HIGH PERFORMANCE FLUORESCENCE IMAGING MODULE FOR GENOMIC TESTING ASSAY

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/076,361, filed September 9, 2020, and U.S. Provisional Application No. 62/962,723, filed January 17, 2020, each of which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] In typical fluorescence-based genomic testing assays, *e.g.*, genotyping or nucleic acid sequencing (using either real time, cyclic, or stepwise reaction schemes), dye molecules that are attached to nucleic acid molecules tethered on a substrate are excited using an excitation light source, a fluorescence photon signal is generated in one or more spatially-localized positions on the substrate, and the fluorescence is subsequently imaged through an optical system onto an image sensor. An analysis process is then used to analyze the images, find the positions of labeled molecules (or clonally amplified clusters of molecules) on the substrate, and quantify the fluorescence photon signal in terms of wavelength and spatial coordinates, which may then be correlated with the degree to which a specific chemical reaction, *e.g.*, a hybridization event or base addition event, occurred in the specified locations on the substrate. Imaging-based methods provide large scale parallelism and multiplexing capabilities, which help to drive down the cost and accessibility of such technologies. However, detection errors that arise from, for example, overly dense packing of labeled molecules (or clonally-amplified clusters of molecules) within a small region of the substrate surface, or due to low contrast-to-noise ratio (CNR) in the image, may lead to errors in attributing the fluorescence signal to the correct molecules (or clonally amplified clusters of molecules).

SUMMARY

[0002a] In one aspect, the present disclosure provides a method of sequencing a nucleic acid molecule, the method comprising:

a) imaging a first surface and an axially-displaced~~axially displaced~~ second surface using a compensation-free optical system which comprises an objective lens and at least one image sensor, wherein ~~said~~ the optical system has a numerical aperture (NA) of less than 0.6 and a field-of-view (FOV) of greater than 1.0 mm^2;

b) processing the images of the first surface and the axially-displaced~~axially displaced~~ second surface to correct for optical aberration such that the images of the first surface and the axially-displaced~~axially displaced~~ second surface have substantially the same optical resolution; and

c) detecting a fluorescently labeled composition comprising the nucleic acid molecule, or a complement thereof, disposed on the first surface or the axially-displaced~~axially displaced~~ second surface to determine an identity of a nucleotide in the nucleic acid molecule.

[0002b] In another aspect, the present disclosure provides a system for sequencing a nucleic acid molecule comprising:

a) an optical system comprising an objective lens and at least one image sensor, wherein ~~said~~ the optical system has a numerical aperture (NA) of less than 0.6 and a field-of-view (FOV) of greater than 3.0 mm^2, and is configured to acquire images of a first surface and an axially-displaced~~axially displaced~~ second surface; and

b) a processor programmed to:

i) process images of the first surface and the axially-displaced~~axially displaced~~ second surface to correct for optical aberration such that the images of the first surface and the axially-displaced~~axially displaced~~ second surface have substantially the same optical resolution; and

ii) detect a fluorescently labeled composition comprising the nucleic acid molecule, or a complement thereof, disposed on the first surface or the axially-displaced~~axially displaced~~ second surface to determine an identity of a nucleotide in the nucleic acid molecule.

[0003] Disclosed herein are imaging systems configured to image a first interior surface and a second interior surface of a flow cell, the imaging systems comprising: a) an objective lens; b) at least one image sensor; and c) at least one tube lens disposed in an optical path between the objective lens and the at least one image sensor; wherein said optical system has a numerical aperture (NA) of less than 0.6 and a field-of-view (FOV) of greater than 1.0 mm^2; and wherein the at least one tube lens is configured to correct imaging performance such that images of the first interior surface of the flow cell and the second interior surface of the flow cell have substantially the same optical resolution.

[0004] In some examples, the flow cell has a wall thickness of at least 700 μm and a fluid-filled gap between the first interior surface and the second interior surface of at least 50 μm. In some examples, the images of the first interior surface and the second interior surface are acquired without moving an optical compensator into an optical path between said objective lens and said at least one image sensor. In some examples, the imaging system has a numerical aperture (NA) of less than 0.6. In some examples, the imaging system has a numerical aperture (NA) of greater than 0.3. In some examples, the imaging system has a field-of-view (FOV) of greater than 1.5 mm^2. In some examples, the optical resolution of images of the first interior surface and the second interior surface are diffraction-limited across the entire field-of-view (FOV). In

CLAIMS

WHAT IS CLAIMED IS:

1. A method of sequencing a nucleic acid molecule, the method comprising:

a) imaging a first surface and an ~~axially displaced~~axially-displaced second surface using a compensation-free optical system which comprises an objective lens and at least one image sensor, wherein ~~said~~ the optical system has a numerical aperture (NA) of less than 0.6 and a field-of-view (FOV) of greater than 1.0 mm^2;

b) processing the images of the first surface and the axially-displaced~~axially displaced~~ second surface to correct for optical aberration such that the images of the first surface and the axially-displaced~~axially displaced~~ second surface have substantially the same optical resolution; and

c) detecting a fluorescently labeled composition comprising the nucleic acid molecule, or a complement thereof, disposed on the first surface or the axially-displaced~~axially displaced~~ second surface to determine an identity of a nucleotide in the nucleic acid molecule.

2. The method of claim 1, wherein the images of the first surface and the axially-displaced~~axially displaced~~ second surface are acquired without moving an optical compensator into an optical path between ~~said~~ the objective lens and ~~said~~ the at least one image sensor.

3. The method of claim 1, wherein the images of the first surface and the axially-displaced~~axially displaced~~ second surface are acquired by just refocusing the optical system.

4. The method of claim 1, further comprising imaging two or more fields-of-view on at least one of the first surface ~~or~~ and the axially-displaced ~~axially displaced~~ second surface.

5. The method of claim 1, wherein the first surface and the axially-displaced~~axially displaced~~ second surface comprise two surfaces of a flow cell.

6. The method of claim 5, wherein ~~said~~ the two surfaces of the flow cell are coated with a hydrophilic coating layer.

7. The method of claim 6, wherein ~~said~~ the hydrophilic coating layer further comprises labeled nucleic acid colonies disposed thereon at a surface density of~~></~~ greater than 10,000 labeled nucleic acid colonies / millimeter (mm)^2.

8. The method of claim 7, wherein an image of a surface of ~~said~~ the two surfaces acquired using ~~said~~ the optical system shows a contrast to noise ratio (CNR) of at least 5 when the nucleic acid colonies are labeled with cyanine dye 3 (Cy3), the optical system comprises a dichroic mirror

and bandpass filter set optimized for Cy3 emission, and the image is acquired under non-signal saturating conditions while the surface is immersed in 25 mM ACES, pH 7.4 buffer.

9. The method of claim 1, wherein ~~said~~ the optical system comprises 1, 2, 3, or 4 imaging channels configured to detect nucleic acid colonies disposed on at least one of the first surface and the axially-displaced~~axially displaced~~ second surface, wherein the nucleic acid colonies ~~that~~ have been labeled with 1, 2, 3, or 4 distinct detectable labels.

10. The method of claim 1, wherein the at least one image sensor comprises pixels having a pixel dimension chosen such that a spatial sampling frequency for the optical system is at least twice an optical resolution of the optical system.

11. The method of claim 1, wherein the optical system comprises at least one tube lens positioned between the objective lens and the at least one image sensor, and wherein the at least one tube lens is configured to correct an imaging performance metric for imaging a first interior surface of a flow cell and a second interior surface of the flow cell.

12. The method of claim 11, wherein the flow cell has a wall thickness of at least 700 μm and a gap between the first interior surface and the second interior surface of at least 50 μm.

13. The method of claim 11, wherein the at least one tube lens comprises, in order, an asymmetric convex-convex lens, a convex-plano lens, an asymmetric concave-concave lens, and an asymmetric convex-concave lens.

14. The method of claim 11, wherein the optical system comprises two or more tube lenses which are designed to provide optimal imaging performance at two or more fluorescence wavelengths.

15. The method of claim 11, wherein a combination of the objective lens and the at least one tube lens is configured to optimize a modulation transfer function in the mid to high spatial frequency range.

16. The method of claim 11, wherein the imaging performance metric comprises a measurement of modulation transfer function (MTF) at one or more specified spatial frequencies, defocus, spherical aberration, chromatic aberration, coma, astigmatism, field curvature, image distortion, image contrast-to-noise ratio (CNR), or any combination thereof.

17. The method of claim 1, wherein the optical resolution of the images of the first surface and axially-displaced~~axially displaced~~ second surface are diffraction-limited across the entire field-of-view (FOV).

18. The method of claim 1, wherein the sequencing of the nucleic acid molecule further comprises performing a sequencing-by-avidity, sequencing-by-nucleotide binding, or sequencing-by-nucleotide incorporation reaction on at least one of the first surface and axially-displaced~~axially displaced~~ second surface and detecting a bound or incorporated nucleotide base.

19. The method of claim 1, further comprising determining a genotype of a sample, wherein determining the genotype of the sample comprises preparing ~~said~~ the nucleic acid molecule for sequencing, and then sequencing ~~said~~ the nucleic acid molecule.

20. A system for sequencing a nucleic acid molecule comprising:

a) an optical system comprising an objective lens and at least one image sensor, wherein ~~said~~ the optical system has a numerical aperture (NA) of less than 0.6 and a field-of-view (FOV) of greater than 3.0 mm^2, and is configured to acquire images of a first surface and an axially-displaced~~axially displaced~~ second surface; and

b) a processor programmed to:

i) process images of the first surface and the axially-displaced~~axially displaced~~ second surface to correct for optical aberration such that the images of the first surface and the axially-displaced~~axially displaced~~ second surface have substantially the same optical resolution; and

ii) detect a fluorescently labeled composition comprising the nucleic acid molecule, or a complement thereof, disposed on the first surface or the axially-displaced~~axially displaced~~ second surface to determine an identity of a nucleotide in the nucleic acid molecule.

21. The system of claim 20, wherein the images of the first surface and the axially-displaced~~axially displaced~~ second surface are acquired without moving an optical compensator into an optical path between ~~said~~ the objective lens and ~~said~~ the at least one image sensor.

22. The system of claim 20, wherein the images of the first surface and the axially-displaced~~axially displaced~~ second surface are acquired by just refocusing the optical system.

23. The system of claim 20, wherein the imaging system has a numerical aperture of greater than 0.3.

24. The system of claim 20, wherein the first surface and axially-displaced~~axially displaced~~ second surface comprise two surfaces of a flow cell.

25. The system of claim 24, wherein ~~said~~ the two surfaces of the flow cell are coated with a hydrophilic coating layer, and wherein ~~said~~ the hydrophilic coating layer further comprises labeled nucleic acid colonies disposed thereon at a surface density of ~~></~~ greater than 10,000 labeled nucleic acid colonies / millimeter (mm)^2.

26. The system of claim 20, wherein ~~said~~ the optical system comprises 1, 2, 3, or 4 imaging channels configured to detect nucleic acid colonies disposed on at least one of the first surface or axially-displaced~~axially displaced~~ second surface that have been labeled with 1, 2, 3, or 4 distinct detectable labels.

27. The system of claim 20, wherein the at least one image sensor comprises pixels having a pixel dimension chosen such that a spatial sampling frequency for the optical system is at least twice an optical resolution of the optical system.

28. The system of claim 20, wherein the system comprises at least one tube lens positioned between the objective lens and the at least one image sensor, and wherein the at least one tube lens is configured to correct an imaging performance metric for imaging a first interior surface of a flow cell and a second interior surface of the flow cell.

29. The system of claim 28, wherein the flow cell has a wall thickness of at least 700 µm and a gap between the first interior surface and the second interior surface of at least 50 µm.

30. The system of claim 28, wherein the optical system comprises two or more tube lenses which are designed to provide optimal imaging performance at two or more fluorescence wavelengths.