

SUMMARY

[0008] The present disclosure discloses, *inter alia*, compositions of beads (such as, polymeric beads) of substantially identical size and shape encoded with unique information-storing identifiers and methods for use of the same in multiplex chemical and biological assays.

[0008a] In one aspect of the present disclosure, there is provided a method for conducting a multiplex assay comprising the steps of:

- a) contacting a sample with beads in an assay system, wherein each bead in the system comprises
 - (1) a substantially transparent polymer layer having a first surface and a second surface, the first and the second surfaces being parallel to each other;
 - (2) a substantially non-transparent polymer layer, wherein the substantially non-transparent polymer layer is affixed to the first surface of the substantially transparent polymer layer and encloses a center portion of the substantially transparent polymer layer, and wherein the substantially non-transparent polymer layer comprises a two-dimensional shape representing an analog code identifier;

wherein the system comprises (i) at least one bead with an analog code identifier that is recognized by an imaging processor as a specific assay, specific analyte, manufacturing lot, individual, locational identifier, or calibration signal, and (ii) a plurality of beads, wherein each bead of the plurality of beads comprises a capture agent that specifically binds to an analyte, wherein the capture agent is immobilized on at least one of the first surface and the second surface of the substantially transparent polymer layer in at least the center portion of the substantially transparent polymer layer, and wherein each bead of the plurality of beads comprises an analog code identifier corresponding to the capture agent; and

simultaneously or sequentially, in any order:

- b) identifying the identifier that is recognized by the imaging processor as the specific assay; and
 - c) detecting the binding of the analyte in the sample by analyzing the binding of the analyte to the capture agent immobilized on the surface of the bead based on the identification of the analog code identifier corresponding to the capture agent;
- wherein optionally the beads are a substantially circular disc;

wherein optionally the center portion of the first substantially transparent polymer layer comprises between about 5% and about 90% of the surface area of the first substantially transparent polymer layer.

[0008b] In another aspect of the present disclosure, there is provided a kit for conducting a multiplex assay comprising:

- a) a set of beads, wherein each bead in the set comprises
 - (1) a substantially transparent polymer layer having a first surface and a second surface, the first and the second surfaces being parallel to each other;
 - (2) a substantially non-transparent polymer layer, wherein the substantially non-transparent polymer layer is affixed to the first surface of the substantially transparent polymer layer and encloses a center portion of the substantially transparent polymer layer, and wherein the substantially non-transparent polymer layer comprises a two-dimensional shape representing an analog code identifier;

and wherein the set of beads comprises (i) at least one bead with an analog code identifier that is recognized by an imaging processor as a specific assay, specific analyte, manufacturing lot, individual, locational identifier, or calibration signal, and (ii) a plurality of additional beads, wherein each bead of the plurality of beads comprises a capture agent that specifically binds to an analyte, wherein the capture agent is immobilized on at least one of the first surface and the second surface of the substantially transparent polymer layer in at least the center portion of the substantially transparent polymer layer, and wherein each bead of the plurality of beads comprises an analog code identifier corresponding to the capture agent; and

- b) a signal-emitting entity capable of directly or indirectly binding to the analyte or capture agent;

wherein optionally the beads are a substantially circular disc;

wherein optionally the center portion of the first substantially transparent polymer layer comprises between about 5% and about 90% of the surface area of the first substantially transparent polymer layer.

[0008c] In a further aspect of the present disclosure, there is provided a multiplex assay system comprising:

- a) a plurality of beads in the kit disclosed herein; and
- b) an apparatus comprising an imaging processor system and a reaction detection system, wherein the identification of an identifier that corresponds to one or more of
 - (i) a specific assay,
 - (ii) a specific analyte,
 - (iii) a manufacturing lot,
 - (iv) an individual,
 - (v) a locational identifier, and/or
 - (vi) a calibration signal

by the imaging processor in the decoding system activates the use of a software analyzing detection signals detected by reaction detection system correlated to the specific assay.

[0009] Also provided herein are methods for conducting a multiplex assay comprising the steps of: a) contacting a sample with beads in an assay system, wherein each bead in the system comprises (1) a substantially transparent polymer layer having a first surface and a second surface, the first and the second surfaces being parallel to each other; (2) a substantially non-transparent polymer layer, wherein the substantially non-transparent polymer layer is affixed to the first surface of the substantially transparent polymer layer and encloses a center portion of the substantially transparent polymer layer, and wherein the substantially non-transparent polymer layer comprises a two-dimensional shape representing an analog code identifier; wherein the system comprises (i) at least one bead with an analog code identifier that is recognized by an imaging processor as a specific assay or assay kit, specific analyte, manufacturing lot, individual, locational identifier, or calibration signal; and (ii) a plurality of beads, wherein each bead of the plurality of beads comprises a capture agent that specifically binds to an analyte, wherein the capture agent is immobilized on at least one of the first surface and the second surface of the substantially transparent polymer layer in at least the center portion of the substantially transparent polymer layer, and wherein each bead of the plurality of beads comprises an analog code identifier corresponding to the capture agent; and simultaneously or sequentially, in any order: b) identifying the identifier that is recognized by the imaging processor as the specific assay; and c) detecting the binding of the analyte in the sample by

analyzing the binding of the analyte to the capture agent immobilized on the surface of the bead based on the identification of the analog code identifier corresponding to the capture agent. In some examples, the system further comprises (iii) at least two beads, at least three beads, at least four beads, at least five beads, or at least six beads, each with an analog code identifier that is recognized by an imaging processor as a specific assay or assay kit, specific analyte, manufacturing lot, individual, locational identifier, or calibration signal. In some examples, the system further comprises (iv) at least one bead recognized by the imaging processor as a positive or negative control. In some examples, the locational identifier corresponds to a hospital, a diagnostic laboratory, an address, a health care professional's office, or a research

laboratory. In some examples, the system further comprises (v) at least one bead that is pre-labeled for monitoring functions of the imaging processor. In some examples, the beads comprise a spherical or rectangular shape. In some examples, the beads are less than about 300 μm in diameter. In some examples, the surface of the bead is smooth and lacks surface irregularities. In some examples, at least one surface of the bead comprises at least one site for chemical attachment of the capture agent. In some examples, the capture agent is selected from the group consisting of: a DNA molecule, a DNA-analog-molecule, an RNA-molecule, an RNA-analog-molecule, a polynucleotide, a protein, an enzyme, a lipid, a phospholipid, a carbohydrate moiety, a polysaccharide, an antigen, a virus, a cell, an antibody, and an antibody fragment. In some examples, the plurality of beads are magnetic or non-magnetic. In some examples, the beads further comprise: (3) a magnetic, substantially non-transparent layer that encloses the center portion of the substantially transparent polymer layer between the substantially non-transparent polymer layer and the center portion of the substantially transparent polymer layer, wherein the magnetic, substantially non-transparent layer is affixed to the first surface or the second surface of the substantially transparent polymer layer. In some examples, the beads further comprise: (4) a second substantially transparent polymer layer aligned with the first substantially transparent polymer layer, the second substantially transparent polymer layer having a center portion that is aligned with the center portion of the first substantially transparent polymer layer, wherein the second substantially transparent polymer layer is affixed to the second surface of the first substantially transparent polymer layer and does not extend beyond the two-dimensional shape of the first substantially transparent polymer layer; and (5) a magnetic, substantially non-transparent layer that encloses the center portion of the first substantially transparent polymer layer between the substantially non-transparent polymer layer and the center portion of the substantially transparent polymer layer, wherein the magnetic, substantially non-transparent layer is affixed between the first and the second substantially transparent polymer layers. In some examples, the beads further comprise an orientation indicator for orienting the analog code identifier of the substantially non-transparent polymer layer. In some examples, the orientation indicator comprises an asymmetry of the magnetic, substantially non-transparent layer. In some examples, the magnetic, substantially non-transparent layer comprises nickel. In some examples, the magnetic, substantially non-transparent layer is between about 50 nm and about 10 μm in thickness. In some examples, the magnetic, substantially non-transparent layer is about 0.1

μm in thickness. In some examples, the two-dimensional shape of the substantially non-transparent polymer layer comprises one or more rings enclosing the center portion of the substantially transparent polymer layer, wherein at least one of the one or more rings comprises a discontinuity. In some examples, the two-dimensional shape of the substantially non-transparent polymer layer comprises a gear shape comprising a plurality of gear teeth, and wherein the analog code identifier is represented by one or more examples selected from the group consisting of the height of one or more gear teeth of the plurality, the width of one or more gear teeth of the plurality, the number of gear teeth in the plurality, and the arrangement of one or more gear teeth within the plurality. In some examples, the plurality of gear teeth comprises one or more gear teeth that are between about 1 μm and about 10 μm wide. In some examples, the plurality of gear teeth comprises one or more gear teeth that are between about 1 μm and about 10 μm tall. In some examples, the plurality of gear teeth comprises two or more gear teeth that are spaced between about 1 μm and about 10 μm apart. In some examples, the beads further comprise: (6) one or more columns projecting from the first surface of the first substantially transparent polymer layer, wherein the one or more columns are not within the center portion of the first substantially transparent polymer layer; and/or (7) one or more columns projecting from the second surface of the first substantially transparent polymer layer or a surface of the second substantially transparent polymer layer that is not affixed to the first substantially transparent polymer layer, wherein the one or more columns are not within the center portions of the first or the second substantially transparent polymer layer. In some examples, the beads are a substantially circular disc. In some examples, the center portion of the first substantially transparent polymer layer comprises between about 5% and about 90% of the surface area of the first substantially transparent polymer layer. In some examples, the center portion of the first substantially transparent polymer layer comprises about 25% of the surface area of the first substantially transparent polymer layer. In some examples, the beads are less than about 200 μm in diameter. In some examples, the beads are about 50 μm in diameter. In some examples, the beads are less than about 50 μm in thickness. In some examples, the beads are about 10 μm in thickness. In some examples, the analyte is selected from the group consisting of a DNA molecule, a DNA-analog-molecule, an RNA-molecule, an RNA-analog-molecule, a polynucleotide, a protein, an enzyme, a lipid, a phospholipid, a carbohydrate moiety, a polysaccharide, an antigen, a virus, a cell, an antibody, a small molecule, a bacterial cell, a cellular organelle, and an antibody

fragment. In some examples, the substantially transparent polymer of the first or the second substantially transparent polymer layer comprises an epoxy-based polymer. In some examples, the epoxy-based polymer is SU-8.

[0010] In other examples, provided herein are kits for conducting a multiplex assay comprising: a) a set of beads, wherein each bead in the set comprises (1) a substantially transparent polymer layer having a first surface and a second surface, the first and the second surfaces being parallel to each other; (2) a substantially non-transparent polymer layer, wherein the substantially non-transparent polymer layer is affixed to the first surface of the substantially transparent polymer layer and encloses a center portion of the substantially transparent polymer layer, and wherein the set of beads comprises (i) at least one bead with an analog code identifier that is recognized by an imaging processor as a specific assay or assay kit, specific analyte, manufacturing lot, individual, locational identifier, or calibration signal, and (ii) a plurality of additional beads, wherein each bead of the plurality of beads comprises a capture agent that specifically binds to an analyte, wherein the capture agent is immobilized on at least one of the first surface and the second surface of the substantially transparent polymer layer in at least the center portion of the substantially transparent polymer layer, and wherein each bead of the plurality of beads comprises an analog code identifier corresponding to the capture agent; and b) a signal-emitting entity capable of directly or indirectly binding to the analyte or capture agent. In some examples, the signal-emitting entity indirectly binds to the analyte or capture agent via a secondary antibody that is conjugated to the signal-emitting entity. In some examples, the signal-emitting entity indirectly binds to the analyte or capture agent via a nucleotide probe that is conjugated to the signal-emitting entity. In some examples, the signal-emitting entity is phycoerythrin, GFP, RFP, CFP, YFP, FITC, horseradish peroxidase, alkaline phosphatase, or a radiolabel. In some examples, the kit further comprises at least two beads, at least three beads, at least four beads, at least five beads, or at least six beads, each with an analog code identifier that is recognized by an imaging processor as a specific assay or assay kit, specific analyte, manufacturing lot, individual, locational identifier, or calibration signal. In some examples, the kit further comprises an imaging processor for (i) identifying the at least one bead with an identifier that is recognized by the imaging processor as a specific assay, (ii) identifying the identifier on the plurality of beads, (iii) detecting the amount of signal produced by the signal-emitting entity, (iv) identifying the at least one bead with an identifier that is recognized by the imaging processor as a manufacturing lot, (v) identifying the at least one bead

with an identifier that is recognized by the imaging processor as an individual, (vi) identifying the at least one bead with an identifier that is recognized by the imaging processor as a locational identifier, or (vii) identifying the at least one bead that is pre-labeled for calibration of the imaging processor. In some examples, the kit further comprises an entity capable of directly or indirectly binding to the analyte or capture agent, wherein the entity comprises an affinity tag. In some examples, the affinity tag is biotin, His₆, or maltose. In some examples of any of the above examples, the kit further comprises at least one bead that is pre-labeled for monitoring functions of the imaging processor. In some examples, the beads comprise a spherical or rectangular shape. In some examples, the beads are less than about 300 μm in diameter. In some examples, the surface of the bead is smooth and lacks surface irregularities. In some examples, at least one surface of the bead comprises at least one site for chemical attachment of the capture agent. In some examples, the capture agent is selected from the group consisting of: a DNA molecule, a DNA-analog-molecule, an RNA-molecule, an RNA-analog-molecule, a polynucleotide, a protein, an enzyme, a lipid, a phospholipid, a carbohydrate moiety, a polysaccharide, an antigen, a virus, a cell, an antibody, a small molecule, and an antibody fragment. In some examples, the plurality of beads are magnetic or non-magnetic. In some examples, the bead further comprises: (3) a magnetic, substantially non-transparent layer that encloses the center portion of the substantially transparent polymer layer between the substantially non-transparent polymer layer and the center portion of the substantially transparent polymer layer, wherein the magnetic, substantially non-transparent layer is affixed to the first surface or the second surface of the substantially transparent polymer layer. In some examples, the bead further comprises: (4) a second substantially transparent polymer layer aligned with the first substantially transparent polymer layer, the second substantially transparent polymer layer having a center portion that is aligned with the center portion of the first substantially transparent polymer layer, wherein the second substantially transparent polymer layer is affixed to the second surface of the first substantially transparent polymer layer and does not extend beyond the two-dimensional shape of the first substantially transparent polymer layer; and (5) a magnetic, substantially non-transparent layer that encloses the center portion of the first substantially transparent polymer layer between the substantially non-transparent polymer layer and the center portion of the substantially transparent polymer layer, wherein the magnetic, substantially non-transparent layer is affixed between the first and the second substantially transparent polymer layers. In some

examples, the bead further comprises an orientation indicator for orienting the analog code identifier of the substantially non-transparent polymer layer. In some examples, the orientation indicator comprises an asymmetry of the magnetic, substantially non-transparent layer. In some examples, the magnetic, substantially non-transparent layer comprises nickel. In some examples, the magnetic, substantially non-transparent layer is between about 50 nm and about 10 μm in thickness. In some examples, the magnetic, substantially non-transparent layer is about 0.1 μm in thickness. In some examples, the two-dimensional shape of the substantially non-transparent polymer layer comprises one or more rings enclosing the center portion of the substantially transparent polymer layer, wherein at least one of the one or more rings comprises a discontinuity. In some examples, the two-dimensional shape of the substantially non-transparent polymer layer comprises a gear shape comprising a plurality of gear teeth, and wherein the analog code identifier is represented by one or more examples selected from the group consisting of the height of one or more gear teeth of the plurality, the width of one or more gear teeth of the plurality, the number of gear teeth in the plurality, and the arrangement of one or more gear teeth within the plurality. In some examples, the plurality of gear teeth comprises one or more gear teeth that are between about 1 μm and about 10 μm wide. In some examples, the plurality of gear teeth comprises one or more gear teeth that are between about 1 μm and about 10 μm tall. In some examples, the plurality of gear teeth comprises two or more gear teeth that are spaced between about 1 μm and about 10 μm apart. In some examples, the beads further comprise: (6) one or more columns projecting from the first surface of the first substantially transparent polymer layer, wherein the one or more columns are not within the center portion of the first substantially transparent polymer layer; and/or (7) one or more columns projecting from the second surface of the first substantially transparent polymer layer or a surface of the second substantially transparent polymer layer that is not affixed to the first substantially transparent polymer layer, wherein the one or more columns are not within the center portions of the first or the second substantially transparent polymer layer. In some examples, the bead is a substantially circular disc. In some examples, the center portion of the first substantially transparent polymer layer comprises between about 5% and about 90% of the surface area of the first substantially transparent polymer layer. In some examples, the center portion of the first substantially transparent polymer layer comprises about 25% of the surface area of the first substantially transparent polymer layer. In some examples, the beads are less than about 200 μm in diameter. In

some examples, the beads are about 50 μm in diameter. In some examples, the beads are less than about 50 μm in thickness. In some examples, the beads are about 10 μm in thickness. In some examples, the analyte is selected from the group consisting of a DNA molecule, a DNA-analog-molecule, an RNA-molecule, an RNA-analog-molecule, a polynucleotide, a protein, an enzyme, a lipid, a phospholipid, a carbohydrate moiety, a polysaccharide, an antigen, a virus, a cell, an antibody, a small molecule, a bacterial cell, a cellular organelle, and an antibody fragment. In some examples, the substantially transparent polymer of the first or the second substantially transparent polymer layer comprises an epoxy-based polymer. In some examples, the epoxy-based polymer is SU-8.

[0011] In other examples, provided herein are multiplex assay systems comprising a) a plurality of beads in the kit according to any one of the above examples; and b) an apparatus comprising an imaging processor system and a reaction detection system, wherein the identification of an identifier that corresponds to one or more of (i) a specific assay or assay kit, (ii) a specific analyte, (iii) a manufacturing lot, (iv) an individual, (v) a locational identifier, and/or (vi) a calibration signal by the imaging processor in the decoding system activates the use of a software analyzing detection signals detected by reaction detection system correlated to the specific assay.

[0012] In other examples, provided herein are methods for conducting a multiplex assay comprising the steps of: a) contacting a sample with beads in an assay system, wherein each bead in the system comprises a substantially non-transparent polymer layer having a first surface and a second surface, the first and the second surfaces being parallel to each other, wherein an outline of the substantially non-transparent polymer layer comprises a two-dimensional shape that represents an analog code identifier; wherein the system comprises (i) at least one bead with an analog code identifier that is recognized by an imaging processor as a specific assay or assay kit, specific analyte, manufacturing lot, individual, locational identifier, or calibration signal, and (ii) a plurality of beads, wherein each bead of the plurality of beads comprises a capture agent that specifically binds to an analyte, wherein the capture agent is immobilized on at least one of the first surface and the second surface of the substantially transparent polymer layer in at least the center portion of the substantially transparent polymer layer, and wherein each bead of the plurality of beads comprises an analog code identifier corresponding to the capture agent; and simultaneously or sequentially, in any order: b) identifying the identifier that is recognized by

the imaging processor as the specific assay; and c) detecting the binding of the analyte in the sample by analyzing the binding of the analyte to the capture agent immobilized on the surface of the bead based on the identification of the analog code identifier corresponding to the capture agent. In some examples, the system further comprises (iii) at least two beads, at least three beads, at least four beads, at least five beads, or at least six beads, each with an analog code identifier that is recognized by an imaging processor as a specific assay or assay kit, specific analyte, manufacturing lot, individual, locational identifier, or calibration signal. In some examples, the system further comprises (iv) at least one bead recognized by the imaging processor as a positive or negative control. In some examples, the locational identifier corresponds to a hospital, a diagnostic laboratory, an address, a health care professional's office, or a research laboratory. In some examples, the system further comprises (v) at least one bead that is pre-labeled for monitoring functions of the imaging processor. In some examples, the beads comprise a spherical or rectangular shape. In some examples, the beads are less than about 300 μm in diameter. In some examples, the surface of the bead is smooth and lacks surface irregularities. In some examples, at least one surface of the bead comprises at least one site for chemical attachment of the capture agent. In some examples, the capture agent is selected from the group consisting of: a DNA molecule, a DNA-analog-molecule, an RNA-molecule, an RNA-analog-molecule, a polynucleotide, a protein, an enzyme, a lipid, a phospholipid, a carbohydrate moiety, a polysaccharide, an antigen, a virus, a cell, an antibody, and an antibody fragment. In some examples, the plurality of beads are magnetic or non-magnetic. In some examples, the beads further comprise: (2) a magnetic, substantially non-transparent layer that encloses the center portion of the substantially transparent polymer layer between the substantially non-transparent polymer layer and the center portion of the substantially transparent polymer layer, wherein the magnetic, substantially non-transparent layer is affixed to the first surface or the second surface of the substantially transparent polymer layer. In some examples, the beads further comprise: (3) a second substantially transparent polymer layer aligned with the first substantially transparent polymer layer, the second substantially transparent polymer layer having a center portion that is aligned with the center portion of the first substantially transparent polymer layer, wherein the second substantially transparent polymer layer is affixed to the second surface of the first substantially transparent polymer layer and does not extend beyond the two-dimensional shape of the first substantially transparent polymer layer; and (4) a magnetic, substantially non-transparent layer

that encloses the center portion of the first substantially transparent polymer layer between the substantially non-transparent polymer layer and the center portion of the substantially transparent polymer layer, wherein the magnetic, substantially non-transparent layer is affixed between the first and the second substantially transparent polymer layers. In some examples, the beads further comprise an orientation indicator for orienting the analog code identifier of the substantially non-transparent polymer layer. In some examples, the orientation indicator comprises an asymmetry of the magnetic, substantially non-transparent layer. In some examples, the magnetic, substantially non-transparent layer comprises nickel. In some examples, the magnetic, substantially non-transparent layer is between about 50 nm and about 10 μm in thickness. In some examples, the magnetic, substantially non-transparent layer is about 0.1 μm in thickness. In some examples, the two-dimensional shape of the substantially non-transparent polymer layer comprises one or more rings enclosing the center portion of the substantially transparent polymer layer, wherein at least one of the one or more rings comprises a discontinuity. In some examples, the two-dimensional shape of the substantially non-transparent polymer layer comprises a gear shape comprising a plurality of gear teeth, and wherein the analog code identifier is represented by one or more examples selected from the group consisting of the height of one or more gear teeth of the plurality, the width of one or more gear teeth of the plurality, the number of gear teeth in the plurality, and the arrangement of one or more gear teeth within the plurality. In some examples, the plurality of gear teeth comprises one or more gear teeth that are between about 1 μm and about 10 μm wide. In some examples, the plurality of gear teeth comprises one or more gear teeth that are between about 1 μm and about 10 μm tall. In some examples, the plurality of gear teeth comprises two or more gear teeth that are spaced between about 1 μm and about 10 μm apart. In some examples, the beads further comprise: (5) one or more columns projecting from the first surface of the first substantially transparent polymer layer, wherein the one or more columns are not within the center portion of the first substantially transparent polymer layer; and/or (6) one or more columns projecting from the second surface of the first substantially transparent polymer layer or a surface of the second substantially transparent polymer layer that is not affixed to the first substantially transparent polymer layer, wherein the one or more columns are not within the center portions of the first or the second substantially transparent polymer layer. In some examples, the beads are a substantially circular disc. In some examples, the center portion of the first substantially transparent polymer layer comprises

between about 5% and about 90% of the surface area of the first substantially transparent polymer layer. In some examples, the center portion of the first substantially transparent polymer layer comprises about 25% of the surface area of the first substantially transparent polymer layer. In some examples, the beads are less than about 200 μm in diameter. In some examples, the beads are about 50 μm in diameter. In some examples, the beads are less than about 50 μm in thickness. In some examples, the beads are about 10 μm in thickness. In some examples, the analyte is selected from the group consisting of a DNA molecule, a DNA-analog-molecule, an RNA-molecule, an RNA-analog-molecule, a polynucleotide, a protein, an enzyme, a lipid, a phospholipid, a carbohydrate moiety, a polysaccharide, an antigen, a virus, a cell, an antibody, a small molecule, a bacterial cell, a cellular organelle, and an antibody fragment. In some examples, the substantially transparent polymer of the first or the second substantially transparent polymer layer comprises an epoxy-based polymer. In some examples, the epoxy-based polymer is SU-8.

[0013] In other examples, provided herein are kits for conducting a multiplex assay comprising: a) a set of beads, wherein each bead in the set comprises a substantially non-transparent polymer layer having a first surface and a second surface, the first and the second surfaces being parallel to each other, wherein an outline of the substantially non-transparent polymer layer comprises a two-dimensional shape that represents an analog code identifier; and wherein the set of beads comprises (i) at least one bead with an analog code identifier that is recognized by an imaging processor as a specific assay or assay kit, specific analyte, manufacturing lot, individual, locational identifier, or calibration signal, and (ii) a plurality of additional beads, wherein each bead of the plurality of beads comprises a capture agent that specifically binds to an analyte, wherein the capture agent is immobilized on at least one of the first surface and the second surface of the substantially transparent polymer layer in at least the center portion of the substantially transparent polymer layer, and wherein each bead of the plurality of beads comprises an analog code identifier corresponding to the capture agent; and b) a signal-emitting entity capable of directly or indirectly binding to the analyte or capture agent. In some examples, the signal-emitting entity indirectly binds to the analyte or capture agent via a secondary antibody that is conjugated to the signal-emitting entity. In some examples, the signal-emitting entity indirectly binds to the analyte or capture agent via a nucleotide probe that is conjugated to the signal-emitting entity. In some examples, the signal-emitting entity is phycoerythrin, GFP, RFP, CFP, YFP, FITC, horseradish peroxidase, alkaline phosphatase, or a

radiolabel. In some examples, the kit further comprises at least two beads, at least three beads, at least four beads, at least five beads, or at least six beads, each with an analog code identifier that is recognized by an imaging processor as a specific assay or assay kit, specific analyte, manufacturing lot, individual, locational identifier, or calibration signal. In some examples, the kit further comprises an imaging processor for (i) identifying the at least one bead with an identifier that is recognized by the imaging processor as a specific assay, (ii) identifying the identifier on the plurality of beads, (iii) detecting the amount of signal produced by the signal-emitting entity, (iv) identifying the at least one bead with an identifier that is recognized by the imaging processor as a manufacturing lot, (v) identifying the at least one bead with an identifier that is recognized by the imaging processor as an individual, (vi) identifying the at least one bead with an identifier that is recognized by the imaging processor as a locational identifier, or (vii) identifying the at least one bead that is pre-labeled for calibration of the imaging processor. In some examples, the kit further comprises an entity capable of directly or indirectly binding to the analyte or capture agent, wherein the entity comprises an affinity tag. In some examples, the affinity tag is biotin, His₆, or maltose. In some examples, the kit further comprises at least one bead that is pre-labeled for monitoring functions of the imaging processor. In some examples, the beads comprise a spherical or rectangular shape. In some examples, the beads are less than about 300 μm in diameter. In some examples, the surface of the bead is smooth and lacks surface irregularities. In some examples, at least one surface of the bead comprises at least one site for chemical attachment of the capture agent. In some examples, the capture agent is selected from the group consisting of: a DNA molecule, a DNA-analog-molecule, an RNA-molecule, an RNA-analog-molecule, a polynucleotide, a protein, an enzyme, a lipid, a phospholipid, a carbohydrate moiety, a polysaccharide, an antigen, a virus, a cell, an antibody, a small molecule, and an antibody fragment. In some examples, the plurality of beads are magnetic or non-magnetic. In some examples, the bead further comprises: (2) a magnetic, substantially non-transparent layer that encloses the center portion of the substantially transparent polymer layer between the substantially non-transparent polymer layer and the center portion of the substantially transparent polymer layer, wherein the magnetic, substantially non-transparent layer is affixed to the first surface or the second surface of the substantially transparent polymer layer. In some examples, the bead further comprises: (3) a second substantially transparent polymer layer aligned with the first substantially transparent polymer layer, the second substantially transparent polymer layer having a center portion that is

aligned with the center portion of the first substantially transparent polymer layer, wherein the second substantially transparent polymer layer is affixed to the second surface of the first substantially transparent polymer layer and does not extend beyond the two-dimensional shape of the first substantially transparent polymer layer; and (4) a magnetic, substantially non-transparent layer that encloses the center portion of the first substantially transparent polymer layer between the substantially non-transparent polymer layer and the center portion of the substantially transparent polymer layer, wherein the magnetic, substantially non-transparent layer is affixed between the first and the second substantially transparent polymer layers. In some examples, the bead further comprises an orientation indicator for orienting the analog code identifier of the substantially non-transparent polymer layer. In some examples, the orientation indicator comprises an asymmetry of the magnetic, substantially non-transparent layer. In some examples, the magnetic, substantially non-transparent layer comprises nickel. In some examples, the magnetic, substantially non-transparent layer is between about 50 nm and about 10 μm in thickness. In some examples, the magnetic, substantially non-transparent layer is about 0.1 μm in thickness. In some examples, the two-dimensional shape of the substantially non-transparent polymer layer comprises one or more rings enclosing the center portion of the substantially transparent polymer layer, wherein at least one of the one or more rings comprises a discontinuity. In some examples, the two-dimensional shape of the substantially non-transparent polymer layer comprises a gear shape comprising a plurality of gear teeth, and wherein the analog code identifier is represented by one or more examples selected from the group consisting of the height of one or more gear teeth of the plurality, the width of one or more gear teeth of the plurality, the number of gear teeth in the plurality, and the arrangement of one or more gear teeth within the plurality. In some examples, the plurality of gear teeth comprises one or more gear teeth that are between about 1 μm and about 10 μm wide. In some examples, the plurality of gear teeth comprises one or more gear teeth that are between about 1 μm and about 10 μm tall. In some examples, the plurality of gear teeth comprises two or more gear teeth that are spaced between about 1 μm and about 10 μm apart. In some examples, the beads further comprise: (5) one or more columns projecting from the first surface of the first substantially transparent polymer layer, wherein the one or more columns are not within the center portion of the first substantially transparent polymer layer; and/or (6) one or more columns projecting from the second surface of the first substantially transparent polymer layer or a surface of the second substantially transparent polymer layer that

is not affixed to the first substantially transparent polymer layer, wherein the one or more columns are not within the center portions of the first or the second substantially transparent polymer layer. In some examples, the bead is a substantially circular disc. In some examples, the center portion of the first substantially transparent polymer layer comprises between about 5% and about 90% of the surface area of the first substantially transparent polymer layer. In some examples, the center portion of the first substantially transparent polymer layer comprises about 25% of the surface area of the first substantially transparent polymer layer. In some examples, the beads are less than about 200 μm in diameter. In some examples, the beads are about 50 μm in diameter. In some examples, the beads are less than about 50 μm in thickness. In some examples, the beads are about 10 μm in thickness. In some examples, the analyte is selected from the group consisting of a DNA molecule, a DNA-analog-molecule, an RNA-molecule, an RNA-analog-molecule, a polynucleotide, a protein, an enzyme, a lipid, a phospholipid, a carbohydrate moiety, a polysaccharide, an antigen, a virus, a cell, an antibody, a small molecule, a bacterial cell, a cellular organelle, and an antibody fragment. In some examples, the substantially transparent polymer of the first or the second substantially transparent polymer layer comprises an epoxy-based polymer. In some examples, the epoxy-based polymer is SU-8.

[0014] In other examples, provided herein are multiplex assay systems comprising a) a plurality of beads in the kit according to any of the above examples; and b) an apparatus comprising an imaging processor system and a reaction detection system, wherein the identification of an identifier that corresponds to one or more of (i) a specific assay or assay kit, (ii) a specific analyte, (iii) a manufacturing lot, (iv) an individual, (v) a locational identifier, and/or (vi) a calibration signal by the imaging processor in the decoding system activates the use of a software analyzing detection signals detected by reaction detection system correlated to the specific assay or assay kit.

[0015] It is to be understood that one, some, or all of the properties of the various examples described above and herein may be combined to form other examples of the present disclosure. These and other examples of the present disclosure will become apparent to one of skill in the art. These and other examples of the present disclosure are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] **FIGS. 1A & 1B** show two views of an exemplary bead.

CLAIMS

What is claimed is:

1. A method for conducting a multiplex assay comprising the steps of:
 - a) contacting a sample with beads in an assay system, wherein each bead in the system comprises
 - (1) a substantially transparent polymer layer having a first surface and a second surface, the first and the second surfaces being parallel to each other;
 - (2) a substantially non-transparent polymer layer, wherein the substantially non-transparent polymer layer is affixed to the first surface of the substantially transparent polymer layer and encloses a center portion of the substantially transparent polymer layer, and wherein the substantially non-transparent polymer layer comprises a two-dimensional shape representing an analog code identifier;wherein the system comprises (i) at least one bead with an analog code identifier that is recognized by an imaging processor as a specific assay, specific analyte, manufacturing lot, individual, locational identifier, or calibration signal, and (ii) a plurality of beads, wherein each bead of the plurality of beads comprises a capture agent that specifically binds to an analyte, wherein the capture agent is immobilized on at least one of the first surface and the second surface of the substantially transparent polymer layer in at least the center portion of the substantially transparent polymer layer, and wherein each bead of the plurality of beads comprises an analog code identifier corresponding to the capture agent; and simultaneously or sequentially, in any order:
 - b) identifying the identifier that is recognized by the imaging processor as the specific assay; and
 - c) detecting the binding of the analyte in the sample by analyzing the binding of the analyte to the capture agent immobilized on the surface of the bead based on the identification of the analog code identifier corresponding to the capture agent;wherein optionally the beads are a substantially circular disc;wherein optionally the center portion of the first substantially transparent polymer layer comprises between about 5% and about 90% of the surface area of the first substantially transparent polymer layer.

2. The method of claim 1, wherein the system further comprises:
 - (iii) at least two beads, at least three beads, at least four beads, at least five beads, or at least six beads, each with an analog code identifier that is recognized by an imaging processor as a specific assay, specific analyte, manufacturing lot, individual, locational identifier, or calibration signal; and/or
 - (iv) at least one bead recognized by the imaging processor as a positive or negative control; and/or
 - (v) at least one bead that is pre-labeled for monitoring functions of the imaging processor.
3. The method of claim 1 or claim 2, wherein the beads are less than about 300 μm in diameter, less than about 200 μm in diameter, or about 50 μm in diameter; and wherein optionally the surfaces of the beads are smooth and lack surface irregularities.
4. The method of any one of claims 1-3, wherein at least one surface of the bead comprises at least one site for chemical attachment of the capture agent.
5. The method of any one of claims 1-4, wherein the capture agent is selected from the group consisting of: a DNA molecule, a DNA-analog-molecule, an RNA-molecule, an RNA-analog-molecule, a polynucleotide, a protein, an enzyme, a lipid, a phospholipid, a carbohydrate moiety, a polysaccharide, an antigen, a virus, a cell, an antibody, and an antibody fragment.
6. The method of any one of claims 1-5, wherein the plurality of beads are magnetic or non-magnetic.
7. The method of claim 1, wherein the beads further comprise an orientation indicator for orienting the analog code identifier of the substantially non-transparent polymer layer; wherein optionally the orientation indicator comprises an asymmetry of a magnetic, substantially non-transparent layer.
8. The method of claim 1, wherein the beads are less than about 50 μm in thickness and optionally about 10 μm in thickness.

9. The method of any one of claims 1-8, wherein the analyte is selected from the group consisting of a DNA molecule, a DNA-analog-molecule, an RNA-molecule, an RNA-analog-molecule, a polynucleotide, a protein, an enzyme, a lipid, a phospholipid, a carbohydrate moiety, a polysaccharide, an antigen, a virus, a cell, an antibody, a small molecule, a bacterial cell, a cellular organelle, and an antibody fragment.
10. The method of claim 1, wherein the substantially transparent polymer of the first or the second substantially transparent polymer layer comprises an epoxy-based polymer.
11. A kit for conducting a multiplex assay comprising:
- a) a set of beads, wherein each bead in the set comprises
 - (1) a substantially transparent polymer layer having a first surface and a second surface, the first and the second surfaces being parallel to each other;
 - (2) a substantially non-transparent polymer layer, wherein the substantially non-transparent polymer layer is affixed to the first surface of the substantially transparent polymer layer and encloses a center portion of the substantially transparent polymer layer, and wherein the substantially non-transparent polymer layer comprises a two-dimensional shape representing an analog code identifier;and wherein the set of beads comprises (i) at least one bead with an analog code identifier that is recognized by an imaging processor as a specific assay, specific analyte, manufacturing lot, individual, locational identifier, or calibration signal, and (ii) a plurality of additional beads, wherein each bead of the plurality of beads comprises a capture agent that specifically binds to an analyte, wherein the capture agent is immobilized on at least one of the first surface and the second surface of the substantially transparent polymer layer in at least the center portion of the substantially transparent polymer layer, and wherein each bead of the plurality of beads comprises an analog code identifier corresponding to the capture agent; and
 - b) a signal-emitting entity capable of directly or indirectly binding to the analyte or capture agent;
- wherein optionally the beads are a substantially circular disc;

wherein optionally the center portion of the first substantially transparent polymer layer comprises between about 5% and about 90% of the surface area of the first substantially transparent polymer layer.

12. The kit of claim 11, wherein the signal-emitting entity indirectly binds to the analyte or capture agent via a secondary antibody or nucleotide probe that is conjugated to the signal-emitting entity; wherein optionally the signal-emitting entity is phycoerythrin, GFP, RFP, CFP, YFP, FITC, horseradish peroxidase, alkaline phosphatase, or a radiolabel.

13. The kit of claim 11 or claim 12, wherein the kit further comprises at least two beads, at least three beads, at least four beads, at least five beads, or at least six beads, each with an analog code identifier that is recognized by an imaging processor as a specific assay, specific analyte, manufacturing lot, individual, locational identifier, or calibration signal.

14. The kit of any one of claims 11-13, further comprising an entity capable of directly or indirectly binding to the analyte or capture agent, wherein the entity comprises an affinity tag; wherein optionally the affinity tag is biotin, His₆, or maltose.

15. A multiplex assay system comprising:

- a) a plurality of beads in the kit of any one of claims 11-14; and
- b) an apparatus comprising an imaging processor system and a reaction detection system, wherein the identification of an identifier that corresponds to one or more of
 - (i) a specific assay,
 - (ii) a specific analyte,
 - (iii) a manufacturing lot,
 - (iv) an individual,
 - (v) a locational identifier, and/or
 - (vi) a calibration signal

by the imaging processor in the decoding system activates the use of a software analyzing detection signals detected by reaction detection system correlated to the specific assay.