Calcium transients

How to visualize calcium transients using Fluo4-AM dye and acquire BF videos to analyze contraction and relaxation speed.

Protocol

Dye loading

1. Change media 24 hours before acquisition
2. 30min before acquistion start heating confocal
3. 30 min before acquisition, load with the Fluo-4AM dye:

- Confluent cells - clusters: 2.5uM (1to1000)[1]
- Single cells: 1.25uM (1to2000)

4. Prepare intermediate stock of dye in RPMI(+): 1to10 dilution
5. Dilute intermediate stock 1to100 (clusters) or 1to200 (single cells) in the final media in the well
6. 15min before acquisition move to 37C confocal, Heating on, CO2 on
7. Let the cells settle and resume beating for 15-30min[2]
8. Find the beating clusters/cells positions using BF and save positions

Imaging

For imaging on ZEISS use live mode with beam splitter NTF 565.
This enables imaging of BF and fluorescence with the same setup but not at the same time. * BF: L1 channel * Fluo4: L2 channel

Acquisition:

- Objective: 40x long
- DImensions: 512x512 pxs
- FPS(frames per second): 60
- Scanning: unidirectional[3]
- Averaging: 2
- Bit depth: 12
- Timeseries: 900 images (30 seconds)

BF imaging settings (channel L1)[4]

- Pinhole: 15
- Transmitted light around 60% (keep the “Display” histogram to the right but avoid saturation)

Fluorescence imaging settings(L2):

- Laser Power: 4-8
- Pinhole: 100

[1]The higher the concentration of the dye, the more likely the cells will stop beating, the confluent cells/clusters are more resistant to the higher concentration of the dye

[2]Fast movement (transfer of the cells, stage movement) can stop the beating, cells usually resume beating in 15min

[3]Bidirectional scanning has the potential to increase fps, but needs to be tuned in order not to mess up the analysis unidirectional is a safe way to go

[4]These videos can be analyzed with the ContractionWave software (Scalzo…Guatimosim; 2021; Cell Rep Methods, link)

Procedure

1. Find the spot in BF Locate mode using okulars

Acquire BF videos:

1. Switch to Acquisition mode
2. Activate Channel L1
3. **Switch off** the laser (489)
4. **Switch on** transmitted light
5. Activate *Continuos* acquisition and set light and focus
6. Acquire BF timeseries
7. Save file

Acquire Fluo-4 videos[5]

1. **Switch off** Channel L2
2. **Switch on** channel L1
3. **Switch off** transmitted light
4. **Switch on** laser 489
5. Acquire FLuo-4 timeseries
6. Move to another beating point

Material

Fluo-4AM

Product

- Cat number: F14201
- Lot: 2528063
- Producer: Thermofisher scientific
- MW =1000
- Amount in vial: 50ug
- Product link
- Datasheet to download link-not-working yet

Stock diluted

- Aliquots: 4uL
- Storage (-20C):
  - MBD cell culture (A.035)
  - Freezer 20F5, shelf E1
  - Falcon with aluminium foil labelled Fluo-4 in the green box for 50mL falcons

Stock not diluted

- Storage (-20C):
  - Freezer room I (A037)
  - Freezer 20F2, shelf A3
  - In a light tight back of Rhod-3 Kit
  - Amount in vial: 50ug

[5]Calcium signal may need to be acquired faster then 30fps, so you can bin the resolution and enable bidirectional scanning to increase temporal resolution

Stock preparation

- Dissolve in DMSO in sterile conditions (in the cultivation hood)
- Final Conc: 2.5mM (20uL of DMSO added to 50ug of dye)
- Aliquot in 4uL aliquots

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