

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 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 objective lens and said 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 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 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 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 objective lens and said 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

surface and second interior surface of the flow cell are coated with a hydrophilic coating layer, and wherein said hydrophilic coating layer further comprises labeled nucleic acid colonies disposed thereon at a surface density of $> 10,000$ nucleic acid colonies / mm^2 . In some examples, an image of the first interior surface or second interior surface acquired using the imaging 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 imaging 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. In some examples, said imaging system comprises 1, 2, 3, or 4 imaging channels configured to detect nucleic acid colonies disposed on at least one of said two distinct surfaces that have been labeled with 1, 2, 3, or 4 distinct detectable labels. In some examples, the imaging system is used to monitor 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 interior surface and the second interior surface and detect a bound or incorporated nucleotide base. In some examples, the imaging system is used to perform nucleic acid sequencing. In some examples, the imaging system is used to determine a genotype of a sample, wherein determining the genotype of the sample comprises preparing a nucleic acid molecule extracted from the sample for sequencing, and then sequencing the nucleic acid molecule. In some examples, 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. In some examples, a combination of the objective lens and the at least one tube lens is configured to optimize a modulation transfer function in the spatial frequency range from 700 cycles per mm to 1100 cycles per mm in the sample plane. In some examples, the at least one tube lens is designed to correct 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, for a combination of the objective lens and the at least one tube lens.

[0005] Also disclosed herein are methods of sequencing a nucleic acid molecule, the methods 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 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 objective lens and said 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 second surface to determine an identity of a nucleotide in the nucleic acid molecule.

[0006] In some examples, a focusing mechanism is utilized to refocus the optical system between acquiring images of the first surface and the axially-displaced second surface. In some examples, the method further comprises imaging two or more fields-of-view on at least one of the first surface or axially-displaced second surface. In some examples, the first surface and the axially-displaced second surface comprise two surfaces of a flow cell. In some examples, said two surfaces of the flow cell are coated with a hydrophilic coating layer. In some examples, said hydrophilic coating layer further comprises labeled nucleic acid colonies disposed thereon at a surface density of $> 10,000$ nucleic acid colonies / mm^2 . In some examples, an image of a surface of said two surfaces acquired using said 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. In some examples, said 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 that have been labeled with 1, 2, 3, or 4 distinct detectable labels. In some examples, 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. In some examples, 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. In some examples, 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 . 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 optical system comprises two or more tube lenses which are designed to provide optimal imaging performance at two or more fluorescence wavelengths. In some examples, a combination of objective lens and tube lens is configured to optimize a modulation transfer function in the mid to high spatial frequency range. In some examples, 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. In some examples, the optical resolution of images of the first surface and axially-displaced second

surface are diffraction-limited across the entire field-of-view (FOV). In some examples, 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 second surface and detecting a bound or incorporated nucleotide base. In some examples, the method further comprises determining a genotype of a sample, wherein determining the genotype of the sample comprises preparing said nucleic acid molecule for sequencing, and then sequencing said nucleic acid molecule.

[0007] Disclosed herein are imaging systems configured to image two distinct, axially-displaced surfaces, the imaging systems comprising an objective lens and at least one image sensor, wherein said 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 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 objective lens and said at least one image sensor.

[0008] In some examples, the imaging system has a numerical aperture of greater than 0.3. In some examples, the imaging system further comprises a focusing mechanism used to refocus the optical system between acquiring images of the two distinct, axially-displaced surfaces. In some examples, the imaging system is configured to image two or more fields-of-view on at least one of said two distinct, axially-displaced surfaces. In some examples, said two distinct, axially-displaced surfaces comprise two surfaces of a flow cell. In some examples, said two distinct surfaces of the flow cell are coated with a hydrophilic coating layer, and wherein said hydrophilic coating layer further comprises labeled nucleic acid colonies disposed thereon at a surface density of $> 10,000$ nucleic acid colonies / mm². In some examples, said imaging system comprises 1, 2, 3, or 4 imaging channels configured to detect nucleic acid colonies disposed on at least one of said two distinct surfaces that have been labeled with 1, 2, 3, or 4 distinct detectable labels. In some examples, 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. In some examples, 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. In some examples, 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 . In some examples, the imaging system comprises two or more tube lenses which are designed to

provide optimal imaging performance at two or more fluorescence wavelengths. In some examples, the optical resolution of images of the two distinct, axially-displaced surfaces are diffraction-limited across the entire field-of-view (FOV).

[0009] Disclosed herein are methods of sequencing a nucleic acid molecule, the method comprising: a) imaging a first surface and an axially-displaced second surface using a compensation-free optical system which comprises an objective lens and 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; b) processing the images of the first surface and the axially-displaced second surface to correct for optical aberration such that the images of the first surface and the 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 second surface to determine an identity of a nucleotide in the nucleic acid molecule.

[0010] In some examples, the images of the first surface and the axially-displaced second 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 images of the first surface and the axially-displaced second surface are acquired by just refocusing the optical system. In some examples, the method further comprises imaging two or more fields-of-view on at least one of the first surface or axially-displaced second surface. In some examples, the first surface and the axially-displaced second surface comprise two surfaces of a flow cell. In some examples, said two surfaces of the flow cell are coated with a hydrophilic coating layer. In some examples, said hydrophilic coating layer further comprises labeled nucleic acid colonies disposed thereon at a surface density of > 10,000 nucleic acid colonies / mm². In some examples, an image of a surface of said two surfaces acquired using said 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. In some examples, said 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 that have been labeled with 1, 2, 3, or 4 distinct detectable labels. In some examples, 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. In some examples, 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. In some examples, 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 . 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 optical system comprises two or more tube lenses which are designed to provide optimal imaging performance at two or more fluorescence wavelengths. In some examples, a combination of objective lens and tube lens is configured to optimize a modulation transfer function in the mid to high spatial frequency range. In some examples, 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. In some examples, the optical resolution of images of the first surface and axially-displaced second surface are diffraction-limited across the entire field-of-view (FOV). In some examples, 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 second surface and detecting a bound or incorporated nucleotide base. In some examples, the method further comprises determining a genotype of a sample, wherein determining the genotype of the sample comprises preparing said nucleic acid molecule for sequencing, and then sequencing said nucleic acid molecule.

[0011] Disclosed herein are systems for sequencing a nucleic acid molecule comprising: a) an optical system comprising an objective lens and 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 is configured to acquire images of a first surface and an axially-displaced second surface; and b) a processor programmed to: i) process images of the first surface and the axially-displaced second surface to correct for optical aberration such that the images of the first surface and the 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 second surface to determine an identity of a nucleotide in the nucleic acid molecule.

[0012] In some examples, the images of the first surface and the axially-displaced second 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 images of the first surface and the axially-displaced second surface are acquired by just refocusing the optical

system. In some examples, the imaging system has a numerical aperture of greater than 0.3. In some examples, the first surface and axially-displaced second surface comprise two surfaces of a flow cell. In some examples, said two surfaces of the flow cell are coated with a hydrophilic coating layer, and wherein said hydrophilic coating layer further comprises labeled nucleic acid colonies disposed thereon at a surface density of $> 10,000$ nucleic acid colonies / mm^2 . In some examples, said 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 second surface that have been labeled with 1, 2, 3, or 4 distinct detectable labels. In some examples, 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. In some examples, 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. In some examples, the flow cell has a wall thickness of at least $700\ \mu\text{m}$ and a gap between the first interior surface and the second interior surface of at least $50\ \mu\text{m}$. In some examples, the optical system comprises two or more tube lenses which are designed to provide optimal imaging performance at two or more fluorescence wavelengths.

[0013] Disclosed herein are fluorescence imaging systems comprising: a) at least one light source configured to provide excitation light within one or more specified wavelength ranges; b) an objective lens configured to collect fluorescence arising from within a specified field-of-view of a sample plane upon exposure of the sample plane to the excitation light, wherein a numerical aperture of the objective lens is at least 0.3, wherein a working distance of the objective lens is at least $700\ \mu\text{m}$, and wherein the field-of-view has an area of at least $2\ \text{mm}^2$; and c) at least one image sensor, wherein the fluorescence collected by the objective lens is imaged onto the image sensor, and wherein a pixel dimension for the image sensor is chosen such that a spatial sampling frequency for the fluorescence imaging system is at least twice an optical resolution of the fluorescence imaging system.

[0014] In some examples, the numerical aperture is at least 0.75. In some examples, the numerical aperture is at least 1.0. In some examples, the working distance is at least $850\ \mu\text{m}$. In some examples, the working distance is at least $1,000\ \mu\text{m}$. In some examples, the field-of-view has an area of at least $2.5\ \text{mm}^2$. In some examples, the field-of-view has an area of at least $3\ \text{mm}^2$. In some examples, the spatial sampling frequency is at least 2.5 times the optical resolution of the fluorescence imaging system. In some examples, the spatial sampling frequency is at least 3 times the optical resolution of the fluorescence imaging system. In some

examples, the system further comprises an X-Y-Z translation stage such that the system is configured to acquire a series of two or more fluorescence images in an automated fashion, wherein each image of the series is acquired for a different field-of-view. In some examples, a position of the sample plane is simultaneously adjusted in an X direction, a Y direction, and a Z direction to match the position of an objective lens focal plane in between acquiring images for different fields-of-view. In some examples, the time required for the simultaneous adjustments in the X direction, Y direction, and Z direction is less than 0.4 seconds. In some examples, the system further comprises an autofocus mechanism configured to adjust the focal plane position prior to acquiring an image of a different field-of-view if an error signal indicates that a difference in the position of the focal plane and the sample plane in the Z direction is greater than a specified error threshold. In some examples, the specified error threshold is 100 nm. In some examples, the specified error threshold is 50 nm. In some examples, the system comprises three or more image sensors, and wherein the system is configured to image fluorescence in each of three or more wavelength ranges onto a different image sensor. In some examples, a difference in the position of a focal plane for each of the three or more image sensors and the sample plane is less than 100 nm. In some examples, a difference in the position of a focal plane for each of the three or more image sensors and the sample plane is less than 50 nm. In some examples, the total time required to reposition the sample plane, adjust focus if necessary, and acquire an image is less than 0.4 seconds per field-of-view. In some examples, the total time required to reposition the sample plane, adjust focus if necessary, and acquire an image is less than 0.3 seconds per field-of-view.

[0015] Also disclosed herein are fluorescence imaging systems for dual-side imaging of a flow cell comprising: a) an objective lens configured to collect fluorescence arising from within a specified field-of-view of a sample plane within the flow cell; b) at least one tube lens positioned between the objective lens and at least one image sensor, wherein the at least one tube lens is configured to correct an imaging performance metric for a combination of the objective lens, the at least one tube lens, and the at least one image sensor when imaging an interior surface of the flow cell, and wherein the flow cell has a wall thickness of at least 700 μm and a gap between an upper interior surface and a lower interior surface of at least 50 μm ; wherein the imaging performance metric is substantially the same for imaging the upper interior surface or the lower interior surface of the flow cell without moving an optical compensator into or out of an optical path between the flow cell and the at least one image sensor, without moving one or more optical elements of the tube lens along the optical path, and without moving one or more optical elements of the tube lens into or out of the optical path.

[0016] In some examples, the objective lens is a commercially-available microscope objective. In some examples, the commercially-available microscope objective has a numerical aperture of at least 0.3. In some examples, the objective lens has a working distance of at least 700 μm . In some examples, the objective lens is corrected to compensate for a cover slip thickness (or flow cell wall thickness) of 0.17 mm. In some examples, the fluorescence imaging system further comprising an electro-optical phase plate positioned adjacent to the objective lens and between the objective lens and the tube lens, wherein the electro-optical phase plate provides correction for optical aberrations caused by a fluid filling the gap between the upper interior surface and the lower interior surface of the flow cell. In some examples, the at least one tube lens is a compound lens comprising three or more optical components. In some examples, the at least one tube lens is a compound lens comprising four optical components. In some examples, the four optical components comprise, in order, a first asymmetric convex-convex lens, a second convex-plano lens, a third asymmetric concave-concave lens, and a fourth asymmetric convex-concave lens. In some examples, the at least one tube lens is configured to correct an imaging performance metric for a combination of the objective lens, the at least one tube lens, and the at least one image sensor when imaging an interior surface of a flow cell having a wall thickness of at least 1 mm. In some examples, the at least one tube lens is configured to correct an imaging performance metric for a combination of the objective lens, the at least one tube lens, and the at least one image sensor when imaging an interior surface of a flow cell having a gap of at least 100 μm . In some examples, the at least one tube lens is configured to correct an imaging performance metric for a combination of the objective lens, the at least one tube lens, and the at least one image sensor when imaging an interior surface of a flow cell having a gap of at least 200 μm . In some examples, the system comprises a single objective lens, two tube lenses, and two image sensors, and each of the two tube lenses is designed to provide optimal imaging performance at a different fluorescence wavelength. In some examples, the system comprises a single objective lens, three tube lenses, and three image sensors, and each of the three tube lenses is designed to provide optimal imaging performance at a different fluorescence wavelength. In some examples, the system comprises a single objective lens, four tube lenses, and four image sensors, and each of the four tube lenses is designed to provide optimal imaging performance at a different fluorescence wavelength. In some examples, the design of the objective lens or the at least one tube lens is configured to optimize the modulation transfer function in the mid to high spatial frequency range. In some examples, 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, contrast-to-noise ratio (CNR), or any combination thereof. In some

examples, the difference in the imaging performance metric for imaging the upper interior surface and the lower interior surface of the flow cell is less than 10%. In some examples, the difference in imaging performance metric for imaging the upper interior surface and the lower interior surface of the flow cell is less than 5%. In some examples, the use of the at least one tube lens provides for an at least equivalent or better improvement in the imaging performance metric for dual-side imaging compared to that for a conventional system comprising an objective lens, a motion-actuated compensator, and an image sensor. In some examples, the use of the at least one tube lens provides for an at least 10% improvement in the imaging performance metric for dual-side imaging compared to that for a conventional system comprising an objective lens, a motion-actuated compensator, and an image sensor.

[0017] Disclosed herein are illumination systems for use in imaging-based solid-phase genotyping and sequencing applications, the illumination system comprising: a) a light source; and b) a liquid light-guide configured to collect light emitted by the light source and deliver it to a specified field-of-illumination on a support surface comprising tethered biological macromolecules.

[0018] In some examples, the illumination system further comprises a condenser lens. In some examples, the specified field-of-illumination has an area of at least 2 mm². In some examples, the light delivered to the specified field-of-illumination is of uniform intensity across a specified field-of-view for an imaging system used to acquire images of the support surface. In some examples, the specified field-of-view has an area of at least 2 mm². In some examples, the light delivered to the specified field-of-illumination is of uniform intensity across the specified field-of-view when a coefficient of variation (CV) for light intensity is less than 10%. In some examples, the light delivered to the specified field-of-illumination is of uniform intensity across the specified field-of-view when a coefficient of variation (CV) for light intensity is less than 5%. In some examples, the light delivered to the specified field-of-illumination has a speckle contrast value of less than 0.1. In some examples, the light delivered to the specified field-of-illumination has a speckle contrast value of less than 0.05.

INCORPORATION BY REFERENCE

[0019] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference in its entirety. In the event of a conflict between a term herein and a term in an incorporated reference, the term herein controls.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0021] FIGS. 1A-1B schematically illustrate non-limiting examples of imaging dual surface support structures for presenting sample sites for imaging by the imaging systems disclosed herein. **FIG. 1A:** illustration of imaging front and rear interior surfaces of a flow cell. **FIG. 1B:** illustration of imaging front and rear exterior surfaces of a substrate.

[0022] FIGS. 2A-2B illustrate a non-limiting example of a multi-channel fluorescence imaging module comprising a dichroic beam splitter for transmitting an excitation light beam to a sample, and for receiving and redirecting by reflection the resultant fluorescence emission to

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 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 objective lens and said 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 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 images of the first surface and the 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 axially displaced second surface.
4. The method of claim 1, wherein the first surface and the axially displaced second surface comprise two surfaces of a flow cell.
5. The method of claim 4, wherein said two surfaces of the flow cell are coated with a hydrophilic coating layer.
6. The method of claim 5, wherein said hydrophilic coating layer further comprises labeled nucleic acid colonies disposed thereon at a surface density of $> 10,000 \text{ nucleic acid colonies} / \text{mm}^2$.
7. The method of claim 6, wherein an image of a surface of said two surfaces acquired using said 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.
8. The method of claim 1, wherein said 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 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 objective lens and 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 images of the first surface and 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 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 said nucleic acid molecule for sequencing, and then sequencing said 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 imaging 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 said 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 objective lens and said 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 images of the two distinct, 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 two distinct, axially displaced surfaces.

23. The imaging system of claim 19, wherein said two distinct, axially displaced surfaces comprise two surfaces of a flow cell.

24. The imaging system of claim 23, wherein said two distinct surfaces of the flow cell are coated with a hydrophilic coating layer, and wherein said hydrophilic coating layer further comprises labeled nucleic acid colonies disposed thereon at a surface density of $> 10,000$ nucleic acid colonies / mm^2 .

25. The imaging system of claim 19, wherein said imaging system comprises 1, 2, 3, or 4 imaging channels configured to detect nucleic acid colonies disposed on at least one of said two distinct surfaces 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 $700 \text{ }\mu\text{m}$ and a gap between the first interior surface and the second interior surface of at least $50 \text{ }\mu\text{m}$.

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 images of the two distinct, axially displaced surfaces are diffraction-limited across the entire field-of-view (FOV).