

CLAIMS

1. A method for measuring cellular responses to differential stimuli using optical and/or fluidic forces, wherein the method comprises:
 - receiving a selection of an initial samples comprising biological cells treated with varying known levels of stimuli or analyte,
 - performing optical force-based measurements on the samples,
 - developing a response metric (RM) to describe the cellular response to the stimuli based on one or more optical or fluidic force-based parameters.
2. The method of claim 1, wherein the response metric is used to measure the response of additional unknown samples.
3. The method of claim 1, further comprising analyzing dilutions of the sample until an accurate measurement of the infectivity is determined, based upon having an RM that falls within the acceptable target value range.
4. The method of claim 1, wherein the samples measured are cell nuclei, mitochondria, or other sub-cellular component or fraction.
5. The method of claim 1, where the optical and fluidic forces are based on laser force cytology.
6. The method of claim 1 further comprising:
 - comparing the response metric of an initial sample to a target value
 - selecting a second sample based on the results of the first and an algorithm governing the expected or known response
 - comparing the response metric of the second sample to a target value
 - selecting subsequent samples in a similar manner until a sample matching the target response metric or other defined endpoint is identified.
7. The method of claim 5, wherein the optical force-based measurements utilize laser force cytology to assess parameters comprising linear velocity, size, perimeter, size (area, diameter, volume, etc.), number of trapped cells per sample, number of beam

ejected cells per sample, number of aggregates (based upon size and/or shape or other parameters), number of debris-sized particles (based upon size and/or shape or other parameters), normalized velocity, minimum x position, optical retention time, optical trapping time, optical force, optical torque, orientation, optical and fluidic dynamics, effective refractive index, eccentricity, minor axis, major axis, deformability, eccentricity deformability, minor and major axis deformability, elongation factor, compactness factor, circularity factor, images including greyscale features, whole images, image components or image derived parameters, morphology characteristics, or other laser force cytology derived parameters.

8. The method of claim 1, wherein the biological cell comprises plant cells (algal cells or others), prokaryotic cells (bacteria), eukaryotic cells, yeast, fungus, mold cells, red blood cells, neurons, egg cell (ovum), spermatozoa, white blood cells, basophils, neutrophils, eosinophils, monocytes, lymphocytes, macrophages, platelets, vesicles, exosomes, stromal cells, multicellular constructs such as spheroids, mesenchymal cells, and induced pluripotent stem cells (iPSC).

9. The method claim 1, wherein the analyte comprises a virus.

10. The method claim 1, wherein the analyte comprises a bacterium.

11. The method of claim 1, wherein the analyte is a virus and a neutralizing serum containing antibodies (neutralization assay).

12. The method of claim 1, wherein the analyte is a bacterium and a neutralizing serum containing antibodies.

13. The method of claim 1, wherein the analyte is a toxin and antibodies in sera capable (or not) of neutralizing the toxin.

14. The method of claim 1, wherein the analyte is a virus and an antiviral compound or material.

15. The method of claim 1, wherein the cells are present in a monolayer, suspension or embedded in a matrix.

16. The method of claim 1, wherein the cells are suspended by alginate, gelatin, or other similar semi-solid suspension.

17. The method of claim 1, wherein the cells are sampled from an ongoing process and analyzed directly with no further incubation.

18. The method of claim 1, further comprising calibration objects.

19. The method of claim 18, wherein the calibration objects comprise beads, particles, biologics, lipids, vesicles, live cells, or fixed cells.

20. The method of claim 19, wherein beads or particles, comprise organic materials, polymers, metals, alloys, glass, sapphire, or diamond.

21. The method of claim 18, wherein the calibration objects are in spherical or non-spherical shapes sized from nanometers to millimeters.

22. The method of claim 18, wherein the calibration objects are mixed with one or more samples and analyzed at the same time.

23. The method of claim 18, wherein the calibration objects can be differentiated from cell samples based on brightfield image analysis of the cells, fluorescence measurements, or one or more optical force-based measurements.

24. A method for generating a calibration curve based on cellular response to varying concentrations of treatments and then using it to predict a sample of an unknown level:

adding treatments and incubating sample cells,
analyzing by fluidic and/or optical force-based measurements a plurality of samples having cells, and a known range of treatments to determine a response metric,

determining optimal response metric and time based on trend with dilution,
using generated data to predict future samples.

25. The method of claim 24, wherein the optical force-based measurements utilize laser force cytology.

26. The method of claim 24, wherein the samples measured are cell nuclei, mitochondria, or other sub-cellular component or fraction.

27. The method of claim 24, wherein the stimulus is viral infection and the concentration is viral titer.

28. The method of claim 24, optionally comprising additional analysis including univariate metrics, total population histogram data, subset population histogram data, K-means clustering, or PLS, PCA, neural network or other multivariate or machine learning algorithms to create a multivariate metric.

29. A method for generating a calibration curve based on cellular changes during the production of a biologic molecule or other ongoing bioprocess that correlates the cellular response to a product or cellular property of interest and then using the calibration to predict the results of a future process:

adding treatments and incubating sample cells,
analyzing by optical force-based measurements a plurality of samples having cells and a known range of product concentrations to determine a response metric;
determining optimal response metric based on trend with dilution,
using generated data to predict future samples.

30. The method of claim 29, wherein the optical force-based measurements utilize laser force cytology.

31. The method of claim 29, wherein the samples measured are cell nuclei, mitochondria, or other sub-cellular component or fraction.

32. The method of claim 29, wherein the product is a viral based product such as a vaccine, oncolytic virus, protein, nucleic acid, or viral vector.

33. The method of claim 29, wherein the product is a non-virally produced protein, nucleic acid, polymer or lipid.

34. The method of claim 29, wherein the cellular property is productivity, viability, or ability to produce a target molecule.

35. The method of claim 29, wherein the cellular property is differentiation state, ability to kill a specific cell type such as a tumor, ability to activate another cell type, or ability to change the biochemical state of another cell type.

36. The method of claim 29, optionally comprising additional analysis including univariate metrics, total population histogram data, subset population histogram data, K-means clustering, or PLS, PCA, neural network or other multivariate or machine learning algorithms to create a multivariate metric.

37. A method for calculating absolute titer/infectivity comprising:
analyzing by optical force-based measurements a plurality of samples having cells and a known range of dilutions of a viral stock to determine an infection metric;
identifying the sample demonstrating a maximum infection metric;
setting the maximum infection metric as 100% infected;
calculating an amount of virus added to each dilution (infectious units/mL) based on the number of cells at a time of infection, the percentage of uninfected cells, a mathematical distribution describing infection, and a volume of viral stock added at the dilution; and
using only the dilutions that fall within specified range, predicting an overall titer of an unknown sample.

38. The method of claim 37, wherein the optical force-based measurements utilize laser force cytology.

39. The method of claim 37, wherein the samples measured are cell nuclei, mitochondria, or other sub-cellular component or fraction.

40. The method of claim 37, wherein the distribution describing infection is Poisson, Bayesian, or other mathematical distribution.

41. The method of claim 37, further comprising determining both an incubation time post infection and laser force cytology parameters used to generate the infection metric when calculating a titer and creating a calibration curve from an unknown viral system with a sample of unknown titer.

42. The method of claim 37, further comprising calibration objects such as beads or particles are used to increase confidence that the instrumentation is behaving in a consistent manner.

43. The method of claim 37, wherein histograms, scatter plots, and/or multivariate data are used in their entirety or in part as the infection metric or a component of a more complex infection metric.

44. The method of claim 37, wherein K-means clustering is used to generate the infection metric.

45. A method for detecting the presence of adventitious agents comprising the use of optical force-based measurements, comprising:

- sampling cells directly from a bioreactor or other vessel without any additional incubation

- making optical force-based measurements on a population of cells

- detecting adventitious agents based on a combination of the measured cellular response and previous data sets describing the process under normal operating conditions.

46. A method for detecting the presence of adventitious agents comprising the use of optical force-based measurements, comprising:

- mixing a test sample with cells growing in suspension or adherent culture and incubating; and

detecting adventitious agents in a test sample media by laser force cytology.

47. The method of claim 46 wherein blank cell samples are used as controls to compare with the test samples.

48. The method of claim 46, wherein the condition media is obtained from a bioreactor, or other manufacturing process

49. The method of claim 46, wherein the amount of time the cells are exposed to the conditioned media can be adjusted as part of assay optimization.

50. The method according to claim 48, wherein the cells are Chinese Hamster Ovary (CHO), Baby Hamster Kidney (BHK), or other related cells or variants thereof.

51. The method according to claim 48, wherein the cells include but are not limited to Vero, HEK-293, MDCK, EB66, AGE1, WI-38, MRC-5, MARC 145, CRFK, A549, HL60, U937, SK-MEL-28, HCC2429, HEp-2, or other related cells or variants thereof.

52. The method according to claim 48, wherein the cells are HeLa cells.

53. The method according to claim 48, wherein the cells are chemically or genetically modified to be susceptible to viral infection either broadly or specifically by adding, changing, or removing genetic material or changing the biological or physical state of the cell.

54. The method according to claim 48, wherein the cells are chemically or genetically modified to be susceptible to bacterial infection either broadly or specifically by adding, changing, or removing genetic material or changing the biological or physical state of the cell.

55. The method according to claim 48, wherein the cells are chemically or genetically modified to be susceptible to viral infection either broadly or specifically by adding, changing, or removing genetic material or changing the biological or physical state of the cell and have good characteristics for measurement using LFC.

56. The method according to claim 48, wherein the cells are chemically or genetically modified to be susceptible to a toxin or specific molecule or molecular class by adding, changing, or removing genetic material or changing the biological or physical state of the cell.

57. The method according to claim 48, wherein the cells are macrophage cells.

58. The method of claim 57 wherein the macrophage cells are treated with a chemical or biochemical to affect its phagocytotic activity or cellular response.

59. The method according to claim 48, further comprising sorting out and collecting cells of interest for further analysis.

60. The method according to claim 59, wherein further analysis includes Raman spectroscopy, fluorescence spectroscopy, mass spectrometry, polymerase chain reaction, single cell sequencing, next generation sequencing, and flow, fluorescence, mass, or image cytometry.

61. The method according to claim 46, further comprising classifying the adventitious agent based on optical force-based measurements data to determine the identity of the adventitious agent.

62. The method according to claim 46, wherein the adventitious agent comprises viruses, bacteria, intracellular bacteria, mycoplasma, fungi, protozoa, parasites, or small molecules.

63. The method according to claim 46, further comprising the step of classifying the agent comprising the use of artificial neural networks, pattern recognition and predictive analytical tools.

64. The method according to claim 63, wherein the artificial neural networks use multiple laser force cytology parameters for classifying the adventitious agent.

65. The method according to claim 64, wherein the multiple laser force cytology parameters comprise linear velocity, size, perimeter, size (area, diameter, volume, etc.), number of trapped cells per sample, number of beam ejected cells per sample, number of aggregates (based upon size and/or shape or other parameters), number of debris-sized particles (based upon size and/or shape or other parameters), normalized velocity, minimum x position, optical retention time, optical trapping time, optical force, optical torque, orientation, optical and fluidic dynamics, effective refractive index, eccentricity, minor axis, major axis, deformability, eccentricity deformability, minor and major axis deformability, elongation factor, compactness factor, circularity factor, images including greyscale features, whole images, image components or image derived parameters, morphology characteristics, or other laser force cytology derived parameters.

66. The method according to claim 46, wherein multiple cell lines may be analyzed simultaneously to speed analysis.

67. A method for adventitious agent testing using optical force-based measurements, comprising:

- growing cell lines in mini bioreactors;
- pumping samples of condition media into the mini bioreactors from a large process bioreactor; and
- detecting adventitious agents in condition media by utilizing optical force-based measurements.

68. The method according to claim 67, wherein the cell lines can be suspension cell lines to spur growth and infection with any adventitious agent present in the large process bioreactor.

69. A method for correlating cell response with biological status comprising:

- receiving cells from a patient or other biological source;
- performing optical force-based measurements on cells from the patient;
- outputting optical force-based measurement data, wherein the data from the analyzed cells is used in conjunction with comparative data to indicate biological status.

70. The method claim 69, wherein the comparative data comes from the same patient under a different condition such as disease or treatment and/or point in time,

71. The method claim 69, wherein the comparative data comes from a different patient or patients.

72. The method claim 69, wherein biological status includes the diagnosis of an infection, and/or identification of a pathogenic agent.

73. The method of claim 69, wherein biological status includes the assessment of therapeutic efficacy, including vaccine or antibody efficacy.

74. The method of claim 69, wherein biological status includes assessment of immunity or drug resistance.

75. The method of claim 69, wherein biological status includes assessment of cancer, metastatic potential of cells.

76. The method of claim 69, wherein samples are collected over time from the same patient to establish a baseline of normal cell characteristics.

77. The method of claim 69, wherein the biological status includes treatment with cell or gene therapy.

78. The method of Claim 69, wherein the laser force cytology comprises the assessment of parameters including linear velocity, size, perimeter, size (area, diameter, volume, etc.), number of trapped cells per sample, number of beam ejected cells per sample, number of aggregates (based upon size and/or shape or other parameters), number of debris-sized particles (based upon size and/or shape or other parameters), normalized velocity, minimum x position, optical retention time, optical trapping time, optical force, optical torque, orientation, optical and fluidic dynamics, effective refractive index, eccentricity, minor axis, major axis, deformability, eccentricity deformability, minor and major axis deformability, elongation factor, compactness factor, circularity

factor, images including greyscale features, whole images, image components or image derived parameters, morphology characteristics, or other laser force cytology derived parameters.