CLAIMS

1. A microfluidic device for multiple single-cell capturing and processing, the microfluidic device comprising:

a plurality of capture configurations coupled in series, wherein each respective capture configuration is configured to capture a single cell; and

a plurality of multi-chamber reaction configurations, wherein each respective multi-chamber reaction configuration is coupled with a respective capture configuration from the plurality of capture configurations and is configured for single-cell processing.

2. The microfluidic device of claim 1, wherein each respective capture configuration comprises one or more a physical barriers sized to hold only a single cell.

3. The microfluidic device of claim 1, wherein each respective capture configuration comprises:

one or more bypass channels coupled with an input channel and an output channel;

a drain coupled with the input channel and the output channel; and

a capture nest situated proximal to a junction of the input channel and the one or more bypass channels and coupled with the drain, wherein the capture nest is configured to capture a single cell from a plurality of cells such that a remaining plurality of cells is diverted into at least one of the one or more bypass channels when the single cell is captured in the capture nest.

4. The microfluidic device of claim 3, wherein the one or more bypass channels comprise a first bypass channel and a second bypass channel.

5. The microfluidic device of claim 4, wherein the first bypass channel and the second bypass channel are symmetrically configured.

6. The microfluidic device of claim 4, wherein the symmetrically configured first bypass channel and second bypass channel comprise a first wing configuration and a second wing configuration.

7. The microfluidic device of claim 3, wherein at least the input channel or the output channel is further configured as a focusing channel.

8. The microfluidic device of claim 7, wherein the focusing channel comprises a narrowing channel in at least a horizontal direction or a vertical direction.

9. The microfluidic device of claim 3, wherein the plurality of multi-chamber reaction configurations are further configured for thermal cycling while one or more valves of a respective multi-chamber reaction configuration is actuated.

10. The microfluidic device of claim 3, further comprising:

one or more imaging features, wherein each respective imaging feature allows for imaging of captured single cells at a respective capture nest site.

11. The microfluidic device of claim 3, further comprising:

a plurality of harvest wells, wherein each respective harvest well is coupled with a respective multi-chamber reaction configuration and configured to deliver reaction products for further analysis.

12. The microfluidic device of claim 3, further comprising:

a genomic analysis configuration coupled with each respective multi-chamber reaction configuration to further analyze the reaction products from each respective multi-chamber reaction configuration.

13. The microfluidic device of claim 1, wherein each respective capture configuration comprises a capture chamber configured to capture a single cell from a limiting dilution.

14. The microfluidic device of claim 1, wherein each capture chamber configured to capture a single cell utilizing a stochastic capture process.

15. The microfluidic device of claim 1, wherein each respective capture configuration comprises:

a capture compartment; and

a binding partner covering a discrete region of the capture compartment, where the discrete portion is sized so that only a single cell binds to the discrete region.

16. The microfluidic device of claim 1, further comprising:

one or more capture supports, wherein each capture support comprises a binding partner distributed over at least a portion of the capture support.

17. The microfluidic device of claim 16, wherein the one or more capture supports comprise one or more bead structures.

18. The microfluidic device of claim 17, further comprising:

a capture feature configured to capture the one or more capture support.

19. A microfluidic device configured for multiple single-cell capturing and processing, the microfluidic device comprising:

a plurality of capture configurations coupled in series, each respective capture configuration comprising:

a plurality of bypass channels coupled with an input channel and an output channel;

a drain coupled with the input channel and the output channel; and

a capture nest situated proximal to a junction of the input channel and the plurality of bypass channels and coupled with the drain, wherein the capture nest is configured to capture an individual cell from a plurality of cells such that a remaining plurality of cells is diverted into at least one of the plurality of bypass channels when the individual cell is captured in the capture nest; and

a plurality of multi-chamber reaction configurations, wherein each respective multi-chamber reaction configuration is coupled with a respective capture configuration from the plurality of capture configurations and configured for single-cell processing.

20. A method for multiple single-cell capturing and processing using microfluidics, the method comprising:

loading a plurality of cells into a microfluidic device;

flowing the plurality of cells to a first capture configuration of the microfluidic device;

capturing a first single cell from the plurality of cells in the first capture configuration;flowing a first remaining plurality of cells from the plurality of cells to a second capture configuration of the microfluidic device;capturing a second single cell from the first remaining plurality of cells in the second capture configuration; and

performing multistage processing of at least the first captured single cell and the second captured single cell to produce respective harvest products with respect to at least the first captured single cell and the second captured single cell on the microfluidic device.

21. The method of claim 20, wherein capturing at least the first single cell or the second single cell comprises:

capturing at least the first single cell or the second single cell utilizing one or more a physical barriers sized to hold only a single cell.

22. The method of claim 20, further comprising:

flowing the first remaining plurality of cells from the plurality of cells through one or more bypass channels of the first capture configuration to a flow channel coupled with a second capture configuration coupled with the first output channel of the first capture configuration.

23. The method of claim 20, further comprising:

flowing a second remaining plurality of cells from the first remaining plurality of cells through one or more second bypass channels to an outlet of the second capture configuration to a third capture configuration through the second output channel.

24. The method of claim 20, wherein the first capture configuration comprises:

one or more bypass channels coupled with a first input channel and a first output channel;

a first drain coupled with the first input channel and the first output channel; and

a first capture nest coupled with the first drain and configured to capture an individual cell from the plurality of cells.

25. The method of claim 24, wherein the second capture configuration includes:

a plurality of bypass channels coupled with a second input channel and a second output channel, wherein the second input channel is coupled with the first output channel of the first capture configuration;

a second drain coupled with the second input channel and the second output channel; and

a second capture nest coupled with a second drain configured to capture an individual cell from the first remaining plurality of cells.

26. The method of claim 20, wherein performing the multistage processing of at least the first captured single cell and the second captured single cell to produce respective harvest products with respect to at least the first captured single cell and the second captured single cell on the microfluidic device comprises:

lysing, on the microfluidic device, each respective individually captured cell to release the one or more constituents of each respective cell.

27. The method of claim 26, wherein performing the multistage processing of at least the first captured single cell and the second captured single cell to produce respective harvest products with respect to at least the first captured single cell and the second captured single cell on the microfluidic device comprises:

flowing the one or more constituents of each respective captured cell into a respective multi-chamber reaction configuration of the microfluidic device for further processing.

28. The method of claim 27, wherein performing the multistage processing of at least the first captured single cell and the second captured single cell to produce respective harvest products with respect to at least the first captured single cell and the second captured single cell on the microfluidic device comprises:

performing a thermal cycling procedure while flowing the one or more constituents through one or more aspects of a respective multi-chamber reaction configuration of the microfluidic device.

29. The method of claim 20, wherein performing the multistage processing of at least the first captured single cell and the second captured single cell to produce respective harvest products with respect to at least the first captured single cell and the second captured single cell on the microfluidic device comprises:

washing, in the microfluidic device, each respective captured cell with one or more reagents.

30. The method of claim 20, wherein performing the multistage processing of at least the first captured single cell and the second captured single cell to produce respective harvest products with respect to at least the first captured single cell and the second captured single cell on the microfluidic device comprises:

dosing, in the microfluidic device, each respective captured cell with one or more reagents.

31. The method of claim 20, further comprising:

imaging the respective captured cells within the microfluidic device.

32. The method of claim 27, wherein performing the multistage processing of at least the first captured single cell and the second captured single cell to produce respective harvest products with respect to at least the first captured single cell and the second captured single cell on the microfluidic device comprises:

performing a preamplification process within the microfluidic device.

33. The method of claim 27, wherein performing the multistage processing of at least the first captured single cell and the second captured single cell to produce respective harvest products with respect to at least the first captured single cell and the second captured single cell on the microfluidic device comprises:

performing a mRNA sequence process within the microfluidic device.

34. The method of claim 27, wherein performing the multistage processing of at least the first captured single cell and the second captured single cell to produce respective harvest products with respect to at least the first captured single cell and the second captured single cell on the microfluidic device comprises:

performing at least a specific target amplification, a whole genome amplification, a whole transcriptome amplification, a real-time PCR preparation, a copy number variation, or a haplotyping within the microfluidic device.

35. The method of claim 20, wherein performing the multistage processing of at least the first captured single cell and the second captured single cell to produce respective harvest products with respect to at least the first captured single cell and the second captured single cell on the microfluidic device comprises:

marking reaction products from the further processing associated with respective captured cells for identification purposes.

36. The method of claim 20, further comprising:

harvesting the harvest products from a plurality of harvest wells of the microfluidic device.

37. The method of claim 36, further comprising:

processing the harvest products.

38. The method of claim 20, wherein capturing at least the first single cell or the second single cell comprises:

capturing at least the first single cell or the second single cell utilizing a capture chamber configured to capture a single cell from a limiting dilution.

39. The method of claim 20, wherein capturing at least the first single cell or the second single cell comprises:

capturing at least the first single cell or the second single cell utilizing a stochastic capture process.

40. The method of claim 20, wherein capturing at least the first single cell or the second single cell comprises:

capturing at least the first single cell or the second single cell utilizing:

a capture compartment; and

a binding partner covering a discrete region of the capture compartment, where the discrete portion is sized so that only a single cell binds to the discrete region.

41. The method of claim 20, wherein capturing at least the first single cell or the second single cell comprises:

capturing at least the first single cell or the second single cell utilizing one or more capture supports , wherein each capture support comprises a binding partner distributed over at least a portion of the capture support.

42. The method of claim 41, wherein the one or more capture supports comprise one or more bead structures.

43. The method of claim 41, further comprising:

a capture feature configured to capture the capture support.

44. A method for multiple single-cell capturing and processing using microfluidics, the method comprising:

loading a plurality of particles or cells into a microfluidic device;

flowing the plurality of cells into at least a first capture configuration of the microfluidic device, wherein the first capture configuration comprises:

a first plurality of bypass channels coupled with a first input channel and a first output channel;

a first drain coupled with the first input channel and the first output channel; and

a first capture nest coupled with the first drain and configured to capture an individual cell from the plurality of cells;

capturing a first individual particle or cell from the plurality of cells at the first capture nest;

flowing a first remaining plurality of cells from the plurality of cells through at least one of the first plurality of bypass channels to at least a second capture configuration coupled with the first output channel of the first capture configuration;

flowing the first remaining plurality of cells into at least the second capture configuration of the microfluidic device, wherein the second capture configuration includes:

a second plurality of bypass channels coupled with a second input channel and a second output channel, wherein the second input channel is coupled with the first output channel of the first capture configuration;

a second drain coupled with the second input channel and the second output channel; and

a second capture nest coupled with a second drain configured to capture an individual cell from the first remaining plurality of cells; and

capturing a second particle or cell from the first remaining plurality of cells at the second capture nest; and

flowing a second remaining plurality of cells from the first remaining plurality of cells through at least one of the plurality of second bypass channels to a third capture configuration through the second output channel.

45. A method of preamplification utilizing a microfluidic device configured to capture and to process individual cells from a plurality of cells, the method comprising:

priming the microfluidic device utilizing one or more solutions;

flowing the plurality of cells through the microfluidic device such that individual cells from the plurality of cells are capture at individual capture sites of the microfluidic device;

lysing the plurality of captured individual cells at the individual capture sites of the microfluidic device;

performing reverse transcription, within the microfluidic device, on the plurality of individual lysed cells to produce reverse transcription products associated with each respective individual cell; and

performing preamplification, within the microfluidic device, on the respective reverse transcription products associated with each respectively lysed individual cell to produce preamplification products associated with each individual capture cell.

46. The method of claim 45, further comprising:

delivering the preamplification products associated with each individual capture cell to a respective harvest inlet from a plurality of harvest inlets of the microfluidic device.

47. The method of claim 45, further comprising:

loading the one or more solutions into the microfluidic device.

48. The method of claim 45, wherein the one or more solutions includes at least one or more reagents or one or more buffers.

49. The method of claim 45, further comprising:

loading the plurality of cells into the microfluidic device.

50. The method of claim 45, further comprising:

imaging one or more of the captured individual cells on the microfluidic device.

51. The method of claim 45, further comprising:

loading at least one or more lysis reagents, one or more reverse transcription reagents, or one or more preamplification reagents into the microfluidic device.

52. The method of claim 45, further comprising:

removing one or more protective layers of one or more harvesting inlets; and

harvesting the preamplification products from each respective harvest inlet from the plurality of harvest inlets of the microfluidic device.

53. The method of claim 45, further comprising:

staining the one or more individual capture cells on the microfluidic device.

54. The method of claim 53, further comprising:

determining whether the one or more individual captured cells are alive or dead based on the staining.

55. The method of claim 50, further comprising:

determining whether the one or more individual captured cells are alive or dead based on the imaging.

56. The method of claim 45, wherein the microfluidic device comprises:

a plurality of capture configurations coupled in series, each respective capture configuration comprising:

a plurality of bypass channels coupled with an input channel and an output channel;

a drain coupled with the input channel and the output channel; and

a capture nest situated proximal to a junction of the input channel and the plurality of bypass channels and coupled with the drain, wherein the capture nest is configured to capture an individual cell from the plurality of cells such that a remaining plurality of cells is diverted into at least one of the plurality of bypass channels when the individual cell is captured in the capture nest, wherein the capture nest comprises one of the individual capture sites; and

a plurality of multi-chamber reaction configurations, wherein each respective multi-chamber reaction configuration is coupled with a respective capture configuration from the plurality of capture configurations and configured for single-cell processing.

57. A method of preamplification utilizing a microfluidic device configured to capture and to process individual cells from a plurality of cells, the method comprising:

loading one or more solutions into the microfluidic device;

priming the microfluidic device utilizing the one or more solutions;

loading the plurality of cells into the microfluidic device;

flowing the plurality of cells through the microfluidic device such that individual cells from the plurality of cells are capture at individual capture sites of the microfluidic device;

imaging one or more of the captured individual cells on the microfluidic device;

loading at least one or more lysis reagents, one or more reverse transcription reagents, or one or more preamplification reagents into the microfluidic device;

lysing the plurality of captured individual cells at the individual capture sites of the microfluidic device;

performing reverse transcription, within the microfluidic device, on the plurality of individual lysed cells to produce reverse transcription products associated with each respective individual cell;

performing preamplification, within the microfluidic device, on the respective reverse transcription products associated with each respectively lysed individual cell to produce preamplification products associated with each individual capture cell;

delivering the preamplification products associated with each individual capture cell to a respective harvest inlet from a plurality of harvest inlets of the microfluidic device;

removing one or more protective layers of one or more harvesting inlets; and

harvesting the preamplification products from each respective harvest inlet from the plurality of harvest inlets of the microfluidic device.

58. A method of mRNA sequencing utilizing a microfluidic device configured to capture and to process individual cells from a plurality of cells, the method comprising:

priming the microfluidic device utilizing one or more solutions;

flowing the plurality of cells through the microfluidic device such that individual cells from the plurality of cells are capture at individual capture sites of the microfluidic device;

lysing the plurality of captured individual cells at the individual capture sites of the microfluidic device;

performing reverse transcription, within the microfluidic device, on the plurality of individual lysed cells to produce reverse transcription products associated with each respective individual cell; and

performing PCR, within the microfluidic device, on the respective reverse transcription products associated with each respectively lysed individual cell to produce PCR products associated with each individual capture cell.

59. The method of claim 58, further comprising:

delivering the PCR products associated with each individual capture cell to a respective harvest inlet from a plurality of harvest inlets of the microfluidic device.

60. The method of claim 58, further comprising:

loading the one or more solutions into the microfluidic device.

61. The method of claim 58, wherein the one or more solutions includes at least one or more reagents or one or more buffers.

62. The method of claim 58, further comprising:

loading the plurality of cells into the microfluidic device;

63. The method of claim 58, further comprising:

imaging one or more of the captured individual cells on the microfluidic device.

64. The method of claim 58, further comprising:

loading at least one or more lysis reagents, one or more reverse transcription reagents, or one or more PCR reagents into the microfluidic device.

65. The method of claim 58, further comprising:

removing one or more protective layers of one or more harvesting inlets; and

harvesting the PCR products from each respective harvest inlet from the plurality of harvest inlets of the microfluidic device.

66. The method of claim 58, further comprising:

staining the one or more individual capture cells on the microfluidic device.

67. The method of claim 66, further comprising:

determining whether the one or more individual captured cells are alive or dead based on the staining.

68. The method of claim 63, further comprising:

determining whether the one or more individual captured cells are alive or dead based on the imaging.

69. The method of claim 58, wherein the PCR products include amplified cDNA.

70. The method of claim 58, further comprising:

preparing one or more libraries utilizing the PCR products associated with each individual captured cell.

71. The method of claim 70, wherein preparing the one or more libraries comprises:

determining a cDNA concentration from each respective harvest products associated with each individual cell; and

diluting each respective cDNA concentration to within a pre-determined concentration range.

72. The method of claim 71, further comprising:

preparing the dilated cDNA concentration for tagmentation to produce tagmentation products.

73. The method of claim 72, further comprising:

performing PCR amplification on the tagmentation products to produce PCR products.

74. The method of claim 73, further comprising:

generating one or more library pools from the PCR products.

75. The method of claim 58, wherein the microfluidic device comprises:

a plurality of capture configurations coupled in series, each respective capture configuration comprising:

a plurality of bypass channels coupled with an input channel and an output channel;

a drain coupled with the input channel and the output channel; and

a capture nest situated proximal to a junction of the input channel and the plurality of bypass channels and coupled with the drain, wherein the capture nest is configured to capture an individual cell from the plurality of cells such that a remaining plurality of cells is diverted into at least one of the plurality of bypass channels when the individual cell is captured in the capture nest, wherein the capture nest comprises one of individual capture sites; and

a plurality of multi-chamber reaction configurations, wherein each respective multi-chamber reaction configuration is coupled with a respective capture configuration from the plurality of capture configurations and configured for single-cell processing.

76. A method of mRNA sequencing utilizing a microfluidic device configured to capture and to process individual cells from a plurality of cells, the method comprising:

loading one or more solutions into the microfluidic device;

priming the microfluidic device utilizing the one or more solutions;

loading the plurality of cells into the microfluidic device;

flowing the plurality of cells through the microfluidic device such that individual cells from the plurality of cells are capture at individual capture sites of the microfluidic device;

imaging one or more of the captured individual cells on the microfluidic device;

loading at least one or more lysis reagents, one or more reverse transcription reagents, or one or more PCR reagents into the microfluidic device;

lysing the plurality of captured individual cells at the individual capture sites of the microfluidic device;

performing reverse transcription, within the microfluidic device, on the plurality of individual lysed cells to produce reverse transcription products associated with each respective individual cell;

performing PCR, within the microfluidic device, on the respective reverse transcription products associated with each respectively lysed individual cell to produce PCR products associated with each individual capture cell;

delivering the PCR products associated with each individual capture cell to a respective harvest inlet from a plurality of harvest inlets of the microfluidic device;

removing one or more protective layers of one or more harvesting inlets; and

harvesting the PCR products from each respective harvest inlet from the plurality of harvest inlets of the microfluidic device.

77. A microfluidic controller configured for multiple single-cell processing using a microfluidic device, the microfluidic controller comprising:

a housing;

a microfluidic device input and output module configured to load and unload the microfluidic device into the housing;

a pressure module configured to couple with the microfluidic device to provide controller pressure to the microfluidic device;

a sealing module configured to provide one or more pressure seals to the microfluidic device; and

a thermal cycling module configured to thermal cycle the microfluidic device.

78. The microfluidic controller of claim 77, further comprising:

an imaging module configured to image one or more aspects of the microfluidic device.

79. The microfluidic controller of claim 78, wherein the imaging module comprises at least a microscope or a camera configured to image one or more captured cells in the microfluidic device.

80. The microfluidic controller of claim 78, wherein the imaging module comprises at least a microscope or a camera configured to image one or more reaction products in the microfluidic device.

81. The microfluidic controller of claim 77, wherein the thermal cycling module is configured to thermal cycle the microfluidic device while the pressure module activates one or more valves within the microfluidic device.

82. The microfluidic controller of claim 77, further comprising:

an input module configured to receive input from a user of the microfluidic controller.

83. The microfluidic controller of claim 77, further comprising:

a display module configured to at least provide information to a user of the microfluidic controller or receive input from the user of the microfluidic controller.

84. The microfluidic controller of claim 77, wherein the pressure module configured to couple with the microfluidic device to provide controller pressure to the microfluidic device is configured to control pressure in the microfluidic device to flow a plurality of cells through the microfluidic device and to capture individual cells at individual capture configurations within the microfluidic device.

85. The microfluidic controller of claim 77, wherein the pressure module configured to couple with the microfluidic device to provide controller pressure to the microfluidic device is configured to control pressure in the microfluidic device to perform multistage processing of multiple single cells captured within the microfluidic device.

86. The microfluidic controller of claim 77, wherein the thermal cycling module configured to thermal cycle the microfluidic device is configured to perform the multistage processing of multiple single cells captured within the microfluidic device.

87. The microfluidic controller of claim 86, wherein the multistage processing comprises preamplification processing.

88. The microfluidic controller of claim 86, wherein the multistage processing comprises mRNA sequence processing.

89. The microfluidic controller of claim 77, wherein the pressure module configured to couple with the microfluidic device to provide controller pressure to the microfluidic device is configured to control pressure in the microfluidic device to prime the microfluidic device.

90. The microfluidic controller of claim 89, wherein the pressure module configured to couple with the microfluidic device to provide controller pressure to the microfluidic device is configured to control pressure in the microfluidic device to load a plurality of cells into the microfluidic device and to capture multiple individual cells from the plurality of cells in the microfluidic device.

91. The microfluidic controller of claim 90, wherein at least the pressure module or the thermal cycling module is configured to facilitate to perform at least lysis, reverse transcription, PCR, or harvesting on the microfluidic device.

92. The microfluidic controller of claim 90, wherein at least the pressure module or the thermal cycling module is configured to facilitate to perform at least lysis, reverse transcription, preamplification, or harvesting on the microfluidic device.

93. A microfluidic system configured for multiple single-cell processing, the microfluidic system comprising:

a microfluidic device comprising:

a plurality of capture configurations coupled in series, each respective capture configuration comprising:

a plurality of bypass channels coupled with an input channel and an output channel;

a drain coupled with the input channel and the output channel; and

a capture nest situated proximal to a junction of the input channel and the plurality of bypass channels and coupled with the drain, wherein the capture nest is configured to capture an individual cell from a plurality of cells such that a remaining plurality of cells is diverted into at least one of the plurality of bypass channels when the individual cell is captured in the capture nest; and

a plurality of multi-chamber reaction configurations, wherein each respective multi-chamber reaction configuration is coupled with a respective capture configuration from the plurality of capture configurations and configured for single-cell processing; and

a microfluidic controller coupled with the microfluidic device, the microfluidic controller comprising:

a housing;

a microfluidic device input and output module configured to load and unload the microfluidic device into the housing;

a pressure module configured to couple with the microfluidic device to provide controller pressure to the microfluidic device;

a sealing module configured to provide one or more pressure seals to the microfluidic device; and

a thermal cycling module configured to thermal cycle the microfluidic device.

CLAIMS

1. An invention not claimed in any divisional application.