CLAIMS

We claim:

1. A microfluidic device comprising:

a flow region configured to contain a flow of a first fluidic medium;

a microfluidic sequestration pen comprising:

(i) an isolation structure comprising an isolation region configured to contain a second fluidic medium, and

(ii) a connection region fluidically connecting said isolation region to said flow region; and

a microfluidic channel comprising at least a portion of said flow region,

wherein said connection region comprises a proximal opening into said microfluidic channel and a distal opening into said isolation region,

wherein while said flow region and said microfluidic sequestration pen are substantially filled with fluidic media:

(i) components of said second medium are able to diffuse into said first medium or components of said first medium are able to diffuse into said second medium, and

(ii) there is substantially no flow of said first medium from said flow region into said isolation region.

2. The device of claim 1, wherein a width of said microfluidic channel at said proximal opening of said connection region is between about 50 microns and about 500 microns.

3. The device of any of claims 1-2, wherein a length L_{con} of said connection region from said proximal opening to said distal opening is greater than or equal to a penetration depth D_p of said first medium into said connection region.

4. The device of any of claims 1-3, wherein a length L_{con} of said connection region from said proximal opening to said distal opening and a width W_{con} of said proximal opening of said connection region are sufficient to keep a micro-object in said isolation region from moving from said isolation region through said connection region into said channel.

5. The device of any of claims 1-4, wherein a length L_{con} of said connection region from said proximal opening to said distal opening is at least equivalent to the width W_{con} of said proximal opening of said connection region.

6. The device of any of claims 1-4, wherein a length L_{con} of said connection region from said proximal opening to said distal opening is at least 1.5 times the width W_{con} of said proximal opening of said connection region.

7. The device of any of claims 1-4, wherein a length L_{con} of said connection region from said proximal opening to said distal opening is at least 2.0 times the width W_{con} of said proximal opening of said connection region.

8. The device of any of claims 1-7, wherein said proximal opening of said connection region has a width W_{con} between about 20 microns and about 100 microns.

9. The device of any of claims 2-8, wherein a length L_{con} of said connection region from said proximal opening to said distal opening is sufficiently short that, at 25°C, components of said second medium are able to diffuse from said isolation region through said connection region and into said microfluidic channel within about ten minutes.

10. The device of any of claims 1-9, wherein a length L_{con} of said connection region from said proximal opening to said distal opening is between about 20 micron and about 500 microns.

11. A process of analyzing a biological micro-object in a microfluidic device, said device comprising a microfluidic channel to which at least one microfluidic sequestration pen is fluidically connected, said at least one sequestration pen comprising a fluidic isolation structure comprising an isolation region, and a connection region fluidically connecting said isolation region to said channel, said process comprising:

loading one or more biological micro-objects into said at least one sequestration pen;

incubating said loaded biological micro-objects for a period of time sufficient to allow said biological micro-objects to produce an analyte of interest;

disposing capture micro-objects in said channel, adjacent to an opening from said connection region of said at least one sequestration pen to said channel, said capture micro-objects comprising at least one type of affinity agent capable of specifically binding said analyte of interest; and

monitoring binding of said capture micro-objects to said analyte of interest.

12. The process of claim 11, wherein loading comprises loading said one or more of said biological micro-objects into said isolation region(s) of said at least one sequestration pen.

13. The process of claim 11 or 12, wherein said biological micro-objects are biological cells.

14. The process of any of claims 11-13, wherein loading said biological micro-objects comprises:

flowing a group of biological micro-objects into said channel of said microfluidic device; and

moving one or more biological micro-objects of said group into each of said at least one sequestration pen.

15. The process of any of claims 11-14, further comprising flushing out any biological micro-objects remaining in said channel after loading said at least one sequestration pen.

16. The process of any of claims 11-14, wherein said flushing removes biological micro-objects from said channel without removing any of said loaded biological micro-objects from said at least one sequestration pen.

17. The process of any of claims 11-16, wherein:

loading said biological micro-objects further comprises selecting individual ones of said biological micro-objects from said group that meet a predetermined criteria; and

selecting is performed while said biological micro-objects are within said channel or said connection region or said isolation region of said at least one sequestration pen.

18. The process of any of claims 11-17, wherein disposing said capture micro-objects comprises:

flowing said capture micro-objects in said channel, and

substantially stopping said flow such that said capture micro-objects are adjacent to said

opening(s) from said connection region(s) of said at least one sequestration pen.

19. The process of any of claims 11-18, wherein said capture micro-objects comprise a label.

20. The process of any of claims 11-19, wherein monitoring binding of said capture micro-objects to said analyte of interest is accomplished by monitoring aggregation of said capture micro-objects.

21. The process of any of claims 11-20, wherein disposing capture micro-objects in said channel comprises disposing a mixture of capture micro-objects and labeling agent into said channel.

22. The process of claim 21, wherein said labeling agent comprises a fluorescent label.

23. The process of any of claims 11-22, wherein:
said analyte of interest is an antibody.

24. The process of claim 23, wherein said at least one type of affinity agent is an antigen specifically recognized by said antibody.

25. The process of claim 24, wherein said antigen is one of a protein, a carbohydrate, a lipid, a nucleic acid, a metabolite, an antibody, or a combination thereof.

26. The process of claim 23, wherein said at least one type of affinity agent is an Fc molecule, an antibody, Protein A, or Protein G.

27. The process of any of claims 11-26, wherein said analyte of interest produced by said one or more biological micro-objects diffuses from said at least one sequestration pen into said channel.

28. The process of any of claims 11-26, wherein said analyte of interest produced by said one or more biological micro-objects diffuses from said isolation region into said connection region of said at least one sequestration pen.

29. The process of any of claims 11-28, wherein:
said microfluidic device comprises a plurality of said sequestration pens, each said sequestration pen comprising a fluidic isolation structure comprising an isolation region, and a connection region fluidically connecting said isolation region to said channel; and
disposing said capture micro-objects comprises substantially filling said channel adjacent to openings from said connection regions of said plurality of sequestration pens to said channel with said capture micro-objects or a mixture of capture micro-objects and labeling agent.

30. The process of any of claims 11-29, wherein:
said connection region of said at least one sequestration pen has a length L_{con} greater than a penetration depth D_p of medium flowing at a maximum permissible flow rate V_{max} in said channel, and
said process further comprises keeping any flow of said channel at less than said maximum permissible flow rate V_{max}.

31. The process of any of claims 11-30 further comprising after monitoring binding of said capture micro-objects to said analyte of interest, flushing said capture micro-objects from said

channel.

32. The process of claim 31, wherein:

said connection region of said at least one sequestration pen has a length L_{con} greater than a penetration depth D_p of medium flowing at a maximum permissible flow rate V_{max} in said channel, and

said flushing comprises flowing a flushing medium in said channel at less than said maximum permissible flow rate V_{max}.

33. A computer program product comprising one or more non-transitory computer-readable media having computer instructions stored therein, the computer program instructions being configured such that, when executed by one or more computing devices, the computer program instructions cause the one or more computing devices to:

load one or more biological micro-objects disposed within a microfluidic channel into an isolation region of a sequestration pen of a microfluidic device via a connection region fluidically coupling the isolation region with the microfluidic channel;

allow for incubation of said loaded biological micro-objects for a period of time sufficient to allow said biological micro-objects to produce an analyte of interest;

dispose capture micro-objects in said microfluidic channel, adjacent to an opening from said connection region of said at least one sequestration pen to said microfluidic channel, said capture micro-objects comprising at least one type of affinity agent capable of specifically binding said analyte of interest; and

monitor binding of said capture micro-objects to said analyte of interest.

34. The computer program product of claim 33, wherein the computer program instructions for loading the one or more biological micro-objects into the isolation region of the sequestration pen includes computer program instructions to cause the one or more computing devices to:

adjust flow of a fluidic medium within the microfluidic channel;

capture an image of the microfluidic channel having the one or more biological micro-objects therein after adjusting the flow of the fluidic medium; and

move the one or more biological micro-objects into the isolation region of the sequestration pen based on the image captured of the flow channel.

35. The computer program product of claim 34, wherein the computer program instructions for moving the one or more biological micro-objects includes computer program instructions to cause the one or more computing devices to project a pattern of light onto an inner surface of the flow region.

36. A system comprising:

a flow controller configured to adjust flow of a first fluidic medium having a micro-object therein that is provided to a flow channel of a microfluidic device;

an imaging device configured to capture images of the microfluidic device; and

a control circuit configured to instruct the flow controller to adjust the flow of the first fluidic medium, instruct the imaging device to capture an image of the flow channel after the flow of the first fluidic medium is adjusted, and move the micro-object from the flow channel to an isolation region of a sequestration pen having an opening into the flow channel based on the image, thereby permitting mixing of components of said second medium with said first medium by diffusion or mixing of components of said first medium with said second medium by diffusion.

37. The system of claim 36, further comprising:

a light source configured to generate patterns of light, and wherein the control circuit is further configured to instruct the light source to generate a pattern of light to move the micro-object from the flow channel to the isolation region of the sequestration pen.

38. The system of claim 37, wherein the pattern of light is based on the image captured after the flow of the first fluidic medium is adjusted.

39. The system of claim 37, wherein the isolation region is a non-flow region of the microfluidic device.