3D-Timelapse imaging of partially photoconverted Histone H2B-mMaple3

Introduction

A major advantage of fluorescent proteins (FPs) is that the fluorescence is an intrinsic property of the protein itself and does not require any cofactor. This also means that the fluorescent properties are entirely encoded in the gene of the fluorescent protein. This is important, because it is very difficult to deliver proteins into cells, whereas introducing DNA is relatively straightforward. Upon delivery of the FP gene, the cell itself will produce the fluorescent protein. Fusion proteins can be made by ligating the FP gene to the gene of a protein of interest.

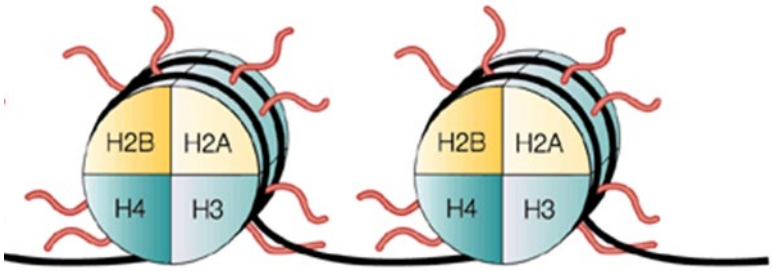


Figure 1. The core proteins of nucleosomes are H2A (histone 2A), H2B (histone 2B), H3 (histone 3) and H4 (histone 4). H2A and H2B form dimers, as well as H3 and H4. Of each dimer two copies are present, together forming an octamer of histones — the core nucleosome.

In this experiment you will use U2OS cells stably expressing Histone H2B – mMaple3 fusion protein and follow these cells through cell division. Since the DNA is wrapped around the histone complex, H2B-mMaple3 acts as a DNA marker. The fluorescent protein mMaple3 is initially green fluorescent. However, upon illumination with 405nm light mMaple3 is photoconverted from green to red fluorescence. This conversion is irreversible, so any H2B-mMaple3 protein that is photoconverted will stay red fluorescent.

The aim of this practical is to setup a 3D multi-color timelapse experiment, during which cells are followed through cell division. You will need to make a 3D confocal Z-stack every 10 minutes. Because recording of the Z-stack only takes a few minutes, you can image cells at multiple locations in the sample to increase the number of cells followed over time.

At the start of the experiment cells are selected and half their nuclei are photoconverted to create nuclei that are half green and half red fluorescent (figure 3). These cells are followed through cell division.

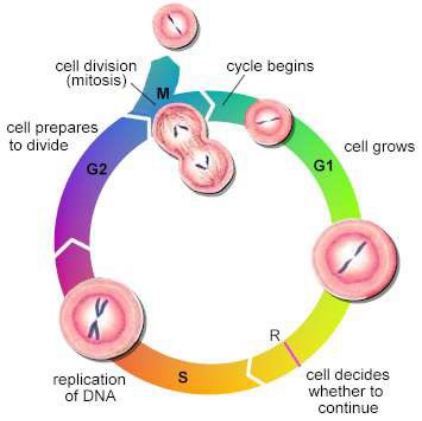


Figure 2. Stages of the cell cycle.

Steps to consider during experimental setup:

- Beam path settings, including laser powers, emission filters and detector sensitivity
- Image settings, including nr of pixels, pixel size, zoom setting, averaging,
- Z-stack settings
- Photoconversion settings
- Timelapse interval
- Multiple positions: how many positions can you image within one time interval?
- Autofocus routine

Example of Imaging settings

40x 1.25NA Oil imm objective

Image size 1024x1024 pixels

Pinhole 1AU or larger?

BiX scanning active

Green fluorescence: Ex 488nm 3%, Em 500-550nm

Gain ???

Red fluorescence: Ex 561nm 3%, Em 570-625nm

Gain ???

Z stack 6 planes, deltaZ 1um

Time interval 10 minutes

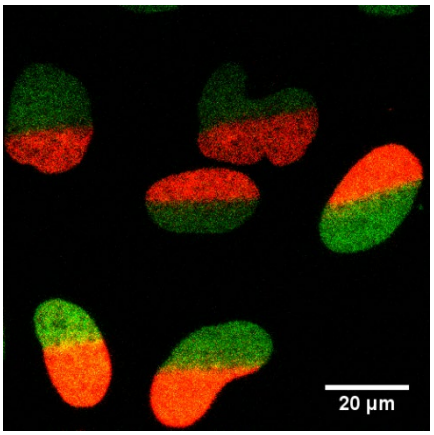


Figure 3. Partially photoconverted nuclei of U2OS cells expressing H2B-mMaple3.

Questions to consider during experimental setup:

1. How can you determine the laser power and detector gain required for imaging the photoconverted red fluorescent H2B-mMaple3?
2. Why do you have to consider the laser powers used for imaging?

Questions to answer in the report:

1. Do you expect the daugther cells to have the same green/red pattern? And if so, will this pattern be similar to the mother cell?
2. Can histone proteins diffuse through the nucleus, or are they immobilized by the DNA wrapped around them?
3. Estimate the duration of the cycle of cell division, based on the number of cell dividing during the time-lapse experiment