

Bio/Chemical Assay Devices and Methods for Simplified Steps, Small Samples, Accelerated Speed, and Ease-of-Use

CROSS-REFERENCING

This application claims the benefit of provisional application serial nos. 62/202,989, filed on August 10, 2015, 62/218,455 filed on September 14, 2015, 62/293,188, filed on February 9, 2016, 62/305,123, filed on March 8, 2016, and 62/369,181, filed on July 31, 2016, all of which applications are incorporated herein in their entireties for all purposes.

FIELD

The present invention is related to the field of bio/chemical sampling, sensing, assays and applications.

BACKGROUND

In many bio/chemical sensing and testing (e.g. immunoassay, nucleotide assay, blood cell counting, etc.), chemical reactions, and other processes, there are needs for the methods and devices that can accelerate the process (e.g. binding, mixing reagents, etc.) and quantify the parameters (e.g. analyte concentration, the sample volume, etc.), that can simplify the sample collection and measurement processes, that can handle samples with small volume, that allow an entire assay performed in less than a minute, that allow an assay performed by a smartphone (e.g. mobile phone), that allow non-professional to perform an assay her/himself, and that allow a test result to be communicated locally, remotely, or wirelessly to different relevant parties. The present invention relates to the methods, devices, and systems that address these needs.

SUMMARY

The following brief summary is not intended to include all features and aspects of the present disclosure. The present disclosure relates to the methods, devices, and systems that make bio/chemical sensing (including, not limited to, immunoassay, nucleic acid assay, electrolyte analysis, etc.) faster, more sensitive, less steps, easy to perform, smaller amount of samples

plates. Such devices do not push liquid onto an open plate surface to produce a thin layer of sample that can be analyzed. Additionally, in other devices, the key function of the pillars is to "filter" or sort nanoparticles (e.g., cells or alike). Hence the inter-pillar distance is determined by the nanoparticles being sorted, not for the goal of making the spacing between the cover plate and the substrate plate uniform. Finally, in devices such as Austin's device, the accuracy of sorting is primarily controlled by the inter-pillar distances not the spacing between the plates, and controlling of the spacing between the plates is not regarded as significant. Hence, such disclosures would not lead one to modify plating spacing uniformity by changing pillar size, shape, inter-pillar spacing, etc.

In view of the above, the present device and method is believed to provide an easy to use, inexpensive, easy to manufacture, and extremely rapid way to determine the absolute concentration of an analyte (or analytes, if the device and method are implemented in a multiplex way) in a liquid sample.

Disclosed is the means that uses a pair of special plates that are movable to each other to manipulate a small volume sample or one or a plurality of reagents or both for a simpler, faster, and/or better assaying. The manipulation includes, but limited to, reshaping a sample, forcing a sample flow, making a contact between the sample and reagent, measuring sample volume, reducing diffusion distance, increasing collision frequency, etc. — all of them have benefit effects to certain assays. In the present disclosure, the special features and properties on the plates, the special methods to handling the plates, and the special ways to handle the reagents and samples provide advantages in assaying.

Disclosed is the means that make at least a portion of a small droplet of a liquid sample deposited on a plate to become a thin film with a thickness that is controlled, predetermined, and uniform over large area. The uniform thickness can be as thin as less than 1 μm . Furthermore, the disclosure allows the same uniform thickness be maintained for a long time period without suffering evaporation to environment.

Disclosed is the means that utilizes the predetermined uniform thin sample thickness formed by the disclosure to determine the volume of a portion or entire of the sample without using any pipette or alike.

Disclosed is an embodiment for the spacers (for controlling the spacing between two plates), that has a pillar shape with a flat top and nearly uniform lateral cross-section. Such spacers offers many advantages in controlling a sample thickness over the spacers of ball (beads) shape.

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Disclosed is the means that make certain chemical reactions (or mixing) occur predominately only in a small portion of the sample, not in the other part of the sample, without using fluidic isolation between the two portion of the sample.

Disclosed is the means that make multiple chemical reactions (or mixing) occur predominately only in each perspective small portion of the sample, not in the other part of the sample, without using fluidic isolation between the different portion of the sample. Thus the disclosure allows multiplexed assaying in parallel using one small drop of sample without fluidic isolation between different reaction sites.

Disclosed is the means that make assay (e.g. immunoassay, nucleic acid assay, etc.) faster. For example, a saturation incubation time (the time for the binding between molecules to reach equilibrium) is reduced from hours to less than 60 seconds.

Disclosed is the means that significantly increase the detection sensitivity by one or a combination of several methods, which including an amplification surface, large or bright labels, etc.

Disclosed is the means that perform assaying using very small amount of sample, for example as small as 0.5 uL (microliter) or less.

Disclosed is the means that simplify an assay by allowing a minute body fluid deposited directly from a subject to the testing or sample area.

Disclosed is the means that simplify and speed up an assay by pre-coating reagents on plates. For example, a capture agent and a detection agent are pre-coated and dried on the plates. Another example is that all required sensing reagents are pre-coated on plates, and a sensing is done by depositing a sample on the pre-coated plates without a need of depositing other reagents.

Disclosed is the means that make reading an assay performed by a mobile phone.

Disclosed is the means that allow a person to test his/her own biomarkers on their own within 60 secs by directly deposit a drop of their own body fluid (e.g. saliva) between a pair of plastics and taking a picture with a mobile phone.

In one aspect, there is provided an *in vitro* method for analyzing a liquid sample, comprising:

- (a) obtaining a first and second plates that are movable relative to each other into different configurations, wherein each plate has a sample contact surface that is substantially planar, one or both plates are flexible, and one or both of the plates comprise spacers that are fixed with a respective sample contacting surface, and wherein the spacers have pillar shape, a substantially flat top surface, a predetermined substantially uniform height and a predetermined constant inter-spacer distance that is at least about 2 times larger than the size of the analyte, and wherein the Young's modulus of the spacers times the filling factor of the spacers is equal or larger than 2 MPa, wherein the filling factor is the ratio of the spacer contact area to the total plate area;
- (b) depositing a sample on one or both of the plates when the plates are configured in an open configuration, wherein the open configuration is a configuration in which the two plates are either partially or completely separated apart and the spacing between the plates is not regulated by the spacers;
- (c), after (b), bringing the two plates together into a closed configuration; in which: at least part of the sample forms a layer of substantially uniform thickness that is confined by the sample contact surfaces of the plates, wherein the uniform thickness of the layer is regulated by the spacers and the plates; and
- (d) analyzing the analyte in the layer of uniform thickness while the plates are in the closed configuration.

In another aspect, there is provided a device for analyzing a liquid sample, comprising:

a first plate, a second plate, and spacers, wherein:

- i. the plates are movable relative to each other into different configurations;
- ii. one or both plates are flexible;
- iii. each of the plates has, on its respective surface, a sample contact area for contacting a sample that contains an analyte,
- iv. one or both of the plates comprise the spacers that are fixed with a respective sample contact area, wherein the spacers have a pillar shape, a substantially flat top surface, a predetermined substantially uniform height and a predetermined constant inter-spacer distance that is at least about 2 times larger than the size of the analyte, wherein at least one of the spacers is inside the sample contact area, and wherein the Young's modulus of the spacers times the filling factor of the spacers is equal or larger than 2 MPa;

wherein the filling factor is the ratio of the spacer contact area to the total plate area;

wherein one of the configurations is an open configuration, in which: the two plates are separated apart, the spacing between the plates is not regulated by the spacers, and the sample is deposited on one or both of the plates; and

wherein another of the configurations is a closed configuration which is configured after the sample deposition in the open configuration; and in the closed configuration: at least part of the sample is compressed by the two plates into a layer of highly uniform thickness, wherein the uniform thickness of the layer is confined by the sample contact surfaces of the plates and is regulated by the plates and the spacers.

In another aspect, there is provided an *in vitro* method for analyzing a blood sample, comprising:

(a) obtaining a first and second plates that are movable relative to each other into different configurations, wherein each plate has a sample contact surface that is substantially planar, one or both plates are flexible, and one or both of the plates comprise spacers that are fixed with a respective sample contacting surface, and wherein the spacers have:

- i. a predetermined substantially uniform height,
- ii. a shape of pillar with substantially uniform cross-section and a flat top surface;
- iii. a predetermined constant inter-spacer distance that is in the range of 10 μm to 200 μm ;
- iv. a filling factor of equal to 1% or larger; and
- v. a product of the filling factor and the Young's modulus of the spacer is 2 MPa or larger; and

(b) depositing the blood sample on one or both of the plates when the plates are configured in an open configuration, wherein the open configuration is a configuration in which the two plates are either partially or completely separated apart and the spacing between the plates is not regulated by the spacers;

(c), after (b), bringing the two plates together into a closed configuration; in which: at least part of the blood sample is compressed into a layer of substantially uniform thickness that is confined by the sample contact surfaces of the plates, wherein the uniform thickness of the layer is regulated by the spacers and the plates, and has an average value in the range of 1.8 μm to 3 μm with a variation of less than 10%; and

(d) analyzing the blood in the layer of uniform thickness while the plates are the closed configuration;
wherein the filling factor is the ratio of the spacer contact area to the total plate area.

In another aspect, there is provided a device for analyzing a liquid sample, comprising: a first plate and a second plate, wherein:

- i. the plates are movable relative to each other into different configurations;
- ii. one or both plates are flexible;
- iii. each of the plates has, on its respective surface, a sample contact area for contacting a blood sample;
- iv. one or both of the plates comprise spacers that are fixed with a respective plate, wherein the spacers have the spacers have pillar shape, a substantially flat top surface, a predetermined substantially uniform height and a predetermined constant inter-spacer distance that is in the range of 7 μm to 200 μm , wherein the Young's modulus of the spacers times the filling factor of the spacers is equal or larger than 2 MPa, wherein the filling factor is the ratio of the spacer contact area to the total plate area; and wherein at least one of the spacers is inside the sample contact area;

wherein one of the configurations is an open configuration, in which: the two plates are separated apart, the spacing between the plates is not regulated by the spacers, and the sample is deposited on one or both of the plates; and

wherein another of the configurations is a closed configuration which is configured after the sample deposition in the open configuration; and in the closed configuration: at least part of the sample is compressed by the two plates into a layer of highly uniform thickness, wherein the uniform thickness of the layer is confined by the inner surfaces of the two plates and is regulated by the plates and the spacers, and has an average value in the range of 1.8 μm to 3 μm with a small variation.

1. An *in vitro* method for analyzing a liquid sample, comprising:

(a) obtaining a first and second plates that are movable relative to each other into different configurations, wherein each plate has a sample contact surface that is substantially planar, one or both plates are flexible, and one or both of the plates comprise spacers that are fixed with a respective sample contacting surface, and wherein the spacers have pillar shape, a substantially flat top surface, a predetermined substantially uniform height and a predetermined constant inter-spacer distance that is at least about 2 times larger than the size of the analyte, and wherein the Young's modulus of the spacers times the filling factor of the spacers is equal or larger than 2 MPa, wherein the filling factor is the ratio of the spacer contact area to the total plate area;

(b) depositing a sample on one or both of the plates when the plates are configured in an open configuration, wherein the open configuration is a configuration in which the two plates are either partially or completely separated apart and the spacing between the plates is not regulated by the spacers;

(c), after (b), bringing the two plates together into a closed configuration; in which: at least part of the sample forms a layer of substantially uniform thickness that is confined by the sample contact surfaces of the plates, wherein the uniform thickness of the layer is regulated by the spacers and the plates; and

(d) analyzing the analyte in the layer of uniform thickness while the plates are in the closed configuration.

2. The method of claim 1, wherein after step (c) and before step (d) the method further comprises, after the plates are in the closed configuration, removing a pressing force, wherein the thickness of the layer of uniform thickness after removal of the pressing force: (i) is substantially the same as of the layer of uniform thickness before removing the pressing force and (ii) deviates from the spacer height by less than 10%.

3. A device for analyzing a liquid sample, comprising:

a first plate, a second plate, and spacers, wherein:

i. the plates are movable relative to each other into different configurations;

ii. one or both plates are flexible;

iii. each of the plates has, on its respective surface, a sample contact area for contacting a sample that contains an analyte,

iv. one or both of the plates comprise the spacers that are fixed with a respective sample contact area, wherein the spacers have a pillar shape, a substantially flat top surface, a predetermined substantially uniform height and a predetermined constant inter-spacer distance that is at least about 2 times larger than the size of the analyte, wherein at least one of the spacers is inside the sample contact area, and wherein the Young's modulus of the spacers times the filling factor of the spacers is equal or larger than 2 MPa;

wherein the filling factor is the ratio of the spacer contact area to the total plate area;
wherein one of the configurations is an open configuration, in which: the two plates are separated apart, the spacing between the plates is not regulated by the spacers, and the sample is deposited on one or both of the plates; and

wherein another of the configurations is a closed configuration which is configured after the sample deposition in the open configuration; and in the closed configuration: at least part of the sample is compressed by the two plates into a layer of highly uniform thickness, wherein the uniform thickness of the layer is confined by the sample contact surfaces of the plates and is regulated by the plates and the spacers.

4. The device of claim 3, wherein the inter-spacer distance is in the range of 1 μm to 120 μm .

5. The device of claim 3 or 4, wherein the flexible plates have a thickness in the range of 20 μm to 250 μm and Young's modulus in the range 0.1 to 5 GPa.

6. An *in vitro* method for analyzing a blood sample, comprising:

(a) obtaining a first and second plates that are movable relative to each other into different configurations, wherein each plate has a sample contact surface that is substantially planar, one or both plates are flexible, and one or both of the plates comprise spacers that are fixed with a respective sample contacting surface, and wherein the spacers have:

- i. a predetermined substantially uniform height,
- ii. a shape of pillar with substantially uniform cross-section and a flat top surface;
- iii. a predetermined constant inter-spacer distance that is in the range of 10 μm to 200 μm ;
- iv. a filling factor of equal to 1% or larger; and
- v. a product of the filling factor and the Young's modulus of the spacer is 2 MPa or larger; and

(b) depositing the blood sample on one or both of the plates when the plates are configured in an open configuration, wherein the open configuration is a configuration in which the two plates are either partially or completely separated apart and the spacing between the plates is not regulated by the spacers;

(c), after (b), bringing the two plates together into a closed configuration; in which: at least part of the blood sample is compressed into a layer of substantially uniform thickness that is confined by the sample contact surfaces of the plates, wherein the uniform thickness of the layer is regulated by the spacers and the plates, and has an average value in the range of 1.8 μm to 3 μm with a variation of less than 10%; and

(d) analyzing the blood in the layer of uniform thickness while the plates are the closed configuration;

wherein the filling factor is the ratio of the spacer contact area to the total plate area.

7. The method of claim 6, wherein the method comprises removing the external force after the plates are in the closed configuration; and imaging the blood cells in the layer of uniform thickness while the plates are the closed configuration; and counting a number of blood cells in an area of the image.
8. The method of claim 6 or 7, wherein the inter-spacer distance is in the range of 20 μm to 200 μm .
9. A device for analyzing a liquid sample, comprising:
a first plate and a second plate, wherein:
 - i. the plates are movable relative to each other into different configurations;
 - ii. one or both plates are flexible;
 - iii. each of the plates has, on its respective surface, a sample contact area for contacting a blood sample;
 - iv. one or both of the plates comprise spacers that are fixed with a respective plate, wherein the spacers have the spacers have pillar shape, a substantially flat top surface, a predetermined substantially uniform height and a predetermined constant inter-spacer distance that is in the range of 7 μm to 200 μm , wherein the Young's modulus of the spacers times the filling factor of the spacers is equal or larger than 2 MPa, wherein the filling factor is the ratio of the spacer contact area to the total plate area; and wherein at least one of the spacers is inside the sample contact area;wherein one of the configurations is an open configuration, in which: the two plates are separated apart, the spacing between the plates is not regulated by the spacers, and the sample is deposited on one or both of the plates; and wherein another of the configurations is a closed configuration which is configured after the sample deposition in the open configuration; and in the closed configuration: at least part of the sample is compressed by the two plates into a layer of highly uniform thickness, wherein the uniform thickness of the layer is confined by the inner surfaces of the two plates and is regulated by the plates and the spacers, and has an average value in the range of 1.8 μm to 3 μm with a small variation.
10. The device of claim 9, wherein the inter-spacer distance is in the range of 14 μm to 200 μm .
11. The device of claim 9 or 10, wherein the inter-spacer distance is in the range of 7 μm to 20 μm .