PRINTED COVERSLIP AND SLIDE FOR IDENTIFYING REFERENCE FOCAL PLANE FOR LIGHT MICROSCOPY

TECHNICAL FIELD

[0001] The present disclosure relates to light microscopy and particularly relates to reference focal planes for use with light microscopy.

BACKGROUND

[0002] Light microscopy uses visible light and a system of lenses to magnify images or small objects. Light microscopy can be performed using an optical microscope. Optical microscopes typically include an eyepiece and/or camera for viewing objects. When a user views an object through the eyepiece, the user can manually change the focus of the microscope when viewing different portions of the object. Further, when object analysis is performed by a computer program in communication with the camera, the computer program may repeatedly change the focus of the microscope when viewing different portions of the object. However, this need to repeatedly change the focus of the microscope can lead to errors in analysis when performed by a human or a computer program.

[0003] Optical microscopes are commonly used to view a sample of material. The sample may include organic matter such as bacteria or small organisms and may include inorganic matter such as crystalline structures or other materials. When the sample is thicker than the optical depth of field, the user or computer program may need to repeatedly adjust the focal depth of the microscope to view different regions of the sample. In some instances, in an effort to ensure the sample is evenly distributed on a slide, a coverslip is placed on top of the sample such that the sample is sandwiched between the slide and the coverslip.

[0004] Ideally, the coverslips and slides used for light microscopy are smooth, flat, and have a uniform thickness. However, coverslips and slides often deviate from this ideal. In many cases, coverslips and slides have a bowing curvature or a non-uniform thickness. In some implementations, the sample consists of a fluid or malleable medium that may have been placed on the slide by hand or other imprecise means. In such an implementation, when the coverslip is placed on top of the sample, the shape and thickness of the sample sandwiched between the slide and the coverslip may change or vary across sample. This variance can be caused by the curvature, tilt, variation in thickness, or surface imperfections of the slide or the coverslip. The variance causes an uneven distribution of the sample and therefore necessitates that the focus of the microscope is repeatedly changed as a person or computer program view different portions of the sample.

[0005] In light of the foregoing, disclosed herein are systems, methods, and devices for reference frames for light microscopy. The reference frames disclosed herein compensate for variances in slides, coverslips, and samples and therefore improve microscopy imaging.

SUMMARY

[0005a] According to an aspect of the present disclosure, there is provided a method including: capturing an overview image of a coverslip or a slide by imaging the coverslip or the slide with a camera, wherein the overview image depicts an entirety of the coverslip or the slide; identifying a fiducial marker captured in the overview image by processing the overview image with one or more computer-executed programs, wherein the fiducial marker is printed on a surface of a coverslip or a surface of a slide; in response to identifying the fiducial marker in the overview image, determining an x-axis coordinate and a y-axis coordinate indicating a location of the fiducial marker; focusing an optical microscope on the fiducial marker; in response to focusing the optical microscope on the fiducial marker, calculating a focal distance to the fiducial marker, wherein the focal distance is a z-axis coordinate indicating the location of the fiducial marker; and calculating, with the one or more computer-executed programs, a reference focal surface defining the surface of the coverslip or the surface of the slide based at least in part on the x-axis coordinate, the y-axis coordinate, and the z-axis coordinate indicating the location of the fiducial marker.

CLAIMS

1. A method comprising:

capturing an overview image of a coverslip or a slide by imaging the coverslip or the slide with a camera, wherein the overview image depicts an entirety of the coverslip or the slide;

identifying a fiducial marker captured in the overview image by processing the overview image with one or more computer-executed programs, wherein the fiducial marker is printed on a surface of a coverslip or a surface of a slide;

in response to identifying the fiducial marker in the overview image, determining an x-axis coordinate and a y-axis coordinate indicating a location of the fiducial marker;

focusing an optical microscope on the fiducial marker;

in response to focusing the optical microscope on the fiducial marker, calculating a focal distance to the fiducial marker, wherein the focal distance is a z-axis coordinate indicating the location of the fiducial marker; and

calculating, with the one or more computer-executed programs, a reference focal surface defining the surface of the coverslip or the surface of the slide based at least in part on the x-axis coordinate, the y-axis coordinate, and the z-axis coordinate indicating the location of the fiducial marker.

2. The method of claim 1, wherein the fiducial marker is printed on the surface of the coverslip such that the method comprises:

identifying the fiducial marker printed on the surface of the coverslip; and

calculating the reference focal plane defining the surface of the coverslip.

3. The method of claim 2, wherein the optical microscope comprises the camera, and:

the fiducial marker is printed on a bottom surface of the coverslip relative to an eyepiece or the camera of the optical microscope, such that the bottom surface is in contact with a sample;

the reference focal plane defining the surface of the coverslip defines the bottom surface of the coverslip; and

the reference focal plane defining the bottom surface of the coverslip further defines a top surface of the sample relative to the eyepiece or the camera of the optical microscope.

4. The method of claim 2, further comprising:

scanning the coverslip with the optical microscope to generate an overview scan;

identifying a plurality of fiducial markers printed on the surface of the coverslip based on the overview scan;

calculating a quantity of fiducial markers printed on the surface of the coverslip based on the overview scan; and

identifying a location of each of the plurality of fiducial markers printed on the surface of the coverslip based on the overview scan.

5. The method of claim 4, wherein calculating the reference focal plane defining the surface of the coverslip comprises:

focusing the optical microscope on each of the plurality of fiducial markers printed on the surface of the coverslip to calculate a focal distance for each of the plurality of fiducial markers;

matching the focal distance for each of the plurality of fiducial markers with a corresponding location for each of the plurality of fiducial markers; and

calculating the reference focal plane based on the corresponding focal distance and location of each of the plurality of fiducial markers.

6. The method of claim 5, wherein calculating the reference focal plane further comprises:

interpolating focal distances for space between two or more fiducial markers based on focal distances for the two or more fiducial markers; and

extrapolating the focal distance for a certain fiducial marker of the plurality of fiducial markers to estimate focal distances for an area surrounding the certain fiducial marker.

7. The method of claim 5, wherein calculating the reference focal plane further comprises:

identifying three fiducial markers of the plurality of fiducial markers;

identifying focal distances for each of the three fiducial markers;

identifying locations for each of the three fiducial markers relative to the overview scan; and

fitting planes to triangles defined by the three fiducial markers based at least in part on the focal distances and the locations for each of the three fiducial markers.

8. The method of claim 5, wherein calculating the reference focal plane further comprises:

identifying four or more fiducial markers of the plurality of fiducial markers;

identifying (x,y,z) coordinates for each of the four or more fiducial markers based on focal distances for each of the four or more fiducial markers and locations of each of the four or more fiducial markers relative to the overview scan; and

fitting curved surfaces to points defined by the (x,y,z) coordinates of each of the four or more fiducial markers to generate a surface topology approximating the entire surface of the coverslip.

9. The method of claim 2, wherein the optical microscope comprises the camera, and wherein the fiducial marker is printed on a bottom surface of the coverslip relative to an eyepiece or the camera of the optical microscope, such that the bottom surface is in contact with a sample, and wherein the method further comprises:

calculating a predicted focal distance to the sample based on results of the focusing of the optical microscope on one or more fiducial markers; and

refining focus on the sample by testing a threshold range of focal distances that are greater than the predicted focal distance to the sample and/or less than the predicted focal distance to the sample.

10. The method of claim 2, further comprising:

scanning the coverslip with the optical microscope to generate the overview scan;

identifying the fiducial marker within the overview scan;

calculating a z-axis location of the fiducial marker based on the focal distance to the fiducial marker; and

calculating an x-axis and a y-axis location of the fiducial marker based on a location of the fiducial marker relative to the overview scan.

11. The method of claim 2, further comprising preparing a sample for imaging with the optical microscope by placing the sample on the slide using a sample transfer tool, wherein the sample transfer tool comprises:

an elongated member;

an outer loop defining an interior space; and

one or more spokes attached to the outer loop and disposed within the interior space of the outer loop;

wherein at least one spoke of the one or more spokes and at least a portion of the outer loop define a sample capture loop for capturing the sample; and

wherein the elongated member is approximately normal to the one or more spokes.

12. The method of claim 2, further comprising identifying a chiral indicator on the coverslip and determining the orientation of the coverslip based on the chiral indicator.

13. The method of claim 2, wherein the optical microscope comprises the camera, and wherein the fiducial marker is printed on a bottom surface of the coverslip relative to an eyepiece or the camera of the optical microscope, such that the bottom surface is in contact with a sample, and wherein the method further comprises locating a particle of interest within the sample by focusing the optical microscope on a predicted particle depth, wherein the predicted particle depth is an estimated depth where the particle is located in the sample relative the bottom surface of the coverslip.

14. The method of claim 13, wherein focusing the optical microscope on the predicted

particle depth comprises determining the predicted particle depth relative to the optical microscope based on the reference focal plane.

15. The method of claim 2, further comprising:

receiving an image of the coverslip captured by the camera, wherein the camera is associated with the optical microscope;

assessing the image to determine if a fiducial marker is captured in the image;

in response to no fiducial marker being visible in the image, altering a field of view for the camera associated with the optical microscope;

receiving a new image of the coverslip captured by the camera with the altered field of view; and

assessing the new image to determine if a fiducial marker is captured in the image.

16. The method of claim 15, further comprising, in response to a fiducial marker being captured in the image and/or the new image:

assessing the image and/or the new image to determine whether an entirety of the captured fiducial marker is present in the image and/or the new image or a portion of the captured fiducial marker is present in the image and/or the new image;

in response to only a portion of the captured fiducial marker being present in the image and/or the new image, identifying a captured edge outline of the captured fiducial marker;

retrieving from memory an expected edge outline of the captured fiducial marker based on an overview image of the coverslip comprising all fiducial markers; and

comparing the captured edge outline with the expected edge outline to calculate an

estimated direction and distance required to move a stage or an objective of the optical microscope such that the entirety of the captured fiducial marker is present in a field of view of the optical microscope.

17. The method of claim 16, further comprising:

causing the stage and/or the objective of the optical microscope to move according to the estimated direction and distance;

determining (x,y) coordinates for the stage and/or the objective of the microscope after movement; and

storing in memory an (x,y) coordinate of the captured fiducial marker based on the (x,y) coordinates of the stage and/or the objective.

18. The method of claim 15, further comprising, in response to a fiducial marker being captured in the image and/or the new image:

assessing the image and/or the new image to determine whether an entirety of the field of view is covered by the captured fiducial marker;

in response to only a portion of the field of view being covered by the captured fiducial marker, identifying a captured edge outline of the captured fiducial marker;

retrieving from memory an expected edge outline of the captured fiducial marker based on an overview image of the coverslip comprising all fiducial markers; and

comparing the captured edge outline with the expected edge outline to calculate an estimated direction and distance required to move a stage or an objective of the optical microscope such that the entirety of the field of view of the optical microscope is covered by the

fiducial marker.

19. The method of claim 18, further comprising:

causing the stage and/or the objective of the optical microscope to move according to the estimated direction and distance;

determining (x,y) coordinates for the stage and/or the objective of the microscope after movement; and

storing in memory an (x,y) coordinate of the captured fiducial marker based on the (x,y) coordinates of the stage and/or the objective.

20. The method of claim 2, further comprising:

retrieving from memory an expected edge outline of a first fiducial marker printed on the surface of the coverslip based on an overview image comprising all fiducial markers printed on the surface of the coverslip;

identifying a captured edge outline of the first fiducial marker based on an image captured with the optical microscope;

comparing the expected edge outline of the first fiducial marker with the captured edge outline of the first fiducial marker; and

determining whether the expected edge outline of the first fiducial marker matches the captured edge outline of the first fiducial marker within an accepted tolerance threshold.

21. The method of claim 2, further comprising:

identifying a printed area of the coverslip; and

optimizing a scan area for a sample covered by the coverslip based on the printed area of the coverslip;

wherein the scan area is optimized based on one or more of scan time, scan file size, or test sensitivity.

22. The method of claim 1, wherein the fiducial marker is printed on the surface of the slide such that the method comprises:

identifying the fiducial marker printed on the surface of the slide; and

calculating the reference focal plane defining the surface of the slide.

23. The method of claim 22, wherein the optical microscope comprises the camera, and wherein:

the fiducial marker is printed on a top surface of the slide relative to an eyepiece or the camera of the optical microscope, such that the top surface of the slide is in contact with a sample;

the reference focal plane defining the surface of the slide defines the top surface of the slide; and

the reference focal plane defining the top surface of the slide further defines a bottom surface of the sample relative to the eyepiece or the camera of the optical microscope.

24. The method of claim 22, further comprising:

scanning the slide with the optical microscope to generate the overview scan;

identifying a plurality of fiducial markers printed on the surface of the slide based on the

overview scan;

calculating a quantity of fiducial markers printed on the surface of the slide based on the overview scan; and

identifying a location of each of the plurality of fiducial markers printed on the surface of the slide based on the overview scan.

25. The method of claim 24, wherein calculating the reference focal plane defining the surface of the slide comprises:

focusing the optical microscope on each of the plurality of fiducial markers printed on the surface of the slide to calculate a focal distance for each of the plurality of fiducial markers;

matching the focal distance for each of the plurality of fiducial markers with a corresponding location for each of the plurality of fiducial markers; and

calculating the reference focal plane based on the corresponding focal distance and location of each of the plurality of fiducial markers.

26. The method of claim 25, wherein calculating the reference focal plane further comprises:

interpolating focal distances for space between two or more fiducial markers based on focal distances for the two or more fiducial markers; and

extrapolating the focal distance for a certain fiducial marker of the plurality of fiducial markers to estimate focal distances for an area surrounding the certain fiducial marker.

27. The method of claim 25, wherein calculating the reference focal plane further comprises:

identifying three fiducial markers of the plurality of fiducial markers;

identifying focal distances for each of the three fiducial markers;

identifying locations for each of the three fiducial markers relative to the overview scan; and

fitting planes to triangles defined by the three fiducial markers based at least in part on the focal distances and the locations for each of the three fiducial markers.

28. The method of claim 25, wherein calculating the reference focal plane further comprises:

identifying four or more fiducial markers of the plurality of fiducial markers;

identifying (x,y,z) coordinates for each of the four or more fiducial markers based on focal distances for each of the four or more fiducial markers and locations of each of the four or more fiducial markers relative to the overview scan; and

fitting curved surfaces to points defined by the (x,y,z) coordinates of each of the four or more fiducial markers to generate a surface topology approximating the entire surface of the slide.

29. The method of claim 22, wherein the optical microscope comprises the camera, and wherein the fiducial marker is printed on a top surface of the slide relative to an eyepiece or the camera of the optical microscope, such that the top surface is in contact with a sample, and wherein the method further comprises:

calculating a predicted focal distance to the sample based on results of the focusing of the optical microscope on one or more fiducial markers; and

refining focus on the sample by testing a threshold range of focal distances that are greater than the predicted focal distance to the sample and/or less than the predicted focal

distance to the sample.

30. The method of claim 22, further comprising:

scanning the slide with the optical microscope to generate the overview scan;

identifying the fiducial marker within the overview scan;

calculating a z-axis location of the fiducial marker based on the focal distance to the fiducial marker; and

calculating an x-axis and a y-axis location of the fiducial marker based on a location of the fiducial marker relative to the overview scan.

31. The method of claim 22, further comprising preparing a sample for imaging with the optical microscope by placing the sample on the slide using a sample transfer tool, wherein the sample transfer tool comprises:

an elongated member;

an outer loop defining an interior space; and

one or more spokes attached to the outer loop and disposed within the interior space of the outer loop;

wherein at least one spoke of the one or more spokes and at least a portion of the outer loop define a sample capture loop for capturing the sample; and

wherein the elongated member is approximately normal to the one or more spokes.

32. The method of claim 22, further comprising identifying a chiral indicator on the slide and determining the orientation of the slide based on the chiral indicator.

33. The method of claim 22, wherein the optical microscope comprises the camera, and wherein the fiducial marker is printed on a top surface of the slide relative to an eyepiece or the camera of the optical microscope, such that the top surface is in contact with a sample, and wherein the method further comprises locating a particle of interest within the sample by focusing the optical microscope on a predicted particle height, wherein the predicted particle height is an estimated vertical distance where the particle is located in the sample relative the top surface of the slide.

34. The method of claim 33, wherein focusing the optical microscope on the predicted particle height comprises determining the predicted particle height relative to the optical microscope based on the reference focal plane.

35. The method of claim 22, further comprising:

receiving an image of the slide captured by the camera, wherein the camera is associated with the optical microscope;

assessing the image to determine if a fiducial marker is captured in the image;

in response to no fiducial marker being visible in the image, altering a field of view for the camera associated with the optical microscope;

receiving a new image of the slide captured by the camera with the altered field of view; and

assessing the new image to determine if a fiducial marker is captured in the image.

36. The method of claim 35, further comprising, in response to a fiducial marker being captured in the image and/or the new image:

assessing the image and/or the new image to determine whether an entirety of the captured fiducial marker is present in the image and/or the new image or a portion of the captured fiducial marker is present in the image and/or the new image;

in response to only a portion of the captured fiducial marker being present in the image and/or the new image, identifying a captured edge outline of the captured fiducial marker;

retrieving from memory an expected edge outline of the captured fiducial marker based on an overview image of the coverslip comprising all fiducial markers; and

comparing the captured edge outline with the expected edge outline to calculate an estimated direction and distance required to move a stage or an objective of the optical microscope such that the entirety of the captured fiducial marker is present in a field of view of the optical microscope.

37. The method of claim 36, further comprising:

causing the stage and/or the objective of the optical microscope to move according to the estimated direction and distance;

determining (x,y) coordinates for the stage and/or the objective of the microscope after movement; and

storing in memory an (x,y) coordinate of the captured fiducial marker based on the (x,y) coordinates of the stage and/or the objective.

38. The method of claim 35, further comprising, in response to a fiducial marker being

captured in the image and/or the new image:

assessing the image and/or the new image to determine whether an entirety of the field of view is covered by the captured fiducial marker;

in response to only a portion of the field of view being covered by the captured fiducial marker, identifying a captured edge outline of the captured fiducial marker;

retrieving from memory an expected edge outline of the captured fiducial marker based on an overview image of the coverslip comprising all fiducial markers; and

comparing the captured edge outline with the expected edge outline to calculate an estimated direction and distance required to move a stage or an objective of the optical microscope such that the entirety of the field of view of the optical microscope is covered by the fiducial marker.

39. The method of claim 38, further comprising:

causing the stage and/or the objective of the optical microscope to move according to the estimated direction and distance;

determining (x,y) coordinates for the stage and/or the objective of the microscope after movement; and

storing in memory an (x,y) coordinate of the captured fiducial marker based on the (x,y) coordinates of the stage and/or the objective.

40. The method of claim 22, further comprising:

retrieving from memory an expected edge outline of a first fiducial marker printed on the surface of the slide based on an overview image comprising all fiducial markers printed on the

surface of the slide;

identifying a captured edge outline of the first fiducial marker based on an image captured with the optical microscope;

comparing the expected edge outline of the first fiducial marker with the captured edge outline of the first fiducial marker; and

determining whether the expected edge outline of the first fiducial marker matches the captured edge outline of the first fiducial marker within an accepted tolerance threshold.

41. The method of claim 22, further comprising:

identifying a printed area of the slide; and

optimizing a scan area for a sample disposed on the slide based on the printed area of the slide;

wherein the scan area is optimized based on one or more of scan time, scan file size, or test sensitivity.