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 from a plurality of cells such that a remaining plurality of cells are passed through one or more bypass channels to the next capture configuration in the series; 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,

wherein each multi-chamber reaction configuration comprises at least two reaction chambers coupled to each other.

2. The device of claim 1 wherein the multi-chamber reaction configurations each comprise

a first reaction chamber coupled to the capture configuration,

a second reaction chamber coupled to the first reaction chamber through two non-overlapping fluid flow paths,

valves positioned to fluidically isolate the first and second reaction chambers from each other, and

a valve positioned to fluidically isolate the capture configuration from the multi-chamber reaction configuration.

3. The device of claim 1 or claim 2 wherein each capture configuration comprises

a capture nest coupled to a drain channel and to the one or more bypass channels, wherein said capture nest is configured to capture a single cell,

a single input channel,

a single output channel,

wherein solution can flow into or out of the capture nest and bypass channel portions of the capture configuration only through the single input channel and the single output channel.

4. The device of any of claims 1- 3 wherein said one or more bypass channels are a symmetrically configured pair of bypass channels.

5. The device of any of claims 1 to 4 wherein each capture configuration comprises

a single input channel,

a single output channel,

a capture nest configured to capture a single cell,

a drain channel, and

a symmetrically configured pair of bypass channels,

wherein in each capture configuration

the capture nest is coupled with the drain channel,

the capture nest is situated proximal to a junction of the input channel and the pair of bypass channels,

the drain channel is coupled with the output channel,

the bypass channels are coupled with the input channel and the output channel,

the capture nest is configured to capture a single cell from a plurality of cells flowing through the input channel, and

the capture configuration is configured so that when the capture nest is not occupied by a cell, fluid may flow through the single input channel, through both the drain channel and the bypass channels, and from both the drain channel and bypass channels into the output channel, and

when the capture nest is occupied by a captured cell, flow through the drain channel is blocked and such that cells that are not captured are diverted into one of the pair of bypass channels and flow to the single output channel,

and wherein solution can flow into or out of the capture nest, drain and bypass channel portions of the capture configuration only through the single input channel and the single output channel.

6. The microfluidic device of claim 4 or claim 5, wherein the symmetrically configured pair of bypass channels first bypass channel and second bypass channel comprise a first wing configuration and a second wing configuration.

7. The microfluidic device of claim 5 or claim 6, wherein at least the input channel or the output channel is further configured as a focusing channel.

8. The microfluidic device of any one of the preceding claims, 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.

9. The microfluidic device of claim 2, wherein the second reaction chamber is adjacent to the first reaction chamber.

10. The microfluidic device of claim 2 or claim 9, wherein the multi-chamber reaction configurations each comprise a third reaction chamber that is coupled to the second reaction chamber through two nonoverlapping fluid flow paths, and valves positioned to fluidically isolate the second and third reaction chambers from each other.

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

a) 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 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

b) 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;

wherein the multi-chamber reaction configurations each comprise

a first reaction chamber coupled to the capture configuration,

a second reaction chamber coupled to the first reaction chamber and coupled to the first reaction chamber through two non-overlapping fluid flow paths,

valves positioned to fluidically isolate the first and second reaction chambers from each other,

a valve positioned to fluidically isolate the capture configuration from the multi-chamber reaction configuration; and

c) 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;

and

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

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

loading a plurality of cells into the microfluidic device of any of claims 1 to 10, and

performing multi-stage processing of at least a single cell.

13. The method of claim 12, further comprising:

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.

14. The method of claim 13 wherein:

a) capturing a first single cell from the plurality of cells in the first capture configuration uses one or more physical barriers sized to hold only a single cell, and 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;

when the capture nest is occupied by a captured cell, flow through the drain channel is blocked and such that cells that are not captured are diverted into one of the pair of bypass channels and flow to the single output channel; and

b) 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; and

c) capturing a second single cell from the first remaining plurality of cells in the second capture configuration uses one or more physical barriers sized to hold only a single cell; and

d) 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 one or more constituents of each respective cell; and

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.

15. The method of claim 13 or claim 14, 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.

16. The method of any one of claims 12 to 15, further comprising: imaging the respective captured cells within the microfluidic device.