Claims

1. A method for detecting ~~and characterizing~~ micro-objects in a~~n~~ microfluidic device, the method comprising:

receiving a first image and one or more second images of a region of interest in the microfluidic device, the first image comprising an illuminated image and the one or more second images comprising one or more non-illuminated images;

pre-processing the first image and the one or more second images ~~to reduce anomalies in the image data~~;

transforming each of the one or more second images to optically align the one or more second image~~(s)~~ with the first image;

processing pixel data in the first image using a machine learning algorithm to detect micro-objects present in the region of interest, wherein detecting each micro-object comprises identifying a boundary of the micro-object; and

detecting a signal located within each boundary of each detected micro-object in each one of the one or more second images, wherein each detected signal is associated with a reagent that specifically binds to a biological molecule comprised by one or more of the detected micro-objects.

2. The method of claim 1, wherein at least one of the one or more second images is a fluorescent image, and wherein the detected signal in the at least one second image is a fluorescent signal.

3. The method of claim 1, wherein each of the one or more second images is a fluorescent image, and wherein the detected signal in each of the one or more second images is a fluorescent signal.

4. The method of claim 2, wherein each fluorescent image represents fluorescent signal from a ~~non-overlapping~~unique portion of the visible light spectrum.

5. ~~The method of claim 1, wherein each detected signal is associated with a reagent that specifically binds to a biological molecule comprised by one or more of the detected micro-~~

~~objects.~~The method of claim 4, wherein each fluorescent image represents fluorescent signal from a non-overlapping portion of the visible light spectrum.

6. The method of any one of claims 1 to 5, wherein processing pixel data in the first image to detect micro-objects present in the region of interest comprises using the machine learning algorithm to generate a plurality of pixel masks from the first image for a corresponding plurality of micro-object characteristics, wherein each pixel mask comprises a set of pixel annotations, each pixel annotation of the set representing a probability that a corresponding pixel in the image represents the corresponding micro-object characteristic.

7. The method of claim 6, wherein detecting the micro-objects comprises using a combination of pixel masks of the plurality of pixel masks.

8. The method of claim 6, wherein the plurality of micro-object characteristics comprises at least: (i) micro-object center; (ii) micro-object edge; and (iii) non-micro-object.

9. The method of claim 8, wherein detecting the micro-objects is based upon the pixel mask corresponding to the micro-object center characteristic or a combination of pixel masks that includes the pixel mask corresponding to the micro-object center characteristic.

10. The method of claim 6, wherein the machine learning algorithm comprises a neural network.

11. The method of any one of claims 1 to 5, wherein there are at least two second images.

12. The method of any one of claims 1 to 5, further comprising: grouping the detected micro-objects into sub-populations of micro-objects that share one or more of the same characteristics.

13. The method of claim 12, wherein the detected micro-objects are grouped into sub-populations based upon their proximity in n-dimensional space, wherein each of the n dimensions is a measurable characteristic of the micro-objects.

14. The method of any one of claims 1 to 5, further comprising: providing a visual display representing a distribution of at least one characteristic of the detected micro-objects.

15. The method of claim 14, wherein the visual display is a two-dimensional graph that represents at least two characteristics of the detected micro-objects.

16. The method of claim 14, wherein the visual display is a three-dimensional graph that represents at least three characteristics of the detected micro-objects (e.g., cross-sectional area and first and second fluorescent signals, or first, second, and third fluorescent signals).

17, The method of claim 14, further comprising providing a user interface that allows the user to select a sub-population of the detected micro-objects and~~, optionally,~~ to provide instruction(s) for repositioning the selected sub-population.

18. The method of any of claims 1 to 5, further comprising increasing or decreasing the identified boundary of the micro-object.

19. The method of any one of claims 1 to 5, wherein the micro-objects are imaged within a microfluidic device, and wherein the pre-processing comprises subtracting out a repeating pattern produced by at least one component of the microfluidic device during imaging.

20. The method of claim 19, wherein the pre-processing comprises applying a Fourier transform to the image to identify the repeating pattern.

21. The method of claim 19, wherein the at least one component of the microfluidic device is a substrate surface.

22. The method of claim 19, wherein the at least one component of the microfluidic device is a substrate surface including a photo-transistor array.

23. The method of any one of claims 1 to 5, wherein the micro-objects are biological cells.

24. The method of claim 23, wherein the ~~living~~ biological cells are: immunological cells; cells from a cell line (e.g., CHO cells) or cancer cells; or oocytes, sperm, or embryos.

25. The method of any one of claims 1 to 24, further comprising identifying each micro-object based at least in part on the one or more second images.

~~25~~26. A system for automatically detecting micro-objects in a microfluidic device, comprising:

an image acquisition unit, comprising:

an imaging element configured to capture a first image and one or more second images of a region of interest in the microfluidic device, the first image comprising an illuminated image and the one or more second images comprising one or more non-illuminated images;

an image pre-processing engine configured to pre-process the first image and the one or more second images~~reduce anomalies in the image data~~; and

an alignment engine configured to transform the second image to optically align the one or more second image~~s~~ with the first image~~,~~; and

~~and~~

a micro-object detection and characterization unit communicatively connected to the image acquisition unit, comprising:

an image processing engine configured to process pixel data in the first image using a machine learning algorithm to detect micro-objects present in the region of interest, wherein detecting the micro-objects comprising identifying a boundary of each detected micro-object; and

a detection engine configured to detect a signal located within each boundary of each detected micro-object in each of the one or more second images, wherein each detected signal is associated with a reagent that specifically binds to a biological molecule comprised by one or more of the detected micro-objects.

~~26~~27. The system of claim ~~25~~26, further comprising: a user interface, wherein the user interface is configured to allow the user to select a sub-population of the detected micro-objects and~~, optionally,~~ to provide instruction(s) for repositioning the selected sub-population.

~~27. A computing device for characterizing and selecting micro-objects in a microfluidic device, the computing device comprising a display screen,~~

~~the computing device being configured to display on the screen a menu for selecting a first parameter, selected from a provided parameter list, for characterizing a set of detected micro-objects, and~~

~~the computing device being configured to display on the screen a plot of the detected micro-object set based on the selected first parameter,~~

~~wherein the provided parameter list is a limited list of parameters offered within the menu, each of the parameters in the list being selectable to characterize the set of detected micro-objects based on the associated parameter, and~~

~~wherein the display screen enables selection of a sub-population of the set of detected micro-objects based on at least one selected threshold value for the selected first parameter, and enables display of the detected micro-object set by visually differentiating the sub-population meeting the at least one selected threshold from the remaining micro-objects of the detected set.~~

~~28. The computing device of claim 27, wherein the provided parameter list provides parameters selected from the group consisting of Circularity, CentroidXPixels, CentroidYPixels, CentroidXMicrons, CentroidYMicrons, CentroidXMicronsPenRelative, CentroidYMicronsPenRelative, NearestNeighborMicrons, DiameterMicrons, VolumeFemtoliters, BackgroundAreaMicrons, MeanBrightness, MinBrightness, MaxBrightness, MedianBrightness, BackgroundMedianBrightness, DeltaMedianBrightness, DeltaMaxBrightness, LogMeanBrightness, LogMaxBrightness, LogMedianBrightness , LogDeltaMaxBrightness, LogDeltaMedianBrightnessCV , BackgroundCV, LogDeltaBrightnessMaxToBackgroundRatio, LogDeltaBrightnessSum, FluidChannelNumber, FieldOfView, CellCount, CellsPerPe, and the change with respect to time of any of the foregoing parameters.~~

~~29. The computing device of claims 27 to 28, wherein the computing device is further configured to accept screen instructions for repositioning one of the set of detected micro-~~

~~objects, sub-population of the set of detected micro-objects, a first subset of the sub-population, or a second subset of the first subset.~~

~~30. The computing device of claims 27 to 28, wherein the computing device is further configured to display on the screen an imaging menu for selecting an imaging parameter, selected from a provided imaging parameter list, for imaging at least a portion of the microfluidic device.~~