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(54) Title: METHOD OF FORMING A RARE EARTH METAL DOPED NANOPARTICLE

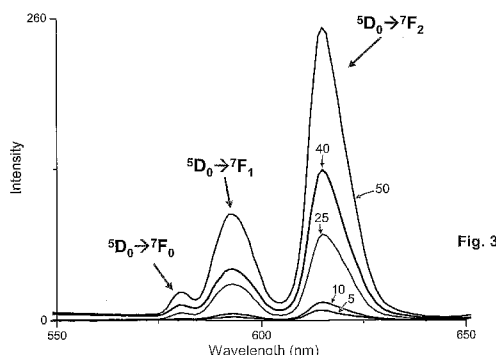


Fig. 3

(57) Abstract: The present invention provides a method of forming a dispersible rare earth metal doped nanoparticle of a metal oxide. The method includes dissolving a salt of a rare earth metal in a mixture of (i) a surfactant and (ii) a suitable solvent. The solvent is at least essentially free of tri-n-octylphosphine oxide and at least essentially free of amines. The method includes adding to said solution a precursor of the metal oxide. The precursor of the metal oxide is added in an excess relative to the rare earth metal. The obtained solution is brought to a temperature selected in the range from about 200 °C to about 400 °C. At the temperature from about 200 °C to about 400 °C an amine is added. The amine is selected from an alkylamine and a dialkylamine.

METHOD OF FORMING A RARE EARTH METAL DOPED NANOPARTICLE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application makes reference to and claims the benefit of priority of an application
5 for "Concentration-effect free rare-earth metal-doped luminescent nanocrystals and bio-
applications" filed on November 25, 2008 with the United States Patent and Trademark Office,
and there duly assigned serial number US Provisional 61/117,658. The contents of said
application filed on November 25, 2008 is incorporated herein by reference for all purposes,
including an incorporation of any element or part of the description, claims or drawings not
10 contained herein and referred to in Rule 20.5(a) of the PCT, pursuant to Rule 4.18 of the PCT.

FIELD OF THE INVENTION

[0002] The present invention relates to a method of forming a rare earth metal doped
nanoparticle. The nanoparticle is dispersible.

BACKGROUND OF THE INVENTION

15 [0003] Luminescent probes can be used to encode chemical information and to detect
particular components of complex bio-systems such as cells with substantial sensitivity and
selectivity. They have diverse applications in drug discovery, catalyst screening, DNA
sequencing, bio-imaging and diagnostics. Photochemical stability and absorption at given
wavelength and bandwidth of the emission spectra are always taken into account in choosing
20 suitable luminescent probes. The common dyes have low photochemical stability, and can be
easily photo-bleached; also they have broad emission spectra.

[0004] Semiconductor nanoparticles, typically nanocrystals, that confine the motion of
conduction band electrons, valence band holes, or excitons (in all three spatial directions) can
serve as "droplets" of electric charge and are termed quantum dots. Quantum dots can be as
25 small as 2 to 10 nanometers, with self-assembled quantum dots typically ranging between 10
and 50 nanometers in size. Quantum dots (QDs) have higher photochemical stability and
narrow emission bandwidths than dyes. However, their cytotoxicity *in vivo* is a limitation.
Moreover, the preparation of high quality QDs requires rigorous synthesis conditions.

[0005] A well established route that can be used to prepare high-quality semiconductor
30 nanoparticles is the decomposition of molecular precursors at high temperatures in a coordinating
solvent (for an overview of previous techniques see e.g. Reed, M.A., *Scientific American*

(1993), January, 118-123), possibly, in the presence of a negative ion source, e.g., TOP/Se, TOP/S, etc. The obtained solution is typically rapidly injected into tri-*n*-octylphosphine oxide (TOPO) at high temperatures (~200 °C-300 °C). Thereby TOP/TOPO capped nanocrystals are obtained. The capping agent allows particle solubility in organic solvents, and plays a crucial
5 role in preventing particle aggregation and electronically passivating the semiconductor surface. This so-called TOPO method permits the production of highly monodisperse nanoparticles in quantities of hundreds of milligrams in one single experiment.

[0006] Rare-earth doped nanocrystals are non-toxic and have large effective Stokes shifts (preventing light scattering), sharp emission spectra, flexibility of excitation wavelengths,
10 suitability for multiphoton and up-conversion excitation. They are furthermore highly resistant to blinking and photobleaching. They can emit various wavelengths by an appropriate choice of colour-center elements instead of varying particle size. In addition, the longer fluorescence lifetime renders it possible to use them as new probes in time-resolved fluoroimmunoassays (TR-FIA) to increase the signal/noise ratio, i.e. the assay sensitivity.

[0007] Ternary rare earth-incorporated metal oxides (RE'M'O) have therefore been continuously receiving tremendous attention in the last decades for the predominant applications in e.g. lighting, display or lasing. Up to date, a low-incorporation level of < 5-10 mol% [RE/ (RE'+M')] rare earth emission centers is achieved in the widely used phosphors including europium-incorporated yttrium oxide. Beyond this point, the well-
20 reported concentration-dependent emission quenching takes place. This long-standing issue is expected to be solved by effective isolation/passivation of more rare earth emission centers in amorphous metal oxide matrices via a uniform incorporation of rare earth elements.

[0008] It is therefore an object of the present invention to provide a method that allows
25 forming nanoparticles that have at least some of the advantages of rare-earth doped nanoparticles but with less, or without, the concentration-dependent quenching. This object is solved by the method of claim 1.

SUMMARY OF THE INVENTION

[0009] In a first aspect the present invention provides a method of forming a dispersible
30 nanoparticle. The nanoparticle is a rare earth metal doped nanoparticle of a metal oxide. The method includes providing a mixture of (i) a surfactant and (ii) a suitable solvent for dissolving a salt of a rare earth metal therein. This suitable solvent is at least essentially free of tri-*n*-octylphosphine oxide. The suitable solvent is furthermore at least essentially free of

amines. The method further includes dissolving in the mixture of the surfactant and the suitable solvent a salt of a rare earth metal. Thereby a first solution is formed. The method also includes adding to the first solution of the salt of the rare earth metal a precursor of the metal oxide. The precursor of the metal oxide is added in an excess relative to the salt of the rare earth metal. Thereby a second solution is formed. Furthermore, the method includes bringing the second solution to a temperature selected in the range from about 200 °C to about 400 °C. The method also includes adding at the temperature selected in the range from about 200 °C to about 400 °C an amine. The amine is selected from an alkylamine and a dialkylamine. By adding the amine the method includes allowing the formation of a dispersible nanoparticle.

[0010] The nanoparticle obtained by a method of the invention is typically homogenous.

[0011] In some embodiments the method further includes dispersing the obtained dispersible nanoparticle in a suitable solvent. Further, the method of such embodiments includes adding an amphiphilic polymer, and allowing the formation of a water-dispersible nanoparticle or microparticle. The water-dispersible nanoparticle or microparticle includes a rare earth metal doped nanoparticle and a hydrophilic or an amphiphilic polymer.

[0012] In a second aspect the invention provides a nanoparticle obtained by the method of the first aspect.

[0013] In a third aspect the invention provides a plurality of nanoparticles obtained by the method of the first aspect. The plurality of dispersible nanoparticles is included in a water-dispersible nanoparticle or microparticle. The water-dispersible nanoparticle or microparticle further includes a hydrophilic or an amphiphilic polymer.

[0014] In a fourth aspect the invention also relates to the use of a nanoparticle obtained by method of the first aspect in the manufacture of an illuminant.

[0015] In a fifth aspect the invention provides a method of monitoring a cell with a colour of a specific wavelength. The method includes contacting the cell with a water-dispersible nanoparticle or microparticle obtained as described above. The method thereby includes allowing the cell to take up the water-dispersible nanoparticle or microparticle. The method also includes irradiating the cell with the wavelength of a $4f \leftrightarrow 4f$ transition of the rare earth metal. This transition may for example be an excitation from the ground level to the first excited level. The method also includes detecting the emission of the water-dispersible nanoparticle or microparticle.

[0016] The invention will be better understood with reference to the detailed description when considered in conjunction with the accompanying drawings, in which:

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Fig. 1A shows an optical photograph depicting photos of (from the left to the right) multicolour Dy-ZrO₂, Tb-ZrO₂, Eu-TiO₂ and Eu-ZrO₂ nanoparticles in glass vials.

[0018] Fig. 1B shows the photograph of Fig. 1A with only the blue channel depicted.

5 [0019] Fig. 1C shows the photograph of Fig. 1A with only the green channel depicted

[0020] Fig. 1D shows the photograph of Fig. 1A with only the red channel depicted.

[0021] Fig. 1E shows the photograph of Fig. 1A with all colours except magenta turned dark.

[0022] Fig. 2A depicts a TEM image of Eu³⁺-doped TiO₂ nanoparticles with 10% Eu³⁺ prepared according to the method of the invention. The white bar corresponds to 50 nm.

10 [0023] Fig. 2B depicts a TEM image of Eu³⁺-doped TiO₂ nanoparticles with 10% Eu³⁺ prepared according to the method of the invention. The white bar corresponds to 50 nm.

[0024] Fig. 3 depicts the effect of the doping concentration on photoluminescence of TiO₂ : Eu nanoparticles. TiO₂ nanoparticles were doped with Europium at 5 molar percentages, indicated next to the corresponding spectrum (5, 10, 25, 40 and 50 %). As can be taken from
15 the effect of the different doping ratios, no concentration-effect of the rare-earth metal occurs at the indicated molar percentages. A RF-5301PC Series Spectrofluorophotometer (Shimadzu) was used for all measurements, and the light intensity was controlled by fixing the slit-width.

[0025] Fig. 4A depicts a TEM image of 10% Eu³⁺ doped SiO₂ nanoparticles. The white bar corresponds to 50 nm.

20 [0026] Fig. 4B depicts RGB colour-tuning of Eu³⁺-doped SiO₂, Eu³⁺ being used for red emission.

[0027] Fig. 4C depicts RGB colour-tuning of Tb³⁺-doped SiO₂, Tb³⁺ being used for green emission.

25 [0028] Fig. 4D depicts RGB colour-tuning of Tm³⁺-doped SiO₂, Tm³⁺ being used for blue emission.

[0029] Fig. 5 depicts TEM images of Eu³⁺-doped Y₂O₃ nanodisks, prepared according to the method of the invention. The white bar corresponds to 100 nm.

[0030] Fig. 6 depicts TEM images of Eu³⁺-doped Gd₂O₃ nanodisks, prepared according to the method of the invention. The black bar corresponds to 50 nm.

30 [0031] Fig. 7 depicts photoluminescence spectra of the rare earth metal oxide particles doped with Eu³⁺ (A, D, E), Tb³⁺ (B, F) and Tm³⁺ (C) as the colour centers.

[0032] Fig. 8 depicts the results of a systematic screening of environmentally stable metal oxide matrices with high availability and low cost for investigating emission properties of rare earth emission centers in different surrounding metal oxide matrices. 50 mol% europium-incorporated metal oxide. With the same incorporated ratio of europium, the samples were excited by light with a wavelength of 396 nm, a trend of $\text{ZrO}_2 > \text{MgO} > \text{Al}_2\text{O}_3 > \text{GeO}_2 > \text{TiO}_2 > \text{SiO}_2 > \text{SrO} > \text{Ga}_2\text{O}_3 \approx \text{Y}_2\text{O}_3 \approx \text{Eu}_2\text{O}_3 > \text{SnO}_2$ was found concerning the corresponding emission intensity. It can be seen that the metal oxides of the invention with good insulating properties can effectively localize photo-excited carriers of Eu (the strong passivation of Eu in matrices), and no efficient self-emission pathways and no internal electron transitions exist for causing emission quenching of Eu.

[0033] Fig. 9 depicts the results of a systematic screening of environmentally stable metal oxide matrices with high availability and low cost for investigating emission properties of rare earth emission centers in different surrounding metal oxide matrices. 50 mol% europium-incorporated metal oxide. A trend of $\text{ZrO}_2 > \text{TiO}_2 > \text{Al}_2\text{O}_3 > \text{MgO} > \text{SrO} > \text{GeO}_2 > \text{SiO}_2 > \text{Ga}_2\text{O}_3 \approx \text{Y}_2\text{O}_3 \approx \text{Eu}_2\text{O}_3 > \text{SnO}_2 > \text{In}_2\text{O}_3$ was found concerning the energy transfer from the hosting matrices to the emission center, when the samples were excited at a wavelength of 320 nm. When the absorption band of the hosting matrix matches the emission center, the energy transfer from the hosting matrix to the emission center is strong, thus the emission intensity is higher.

[0034] Fig. 10 illustrates the preparation of PLGA particles with $\text{TiO}_2\text{:Eu}$ nanoparticles encapsulated therein.

[0035] Fig. 11A depicts an SEM image of $\text{TiO}_2\text{:Eu}$ -embedded PLGA nanoparticles. The white bar represents 2 μm .

[0036] Fig. 11B depicts a confocal image of $\text{TiO}_2\text{:Eu}$ -embedded PLGA nanoparticles. The white bar represents 10 μm .

[0037] Fig. 12A depicts a confocal transmitted light image of PLGA particles with $\text{SiO}_2\text{:Tb}$ nanoparticles embedded therein, showing the morphology of the PLGA particles. The white bar represents 20 μm .

[0038] Fig. 12B depicts a confocal fluorescent image of PLGA particles with $\text{SiO}_2\text{:Tb}$ nanoparticles embedded therein, showing the fluorescent intensity of the PLGA particles. The white bar represents 20 μm .

[0039] Fig. 13A depicts a confocal fluorescent image of the internalization of the red PLGA microparticles into the cytoplasm of MCF-7 cells after 4-h incubation at 37 °C, followed by counterstaining of nucleus with blue DAPI.

[0040] Fig. 13B depicts a confocal image of the internalization of the red PLGA microparticles into the cytoplasm of MCF-7 cells after 4-h incubation at 37 °C, followed by counterstaining of nucleus with blue DAPI. The image is an overlay of the nucleus stained cell with the corresponding transmitted light image, allowing the distribution of microparticles in the cell to be located.

DETAILED DESCRIPTION OF THE INVENTION

[0041] The method of the present invention yields one or more dispersible nanoparticles of a metal oxide. The metal oxide of the nanoparticle(s) is doped with a rare-earth metal. The dispersible nanoparticle has an emission at room temperature that is generally higher than the emission of the corresponding non-doped nanoparticle. The method, and accordingly the nanoparticles according to the invention, provides the unexpected advantage that up to a doping amount of roughly about 50% no quenching is observed. Accordingly, it is possible to increase photoluminescence, including at the temperature range around typical outdoor temperatures (in any region of the earth) and ambient temperature/room temperature (i.e. around 18 °C), by increasing the doping ratio up to much higher doping ratios, and thereby providing significantly higher photoluminescence values than previously achieved. Furthermore, the method of the invention can be carried out as a one-pot synthesis in a conveniently straightforward and facile manner.

[0042] The nanoparticles are dispersible and can be termed solvable in a non-polar solvent. Examples of such a non-polar solvent include, but are not limited to, hexane, heptane, octane, cyclohexane, benzene, toluene, pyridine, dichloromethane, chloroform, carbon tetrachloride, carbon disulfide, dioxane, diethyl ether, diisopropylether, ethylene glycol monobutyl ether and tetrahydrofuran. In terms of their handling, nanoparticles of the invention have thus properties that are comparable to typical high-quality quantum dots. Respective quantum dots in the art are usually coated with a monolayer of TOPO, a hydrophobic molecule with inherent health and environmental risks. The nanoparticles of the invention do per se not carry a monolayer of TOPO and it is not required for easy handling to provide them with such a layer (cf. also below).

[0043] The nanoparticle(s) can be rendered dispersible (and can then be termed solvable) in a polar solvent, such as a polar protic solvent, by treatment with an amphiphilic polymer as explained below. Typically such an amphiphilic polymer defines a coating on the surface of a nanoparticle. A protic solvent is a solvent that has, for example, a hydrogen atom bound to an oxygen as in a hydroxyl group or a nitrogen as in an amine group. More generally, any

molecular solvent which contains dissociable H^+ , such as hydrogen fluoride, is called a protic solvent. The molecules of such solvents can donate an H^+ (proton). Examples of polar protic solvents include, but are not limited to, water, methanol, ethanol or acetic acid. Thus water and aqueous media may be used to handle such nanoparticle(s).

5 [0044] The nanoparticles have a longer fluorescence lifetime following excitation, i.e. irradiation when compared to typical quantum dots that do not include rare earth elements. This property renders the nanoparticles particularly useful in detection and sensing, for example in the context of in vivo or in vitro, including cellular, systems. Using the nanoparticle(s) of the present invention it is furthermore possible to induce emission at a
10 particular colour, defined by a wavelength range of a few nanometers, including emission at a specific wavelength.

[0045] Using the method of the invention no fine tuning and control of parameters is required for achieving desired optical properties of the obtained nanoparticles (cf. also below). Rather by selecting a certain rare earth metal, in the form of a corresponding metal compound, a
15 specific colour of emission is selected. Further adjustment of colours can be performed by combining different nanoparticles, generally different nanoparticles that have emissions at different wavelengths. As an example, a nanoparticle obtained by the method of the present invention may be used in an illuminant, an amplifier, in a biological sensor or for computation methods. When used in an illuminant, i.e. a light emitting device such as a lamp, a light
20 emitting diode, a laser diode, a fluorophore (for instance in the detection of tumours), a TV-screen or a computer monitor, the wavelength range, including the peak of light emission, can be adjusted by selecting one or more appropriate dopants.

[0046] One such embodiment of the invention is a plurality of nanoparticles that emit white light. Accordingly, the present invention also relates to the use of a nanoparticle obtainable or
25 obtained by the method of the invention. As can be taken by the illustrative figures, the respective wavelength range, including the emission peak, can be controlled by factors such as the temperature at which the element A is added, the reaction time, the solvent used, the surfactant used, and the amount of surfactant added.

[0047] The nanoparticle according to the first aspect of the invention is a rare earth metal
30 doped nanoparticle. The rare earth metal (also abbreviated as RE) may be any element of the lanthanide and of the actinide series. In some embodiments the rare earth metal is a lanthanoid. The rare earth metal is in some embodiments selected to be no trans-uranium element. The rare earth metal may for instance be selected from the group of cerium, praseodymium, neodymium, promethium, samarium, europium, gadolinium, terbium, dysprosium, holmium,

erbium, thulium, ytterbium and combinations thereof.

[0048] The rare earth metal doped nanoparticle is a nanoparticle of a metal oxide. Any metal oxide may be used as long as it differs from the dopant and as long as it can be doped with the selected rare earth metal. In some embodiments the metal oxide differs from a rare earth metal oxide. In some embodiments the metal oxide is an oxide of a rare earth metal, e.g. Y_2O_3 or Gd_2O_3 . Examples of metals include, but are not limited to, Zn, Sn, Si, Ti, Y, Cd, Hg, Mg, Zr, Sc, Mn, Ga, In, Sr, Al, Ge, Fe, Co, Ni, Cu, Ag, Au and Au.

[0049] In the method of forming the nanoparticle according to the first aspect of the invention a suitable solvent is provided. Any suitable solvent may be used in the method of the present invention as long as it is at least essentially free of tri-*n*-octylphosphine oxide (see also below). The term "at least essentially free of" as used herein for a solvent refers to the use of amounts of a solvent that do not significantly affect the total fluid content. This term thus includes the complete absence and the presence of traces of the solvent, for example in the range from 0 to about 5 %, 0 to about 2.5 %, 0 to about 1 %, 0 to about 0.1 % or 0 to about 0.01 %, e.g. about 0.01%, about 0.1%, about 0.5%, about 1 %, about 2 %, about 3%, about 4% or about 5 % (in relation to the total volume of the solvent used). Accordingly the main solvent (which can also be a mixture of different solvents other than tri-*n*-octylphosphine oxide) provided in the method of the present invention, is, or is dominated and governed, by a solvent that differs from tri-*n*-octylphosphine oxide.

[0050] Typically the solvent is a non-coordinating solvent such as an alkane or an alkene. Illustrative examples of an alkene include, but are not limited to, 1-dodecene (CAS-No 112-41-4), 1-tetradecene (CAS-No 1120-36-1), 1-hexadecene (CAS No. 629-73-2), 1-heptadecene (CAS No. 6765-39-5), 1-octadecene (CAS No. 112-88-9), 1-eicosene (CAS No. 3452-07-1), 7-tetradecene (CAS-No 10374-74-0), 9-hexacosene (CAS-No 71502-22-2), 1,13-tetradecadiene (CAS-No 21964-49-8) or 1,17-octadecadiene (13560-93-5). Illustrative examples of an alkane are decane (CAS-No 124-18-5), undecane (CAS-No 1120-21-4), tridecane (CAS-No 629-50-5), hexadecane (CAS-No 544-76-3), octadecane (CAS-No 593-45-3), dodecane (CAS-No 112-40-3) and tetradecane (CAS-No 629-59-4). In some embodiments the solvent may also be or include a weak coordinating solvent such as an ether. Examples of a suitable ether include, but are not limited to, dioctylether (CAS-No. 629-82-3), didecyl ether (CAS-No. 2456-28-2), diundecyl ether (CAS-No. 43146-97-0), didodecyl ether (CAS-No. 4542-57-8), 1-butoxy-dodecane (CAS-No. 7289-38-5), heptyl octyl ether (CAS-No. 32357-84-9), octyl dodecyl ether (CAS-No. 36339-51-2), and 1-propoxy-heptadecane (CAS-No. 281211-90-3).

[0051] The solvent used in the method of the invention is at least substantially void of amines,

i.e. amine-free. The term "amine" is used herein in its regular meaning to refer to compounds having at least one primary, secondary or tertiary amine group (compound of the general formula $(R_1R_2R_3N)$ with R_1 , R_2 , and R_3 being hydrogen or an alkyl group, for example) which would be able to react with a metal such as Cd or Zn that may be used in the present invention.

5 It is noted that an amine is only later on added during the method of the invention. The term "at least essentially free of amines" thus includes the complete absence and the presence of traces of an amine, for example in the range from 0 to about 5 %, 0 to about 2.5 %, 0 to about 1 %, 0 to about 0.1 % or 0 to about 0.01 %, e. about 0.01 %, about 0.1 %, about 0.5 %, about 1 %, about 2 %, about 3 %, about 4 % or about 5 % (in relation to the total volume of the solvent
10 used). Accordingly the main solvent (which can also be a mixture of different solvents other than an amine) in these embodiments of the process of the invention, in which a solution or a reaction mixture as defined herein is prepared, is, or is dominated and governed, by a solvent that differs from an amino compound.

[0052] The solvent used in the method of the invention is typically a high-boiling solvent, e.g.
15 with a boiling point above about 120 °C, 150 °C, 180 °C or above about 220 °C. In some embodiments a combination of solvent components is selected, which has a boiling point above the highest selected temperature during the method of the invention (e.g. for dissolving cadmium or a cadmium compound). Noteworthy, the method of the present invention can be performed in the absence of phosphines or phosphine oxides. Such solvents, which are
20 continuously being used in approaches to provide passivation to nanoparticles are generally cost intensive and thus provide an obstacle to upscaling and economic production.

[0053] Furthermore, a dispersing agent, e.g. a surfactant, is provided. The dispersing agent generally includes a polar head group, which may be a hydrogen containing group. Any surfactant may for instance be used as the dispersing agent. Without being bound by theory,
25 the surfactant is believed to help to control the particle growth. It is also thought to reduce the oxygen bridge bonds between particles and to prevent agglomeration. It is thereby believed to act as a stabilizer to achieve high dispersibility in a high boiling solvent. The surfactant may for instance be an organic carboxylic acid, an organic phosphate, an organic phosphonic acid or a mixture thereof. Illustrative examples of suitable organic carboxylic acid include, but are
30 not limited to, stearic acid (octadecanoic acid), lauric acid, oleic acid ([Z]-octadec-9-enoic acid), n-undecanoic acid, linoleic acid, ((Z,Z)-9,12-octadecadienoic acid), arachidonic acid ((all-Z)-5,8,11,14-eicosatetraenoic acid), linoleic acid ((E,E)-9,12-octadecadienoic acid), myristoleic acid (9-tetradecenoic acid), palmitoleic acid (cis-9-hexadecenoic acid), myristic acid (tetradecanoic acid), palmitic acid (hexadecanoic acid) and γ -homolinolenic acid

((Z,Z,Z)-8,11,14-eicosatrienoic acid). Examples of other surfactants (an organic phosphonic acid, for example) include hexylphosphonic acid and tetra decylphosphonic acid. It has previously been observed that oleic acid is capable of stabilising nanoparticles and allows the usage of octadecene as a solvent (Yu, W.W., & Peng, X., *Angew. Chem. Int. Ed.* (2002) 41, 13, 2368-2371). In the synthesis of other nanoparticles surfactants have been shown to affect the crystal morphology of the nanoparticles formed (Zhou, G., et al., *Materials Lett.* (2005) 59, 2706-2709).

[0054] In the method of the invention a mixture of the dispersing agent and the solvent is provided. In this mixture a salt of a rare earth metal is dissolved. Any salt of the rare earth metal may be used. The counter ion may be an inorganic or an organic anion. Examples of inorganic anions are carbonate, sulphate, nitrate, bromide or chloride. Examples of organic anions are carboxylic acids such as acetate, acetylacetonate, oleate or stearate. As illustrative examples may serve samarium acetate (SmAc_3), samarium acetylacetonate ($\text{Sm}(\text{acac})_3$); europium acetate (EuAc_3), europium acetylacetonate ($\text{Eu}(\text{acac})_3$); terbium acetate (TbAc_3), terbium acetylacetonate ($\text{Tb}(\text{acac})_3$); erbium acetate (ErAc_3), erbium acetylacetonate ($\text{Er}(\text{acac})_3$); gadolinium acetate (GdAc_3), gadolinium acetylacetonate ($\text{Gd}(\text{acac})_3$); thulium acetate (TmAc_3), and thulium acetylacetonate ($\text{Tm}(\text{acac})_3$). Forming a solution of the rare earth metal salt, respectively, may in some embodiments include bringing the mixture to an elevated temperature, such as in the range from about 30 °C to about 120 °C, about 40 °C to about 100 °C or about 50 °C to about 100 °C, e.g. about 50 °C, about 60 °C, about 70 °C, about 80 °C, about 90 °C or about 100 °C. Forming a solution of the rare earth metal salt may in some embodiments take one or several hours, including about 2, about 3, about 4, about 5, about 6, about 12 or more hours. In some embodiments vacuum is applied during the formation of the solution, for example to remove oxygen and water vapour. By dissolving the salt of a rare earth metal a first solution is formed. After dissolving the rare earth metal salt, the temperature of the solution may be changed, such as reduced to a selected temperature. The temperature may also be raised. In some embodiments the temperature is maintained.

[0055] To the first solution a precursor of the metal oxide is added. In some embodiments the precursor of the metal oxide is added rapidly, e.g. within less than a minute or several minutes or, depending on the dimensions used, in a matter of a few seconds, about 10, about 20, about 30 or about 40 seconds. In one embodiment the precursor of the metal oxide is injected, e.g. swiftly injected, into the first solution. The precursor of the metal oxide is generally formed from a compound of the metal of the metal oxide or from the respective elemental metal. Any metal compound may be used that can be dissolved in the selected mixture. The metal compound

may for example be an inorganic metal salt such as a carbonate, a nitrate or a chloride or an organic compound (e.g. salt) such as a carboxylic acid salt, e.g. an acetate or an acetylacetonate, or an alkoxide. As five illustrative examples may serve titanium isopropoxide, titanium butaoxide, tetraethyl orthosilicate, propyltrimethoxysilane or zirconium acetylacetonate. The compound may also be a metal oxide or a metal hydroxide. The precursor of the metal oxide is added in a molar excess relative to the salt of the rare earth metal. The amount of the precursor of the metal oxide added may for instance be added in an amount (on a molar basis) of about 1.05 to about 100-fold in relation to the rare earth metal salt, e.g. in an amount of about 2- to about 50-fold, in an amount of about 2 to about 10-fold, in an amount of about 3 to about 10-fold or in an amount of about 4 to about 10-fold, such as about 10-fold, about 9-fold, about 8-fold, about 7-fold, about 6-fold, about 5-fold, about 4-fold, about 3-fold, about 2-fold, about 1.5-fold, about 1.25-fold or about 1.1-fold. By adding the metal compound a second solution is formed.

[0056] This second solution is brought to a temperature selected in the range from about 180 °C to about 450 °C, such as about 200 °C to about 400 °C. Typically the temperature of the second solution is increased. The second solution may for example be brought to a temperature from about 200 °C to about 350 °C, such as about 200 °C to about 300 °C, about 200 °C to about 280 °C, about 220 °C to about 280 °C, or about 200 °C to about 270 °C.

[0057] At this temperature, i.e. at the temperature selected in the range from about 200 °C to about 400 °C, an amine is added to the second solution. The amine is an alkylamine or a dialkylamine. The term "alkyl" refers, unless otherwise stated, to a saturated aliphatic or alicyclic hydrocarbon chain, which may be straight or branched and include heteroatoms. A heteroatom is any other atom than carbon and hydrogen, such as N, O, S, Se and Si. The branches of the hydrocarbon chain may include linear chains as well as non-aromatic saturated cyclic elements. The (main) chain of an alkyl moiety, may, unless otherwise stated, be of any length, and contain any number of branches. Typically, the hydrocarbon (main) chain includes 1 to about 5, to about 10, to about 15, to about 20, to about 30 or to about 40 carbon atoms. Illustrative examples of non-cyclic, i.e. alkyl groups are methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, the n isomers of these radicals, isopropyl, isobutyl, isopentyl, neopentyl, sec.-butyl, tert.-butyl, neopentyl and 3,3-dimethylbutyl. Both the main chain as well as the branches may furthermore contain heteroatoms as for instance N, O, S, Se or Si or carbon atoms may be replaced by these heteroatoms. A cyclic alkyl moiety, i.e. an alicyclic moiety, is a non-aromatic cyclic moiety (e.g. hydrocarbon moiety), which is saturated. The cyclic hydrocarbon moiety may also include fused cyclic ring systems such as

decalin and may also be substituted with non-aromatic cyclic as well as chain elements. The main chain of the cyclic hydrocarbon moiety may, unless otherwise stated, be of any length and contain any number of saturated non-aromatic cyclic and chain elements. Typically, the hydrocarbon (main) chain includes 3, 4, 5, 6, 7 or 8 main chain atoms in one cycle. Examples of such moieties include, but are not limited to, cyclopentyl, cyclohexyl, cycloheptyl, or cyclooctyl. Both the cyclic hydrocarbon moiety and, if present, any cyclic and chain substituents may furthermore contain heteroatoms, as for instance N, O, S, Se or Si, or a carbon atom may be replaced by these heteroatoms.

[0058] Without being bound by theory, the amine is believed to act as an accelerator that promotes the formation of nanoparticles. The amine itself may be a high-boiling solvent. Examples of a suitable amine include, but are not limited to, 1-amino-9-octadecene (oleylamine) (CAS-No. 112-90-3), 1-amino-4-nonadecene (CAS-No. 25728-99-8), 1-amino-7-hexadecene (CAS-No. 225943-46-4), 1-amino-8-heptadecene (CAS-No. 712258-69-0, CAS-No of the pure Z-isomer: 141903-93-7), 1-amino-9-heptadecene (CAS-No. 159278-11-2, CAS-No of the Z-isomer: 906450-90-6), 1-amino-9-hexadecene (CAS-No. 40853-88-1), 1-amino-9-eicosene (CAS-No. 133805-08-0), 1-amino-9,12-octadecadiene (CAS-No. 13330-00-2), 1-amino-8,11-heptadecadiene (CAS-No. 141903-90-4), 1-amino-13-docosene (CAS-No. 26398-95-8), N-9-octadecenyl-propanediamine (CAS-No. 29533-51-5), N-octyl-2,7-octadienyl-amine (CAS-No. 67363-03-5), N-9-octadecen-1-yl-9-octadecen-1-amine (dioleylamine) (CAS-No. 40165-68-2), bis(2,7-octadienyl)amine (CAS-No. 31334-50-6), and N,N-Dibutyl-2,7-octadienylamine (CAS-No. 63407-62-5).

[0059] The amount of the amine added may in some embodiments be a molar excess compared to the amount of the precursor of the metal oxide. The amount of amine added may for instance be an amount of about 1.05 to about 15-fold in relation to precursor of the metal oxide, e.g. in an amount of about 1.5- to about 10-fold or in an amount of about 1.5 to about 8-fold, such as about 10-fold, about 9-fold, about 8-fold, about 7-fold, about 6-fold, about 5-fold, about 4-fold, about 3-fold, about 2-fold, about 1.5-fold or about 1.25-fold.

[0060] By adding the amine the formation of a dispersible nanoparticle is allowed. The reaction may be carried out for any desired period of time, ranging from milliseconds to a plurality of hours. Typically, the formation of the dispersible nanoparticle takes at least 30 minutes, such as about 1 to 10 hours, e.g. about 1, about 2, about 3 or about 4 hours. Where desired, the reaction is carried out in an inert atmosphere, i.e. in the presence of gases that are not reactive, or at least not reactive to a detectable extent, with regard to the reagents and solvents used. Examples of a reactive inert atmosphere are nitrogen or a noble gas such as

argon or helium. It is however noteworthy that an inert gas atmosphere was found to be generally unnecessary. The only step that may require an inert gas atmosphere is the reaction of the metal oxide precursor with the surfactant, typically the formation of a metal carboxylate, following adding the precursor of the metal oxide.

5 [0061] The method of the invention may further include nanoparticle post-processing. Although the nanoparticles obtained by the method of the invention are generally at least essentially or at least almost monodisperse, if desired a step may be performed to narrow the size-distribution (for example as a precaution or a safety-measure). Such techniques, e.g. size-selective precipitation, are well known to those skilled in the art. The surface of the
10 nanoparticle may also be altered, for instance coated.

[0062] In some embodiments the nanoparticle is incorporated into a water-soluble nanoparticle or microparticle. Such a water-soluble nanoparticle or microparticle further includes an amphiphilic or a hydrophilic, i.e. a polar, polymer. The term amphiphilic refers to a polymer that is soluble in both polar and non-polar fluids. The amphiphilic properties of the
15 polymer are due to the presence of both polar and non-polar moieties within the same polymer. Polar properties of a respective polymer are based on polar moieties. Such moieties are for instance -COOH, -NH₂ or -OH side groups, in particular in the form of charged COO⁻ or NH₃⁺ groups, which may for example be carried by a polar, or in the case of an amphiphilic polymer for example by a non-polar backbone such as a hydrocarbon backbone of the polymer.

20 [0063] The nanoparticle of a doped metal oxide may for example be provided in a suitable solvent or mixtures of such solvents. Suitable in this respect as used herein means that the nanoparticle should be soluble in the respective solvent. Examples of such solvents are, but not limited to, aprotic solvents and/or non-polar solvents, such as an aprotic non-polar solvent. Examples of non-polar liquids include, but are not limited to mineral oil, hexane, heptane,
25 cyclohexane, benzene, toluene, pyridine dichloromethane, chloroform, carbon tetrachloride, carbon disulfide, dioxane, diethyl ether, diisopropylether, methyl propyl ketone, methyl isoamyl ketone, methyl isobutyl ketone, cyclohexanone, isobutyl isobutyrate, ethylene glycol diacetate, and a non-polar ionic liquid. Examples of a non-polar ionic liquid include, but are not limited to, 1-ethyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]amide bis(triflyl)-
30 amide, 1-ethyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]amide trifluoroacetate, 1-butyl-3-methylimidazolium hexafluorophosphate, 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, trihexyl(tetradecyl)phosphonium bis[oxalato(2-)]borate, 1-hexyl-3-methyl imidazolium tris-(pentafluoroethyl)trifluorophosphate, 1-butyl-3-methyl-imidazolium hexafluorophosphate,

tris(pentafluoroethyl)trifluorophosphate, trihexyl(tetradecyl)phosphonium, N"-ethyl-N,N,N', N'-tetramethylguanidinium, 1-butyl-1-methyl pyrroledinium tris(pentafluoroethyl) trifluorophosphate, 1-butyl-1-methyl pyrrolidinium bis(trifluoromethylsulfonyl) imide, 1-butyl-3-methyl imidazolium hexafluorophosphate, 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide and 1-n-butyl-3-methylimidazolium. The solvent may be removed after non-covalent or covalent interaction between the amphiphilic or hydrophilic polymer and the nanocrystal has been allowed to occur.

[0064] In the method the nanoparticle is contacted with an amphiphilic or with a hydrophilic polymer. As a result, the amphiphilic or hydrophilic material is wrapped around the nanoparticle. An amphiphilic polymer, and in some embodiments also a hydrophilic polymer, may be fixed to the nanoparticles via non-covalent or covalent interaction. Such interaction may be, but is not limited to, a coordinative bond, a Casimir interaction, a hydrophobic interaction, hydrogen bonding, a solvation force and a Van-der-Waals interaction. A hydrophilic polymer or an amphiphilic polymer may in some embodiments also form a shell entrapping the nanoparticle without being fixed thereto.

[0065] An amphiphilic or a hydrophilic polymer may be added to the nanoparticle in a suitable solvent, or the respective polymer may be provided in a suitable solvent and the nanoparticle may be added thereto. A suitable solvent may be a polar solvent, such as a polar protic solvent. A protic solvent is a solvent that has, for example, a hydrogen atom bound to an oxygen as in a hydroxyl group or a nitrogen as in an amine group. More generally, any molecular solvent which contains dissociable H^+ , such as hydrogen fluoride, is called a protic solvent. The molecules of such solvents can donate a H^+ (proton). Examples for polar protic solvents may be, but are not limited to, water, methanol, ethanol or acetic acid. In one embodiment of the present invention water may be used.

[0066] In the above procedure any organic solvent provided with the nanoparticle may be replaced by an aqueous solution after being contacted with the amphiphilic or hydrophilic polymer. In one embodiment the organic solvent is removed by evaporation.

[0067] Any hydrophilic polymer may be used, in particular if it can form a solid or a semi-solid particle. In some embodiments a hydrophilic polymer is a biocompatible polymer. The term "biocompatible polymer" (which also can be referred to as "tissue compatible polymer"), as used herein, is a polymer that produces little if any adverse biological response when used in vivo. The term thus generally refers to the inability of a polymer to promote a measurably adverse biological response in a cell, including in the body of an animal, including a human. A biocompatible polymer can have one or more of the following properties: non-toxic, non-

mutagenic, non-allergenic, non-carcinogenic, and/or non-irritating. A biocompatible polymer, in the least, can be innocuous and tolerated by the respective cell and/or body. A biocompatible polymer, by itself, may also improve one or more functions in the body. A variety of biocompatible polymers is suitable for the formation of a microparticle according to the invention. The biocompatible polymers can be synthetic polymers, naturally occurring polymers or combinations thereof. As used herein the term "synthetic polymer" refers to polymers that are not found in nature, including polymers that are made from naturally occurring biomaterials. Examples of suitable biocompatible polymers include non-absorbable polymers such as polypropylene, polyethylene, polyethylene terephthalate, polytetrafluoroethylene, polyaryletherketone, nylon, fluorinated ethylene propylene, polybutester, and silicone, or copolymers thereof (e.g., a copolymer of polypropylene and polyethylene); absorbable polymers such as polyglycolic acid (PGA), polylactic acid (PLA), polycaprolactone, and polyhydroxyalkanoate, copolymers thereof (e.g., a copolymer of PGA and PLA), and mixtures thereof.

[0068] A wide variety of biodegradable polymers is also suitable for a water-soluble microparticle or nanoparticle. Biodegradable polymers, as defined herein, are a subset of biocompatible polymers that gradually disintegrate or are absorbed in vivo over a period of time (e.g., within months or years). Disintegration may for instance occur via hydrolysis, may be catalysed by an enzyme and may be assisted by conditions to which the microparticles are exposed in the cell. Examples of biodegradable polymers suitable for a microparticle according to the invention include, but are not limited to, polyesters, polyanhydrides, polyorthoesters, polyphosphazenes, polyphosphates, polyphosphoesters, polyphosphonates, polydioxanones, polyhydroxyalkanoates, polycarbonates, polyalkylcarbonates, polyorthocarbonates, polyesteramides, polyamides, polyamines, polypeptides, polyurethanes, polyetheresters, or combinations thereof. An illustrative example of a biodegradable polymer is poly(α -hydroxy acid), for example polylactic acid, polyglycolic acid and copolymers and mixtures thereof such as poly(L-lactide) (PLLA), poly(D,L-lactide) (PLA); poly(glycolide) (PGA), poly(L-lactide-co-D,L-lactide) (PLLA/PLA), poly(L-lactide-co-glycolide) (PLLA/PGA), poly(D,L-lactide-co-glycolide) (PLA/PGA), poly(glycolide-co-trimethylene carbonate) (PGA/PTMC), poly(D,L-lactide-co-caprolactone) (PLA/PCL), poly(glycolide-co-caprolactone) (PGA/PCL); polyethylene oxide (PEO); polydioxanone (PDS); polypropylene fumarate; poly(ethyl glutamate-co-glutamic acid); poly(tert-butyloxy-carbonylmethyl glutamate); poly(carbonate-esters). Further examples of suitable biodegradable polymers include a polylacton such as a poly(ϵ -caprolactone) (PCL) and copolymers thereof such as polycaprolactone co-butylacrylate; polyhydroxybutyrate (PHBT) and copolymers of polyhydroxybutyrate;

poly(phosphazene); poly(phosphate ester); a polypeptide; a polydepsipeptide, a maleic anhydride copolymer; a poly-phosphazene; a polyiminocarbonate; poly(dimethyl-trimethylene carbonate-co-trimethylene carbonate); a polydioxanone, polyvalerolactone, a polyorthoester, a polyanhydride, polycyanoacrylate; a tyrosine-derived polycarbonate or polyester-amide; a polysaccharide such as hyaluronic acid; and copolymers and mixtures of the above polymers, among others. In some embodiments the biocompatible polymer may be crosslinked, for example to improve mechanical stability of the microparticle.

[0069] A further illustrative example of a suitable biocompatible polymer is a crosslinked dextrane polymer. The formation of nanoparticles of a crosslinked dextrane polymer has been described by Li et al. (*Angew. Chem. Int. Ed.* (2009) 48, DOI: 10.1002/anie.200904260). Such nanoparticles can be prepared from dextran-lipoic acid derivatives and can be crosslinked using a catalytic amount of dithiothreitol. By the degree of substitution, the ratio of lipoic acid and anhydroglucosidic units, the particle size can be controlled. Using particularly low degrees of substitution microparticles can be obtained following the protocol of Li et al. A further factor controlling particle size is the amount of crosslinking by dithiothreitol. High amounts of dithiothreitol lead to the formation of microparticles.

[0070] In certain embodiments, the water-soluble microparticle or nanoparticle is formed from a biocompatible polymer, such as a biodegradable polymer. It may for instance be formed from a poly(α -hydroxy acid), such as a poly(lactide) ("PLA"), a copolymer of lactide and glycolide, such as a poly(D,L-lactide-co-glycolide) ("PLG"), or a copolymer of D,L-lactide and caprolactone. Poly(D,L-lactide-co-glycolide) polymers include those having a lactide/glycolide molar ratio ranging, for example, from 20:80 to 25:75 to 40:60 to 45:55 to 55:45 to 60:40 to 75:25 to 80:20, and having a molecular weight ranging, for example, from 5,000 to 10,000 to 20,000 to 40,000 to 50,000 to 70,000 to 100,000 to 200,00 Daltons. The microparticle may also include or be formed from poly (lactic acid)-d- α -tocopheryl polyethylene glycol 1000 succinate (e.g. Pan, J., et al., *Biotechnology & Bioengineering* (2008) 101, 622-633). Yet further illustrative examples of a biocompatible polymer are a collagen, a chitosan, an alginate, heparin, gelatin and hyaluronic acid, six naturally occurring polymers. In this regard polyhydroxybutyrate (supra) is a polyester produced as granules by microorganisms.

[0071] In some embodiments the water-soluble microparticle or nanoparticle includes a plurality of nanoparticles of a metal oxide. The nanoparticles of a metal oxide may be included in the water-soluble microparticle(s) or nanoparticle(s) in an amount of about 0.01 to about 60 wt % of the amphiphilic or hydrophilic (including biocompatible) polymer, such as about 0.1

to about 60 wt %, about 0.1 to about 50 wt %, about 0.1 to about 45 wt %, about 0.1 to about 40 wt %, about 1 to about 40 wt %, about 2 to about 40 wt %, about 5 to about 40 wt %, about 10 to about 40 wt %, about 1 to about 35 wt %, about 1 to about 30 wt %, about 5 to about 30 wt %, about 10 to about 30 wt %, about 5 to about 25 wt %, about 10 to about 25 wt % or about 10 to about 20 wt % of the biocompatible polymer.

[0072] The water-soluble microparticle or nanoparticle may in some embodiments be a micro- or nanosphere, i.e. a matrix-filled system without a void or cavity. In some embodiments the microparticle or nanoparticle may be of non-homogenous structure. As an illustrative example, the microparticle or nanoparticle may have a core loaded with further matter. In some
10 embodiments the microparticle or nanoparticle may have a shell loaded with further matter. In some embodiments the water-soluble microparticle or nanoparticle includes a pharmaceutically active compound. The pharmaceutically active compound may be a low molecular weight organic compound. In some embodiments the pharmaceutically active compound is or includes a peptide, a protein, a lipid, a saccharide or a polysaccharide. The
15 pharmaceutically active compound may be more or less homogeneously distributed, e.g. dispersed, within the water-soluble microparticle or nanoparticle. In some embodiments the pharmaceutically active compound is located within a certain portion of the water-soluble microparticle or nanoparticle, such as a core or a shell.

[0073] Where desired, the water-soluble microparticle or nanoparticle may be designed for
20 sustained and for controlled delivery. In a sustained system the pharmaceutically active compound is delivered over a prolonged period of time, which overcomes the highly periodic nature of tissue levels associated with conventional (e.g. enteral or parenteral) administration of single doses of free compounds. The term 'controlled' indicates that control is exerted over the way in which the pharmaceutically active compound is delivered to the tissues once it has
25 been administered to the organism to be treated, e.g. the patient. The use of standard biocompatible polymers is particularly useful for sustained release. Additional tissue specific ligands such as antibodies may be used for controlled release.

[0074] Accordingly, a rare earth metal doped nanoparticle of the invention may be used as a fluorescent label in a variety of applications. In some embodiments the dispersible
30 nanoparticles of a metal oxide (or the plurality thereof) formed by the method of the invention is coupled to a molecule with binding affinity for a selected target molecule, such as a microorganism, a virus particle, a peptide, a peptoid, a protein, a nucleic acid, a peptide, an oligosaccharide, a polysaccharide, an inorganic molecule, a synthetic polymer, a small organic molecule or a drug.

[0075] The term "nucleic acid molecule" as used herein refers to any nucleic acid in any possible configuration, such as single stranded, double stranded or a combination thereof. Nucleic acids include for instance DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), analogues of the DNA or RNA generated using nucleotide analogues or using nucleic acid chemistry, locked nucleic acid molecules (LNA), and protein nucleic acids molecules (PNA). DNA or RNA may be of genomic or synthetic origin and may be single or double stranded. In the present method of the invention typically, but not necessarily, an RNA or a DNA molecule will be used. Such nucleic acid can be e.g. mRNA, cRNA, synthetic RNA, genomic DNA, cDNA synthetic DNA, a copolymer of DNA and RNA, oligonucleotides, etc. A respective nucleic acid may furthermore contain non-natural nucleotide analogues and/or be linked to an affinity tag or a label. In some embodiments the nucleic acid molecule may be isolated, enriched, or purified. The nucleic acid molecule may for instance be isolated from a natural source by cDNA cloning or by subtractive hybridization. The natural source may be mammalian, such as human, blood, semen, or tissue. The nucleic acid may also be synthesized, e.g. by the triester method or by using an automated DNA synthesizer.

[0076] Many nucleotide analogues are known and can be used in nucleic acids and oligonucleotides used in the methods of the invention. A nucleotide analogue is a nucleotide containing a modification at for instance the base, sugar, or phosphate moieties. Modifications at the base moiety include natural and synthetic modifications of A, C, G, and T/U, different purine or pyrimidine bases, such as uracil-5-yl, hypoxanthin-9-yl, and 2-aminoadenin-9-yl, as well as non-purine or non-pyrimidine nucleotide bases. Other nucleotide analogues serve as universal bases. Universal bases include 3-nitropyrrole and 5-nitroindole. Universal bases are able to form a base pair with any other base. Base modifications often can be combined with for example a sugar modification, such as for instance 2'-O-methoxyethyl, e.g. to achieve unique properties such as increased duplex stability.

[0077] A peptide may be of synthetic origin or isolated from a natural source by methods well-known in the art. The natural source may be mammalian, such as human, blood, semen, or tissue. A peptide, including a polypeptide may for instance be synthesized using an automated polypeptide synthesizer. Illustrative examples of polypeptides are an antibody, a fragment thereof and a proteinaceous binding molecule with antibody-like functions. Examples of (recombinant) antibody fragments are Fab fragments, Fv fragments, single-chain Fv fragments (scFv), diabodies, triabodies (Iliades, P., et al., *FEBS Lett* (1997) 409, 437-441), decabodies (Stone, E., et al., *Journal of Immunological Methods* (2007) 318, 88-94) and other domain antibodies (Holt, L.J., et al., *Trends Biotechnol.* (2003), 21, 11, 484-490). An example of a

proteinaceous binding molecule with antibody-like functions is a mutein based on a polypeptide of the lipocalin family (WO 03/029462, Beste et al., *Proc. Natl. Acad. Sci. U.S.A.* (1999) 96, 1898-1903). Lipocalins, such as the bilin binding protein, the human neutrophil gelatinase-associated lipocalin, human Apolipoprotein D or glycodefin, possess natural ligand-binding sites that can be modified so that they bind to selected small protein regions known as haptens. Examples of other proteinaceous binding molecules are the so-called glubodies (see e.g. international patent application WO 96/23879), proteins based on the ankyrin scaffold (Mosavi, L.K., et al., *Protein Science* (2004) 13, 6, 1435-1448) or crystalline scaffold (e.g. international patent application WO 01/04144) the proteins described in Skerra, *J. Mol. Recognit.* (2000) 13, 167-187, AdNectins, tetranectins and avimers. Avimers contain so called A-domains that occur as strings of multiple domains in several cell surface receptors (Silverman, J., et al., *Nature Biotechnology* (2005) 23, 1556-1561). Adnectins, derived from a domain of human fibronectin, contain three loops that can be engineered for immunoglobulin-like binding to targets (Gill, D.S. & Damle, N.K., *Current Opinion in Biotechnology* (2006) 17, 653-658). Tetranectins, derived from the respective human homotrimeric protein, likewise contain loop regions in a C-type lectin domain that can be engineered for desired binding (ibid.). Peptoids, which can act as protein ligands, are oligo(N-alkyl) glycines that differ from peptides in that the side chain is connected to the amide nitrogen rather than the α carbon atom. Peptoids are typically resistant to proteases and other modifying enzymes and can have a much higher cell permeability than peptides (see e.g. Kwon, Y.-U., and Kodadek, T., *J. Am. Chem. Soc.* (2007) 129, 1508-1509).

[0078] As a further illustrative example, a linking moiety such as an affinity tag may be used to immobilise the respective molecule. Such a linking moiety may be a molecule, e.g. a hydrocarbon-based (including polymeric) molecule that includes nitrogen-, phosphorus-, sulphur-, carben-, halogen- or pseudohalogen groups, or a portion thereof. As an illustrative example, the selected surface may include, for instance be coated with, a brush-like polymer, for example with short side chains. The immobilisation surface may also include a polymer that includes a brush-like structure, for example by way of grafting. It may for example include functional groups that allow for the covalent attachment of a biomolecule, for example a molecule such as a protein, a nucleic acid molecule, a polysaccharide or any combination thereof. Examples of a respective linking moiety functional group include, but are not limited to, an amino group, an aldehyde group, a thiol group, a carboxy group, an ester, an anhydride, a sulphonate, a sulphonate ester, an imido ester, a silyl halide, an epoxide, an aziridine, a phosphoramidite and a diazoalkane.

[0079] Examples of an affinity tag include, but are not limited to biotin, dinitrophenol or digoxigenin, oligohistidine, polyhistidine, an immunoglobulin domain, maltose-binding protein, glutathione-S-transferase (GST), calmodulin binding peptide (CBP), FLAG'-peptide, the T7 epitope (Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly), maltose binding protein (MBP), the HSV epitope of the sequence Gln-Pro-Glu-Leu-Ala-Pro-Glu-Asp-Pro-Glu-Asp of herpes simplex virus glycoprotein D, the hemagglutinin (HA) epitope of the sequence Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala, the "myc" epitope of the transcription factor c-myc of the sequence Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu, or an oligonucleotide tag. Such an oligonucleotide tag may for instance be used to hybridise to an immobilised oligonucleotide with a complementary sequence. A further example of a linking moiety is an antibody, a fragment thereof or a proteinaceous binding molecule with antibody-like functions (see also above).

[0080] A further example of linking moiety is a cucurbituril or a moiety capable of forming a complex with a cucurbituril. A cucurbituril is a macrocyclic compound that includes glycoluril units, typically self-assembled from an acid catalyzed condensation reaction of glycoluril and formaldehyde. A cucurbit[n]uril, (CB[n]), that includes n glycoluril units, typically has two portals with polar ureido carbonyl groups. Via these ureido carbonyl groups cucurbiturils can bind ions and molecules of interest. As an illustrative example cucurbit[7]uril (CB[7]) can form a strong complex with ferrocenemethylammonium or adamantylammonium ions. Either the cucurbit[7]uril or e.g. ferrocenemethylammonium may be attached to a biomolecule, while the remaining binding partner (e.g. ferrocenemethylammonium or cucurbit[7]uril respectively) can be bound to a selected surface. Contacting the biomolecule with the surface will then lead to an immobilisation of the biomolecule. Functionalised CB[7] units bound to a gold surface via alkanethiolates have for instance been shown to cause an immobilisation of a protein carrying a ferrocenemethylammonium unit (Hwang, I., et al., *J. Am. Chem. Soc.* (2007) 129, 4170-4171).

[0081] Further examples of a linking moiety include, but are not limited to an oligosaccharide, an oligopeptide, biotin, dinitrophenol, digoxigenin and a metal chelator (cf. also below). As an illustrative example, a respective metal chelator, such as ethylenediamine, ethylenediaminetetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), diethylenetriaminepentaacetic acid (DTPA), N,N-bis(carboxymethyl)glycine (also called nitrilotriacetic acid, NTA), 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), 2,3-dimercapto-1-propanol (dimercaprol), porphine or heme may be used in cases where the target molecule is a metal ion. As an example, EDTA forms a complex with most monovalent, divalent, trivalent and tetravalent metal ions, such as e.g. silver (Ag^+), calcium (Ca^{2+}),

manganese (Mn^{2+}), copper (Cu^{2+}), iron (Fe^{2+}), cobalt (Co^{3+}) and zirconium (Zr^{4+}), while BAPTA is specific for Ca^{2+} . In some embodiments a respective metal chelator in a complex with a respective metal ion or metal ions defines the linking moiety. Such a complex is for example a receptor molecule for a peptide of a defined sequence, which may also be included in a protein. As an illustrative example, a standard method used in the art is the formation of a complex between an oligohistidine tag and copper (Cu^{2+}), nickel (Ni^{2+}), cobalt (Co^{2+}), or zinc (Zn^{2+}) ions, which are presented by means of the chelator nitrilotriacetic acid (NTA).

[0082] Avidin or streptavidin may for instance be employed to immobilise a biotinylated nucleic acid, or a biotin containing monolayer of gold may be employed (Shumaker-Parry, J.S., et al., *Anal. Chem.* (2004) 76, 918). As yet another illustrative example, the biomolecule may be locally deposited, e.g. by scanning electrochemical microscopy, for instance via pyrrole-oligonucleotide patterns (e.g. Fortin, E., et al., *Electroanalysis* (2005) 17, 495). In other embodiments, in particular where the biomolecule is a nucleic acid, the biomolecule may be directly synthesised on the surface of the immobilisation unit, for example using photoactivation and deactivation. As an illustrative example, the synthesis of nucleic acids or oligonucleotides on selected surface areas (so called "solid phase" synthesis) may be carried out using electrochemical reactions using electrodes. An electrochemical deblocking step as described by Egeland & Southern (*Nucleic Acids Research* (2005) 33, 14, e125) may for instance be employed for this purpose. A suitable electrochemical synthesis has also been disclosed in US patent application US 2006/0275927. In some embodiments light-directed synthesis of a biomolecule, in particular of a nucleic acid molecule, including UV-linking or light dependent 5'-deprotection, may be carried out.

[0083] The molecule that has a binding affinity for a selected target molecule may be immobilised on the nanoparticles by any means. As an illustrative example, an oligo- or polypeptide, including a respective moiety, may be covalently linked to the surface of nanoparticles via a thio-ether-bond, for example by using ω functionalized thiols. Any suitable molecule that is capable of linking a nanoparticle of the invention to a molecule having a selected binding affinity may be used to immobilise the same on a nanoparticle. For instance a (bifunctional) linking agent such as ethyl-3-dimethylaminocarbodiimide, N-(3-aminopropyl) 3-mercapto-benzamide, 3-aminopropyl-trimethoxysilane, 3-mercaptopropyl-trimethoxysilane, 3-(trimethoxysilyl) propyl-maleimide, or 3-(trimethoxysilyl) propyl-hydrazide may be used. Prior to reaction with the linking agent, the surface of the nanoparticles can be modified, for example by treatment with glacial mercaptoacetic acid, in order to generate free mercaptoacetic groups which can then be employed for covalently coupling with an analyte

binding partner via linking agents.

[0084] As already mentioned above, rare-earth metals have certain unique properties in terms of their photoluminescence behaviour. Rare earth metal ions have a partially filled 4f-electron shell, proving them with unique characteristics. 5s and 5p shells are filled in atoms of these elements (Aufbau principle). The 4f shell, however, does not have a larger radius than the 5s and 5p shells, but is rather contracted and bounded by the 5s and 5p electron shells. Hence, the 4f-electron shell is shielded from the ambience by the outer 5s and 5p electron shells. The average radius of the 4f shell is about 0.7 times the Bohr radius, with a slight decrease e.g. along the Lanthanide series. The electrons in the 4f subshell are strongly localized and do not participate in chemical bonding. Ions of e.g. the Lanthanides lack two electrons of the 6s shell and either a 4f or a 5d electron. As a result, rare earth metal ions have the unique property of sharp spectral lines in the solid phase. The energy spectrum of a rare earth doped material accordingly has a series of narrow lines rather than a broad energy spectrum. A continuous broad spectrum is, in contrast thereto, found for the majority of inorganic solids, due to interactions between ions that cause line broadening and overlapping of spectral lines.

[0085] A 4f electron of a rare earth metal ion can be excited, upon irradiation with light. It can firstly be excited to the 5d and 5g orbitals, orbitals much farther removed from the atom nucleus, and in a solid overlapping with neighbouring ions. Secondly, and of particular practical relevance in the context of the invention, intra-ionic coulomb interactions split the energy levels of the 4f-electronic states of rare earth metal ions, with the 7F term lying lowest. Spin-orbit splittings further result in an atomic-like level structure. Therefore the energy levels of the 4f shell that are of interest here can be described as a 7F multiplet. In describing the properties of a particular state, only the J total angular quantum number is required due to the strong spin-orbit coupling characteristic of rare earth metal ions, such as the lanthanides. Hence, the energy level is described as a 7F_J level ($J = 0, 1, 2, 3, 4, 5, 6$). 7F_0 is the ground state or ground level. Due to these different energy levels, intra-4f shell transitions occur upon irradiation. Such $4f \leftrightarrow 4f$ transitions are very sharp in energy (narrow absorption and emission bands), since the 4f electrons are effectively shielded by the filled 5s and 5p shells (supra). As a result, nearly atom-like narrow-line absorption and emission spectra are observed, with excited state lifetimes reaching the millisecond timescale.

[0086] It has however been found that in pure rare earth metal ions, including oxides, excitation energy can migrate to traps where it can be quenched nonradiatively. This process of energy transfer to traps is called concentration quenching, and renders luminescence from rare earth metal oxides such as Eu_2O_3 too inefficient for use as a commercial phosphor. In Eu_2O_3

about 97% of absorbed radiation is quenched at luminescence traps, for example. Eu_2O_3 is however commonly used as a source of Eu^{3+} ions in the production of other phosphors and laser materials. In these materials the Eu^{3+} ions are dispersed within an insulating host to eliminate energy transfer and concentration quenching. As noted above, at least as far as nanoparticles are concerned, quenching can so far only be avoided if the concentration of rare earth metal ions is kept low, namely at molar percentages of the rare earth metal ion in the host of less than about 5-10 mol%. At higher amounts of the rare earth metal ion, concentration quenching occurs, resulting in an overall decrease in luminescence output.

[0087] A host metal oxide such as titanium oxide can be irradiated with light to excite electrons in a valence band to move to a conduction band. Electrons that are excited to move to the conduction band are not relaxed to move back directly to the valence band but trapped temporarily in a defect level. Subsequently such electrons move to the excitation level of the rare earth metal ion, e.g. Eu^{3+} , without being recombined with holes in the valence band.

[0088] Emission spectra of Eu^{3+} ions have been researched extensively, e.g. in glass crystals. The spectra consist of sharp lines due to transitions from the excited $^5\text{D}_0$ level to lower $^7\text{F}_j$ levels, two of which are the transitions $^5\text{D}_0 \rightarrow ^7\text{F}_1$ and $^5\text{D}_0 \rightarrow ^7\text{F}_2$ (cf. Fig. 3). For Terbium as a further example, transition is from the excited $^5\text{D}_4$ level. These transitions are the basis of a temperature dependent luminescence. As explained above, rare earth metal ions such as Eu^{3+} generate luminescence due to the transition from an excited $^5\text{D}_j$ level to the $^7\text{F}_j$ levels. This results in sharp emissions of distinct energy and high intensity. For the example of Eu^{3+} the transition $^5\text{D}_0 \rightarrow ^7\text{F}_1$ is a magnetic dipole transition that is nearly independent of the matrix. The transitions $^5\text{D}_0 \rightarrow ^7\text{F}_2$, $^5\text{D}_0 \rightarrow ^7\text{F}_4$ and $^5\text{D}_0 \rightarrow ^7\text{F}_6$ are electric dipole transitions with intensities that are, at least to a certain degree, sensitive to the chemical environment.

[0089] Based on these properties of a dispersible nanoparticle of a metal oxide according to the invention there is also provided a method of detection and/or of monitoring. In the method matter that can encompass the dispersible nanoparticle is labelled therewith. In particular, such a method can be used in *in-vivo*, *ex-vivo* or *in-vitro* systems. In such embodiments a water-dispersible nanoparticle or microparticle as described above is used. Such a water-dispersible nanoparticle or microparticle includes an amphiphilic or a hydrophilic polymer and one or more rare earth metal doped nanoparticles (supra). Matter or compartments such as a vesicle, a cell, an intracellular organelle or a tissue is labelled with a respective water-dispersible nanoparticle or microparticle. In some embodiments a cell is contacted with a water-dispersible nanoparticle or microparticle. Thereby the cell is allowed to take up the water-dispersible nanoparticle or microparticle. In the nanoparticle(s) a $4f \leftrightarrow 4f$ transition is induced

by irradiation. Hence, the matter or compartment, e.g. the cell, is irradiated with the wavelength of a $4f \leftrightarrow 4f$ transition of the rare earth metal that is included in the nanoparticle of a metal oxide according to the invention.

[0090] The irradiation may be selected to be of a wavelength of any desired $4f \leftrightarrow 4f$ transition. In some embodiments the wavelength is the wavelength of an excitation from the ground state to the first excited level. In the case of an Europium doped nanoparticle this $4f \leftrightarrow 4f$ transition is the excitation ${}^7F_0 \rightarrow {}^5D_0$. The wavelength of this transition is typically about 590 nm. A large variety of spectra with the respective wavelengths and patterns have been published and numerous theoretical approximations have been disclosed. While the whereabouts of a wavelength of a desired $4f \leftrightarrow 4f$ transition can thus easily be identified, the wavelengths of each particular composition and batch of particles may differ slightly, i.e. by 1 or a few nanometers. It may thus be advantageous to determine the photoluminescence properties of a nanoparticle or of a plurality of nanoparticles before carrying out the method of detection and/or of monitoring.

[0091] In the method, the emission of the water-dispersible nanoparticle or microparticle is further detected. In the case of an Europium doped nanoparticle an emission at about 615 nm corresponds to the ${}^5D_0 \rightarrow {}^7F_2$ transition. An emission at about 592 nm corresponds in the case of an Europium doped nanoparticle to the ${}^5D_0 \rightarrow {}^7F_1$ transition. In the case of a Terbium doped nanoparticle an emission at about 545 nm corresponds to the ${}^5D_4 \rightarrow {}^7F_5$ transition.

[0092] Accordingly, the wavelength of emission can be selected by doping the nanoparticle of the invention with a selected rare earth metal. Europium, erbium and thulium, respectively, can for instance generate red, green and blue emissions. Therefore different nanoparticles can be detected and/or monitored at the same time in the same matter or in parallel in several e.g. vesicles, cells, intracellular organelles or tissue portions. As an illustrative example, cells of different type or origin may be labelled with different water-dispersible nanoparticles or microparticles, including individually. The different water-dispersible nanoparticles or microparticles may include different rare earth metals as dopants. Since the labelled cells emit light of different wavelengths, they can be monitored and/ or detected at the same time, for instance within the same cell population.

[0093] Rare earth metal doped nanoparticles of the invention may for instance be used in an illuminant and displays, where they provide a particularly high brightness as explained above. Due to the temperature dependence of their luminescence (supra) they may also be included in or serve as temperature-sensitive probes. In some embodiments a respective rare earth metal doped nanoparticle may also be used as a photo-catalysts, for instance in form of a surface

coating, as which it may provide matter with self-cleansing properties.

EXEMPLARY EMBODIMENTS OF THE INVENTION

[0094] Exemplary embodiments of methods according to the invention as well as reactants and further processes that may be used are shown in the appending figures.

[0095] According to the method of the present invention europium-incorporated titanium oxide (Eu-TiO₂) nanoparticles have been prepared with a tunable molar percentage of europium from 0 to 90 mol%, and a drastic incorporation-level increase up to ≥ 50 mol% was achieved before self-quenching occurred. More than 65 times increase of emission was demonstrated while increasing europium molar percentage from 5 to 50 mol%, and obvious concentration quenching effect only occurred beyond the value.

[0096] All the chemically/environmentally stable metal oxides with high availability from the periods of 3 to 5 were systematically screened as potential matrix candidates to host an increased amount of emission centers for greatly improving emission properties of rare earth-incorporated metal oxides. White full-shelled metal oxides are usually suitable matrices because of good insulating properties to effectively localize photo-excited carriers of RE (the strong passivation of RE in matrices), and no efficient self-emission pathways and no internal electron transitions for causing emission quenching of RE. As a consequence, a list of different 50 mol% europium-incorporated metal oxides was synthesized and their emission intensity (used wavelength 396nm) under direct $^7F_0 \rightarrow ^5L_6$ excitation of Eu³⁺ was observed with a sequence of:



For example, the Eu-ZrO₂ nanoparticles exhibited high luminescence of 7 and 18 times as strong as the Eu-TiO₂ and the Eu-Y₂O₃ nanoparticles, respectively.

General Procedure of Forming Earth Metal doped Nanoparticles of a Metal Oxide

[0097] In this example the red-emission europium doped titanium oxide (for example, TiO₂:Eu 15%) is used as a demonstration. In a typical experiment, 0.15 mmol europium acetate (EuAc₃) was dissolved in 5 mmol oleic acid together with 6 ml 1-octadecene (ODE) at 80 °C under vacuum for 1 hour. Subsequently 1 mmol titanium isopropoxide (TIP) was injected to the solution and maintained at 80 °C for 20 mins. After that the temperature was increased to 260 °C, appropriate oleylamine (3-6 mmol) was injected. The reaction lasts 1-2 hours including aging at higher temperature (300 °C).

[0098] The general synthetic procedure is described as follows: In a typical experiment, a certain amount of rare-earth metal salt(s) is dissolved in a mixture of an alkyl chain acid and a high-boiling organic solvent at a temperature ranging from 25 to 200 °C for about 1 hour. Subsequently a predetermined amount of titanium isopropoxide (TIP) at a molar ratio range up to 1000 x is then injected to the solution and maintained at the same temperature for 20 min. Subsequently the temperature is increased to a value in the range from 200 to 350 °C, and an appropriate amount of oleylamine is injected. The reaction lasts 1-2 hours including aging at a higher temperature.

General Procedure of Forming Water-soluble Micro- or Nanoparticles that include the Earth Metal doped Nanoparticles of a Metal Oxide

[0099] After purification, TiO₂:Eu particles (~20 mg) were dispersed in DCM (dichloromethane) and mixed with PLGA (Poly (D,L-lactic-co-glycolic acid ~50 mg) in DCM solution. Nanoparticle-incorporated PLGA particles were further prepared by a modified emulsion-solvent evaporation method by using PVA (polyvinyl alcohol) as an emulsifier. Briefly, the obtained solution (4 ml) of nanoparticles and PLGA was added dropwise into 24 ml aqueous solution of 2 wt% PVA, under magnetic stirring followed by emulsification for 90 s with a homogenizer. Solid nanoparticle-incorporated PLGA particles were successfully obtained by solvent evaporation of DCM from oil-in-water droplets. The resulting water-dispersible PLGA particles were collected by centrifugation and further washed with water for 3 times to remove excessive emulsifier. As-purified particles were dispersed in water, followed by freeze-drying into fine powder for use.

[0100] Highly luminescent, multicolour rare earth-incorporated metal oxide nanophosphors were prepared by a non-hydrolytic aminolysis process of two metal precursors at high temperature together. As used, herein the term phosphor refers to photoluminescent material, i.e., material that converts photons of one energy to photons of a different energy. A great advantage of this high-temperature wet-chemistry approach over conventional low-temperature sol-gel processes is to avoid post-annealing of products at temperatures of ~1,000 °C, which is undesirable for bio-applications due to bulky size and low suspension ability in aqueous solution. The first multicolour cellular imaging has been successfully demonstrated using colloidal rare earth-incorporated metal oxide nanoparticles through effectively passivation of various rare earth elements into non-toxic host matrices including TiO₂, SiO₂ and ZrO₂. In addition to greatly reduced cytotoxicity, greatly improved chemical stability high resistance to photobleaching and large Stokes shifts (preventing light scattering), these new

multicolour nanophosphors also present ultranarrow fixed lines determined by the electronic structure of RE and are almost independent of the host matrix for high reliability biological detection, as compared to quantum dots by varying particle sizes and/or compositions. For the first time, one of the multi-lines is selectively chosen to emit specific colours upon the direct excitation at well-defined longer wavelength (Eu^{3+} : ${}^7\text{F}_0 \rightarrow {}^5\text{D}_0$, an excitation from the ground level/state to the first excited state/level at 590 nm to give single emission line at 615 nm from ${}^5\text{D}_0 \rightarrow {}^7\text{F}_1$) for bio-imaging applications, and the short wavelength excitation only produces multi-lines for causing complexity of bio-applications.

[0101] In addition, the longer fluorescence lifetime also renders the obtained nanoparticles a new type of nanoprobe in time-resolved fluoroimmunoassays with increased S/N ratio (*i.e.* the assay sensitivity). Overall, the resulting colloidal rare-earth-incorporated metal oxide nanoparticles are also identified here as a promising alternative of prevailing luminescent probes like organic dyes and semiconductor quantum dots as bioprobes/nanotags/nanolabels for imaging, sensing, and diagnostics besides lighting applications.

Cell uptake of PLGA particles – Confocal microscopy

[0102] To study cell uptake of particles using confocal laser scanning microscopy, cells were seeded at 2.0×10^4 cells/cm² in Lab-Tek chambered cover glasses and cultured as a monolayer at 37 °C in a humidified atmosphere containing 5% CO₂. The cell uptake was started when the culture medium was replaced by particles suspension/dispersion (500 µg/mL in culture medium) and the monolayer was further incubated for 4 h at 37 °C. At the end of experiment, the cell monolayer was washed 3 times with fresh pre-warmed PBS buffer to eliminate excess nanoparticles which were not associated to the cells. Cells were then fixed with 70% ethanol. Nucleus staining was carried out using DAPI to facilitate determine the location of the particles inside the cells. The samples were then mounted in the fluorescent mounting medium (Dako). Confocal fluorescent microscopy was performed using an Olympus FV500 system supported with a 60x water-immersion objective. TiO₂:Eu-embedded PLGA microparticles were excited by 405 nm diode laser and fluorescent emission image were collected using 560IF filter while SiO₂:Tb-embedded PLGA particles were excited by 488 nm Ar laser and fluorescent emission images were collected using 505-560IF filter. Images were processed by FV10-ASW 1.3 Viewer.

[0103] Other RE-doped metal oxide nanoparticles are prepared in the similar manner.

[0104] The listing or discussion of a previously published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state

of the art or is common general knowledge. All documents listed are hereby incorporated herein by reference in their entirety for all purposes as if each individual document were specifically and individually indicated to be incorporated by reference.

[0105] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0106] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising”, “including,” “containing”, etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognised that various modifications are possible within the scope of the invention claimed. Additional objects, advantages, and features of this invention will become apparent to those skilled in the art upon examination of the foregoing examples and the appended claims. Thus, it should be understood that although the present invention is specifically disclosed by exemplary embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognise that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

Claims

What is claimed is:

1. A method of forming a dispersible rare earth metal doped nanoparticle of a metal oxide, the method comprising:
 - (a) dissolving a salt of a rare earth metal in a mixture of (i) a surfactant and (ii) a suitable solvent, wherein the solvent is at least essentially free of tri-*n*-octylphosphine oxide and at least essentially free of amines, thereby forming a first solution,
 - (b) adding to the first solution of the rare earth metal a precursor of the metal oxide, wherein the precursor of the metal oxide is added in an excess relative to the rare earth metal salt, thereby forming a second solution,
 - (c) bringing the second solution to a temperature selected in the range from about 200 °C to about 400 °C
 - (d) adding at the temperature selected in the range from about 200 °C to about 400 °C an amine selected from an alkylamine and a dialkylamine, thereby allowing the formation of a dispersible nanoparticle.
2. The method of claim 1, wherein the solvent, in a mixture of which with the surfactant the salt of a rare earth metal is dissolved, is a high-boiling solvent.
3. The method of claims 1 or 2, wherein the solvent is a non-coordinating solvent.
4. The method of claim 3, wherein the non-coordinating solvent is an alkene.
5. The method of claim 4, wherein the alkene is selected from the group consisting of 1-hexadecene, 1-heptadecene, 1-octadecene, 1-eicosene, 1,17-octadecadiene and mixtures thereof.
6. The method of any one of claims 1-5, wherein the amine is added in an excess relative to the precursor of the metal oxide.
7. The method of any one of claims 1-6, wherein the amine is one of 1-amino-9-octadecene (oleylamine), 1-amino-4-nonadecene, 1-amino-7-hexadecene, 1-amino-8-heptadecene, 1-amino-9-heptadecene 1-amino-9-hexadecene, 1-amino-9-eicosene, 1-amino-9,12-octadecadien, 1-amino-8,11-heptadecadiene, 1-amino-13-docosene, N-9-octadecenyl-propanediamine, N-octyl-2,7-octadienyl-amine, N-9-octadecen-1-yl-9-octadecen-

1-amine (dioleylamine), bis(2,7-octadienyl)amine, and N,N-dibutyl-2,7-octadienylamine.

5 8. The method of any one of claims 1-7, wherein the precursor of the metal oxide is selected from the group consisting of a metal oxide, an inorganic metal salt and an organic metal salt.

9. The method of any one of claims 1-8, wherein the surfactant is selected from the group consisting of an organic carboxylic acid, an organic phosphate, an organic phosphonic acid and mixtures thereof.

10 10. The method of claim 9, wherein the carboxylic acid has a main chain length of at least 6 carbon atoms.

15 11. The method of claims 9 or 10, wherein the organic carboxylic acid is selected from the group consisting of stearic acid (octadecanoic acid), lauric acid, oleic acid ([Z]-octadec-9-enoic acid), n-undecanoic acid, linoleic acid, ((Z,Z)-9,12-octadecadienoic acid), arachidonic acid ((all-Z)-5,8,11,14-eicosatetraenoic acid), linelaidic acid ((E,E)-9,12-octadecadienoic acid), myristoleic acid (9-tetradecenoic acid), palmitoleic acid (cis-9-hexadecenoic acid), myristic acid (tetradecanoic acid), palmitic acid (hexadecanoic acid), γ -homolinolenic acid ((Z,Z,Z)-8,11,14-eicosatrienoic acid) and mixtures thereof.

20 12. The method of any one of claims 1-11, wherein dissolving the salt of a rare earth metal is carried out under vacuum.

13. The method of any one of claims 1-12, wherein dissolving the salt of a rare earth metal is carried out at a temperature in the range from about 18 °C to about 80 °C.

25 14. The method of any one of claims 1-13, wherein forming a solution of cadmium or a compound thereof comprises heating the solvent to a temperature from about 100 °C to about 250 °C.

15. The method of any one of claims 1-14, further comprising:

(e) dispersing the obtained dispersible nanoparticle in a suitable solvent, adding an amphiphilic polymer, and allowing the formation of a water-dispersible nanoparticle or

microparticle, the water-dispersible nanoparticle or microparticle comprising a rare earth metal doped nanoparticle and a hydrophilic or an amphiphilic polymer.

- 5 **16.** A dispersible nanoparticle of a metal oxide, the metal oxide being doped with a rare-earth metal, wherein the nanoparticle is obtained by a method of any one of claims 1-15.

- 17.** A plurality of dispersible nanoparticles of a metal oxide according to claim 16, wherein the plurality of dispersible nanoparticles is comprised in a water-dispersible nanoparticle or microparticle, the water-dispersible nanoparticle or microparticle further comprising a hydrophilic or an amphiphilic polymer.

- 10 **18.** The plurality of dispersible nanoparticles of a metal oxide of claim 17, wherein the percentage of dispersible nanoparticles of a metal oxide in the water-dispersible nanoparticle or microparticle is selected in the range from about 0.1 to about 50 wt % of the hydrophilic or the amphiphilic polymer.

- 15 **19.** The use of a nanoparticle obtained by the method according to any one of claims 1-14 in the manufacture of an illuminant.

- 20.** A method of monitoring a cell with a colour of a specific wavelength, the method comprising:

- 20 (a) contacting the cell with a water-dispersible nanoparticle or microparticle obtained according to the method of claim 15, thereby allowing the cell to take up the water-dispersible nanoparticle or microparticle,
 (b) irradiating the cell with the wavelength of a $4f \leftrightarrow 4f$ transition of the rare earth metal, and
 (c) detecting the emission of the water-dispersible nanoparticle or microparticle.

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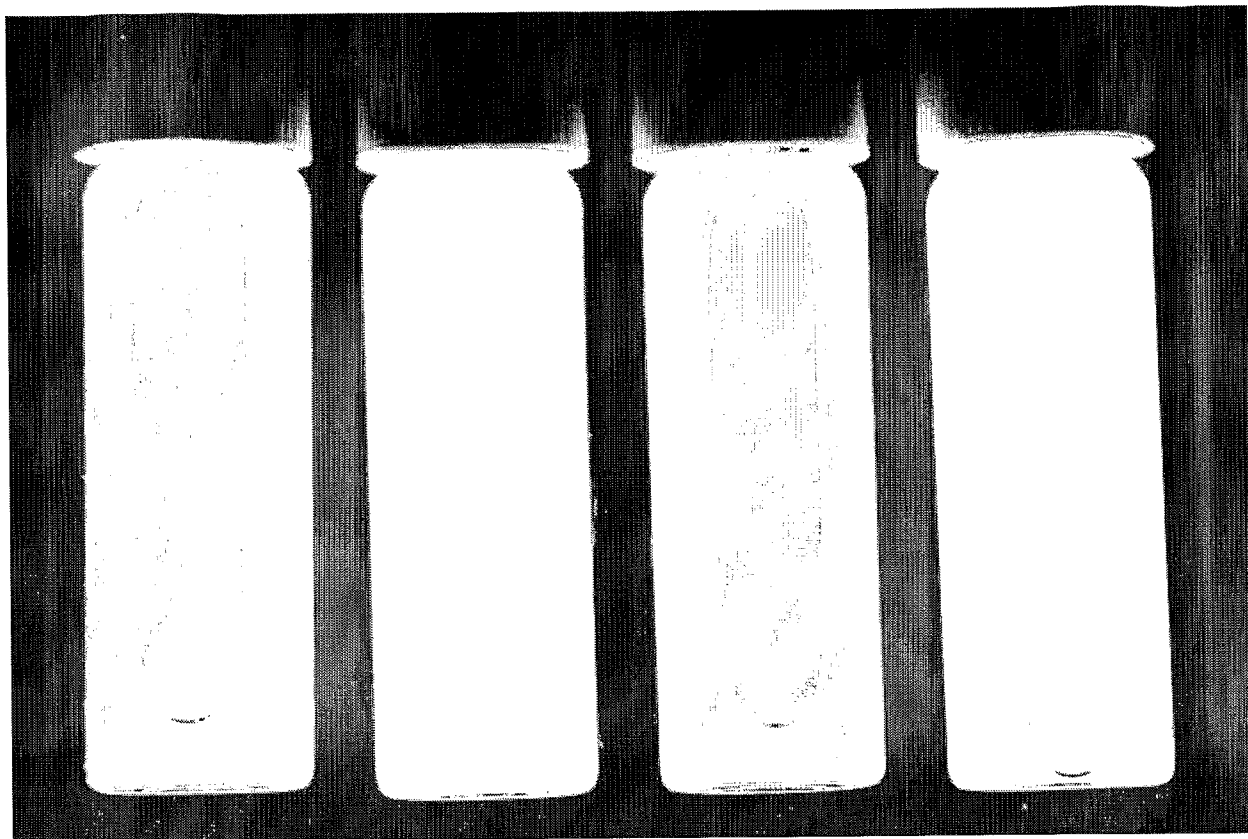
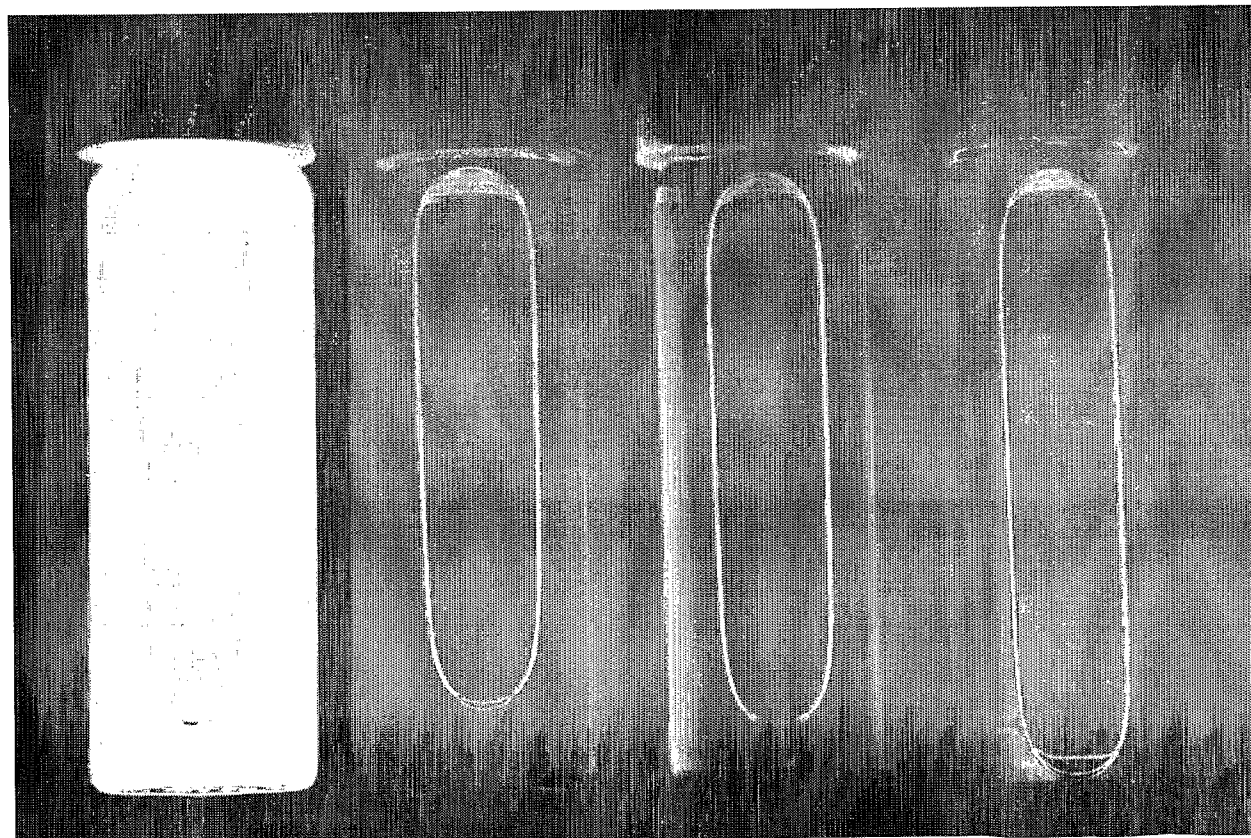


Fig. 1A

Fig. 1B



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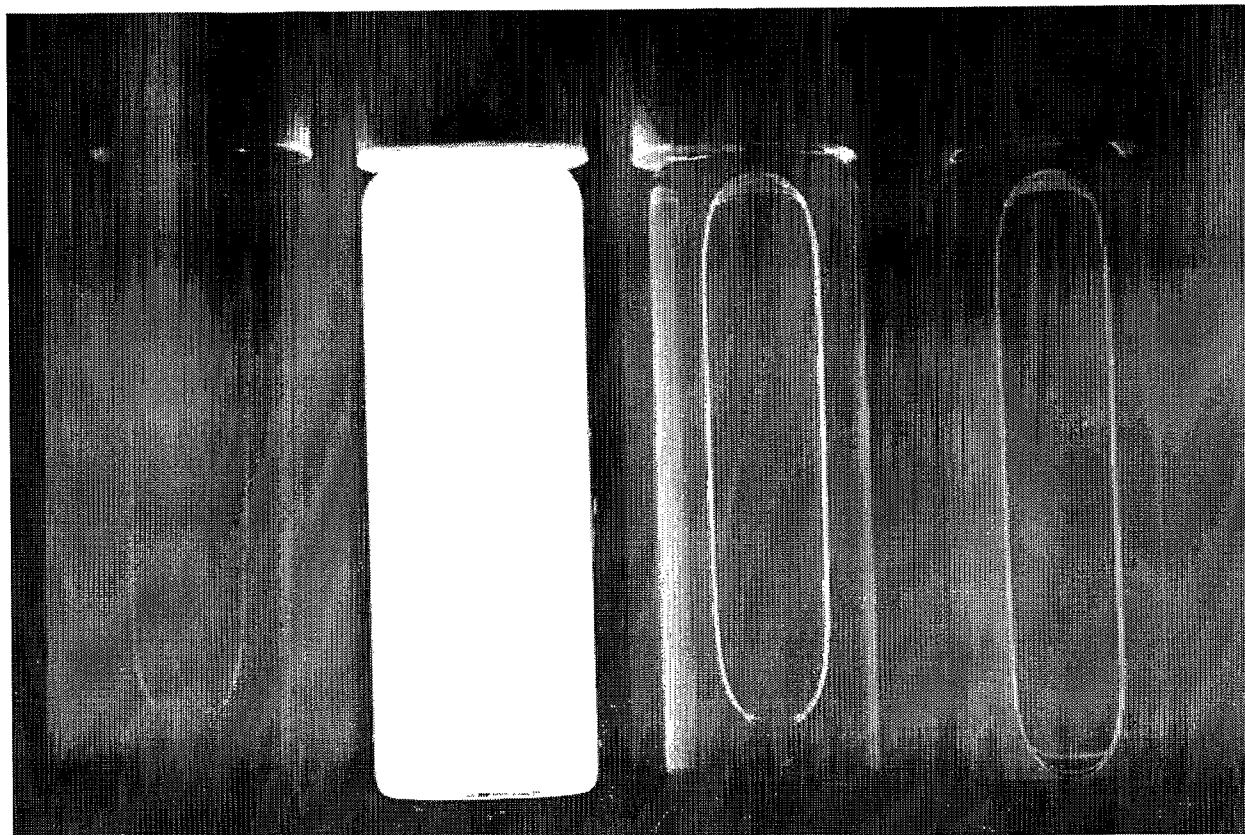
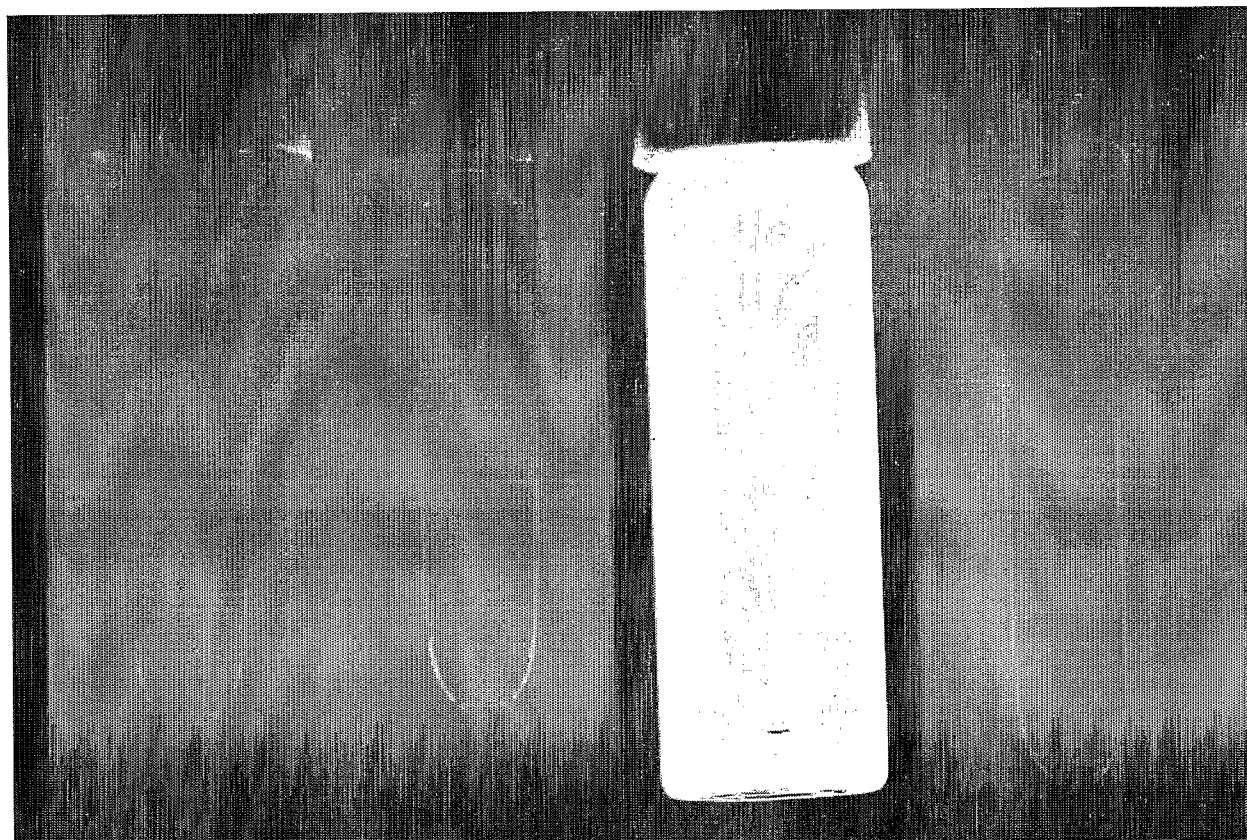
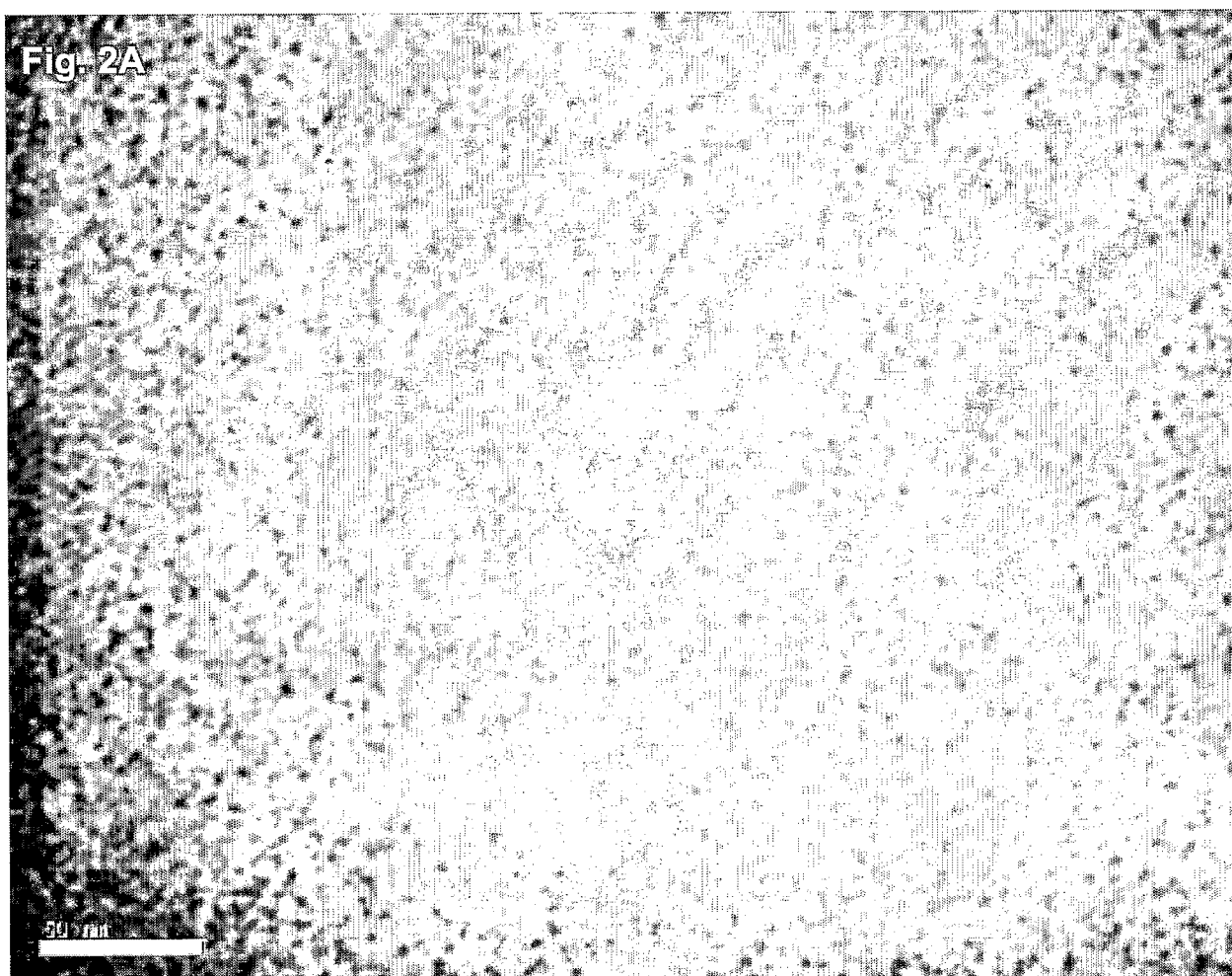
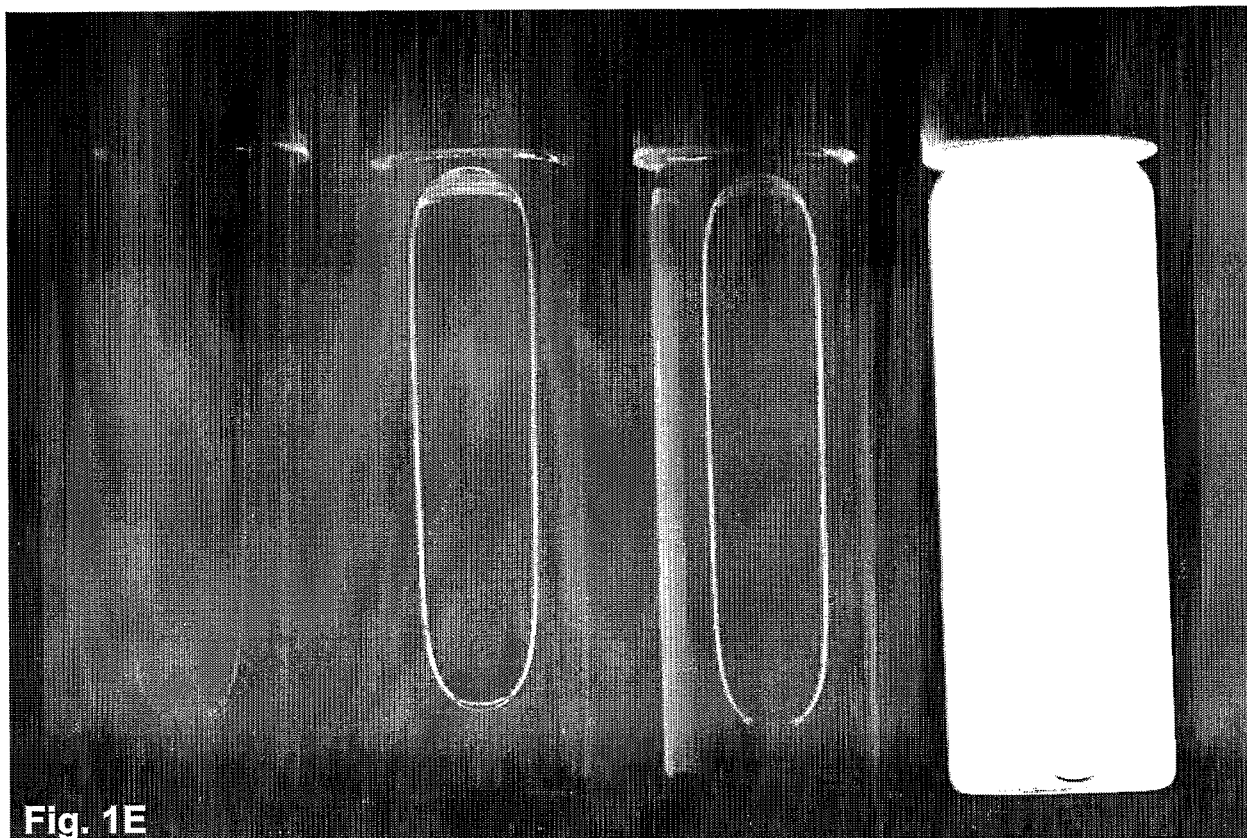


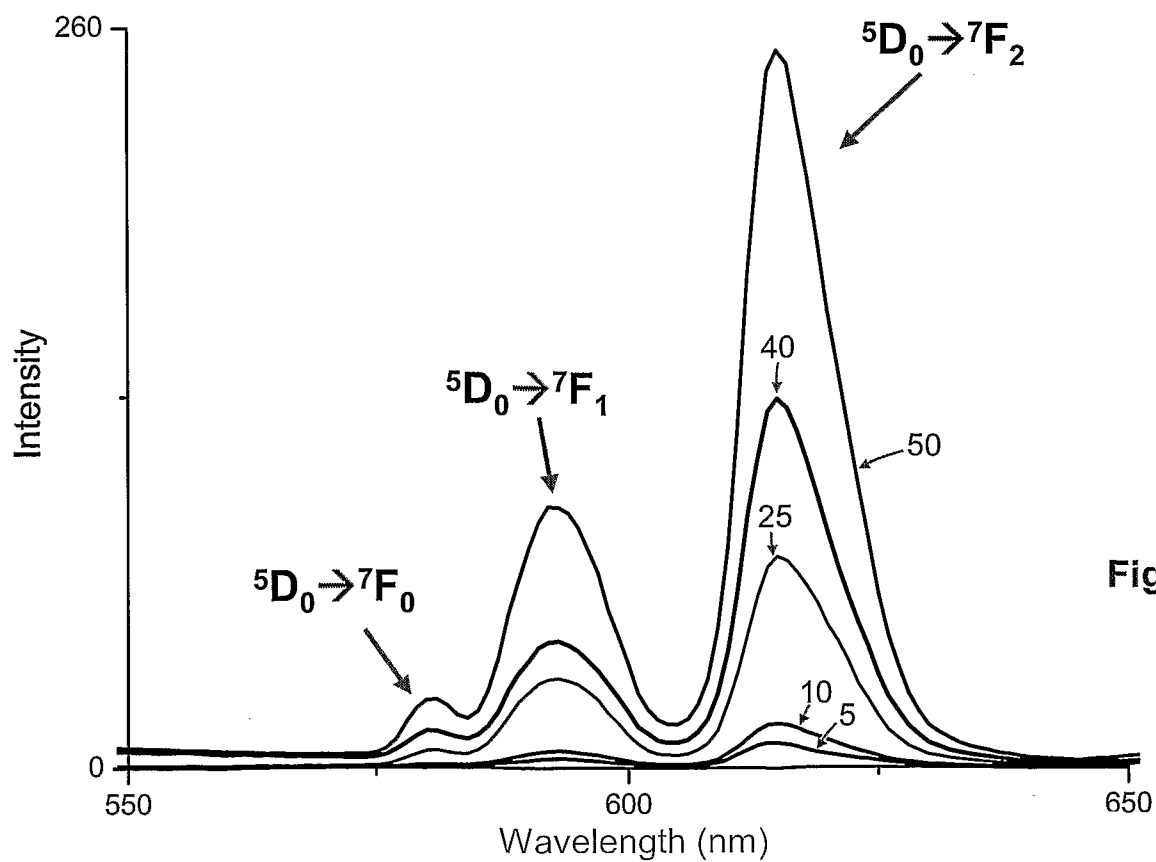
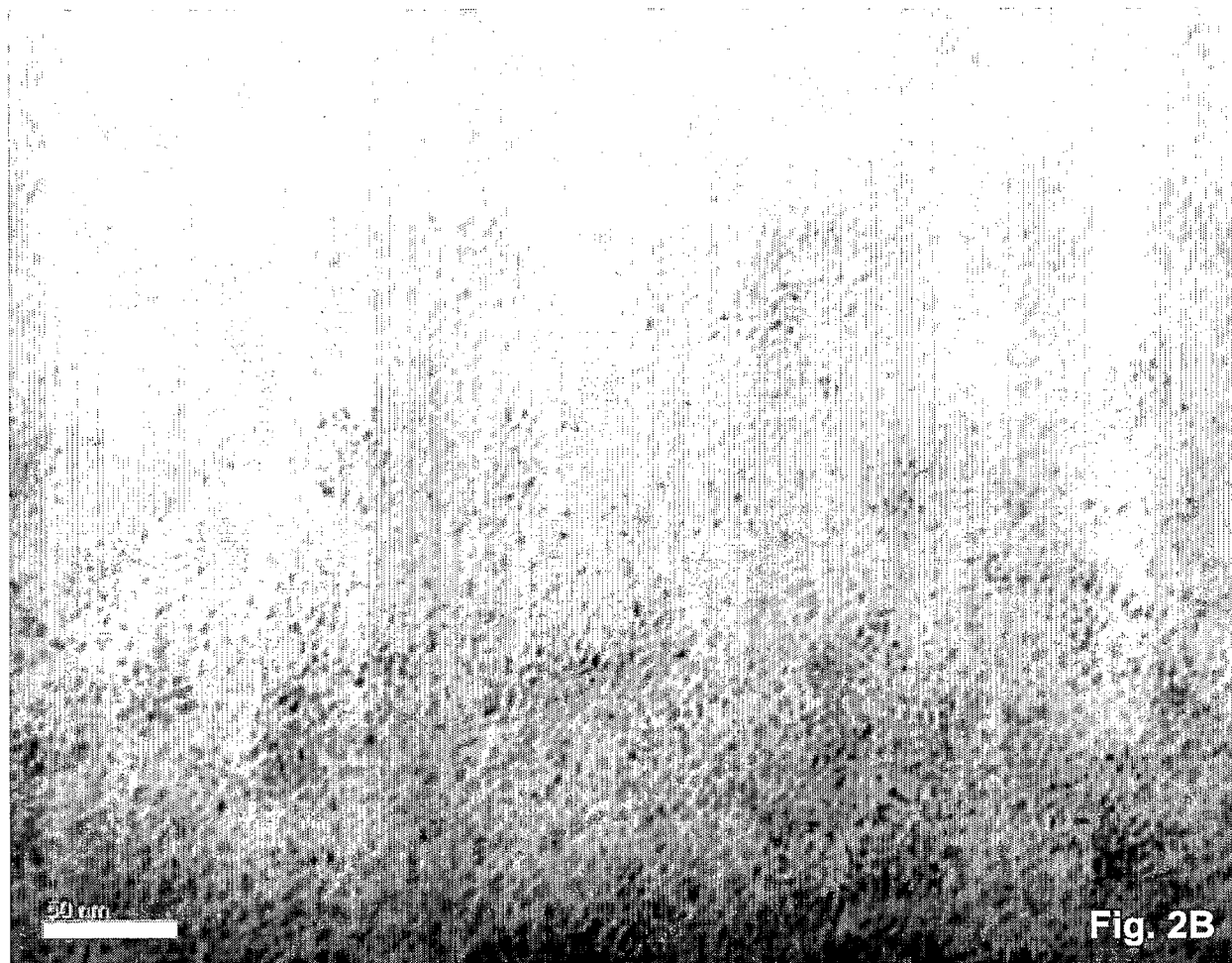
Fig. 1C

Fig. 1D

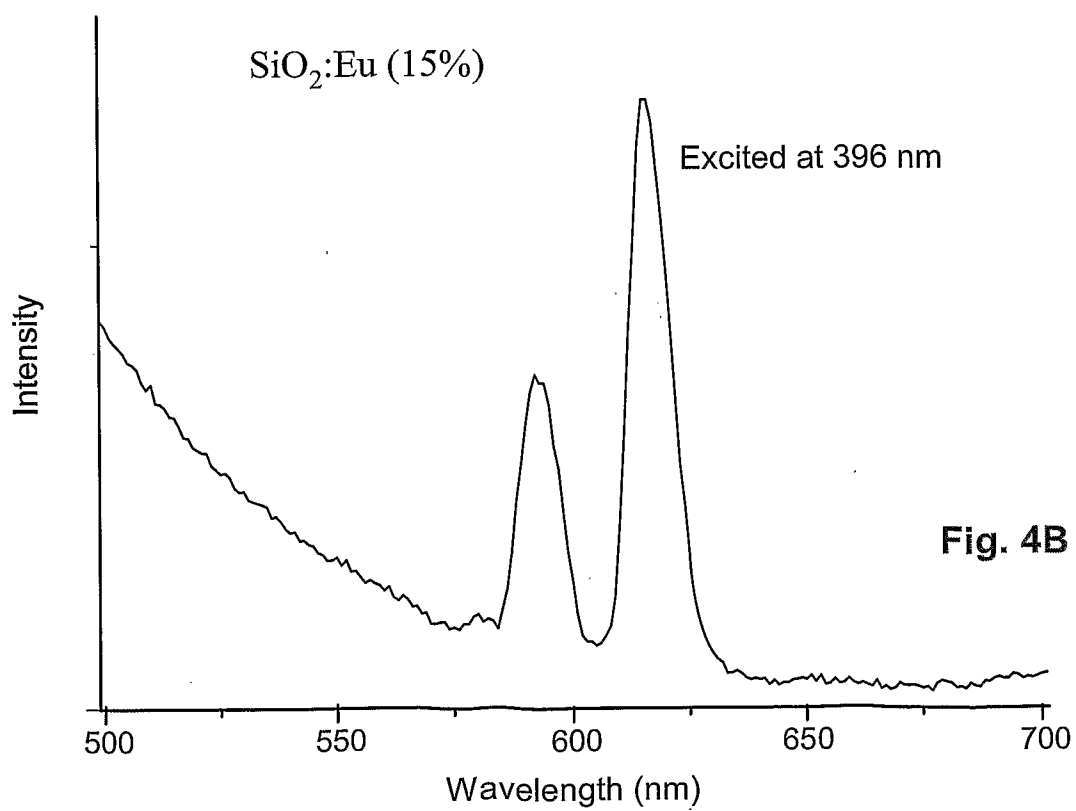
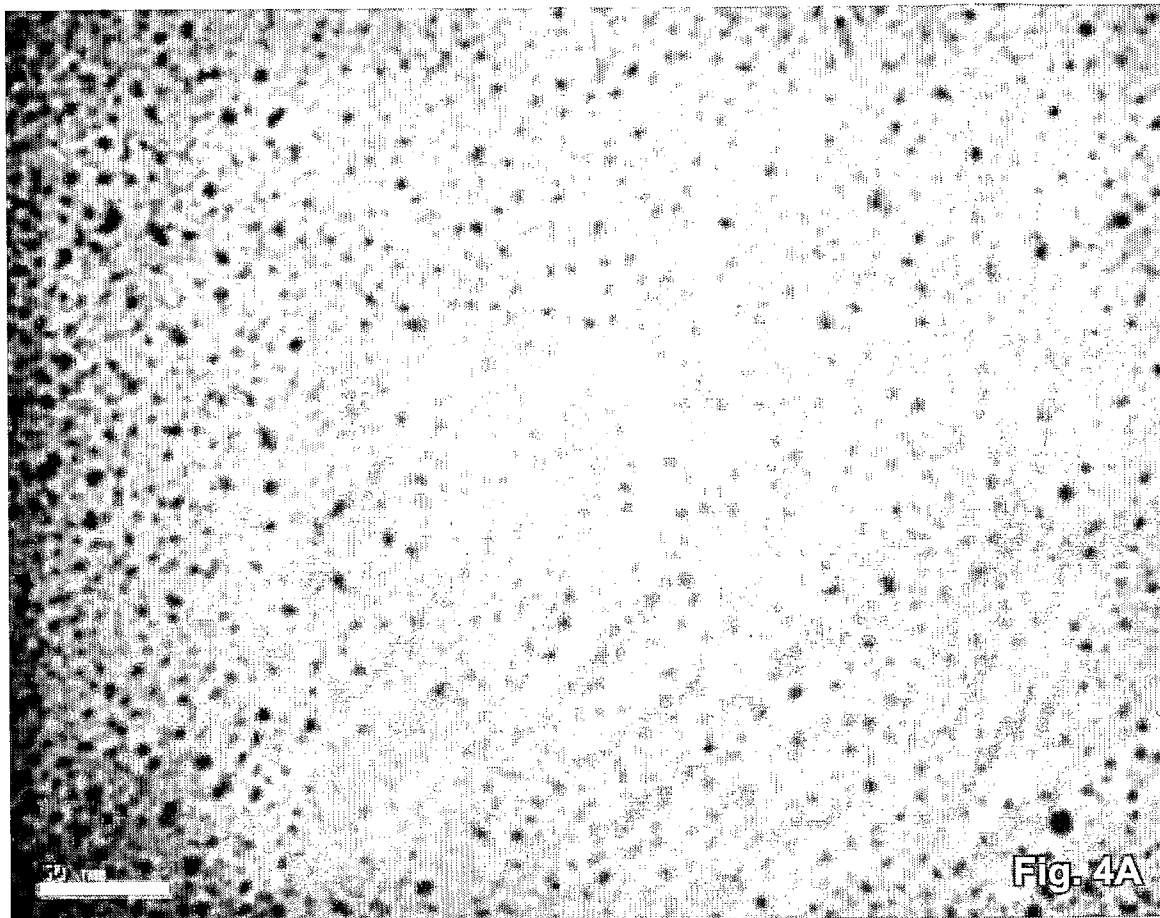


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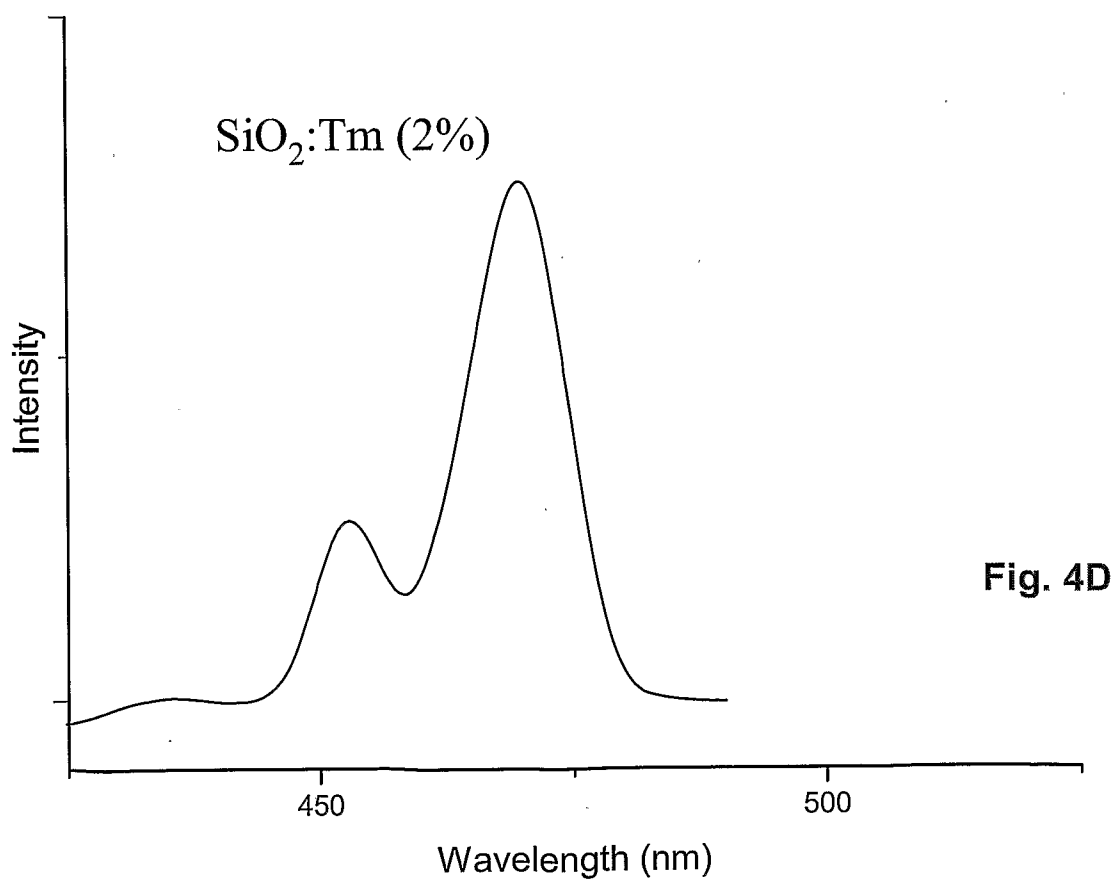
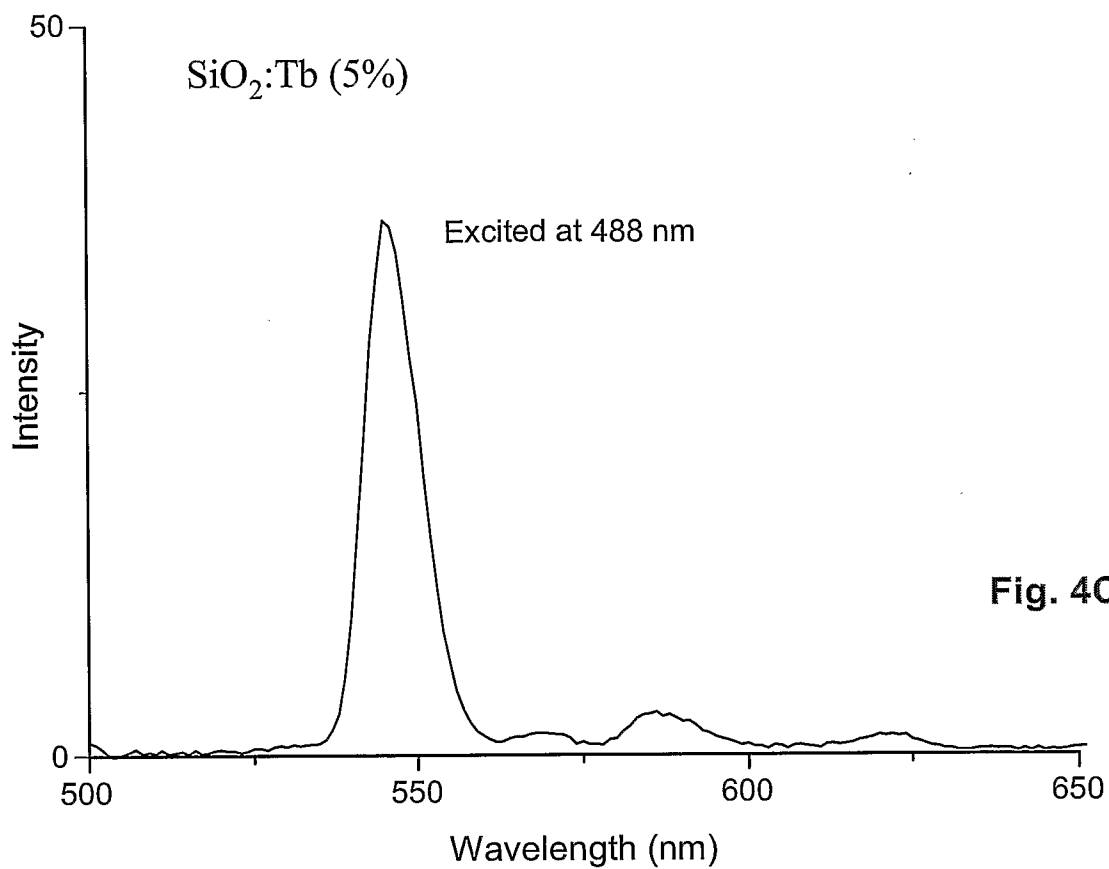


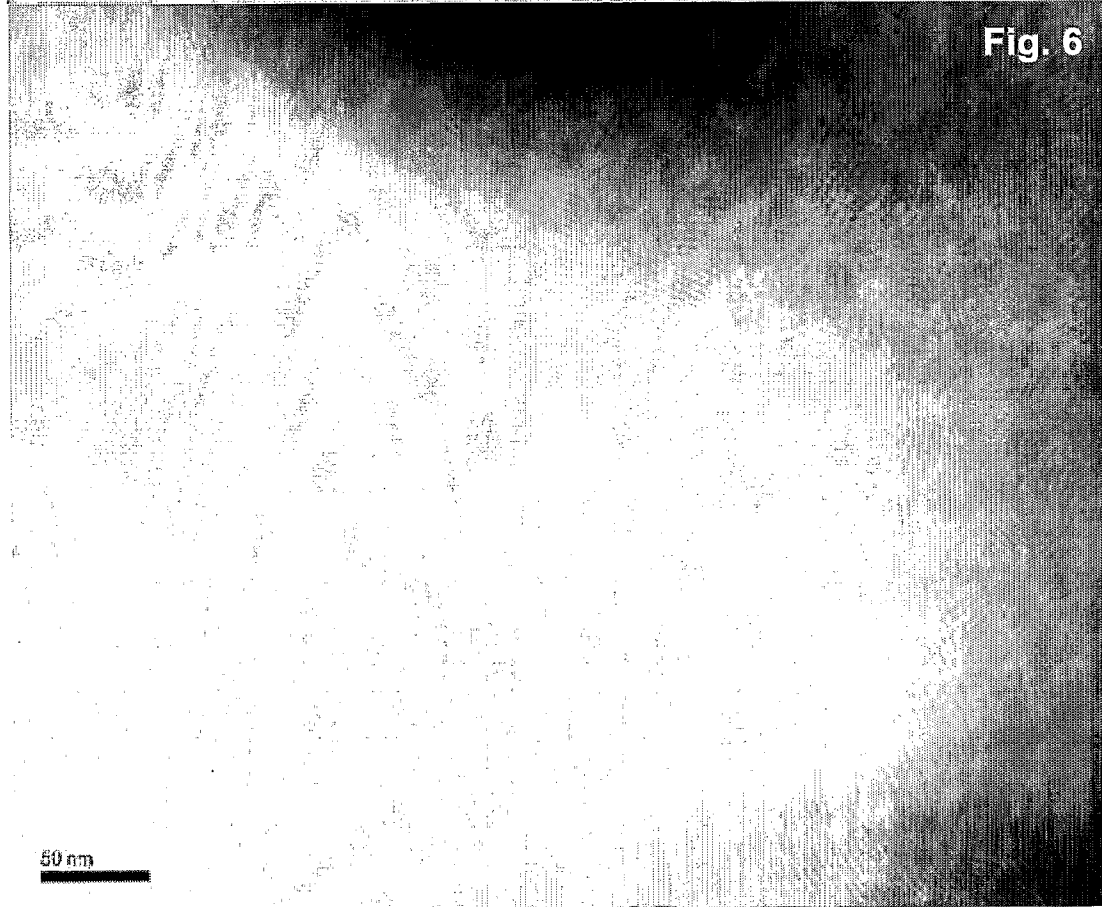
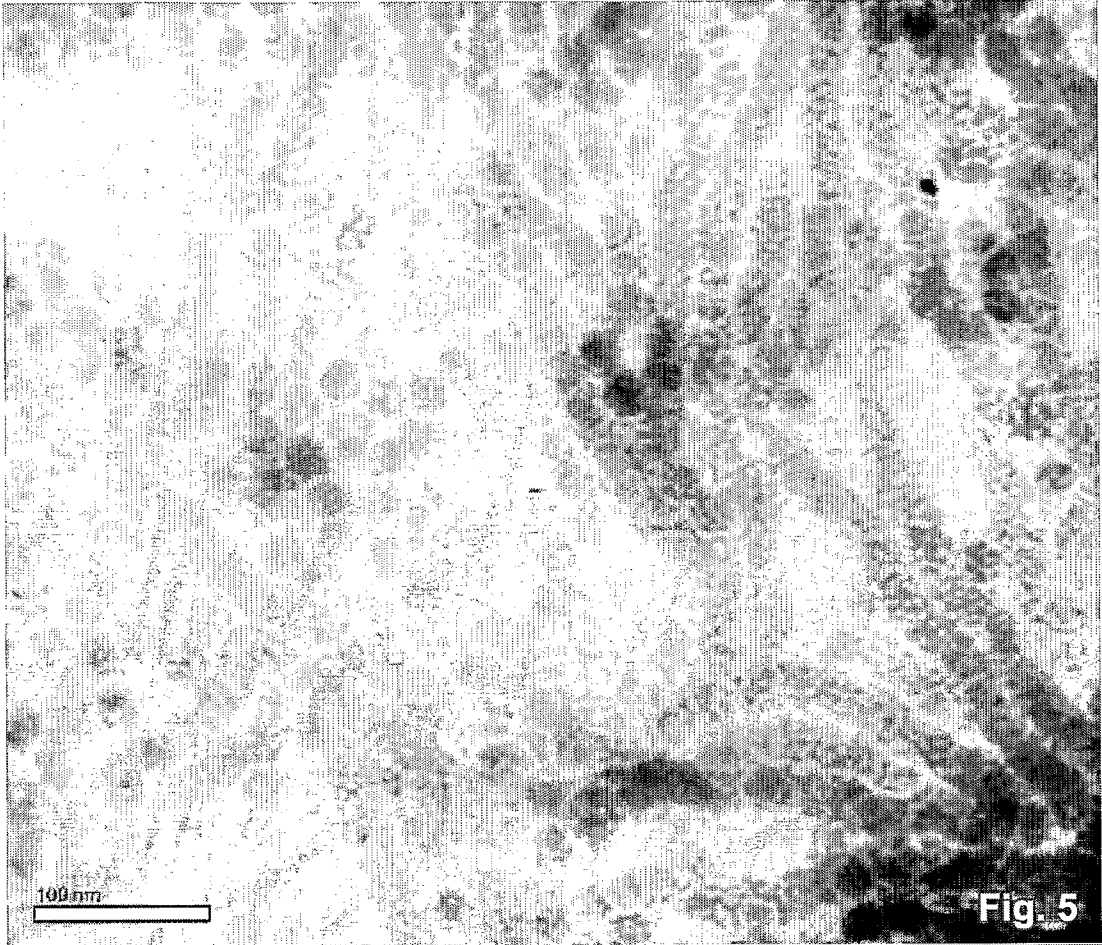


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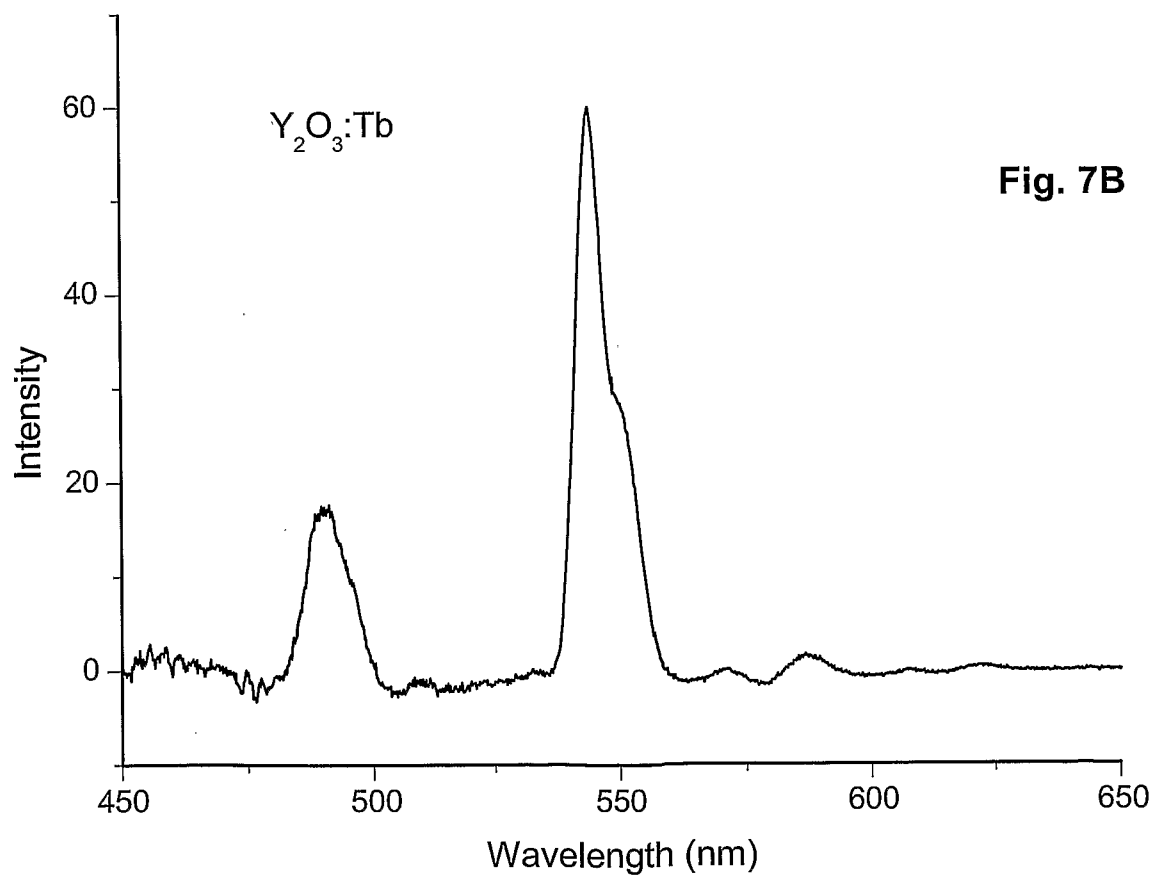
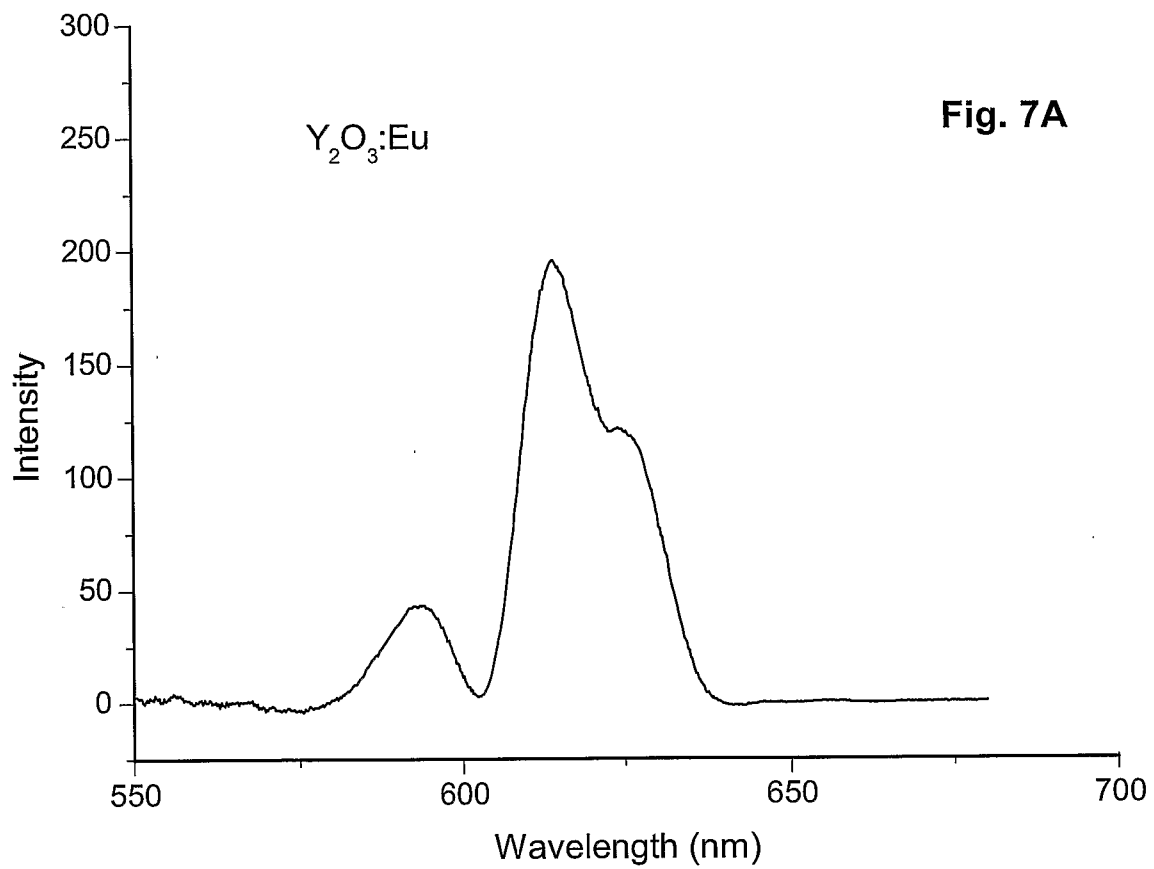


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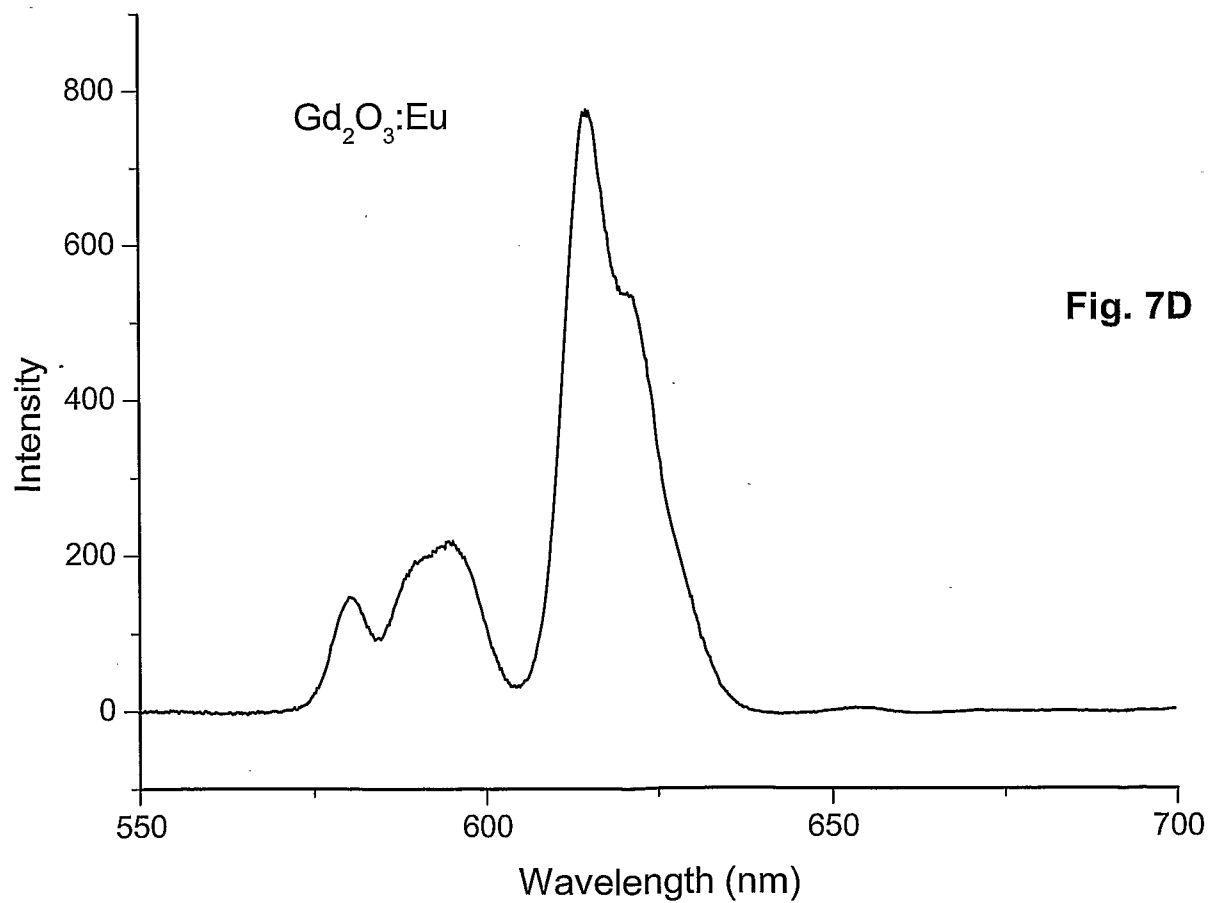
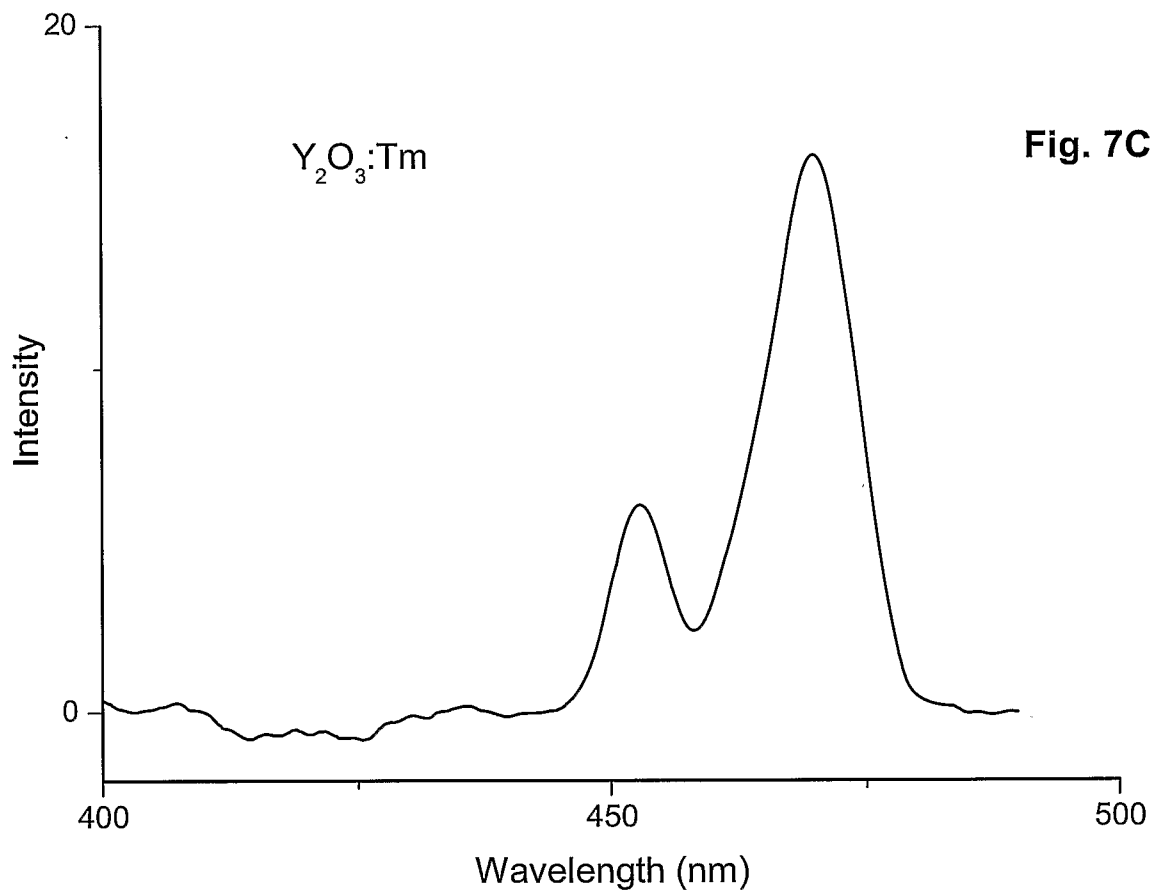




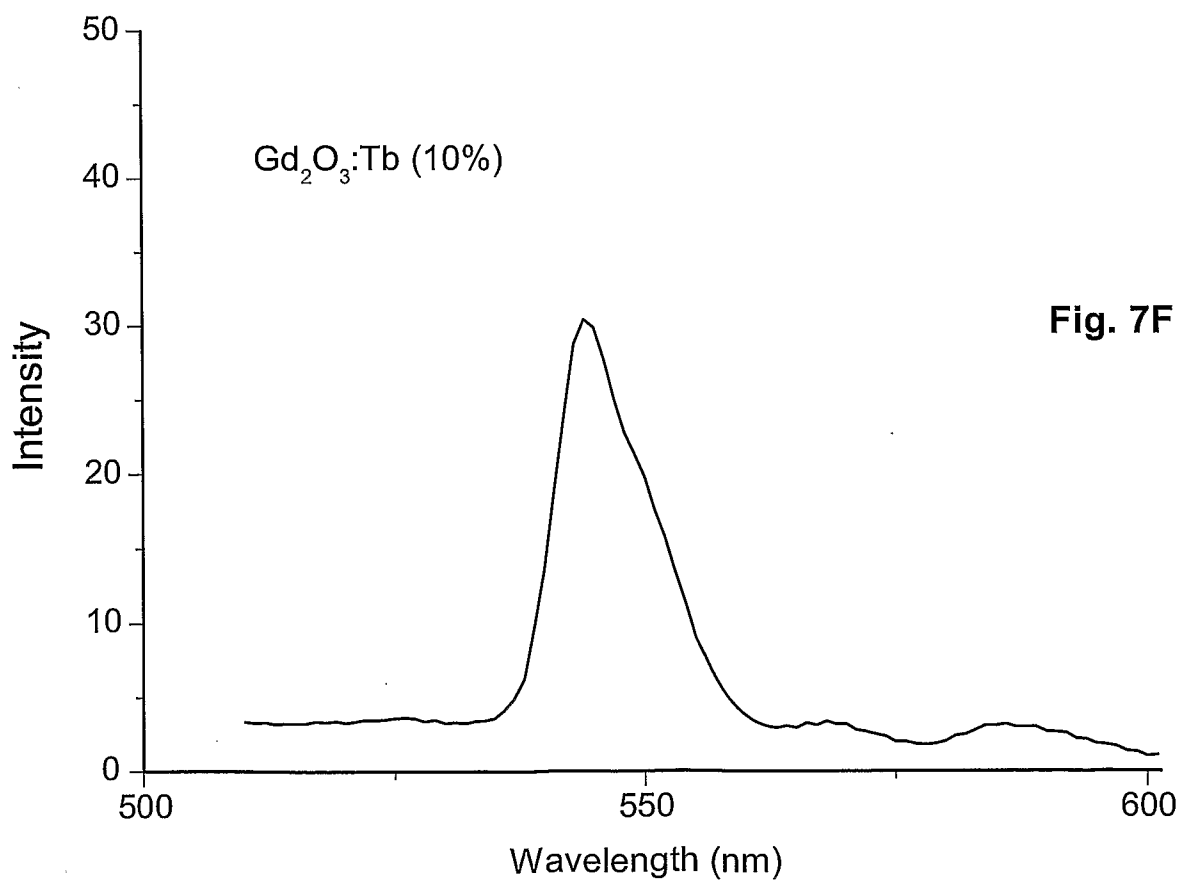
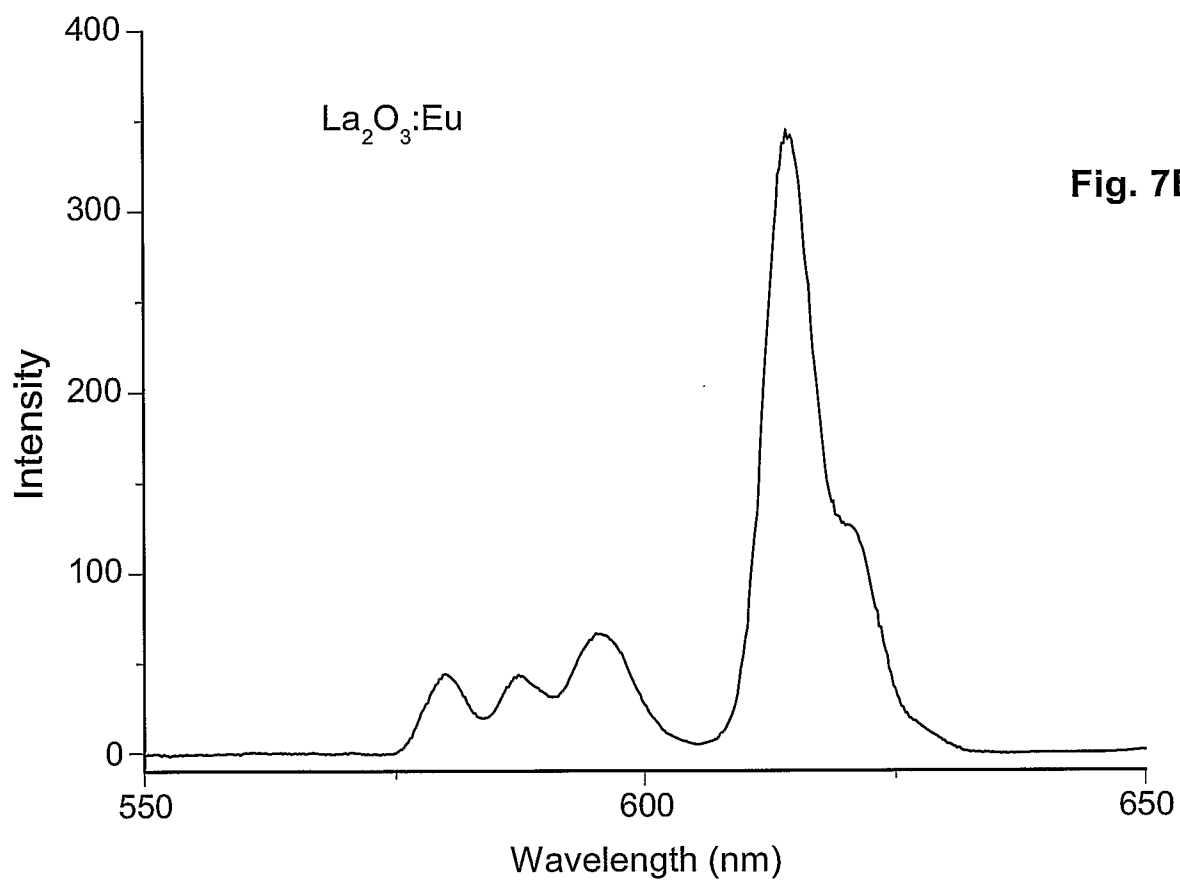
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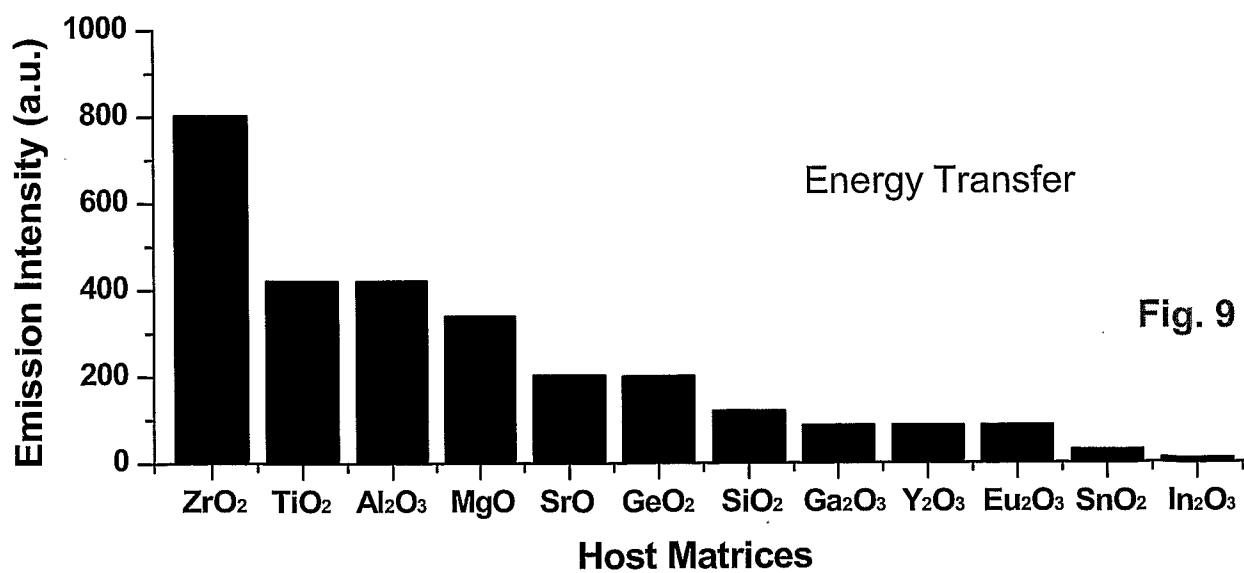
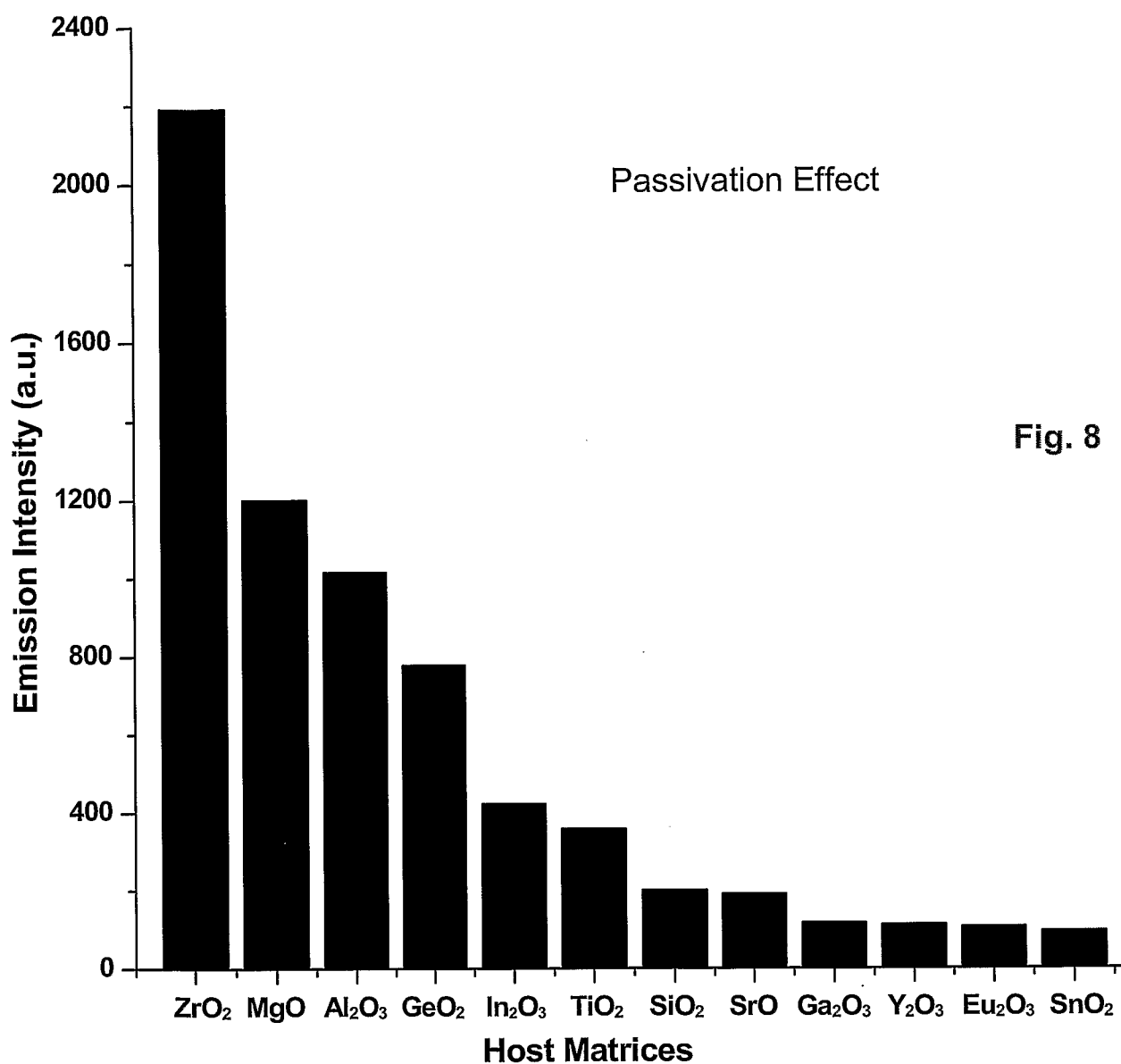
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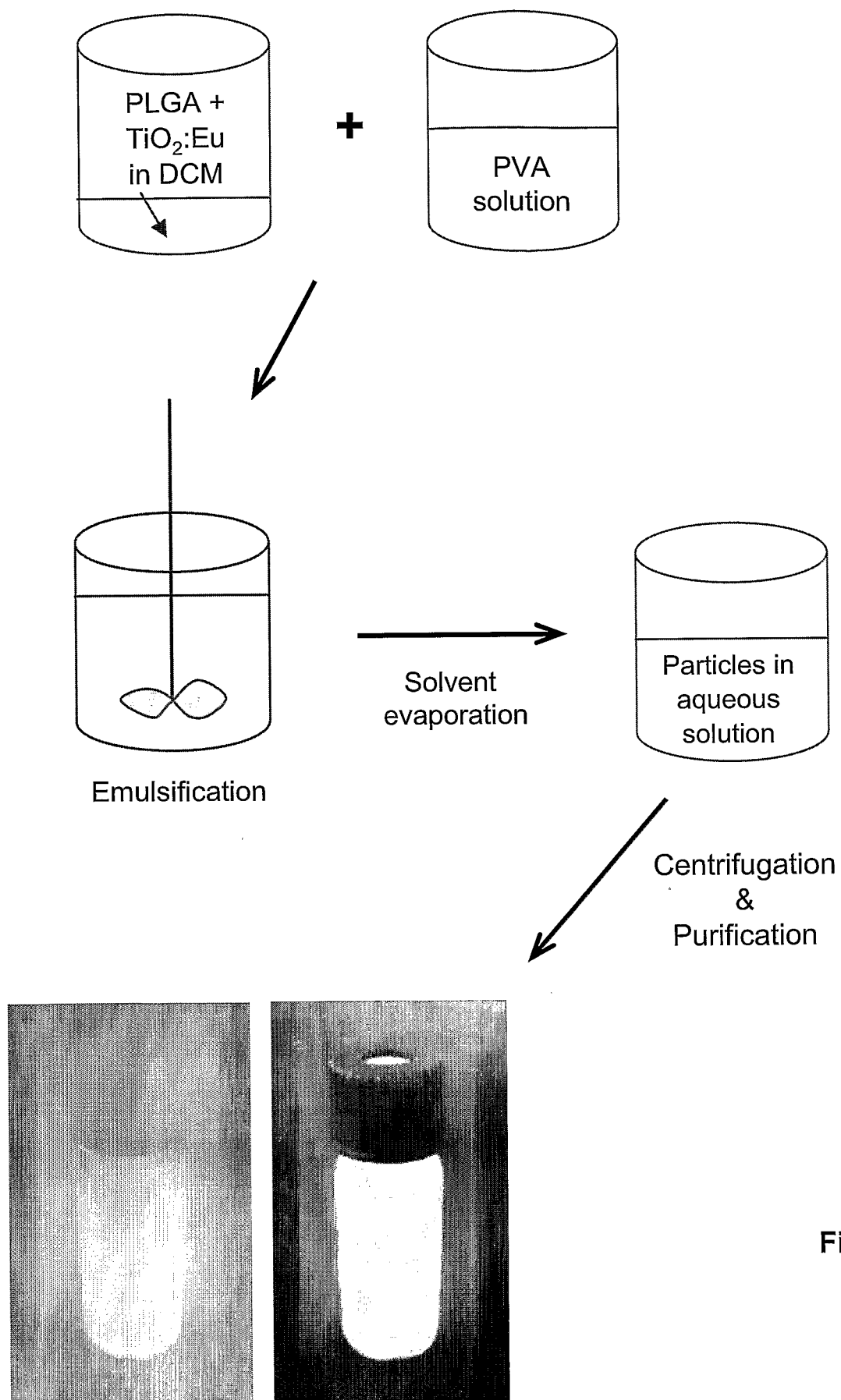
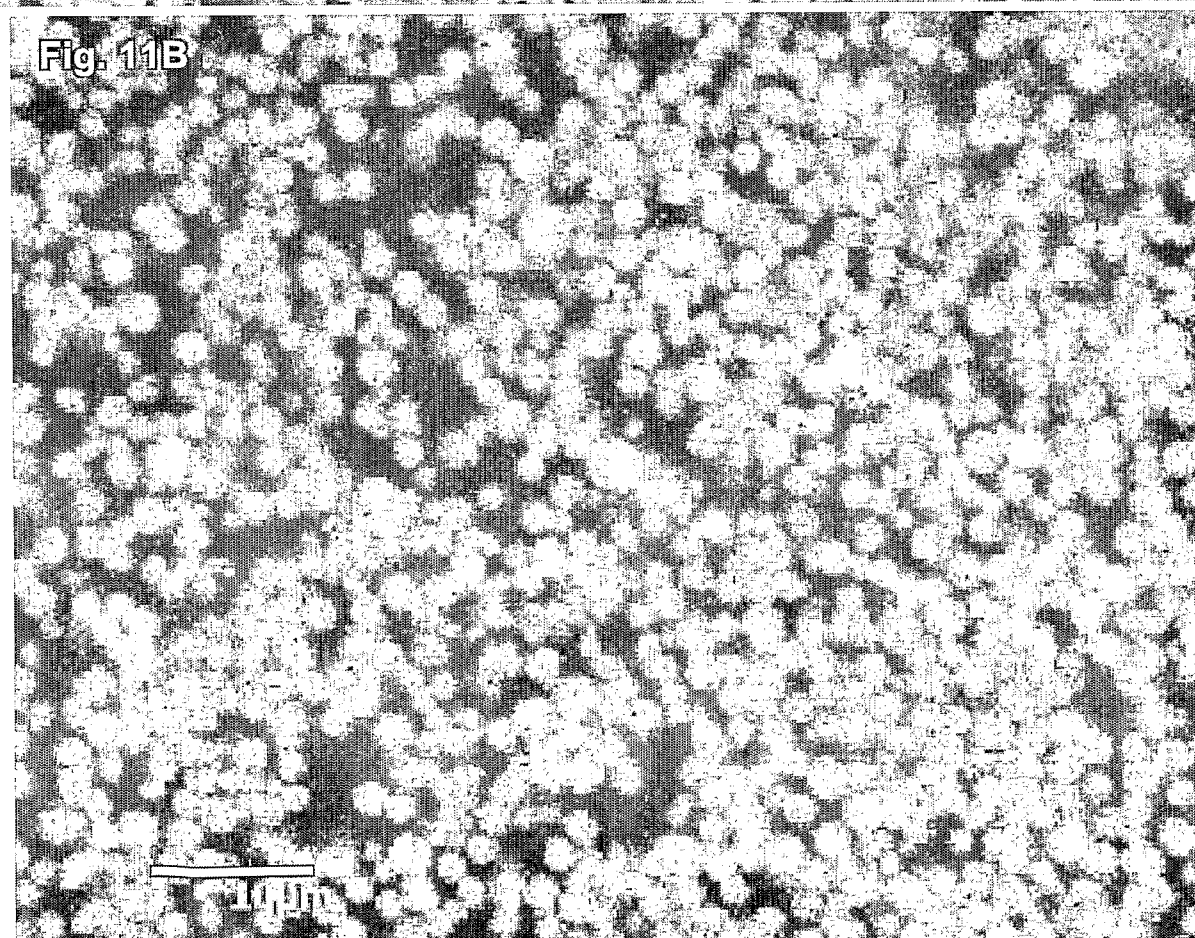
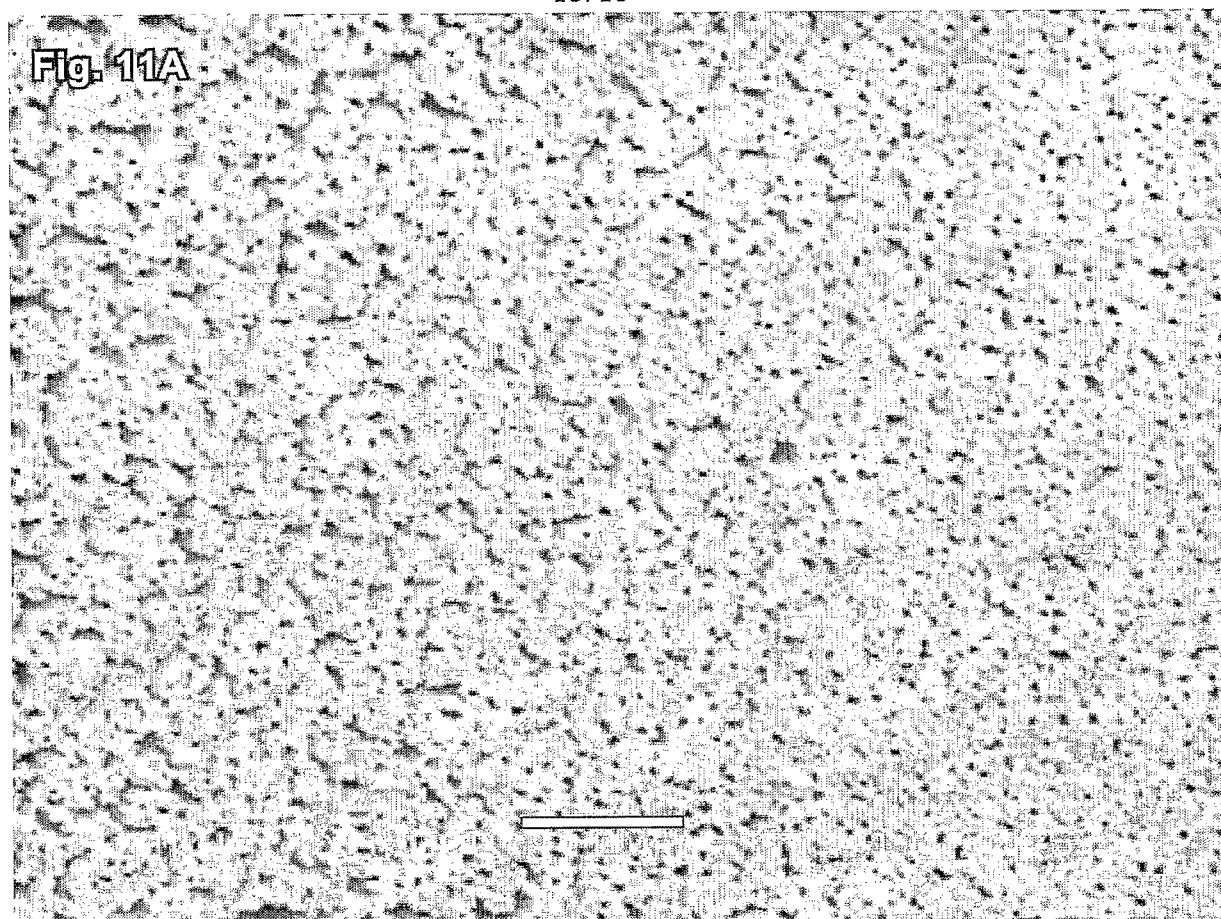
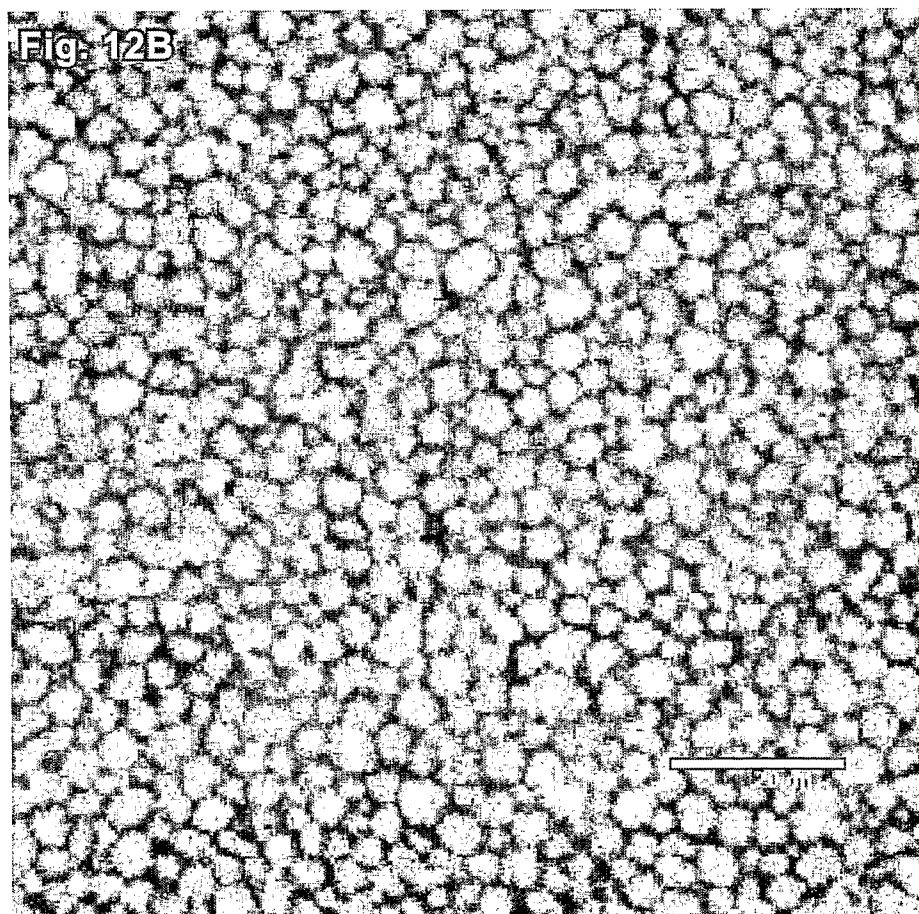
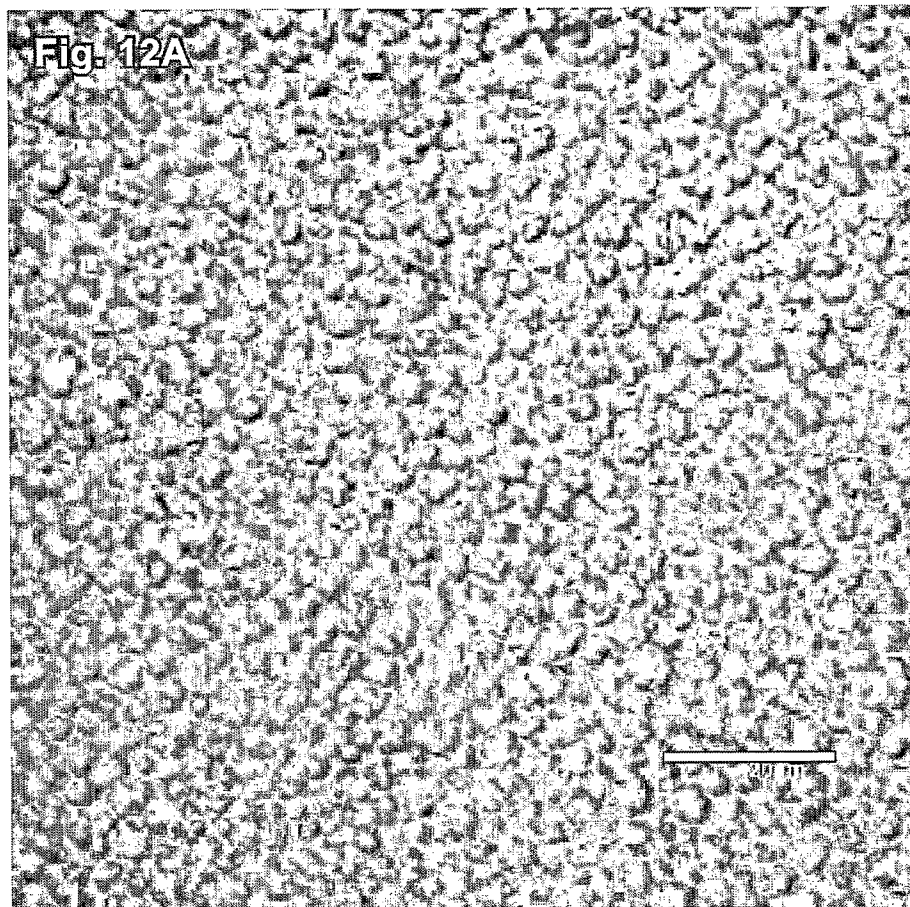


Fig. 10

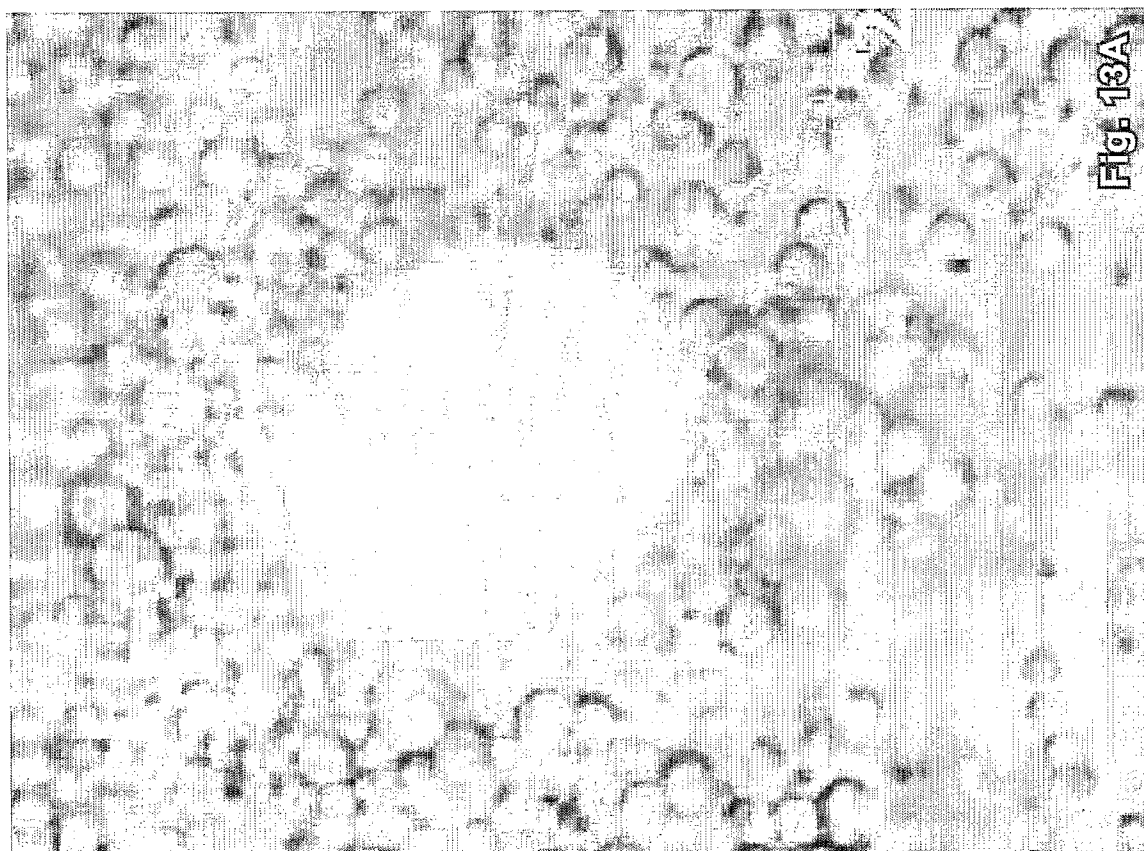
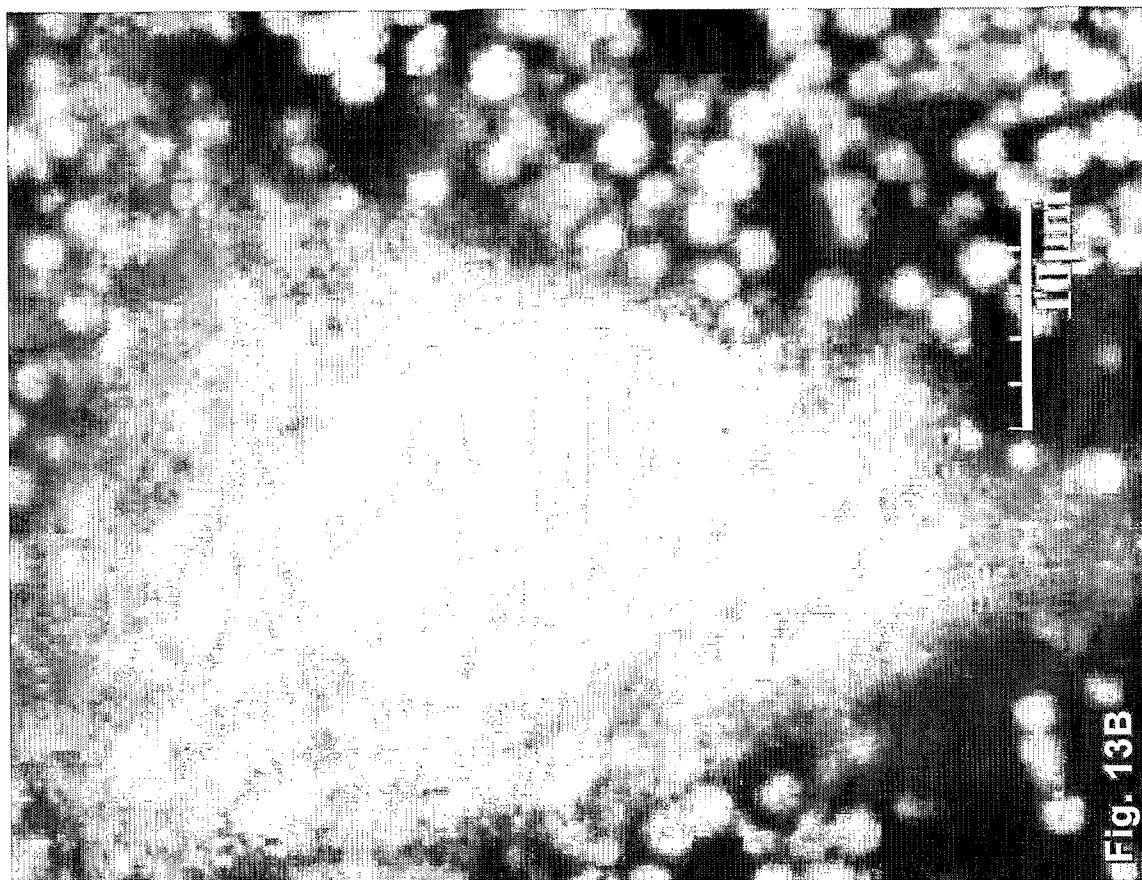
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2009/000451

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C09K 11/78 (2006.01)

C08K 3/22 (2006.01)

C09K 11/79 (2006.01)

B82B 3/00 (2006.01)

C09K 11/77 (2006.01)

C09K 11/80 (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

WPI, EPODOC, CAPLUS, INSPEC & Keywords rare-earth, nanoparticle, dopant, dispersion, luminescent and the like

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WANG ET AL. "Synthesis of Well-Dispersed Y ₂ O ₃ :Eu Nanocrystals and Self-Assembled Nanodisks Using a Simple Non-hydrolytic Route" <i>Adv. Mater.</i> , 2005, 17, pp. 2506-2509. See pg 2506, col 1, para 1; pg 2508-2509, experimental section. See pg 2506, col 1, para 1; pg 2508-2509, experimental section.	1-14 15-20
X Y	Patent Abstracts of Japan, JP 2007-046022 A (NATIONAL INSTITUTE OF ADVANCED INDUSTRIAL & TECHNOLOGY) 22 February 2007, (machine translation, retrieved 15 January 2010 from Internet) URL: http://www4.ipdl.inpit.go.jp/Tokujitu/PAJdeta...!N0000=60&N0120=01&N2001=2&N3001=2007-046022 See para [0001]-[0002]; Example 2. See para [0001]-[0002]; Example 2.	1-14 15-20



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
12 January 2010

Date of mailing of the international search report

22 JAN 2010

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2009/000451

C (Continuation).

DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KONG ET AL. "One-Step Synthesis and Luminescent Properties of Nanocrystalline YVO ₄ :Eu ³⁺ Powders" <i>Journal of Nanoscience and Nanotechnology</i> , March 2008, Vol. 8, No. 3, pp. 1228-1233.	1-14
Y	See abstract; pg 1229, Section 2.2, Synthesis of YVO ₄ :Eu ³⁺ Nanocrystalline Particles.	15-20
	See abstract; pg 1229, Section 2.2, Synthesis of YVO ₄ :Eu ³⁺ Nanocrystalline Particles.	
	LI ET AL. "Facile synthesis of size-tunable lanthanum phosphate nanocrystals monodispersible in both organic and aqueous solutions" <i>J. Mater. Res.</i> , Oct 2008, Vol. 23, No. 10, pp. 2796-2803.	
X	See abstract; see pg 2797, Experimental, Section IIA	1-14
Y	See abstract; see pg 2797, Experimental, Section IIA	15-20
	US 7108915 B2 (ADAMS ET AL.) 19 September 2006	
Y	See whole document and in particular Examples 1-6	15-20
	US 7241399 B2 (HAUBOLD ET AL.) 10 July 2007	
A	See whole document	1-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG2009/000451

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report			Patent Family Member					
JP	2007046022	NONE						
US	7108915	AU	2002243207	CA	2424082	EP	1326704	
		US	6649138	US	7147917	US	2002045045	
		US	2004101465	US	2004101621	US	2005003187	
		US	2008241375	WO	02055186			
US	7241399	AU	10036/02	CA	2388094	CA	2439381	
		CN	1388827	CN	1491118	DE	10111321	
		EP	1232226	EP	1368065	EP	1707611	
		HK	1064297	US	2003032192	US	2004156784	
		WO	0220695	WO	0220696	WO	02072154	
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.								
END OF ANNEX								