

MEMBRANE FOR RETAINING A MICROSPHERE

Field of the invention

5 The present invention relates to a novel optical nano-imaging scheme by using a transparent particle as an optical lens in order to achieve super-resolution nano-imaging. In particular, the present invention relates to a membrane for retaining a microsphere. The present invention relates to the membrane, and its application in super-resolution nano-imaging.

10 Background of the invention

The optical microscope, often referred to as the "light microscope", is a type of microscope which uses visible light and a system of lenses to magnify images of small samples. Optical microscopes are the oldest design of microscope and were possibly invented in their present
15 compound form in the 17th century. Basic optical microscopes can be very simple, although there are many complex designs which aim to improve resolution and sample contrast.

Alternatives to optical microscopy which do not use visible light include scanning electron microscopy and transmission electron microscopy. At very high magnifications with
20 transmitted light, point objects are seen as fuzzy discs surrounded by diffraction rings. These are called Airy disks. The resolving power of a microscope is taken as the ability to distinguish between two closely spaced Airy disks (or, in other words the ability of the microscope to reveal adjacent structural detail as distinct and separate). It is these impacts of diffraction that limit the ability to resolve fine details. The extent and magnitude of the diffraction
25 patterns are affected by both the wavelength of light (λ), the refractive materials used to manufacture the objective lens and the numerical aperture (NA) of the objective lens. There is therefore a finite limit beyond which it is impossible to resolve separate points in the objective field, known as the diffraction limit.

30 For centuries, optical lens maker and researchers work diligently to pursue smaller and smaller resolution of optical microscopes. It is believed that traditional optics confines the resolution physically to about a half of the incident wavelength by the Abbe diffraction limit.

However, as modern techniques develop over the years, breakthrough of the resolution limit can be achieved. One of the most promising approaches is to observe subwavelength features through transparent microspheres.

5 This breakthrough further leads to a broad interest of microsphere imaging study. These research have confirmed the resolution performance of microspheres. In general, the function of the microsphere is to magnify the virtual image of the sample to a size that can be resolved by conventional optical microscopes. Microspheres are able to focus the incident light into a sharp point. By introducing a microsphere above the sample surface,
10 subwavelength features on the sample can be illuminated by the focused light and an enlarged virtual image can be formed and captured by eyes or CCDs. In current development of this technique, most of the experiments are conducted by placing the microspheres directly on top of the samples, which have many disadvantages, such as pollution or destruction to fragile samples, inability to scan the surface, difficulty in separating the sample and
15 microspheres apart and difficulty in achieving the optimal image plane. To further promote the development of this field for practical applications, separating the microspheres from the sample surface is essential.

Current particle lens nano-imaging suffers from some fundamental limitations for its practical
20 applications, such as direct contact of the particle lens with the object, slow imaging speed and image distortion due to chromatic aberration.

Hence, there exists a need for an improved super-resolution optical imaging system.

25 The listing or discussion of an apparently prior-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.

Any document referred to herein is hereby incorporated by reference in its entirety.

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Summary of the invention

The present invention, making use of a microsphere as an optical element for super-resolution imaging, offers the possibility to restore the nanoscale information in the far-field. In a suitable apparatus in accordance with the invention, incident light can be trapped inside a tiny transparent sphere by bouncing the photons around the interior through total internal reflection. At its resonance, the enhanced energy only emits out through a small window. This preference allows a number of interesting applications. For example, a transparent microsphere can form an efficient laser cavity.

Thus, this leads to a novel lens assembly chip has been contemplated which can be integrated with a traditional optical microscope to solve the problems mentioned above. In particular, the lens assembly chip includes a microsphere that is retained by a thin membrane, avoiding contamination and destruction to the sample. Another key advantage is that by introducing the distance between the objective lens and microsphere, the optimal image plane can be found flexibly by adjusting the vertical position of the membrane, ensuring the best performance of this experimental setup. Also, as the microsphere and the sample are separated, scanning of the whole surface can be achieved by either moving the sample using the stage installed on a traditional microscope or moving the microsphere located on the membrane. In addition, the low fabrication price and simple setup make our design to be an excellent candidate which can be coupled with commercialized microscopes.

In a first aspect of the present invention, there is provided a membrane having a first surface and a second surface, the first and second surfaces oppose each other, the membrane comprising: (a) an opening penetrating through the membrane and is sized to retain a microsphere, wherein the opening is tapered, the size of the opening on the first surface is larger than the size of the opening on the second surface.

By "membrane", it is meant to refer to any thin sheet material suitable for holding and retaining the microsphere within it. The membrane may be of any suitable thickness and may be material of any suitable material. Accordingly, first and second surface that oppose each other and those two surfaces on either side of the membrane. The opening penetrating through the membrane may include any hole, perforation, aperture of the like that allows the reception and the retention of the microsphere within the membrane.

The inner profile of the opening may be an inverted pyramid. Alternatively, the inner profile of the opening is frusto-conical in shape.

5 In an embodiment, the opening in the membrane is tapered, i.e. the size of the hole penetrating through the membrane is diminished or reduced in size from one end towards the other opposite end. Such a configuration allows for a microsphere to be inserted into the opening at one end, but not allowed through the opening. Thus, being retained within the opening. Preferably, the opening is sized to retain a microsphere having a diameter between
10 4 μm to 50 μm , the size of the opening of the first surface is larger than the diameter of the microsphere, and the size of the opening on the second surface is smaller than the diameter of the microsphere. In alternative embodiments, the diameter of the microsphere may be between 5 μm to 30 μm . The opening in the membrane may be fabricated in any suitable method, for example by an etching method (chemically or mechanically), or puncturing the
15 membrane, or the like.

By "microsphere", it is meant to include any small spherical particles with diameters in the micrometer range. In an embodiment of the present invention, the diameter of the microsphere may be about between 4 μm to 10 μm . The term may also be used to include
20 any microsphere lens array that may be used over a substrate of a microfluidic device, and may be used as part of an optical detection method based on the photonic nanojet phenomenon which transports nano-objects in the microfluidic channel with a depth comparable to the longitudinal dimension of the photonic nanojet. The microsphere may be a dielectric microsphere. In the present application, the microsphere may interchangeably
25 referred to as a particle lens.

Preferably, when in use with a microscope for imaging or magnifying a sample, the angle formed between a central axis of the opening and a side of the opening is larger than the numerical aperture of an objective lens of a microscope when the microsphere is placed
30 between the objective lens and a surface of a sample. The numerical aperture of the objective lens is different for each magnification. As such, the angle is suggested to be larger than the highest numerical aperture of the objective lenses in the applied imaging system. Typically,

when imaging in ambient air environment, the numerical aperture of an objective lens is usually 0.3 to 0.9, corresponding to an angle of between 17 to 64 degrees. Hence, in an embodiment, the angle is larger than 64 degrees to optimise the imaging quality.

5 In an embodiment on the present invention, the surface of the membrane is coated with any suitable reflective material. Preferably, the material is a metal. More preferably, the metal may be selected from the group comprising: gold, silver, chromium, aluminium, copper, or combinations thereof.

10 Preferably, the thickness of the membrane is equal to or less than the diameter of the microsphere.

Preferably, the membrane is made from a material selected from the group comprising: silicon, silica, sapphire, a polystyrene, a poly(methyl methacrylate), a polycarbonate, a
15 poly(ethylene terephthalate), or combinations thereof.

In a second aspect of the present invention, there is provided a device comprising the membrane according to the first aspect of the invention, wherein the membrane is coupled to a support structure configured to position the microsphere between the objective lens of
20 a microscope and a surface of a sample for imaging the sample.

Preferably, the support structure is configured to position the microsphere at a pre-determined distance above the surface of the sample. In an embodiment, the pre-determined distance is any distance that is less than 1 μ m.

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In an embodiment, the support structure is a nano-stage comprising a cantilever, the membrane retaining the microsphere is coupled to the distal end of the cantilever.

Alternatively, the support structure is a frame comprising at least one arm extending from a
30 side of the frame into the interior space of the frame, the membrane retaining the microsphere is coupled to the at least one arm.

By "frame", it is meant to refer to any structure that has a border or perimeter surrounding an interior space. Preferably, the frame comprising a least one arm extending from opposing sides of the frame into the interior space of the frame, the membrane retaining the microsphere is disposed between the opposing arms. The arms may be attached to the frame
5 by screw threads, and the screwing action on these threads adjusts the position of the membrane above the surface of the sample.

In any case, the support structure is configured to position the microsphere anywhere along an x-, y- or z-axis direction. This movement may either be carried out manually, e.g. by hand
10 actions of the screw threads, or by a control unit.

In a third aspect of the present invention, there is provided a system for imaging a sample, the system comprising: (a) an optical component; and (b) the device according to any one of claims 9 to 13.

By "optical component", it is meant to refer to any component for imaging and/or magnifying a sample, such component includes an objective lens of a microscope.

In a fourth aspect of the present invention, there is provided a method for imaging a sample, the method comprising: (a) retaining a microsphere in a membrane according to the first
20 aspect of the present invention; (b) positioning the microsphere above the surface of the sample; (c) illuminating the surface of the sample, whereby light reflected from the surface is reflected through the microsphere and focused through an optical component; and (d) detecting the light to form an image of the surface.

Preferably, the microsphere is positioned at a predetermined distance of less than 1 μ m above the surface of the sample.

Advantageously, to overcome the imaging resolution limit of conventional microscopies in air
30 (about a half of the illuminating light wavelengths due to diffraction limit), the invention provides a novel super-resolution imaging scheme by positioning a transparent particle close but not in contact with the samples' surfaces as a unique optical lens. The optimized particle

lens are designed for optical nano-imaging in ambient air and have experimentally shown a resolution of 50 nm.

In addition, the ability of manipulating the location of the microsphere is important in most microscope observation experiments. Current methods are complex and bulky. As such, the present invention realises a space-saving microscope accessories with pre-integrated microspheres which can be assembled to most conventional optical microscopes and achieve super-resolution at low cost and high efficiency.

Thus, in the present invention, the problems faced by existing art are addressed by the following methods:

1) A user-friendly nanoscope is constructed by designing the particle lens holder and its nano-manipulation system to avoid physical contact with the object; and

2) The particle lens nanoscope is coupled with a laser source to filter away the distorted image information due to the broadband wavelength of the illumination light. In such illumination light, each wavelength will generate an image which is captured by the CCD camera. These images overlap with each other and form the composite picture observed on CCD, which may be distorted. However, when a laser source is used, it offers a narrow wavelength range and therefore the image quality is higher.

In order that the present invention may be fully understood and readily put into practical effect, there shall now be described by way of non-limitative examples only preferred embodiments of the present invention, the description being with reference to the accompanying illustrative figures.

Brief description of the Figures

In the Figures:

Figure 1 (a) shows the physics behind particle lens nanoscope, **(b)** 50 nm resolution images being captured, and **(c)** light energy enhancement by the particle lenses with different refractive indices.

5 **Figure 2 (a)** SEM images of a single hole in the lens holder, **(b)** a hole with a microsphere inside.

Figure 3 (a) shows a cross-sectional view of the membrane retaining a microsphere in accordance with an embodiment of the present invention, **(b)** shows a setup of microsphere-assisted nanoscope using a device in accordance with an embodiment of the present invention.

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Figure 4(a) show samples fabricated by a LIL technique that consists of a nano-dot array with diameter of 200 nm and period of 700 nm, **(b)** shows an image resolved under a 10 μm diameter microsphere in accordance with an embodiment of the present invention.

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Figures 5 (a) and (b) show an overview of the setup of a conventional optical microscope integrated with a device according to an embodiment of the present invention.

20 **Figure 6** are SEM images of the lens assembly chip: (a) a hole with a microsphere inside and (b) without the microsphere.

Figure 7 shows the scanning path of the microsphere over the sample surface.

25 **Figure 8** are SEM image and optical image of the sample obtained by the microsphere.

Figure 9 shows the scanning process of the microsphere nanoscope above the sample surface to indicate the non-contact imaging mode

30 **Detailed description of the preferred embodiments**

In accordance with the present invention, the microsphere particle lens enables one to enlarge the object into a magnified virtual image, which is then captured by a conventional microscope, providing new opportunities to image biomolecules and material cracks in real time and in ambient air.

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The problem of diffraction limit in nano-imaging stems from the loss of evanescent waves in the far-field, which carry high spatial frequency subwavelength information of an object and decay exponentially with distance. The present invention, making use of a microsphere as an optical element for super-resolution imaging, offers the possibility to restore the nanoscale
10 information in the far-field. In a suitable apparatus in accordance with the invention, incident light can be trapped inside a tiny transparent sphere by bouncing the photons around the interior through total internal reflection. At its resonance, the enhanced energy only emits out through a small window. This preference allows a number of interesting applications. For example, a transparent microsphere can form an efficient laser cavity.

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In the present invention, a technical breakthrough at 50 nm resolution by coupling a microsphere (particle lens) with a conventional microscope is achieved. A 50 nm ($\lambda/8$) super-resolution imaging under white-light illumination is realized by using a particle lens as the far-field superlens. It is shown that the microsphere is a perfect candidate to transmit the near-
20 field image information to the far field, which is a key point to address the diffraction limit. The magnification ratio depends on the lens' material (refractive index) and size. In accordance with the present invention, light energy is confined inside a small area, instead of being dissipated away. This is a significant advantage over near-field scanning optical microscope (NSOM) etc. techniques. In a suitable apparatus in accordance with the invention,
25 this particle lens nanoscope can work in both transmission and reflection modes, providing the flexibility to *in-vivo* observe viruses and molecules in ambient air.

FIG. 1a shows the physics behind a microsphere (particle lens) nanoscope. In this figure, a sample object being imaged or magnified is placed on a stage under an optical component
30 such as an objective lens of a conventional microscope. Here, microspheres are placed on the surface of the sample object. FIG. 1b shows SEMs of 50nm resolution images being captured. FIG. 1c illustrates that the physics behind this particle lens nano-imaging is attributed to light

energy enhancement in the near field when light transmits through the particle lens. In our previous research, a SiO₂ particle lens (refractive index: ~1.4 in optical range) was used. Basically, the higher field enhancement and smaller focused spot can be achieved by designing new particles lenses with higher refractive index, especially for the material with refractive index close to 2 according to ray tracing under the approximation of geometrical optics. Sapphire particle lens is a good candidate (refractive index: ~1.8). In accordance with the present invention, the particle lens made of these materials can further reduce current imaging capability down to 20 nm.

The design of the particle lens with the refractive index higher than 2.0 is investigated as their energy localization in near field is more significant. It shows that this new approach can achieve 20 nm resolution under white-light illumination. However, there exists a technical issue: FIG. 1c shows that its focus point shrinks inside the sphere. Hence, this particle lens is truncated into a partial sphere so that the focus point emerges out of the lens for imaging applications.

On the other hand, particle lens size also determines its focusing. Figure 2 shows one single hole on the lens holder with a 10 μm diameter microsphere inside. Extensive theoretical investigation is carried out to study the relationship between the lens size and its focusing capability, as a guideline for 20 nm resolution particle lens nanoscope's design and experiment. In the invention, there are rich physics behind for the light focusing in optical meso-field, which is seldom studied in advanced optics. Both evanescent waves and propagating waves exist in this unique regime between the optical far field and near field.

FIG. 3a shows a membrane for holding and retaining a microsphere particle lens in accordance with an embodiment of the present invention. The membrane 5 may be made from a material selected from the group comprising: silicon, silica, sapphire, a polystyrene, a poly(methyl methacrylate), a polycarbonate, a poly(ethylene terephthalate), or combinations thereof. In particular, the membrane 5 has two surfaces that oppose each other – a first 10 and a second 15 surface. Alternatively, first 10 and second 15 surfaces may be just the top and bottom surfaces of the membrane 15. An opening 20 penetrates through the membrane 5 and is sized to retain a microsphere 25. As can be seen from the cross-sectional view of the membrane 5,

the opening 20 is tapered, i.e. the size of the opening on the first surface 10 is larger than the size of the opening on the second surface 15. This configuration allows the opening 20 to receive and retain the microsphere 25. In an embodiment, the profile of the opening 20 resembles an inverted pyramid. Alternatively, the profile of the opening 20 is frusto-conical in shape.

In an embodiment of the present invention, the diameter of the microsphere may be between 4 μm to 50 μm . As such, the opening 20 may be suitably sized to receive and retain such a microsphere, i.e. the size of the opening 20 of the first surface 10 is larger than the diameter of the microsphere 25, and the size of the opening 20 on the second surface 15 is smaller than the diameter of the microsphere 25. The thickness of the membrane 5 may be equal to or less than the diameter of the microsphere 25.

The angle formed between a central axis of the opening 20 (not shown in the figure but runs parallel to the incident light) and a side of the opening 20 (i.e. the angled side of the opening 20) is larger than the numerical aperture of an objective lens of a microscope when the microsphere is placed between the objective lens and a surface of a sample 30 for imaging the sample 30. The sample 30 may be placed on a stage 35.

The surface of the membrane 5 may be coated with a reflective layer 40. For example, a metallic layer may be coated on the surface of the membrane 5. Any suitable metal may be used but, in an embodiment, any one selected from the following group may be used: gold, silver, chromium, aluminium, copper, or combinations thereof.

In accordance with the invention, in order to develop the particle lens nanoscope for practical applications, the dynamic optical control systems are set up to manipulate the particle lens and adjust its distance from the sample surface in non-contact mode. The size of the particle lens is typically a few microns, so that accurate control of the distance between the particle lens and object in FIG. 3a is critical. In the invention, the particle lens holder is made by etching inverted pyramids onto a silicon wafer followed by metallic layer coating and the particle lens deposition on the wafer in sequence. Then, the lens assembly is installed on a nano-positioning stage to flexibly tune the lens position for the sharp focusing of the object. The

sample can be mounted on an XYZ piezo-stage, which keeps a distance away from the particle lens. The sample is scanned for whole surface imaging.

In accordance with another aspect of the present invention, the membrane 5 may be coupled to a support structure that is configured to position the microsphere 5 between the objective lens of a microscope 45 and the surface of a sample 30 when the sample 30 is being imaged. This support structure allows for the positioning of the microsphere at a pre-determined distance above the surface of the sample 30. In a particular embodiment, the pre-determined distance is less than 1 μm .

As shown in FIG. 3b, the support structure may be a nano-stage 50 comprising a bridge or a cantilever 55, the membrane 5 retaining the microsphere 25 is coupled to the distal end 60 of the cantilever 55. The nano-stage 50 allows for the movement of the cantilever 55 in the x-, y- and z-axis direction.

In an alternative embodiment, as shown in FIG. 5b, the support structure is integral with a traditional conventional optical microscope and comprises a frame 65 which has at least one arm 70 extending from a side of the frame 65 into the interior space A of the frame 65, the membrane 5 retaining the microsphere 25 is coupled to the at least one arm 70. As shown in the figure, the frame 65 has a least one arm 70 extending from opposing sides of the frame 65 into the interior space A of the frame 65, the membrane 5 retaining the microsphere 25 is disposed between the opposing arms 70. The arms may be made of metal. The arms 70 thus allow for the movement of the membrane 5 anywhere along an x-, y- or z-axis direction (i.e. 3 dimensional movement).

FIG. 5a shows perspective views of the integrated microscope.

The support structure can be mounted stably onto the objective lens tube through a metal arm 70 and located between the sample 30 and the objective lens 45. This allows the realization of far-field super-resolution imaging. This allows the realization of far-field super-resolution imaging. In an embodiment, the membrane 5 which carries the microsphere 25 is held by four thin metal bars 70. The microsphere 25 possesses a small travel range within the

frame 65. An electrical controller, which precisely controls the movement of the membrane 5 in x, y and z directions, is linked to this chip (membrane 5). This design ensures the relative movements of the microsphere 25 and the sample 30, which is beneficial for the imaging process. After the alignment of the microsphere 25 and the objective lens 45, scanning of the sample surface 30 can be done by adjusting the location of the sample 30. Therefore, the area of interests can be imaged at a higher resolution.

The arms 70 are attached to the frame by screw threads 75, and the screw action of these threads adjusts the position of the membrane 5 above the surface of the sample 30.

The method of operation is now described. The dimensions of the membrane and its edges are specially designed so as not to interrupt the switching between different objective lenses 45. To locate the microsphere 25 vertically above the sample 30, four thin metal bars 70 are connected to the edges of this membrane 5 to precisely control the position of the microsphere 25. To move the microsphere 25 along the y-axis direction, the metal bars 70 can be carried horizontally by the mechanical force from the nano-stage to rotate the screw threads 75. The microsphere 25 is also able to move along the x-axis direction by moving the metal bars 70 along the lower layer 80 of the frame 65, which is also controlled by the electrical controller. The position of the surface layer 85 of the frame 65 is fixed once the lens assembly chip is mounted onto the microscope, however the z-axis position of the lower layer of the frame 65 is tunable. The z-axis position of the microsphere 25 is controlled by tuning the gap between the lower layer 80 and surface layer 85 of the frame 65.

During the optical super-resolution imaging, after integrating the lens assembly chip onto a conventional optical microscope, the user needs to ensure that the membrane 5 is not directly under objective lens. The purpose is to make sure that the microsphere 25 does not interrupt the normal imaging process. The next step is to place the sample 30 on the microscope load. The switching from low magnification objective lens gradually to a high magnification objective lens is similar to the conventional imaging process. The only difference occurs after the user has located the area of interest and switched the objective lens. At this time, the thin membrane 5 which carries the microsphere 25 is moved slowly into the gap between the objective lens 45 and sample 30 by the electrical controller. After ensuring the microsphere

25 is right above the area of interest, control the movements in z-axis can adjust the focal plane of the microsphere 25, optimal image can be achieved. In this way, a magnified image is recorded by the computer, which exceeds the resolution limit of the original optical microscope. In addition, this lens assembly chip can be both electrically and manually
5 controlled, enabling free movements based on the individual needs. A basic movement of three dimensions (x, y and z) is advised to ensure the functionality of this thin chip. To ensure the imaging plane can be captured, maximum step movement of the membrane is suggested to be less than 1 μm in z-axis. When more dimensions are available, this chip can be tilted or rotated to satisfy different needs in experiments, including the calibration of the stage and
10 parallelism between the microsphere and the sample.

As such, together with a conventional microscope, the membrane and its support structure provides a system for imaging / magnifying an object or sample. Accordingly, in summary, the present invention provides for a method for imaging a sample, the method comprising (a)
15 retaining a microsphere 25 in a membrane 5; (b) positioning the microsphere 25 above the surface of the sample 30; (c) illuminating the surface of the sample 30, whereby light reflected from the surface is reflected through the microsphere 25 and focused through an optical component (e.g. objective lens 45); and (d) detecting the light to form an image of the sample 30 or surface of the sample 30.

20 In this invention, in order to address the image distortion issue due to the particle lens' aberration, the particle lens nanoscope can be coupled with a laser source or a narrow wavelength LED light source. The incident beam goes through a conventional microscope lens, particle lens and then illuminate on the object. The particle lens forms a magnified virtual
25 image of the object, which is collected by the microscope lens.

In FIG. 4, experiment results of imaging by means of nanoscope is demonstrated. The sample which is patterned with a nano-dot array with a diameter of 200 nm and period of 700 nm is observed. The image under the microsphere is significantly improved by the magnification of
30 around 6 times.

In accordance with the invention, the laser filters away the distorted image information at edges. Therefore, the PMT only collects the image information at the centre. By this means, we can obtain 200 nm resolution high quality images by scanning the object surface. Also, it is reported that direct optical super-resolution imaging under microsphere can reach 25 nm
5 under 408 nm wavelength illumination with a conventional scanning laser confocal microscope (SLCM).

Besides improving the resolution of a 2D image, the research on 3D imaging is also carried out by this laser particle lens nanoscope. By accurately tuning the positions of the particle
10 lens by adjusting the nano-stage, the laser beam can be focused at different layers of the sample to obtain a series of 2D high resolution images. In accordance with the present invention, image reconstruction combines these 2D images together to provide us a 3D image.

As shown in Figs. 6 (a) and (b), a microsphere 25 with the diameter of 10 μm is located inside a hole fabricated with the same diameter. It can be observed that the edges of microsphere 25 are in close contact with the side walls of the opening 20. With the frictional force, the microsphere 25 is seated stably in the opening 20 and can move together with the membrane
5. Different microspheres with diameter from 4 μm to 50 μm can be applied in optical
20 microscope imaging experiments. The microsphere 25 is trapped inside the opening 20 with the same dimension as its diameter to ensure the stabilization during transportation and movement for imaging. For imaging of different samples, both reflection and transmission modes are available using our designed lens assembly chip. With this invention, most traditional optical microscopes in market can be upgraded to a nanoscope with a sub-100 nm
25 resolution flexibly. An impressive improvement in resolution is now possible with a comparably low price.

To prove the concept, the far-field super-resolution imaging with a high refractive microsphere 25 which possesses the ability to resolve small feature at the size of 74 nm was
30 demonstrated. The refractive index of the microsphere is 1.9, at a diameter of around 20 μm . The contrast of refractive index between microspheres and ambient should be around 1.4 for better resolution. Microspheres with different refractive indexes need to be chosen carefully.

It is also noted that the diameter of the microsphere has an impact on the resolution ability and field-of-view. In this demonstration, the microsphere is located on a glass substrate and controlled by a 3-dimensional nanostage. The optical microscope is a conventional upright microscope with white light illumination. We employ the reflection mode for demonstration.

5 As shown in Fig. 7, the microsphere is moved in a designed path above the sample surface. Therefore, in case of large sample, a fine resolution can still be achieved.

To demonstrate the super-resolution ability of this optical microscope, samples with designed features are fabricated. As shown in Fig. 8(a), different structures with the size of 98 nm and
10 as small as 74 nm are applied in the experiment. When the microsphere is located at around 2 μm above the sample surface, a clear image can be observed as shown in Fig. 8(b). The fine features on the sample can be distinguished clearly via the microsphere nano-imaging. This experiment verifies that by locating a microsphere between the objective lens and the sample, a high resolution can be achieved. Most importantly, the microsphere is not in
15 contact with the sample. Therefore, it enables the free movement of the microsphere and achieve the flexibly scanning during the imaging process. Also, it protects the sample from contamination.

To demonstrate the scanning mode of the microsphere nanoscope, a movement designed as
20 Fig. 7 is recorded and the video chips are shown in Fig. 9. The microsphere is carried by a glass substrate and located above the sample surface. The movement speed and distance above the sample can be controlled by the nanostage. The optimal viewing window is at the center of the microsphere, where the spherical aberration is the least significant. The minimum step of the movement is determined by the nanostage. To achieve a high performance with this
25 setup, a moving step smaller than 1 μm is advised.

In summary, the present invention provides for a new nanoscope that can observe 50 nm structures with the assist of microspheres. By further introducing a gap between the sample and the microsphere, this non-physical-contact mode imaging method benefits in many ways,
30 such as no damage to fragile samples (biological sample friendly), best focal plane can be captured, ability to scan over the whole surface in a quick speed, combine with laser source

to increase the resolution and reduce the cost significantly (compare to confocal microscope and NSOM).

Conventional optical microscopes suffer from diffraction limit at about a half of the incident wavelength, resulting in the highest resolution around 200 nm. This invention offers a solution to flexible realization of super-resolution in optical microscopes, achieving the sub-100 nm resolution (down to 25 nm). Current methods requires high cost and strict condition to achieve this resolution. Reducing the fabrication price and simplifying the experimental procedures are also the key advantages of this patent for practical applications.

Other key features include:

- An optical super-resolution nano-imaging scheme by coupling a microsphere (particle lens) with a conventional microscope.
- The microsphere (particle lens) is positioned close but in non-physical-contact mode with the samples' surfaces as an optical lens.
- A lens assembly designed to control the microsphere a distance away from the sample.
- The particle lens assembly is installed on a nano-positioning stage to flexibly tune the lens position for the sharp focusing of the object.
- The particle lens nanoscope can be coupled with either white light source, LED light source, or laser source.
- The incident beam goes through a conventional microscope lens, particle lens and then illuminate on the object, the particle lens forms a magnified virtual image of the object, which is collected by the microscope lens.

- By introducing a gap between the sample and the microsphere, this non-physical-contact mode imaging method provides no damage to fragile samples (biological sample friendly), best focal plane can be captured, ability to scan over the whole surface in a quick speed, combine with either white light source, LED light source, or laser source to increase the resolution and reduce the cost significantly.

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Whilst there has been described in the foregoing description preferred embodiments of the present invention, it will be understood by those skilled in the technology concerned that many variations or modifications in details of design or construction may be made without departing from the present invention.

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Claims

1. A membrane having a first surface and a second surface, the first and second surfaces oppose each other, the membrane comprising:
 - (a) an opening penetrating through the membrane and is sized to retain a microsphere,
wherein the opening is tapered, the size of the opening on the first surface is larger than the size of the opening on the second surface; and
wherein the surface of the membrane is coated with a metal.
2. The membrane according to claim 1, wherein an angle formed between a central axis of the opening and the side of the opening is larger than a maximal half-angle θ of a cone of light that can exit an objective lens of a microscope, the maximal half-angle θ which is derived from the numerical aperture of the objective lens of a microscope, when the microsphere is placed between the objective lens and a surface of a sample for imaging the sample.
3. The membrane according to claim 2, wherein the angle is larger than 64 degrees.
4. The membrane according to any one of the preceding claims, wherein the inner profile of the opening is frusto-conical.
5. The membrane according to claim 1, wherein the metal is any one selected from the group comprising: gold, silver, chromium, aluminium, copper, or combinations thereof.
6. The membrane according to any one of the preceding claims, wherein the opening is sized to retain a microsphere having a diameter between 4 μm to 50 μm , the size of the opening of the first surface is larger than the diameter of the microsphere, and the size of the opening on the second surface is smaller than the diameter of the microsphere.

7. The membrane according to claim 6, wherein the thickness of the membrane is equal to or less than the diameter of the microsphere.
8. The membrane according to any one of the preceding claims, wherein the membrane is made from a material selected from the group comprising: silicon, silica, sapphire, a polystyrene, a poly(methyl methacrylate), a polycarbonate, a poly(ethylene terephthalate), or combinations thereof.
9. A device comprising the membrane according to any one of claims 1 to 8, wherein the membrane is coupled to a support structure configured in such a way that is suitable for positioning the microsphere between the objective lens of a microscope and a surface of a sample for imaging the sample.
10. The device according to claim 9, wherein the support structure is configured in such a way that is suitable for positioning the microsphere at a pre-determined distance above the surface of the sample.
11. The device according to any one of claims 9 or 10, wherein the pre-determined distance is less than 1 μm .
12. The device according to any one of claims 9 to 11, wherein the support structure is a nano-stage comprising a cantilever, the membrane retaining the microsphere is coupled to the distal end of the cantilever.
13. The device according to any one of claims 9 to 11, wherein the support structure is a frame comprising at least one arm extending from a side of the frame into the interior space of the frame, the membrane retaining the microsphere is coupled to the at least one arm.
14. The device according to claim 13, wherein the frame comprising a least one arm extending from opposing sides of the frame into the interior space of the frame, the membrane retaining the microsphere is disposed between the opposing arms.

15. The device according to claim 14, wherein the arms are attached to the frame by screw threads, the screw action of these threads adjusts the position of the membrane above the surface of the sample.
16. The device according to any one of claims 9 to 15, wherein the support structure is configured to position the microsphere anywhere along an x-, y- or z-axis direction.
17. A system for imaging a sample, the system comprising:
- (a) an optical component; and
 - (b) the device according to any one of claims 9 to 16.
18. A method for imaging a sample, the method comprising:
- (a) retaining a microsphere in a membrane according to any one of claims 1 to 8;
 - (b) positioning the microsphere above the surface of the sample;
 - (c) illuminating the surface of the sample, whereby light reflected from the surface is reflected through the microsphere and focused through an optical component; and
 - (d) detecting the light to form an image of the surface.
19. The method according to claim 18, wherein the microsphere is positioned at a predetermined distance of less than 1 μm above the surface of the sample.