Cell Labeling via Photobleaching

Loïc Binan (loic651@hotmail.fr)
Université de Montréal
Santiago Costantino (santiago.costantino@umontreal.ca)
Hôpital Maisonneuve-Rosemont, Université de Montréal
Javier Mazzaferri
Hôpital Maisonneuve-Rosemont
Claudia Kleinman
Lady Davis Institute, McGill University

Method Article

Keywords: CLaP, Imaging, labelling, tagging, fluorescence marker

Posted Date: June 28th, 2016

DOI: https://doi.org/10.1038/protex.2016.018

Abstract

This protocol describes the technique Cell Labeling via Photobleaching \(CLaP) for adding fluorescent tags to individual cells in a microscopy field based on their shape, position, behavior, or any other visual feature. The procedure tethers biotin molecules to the cell membrane using a focused laser beam. Biotinylated cells are subsequently tagged by adding fluorescent streptavidin conjugates to the culture medium. The method can be used for tracking cells, and it can be combined with flow cytometry or microfluidics-based single-cell capture for genomic or other biochemical analysis. The whole procedure typically takes one hour, plus one extra minute for each tagged cell.

Introduction

Most methods for labeling cells with fluorescence rely on previous knowledge of cell markers, transfection of reporter genes, or biochemical characteristics that are common to a subset of cells within a sample. CLaP, on the other hand, allows to label cells based only on visual features; fluorescent tags can be added to any cell of choice with single cell precision. The method permits combining image-based selection with automated cell sorting methods, thus allowing experiments accounting for cell context or time evolution, such as transcriptomic profiling preserving spatial information. The technique can be implemented using off-the-shelf reagents, and may be performed on a standard confocal microscope.

Reagents

• Biotin-4-fluorescein, B4F \(Sigma Aldrich, B9431-5MG). Prepare the stock solution at 2mg/mL in PBS. Store at -20ºC. • Alexa-647-Strepatavidin \(ThermoFisher Scientific, S-21374). Prepare the stock solution at 1.8mg/mL in PBS. Store at -20ºC. • Cell culture media. Mix 45% DMEM \(ThermoFisher Scientific, 11995073), 45% F12 \(ThermoFisher Scientific, 11765-062), 10% FBS \(ThermoFisher Scientific, 2483-020). Add penicillin neomycin gentamicin mix \(ThermoFisher Scientific, PSN 15640-055) to a final concentration of 1%. • Glass bottom dishes \(Mattek p35GC-1.0-14-C)

Equipment

• Confocal inverted microscope that allows brightfield and epifluorescence imaging. • Top stage incubator for microscope \(Tokai hit) • Objective with 0.7 numerical aperture \(Zeiss LD Plan-NEOFLUAR, 441370-9970) • Power meter \(Thorlabs PM204)

Procedure

1. Find the location of the confocal scanning region relative to the camera coordinates: let dry a drop of B4F solution on a glass bottom dish to produce a fluorescent thin layer on the cover slip. After focusing the sample with epifluorescence, set the confocal microscope to scan a square region of $_{10 \times 10 \mu m2 \text{ with}}$ 1mW power after the objective for 3-10 seconds. Switch back to epifluorescence mode, and note the position of the photobleached area within the camera image. TIP: Alternatively, a fluorescent thin layer can be fabricated using a mix of fluorescein and transparent nail polish. Spread the mix on a cover glass using a razor blade to

obtain a thin coating. After drying, the cover glass can be mounted on a microscope slide using aqueous mounting medium for long term use. 2. Use a power meter to adjust the power of the confocal laser between 100μW to 350μW at the exit of the objective. 3. Plate cells on a glass bottom dish and allow them to attach and spread for 12-24h. Keep the culture healthy since unstable cell membranes can uptake the tag non-specifically. 4. Dilute 20 μL of B4F stock solution into 980 μL of cell culture media, and add to the cells. 5. Use bright field microscopy to find the cell of interest, and move the stage until the scanning region \(step 1) falls within the cell. Refine the cell position ensuring the scanning region does not overlap with neighboring cells. Focus near the cell top membrane. 6. Irradiate with 100 μW to 350 μW during 2 seconds. 7. Go back to step 5 to tag other cells. 8. Rinse 3 times with pre-warmed cell culture media at 37ºC. 9. Dilute 20μL of Alexa-647-streptavidin stock solution into 680μL of cell culture media, add to the cells and incubate at 37ºC, 5% CO2 during 15 minutes. 10. Rinse 3 to 6 times with pre-warmed cell culture media. 11. For multi-color staining, go back to step 4. At step 9, replace Alexa-647-streptavidin with a different color streptavidin-conjugate. TIP: In order to observe also non-CLaPped cells, the sample can be incubated with wheat germ agglutinin \(WGA) fluorescent conjugates to stain the cell membrane of all cells.

Timing

The whole procedure requires 30 minutes of preparation, 30 minutes of incubations and rinsing, and approximately one extra minute per cell for aiming the laser and irradiating.

Troubleshooting

The Alexa-647 tag is not visible with epifluorescence microscopy. • Improve the focus on the top cell membrane during irradiation. • Increase laser power. • Increase irradiation time. Non-specific fluorescent puncta are visible in the Alexa-647 channel. This is usually due to poor cell viability.

Anticipated Results

Tagged cells can be observed using fluorescence microscopy, as shown in Figure 1. Tags are generally distributed along the cell membrane; they can sometimes be observed on the substrate surface as well \(e.g. green square in Fig. 1). See figure in Figures section.:http://www.nature.com/protocolexchange/system/uploads/4327/original/20150314Figure1_JM.jpg?1458134443

Acknowledgements

We thank all the previous undergraduate and graduate students who have completed a project or a thesis in the laboratory and helped to build the foundations of these protocols. The project was partly supported by the Natural Sciences and Engineering Research Council of Canada, the Canadian Institutes of Health Research, the NSERC CREATE program in NeuroEngineering, and the Canada Foundation for Innovation grants awarded to S.C.

Figures

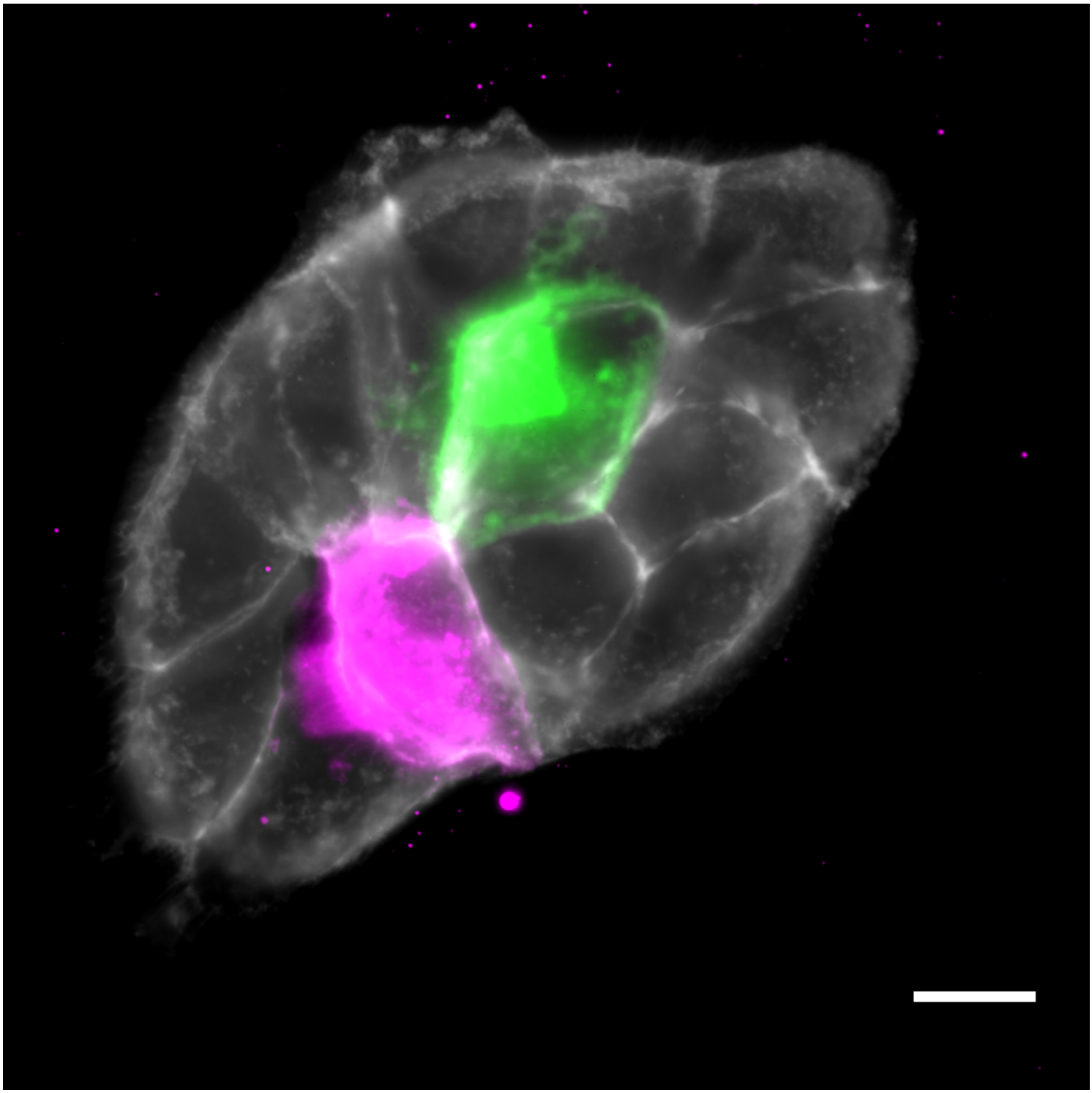

Figure 1

Two-color CLaP Figure 1 Two cells tagged with different fluorophores observed using epifluorescence. The gray labeling corresponds to Alexa-350-WGA membrane staining. Scale bar is 25 μm.