What is claimed:

1. A microfluidic device comprising:

an enclosure comprising:

a substrate;

a flow region comprising a microfluidic channel located within the enclosure,

~~at least one~~a sequestration pen fluidically connected to the flow region and comprising a single opening ~~to the microfluidic channel~~; and

~~at least one~~an in situ-generated isolation structure disposed at least in part in the sequestration pen, wherein the ~~at least one~~ in situ-generated structure comprises a solidified polymer network;

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, the connection region comprising a fluid flow pathway having a proximal opening to the microfluidic channel and a distal opening to the isolation region; and wherein the proximal opening is oriented substantially parallel to a flow of fluidic medium in the flow region~~.

2. The microfluidic device of claim 1, wherein the solidified polymer network comprises a photoinitiated polymer.

3. The microfluidic device of claim 1 or claim 2, wherein at least a portion of the ~~at least one~~ in situ-generated isolation structure extends into the microfluidic channel.

4. The microfluidic device of any one of claims 1 to 3, wherein the microfluidic device comprises a plurality of sequestration pens.

5. The microfluidic device of any one of claims 1 to 4, wherein the ~~at least one~~ in situ-generated isolation structure is configured to retain one or more of a plurality of micro-objects.

6. The microfluidic device of any one of claims 1 to 5, 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~~ the at least one in situ-generated isolation structure comprises a plurality of in situ-generated isolation modules disposed in the flow region, wherein the plurality of in situ-generated isolation modules are configured to substantially restrict passage of micro-objects into, out of, and/or through the at least one in situ generated isolation structure in a size-dependent manner~~.

7. The microfluidic device of claim 6, 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 ~~each in situ-generated isolation module of the plurality is spaced apart from any other in situ-generated isolation module of the plurality such that micro-objects having a diameter of 5 microns or greater are substantially prevented from passing into, out of, and/or through the at least one in situ-generated isolation structure~~.

8. The microfluidic device of claim 6 or claim 7, wherein a height H_{ch} of the microfluidic channel at the single opening of the sequestration pen is 20-100 microns.

9. The microfluidic device of any one of claims 1 to 8, wherein the solidified polymer network comprises a synthetic polymer, a modified synthetic polymer, or a biological polymer.

10. The microfluidic device of any one of claims 1 to 9, wherein the solidified polymer network comprises a modified polyethylene glycol polymer.

11. The microfluidic device of any one of claims 1 to 10, wherein the solidified polymer network is at least partially removable by application of increased fluid flow through the flow region, hydrolysis, proteolysis, osmotic change, temperature change, or optical illumination.

12. The microfluidic device of any one of claims 1 to 11, wherein at least one inner surface of the enclosure comprises a conditioned surface.

13. The microfluidic device of any one of claims 1 to 12, wherein the substrate is configured to generate dielectrophoresis (DEP) forces within the enclosure.

~~8~~14. The microfluidic device of any one of claims 1~~-7~~ to 13, wherein the microfluidic device further comprises a plurality of in situ-generated isolation structures.

15. The microfluidic device of any one of claims 1 to 14, wherein the in situ-generated isolation structure is porous.

~~9~~16. A microfluidic device comprising:

an enclosure comprising:

a flow region comprising a microfluidic channel;

a sequestration pen fluidically connected to the flow region and comprising a single opening to~~, wherein the sequestration pen opens off of the microfluidic channel~~; and

an in situ-generated isolation structure comprising an in situ-generated barrier disposed at least in part in the microfluidic channel, the in situ-generated barrier comprising a solidified polymer network;

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~~comprises an isolation structure defining an isolation region with a single opening to a connection region, the connection region comprising a fluid flow pathway 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~~.

~~10~~17. The microfluidic device of claim ~~9~~16, wherein the in situ-generated barrier at least partially blocks the microfluidic channel and/or the sequestration pen.

~~11~~18. The microfluidic device of claim ~~9~~16 or claim 17, wherein the in situ-generated barrier is disposed at least in part within a connection region of the sequestration pen.

~~12~~19. The microfluidic device of ~~claim 9~~any one of claims 16 to 18, wherein the in situ-generated barrier is located adjacent to one edge of a proximal opening of the sequestration pen.

~~13~~20. The microfluidic device of ~~claim 1~~any one of claims 16 to 19, wherein the solidified polymer network comprises a synthetic polymer, a modified synthetic polymer, or a biological polymer.

~~14~~21. The microfluidic device of ~~claim 1~~any one of claims 16 to 20, wherein the solidified polymer network comprises a modified polyethylene glycol polymer.

~~15~~22. The microfluidic device of ~~claim 1~~any one of claims 16 to 21, wherein the solidified polymer network is at least partially removable by application of increased fluid flow through the flow region, hydrolysis, proteolysis, osmotic change, temperature change, or optical illumination.

~~16~~23. The microfluidic device of ~~claim 1~~any one of claims 16 to 22, wherein at least one inner surface of the enclosure comprises a conditioned surface.

~~17~~24. The microfluidic device of ~~claim 1~~any one of claims 16 to 23, wherein the substrate is configured to generate dielectrophoresis (DEP) forces within the enclosure.

25. The microfluidic device of any one of claims 16 to 24, 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.

26. The microfluidic device of claim 25, 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.

27. The microfluidic device of claim 25 or claim 26, wherein a height H_{ch} of the microfluidic channel at the single opening of the sequestration pen is 20-100 microns.

28. The microfluidic device of any one of claims 16 to 27, wherein the in situ-generated barrier is porous to a flow of fluidic medium within the enclosure.

~~18~~29. A method of isolating a micro-object in a microfluidic device, comprising:

introducing a first fluidic medium comprising a plurality of micro-objects into an enclosure of the microfluidic device, the enclosure comprising a substrate and a flow region fluidically connected to the flow region and comprising a microfluidic channel;

introducing a solution comprising a flowable polymer into the enclosure;

activating solidification of the flowable polymer in ~~at least one~~a selected area ~~of the flow region~~within the enclosure, thereby forming an in situ-generated isolation structure; and

isolating at least one micro-object of the plurality of micro-objects with the in situ-generated isolation structure;

wherein the enclosure of the microfluidic device further comprises ~~at least one~~a sequestration pen comprising 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

wherein the in situ-generated isolation structure is disposed at least in part in the sequestration pen, wherein the in situ-generated structure comprises a solidified polymer network ~~comprising an isolation structure defining an isolation region having a single opening to a connection region, the connection region comprising a fluid flow pathway 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~~.

~~19~~30. The method of claim ~~18~~29, wherein the activating solidification of the flowable polymer comprises optically illuminating the ~~at least one~~ selected area of the flow region, and thereby initiating polymerization of the flowable polymer to form a polymer network.

~~20~~31. The method of claim ~~18~~29 or claim 30, wherein the introducing the solution comprising a flowable polymer further comprises introducing a photoactivatable polymerization initiator.

~~21~~32. The method of ~~claim 18~~any one of claims 29 to 31, further comprising processing a remainder of the plurality of micro-objects or exporting a remainder of the plurality of micro-objects from the microfluidic device.

~~22~~33. The method of ~~claim 18~~any one of claims 29 to 32, further comprising:

reducing or removing the in situ-generated isolation structure by:

increasing flow of the first fluidic medium through the flow region;

introducing a hydrolytic agent into the flow region;

introducing a proteolytic agent into the flow region;

introducing a different fluidic medium into the flow region that increases/decreases an osmolality within the flow region;

changing a temperature of the in situ-generated isolation structure; or

optically illuminating the in situ-generated isolation structure; and

thereby releasing the at least one micro-object from the in situ-generated isolation structure.

~~23~~34. The method of claim ~~22~~33, further comprising exporting the at least one released micro-object from the microfluidic device.

~~24~~35. The method of ~~claim 18~~any one of claims 29 to 34, wherein the in situ-generated isolation structure comprises a plurality of in situ-generated isolation modules disposed in the flow region, wherein the plurality of in situ-generated isolation modules are configured to substantially restrict passage of micro-objects into, out of, and/or through the ~~at least one~~ in situ generated isolation structure in a size dependent manner.

~~25~~36. The method of ~~claim 18~~any one of claims 29 to 35, wherein the activating solidification forms an in situ-generated isolation structure comprising an in situ-generated barrier inside the ~~at least one~~ sequestration pen.

~~26. The method of claim 18, wherein activating solidification of the flowable polymer forms an in situ-generated isolation structure comprising an in situ-generated barrier in the flow region.~~

37. The method of any one of claims 29 to 36, 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.

38. The method of claim 37, 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.

39. The method of claim 37 or claim 28, wherein a height H_{ch} of the microfluidic channel at the single opening of the sequestration pen is 20-100 microns.

~~27~~40. The method of any one of claims ~~18-26~~29 to 39, wherein the flowable polymer comprises a synthetic polymer, a modified synthetic polymer, or a biological polymer.

~~28~~41. A kit comprising:

a microfluidic device comprising an enclosure comprising:

a substrate;

a flow region located within the enclosure and comprising a microfluidic channel; and

a sequestration pen fluidically connected to the flow region and comprising 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~~an isolation structure defining an isolation region having a single opening to a connection region, the connection region comprising a fluid flow pathway 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 flowable polymer solution, wherein the polymer is capable of polymerization and/or thermally induced gelling.

~~29~~42. The kit of claim ~~28~~41, further comprising a photoactivatable polymerization initiator.

~~30~~43. The kit of claim ~~28~~41 or claim 42, wherein the flowable polymer comprises a synthetic polymer, a modified synthetic polymer, or a biological polymer.

~~31~~44. The kit of ~~claim 28~~any one of claims 41 to 43, wherein the flowable polymer comprises at least one of a polyethylene glycol, modified polyethylene glycol, polylactic acid (PLA), modified polylactic acid, polyglycolic acid (PGA), modified polyglycolic acid, polyacrylamide (PAM), modified polyacryla mide, poly-N-isopropylacrylamide (PNIPAm), modified poly-N-isopropylacrylamide, polyvinyl alcohol (PVA), modified polyvinyl alcohol, polyacrylic acid (PAA), modified polyacrylic acid, polycaprolactone (PCL), modified polycaprolactone, fibronectin, modified fibronectin, collagen, modified collagen, laminin, modified laminin, polysaccharide, modified polysaccharide, or a co-polymer in any combination.

45. The kit of any one of claims 41 to 45, 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.

46. The kit of claim 45, 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.

47. The kit of claim 45 or claim 46, wherein a height H_{ch} of the microfluidic channel at the single opening of the sequestration pen is 20-100 microns.

~~32~~48. The kit of any one of claims ~~28-31~~41 to 47, further comprising a reagent configured to covalently modify at least one internal surface of the microfluidic device, wherein the reagent provides an

alkyl-terminated, a fluoroalkyl terminated, a PEG- terminated, a dextran terminated, or a positively charged or negatively charged terminated moiety to the covalently modified surface.