IMAGE ANALYSIS AND MEASUREMENT OF BIOLOGICAL SAMPLES

BACKGROUND

[0001] Analysis of biological samples from a subject may be important for health-related diagnosing, monitoring and/or treating of the subject. A variety of methods are known for the analysis of biological samples. However, in order to provide better diagnosing, monitoring, and/or treating of subjects, improvements in the analysis of biological samples are desired.

INCORPORATION BY REFERENCE

[0002] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

SUMMARY

[0003] Methods, devices, systems, and apparatuses described herein are useful for optical and image analysis and/or measurement of biological samples. In a first aspect, the present invention refers to a system for analyzing a sample, the system comprising: a sample holder comprising a sample chamber comprising an elongated channel configured to hold said sample, at least a portion of said sample holder comprising an optically transmissive material, said optically transmissive material comprising an optically transmissive surface and at least one flat reflective surface; and an illumination source configured to provide light that illuminates and passes through said optically transmissive surface; wherein said sample holder is configured effective that said light from said illumination source simultaneously provides both epi-illumination and trans-illumination to a sample in the sample holder, where epi-illumination comprises light traveling from said illumination source to said sample without reflection at a flat reflective surface of the optically transmissive material of the sample holder, and where trans-illumination comprises light traveling within the optically transmissive material and to the sample following at least one reflection from at least one flat reflective surface of said optically transmissive material. In a second aspect, the present invention refers to a cuvette for illumination by an illumination source, said cuvette comprising a sample chamber comprising en elongated channel configured to hold a sample, at least a portion of said cuvette comprising an optically transmissive material, said optically transmissive material comprising an optically transmissive surface and at least one flat reflective surface, wherein said optically transmissive surface and said at least one flat reflective surface are configured effective that light passing through the optically transmissive surface simultaneously provides both epi-illumination and trans-

illumination to said sample in the sample chamber, where epi-illumination comprises light traveling from said illumination source to the sample without reflection at a surface of the optically transmissive material, and where trans-illumination comprises light traveling within the optically transmissive material and to the sample following at least one reflection from at least one flat reflective surface of said optically transmissive material. ~~In a third aspect, the present invention refers to a cuvette comprising a sample chamber having an optically transmissive floor, said cuvette having an outer surface comprising at least one concave or convex structure configured to provide mechanical support to the cuvette.~~ In a ~~fourth~~ third aspect, the present invention refers to a method of identifying a cell in a sample containing a plurality of cells, comprising: (a) placing said sample in a sample holder comprising a sample chamber comprising an elongated channel configured to hold the sample, at least a portion of said sample holder comprising an optically transmissive material, said optically transmissive material comprising an optically transmissive surface and at least one flat reflective surface, wherein said optically transmissive surface and said at least one flat reflective surface are configured effective that light passing through the optically transmissive surface simultaneously provides both epi-illumination and trans-illumination to the sample in the sample chamber, where epi-illumination comprises light traveling from said illumination source to the sample without reflection at a flat reflective surface of the optically transmissive material, and where transillumination comprises light traveling within the optically transmissive material and to the sample following at least one reflection from at least one flat reflective surface of said optically transmissive material; (b) illuminating said sample holder effective to simultaneously provide both epi-illumination and trans-illumination of the sample; and (c) identifying a cell in the sample.

[0004] Examples disclosed herein include sample holders suitable for holding samples, including biological samples, for optical examination, for optical measurement, and for other examinations and measurements. In examples, a sample holder having an optically transmissive portion and a portion configured to provide internal reflection of light within the sample holder is provided. In examples, internal reflections may include partial internal reflection and may include total internal reflection of light. Incident light from an external light source, and directed from one side of the sample holder, is effective to illuminate a sample within the sample holder from a plurality of directions. In examples, an external light source disposed on one side of the sample holder may provide epi-illumination of a sample within the sample holder; may provide trans-illumination of a sample within the sample holder; and/or may provide both epi-illumination and trans-illumination of a sample within the sample holder.

within the sample holder. In examples, a sample holder is configured to provide trans-illumination of a sample by reflection of light within the sample holder; such reflectance may include partial internal reflection (PIR), and such reflectance may include total internal reflectance (TIR). In examples, a sample holder is configured to provide trans-illumination of a sample by reflection of light within the sample holder, wherein the source of the reflected light is disposed on the same side of the sample holder as the optics used to detect or measure the light ~~(i.e., the light source is an epi-illumination light source)~~.

[0015] The system herein can simultaneously use both epi (direct) and trans (reflected) illumination in darkfield imaging. This differs from traditional darkfield imaging which uses either epi-illumination, or trans-illumination, but not both types of illumination, and not both types of illumination from a single source or single direction or location. Thus, the combination of epi- and trans-illumination disclosed herein, wherein the trans-illumination originates from the same light source as the epi-illumination, differs from known systems. Optionally, the use of a shaped sample holder such as the cuvette can be used to provide the trans-illumination. In examples, a shaped sample holder is configured to provide trans-illumination by reflection of light. In examples, a shaped sample holder is configured to provide trans-illumination by reflection of light within the sample holder. In examples, one or more of the size, shape, surface, materials, or other feature of a shaped sample holder is effective to provide internal reflection of light within the shaped sample holder. In examples, one or more of the size, shape, surface, materials, or other feature of a shaped sample holder is effective to provide partial internal reflection (PIR) of light within the shaped sample holder. In examples, one or more of the size, shape, surface, materials, or other feature of a shaped sample holder is effective to provide total internal reflection (TIR) of light within the shaped sample holder. Optionally, the intensity of trans-illumination is non-negligible. In examples, a shaped sample holder may include a reflective surface effective to increase trans-illumination light intensity. The dark field light source may be a light-emitting diode (LED), laser, or other illumination source that can provide the desired illumination and/or excitation wavelength(s).

[0016] In one example, the combination of the microscope objective and light source such as but not limited to a ringlight (for darkfield microscopy) is at a physical distance between them that enables a compact size for the detector assembly. In one example, only light at a desired wavelength or within a desired range of wavelengths are directed to the sample. In one example, the light is non-polarized light. In another embodiment, the light is polarized light.

[0017] In yet another example, information from the cytometry assay, either from the sample preparation phase and/or from the analysis phase, is used to guide and/or trigger a secondary procedure. In examples, such a secondary procedure may be to provide an alert

WHAT IS CLAIMED IS:

1. A system for analyzing a sample, the system comprising:

a sample holder comprising a sample chamber comprising an elongated channel configured to hold said sample, at least a portion of said sample holder comprising an optically transmissive material, said optically transmissive material comprising an optically transmissive surface and at least one flat reflective surface; and

an illumination source configured to provide light that illuminates and passes through said optically transmissive surface;

wherein said sample holder is configured effective that said light from said illumination source simultaneously provides both epi-illumination and trans-illumination to a sample in the sample holder, where epi-illumination comprises light traveling from said illumination source to said sample without reflection at a flat reflective surface of the optically transmissive material of the sample holder, and where trans-illumination comprises light traveling within the optically transmissive material and to the sample following at least one reflection from at least one flat reflective surface of said optically transmissive material.

2. The system of claim 1, wherein the sample holder comprises one or more optically non-transmissive surfaces.

3. The system of claim 1, wherein said trans-illumination is provided at least in part by total internal reflection of light at a flat reflective surface.

4. The system of claim 1, wherein said trans-illumination is provided at least in part by total internal reflection of light within the sample holder, wherein the sample holder comprises a cuvette.

5. The system of claim 1, wherein the sample holder comprises two or more sample chambers for holding sample.

6. The system of claim 1, wherein the sample holder comprises a cuvette and wherein the cuvette has a rectangular horizontal, cross-sectional shape.

7. The system of claim 1, wherein the sample holder comprises a cuvette and wherein the cuvette has a circular horizontal, cross-sectional shape.

8. The system of claim 1, wherein the sample holder comprises a cuvette and wherein the cuvette has a saw tooth vertical cross-sectional shape.

9. The system of claim 1, wherein the sample holder comprises a cuvette and wherein the cuvette has a step-shaped vertical cross-sectional shape.

10. The system of claim 1, wherein said sample holder is movable relative to said illumination source to a plurality of locations, wherein said optically transmissive surface of the sample holder may be illuminated by the illumination source at each of said locations.

11. The system of claim 1, wherein said illumination source comprises a ringlight.

12. The system of claim 11, wherein said ringlight is selected from a light emitting diode (LED)-based ringlight and a laser-based ringlight.

13. The system of claim 1, further comprising a support structure comprising an optically transmissive surface shaped to engage an optically transmissive surface of the sample holder.

14. The system of claim 1, further comprising a compression device configured to retain the sample holder in a desired location for illumination by the illumination source.

15. The system of claim 1, further comprising a detector configured to image at least a portion of a channel in the sample holder.

16. The system of claim 15, wherein said sample holder comprises an elongated channel configured to contain at least a portion of the sample, and wherein said detector is configured to image an entire elongated channel in the sample holder.

17. The system of claim 15, wherein the sample holder is configured to hold the sample in a static, non-flowing manner during imaging.

18. The system of claim 15, wherein during imaging, the sample holder is configured to hold one portion of the sample in a static, non-flowing manner and another portion in a flowing manner.

19. The system of claim 15, wherein said illumination source is movable relative to the sample holder.

20. The system of any one of the foregoing claims wherein during imaging, the sample holder is configured to hold the sample in a flowing manner.

21. The system of claim 15, wherein said sample holder further comprises a fluid circuit fully confined in the sample holder, and wherein the sample is located in said fluid circuit, effective that the sample remains separate from said detector.

22. The system of claim 21, wherein said sample holder is movable relative to the detector.

23. The system of claim 21, wherein said detector is movable relative to the sample holder.

24. The system of claim 1, wherein said sample holder and said illumination source comprise at least part of an optical analysis unit, said system further comprising a clinical analysis unit configured to perform clinical analysis on said sample.

25. The system of claim 24, wherein said system is configured to provide an aliquot of a single sample to each of said optical analysis unit and said clinical analysis unit, effective that said clinical analysis unit and said optical analysis unit may perform optical analysis and clinical analysis on portions of a sample at the same time.

26. The system of claim 24, wherein said clinical analysis is selected from general chemical analysis, nucleic acid analysis, and enzyme-linked binding analysis.

27. The system of claim 24, comprising a plurality of clinical analysis units, wherein each clinical analysis unit of said plurality of clinical analysis units is configured to provide a clinical analysis selected from general chemical analysis, nucleic acid analysis, and enzyme-linked binding analysis.

28. A cuvette for illumination by an illumination source, said cuvette comprising a sample chamber comprising an elongated channel configured to hold a sample, at least a portion of said cuvette comprising an optically transmissive material, said optically transmissive material comprising an optically transmissive surface and at least one flat reflective surface, wherein said optically transmissive surface and said at least one flat reflective surface are configured effective that light passing through the optically transmissive surface simultaneously provides both epi-illumination and trans-illumination to said sample in the sample chamber, where epi-illumination comprises light traveling from said illumination source to the sample without reflection at a surface of the optically transmissive material, and where trans-illumination comprises light traveling within the optically transmissive material and to the sample following at least one reflection from at least one flat reflective surface of said optically transmissive material.

29. The cuvette of claim 28, further comprising one or more optically non-transmissive surfaces.

30. The cuvette of claim 28, wherein said trans-illumination is provided at least in part by partial internal reflection of light at a flat reflective surface.

31. The cuvette of claim 28, wherein said trans-illumination is provided at least in part by total internal reflection of light at a flat reflective surface.

32. The cuvette of claim 28, wherein the sample holder comprises two or more sample chambers for holding sample.

33. The cuvette of claim 28, comprising a cross-sectional shape selected from rectangular horizontal cross-sectional shape and a circular horizontal cross-sectional shape.

34. The cuvette of claim 28, comprising a cross-sectional shape selected from a saw tooth vertical cross-sectional shape and a step-shaped vertical cross-sectional shape.

35. A method of identifying a cell in a sample containing a plurality of cells, comprising:

(a) placing said sample in a sample holder comprising a sample chamber comprising an elongated channel configured to hold the sample, at least a portion of said sample holder comprising an optically transmissive material, said optically transmissive

material comprising an optically transmissive surface and at least one flat reflective surface, wherein said optically transmissive surface and said at least one flat reflective surface are configured effective that light passing through the optically transmissive surface simultaneously provides both epi-illumination and trans-illumination to the sample in the sample chamber, where epi-illumination comprises light traveling from said illumination source to the sample without reflection at a flat reflective surface of the optically transmissive material, and where trans-illumination comprises light traveling within the optically transmissive material and to the sample following at least one reflection from at least one flat reflective surface of said optically transmissive material;

(b) illuminating said sample holder effective to simultaneously provide both epi-illumination and trans-illumination of the sample; and

(c) identifying a cell in the sample.

36. The method of claim 35, wherein said identifying comprises identifying said cell with a detector configured to image at least a portion of said sample chamber.

WHAT IS CLAIMED IS:

1. A system for analyzing a sample, the system comprising:

a sample holder comprising a sample chamber configured to hold said sample, at least a portion of said sample holder comprising an optically transmissive material, said optically transmissive material comprising an optically transmissive surface and a reflective surface; and

an illumination source configured to provide light that illuminates and passes through said optically transmissive surface;

wherein said sample holder is configured effective that said light from said illumination source simultaneously provides both epi-illumination and trans-illumination to a sample in the sample holder, where epi-illumination comprises light traveling from said illumination source to said sample without reflection at a surface of the optically transmissive material of the sample holder, and where trans-illumination comprises light traveling within the optically transmissive material and to the sample following at least one reflection from at least one surface of said optically transmissive material.

2. The system of claim 1, wherein the sample holder comprises a cuvette having an elonagated channel configured for holding a sample.

3. The system of claim 1 or 2, wherein the sample holder comprises one or more optically non-transmissive surfaces.

4. The system of claim 1 or 2, wherein said trans-illumination is provided at least in part by total internal reflection of light at a surface.

5. The system of claim 2, wherein said trans-illumination is provided at least in part by total internal reflection of light within the cuvette.

6. The system of claim 1, wherein the sample holder comprises two or more sample chambers for holding sample.

7. The system of claim 2, wherein the cuvette has a rectangular horizontal, cross-sectional shape.

8. The system of claim 2, wherein the cuvette has a circular horizontal, cross-sectional shape.

9. The system of claim 2, wherein the cuvette has a saw tooth vertical cross-sectional shape.

10. The system of claim 2, wherein the cuvette has a step-shaped vertical cross-sectional shape.

11. The system of claim 1, wherein said sample holder is movable relative to said illumination source to a plurality of locations, wherein said optically transmissive surface of the sample holder may be illuminated by the illumination source at each of said locations.

12. The system of claim 1, wherein said illumination source comprises a ringlight.

13. The system of claim 12, wherein said ringlight is selected from a light emitting diode (LED)-based ringlight and a laser-based ringlight.

14. The system of claim 1, further comprising a support structure comprising an optically transmissive surface shaped to engage an optically transmissive surface of the sample holder.

15. The system of claim 1, further comprising a compression device configured to retain the sample holder in a desired location for illumination by the illumination source.

16. The system of claim 1, further comprising a detector configured to image at least a portion of a channel in the sample holder.

17. The system of claim 16, wherein said sample holder comprises an elongated channel configured to contain at least a portion of the sample, and wherein said detector is configured to image an entire elongated channel in the sample holder.

18. The system of claim 16, wherein the sample holder is configured to hold the sample in a static, non-flowing manner during imaging.

19. The system of claim 16, wherein during imaging, the sample holder is configured to hold one portion of the sample in a static, non-flowing manner and another portion in a flowing manner.

20. The system of claim 16, wherein said illumination source is movable relative to the sample holder.

21. The system of any one of the foregoing claims wherein during imaging, the sample holder is configured to hold the sample in a flowing manner.

22. The system of claim 16, wherein said sample holder further comprises a fluid circuit fully confined in the sample holder, and wherein the sample is located in said fluid circuit, effective that the sample remains separate from said detector.

23. The system of claim 22, wherein said sample holder is movable relative to the detector.

24. The system of claim 22, wherein said detector is movable relative to the sample holder.

25. The system of claim 1, wherein said sample holder and said illumination source comprise at least part of an optical analysis unit, said system further comprising a clinical analysis unit configured to perform clinical analysis on said sample.

26. The system of claim 25, wherein said system is configured to provide an aliquot of a single sample to each of said optical analysis unit and said clinical analysis unit, effective that said clinical analysis unit and said optical analysis unit may perform optical analysis and clinical analysis on portions of a sample at the same time.

27. The system of claim 25, wherein said clinical analysis is selected from general chemical analysis, nucleic acid analysis, and enzyme-linked binding analysis.

28. The system of claim 25, comprising a plurality of clinical analysis units, wherein each clinical analysis unit of said plurality of clinical analysis units is configured to provide a clinical analysis selected from general chemical analysis, nucleic acid analysis, and enzyme-linked binding analysis.

29. A cuvette comprising a sample chamber configured to hold a sample, at least a portion of said cuvette comprising an optically transmissive material, said optically transmissive material comprising an optically transmissive surface and a reflective surface, wherein said optically transmissive surface and said reflective surface are configured effective that light passing through the optically transmissive surface simultaneously provides both epi-illumination and trans-illumination to said sample in the sample chamber, where epi-illumination comprises light traveling from said illumination source to the sample without reflection at a surface of the optically transmissive material, and where trans-illumination comprises light traveling within the optically transmissive material and to the sample following at least one reflection from at least one surface of said optically transmissive material.

30. The cuvette of claim 29, wherein the sample chamber comprises an elongated channel.

31. The cuvette of claim 29, further comprising one or more optically non-transmissive surfaces.

32. The cuvette of claim 29, wherein said trans-illumination is provided at least in part by partial internal reflection of light at a surface.

33. The cuvette of claim 29, wherein said trans-illumination is provided at least in part by total internal reflection of light at a surface.

34. The cuvette of claim 29, wherein the sample holder comprises two or more sample chambers for holding sample.

35. The cuvette of claim 29, comprising a cross-sectional shape selected from rectangular horizontal cross-sectional shape and a circular horizontal cross-sectional shape.

36. The cuvette of claim 29, comprising a cross-sectional shape selected from a saw tooth vertical cross-sectional shape and a step-shaped vertical cross-sectional shape.

37. A cuvette comprising a sample chamber having an optically transmissive floor, said cuvette having an outer surface comprising at least one concave or convex structure configured to provide mechanical support to the cuvette.

38. The cuvette of claim 37, wherein said at least one concave or convex structure has a cross-sectional shape selected from rectangular, triangular, circular, and semi-circular.

39. The cuvette of claim 37, wherein said at least one concave or convex structure is configured to provide a pathway for internally reflected light within the cuvette.

40. The cuvette of claim 37, wherein said at least one concave or convex structure comprises a surface, and wherein said surface is configured to reflect light within the cuvette.

41. A method of identifying a cell in a sample containing a plurality of cells, comprising:

(a) placing said sample in a sample holder comprising a sample chamber configured to hold the sample, at least a portion of said sample holder comprising an optically transmissive material, said optically transmissive material comprising an optically transmissive surface and a reflective surface, wherein said optically transmissive surface and said reflective surface are configured effective that light passing through the optically transmissive surface simultaneously provides both epi-illumination and trans-illumination to the sample in the sample chamber, where epi-illumination comprises light traveling from said illumination source to the sample without reflection at a surface of the optically transmissive material, and where trans-illumination comprises light traveling within the optically transmissive material and to the sample following at least one reflection from at least one surface of said optically transmissive material;

(b) illuminating said sample holder effective to simultaneously provide both epi-illumination and trans-illumination of the sample; and

(c) identifying a cell in the sample.

42. The method of claim 41, wherein said identifying comprises identifying said cell with a detector configured to image at least a portion of said sample chamber.

43. The method of claim 43, wherein said sample chamber comprises an elongated channel.