likelihood of non-uniform illumination. Another common problem during imaging (irrespective of imaging mode) is image non-uniformity. For example, when identical samples are placed at different locations of an imaging surface or a field of view, the corresponding image appears to be non-uniform based on the location, even though the identical samples emits identical signal. There is a need in the art to address image non-uniformity.

SUMMARY

[0005] According to a first aspect of the present disclosure, there is provided an illumination system, including: a surface configured to have an imaging target placed thereon; a light source configured to emit a beam of light; a beam splitter configured to split the beam of light from the light source into a first beam and a second beam; a first mirror configured to reflect the first beam to provide a reflected first beam that illuminates the surface; and a second mirror configured to reflect the second beam to provide a reflected second beam that illuminates the surface, wherein the reflected first beam and the reflected second beam provide off-axis illumination of the surface; and wherein the reflected first beam and the reflected second beam provide substantially symmetrical illumination of the surface.

[0006] Disclosed herein is an illumination system that includes a surface, a light source, a beam splitter, and a first mirror. The surface is configured to have an imaging target placed thereon. The light source is configured to emit a beam of light. The beam splitter is configured to split the beam of light from the light source into a first beam and a second beam. The second beam illuminates the surface. The first mirror is configured to reflect the first beam from the beam splitter to provide a reflected first beam that illuminates the surface.

[0007] According to a second aspect of the present disclosure, there is provided an illumination method, including: providing a surface with an imaging target placed thereon; providing a beam of light with a light source; splitting the beam of light into a first beam and a second beam; and illuminating the surface, wherein illuminating comprises: (i) using a first mirror to reflect the first beam to produce a reflected first beam that illuminates the surface, and (ii) using a second mirror to reflect the second beam to produce a reflected second beam that illuminates the surface, wherein the reflected first beam and the reflected second beam provide off-axis illumination of

the surface; and wherein the reflected first beam and the reflected second beam provide substantially symmetrical illumination of the surface.

[0008] Disclosed herein is an illumination method that includes providing a beam of light with a light source. The method also includes splitting the beam of light into a first beam and a second beam. The method further includes illuminating a surface with an imaging target placed thereon. Illuminating includes using a first mirror to reflect the first beam to produce a reflected first beam that illuminates the surface. The second beam is split from the beam of light such that it illuminates the surface.

[0009] An imaging system is also disclosed. The imaging system includes an imaging surface, a mirror, and a capturing device. The imaging surface is configured to have an imaging target placed thereon. The capturing device is configured to capture an image of the imaging target through a path of emitted light that extends from the imaging target, reflects off of the mirror, and to the capturing device. The mirror, the capturing device, or both are configured to move in a diagonal direction with respect to the imaging surface to reduce a length of the path of emitted light.

[0010] Disclosed herein is an imaging system that includes an imaging surface, a mirror, a mirror shaft, a capturing device, a capturing device shaft, and a transmission block. The imaging surface is configured to have an imaging target placed thereon. The mirror is configured to move in a first diagonal direction along the mirror shaft. The capturing device is configured to capture an image of the imaging target through a path of emitted light that extends from the imaging target, reflects off of the mirror, and to the capturing device. The capturing device is configured to move in a second diagonal direction along the capturing device shaft. The transmission block transmits movement between the mirror and the capturing device, thereby causing the mirror and the capturing device to move simultaneously.

[0011] An imaging method is also disclosed. The method includes placing an imaging target on an imaging surface. The method also includes causing a capturing device, a mirror, or both to move in a diagonal direction with respect to the imaging surface. The method further includes capturing an image of the imaging target, using the capturing device, through a path of emitted light that extends from the imaging target, reflects off of the mirror, and to the capturing device.

[0012] An illumination and imaging system is also disclosed. The system includes a surface configured to have an imaging target placed thereon. A light source is configured to emit a beam of light. A beam splitter is configured to split the beam of light from the light source into a first beam and a second beam. A first illumination mirror is configured to reflect the first beam to provide a reflected first beam that illuminates the surface. A second illumination mirror is configured to reflect the second beam to provide a reflected second beam that illuminates the surface. A capturing device is configured to capture an image of the imaging target through a path that extends from the imaging target, reflects off of an emission mirror, and to the capturing device. The emission mirror, the capturing device, or both are configured to move in a diagonal direction with respect to the surface to reduce a length of the path.

[0013] An illumination and imaging method is also disclosed. The method includes placing an imaging target on a surface. The method also includes emitting a beam of light from a light source. The method further includes splitting the beam of light into a first beam and a second beam. The method further includes illuminating the imaging target. Illuminating includes: (i) using a first illumination mirror to reflect the first beam to produce a reflected first beam that illuminates the surface, and (ii) using a second illumination mirror to reflect the second beam to produce a reflected second beam that illuminates the surface. The method further includes capturing an image of the imaging target, using a capturing device, through a path that extends from the imaging target, reflects off of an emission mirror, and to the capturing device.

[0014] The disclosure, in some embodiments, describes methods for generating an image corrected for a non-uniformity. In some embodiments, a non-uniformity is displayed as images with signals of varying intensity for an identical signal measured at different locations on the field of view.

[0015] A non-uniformity correction method of the present disclosure can be applied to images obtained from a variety of samples, including biological samples that comprise biological molecules such as proteins, peptides, glycoproteins, modified proteins, nucleic acids, DNA, RNA, carbohydrates, lipids, lipidoglycans, biopolymers and other metabolites generated from cells and tissues and combinations thereof. A bimolecule or biological sample having a biomolecule can be imaged alone or can be imaged while it is dispersed, located or embedded in a membrane, a gel, a filter paper, slide glass, microplate, or a matrix, such as a polyacrylamide

gel or nitrocellulose or PDVF membrane blot, an agarose gel, an agar plate, a cell culture plate or a tissue section slide. A non-uniformity correction method of the present disclosure can be applied to images obtained from any of the samples described above.

[0016] A non-uniformity correction method of the present disclosure can be applied to an image generated by a chemiluminescence change to a biological sample or to an image generated by a fluorescence change to the sample. A non-uniformity correction method of the present disclosure can be applied to an image generated by bioluminescent imaging, transillumination or reflective light imaging.

[0017] Disclosed herein is a method for generating an image corrected for a non-uniformity including: calculating a relative illumination of an imaging lens for a plurality of pixels on an imaging sensor; generating a flat fielding matrix based upon the relative illumination; capturing or acquiring an image of one or more biological samples, wherein the image has a non-uniformity; and adjusting the captured image with the flat fielding matrix to generate an image corrected for the non-uniformity.

[0018] In the above disclosure, generating a flat fielding matrix comprises inverting relative illumination to generate a flat fielding matrix. In some embodiments, relative illumination is calculated using an equation obtained by a linear or a non-linear curve fitting regression. The curve can be a first degree polynomial, a second degree polynomial, a third degree polynomial, or the like. Calculations of flat fielding matrix generate a flat fielding matrix value.

[0019] Adjusting the captured image with the flat fielding matrix comprises multiplying the captured or acquired image of one or more biological samples by the value of flat fielding matrix. In some embodiments, adjusting the captured or acquired image with the flat fielding matrix further comprises multiplying the captured or acquired image of the one or more biological samples by the value of the flat fielding matrix on a pixel-to-pixel basis to generate a flat fielded image. In some embodiments, the flat fielded image displays a correct ratio of a signal level of each captured or acquired image of the one or more biological sample irrespective of its location on a field of view.

[0020] Disclosed herein is a method for generating an image corrected for a non-uniformity, including: calculating a relative illumination of an imaging lens of a plurality of pixels on an

imaging sensor; inverting the relative illumination to generate a flat fielding matrix; capturing or acquiring an image of a biological sample; and multiplying the captured or acquired image of a biological sample by the value of the flat fielding matrix on a pixel-to-pixel basis to generate a flat fielded image.

[0021] Disclosed herein are methods for generating an image corrected for a non-uniformity, including: capturing or acquiring an image of one or more biological samples, wherein the image has a non-uniformity; and adjusting the captured or acquired image with a flat fielding matrix to generate an image corrected for the non-uniformity. Adjusting the captured or acquired image can comprise multiplying the captured image of the one or more biological samples by the value of the flat fielding matrix on a pixel-to-pixel basis to generate a flat fielded image.

[0022] In the above disclosure, the flat fielding matrix is in an imager or imaging system. The flat fielding matrix is available to a user using the imager. The flat fielding matrix value can be stored in an imaging device. In some embodiments, the flat fielding matrix value can be stored in a software component or computer component of the imaging device. The flat fielding matrix value is available to a user using the imaging system performing the non-uniformity correction. In some embodiments, the flat fielding matrix is a flat fielding master matrix.

[0023] Disclosed herein is a method for generating a flat fielding matrix for correcting images for a non-uniformity, comprises: calculating a relative illumination of an imaging lens for a plurality of pixels on an imaging sensor; generating a flat fielding matrix based upon the relative illumination and normalizing flat fielding matrix based on the maximum pixel intensity value in the matrix. In one embodiment, generating the flat fielding matrix comprises inverting the relative illumination and normalization of the values in the matrix. For example, a manufacturer or a user can generate a flat fielding matrix for future use.

[0024] Additional aspects and advantages of the disclosure will be set forth in part in the description which follows, and in part will be obvious from the description, or can be learned by practice of the disclosure. The aspects and advantages of the disclosure will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

CLAIMS

1. An illumination system, comprising:

a surface configured to have an imaging target placed thereon;

a light source configured to emit a beam of light;

a beam splitter configured to split the beam of light from the light source into a first beam and a second beam;

a first mirror configured to reflect the first beam to provide a reflected first beam that illuminates the surface; and

a second mirror configured to reflect the second beam to provide a reflected second beam that illuminates the surface,

wherein the reflected first beam and the reflected second beam provide off-axis illumination of the surface; and

wherein the reflected first beam and the reflected second beam provide substantially symmetrical illumination of the surface.

2. The illumination system of claim 1, wherein the beam of light has a beam of light optical power, the first beam has a first beam optical power and the second beam has a second beam optical power, and wherein the first beam optical power and the second beam optical power are each at least 40% of the beam of the light optical power.

3. The illumination system of claim 2, wherein the first beam optical power and the second beam optical power are each at least 45% of the beam of the light optical power.

4. The illumination system of claim 3, wherein the first beam optical power and the second beam optical power are substantially equal.

5. The illumination system of claim 1, further comprising a third mirror, wherein the reflected first beam or the reflected second beam is configured to reflect off of the third mirror prior to illuminating the surface.

6. The illumination system of claim 1, wherein the beam splitter is configured to split the beam of light from the light source into the first beam, the second beam, and a third beam.

7. The illumination system of claim 1, wherein the beam splitter comprises a prism, a plate, a dielectric mirror, a metal coated mirror, a beam splitter cube, a fiber optic beam splitter, or optical fibers configured to collimate light into a bundle before producing two or more output beams.

8. The illumination system of claim 1, wherein the first beam reflects off of the beam splitter, and wherein the second beam passes through the beam splitter.

9. The illumination system of claim 1, further comprising a second beam splitter configured to split the reflected first beam into two reflected beams that provide different degrees of off-axis illumination of the surface.

10. The illumination system of claim 9, further comprising a third beam splitter configured to split the reflected second beam into two reflected beams that provide different degrees of off-axis illumination of the surface.

11. The illumination system of claim 1, wherein an angle between a center of the first beam and a center of the second beam is from 62° to 68°.

12. The illumination system of claim 1, wherein an angle between a center of the reflected first beam and a center of the reflected second beam is from 106° to 114°.

13. The illumination system of claim 1, wherein a first distance from the beam splitter to the first mirror to the surface is substantially equal to a second distance from the beam splitter to the second mirror to the surface.

14. An illumination method, comprising:

providing a surface with an imaging target placed thereon;

providing a beam of light with a light source;

splitting the beam of light into a first beam and a second beam; and

illuminating the surface, wherein illuminating comprises: (i) using a first mirror to reflect the first beam to produce a reflected first beam that illuminates the surface, and (ii) using a second mirror to reflect the second beam to produce a reflected second beam that illuminates the surface,

wherein the reflected first beam and the reflected second beam provide off-axis illumination of the surface; and

wherein the reflected first beam and the reflected second beam provide substantially symmetrical illumination of the surface.

15. The method of claim 14, wherein the beam of light has a beam of light optical power, the first beam has a first beam optical power and the second beam has a second beam optical power, and wherein the first beam optical power and the second beam optical power are each at least 40% of the beam of the light optical power.