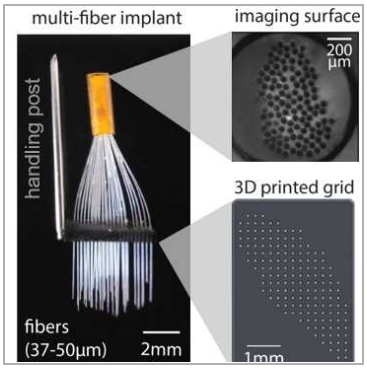

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Multifiber Array Fabrication

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ABSTRACT

We have developed a multi-fiber photometry approach to monitor dopamine release with sub-millimeter spatial resolution and sub-second temporal resolution at over 50 locations simultaneously throughout the striatum in awake, behaving mice expressing a fluorescent sensor. This protocol includes the array fabrication and pre-implant mapping steps. Please contact us (mwhowe@bu.edu) if you are interested in using this technique.

OPEN ACCESS

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

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PROTOCOL integer ID: 84787

MATERIALS

Equipment:

- Fibers (Fiber Optics Tech, 0.66 NA)
  - 37µm (34µm core, 3µm cladding) or
  - 50µm (46µm core, 4µm cladding)
- Custom 3D printed biocompatible plastic grid (Boston Micro Fabrication)
  - 3mm x 5mm
  - our holes were spaced radially by 220 microns
- UV glue (Norland Optical Adhesive 61)
- UV curing system (ThorLabs, CS20K2)
- Instant glue (Pacer Zap CA, Thin CA)
- Polyamide tubing (Cole-Parmer or MicroLumen)
  - 0.8-1.3mm diameter
- Fine-grained polishing paper (ThorLabs)
  - 5- or 6-micron
  - 3-micron
- Razor blades
- ThorLabs Ruby Fiber Scribe (S90R)
- Dissection microscope for fabrication
- Helping Hands Soldering Aid
- LED pen light

Helpful but not necessary:

- USB digital microscope camera
- Smooth thin paper for the calibration step; we have found that a tiny piece (5mm x 10mm) of Tyvek USPS mailing envelope colored black with a marker works well

Fabrication steps

1 Cut fibers into several ~3 cm pieces using the ThorLabs fiber scribe

2 Secure the grid to the Helping Hands using one of the clamps and paper tape

3 Under the microscope, load fibers into the desired holes in the first row of the grid. The design of these implants is completely flexible; our designs are meant to target the striatum with maximal coverage with no overlap in the collection fields of individual fibers.

4 Rotate the grid such that the fibers are now parallel to the lab bench.

5 Under the microscope, push/pull the fibers to the desired depths on the implanted side beneath the grid.

6 Rotate the grid such that the top is visible, and apply UV glue to the loaded holes

7 Under the microscope, push/pull the fibers to the desired depths on the implanted side beneath the grid.

8 Verify that the lengths are correct, and then cure.

9 Repeat steps 2-7 for the remainder of the rows. Note that we do this row by row, but column-by-column or hole-by-hole is possible.

10 Once all fibers are loaded and secured, gather the fiber distal ends into a ~1cm section of polyamide tube.

11 Fill the tube with instant glue and let dry.

12 Cut with a fresh razorblade – this cross-section is the imaging surface.

13 Polish first with the 6 micron polishing paper, and then the 3 micron to create a smooth, uniform fiber bundle surface for imaging.

14 Mount a large diameter post (this can be a blunted, sanded section of a needle) on one side of the grid to facilitate holding during implantation.

Calibration steps (mapping the fiber grid location to imagin..

15 The overall idea will be to use the LED penlight to illuminate the implanted ends and note which fibers in the imaging surface are lit. Do this by sequentially uncovering one row at a time, and then one column at a time, such that each fiber in the imaging surface has a row and column coordinate in the grid. An example way to do this is as follows:

15.1 Flip the multifiber array upside down, so the implanted ends are upwards.

15.2 Mount the USB digital microscope camera underneath so you have a clear view of the imaging surface

15.3 Under the dissection microscope, using a piece of paper (material #14 above) bent to 90 degrees, slot it between the first and second rows such that rows 2 onwards are covered.

15.4 Shine the LED light on the exposed fibers and note (taking a picture is useful) the illuminated ends in the imaging surface

15.5 Now slot the piece of paper between rows 2 and 3, such that rows 1 and 2

are exposed, and rows 3 onward are covered, and repeat step **16.4**

15.6 Repeat for the remainder of the rows

15.7 Repeat for the columns.