Live and Fixed Cell Imaging using JuLI™ Stage Real-Time CHR (Cell History Recorder)

A. Fixed Cells

The goal of fixation is to halt your cells decomposition and freeze cellular proteins and subcellular structures in place.

Treating cells with **paraformaldehyde (PFA)** leads to the establishment of chemical cross-links between free amino groups. When the cross-links join different molecules, a latticework of interactions occurs that holds the overall architecture of the cell together.

METHOD

1. Prepare fresh solution of paraformaldehyde 4% in PBS
   Add 4mg of PFA in 10mL PBS and mix for 45 minutes at 65^{o}C.
2. Aspirate media from the wells
3. Wash 3x with 50µL PBS
4. Add 50µL PFA per well for 20 minutes at room temperature
5. Aspirate PFA
6. Wash 3x with 50µL PBS
7. Add 20µL of first fluorescent dye diluted at desired concentration in PBS
   e.g. Add 1µL of Hoechst stock solution 10mg/mL in 5mL PBS for final concentration 2µg/mL
8. Wash 3x with 50µL PBS
9. Add 20µL of second fluorescent dye diluted at desired concentration in PBS
10. Wash 3x with 50µL PBS
11. Add 20µL of third fluorescent dye diluted at desired concentration in PBS
12. Wash 3x with 50µL PBS
13. Screen using JuLI™ Stage

B. LIVE CELLS

The study of living cells using time-lapse imaging is frequently referred to as “live-cell imaging”. The time lapses between the pictures can be anywhere from milliseconds to days, depending on what processes are being observed during the experiment. By using a live-cell imaging technique, you can study the dynamic biological processes your target is involved in; this is in contrast to fixed-cell imaging, where the cellular structures are frozen at a single point in time and cellular activity is at a standstill. In order to study active biological processes, you will need to create and maintain the conditions that keep cells functioning and relatively healthy during their time on the microscope.

METHOD

1. Prepare Imaging culture media buy adding fluorescent dyes to cell culture media at desired final concentrations
2. Incubate for appropriate time for each dye
3. Screen using JuLI™ Stage

C. LIVE/DEAD imaging protocol

1. Prepare staining solution. For each well of a 96-well plate, you need 30 ul of staining solution. Staining solution contains Calcein AM (0.5 to 1 uM), PI (1 to 2 uM) and Hoechst33342 (1-2 ug/ml). Dyes should be dissolved fresh in serum-free medium. CAUTION: PI and Hoechst are mutagens!!!
2. Aspirate the medium from each well and add 30 ul staining solution
3. Incubate 30 to 45 min in the 37oC incubator
4. Aspirate and discard staining solution from each well. add 50-100 ul fresh medium
5. Image at JULI. CalceinAM is at the GFP channel, PI is on the RFP channel and Hoechst at DAPI
6. Process cells. i) Use some image processing algorithm to identify nuclei (e.g. julistat, imagej). ii) For each nucleus calculate the mean PI, and the mean hoechst signal. Use the [mean PI]/[mean hoechst] ration to pick cells that are dead (significant PI).

Fluorescent dyes available in our lab:

Dye	What stains	Ex/Em	Channel	Stock Concentration	Fixed/Live Cell Imaging
Hoechst	ds DNA	350/461	DAPI	10mg/mL and 0,2 mg/mL	Both
Nile Red	Lipids/neutral lipid droplets	552/636	RFP	1mg/mL and 4mg/mL	Both
Calcein AM	Cell viability	493/516	GFP	1mM	Live
DAF-FM	Nitric Oxide	495/518	GFP	1mM	Live
Mitotracker	Mitochondria	578/600	RFP	1mM	Both
CM-H2DCFDA	Reactive Oxygene Species (ROS)	492–495/517–527	GFP	20x50g	Live
Monochlorobimane (mBCl)	Thiols (glutathione, N-acetylcysteine, mercaptopurine, peptides and plasma thiols)	394/490	DAPI	25mg	Live
PI (Propidium iodide)	DNA, RNA (membrane impermeant)	535/617	RFP	1.0 mg/mL	Both