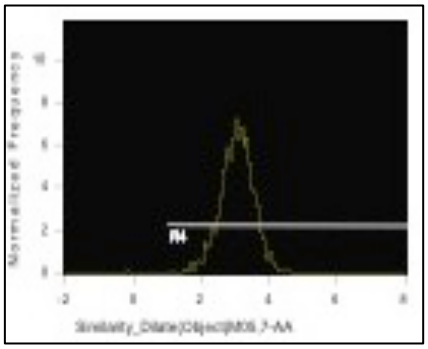
CyTRAK Orange™ in Imaging Flow Cytometry

Orange Fluorescent Live-Cell Permeant DNA and Cytoplasmic Dye

CO5.APPNOTE: IMAGING FCM 003 010814

1. NUCLEATED CELL LABELLING & NUCLEAR SEGMENTATION

BACKGROUND



Imaging flow cytometers (Amnis Imagestream and Flowsight) combine cell-by-cell multi-parameter analysis of a flow cytometer with morphological or textural analysis from a fluorescence microscope. A key feature is the ability to cross-compare phenotypically distinct cell types with morphological characteristics and so-called "similarity" analyses. It is possible to classify individual cell events from a dot-plot to further confirm their identities using bright field and individual fluorescence images and composites.

It is possible to analyse any sample suitable for an equivalent conventional flow cytometer, including blood and bone marrow samples that, of course, contain a mixture of nucleated and enucleated cells. When analysing the nucleated cells it is common to lyse the enucleated cells with NH_4Cl or similar. This is mostly preferable since the event analysis rate is somewhat lower than a conventional flow cytometer.

WHAT IS THE PROBLEM?

As in fluorescence microscopy one is able to view individual cells. One can potentially segment the cells into the nuclear and cytoplasmic compartments. However, this requires the addition of a fluorescent DNA counterstain. The ideal DNA counterstain to segment nuclei should meet all of the following criteria: show discrete nuclear staining; be spectrally separated from commonly-used chromophores; work in live or fixed cells; report DNA content; be cross-platform compatible for higher resolution or routine applications.

Despite the relatively slow event analysis rate it may be inappropriate to perform red cell lysis (RBC) lysis on blood / bone marrow e.g. for small samples, rare event analysis or studying erythropoietic / thrombopoietic pathways or leukocyte-platelet aggregation.

HOW DOES CyTRAK Orange™ HELP?

Orange/red fluorescing cell-permeant DNA-binding probe CyTRAK Orange™ discretely segments the nucleus from the cytoplasm in imaging flow cytometry in live or fixed cell experiments, for detailed morphometric analyses. With high gain settings it is possible to achieve differential counterstaining of both nucleus and cytoplasm with this single probe. It can be combined with UV/violet-excited, FITC/GFP-like and red-excited chromophores for multi-colour experiments, also making it cross-platform compatible.

CyTRAK Orange™ is cell permeant. It can be added to a complex sample such as blood / bone marrow to rapidly label nucleated cells allowing these to be identified from enucleated cells without time-consuming and risky RBC lysis – especially for precious or fragile samples.

CyTRAK Orange™ Product Features:

- Orange-fluorescing cell-permeant dsDNA probe
- rapidly and clearly labels all nucleated cells (live or fixed)
- single-channel dual compartment (nucl:cyto) segmentation
- compatible with Horizon BV / BUV, FITC/GFP & red-excited dyes
- water-soluble; ready-to-use from the fridge

For a full price list and further information see **www.biostatus.com** or contact us at:
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