Systems and Methods for Containing Biological Samples

Background of the Invention

Field of the Invention

The present invention relates generally to devices, systems, and methods for containing biological samples, and more specifically to devices, systems, and methods for containing biological samples in a plurality of reaction sites for assessment.

Description of the Related Art

The use of microtiter plates have been used for monitoring, measuring, and/or analyzing multiple biological and biochemical reactions during a single experiment or assay. Such plates are commonly used in sequencing, genotyping, polymerase chain reactions (PCR), and other biochemical reactions to monitor progress and provide quantitative data. For example, an optical excitation beam may be used during real-time PCR (qPCR) processes to illuminate fluorescent DNA-binding dyes or fluorescent probes to produce fluorescent signals indicative of the amount of a target gene or other nucleotide sequence. Increasing demands to provide greater numbers of reactions per experiment or assay have resulted in instruments that are able to conduct much large numbers of reactions simultaneously.

Newer approaches such as digital PCR (dPCR) have increased the demand for devices, systems, and methods involving ever greater numbers of reaction sites that are much smaller than those used in more traditional quantitative PCR (qPCR). There is a need for systems and sample formats that will provide reliable, high quality, data in high-density sample formats with sample sites having volumes on the order of nanoliters or picoliters or even smaller.

Summary of the Invention

According to a first aspect of the present invention, there is provided an article for holding a plurality of biological samples, the article comprising:

a substrate comprising a first surface and an opposing second surface; and

a plurality of reaction sites in the substrate, each of the reaction sites extending from an opening in the first surface to an opening in the second surface, the reaction sites being configured to provide sufficient surface tension by capillary action to hold respective biological samples;

wherein a density of reaction sites over at least a portion of one of surfaces is at least 170 holes per square millimeter.

According to a second aspect, there is provided an article for holding biological samples for analysis, the article comprising:

a substrate having a pair of opposing surfaces; and

a plurality of reaction sites in the substrate, each of the reaction sites extending from an opening in one of the opposing surfaces of the substrate to an opening in the other one of the opposing surfaces, the reaction sites being configured to provide sufficient surface tension by capillary action to hold respective biological samples;

wherein at least some of the reaction sites have a volume between the opposing surfaces that is less than or equal to 1 nanoliter.

According to a third aspect of the present invention, there is provided an apparatus, comprising:

the article as defined in the first aspect; and

a carrier comprising:

a first cover comprising a bottom surface; and

a second cover comprising a top surface;

wherein the article is disposed between the top surface and the bottom surface;

wherein there is a space between the first surface of the substrate and the top surface;

wherein there is a space between the second surface of the substrate and the bottom surface.

According to a fourth aspect of the present invention, there is provided a system comprising:

the apparatus as defined in the third aspect;

a light source configured to provide an excitation beam;

a photodetector configured to receive emission light from two or more of the reaction sites when the excitation beam interacts with a biological solution contained in the two or more of the reaction sites;

an optical system comprising an excitation optical system configured to deliver at least a portion of the excitation beam to the two or more of the reaction sites and emission optical

system to deliver at least some of the emission light from the two or more of the reaction sites to the detector.

According to a fifth aspect of the present invention, there is provided a method of making an article for holding biological samples for analysis, the method comprising:

providing a substrate comprising glass and having a first surface and an opposing second surface;

covering the glass with a mask comprising a pattern;

passing an ultraviolet beam through the mask and onto at least portions of the substrate;

exposing the at least portion of the substrate to a corrosive agent;

removing material to provide a plurality of reaction sites extending from one of the surfaces of the substrate to the other one of the surfaces;

subsequently exposing the substrate to an ultraviolet beam so as to change a characteristic of the glass.

According to a sixth aspect of the present invention, there is provided a method of making an article for holding biological samples for analysis, the method comprising:

providing a substrate comprising silicon and having a first surface and an opposing second surface;

applying a pattern to one of the surfaces;

etching a plurality of wells the first surface;

removing material from the second surface to form at least one through-hole from a corresponding well of the plurality of wells;

forming a surface roughness on at least one of the surfaces, the surface roughness characterized by one or more of an arithmetic average roughness (Ra) that is less than or equal to 5 nanometers, a maximum valley depth roughness (Rv) that is less than or equal to 15 nanometers, a maximum peak height roughness (Rp) that is less than or equal to 9 nanometers, or a maximum peak to trench roughness (Rt) that is less than or equal to 24 nanometers.

According to a seventh aspect of the present invention, there is provided a method of preparing a plurality of biological samples, the method comprising:

providing the article as defined in the first aspect;

providing a carrier comprising:

a first cover comprising a bottom surface; and

a second cover comprising a top surface;

an aperture sized to receive the article;

providing an insertion tool comprising:

a U-shaped body;

a pair of arms configured to slideably engage the carrier;

attaching the article within the U-shaped body;

sliding the insertion tool by an amount sufficient to dispose the article between the covers.

Brief Description of the Drawings

Embodiments of the present invention may be better understood from the following detailed description when read in conjunction with the accompanying drawings. Such embodiments, which are for illustrative purposes only, depict novel and non-obvious aspects of the invention. The drawings include the following figures:

Figure 1 is a top view of an article of manufacture according to an embodiment of the present invention.

WHAT IS CLAIMED IS:

1. An article for holding a plurality of biological samples, the article comprising:

a substrate comprising a first surface and an opposing second surface; and

a substrate comprising a first surface and an opposing second surface; and

a plurality of reaction sites in the substrate, each of the reaction sites extending from an opening in the first surface to an opening in the second surface, the reaction sites being configured to provide sufficient surface tension by capillary action to hold respective biological samples;

wherein a density of reaction sites over at least a portion of one of surfaces is at least 170 holes per square millimeter.

2. The article of claim 1, wherein at least one of the surfaces has a surface roughness characterized by one or more of an arithmetic average roughness (Ra) that is less than or equal to 5 nanometers, a maximum valley depth roughness (Rv) that is less than or equal to 15 nanometers, a maximum peak height roughness (Rp) that is less than or equal to 9 nanometers, or a maximum peak to trench roughness (Rt) that is less than or equal to 24 nanometers.

3. The article of claim 1, wherein two or more of the reaction sites comprise a non-circular shape at one of the surfaces or at each of the surfaces.

4. The article of claim 1, wherein two or more of the reaction sites comprise a hexagonal shape at one of the surfaces or at each of the surfaces.

5. The article of claim 1, wherein the reaction site is a through-hole, a well, an indentation in one of the surfaces, a chemically modified spot on one of the surface, a hydrophilic spot on an otherwise hydrophobic surface.

6. The article of claim 1, wherein the substrate comprises a flat plate.

7. The article of claim 1, wherein each of the opposing surfaces comprises a hydrophobic material and two or more of the reaction sites comprise a wall comprising a hydrophilic material.

8. The article of claim 1, wherein the openings of each surface are arranged in a close-packed hexagonal matrix.

9. The article of claim 1, wherein the substrate has a thickness that is less than or equal to 300 micrometers.

10. The article of claim 1, wherein each of the plurality of reaction sites is characterized by a diameter that is less than or equal to 60 micrometers.

11. The apparatus of claim 1, wherein a reaction site diameter of at a first plurality of the reaction sites is different than a reaction site diameter of at a second plurality of the reaction sites.

12. The article of claim 1, wherein each of the walls of the two or more of the reaction sites is tapered between the surfaces.

13. The article of claim 12, wherein the taper is greater than 3 degrees.

14. The article of claim 1, wherein each of the walls of the two or more of the reaction sites includes a chamfer located at one of the surfaces or at each of the surfaces.

15. The article of claim 1, wherein at least some of the reaction sites have a volume between the opposing surfaces that is less than or equal to 10 nanoliter.

16. The article of claim 1, wherein at least some of the reaction sites have a volume between the opposing surfaces that is less than or equal to 1 nanoliter.

17. The article of claim 1, wherein at least some of the reaction sites have a volume between the opposing surfaces that is less than or equal to 100 picoliters.

18. The article of claim 1, wherein at least some of the reaction sites have a volume between the opposing surfaces that is less than or equal to 30 picoliters.

19. The apparatus of claim 1, wherein a reaction site volume of at a first plurality of the reaction sites is different than a reaction site volume of at a second plurality of the reaction sites.

20. The article of claim 1, wherein the density of reaction sites is at least 170 holes per square millimeter.

21. The article of claim 1, wherein the density of reaction sites is at least 200 holes per square millimeter.

22. The article of claim 1, wherein a minimum spacing between adjacent reaction sites of the plurality of reaction sites is less than or equal to 85 micrometers.

23. The article of claim 1, wherein surfaces of the substrate have a dimension of at least 14 millimeters by 14 millimeters, the surfaces defining an active area containing the plurality of reaction sites, the active area having an extent of at least 13 millimeters by 13 millimeters.

24. The article of claim 1, wherein the substrate contains at least 20,000 reaction sites.

25. The article of claim 1, wherein the substrate contains at least 30,000 reaction sites.

26. The article of claim 1, wherein the substrate contains at least 100,000 reaction sites.

27. The article of claim 1, wherein the substrate contains at least 1,000,000 reaction sites.

28. The article of claim 1, wherein the substrate comprises a photo-structured glass ceramic material.

29. The article of claim 1, wherein the substrate comprises a silicon material.

30. The apparatus of claim 1, further comprising a fiducial disposed on at least one of the surfaces.

31. An article for holding biological samples for analysis, the article comprising:

a substrate having a pair of opposing surfaces; and

a plurality of reaction sites in the substrate, each of the reaction sites extending from an opening in one of the opposing surfaces of the substrate to an opening in the other one of the opposing surfaces, the reaction sites being configured to provide sufficient surface tension by capillary action to hold respective biological samples;

wherein at least some of the reaction sites have a volume between the opposing surfaces that is less than or equal to 1 nanoliter.

32. An apparatus, comprising:

the article of claim 1; and

a carrier comprising:

a first cover comprising a bottom surface; and

a second cover comprising a top surface;

wherein the article is disposed between the top surface and the bottom surface;

wherein there is a space between the first surface of the substrate and the top surface;

wherein there is a space between the second surface of the substrate and the bottom surface.

33. The apparatus of claim 32, further comprising a gel material disposed within the spaces.

34. The apparatus of claim 32, further comprising a PDMS material disposed within the spaces.

35. The article of claim 32, wherein at least one of the covers comprise a window configure to provide optical access to the reaction sites.

36. The article of claim 32, wherein the carrier comprises an aperture disposed perpendicular to the surfaces and sized to allow passage of the substrate.

37. The article of claim 36, wherein the aperture comprises a piercable membrane that is broken when the substrate is moved into the carrier.

38. The apparatus of claim 32, further comprising:

an insertion tool comprising:

a U-shaped body configured to hold the article for loading into the carrier;

a pair of arms configured to slideably engage the carrier;

wherein the insertion tool is configured for transferring the article into the carrier.

39. The apparatus of claim 38, wherein the carrier comprises a wiper blade configured contact at least one of the surfaces of the article during loading of the article into the carrier.

40. A system comprising:

the apparatus of claim 32;

a light source configured to provide an excitation beam;

a photodetector configured to receive emission light from two or more of the reaction sites when the excitation beam interacts with a biological solution contained in the two or more of the reaction sites;

an optical system comprising an excitation optical system configured to deliver at least a portion of the excitation beam to the two or more of the reaction sites and emission optical system to deliver at least some of the emission light from the two or more of the reaction sites to the detector.

41. The system of claim 40, further comprising a thermal control unit to control a temperature of the substrate or within the substrate.

42. The article of claim 40, further comprising a microprocessor configure to provide one or more of:

control of the light source;

control of the photodetector;

control of an optical element of the optical system; or

control a temperature of one or more of the apparatus or a solution contained inside at least one of the reaction sites; or

processing of data obtained by the photodetector of .

43. The article of claim 40, wherein the photodetector comprises a CCD array or a CMOS array.

44. The article of claim 43, photodetector comprises at least 4,000,000 pixels.

45. The article of claim 43, photodetector comprises at least 10,000,000 pixels.

46. A method of making an article for holding biological samples for analysis, the method comprising:

providing a substrate comprising glass and having a first surface and an opposing second surface;

covering the glass with a mask comprising a pattern;

passing an ultraviolet beam through the mask and onto at least portions of the substrate;

exposing the at least portion of the substrate to a corrosive agent;

removing material to provide a plurality of reaction sites extending from one of the surfaces of the substrate to the other one of the surfaces;

subsequently exposing the substrate to an ultraviolet beam so as to change a characteristic of the glass;

47. The method of claim 46, wherein the glass is a photostructurable material.

48. The method of claim 46, wherein the characteristic is an opacity of the glass and the change is an increase in the opacity of the glass by an amount sufficient prevent light emitted in one reaction site of the plurality of reaction sites from being transmitted through the glass and into an adjacent reaction site of the plurality of reaction sites.

49. The method of claim 46, further comprising removing material from the substrate by an amount sufficient to reduce thickness between the surfaces of the substrate.

50. The method of claim 46, further heating the substrate with reaction sites to a temperature of at least 500 degrees Celsius.

51. The method of claim 46, further comprising removing material from the substrate by an amount sufficient to reduce thickness between the surfaces of the substrate by at least 30% over an initial thickness between the surfaces.

52. The method of claim 46, wherein the mask comprises a quartz plate with chrome pattern.

53. The method of claim 46, wherein the corrosive material is hydrofluoric acid.

54. The method of claim 46, further comprising removing the mask prior to exposing the at least portion of the substrate to the corrosive agent.

55. A method of making an article for holding biological samples for analysis, the method comprising:

providing a substrate comprising silicon and having a first surface and an opposing second surface;

applying a pattern to one of the surfaces;

etching a plurality of wells the first surface;

removing material from the second surface to form at least one through-hole from a corresponding well of the plurality of wells;

forming a surface roughness on at least one of the surfaces, the surface roughness characterized by one or more of an arithmetic average roughness (Ra) that is less than or equal to 5 nanometers, a maximum valley depth roughness (Rv) that is less than or equal to 15 nanometers, a maximum peak height roughness (Rp) that is less than or equal to 9 nanometers, or a maximum peak to trench roughness (Rt) that is less than or equal to 24 nanometers.

56. The method of claim 55, further comprising at least partially filling at least one of the at least one through-hole with a liquid solution containing a nucleotide sequence and a fluorescent dye.

57. A method of preparing a plurality of biological samples, the method comprising:

providing the article of claim 1;

providing a carrier comprising:

a first cover comprising a bottom surface; and

a second cover comprising a top surface;

an aperture sized to receive the article;

providing an insertion tool comprising:

a U-shaped body;

a pair of arms configured to slideably engage the carrier;

attaching the article within the U-shaped body;

sliding the insertion tool by an amount sufficient to dispose the article between the covers;

58. The method of claim 57, further comprising:

providing a wiper blade configured contact at least one of the surfaces of the article;

placing an amount of liquid onto one of the surfaces of the article, the liquid containing a biological sample to be deposited into the reaction sites of the article;

depositing the liquid into at least some of the plurality of reaction sites while sliding the insertion tool by wiping the wiper blade across the at least one of the surfaces.

59. The method of claim 57, further comprising removing the insertion tool from the article and the carrier.

60. The method of claim 57, further comprising attaching the insertion tool onto the carrier, then subsequently attaching the article within the U-shaped body.

61. The method of claim 57, further comprising attaching the insertion tool and the article together onto the carrier.

62. The method of claim 57, wherein at least 99 percent of the liquid sample is received by at least some of reaction sites.

63. The method of claim 57, wherein the at least 99 percent of the liquid sample is received by less than 95 percent of the reaction sites.

CLAIMS

1. An article for holding a plurality of biological samples, the article comprising:

a substrate comprising a first surface and an opposing second surface; and

a plurality of reaction sites in the substrate, each of the reaction sites extending from an opening in the first surface to an opening in the second surface, the reaction sites being configured to provide sufficient surface tension by capillary action to hold respective biological samples;

wherein a density of reaction sites over at least a portion of one of surfaces is at least 170 holes per square millimeter.

2. The article of claim 1, wherein at least one of the surfaces has a surface roughness characterized by one or more of an arithmetic average roughness (Ra) that is less than or equal to 5 nanometers, a maximum valley depth roughness (Rv) that is less than or equal to 15 nanometers, a maximum peak height roughness (Rp) that is less than or equal to 9 nanometers, or a maximum peak to trench roughness (Rt) that is less than or equal to 24 nanometers.

3. The article of claim 1, wherein two or more of the reaction sites comprise a hexagonal shape at one of the surfaces or at each of the surfaces.

4. The article of claim 1, wherein the openings of each surface are arranged in a close-packed hexagonal matrix.

5. The article of claim 1, wherein each of the walls of the two or more of the reaction sites is tapered between the surfaces, wherein the taper is greater than 3 degrees.

6. The article of claim 1, wherein each of the walls of the two or more of the reaction sites includes a chamfer located at one of the surfaces or at each of the surfaces.

7. The article of claim 1, wherein at least some of the reaction sites have a volume between the opposing surfaces that is less than or equal to 1 nanoliter.

8. The apparatus of claim 1, wherein a reaction site volume of at a first plurality of the reaction sites is different than a reaction site volume of at a second plurality of the reaction sites.

9. The article of claim 1, wherein the substrate comprises a photo-structured glass ceramic material.

10. The article of claim 1, wherein the substrate comprises a silicon material.

11. The apparatus of claim 1, further comprising a fiducial disposed on at least one of the surfaces.

12. An article for holding biological samples for analysis, the article comprising:

a substrate having a pair of opposing surfaces; and

a plurality of reaction sites in the substrate, each of the reaction sites extending from an opening in one of the opposing surfaces of the substrate to an opening in the other one of the opposing surfaces, the reaction sites being configured to provide sufficient surface tension by capillary action to hold respective biological samples;

wherein at least some of the reaction sites have a volume between the opposing surfaces that is less than or equal to 1 nanoliter.

13. An apparatus, comprising:

the article of claim 1; and

a carrier comprising:

a first cover comprising a bottom surface; and

a second cover comprising a top surface;

wherein the article is disposed between the top surface and the bottom surface;

wherein there is a space between the first surface of the substrate and the top surface;

wherein there is a space between the second surface of the substrate and the bottom surface.

14. The apparatus of claim 13, further comprising a PDMS material disposed within the spaces.

15. The article of claim 13, wherein the carrier comprises an aperture disposed perpendicular to the surfaces and sized to allow passage of the substrate, wherein the aperture comprises a piercable membrane that is broken when the substrate is moved into the carrier.

16. A system comprising:

the apparatus of claim 13;

a light source configured to provide an excitation beam;

a photodetector configured to receive emission light from two or more of the reaction sites when the excitation beam interacts with a biological solution contained in the two or more of the reaction sites;

an optical system comprising an excitation optical system configured to deliver at least a portion of the excitation beam to the two or more of the reaction sites and emission optical system to deliver at least some of the emission light from the two or more of the reaction sites to the detector.

17. The system of claim 16, further comprising a thermal control unit to control a temperature of the substrate or within the substrate.

18. A method of making an article for holding biological samples for analysis, the method comprising:

providing a substrate comprising glass and having a first surface and an opposing second surface;

covering the glass with a mask comprising a pattern;

passing an ultraviolet beam through the mask and onto at least portions of the substrate;

exposing the at least portion of the substrate to a corrosive agent;

removing material to provide a plurality of reaction sites extending from one of the surfaces of the substrate to the other one of the surfaces;

subsequently exposing the substrate to an ultraviolet beam so as to change a characteristic of the glass.

19. The method of claim 18, wherein the glass is a photostructurable material.

20. The method of claim 18, further comprising removing material from the substrate by an amount sufficient to reduce thickness between the surfaces of the substrate by at least 30% over an initial thickness between the surfaces.

21. A method of making an article for holding biological samples for analysis, the method comprising:

providing a substrate comprising silicon and having a first surface and an opposing second surface;

applying a pattern to one of the surfaces;

etching a plurality of wells the first surface;

removing material from the second surface to form at least one through-hole from a corresponding well of the plurality of wells;

forming a surface roughness on at least one of the surfaces, the surface roughness characterized by one or more of an arithmetic average roughness (Ra) that is less than or equal to 5 nanometers, a maximum valley depth roughness (Rv) that is less than or equal to 15 nanometers, a maximum peak height roughness (Rp) that is less than or equal to 9 nanometers, or a maximum peak to trench roughness (Rt) that is less than or equal to 24 nanometers.

22. A method of preparing a plurality of biological samples, the method comprising:

providing the article of claim 1;

providing a carrier comprising:

a first cover comprising a bottom surface; and

a second cover comprising a top surface;

an aperture sized to receive the article;

providing an insertion tool comprising:

a U-shaped body;

a pair of arms configured to slideably engage the carrier;

attaching the article within the U-shaped body;

sliding the insertion tool by an amount sufficient to dispose the article between the covers.

23. The method of claim 22, further comprising:

providing a wiper blade configured contact at least one of the surfaces of the article;

placing an amount of liquid onto one of the surfaces of the article, the liquid containing a biological sample to be deposited into the reaction sites of the article;

depositing the liquid into at least some of the plurality of reaction sites while sliding the insertion tool by wiping the wiper blade across the at least one of the surfaces.

24. The method of claim 22, further comprising attaching the insertion tool onto the carrier, then subsequently attaching the article within the U-shaped body.

25. The method of claim 22, wherein at least 99 percent of the liquid sample is received by at least some of reaction sites.