

CLAIMS

What is claimed is:

1. A plug comprising:
A body with a bottom surface:
5 A central opening in the body; and
A dried reagent on the bottom surface, wherein the body is formed from a material transmissive to excitation wavelengths and emission wavelengths in at least one of a red spectrum, a blue spectrum and a green spectrum.
2. The plug of claim 1 wherein the dried reagent is on a portion of the bottom surface wider
10 than a width of the central opening in the body.
3. The plug of claim 1 wherein a width of the central opening is wider than a portion of the bottom surface containing the dried reagent.
4. The plug of claim 1 further comprising a cavity in the bottom surface with the dried reagent within the cavity.
- 15 5. The plug of claim 4 further comprising a plug thickness between a central opening bottom and the plug body bottom wherein a depth of the cavity is less than 90% of the plug thickness.
6. The plug of claim 4 further comprising a plug thickness between a central opening bottom and the plug body bottom wherein a depth of the cavity is less than 70% of the plug
20 thickness.
7. The plug of claim 4 further comprising a plug thickness between a central opening bottom and the plug body bottom wherein a depth of the cavity is less than 50% of the plug thickness.
8. The plug of claim 4 further comprising an annulus on the plug bottom surface from an
25 outer edge of the plug body to a perimeter of the cavity.
9. The plug of claim 6 wherein the annulus completely encircles the perimeter of the cavity.
10. The plug of claim 4 the cavity further comprising a perimeter in the bottom surface wherein an angle of initiation of the cavity is measured from the perimeter relative to the bottom surface and the angle of initiation is 60 degrees or less.

11. The plug of any of claims 4-8 wherein the cavity is wider than the plug body central opening.
12. The plug of any of claim 4-8 wherein the plug body central opening is wider than the cavity.
- 5 13. The plug of claim 1 the plug body bottom surface further comprising a bounded area on the plug body bottom surface wherein the dried reagent is within the bounded area.
14. The plug of claim 11 wherein the bounded area on the plug bottom surface is provided by a feature on the plug body bottom surface.
15. The plug of claim 12 wherein the feature is raised above the plug bottom surface or
10 recessed into the plug bottom surface.
16. The plug of the claim 13 wherein the feature has a curved cross section or a rectangular cross section.
17. The plug of claim 11 wherein a width of the bounded area is greater than a width of the body central opening, a width of the bounded area is less than a width of the body central
15 opening, or a width of the bounded area is about the same as a width of the body central opening.
18. The plug of any of claims 1-15 wherein the plug has a polished or smooth finish facilitating the transmissivity of the excitation wavelengths and the emission wavelengths.
19. The plug of any of claims 1-16 further comprising a flange on the plug body around the central opening in the plug body.
- 20 20. The plug of any of claims 1-17 wherein the dried reagent is selected from the group consisting of nucleic acid synthesis reagents, peptide synthesis reagents, polymer synthesis reagents, nucleic acids, nucleotides, nucleobases, nucleosides, peptides, amino acids, monomers, detection reagents, catalysts or combinations thereof.
21. The apparatus of any of claims 1-18 wherein the dried reagent is a continuous film
25 adhering to the plug bottom surface.
22. The apparatus of any of claims 1-18, wherein the dried reagent is a lyophilized reagent.

23. The apparatus of any of claim 1-18 wherein the dried reagent comprises a plurality of droplets adhering to the plug bottom surface.
24. An assay chamber, comprising:
- a. a tapered inlet;
 - 5 b. a tapered outlet;
 - c. a plug comprising a bottom surface and a central opening in the body, wherein the body is formed from a material transmissive to excitation wavelengths and emission wavelengths in at least one of an ultraviolet spectrum, a blue spectrum, a green spectrum and a red spectrum;
 - 10 d. two curved boundaries, wherein each curved boundary extends from the tapered inlet to the tapered outlet such that together, the two curved boundaries and the plug enclose a volume of the assay chamber; and
 - e. a shoulder extending from each curved boundary wherein the plug contacts each shoulder such that a boundary of the assay chamber is provided by the two curved
 - 15 boundaries, the shoulders extending from each of the curved boundaries and the plug.
25. The assay chamber of claim 24, wherein plug within the assay chamber has a dried reagent thereon.
26. The assay chamber of claim 25, wherein a cavity on the plug is positioned between each of the shoulders and the dried reagent is in the cavity.
- 20 27. The assay chamber of claim 26, wherein a portion of the curved boundaries or of the shoulders is shaped to conform to a perimeter of the cavity.
28. The assay chamber of claim 25, wherein the dried reagent on the plug is positioned between each of the shoulders.
29. The assay chamber of claim 24, wherein a flat portion of the bottom of the plug body
- 25 contacts the shoulders.
30. The assay chamber of claim 24, wherein a height of each of the shoulders is used to adjust the volume of the assay chamber.
31. The assay chamber of claim 30, wherein the height of each of the shoulders is 100 micrometers or more.
- 30 32. The assay chamber of claim 30, wherein the height of each of the shoulders is no greater than a distance of the two curving boundaries from each other at a point of greatest separation.

33. The assay chamber of any of claims 24-32, wherein the shoulders are shaped to maintain an overall curved boundary of the assay chamber from the tapered inlet to the tapered outlet.
34. The assay chamber of claim 24, wherein the two curved boundaries and the shoulders are formed in a monolithic substrate.
- 5 35. The assay chamber of 34, further comprising a film adhered to a surface the monolithic substrate, wherein the film forms one wall of the assay chamber.
36. The assay chamber of any of claims 24-35 having a plug as in any of claims 1-23.
37. An apparatus, comprising:
- a. a common fluid pathway, and
- 10 b. a plurality of independent, continuous fluidic pathways connected to the common fluid pathway, wherein each independent, continuous fluidic pathway comprising:
- i. an assay chamber, and
- ii. a pneumatic compartment;
1. wherein the assay chamber is connected to the common fluid pathway, the
- 15 assay chamber having a fluid volume defined in part by a plug having a dried reagent thereon; and
2. the pneumatic compartment, having a pneumatic volume, is connected to the common fluid pathway via the assay chamber;
- wherein, each fluidic pathway of the plurality of independent, continuous fluidic
- 20 pathways is a closed system excluding the connection between the assay chamber and common fluid source, wherein each assay chamber further comprises:
- c. a double tapered chamber, the double tapered chamber comprising:
- iii. a tapered inlet in fluidic communication with a terminus of the entry conduit
- 25 of the fluidic pathway,
- iv. a tapered outlet in fluidic communication with a terminus of the pneumatic compartment, and
- v. two curved boundaries, wherein each curved boundary extends from the
- 30 tapered inlet to the tapered outlet such that together, the two curved boundaries enclose the volume of the assay chamber;

- d. a shoulder extending from each curved boundary wherein the plug contacts each shoulder such that a boundary of the assay chamber is provided by the two curved boundaries, the shoulders extending from each of the curved boundaries and the plug.
38. The apparatus of claim 37 wherein a cavity on the plug is positioned between each of the shoulders and the dried reagent is in the cavity.
39. The apparatus of claim 37 wherein a dried reagent on the plug is positioned between each of the shoulders.
40. The apparatus of claim 37 wherein a flat portion of the bottom of the plug body contacts the shoulders.
41. The apparatus of claim 37 wherein a height of each of the shoulders is used to adjust the volume of the assay chamber.
42. The apparatus of claim 41 wherein the height of each of the shoulders is 100 micrometers or more.
43. The apparatus of any of claims 37-42 wherein the shoulders are shaped to maintain an overall curved boundary of the assay chamber from the tapered inlet to the tapered outlet.
44. The apparatus of claim 37 wherein the two curved boundaries are formed in a monolithic substrate.
45. The apparatus of claim 44, wherein the body of the plug protrudes into the monolithic substrate of the assay chamber at a depth such that the assay chamber volume can be readily changed by altering the depth at which the body of the plug protrudes into the monolithic substrate of the assay chamber.
46. The apparatus of claim 38 wherein a portion of the curved boundaries or of the shoulders is shaped to conform to a perimeter of the cavity.
47. The apparatus of any of claims 37-46, further comprising a first film adhered to a surface of at least a portion of the apparatus, wherein the first film forms one wall of one or more chambers, compartments, or conduits of the apparatus.
48. The apparatus of claim 47, further comprising a second film adhered to the first film, wherein the second film has a higher melting temperature than the first film.

49. The apparatus of claim 48 further comprising a heat staked region formed in each of the fluidic pathways using the first film or the second film wherein the heat staked region seals off the common fluid pathway from the assay chamber and the pneumatic chamber.
50. The apparatus of claim 49 further comprising a raised platform within each of the plurality of independent, continuous fluidic pathways the raised platform positioned between an inlet to the assay chamber and the common fluid pathway wherein the heat staked region is formed using a portion of the raised platform.
51. The apparatus of any of claims 37-50 having a plug as in any of claims 1-23.
52. A method of simultaneously filling a plurality of sample chambers, the method comprising:
- Pressurizing a fluid sample within a common fluid pathway;
 - introducing the fluid sample into a plurality of entry conduits from the common fluid pathway;
 - flowing the fluid sample along each of the entry conduits towards an entry conduit terminus in each of the entry conduits, each entry conduit connected to a sample chamber;
 - flowing the fluid sample along a tapered inlet portion of each sample chamber;
 - flowing the fluid sample adjacent a pair of shoulders and along a plug within each sample chamber;
 - flowing the fluid sample along a tapered outlet portion of each sample chamber towards a pneumatic compartment terminus; and
 - displacing a gas contained within each entry conduit and each sample chamber into a pneumatic chamber in communication with each pneumatic compartment terminus.
53. The method of claim 52, wherein pressuring the fluid sample step is performed at a constant pressure.
54. The method of claim 53 wherein the constant pressure is one of 5, 10, 20, 40 or 60 psi.
55. The method of claim 52 wherein the pressurizing the fluid step further comprises pressuring the fluid sample at a series of increasing pressure levels.
56. The method of claim 55 wherein each increasing level of pressure is applied for a consistent duration.

57. The method of claim 55 wherein each increasing level of pressure is increased by a constant amount.
58. The method of claim 55 wherein the pressurizing the fluid sample applies a series of pressure levels from a lower pressure level to a higher pressure level.
- 5 59. The method of claim 52, wherein in use the pneumatic chamber is above the sample chamber such that the steps of flowing the fluid sample along a tapered outlet portion of the sample chamber towards a pneumatic compartment terminus and displacing a gas contained within each entry conduit are performed against a gravitational force.
60. The method of claim 52 or claim 59 wherein, in use, the plurality of sample chambers are
10 oriented such that each pneumatic chamber associated with a specific sample chamber of the plurality of sample chamber is positioned above the sample chamber.
61. The method of claim 52, wherein flowing the fluid sample into the sample chamber of each fluidic pathway of the apparatus compresses the gas within the fluidic pathways toward the pneumatic compartments of the fluidic pathways
- 15 62. The method of claim 52 further comprising maintaining the pressure reached during the pressurizing a fluid sample step when an internal pressure in each of the pneumatic compartments equals the pressure applied to the common fluid pathway.
63. The method of claim 52 further comprising; increasing a pressure within each pneumatic compartment during the displacing a gas step; and stopping increasing the pressure when a
20 pressure applied to the common fluid pathway equals the pressure within each pneumatic compartment.
64. The method of claim 52 further comprising stopping each of the flowing the sample steps when the internal pressure in each of the pneumatic compartments equals a pressure applied to the common fluid pathway.
- 25 65. The method of claim 52, wherein at least two sample chambers of the plurality of sample chambers differ in volume.
66. The method of claim 65 wherein a flowrate from the common fluid pathway into each sample chamber of the plurality of sample chambers is proportional to a fluid volume of the sample chamber and there are at least two different flowrates.

67. The method of claim 52 further comprising: simultaneously filling each sample chamber of the plurality of sample chambers.
68. The method of claim 52 further comprising: flowing the fluid sample along two diverging curved boundaries within the sample chamber during or after the flowing the fluid sample along the tapered inlet step.
69. The method of claim 52 further comprising: flowing the fluid sample along two converging curved boundaries within the sample chamber after or during the flowing the fluid sample along the pair of shoulders step.
70. The method of claim 69, wherein convergence of the two curved boundaries slow the rate of fluid advance at a leading front of a meniscus of the fluid sample, such that when the fluid sample reaches the tapered outlet, the meniscus of the fluid sample is substantially symmetric with respect to the largest dimension of the assay chamber, thereby minimizing the trapping of bubbles within the assay chamber during filling.
71. The method of claim 52 further comprising: positioning a meniscus in each sample chamber adjacent to the pneumatic chamber terminus.
72. The method of claim 52 further comprising: performing one or more of the steps so as to position one or more bubbles formed within the fluid sample adjacent to a meniscus of the fluid sample within the sample chamber.
73. The method of claim 72 wherein the meniscus is proximate to the pneumatic chamber terminus.
74. The method of claim 52, further comprising: sealing each one of the plurality of entry conduits while performing the step of pressurizing a fluid sample within the common fluid pathway.
75. The method of claim 52, further comprising: sealing each one of the plurality of entry conduits when the step of flowing the fluid sample along a tapered portion of the sample chamber stops.
76. The method of claim 52, further comprising: sealing each one of the plurality of entry conduits when the step of flowing the fluid sample along each of the entry conduits from the common fluid pathway stops.

77. The method of any of claims 74-76 wherein the step of sealing further comprising heat staking a portion of the entry conduit closed.
78. The method of any of claims 74-76 further comprising: heating a portion of a first film adjacent to each of the entry conduits; melting the first film to seal off the each one of the entry
5 conduits.
79. The method of claim 77 further comprising simultaneously sealing all entry conduits.
80. The method of claim 77 further comprising: heating without melting a second film separated from the entry conduit by the first film.
81. The method of claim 80 further comprising fusing a portion of the entry conduit to a
10 portion of the first film without melting the second film.
82. The method of any of claims 74-81 wherein after sealing each one of the entry conduits a portion of a first film or a second film is fused to a raised platform formed in each one of the entry conduits.