Multicolor Fluorescent Imaging of Cultured Cells on the Sapphire FL Biomolecular Imager: Live/Dead (AO/PI) Assays

Background/Introduction

Cell culture is an essential technique used in many types of laboratories, spanning fields including cell biology, biochemistry, molecular biology, cancer biology, immunology, and more. Mammalian cells may be grown in culture to study basic cell functions or to assess the reaction of cells to drugs, irradiation, or environmental manipulations. Alternatively, cells may be engineered to express a specific protein and cultured to achieve a sufficient number of cells to purify the protein for downstream experiments such as enzymology or structural studies.

Most cell culture experiments will require that the viability of the cells under specific experimental conditions be determined. Even if assessing the toxicity of a treatment or manipulation is not part of the experimental design, it is important to confirm that culture conditions are optimized and not unnecessarily stressful such that they cause cell death.

A common assay to measure cell viability is a dual fluorescence assay in which cells are stained with two nucleic acid–binding dyes: acridine orange (AO) and propidium iodide (PI).[1,2] As AO is cell permeable, it will enter both living and dead cells, causing them to fluoresce green. PI, however, can only enter cells with compromised membranes, such as dead, dying, and necrotic cells. Cells stained with PI fluoresce red. When both PI and AO are used, dead cells will be stained by both dyes, but will only fluoresce red because the green fluorescence emitted by AO is absorbed by PI.[3]

This application note demonstrates using the Sapphire FL Biomolecular Imager to image cells stained with AO/PI and visualize individual live and dead cells.

Materials and Methods

Cell preparation and staining

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood and used immediately. The suspension of PBMCs in EMEM was titrated in a series of 2-fold dilutions in tissue culture plates using PBS containing 2% FBS. Cells were stained by adding an equal volume of dye solution (from the CTL-LDC™ Live/Dead Cell Counting Kit, ImmunoSpot, catalog # CTL-LDC-100-2) to 10 µl of cell suspension.

After staining, 10 µl of the stained cells were transferred to a cell counting slide (from the CTL-LDC™ Live/Dead Cell Counting Kit, ImmunoSpot, catalog # CTL-LDC-100-2) for imaging.

Imaging

The cell counting slide was imaged on the Sapphire FL Biomolecular Imager using the 488 Standard Optical Module at intensity 8 to detect AO fluorescence and the 532-PI (Propidium Iodide) Standard Optical Module at intensity 5 to detect PI fluorescence. Resolution was set to 10 µm, the focus setting was set to Slide (+1.00 mm), and Image Quality was set to Highest.

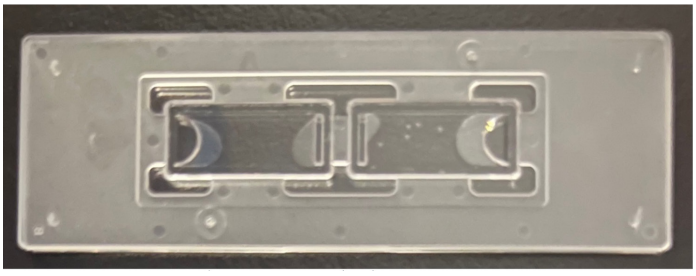

Figure 1. A cell counting slide containing two chambers.

Results and Discussion

Cells stained with AO/PI were imaged in a cell counting slide (Figure 1). Due to capillary action, the cell suspension spreads out within the chamber so individual cells can be visualized. This allows cells to be counted under a microscope and the number of cells present in the chamber calculated. For live/dead assays such as AO/PI, the number of green- and red-fluorescing cells can be counted and the proportion of cells that are alive and dead calculated.

Figure 2 shows a two-color fluorescent image of AO/PI-stained PBMCs captured on the Sapphire FL Biomolecular Imager. Living cells are stained with AO and fluoresce green. Dead or dying cells are stained with PI and fluoresce red.

In Figure 2, it is easy to distinguish individual cells and to differentiate live (green) from dead/dying cells (red).

The high-resolution and multi-channel fluorescent imaging abilities of the Sapphire FL Biomolecular Imager allow it to image single cells stained with a variety of colorimetric and fluorescent dyes. Due to the large imaging surface of the Sapphire FL, the entire cell counting slide can be imaged for archival purposes, in addition to imaging the cell suspension chamber itself at 10 µm resolution sufficient to visualize individual cells. The capacity to image one sample under up to three different fluorescence channels simultaneously allows differential staining assays like AO/PI to be carried out quickly and simply. There is no need to readjust focus or move the slide between imaging wavelengths, resulting in increased accuracy when counting and calculating ratios of differently stained cells within a single region of the slide.

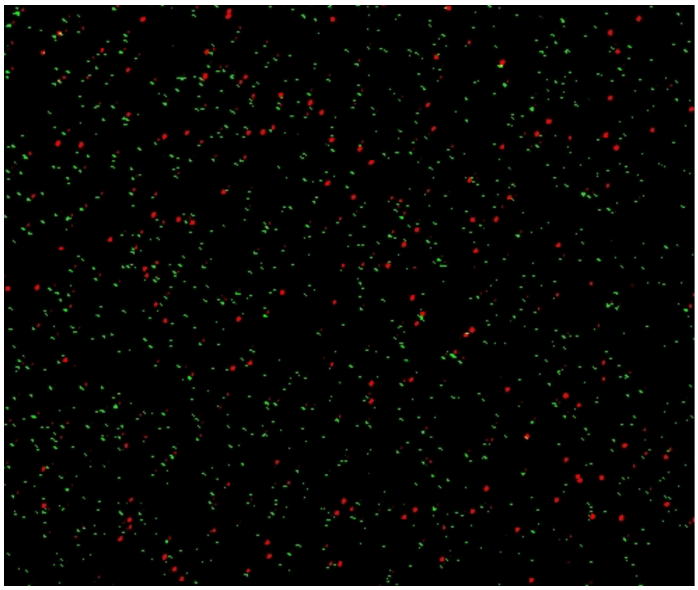

Figure 2. PBMCs stained with AO/PI were imaged on the Sapphire FL Biomolecular Imager. Living AO-stained cells appear green while dead or dying PI-stained cells appear red. Resolution 10 µm.

Figure 3. Sapphire FL is a laser scanner designed for multicolor fluorescent imaging, NIR fluorescent imaging, chemiluminescence, phosphor imaging, and more.

References

1. Bank HL. Rapid assessment of islet viability with acridine orange and propidium iodide. *In Vitro Dev Bio.* 1988;24(4):266–273.
2. Bank HL. Assessment of islet cell viability using fluorescent dyes. *Diabetologia.* 1987;30:812–816.
3. Chan LLY, Smith T, Kumph KA, et al. A high-throughput AO/PI-based cell concentration and viability detection method using the Celigo image cytometry. *Cytotechnology.* 2016;68(5):2015–2025.

MM-0151 R2