

CLAIMS

1. A microfluidic device comprising:

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

wherein the ~~at least one~~ 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 ~~an isolation structure defining an isolation region having a single opening to a connection region, wherein the connection region fluidically connects the isolation region and the flow region, the connection region having a proximal opening to the flow region and a distal opening to the isolation region; wherein the proximal opening is oriented substantially parallel to a flow of fluidic medium in the flow region~~; and

~~at least one~~ an in situ-generated capture structure disposed within the ~~at least one~~ sequestration pen, wherein the ~~at least one~~ 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 ~~at least one~~ 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 ~~at least one~~ 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.

~~10~~12. The microfluidic device of any of claims 1-~~9~~11, wherein the solidified polymer network comprises a photoinitiated polymer.

~~11~~13. The microfluidic device of any of claims 1-~~10~~12, wherein the solidified polymer network comprises a synthetic polymer, a modified synthetic polymer, a biological polymer, or any combination thereof.

~~12~~14. The microfluidic device of any of claims 1-~~11~~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.

~~13~~19. A method of assaying a micro-object in a microfluidic device comprising ~~at least~~ a first in situ-generated capture structure comprising:

disposing a micro-object within the microfluidic device in a region proximal to the ~~at least~~ first in situ-generated capture structure comprising a first solidified polymer network, wherein the solidified polymer network comprises ~~an 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 ~~at least one~~ a sequestration pen fluidically connected to the flow region and having a single opening, each located within the enclosure,

wherein the ~~at least one~~ 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~~an isolation region with a single opening to a connection region, wherein the connection region fluidically connects the isolation region and the flow region, the connection region having a proximal opening to the flow region and a distal opening to the isolation region; wherein the proximal opening is oriented substantially parallel to a flow of fluidic medium in the flow region; and~~

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

~~14. The method of claim 13, wherein the microfluidic device comprises: an enclosure comprising a substrate and microfluidic circuit material, the enclosure defining a flow region and at least one sequestration pen; and~~

~~wherein the at least first in situ-generated capture structure is disposed within the at least one sequestration pen.~~

~~15. The method of claim 13 or 14, wherein the at least one sequestration pen comprises an isolation region and with a single opening to a connection region, wherein the connection region fluidically connects the isolation region and the flow region, the connection region having a proximal opening to the flow region and a distal opening to the isolation region, and further wherein the at least first in situ-generated capture structure is disposed within the isolation region of the sequestration pen.~~

~~16~~20. The method of ~~any one of claims 13-15~~claim 19, wherein the first assay reagent or assay analyte is non-covalently attached to the solidified polymer network.

~~17~~21. The method of ~~any one of claims 13-16~~claim 19 or claim 20, wherein the biological product comprises a protein, an oligonucleotide, an organic molecule, or a saccharide.

~~18~~22. The method of any one of claims ~~13-17~~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.

~~19~~23. The method of any one of claims ~~13-18~~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 ~~at least~~ first in situ-generated capture structure.

~~20~~24. The method of claim ~~19~~23, wherein the detectable label is configured to be concentrated to the ~~at least~~ first in situ-generated capture structure when the first assay reagent or assay analyte interacts with the biological product or the micro-object.

~~21~~25. The method of any one of claims ~~13-20~~19-24, wherein the step of detecting comprises detecting a fluorescent signal from the ~~at least~~ first in situ-generated capture structure.

~~22~~26. The method of any one of claims ~~13-21~~19-25, wherein the microfluidic device further comprises a second in situ-generated capture structure disposed within the ~~at least~~ 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~~wherein each of the first and second in situ-generated capture structures comprises a different assay reagent or assay analyte, and wherein the step of detecting comprises detecting a first biological product of the micro-object and a second biological product of the micro-object, wherein the first biological product is different from the second biological product.~~

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.

~~23~~37. The method of any one of claims ~~13-22~~19-36, wherein the step of disposing the micro-object within the microfluidic device in the region proximal to the ~~at least~~ first in situ-generated capture structure comprises moving the micro-object using dielectrophoresis force.

~~24~~38. The method of claim ~~13~~19, wherein the solidified polymer network comprises ~~an~~ the first assay reagent, and wherein the first assay reagent comprises a protein.

2539. The method of claim 2438, wherein the assay reagent comprises an antigen or an antibody.

2640. The method of ~~claim 13~~ any one of claims 19-39, wherein the biological product of the micro-object is an antibody or an antigen.

2741. A kit comprising a microfluidic device comprising:

an enclosure comprising a substrate and microfluidic circuit material, the enclosure defining a flow region and ~~at least one~~ a sequestration pen fluidically connected to the flow region and having a single opening, wherein the ~~at least one~~ 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~~an isolation region with a single opening to a connection region, wherein the connection region fluidically connects the isolation region and the flow region, the connection region having a proximal opening to the flow region and a distal opening to the isolation region; wherein the proximal opening is oriented substantially parallel to a flow of fluidic medium in the flow region; and~~

a functionalized pre-polymer that can be controllably activated to form a solidified polymer network.

2842. The kit of claim 2741, further comprising an assay reagent or assay analyte.

2943. A method of preparing a microfluidic device comprising ~~at least~~ 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 ~~at least one~~ 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 ~~at least one~~ a first selected area of the ~~at least one~~ sequestration pen, thereby forming the ~~at least a~~ first in situ-generated capture structure therein;

wherein the ~~at least one~~ 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~~an isolation region with a single opening to a connection region, wherein the connection region fluidically connects the isolation region and the flow region, the connection region having a proximal opening to the flow region and a distal opening to the isolation region; wherein the proximal opening is oriented substantially parallel to a flow of fluidic medium in the flow region.~~

~~3044~~. The method of claim ~~2943~~, wherein the ~~at least a~~ 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.

~~3145~~. The method of claim ~~2943~~ or ~~3044~~ further comprising: introducing a first functionalized assay reagent or assay analyte to the ~~at least~~ first in situ-generated capture structure within the ~~at least one~~ sequestration pen; and associating the first functionalized assay reagent or assay analyte to the functionalized sites of the solidified polymer network of the ~~at least~~ first in situ-generated capture structure.

~~3246~~. The method of any one of claims ~~29-3143-45~~, further comprising: introducing a second functionalized assay reagent or assay analyte.

~~3347~~. The method of claim ~~3246~~, wherein the second functionalized assay reagent or assay analyte is detectably distinguishable from the first functionalized assay reagent or assay analyte.

~~3448~~. The method of claim ~~3246~~ 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.

~~3549~~. The method of claim ~~3448~~, further comprising introducing ~~at the~~ second in situ-generated capture structure in the ~~at least one~~ 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 ~~at least~~ a second selected area of the ~~at least one~~ sequestration pen, thereby forming the second in situ-generated capture structure therein.