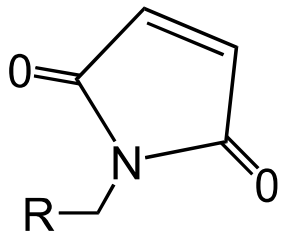
[0005] There is a continuing need to develop new and improved rapid, high throughput cell viability assays, such as those using flow cytometry. The identification of reagent compositions useful in these applications is of particular importance. The present invention addresses these and other needs.

BRIEF SUMMARY

[0006] In one aspect, the present disclosure provides methods for distinguishing dead cells from live cells in a cell sample. The methods comprise contacting the cell sample with a labelling compound of Formula I:



wherein R is a label or a label attached through a linker, wherein the labelling compound has a molecular weight of 714.69 g/mol and an excitation/emission wavelength of 402 nm/425 nm, a molecular weight of 836.78 g/mol and an excitation/emission wavelength of 556 nm/569 nm, a molecular weight of 1186.27 g/mol and an excitation/emission wavelength of 638 nm/652 nm, or a molecular weight of 1647.66 g/mol and an excitation/emission wavelength of 852 nm/877 nm, under conditions whereby the labelling compound binds free thiol groups on cell surface proteins on dead cells and live cells and preferentially crosses cell membranes of dead cells and binds free thiol groups on intracellular proteins in the dead cells; and detecting the label in the cell sample, thereby distinguishing dead cells from live cells in the cell sample by detecting increased labelling of the dead cells, as compared to live cells.

[0007] The method may further comprise labelling intracellular targets in the cells after the step of contacting the cell sample with the labelling compound. The labelling of intracellular targets can include fixing and permeabilizing the cells. In some examples, the method further comprises labelling extra cellular targets.

[0008] A number of labels can be used in the methods of the disclosure. The label may be a direct label., such as a fluorescent label.

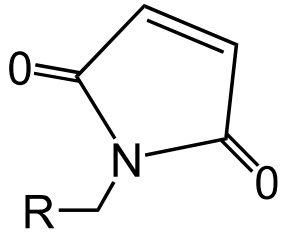
[0009] In some examples, the methods are carried under conditions that include a pH of between about 6.5 and about 7.5.

[0010] The methods of the disclosure can result in labeling of dead cells that is at least about 20 times the labeling of live cells, often at least 500 time the labeling of live cells.

[0011] In some examples, the step of contacting includes incubating the cell sample with the labelling compound for between about 10 and about 60 minutes. The step of detecting the label in the cell sample can be carried out using a flow cytometer.

[0012] The cell sample can be any of a number of types. In some examples, the cell sample is a blood cell sample, for example, from a human.

[0013] In another aspect, the disclosure also provides kits for distinguishing dead cells from live cells in a cell sample. The kits comprise a container comprising a labelling compound of Formula I:



wherein R is a label or a label attached through a linker, wherein the labelling compound has a molecular weight of 714.69 g/mol and an excitation/emission wavelength of 402 nm/425 nm, a molecular weight of 836.78 g/mol and an excitation/emission wavelength of 556 nm/569 nm, a molecular weight of 1186.27 g/mol and an excitation/emission wavelength of 638 nm/652 nm, or a molecular weight of 1647.66 g/mol and an excitation/emission wavelength of 852 nm/877 nm, one or more containers comprising reagents for fixing and permeabilizing cells in the sample, and instructions on how to distinguish dead cells from live cells in a cell sample using the labelling compound.

[0014] The label may be a direct label, for example a fluorescent label.

[0015] In some examples, the labelling compound is provided in dry form. The kit may be suitable for distinguishing dead cells from live cells in a cell sample using a flow cytometer. The kit may further comprise labelled binding agents for detecting intracellular and/or extra cellular targets in the cell sample.

BRIEF DESCRIPTION OF THE DRAWINGS

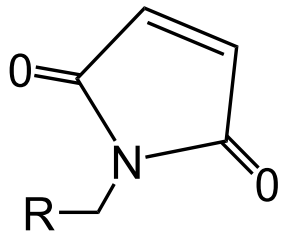
[0016] FIG. 1A shows histograms demonstrating greater fluorescence of dead Jurkat cells, as compared to live Jurkat cells as detected by flow cytometry using labelling compounds of the invention, i.e., ViaKrome 405, ViaKrome 561, ViaKrome 638, or ViaKrome 808. FIG. 1B shows the results of the same samples that were stained with NHS-ester labeled with Violet Stain, Red Stain, or Far Red Stain.

[0017] Fig. 2 shows that greater fluoresce from dead peripheral blood mononuclear cells (PBMCs) were detected as compared to live PBMCs, when the PBMCs were stained with labeling compounds of the invention, ViaKrome 405, ViaKrome 561, ViaKrome 638, or ViaKrome 808.

WHAT IS CLAIMED IS:

1. A method for distinguishing dead cells from live cells in a cell sample, the method comprising

contacting the cell sample with a labelling compound of Formula I:



wherein R is a label or a label attached through a linker, wherein the labelling compound has

a molecular weight of 714.69 g/mol and an excitation/emission wavelength of 402 nm/425 nm,

a molecular weight of 836.78 g/mol and an excitation/emission wavelength of 556 nm/569 nm,

a molecular weight of 1186.27 g/mol and an excitation/emission wavelength of 638 nm/652 nm, or

a molecular weight of 1647.66 g/mol and an excitation/emission wavelength of 852 nm/877 nm,

under conditions whereby the labelling compound binds free thiol groups on cell surface proteins on dead cells and live cells and preferentially crosses cell membranes of dead cells and binds free thiol groups on intracellular proteins in the dead cells; and

detecting the label in the cell sample, thereby distinguishing dead cells from live cells in the cell sample by detecting increased labelling of the dead cells, as compared to live cells.

2. The method of claim 1, wherein the method further comprises labelling intracellular targets in the cells after the step of contacting the cell sample with the labelling compound.

3. The method of claim 2, wherein the step of labelling intracellular targets includes fixing and permeabilizing the cells.

4. The method of claim 2 or claim 3, wherein the method further comprises labelling extra cellular targets.

5. The method of any of the preceding claims, wherein the label is a direct label.

6. The method of claim 5, wherein the direct label is a fluorescent label.

7. The method of any of the preceding claims, wherein the conditions include a pH of between 6.5 and 7.5.

8. The method of any of the preceding claims, wherein the labeling of dead cells is at least 20 times the labeling of live cells.

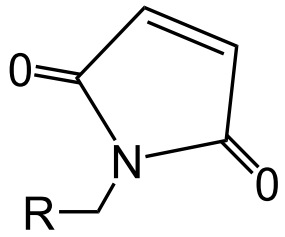
9. The method of any of the preceding claims, wherein the step of contacting includes incubating the cell sample with the labelling compound for between 10 and 60 minutes.

10. The method of any of the preceding claims, wherein the step of detecting the label in the cell sample is carried out using a flow cytometer.

11. The method of any of the preceding claims, wherein the cell sample is a blood cell sample.

12. The method of any of the preceding claims, wherein the cell sample is from a human.

13. A kit for distinguishing dead cells from live cells in a cell sample, the kit comprising a container comprising a labelling compound of Formula I:



wherein R is a label or a label attached through a linker, wherein the labelling compound has

a molecular weight of 714.69 g/mol and an excitation/emission wavelength of 402 nm/425 nm,

a molecular weight of 836.78 g/mol and an excitation/emission wavelength of 556 nm/569 nm,

a molecular weight of 1186.27 g/mol and an excitation/emission wavelength of 638 nm/652 nm, or

a molecular weight of 1647.66 g/mol and an excitation/emission wavelength of 852 nm/877 nm,

one or more containers comprising reagents for fixing and permeabilizing cells in the sample, and

instructions on how to distinguish dead cells from live cells in a cell sample using the labelling compound.

14. The kit of claim 13, wherein the label is a direct label.

15. The kit of claim 14, wherein the direct label is a fluorescent label.

16. The kit of any of claims 13 to 15, wherein the labelling compound is in dry form.

17. The kit of any of claims 13 to 16, which is suitable for distinguishing dead cells from live cells in a cell sample using a flow cytometer.

18. The kit of any of claims 13 to 17, further comprising labelled binding agents for detecting intracellular and/or extra cellular targets in the cell sample.