MICRODISSECTION WITH COUNTER-CHANGE-IN-TILT INTERFEROMETRY

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Related Applications

[0001] This application claims the benefit of priority of U. S. Provisional Application Nos. 62/053,785, filed September 22, 2014 and 62/054,359, filed September 23, 2014, the entire contents of which application(s) are incorporated herein by reference.

Field of the Invention

[0002] The present invention relates generally to microdissection with counter-change-in-tilt interferometry and more particularly, but not exclusively, to microdissection with rotational-shear interferometry.

Background of the Invention

[0003] Microdissection is an important tool in the life sciences. A good introduction is given by Emily Willingham in an article titled "Laser Microdissection Systems"; an excerpt is as follows:

> Years ago, tissue samples mounted on glass slides were consigned to a future of histological or immunohistological examination, and then, to the trash bin. Postmicroscopic polymerase chain reaction (PCR) analyses of interesting cellular colonies were impossible, because there was no way to get those cells off the slides, or to isolate those few cells from the vast majority of uninteresting ones. But thanks to laser microdissection (LMD), that is no longer the case.
>
> LMD allows researchers to target a specific cell—or even chromosome—and excise it from the cells that surround it on a slide, or in some cases, on a culture dish. Researchers can use some LMD systems to destroy a particular cell in the midst of surrounding tissue of interest, a process referred to as negative selection. These instruments have other applications in microsurgery, microinjection, in vitro fertilization, and cell fusion research.
>
> LMD dates to the 1970s, when researchers used lasers to trap and manipulate cells. In the mid-1990s, under the National Institutes of Health at the National Institute of Child Health

> and Human Development and the National Cancer Institute, researchers developed the first LMD system as a research prototype. The NIH later worked in partnership with Mountain View, Calif.-based Arcturus Engineering, to develop a commercial system.
>
> The field has matured since that first venture, and modern LMD systems vary in the cell-capture method, system configuration, and applications. Some systems offer the possibility of working with cells straight from a culture dish. Others allow researchers to avoid touching a specimen by catapulting or dropping the untouched, dissected cells straight into a capture tube or lifting the protected specimen directly into the lid of a micro-centrifuge tube.

(E. Willingham, "Laser Microdissection Systems," The Scientist, May 13, 2002.)

[0004] Various difficulties exist with current microdissection systems and imagers used therewith. For example, the region of focus of an imager used to guide the microdissection may not encompass a sufficiently large portion of a sample of interest. A larger portion of the sample may be of interest since the other portions (not in the region of focus) may contain material that could be dissected to improve the yield and thus improve the reliability of analyses of the material recovered during the dissection. Tilt misalignment between the imager and the sample may contribute to this difficulty, but its correction can require exacting effort if a mechanical adjustment is employed. In addition, the choice of construction materials is constrained to materials able to maintain shape and dimension against even small thermal and mechanical disturbances. Constraints such as these impede system design.

[0005] Moreover efforts to mitigate the effects of tilt misalignment may introduce a simultaneous degradation of lateral spatial resolution. A degradation of lateral spatial resolution is undesirable as this may interfere with an analyst's ability to locate and identify desired targets within the sample under study. A degradation of lateral spatial resolution may also interfere with efforts to automate the microdissection process. Some microdissectors use software algorithms to automate the localization and selection of regions of interest within a sample for dissection. The software may retrieve an image from the microdissector's imaging system,

analyze the image, and send commands to the cutting tool (such as a laser or a mechanical cutter) to perform the dissection. Automation is beneficial, in part because less human intervention is required, which reduces cost, increases speed, and minimizes errors. However a limit to image sharpness may burden the software algorithms, reducing the range of samples the software may be able to process successfully.

[0006] A related difficulty with current microdissector systems may occur if the sample under examination is thick, as the imaging system then may not have a large enough depth of field to image the entire sample at once, even in the absence of any tilt misalignment. In this case the imaging system may be refocused to multiple depths within the sample, with an image recorded after each refocusing, and the images assembled to determine where to dissect. This takes extra time and slows the measurement. Alternatively, the microdissection system may be limited to use with thin samples, which is a constraint that is undesirable.

[0007] A further difficulty with current microdissector systems is degradation of image quality due to turbidity in the sample medium. Turbid media distort light as it travels from the source to the imager, which may result in a poor quality image.

[0008] In addition, even in the absence of any tilt misalignment, the imager or imagers of current microdissector systems may be limited to the Rayleigh limit for lateral spatial resolution. This limits an analyst's ability to locate and identify the desired targets within the sample under study. A system designer may make a tradeoff between lateral spatial resolution and depth of field, but both parameters are important. To further quote Willingham: "Regardless of the cell-cutting method, researchers will want to know whether the cutting process actually worked. All systems offer an inspection mode so that when cutting is complete, the operator can inspect both the fragment that has been removed and the area from which it was excised." As emphasized, post-cut inspection is important, and limits to lateral spatial resolution and depth of field pose difficulties here as well.

[0009] Thus, there is a need in the art for improved microdissection systems that may address, for example, one or more of the above-noted difficulties or additional difficulties. Outcomes may include better diagnosis of disease, improved patient outcome, and improvements in biomedical research.

Summary of the Invention

[0010] As used herein, and further explained below, the term "counter-change-in-tilt" refers to interferometer systems in which the angle between the two interfering beams of the interferometer increases or decreases as a point source at the input of the interferometer is moved laterally across the scene. Moreover, while devices and methods of the present invention relate to all counter-change-in-tilt interferometers, rotational-shear interferometers (RSI's) are non-limiting examples which can have 100% beam overlap on the detector, allowing potentially maximal resolution enhancement from the counter-change-in-tilt configuration, and a particularly long depth-of-field.

[0011] In one of its aspects, the present invention may provide a microdissection counter-change-in-tilt interferometer apparatus, comprising a microdissector having a sample holder for mounting a sample under study thereto, and a counter-change-in-tilt interferometer positioned relative to the sample holder to receive light from a sample placed thereon. The counter-change-in-tilt interferometer may include a rotational-shear interferometer; the microdissector may include a laser and/or mechanical cutter for dissecting a sample under study. The counter-change-in-tilt interferometer may include an optical element having optical power in at least one arm thereof. In addition, the counter-change-in-tilt interferometer may be configured to provide overlap between two of the beams at the output thereof, and the area of overlap maybe 100%, at least 95%, at least 80%, or at least 65%. Also, the counter-change-in-tilt interferometer may be configured such that the fringe pattern from a point source does not change by more than one wave when the object point is moved in the longitudinal direction, or may be configured such that the fringe pattern from a point source changes by more than one wave when the object point is moved in the longitudinal direction.

[0012] In another of its aspects, the present invention may provide a method for providing microdissection of a sample under study, comprising imaging the sample with a counter-change-in-tilt interferometer, identifying in the image a region of sample to dissect, and then dissecting identified region of interest. The identified region of interest may be dissected with a laser and/or a mechanical cutter. The counter-change-in-tilt interferometer may include a rotational-shear interferometer.

Brief Description of the Drawings

[0013] The foregoing summary and the following detailed description of exemplary embodiments of the present invention may be further understood when read in conjunction with the appended drawings, in which:

[0014] Figure 1 schematically illustrates a side elevational view of tilt misalignment between a region of focus and a sample under study along with the advantage of an increased depth of field provided by a rotational-shear interferometer of the present invention;

[0015] Figure 2 schematically illustrates a side elevational view of a sample that is thicker than a region of focus of a conventional imager and illustrates the advantage of an increased depth of field provided by a rotational-shear interferometer of the present invention;

[0016] Figure 3 schematically illustrates an exemplary microdissection rotational-shear interferometer (a type of counter-change-in-tilt interferometer) apparatus in accordance with the present invention;

[0017] Figure 4 schematically illustrates another exemplary microdissection rotational-shear interferometer apparatus in accordance with the present invention;

[0018] Figure 5 schematically illustrates Modulation Transfer Functions (MTFs) qualitatively showing a potential benefit of a microdissection rotational-shear interferometer over a conventional imager; and

[0019] Figure 6 schematically illustrates a procedural flow for microdissection in accordance with the present invention.

Detailed Description of the Invention

[0020] Referring now to the figures, wherein like elements are numbered alike throughout, Figures 1 and 2 schematically illustrate exemplary advantages provided by microdissection counter-change-in-tilt interferometer systems in accordance with the present invention, such as the benefits obtained from an increased depth of field. (A rotational-shear interferometer (RSI) is an example of a counter-change-in-tilt interferometer.) For instance, with reference to Fig. 1, systems of the present invention may provide an advantage in overcoming problems associated with tilt misalignment. A sample 102 under study may be tilted by an angle 112 relative to a

conventional region of focus 110 provided by a conventional (non-counter-change-in-tilt/non-RSI) imager, *e.g.*, a conventional microscope, (not shown) which is used to guide the microdissection. As illustrated, only a portion of the sample 102 may be in focus and thus able to be imaged in sharp focus when using a conventional imager having a relatively small depth of field 104, which limits the region of focus 110, even though the entire sample 102 may be of interest. The problem may be more acute when the region of focus 110 is narrow and the size of the sample 102 is large, such as when the lateral extent 106 of the sample 102 is larger than the region of focus 110.

[0021] To mitigate the effect of an uncorrected tilt misalignment 112, the depth of field 104 of the conventional imager may be increased enough that the entire sample 102 is in focus despite the tilt misalignment 112. This may be achieved by decreasing the numerical aperture of the conventional system. However a decrease in numerical aperture may be accompanied by a simultaneous degradation of lateral spatial resolution, which is undesirable as this may interfere with an analyst's ability to locate and identify the desired targets within the sample 102 under study.

[0022] In contrast, microdissection counter-change-in-tilt interferometer systems in accordance with the present invention may include a rotational-shear interferometer as an imager, providing a relatively larger depth of field 108, *i.e.*, the depth of field 108 of systems of the present invention may be significantly larger than depth of field 104 of a conventional imager. For instance, the depth of field 108 of the systems of the present invention may be large enough such that all of sample 102 is in focus despite the tilt misalignment 112.

[0023] A further exemplary advantage of systems of the present invention is illustrated in Fig. 2. A conventional imaging system may not have a large enough depth-of-field to image the entirety of a sample at once, even in the absence of any tilt misalignment, such as when a sample 202 is thicker than a depth of field 204 provided by a conventional imager (not shown). In this example, the sample 202 contains four cells of interest, as indicated. Again, the depth of field 208 of microdissection counter-change-in-tilt interferometer systems of the present invention may be significantly larger than the conventional depth of field 204, and may be large enough such that all of sample 202 is in focus. Thus, microdissection

counter-change-in-tilt interferometer systems of the present invention may be particularly well suited for thicker samples and may be less sensitive to problems associated with tilt misalignment.

[0024] Without intending to be bound by any theory, the increased depth of field may be understood from the manner in which an RSI type of counter-change-in-tilt interferometer operates, for example. In an RSI, a beam of light from the scene under study may be split into two beams. (As used herein the word "light" refers to electromagnetic radiation of any wavelength.) The two beams may be arranged prior to recombination in such a way that any defocus aberration and/or change in defocus aberration is common-mode between the two beams and mostly cancels out when the interference fringes are formed on the detector. An improvement in the depth-of-field of an imaging system is often believed to come at the cost of a corresponding degradation in image sharpness. But an RSI is an exception due to the cancellation between the two arms. Thus an RSI can exhibit the property that a fringe pattern on a detector due to a point source does not change by more than one wave when the object point is moved in the longitudinal direction by a distance equal to the longitudinal depth of the sample under study. (The image inferred by an RSI is a conical projection with the tip of the cone located at the center of the RSI entrance pupil. Thus technically in an RSI the longitudinal direction is along an axis from the center of the RSI entrance pupil to the object.)

[0025] A further, remarkable property of microdissection counter-change-in-tilt interferometer systems of the present invention can be sharp lateral spatial resolution, at least in cases where the scene under study emits spatially incoherent electromagnetic radiation. In a counter-change-in-tilt interferometer, such as an RSI, for example, a beam of light from the scene under study may be split into two beams and the two beams arranged prior to recombination in such a way that, as a point source in front of the RSI is moved laterally across the scene, the angle between the two interfering beams will increase or decrease. I refer to this as "counter-change-in-tilt," since the tilt angles of the two beams change in opposite directions to each other (as a point source in front of the RSI is moved laterally). One way to achieve this arrangement is to send the beam in one arm of the interferometer through an odd number of reflections and the beam in the other arm through an even number.

Another way to achieve this arrangement is to send the beam in one arm of the interferometer through an intermediate focus.

[0026] Conventional wisdom teaches away from the possibility that an imaging system may outperform the Rayleigh limit for resolution in any sort of fundamental way. But counter-change-in-tilt interferometers differ, due to the counter-change-in-tilt. In a single-beam imager, such as a conventional microscope, the Lagrange Invariant limits lateral spatial resolution (to the Rayleigh limit). It can be shown theoretically that if a device existed that could violate the Lagrange Invariant by a factor of "x", that the device could be incorporated into a conventional imager to improve the lateral spatial resolution by the same factor "x". A single-beam device generally cannot violate the Lagrange Invariant. In a counter-change-in-tilt system there are two beams. The light in each of the two beams conforms to the Lagrange Invariant. But working together, the two beams are able to beat the Lagrange Invariant by up to a factor of two. Any system that breaks a beam of light in two and arranges the two beams for counter-change-in-tilt has an extremely-fundamental, factor-of-two advantage for achieving lateral spatial resolution. (The degree to which such a system makes use of this advantage may vary from one instrument to another.)

[0027] This advantage for lateral spatial resolution is illustrated in Fig. 5, which schematically illustrates the Modulation Transfer Functions (MTF's) of a conventional imager and of an exemplary rotational-shear interferometer with a 180-degree rotation angle and full overlap of the two beams on the detector. For such a counter-change-in-tilt interferometer, the area under the MTF is approximately a factor of two larger than the area under the same curve for a conventional imager. This indicates sharper lateral spatial resolution. In a microdissection system, sharper lateral spatial resolution may allow for a more-accurate selection of the regions of interest to cut. Sharper resolution may also allow for better inspection post-cut to confirm the appropriate cut was made. Sharper lateral spatial resolution may also facilitate the robotic automation of a microdissection system.

[0028] Apart from an improvement in the numeric value of the area below the MTF curve, note also the difference in shapes of the two MTF curves. An RSI has good response at spatial frequencies (those just below the cutoff frequency) that a

conventional imager cannot easily access, even if the aperture on the conventional imager were, say, to double in size. This is significant because different morphological features in an image are composed of different spatial frequencies. Morphological features with significant spatial frequency content just below the cutoff frequency may be more recognizable to an analyst when the imaging system transmits those frequencies more faithfully, such as an RSI does as compared to a conventional imager. This may allow for more visual cues to identify regions of interest in the sample, which may help with target selection.

[0029] Another remarkable capability of counter-change-in-tilt interferometers, also rarely made use of, is their ability to image through certain turbid media. One counter-change-in-tilt interferometer with this capability is called a quadrature-phase rotational-shear interferometer. Phase closure is the basic means by which this system is able to image through certain turbid media. Sharper images may be achieved when the deleterious effects of turbidity are mitigated.

[0030] Turning then to exemplary configurations of the microdissection counter-change-in-tilt interferometer, Fig. 3 schematically illustrates a microdissection counter-change-in-tilt interferometer apparatus 300 in accordance with the present invention having a microdissector 380 and a counter-change-in-tilt interferometer, a rotational-shear interferometer 390. The microdissector 380 may include a mechanical cutter 304 to slice tissue from a slide surface along with a fluidics system 314 to recover the dissected fragments for further testing. In this example, a sample under study (not shown) may be placed on a transparent substrate 302. The sample rests on the "positive z" side of the substrate 302, beneath a cutting tip 304. The cutting tip 304 may be located at the end of an extraction device 306, and a motor 308 may be used to rotate the cutting tip 304. A positional movement system 310 may be coupled to an extraction device 306 to move the cutting tip 304 relative to the substrate 302. A separate substrate movement system 316 may be used to move the substrate 302 relative to the cutting tip 304. A computer 318 may be operably coupled to the movement systems 310 and 316 to control the respective motions thereof. A fluidics system 314 may be coupled to the extraction device 306 to deliver and withdraw fluid so as to retrieve dissected tissue fragments. A microscope objective 320 may be aimed at the sample under study.

[0031] The RSI 390 may be positioned to receive light from the sample on the substrate 302 through the objective 320. (Though the RSI 390 is drawn as 2-dimensional for simplicity of illustration, it is understood that the RSI 390 is a 3-dimensional structure which can image a scene in both lateral dimensions. The present disclosure is not meant to be limited to 2-dimensional counter-change-in-tilt interferometers.)

[0032] A light beam collected from the sample on the substrate 302 leaves microscope objective 320 and travels in the "minus z" direction. The light propagates to a lens 322 which may focus the light to the plane of a field stop 340 which may limit the field-of-view of the system, if desired. The light then further propagates to a lens 324, which in this example collimates the beam.

[0033] In this example the microscope objective 320 contains the limiting aperture (not shown) that is commonly referred to as the system "stop." The lenses 322, 324, 342, 344, and 346 may work together to image the system stop onto detector 348. Likewise, lenses 322, 324, 350, 352, and 354 may work together to image the system stop onto detector 356. The lenses 322, 324, 342, 344, 346, 350, 352, and 354 may be further configured according to the design principles discussed herein to maximize the field-of-view over as wide of a spectral bandwidth as possible. In addition, the lenses 322, 324, 342, 344, 346, 350, 352, 354 of Figure 3 are illustrated as singlets. Those skilled in the art will appreciate that these and all other lenses disclosed herein may contain multiple elements, cemented or otherwise, and need not comprise only a single element. Additional elements may be provided as desired to correct aberrations and improve image quality, for example. In some cases, fewer elements may be desired. Also, the spacings between elements and the optical power of each element may be adjusted to further correct aberrations and improve image quality, for example. Those skilled in the art will further appreciate that lenses often may be replaced with mirrors with corresponding optical power, so long as associated changes in beam directions are accounted for in the optical layout of the system.

[0034] After leaving lens 324 the light propagates to beamsplitter 326, where it is split into first and second beams. The first beam travels to fold mirrors 328 and 330, then to beamsplitter 332. The second beam travels to fold mirrors 334, 336, and 338,

then to beamsplitter 332, where it is combined with the first beam. A portion of the combined first and second beams leaves beamsplitter 332 and passes through lenses 342, 344, and 346 to provide an interference pattern on detector 348. Another portion of the combined first and second beams leaves beamsplitter 332 and passes through lenses 350, 352, and 354 to provide an interference pattern on detector 356. The combined first and second beams at either or both of the detectors 348, 356 may overlap exactly (100%) or less than 100%. For instance, the area of overlap between the first and second beams may be at least 95%, at least 80%, or at least 65%, for example. Detectors 348 and 356 may be electronically connected to the computer 318, as shown, and the computer may process interference patterns on the detectors 348, 356 to provide an image of the sample.

[0035] Figure 4 schematically illustrates another a microdissection counter-change-in-tilt interferometer apparatus 400 in accordance with the present invention having a microdissector 480 and a counter-change-in-tilt interferometer, rotational-shear interferometer 490. In this example, a sample under study (not shown) may be attached to the "negative z" side of a membrane 402. The membrane 402 may be stretched across an opening in a slide 404. The slide 404 may sit on a sample movement system 416. A laser 414 may be used to dissect one or more regions of the sample. Light from laser 414 may travel through fold mirrors 410, 412, which may be actuated with tip/tilt mechanisms to allow dynamic steering of the direction of the laser beam toward desired regions of the sample under study. The laser light that leaves fold mirror 410 may then reflect off a beamsplitter 408 into a microscope objective 420. The microscope objective 420 may be configured to focus the laser light onto the sample under study. The fold mirrors 410, 412 may steer the laser light to carve out a region of the sample to be collected for further analysis. A computer 418 may be operably coupled to the fold mirrors 410, 412, movement system 416, and laser 414 to control the steering of the mirrors 410, 412, movement of the sample under study, and operation of the laser 414. The laser 414 may cut through both the sample under study and the membrane 402 that supports the sample. The region or regions of the sample under study that are carved out may then fall under gravity into a collection cup 406. The samples in collection cup 406 may be processed for further analysis.

[0036] As with Figure 3, the RSI 490 is drawn as 2-dimensional for simplicity of illustration, and not to limit the disclosure to counter-change-in-tilt interferometers that are 2-dimensional. The RSI is used to generate interference patterns, from which an image of the sample under study may be inferred. (While RSI's may be referred to herein as imaging devices, or devices that provide an image of a sample, strictly speaking RSI's most directly provide fringe patterns from which an image of the sample under test can be inferred.) A light beam from the sample under study leaves microscope objective 420 and travels in the "positive z" direction. (Not shown in Figure 4 is the light beam from the sample under study to the entrance of microscope objective 420.) The light propagates to beamsplitter 408 and transmits through. The light then propagates to a lens 422 which may focus the light to the plane of a field stop 440 which may limit the field-of-view of the system, if desired. The light then further propagates to a lens 424, which in this example collimates the beam.

[0037] In this example the microscope objective 420 contains the limiting aperture (not shown) that is commonly referred to as the system "stop," and lenses 422, 424, 442, 444, and 446 work together to image the system stop onto detector 448. Likewise, lenses 422, 424, 450, 452, and 454 work together to image the system stop onto detector 456. The lenses 422, 424, 442, 444, 446, 450, 452, and 454 may be further configured according to the design principles discussed herein to maximize the field-of-view over as wide of a spectral bandwidth as possible.

[0038] After leaving lens 424 the light propagates to beamsplitter 426, where it is split into first and second beams. The first beam travels to fold mirrors 428 and 430, then to beamsplitter 432. The second beam travels to fold mirrors 434, 436, and 438, then to beamsplitter 432, where it is combined with the first beam. A portion of the combined first and second beams that leaves beamsplitter 432 passes through lenses 442, 444, and 446 to provide an interference pattern on detector 448. In other portion of the combined first and second beams leaves beamsplitter 432 passes through lenses 450, 452, and 454 to provide an interference pattern on detector 456. The combined first and second beams at either or both of the detectors 448, 456 may overlap exactly (100%) or less than 100%. For instance, the area of overlap between the first and second beams may be at least 95%, at least 80%, or at least 65%, for

example. Detectors 448 and 456 may be connected electronically to a computer 418 as shown, and the computer may process interference patterns on the detectors 448, 456 to provide an image of the sample.

[0039] As a further option, a microdissection counter-change-in-tilt interferometer (such as 300 or 400, for example) may be configured so the two beams incident on a given detector have different wavefront curvature from each other at the detector. This difference in curvature may be achieved by introducing optical power into one or both of the two arms of the interferometers 390, 490. Such optical power may be introduced, for example, by adding optical power to one or more of the mirrors 328–338, 428–438, in the interferometer 390, 490. Alternatively or additionally optical power may be introduced in the interferometer 390, 490 by one or more lenses positioned within the arms of the interferometer, for example. Thus, microdissection RSI's of the present invention may differ from conventional RSI's in that the two wavefronts incident on a given detector 348, 356, 448, 456 may have different wavefront curvature from each other. A Fourier Incoherent Single Channel Holography (FISCH) configuration is an example of such an arrangement. In a FISCH system the longitudinal coordinate of a point source (the distance from the imager to the point source) is encoded in the difference in curvature of the two wavefronts incident on one or both of the two detectors. Thus, as used herein the terms "counter-change-in-tilt interferometer" and "rotational-shear interferometer" include configurations in which the two wavefronts incident on each detector have different curvatures from each other.

[0040] Figure 6 schematically illustrates a sequence of steps one may take to perform microdissection with a counter-change-in-tilt interferometer system of the present invention. Namely, one may first image a sample with a counter-change-in-tilt interferometer, identify region(s) of the sample to dissect, and then dissect target region(s) of interest.

[0041] These and other advantages of the present invention will be apparent to those skilled in the art from the foregoing specification. Accordingly, it will be recognized by those skilled in the art that changes or modifications may be made to the above-described embodiments without departing from the broad inventive concepts of the invention. It should therefore be understood that this invention is not limited to the

particular embodiments described herein, but is intended to include all changes and modifications that are within the scope and spirit of the invention as set forth in the claims.

Claims

What is claimed is:

1. A microdissection counter-change-in-tilt interferometer apparatus, comprising:
   a microdissector having a sample holder for mounting a sample under study thereto; and
   a counter-change-in-tilt interferometer positioned relative to the sample holder to receive light from a sample placed thereon.
2. The microdissection counter-change-in-tilt interferometer apparatus of claim 1, wherein the microdissector includes a laser for dissecting a sample under study.
3. The microdissection counter-change-in-tilt interferometer apparatus of claim 1, wherein the microdissector includes a mechanical cutter for dissecting a sample under study.
4. The microdissection counter-change-in-tilt interferometer apparatus of any one of the preceding claims, wherein the counter-change-in-tilt interferometer includes an optical element having optical power in at least one arm thereof.
5. The microdissection counter-change-in-tilt interferometer apparatus of claim 4, wherein the optical element comprises a mirror.
6. The microdissection counter-change-in-tilt interferometer apparatus of claim 4, wherein the optical element comprises a lens.
7. The microdissection counter-change-in-tilt interferometer apparatus of any one of the preceding claims, wherein the interferometer is configured to provide overlap between two of the beams at the output thereof, and the area of overlap is at least 65%.
8. The microdissection counter-change-in-tilt interferometer apparatus of any one of the preceding claims, wherein the interferometer is configured to provide overlap between two of the beams at the output thereof, and the area of overlap is at least 80%.
9. The microdissection counter-change-in-tilt interferometer apparatus of any one of the preceding claims, wherein the interferometer is configured to provide overlap between two of the beams at the output thereof, and the area of overlap is at least 95%.
10. The microdissection counter-change-in-tilt interferometer apparatus of any one of the preceding claims, wherein the interferometer is configured to provide overlap between two of the beams at the output thereof, and the area of overlap is 100%.

11. The microdissection counter-change-in-tilt interferometer apparatus of any one of the preceding claims, wherein the interferometer is configured to provide two beams that travel separate physical paths in the interferometer.
12. The microdissection counter-change-in-tilt interferometer apparatus of any one of the preceding claims, comprising a computer operably coupled to the microdissector and to the counter-change-in-tilt interferometer.
13. The microdissection counter-change-in-tilt interferometer apparatus of any one of the preceding claims, wherein the counter-change-in-tilt interferometer comprises a rotational-shear interferometer.
14. The microdissection counter-change-in-tilt interferometer apparatus of any one of the preceding claims, wherein the interferometer is configured such that the fringe pattern from a point source does not change by more than one wave when the object point is moved in the longitudinal direction.
15. The microdissection counter-change-in-tilt interferometer apparatus of any one of the preceding claims, wherein the interferometer is configured such that the fringe pattern from a point source changes by more than one wave when the object point is moved in the longitudinal direction.
16. A method for providing microdissection of a sample under study comprising imaging the sample with a counter-change-in-tilt interferometer, identifying in the image a region of sample to dissect, and then dissecting identified region of interest.
17. The method of claim 16, comprising dissecting identified region of interest with a laser.
18. The method of claim 16, comprising dissecting identified region of interest with a mechanical cutter.
19. The method of claim 16, wherein the counter-change-in-tilt interferometer includes an optical element having optical power in at least one arm thereof.
20. The method of claim 19, wherein the optical element comprises a mirror.
21. The method of claim 19, wherein the optical element comprises a lens.
22. The method of any one of claims 16–21, wherein the counter-change-in-tilt interferometer comprises a rotational-shear interferometer.

Abstract

Microdissection with counter-change-in-tilt interferometry, such as rotational-shear interferometry, and method.

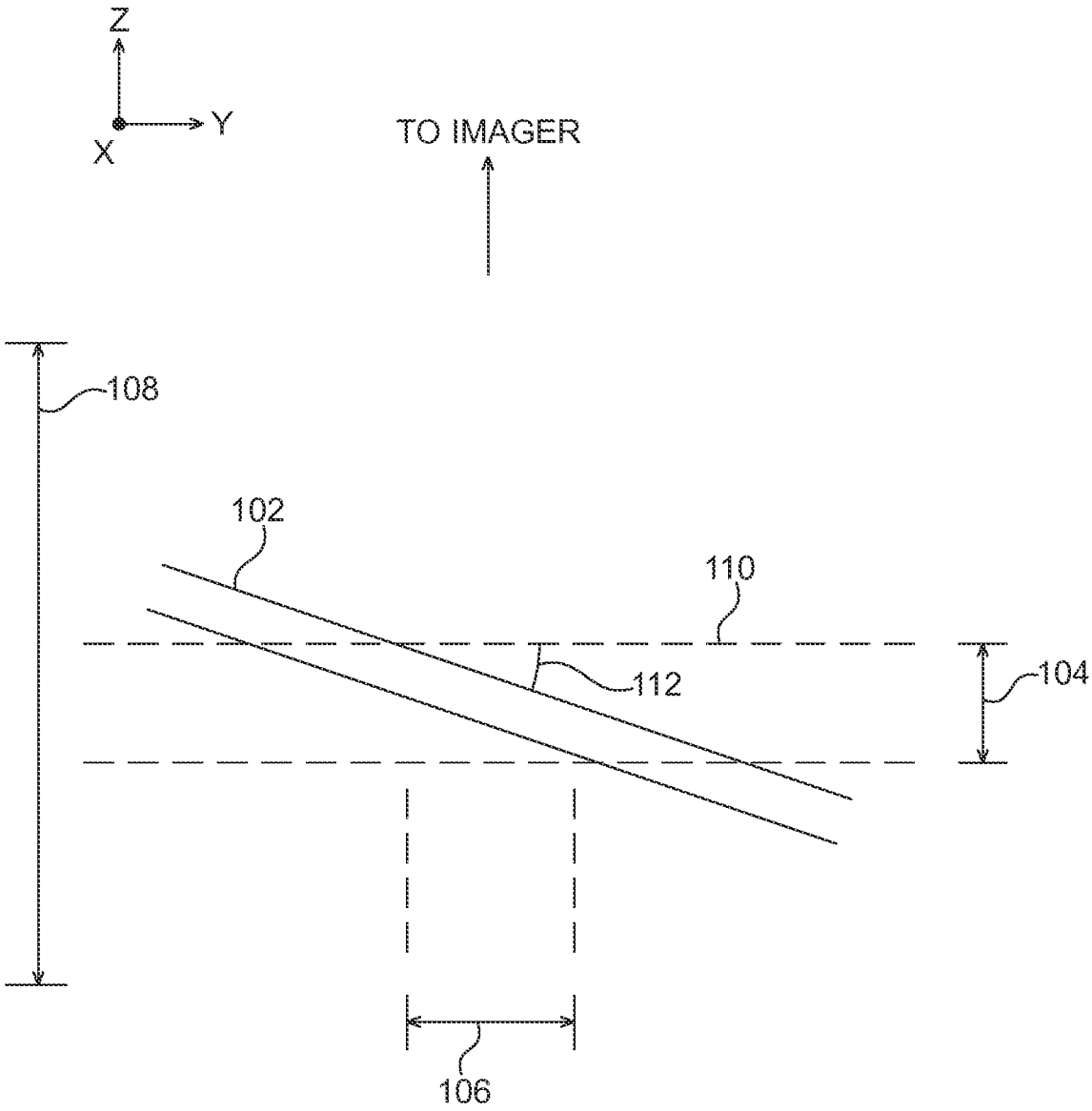


FIG. 1

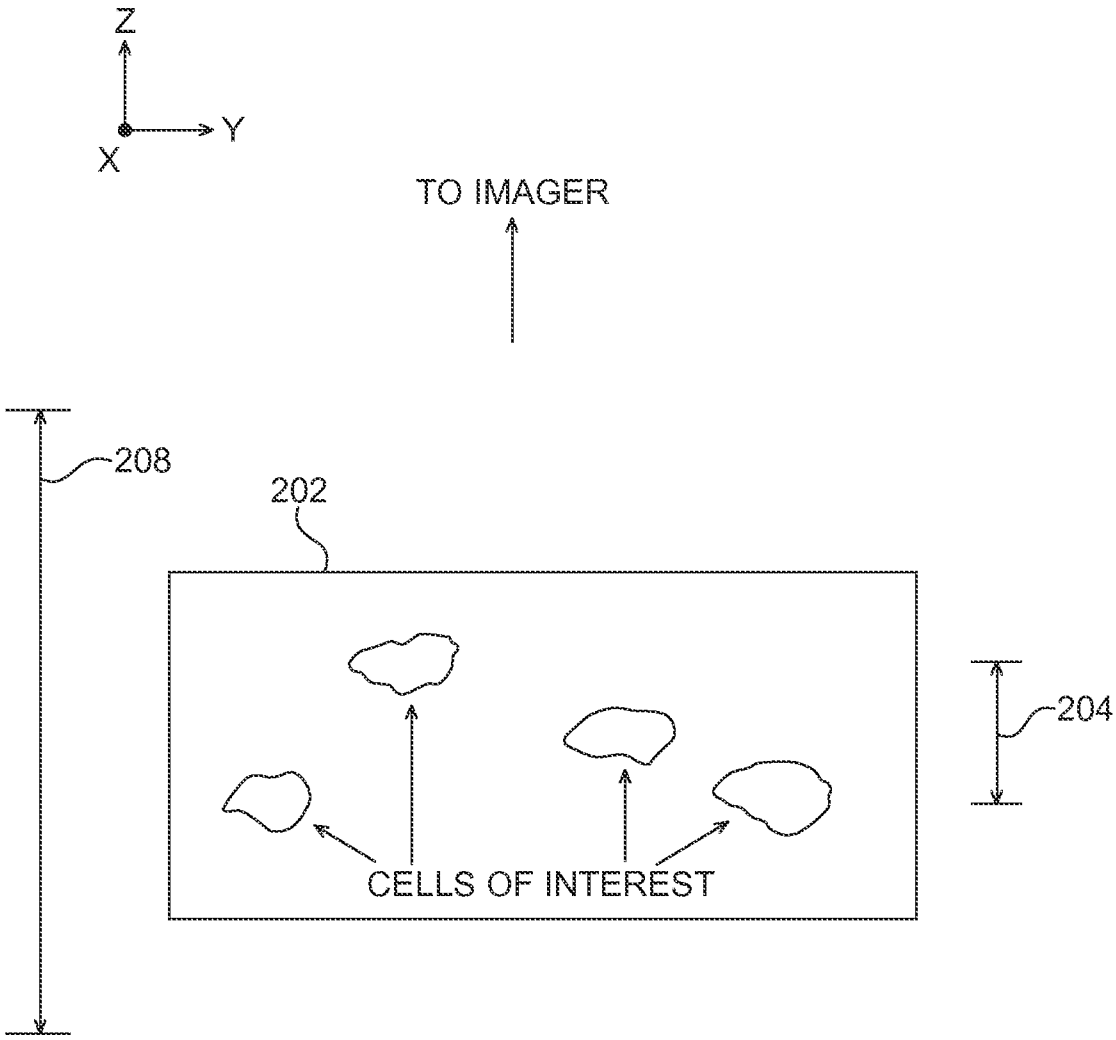
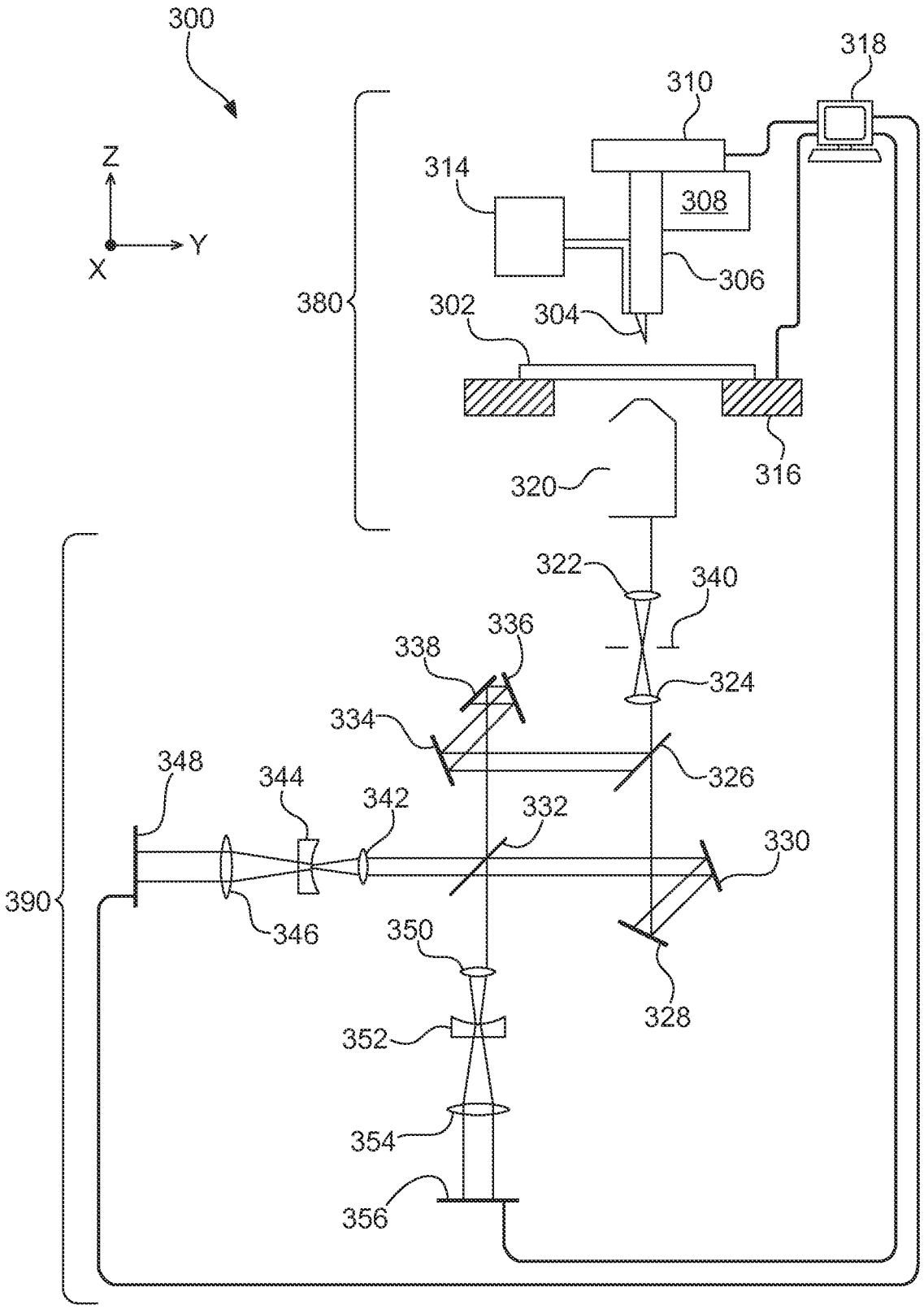


FIG. 2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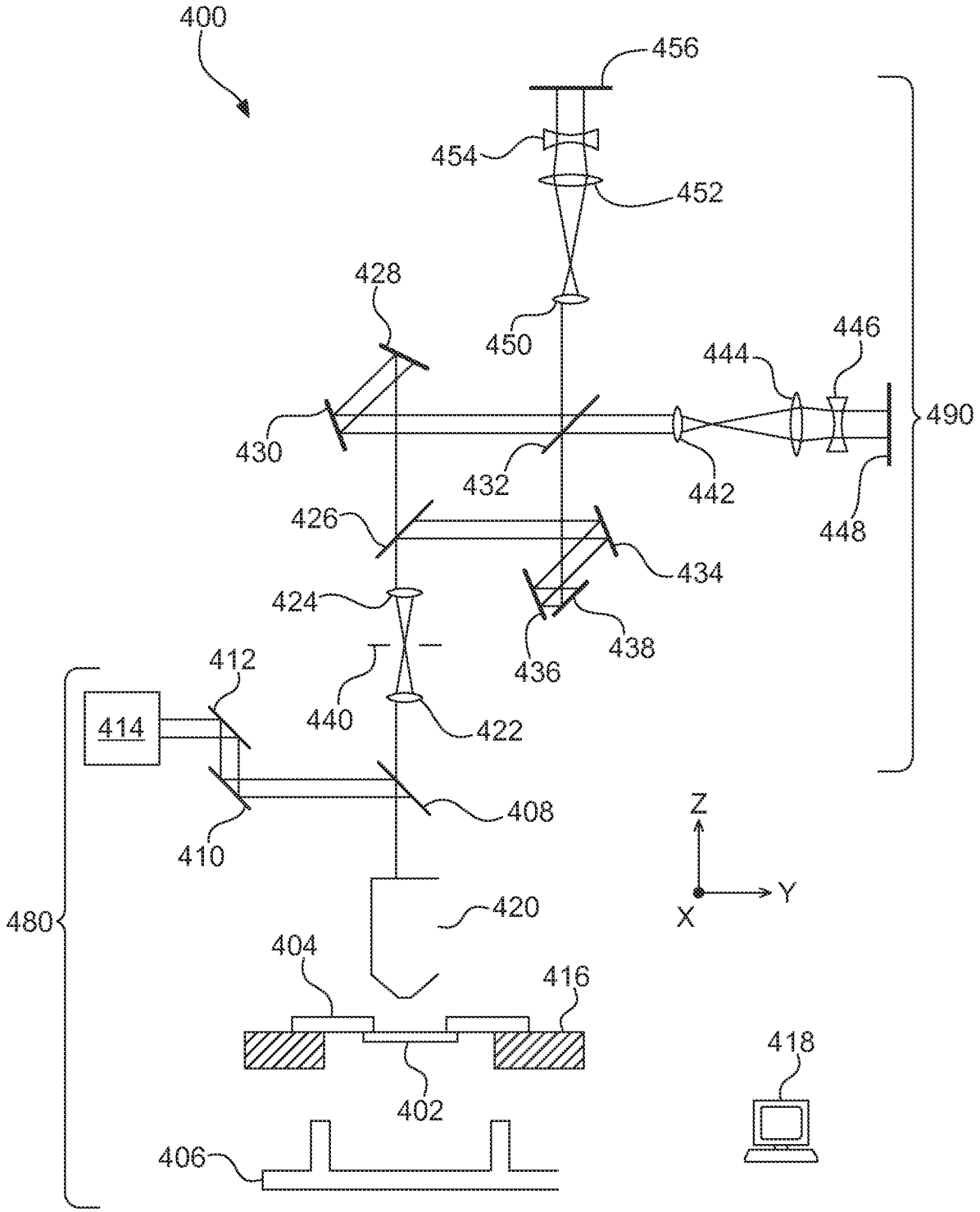
356

FIG. 3


400
456
454
452
428
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422
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404
416
418
402
406
Z
Y
X

FIG. 4

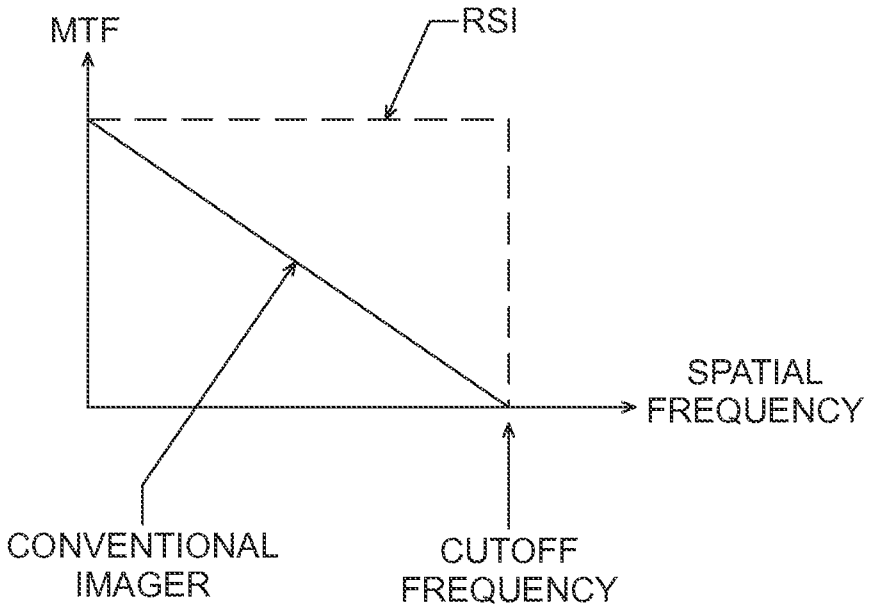
MTF
RSI
SPATIAL
FREQUENCY
CONVENTIONAL
IMAGER
CUTOFF
FREQUENCY

FIG. 5

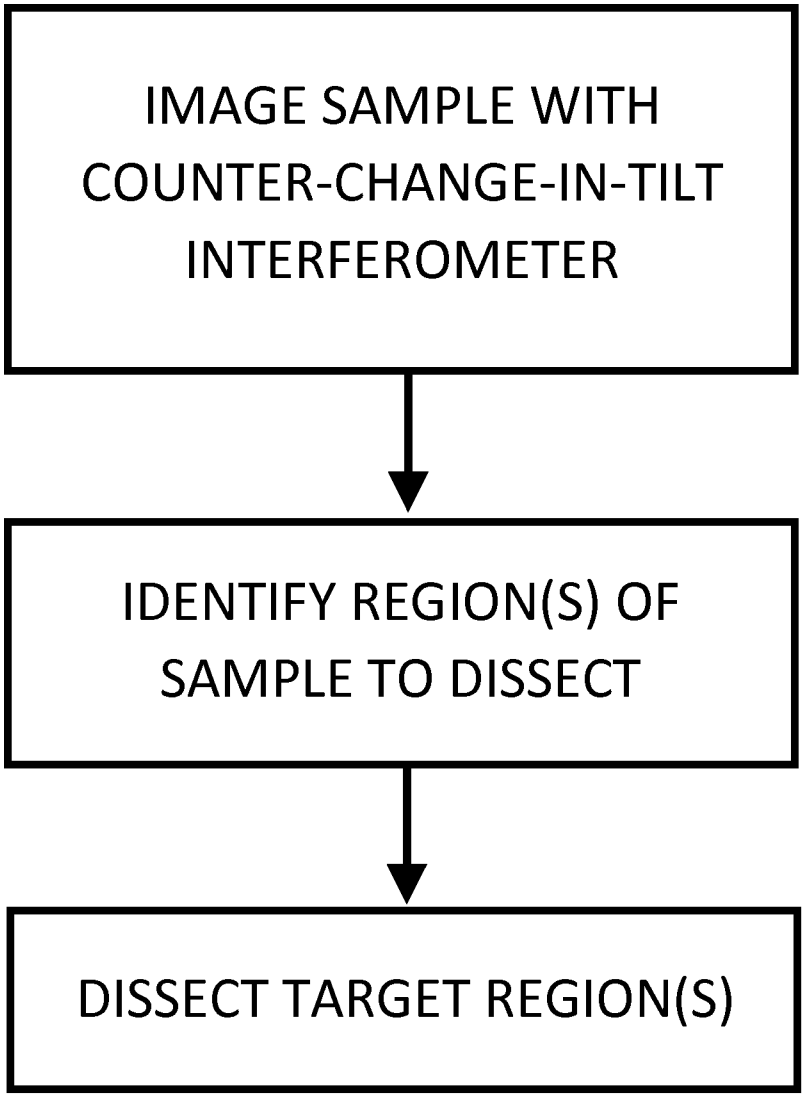
IMAGE SAMPLE WITH COUNTER-CHANGE-IN-TILT INTERFEROMETER
IDENTIFY REGION(S) OF SAMPLE TO DISSECT
DISSECT TARGET REGION(S)

FIG. 6