CLAIMS

What is claimed is:

1. A method for detecting a set of targets comprising biomarkers of a sample, the method comprising:

capturing a set of cells of the sample in proximity to a set of functionalized particles, in single cell format, at a first set of microwells of a sample processing substrate;

performing lysis of the set of cells, at the sample processing substrate, thereby allowing binding of released biomarkers from the set of cells to the set of functionalized particles;

performing a reverse transcription operation, at the first set of microwells of the sample processing substrate, with said biomarkers ~~content~~ bound to the set of functionalized particles;

with products of the reverse transcription operation, performing a second strand synthesis operation with ~~content bound to~~ the set of functionalized particles;

with products of the second strand synthesis operation, performing an amplification operation at the set of functionalized particles;

hybridizing each of a set of probes, corresponding to a set of fluorophores, to products of the amplification operation; and

generating an image dataset of light emitted by the set of probes, thereby enabling detection of the set of targets of the sample.

2. The method of Claim 1, wherein the set of targets comprises a panel of antibodies for assessment of immune responses from a subject, the panel of antibodies comprising one or more of: IgM antibodies, IgG antibodies, IgD antibodies, IgA antibodies, and IgE antibodies.

3. The method of Claim 2, wherein the panel of antibodies comprises antibodies for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).

4. The method of Claim 1, wherein the set of targets comprises a panel of mRNA

molecules.

5. The method of Claim 4, further comprising performing a spatial transcriptomics analysis of the panel of mRNA molecules, with the sample distributed across the sample processing substrate.

6. The method of Claim 1, wherein capturing the set of cells of the sample in proximity to the set of functionalized particles comprises delivering the set of cells to thea first set of microwells of the sample processing substrate, and positioning the set of functionalized particles within a second set of microwells of a second substrate aligned with and facing the first set of microwells.

7. The method of Claim 6, further comprising compressing the second substrate, with the second set of microwells, against the first set of microwells, thereby partitioning individual wells of the sample processing substrate and allowing content said biomarkers derived from the set of cells to diffuse toward the set of functionalized particles at the second substrate.

8. The method of Claim 7, wherein compressing the second substrate, with the second set of microwells, against the first set of microwells, comprises actuating a heating object against the second substrate, thereby providing compression and heating contents of at least one of the sample processing substrate and the second substrate.

9. The method of Claim 1, wherein hybridizing each of the set of probes comprises hybridizing a first subset of the set of probes, corresponding to a first fluorophore, and performing a first detection operation; de-hybridizing and washing the first subset of probes; and hybridizing a second subset of the set of probes, corresponding to a second fluorophore, and performing a second detection operation.

10. The method of Claim 1, wherein the set of probes (P) is configured for multiplexed detection of the set of targets, where the set of fluorophores (F) has a set of

melting points (M), thereby enabling multiplexing of M^*F^P targets at the sample processing substrate.

11. The method of Claim 1, further comprising performing an mRNA quantitation operation with estimation of initial concentrations of mRNAs captured at the set of functionalized particles, based upon a set of fluorescence values from the image data set and a number of amplification cycles performed.

~~12. A system for automatically processing a sample, the system comprising:~~

~~a deck configured to support and position:~~

~~a reagent cartridge at a first region of the first side of the deck; and~~

~~a sample processing substrate at a second region of the first side; and~~

~~a gantry coupled to the deck and comprising:~~

~~a set of tracks defining paths of movement along a set of axes, within a three dimensional volume bound by the first side of the deck, for a pipette interface; and~~

~~a base opposite the first side of the deck and supporting:~~

~~a heating and cooling subsystem comprising a set of thermal bodies;~~

~~a pumping subsystem comprising a vacuum port into the second region; and;~~

~~and imaging subsystem comprising:~~

~~an illumination subsystem comprising a set of a set of emitters configured for light transmission through a set of filters and a set of optical elements, for uniform illumination of the sample processing substrate at the deck, and~~

~~a detection subsystem comprising a set of lenses for shaping light from the sample processing substrate onto a detector.~~

~~13. The system of Claim 12, further comprising a processing and control subsystem, supported between the deck and the base, the processing and control subsystem comprising non-transitory instructions stored in computer readable media for executing~~

automatic sample processing operations comprising at least one of:

using the pipette interface and the pumping subsystem:

capturing a set of cells of the sample in proximity to a set of functionalized particles, in single cell format, at a set of microwells of a sample processing substrate;

performing lysis of the set of cells, at the sample processing substrate, thereby allowing binding of released biomarkers from the set of cells to the set of functionalized particles;

performing a reverse transcription operation and a second strand synthesis operation, at the set of microwells of the sample processing substrate, with content bound to the set of functionalized particles; and

hybridizing each of a set of probes, corresponding to a set of fluorophores, to products of the amplification operation; and

with the imaging subsystem, generating an image dataset of light emitted by the set of probes, thereby enabling detection of the set of targets of the sample.

14. The system of Claim 12, wherein the sample processing substrate comprises a microwell region comprising the set of microwells, the sample processing substrate retained within a base defining a flow path from the set of microwells.

15. The system of Claim 14, further comprising a second substrate comprising a second set of microwells aligned with and facing the set of microwells of the sample processing substrate, the second substrate partially separated from the sample processing substrate by a compressible layer.

16. The system of Claim 15, wherein the heating and cooling subsystem further comprises a heating object configured to couple to the pipette interface of the gantry.

17. The system of Claim 16, further comprising non-transitory instructions stored in computer readable media for moving the heating object against the second substrate, thereby compressing the second substrate against the sample processing substrate,

~~partitioning individual wells of the sample processing substrate, and transmitting heat to contents of at least one of the sample processing substrate and the second substrate.~~

~~18. A sample processing assembly comprising:~~

~~a sample processing substrate comprising a microwell region comprising a set of microwells configured to capture a set of cells in single-cell format;~~

~~a base coupled to a sample processing substrate and defining:~~

~~an inlet reservoir fluidly coupled to the sample processing substrate, and~~

~~an access region into the microwell region of the sample processing substrate;~~

~~a particle capture region, proximal to the access region and configured for co-capture of a set of functionalized particles in proximity to the set of cells; and~~

~~an elastomeric valve coupling the sample processing substrate to the base along a flow path from the microwell region to an outlet of the sample processing substrate.~~

~~19. The sample processing assembly of Claim 18, further comprising a second substrate comprising a second set of microwells defining the particle capture region, wherein the second set of microwells is aligned with and faces the set of microwells of the sample processing substrate, the second substrate partially separated from the sample processing substrate by a compressible layer.~~

~~20. The sample processing assembly of Claim 18, wherein the second substrate is transitionable between a first mode, in which the second substrate is displaced from the sample processing substrate by the compressible layer, thereby defining a cavity adjacent to the microwell region, and a second mode, in which the second substrate is displaced toward the sample processing substrate with compression of the compressible layer, thereby partitioning individual wells of the microwell region.~~

~~21~~12. A method for detecting a set of targets comprising biomarkers of a sample, the method comprising:

capturing a tissue sample in proximity to a set of functionalized particles, in

single array format, at a set of microwells of a sample processing substrate;

performing lysis of the tissue sample, at the sample processing substrate, thereby allowing binding of released biomarkers from the tissue section to the set of functionalized particles;

performing a reverse transcription operation, at the set of microwells of the sample processing substrate, with ~~content~~ said biomarkers bound to the set of functionalized particles;

with products of the reverse transcription operation, performing a second strand synthesis operation with ~~content bound to~~ the set of functionalized particles;

with products of the second strand synthesis operation, performing an amplification operation at the set of functionalized particles;

hybridizing each of a set of probes, corresponding to a set of fluorophores, to products of the amplification operation; and

generating an image dataset of light emitted by the set of probes, thereby enabling detection of the set of targets of the sample.

~~22~~13. The method of Claim ~~21~~12, wherein the set of targets comprises a panel of antibodies for assessment of immune responses from a subject, the panel of antibodies comprising one or more of: IgM antibodies, IgG antibodies, IgD antibodies, IgA antibodies, and IgE antibodies.

~~23~~14. The method of Claim ~~22~~13, wherein the panel of antibodies comprises antibodies for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).

~~24~~15. The method of Claim ~~21~~12, wherein the set of targets comprises a panel of mRNA molecules.

~~25~~16. The method of Claim ~~24~~15, further comprising performing a spatial transcriptomics analysis of the panel of mRNA molecules, with the sample distributed across the sample processing substrate.