

CLAIMS

1. A microfluidic device comprising:

an enclosure comprising a substrate and microfluidic circuit material, the enclosure defining a flow region and a sequestration pen fluidically connected to the flow region and having a single opening, each located within the enclosure,

wherein the sequestration pen comprises an isolation region and a connection region fluidically connecting the isolation region to the microfluidic channel; wherein the isolation region is an unswept region; and

an in situ-generated capture structure disposed within the sequestration pen, wherein the in situ-generated capture structure comprises a solidified polymer network.
2. The microfluidic device of claim 1, wherein the solidified polymer network comprises one or more functionalized sites.
3. The microfluidic device of claim 1 or 2, wherein the solidified polymer network comprises an assay reagent or assay analyte.
4. The microfluidic device of any of claims 1-3, wherein the in situ-generated capture structure is disposed within the isolation region of the sequestration pen.
5. The microfluidic device of claim 3 or 4, wherein the assay reagent is non-covalently attached to the solidified polymer network.
6. The microfluidic device of any of claims 3-5, wherein the assay reagent comprises a protein, a nucleic acid, an organic molecule, and/or a saccharide.
7. The microfluidic device of claim 6, wherein the assay reagent comprises an antibody or an antigen.
8. The microfluidic device of claim 3 or 4, wherein the assay analyte comprises an antibody or a cytokine.
9. The microfluidic device of any of claims 1-8, wherein two or more in situ-generated capture structures are disposed in the sequestration pen.
10. The microfluidic device of claim 9, comprising

a first in situ-generated capture structure disposed at a first location within the chamber and comprising a first solidified polymer network, wherein the first solidified polymer network comprises a first assay reagent; and

a second in situ-generated capture structure disposed at a second location within the chamber and comprising a second solidified polymer network, wherein the second solidified polymer network comprises a second assay reagent;

wherein the first assay reagent is different from the second assay reagent, and further wherein the first location and the second location are physically distinguishable.

11. The microfluidic device of claim 10, wherein the first assay reagent or assay analyte is different from the second assay reagent or assay analyte.
12. The microfluidic device of any of claims 1-11, wherein the solidified polymer network comprises a photoinitiated polymer.
13. The microfluidic device of any of claims 1-12, wherein the solidified polymer network comprises a synthetic polymer, a modified synthetic polymer, a biological polymer, or any combination thereof.
14. The microfluidic device of any of claims 1-13, wherein the substrate is configured to generate dielectrophoresis (DEP) forces within the enclosure.
15. The microfluidic device of any of claims 1-14, wherein the in situ-generated capture structure permits entrance of a micro-object into the sequestration pen and export of a micro-object from the sequestration pen.
16. The microfluidic device of any one of claims 1-15, wherein the connection region has the single opening of the sequestration pen, and the single opening opens to the microfluidic channel; further wherein a width W_{con} of the connection region at the single opening is from 20 microns to 100 microns.
17. The microfluidic device of claim 16, wherein the connection region has a distal opening to the isolation region, and a length L_{con} of the connection region from the single opening to the distal opening is at least 1.0 times the width W_{con} of the connection region.
18. The microfluidic device of claim 16 or claim 17, wherein a height H_{ch} of the microfluidic channel at the single opening of the sequestration pen is 20-100 microns.
19. A method of assaying a micro-object in a microfluidic device comprising a first in situ-generated capture structure comprising:
 - disposing a micro-object within the microfluidic device in a region proximal to the first in situ-generated capture structure comprising a first solidified polymer network, wherein the solidified polymer network comprises a first assay reagent or assay analyte;
 - contacting the first assay reagent or assay analyte with the micro-object or a biological product of the micro-object; and
 - detecting an interaction of the first assay reagent or assay analyte with the micro-object or the biological product;
 wherein the microfluidic device comprises an enclosure comprising a substrate and microfluidic circuit material, the enclosure defining a flow region and a sequestration

pen fluidically connected to the flow region and having a single opening, each located within the enclosure,

wherein the sequestration pen comprises an isolation region and a connection region fluidically connecting the isolation region to the microfluidic channel; wherein the isolation region is an unswept region; and

wherein the first in situ-generated capture structure is disposed within the sequestration pen.

20. The method of claim 19, wherein the first assay reagent or assay analyte is non-covalently attached to the solidified polymer network.

21. The method of claim 19 or claim 20, wherein the biological product comprises a protein, an oligonucleotide, an organic molecule, or a saccharide.

22. The method of any one of claims 19-21, wherein the step of contacting the first assay reagent or assay analyte with the biological product or the micro-object further comprises forming a noncovalent complex.

23. The method of any one of claims 19-22, wherein the step of detecting the interaction further comprises introducing a detection reagent having a detectable label to the region proximal to the first in situ-generated capture structure.

24. The method of claim 23, wherein the detectable label is configured to be concentrated to the first in situ-generated capture structure when the first assay reagent or assay analyte interacts with the biological product or the micro-object.

25. The method of any one of claims 19-24, wherein the step of detecting comprises detecting a fluorescent signal from the first in situ-generated capture structure.

26. The method of any one of claims 19-25, wherein the microfluidic device further comprises a second in situ-generated capture structure disposed within the one sequestration pen, wherein the second in situ-generated capture structure comprises a second solidified polymer network and further wherein the second solidified polymer network comprises a second assay reagent or assay analyte,

wherein the first in situ-generated capture structure is disposed at a first location within the sequestration pen, and the second in situ-generated capture structure is disposed at a second location within the sequestration pen; wherein the first assay reagent or assay analyte is different from the second assay reagent or assay analyte, and first location and the second location are physically distinguishable; and

wherein the method further comprises detecting a first interaction of the first assay reagent with the micro-object or the biological product thereof and/or a second interaction of the second assay reagent with the micro-object or the biological product thereof.

27. The method of claim 26, wherein detecting the first interaction and/or detecting the second interaction comprises detecting a first signal from the first location and/or a second signal from the second location.
28. The method of claim 26 or claim 27,
wherein detecting a first interaction comprises detecting the first interaction between the first assay reagent with a first biological product; and wherein detecting a second interaction comprises detecting the second interaction between the second assay reagent with a second biological product; or
wherein detecting a first interaction comprises detecting the first interaction between the first assay reagent with the biological product; and wherein detecting a second interaction comprises detecting the second interaction between the second assay reagent with the micro-object.
29. The method of any one of claims 26-28,
wherein detecting a first interaction comprises detecting a first characteristic of the biological product; and
wherein detecting a second interaction comprises detecting a second characteristic of the biological product.
30. The method of any one of claims 26-29,
wherein detecting the first interaction comprises introducing a first detection reagent comprising a first detectable label to a region adjacent to the first in situ-generated capture structures; and
wherein detecting the second interaction comprises introducing a second detection reagent comprising a second detectable label to a region adjacent to the second in situ-generated capture structures.
31. The method of claim 30, wherein the first detectable label and the second detectable label are independently a fluorescent label, a colorimetric label, or a luminescent label.
32. The method of claim 30 or claim 31, wherein the first detectable label and the second detectable label are spectrally distinct.
33. The method of any of claims 19 to 32, wherein the in situ-generated capture structure permits entrance of a micro-object into the sequestration pen and export of a micro-object from the sequestration pen.
34. The method of any of claims 19 to 33, wherein the connection region has the single opening of the sequestration pen, and the single opening opens to the microfluidic channel; further wherein a width W_{con} of the connection region at the single opening is from 20 microns to 100 microns.

35. The method of claim 34, wherein the connection region has a distal opening to the isolation region, and a length L_{con} of the connection region from the single opening to the distal opening is at least 1.0 times the width W_{con} of the connection region.
36. The microfluidic device of claim 34 or claim 35, wherein a height H_{ch} of the microfluidic channel at the single opening of the sequestration pen is 20-100 microns.
37. The method of any one of claims 19-36, wherein the step of disposing the micro-object within the microfluidic device in the region proximal to the first in situ-generated capture structure comprises moving the micro-object using dielectrophoresis force.
38. The method of claim 19, wherein the solidified polymer network comprises the first assay reagent, and wherein the first assay reagent comprises a protein.
39. The method of claim 38, wherein the assay reagent comprises an antigen or an antibody.
40. The method of any one of claims 19-39, wherein the biological product of the micro-object is an antibody or an antigen.
41. A kit comprising a microfluidic device comprising:
- an enclosure comprising a substrate and microfluidic circuit material, the enclosure defining a flow region and a sequestration pen fluidically connected to the flow region and having a single opening, wherein the sequestration pen comprises an isolation region and a connection region fluidically connecting the isolation region to the microfluidic channel; wherein the isolation region is an unswept region; and
 - a functionalized pre-polymer that can be controllably activated to form a solidified polymer network.
42. The kit of claim 41, further comprising an assay reagent or assay analyte.
43. A method of preparing a microfluidic device comprising a first in situ-generated capture structure, the method comprising:
- providing the microfluidic device, wherein the microfluidic device comprises an enclosure comprising a substrate and microfluidic circuit material, the enclosure defining a flow region and a sequestration pen fluidically connected to the flow region and having a single opening;
 - introducing a first flowable functionalized pre-polymer into the flow region; and
 - activating solidification of the first flowable functionalized pre-polymer at a first selected area of the sequestration pen, thereby forming the first in situ-generated capture structure therein;

wherein the sequestration pen comprises an isolation region and a connection region fluidically connecting the isolation region to the microfluidic channel; wherein the isolation region is an unswept region.

44. The method of claim 43, wherein the first in-situ generated capture structure comprises a solidified polymer network comprising one or more functionalized sites, wherein the one or more functionalized sites comprise a biotin, avidin or streptavidin moiety.

45. The method of claim 43 or 44 further comprising: introducing a first functionalized assay reagent or assay analyte to the first in situ-generated capture structure within the sequestration pen; and associating the first functionalized assay reagent or assay analyte to the functionalized sites of the solidified polymer network of the first in situ-generated capture structure.

46. The method of any one of claims 43-45, further comprising: introducing a second functionalized assay reagent or assay analyte.

47. The method of claim 46, wherein the second functionalized assay reagent or assay analyte is detectably distinguishable from the first functionalized assay reagent or assay analyte.

48. The method of claim 46 or claim 47, wherein the second functionalized assay reagent or assay analyte associates with a second functionalized site on a second in situ-generated capture structure in the at least one sequestration pen.

49. The method of claim 48, further comprising introducing a second in situ-generated capture structure in the sequestration pen, wherein introducing the second in situ-generated or more capture structure comprises the steps of:

introducing a second flowable functionalized pre-polymer into the flow region; and

activating solidification of the second flowable functionalized pre-polymer at a second selected area of the sequestration pen, thereby forming the second in situ-generated capture structure therein.