BACKGROUND OF THE INVENTION

[0001] 1. Field of the Invention

[0002] The present invention relates to an optical device for light collection and imaging of microscopic particles such as those present in flow cytometers.

[0003] 2. Description of the Related Art

[0004] Flow cytometers are used for characterizing and sorting cells or other types of microscopic particles. In a typical flow cytometer, the particles are carried by fluid into a viewing zone illuminated by intense excitation light sources such as focused laser beam or arc lamp. Scatter and fluorescence light emission from the particles are then collected by a microscope objective for further analysis. In many modern flow cytotemters, a spatial filter, made of either a mechanical pinhole or a large core optical fiber, is often placed at the image location of the said objective to prevent undesired background light from entering detectors. Because particles only stay in the viewing zone for a few microseconds, microscope objectives with large neumerical aperature must be used to maximize light collection efficiency. To support multiple spatially separated excitation laser beams in a flow cytometer, as suggested by US patent No. 4727020, it is also desirable to use an objective with large field of view. In order to achieve these goals, US Pat. No. 6510007 and No. 7110192 proposed an objective design using modified apochromat with a gel-coupled or epoxy bonded near hemisphere lens as the optical element closest to the sample and followed it with multiple miniscus lenses. While these work did achieve satisfactory numerical aperture and field of view, they significantly sacrificed image quality, therefore limited the effective use of the spatial filter, resulting in poor background light discrimination. Futher, these refractive microscope objective are bulky, expensive to manufacture and often suffer severe chromatic aberration. To overcome the dificiency, WIPO application No. WO0127590 proposed an alternative objective design based on aspheric concave mirror. The design offers large numerical aperature and good image quality along the optical axis. However, due to its poor off-axis characteristics, such design is unsuitable for flow cytometer applications requiring multiple spatially separated laser beams.

SUMMARY OF THE INVENTION

[0005] Consequently, it is desirable to develop an imaging quality microscope objective that is easy to manufacture, has long working distance, large numerical aperature, large field of view and minimal chromatic aberration.

[0006] A microscope objective in accordance with the present invention, generally includes a concave spherical mirror, a transparent aberration compensation plate and a viewing zone placed in between the said mirror and the said plate. Scatter and fluorescence light emitted from particles in the said viewing zone is collected by the said mirror and reflected back toward the said compensation plate. Optical aberrations originating from the mirror is signigicantly reduced after light passes through the said compensation plate. In one exemplary embodiment of the present invention, the said viewing zone is inside a rectangular glass cuvette with a small rectangular channel for the particle carrying fluid to pass through. The said mirror is made of an optically transparent material, such as glass or optical quality plastics, of plano-convex shape, with a highly-reflective coating on the convex side for internal reflection. The plano-side of the said mirror is either gel-coupled or bonded to one side surface of the said cuvette. The said plano-aspheric compensation plate is made of a transparent material, such as glass or optical quality plastics, with the plano side gel-coupled or bonded to the opposite side of the said cuvette. The said plano-convex shaped mirror and the said aspheric compensation plate may also be made to be an integral part of the said cuvettte. In yet another examplary embodiment of the present invention, the viewing zone is in a jet stream, both the said concave mirror and the said compensation plate are free standing from the viewing zone and the said mirror is preferably a front surface concave mirror.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] Fig. 1 shows a three dimensional view for an examplary composite reflective objective of the present invention for a flow cytometer with the viewing zone inside a cuvette with a fluid-passing channel.

[0008] Fig. 2a shows a cross sectional view for the examplary reflective objective of the present invention of figure 1. Ray traces are shown to illustrate the propagation of scatter and fluorescence emission from 3 spatially separated spots in the viewing zone to the image plane.

[0009] Fig. 2b shows the spot diagrams near the image plane corresponding to the 3 spatially separated light emission spots originated near the viewing zone for the examplary composite reflective objective of the present invention shown in figure 1.

[0010] Fig. 3 shows a cross sectional view for another examplary reflective objective of the present invention. Ray traces are shown to illustrate the propagation of scatter and fluorescence emission from 3 spatially separated spots in the viewing zone to the image plane.

[0011] Fig. 4 shows a three dimensional view for another examplary composite reflective objective of the present invention for a viewing zone contained inside a cuvette with a fluid-passing channel.

[0012] Fig. 5 shows a three dimensional view for an examplary composite reflective objective of the present invention for a viewing zone inside a jet stream.

[0013] Fig 6 shows a three dimensional view for an examplary composite reflective objective of the present invention for a viewing zone on the surface of a microscope slide.

DETAILED DESCRIPTION

[0014] One exemplary embodiment of the composite microscope objective of the present invention is shown in figure 1. As illustrated in the figure, the said objective is designed for a viewing zone inside a rectangular glass cuvette 3 with a small rectangular channel 4 for the particle carrying fluid to pass through. The plano-concave back surface mirror 1 of the said objective is made of a transparent substrate, preferably of similar refractive index as the glass cuvette 3, such as glass or optical quality plastics. To minimize optical loss, the flat front surface of the said mirror 1 is optically coupled to the flat surface of the said cuvette 3. The coupling may be realized by an index-matching gel, or optical adhesive, or direct optical bonding. The said mirror 1 may also be made an integral part of the said cuvette 3.

The plano-ashperic compensation plate 2 of the said objective is also made of an optically transparent substrate of preferably similar refractive index as the said glass cuvette 3, such as glass or optical quality plastics. To reduce optical loss, the flat side of the said compensation plate maybe optically coupled to the side of the said rectangular cuvette 3 opposite of mirror 1, using index-matching gel, or optical adhesive, or direct optical bonding, and the said aspheric surface may be coated with an anti-reflective coating to reduce optical transmission loss, although the coating is not a mandatory requirement of the present invention. The shape of the said aspheric surface of the compensation plate is similar to that in a classical Schmidt camera, (Schmidt, B ., *Mitt . Hamburg . Sternwart* 7 (36) 1932), with the zone of the said compensation plate 2 outside of the neutral zone, where the plate thickness is thinnest, having negative optical power, and the zone inside the neutral zone having positive optical power. The exact prescription of the aspheric compensation plate may be readily obtained using any commercially available optical ray tracing tool by any person having ordinary skill in the art. For example, figure 2a is the ray tracing result for the exemplary embodiment of the present invention illustrated in figure 1. As shown, the scatter and fluorescence emission from 3 spatially separated spots along channel 4 near the center of the cuvette 3 propagates toward mirror 1, is internally reflected by the mirror 1, traverses through the cuvette 3, then passes through the aspheric compensation plate 2, and finally forms 3 distinct images near the image plane 5. Notice that the composite microscope objective is nearly optically-uniform and the light emission traverses the compensation plate 2 at near normal incidence. Consequently, the said objective introduces very little chromatic dispersion. Further, it is well known in the astrophysics community that Schmidt camera offers the unparalleled combination of a fast focal ratio and a large field of view with near diffraction limited optical performance. The principal drawback in a conventional Schmidt camera is that the image surface lies inside the instrument. In the exemplary embodiment of the present invention, the direction of light propagation is reversed in comparison to the Schmidt camera and the image surface naturally lies outside the objective. Consequently, the present invention takes full advantage of the optical performance of the Schmidt camera without suffering its limitation. Figure 2b shows the spot diagrams near the image plane corresponding to 3 emission spots spaced 150 micron apart from each other at the viewing zone. The diameters of all images are less than 35 microns.

[0015] When light emission exits the aspheric compensation plate 2, small amount of chromatic aberration is introduced. Figure 3 shows another exemplary embodiment of the composite microscope objective of the present invention. The shapes of the mirror 1 and the aberration compensation plate 2 are modified slightly to produce collimated afocal images of the emission spots near the viewing zone. A doublet lens 9 is then inserted between the said compensation plate 2 and the image plane 5. In addition to focus the collimated light emission from the said compensation plate 2 onto the image plane 5, the said doublet lens 9 also serves to further reduce the residue chromatic aberration introduced by the aspheric compensation plate 2.

[0016] It is not necessary for the flat surface of the said compensation plate 2 to be optically coupled to the said cuvette 3. Figure 4 shows an alternative exemplary embodiment of the composite microscope objective of the present invention. Here the aberration compensation plate 2 is optically decoupled from the cuvette. To improve the light transmission efficiency, both surfaces of the compensation plate 2 and the exposed flat surface of the cuvette 3 may be anti-reflectively coated, although the coating is not mandatory for the present invention. It is understood that the said compensation plate 2 shown in figure 4 will be held in place by mechanical means not shown in the drawing. Similar to the designs shown in Figure 2a and Figure 3, the composite microscope objective with detached compensation plate may be designed for either finite focal length image, or for an afocal system which in turn is focused to a finite distance image plane by a chromatic compensating doublet lens.

[0017] Another exemplary embodiment of the composite microscope objective of the present invention is shown in figure 5. The said objective is designed for collecting scatter and fluorescence emission from cells or other microscopic particles carried in a jet stream 14 from the nozzle 13. The said objective consists of a concave spherical shaped front surface mirror 10 and an aberration compensation plate 12. The said front surface mirror 10 may be made of glass or other types of hard material with a highly reflective coating on the concave surface 11, or made of metal with polished concave surface 11. The plano-aspheric aberration compensation plate 12 is made of a thin piece of transparent material, such as glass or optical quality plastics. The aspheric surface may be on either side of the said plate

12. Preferably, both surfaces of the plate 12 are coated with anti-reflective coating to reduce light transmission loss, although the said anti-reflective coating is not mandatory to the present invention. It is understood that the said mirror 10 and the said compensation plate 12 will be held in place by mechanical means not shown in the drawing. Scatter and fluorescence light emitted from cells or other types of microscopic particles in the viewing zone inside the jet stream 14 is reflected by the concave front surface 11 of the said mirror 10. The aberration of the surface 11 is corrected by the said aberration compensation plate 12 after light traverses through the plate 12. It is understood to any person having ordinary skill in the art that the said composite microscope objective may be designed for either a finite-focused image, similar to that shown in Figure 2a, or for collimated afocal image which are then focused at finite distance from the said objective by a chromatic aberration correction doublet, similar to the design shown in Figure 3.

[0018] The composite microscope objective of the present invention may also be adapted for microscopic studies of specimens fixed to the surface of a transparent substrate, such as a glass slide. Figure 6 shows an exemplary embodiment of the present invention for such applications. The said composite objective is made of two optical elements, one plano-concave back surface mirror 17 made of a transparent substrate, such as glass or optical quality plastics, and an aberration compensation plate 18. As shown in the figure, the species is fixed to the front surface 15 of the glass slide 16. The said slide 16 is optically coupled, preferably using a thin layer of index matching fluid, to the flat surface of the said concave mirror 17. Scatter and fluorescence light emitted by the species propagates through the slide 16 and the said concave mirror 17, is internally reflected by the said mirror 17, back through the slide 16 then through the said compensation plate 18, eventually forms an image at the image plane beyond the said compensation plate.

[0019] Although several examplary embodiments of the present invention have been described in some detail, it will be apparent to those ordinary skilled in the art that many modifications and variations of the described embodiments are possible in the light of the above teachings without departing from the principles and concepts of the invention as set forth in the claims.

What is claimed is:

1. A microscope objective device, comprising:
   a) A concave spherical mirror means;
   b) An abberation compensation plate means made of optically transparent material, wherein the said compensation plate means is an ashperic lens with the zone of the said plate means outside of the neutral zone, wherein the thickness of the said plate means is thinnest, having negative optical power, and the zone inside the neutral zone having positive optical power.
   c) A viewing zone located in between the said concave mirror means and the said abberation compensation plate means.
2. A device in accordance with claim 1, wherein the optical image of the said viewing zone is formed outside the device.

3. A device in accordance with claim 2, wherein the said viewing zone is inside a fluid carrying channel contained in a reacangular cuvette means made of optically transparent material.
4. A device in accordance with claim 3, wherein the said concave mirror means is a plano-concave back surface mirror made of an optically transparent substrate.
5. A device in accordance with claim 4, wherein the plano-surface of the said plano-concave mirror means is optically coupled to one of the flat surfaces of the said cuvette means.
6. A device in accordance with claim 5, wherein the optical coupling is realized using optical adhesive.
7. A device in accordance with claim 5, wherein the optical coupling is realized using index matching gel.
8. A device in accordance with claim 5, wherein the optical coupling is realized using index matching fluid.
9. A device in accordance with claim 5, wherein the optical coupling is realized using optical contact bonding.
10. A device in accordance with claim 5, wherein the plano-concave mirror means is an integral part of the said cuvette means.
11. A device in accordance with claim 3, wherein the said aberration compensation plate means is a plano-aspherical lens.
12. A device in accordance with claim 11, wherein the plano-side of the said

aberration compensation plate is optically coupled to the flat surface of said cuvette means opposite of the said concave mirror means.

13. A device in accordance with claim 12, wherein the optical coupling is realized using index matching gel.

14. A device in accordance with claim 12, wherein the optical coupling is realized using index matching fluid.

15. A device in accordance with claim 12, wherein the optical coupling is realized using optical contact bonding.

16. A device in accordance with claim 12, wherein the plano- aspherical lens means is an integral part of the said cuvette means.

17. A device in accordance with claim 11, wherein the said aberration compensation plate means is detached from the said cuvette means.

18. A device in accordance with claim 2, wherein the said viewing zone is inside a fluid jet stream.

19. A device in accordance with claim 17, wherein the said concave mirror means is a front surface mirror.

20. A device in accordance with claim 2, wherein the said viewing zone is on the surface of a flat substrate made of transparent material.

21. A device in accordance with claim 20, wherein the said concave mirror means is a plano-concave back surface mirror made of an optically transparent substrate.

22. A device in accordance with claim 21, wherein the plano-surface of the said

plano-concave mirror means is optically coupled to the said flat substrate.

23. A device in accordance with claim 22, wherein the optical coupling is realized using optical adhesive.

24. A device in accordance with claim 22, wherein the optical coupling is realized using index matching gel.

25. A device in accordance with claim 22, wherein the optical coupling is realized using index matching fluid.

26. A device in accordance with claim 22, wherein the optical coupling is realized using optical contact bonding.

27. A device in accordance with claim 21, wherein the said plano-concave mirror means is an integral part of the said flat substrate.

28. A device in accordance with claim 20, wherein the said aberration compensation plate means is detached from the said flat substrate.

29. A method for characterizing microscopic species using a microscope objective device comprising

   a) A concave spherical mirror means;

   b) An abberation compensation plate means made of optically transparent material, wherein the said compensation plate means is an ashperic lens with the zone of the said plate means outside of the neutral zone, wherein the thickness of the said plate means is thinnest, having negative optical power, and the zone inside the neutral zone having positive optical power.

c) A viewing zone located in between the said concave mirror means and the said abberation compensation plate means.

30. The method of claim 29, wherein the optical image of the said viewing zone is formed outside the device.

31. The method of claim 30, wherein the said viewing zone is inside a fluid carrying channel contained in a reacangular cuvette means made of optically transparent material.

32. The method of claim 31, wherein the said concave mirror means is a plano-concave back surface mirror made of an optically transparent substrate.

33. The method of claim 32, wherein the plano-surface of the said plano-concave mirror means is optically coupled to one of the flat surfaces of the said cuvette means.

34. The method of claim 33, wherein the optical coupling is realized using optical adhesive.

35. The method of claim 33, wherein the optical coupling is realized using index matching gel.

36. The method of claim 33, wherein the optical coupling is realized using index matching fluid.

37. The method of claim 33, wherein the optical coupling is realized using optical contact bonding.

38. The method of claim 33, wherein the plano-concave mirror means is an integral part of the said cuvette means.

39. The method of claim 31, wherein the said aberration compensation plate means is a plano-aspherical lens.

40. The method of claim 39, wherein the plano-side of the said aberration compensation plate is optically coupled to the flat surface of said cuvette means opposite of the said concave mirror means.

41. The method of claim 40, wherein the optical coupling is realized using index matching gel.

42. The method of claim 40, wherein the optical coupling is realized using index matching fluid.

43. The method of claim 40, wherein the optical coupling is realized using optical contact bonding.

44. The method of claim 40, wherein the plano- aspherical lens means is an integral part of the said cuvette means.

45. The method of claim 39, wherein the said aberration compensation plate means is detached from the said cuvette means.

46. The method of claim 29, wherein the said viewing zone is inside a fluid jet stream.

47. The method of claim 46, wherein the said concave mirror means is a front surface mirror.

48. The method of claim 29, wherein the said viewing zone is on the surface of a flat substrate made of transparent material.

49. The method of claim 48, wherein the said concave mirror means is a

plano-concave back surface mirror made of an optically transparent substrate.

50. The method of claim 49, wherein the plano-surface of the said plano-concave mirror means is optically coupled to the said flat substrate.

51. The method of claim 50, wherein the optical coupling is realized using optical adhesive.

52. The method of claim 50, wherein the optical coupling is realized using index matching gel.

53. The method of claim 50, wherein the optical coupling is realized using index matching fluid.

54. The method of claim 50, wherein the optical coupling is realized using optical contact bonding.

55. The method of claim 50 The method of claim 49, wherein the said plano-concave mirror means is an integral part of the said flat substrate.

56. The method of claim 55, wherein the said aberration compensation plate means is detached from the said said flat substrate.

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Title

A Composite Microscope Objective with a Dispersion Compensation Plate

Abstract

An optical device for use in the characterization of microscopic species is provided that comprises a viewing zone located in between a concave spherical mirror and an ashperical aberration compensation plate with the zone of the said compensation plate outside of the neutral zone, where the plate thickness is thinnest, having negative optical power, and the zone inside the neutral zone having positive optical power.