(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 September 2002 (06.09.2002)

PCT

(10) International Publication Number
WO 02/069015 A2

(51) International Patent Classification[7]: G02B 21/00, 7/04

(21) International Application Number: PCT/US02/05370

(22) International Filing Date: 21 February 2002 (21.02.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/788,666 21 February 2001 (21.02.2001) US

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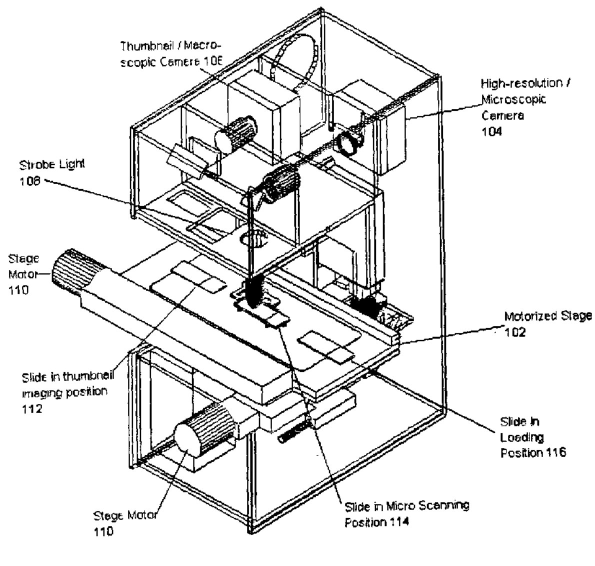
(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: A METHOD FOR MAINTAINING HIGH-QUALITY FOCUS DURING HIGH-THROUGHPUT, MICROSCOPIC DIGITAL MONTAGE IMAGING



(57) Abstract: Today's technology allows montage images of large areas to be captured, stored and displayed at the resolution limit of the microscope optics. The invention reduces the overall time to capture a microscope slide by reducing the overhead associated with refocusing the optics at each tile location. Using a macroscopic image of the region to be scanned, representative focus positions are selected based on a predefined set of image characteristics. Prior to montage scanning, these focus positions are placed under the microscope optics and a best-focus position determined. A surface is fit to the resulting three-dimensional data. The parameters that define this surface are feed into the scanning control component to allow high-quality focused images to be acquired throughout the scanning process, eliminating the required stop, refocus, acquire image processing steps used in traditional montage imaging systems.

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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

A Method for Maintaining High-quality Focus During High-throughput, Microscopic Digital Montage Imaging

FIELD OF THE INVENTION

The present invention relates to microscopic digital imaging of complete tissue sections for medical and research use. In particular it describes a method for maintaining high quality focus during high throughput montage imaging of microscope slides.

BACKGROUND OF THE INVENTION

Laboratories in many biomedical specialties, such as anatomic pathology, hematology, and microbiology, examine tissue under a microscope for the presence and the nature of disease. In recent years, these laboratories have shown a growing interest in microscopic digital imaging as an adjunct to direct visual examination. Digital imaging has a number of advantages including the ability to document disease, share findings, collaborate (as in telemedicine), and analyze morphologic findings by computer. Though numerous studies have shown that digital image quality is acceptable for most clinical and research use, some aspects of microscopic digital imaging are limited in application. Perhaps the most important limitation to microscopic digital imaging is a "sub-sampling" problem encountered in all single frame images. The sub-sampling problem has two components: a field of view problem and a resolution-based problem. The field of view problem occurs when an investigator looking at a single frame cannot determine what lies outside the view of an image on a slide. The resolution-based problem occurs when the investigator looking at an image is limited to the resolution of the image. The investigator cannot "zoom in" for a closer examination or "zoom out" for a bird's eye view. Significantly, the field of view and resolution-based problems are inversely related. Thus, as one increases magnification to improve resolution, one decreases the field of view.

To get around the limitation of single frame imaging, several neighboring images can be tiled together to form a montage image or "virtual slide". To accomplish this, a robotic microscope systematically scans the entire slide, taking an image at every field. The individual images are then "knitted" together in a software application to form a very large data set with very appealing properties. The robotic microscope can span the entire slide area at a resolution limited only by the power of the optical system and camera. Software exists to display this data set at any resolution on a computer screen, allowing the user to zoom in, zoom out, and pan around the data set as if using a physical microscope. The data set can be stored for documentation, shared over the Internet, or analyzed by computer programs.

The "virtual slide" option has some limitations, however. One of the limitations is file size. For an average tissue section, the data generated at 0.33 um/pixel can be between two and five gigabytes uncompressed. In a extreme case, the data generated from one slide can be up to thirty-six gigabytes.

A much more difficult limitation with the prior systems is an image capture time problem. Given an optical primary magnification of twenty and a two-third inch CCD, the system field of view is approximately (8.8 mm x 6.6 mm) / 20 = 0.44x0.33 mm. A standard tissue section of approximately 2.25 square centimeters, therefore, requires approximately fifteen hundred fields to cover the tissue alone.

Field rate in montage systems is limited by three factors – camera frame rate, image processing speed, and the rate of slide motion between fields. Given today's technology, the rate of slide motion is a significant limiting factor largely because the existing imaging systems require the slide to come to a stop at the center of each field to capture a blur free image of the field.

The three dimensional characteristic of the tissue sample and the slide places additional limitations on the imaging system. Like all lenses, microscope optics have a finite depth of field, the distance within which objects will appear to be focused. A typical depth of field is about 8 microns for a 10x objective, and in general, as the magnification increases, the depth of field decreases. While microscope slides are polished glass, the flatness of the slide can vary on the order of 50 microns or more across the slide. The variations in the tissue sample thickness and any defects associated with placing the sample on the slide such as folds in the tissue, also affect the optimal position of the slide with respect to the imaging optics. The magnitude of the optimal position and the limited depth of field of the microscope optics require the focus to be adjusted as the system moves from field to field. In order to determine the optimal focal position, multiple images must be acquired as the slide is displaced along the optical axis (perpendicular to the scanning plane) and a quantitative value such as contrast calculated for each image. The direction and the amount of displacement required to maintain high-quality focus is dependent on the three dimensional structure of the slide and tissue specimen. Given that the average tissue section mentioned above requires fifteen hundred image fields, the time required to refocus at each tile can contribute substantially to the overall scan time.

Thus, a system is needed to reduce the overhead associated with refocusing while maintaining efficiency and image quality. This invention relates to maintaining high-quality focus as it is scanned without having to rely on refocusing during the scanning process.

SUMMARY OF THE INVENTION

Accordingly the invention relates to a method and system for ensuring that a scanning process captures a high-quality montage image of a slide by enabling accurate focus control of raw image tiles of the slide. The system includes a focus point selection component, a focal surface determination component, and a scan control component. The focus point selection component evaluates tissue regions of a thumbnail image and selects several points to initially focus microscope optics on a point-by-point basis. The focal surface determination component uses focus positions to generate a three-dimensional data set corresponding to optical specimen distance at each stage location, wherein data points in the data set are used as input to a routine that generates control parameters for a slide scanning process. The scan control component captures the high-quality montage image by maintaining motion of a stage and synchronization of a microscopic imaging system during montage image acquisition. The scan control component thus, enables accurate focus control of optical elements without requiring stopping and refocusing of the stage at each tile location and substantially reduces montage acquisition time. The system also includes means for placing focus positions from the focus point selection component into microscope optics in the focal surface determination component and for passing tissue information and surface parameters to the scan control component.

It is therefore an object of the invention to provide an automated, microscopic imaging system for whole slide montage in which standard microscope optics, an off-the-shelf camera and a simple motorized stage can be used to produce perfectly aligned, well-focused image tiles, and acquire these images at a speed limited by the camera frame rate. The present invention uses the fact that the scale length associated with the three dimensional nature of the slide and tissue specimen is large compared to the field of view of a single camera image. By pre-sampling key regions on the slide, a focal surface can be calculated, control parameters generated based on this surface, and the optimal stage position with respect to the optical elements maintained throughout the scanning process, insuring high-quality images with minimal overhead.

Additional features and advantages of the invention will be set forth in the description that follows, and in part will be apparent from the description, or may be learned by practice of the invention. The objectives and advantages of the invention to be realized and attained by the microscopic image capture system will be pointed out in the written description and claims hereof as well as the appended drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are included to provide a further understanding of the invention and are incorporated in and constitute a part of this specification, illustrate embodiments of the invention that together with the description serve to explain the principles of the invention.

Fig 1 illustrates a side view of the system in a preferred embodiment;

Fig 2 illustrates the results of the focus point selection component on a sample image; and

Fig. 3 illustrates a generated three-dimensional data set.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Reference will now be made in detail to the preferred embodiments of the present invention, an example of which is illustrated in the accompanying drawing. The following paragraphs describe the functionality of the inventive system and method for focus controlled, high throughput montage imaging of microscope slides using a standard microscope, camera, and a motorized mechanical stage.

Fig. 1 illustrates a preferred embodiment of the invention. In this embodiment, a slide to be imaged is placed in a slide holder on a motorized stage. A low-resolution image obtained from the macroscopic camera is processed to determine tissue locations on the slide. As is obvious to one skilled in the art, a single image or multiple low-resolution images can also be acquired through the microscopic optics with the proper objectives. All of which can be processed to determine tissue locations on the slides.

The tissue locations can then be used to generate control parameters for the stage and microscopic camera to ensure that the scanning process captures high quality images of only the tissue regions. Specifically in a preferred embodiment, a pre-scan processing of the tissue locations includes a focus point selection component, a focal surface determination component and a scan control component. The first two components ensure that the last component captures a high-quality montage image, by enabling accurate focus control of the optical elements without requiring the stage to be stopped and refocused at each tile location, substantially reducing the acquisition time.

To achieve good focus for an entire slide, the surface that best represents the focal position of the sample with respect to the optical elements is determined and used to automatically control the focus as the tissue is scanned under the microscope optics.

In the preferred embodiment, the focus point selection component evaluates the tissue regions of the thumbnail image and selects several points to initially focus the microscope optics on a point-by-point basis. These positions are selected based on their relative contrast within the thumbnail image, or an alternative selection criteria, and their overall distribution of points with respect to the tissue coverage area.

In alternative embodiments, the focus points are either user definable through an input file or through a suitable user interface. In addition, for cases where the specimen locations are reproducible on the slides, the focus points can be predefined and repeated for each slide without the use of a macroscopic image or any preprocessing to find the tissue regions.

Once selected, each focus position is placed under the microscope optics in the focal surface determination component, and an auto-focus program determines the best-fit focus at each position. This generates a three dimensional data set corresponding to the optimal specimen distance at each stage location. These data points are used as input to a surface fitting routine that generates the necessary control parameters for the slide scanning process. The number of data points required will depend on the actual three-dimensional structure defined by the specimen and slide, and the geometrical dimension of the surface to be fit. Once the surface has been determined, an error function can be calculated to determine the fit accuracy. If the accuracy exceeds expected limits, additional points can be acquired and the surface recalculated.

At the completion, the tissue information and the surface parameters are passed to the scan control component. This component is responsible for the motion of the stage and synchronization of the microscopic imaging system during montage image acquisition. To achieve accurate, well-aligned tiled images, the specimen must be positioned such that each camera image is aligned within the equivalent single pixel spacing in real or stage space (camera pixel size divided by the overall optical magnification). This usually entails a stage step of δx and δy where each step is directly related to the effective pixel size and the number of image pixels in the x and y directions respectively. For example, a 1300x1030 pixel, 10 μm square pixel camera operated at 20^x magnification results in $\delta x = 10\ \mu m * 1300 / 20 = 650\ \mu m$ and $\delta y = 515\ \mu m$. To maintain focus during the scanning process, the stage must be positioned at the proper focal position as determined by the focus surface parameters: $z_{ij} = f(x_i, y_j)$ where $x_i = x_o + i * \delta x$ and $y_i = y_o + i * \delta y$.

Image montage scanning is traditionally accomplished by either scanning by rows or columns. Assuming that tiling is completed by scanning across row and stepping in the y-direction, if the scanning process is completed by stepping in the row or x-direction, the stage is simply positioned at the appropriate position given by $z_{,ij} = f(x_i, y_j)$. Thereafter, the stage is stopped and an image is acquired. If imaging is accomplished during continuous motion of the stage in the x-direction via a line scan camera or alternative imaging arrangement, the vertical velocity as a function of x_i and subsequently of time, can be computed from the derivative of the focal surface. This velocity can be used to control the optical position and maintain focus as images are acquired continuously across the row. Depending on the surface, a new velocity function may be required for each row scanned based on the stepped y-position.

Fig 2 represents the results of the focus point selection component. This figure shows the thumbnail or macroscopic image of the region to be scanned. The dark spots overlaid on the figure represent the positions selected by the focus point selection component. These positions are placed under the microscope and auto-focused on each location. Fig. 3 illustrates the three-dimensional data set generated from Fig. 2. For this slide, the best fit was planar ($z(x,y) = dz/dx\ x + dz/dy\ y + z0$) with the parameters shown in Fig. 3.

The foregoing description has been directed to specific embodiments of this invention. It will be apparent, however, that other variations and modifications may be made to the described embodiments, with the attainment of some or all of their advantages. Therefore, it is the object of the appended claims to cover all such variations and modifications as come within the true spirit and scope of the invention.

What is Claimed

1. A method for ensuring that a scanning process captures a high-quality montage image of a slide by enabling accurate focus control of image tiles of the slide, the method comprising the steps of:

evaluating tissue regions of a thumbnail image and selecting several focus positions to initially focus microscope optics on a point-by-point basis;

placing under the microscope optics each focus position, wherein the best-fit focus at each position is determined;

generating a three-dimensional data set corresponding to an optimal specimen distance at each stage location, wherein data points in the data set are used as input to a routine that generates necessary control parameters for a slide scanning process; and

passing the tissue information and control parameters to a component that is responsible for the motion of a stage and synchronization of a microscopic imaging system during a montage image acquisition.

2. The method of claim 1, further comprising the step of determining a surface, on the slide, that represents a focal position of a tissue sample and using information from the surface to automatically control focus as the tissue sample is scanned under microscope optics.

3. The method of claim 1, wherein the step of evaluating further comprises the step of selecting positions based on their relative contrast within the thumbnail image and their overall distribution of points with respect to a tissue coverage area.

4. The method of claim 1, wherein the step of evaluating further comprises the step of allowing a user to define focus points through an input file.

5. The method of claim 1, wherein the step of evaluating further comprises the step of allowing a user to define focus points through a user interface.

6. The method of claim 1, wherein in the step of generating the number of data points required depends on the actual three-dimensional structure defined by the specimen and the slide and the geometrical dimension of the surface to be fit.

7. The method of claim 6, further comprising the step of calculating an error function to determine a fit accuracy once the surface has been determined, wherein if the accuracy exceeds expected limits additional points can be acquired and the surface is recalculated.

8. The method of claim 1, further comprising the step of positioning the specimen such that each camera image is aligned within the equivalent single pixel spacing.

9. The method of claim 1, further comprising the step of positioning the stage at a proper focal position as determined by focus surface parameters.

10. The method of claim 1, further comprising the step of computing a vertical velocity, as a function of a parameter and subsequently of time, from the derivative of the focal surface if imaging is accomplished during continuous motion of the stage in a x-direction via an imaging arrangement.

11. The method of claim 10, further comprising the step of using the velocity to control the optical position and maintain focus as images are acquired continuously across a row.

12. The method of claim 11, further comprising the step of requiring a new velocity function for each row scanned based on a stepped y-position.

13. A system for ensuring that a scanning process captures a high-quality montage image of a slide by enabling accurate focus control of raw image tiles of the slide, the system comprising:

a focus point selection component that evaluates tissue regions of a thumbnail image and selects several points to initially focus microscope optics on a point-by-point basis;

a focal surface determination component that uses focus positions to generate a three-dimensional data set corresponding to optical specimen distance at each stage location, wherein data points in the data set are used as input to a routine that generates control parameters for a slide scanning process;

a scan control component that captures the high-quality montage image by maintaining motion of a stage and synchronization of a microscopic imaging system during montage image acquisition, thereby enabling accurate focus control of optical elements without requiring stopping and refocusing of the stage at each tile location and substantially reducing montage acquisition time; and

means for placing focus positions from the focus point selection component into microscope optics in the focal surface determination component and for passing tissue information and surface parameters to the scan control component.

14. The system of claim 13, wherein focus point selection component selects positions based on their relative contrast within the thumbnail image and their overall distribution of points with respect to a tissue coverage area.

15. The system of claim 13, wherein focus points are definable through an input file.

16. The system of claim 13, wherein focus points are definable through a user interface.

17. The system of claim 13, wherein focus points are predefined and repeated for each slide without any preprocessing to find tissue regions, when specimen locations are reproducible on the slides.

18. The system of claim 13, wherein the number of data points required depends on the actual three-dimensional structure defined by the specimen and the slide and the geometrical dimension of the surface to be fit.

19. The system of claim 18, wherein once the surface is determined, an error function is calculated to determine a fit accuracy, wherein if the accuracy exceeds expected limits additional points can be acquired and the surface is recalculated.

20. The system of claim 13, wherein specimen are positions such that each camera image is aligned within the equivalent single pixel spacing.

21. The system of claim 13, wherein to maintain focus during the scanning process, the stage is position at a proper focal position as determined by the focus surface parameters.

22. The system of claim 13, wherein a vertical velocity is computed, as a function of a parameter and subsequently of time from the derivative, of the focal surface if imaging is accomplished during continuous motion of the stage in the x-direction via an imaging arrangement.

23. The system of claim 22, wherein the velocity is used to control the optical position and maintain focus as images are acquired continuously across a row.

24. The system of claim 23, wherein a new velocity function is required for each row scanned based on a stepped y-position.

25. A system for ensuring that a scanning process captures a high-quality montage image of a slide by enabling accurate focus control of raw image tiles of the slide, the system comprising:

a plurality of first components for evaluating tissue regions of a thumbnail image, selecting several points to initially focus microscope optics on a point-by-point basis and generating a three-dimensional data set corresponding to optical specimen distance at each stage location, wherein data points in the data set are used as input to a routine that generates control parameters for a slide scanning process;

a second component that captures the high-quality montage image by maintaining motion of a stage and synchronization of a microscopic imaging system during montage image acquisition, thereby enabling accurate focus control of optical elements without requiring stopping and refocusing of the stage at each tile location and substantially reducing montage acquisition time; and

means for passing information between the plurality of first components and for passing information between the plurality of first components and the second component.

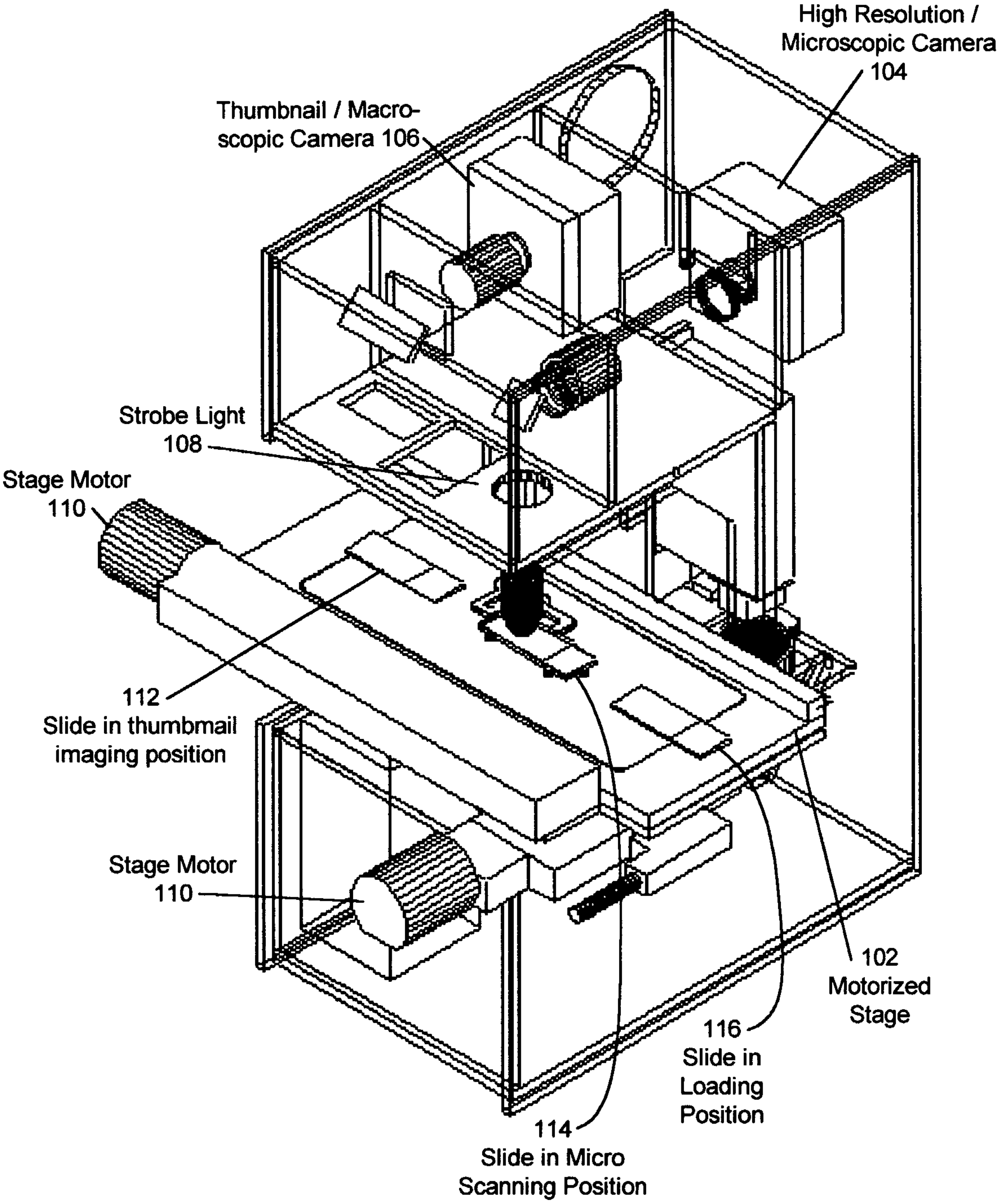
High Resolution / Microscopic Camera 104
Thumbnail / Macro-scopic Camera 106
Strobe Light 108
Stage Motor 110
112 Slide in thumbmail imaging position
Stage Motor 110
102 Motorized Stage
116 Slide in Loading Position
114 Slide in Micro Scanning Position

Fig. 1

Fig. 2

Table 1 Representative focus position from Fig 2

x-position	y-position	z-position	error from fit
-560176	-242615	7780	0.71
-509376	-234148	7660	13.43
-577109	-205362	7430	-100.53
-522076	-203668	7390	-51.43
-577109	-188428	7400	-6.72
-539009	-173189	7250	7.79
-562293	-220602	7640	18.70
-547476	-220602	7610	9.35
-510000	-220000	7570	26.00
-540000	-200000	7430	-9.60
-535000	-230000	7710	58.04
-558059	-205362	7460	-43.97
-507259	-205362	7340	-93.16
-572876	-178268	7400	73.45
-545359	-171495	7280	41.32
-524193	-218908	7610	54.19

Best fit is planar with parameters:
dz/dx = -0.00139400 dz/dy = -0.00731100 z0 = 5224.64

Fig. 3