

WHAT IS CLAIMED IS:

1. A cell observation method comprising:
a detection step of detecting radiation light that is radiated from an observation target when the observation target is irradiated with irradiation light; and
a determination step of determining whether a cell included in the observation target is alive or dead based on the radiation light.

2. A cell observation method comprising:
a detection step of detecting radiation light that is radiated from an observation target when the observation target is irradiated with irradiation light; and
a detection step of detecting the number of live cells included in the observation target based on the radiation light.

3. A cell observation method comprising:
an acquisition step of acquiring a spectrum of irradiation light that irradiates an observation target when the observation target is irradiated with the irradiation light; and
a detection step of detecting a state of at least one of lipid and protein included in the observation target based on the spectrum.

4. The cell observation method according to Claim 1, wherein
the detection step includes detecting Raman-scattered light radiated from the observation target when the observation target is irradiated with first excitation light having a first wavelength and second excitation light having a second wavelength that has a wavelength difference relative to the wavelength of the first excitation light corresponding to normal mode oscillation of molecules included in a cell of the observation target, and
the determination step includes determining whether a cell included in the observation target is dead or alive based on the Raman-scattered light detected in the detection step.

5. The cell observation method according to Claim 4, wherein
the Raman-scattered light includes at least one of coherent anti-Stokes Raman-scattered light and stimulated Raman-scattered light.

6. The cell observation method according to Claim 4 or 5, wherein the determination step includes comparing the detection result of the detection step to at least one of a detection result of Raman-scattered light generated from a cell determined in advance to be a live cell and a detection result of Raman-scattered light generated from a cell determined in advance to be a dead cell, to determine whether a cell of the observation target is dead or alive.

7. The cell observation method according to any one of Claims 4 to 6, wherein the detection step includes an image generation step of generating a detection image showing a frequency distribution of the Raman-scattered light in the observation target.

8. The cell observation method according to any one of Claims 4 to 7, wherein the detection step includes a step of detecting a two-dimensional distribution of Raman-scattered light in one observation plane in the observation target.

9. The cell observation method according to any one of Claims 4 to 8, wherein the detection step includes a step of detecting a three-dimensional distribution of Raman-scattered light in a plurality of observation planes in the observation target.

10. The cell observation method according to Claim 8 or 9, wherein the detection step includes a step of detecting a distribution of lipid in the observation target.

11. The cell observation method according to Claim 10, wherein the determination step includes a step of counting the number of dead cells according to the number of cell nuclei distributed near clumps of lipid.

12. The cell observation method according to Claim 8 or 9, wherein the detection step includes a step of generating a detection image that reflects detection of a distribution of protein in the observation target.

13. The cell observation method according to any one of Claims 4 to 12, wherein the detection step includes a selection step of selecting a type of molecule to

generate Raman-scattered light, according to a type of cell included in the observation target.

14. The cell observation method according to any one of Claims 4 to 13, wherein

the detection step includes a step of extracting a portion of the observation target as an observation target region and detecting Raman-scattered light in the observation target region.

15. The cell observation method according to Claim 14, wherein

the detection step includes a step of scanning a predetermined observation target region in the observation target with the first excitation light and the second excitation light, and detecting Raman-scattered light generated by the observation target region.

16. The cell observation method according to Claim 13 or 14, wherein

the detection step includes a step of displacing the observation target in a direction intersecting an irradiation direction of the first excitation light and the second excitation light, and detecting Raman-scattered light generated by a predetermined observation target region in the observation target.

17. The cell observation method according to Claim 7, wherein

the determination step includes a calculation step of performing image analysis on the detection image and calculating a ratio of at least one of live cells and dead cells.

18. The cell observation method according to Claim 17, wherein

the image analysis includes performing a Fourier transform on the detection image.

19. The cell observation method according to any one of Claims 4 to 18, wherein

the determination step further includes an output step of outputting a determination result to the outside, and

the output step includes a step of outputting a ratio of at least one of live cells and dead cells included in the observation target.

20. The cell observation method according to any one of Claims 4 to 19, wherein

the determination step includes a step of determining whether a cell is dead or alive based on a difference between detection images detected at different times for the same detection target.

21. The cell observation method according to any one of Claims 4 to 20, wherein

the determination step includes a step of determining whether a cell is dead or alive based on a distribution of shapes smaller than nuclei of cells appearing in a detection image.

22. The cell observation method according to any one of Claims 4 to 21, wherein

the determination step further includes a step of determining whether a dead cell included in a detection target died of apoptosis or died of necrosis.

23. The cell observation method according to any one of Claims 4 to 21, wherein

the determination step further includes a step of determining whether a dead cell included in a detection target died of apoptosis or died of necrosis, based on a distribution of shapes smaller than nuclei of cells appearing in a detection image.

24. The cell observation method according to any one of Claims 4 to 18, wherein

the determination step further includes an output step of outputting a determination result to the outside, and

the output step includes a step of outputting at least one of the number of live cells and the number of dead cells included in the observation target.

25. The cell observation method according to Claim 24, wherein

the output step includes a step of displaying whether each cell in the observation target is dead or alive.

26. The cell observation method according to any one of Claims 4 to 25, wherein

the determination step further includes an output step of outputting a determination result to the outside, and

the output step includes a step of sending a warning to the outside when at least one of the number of dead cells and a percentage of live cells in the observation target is greater than a predetermined threshold value.

27. The cell observation method according to any one of Claims 24 to 26, wherein

the output step includes outputting whether or not the observation target satisfies a predetermined quality standard.

28. The cell observation method according to any one of Claims 4 to 6, wherein

the detection step includes a step of generating a character sequence indicating a frequency distribution of the Raman-scattered light in the observation target.

29. The cell observation method according to any one of Claims 4 to 28, wherein

the detection step includes a first detection step of detecting a spectrum of Raman-scattered light generated from the observation target and a second detection step of detecting Raman-scattered light generated by irradiating with the first excitation light and the second excitation light corresponding to a predetermined region in a detection region of the spectrum detected in the first detection step.

30. The cell observation method according to Claim 29, wherein

the observation target includes a plurality of cell groups having a common point as a culture condition, and

the first detection step includes detecting a spectrum of Raman-scattered light generated from one cell group among the plurality of cell groups and the second detection step includes detecting Raman-scattered light generated by irradiating each of the other cell groups among the plurality of cell groups with the first excitation light and the second excitation light corresponding to a peak of the spectrum detected in the first detection step.

31. The cell observation method according to any one of Claims 4 to 30, wherein

the determination step includes determining that the observation target includes a dead cell when lipid droplets that have entered into cytoplasm or a cell nucleus are detected in the observation target.

32. The cell observation method according to any one of Claims 4 to 31, wherein

the detection step further includes an empty space detection step of detecting a refractive index distribution of the observation target and distinguishing empty spaces included in the observation target from intended detection targets in the observation target.

33. A cell observation apparatus comprising:

a detecting section that detects radiation light that is radiated from an observation target when the observation target is irradiated with irradiation light; and

a determining section that determines whether a cell included in the observation target is alive or dead based on the radiation light.

34. A cell observation apparatus comprising:

a first detecting section that detects radiation light that is radiated from an observation target when the observation target is irradiated with irradiation light; and

a second detecting section that detects the number of live cells included in the observation target based on the radiation light.

35. A cell observation apparatus comprising:

an acquiring section that acquires a spectrum of irradiation light that irradiates an observation target when the observation target is irradiated with the irradiation light; and

a detecting section that detects a state of at least one of lipid and protein included in the observation target based on the spectrum.

36. A cell observation apparatus comprising:

a detecting section that detects Raman-scattered light radiated from an observation target when the observation target is irradiated with first excitation light having a first wavelength and second excitation light having a second wavelength that has

a wavelength difference relative to the wavelength of the first excitation light corresponding to normal mode oscillation of molecules included in a cell of the observation target; and

a determining section that determines whether a cell included in the observation target is dead or alive based on the Raman-scattered light detected by the detecting section.

37. A cell observation program that causes a calculator to:

detect Raman-scattered light radiated from an observation target when the observation target is irradiated with first excitation light having a first wavelength and second excitation light having a second wavelength that has a wavelength difference relative to the wavelength of the first excitation light corresponding to normal mode oscillation of molecules included in a cell of the observation target, and

determine whether a cell included in the observation target is dead or alive based on the detected Raman-scattered light.

38. A cell sheet manufacturing method comprising:

a preparation step of dividing cells to prepare a cell line;

a culture step of culturing the cell line in a cell sheet; and

an observation step of observing the cells using the cell observation method according to any one of Claims 1 to 32.

39. The cell sheet manufacturing method according to Claim 38, wherein

the observation step includes a determination step of determining pass/fail of the cell sheet.

40. A cell sheet manufacturing apparatus comprising:

a preparing section that divides cells to prepare a cell line;

a culturing section that cultures the cell line in a cell sheet; and

an observing section that observes the cells using the cell observation method according to any one of Claims 1 to 32.

41. The cell sheet manufacturing apparatus according to Claim 40, wherein

the observing section includes a determining section that determines pass/fail of the cell sheet.

WHAT IS CLAIMED IS:

1. A cell observation method comprising:
acquiring information identifying a cell included in an observation target;
acquiring an image showing a prescribed component included the cell, based on Raman-scattered light radiated from the observation target irradiated by irradiation light; and
detecting the prescribed component in the cell from among the prescribed components shown in the image, based on the information.
2. The cell observation method according to Claim 1, wherein
the information acquisition includes acquiring a composite image having four wave mixing of the observation target, as the information.
3. The cell observation method according to Claim 1 or 2, wherein
the information acquisition includes detecting a refractive index distribution of the observation target, as the information.
4. The cell observation method according to any one of Claims 1 to 3, further comprising:
peak extraction of acquiring a frequency distribution of optical intensity of Raman-scattered light radiated from the observation target irradiated with irradiation light in a predetermined frequency range and extracting a peak corresponding to the prescribed component based on the frequency distribution, wherein
the Raman-scattered light detection includes irradiating the observation target with irradiation light corresponding to a frequency of the peak extracted during the peak extraction.
5. The cell observation method according to any one of Claims 1 to 4, wherein
the image acquisition includes acquiring an image showing a distribution of lipid and an image showing a distribution of protein, as the distributions of the prescribed components, and
the method further comprises generating a differential image by subtracting one of the image showing the distribution of lipid and the image showing the distribution of protein from the other.

6. The cell observation method according to any one of Claims 1 to 5, wherein the Raman-scattered light detection includes selecting a type of molecule to generate the Raman-scattered light, according to a type of the cell included in the observation target.

7. The cell observation method according to any one of Claims 1 to 6, comprising:

determination of determining a state of the cell based on a state of the extracted prescribed component.

8. The cell observation method according to Claim 7, wherein the determination includes determining the state of the cell by comparing at least one of a detection result of Raman-scattered light generated from a cell determined in advance to be a live cell and a detection result of Raman-scattered light generated from a cell determined in advance to be a dead cell to a detection result obtained during the Raman-scattered light detection.

9. The cell observation method according to Claim 7 or 8, wherein the image acquisition includes acquiring an image showing a distribution of lipid as a distribution of the prescribed component, and

the determination includes counting the number of dead cells based on cell nuclei distributed near clumps of lipid.

10. The cell observation method according to any one of Claims 7 to 9, wherein

the determination includes analyzing the image and calculating a ratio of at least one of dead cells and live cells.

11. The cell observation method according to any one of Claims 7 to 10, wherein

the determination includes determining a state of the cell based on a distribution of shapes smaller than cell nuclei that appear in the image.

12. The cell observation method according to any one of Claims 7 to 11,

wherein

the determination further includes determining whether a dead cell included in a detection target died of apoptosis or died of necrosis, based on a distribution of shapes smaller than cell nuclei that appear in the image.

13. The cell observation method according to any one of Claims 7 to 12, wherein

the determination includes determining that the observation target includes a dead cell if a lipid droplet that has entered into cytoplasm or a cell nucleus is detected in the observation target.

14. The cell observation method according to any one of Claims 7 to 13, wherein

the identifying the cell includes using a plurality of images acquired by detecting the same observation target at different times.

15. The cell observation method according to any one of Claims 1 to 14, further comprising:

determining whether the observation satisfies a predetermined quality standard.

16. The cell observation method according to any one of Claims 1 to 15, wherein

the Raman-scattered light detection includes irradiating the observation target with first excitation light having a first wavelength and second excitation light having a second wavelength that has a frequency difference relative to a frequency of the first excitation light corresponding to normal mode oscillation of molecules included in the prescribed component.

17. The cell observation method according to Claim 16, wherein

the Raman-scattered light includes at least one of coherent anti-Stokes Raman-scattered light and dstimulated Raman-scattered light.

18. A cell observation apparatus comprising:

an information acquiring section that acquires information identifying a cell included in an observation target;

an image acquiring section that acquires an image showing a prescribed component included in the cell, based on Raman-scattered light radiated from the observation target irradiated by irradiation light; and

a detecting section that detects the prescribed component in the cell from among the prescribed components shown in the image, based on the information.

19. A program that causes a computer to:

acquire information identifying a cell included in an observation target;

acquire an image showing a prescribed component included in the cell, based on Raman-scattered light radiated from the observation target irradiated by irradiation light; and

detect the prescribed component in the cell from among the prescribed components shown in the image, based on the information.

20. A cell sheet manufacturing method comprising:

preparation of dividing cells to prepare a cell line;

culture of culturing the cell line in a cell sheet; and

observation of observing the cells using the cell observation method according to any one of Claims 1 to 17.

21. The cell sheet manufacturing method according to Claim 20, wherein the observation includes determining pass/fail of the cell sheet.

22. A cell sheet manufacturing apparatus comprising:

a preparing section that divides cells to prepare a cell line;

a culturing section that cultures the cell line in a cell sheet; and

an observing section that observes the cells using the cell observation method according to any one of Claims 1 to 17.

23. The cell sheet manufacturing apparatus according to Claim 22, wherein

the observing section includes a determining section that determines pass/fail of the cell sheet.