



Method and Apparatus for Imaging a Sample

Technical Field

This invention relates generally to a method and apparatus for imaging a sample and
5 relates more particularly, though not exclusively to such a method and apparatus for
imaging a cell sample.

Background

Dark field illumination is commonly used when imaging low contrast objects against a
10 reflective background. With dark field illumination, light is provided directionally in
such a way that only light that is scattered by features of interest is recorded by an
image recorder such as a camera, while light interacting with other regions is
transmitted or deflected away.

15 In some cases, anisotropically reflective or emissive features and isotropic features are
both found within the same field of view. An example is high-contrast imaging of cell
samples within microwell arrays. As shown in the cell sample 10 of Fig. 1, cells 12 are
relatively weak and isotropic in their reflection (scattering) of incident light when
compared to the microwell walls 14 which reflect significantly more strongly and
20 directionally . Artifacts such as a bright ring 16 arise due to reflection from the corner
where the wall meets the base in the microwell. Such strongly reflective features tend to
dominate the image recorder's dynamic range, making it very difficult to image the
relatively low intensity cells 18 near the microwell walls 14.



The microwell walls 14 and meniscus of the liquid medium in which the cells are immersed also have circularly symmetrical surfaces at least some of which will reflect any given direction of incident light into the image recorder . These surfaces also back-reflect a proportion of the illumination onto cell areas in their proximity 18, resulting in
 5 poor uniformity of intensity and contrast in the recorded image.

Loss of uniformity makes automated segmentation of images difficult. This is because cells in the central region 20 of a microwell are less brightly lit than cells 18 near the microwell walls 14 which in this example receive additional illumination from reflected
 10 and scattered light from the microwell walls 14 and corners. As a result using a decision threshold chosen to correctly segment regions occupied by cells from regions not occupied by cells in the central region 20 of a microwell would lead to poor segmentation of the cells 18 near the microwell walls 14 if it were applied globally. Calibration methods might be used to select a spatially variant threshold, but in many
 15 cases this is not practical due to variability in factors such as well construction, support layers under the cells, volume and type of liquid covering the cells. It is preferable in terms of reliability and accuracy to derive an imaging approach that can generate uniform images.

20 **Summary**

According to one aspect, there may be provided method of imaging a stationary sample having features with anisotropic light scattering characteristics. The method comprises performing a plurality of angular illuminations of the sample according to a pattern of angular illumination controlled by a controller so as to specify an optical path for light
 25 scattered by the sample; recording images of the sample corresponding to the plurality

of angular illuminations of the sample; and processing the recorded images to form a computed image.

5 The optical path may be selected from the group consisting of: direct, back scattered and secondarily reflected. At least one of the features may be selectively eliminated from at least a portion of the computed image. The pattern of angular illuminations may be controlled such that light secondarily reflected by at least one of the features may be selectively eliminated from at least a portion of the computed image.

10 The processing may comprise processing corresponding image pixels among the recorded images by an algorithm.

The method may further comprise aligning the polar centre of an angular illuminator with an approximate centre of a sample holder supporting the sample before the step of
15 performing a plurality of angular illuminations of the sample.

The performing a plurality of angular illuminations may comprise applying illumination at angular intervals around the sample, such as by rotating a mask at angular intervals.

20 According to another aspect, there is provided apparatus for imaging a stationary sample with features having anisotropic light scattering characteristics. The apparatus may comprise an angular illuminator for angularly illuminating the sample; an image recorder for recording images of the sample corresponding to a plurality of angular illuminations of the sample; a controller for controlling the pattern of angular
25 illuminations so as to specify an optical path for light to travel from the angular illuminator to the image recorder when scattered by the sample; and a processor for processing the recorded images to form a computed image.

The image recorder may be an image sensor array with a lens.
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The pattern of angular illuminations may be controlled such that light secondarily reflected by at least one of the features may be selectively eliminated from at least a portion of the computed image.

5 The processor may form a computed image such that at least one of the features may be selectively eliminated from at least a portion of the computed image. The feature may be a wall of a microwell array. The corresponding image pixels from the recorded images may be processed by an algorithm in the processor.

10 The angular illuminator may include a mask. The mask may be between the light source and the sample. The mask may be controlled by the controller. The mask may be of a sector shape having an angular size.

The imaging may be of at least one weakly scattered feature of the sample. The sample
15 may be a cell sample. The apparatus may further comprise an image processing application on a computer for combining the images recorded by the image recorder.

For all aspects, the pattern of angular illumination may range within a three dimensional space surrounding the sample and varying in one or more of azimuth and elevation
20 angles relative to the sample plane, and it may be selected from the group consisting of: symmetrical and asymmetrical about the sample. The pattern of angular illumination may further be automatically optimised based on analysis of specularly reflective regions of the sample obtained from a prior scan.

25 The algorithm may include an analysis operator for differentiating features based on their anisotropy, an isotropy operator for quantifying relative amplitude and directionality of feature anisotropy, or a combining operator for producing a combined computed image. The combining operator may be a non-linear operator for combining the features with anisotropic light scattering characteristics in a single computed image
30 independent of their orientation. The non-linear operator may be selected from the group consisting of: minimum and maximum.

The angular intervals may be equal to or less than an angular size of the mask to form an overlap region of images. The mask may be rotated about an axis that may be perpendicular to the sample. The axis may be coincident with a longitudinal axis. Each of the recorded images may have an overlap region with adjacent recorded images.

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The angular illuminator may comprise a light source selected from the group consisting of: a ring light, and an array of light emitting diodes. The plurality of angular illuminations may be performed by appropriately activating portions of the array of light emitting diodes. The array of light emitting diodes may be of multiple wavebands that are directional in illumination and sensed independently by spectrally selective channels in the image recorder.

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Brief Description of the Drawings

In order that the invention may be fully understood and readily put into practical effect

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there shall now be described by way of non-limitative example only exemplary embodiments, the description being with reference to the accompanying illustrative drawings.

In the drawings:

Fig. 1 is an image of a cell sample in a microwell array taken using conventional dark

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field illumination (prior art);

Fig. 2 is a schematic representation of an exemplary embodiment of an apparatus for partial directional illumination and imaging;

Fig. 3 is an image of the cell sample of Fig. 1 taken using directional illumination of a portion of the cell sample using the exemplary embodiment of Fig. 2;

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Fig. 4 is an image of the cell sample of Fig. 1 taken using directional illumination of another portion of the cell sample using the exemplary embodiment of Fig. 2;

Fig. 5 is a computed image of the cell sample of Fig. 1 formed by combining a plurality of images including those of Fig. 3 and Fig. 4;

Fig. 6a is an image of a cell sample in a microwell array taken using conventional dark field illumination (prior art);

5 Fig. 6b is an image obtained using an exemplary embodiment;

Fig. 6c is a computed image of the difference between the images of Fig. 6a and Fig. 6b; and

Fig. 7 shows a flowchart of an exemplary embodiment of the method.

10 Detailed Description of Exemplary Embodiments

According to one aspect, there is provided a method for imaging a sample 10, for example, a cell sample. In this method, light passes from an angular illuminator 30 through the cell sample 10 into an image recorder such as a digital camera 32, as shown in Fig. 2.

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In a preferred embodiment, the angular illuminator may comprise a mask 34 between a light source 31 and the cell sample 10. The light source 31 is preferably a ring light. Alternatively, it may be a ring of light emitting diodes, or a spatial light modulator.

20 The mask 34 is preferably of a sector shape and rotatable about an axis that may be perpendicular to the sample 10 and which preferably is coincident with the longitudinal axis 36. The longitudinal axis 36 may pass through the centre of the angular illuminator 30. Alternatively, the axis of rotation may be spaced from but parallel to the longitudinal axis 36. Angular size of a sector-shaped mask typically depends on how

much angular illumination is ideal to obtain the best images. However, it may be in the range of 10° to 90° . As shown it is 60° . Alternatively, other suitable shapes may be used for the mask. Instead of rotating, the mask may make appropriate translational movements to shade portions of the cell sample.

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As part of an exemplary method, angular illuminations of the sample 10 are performed according to a pattern of angular illumination controlled by a controller 38 so as to specify an optical path for light scattered by the sample. Depending on the light scattering characteristics of features in the sample, the optical path may be direct, back
10 scattered, or secondarily reflected, among other possible optical paths.

In the preferred embodiment, the cell sample 10 is angularly illuminated with the mask 34 sharing a portion of the cell sample (100, Fig. 7). An image of the cell sample 10 under such angular illumination is recorded (102, Fig. 7). As shown in Fig. 3, a portion
15 22 (indicated by white dotted lines) is shaded by the mask 34. Cells in the portion 22 therefore do not experience unwanted illumination from back-reflection due to the microwell walls 14. As a result, the image of portion 22 is mainly of only light scattered by the cells in the sample 10. The portion 22 is readily identified by the absence of the bright ring 16 due to reflection from the corner between the wall 14 and base of the well
20 which is visible in the rest of the image. Imaging of cells 18 near the microwell walls 14 in the portion 22 is improved, since direct reflection to the image recorder 32 and back-reflection of light by the microwell walls 14 is eliminated.

The mask 34 is then rotated by an angular interval so that another portion of the microwell is shaded (104, Fig. 7). Another image is then recorded (106, Fig. 7), as shown in Fig. 4. To automate the process, the controller 38 controls the rotation of the mask and synchronises it with the recording of images. The image in Fig. 4 shows a
5 second portion 24 (indicated by white dotted lines) at a separate angular location from the portion 22 in Fig. 3. Again, the second portion 24 is readily identified by the absence of the bright ring 16.

Until all portions of the cell sample 10 have been shaded and imaged (108, Fig. 7) at
10 least once, the process is repeated by rotating the mask 34 at angular intervals around the cell sample 10 and recording an image of the cell sample 10 at each angular interval. In this way, each of the recorded images has a separate shaded portion, and all the recorded images together include shaded portions encompassing the entire cell sample
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Preferably, the angular interval is equal to or less than the angular size of the mask 34. More preferably, the angular interval is significantly less than the angular size of the mask so each of the plurality of images has an overlap region with each adjacent image. For example, if the angular size is 60° , the angular interval may be 50° .

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A processor 39 processes corresponding image pixels among the recorded images by an algorithm. In this embodiment, the algorithm includes a non-linear operator used to combine corresponding image pixels using a per-pixel mathematical minimum function

over the entirety of each image (110, Fig. 7) to form a combined computed image. This is preferably performed using an image-processing application on the computer 40.

For example, a first pixel from a first image is compared to a second pixel from a
5 second image. The first pixel and the second pixel correspond to a single location on the cell sample. The lower value of the two pixels is taken. The same comparison is done between each image for all the recorded images, for all the pixels on each image, so that the lowest pixel value for every location on the cell sample 10 is eventually obtained. This is made possible by keeping the angular illuminator 30, the cell sample 10 and the
10 camera 32 stationary during image recording, so that the recorded images match up with each other. Only the angular illumination is varied by rotating the mask 34 for each image.

Similar angular illumination may also be achieved by other methods and apparatus such
15 as having the controller 38 appropriately activating portions of a programmable array of light emitting diodes. This may be in addition, or as an alternative, to use of the mask 34. When the angular illuminator 30 is a programmable series of light emitting diodes, the light emitting diodes may be sequentially illuminated and switched off around the ring by the controller 38 to provide the same effect of reduced illumination as shading by the
20 mask 34.

By performing the minimum function between all images, a computed image as shown in Fig. 5 is formed. This allows weak isotropic reflectors of the cell sample 10 to be imaged independently of directionally reflecting features. As can be seen, the bright ring

16 visible in Figs. 1, 3 and 4 has been entirely eliminated in Fig. 5. Cells 18 near the microwell walls 14 and cells 20 in the central region have relatively uniform illumination without back-reflection effects.

5 Where directional defects such as, for example, surface scratches, are to be detected, the images obtained may be combined by use of another non-linear operator, e.g., a mathematical maximum function to obtain a single computed image comprising all defect locations independent of orientation of the defects. This allows features with anisotropic light scattering characteristics to be combined in a single computed image of
10 the sample.

The recorded images may be processed by yet other operators depending on imaging requirements for different samples. For example, an analysis operator may be used to differentiate between features based on their degree of anisotropy based on comparison
15 of a pixel's brightness when shaded to that when it is unshaded. Additionally knowledge of the rotation angle of the shade and the brightness of a feature at each rotation may be used to quantify the directionality and relative amplitude of a feature's anisotropy in the sample.

20 In combination with varying operators in the algorithm during processing, depending on imaging requirements, the pattern of angular illumination can also be varied by the controller 38. The pattern of angular illumination may have a range within a three dimensional space surrounding the sample, varying in azimuth angle and/or elevation angle relative to the sample plane, and it may be either symmetrical or asymmetrical

about the sample as the imaging outcome may require. In addition, the pattern of angular illumination may be automatically optimised based on analysis of specularly reflective regions of the sample obtained from a prior scan.

5 By combining various operators in the processing algorithm with various patterns of angular illuminations, specific features or light secondarily reflected by specific features may be selectively eliminated from one or more of the recorded images. Both isotropic and anisotropic features may be separated to allow optimal independent imaging as desired.

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Alternatively or additionally, variable direction illumination may be used. This may be by the use of multiple wavebands that are directional in illumination and sensed independently by spectrally selective channels in the image recorder. This covers variations in which, for example, a colour camera which has red, green and blue

15 responsive pixels could collect data on three directions at once when there are three sets of LEDs of different colours each pointing in a different direction. The wavelength response of the features should be consistent for the colours used. This may be extended to 'n' wavelengths and multiple digital cameras. This allows the information to be recorded in a single instant and may be faster. This may be of advantage for samples that
20 move or vary over time.

Fig. 6c shows a computed image of the difference between an image obtained using conventional full-angle illumination (Fig. 6a) and an image obtained using an exemplary method (Fig. 6b). As Fig. 6c shows, the method eliminates the effect of

radially-increasing excessive illumination (indicated by the white arrow) due to back-reflection by the microwell walls 14 as well as unwanted reflections that lead to a bright ring at the well boundary and so reduce image contrast. Compared to Fig. 6a, a complete image having improved cell to background contrast and uniformity is obtained in Fig. 6b.

Whilst there has been described in the foregoing description exemplary embodiments of the present invention, it will be understood by those skilled in the technology concerned that many variations in details of design, construction and/or operation may be made without departing from the present invention.

The Claims

1. A method of imaging a stationary sample having features with anisotropic light scattering characteristics, the method comprising:
performing a plurality of angular illuminations of the sample according to a
5 pattern of angular illumination controlled by a controller so as to specify an optical path for light scattered by the sample;
recording images of the sample corresponding to the plurality of angular illuminations of the sample;
processing the recorded images to form a computed image.
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2. The method of claim 1, wherein the optical path is selected from the group consisting of: direct, back scattered and secondarily reflected.
3. The method of any one of the preceding claims, wherein at least one of the
15 features is selectively eliminated from at least a portion of the computed image.
4. The method of any one of the preceding claims, wherein the processing comprises processing corresponding image pixels among the recorded images by an algorithm comprising at least one operator.
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5. The method of claim 4, wherein the operator is an analysis operator for differentiating features based on their anisotropy.
6. The method of claim 4, wherein the operator is an isotropy operator for
25 quantifying relative amplitude and directionality of feature anisotropy.
7. The method of claim 4, wherein the operator is a combining operator for producing a combined computed image.

8. The method of claim 7, wherein the combining operator is a non-linear operator for combining the features with anisotropic light scattering characteristics in a single computed image independent of their orientation.
- 5 9. The method of claim 8, wherein the non-linear operator is selected from the group consisting of: minimum and maximum.
10. The method of any one of the preceding claims, further comprising aligning the polar centre of an angular illuminator with an approximate centre of a sample
10 holder supporting the sample before the step of performing a plurality of angular illuminations of the sample.
11. The method of any one of the preceding claims, wherein the pattern of angular illumination ranges within a three dimensional space surrounding the sample and
15 varying relative to the sample plane in one or more of: azimuth and elevation angles.
12. The method of any one of the preceding claims, wherein the pattern of angular illumination is selected from the group consisting of: symmetrical and
20 asymmetrical about the sample.
13. The method of any one of the preceding claims, wherein the pattern of angular illumination is automatically optimised based on analysis of specularly reflective regions of the sample obtained from a prior scan.
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14. The method of any one of the preceding claims, wherein the performing a plurality of angular illuminations comprises applying illumination at angular intervals around the sample.
- 30 15. The method of any one of the preceding claims, wherein the plurality of angular illuminations is performed by rotating a mask at angular intervals.

16. The method of claim 15, wherein the angular intervals are equal to or less than an angular size of the mask.
17. The method of any one of the preceding claims, wherein each of the recorded
5 images has an overlap region with adjacent recorded images.
18. The method of any one of claims 15 to 8, wherein the mask is rotated about an axis that is perpendicular to the sample.
- 10 19. The method as claimed in claim 18, wherein the axis is coincident with a longitudinal axis.
20. The method of any one of the preceding claims, wherein the angular illuminator comprises a light source selected from the group consisting of: a ring light, and
15 an array of light emitting diodes.
21. The method of claim 20, wherein the plurality of angular illuminations is performed by appropriately activating portions of the array of light emitting diodes.
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22. The method of claim 20 or claim 21, wherein the array of light emitting diodes are of multiple wavebands that are directional in illumination and sensed independently by spectrally selective channels in the image recorder.
- 25 23. Apparatus for imaging a stationary sample with features having anisotropic light scattering characteristics, the apparatus comprising:
an angular illuminator for angularly illuminating the sample;
an image recorder for recording images of the sample corresponding to a plurality of angular illuminations of the sample;
30 a controller for controlling the pattern of angular illuminations so as to specify an optical path for light to travel from the angular illuminator to the image recorder when scattered by the sample;

and

a processor for processing the recorded images to form a computed image.

24. The apparatus of claim 23, wherein the processor forms a computed image such
5 that at least one of the features is selectively eliminated from at least a portion of
the computed image.
25. The apparatus of claim 23 or 24, wherein the pattern of angular illuminations is
controlled such that light secondarily reflected by at least one of the features is
10 selectively eliminated from at least a portion of the computed image.
26. The apparatus of claim 25, wherein the feature is a wall of a microwell array.
27. The apparatus of any one of claims 23 to 26, wherein corresponding image
15 pixels from the recorded images are processed by an algorithm in the processor,
the algorithm comprising at least one operator.
28. The apparatus of claim 27, wherein the operator is an analysis operator for
differentiating features based on their anisotropy.
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29. The apparatus of claim 27, wherein the operator is an isotropy operator for
quantifying relative amplitude and directionality of feature anisotropy.
30. The apparatus of claim 27, wherein the operator is a combining operator for
25 producing a combined computed image.
31. The apparatus of claim 30, wherein the combining operator is a non-linear
operator for combining the features with anisotropic light scattering
characteristics in a single computed image independent of their orientation.
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32. The apparatus of claim 31, wherein the non-linear operator is selected from the
group consisting of: minimum and maximum.

33. The apparatus of any one of claims 23 to 32, wherein the pattern of angular illumination ranges within a three dimensional space surrounding the sample and varying relative to the sample plane in one or more of: azimuth and elevation angles.
34. The apparatus of any one of claims 23 to 33, wherein the pattern of angular illumination is selected from the group consisting of: symmetrical and asymmetrical about the sample.
35. The apparatus of any one of claims 23 to 34, wherein the pattern of angular illumination is automatically optimised based on analysis of specularly reflective regions of the sample obtained from a prior scan.
36. The apparatus of any one of claims 23 to 35, wherein the image recorder is an image sensor array with a lens.
37. The apparatus of any one of claims 23 to 36, wherein the angular illuminator includes a light source selected from the group consisting of: a ring light, an array of light emitting diodes and a spatial light modulator.
38. The apparatus of claim 37, wherein the array of light emitting diodes is controlled by the controller to appropriately activate portions of the array of light emitting diodes.
39. The apparatus of claim 37 or claim 38, wherein the light emitting diodes of the array of light emitting diodes are of multiple wavebands that are directional in illumination and able to be sensed independently by spectrally selective channels in the image recorder.
40. The apparatus of any one of claims 37 to 39, wherein the angular illuminator further includes a mask.

41. The apparatus of claims 40, wherein the mask is between the light source and the sample.
- 5 42. The apparatus of claim 40 or 41, wherein the mask is controlled by the controller.
43. The apparatus of any one of claims 40 to 42, wherein the mask is rotatable at a plurality of angular intervals about an axis perpendicular to the sample.
- 10 44. The apparatus as claimed in claim 43, wherein the axis passes through a centre of the angular illuminator.
45. The apparatus as claimed in claim 43 or claim 44, wherein the axis is coincident with a longitudinal axis.
- 15 46. The apparatus of any one of claims 40 to 45, wherein the mask is of a sector shape having an angular size.
47. The apparatus of claim 46 when dependent on claim 43, wherein the angular interval is equal to or less than the angular size to form an overlap region of images.
- 20 48. The apparatus of any one of claims 23 to 47, wherein the imaging is of at least one weakly scattered feature of the sample.
- 25 49. The apparatus of any one of claims 23 to 48, wherein the sample is a cell sample.
50. The apparatus of any one of claims 23 to 49, further comprising an image processing application on a computer for combining the images recorded by the image recorder.
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