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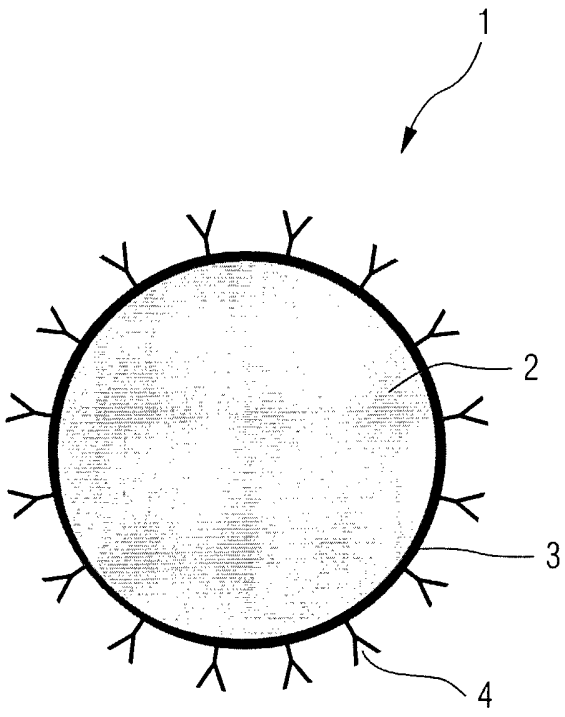
(54) **Title:** CONTRAST AGENT FOR OPTICAL IMAGING



(57) **Abstract:** A contrast agent for optical imaging comprising particles (1) consisting of at least a core (2), wherein the core comprises luminescent substances absorbing and emitting electromagnetic radiation at different wavelengths.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Contrast Agent for Optical Imaging

The present invention generally pertains to the field of medicine and optical imaging. The invention provides compositions and methods for imaging cells, tissues and organs *in vivo* and *in vitro*. In particular, compositions and methods are provided to enhance the imaging of tissue of throat, gullet, stomach or intestine by optical imaging techniques.

Medical imaging techniques have recently gained in importance in the field of diagnostics and treatment. The most important imaging techniques that are recently used are positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), computed tomography (CT) and ultrasound (US). All these techniques except ultrasound imaging are characterised by a significant complexity with respect to the employed equipment. Besides the huge purchase costs of such apparatuses, the high running costs negatively influence the expenses per examination and patient. Furthermore, the evaluation and interpretation of obtained data of such tomography apparatuses is complex and can only performed by experts.

It has been found that optical imaging techniques involve significantly less technical and personal effort compared to the above mentioned techniques. At present, however, optical imaging suffers from low sensitivity which complicates a diagnosis. It is of major interest to obtain results that enable the diagnosis of clinical pictures with high sensitivity and high specificity, preferably at an early stage. High sensitivity means that false negative diagnoses are excluded. High specificity means a reliable detection of a disease pattern, i.e. the exclusion of false positive diagnoses.

It therefore remains the need for means that enhance the sensitivity and specificity of optical imaging and which are available at a reasonable price as well as easy to handle.

The aim of the present invention is to overcome the drawbacks of prior art and to provide compositions and methods for imaging cells, tissues and organs *in*

vivo and *in vitro* at a high sensitivity and with high contrast.

This aim is solved by the compositions and methods according to the independent claims of the present invention, while useful embodiments are described by the features as contained in the dependent claims.

In general, the invention provides a contrast agent for optical imaging comprising particles consisting of at least a core, wherein the core comprises luminescent substances absorbing and emitting electromagnetic radiation at different wavelengths. The application of such a contrast agent for optical imaging techniques increases the sensitivity of the measurement. Although the usage of contrast agents is common in the field of PET, SPECT, MRI, CT or US, a person skilled in the art was not provided with such means for optical imaging techniques.

In a first aspect, the contrast agent according to the present invention is available in the form of nanoparticles, which facilitates the handling and administration thereof. The fact that the core of these particles comprises luminescent substances, renders it possible that a relatively high amount of such substances may be processed in the agent. This means that a high number of active centers may be present in the agent while concentrated to a small volume. As a particular advantage, these active centers in the form of luminescent substances absorb and emit electromagnetic radiation at different wavelengths. This leads to a clear distinguishability between the radiation applied to the agent and the radiation obtained therefrom. As a consequence, it is possible to detect radiation emitted from the particles despite excitation radiation which is used to excite the luminescence even in the presence of background light. It is understood that this positive effect increases with the distance between the wavelength ranges in which the absorption and emission take place as well as with the accuracy and precision of the spectral areas in which absorption and emission take place.

Preferably, the luminescent substances absorb electromagnetic radiation in the wavelength range between about 350 nm and about 480 nm. Accordingly, the excitation may easily be achieved by using a GaN- or InGaN-based LED (Light Emitting Diode), which may be mounted to the end of an endoscope. Alternatively, the light of such a source may be directed through an optical fiber to the head of an endoscope.

As a particular advantage the exciting radiation in this wavelength range

is identified and characterised by its blue or violet appearance. Accordingly, any light coming from the probe which is not blue or violet may be identified as some result or effect. It is thereby particularly advantageous if the exciting radiation lies in the range beneath the visible wavelength range, i.e. about 350 to 400nm. This would cause that any visible light obtained from the probe would allow the conclusion on an effect.

Furthermore, the luminescent substances preferably emit electromagnetic radiation in the wavelength range between about 480 nm and about a 750 nm. Within this range it is advantageously ensured that the emitted light is clearly distinguishable from the introduced light. The emitted light may be detected spectroscopically or, since said wavelength range lies in the visible spectrum, with the human eye. A detection and evaluation via the eye of a diagnostician without the need of any further apparatuses is a major contribution to the simplicity of this technique. In particular, the fact that an analyst who conducts an examination using the means provided by the present application may evaluate the measurement results and be able to obtain a diagnose without the need of employing complex equipment or assemblies. This helps to keep such treatments feasible and the costs low.

In a preferred embodiment the luminescent substances are formed of inorganic solids. These are easy to handle and in particular not sensitive to heat or pressure. Furthermore, inorganic solids are easy to obtain and process which makes the resulting contrast agents available at a reasonable price.

Preferably, the luminescent substances are selected from the group consisting of the compounds and compositions as listed in table 1.

Table 1

Luminescent substance	**Excitation (nm)**	**Maximum of emission (nm)**
$LaVO_4$:Eu	350-480	620/710
YVO_4:Eu	350-480	620/710
Mg_2TiO_4:Mn	350-480	660
$CaTiO_3$:Pr	350-480	620
$Y_3Al_5O_{12}$:Ce	350-480	560
$(Y,Gd)_3(Al,Ga)_5O_{12}$:Ce	350-480	560

Y_2O_3:Bi	350-480	530
$Ca_2MgSi_2O_7$:Eu,Mn	350-480	690
$Ca_2MgSi_2O_7$:Eu	350-480	550
Ba_2SiO_4:Eu	350-480	520
$Ca_2P_2O_7$:Eu,Mn	350-480	620
$Ca_5(PO_4)Cl$:Eu,Mn	350-480	600
$Sr_5(PO_4)Cl$:Ce,Mn	350-480	580
$Sr_5(PO_4)F$:Eu,Mn	350-480	580
$CaSO_4$:Bi	350-480	630
$SrSO_4$:Bi	350-480	620
$CaSO_4$:Eu,Mn	350-480	520
$CaSO_4$:Ce,Mn	350-480	540
$CaGa_2S_4$:Mn	350-480	710
$ZnGa_2S_4$:Mn	350-480	650
$CaGa_2S_4$:Eu	350-480	650
$CaGa_2O_4$:Eu	350-480	560
$CaGa_2S_4$:Ce	350-480	460/520
Y_2O_2S:Eu	350-480	710
$SrSi_5N_8$:Eu	350-480	650
$LaSi_3N_5$:Eu,O	350-480	550
SrS:Eu	350-480	620
SrS:Mn	350-480	550
SrS:Cu,Na	350-480	540
ZnS:Sn	350-480	700
ZnS:Cu,Au,Al	350-480	560
ZnS:Au,In	350-480	540

ZnS:Cu,Al	350-480	530
$(M_1,M_2)_xSi_{a-y}Al_yO_zN_{b-z}$:SE	350-480	350-750

with M_1: Li, Na, K, Rb, Cs, Cu, Ag, Be, Mg, Ca, Sr, Ba, Zn, Sn, Pb, Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, B, Ga or In;

with M_2: Li, Na, K, Rb, Cs, Cu, Ag, Be, Mg, Ca, Sr, Ba, Zn, Sn, Pb, Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, B, Ga or In;

with SE: Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, or Lu, most preferable is: Ce, Pr, Sm, or Eu;

with x, y, z, a, b = 0-20, most preferable is: x, y, z, a, b = 0-8; and

with a concentration of SE of 0.01 mol-% to 20 mol-%, most preferable is SE with a concentration of 0.5 to 5 mol-%.

These preferred luminescent substances show an absorption of electromagnetic radiation which is clearly distinguished from the resulting emission. As can be seen from table 1, the excitation of these substances takes place in the wavelength range between 350 nm and 480 nm. The maximum of emission in turn usually occurs in the range between 520 nm and 710 nm, respectively. Thereby it is ensured in a favourable manner that light emitted from these materials is sufficiently different from the excitation radiation so that a contrast generation is possible. It is understood that the listing of luminescent materials as given in table 1 is not to be considered as exhaustive. The listed compounds and compositions rather represent preferred materials that can be used as luminescent substances in connection with the present invention.

According to a particularly preferred embodiment of the present invention, the contrast agent further comprises at least an optional shell around the particle core. By introducing a shell, different advantageous effects can be reached. Firstly, additional materials can be processed in said shell, which may be specifically effective for one or more additional imaging techniques or wavelength ranges, thus leading to the possibility of an examination using more than one imaging technique or different wavelengths for excitation or detection. Furthermore, high compatibility can

be established by choosing shell material that prevents an immune reaction of the examined body against the contrast agent particles. Moreover, a shell may be established containing biological active compounds, such as antibodies, thereby supporting a favorable distribution of the contrast agent in the examined tissue.

Moreover, by using a shell around the particle core, the solvation or hydrolysis of the core could be prevented if the core is formed of material that is sensitive to hydrolysis such as SrS.

According to another preferred embodiment of the present invention, at least one further shell is present, providing biocompatibility. This ensures, that after administering the contrast agent to a living organism, no immune reaction against this agent takes place, which allows the application *in vivo*. This at least one biocompatibility shell may particularly consist of gold, SiO_2, a polyphosphate (e.g. calcium polyphosphate), an amino acid (e.g. asparagin acid), an organic polymer (e.g. polyethylene glycol/PEG, polyvinyl alcohol/PVA, polyamide, polyacrylate, polyurea), a biopolymer (e.g. polysaccharide, such as dextrane, xylane, glycogene, pectine, cellulose, or polypeptide, such as collagene, globuline), cysteine, or a peptide with a high amount of asparagine, or a phospholipide.

By providing a biocompatibility shell, it is advantageously ensured that the contrast agents of the present invention may be administered to a living organism without the risk of immune reaction or toxic interaction. The expulsion of these contrast agents may furthermore take place via the kidneys if the particle diameter is small or alternatively after solvation of the solids in blood or spleen or liver. According to an imaging of throat, gullet, stomach or intestine, the contrast agent is removed by bowel movements/stool.

It is thereby preferred if at least one of the biocompatibility shells covers the core completely in order to efficiently provide biocompatibility and prevent the core from being dissolved or hydrolysed.

By using gold as shell material, some further positive effects may be achieved. On one hand, further active or inactive compounds may be applied and immobilised on the gold surface in an easy manner, for example by thiole linkers. This means that certain polypeptides, proteins or even antibodies may be bound to the gold shell without major effort.

On the other hand, a thin gold layer such as the one on top of the core may under certain circumstances show an SPR (surface plasmon resonance) effect if exposed to electromagnetic radiation. This additional effect may be employed for the purpose of this invention, namely for the extinction and emission of certain wavelength ranges. Accordingly, depending on the thickness of the gold shell, a contrast generation in certain spectral areas is enhanced. This applies in particular if the gold shell is formed of discrete gold nanoparticles attached to the surface of the core or underlying shell rather than covering the core or underlying shell in a sealing manner.

Last but not least, by using a gold shell of a certain thickness, the particles according to the preferred embodiment may be used as contrast agent for ultrasound (US) measurements. The particles thereby provide reflection capabilities for ultrasound (US) comparable to gas microbubbles as conventionally used.

The at least one optional shell preferably has at thickness of 1 nm to 200 nm, and more preferably 20 nm to 100 nm. It is thereby ensured that the adhesion characteristics of said shell to the core are convenient. This helps to prevent any immune reactions as well as the solvation or hydrolysis of the core. If the shell is formed of gold, it is particularly advantageous if the thickness lies between 1 nm and 50 nm and most preferably between 1 nm and 30 nm so that the above mentioned advantageous effects may be achieved.

According to a further preferred embodiment of the present invention, at least one further shell is present, containing at least one antibody. By immobilizing antibodies on the surface of the nanoscale particles, a specific antibody-antigene reaction can be established. This leads to specific adsorption/concentration of the contrast agent in infected tissue (e.g. cancer cells, coronar plaques). As a result, the contrast agent and the imaging process are highly specific to the respective case. Moreover, medical imaging is possible on a cellular or even molecular level. Dependent on the desired purpose, one or more antibodies may be employed. In the following, several examples of antibodies are given, that may be used for the described application. However, this list is not intended to be exhaustive, since other antibodies are also applicable, in particular, antibodies that are available at some future date only.

Trastuzumab (detection of breast cancer)

Rituximab (detection of Non-Hodgkin lymphome)

Alemtuzumab (detection of chronical-lymphocytic leukemia)

Gemtuzumab (detection of acute myelogene leukemia)

Edrecolomab (detection of bowel cancer)

Ibritumomab (detection of Non-Hodgkin-lymphome)

Cetuximab (detection of bowel cancer)

Tositumomab (detection of Non-Hodgkin-lymphome)

Epratuzumab (detection of Non-Hodgkin-lymphome)

Bevacizumab (detection of lung and bowel cancer)

anti-CD33 (detection of acute myelogene leukemia)

Pemtumomab (detection of ovary and stomach cancer)

Mittumomab (detection of lung and skin cancer)

anti-MUC 1 (detection of Adenocarcinoma)

anti-CEA (detection of Adenocarcinoma)

anti-CD 61 (detection of coronar deposits/plaques)

Since the contrast agent according to the present invention is particularly useful for optical imaging and diagnosis of malign changes of throat, gullet, stomach or intestine, Edrecolomab, Cetuximab, Bevacizumab and Pemtumomab are particularly preferred antibodies. Furthermore, the contrast agent of the present invention may be used for examination of biopsates.

Preferably, the at least one antibody is a tumor specific antibody. This allows for the usage of the contrast agents for tumor prevention and treatment, involving the identification and localization of specific tumors.

The at least one antibody containing shell may further contain one or more proteins, preferably the HIV-tat protein. This facilitates the passage of these agents through e.g. a cell membrane. This advantageously enables examinations involving intracellular procedures and metabolisms.

According to a preferred embodiment of the present invention, the core of the contrast agents has a spherical, oval or lens-shape. Thereby, an optimized volume to surface ratio is provided. Furthermore, the distribution of said particles in the examined tissue or body is facilitated. Preferably, the core has a diameter of 1 nm to 1000 nm, preferably 50 nm to 500 nm. This comes up to the size of several proteins and bioorganic compounds as present in human and animal organisms. Thereby, these

particles are easily involved in metabolism processes, as for example intercellular exchange reactions, thereby facilitating the transport and adsorption of the contrast agents at areas of interest.

The invention further provides pharmaceutical formulations comprising the contrast agent of the invention and a pharmaceutically acceptable excipient wherein the contrast agent is formed according to any of the above described embodiments, and wherein the formulation is suitable for administration as an imaging enhancing agent and the contrast agent is present in an amount sufficient to enhance a optical imaging image.

The formulations of the invention can include pharmaceutically acceptable carriers that can contain a physiologically acceptable compound that acts, e.g. to stabilize the composition or to increase or to decrease the absorption of the agent and/or pharmaceutical composition. Physiologically acceptable compounds can include, for example, carbohydrates, such as glucose, sucrose, or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins, compositions that reduce the clearance or hydrolysis of any co-administered agents, or excipients or other stabilizers and/or buffers. Detergents can also be used to stabilize the composition or the increase or decrease the absorption of the pharmaceutical composition. Other physiologically acceptable compounds include wetting agents, emulsifying agents, dispersing agents or preservatives that are particularly useful for preventing the growth or action of microorganisms. Various preservatives are well known, e.g. ascorbic acid. One skilled in the art would appreciate that the choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound depends, e.g. on the route of administration and on the particular physio-chemical characteristics of any co-administered agent.

In one aspect, the composition for administration comprises a contrast agent of the invention in a pharmaceutically acceptable carrier, e.g., an aqueous carrier. A variety of carriers can be used, e.g., buffered saline and the like. These solutions are sterile and generally free of undesirable matter. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride,

calcium chloride, sodium lactate and the like. The concentration of active agent in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration and imaging modality selected.

The invention may be applied according to a method for *in vivo* or *in vitro* imaging a cell, a tissue, an organ or a full body comprising the following steps:

a) providing a pharmaceutical formulation comprising the contrast agent of the invention and a pharmaceutically acceptable excipient, wherein the contrast agent is formed according to any of the above described embodiments, and wherein the formulation is suitable for administration as an imaging enhancing agent and the contrast agent is present in an amount sufficient to enhance an optical imaging image;

b) providing an optical imaging device or equivalent;

c) administering the pharmaceutical formulation in an amount sufficient to generate the cell, tissue or body image; and

d) imaging the distribution of the pharmaceutical formulation of step a) with the imaging device, thereby imaging the cell, tissue or body.

The pharmaceutical formulations of the invention can be administered in a variety of unit dosage forms, depending upon the particular cell or tissue or cancer to be imaged, the general medical condition of each patient, the method of administration, and the like. Details on dosages are well described on the scientific and patent literature. The exact amount and concentration of contrast agent or pharmaceutical of the invention and the amount of formulation in a given dose, or the "effective dose" can be routinely determined by, e.g. the clinician. The "dosing regimen" will depend upon a variety of factors, e.g. whether the cell or tissue or tumour to be imaged is disseminated or local, the general state of the patient's health, age and the like. Using guidelines describing alternative dosing regimens, e.g. from the use of other imaging contrast agents, the skilled artisan can determine by routine trials optimal effective concentrations of pharmaceutical compositions of the invention.

The pharmaceutical compositions of the invention can be delivered by any means known in the art systematically (e.g. intra-venously), regionally or locally (e.g. intra- or peri-tumoral or intra-cystic injection, e.g. to image bladder cancer) by e.g.

intra-arterial, intra-tumoral, intra-venous (iv), parenteral, intra-pneural cavity, topical, oral or local administration, as sub-cutaneous intra-zacheral (e.g. by aerosol) or transmucosal (e. g. voccal, bladder, vaginal, uterine, rectal, nasal, mucosal), intra-tumoral (e.g. transdermal application or local injection). For example, intra-arterial injections can be used to have a "regional effect", e.g. to focus on a specific organ (e.g. brain, liver, spleen, lungs). For example intra-hepatic artery injection or intra-carotid artery injection. If it is decided to deliver the preparation to the brain, it can be injected into a carotid artery or an artery of the carotid system of arteries (e.g. ocipital artery, auricular artery, temporal artery, cerebral artery, maxillary artery etc.). Aiming at an imaging of throat, gullet, stomach or intestine, oral or anal application of the contrast agent is most preferred.

These and other aspects of the invention will be apparent from and elucidated with reference to the embodiments described hereinafter.

Fig. 1 is a cross-sectional view of a particle according to the present invention. This particle 1 comprises a core 2, optionally covered by shells 3 to 4.

The core 2 comprises a luminescent solid as described above. The core 2 can be excited with 350 to 480 nm light of, for instance, a GaN- or InGaN-based light emitting diode. Such a diode can be located on the top-end of an endoscope, or the light can be sent via a glass fiber to the top-end of an endoscope. While being excited, the luminescent core 2 emits visible light which is with a different wavelength compared to the incoming light. The emitted light can be detected endoscopically via spectroscopic measures or via the human eye.

On top of the core 2, a first optional shell 3 is applied. This optional shell 3 mainly serves to provide biocompatibility and is built of for example dextrane. It preferably covers the core completely and has a thickness greater than 1 nm. Additionally, if shell 3 covers the core completely it may serve to prevent the core from being dissolved or hydrolysed, for instance by applying SiO_2. Optionally, shell 3 can also contain nanoscale gold particles, which may serve as linkers to e.g. thiol functionalities of a biopolymer or antibody and/or as an antenna for incoming light.

On top of this first optional shell 3, a second optional shell 4 is applied which contains a suitable antibody. This antibody containing shell 4 does not cover the core completely. It causes that the contrast agent will be attached specifically to infected tissue due to a specific antibody-antigen interaction of the antibodies. Thereby, the optional antibody containing shell 4 allows to diagnose cancel infected tissue, in particular of throat, gullet, stomach or intestine.

In the following examples are given, according to which the invention may be accomplished.

Example 1:

5,5 g $Zn(CH_3COO)_3 \times 2H_2O$, 8,8 g $GaCl_3$, 11,4 g thiourea and 67 mg $Mn(CH_3COO)_2 \times 2H_2O$ are suspended in 50ml diethylenglycole. The suspension is stirred steadily and heated to 190°C for 2 hours. A suspension is obtained which contains nanoscaled $ZnGa_2S_4$:Mn with a particle diameter of about 90 nm. After cooling down, the $ZnGa_2S_4$:Mn-particles may be separated from the primary suspension by centrifugation, followed by suitable washing processes (e.g. repeated resuspending of the solid in ethanol and/or acetone, repeated centrifugation) and transferred to an aqueous suspension (e.g. isotonic solution or phosphate buffer).

Starting from the diethylenglycole based primary suspension as well as a secondary, aqueous suspension, the nanoscaled $ZnGa_2S_4$:Mn-particles may be further modified. 10 ml of an aqueous solution, containing 0,2 g cysteine and 1,5 tetraethylorthosilicate may be added. Thereby, a cysteine containing shell of SiO_2 may be built on top of the phosphor material. The thickness of this shell amounts approximately 30 nm. Finally, 5 ml of an aqueous 10^{-4} molar solution of an antibody (e.g. pemtumomab) or histidine-modified antibody (e.g. hystidine-modified pemtumomab) may be added and the antibody may be attached to the cysteine/SiO_2-layer by amide-bridging as a first shell. The product may be used as contrast agent for optical imaging.

Example 2:

1,2 g $Ca(CH_3COO)_2 \times H_2O$ and 23 mg $BiCl_3$ are suspended in 50 ml diethylenglycole. The suspension is stirred steadily and heated to 190°C. At this temperature 1,2 g Na_2SO_4 dissolved in 20 ml diethylenglycole are added. Thereafter, the solution is heated at 190°C for 2 hours. A suspension is obtained which contains

nanoscaled $CaSO_4$:Bi with a particle diameter of about 60 nm. To this suspension, a solution of 1,3 g $NaAuCl_4$ x $2H_2O$ in water is added at 180°C during a period of time of 1 hour. Thereby nanoscale gold particles are precipitated and adhered on the surface of $CaSO_4$:Bi. The gold particles are about 5 to 10 nm in diameter. After cooling down, the gold covered $CaSO_4$:Bi-particles may be separated from the primary suspension by centrifugation, followed by suitable washing processes (e.g. repeated resuspending of the solid in ethanol and/or acetone, repeated centrifugation) and transferred to an aqueous suspension (e.g. isotonic solution or phosphate buffer).

Starting from the diethylenglycole based primary suspension as well as a secondary, aqueous suspension, the nanoscaled gold covered $CaSO_4$:Bi-particles may be further modified. 10 ml of an aqueous solution, containing 0,2 g cysteine and 1,5 tetraethylorthosilicate may be added. Thereby, a cysteine containing shell of SiO_2 may be built on top of the gold shell. The thickness of this shell amounts to approximately 30 nm. Finally, 5 ml of an aqueous 10^{-4}- molar solution of an antibody (e.g. pemtumomab) or histidine-modified antibody (e.g. hystidine-modified pemtumomab) may be added and the antibody may be attached to the cysteine/SiO_2-layer by amide-bridging as a second shell. The product may be used as contrast agent for optical imaging. Due to the gold layer, the contrast agent may further be employed in ultrasound (US) measurements.

Example 3:

1,00 g $Al(OCH(CH_3)_2)_3$, 0,78 g $Y(OCH(CH_3)_2)_3$ and 10 mg $Ce(CH_3COO)_3$ x H_2O are suspended in 50 ml diethylenglycole. The suspension is stirred steadily and heated to 140°C. 0,5 ml of a 2-molar caustic soda are added. In the following, it is heated to 190°C for 4 hours. A suspension is obtained containing nanoscaled $Y_3Al_5O_{12}$:Ce with a particle diameter of approximately 30 nm. By centrifugation followed by adequate washing processes (e.g. repeated resuspending of the solid in ethanol and/or acetone, repeated centrifugation) the nanoscaled particles may be separated from the primary suspension and transferred to a aqueous suspension (i.e. isotonic solution or phosphate buffer).

Starting from the diethylenglycole based primary suspension as well as from a secondary aqueous suspension, the nanoscaled $Y_3Al_5O_{12}$:Ce-particles may be further modified. 10 ml of an aqueous solution containing 50 ml asparagine acid and

100 mg tetraethylorthosilicate may be added to the suspensions over a time span of 1 hour, respectively. Thereby an asparagine acid containing shell of SiO_2 may be built upon the nanoparticles. The thickness of this shell amounts to approximately 15 nm. Finally, 2 ml of an aqueous 10^{-4} molar solution of an antibody (e.g. cetuximab or a histidine modified antibody (e.g. histidine modified cetuximab) may be added and the antibody may be attached to the asparagine acid/SiO_2-layer by amide bridging as a second shell. The product may be used as a contrast agent for optical imaging.

The invention has been described herein with reference to certain preferred embodiments. However, as obvious variations thereon will become apparent to those skilled in the art, the invention is not to be considered as limited thereto. In particular, other combinations and preparations of metal oxides than described in one of the examples may serve as contrast agents according to the present invention. Furthermore, the given examples of antibodies that may be used according to the present invention are not intended to be exhaustive, since other antibodies are also applicable, in particular, antibodies that are available at some future date only. Any reference signs in the claims do not limit the scope of the invention. The term „comprising" is to be understood as not excluding other elements or steps and the term „a" or „an" does not exclude a plurality.

CLAIMS:

1. A contrast agent for optical imaging comprising particles (1) consisting of at least a core (2), wherein the core comprises luminescent substances absorbing and emitting electromagnetic radiation at different wavelengths.

2. The contrast agent according to claim 1, wherein the luminescent substances absorb electromagnetic radiation in the wavelength range between about 350 nm and about 480 nm, preferably between about 350 nm and 400 nm.

3. The contrast agent according to any of the foregoing claims, wherein the luminescent substances emit electromagnetic radiation in the wavelength range between about 480 nm and about 750 nm.

4. The contrast agent according to any of the foregoing claims, wherein the luminescent substances are formed of inorganic solids.

5. The contrast agent according to any of the foregoing claims, wherein the luminescent substances are selected from the group consisting of $LaVO_4$:Eu; YVO_4:Eu; Mg_2TiO_4:Mn; $CaTiO_3$:Pr; $Y_3Al_5O_{12}$:Ce; $(Y,Gd)_3(Al,Ga)_5O_{12}$:Ce; Y_2O_3:Bi; $Ca_2MgSi_2O_7$:Eu,Mn; $Ca_2MgSi_2O_7$:Eu; Ba_2SiO_4:Eu; $Ca_2P_2O_7$:Eu,Mn; $Ca_5(PO_4)Cl$:Eu,Mn; $Sr_5(PO_4)Cl$:Ce,Mn; $Sr_5(PO_4)F$:Eu,Mn; $CaSO_4$:Bi; $SrSO_4$:Bi; $CaSO_4$:Eu,Mn; $CaSO_4$:Ce,Mn; $CaGa_2S_4$:Mn; $ZnGa_2S_4$:Mn; $CaGa_2S_4$:Eu; $CaGa_2O_4$:Eu; $CaGa_2S_4$:Ce; Y_2O_2S:Eu; $SrSi_5N_8$:Eu; $LaSi_3N_5$:Eu,O; SrS:Eu; SrS:Mn; SrS:Cu,Na; ZnS:Sn; ZnS:Cu,Au,Al; Zns:Au,In; ZnS:Cu,Al; and $(M_1,M_2)_xSi_{a-y}Al_yO_zN_{b-z}$:SE, wherein M_1 and M_2 are independently selected from: Li, Na, K, Rb, Cs, Cu, Ag, Be, Mg, Ca, Sr, Ba, Zn, Sn, Pb, Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, B, Ga or In; and SE is selected from: Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, or Lu, and most preferably from: Ce, Pr, Sm, or Eu.

6. The contrast agent according to any of the foregoing claims, wherein the particles (1) further comprise at least one optional shell (3-4) on the core (2).

7. The contrast agent according to claim 6, wherein at least one of the shells (3-4) provides bio-compatibility.

8. The contrast agent according to claim 7, wherein the at least one optional shell (3-4) has a thickness of 1 to 200 nm, preferably 20 to 100 nm.

9. The contrast agent according to any of the claims 6 to 8, wherein at least one further shell (3-4) is present containing at least one antibody.

10. The contrast agent according to claim 9, wherein the at least one antibody is a tumor-specific antibody.

11. The contrast agent according to claim 9, wherein the at least one antibody containing shell (3-4) further contains one or more proteins, preferably the HIV-tat protein.

12. The contrast agent according to any of the foregoing claims, wherein the core (2) has a spherical, oval or lens shape.

13. The contrast agent according to any of the foregoing claims, wherein the core (2) has a diameter of 1 to 1000 nm, preferably 50 to 500 nm.

14. A pharmaceutical formulation comprising a contrast agent and a pharmaceutically acceptable excipient, wherein the contrast agent is formed according to any of the foregoing claims; and wherein the formulation is suitable for administration as an imaging enhancing agent and the contrast agent is present in an amount sufficient to enhance a optical imaging image.

15. The pharmaceutical formulation of claim 14, wherein the pharmaceutical acceptable excipient is a buffered saline.

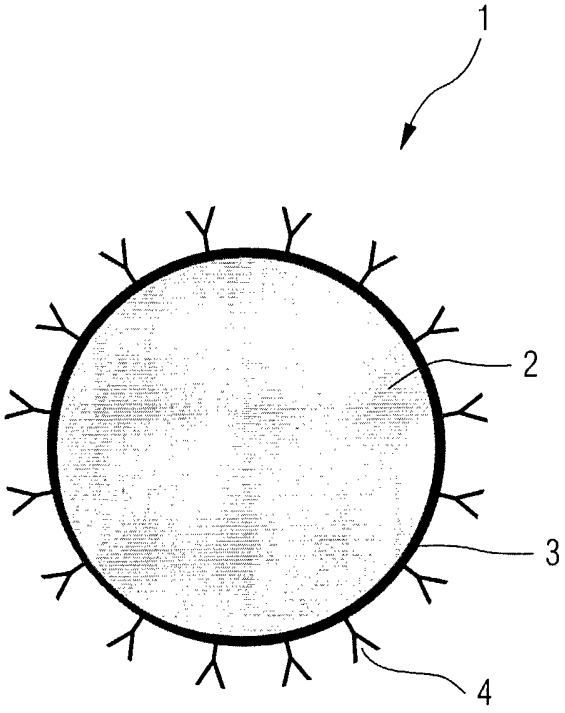

FIG.1