

CLAIMS

What is claimed is:

- ~~1. A method of pooled screening of cells from different genetic backgrounds, comprising:
a) labeling two or more populations of cells of different genetic backgrounds with two or more unique nucleic acid barcode sequences, each unique nucleic acid barcode sequence corresponding to a different population of cells;
b) combining the two or more populations of cells to obtain a single mixed population of cells;
c) performing *in situ* single cell sequencing on the cells; and
d) analyzing known or identifying new phenotypes of the cells in the mixed population.~~
- ~~2. The method of claim 1, wherein the two or more populations of cells are from different cell lines.~~
- ~~3. The method of claim 2, wherein the different cell lines are healthy cell lines.~~
- ~~4. The method of claim 2, wherein the different cell lines are patient cell lines.~~
- ~~5. The method of claim 2, wherein the different cell lines are isogenically engineered cell lines.~~
- ~~6. The method of claim 2, wherein the different cell lines include any combination of healthy, patient, and isogenically engineered cell lines.~~
- ~~7. The method of any one of claims 1-6, wherein the cells are induced pluripotent stem cells (iPSCs).~~
- ~~8. The method of claim 7, wherein the iPSCs are differentiated prior to analyzing the phenotype.~~
- ~~9. The method of claim 8, wherein the method further comprises culturing the cells prior to analyzing the phenotype.~~

- ~~10. The method of any one of claims 1-9, wherein the single mixed population of cells is on a substrate or in three-dimensional culture.~~
- ~~11. The method of claim 10, wherein the substrate is a cell culture dish.~~
- ~~12. The method of any one of claims 1-11, wherein the method further comprises performing single cell RNAseq.~~
- ~~13. The method of any one of claims 1-12, wherein the method further comprises growing the two or more populations of cells for two or more generations prior to step b).~~
- ~~14. The method of any one of claims 1-13, wherein the method comprises stably integrating the unique nucleic acid barcode sequences into the genomes of the two or more populations of cells.~~
- ~~15. The method of claim 14, wherein the unique nucleic acid barcode sequences are delivered into the cell using a virus.~~
- ~~16. The method of claim 15, wherein the virus is a lentivirus.~~
- ~~17. The method of claim 15 or claim 16, wherein the virus encodes a selectable marker.~~
- ~~18. The method of claim 17, wherein the selectable marker is an antibiotic resistance gene.~~
- ~~19. The method of any one of any one of claims 15-18, wherein the virus encodes a fluorescent protein.~~
- ~~20. The method of any of claims 1-19, wherein each unique nucleic acid barcode sequence is at least 1 base pair in length.~~
- ~~21. The method of claim 20, wherein each unique nucleic acid barcode sequence is 1 to about 18 base pairs in length.~~

22. The method of claim 20, wherein each unique nucleic acid barcode sequence is 8 base pairs in length.

23. The method of any one of claims 1-22, wherein the two or more populations of cells are sequenced prior to labeling with the unique nucleic acid barcode sequences.

24. The method of claim 23, wherein the sequencing is whole genome sequencing.

25. The method of any one of claims 1-24, wherein the two or more populations of cells were obtained from related individuals.

26. The method of claim 25, wherein the two or more populations of cells were obtained from humans.

27. The method of any one of claims 1-26, comprising labeling ten or more populations of cells of different genetic backgrounds with ten or more unique nucleic acid barcode sequences, each unique nucleic acid barcode sequence corresponding to a different population of cells.

28. The method of any one of claims 1-27, wherein analyzing the phenotype of the cells comprises an assay selected from the group consisting of high content imaging, calcium imaging, immunohistochemistry, cell morphology imaging, protein aggregation imaging, cell-cell interaction imaging, live cell imaging, and any other imaging-based assay modality.

29. The method of any one of claims 1-28, wherein step d) comprises analyzing the phenotype of the cells by capturing a microscopic image or a time series of microscopic images of the cells and evaluating phenotypic features presented in the image or images.

30. The method of claim 29, wherein the method further comprises a computer-implemented technique for aligning between a first plurality of images and a second plurality of images, comprising:

~~generating a first reference coordinate space of the first plurality of images, wherein the first plurality of images is of a well on a culture plate;~~
~~extracting a first patch of the first plurality of images;~~
~~generating a second reference coordinate space of the second plurality of images, wherein the second plurality of images is of the well on the culture plate;~~
~~extracting a second patch of the second plurality of images;~~
~~computing an affine transformation function between the first patch and the second patch to obtain a plurality of transformation parameters; and~~
~~generating a coordinate transformation function between the first reference coordinate space and the second reference coordinate space based on the plurality of transformation parameters.~~

341. A computer-implemented method of pooled screening of cells from different genetic backgrounds for aligning between a first plurality of images and a second plurality of images, comprising:

labeling two or more populations of cells of different genetic backgrounds with two or more unique nucleic acid barcode sequences, each unique nucleic acid barcode sequence corresponding to a different population of cells;
combining the two or more populations of cells to obtain a single mixed population of cells in a well on a culture plate;
performing *in situ* single-cell sequencing on the cells;
obtaining a first plurality of images, wherein the first plurality of images comprises one or more barcoding images of the well on the culture plate;
obtaining a second plurality of images, wherein the second plurality of images comprises one or more marker-based or marker-free readout images of the well on the culture plate;
~~generating a first reference coordinate space of the first plurality of images, wherein the first plurality of images is of a well on a culture plate;~~
~~extracting a first patch of the first plurality of images;~~
~~generating a second reference coordinate space of the second plurality of images, wherein the second plurality of images is of the well on the culture plate;~~
~~extracting a second patch of the second plurality of images;~~

computing an affine transformation function between the first patch and the second patch to obtain a plurality of transformation parameters; and

generating a coordinate transformation function between the first reference coordinate space and the second reference coordinate space based on the plurality of transformation parameters;

identifying cell-specific image data for one or more cells in the single mixed population of cells by correlating at least one image of the first plurality of images with at least one image of the second plurality of images based on the coordinate transformation function; and

identifying one or more phenotypes of the one or more cells based on the cell-specific image data.

~~32. The method of claim 31, wherein the first plurality of images is a plurality of barcoding images.~~

~~33. The method of claim 31 or claim 32, wherein the second plurality of images is a plurality of marker-based/marker-free readout image.~~

~~34. The method of any one of claims 31-33, wherein the first plurality of images and the second plurality of images provide different coverage of the well, are taken at different times, and/or have different resolutions.~~

~~35. The method of any one of claims 33-34, wherein the first plurality of images and the second plurality of images are taken at different times.~~

~~36. The method of any one of claims 31-35, wherein the first plurality of images and the second plurality of images have different resolutions.~~

~~37. The method of any one of claims 31-36, wherein the first plurality of images is taken by a first microscope and the second plurality of images is taken by a second microscope.~~

~~38. The method of claim 37, wherein the first microscope is a fluorescent microscope.~~

~~39. The method of claim 37, wherein the second microscope is a non-fluorescent microscope.~~

~~403.~~ The method of ~~any one of claim 31-39~~claim 1 or 2, wherein the first plurality of images is captured by a first imager.

~~414.~~ The method of claim ~~403~~, further comprising:

detecting one or more physical characteristics of the well in the first plurality of images, wherein the one or more physical characteristics of the well comprises: a shape of the well, an edge of the well, a location of the well; and

generating the first reference coordinate space based on the detected one or more physical characteristics.

~~42. The method of claim 41, wherein the one or more physical characteristics of the well comprises: a shape of the well, an edge of the well, a location of the well.~~

~~435.~~ The method of ~~any one of claims 40-42~~claim 3 or 4, wherein two or more images in the first plurality of images are offset from each other by an overlap ratio, and wherein the first reference coordinate space is generated based on the overlap ratio.

~~44. The method of any one of claims 40-43, wherein the first reference coordinate space is generated based on metadata of the first imager.~~

~~456.~~ The method of any one of claims ~~40-44~~2-5, further comprising: selecting one or more marker images from the first plurality of images based on one or more landmarks captured in the one or more images, wherein the first patch is obtained from the one or more marker images from the first plurality of images, and wherein the one or more landmarks comprise: one or more cells, one or more well boundaries, one or more beads, one or more nuclei, or any combination thereof.

~~46. The method of claim 45, wherein the one or more landmarks comprise: one or more cells, one or more well boundaries, one or more beads, one or more nuclei, or any combination thereof.~~

~~477.~~ The method of any one of ~~claim 31-46~~claims 1-6, wherein the second plurality of images captured by a second imager.

~~488.~~ The method of claim ~~477~~, further comprising:

detecting one or more physical characteristics of the well in the second plurality of images, wherein the one or more physical characteristics of the well comprises: a shape of the well, an edge of the well, a location of the well; and

generating the second reference coordinate space based on the detected one or more physical characteristics.

~~49. The method of claim 48, wherein the one or more physical characteristics of the well comprises: a shape of the well, an edge of the well, a location of the well.~~

~~509.~~ The method of ~~any one of claims 47-49~~claim 7 or 8, wherein two or more images in the second plurality of images are offset from each other by an overlap ratio, and wherein the second reference coordinate space is generated based on the overlap ratio.

~~51. The method of any one of claims 47-50, wherein the second reference coordinate space is generated based on metadata of the second imager.~~

~~5210.~~ The method of any one of claims ~~47-51~~7-9, further comprising: selecting one or more marker images from the second plurality of images based on one or more landmarks captured in the one or more images, wherein the second patch is obtained from the one or more marker images of the second plurality of images, and wherein the one or more landmarks comprise: one or more cells, one or more well boundaries, one or more beads, one or more nuclei, or any combination thereof.

~~53. The method of claim 52, wherein the one or more landmarks comprise: one or more cells, one or more well boundaries, one or more beads, one or more nuclei, or any combination thereof.~~

~~54~~11. The method of any one of claims ~~1-10~~31-53, wherein the first patch covers a center of the first image and/or wherein the second patch covers a center of the second image.

~~55. The method of any one of claims 31-54, wherein the second patch covers a center of the second image.~~

~~56~~12. The method of any one of claims ~~31-55~~1-11, wherein at least a portion of the first patch and at least a patch of the second patch correspond to the same subject.

~~57~~13. The method of any one of claims ~~31-56~~1-12, wherein the transformation parameters comprise one or more of: a translation parameter, a scaling parameter, and a rotation parameter.

~~58. The method of any one of claims 31-57, wherein the first and second images were obtained from an assay of pooled screening of cells from different genetic backgrounds, comprising:~~
~~a) labeling two or more populations of cells of different genetic backgrounds with two or more unique nucleic acid barcode sequences, each unique nucleic acid barcode sequence corresponding to a different population of cells;~~
~~b) combining the two or more populations of cells to obtain a single mixed population of cells;~~
~~c) performing *in situ* single cell sequencing on the cells; and~~
~~d) analyzing known or identifying new phenotypes of the cells in the mixed population.~~

~~59~~14. The method of any one of claims ~~1-13~~1-58, further comprising utilizing a classifier configured to receive an image and output a classification result.

~~60. The method of claim 59, wherein the classifier comprises a plurality of layers.~~

~~61. The method of claim 59 or 60, wherein the classifier is a convolutional neural network.~~

~~62. The method of any one of claims 59-61, wherein the classifier is a DenseNet classifier.~~

~~63~~15. The method of ~~any one of claims 59-62~~claim 14, wherein the classification result is a classification based on the genetic background of each single cell captured in the image.

~~64~~16. The method of ~~any one of claims 59-63~~claim 14 or 15, further comprising generating an embedding of the image.

~~65. The method of claim 64, wherein the embedding is generated from an activation output layer prior to the last layer of the classifier.~~

~~66. The method of claim 64 or 65, wherein the embedding is reduced in dimension using a linear dimensionality reduction method.~~

~~67~~17. The method of ~~any one of claims 64-66~~claim 16, wherein the embedding is reduced in dimension using a UMAP (Uniform Manifold Approximation and Projection for Dimension Reduction) algorithm to obtain one or more UMAP plots for visualization of the embedding.

~~68~~18. The method of ~~any one of claims 64-67~~claim 16 or 17, further comprising evaluating a treatment based on at least a portion of the embedding.

~~69~~19. The method of claim 17 or 18~~67 or 68~~, further comprising evaluating a treatment based on at least a portion of the one or more UMAP plots.