CLAIMS

~~What is claimed is:~~

1. A fluorescent in-situ hybridization imaging system, comprising:

a flow cell to contain a sample to be exposed to fluorescent probes in a reagent;

a valve to control flow from one of a plurality of reagent sources the flow cell;

a pump to cause fluid flow through the flow cell;

a fluorescence microscope including a variable frequency excitation light source and a camera positioned to receive fluorescently emitted light from the sample;

an actuator to cause relative vertical motion between the flow cell and the fluorescence microscope;

a motor to cause to cause relative lateral motion between the flow cell and the fluorescence microscope; and

a control system configured to, as nested loops,

cause the valve to sequentially couple the flow cell to a plurality of different reagent sources to expose the sample to a plurality of different reagents,

for each reagent of the plurality of different reagents, cause the motor to sequentially position the fluorescence microscope relative to sample at a plurality of different fields of view,

for each field of view of the plurality of different fields of view, cause the variable frequency excitation light source to sequentially emit a plurality of different wavelengths,

for each wavelength of the plurality of different wavelengths, cause the actuator to sequentially position the fluorescence microscope relative to sample at a plurality of different vertical heights, and

for each vertical height of the plurality of different vertical heights, obtain an image at the respective vertical height covering the respective field of view of the sample having respective fluorescent probes of the respective regent as excited by the respective wavelength.

2. The system of claim 1, wherein the variable frequency excitation light source comprises a plurality of laser modules and the control system is configured to sequentially activate the laser modules to sequentially emit the plurality of different wavelengths.

3. The system of claim 2, wherein the control system includes a computer to control the valve and the motor and a microcontroller to control the laser module and the actuator.

4. The system of claim 1, comprising a stage to support the sample in the flow cell, and wherein the motor comprises a pair of linear actuators configured to drive the stage along a pair of perpendicular axes.

5. The system of claim 1, further comprising a data processing system configured to, for each field of view of at least two fields of view of the plurality of different fields of view and for each pixel of at least two pixels shared in images of the field of view, decode the pixel by identifying a code word from a plurality of code words in a code book that provides a best match to data values for the pixel in the plurality of images for the field of view.

6. A fluorescent in-situ hybridization imaging and analysis system, comprising:

a flow cell to contain a sample to be exposed to fluorescent probes in a reagent;

a fluorescence microscope to obtain sequentially collect a plurality of images of the sample at a plurality of different combinations of imaging parameters; and

a data processing system including

an online pre-processing system configured to sequentially receive the images from the fluorescence microscope as the images are collected and perform on-the-fly image pre-processing to remove experimental artifacts of the image and to provide RNA image spot sharpening, and

an offline processing system configured to, after the plurality of images are collected, perform registration of images having a same field of view and to decode intensity values in the plurality of images to identify expressed genes.

7. The system of claim 6, wherein the data processing system is configured to determine an image quality value for each image and to screen the image from pre-processing if the image quality value fails to meet a quality threshold.

8. The system of claim 6, wherein the data processing system is configured to determine a plurality of image quality values for each image and to screen the image from pre-processing if any of the image quality value fail to meet an associated quality threshold.

9. A fluorescent in-situ hybridization imaging system, comprising:

a flow cell to contain a sample to be exposed to fluorescent probes in a reagent;

a fluorescence microscope including

a variable frequency excitation light source to illuminate the sample,

a plurality of emission bandpass filters on a filter wheel,

an actuator to rotate the filter wheel,

a camera positioned to receive fluorescently emitted light from the sample that has passed through a filter on the filter wheel;

a control system configured to

cause the variable frequency excitation light source to emit a light beam having a selected wavelength,

cause the actuator to rotate the filter wheel to position a selected filter in a light path between the sample and the camera,

obtain an image from the camera, and

coordinate the variable frequency excitation light source and filter wheel such that the selected filter has an emission bandpass associated with emission by the fluorescent probes when excited by the selected wavelength when the image is obtained.

10. The system of claim 9, wherein the control system comprises a first microcontroller to cause the actuator to rotate the filter wheel in response to a first trigger signal.

11. The system of claim 10, wherein the control system comprises a second microcontroller to cause the variable frequency excitation light source to emit the light beam having the selected wavelength in response to a second trigger signal.

12. The system of claim 11, wherein the first microcontroller is configured to generate the second trigger signal.

13. The system of claim 12, wherein the second microcontroller is configured to send a third trigger signal to the camera to cause image acquisition.

~~14. The system of claim 9, comprising a dichroic filter in the light path between the sample and the camera or in the light path between the sample and the filter wheel.~~

~~15. The system of claim 14, wherein the dichroic mirror is a multi-pass dichroic mirror having a pass band for each emission wavelength for the fluorescent probes.~~

~~16~~14. The system of claim 9, wherein each filter of the plurality of emission bandpass filters has a unique band and is associated with a unique wavelength for the variable frequency excitation light source.

~~17. The system of claim 9, wherein the variable frequency excitation light source is configured to illuminate the sample with excitation light normal to the sample.~~

~~18~~15. A computer program product, comprising a non-transitory computer readable media having instructions to cause one or more processors to:

apply one or more filters to ~~and~~ perform deconvolution on each image of the plurality of images to generate a plurality of filtered images;

perform registration of the plurality of filtered images to generate a plurality of registered and filtered images;

for each pixel of at least two pixels of the plurality of registered and filtered images, decode the pixel by identifying a code word from a plurality of code words in a code book that

provides a best match to data values in the plurality of registered and filtered images for the pixel; and

for each pixel of at least two pixels, determine a gene associated with the code word and store an indication that the gene is expressed at the pixel.

~~19~~16. The computer program product of claim ~~18~~15, wherein the instructions to perform registration comprise instructions to

for each filtered image of the plurality of filtered images

split the filtered image into a plurality of sub-images of equal size,

calculate from the plurality of sub-images a combined image of size equal to that of a sub-image and thereby generate a plurality of reduced-size images with each image of the plurality of images having a corresponding reduced-size image,

for at least one reduced-size images of the plurality of reduced-size images, determine a transform to register the plurality of reduced-size images;

for each of the at least one reduced-size images, apply the transform determined for the reduced-size image to the corresponding filtered image to generate a transformed image of size equal to the initial image and thereby generate a plurality of transformed images.

~~20~~17. The computer program product of claim ~~18~~15, further comprising instructions to, for each image of the plurality of images, set brightness values above a threshold to a maximum brightness value and scale remaining brightness values below the threshold based on the maximum value.

~~21~~18. The computer program product of claim ~~18~~15, further comprising instructions to, for each image of the plurality of images, perform field flattening and/or chromatic aberration correction.

~~22. A computer program product, comprising a non-transitory computer readable media having instructions to cause one or more processors to:~~

~~receive a plurality of initial images of a sample, the plurality of initial images sharing a common field of view;~~

~~for each initial image of the plurality of initial images~~

split the initial image into a plurality of sub-images of equal size,

calculate from the plurality of sub-images a combined image of size equal to that of a sub-image and thereby generate a plurality of reduced-size images with each image of the plurality of images having a corresponding reduced-size image,

for at least one reduced-size images of the plurality of reduced-size images, determine a transform to register the plurality of reduced-size images;

for each of the at least one reduced-size images, apply the transform determined for the reduced-size image to the corresponding initial image to generate a transformed image of size equal to the initial image and thereby generate a plurality of transformed images; and

for each pixel of at least two pixels of the plurality of transformed images, decode the pixel by identify a code-word from a plurality of code-words in a code-book that provides a best match to data values in the plurality of transformed images for the pixel; and

for each pixel of at least two pixels, determine a gene associated with the code-word and store an indication that the gene is expressed at the pixel.

23. The computer program product of claim 22, wherein the instructions to calculate the combined image comprise instructions to phase shift the reduced-size images by a different phase shift amounts to generate phase-shifted reduced-sized images.

24. The computer program product of claim 23, wherein the instructions to calculate the combined image comprise instructions sum the phase shifted reduced-sized image to generate a complex image.

25. The computer program product of claim 24, wherein the instructions to calculate the combined image comprise instructions to sum real and imaginary portions of the complex image to form the combined image.

26. A computer program product, comprising a non-transitory computer readable media having instructions to cause one or more processors to:

receive a plurality of images of a sample, the plurality of images representing a common field of view;

perform registration of the plurality of images to generate a plurality of registered images;

for each pixel of a plurality of pixels of the registered images, decode the pixel by identifying a code word from a plurality of code words in a code book that provides a best match to data values in the plurality of registered images for the pixel; and

for each code word identified as a best match for one or more pixels and each pixel of the one or more pixels, determine whether a bit ratio for an image word for the pixel meets a threshold for the code word, the image word being formed from the data values in the plurality of registered images for the pixel; and

for at least one pixel that is determined to meet the threshold determine a gene associated with the code word and store an indication that the gene is expressed at the pixel, and screen pixels for which the bit ratio does not meet the threshold.

27. The computer program product of claim 26, wherein the code word comprises a sequence of code values and the image word comprises a sequence of data values with each data value in the sequence having a corresponding code value in the sequence of code values.

28. The computer program product of claim 27, wherein the instructions to determine the bit ratio comprise instructions to determine which code values in the code word indicate an on status and which code values in the code word indicate an "off" status.

29. The computer program product of claim 28, wherein the instructions to determine the bit ratio comprise instructions to determine a first average of data values in the image word that correspond to code values in the code word that indicate an on status.

30. The computer program product of claim 29, wherein the instructions to determine the bit ratio comprise instructions to determine a second average of data values in the image word that correspond to code values in the code word that indicate an off status.

31. The computer program product of claim 30, wherein the instructions to determine the bit ratio comprise instructions to divide the first average by the second average.

32. A computer program product, comprising a non-transitory computer readable media having instructions to cause one or more processors to:

receive a plurality of images of a sample, the plurality of images representing a common field of view;

perform registration of the plurality of images to generate a plurality of registered images;

for each pixel of a plurality of pixels of the registered images, decode the pixel by identifying a code word from a plurality of code words in a code book that provides a best match to data values in the plurality of registered images for the pixel; and

for each code word identified as a best match for one or more pixels and each pixel of the one or more pixels, determine whether a bit brightness for an image word for the pixel meets a threshold for the code word, the image word being formed from the data values in the plurality of registered images for the pixel; and

for each pixel that is determined to meet the threshold determine a gene associated with the code word and storing an indication that the gene is expressed at the pixel, and screen pixels for which the bit ratio does not meet the threshold.

33. The computer program product of claim 32, wherein the code word comprises a sequence of code values and the image word comprises a sequence of data values with each data value in the sequence having a corresponding code value in the sequence of code values.

34. The computer program product of claim 33, wherein the instructions to determine the bit brightness comprise instructions to determine which code values in the code word indicate an on status and which code values in the code word indicate an off status.

35. The computer program product of claim 34, wherein the instructions to determine the bit brightness comprise instructions to determine an average of data values in the image word that correspond to code values in the code word that indicate an on status.

~~36. The computer program product of claim 32, comprising instructions to for each code word identified as a best match for one or more pixels and each pixel of the one or more pixels, determine whether a bit ratio for an image word for the pixel meets a second threshold for the code word, and screen pixels for which the bit ratio does not meet the second threshold.~~

~~37. The computer program product of claim 36, comprising instructions to determine the threshold by determining a value at a first quantile limit in a histogram of bit values from the plurality of images of the sample, and to determine the second threshold by determining a second value at a second quantile limit in a histogram of bit values from the plurality of images of the sample, wherein the second quantile limit is higher than the first quantile limit.~~

~~38. A computer program product, comprising a non-transitory computer readable media having instructions to cause one or more processors to:~~

~~receive a plurality of initial images of a sample, the plurality of initial images including a multiplicity of images for each field of view of a plurality of different fields of view;~~

~~store instructions to perform an image processing algorithm in which one or more of image filtering of the multiplicity of images for the field of view or registration of the multiplicity of images for the field of view;~~

~~for each field of view, determine one or more initial parameter values, wherein different fields of view have different initial parameter values;~~

~~perform the image processing algorithm for each field of view using the different initial decoding parameter values for the different fields of view;~~

~~determine one or more subsequent decoding parameter values; and~~

~~perform the image processing algorithm and a decoding algorithm for each field of view using the same subsequent parameter values for the different fields of view to generate a set of spatial transcriptomic data.~~

~~39. The computer program product of claim 38, wherein the instructions to determine the one or more subsequent parameter values include instructions to determine the one or more subsequent parameter values the based on at least one of the one or more initial parameter values for each field of view.~~

~~40. The computer program product of claim 38, comprising instructions to determine a best average goodness of fit from a plurality of average goodnesses of fit, and wherein the instructions to determine the one or more subsequent parameter values comprise instructions to select one or more decoding parameters from the initial coding parameters for a field of view associated with the best average goodness of fit.~~

~~41. The computer program product of claim 38, comprising instructions to select a field of view at a predetermined position in an array of fields of view, wherein the instructions to determine the one or more subsequent decoding parameter values comprise instructions to select one or more decoding parameters from the initial coding parameters for the field of view at the predetermined position.~~

~~42. The computer program product of claim 38, wherein the instructions to determine the one or more subsequent decoding parameter values comprise instructions to average decoding parameters from the plurality of fields of view.~~

~~43~~19. A computer program product, comprising a non-transitory computer readable media having instructions to cause one or more processors to:

receive a plurality of initial images of a sample, the plurality of initial images including a multiplicity of images for each field of view of a plurality of different fields of view;

for each field of view, generate a set of spatial transcriptomic data based on the multiplicity of images for the field of view, each set of spatial transcriptomic data including one or more data pairs, each data pair of the one or more data pairs including a position and an expressed gene for that position;

determine regions of overlap of multiplicities of images from adjacent fields of view; and

from the set of spatial transcriptomic data for each field of view, generate a combined set of spatial transcriptomic data for a combined image covering the plurality of different fields of view, wherein the instructions to generate the combined set of spatial transcriptomic data include instructions to screen in a region of overlap at least one double-counted data pair from different fields of view that represents the same expressed gene at the same location within the sample.

~~44~~20. The computer program product of claim ~~43~~19, wherein the instructions to screen include instructions to determine a distance between positions for expressions of a same gene and to determine whether the distance is below a threshold value.

~~45~~21. A computer program product, comprising a non-transitory computer readable media having instructions to cause one or more processors to:

receive a plurality of images of a sample, the plurality of images including a multiplicity of images for each field of view of a plurality of different fields of view;

on each image of the plurality of images, perform an image quality check on the image;

after performing the image quality check, on each image of the plurality of images, remove artifacts from the image;

after removing artifacts, on each image of the plurality of images, sharpen image spots in the image;

after sharpening the image spots, for each multiplicity of images, performing registration of the multiplicity of images to generate a registered images; and

after performing registration, for each multiplicity of images, for each pixel of a plurality of pixels of the registered images of a respective multiplicity, decode the pixel by identifying a code word from a plurality of code words in a code book that provides a best match to data values in the respective multiplicity of registered images for the pixel.