

What is claimed is:

1. A method comprising:
directing, using an objective and a first reflective surface, first autofocus light toward a sensor, the first autofocus light reflected from a first surface of a substrate;
preventing second autofocus light from reaching the sensor, the second autofocus light reflected from a second surface of the substrate; and
directing, using the objective and a second reflective surface, emission light toward the sensor, the emission light originating from a sample at the substrate.
2. The method of claim 1, further comprising directing the first autofocus light toward the second reflective surface, the second reflective surface transparent to the first autofocus light, wherein the first reflective surface is positioned behind the second reflective surface relative to a travel direction of the first autofocus light.
3. The method of claim 2, further comprising directing also the second autofocus light toward the second reflective surface, the second reflective surface transparent to the second autofocus light, wherein the first reflective surface is transparent to the second autofocus light to prevent the second autofocus light from reaching the sensor.
4. The method of claim 1, wherein the first reflective surface is positioned on a first reflective component, wherein the second reflective surface is positioned on a second reflective component, and wherein the first reflective component is separate from the second reflective component, the method further comprising orienting the first reflective component independently of an orientation of the second reflective component.
5. The method of claim 4, wherein orienting the first reflective component comprises steering the first autofocus light on the sensor independently of a position of the emission light on the sensor.
6. The method of claim 1, further comprising:
forming, using a lateral displacement prism, left autofocus light and right autofocus light that diverge at a predetermined angle from each other, wherein the first autofocus light comprises first left autofocus light from reflection of the left autofocus light off the first surface of the substrate, wherein the first autofocus light further comprises first right autofocus light from reflection of the right autofocus light off the first surface of the substrate, wherein the second autofocus light comprises second left autofocus light from

reflection of the left autofocus light off the second surface of the substrate, and wherein the second autofocus light further comprises second right autofocus light from reflection of the right autofocus light off the second surface of the substrate;

wherein directing the first autofocus light toward the sensor comprises directing, using the objective and the first reflective surface, the first left autofocus light and the first right autofocus light toward the sensor; and

wherein preventing the second autofocus light from reaching the sensor comprises preventing the second left autofocus light and the second right autofocus light from reaching the sensor.

7. The method of claim 6, wherein the substrate further comprises a third surface, wherein the left autofocus light forms third left autofocus light upon reflection off the third surface, wherein the right autofocus light forms third right autofocus light upon reflection off the third surface, the method further comprising directing, using the objective and the first reflective surface, the third left autofocus light and the third right autofocus light toward the sensor.

8. The method of any of claims 1 to 7, further comprising adjusting a distance between the objective and the substrate based on the first autofocus light.

9. A system comprising:

a substrate to hold a sample for analysis;

a sensor;

an objective;

a first reflective surface to direct first autofocus light to the sensor, the first autofocus light reflected from a first surface of the substrate and conveyed by the objective;

a second reflective surface to direct emission light to the sensor, the emission light originating from the sample and conveyed by the objective; and

a structure that prevents second autofocus light from reaching the sensor, the second autofocus light reflected from a second surface of the substrate and conveyed by the objective.

10. The system of claim 9, wherein the first reflective surface is positioned behind the second reflective surface relative to a travel direction of the first autofocus light, and wherein the second reflective surface is transparent to the first autofocus light.

11. The system of claim 10, wherein the first reflective surface is positioned on a first reflective component, wherein the second reflective surface is positioned on a second reflective component, and wherein the first reflective component is separate from the second reflective component.

12. The system of claim 10, wherein the second reflective surface is positioned on a front surface of a reflective component relative to the travel direction of the first autofocus light, wherein the first reflective surface covers a first portion of a rear surface of the reflective component relative to the travel direction of the first autofocus light, and wherein the structure covers a second portion of the rear surface of the reflective component.

13. The system of any of claims 9 to 12, further comprising a lateral displacement prism, the lateral displacement prism forming left autofocus light and right autofocus light that diverge at a predetermined angle from each other, wherein the first autofocus light comprises first left autofocus light from reflection of the left autofocus light off the first surface of the substrate, wherein the first autofocus light further comprises first right autofocus light from reflection of the right autofocus light off the first surface of the substrate, wherein the second autofocus light comprises second left autofocus light from reflection of the left autofocus light off the second surface of the substrate, and wherein the second autofocus light further comprises second right autofocus light from reflection of the right autofocus light off the second surface of the substrate.

14. The system of claim 13, wherein the lateral displacement prism includes exit surfaces having a non-zero angle relative to each other.

15. The system of claim 13, wherein the lateral displacement prism comprises:

- a first surface;
- a second surface that is parallel to the first surface;
- a third surface;
- a fourth surface;
- a fifth surface having a boundary with the fourth surface, wherein each of the fourth surface and the fifth surface forms a common angle with the third surface; and
- a partially reflective layer extending between the third surface and the boundary of the fourth surface and the fifth surface.

16. The system of claim 15, wherein:

the first surface has boundaries with the third surface, the fourth surface, and the fifth surface; and

the second surface has boundaries with the third surface, the fourth surface, and the fifth surface.

17. The system of any of claims 15 to 16, wherein the third surface is an entry surface, wherein the fourth surface is an exit surface for the left autofocus light, and wherein the fifth surface is an exit surface for the right autofocus light.

18. The system of claim 13, wherein the lateral displacement prism comprises:

a first prism having a first wedge profile, the first wedge profile including a first side forming a non-zero angle with regard to a first exit side;

a second prism having a second wedge profile, the second wedge profile including a second side forming a non-zero angle with regard to a second exit side; and

a third prism having a parallelogram profile, the parallelogram profile including a third side parallel to a fourth side, and a fifth side parallel to a sixth side, the third side of the parallelogram profile being part of an entry surface of the lateral displacement prism;

wherein each of the first side of the first prism and the second side of the second prism faces toward the fourth side of the third prism.

19. The system of any of claims 9 to 18, configured for analysis of nucleic material at the substrate.

20. A method comprising:

forming left autofocus light and right autofocus light that diverge at a predetermined angle from each other;

directing the left autofocus light and the right autofocus light through an objective toward a first surface of a substrate; and

after reflection off the first surface, directing at least a first part of the left autofocus light and at least a first part of the right autofocus light toward a sensor, wherein a predefined separation between the first part of the left autofocus light and the first part of the right autofocus light at the sensor indicates that the substrate is in focus of the objective.

21. The method of claim 20, wherein the substrate further includes a second surface, wherein the reflection of the left autofocus light off the first surface forms first left autofocus light, wherein a reflection of the left autofocus light off the second surface forms

second left autofocus light, wherein at the sensor the first part of the left autofocus light comprises the first left autofocus light and the second left autofocus light, wherein the reflection of the right autofocus light off the first surface forms first right autofocus light, wherein a reflection of the right autofocus light off the second surface forms second right autofocus light, wherein at the sensor the first part of the right autofocus light comprises the first right autofocus light and the second right autofocus light.

22. The method of claim 21, wherein a first predefined separation between the first left autofocus light and the first right autofocus light at the sensor indicates that the first surface of the substrate is in focus of the objective.

23. The method of claim 22, wherein a second predefined separation between the second left autofocus light and the second right autofocus light at the sensor indicates that the second surface of the substrate is in focus of the objective.

24. The method of claim 20, wherein directing the first part of the left autofocus light and the first part of the right autofocus light toward the sensor comprises directing, using a first reflective surface, the first part of the left autofocus light and the first part of the right autofocus light toward the sensor.

25. The method of claim 24, further comprising directing, using the objective and a second reflective surface, emission light toward the sensor, the emission light originating from a sample at the substrate.

26. The method of claim 25, further comprising directing the first part of the left autofocus light and the first part of the right autofocus light toward the second reflective surface, the second reflective surface transparent to the first part of the left autofocus light and the first part of the right autofocus light, wherein the first reflective surface is positioned behind the second reflective surface relative to a travel direction of the first part of the left autofocus light and the first part of the right autofocus light.

27. The method of claim 26, wherein the substrate further comprises a second surface, wherein a second part of the left autofocus light is formed upon reflection of the left autofocus light off the second surface, and wherein a second part of the right autofocus light is formed upon reflection of the right autofocus light off the second surface, the method further comprising directing the second part of the left autofocus light and the second part of the right autofocus light toward the second reflective surface, the second reflective surface

also transparent to the second part of the left autofocus light and the second part of the right autofocus light, wherein the first reflective surface is transparent to the second part of the left autofocus light and the second part of the right autofocus light to prevent the second part of the left autofocus light and the second part of the right autofocus light from reaching the sensor.

28. The method of claim 25, wherein the first reflective surface is positioned on a first reflective component, wherein the second reflective surface is positioned on a second reflective component, and wherein the first reflective component is separate from the second reflective component, the method further comprising orienting the first reflective component independently of an orientation of the second reflective component.

29. The method of claim 28, wherein orienting the first reflective component causes steering of the first part of the left autofocus light and the first part of the right autofocus light on the sensor independently of a position of the emission light on the sensor.

30. The method of any of claims 20 to 29, further comprising adjusting a distance between the objective and the substrate based on the first part of the left autofocus light and the first part of the right autofocus light.

31. A system comprising:

a beam splitter to form left autofocus light and right autofocus light that diverge at a predetermined angle from each other;

an objective to convey the left autofocus light and the right autofocus light toward a first surface of a substrate; and

a sensor to receive at least a first part of the left autofocus light and at least a first part of the right autofocus light, after reflection off the first surface, wherein a predefined separation between the first part of the left autofocus light and the first part of the right autofocus light at the sensor indicates that the substrate is in focus of the objective.

32. The system of claim 31, wherein the beam splitter is part of a lateral displacement prism.

33. The system of claim 32, wherein the lateral displacement prism includes exit surfaces having a non-zero angle relative to each other.

34. The system of claim 32, wherein the lateral displacement prism comprises:

a first surface;
a second surface that is parallel to the first surface;
a third surface;
a fourth surface;
a fifth surface having a boundary with the fourth surface, wherein each of the fourth surface and the fifth surface forms a common angle with the third surface; and
a partially reflective layer extending between the third surface and the boundary of the fourth surface and the fifth surface.

35. The system of claim 34, wherein:

the first surface has boundaries with the third surface, the fourth surface, and the fifth surface; and

the second surface has boundaries with the third surface, the fourth surface, and the fifth surface.

36. The system of any of claims 34 to 35, wherein the third surface is an entry surface, wherein the fourth surface is an exit surface for the left autofocus light, and wherein the fifth surface is an exit surface for the right autofocus light.

37. The system of claim 32, wherein the lateral displacement prism comprises:

a first prism having a first wedge profile, the first wedge profile including a first side forming a non-zero angle with regard to a first exit side;

a second prism having a second wedge profile, the second wedge profile including a second side forming a non-zero angle with regard to a second exit side; and

a third prism having a parallelogram profile, the parallelogram profile including a third side parallel to a fourth side, and a fifth side parallel to a sixth side, the third side of the parallelogram profile being part of an entry surface of the lateral displacement prism;

wherein each of the first side of the first prism and the second side of the second prism faces toward the fourth side of the third prism.

38. The system of claim 31, wherein the beam splitter comprises:

a first reflective surface at which initial autofocus light is incident;

a partially reflective layer at which the initial autofocus light is incident after being reflected at the first reflective surface, the partially reflective layer forming the left autofocus light and the right autofocus light; and

a second reflective surface at which one of the left autofocus light or the right

autofocus light is incident after being formed at the partially reflective layer.

39. The system of claim 31, further comprising a first reflective surface to direct the first part of the left autofocus light and the first part of the right autofocus light to the sensor.

40. The system of claim 39, further comprising a second reflective surface to direct emission light to the sensor, the emission light originating from a sample at the substrate and conveyed by the objective.

41. The system of claim 40, wherein the substrate further includes a second surface, wherein a second part of the left autofocus light is formed upon reflection of the left autofocus light off the second surface of the substrate, and wherein a second part of the right autofocus light is formed upon reflection of the right autofocus light off the second surface of the substrate, the system further comprising a structure to prevent the second part of the left autofocus light and the second part of the right autofocus light from reaching the sensor.

42. The system of claim 41, wherein the first reflective surface is positioned behind the second reflective surface relative to a travel direction of the first part of the left autofocus light, the second part of the left autofocus light, the first part of the right autofocus light, and the second part of the right autofocus light, wherein the second reflective surface is transparent to the first part of the left autofocus light, the second part of the left autofocus light, the first part of the right autofocus light, and the second part of the right autofocus light.

43. The system of claim 42, wherein the first reflective surface is positioned on a first reflective component, wherein the second reflective surface is positioned on a second reflective component, and wherein the first reflective component is separate from the second reflective component.

44. The system of claim 42, wherein the second reflective surface is positioned on a front surface of a second reflective component relative to the travel direction of the first part of the left autofocus light, the second part of the left autofocus light, the first part of the right autofocus light, and the second part of the right autofocus light, wherein the first reflective surface covers a first portion of a rear surface of the second reflective component relative to the travel direction of the first part of the left autofocus light, the second part of the left autofocus light, the first part of the right autofocus light, and the second part of the right autofocus light, and wherein the structure covers a second portion of the rear surface of the second reflective component.

45. The system of any of claims 31 to 44, configured for analysis of nucleic material at the substrate.
46. An autofocus assembly comprising:
- a prism comprising:
 - a first surface;
 - a second surface that is parallel to the first surface;
 - a third surface;
 - a fourth surface;
 - a fifth surface having a boundary with the fourth surface, wherein each of the fourth surface and the fifth surface forms a common angle with the third surface; and
 - a partially reflective layer extending between the third surface and the boundary of the fourth surface and the fifth surface; and
 - a light source to direct light at the prism, the prism to form first autofocus light and second autofocus light from the light, the first autofocus light and the second autofocus light diverging at a predetermined angle from each other.
47. The autofocus assembly of claim 46, wherein the fourth surface and the fifth surface form exit surfaces having a non-zero angle relative to each other.
48. The autofocus assembly of claim 46, wherein:
- the first surface has boundaries with the third surface, the fourth surface, and the fifth surface; and
 - the second surface has boundaries with the third surface, the fourth surface, and the fifth surface.
49. The autofocus assembly of any of claims 46 to 48, wherein the third surface is an entry surface.
50. The autofocus assembly of claim 46, wherein the prism comprises:
- a first prism having a first wedge profile, the first prism forming the fourth surface, the first wedge profile including a first side forming a non-zero angle with regard to the fourth surface;
 - a second prism having a second wedge profile, the second prism forming the fifth surface, the second wedge profile including a second side forming a non-zero angle with regard to the fifth surface; and

a third prism having a parallelogram profile, the parallelogram profile including a third side parallel to a fourth side, the third side defining the third surface, and a fifth side parallel to a sixth side;

wherein each of the first side of the first prism and the second side of the second prism faces toward the fourth side of the third prism.

51. A structured illumination microscopy assembly comprising:

a light source providing first light;

an anamorphic prism to provide second light by transforming the first light;

a first grating;

a second grating; and

a rotatable mirror configured to assume a first position for redirecting the second light toward the first grating, and a second position for receiving the second light from the second grating.