

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 refers to a method of sequencing a nucleic acid molecule, the method comprising: a) imaging a first surface and an axially-displaced second surface using an 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 mm², and wherein images of the first surface and the axially-displaced second surface having substantially the same optical resolution are acquired without moving an optical compensator into an optical path between ~~said-the~~ objective lens and ~~said-the~~ at least one image sensor; and b) 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 refers to an imaging system configured to image two distinct, axially-displaced surfaces, the imaging system comprising an objective lens and at least one image sensor, wherein ~~said-the~~ imaging system has a numerical aperture (NA) of less than 0.6 and a field-of-view (FOV) of greater than 1.0 mm², and wherein ~~said-the~~ imaging system is capable of acquiring images of the two distinct, axially-displaced surfaces that have substantially the same optical resolution without moving an optical compensator into an optical path between ~~said-the~~ objective lens and ~~said-the~~ at least one image sensor.

[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 mm²; 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 mm². 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 some examples, 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. In some examples, the imaging system comprises two or more tube lenses which are designed to provide optimal imaging performance for the first interior surface and the second interior surface at two or more fluorescence wavelengths. In some examples, the imaging system further comprises a focusing mechanism configured to refocus the optical system between acquiring images of the first interior surface and the second interior surface. In some examples, the imaging system is configured to image two or more fields-of-view on at least one of the first interior surface or the second interior surface. In some examples, the first interior

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 second surface using an 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 , and wherein images of the first surface and the axially-displaced second surface having substantially the same optical resolution are acquired without moving an optical compensator into an optical path between ~~said-the~~ objective lens and ~~said-the~~ at least one image sensor; and
 - b) 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 a focusing mechanism is utilized to refocus the optical system between acquiring the images of the first surface and the ~~axially-displaced~~axially-displaced second surface.
3. 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.
4. The method of claim 1, wherein the first surface and the ~~axially-displaced~~axially-displaced second surface comprise two surfaces of a flow cell.
5. The method of claim 4, wherein ~~said-the~~ two surfaces of the flow cell are coated with a hydrophilic coating layer.
6. The method of claim 5, 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.
7. The method of claim 6, 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 labeled 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.
8. 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 second surface, ~~wherein the nucleic acid colonies that~~ have been labeled with 1, 2, 3, or 4 distinct detectable labels.

9. 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.

10. 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.

11. The method of claim 10, 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 .

12. The method of claim 10, 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.

13. The method of claim 10, wherein the optical system comprises two or more tube lenses which are designed to provide optimal imaging performance at two or more fluorescence wavelengths.

14. The method of claim 10, 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.

15. The method of claim 10, 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.

16. 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).

17. The method of claim 1, wherein the sequencing of the nucleic acid molecule further comprises performing a sequencing-by-avidity, sequencing-by-nucleotide base-pairing, 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.

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

19. An imaging system configured to image two distinct, axially-displaced surfaces, the imaging system comprising an objective lens and at least one image sensor, wherein ~~said-the~~ imaging system has a numerical aperture (NA) of less than 0.6 and a field-of-view (FOV) of greater than 1.0 mm², and wherein ~~said-the~~ imaging system is capable of acquiring images of the two distinct, axially-displaced surfaces that have substantially the same optical resolution without moving an optical compensator into an optical path between ~~said-the~~ objective lens and ~~said-the~~ at least one image sensor.
20. The imaging system of claim 19, wherein the imaging system has a numerical aperture of greater than 0.3.
21. The imaging system of claim 19, further comprising a focusing mechanism used to refocus the optical system between acquiring the images of the two distinct, ~~axially-displaced~~~~axially-displaced~~ surfaces.
22. The imaging system of claim 19, wherein the imaging system is configured to image two or more fields-of-view on at least one of ~~said-the~~ two distinct, ~~axially-displaced~~~~axially-displaced~~ surfaces.
23. The imaging system of claim 19, wherein ~~said-the~~ two distinct, ~~axially-displaced~~~~axially-displaced~~ surfaces comprise two surfaces of a flow cell.
24. The imaging system of claim 23, wherein ~~said-the~~ two distinct 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.
25. The imaging system of claim 19, wherein ~~said-the~~ imaging system comprises 1, 2, 3, or 4 imaging channels configured to detect nucleic acid colonies disposed on at least one of ~~said-the~~ two distinct surfaces, ~~wherein the nucleic acid colonies that~~ have been labeled with 1, 2, 3, or 4 distinct detectable labels.
26. The imaging system of claim 19, wherein the at least one image sensor comprises pixels having a pixel dimension chosen such that a spatial sampling frequency for the imaging system is at least twice an optical resolution of the imaging system.
27. The imaging system of claim 19, wherein the imaging 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.

28. The imaging system of claim 27, 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 .

29. The imaging system of claim 27, wherein the imaging system comprises two or more tube lenses which are designed to provide optimal imaging performance at two or more fluorescence wavelengths.

30. The imaging system of claim 19, wherein the optical resolution of the images of the two distinct, axially-displaced~~axially-displaced~~ surfaces are diffraction-limited across the entire field-of-view (FOV).