Claims

1. An imaging system comprising:

an analysis substrate comprising:

a localization layer to be provided with a sample comprising a nucleotide provided with a fluorescent dye; and

a sensor layer, the localization layer being on-chip relative to the sensor layer; and

imaging circuitry to image the sample, the imaging circuitry comprising:

an array of sensor pixels at the sensor layer, one or more of the array of sensor pixels to receive a propagation of fluorescence from the fluorescent dye; and

fitting circuitry to identify, using a signal of the sensor layer, a pixel intensity distribution due to a point spread function, and determine a centroid of the pixel intensity distribution by fitting a function to the pixel intensity distribution.

2. The imaging system of claim 1, wherein the analysis substrate is part of a flowcell.

3. The imaging system of any of claims 1 or 2, wherein the localization layer comprises nanowells.

4. The imaging system of any of claims 1 or 2, wherein the localization layer comprises a zero-mode waveguide.

5. The imaging system of any of claims 1 to 4, wherein the localization layer comprises a surface passivation layer.

6. The imaging system of any of claims 1 to 5, wherein the sensor layer comprises a complementary metal-oxide-semiconductor chip.

7. The imaging system of any of claims 1 to 6, wherein the array of sensor pixels comprises an array of photodiodes.

8. The imaging system of any of claims 1 to 7, wherein the sensor layer comprises a charge-coupled device.

9. The imaging system of any of claims 1 to 8, wherein the analysis substrate further comprises a spacer layer between the localization layer and the sensor layer, the spacer layer having a thickness based on the propagation of fluorescence from the fluorescent dye.

10. The imaging system of claim 9, wherein the spacer layer comprises a planar waveguide.

11. The imaging system of any of claims 9 to 10, wherein the thickness of the

spacer layer corresponds to the propagation of fluorescence from the fluorescent dye being received by a predefined proportion of the array of sensor pixels.

12. The imaging system of any of claims 9 to 11, wherein the thickness of the spacer layer is selected based on a predefined full width at half maximum of the propagation of fluorescence from the fluorescent dye.

13. The imaging system of any of claims 1 to 12, wherein the imaging circuitry further comprises restriction circuitry restricting the fitting circuitry to fit the function to the pixel intensity distribution only in at least one area indicated by a well distribution pattern.

14. The imaging system of claim 13, wherein the imaging circuitry further comprises rejection circuitry to reject at least one localization outside the area indicated by the well distribution pattern.

15. The imaging system of any of claims 1 to 14, wherein the sensor layer provides a single field of view of the localization layer.

16. The imaging system of any of claims 1 to 15, further comprising a filter layer between the localization layer and the sensor layer, the filter layer including at least one color filter.

17. The imaging system of any of claims 1 to 16, wherein the analysis substrate does not have a filter between the localization layer and the sensor layer, and wherein the analysis substrate does not have a waveguide between the localization layer and the sensor layer.

18. The imaging system of any of claims 1 to 17, further comprising:

an illumination light source;

illumination light timing circuitry to generate discrete light pulses using the illumination light source; and

image sensor timing circuitry to time-gate the array of sensor pixels based on the discrete light pulses of the illumination light.

19. An analysis substrate comprising:

a localization layer to be provided with a sample comprising a nucleotide provided with a fluorescent dye;

a sensor layer comprising an array of sensor pixels, the localization layer being on-chip relative to the sensor layer, one or more of the array of sensor pixels to receive a propagation of fluorescence from the fluorescent dye; and

a spacer layer between the localization layer and the sensor layer, the spacer layer having a thickness based on the propagation of fluorescence from the fluorescent dye.

20. The analysis substrate of claim 19, wherein the analysis substrate is part of a flowcell.

21. The analysis substrate of any of claims 19 or 20, wherein the localization layer comprises nanowells.

22. The analysis substrate of any of claims 19 or 20, wherein the localization layer comprises a zero-mode waveguide.

23. The analysis substrate of any of claims 19 to 22, wherein the localization layer comprises a surface passivation layer.

24. The analysis substrate of any of claims 19 to 23, wherein the sensor layer comprises a complementary metal-oxide-semiconductor chip.

25. The analysis substrate of any of claims 19 to 23, wherein the array of sensor pixels comprises an array of photodiodes.

26. The analysis substrate of any of claims 19 to 23, wherein the sensor layer comprises a charge-coupled device.

27. The analysis substrate of any of claims 19 to26, wherein the spacer layer comprises a planar waveguide.

28. The analysis substrate of any of claims 19 to 27, wherein the thickness of the spacer layer corresponds to the propagation of fluorescence from the fluorescent dye being received by a predefined proportion of the array of sensor pixels.

29. The analysis substrate of any of claims 19 to 28, wherein the thickness of the spacer layer is selected based on a predefined full width at half maximum of the propagation of fluorescence from the fluorescent dye.

30. The analysis substrate of any of claims 19 to 29, wherein the sensor layer provides a single field of view of the localization layer.

31. The analysis substrate of any of claims 19 to 30, further comprising a filter layer between the localization layer and the sensor layer, the filter layer including at least one color filter.

32. The analysis substrate of any of claims 19 to 31, wherein the analysis substrate does not have a filter between the localization layer and the sensor layer, and wherein the analysis substrate does not have a waveguide between the localization layer and the sensor layer.