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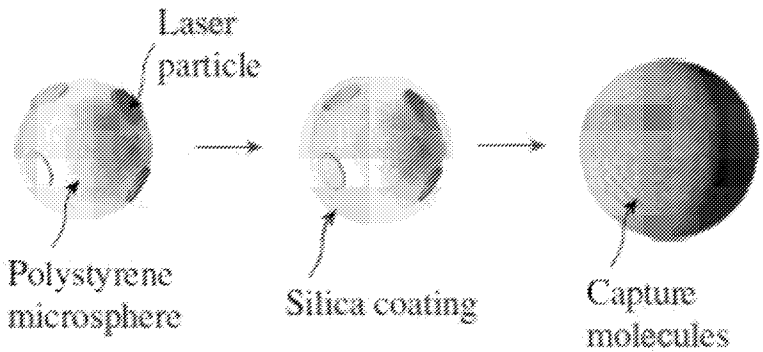

FIG. 3A

(57) Abstract: An apparatus for optical barcoding including: a microbead comprising one or more laser particles, the microbead being configured to generate one or more stimulated emission peaks hen energetically excited.

WO 2024/258932 A1

SPECTRALLY DISTINCT MICROBEADS USING LASER PARTICLES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is based on and claims priority from U.S. Patent Application Serial No. 63/507,506, filed on June 12, 2023. The entire disclosure of which is incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under grant numbers EB033155 and EB034687 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0003] Laser-emitting microparticles or laser particles (both referred to herein as "LPs") have been shown to be capable of generating stimulated emission when stimulated by suitable excitation energy. While existing LPs are proving to be useful, there is nevertheless room for improvement in this area.

SUMMARY OF THE INVENTION

[0004] Accordingly, new systems, methods, and apparatus for optical barcoding are desirable.

[0005] The output spectrum of LPs typically includes or consists of one or a few discrete narrowband laser lines or peaks. The wavelengths and frequencies of the lasing peaks can serve as an optical barcode of an object associated with the LPs. LPs can have various shapes including discoidal, spherical, rod-like, and cuboidal shapes as well as arbitrary shapes. The typical size of an LP is between 50 nm and 100 μm.

[0006] To expand the usefulness of LPs, the present disclosure provides embodiments of microbeads that contain one or more laser particles, each of which is capable of generating narrowband spectra and which thereby can be spectrally distinctive. The emission properties of laser particles provide an "optical barcode" of each microbead, which can be read optically and serve as an identification of the object. The disclosure provides embodiments of the design,

fabrication, and utilities of such microbeads.

[0007] Microbeads typically have a spherical shape, but herein we include non-spherical, spheroidal, discoidal, or cubic shapes as microbeads. More complex shapes are also possible, depending on the specific application. For example, in some embodiments droplets of resin containing LPs may be drop-casted on objects and solidified to provide a form of "microbeads". The objects used for drop-casting may be macro-sized objects, such as microwell plates, goods, electronic devices, and drug packages. The size of a microbead typically ranges from 1 μm to 1000 μm. However, in some embodiments, larger sizes on the order of millimeters or centimeters are also possible.

[0008] Microbeads with LPs may be used in screening of compounds. Major drug discovery programs screen millions of compounds to identify a handful of "hits" or lead compounds that proceed further along the drug development pathway. While state-of-the-art, ultrahigh-throughput machines can process more than 100,000 samples a day, they are restricted to one or two readouts per sample for measuring simple binary events, such as drug binding or cell viability. These simplistic screens can be often misleading and ultimately impede drug discovery by identifying unsuccessful or suboptimal candidates. Recently, there have been growing needs for multiplexed analysis early in the drug discovery process to increase the probability of identifying successful drug candidates. For example, antibody screening benefits from multiparameter analysis; phenotypic screening encompasses multiple parameters to assess efficacy and toxicity; and immunotherapy screening involves multi-marker functional assays.

[0009] Multiplexed high-throughput screening relies on some sort of encoding or barcoding strategy that allows different analytes or different samples to be identified during or post data acquisition. Fluorescence barcoding is a well-established technique, but it only allows for small-scale multiplexing of analytes. DNA barcoding is highly scalable and well suited for encoding a large number of compounds, genes, and samples, but it requires post-acquisition sequencing for its readout, which is slow and typically resorts to binary sorting based on the fluorescence data.

[0010] Accordingly, various embodiments disclosed herein include LPs embedded inside microbeads or attached on the surface of microbeads. The microbeads may further include various inorganic, organic, and biological materials, such as antibodies, oligonucleotides, DNA,

and drug candidates. The optical barcodes of microbeads may be related to the materials associated with the microbeads. The relationship allows one to identify the particular materials associated with individual microbeads by interrogating the optical barcodes. This ability is particularly useful for multiplexed molecular assays and high throughput drug screening in life sciences. Other applications include supply chain management, anti-counterfeiting, and DNA-data storage management.

[0011] The number of unique optical barcodes that can be created depends on the number of spectrally distinct stimulated emission peaks that are possible using the particular number of LPs associated with each microbead. Thus, in various embodiments the unique number of optical barcodes can be well over 1 million, and in particular embodiments greater than 1 billion unique barcodes are possible.

[0012] The disclosure also provides embodiments of optical barcoding technology that overcome the bottlenecks of the current encoding methods and which have the potential for making breakthroughs in high-throughput screening. One embodiment of LPs includes semiconductor microdisks with a diameter of between 1 μm and 2 μm which, together with the fact that the laser emission spectra from LPs are typically 1000-times narrower than fluorescence, makes them well suited for tagging micro-objects including cells, microbeads, and microcarriers, which can enable massive sample barcoding and pooled screening. Furthermore, the bright LP emission is readily readable using a near-infrared spectrometer integrated into a high-throughput platform, such as flow cytometry and droplet microfluidics. Because its readout can be performed simultaneously with fluorescence assays of each sample, this technology is particularly well suited for multiparameter and multifunctional screening.

[0013] Assays including homogeneous time resolved fluorescence (HTRF), enzyme-linked immunosorbent assays (ELISA), and amplified luminescent proximity homogeneous assays (Alpha) have been the workhorses of the pharmaceutical industry. Major drug discovery programs screen millions of compounds to identify a handful of "hits" or lead compounds that proceed further along the drug development pipeline. While current automated machines can process more than 100,000 samples per day, most measurements and analyses are restricted to one or two readouts per sample, measuring simple events such as drug binding or cell viability. These simplistic screens can often be misleading and ultimately impede drug discovery by

identifying unsuccessful or suboptimal candidates. Recently, there has been a growing need for multiparameter analysis early in the drug discovery process to increase the probability of identifying successful drug candidates. New technologies are needed to meet this compelling need and to continue to keep up with the ever-increasing demand for more biological information at higher rates of throughput.

[0014] Another use for microbead-LP complexes is in the area of sample multiplexing or barcoding. Sample multiplexing or sample barcoding is a technique that facilitates high-throughput analysis by allowing multiple target samples, such as cells in micro-wells and beads carrying analyte-capture molecules or drug compounds, to be combined into a single tube for multicolor staining and analysis. By mixing samples (cells or beads) prior to staining, reagent consumption is typically reduced 10- to 100-fold. Data robustness is increased through the combination of control and treated samples in the same batch, minimizing pipetting error, staining variation, and the need for normalization. Furthermore, data acquisition speed is enhanced. Unlike DNA, protein, or isotope-based multiplexing, optical multiplexing is compatible with fluorescence data acquisition, enabling multiparameter analysis via real-time demultiplexing (decoding).

[0015] The massive scalability of the technology disclosed herein comes from the unique laser emission provided through the use of many LPs (n), each having a unique wavelength or color and each of which serves as a base unit for multiplexing. By combining m LPs per sample, the number of unique spectral barcodes (N) scales up with $N = C(n, m)$, where C represents combination and where having a large value for n is important. For example: when n=20, $C(20,10) = 184,756$; when n=40, $C(40,10) = 8.48 \times 10^8$; and when n=80, $C(80,10) = 1.65 \times 10^{12}$.

[0016] Luminex xMAP bead technology is an industry standard for multiplex protein assays. Multiplexing is achieved by doping polystyrene beads with 2 or 3 different fluorescent dyes at various concentrations. The color-intensity combination encodes specific captured proteins and nucleic acids and can be decoded using flow cytometry. Although up to 80 multiplex beads are commercially available, this limited number of color-intensity levels does not permit users to scale up sufficiently to perform sample multiplexing for high-throughput screening applications.

[0017] On the other hand, using LP-tagged microbeads will increase the multiplexing

capability from 80 to well over 10,000. This massive multiplicity can be used for encoding either or both analytes and samples without having to resort to using time-consuming DNA-barcode sequencing readout procedures. A pooled optical assay can reduce the required sample volume, thereby reducing reagent costs and batch errors as well as flow acquisition time. This advance can provide significant benefits in drug screening, biomarker discovery, and immunoassays.

[0018] Microbeads have been extensively adopted in high-throughput screening as carriers of molecular diversity. Compound screening requires ~100 atto-mole, which is within the capacity of a single bead with a diameter in a range of 10-20 μm. Droplet microfluidic systems are ideally suited to handle and analyze bead-based libraries with high precision at minute volume scales. Currently, DNA barcoding is the standard choice for encoding. To generate DNA-encoded libraries, combinatorial chemistry or 'split-and-mix' methods are commonly used. The so-called One-Bead One-Compound (OBOC) synthesis can produce a large number (typically 10^6) of compounds, each bead having a specific compound and corresponding oligonucleotides.

[0019] One drawback of DNA encoding is that it puts a constraint on chemistry to generate libraries, as they must be able to tolerate water because DNA requires an aqueous solution. Removing the need to use DNA opens up more possibilities for drug screening. More importantly, DNA barcoding strategy relies on sorting positive phenotypes followed by sequencing the DNA barcodes for the counting and rank order of 'hit' compounds. While such a binding assay has been widely used for drug screening, its simple binary nature is insufficient in many emerging applications. A method to enable multiparameter analysis in the OBOC screening platforms has been lacking, however.

[0020] LP-based optical barcoding of beads offers an attractive solution overcoming the drawbacks of DNA barcoding in OBOC screening. Optical decoding of LPs can be simultaneously performed alongside the fluorescence analysis of each sample (e.g., a bead-containing droplet). This makes it possible to acquire high-parameter data and analyze the sample in real time at the resolution of a single OBOC. This innovation will have significant impacts on high-throughput drug screening, particularly for peptide screening, for several diseases including diabetes and cancer.

[0021] In addition to these applications in life sciences, LP-tagged microbeads can be useful for barcoding a wide range of objects, such as apparel and other goods, electronic chips, and

pharmacy pill blister packs. The microbeads may be formed from droplets of resin containing LPs, which can be dropped onto the objects to be barcoded and solidified to form microbeads. The size of the microbeads may be smaller than 1 mm, although larger sizes are possible. Besides the lasing wavelengths emitted from LP-tagged microbeads, the spatial positions associated with individual lasing wavelengths may also be used as the emission properties that constitute optical barcodes.

[0022] Accordingly, one embodiment provides an apparatus for optical barcoding including: a microbead including one or more laser particles, the microbead being configured to generate one or more stimulated emission peaks when energetically excited.

[0023] Another embodiment provides a method for using optical barcoding to evaluate a sample, including: providing a plurality of microbeads, each microbead including one or more laser particles, and each of the plurality of microbeads being configured to generate one or more stimulated emission peaks when energetically excited; combining the plurality of microbeads with a sample; and energetically exciting, based on combining the plurality of microbeads with the sample, each of the plurality of microbeads to evaluate the sample.

[0024] Yet another embodiment provides a method of attaching a laser particle to a microbead for optical barcoding, including: treating the laser particle with a negatively charged compound to provide a negatively charged laser particle to improve adhesion to the microbead; and combining the negatively charged laser particle with the microbead to produce a laser particle-labeled microbead, the laser particle-labeled microbead being configured to generate one or more stimulated emission peaks when energetically excited.

[0025] Still another embodiment provides a system to capture biomolecules, including: microbeads tagged with laser particles and tagged with capture molecules, wherein the capture molecules are configured to capture target biomolecules, and wherein the laser particles are configured to generate one or more stimulated emission peaks when energetically excited; a laser particle barcode reader configured to read stimulated emission spectra from the laser particles; and a fluorescence assay configured to detect the captured target biomolecules.

[0026] Another embodiment provides a method of drug screening, including: providing a plurality of microbeads each including one or more laser particles, each microbead of the plurality of microbeads being configured to generate one or more stimulated emission peaks

when energetically excited; contacting the plurality of microbeads with a plurality of compounds such that each microbead of the plurality of microbeads conjugates to only one compound of the plurality of compounds; combining the plurality of microbead conjugated compounds with one or more target biomolecules; and detecting the one or more stimulated emission peaks of each conjugated microbead in the plurality of conjugated microbeads.

[0027] Yet another embodiment provides a microfluidic screening system, including: a plurality of microbeads each including a plurality of laser particles configured to generate one or more stimulated emission peaks when energetically excited, the microbeads each being conjugated to one or more screening compounds; one or more assay reagents; and a microfluidic circuit configured to contact the plurality of microbeads to the one or more assay reagents to form droplets.

[0028] Still another embodiment provides a method of generating a microbead including a laser particle, the method including: mixing the laser particle with a solution including at least one polymer and at least one solvent to create a laser particle suspension; generating a microdroplet from the laser particle suspension; drying the microdroplet to generate the microbead including the laser particle.

[0029] Another embodiment provides a method of optical barcoding, including: providing a plurality of microbeads; conjugating a first molecule to each of the plurality of microbeads; attaching a respective first laser particle to each of the plurality of microbeads, wherein the first laser particle attached to each of the plurality of microbeads corresponds to the first molecule attached to each of the plurality of microbeads; conjugating a second molecule to each of the plurality of microbeads; attaching a respective second laser particle to each of the plurality of microbeads, wherein the second laser particle attached to each of the plurality of microbeads corresponds to the second molecule attached to each of the plurality of microbeads; and performing an assay using the plurality of microbeads, wherein each of the plurality of microbeads is configured to generate one or more stimulated emission peaks when energetically excited.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] Various objects, features, and advantages of the disclosed subject matter can be more fully appreciated with reference to the following detailed description of the disclosed

subject matter when considered in connection with the following drawings, in which like reference numerals identify like elements.

[0031] FIGS. 1A-1D show several optically luminescent microbeads known in the art. FIG. 1A shows a fluorescent dye-doped polystyrene microsphere. FIG. 1B shows a dye-doped polystyrene microsphere (green) inside a biological cell. FIG. 1C shows a hyperspectral image of WGM emission from a dye-doped polystyrene microbead. FIG. 1D shows a 100% TPEQBN dye aggregate microbead.

[0032] FIGS. 2A-2B. 2A shows 45 μm-sized hydrogels encapsulating one or more dye-doped polystyrene microspheres. FIG. 2B shows a schematic of a cell tagged with discoidal LPs.

[0033] FIGS. 3A-3B. 3A shows a schematic embodiment of a polystyrene microbead with LPs attached on the surface and further coated with protective silica coating and then capture molecules. FIG. 3B shows a scanning electron microscopy image of a polystyrene microsphere tagged with a discoidal semiconductor LP (arrow).

[0034] FIG. 4 shows a microfluidic system for producing oil-in-water emulsions. After the solvent has evaporated, the droplet emulsions are turned into LP-embedded polystyrene microbeads.

[0035] FIG. 5 shows optical transmission images of two polystyrene LP-embedded microbeads formed using a system such as that shown in FIG. 4.

[0036] FIG. 6 shows experimentally measured spectra of stimulated emission from different microbeads, showing: Panel (a): a single laser peak; Panels (b-c): three laser peaks; Panels (d-h): more than four peaks above the background noise floor. The x-axis is photon energy, $E = hf$, where h is the Planck constant and f is optical frequency.

[0037] FIG. 7 shows an experimentally measured histogram for the number of LPs per microbead formed using a system such as that shown in FIG. 4. The dashed curve shows a theoretical Poisson distribution.

[0038] FIGS. 8A-8C. FIG. 8A Diagram of a streptavidin-conjugated bead designed to capture biotin-conjugated dye. FIG. 8B Zeta potential comparison between acrylic acid-functionalized microbeads and control. FIG. 8C Fluorescence intensity of dye-captured versus control LP-encoded beads (Exp), alongside measured values from commercial streptavidin-conjugated beads for comparison (Comm).

[0039] FIG. 9 shows a calculation of barcode errors as a function of the matching threshold δ for the case of $N_0 = 100{,}000$, $m = 3$, and $\sigma = 0.1$ meV.

[0040] FIGS. 10A-10B. FIG. 10A shows normalized stimulated emission lasing spectra of 400 "random" LPs with a uniform spacing in frequency. FIG. 10B provides an illustration showing the lasing spectra of ~200 LPs in 50 batches or frequency channels, with 50% frequency usage efficiency.

[0041] FIGS. 11A-11C shows a 10-plex, 384 sample multiplexing scheme. FIG. 11A depicts tagging core beads with pairs of LPs in wells. FIG. 11B depicts placing multiplex beads on wells. FIG. 11C depicts analysis of pooled multiplex beads.

[0042] FIGS. 12A-12B show y(x) and P(x)p1(x) curves for two exemplary cases. FIG. 12A shows the curves for using 5.5 μm diameter beads for assays, with 3-15 LPs per bead. FIG. 12B shows the curves for 15 μm diameter beads, with 10-33 LPs per bead.

[0043] FIG. 13 provides a schematic showing a known procedure for the attachment of DNA barcodes onto beads through iterative cycles alongside the in-situ synthesis of drug candidate chemicals.

[0044] FIG. 14 provides a schematic showing a procedure for using LPs for labeling or barcoding purposes. As a result of adding a particular bead at each step of a synthesis process, the specific compound on each bead is readily identified by measuring the optical emission of the attached LPs which enables real-time assessment of the function of the compound.

[0045] FIGS. 15A-15C. FIG. 15A shows a procedure in which LPs are added and attached via click chemistry using alkyne-PEG-linkers, where peptides are attached to Fmoc-protected amine photocleavable (PC) linkers. The LP tagging and peptide synthesis are orthogonal and performed serially. FIG. 15B shows a procedure in which LPs are added and attached via coulomb interaction, where the deprotected microbead and the polyelectrolyte coated LPs have opposite charges. Following the procedure, LPs remain attached on the microbead. FIG. 15C shows a barcoding scheme for 64 blocks in each round using a pair of two LPs from different batches. Numbers along the tops and sides of the array of wells (shown in red) indicate assigned LP channels. An exemplary bead is shown, which has gone through the three wells marked in blue.

[0046] FIGS. 16A-16C. FIG. 16A shows SEM images of Tentagel resin beads, each

tagged with 10-15 LPs. FIGS. 16B-16C show bright-field (Transmission) and fluorescence (FL) images which show that LP tagging and FITC-dye conjugation work well regardless of the sequence. The size of beads is larger in FIGS. 16B-16C since they are swollen inside DMSO while the beads in FIG. 16A have been dried prior to SEM visualization. LPs remained firmly attached in the presence of dyes, during drying and swelling, and for several days in DMSO.

[0047] FIGS. 17A-17C. FIG. 17A shows a schematic of an epoxy droplet microbead containing multiple LPs. FIG. 17B shows a barcoding microbead formed on an object. FIG. 17C shows an exemplary hyperspectral barcode of a microbead.

DETAILED DESCRIPTION

[0048] In accordance with some embodiments of the disclosed subject matter, mechanisms (which can include systems, methods, and apparatus) for optical barcoding are provided.

[0049] Embodiments of an apparatus for optical barcoding are described herein. The apparatus may include a microbead including one or more laser particles, in which the microbead is configured to generate one or more stimulated emission peaks when energetically excited. The one or more stimulated emission peaks may be generated by a respective laser particle in the microbead. Each laser particle in the microbead may be configured to have a different stimulated emission peak when energetically excited. A microbead can have a unique signature of one or more emission peaks based on the laser particles attached to the microbead.

[0050] The number of laser particles in a microbead can be adjusted for the intended purposes of the optical barcoding. For instance, if the aim is to label a small number of biomolecules with a unique microbead, a small number of laser particles may be used to label each microbead. Alternatively, if the aim is to label a large number of biomolecules with a unique microbead, a larger number of laser particles may be used to label each microbead. Increasing the number of laser particles in each microbead increases the number of possible signature emission peaks. The methods to determine the optimal number of laser particles for a given purpose are described in the examples below. Each emission peak may have a spectral width smaller than 1 nm. The stimulated emission peaks may also fall within a range of emission channels.

[0051] The apparatus for optical barcoding may also include at least one molecule that

can induce specific biological effects (e.g., binding, activity assays, etc.). The molecule can include a peptide, an amino acid, a protein, a small molecule, an antibody, an oligonucleotide, an RNA molecule, or a DNA molecule, or some combination thereof.

[0052] The laser particles may include a semiconductor disk, which can have a diameter ranging from 100 nm and 2.5 μm. More specifically, the diameter may range from 1.6 μm and 2.5 μm.

[0053] The laser particles may be embedded within the microbead or attached to an outer surface of the microbead. Various methods of attached laser particles to the microbeads are described in further detail in the examples below. The microbeads may be spherically shaped and have a diameter between 1 μm and 20 μm. Alternatively, the microbeads can have a non-spherical shape such as a dispensed droplet, a drop-cast shape, or an arbitrary three-dimensional shape. The microbead may include a polymer, hydrogel, or resin. The microbead may also include a silica coating. The microbead may also include a ferromagnetic material.

[0054] Embodiments of methods of using optical barcoding to evaluate a sample are also described herein. The method may include providing a plurality of microbeads, each including one or more laser particles such that the microbead is configured to generate one or more stimulated emission peaks when energetically excited. The plurality of microbeads may be combined with a sample, and then energetically excited. Each microbead may have a unique signature of emission peaks caused by the laser particles. Each laser particle in the microbeads may have a different stimulated emission peaks when energetically excited. The method may also include measuring the stimulated emission peaks of the microbeads when they are energetically excited to uniquely identify each of the microbeads. In addition, the sample may be evaluated based on the interaction of the sample with the assay compound.

[0055] Energetically exciting each of the microbeads may further include using near-infrared light to energetically excite the particles and measuring the fluorescence light emitted from each of the plurality of microbeads. The method may also include identifying each of the microbeads based on the measured one or more stimulated emission peaks.

[0056] In some embodiments, the sample may include a plurality of samples. Depositing each microbead into a respective separate container may include depositing each sample in plurality of samples into a respective separate container. Evaluating the plurality of samples then

includes evaluating the fluorescence light emitted from each of the respective microbeads.

[0057] The microbeads used in some embodiments may be sequentially conjugated to a plurality of compounds and a plurality of laser particles. This creates a library of uniquely identifiable microbeads, each of which has a different combination of compounds. Combining these microbeads with the sample may then include combining each microbead in the library with the sample. Energetically exciting the samples then includes exciting each microbead in the library. Since each microbead is uniquely identifiable, they can be used to evaluate the interaction of the sample and the microbead.

[0058] In some embodiments, the sample can include a plurality of objects. Similar to the embodiments described above, each microbead can be combined to a specific object and used to evaluate the plurality of objects. The object may include a microwell plate, an electronic device, or a drug package.

[0059] Also disclosed herein are embodiments of methods of attaching a laser particle to a microbead for optical barcoding. The laser particle may be treated with a negatively charged compound to provide a negatively charged laser particle to improve adhesion to the microbead. The negatively charged laser particle may be combined with the microbead to produce a laser particle-labeled microbead, where the laser particle-labeled microbead may be configured to generate one or more stimulated emission peaks when energetically excited.

[0060] In various embodiments, the laser particle may be a discoidal laser particle. The discoidal laser particle maybe coated on one side with the negatively charged compound to produce the negatively charged laser particle, which may include a single negatively charged face. In particular embodiments, the negatively charged compound may include a negatively charged polyelectrolyte.

[0061] In some embodiments, the laser particle may be coated with a silane compound to create a silanated laser particle, and then coating the silanated laser particle with a negatively charge polyelectrolyte to produce a negatively charged laser particle. The negatively charged laser particle can then be combined with a microbead to produce a microbead labeled with a laser particle. Each microbead can be configured to generate one or more stimulated emission peaks.

[0062] In some embodiments, the silanated laser particle may include a silanated discoidal laser particle. Coating the silanated laser particle may further include coating one side

of the discoidal silanated laser particle with a negatively charged polyelectrolyte to produce a silanated laser particle with a negatively charged face. Coating one side of the discoidal silanated laser particle may also include adhering the discoidal salinated laser particle on a dish in saline solution to expose a side of the discoidal salinated laser particle. The method may further include applying the negatively charged polyelectrolyte to the exposed side of the laser particle and removing the coated discoidal silanated laser particle from the dish by applying ethanol.

[0063] The silane compound may include (3-aminopropyl)triethoxysilane. The polyelectrolyte may include poly(sodium 4-styrenesulfonate). The laser particle-labeled microbead may include a silica coating. The laser particle-labeled microbead may also include a ferromagnetic material.

[0064] A system to capture biomolecules is also described herein. The system may include beads that are tagged with both laser particles and capture molecules that are configured to capture target biomolecules. The system may further include a laser particle barcode reader and a fluorescence assay to detect the captured target biomolecules. The microbeads may include more than one laser particle, each of which has a different stimulated emission peak when energetically excited. The combination of laser particles in a microbead can be used to uniquely label individual microbeads. In some embodiments, the capture molecules are configured to capture biotinylated dyes.

[0065] Methods and systems for high-throughput drug screening are described herein. Microbeads that are uniquely labeled with one or more laser particles are configured to generate one or more emission peaks when energetically excited. The microbeads may then contact a plurality of compounds so each compound is uniquely labeled with a microbead. This "one bead, one compound" method is described in more detail in the examples below. The microbead conjugated compounds may then be mixed with target biomolecules, and the one or more stimulated emission peaks of each conjugated microbead may be detected. This method can also use the split and pool technique, which is also described in more detail in the examples below.

[0066] Each microbead may include at least three laser particles, each of which has a different stimulated emission peak when energetically excited. In some embodiments, up to seven laser particles may be added to each microbead. The stimulated emission peaks of the laser particles may call within an emission channel.

[0067] The microbeads may have a diameter of between 10 μm and 20 μm. The laser particles may be attached to a microbead using one or more linkers, such as alkyne terminated PEG-connected linker and a photocleavable linker. Different linkers are described throughout the examples below, including discussions of benefits of specific linkers or specific combinations of linkers. In some embodiments, the compounds may be conjugated to the microbeads using a photocleavable linker such that the compounds may be released from the microbeads by applying light (e.g., UV light) to the photocleavable linker; the released compounds may then be in solution where they may react with other materials such as cells.

[0068] In some embodiments, the target biomolecules may be one or more of a peptide, an amino acid, a protein, a small molecule, an antibody, an oligonucleotide, an RNA molecule, or a DNA molecule.

[0069] In some embodiments, a microfluidic setup is used for detecting the stimulated emission peaks of the microbeads. The microfluidic set up may include excitation light sources, fluorescence detectors, and a spectrometer. Microfluidic set ups are described in more detail in the examples below.

[0070] A microfluidic screening system is described herein. The examples below describe an illustrative embodiment of a system configured for a CatD protease activity assay using a droplet-based screening system. The screening system may include microbeads including laser particles configured to generate one or more stimulated emission peaks when energetically excited. The microbeads may be distributed in individual wells of a multiwell plate. The system may also include one or more assay reagents, and a microfluidic circuit that is configured to contact the microbeads to the assay reagents to form droplets.

[0071] Each microbead may include at least three laser particles, each of which has a different stimulated emission peak when energetically excited. The laser particles may be attached to the microbeads using at least one linker. The linker may be a lysine linker or a photocleavable linker. In some embodiments, more than one linker may be used. In some embodiments, the system may include control microbeads.

[0072] In some embodiments, the assay reagents may be configured for an activity assay, such as a protease activity assay.

[0073] The system may further include a capillary-based flow instrument configured to

record the one or more stimulated emission peaks of the droplets. In some embodiments, the concentration of the assay reagents in the droplets is between 0.1 to 10 μM.

[0074] Also provided herein are methods of generating a microbead which includes a laser particle. The procedures, which are disclosed in further detail herein, may include: mixing the laser particle with a solution including at least one polymer and at least one solvent to create a laser particle suspension; generating a microdroplet from the laser particle suspension; and drying the microdroplet to generate the microbead. In some embodiments, the laser particle may be embedded within the microbead and/or may be on an outer surface of the microbead.

[0075] The following provides non-limiting examples of apparatus, methods, and systems for optical barcoding according to various embodiments of the disclosure.

[0076] Photoluminescent microbeads have been widely used in life sciences and other industries. One type of microbeads are dye-doped fluorescent microbeads or microspheres (FIG. 1A), however these microbeads do not contain LPs and as a result are limited in their ability to uniquely identify objects.

[0077] Microbeads capable of generating stimulated laser emission have also been known. Such microbead lasers are typically made of gain materials, such as conjugated polymer, quantum dots, and dye-doped resins. FIGS. 1B-1D show images of exemplary microbeads. The microbeads themselves serve as optical cavities, and the laser emission stems directly from the whispering gallery modes (WGMs) of the spherical cavities. While the output emission can exhibit multiple lasing peaks associated with one or more WGMs, since the modes are related to the dimension of the microbead, the number of unique spectral patterns is limited by the number of distinctive diameters of microbeads. This number is typically no more than 1,000.

[0078] By contrast, the emission from microbeads in this invention is based on the individual LPs embedded within and/or attached on the microbeads. This unique design allows the microbeads to be made of optically inactive materials without needing to provide optical gain. Also, multiple LPs coving a wide spectral range can be associated with each microbead. These advantages result in a far greater number of optical barcodes, easily over 1 million and far greater than 1 billion and even 1 trillion.

[0079] Hydrogels containing one or more smaller, dye-doped polystyrene microspheres have also been demonstrated (FIG. 2A). The combined WGM emission spectra from the internal

microspheres serve as the optical barcode of the encapsulating hydrogel microbead. However, the optical emission from microbeads is spontaneous, not stimulated, emission. Furthermore, due to the relatively small difference in the refractive index between the embedded microspheres and the surrounding hydrogels, the number of barcodes was limited to < 1 million for three microspheres per hydrogel.

[0080] Recently, optical barcoding of biological cells using semiconductor-based discoidal LPs have been demonstrated (FIG. 2B). One or more individual LPs are inserted into live cells or attached on the cell membrane. The combined stimulated emission spectra from the LPs provided the optical barcodes of the LP-tagged cells.

[0081] As an improvement over present technologies, the present disclosure provides microbeads containing one or more LPs, each capable of generating one or more spectral peaks of stimulated emission when energetically excited. While the LP-labeled microbeads can be made in multiple ways, one particular method to fabricate such microbeads uses surface tagging.

[0082] In one embodiment, the fabrication may begin with polystyrene cores (FIG. 3A). The core microspheres are tagged with one or more LPs (e.g., discoidal LPs) and may be optionally further encapsulated with a silica layer. The polystyrene core or silica coating layer may optionally contain magnetized iron oxide nanoparticles. The magnetite will allow the beads to be immobilized during washing. Further, biological molecules, such as antibodies and oligonucleotides that are capable of capturing specific molecules, may be attached to the microbeads. For nucleic acid assays, specific capture sequences, such as TAG™ or feature barcodes, can be attached using the same linker strategy as used in the industry. Alternatively, drug molecules, such as peptides, amino acids, proteins, or small molecules, that are capable of inducing specific biological effects may be attached to the microbeads. Fluorescent molecules, such as dyes that are capable of emitting fluorescence light when energetically excited, may also be attached to the microbeads.

[0083] To demonstrate one embodiment of a procedure for making LP-coated microbeads, we treated the LPs with compounds to improve their adhesion to microbeads. In one embodiment, the treatment first included treating the LPs with a silane compound on all sides followed by treatment of one side of the LPs with a negatively-charged compound to improve adhesion of the LPs to the beads. We first coated discoidal InGaAsP-based LPs with aminated

silica using (3-aminopropyl)triethoxysilane and then coated the particles with poly(sodium 4-styrenesulfonate) (PSS), which is a negatively charged polyelectrolyte when dissociated. To avoid bead aggregation, we coated only one side of the laser microdisks with PSS using the following technique. We found that aminated LPs easily adhere to the bottom of a plastic dish when they are in PBS, whereas they easily fall off in ethanol. Aminated LPs in ethanol (~50 μl) were put into a dish, followed by adding 1 ml PBS. The dish was centrifuged down to ensure LP adhesion to the bottom, then 1 ml of 1 wt% PSS solution was gently introduced and the sample was incubated for 5 hrs. To harvest the LPs, the dish was washed with PBS, and the LPs were harvested in ethanol with pipetting and sonication. The one-side PSS-coated LPs were then mixed with aminated polystyrene microbeads. FIG. 3B shows a representative microbead containing an LP made according to the method above.

[0084] Another embodiment of a method to fabricate such microbeads is provided using a microfluidic setup. FIG. 4 shows a microfluidic setup for producing LP-containing polystyrene microbeads. We used silica coated, random-wavelength LPs with diameters ranging between 1.6 and 2.3 μm and having a thickness of 230 to 300 nm, which were fabricated from three different compositions of $In_xGa_{1-x}As_yP_{1-y}$ on an InP substrate. The LPs were suspended in polystyrene solution containing 0.5 wt% polystyrene in methylene chloride (2M LPs in 100 μl). Polystyrene microdroplets containing LPs were produced in a glass microfluidic chip using an oil-in-water emulsion method. The microdroplets were then solidified by evaporating the methylene chloride solvent. We used polystyrene with a large molecular weight of 3,000,000 to prevent the encapsulated LPs from escaping the microdroplets and kept them embedded while microdroplets were being solidified. FIG. 5 shows microscope image of fabricated LP-beads, where it can be seen that several LPs are embedded in solidified polystyrene microbeads.

[0085] The microbeads were further coated with aminated silica using a modified Stöber process. Various methods are available to attach other chemical groups, chemicals, dyes, or biological molecules. For example, the Carboxyl group can be attached onto the surface. Antibodies can be attached using EDC chemistry. We have conjugated fluorescein isothiocyanate (FITC) and confirmed fluorescence emission from the dyes on the surface.

[0086] We measured lasing emission spectra of individual LP-tagged beads using a custom-built flow cytometer equipped with a pump laser providing 1064 nm nanosecond pulses

and a high-resolution spectrometer. FIG. 6 shows representative lasing spectra from different microbeads, which we measured using the LP-reading flow cytometer, where the spectral acquisition time was about 4 μs per bead. The ratio of the signal peak to the background noise floor varied depending on the output power and, to some extent, the orientation of the discoidal LPs. The dynamic range of the spectrometer was about 30 dB. In one embodiment, we chose to use a signal-to-background ratio of about 5:1 as a criterion for identifying lasing peaks, although other ratios may also be used. Using a ratio of 5:1, FIG. 6E exhibits six well defined peaks whose wavelengths or frequencies serve as the optical barcode of the particular microbead. The 7th largest peak (small peak to the right of the three peaks on the left) may be discarded.

[0087] FIG. 7 shows a histogram of the number of spectral peaks or number of LPs we have measured per bead for a large number of beads, where the mean number was about 5. Based on this histogram, about 76% microbeads contained 3 or more LPs.

[0088] To facilitate the microfluidic production of LP-encoded polystyrene microbeads, we may use LPs pre-coated with polystyrene. Bare or silica-coated LPs tend to stick to silica-based microfluidic channels, and bare LPs are prone to aggregation and may escape from polystyrene microdroplets during the solvent drying process. We conducted experiments with InGaAsP LPs with diameters between 1.6 and 2.3 μm and thicknesses between 230 to 300 nm. The process began by coating the bare LPs with polydopamine. This was achieved by immersing the LPs in a dopamine hydrochloride solution, followed by agitation under 254 nm UV light for six hours, resulting in a 10 nm thick polydopamine layer. This layer serves as a foundation, offering radical initiators and chemical functional groups necessary for the subsequent radical polymerization reaction. The polydopamine-coated LPs were transferred to a degassed styrene solution and agitated under 365 nm UV light for 12 hours. This step led to the formation of a polystyrene layer, 20 to 30 nm thick, on top of the polydopamine, resulting in a total coating thickness of 30 to 40 nm. LP-embedded polystyrene microbeads were successfully produced using a microfluidic fabrication method (e.g., see FIG. 4). This process involved dispersing polystyrene-coated LPs in a polystyrene solution (0.5 wt% in chloroform, with 2 million LPs per 100 μl). The solution was then used to create an oil-in-water emulsion inside a silica microfluidic chip, forming microdroplets containing the LPs. These droplets were solidified by evaporating the chloroform, effectively embedding the LPs within the polystyrene microbeads. To ensure that

the LPs remained encapsulated during the solidification process, a high molecular weight polystyrene (1.5 to 3 MDa) was used.

[0089] To enable bead-based assays, the beads are modified to attach antibodies or nucleic acid sequences or other molecules that can capture targets such as proteins, DNAs, and/or RNAs. Initially, the challenge with pure polystyrene microbeads is their lack of chemical functional groups necessary for biomolecule conjugation. One solution to this issue is integrating a 10% polystyrene-acrylic acid copolymer into the polystyrene solution during microbead fabrication. This copolymer introduces carboxyl groups to the bead surface, which facilitating biomolecule conjugation through NHS-EDC chemistry, as illustrated in FIG. 8A. The introduction of the polystyrene-acrylic acid copolymer significantly alters the surface charge of the microbeads, evidenced by their zeta potential. Microbeads containing the copolymer exhibited a zeta potential of -37 mV, in contrast to -21 mV for pure polystyrene microbeads (FIG. 8B). This decrease in zeta potential is attributed to the negatively charged acrylic acid groups present on the microbead surface.

[0090] To validate the effectiveness of this approach for protein conjugation, streptavidin was attached to the microbeads using NHS-EDC chemistry. The beads were then immersed in a CF568 dye-biotin solution, allowing for the capture of biotinylated dyes. The resulting fluorescence from these microbeads was measured using a previously developed flow cytometer from LASE Innovation (Woburn, MA). The mean fluorescence intensity (MFI) observed was 6×10^5 for the dye-soaked beads, with pristine beads displaying a low fluorescence background (FIG. 8C). For comparative purposes, commercially available 10-μm-diameter carboxyl-polystyrene microbeads were tested, yielding an MFI of 1.8×10^6 —three times higher than that of our experimental beads. The coefficient of variation for our beads was measured at 46, compared to 52 for the commercial beads, indicating a comparable level of uniformity in fluorescence intensity. With further refinement of the conjugation process, we aim to enhance the density of biomolecule attachment on our LP-encoded microbeads, potentially matching or exceeding that of commercially available beads.

[0091] To understand the efficiency of combinatorial barcoding, let us consider a pool of N_0 beads each tagged with m number of LPs randomly chosen. The sample is measured twice in subsequent experiments (A and B), yielding two sets of barcodes. To match the two sets, we look

for pairs of barcodes whose emission lines are within a certain threshold δ. When matching the two barcodes sets, two issues can arise. Due to the presence of noise in the laser frequency or energy measurements, some pairs of barcodes may not be matched correctly. Having a wider threshold δ can reduce the incidence of this event. In case of Gaussian noise with standard deviation σ, the probability of such unmatched barcodes is given by $P_1 = 1 - \left(erf\left(\frac{\delta}{\sqrt{2}\sigma}\right)\right)^m$. On the other hand, two different barcodes may have similar emission lines (within the threshold δ) and, as a consequence, may not be distinguishable. Decreasing δ can reduce the number of these duplicate events. In the case in which the LPs are taken from a uniform distribution in a range Δ, the probability of duplicate barcodes is $P_2 = 1 - \left(\frac{B-1}{B}\right)^{N_0-1}$, where $B = \left(\frac{\Delta/2\delta}{m}\right)$. For specific values of the problem parameters (N_0, m, σ), we can find an optimal value of δ that minimizes the percentage of "unreliable barcodes," i.e., barcodes that are not reliably matched. FIG. 9 shows the theoretical result for N_0 = 100,000, m = 3, and σ = 0.1 meV, which indicates that δ = 0.3-0.4 meV could yield the lowest total error, $P_1 + P_2$.

[0092] For massive scale barcoding, a large value for m, e.g., greater than 10, can be used. In many cases, the number of LPs per bead is not a single value but distributed in a range. For example, m may follow the Poisson distribution with a mean value $\langle m \rangle$.

[0093] The LPs used in the above experiment had "random" lasing wavelengths that were unknown a priori. This class of LPs is called "random" LPs. On the other hand, it is possible to make LPs with predetermined wavelengths and batch LPs may be produced which have a "nominally identical" lasing wavelength. FIG. 10 show exemplary emission spectra expected from the two different classes of LPs: (FIG. 10A) random and (FIG. 10B) batch LPs. The spectra of a large number of random LPs shown in FIG. 10A shows that the wavelengths are distributed more or less evenly across the spectrum (where the spectrum is represented in THz, ranging from 256 THz (1170 nm) to less than 190 THz (1580 nm)) whereas the batch LPs in FIG. 10B fall within wavelength ranges with empty space in between the various "channels"; the spectrum in FIG. 10B includes LPs that fall within one of 50 distinct channels.

[0094] FIG. 10B illustrates a design for 50 "batch" based on 50 spectral bins or color channels. The color channel is defined based on frequency division rather than the wavelength division because the gain bandwidth of semiconductors and the stimulated emission linewidths

tend to be more uniform in optical frequency than wavelength. Over a span of 66 THz (400 nm from 1170 nm to 1570 nm), 50 channels can be positioned with uniform spacing. The diagram in FIG. 10B has a constant channel width of 660 GHz (~3 nm at 1170 nm, ~5.4 nm at 1570 nm). The choice of 50 channels is reasonable for two reasons. First, it provides a large "n" number for high scalability in multiplexing. Second, the 660 GHz (~ 4 nm) gap between channels is sufficient to accommodate small wavelength shifts of LPs, for example, due to variations in temperature (~0.08 nm/°C) and surrounding index (<0.1 nm per % refractive index change) while still maintaining space between the channels.

[0095] Batch LPs may be produced using a microfluidic sorter. For example, a single-composition 9-layer 4-inch wafer can have ~3 billion disks with an interpillar spacing of 5 μm. With our current yield of 50% from wafer to particles, we expect to produce 1.5 billion microdisks per wafer, but about a half of these will fall at frequencies between channels, leaving 750 million usable microdisks. Assuming 10% loss in each sorting stage, a total 490 million microdisks in 8 channels may be harvested.

[0096] The maximum number of unique barcodes depends on how many colors are assigned to each bead. With n types of LPs from the 50 colors, we can in principle obtain C(50, n) combinations. For example, we may explore n = 3, which gives a full capacity of C(50,3) = 19,600. FIG. 11 illustrates one approach for a 10-plex pooled assay of 384 samples. LP-tagging on microbeads is performed in a 384 deep-well plates *in situ* (FIG. 11A). About 100,000 polystyrene core beads in ~50 μL water are added into each well and are labeled with two types of LPs (1 to 24 in rows, and 25 to 40 in columns) with a 4:1 LP-to-bead ratio. A third type of LPs (4:1 ratio) is also added to each of the wells. The third type is associated with specific antibodies, relating Channels 41 to 50 to 10 different target analytes. Capture antibodies are added to the wells and conjugated to the beads. For assay, diluents of LP-tagged microbeads are preloaded in a 384-well plate (FIG. 11B). Samples are added into the wells and incubated. After that, all the beads from the 384 wells are pooled into a tube, washed, and analyzed by a sandwich assay with detection antibodies and fluorophores in the tube (FIG. 11C). The pooled sample will then be acquired by an appropriate instrument such as an LP-reading flow cytometer or microfluidic system which identifies the sample indices (from LP 1-40) and analytes (from LPs 41-50) as well as the analyte concentrations based on mean fluorescence intensity.

[0097] For cytokine assays, microbeads may be coupled with antibodies against cytokines at saturated concentration (~5 μg per 1 million microbeads). LP-tagged microbeads in assay buffer may be added to a 384-well plate by a liquid handler at a concentration of ~100 beads per well for each type. Test samples are transferred to the assay plate, which are then sealed and shaken on a plate shaker at 2,000 rpm for 2 h at room temperature. After incubation, the content is washed by a magnetic plate washer thrice. Next, all of the beads in assay buffer in the wells are transferred to a tube using the liquid handler. After that, ~300 μL of detection antibodies are added into the tube, incubated for 1 hour in a shaker, and then washed twice. Next, ~300 μL of streptavidin-phycoerythrin is added to the tube, incubated for 30 min in a shaker, and washed twice, and ~500 μL of sheath fluid is added to the tube. The LP emission and fluorescence intensity from individual LP-tagged microbeads are read at ~120 μL/min until at least 50 beads per analyte per sample is measured.

[0098] The optimum number of LPs per microbead depends on applications and the sizes of the core microbead and LPs. The probability for the random binding of an LP to a bead to occur x times follows the Poisson statistics: $y(x) = \lambda^x e^{-\lambda}/x!$, where λ is the average number of events. When n types of LPs are used with a mean LP-to-bead ratio of λ_i for each type i, the number of LPs per bead will follow the Poisson distribution with $\lambda = n\lambda_i$ and $x = \sum_{i=1}^{n} x_i$. A tagging error occurs when any type of LP is missing from a microbead. The probability of this null error is $p_1(x) = \sum_{j=1}^{n} C(n,j)(-1)^{j-1}(n-j)^x/n^x$, where j is the number of missing types. As x increases and the available surface area gets depleted, it is increasingly difficult for the next event to happen, deviating from the Poisson statistics. To calculate this size effect, we use a model that the probability of the k-th event is reduced by $1-(k-1)r$, where r is the ratio of the effective area of an LP to the bead's surface area. We may approximate $r = 2d^2/D^2$ where D is the bead diameter, and d is the diameter of an LP (1.6 μm). Then, the event probability function is $P(x) = y(x)\,(1 - p_2(x))$, where $p_2(x) = \prod_{k=1}^{x}(1-(k-1)r)/\sum_{k=0}^{\infty} p_2(k)$. Finally, the barcoding efficiency R is then given by $1 - \sum_{x=1}^{\infty} y(x)p_1(x)$.

[0099] FIGS. 12A-12B depicts $y(x)$ (red curves) and $P(x)p_1(x)$ (blue curves) for two exemplary cases. FIG. 12A is directed to using 5.5 μm beads for assays, where the number of LPs per bead ranges between 3 and 15. This range of LPs can cover up to 32% of the total surface of a 5.5 μm bead. FIG. 12B is directed to using beads with a diameter of 15 μm, where

the number of LPs per barcoded bead ranges from 10 to 33. The total area that LPs occupy for a 15 μm diameter bead ranges between 3% to 9% of the bead's surface, leaving the remaining 91% to 97% of the surface area intact.

[0100] As an alternative to targeted tagging of microbeads using batch LPs, microbeads tagged with random LPs may be used for multiplexed assays. In this case, LP-tagged microbeads are loaded into a multi-well plate, then the optical barcodes of the microbeads in individual wells are measured and recorded in a storage medium. Then, biological molecules such as antibodies and oligonucleotides are loaded into the wells and linked to the microbeads. During fluorescence analysis of the microbeads, the optical barcodes are acquired simultaneously with fluorescence acquisition. From the barcodes, the biological molecules are identified and the assay molecules related to the biological molecules are measured based on the fluorescence information.

[0101] Combinatorial solid-phase peptide synthesis (SPPS) and DNA-encoded libraries (DELs) are established technologies for generating large molecular libraries and widely used by the pharmaceutical industry to discover small molecules capable of modulating biologically relevant targets. As an example, FIG. 13 illustrates the generation of DEL on the OBOC platform via the combinatorial split-and-pool technique. The effect of compounds on the target molecule(s) is assessed typically using fluorescence techniques, such as quenching (negative signal), time-resolved Forster resonant energy transfer (positive signal), amplified luminescent proximity homogeneous assay, and fluorescent protein expression. Microbeads that make "hits" or microdroplets containing such "hit" microbeads are sorted out. Then, the DNA barcodes on the microbeads or in their solution are amplified by polymerase chain reaction (PCR) and identified by bulk sequencing. The rank order in abundance indicates lead compounds.

[0102] LP-tagged microbeads allow optical barcoding to replace DNA barcoding. FIG. 14 illustrates this new approach. In each round of split-pool synthesis, both batch LPs and chemical building blocks are attached to microbeads. Once the LP-encoded library is created, high-throughput screening is possible in a microfluidic setup equipped with excitation light sources, fluorescence detectors, and a spectrometer. The ability to simultaneously measure fluorescence and optical barcodes makes it possible to align the quantitative fluorescence data (over a dynamic range) to individual beads and thereby measure the function of individual compounds in

greater detail with less ambiguity than conventional DEL-based screening. The real-time, quantitative, and high-throughput assessment can be a major step forward, breaking the limitations imposed by binary decision and sequencing in the *status quo*.

[0103] For library synthesis, one potential starting material is TentaGel® resin beads, which are copolymers which include a low crosslinked PS matrix on which polyethylene glycol (PEG, 3000 Da) is grafted. A bead diameter of 10 μm in a dry condition, or 15 μm when fully swollen, may be used. The gel volume can harbor ~100 fmol of a library member. This loading capacity is adequate for functional screening. LPs can be attached without interfering with compound synthesis. In one method, which is illustrated in FIG. 15A, we can utilize "click" chemistry which is inert to peptide synthesis for LP attachment (and vice versa). LPs are added and attached via copper-catalyzed azide-alkyne click chemistry using Alkyne terminated PEG connected to a linker. The LP binding linker is without a photocleavable moiety, so that LPs remain attached to the beads. For SPPS, an o-nitrobenzyl photocleavable linker may be introduced so that the coupled compounds are released into solution inside droplets (which may contain assay samples, e.g., cells) by illuminating UV light (365 nm). The amount of compound release is controllable by the UV intensity. This dual-linker design uses standard Fmoc chemistry (piperidine for deprotection) to attach peptide building blocks via standard amide bond formation and nucleophilic substitution reactions. It is also compatible with Boc, tBu, Cbz chemistry to protect side chains. The ratio of the two different linkers may be optimized.

[0104] An alternative approach is illustrated in FIG. 15B, which uses cationic polyelectrolyte coating to promote the adherence of LPs to the resin bead surface through Coulomb interaction. Bifunctional hybrid Tentagel® resin beads contain 20% non-cleavable sites and 80% cleavable sites. Even if 80% of ligands are cleaved to harvest compounds, LPs can remain attached on the bead surface via the interaction with the remaining ligands. All the reactions can be performed in an organic solvent, such as methylene chloride (DCM), without needing a series of alternating organic phase SPPS and aqueous phase enzymatic ligation steps for DNA encoding.

[0105] FIG. 15C depicts an exemplary barcoding scheme for 3-stage combinatorial libraries. The design accommodates 64 combinations each stage or round, suitable for mid-sized libraries of up to $64 \times 64 \times 64 = 262{,}144$ compounds. The 64 blocks in each round are barcoded

with a pair of LPs of different types. A 4:1 LP-to-bead ratio per channel can provide 85% barcoding efficiency. The small surface of LPs will not affect the total volumetric payload of the Tentagel resin beads. The presence of LPs can affect fluorescence intensity for assays, as the semiconductor absorbs visible light. However, the coefficient of variation (CV) would be < +/- 3%, which is smaller than the typical requirement of 15% CV in drug screening and fluorescence assays. The encoding number is increased by using more types of LPs per stage. For example, 3 types of LPs per stage gives $C(16,3) = 560$, which can generate $256 \times 256 \times 256 = 16,777,216$ combinations.

[0106] For experimental demonstration, we performed amine-functionalized silica coating on LPs and then single-side coating with PSS. FIG. 16A show SEM images of two representative LP-tagged Tentagel beads. To test compatibility with chemical conjugation, we conjugated FITC to LP-tagged beads and confirmed efficient conjugation (FIG. 16B). We also conjugated dyes first, followed by adding PSS-coated LPs (FIG. 16C). Both cases showed successful LP attachment and FITC-dye conjugation on the gel microbeads.

[0107] For illustration, let us consider a Cathepsin D (CatD) protease activity assay using a microfluidic droplet-based screening system. For bead screening and identification with analog criteria with real-time optical quantification, we may couple a lysine linker, a photocleavable linker, and Glu sequentially on TentaGel resin beads in 64 wells. In 48 of 64 wells, we may couple pepstatin A to Glu, and in the remaining 18 wells negative control beads are prepared by acetylating the Glu α amine and linker amine. The droplet-generating microfluidic circuit distributes the library beads into droplets containing activity assay reagents in a ~100 pL volume. The droplets are harvested and then split into 10 vials. The vials are illuminated by UV at different, pre-calibrated UV intensity levels so that the concentration of released pepstatin A in droplets ranges from 0.1 to 10 μM. After incubation for 15 min, the droplets in vials are pooled and loaded into the capillary-based flow instrument. The fluorescence intensity from a CatD-sensitive probe and the optical barcode of each droplet is recorded. From the acquired data, the standard curves of CatD activity over different dose levels, and the half maximal inhibitory concentration (IC50) can be determined (literature value is 10 nM).

[0108] The LP-based optical multiplexing is further scalable, and much greater than 3840-plex bead assays are possible. Seven (7) LP colors per bead (10 μm dia.) could make

$C(50,7)$ = 100 million combinations, which for instance can handle 200 proteins times a 1536-well plate ("307k-plex", ~30 ml with 20 µl/sample). Likewise, OBOC screening can be easily extended to 256 × 256 × 256 = 16.8 million. To create more LP channels, one hundred (100) LP colors may be possible by halving the channel width.

[0109] Instead of batch LPs, random LPs can be used. In this case, random LP-tagged microbeads are used as a starting material. LP-tagged microbeads, such as TentaGels, are split to different wells in a multi-well plate. The optical barcodes of the microbeads in each well are acquired and recorded in a storage medium. Different chemicals are added into different wells and coupled to the microbeads in the particular wells using chemical bonding or other appropriate reactions. Then, the microbeads from multiple or the entire wells are pooled. This completes a single stage in the combinatorial split-pool synthesis. This process is repeated for more stages until a library of desired compounds is created. The optical barcode reading allows the optical identification of the specific compound on each microbead.

[0110] Microbeads can also be made by using a drop cast method. FIG. 17A shows a resin or epoxy droplet containing LPs. Microdroplets are dispensed from a nozzle onto an object and then cured on the sample. The specific shape of the solidified resin or epoxy microbeads can be determined by the surface tension and viscosity of the droplet solution. FIG. 17B shows an example. Each microdroplet microbead is encoded by the stimulated emission properties of the LPs within it. The lasing peak wavelengths are the primary elements for the optical barcode. Optionally, the relative locations of LPs or the points of maximum spectral intensities can also serve as additional elements that can be used to constitute optical barcodes in addition to the spectral peaks. Such a hyper-spectral image barcode is shown in FIG. 17C. As long as the microbead is physically associated with, attached to, or linked to the object, the particular optical barcode of the microbead provides an identification of the particular object.

[0111] Thus, while the invention has been described above in connection with particular embodiments and examples, the invention is not necessarily so limited, and that numerous other embodiments, examples, uses, modifications and departures from the embodiments, examples and uses are intended to be encompassed by the claims attached hereto.

CLAIMS

What is claimed is:

1. An apparatus for optical barcoding comprising:
a microbead comprising one or more laser particles,
the microbead being configured to generate one or more stimulated emission peaks when energetically excited.

2. The apparatus of claim 1, further comprising at least one molecule capable of inducing specific biological effects.

3. The apparatus of claim 2, wherein the molecule comprises an assay compound comprising at least one of a peptide, an amino acid, a protein, a small molecule, an antibody, an oligonucleotide, an RNA molecule, or a DNA molecule.

4. The apparatus of claim 3, wherein the one or more stimulated emission peaks are related to at least one of the compounds.

5. The apparatus of claim 1, wherein each of the one or more laser particles comprises a semiconductor disk.

6. The apparatus of claim 5, wherein each of the semiconductor disks has a diameter between 100 nm and 2.5 μm.

7. The apparatus of claim 6, wherein each of the semiconductor disks has a diameter between 1.6 μm and 2.3 μm.

8. The apparatus of claim 1, wherein each microbead comprises at least three laser particles.

9. The apparatus of claim 8, wherein each of the at least three laser particles has a different stimulated emission peak when energetically excited.

10. The apparatus of claim 1, wherein the microbead comprises a plurality of laser particles, wherein at least two of the plurality of laser particles have different stimulated emission peaks when energetically excited.

11. The apparatus of claim 10, wherein the stimulated emission peaks of the plurality of laser particles fall within one of a plurality of emission channels.

12. The apparatus of any one of claims 1-11, wherein the one or more laser particles are embedded within the microbead.

13. The apparatus of any one of claims 1-11, wherein the one or more laser particles are attached on an outer surface of the microbead.

14. The apparatus of any one of claims 1-11, wherein the microbead comprises a spherical shape having a diameter of between 1 μm and 20 μm.

15. The apparatus of any one of claims 1-11, wherein the microbead comprises a substantially non-spherical shape comprising at least one of a dispensed droplet, a drop-cast shape, or an arbitrary three-dimensional shape.

16. The apparatus of any one of claims 1-11, wherein each of the stimulated emission peaks has a spectral width smaller than 1 nm.

17. The apparatus of any one of claims 1-11, wherein the microbead comprises at least one of a polymer, a hydrogel, or a resin.

18. The apparatus of any one of claims 1-11, wherein the microbead comprises at least one molecule capable of emitting fluorescence light when energetically excited.

19. The apparatus of any one of claims 1-11, wherein the microbead comprises a silica coating.

20. The apparatus of any one of claims 1-11, wherein the microbead further comprises ferromagnetic material.

21. The apparatus of any one of claims 1-11, wherein each of the one or more stimulated emission peaks of the microbead is generated by a respective laser particle of the one or more laser particles.

22. A method for using optical barcoding to evaluate a sample, comprising:

providing a plurality of microbeads,

each microbead comprising one or more laser particles, and

each of the plurality of microbeads being configured to generate one or more stimulated emission peaks when energetically excited;

combining the plurality of microbeads with a sample; and

energetically exciting, based on combining the plurality of microbeads with the sample, each of the plurality of microbeads to evaluate the sample.

23. The method of claim 22, wherein each of the plurality of microbeads comprises a plurality of laser particles,

wherein at least two of the plurality of laser particles emit different stimulated emission peaks when energetically excited,

wherein each of the plurality of microbeads emits a different combination of stimulated emission peaks when energetically excited such that each of the plurality of microbeads is uniquely identifiable, and

wherein energetically exciting each of the plurality of microbeads to evaluate the sample further comprises:

measuring the one or more stimulated emission peaks generated when the plurality of microbeads are energetically excited to uniquely identify each of the plurality of microbeads.

24. The method of claim 23, further comprising:

depositing each microbead of the plurality of microbeads into a respective separate container of a plurality of containers,

wherein combining the plurality of microbeads with the sample further comprises:

combining the sample with each of the plurality of microbeads in each of the plurality of containers, and

wherein energetically exciting each of the plurality of microbeads further comprises:

energetically exciting each of the plurality of microbeads to evaluate the sample.

25. The method of claim 24, further comprising, prior to energetically exciting each of the plurality of microbeads to evaluate the sample:

pooling the microbeads of each of the plurality of containers into a single container, and

energetically exciting each of the plurality of pooled microbeads to evaluate the sample.

26. The method of claim 25, wherein each of the plurality of microbeads comprises an assay compound,

wherein combining the plurality of microbeads with the sample further comprises:

combining the plurality of microbeads with the sample such that the sample interacts with the assay compound, and

wherein energetically exciting each of the plurality of microbeads to evaluate the sample further comprises:

energetically exciting each of the plurality of microbeads to evaluate the sample,

wherein the sample is evaluated based on interaction of the sample with the assay compound.

27. The method of claim 26, wherein energetically exciting each of the plurality of microbeads to evaluate the sample further comprises:

energetically exciting each of the plurality of laser particles using near-infrared light and measuring the one or more stimulated emission peaks,

energetically exciting each of the plurality of microbeads with excitation light and measuring fluorescence light emitted from each of the plurality of microbeads,

identifying each of the plurality of microbeads based on measuring the one or more stimulated emission peaks, and

evaluating the sample based on measuring the fluorescence light emitted from each of the plurality of microbeads.

28. The method of claim 27, wherein the sample comprises a plurality of samples,

wherein depositing each microbead of the plurality of microbeads into the respective separate container of the plurality of containers further comprises:

depositing each sample of the plurality of samples into the respective separate container of the plurality of containers, and

wherein evaluating the sample based on measuring the fluorescence light emitted from each of the plurality of microbeads further comprises:

evaluating each of the plurality of samples based on measuring the fluorescence light emitted from each of the respective plurality of microbeads.

29. The method of claim 24, wherein providing the plurality of microbeads further comprises:

sequentially conjugating each of a plurality of compounds and a corresponding plurality of laser particles to each of the microbeads to create a library of uniquely identifiable microbeads each comprising a different combination of the plurality of compounds,

wherein combining the plurality of microbeads with the sample further comprises:

combining each of the library of uniquely identifiable microbeads with the sample, and

wherein energetically exciting each of the plurality of microbeads to evaluate the sample further comprises:

energetically exciting each of the library of uniquely identifiable microbeads to evaluate an interaction of the sample with each microbead of the library of uniquely identifiable microbeads.

30. The method of claim 22, wherein the sample comprises a plurality of objects,

wherein combining the plurality of microbeads with the sample further comprises:

combining each of the plurality of microbeads with a respective object of the plurality of objects, and

wherein energetically exciting each of the plurality of microbeads to evaluate the sample further comprises:

energetically exciting each of the plurality of microbeads to identify each of the plurality of objects.

31. The method of claim 30, wherein the object comprises at least one of a microwell plate, an electronic device, or a drug package.

32. A method of attaching a laser particle to a microbead for optical barcoding, comprising:

treating the laser particle with a negatively charged compound to provide a negatively charged laser particle to improve adhesion to the microbead; and

combining the negatively charged laser particle with the microbead to produce a laser particle-labeled microbead,

the laser particle-labeled microbead being configured to generate one or more stimulated emission peaks when energetically excited.

33. The method of claim 32, wherein the laser particle comprises a discoidal laser particle, and

wherein treating the laser particle with the negatively charged compound further comprises:

coating one side of the discoidal laser particle with the negatively charged compound to produce the negatively charged laser particle comprising a single negatively charged face.

34. The method of claim 33, wherein the microbead comprises aminated polystyrene.

35. The method of claim 34, wherein coating one side of the discoidal laser particle with the negatively charged compound further comprises:

adhering the discoidal laser particle on a dish in a saline solution to expose a side of the discoidal laser particle,

applying the negatively charged compound to the dish to coat the exposed side of the discoidal laser particle with the negatively charged compound, and

removing the coated silanated laser particle from the dish by applying ethanol.

36. The method of any one of claims 32-35, wherein the negatively charged compound comprises a negatively charged polyelectrolyte.

37. The method of claim 36, wherein the negatively charged polyelectrolyte comprises poly(sodium 4-styrenesulfonate).

38. The method of any one of claims 32-35, wherein, prior to treating the laser particle with the negatively charged compound, the method further comprises:

treating the laser particle with silane.

39. The method of clam 38, wherein the silane compound comprises (3-aminopropyl)triethoxysilane.

40. The method of any one of claims 32-35, further comprising applying a silica coating to the laser particle-labeled microbead.

41. The method of any one of claims 32-35, wherein the laser particle-labeled microbead comprises ferromagnetic material.

42. The method of any one of claims 32-35, further comprising combining a plurality of negatively charged laser particles with the microbead.

43. The method of any one of claims 32-35, further comprising attaching at least one molecule to the microbead which is capable of inducing specific biological effects,

wherein the molecule comprises an assay compound comprising at least one of a peptide, an amino acid, a protein, a small molecule, an antibody, an oligonucleotide, an RNA molecule, or a DNA molecule.

44. The method of claim 43, wherein the microbead comprises a polystyrene-acrylic acid copolymer.

45. A system to capture biomolecules, comprising:

microbeads tagged with laser particles and tagged with capture molecules,

wherein the capture molecules are configured to capture target biomolecules, and

wherein the laser particles are configured to generate one or more stimulated emission peaks when energetically excited;

a laser particle barcode reader configured to read stimulated emission spectra from the laser particles; and

a fluorescence assay configured to detect the captured target biomolecules.

46. The apparatus of claim 45, wherein the capture molecules comprise an assay compound comprising at least one of a peptide, an amino acid, a protein, a small molecule, an antibody, an oligonucleotide, an RNA molecule, or a DNA molecule.

47. The apparatus of claim 45, wherein each microbead comprises at least three laser particles.

48. The apparatus of claim 47, wherein each of the at least three laser particles has a different stimulated emission peak when energetically excited.

49. The apparatus of claim 45, wherein each of the stimulated emission peaks has a spectral width smaller than 1 nm.

50. The apparatus of claim 45, wherein the capture molecules are configured to capture biotinylated dyes.

51. A method of drug screening, comprising:

providing a plurality of microbeads each comprising one or more laser particles, each microbead of the plurality of microbeads being configured to generate one or more stimulated emission peaks when energetically excited;

contacting the plurality of microbeads with a plurality of compounds such that each microbead of the plurality of microbeads conjugates to only one compound of the plurality of compounds;

combining the plurality of microbead conjugated compounds with one or more target biomolecules; and

detecting the one or more stimulated emission peaks of each conjugated microbead in the plurality of conjugated microbeads.

52. The method of claim 51, wherein each microbead of the plurality of microbeads comprises at least three laser particles.

53. The method of claim 52, wherein each of the at least three laser particles has a different stimulated emission peak when energetically excited.

54. The method of claim 51, wherein each microbead of the plurality of microbeads has a diameter of between 10 μm and 20 μm.

55. The method of claim 51, wherein the stimulated emission peaks of the plurality of laser particles fall within one of a plurality of emission channels.

56. The method of claim 51, wherein the laser particles are at least one of attached to or embedded within each microbead in the plurality of microbeads.

57. The method of claim 51, wherein the laser particles are at least one of attached to or embedded within each microbead in the plurality of microbeads using one or more linkers,

wherein the one or more linkers comprise at least one of alkyne terminated PEG connected to a linker or a photocleavable linker.

58. The method of claim 51, wherein each compound in the plurality of compounds is a pharmaceutical compound.

59. The method of claim 51, wherein the target biomolecules comprise an assay compound comprising at least one of a peptide, an amino acid, a protein, a small molecule, an antibody, an oligonucleotide, an RNA molecule, or a DNA molecule.

60. The method of claim 51, wherein a microfluidic setup is used for detecting the one or more stimulated emission peaks.

61. The method of claim 56, wherein the microfluidic setup comprises at least one of an excitation light source, a fluorescence detector, or a spectrometer.

62. The method of claim 51, wherein the compounds are conjugated to the microbeads using a photocleavable linker, and

wherein the method further comprises:

releasing the compounds from the microbeads by applying light to the photocleavable linker.

63. A microfluidic screening system, comprising:

a plurality of microbeads each comprising a plurality of laser particles configured to generate one or more stimulated emission peaks when energetically excited,

the microbeads each being conjugated to one or more screening compounds;

one or more assay reagents; and

a microfluidic circuit configured to contact the plurality of microbeads to the one or more assay reagents to form droplets.

64. The system of claim 63, wherein each microbead in the plurality of microbeads comprises at least three laser particles.

65. The system of claim 64, wherein each of the at least three laser particles has a different stimulated emission peak when energetically excited.

66. The system of claim 63, wherein the laser particles are attached to or embedded within the plurality of microbeads using at least one linker, wherein the at least one linker includes a lysine linker or a photocleavable linker.

67. The system of claim 63, wherein the plurality of microbeads comprises control microbeads.

68. The system of claim 63, wherein the assay reagents are configured for an activity assay.

69. The system of claim 63, wherein the assay reagents are configured for a binding assay.

70. The system of claim 63, wherein the assay reagents are produced by cells, wherein the cells are contained in the droplets.

71. The system of claim 63, further comprising a capillary-based flow instrument configured to record the one or more stimulated emission peaks of the droplets.

72. The system of claim 63, wherein the concentration of the assay reagents in the droplets is between 0.1 to 10 μM.

73. The system of claim 63, wherein the screening compounds are conjugated to the microbeads using a photocleavable linker, and

wherein the system further comprises a light configured to release the screening compounds from the microbeads by applying light to the photocleavable linker.

74. A method of generating a microbead comprising a laser particle, the method comprising:

mixing the laser particle with a solution comprising at least one polymer and at least one solvent to create a laser particle suspension;

generating a microdroplet from the laser particle suspension;

drying the microdroplet to generate the microbead comprising the laser particle.

75. The method of claim 74, wherein the laser particle is embedded within the microbead.

76. The method of claim 74, wherein the at least one polymer comprises a polystyrene solution.

77. The method of claim 74, wherein generating the microdroplet further comprises:

generating the microdroplet using a microfluidic chip.

78. The method of claim 77, wherein generating the microdroplet using the microfluidic chip further comprises:

generating the microdroplet using the microfluidic chip based on an oil-in-water emulsion method.

79. The method of claim 74, wherein drying the microemulsion is done using solvent drying.

80. The method of claim 74, wherein the laser particle comprises a coating.

81. The method of claim 80, wherein the coating comprises at least one of silica or polystyrene.

82. The method of claim 74, wherein the laser particle comprises a diameter between 1.6 μm and 2.3 μm.

83. The method of claim 74, wherein the laser particle comprises a thickness between 230 to 300 nm.

84. The method of claim 74, wherein the at least one polymer has a large molecular weight.

85. The method of claim 74, wherein the laser particle comprises a plurality of laser particles.

86. A method of optical barcoding, comprising:

providing a plurality of microbeads;

conjugating a first molecule to each of the plurality of microbeads;

attaching a respective first laser particle to each of the plurality of microbeads,

wherein the first laser particle attached to each of the plurality of microbeads corresponds to the first molecule attached to each of the plurality of microbeads;

conjugating a second molecule to each of the plurality of microbeads;

attaching a respective second laser particle to each of the plurality of microbeads,

wherein the second laser particle attached to each of the plurality of microbeads corresponds to the second molecule attached to each of the plurality of microbeads; and performing an assay using the plurality of microbeads,

wherein each of the plurality of microbeads is configured to generate one or more stimulated emission peaks when energetically excited.

87. The method of claim 86, wherein the one or more stimulated emission peaks are related to the first molecule and the second molecule conjugated to the microbeads.

88. The method of claim 86, wherein the first molecule is coupled to the second molecule, and

wherein the first molecule or the second molecule comprises at least one of a peptide, an amino acid, a protein, a small molecule, an antibody, an oligonucleotide, an RNA molecule, or a DNA molecule.

89. The method of claim 86, wherein at least two microbeads of the plurality of microbeads comprise different conjugated molecules from one another,

wherein each of the at least two microbeads comprise different attached laser particles from one another,

wherein each of the at least two microbeads is configured to generate different stimulated emission peaks from one another when energetically excited based on the different attached laser particles, and

wherein the different stimulated emission peaks correspond to the different conjugated molecules.

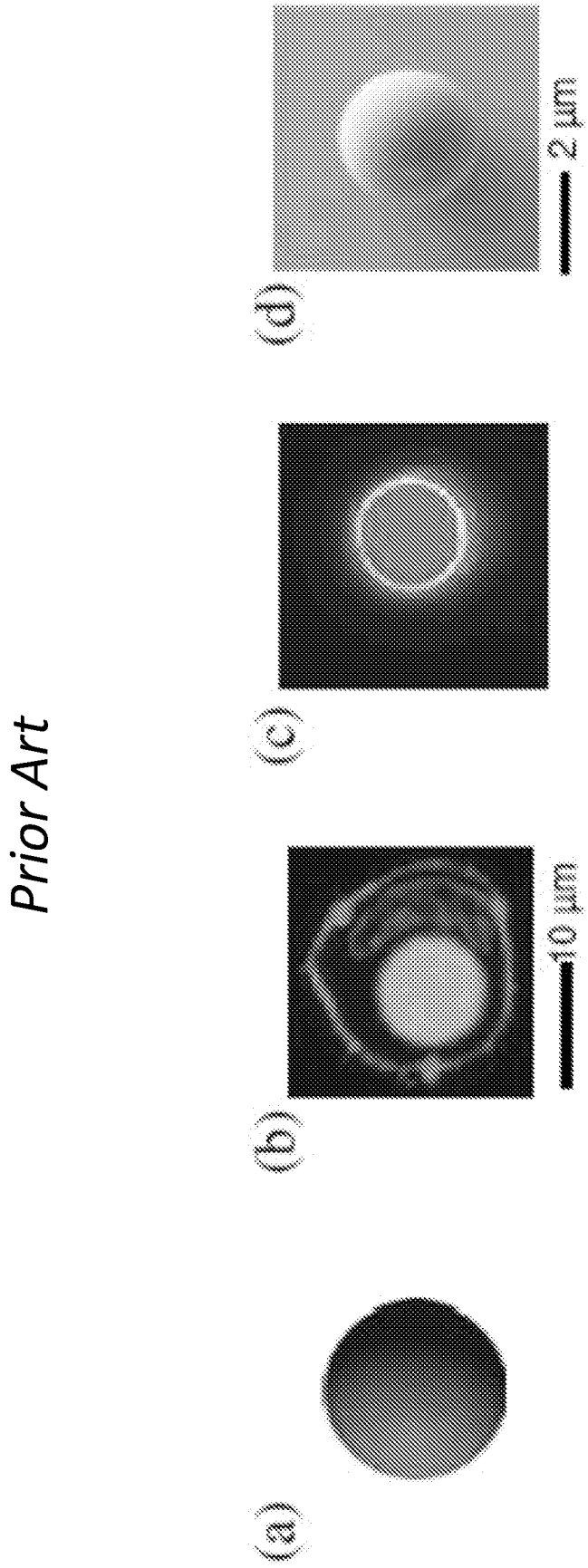

FIG. 1A

FIG. 1B

FIG. 1C

FIG. 1D

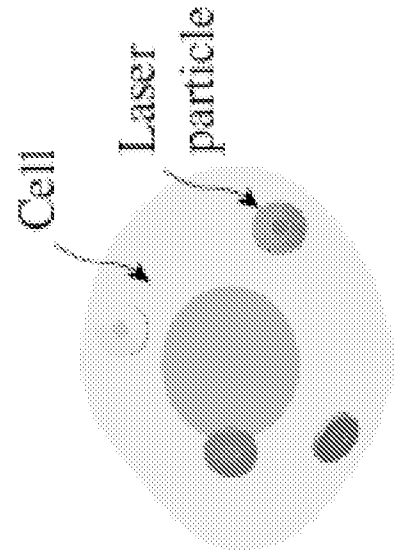

FIG. 2B

Prior Art

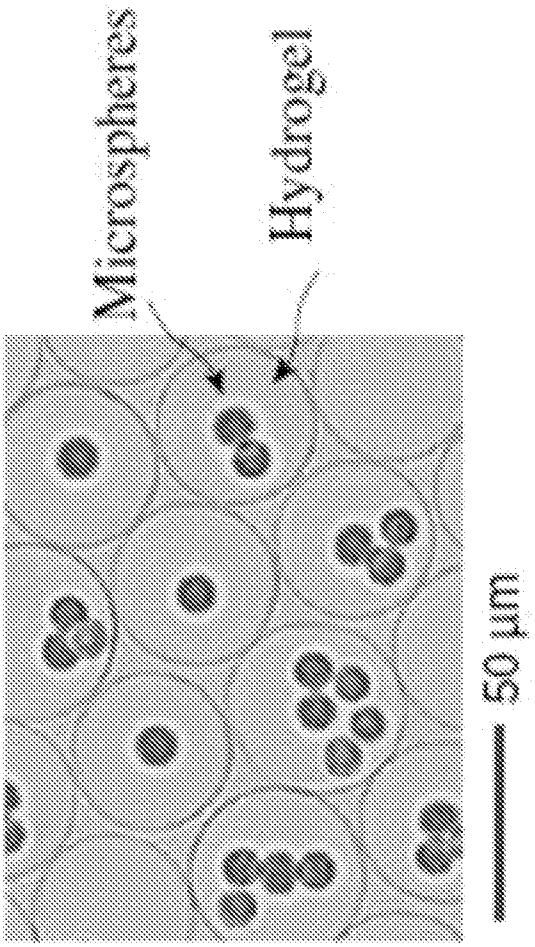

FIG. 2A

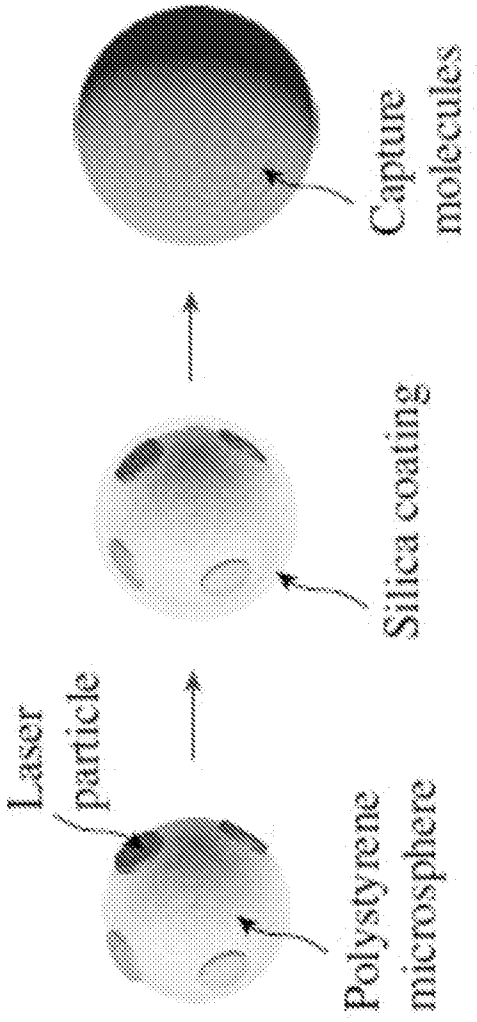
Laser particle
Polystyrene microsphere
Silica coating
Capture molecules

FIG. 3A

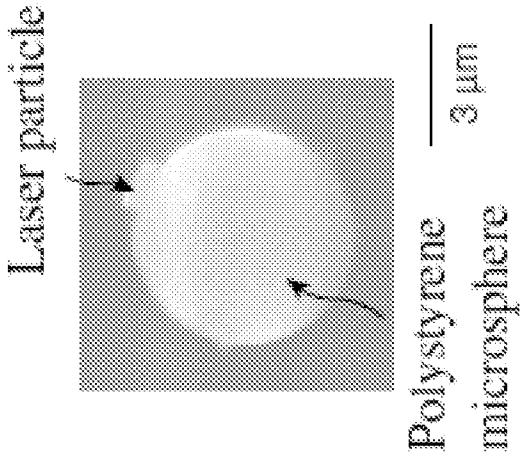
Laser particle
Polystyrene microsphere
3 μm

FIG. 3B

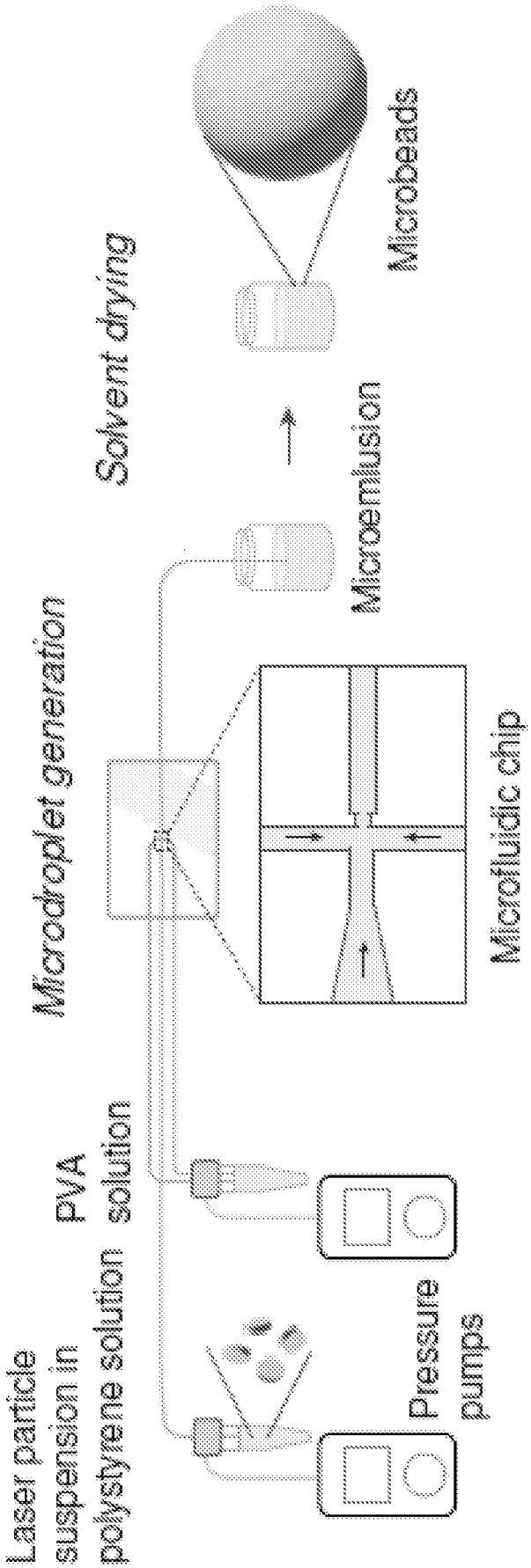
Laser particle suspension in polystyrene solution
PVA solution
Pressure pumps
Microdroplet generation
Microfluidic chip
Microemulsion
Solvent drying
Microbeads

FIG. 4

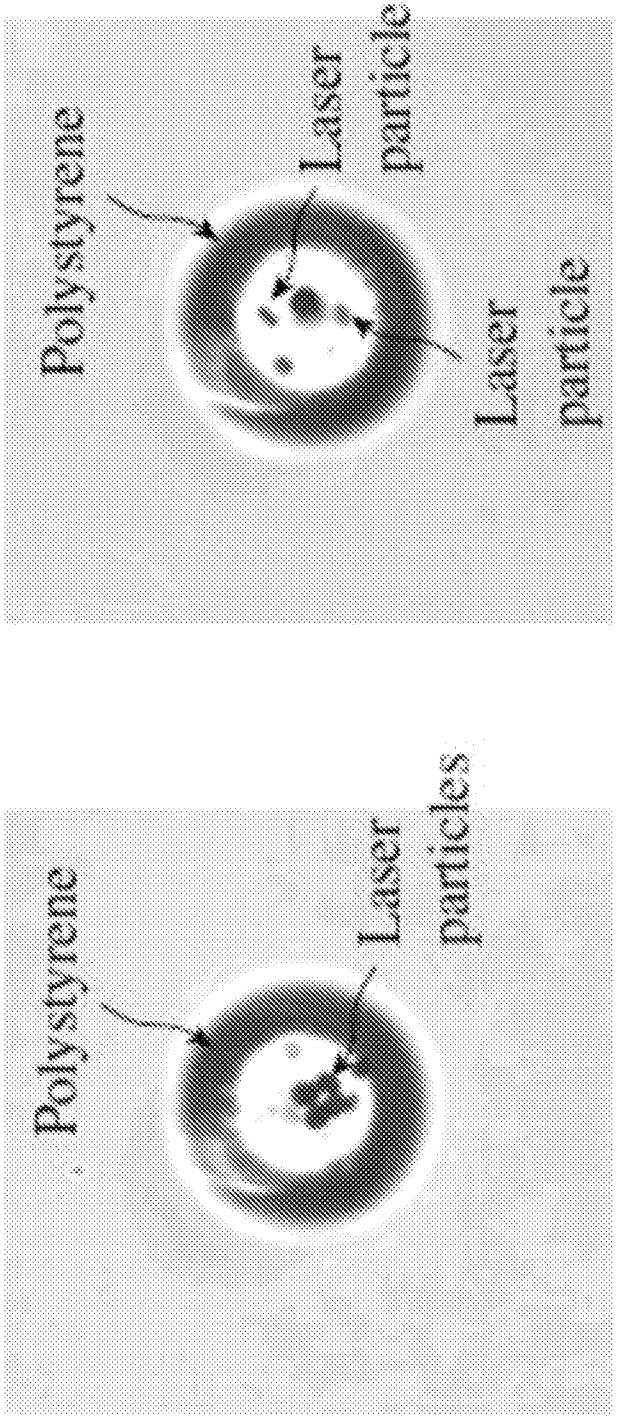
Polystyrene
Laser particle
Laser particle
Polystyrene
Laser particles
20 μm

FIG. 5

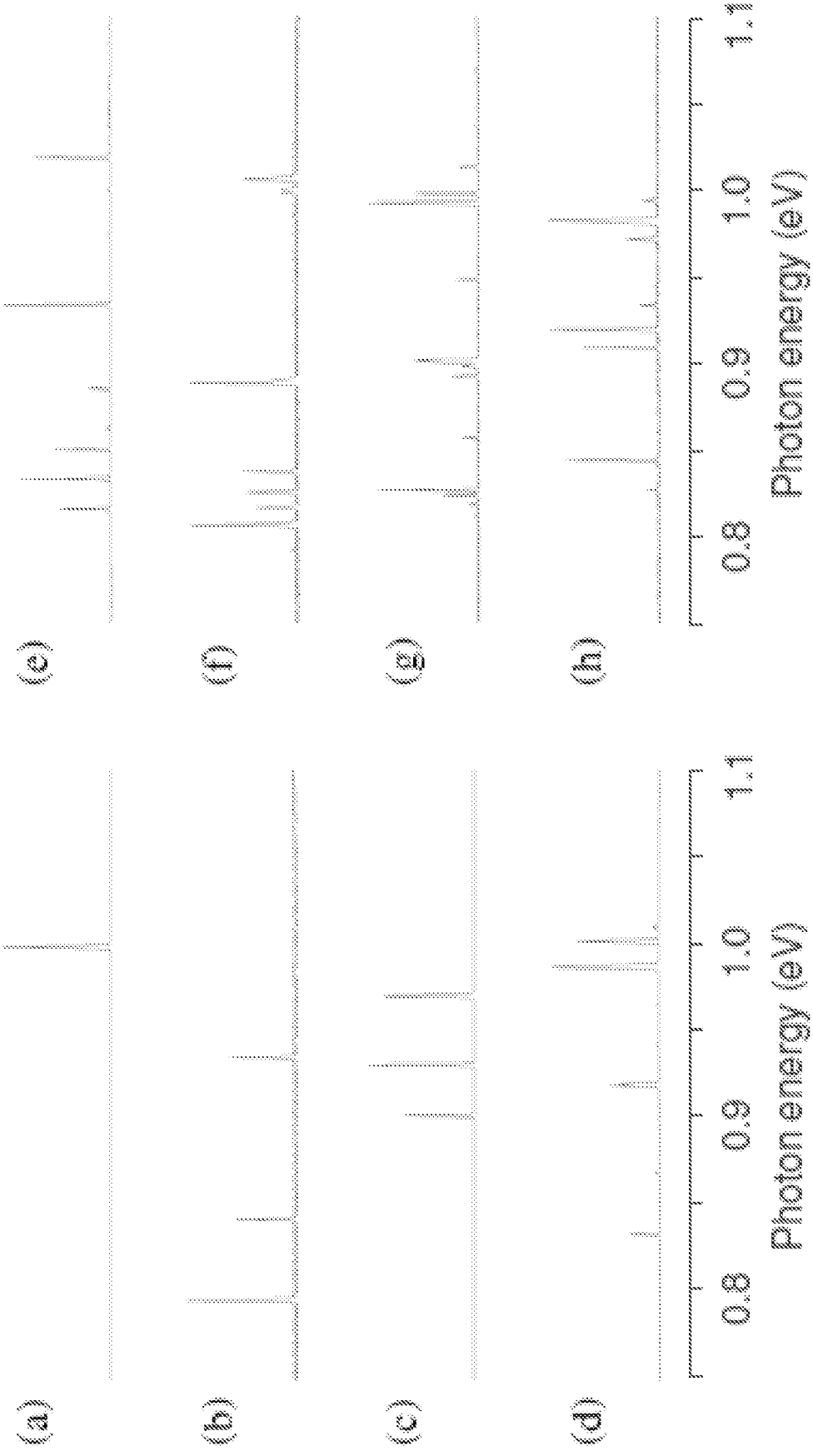
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
0.8
0.9
1.0
1.1
Photon energy (eV)
0.8
0.9
1.0
1.1
Photon energy (eV)

FIG. 6

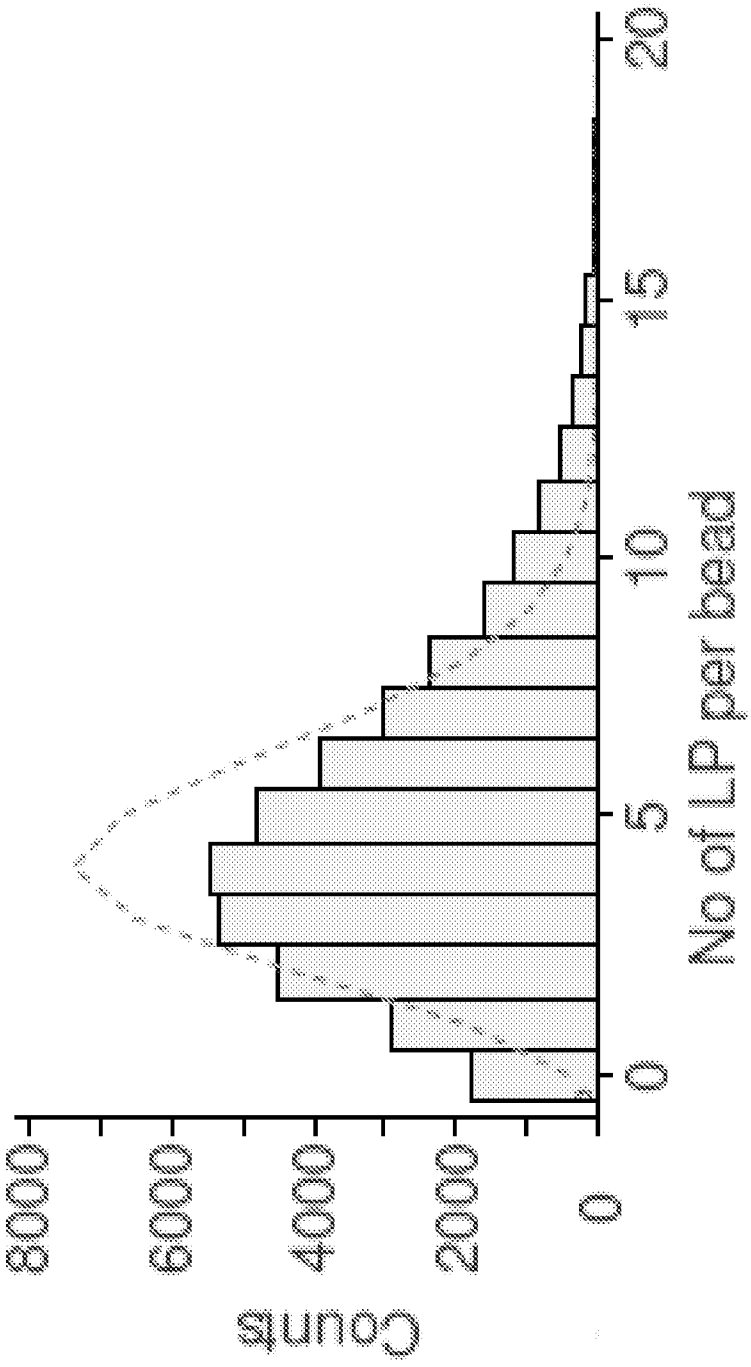

FIG. 7

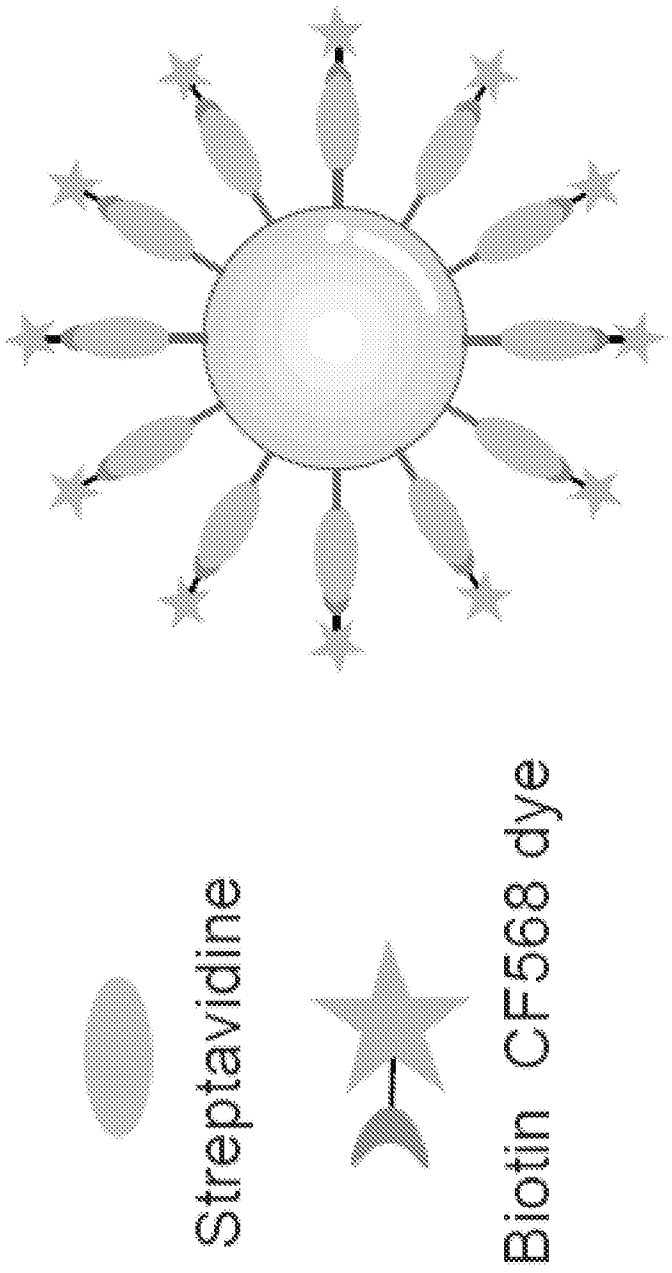

FIG. 8A

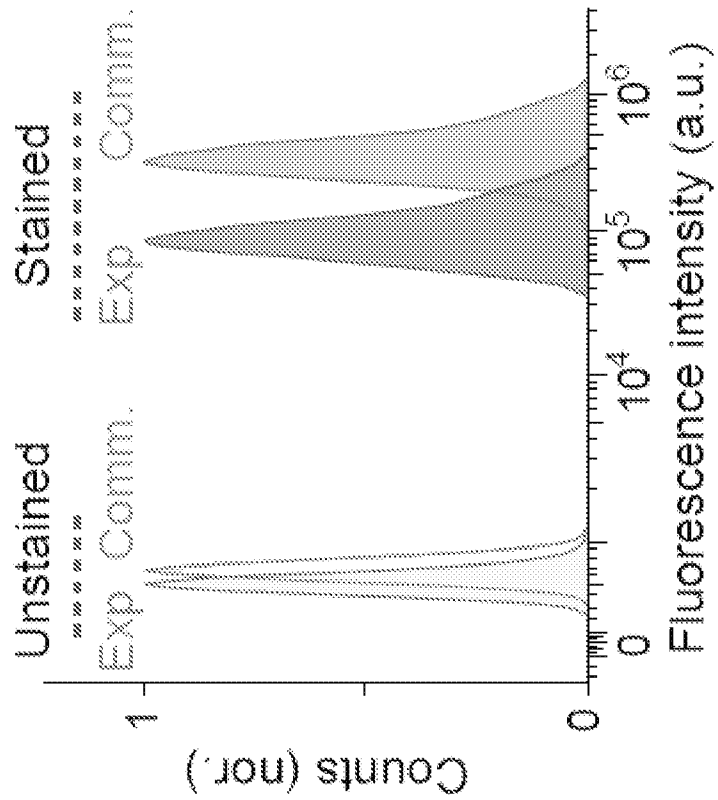
Unstained
Exp.
Comm.
Stained
Exp.
Comm.
Counts (nor.)
1
0
0
10^4
10^5
10^6
Fluorescence intensity (a.u.)

FIG. 8C

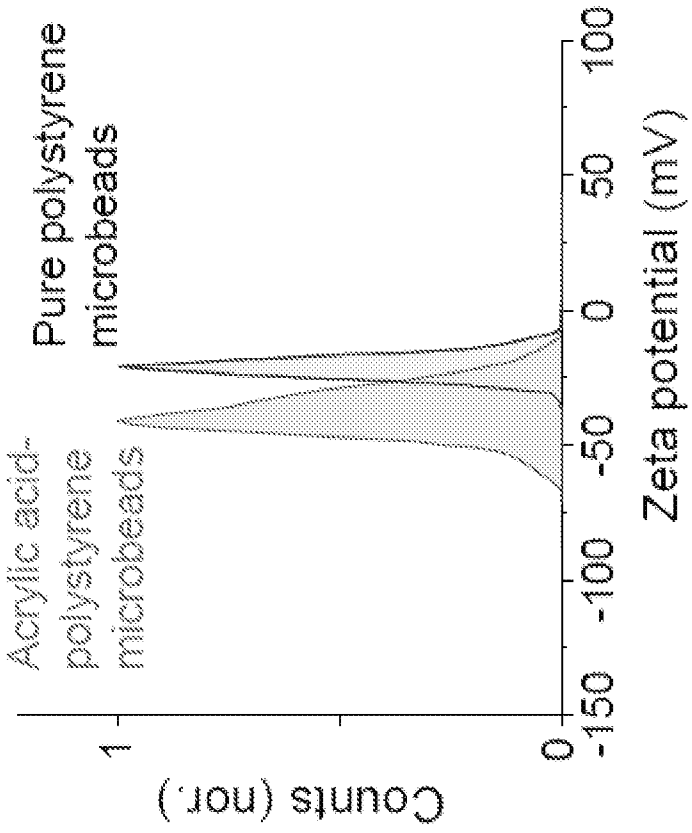
Acrylic acid-polystyrene microbeads
Pure polystyrene microbeads
Counts (nor.)
1
0
-150
-100
-50
0
50
100
Zeta potential (mV)

FIG. 8B

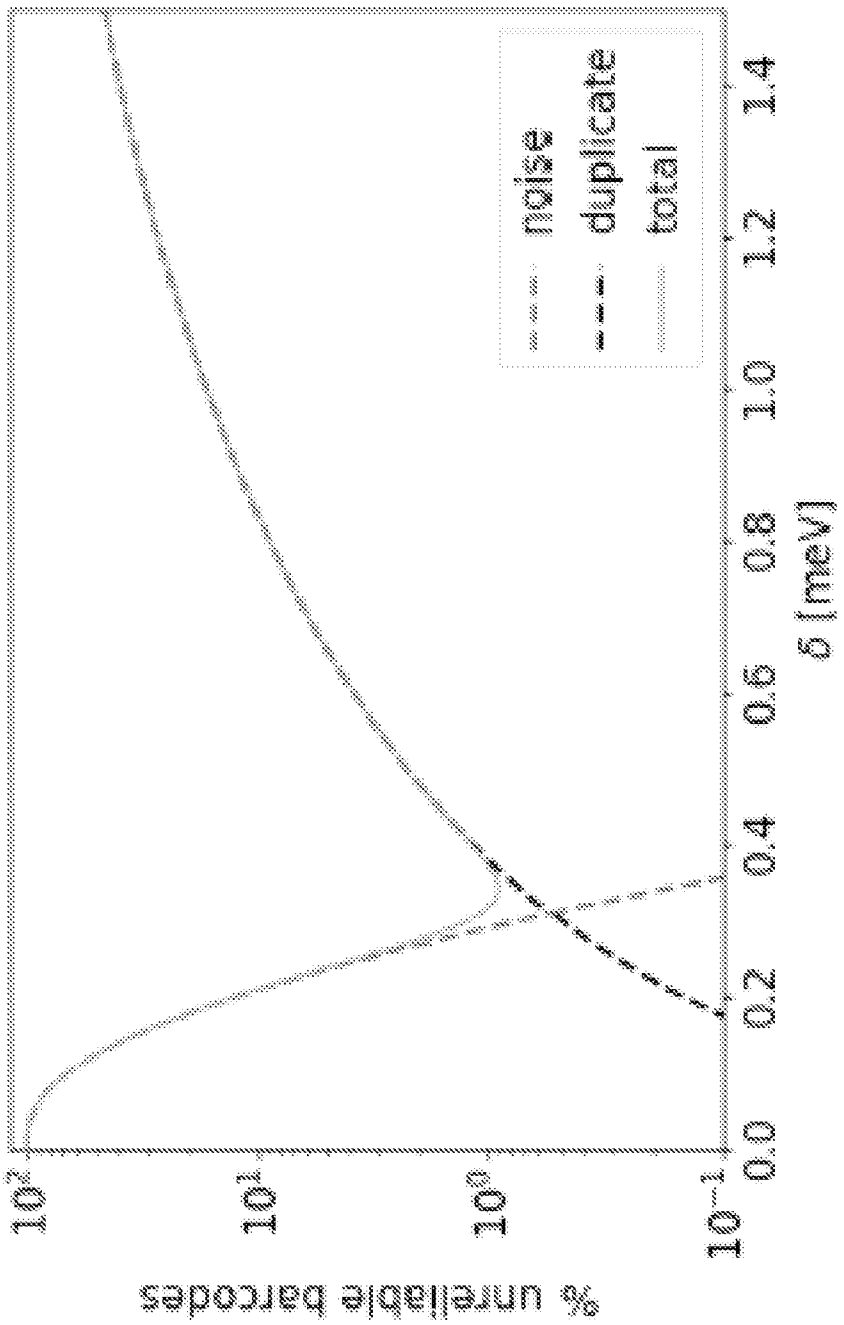
% unreliable barcodes
10^2
10^1
10^0
10^{-1}
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
δ [meV]
noise
duplicate
total

FIG. 9

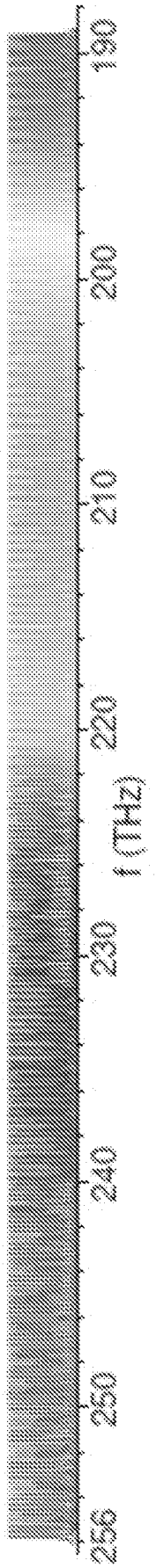

FIG. 10A

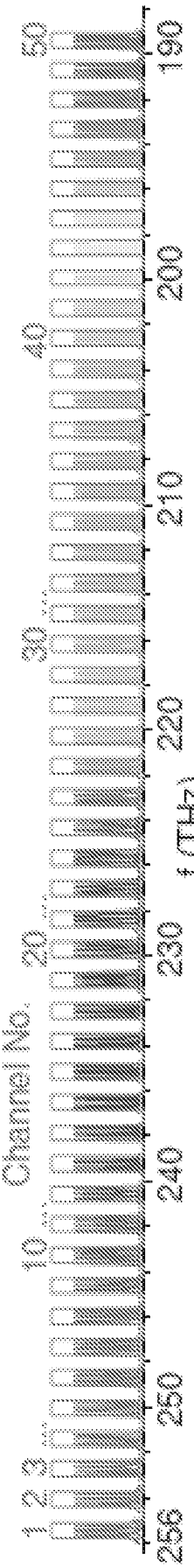

FIG. 10B

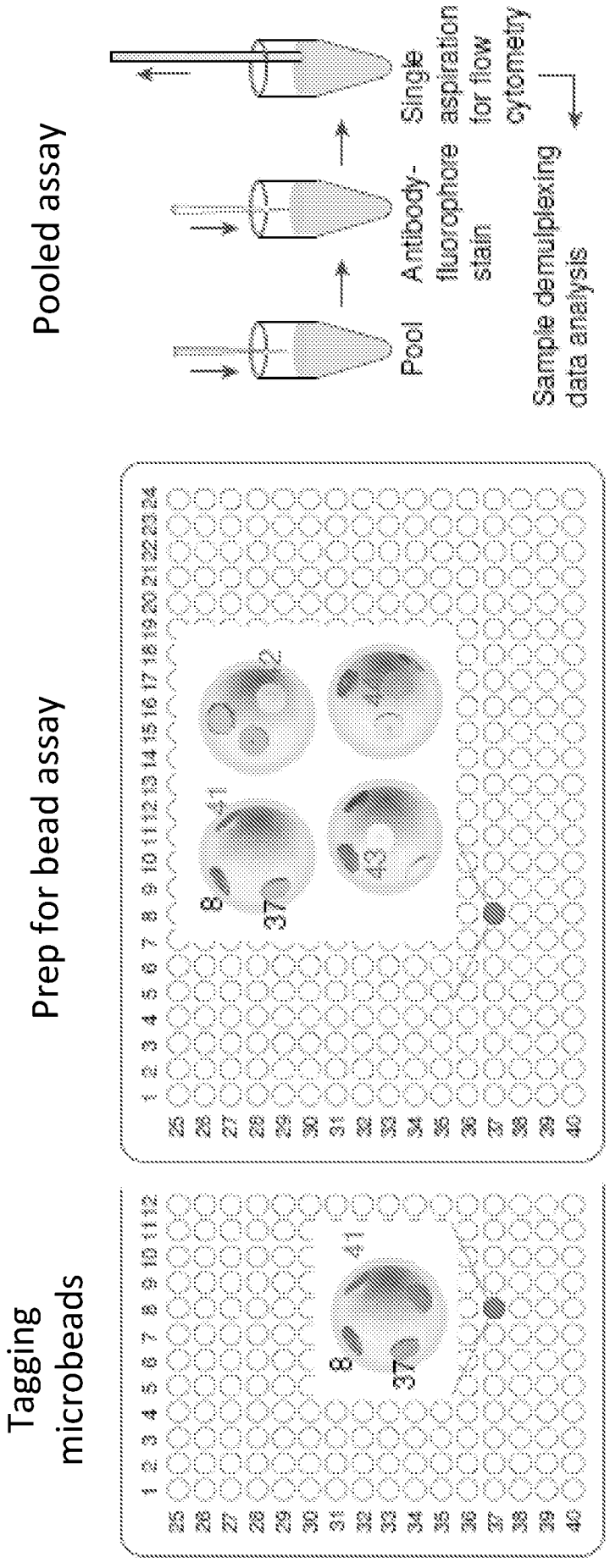

FIG. 11A FIG. 11B FIG. 11C

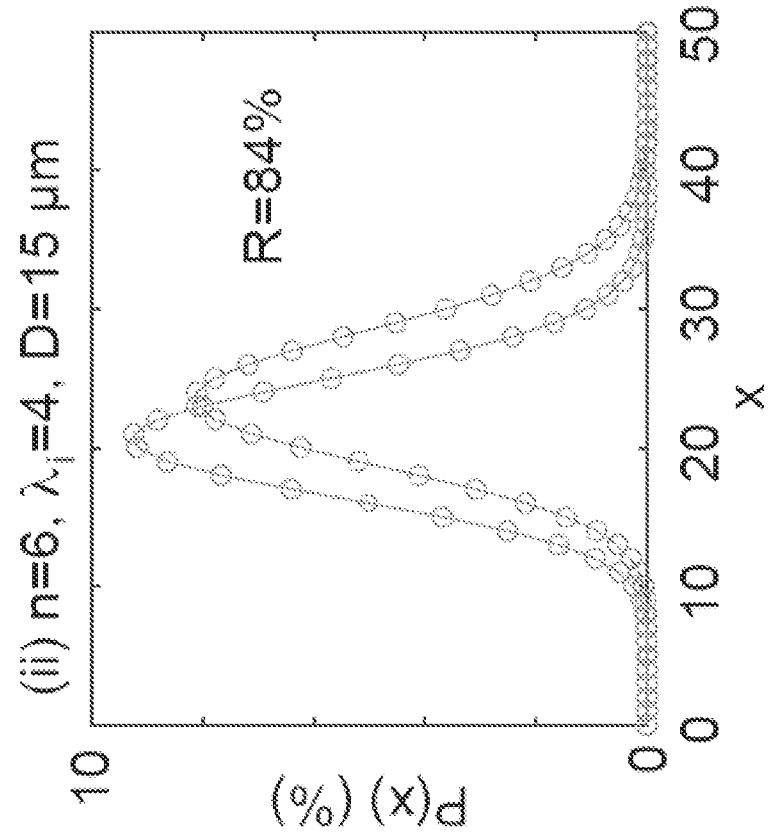

FIG. 12B

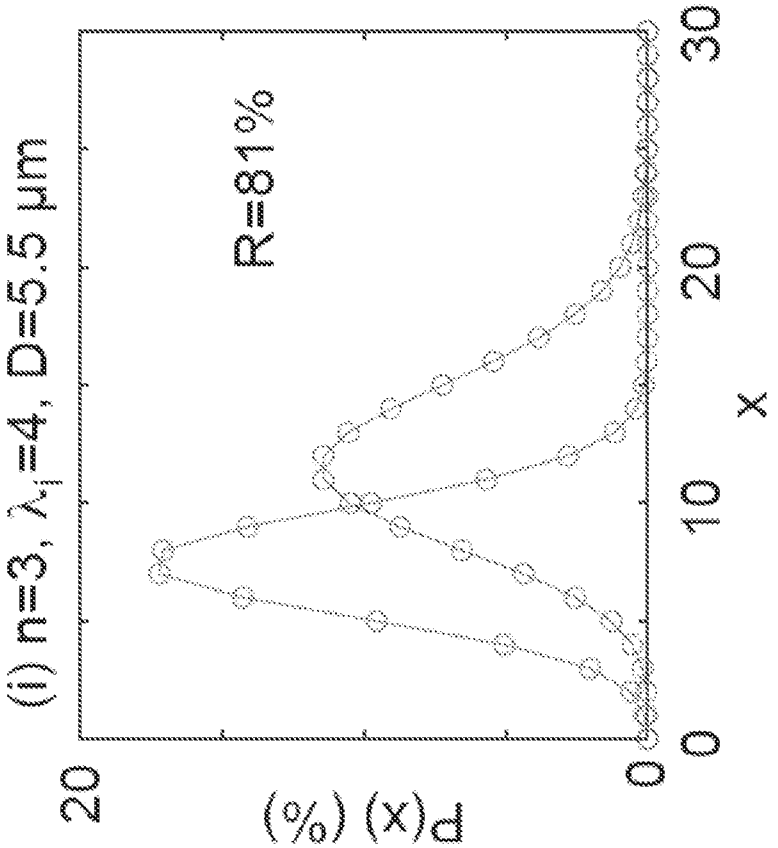

FIG. 12A

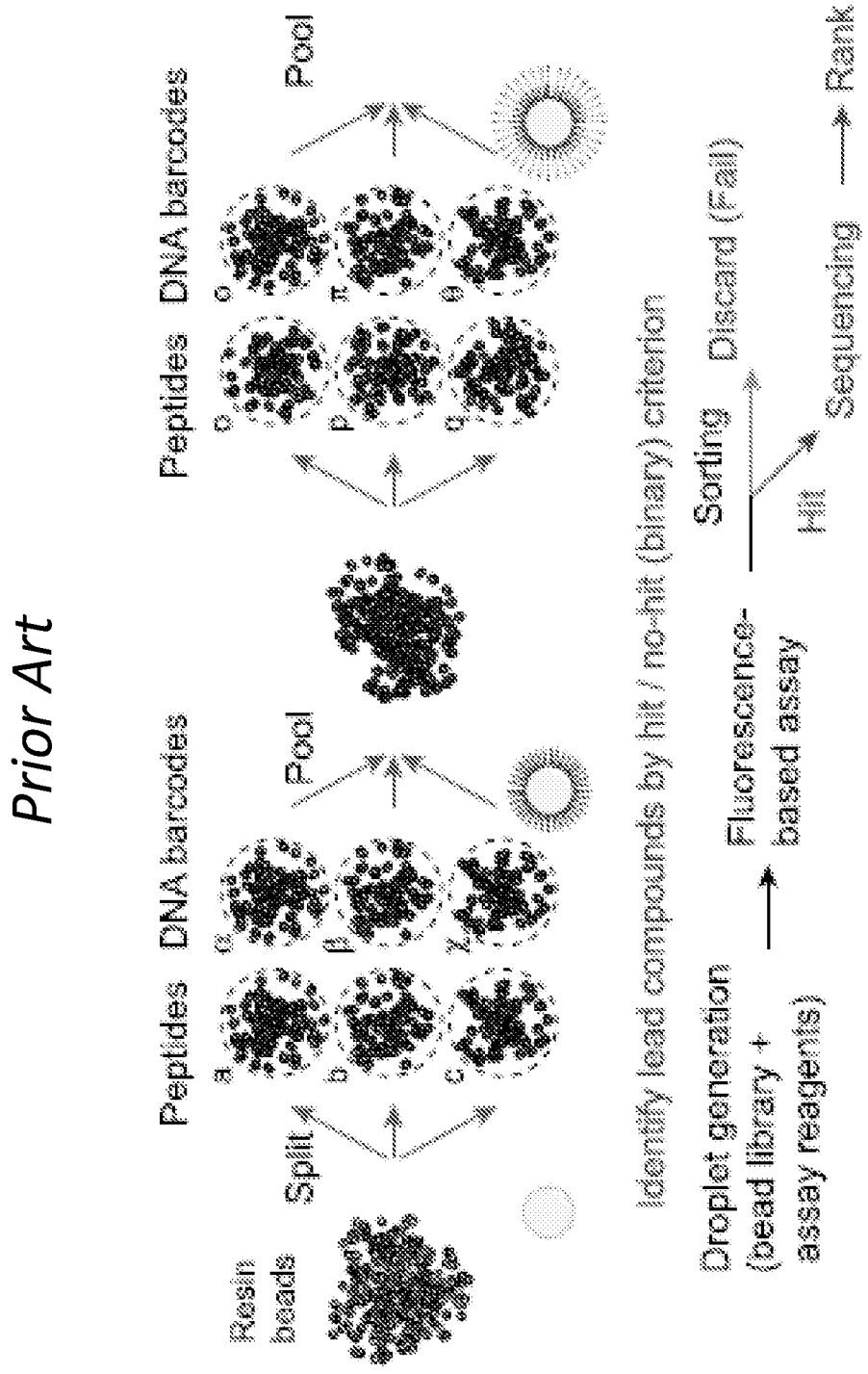
Prior Art
Resin beads
Split
Peptides
DNA barcodes
Pool
Peptides
DNA barcodes
Pool
Identify lead compounds by hit / no-hit (binary) criterion
Droplet generation (bead library + assay reagents)
Fluorescence-based assay
Sorting
Discard (Fail)
Hit
Sequencing
Rank

FIG. 13

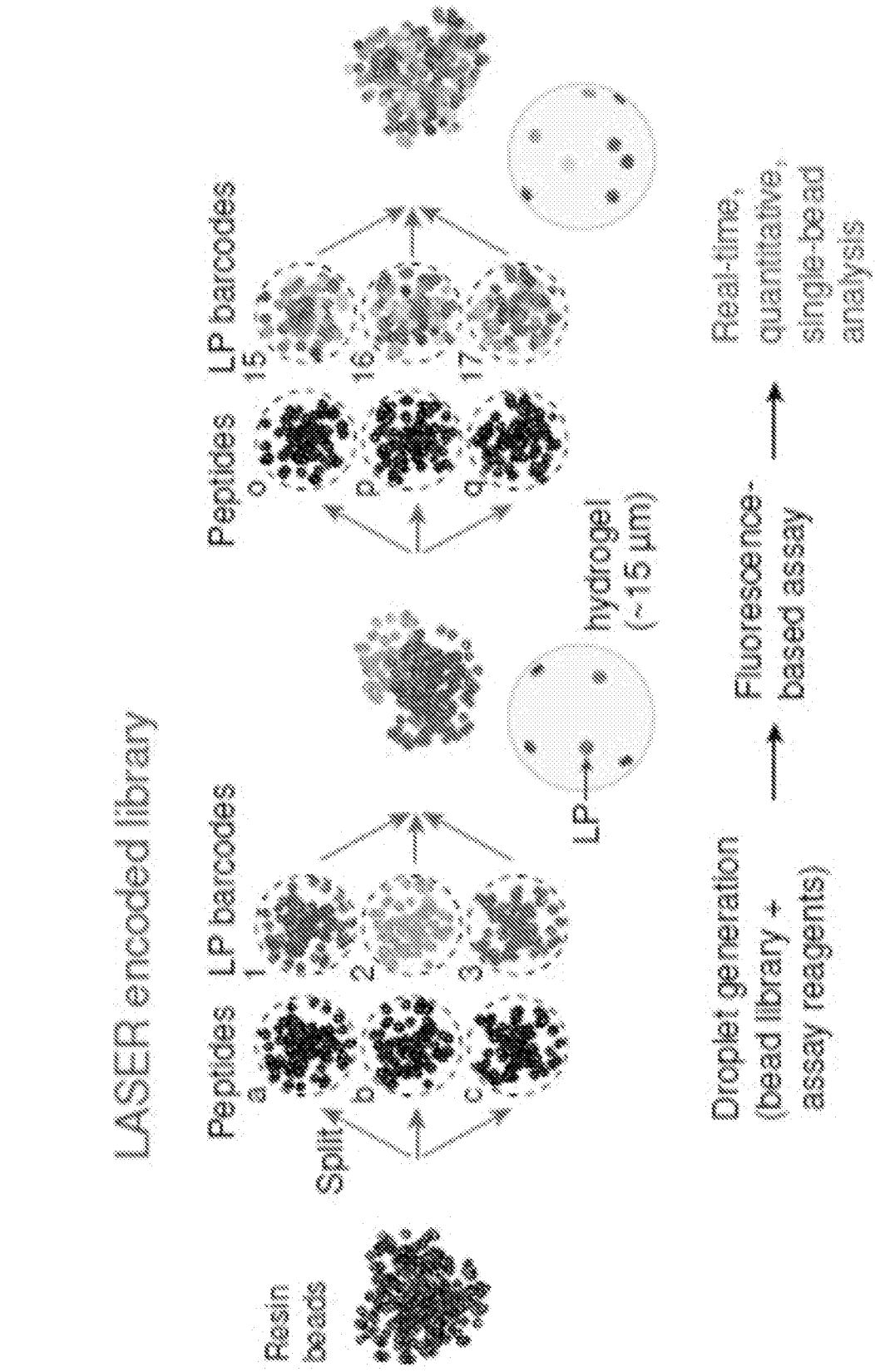
LASER encoded library
Resin beads
Split
Peptides
LP barcodes
a
b
c
1
2
3
LP
hydrogel (~15 µm)
Peptides
LP barcodes
o
p
q
15
16
17
Droplet generation (bead library + assay reagents)
Fluorescence-based assay
Real-time, quantitative, single-bead analysis

FIG. 14

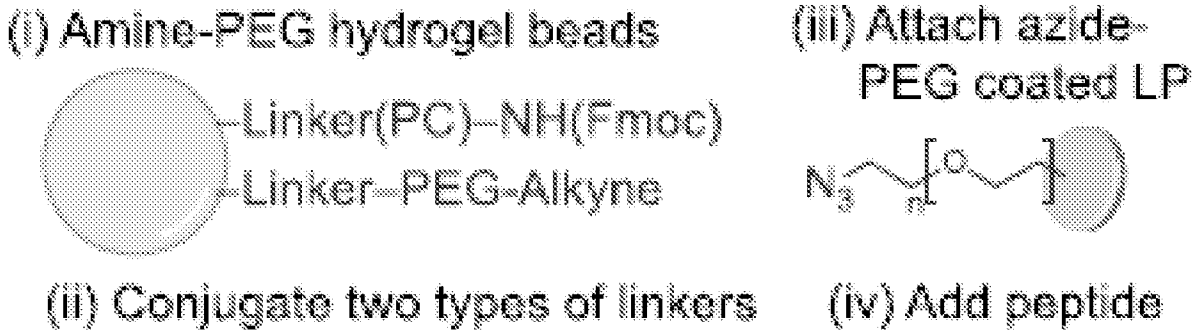

FIG. 15A

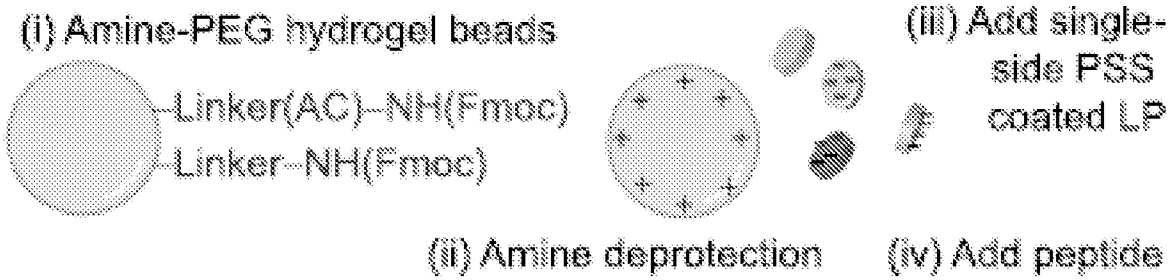

FIG. 15B

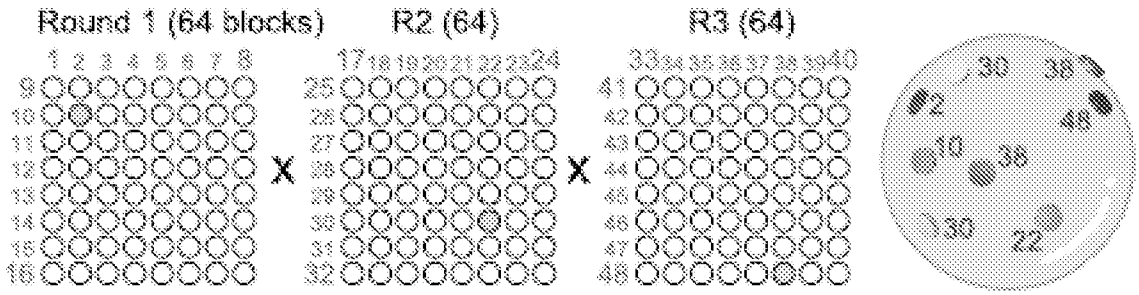

FIG. 15C

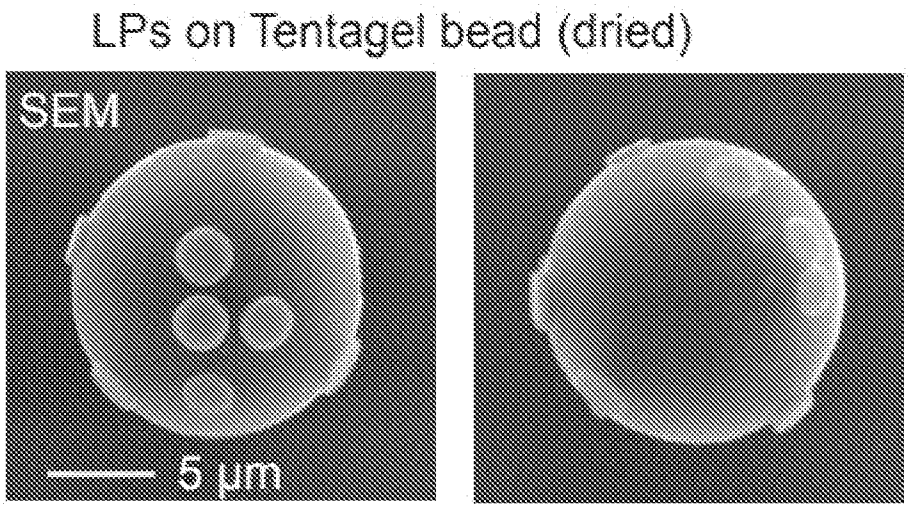

FIG. 16A

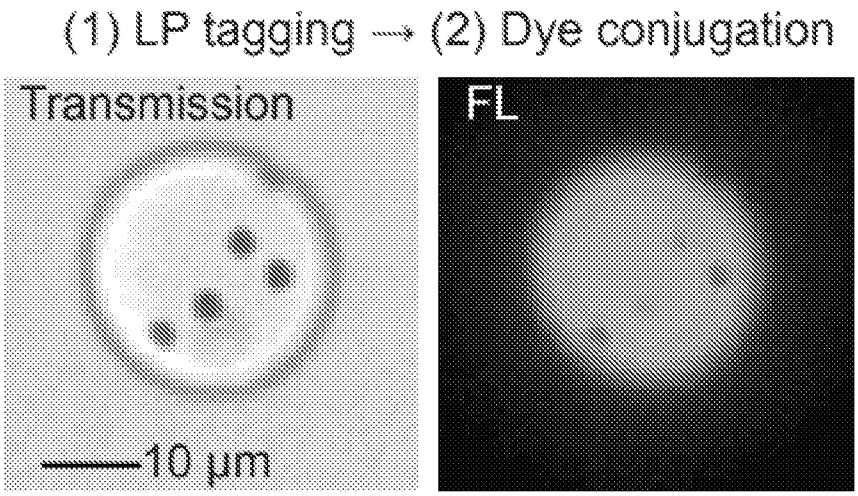

FIG. 16B

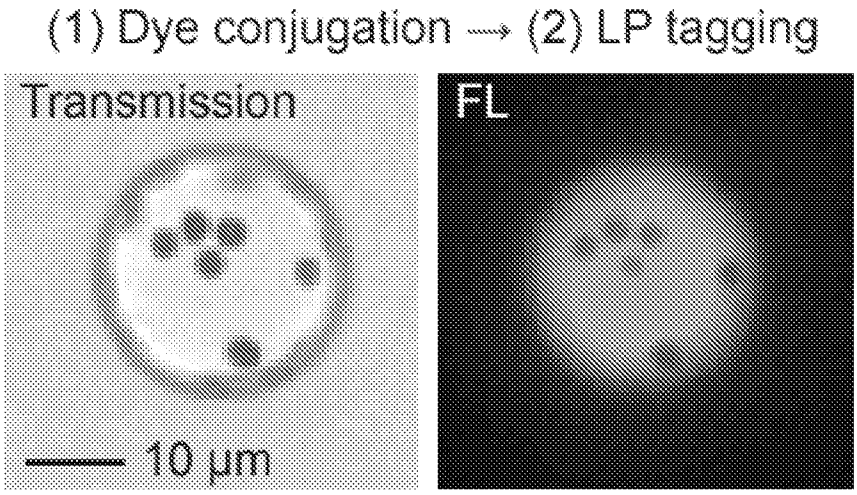

FIG. 16C

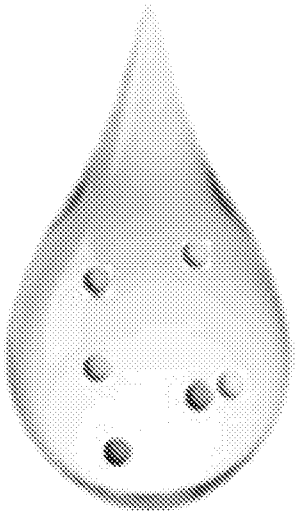

FIG. 17A

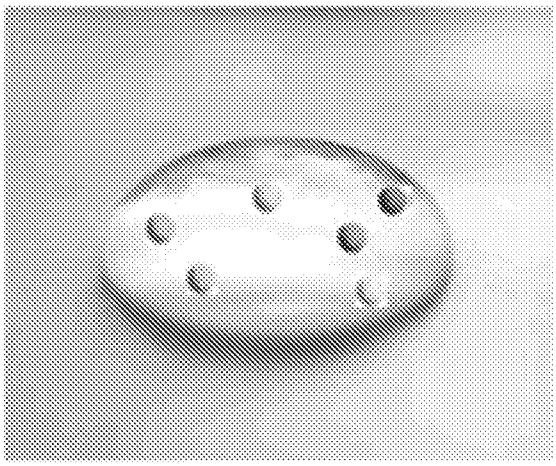

FIG. 17B

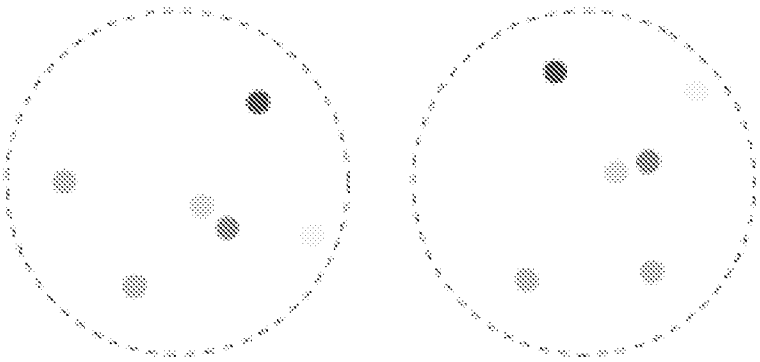

FIG. 17C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/033556

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***G01N 15/14*** (2024.01); ***G01N 21/64*** (2024.01); ***G01N 33/533*** (2024.01); *C12N 15/10* (2024.01)

CPC: ***G01N 15/14; G01N 21/64; G01N 33/533;*** *C12N 15/10*

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.
	US 2021/0382061 A1 (THE GENERAL HOSPITAL CORPORATION) 09 December 2021 (09.12.2021)	
X	entire document	1-5, 8-12, 14, 15, 17, 18, 21
Y	entire document	6, 7, 13, 16, 19, 20
	US 2020/0303900 A1 (THE GENERAL HOSPITAL CORPORATION) 24 September 2020 (24.09.2020)	
Y	entire document	6, 7, 13, 16
	WO 2023/023153 A1 (LASE INNOVATION INC. et al.) 23 February 2023 (23.02.2023)	
Y	entire document	19, 20
	US 2021/0239590 A1 (LASE INNOVATION INC.) 05 August 2021 (05.08.2021)	
A	entire document	1-21
	US 2021/0364407 A1 (LIFE TECHNOLOGIES CORPORATION) 25 November 2021 (25.11.2021)	
A	entire document	1-21

☑ 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	Date of mailing of the international search report
17 August 2024 (17.08.2024)	**26 September 2024 (26.09.2024)**

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US **Commissioner for Patents** **P.O. Box 1450, Alexandria, VA 22313-1450**	**MATOS** **TAINA**
Facsimile No. **571-273-8300**	Telephone No. **571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/033556

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,073,497 A (SCHWARTZ) 17 December 1991 (17.12.1991) entire document	1-21
A	US 2009/0108214 A1 (SHINODA et al.) 30 April 2009 (30.04.2009) entire document	1-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/033556

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:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-21, is drawn to an apparatus for optical barcoding.

Group II, claims 22-31, is drawn to a method for using optical barcoding to evaluate a sample.

Group III, claims 32-44, is drawn to a method of attaching a laser particle to a microbead for optical barcoding.

Group IV, claims 45-50, is drawn to a system to capture biomolecules.

Group V, claims 51-62, is drawn to a method of drug screening.

Group VI, claims 63-73, is drawn to a microfluidic screening system.

Group VII, claims 74-85, is drawn to a method of generating a microbead comprising a laser particle.

Group VIII, claims 86-89, is drawn to a method of optical barcoding.

The inventions listed as Groups I-VIII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical feature of the Group I invention: a microbead comprising one or more laser particles, the microbead being configured to generate one or more stimulated emission peaks when energetically excited as claimed therein is not present in the invention of Groups II-VIII. The special technical feature of the Group II invention: combining the plurality of microbeads with a sample; and energetically exciting, based on combining the plurality of microbeads with the sample, each of the plurality of microbeads to evaluate the sample as claimed therein is not present in the invention of Groups I, III-VIII. The special technical feature of the Group III invention: treating the laser particle with a negatively charged compound to provide a negatively charged laser particle to improve adhesion to the microbead; and combining the negatively charged laser particle with the microbead to produce a laser particle-labeled microbead as claimed therein is not present in the invention of Groups I, II, IV-VIII. The special technical feature of the Group IV invention: a laser particle barcode reader configured to read stimulated emission spectra from the laser particles; and a fluorescence assay configured to detect the captured target biomolecules as claimed therein is not present in the invention of Groups I, II, III or V-VIII. The special technical feature of the Group V invention: contacting the plurality of microbeads with a plurality of compounds such that each microbead of the plurality of microbeads conjugates to only one compound of the plurality of compounds; combining the plurality of microbead conjugated compounds with one or more target biomolecules; and detecting the one or more stimulated emission peaks of each conjugated microbead in the plurality of conjugated microbeads as claimed therein is not present in the invention of Groups I-IV, VI-VIII. The special technical feature of the Group VI invention: the microbeads each being conjugated to one or more screening compounds; one or more assay reagents; and a microfluidic circuit configured to contact the plurality of microbeads to the one or more assay reagents to form droplets as claimed therein is not present in the invention of Groups I-V, VII-VIII. The special technical feature of the Group VII invention: mixing the laser particle with a solution comprising at least one polymer and at least one solvent to create a laser particle suspension; generating a microdroplet from the laser particle suspension; drying the microdroplet to generate the microbead comprising the laser particle as claimed therein is not present in the invention of Groups I-VI or VIII. The special technical feature of the Group VIII invention: conjugating a first molecule to each of the plurality of microbeads; attaching a respective first laser particle to each of the plurality of microbeads, wherein the first laser particle attached to each of the plurality of microbeads corresponds to the first molecule attached to each of the plurality of microbeads; conjugating a second molecule to each of the plurality of microbeads; attaching a respective second laser particle to each of the plurality of microbeads, wherein the second laser particle attached to each of the plurality of microbeads corresponds to the second molecule attached to each of the plurality of microbeads; and performing an assay using the plurality of microbead as claimed therein is not present in the invention of Groups I-VII.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/033556

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

Groups I-VIII lack unity of invention because even though the inventions of these groups require the technical feature of a microbead comprising one or more laser particles, the microbead being configured to generate one or more stimulated emission peaks when energetically excited, this technical feature is not a special technical feature as it does not make a contribution over the prior art.

Specifically, US 2020/0303900 to THE GENERAL HOSPITAL CORPORATION teaches a microbead comprising one or more laser particles, the microbead being configured to generate one or more stimulated emission peaks when energetically excited (Example laser particles have an output emission spectra with one or plural peaks, para. 0007. FIG. 14 illustrates the principle of high-resolution optical sectioning by detecting stimulated emission from laser particles. At top left, miniature laser particles (circles) excited by a tightly focused optical pump beam (gray), para. 0041. Stand-alone laser particles, once injected into biological systems such as soft tissues, may be detected and localized by an optical imaging instrument such as fluorescence microscope. However, a novel microscope configuration may be devised to detect the stimulated emission from the laser particles, para. 0209).

Since none of the special technical features of the Group I-VIII inventions are found in more than one of the inventions, unity of invention is lacking.

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.: **1-21**

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.