image, magnified image) referred to as scanned images of slides that are no longer present.

[0058] Referring to profile selection section 750 of the GUI, the section allows the user to select specific, predefined scanned profiles that are optimized and customized image acquisitions for a specific stain or, in an embodiment utilizing fluorescent image, a fluorescence number. For example, a specific profile for an H&E stain might be a 20x image. Section 750 allows a user to select the tool bar and be offered a choice of a 20x view and scan. Slide and label information is presented in slide information section 760 and provides, in one embodiment, identifying information about the patient as well as the process step(s) that a sample was subjected to in preparing it for microscopic analysis.

[0059] Referring to the screen shot in **Figure 10**, options to proceed include starting a "live image mode" of a single slide or a group of slides or ejecting the slide carrier (slide carrier 180). In a "live image mode," further images (e.g., magnified images) of a slide or a group of slides may be sensed and/or captured (saved).

[0060] In an instance where a user selects a "live image mode," a user may select a particular slide or slides based on the displayed overview images (block 540, **Figure 8**). **Figure 11** and **Figure 12** show typical user interface screens. While **Figure 11** illustrates the screen with a single image or scan, **Figure 12** shows views of three different images or scans. The image in **Figure 11** is a live image (indicated video icon in right corner, as viewed). The screen is divided into different areas. The two upper images (as viewed) are live images (see video icon) and the lower image is a saved (scanned) image (see camera icon). System 100 is able to show saved tissue images (tissue scans) and live images of slides that are in digital microscope 150 as well as stored tissue scans of pre-scanned slides. In one embodiment, for "live" images of slides present in digital microscope 150, sensor 160 continuously acquires (refreshes) images at the current position of the microscope at a rate of several

images/second (block 545, **Figure 8**). For example, if a sensor last sensed a sample on a slide in slide cavity 220A and no further activity by a user or system 100 has been dictated, sensor 160 will remain over the slide in slide cavity 220A and refresh that image at a rate of, for example, six images/second. In one embodiment, only the sample of the image is refreshed, not the associated label. Although **Figure 11** shows a single scan and **Figure 12** shows three scans, up to 16 scans can be viewed simultaneously. In such case, the view is split to show all selected scans. In another embodiment, images from a case can be displayed on more than one screen.

[0061] Views can be added and removed from the screen via selection in a journal. The journal is a collection of all objects in a case and can be found on the right hand side of the screen. At the same time, only one view is active. All controls reflect the setting of the active view. Any operation will only affect this specific image. The images from the other scans are "frozen." To switch focus to another slide, the user needs to click on one of the displayed images or a label in the journal.

[0062] In title bars 810 and 910 of the screen shots shown in **Figure 11** and **Figure 12**, respectively, system 100 offers the following controls:

CONTROL		DESCRIPTION
Start/stop acquisition 815/915	:	Image acquisition gets stopped/continued.
Snap 820/920	:	Capture current image from the selected slide. The image can be saved or exported.
Color channel selection 830/930	:	Switch on/off color channels.
Distance/area measurement tools 840/940	:	Calibrated tools to measure actual distances and areas on the sample.
Annotations and annotation setting 845/945	:	Using these tools, the user is able to add comments to the image with different object (e.g., text, square, arrow).
HR scan regions	:	Defines regions which will be scanned using the selectable high resolution (HR) optics. For regions smaller than a camera tile, the software immediately acquires and displays the image (i.e., no stitching required). However, if the selected region is larger than a camera tile, the region will be stitched and scanned.

[0063] In each view, the magnification of the image, a scale indicator and a focus control may be displayed.

[0064] **Figure 11** shows a single image at 2.5x magnification. A user can indicate regions of interest or desired scans by maneuvering mouse 1400A or mouse 1400B over an area (see e.g., Scan 1, Region 1, Region 2). **Figure 12** shows these areas at 20x.

[0065] Using keyboard 130, mouse 1400A or 3D mouse 1400B, the user is able to navigate within the scanned image data (block 550, **Figure 8**). The following actions are possible:

ACTION	DESCRIPTION
Zoom :	Digitally zoom in/out of the displayed image. For slides, which are physically in the instrument, a user may digitally zoom in and out of the displayed image. The zooming in and out initially causes system 100 to modify the sensor to display pixel ratio (block 565, Figure 8). As soon as a microscope objective can be used to obtain the view (block 570, Figure 8), the system will automatically use the optimal magnification for a specific zoom level. At this point, a user may again zoom in and out by initially changing a sensor to display pixel ratio and then an objective where a higher magnification is desired.
Pan :	Pan around the sample to display neighboring areas. If a user examines different slides in digital microscope 150, system 100 will move the sample accordingly.
Focus :	In the live image mode, a user is able to change the focus of the instrument. It is possible to trigger an autofocus and to manually adjust the focus with respect to a nominal position.
	In the case of a pre-scanned z-stack scan (i.e., scans of different depths into the tissue, a user can change the focal plane within the z-stack.

[0066] Each view can be viewed in a full screen mode. In this mode, the total screen is covered by the view. Only the magnification of the image, scale indicator and the navigation control are displayed in the image. A user can leave this total screen view mode by selecting (pushing) the "esc" key on keyboard 130.

[0067] At any time during the navigation, a user may save the displayed image (block 560, **Figure 8**).

[0068] On the right hand side of the GUI in **Figure 11** and **Figure 12**, display 120 shows navigation control 850/950, journal 860/960 and image parameter control 870/970.

[0069] Navigation control 850/950 shows an overview image of the active slide. The control indicates the position of the active view (e.g., the live image that can be refreshed at a desired refresh rate). By clicking on the control, the position can be moved. The control also indicates the positions of the annotations and scans which have been added to the slide.

[0070] Below navigation control 850/950, journal 860/960 is located. Journal 860/960 represents the structure of a case. In one embodiment, journal 860/960 contains slide

information, annotations and comments of all slides in a case. Slides can be added and removed from the case. The slides are grouped into two parts. In the upper part of the list, slides in the instrument can be found. A user can examine these slides live. In this case, the user can add scans to these slides by scanning selected areas of the slides. In the lower part of the list (as viewed), pre-scanned slides from the memory in computer are presented.

[0071] In the structure of journal 860/960, each slide has by default three objects: a label image, the slide information and an overview image. The user can add additional objects (annotations, comments, bookmarks and high-resolution scans) to the journal. A user can choose one of the entries in journal 860/960 to jump to a specific image position.

[0072] In one embodiment, journal 860/960 will be a starting point for evaluation of specific regions or for the creation of a report of the considered case. Journal 860/960 also contains the "save session" button. By selecting this button, system 100 will store the session (including all slides, annotations and setting) to memory in computer 110. The file contains the labels, the overview image and the high-resolution scans, which are defined in the journal. A user can restore the session at a later time.

[0073] Below journal 860/960 in **Figure 11** and **Figure 12**, image parameter control 870/970, in one embodiment, is located. Image parameter control 870/970 allows the acquisition parameters of the camera (exposure time, camera gain, etc.) and the image settings (brightness, contrast, color balance, black/white balance, image filters (sharpness, etc.)) to be modified. A user can save settings, for example, a specific staining. Afterwards, the user can access these settings without unfolding the controls.

[0074] Following optional modification of image parameters, a user may save the session. A user may also abort the session. If a user decides to abort the session, the user exits the live image mode. If the user has modified entries in journal 860/960, which are not saved yet, system 100 will ask the user, if the user wants to save the session. Subsequently, system 100 moves to the "slide (group) selection" screen. The user can continue with the next slide(s).

[0075] In a viewing mode, a user can retrieve and view stored images of previously scanned samples (slides) including multiple slides that may make up a case. In this manner, a user can view the images from display 120 directly connected to computer 110 and optical microscope 150. Alternatively, through intranet/internet connection 145 (**Figure 1**), a user can access a memory of system 100 and view images remotely. Intranet/internet connection 145 of system 100 also allows images to be sent from one location to another (e.g., via email).

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US61375703A [0001]
- US4760385A [0005]

Patentkrav**1.** Fremgangsmåde omfattende at:

detektere et live-billede ved en første optisk forstørrelse af én eller mindre af en del af en vævsprøve på et substrat med en sensor (160, 165);

5 uden lagring af det detekterede live-billede,

vise mindst en del af det detekterede live-billede på en skærm (120); og
som reaktion på brugerinput,

10 elektronisk modificere en sensor til at vise pixelforhold ved hvilke det detekterede live-billede vises på skærmen (120);

og

når en tærskelværdiopløsning er nået over hvilken en sensor til at vise pixelforhold ikke længere kan modificeres,
15 automatisk detektere et live-billede af et område af delen af vævsprøven ved en anden optisk forstørrelse der er højere end den første optiske forstørrelse som reaktion på et forsøg på at modificere sensoren til at vise pixelforhold over grænseværdiopløsningen.

20 **2.** Fremgangsmåden ifølge krav 1, yderligere omfattende lagring af det detekterede live-billede som reaktion på brugerinput.

3. Fremgangsmåden ifølge krav 1, hvor modificering af sensoren til at vise pixelforhold omfatter overgang fra et forhold større end én-til-én til et forhold på
25 én-til-én som reaktion på brugerinput.

4. Fremgangsmåden ifølge krav 1, yderligere omfattende visning af det detekterede live-billede ved den anden optiske forstørrelse på skærmen (120).

5. Fremgangsmåden ifølge krav 1, hvor et område af delen af vævet detekteret ved en anden optisk forstørrelse svarer til et område valgt af brugerinputtet.

6. Fremgangsmåden ifølge krav 1, yderligere omfatter at detektere et billede af en markør på substratet og vise billedet på skærmen (120).

7. Fremgangsmåden ifølge krav 6, hvor at detektere et billede af en vævsprøve på et substrat omfatter at detektere et lysfeltbillede og detektere et billede af markøren omfatter at detektere et billede under anvendelse af reflektans.

10

8. Apparat omfattende:

et digitalt mikroskop (150) omfattende:

en første billedsensor (160) og en anden billedsensor (165);

et bord (180) konfigureret til at bære mindst et objektglas;

15

et første optisk billeddannende under-system (168) indrettet mellem den første billedsensor (160) og bordet (180), det første optiske billeddannende under-system (168) konfigureret til at projicere et billede med en optisk forstørrelse på én eller mindre;

20

et andet optisk billeddannende under-system (170) indrettet mellem den anden billedsensor (165) og bordet (180), det andet optiske billeddannende under-system (170) konfigureret til at projicere et billede med en optisk forstørrelse større end én; og

et belysnings-under-system omfattende mindst en lyskilde (195, 196);

kendetegnet ved at apparatet yderligere omfatter:

25

en computer (110) koblet til det digitale mikroskop (150) og operabel til at lede et live-billede taget af den første billeddannende sensor (160) eller den anden billedsensor (165) af en del af et objektglas på bordet (180) projiceret gennem det første optiske billeddannende under-system (168) eller det andet optiske

5 bileddannende under-system (170), og hvor computeren (110) er operabel til automatisk at lede et live-billede taget gennem det andet optiske bileddannende under-system (170) som reaktion på brugerinput til at modificere en sensor til at vise pixelforhold over en grænseværdi-opløsning af et live-billede opnået gennem det første optiske bileddannende under-system (168); og

10 en skærm (120) koblet til computeren (110) og, uden at lagre et billede, er computeren (110) operabel til at vise det live-billede taget af den første billedsensor (160) eller den anden billedsensor (165) og til at opdatere billedet ved en forudbestemt opdateringshastighed.

9. Apparatet ifølge krav 8, hvor computeren (110) er operabel til at lede et billede taget af konsekutive multiple billeder af et objektglas og til at binde de multiple
15 billeder sammen for at danne et samlet billede, et første af de tagne multiple billeder designeres en fast reference til hvilken de andre af de tagne multiple billeder bindes for at danne det samlede billede.

10. Apparatet ifølge krav 9, hvor computeren (110) er konfigureret således at de
20 konsekutive multiple billeder gemmes.

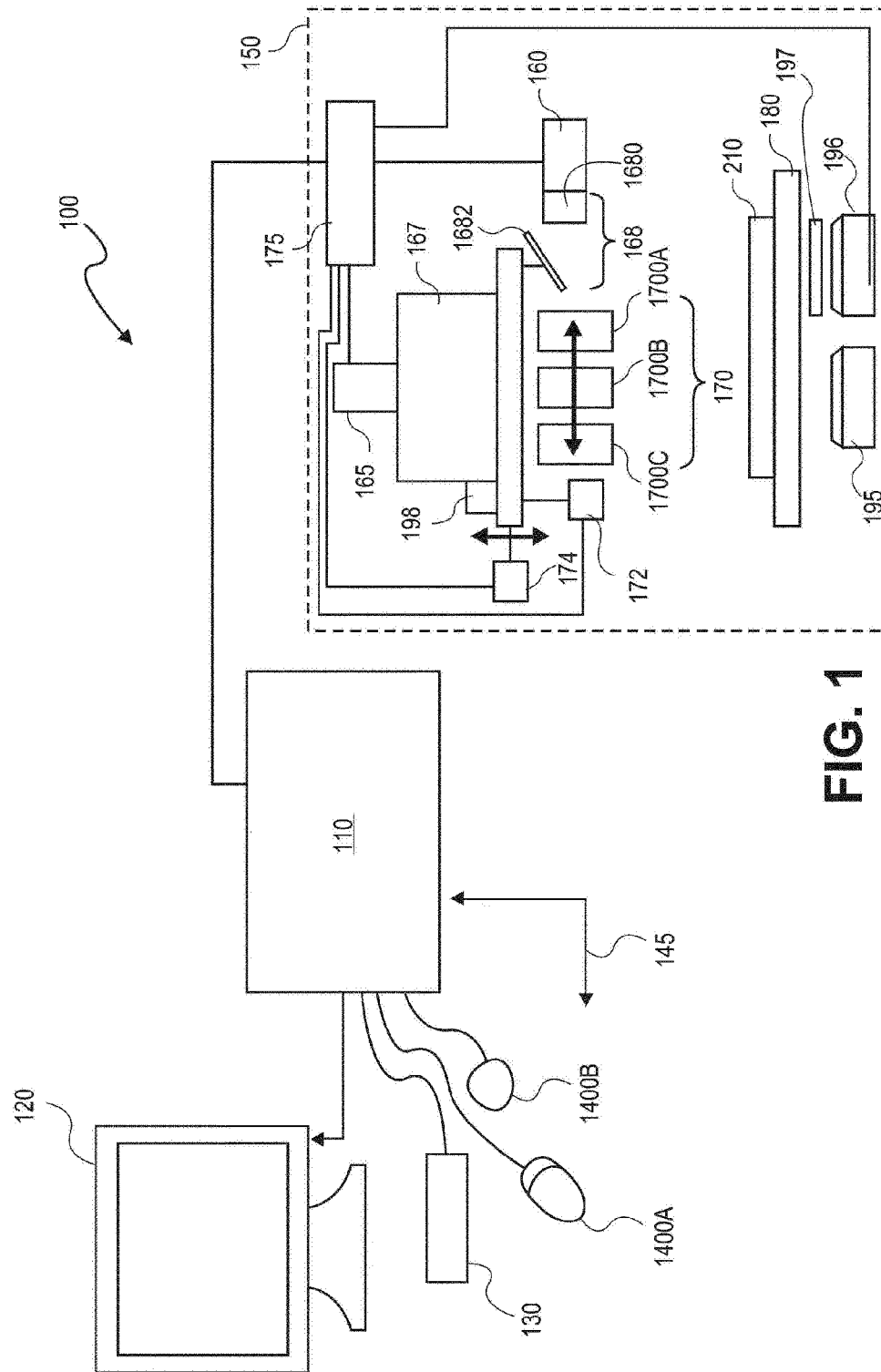
11. Apparatet ifølge krav 9, hvor retningen af billedoptagelsen af multiple billeder omfatter retning til at fange billeder af respektive områder af et objektglas og tilgrænsende tagne billeder bindes sammen baseret på opretning af fælles
25 landmarks mellem billederne.

12. Apparatet ifølge krav 11, hvor opretningen er langs multiple akser.

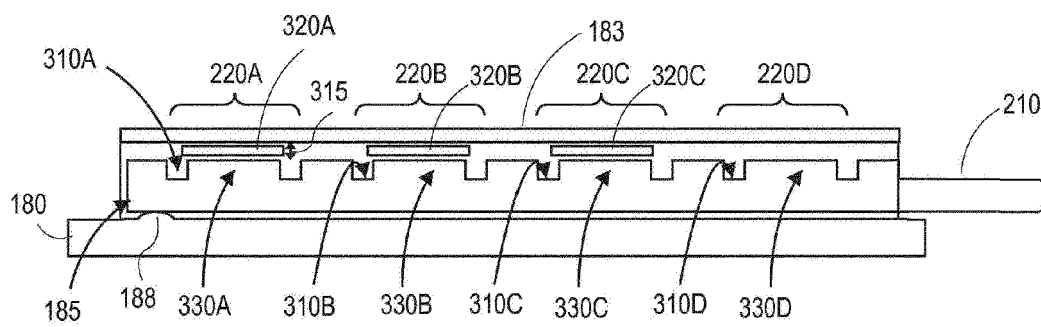
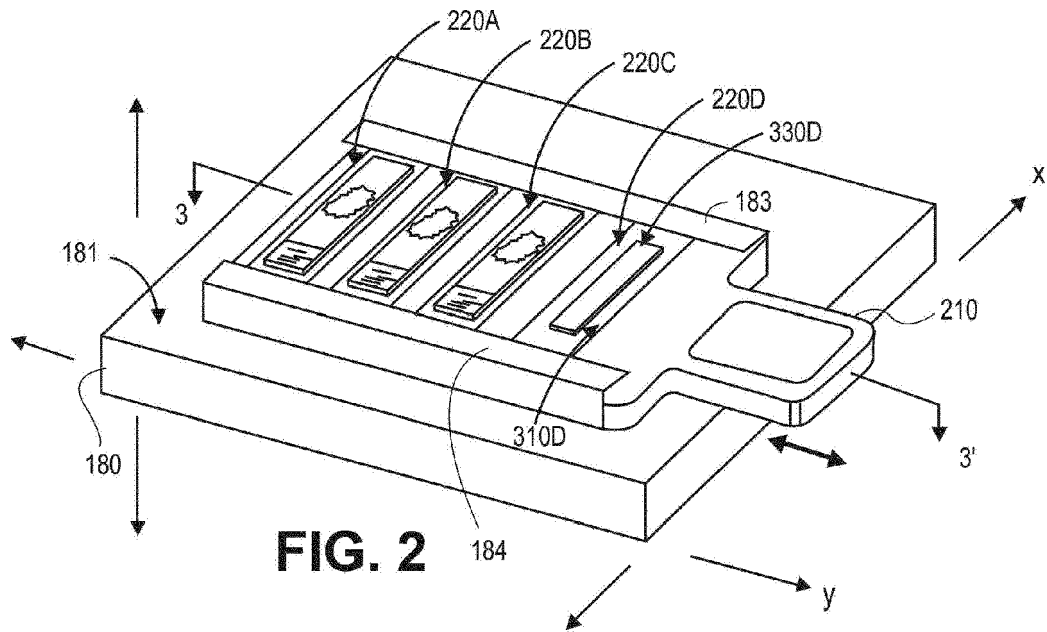
13. Apparatet ifølge krav 9, hvor computeren (110) er operabel til at lede et
30 billede taget af en markør på objektglasset og til at vise det samlede billede og markøren på skærmen (120), hvor markørbilledet er tilgrænsende det samlede billede.

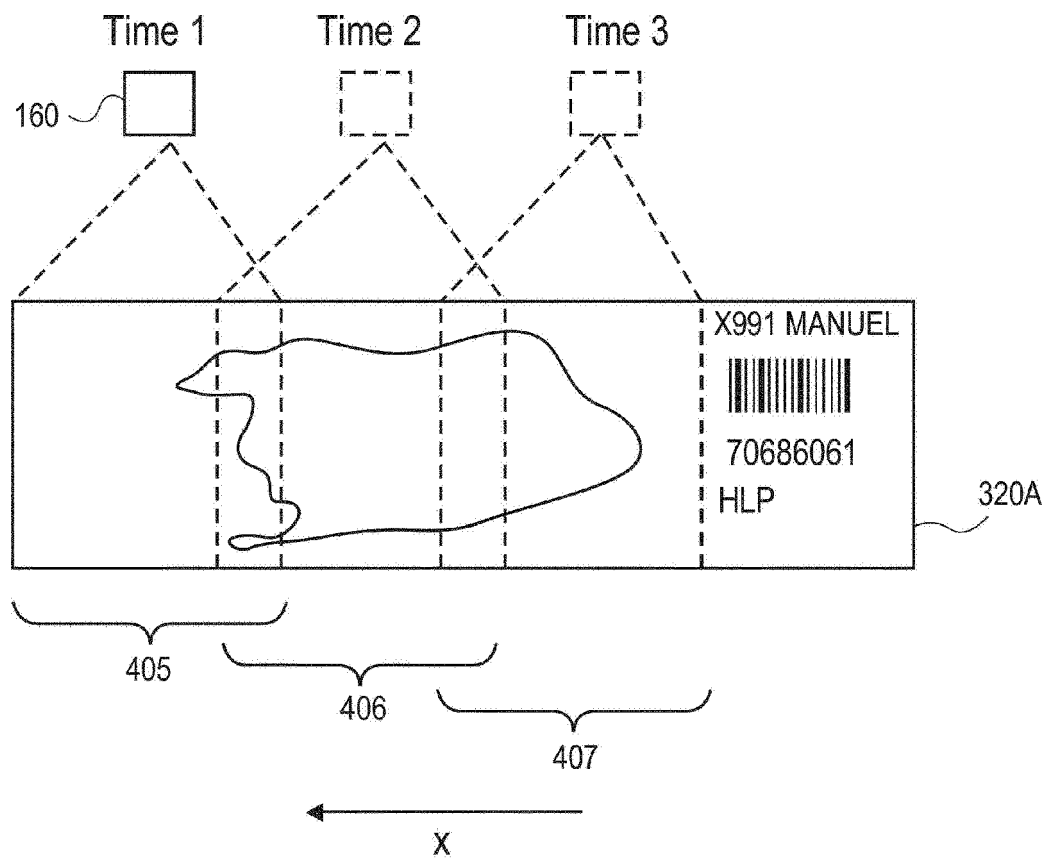
14. Apparatet ifølge krav 13, hvor computeren (110) er operabel til at udføre at det samlede billede og markørbillede bliver gemt.

DRAWINGS



FILE



**FIG. 4**

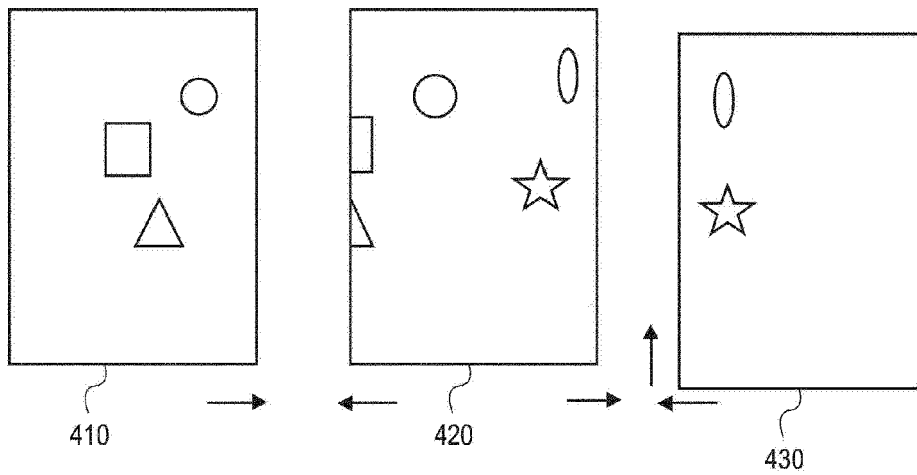


FIG. 5

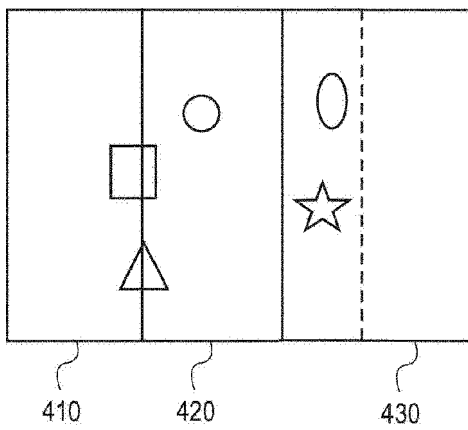


FIG. 6

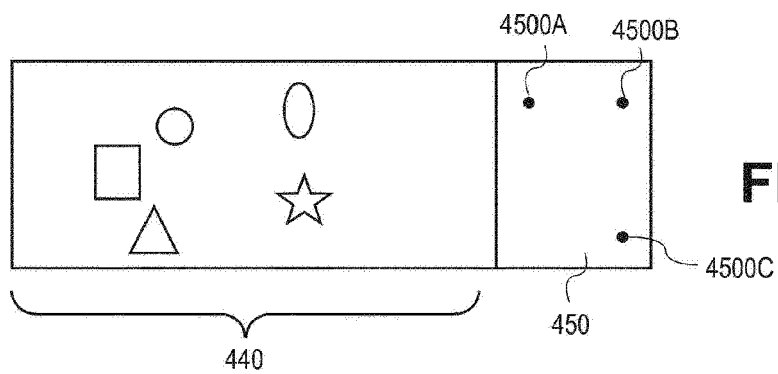
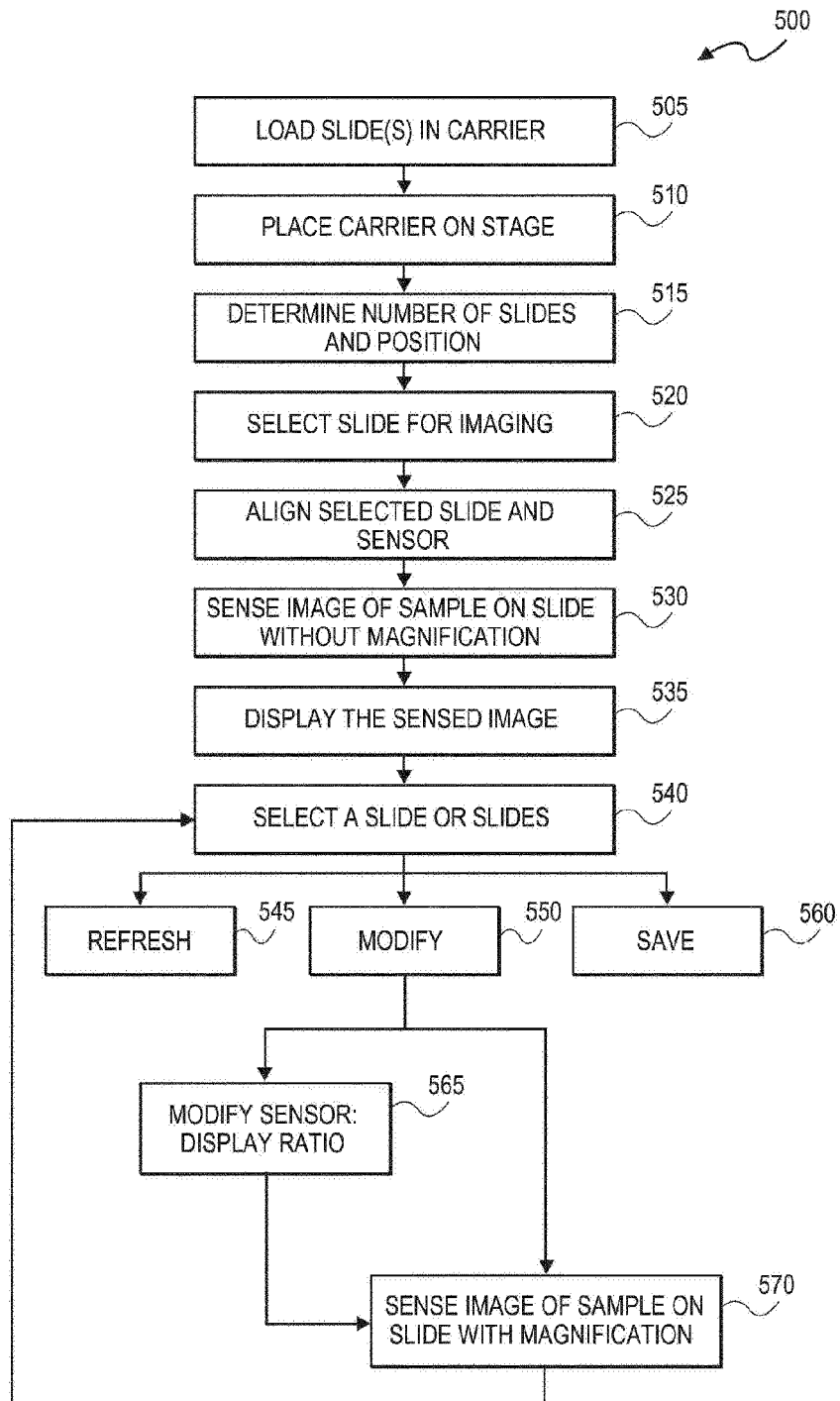


FIG. 7

**FIG. 8**

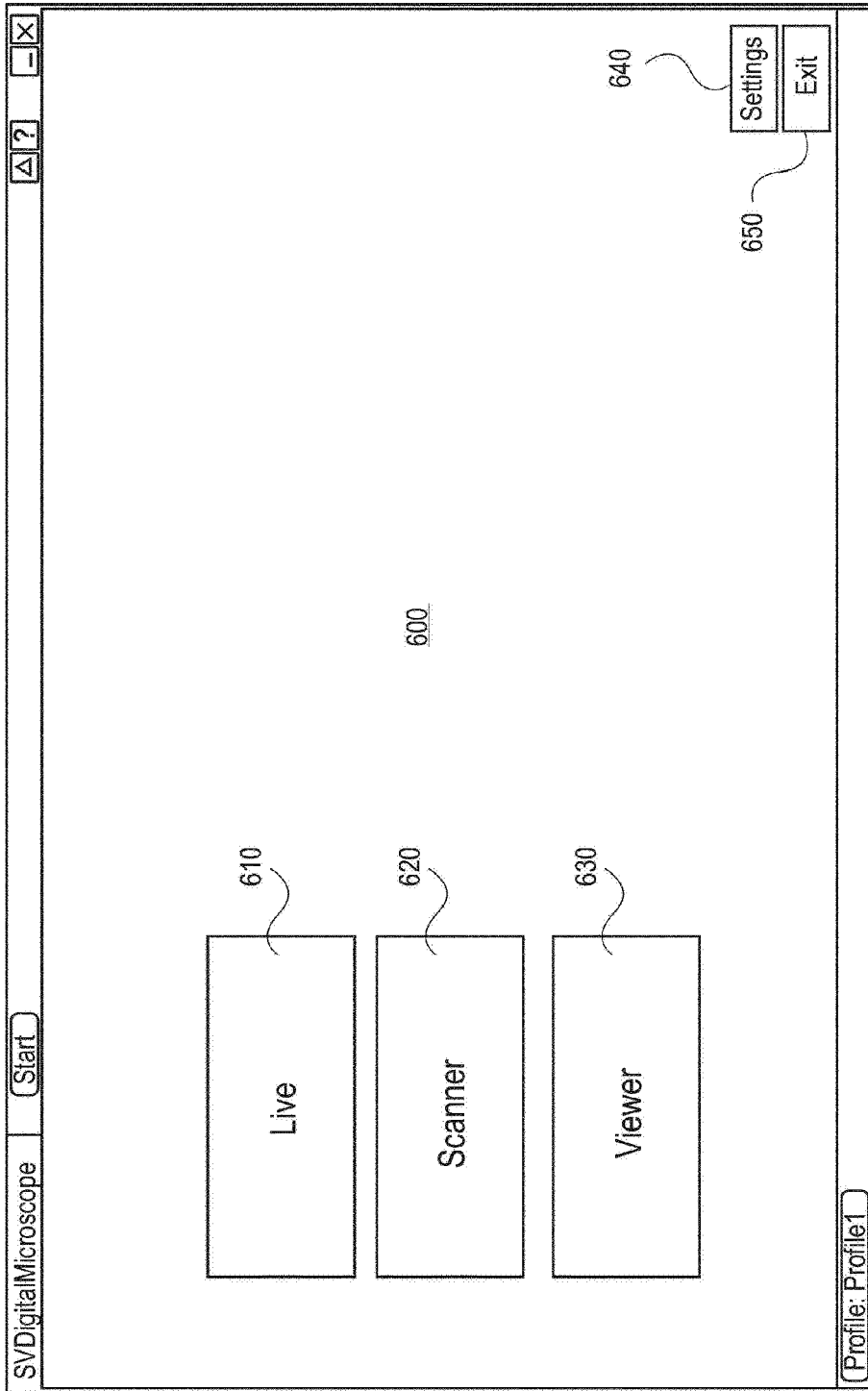
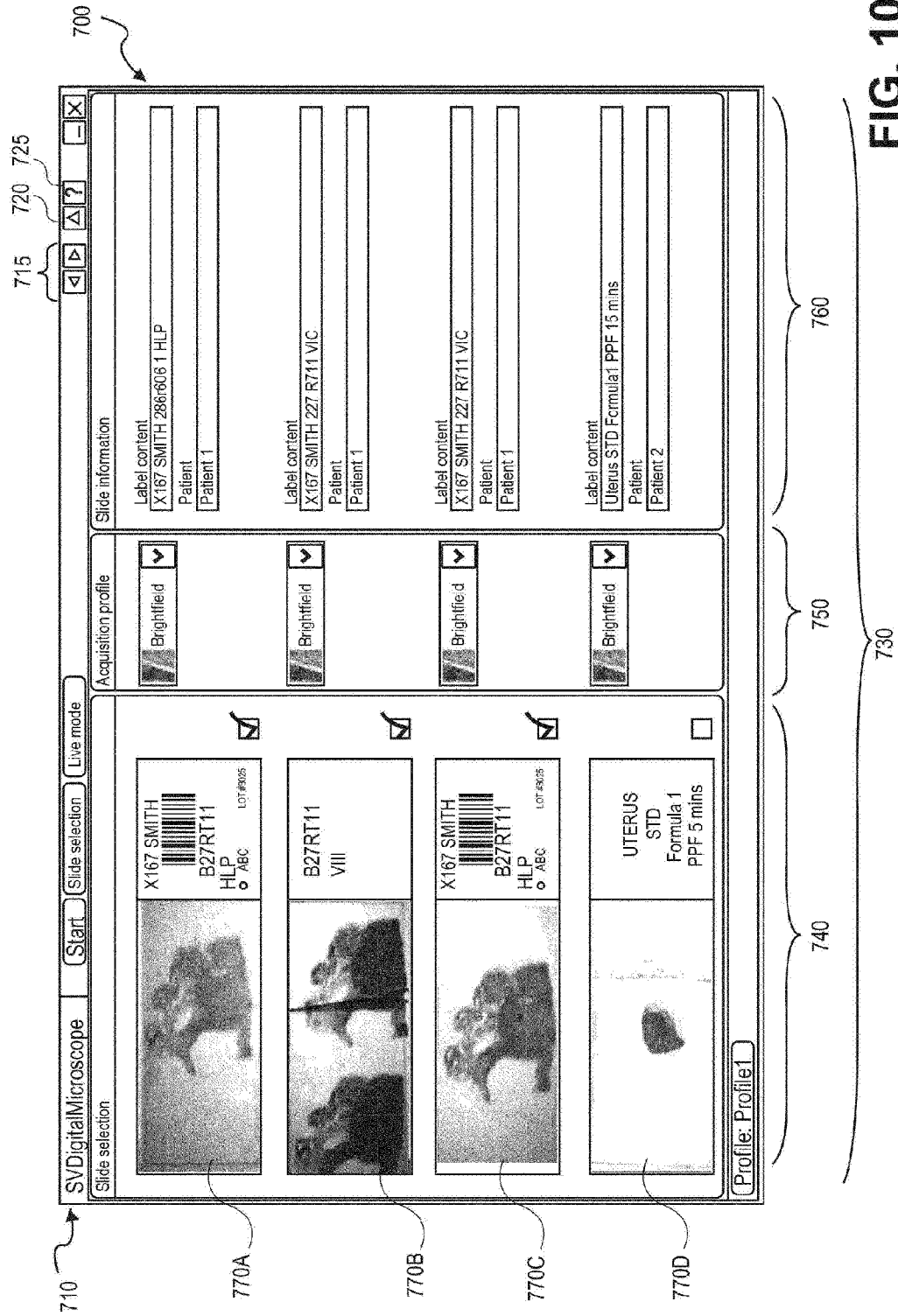


FIG. 9



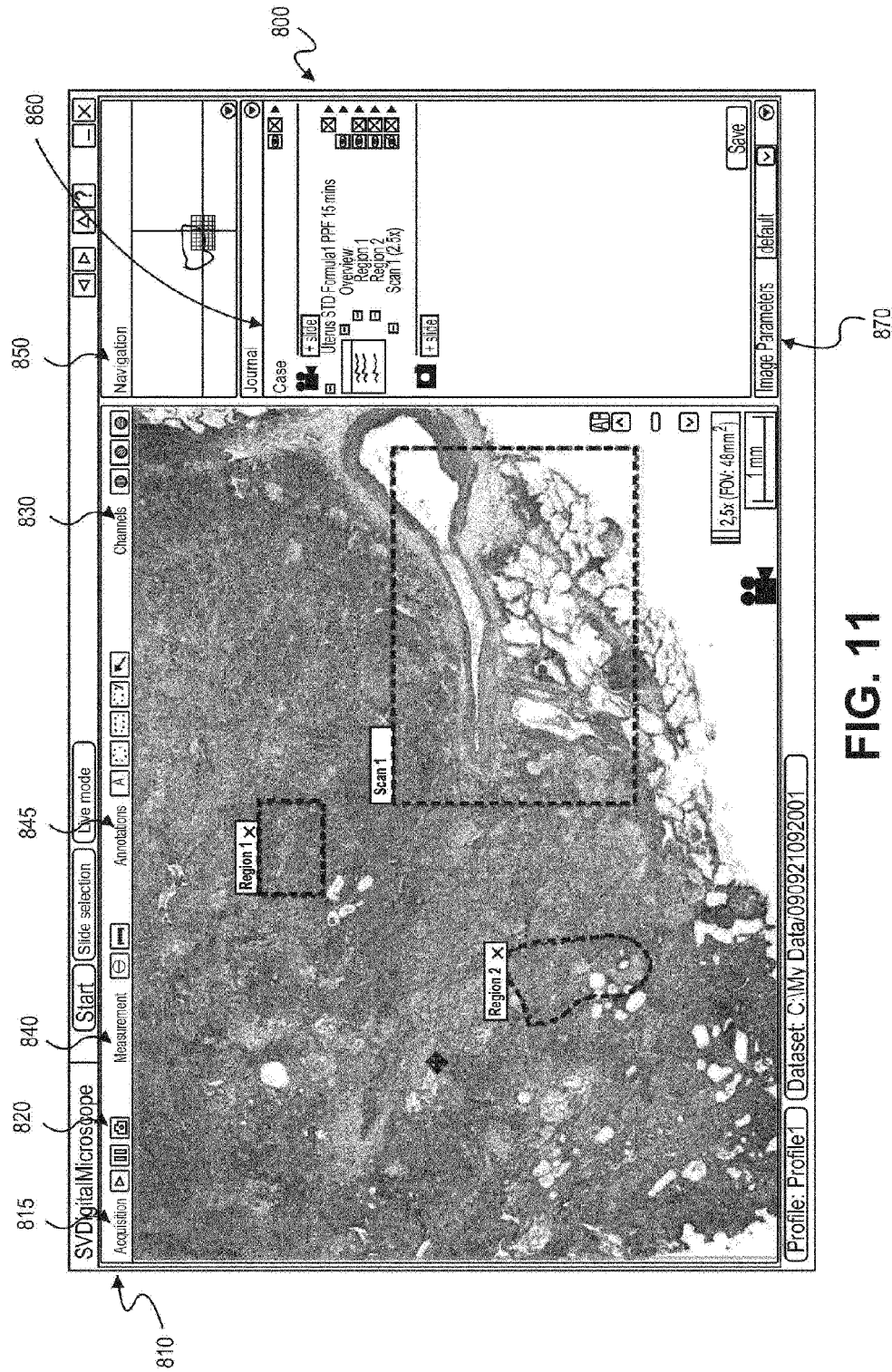


FIG. 11

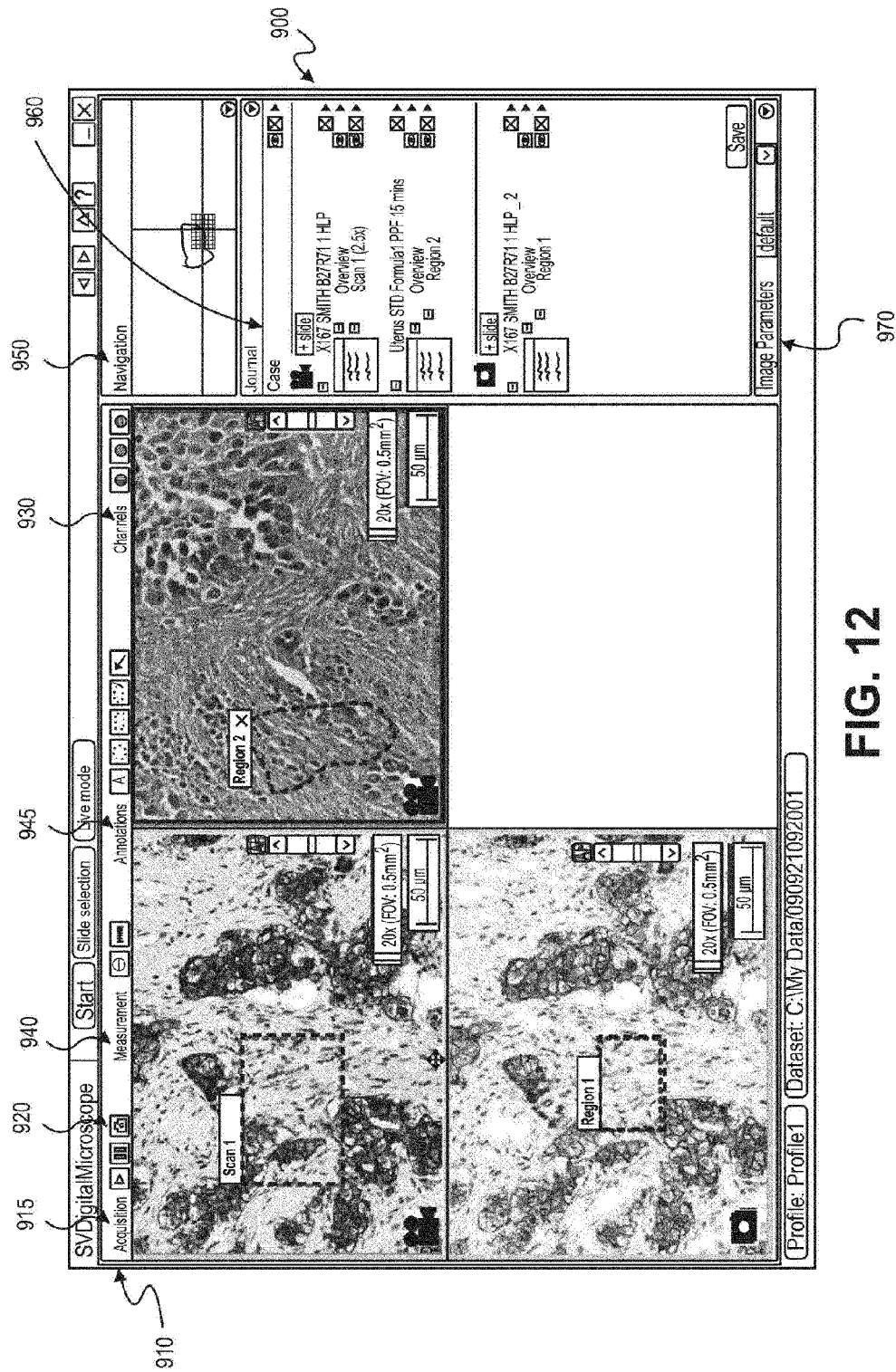


FIG. 12