Rapid Characterisation of Cells by Fluorescence Microscopy and Hyperspectral Analysis

The existing problem or issue

There is a shortage of methods to rapidly identify specific cell subpopulations without the use of intrusive chemical procedures. Current methods based on biochemical, and physiological criteria, molecular DNA analysis or fluorescence labelling are complex, time consuming and/or reagent intensive.

Our solution:

We have developed an automated reagent-free method for cellular characterization able to distinguish between subpopulations of cells based on intrinsic fluorescence microscopy. The method provides a more rapid, cheaper and significantly more sensitive alternative to current approaches.

Our method uses autofluorescence, light emitted by native molecules found in all cells. In our approach the fluorescence images of live cells are obtained at a number of selected excitation wavelengths and their emission is captured in a specified, longer wavelength range. These data are obtained using a standard microscope with a CCD camera and a customised inexpensive light source comprising multiple light emitting diodes. Autofluorescence micrographs of cell populations are then analysed using custom-developed software to gather information about hundreds of quantitative features including size, circularity, intensity at each wavelength and texture. Multiple types of data analysis developed within our software enable us to:

1) Capture statistically meaningful differences between cell subpopulations. For example by using these specialised statistical tests we have been able to differentiate between healthy and diseased cells from patients suffering from a mitochondrial disease MELAS. We have also been able to distinguish the diseased cells from the cells treated with a specific drug and compare the treated cells to the healthy controls, where our method enabled us to quantify the progress towards the complete cure for MELAS.

2) Our method provides detailed insights into cell biochemistry, and we have been able to identify the abundances of several native fluorophores including free and bound NADH, flavins, retinoids and tryptophan. Their cellular maps and average cell values enabled us to detect subtle physiological features such as the metabolic rate. Trends over time, for example in stem cell differentiation experiments have been obtained.

3) We have been able to identify previously undetected cell subpopulations with respect to these abundances. These subpopulations of the cells previously regarded as homogeneous are sometimes completely separate, with no overlap.
4) The method is extremely sensitive, for example it is able to detect stem cell differentiation as early as 24 h after onset.

5) A suitable combination of autofluorescence features is highly (87%) correlated with surface expression of a commonly used surface biomarker for stem cells. Hence cells that are positive with respect to this marker do not need to be stained to be positively identified, autofluorescence alone will suffice. This is important when stem cells are selected for therapeutic use.

Diagram A distinguishes between healthy (green), MELAS (purple and cyan), and MELAS treated (red and blue) cells. Data from 3 patients.

Diagram B is a histogram of the most relevant spectral features for healthy (red) and 6% MELAS (blue) cells. Statistical methods have been applied to differentiate the distributions.

**Advantages**

1. A supremely sensitive, fully quantitative diagnostic method for distinction of cell subpopulations
2. The method provides cellular level insight into cell metabolism.
3. No cell preparation whatsoever, live cell imaging
4. Method is rapid, image collection is several minutes, data analysis speed depends on computer speed and analysis complexity, feasible using a PC.
5. Method is applicable to live tissues

**Applications**

- This invention is applicable to high throughput screening and it can be used to automatically analyse cells in cell cultures.
- We demonstrated that this method is suitable to study the effect of pharmaceutical agents.
- The method can be applied to find and/or isolate “most potent” stem cells or other cells with desirable metabolic characteristics, including most viable sperm, embryo, detect metabolic competency of neurons important in neurodegenerative diseases, etc.

**Inventors**

Prof Ewa Goldys, Martin Gosnell, Dr Ayad Anwer, Sandeep Perinchery, Dr David Inglis.

**Intellectual Property position**

“Cell Characterisation Method” provisional patent application has been filed in Australia. No. 2012904896.

**Publications**

Nil

**Would you like to know more?** Contact [Warren Bailey](mailto:warren.bailey@mq.edu.au) +61(0) 417 221 603 or warren.bailey@mq.edu.au