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, the sequestration pen oriented so that no opening into the sequestration pen from the microfluidic channel faces directly into the flow of the first fluidic medium,
wherein said connection region comprises a proximal opening into said microfluidic channel having a width Wcon ranging from 20 microns to 100 microns and a distal opening into said isolation region,
wherein the isolation region has a single opening into the connection 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 50 microns and 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 2-7, 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 ten minutes.

9. The device of any of claims 1-8, wherein a length L_{con} of said connection region from said proximal opening to said distal opening is between 20 micron and 500 microns.