OPTICAL SYSTEMS FOR NUCLEIC ACID SEQUENCING AND METHODS THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/224,351, filed July 21, 2021, U.S. Provisional Application No. 63/334,613, filed April 25, 2022, and U.S. Provisional Application No. 63/334,609, filed April 25, 2022, 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, provided herein is a method of imaging a sample, said method comprising:

(a) providing an optical system, wherein the optical system comprises:

(1) a light source that illuminates said sample with a first light;

(2) a sensor that obtains an image of said sample while said sample is illuminated by said first light from said light source; and

(3) a focusing element assembly disposed along an optical path from said light source to said sensor, wherein said focusing element assembly comprises (i) a first focusing element, and (ii) a second focusing element, wherein said first focusing element moves relative to said second focusing element without removal of said first focusing element from said optical path;

(b) illuminating said sample with said first light from said light source;

(c) focusing a second light emitted from said sample with said focusing element assembly; and

(d) receiving said second light from (c) and obtaining one or more images of said sample by said sensor.

[0003] Aspects disclosed herein provide systems, comprising: a substrate comprising a curved surface, wherein said curved surface comprises at least one binding moiety configured to bind to an analyte; and an optical system comprising a light source, wherein said light source is configured to direct light to said curved surface and wherein said light is configured to probe a presence or absence of said analyte bound to said at least one binding moiety. In some embodiments, said analyte comprises a nucleic acid. In some embodiments, said at least one binding moiety comprises at least one nucleic acid configured to bind to said nucleic acid. In some embodiments, said curved surface is a component of a flow cell. In some embodiments, said

CLAIMS

WHAT IS CLAIMED IS:

1. A method of imaging a sample, said method comprising:

    (a) providing an optical system, wherein the optical system comprises:

        (1) a light source that illuminates said sample with a first light;

        (2) a sensor that obtains an image of said sample while said sample is illuminated by said first light from said light source; and

        (3) a focusing element assembly disposed along an optical path from said light source to said sensor, wherein said focusing element assembly comprises (i) a first focusing element, and (ii) a second focusing element, wherein said first focusing element moves relative to said second focusing element without removal of said first focusing element from said optical path;

    (b) illuminating said sample with said first light from said light source;

    (c) focusing a second light emitted from said sample with said focusing element assembly; and

    (d) receiving said second light from (c) and obtaining one or more images of said sample by said sensor.

2. The method of claim 1, wherein said optical system further comprises a plurality of said light sources, wherein two or more light sources of said plurality emits a different wavelength of said light.
3. The method of claim 1, wherein said optical system further comprises a plurality of sensors comprising said sensor, wherein two or more sensors of said plurality of sensors obtain said one or more images of said sample at different times.
4. The method of claim 1, wherein said optical system further comprises a filter disposed along said optical path from said light source to said sensor, wherein said filter is configured to receive said second light from said sample and transmit another light to said sensor.
5. The method of claim 4, wherein said filter comprises a multi-band filter.
6. The method of claim 5, wherein said multi-band filter comprises a tri-band stopband filter.
7. The method of claim 1, wherein said optical system further comprises a piezo drive coupled to said first focusing element, wherein said piezo drive controls movement of said first focusing element relative to said second focusing element.
8. The method of claim 1, wherein said optical system further comprises a housing, wherein said housing comprises said focusing element assembly.
9. The method of claim 1, wherein said first focusing element and said second focusing element each have a refractive index of about 1.5.

10. The method of claim 1, wherein said optical system further comprises a gap between said first focusing element and said second focusing element, wherein said gap has a width that remains constant when said first focusing element moves relative to said second focusing element.
11. The method of claim 1, wherein said focusing element assembly comprises a wedge block assembly, and wherein said first focusing element comprises a first wedge piece of said wedge block assembly and said second focusing element comprises a second wedge piece of said wedge block assembly.
12. The method of claim 1, wherein said sample comprises a plurality of biological polymers, wherein a first subset of said plurality of biological polymers is coupled to a first interior surface of a flow cell, and a second subset of said plurality of biological polymers is coupled to a second interior surface of said flow cell, and wherein said obtaining said one or more images of said sample comprises obtaining said one or more images of said first interior surface and said second interior surface of said flow cell.
13. The method of claim 12, wherein said obtaining said one or more images of said first interior surface comprises:
    (a) obtaining a first image of said first interior surface;
    (b) moving first focusing element relative to said second focusing element without removal of said first focusing element from said optical path, thereby adjusting a focal depth of said optical system to focus on said second interior surface; and
    (c) obtaining a second image of said second interior surface.
14. The method of claim 12, wherein said first interior surface and said second interior surface of said flow cell comprise a hydrophilic polymer layer coupled thereto.
15. The method of claim 14, wherein said plurality of biological polymers is coupled to said hydrophilic polymer layer.
16. The method of claim 1, wherein said optical system has a field-of-view (FOV) of greater than 1 square millimeter (mm^2).
17. The method of claim 16, wherein said optical system has a field-of-view (FOV) of greater than 2.5 mm^2.
18. The method of claim 1, wherein said optical system comprises a numerical aperture (NA) that is less than 0.6.
19. The method of claim 1, wherein said first focusing element and said second focusing element comprise fused silica.
20. The method of claim 1, where said optical system is a fluorescence imaging system.