

CLAIMS

1. A method to produce an enhanced resolution image from images of a target captured under structured illumination, the method including:
transforming at least three images of the target, captured by a sensor in a spatial domain from at least three phase displacements of the structured illumination along one angle, into a Fourier domain to produce at least three frequency domain matrices that include at least non-redundant components and redundant conjugate components, which are conjugates, in complementary matrix positions, of the non-redundant components;
using estimated reconstruction parameters, applying an inverse mixing matrix to at least the non-redundant components to produce at least three phase-separated matrices in the Fourier domain, which are unshifted and shifted matrices, and which include non-redundant unshifted components and non-redundant shifted spatial frequency components, derived from the non-redundant components of the frequency domain matrices;
performing one or more intermediate transformations on at least the non-redundant components of the phase-separated matrices, including:
 subpixel shifting, by transforming from the Fourier domain into the spatial domain at least the non-redundant shifted spatial frequency components of the shifted matrices, applying a translation vector to data in the spatial domain, and transforming the translated data from the spatial domain data into one or more realigned shifted matrices in the Fourier domain, wherein applying the translation vector effectively realigns overlapping values in the shifted matrices in the Fourier domain with the unshifted matrix more precisely than whole matrix positions of discrete Fourier transforms and
recovering the redundant conjugate components, after the intermediate transformations, from the non-redundant components by copying transformed values from the non-redundant components to the complementary matrix positions of the redundant conjugate components and changing a sign of imaginary parts of the copied values to produce transformed redundant conjugate components;
aligning and summing overlapping values of the unshifted matrix and the 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 image in the spatial domain.
2. The method of claim 1, further including using a sequence of enhanced resolution images, produced by repeatedly applying actions of claim 1, to sequence samples imaged by the sensor over multiple cycles.
3. The method of any of claims 1-2, wherein the complementary matrix positions of the redundant conjugate components, when arranged in a corner shifted matrix, are rotated 180 degrees from positions of the non-redundant components.

4. The method of any of claims 1-3, further including applying, during the intermediate transformations, of one or two-step Wiener filtering to reduce noise.
5. The method of claim 4, further including applying the two-step Wiener filtering, including:
applying an independent modulation transfer function to the phase separated matrices to compensate for optical transfer of contrast, which decreases with increasing spatial frequency, by an objective lens transferring the target under the structured illumination to a sensor; and
then later applying noise removal components of the Wiener filtering.
6. The method of any of claims 1-3, further including applying Apodization filtering during the intermediate transformations.
7. The method of any of claims 1-6, wherein the phase-separated matrices in the Fourier domain include an even number of rows and an even number of columns, further including setting equal between the shifted matrices a DC column, of DC components, and a middle column, of other components.
8. The method of claim 7, further including averaging, over the shifted matrices, values in the DC column, of DC components, and the middle column, of other components before the setting equal.
9. The method of claim 7, further including copying values in the DC column, of DC components, and the middle column, of other components from one of the shifted matrices to another of the shifted matrices.
10. The method of claim 7, further including calculating a weighted average, over the shifted matrices, of values in the DC column, of DC components, and the middle column, of other components before the setting equal.
11. The method of claim 7, wherein the column averaging precedes the subpixel shifting.
12. The method of any of claims 1-6, wherein at least one of the phase-separated matrices in the Fourier domain is corner shifted, DC components appear in a top row and a left column of the matrices and the redundant conjugate components appear in a block rotated 180 degrees from its orientation as the non-redundant components.
13. The method of claim 12, wherein the phase-separated matrices in the Fourier domain include an odd number of rows and an odd number of columns without a middle column between blocks of the non-redundant components and the redundant conjugate components.
14. The method of any of claims 1-6 or 13, further including applying the method to sets of at least three images of the target captured through a lens by a sensor in the spatial domain, from phase displacements along two angles of the structured illumination of the target, wherein the target includes regularly spaced nanowells and the two angles of the structured illumination are along diagonals between opposing corners of quadrilaterals among the regularly spaced nanowells.

15. The method of any of claims 1-13, further including applying the method to sets of at least three images of the target captured through a lens by a sensor in the spatial domain, from phase displacements along three angles of the structured illumination of the target, wherein the target includes regularly spaced nanowells and the three angles are along diagonals between opposing corners of hexagons among the regularly spaced nanowells.

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

transforming at least three images of the target, captured by a sensor in a spatial domain from at least three phase displacements of the structured illumination along one angle, into a Fourier domain to produce at least three frequency domain matrices that include at least non-redundant components and redundant conjugate components, which are conjugates, in complementary matrix positions, of the non-redundant components;

using estimated reconstruction parameters, applying an inverse mixing matrix to at least the non-redundant components to produce at least three phase-separated matrices in the Fourier domain, which are unshifted and shifted matrices, and which include non-redundant unshifted components and non-redundant shifted spatial frequency components, derived from the non-redundant components of the frequency domain matrices;

performing one or more intermediate transformations on at least the non-redundant components of the phase-separated matrices, including:

subpixel shifting, by transforming from the Fourier domain into the spatial domain at least the non-redundant shifted spatial frequency components of the shifted matrices, applying a translation vector to data in the spatial domain, and transforming the translated data from the spatial domain data into one or more realigned shifted matrices in the Fourier domain, wherein applying the translation vector effectively realigns overlapping values in the shifted matrices in the Fourier domain with the unshifted matrix more precisely than whole matrix positions of discrete Fourier transforms and

recovering the redundant conjugate components, after the intermediate transformations, from the non-redundant components by copying transformed values from the non-redundant components to the complementary matrix positions of the redundant conjugate components and changing a sign of imaginary parts of the copied values to produce transformed redundant conjugate components; aligning and summing overlapping values of the unshifted matrix and the 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 image in the spatial domain.

17. The computer readable medium of claim 16, further impressed with program instructions, the method further including using a sequence of enhanced resolution images, produced by repeatedly applying actions of claim 16, to sequence samples imaged by the sensor over multiple cycles.

18. The computer readable medium of any of claims 16-17, wherein the complementary matrix positions of the redundant conjugate components, when arranged in a corner shifted matrix, are rotated 180 degrees from positions of the non-redundant components.

19. The computer readable medium of any of claims 16-18, further impressed with program instructions, the method further including applying, during the intermediate transformations, of one or two-step Wiener filtering to reduce noise.

20. The computer readable medium of claim 19, further impressed with program instructions, the method further including applying the two-step Wiener filtering, including:
applying an independent modulation transfer function to the phase separated matrices to compensate for optical transfer of contrast, which decreases with increasing spatial frequency, by an objective lens transferring the target under the structured illumination to a sensor; and
then later applying noise removal components of the Wiener filtering.

21. The computer readable medium of any of claims 16-18, further impressed with program instructions, the method further including applying Apodization filtering during the intermediate transformations.

22. The computer readable medium of any of claims 16-21, wherein the phase-separated matrices in the Fourier domain include an even number of rows and an even number of columns, further including setting equal between the shifted matrices a DC column, of DC components, and a middle column, of other components.

23. The computer readable medium of claim 22, further impressed with program instructions, the method further including averaging, over the shifted matrices, values in the DC column, of DC components, and the middle column, of other components before the setting equal.

24. The computer readable medium of claim 22, further impressed with program instructions, the method further including copying values in the DC column, of DC components, and the middle column, of other components from one of the shifted matrices to another of the shifted matrices.

25. The computer readable medium of claim 22, further impressed with program instructions, the method further including calculating a weighted average, over the shifted matrices, of values in the DC column, of DC components, and the middle column, of other components before the setting equal.

26. The computer readable medium of claim 22, wherein the column averaging precedes the subpixel shifting.

27. The computer readable medium of any of claims 16-21, wherein at least one of the phase-separated matrices in the Fourier domain is corner shifted, DC components appear in a top row and a left column of the matrices and the redundant conjugate components appear in a block rotated 180 degrees from its orientation as the non-redundant components.

28. The computer readable medium of claim 27, wherein the phase-separated matrices in the Fourier domain include an odd number of rows and an odd number of columns without a middle column between blocks of the non-redundant components and the redundant conjugate components.

29. The computer readable medium of any of claims 16-21 or 28, further impressed with program instructions, the method further including applying the method to sets of at least three images of the target captured through a lens by a sensor in the spatial domain, from phase displacements along two angles of the structured illumination of the target, wherein the target includes regularly spaced nanowells and the two angles of the structured illumination are along diagonals between opposing corners of quadrilaterals among the regularly spaced nanowells.

30. The computer readable medium of any of claims 16-28, further impressed with program instructions, the method further including applying the method to sets of at least three images of the target captured through a lens by a sensor in the spatial domain, from phase displacements along three angles of the structured illumination of the target, wherein the target includes regularly spaced nanowells and the three angles are along diagonals between opposing corners of hexagons among the regularly spaced nanowells.

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

transforming at least three images of the target, captured by a sensor in a spatial domain from at least three phase displacements of the structured illumination along one angle, into a Fourier domain to produce at least three frequency domain matrices that include at least non-redundant components and redundant conjugate components, which are conjugates, in complementary matrix positions, of the non-redundant components;

using estimated reconstruction parameters, applying an inverse mixing matrix to the non-redundant components to produce at least three phase-separated matrices in the Fourier domain, including one unshifted and at least two shifted matrices, derived from the non-redundant components of the frequency domain matrices;

performing a subpixel shifting transformation of the non-redundant components of the phase-separated matrices, including transforming from the Fourier domain into the spatial domain the non-redundant components of the shifted matrices, applying a translation vector to data in the spatial domain, and transforming the translated data back a realigned shifted matrix in the Fourier domain;

recovering the redundant conjugate components from the non-redundant components of the realigned shifted matrix by copying transformed values from the non-redundant components to the

complementary matrix positions of the redundant conjugate components and changing a sign of imaginary parts of the copied values;
aligning and summing overlapping values of the unshifted matrix and the shifted matrices to produce an expanded frequency coverage matrix; and
inversely transforming the expanded frequency coverage matrix into the spatial domain to produce an enhanced resolution image.

32. The method of claim 31, further including using a sequence of enhanced resolution images, produced by repeatedly applying actions of claim 31, to sequence samples imaged by the sensor over multiple cycles.

33. The method of any of claims 31-32, wherein the complementary matrix positions of the redundant conjugate components, when arranged in a corner shifted matrix, are rotated 180 degrees from positions of the non-redundant components.

34. The method of any of claims 31-33, further including applying, before the subpixel shifting transformation, a one or two-step Wiener filtering to reduce noise.

35. The method of claim 34, further including applying the two-step Wiener filtering, including:
applying an independent modulation transfer function to the phase separated matrices to compensate for optical transfer of contrast, which decreases with increasing spatial frequency, by an objective lens transferring the target under the structured illumination to a sensor; and
then later applying noise removal components of the Wiener filtering.

36. The method of any of claims 31-33, further including applying, before the subpixel shifting transformation, Apodization filtering.

37. The method of any of claims 31-36, wherein the phase-separated matrices in the Fourier domain include an even number of rows and an even number of columns, further including setting equal between the shifted matrices a DC column, of DC components, and a middle column, of other components.

38. The method of claim 37, further including averaging, over the shifted matrices, values in the DC column, of DC components, and the middle column, of other components before the setting equal.

39. The method of claim 37, further including copying values in the DC column, of DC components, and the middle column, of other components from one of the shifted matrices to another of the shifted matrices.

40. The method of claim 37, further including calculating a weighted average, over the shifted matrices, of values in the DC column, of DC components, and the middle column, of other components before the setting equal.

41. The method of any of claims 31-40, wherein at least one of the phase-separated matrices in the Fourier domain is corner shifted, DC components appear in a top row and a left column of the matrices and the redundant conjugate components appear in a block rotated 180 degrees from its orientation as the non-redundant components.

42. The method of any of claims 31-36 or 41, wherein the phase-separated matrices in the Fourier domain include an odd number of rows and an odd number of columns without a middle column between blocks of the non-redundant components and the redundant conjugate components.

43. The method of any of claims 31-42, further including applying the method to sets of at least three images of the target captured through a lens by a sensor in the spatial domain, from phase displacements along two angles of the structured illumination of the target, wherein the target includes regularly spaced nanowells and the two angles are along diagonals between opposing corners of quadrilaterals among the regularly spaced nanowells.

44. The method of any of claims 31-42, further including applying the method to sets of at least three images of the target captured through a lens by a sensor in the spatial domain, from phase displacements along three angles of the structured illumination of the target, wherein the target includes regularly spaced nanowells and the three angles are along diagonals between opposing corners of hexagons among the regularly spaced nanowells.