

limited available high-throughput and sensitive methods to detect these events without the need of DNA amplification, which amplification could conceal those modifications. Accordingly, there is a need in the art for methods and systems for detection of polynucleotide damage.

SUMMARY

[0006] In meeting the described challenges, the present disclosure first provides methods, the methods including converting a first site on a polynucleotide to a first moiety capable of supporting polymerase extension; effecting extension at the first moiety so as to incorporate a first label at or proximate to the first site; linearizing a portion of the polynucleotide that includes the first label; and imaging the first label.

[0007] The disclosure also provides analysis systems, the systems suitably including a sample stage configured to receive a fluidic chip that comprises one or more nanochannels having a characteristic dimension in the range of from 1 nm to about 250 nm, an illumination source configured to illuminate a sample disposed within the fluidic chip; and an image collector configured to collect an image of an illuminated sample disposed within the fluidic chip. In one aspect, the present disclosure refers to an analysis system, comprising:

- a sample stage configured to receive a fluidic chip, wherein the fluidic chip comprises one or more nanochannels for passing through one or more polynucleotides that are optically labeled at or proximate to one or more sites of damage, wherein the one or more nanochannels have at least one dimension in a range of from about 1 nm to about 250 nm,

- two or more illumination sources configured to illuminate a sample disposed within the fluidic chip, wherein each of the two or more illumination sources is configured to provide a different illumination wavelength; and

- an image collector configured to collect one or more images of an illuminated sample disposed within the fluidic chip,

- wherein the analysis system is configured to assess the presence, type, and extent of one or more damages that are present on the one or more polynucleotides, and wherein the damage is caused by one or more of ultraviolet radiation, ionizing radiation, and oxidation.

[0007a] In another aspect, the present disclosure refers to a method for analyzing a polynucleotide sample, comprising:

- labeling multiple polynucleotides in the sample with at least two types of optically-detectable labels, wherein at least one of the optically-detectable labels is incorporated at or proximate to one or more sites of damage on the polynucleotides, and wherein the damage is caused by one or more of ultraviolet radiation, ionizing radiation, and oxidation;

- loading the labeled sample into a fluidic chip;

- moving at least a portion of the labeled polynucleotides into a plurality of nanochannels in the fluidic chip and maintaining the labeled polynucleotides in the nanochannels in a linearized form;

- illuminating the sample with a first light source and then imaging the labeled polynucleotides in the nanochannels;

illuminating the sample with a second light source and then imaging the labeled polynucleotides in the nanochannels; and

correlating the presence or location of the labels to assess the presence, type, and extent of one or more damages that are present on the polynucleotides.

[0008] Also provided are methods, the methods including contacting a first single-strand break in a polynucleotide with an alkaline phosphatase so as to give rise to a first moiety capable of supporting polymerase extension; contacting the moiety with a polymerase and a labeled nucleotide so as to incorporate a label into the polyoligonucleotide; linearizing at least a portion of the polynucleotide by confining the first label within a nanochannel; and imaging the first label.

[0009] Additionally provided are methods, the methods including applying to a single-strand break in a polynucleotide a DNA polymerase having 3' to 5' exonuclease activity so as to convert the non-extendable single strand break into a polymerase-extendable sites; and applying a DNA polymerase and a labeled deoxynucleotide so as to incorporate a label into the polynucleotide.

[0010] The disclosure also provides methods, the methods including disposing a polynucleotide having an abasic site within a porous matrix material; contacting the polynucleotide with an alkaline material so as to convert the abasic site to a single strand break in the polynucleotide, so as to convert a single strand break in the polynucleotide to a double strand break in the polynucleotide, or both; converting a single strand break in the polynucleotide, a double strand break in the polynucleotide, or both, to a moiety capable of

What is claimed is:

1. An analysis system, comprising:
a sample stage configured to receive a fluidic chip, wherein the fluidic chip comprises one or more nanochannels for passing through one or more polynucleotides that are optically labeled at or proximate to one or more sites of damage, wherein the one or more nanochannels have at least one dimension in a range of from about 1 nm to about 250 nm,

two or more illumination sources configured to illuminate a sample disposed within the fluidic chip, wherein each of the two or more illumination sources is configured to provide a different illumination wavelength; and

an image collector configured to collect one or more images of an illuminated sample disposed within the fluidic chip,

wherein the analysis system is configured to assess the presence, type, and extent of one or more damages that are present on the one or more polynucleotides, and wherein the damage is caused by one or more of ultraviolet radiation, ionizing radiation, and oxidation.
2. The system of claim 1, further comprising a detector capable of detecting positions of one or more beams of illumination reflected from the sample disposed within the fluidic chip.
3. The system of claim 1, further comprising a controller configured to translate the sample stage in response to a position of a beam of illumination reflected from the fluidic chip.
4. The system of claim 1, further comprising at least one filter capable of being disposed in an illumination path so as to provide a desired illumination wavelength to the sample disposed within the fluidic chip.
5. The system of claim 1, further comprising a beam expander disposed in an illumination path between the illumination source and the sample.
6. The system of claim 1, wherein the system comprises a source of electric field configured to move one or more macromolecules into nanochannels of the fluidic chip.
7. The system of claim 1, further comprising a reader configured to recognize the system in accordance with one or more indicia disposed on the fluidic chip.
8. The system of claim 1, wherein the system is configured to utilize wide-field illumination to achieve single molecule fluorescent detection in the nanochannel.
9. The system of claim 1, wherein the illumination source is selected from the group

consisting of laser, light emitting diode, incandescent bulb, ultraviolet source, or any combinations thereof.

10. The system of claim 1, wherein the system includes an integrated autofocus unit configured to maintain automated optical focus of the sample.

11. The system of claim 10, wherein the integrated autofocus unit comprises at least one additional illumination source, wherein the additional illumination source shares an optical path of other illumination sources and is aligned to accommodate focus tracking of a nanostructured imaging surface.

12. The system of claim 1, wherein the system is partially or fully automated.

13. The system of claim 1, further comprising an evaporation control system for mitigating or controlling sample reservoir evaporation.

14. The system of claim 1, wherein the system is capable of determining one or more structural characteristics of a macromolecule.

15. The system of claim 1, wherein the system is capable of correlating the one or more structural characteristics of the polynucleotide to whether a damage has occurred within a particular location within at least one of the one or more polynucleotides.

16. A method for analyzing a polynucleotide sample, comprising:

labeling multiple polynucleotides in the sample with at least two types of optically-detectable labels, wherein at least one of the optically-detectable labels is incorporated at or proximate to one or more sites of damage on the polynucleotides, and wherein the damage is caused by one or more of ultraviolet radiation, ionizing radiation, and oxidation;

loading the labeled sample into a fluidic chip;

moving at least a portion of the labeled polynucleotides into a plurality of nanochannels in the fluidic chip and maintaining the labeled polynucleotides in the nanochannels in a linearized form;

illuminating the sample with a first light source and then imaging the labeled polynucleotides in the nanochannels;

illuminating the sample with a second light source and then imaging the labeled polynucleotides in the nanochannels; and

correlating the presence or location of the labels to assess the presence, type, and extent of one or more damages that are present on the polynucleotides.

17. The method of claim 16, further comprising autofocusing on the sample disposed within the nanochannels by automatically finding a primary focus position and then tracking that focus position once found.

18. The method of claim 16, further comprising recording one or more images of the sample.

19. The method of claim 16, comprising labeling a backbone of the polynucleotides with a first label and labeling a plurality of sites of the polynucleotide with a second label in a site-specific manner.

20. The method of claim 16, further comprising characterizing at least one structure feature of the sample based on locations of the labels on the polynucleotides.

21. The method of claim 16, further comprising preventing or mitigating evaporation of the sample after loading.

22. The method of claim 16, further comprising using wide-field illumination for imaging the sample.

23. The method of claim 16, further comprising converting the site of damage to an intermediate capable of supporting polymerase extension.