Claims:

1. A method for determining a quantity of analyte produced by a biological micro-object, comprising:

receiving imaging data of a microfluidic device that includes a flow region and a plurality of sequestration pens that are fluidically connected to the flow region, wherein the imaging data includes an analyte assay image and one or both of a background noise image and a signal reference image;

defining an area of interest for each sequestration pen, wherein the area of interest includes an image area within the sequestration pen that is most sensitive for measuring analyte concentration fluctuations, is least sensitive to the position of biological micro-objects in the sequestration pen when analyte fluctuations are measured, and extends along an axis of diffusion between the sequestration pen and the flow region, and

determining scores that are indicative of the quantity of analyte in each sequestration pen by analyzing at least a portion of the image area of the area of interest for each sequestration pen.

2. The method of claim 1, wherein the imaging data comprises light emission data determined from light emitted from a reporter molecule that binds to the analyte produced by the biological micro-objects.

3. The method of claim 1 or 2, further comprising:

normalizing at least the image area of the area of interest for each of the sequestration pens in the analyte assay image by subtracting out background noise captured in the background noise image; and/or normalizing at least the image area of the area of interest for each of the sequestration pens in the analyte assay image by accounting for image acquisition distortions captured in the signal reference image,

wherein determining scores that are indicative of the quantity of analyte in each sequestration pen further comprises analyzing at least a portion of the normalized image area of the area of interest for each sequestration pen.

4. The method of claim 3, wherein determining scores that are indicative of the quantity of analyte in each sequestration pen further comprises applying a linear regression analysis to light

emission data over at least a portion of the normalized image area of the area of interest of each sequestration pen.

5. The method of claim 3, wherein determining scores that are indicative of the quantity of analyte in each sequestration pen further comprises integrating light emission data over at least a portion of the normalized image area of the area of interest of each sequestration pen.

6. The method of any one of claims 1 to 3 and 4 to 5, wherein the analyte comprises a protein.

7. A non-transitory computer-readable medium in which a program is stored for causing a computer to perform an image processing method for determining a quantity of analyte produced by a biological micro-object, the method comprising:

receiving imaging data of a microfluidic device that includes a flow region and a plurality of sequestration pens that are fluidically connected to the flow region, wherein the imaging data includes an analyte assay image and one or both of a background noise image and a signal reference image;

defining an area of interest for each sequestration pen, wherein the area of interest includes an image area within the sequestration pen that is most sensitive for measuring analyte concentration fluctuations, is least sensitive to the position of biological micro-objects in the sequestration pen when analyte fluctuations are measured, and extends along an axis of diffusion between the sequestration pen and the flow region, and

determining scores that are indicative of the quantity of analyte in each sequestration pen by analyzing at least a portion of the image area of the area of interest for each sequestration pen.

8. The method of the non-transitory computer-readable medium of claim 7, wherein the imaging data comprises light emission data determined from light emitted from a reporter molecule that binds to from the analyte produced by the biological micro-objects.

9. The method of the non-transitory computer-readable medium of claims 7 and 8, further comprising:

normalizing at least the image area of the area of interest for each of the sequestration pens in the analyte assay image by subtracting out background noise captured in the background

noise image; and/or normalizing at least the image area of the area of interest for each of the sequestration pens in the analyte assay image by accounting for image acquisition distortions captured in the signal reference image,

wherein determining scores that are indicative of the quantity of analyte in each sequestration pen further comprises analyzing at least a portion of the normalized image area of the area of interest for each sequestration pen.

10. The method of the non-transitory computer-readable medium of claim 9, wherein determining scores that are indicative of the quantity of analyte in each sequestration pen further comprises applying a linear regression analysis to the light emission data from at least a portion of the normalized image area of the area of interest of each sequestration pen.

11. The method of the non-transitory computer-readable medium of claim 9, wherein determining scores that are indicative of the quantity of analyte in each sequestration pen further comprises integrating light emission data over at least a portion of the normalized image area of the area of interest of each sequestration pen.

12. The method of the non-transitory computer-readable medium of any one of claims 7 to 9 and 10 to 11, wherein the analyte comprises a protein.

13. A method of assessing a level of secretion of an analyte by a biological micro-object, or a population of biological micro-objects generated therefrom, the method comprising:

introducing the biological micro-object into a sequestration pen of a microfluidic device, wherein the microfluidic device comprises an enclosure having a flow region, wherein the sequestration pen is fluidically connected to the flow region, and wherein the sequestration pen contains a first fluidic medium;

allowing the biological micro-object, or the population of biological micro-objects generated therefrom, to secrete the analyte into the first fluidic medium within the sequestration pen;

introducing a second fluidic medium into the flow region for a first period of time, wherein the second fluidic medium comprises a plurality of reporter molecules, and wherein each reporter molecule comprises:

a binding component configured to bind the secreted analyte; and

a detectable label;

allowing a portion of the plurality of reporter molecules to diffuse into the sequestration pen and bind to the analyte secreted therein, thereby producing a plurality of reporter molecule: secreted analyte (RMSA) complexes; and

detecting reporter molecules located within an area of interest within the microfluidic device, wherein the area of interest includes at least a portion of the sequestration pen.

14. The method of claim 13, wherein the flow region comprises a microfluidic channel, wherein the sequestration pen has an isolation region and a connection region fluidically connecting the isolation region to the microfluidic channel, wherein the connection region comprises a proximal opening to the microfluidic channel having a width W_{con} of about 20 microns to about 100 microns and a distal opening to the isolation region, and wherein a length L_{con} of the connection region from the proximal opening to the distal opening is at least 1.0 times the width W_{con} of the proximal opening of the connection region.

15. The method of claim 14 further comprising expanding the biological micro-object within the sequestration pen into a clonal population of biological micro-objects.

16. The method of claim 15, further comprising perfusing the flow region with a culturing medium, wherein the perfusing occurs after introducing the biological micro-object into the sequestration pen and before introducing the second fluidic medium into the flow region.

17. The method of claim 16, wherein the culturing medium comprises one or more of a soluble feeder cell component, a defined dissolved oxygen component, defined pH component, an exhausted growth medium component, and/or a soluble stimulatory component.

18. The method of any one of claims 13 to 17, wherein the first period of time is about 30 to about 60 minutes.

19. The method of any one of claims 13 to 17 or 18, further comprising:

introducing a third fluidic medium into the flow region for a second period of time, wherein the third fluidic medium does not comprise reporter molecules; and

allowing at least a portion of unbound reporter molecules to diffuse out of the sequestration pen,

wherein detecting the reporter molecules located within the area of interest occurs at a time selected such that an amount of unbound reporter molecules that have diffused out of the sequestration pen is at least 2X greater than an amount of RMSA complexes that have diffused out of the sequestration pen.

20. The method of claim 19, wherein the second period of time is selected based on modelling of diffusion profiles for unbound reporter molecules and RMSA complexes.

21. The method of claim 19, wherein the second period of time is about 20 to about 50 minutes.

22. The method of any one of claims 13 to 17, 18, or 20 to 21, wherein the area of interest comprises at least a portion of the sequestration pen aligned along an axis of diffusion from within the sequestration pen to out into the flow region.

23. The method of any one of claims 13 to 17, 18, or 20 to 22, wherein detecting the reporter molecules located within the area of interest comprises measuring an intensity of a detectable signal coming from the area of interest, wherein at least some of the detectable signal emanates from the detectable label of reporter molecules located within the area of interest.

24. The method of claim 23, wherein detecting the reporter molecules located within the area of interest further comprises:

measuring an intensity of a background signal within the area of interest; and

determining a background-subtracted signal intensity by subtracting the intensity of the background signal from the measured intensity of the detectable signal.

25. The method of claim 24, wherein measuring the intensity of the background signal within the area of interest is performed at a time prior to introducing the biological micro-object into the sequestration pen.

26. The method of claim 23, wherein the measured intensity of the detectable signal is normalized for a number of cells observed within the sequestration pen.

27. The method of any one of claims 13 to 17, 18, or 20 to 26 further comprising quantifying the level of secretion of the analyte and/or providing a secretion score for the sequestration pen.

28. The method of claim 27, wherein the secretion score is determined according to the method of claim 1.

29. The method of any one of claims 13 to 17, 18, 20 to 27, or 28, wherein the secreted analyte has a molecular weight at least twice as great as a molecular weight of the reporter molecules.

30. The method of any one of claims 13 to 17, 18, 20 to 27, or 28 to 29, wherein the binding component of the reporter molecule comprises at least one amino acid and/or at least one nucleic acid.

31. The method of claim 30, wherein the binding component of the reporter molecule comprises a peptide or protein.

32. The method of claim 31, wherein the binding component of the reporter molecule comprises a peptide having the sequence of any one of SEQ ID NOs: 1 to 10.

33. The method of claim 31, wherein the binding component of the reporter molecule comprises protein A, protein G, or an IgG-binding fragment of protein A or protein G.

34. The method of any one of claims 13 to 17, 18, 20 to 27, 28 to 29 or 30 to 33, wherein the binding component of the reporter molecule comprises an aptamer.

35. The method of any one of claims 13 to 17, 18, 20 to 27, 28 to 29, or 30 to 34, wherein the detectable label is a fluorescent label.

36. The method of any one of claims 13 to 17, 18, 20 to 27, 28 to 29, 30 to 34, or 35, wherein the analyte secreted by the biological micro-object comprises a protein.

37. The method of any one of claims 13 to 17, 18, 20 to 27, 28 to 29, 30 to 34, or 35 to 36, wherein the analyte secreted by the biological micro-object is an antibody.

38. The method of any one of claims 13 to 17, 18, 20 to 27, 28 to 29, 30 to 34, or 35 to 37, wherein the analyte secreted by the biological micro-object is a protein other than an antibody.

39. The method of any one of claims 13 to 17, 18, 20 to 27, 28 to 29, 30 to 34, or 35 to 38, wherein the microfluidic device comprises a plurality of sequestration pens, wherein a biological micro-object is introduced into at least two sequestration pens of the plurality, and wherein the remainder of the method is carried out with respect to each of the at least two sequestration pens, and

comparing a level of secretion for sequestration pens of the at least two sequestration pens of the plurality.

40. The method of claim 39, further comprising:

selecting one or more of the at least two sequestration pens; and

exporting one or more biological micro-objects from each of the selected sequestration pens,

wherein the one or more biological micro-objects from each of the selected sequestration pens are further exported out of the microfluidic device.

41. The method of any one of claims 13 to 17, 18, or 20 to 27, 28 to 29, 30 to 34, 35 to 39, or 40, wherein the area of interest comprises an image area corresponding to an area within the sequestration pen that is most sensitive for measuring analyte concentration fluctuations, is least sensitive to the position of biological micro-objects in the sequestration pen when analyte fluctuations are measured, and extends along an axis of diffusion between the sequestration pen and the flow region.

42. The method of any one of claims 13 to 17, 18, or 20 to 27, 28 to 29, 30 to 34, 35 to 39, 40, or 41, wherein the method is automated.

43. A non-transitory computer-readable medium in which a program is stored for causing a computer to direct a system to perform a method for determining a quantity of analyte produced by a biological micro-object, wherein the method is the method of claim 41.

44. The method of claim 14, wherein the length L_{con} of the connection region is at least 1.5 times the width W_{con} of the proximal opening of the connection region.

45. The method of claim 14, wherein the length L_{con} of the connection region is at least 2.0 times the width W_{con} of the proximal opening of the connection region.

46. The method of claim 14, wherein the width W_{con} of the proximal opening of the connection region is about 20 microns to about 60 microns.

47. The method of claim 14, wherein the length L_{con} of the connection region is between about 20 microns and about 500 microns.

48. The method of claim 14, wherein the flow region comprises a microfluidic channel from which the sequestration pen opens, and wherein a width of the microfluidic channel at the proximal opening of the connection region is between about 50 microns and about 500 microns.

49. The method of claim 14, wherein the flow region comprises a microfluidic channel from which the sequestration pen opens, and wherein a height of the microfluidic channel at the proximal opening of the connection region is between 20 microns and 100 microns.

50. The method of claim 14, wherein the volume of the sequestration pen ranges from about 5×10^5 to about 1×10^7 cubic microns.

51. The method of any one of claims 13 to 17, 18, or 20 to 27, 28 to 29, 30 to 34, 35 to 39, 40, 41, or 44 to 50, wherein the biological micro-object is a eukaryotic cell.

52. The method of claim 51, wherein the eukaryotic cell is a mammalian cell.

53. The method of claim 51, wherein the eukaryotic cell is a fungal cell.