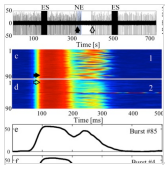

# Calcium imaging

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Working

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ABSTRACT

Calcium imaging - recording the calcium dynamics of cultures using fluorescent calcium dye, fluorescence microscopy and a camera.

PROTOCOL STATUS

**Working**

We use this protocol in our group and it is working

## Calcium imaging set up

1 Prepare the set up as follows:
Perform time lapse data at 2x2 binning mode with a resolution of 500x502 and 51.948 frames per second, taken with an Olympus upright microscope (BX51WI) fitted with an EMCCD camera (Andor Ixon-885) and a 20x water immersion objective (Olympus, UMPLFLN 20XW NA 0.5). Fluorescent excitation is delivered by a 120-W mercury lamp (EXFO x-cite 120PC) coupled with a dichroic mirror with a filter to match the dye spectrum (Chroma T495LP). Camera control utilizes the Andor propriety SOLIS software (to download see http://www.andor.com/downloads).

## Wash culture before insertion of calcium dye

2 Replace 1 ml of growth medium with 1 ml ACSF, and then replace again

NOTE

ACSF medium containing 10 mM HEPES (pH 7.4), 4 mM KCl, 1.5 CaCl2, 0.75 mM MgCl2, 129 mM NaCl, and 10 mM D-glucose.

1 ml the ACSF.

## Insertion of calcium dye

3 Incubate the cultures for 35 min in 1 ml ACSF supplemented with 1 µl of 10% pluronic acid F-127 (Biotium59000) and 1 µl Oregon-Green BAPTA-I AM previously diluted with 6.7 µl anhydrous DMSO.

## Wash culture after incubation

4 Wash 3 times with ACSF medium

## Recording

5 Place the culture under the microscope, adjust the focus and the intensity of light needed for good vision of the calcium dye. Start recording.

## Pharmacology - chemical appliacation

6 Optional: during recording replace 1 ml of the recording medium with a ACSF containing the chemical in the wanted concentration, e.g. 50µM norepinephrine. Timing of the pharmacological treatment can be recorded as a manual trigger by MC-Rack software when the set-up is combined with MEA set-up (see MEA protocol above). See MC-Rack manual for detailed explanation of manual triggers.

## Electrical stimulation

7 For a set up combined with MEA system (see electrophysiology protocol) the culture is plated on MEA and during recording it is placed under the microscope on MEA amplifier. Electrical stimulation can be applied as detailed in the MEA recording and stimulation protocol (electrophysiology protocol).