

the particle and said wave becomes coherent to the circumference of the particle. Particle electrons resonate in phase with the incident light forming an electromagnetic dipole that emits energy as scattered light. The wavelength of the reflected (scattered) light is a function of the composition, shape, and particle size. Often, the composition of the particle is a noble metal, such as gold or silver. Often, the size of the particle is below the wavelength of white light (below 200 nm). Particles under 1000 nm in size are often referred to as “nanoparticles.”

[0006] The advantages of using particles with a wavelength less than the wavelength of light for cellular analysis using the resonant light scattering properties of the particles are: (a) the nanoparticles can be detected and imaged at magnifications as low as 10X using a simple illuminator, such as a white light illuminator, with dark field illumination, (b) the nanoparticles provide a non-bleaching signal, (c) the color of scattered light can be changed by changing nanoparticle size and/or composition for multicolor multiplexing, (d) the nanoparticles can be conjugated with biomarker-binding moieties for specific analyte detection to create functionalized nanoparticles, (e) biological samples contacted with the functionalized nanoparticles are archivable, and (f) the functionalized nanoparticles exhibit a greater range of linearity of detection when present on a cell because the particles do not self-quench. In some examples, the methods of this invention are useful in obtaining images of cell-functionalized nanoparticle complexes under ambient conditions which do not require use of a darkroom, in contrast to fluorescent labeling systems. In some examples, the samples may be viewed on a microscope in a doctor’s or pathologist’s office.

[0006a] In one aspect, the present invention provides an *in vitro* method for detecting the biomarker-morphological profile of a cell, the method comprising:

- (a) providing a sample comprising cells from a subject;
- (b) contacting the cells with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety, and forming nanoparticle- cell complexes through binding of the functionalized nanoparticle species comprising a biomarker-binding moiety to its respective biomarker;
- (c) adhering the functionalized nanoparticle-cell complexes to a substrate;
- (d) illuminating the functionalized nanoparticle-cell complexes with epi-illumination or evanescent light and detecting the resonant light scattering from each observed complexed functionalized nanoparticle, to obtain a biomarker signature of each imaged cell;

- (e) contacting the substrate-adhered cells with an optical contrast agent;
- (f) imaging morphological features of the contacted cells; and
- (g) associating the morphological features of the contacted cells with the biomarker signature of each substrate-adhered cell to detect the biomarker-morphological profile of each cell.

[0006b] In another aspect, the present invention provides an *in vitro* method for detecting the biomarker-morphological profile of a cell, the method comprising:

- (a) providing a sample comprising cells from a subject;
- (b) contacting the cells with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety, and forming nanoparticle- cell complexes through binding of the nanoparticle species comprising a biomarker-binding moiety to its respective biomarker;
- (c) adhering the functionalized nanoparticle-cell complexes to a substrate;
- (d) illuminating the functionalized nanoparticle-cell complexes with non-evanescent light and detecting the resonant light scattering from each observed complexed nanoparticle, to obtain a biomarker signature of each observed cell;
- (e) contacting the substrate-adhered cells with an optical contrast agent;
- (f) imaging morphological features of the contacted cells; and
- (g) associating the morphological features of the contacted cells with the biomarker signature of each substrate-adhered cell to detect the biomarker-morphological profile of each cell.

[0006c] In yet another aspect, the present invention provides an *in vitro* method for detecting the biomarker-morphological profile of a cell, the method comprising:

- (a) providing a sample comprising cells from a subject;
- (b) adhering the cells to a substrate;
- (c) contacting the substrate-adhered cells with an optical contrast agent;
- (d) imaging morphological features of the contacted cells;
- (e) converting the optical contrast agent to a colorless form;

- (f) contacting the cells with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety to form nanoparticle- cell complexes;
- (g) illuminating the nanoparticle-cell complexes with evanescent light and detecting the resonant light scattering from each observed complexed nanoparticle, to obtain a biomarker signature of each observed cell; and
- (h) associating the morphological features of the contacted cells with the biomarker signature of each substrate-adhered cell to detect the biomarker-morphological profile of each cell.

[0006d] In yet another aspect, the present invention provides a homogeneous assay for detecting functionalized nanoparticle cell complexes, the assay comprising:

- (a) providing a sample comprising cells from a subject;
- (b) contacting the cells with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety, and forming functionalized nanoparticle- cell complexes through binding of the nanoparticle species comprising a biomarker-binding moiety to its respective biomarker;
- (c) adhering the functionalized nanoparticle-cell complexes to a substrate;
- (d) illuminating the functionalized nanoparticle-cell complexes with evanescent light and detecting the resonant light scattering from each observed complexed nanoparticle, to obtain a biomarker signature of each observed cell,

wherein unbound functionalized nanoparticles are not removed from the field of view.

[0006e] In yet another aspect, the present invention provides an *in vitro* method for detecting functionalized nanoparticle cell complexes, the method comprising:

- (a) providing a sample comprising cells from a subject;
- (b) contacting cells which have been fixed with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety, and forming nanoparticle- cell complexes through binding of the nanoparticle species comprising a biomarker-binding moiety to its respective biomarker;

(c) adhering the nanoparticle-cell complexes to a substrate, wherein the adhered functionalized nanoparticle-cell complexes are placed in contact with a mountant, wherein the refractive index of the mountant is within about 0.1 of the refractive index of the fixed cells;

(d) detecting the functionalized nanoparticle cell complexes by illuminating the nanoparticle-cell complexes with evanescent light and detecting the resonant light scattering from each observed functionalized nanoparticle cell complex, to obtain a biomarker signature of each observed cell; and

(e) associating the biomarker signature of each substrate-adhered cell to a known disease, condition, or state of a cell exhibiting substantially the same biomarker signature to identify the disease, condition, or state of the cell from a subject.

[0006f] In yet another aspect, the present invention provides an *in vitro* method for detecting functionalized nanoparticle cell complexes to obtain a biomarker signature, the method comprising:

(a) providing a sample comprising cells from a subject;

(b) contacting the cells with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety, and forming nanoparticle-cell complexes through binding of the nanoparticle species comprising a biomarker-binding moiety to its respective biomarker;

(c) adhering the functionalized nanoparticle-cell complexes to a substrate;

(d) illuminating the functionalized nanoparticle-cell complexes with evanescent light and detecting the resonant light scattering from each observed complexed nanoparticle, to obtain a biomarker signature of each observed cell; and

(e) associating the biomarker signature of an imaged cell from the subject with a biomarker signature of a reference cell exhibiting substantially the same biomarker signature as the imaged cell biomarker signature, wherein a diagnostic concordance has been established between the reference cell biomarker signature and a disease, disorder, condition or state of the reference subject.

[0007] Disclosed are methods and compositions for detecting cell-functionalized nanoparticle binding moiety complexes useful in detecting a biomarker signature of a cell. Also disclosed is that the methods

and compositions are also useful in detecting the biomarker-morphological profile of an imaged cell.

[0008] Also disclosed is that the nanoparticles functionalized with biomarker-binding moieties can be used for detecting functionalized nanoparticle cell complexes, which are useful, for example, for identifying and quantifying biomarkers present on cells. In some examples, the cells may be imaged to detect morphological features of the cells complexed with functionalized nanoparticles. In some examples, the functionalized nanoparticles can be contacted with the same cells analyzed by a morphological imaging analysis.

[0009] In some examples, the methods of this invention are useful for improving signal generation, detection limits, dynamic range, automation and/or performance characteristics of the biomarker signature assays. For example, in some examples, the present disclosure relates to methods for increasing the loading amount of a biomarker binding moiety onto a cell by using an external force to increase the local concentration of the functionalized nanoparticles and cells. Also disclosed is that the biomarker binding moiety may be a functionalized nanoparticle, or a biomarker binding moiety comprising a label other than a nanoparticle. A “functionalized nanoparticle” is a nanoparticle presenting a functional group, directly or indirectly. Also disclosed is that the external force may be a centrifugal, electromagnetic, or magnetic force.

[0010] Also disclosed is that the method of detecting functionalized nanoparticle cell complexes can comprise:

- (a) providing a sample comprising cells from a subject;
- (b) contacting the cells with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety, wherein an external force is used to accelerate the formation of nanoparticle-cell complexes through binding of the nanoparticle species comprising a biomarker-binding moiety to its respective biomarker;
- (c) adhering the functionalized nanoparticle-cell complexes to a substrate;
- (d) detecting the functionalized nanoparticle cell complexes by illuminating the nanoparticle-cell complexes with evanescent light and detecting the resonant light scattering from each observed functionalized nanoparticle cell complex, to obtain a biomarker signature of each observed cell; and
- (e) associating the biomarker signature of each substrate-adhered cell to a known disease, condition, or state of a cell exhibiting substantially the same or a similar biomarker signature to identify the disease, condition, or state of the cell from a subject.

[0011] In any of the methods disclosed herein, where a biomarker or biomarker morphological profile is obtained, detecting the cell-functionalized nanoparticle complexes on the imaged cell optionally includes storing the positional information for each imaged cell.

[0012] In some examples, the disease, condition, or state of a cell can be identified by forming and detecting complexes between the functionalized nanoparticles and cells. The method can comprise associating a biomarker signature of each substrate-adhered cell to a known disease, condition, or state of a cell exhibiting substantially the same or a similar biomarker signature to identify the disease, condition, or state of the cell from a subject.

[0013] In some examples, the biomarker signature of a cell can be detected in a homogeneous assay, the assay comprising the steps:

- (a) providing a sample comprising cells from a subject;
- (b) contacting the cells with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety, and forming functionalized nanoparticle- cell complexes through binding of the nanoparticle species comprising a biomarker-binding moiety to its respective biomarker;
- (c) adhering the functionalized nanoparticle-cell complexes to a substrate;
- (d) illuminating the functionalized nanoparticle-cell complexes with evanescent light and detecting the resonant light scattering from each observed complexed nanoparticle, to obtain a biomarker signature of each observed cell,

where unbound functionalized nanoparticles are not removed from the field of view.

[0014] Often, unbound species are washed from a target to reduce background noise. In some examples wash steps are omitted, leaving unbound functionalized nanoparticles in the field of view. This example may be used in some examples, for example, for high throughput assays, and/or automated assays. The functionalized nanoparticles are specific to the biomarker on the cell, and can substantially contact the cell such that little to no signal is observed for the unbound functionalized nanoparticles.

[0015] Also disclosed is that, where biomarker signatures are detected, the detecting of the resonant light scattering from each observed complexed-nanoparticle comprises imaging the cell-functionalized nanoparticle complexes in contact with a mountant. Also disclosed is that the mountant can comprise a solution with about the refractive index of the cells. Also disclosed is that the mountant can be within about 0.1 of the refractive index of cells, where the cells are fixed. In some examples, the refractive index of fixed cells is about 1.52, or 1.52. Also disclosed is that the index of

refraction of the mountant is from 1.51 to 1.54. Also disclosed is that using a mountant having an RI within 0.1 of the refractive index of fixed cells is useful for reducing the amount of white light scatter, and obtaining better images of resonance scattering from the cell-functionalized nanoparticle complexes. Also disclosed is a method for detecting functionalized nanoparticle cell complexes, the method comprising:

- (a) providing a sample comprising cells from a subject;
- (b) contacting cells which have been fixed with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety, and forming nanoparticle- cell complexes through binding of the nanoparticle species comprising a biomarker-binding moiety to its respective biomarker;
- (c) adhering the nanoparticle-cell complexes to a substrate, wherein the adhered nanoparticle-cell complexes are placed in contact with a mountant, wherein the refractive index of the mountant is within about 0.1 of the refractive index of the fixed cells;
- (d) detecting the functionalized nanoparticle cell complexes by illuminating the nanoparticle-cell complexes with evanescent light and detecting the resonant light scattering from each observed functionalized nanoparticle cell complex, to obtain a biomarker signature of each observed cell; and
- (e) associating the biomarker signature of each substrate-adhered cell to a known disease, condition, or state of a cell exhibiting substantially the same biomarker signature to identify the disease, condition, or state of the cell from a subject.

Also disclosed is that the mountant may have a refractive index of about 1.52.

[0016] Also disclosed is the ability to use the compositions and methods of this disclosure to associate the biomarker signature of an individual cell, and in some examples, with its morphological image/features greatly enhances the ability to diagnose and monitor abnormal conditions or disorders.

[0017] As set forth herein, the inventors have surprisingly determined that nanoparticles functionalized with biomarker-binding moieties can be used for detecting cell-functionalized nanoparticle complexes and identifying and quantifying biomarkers present on imaged cells for example, when the functionalized nanoparticles are contacted with the same

cells analyzed by a morphological imaging analysis. The ability to use the compositions and methods of this disclosure to associate the biomarker signature of an individual cell with its morphological image/features greatly enhances the ability to diagnose and monitor abnormal conditions or disorders.

[0018] Also disclosed are compositions and methods for obtaining a biomarker signature for an imaged cell, which is used, in some examples, in combination with detected morphological features of the cell obtained from imaging the cell. Also disclosed are compositions comprising functionalized nanoparticle species, each comprising a specific biomarker-binding moiety, are used to detect the biomarker signature of the imaged cell. Also disclosed are combinations of such compositions are made or used in the methods of this disclosure. The combinations and kits comprising the combinations can be mixtures of such compositions, or may comprise compositions segregated before use.

[0019] Also disclosed is that the method comprises the steps of:

- (a) providing a sample comprising cells from a subject;
- (b) contacting the cells with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety, and forming nanoparticle-cell complexes through binding of the nanoparticle species comprising a biomarker-binding moiety to its respective biomarker on the cell;
- (c) adhering the functionalized nanoparticle-cell complexes to a substrate;
- (d) illuminating the functionalized nanoparticle-cell complexes with evanescent light and detecting the resonant light scattering from each observed complexed functionalized nanoparticle, to obtain a biomarker signature of each observed cell;
- (e) contacting the substrate-adhered cells with an optical contrast agent;
- (f) imaging morphological features of contacted cells; and
- (g) associating the morphological features of the contacted cells with the biomarker signature of each substrate-adhered cell to detect the biomarker-morphological profile of each cell.

[0020] Also disclosed is that biomarker-morphological profiles can be used to obtain a diagnostic concordance between a reference cell biomarker signature and a disease, disorder,

condition or state of a reference subject. In some examples, a concordance database of biomarker signatures with diseases, disorders, conditions or states of diagnosed subjects can be obtained. A diagnostic concordance includes an association based on an association between a reference biomarker signature and/or a reference biomarker-morphological profile, and a disease, disorder, condition or state of the reference subject. In some examples the diagnostic concordance or association, may be made independent of any treatment decision, for example, using data obtained from an autopsy and/or based on archived tissue samples and patient records.

[0021] Also disclosed is a method for detecting functionalized nanoparticle cell complexes to obtain a biomarker signature, the method comprising:

- (a) providing a sample comprising cells from a subject;
- (b) contacting the cells with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety, and forming nanoparticle-cell complexes through binding of the functionalized nanoparticle species comprising a biomarker-binding moiety to its respective biomarker;
- (c) adhering the functionalized nanoparticle-cell complexes to a substrate;
- (d) illuminating the functionalized nanoparticle-cell complexes with evanescent light and detecting the resonant light scattering from each observed complexed nanoparticle, to obtain a biomarker signature of each observed cell; and
- (e) associating the biomarker signature of an imaged cell from the subject with a biomarker signature of a reference cell exhibiting substantially the same biomarker signature as the imaged cell biomarker signature, wherein a diagnostic concordance has been established between the reference cell biomarker signature and a disease, disorder, condition or state of the reference subject.

[0022] Also disclosed is that the sample can be a biological sample. For example, in some examples, the sample may be any sample containing cells. In some examples, the sample may be from blood, bone marrow, a fine needle aspirate, or tissue. The tissue sample can be obtained, for example, from a biopsy. In some examples the tissue sample may be obtained from a FFPE (formalin-fixed, paraffin-embedded) tissue

sample. Also disclosed is that a biological sample may be processed. For example, the sample may comprise white blood cells. In some examples, at least 50% of the red blood cells are removed before contacting the cells with the plurality of functionalized nanoparticle species.

[0023] Also disclosed is that the cell may be alive, fixed and/or substantially intact. In some examples, the detected cells interrogated can be the same type or different types. When the cells are different types, they may be of different tissue or tumor origin, different stages of cancer progression, metastatic and non-metastatic cancer cells, and may comprise infectious agents or cells, infected cells, and uninfected cells, or express different levels, types, variants, mutants, forms, and/or post-translationally modified forms of biomarkers found on normal or reference cells. In some examples, the different cells may exhibit different pathologies, and/or different morphologies from normal or reference cells.

[0024] Also disclosed is that the cell is fixed with a fixing agent. The fixing agent may be, for example, formaldehyde, glutaraldehyde, or another cross-linking agent. In other examples water-soluble preservatives, for example, methyl or propyl paraben, dimethylolurea, sorbic acid, 2-pyridinethiol-1-oxide, or potassium sorbate may be used. In some examples the cell can be permeabilized by surfactants.

[0025] Also disclosed is that the functionalized nanoparticle cell complexes adhered to a substrate can be placed in contact with a mountant. The volume of the mountant can be from about 2 microliters to about 15 microliters.

[0026] Also disclosed is that the detected biomarker can be present on the cell surface, within the cell, or both on the surface and within the cell. In some examples the biomarker in the cell may be present in or on one or more cellular features, for example, the cytosol, the nucleus, the nuclear membrane, nucleoli, the endoplasmic reticulum, Golgi apparatus, mitochondria, or other cellular structure, compartment, or feature.

[0027] Also disclosed is that the detected biomarker can be a biomolecule identified by the Cluster Determinant antigen (CD) and or other molecules /antigenic sites. For example, the biomarker can include or exclude any of the from the following biomarkers: CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD21, CD22, CD23, CD25, CD30, CD33, CD34, CD38, CD41, C43, CD45, CD56, CD57, CD58, CD61, CD64, C71, CD79a, CD99, CD103, CD117, CD123, CD138, CD138, CD163, CD235a, HLA-DR, Kappa,

Lambda, Pax-5, BCL-2, Ki-67, ZAP-70, MPO, TdT, and FMC-7. In some examples, for example, the biomarker can include or exclude markers expressed by kidney cells, infectious agents, solid tumor cells, circulating tumor cells, or any other cell useful for diagnosis or prognosis. In some examples, for example, the biomarker can include or exclude biomarkers expressed on the surface or within kidney cells, infectious agents (*e.g.*, bacteria or virus), solid tumor cells, or circulating tumor cells. In some examples, for example the biomarker may include or exclude HER2, NEU, Prostate stem cell antigen (PSCA), epithelial-specific antigen (ESA), epithelial cell adhesion molecule (EpCAM), $\alpha 2\beta 1$, VEGFR-1, VEGFR-2, CD133, or AC133 antigen.

[0028] Also disclosed is that the biomarker-binding moiety can be selected from or comprise the following: an antibody or antibody fragment, nanobody, receptor fragment, DNA aptamer, DNA/RNA oligonucleotide, RNA aptamer, PNA aptamer, peptide aptamer, LNA aptamer, carbohydrate, and a lectin.

[0029] In examples where the biomarker binding moiety comprises an antibody, the antibody can be a monoclonal or polyclonal antibody. In some examples, the biomarker binding moiety may comprise an antibody fragment, ScFv, or single-domain antibody (nanobody). The biomarker binding moiety may bind to a protein, protein fragment, glycosylation moiety or pattern, or a carbohydrate. The biomarker binding moiety can include or exclude a biomarker binding moiety, *e.g.*, an antibody or fragment thereof or other biomarker binding moiety that binds to, for example: CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD21, CD22, CD23, CD25, CD30, CD33, CD34, CD38, CD41, C43, CD45, CD56, CD57, CD58, CD61, CD64, C71, CD79a, CD99, CD103, CD117, CD123, CD138, CD138, CD163, CD235a, HLA-DR, Kappa, Lambda, Pax-5, BCL-2, Ki-67, ZAP-70, MPO, TdT, and FMC-7. In some examples, when the biomarker-binding moiety is anti-CD45, the biomarker signature obtained is the white blood cell count. The white blood cell count can be measured as a function of the mass or volume of the sample mass or volume, respectively.

[0030] In some examples, the optical contrast agent can be a leuco dye, cell stain, or any dye useful for imaging for morphological analysis including, for example, any dye useful for histological, cytological, cytopathological, or histopathological imaging. In some examples, the optical contrast agent provides visual classification and identification of cells by differentially staining cells. The leuco dye can be red leuco dye, methylene blue, crystal violet, phenolphthalein, thymolphthalein, or methylene green.

[0031] In some examples, the optical contrast agent can include or exclude a cell stain selected from, for example: Giemsa stain, Wright stain, Wright-Giemsa stain, May-Grünwald stain, Mallory trichrome, Periodic acid-Schiff reaction stain, Weigert's elastic stain, Heidenhain's AZAN trichrome stain, Orcein stain, Masson's trichrome, Alcian blue stain, May-Grünwald-Giemsa, van Gieson stain, Hansel stain, Reticulin Stain, Gram stain, Bielschowsky stain, Ferritin stain, Fontana-Masson stain, Hales colloidal iron stain, Pentachrome stain, Azan stain, Luxol fast blue stain, Golgi's method (reduced silver), reduced gold, chrome alum/haematoxylin stain, Isamin blue stain, Argentaffin stains, Warthin-Starry silver stain, Nissl stain, Sudan Black and osmium stain, osmium tetroxide stain, hematoxylin stain, uranyl acetate stain, lead citrate stain, Carmine stain, safranin stain, and Ziehl-Neelsen stain.

[0032] In some examples, the optical contrast agent can be a dye or colorant that can include or exclude, for example: eosin Y, eosin B, azure B, pyronin G, malachite green, toluidine blue, copper phthalocyanin, alcian blue, auramine-rhodamine, acid fuchsin, aniline blue, orange G, acid fuchsin, neutral red, Sudan Black B, acridine orange, Oil Red O, Congo Red, Fast green FCF, Perls Prussian blue reaction, nuclear fast red, alkaline erythrocin B, and naphthalene black.

[0033] When the sample is from tissue, the optical contrast agent can be a H&E (hematoxylin and eosin) stain. Also disclosed is that the optical contrast agent may be suitable for supravital staining.

[0034] In some examples, the cells can be contacted with one or a plurality of functionalized nanoparticle species by subjecting the cells and functionalized nanoparticles to an external force to increase the local concentration of the functionalized nanoparticles and cells. The external force can be a gravitational, electric, or magnetic force. The gravitational force can be generated by centrifugation. The magnetic force can be effected by paramagnetic nanoparticles. The core of a paramagnetic functionalized nanoparticle comprises a paramagnetic region and the shell of the nanoparticle can include or exclude Ag, Au, Pt, Pd, Rh, Ru, Al, Cu, Ru, Cr, Cd, Zn, Si, Se or mixtures or alloys thereof. In some examples, charged polymers can be added to the cells after first providing a sample comprising cells from a subject. These methods may be useful, for example, for methods of detecting cell-biomarker binding moieties, for example, in any examples of detecting cell-functionalized nanoparticle complexes disclosed herein. In some examples the sample may be re-mixed between two or more applications of the external force. As one non-limiting example, where the force is a centrifugal force, the force can be applied in a forward direction

to concentrate the cells and functionalized nanoparticles, and then applied in the reverse direction to resuspend the cells and functionalized nanoparticles. The centrifugal force can also be applied and reversed two or more times. As one non-limiting example where the force is a electric force, the force can be applied by electrophoresis on a conductive or semiconductive surface, where the functionalized nanoparticles and cells are mixed by their different relative electrophoretic mobilities when under a potential bias (see, Su, H., *et al.*, *Electrophoresis*, 23 1551-1557 (2002) and U.S. Patent Application Publication No. US 2003/0119028). The electrical force can also be applied and reversed two or more times.

[0035] In some examples, the imaging of the morphological features of the contacted cells can comprise measuring an optical property of the optical contrast agent. The optical property of the optical contrast agent can include or exclude, for example: absorbance, scattering, fluorescence, photoluminescence, Raman emission, and photoluminescent lifetime. The optical property of the optical contrast agent can be measured under a microscope with either a light field illumination or dark field illumination.

[0036] In some examples, the morphological features identified from the cell can include or exclude, for example: the shape of cellular features, for example, the cell surface shape, the cell nucleus shape, the chromatin shape, the nucleolar shape, the number of cellular features, such as the number of nucleoli or mitochondria, the density of staining of cellular features, or any combination of any of the foregoing, or any other imaged cellular feature or compartment.

[0037] In some examples, the method for detecting the biomarker-morphological profile of a cell can further comprise: (h) diagnosing the subject's condition based on the biomarker-morphological profile of each cell. Also disclosed is that the subject's condition may include or exclude, for example, the presence of a hematological cancer, non-malignant hematological disorder, solid tumor, kidney disease, bladder disease, liver disease, or infectious disease. The hematological cancer can include or exclude leukemia, lymphoma, or multiple myeloma. The non-malignant hematological disorder can be anemia or sickle cell disease. The solid tumor can include or exclude breast cancer, lung cancer, prostate cancer, bone cancer, colorectal cancer, or bladder cancer. When the solid tumor is breast cancer, the biomarkers can include or exclude, for example, Her2 or Neu. In some examples, the kidney disease can include or exclude acute kidney injury, chronic kidney disease, lupus nephritis, kidney rejection, or preeclampsia. In some examples, the infectious disease can include or exclude, for example: HIV, hepatitis, sexually transmitted diseases, or sepsis. In some examples, the hematological cancer can further comprise circulating cancer cells.

[0038] In some examples, when the subject's condition is a cancer, the subject's condition can be further identified by the lineage of the malignancy, the stage, or state of remission. For example, the lineage of the malignancy can include or exclude, for example: negative, Myeloid line, Lymphoid T cell line, or Lymphoid B cell line.

[0039] Also disclosed is that the resonant light scattering from each observed complexed nanoparticle can be detected using evanescent or non-evanescent light. Also disclosed is that the non-evanescent light can be transmitted light. The resonant light scattering of the complexed nanoparticle can be detected when imaging under a dark field illumination. Also disclosed is that an illuminated slide holder can replace the darkfield condenser in the microscope. The illuminated slide holder can use total internal reflection to illuminate the slide holder. The illuminated slide holder can comprise optical fibers to deliver light to the edge to the slide.

[0040] In some examples, the detection of the resonant light scattering from each observed complexed nanoparticle can be completed in under, for example, 1 second, 500 milliseconds, or 200 milliseconds.

[0041] In some examples, the one or a plurality of functionalized nanoparticle species can be comprised from nanoparticles from 10 to 200 nm in diameter. In some examples, the nanoparticles can be comprised of, for example, Ag, Au, Pt, Pd, Rh, Ru, Al, Cu, Ru, Cr, Cd, Zn, Si, Se or mixtures or alloys thereof. The alloy can be an alloy of gold (Au) and silver (Ag). The alloy can be of copper (Cu) and Gold (Au). The nanoparticles can comprise mixtures of the listed metals in discrete shells or layers. When the nanoparticles comprise Si, the nanoparticles can have a Si shell, SiO₂ shell (silica) or Si core. In some examples, the nanoparticles can be spherical, tubular, cylindrical, pyramidal, cubic, egg-shaped, t-bone-shaped, urchin- or rose-like (with spiky uneven surfaces) or hollow shaped. In some examples, the nanoparticles may have a round, oval, triangular, square, egg-shaped, or a t-bone-shaped cross-section.

[0042] In some examples, the plurality of functionalized nanoparticle species can be from 2 to 50 different species of functionalized nanoparticle species. Each species of functionalized nanoparticle species can be functionalized with a different species of biomarker-binding moiety. For example, also disclosed is that each species of functionalized nanoparticle species is functionalized with a different biomarker binding moiety. Also disclosed is that different biomarker binding moieties used in successive contact of the cell with different pluralities of functionalized nanoparticle species, may be bound to the same functionalized nanoparticle, as disclosed herein.

[0043] In some examples, when the nanoparticle species are functionalized with a biomarker binding moiety, *e.g.*, an antibody or antibody fragment or other biomarker binding moiety that binds to one of the following: CD3, CD22, CD79a, Kappa, Lambda, Pax-5, ZAP-70, MPO, or TdT; the functionalized nanoparticle species can enter the cell and bind to its respective intracellular biomarker. The intracellular biomarker can be in a cellular region which can include or exclude, for example, the cytosol, nucleus, on the nuclear membrane, or in or on another cellular compartment or structure. In some examples, the functionalized nanoparticles are small enough to enter the cell without disrupting the cell membrane. The cells can be treated with a permeabilizer so as to allow the functionalized nanoparticles to enter the cell without disrupting the cell membrane. In some examples, the biomarker signature can be obtained by counting the number or proportion of each of the functionalized nanoparticle species per cell. The number of cells having identified normal or abnormal morphological profiles in the sample can be totaled, weighted, or otherwise determined.

[0044] In some examples, the step of (d) illuminating the nanoparticle-cell complexes with evanescent light and detecting the resonant light scattering from each observed complexed nanoparticle, to obtain a biomarker signature of each observed cell in the method of detecting the biomarker-morphological profile of a cell can further comprise:

- (i) using a software program that counts the number of each of the functionalized nanoparticle species per cell and processes images in each cell in the field of view;
- (ii) moving the field of view digitally;
- (iii) using a software program to count the number of each of the functionalized nanoparticle species per imaged cell in the next field of view and repeating steps (ii) and (iii) until the entire substrate area is analyzed;
- (iv) digitally combining all images obtained to generate a single image covering the entire substrate area; and
- (v) generating from the data obtained for the entire substrate area the number of each of the functionalized nanoparticle species per cell, the biomarker signature, of each substrate-adhered cell.

[0045] In some examples, the software program also stores the positional information for each observed and/or imaged cell.

[0046] In some examples, the field of view is from about $0.25\ \mu\text{m}^2$ to about $2.5\ \text{cm}^2$. In some examples, the field of view can be from about $100\ \mu\text{m}^2$ to about $1000\ \text{mm}^2$. In some examples, the field of view is a 5 microns by 5 microns. In some examples, the field of view is 100 mm by 100 mm. In some examples, the field of view is square-shaped. The sides of the square-shaped field of view can be from 0.25 microns up to 2.5 centimeters. The field of view can cover one cell, or a plurality of cells. In some examples, the field of view can cover the area of the entire slide.

[0047] In some examples, the step of (f) imaging morphological features of the contacted cells in the method of detecting the biomarker-morphological profile of a cell can further comprise:

- (i) using a software program that processes images of morphological features of each cell in the field of view;
- (ii) moving the field of view digitally;
- (iii) using a software program to process images of morphological features of each cell in the next field of view and repeating steps (ii) and (iii) until the entire substrate area is analyzed;
- (iv) digitally combining all images obtained to generate a single image covering the entire substrate area; and
- (v) generating from the data obtained for the entire substrate area the morphological features of each substrate-adhered cell to detect the biomarker-morphological profile of each cell.

[0048] In some examples, a method for detecting the biomarker-morphological profile of a cell can comprise:

- (a) providing a sample comprising cells from a subject;
- (b) adhering the cells to a substrate;
- (c) contacting the substrate-adhered cells with an optical contrast agent;
- (d) imaging morphological features of the contacted cells;
- (e) converting the optical contrast agent to a colorless form;

(f) contacting the cells with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety to form nanoparticle- cell complexes;

(g) illuminating the nanoparticle-cell complexes with evanescent light and detecting the resonant light scattering from each imaged cell nanoparticle complex, to obtain a biomarker signature of each observed cell; and

(h) associating the morphological features of the contacted cells with the biomarker signature of each substrate-adhered cell to detect the biomarker-morphological profile of each cell.

[0049] In some examples, the optical contrast agent can be a leuco dye. The leuco dye can be methylene blue, methylene green, red leuco dye, crystal violet, phenolphthalein, or thymolphthalein. The leuco dye can be converted to a colorless form by the addition of one or more electrons to the dye. Electrons can be added to the dye via a reduction method. The reduction method can be effected by an electrochemical reduction, photoreduction, or reaction with a reducing agent. In some examples, the leuco dye can be converted to a colored form by the removal of one or more electrons from the dye. One or more electrons can be removed from the dye by an oxidation method. The oxidation method can be effected by an electrochemical oxidation, photooxidation, or reaction with an oxidation agent.

[0050] In some examples, the method for detecting the biomarker-morphological profile of a cell can further comprise: (d)(2) removing a first plurality of functionalized nanoparticles; and (d)(3) contacting the cells with a second plurality of functionalized nanoparticle species. In some examples, removing a first plurality of functionalized nanoparticles can be achieved by cleaving a linker between each species of functionalized nanoparticle and each species of a functionalized nanoparticle-associated biomarker-binding moiety. Also disclosed is that different biomarker binding moieties used in successive contact of the cell with different pluralities of functionalized nanoparticle species, may be bound to the same functionalized nanoparticle. For example, an anti-CD3 binding moiety may be bound to a 10nm gold particle for use in contacting a first plurality of functionalized nanoparticle species with a cell, and an anti-CD8 antibody may be bound to a 10nm gold particle for use in a second plurality of functionalized nanoparticle species, after the first plurality of functionalized nanoparticles has been released. Also disclosed is that

different pluralities of functionalized nanoparticle species may detect biomarkers indicative of two or more diseases or conditions.

[0051] In some examples of this disclosure, the cells may be the same type or different types from each other. In some examples, the cells may be associated with different conditions. For example, cells may be associated with one or more of the following conditions: hematological cancer, non-malignant hematological disorder, solid tumor, bladder disease, liver disease, kidney disease, or infectious disease. Also disclosed is that the cells may be associated with two or more types of solid tumors.

[0052] In some examples, the removal of a first plurality of functionalized nanoparticles can be achieved by releasing the first plurality of functionalized nanoparticles from the biomarker binding moieties. The functionalized nanoparticles can be released by displacing, cleaving, separating, disconnecting, hydrolyzing, or dissociating the nanoparticles from the biomarker-binding moieties. In some examples, the linker between each nanoparticle species in the first plurality of nanoparticles and its respective biomarker binding moiety comprises a first oligonucleotide bound to a first nanoparticle species and a second oligonucleotide bound to its respective biomarker binding moiety, where the second oligonucleotide comprises a portion complementary to at least a portion of the first oligonucleotide, and hybridization of the first oligonucleotide to the second oligonucleotide forms a linker comprising a double-stranded nucleic acid in these oligonucleotide-linker functionalized nanoparticle species. In some examples the first, second and third oligonucleotides may be the same for each of the nanoparticle species and respective biomarker binding moiety in the first plurality of nanoparticles. Each nanoparticle species can be released from its respective biomarker binding moiety by binding of a third oligonucleotide to the first oligonucleotide with the hybrid formed by hybridization of the third oligonucleotide and the first oligonucleotide exhibiting a melting temperature higher than the melting temperature of the double-stranded nucleic acid formed by hybridization of the first and second oligonucleotide. In other examples, first, second and third oligonucleotides associated with each nanoparticle species and its respective biomarker binding moiety may be different for each nanoparticle species and its respective biomarker binding moiety. For example, in the first plurality of nanoparticles, the second nanoparticle species may comprise a fourth oligonucleotide, its respective biomarker binding moiety may comprise a fifth oligonucleotide, and the displacing oligonucleotide may be a sixth oligonucleotide

[0053] Also disclosed is that each species of functionalized nanoparticle species can be functionalized with a different DNA oligonucleotide releasing system. Also disclosed is that all of the functionalized nanoparticle species in a plurality of functionalized nanoparticle species may be functionalized with the same DNA oligonucleotide releasing system. Also disclosed is that all of the functionalized nanoparticle species used may be functionalized with the same or different DNA oligonucleotide releasing system.

[0054] In some examples, one or more iterations of interrogating cells and detecting biomarkers can be achieved by successive contacts with at least a second, third, up to ten or more plurality of nanoparticle species. In some examples, the one or more iterations of interrogating biomarkers can be one, two, three, four, five, six, seven, eight, nine, ten, fifteen, twenty, thirty, forty, fifty, sixty, seventy, or more times. In examples where oligonucleotide-linker functionalized nanoparticles are used, each plurality of nanoparticle species and respective biomarker binding moiety may comprise the same first, second third oligonucleotide for each oligonucleotide-linker functionalized nanoparticle species in a given plurality of oligonucleotide-linker functionalized nanoparticle species. Alternatively, each oligonucleotide-linker functionalized nanoparticle species and its respective biomarker binding moiety in each plurality of oligonucleotide-linker functionalized nanoparticle species may comprise a unique set of first, second and third oligonucleotides such that each biomarker binding moiety is associated with a unique set of first, second and third oligonucleotides. In this example, from one, to ten or more successive rounds of displacement and contact with a new plurality of oligonucleotide-linker functionalized nanoparticle species can take place.

[0055] In some examples, after releasing the second or previous plurality of functionalized nanoparticles from the biomarker binding moieties, the following steps are performed:

- (i) the biomarkers bound on or in the cell by the biomarker-binding moieties of the functionalized nanoparticles are classified, and
- (ii) the cells are contacted with a next plurality of nanoparticles functionalized with different biomarker binding moieties, where each nanoparticle species of the next plurality of nanoparticles is functionalized with a biomarker binding moiety that binds to a biomarker suspected of being associated with samples or conditions, diseases, or disorders that are also associated with the first biomarker.

Also disclosed is that the methods of this disclosure are useful in detecting whether the associated biomarkers are present on the same or different cell or populations of cells.

[0056] In some examples, the removing a first plurality of functionalized nanoparticles can be achieved by cleaving a linker between the nanoparticle and the biomarker-binding moiety. The linker can comprise a polynucleotide, modified polynucleotide, polyribonucleotide, modified polyribonucleotide, peptide, dextran or glycan. The polynucleotide can comprise a DNA restriction enzyme sequence. The modified polynucleotide can comprise a di-thiol, diol, abasic, or uracil moiety within the polynucleotide sequence.

[0057] In some examples, the linker can comprise a peptide that further comprises a protease sequence. The protease sequence can be a trypsin or chymotrypsin protease recognition sequence. In some examples, the linker can comprise a glycan that further comprises an alpha-fucosidase recognition site. The alpha-fucosidase recognition site can be an alpha-1,2 fucoside bond. Also disclosed is that the linker can be cleaved with a peptidase, DNAase, and/or RNAse.

[0058] In some examples, the substrate can be glass silica, clear polymer, gold, or alumina. The substrate can be functionalized. The substrate functionalization can be patterned. The substrate functionalization can be a silane-linked cell biomarker, polymer-linked cell biomarker, silane-linked amine, silane-linked carboxylic acid, polymer-linked amine, polymer-linked carboxylic acid, polyethylene glycol (PEG), gold, silver, alumina, dextran, or glass silica.

[0059] A combination or kit is described for the detection of a cellular biomarker signature, the combination comprising a plurality of biomarker-binding-moiety-functionalized nanoparticles.

[0060] A combination or kit is described for the detection of a cellular biomarker signature. In some examples, the combination can comprise a plurality of biomarker-binding moiety functionalized nanoparticles where the functionalized nanoparticles can comprise a mixture, or can be segregated. In some examples, the functionalized nanoparticles can further comprise: a nanoparticle functionalized with a first oligonucleotide; a biomarker-binding moiety functionalized with a second oligonucleotide, and the first oligonucleotide is complementary to a portion of the second oligonucleotide, and the first and second oligonucleotide form a hybridized duplex. In an alternative example, the functionalized nanoparticles can further comprise a nanoparticle functionalized with a first

oligonucleotide; a biomarker-binding moiety functionalized with a second oligonucleotide; and a third oligonucleotide, where the first oligonucleotide is complementary to a portion of the third oligonucleotide, the second oligonucleotide is complementary to a separate portion of the third oligonucleotide, and the first and second oligonucleotides form a hybridized duplex to the third oligonucleotide.

[0061] Also disclosed is a kit for the detection of a biomarker signature, the kit comprising a combination comprising a plurality of functionalized nanoparticle species, each comprising a biomarker binding moiety. Also disclosed is that a kit may comprise a plurality of functionalized nanoparticles and an optical contrast agent. The optical contrast agent can be the optical contrast agents described herein.

[0062] Also disclosed is that the kit for the detection of a biomarker signature, comprising a combination comprising a plurality of functionalized nanoparticle species, may also comprise a mountant having substantially the same refractive index as the cells to be imaged. Also disclosed is that the mountant may have a refractive index within 0.1 of the refractive index of the cells to be imaged. The mountant may be any of the mountants described herein. As a non-limiting example, the mountant may have a refractive index of 1.52.

[0063] In some examples, the kit may comprises a plurality of biomarker-binding moiety functionalized nanoparticles where the nanoparticles can comprise mixture, or can be segregated. In some examples, for example, the functionalized nanoparticles can further comprise a functionalized nanoparticle, where the nanoparticle is releasable from the biomarker binding moiety. Also disclosed is that a first nanoparticle species is functionalized with a first oligonucleotide; a biomarker-binding moiety functionalized with a second oligonucleotide, and the first oligonucleotide is complementary to a portion of the second oligonucleotide, and the first and second oligonucleotide form a hybridized duplex. In an alternative example, the functionalized nanoparticles can further comprise a nanoparticle functionalized with a first oligonucleotide; a biomarker-binding moiety functionalized with a second oligonucleotide; and a third oligonucleotide, where the first oligonucleotide is complementary to a portion of the third oligonucleotide, the second oligonucleotide is complementary to a separate portion of the third oligonucleotide, and the first and second oligonucleotides form a hybridized duplex to the third oligonucleotide.

[0064] A kit is described for the detection of a cellular biomarker signature. In some examples, the kit can comprise a plurality of functionalized nanoparticles, an optical contrast agent, and a mountant.

BRIEF DESCRIPTION OF THE FIGURES

[0065] This patent file contains several drawings executed in color. Copies of this patent publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

[0066] **FIG. 1** depicts the general process for the successive displacements of functionalized nanoparticles.

[0067] **FIG. 2** depicts one embodiment for the displacement of a functionalized nanoparticle.

[0068] **FIG. 3** depicts one embodiment for the displacement of a functionalized nanoparticle using a bridging oligonucleotide.

[0069] **FIG. 4** depicts the process for the preparation of the functionalized nanoparticle with a biomarker-binding moiety. In the figure “b” denotes biotin, “SAv” denotes streptavidin.

[0070] **FIGS. 5A and 5B:** Fig 5a depicts the process for the preparation of the functionalized nanoparticle with an oligonucleotide, and the preparation of a functionalized nanoparticle with a biomarker-binding moiety via a displaceable oligonucleotide overlap. Fig 5a. depicts the formation of a functionalized nanoparticle by the coupling of an amine-functionalized oligonucleotide with a carboxylic acid-functionalized nanoparticle in the presence of EDC catalyst. Fig 5a also depicts the formation of a functionalized nanoparticle by the coupling of streptavidin to a carboxylic acid-functionalized nanoparticle in the presence of EDC catalyst, followed by the subsequent coupling of a biotin-functionalized oligonucleotide to the streptavidin-coated nanoparticle. Fig. 5b depicts the formation of a functionalized nanoparticle by the reaction of a maleimide-functionalized oligonucleotide with a thiol (from a cysteine amino acid) on an antibody, followed by hybridization of the oligonucleotide-functionalized antibody to an oligonucleotide-functionalized nanoparticle containing a partial reverse complement sequence to the oligonucleotide sequence connected to the antibody.

CLAIMS

1. An *in vitro* method for detecting the biomarker-morphological profile of a cell, the method comprising:

- (a) providing a sample comprising cells from a subject;
 - (b) contacting the cells with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety, and forming nanoparticle- cell complexes through binding of the functionalized nanoparticle species comprising a biomarker-binding moiety to its respective biomarker;
 - (c) adhering the functionalized nanoparticle-cell complexes to a substrate;
 - (d) illuminating the functionalized nanoparticle-cell complexes with epi-illumination or evanescent light and detecting the resonant light scattering from each observed complexed functionalized nanoparticle, to obtain a biomarker signature of each imaged cell;
 - (e) contacting the substrate-adhered cells with an optical contrast agent;
 - (f) imaging morphological features of the contacted cells; and
 - (g) associating the morphological features of the contacted cells with the biomarker signature of each substrate-adhered cell to detect the biomarker-morphological profile of each cell.
2. The method of claim 1, wherein the biomarker is present on the cell surface.
3. The method of claim 1, wherein the biomarker is present within the cell.
4. The method of claim 1, wherein the biomarker is selected from the following: CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD21, CD22, CD23, CD25, CD30, CD33, CD34, CD38, CD41, C43, CD45, CD56, CD57, CD58, CD61, CD64, C71, CD79a, CD99, CD103, CD117, CD123, CD138, CD138, CD163, CD235a, HLA-DR, Kappa, Lambda, Pax-5, BCL-2, Ki-67, ZAP-70, MPO, TdT, and FMC-7.
5. The method of claim 1, wherein the optical contrast agent is a leuco dye.

74. The method of claim 73, wherein the different conditions are selected from: having a hematological cancer, non-malignant hematological disorder, solid tumor, bladder disease, liver disease, kidney disease, or infectious disease.
75. The method of claim 74, wherein the different conditions are having a different type of solid tumor.
76. The method of claim 1, wherein the biomarker-binding moiety is anti-CD45, and the biomarker signature obtained is the white blood cell count.
77. The method of claim 1, wherein the cells are live.
78. The method of claim 1, wherein the cells are fixed.
79. The method of claim 78, wherein the cells are fixed with formaldehyde.
80. The method of claim 1, wherein the substrate is selected from: glass silica, clear polymer, gold, or alumina.
81. The method of claim 1, wherein the substrate is functionalized.
82. The method of claim 81, wherein the substrate functionalization is patterned.
83. The method of claim 82, wherein the functionalization is a silane-linked cell biomarker, polymer-linked cell biomarker, silane-linked amine, silane-linked carboxylic acid, polymer-linked amine, polymer-linked carboxylic acid, polyethylene glycol (PEG), amino-functionalized dextran, gold, silver, alumina, or glass silica.
84. The method of claim 1, wherein the sample is from blood, bone marrow, fine needle aspirate, or tissue.
85. The method of claim 84, wherein the tissue sample is FFPE (formalin-fixed, paraffin-embedded) tissue samples.
86. The method of claim 84, wherein when the sample is tissue, the optical contrast agent is H&E (hematoxylin and eosin) stain.
87. An *in vitro* method for detecting the biomarker-morphological profile of a cell, the method comprising:
- (a) providing a sample comprising cells from a subject;
 - (b) contacting the cells with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety, and

forming nanoparticle- cell complexes through binding of the nanoparticle species comprising a biomarker-binding moiety to its respective biomarker;

(c) adhering the functionalized nanoparticle-cell complexes to a substrate;

(d) illuminating the functionalized nanoparticle-cell complexes with non-evanescent light and detecting the resonant light scattering from each observed complexed nanoparticle, to obtain a biomarker signature of each observed cell;

(e) contacting the substrate-adhered cells with an optical contrast agent;

(f) imaging morphological features of the contacted cells; and

(g) associating the morphological features of the contacted cells with the biomarker signature of each substrate-adhered cell to detect the biomarker-morphological profile of each cell.

88. The method of claim 87, wherein the non-evanescent light is transmitted light.

89. An *in vitro* method for detecting the biomarker-morphological profile of a cell, the method comprising:

(a) providing a sample comprising cells from a subject;

(b) adhering the cells to a substrate;

(c) contacting the substrate-adhered cells with an optical contrast agent;

(d) imaging morphological features of the contacted cells;

(e) converting the optical contrast agent to a colorless form;

(f) contacting the cells with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety to form nanoparticle- cell complexes;

(g) illuminating the nanoparticle-cell complexes with evanescent light and detecting the resonant light scattering from each observed complexed nanoparticle, to obtain a biomarker signature of each observed cell; and

(h) associating the morphological features of the contacted cells with the biomarker signature of each substrate-adhered cell to detect the biomarker-morphological profile of each cell.

90. The method of claim 89, wherein the optical contrast agent is a leuco dye.
91. The method of claim 90, wherein the leuco dye is methylene blue, methylene green, red leuco dye, crystal violet, phenolphthalein, or thymolphthalein.
92. The method of claim 90, wherein the leuco dye is converted to a colorless form by the addition of one or more electrons to the dye.
93. The method of claim 91, wherein the leuco dye is converted to a colored form by the removal of one or more electrons from the dye.
94. A kit for the detection of a biomarker signature, the combination comprising a plurality of biomarker-binding moiety functionalized nanoparticle species.
95. The kit of claim 94, wherein the functionalized nanoparticle species further comprise:
- (a) a nanoparticle species bound to a first oligonucleotide; and
 - (b) a biomarker-binding moiety bound to a second oligonucleotide,
- wherein the first oligonucleotide is complementary to a portion of the second oligonucleotide, and the first and second oligonucleotide form a hybridized duplex.
96. The kit of claim 94, wherein the functionalized nanoparticle species further comprise:
- (a) a nanoparticle functionalized with a first oligonucleotide;
 - (b) a biomarker-binding moiety functionalized with a second oligonucleotide; and
- a third oligonucleotide,
- wherein the first oligonucleotide is complementary to a portion of the third oligonucleotide, the second oligonucleotide is complementary to a separate portion of the third oligonucleotide, and the first and second oligonucleotides form a hybridized duplex to the third oligonucleotide.
97. A kit for the detection of a biomarker morphological profile, the combination comprising a plurality of functionalized nanoparticle species and an optical contrast agent.
98. The kit of any one of claims 94 or 97, wherein the plurality of functionalized nanoparticle species comprise a mixture.
99. The kit of any one of claims 94 or 97, wherein the plurality of functionalized nanoparticle species are segregated before use.
100. A kit comprising a plurality of functionalized nanoparticle species, and a mountant.

101. The kit of claim 100, wherein the mountant has a refractive index of within 0.1 of the refractive index of fixed cells.
102. The method of claim 56, wherein the mountant has a refractive index of within 0.1 of the refractive index of fixed cells.
103. The kit of any one of claims 100-102, further comprising an optical contrast agent.
104. The method of claim 1, wherein the plurality of nanoparticles exhibit a peak resonance wavelength of the nanoparticle plasmon resonance between 400 to 900 nm.
105. A homogeneous assay for detecting functionalized nanoparticle cell complexes, the assay comprising:
- (a) providing a sample comprising cells from a subject;
 - (b) contacting the cells with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety, and forming functionalized nanoparticle- cell complexes through binding of the nanoparticle species comprising a biomarker-binding moiety to its respective biomarker;
 - (c) adhering the functionalized nanoparticle-cell complexes to a substrate;
 - (d) illuminating the functionalized nanoparticle-cell complexes with evanescent light and detecting the resonant light scattering from each observed complexed nanoparticle, to obtain a biomarker signature of each observed cell,
- wherein unbound functionalized nanoparticles are not removed from the field of view.
106. An *in vitro* method for detecting functionalized nanoparticle cell complexes, the method comprising:
- (a) providing a sample comprising cells from a subject;
 - (b) contacting cells which have been fixed with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety, and forming nanoparticle- cell complexes through binding of the nanoparticle species comprising a biomarker-binding moiety to its respective biomarker;
 - (c) adhering the nanoparticle-cell complexes to a substrate, wherein the adhered functionalized nanoparticle-cell complexes are placed in contact with a mountant,

wherein the refractive index of the mountant is within about 0.1 of the refractive index of the fixed cells;

(d) detecting the functionalized nanoparticle cell complexes by illuminating the nanoparticle-cell complexes with evanescent light and detecting the resonant light scattering from each observed functionalized nanoparticle cell complex, to obtain a biomarker signature of each observed cell; and

(e) associating the biomarker signature of each substrate-adhered cell to a known disease, condition, or state of a cell exhibiting substantially the same biomarker signature to identify the disease, condition, or state of the cell from a subject.

107. The method of claim 106, wherein the mountant has a refractive index of from 1.51 to 1.54.

108. An *in vitro* method for detecting functionalized nanoparticle cell complexes to obtain a biomarker signature, the method comprising:

(a) providing a sample comprising cells from a subject;

(b) contacting the cells with one or a plurality of functionalized nanoparticle species, each functionalized nanoparticle species comprising a biomarker-binding moiety, and forming nanoparticle-cell complexes through binding of the nanoparticle species comprising a biomarker-binding moiety to its respective biomarker;

(c) adhering the functionalized nanoparticle-cell complexes to a substrate;

(d) illuminating the functionalized nanoparticle-cell complexes with evanescent light and detecting the resonant light scattering from each observed complexed nanoparticle, to obtain a biomarker signature of each observed cell; and

(e) associating the biomarker signature of an imaged cell from the subject with a biomarker signature of a reference cell exhibiting substantially the same biomarker signature as the imaged cell biomarker signature, wherein a diagnostic concordance has been established between the reference cell biomarker signature and a disease, disorder, condition or state of the reference subject.

109. The method of any one of claims 1, 87, 89, 105, 106, or 108, wherein the steps of providing a sample comprising cells from a subject, contacting the cells with one or a plurality of functionalized nanoparticle species, and adhering the functionalized nanoparticle-cell complexes to a substrate, are performed by an automated liquid handling system.

110. The method of any one of claims 105, 106, or 108, further comprising obtaining and storing the positional information for each observed cell is stored.
111. The method of any one of claims 1, 87, or 89, further comprising obtaining and storing the positional information for each observed and imaged cell is stored.
112. The method of claim 1, wherein the nanoparticle preparations are of a narrow size distribution, such that an individual nanoparticle preparation has a scattering spectrum whose full-width half maximum ranges from 5 to 150 nm.
113. The method of claim 1, wherein the functionalized nanoparticle is present on the cell surface.
114. The method of claim 1, wherein the functionalized nanoparticle is present within the cell.