Glass Pipette Preparation

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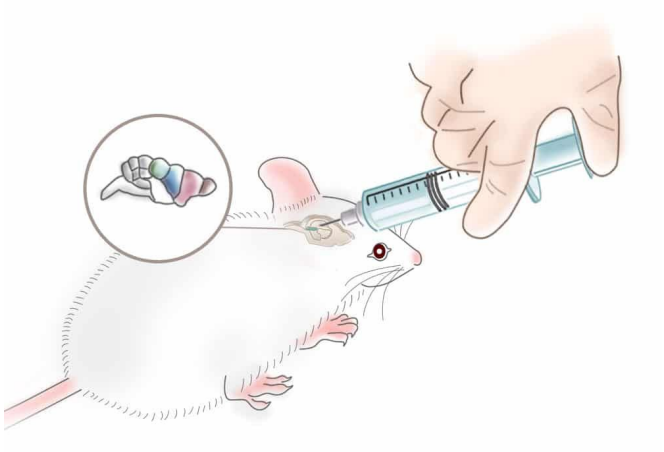
Introduction

Many methods of injecting therapeutics to the brain, such as

- Intracerebral injections

Has its upsides, such as its precise targeting, however one major negative is the its invasive nature



Motivations

Thin tipped tools

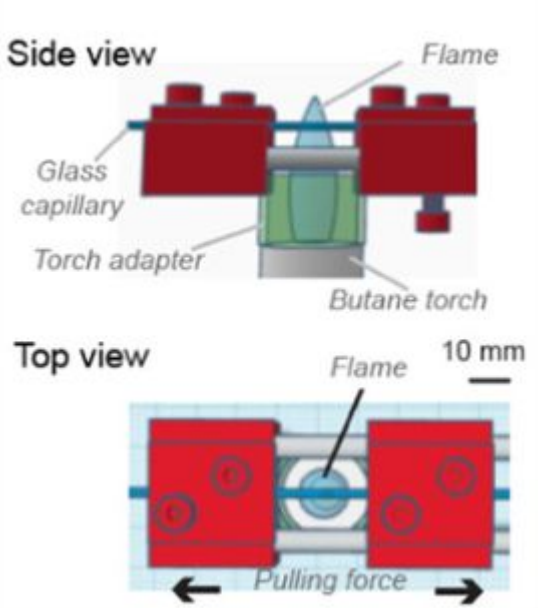
- Thin-tip glass pipette

Problems

- Affordability
  - Price of obtaining glass pipettes
    - Requires conversion of professional programmable pipette pullers (thousands of dollars)
- Accessibility
  - Non electrophysiological labs lack access to pipette pullers

3D Printing can drastically reduce the price

Pulling Device



https://onlinelibrary.wiley.com/doi/full/10.1002/nep3.20

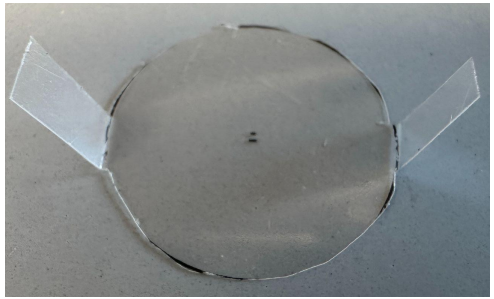
My Project: Processing and Calibrating Glass Pipettes

- Calibrating
- Trimming
- Beveling

Calibrating

- Different therapeutics to be injected are different sizes, so we must cater our pipettes to these needs
  - Liquid delivery - 20 µm
  - Cell delivery - 40-50 µm
- Determining where to cut pipette
  - Visible measuring scale in the microscope lens
    - Reticle
  - Allows measurement of items visible in the microscope

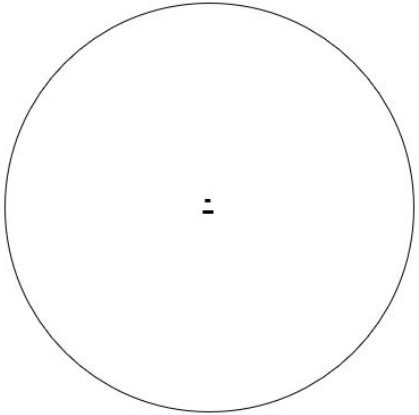
Reticle

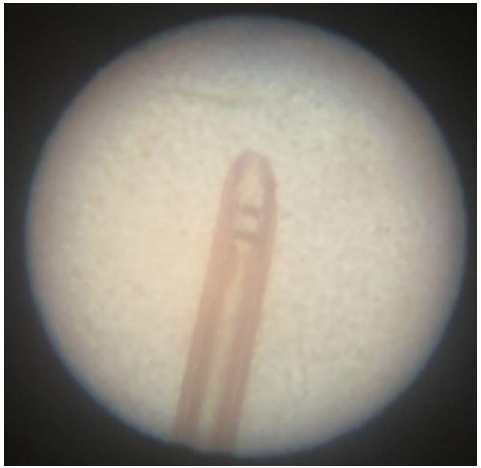


Reticle

Microsoft Word used to create the reticle

1. Measure the diameter of the microscope lens
2. Create a circle of that diameter in word
    - Set height and width equal to the diameter
3. Convert desired length in microscope to actual length on the reticle
    - For 100x magnification, 20 µm * 100 = 2 mm
    - 40 µm * 100 = 4 mm
4. Create line of that length and center it in the circle with align tool
    - Multiple lines -> group first
5. Print out reticle on a clear film
6. Cut out the reticle
    - Cut out “ears” on the reticle so it's easy to take out of the microscope





Problem with Old Reticle

Problem:

- Intended lengths obtained from calculations were off
  - Ex: The line for 20 µm was actually 150 µm through the microscope
  - Hidden lens within the microscope

Solution:

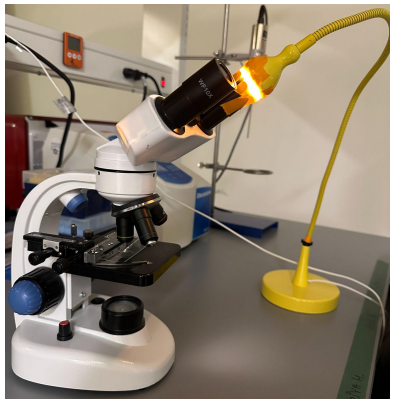
1. Calculate magnification factor
   - Ex: 150/20 =7.5
2. Divide length of the line on the reticle by magnification
   - 2 mm/7.5 = 0.2667 mm

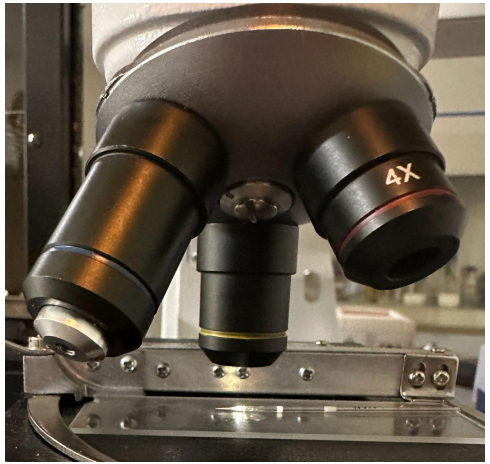
Trimming

Shining a bright light through one of the lenses projects a bright dot

Trimming Procedure:

1. Match the line on the reticle with the pipette to measure desired diameter
2. Turn off the microscope light and turn on the flashlight
3. Make a cut on the pipette where the bright dot is





Beveling

We can bevel the pipettes with a spinning hard drive disk

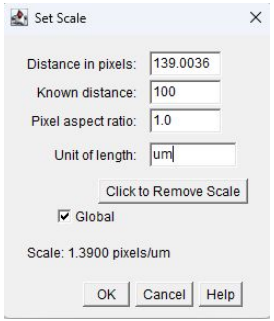
Put the tip on the surface of the disk very carefully at a small angle

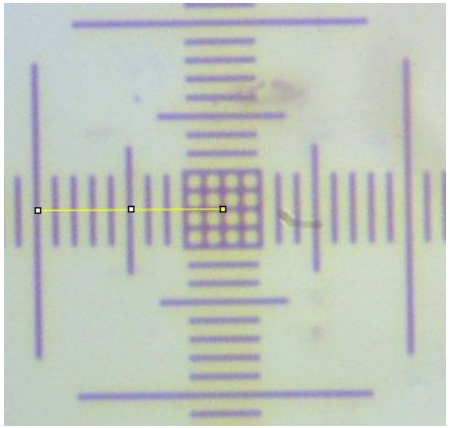
- Too much force could break the tip
- Too large of an angle could result in a dull tip

Software

We can confirm if our measurements were correct using an app called imageJ.

1. Take screenshot of the grid on the slide
    - Each space is 10 µm
2. Draw a line from origin to any line on the scale
3. Calculate that distance in µm
4. Analyze -> Set Scale
    - Change known distance to calculated length
    - Change units to um
    - Check global





Thank You

Questions?