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Calcium imaging in human dorsal root ganglia neurons

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We use this protocol and it's working

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Abstract

Recording capsaicin and high K^+ induced calcium dynamics using Fluo-4 AM dye in cultured human dorsal root ganglia neurons treated with human interferon alpha (hIFN-α) or human interferon beta (hIFN-β).

Guidelines

All steps described in the protocol must be performed while wearing appropriate PPE, including a lab coat and gloves. Human neuronal cultures should be handled with proper safety precautions while handling and disposing.

Materials

Solutions:

Recording solution (pH 7.4; 300-310 mOsm):

- 130 mM NaCl
- 4.2 mM KCl
- 1.1 mM $CaCl_2$
- 1 mM $MgSO_4$
- 0.45 mM $NaH_2PO_4.H_2O$
- 0.5 mM $NaH_2PO_4.7H_2O$
- 20 mM Glucose
- 10 mM HEPES

High K^+ solution (pH 7.4; 300-310 mOsm):

- 84.2 mM NaCl
- 50 mM KCl
- 1.1 mM $CaCl_2$
- 1 mM $MgSO_4$
- 0.45 mM $NaH_2PO_4.H_2O$
- 0.5 mM $NaH_2PO_4.7H_2O$
- 20 mM Glucose
- 10 mM HEPES

Materials and equipment:

- Fluo-4 AM - Invitrogen (Cat no. F14201)
- Pluronic F-127 - Invitrogen (Cat. no. P3000MP)
- HBSS - Thermofisher Scientific (Cat. no. 14170161)
- Recombinant human interferon alpha A protein - Sigma Aldrich (Cat. no. IF007)
- Recombinant human interferon beta 1a protein - Sigma Aldrich (Cat. no. IF014)
- Capsaicin - Sigma Aldrich (Cat. no. M2028)
- Bath imaging chamber - Warner Instruments (Cat. no. RC-25)
- Forceps - Fine Science Tools (Cat. no. 11252-00)
- Elve Flow MUX Distribution 12-way Bidirectional Valve perfusion system
- Olympus IX83 inverted microscope
- Vapor Pressure Osmometer - Vapro (Cat. no. 5600)
- Pipettes and corresponding pipette tips

Software/tools:

- MetaFluor for Olympus imaging software (version 7.10.5.476)

- Elveflow Smart Interface (version 3.07.03)

Solutions and dye preparation

1 Prepare the recording solution with 130 mM NaCl, 4.2 mM KCl, 1.1 mM $CaCl_2$, 1 mM $MgSO_4$, 0.45 mM $NaH_2PO_4.H_2O$, 0.5 mM $NaH_2PO_4.7H_2O$, 20 mM glucose, and 10 mM HEPES. Using a vapor pressure osmometer, adjust the osmolarity to 300-310 mOsm. Adjust the pH to 7.4 using N-methyl-D-glucamine.

Note

The recording solution can be prepared and stored at 4 ℃. Equilibrate the recording solution to RT before imaging.

2 Prepare high K^+ solution by adjusting KCl to 50 mM and NaCl to 84.2 mM in the recording solution, osmolarity 300-310 mOsm. Adjust the pH to 7.4 using N-methyl-D-glucamine.

Note

High K^+ can be prepared and stored at 4 ℃. Equilibrate high K^+ solution to RT before imaging.

3 Prepare 1 mL of 10 µM working solution of Fluo-4 AM with 0.04% Pluronic F-127 surfactant in HBSS. Store it away from light.

Treatment of hDRG neurons

4 Treat cultured DIV 1-4 hDRG neurons with 500 U/mL hIFN-α2 or hIFN-β 1a or their corresponding vehicles (0.002% BSA in PBS or 50 mM NaOAc, pH 5.5, containing 0.1% BSA, respectively) 02:30:00 .

2h 30m

Note

Preparation of hDRG cultures followed previously described methods (see references).

Disclaimer: The duration of treatment depends on the experimental designs and cytokines used, and should be optimized accordingly.

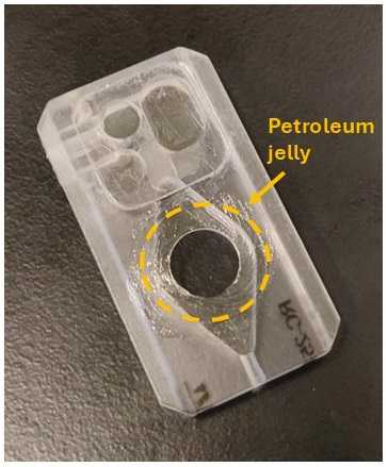
Setting up calcium imaging

5 Attach the recording solution, 200 nM capsaicin dissolved in the recording solution, and the high K^+ solution to the Elve Flow MUX Distribution 12-way Bidirectional Valve system for perfusion and set the flow rate to 1 mL/min.

Note

Disclaimer: The concentration of capsaicin can vary between experimental designs and hence, should be optimized accordingly.

6 Using a brush, coat the bottom of a bath imaging chamber with petroleum jelly.

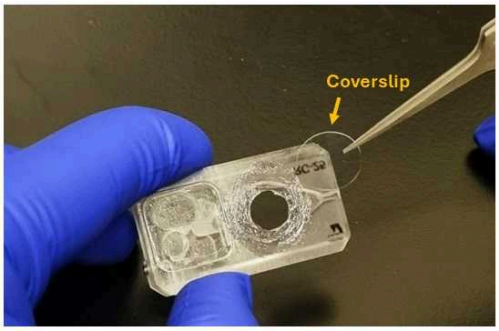


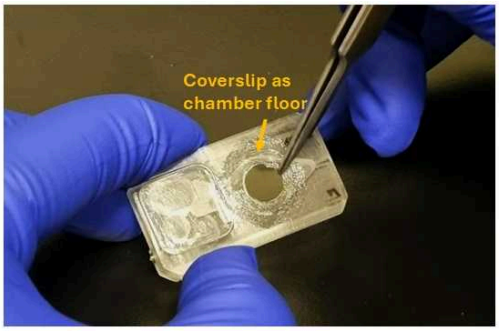
7 Wash cells once with growth media.

8 Incubate cells at 37 °C with 10 µM Fluo-4 AM 00:30:00 . 30m

9 Remove the dye and wash the cells with 1 mL of HBSS.

10 Retrieve the coverslip with cultured hDRG neurons from the plate well with forceps and mount it to the imaging chamber coated with petroleum jelly to form a tight seal.

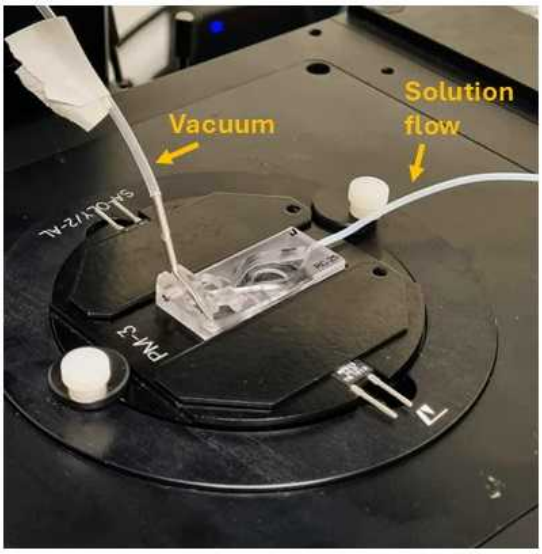




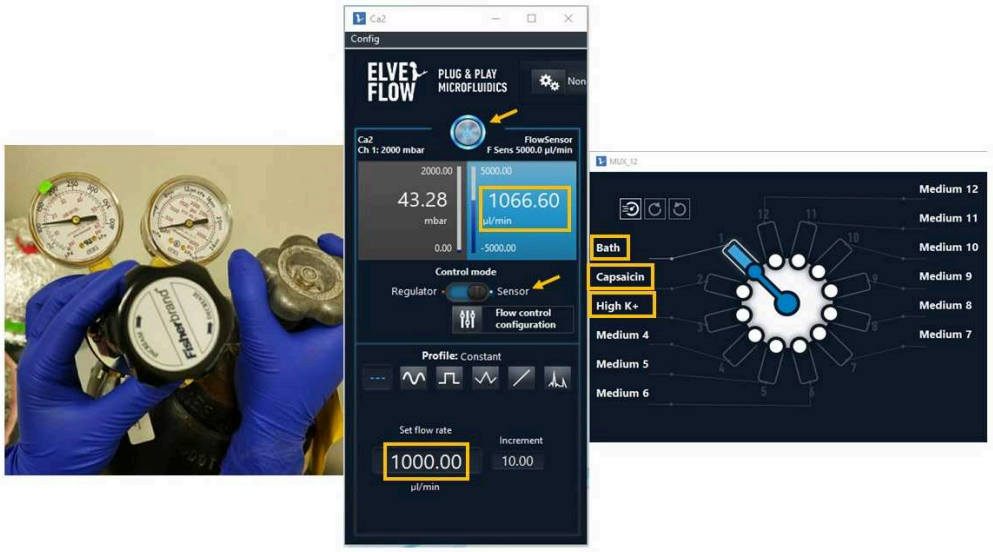
Note

Gently pipette ~50 µL of recording solution onto the cells to prevent them from drying out.

11 Place the imaging chamber on an Olympus IX83 inverted microscope at 10x magnification.



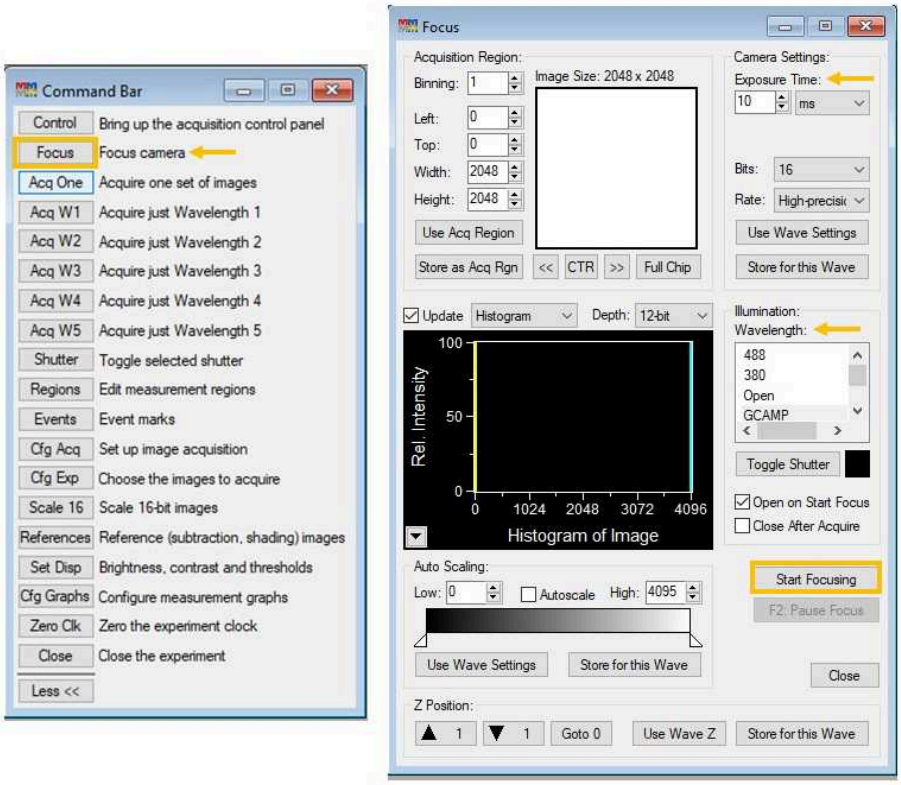
12 Begin perfusion of recording solution at a flow rate of 1 mL/min achieved by 100-120 kPa of nitrogen 00:05:00 . 5m



Flow rate adjustment and channels on Elveflow Smart Interface (ESI) for the Elve Flow MUX Distribution 12-way Bidirectional Valve perfusion system.

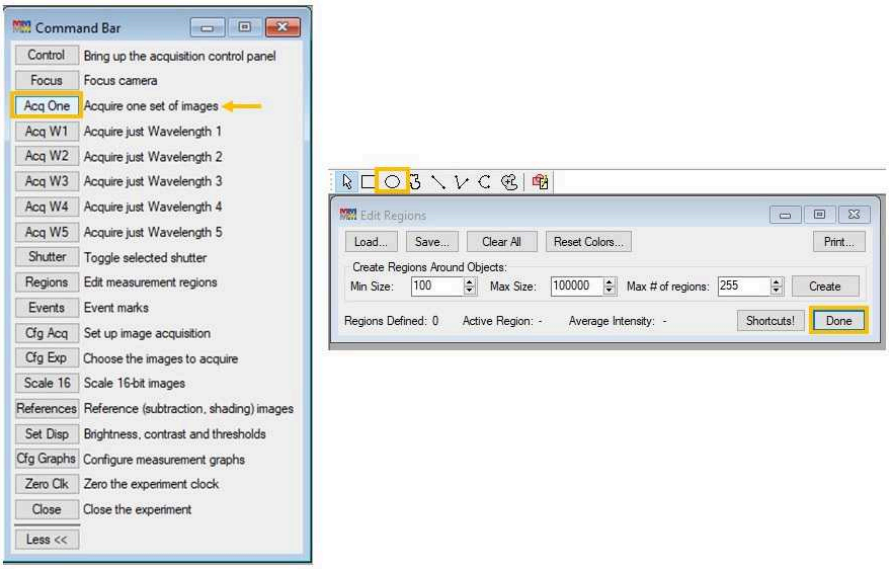
Capturing intracellular calcium changes in hDRG neurons

13 Open MetaFluor for Olympus imaging software. Choose the field of view to be imaged and bring it to focus.

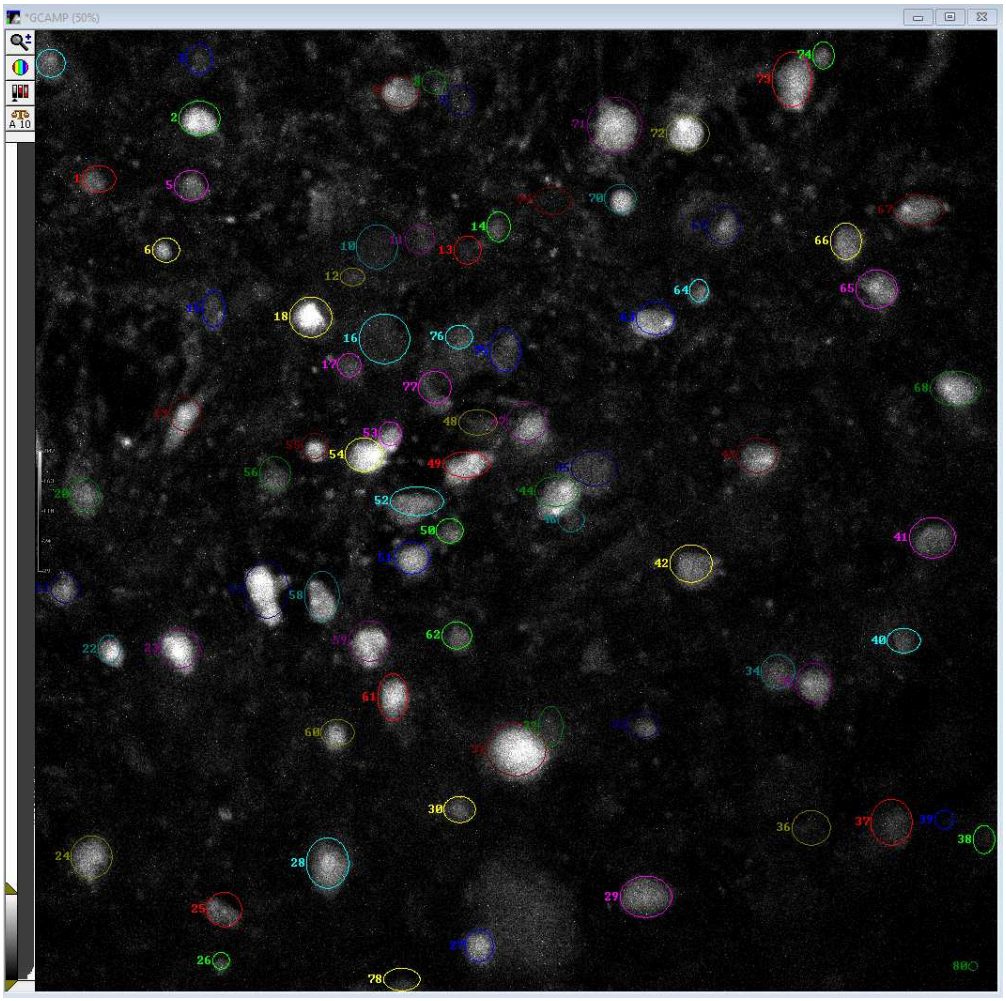


Focusing field of view for imaging on MetaFluor.

14 Acquire an image and select all neurons of interest by drawing a circular ROI around them. Keep in mind that at least one ROI in the background should be drawn to account for background fluorescence.

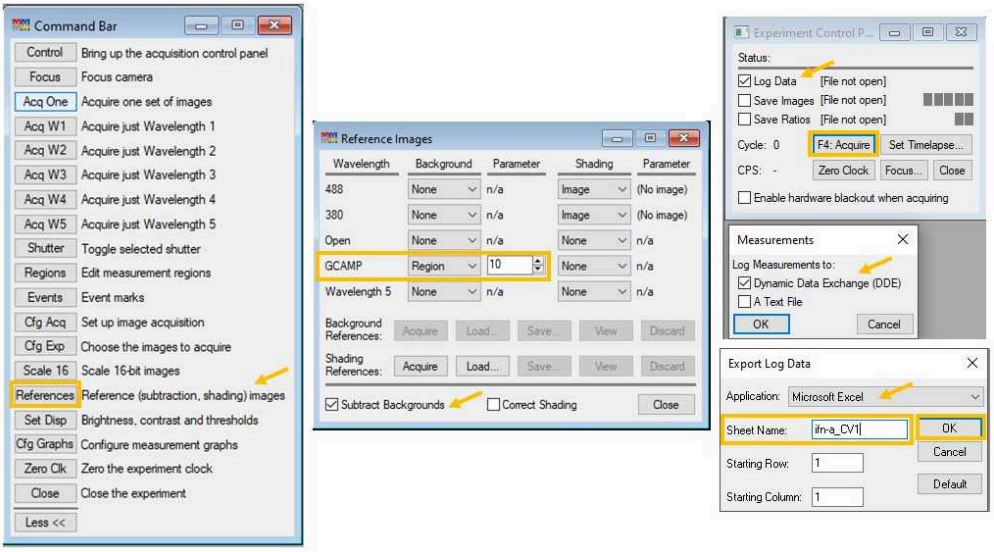


Regions tool on MetaFluor.



Circular ROI around hDRG neurons loaded with Fluo4-AM to read calcium dynamics.

15 Select the background ROI drawn for the background fluorescence to be accounted for as a reference for subtraction.
Check "Log Data" to open an active spreadsheet that logs the fluorescence readout of each frame recorded.



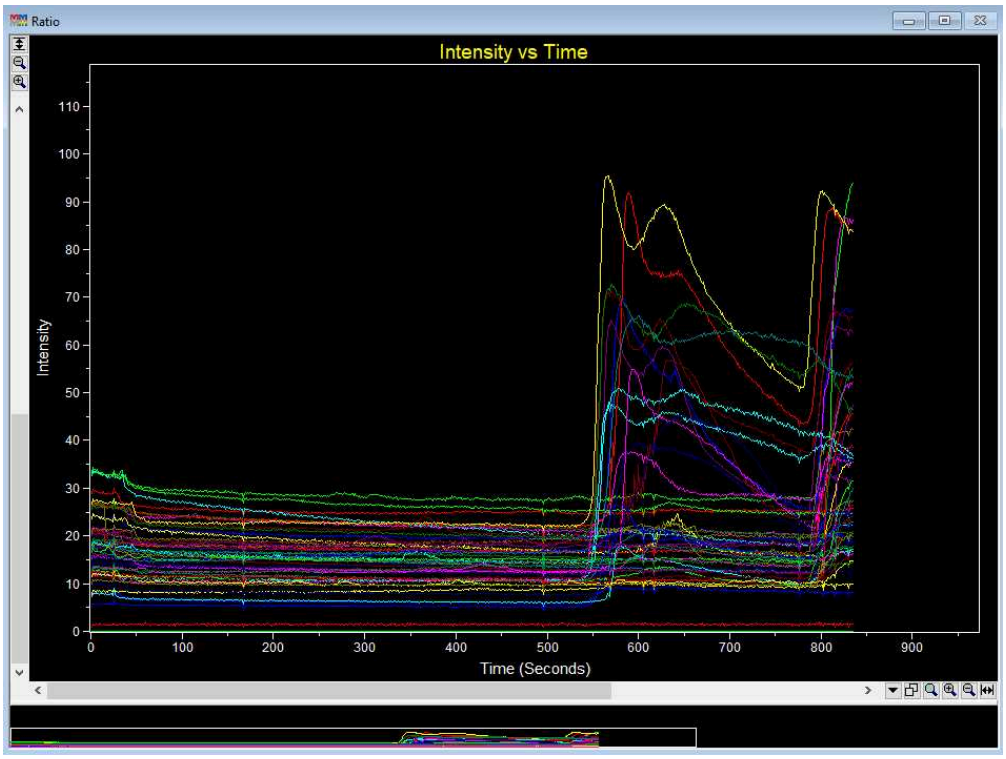
Acquisition and data logging on MetaFluor.

16 Start acquiring images and recording baseline fluorescence indicative of baseline intracellular calcium levels 00:10:00 . 10m

17 After recording the baseline, switch to perfusion with 200 nM capsaicin solution to record capsaicin-induced intracellular calcium changes in neurons 00:02:00 . 2m

18 Wash out capsaicin by perfusing the recording solution 00:03:00 . 3m

19 Finally, perfuse 50 mM high K^+ solution 00:02:00 . 2m



Graph representing varying intracellular calcium levels in hDRG neurons induced by capsaicin and high K^+ as fluorescence intensity over time.

20 Stop the solution perfusion and acquisition of images. Export the spreadsheet for further data analysis.

Note

Analysis of peak fold-change in fluorescence, latency to peak, and other metrics can be performed using Python, R, MATLAB, or other open-source or commercially available packages.

Protocol references

Yousuf MS, *et al*. (2023) Highly specific σ_2R/TMEM97 ligand FEM-1689 alleviates neuropathic pain and inhibits the integrated stress response. *Proc Natl Acad Sci USA* 120(52):e2306090120.

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Acknowledgements

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