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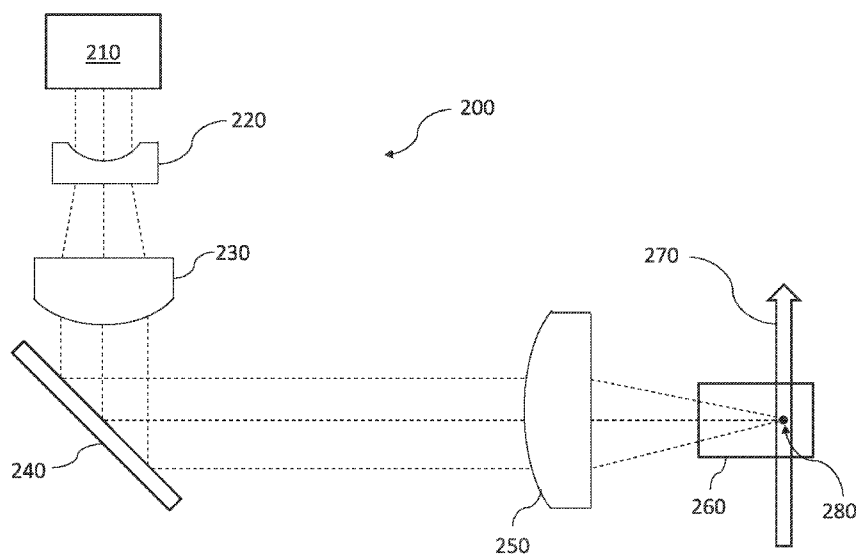


Fig. 2

(57) Abstract: A detection system of a flow cytometer and a method of using the detection system are provided. The detection system comprises a light-emitting unit; a lens assembly comprising a focussing lens; and a light collection unit configured to collect light scattered from a nanoparticle at the interrogation point. The method comprises using the lens assembly to focus a laser beam emitted by the light-emitting unit at an interrogation point. A profile of the incident laser beam incident on the focussing lens has a first dimension parallel to the trajectory of the nanoparticle. A first ratio of a first diameter of the focussing lens to the first dimension of the incident laser beam is higher than a first threshold such that light fringes of the focussed laser beam at the interrogation point are suppressed. An intensity of the focused laser beam at the interrogation point is higher than a second threshold.

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DETECTION OF NANOPARTICLES

Cross-Reference to Related Application

[0001] This application is being filed on June 6, 2024, as a PCT International application and claims the benefit of and priority to U.S. Application No. 63/506,525, filed on June 6, 2023, entitled DETECTION OF NANOPARTICLES, the disclosure of which is hereby incorporated by reference in its entirety.

Field of the disclosure

[0002] The disclosure relates to the field of detection systems of flow cytometers, for detecting nanoparticles.

Background

[0003] Sample processing instruments are known to be used to detect small particles by using a light source to scatter light from a small particle, and detecting the scattered light. Such sample processing instruments may be configured to analyse a liquid sample having small suspended particles. For example, a flow cytometer comprises a light source focussed on an interrogation point of a detection channel of a flow cell. The particles to be detected flow through the detection channel and pass through the interrogation point such that the light source is incident on the particle. Conventionally, these instruments are suitable for detecting microparticles such as cells, biological particles or non-biological particles with sizes of the order of micrometres.

[0004] It would be beneficial to use such instruments to detect very small biological or non-biological nanoparticles. The intensity of scattered light is significantly lower for nanoparticles than for microparticles, making it difficult to detect nanoparticles with detection systems of conventional sample processing instruments, which lack the sensitivity to detect scattered light from such small particles. Although tightening the focus of the light source at the interrogation point can help to increase the intensity of the scattered light, aberrations can introduce light fringes in the excitation profile of the light beam at the interrogation point. These light fringes can introduce noise and artefacts into the captured scattered light.

[0005] It is an object of the present disclosure to provide a method of detecting nanoparticles using a light source.

Summary of the disclosure

[0006] Against this background, there is provided a method of using a detection system of a flow cytometer, wherein the detection system is configured to detect nanoparticles. The detection system comprises a light-emitting unit configured to emit a laser beam. The detection system further comprises a lens assembly comprising a focussing lens, wherein the focussing lens is configured to focus an incident laser beam at an interrogation point, wherein a trajectory of a nanoparticle passes through the interrogation point perpendicular to a central axis of the laser beam and wherein the focussing lens has a first diameter in a direction parallel to the trajectory of the nanoparticle. The detection system further comprises a light collection unit configured to collect light scattered from a nanoparticle at the interrogation point. The method comprises using the lens assembly to focus a laser beam emitted by the light-emitting unit at the interrogation point, wherein a profile of the incident laser beam incident on the focussing lens has a first dimension parallel to the trajectory of the nanoparticle and a second dimension perpendicular to the trajectory of the nanoparticle. A first ratio of the first diameter of the focussing lens to the first dimension of the incident laser beam is higher than a first threshold such that light fringes of the focussed laser beam at the interrogation point are suppressed. An intensity of the focused laser beam at the interrogation point is higher than a second threshold.

[0007] In this way, it is possible to detect nanoparticles using a flow cytometer, by suppressing light fringes of the focussed laser beam and by having an intensity of the focused laser beam at the interrogation point that is higher than a second threshold. Light fringes of the focussed laser beam arising from optical aberrations may result in associated artefacts when the scattered light is detected. For example, light fringes may show as an additional peak in intensity in the spectrum of collected light, falsely appearing as an unexpected additional population of nanoparticles. Suppressing optical aberrations may prevent such artefacts from appearing. An intensity of the focused

laser beam at the interrogation point is higher than a second threshold. In this way, the intensity of the light scattered from the nanoparticle may be high enough to be detected.

[0008] The first threshold may be 6.6.

[0009] Advantageously, a first ratio that is higher than 6.6 may suppress light fringes of the focussed laser beam at the interrogation point.

[0010] The second threshold may be proportional to a laser power of the laser beam.

[0011] The second threshold may be inversely proportional to the area of the laser beam.

[0012] The second threshold may be inversely proportional to a power of a wavelength of the laser beam.

[0013] The second threshold may be proportional to a power of a diameter of the nanoparticle.

[0014] The second threshold may be 2.7, wherein the second threshold has units of mWatts/micrometres squared.

[0015] Advantageously, an intensity of the focused laser beam at the interrogation point that is higher than 2.7 may result in an intensity of the light scattered from the nanoparticle may be high enough to be detected.

[0016] The focussing lens may be a convex spherical lens.

[0017] The first dimension of the profile of the laser beam at the detection position may be smaller than the second dimension of the profile of the laser beam at the interrogation point.

[0018] A diameter of the nanoparticle may be less than 70 nm.

[0019] There is also provided a detection system of a flow cytometer, wherein the detection system is configured to detect nanoparticles. The detection system comprises a light-emitting unit configured to emit the laser beam. The detection system further comprises a lens assembly comprising a focussing lens, wherein the focussing lens is configured to focus an incident laser beam at an interrogation point, wherein a trajectory of a nanoparticle passes through the interrogation point perpendicular to a central axis of the laser beam and wherein the focussing lens has a first diameter in a direction parallel to the trajectory of the nanoparticle. The detection system further comprises a light collection unit configured to collect light scattered from a nanoparticle at the interrogation point. The lens assembly is configured to focus a laser beam emitted by the light-emitting unit such that a profile of the incident laser beam incident on the focussing lens has a first dimension parallel to the trajectory of the nanoparticle and a second dimension perpendicular to the trajectory of the nanoparticle. A first ratio of the first diameter of the focussing lens to the first dimension of the incident laser beam is higher than a first threshold such that light fringes of a laser beam at the interrogation point are suppressed. An intensity of the focused laser beam at the interrogation point is higher than a second threshold.

Brief description of the drawings

[0020] A specific embodiment of the disclosure will now be described, by way of example only, with reference to the accompanying drawings in which:

[0021] Figure 1 shows a block diagram illustrating a flow cytometer.

[0022] Figure 2 shows a schematic diagram of a lens assembly of a detection system according to an embodiment of the present disclosure.

[0023] Figure 3 shows a schematic diagram of a detection system according to an embodiment of the present disclosure.

[0024] Figure 4 (including FIGS. 4A to 4E) shows beam profiles of a laser beam affected by optical aberrations. Figure 4A shows the beam profile of the incident laser beam; Figure 4B shows a ray diagram of the laser beam passing through a lens; Figure 4C shows the beam profile of the focused laser beam; Figure 4D shows a plot of irradiance of the focused beam plotted against y; Figure 4E shows a plot of irradiance of the focused beam plotted against x.

[0025] Figure 5 (including FIGS. 5A to 5C) shows beam profiles of a laser beam affected by optical aberrations. Figure 5A shows a ray diagram of the laser beam passing through a lens; Figure 5B shows the beam profile of the incident laser beam; Figure 5C shows a plot of irradiance of the focused beam plotted against y.

[0026] Figure 6 (including FIGS. 6A to 6C) shows beam profiles of a laser beam with the effects of optical aberrations suppressed, according to an embodiment of the present disclosure. Figure 6A shows a ray diagram of the laser beam passing through a lens; Figure 6B shows the beam profile of the incident laser beam; Figure 6C shows a plot of irradiance of the focused beam plotted against y.

[0027] Figure 7 (including FIGS. 7A to 7C) shows beam profiles of a laser beam with the effects of optical aberrations suppressed, according to an embodiment of the present disclosure. Figure 7A shows a ray diagram of the laser beam passing through a lens; Figure 7B shows the beam profile of the incident laser beam; Figure 7C shows a plot of irradiance of the focused beam plotted against y.

[0028] Figure 8 (including FIGS. 8A to 8D) shows beam profiles of a laser beam affected by optical aberrations. Figure 8A shows a ray diagram of the laser beam passing through a lens; Figure 8B shows the beam profile of the incident laser beam; Figure 8C shows a plot of irradiance of the focused beam plotted against y and Figure 8D shows a plot of irradiance of the focused beam plotted against x.

[0029] Figure 9 (including FIGS. 9A to 9D) shows beam profiles of a laser beam with the effects of optical aberrations suppressed, according to an embodiment of the present

disclosure. Figure 9A shows a ray diagram of the laser beam passing through a lens; Figure 9B shows the beam profile of the incident laser beam; Figure 9C shows a plot of irradiance of the focused beam plotted against y and Figure 9D shows a plot of irradiance of the focused beam plotted against x.

[0030] Figure 10 shows scattering intensity plotted against scattering angle for lasers of different wavelengths.

[0031] Figure 11 shows scattering intensity plotted against scattering angle for different nanoparticles.

Detailed description

[0032] The present disclosure relates to detection of nanoparticles using a light source, wherein a light source is incident on an interrogation point. A nanoparticle to be detected pass through the interrogation point, and light from the light source is scattered from the nanoparticle. The scattered light is detected. For the purposes of illustration, a flow cytometer is described in the following. However, the present disclosure may be applied to any sample processing unit for detecting, sorting or processing nanoparticles, wherein the sample processing unit uses a light source to scatter light from the nanoparticles.

[0033] Flow cytometry is used in the investigation of particles of various sizes. A particle in motion undergoes spectral or other analytical interrogation. Sample particles may be suspended in a stream of fluid and may scatter light which is detected by the flow cytometer. Flow cytometry allows analysis of physical and/or chemical characteristics of particles, and can accommodate a throughput of up to thousands of cells or particles per second. Samples can be characterized according to size, concentration, and phenotyping. Examples of particles that it may be useful to characterise or identify using flow cytometry includes biological and non-biological nanoparticles. For example, it may be useful to identify cell subcomponents, such as vesicles, mitochondria, proteins, lipids, and nucleic acids. As described herein,

nanoparticles refer to particles having a size (such as diameter, maximum size or average size) less than or equal to 1000 nm.

[0034] With reference to Figure 1, a simplified block diagram of a flow cytometer 100 is illustrated. The flow cytometer 100 may comprise a flow cell 110, a fluid system 120, a light source 130, an optical detection system 140 and a sample analysis system. A sample of nanoparticles 150 and a fluid 160 may be transported to the flow cell 110 by the fluid system 120, such that the sample nanoparticles pass through the flow cell 110 linearly and in a single row. A light source 130 irradiates an interrogation area or interrogation point of the flow cell 110, such that the nanoparticles passing through the flow cell 110 are irradiated by light 131 from the light source 130. The light source 130 is usually a laser light source. The irradiation of the sample particles may cause the sample nanoparticles to scatter light. The scattered light 141 is collected by the optical detection system 140. In an event that the nanoparticles are fluorescent, or are tagged with a fluorescent material, the optical detection system 140 may be further configured to collect fluorescence from the nanoparticles. The sample analysis system may analyse the collected signals to obtain information relating to the detected nanoparticles.

[0035] With reference to Figure 2, an example of an optical system 200 used to irradiate the interrogation point is illustrated. By irradiating the interrogation point, light is scattered from any nanoparticle passing through the interrogation point. In an event that a nanoparticle is fluorescent or is tagged with a fluorescent material, the irradiation may also excite the fluorescent material. The optical system may be part of a detection system of a flow cytometer. A light source 210, such as a laser, is used. The light beam from the light source 210 is directed to and focused at the interrogation point 280. The sample nanoparticles follow path 270 through flow cell 260, wherein the path 270 passes through the interrogation point 280. The light beam may be directed to and focussed at the interrogation point 280 by any optical components. An example is shown in Figure 2, but it will be understood that any suitable combination of lenses or other optical components may be used, and that the orientation of the light source with respect to the flow cell may be different. The path of the light beam is indicated by the dashed lines. In this example, the light beam passes through optical components 220

and 230 such that the light beam is incident on a reflector 240. Optical components 220 and 230 may be configured to collimate or expand the light beam to a particular shape or beam size. The reflector 240 reflects the light beam towards the flow cell 260. The reflector 240 may be configured to reflect light of a specific wavelength. For example, the reflector 240 may comprise a mirror or a dichroic mirror. The light beam passes through optical component 250 such that the light beam is focused at the interrogation point 280. The optical component 250 is configured to focus the collimated laser beam at the interrogation point 280. Optical component 250 is shown as being a spherical lens. However, other types, geometries and sizes of lens may be used as the optical component 250. The optical component 250 may comprise a combination of more than one lens. For example, optical component 250 may comprise a spherical convex lens and a cylindrical convex lens, or two convex cylindrical lenses, or a spherical convex lens and a cylindrical concave lens, or other combination of lenses. The positions of the lenses may vary.

[0036] According to an embodiment of the present disclosure, a method is provided of suppressing optical aberrations of a laser beam of a detection system for nanoparticles. With reference to Figure 3, the detection system 300 comprises a light-emitting unit 310 configured to emit the laser beam 320. The detection system further comprises a lens assembly 330 comprising a focussing lens, wherein the focussing lens is configured to focus an incident laser beam 320 at an interrogation point 340, wherein a trajectory 350 of a nanoparticle passes through the interrogation point 340 perpendicular to a central axis of the laser beam 320. The focussing lens has a first diameter in a direction parallel to the trajectory 350 of the nanoparticle. The lens assembly may comprise one or more additional lenses. The additional lens(es) may also focus the incident laser beam, and/or the additional lens(es) may collimate the laser beam before it is incident on the focussing lens. The interrogation point 340 may be proximal to the lens assembly. The detection system 300 further comprises a light collection unit 360 configured to collect light 370 scattered from a nanoparticle at the interrogation point 340. The nanoparticle may pass through a flow cell 380, wherein the interrogation point 340 is within the flow cell.

[0037] The method comprises using the lens assembly to focus a laser beam emitted by the light-emitting unit at the interrogation point, wherein a profile of the incident laser beam incident on the focussing lens has a first dimension parallel to the trajectory of the nanoparticle and a second dimension perpendicular to the trajectory of the nanoparticle. A first ratio of the first diameter of the focussing lens to the first dimension of the incident laser beam is higher than a first threshold, such that light fringes of a laser beam at the interrogation point are suppressed. An intensity of the focused laser beam at the interrogation point is higher than a second threshold.

[0038] As discussed above, the intensity of scattered light from nanoparticles is significantly lower than the intensity of scattered light from microparticles. To achieve an intensity of the scattered light 360 from nanoparticles that is detectable by the light collection unit 350, the intensity of the laser beam 320 at the interrogation point is higher than the second threshold. The intensity I_0 of the laser beam 320 at the interrogation point is:

$$I_0 = \frac{P}{A} = \frac{P}{\pi ab}$$

where P is the power of the laser beam and A is the cross-sectional area of the laser beam. Assuming that the laser beam has an elliptical profile with major axis a and minor axis b , the area A of the laser beam is πab .

[0039] Decreasing the area of the laser beam therefore increases the intensity of the laser beam for a given power. Focussing the laser beam more tightly in order to achieve the higher intensity can result in fringes in the beam profile of the laser beam at the interrogation point. These occur due to aberrations of the optical system. With reference to Figure 4A, this effect is illustrated for an incident laser beam 420 having a Gaussian beam profile 410. In the example shown in Figure 4A, the beam profile 410 is elliptical, with major axis a parallel to the trajectory of the nanoparticles and minor axis b perpendicular to the trajectory of the nanoparticles. As shown in Figure 4B, the laser beam 420 passes through the lens 430 and is focussed at the interrogation point 440. At the interrogation point 440, the focused laser beam has a beam profile 450, with fringes

451 in the y direction as shown in Figure 4C. The beam profiles 410 and 450 are shown in the x - y plane, i.e. perpendicular to the central axis of the laser beam. The diagram illustrating the laser beam 420 passing through the lens 430 and being focused at the interrogation point 440 is shown in the y - z plane, where the x -axis is out of the page. The trajectory of the nanoparticle would be along the y direction. Figure 4D shows a 2D plot of the intensity across the centre of the beam profile, showing intensity against the y axis. Fringes 451 resulting from optical aberrations are visible to either side of the main peak 452. Figure 4E shows a 2D plot of the intensity across the centre of the beam profile, showing intensity against the x axis. No fringes are present. Fringes such as those illustrated in Figure 4D can result in artefacts when the scattered light is detected. For example, the light fringes may show as an additional peak in intensity in the spectrum of collected light, falsely appearing as an unexpected additional population of nanoparticles.

[0040] The method of this disclosure achieves a beam profile of the laser beam at the interrogation point without light fringes along either axis, by having a first ratio of the first diameter of the focussing lens to the first dimension of the laser beam that is higher than a first threshold.

[0041] There are several types of aberrations of optical systems, referred to herein as optical aberrations. Two of these types of optical aberrations, spherical aberrations and curvature of field, can cause the light fringes of the beam profile shown in Figure 4D. Spherical aberrations occur when light passing through edges of a lens focuses at a slightly different focal point than light that passes through the centre of the lens. Curvature of field is present when the lens focuses a flat subject normal to its optical axis onto a curved image plane. In conventional optical systems, spherical aberrations and curvature of field can be reduced by stopping down an aperture (decreasing the size of an aperture) through which the light passes. Here, a laser is used and the beam size can be controlled. Reduction of the beam size relative to the diameter of the focussing lens has a similar effect to reducing the aperture size and so is beneficial in reducing spherical aberrations and curvature of field. Reducing the beam relative to the diameter of the focussing lens therefore reduces or eliminates light fringes of the beam profile.

[0042] Examples that show the effect of varying the first ratio of the first diameter of the focussing lens to the first dimension of the incident laser beam are shown in Figures 5 to 7. For the examples shown in Figures 5 to 7, a spherical lens having a diameter of 12.5 mm is used as the focussing lens.

[0043] Figure 5A shows an incident laser beam 510 passing through a lens assembly comprising focussing lens 520. The incident laser beam is focused at the interrogation point 530 by the lens assembly comprising the focussing lens 520. The interrogation point is shown in this example as being in a flow cell 540. In the example shown in Figure 5, the lens assembly further comprises a convex cylindrical lens 550 with an axis parallel to the y axis. The trajectory of the nanoparticles to be detected is parallel to the y axis. Figure 5B illustrates a beam profile 560 of the incident laser beam 510 before it passes through the focussing lens 520. The beam profile 560 of the incident laser beam is elliptical in shape. The beam profile 560 has a major axis a parallel to the y axis and to the trajectory of the nanoparticles to be detected. The beam profile 560 has a minor axis b parallel to the x axis and perpendicular to the trajectory of the nanoparticles to be detected. Figure 5C shows a plot of a 2D section of the beam profile of the focused laser beam at the interrogation point 530, with irradiance plotted against the y axis at the centre of the focused beam. For beam profiles 560 of the incident laser beam 510 prior to the focussing lens 520 having a size in the y direction between $a = 1.9$ mm and $a = 2.8$ mm, fringes are present in the beam profile of the focused laser on either side of the main peak. The fringes have a height of up to 5% of the height of the main peak. The ratio of the diameter of the focussing lens 520 to the y dimension of the beam profile 560 of the incident laser beam 510 is between 4.4 and 6.6.

[0044] Figure 6A shows an incident laser beam 610 passing through a lens assembly comprising focussing lens 620. The laser beam is focused at the interrogation point 630 by the lens assembly comprising the focussing lens 620. The interrogation point is shown as being in a flow cell 640. In this example, the lens assembly further comprises a convex cylindrical lens 650 with an axis parallel to the y axis. The trajectory of the nanoparticles to be detected is parallel to the y axis. Figure 6B illustrates a beam profile

660 of the incident laser beam 610 before it passes through the focussing lens 620. The beam profile 660 of the incident laser beam is elliptical in shape. The beam profile 660 has a minor axis b parallel to the y axis and to the trajectory of the nanoparticles to be detected. The beam profile 660 has a major axis a parallel to the x axis and perpendicular to the trajectory of the nanoparticles to be detected. Figure 6C shows a plot of a 2D section of the beam profile of the focused laser at the interrogation point 630, with irradiance plotted against the y axis at the centre of the focused laser beam. For beam profiles 660 of the incident laser beam 610 prior to the focussing lens 620 having a size in the y direction between $b = 0.8$ mm and $b = 1.5$ mm, fringes in the beam profile of the focused laser beam either side of the main peak have been reduced such that the height of the fringes is approximately 0.01 % of the height of the main peak. The ratio of the diameter of the focussing lens 620 to the y dimension of the beam profile 660 of the incident laser beam 610 is between 8.3 and 15.6.

[0045] Figure 7A shows an incident laser beam 710 passing through a lens assembly comprising focussing lens 720. The laser beam is focused at the interrogation point 730 by the lens assembly comprising focussing lens 720. The interrogation point is shown as being in a flow cell 740. In this example, the lens assembly further comprises a convex cylindrical lens 750 with an axis parallel to the y axis. The trajectory of the nanoparticles to be detected is parallel to the y axis. Figure 7B illustrates a beam profile 760 of the incident laser beam 710 before it passes through the focussing lens 720. The beam profile 760 of the incident laser beam is circular in shape. Figure 7C shows a plot of a 2D section of the beam profile of the focused laser beam at the interrogation point 730, with irradiance plotted against the y axis at the centre of the focused laser beam. For beam profiles 760 of the incident laser beam 710 prior to the focussing lens 720 having a diameter (and, therefore, a size in the y direction) of between 0.8 mm and 1.5 mm, fringes in the beam profile of the focused laser beam either side of the main peak have been reduced such that the height of the fringes is approximately 0.01 % of the height of the main peak. The ratio of the diameter of the focussing lens 720 to the y dimension of the beam profile 760 of the incident laser beam 710 is between 8.3 and 15.6.

[0046] For ratios of the diameter of the focussing lens to the y dimension of the beam profile of the incident laser beam that are above 6.6, there are no fringes present in the beam profile of the focused laser beam.

[0047] The presence or absence of fringes in the y direction of the beam profile at the interrogation point affect the collected scattered light to a significantly higher degree than the presence or absence of fringes in the x direction of the beam profile at the interrogation point (perpendicular to the trajectory of the nanoparticles being detected). This is because the pulse generated in the Y direction, along the nanoparticle trajectory, is taken into account during signal acquisition. Therefore quality of the beam profile in Y direction at interrogation point is most important. Notably, as shown in Figures 5 to 7, the dimension of the incident laser beam parallel to the y -axis relative to the diameter of the focussing lens affects whether the fringes are present in the beam profile, irrespective of the dimension parallel to the x dimension or the area of the laser beam. Figures 5B and 6B show beam profiles 560, 660 of incident laser beams 510, 610 having the same area and same aspect ratio. However, in Figure 5B the major axis is parallel to the y -axis whereas in Figure 6B the major axis is parallel to the x -axis. The beam profile of the focussed beam has fringes in Figure 5C, but not in Figure 6C. Figures 6B and 7B show beam profiles 660, 760 of incident laser beams 610, 710 having the same dimension in the y direction but different dimensions in the x direction. However, neither the beam profile of the focused laser beam shown in Figure 6C nor the beam profile of the focused laser beam shown in Figure 7C has fringes.

[0048] This is further illustrated in the examples shown in Figures 8 and 9. Figure 8A shows an incident laser beam 810 passing through a lens assembly comprising focussing lens 820. The incident laser beam is focused at the interrogation point 830 by the lens assembly. The interrogation point is shown as being in a flow cell 840. In this example, the lens assembly further comprises a convex cylindrical lens 850. The trajectory of the nanoparticles to be detected is parallel to the y axis. Figure 8B illustrates a beam profile 860 of the incident laser beam 810 before it passes through the focussing lens 820. The beam profile 860 of the incident laser beam is elliptical in shape. The beam profile 860 has a major axis a parallel to the y axis and to the

trajectory of the nanoparticles to be detected. The beam profile 860 has a minor axis b parallel to the x axis and perpendicular to the trajectory of the nanoparticles to be detected. In this example, $a = 2.8$ mm and $b = 1.15$ mm. Figure 8C shows a 2D section of the beam profile of the focused laser beam at the interrogation point 830, with irradiance plotted against the y axis at the centre of the focused beam. Fringes are present in the beam profile of the focused laser on either side of the main peak. Figure 8D shows a 2D section of the beam profile of the focused laser beam at the interrogation point 830, with irradiance plotted against the x axis at the centre of the focused beam. There are no fringes present in the beam profile of the focused laser.

[0049] Figure 9A shows an incident laser beam 910 passing through a lens assembly comprising focussing lens 920. The laser beam is focused at the interrogation point 930 by the lens assembly. The interrogation point is shown as being in a flow cell 940. In this example, the lens assembly further comprises a convex cylindrical lens 950. The trajectory of the nanoparticles to be detected is parallel to the y axis. Figure 9B illustrates a beam profile 960 of the incident laser beam 910 before it passes through the focussing lens 920. The beam profile 960 of the incident laser beam is elliptical in shape. The beam profile 960 has a minor axis b parallel to the y axis and to the trajectory of the nanoparticles to be detected. The beam profile 960 has a major axis a parallel to the x axis and perpendicular to the trajectory of the nanoparticles to be detected. In this example, $a = 2.8$ mm and $b = 1.15$ mm. Figure 9C shows a 2D section of the beam profile of the focused laser at the interrogation point 930, with irradiance plotted against the y axis at the centre of the focused laser beam. There are no fringes present in the beam profile of the focused laser. Figure 9D shows a 2D section of the beam profile of the focused laser at the interrogation point 930, with irradiance plotted against the x axis at the centre of the focused laser beam. There are no fringes present in the beam profile of the focused laser.

[0050] In both the examples illustrated in Figure 8 and Figure 9, the incident laser beam 810, 910 has an elliptical beam profile with $a = 2.8$ mm and $b = 1.15$ mm. However, the incident laser beam 810 has its major axis parallel to the y axis and to the trajectory of the nanoparticles to be detected, whereas incident laser beam 910 has its

major axis parallel to the x axis and perpendicular to the trajectory of the nanoparticles to be detected. At the interrogation point, the beam profile of the focused laser beam for the example in Figure 8 has fringes in the y direction, but not in the x direction. The beam profile of the focused laser beam for the example of Figure 9 does not have fringes in either the x or y direction, even though the incident laser beam is effectively only rotated with respect to the incident laser beam of Figure 8. Reducing the dimension of the incident laser beam in the y direction with respect to the first diameter of the focussing lens removes fringes from the y direction of the beam profile of the focused laser. However, changing the dimension of the incident laser beam in the x direction with respect to the first diameter of the focussing lens does not affect whether fringes are present in the x direction of the beam profile of the focused laser, with neither example showing fringes in the x direction of the beam profile of the focused laser.

[0051] The incident laser beams shown in Figures 5 to 9 may have passed through other optical elements prior to being incident on the focussing lens. For example, the incident laser beams may be collimated by other optical components to achieve the beam profile of the incident laser beam shown in Figures 5B, 6B, 7B, 8B and 9B.

[0052] In addition to removing fringes from the beam profile of the focused laser beam, in order to detect nanoparticles the intensity of the focused laser beam at the interrogation point should be higher than a second threshold. In certain embodiments, the second threshold may be 2.7 mWatts/micrometres squared. To achieve this, the size of the incident laser beam should be higher than a threshold such that the incident laser beam is focused into a tighter beam with an intensity above the second threshold at the interrogation point.

[0053] As an example, when detecting 40nm polystyrene nanoparticles using a laser having a wavelength of 405 nm, the second threshold for the intensity of the focused laser beam may be approximately $4 \text{ mW } \mu\text{m}^{-2}$. For a 100 mW laser, the area of the beam profile of the focused laser beam may be at or below $28 \text{ } \mu\text{m}^2$ to achieve an intensity at or above the second threshold of $4 \text{ mW } \mu\text{m}^{-2}$. The area of the beam profile

of the incident beam (prior to passing through the focussing lens) may be greater than approximately 2.8 mm².

[0054] In order to successfully detect nanoparticles, there is a trade off between having a small enough y dimension of the beam profile of the incident laser beam to suppress the effects of optical aberrations, and having a large enough area of the beam profile of the incident laser beam to achieve a high enough intensity of the focused laser beam.

[0055] The intensity of the scattered light may depend on other factors, in addition to the intensity of the focused laser beam at the interrogation point. The intensity of scattered light may be given according to the following equation:

$$I = I_0 \left(\frac{1 + \cos^2 \theta}{2R^2} \right) \left(\frac{2\pi}{\lambda} \right)^4 \left(\frac{n^2 - 1}{n^2 + 2} \right)^2 \left(\frac{d}{2} \right)^6$$

I = intensity of scattered light

I₀ = intensity of focused laser beam at interrogation point

d = diameter of nanoparticle

θ = scattering angle

R = distance between particle and observer

n = refractive index of particle

As described above, the intensity I₀ of the focused laser beam at the interrogation point is given by Power/Area of the focused laser beam.

[0056] Based on this equation, the intensity of scattered light may be proportional to a sixth power of the diameter of the nanoparticle being detected. The scattered intensity from a 40nm particle is likely to be of the order of 100 times lower than the scattered intensity from an 80 nm particle.

[0057] Lowering the wavelength of the laser beam may increase the intensity of the scattered light. With reference to Figure 10, intensity of scattered light is plotted against scattering angle for four wavelengths of laser beam. The highest scattered intensity is for $\lambda = 405$ nm (line 1010), the second highest scattered intensity is for $\lambda = 488$ nm (line 1020), the third highest scattered intensity is for $\lambda = 561$ nm (line 1030) and the lowest scattered intensity is for $\lambda = 632$ nm (line 1040).

[0058] With reference to Figure 11, intensity of scattered light is plotted against scattering angle for three particle types. Line 1110 is for 40nm polystyrene particles, line 1120 is for 50 nm polystyrene particles, and line 1130 is for 40 nm Silica particles. The refractive index of polystyrene is 1.6, whereas the refractive index of silica is 1.44. It can be seen that the 40nm silica particles provide a lower scattering intensity than the 40 nm polystyrene particles. The 50 nm polystyrene particles provide a lower scattering intensity than the 40 nm polystyrene particles.

[0059] In the examples provided above, the focussing lens comprises a spherical lens. The lens assembly may further comprise a cylindrical lens. The radii of curvatures of the focussing lens and the cylindrical lens may be between 5 and 15 mm. The distance between the focussing lens and the cylindrical lens may be between 15 and 20 mm. however, other lens types, configurations, geometries and sizes may be used. For example, two convex cylindrical lenses may be used, or one convex spherical lens and one concave cylindrical lens. The positions of the lenses relative to the incident laser beam may vary.

[0060] The structure of the cuvette may also affect how much scattered light is collected. In certain embodiments, scattered light with a scattering angle of $\pm 54^\circ$ may be collected.

WHAT IS CLAIMED IS:

1. A method of using a detection system of a flow cytometer, wherein the detection system is configured to detect nanoparticles and wherein the detection system comprises:
 - a light-emitting unit configured to emit a laser beam;
 - a lens assembly comprising a focussing lens, wherein the focussing lens is configured to focus an incident laser beam at an interrogation point, wherein a trajectory of a nanoparticle passes through the interrogation point perpendicular to a central axis of the laser beam and wherein the focussing lens has a first diameter in a direction parallel to the trajectory of the nanoparticle; and
 - a light collection unit configured to collect light scattered from a nanoparticle at the interrogation point;wherein the method comprises:
 - using the lens assembly to focus a laser beam emitted by the light-emitting unit at the interrogation point, wherein a profile of the incident laser beam incident on the focussing lens has a first dimension parallel to the trajectory of the nanoparticle and a second dimension perpendicular to the trajectory of the nanoparticle, wherein:
 - a first ratio of the first diameter of the focussing lens to the first dimension of the incident laser beam is higher than a first threshold such that light fringes of the focussed laser beam at the interrogation point are suppressed; and
 - an intensity of the focused laser beam at the interrogation point is higher than a second threshold.
2. The method of claim 1 wherein the first threshold is 6.6.
3. The method according to any one of claims 1-2 wherein the second threshold is proportional to a laser power of the laser beam.

4. The method according to any one of claims 1-2 wherein the second threshold is inversely proportional to the area of the laser beam.
5. The method according to any one of claims 1-2 wherein the second threshold is inversely proportional to a power of a wavelength of the laser beam.
6. The method according to any one of claims 1-2 wherein the second threshold is proportional to a power of a diameter of the nanoparticle.
7. The method of claim 3 wherein the second threshold is 2.7, wherein the second threshold has units of mWatts/micrometres squared.
8. The method according to any one of claims 1-7 wherein the focussing lens is a convex spherical lens.
9. The method according to any one of claims 1-8 wherein the first dimension of the profile of the laser beam at the detection position is smaller than the second dimension of the profile of the laser beam at the interrogation point.
10. The method according to any one of claims 1-9 wherein a diameter of the nanoparticle is less than 70 nm.
11. A detection system of a flow cytometer, wherein the detection system is configured to detect nanoparticles and wherein the detection system comprises:
 - a light-emitting unit configured to emit the laser beam;
 - a lens assembly comprising a focussing lens, wherein the focussing lens is configured to focus an incident laser beam at an interrogation point, wherein a trajectory of a nanoparticle passes through the interrogation point perpendicular to a central axis of the laser beam and wherein the focussing lens has a first diameter in a direction parallel to the trajectory of the nanoparticle; and
 - a light collection unit configured to collect light scattered from a nanoparticle at the interrogation point;

wherein the lens assembly is configured to focus a laser beam emitted by the light-emitting unit such that a profile of the incident laser beam incident on the focussing lens has a first dimension parallel to the trajectory of the nanoparticle and a second dimension perpendicular to the trajectory of the nanoparticle, wherein:

- a first ratio of the first diameter of the focussing lens to the first dimension of the incident laser beam is higher than a first threshold such that light fringes of a laser beam at the interrogation point are suppressed; and
- an intensity of the focused laser beam at the interrogation point is higher than a second threshold.

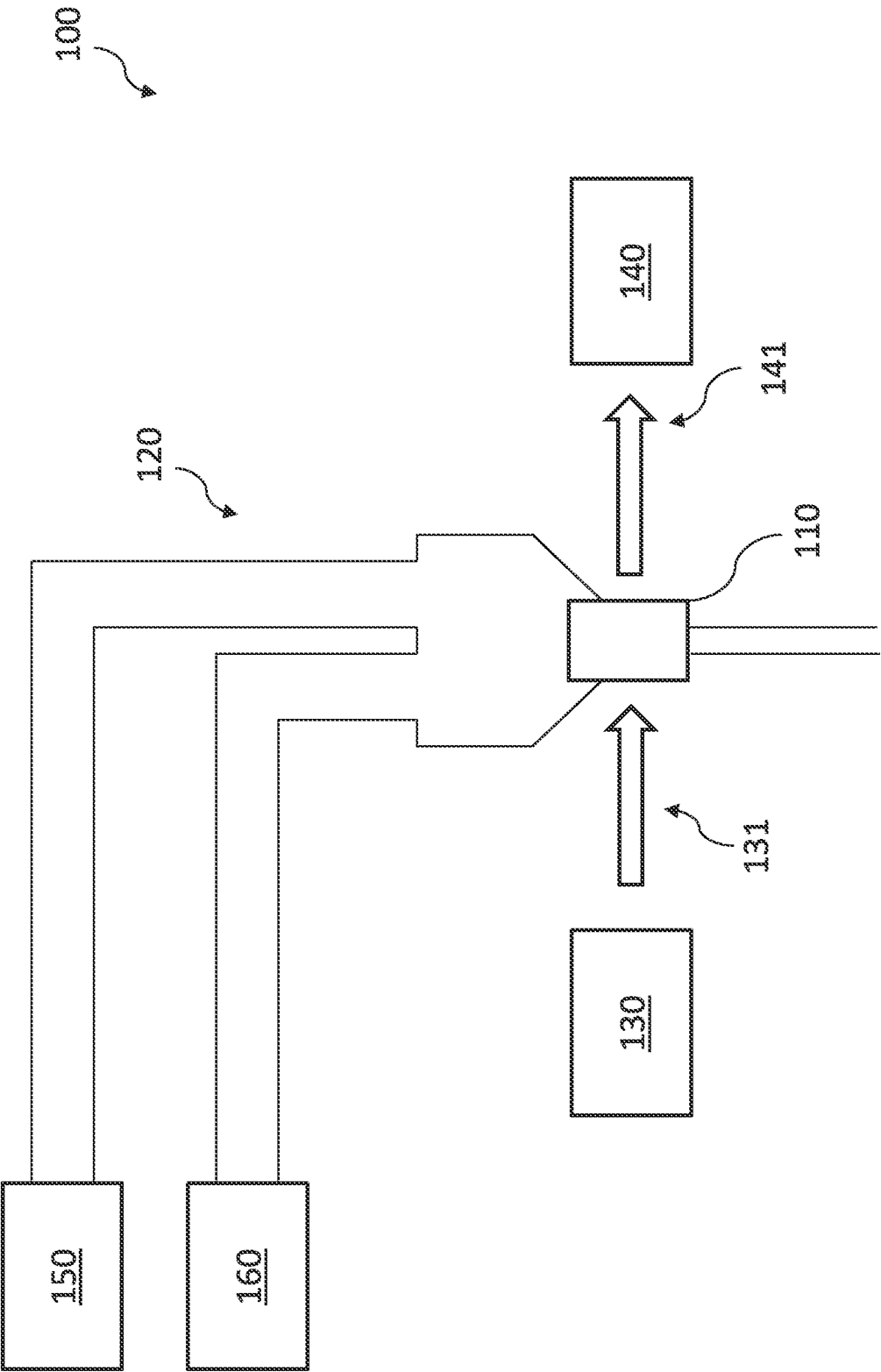


Fig. 1

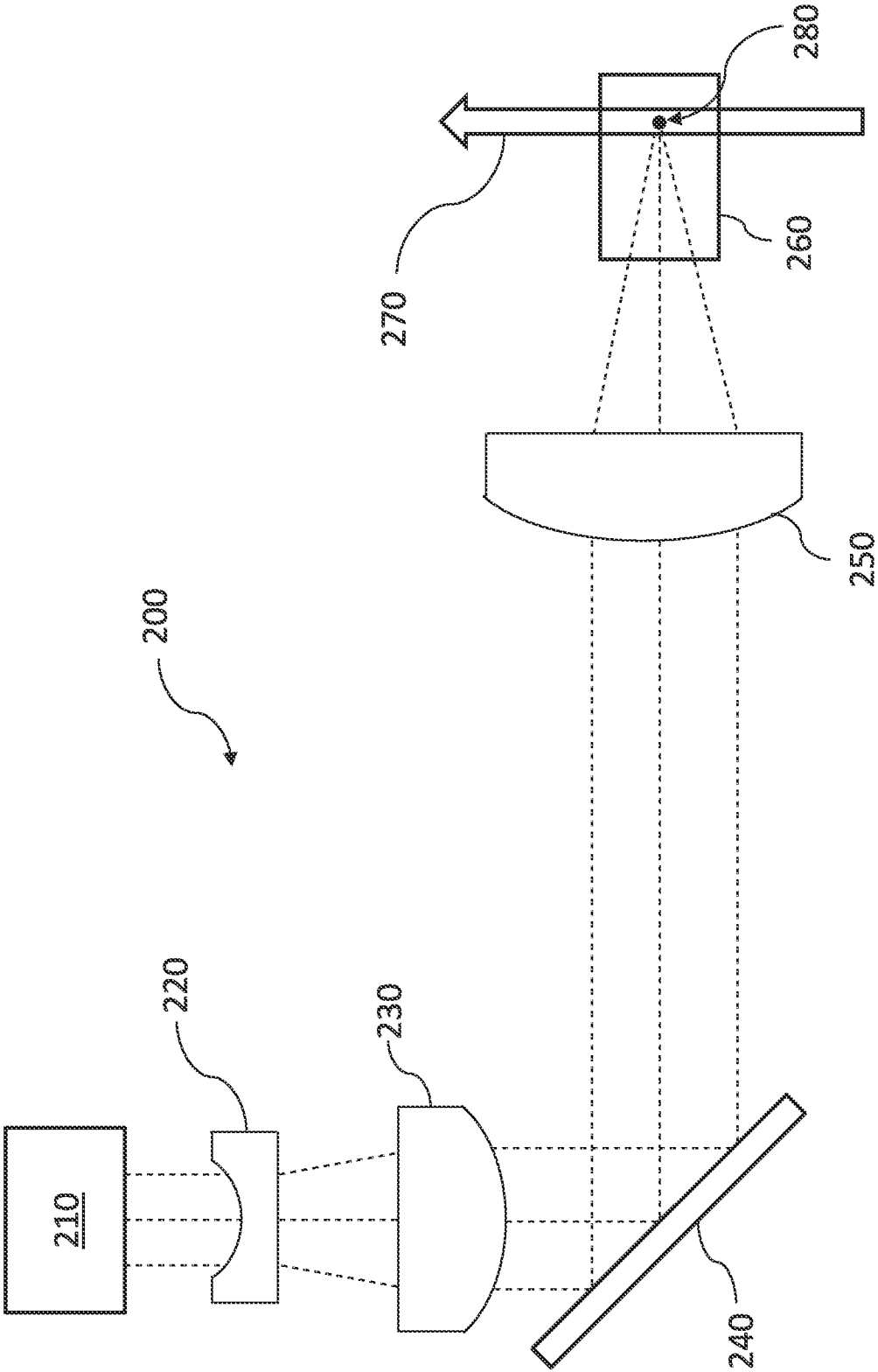


Fig. 2

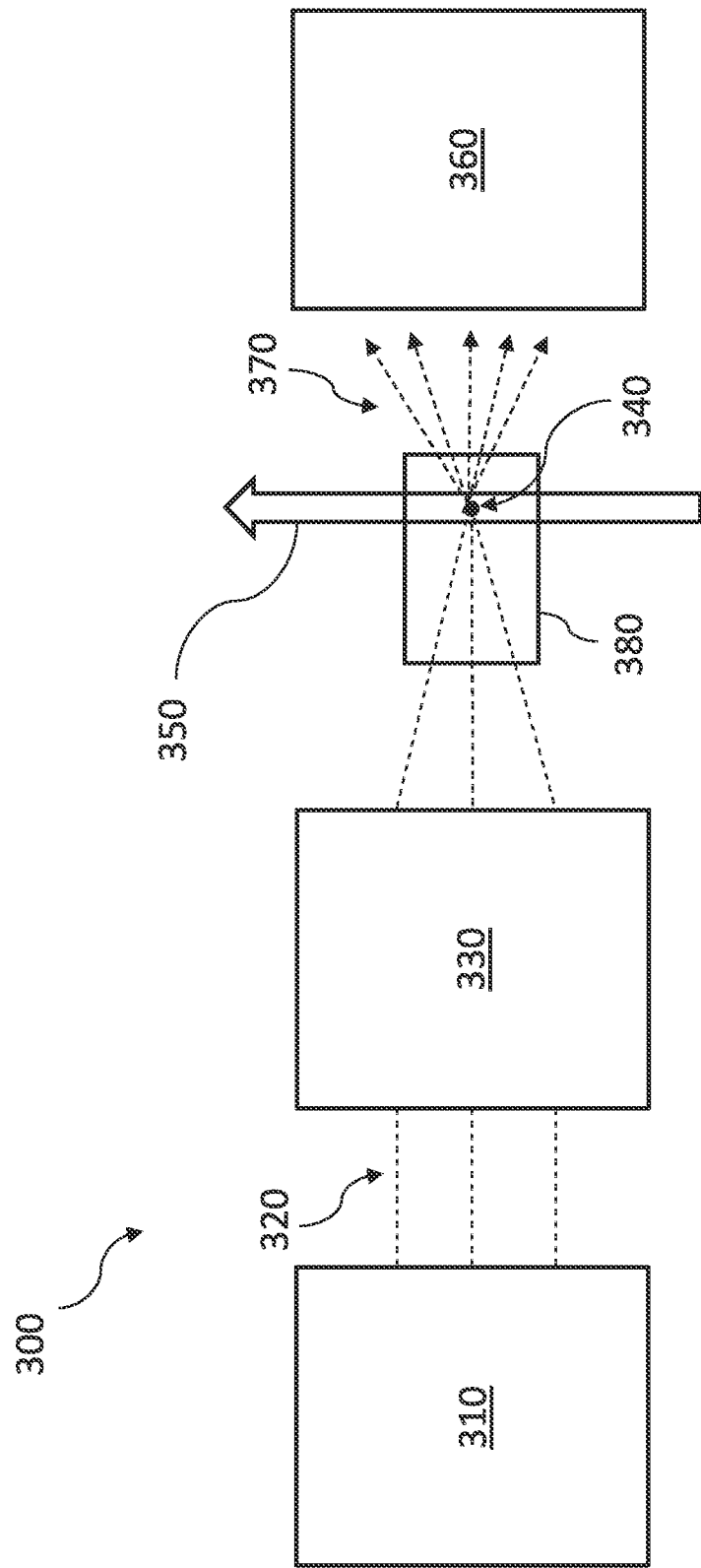


Fig. 3

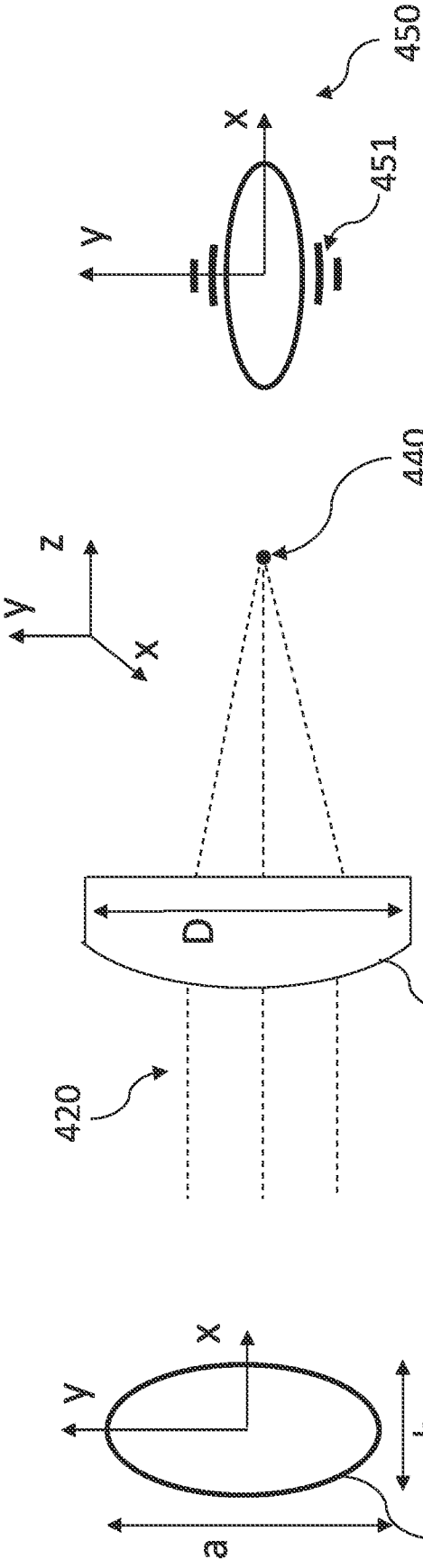


Fig. 4C

Fig. 4B

Fig. 4A

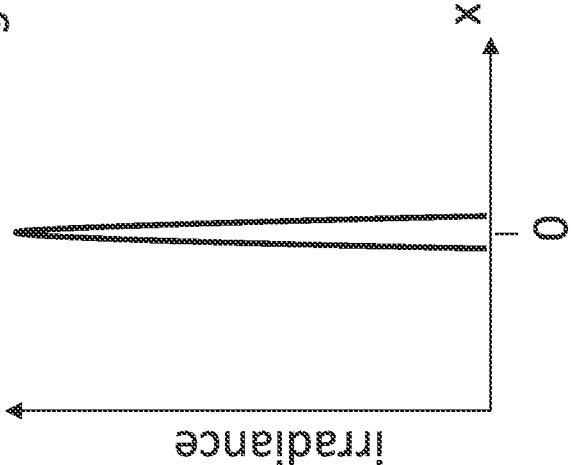


Fig. 4E

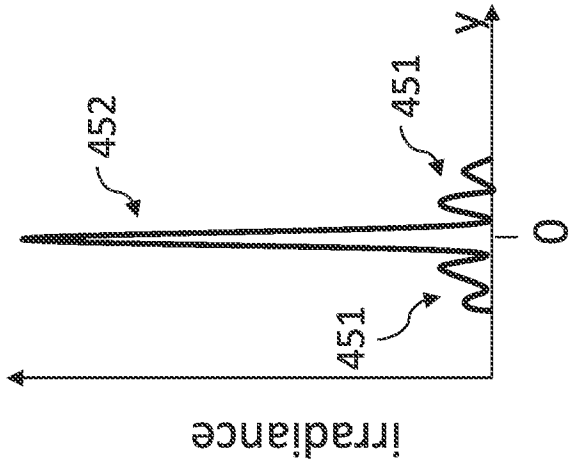


Fig. 4D

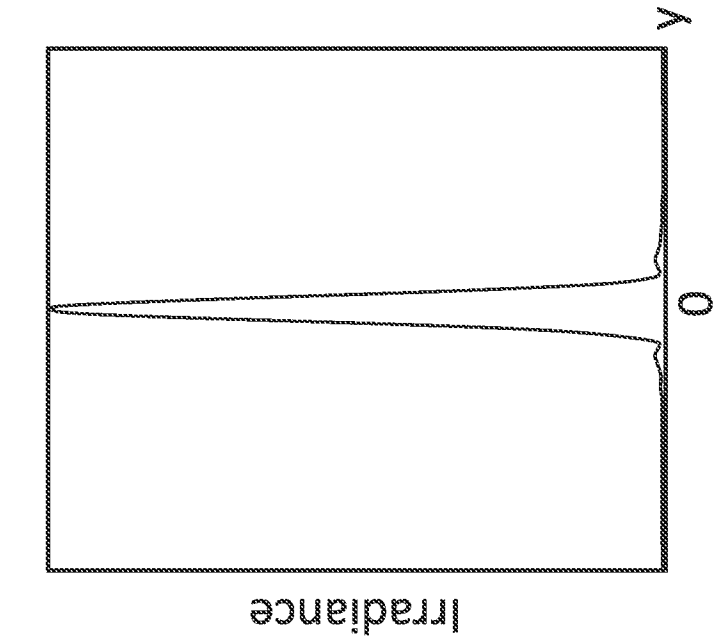


Fig. 5C

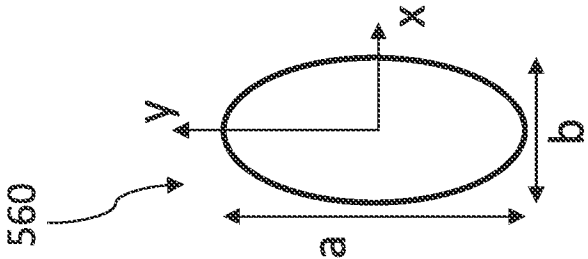


Fig. 5B

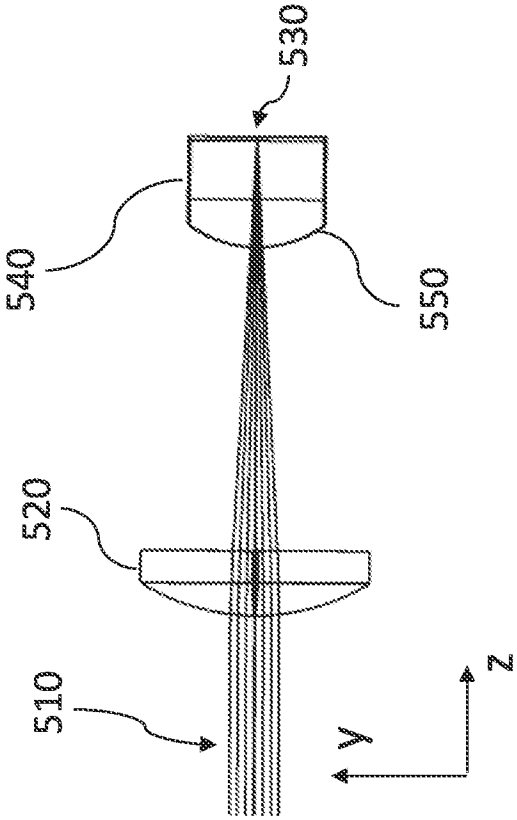


Fig. 5A

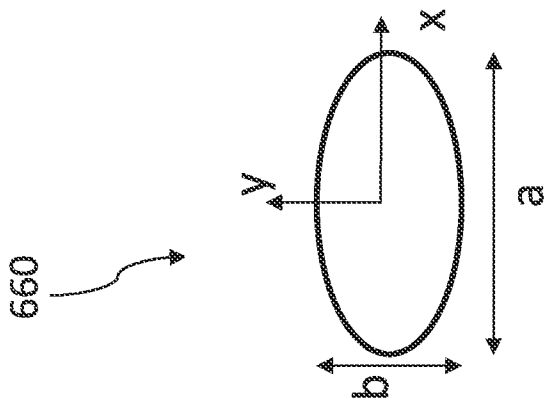


Fig. 6B

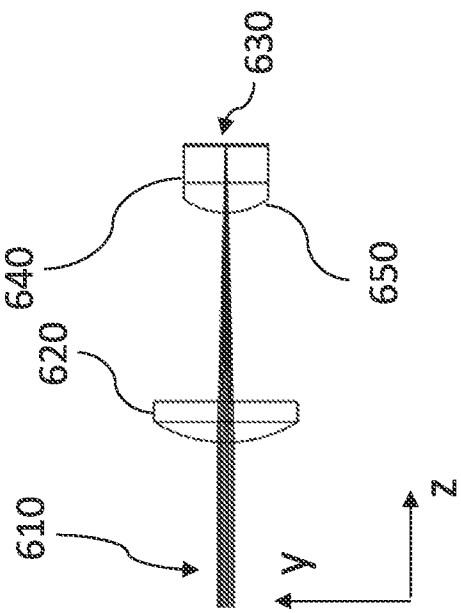


Fig. 6A

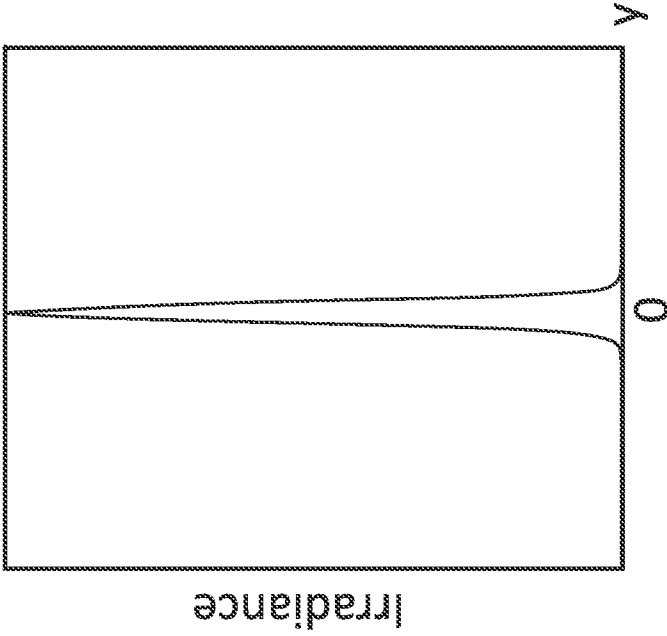


Fig. 6C

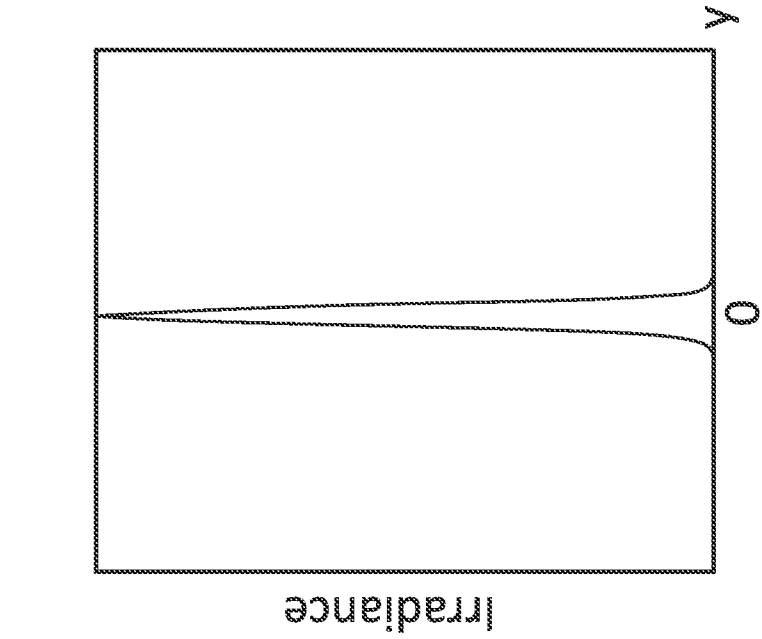


Fig. 7C

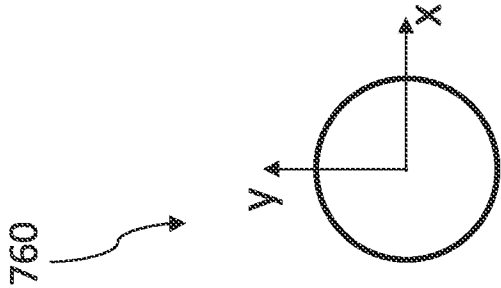


Fig. 7B

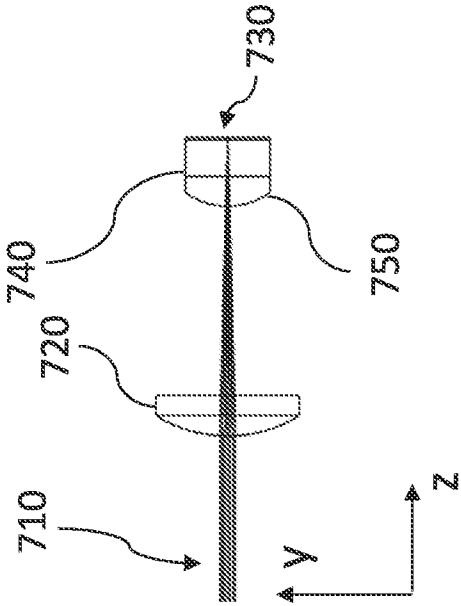


Fig. 7A

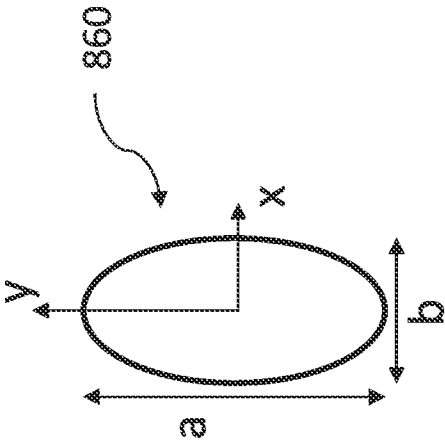


Fig. 8B

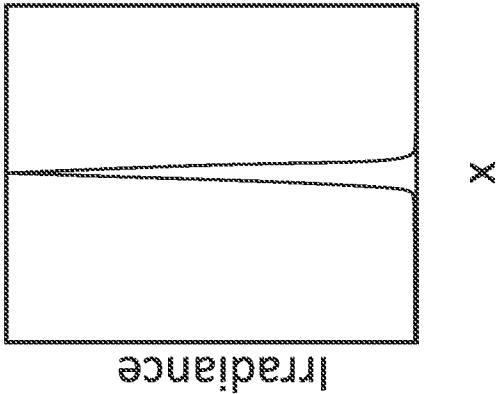


Fig. 8D

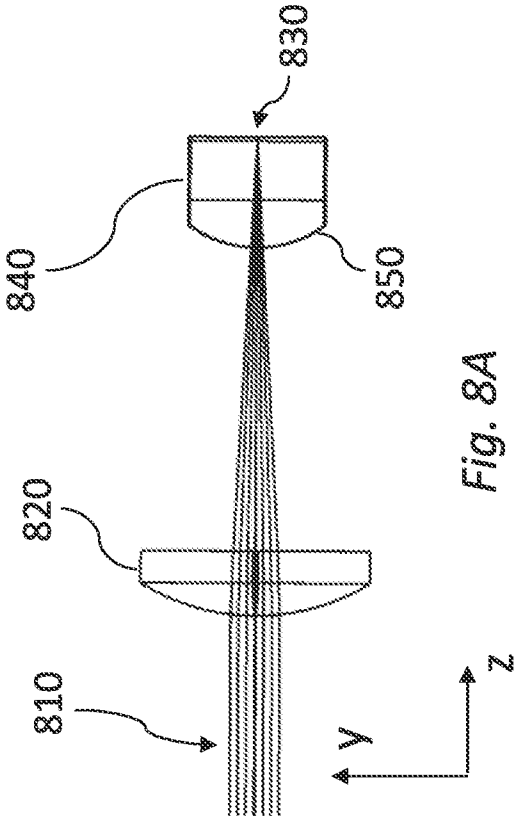


Fig. 8A

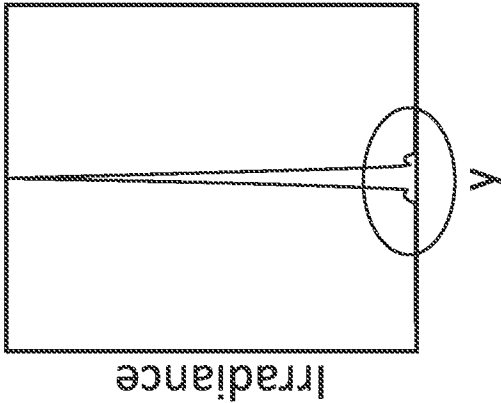


Fig. 8C

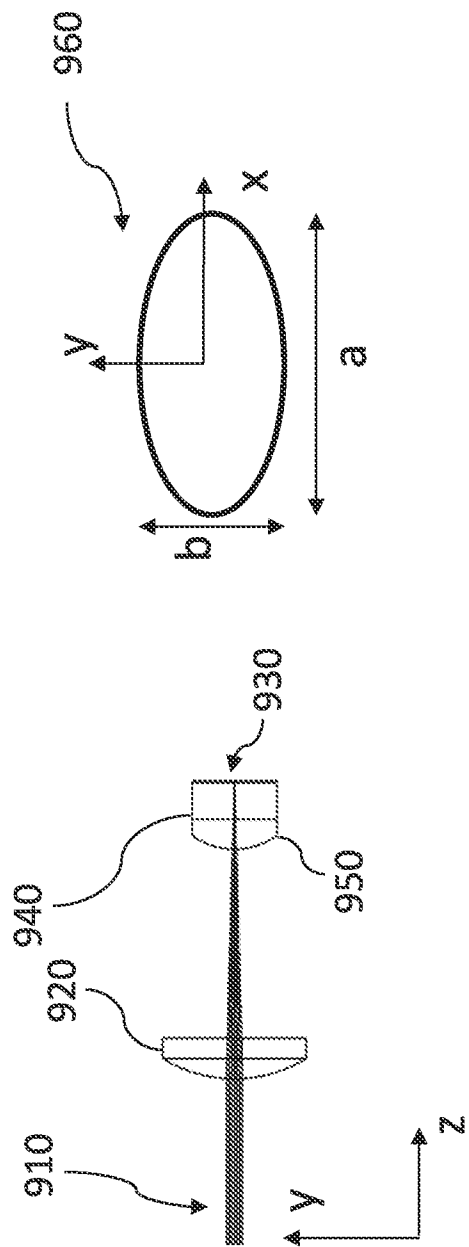


Fig. 9B

Fig. 9D

Fig. 9C

Fig. 9A

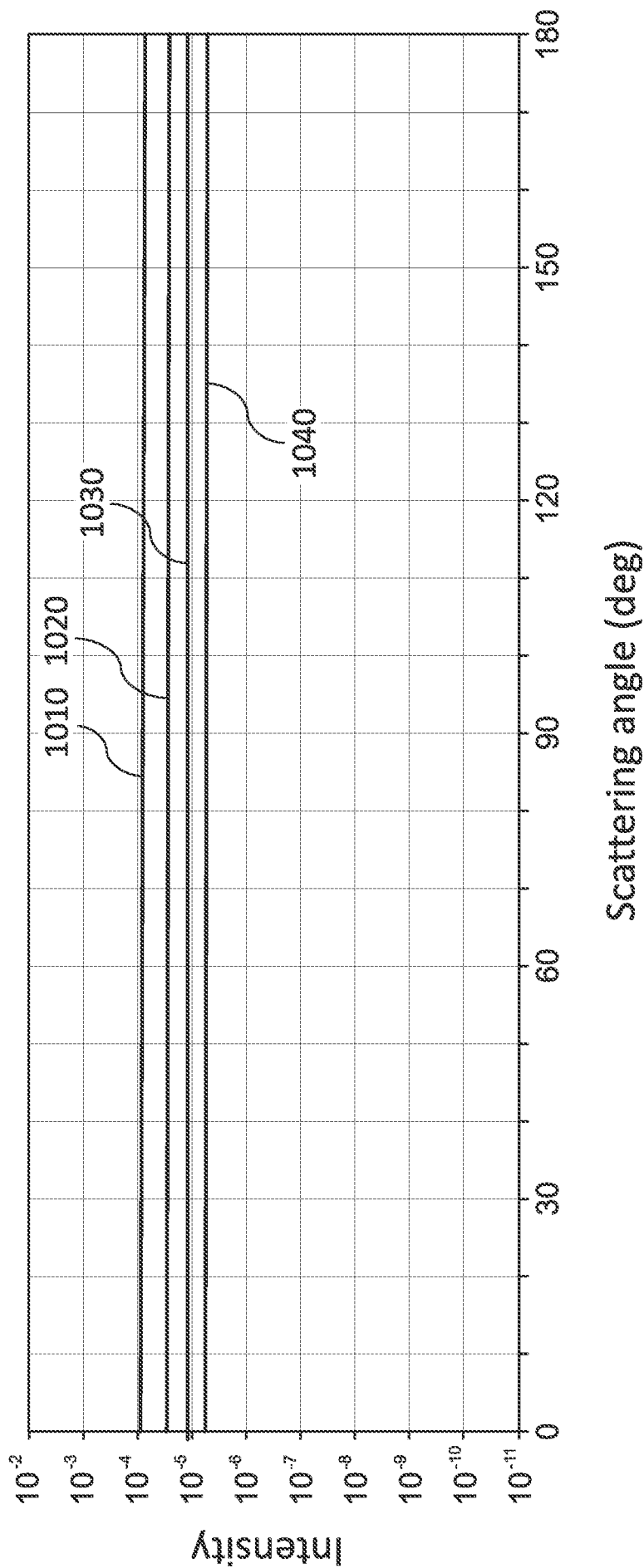


Fig. 10

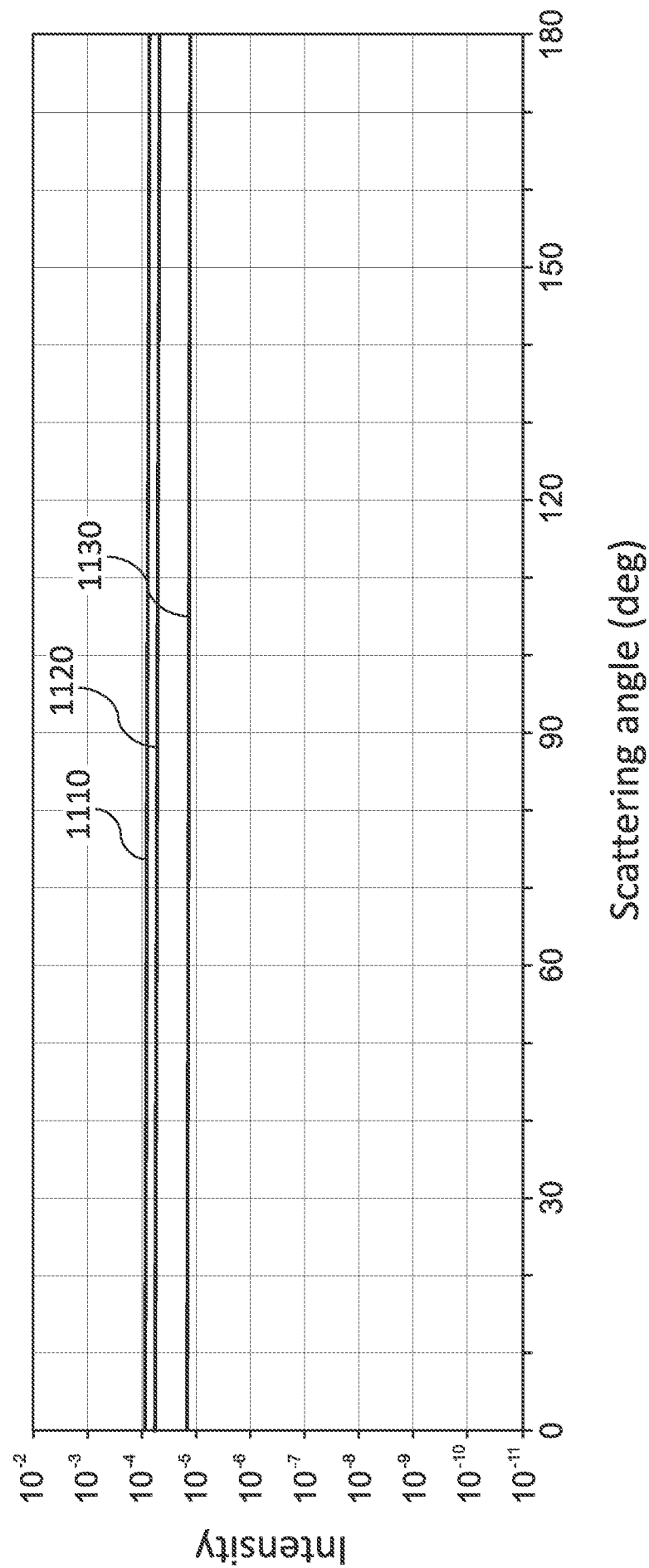


Fig. 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/032813

A. CLASSIFICATION OF SUBJECT MATTER INV. G01N15/14 G01N15/1434 G01N15/00 G01N15/10 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) 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) EPO-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 2023/073269 A1 (CHEN YONG QIN [US]) 9 March 2023 (2023-03-09) paragraph [0002] paragraph [0046] paragraphs [0325] - [0328] paragraph [0347] figures 1-8, 31-38 -----	1 - 11
X	US 2007/159619 A1 (CHU JIANJUN [CN] ET AL) 12 July 2007 (2007-07-12) paragraph [0009] paragraphs [0045] - [0049] pages 1-13 -----	1 - 11
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☒ 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 "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance;: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "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 5 September 2024		Date of mailing of the international search report 13/09/2024
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Sánchez Rivas, S

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2024/032813

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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Information on patent family members

International application No

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