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Protocol status: Working
 We use this protocol and it's working

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Localised axotomy of human Cortical Neurons (CNs) from induced pluripotent stem cells (iPSCs)

In 2 collections

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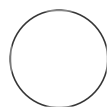
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ABSTRACT

This protocol describes the process followed to perform localised axotomy of iPSC-derived human cortical axons cultured within fluid-walled dumbbells in 6 cm TCT-treated Petri dishes. A similar protocol was first described by [Soitu et al., 2020](#) to perform wounds assays in monolayer cell cultures.

MATERIALS

Reagents:

- [B-27™ Supplement \(50X\), serum free](#) (ThermoFisher Scientific, CAT# 17504044)
- [FC40](#) (iotaSciences Ltd, CAS# 51142-49-5)
- Neurobasal (ThermoFisher Scientific, CAT#2113049)
- [Phosphate-buffered saline](#), pH 7.4 (PBS) (Life Technologies, CAT# 10010056)

Equipment:

- In-house Fluid Printer (iotaSciences Ltd.)

Preparation of coating-prep medium:

- Neurobasal
- 1x B-27 supplement

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Localised axotomy assay

- 1 On Day 20, remove the FC40 overlay from the Petri dish where human Cortical Neurons (CNs) were cultured inside fluid-walled dumbbells.
- 2 Gently add ~2 ml of coating-prep medium (see **Materials**) to destroy every fluid wall without peeling cells off the substrate.
- 3 Remove medium and wash with PBS twice. **Be careful with cells peeling.**
- 4 After last wash, add ~5 ml of fresh medium (Neurobasal supplemented with B27).

Note

Cells can now be stored in incubator for several minutes if needed.
- 5 Equip the fluid printer with a 1 mL glass syringe filled with medium.
- 6 Place dish into the fluid printer.

- 7 Fluid printer automatically perform axotomy by means of a submerged medium jet. Such jet is held at a fixed height above cells and it is moved around the dish by the printer traverse to cross perpendicularly across axon bundles at the midpoint.

Key parameters:

- jet height = 300 μm
- jet flow rate = 480 $\mu\text{l}/\text{min}$
- traverse speed = 960 mm/min

Note


Parameters must be finely tuned, depending on cells maturation and concentration.

Fluid printer is controlled by scripts written in G-code.

- 8 Remove medium and overlay fresh FC40.
- 9 New fluid-walled dumbbells can be fabricated around axotomized cultures following **steps 1.5 and 1.6** in [Protocol: Fabrication of fluid-walled dumbbells and generation of the human corticostriatal pathway](#)

Live-imaging of axonal regeneration

- 10 On DIV 0, fluorescent live images of all dumbbell conduits were taken just prior to CNs replating to serve as initial normalising timepoint.
- 11 Images were taken on a digital SLR camera (Nikon D7100 DSLR) connected to an epi-fluorescence microscope (Olympus IX53; 1.25 \times , 4 \times , 10 \times , 25 \times objectives) equipped with a translation stage and an overhead illuminator (Olympus IX3 with filters).
- 12 Medium was changed every other day from now on for the next 20 days.

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- 13** Similar live images were taken again on day 20 prior to axotomy, and subsequently 36, 60, and 84 hours post-axotomy.