Immunofluorescence Labeling of Cardiomyocytes and Image Acquisition (Kranias laboratory)

Seeding the cells:

-Laminin covered slides/chamber preparation:

-add 100-200 ul of laminin stock to 10 ml of cold phosphate-buffered saline (PBS).
-Cover coverslips or chambers with 1ml of laminin buffer.
-Incubate at room temperature (RT) for 1 h.

-**Isolated heart myocytes from adult mouse hearts**

-Add cardiomyocytes to the chambers, incubate for 30 minutes at RT.
NOTE: Make sure that the cells are suspended in Tyrode buffer + 1.2mM Cacl with either BDM or blebbistatin to relax the cells for better attachment.

-NO WASH!! Go to next step.

Fixing:

-Fix the isolated cells with 4% paraformaldehyde in PBS for 15 min at RT.
-NO WASH!! Go to next step.

Quenching paraformaldehyde:

-Quench the cells with 100mM glycine +PBS for 10 minutes at RT.
-Wash 5X with PBS

Permeabilization :
-Permeabilize the cells with 1% Triton-X 100 plus PBS for 10 minutes in 37 degree C.

-Wash 5X with PBS.

Blocking:

From here on: the slides should be kept in a humid chamber, which is basically a covered container or petri dishes etc with water wetted filter paper or paper towel to keep the slides in "humid environment".

- Incubate the cells (for blocking of the nonspecific binding) for 1 hour at RT or O/N at 4 degree C using the blocking buffer containing:

  -2% goat or donkey serum
  -2% BSA
  -0.1% Triton X-100

- In PBS

-Wash 3X with PBS

Double labeling with primary antibodies:

-Incubate the cells with specific primary polyclonal and monoclonal antibodies in blocking buffer overnight at 4 degree C. Antibody amount needs to be determined for every single antibody!! Usually, 1:50 to 1:100 concentration is a good combination.

- Wash 5X with PBS.

Double labeling with secondary antibodies

From here on: every step must be done in the DARK.

-Incubate with secondary Abs Alexa 488 anti-rabbit IgG and Alexa 594 anti-mouse IgG (or any other secondary antibody depending on the primary antibody) in blocking buffer. Usually 2 mg/mL or about 1:500 works well.

-Incubate O/N at 4 degree C.

-Wash 5X with PBS

Mounting the slides

- Add drop of Vectashield (with DAPI) and place coverslip on top.

-Seal them with the clear nail polish.

Acquire images by confocal-microscope.