

CLAIMS

1. A method to improve performance of a scanner detecting florescence of millions of samples distributed across a flow cell, in images collected over multiple cycles, the samples positioned closer together than an Abbe diffraction limit for optical resolution between at least some adjoining pairs of the samples, including:
calibrating the scanner using substantially a full field of view projected by a lens onto an optical sensor in the scanner, including:
 - capturing at least one image tile under a structured illumination at multiple angles and phase displacements of the structured illumination;
 - calculating optical distortion across the image tile, including measuring spacing between intensity peaks and angle of the intensity peaks in at least 9 subtiles of the image tile, including a near-center subtile, and fitting a spacing surface and an angle surface to the measured spacing and angle, respectively, wherein the spacing surface and the angle surface both express corrections for distortions across the subtiles of the image tile, and saving fitting results; and
 - measuring phase displacements of the structured illumination at the multiple angles and phase displacements within the subtiles and saving a look-up table that expresses differences between the phase displacement in the near-center subtile and the phase displacements in other subtiles of the image tile;processing captured image tiles covering positions across the flow cell, captured at the multiple angles and phase displacements of the structured illumination, over the multiple cycles, including:
 - combining the saved fitting results and the saved look-up table with measurements, for a near-center subtile of each captured image tile, of the spacing between intensity peaks, the angle of the intensity peaks, and the phase displacements of the structured illumination, to determine reconstruction parameters for each captured image tile, on a per-subtile basis;
 - producing enhanced resolution images for the subtiles at the positions across the flow cell, over the multiple cycles, with resolving power better than the Abbe diffraction limit; andusing the enhanced resolution images to sequence the samples over multiple cycles.
2. The method of claim 1, wherein the samples are distributed in millions of nanowells across the flow cell and at least some adjoining pairs of nanowells are positioned closer together than an Abbe diffraction limit for optical resolution.
3. The method of any of claims 1-2, wherein the subtiles each include at least 512 by 512 pixels of the optical sensor.
4. The method of any of claims 1-3, wherein the subtiles overlap by at least 2 pixels of the optical sensor.

5. The method of any of claims 1-4, further including saving the fitting results of the spacing surface and the angle surface as coefficients of a quadratic surface.
6. The method of any of claims 1-4, further including saving the fitting results of the spacing surface and the angle surface as coefficients of a cubic surface.
7. The method of any of claims 1-4, further including saving the fitting results in a look-up table calculated from quadratic fits of the spacing surface and the angle surface.
8. The method of any of claims 1-4, further including saving the fitting results in a look-up table calculated from cubic fits of the spacing surface and the angle surface.
9. The method of any of claims 1-8, further including, at an intermediate cycle among the multiple cycles, re-measuring the spacing between intensity peaks and angle of the intensity peaks in the subtiles, refitting the spacing surface and the angle surface, and saving results of the refitting for use in processing subsequently captured image tiles.
10. The method of claim 9, wherein the intermediate cycle is a 50th cycle or later among at least 100 cycles.
11. The method of claim 2, wherein the nanowells are arranged in a repeating rectangular pattern with two diagonals connecting opposing corners of a rectangle in the pattern, further including using two structured illumination angles at which the intensity peaks are oriented substantial normal to the two diagonals.
12. The method of claim 2, wherein the nanowells are arranged in a repeating hexagonal pattern with three diagonals connecting opposing corners of a hexagon in the pattern, further including using three structured illumination angles at which the intensity peaks are oriented substantial normal to the three diagonals.
13. The method of any of claims 1-12, further including aggregating the enhanced resolution images for the subtiles into an enhance resolution image for the tile and using the enhanced resolution image tile to sequence the samples over the multiple cycles.
14. The method of any of claims 1-13, further including applying a cropping margin to eliminate pixels around edges of the sensor from use in fitting the spacing surface and the angle surface.
15. The method of any of claims 1-14, further including measuring spacing between intensity peaks and angle of the intensity peaks over at least 8 by 11 subtiles of the image tile.
16. A computer readable medium impressed with program instructions that, when executed on one or more processors, are configurable to carry out a method to improve performance of a scanner detecting

fluorescence of millions of samples distributed across a flow cell, in images collected over multiple cycles, the samples positioned closer together than an Abbe diffraction limit for optical resolution between at least some adjoining pairs of the samples, including:

calibrating the scanner using substantially a full field of view projected by a lens onto an optical sensor in the scanner, including:

capturing at least one image tile under a structured illumination at multiple angles and phase displacements of the structured illumination;

calculating optical distortion across the image tile, including measuring spacing between intensity peaks and angle of the intensity peaks in at least 9 subtiles of the image tile, including a near-center subtile, and fitting a spacing surface and an angle surface to the measured spacing and angle, respectively, wherein the spacing surface and the angle surface both express corrections for distortions across the subtiles of the image tile, and saving fitting results; and

measuring phase displacements of the structured illumination at the multiple angles and phase displacements within the subtiles and saving a look-up table that expresses differences between the phase displacement in the near-center subtile and the phase displacements in other subtiles of the image tile;

processing captured image tiles covering positions across the flow cell, captured at the multiple angles and phase displacements of the structured illumination, over the multiple cycles, including:

combining the saved fitting results and the saved look-up table with measurements, for a near-center subtile of each captured image tile, of the spacing between intensity peaks, the angle of the intensity peaks, and the phase displacements of the structured illumination, to determine reconstruction parameters for each captured image tile, on a per-subtile basis;

producing enhanced resolution images for the subtiles at the positions across the flow cell, over the multiple cycles, with resolving power better than the Abbe diffraction limit; and

using the enhanced resolution images to sequence the samples over multiple cycles.

17. The computer readable medium of claim 16, wherein the samples are distributed in millions of nanowells across the flow cell and at least some adjoining pairs of nanowells are positioned closer together than an Abbe diffraction limit for optical resolution.

18. The computer readable medium of any of claims 16-17, wherein the subtiles each include at least 512 by 512 pixels of the optical sensor.

19. The computer readable medium of any of claims 16-18, wherein the subtiles overlap by at least 2 pixels of the optical sensor.

20. The computer readable medium of any of claims 16-19, further impressed with program instructions, the method further including saving the fitting results of the spacing surface and the angle surface as coefficients of a quadratic surface.
21. The computer readable medium of any of claims 16-19, further impressed with program instructions, the method further including saving the fitting results of the spacing surface and the angle surface as coefficients of a cubic surface.
22. The computer readable medium of any of claims 16-19, further impressed with program instructions, the method further including saving the fitting results in a look-up table calculated from quadratic fits of the spacing surface and the angle surface.
23. The computer readable medium of any of claims 16-19, further impressed with program instructions, the method further including saving the fitting results in a look-up table calculated from cubic fits of the spacing surface and the angle surface.
24. The computer readable medium of any of claims 16-23, further impressed with program instructions, the method further including, at an intermediate cycle among the multiple cycles, re-measuring the spacing between intensity peaks and angle of the intensity peaks in the subtiles, refitting the spacing surface and the angle surface, and saving results of the refitting for use in processing subsequently captured image tiles.
25. The computer readable medium of claim 24, wherein the intermediate cycle is a 50th cycle or later among at least 100 cycles.
26. The computer readable medium of claim 17, wherein the nanowells are arranged in a repeating rectangular pattern with two diagonals connecting opposing corners of a rectangle in the pattern, further impressed with program instructions, the method further including using two structured illumination angles at which the intensity peaks are oriented substantial normal to the two diagonals.
27. The computer readable medium of claim 17, wherein the nanowells are arranged in a repeating hexagonal pattern with three diagonals connecting opposing corners of a hexagon in the pattern, further impressed with program instructions, the method further including using three structured illumination angles at which the intensity peaks are oriented substantial normal to the three diagonals.
28. The computer readable medium of any of claims 16-27, further impressed with program instructions, the method further including aggregating the enhanced resolution images for the subtiles into an enhance resolution image for the tile and using the enhanced resolution image tile to sequence the samples over the multiple cycles.

29. The computer readable medium of any of claims 16-28, further impressed with program instructions, the method further including applying a cropping margin to eliminate pixels around edges of the sensor from use in fitting the spacing surface and the angle surface.

30. The computer readable medium of any of claims 16-29, further impressed with program instructions, the method further including measuring spacing between intensity peaks and angle of the intensity peaks over at least 8 by 11 subtiles of the image tile.

31. A method of generating subtile calibration parameters, used with a scanner to produce enhanced resolution images of florescence of samples positioned closer together than an Abbe diffraction limit for optical resolution between at least some adjoining pairs of the samples, including:
calibrating the scanner using substantially a full field of view projected by a lens onto an optical sensor in the scanner, including:

- capturing at least one image tile under a structured illumination at multiple angles and phase displacements of the structured illumination;

- calculating optical distortion across the image tile, including

- measuring spacing between intensity peaks and angle of the intensity peaks in at least 9 subtiles of the image tile, including a near-center subtile, and

- fitting a spacing surface and an angle surface to the measured spacing and angle, respectively, wherein the spacing surface and the angle surface both express corrections for distortions across the subtiles of the image tile; and

- measuring phase displacements of the structured illumination at the multiple angles and phase displacements within the subtiles and generating a look-up table that expresses differences between the phase displacement in the near-center subtile and the phase displacements in other subtiles of the image tile;

saving the subtile calibration parameters, including the fitting results that relate the spacing of the intensity peaks and the angle of the intensity peaks across the at least 9 subtiles to the near-center subtile, and the look-up table of the differences in the phase displacement across the at least 9 subtiles, relative to the near-center subtile, for use by the scanner in production.

32. The method of claim 31, wherein the samples include millions of samples distributed across a flow cell, in images collected over multiple cycles.

33. The method of any of claims 31-32, wherein the subtiles each include at least 512 by 512 pixels of the optical sensor.

34. The method of any of claims 31-33, wherein the subtiles overlap by at least 2 pixels of the optical sensor.

35. The method of any of claims 31-34, further including saving results of the fitting of the spacing surface and the angle surface as coefficients of a quadratic surface.
36. The method of any of claims 31-34, further including saving results of the fitting of the spacing surface and the angle surface as coefficients of a cubic surface.
37. The method of any of claims 31-34, further including saving results of the fitting in a look-up table calculated from quadratic fitting of the spacing surface and the angle surface.
38. The method of any of claims 31-34, further including saving results of the fitting in a look-up table calculated from cubic fitting of the spacing surface and the angle surface.
39. The method of any of claims 31-38, further including applying a cropping margin to eliminate pixels around edges of the sensor from use in fitting the spacing surface and the angle surface.
40. The method of any of claims 31-39, further including measuring spacing between intensity peaks and angle of the intensity peaks over at least 8 by 11 subtiles of the image tile.
41. A method of using a calibrated scanner to produce enhanced resolution images of samples positioned closer together than an Abbe diffraction limit for optical resolution between at least some adjoining pairs of the samples, including:
- for a near-center subtile of an image tile captured by an optical sensor, accessing or generating estimated reconstruction parameters, including
- spacing parameters, for the spacing between intensity peaks, and pattern angle parameters, for the angle of the intensity peaks, the spacing parameters generated from measurements of imaging a regularly spaced and angled structured illumination pattern projected onto the near-center subtile, and
 - phase displacement parameters for relative phase relationship among the subtiles, at a subtile reference point, generated from further measurements of imaging multiple phase displacements along one angle of the structured illumination pattern projected onto the near-center subtile;
- deriving estimated reconstruction parameters for at least 8 additional subtiles of the image tile, in addition to the near-center subtile, by combining the accessed or estimated reconstruction parameters for the near-center subtile with:
- saved fitting results that relate the spacing parameters and the angle parameters across the at least 8 additional subtiles to the near-center subtile, and
 - a saved look-up table for the with phase displacement measurements across the at least 8 additional subtiles, relative to the near-center subtile; and

- applying the estimated reconstruction parameters for the near-center subtitle and for the at least 8 additional subtitles to produce enhanced resolution images for the subtitles with resolving power better than the Abbe diffraction limit.
42. The method of claim 41, further including repeatedly applying actions of claim 41 to produce a sequence of the enhanced resolution images for the subtitles and using the sequence of the enhanced resolution images to sequence samples imaged by the sensor over multiple cycles.
43. The method of any of claims 41-42, wherein the subtitles each include at least 512 by 512 pixels of the optical sensor.
44. The method of any of claims 41-43, wherein the subtitles overlap by at least 2 pixels of the optical sensor.
45. The method of any of claims 41-44, further including aggregating the enhanced resolution images for the subtitles into an enhanced resolution image for the tile and using the enhanced resolution image for the tile to sequence the samples over multiple cycles.
46. The method of any of claims 41-45, further including, at an intermediate cycle among the multiple cycles, re-measuring the spacing between intensity peaks and angle of the intensity peaks in the subtitles, refitting the spacing surface and the angle surface, and saving results of the refitting for use in processing subsequently captured image tiles.
47. The method of claim 46, wherein the intermediate cycle is a 50th cycle or later among at least 100 cycles.
48. The method of any of claims 41-47, further applied to image tiles at positions across a flow cell that capture florescence of millions of samples distributed across the flow cell, including producing the enhanced images for the flow cell.
49. The method of claim 48, wherein the samples are distributed in millions of nanowells across the flow cell and at least some adjoining pairs of nanowells are positioned closer together than an Abbe diffraction limit for optical resolution.
50. The method of any of claims 41-49, further including producing sequences of the enhanced images for the flow cell, and using the sequences of the enhanced images to call sequences of the samples.
51. The method of claim 50, wherein the nanowells are arranged in a repeating rectangular pattern with two diagonals connecting opposing corners of a rectangle in the pattern, further including using two structured illumination angles at which the intensity peaks are oriented substantial normal to the two diagonals.

52. The method of claim 50, wherein the nanowells are arranged in a repeating hexagonal pattern with three diagonals connecting opposing corners of a hexagon in the pattern, further including using three structured illumination angles at which the intensity peaks are oriented substantial normal to the three diagonals.

53. A method to produce an enhanced resolution image from images of a target captured under structured illumination, the method including:

dividing a captured image of the target captured by an optical sensor into 9 or more subtiles and operating independently on each of the 9 or more subtiles, including:

transforming, in a respective subtile, at least three images of the target, captured by a sensor in a spatial domain while applying at least three phase displacements of the structured illumination along one angle, into a Fourier domain to produce at least three frequency domain matrices of the respective subtile;

using estimated reconstruction parameters, applying an inverse mixing matrix to the frequency domain matrices to produce at least three phase-separated matrices in the Fourier domain, which are unshifted and shifted matrices; and

performing subpixel shifting on at least one shifted matrix, including transforming the shifted matrix from the Fourier domain into the spatial domain, applying a translation vector to data in the spatial domain, transforming the translated data from the spatial domain data and producing two or more realigned shifted matrices in the Fourier domain;

aligning and summing overlapping values of the unshifted matrix and the realigned shifted matrices to produce an expanded frequency coverage matrix; and

inversely transforming the expanded frequency coverage matrix from the Fourier domain to produce an enhanced resolution subtile in the spatial domain; and

merging the enhance resolution subtiles for the 9 or more subtiles into an enhanced resolution image.

54. The method of claim 53, further including repeatedly applying actions of claim 53 to produce a sequence of the enhanced resolution images and using the sequence of the enhanced resolution images to sequence samples imaged by the sensor over multiple cycles.

55. The method of any of claims 53-54, wherein the subtiles each include at least 512 by 512 pixels of captured by the optical sensor.

56. The method of any of claims 53-55, wherein the subtiles overlap by at least 2 pixels of the optical sensor.

57. The method of any of claims 53-56, further including aggregating the enhanced resolution images for the subtiles into an enhanced resolution aggregate image and using the enhanced resolution aggregate image to sequence the samples over multiple cycles.

58. The method of any of claims 53-57, further applied to image tiles at positions across a flow cell that capture florescence of millions of samples distributed across the flow cell, including producing the enhanced images for the flow cell.

59. The method of claim 58, wherein the samples are distributed in millions of nanowells across the flow cell and at least some adjoining pairs of nanowells are positioned closer together than an Abbe diffraction limit for optical resolution.

60. The method of any of claims 53-58, further including producing sequences of the enhanced images for the flow cell, and using the sequences of the enhanced images to call sequences of the samples.