

What is claimed:

1. A method of detecting a B cell lymphocyte expressing an antibody that specifically binds to an antigen of interest, the method comprising:
 - introducing a sample comprising B cell lymphocytes into a microfluidic device, the microfluidic device comprising:
 - an enclosure having a flow region and a sequestration pen,
 - wherein said sequestration pen comprises an isolation region having a single opening and a connection region, said connection region providing a fluidic connection between said isolation region and said flow region, and wherein said isolation region of said holding pen is an unswept region of said micro-fluidic device;
 - loading a B cell lymphocyte from said sample into said isolation region of said sequestration pen;
 - introducing said antigen of interest into said flow region of said enclosure such that said antigen of interest is proximal to said B cell lymphocyte; and
 - monitoring binding of said antigen of interest to said antibody expressed by said B cell lymphocyte,
 - wherein said isolation region of said sequestration pen comprises at least one conditioned surface.
2. The method of claim 1, wherein said at least one conditioned surface comprises a layer of covalently linked hydrophilic molecules.
3. The method of claim 2, wherein said hydrophilic molecules comprise polyethylene glycol (PEG)-containing polymers.
4. The method of any one of claims 1 to 3, wherein said enclosure of said microfluidic device further comprises a dielectrophoresis (DEP) configuration.
5. The method of claim 4, wherein said enclosure of said microfluidic device further comprises a base, a microfluidic circuit structure, and a cover which together define a microfluidic circuit, and wherein said microfluidic circuit comprises said flow region and said sequestration pen.
6. The method of claim 5, wherein:
 - said base comprises a first electrode;
 - said cover comprises a second electrode; and

said base or said cover comprises an electrode activation substrate,
wherein said electrode activation substrate has a surface comprising a plurality of DEP electrode regions, and wherein said surface of said electrode activation substrate provides an inner surface of said flow region.

7. The method of claim 1, wherein said connection region has a width W_{con} of about 20 microns to about 60 microns.
8. The method of claim 1, wherein the connection region has a length L_{con} , and wherein a ratio of said length L_{con} of said connection region to said width W_{con} of said connection region has a value of at least 1.5.
9. The method of claim 7 or 8, wherein said isolation region has a width W_{iso} that is greater than the width W_{con} of said connection region.
10. The method of claim 9, wherein said isolation region has a width W_{iso} that is about 50 microns to about 250 microns.
11. The method of claim 1, wherein said sequestration pen comprises a volume of about 0.5 nL to about 2.5 nL.
12. The method of claim 1, wherein said isolation region of said sequestration pen comprises at least one surface coated with a coating material.
13. The method of claim 12, wherein said coating material comprises polyethylene glycol (PEG)-containing polymers.
14. The method of claim 1, wherein said sample comprising B cell lymphocytes is a sample of peripheral blood, a spleen biopsy, a bone marrow biopsy, a lymph node biopsy, or a tumor biopsy.
15. The method of claim 14, wherein said sample comprising B cell lymphocytes is a sample of peripheral blood.
16. The method of claim 14, wherein said sample comprising B cell lymphocytes is a bone marrow biopsy.
17. The method of claim 14 or 15, wherein said B cell lymphocyte is a memory B cell.

18. The method of claim 14 or 16, wherein said B cell lymphocyte is a plasma B cell.
19. The method of claim 1, wherein said sample comprising B cell lymphocytes is obtained from a mammal or avian animal.
20. The method of claim 19, wherein said sample comprising B cell lymphocytes is obtained from a human, mouse, rat, guinea pig, gerbil, hamster, rabbit, goat, sheep, llama, or chicken.
21. The method of claim 19 or 20, wherein said mammal has been immunized against said antigen of interest, wherein said mammal has been exposed to or immunized against a pathogen associated with said antigen of interest, wherein said mammal has cancer and said cancer is associated with said antigen of interest, or wherein said mammal has an auto-immune disease and said auto-immune disease is associated with said antigen of interest.
22. The method of claim 1, wherein said sample comprising B cell lymphocytes has been depleted of cell types other than B cell lymphocytes.
23. The method of claim 1 or 22, wherein said sample comprising B cell lymphocytes has been enriched for B cell lymphocytes expressing CD27.
24. The method of claim 1 or 22, wherein said sample comprising B cell lymphocytes has been enriched for B cell lymphocytes expressing CD138.
25. The method of claim 1, wherein said sample comprising B cell lymphocytes has been contacted with DNase prior to being introduced into said microfluidic device.
26. The method of claim 1, wherein a single B cell lymphocyte is loaded into said isolation region.
27. The method of claim 1, wherein a plurality of B cell lymphocytes are loaded into said isolation region.
28. The method of claim 1, further comprising:
contacting said B cell lymphocyte with a stimulating agent that stimulates B cell activation.
29. The method of claim 28, wherein said stimulating agent comprises a CD40 agonist.

30. The method of claim 29, wherein said stimulating agent comprises one or more CD40L⁺ feeder cells.
31. The method of claim 29 or 30, wherein said stimulating agent further comprises a B cell receptor (BCR)-ligating molecule, wherein said BCR-ligating molecule comprises Protein A or Protein G.
32. The method of claim 31, wherein said BCR-ligating molecule is linked to a micro-object.
33. The method of claim 28, wherein contacting said B cell lymphocytes with a stimulating agent comprises contacting said B cell lymphocytes with a mixture of CD40L⁺ feeder cells and Protein A conjugated to beads, wherein contacting said B cell lymphocytes with a stimulating agent comprises loading said mixture into said isolation region of said sequestration pen.
34. The method of claim 29, wherein said B cell lymphocyte is contacted substantially continuously with said stimulating agent for a period of 3 to 5 days.
35. The method of claim 29, further comprising:
providing culture medium to said B cell lymphocyte, wherein said culture medium comprises one or more agents that promote B cell expansion and/or activation.
36. The method of claim 35, wherein said culture medium comprises IL-2, IL-4, IL-6, IL-21, and BAFF or APRIL.
37. The method of claim 35, wherein said culture medium comprises a TLR agonist.
38. The method of claim 37, wherein said TLR agonist is CpG oligonucleotides.
39. The method of claim 35, wherein said B cell lymphocyte is provided culture medium for a period of one to 3 to 5 days.
40. The method of claim 35, wherein said contacting with a stimulating agent and said providing of culture medium are preformed over a substantially coextensive period of time.
41. The method of claim 4, wherein loading said B cell lymphocyte into said isolation region of said sequestration pen comprises using DEP force to move said B cell lymphocyte into said isolation region.

42. The method of claim 41, wherein said B cell lymphocyte is moved from said flow region to said isolation region.
43. The method of claim 1, wherein providing said antigen of interest comprises flowing a solution comprising soluble antigen of interest into or through said flow region.
44. The method of claim 43, wherein said antigen of interest is covalently bound to a first detectable label.
45. The method of claim 43 or 44 further comprising providing a micro-object comprising a first antibody-binding agent, wherein said first antibody-binding agent binds to said antibody expressed by said B cell lymphocyte without inhibiting the binding of antigen of interest to said antibody expressed by said B cell lymphocyte, and wherein monitoring of binding of said antigen of interest to said antibody expressed by said B cell lymphocyte comprises detecting indirect binding of said antigen of interest to said micro-object.
46. The method of claim 45, wherein said first antibody-binding agent binds to an Fc domain of said antibody expressed by said B cell lymphocyte.
47. The method of claim 45, wherein providing said micro-object comprises flowing a solution comprising said micro-object into said flow region and stopping said flow when said micro-object is located proximal to said sequestration pen.
48. The method of claim 45, wherein said solution comprising said micro-object and said solution comprising said soluble antigen of interest are the same solution.
49. The method of claim 45 further comprising:
providing a second antibody-binding agent, wherein said second antibody-binding agent comprises a second detectable label; and
monitoring indirect binding of said second antibody-binding agent to said micro-object, wherein said first detectable label is different from said second detectable label.
50. The method of claim 49, wherein said second antibody-binding agent binds to IgG antibodies.
51. The method of claim 1, wherein providing said antigen of interest comprises providing a micro-object that comprises said antigen of interest, wherein said micro-object is a cell, a liposome, a lipid nanoraft, or a bead.

52. The method of claim 51 further comprising:
providing a labeled antibody-binding agent prior to or concurrently with said antigen of interest,
wherein said monitoring of binding of said antigen of interest to said antibody expressed by said B cell lymphocyte comprises detecting indirect binding of said labeled antibody-binding agent to said antigen of interest.
53. The method of claim 52, wherein said labeled antibody-binding agent binds to anti-IgG antibodies.
54. The method of claim 1, wherein monitoring binding of said antigen of interest to said antibody expressed by said B cell lymphocyte comprises imaging all or part of said sequestration pen of said microfluidic device.
55. The method of claim 54, wherein said imaging comprises fluorescence imaging.
56. The method of claim 54, wherein said imaging comprises taking a plurality of images.
57. The method of claim 1, wherein said microfluidic device comprises a plurality of said sequestration pens, each having an isolation region and a connection region, each said connection region providing a fluidic connection between said isolation region and said flow region, said method further comprising:
loading one or more of said plurality of B cell lymphocytes into said isolation region of each of two or more sequestration pens of said plurality;
introducing said antigen of interest into said microfluidic device such that said antigen of interest is proximal to each of said two or more sequestration pens loaded with one or more B cell lymphocytes; and
monitoring of binding of said antigen of interest to said antibody expressed by each of said loaded B cell lymphocytes.
58. The method of claim 57, wherein a single B cell lymphocyte is loaded into said isolation region of each of said two or more sequestration pens of said plurality.
59. The method of claim 57, further comprising:
detecting binding of said antigen of interest to said antibody expressed by said loaded B cell lymphocyte, or ones of said loaded B cell lymphocytes;

identifying said loaded B cell lymphocyte, or said ones of said loaded B cell lymphocytes, as expressing an antibody that specifically binds to said antigen of interest.

60. A method of characterizing an antibody that specifically binds to an antigen of interest, the method comprising:

identifying a B cell lymphocyte, or a clonal population thereof, that expresses an antibody that specifically binds to said antigen of interest, wherein said identifying is performed according to the method of claim 59;

isolating from said B cell lymphocyte, or said clonal population thereof, a nucleic acid encoding an immunoglobulin heavy chain variable region (V_H) and/or an immunoglobulin light chain variable region (V_L); and

sequencing at least a portion of said nucleic acid encoding said immunoglobulin heavy chain variable region (V_H) and/or at least a portion of said nucleic acid encoding said immunoglobulin light chain variable region (V_L).

61. The method of claim 60, wherein sequencing said immunoglobulin heavy chain variable region (V_H) comprises:

lysing said identified B cell lymphocyte, or B cell lymphocyte(s) of said clonal population thereof;

reverse transcribing mRNA isolated from said B cell lymphocyte, or said B cell lymphocyte(s) of said clonal population thereof, wherein said mRNA encodes said immunoglobulin heavy chain variable region (V_H), thereby forming V_H cDNA; and

sequencing at least a portion of said V_H cDNA.

62. The method of claim 60, wherein sequencing said immunoglobulin light chain variable region (V_L) comprises:

lysing said identified B cell lymphocyte, or B cell lymphocyte(s) of said clonal population thereof;

reverse transcribing mRNA isolated from said B cell lymphocyte, or said clonal population thereof, wherein said mRNA encodes said immunoglobulin light chain variable region (V_L), thereby forming V_L cDNA; and

sequencing at least a portion of said V_L cDNA.

63. The method of claim 61 or 62, wherein reverse transcribing said mRNA comprises contacting said mRNA with a capture/priming oligonucleotide.

64. The method of claim 63, wherein said reverse transcribing is performed in the presence of a transcript switching oligonucleotide.

65. The method of claim 61 or 62, wherein said identified B cell lymphocyte, or said B cell lymphocyte(s) of said clonal population thereof, is(are) exported from said microfluidic device prior to being lysed.

66. The method of claim 65, wherein exporting said identified B cell lymphocyte, or said clonal population thereof, comprises:

moving said identified B cell lymphocyte, or said B cell lymphocyte(s) of said clonal population thereof, from said isolation region of said sequestration pen into said flow region of said microfluidic device; and

flowing said identified B cell lymphocyte, or said B cell lymphocyte(s) of said clonal population thereof, through said flow region and out of said microfluidic device.

67. The method of claim 66, wherein moving said identified B cell lymphocyte, or said B cell lymphocyte(s) of said clonal population thereof, from said isolation region of said sequestration pen comprises capturing and moving said identified B cell lymphocyte, or said clonal population thereof, using DEP force.

68. The method of claim 61 or 62, wherein said identified B cell lymphocyte, or said B cell lymphocyte(s) of said clonal population thereof, is(are) lysed within said microfluidic device.

69. The method of claim 68, further comprising:

providing one or more capture beads in close proximity to said identified B cell lymphocyte, or said B cell lymphocyte(s) of said clonal population thereof, where said one or more capture beads each comprises oligonucleotides capable of binding said V_H mRNA and/or said V_L mRNA;

lysing said identified B cell lymphocyte, or said clonal population thereof; and

allowing said V_H mRNA and/or said V_L mRNA from said lysed B cell lymphocyte, or from said lysed B cell lymphocyte(s) of said clonal population thereof, to be bound by said one or more capture beads.

70. The method of claim 69, wherein each capture bead of said one or more capture beads comprises a plurality of capture/priming oligonucleotides.

71. The method of claim 69, wherein said bound V_H mRNA and/or said bound V_L mRNA is reverse transcribed into V_H cDNA and/or V_L cDNA while bound to said one or more capture beads.

72. The method of claim 71, wherein said V_H cDNA and/or V_L cDNA is exported from said microfluidic device while bound to said one or more capture beads.

73. The method of claim 61 or 62, further comprising amplifying said V_H cDNA and/or said V_L cDNA prior to said sequencing.

74. The method of claim 73, wherein said amplifying comprises increasing the representation of V_H cDNA and/or V_L cDNA, or fragments thereof, in the reverse transcribed mRNA isolated from said B cell lymphocyte.

75. The method of claim 74, wherein said amplifying comprises:

a first round of amplification which increases the representation of V_H cDNA and/or V_L cDNA, or fragments thereof, in the reverse transcribed mRNA isolated from said B cell lymphocyte; and

a second round of amplification which introduces barcode sequences into the V_H cDNA and/or V_L cDNA, or fragments thereof, amplified in the first round.