

Jun 09, 2023

Version 1

Making CFM V.1

 In 2 collections

DOI

<https://dx.doi.org/10.17504/protocols.io.n2bvj84qpgk5/v1>



Katherine Brimblecombe^{1,2,3}, Stephanie J Cragg^{1,2,3}

¹Department of Physiology, Anatomy and Genetics, University of Oxford, OX1 3PT, UK;

²Oxford Parkinson's Disease Centre, University of Oxford, Oxford, United Kingdom;

³Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815



Cláudia C. Mendes

University of Oxford

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.n2bvj84qpgk5/v1>

Protocol Citation: Katherine Brimblecombe, Stephanie J Cragg 2023. Making CFM. protocols.io

<https://dx.doi.org/10.17504/protocols.io.n2bvj84qpgk5/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: April 24, 2023

Last Modified: June 09, 2023

Protocol Integer ID: 80963

Keywords: making cfm tbd, cfm tbd

Funders Acknowledgements:

Parkinson's UK

Grant ID: G-1803

Aligning Science Across Parkinson's (ASAP)

Grant ID: ASAP-020370

Abstract

TBD

Materials

Reagents:

- Acetone
- Conductive silver paint (RS 186-3600)
- Cyanoacrylate glue (Loctite superglue)

Equipment:

- Light box
- Test tube
- Forceps with rubber ends (push small piece of rig tubing over each prong of forceps to stop snapping carbon fibre)
- Small scissors
- Scalpel
- Microscope with 10X objective and graticule eye piece
- Puller
- Capillary tubes 2.0 OD 1.16 X 100 mm (Harvard Apparatus, CAT# 30-0074)
- Wire (RS PRO Data Cable, 9 cores (7/0.1 mm), 30 AWG, PVC coated)
- Glass Slides
- Blu Tack

For each electrode:

- Capillary tubes
- Wire (use 1 core per electrode)
- Conductive silver paint
- Cyanoacrylate glue

Making a cutting block:

- Stick two capillary tubes to a glass slide, slightly further apart than a capillary tube.
- Cut glass slide in half and on one half score a single groove the full width of the slide with a diamond knife and a metal ruler, sufficiently deep to hold a scalpel in place when drawn down it.
- Stick this half slide to the first glass slide (see **Figure A** for clarification).



Threading Carbon Fibre

1 Fill test tube with acetone (flammables cupboard) and add capillary tubes.

2 Stick test tube to light box with Blu Tack.

Note

Careful not to spill acetone on light box.

3 Select a single carbon fibre using rubber tipped forceps.

4 Thread carbon fibre down capillary tube.

Note

Ensure capillary tube is completely filled with acetone.

Ensure ~ 0.5 cm carbon fibre is exposed at the end of the capillary tube to stop it from falling out of the end of the tube.

5 Lift the tube out of the acetone and allow acetone to evaporate.

Pulling Carbon Fibre

6 Place capillary tube into puller.

7 Tighten holder "finger-tight" and raise tube for 3 clicks and secure in place "finger tight".

8 **Settings:**

- Magnet: off
- Heat: ~6.7

**Note**

The higher the heat, the longer/more gradual the seal will be.

Golden Rule:

- Too cold or short seal: easier to see the capillary tube being cut, but it can make it leaky.
- Too hot or long seal: harder to see the capillary tube being cut, but also fine class seal can break during an experiment suddenly exposing extra carbon fibre, leading to a noisy and unreliable electrode.

- 9 Check which end of the tube the carbon is in and carefully remove from puller.
- 10 Trim excess exposed fibre with fine scissors to stop waving extended fibre from stressing the glass seal.

Cutting Carbon Fibre

- 11 Carefully place electrode between capillary tubes on cutting block.
- 12 Approximately line up seal with cut ridge.

Note

If you have problems seeing the seal, breathe on the fibre and its flex point will indicate the approximate point it joins the glass.

Roll the capillary tube, often one side is more clear.

When looking, start at the fully exposed carbon fibre end and travel towards the capillary tube, your eye finds spotting the increase from a constant easier than a change to a constant (IMO).

- 13 Make a cut at ~ 300 μm from seal, by running scalpel down cut ridge, mark this as 0 on the graticule.
- 14 Move electrode so there is 50-100 μm exposed carbon fibre and run scalpel down cut ridge.

Note

This step takes practice. Don't be discouraged and take your time!

The length of exposed carbon fibre is another golden rule.

- The longer the exposed carbon fibre, the more sensitive your electrode will be (bigger surface to detect dopamine), however, there is more area to detect noise too.
- A shorter electrode will be less sensitive, but also often quieter. We can amplify the signal from a shorter electrode, however, remember it will also amplify the noise.

Given we record dopamine from ~100 μm sphere, it makes sense to try to sample the most amount of dopamine available, but not over-sample. An electrode length of 50-100 μm will allow an amplification of 3-10 nA/mV, which we have determined to be optimal for our set up.

- 15 Carefully remove electrode from cutting block.

Wiring Electrode

- 16 Cut wire to slightly longer than your capillary tube.
- 17 Strip off ~1cm of the plastic coating from each end.
- 18 On one end trim exposed wires so only 1 is sticking out.
- 19 Dip this end in the conductive silver paint and gently thread down the capillary so the paint makes contact with the carbon fibre.
- 20 Glue exposed end of wire in place by dripping glue down capillary tube, exposed wire can be connected to voltammetry head-stage using croc-clip.
- 21 Allow to set overnight before use.