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(54) Title: METHODS AND SYSTEMS FOR SCREENING USING MICROCAPILLARY ARRAYS

(57) Abstract: High-throughput methods for screening large populations of variant proteins are provided. The methods utilize large-scale arrays of microcapillaries, where each microcapillary comprises a solution containing a variant protein, an immobilized target molecule, and a reporter element. Immobilized target molecules may include any molecule of interest, including proteins, nucleic acids, carbohydrates, and other biomolecules. The association of a variant protein with a molecular target is assessed by measuring a signal from the reporter element. The contents of microcapillaries identified in the assays as containing variant proteins of interest can be isolated, and cells expressing the variant proteins of interest can be characterized. Also provided are systems for performing the disclosed screening methods.

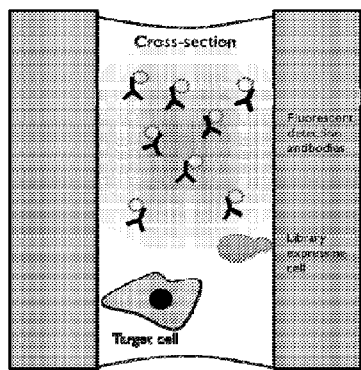


FIG. 1A

METHODS AND SYSTEMS FOR SCREENING USING MICROCAPILLARY ARRAYS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] The present application claims the benefit of U.S. Provisional Application No. 62/830,978, filed April 8, 2019, which is expressly incorporated by reference in its entirety for all purposes.

TECHNICAL FIELD

[0002] The present disclosure provides methods and systems for screening using microcapillary arrays.

BACKGROUND OF THE INVENTION

[0003] The analysis of biological samples, including the identification, characterization, and re-engineering of proteins, nucleic acids, carbohydrates, and other important biomolecules, has benefited greatly from the scaling up of sample numbers and the scaling down of sample sizes. For example, the two-dimensional microarrays of biological materials, such as DNA microarrays, have enabled the development of high-throughput screening methods involving multiplexed approaches for processing samples and detecting results.

[0004] The above approaches have, in some cases, benefited from their combination with optical sensing technology to identify specimens of interest using fluorescent or other corresponding specific and sensitive labeling approaches.

[0005] While such techniques provide analytical information about a particular sample, for example the presence and potentially the amount of a particular biomolecule in a solution or the sequence of a particular nucleic acid or polypeptide, they typically do not allow for the recovery of a biological sample identified by the assay without inactivating or otherwise damaging the sample of interest.

[0006] There is therefore a continuing need to develop improved microscale screening and analysis methods and systems with high throughput capabilities, and particularly methods and systems that enable recovery of samples identified in the screening and analysis using additional assays with the claimed system, including a calcium dye assay, a T cell activation assay, a B cell assay, and a GFP assay.

SUMMARY OF THE INVENTION

[0007] The present disclosure addresses these and other needs by providing in one aspect methods of screening a population of variant proteins comprising the steps of:

providing a microcapillary array comprising a plurality of microcapillaries, each microcapillary comprising a variant protein, an immobilized target molecule, and a reporter element, wherein the variant protein associates with the immobilized target molecule with a particular affinity; and

measuring a signal from at least one reporter element in a reporter assay that indicates association of at least one variant protein with at least one immobilized target molecule to identify at least one microcapillary of interest wherein said reporter assay is selected from the group consisting of a calcium dye assay, a T cell activation assay, a B cell assay, and a GFP assay.

[0008] In some embodiments, the methods further comprise the step of isolating the contents of the microcapillary of interest.

[0009] In another aspect are provided systems for screening a population of variant proteins comprising:

an array comprising a plurality of microcapillaries, each microcapillary comprising a variant protein, an immobilized target molecule, and a reporter element, wherein the variant protein associates with the immobilized target molecule with a particular affinity.

[0010] In some embodiments, the systems further comprise a microscope.

[0011] In some embodiments, the systems further comprise an optical source and a detector.

[0012] In some embodiments, the systems further comprise an extraction device.

[0013] In some embodiments, the systems further comprise a two-stage sample recovery element.

[0014] In some embodiments, the present invention provides a method of screening a population of variant proteins comprising the steps of:

providing a microcapillary array comprising a plurality of microcapillaries, each microcapillary comprising a variant protein, an immobilized target molecule, and a reporter element, wherein the variant protein associates with the immobilized target molecule with a particular affinity; and

measuring a signal from at least one reporter element that indicates association of at least one variant protein with at least one immobilized target molecule to identify at least one microcapillary of interest.

- [0015] In some embodiments, the variant protein is expressed by an expression system.
- [0016] In some embodiments, the expression system is a cell-free expression system.
- [0017] In some embodiments, the expression system is a cellular expression system.
- [0018] In some embodiments, the cellular expression system is an animal system, an avian system, a fungal system, a bacterial system, an insect system, or a plant system.
- [0019] In some embodiments, the cellular expression system is an avian system.
- [0020] In some embodiments, the avian expression system is an chicken system.
- [0021] In some embodiments, the variant protein is a soluble protein.
- [0022] In some embodiments, the target molecule is a target antibody, target protein or polypeptide, a target nucleic acid, a target carbohydrate, or a combination of each.
- [0023] In some embodiments, the target molecule is immobilized on a surface.
- [0024] In some embodiments, the surface is a surface of a cell.
- [0025] In some embodiments, the target molecule is a native protein.
- [0026] In some embodiments, the surface is a surface of a bead.
- [0027] In some embodiments, the surface is a surface of a microcapillary wall.
- [0028] In some embodiments, the surface is a surface configured to settle in the microcapillary by gravitational sedimentation.
- [0029] In some embodiments, the reporter element is a labeled antibody or other binding molecule.
- [0030] In some embodiments, the labeled antibody or other binding molecule is a fluorescently-labeled antibody or other binding molecule.
- [0031] In some embodiments, the labeled antibody is a primary or a secondary antibody.
- [0032] In some embodiments, the labeled antibody or other binding molecule is an enzyme-linked antibody or other binding molecule.
- [0033] In some embodiments, the reporter element is activated within a cell, and the target molecule is immobilized on a surface of the cell.
- [0034] In some embodiments, the reporter element comprises a green fluorescent protein or variant.
- [0035] In some embodiments, the signal is a fluorescent signal, an absorbance signal, a bright-field signal, or a dark-field signal.
- [0036] In some embodiments, each microcapillary in the microcapillary array comprises 0 to 5 variant proteins from the population of variant proteins.
- [0037] In some embodiments, the microcapillary array comprises at least 100,000, at least 300,000, at least 1,000,000, at least 3,000,000, or at least 10,000,000 microcapillaries.

- [0038] In some embodiments, each microcapillary further comprises an agent to improve viability of the cellular expression system.
- [0039] In some embodiments, the agent is methylcellulose, dextran pluronic F-68, polyethylene glycol, or polyvinyl alcohol.
- [0040] In some embodiments, the agent is a growth medium.
- [0041] In some embodiments, the signal is measured by an optical detector.
- [0042] In some embodiments, the signal is measured by a microscope.
- [0043] In some embodiments, the further comprises the step of isolating the contents of the microcapillary of interest.
- [0044] In some embodiments, the contents of the microcapillary of interest are isolated by pulsing the microcapillary of interest with a laser.
- [0045] In some embodiments, the laser is a diode-pumped Q-switched laser.
- [0046] In some embodiments, the laser is directed at the water-glass interface between the microcapillary wall and the sample contained in the microcapillary.
- [0047] In some embodiments, the contents of the microcapillary of interest are isolated using a two-stage sample recovery element.
- [0048] In some embodiments, the microcapillary does not comprise a microparticle capable of inhibiting the transmission of electromagnetic radiation, a magnetic microparticle, a magnetic bead, or an electromagnetic radiation absorbent material.
- [0049] The present invention also provides a system for screening a population of variant proteins comprising:
- an array comprising a plurality of microcapillaries, each microcapillary comprising a variant protein, an immobilized target molecule, and a reporter element, wherein the variant protein associates with the immobilized target molecule with a particular affinity.
- [0050] In some embodiments, the variant protein is expressed by an expression system.
- [0051] In some embodiments, the expression system is a cell-free expression system.
- [0052] In some embodiments, the expression system is a cellular expression system.
- [0053] In some embodiments, the cellular expression system is an animal system, an avian system, a fungal system, a bacterial system, an insect system, or a plant system.
- [0054] In some embodiments, the cellular expression system is an avian system.
- [0055] In some embodiments, the avian expression system is a chicken system.
- [0056] In some embodiments, the variant protein is a soluble protein.
- [0057] In some embodiments, the target molecule is a target protein or polypeptide, a target nucleic acid, a target carbohydrate, or a combination of each.

- [0058] In some embodiments, the target molecule is immobilized on a surface.
- [0059] In some embodiments, the surface is a surface of a cell.
- [0060] In some embodiments, the target molecule is a native protein.
- [0061] In some embodiments, the surface is a surface of a bead.
- [0062] In some embodiments, the surface is a surface of a microcapillary wall.
- [0063] In some embodiments, the surface is a surface configured to settle in the microcapillary by gravitational sedimentation.
- [0064] In some embodiments, the reporter element is a labeled antibody or other binding molecule.
- [0065] In some embodiments, the labeled antibody or other binding molecule is a fluorescently-labeled antibody or other binding molecule.
- [0066] In some embodiments, the labeled antibody is a primary or a secondary antibody.
- [0067] In some embodiments, the labeled antibody or other binding molecule is an enzyme-linked antibody or other binding molecule.
- [0068] In some embodiments, the reporter element is activated within a cell, and the target molecule is immobilized on a surface of the cell.
- [0069] In some embodiments, the reporter element comprises a green fluorescent protein or variant.
- [0070] In some embodiments, the signal is a fluorescent signal, an absorbance signal, a bright-field signal, or a dark-field signal.
- [0071] In some embodiments, each microcapillary in the microcapillary array comprises 0 to 5 variant proteins from the population of variant proteins.
- [0072] In some embodiments, the microcapillary array comprises at least 100,000, at least 300,000, at least 1,000,000, at least 3,000,000, or at least 10,000,000 microcapillaries.
- [0073] In some embodiments, each microcapillary further comprises an agent to improve viability of the cellular expression system.
- [0074] In some embodiments, the agent is methylcellulose, dextran pluronic F-68, polyethylene glycol, or polyvinyl alcohol.
- [0075] In some embodiments, the agent is a growth medium.
- [0076] In some embodiments, the system further comprises an optical source and a detector.
- [0077] In some embodiments, the system further comprises a microscope.
- [0078] In some embodiments, the system further comprises an extraction device.

[0079] In some embodiments, the extraction device comprises a diode-pumped Q-switched laser.

[0080] In some embodiments, the system further comprises a two-stage sample recovery element.

[0081] In some embodiments, the microcapillary does not comprise a microparticle capable of inhibiting the transmission of electromagnetic radiation, a magnetic microparticle, a magnetic bead, or an electromagnetic radiation absorbent material.

[0082] The present invention also provides a method of screening a population of variant proteins comprising the steps of:

providing a microcapillary array comprising a plurality of microcapillaries, each microcapillary comprising a cellular expression system expressing a variant protein, a target molecule immobilized on the surface of a cell, and a reporter element, wherein the variant protein associates with the immobilized target molecule in the microcapillary with a particular affinity, wherein the cellular expression system is an avian system; and

measuring a signal from at least one reporter element in a reporter assay that indicates association of at least one variant protein with at least one immobilized target molecule to identify at least one microcapillary of interest.

[0083] In some embodiments, the avian system is a chicken system.

[0084] In some embodiments, the target molecule is a target protein or polypeptide, a target nucleic acid, a target carbohydrate, or target antibody, or a combination of each.

[0085] In some embodiments, the target molecule is a target antibody.

[0086] In some embodiments, the reporter assay is selected from the group consisting of a calcium dye assay, a T cell activation assay, a B cell assay, and a GFP assay.

[0087] In some embodiments, the reporter element is a labeled antibody or other binding molecule, wherein the labeled antibody or other binding molecule localizes to an epitope on the variant protein.

[0088] In some embodiments, the labeled antibody or other binding molecule is a fluorescently-labeled antibody or other binding molecule.

[0089] In some embodiments, the B cell assay comprises B cells sourced from the spleen.

[0090] In some embodiments, the T cell activation assay is used to evaluate the cell signaling induction capability of an antibody.

[0091] In some embodiments, the T cell activation assay is used to measure the internal signaling or cell surface marker induction capability of an antibody.

- [0092] In some embodiments, the T cell activation assay is used to evaluate the cell expansion induction capability of an antibody.
- [0093] In some embodiments, the T cell activation assay is used to evaluate the cytokine secretion induction capability of an antibody.
- [0094] In some embodiments, the T cell activation assay measures CD25 expression to determine activation capability of an antibody.
- [0095] In some embodiments, the CD25 expression is measured with a fluorescently labeled anti-CD25 antibody.
- [0096] In some embodiments, the T cell activation assay measures calcium signaling to determine activation capability of an antibody.
- [0097] In some embodiments, the T cell activation assay uses a calcium-sensitive fluorophore to measure the calcium signaling.
- [0098] In some embodiments, the calcium-sensitive fluorophores are selected from the group consisting of Fluo-4 AM, Fura-2 AM, and Indo-1 AM.
- [0099] In some embodiments, the T cell activation assay comprises a mixture of T cells and antibody secreting cells (ASC).
- [0100] In some embodiments, the mixture of T cells and ASC varies from a ratio of 2:1 to 12:1.
- [0101] In some embodiments, the mixture of T cells and ASC is a ratio of 5:1.
- [0102] In some embodiments, the ASC are B cells.
- [0103] In some embodiments, the mixture of T cells and ASC further comprises T cell activating beads and antibody capture beads.
- [0104] In some embodiments, the T cells are purified from peripheral blood.
- [0105] In some embodiments, the signal used to identify the least one microcapillary of interest is an increase of at least 10% to 10,000% or greater than the signal as compared to a baseline and/or a control sample.
- [0106] In some embodiments, the signal used to identify the least one microcapillary of interest is an increase of at least 10% or greater than the signal as compared to a baseline and/or a control sample.
- [0107] In some embodiments, the increase in the signal used to identify the least one microcapillary of interest represents a statistically significant increase when compared to baseline and/or a control.

BRIEF DESCRIPTION OF THE DRAWINGS

[0108] FIG. 1A- FIG. 1C schematically illustrate the steps of an exemplary microcapillary screening assay. The illustration on the left in each panel is a cross-sectional view from the side of a single microcapillary. The illustration on the right in each panel is a bottom view of a subsection of the array of microcapillaries. The shading in each case is intended to illustrate an electromagnetic signal, such as fluorescence.

[0109] FIG. 2A-FIG. 2C show the bottom view of a subsection of a microcapillary array illustrating hybridoma screening against mammalian cells, where the cells are imaged using either bright-field (FIG. 2A), LiveGreen (FIG. 2B), or a fluorescent anti-mouse secondary antibody (FIG. 2C).

[0110] FIG. 3 shows images of a microcapillary containing both an A431 target cell and a hybridoma cell over the course of a 4 hour incubation.

[0111] FIG. 4A - FIG. 4B show images of a subsection of a microcapillary array highlighting expressing and non-expressing yeast cells against mammalian cells, where the cells are imaged using either bright-field (FIG. 4A) or a fluorescent antibody (FIG. 4B).

[0112] FIG. 5A- FIG. 5G illustrate the growth of an immortalized human cell in a microcapillary array over the course of 6 days.

[0113] FIG. 6A- FIG. 6E are different views of a microscope system designed to carry out the screening methods of the instant disclosure.

[0114] FIG. 7 shows an exemplary embodiment of cells (including mammalian or yeast) expression and binding to mammalian cells using the present invention. Each one of the four panels represents one microcapillary microcavity over time.

[0115] FIG. 8A – FIG. 8B shows an exemplary embodiment of cells (including mammalian or yeast) expression and binding to 2 or more mammalian cell types using the present invention. 8A) Each one of the four panels represents one microcapillary microcavity over time. 8B) Provides potential readouts from the exemplary assay embodiment.

[0116] FIG. 9 shows exemplary fluorescence and bright-field data generated by the exemplary assay described in Example 6.

[0117] FIG. 10 shows an exemplary embodiment of cells (including mammalian or yeast) expression and binding to immobilized targets on a solid support (such as a bead) using the present invention. Each one of the four panels represents one microcapillary microcavity over time.

[0118] FIG. 11 shows an exemplary embodiment of cells (including mammalian or yeast) expression and functional reporter response using the present invention. Each one of the four panels represents one microcapillary microcavity over time. Reporters can include any detectable reporter, including for example GFP, YFP, and/or RFP, as well as any fluorophores described herein or known in the art.

[0119] FIG. 12 provides examples of IgG1, IgG2, IgG3, and IgG4 sequences.

[0120] FIG. 13A – FIG. 13B. Data provides A) image of beads from Example 7 titration experiment. B) Graph showing the optimum range of signal was around 1:500-1:5000 (lower range of manufacturer recommended ranges).

[0121] FIG. 14. Summary of screening full-length, soluble antibodies against cell surface targets.

[0122] FIG. 15. Data from high-throughput morphological and fluorescence screens.

[0123] FIG. 16. Three functional screens for use with the present system.

[0124] FIG. 17. Data showing that the integrated platform system described herein enables precise identification of rare cells.

[0125] FIG. 18. Data from antigen specific B cell profiling of a single OmniChicken. OmniChicken expresses fully human, highly diversified antibody repertoire. Genetic divergence from human and mouse allows for more diverse epitope coverage. Allows for deep immune profiling which will potentially lead to a more diverse panel of functional antibodies. Exemplary assay shown: Profiling the antigen specific antibody repertoire after immunization with progranulin.

[0126] FIG. 19. B Cell Assay. Top: assay conditions. Bottom: Exemplary data/readout.

[0127] FIG. 20. Binding analysis related to B cell assay.

[0128] FIG. 21. Quantification and sorting relate to B cell assay.

[0129] FIG. 22. Single cell VH/VK isolation and sequencing. Pairwise distance between each cell's concatenated HCDR3-LCDR3.

[0130] FIG. 23. Deeper characterization identifies new clonotype families. Screen identifies majority of clones identified by GEM assay. Multiple new clonotype families identified.

[0131] FIG. 24. Antigen specific clones have high affinity and broad epitopic coverage. Ligand expressed subset of discovered clones as scFv-Fc. Characterization via Carterra LSA (affinity and epitope binning data). Clusters map to distinct subdomains of progranulin, with broad coverage of the seven subdomains. Further validation in progress with newly discovered clonal clusters.

- [0132] FIG. 24. Antigen specific clones have high affinity and broad epitopic coverage. Ligand expressed subset of discovered clones as scFv-Fc. Characterization via Carterra LSA (affinity and epitope binning data). Clusters map to distinct subdomains of progranulin, with broad coverage of the seven subdomains. Further validation in progress with newly discovered clonal clusters.
- [0133] FIG. 25. Representative B cell screening results.
- [0134] FIG. 26. General xPloration T cell activating assay classes.
- [0135] FIG. 27. T cell activation (CD25 surface expression) with B cells.
- [0136] FIG. 28. T cell activation (calcium signaling) with B cells. Human T cell activation when measured via calcium signaling.

DETAILED DESCRIPTION OF THE INVENTION

[0137] Microcapillary arrays have recently been employed in approaches for high-throughput analysis and protein engineering with large numbers of biological samples, for example in an approach that has been termed “microcapillary single-cell analysis and laser extraction” or “ μ SCALE”. See Chen *et al.* (2016) *Nature Chem. Biol.* 12:76–81; DOI: 10.1038/NCHEMBIO.1978. This approach relies on the spatial segregation of single cells within a microcapillary array, and thus enables repeated imaging, cell growth, and protein expression of the separate samples within each microcapillary of the microcapillary array. Accordingly, the technique enables massively parallel, quantitative biochemical and biophysical measurements on millions or multi-millions of samples within a microcapillary array, for example, in the analysis of millions or multi-millions of protein variants expressed from yeast, bacteria, or other suitable cells distributed throughout the array. Advantageously, the approach has allowed the simultaneous time-resolved kinetic analysis of the multiplexed samples, as well as the sorting of those cells based on targeted phenotypic features.

[0138] The development of μ SCALE methods and apparatus for the quantitative biochemical and biophysical analysis of populations of biological variants has also been reported in U.S. Patent Application Publication No. 2016/0244749 A1, which is incorporated by reference herein in its entirety. Extraction of the contents of a desired microcapillary according to the μ SCALE approach requires, however, the inclusion of a radiation-absorbing material in each sample and the directing of electromagnetic radiation from a pulsed laser into this material, thus adding complexity to the extraction methods. In addition, earlier methods of screening of biological variants in arrays of microcavities relied on the addition of microparticles to the arrayed samples to partially or completely inhibit the transmission of

electromagnetic radiation into and out of the sample in order to minimize signal emitted from microcavities lacking a desired binding activity. See U.S. Patent Application Publication No. U.S. 2014/0011690 A1. In some aspects of the instant disclosure, the screening methods do not rely on these additional sample components or manipulations, thus simplifying and improving the efficiency of the screening techniques.

[0139] In specific applications of these approaches, and as will be disclosed in more detail herein, the target molecule can be immobilized on a surface, such as the surface of a particle (*e.g.*, a magnetic particle), a cell, or a microcapillary wall. The interaction between a variant protein and a target molecule in these approaches can then be measured by several methods, including methods utilizing detectable antibodies and methods of measuring detectable signals generated within the target cells. It will be understood that such methods can be used in high-throughput screens to discover protein variants that bind to target molecules, for example a target molecule on a cell or other surface.

Methods of Screening

[0140] Accordingly, in some aspects, the instant disclosure provides methods of screening a population of variant proteins comprising the steps of:

providing a microcapillary array comprising a plurality of microcapillaries, each microcapillary comprising a variant protein, an immobilized target molecule, and a reporter element, wherein the variant protein associates with the immobilized target molecule with a particular affinity; and

measuring a signal from at least one reporter element that indicates association of at least one variant protein with at least one immobilized target molecule to identify at least one microcapillary of interest.

[0141] In these methods, the microcapillary arrays preferably comprise a plurality of longitudinally fused capillaries, for example fused silica capillaries, although any other suitable material may be utilized in the arrays. See, *e.g.*, PCT International Patent Publication Nos. WO2012/007537 and WO2014/008056, the disclosures of which are incorporated by reference herein in their entireties. Such arrays can be fabricated, for example, by bundling millions or billions of silica capillaries and fusing them together through a thermal process, although other suitable methods of fabrication may also be employed. The fusing process may comprise, for example, the steps of i) heating a capillary single draw glass that is drawn under tension into a single clad fiber; ii) creating a capillary multi draw single capillary from the single draw glass by bundling, heating, and drawing; iii)

creating a capillary multi-multi draw multi capillary from the multi draw single capillary by additional bundling, heating, and drawing; iv) creating a block assembly of drawn glass from the multi-multi draw multi capillary by stacking in a pressing block; v) creating a block pressing block from the block assembly by treating with heat and pressure; and vi) creating a block forming block by cutting the block pressing block at a precise length (*e.g.*, 1 mm).

[0142] In some embodiments, the fabrication method further comprises slicing the silica capillaries, thereby forming very high-density glass microcapillary arrays. In some embodiments, the microcapillary arrays may be cut to approximately 1 millimeter in height, but even shorter microcapillary arrays are contemplated, including arrays of 10 μm in height or even shorter. In some embodiments, even longer microcapillary arrays are contemplated, including arrays of 10 mm or even longer.

[0143] Such processes form very high-density microcapillary arrays that are suitable for use in the present methods. In an exemplary array, each microcapillary has an approximate 5 μm diameter and approximately 66% open space (*i.e.*, representing the lumen of each microcapillary). In some arrays, the proportion of the array that is open ranges between about 50% and about 90%, for example about 60 to 75%, such as a microcapillary array provided by Hamamatsu that has an open area of about 67%. In one particular example, a 10x10 cm array having 5 μm diameter microcapillaries and approximately 66% open space has about 330 million total microcapillaries.

[0144] In various embodiments, the internal diameter of each microcapillary in the array ranges from between approximately 1 μm and 500 μm . In some arrays, each microcapillary can have an internal diameter in the range between approximately 1 μm and 300 μm ; optionally between approximately 1 μm and 100 μm ; further optionally between approximately 1 μm and 75 μm ; still further optionally between approximately 1 μm and 50 μm ; and still further optionally between approximately 5 μm and 50 μm .

[0145] In some microcapillary arrays, the open area of the array comprises up to 90% of the open area (OA), so that, when the pore diameter varies between 1 μm and 500 μm , the number of microcapillaries per cm of the array varies between approximately 460 and over 11 million. In some microcapillary arrays, the open area of the array comprises about 67% of the open area, so that, when the pore size varies between 1 μm and 500 μm , the number of microcapillaries per square cm of the array varies between approximately 340 and over 800,000.

In some embodiments, the pore size is 1 μm , 5 μm , 10 μm , 50 μm , 100 μm , 250 μm , 350 μm or 500 μm . In some embodiments, the pore size is between 5 μm and 500 μm . In some

embodiments, the pore size is between 10 μm and 450 μm . In some embodiments, the pore size is between 50 μm and 500 μm . In some embodiments, the pore size is between 100 μm and 500 μm . In some embodiments, the pore size is between 250 μm and 500 μm . In some embodiments, the pore size is between 350 μm and 500 μm . In some embodiments, the pore size is between 100 μm and 450 μm . In some embodiments, the pore size is between 250 μm and 450 μm .

[0146] In some embodiments, the number of microcapillaries per square cm of the array is approximately 400; 500; 1000; 2,000; 3,000; 4,000; 5,000; 6,000; 7,000; 8,000; 9,000; 10,000; 20,000; 50,000; 100,000; 200,000; 300,000; 400,000; 500,000; 600,000; 700,000; or 800,000. In some embodiments, the number of microcapillaries per square cm of the array varies between approximately 500 and 800,000. In some embodiments, the number of microcapillaries per square cm of the array varies between approximately 1000 and 700,000. In some embodiments, the number of microcapillaries per square cm of the array varies between approximately 2000 and 600,000. In some embodiments, the number of microcapillaries per square cm of the array varies between approximately 10,000 and 800,000. In some embodiments, the number of microcapillaries per square cm of the array varies between approximately 10,000 and 700,000. In some embodiments, the number of microcapillaries per square cm of the array varies between approximately 50,000 and 800,000. In some embodiments, the number of microcapillaries per square cm of the array varies between approximately 50,000 and 700,000. In some embodiments, the number of microcapillaries per square cm of the array varies between approximately 100,000 and 700,000. In some embodiments, the number of microcapillaries per square cm of the array varies between approximately 100,000 and 600,000. In some embodiments, the number of microcapillaries per square cm of the array varies between approximately 100,000 and 500,000. In some embodiments, the number of microcapillaries per square cm of the array varies between approximately 500,000 and 800,000.

[0147] In one particular embodiment, a microcapillary array can be manufactured by bonding billions of silica capillaries and then fusing them together through a thermal process. After that slices (0.5 mm or more) are cut out to form a very high aspect ratio glass microcapillary array. Arrays are also commercially available, such as from Hamamatsu Photonics K. K. (Japan), Incom, Inc. (Massachusetts), Photonis Technologies, S.A.S. (France) Inc., and others. In some embodiments, the microcapillaries of the array are closed at one end with a solid substrate attached to the array.

[0148] The microcapillary arrays of the instant screening methods can comprise any number of microcapillaries within the array. In some embodiments, the microcapillary array comprises at least 100,000, at least 300,000, at least 1,000,000, at least 3,000,000, at least 10,000,000, or even more microcapillaries. In some embodiments, the array comprises at least 100,000, at least 200,000, at least 300,000, at least 400,000, at least 500,000, at least 600,000, at least 700,000, at least 800,000, at least 1,000,000, at least 1,500,000, at least 2,000,000, at least 2,500,000, or at least 3,000,000 or more microcapillaries. The number of microcapillaries within an array is preferably chosen in view of the size of the variant protein library to be screened.

[0149] As described above, each capillary in the microcapillary arrays used in the instant screening methods comprises a variant protein, an immobilized target molecule, and a reporter element, where the variant protein is one of the population of variant proteins that is being subjected to the screening method. The population of variant proteins can be any population of proteins that can be suitably distributed within a microcapillary array. Ideally, the population of variant proteins is distributed in the microcapillary array so that each microcapillary comprises a small number of different variant proteins, preferably just a single different variant protein per microcapillary. Importantly, the population of variant proteins is chosen in combination with the immobilized target molecule, such that at least some of the proteins in the population can associate with the immobilized target molecule with a particular affinity, such that the association is detectable by measuring a signal from a reporter element.

[0150] The term “protein”, as used herein, refers both to full-length proteins or polypeptide sequences and to fragments thereof. Such fragments may include fragments that retain a functional activity, such as, for example, a binding activity. The terms “protein” and “polypeptide” are used interchangeably throughout the disclosure and include chains of amino acids covalently linked through peptide bonds, where each amino acid in the polypeptide may be referred to as an “amino acid residue”. Use of the terms “protein” or “polypeptide” should not be considered limited to any particular length of polypeptide, *e.g.*, any particular number of amino acid residues. The subject proteins may include proteins having non-peptidic modifications, such as post-translational modifications, including glycosylation, acetylation, phosphorylation, sulfation, or the like, or other chemical modifications, such as alkylation, acetylation, esterification, PEGylation, or the like. Additional modifications, such as the inclusion of non-natural amino acids within a

polypeptide sequence or non-peptide bonds between amino acid residues should also be considered within the scope of the definition of the term “protein” or “polypeptide”.

[0151] The population of variant proteins is preferably a population of proteins having minor variations, for example a population of proteins where each protein has a slightly different amino acid sequence. The screening assays can, therefore, identify variant protein sequences having desirable properties. Because the screens can be performed in such large numbers at microscopic scale, huge numbers of variant proteins can be assayed in relatively short times.

[0152] The term “antibody” is used in the broadest sense and includes, for example, an intact immunoglobulin or an antigen binding portion. Antigen binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Thus the term antibody includes traditional tetrameric antibodies of two heavy chains and two light chains, as well as antigen binding fragments such as Fv, Fab and scFvs. In some cases, the invention provides bispecific antibodies that include at least one antigen binding domain as outlined herein.

[0153] Variant proteins and/or variant polypeptides can include but are not limited to secreted proteins. In some embodiments, the secreted proteins are from a recombinant protein and/or polypeptide library. In some embodiments, the secreted proteins are from a recombinant protein and/or polypeptide library. In some embodiments, the secreted proteins are from a recombinant protein and/or polypeptide library from a mammalian cell line. In some embodiments, the recombinant protein and/or polypeptide library comprises full length mammalian antibodies. In some embodiments, the recombinant protein and/or polypeptide library comprises full length mammalian antibodies, including IgG1, IgG2, and IgG4 antibodies and variants thereof. In some embodiments, the recombinant protein and/or polypeptide library comprises full length human antibodies. In some embodiments, the recombinant protein and/or polypeptide library comprises full length human antibodies, including IgG1, IgG2, and IgG4 antibodies. In some embodiments, the recombinant protein and/or polypeptide library comprises full length mouse antibodies. In some embodiments, the recombinant protein and/or polypeptide library comprises full length mouse antibodies, including IgG1, IgG2, and IgG4 antibodies. In some embodiments, the recombinant protein and/or polypeptide library comprises full length rat antibodies. In some embodiments, the recombinant protein and/or polypeptide library comprises full length rat antibodies, including IgG1, IgG2, and IgG4 antibodies. In some embodiments, the recombinant protein and/or polypeptide library comprises antibody fragments (Fab). In some embodiments, the

recombinant protein and/or polypeptide library comprises single chain variable fragments (scFv). In some embodiments, the recombinant protein and/or polypeptide library comprises natural protein ligands. In some embodiments, the recombinant protein and/or polypeptide library comprises natural protein ligands to a defined target protein and/or polypeptide. In some embodiments, the recombinant protein and/or polypeptide library comprises target antibodies and/or fragments thereof. In some embodiments, the recombinant protein and/or polypeptide library comprises target antibody heavy chains and/or fragments thereof, such as variable heavy chains. In some embodiments, the recombinant protein and/or polypeptide library comprises target antibody light chains and/or fragments thereof, such as variable light chains. In some embodiments, the system of the present invention allows for accurate pairing of VH/VL (variable heavy chains and variable light chains).

[0154] In some embodiments, each microcapillary in the microcapillary array comprises 0 to 5 different variant proteins from the population of variant proteins. In specific embodiments, each microcapillary in the microcapillary array comprises 0 to 4, 0 to 3, 0 to 2, or even 0 to 1 different variant proteins from the population of variant proteins. It should be understood that the different variant proteins in the population of variant proteins differ in their molecular structure, whether the difference is in their amino acid sequence or in some other chemical modification of the protein.

[0155] It should be understood that each microcapillary will typically comprise many multiple copies of the same variant protein, depending on the source and expression level of the particular variant protein (see below). In some embodiments, each microcapillary will comprise thousands, tens of thousands, hundreds of thousands, millions, billions, or even more molecules of a particular variant protein, depending on how the variant protein is delivered to or expressed within the microcapillary. In some embodiments, the variant protein can bind to one, two, three, or four or more target molecules. In some embodiments, the variant protein can bind to one target molecule. In some embodiments, the variant protein can bind to two target molecules. In some embodiments, the variant protein can bind to three target molecules. In some embodiments, the variant protein can bind to four target molecules. In some embodiments, the variant protein can bind to more than four target molecules. In some embodiments, this assay can alternatively be used to screen for antibodies that bind both a mouse and human (or other combination of animals) variant of a target protein, i.e. finding “cross-reactive” antibodies. For example, the presence of stained cells and the presence of stained beads within a microcapillary indicates the presence of an antibody which binds to the “target protein” (for example, a mouse target) and which also binds to the “target

protein analog” (for example, a human target), in order to identify antibodies which bind to both a mouse and human target. For example, the presence of stained cells and the presence of stained beads within a microcapillary indicates the presence of an antibody which binds to the “target protein” (for example, a cynomolgus target) and which also binds to the “target protein analog” (for example, a human target), in order to identify antibodies which bind to both a cynomolgus and human target.

[0156] The population of variant proteins is typically generated using a genetic library in a biological expression system, for example in an *in vitro* (*i.e.*, cell-free) expression system or in an *in vivo* or cellular expression system. Exemplary cellular expression systems include, for example, animal systems (*e.g.*, mammalian systems), fungal systems (*e.g.*, yeast systems), bacterial systems, insect systems, or plant systems. In specific embodiments, the expression system is a mammalian system or a yeast system. In specific embodiments, the expression system is an avian system (for example, a chicken system). The expression system, whether cellular or cell-free, typically comprises a library of genetic material encoding the population of variant proteins. Cellular expression systems offer the advantage that cells with a desirable phenotype, for example cells that express a particular variant protein of interest, such as a variant protein capable of associating with an immobilized target molecule with high affinity, can be grown and multiplied, thus facilitating and simplifying the identification and characterization of the proteins of interest expressed by the cells. In some embodiments, the biological expression system comprises a mammalian cell line. In some embodiments, the mammalian cell line is selected from the group consisting of CHO-K1, CHO-S, HEK293T, and/or any derivatives of these cell types. In some embodiments, the mammalian cell line is CHO-K1. In some embodiments, the mammalian cell line is CHO-S. In some embodiments, the mammalian cell line is HEK293T. In some embodiments, the mammalian cell line is selected from the group consisting of human, mouse, and/or rat hybridoma cell lines. In some embodiments, the mammalian cell line is a human hybridoma cell line. In some embodiments, the mammalian cell line is a mouse hybridoma cell line. In some embodiments, the mammalian cell line is a rat hybridoma cell line.

[0157] Genetic libraries encoding large populations of variant proteins are well known in the art of bioengineering. Such libraries are often utilized in systems relying on the process of directed evolution to identify proteins with advantageous properties, such as high-affinity binding to target molecules, stability, high expression, or particular spectroscopic, *e.g.*, fluorescence, or enzymatic activities. Often the libraries include genetic fusions with sequences from the host expression system, for example fragments of proteins directing

subcellular localization, where the expressed population of variant fusion proteins are directed by the targeting fragment to a particular location of the cell or virus particle for purposes of activity screening of the variant protein population. Large numbers of variant proteins (*e.g.*, 10^6 variants, 10^8 variants, 10^{10} variants, 10^{12} variants, or even more variants) can be generated using routine bioengineering techniques, as is well known in the art. Such libraries can include any of the variant proteins described herein, including antibodies, antibody fragments, single chain variable fragments, or natural protein ligands. In some embodiments, the system of the present invention allows for accurate pairing of VH/VL (variable heavy chains and variable light chains).

[0158] Accordingly, in some embodiments, the variant proteins are soluble proteins, for example soluble proteins that are secreted by a cellular expression system. Exemplary soluble variant proteins include antibodies and antibody fragments, alternative protein scaffolds, such as disulfide-bonded peptide scaffolds, extracellular domains of cell-surface receptor proteins, receptor ligands, such as, for example, G-protein coupled receptor ligands, other peptide hormones, lectins, and the like. Advantageously, the variant proteins screened for binding activity in the instant methods do not need to be covalently attached to the cell or virus that expresses them in order to be identified following a screening assay, since a variant protein with a desired binding activity and the cell that expressed it remain co-localized within the same microcapillary throughout the assay. Isolation of the contents of the desired microcapillary, followed by propagation of the cell or virus clone responsible for expression of the desired variant protein, thereby enables the identification and characterization of that protein. Unlike screening assays where a variant protein of interest is displayed by fusion of the protein to a molecule on the surface of a cell or virus particle, the variant proteins identified in the instant screening methods need not be altered in any way following their identification. The observed activities of the variant proteins in the screens are thus more likely to represent the actual activities of those proteins in their subsequent applications.

[0159] In other embodiments, however, it may be desirable for the variant proteins to be membrane-associated proteins, for example proteins remaining associated with the surface of a cell or a viral particle in an expression system. Screening of cell-associated variant proteins may be desirable where the variant protein and its target molecule mediate interactions between two cells within a biological tissue. The ability to screen against cell-associated variant proteins may also be desirable in screening for interactions with traditionally “non-druggable” protein targets, such as, for example, G-protein coupled receptors or ion channels.

[0160] In addition to a variant protein, each microcapillary in the microcapillary arrays of the instant screening methods also comprises an immobilized target molecule. The immobilized target molecule serves as the potential binding partner for the variant protein of the screening assay. Unlike the population of variant proteins, where each microcapillary ideally contains a variant protein of slightly different sequence, the immobilized target molecules ideally have the same molecular structure in each microcapillary of the array. In some embodiments, there is no binding or other interaction between the variant protein and another agent or molecule (*e.g.*, the target molecule) prior to the addition of the variant protein to the microcapillary. In some embodiments, the interaction between the variant protein and the target molecule occurs within the microcapillary and/or microcavity.

[0161] In some embodiments, the target molecule is a target protein or polypeptide, a target nucleic acid, a target carbohydrate, a target lipid, or a combination of two or more of these target molecules. For example, in some embodiments the target molecule can be a lipid-modified or glycosylated protein. In some embodiments, the target molecule is immobilized on a surface. In more specific embodiments, the target molecule is immobilized on the surface of a cell, such as a target cell, the surface of a bead, the surface of a microcapillary wall, or another suitable surface. In other more specific embodiments, the target molecule is a native protein, for example a native protein immobilized on the surface of a cell. In still other more specific embodiments, the target molecule is immobilized on a surface configured to settle in the microcapillary by gravitational sedimentation. In some embodiments, one, two, three, or four, or more target molecules are employed, in order to identify variants that bind to one, two, three, or four, or more target molecules. In some embodiments, the target molecules are contained separately in separate and different microcapillaries. In some embodiments, the target molecules are contained separately in separate and different microcapillaries within a single array. In some embodiments, the target molecules are contained separately in separate and different microcapillaries within one or more arrays. In some embodiments, the target molecules are contained together in a single microcapillary. In some embodiments, the target molecules are contained together in a single microcapillary within a single array. In some embodiments, the one, two, three, or four, or more target molecules to which the variant binds are derivatives or variants of an original target molecule, including chemical modifications, secondary post-translational modifications, or sequence identity variants (including, for example, variants with 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to an original nucleic acid or amino acid target sequence).

[0162] As previously noted, in the methods of the instant disclosure, the variant protein associates with the immobilized target molecule with a particular affinity within a microcapillary. Importantly, such affinities should be sufficiently strong for variant proteins of interest that the association can be measured by a signal from a reporter element. Binding affinities are typically assessed by a dissociation constant (K_d), as is well understood by those of ordinary skill in the art, where the lower the dissociation constant, the higher the affinity. In some embodiments, the association between the variant protein of interest and the immobilized target molecule displays a dissociation constant in the millimolar to micromolar range. In specific embodiments, the association displays a dissociation constant from micromolar to high nanomolar (*i.e.*, 10^{-6} M to 10^{-8} M). In more specific embodiments, the association displays a dissociation constant from lower nanomolar to high picomolar (*i.e.*, 10^{-8} M to 10^{-10} M). In even more specific embodiments, the association displays a dissociation constant in the picomolar range (*i.e.*, 10^{-10} M to 10^{-12} M), or even lower. In some embodiments, a first cell expresses and secretes the variant protein or polypeptide and a second cell comprises the target, such that the first cells binds to the second cell. In some embodiments, the second cell expresses the target. In some embodiments, the second cell is labeled with the target. In some embodiments, the first cell binds to the second cell in the microcapillary. In some embodiments, the first cell binds to the second cell in the microcapillary and/or microcavity.

[0163] In addition to a variant protein and an immobilized target molecule, each microcapillary in the microcapillary array of the instant screening methods also comprises a reporter element. Importantly, the reporter element provides a measureable signal indicative of the association of a variant protein with an immobilized target molecule and thus serves to identify a microcapillary containing variant proteins of interest.

[0164] In some embodiments, the reporter element is a labeled antibody or other molecule capable of binding to each variant protein in the population of variant proteins. More specifically, the reporter element is a fluorescently-labeled antibody or other binding molecule.

[0165] In some embodiments, the labeled antibody is a labeled primary antibody or a labeled secondary antibody. For purposes of this disclosure, a primary antibody is typically considered to be an antibody that binds directly to an antigen of interest, whereas a secondary antibody is typically considered to be an antibody that binds to a constant region on a primary antibody for purposes of labeling the primary antibody. Accordingly, secondary antibodies are frequently labeled with fluorophores or other detectable labels or are labeled with

enzymes that are capable of generating detectable signals. They are generally specific for a primary antibody from a different species. For example, a goat or other animal species may be used to generate secondary antibodies against a mouse, chicken, rabbit, or nearly any primary antibody other than an antibody from that animal species, as is understood by those of ordinary skill in the art. In specific embodiments, the labeled antibody is a fluorescent antibody or an enzyme-linked antibody.

[0166] In some of the method embodiments, for example in the screening methods illustrated in FIGs. 1A-1C, the variant protein mediates the association of a reporter element with a target molecule, in this example, a target molecule on the surface of a target cell. As shown in FIG. 1B, where the variant protein (here designated as a “secreted protein”) has sufficient affinity for its target molecule on the target cell that the variant proteins associate with the target cell under the conditions of the microcapillary solution. The reporter element (here designated as “fluorescent detection antibodies”) binds to the variant protein, ideally at an epitope that does not affect the affinity of the variant protein for the target molecule, as shown in FIG. 1C.

[0167] As would be understood by those of ordinary skill in the art, when a soluble reporter element, such as a fluorescent antibody, is used in the instant screening methods, the signal emitted by any excess reporter element remaining free in solution (*i.e.*, either not bound to a variant protein or bound to a variant protein that is not bound to a target molecule) within the microcapillary should not be so high that it overwhelms the signal of reporter elements associated with a target molecule via a variant protein (see, *e.g.*, the unassociated fluorescent detection antibodies illustrated in FIG. 1C). Such background signals can be minimized, however, by limiting the concentration of labeled antibody or other reporter element within the microcapillary solution. In addition, where signals from the screening methods are measured using a fluorescent microscope, configuring the microscope to image a relatively narrow depth of field bracketing the location of the target molecules (*e.g.*, the bottom of the microcapillaries when target cells have settled there by gravitational sedimentation) can minimize the background signal from reporter elements not associated with the target molecule.

[0168] In other embodiments, the reporter element is an intracellular reporter element that generates a detectable signal in connection with a binding event, such as, for example, the association of a variant protein with an immobilized target molecule, for example, a receptor or other target molecule on the surface of the cell. In these embodiments, the reporter element may comprise an entire cellular pathway, such as, for example, an intracellular

signaling pathway. Such a pathway should include, or be engineered to include, a detectable signal as the downstream readout of the pathway. In contrast to the assays illustrated in FIGs. 1A-1C, where the detectable signal is bound to the outer surface of the target cell, the detectable signal in these embodiments would typically be generated inside the target cell.

[0169] Many intracellular signaling pathways have been developed for use in high throughput screening assays, in particular in drug discovery screens, and can be adapted for use in the instant assays. See, *e.g.*, Michelini *et al.* (2010) *Anal. Bioanal. Chem.* 398:227-38. In particular, any cellular assay where a binding event with a target molecule on the surface of a cell results in the generation of a measurable signal, in particular a fluorescent signal, can be used as a reporter element in the instant assays. Preferably, the cells can be engineered to express a target molecule of interest on their surface, so that the binding of a particular variant protein to the target molecule and the consequent activation of the intracellular signaling pathway result in the production of a detectable signal from the reporter element, thus enabling the identification of the microcapillary as a positive hit. The expression of a green fluorescent protein (GFP), or any of a wide variety of variant fluorescent proteins, is often used as a readout in such cellular assays and can serve as the reporter element endpoint in the instant methods. Alternatively, the signaling readout can be provided by luciferase or other related enzymes that produce bioluminescent signals, as is well understood by those of ordinary skill in the art. See, *e.g.*, Kelkar *et al.* (2012) *Curr. Opin. Pharmacol.* 12:592-600. Reporter elements can also include RFP (red fluorescent protein) as well as YFP (yellow fluorescent protein), and variants thereof. Other well-known enzymatic reporters from bacterial and plant systems include β -galactosidase, chloramphenicol acetyltransferase, β -glucuronidase (GUS), and the like, which can be adapted for use in the instant screening assays with suitable colorogenic substrates. Transcriptional reporters using firefly luciferase and GFP have been used extensively to study the function and regulation of transcription factors. They can likewise be adapted for use in the instant screening assays. Exemplary intracellular signaling systems are available commercially, for example the Cignal™ Reporter Assay kits from Qiagen (see, *e.g.*, www.sabiosciences.com/reporterassays.php), which are available with either luciferase or GFP readouts. Such systems can be suitably re-engineered for use in the instant screening methods.

[0170] It should be understood that a variant protein expression system, in particular where the expression system is a cellular expression system, can be combined with the immobilized target molecule and the reporter element (or suitable components, such as cellular components, responsible for generating the immobilized target molecule and/or reporter

element) prior to the expression of the variant proteins and/or prior to delivery of an assay mixture into the array of microcapillaries. Such approaches advantageously allow for flexibility and control in the timing of interactions between the components compared to prior art microcapillary screening systems, where all of the components of the screening assays are typically mixed and loaded into the microcapillaries in static form. In contrast, the instant methods enable some or all of the components of a binding assay to be generated *in situ* within the microcapillaries, either by allowing for the growth of cellular components, the expression of genetic components, or both. In some embodiments, cell culture media and/or growth agents are employed in order to maintain the health of the cells during the assay process. In some embodiments, components are included that facilitate the metabolic health of the cells.

[0171] It should also be understood that the concentrations of each component of the screening assay within a microcapillary, including the concentration of the variant protein, the concentration of the immobilized target molecule, and the concentration of the reporter element, can be modulated as desired in an assay in order to achieve an optimal outcome. In particular, it may be desirable to modulate the concentration of variant protein and/or immobilized target molecule to achieve the desired level of association between these components. The level of association will also depend on the particular affinity between these components, wherein a higher affinity results in a higher level of association for a given concentration of the components, and a lower affinity results in a lower level of association of the components for a given concentration. Concentration of the reporter element may likewise be modulated in order to achieve optimum levels of signal output, as would be understood by those of ordinary skill in the art. In some embodiments, the reporter element employed includes a secondary antibody, including those commercially available. In some embodiments the dilution range is 1:200-1:2000. In some embodiments the dilution range is 1:300-1:2000. In some embodiments the dilution range is 1:300-1:1500. In some embodiments the dilution range is 1:400-1:1500. In some embodiments the dilution range is 1:500-1:1500. In some embodiments the dilution range is 1:200-1:1000. In some embodiments the dilution range is 1:500-1:1000. In some embodiments the dilution range is 1:1000-1:2000. In some embodiments the dilution range is 1:1500-1:2000. In some embodiments, the dilution is 1:200, 1:300, 1:400, 1:500, 1:600, 1:700, 1:800, 1:900, 1:1000, 1:1500, or 1:2000. In some embodiments, the fluorophore can include but is not limited to AlexaFluor 3, AlexaFluor 5, AlexaFluor 350, AlexaFluor 405, AlexaFluor 430, AlexaFluor 488, AlexaFluor 500, AlexaFluor 514, AlexaFluor 532, AlexaFluor 546, AlexaFluor 555,

AlexaFluor 568, AlexaFluor 594, AlexaFluor 610, AlexaFluor 633, AlexaFluor 647, AlexaFluor 660, AlexaFluor 680, AlexaFluor 700, and AlexaFluor 750 (Molecular Probes AlexaFluor dyes, available from Life Technologies, Inc. (USA)). In some embodiments, the fluorophore can include but is not limited to Cy dyes, including Cy2, Cy3, Cy3B, Cy3.5, Cy5, Cy5.5 and Cy7 (available from GE Life Sciences or Lumiprobe). In some embodiments the fluorophore can include but is not limited to DyLight 350, DyLight 405, DyLight 488, DyLight 550, DyLight 594, DyLight 633, DyLight 650, DyLight 680, DyLight 750 and DyLight 800 (available from Thermo Scientific (USA)). In some embodiments, the fluorophore can include but is not limited to a FluoProbes 390, FluoProbes 488, FluoProbes 532, FluoProbes 547H, FluoProbes 594, FluoProbes 647H, FluoProbes 682, FluoProbes 752 and FluoProbes 782, AMCA, DEAC (7-Diethylaminocoumarin-3-carboxylic acid); 7-Hydroxy-4-methylcoumarin-3; 7-Hydroxycoumarin-3; MCA (7-Methoxycoumarin-4-acetic acid); 7-Methoxycoumarin-3; AMF (4'-(Aminomethyl)fluorescein); 5-DTAF (5-(4,6-Dichlorotriazinyl)aminofluorescein); 6-DTAF (6-(4,6-Dichlorotriazinyl)aminofluorescein); 6-FAM (6-Carboxyfluorescein), 5(6)-FAM cadaverine; 5-FAM cadaverine; 5(6)-FAM ethylenediamme; 5-FAM ethylenediamme; 5-FITC (FITC Isomer I; fluorescein-5-isothiocyanate); 5-FITC cadaverin; Fluorescein-5-maleimide; 5-IAF (5-Iodoacetamidofluorescein); 6-JOE (6-Carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein); 5-CRI 10 (5-Carboxyrhodamine 110); 6-CRI 10 (6-Carboxyrhodamine 110); 5-CR6G (5-Carboxyrhodamine 6G); 6-CR6G (6-Carboxyrhodamine 6G); 5(6)-Carboxyrhodamine 6G cadaverine; 5(6)-Carboxyrhodamine 6G ethylenediamme; 5-ROX (5-Carboxy-X-rhodamine); 6-ROX (6-Carboxy-X-rhodamine); 5-TAMRA (5-Carboxytetramethylrhodamine); 6-TAMRA (6-Carboxytetramethylrhodamine); 5-TAMRA cadaverine; 6-TAMRA cadaverine; 5-TAMRA ethylenediamme; 6-TAMRA ethylenediamme; 5-TMR C6 maleimide; 6-TMR C6 maleimide; TR C2 maleimide; TR cadaverine; 5-TRITC; G isomer (Tetramethylrhodamine-5-isothiocyanate); 6-TRITC; R isomer (Tetramethylrhodamine-6-isothiocyanate); Dansyl cadaverine (5-Dimethylaminonaphthalene-1-(N-(5-aminopentyl))sulfonamide); EDANS C2 maleimide; fluorescamine; NBD; and pyrromethene and derivatives thereof. In some embodiments, the reporter element used can be a donkey anti-goat IgG secondary antibody labeled with AlexaFluor 633.

[0172] In some embodiments, each microcapillary in the microcapillary arrays of the instant screening methods further comprises an agent or agents to improve viability of the cellular expression system. Specifically, the agent or agents is included to prevent cell damage during the step of isolating the contents of the microcapillary of interest, for example

by a laser pulse (see below). In preferred embodiments, the agent is methylcellulose (for example at 0.001 to 10 wt %), dextran (for example at 0.5 to 10 wt %), pluronic F-68 (for example at 0.01 to 10 wt %), polyethylene glycol ("PEG") (for example at 0.01 to 10 wt %), polyvinyl alcohol ("PVA") (for example at 0.01 to 10 wt %), or the like. Alternatively, or in addition, each microcapillary in the microcapillary arrays of the instant screening methods can further comprise a growth additive, such as, for example, 50% conditioned growth media, 25% standard growth media, or 25% serum. In some embodiments, the conditioned growth media is conditioned for 24 hours. In some embodiments, the added agent is insulin, transferrin, ethanolamine, selenium, an insulin-like growth factor, or a combination of these agents or any of the agents recited above.

[0173] The screening methods of the instant disclosure preferably include the further step of measuring a signal from at least one reporter element that indicates association of at least one variant protein with at least one immobilized target molecule to identify at least one microcapillary of interest. In some embodiments, the signal measured is a fluorescent signal, an absorbance signal, a bright-field signal, a dark-field signal, a phase contrast signal, or the like. Accordingly, the measuring step can be performed by an appropriate detector device, for example a device capable of detecting electromagnetic radiation or any other suitable signal. In specific embodiments, the measuring step is performed by a microscope, such as a fluorescence microscope or any other microscope configured to detect the above-mentioned signals.

[0174] It should be understood that in preferred embodiments, the microcapillaries utilized in the instant screening methods do not comprise microparticles capable of inhibiting the transmission of electromagnetic radiation. In other words, the microcapillaries are preferably fully transparent to electromagnetic radiation incident on the microcapillary array, in particular along the longitudinal axes of the microcapillaries. In other preferred embodiments, the microcapillaries of the instant screening methods do not comprise magnetic microparticles or beads. In still other preferred embodiments, the microcapillaries of the instant screening methods do not comprise microparticles capable of inhibiting the transmission of electromagnetic radiation, magnetic microparticles, or magnetic beads.

[0175] In other preferred embodiments, the microcapillaries utilized in the instant screening methods do not comprise an electromagnetic radiation absorbent material. It should be understood, however, that the component of a reporter element responsible generating a measurable signal in the screening method, for example the fluorophore on a fluorescent

antibody, should not be considered an electromagnetic radiation absorbent material for purposes of this aspect of the invention.

[0176] In some embodiments, the instant screening methods further comprise the step of isolating the contents of the microcapillary of interest. In specific embodiments, the contents of the microcapillary of interest are isolated by pulsing the microcapillary of interest with a laser. More specifically, the laser can be a diode laser or a diode-pumped Q-switched Nd:YLF laser. In some embodiments, the laser can be directed at the water-glass interface between the microcapillary wall and the sample contained in the microcapillary. Without intending to be bound by theory, it is believed that firing a UV laser at this interface can break the meniscus/water surface tension that normally holds a sample in the microcapillary, thus allowing the sample to fall out of the array via the force of gravity. In other embodiments, the contents of the microcapillary of interest are isolated by laser-triggered vapor force expansion. In some embodiments, the contents of the microcapillary are isolated by breaking the glass of the microcapillary itself.

[0177] In some embodiments, the microcapillary screening methods of the instant invention allow for screening reactions and/or interactions (including binding interactions) that occur between the variant protein and the target molecule within minutes of the addition of the components to the microcapillary. In some embodiments, the reactions and/or interactions between the variant protein and the target molecule occur and/or are detectable within about 1 minute to about 10 minutes. In some embodiments, the reactions and/or interactions between the variant protein and the target molecule occur and/or are detectable within about 1 hour to about 6 hours. In some embodiments, the reactions and/or interactions occur within about 1 hour, about 2 hours, about 4 hours, about 6, about 10 hours, about 12 hours, about 16 hours, about 24 hours, about 36 hours, or about 48 hours. In some embodiments, the reactions and/or interactions between the variant protein and the target molecule occur and/or are detectable within a period of time such that the cells within the microcapillary are alive and healthy. In some embodiments, the reactions and/or interactions between the variant protein and the target molecule occur and/or are detectable within a period of time such that the cells within the microcapillary are viable. In some embodiments, the cells can be grown after removal from the microcapillary and/or microcavity. In some embodiments, the cells are viable after removal from the microcapillary and/or microcavity. In some embodiments, the reactions and/or interactions between the variant protein and the target molecule occur within the microcapillary.

Systems for Screening

[0178] According to another aspect of the invention are provided systems for screening a population of variant proteins comprising:

an array comprising a plurality of microcapillaries, each microcapillary comprising a variant protein, an immobilized target molecule, and a reporter element, wherein the variant protein associates with the immobilized target molecule with a particular affinity. The components of these screening devices are described in detail above and any of those can be incorporated into a system for screening.

[0179] In some embodiments, the screening systems further comprise an optical source and a detector. The optical source and detector are chosen according to the particular reporter element used in the screening system. For example, where the reporter element generates a fluorescent signal, the optical source provides excitation light of an appropriate wavelength to excite the fluorescent probe. Likewise, the detector is chosen to be sensitive to the wavelength of light emitted by the fluorescent probe. The optical source and the detector may, for example, be components of a microscope, such as a fluorescent microscope, or they may be separate devices, as would be understood by those of ordinary skill in the art.

[0180] In some embodiments, the screening system further comprises an extraction device, for example a diode laser, a diode-pumped Q-switched laser, or other appropriate component for isolating the contents of a microcapillary of interest.

[0181] An exemplary microscope for screening populations of variant proteins according to the instant methods is illustrated in the drawings of FIGs. 6A-6E. FIG. 6A shows a perspective view from above the microscope, illustrating both the array-holding stage and the sample-recovery stage. FIG. 6B shows a front view of the device. FIG. 6C shows a view from the right side. A magnified view of the right side of the device is provided in FIG. 6D, which illustrates in detail the relationship between the array-holding stage and the sample-recovery stage. FIG. 6E provides an exploded view of various components of a multi-stage sample-recovery microscope suitable for use in the instant screening systems.

[0182] It will be readily apparent to one of ordinary skill in the relevant arts that other suitable modifications and adaptations to the methods and applications described herein can be made without departing from the scope of the invention or any embodiment thereof.

Having now described the present invention in detail, the same will be more clearly understood by reference to the following Examples, which are included herewith for purposes of illustration only and are not intended to be limiting of the invention. In some embodiments, any aspects disclosed under the methods section above are also readily

applicable to the systems of the instant invention. The systems of the invention can be employed with any of the methods described herein.

Microcavity Array

[0183] In these methods, the microcavity arrays comprise any array which comprises individual chambers and which allows for the transmission of light through the array and onto a detector. In some embodiments, the arrays are microcapillary arrays. In some embodiments the microcapillary arrays comprise a plurality of longitudinally fused capillaries, for example fused silica capillaries, although any other suitable material may be utilized in the arrays. See, *e.g.*, the arrays described U.S. Application No. 62/433,210, filed December 12, 2016, US Application No. 15/376,588, filed on December 12, 2016, PCT International Patent Publication Nos. WO 2012/007537 and WO 2014/008056, the disclosures all of which are incorporated by reference herein in their entireties.

[0184] Such arrays can be fabricated, for example, by bundling millions or billions of silica capillaries and fusing them together through a thermal process, although other suitable methods of fabrication may also be employed. The fusing process may comprise, for example, the steps of i) heating a capillary single draw glass that is drawn under tension into a single clad fiber; ii) creating a capillary multi draw single capillary from the single draw glass by bundling, heating, and drawing; iii) creating a multi-draw multi-capillary from the multi-draw single capillary by additional bundling, heating, and drawing; iv) creating a block assembly of drawn glass from the multi-multi-draw multi-capillary by stacking in a pressing block; v) creating a block pressing block from the block assembly by treating with heat and pressure; and vi) creating a block forming block by cutting the block pressing block at a precise length (*e.g.*, 1 mm).

[0185] In some embodiments, the fabrication method further comprises slicing the silica capillaries, thereby forming very high-density glass microcapillary arrays. In some embodiments, the microcapillary arrays may be cut to approximately 1 millimeter in height, but even shorter microcapillary arrays are contemplated, including arrays of 10 μm in height or even shorter. In some embodiments, even longer microcapillary arrays are contemplated, including arrays of 10 mm or even longer.

[0186] Such processes form very high-density microcapillary arrays that are suitable for use in the present methods. In an exemplary array, each microcapillary has an approximate 5 μm diameter and approximately 66% open space (*i.e.*, representing the lumen of each

microcapillary). In some arrays, the proportion of the array that is open ranges from between about 50% and about 90%, for example about 60% to about 75%, such as a microcapillary array provided by Hamamatsu that has an open area of about 67%. In one particular example, a 10x10 cm array having 5 μm diameter microcapillaries and approximately 66% open space has about 330 million total microcapillaries.

[0187] In various embodiments, the internal diameter of each microcapillary in the array ranges from between approximately 1 μm and 500 μm . In some arrays, each microcapillary can have an internal diameter in the range between approximately 1 μm and 300 μm ; optionally between approximately 1 μm and 100 μm ; further optionally between approximately 1 μm and 75 μm ; still further optionally between approximately 1 μm and 50 μm ; and still further optionally between approximately 5 μm and 50 μm .

[0188] In some microcapillary arrays, the open area of the array comprises up to 90% of the open area (OA), so that, when the pore diameter varies between 1 μm and 500 μm , the number of microcapillaries per cm of the array varies between approximately 460 and over 11 million. In some microcapillary arrays, the open area of the array comprises about 67% of the open area, so that, when the pore size varies between 1 μm and 500 μm , the number of microcapillaries per square cm of the array varies between approximately 340 and over 800,000. In some embodiments, the number of microcapillaries per square cm of the array is approximately 400; 800; 1000; 2000; 4000; 5000; 10,000; 25,000; 50,000; 75,000; 100,000; 200,000; 300,000; 400,000; 500,000; 600,000; 700,000; 800,000; 900,000; 1,000,000 or more.

[0189] In one particular embodiment, a microcapillary array can be manufactured by bonding billions of silica capillaries and then fusing them together through a thermal process. After that slices (0.5 mm or more) are cut out to form a very high aspect ratio glass microcapillary array. Arrays are also commercially available, such as from Hamamatsu Photonics K. K. (Japan), Incom, Inc. (Massachusetts), Photonis Technologies, S.A.S. (France) Inc., and others. In some embodiments, the microcapillaries of the array are closed at one end with a solid substrate attached to the array.

[0190] The microcapillary arrays of the instant screening methods can comprise any number of microcapillaries within the array. In some embodiments, the microcapillary array comprises at least 100,000, at least 300,000, at least 1,000,000, at least 3,000,000, at least 10,000,000, or even more microcapillaries. In some embodiments, the microcapillary array comprises at least 100,000. In some embodiments, the microcapillary array comprises at least 200,000 microcapillaries. In some embodiments, the microcapillary array comprises at least

300,000 microcapillaries. In some embodiments, the microcapillary array comprises at least 400,000 microcapillaries. In some embodiments, the microcapillary array comprises at least 500,000 microcapillaries. In some embodiments, the microcapillary array comprises at least 600,000 microcapillaries. In some embodiments, the microcapillary array comprises at least 700,000 microcapillaries. In some embodiments, the microcapillary array comprises at least 800,000 microcapillaries. In some embodiments, the microcapillary array comprises at least 900,000 microcapillaries. In some embodiments, the microcapillary array comprises at least 1,000,000 microcapillaries. In some embodiments, the microcapillary array comprises at least 2,000,000 microcapillaries. In some embodiments, the microcapillary array comprises at least 3,000,000 microcapillaries. In some embodiments, the microcapillary array comprises at least 4,000,000 microcapillaries. In some embodiments, the microcapillary array comprises at least 5,000,000 microcapillaries. In some embodiments, the microcapillary array comprises at least 10,000,000 microcapillaries. In some embodiments, the microcapillary array comprises at least 15,000,000 microcapillaries. In some embodiments, the microcapillary array comprises at least 20,000,000 microcapillaries. The number of microcapillaries within an array is preferably chosen in view of the size of the variant protein library to be screened.

[0191] The microcavity arrays are about 0.2 mm (200 μ m) to about 1 mm thick and about 50 μ m to about 200 μ m in diameter. In some embodiments, the microcavity arrays are about 1.5 mm thick and about 150 μ m in diameter. In some embodiments, the microcavity arrays are about 2 mm thick and about 200 μ m in diameter. In some embodiments, the microcavity arrays are about 1 mm thick and about 100 μ m in diameter. In some embodiments, the microcavity arrays are about 1 mm thick and about 10 μ m in diameter. In some embodiments, the microcavity arrays are about 1 μ m, 5 μ m, and/or 10 μ m in diameter. In some embodiments, the microcavity arrays are about 10 μ m in diameter.

[0192] A variety of microcavity arrays can find use in the present methods. Exemplary microcavity array sizes are provided herein. In some embodiments, the microcavities within the arrays are about 50 μ m to about 200 μ m in diameter. In some embodiments, the microcavities within the arrays are about 75 μ m to about 150 μ m in diameter. In some embodiments, the microcavities within the arrays are about 75 μ m to about 125 μ m in diameter. In some embodiments, the microcavities within the arrays are about 75 μ m to about 110 μ m in diameter. In some embodiments, the microcavities within the arrays are about 80 μ m to about 110 μ m in diameter. In some embodiments, the microcavities within the arrays are about 75 μ m to about 150 μ m in diameter. In some embodiments, the microcavities

within the arrays are about 80 μm , about 90 μm , about 100, or about 110 μm in diameter. In some embodiments, the microcavities within the arrays are about 100 μm in diameter

[0193] A variety of microcavity arrays can find use in the present methods. Exemplary sample volumes are provided herein. In some embodiments, the sample volume in each microcavity is less than about 500 nL. In some embodiments, the sample volume in each microcavity is about 5 nL to about 500 nL. In some embodiments, the volume in each microcavity is about 5 nL to about 400 nL. In some embodiments, the volume in each microcavity is about 5 nL to about 300 nL. In some embodiments, the volume in each microcavity is about 5 nL to about 200 nL. In some embodiments, the volume in each microcavity is about 5 nL to about 100 nL. In some embodiments, the volume in each microcavity is about 5 nL to about 90 nL. In some embodiments, the volume in each microcavity is about 5 nL to about 80 nL. In some embodiments, the volume in each microcavity is about 5 nL to about 70 nL. In some embodiments, the volume in each microcavity is about 5 nL to about 60 nL. In some embodiments, the volume in each microcavity is about 5 nL to about 50 nL. In some embodiments, the volume in each microcavity is about 5 nL to about 40 nL. In some embodiments, the volume in each microcavity is about 5 nL to about 30 nL. In some embodiments, the volume in each microcavity is about 5 nL to about 20 nL. In some embodiments, the volume in each microcavity is about 5 nL to about 10 nL. In some embodiments, the volume in each microcavity is about 5 nL to about 8 nL. In some embodiments, the volume in each microcavity is about 7 nL to about 8 nL. In some embodiments, the volume in each microcavity is about 7.8 nL. In some embodiments, the volume in each microcavity is about 70 pL to about 100 pL. In some embodiments, the volume in each microcavity is about 70 pL to about 90 pL. In some embodiments, the volume in each microcavity is about 70 pL to about 80 pL. In some embodiments, the volume in each microcavity is about 78.5 pL. In some embodiments, the volume in each microcavity is about 150 fL to about 1000 fL. In some embodiments, the volume in each microcavity is about 200 fL to about 1000 fL. In some embodiments, the volume in each microcavity is about 300 fL to about 1000 fL. In some embodiments, the volume in each microcavity is about 400 fL to about 900 fL. In some embodiments, the volume in each microcavity is about 500 fL to about 800 fL. In some embodiments, the volume in each microcavity is about 150 fL to 200 fL. In some embodiments, the volume in each microcavity is about 157 fL.

[0194] In some embodiments, each microcavity in the microcavity arrays of the instant screening methods further comprises an agent or agents to improve viability of the cellular

expression system when cellular expression assays are used. Specifically, the agent or agents is included to prevent cell damage during the step of isolating the contents of the microcapillary of interest, for example by a laser pulse (see below). In preferred embodiments, the agent is methylcellulose (for example at 0.001 wt % to 10 wt %), dextran (for example at 0.5 wt % to 10 wt %), pluronic F-68 (for example at 0.01 wt % to 10 wt %), polyethylene glycol ("PEG") (for example at 0.01 wt % to 10 wt %), polyvinyl alcohol ("PVA") (for example at 0.01 wt % to 10 wt %), or the like. Alternatively, or in addition, each microcapillary in the microcapillary arrays of the instant screening methods can further comprise a growth additive, such as, for example, 50% conditioned growth media, 25% standard growth media, or 25% serum. In some embodiments, the conditioned growth media is conditioned for 24 hours. In some embodiments, the added agent is insulin, transferrin, ethanolamine, selenium, an insulin-like growth factor, or a combination of these agents or any of the agents recited above.

It should also be understood that the concentrations of each component of the screening assay within a microcavity can be modulated as desired in an assay in order to achieve an optimal outcome. In particular, it may be desirable to modulate the concentration of proteins, polypeptides, nucleic acids, small molecules, and/or cells to achieve the desired level of association between these components. The level of association will also depend on the particular affinity between these components, wherein a higher affinity results in a higher level of association for a given concentration of the components, and a lower affinity results in a lower level of association of the components for a given concentration. Concentration of various components may likewise be modulated in order to achieve optimum levels of signal output, as would be understood by those of ordinary skill in the art.

Samples and/or Library Components

[0195] Libraries that can be screened according to the present methods include any library comprising a plurality of molecules as well as mixtures and/or combinations thereof. In some embodiments, libraries comprise samples comprising biological material. In some embodiments, the library comprises samples comprising a plurality of one or more molecules and/or cells as well as mixtures and/or combinations thereof. In some embodiments, the library comprises samples comprising a plurality of one or more proteins (including antibodies), polypeptides, nucleic acids, small molecules, dyes, and/or cells as well as mixtures and/or combinations thereof. In some embodiments, molecules include any

molecule. In some embodiments, molecules include but are not limited to proteins, polypeptides, nucleic acids, small molecules, and/or dyes as well as mixtures and/or combinations thereof. In some embodiments, libraries comprise samples comprising biological materials that comprise polypeptides, nucleic acids, small molecules, and/or cells as well as mixtures and/or combinations thereof. In some embodiments, libraries comprise samples. In some embodiments, samples include but are not limited to biological materials that comprise polypeptides, nucleic acids, small molecules, dyes, and/or cells as well as mixtures and/or combinations thereof. In some embodiments, samples contain a least one molecules and/or cell to be screened. In some embodiments, samples contain a least one to ten molecules and/or cells to be screened as well as mixtures and/or combinations thereof. In some embodiments, samples contain a plurality of molecules and/or cells to be screened as well as mixtures and/or combinations thereof. In some embodiments, the molecule to be screened is termed a target molecule. In some embodiments, the cell to be screened is termed a target cell.

[0196] The arrays provided herein allow for screening of libraries made up of proteins, polypeptides, nucleic acid, small molecules, dyes, and/or cells as well as mixtures and/or combinations thereof. In some embodiments, the target molecule to be screened is a protein, polypeptide, nucleic acid, small molecule, dye, carbohydrate, lipid, or a combination of two or more of these target molecules. In some embodiments, the proteins and/or polypeptides are selected from the group consisting of enzymes, ligands, and receptors. For example, in some embodiments the target molecule can be a lipid-modified or glycosylated protein. In some embodiments, the target molecule is a native protein.

[0197] As described above, each capillary in the microcavity array used in the instant screening methods will contain different sample components. Such sample components can include, but are not limited to proteins, polypeptides, nucleic acids, small molecules, dyes, and/or cells (*i.e.*, target molecules and/or target cells) as well as mixtures and/or combinations thereof. In some embodiments, the library for screening comprises the variant protein, variant polypeptide, variant nucleic acid, variant small molecule, variant dye, and/or variant cells exhibiting distinguishing characteristics. In some embodiments, the variant protein, variant polypeptide, variant nucleic acid, variant small molecule, variant dye, and/or variant cells exhibit distinguishing characteristics, such that each microcavity comprises a sample that comprises a different target molecule and/or target cell from the sample found in each of the other microcavities within the array. In some embodiments, one or more microcavities within the array comprise a sample that comprises the same target molecule and/or target cell as a

sample found in at least one other microcavity within the array (*e.g.*, as duplicates for comparison).

In some embodiments, the proteins and/or polypeptides in the library to be screened in the microcavity array can be variant proteins and/or polypeptides. Variant proteins include proteins and polypeptides which are distinguishable from one another based on at least one characteristic or feature. In some embodiments, the variant proteins and/or polypeptides exhibit different amino acid sequences, exhibit different amino acid sequence lengths, are produced/generated by different methods, exhibit different activities, exhibit different chemical modifications, and/or exhibit different post-translational modifications. In some embodiments, the variant proteins and/or polypeptides exhibit different amino acid sequences. In some embodiments, the variant proteins and/or polypeptides exhibit different amino acid sequence lengths. In some embodiments, the variant proteins and/or polypeptides are produced/generated by different methods. In some embodiments, the variant proteins and/or polypeptides exhibit different activities. In some embodiments, the variant proteins and/or polypeptides exhibit different chemical modifications. In some embodiments, the variant proteins and/or polypeptides exhibit different post-translational modifications. In some embodiments, the variant protein is one of a population of variant proteins and/or polypeptides that is being subjected to the screening method and analyzed using the microcavity arrays disclosed herein. The population of variant proteins and/or polypeptides can be any population of proteins that can be suitably distributed within a microcapillary array.

[0198] In some embodiments, the nucleic acids in the library to be screened in the microcavity array can be variant nucleic acids. Variant nucleic acids include nucleic acids which are distinguishable from one another based on at least one characteristic or feature. In some embodiments, the variant nucleic acids have different nucleotide sequences, have different nucleotide sequence lengths, have been produced/generated by different methods, have different methylation patterns, have different chemical modifications, and/or exhibit other distinguishing modifications. In some embodiments, the variant nucleic acids have different nucleotide sequences. In some embodiments, the variant nucleic acids have different nucleotide sequence lengths. In some embodiments, the variant nucleic acids have been produced/generated by different methods. In some embodiments, the variant nucleic acids have different methylation patterns. In some embodiments, the variant nucleic acids have different chemical modifications. In some embodiments, the variant nucleic acids exhibit other distinguishing modifications. In some embodiments, the nucleic acid is one of a

population of variant nucleic acids that is being subjected to the screening method and analyzed using the microcavity arrays disclosed herein. The population of variant nucleic acids can be any population of nucleic acids that can be suitably distributed within a microcapillary array.

[0199] In some embodiments, the small molecules in the library to be screened in the microcavity array can be variant and/or different small molecules. Variant small molecules include small molecules which are distinguishable from one another based on at least one characteristic or feature. In some embodiments, the variant small molecules have different structures, have been produced/generated by different methods, have different chemical modifications, and/or exhibit other distinguishing different features. In some embodiments, the variant small molecules have different structures. In some embodiments, the variant small molecules have been produced/generated by different methods. In some embodiments, the variant small molecules have different chemical modifications. In some embodiments, the variant small molecules exhibit other distinguishing different features. In some embodiments, the small molecules are derivatives of one another. In some embodiments, the small molecule is one of a population of small molecules that is being subjected to the screening method and analyzed using the microcavity arrays disclosed herein. The population of small molecules can be any population of small molecules that can be suitably distributed within a microcapillary array.

[0200] In some embodiments, the cells in the library to be screened in the microcavity array can be variant cells and/or cells of varying types. Variant cells include cells which are distinguishable from one another based on at least one characteristic or feature. In some embodiments, the cells are derived from different samples, are derived from different patients, are derived from different diseases, have different chemical modifications, and/or have been genetically modified. Cells can include eukaryotic and prokaryotic cells. In some embodiments, the cells are derived from different samples. In some embodiments, the cells are derived from different patients. In some embodiments, the cells are derived from different diseases. In some embodiments, the cells have different chemical modifications. In some embodiments, the cells have been genetically modified. In some embodiments, the cells can include human cells, mammalian cells, bacterial cells, avian cells, including chicken cells, and fungal cells, including yeast cells. In some embodiments, the cells can include human cells. In some embodiments, the cells can include mammalian cells. In some embodiments, the cells can include avian cells. In some embodiments, the cells can include chicken cells. In some embodiments, the cells can include bacterial cells. In some embodiments, the cells can

include fungal cells. In some embodiments, the cells can include yeast cells. In some embodiments, the cells can include chicken cells. In some embodiments, the cell is one of a population of cells that is being subjected to the screening method and analyzed using the microcavity arrays disclosed herein. The population of cells can be any population of cells that can be suitably distributed within a microcapillary array.

[0201] In some embodiments, the population of proteins, polypeptides, nucleic acid, and/or cells is distributed in the microcavity array so that each microcavity comprises a small number of different variant proteins, variant polypeptides, variant nucleic acid, and/or cells. In some embodiments, each microcavity comprises a single different variant protein, variant polypeptide, variant nucleic acid, and/or cell per microcavity. In some embodiments, each microcavity comprises a single different variant protein per microcavity. In some embodiments, each microcavity comprises a single different variant polypeptide per microcavity. In some embodiments, each microcavity comprises a single different variant nucleic acid per microcavity. In some embodiments, each microcavity comprises a single different cell per microcavity. The population of variant proteins, variant polypeptides, variant nucleic acid, and/or cells is chosen in combination with the other components within the composition.

[0202] In some embodiments, each microcavity in the microcavity array comprises 0 to 5 different variant proteins, variant polypeptides, variant nucleic acid, and/or cells from the population of variant proteins. In some embodiments, each microcavity in the microcavity array comprises 0 to 4, 0 to 3, 0 to 2, or even 0 to 1 different variant proteins from the population of variant proteins, variant polypeptides, variant nucleic acid, and/or cells. Accordingly, in some embodiments, the variant proteins are soluble proteins, for example soluble proteins that are secreted by a cellular expression system. Exemplary soluble variant proteins include antibodies and antibody fragments, alternative protein scaffolds, such as disulfide-bonded peptide scaffolds, extracellular domains of cell-surface receptor proteins, receptor ligands, such as, for example, G-protein coupled receptor ligands, other peptide hormones, lectins, and the like. In some embodiments, the variant proteins screened using the instant methods do not need to be covalently attached to the cell or virus that expresses them in order to be identified following a screening assay. Isolation of the contents of the desired microcapillary, followed by propagation of the cell or virus clone responsible for expression of the desired variant protein, thereby enables the identification and characterization of that variant protein. Unlike screening assays where a variant protein of interest is displayed by fusion of the protein to a molecule on the surface of a cell or virus particle, the variant

proteins identified in the instant screening methods need not be altered in any way either before or after their identification. The observed activities of the variant proteins in the screens are thus more likely to represent the actual activities of those proteins in their subsequent applications. Not needing to alter variant proteins or polypeptides prior to screening also allows for more efficient screening, saving costs and time for library preparation.

[0203] In some embodiments, the variant proteins to be screened are membrane-associated proteins, for example proteins typically associated with the surface of a cell or a viral particle in an expression system. Screening of cell-associated variant proteins may be desirable where the variant protein and its target molecule mediate interactions between two cells within a biological tissue. The ability to screen cell-associated variant proteins may also be desirable in screening for interactions with traditionally “non-druggable” protein targets, such as, for example, G-protein coupled receptors or ion channels. Again, not needing to alter variant proteins or polypeptides prior to screening also allows for more efficient screening, saving costs and time for library preparation.

[0204] In some embodiments, the variant nucleic acids to be screened include any nucleic acid or polynucleotide, including nucleic acids or polynucleotides that bind to or interact with proteins. Again, not needing to alter the nucleic acids or polynucleotides prior to screening also allows for more efficient screening, saving costs and time for library preparation.

[0205] In some embodiments, the protein to be screened is an antibody, antibody fragment, such as an Fc, or an antibody fusion, including for example Fc fusions. In some embodiments, the antibody or antibody fragment can be labeled.

In some embodiments, the method employs the use of an antibody to bind to the target molecule to be screened. In some embodiments, the antibody is a labeled primary antibody or a labeled secondary antibody as is used to bind to the target molecules. A primary antibody is typically considered to be an antibody that binds directly to an antigen of interest, whereas a secondary antibody is typically considered to be an antibody that binds to a constant region on a primary antibody for purposes of labeling the primary antibody. Accordingly, secondary antibodies are frequently labeled with fluorophores or other detectable labels or are labeled with enzymes that are capable of generating detectable signals. They are generally specific for a primary antibody from a different species. For example, a goat or other animal species may be used to generate secondary antibodies against a mouse, chicken, rabbit, or nearly any primary antibody other than an antibody from that animal species, as is understood by those of ordinary skill in the art. In some embodiments, the labeled antibody is a primary or

secondary antibody. In some embodiments, the labeled antibody is a fluorescent antibody or an enzyme-linked antibody.

[0206] As would be understood by those of ordinary skill in the art, when a fluorescent antibody, for example is used in the instant screening methods, the signal emitted by any excess reporter element remaining free in solution (*i.e.*, either not bound to a variant protein or bound to a variant protein that is not bound to a target molecule) within the microcavity should not be so high that it overwhelms the signal of reporter elements associated with a target molecule via a variant protein (see, *e.g.*, the unassociated fluorescent antibodies). Such background signals can be minimized, however, by limiting the concentration of labeled antibody or other reporter element within the microcapillary solution. In addition, where signals from the screening methods are measured using a fluorescent microscope, configuring the microscope to image a relatively narrow depth of field bracketing the location of the target molecules (*e.g.*, the bottom of the microcapillaries when target cells have settled there by gravitational sedimentation) can minimize the background signal from reporter elements not associated with the target molecule.

[0207] In some embodiments, the reporter element is part of a reporter assay. In some embodiments, the reporter assay can include but is not limited to a calcium dye assay, a T cell activation assay, a B cell assay, and a GFP assay. In some embodiments, the reporter assay is selected from the group consisting of a calcium dye assay, a T cell activation assay, a B cell assay, and a GFP assay. In some embodiments, the reporter assay is a calcium dye assay. In some embodiments, the reporter assay is a T cell activation assay. In some embodiments, the reporter assay is a B cell assay. In some embodiments, the reporter assay is a GFP assay.

[0208] In some embodiments, purified or enriched populations of cells may be used in the reporter assays. In some embodiments, the source of the cells may be an organ, tissue, tumor, or liquid from an animal, including but not limited to a human. The cell source may be from a variety of sources with different preferences for each animal. In some embodiments, the cells are immune cells. In some embodiments, the cells are T cells. In some embodiments, the cells are B cells. In some embodiments, the cells are subtypes of T cells. In some embodiments, the cells are subtypes of B cells. In some embodiments, the cells are NK cells. In some embodiments, the cells are phagocytes. In some embodiments, the cells are macrophages. In some embodiments, the cells are neutrophils. In some embodiments, the cells are mast cells. In some embodiments, the cells are monocytes. In some embodiments, the cells are subtypes of any of the cell types listed herein. Purification or enrichment of cell populations can be performed using a variety of methods, including, but not limited to density gradient

separation, immunodensity cell isolation, immunomagnetic cell separation, fluorescent activated cell sorting (FACS), and microfluidic cell sorting. Other forms of purification or enrichment may include treating a heterogeneous cell population with factors which modify cell growth or proliferation of one cell type over another. In some embodiments, for example, in isolating cells from tissues or tumors, a prior step in which the tissue is disaggregated, homogenized and/or filtered may be necessary to assist in purification.

[0209] For example, in one embodiment, B cells are enriched from mouse splenocytes by isolating CD138⁺ cells using a commercially available kit. Splenocytes were incubated with CD138-coated magnetic microbeads. In some embodiments, the mixture was loaded onto a magnetic column and unbound cells were washed out. The magnetically labeled cells were then eluted from the column and washed with warmed PBMC media. The washed CD138⁺ cells were immediately used for binding screening. In some embodiments, a Pan B cell kit may be used (commercially available from Miltenyi Biotec).

[0210] In some embodiments, the reporter assay is a B cell assay. In some embodiments, the source of the B cells may be an organ, tissue, tumor, or liquid from an animal, including but not limited to a human. The B cell source used for providing cells for a B cell assay may be from a variety of sources with different preferences for each animal. In some embodiments, the source providing the B cells for a B cell assay is the spleen. Representative hits from an example of a B cell assay are found in Figure 25.

[0211] In some embodiments, the reporter assay is a T cell activation assay. In some embodiments, the source of the T cells may be an organ, tissue, tumor, or liquid from an animal, including but not limited to a human. The T cell source used for providing cells for a T cell activation assay may be from a variety of sources with different preferences for each animal. In some embodiments, the source providing the T cells for a T cell assay is peripheral blood. In some embodiments, the source providing the T cells can include but is not limited to spleen, tonsil, bone marrow or expanded lymphoid progenitors, and induced pluripotent stem cell (iPSC)-derived T lymphocytes. In some embodiments, the source providing the T cells are selected from the group consisting of spleen, tonsil, bone marrow or expanded lymphoid progenitors, and induced pluripotent stem cell (iPSC)-derived T lymphocytes.

In some embodiments, the source providing the T cells is the spleen. In some embodiments, the source providing the T cells is the tonsil. In some embodiments, the source providing the T cells is the bone marrow. In some embodiments, the source providing the T cells are expanded lymphoid progenitors. In some embodiments, the source providing the T cells are induced pluripotent stem cell (iPSC)-derived T lymphocytes. Some embodiments of T cell

activation assays are found in Figure 26, Figure 27, and Figure 28. In some embodiments, T cell activation assays are used to evaluate the cell signaling induction capability of an antibody. In some embodiments, capability is determined by looking at an antibody-induced response compared to baseline. In some embodiments, capability is determined by looking at an antibody-induced response compared to a control. In some embodiments, baseline is determined using a negative control. In some embodiments, a negative control is a control with no stimulation. In some embodiments, a negative control is a non-functional antibody. In some embodiments, an antibody-induced response is an increase of about 10% or greater. In some embodiments, an antibody-induced response is an increase greater than or equal to 10%. In some embodiments, an antibody-induced response is an increase greater than or equal to 10% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 20% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 30% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 40% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 50% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 60% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 70% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 80% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 90% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 100% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 200% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 300% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 400% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 500% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 600% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 700% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or

equal to 800% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 900% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 1000% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 2,000% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 3,000% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 4,000% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 5,000% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 6,000% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 7,000% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 8,000% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 9,000% as compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase greater than or equal to 10,000% as compared to baseline and/or a control. In some embodiments, antibody-induced response is an increase between 10% and 10,000% when compared to baseline and/or a control. In some embodiments, antibody-induced response is an increase between 100% and 1,000% when compared to baseline and/or a control. In some embodiments, antibody-induced response is an increase between 10% and 100% when compared to baseline and/or a control. In some embodiments, antibody-induced response is an increase between 100% and 5,000% when compared to baseline and/or a control. In some embodiments, an antibody-induced response is an increase between 10% and 1000%. In some embodiments, the increase in the signal used to identify the least one microcapillary of interest represents a statistically significant increase when compared to baseline and/or a control. In some embodiments, the response is a statistically significant response, as measured using for example, a one-way ANOVA or T test or other standard statistical parameter, and when compared to baseline and/or a control. In some embodiments, T cell activation assays are used to measure internal signaling or cell surface marker induction capability of an antibody. In some embodiments, T cell activation assays are used to evaluate an antibody's ability to induce cell expansion. In some embodiments, T cell activation assays are used to evaluate cytokine secretion induction capability of an antibody. In some embodiments, T cell

activation assays measure CD25 expression to determine activation. In some embodiments, T cell activation assays use a fluorescently labeled anti-CD25 antibody to measure CD25 expression. In some embodiments, T cell activation assays measure calcium signaling to determine activation capability of an antibody. In some embodiments, T cell activation assays use a calcium-sensitive fluorophore to measure calcium signaling. Some examples of calcium-sensitive fluorophores include Fluo-4 AM, Fura-2 AM, and Indo-1 AM. In some embodiments, calcium dye assays have a dynamic range of 1:20000. In some embodiments, calcium dye assays have a dynamic range of 1:5000. In some embodiments, calcium dye assays have a dynamic range of 1:10000. In some embodiments, calcium dye assays have a dynamic range of 1:15000. In some embodiments, the T cell activation assay comprises a mixture of T cells and antibody secreting cells (ASC). In some embodiments, the mixture of T cells and ASC varies from a ratio of 2:1 to 12:1. In some embodiments, the mixture of T cells and ASC is a ratio of 5:1. In some embodiments, the T cells are purified from peripheral blood. In some embodiments, the ASC are B cells. In some embodiments, the mixture of T cells and ASC further comprises T cell activating beads and antibody capture beads.

EXEMPLARY EMBODIMENTS:

[0212] The present application provides a method of screening a population of variant proteins comprising the steps of:

providing a microcapillary array comprising a plurality of microcapillaries, each microcapillary comprising a variant protein, an immobilized target molecule, and a reporter element, wherein the variant protein associates with the immobilized target molecule with a particular affinity; and

measuring a signal from at least one reporter element that indicates association of at least one variant protein with at least one immobilized target molecule to identify at least one microcapillary of interest.

[0213] In some embodiments, the variant protein is expressed by an expression system.

[0214] In some embodiments, the expression system is a cell-free expression system.

[0215] In some embodiments, the expression system is a cellular expression system.

[0216] In some embodiments, the cellular expression system is an animal system, an avian system, a fungal system, a bacterial system, an insect system, or a plant system.

[0217] In some embodiments, the cellular expression system is an avian system.

[0218] In some embodiments, the cellular expression system is a chicken system.

- [0219] In some embodiments, the variant protein is a soluble protein.
- [0220] In some embodiments, the target molecule is a target protein or polypeptide, a target nucleic acid, a target carbohydrate, or a combination of each.
- [0221] In some embodiments, the target molecule is immobilized on a surface.
- [0222] In some embodiments, the surface is a surface of a cell.
- [0223] In some embodiments, the target molecule is a native protein.
- [0224] In some embodiments, the surface is a surface of a bead.
- [0225] In some embodiments, the surface is a surface of a microcapillary wall.
- [0226] In some embodiments, the surface is a surface configured to settle in the microcapillary by gravitational sedimentation.
- [0227] In some embodiments, the reporter element is a labeled antibody or other binding molecule.
- [0228] In some embodiments, the labeled antibody or other binding molecule is a fluorescently-labeled antibody or other binding molecule.
- [0229] In some embodiments, the labeled antibody is a primary or a secondary antibody.
- [0230] In some embodiments, the labeled antibody or other binding molecule is an enzyme-linked antibody or other binding molecule.
- [0231] In some embodiments, the reporter element is activated within a cell, and the target molecule is immobilized on a surface of the cell.
- [0232] In some embodiments, the reporter element comprises a green fluorescent protein or variant.
- [0233] In some embodiments, the signal is a fluorescent signal, an absorbance signal, a bright-field signal, or a dark-field signal.
- [0234] In some embodiments, each microcapillary in the microcapillary array comprises 0 to 5 variant proteins from the population of variant proteins.
- [0235] In some embodiments, microcapillary array comprises at least 100,000, at least 300,000, at least 1,000,000, at least 3,000,000, or at least 10,000,000 microcapillaries.
- [0236] In some embodiments, each microcapillary further comprises an agent to improve viability of the cellular expression system.
- [0237] In some embodiments, the agent is methylcellulose, dextran pluronic F-68, polyethylene glycol, or polyvinyl alcohol.
- [0238] In some embodiments, the agent is a growth medium.
- [0239] In some embodiments, the signal is measured by an optical detector.
- [0240] In some embodiments, the signal is measured by a microscope.

[0241] In some embodiments, the method further comprises the step of isolating the contents of the microcapillary of interest.

[0242] In some embodiments, the contents of the microcapillary of interest are isolated by pulsing the microcapillary of interest with a laser.

EXAMPLES

Example 1. Screening for a Secreted EGFR-binding Protein

[0243] FIG. 1A-FIG. 1C illustrate an exemplary screening method for a soluble protein capable of associating with a cell-surface protein (*e.g.*, the epidermal growth factor receptor (“EGFR”)) as the immobilized target molecule, in this case an immobilized target protein. FIG. 1A (left panel) shows the target cell, which expresses EGFR on its surface. Also shown is a “library expressing cell”, which expresses a population of variant proteins, and a number “fluorescent detection antibodies” in the microcapillary solution. A bottom view of the microcapillary array is illustrated in the right panel.

[0244] Components of each microcapillary according to this screening assay:

1. Cells secreting the variant protein of interest (the “library expressing cell”). The variant protein of interest is preferably a member of a population of variant proteins, *i.e.*, a protein library.
2. Target protein immobilized on a surface of a “target cell”. In this example, the target protein is a native, cell-surface receptor (*i.e.*, EGFR). Alternatively, however, the target protein could be immobilized on another surface, such as a bead surface or a surface of the microcapillary itself.
3. Reporter element
 - a. In this example, the reporter element corresponds to a fluorescently-labeled antibody specific for the secreted protein (*i.e.*, the “fluorescent detection antibodies”). The antibody specifically localizes to an epitope on the secreted protein but ideally does not interfere with the binding of the secreted protein to the target protein on the target cell.
 - b. Alternatively, the reporter element can be a signaling pathway within the cells that express the target protein. If a secreted variant protein binds the target protein on the cell surface and activates the signaling pathway within the target cell, the binding interaction will generate a fluorescent signal within the cell (not shown).

4. Reaction buffer:

- a. Can be media for the library-expressing cells or for the target cells (for example, such media could be hybridoma medium: such as one commercially available, including for example from ThermoFisher, see, the World Wide Web at thermofisher.com/order/catalog/product/11279023; CD Hybridoma Medium).
- b. Can be a mammalian imaging solution (for example, such an imaging solution can be optically clear, physiological solution buffered with HEPES at pH 7.4).

Illustration of method:

[0245] Step 1: Add all components into microcapillary (see FIG. 1A).

[0246] Step 2: A specific “secreted protein” is expressed by the library-expressing cell into the microcapillary. Secreted protein variants capable of binding to the target protein are localized to the target cell surface as shown (see FIG. 1B).

[0247] Step 3: Fluorescent detection antibodies associated with the bound secreted protein variants are observed in association with target cells in specific microcapillaries (see FIG. 1C).

Detailed description and sample data:

[0248] To demonstrate this method, a yeast vector library expressing a protein designed to bind to EGFR on human cancer cells was created. In this library, some yeast variants were capable of expressing the protein, while other variants were not able to express the protein. Yeast cells, cancer cells, and a fluorescent antibody against the expressed protein were added to the microcapillary. After 18 hours, the microcapillary array was imaged. Further details and results of the screen are provided in Example 3 below.

Example 2. Hybridoma Screening Against Mammalian Cells

General background

[0249] Current methods to screen binding interactions between proteins or other target molecules typically rely on the use of “display” methods, *e.g.*, phage display, bacterial display, yeast display, mammalian display, or virus display. In the display methods, a library of genes encoding protein variants is expressed at the surface of the cell or phage. The protein variants are incubated with a soluble version of the target molecule in order to identify protein variants capable of binding to the target. The library can be screened by panning or by fluorescence-activated cell sorting (“FACS”). Such assays have two primary

limitations: 1) the engineered protein is typically tethered to the display platform; and 2) it is usually advantageous for a soluble form of the target molecule to exist. Therefore, it can be difficult to develop reliable assays for variant proteins that bind to many target molecules, in particular membrane proteins, such as G-protein coupled receptors and other such receptors.

Hybridoma screening against mammalian cells

[0250] To identify antibody variants with specific binding to a target molecule, hybridomas (which secrete antibody variants) were added to a cancer cell line that expressed high levels of EGFR as the target molecule. Labeled antibodies specific for the secreted antibodies were then added.

Materials:

[0251] Cells:

[0252] Mouse hybridoma

[0253] A431 target cells (human cancer cell line expressing high levels of EGFR)

Detection antibodies:

[0254] Anti-mouse secondary antibody labeled with Alexa488 (a fluorophore)

Media for cell culture:

[0255] DMEM-10% fetal bovine serum

[0256] DMEM-10% horse serum

[0257] Cell line growth and preparation. Mouse hybridoma cells were cultured in complete media (Dulbecco's Modified Eagle's Medium with 10% horse serum). The hybridoma cells were washed twice with PBSA and suspended in complete media at 600 cells /uL. The A431 cells were cultured in complete media (Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum). The A431 cells were washed twice with PBSA and stained with a LiveGreen fluorescent signal. The A431 cells were then suspended in the complete media containing hybridoma at a final concentration of 1800 cells /uL.

[0258] Assay setup. Following mixing of the two cell types, detection antibodies were added to the reaction mixture: 1:100 dilution of secondary (anti-mouse Alexa488). This reaction mixture was then loaded into an ethanol-sterilized, corona-treated microcapillary array (40 μ m diameter, 1 mm thick). A 2 mm slab of 1% weight/volume agarose was placed on the array to help prevent evaporation. After each hour, the sample was imaged under fluorescence and bright-field microscopy.

Sample data:

[0259] FIGs. 2A-2C show images of a subsection of the microcapillary array showing either all of the cells (FIG. 2A, bright-field signal), A431 target cells (FIG. 2B, LiveGreen signal), or cells labeled with the fluorescent anti-mouse secondary antibody (FIG. 2C, Ab-a555 signal). Microcapillaries containing hybridoma cells that express antibodies specific for EGFR are indicated with two arrows in each image.

[0260] FIG. 3 shows images of a microcapillary containing both an A431 target cell and a hybridoma cell over the course of a 4 hour incubation, where the antibody binding signal to the A431 target cells increased during the time course of the assay as mouse antibodies specific to EGFR are produced (middle column). LiveGreen staining of the A431 target cells declined over the same time period (right column).

Example 3. Yeast Library Screening Against Mammalian Cells

[0261] To determine the best secretion yeast plasmid vectors, a yeast vector library expressing scaffold proteins designed to bind to EGFR on a cancer cell surface was created. This library contained yeast cells with various soluble expression levels of a scaffold protein. Using the described assay, the variant expression library was screened to recover the plasmid vectors with high expression of the desired scaffold protein. In this experiment, the secreted scaffold has a c-Myc tag, which can be labeled with fluorescently-labeled antibodies.

Materials:

[0262] Cells:

[0263] Yeast secretion library of scaffold proteins

A431 cells (human cancer cell line expressing high levels of EGFR)

[0264] Detection antibodies:

[0265] Chicken anti-c-Myc

[0266] Anti-chicken secondary antibody labeled with Alexa488

[0267] Media for cell culture:

[0268] DMEM-10% FBS

[0269] SD-CAA minimal yeast media

[0270] Reaction buffer:

[0271] SD-CAA minimal yeast media

Methods:

[0272] Cell line growth and preparation. The yeast library was grown in SD-CAA minimal yeast media (20 g dextrose; 6.7 g Difco yeast nitrogen base; 5 g Bacto casamino acids; 5.4 g Na_2HPO_4 ; 8.56 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$; dissolved in deionized H_2O to a volume of 1 liter). After growth, the yeast cells were washed twice with PBSA (phosphate-buffered saline + 1 mg/ml BSA) and suspended in SD-CAA at a final concentration of 2,400 cells/ μL .

[0273] The A431 cells were cultured in complete media (Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum). The A431 cells were washed twice with PBSA and suspended in the SD-CAA containing yeast cells at a final concentration of 600 cells / μL .

[0274] Assay setup. Following mixing of the two cell types, two antibodies were added to the reaction mixture: 1:250 dilution of an unlabeled primary antibody (chicken anti-c-Myc) and 1:200 dilution of a labeled secondary antibody (anti-chicken Alexa488). This reaction mixture was then loaded into an ethanol-sterilized, corona-treated microcapillary array (40 μm diameter, 1 mm thick). A 2 mm slab of 1% weight/volume agarose was placed on the array to help prevent evaporation. After 18 hours of growth, the sample was imaged under fluorescence and bright-field microscopy.

[0275] Microcapillary array extraction. A Triton UV laser was used to extract the contents of desired capillaries. The laser operates for 18 ± 2 ms ($n = 5$ measurements), delivering a train of pulses at 2.5 kHz with a total energy of approximately 100 μJ . The microcapillary contents were extracted onto a glass coverslip, which was then placed in yeast growth media (liquid medium or agar plates) to propagate the extracted cells.

Sample data:

[0276] FIGs. 4A and 4B show images of a subsection of the microcapillary array that identifies microcapillaries with expressing and non-expressing cells using bright-field imaging (FIG. 4A) and fluorescence imaging (FIG. 4B).

Example 4. Growth of Cultured Human Cells in a Microcapillary Array

[0277] FIGs. 5A-5G demonstrate the growth of K562 cells (a human immortalized myelogenous leukemia cell-line) in growth media over the course of 6 days within an array of microcapillaries. A bright-field image of the same section of the array was taken every 24 hours. FIG. 5A: Day 0; FIG. 5B: Day 1; FIG. 5C: Day 2; FIG. 5D: Day 3; FIG. 5E: Day 4; FIG. 5F: Day 5; and FIG. 5G: Day 6. A 40 μm scale bar is shown in each image.

Example 5. Hybridoma Screening Against Mammalian Reporter Cells

[0278] To identify antibody variants that activate specific signaling pathways, hybridomas secreting different antibody variants were added into a microcapillary array with a reporter cell. For example, the reporter cell can be from Qiagen (see

http://www.sabiosciences.com/reporter_assay_product/HTML/CCS-013L.html).

If a protein variant binds the reporter cell and activates the signaling pathway, the reporter cell expresses a fluorescent protein. The signal fluorescence of activated cells is observed in microcapillaries that contain desirable protein variants and used to isolate the contents of those microcapillaries.

Example 6. Sample Data for Multiple Target Binding**Study goal:**

[0279] Identify hybridoma secreting antibody that would specifically bind target protein, but not bind to a similarly structured protein. See, for example, FIG. 9. This method allowed for and will continue to allow for screening of antibody libraries.

Materials:

[0280] A hybridoma library.

[0281] The target protein would be displayed on a cancer cell type. See, for example, FIG. 9.

[0282] The target protein analog is immobilized on beads. See, for example, FIG. 9.

Protocol:

1. Cultured cells that displayed **target protein A** on surface.
2. Labeled protein Dynabeads Biotin Binder with **target protein B** following manufacturing directions.
3. Cultured rat **hybridoma library**.
4. Diluted cells, beads, and hybridoma so that there was an average of 1 hybridoma per microcapillary, ~2 cells of target protein A cells, and ~10 beads covered of target protein B.
5. Added anti-rat secondary antibody (**reporter element**) to prepared cell sample.
6. Loaded sample into array.
7. Incubated 1 hours before imaging.
8. Recovered cells that bind cells with **target protein A** but not **beads with target protein B**.

Sample result:

[0283] Identified microcapillaries that have properly stained cells but no stained beads. The presence of stained cells and the absence of stained beads within a microcapillary indicated the presence of an antibody which bound to the target protein but did not bind to the target protein analog.

[0284] In some embodiments, the assay tested in this example can alternatively be adapted used to screen for antibodies that bind both a mouse and human (or other combination of animals) variant of a target protein, i.e. finding “cross-reactive” antibodies. For example, the presence of stained cells and the presence of stained beads within a microcapillary indicates the presence of an antibody which binds to the “target protein” (for example, a mouse target) and which also binds to the “target protein analog” (for example, a human target), in order to identify antibodies which bind to both a mouse and human target. For example, the presence of stained cells and the presence of stained beads within a microcapillary indicates the presence of an antibody which binds to the “target protein” (for example, a cynomolgus target) and which also binds to the “target protein analog” (for example, a human target), in order to identify antibodies which bind to both a cynomolgus and human target.

Example 7. Titration of reporter element for optimum signal output

[0285] A frequent reporter element used is a fluorescently labeled secondary antibody. In this assay format, the secondary antibody was added in the beginning of the assay, and over time this antibody bound to the secreted antibody (the variant protein), which was bound to the target protein.

[0286] A key consideration was the amount of secondary antibody used. If there was too much secondary antibody, the background noise would be too high. If too little secondary antibody was used, the signal would be too low. In this experiment, beads were labeled with the primary antibody and then titrated with various levels of the secondary antibody to determine the optimal signal to noise ratio.

[0287] Reporter element used was Donkey anti-Goat IgG Secondary Antibody labeled with Alexa Fluor 633.

[0288] Manufacturer recommended range of usage: 1:200-1:2000 dilution range.

[0289] Dilution series tested was 1:100, 1:200, 1:500, 1:1000, 1:2000, 1:5000. Image of beads from the experiment is shown in FIG. 13.

Example 8. Reporter Cell Assays**Calcium dye assay**

[0290] Jurkat cells were loaded with 10 μ M Fluo-4 AM in IMDM with 10% FBS at 37 °C for 25 min and washed twice with imaging buffer (PBS with 1 mM MgCl₂, 1 mM CaCl₂, 1 mM HEPES, and 0.1% BSA). Cells were resuspended at 5×10^6 /mL in imaging buffer. Dye-loaded cells were activated by adding α -CD28 (28.2) and α -CD3 (OKT3), both of which were mouse IgG antibodies that were crosslinked by preincubation with goat anti-mouse IgG. The final concentrations of the antibodies were 5 μ g/mL, 2.5 μ g/mL, and 5 μ g/mL, respectively. The activated cells were immediately loaded onto a 40 μ m μ Pore array and the sample was overlaid with PBS 1% agarose gel for imaging of Ca²⁺ signaling.

T cell activation assays

[0291] Human T cells were isolated from peripheral blood mononuclear cells (PBMCs) via MACS (Pan T Cell Isolation Kit, human, Miltenyi Biotec). Cells were mixed with beads coated with agonistic antibodies. In this case, we used Dynabead Humans T-Activator beads that are coated with anti-CD3/CD28. The beads and cells mixture is loaded into the 40 μ m μ Pore array and the sample was overlaid with 1% agarose gel (containing media). The T cells are activated by the antibodies and express activation markers on their surface. After 24-72 hours, the cells are stained with fluorescently-labeled antibodies via our flow cell system against activation markers and imaged.

GFP reporter cells

[0292] GLP-1 receptor expressing cells were transfected with a cAMP response element (CRE) reporter, which expresses GFP when the CRE signalling pathway is activated. These GLP-1 reporter cells were placed into the 40 μ m μ Pore array along with 10uM of its cognate ligand: GLP-1. After 24 hours, the cells were imaged for fluorescence. In an actual screen, we were co-incubate the GLP-1 CRE reporter cell with an antibody secreting cell in the μ Pore array. The antibody secreting cell will secrete antibodies that bind and activate the GLP-1 reporter cell, activating a GFP or other fluorescent protein variant signal.

[0293] In some instances, the reporter cell assays can use chicken cells.

Example 9. Chicken Cell Assays

[0294] OmniChicken expresses fully human, highly diversified antibody repertoire. Genetic divergence from human and mouse allows for more diverse epitope coverage. Deep immune

profiling can lead to a more diverse panel of functional antibodies. Can also be referred to as a B cell assay.

[0295] Test case:

Profiling the antigen specific antibody repertoire after immunization with progranulin. *See*, Figures 18-19.

[0296] Assay conditions – see Figure 19.

Protocol:

[0297] Combine mAb secretor and target beads. Load cells + detection antibody into array (homogenous assay). Incubate for 3 hours. Image and quantify data.

Example 10. T cell activation (CD25 surface expression) with B cells

[0298] Goal: Find antibodies that can activate T cells as measured by induction of CD25 expression on the T cell surface. See Figure 27.

[0299] Protocol:

1. Isolate CD3⁺ T cells from human peripheral blood mononuclear cells (PBMCs) using the MACS Pan T Cell Isolation Kit following the supplier's procedure.
2. Label the antibody secreting cells (ASCs) with CellTrace Far Red following the supplier's protocol.
3. Prepare a 1:1 mixture of CD3 T cell activating beads and antibody capture beads and wash the mixture with PBMC media.
4. Wash Dynabeads MyOne Silane beads with PBMC media.
5. Combine the beads and resuspend them in PBMC media with 1:200 anti-human CD25 Alexa Fluor 488 detection antibody.
6. Mix T cells and ASCs at a ratio of 5:1 (could be 2:1 to 12:1).
7. Wash the cell mixture with PBMC media. Pellet and resuspend the cells with the bead mixture.
8. Load this assay mixture onto a 40 μ m μ Pore array, overlay the sample with 1% RPMI agarose gel, and incubate at 37 °C with humidity for 2 days.
9. After the 2-day incubation, image the chip on xPloration and screen for activated T cells labeled with α CD25.

[0300] B cell isolation protocol:

[0301] B cells were enriched from mouse splenocytes by isolating CD138⁺ cells using a commercially available kit. Splenocytes were incubated with CD138-coated magnetic microbeads. The mixture was loaded onto a magnetic column and unbound cells were washed out. The magnetically labeled cells were then eluted from the column and washed with warmed PBMC media. The washed CD138⁺ cells were immediately used for binding screening.

[0302] B cells can also be obtained using Pan B cells beads. For example, using the Pan B Cell Isolation Kit from Miltenyi Biotec).

Example 11. T cell activation (calcium signaling) with B cells

[0303] Goal: Find antibodies that can activate T cells as measured by induction of calcium signaling. See Figure 28.

[0304] Protocol:

1. Isolate CD3⁺ T cells from human peripheral blood mononuclear cells (PBMCs) using the MACS Pan T Cell Isolation Kit following the supplier's procedure.
2. Label the antibody secreting cells (ASCs) with CellTrace Far Red following the supplier's protocol.
3. Stain T cells with 10 μ M of a calcium-sensitive fluorophore, such as Fluo-4 AM, Fura-2 AM, Indo-1 AM, in AIM V media with 10% FBS at 37 °C for 25 min.
4. Wash the T cells twice with imaging buffer (BPS with 1 mM MgCl₂, 1 mM CaCl₂, 1 mM HEPES, and 0.1% BSA).
5. Mix T cells and ASCs at a ratio of 5:1 (could be 2:1 to 12:1).
6. Wash the cell mixture with PBMC media. Pellet and resuspend the cells with the bead mixture.
7. Load this assay mixture onto a 40 μ m μ Pore array, overlay the sample with 1% RPMI agarose gel, and incubate at 37 °C for 0-6 hour.
8. Image the chip on xPloration and screen for activated T cells via calcium signaling.

[0305] All patents, patent publications, and other published references mentioned herein are hereby incorporated by reference in their entireties as if each had been individually and specifically incorporated by reference herein.

[0306] While specific examples have been provided, the above description is illustrative and not restrictive. Any one or more of the features of the previously described embodiments can be combined in any manner with one or more features of any other embodiments in the present invention. Furthermore, many variations of the invention will become apparent to those skilled in the art upon review of the specification. The scope of the invention should, therefore, be determined by reference to the appended claims, along with their full scope of equivalents.

[0307] The examples set forth above are provided to give those of ordinary skill in the art a complete disclosure and description of how to make and use the embodiments of the compositions, systems and methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Modifications of the above-described modes for carrying out the invention that are obvious to persons of skill in the art are intended to be within the scope of the following claims. All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

[0308] All headings and section designations are used for clarity and reference purposes only and are not to be considered limiting in any way. For example, those of skill in the art will appreciate the usefulness of combining various aspects from different headings and sections as appropriate according to the spirit and scope of the invention described herein.

[0309] All references cited herein are hereby incorporated by reference herein in their entireties and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[0310] Many modifications and variations of this application can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments and examples described herein are offered by way of example only, and the application is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which the claims are entitled.

What is Claimed is:

1. A method of screening a population of variant proteins comprising the steps of:
providing a microcapillary array comprising a plurality of microcapillaries, each microcapillary comprising a variant protein, an immobilized target molecule, and a reporter element, wherein the variant protein associates with the immobilized target molecule with a particular affinity; and
measuring a signal from at least one reporter element in a reporter assay that indicates association of at least one variant protein with at least one immobilized target molecule to identify at least one microcapillary of interest, wherein said reporter assay is selected from the group consisting of a calcium dye assay, a T cell activation assay, a B cell assay, and a GFP assay.
2. The method of claim 1, wherein the variant protein is expressed by an expression system.
3. The method of claim 2, wherein the expression system is a cell-free expression system.
4. The method of claim 2, wherein the expression system is a cellular expression system.
5. The method of claim 4, wherein the cellular expression system is an animal system, an avian system, a fungal system, a bacterial system, an insect system, or a plant system.
6. The method of claim 5, wherein the cellular expression system is an avian system.
7. The method of claim 6, wherein the avian expression system is a chicken system.
8. The method of claim 1, wherein the variant protein is a soluble protein.
9. The method of claim 1, wherein the target molecule is a target protein or polypeptide, a target nucleic acid, a target carbohydrate, or a combination of each.

10. The method of claim 1, wherein the target molecule is immobilized on a surface.
11. The method of claim 9, wherein the surface is a surface of a cell.
12. The method of claim 9, wherein the target molecule is a native protein.
13. The method of claim 9, wherein the surface is a surface of a bead.
14. The method of claim 9, wherein the surface is a surface of a microcapillary wall.
15. The method of claim 9, wherein the surface is a surface configured to settle in the microcapillary by gravitational sedimentation.
16. The method of claim 1, wherein the reporter element is a labeled antibody or other binding molecule.
17. The method of claim 15, wherein the labeled antibody or other binding molecule is a fluorescently-labeled antibody or other binding molecule.
18. The method of claim 15, wherein the labeled antibody is a primary or a secondary antibody.
19. The method of claim 15, wherein the labeled antibody or other binding molecule is an enzyme-linked antibody or other binding molecule.
20. The method of claim 1, wherein the reporter element is activated within a cell, and the target molecule is immobilized on a surface of the cell.
21. The method of claim 20, wherein the reporter element comprises a green fluorescent protein or variant.

22. The method of claim 1, wherein the signal is a fluorescent signal, an absorbance signal, a bright-field signal, or a dark-field signal.
23. The method of claim 1, wherein each microcapillary in the microcapillary array comprises 0 to 5 variant proteins from the population of variant proteins.
24. The method of claim 1, wherein the microcapillary array comprises at least 100,000, at least 300,000, at least 1,000,000, at least 3,000,000, or at least 10,000,000 microcapillaries.
25. The method of claim 1, wherein each microcapillary further comprises an agent to improve viability of the cellular expression system.
26. The method of claim 25, wherein the agent is methylcellulose, dextran pluronic F-68, polyethylene glycol, or polyvinyl alcohol.
27. The method of claim 25, wherein the agent is a growth medium.
28. The method of claim 1, wherein the signal is measured by an optical detector.
29. The method of claim 1, wherein the signal is measured by a microscope.
30. The method of claim 1, further comprising the step of isolating the contents of the microcapillary of interest.
31. The method of claim 30, wherein the contents of the microcapillary of interest are isolated by pulsing the microcapillary of interest with a laser.
32. The method of claim 31, wherein the laser is a diode-pumped Q-switched laser.
33. The method of claim 31, wherein the laser is directed at the water-glass interface between the microcapillary wall and the sample contained in the microcapillary.

34. The method of claim 30, wherein the contents of the microcapillary of interest are isolated using a two-stage sample recovery element.
35. The method of claim 1, wherein the microcapillary does not comprise a microparticle capable of inhibiting the transmission of electromagnetic radiation, a magnetic microparticle, a magnetic bead, or an electromagnetic radiation absorbent material.
36. A system for screening a population of variant proteins comprising:
an array comprising a plurality of microcapillaries, each microcapillary comprising a variant protein, an immobilized target molecule, and a reporter element, wherein the variant protein associates with the immobilized target molecule with a particular affinity.
37. The screening system of claim 36, wherein the variant protein is expressed by an expression system.
38. The screening system of claim 37, wherein the expression system is a cell-free expression system.
39. The screening system of claim 38, wherein the expression system is a cellular expression system.
40. The method of claim 39, wherein the cellular expression system is an animal system, an avian system, a fungal system, a bacterial system, an insect system, or a plant system.
41. The method of claim 40, wherein the cellular expression system is an avian system.
42. The method of claim 41, wherein the avian expression system is a chicken system.
43. The screening system of claim 36, wherein the variant protein is a soluble protein.

44. The screening system of claim 36, wherein the target molecule is a target protein or polypeptide, a target nucleic acid, a target carbohydrate, or a combination of each.
45. The screening system of claim 36, wherein the target molecule is immobilized on a surface.
46. The screening system of claim 45, wherein the surface is a surface of a cell.
47. The screening system of claim 46, wherein the target molecule is a native protein.
48. The screening system of claim 45, wherein the surface is a surface of a bead.
49. The screening system of claim 45, wherein the surface is a surface of a microcapillary wall.
50. The screening system of claim 45, wherein the surface is a surface configured to settle in the microcapillary by gravitational sedimentation.
51. The screening system of claim 36, wherein the reporter element is a labeled antibody or other binding molecule.
52. The screening system of claim 51, wherein the labeled antibody or other binding molecule is a fluorescently-labeled antibody or other binding molecule.
53. The screening system of claim 51, wherein the labeled antibody is a primary or a secondary antibody.
54. The screening system of claim 51, wherein the labeled antibody or other binding molecule is an enzyme-linked antibody or other binding molecule.

55. The screening system of claim 36, wherein the reporter element is activated within a cell, and the target molecule is immobilized on a surface of the cell.
56. The screening system of claim 55, wherein the reporter element comprises a green fluorescent protein or variant.
57. The screening system of claim 36, wherein the signal is a fluorescent signal, an absorbance signal, a bright-field signal, or a dark-field signal.
58. The screening system of claim 36, wherein each microcapillary in the microcapillary array comprises 0 to 5 variant proteins from the population of variant proteins.
59. The screening system of claim 36, wherein the microcapillary array comprises at least 100,000, at least 300,000, at least 1,000,000, at least 3,000,000, or at least 10,000,000 microcapillaries.
60. The screening system of claim 36, wherein each microcapillary further comprises an agent to improve viability of the cellular expression system.
61. The screening system of claim 60, wherein the agent is methylcellulose, dextran pluronic F-68, polyethylene glycol, or polyvinyl alcohol.
62. The screening system of claim 60, wherein the agent is a growth medium.
63. The screening system of claim 36, wherein the system further comprises an optical source and a detector.
64. The screening system of claim 36, wherein the system further comprises a microscope.

65. The screening system of claim 36, wherein the system further comprises an extraction device.
66. The screening system of claim 65, wherein the extraction device comprises a diode-pumped Q-switched laser.
67. The screening system of claim 36, wherein the system further comprises a two-stage sample recovery element.
68. The screening system of claim 36, wherein the microcapillary does not comprise a microparticle capable of inhibiting the transmission of electromagnetic radiation, a magnetic microparticle, a magnetic bead, or an electromagnetic radiation absorbent material.
69. A method of screening a population of variant proteins comprising the steps of:
providing a microcapillary array comprising a plurality of microcapillaries, each microcapillary comprising a cellular expression system expressing a variant protein, a target molecule immobilized on the surface of a cell, and a reporter element, wherein the variant protein associates with the immobilized target molecule in the microcapillary with a particular affinity, wherein the cellular expression system is an avian system; and
measuring a signal from at least one reporter element in a reporter assay that indicates association of at least one variant protein with at least one immobilized target molecule to identify at least one microcapillary of interest.
70. The method of claim 69, wherein the avian system is a chicken system.
71. The method of claim 69, wherein the target molecule is a target protein or polypeptide, a target nucleic acid, a target carbohydrate, or target antibody, or a combination of each.
72. The method of claim 69, wherein the target molecule is a target antibody.

73. The method of claim 69, wherein the reporter assay is selected from the group consisting of a calcium dye assay, a T cell activation assay, a B cell assay, and a GFP assay.
74. The method of claim 69, wherein the reporter element is a labeled antibody or other binding molecule, wherein the labeled antibody or other binding molecule localizes to an epitope on the variant protein.
75. The method of claim 74, wherein the labeled antibody or other binding molecule is a fluorescently-labeled antibody or other binding molecule.
76. The method of claim 75, wherein the signal is a fluorescent signal, an absorbance signal, a bright-field signal, or a dark-field signal.
77. The method of any one of claims 73-76, wherein the B cell assay comprises B cells sourced from the spleen.
78. The method of any one of claims 73-76, wherein the T cell activation assay is used to evaluate to the cell signaling induction capability of an antibody.
79. The method of any one of claims 73-76, wherein the T cell activation assay is used to measure the internal signaling or cell surface marker induction capability of an antibody.
80. The method of any one of claims 73-76, wherein the T cell activation assay is used to evaluate the cell expansion induction capability of an antibody.
81. The method of any one of claims 73-76, wherein the T cell activation assay is used to evaluate the cytokine secretion induction capability of an antibody.
82. The method of any one of claims 73-76, wherein the T cell activation assay measures CD25 expression to determine activation capability of an antibody.

83. The method of claim 82, wherein the CD25 expression is measured with a fluorescently labeled anti-CD25 antibody.

84. The method of any one of claims 73-76, wherein the T cell activation assay measures calcium signaling to determine activation capability of an antibody.

85. The method of claim 84, wherein the T cell activation assay uses a calcium-sensitive fluorophore to measure the calcium signaling.

86. The method of claim 85, wherein the calcium-sensitive fluorophores are selected from the group consisting of Fluo-4 AM, Fura-2 AM, and Indo-1 AM.

87. The method of any one of claims 73-76 and 78-86, wherein the T cell activation assay comprises a mixture of T cells and antibody secreting cells (ASC).

88. The method of claim 87, wherein the mixture of T cells and ASC varies from a ratio of 2:1 to 12:1.

89. The method of claim 88, wherein the mixture of T cells and ASC is a ratio of 5:1.

90. The method of any one of claims 87-89, wherein the ASC are B cells.

91. The method of any one of claims 87-90, wherein the mixture of T cells and ASC further comprises T cell activating beads and antibody capture beads.

92. The method of any one of claims 87-91, wherein the T cells are purified from peripheral blood.

93. The method of any one of the preceding claims, wherein the signal used to identify the least one microcapillary of interest is an increase of at least 10% to 10,000% or greater than the signal as compared to a baseline and/or a control sample.

94. The method of any one of the preceding claims, wherein the signal used to identify the least one microcapillary of interest is an increase of at least 10% or greater than the signal as compared to a baseline and/or a control sample.

95. The method of any one of the preceding claims, wherein the increase in the signal used to identify the least one microcapillary of interest represents a statistically significant increase when compared to baseline and/or a control.

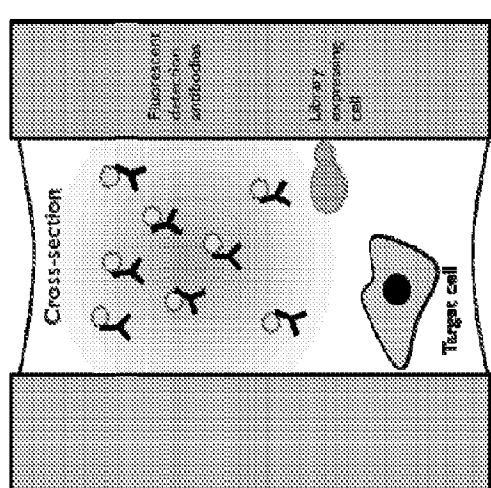


FIG. 1A

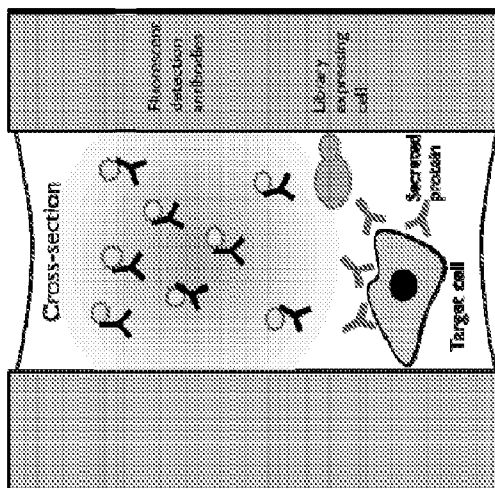


FIG. 1B

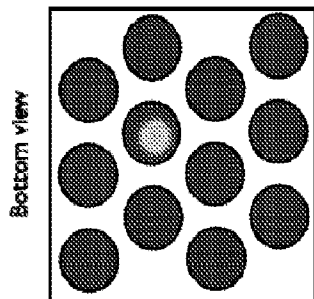
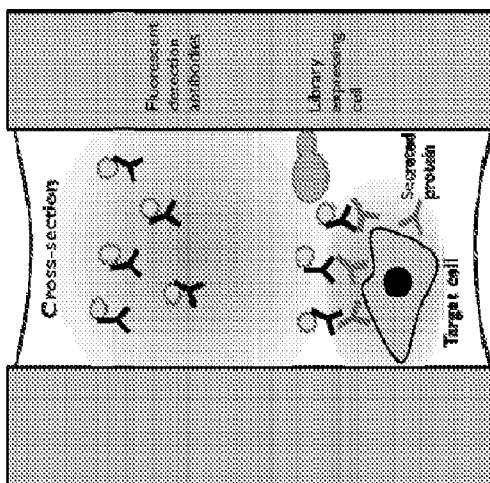
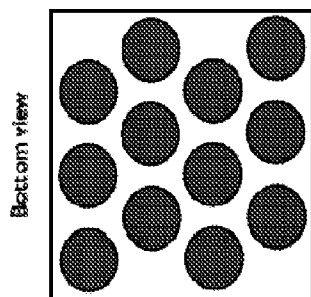


FIG. 1C

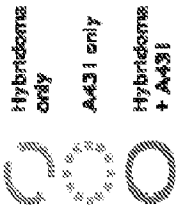
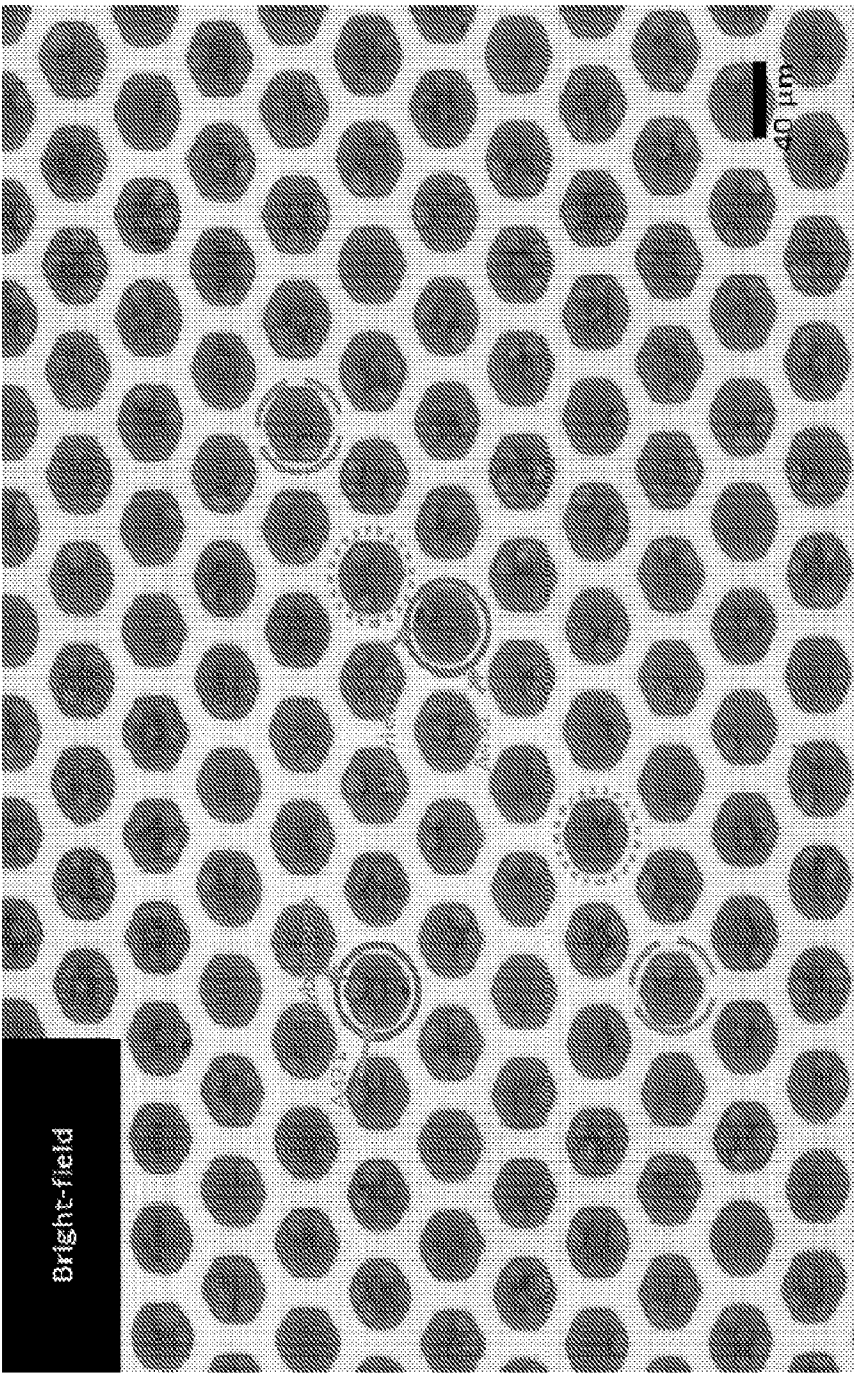


FIG. 2A

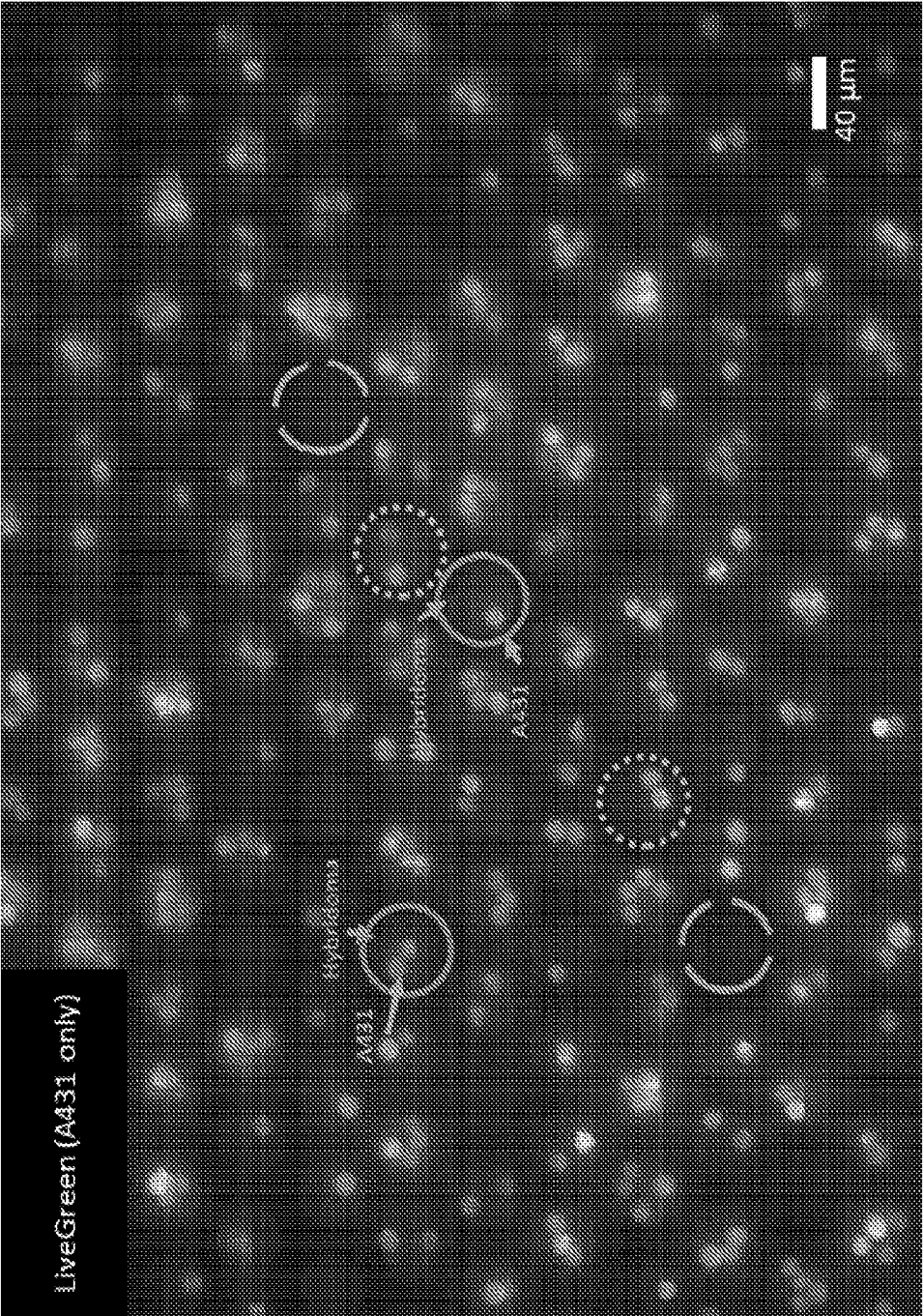
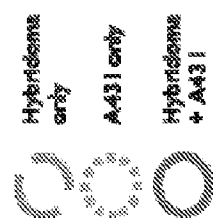
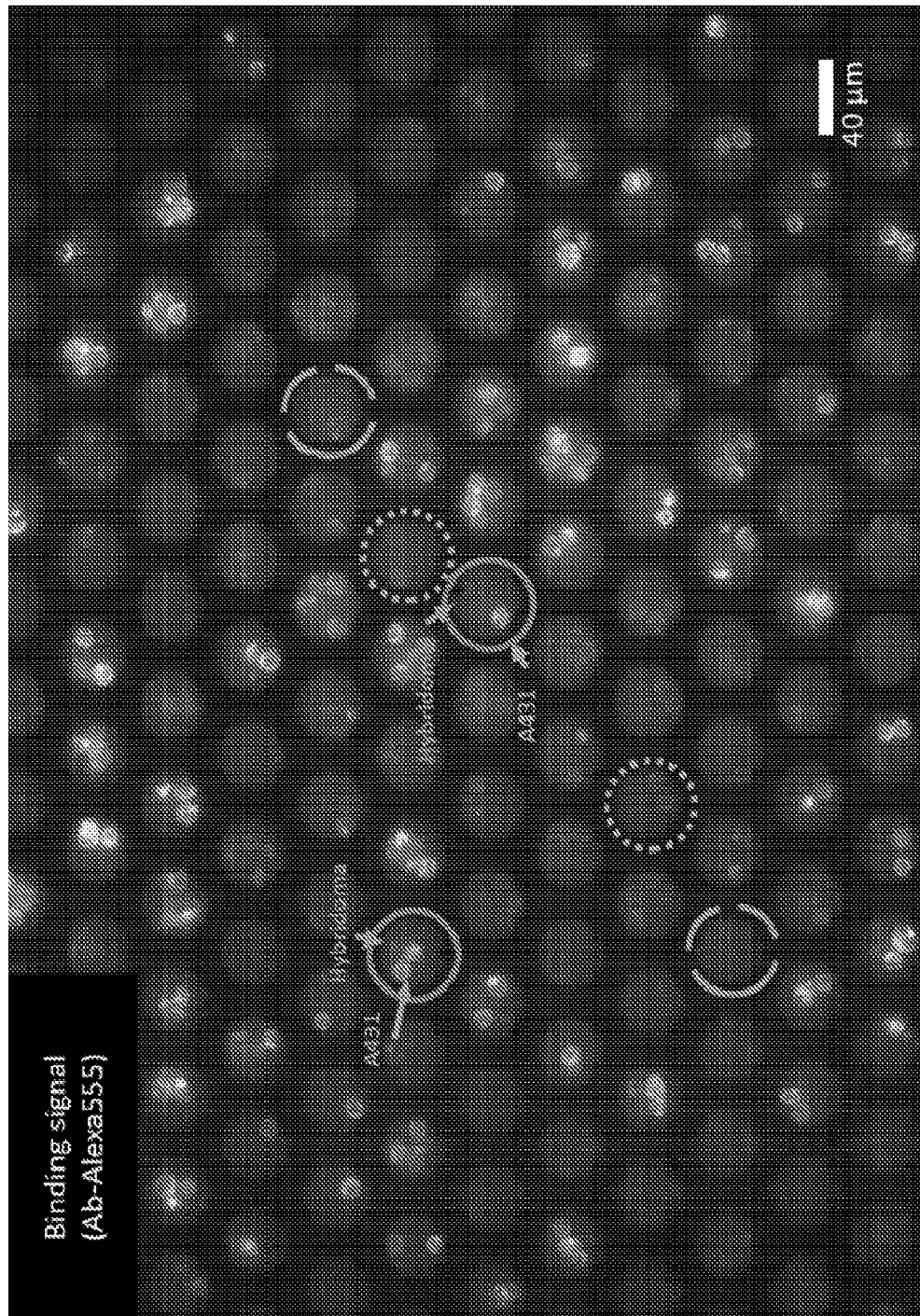


FIG. 2B

FIG. 2C



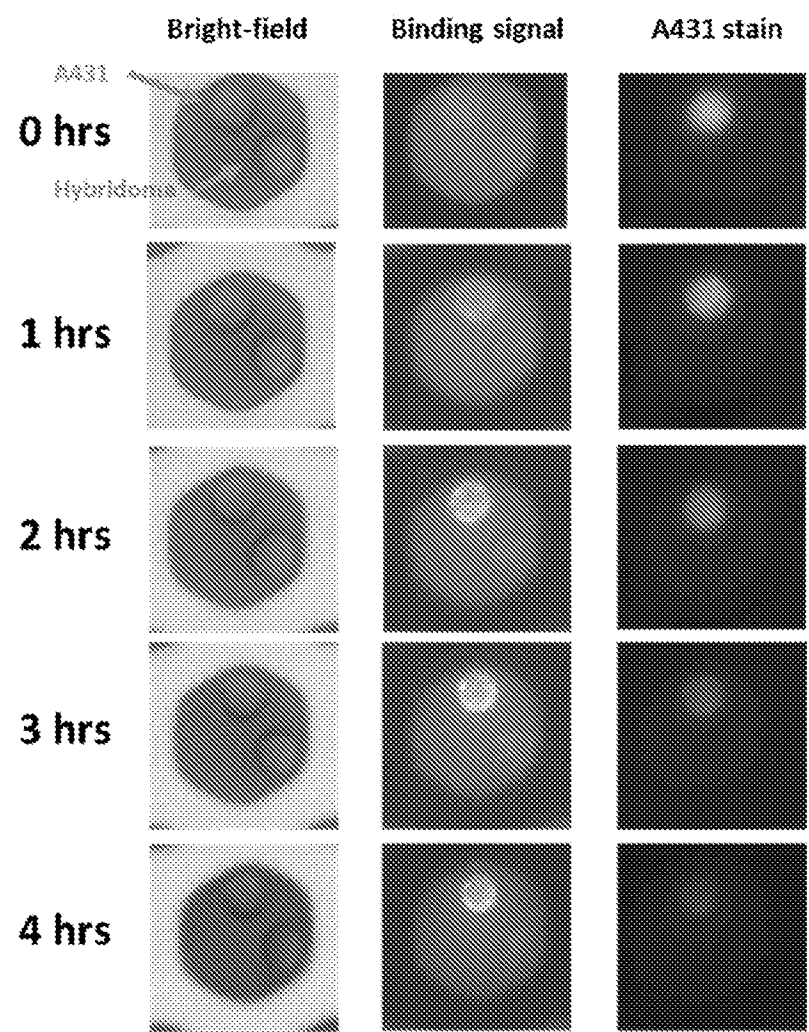


FIG. 3

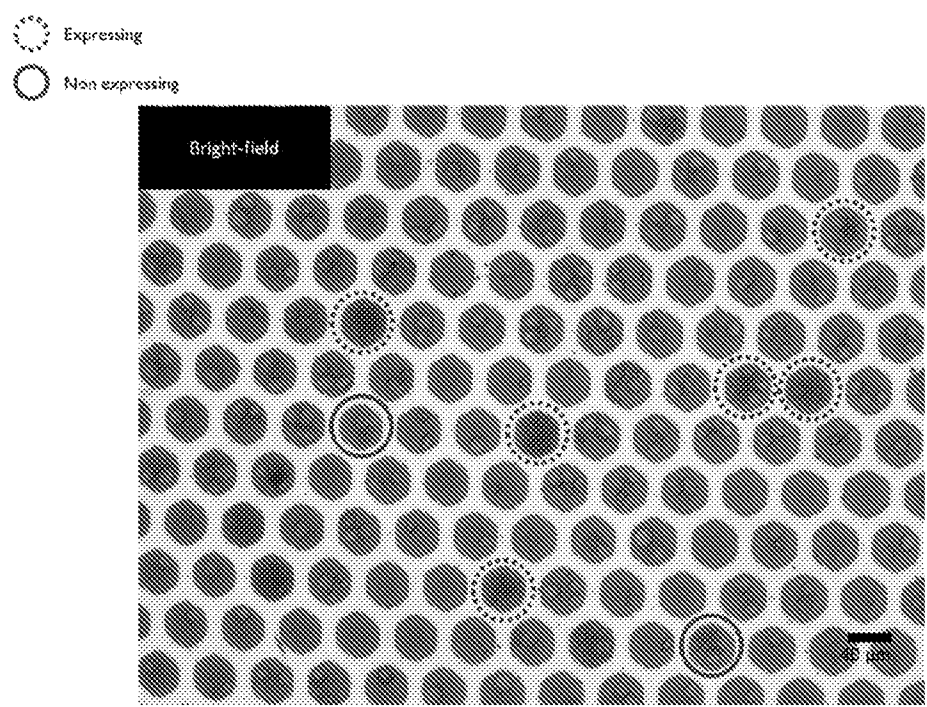


FIG. 4A

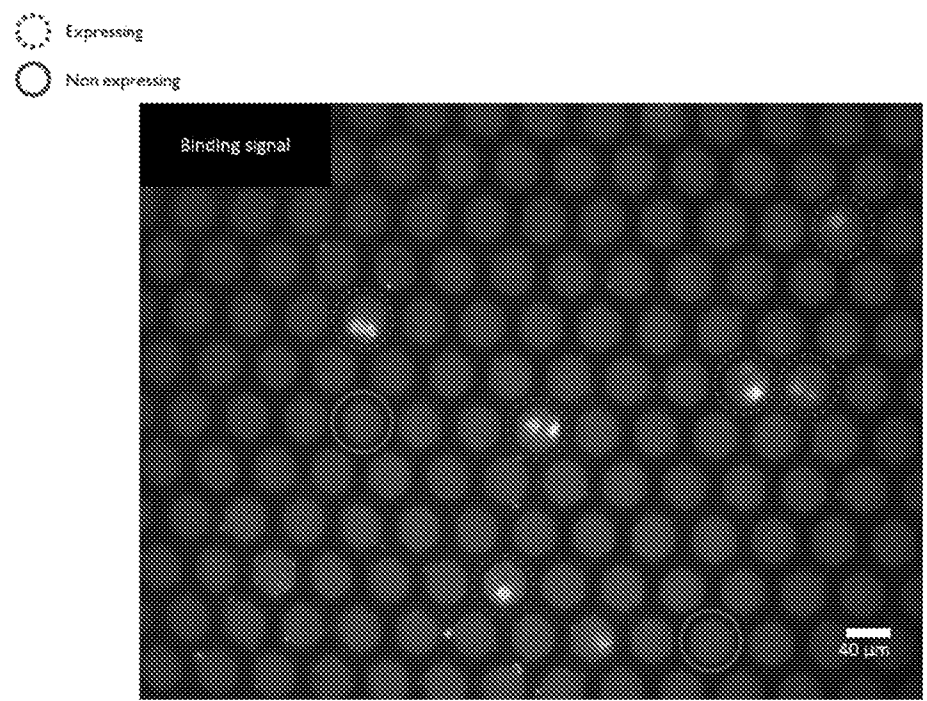


FIG. 4B

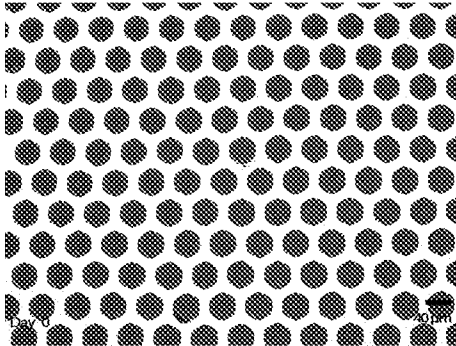


FIG. 5A

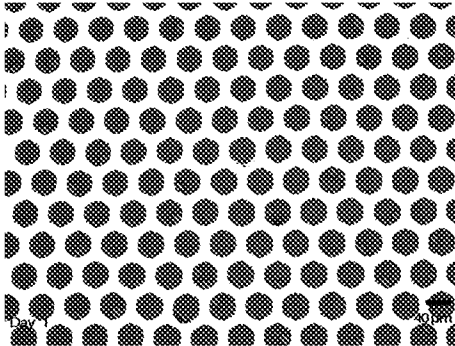


FIG. 5B

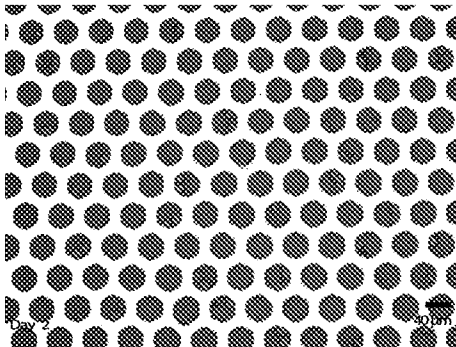


FIG. 5C

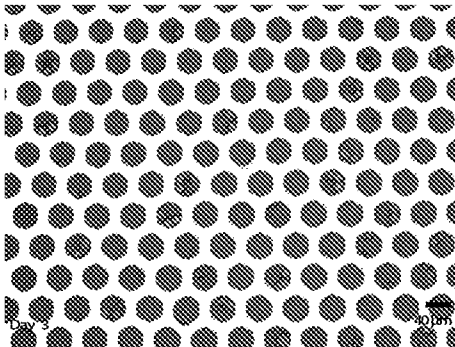


FIG. 5D

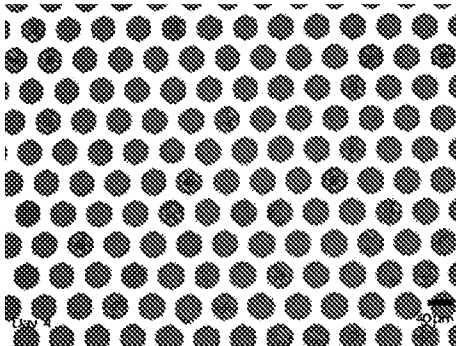


FIG. 5E

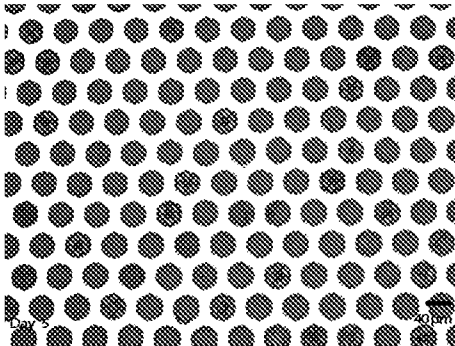


FIG. 5F

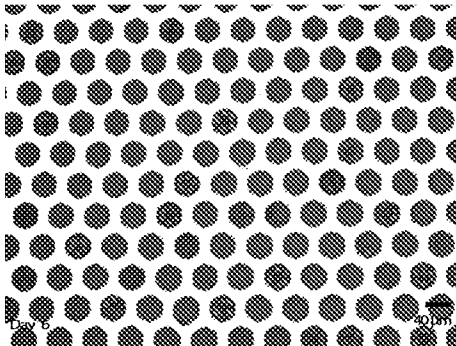


FIG. 5G

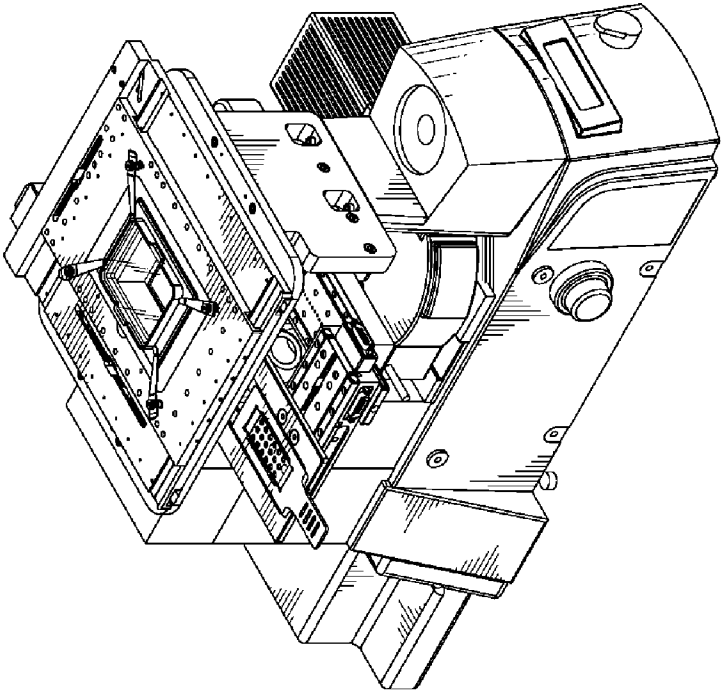


FIG. 6A

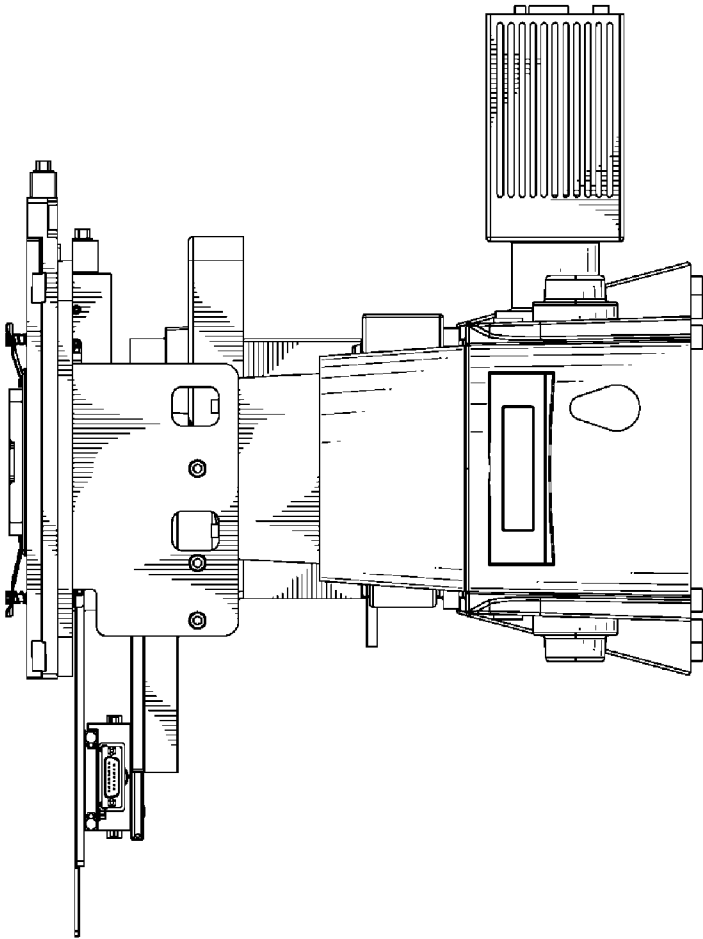


FIG. 6B

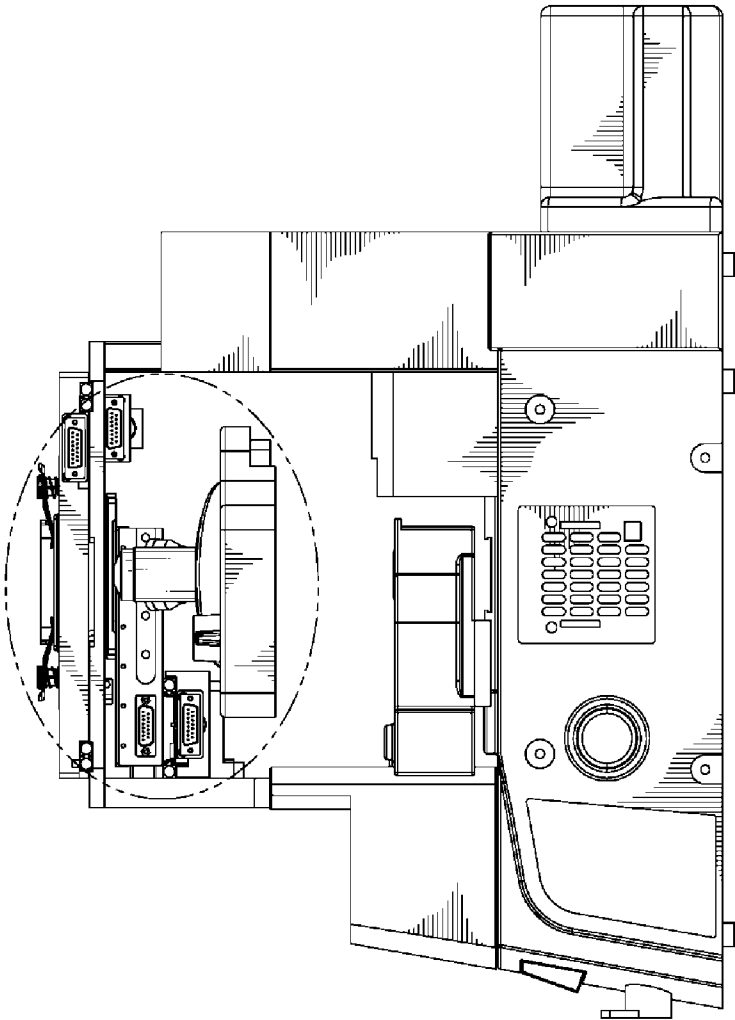


FIG. 6C

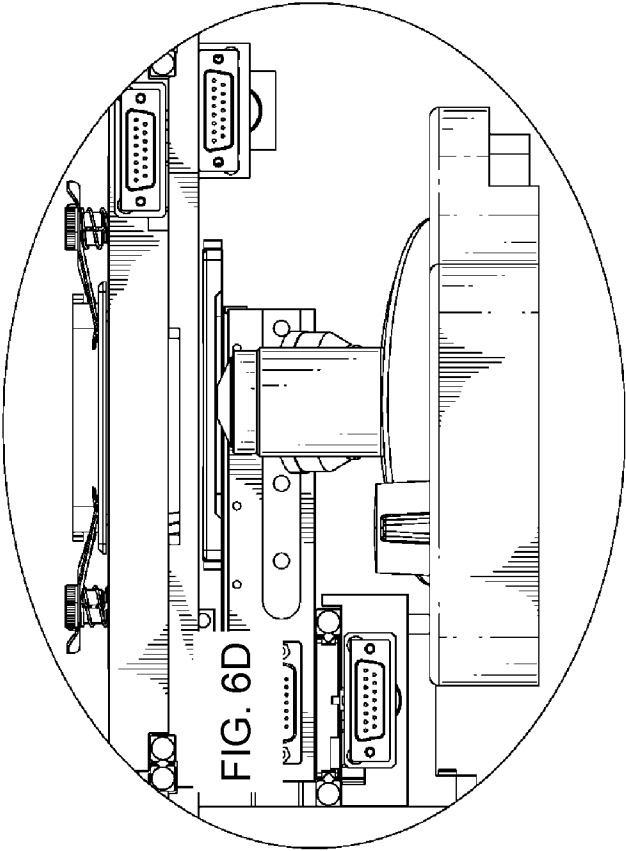


FIG. 6D

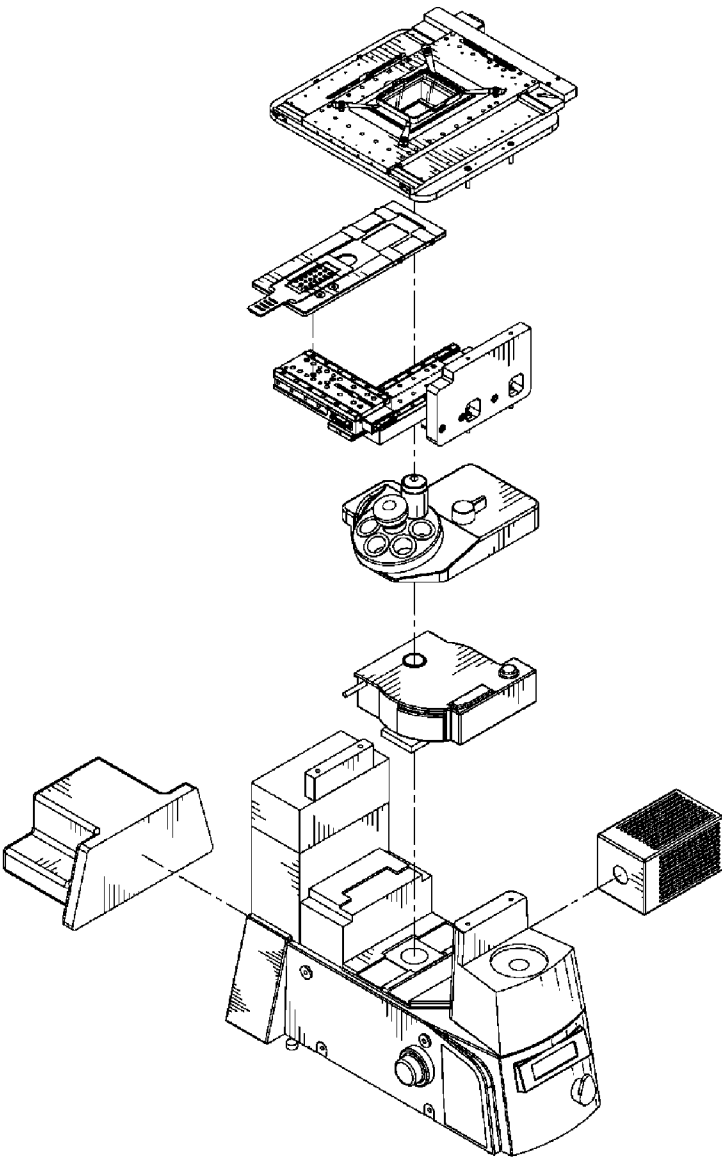


FIG. 6E

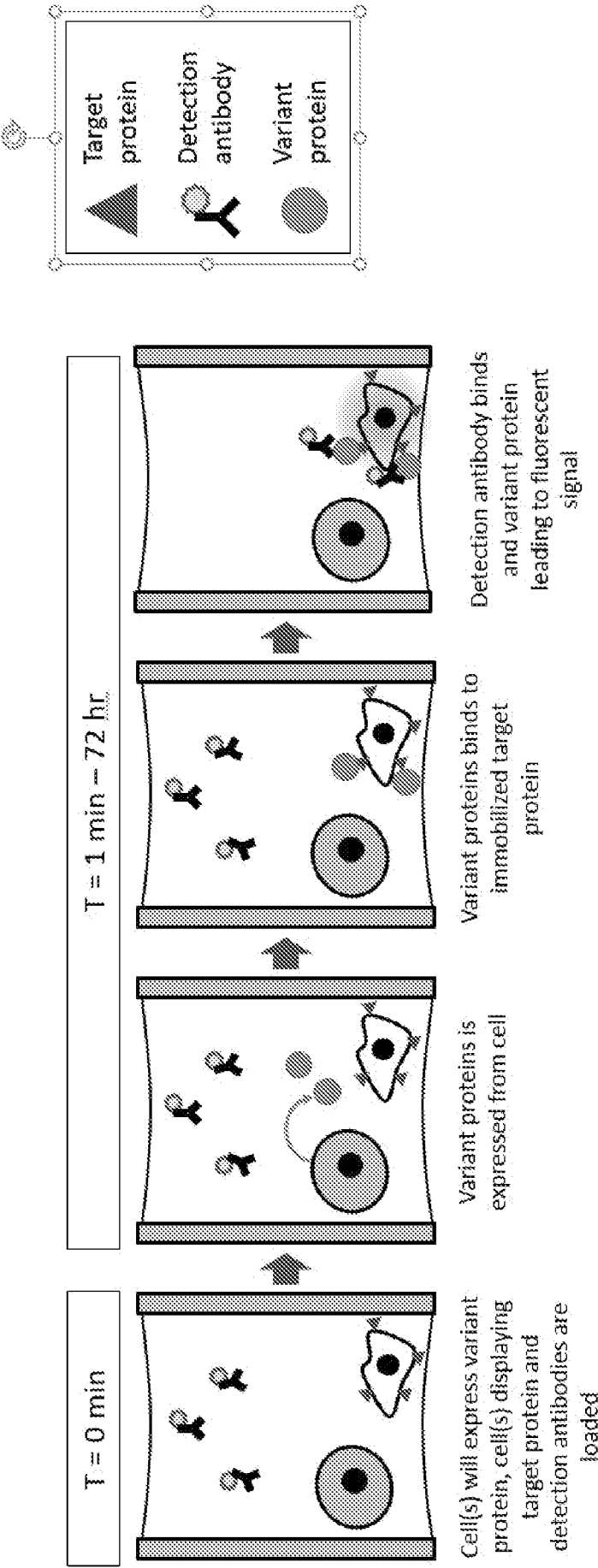
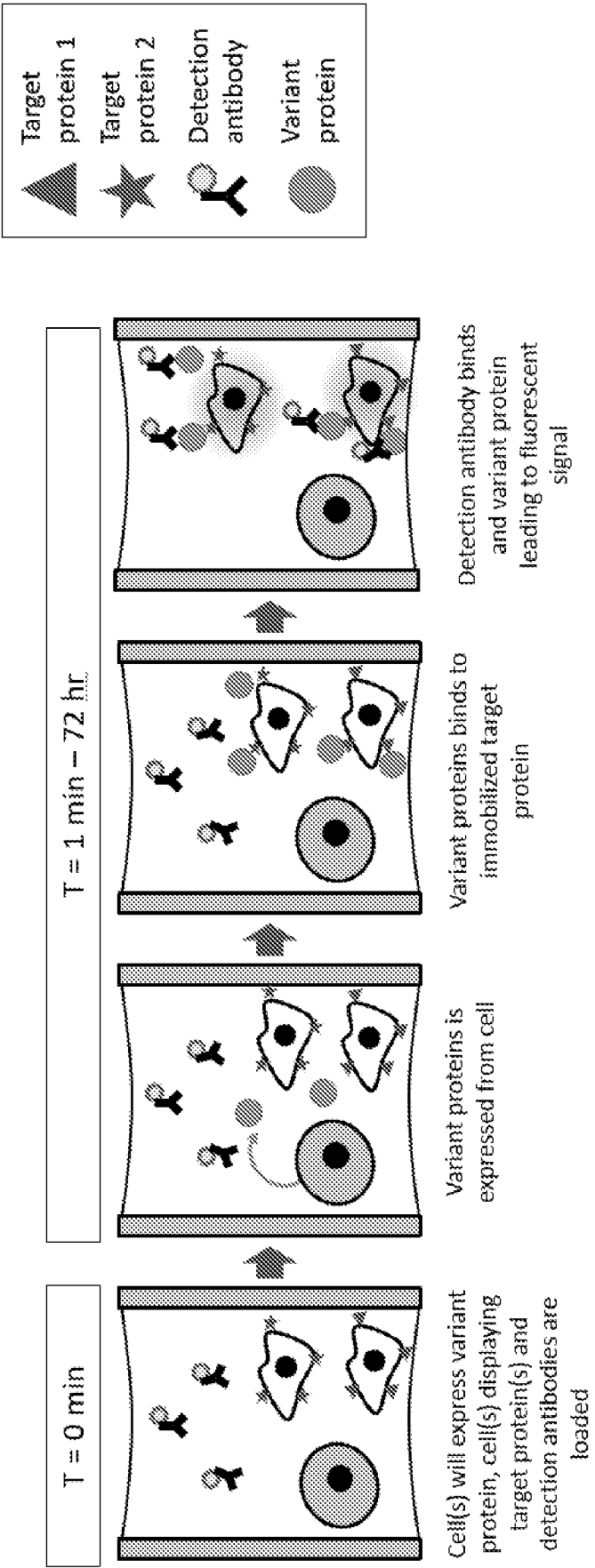


FIG. 7



Potential readouts:
Scales with more target proteins in well

Potential outcomes	Target Protein 1	Target Protein 2
1	X	X
2	X	
3		X

FIG. 8B

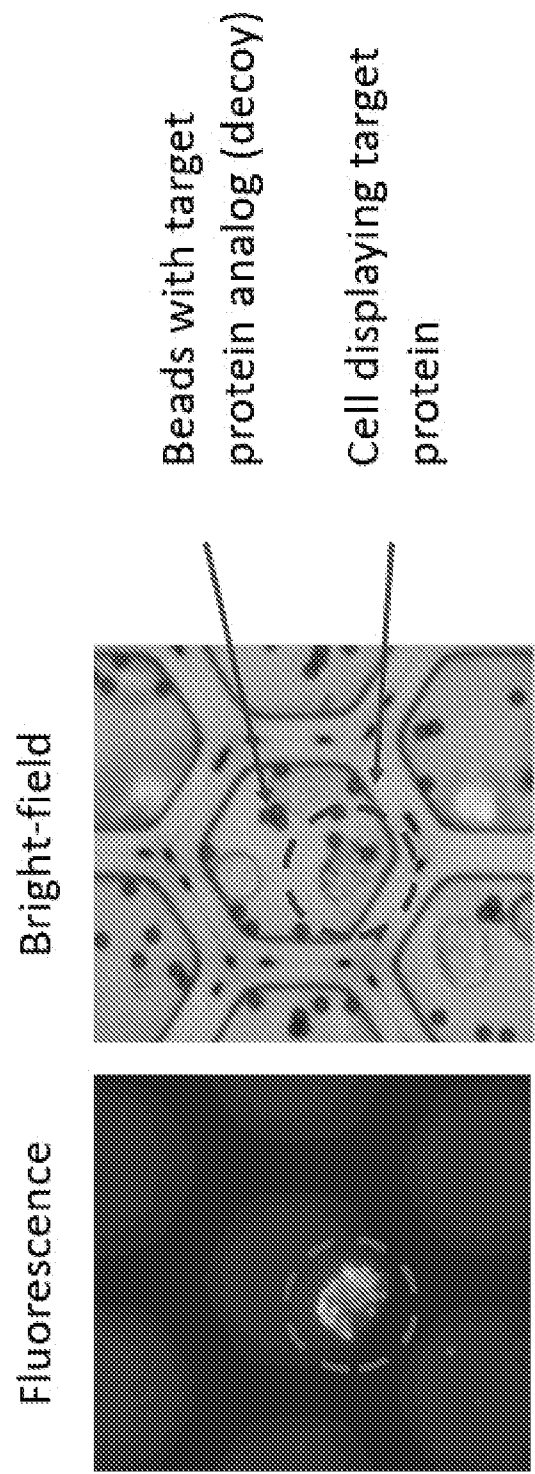


FIG. 9

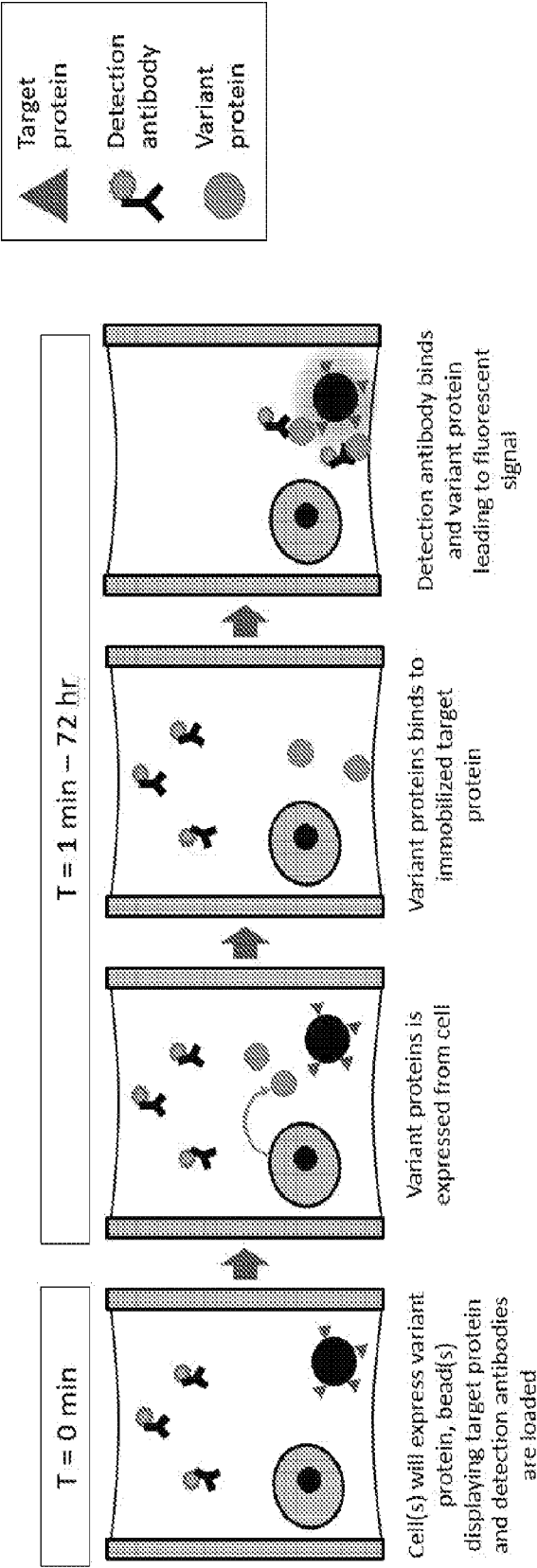


FIG. 10

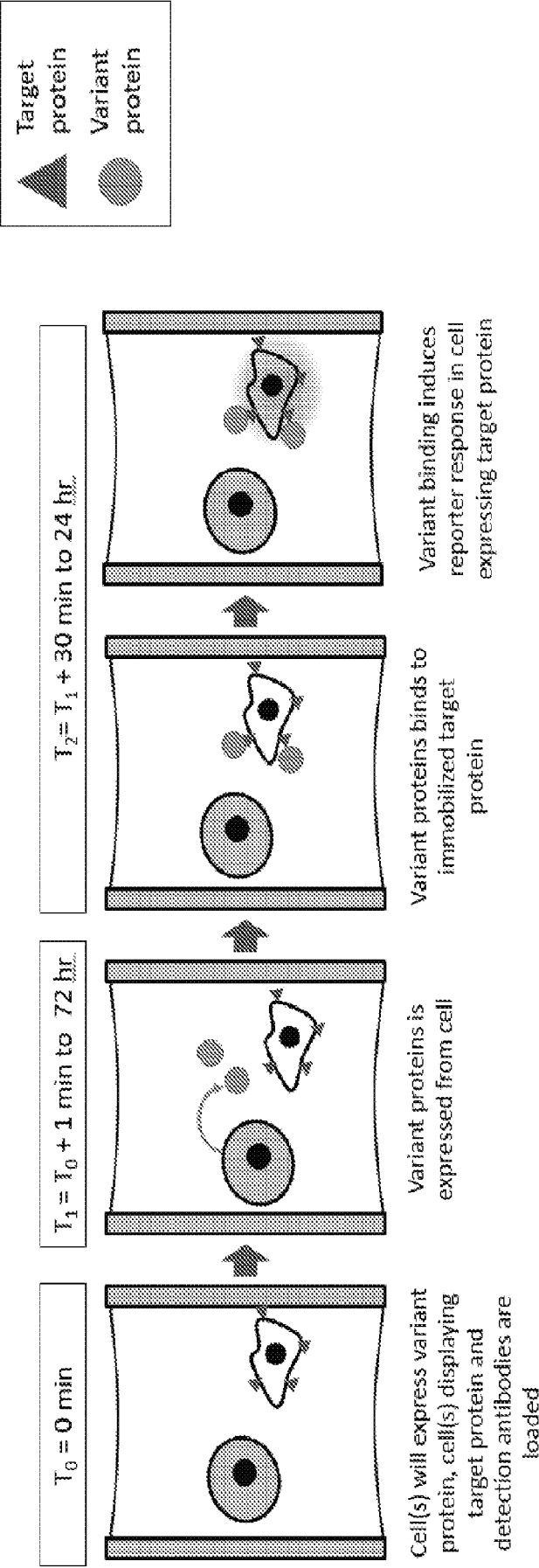


FIG. 11

CH1																	
EU Index	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134
IgG1	A	S	T	K	G	P	S	V	F	P	L	A	P	S	S	K	S
IgG2	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S
IgG3	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S
IgG4	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S
EU Index	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155
IgG1	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T
IgG2	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T
IgG3	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T
IgG4	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T
EU Index	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176
IgG1	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S
IgG2	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S
IgG3	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S
IgG4	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S
EU Index	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197
IgG1	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q	T
IgG2	S	L	S	S	V	V	T	V	P	S	S	N	F	G	T	Q	T
IgG3	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q	T
IgG4	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	K	T
EU Index	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218
IgG1	V	N	H	K	P	S	N	T	K	V	D	K	K	V	E	P	K
IgG2	V	D	H	K	P	S	N	T	K	V	D	K	T	V	E	R	K
IgG3	V	N	H	K	P	S	N	T	K	V	D	K	R	V	E	L	K
IgG4	V	D	H	K	P	S	N	T	K	V	D	K	R	V	E	S	K
Hinge																	
EU Index	221																
IgG1	D				K	T	H	T	C	P	P						
IgG2					V			E		C	P	P					
IgG3	L	G	D	T	T	H	T	C	P	R	C	P	E	P	K	S	C
IgG4							P	P	C	P	S						
EU Index																	
IgG1																	
IgG2																	
IgG3	P	C	P	R	C	P	E	P	K	S	C	D	T	P	P	C	P
IgG4																	
Fc >																	
EU Index	229	230	231	232	233	234	235	236									
IgG1	C	P	A	P	E	L	L	G									
IgG2	C	P	A	P	P	V	A										
IgG3	E	P	K	S	C	D	T	P	P	P	C	P	R				
IgG4	C	P	A	P	E	F	L	G									

FIG. 12A

C302																													
EU Index	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257								
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IgG2	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	N	I	S	R	T	P								
IgG3	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	N	I	S	R	T	P								
IgG4	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	N	I	S	R	T	P								
EU Index	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278								
IgG1	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	K	F	N	W	Y								
IgG2	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	K	F	N	W	Y								
IgG3	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	K	F	N	W	Y								
IgG4	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	K	F	N	W	Y								
EU Index	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299								
IgG1	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	G	Y	N	S	T								
IgG2	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	G	Y	N	S	T								
IgG3	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	G	Y	N	S	T								
IgG4	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	G	Y	N	S	T								
EU Index	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320								
IgG1	Y	R	V	V	S	V	L	T	V	L	H	G	D	W	L	N	G	K	E	Y	K								
IgG2	Y	R	V	V	S	V	L	T	V	L	H	G	D	W	L	N	G	K	E	Y	K								
IgG3	Y	R	V	V	S	V	L	T	V	L	H	G	D	W	L	N	G	K	E	Y	K								
IgG4	Y	R	V	V	S	V	L	T	V	L	H	G	D	W	L	N	G	K	E	Y	K								
EU Index	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340									
IgG1	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K									
IgG2	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K									
IgG3	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K									
IgG4	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K									
C303																													
EU Index	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361								
IgG1	G	G	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L	T	K	N								
IgG2	G	G	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L	T	K	N								
IgG3	G	G	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L	T	K	N								
IgG4	G	G	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L	T	K	N								
EU Index	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382								
IgG1	G	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E								
IgG2	G	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E								
IgG3	G	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E								
IgG4	G	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E								
EU Index	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403								
IgG1	S	N	G	G	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S								
IgG2	S	N	G	G	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S								
IgG3	S	N	G	G	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S								
IgG4	S	N	G	G	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S								
EU Index	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424								
IgG1	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S								
IgG2	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S								
IgG3	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S								
IgG4	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S								
EU Index	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445								
IgG1	C	S	V	M	H	E	A	L	H	N	H	Y	T	G	K	S	L	S	L	S	P								
IgG2	C	S	V	M	H	E	A	L	H	N	H	Y	T	G	K	S	L	S	L	S	P								
IgG3	C	S	V	M	H	E	A	L	H	N	H	Y	T	G	K	S	L	S	L	S	P								
IgG4	C	S	V	M	H	E	A	L	H	N	H	Y	T	G	K	S	L	S	L	S	P								
EU Index	446	447																											
IgG1	G	K																											
IgG2	G	K																											
IgG3	G	K																											
IgG4	G	K																											

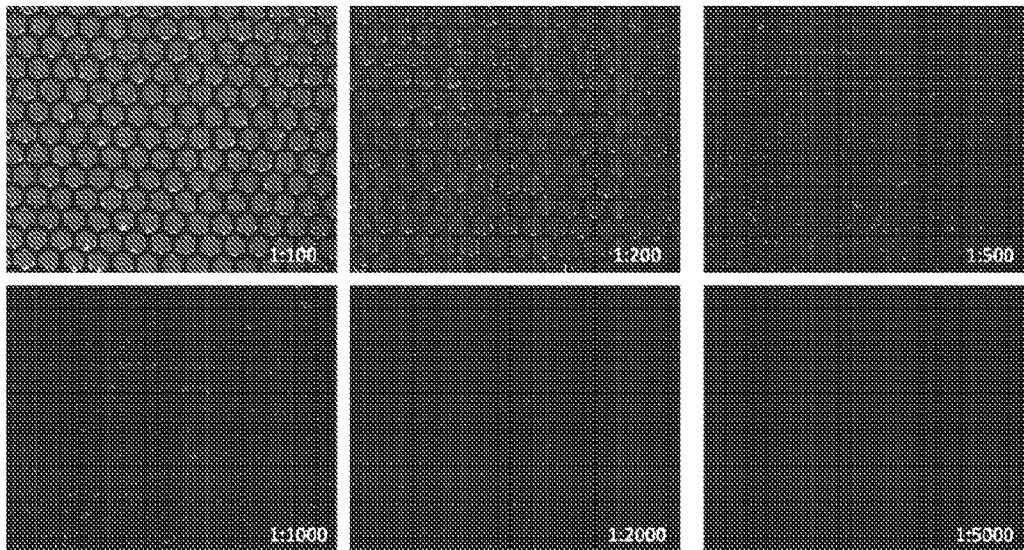


FIG. 13A

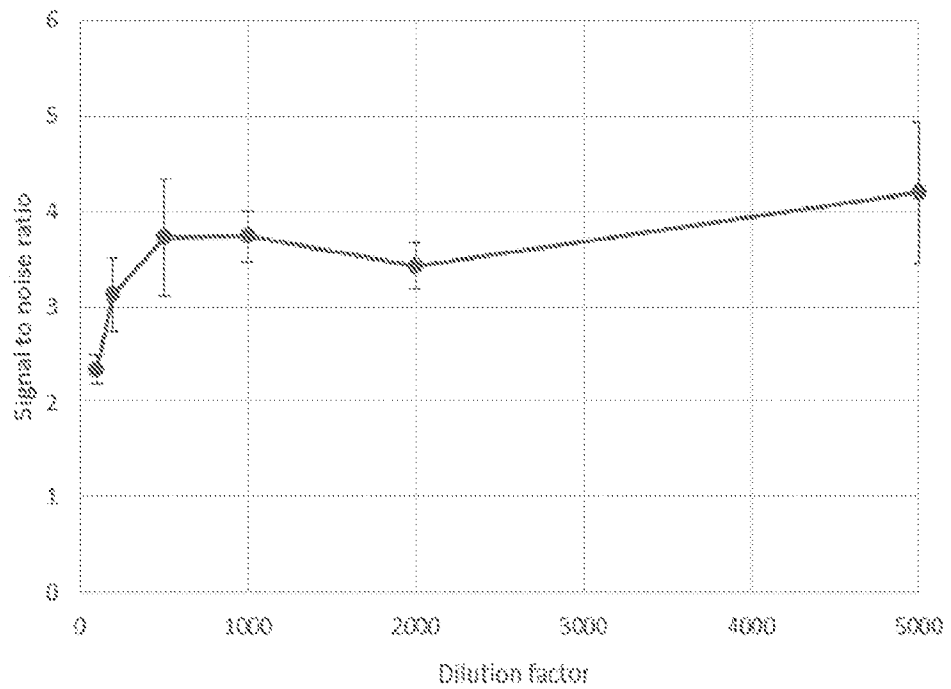


FIG. 13B

FIG. 14

Screen throughput (per array)	~1.5-6 x 10 ⁶ μ Pores
Recovery throughput	Up to 1000 single cells
Total screen duration	1-4 hr
B cell applications tested	WT mouse, OmniAb (humanized rat, chicken), Human PBMCs
Readouts	IgG secretion, bead binding, cell surface binding, cell activation (in development)

FIG. 15

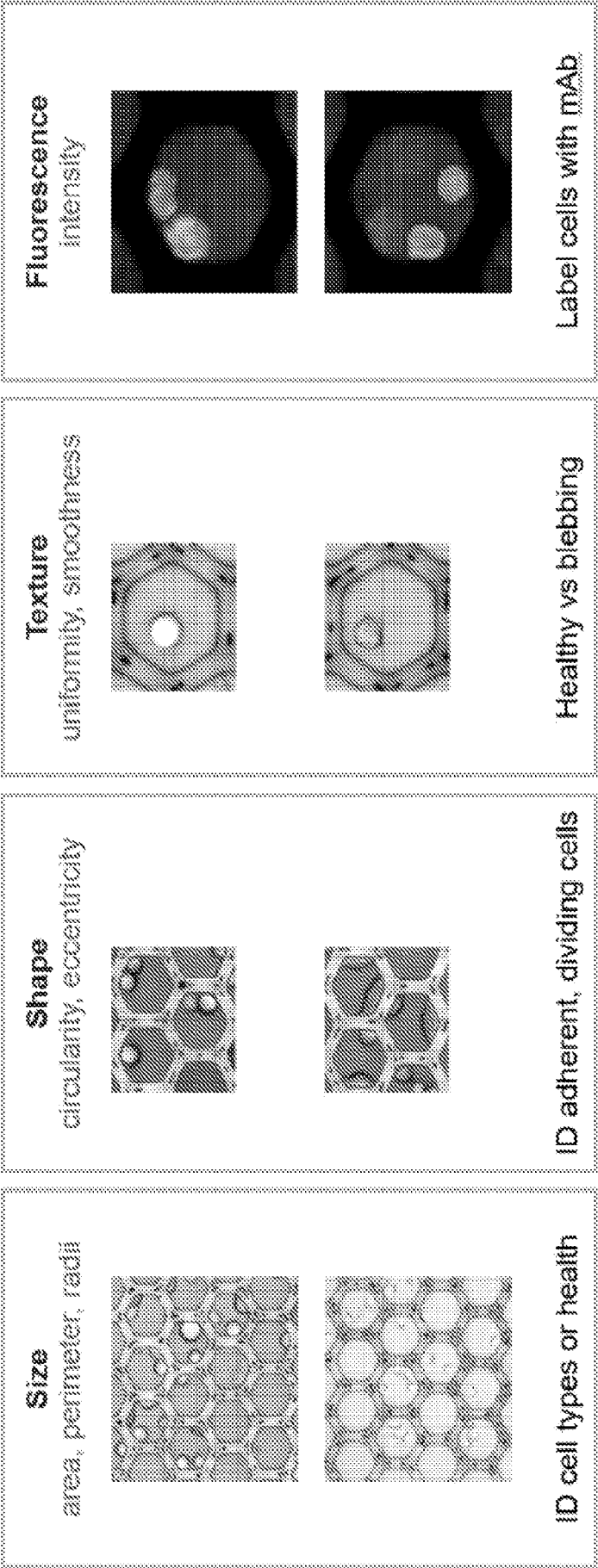
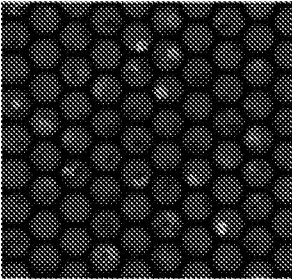
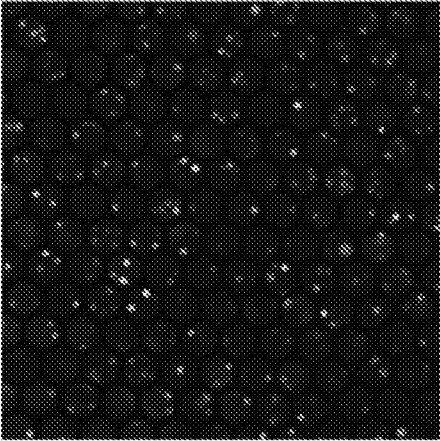


FIG. 16

Activation of primary
immune cells (24 hr)



Calcium mobilization
assay (2 min)



cAMP responsive GFP
reporter assay (20 hr)

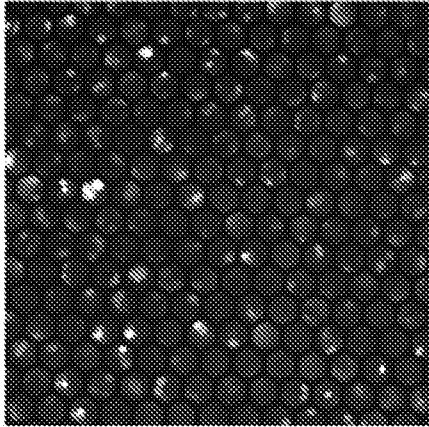


FIG. 17

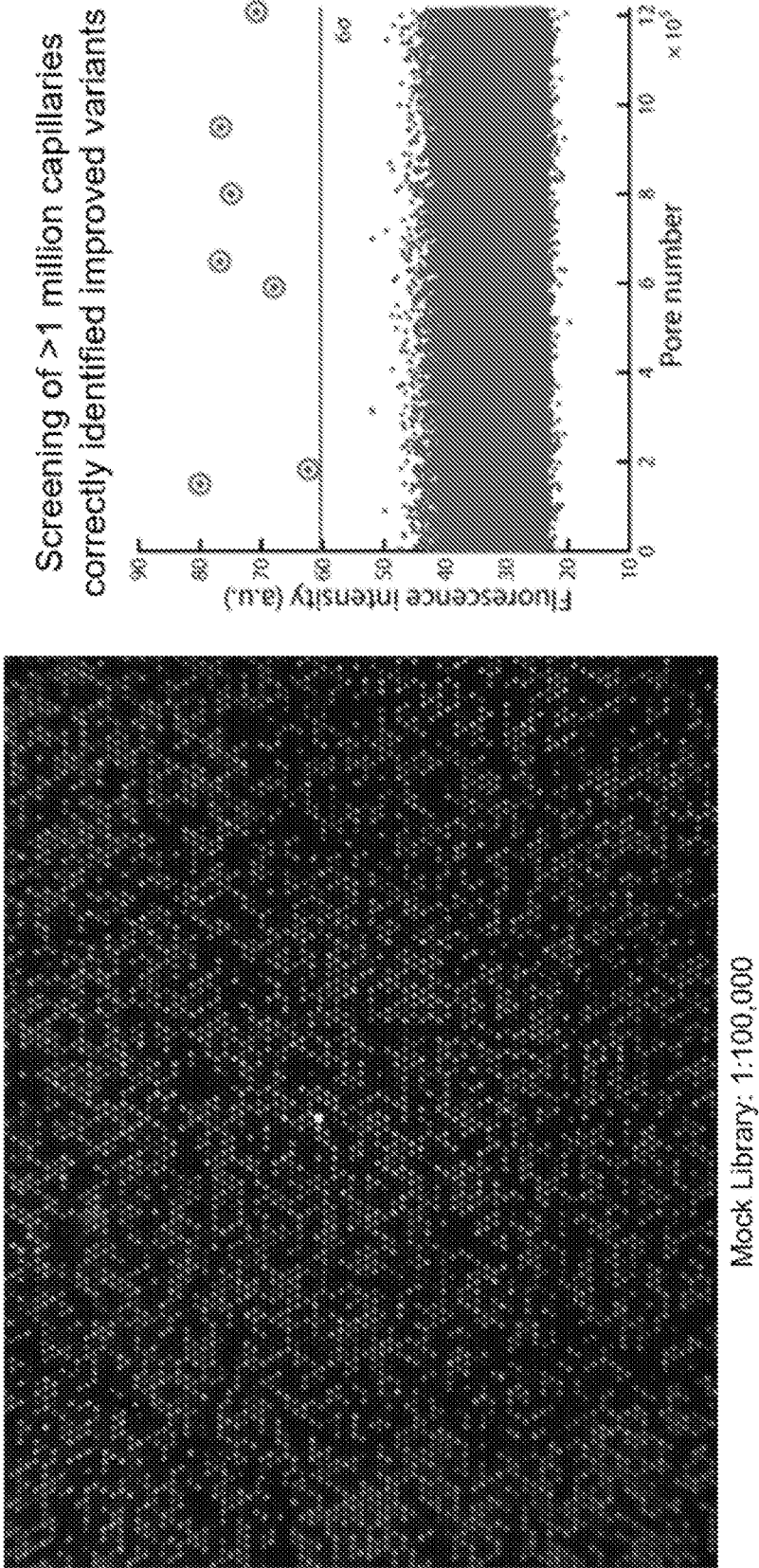


FIG. 18

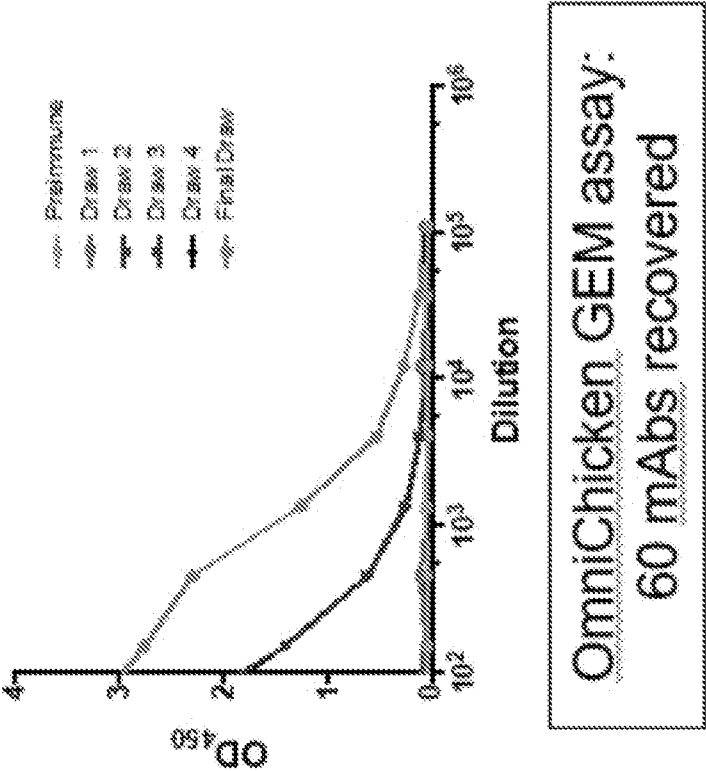
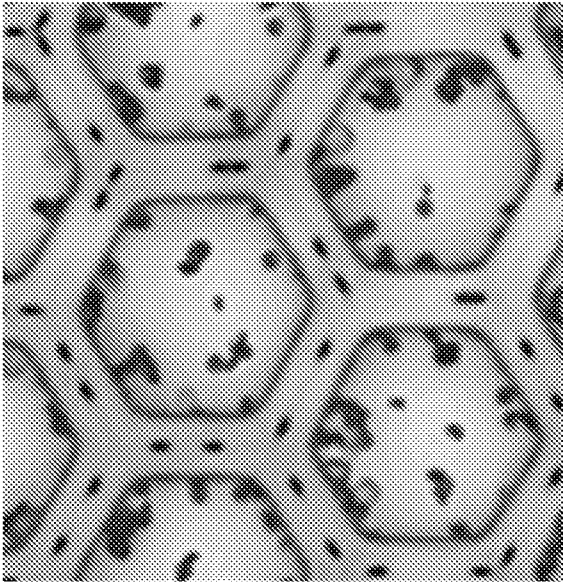


FIG. 19

mAb secretor	OmniChicken splenocytes (~1 cell / μ Pore)
Target	Progranulin-biotin on streptavidin beads (2.8 μ m)
Detection	Goat anti-Chicken IgY (H+L) - Alexa Fluor 488



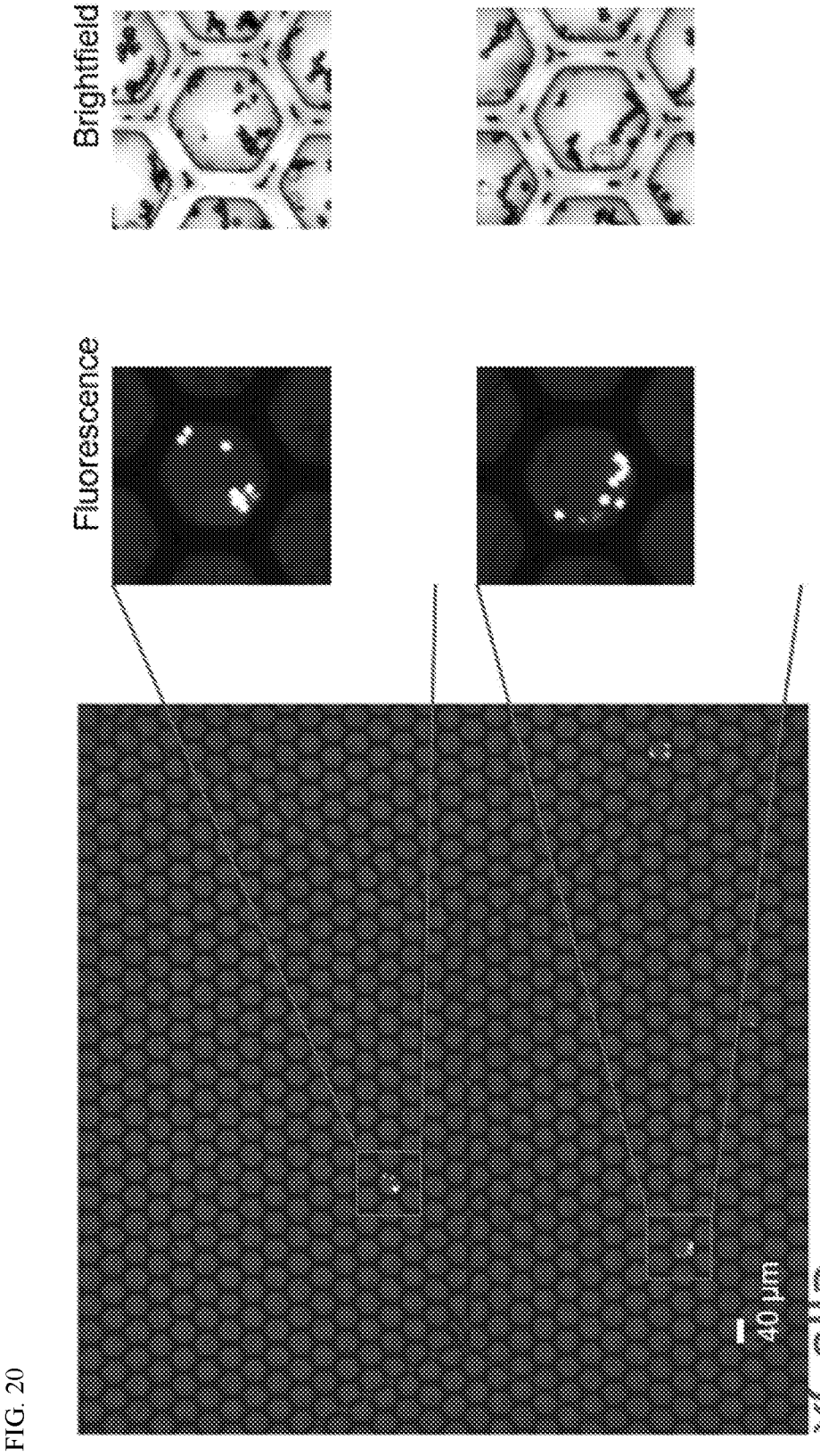
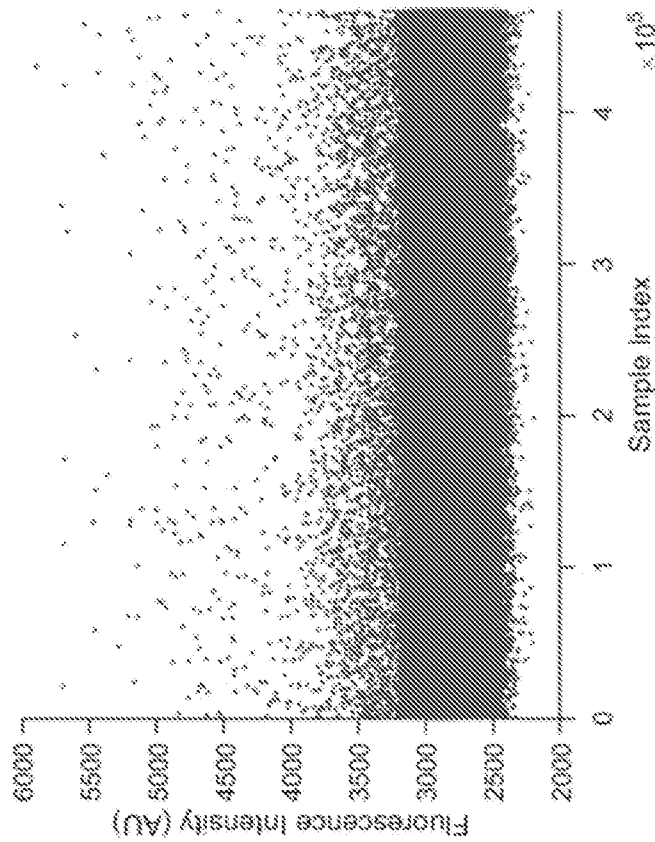


FIG. 21



Cells screened	470,000
% IgY secreting	0.85%
% Antigen binding	0.4%
% Specific of secreting	47%

■ Extracted ~1000 hits above cutoff

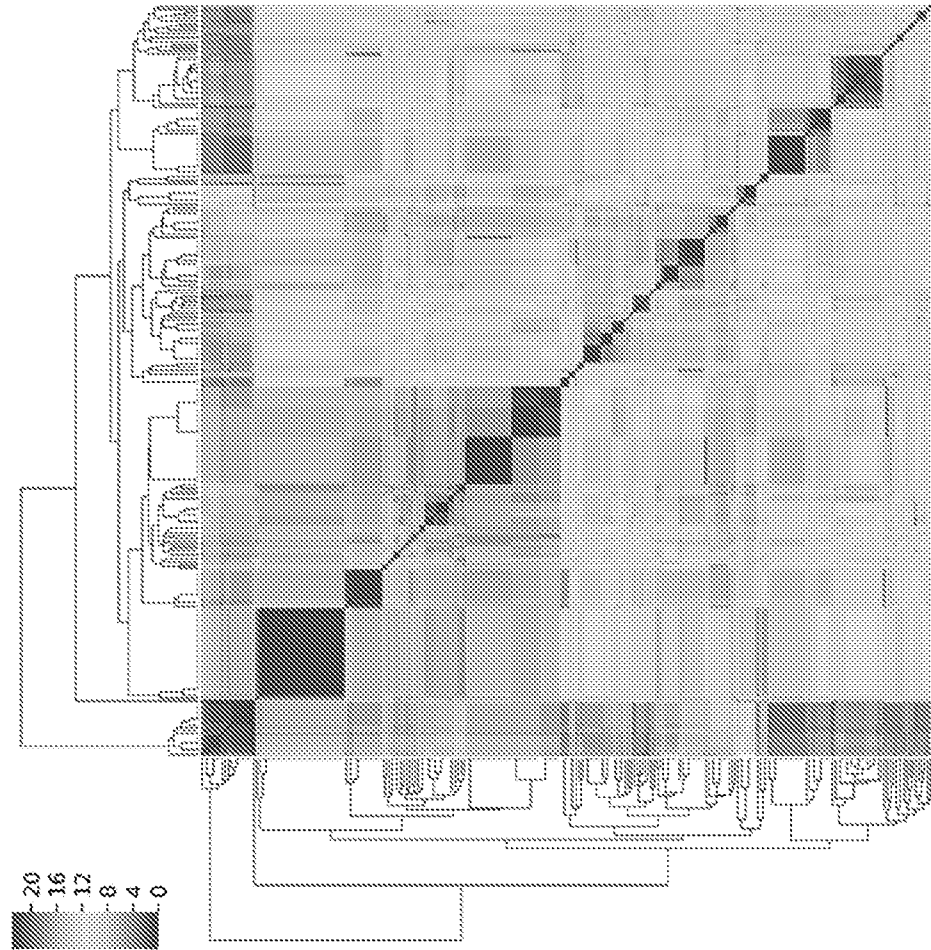


FIG. 22

FIG. 23

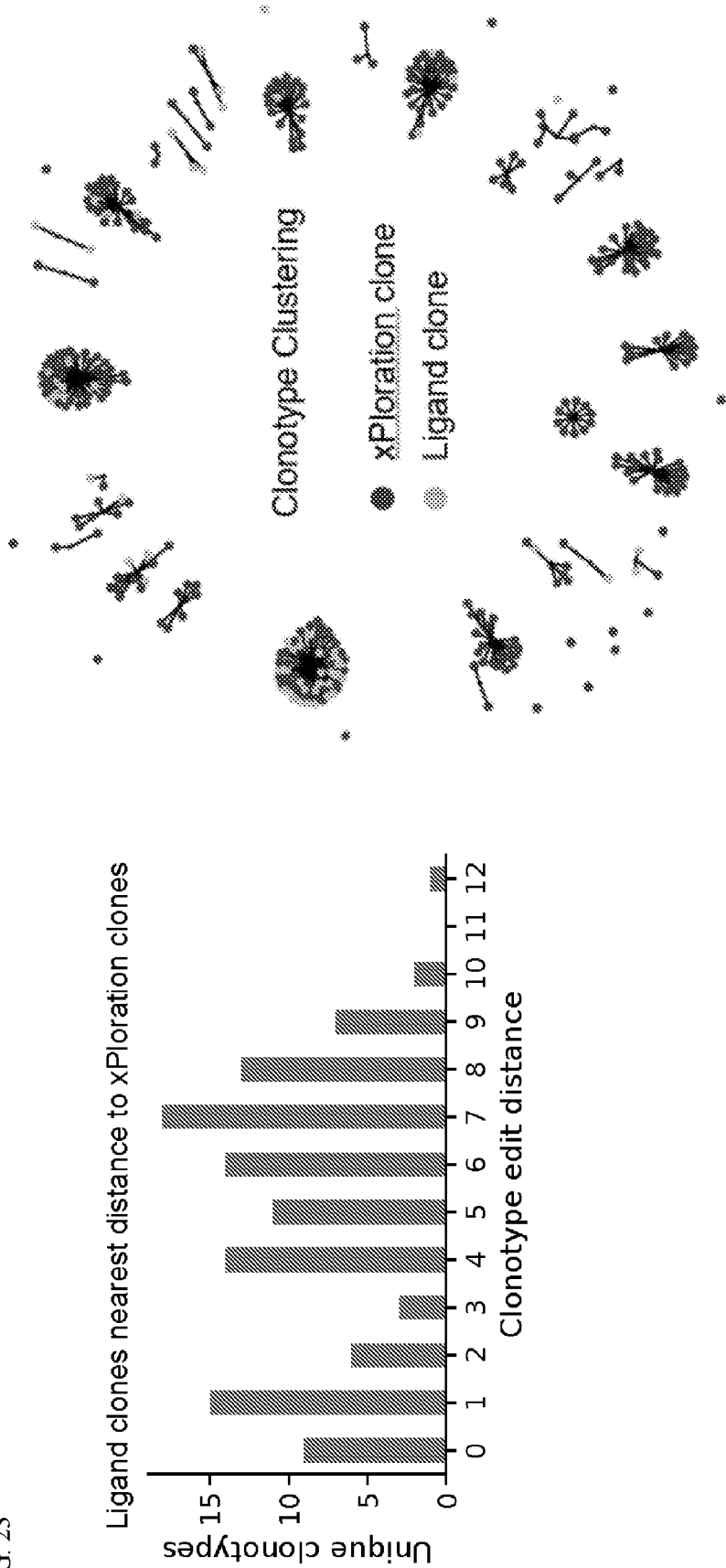
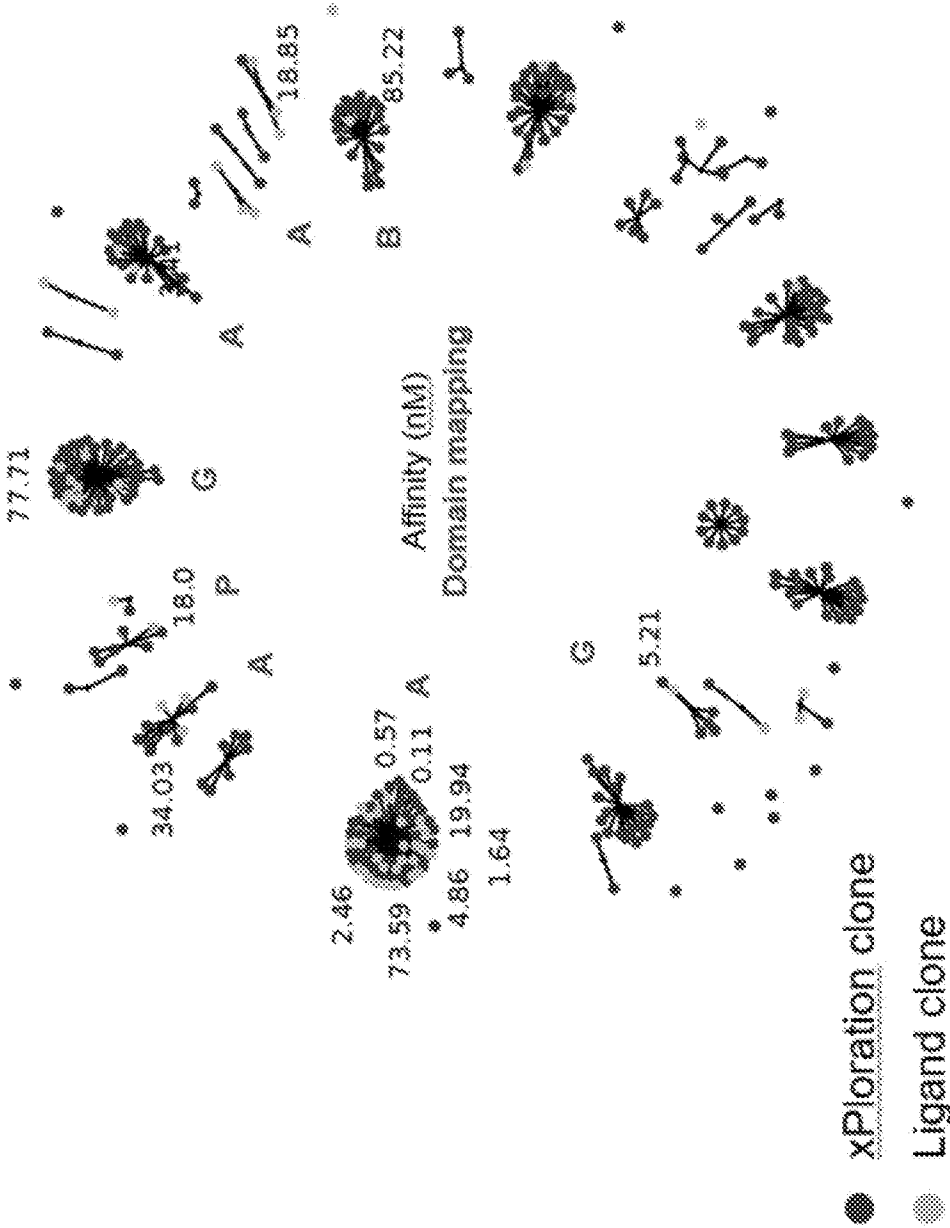


FIG. 24



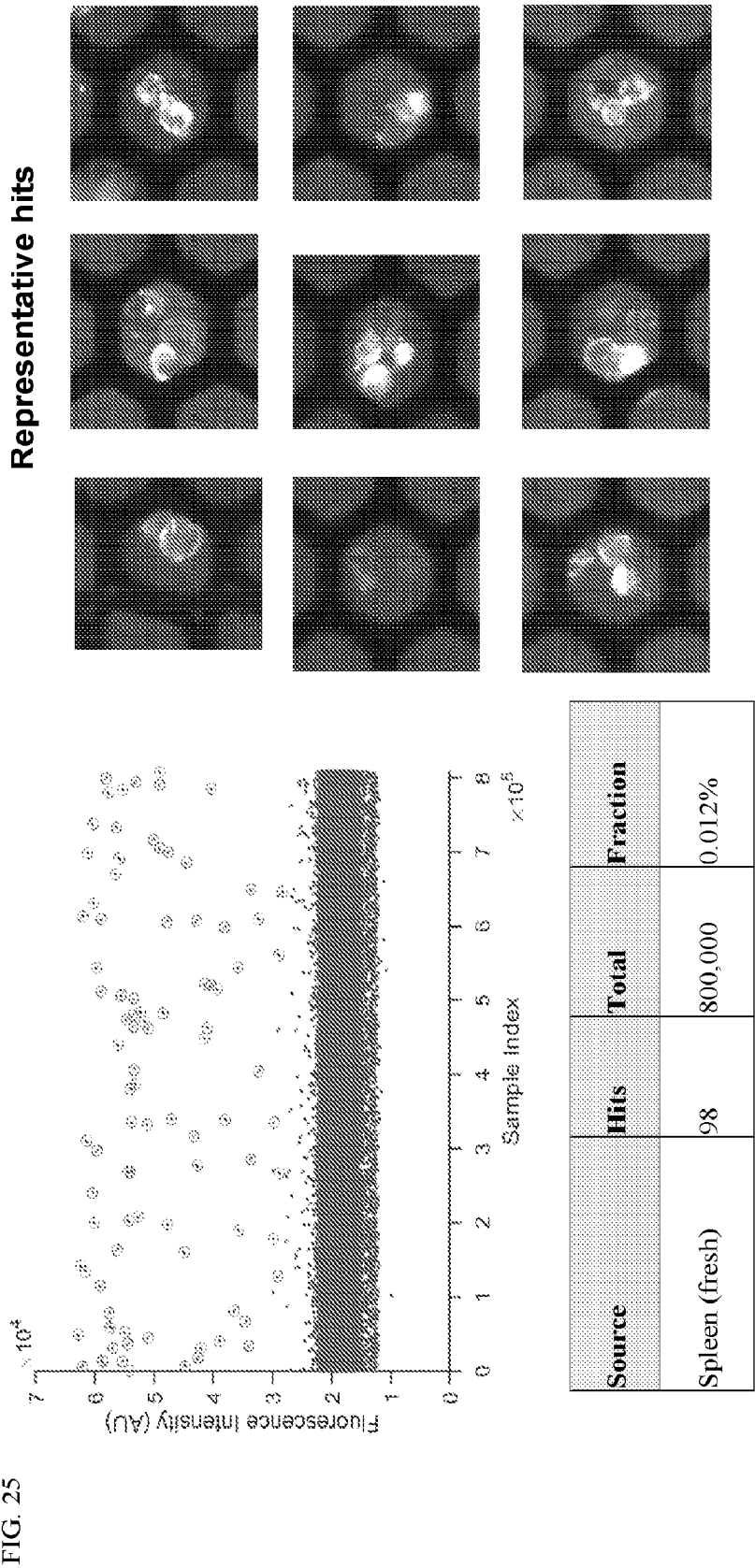


FIG. 26

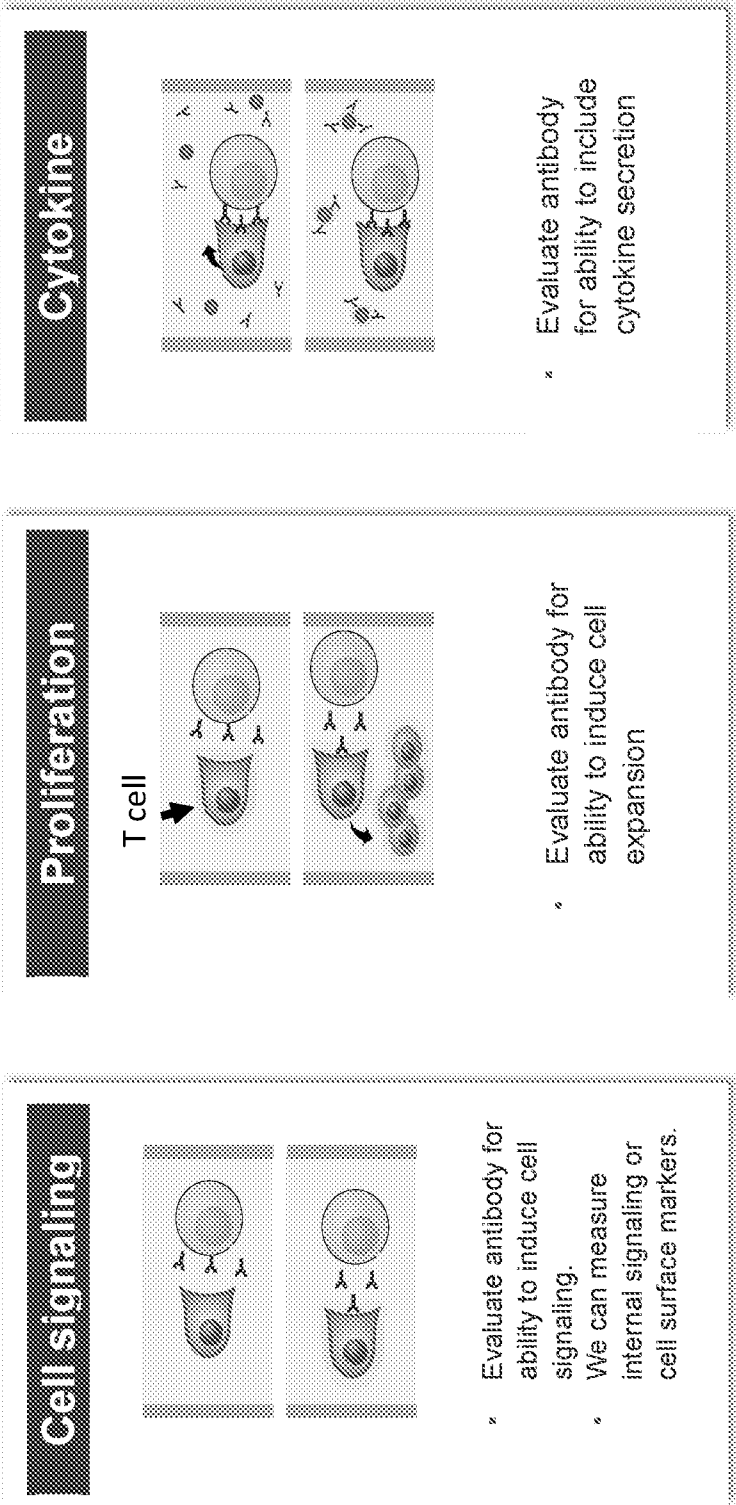
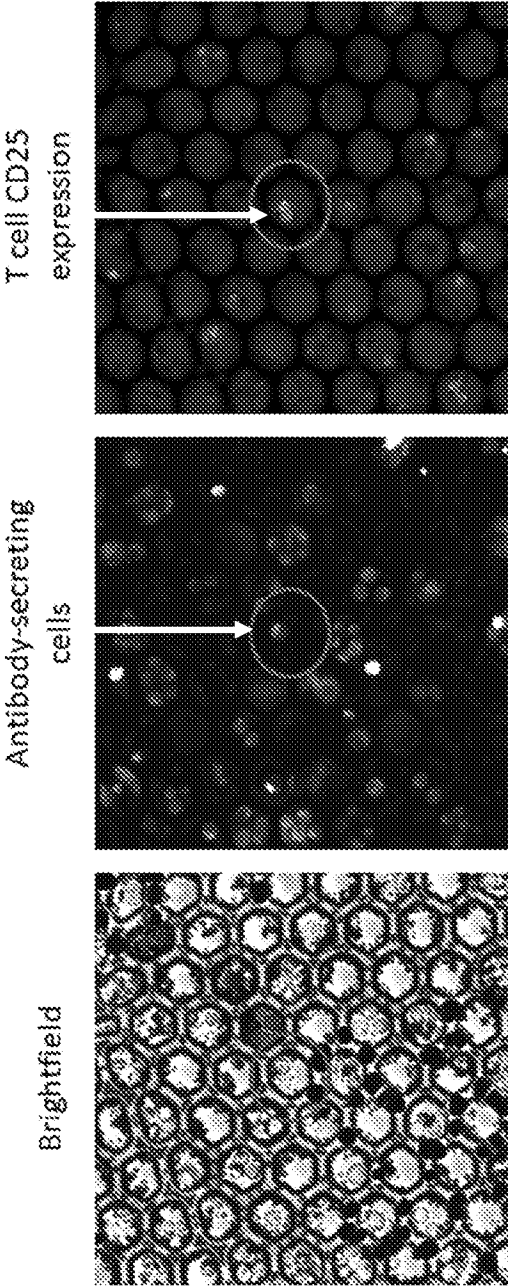


FIG. 27



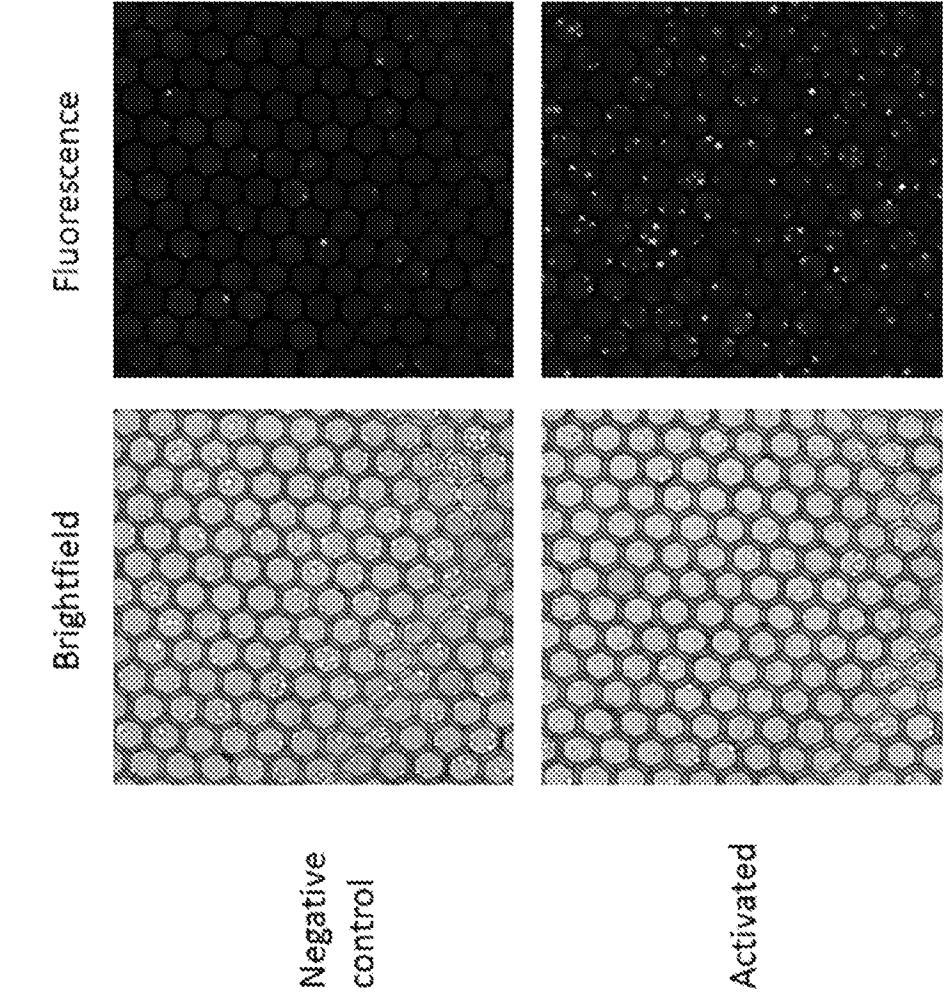


FIG. 28

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/026848

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - B01L 3/00; C12N 15/10; G01N 33/68 (2020.01)

CPC - B01L 3/50857; B01L 2300/0838; C12N 15/1055; G01N 33/6845; G01N 2500/10; G01N 2500/20 (2020.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2018/0163198 A1 (XCELLA BIOSCIENCES, INC.) 14 June 2018 (14.06.2018) entire document	1-5, 8-40, 43-68 ----- 6, 7, 41, 42, 69-89
Y	CHING et al. "Chickens with humanized immunoglobulin genes generate antibodies with high affinity and broad epitope coverage to conserved targets," mAbs, 02 November 2017 (02.11.2017), Vol. 10, No. 1, Pgs. 71-80. entire document	6, 7, 41, 42, 69-89
Y	US 2013/0236905 A1 (MARSHALL) 12 September 2013 (12.09.2013) entire document	77
Y	US 2010/0008892 A1 (SHANKAR et al) 14 January 2010 (14.01.2010) entire document	78-83, 87-89
Y	WO 2015/017889 A1 (UNIVERSITY OF SOUTH AUSTRALIA et al) 12 February 2015 (12.02.2015) entire document	84-86
A	US 10,227,583 B2 (COCHRAN et al) 12 March 2019 (12.03.2019) entire document	1-89
A	US 2016/0245805 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 25 August 2016 (25.08.2016) entire document	1-89

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

02 June 2020

Date of mailing of the international search report

09 JUL 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/026848

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 90-95
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.