PARALLELIZED SAMPLE HANDLING

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 61/814,090, filed April 19, 2013, and the benefit of U.S. Provisional Application No. 61/903,156, filed November 12, 2013, which applications are incorporated herein by reference.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with the support of the United States government under Contract number HG005826 by the National Institutes of Health. The United States government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Cultivation methods that employ miniaturization and compartmentalization, including but not limited to Gel MicroDroplets (GMDs), miniaturized Petri dishes, and microfluidics, can increase throughput, initiate high-density behavior, and eliminate competition from rapidly growing "weed" cells. However, the equipment needed for these methods are often not accessible to most laboratories and operation is complicated, limiting their use for biological assays in the real world.

[0004] Microbes play critical roles in environments ranging from soil and oceans to the human gut. Although culture-independent methods such as metagenomics and single-cell genomics have revealed rich information about the composition and function of microbial communities, obtaining pure cultures of these microbes is still fundamental to understanding bacterial genomics and physiology. As bacteria are highly abundant and diverse, it is impractical to cultivate and isolate every strain from the environment.

SUMMARY OF THE INVENTION

[0005] Disclosed herein are methods, devices, systems, and kits for the creation and manipulation of small volumes. In some cases, these are used for control of the micro-environments, including both liquid and gas environments. In some cases, the methods, devices, systems, and kits disclosed herein are used for parallelized sample handling. In some cases, these are used for isolation or cultivation of organisms.

[0005a] In one aspect, the present invention provides a method of selecting a subset of sample volumes, the method comprising:

(a) providing a device comprising (i) a first substrate comprising a plurality of first defined volumes and (ii) a second substrate comprising a plurality of second defined volumes, said first substrate coupled to said second substrate;

(b) dispersing a sample among the plurality of first defined volumes;

(c) bringing each of the plurality of first defined volumes into fluidic contact with one of the plurality of second defined volumes to form a plurality of combined volumes;

(d) splitting the plurality of combined volumes into a plurality of matched pairs of daughter volumes comprising a plurality of first daughter volumes and a plurality of matched second daughter volumes;

(e) conducting one or more analysis on the plurality of the first daughter volumes; and

(f) selecting a subset of the plurality of matched second daughter volumes based on the analysis.

[0005b] In one aspect, the present invention provides a method of sample processing, the method comprising:

(a) loading a first sample comprising one or more cells into one or more first defined volumes of a first substrate;

(b) bringing the one or more first defined volumes into fluidic contact with one or more second defined volumes of a second substrate coupled to the first substrate;

(c) mixing contents of the one or more first defined volumes with contents of the one or more second defined volumes, thereby producing a mixed sample comprising one or more of the cells;

(d) separating the one or more first defined volumes from the one or more second defined volumes, wherein a first portion of the mixed sample is contained by the one or more first defined volumes and a second portion of the mixed sample is contained by the one or more second defined volumes, wherein the first substrate remains coupled to the second substrate;

(e) conducting at least one analysis on one or more cells in the first portion of the mixed sample; and

(f) determining whether to preserve one or more cells in the second portion of the mixed sample based on the at least one analysis.

[0006] In some embodiments the method comprises: a) providing a substrate comprising a first defined volume, said first defined volume comprising a first sample, said first volume covered by

a second substrate; b) providing said second substrate comprising a second defined volume, said second defined volume comprising a second sample, said second defined volume covered by said first substrate; and c) separating said first substrate from said second substrate, whereby said

WHAT IS CLAIMED IS:

1. A method, comprising:
   (a) dispersing a sample among a plurality of defined volumes;
   (b) splitting said plurality of defined volumes, essentially simultaneously, into a plurality of matched pairs of daughter volumes comprising a plurality of first daughter volumes and a plurality of matched second daughter volumes, wherein said splitting is performed without the application of a pumping force to said defined volumes;
   (c) conducting at least one analysis on said plurality of said first daughter volumes; and
   (d) selecting a subset of said plurality of matched second daughter volumes based on said analysis.

2. The method of claim 1, wherein said analysis comprises a genetic assay or a functional assay.

3. The method of claim 1, wherein said sample comprises cells.

4. The method of claim 1, wherein said sample comprises viruses.

5. The method of claim 1, wherein said sample comprises nucleic acids.

6. The method of claim 1, wherein said sample comprises multiple species of cells.

7. The method of claim 1, wherein said sample comprises antibiotics, chemotherapy agents, growth media, growth factors or inhibitors.

8. A method, comprising:
   (a) providing a first substrate comprising a first defined volume;
   (b) providing a second substrate comprising second defined volume, said second substrate coupled to said first substrate;
   (c) loading a first sample into said first defined volume;
   (d) bringing said first defined volume into fluidic contact with said second defined volume, wherein said first substrate remains coupled to said second substrate;
   (e) mixing contents of said first defined volume with contents of said second defined volume, thereby producing a mixed sample;

(f) separating said first defined volume from said second defined volume, wherein a first part of said mixed sample is contained by said first defined volume and a second part of said mixed sample is contained by said second defined volume, wherein said first substrate remains coupled to said second substrate; and

(g) decoupling said first substrate from said second substrate, wherein said first part of said mixed sample remains contained by said first defined volume and said second part of said mixed sample remains contained by said second defined volume.

9. The method of claim 8, wherein said mixed sample further comprises a gelling agent.

10. The method of claim 8, wherein said mixing comprises diffusion.

11. The method of claim 8, wherein said mixing comprises sonication.

12. The method of claim 8, wherein said bringing said first defined volume into fluidic contact with said second defined volume is performed on an apparatus.

13. A method, comprising:

(a) providing a plurality of defined volumes, each of said plurality of defined volumes comprising one of a plurality of samples;

(b) subjecting said plurality of samples to a set of conditions;

(c) conducting a process on said plurality of samples;

(d) transferring said plurality of samples from said plurality of defined volumes to a shared container, thereby creating a pooled sample;

(e) conducting an analysis on said pooled sample; and

(f) determining from said analysis the extent to which said set of conditions enabled or did not enable said process.

14. The method of claim 13, wherein said set of conditions comprises the presence of an antibiotic or of a chemotherapy agent.

15. The method of claim 13, wherein said set of conditions comprises a given temperature or a given atmospheric composition.

16. The method of claim 13, wherein said set of conditions comprises the presence of a given organism, growth factor or inhibitor.

17. The method of claim 13, wherein said set of conditions comprises the absence of a nutrient.

18. The method of claim 13, wherein said process comprises cell growth.

19. The method of claim 13, wherein said process comprises nucleic acid amplification.

20. The method of claim 13, wherein said analysis comprises a genetic assay or a functional assay.

CLAIMS

1. A method of selecting a subset of sample volumes, the method comprising:

(a) providing a device comprising (i) a first substrate comprising a plurality of first defined volumes and (ii) a second substrate comprising a plurality of second defined volumes, said first substrate coupled to said second substrate;

(b) dispersing a sample among the plurality of first defined volumes;

(c) bringing each of the plurality of first defined volumes into fluidic contact with one of the plurality of second defined volumes to form a plurality of combined volumes;

(d) splitting the plurality of combined volumes into a plurality of matched pairs of daughter volumes comprising a plurality of first daughter volumes and a plurality of matched second daughter volumes;

(e) conducting one or more analysis on the plurality of the first daughter volumes; and

(f) selecting a subset of the plurality of matched second daughter volumes based on the analysis.

2. The method of claim 1, wherein the one or more analyses are destructive or consumptive of the sample.

3. The method of claim 1, wherein said sample comprises cells.

4. The method of claim 1, wherein said sample comprises bacterial cells.

5. The method of claim 1, wherein said sample comprises multiple species of cells.

6. The method of claim 3, further comprising a step of cultivating the cells in the plurality of volumes.

7. The method of claim 1, wherein dispersing the sample among the plurality of first defined volumes comprises stochastically confining the cells.

8. The method of claim 1, wherein the sample comprises a gelling agent.

9. The method of claim 1, further comprising dispersing a gelling agent in the plurality of second volumes prior to bringing each of the plurality of first defined volumes into fluidic contact with one of the plurality of second defined volumes.

10. The method of claim 1, wherein splitting the plurality of combined volumes comprises decoupling the first substrate from the second substrate.

11. The method of claim 7, wherein stochastically confining the cells comprises actuating the first substrate relative to the second substrate.

12. The method of claim 1, wherein splitting the plurality of combined volumes comprises actuating the first substrate relative to the second substrate.

13. The method of claim 1, wherein the splitting is performed without the application of a pumping force to the volumes.

14. The method of claim 1, wherein the one or more analysis comprises a genetic assay.

15. The method of claim 1, wherein the one or more analysis comprises a functional assay.

16. The method of claim 1, wherein the sample comprises viruses.

17. The method of claim 1, wherein the sample comprises nucleic acids.

18. The method of claim 1, wherein the sample comprises antibiotics.

19. The method of claim 1, wherein the sample comprises chemotherapy agents.

20. The method of claim 1, wherein the sample comprises growth media.

21. The method of claim 1, wherein the sample comprises growth factors.

22. The method of claim 1, wherein the sample comprises inhibitors.

23. The method of claim 3, wherein conducting one or more analysis on the plurality of the first daughter volumes comprises analyzing one or more of the cells.

24. A method of sample processing, the method comprising:

(a) loading a first sample comprising one or more cells into one or more first defined volumes of a first substrate;

(b) bringing the one or more first defined volumes into fluidic contact with one or more second defined volumes of a second substrate coupled to the first substrate;

(c) mixing contents of the one or more first defined volumes with contents of the one or more second defined volumes, thereby producing a mixed sample comprising one or more of the cells;

(d) separating the one or more first defined volumes from the one or more second defined volumes, wherein a first portion of the mixed sample is contained by the one or more first defined volumes and a second portion of the mixed sample is contained by the one or more second defined volumes, wherein the first substrate remains coupled to the second substrate;

(e) conducting at least one analysis on one or more cells in the first portion of the mixed sample; and

(f) determining whether to preserve one or more cells in the second portion of the mixed sample based on the at least one analysis.

25. The method of claim 24, wherein the one or more first defined volumes comprise a plurality of volumes.

26. The method of claim 24, wherein the one or more second defined volumes comprise a plurality of volumes.