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(54) Title: PHOTOTHERMAL DETECTION

(57) Abstract: The present invention provides a system for in-vivo imaging a tissue with gold nanoparticles inserted therein, the system includes: a composition of cellular gold nanoparticles; and a thermal imaging camera configured to in-vivo image the tissue. The invention further provides a method for in-vivo imaging a tissue or a portion thereof by utilizing the system of the invention.

PHOTOTHERMAL DETECTION

FIELD OF THE INVENTION

The invention relates to photothermal detection of cell clusters utilizing gold nanoparticles and to a system which includes a thermal imaging camera, laser irradiating device, and gold nanoparticles.

BACKGROUND

Histopathologic evaluation of surgical specimens is a well established technique for disease identification, and has remained relatively unchanged since its clinical introduction. Although it is essential for clinical investigation, histopathologic identification of tissues remains a time consuming and subjective technique, with unsatisfactory levels of inter- and intra-observer discrepancy.

Cancer is a leading cause of death worldwide. One of the critical problems in cancer management is local cancer recurrence. Between 20-30 percent of patients who undergo tumor resection surgery require re-operation because of incomplete excision. Currently, tumor margins are examined using conventional histological tests which are being mostly performed as a post-surgical procedure. During the last decade, few methods for real time intra-operative tumor margin detection were developed with relative success, such as diffusive reflectance methods radiofrequency based detection and targeted fluorescent imaging.

However, there is still an urgent need to develop a highly specific and sensitive intra-operative real time tumor margin detection method, which will reduce reoperation and cancer recurrence.

During the last decade, much research has focused on developing nanoparticles which enhance scattering or absorption in the near infrared (NIR), because of the relatively high transmittance of tissue in that region. Particles such as fluorescent dyes, gold nanoshells and gold nanoparticles (GNP) were utilized either as diagnostic tool or as photothermal therapy mediated agents.

As a diagnostic tool, GNP gained much attention due to their biocompatibility, their relatively easy fabrication and bioconjugation with biomolecules for targeting, and their unique optical properties; they have an enhanced optical extinction

coefficient as compared to conventional fluorescent dyes, and by changing their size and aspect ratio, their surface plasmon resonance (SPR) wavelength and absorption to scattering coefficient ratio can be tuned and controlled. Therefore, nanoparticles have been vastly utilized as NIR, photoacoustic, Raman scattering and diffusion reflection imaging contrast agents.

In addition to research regarding early detection of cancer, GNP were utilized as photothermal therapy mediated agents. In those studies, the absorption properties of GNP in the SPR wavelength are used to elevate temperature to 50°C and above, usually by using lasers with high optical outputs (above 10W/cm^2) for about 10 minutes, in order to achieve effective denaturation and coagulation of cellular proteins and cell death.

The foregoing examples of the related art and limitations related therewith are intended to be illustrative and not exclusive. Other limitations of the related art will become apparent to those of skill in the art upon a reading of the specification and a study of the figures.

SUMMARY

In one embodiment, the present invention provides a method for in-vivo imaging a tissue, comprising the steps of: (a) introducing a composition comprising gold nanoparticles into the tissue; (b) selectively increasing the temperature of the nanoparticles by up to 5°C comprising irradiating the tissue by laser; and (c) in-vivo imaging the tissue comprising the nanoparticles by a thermal imaging camera, thereby imaging a tissue. In one embodiment, irradiating the tissue by laser is irradiating at 500 to 900nm wavelength. In one embodiment, irradiating is irradiating at 10 to 100 joules per square cm (J/cm^2). In one embodiment, irradiating is irradiating at 1 to 10 Watts per square cm (W/ cm^2) for about 1-10 seconds.

In one embodiment, the laser operates in pulsed beam having temporal width less than or equal to 0.8 psec. In another embodiment, the method further includes interfering the back scattered light with a reference, wherein the reference is the reference used for irradiating the tissue.

In another embodiment, the present invention further provides that the gold nanoparticles comprise a gold nucleus coated with thiol-polyethylene-glycol (SH-PEG-COOH) and a heterofunctional polyethylene-glycol.

In another embodiment, the present invention further provides that thermal imaging camera comprises temperature sensitivity of at least 0.1 degree Celsius, spatial resolution of 0.9 mm to 0.1 mm, thermal radiation sensitivity at a wavelength range of 8 to 14 μm, or any combination thereof.

In another embodiment, the present invention further provides that the tissue comprises cancerous cells.

In another embodiment, the present invention further provides a system for in-vivo imaging a tissue comprising gold nanoparticles, comprising: (a) a composition comprising cellular gold nanoparticles; (b) an irradiation device configured to irradiate at 10 to 100 joules per square cm (J/cm^2); and (c) a thermal imaging camera configured to in-vivo image the tissue comprising the nanoparticles.

BRIEF DESCRIPTION OF THE FIGURES

Exemplary embodiments are illustrated in referenced figures. Dimensions of components and features shown in the figures are generally chosen for convenience and clarity of presentation and are not necessarily shown to scale. The figures are listed below.

Figure 1: Optical setup scheme. The laser radiation raises the temperature by **ΔT** and the thermal camera read the temperature field by sensing the thermal radiation in the long IR region.

Figure 2: A graph showing the absorbance spectra of GNS (line) and GNR (dashed line) (2A). Micrographs showing TEM image of 30 nm GNS (2B) and 25 nm X 65 nm GNR (2C, scale bar 100nm).

Figure 3: Is a graph showing the temperature elevation as a function of the irradiation time with 663nm laser, for different concentration of GNR in aqueous solution. The graph reaches a plateau after approximately 5 seconds.

Figure 4: Are graphs showing temperature difference as a function of the concentration of GNS (4A) and GNR (4B), for the two lasers. The temperature is defined after 5 seconds of laser irradiation, when the temperature curves have reached a plateau. The laser optical intensity on the sample was $10W/cm^2$ for both lasers.

Figure 5: Are thermal images of a sample made of two halves of cylindrical solid phantoms joint together, one with GNR and the other without. Image (5A) was taken before the laser incitation. The border in-between the phantoms are shown by a dashed strait line. The elliptical contour shows the place where the laser beam aims to be on the sample. Images (5B) and (5C) were taken after 10 seconds and five minutes of laser irradiance, respectively. It can be seen that the temperature of the part of the sample with the GNR, rise up compare to that of the part without GNR.

Figure 6: Is a graph showing temperature elevation of GNR bioconjugated to A431 cancer cell line (GNR-A431) compared to A431 cells without GNR, in solution. It can be seen that the bioconjugated GNR-A431 has a distinct heating profile compared to the A431 cells.

Figure 7: Is a schematic sketch of a proposed configuration.

DETAILED DESCRIPTION

In one embodiment, the present invention provides a method for in-vivo imaging a tissue in a subject in need thereof, comprising the steps of: (a) introducing gold nanoparticles to the tissue; (b) selectively increasing the temperature of the nanoparticles by 1-7^{O}C comprising irradiating the tissue by laser; and (c) in-vivo imaging the tissue comprising the nanoparticles by a thermal imaging camera, thereby imaging a tissue.

In another embodiment, nanoparticles as described herein can be of any shape known to one of skill in the art. In another embodiment, nanoparticles are nanorods. In another embodiment, nanoparticles are nanospheres. In another embodiment, nanoparticles of the present invention are gold nanospheres (GNS). In another embodiment, nanoparticles of the present invention comprise a diameter of up to 90 nm. In another embodiment, nanoparticles of the present invention comprise a diameter of up to 80 nm. In another embodiment, nanoparticles of the present invention comprise a diameter of up to 70 nm. In another embodiment, nanoparticles of the present invention comprise a diameter of up to 60 nm. In another embodiment, nanoparticles of the present invention comprise a diameter of up to 50 nm. In another embodiment, nanoparticles of the present invention comprise a diameter of up to 40 nm. In another embodiment, nanoparticles of the present invention comprise a diameter of up to 30 nm. In another embodiment, nanoparticles of the present

invention comprise a diameter of 15-100 nm. In another embodiment, nanoparticles of the present invention comprise a diameter of 15-90 nm. In another embodiment, nanoparticles of the present invention comprise a diameter of 20-80 nm. In another embodiment, nanoparticles of the present invention comprise a diameter of 20-60 nm.

In another embodiment, the method of the present invention provides in-vivo tissue or tumor targeting using a passive targeting mechanism known as enhanced permeability and retention (EPR). In another embodiment, the present invention provides that a pathological tissue such as a tumor or a degenerative tissue, comprise leaky vasculature which enables systemically circulating nanoparticles to extravasate and accumulate. In another embodiment, the method of the present invention provides in-vivo tissue or tumor active targeting which exploits, for example, the over-expression of surface receptors on cancer cells by providing targeting ligands that can engage these receptors. In another embodiment, the targeting ligand or molecule is an antibody. In another embodiment, the targeting ligand or molecule is anti-VEGF antibody. In another embodiment, the targeting ligand or molecule is anti-EGFR antibody. In another embodiment, the targeting ligand is any protein targeting a cancer cell.

In another embodiment, gold nanoparticles comprise a gold nucleus. In another embodiment, gold nanoparticles comprise a gold nucleus coated with a biocompatible polymer. In another embodiment, gold nanoparticles comprise a gold nucleus coated with a non-toxic polymer. In another embodiment, gold nanoparticles comprise a gold nucleus coated with a non-toxic polymer that prevents agglomeration. In another embodiment, gold nanoparticles comprise a gold nucleus coated with a non-toxic polymer utilized as anchoring means for selective targeting molecules such as but not limited to antibodies. In another embodiment, gold nanoparticles comprise a gold nucleus coated with a biopolymer. In another embodiment, gold nanoparticles are pegylated gold nanoparticles. In another embodiment, the selective targeting molecules are covalently conjugated to the coating.

In another embodiment, gold nanoparticles comprise a coating aimed at increasing the in vivo tumor targeting competence. In another embodiment, gold nanoparticles of the present invention comprise optical and thermal properties that make them effective contrast and photo-thermal agents. In another embodiment, gold nanoparticles of the present invention are neutral or slightly negatively charged. In

another embodiment, gold nanoparticles of the present invention are slightly positively charged.

In another embodiment, the coating layer of the gold nanoparticles comprises thiol-polyethylene-glycol, a heterofunctional polyethylene-glycol, or a combination thereof. In another embodiment, thiol-polyethylene-glycol is about 5 kDa. In another embodiment, the coating layer of the gold nanoparticles comprises at least 65% thiol-polyethylene-glycol. In another embodiment, the coating layer of the gold nanoparticles comprises at least 70% thiol-polyethylene-glycol. In another embodiment, the coating layer of the gold nanoparticles comprises at least 75% thiol-polyethylene-glycol. In another embodiment, the coating layer of the gold nanoparticles comprises at least 80% thiol-polyethylene-glycol. In another embodiment, the coating layer of the gold nanoparticles comprises at least 85% thiol-polyethylene-glycol. In another embodiment, the coating layer of the gold nanoparticles comprises at least 90% thiol-polyethylene-glycol.

In another embodiment, heterofunctional polyethylene-glycol is about 3.4 kDa. In another embodiment, the coating layer of the gold nanoparticles comprises up to 35% heterofunctional polyethylene-glycol. In another embodiment, the coating layer of the gold nanoparticles comprises up to 30% heterofunctional polyethylene-glycol. In another embodiment, the coating layer of the gold nanoparticles comprises up to 25% heterofunctional polyethylene-glycol. In another embodiment, the coating layer of the gold nanoparticles comprises up to 20% heterofunctional polyethylene-glycol. In another embodiment, the coating layer of the gold nanoparticles comprises up to 15% heterofunctional polyethylene-glycol.

In another embodiment, gold nanoparticles of the invention are functionalized with hybrid biomolecules. In another embodiment, gold nanoparticles are conjugated with tumor-avid peptides or other biomolecules. In another embodiment, gold nanoparticles are labeled. In another embodiment, the term "labeling" is intended to be broadly interpreted, and may include attachment or conjugation.

In another embodiment, gold nanoparticles have high reactivity with sulfhydryl (SH) groups; example methods of the invention utilize a biomolecule having a sulfhydryl group. In another embodiment, cysteine is used to label gold nanoparticles according to methods known to one of skill in the art.

In another embodiment, the invention includes conjugating of the gold nanoparticles with a biomolecule such as a protein to develop a viable gold nanoparticle labeling approach for potential applications such as labeling target specific biological proteins or peptides with gold nanoparticles. In another embodiment, gold nanoparticles are directed to tumor sites for potential applications in the development of cancer diagnostic/therapeutic agents. In another embodiment, methods of the invention include steps of conjugating gold nanoparticles with a peptide. In another embodiment, conjugating to link gold nanoparticles with a tumor-avid peptide are beneficial and advantageous for the design and development of cancer specific diagnostic agent.

In another embodiment, conjugation protocols for labeling nanoparticles of gold are known in the art. In another embodiment, methods of the invention include introduction of additional spacer functions (for example, in the form of 5-aminopentanoic acid) to the N-terminal region of a peptide to avoid interference of the chelating moiety with the receptor binding C-terminus of the peptide. In another embodiment, steps that maximize binding of gold nanoparticle labeled with a peptide with receptors over expressed on cancer cells are used.

In another embodiment, the term "tissue" includes a cluster of cells. In another embodiment, the term "tissue" includes an aggregate of cells in an organism that have similar structure and function. In another embodiment, tissue is an epithelial tissue, a nerve tissue, a connective tissue, a muscle, a vascular tissue, a cancerous tissue, a diseased tissue, or a pathological tissue. In another embodiment, the term "tissue" includes a cluster of cells of at least 2 mm in size. In another embodiment, the term "tissue" includes a cluster of cells of at least 5 mm in size. In another embodiment, the term "tissue" includes a cluster of cells of at least 8 mm in size. In another embodiment, the term "tissue" includes a cluster of cells of at least 10 mm in size. In another embodiment, the term "tissue" includes a cluster of cells of at least 15 mm in size.

In another embodiment, a diseased tissue or a pathological tissue includes cellular abnormalities used as disease indicators. In another embodiment, a diseased tissue or a pathological tissue is a disorganized tissue such as but not limited to a neoplastic tissue, degenerative tissue, or a hypoxic tissue. In another embodiment, a diseased tissue or a pathological tissue is characterized by elevated cell death or

apoptosis. In another embodiment, a diseased tissue or a pathological tissue is an injured tissue. In another embodiment, a diseased tissue or a pathological tissue is a damaged tissue. In another embodiment, a diseased tissue or a pathological tissue is an inflamed tissue. In another embodiment, a diseased tissue or a pathological tissue is a tissue undergoing degeneration.

In another embodiment, the method and system of the present invention provide a long desired, non-invasive, fast solution for imaging a tissue or a portion thereof. In another embodiment, the method and system of the present invention provide a long desired, non-invasive, fast solution for imaging a tissue or a portion thereof during a medical procedure. In another embodiment, the method and system of the present invention provide a long desired, non-invasive, fast solution for imaging a tissue or a portion thereof during surgery. In another embodiment, the method and system of the present invention provide a long desired, non-invasive, fast solution for imaging a tumor or tumor margins. In another embodiment, the method and system of the present invention provide a long desired, non-invasive, fast solution for imaging metastasis. In another embodiment, the method and system of the present invention provide a long desired, non-invasive, fast solution for imaging clusters of inflammatory and/or immune cells. In another embodiment, the method and system of the present invention provide a long desired, non-invasive, fast solution for imaging disorganized, pathological cellular structures such as but not limited to the CNS in Alzheimer's disease or muscle atrophy.

In another embodiment, the term "introducing" is injecting the nanoparticles to the tissue. In another embodiment, the term "introducing" is injecting the nanoparticles to the tissue's surrounding. In another embodiment, the term "introducing" is systemically introducing the nanoparticles. In another embodiment, the term "introducing" is administering the nanoparticles according to the desired medical procedure.

In another embodiment, selectively increasing the temperature of the nanoparticles is selectively increasing the temperature of the nanoparticles present within the target tissue. In another embodiment, selectively increasing the temperature of the nanoparticles is selectively increasing the temperature of the nanoparticles present within the cells or bound to the cells or to the target tissue. In another embodiment, selectively increasing the temperature of the nanoparticles is by 1-7^{O}C.

In another embodiment, selectively increasing the temperature of the nanoparticles is by 1-5^{O}C. In another embodiment, selectively increasing the temperature of the nanoparticles is by 1-4^{O}C. In another embodiment, selectively increasing the temperature of the nanoparticles is by 2-5^{O}C. In another embodiment, selectively increasing the temperature of the nanoparticles is by 2-4^{O}C.

In another embodiment, selectively increasing the temperature lasts 1-40 seconds. In another embodiment, selectively increasing the temperature lasts 10-35 seconds. In another embodiment, selectively increasing the temperature lasts 1-20 seconds. In another embodiment, selectively increasing the temperature lasts 1-15 seconds. In another embodiment, selectively increasing the temperature lasts 2-12 seconds. In another embodiment, selectively increasing the temperature lasts 2-10 seconds. In another embodiment, selectively increasing the temperature lasts 2-8 seconds. In another embodiment, selectively increasing the temperature lasts 3-6 seconds.

In another embodiment, selectively increasing the temperature is irradiating at a wavelength of 500 to 900nm. In another embodiment, selectively increasing the temperature is irradiating at a wavelength of 530 to 900nm. In another embodiment, selectively increasing the temperature is irradiating at a wavelength of 650 to 900nm. In another embodiment, selectively increasing the temperature is irradiating at a wavelength of 650 to 850nm. In another embodiment, selectively increasing the temperature is irradiating at a wavelength of 650 to 750nm. In another embodiment, selectively increasing the temperature is irradiating at a wavelength of 700 to 850nm.

In another embodiment, irradiating is irradiating for 1-15 seconds. In another embodiment, irradiating is irradiating for 2-12 seconds. In another embodiment, irradiating is irradiating for 2-10 seconds. In another embodiment, irradiating is irradiating for 2-8 seconds. In another embodiment, irradiating is irradiating for 3-6 seconds.

In another embodiment, irradiating is irradiating at 1 to 100 joules per square cm (J/cm^2). In another embodiment, irradiating is irradiating at 10 to 50 J/cm^2. In another embodiment, irradiating is irradiating at 20 to 70 J/cm^2. In another embodiment, irradiating is irradiating at 30 to 80 J/cm^2. In another embodiment, irradiating is irradiating at 20 to 40 J/cm^2.

In another embodiment, irradiating is irradiating at 1 to 20 Watts per square cm (W/ cm^2). In another embodiment, irradiating is irradiating at 1 to 10 W/ cm^2. In another embodiment, irradiating is irradiating at 1 to 5 W/ cm^2. In another embodiment, irradiating is irradiating at 2 to 8 W/ cm^2. In another embodiment, irradiating is irradiating at 3 to 6 W/ cm^2.

In another embodiment, irradiating is laser irradiating. In another embodiment, the laser irradiation is two-photon laser irradiation. In another embodiment, the laser is a swept laser source. In another embodiment, the laser source comprises a polygon-based tunable filter. In another embodiment, the laser source has a sweeping range of at least 100 nm centered at about 1300 nm. In another embodiment, the sweeping range is of at least 70 nm centered at 1000 to 1500 nm. In another embodiment, the laser is pulsed laser.

In another embodiment, laser is a a red diode laser. In another embodiment, laser is a green Nd:YAG diode.

In another embodiment, the laser source has an axial resolution of 3-25 μm in a tissue. In another embodiment, the laser source has an axial resolution of 3-10 μm in a tissue. In another embodiment, the laser source has an axial resolution of 6-12 μm in a tissue. In another embodiment, the laser source has an axial resolution of 7-9 μm in a tissue.

In another embodiment, the laser source has an average output power of 30-80 mW. In another embodiment, the laser source has an average output power of 30-50 mW. In another embodiment, the laser source has an average output power of 45-55 mW.

In another embodiment, the laser source is a Cladding-pumped Fiber Laser. In another embodiment, the laser source is a diode-laser-based.

In another embodiment, the imaging scan area is 1×1 mm^2 to 20×20 mm^2. In another embodiment, the imaging scan area is 3×3 mm^2 to 10×10 mm^2. In another embodiment, the imaging scan area is 6×6 mm^2 to 10×10 mm^2.

In another embodiment, the imaging depth in tissue is 0.2 to 5 mm. In another embodiment, the imaging depth in tissue is 0.5 to 5 mm. In another embodiment, the imaging depth in tissue is 0.5 to 3 mm. In another embodiment, the imaging depth in tissue is 1 to 3 mm. In another embodiment, the imaging depth in tissue is 0.5 to 5 mm.

In another embodiment, in-vivo imaging a tissue is non-invasive imaging. In another embodiment, in-vivo imaging is performed utilizing a thermal imaging device. In another embodiment, a thermal imaging device is a thermal imaging camera.

In another embodiment, the present invention utilizes the absorption properties of GNPs and not their scattering properties. In another embodiment, the present invention provides an imaging technique utilizing the absorption properties of GNPs rather than their scattering properties, leading to high contrast between labeled structures (such as targeted cancer cells) and normal background tissue.

In another embodiment, a thermal imaging device comprises temperature sensitivity of at least 0.8 degree Celsius. In another embodiment, a thermal imaging device comprises temperature sensitivity of at least 0.6 degree Celsius. In another embodiment, a thermal imaging device comprises temperature sensitivity of at least 0.5 degree Celsius. In another embodiment, a thermal imaging device comprises temperature sensitivity of at least 0.3 degree Celsius. In another embodiment, a thermal imaging device comprises temperature sensitivity of at least 0.1 degree Celsius.

In another embodiment, a thermal imaging device comprises spatial resolution of at least 2 mm. In another embodiment, a thermal imaging device comprises spatial resolution of at least 1.5 mm. In another embodiment, a thermal imaging device comprises spatial resolution of at least 1 mm. In another embodiment, a thermal imaging device comprises spatial resolution of at least 0.8mm. In another embodiment, a thermal imaging device comprises spatial resolution of at least 0.6 mm. In another embodiment, a thermal imaging device comprises spatial resolution of at least 0.5 mm. In another embodiment, a thermal imaging device comprises spatial resolution of at least 0.3 mm. In another embodiment, a thermal imaging device comprises spatial resolution of at least 0.2 mm. In another embodiment, a thermal imaging device comprises spatial resolution of 1 to 0.1 mm.

In another embodiment, a thermal imaging device comprises radiation sensitivity at a wavelength range of 1 to 20 μm. In another embodiment, a thermal imaging device comprises radiation sensitivity at a wavelength range of 2 to 16 μm. In another embodiment, a thermal imaging device comprises radiation sensitivity at a wavelength

range of 4 to 12 μm. In another embodiment, a thermal imaging device comprises radiation sensitivity at a wavelength range of 5 to 10 μm.

In another embodiment, a thermal imaging device comprises magnification of up to x2 to x100. In another embodiment, a thermal imaging device comprises magnification of x2 to x50. In another embodiment, a thermal imaging device comprises magnification of x10 to x50. In another embodiment, a thermal imaging device comprises magnification of up to x100. In another embodiment, a thermal imaging device comprises magnification of up to x80. In another embodiment, a thermal imaging device comprises magnification of up to x50. In another embodiment, a thermal imaging device comprises magnification of up to x40. In another embodiment, a thermal imaging device comprises magnification of up to x25.

In another embodiment, the invention further provides analysis of data including the extraction of the non scattered data via interference. In another embodiment, the invention further provides analysis of data in scattering resonance of the nanoparticles rather than only in absorption resonance. In another embodiment, image analysis programs that reduce interference are utilized according to the method and system of the invention.

In another embodiment, the present invention further provides a system for in-vivo imaging a tissue comprising gold nanoparticles, comprising (a) a composition comprising cellular gold nanoparticles; (b) an irradiation device configured to irradiate at 10 to 100 joules per square cm (J/cm^2); and (c) a thermal imaging camera configured to in-vivo image the tissue comprising the nanoparticles. In another embodiment, cellular gold nanoparticles are nanoparticles that can be engulfed by a cell. In another embodiment, cellular gold nanoparticles are nanoparticles that can be internalized by a cell. In another embodiment, cellular gold nanoparticles are intracellular gold nanoparticles. In another embodiment, cellular gold nanoparticles are nanoparticles that are capable of binding a cell. In another embodiment, cellular gold nanoparticles are nanoparticles that are capable of binding an extra-cellular matrix component. In another embodiment, cellular gold nanoparticles are cellular nanoparticles. In another embodiment, cellular gold nanoparticles are attached to a cell. In another embodiment, cellular gold nanoparticles are attached to a specific molecule present on the cell's surface cell. In another embodiment, cellular gold nanoparticles are intracellular particles present within the cell's cytoplasm.

In another embodiment, the present invention further provides a kit for in-vivo imaging a tissue wherein the kit comprises (a) a composition comprising cellular gold nanoparticles; (b) an irradiation device configured to irradiate at 10 to 100 joules per square cm (J/cm^2); (c) a thermal imaging device configured to in-vivo image the tissue comprising the nanoparticles (d) an instruction manual for: (1) administering the nanoparticles to a subject; (2) operating the irradiating device and the thermal imaging device.

In another embodiment, methods of the present invention enable an accurate photo-thermal detection of cancerous tumor margins. In another embodiment, this detection allows to in-vivo distinguish between cancerous and non-cancerous cells within a target tissue, for example during, before, or after a surgical tumor excision procedure. Then, advantageously, the cancerous cells are excised in a relatively accurate manner.

To facilitate the detection, nano-particles, such as gold nano-particles (GNPs), are introduced into the target tissue. In some embodiments, the nanoparticles are free of specific targeting molecules. In some embodiments, nanoparticles free of specific targeting molecules are primarily internalized by cancerous cells. In some embodiments, the nanoparticles comprise a targeting molecule which facilitates the specific association between the nanoparticle and a cell comprising the specific ligand recognizing the targeting molecule.

In some embodiments, the concentration of the nanoparticles within the tumor, as opposed to its non-cancerous surroundings, will be relatively high. In some embodiments, the concentration of the nanoparticles within the tumor, as opposed to its non-cancerous surroundings, will be at least x2. In some embodiments, the concentration of the nanoparticles within the tumor, as opposed to its non-cancerous surroundings, will be at least x3. In some embodiments, the concentration of the nanoparticles within the tumor, as opposed to its non-cancerous surroundings, will be at least x4. In some embodiments, the concentration of the nanoparticles within the tumor, as opposed to its non-cancerous surroundings, will be at least x5. In some embodiments, the concentration of the nanoparticles within the tumor, as opposed to its non-cancerous surroundings, will be at least x7. In some embodiments, the

concentration of the nanoparticles within the tumor, as opposed to its non-cancerous surroundings, will be at least x10. In some embodiments, the concentration of the nanoparticles within the tumor, as opposed to its non-cancerous surroundings, will be at least x25. In some embodiments, the concentration of the nanoparticles within the tumor, as opposed to its non-cancerous surroundings, will be at least x50.

In one embodiment, the difference in nanoparticles concentrations between a cluster of diseased cells and healthy cells is readily visible according to the methods disclosed herein. The target tissue is then irradiated for few seconds, for example using laser, so as to selectively raise the temperature of the nano-particles. An infrared and/or near-infrared imaging device may be used to receive photons emitted from the nano-particles, thereby precisely detecting the margins of the cancerous tumor.

Advantageously, the detection is conducted whilst the temperature of the target tissue is slightly elevated (1-5^{O}C). This feature prevents or at least mitigates damage to the irradiated non-cancerous tissue or to the surrounding tissue.

In some embodiments, the target tissue is heated to a temperature of approximately (namely, ±5%) 40°C or less, such that cell viability of non-diseased (such as cancerous) cells is not affected or is minimally affected.

Some embodiments utilize a photothermal imaging technique which overcomes the inevitable background signal caused by light scattering from the target tissue. This imaging technique optionally uses the absorption properties of the nano-particles rather than their scattering properties, which scattering properties are known to suffer from relatively high background noise and low contrast. By utilizing, in some embodiments, only the absorption properties of the nano-particles, higher contrast between the targeted cancer cells and normal background tissue can be achieved.

In some embodiments, the photothermal imaging is combined with photothermal therapy, instead of or in addition to surgical excision of the tumor or parts thereof. Irradiation of the target tissue, for example using laser, may selectively destruct the cancer cells that were targeted with GNPs. Advantageously, this photothermal therapy may be conducted within the same clinical setting as the photothermal imaging, namely – in the same surgical procedure.

In some embodiments, the method and system of the invention include a new optical configuration allowing to image in high resolution clusters of cells and or

borders of a tumor, in-vivo, in a non-invasive manner. In some embodiments, the method and system of the invention include heating the nanoparticles of the invention with pulsed laser. In some embodiments, imaging is performed with infra-red camera. In some embodiments, the invention further provides means for avoiding blurring the tissue structure or the border of a tumor due to the scattering of the tissue. In some embodiments, the invention further provides extracting the first arriving photons scattered from the inspected tissue. In some embodiments, scattering of light in a tissue is provided in example 4.

In another embodiment, a femto second laser is used such that the temporal width of its pulses is less than 0.8 [psec] thus even the first scattering photons are filtered out and only the first arriving light i.e. the photons that passed the tissue without being scattered at all arrive the camera/detector. In another embodiment, a femto second laser is used such that the temporal width of its pulses is less than 0.6 [psec] thus even the first scattering photons are filtered out and only the first arriving light i.e. the photons that passed the tissue without being scattered at all arrive the camera/detector. In another embodiment, a femto second laser is used such that the temporal width of its pulses is less than 0.5 [psec] thus even the first scattering photons are filtered out and only the first arriving light i.e. the photons that passed the tissue without being scattered at all arrive the camera/detector. In another embodiment, a femto second laser is used such that the temporal width of its pulses is less than 0.44 [psec] thus even the first scattering photons are filtered out and only the first arriving light i.e. the photons that passed the tissue without being scattered at all arrive the camera/detector. In another embodiment, the first arriving light photons are the one that have the high spatial resolution and they are separated from the photons that are spatially blurred due to multiple scattering effects.

In another embodiment, the nanoparticles illuminating the tissue or the tumor from a distance and through other body tissues (such as the skin) using the pulsed laser configuration at the absorption resonance of the nanoparticles, results in controlled heating of the nanoparticles. In another embodiment, 0.5-7^{O}C degrees heating and inspecting the nanoparticles with infra red (IR) camera allows high quality detection of the boundaries of the tissue/tumor.

In another embodiment, as the tissue/tumor is internal (body wise) the IR photons are scattered on their way back to the detector/camera and thus although the heating

process of the nanoparticles is used to detect the boundaries, at reality the boundaries are blurred due to this scattering process. In another embodiment, proper adaptation of the irradiation/illumination and detection scheme is performed to overcome this drawback.

In another embodiment, a pulsed beam having temporal width less than 0.4 psec is generated and then interfering the back scattered light with the reference that was also used for the illumination/irradiation of the tissue (see Fig. 7). In another embodiment, the first detection (arriving light information) is extracted not only via pulsed laser and holography/interference but also by sending non-pulsed light having very short coherence length corresponding to the temporal length mentioned above (less than 0.4 psec). In another embodiment, a non pulsed broad band source (to have sufficiently short coherence length) is used and the extracted energy is very low and the SNR of the captured image is not sufficient.

In another embodiment, nanoparticles are intravenously injected in doses of 1-50 μL/g of nanoparticles (nanoparticles: 2.5×10^8 -2.5×10^{12} particles/μL). In another embodiment, nanoparticles are administered in a single dose. In another embodiment, nanoparticles are administered in multiple-doses. In another embodiment, nanoparticles are administered in 1-10 doses. In another embodiment, nanoparticles are administered in 3-6 doses. In another embodiment, nanoparticles are administered once a day. In another embodiment, nanoparticles are administered once every 2 days. In another embodiment, nanoparticles are administered twice a day.

In one embodiment, a composition comprising nanoparticles is a "pharmaceutical composition" and includes chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of the nanoparticles to an organism.

In one embodiment, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases. In one embodiment, one of the ingredients included in the pharmaceutically acceptable carrier can be for example polyethylene

glycol (PEG), a biocompatible polymer with a wide range of solubility in both organic and aqueous media (Mutter et al. (1979).

In one embodiment, "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of the nanoparticles. In one embodiment, excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs are suitable for the nanoparticles and are found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

In one embodiment, suitable routes of administration, for example, include oral, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

Peroral compositions, in some embodiments, comprise liquid solutions, emulsions, suspensions, and the like. In some embodiments, pharmaceutically-acceptable carriers suitable for preparation of such compositions are well known in the art. In some embodiments, liquid oral compositions comprise from about 0.0012% to about 0.933% of the nanoparticles, or in another embodiment, from about 0.033% to about 0.7%.

In some embodiments, compositions for use in the methods of this invention comprise solutions or emulsions, which in some embodiments are aqueous solutions or emulsions comprising a safe and effective amount of the nanoparticles of the present invention.

In another embodiment, the pharmaceutical compositions are administered by intravenous, intra-arterial, or intramuscular injection of a liquid preparation. In some embodiments, liquid formulations include solutions, suspensions, dispersions, emulsions, oils and the like. In one embodiment, the pharmaceutical compositions are administered intravenously, and are thus formulated in a form suitable for intravenous administration. In another embodiment, the pharmaceutical compositions are administered intra-arterially, and are thus formulated in a form suitable for intra-arterial administration. In another embodiment, the pharmaceutical compositions are

administered intramuscularly, and are thus formulated in a form suitable for intramuscular administration.

In one embodiment, pharmaceutical compositions of the present invention are manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

In one embodiment, pharmaceutical compositions for use in accordance with the present invention is formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. In one embodiment, formulation is dependent upon the route of administration chosen.

In one embodiment, injectables, of the invention are formulated in aqueous solutions. In one embodiment, injectables, of the invention are formulated in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. In some embodiments, for transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

In one embodiment, the preparations described herein are formulated for parenteral administration, e.g., by bolus injection or continuous infusion. In some embodiments, formulations for injection are presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. In some embodiments, compositions are suspensions, solutions or emulsions in oily or aqueous vehicles, and contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Also comprehended by the invention are particulate compositions coated with polymers (e.g. poloxamers or poloxamines) and the compound coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors.

In some embodiments, preparation of effective amount or dose can be estimated initially from in vitro assays. In one embodiment, a dose can be formulated in animal

models and such information can be used to more accurately determine useful doses in humans.

In one embodiment, the dosages vary depending upon the dosage form employed and the route of administration utilized.

In one embodiment, compositions of the present invention are presented in a pack or dispenser device, such as an FDA approved kit, which contain one or more unit dosage forms containing the nanoparticles. In one embodiment, the pack, for example, comprise metal or plastic foil, such as a blister pack. In one embodiment, the pack or dispenser device is accompanied by instructions for administration. In one embodiment, the pack or dispenser is accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, in one embodiment, is labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert.

In the description and claims of the application, each of the words “comprise” “include” and “have”, and forms thereof, are not necessarily limited to members in a list with which the words may be associated. In addition, where there are inconsistencies between this application and any document incorporated by reference, it is hereby intended that the present application controls.

As used herein, the singular forms "a", "an", and "the" include plural forms unless the context clearly dictates otherwise. Thus, for example, reference to "a therapeutic agent" includes reference to more than one therapeutic agent.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive.

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to."

As used herein, the terms "comprises," "comprising," "containing," "having" and the like can have the meaning ascribed to them in U.S. patent law and can mean "includes," "including," and the like; "consisting essentially of or "consists essentially" likewise has the meaning ascribed in U.S. patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel

characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments. In another embodiment, the term "comprise" includes the term "consist".

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXPERIMENTAL RESULTS

Materials and Methods

Nanoparticle synthesis and characterization

Gold nanospheres (GNS) with diameter of 30 nm, were synthesized using Sodium Citrate, according to the methodology described by Enüstün & Turkevich (B. V. Enüstün JT. Coagulation of Colloidal Gold. *J. Am. Chem. Soc.* 1963;85 (21):3317–3328) GNR (25 nm X 65 nm) were synthesized using the seed mediated growth method. Particle size, shape and uniformity were measured using transmission electron microscopy (TEM). For the bioconjugation process, a protective layer of polyethylene-glycol (PEG) was adsorbed on the surface of the GNS in order to prevent aggregation. The PEG layer consisted of a mixture of thiol-polyethylene-glycol (SH-PEG-COOH) (~85%, MW ~ 5 kDa) and a heterofunctional polyethylene-glycol (SH-PEG-COOH) (~15%, MW ~ 3.4 kDa) (creative PEGWorks, Winston Salem, USA). For cancer cell targeting, the heterofunctional PEG was covalently conjugated to an anti-EGFR monoclonal antibody (Erbitux®, Merck KGaA), using 1-ethyl-3-(3'-(dimethylamino)propyl)carbodiimide (EDC) and Sulfo-NHS (Thermo Scientific). The bioconjugation of the GNR to the anti-EGFR antibody was achieved according to the

method described (Ai H, Fang M, Jones SA, Lvov YM. Electrostatic layer-by-layer nanoassembly on biological microtemplates: Platelets. *Biomacromolecules.* May-Jun 2002;3(3):560-564) using polystyrene sulfonate (PSS).

Phantoms

Solid phantoms were prepared in order to simulate the optical properties of skin tissue. Phantoms were prepared using $3x10^{-3}$ % of India Ink as an absorbing component and 2% of Intralipid (Lipofundin MCT/LCT 20%, B.Braun Melsungen AG, Germany) as a scattering component. GNS (30 mg/mL) or GNR (5 mg/mL) were added to the phantom solutions and achieved final concentrations of 0.002, 0.02, 0.022, 0.03, 0.04, and 0.1 mg/ml of gold.

In order to prepare solid pantoms, the solutions were heated and mixed at a temperature of approximately 90°C while the 1% Agarose powder (SeaKem LE Agarose, Lonza, USA) was slowly added. The heated phantom solutions were cooled under vacuum conditions (to avoid bubbles). All phantoms were poured into a 24-well plate (16 mm diameter wells), each well containing different concentrations of gold.

Cell preperation and bioconjugation

A431 human head and neck cancer cells ($2.5x10^6$), which are known to express an extremely high level of EGFR,[30] were cultured in 5 ml DMEM medium containing 5% FCS, 0.5% Penicillin and 0.5% Glutamine at 37 °C under 5% CO_2.

Cell-GNP conjugation: 1 mL of cell suspension ($2.5x10^6$/mL) was mixed with 1 mL of antibody-coated GNR solution (5 mg/mL), and allowed to interact for 30 min at room temperature. Then, the solution was centrifuged 3 times at 1000 rpm for 5 min, to wash out unbound GNR; after each centrifugation the mixture was redispersed in PBS solution (1 mL total volume).

Experimental Setup

This experiment was designed to image the distribution of temperature over the sample area under laser illumination. The laser beam was directed at the sample from above as shown in Figure 1. Two different lasers were used: one, a red diode laser at wavelength of 663nm (custom built), and the other, a green Nd:YAG diode pumped solid state laser at 532nm (Suwtech Laser, DPGL2200 Photop, Fuzhou, China). In

accordance with the peak at the absorption spectrum of the GNPs, the red laser was used for samples with GNR, and the green one for samples with GNS.

Temperature elevation over the sample was imaged by radiometric thermal imaging camera (FLIR Systems, Inc., Boston,MA, model A325). The camera has 320X240 pixels and temperature sensitivity of 0.07 degree Celsius. The spatial resolution of the camera is 0.5mm. By adding an extra lens it can achieve a spatial resolution of 0.1 mm, at a working distance of 80 mm. This kind of camera is sensitive to thermal radiation at a wavelength range of 8 to 14 μm and completely blind to lasers and others light sources at the visible or near infrared spectral range.

For each experiment a few seconds of ambient temperature was recorded (at the center of the laser spot), following irradiation until the sample reached a saturation temperature. Irradiance was measured to be 10 W/cm^2 at the center of the beam.

Example 1: GNP synthesis and characterization

Figure 2 shows the absorbance spectra (UV-vis spectrometer, Shimadzu, UV1650 PC, Japan) of the GNR and GNS, and the wavelength of the lasers used (532 nm and 663 nm, spectrophotometer, USB2000, Ocean Optics Inc., USA). The absorption peak of GNS and GNR is around 515 nm and 690 nm, respectivley. Particle size, shape and uniformity were measured using transmission electron microscopy (TEM) and proved to be 30 nm GNS and 25 nm X 65 nm GNR, with a narrow size distribution (10%) (Figure 2, right).

In order to evaluate the potential of tumor margin delineation using targeted GNP with photo-thermal imaging technique, phantoms containing different concentrations of GNS and GNR, and cancer cells (A431) that were specifically targeted with GNR were irradiated, and their heating profiles were measured.

Example 2: Photothermal imaging of phantoms with GNPs

Figure 3 shows temperature difference profiles of irradiated GNR solutions as a function of time for different concentrations of GNR. As demonstrated, after less than 5 seconds of laser irradiation, the temperature profiles of the irradiated GNR solutions are significantly different than those of the control (solution without GNR). The temperature of the control sample remained unchanged during the experiment, while the temperature of the GNR samples was elevated. In addition, it can be seen that there is a positive correlation between the GNR concentrations and temperature elevation.

For the highest GNR concentration (0.1 mg/mL), a temperature difference of about 14°C has been observed after 10 seconds of irradiation, while for the lowest detectable concentration (0.02 mg/ml), a concentration that is clinically relevant, the temperature has been elevated by about 1°C after the same period of time.

The present invention provides that a low concentration of GNPs (0.02 mg/mL can be easily detected.

The effect of laser wavelength on the heating properties of the GNS and GNR has been further investigated using green and red lasers. Red laser was used since the NIR region of the spectrum provides maximal penetration of light due to relatively lower scattering and absorption from intrinsic tissue chromophores. In this region, penetration depth of red light is up to 10 cm, depending on the type of tissue. In comparison, tissue penetration depth of green light (532 nm) is very low (less than 500 μm), which could be useful for superficial lesions and margin detection during surgery.

Different concentrations of GNS (absorption peak at 515 nm) and GNR (absorption peak at 690 nm) were irradiated both with 663 nm and 532 nm lasers, for 30 seconds. As expected, the temperature profile is strongly affected by the optical properties of the nanoparticles. When GNS (0.1 mg/mL) were irradiated with 532 nm the temperature increased by 5.5°C after 5 seconds, while following irradiation with the 663 nm laser, for the same period of time, temperature elevation of only 2.5°C was observed (Figure 4, left). Figure 4 represents similar results for GNR; when GNR (0.1 mg/mL) were irradiated with 663 nm after 5 seconds the temperature increased by 14°C, while following irradiation with the 532 nm laser, for the same period of time, temperature elevation of only 4.5°C was observed. Temperature elevation of the samples when irradiated with lasers not at the GNPs resonance peak, can be explained by the tail of the resonance peak of the GNS at 690 nm and the "short axis" GNR peak, at 510 nm. In addition, because of the larger cross section of the GNR compared to the GNS, the GNR have higher absorption efficiency that being converted to thermal energy (Figure 4A and 4B).

The potential of this imaging modality to delineate tumor margins during surgery has been further investigated. Two halves circular solid phantoms, one with GNR (0.04 mg/mL) and the other, a homogenous phantom (without GNR), were bonded and irradiated with the 663 nm laser. As demonstrated in Figure 5, after 10 seconds of

irradiation, the GNR phantom region and the homogenous phantom are easily distinguishable. The temperature in the GNR region was elevated by 3.5°C after 10 seconds of irradiation (Figure 5B), and by 9°C after 5 minutes of irradiation (Figure 5C). This concentration of GNR was found to be clinically relevant in several *in vivo* studies and therefore demonstrates the ability to sensitively delineate tumor margins during surgery.

Example 3: Photothermal imaging of GNR targeted cancer cells

In order to demonstrate the ability to perform photo-thermal imaging using targeted GNR, first the specificity of the interaction between the antibody-coated GNR and the A431 SCC cancer cells (which highly express the EGF receptor) has been evaluated. Two types of GNR were introduced to the cells; the first was specifically coated with anti-EGFR antibody, while the second, which was used as a negative control, was coated with a non-specific antibody (anti-Rabbit IgG). Flame atomic absorption spectroscopy measurements (SpectrAA 140, Agilent Technologies) quantitatively demonstrated that the active tumor targeting (anti-EGFR coated GNR) was significantly more specific than the control experiment (anti-Rabbit IgG coated GNR). The A431 cells took up 26.3±2.3 μg of targeted GNR ($3.9x10^4$ GNR per A431 cell), while parallel cells in the negative control experiment took up only 0.2±0.01 μg of GNR ($3.4x10^3$ GNR per cell). These results correlated with previously published studies, which report that head and neck SCC express from 2×10^4 to 2×10^6 EGFRs/cell.

For photothermal imaging studies, GNR-A431 bioconjugated cancerous cells ($2.5x10^6$/mL) and control samples (A431 cells without GNR) were irradiated for 6 minutes (mins). As shown in Figure 6, in about 5 seconds there is a distinct difference of 1°C between the Au targeted cells and the non-targeted cells. Following 30 seconds of laser irradiation, a temperature difference of 3°C has been observed, which is an appropriate temperature for imaging, without affecting cell viability.

In summary, this study demonstrates that photothermal imaging of a tissue or a cell mass such as tumor can be effectively used for viewing tumor margins when targeted with GNPs.

By reducing the laser irradiation, GNP-targeted cancer cells can be detected without affecting cell viability. It has been shown that a low concentration of GNPs

(0.02 mg/mL), lower than that found in several in vivo studies, can be easily detected. An important advantage of this imaging technique is the use of the absorption properties of GNPs rather than their scattering properties, leading to high contrast between targeted cancer cells and normal background tissue.

The possibility of combining photothermal imaging with photothermal therapy is an exciting approach toward theranostics. Additional laser irradiation following the detection of residual tumors adjacent to the primary tumor would lead to selective destruction of the cancer cells targeted with GNPs within the same clinical setting. This photothermal imaging technique is long desired by surgeons that can use the present system and method for accurately determining tumor margins during surgery, leading to complete tumor resection and improved patient outcome.

Example 4: Imaging of cancer tissue borders in-vivof from outside the tissue

The presently developed a new optical configuration allows to image in high resolution the borders of a tumor from outside the tissue. The concept involves heating with pulsed laser nano particles that are concentrated at the edges of the tumor and then imaging it with infra-red camera. Avoiding the blurring of the borders of the tumor due to the scattering of the tissue is obtained by properly extracting the first arriving photons scattered from the inspected tissue.

Scattering of light in a tissue

The mean free path (MFP) in tissue is defined as $1/\mu_t$, in which μ_t is the transport coefficient, typically expressed as a sum of the tissue's absorption coefficient μ_a and the tissue's scattering coefficient μ_s, that is, $\mu_t = \mu_a + \mu_s$. As $\mu_s >> \mu_a$ in most tissues, MFP can be simply written as:

$$MFP = 1/\mu_s \quad (1)$$

When considering a photon undergoing several scattering events, the reduced scattering coefficient can be defined to describe this multiple scattering process as:

$$\mu_s' = \mu_s(1 - g) \quad (2)$$

in which g is the anisotropy function defining the degree of forward scattering, expressed as a probability function of scattering in the forward direction:

$$g = \cos\left(\theta_{scatter}\right)$$

(3)

Where the scattering events redirect the photon in a new direction that is specified by the scattering angle $\theta_{scatter}$ and an azithmul angle $\theta_{scatter}$. The azithmul angle is uniformly distributed [0,2π] and $\theta_{scatter}$ is given by the anisotropy factor of the tissue g. Thus, g specifies the likelihood that a photon is being forward scattered (g=1), backwards scattered (g=-1) or isotropically scattered (g=0). For photon scattering in tissue, g is typically 0.8–1.

The transport mean free path (TMFP) can then be defined as:

$$MFP = 1/\mu_s ,$$

(4)

also assuming that scattering is dominant over absorption, that is, $\mu_s' >> \mu_a$, which generally holds for most tissues. Therefore:

$$\mathrm{TMFP} = \mathrm{MFP}/(1-g) = 1/\mu_s(1-g)^2$$

(5)

The diffusion length in a tissue is defined as:

$$D = TMFP/3$$

(6)

and the optical penetration depth is:

$$\delta = \sqrt{D/\mu_a}$$

(7)

The translation between the TMFP and the multiple scattering time is as follows:

$$T_{ms} = \frac{TMFP}{c/n} = \frac{n}{c\mu_s(1-g)^2}$$

(6)

Where n is the refractive index of the tissue and c is the speed of light. For typical tissue one may assume that n=1.33, μ_s=10 [1/mm], g=0.9 and thus we obtain that T_{ms}=44 [psec]. The time for the first scattering is:

$$T_{fs} = \frac{TFP}{c/n} = \frac{n}{c\mu_s}$$

(7)

Which yields T_{fs}=0.44 [psec]. Thus, if a femto second laser is used such that the temporal width of its pulses is less than 0.44 [psec]=440 [femto sec] then even the first scattering photons will be filtered out and only the first arriving light i.e. the photons that passed the tissue without being scattered at all will arrive the detector. The first arriving light photons are the one that have the high spatial resolution and they are separated from the photons that are spatially blurred due to multiple scattering effects.

Usage of nano particles

Nano particles (NP) can either be directed by labeling towards a specific tumor or as there is more blood vessels surrounding a tumor they will be concentrated at higher concentrations at its boundaries. By illuminating the tumor from a distance and even through a tissue using the pulsed laser configuration at the absorption resonance of the NP can heat them up. Heating them even by a few degrees and inspecting them with infra red (IR) camera can allow high quality detection of the boundaries of the tumor. However, as the tumor is located inside a tissue the IR photons will be scattered on their way back to the camera and thus although the heating process of the NP can be used to detect the boundaries, at reality the boundaries will be blurred due to this scattering process unless proper adaptation of the illumination and detection scheme is done. The setup we propose aims to overcome this problem.

The proposed optical configuration

It is aimed to send pulsed beam having temporal width less than 0.4 psec and then interfering the back scattered light with the reference that was also used for the illumination of the tissue. The proposed configuration is described in Fig. 7.

The first arriving light information can be extracted not only via pulsed laser and holography/interference but also by sending not pulsed light having very short coherence length corresponding to the temporal length mentioned above (less than 0.4 psec). However, if a non pulsed broad band source (to have sufficiently short coherence length) is used then the extracted energy will be very low and the SNR of the captured image might not be sufficient.

CLAIMS

What is claimed is:

1. A method for in-vivo imaging a tissue, comprising the steps of:

   a. introducing a composition comprising gold nanoparticles into said tissue;

   b. selectively increasing the temperature of said nanoparticles by up to $5^{O}C$ within 1 to 30 seconds; and

   c. in-vivo imaging said tissue comprising said nanoparticles by a thermal imaging camera,

   thereby imaging a tissue.

2. The method of claim 1, wherein said selectively increasing the temperature comprises irradiating at a wavelength of 650 to 900nm.

3. The method of claim 1, wherein said selectively increasing the temperature comprises irradiating at 10 to 100 joules per square cm (J/cm^2).

4. The method of claim 3, wherein said irradiating comprises laser irradiating.

5. The method of claim 4, wherein said laser operates in pulsed beam having temporal width equals or less than 0.8 psec.

6. The method of claim 5, further comprising interfering the back scattered light with a reference, said reference is the reference used for irradiating said tissue.

7. The method of claim 3, wherein said irradiating is irradiating at 1 to 10 Watts per square cm (W/ cm^2).

8. The method of claim 1, wherein said gold nanoparticles comprise a gold nucleus coated with thiol-polyethylene-glycol (SH-PEG-COOH) and a heterofunctional polyethylene-glycol.

9. The method of claim 1, wherein said laser is a a red diode laser or a green Nd:YAG diode.

10. The method of claim 1, wherein said thermal imaging camera comprises temperature sensitivity of at least 0.1 degree Celsius, spatial resolution of at least

0.8mm, thermal radiation sensitivity at a wavelength range of 2 to 16 μm, magnification of up to x50, or any combination thereof.

11. The method of claim 1, wherein said tissue comprises cancerous cells.

12. The method of claim 11, wherein said gold nanoparticles comprise a gold nucleus coated with thiol-polyethylene-glycol (SH-PEG-COOH), a heterofunctional polyethylene-glycol, and an anti-EGFR monoclonal antibody.

13. A system for in-vivo imaging a tissue comprising gold nanoparticles, comprising:

a. a composition comprising cellular gold nanoparticles;

b. an irradiation device configured to irradiate at 10 to 100 joules per square cm (J/cm^2); and

c. a thermal imaging camera configured to in-vivo image said tissue comprising said nanoparticles.

14. The system of claim 13, wherein said irradiation device irradiates at a wavelength of 650 to 900nm.

15. The system of claim 13, wherein said irradiation device is laser.

16. The system of claim 15, wherein said laser operates in pulsed beam having temporal width less than 0.8 psec.

17. The system of claim 16, further comprising interfering the back scattered light with a reference, said reference is the reference used for irradiating said tissue.

18. The system of claim 13, wherein said irradiation device irradiates at 0.1 to 0.5 Watts.

19. The system of claim 13, wherein each nanoparticle of said gold nanoparticles comprises a gold nucleus coated with thiol-polyethylene-glycol (SH-PEG-COOH) and a heterofunctional polyethylene-glycol.

20. The system of claim 15, wherein said laser is a a red diode laser or a green Nd:YAG diode.

21. The system of claim 13, wherein said thermal imaging camera comprises temperature sensitivity of at least 0.1 degree Celsius, spatial resolution of at least

0.8mm, thermal radiation sensitivity at a wavelength range of 2 to 16 μm, magnification of up to x50, or or any combination thereof.

22. The system of claim 13, wherein said tissue comprises a cancerous cell.

23. The system of claim 22, wherein said gold nanoparticles comprise a gold nucleus coated with thiol-polyethylene-glycol (SH-PEG-COOH), a heterofunctional polyethylene-glycol, and an anti-EGFR monoclonal antibody.

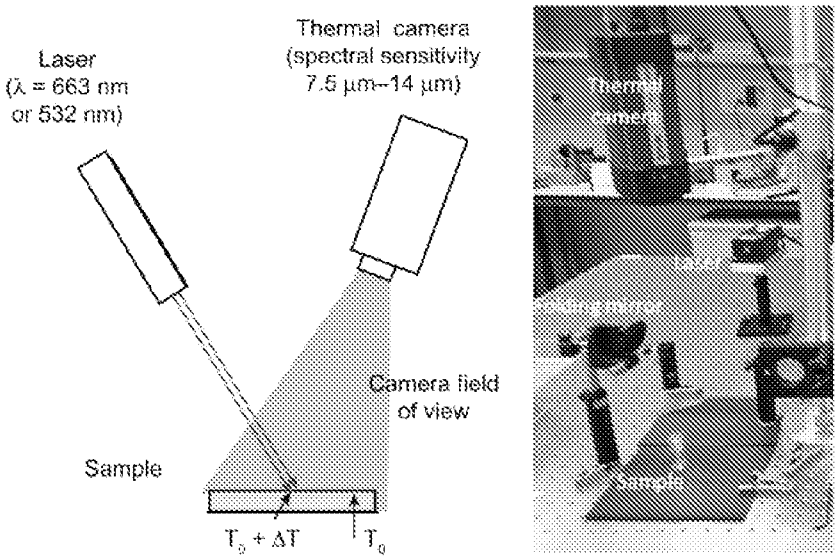

Figure 1

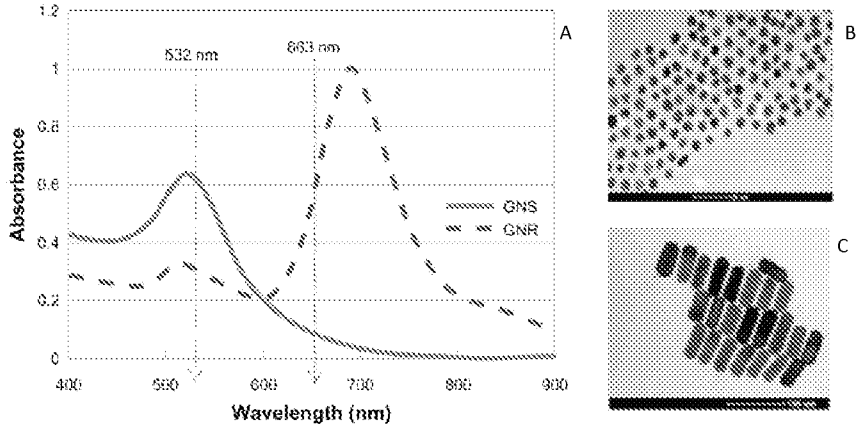

Figure 2

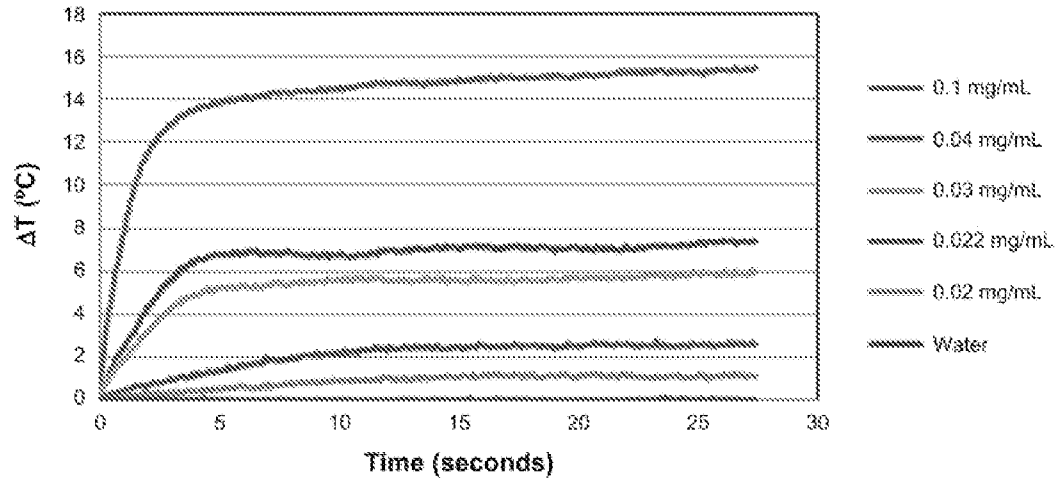

Figure 3

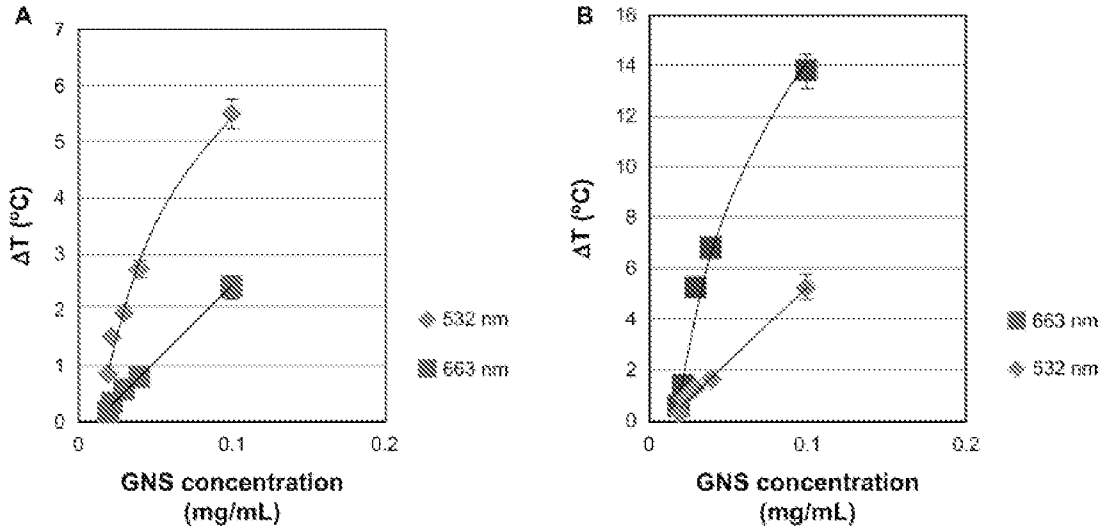

Figure 4

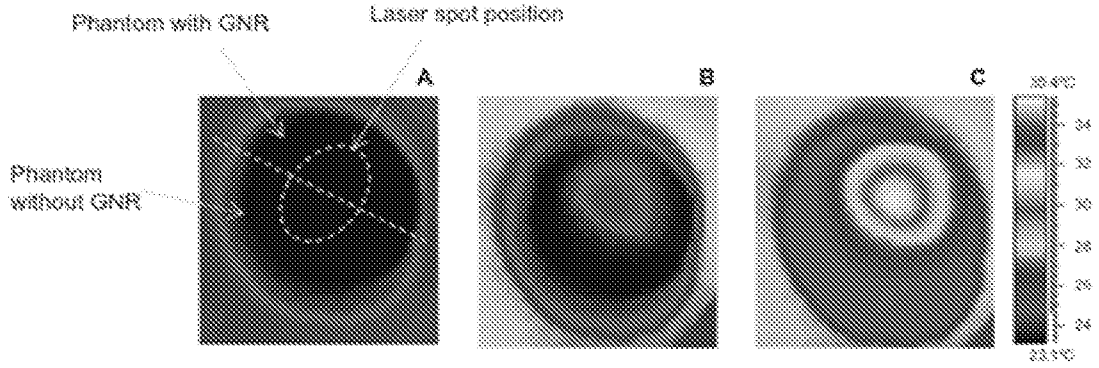

Figure 5

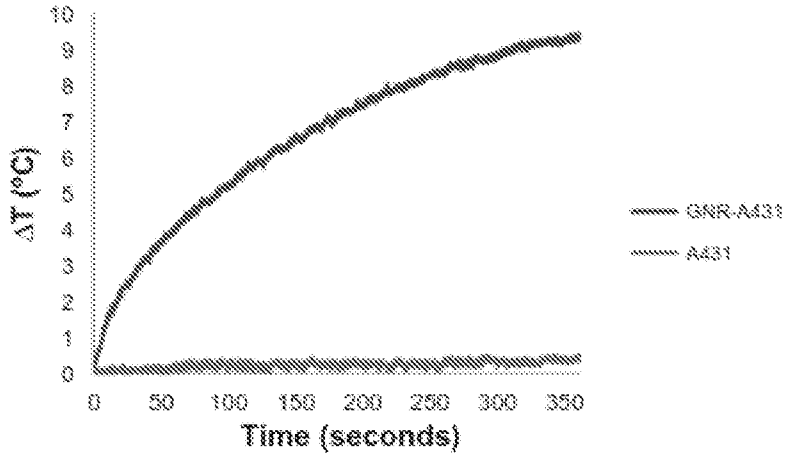

Figure 6

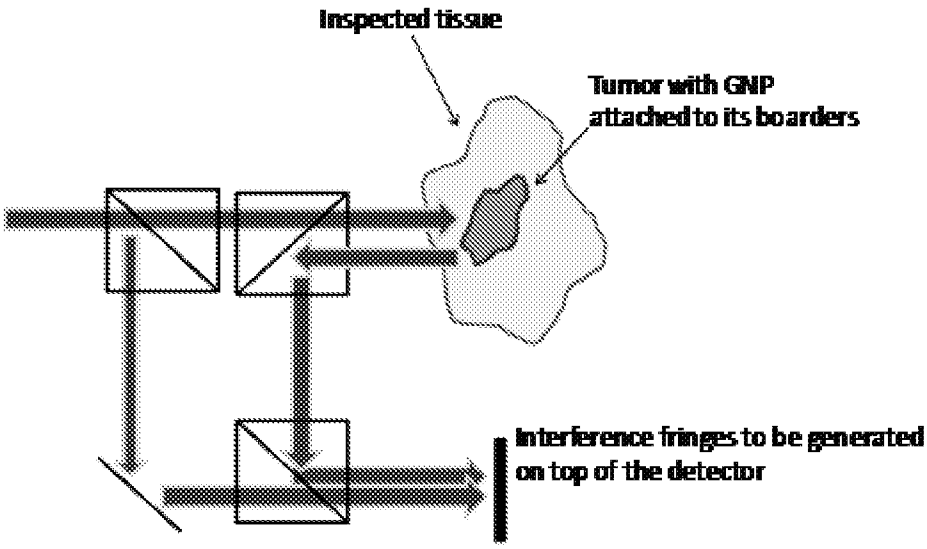
Inspected tissue
Tumor with GNP
attached to its boarders
Interference fringes to be generated
on top of the detector

Figure 7