Application note No. 4001

Xcyto®

Mask staining of adherent cells

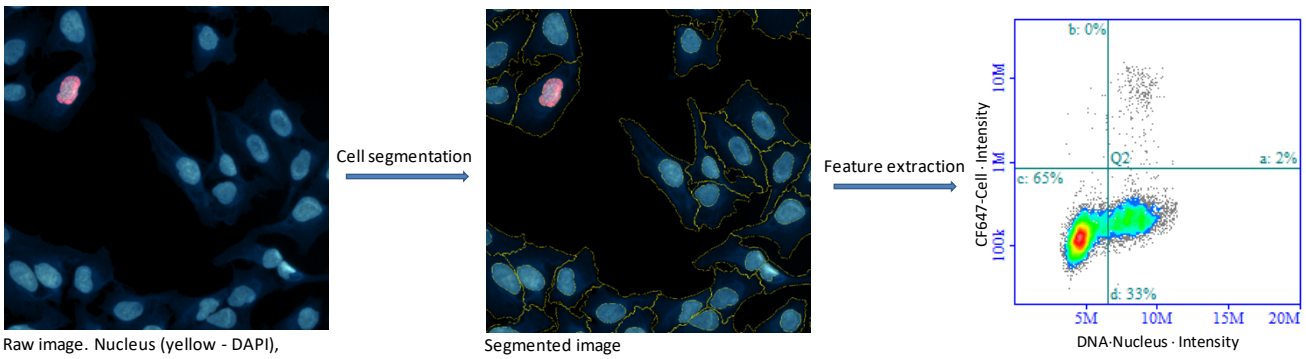
Application

This protocol provides a simple and robust method for fluorescently labeling nuclei and cytoplasm of adherent cells, thereby enabling easy detection and segmentation of cells and their nuclei (see figure 1). Information about morphology and user-defined fluorophore intensities are provided for each individual cell. Furthermore, this protocol enables the user to quantify DNA content of mammalian cells, thus allowing determination of G_0/G_1, S and G_2/M cell cycle phases.

Introduction

Fluorescent microscopy is an essential tool in the field of biology. The ability to visualize the precise distribution of biomolecules within a single cell and the nucleus has provided scientists with vital information of cellular mechanisms for many decades. However, most fluorescent microscopy based assays are very labor intensive and demand operators with specialized training. Thus a high demand for automated fluorescent microscopes (image cytometers) and automated analysis exist. Several systems for automated image acquisition have been developed and they can readily generate vast amounts of image data. The major bottleneck within the field of image cytometry is correct segmentation of single cells and their nuclei in the acquired images. Several studies have been focusing on this problem and can in general be divided into two approaches. The first approach is based on staining the cytoplasm and the nuclei with two spectrally different fluorescent dyes. This method readily enables segmentation of the cells and nuclei. However, an often limiting parameter in fluorescent microscopy is the number of available excitation light sources and emission filters. Thus the use of two spectrally different fluorescent dyes to detect the cytoplasm and the nuclei, limits the available fluorescent channels to visualize the biomolecules of interest. The second approach relies on determining the cell outline by light microscopy images such as bright field, phase contrast and differential interference contrast (DIC). For suspension cells this approach is reliable, however images of adherent cells often have strongly reduce contrast at the cell borders. This is further complicated in many assays depending on fixation of the cells that further reduces the cellular contrast.

This protocol describes a segmentation method based on fluorescent microscopy, which only requires one excitation channel and, thus, maximizes the number of available channels. This is possible due to the broad emission spectrum of the nuclei stain and the narrow emission spectrum of the cytoplasm stain used in this protocol. The whole cell is imaged in a channel using LED405 nm as excitation and detecting blue light with the 430-475 nm emission filter. The nucleus is imaged in another channel using LED405 nm as excitation and detecting yellow light with the 573-613 nm emission filter.



Raw image. Nucleus (yellow - DAPI), cytosol (dim blue – BlueMask), phospho-histone H3 Ser10 (red – CF647)

Segmented image

Fig. 1. HeLa cells stained with BlueMask-1™, DAPI and anti phospho-histone H3 Ser10 (CF647). Stained cells were imaged and analysed in Xcyto® 10 at 20x magnification.

Procedures

Mask staining is performed on permeabilized fixed adherent cells grown either on a Millicell EZ SLIDE or on a round cover slip. The cover slip has to measure Ø≥15mm and the cell confluence should be in the range 10% to 80%. Methanol, NBF or paraformaldehyde can be used as fixative. Permeabilization of the cells is necessary for optimal mask staining. The fixed cells can be stained by a user defined protocol prior to mask staining e.g. immunofluorescence staining. If protein degrading steps have been used prior to the mask staining, increase the concentrations of the BlueMask-1™ dye.

Important notes! Do not store samples more than one month prior to mask staining, and analyze samples within 2 days after mask staining. Moreover, avoid repeated thaw freeze cycles of the masking stain. Thus, it is strongly recommended to aliquot the BlueMask-1™ dye in order to ensure activity and assay performance.

Materials

- Permeabilized fixed cells on a round cover slip (Ø ≥ 15mm, #1.5 thickness) or on a Millicell EZ SLIDE*
- Phosphate buffered saline (PBS)*
- Mounting media*
- Cover slips for the Millicell EZ SLIDE (≥ 50mm X 22mm, #1.5 thickness)*
- Xcyto® 2-Sample Slide Type 15-A
- Solution 21 (BlueMask-1™ 1000X)
- Solution 12 (500 µg/ml DAPI)

* provided by the user

Mask staining protocol

1. Wash the permeabilized fixed cells 3 times for 2 min in PBS. (**NB! It is important to remove excess of blocking protein from user defined staining protocols**)
2. Prepare the mask staining solution. Dilute Solution 21 (BlueMask-1™ stock) 1:1000 and Solution 12 1:250 (final concentration 2 µg/ml) in PBS (**NB! Do not add any blocking protein to the mask staining solution**).
3. Apply the mask staining solution so it completely covers the cells and incubate in the dark for 30 minutes at room temperature.
4. Wash the fixed cells 3 times for 2 min in PBS.
5. Mount cover slips on the Xcyto® 2-Sample Slide Type 15-A or on the Millicell EZ SLIDE and seal with nail polish.
6. Prior to analysis clean the cover slip and slide carefully with ethanol.
7. Engage the Xcyto® image cytometer by starting the XcytoView™ software.
8. Place the mounted slide on the tray of Xcyto® and set-up the analysis
    a. In the setup window select “Adherent – LED405 Fluorescent” as image analysis method and add the channel(s) required for detection of the user-defined fluorophores. Save protocol.
    b. In the main window select your sample media and press the run button.
9. Cells are automatically imaged and analysed. Numerical cell data are presented in the Plot Manager.

Handling and storage

For handling and storage of ChemoMetec instruments, reagents and slides refer to the corresponding product documentation. For other reagents refer to the material data sheet from the manufacturer of the reagents and chemicals.

Warnings and precautions

For safe handling and disposal of the ChemoMetec reagents and slides refer to the corresponding product documentation and the Xcyto® image cytometer user guide. For other reagents refer to the safety data sheet from the manufacturer of the reagents and chemicals required for this protocol. Wear suitable eye protection and protective clothes and gloves when handling biologically active materials.

Limitations

The Xcyto® instruments are FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE. The results presented by the Xcyto® system depend on correct use of the reagents, slides and the Xcyto® instrument and might depend on the type of cells being analyzed. Refer to the Xcyto® user guides for instructions and limitations.

Liability disclaimer

This application note is for RESEARCH PURPOSES ONLY. It is not intended for food, drug, household, or cosmetic use. Its use must be supervised by a technically qualified individual experienced in handling potentially hazardous chemicals. The above information is correct to the best of our knowledge. Users should make independent decisions regarding completeness of the information based on all sources available. ChemoMetec A/S shall not be held liable for any damage resulting from handling or contact with the above product.

Product disclaimer

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