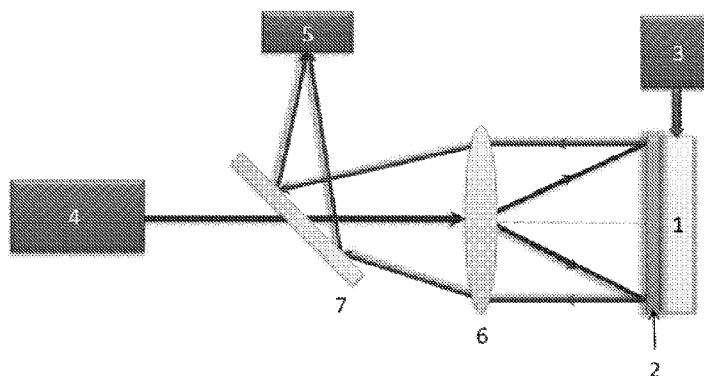




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(54) **Title:** MEASURING A SPATIO-TEMPORAL TEMPERATURE PROFILE OF AN OBJECT

Figure 1



(57) **Abstract:** The invention is directed to a microscopic method for measuring a spatio-temporal temperature profile of an object. The microscopic method of the invention comprises: i) providing a coating onto said object, wherein the coating comprises at least one luminophore having a temperature dependent luminescence quantum yield and is in thermal contact with the surface of said object; iii) performing the sequence of a) subjecting at least part of the coated object to heat, wherein said heat defines a time, t_0 ; b) waiting for a delay period p ; c) subjecting at least part of the coated object to a photoexcitation pulse at a time, t_1 , after t_0 , thereby photoexciting at least part of the luminophores, wherein $t_1 = t_0 + p$; d) collecting a luminescence image from the photoexcited luminophores during a time interval Δt , starting at a time t_2 , at or after t_1 ; and e) optionally repeating steps a)-d) for a number of times to improve signal to noise ratio; iv) repeating step iii) for a number of times wherein in each step iii) a different delay period p is applied; and vi) converting the collected luminescence image at each delay period p to a temperature image using a pre-determined calibrated relation between luminescence intensity and temperature.

Title: Measuring a spatio-temporal temperature profile of an object

The invention is directed to a microscopic method for measuring a spatio-temporal temperature profile of an object.

The measurement of surface temperature profiles is a valuable diagnostic tool, for instance, in the microelectronic industry and in other areas.

5 Such measurements provide information on the device physics and functionality which would then facilitate optimisation processes and increase device yields. As the functional devices become ever smaller, their density increases and clock speeds become ever faster, local heat generation degrades their speed, reliability, and, hence, the lifecycle. The increase of speed and
10 productivity or functionality of modern micro- and nanotechnology crucially depends therefore on efficient thermal management.

Thermal management in innovative technologies is a major problem to be solved, especially in novel electronic devices, such as further miniaturised microchips, memory devices, and interfaces between biological structures and
15 semiconductor microstructures. Therefore, the semiconductor industry is highly interested in new thermal imaging technologies which allow high space (*viz.* nanometer scale) and time resolution.

However, measurement of temperature profiles is difficult, in particular because of the high spatial, thermal, and temporal resolution which
20 would be necessary to adequately detect and map such temperature profiles.

Some previously reported techniques for measuring surface temperature profiles of objects include infrared thermal microscopy and photo emission microscopy in combination with Raman microscopy.

These methods, however, fail to provide the required spatial,
25 thermal, and/or temporal resolution in order to acquire the desired details regarding the temperature profile.

US-A-4 455 741 describes a method of measuring surface temperature profiles of a device by applying a thin film of material to the device, which material luminesces when irradiated with appropriate energy.

This document does not teach using a different delay period for each pulse sequence.

Kolodner *et al.* (*Appl. Phys. Lett.* 1982, *40*(9), 782-784) describe the measurement of surface temperature profiles by depositing a film whose
5 fluorescence quantum yield depends on the temperature. At low temperatures, the film exhibits bright fluorescence under ultraviolet excitation, while the fluorescence intensity decreases with temperature due to temperature-dependent quenching. The spatial resolution of the method in this document is limited by the diffraction limit (*i.e.* it is inversely proportional
10 to the wavelength of the emitted light). For fluorescent materials this, typically, leads to a spatial resolution in the range of from 500 to 1000 nm. In addition, the technique in this document does not allow a high temporal resolution.

US-A-4 819 658 describes a method of measuring surface
15 temperature profiles by applying a thin luminescent film to a device, wherein the intensity of the luminescence is dependent upon the temperature of the material. This document further suggests applying heat pulses and photo-excitation pulses to reduce the time resolution, such as down to about 10 ns or less.

20 EP-A-1 936 345 describes a method of measuring a temperature distribution with high spatial and temporal resolution. The method uses a layer that contains molecules which exhibit a temperature dependent luminescence ratio at a first and second wavelength. In order to reduce the spatial resolution beyond the diffraction limit, it is suggested to apply
25 stimulated emission depletion microscopy.

Motosuke *et al.* (*Journal of Mechanical Science and Technology* 2009, *23*, 1821-1828) describe the measurement of the temperature of a fluid in a microfluidic device with high temporal and spatial resolution using laser-induced fluorescence. Temperature dependency is induced by employing
30 a temperature dependent fluorophore.

Despite these reported efforts, there remains a need for improving the visualisation of a spatio-temporal temperature distribution of an object with excellent spatial, thermal, and temporal resolution.

Objective of the invention is to provide a solution for this existing
5 need in the art.

The inventors found that this objective can, at least in part, be met by providing the object with a specific coating and repetitively subjecting the coated object to a defined sequence of irradiation pulses.

Accordingly, in a first aspect the invention is directed to a
10 microscopic method for measuring a spatio-temporal temperature profile of an object, comprising:

- i) providing a coating onto said object, wherein the coating comprises at least one luminophore having a temperature dependent luminescence quantum yield and is in thermal contact with the surface of said object;
- 15 iii) performing the sequence of
 - a) subjecting at least part of the coated object to heat, wherein said heat defines a time, t_0 ;
 - b) waiting for a delay period p ;
 - c) subjecting at least part of the coated object to a photoexcitation
20 pulse at a time, t_1 , after t_0 , thereby photoexciting at least part of the luminophores, wherein $t_1 = t_0 + p$;
 - d) collecting a luminescence image from the photoexcited luminophores during a time interval Δt , starting at a time t_2 , at or after t_1 ; and
 - e) optionally repeating steps a)-d) for a number of times to improve
25 signal to noise ratio;
- iv) repeating step iii) for a number of times wherein in each step iii) a different delay period p is applied; and
- vi) converting the collected luminescence image at each delay period p to a temperature image using a pre-determined calibrated relation between
30 luminescence intensity and temperature.

The method of the invention allows the measurement of dynamic spatio-temporal temperature profiles, such as in micro-electromechanical systems or any object on which the coating can be applied. The temperature profiles are advantageously translated into one or more microscopic images with excellent thermal, spatial and temporal resolution. The inventors found that the method of the invention allows a thermal resolution of better than 0.01 K, a spatial resolution of better than 20 nm, and a temporal resolution of better than 500 ps. The combination of these excellent resolutions is unique to the method of this invention.

The object of which the spatio-temporal temperature profile is to be determined can be an electronic device, including a micro-electromechanical system, a biological object, or combinations thereof. Preferably, the object is a solid state object, or at least comprises a solid state surface onto which the coating can suitably be applied.

In step i) of the method the object is provided with a coating. The coating may be applied onto the object using conventional techniques, including spin-coating, dip-coating, drop casting, doctor blading, Langmuir-Blodgett techniques, spraying, thermal evaporation, molecular beam deposition, lamination and adhering, *e.g.* gluing.

The coating can be applied to the surface of the object in the form of a thin film. The coating can suitably have a thickness of 10 nm or more, such as 100 nm or more, or 500 nm or more. Normally, the coating will have a thickness of 10 μm or less, preferably 5 μm or less, such as 2 μm or less. If the coating thickness is more than 10 μm , then this will have an adverse effect on the spatial and thermal resolution. For example, if the heat capacity of the coating is relatively large, the layer itself will distort the observed temperature profile. The coating may optionally be a multilayer.

The coating comprises a material which luminesces upon exposure to radiation. For example, such materials, upon exposure to ultraviolet radiation (having a wavelength ranging from 200 to 400 nm), may be excited,

and upon de-excitation will emit ultraviolet, visible (wavelength ranging from 400 to 700 nm), or infrared (wavelength ranging from 700 to 1000 nm) radiation. In this application, such materials are generally referred to as luminophores.

5 The term “luminophore” as used in this application is meant to refer to a species that upon photoexcitation can emit light through fluorescence or phosphorescence. The term “luminophore” in the context of this application is meant to include a fluorophore, a chromophore, a phosphorescent compound, a quantum dot, a luminescent nanoparticle (that for instance comprise
10 multiple luminescent molecules), and the like. Nanoparticles in this respect can have an average particle size (defined as the diameter of an equivalent sphere having the same volume) in the range of 1-500 nm, such as 10-200 nm. The equivalent spherical diameter of nanoparticles can be determined by dynamic light scattering or by high resolution imaging techniques such as
15 electron microscopies or by coherent X-ray imaging.

 The term “luminescence” as used in this application is meant to refer to the emission of electromagnetic radiation (light) from a material. In particular, this term is meant to include fluorescence and phosphorescence.

 In order to achieve the required thermal accuracy, the luminophores
20 must emit with an intensity that is characteristic of the temperature of the underlying object. Therefore, it is required that the coating is in thermal contact with the surface of the object. In addition, the luminophore must have a temperature dependent luminescence quantum yield so that it is capable of translating the temperature of the surface of the object to a luminescence
25 intensity that is correlated with said temperature.

 The luminophores in the coating can for example comprise one or more rare earth metal chelate luminophores. The rare earth metal is preferably europium or terbium. The chelate can, for instance, comprise one or more selected from bidentate ligands, tridentate ligands, and tetradentate
30 ligands. Examples of luminophores that can be used for this purpose include,

but are not limited to, europium thenoyltrifluoro-acetate (EuTTA) and perdeutero(tris-6,6,7,7,7,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionate) europium (EuFOD).

Suitably, the luminophore can be incorporated into a polymer film which is applied onto a surface of the object. The luminophore and polymer can, for example, be dissolved in an appropriate solvent, and the solution can be spun onto the surface of the object. Optionally, the solution can be filtered prior to spinning in order to remove aggregates or crystallites. The solvent evaporates during the spinning step, leaving a thin film of polymer homogeneously doped with the luminophore. Examples of polymers that can be used for incorporation of the luminophore include polystyrenes, polymethylmethacrylates, polycarbonates, polysilicones (such as poly(dimethylsiloxane), and polyolefins (such as polyethylene or polypropylene). Preferably, the polymer is optically transparent (*i.e.* the thin film of polymer without the luminophore is preferably optically transparent). The term “optically transparent” as used herein is meant to refer to transparency (transmission of 50 % or more, such as 60 % or more, or 80 % or more, preferably 90 % or more) in the range of emission of the luminophore. It is further preferred that the polymer is inert. The term “inert” as used herein is meant to signify that such polymer is chemically inert, *i.e.* it neither undergoes chemical reactions with the fluorophore embedded in the polymer, nor with the sample.

In a first embodiment, the method of the invention is used to perform excellent temporal resolution (such as better than 1000 ps, smaller than 100 ps, or smaller than 10 ps) and temperature resolution (such as better than 1 K, better than 0.1 K, or better than 0.01 K) with good spatial resolution (such as better than 300 nm).

In accordance with this first embodiment, the luminophores can have a temperature dependent quantum efficiency wherein preferably dI/dT is at least 1 % per degree K, more preferably at least 2 % per degree K, or even

more preferably 5 % per degree K. The coating can suitably have a luminophore concentration in the range of 0.01-100 luminophores per 10 nm³ of coating, such as in the range of 0.1-10 luminophores per 10 nm³ of coating. In an embodiment, the luminophores are present in the coating at a
5 concentration in the range of from 20-50 mol% based on the total coating, preferably from 50-80 mol%.

In accordance with this first embodiment of the invention, the object is subjected to heat, after which multiple luminescence images are collected at variable delay time p in order to obtain the time development of the heat
10 profile. Consequently, each of the luminescence images at a specific delay time can be converted into a temperature image using a calibrated relation between the luminescence intensity and temperature. This calibrated relation depends on the luminophore that is applied in the method of the invention and can be readily determined by the person skilled in the art.

15 In accordance with step iii)a), at least part of the coated object is subjected to heat. The temporal behaviour of this heat defines a time t_0 . This may be achieved, for instance, by applying a heat pulse (thereby defining a starting point), or by subjecting the object to a heat wave, which can define a time t_0 using the wave phase. Hence, the heat may be in pulsed or modulated
20 mode. The time scale of the induced transient heat variations may be in the range of 5 ps to 1 s. The heat may, for example, be generated using a laser, a heater, an electronic element, or the like. The coated object may be subjected to the heat as a whole, but also a part of the coated object can be subjected to the heat.

25 After having applied the heat, a delay time p is applied, after which at least part of the coated object is subjected to a photoexcitation pulse at time t_1 . The photoexcitation pulse serves to photoexcite luminophores in the irradiated area. After photoexcitation, these photoexcited luminophores will return to their ground state while emitting light. The photoexcitation pulse
30 can, for example, be generated by means of a laser or diode. The power of the

source should be sufficient to excite at least part of the luminophores, such as at least 1 %, at least 10 %, or substantially all luminophores. Typically, the photoexcitation pulse will be shorter than the time resolution that is intended. For, example the photoexcitation pulse can last for 100 fs to 200 ps, such as 100
5 fs to 100 ps. The photoexcitation pulse will normally be such that it illuminates the whole area of interest of the coated object.

The wavelength of the photoexcitation pulse should be suitable for photoexcitation of the applied luminophores. Typically, the wavelength of the photoexcitation pulse will be in the ultraviolet (200-400 nm) or in the visible
10 (400-700 nm) part of the spectrum. The photoexcitation pulse can, for example, have a wavelength in the range of 250-700 nm, preferably in the range of 300-500 nm.

The photoexcitation pulse is applied a delay time p after the heat is applied, such that $t_1 = t_0 + p$. The lower limit of the delay time p is defined by
15 the duration of the photoexcitation pulse, which can for instance be 10 ps, or preferably 5 ps. The upper limit of the delay time p can, for example be in the range of 10 ps to 1 s and even longer, such as 10 ps, 1 ns, or 1 μ s. This delay time p is varied in step iv) of the method of the invention as will be discussed hereinafter.

20 After photoexcitation of luminophores by the photoexcitation pulse, at least some of the luminophores will luminesce. This luminescence is collected as a luminescence image in step iii)d) of the method of the invention during a time interval Δt , starting at a time t_2 , at or after t_1 . The luminescence intensity can be collected as a function of the location. Hence, a (x,y,I) image is
25 obtained, wherein x is the location on the x-axis, y is the location on the y-axis, and I is the luminescence intensity. The luminescence can be measured using a suitable detector, such as a charge coupled device (CCD) camera, or a photomultiplier. It is preferred to use a CCD camera.

In a preferred embodiment, a reference luminescence image that has
30 been collected without subjecting the object to the heat is subtracted from the

luminescence images at each delay period p . Hence, in this embodiment, each collected luminescence image in fact represents a difference luminescence image as a function of location $(x,y,\Delta I)$. In this way, the temperature resolution of the method of the invention is improved.

5 The time interval Δt can vary widely, from 100 ns to 1 s, such as 500 ns to 500 μ s, and strongly depends on the lifetime of the luminescent state.

The luminescence collection will normally be focussed on the field of interest.

 The luminescence can have varying wavelengths, depending on the applied luminophores. This may comprise wavelengths in the ultraviolet,
10 visible or infrared range of the spectrum.

 Optionally, a step iii)e) may be performed wherein steps iii)a)-iii)d) are repeated for a number of times to improve the signal to noise ratio. Good results in respect of improved signal to noise ratio can already be achieved by repeating steps iii)a)-iii)d) for 4 times or more, such as 9 times or more, or 16
15 times or more. Although the signal to noise ratio can be improved by multiple repetitions, of course this involves additional measuring time. Depending on the application and the intended purpose, the skilled person will be able to balance the signal to noise ratio to the measuring time.

 After having performed the sequence of step iii), the method of the
20 invention comprises a step iv) wherein step iii) is repeated for a number of times with variable delay time p (*i.e.* using for each step iii) a different delay time p). By applying a variable delay time p , for each sequence of step iii), a time development of the heat profile of the object is obtained. Step iii) can, for example, be repeated for 5 times or more, preferably for 7 times or more, such
25 as 10-20 times.

 Thereafter, in step vi) of the invention, the (x,y,I) images, that contain spatially resolved information on the luminescence intensity at different delay times p , are converted into (x,y,T) images, that contain spatially resolved information on the temperature at different delay times p .
30 This can be achieved by using a calibrated relation between the luminescence

intensity of the applied luminophore and temperature. Such relation may be known for the respective luminophore, or it may be pre-determined. This may, for instance, be done using the procedure as described by Otter *et al.* in *Journal of Magnetism and Magnetic Materials* 2009, 321, 796. In the preferred
5 embodiment described above, wherein each luminescence image represents a difference luminescence image, the (x,y, ΔI) images are converted into (x,y, ΔT) images.

In a second embodiment, the method of the invention is used to perform excellent temporal resolution (such as better than 1000 ps, smaller
10 than 100 ps, or smaller than 10 ps), temperature resolution (such as better than 1 K, better than 0.1 K, or better than 0.01 K) and spatial distance resolution (such as better than 20 nm).

In this second embodiment, the luminophores (like in the first embodiment described above) can have a temperature dependent quantum
15 efficiency wherein preferably dI/dT is at least 1 % per degree K, more preferably at least 2 % per degree K, or even more preferably 5 % per degree K. However, in accordance with this second embodiment, the coating has a very low concentration of luminophores in the coating, such as 1-10 luminophores per $1 \mu\text{m}^2$ of coating. It is preferred in this embodiment, that the luminophores
20 are nanoparticles, such as nanoparticles comprising multiple luminescent molecules.

An example of an instrumental setup for carrying out the first or second embodiment of the invention is shown in figure 1, wherein 1 is a the object of which the spatio-temporal temperature profile is to be determined, 2
25 is a coating with a temperature-dependent luminescence material applied to the object, 3 is a heat control unit, 4 is a source of photo-excitation radiation, 5 is an image detector, 6 is a collection lens, and 7 is a beam splitter.

Steps iii) and iv) in this second embodiment are the same as for the first embodiment, described hereinabove. Suitably, in step vi) the collected
30 luminescence images at each delay period p are fitted to two dimensional

peaked intensity distributions at the positions of the luminophores prior to converting the collected luminescence image at each delay period p to a temperature image using integrated peak intensities of single luminophores. Thereby, the spatial resolution of the method will be improved.

5 In a third embodiment, the method of the invention is used to perform excellent temporal resolution (such as better than 1000 ps, smaller than 100 ps, or smaller than 10 ps), temperature resolution (such as better than 1 K, better than 0.1 K, or better than 0.01 K) and spatial resolution (such as better than 20 nm). Contrary to the second embodiment, however, this third
10 embodiment does not require such low concentration of luminophores.

 In this third embodiment, the luminophores are single molecules that can have a temperature dependent quantum efficiency wherein preferably dI/dT is at least 1 % per degree K, more preferably at least 2 % per degree K, or even more preferably 5 % per degree K. In this embodiment, however, the
15 luminophores have an active state (in which they are capable of luminescence when excited at a specific wavelength) and an inactive state (in which they are incapable of luminescence when excited at the same wavelength). The transition of the active state to the inactive state (or *vice versa*) may be reversible or irreversible. It can, for instance, be a photoisomerisation process,
20 wherein one photoisomer represents the active state, while the other photoisomer represents the inactive state. It is preferred in this embodiment, that the luminophores are photoisomerising luminescent molecules. For instance, the molecules can be rare earth metal chelates wherein the ligand can photoisomerise thereby allowing a transition from an active (luminescent)
25 state to an inactive (non-luminescent) state of the molecule.

 An example of an instrumental setup for carrying out the third embodiment of the invention is shown in figure 2, wherein 1 is a the object of which the spatio-temporal temperature profile is to be determined, 2 is a coating with a temperature-dependent luminescence material applied to the
30 object, 3 is a heat control unit, 4 is a source of photo-excitation radiation, 5 is

an image detector, 6 is a collection lens, 7 and 8 are a beam splitters, and 9 is a source of activation/inactivation radiation.

The coating, in accordance with this third embodiment, can suitably have a luminophore concentration in the range of 0.01-1 luminophores per 10
5 nm³ of coating, such as in the range of 0.05-0.5 luminophores per 10 nm³ of coating, or 0.1-0.5 luminophores per 10 nm³. In an embodiment, the luminophores are present in the coating at a concentration in the range of from 20-50 mol% based on the total coating, preferably from 50-80 mol%.

In this third embodiment, prior to step iii) at least part of the coated
10 object is subjected to an activation or inactivation radiation pulse, wherein part of the luminophores are at least temporarily activated or inactivated. Preferably, 1-10 % of the luminophores (more preferably 1-5 % of the luminophores) in the area of interest should become or remain active after being subjected to the activation or inactivation radiation pulse, respectively.

15 If an inactivation radiation pulse is applied, then this inactivation radiation pulse can have the effect that at least 90 % of the luminophores that are subjected to the inactivation radiation pulse is inactivated, preferably at least 95 %, such as at least 98 %. Alternatively, if an activation radiation pulse is applied, then this activation radiation pulse can have the effect that at most
20 10 %, of the luminophores that are subjected to the activation radiation pulse are activated, preferably at most 5 %, such as at most 2 %.

This activation or inactivation radiation pulse may typically be generated by a laser having a wavelength in the ultraviolet, visible or (near) infrared range of the spectrum. The activation or inactivation radiation pulse
25 can, for example, have a wavelength in the range of 250-1000 nm, preferably 350-800 nm. Suitable lasers include, for instance, dye lasers, solid-state lasers or gas lasers. The pulse can, for example, have a duration in the range of from 10 ps to 1 ms, such as in the range of from 100 ps to 100 μ s. Normally, the entire area of interest will be irradiated. The exact timing of this activation or

inactivation radiation pulse is not critical, as long as it is performed prior to step iii) of the method of the invention.

Because after the activation or inactivation radiation pulse only a relatively small part of the luminophores is in the active state, a situation is created wherein detection of luminescence from single luminophores becomes possible. Contrary to the second embodiment described above, however, the concentration of luminophores can be at a reasonably high level, and in any case significantly higher than in the second embodiment.

Steps iii) and iv) in this third embodiment are the same as for the first and second embodiments, described hereinabove.

The third embodiment of the method of the invention, further comprises a step v), after step iv), wherein previously activated luminophores are inactivated and/or destroyed, or wherein previously inactivated luminophores are activated. This step may be performed by application of a laser pulse, such as an ultraviolet, visible or (near) infrared laser pulse. The duration of the pulse may be from 10 ps to 1 ms, such as from 100 ps to 10 μ s, or from 1 ns to 1 μ s. In an embodiment, this step involves a back transition of previously converted photoisomers to their original state (before the activation or inactivation radiation pulse). Normally, this step will involve irradiation of the whole field of interest.

Suitably, in step vi) of the third embodiment, the collected luminescence images at each delay period p can be fitted to two dimensional peaked intensity distributions at the positions of the luminophores prior to converting the collected luminescence image at each delay period p to a temperature image using integrated peak intensities of single luminophores. Thereby, the spatial resolution of the method will be improved.

In accordance with the third embodiment, steps ii) to v) are repeated for a number of times in order to increase the spatial resolution. For example, the steps ii) to v) can be repeated for 2-50 times, such as 5-30 times, or 10-25 times. The images at each specified delay time p from the repeated cycles are

then combined to arrive at the desired spatio-temporal temperature profile of the object.

Claims

1. Microscopic method for measuring a spatio-temporal temperature profile of an object, comprising:
 - i) providing a coating onto said object, wherein the coating comprises at least one luminophore having a temperature dependent luminescence quantum yield and is in thermal contact with the surface of said object;
 - 5 iii) performing the sequence of
 - a) subjecting at least part of the coated object to heat, wherein said heat defines a time, t_0 ;
 - b) waiting for a delay period p ;
 - 10 c) subjecting at least part of the coated object to a photoexcitation pulse at a time, t_1 , after t_0 , thereby photoexciting at least part of the luminophores, wherein $t_1 = t_0 + p$;
 - d) collecting a luminescence image from the photoexcited luminophores during a time interval Δt , starting at a time t_2 , at or after t_1 ; and
 - 15 e) optionally repeating steps a)-d) for a number of times to improve signal to noise ratio;
 - iv) repeating step iii) for a number of times wherein in each step iii) a different delay period p is applied; and
 - vi) converting the collected luminescence image at each delay period p to a temperature image using a pre-determined calibrated relation between
20 luminescence intensity and temperature.
2. Method according to claim 1, wherein the concentration of the luminophores in the coating is in the range of 1-10 luminophores per $1 \mu\text{m}^2$ of
25 coating, and wherein the collected luminescence images at each delay period p are fitted to two dimensional peaked intensity distributions at the positions of the luminophores prior to converting the collected luminescence image at each

delay period p to a temperature image using integrated peak intensities of single luminophores.

3. Method according to claim 1, wherein said luminophores are
5 luminescent molecules and wherein said method further comprises a step ii) prior to step iii), and a step v) after step iv):

- ii) subjecting at least part of the coated object to an activation or inactivation radiation pulse, thereby at least temporarily activating or inactivating part of the luminophores,
- 10 v) inactivating previously activated luminophores, activating previously inactivated luminophores, or destroying previously activated luminophores using a radiation pulse, and

wherein the collected luminescence images at each delay period p are fitted to two dimensional peaked intensity distributions at the positions of the
15 luminophores prior to converting the collected luminescence image at each delay period p to a temperature image, and
wherein steps ii) to v) are repeated for a number of times.

4. Microscopic method according to claim 3, wherein said activation or
20 inactivation radiation pulse has a wavelength in the range of 250-1000 nm, preferably 350-800 nm.

5. Microscopic method according to any one of claims 3 or 4, wherein
- the part of the luminophores that is inactivated in step ii) is at least 90 %
25 of the luminophores that are subjected to activation or inactivation radiation pulse, preferably at least 95 %, such as at least 98 %; or
- the part of the luminophores that is activated in step ii) is at most 10 %, of the luminophores that are subjected to said activation or inactivation radiation pulse, preferably at most 5 %, such as at most 2 %.

6. Microscopic method according to any one of claims 1-5, wherein the object is an electronic device, including a micro electro-mechanical system, or a biological object.
- 5 7. Microscopic method according to any one of claims 1-6, wherein said coating comprises an optically transparent polymer, preferably one or more selected from the group consisting of polystyrene, polymethylmethacrylate, polycarbonate, polysilicone, and polyolefin.
- 10 8. Microscopic method according to any one of claims 1-7, wherein said luminophore comprises a rare earth metal chelate luminophore, wherein the rare earth metal is preferably europium or terbium, such as one or more selected from the group consisting of europium-thenoyltrifluoroacetate, perdeutero(tris-6,6,7,7,7,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionate)
- 15 europium.
9. Microscopic method according to any one of claims 1-8, wherein the coating has a thickness of from 50 nm to 10 μm , preferably from 100 nm to 5 μm , such as from 500 nm to 2 μm .
- 20 10. Microscopic method according to any one of claims 1-9, wherein the luminescence image collected in step iii)d) represents a difference luminescence image obtained by subtracting a reference luminescence image from the collected luminescence image, wherein said reference luminescence
- 25 image is collected without subjecting the object to said heat.
11. Microscopic method according to any one of claims 1-10, wherein said photoexcitation pulse is a pulse of 5-200 ps, preferably 10-100 ps.

12. Microscopic method according to any one of claims 1-11, wherein said photoexcitation pulse has a wavelength in the range of 250-700 nm, preferably in the range of 300-500 nm.
- 5 13. Microscopic method according to any one of claims 1-12, wherein Δt is in the range of 100 ns to 1 s, and wherein said luminescence image is preferably collected using a charge-coupled device camera.
14. Microscopic method according to any one of claims 1 or 3-13, wherein
10 the luminophores are present in said coating at a concentration in the range of from 20-50 mol% based on the total weight of the coating, preferably from 50-80 mol%.
- 15 15. Microscopic method according to any one of claims 1-14, wherein step iv) involves repeating step iii) for 4 times or more, preferably for 9 times or more, such as 10-100 times.

1/1

Figure 1

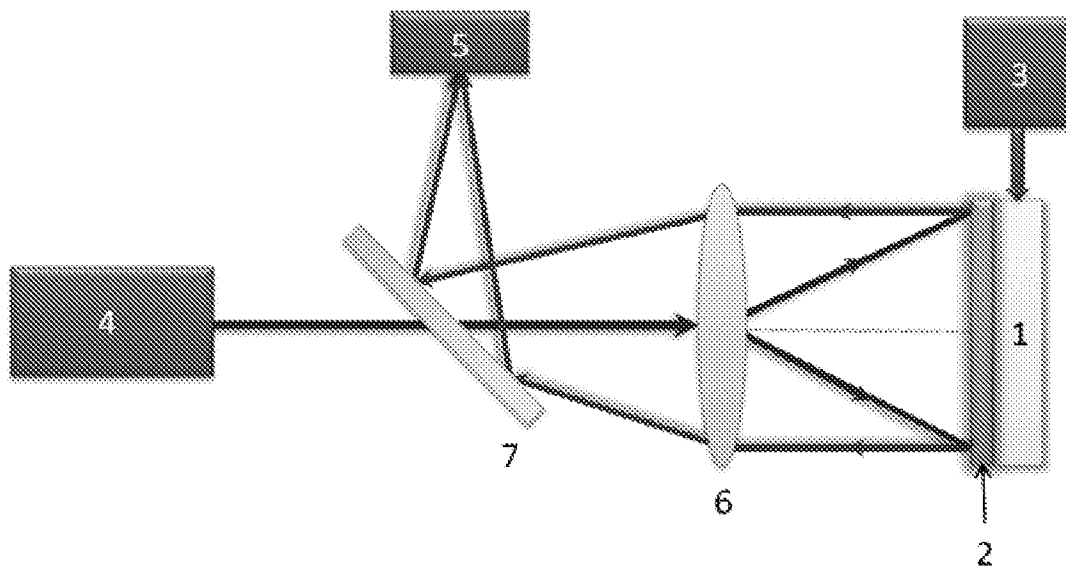
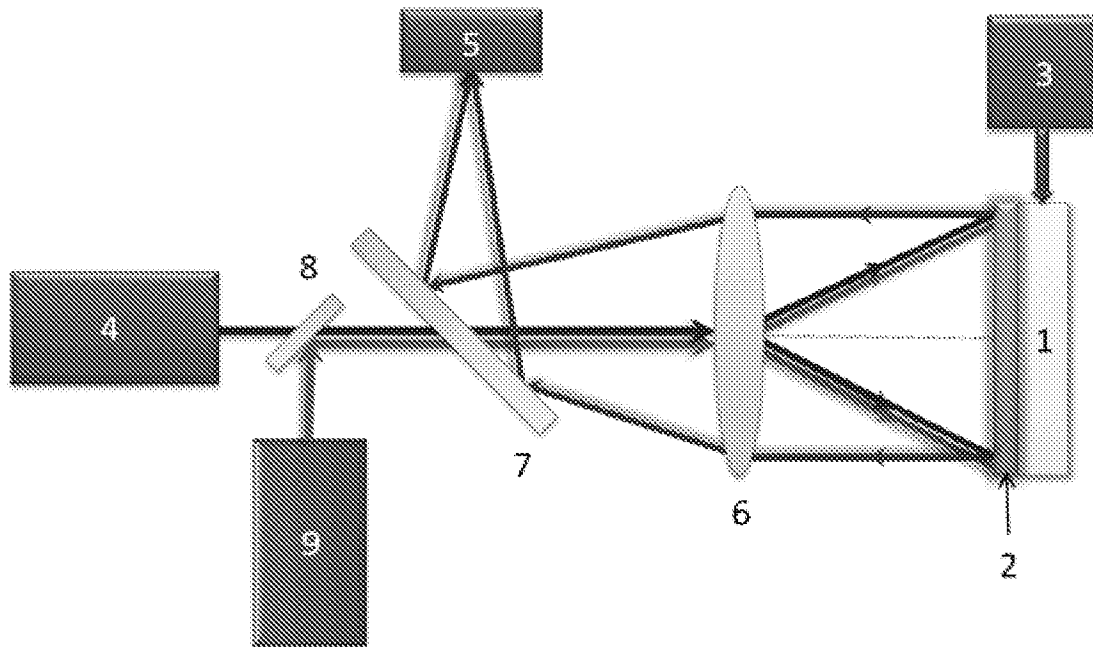


Figure 2



INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2012/050257

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01K11/20
ADD.

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)
G01K G01N

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)

EP0-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 455 741 A (KOLODNER) 26 June 1984 (1984-06-26) cited in the application abstract; figures columns 2-5,9,11 column 6, line 46 - column 9, line 18 -----	1-15
A	KOLODNER ET AL.: "Microscopic fluorescent imaging of surface temperature profiles with 0,01°C resolution", APPL. PHYS. LETT., vol. 40, no. 9, 1982, pages 782-784, XP055008469, cited in the application abstract -----	1-15

☐

Further documents are listed in the continuation of Box C.

☒

See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"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

"&" document member of the same patent family

Date of the actual completion of the international search

4 July 2012

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/NL2012/050257

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4455741	A	26-06-1984	NONE
