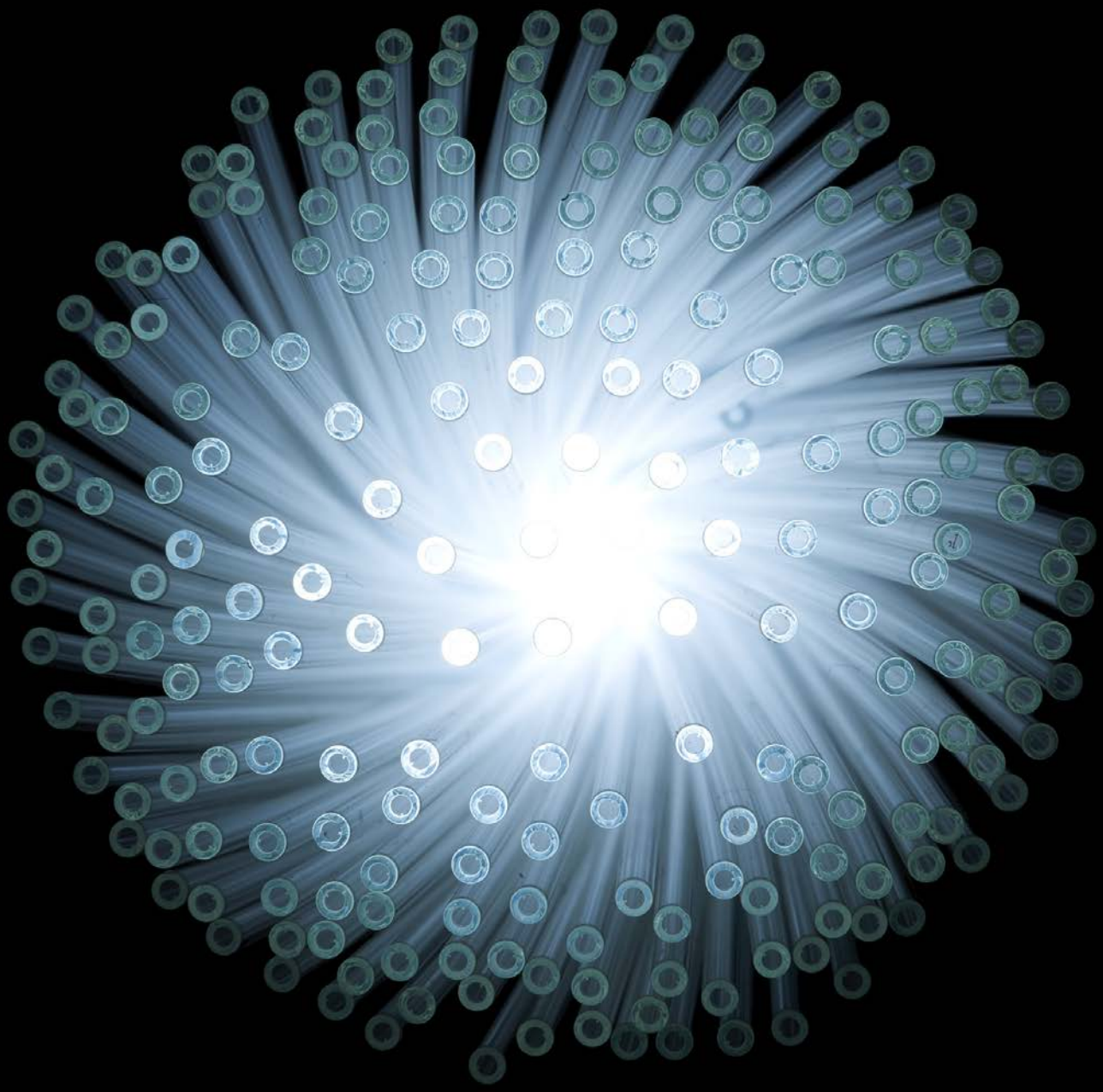

Pipette Cookbook 2018

P-97 & P-1000 Micropipette Pullers

Rev. F

SUTTER INSTRUMENT

The Pipette Cookbook was written, compiled and created by Adair Oesterle with Sutter Instrument Company. A printed version of the Pipette Cookbook is included with all new P-97 and P-1000 Pullers and the pdf can found and downloaded for free through the Sutter web site.
http://www.sutter.com/contact/faqs/pipette_cookbook.pdf

Revised and expanded versions of the Pipette Cookbook are released every two to three years. Please contact Sutter if you have any recommendations, comments and/or corrections to offer.
We welcome your feedback!

Adair Oesterle at the Getty Museum – not pulling pipettes!

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**New* P-1000 Pipette Puller

*The New P-1000 Pipette Puller has the "Pipette Cookbook" programs incorporated into its memory. The new Cookbook feature for the P-1000 can be used to look up and install a majority of the programs found in this text. By selecting the filament type, glass dimension, and application you can install a pre-written program. To fine-tune the program you installed from the cookbook directory, please refer to pg 11 for instructions on how to adjust the parameter settings.

Introduction

The Sutter Instrument Pipette Cookbook is organized according to application and describes the required pipette needed for that application. While we have attempted to cover a wide range of applications and types of pipettes, it is possible we have yet to include what you might be looking for. Additional applications are occasionally added to new revisions of the cookbook and if you have suggestions for future revisions, please contact Sutter Instrument Company.

At the beginning of each chapter you will find a general discussion of each application and a detailed description of the morphology of the pipette (taper length, tips size, resistance, etc) needed for your application. In each chapter we provide the "ideal" combination of glass size (outer and inner diameters) and filament type for your application, along with the recommended parameter settings with which to start. We then provide suggestions on how to adjust and "tweak" the recommended parameter settings in case you need to modify the morphology of the resulting pipette. When applicable, we have also provided information on the XenoWorks microinjection equipment, the set up and the settings that would be required for that application.

The "ideal" filament and glass combination, and the associated recommended parameter settings we have provided in each chapter have been established over years of experience, and are a result of in-depth research results and customer feedback. If you are unable to match the filament and glass combination that is provided for a specific application or you lack the "recommended ingredients," you can refer to the "General Look Up Tables" to find an alternate program. The General Look Up Tables are organized according to the type of filament installed in your puller and the dimension of glass (OD and ID). Programs are listed in the Tables as Type A, B, C, D, and E. Each "Type" classification is explained on page 97. It is important to keep in mind that what is provided in the General Look Up Tables might not be "ideal" for your application. There are some combinations of filament and glass that do not work well for a given application and can create very unstable results. So, we would like to emphasize again that what has been provided in each chapter is the best approach we have come to recognize.

The most common sources of difficulty in producing the right shape of pipette can be attributed to the use of poor parameter settings. Just one poorly adjusted parameter setting in the program - too high or too low - can lead to a lot of variability in tip size, taper length and resistance. These very high or low settings can be seen as "bad ingredients" in your recipe. For a general guideline on the parameter settings and the range of values we recommend, please refer to page 11. If you stay within the suggested range for each parameter, it will be less likely that you will "get lost" or off track when trying to write a program or adjust your settings. It is possible that the not-so-ideal parameter settings were established in a somewhat haphazard manner by various well-intentioned researchers who are possibly no longer in the lab. It is also conceivable that the program one is using had been adopted from someone who had a different model of Sutter puller and/or a different combination of filament and glass.

Please be aware that simply using a program recommended by someone else can often lead to undesirable tip sizes, and in worse cases, the unfortunate event of burning out your filament. If you have acquired your settings from someone outside of Sutter, it is best to run a ramp test to make sure the heat settings you have been advised to use will not damage or burn out your filament. Information about the Ramp Test can be found on page 12.

If a program in this Cookbook does not produce the proper pipette, this might be a result of a poor alignment or mechanical adjustment on your puller. It is important to make sure your puller is in good working order. Be sure to check that the filament is perfectly centered over the air jet, the air jet is set 2 to 3mm below the base of the filament, the glass capillary is properly positioned within the filament, and the filament shape is correct. If you find that the program provided and the suggested changes to the parameter settings do not produce good results, please refer to page 11, "General Guideline for Parameter Settings" and Chapter 15, "Variability." If you feel your puller might be in need of a tune up or repair, please contact Sutter Instrument and inquire about having your puller refurbished.

Using the Pipette Cookbook with a P-87 or a P-80/PC

To adapt the P-97/P-1000 Pipette Cookbook programs to an earlier model Sutter Micropipette Puller, including the P-87 and P-80/PC, please do the following:

- If you have a **P- 87**, reduce the heat setting listed in the chart by 5-10 units. The power available to the filament in the P-97 is 25% higher than the P-87 and the rate of current increase during a ramp test on the P-97 is more gradual. These differences require one to alter the heat setting when using a P-97 program on a P-87. If a Delay Value is provided, you will need to install a Time Value between 175 and 250 in place of the Delay Value.

- If you have a **P-80/PC,** reduce the heat setting listed in the table by 10 units. The Time Mode on the P-80/PC functions just like the Delay Mode on the P-97 and P-1000. If a Delay Value is provided in Pipette Cookbook program, install this same number as the Time Value on your P-80/PC. If a Time Value is provided in the Pipette Cookbook program, please install a Time Value between 50 to 110 on the P-80/PC.

- The **Pressure** of the out-going regulator of the P-80/PC should be set to 50psi. The **airflow** of the Nitrogen is controlled by adjusting the valve opening of the air solenoid, which is a vertical micrometer dial behind the left puller bar. Micrometer settings between 1 and 1.25 are recommended.

  * For detailed information about the cooling modes, Time & Delay, please refer to your P-97 or P-1000 manual.

Using the Pipette Cookbook with a P-1000

The New P-1000 Pipette Puller has the "Pipette Cookbook" programs incorporated into its memory. The new Cookbook feature for the P-1000 can be used to look up and install a majority of the programs found in this text. By selecting the filament type, glass dimension, and application you can find and install a pre-written program. To fine tune your program, please refer to page 11 for instructions on how to adjust the parameter settings. Aluminosilicate programs and Type E programs were not incorporated into the P-1000. Programs for pulling Aluminosilicate glass can be found in Chapter 12. You might also want to try using the new Pre-Heat Feature on your P-1000 puller to help stabilize your programs. For additional advice, please contact Sutter Technical Support.

SUTTER CAPILLARY GLASS

Standard Wall Borosilicate Tubing (With Filament)

Catalog Number	Outside Diameter	Inside Diameter	Overall Length	Pieces per Package
BF100-50-10	1.00mm	0.50mm	10cm	225
BF100-50-15	1.00mm	0.50mm	15cm	225
BF100-58-10	1.00mm	0.58mm	10cm	250
BF100-58-15	1.00mm	0.58mm	15cm	250
BF120-60-10	1.20mm	0.60mm	10cm	225
BF120-69-10	1.20mm	0.69mm	10cm	250
BF120-69-15	1.20mm	0.69mm	15cm	250
BF150-75-10	1.50mm	0.75mm	10cm	225
BF150-86-7.5	1.50mm	0.86mm	7.5cm	250
BF150-86-10	1.50mm	0.86mm	10cm	250
BF150-86-15	1.50mm	0.86mm	15cm	250
BF200-100-10	2.00mm	1.00mm	10cm	225
BF200-116-10	2.00mm	1.16mm	10cm	250
BF200-116-15	2.00mm	1.16mm	15cm	250

Standard Wall Borosilicate Tubing (No Filament)

Catalog Number	Outside Diameter	Inside Diameter	Overall Length	Pieces per Package
B100-50-10	1.00mm	0.50mm	10cm	225
B100-50-15	1.00mm	0.50mm	15cm	225
B100-58-10	1.00mm	0.58mm	10cm	250
B100-58-15	1.00mm	0.58mm	15cm	250
B120-69-10	1.20mm	0.69mm	10cm	250
B120-69-15	1.20mm	0.69mm	15cm	250
B150-86-7.5	1.50mm	0.86mm	7.5cm	250
B150-86-10	1.50mm	0.86mm	10cm	250
B150-86-15	1.50mm	0.86mm	15cm	250
B200-116-10	2.00mm	1.16mm	10cm	250
B200-116-15	2.00mm	1.16mm	15cm	250

Thin Wall Borosilicate Tubing (With Filament)

Catalog Number	Outside Diameter	Inside Diameter	Overall Length	Pieces per Package
BF100-78-10	1.00mm	0.78mm	10cm	250
BF100-78-15	1.00mm	0.78mm	15cm	250
BF120-94-10	1.20mm	0.94mm	10cm	250
BF120-94-15	1.20mm	0.94mm	15cm	250
BF150-110-7.5	1.50mm	1.10mm	7.5cm	250
BF150-110-10	1.50mm	1.10mm	10cm	225
BF150-117-10	1.50mm	1.17mm	10cm	250
BF150-117-15	1.50mm	1.17mm	15cm	100
BF165-120-10	1.65mm	1.20mm	10cm	250
BF200-156-10	2.00mm	1.56mm	10cm	250
BF200-156-15	2.00mm	1.56mm	15cm	100

Thin Wall Borosilicate Tubing (No Filament)

Catalog Number	Outside Diameter	Inside Diameter	Overall Length	Pieces per Package
B100-75-10	1.00mm	0.75mm	10cm	225
B100-75-15	1.00mm	0.75mm	15cm	225
B120-90-10	1.20mm	0.90mm	10cm	225
B150-110-7.5	1.50mm	1.10mm	7.5cm	225
B150-110-10	1.50mm	1.10mm	10cm	225

For a list of our Aluminosilicate & Multibarrel Borosilicate Glass, please refer to our web site (www.sutter.com)

Filaments & Accessories

Box Filaments

P-1000, P-97, P-87, P80PC, P80C, PC-84, P-77B

FB215B 2.0mm square box filament, 1.5mm wide…….

FB220B 2.0mm square box filament, 2.0mm wide…….

FB230B 2.0mm square box filament, 3.0mm wide…….

FB255B 2.5mm square box filament, 2.5mm wide…….

FB245B 2.5mm square box filament, 4.5mm wide…….

FB315B 3.0mm square box filament, 1.5mm wide…….

FB320B 3.0mm square box filament, 2.0mm wide…….

FB330B 3.0mm square box filament, 3.0mm wide…….

Trough Filaments

P-1000, P-97, P-87, P80PC, P80C, PC-84, P-77B

FT315B 1.5mm wide trough filament…….

FT320B 2.0mm wide trough filament…….

FT330B 3.0mm wide trough filament…….....

FT345B 4.5mm wide trough filament…….....

Accessories

BX-10: Pipette Storage Box (Holds 10 pipettes)…….

BX-20: Pipette Storage Box (Holds 20 pipettes)…….

Custom Filament: Custom platinum/iridium filament……...

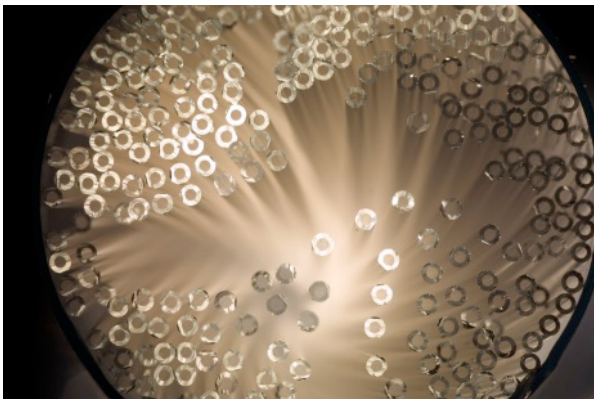
FPS Fire polishing spacer for P97, P87, and P2000 pullers…….

FS1875 Platinum/iridium sheet, 18mm x 75mm x 0.05mm (0.002in)……....

CTS Ceramic tile for scoring glass (large tips 20 to 200 microns)……..

IMOXAB Instruction manual (specify product when ordering)……..

Pipette Storage Boxes
Item# BX10 or BX20

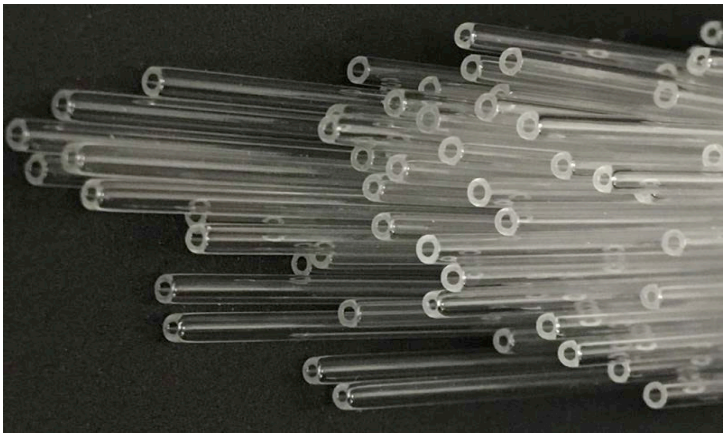


Sutter Capillary Glass
Page 7 for Parts Numbers

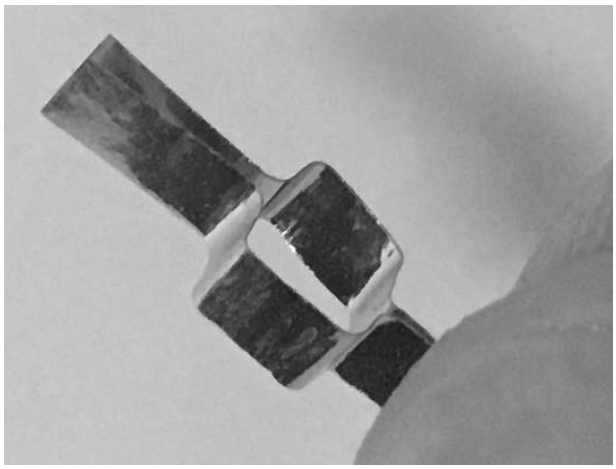
Ceramic Tiles (See Chapter 10)
Item # CTS

THREE MAIN INGREDIENTS:

- GLASS



- FILAMENT TYPE & SIZE (Heating Element)



2.5mm x 2.5mm Box Filament

Box Filament Centered Over Air Jet

- PARAMETER SETTINGS = PROGRAM

HEAT	PULL	VEL	DEL	PRESSURE
Ramp +10	60-90	80	90	200

GLASS

Within each Chapter describing a specific application, Slice Patch Recording for example, a specific glass type, a specific wall thickness, and filamented or non-filamented glass is recommended. Quartz glass is not discussed much in this cookbook because the filament-based pullers like the P-97 and P-1000 cannot melt Quartz glass, but it is mentioned (and recommended if you have a P-2000 Puller) in Chapter 13 – Zika Research and Mosquito Egg Injections. More information on the glass can be found in Chapter 14– Crazy Lab Lore.

FILAMENTS

The filament in your puller is the heating element used to melt the glass. The filaments come in two basic shapes, box or trough. See page 8 for list of filaments. They also come in various widths and IDs (inner diameter or opening of the box or trough). The filament is made of Platinum:Iridium (90:10) and the proper heat setting to use for your filament is determined by running a RAMP TEST. The ramp test is further discussed on page 12.

PARAMETER SETTINGS

The P-97 and P-1000 puller use the following parameters to control the melting and pulling of the glass: Each application described in the Pipette Cookbook will offer a starting program where the heat, pull, velocity, time/delay and pressure settings are suggested. Please see the SutterInstrument YouTube Channel to find the Scientists Empowering Scientists Webinar, "How to Make Better Pipettes", which describes the parameters settings in more depth. The manual that came with your puller will also have a description of the parameter settings. A brief description of each setting is discussed below:

HEAT – This is the amount of current supplied to the filament. The value does not represent the temperature, but indicates how much current is delivered to the filament. The filament needs to get hot enough to melt the glass, and this heat value is dependent on the filament shape, the filament size and the size and wall thickness of the glass. If you use a heat setting that is too high or too low, you risk burning out the filament or damaging the puller. So it is not a good idea to guess at the heat setting. You can also introduce a lot of variability if the heat setting is not ideal. To determine the proper and safe HEAT value to use, one should run a RAMP TEST. For more information about the Ramp TEST, see page 12.

PULL – This is the hard pull introduced to the glass after the glass has softened.
This pull value determines the amount of current supplied to the pull solenoid to create the hard pull. The puller can pull the glass out with a hard pull (Pull = < 0 >) where the puller only uses the gravitational weight of the plunger inside the puller to pull on the glass. A pull of < 0 > is typical when pulling a patch pipette where you want a 3-5mm short taper and a 1-3μm tip. A pull of 50-150 is typical when making a sharp electrode of 30 to100 MΩ or when making a microinjection needle.

VELOCITY – This is the rate of separation of the puller bars when the glass first starts to melt. The velocity is detected by a transducer inside the puller, a patented feature, and the velocity has a direct correlation to the viscosity of the glass. The velocity is the "trip point" for turning off the heat and starting the cooling and the hard pull. When the velocity is low, the pipette taper will be shorter. When the velocity value is low enough, as when pulling a patch pipette, the puller will pull in multiple cycles or "LOOPS". A velocity of 18 - 65 is typical when pulling a patch pipette, and when using a one-line program, the lower the velocity is, the more times the program will loop. LOOPING is further discussed on page 29. A pull of 50-150 is typical when making a sharp electrode of 30 to100 MΩ or when making a microinjection needle.

TIME – This is the duration of air used to cool the glass and the filament as the glass is being pulled. When using the Time mode, the glass softens, the velocity trip point is reached, and then the glass is cooled at pulled simultaneously. The duration of cooling is determined by the value of the Time where each unit of time is equivalent to 0.5 milliseconds of cooling air. Traditionally a Time of 150 (75ms) is recommended when using a trough filament or when using thin walled glass and making a pipette for slice patch recording. A higher Time value of 250 (125ms) is recommended when using a box filament and pulling thick walled glass.

DELAY – This is the alternative mode of cooling which provides a longer duration of cooling (300ms) than the Time mode (max of 127.5ms). In the Delay mode, the glass softens, the velocity trip point is reached, and then 300ms of cooling is initiated. The Delay value determines how long the glass is cooled before the hard pull is engaged and continues cooling the glass as the glass is pulled. So by cooling the glass while delaying the hard pull, you can determine how viscous the glass is when it is being pulled. I find this to be a more sophisticated way of controlling the glass when making electrodes. A low Delay value of 40-90 units will expose the glass to less cooling before the hard pull, the glass will be more molten when the hard pull engages and this will result

in a longer tapered pipette. A higher delay value of 100-250 will provide more cooling to the glass before the hard pull engages, the glass will be more viscous, and this will result in a shorter tapered pipette. The delay mode of cooling is also most often used when pulling patch pipettes to deliver a longer duration of cooling to the glass which helps produce shorter tapered patch pipettes. As demonstrated on pages 30 and 31, a Delay mode of <1> with a Pull value of <0> results in short tapered pipettes.

PRESSURE – This is the pressure of air used to cool the filament and the glass. The default Pressure setting is 500 units, which represents 2psi of cooling air. Both the Time and Delay modes (duration of cooling) work in conjunction with the Pressure to cool the glass. The higher the Pressure, the more robust the cooling is to the glass and filament. An increase in Pressure will cool the glass faster and shorten the taper. A decrease in the Pressure will reduce the cooling to the glass and allow for longer and more gradual tapers.

General Guideline for the Parameter Settings

When designing a program or adjusting your existing parameter settings, it is quite easy to end up "lost" and with very unstable settings if you do not know where to start or when a setting is considered too high or too low. If you are trying to design a program from scratch, please refer to the "cookbook" programs provided for various applications. There you will find what we hope proves to be a good starting point. If you are in the midst of adjusting and fine-tuning your existing parameter settings, below is a general guideline suggesting the range of settings to stay within for each parameter. These ranges are a general rule of thumb, and there could often be exceptions depending on the filament and glass combination, the OD and ID of glass you are using, and the final morphology of the pipette you are aiming for.

HEAT:	**Ramp - 5 to Ramp + 15** for most applications!	
PULL:	**0**	**Patch,** 3-5mm taper and 1-3μm tips
	30 -70	**Microinjection**, 6-8mm tapers, 0.9 to 0.5μm tips
	70 - 150	**High MΩ**, 9-15mm tapers, 0.5 to 0.06μm tips
VELOCITY:	**20 - 60**	**Patch,** 3-5mm taper and 1-3μm tips
	50 - 80	**Microinjection**, 6-8mm tapers, 0.9 to 0.5μm tips
	70 - 100	**High MΩ**, 9-15mm tapers, 0.5 to 0.06μm tips
TIME:	**250 box, 150 trough**	**Patch,** 3-5mm taper and 1-3μm tips
	250 box, 150 trough	**Microinjection**, 6-8mm tapers, 0.9 to 0.5μm tips
	250 box, 150 trough	**High MΩ**, 9-15mm tapers, 0.5 to 0.06μm tips
DELAY:	**1**	**Patch,** 3-5mm taper and 1-3μm tips
	60 - 110	**Microinjection**, 6-8mm tapers, 0.9 to 0.5μm tips
	40 - 90	**High MΩ**, 9-15mm tapers, 0.5 to 0.06μm tips
PRESSURE:	**200 - 500**	**Thin Walled Glass**
	200 - 700	**Thick Walled Glass**

RAMP TEST

To choose an appropriate heat setting, you must first determine the amount of heat required to melt your glass by running a RAMP TEST. The heat value established by the ramp test will depend on the type of heating filament installed in your puller and the type and dimension of glass you are using. The ramp test value for a box filament will traditionally be 1.5 to 2 times higher than the value of a trough filament.

When to Run a Ramp Test

- Using the Puller for the First Time
- Whenever you Change the Filament
- Whenever you Change Glass
- Before Writing or Editing a Program

How to Run a Ramp Test on a P-97 (for the P-1000, press "Ramp" on upper right of display)

- Enter any program number <0-99> when using a P-97
- Press clear <CLR> to enter the control functions
- Press <0> to not clear all parameter values
- Press <1> to run a RAMP TEST
- Install glass and press <PULL>

To interrupt the RAMP TEST or reset the display after a ramp test, press <RESET>

When a ramp test is executed, the following events take place

1. The puller increments the HEAT
2. Once the HEAT output allows the glass to soften, the puller bars will drift apart
3. When the factory-set ramp velocity is reached (trip-point) the heat is turned off
4. The Ramp Test value will be shown on the display

Expected ramp test values

Filament #	Filament Dimensions	Expected Ramp Test Values	Maximum Heat
FT330B	3mm x 3mm TROUGH	250 – 300 **(see warning below)**	Ramp + 20
FB255B	2.5mm x 2.5mm BOX	~ 480 to 540	Ramp + 30
FB330B	3.0mm x 3.0mm BOX	~ 550 to 650	Ramp + 40
FB245B	2.5mm x 4.5mm BOX	~ 750 to 880	Ramp + 75

* **Warning -** If the ramp test value for your trough filament is OVER 300 units, this might be too high and could indicate that the filament shape is poor and therefore inefficiently heating the glass. Please remove your filament and reshape it according to the instructions in Chapter 14.

Recommended Heat Settings for Each Filament Type

Filament	Recommended Heat Setting
3mm TROUGH	Ramp +15
2.5mm x 2.5mm BOX	Ramp
3.0mm x 3.0mm BOX	Ramp
2.5mm x 4.5mm BOX	Ramp

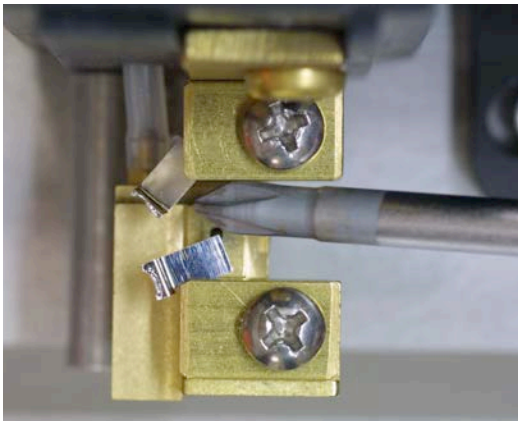
* **Caution -** If your Heat setting is greater than 20 to 50 units above the ramp value, depending on the filament shape and size, (see above table), you will risk burning out the filament!

“INSTALLING A FILAMENT” - STEP by STEP

Video Available on SutterInstrument YouTube Channel

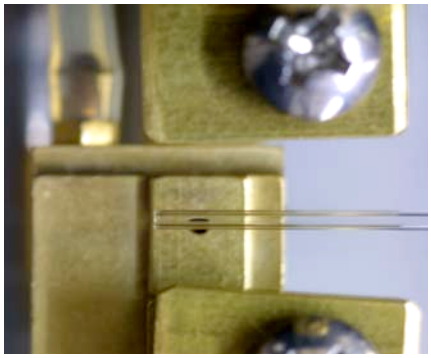
Installing a Filament - Step 1 of 11

Remove the Humidity Control Chamber, loosen the filament clamp screws and remove the old or damaged filament.



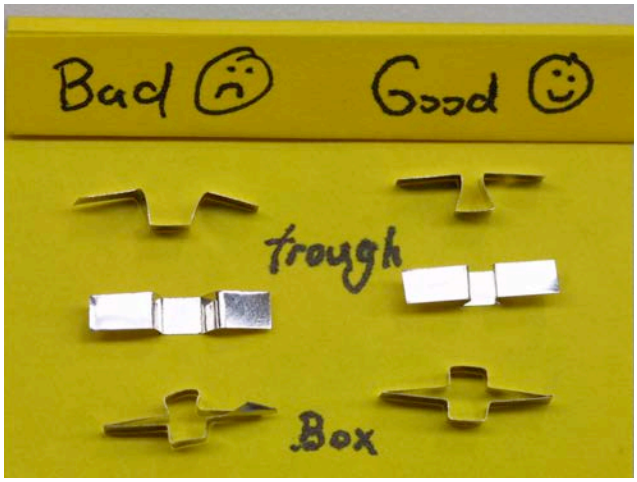
Installing a Filament - Step 2 of 11

Install a piece of glass in the right puller bar. Slide the glass to the left edge of the brass jaws and clamp the glass as depicted below.



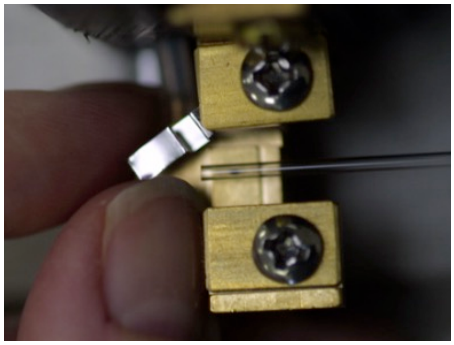
Installing a Filament - Step 3 of 11

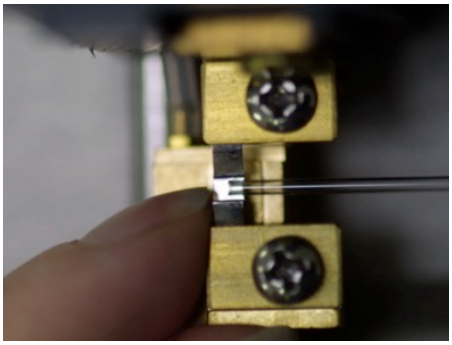
Before installing a trough filament, you must shape it.
Use your fingers and needle-nose pliers to produce a good shape.



Installing a Filament - Step 4 of 11

Hold the filament as shown and install the filament so the glass runs through the center of the filament. Using your fingers will not damage the filament and any oils from your hands will burn off during the first ramp test.

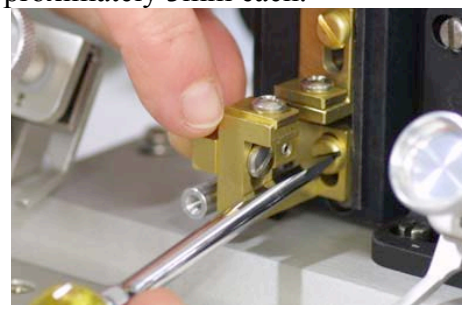




Installing a Filament - Step 5 of 11

If replacing a trough with a box – move both jaws DOWN approximately 3mm each.
If replacing a box with a trough – move both jaws UP approximately 3mm each.

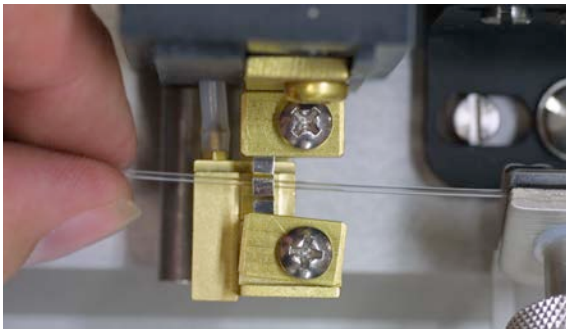
Loosen screw to move top jaw

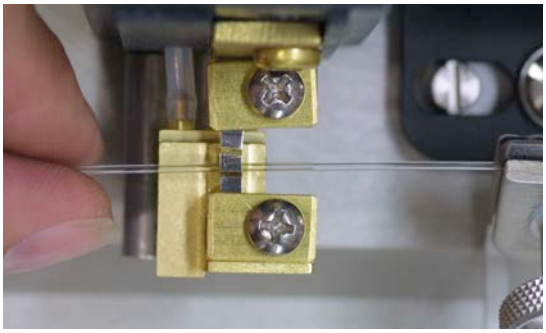


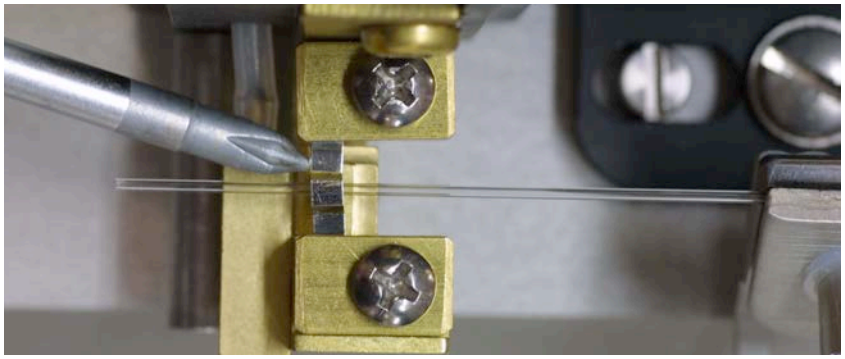
Loosen screw to moving bottom jaw

Installing a Filament - Step 6 of 11

Use your glass as a tool. Hold the left end of the glass and bend it front to back to gently position the filament front to back so it is centered around the glass. Then use the tip of a screw driver to **nudge the filament right/left to center it over the air jet (VERY IMPORTANT). See Page 17**

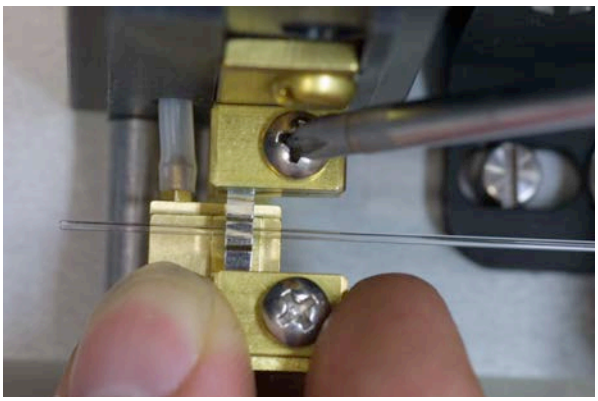






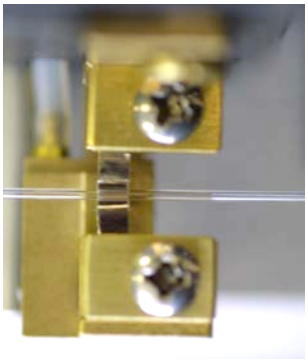
Installing a Filament - Step 7 of 11

Once the filament is centered to the glass and to the air jet, place your finger on the brass clamp before tightening the clamp screw. This prevents the filament from moving out of place when tightening the screw and will make your life easier.

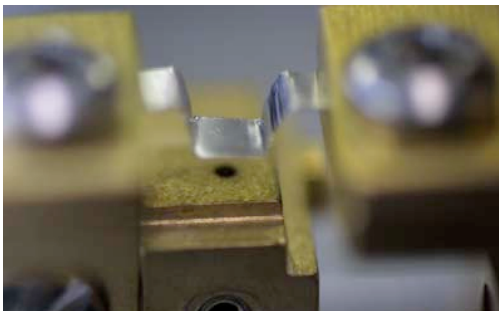


Trough Installation:

BAD – not centered over air jet



GOOD – centered over air jet & centered around glass



BAD Shape – walls angled outward

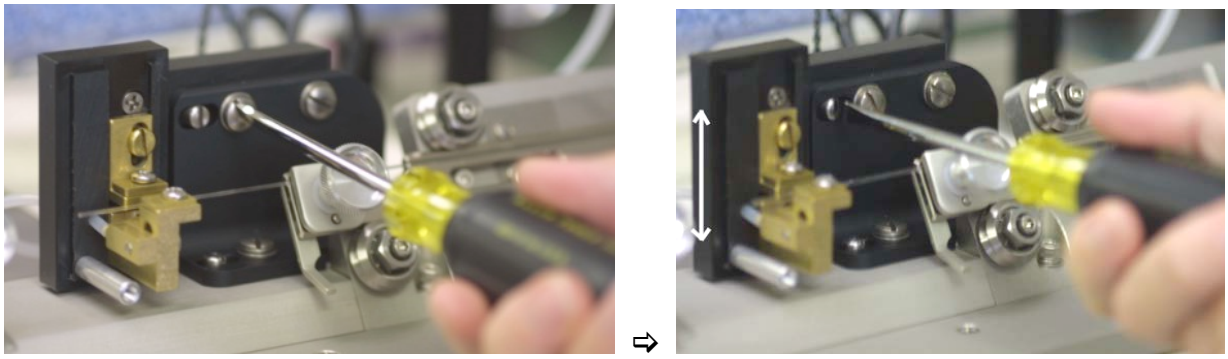
GOOD Shape – walls angled inward

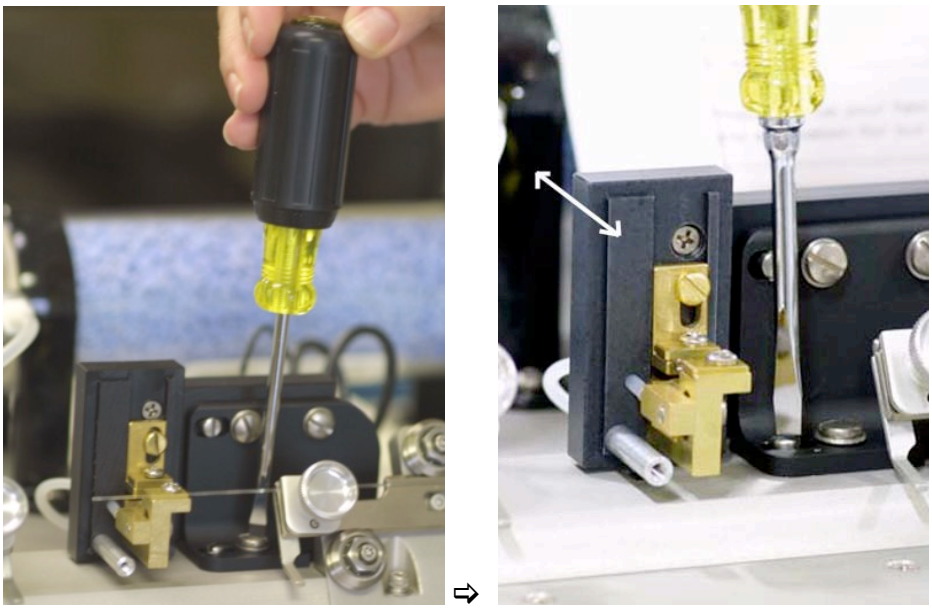
Box Installation: BAD & GOOD

Installing a Filament - Step 8 of 11

With the glass still in place, examine the alignment of the filament from above and horizontally.
If the filament is not properly positioned, fix the alignment.
For **LARGE** adjustments, repeat steps 4-7.
For **SMALL** adjustments, use the filament block assembly eccentrics (seen below)

ECCENTRIC ADJUSTMENTS
Loosen locking screw, turn eccentric to adjust jaw assembly **Up and Down** then tighten locking screw





Loosen locking screw, turn eccentric to adjust **Front to Back**, then tighten locking screw

Installing a Filament - Step 9 of 11

Once the filament is properly installed, replace the humidity control chamber.
Install a piece of glass making sure it does not hit either side of the chamber, or the filament.
If the glass runs into the chamber or the filament, contact Sutter for additional instructions.

Installing a Filament - Step 10 of 11

Open an existing or new program.
Bring the puller bars together.
Clamp the glass and **run a new Ramp Test.**
Any time you install a new filament, open a new vial of glass,
change the alignment, position or shape of the filament, you must run a new ramp test.

The Last Step! Check to make sure your filament is CENTERED over air jet. See pages 17 - 18.

Centering Filament Over Air Jet – Step 11 of 11

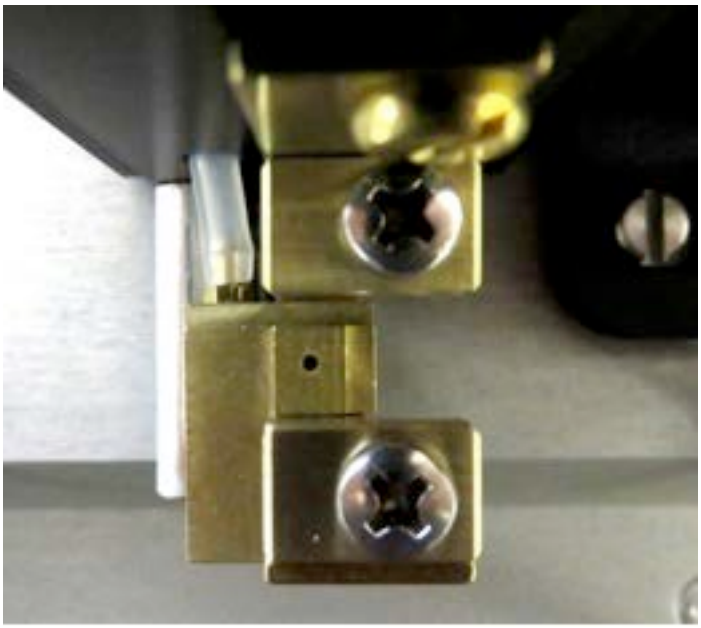
If your filament is not properly centered over the air jet (right to left), this will not only make one pipette longer than the other and the right and left pipettes will have different resistances, it will also cause a stable program **to become very unstable. This is ESPECIALLY true for PATCH PIPETTE PROGRAMS!**

After installing a new filament, it is very important to first pull a pair of pipettes with a long taper and compare the right and left pipettes to see if they are identical in length from taper to tip. Use the following settings to pull a long taper:

HEAT	PULL	VEL	TIME	PRESSURE
Ramp	70	70	250	500

Then take the right and left pipettes out of the puller, line up the shoulders of the tapers to compare them to each other. If the right pipette is longer, this indicates that the filament is positioned too far to the right. In this case the right pipette "sees" more heat and the left pipette "sees" less heat and more cooling. To correct this, you will need to loosen the filament clamp screws and **gently nudge the filament 0.1 to 0.5mm in the direction of the shorter pipette.**

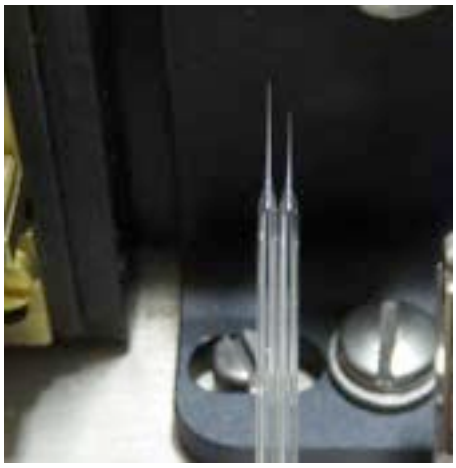
If the right pipette is longer and the **left pipette is shorter**, **shift the filament to the LEFT.**
If the left pipette is longer and the **right pipette is shorter**, **shift the filament to the RIGHT.**
You then need to pull another pair of pipettes to check to see if they are now the same length. If they are not, continue to adjust the filament right to left until they are the exact length. **It is not uncommon to have to adjust the filament a number of times until it is perfectly centered over the air jet and both pipette tapers are the same length.**



Between the front and back filament clamps you will see the air jet below. You must make sure the filament is perfectly centered over the air jet hole. Also observe that the air jet hole is drilled into a slightly raised square of brass.

When the filament is perfectly centered over the air jet hole, there will be an equal amount of the raised brass exposed to the right and left of the heating filament.

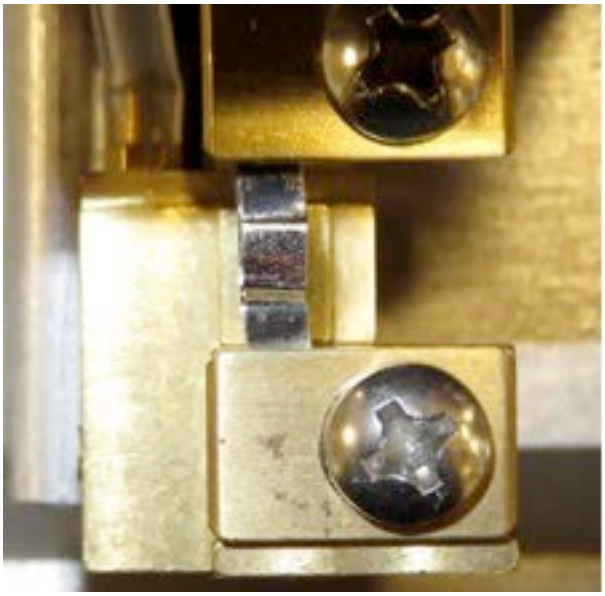




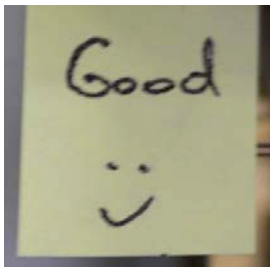
When the filament is installed too far to the left . . . . . the left taper will be longer.
MOVE the filament to the RIGHT.



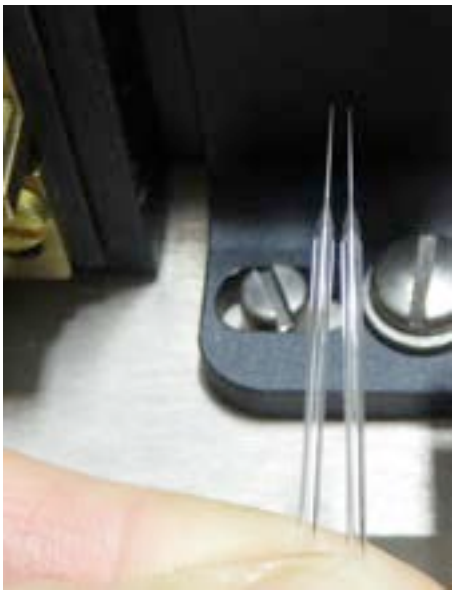
When the filament is installed too far to the right . . . . . the right taper will be longer.
MOVE the filament to the LEFT



When the filament is perfectly centered over the AIR JET HOLE and centered over the raised brass of the air jet . . . . .



⇨



Both right and left pipettes will be the SAME LENGTH. This is GOOD!

CHAPTER 1

Electrophysiology Overview

Electrophysiology studies the flow of charges (ions) in biological tissues and relies on the electrical recording techniques that enable the measurement of this flow. The most common recording techniques use glass electrodes, referred to as patch pipettes and sharp electrodes, to establish electrical contact with the inside or outside of a cell or tissue and measure this flow of ions. The glass electrode is most commonly fabricated from 1.0mm, 1.2mm, or 1.5mm outer diameter thin or thick-walled capillary glass. The tip size, taper length and resistance needed for the application are determined by the type of recording (intracellular or extracellular), the type and size of the tissue or cells, and the types and concentrations of your filling and bath solutions. After the pipette is made to the specifications needed by the researcher, the pipette is filled with a salt solution and a chloride coated silver or platinum wire is inserted in the back end of the pipette to establish an electrochemical junction with the pipette solution and the tissue or cell. The chloride coated silver wire connects back to the amplifier which measures and records the currents.

The two main recording techniques include *Extracellular* or passive recording and *Intracellular* or voltage and patch clamp recording techniques, the latter of which can clamp or maintain the cell potential at a level determined by the experimenter.

Extracellular Recording measures:

- changes in current density using single unit recording, field potential recording, and single channel recording techniques

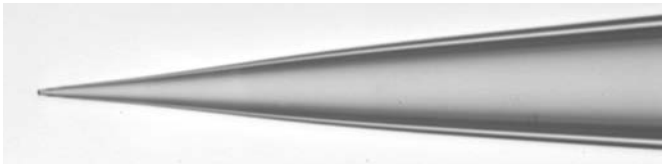
Intracellular Recording measures:

- the current flowing across that membrane using voltage clamp
- the voltage across a cell's membrane using current clamp
- the intracellular potential of the cell

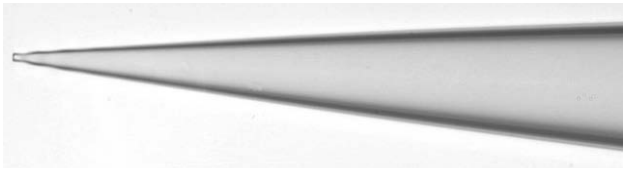
Extracellular Recording

Extracellular recording, currently referred to as loose-patch recording, is the precursor approach to the modern patch-clamp technique used commonly today. Extracellular recordings measure changes in the voltage potential in the extracellular space surrounding a neuron or axon and are detected by the use of extracellular microelectrodes (glass pipettes). The seals created between the glass pipette tip and the cell in this loose patch configuration have low resistances, so minimal interaction occurs between the recording electrode and the cell membrane. In extracellular recording, the cell membrane is neither broken nor penetrated, and the contents of the cell remain undisturbed. The greatest advantage of extracellular recording is that it is the least invasive electrophysiological method that allows for repeated recordings from the same cell without having to impale and consequently damage the cell. Applications include exploring the distribution of ion channels throughout the surface of a cell, recording from fragile membranes, and making stable long-term recordings. The pipettes required for this application tend to be in the 1-3 MΩ range and have a 3-6mm taper and a 1-3 micron tip. If the pipette is too small (under 1μm or 1MΩ) one can inadvertently and spontaneously form a GΩ seal to the cell membrane. If the pipette is too large (over 3μm or 3MΩ) one might change the cell morphology or aspirate the cell into the pipette during the recording.

Extracellular Microelectrode Images



400X mag., 1.5mm x 0.86mm Thick Walled Glass



400X mag., 1.5mm x 1.1mm Thin Walled Glass

Extracellular Microelectrodes - Recommended Programs

Goal = 1 - 3µm, 1 - 10MΩ, 3-5mm Short and Gradual Taper

3mm Box (FB330B) Filament & **1.5mm x 1.1mm** (B150-110-10) Thin-Walled Glass

Heat	Pull	Velocity	Time	Pressure	Loops
Ramp	0	65	250	500	2-3

3mm Box (FB330B) Filament & **1.5mm x .86mm** (B150-86-10) Thick-Walled Glass

Heat	Pull	Velocity	Delay	Pressure	Loops
Ramp + 5	0	19-26	1	500	4-5

3mm Trough (FT330B) Filament & **1.5mm x 1.1mm** (B150-110-10) Thin-Walled Glass

Heat	Pull	Velocity	Time	Pressure	Loops
Ramp + 15	0	90	150	500	3

3mm Trough (FT330B) Filament & **1.5mm x .86mm** (B150-86-10) Thick-Walled Glass

Heat	Pull	Velocity	Time	Pressure	Loops
Ramp + 10	0	55 -65	150	500	4

- For a larger tip and lower resistance, decrease the velocity to allow one more loop
- For a smaller tip and higher resistance, increase the velocity to allow one less loop

Since the pipette tip in extracellular recording is in proximity to, but not in tight contact with the cell or tissue, the resulting pipette can afford a wider range of taper lengths, tip sizes, and resistances. For those working within tighter tolerances please do the following:

- To achieve lower resistances and larger tips, use thin-walled glass and a box filament
- To achieve higher resistances and smaller tips, use thick-walled glass and a trough filament

* *For different filament and glass combinations, please refer to the " General Look Up Table for Patch Pipettes" and increase the velocity to allow one more loop than what is indicated in the last column.*

Intracellular Recording

Resistance & Geometry
Intracellular recording is used to study resting membrane potentials, intracellular potentials, and evoked potentials. Intracellular recording involves using a single glass electrode to puncture the cell membrane and enter the intracellular space. A reference electrode is then placed in the extracellular space within the bath, and the difference in electrical potential (voltage) between the two electrodes is measured.

A wide range of microelectrodes is used for intracellular recordings. Microelectrodes are drawn from many different types and sizes of glass capillaries, and Borosilicate is the most common glass used for intracellular recording. Quartz (or fused silica) and, to a lesser degree, Aluminosilicate, are superior to Borosilicate in strength, stiffness and the ability to form a small tip, but are also somewhat more expensive. Aluminosilicate can be pulled using the P-97 and P-1000 Pipette Pullers, but Quartz or Fused Silica must be pulled using the P-2000 laser-based Pipette Puller.

The intracellular approach requires the use of either a sharp or a blunt patch-clamp type electrode. The sharp electrodes will have a long taper and small tip with high resistances between 30 to100 MΩ and higher. Blunt electrodes will have a short stubby taper and a larger tip with low resistances in the1-20 MΩ range.

Low Resistance Microelectrodes, Blunt & Short
For the low 1-20 MΩ resistance electrodes, please refer to the Patch Pipette section to find glass, filament and program suggestions. If you need settings specific to an existing filament or glass, please refer to the General Look Up Tables at the end of the Cookbook. The patch pipette programs will produce a very short 3-4mm taper. If you need a slightly longer taper, install the suggested parameters, but use a higher range of velocity settings to allow the puller to loop one less time than what is indicated.

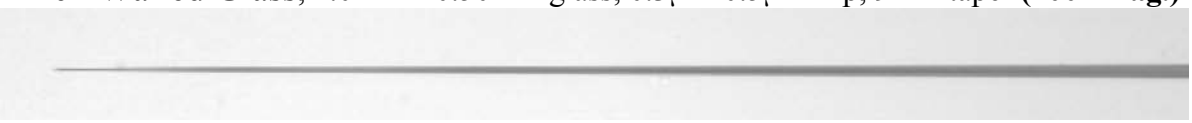
High Resistance Microelectrodes, Sharp & Long
For sharp microelectrodes with 30 to100 MΩ resistances and higher, the tip size and the geometry of the taper are usually the key factors determining if an electrode can successfully impale a cell. Small tips and gradual, uniform tapers have an obvious advantage in terms of causing less damage when a cell is impaled. They also tend to produce a high electrical resistance, which can add noise and make current recording more difficult. Injection of dyes etc. may also be effected.

The gradual uniform taper also has the advantage that it produces less dimpling of the tissue. When a microelectrode is advanced into the tissue, it tends to cause a local compression of the tissue. After the microelectrode stops, the tissue will gradually expand back to its original form, causing any cell that may have been impaled to be carried up the microelectrode along with the rest of the expanding tissue. It is important that the taper behind the tip has a slender and gradual slope and does not have an inflection or "shoulder" where the taper is suddenly larger. The inflection in the taper can cause excessive damage as it advances into the cell or tissue, or as the cell or tissue expands around the taper. Therefore, it is advisable to use gradually tapered electrodes in situations where the tip will be advanced into a cell or tissue and use blunt electrodes with a dramatic inflection near the tip only when you are recording from cells on the surface of a tissue. The overall shape and taper length of the microelectrode can also be critical. If the micromanipulator guiding the electrode into the cell or tissue does not produce a pure straight-ahead advance, long flexible tapers can be a big advantage in that they allow the tissue to stabilize the tip and this reduces the unwanted lateral motion. On the other hand, a shorter tapered and very stiff microelectrode is required to penetrate very tough and rigid membranes.

- *"Additional Concerns about Intracellular Recording" see pages 23-26.*

Intracellular Microelectrode Image

Thick Walled Glass, 1.0mm x 0.50mm glass, 0.3µm-0.5µm Tip, 9mm taper **(400x mag.)**



Intracellular Microelectrodes - Recommended Programs

- Programs using a **2.5mm x 2.5mm Box** (FB255B) Filament

  1.0mm x 0.5mm (BF100-50-10) Thick Walled Glass w/ Filament

Heat	Pull	Velocity	Delay	Pressure
Ramp	55-95	75	90	400

  1.2mm x 0.69mm (BF120-69-10) Thick Walled Glass w/ Filament

Heat	Pull	Velocity	Delay	Pressure
Ramp	60-80	80	70-90	300-500

  1.5mm x 0.86mm (BF150-86-10) Thick Walled Glass w/ Filament

Heat	Pull	Velocity	Delay	Pressure
Ramp	70-90	80	100-200	500

- Programs using a **3.0mm x 3.0mm Box** (FB330B) Filament

  1.0mm x 0.5mm (BF100-50-10) Thick Walled Glass w/ Filament

Heat	Pull	Velocity	Delay	Pressure
Ramp	55-125	75	90	500

  1.2mm x 0.69mm (BF120-69-10) Thick Walled Glass w/ Filament

Heat	Pull	Velocity	Delay	Pressure
Ramp + 5	55-80	90	60-90	500

  1.5mm x 0.86mm (BF150-86-10) Thick Walled Glass w/ Filament

Heat	Pull	Velocity	Delay	Pressure
Ramp	45-75	80-95	110-150	500

- For lower resistances and larger tips, use lower pull values or higher time/delay values
- For higher resistances and smaller tips, use higher pull values or lower time/delay values

* *For different filament and glass combinations, please refer to the "General Look Up Tables" and use* ***Type C or D*** *programs appropriate for your filament and glass combination.* ***Type D*** *programs will provide longer tapers, smaller tips, and higher resistances than* ***Type C****.*

Additional Concerns about Intracellular Recording

Intracellular recording is a demanding technique. Perhaps the most difficult aspect is that, while failure to obtain successful recordings might be due to any of a variety of causes, there is often no clue available as to the cause. This note is intended as an introduction to the requirements for successful intracellular recording. This is not a complete how-to guide, but rather an appreciation of the minimum standards for the key elements.

The first requirement is a good understanding of the technique and equipment required. Most individuals develop this understanding through working in a lab that is already successfully using intracellular recording or by taking one of the fine courses available. The ideal situation is to work in a lab with people who are successfully doing just what you want to do on your preparation. If you learn in this manner, make the most of it. In a big lab that has been successful, you may be able to make nice recordings without learning all the details. So you must guard against that. Take massive amounts of notes about every detail. Ask questions.

It is possible to set up a lab and teach yourself the technique independently. You clearly need to consult the methods sections of papers in your field, but it is also wise to obtain a lab manual from one of the many courses. In addition to the relevant biology, you must learn the basics of electronics. You need to know basic circuits and instrumentation. You should make quite sure that you have decent access to people with a lot of experience before you get started. When you think you have done everything perfectly but you get no results, you are going to need help.

Biology

It is obvious that you need a healthy preparation. Is there adequate oxygen? Is the tissue losing too much moisture? In addition, it must be very stable mechanically. If it is an isolated tissue, is the mounting scheme stable? If there is a superfusion, is the flow perfectly stable? If the preparation is most or all of an animal, you must make sure that there is no movement at the recording site while still allowing respiration and circulation. There are so many other concerns in relation to the preparation that we could not cover them all in a book, let alone in this short note.

You need to know what to expect when you run an electrode into the preparation. How will you know when you have made contact with the tissue? How will you know if you have got a penetration? Are there clues available that will help you get a superior yield? Can you tell where your electrode is within the tissue based on what you see? If you cannot get this information from someone with experience in your field, then you must become a good observer and determine the clues for yourself. There must be some hard surfaces, so do not be surprised if you break a lot of pipettes. This will improve with time, especially when you start getting cells before you hit the bottom of your recording chamber.

Electronics

The amplifier for intracellular recording can be any of a range of designs, but it must have a reasonably high input resistance and a low bias current. Most microelectrodes for intracellular recording have a resistance with the range of 10 to 500 MΩ. If the input resistance of the amplifier is not considerably greater than that of the recording electrode, the voltage measured at the amplifier will be reduced by a voltage drop across the resistance of the microelectrode. This is a simple voltage divider, and you need to understand electronics at this level. The ideal amplifier for intracellular recording would not source or sink any current when you are simply measuring voltage. Any current that does flow under these conditions will create a voltage drop across the microelectrode and also across the cell membrane, thus altering both the real and the measured membrane potentials. The amount of bias current that is tolerable depends on the electrode resistance and the target cells. A nanoamp of current (which is pretty massive for a bias current) produces one millivolt of voltage drop with a 1 megohm resistance. It is not difficult to design an amplifier that has a bias current of just a few picoamps, but the user should check bias current from time to time. It is a simple matter of looking at

the difference in output voltage from the amplifier when the input is grounded directly and when grounded through a high value resistor (100 megohms is a good value). The bias current can then be determined from Ohms law. (You must know and understand that one very well).

If the input resistance is sufficiently high and the bias current sufficiently low, D.C. voltages can be measured accurately. A device with just these features might be called an electrometer. Because of the high resistance of microelectrodes, even a modest capacitance at the input to the amplifier can attenuate higher frequency signals. For that reason most amplifiers designed for intracellular recording feature "capacitance neutralization". This is usually an adjustable, non-inverting, feedback from the output of the amplifier back to the input through a capacitor. A final feature that is fairly essential is a means of passing a controlled current through the electrode. This allows for measurement of electrode resistance and injection of current into the cell.

Microelectrode Resistance

Electrode resistance is the common means for comparing and screening microelectrodes, but resistance depends on many factors, and this should be taken into consideration. It is important that you realize that the same glass micropipette may have a wide range of resistances as a microelectrode, depending on the filling solution and the means of resistance measurement. This is the reason that we rely so heavily on electron microscopy to evaluate fine micropipettes.

Resistance is determined from Ohm's law (V=I*R or R=V/I) by injecting a known D.C. current through the electrode and measuring the resulting voltage drop across the electrode. You must measure the voltage drop after it reaches a stable value. If the electrode has a long time constant and the voltage does not reach a stable level before the end of the current application, extend the period the current is left on. If there is a built-in current measurement, make sure that it allows time for the voltage drop to stabilize.

The amplitude and sign of the current can have a dramatic effect on the voltage drop. Microelectrodes are not automatically all pure Ohmic resistors and can exhibit dramatic rectification. For example, K acetate microelectrodes have a much lower voltage drop for strong depolarizing currents than they do for hyperpolarizing currents. Microelectrodes that rectify cannot be properly characterized by a single resistance value. You must at least specify the sign and magnitude of the measuring current in order to be useful. If you want to compare the pipettes you are pulling now with pipettes pulled in another lab, you would be wise to fill the pipettes and measure resistance in a comparable manner. With the direct comparison established, you can do things your own way.

The Micromanipulator and Other Mechanical Considerations

The tip of the microelectrode must have a stable position in relationship to the tissue. If not, you have serious problems. The micromanipulator must be stable, but the stability of the electrode tip in the tissue is not just a function of the micromanipulator. The setup must be installed in a place and in a manner that reduces the potential for introduction of undesirable vibrations. The setup must be put together to minimize the effects of any residual vibrations or disturbances.

Mechanical Considerations

The first mechanical consideration is location. The more stable the location of the recording setup, the better. A solid concrete floor built directly on bedrock is one ideal. Stay away from springy floors. Put a dish of water on the floor, jump up and slam your feet down. If you cannot see any ripples in the water you have a good spot. If you are not on the ground floor, look for corners of the building or at least an exterior wall. Check for sources of vibration such as nearby ventilator fans, elevators and other equipment. Try to stay away from busy streets that have significant bus or truck traffic.
Check for heavy doors that can slam shut. Set door closers to be slow and soft in action.
Once you have found your best possible location, you will want to build your setup on something

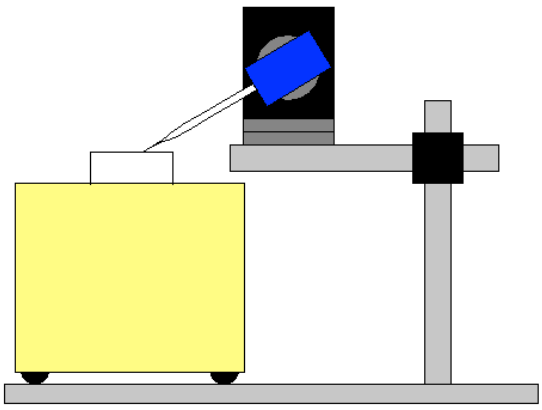
that can isolate it from any vibration that does exist. There are many ways to do this. It is a common practice to use an air table or a similar commercial product. In many cases a heavy balance table or a simple table made from concrete slabs will do very well by using mass and damping materials to attenuate vibration. Make sure the floor can handle the weight. Avoid mounting any source of vibration on the setup. Take care to avoid stiff cables or tubes that can bring vibration into the setup.

If the setup is mounted on a support that is perfectly free from vibration or disturbances, drift would be the only remaining mechanical concern. However, try as we might, there will likely still be little disturbances. As long as the tissue and the tip of the microelectrode move in perfect concert, the relative position of the tip and the tissue will remain unchanged. Consider the nature of every mechanical element that is involved in connecting the microelectrode back through the micromanipulator to the tissue. This connection should be as solid and stiff as possible. If there is any movement of the tissue or the microelectrode, you want them to move together so that there is no change in their relative position.

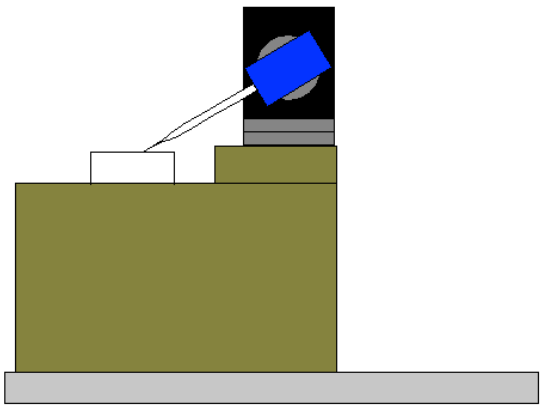
How do you make the connection from the tissue to the microelectrode stiff? Obviously we cannot expect the tissue to be rigid, but it should be constrained so that it does not move around easily. You will need to be especially attentive to this issue if the tissue is located in moving fluid. Each mechanical element between the chamber or container that holds the tissue and the micromanipulator is worthy of careful attention. The ideal situation might be thought of as a large solid block of steel or stone to which both the chamber and the manipulator directly attach.

The worst case would be a long, thin flexible connection. Watch out for long thin elements, however rigid they seem. Thin, flexible elements become even more of a problem when they connect items with significant mass. Imagine you move one end of a spring which has a weight attached to the other end. When you move the spring at one end, the weight at the other end will move with a delay and eventually reestablish its original relationship with the other end of the spring. The spring and weight also act as a resonator, which can enhance vibrations.

Watch out for unstable mechanical connections. It is important to realize that a loose connection that allows a small amount of rocking can produce a much larger movement at the end of a long connecting piece. Large flat surfaces mated together seem ideal, but it does not take much to cause problems. If one of the surfaces is slightly convex or if there are high spots, rocking can occur. Toe clamps can be ideal, but be careful that the force applied is not on the edge where it will have a tipping effect. A round rod clamped in a hole with a setscrew is also prone to rock, even if the fit is fairly tight.



Can you see the problems in this setup? Rubber feet, long, thin elements, weight on the end of a springy rod.



This is a more ideal configuration.

Buckets of sand, tennis balls and motorcycle inner-tubes have all been used as vibration isolation elements. Go ahead and try anything you like. Just keep the tissue-manipulator connection short, direct and rigid, and it will likely work.

Micromanipulator

When advancing the microelectrode, it must be under very good control, and the movement should be as close to a pure movement on a line as possible. This is the job of the micromanipulator. How do you make sure your micromanipulator is up to the job? Let's first consider typical specifications. Resolution, backlash, accuracy, repeat accuracy, stability and range of travel are most frequently considered, but what really matters? Good stability and resolution are generally much more significant considerations than superior accuracy, backlash and range in terms of getting successful recordings. Range of movement can be fairly important. You must have enough travel to get the microelectrode from the loading position into the desired location in the tissue. It is very frustrating to have a series of promising penetrations only to run out of fine travel before you get to the bottom of the tissue.

A very high degree of absolute accuracy or repeat accuracy is seldom critical. The tissue is likely not very consistent from session to session, and it is unlikely that the angle of attack relative to the tissue is always the same. It is handy to know relative position within a few microns, and it helps if backlash is a few microns or less. You would like to know about how deep you have gone in the tissue so that you know if you have gone through various layers of your tissue. You certainly want to know if you have gone completely through the tissue, but knowing position to better than a few microns is really not of much help.

If you were driving a car, an odometer that had absolute accuracy to 1 meter would be of little advantage if your directions say to turn right in about 1 kilometer. You simply want to know when to look for the turn-off and when to start thinking you missed the turnoff. You do not expect to measure the distance between points and turn without looking. If you are trying to penetrate a cell, you are not going to say to yourself, "I have now gone exactly 37.5 microns into the tissue. I will now go an additional 0.5 microns and I will have my cell." A much more likely thought would be, "I have gone over 300 microns past the point where I thought I hit the tissue, and I still have not seen any activity. Since my slice is only 200 microns thick, I better pull out and try again."

Some feel that good speed of movement over short distances (>2 mm per second) is a significant asset in penetrating cells, but experience shows that you can get excellent results with a manipulator that does not have any real claim to a particular speed. Techniques such as "buzzing-in" and tapping the table can be used in place of fast movement. It is also important to know that fast manipulators that use piezoelectric-based movements tend to be very poor in terms of producing pure, straight-ahead movement.

Although the distance and speed of movement might be well specified, what is seldom specified is what happens during the move. If the microelectrode is at all stiff, it is essential that movement is straight ahead without any lateral excursions. If you take a step move, does the tip of the microelectrode seem to disappear and reappear in the new position? Does the tip appear fuzzy as it moves? These are not good signs. It is fine to have the movement appear instantaneous, but the tip should always be visible and should appear in crisp focus during the entire move. Don't worry if you find that there are speeds that produce vibration due to mechanical resonances, just don't use those speeds for critical movements.

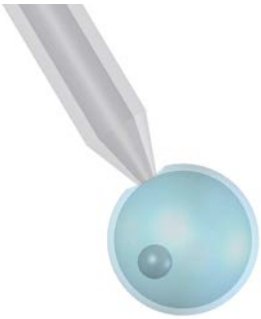
The micromanipulator is of little use without the microelectrode and amplifier. The microelectrode must be mounted close to the head stage of the amplifier so that the electrical connection is as short as possible. It is common practice to mount the headstage to the micromanipulator and then the microelectrode in a small plastic holder that connects directly to the head stage mechanically and electrically. If you use this approach, mount the head stage as close as possible to the micromanipulator to avoid additional weight and spring problems. Use an electrode holder that fits your glass outer diameter so that it can be clamped in place securely. If the headstage cannot be mounted right on the manipulator, be careful to avoid a springy rod or a plastic material that moves with small temperature changes.

* *For Micromanipulators (MP-285, MP-225, MPC-200, MP-265 and QUAD), Micromanipulator Stands and Platforms (MT and MD Series), please see the Sutter Instrument Catalog.*

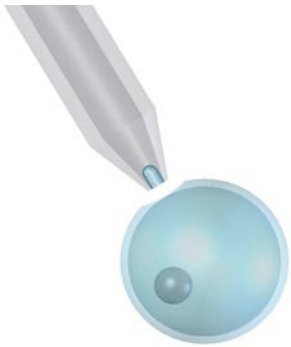
Patch Pipettes

The patch clamp technique is used in electrophysiological research to study the electrical activity of neurons at the cellular level. The technique requires using a blunt pipette with a 3-4mm short taper and a 1-3μm tip to isolate a patch of membrane. In general, patch pipettes are used to electrically isolate and study the movement of charges (ions) through the pores (ion channels) of the neuronal surface membrane. There are basically four different approaches to the patch technique: cell-attached patch, whole cell recording, and excised patch (outside-out patch and inside-out patch).

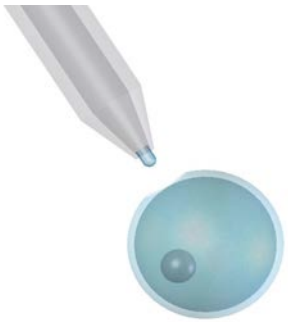
Cell-Attached Patch



Whole Cell Patch



Inside-Out Patch



Outside-Out Patch

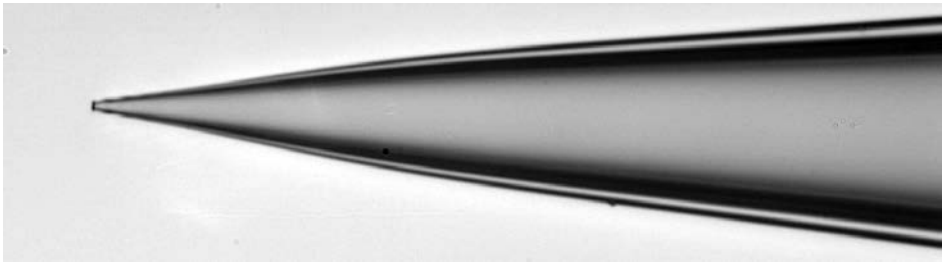
The patch technique is based on the electrical isolation of a small patch of membrane from the rest of the cell. To achieve this isolation, the patch pipette is placed against the cell membrane, and a slight suction or negative pressure is generated within the pipette. A tight seal is created between the pipette and the lipids of the cell membrane which is referred to as a "giga-seal" due to the high resistances (in the GΩ range) created between the outside of the patch pipette and the surrounding bath solution. The cell-attached patch configuration is a non-invasive approach which is used to measure the currents (current clamp) of single ion channels of the intact cell. The whole cell patch configuration is achieved when additional negative pressure is applied to the cell membrane through the pipette as it is in the cell-attached configuration. The suction through the pipette causes the cell membrane to rupture and create the whole cell patch where the cell is perfused by the solution in the pipette. In this case, the interior of the cell and the solution of the pipette become contiguous and the currents passing through the entire cell membrane are recorded. This whole cell recording configuration is equivalent to intracellular recording with sharp microelectrodes and has the advantage that it can be applied to very tiny or flat cells that in most other situations would be impossible to impale.

Patch Pipette Images

Whole Cell Recording, Cultured and Dissociated Cells

The ideal morphology for a patch pipette intended for dissociated/cultured cells is a short stubby taper, a high cone angle, and a 2-3 micron tip. This is best generated by using **Thick Walled Glass** and a 2.5mm or 3.0mm box filament.

FIVE LOOPS

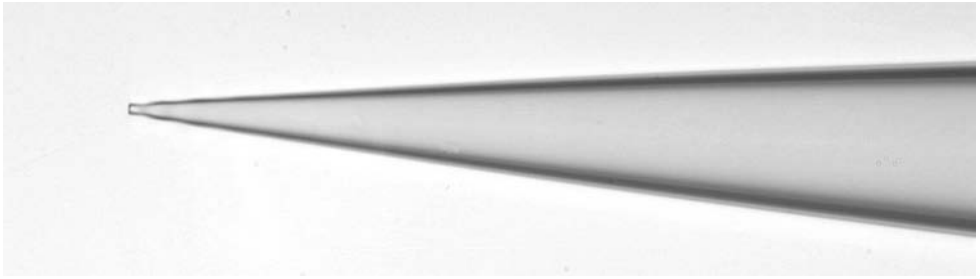


1.5mm x 0.86mm thick walled glass, ~2μm Tip, 3-4mm taper (400X mag)

Tissue Slice Recording

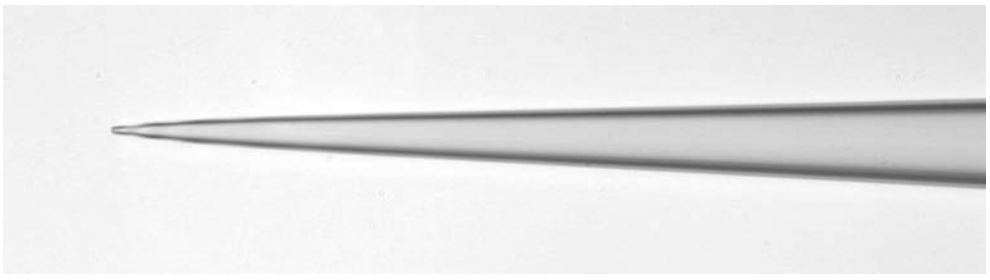
The ideal morphology for slice recording is a gradual slender taper, a low cone angle, and a 1-2 micron tip. This is best generated by using **Thin Walled Glass**, a 2.5mm or 3.0mm box filament and a program that allows the puller to loop 2-3 times. If you are going deep into the slice and find you are losing your seal or "chasing your cells," often due to a final taper that is too short and wide, select a velocity setting that allows two loops instead of three loops. This will reduce the cone angle and allow for a more slender taper.

THREE LOOPS



1.5mm x 1.1mm thin walled glass, 2-3μm Tip, 4mm taper (400X mag)

TWO LOOPS



1.5mm x 1.1mm thin walled glass, 1-2μm Tip, 5mm taper (400X mag)

LOOPING

The programs provided for making patch pipettes traditionally start off as one-line programs where the puller will pull the glass in multiple stages using this one line of programming. When the program has (0)pull, a low velocity (18-65) and only one line of programming, the glass will not separate on the first heat cycle. When the glass does not separate in one stage and there are no values on line two, the puller will return back to line one, also known as "LOOPING", and read line one over and over again until the glass separates.

Whole cell patch applications often require a tip between 2-3μm, a short 3-4mm taper, and a resistance between 1-5 MΩ. To achieve this morphology, it is best to use thick walled 1.5 x 0.86 glass, a **2.5mm or 3.0mm box filament (FB255B, FB330B)** and a program that allows 4-5 LOOPS.
For those requiring higher resistances between 5-10MΩ, using a higher velocity to allow 4 loops instead of 5 loops will help achieve this goal.

Those working within a slice preparation often require a slightly smaller tip of 1-2μm and more gradual and long taper. In this case it is best to use thin walled glass (1.5mm x 1.1mm, 1.2 x 0.96mm, and 1.0mm x 0.78mm) and a trough or box filament.

If you are aiming for pipette resistance between 1-10 MΩ and a tip size between 1-3 μm, please see the following section, "Writing a Stable Patch Program," to get started.

If you have a **3mm trough filament (FT330B)** installed in your puller, please refer to the general look up tables to find an appropriate program.

If you are using 1.5mm x 0.86mm glass, it is best to use a 2.5mm x 2.5mm box filament or a 3mm x 3mm box filament and aim for **4 to 5 loops**. Using a 2.5mm or 3mm box filament in combination with the delay mode for cooling will produce the shortest, most stubby taper and highest cone angle.

If you are using 1.5mm x 1.1mm glass, it is best to use a 2.5mm x 2.5mm box filament or a 3mm trough filament and aim for **2 to 3 loops**. Using a trough filament and the time mode for cooling will produce the most stable results, while using a 2.5mm or 3mm box and the delay mode for cooling will produce the shortest, most stubby taper and highest cone angle. When more than 3 loops or 3 lines are used to pull thin walled glass, it can often lead to variability in tip size, and the tips will more likely have an uneven, flared tip, splintered or cracked tip.

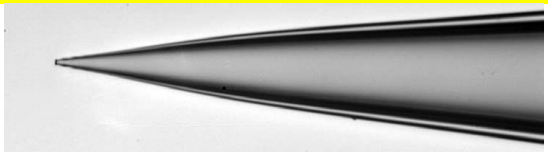
Pre-Heat Mode

When using the P-1000 Puller, you can also use the **THERMOLOCK**™ feature which pre-heats and maintains the jaw temperature at 70° C (Please see P-1000 manual for instructions). The variation of heat retained in the brass jaws (which hold the filament) can introduce variability if you have an "unstable" velocity setting. By choosing the **stable mid-point velocity** value, you will buffer these slight changes in heat, and your puller will loop the number of times you have intended. Using a **threshold velocity will result in variability** in cone angle and number of loops. You will find that there is not much leeway on each side of the mid-point velocity, so it is important to identify the exact range of velocities.

WRITING A STABLE PATCH PROGRAM: Mid-Point Velocity = Stable Velocity

To make a program with consistent results, you must find the mid-point velocity and this value will be your stable velocity setting. To do this you must find the entire range of velocity settings that loop the number of times recommended below. By using the absolute mid-point of the velocity range, you will create the most stable and reproducible program. After you establish and use the mid-point velocity, you find the resistance too high or too low, please see pages 32 and 33 to fine tune the program settings.

WHOLE CELL RECORDING - THICK WALLED GLASS



Using 1.5 x .86 glass and a 2.5 x 2.5 box filament install the following settings:

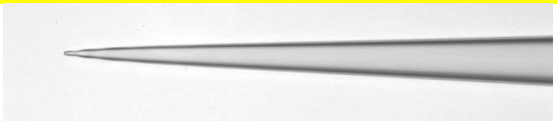
Heat	Pull	Velocity	Delay	Pressure	Ideal # of Loops
Ramp	0	22	1	500	5 times

Using the above **one line program**, pull a series of pipettes and only change the velocity to find the entire range which allows for **five loops**. To find the entire range, gradually increase the velocity in ONE UNIT increments until the puller loops four times and then gradually decrease the velocity until the puller loops six times. See example below:

Velocity	17	18	19	20	**21**	22	23	24	25
Loops	7	6	6	5	**5**	5	4	4	4

(Mid-Point Velocity)

SLICE RECORDING - THIN WALLED GLASS



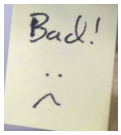
Using 1.5 x 1.1 glass and a 2.5 x 2.5 box filament install the following settings:

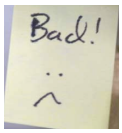
Heat	Pull	Velocity	Time	Pressure	Ideal # of Loops
Ramp	0	65	250	500	2 times

Using the above **one line program**, pull a series of pipettes and only change the velocity to find the entire range which allows for **two loops**. To find the entire range, gradually increase the velocity in THREE UNIT increments until the puller loops one time and gradually decrease the velocity until the puller loops three times. See example below:

Velocity	53	56	**59**	**62**	**65**	**68**	**71**	74	77
Loops	3	3	**2**	**2**	**2**	**2**	**2**	1	1

Threshold **(Mid-Point Velocity)** **Threshold**





WRITING A STABLE PATCH PROGRAM: CONTINUED

SUPERFICIAL SLICE RECORDING - THICK WALLED GLASS

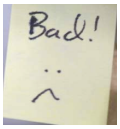
Using 1.5 x .86 glass and a 2.5 x 2.5 box filament install the following settings:

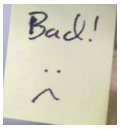
Heat	Pull	Velocity	Delay	Pressure	Loops
525	0	**26**	1	500	**4**

Using the above **one line program**, pull a series of pipettes and only change the velocity (up and down in TWO unit increments) to find the entire range which allows for **four loops**. To find the entire range, gradually increase the velocity in 2 unit increments until the puller loops three times and gradually decrease the velocity until the puller loops five times. See example below:

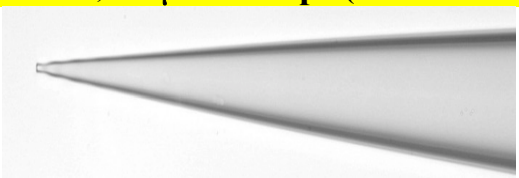
Velocity	21	**23**	**25**	**27**	**29**	**30**	**32**	**34**	36
Loops	5	**4**	**4**	**4**	**4**	**4**	**4**	**4**	3

Threshold **(Mid-Point Velocity)** **Threshold**





LARGE PATCH PIPETTE, 3-5µm ID tip (Whole Cell or Slice Patch Recording)



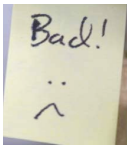
Using 1.65 x 1.20 or 2.0 x 1.56 glass and a 2.5 x 2.5 box filament install the following settings:

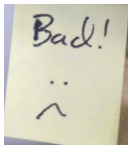
Heat	Pull	Velocity	Time	Pressure	Ideal # of Loops
Ramp	0	45	250	500	3 - 4 times

Using the above **one line program,** pull a series of pipettes and only change the velocity (up and down in THREE unit increments) to find the entire range which allows for **three loops**. To find the entire range, gradually increase the velocity in 3 unit increments until the puller loops two times and gradually decrease the velocity until the puller loops four times. See example below:

Velocity	**33**	**36**	**39**	**42**	**45**	**48**	**51**	**54**	57
Loops	**4**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	2

Threshold **(Mid-Point Velocity)** **Threshold**





A. To make a pipette with a **SMALLER TIP & HIGHER RESISTANCE . . .**
Increase the velocity to allow the puller to loop one less time. Aim for 2 loops instead of 3 loops for thin walled glass and aim for 4 loops instead of 5 loops for thick walled glass.

Find the range of velocities that loops 4 times (instead of 5) by gradually increasing the velocity and choosing the mid-point value. For example, using a 2.5mm box filament, 1.5 x .86 glass and the following settings:

Find the range of velocities that allows 4 loops and install the mid-point value…

Heat	**Pull**	**Velocity**	**Delay**	**Pressure**	**Loops**
525	0	**26**	1	500	**4 (**instead of 5)

If you would like a **SMALLER TIP but NOT A LONGER TAPER**, write out your program into five identical lines based on your one-line five-looping program you previously established. Then reduce the velocity on the fourth line by three units (which "saves" a little more glass for line five when the tip is forming. You can also add a slight amount of pull on the last line and/or increase the heat on the last line by 5-20 units. So as not to get "lost and confused" make only one modification at a time.

Line	**Heat**	**Pull**	**Velocity**	**Delay**	**Pressure**
1	525	0	21	1	500
2	525	0	21	1	500
3	525	0	21	1	500
4	525	0	**18**	1	500
5	**525+**	**10-40**	**21-25**	1	500

If you are using *thin walled glass and require a smaller tip* but do not want to increase the taper length, write the program out into multiple lines and *reduce the heat on the last line* instead of increasing it. Using a lower heat to produce a smaller tip might appear counter-intuitive but, if there is too much heat or too little cooling supplied to thin walled glass when the tip is forming, on the last line, one will produce a blunt tube-like tip instead of a fine tip.

B. To make a pipette with a **LARGER TIP, SHORTER TAPER & LOWER RESISTANCE . . .**
Lower the velocity to allow the puller to loop one additional time. Aim for 3 loops instead of 2 loops for thin walled glass and aim for 5 to 6 loops instead of 4 loops for thick walled glass.

Heat	Pull	Velocity	Delay	Pressure	Loops
525	0	24	1	500	4
		(change to 19-21) → → → →			**(now looping 5 times)**

Another way to make a **LARGER TIP** is to write the program out into multiple lines and lower the heat and/or the velocity on the last line. So if you have a one-line program that looped 5 times, you would write the program out into 5 identical lines, and change the parameters on the last line only when the tip is forming. It is best to make one change at a time and see the result.

Line	**Heat**	**Pull**	**Velocity**	**Delay**	**Pressure**
1	525	0	21	1	500
2	525	0	21	1	500
3	525	0	21	1	500
4	525	0	21	1	500
5	**520-500**	0	**19**	1	500

NOTE: If the velocity or the heat are reduced too far on the last line, the glass will not separate on Line 5 and will loop back to Line 1 to complete the pulling of the glass. If this happens, do not make such a large reduction in the value.

Cheat Sheet – a.k.a The Handy Dandy Parameter Adjustment Guide

SUTTER INSTRUMENT
P-97 & P-1000 PARAMETER CHEAT SHEET

	INCREASE	DECREASE
HEAT		
Δ 5 units	Smaller Tip, Longer Taper, ↑ MΩ	Larger Tip, Shorter Taper, ↓ MΩ
PULL		
Δ 10 units	Smaller Tip, Longer Taper, ↑ MΩ	Larger Tip, Shorter Taper, ↓ MΩ
VELOCITY		
Δ 1 - 3 (patch) Δ 10 (sharps)	Smaller Tip, Longer Taper, ↑ MΩ	Larger Tip, Shorter Taper, ↓ MΩ
TIME (cooling)		
Δ 25 units	Larger Tip, Shorter Taper, ↓ MΩ	Smaller Tip, Longer Taper, ↑ MΩ
DELAY (w/ Hard Pull)		
Δ 10 units	Larger Tip, Shorter Taper, ↓ MΩ	Smaller Tip, Longer Taper, ↑ MΩ
DELAY =1 (w/ Pull = 0)	**FOR PATCH PIPETTES USING THICK WALLED GLASS**	
	Delay (1) = 300ms of cooling. Do NOT increase or decrease the Delay!	
PRESSURE (cooling)		
Δ 100 units	Larger Tip, Shorter Taper, ↓ MΩ	Smaller Tip, Longer Taper, ↑ MΩ

References for Electrophysiology

- Advanced Micropipette Techniques for Cell Physiology, K.T Brown, D.G. Flaming
- The Axon Guide – For Electrophysiology & Biophysics: Laboratory Techniques http://www.moleculardevices.com/pdfs/Axon_Guide.pdf
- Patch Clamping An Introductory Guide to Patch Clamp Recording, Areles Molleman
- The American College of Neuropsychopharmacology web site http://www.acnp.org/g4/GN401000005/CH005.html
- Curtis, H.J. & Cole, K. S. Membrane action potentials from the squid giant axon. J. Cell. & Comp. Physiol. 15: 147-157, 1940
- Huxley AL and Hodgkin AF. Measurement of Current-Voltage Relations in the Membrane of the Giant Axon of Loligo. *Journal of Physiology* 1: 424-448, 1952(a).
- Neher E and Sakmann B. Noise analysis of drug induced voltage clamp currents in denervated frog muscle fibers. *J Physiol* 258: 705–729, 1976
- General descriptions about electrophysiology and various approaches: http://www.answers.com/topic/electrophysiology
- "Single Channel Recording" 2nd edition 1995 by Sakmann & Neher

Where to take a concentrated short course to learn electrophysiology techniques

- Marine Biological Lab, Woods Hole, MA http://www.mbl.edu/education/courses/
- Cold Spring Harbor Lab, Cold Spring Harbor, NY http://meetings.cshl.edu/courses.html

CHAPTER 2

Adherent Cell, *C.elegans, & Drosophila* – Recommended Programs

The pipettes required for these applications are most commonly made using thick or thin walled filamented glass; Sutter Part Number BF100-50-10, BF100-58-10 and BF100-78-10. These applications require a fairly short taper that is approximately 6-8mm in length and a tip that is just under 1μm. The tip and taper need to be fine enough not to cause damage, and the taper should not be so long that it becomes wispy and not durable. To maintain good durability in the pipette, it is best to keep the taper on the shorter side by using a 2.5mm x 2.5mm box filament. Any time you are making needles with tip sizes under 1μm, filamented glass is used to allow for easy loading of solution into the needle. The best ingredients to start with would be 1mm x 0.58mm or 1mm x 0.78 filamented glass and a 2.5mm x 2.5mm box filament (FB255B). Although a 3mm x 3mm box filament (FB330B) will also work well for pulling these types of pipettes, the more narrow 2.5mm box filament will keep the taper from becoming too long and wispy. If you are using a puller where the filament cannot be changed, please find the filament shape and size in the General Look Up Table and use the program designed for your 1mm OD glass.

Programs for a 2.5 x 2.5 box filament (FB255B)

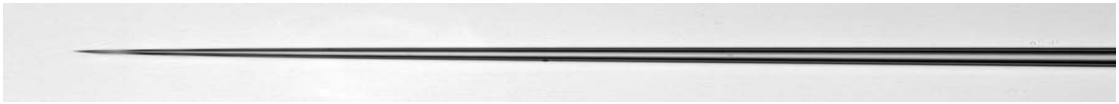
1. **THICK WALLED GLASS - BF100-58-10 (1.0 x 0.58) or BF100-50-10 (1.0 x 0.5)**

HEAT	PULL	VEL	DEL	PRESSURE
Ramp -10	60-80	80	130-170	500

2. **THIN WALLED GLASS - BF100-78-10 (1.0 x 0.78)**

HEAT	PULL	VEL	DEL	PRESSURE
Ramp +10	60-90	80	90	200

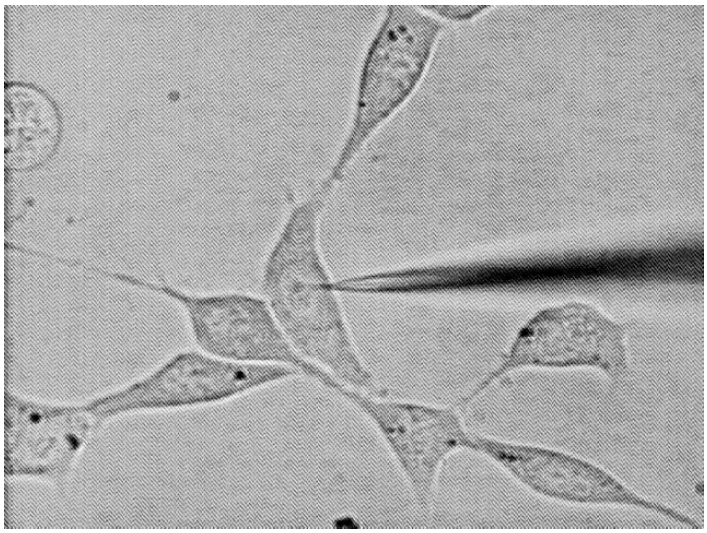
- **Larger Tip & Shorter Taper** = Increase the Delay or Pressure in 10 unit increments
- **Smaller Tip & Longer Taper** = Decrease the Delay or Pressure in 10 unit increments
- **Reduce Shoulder** to make tip more narrow and gradual = Increase Pull in 10 unit increments



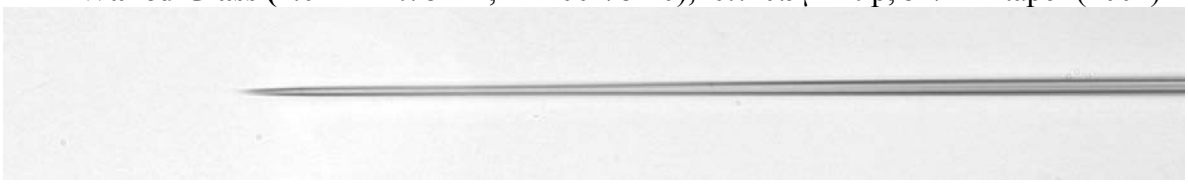
Depending on your specific application and technique, these pipettes can be used "as is," broken back, or beveled. If you will be using them "as is," it is best to use a lower pull value so the tips do not become too small. If you will be breaking back or "tapping off" the tip to "open it up", it is best to introduce 10-20 additional units of pull strength so the tip does not break off too big or become too large too fast. If you will be beveling the tip back to make it sharper and more open, it is best to introduce 5-10 additional units of pull so your final tip retains the same outer diameter.

Using the P-97 or P-1000, the **most durable and sharp needles** for this application can be made with **Aluminosilicate glass**, AF100-64-10. Even sharper needles can be achieved by beveling the tips using the Sutter BV-10 Beveler (Chapter 19). For a shorter and quick taper, you might want to try a "Bee Stinger Needle" for this application (Chapter 3).

Adherent Cell, *C.elegans & Drosophila* microinjection

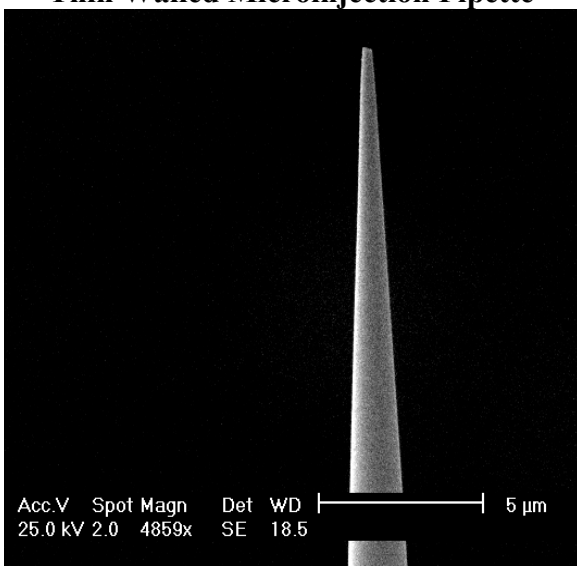


Thin Walled Glass (1.0mm x .78mm, BF100-78-10)**,** 0.7-0.9µm tip, 5-7mm taper (400x)



Thick Walled Glass (1.0mm x 0.50mm, BF100-50-10), 0.6-0.9µm tip, 6-8mm taper (400x)

Thin Walled Microinjection Pipette



Scanning Electron Microscopy of a Microinjection Pipette (~5,000x mag)

XenoWorks Digital Microinjector for Adherent Cell, *C.elegans, & Drosophila*.

XENOWORKS DIGITAL INJECTOR SETTINGS:

- **RANGE SETTING: 1**
- **TRANSFER PRESSURE (compensation) = 10-40 hPa**
- **INJECTION PRESSURE: (PULSE or CONTINUOUS)**

Recommended Approach…….

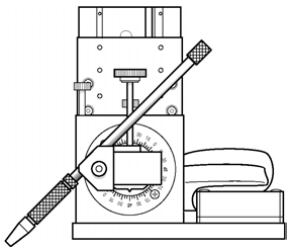
PULSE MODE: Adherent cell = 200-1000 hPa
Worms & Flies = 50-200 hPa
Pulse Width = 0.20 seconds

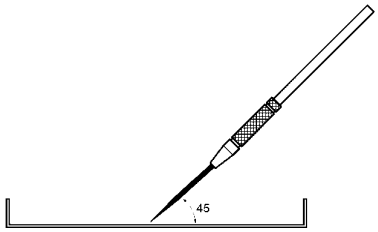
Alternate Approach…….

CONTINUOUS MODE: Adherent cell = 200-1000 hPa
Worms & Flies = 50-200 hPa
Duration of injection determined by how long the inject key/foot switch is held down.

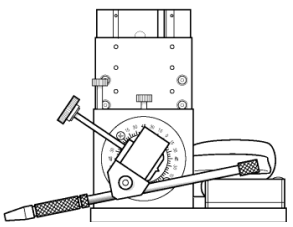
Alternate Method of Continuous Inj. using Transfer/Compensation Pressure:
Increase transfer pressure until desired flow rate is created. For higher pressures, switch to Range Setting 2 which will double available pressure.

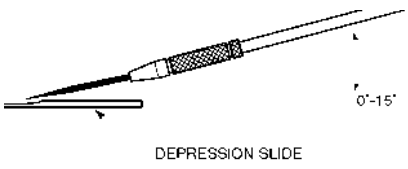
XENOWORKS MICROMANIPULATOR (BRMR) POSITION:





Adherent Cell



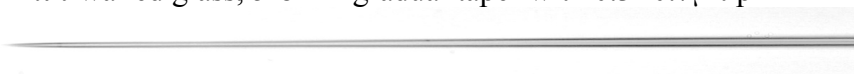


C.elegans & Drosophila

Pipette Morphology

Preferred Morphology – Gradual Taper

A. ***Thin*** walled glass, 5-8mm gradual taper with 0.3- 0.7µ tip



B. ***Thick*** walled glass, 5-8mm gradual taper with 0.3- 0.7µ tip

Alternate Morphology – Stubby Taper∗

C. Bee-Stinger - Thick walled glass with stubby 2-3mm taper and 0.5µ tip

D. Bee-Stinger - Thin walled glass with stubby 4-5mm taper and 0.5µ tip

∗ See Chapter Three of the Pipette Cookbook or contact Sutter for instructions on how to make a bee-stinger type pipette. This stubby taper is usually **not recommended** since this morphology of pipette is more likely to clog.

Unclogging or Clearing a Pipette

If the pipette clogs it is best to use the CLEAR Button first to attempt to clean out the tip of debris. If it does not clear, you can attempt to gently break the pipette to remove the clogged portion of the tip. The more gradual taper of the pipettes, which are pulled in one stage, can usually be broken back a few times before they get too big to use.

Tapping Off /Breaking Back /Opening up - your pipette tip:
It is best to use an uncoated glass or plastic surface upon which to drag or tap the tip of the glass. If you use the bottom of the coated dish, it will not break easily and you are more likely to further clog the tip.

Clogging – Pipette Tips will clog due to:

- ➲ Concentration of the DNA is too high
- ➲ Contamination or poor filtration of injection solutions
- ➲ Lowering the tip down into the dish/media without adequate compensation pressure
- ➲ Dirty glass – small particles of debris along the inner wall of the glass capillary
- ➲ Extended use - the pipette tip will eventually get dull and/or permanently clog after multiple injections (~20-50) due to lipids and proteins from the membrane coating the tip.

Clearing/Unclogging:

- ⇨ Use the clear button to flush out the tip and/or tap/break the tip to create a new sharp opening.
- ⇨ If you establish a compensation pressure that allows for a slight "bleeding" or leaking from the tip, this could extend the "life" of the pipette and help prevent it from clogging while you are injecting your cells.

Using Purchased/Pre-Made Needles for Adherent Cell Microinjection

Not Recommended! Why? See Pg 39-40.

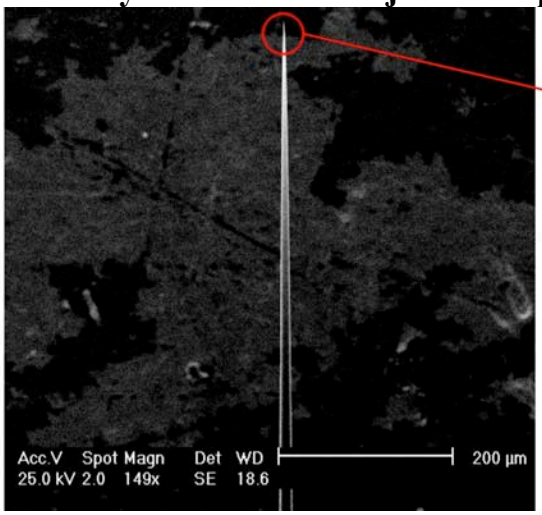
Pre-Pulled Microinjection Pipettes – Do you really know what you are buying?

Pipettes made for microinjection, specifically those for adherent cell inj., pronuclear inj., C. elegans, Drosophila, Zebrafish and Xenopus are in best condition when they are freshly pulled and used within minutes to hours of making the pipette. Microinjection pipettes that are pre-pulled and stored are much more likely to become contaminated.

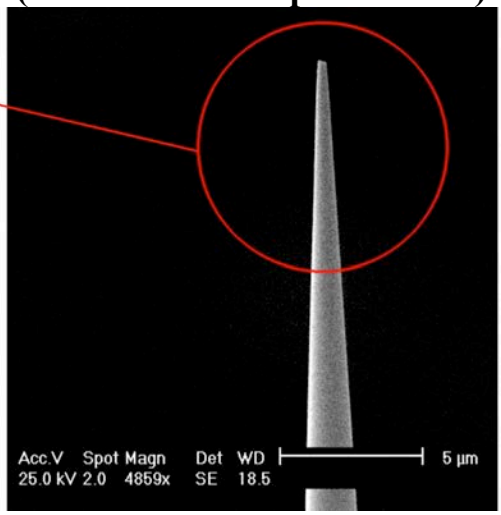
As a quality control measure, Sutter Instrument routinely performs scanning electron microscopy on pipettes pulled from each and every pipette puller we sell. During the past ten years we have, on occasion, been asked by customers to examine the pre-pulled pipettes they had purchased from a manufacturer. Throughout this time over hundreds of pre-pulled pipettes have been examined and we have seen a trend where these pipettes exhibit some unique artifacts. Provided to us in their sterile packaging, the pre-pulled pipettes were opened in a clean, dust-free environment, mounted on stubs, sputter coated with 10nm of gold (Polaron) and then observed under Scanning Electron Microscopy (SEM, Philips XL30).

The comparisons between freshly pulled microinjection pipettes made on a Sutter P-1000 Pipette Puller, to those microinjection pipettes sold by a manufacturer of pre-pulled pipettes are depicted below.

Freshly Pulled Microinjection Pipette (P-1000 Sutter Pipette Puller)

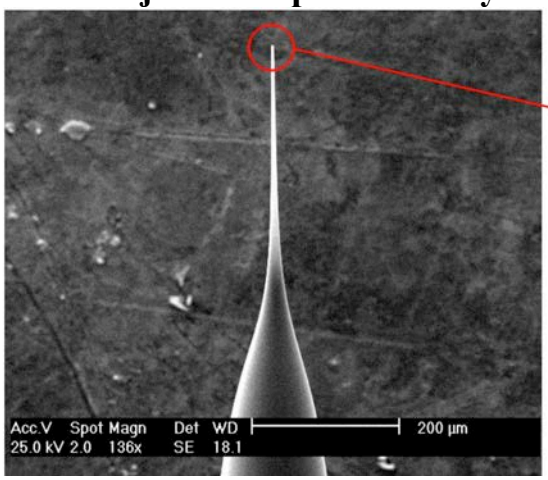


1a. SEM analysis of freshly pulled pipette at low magnification (~140x).

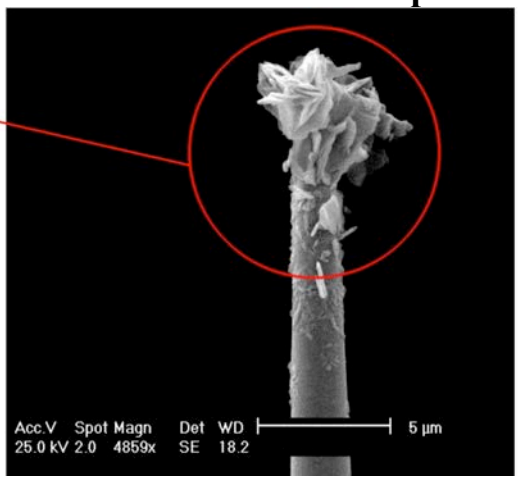


1b. SEM analysis of freshly pulled pipette at high magnification (~5,000x).

Microinjection Pipette Sold by a Manufacturer of Pre-Pulled Pipettes



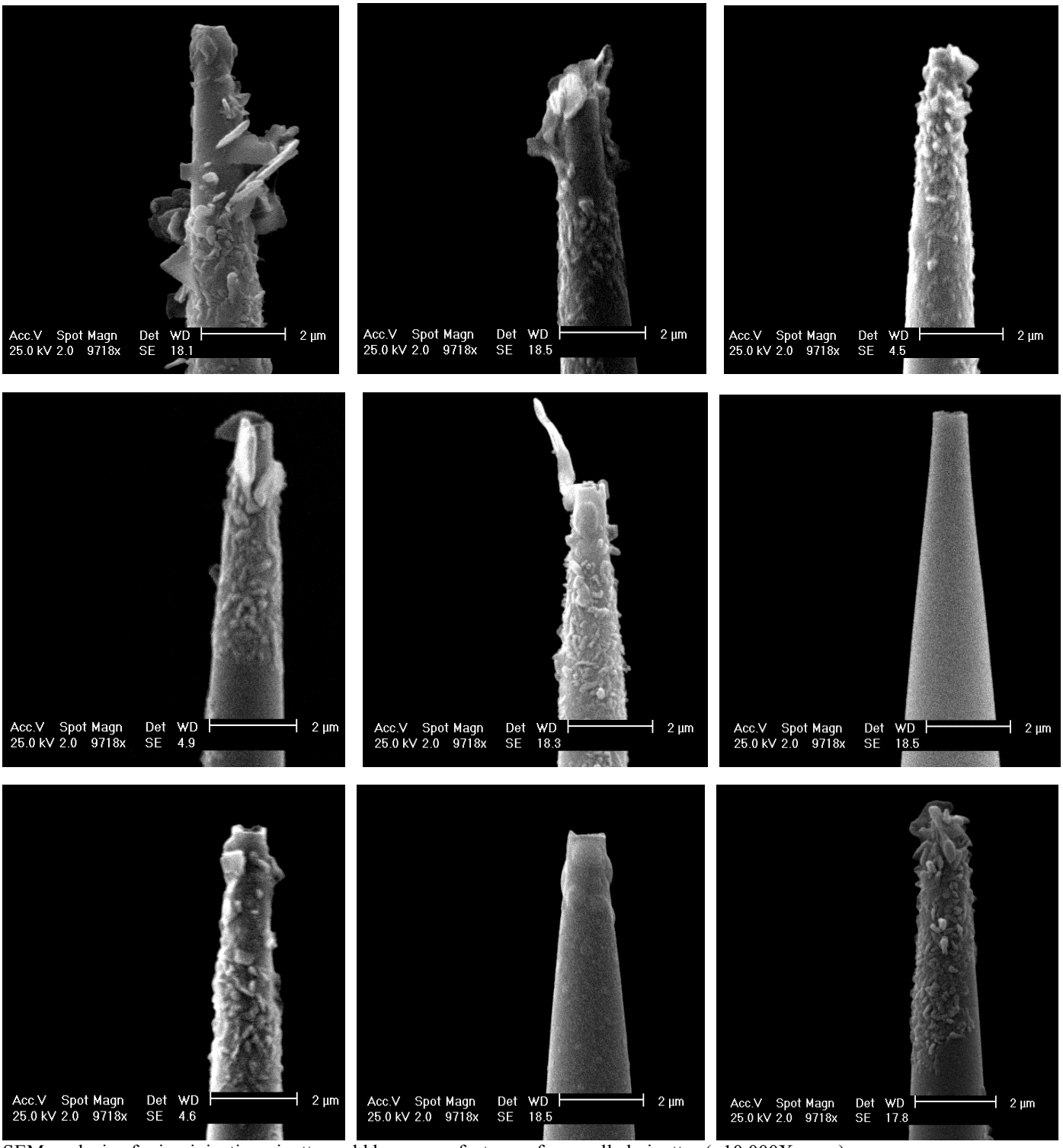
2a. SEM of pipette sold by a manufacturer of pre-pulled pipettes (~140x).



2b. SEM of pipette sold by a manufacturer of pre-pulled pipettes reveals contamination (~5,000x).

Pre-Pulled Microinjection Pipettes (continued)

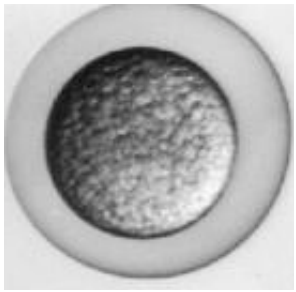
Analysis at high magnification found various levels of contamination on more than 80% of the pre-pulled pipettes inspected. Representative images of pre-pulled pipettes examined at 10,000x mag. with a scanning electron microscope are presented below.

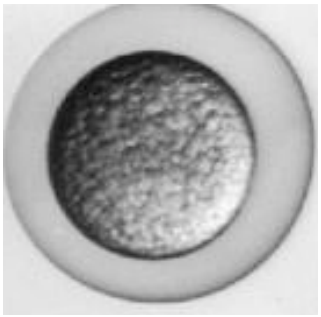


SEM analysis of microinjection pipettes sold by a manufacturer of pre-pulled pipettes (~10,000X mag).

While there are various possible sources of contamination for pre-pulled pipettes, these particulates are atypical of dust and moisture. One could hypothesis as to where this contamination originates, but other types and levels of analysis would need to be preformed to identify these artifacts.

Zebrafish Embryo Injection





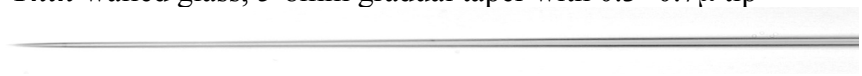
Pipette Morphology:

Zebrafish microinjection pipettes traditionally start off having a gradual 6-8mm taper. The ideal pipette should have a very slender inner diameter for the last 1-2mm behind the tip. This gradual taper allows one to be able to break back the tip and still maintain a small opening (4-10 microns) at the end of the pipette. To make sure your pipette has a slender taper, it is best to use Pull settings between 45-110 units.

Use the settings provided below to make your microinjection needles. The expected tip size will be between 0.3 – 0.7 microns before the tip is broken back. To break back the tip, use a dissection scope and a pair of tweezers to break back and remove the last 1-2mm of the taper.

Preferred Morphology – Gradual Taper, then broken back to have a 4-10µm tip

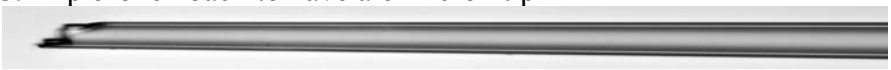
A. ***Thin*** walled glass, 5-8mm gradual taper with 0.3- 0.7µ tip



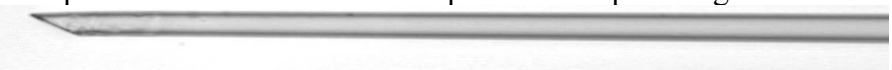
B. ***Thick*** walled glass, 5-8mm gradual taper with 0.3- 0.7µ tip

Tip is then broken back or beveled viewed at 20X magnification

C. Tip broken back to have a 6 micron tip



D. Tip beveled to have a clean sharp 6 micron tip – using BV-10 Beveler



Programs for a 2.5 x 2.5 box filament (FB255B)

1. **THICK WALLED GLASS - BF100-58-10 (1.0 x 0.58) or BF100-50-10 (1.0 x 0.5)**

HEAT	PULL	VEL	DEL	PRESSURE
Ramp -10	60-80	70	130-170	500

2. **THIN WALLED GLASS - BF100-78-10 (1.0 x 0.78)**

HEAT	PULL	VEL	DEL	PRESSURE
Ramp +10	60-90	70	90-200	200

- Larger Tip & Shorter Taper = Increase the Delay or Pressure
- Smaller Tip & Longer Taper = Increase Pull or Heat in 5 unit increments

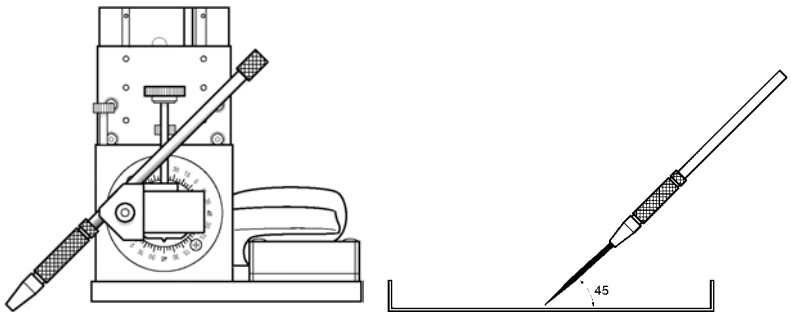
XenoWorks Digital Microinjector for Zebrafish Embryo Injection.

A. BRE XenoWorks Digital Injector Settings:

- RANGE SETTING = **1**
- TRANSFER PRESSURE (compensation) = **5-15**
- INJECTION MODE = **PULSE**
- PULSE WIDTH = **0.04 seconds**
- PRESSURE SETTING = Start with **4,500hPa** - Avg, range = 3,500 to 5000hPa

After you have back filled the pipette and broken back the taper to create a 4 to 10 micron tip, the goal is to establish a pressure and duration that provides the proper injection volume. To calibrate the pressure and duration required for your pipette opening, back fill the pipette with the injection the solution and inject this solution into a drop of oil that has been placed on a slide with a stage micrometer (S8 stage micrometer graticule with 1mm/0.01mm div.) Inject a bead of solution into the oil and adjust the pressure to produce a 0.1mm diameter bead, which is equivalent to 500 picoliters. Typical volumes range from 500 picoliters to 1 nanoliter and should represent 10% or less of the egg volume.

MP-225 SUTTER MICROMANIPULATOR POSITION for Zebrafish Embryo Injection



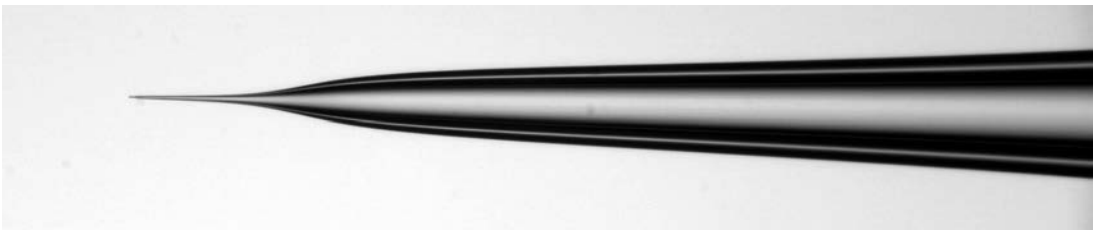
Zebrafish embryo approached at a 30-45 degree angle

CHAPTER 3

Bee-Stinger Needle – mainly recommended for C. elegans (worm injections)

0.5µm Tip ID
4 to 5mm Overall Taper Length
50-500µm final "bee stinger" taper and tip

- Using a 2.5mm x 4.5mm Box filament (FB245B)
  (This type of pipette cannot be made with a trough filament)
- Using 1.0mm x 0.78mm or 1.0mm x 0.50mm Borosilicate Glass



This program will work with either 1mm x .78mm (BF100-78-10) thin walled glass or 1mm x 0.5mm (BF100-50-10) thick walled glass. Below we have provided a one-line program which is intended to loop three times when using a 2.5mm x 4.5mm box filament.

Heat	Pull	Velocity	Time	Pressure
Ramp	100	10	250	500

Your Goal is 3 Loops!

The velocity setting in this one line program is the determining factor which controls the number of times the program will loop. Three loops appears to work best and produce the most stable results. If the program does not run as expected (loops too many or too few times), please make small adjustments in 1-2 unit increments up or down in the velocity setting to insure that it loops three times. An increase in velocity will cause the program to loop fewer times, and a decrease in velocity will cause it to loop more times. If you need to fine-tune the final length of the "bee stinger" like projected tip, write the program out into three identical lines and make small adjustments to the velocity on the last line only (Image B, pg. 40) A slight reduction in the velocity will reduce the final taper and a slight increase in the velocity will lengthen the final taper.

Smaller and shorter box filaments (2.5mm and 3.0mm box filaments) do not seem to work as well when trying to fabricate this morphology of pipette. The 2.5 x 4.5 wide box filament is the most reliable filament to make a Bee-Stinger shape. Additional programs listed in the "General Look Up Tables" will not generate this exact morphology of pipette, so if you are encountering difficulty making a Bee-Stinger Needle, please contact Sutter Technical Support.

SPECIAL NOTE: Although this morphology is sometimes advertised as being good for adherent cell microinjection, and while this might prove to be true with some protocols, often a more gradual tapered pipette is a superior morphology to aim for.

Bee-Stinger Needle
(Primarily Recommended for C. elegans)

Image A **(40x mag.)** 1.0 x 0.5 thick walled glass (BF100-50-10)
~0.5µm Tip, 4-5mm taper, one line program with 3 loops

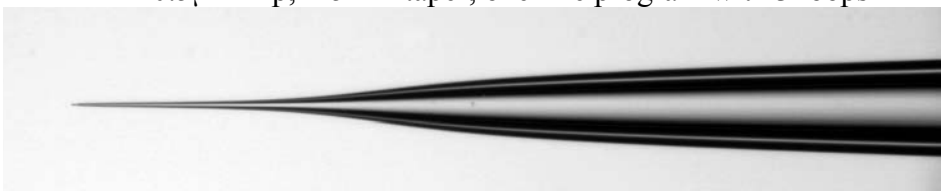
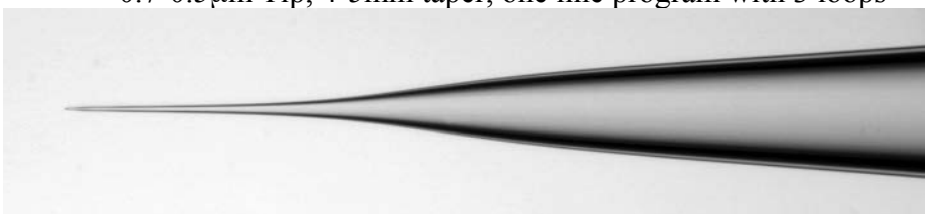
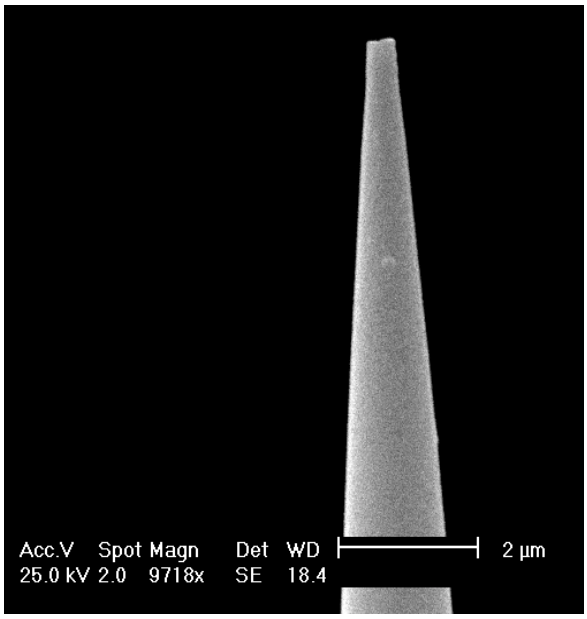


Image B **(40x mag.)** 1.0 x 0.5 thick walled glass (BF100-50-10)
~0.5µm Tip, w/ a shorter taper than that shown above.
Three line program with a reduced velocity on Line 3

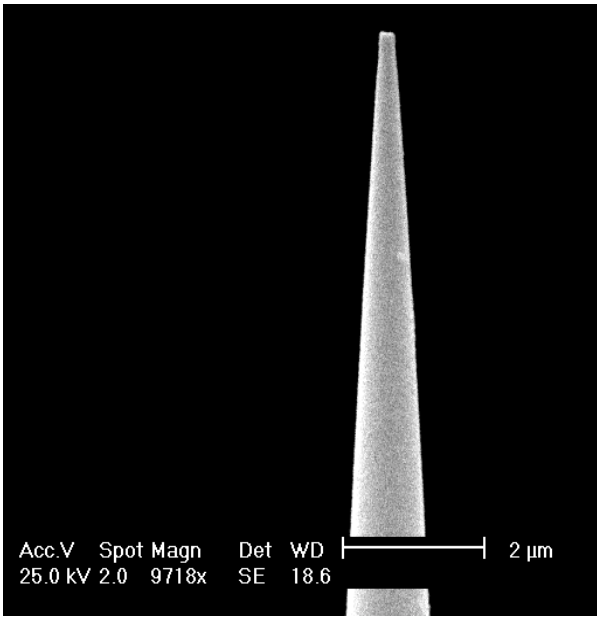
Image C **(100x mag.)** 1.0 x 0.78 thin walled glass (BF100-78-10)
0.7-0.5µm Tip, 4-5mm taper, one line program with 3 loops



THICK WALLED INJECTION PIPETTE



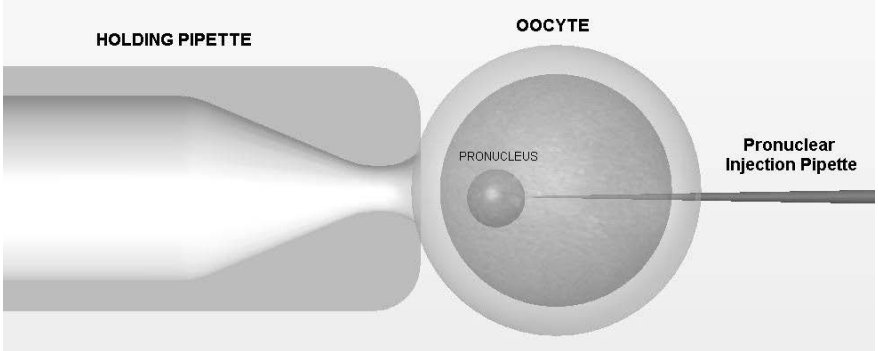
THIN WALLED INJECTION PIPETTE



Scanning Electron Microscopy of Bee-Stinger Type Injection Pipette (~10,000x mag)

CHAPTER 4

Pronuclear, Cytoplasmic and CRISPR Injection



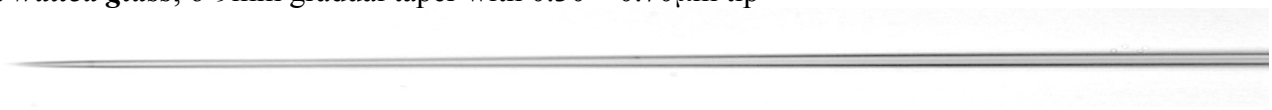
Microinjection into the pronucleus (Pronuclear Injection) is one of the most commonly used techniques for the production of transgenic animals, including the development of transgenic mice which are among the most useful research tools in the biological sciences. Pronuclear injection involves the mechanical introduction of DNA (transgene) into the pronucleus of a fertilized mammalian oocyte. After the DNA is injected into the egg, the egg is then implanted into the surrogate animal. The DNA is then integrated into the existing genetic sequence and this integration causes the animal to be born with a copy of the new sequence in every cell. This technique of making transgenic animals provides an excellent method for studying mammalian growth and pathology.

Pipette Morphology

The pipette needed for pronuclear injection typically has a 0.7 to 0.3μm tip and a 6-8mm long taper. The taper of the pronuclear injection needle should be gradual so the pipette comes to a fine tip without having a distinct "shoulder" behind the tip. If the inner diameter behind the tip of the pipette increases rapidly and has a "shoulder," this can cause excessive damage to the oocyte. In addition, if the final tip of the pipette is tapped off and broken back to "open it up," the ID of the resulting pipette should remain under 1μm .

Preferred Morphology – Gradual Taper with Low Shoulder Behind Tip

Thin walled glass, 6-9mm gradual taper with 0.30 – 0.70μm tip



Using the Pipette "As Is" or "Tapping Off" the Tip

It appears that about half the population of microinjectionists doing pronuclear injection use the injection pipette "as is" and do not break back the tip until it becomes clogged and they are unable to flush it out. The other half of microinjectionists are in the habit of breaking back, or tapping off the tip before using it to be able to have a good flow rate through the needle. While the technique being used is often determined by your predecessor, it is also possible that the method of injection is determined by how well you are able to control the outcome of the pipette morphology. Therefore, the technique of tapping off the tip is sometimes being done due to the inability to find proper parameter settings. The program settings provided below are intended to make a pipette that has a tip inner diameter and taper length appropriate for performing the injection without tapping off or breaking back the tip. If you prefer to tap off the tip, please use a pull setting that is 10 to 30 units higher than what is suggested or use lower delay values.

Pronuclear Injection – Recommended Programs

Pronuclear injection pipettes are most commonly made using 1.0mm x 0.78mm thin walled filamented glass (BF100-78-10). This type of pipette is quite specific with very tight tolerances and requires a fairly gradual taper that is approximately 7-8mm long and a tip size under 1μm. The tip and taper need to be fine enough to not cause excessive damage, therefore a gradual taper with a minimal "shoulder" behind the tip is desired. A taper length that is too short (under 6mm) and a tip size that is too big (over 1μm) will cause too much damage. On the other hand, a taper that is too long (over 8mm) and a tip that is too small (under 0.2μm) will make it difficult to inject the dilution of DNA through the tip.

Goal = 7-8mm taper, 0.5 to 0.9μm tip, gradual taper and no distinct shoulder behind the tip.

Pronuclear Injection Pipette (400x mag) 1.0mm x 0.78mm filamented glass 0.5-0.9μm Tip, 7-8mm taper. Final taper is gradual with minimal "shoulder" behind the final tip .

Recommended Programs using a box filament and thin walled glass:

2.5 x 2.5 Standard Box Filament (FB255B) and BF100-78-10 Glass

Line 1	Heat	Pull	Velocity	Delay	Pressure
	Ramp	90	70	80	200

2.5 x 4.5 Wide Box Filament (FB245B) and BF100-78-10 Glass

	Heat	Pull	Velocity	Time	Pressure
Line 1	Ramp	0	20	200	500
Line 2	Ramp+10	80-100	70-80	200	500

If the ***taper is too long and the tip too small***, increase the delay or time in 10 unit increments and then gradually decrease the pull in 5 unit increments.

If the ***taper is too short and the tip too large*****,** increase the pull in 5 unit increments then begin to drop the delay or time (cooling) in 5 unit increments.

CRISPR Injection Pipettes – For these types of injection needles, the tip must be broken back slightly larger than those required for pronuclear and cytoplasmic injection. Therefore, it is best to use slightly higher pull settings to make the pipette a little longer and more slender at the tip. This will prevent the pipette opening from being too large when broken back and will maintain a narrow taper behind the tip.

Recommended Range for Parameter Settings

Heat = Ramp -5 to +10
Pull = 40 - 120
Velocity = 60 - 80
Time = 175 – 250
Delay = 60 - 100
Pressure = 200 - 400

XenoWorks Digital Injector for Pronuclear, Cytoplasmic and CRISPR Injection

XENOWORKS DIGITAL INJECTOR SETTINGS:

- **RANGE SETTING: 1**
- **TRANSFER PRESSURE (compensation) = 20-40 hPa**
- **INJECTION PRESSURE: (PULSE or CONTINUOUS) = 500-1000 hPa**

Recommended Approach……

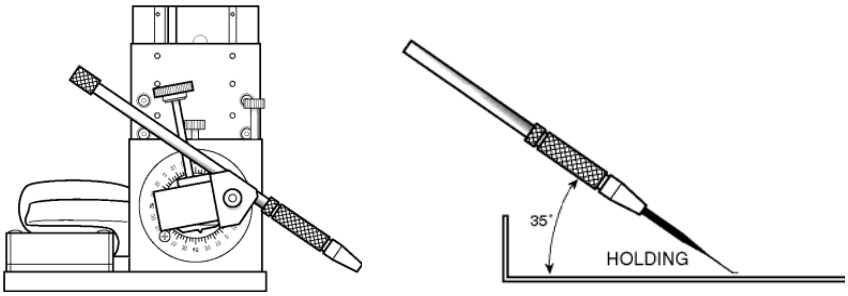
PULSE MODE: Pressure = 500-1000 hPa
Pulse Width = 0.20 seconds

Alternate Approach……

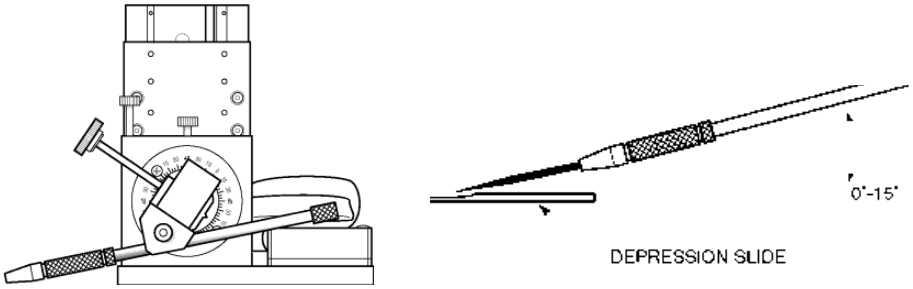
CONTINUOUS MODE: Pressure = 500-1000 hPa
Duration of injection determined by how long the inject key/foot switch is held down.

Alternate Method of Continuous Injection using Transfer/Compensation Pressure:
Increase transfer pressure until desired flow rate is created. For higher pressures, switch to Range Setting 2 which will double available pressure.

XenoWorks Micromanipulator (BRMR & BRML) Positions for Pronuclear, Cytoplasmic and CRISPR Injection



Holding Pipette angled to 25-35 degrees - Left Side (BRML) XenoWorks Manipulator



Injection Pipette angled to 15-35 degrees - Right Side (BRMR) XenoWorks Micromanipulator
Low angle is ideal. Degree of angle will depend on side walls of dish (shallow is best) or depth of well.

Pipette Clogging

The eventual clogging of pipettes during pronuclear injection is inevitable, but one hopes to be able to do multiple injections before encountering this event. After a series of injections the pipette will clog, and, at this stage, one can clear out the pipette using the "clearing" function on the microinjector. When or if this fails, one can then tap off the tip to remove the clogged region and continue injecting. If one encounters immediate or premature clogging of the injection needle, it is very rarely due to the pipette itself, but can be caused by a number of factors which are listed below:

- The media used to dilute the DNA is not clean
- The DNA is not clean and has residual aggregates
- The final injection concentration has not been "spun down"
- Dilution of DNA is insufficient (1:100 dilution is often recommended)
- The internal bore of the glass is dirty, usually a result of dust and glass particles

Injection System Considerations

The need for a smaller or larger tip opening is not only determined by the DNA construct and dilution, it is also dependent on the type of microinjection system you have in your facility. Some injectors cannot supply sufficient injection pressures (old equipment, leaky gaskets, low end device) and this can greatly influence the size of pipette tip that "seems" usable. It is best to pull the proper morphology
of pipette (small tip and gradual taper which will maintain high survival rates) and use an injector properly designed to do the job. Please see Chapter 18 describing the Sutter XenoWorks microinjection system commonly used in Transgenic facilities around the world.

For successful repair of the oocyte and incorporation of the DNA, some believe a small amount of trauma to the plasma membrane is considered a key ingredient. As a result, tapping off the tip to further open it up can often be seen as good practice and preferred technique.

The YAC (yeast artificial chromosome) DNA is very large and often requires a pipette with a shorter taper and larger tip. Often it is necessary to cut or tap off the final tip to provide a larger bore so as to prevent clogging or shearing of the DNA.

CHAPTER 5

Embryonic Stem (ES) Cell Microinjection

Embryonic stem cells have been used to either add gene copies (transgenesis) or disrupt the genes (knockout) in the mouse genome. The ES Cell microinjection technique has made a significant impact on the study of gene function, altered gene expression, and gene regulation. Embryos for microinjection are collected from pregnant mice on the 3rd day of gestation. The holding pipette (usually positioned on the left side of the preparation) holds the embryo in place while an opposing injection needle (on the right side) introduces the ES cells into the embryo. The injection needle is pushed into the cavity of the immobilized embryo, and the cells are expelled with a slight positive pressure into the embryo. Following microinjection, the embryos are surgically transferred back into the surrogate mother. Embryonic Stem (ES) Cell Microinjection requires a long and fine micropipette to transfer the embryonic stem cells into blastocysts.

Pipette Morphology

The ES cell injection needle has a long 8-12mm taper, where the taper at the tip is as parallel as possible, and the final tip has an internal diameter slightly larger than the ES cells (15-25µm inner diameter). Using a manual microinjector like the XenoWorks Analog Microinjector, 10-15 cells are collected into the injection needle by slight suction and then with slight positive pressure the ES cells are transferred into the blastocysts.

ES Cell micropipettes often have a 35 to 45 degree bevel which can be produced by using the BV-10 micropipette beveler and a course diamond plate (Chapter 19). It is best to remove some of the final taper of the glass by break back or clipping off the tip to create a 10 to 15 micron opening before beveling the tip. These needles also sometimes require a short spike at the tip of the bevel which can be performed using a microforge.

ES Cell – Recommended Programs

These pipettes are most often made from 1mm x 0.75mm (B100-75-10) capillary glass. The 2.5 x 4.5 (FB245B) or 3.0 x 3.0 (FB330B) box filament is recommended. With this glass and either the 4.5mm or the 3mm wide box filament, please install the following settings:

Heat	Pull	Velocity	Time	Pressure
Ramp + 20	30	120	200	200

- If you find the *taper too long*, reduce the heat in 5 unit increments (but do not go below the ramp value) and then gradually increase the time in 10 unit increments.
- If you find the *taper too short*, reduce the time in 25 unit increments and then increase the pull in 10 unit increments.

Technical Notes:
The injection of ES cells into embryos requires a micromanipulation system similar to that used for pronuclear microinjection of DNA. Please refer to Chapter 18 which describes the XenoWorks Microinjection System.

* *If you are using a puller where the filament cannot be changed, please find the filament type that is installed in your puller in the General Look Up Tables and use the program designed for the appropriate glass.* ***Type D or E*** *programs are recommended for this application.*

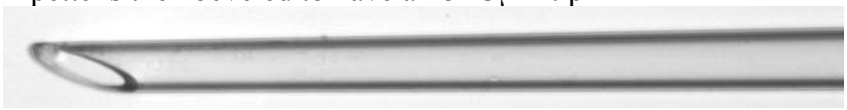
Embryonic Stem (ES) Cell Injection

Embryonic Stem Cell Pipette (400x mag.)

Pipette has been scored and broken back using a ceramic tile (CTS) 18-23µm tip, 8-10mm final taper, with 30 degree bevel (BV-10 Beveler & 104D fine plate)

Pipette is forged 4-5mm back from the initial tip

Pipette is then beveled to have a 18-23µm tip ID



XenoWorks Analog Injector (BRI) for ES Cell Transfer

Recommended Set-up

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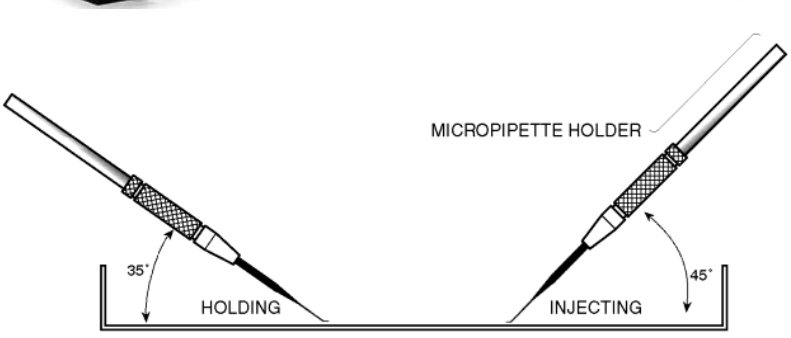
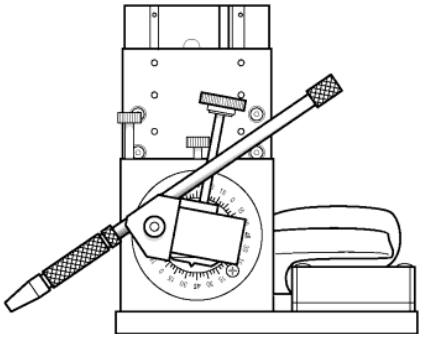
Recommended Set-up – Contact Sutter or see BRE manual for additional technical support

Syringe Size: 100µl or 250µl
Oil or water: Fill tubing & syringe with light mineral oil or water (oil preferred for finer control)
Fluorinert ™ FC-77: Optional filling solution in pipette. Often helps provide better control of cells
Course/Fine Dial: Use fine dial to control collection and transfer/injection of Stem Cells

Xenoworks Micromanipulator (BRMR & BRML) Positions for ES Cell Transfer





Suggested set up for **suspended cells using angled pipettes** (ICSI, Stem Cell, Nuclear Transfer)

CHAPTER 6

ICSI (Intracytoplasmic Sperm Injection)

ICSI is an advanced form of in-vitro fertilization (IVF), which was traditionally performed in a test tube. ICSI pipettes are used to aspirate and inject a single sperm cell directly into a single oocyte in order to achieve fertilization. It has been declared one of the most important advancements in the research of reproductive medicine. In this technique the oocytes and sperm are placed on a slide and viewed under an inverted microscope. Mounted on the microscope stage is a system of two micromanipulators to which the micropipettes are attached. Commonly the holding pipette is positioned on the left to maintain the position of the oocyte, while the ICSI pipette is positioned on the right to microinject the sperm into the egg. The movement of the pipettes is controlled by joysticks as demonstrated by the XenoWorks Microinjection System (Chapter 18).

General considerations

The most important factor to consider in the fabrication of a sperm injection micropipette is the inner diameter of the pipette tip. By definition, a sperm injection pipette must penetrate the cell membrane of an oocyte, and therefore must present the smallest cross-section to the oocyte to minimize damage during the injection process. The inner diameter of the pipette is determined by the size and morphology of the sperm cell, therefore the ID of the pipette should be large enough to easily aspirate the sperm, but not be so large as to allow the sperm to bunch up in the pipette. A typical range for the ID of a pipette for this application is between 4-7μm.

Pipette Morphology

Thin-walled, non-filamented borosilicate capillary glass (B100-75-10) should be used with parameter settings which are sufficient to draw out a 10-15mm long, gently-tapering pipette. The resulting tip diameter is irrelevant since the pipette tip will be broken back and then beveled to create the appropriate tip size between 4 and 7μm.This process is performed by first clipping back the final tip to create a 3-5μm tip and then beveling the tip using the Sutter BV-10 Beveler (Chapter19). The pipette tip should be beveled back to the proper ID and beveled at the desired angle, which is usually between 30 and 40 degrees. If required, creating a small spike on the pipette tip and bending the pipette is performed using a microforge.

ICSI – Recommended Program

These pipettes are made from 1mm x 0.75mm **(B100-75-10)** capillary glass. The 2.5 x 4.5 **(FB245B)** or 3.0 x 3.0 **(FB330B)** box filament is recommended. With this glass and either the 4.5mm or the 3mm wide box filament, please install the following settings:

	Heat	Pull	Velocity	Time	Pressure
Line 1	Ramp	0	20-30	200	500
Line 2	Ramp	30	120	200	500

- If the *taper is too long*, reduce the heat in 5 unit increments and then gradually increase the time in 10 unit increments.
- If the *taper is too short*, reduce the time in 25 unit increments and then increase the pressure in 50 unit increments.

* *If you are using a puller where the filament cannot be changed, please find the filament type that is installed in your puller in the General Look Up Tables and use the program designed for the appropriate glass.* ***Type D or E*** *programs are recommended for this application.*

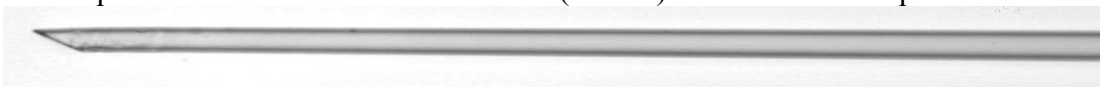
ICSI Pipettes - Intracytoplasmic Sperm Injection

ICSI Pipettes (400x mag)

Pipette has been scored and broken 1 to 2mm back from the initial tip using a microforge. A blunt end is used for Mouse Piezo ICSI or the tip can be beveled for Mouse, Bovine and Equine ICSI (~4-7µm ID)

The pipette above is the type needed for **PIEZO ICSI (PMM4G)**

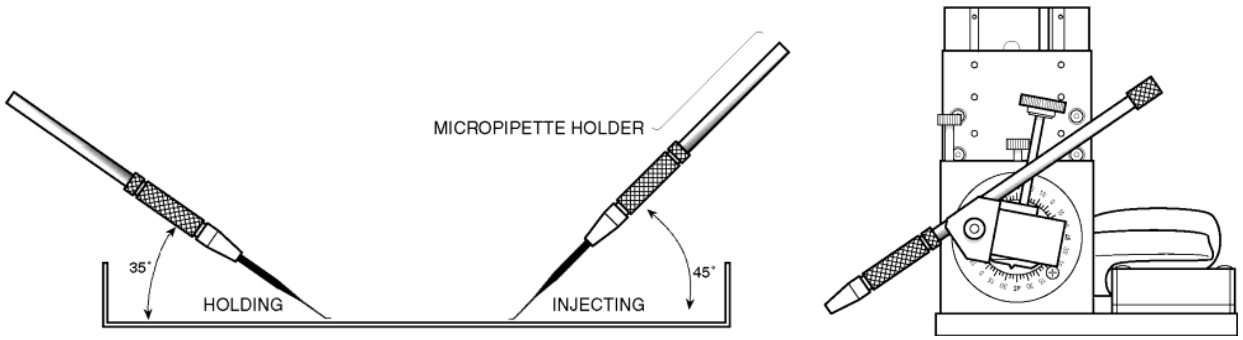
Same tip as above after it has been beveled (BV-10) with a 104D fine plate for 30-60s



XenoWorks Analog Injector (BRI) for ICSI

Recommended Set-up
Syringe Size: 50µl or 100µl
Oil or water: Fill tubing & syringe with light mineral oil or water (oil preferred for finer control)
Fluorinert ™ FC-770: Optional pipette filling solution. Often helps provide better control of sperm
Course/Fine Dial: Use fine dial to control collection and transfer/injection of sperm

Xenoworks Micromanipulator (BRMR & BRML) Positions for ICSI



Suggested set up for **suspended cells using angled pipettes** (ICSI, Stem Cell, Nuclear Transfer)

CHAPTER 7

Nuclear Transfer

Nuclear Transfer is one of the methods used for the cloning animals. It involves removing the nucleus from a donor cell of an animal and then placing the donor cell's nucleus inside an enucleated oocyte through cell fusion or transplantation. The oocyte is then stimulated to begin forming an embryo. After this occurs, the embryo is transplanted into a surrogate mother, and occasionally a perfect replica of the donor animal will be born.

General considerations

The most important factor to consider in the fabrication of a nuclear transfer micropipette is the inner diameter of the pipette tip. The pipette must penetrate the cell membrane and be small enough to minimize damage during the injection process. The inner diameter of the pipette is determined by the size of the nucleus; therefore, the ID of the pipette should be large enough to easily transfer the nucleus. A typical range for the ID of a pipette for this application is between 15-21μm.

Pipette Morphology

Thin-walled, non-filamented borosilicate capillary glass (B100-75-10) is recommended using parameter settings which are sufficient to draw out a 10-15mm long, gently-tapering pipette. The resulting tip diameter is irrelevant since the pipette tip will be broken back clean or it will be broken back and beveled to create a tip size between 5 to15μm. Breaking the glass back to have a clean blunt end is performed using a microforge. The resulting blunt needle is used in conjunction with a Piezo device like the Primetech PMM4G (See Sutter Instrument Catalog). If a beveled pipette is needed, rather than a blunt tip, it is created by first clipping back the pipette to make a 3-5μm tip and then beveling the tip with the Sutter BV-10 (Chapter 19). The pipette tip should be beveled back to a diameter appropriate to the sperm being injected and beveled at the desired angle, which is usually between 30 and 40 degrees. If required, creating a small spike on the pipette tip is performed using a microforge.

Nuclear Transfer – Recommended Program

These pipettes are usually made from 1mm x 0.75mm **(B100-75-10)** capillary glass. The 2.5 x 4.5 **(FB245B)** and the 3.0 x 3.0 **(FB330B)** box filaments are recommended. With thin walled glass and either the 4.5mm or the 3mm wide box filament, please install the following settings:

Heat	Pull	Velocity	Time	Pressure
Ramp + 30	30	120	200	200

If the *taper is too long*, reduce the heat in 10 unit increments, the gradually increase the pressure in 25 unit increments.

If the *taper is too short*, reduce the time in 25 unit increments, then gradually increase the pull in 20 unit increments.

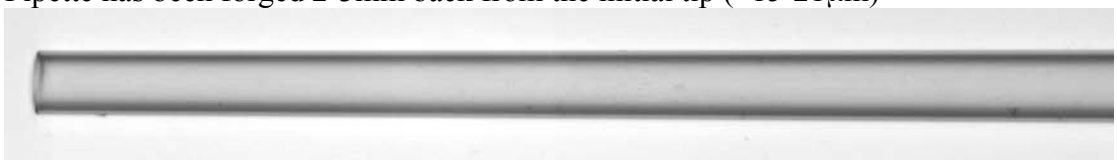
Additional Information on Embryonic Stem (ES) Cells, Nuclear Transfer (NT), and Cloning:

- The following PBS/Nova web site
  http://www.pbs.org/wgbh/nova/sciencenow/3209/04-clon-nf.html
- Institute for Laboratory Animal Research
  http://dels.nas.edu/ilar_n/journal/33_4/v33_4Nuclear.shtml
- Nuclear Transfer: Bringing in the Clones
  http://www.pnas.org/misc/classics4.shtml

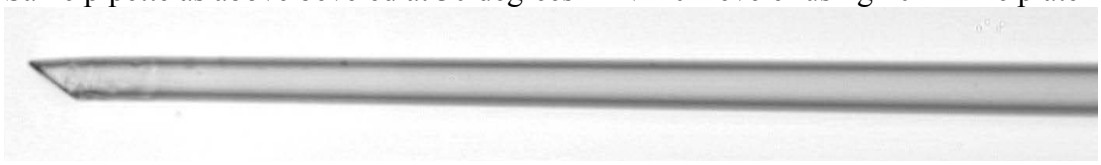
Nuclear Transfer (NT)

Nuclear Transfer Pipette (400x mag)

Pipette has been forged 2-3mm back from the initial tip (~15-21μm)



Same pipette as above beveled at 30 degrees - BV-10 Beveler using 104D fine plate



XenoWorks Analog Injector (BRI) for Nuclear Transfer

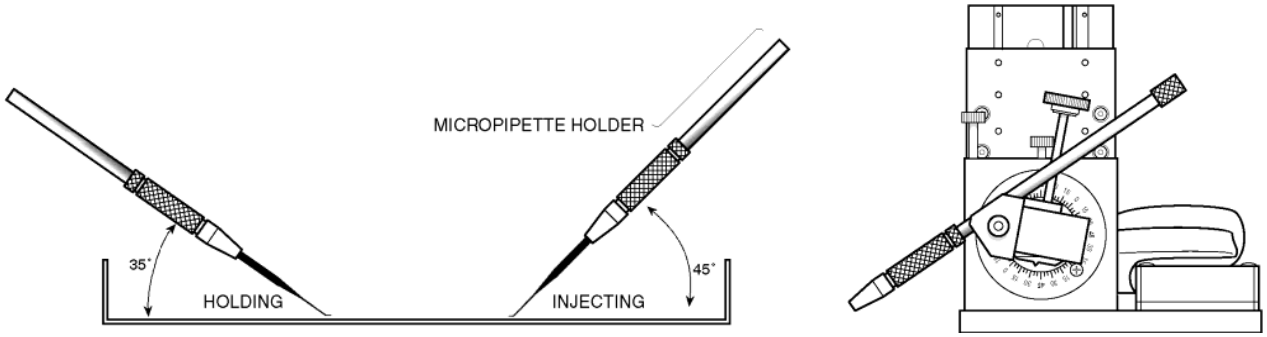
Recommended Set-up

Syringe Size: 100μl or 250μl

Oil or Water: Fill tubing & syringe with light mineral oil or water (oil preferred for finer control)

Course/Fine Dial: Use fine dial to control aspiration and transfer

Xenoworks Micromanipulator (BRMR & BRML) Positions for Nuclear Transfer



Suggested set up for **suspended cells using angled pipettes** (ICSI, Stem Cell, Nuclear Transfer)

CHAPTER 8

Holding Pipettes

When performing techniques such as pronuclear injection, ICSI, and ES Cell injection, the holding pipette is used to hold and immobilize the cell or blastocyst during the microinjection procedure. Holding pipettes have a clean 90-degree break at the tip which is fire-polished to create a smooth surface to interface with the cell. Holding pipettes are usually made to have a fairly large inner and outer diameter, which provides better support and reduces any possible distortion of the oocyte during the micromanipulation. Depending on the size of the oocyte, the extent of polish, and the thickness of the capillary glass used to make the pipette, holding pipettes traditionally have an inner diameter of 5-15µm and an outer diameter between 75-125µm.

Holding Pipettes – Recommended Program

These pipettes are usually made from 1mm x 0.75mm **(B100-75-10)** capillary glass and a 2.5 x 4.5 **(FB245B)** or a 3.0 x 3.0 **(FB330B)** box filament is recommended. With this glass and either the 4.5mm or the 3mm wide box filament, please install the following settings:

Heat	Pull	Velocity	Time	Pressure
Ramp + 30	0	150	0	200

This will generate a long, even taper with parallel walls

If the *taper is too long*, reduce the heat in 10 unit increments, then gradually increase the pressure in 25 unit increments.

If the *taper is too short*, reduce the time in 25 unit increments, or use a wider 4.5mm box filament (FB245B)

Additional Steps Needed to Fabricate a Holding Pipette

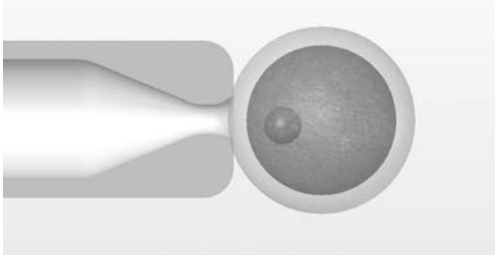
Breaking back the glass – The pipette will need to be broken back to have an inner diameter between 50 and 75 microns. This will depend on the size of cell you intend to "hold" and the extent of fire polish you will need to provide to the tip. To break the glass back clean at tip sizes of 20 microns and larger, it is best to use a **ceramic tile or "glass-on-glass"** to score the glass at the location the glass needs to be broken. See Chapter 10 for instructions. A microforge can also be used, but when breaking glass with a microforge at tip sizes over 25 microns, the glass tends to break at an angle or cause distortion and bending of the forge heating element.

Fire-Polishing the Tip – Fire-polishing is done to create a smooth surface to interface with the cell, and produce an inner and outer diameter best suited to hold your cell. If the holding pipette is too small, the cell will roll off the pipette tip as one tries to inject the cell from the other side. If the holding pipette is too large, one can either distort the cell or aspirate the cell into the pipette. A microforge is required to fire-polish the holding pipette.

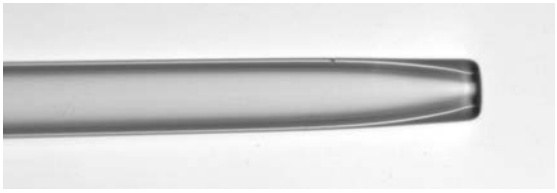
Bending the Pipette – Depending on your application and set up, it is sometimes necessary to create a 20 to 35 degree bend in the taper of the pipette. The bending of the pipette is most often needed when your cells are in a dish rather than on a slide and a specific incident angle is preferred. A microforge is required for this step.

* *If you are using a puller where the filament cannot be changed, please find the filament type that is installed in your puller in the General Look Up Tables and use the program designed for the appropriate glass.* ***Type E*** *programs are recommended for this application.*

Holding Pipette Images



Small Holding Pipette (400x mag.)
Forged & fire-polished
5µm ID x 15 µm OD



Small Holding Pipette (40x mag.)
Viewed at lower mag. to visualize bend
5µm ID x 15 µm OD, forged, polished, and bent

Medium/Large Holding Pipette (400x mag.)
Scored and cut with clean break using a ceramic tile, then fire-polished. 15µm ID x 80 µm OD

XenoWorks (BRI) Analog Injector or (BRE) Digital Injector for Holding

<u>Recommended Set-up</u>

↓

<u>Recommended Set-up</u> – Contact Sutter

Syringe Size: 500µl or 1000µl
Air or Water: Fill tubing & syringe with air or water (use water for finer control)
Course/Fine Dial: Use fine dial to control holding

CHAPTER 9

Xenopus (frog egg) Microinjection

Embryos of the frog Xenopus are used to study how nervous systems work at the cellular level, how the nerves develop to form the correct connections, and how the nerves are organized to allow animals to behave. Xenopus embryos, including those from *Xenopus laevis* and *Xenopus tropicalis*, are significant models for the study of embryonic development due to many advantages, including the large size of the eggs, easily identifiable blastomeres, and their ability to withstand extensive surgical intervention and culture (*in vitro).* As a result, Xenopus embryos are important and unique resources in the research in early embryonic development and cell biology. The techniques used with Xenopus embryos include whole-cell patch recording, neuron imaging, and network modeling.

Xenopus Microinjection – Recommended Program

These pipettes are usually made from 1 x 0.75 **(B100-75-10)** or 1 x 0.5 **(B100-50-10)** capillary glass. The 2.5 x 4.5 **(FB245B)** or 3.0 x 3.0 **(FB330B)** box filament is recommended. With either glass and the 4.5mm or the 3mm wide box filament, please install the following settings:

Heat	Pull	Velocity	Time	Pressure
Ramp	30	120	200	200 – 300

If the *taper is too long*, reduce the velocity in 10 unit increments, then gradually increase the pressure in 25 unit increments.

If the *taper is too short*, reduce the pressure in 25 unit increments, then gradually increase the heat in 10 unit increments. Do not exceed Ramp + 30 unless you know your filament can withstand heat settings beyond this value.

Breaking back the pipette to make a 3-5µm tip

These settings will create a 10-12 mm long taper and a small fine tip. For Xenopus microinjection you will need to cut back the final tip using tweezers, scissors or a razor blade to make a 3-5µm opening at the tip. A pipette tip that has a somewhat rough, jagged break is ideal for cutting through the membrane of the Xenopus oocyte. Some folks will take the extra effort to bevel the pipette to make a clean, sharp, hypodermic-like needle.

* *If you are performing intracellular recording you will need a pipette that is sharper and not broken back. Please refer to the General Look Up Tables, locate the chart with matches the type of filament in your puller and the glass you are using, and try* ***Type B or C programs*** *to make a sharp electrode.*

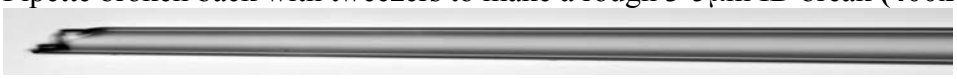
* *If you are using a puller where the filament cannot be changed, please find the filament type that is installed in your puller in the General Look Up Tables and use the program designed for the appropriate glass.* ***Type D*** *programs are recommended for this application.*

Xenopus Microinjection Pipettes, 3µm - 5µm Tips

The capillary glass is first pulled out to have a long even gradual taper **(100x mag.)**

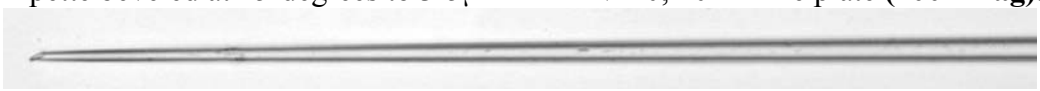
To make the proper tip opening, you must break off the final tip

Broken with Tweezers
Pipette broken back with tweezers to make a rough 3-5µm ID break **(400x mag)**.



Broken by gently dragging tip on taught piece of Kimwipe
3-5µm Tip, thin or thick walled glass **(400x mag.)**

Beveled with the BV-10 Beveler
Pipette beveled at 45-degrees to 3-5µm ID - BV-10, 104D fine plate **(200x mag)**.



XenoWorks Digital Microinjector (BRE) for Xenopus Injection

XENOWORKS DIGITAL INJECTOR SETTINGS

- **RANGE SETTING: 1 or 2**
- **TRANSFER PRESSURE (compensation) = 5-15 hPa**
- **INJECTION PRESSURE: (PULSE or CONTINUOUS) = 50-200 hPa**

Recommended Approach……
PULSE MODE: Pressure = 50-200 hPa
Pulse Width = 0.20 seconds

Alternate Approach……
CONTINUOUS MODE: Pressure = 50-200 hPa
Duration of injection determined by how long the inject key/foot switch is held down.

Alternate Method of Continuous Injection using Transfer/Compensation Pressure:
Increase transfer pressure until desired flow rate is created. For higher pressures, switch to Range Setting 2 which will double available pressure.

CHAPTER 10

Large Pipettes, 20µm - 200µm Tips: Using the Ceramic Tile (CTS) or "Glass-on-Glass"

Micropipettes with tip sizes over 20µm are often difficult to create without using a mechanical device to score and break back the glass. Microforges are often used to create tip sizes between 5 and 20µm, but once a larger tip is needed, the delicate filament on a Microforge is often too fragile to effectively break the glass back or produce a clean break. It is in these circumstances that we recommend using thick walled glass and the ceramic tile or "Glass on Glass" to create a tip between 20-200µm and a clean 90-degree break (See pg 60).

The front of the ceramic tile (pg 60) is the surface with the Sutter logo. The front edges of the tile are rough and should be used for scoring the glass. You can use a microscope at 100 to 200 times magnification to examine the taper of the pipette and determine where the pipette needs to be scored to create a specific tip ID. Marking the glass with a Sharpie will give you a general target to aim for. You can also "blindly" score the glass by starting high and moving down the taper and use a little "trial and error" to find the proper location. You will find that the flexibility of the glass decreases as the diameter of the taper increases, and this tactile feedback can sometimes help one determine where the glass should be scored to achieve a specific tip size.

Using the P-97 or P-1000 Micropipette Puller, any heating filament, and thick walled glass, 1mm x 0.5mm (B100-50-10), 1.2mm x .69mm (B120-69-10), or 1.5mm x 0.86mm (B150-86-10), please try the following parameter settings:

Example program using 1.5mm x 0.86mm OD/ID glass and a 3mm x 3mm box filament:

Heat	Pull	Velocity	Time	Pressure
Ramp + 25	0	150	0	200

Remove the resulting long tapered pipette and hold it vertically up to the light or against a dark background. Using a front edge of the ceramic tile, score the glass in a perpendicular fashion (90 degrees to the taper) and then use the tile to push and bend the glass over, just above the location of the "score", to break back the glass. You might find that it takes a number of trials to be able to consistently break the glass cleanly at the proper location. Refer to images on pg 58.

Additional "Tricks" in making 15-75µm Tips

Clean Break

I have found that by simply bending over the finest aspect of the taper, by means of brushing and bending the glass over using my index finger, the glass will naturally break between **15-30µm** and will surprisingly have a clean break about 70% of the time.

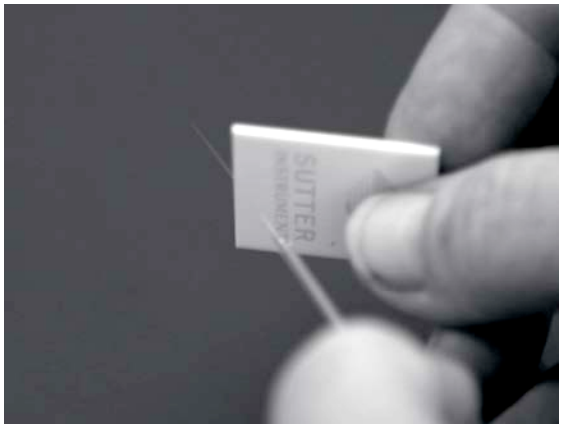
Angled Break

To create a beveled/angled semi-rough break, and a 20-25µm open tip, pull a long tapered pipette, stretch out a piece of Kimwipe taut over a beaker, and quickly and gently poke the needle through the Kimwipe. For what has yet to be explained to me, this technique ends up producing a **20-25µm angled break** in the glass and about a 90% yield of good usable pipettes. If you use 1 or 2-ply tissue, you can often get a **10-20µm angled break**, and if you use a paper towel, you can often get a **50-75µm angled break**. Additional paper products remain to be tested!
See page 61 for images.

IMPORTANT: We recommend you use protective eyewear and break the glass in an outward direction, away from your eyes.

Ceramic Tile (CTS) Images

Ceramic Tiles, Item # CTS, $15.00 each

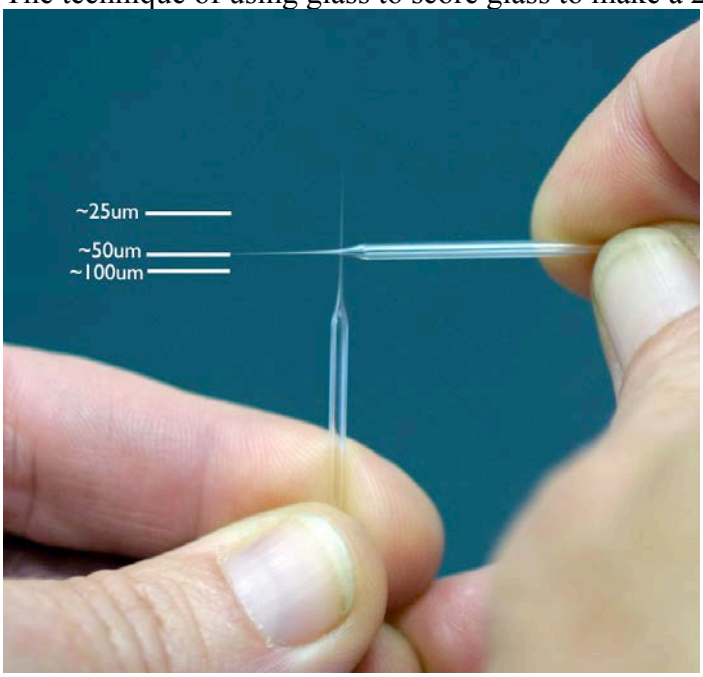


Score the glass at a 90-degree angle to the taper using the front edge (logo side) of the tile.

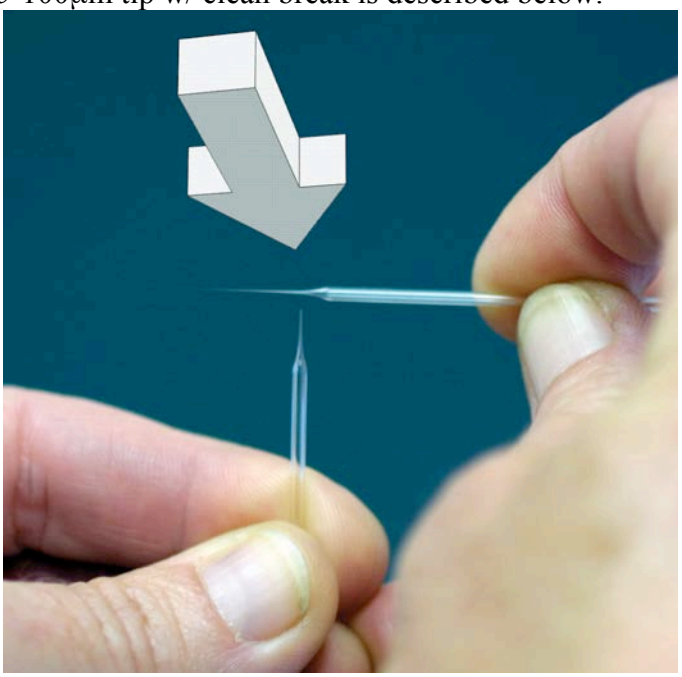
"Glass-on-Glass"

(© Adair L. Oesterle)

The technique of using glass to score glass to make a 25-100µm tip w/ clean break is described below.



Step .1 *Score only, do not break!*



Step 2. *Move above the score location & push with a smooth, continuous motion to break the glass.*

To score the glass held in the left hand, use the thicker region (near shoulder of the taper) of the pipette held in the right hand. A light contact of glass-on-glass with a very slight (1mm) horizontal movement of the scoring pipette (right hand) is all that is required to score the glass. The interaction of the two pieces of glass at 25µm and larger will feel rough like sand paper. One motion sideways is sufficient. Many back and forth sawing motions is bad! You DO NOT want the glass to break while scoring the taper since this will create a bad break. After scoring, move the scoring pipette above the location of the score, and in a fluid motion, push the top of the taper back and away (*Step 2)* to break the glass off at the desired location.

General Rule of Thumb: **¼**th back from the tip = 25µm **½** back from the tip = 50µm

2/3rd back from the tip = 75µm **3/4**th back from the tip = 100-200µm

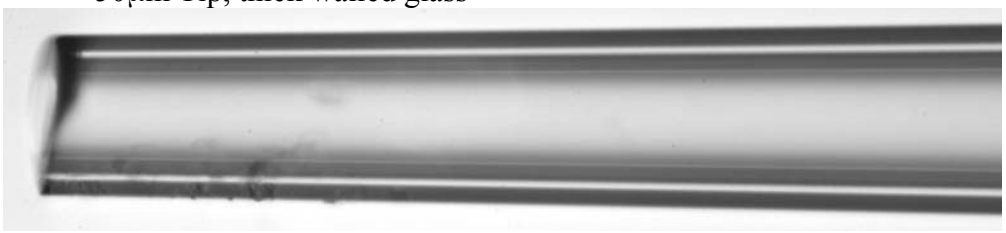
SutterInstrument YouTube Channel, Scientists Empowering Scientists Webinar "Achieving the Impossible" (17:55) for video demonstration. https://www.youtube.com/watch?v=gC4Ktmb6ndk

Large Pipette Images, 20µm - 200µm Tips

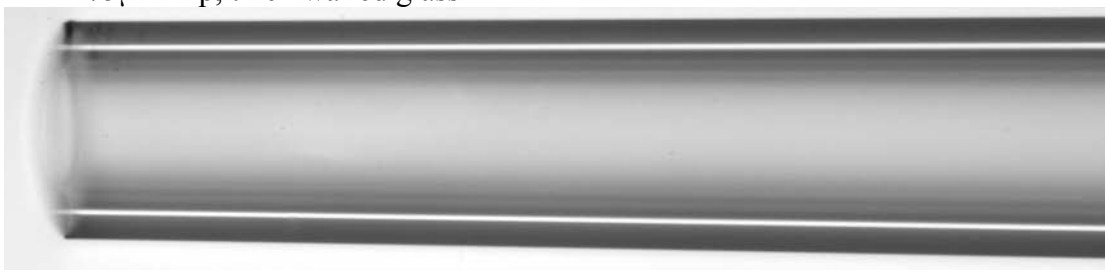
Using Ceramic Tile or Glass-on-Glass Technique:

- 25µm Tip, thick walled glass

- 50µm Tip, thick walled glass



- 75µm Tip, thick walled glass



Stabbing through Kimwipe, Kleenex, or other tissues:

- 15µm Tip, thick walled glass, created by stabbing through Kleenex

- 30µm Tip, thick walled glass, created by stabbing through Kimwipe

- 75µm Tip, thick walled glass, created by stabbing through Paper Towel

Table of Techniques for making Pipettes with a Large Tip and Long Taper

Tip Size	Glass Dimension	Method
1-5μm	Any Sutter Glass (thin or thick walled)	1) Very gently touch pipette tip to a taught or loose piece of Kimwipe a.k.a. "Tickle the Kimwipe" 2) Break on the edge of a cover slip in the dish on microscope stage
6-15μm	Any Sutter Glass (Thin or Thick Walled) using a Microforge Aluminosilicate Glass using modified "Glass-on-Glass" technique	1) Use a Microforge – Contact Sutter for more information 2) Use Aluminosilicate Glass and perform a modified version of the Glass-on-Glass technique. Contact Sutter for additional instructions.
15-50 μm	Sutter Standard Glass and Thick Walled Glass B100-50-10, B120-69-10, B150-86-10, B150-75-10 and Aluminosilicate Glass	1) Use Glass-on-Glass technique. 2) Use Ceramic Tile (Part # CTS) *See Chapter 10
50-100 μm	Sutter Standard Glass and Thick Walled Glass B100-50-10, B120-69-10, B150-86-10, B150-75-10 and Aluminosilicate Glass Note: Thin Walled Glass is more challenging and is not recommended!	1) Use Ceramic Tile (Part # CTS) *See Chapter 10 2) Diamond Knife – Not advised! These are bulky and heavy for scoring the thin taper of glass. The ceramic tile is more delicate and provides better physical feedback 3) Glass-on-Glass technique might work, but the ceramic tile works better on wider/thicker regions of the pipette taper
100-175 μm	Sutter Standard Glass and Thick Walled Glass B100-50-10, B120-69-10, B150-86-10, B150-75-10 and Aluminosilicate Glass Note: Thin Walled Borosilicate glass is not recommended!	1) Use a (tricky) one-sided manual pull using the pipette puller. Contact Sutter for instructions 2) Use higher heats (careful!) and no cooling to allow a larger ID to be further from the shoulder of the taper. Contact Sutter for further instructions.

CHAPTER 11

Aspiration Pipettes: Cell Transfer, Single Cell Mechanics, & Blastomere Biopsy

Aspiration pipettes are most often used for the collection and micromanipulation of small cells, beads or particles, and also used for studying the viscoelastic properties of a cell. They are also used for ICSI and ES Cell procedures.

The ideal morphology for an aspiration pipette is to have a clean 90 degree break at the tip and a long even taper where the walls are fairly parallel. The required tip size will depend on the size of the cell and whether a portion of the cell membrane, or the entire cell, is to be drawn into the pipette. Most pipettes used for Aspiration are between 10-50 microns.

For tips 20µ and larger, one can pull the glass out long, then score and break the glass according to the instructions in Chapter 10. The Glass-on-Glass technique works well for tip sizes between 20-50µ, and for tips over 50µ, it is best to use the ceramic tile. To make pipette tips between 10-20µ, one can use a microforge and/or purchase Aluminosilicate glass which is stiffer and stronger that allows the glass to be scored and broken at locations closer to the tip where the ID is smaller. For pipettes tips 5-10µ, it is best to use a microforge. For more information concerning the use of microforges, please contact Sutter.

It is most ideal to have a wide 2.5mm x 4.5mm box filament (FB245B) to heat and pull the glass to create a long even taper with parallel walls. We recommend using thick/standard walled glass since this optimizes one's ability to make a clean break at the tip. It is best to use Sutter Borosilicate glass: B100-50-10, B120-69-10, B150-86-10. Thin walled Borosilicate glass can also be used, but it is more challenging to break the glass clean at the larger tips sizes. If you would prefer to use thin walled glass, try our Aluminosilcate glass A100-64-10, A120-87-10 and A150-100-10. Please see Chapter 12 for more information on Aluminosilicate glass

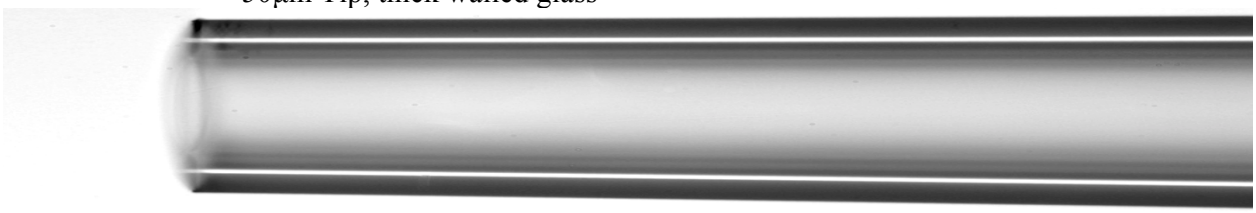
Please install the following parameter settings and follow the instructions in Chapter 10 to score and break the glass back to the proper tip size.

Heat	Pull	Vel	Time	Pressure
Ramp + 30	0	120-150	200	200

▼ 10µm Tip, thick walled glass

▼ 25µm Tip, thick walled glass

▼ 50µm Tip, thick walled glass



* For very long 1-2cm tapers with 75-150µm ID tips, please contact Sutter Technical Support to receive special instructions on how to extrude the glass using your puller.

XenoWorks Injectors (BRI & BRE) for Aspiration

XenoWorks Analog Injector (BRI) for Aspiration

Recommended Set-up

Syringe Size: 10µl, 50µl, or 100µl - depending on size & number of cells/particles being collected.
Oil or Water: Fill tubing & syringe with light mineral oil or water (oil preferred for finer control)
Course/Fine Dial: Use fine dial to control Aspiration
Pipette Morphology: Application dependent, contact Sutter Technical Support for advice

XenoWorks Digital Injector (BRE) for Aspiration

Recommended Set-up

Range Setting: 1 or 2 (Injector can be customized if lower pressure ranges are required)
Transfer Channel: Use transfer dial in remote user interface to control Aspiration
Pipette Morphology: Application dependent, contact Sutter Technical Support for advice

NOTE: Please Contact Sutter Technical Support for recommended protocol best suited for your application (pipette morphology, injector type, & injector set up).

CHAPTER 12

Aluminosilicate Glass & Custom Programs

Because of the **durability and hardness**, there has been an increased interest in fabricating micropipettes from aluminosilicate glass. In comparison with borosilicate glass, aluminosilicate provides increased hardness, improved chemical durability, and reduced electrical conductivity.

While the original ratio of a borosilicate capillary's inner to outer diameter remains unchanged over its total taper length, aluminosilicate glass demonstrates a marked tendency to thin as it is drawn to a tip. This behavior allows **extremely fine tips** to be formed when pulling sharp pipettes that have a long taper and small tip.

The P-97 and P-1000 can pull both Borosilicate and Aluminosilicate glass, but Aluminosilicate has a higher melting temperature and therefore you will see higher ramp values when using this glass. The most common heating filaments used on the P-97 and P-1000 are the 3mm trough filament (FT330B) and the 2.5mm x 2.5mm (FB255B), 3mm x 3mm (FB330B) and 2.5mm x 4.5mm (FB245B) box filaments. If you have one of these four filaments installed in your puller, you can use this glass without changing the filament. If you have a filament less than 2.5mm wide and you attempt to run and ramp test with Aluminosilicate glass, you will risk burning out the filament since the higher levels of heat required to melt this glass could exceed the levels of heat your filament is able to produce.

Page 66 provides custom programs design specifically for making pipettes using Aluminosilicate glass. To locate the recommended program and parameter settings with which to get started, please follow steps 1-4 to locate the program.

1) *Verify the type of filament installed in your puller:*

   3mm trough (FT330B)
   2.5mm x 2.5mm Box (FB255B)
   3mm x 3mm box (FB330B)
   2.5mm x 4.5mm Box (FB245B)
   If the filament installed in your puller that is not listed above, please contact Sutter technical service for additional help.

2) *Verify the OD and ID of the Aluminosilicate Glass:*

Aluminosilicate Glass: 1.0mm OD x 0.64mm ID	AF100-64-10 and A100-64-10
Aluminosilicate Glass: 1.2mm OD x 0.77mm ID	AF120-77-10 and A120-77-10
Aluminosilicate Glass: 1.2mm OD x 0.87mm ID	AF120-87-10 and A120-87-10
Alunimosilicate Glass: 1.5mm OD x 1.00mm ID	AF150-100-10 and A150-100-10

3) *Select your "Type" of pipette:*

   Type A = Patch type pipette, 2-4mm taper, 1 - 3μm tip, 1-10 MΩ
   Type B = Short microinjection needle, 5-7mm taper, 0.9 - 0.5μm tip, 10-50MΩ
   Type C = Pronuclear inj. or Intracellular recording, 6-8mm taper, 0.5 - 0.1μm tip, 60-150MΩ
   Type D = Long taper, Intracellular recording, 0.3-0.06μm tip, 100-200MΩ
   Type E = Very long taper, Holding, ICSI, NT, Stem Cell, Xenopus, and Aspiration, to be scored & broken. Please see Chapter 5-10 for additional details.

4) *Find the page that provides programs intended for your filament...*

   Select the table that is intended for the OD and ID of your Aluminosilcate glass…

   Then select the "Type" A, B, C, D or E program that best describes your application.

Aluminosilicate Glass w/ 3mm Trough Filament (FT330B)

Aluminosilicate Glass: AF100-64-10 and A100-64-10, AF120-77-10 and A120-77-10
AF120-87-10 and A120-87-10, AF150-100-10 and A150-100-10

Type	Heat	Pull	Vel	Time	Pressure	Loops
A	Ramp	0	30	150	400	3
B	Ramp	70	70	200	400	1
C	Ramp + 5	80	80	175	400	1
D	Ramp + 10	90	90	175	400	1
E	Ramp + 10	0	120	100	200	1

Aluminosilicate Glass w/ 2.5mm x 2.5mm Box Filament (FB255B) or w/ 3.0mm x 3.0mm Box Filament (FB330B)

Aluminosilicate Glass: AF100-64-10 and A100-64-10, AF120-77-10 and A120-77-10
AF120-87-10 and A120-87-10, AF150-100-10 and A150-100-10

Type	Heat	Pull	Vel	Time/Delay	Pressure	Loops
A	Ramp - 10	0	25-35	250	500	3-4
B	Ramp - 5	60	70	200	400	1
C	Ramp - 10	90	80	40 (delay)	200	1
D	Ramp	120	70	60 (delay)	400	1
E	Ramp + 20	0	150	100	100	1

Aluminosilicate Glass w/ 2.5mm x 4.5mm Box Filament (FB245B)

Aluminosilicate Glass: AF100-64-10 and A100-64-10, AF120-77-10 and A120-77-10
AF120-87-10 and A120-87-10, AF150-100-10 and A150-100-10

Type	Heat	Pull	Vel	Time/Delay	Pressure	Loops
A	Ramp - 20	0	20	200	400	3
B	Ramp - 20	40	80	200 (delay)	500	1
C	Ramp - 15	80	80	200 (delay)	400	1
D	Ramp - 20	80	80	80 (delay)	400	1
E	Ramp	0	150	200	400	1

Special Advantages and Considerations using Aluminosilicate Glass

1) Higher Durability and Hardness – There has been an increased interest in fabricating micropipettes from aluminosilicate glass since it provides increased hardness, improved chemical durability, and reduced electrical conductivity when compared to borosilicate glass.

2) Extremely fine tips - While the original ratio of a borosilicate capillary's inner to outer diameter remains unchanged over its total taper length, aluminosilicate glass demonstrates a marked tendency to thin as it is drawn to a tip. This behavior allows extremely fine tips to be formed when pulling sharp pipettes that have a long taper and small tip.

3) Shorter Tapers – Aluminosilicate glass cools down more rapidly than borosilicate glass and therefore can often produce much shorter tapered pipettes. When pulling microinjection and intracellular recording pipettes (Type B and C in the tables above), the taper length will be about 1/2 to 2/3 to what one would normally find when using borosilicate glass. When pulling patch type pipettes (Type A in the tables above) it is best to choose a <Velocity> setting that allows three loops. If the <Velocity> setting is too low, the program will loop 4 or 5 times. Often you can get larger 3-6μ tips in these circumstances, but the tip will not always have a clean and smooth break.

4) Higher Melting Temperatures – Compared to borosilicate glass, aluminosilicate glass requires higher heat settings. Please do not use heating filaments that are less than 2.5mm wide and always run a ramp test before installing a heat setting. If you see your filament is getting very bright orange to white in color, it might be close to burning out and you will need to change to a larger sized heating filament.

5) Looping – Aluminosilicate glass will thin out as it is pulled and due to this behavior, a one line program that loops over 3 times is more likely to give larger tips than one would expect when using borosilicate glass. A program that loops over 4-6 times will generate larger 3-6μ tips, but you will experience increased variability and the tip will not always have a smooth break.

6) Sensitivity to Parameter Settings – Since Aluminosilicate glass has a shorter range of workable temperatures, you will find that this glass is more sensitive to small adjustments in the parameter settings. Small changes to the heat settings will make large changes to the morphology of the pipette. It is our recommendation that you adjust the <Pull> and <Velocity> settings to make gradual changes and leave the heat settings alone.

7) Fine Tip vs. Long Tube – If you introduce too much heat and/or too little cooling, the glass will have the tendency to extrude and form a blunt tube instead of making a fine sharp tip. To avoid pulling tubes, make sure the heat setting is between <Ramp> and <Ramp -30> and the cooling is not below a Delay of 40 or a Time of 175.

8) Straight vs. Curved Taper – When pulling Type C, D and E pipettes that have longer tapers, one can sometimes run into problems with curved tapers or a slight bend in the final taper behind the tip. This is especially the case when working with filamented glass. This bending is most likely caused by a greater sensitivity to balance of heat within the heating filament. To "straighten out" the taper, one can use the eccentric adjustments (see page 88) to adjust the position of the glass within the filament. Most often LOWERING the glass within the filament will remove or reduce the incidence of bending.

Technical Data for Aluminosilicate Glass

Aluminosilicate Glass (Schott 8252)

Aluminosilicate glass is similar in composition to borosilicate glass, but is a much harder glass and has greater chemical durability. Compared to borosilicate, aluminosilicates are appreciably more difficult to fabricate and costs for materials and manufacturing are about three times more than borosilicates. Aluminosilicate glass has a higher percent of aluminum oxide composition (14% Al_2O_3) than borosilicate (2.3% Al_2O_3) and additionally has relatively small amounts of calcium oxide, magnesium oxide and boric oxide. See tables below for details.

GLASS TYPE	SiO_2%	B_2O_3%	Na_2O %	$A1_2O$%	BaO%	CaO%	MgO%
Borosilicate Pyrex Corning 7740 Schott COE 33	80.6	13.0	4.0	2.3			
Aluminosilicate Schott 8252	60.0	4.5	<0.02	14.0	9	10	2.5

GLASS TYPE	Annealing Point	Softening Point
Borosilicate Pyrex Corning 7740 Schott COE 33	560	821
Aluminosilicate Schott 8252	725	935

Technical Data Sheet for 7740 Borosilicate Glass
http://www.corning.com/Lifesciences/technical_information/techDocs/descglasslabware.asp?region=na&language=en#7740LowExpansion

Technical Data Sheet for 8252 Aluminosilicate Glass
http://www.schott.com/tubing/english/download/8252_data_sheet.pdf?PHPSESSID=91

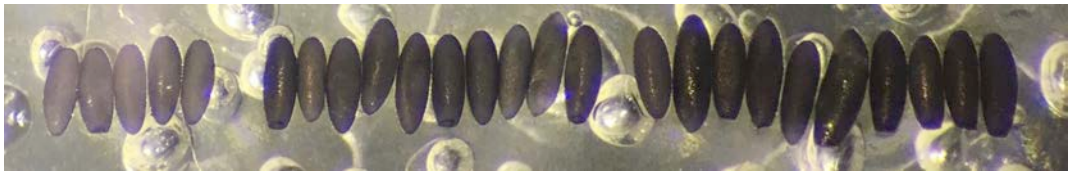
Quartz glass (Heraeus HSQ300) - The P-97 and P-1000 Pullers CANNOT melt QUARTZ!

The purest and hardest glass for making micropipettes and microelectrodes is Quartz glass. Quartz has a melting temperature of approx.1600 °C and this level of heat is beyond what your P-97 and P-1000 can provide. The higher temperatures required to melt quartz can be achieved using the **Sutter P-2000 Puller**, http://www.sutter.com/products/product_sheets/p2000.html

Quartz Heraeus HSQ 300	Fe	Cu	Na	Al	K	Ca	Mg
parts per million (ppm)	**0.1**	**<0.05**	**0.3**	**15**	**0.4**	**0.05**	**0.05**

CHAPTER 13

ZIKA Research and Mosquito Egg Injections



The pipettes required for mosquito egg injections are most commonly made using thin-walled Aluminosilicate glass with a filament, Sutter Part Number AF100-64-10. Aluminosilicate glass is 10X stronger and stiffer than Borosilicate glass and is recommended in situations where the pipette is inserted through a chorion or tough fibrous membranes. Mosquito eggs injections require a fairly short taper that is approximately 6-8mm in length and a tip that is just under 1μm. The tip and taper need to be fine enough not to cause damage to the chorion, and the taper should not be so long that it becomes wispy and not durable. To maintain good durability in the pipette, it is best to keep the taper on the shorter side by using a 2.5mm x 2.5mm box filament. Any time you are making needles with tip sizes under 1μm, filamented glass is used to allow for easy loading of solution into the needle. The best ingredients to start with are AF100-64-10 filamented glass and a 2.5mm x 2.5mm box filament (FB255B).

Programs for a 2.5 x 2.5 box filament (FB255B)

3. **ALUMINOSILICATE GLASS – AF100-64-10**

HEAT	PULL	VEL	DEL	PRESSURE
Ramp - 10	50	60	60-90	300

Parameter Setting Adjustments

- **Larger Tip & Shorter Taper** = Increase the Delay or Pressure in 10 unit increments
- **Smaller Tip & Longer Taper** = Decrease the Delay or Pressure in 10 unit increments
- **Reduce Shoulder** to make tip more narrow and gradual = Increase Pull in 10 unit increments

The pipettes can be used “as is,” broken back, or beveled. If you will be using them “as is,” it is best to use a lower pull value so the tips do not become too small. If you will be breaking back or “tapping off” the tip to “open it up”, it is best to introduce 10-20 additional units of pull strength so the tip does not break off too big or become too large, too fast. If you will be beveling the tip back to make it sharper and more open, it is best to introduce 5-10 additional units of pull so your final tip retains the same inner diameter.

IAEA & Sutter Instrument Microinjection Suite for Zika Eradication Research

Sutter Instrument has consulted and worked in conjunction with the International Atomic Energy Agency (IAEA) in Austria to help develop and optimize a microinjection suite for Zika Eradication Research. The following protocol was developed and improved for DNA microinjection and for cytoplasmic transfers.

P-2000 Laser-Based Puller

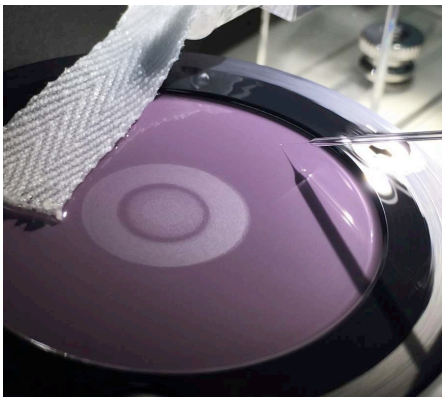
Using the P-2000 Laser-based puller and Quartz glass, the most durable pipettes for this application can be made. The chorion of the mosquito egg is exceptionally difficult to penetrate and we have found that Quartz pipettes are the most durable and long lasting compared to the pipettes made with Borosilicate or Aluminosilicate glass. If a P-2000 is not available, we recommend a P-1000 filament-based puller and AF100-64-10 Aluminosilicate glass.

Using the Sutter Instrument P-2000 CO_2 laser-based puller and QF100-70-10 Quartz glass, sharp micropipettes were pulled to have a 9-11mm taper using the following settings:

HEAT	FIL	VEL	DEL	PULL
800	5	50	145	200

BV-10 Beveler

The pipettes were then bevelled using two different bevelling surfaces, the 104D (fine) and 104E (very fine) bevelling plates, to determine which would produce better pipettes. It was determined that the **104E** (very fine) bevelling plate provided better control over the tip size and a smoother bevel when bevelling the pipettes. A bevelling time of 15-30 seconds is sufficient to make a 1-3 micron tip opening.



BRE Xenoworks Digital Injector

An analog oil-based injector and the air-based Sutter BRE Digital injector were tested to determine which type of injector was more effective and efficient for the microinjection and for DNA microinjection and for cytoplasmic transfers. It was found to be slightly easier to collect the cytoplasm using the air-based Sutter Xenoworks BRE Digital Injector compared to the Analog injector, and it was found that the high degree of negative pressure required to aspirate the cytoplasm tended to out-gassed the oil in the analog injector and create air bubbles in the air line. Once there is air in the oil-filled injection line of an analog injector, it will greatly reduce the control one has over the negative and positive pressures. When using the Sutter Digital Injector, we did not run into these difficulties since the entire system is air-based and no oil is required. When trying to transfer and distribute the collected cytoplasm in equal volumes into the recipient mosquito eggs using the analog system, poor control was experienced in the delivery if cytoplasm and unequal volumes were dispensed. Alternatively, when using the Sutter Xenoworks BRE Air-based Digital injector, it was found to offer good control when aspirating the cytoplasm, and had far superior control, compared to the Analog oil-based injector when delivering equal volumes of embryonic cytoplasm into the recipient eggs. Both the continuous and the pulse mode for injecting the cytoplasm was tested and both modes worked quite well for the transfers. For the DNA injections, we found the pulse mode with a slightly higher compensation pressure was preferred.

BRE Xenoworks Digital Injector

PrimeTech PMM4G Piezo Drive w/ FC-770 Fluorinert™

Because smaller and less damaging pipettes are being used at the IAEA, and because the cytoplasm of the mosquito eggs have a very high viscosity and is very sticky, these factors tend to cause the pipette tips to readily clog. As a result, the PMM4G Piezo drive was introduced to create very finely controlled microvibration to the pipette to help facilitate the movement of the highly viscous and sticky cytoplasm into and out of the pipette during both aspiration and transfer. The quartz glass was pre-treated with Sigmacote®, pulled on the P-2000 Sutter Puller, bevelled on the BV-10 Beveler with a 104F plate, and then pre-filled with FC-770 Fluorinert which is traditionally used in mouse ICSI procedures to "carry" the microvibration to the pipette tip. It was also realized that the cytoplasm would quickly solidify when exposed to air, so it was very important to keep the pipette under oil when transferring the cytoplasm.

It was determined that when using the smaller and less damaging 3-7 µm ID pipettes, which lead to higher survival rates, it was very difficult and sometimes impossible without the Piezo drill to withdraw the cytoplasm from the donor eggs. When using these smaller bevelled pipettes in conjunction with the PMM4G Piezo drive to introduce microvibration to the pipette (Intensity: 5, Speed: 6), the withdrawal and transfer of the cytoplasm became possible and we also found we had far more control over the negative and positive pressures of the injector when using in conjunction with the Piezo drive. The PMM4G Piezo drive, in combination with the glass pre-treated with Sigmacote® turned out to be two key ingredients allowing us to precisely control the aspirations and transfers using the smaller 3-7 µm bevelled pipettes.

PrimeTech PMM4G Piezo Drill

If only DNA injections are being performed and no cytoplasmic transfers will be performed, it is not necessary to use a Piezo drill. But if a Piezo drill is part of the set up, it can be used to clean the pipette and allow one to use the pipette for a longer period of time without it clogging.

Sigmacote®

Due to the high viscosity and stickiness of the embryonic cytoplasm, often the pipette tips would clog and only 5-10 injections were possible before having to change out the pipette tip. When bevelling the pipettes to create a 1-3µm tip ID, the clogging was greatly reduced. In addition, the capillary glass was then coated with a siliconizing reagent known as Sigmacote® to make the pipettes less sticky.

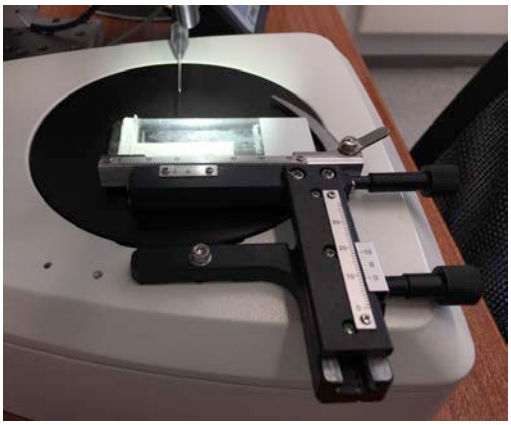
- Coating the Capillary Glass - GOOD
  Approximately 10ml of Sigmacote® was introduced inside the glass capillary, the glass was tilted end-to-end 4X to allow the solution to travel from one end to the other to coat the lumen of the glass and then the solution was blown out. The capillary glass was then dried in the oven at 200*C overnight. The capillary glass was then allowed to cool and placed in a container to be used for making the injection pipettes. It was observed that the pipettes made from the siliconized pre-coated glass, the injection pipettes could be used for over 50 injections without getting clogged.

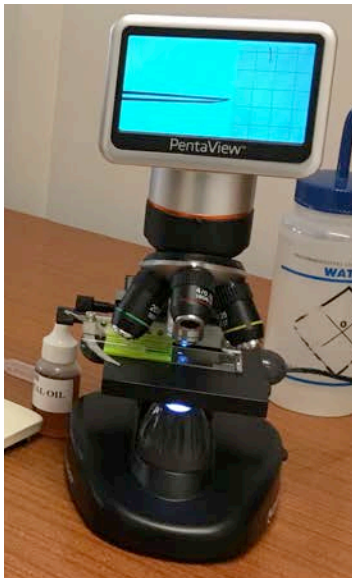
- Coating the Pipettes & Baking Dry - BAD
  After the pipettes were pulled, they were treated with Sigmacote® after they were pulled and bevelled was also tested, but upon baking dry the pipettes, the remaining nanoliters or picoliters of solution in the shaft of the pipette would travel to the tip and bake into a solid gel and clog the taper and tip of the pipette.

- Coating the Pipettes & Air Drying - GOOD
  The ID of the injection pipettes were also coating with Sigmacote® just after pulling and bevelling, and right before injecting, and this was found to be successful in making the pipettes less sticky. Approximately 0.5 microliters of Sigmacot® was back-loaded into the pipette and it was then allowed to fill the tip via capillary action. The tip was introduced into a beaker of ETOH and using high air pressure with the BRE Digital Injector the remaining Sigmacote® was cleaned out and the pipette tip was dried.

Additional Tools

- Graduated Microscope Mechanical Stage
- "Nail Art" Brushes for Manipulation of Eggs
- Pipette Examining Scope with Monitor





CHAPTER 14

CRAZY LAB LORE - Misconceptions, Old Wives' Tales & Superstitious Behavior

Crazy Lab Lore #1: Never touch the filament.
Answer: FALSE!

Crazy Lab Lore #2: Never touch the center of the glass with your fingers.
Answer A: FALSE if using the P-97 and P-1000 and other filament-based pullers.
Answer B: TRUE if using the P-2000 laser-based puller or for RNase free conditions

Crazy Lab Lore #3: You always need to wash the glass.
Answer: FALSE

Crazy Lab Lore #4: It is best to fire-polish the back ends of the glass by hand, one at a time, piece by piece, over a flame… in the lab, wasting your precious research time!
Answer A: FALSE…If you are using Sutter Glass which is all pre-polished
Answer B: TRUE (possibly) if you are using Non-Sutter Glass.

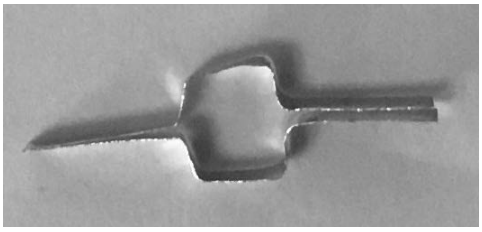
Crazy Lab Lore #5: It does not matter if the Drierite is PINK plus, pink is pretty.
Answer: FALSE

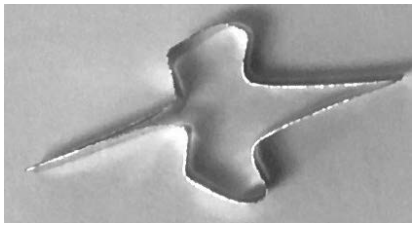
Crazy Lab Lore #1: Never Touch the Filament.

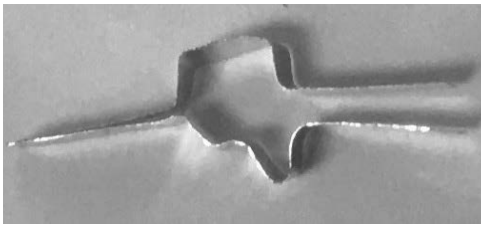
Answer: FALSE!

You CAN touch the filament when installing it, reshaping it or realigning it….but obviously not while it is hot! On the other hand, once the filament is installed, it is best to avoid touching the filament with the glass since this can bend or dent the filament. If the filament shape is altered by smashing it or denting it with the glass, the heating characteristics of the filament also change.

The filament will sometimes become dented or bent due to collisions with the glass. And, on occasion, when tightening the filament clamp screws, the wings of the filament can be pushed inward and the filament can become compressed (squashed) into a tall rectangle. In this case, one needs to pull the wings of the filament outward to reshape it. In these situations, you can handle the filament with your fingers and use small flat needle-nosed forceps to help reshape it or to flatten the bottom, top and side walls of the filament.


GOOD = Uniform Square

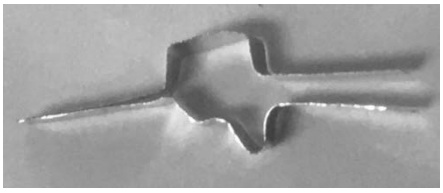
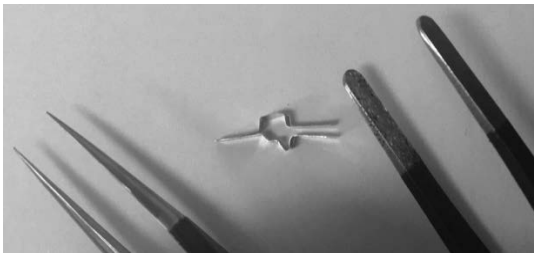
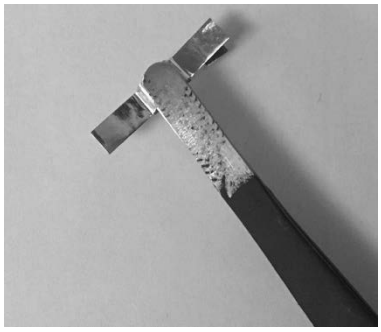

Squashed = Tall Rectangle


Smashed = Collision with glass

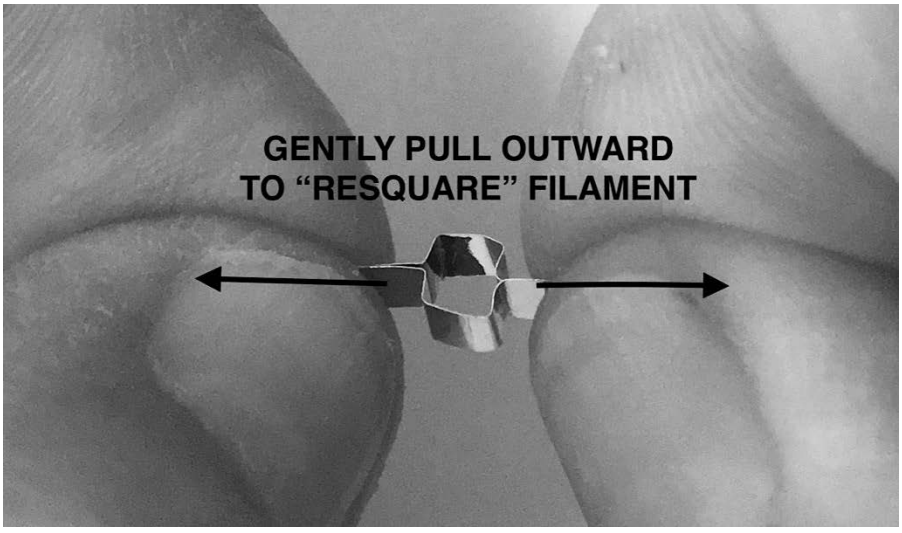
When handling the filament, you just need to be gentle. It is not necessary to use forceps or gloves, but you do need to be very careful not to smash or bend the filament while holding onto it.

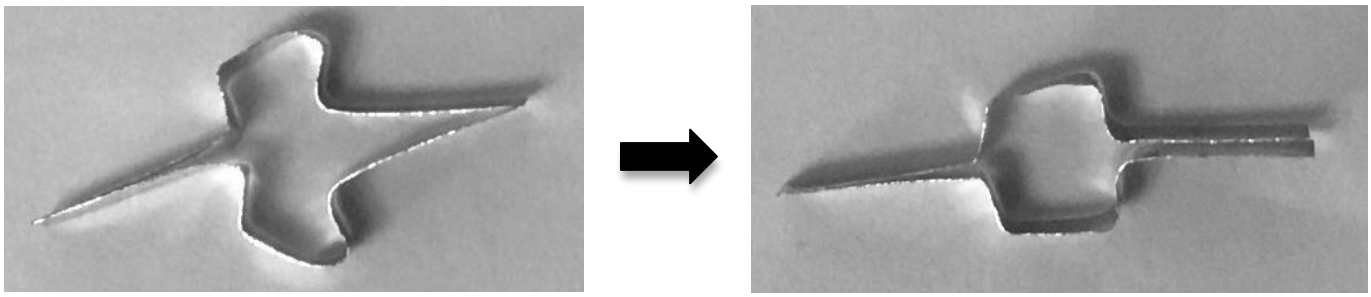
It does not matter if the oils from your fingers get onto the filament since any oils or debris on the filament will simply burn off the first time it heats up (when running a ramp test or when pulling a pipette). In fact, the oils or debris on the filament will burn off even before the glass softens.

RESHAPING the FILAMENT

Use flat needle-nosed forceps to flatten the top or bottom wall.





When tightening the filament clamp screws, the clamp can pivot and push the wing of the filament inward and deform the filament. If the filament has been squashed and is now a tall rectangle, remove the filament and gently pull outward on the wings to "re-square" the filament

Crazy Lab Lore #2: Never touch the center of the glass with your fingers.

Answer: FALSE when using the P-97 and P-1000 and other filament-based pullers.
Answer: TRUE when using the P-2000 laser-based puller or for RNase free conditions.

The P-97 and P-1000 pullers use a Platinum:Iridium filament to melt the glass. When installing the glass in the puller bars, many researchers are concerned the oils from ones fingers that get transferred to the glass will somehow damage the filament, or the puller, or will introduce a contaminant into the experiment. The oils on the glass will burn off before the glass is softened and this virtually sterilizes the glass along the taper and to the tip.

The P-2000 pullers use a CO_2 laser and a retro mirror to provide heat to the front and back sides of the glass. If you handle the center of the glass with your fingers when using a P-2000 puller, these oils get liberated when the glass is heated and are transferred onto the retro mirror. When the retro mirror gets coated with these oils, it becomes less effective in reflecting the heat to the other side of the glass. The compromised reflective surface of the mirror will introduce variability and, if not cleaned off, can burn into the surface and permanently damage the retro mirror.

Since the P-97 and P-1000 pullers use a filament as their heat source and there is no mirror involved in the transfer of heat to the glass, the oils burned off the capillary glass do not damage the filament, the glass or the puller.

In situations where one requires RNase free conditions, one needs to perform their own wash and sterilization to the glass and one should handle the glass with gloves or sterile forceps. This is true no matter what type of pipette puller the researcher is using.

RNases are enzymes found in all cell types and organisms and have very high specific activity, meaning tiny amounts of contamination in an RNA sample is sufficient to destroy the RNA. The major sources of RNase contamination in a typical laboratory include Human contact with hands and skin, aqueous solutions and reagents, and environmental exposure to air, dust and most surfaces.

Sutter glass is acid-washed, then rinsed in D_2O and ETOH, and baked dry. While this does render the glass cleaner than what other suppliers provide, the glass is not considered sterile. One can decontaminate capillary glass by baking it at 180°C or higher for several hours or by soaking it in freshly prepared 0.1% (v/v) DEPC in water or ethanol for 1 hour, followed by draining and autoclaving. Autoclaving will destroy any unreacted DEPC which can otherwise react with other proteins and RNA.

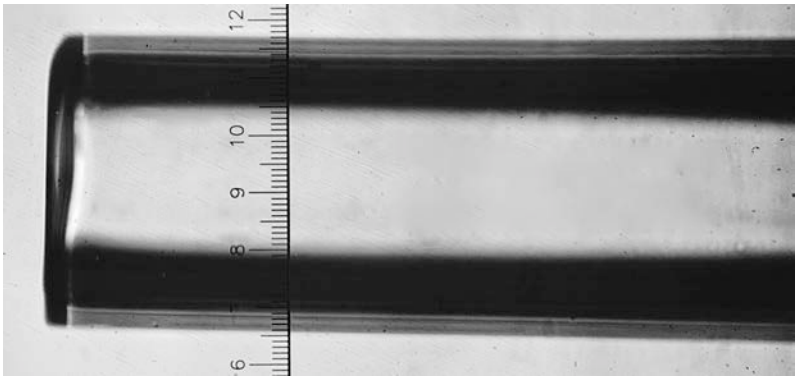
Crazy Lab Lore #3: You always need to wash the glass.

Answer: FALSE

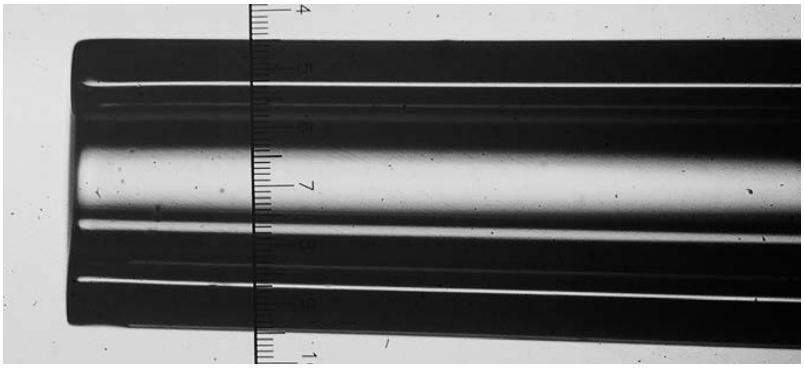
Sutter capillary glass is pre-washed and does not need to be washed in the lab unless you are requiring RNase free conditions. For more details on RNase and decontamination of the capillary glass, please see the last section of "Crazy Lab Lore #2"

Sutter glass is acid-washed, ultrasonically rinsed in D2O, then rinsed in ETOH, drained and baked dry. This renders the glass clean and free of most particles and dust. While this does not render the glass sterile, it usually renders the glass cleaner than what other companies provide.

CLEAN GLASS



Sutter Instrument Thin Walled Glass – Pre-cleaned by manufacturer

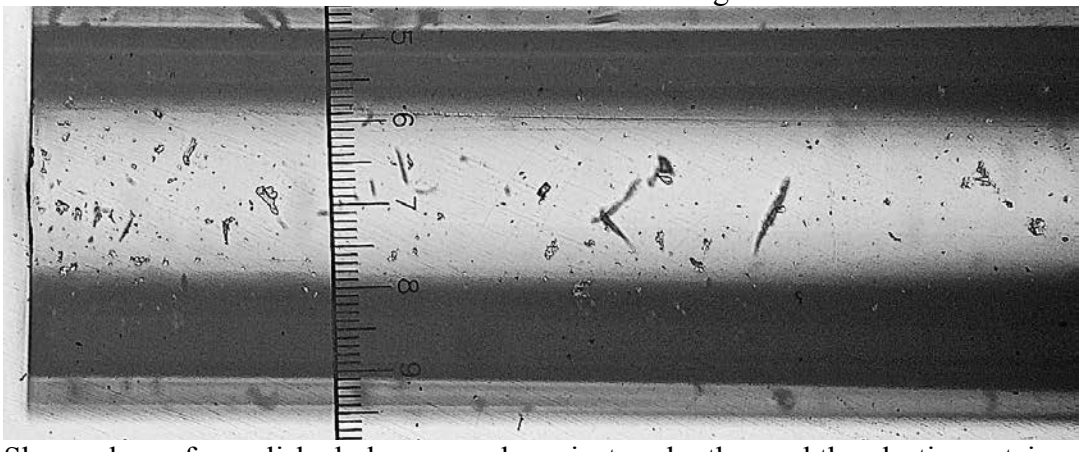


Sutter Instrument Thick Walled Glass – Pre-cleaned by manufacturer

DIRTY GLASS

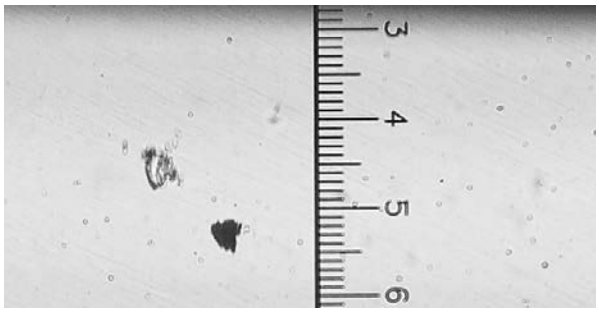
If your glass is old, has been open for a long time or exposed to the air, it is a good idea to check the glass at 50X magnification under a light microscope to see if the glass is contaminated with particles, dust or oils. Normally these oils and particles will burn off the outside of the capillary glass when it is heated and pulled, but when these particles or oils get into the lumen of the glass, especially on the back ends or the regions of the glass where it is not heated, this can lead to the clogging of the pipette.

Debris in the Glass Lumen – Glass and Plastic shavings

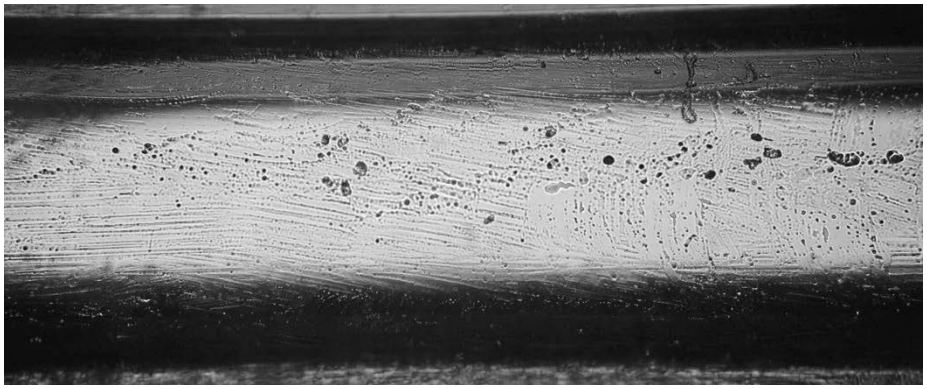


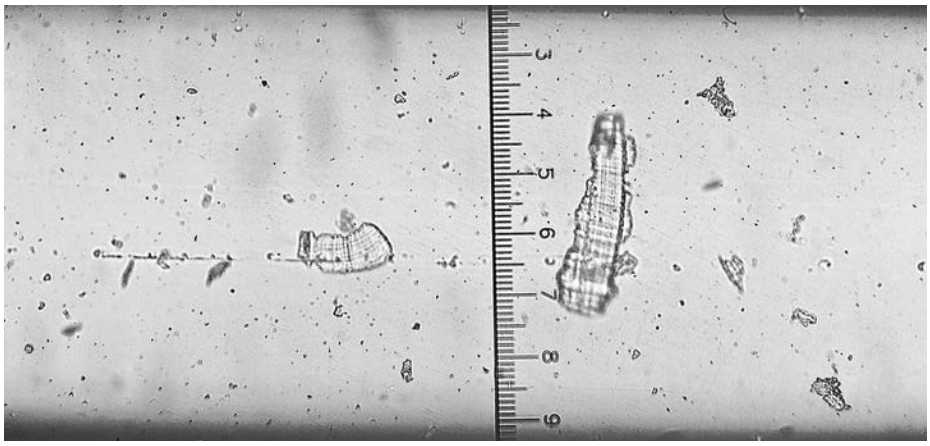
Sharp edges of unpolished glass can rub against each other and the plastic container and create a lot of debris in the glass.

DIRTY GLASS - CONTINUED

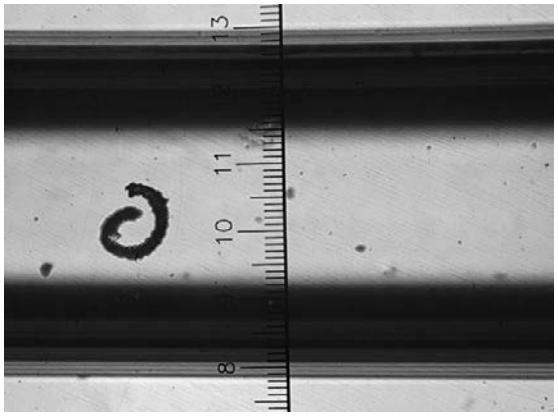


Glass Particle (clear) & Carbon Particle (black) from air in factory.


Finger Oils and Skin Dander



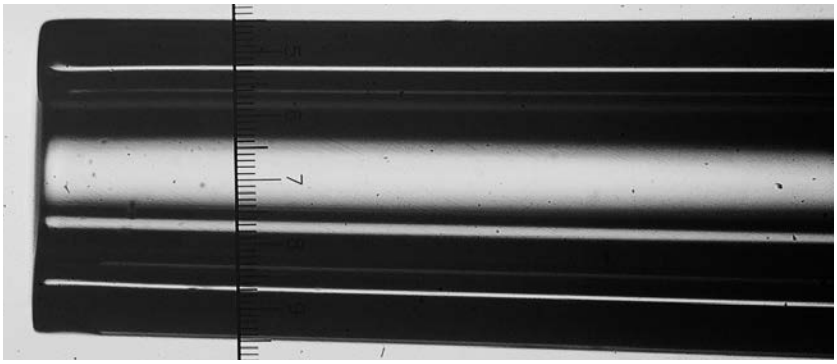
Chipped Glass and Skin Dander



OMG! Who knows! And Obviously NOT clean!

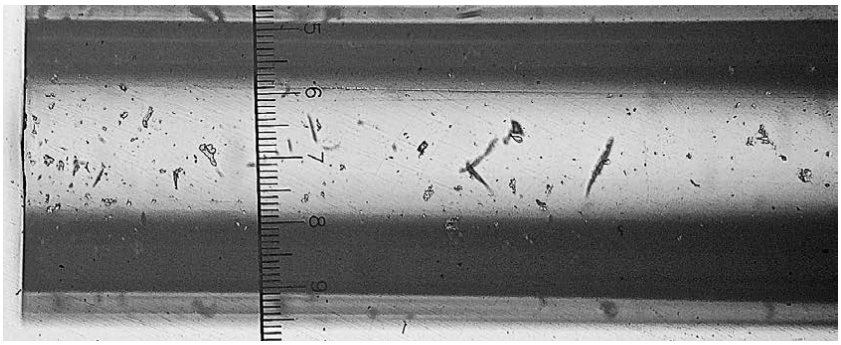
Crazy Lab Lore #4: It is best to fire-polish the back ends of the glass by hand, one at a time, piece by piece, over a flame… in the lab, wasting your precious research time!
Answer: FALSE when using Sutter Glass.
Answer: TRUE (most likely) when using Non-Sutter Glass.

Sutter glass is not only pre-washed, the back ends of the capillary glass are also PRE-POLISHED so the glass does not need to be polished in the lab. This is a free service and we do not charge extra to polish the back ends of the glass. Some Sutter glass is also provided with Heavy Polish. The item number of the glass with a heavy polish will end with "HP", for example, BF150-86-10HP. All other glass has a moderate degree of polish on the back end of the glass.

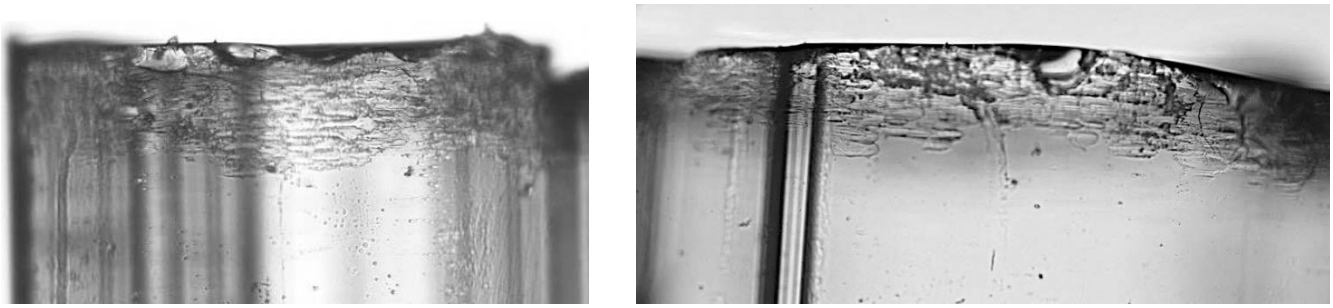


Sutter Glass with Smooth Polished Ends

Capillary glass from other suppliers (as shown below) might not have polished ends. The ends of the glass in this condition will have a sharp or rough edge which, over time, will damage the O-rings, gaskets and the silver chloride wire of the head stage.



Glass from other Supplier – Sharp edge with no polish



Glass from other Supplier - Badly Cut with Non-Polished Ends

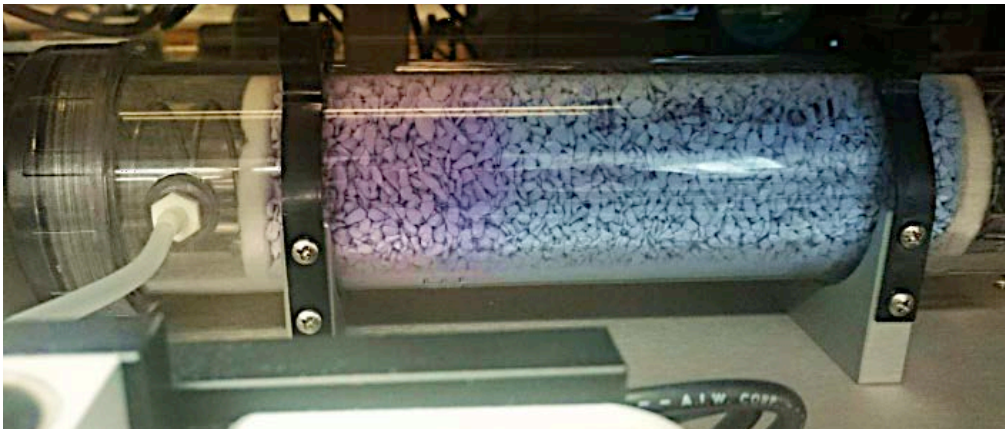
Above the glass is scored and cut in a manner where the back ends are sharp or galled and rough. When the back ends of the glass are not cut clean and then "treated" with fire-polishing, the rough sharp edges will generate small glass fragments which will adhere to the inner wall of the glass and potentially clog the pipette tip when it is filled with solution. The back ends of the glass will also damage the O-rings and gaskets that are inside the head stages and pipette holders.

Crazy Lab Lore #5: It does not matter if the Drierite turns PINK plus, pink is pretty.
Answer: FALSE on both accounts . . . :-)

The Drierite in the canister has an indicator (Calcium Chloride) which is blue when it is dry and turns pink when the Drierite becomes moist. The air used to cool the glass and filament is filtered through the Drierite canister to remove moisture from the cooling air. The cooling air should be dry since humidity will introduce variability to the cooling rate and this will effect how the glass is pulled. In addition, humidity in the presence of cooling glass is also thought to introduce the formation of Hydroxyl ions* on the tip of the glass, and some think this could interfere with forming a good seal to the cell membrane in patch clamp experiments.

This dry air from the Drietite canister is also used to purge the humidity control chamber (rectangular chamber around the filament and jaws) for 5 seconds before and after the pull, so the humidity is removed before the glass is heated. During humid times of the year, or in humid environments, we recommend that you use the default setting of 5 seconds to purge the chamber or increase the purge time to 10-15 seconds to drive out the humidity. On the P-97, you can by going to the control settings and selecting functions 4 & 5 to change the purge time before and after the pull. On the P-1000 you can go to the menu of the program and change the purge time.

If the Drierite canister still has blue granules in it on the far right, you still have active Drierite and you do not need to change it out. Once ALL the Drieirite in the canister is lavender or pink, it has all been expended and is moist and should be regenerated or replaced. Please find the SutterInstrument YouTube Video for instructions on how to replace the Drierite. https://www.youtube.com/watch?v=BLvYLLJmcnY


Drierite 1/3 expended….Still good. No need to replace it.


Drierite completely expended… all moist. Time to replace it!

*Properties of Glass-Forming Melts 1st Edition
by David Pye (Editor), Innocent Joseph (Editor)m Angelo Montenero (Editor)

CHAPTER 15

Problems with Variability

Variability in the pipette taper length, tip size, or resistance is most often a result of unstable parameter settings. If you have tried the recommended filament, glass and program settings provided in this cookbook and are still unable to achieve stable and reliable results, please review the following topics.

- **Pulleys (black or silver wheels) not freely rotating or rolling smoothly:** Push both puller bars away from the filament and into their "clip-locked" position. In this position the cable will no longer ride in the groove of the pulley and the pulley is free of friction. Spin the pulleys and see if they rotate and spin freely. If there is resistance or it feels rough, contact Sutter for technical support ASAP.

- **Old or Damaged Filament**: If your platinum filament (box or trough) is over 2 years old or the puller is in high use, the filament can be worn thin and will provide uneven heating to the glass. If your filament is old it will have a matte-like finish, look very dull and similar to very old aluminium foil. If it is in good condition it will have a clean and shiny surface. It might also be possible that your filament has survived a collision with the glass and is now bent or misshapen. *SOLUTION:* Replace your filament.

- **Filament Shape and Alignment**: If you have just replaced your filament and are experiencing variability, check the shape of the filament and make sure the filament is centered over the air jet. To make sure your filament is perfectly centered over the air jet, pull a pipette with a long taper and compare the taper length of the right and left pipettes. If they are NOT identical in length, loosen the filament clamp screws and "nudge" the filament in the direction of the shorter pipette. Repeat this procedure until both pipettes are the same length. A trough filament should be shaped such that its walls angle inward by 80 degrees to the base. The glass should run through the center of a box filament or through the bottom 1/3 of a trough filament. Refer to pages 13-18 and contact Sutter Instrument for further instructions about aligning the filament and the glass.

- **New Filament = New Ramp Test!** If you have replaced your filament, you need to run a new ramp test. If you are changing from a trough filament to a box filament (see page 87), your new ramp value could increase two-fold. Run a ramp test (see page 12) and adjust your heat values accordingly.

- **Built Up of Dirt and Oils on the Puller Bars and Bearings**: Check the beveled edge of the puller bars and the V-groove in the bearings (where the puller bars reside) for dirt. The bearing groove can be cleaned with 70% Ethanol on a Q-tip or applicator. To check for obstructions, depress the spring stop and insure that the puller bars slide smoothly from left to right. You should also be able to rotate the bearings by holding the puller bar stable and rolling your thumb or finger over the bearing. DO NOT OIL THE BEARINGS!

- **Cable Tension**: To check the tension, hold both puller bars together and tap the cable between the bumper and the pulley with your forefinger. The cables should have about 1 to 2mm of slack and should not be taut. You should be able to tap on each cable and hear the pull solenoid plunger hit its stop with a "knocking" or "clunking" sound inside the cabinet.

- **Drierite Granules**: The rear right canister on the base plate contains a desiccant (Drierite) which should be light blue in color. If the granules have turned lavender or pink, this indicates that the granules are saturated with moisture. If the air cooling your glass has a high level of humidity, this can introduce variability in cooling and cause the puller to generate inconsistent tip sizes. *SOLUTION:* Remove and refill the canister with new or regenerated Drierite.

REFURBISH – You can send your P-97 Puller to Sutter Instrument to be refurbished! The main board will be upgraded, all worn parts will be replaced, and you will get an extended 1-year warranty. This is often recommended for pullers that are 10-15 years old. Contact Sutter for details.

"The 15 Questions"

When working with the Sutter P-87, P-97 or P-1000 Pipette Pullers, about 95% of all difficulties one might experience with the puller are a result of unstable parameter settings, poor alignment of the filament, misalignment of the glass, a poorly shaped filament, and/or an incorrect placement of the air-jet. Please provide the following details so we can let you know if any of these foundational concerns are an ingredient in the difficulties you are experiencing.

1) Which model puller do you have (P-87, P-97, or P-1000) and what is the serial number?
   The serial number on newer pullers will be "P97-_ _ _ _" or "P1000- _ _ _"
   The serial number for older pullers will be a series of 4 - 5 numbers.

2) Ramp Test Value of your filament using your glass?

3) Trough or Box filament installed in the puller?

4) Width of the filament or the item number of the filament?

5) Application: slice patch, whole cell patch, dissociated/cultured cell patch, microinjection (and type), extracellular recording, or high resistance intracellular recording?

6) What taper length, tips size and/or resistance do you aim for?

7) OD and ID of the glass (outer and inner diameter)?

8) Item number of the glass?

9) Parameter settings (Heat, Pull, Velocity, Time/Delay, and Pressure)?

10) Are you using a one-line program?

11) If a one-line program, check line 2 to make sure it is blank. Let us know how many times it loops.

12) If it is a multi-line program, provide all lines of the program and indicate on what line the glass separates.

13) Send images of the filament installed in the puller (top view and a horizontal view from the left looking into the right). Provide two images of each, one with the glass in the right puller bar, and one without the glass in place. We need 4 pictures total.

14) Push the puller bars all the way back (in their position after a pull) and this will lift the cable off the black or silver pulley (to the far right and left of the puller). Now spin the black or silver pulley and let us know if it spins freely and many times around. Is there resistance and uneven friction?

15) There is also a pair of pulleys inside the puller and these too might need to be examined. Remove the front panel screws, drop down the front panel and the pulleys are mounted center and right below the base plate.

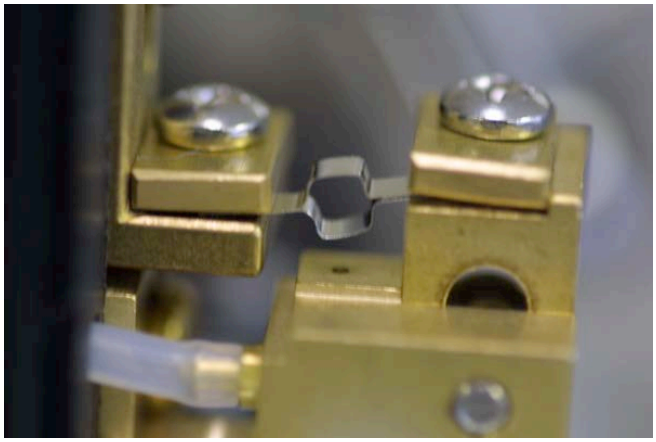
Providing a response to each and every question will rapidly accelerate our ability to trouble shoot and resolve problems you are now encountering. If it is determined that the puller has an electronic and/or mechanical issue that cannot easily be addressed on-site, we recommend you return the puller to the Sutter Factory for a REPAIR or a REFURBISH.

Installing a Box or Trough Filament – Review

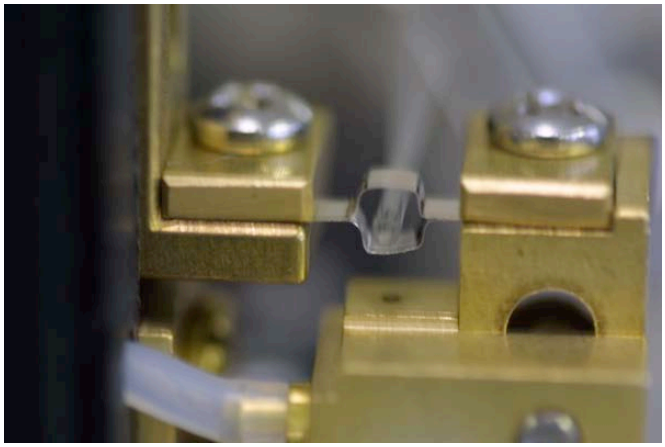
The box filament does not require shaping, but needs to be installed so it is centered right to left over the air jet and also centered around the glass. The glass should run through the middle of the filament.

Box filament not centered over air jet & crooked
This is BAD!

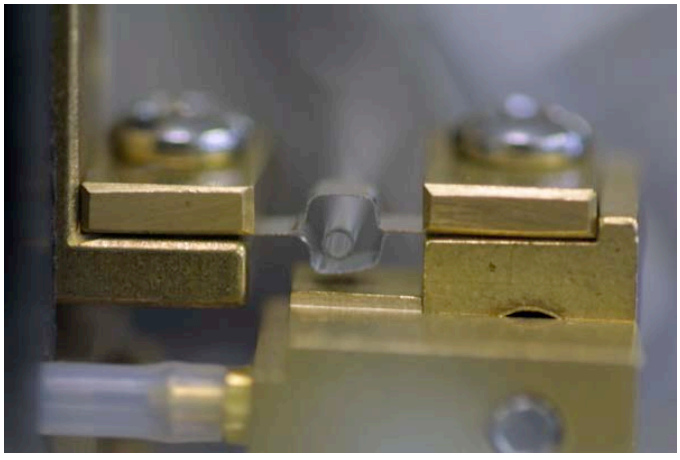
Box filament is centered over the air jet and is 1.0mm in from the left edge of the brass jaws.
This is GOOD!



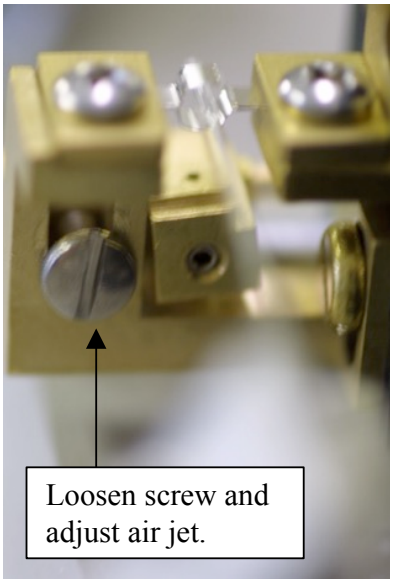
BAD…adjust jaws to make them even (page 10)



GOOD…jaws even, glass centered, air jet 3mm below the filament.



BAD…Air Jet too close



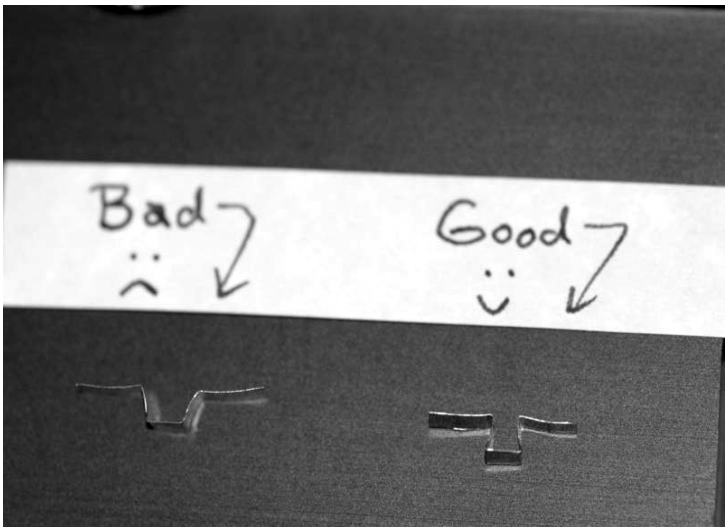
BAD…Air Jet too far and tilted

Trough Filament Shape & Alignment

3mm Trough Filament (FT330B)

Top View

BAD shape **GOOD shape**



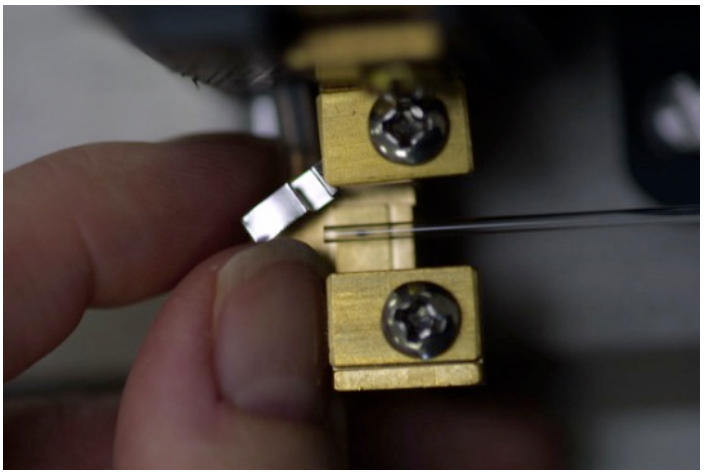
Side View

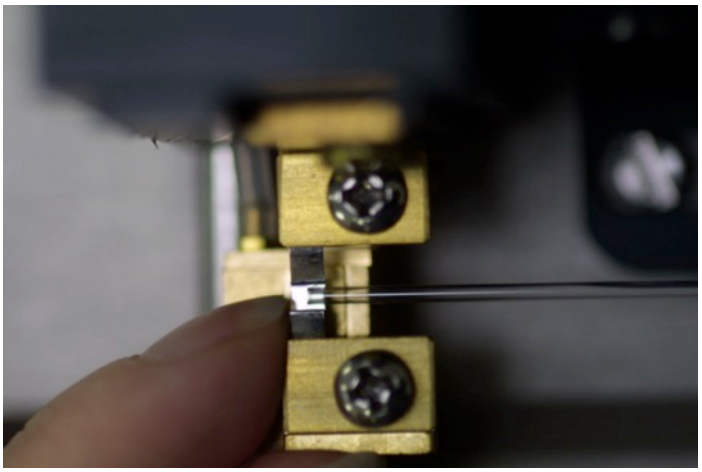
BAD shape **GOOD shape**

BAD filament shape: A "Bad" filament shape for the 3mm trough (FT330B) pictured above with the walls angled outward (this is bad!) will provide inefficient heating, ramp values over 300 units, and a greater chance of burning out your filament. If your ramp test values or heat values are above 300 units, it is best to remove the filament and reshape it according to what is shown as a "good shape. After you have reshaped the filament, run a new ramp test and adjust your heat settings to be no greater than 15 units over the ramp test value and stay under 300 units for your heat.

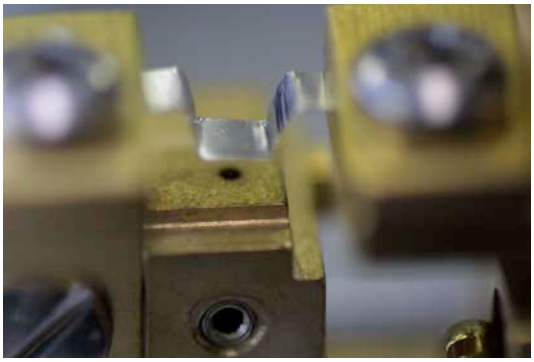
GOOD filament shape: A "Good" filament shape for the 3mm trough (FT330B) pictured above will provide efficient heating of the glass, a ramp value between 240 - 290 units, and a long filament life span of one to two years. The walls should angle inward at 80 degrees and there should be a 2mm opening at the top of the filament.

Installing your filament:
Place a piece of glass in the right puller bar and install the filament around the glass. Filament should be positioned over your air jet. Usually the filament is centered over the air jet when it is sitting 0.5 to 1mm in from the left edge of the brass jaws.

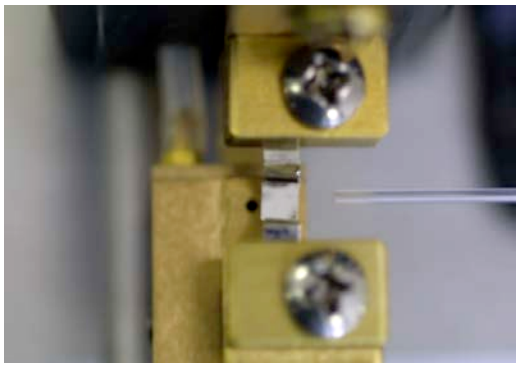




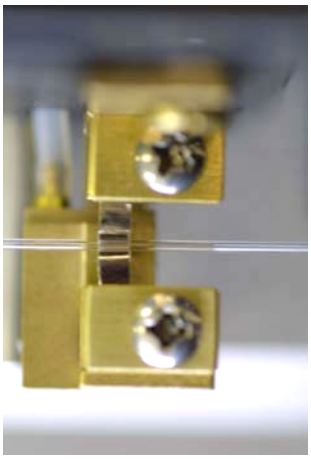
Trough Filament continued….



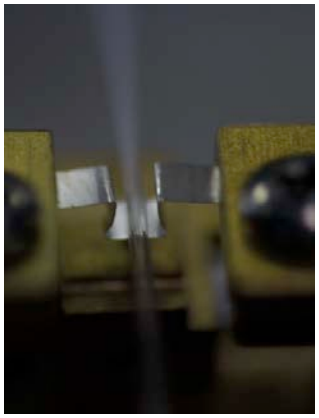
BAD SHAPE



BAD ALIGNMENT



GOOD shape & Alignment

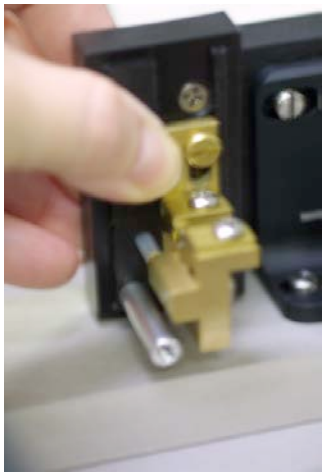


GOOD

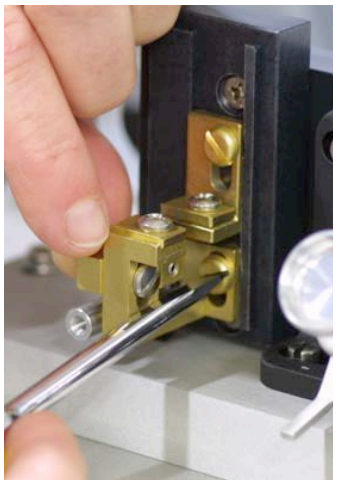
GOOD

Changing from a Trough to a Box Filament: If you are changing from a trough filament to a box filament, you will need to adjust the position of the brass jaws. The trough sits higher than the box, so you will need to loosen the brass screws (one at a time) and slide each jaw **down** about 3mm.

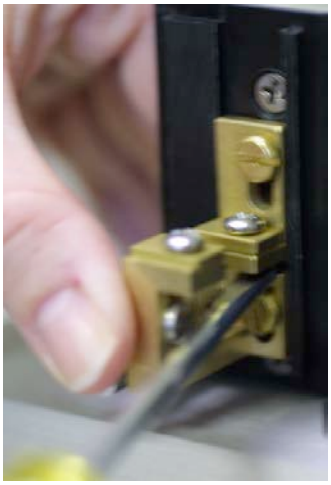
STEP 1.
Loosen top brass screw then…



STEP 2. Slide jaw down 3mm, then tighten the brass screw.



STEP 3. Loosen bottom brass screw and then…



STEP 4. Use the screw driver to pry jaw down 3mm, then tighten the brass screw.

ECCENTRIC ADJUSTMENTS

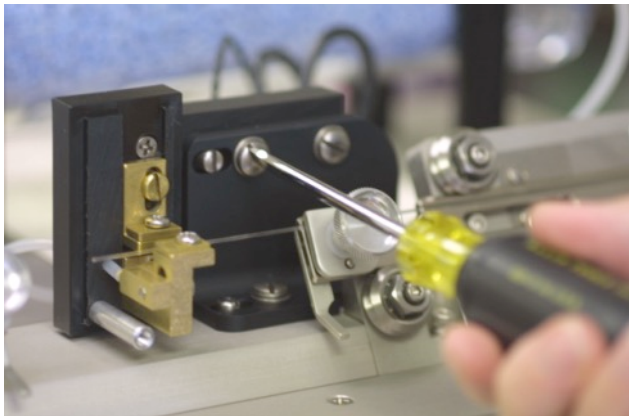
Large adjustments to Box or Trough Filament Position (more than 1 to 2mm)

If you find that the glass is not centered in the filament from top to bottom, it is best to make large adjustments by adjusting the brass jaws (as seen on pg.87). If the filament is not centered in the filament front to back (the glass is sitting closer to the front or back wall of the filament), it is best to loosen the clamp screws and move the filament forward or back.

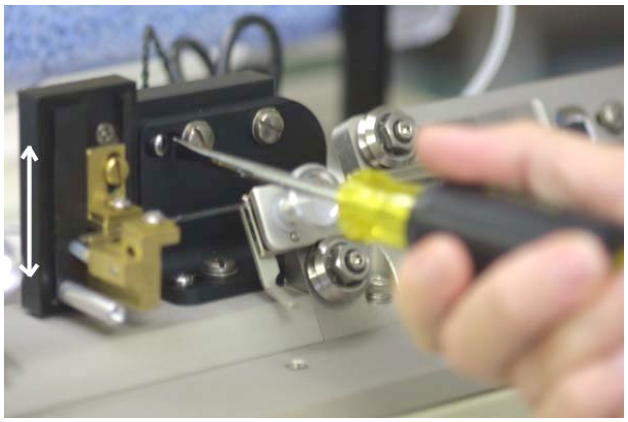
Eccentric Adjustments (Fine - less than 1 to 2mm change of position)

To fine-tune the position of the glass within the filament, you can use the eccentric adjustments to fix the vertical and horizontal alignment. The eccentrics allow you to adjust the filament position in relation to the glass. For a trough filament, the glass should sit centered and low within the filament. For a box filament, the glass should sit centered in both the horizontal and vertical axis.

Vertical Eccentric Adjustment (Moving Filament Up & Down)



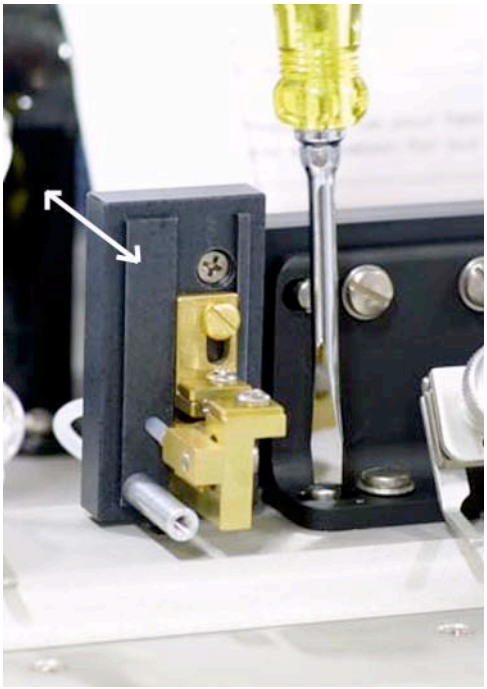
Loosen the locking screw



Turn Eccentric Screw

Horizontal Eccentric Adjustment (Moving Filament Forward and Back)

Loosen the locking screw



Turn Eccentric Screw

***Always remember to tighten the locking screw after you adjust the eccentric!!**

CHAPTER 17

FILAMENTED GLASS - WHAT IS "FILAMENTED" GLASS & WHO NEEDS IT?

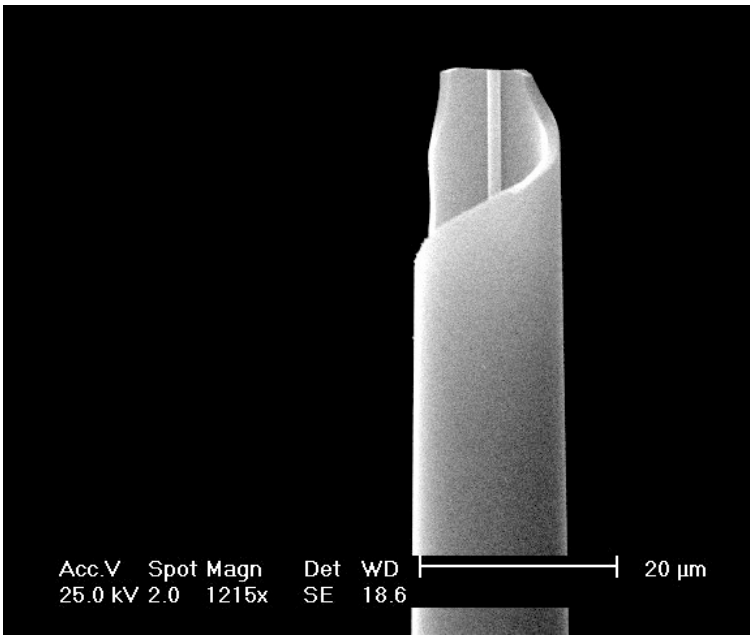
You have a choice of using "filamented" or "non-filamented" capillary glass. The glass is filamented if the Sutter Item number for Borosilicate, Aluminosilicate or Quartz glass starts with BF, AF or QF. For Example, BF150-86-10 indicates the following: Borosilicate Glass with Filament, 1.5mm outer diameter, 0.86mm inner diameter and a 10cm in length.

Any time you are making a micropipette that has a tip at or under 3µm, as needed when performing microinjection or electrophysiology applications, it is best to buy filamented glass. Without a filament, back-filling pipettes can become difficult to impossible and this will most often cause poor filling and introduce air bubbles within the solution of the pipette.

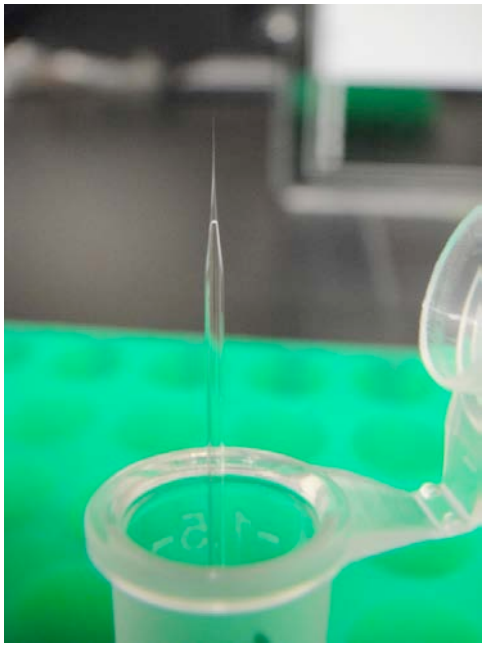
The "filament" refers to a ~160 micron rod of glass that is annealed to the inner wall of the tubing. This filament in the lumen acts as a wick, creating capillary action, which allows for the easy back filling of solution into the pipette taper and tip. Filamented glass is absolutely required for microelectrodes which have a tip size less than 1 micron, but most researchers choose to use filamented glass even for patch pipettes with 1-3µm tips. The filament will not interfere with establishing a gigolm seal and will help reduce the development of air bubbles in the pipette when it is being filled.

When back-filling a patch pipette, the tip and most of the taper will fill within 30-60 seconds. When back-filling a sharp and long microelectrode or a microinjection pipette, the tip and most of the taper will fill within 2-3 minutes.

As your capillary glass is heated and drawn out on your Sutter Pipette Puller to create a microelectrode or microinjection pipette, the inner diameter of the glass and this filament will gradually reduce in size. If you make a pipette that has a 0.5µm tip ID, this rod (a.k.a. filament) inside the glass will end up being around 80 nanometers at the tip.



SEM image of filamented capillary glass where the tip has been broken back to reveal the "filament" (Borosilicate glass rod) that is annealed to the inner wall of the lumen.



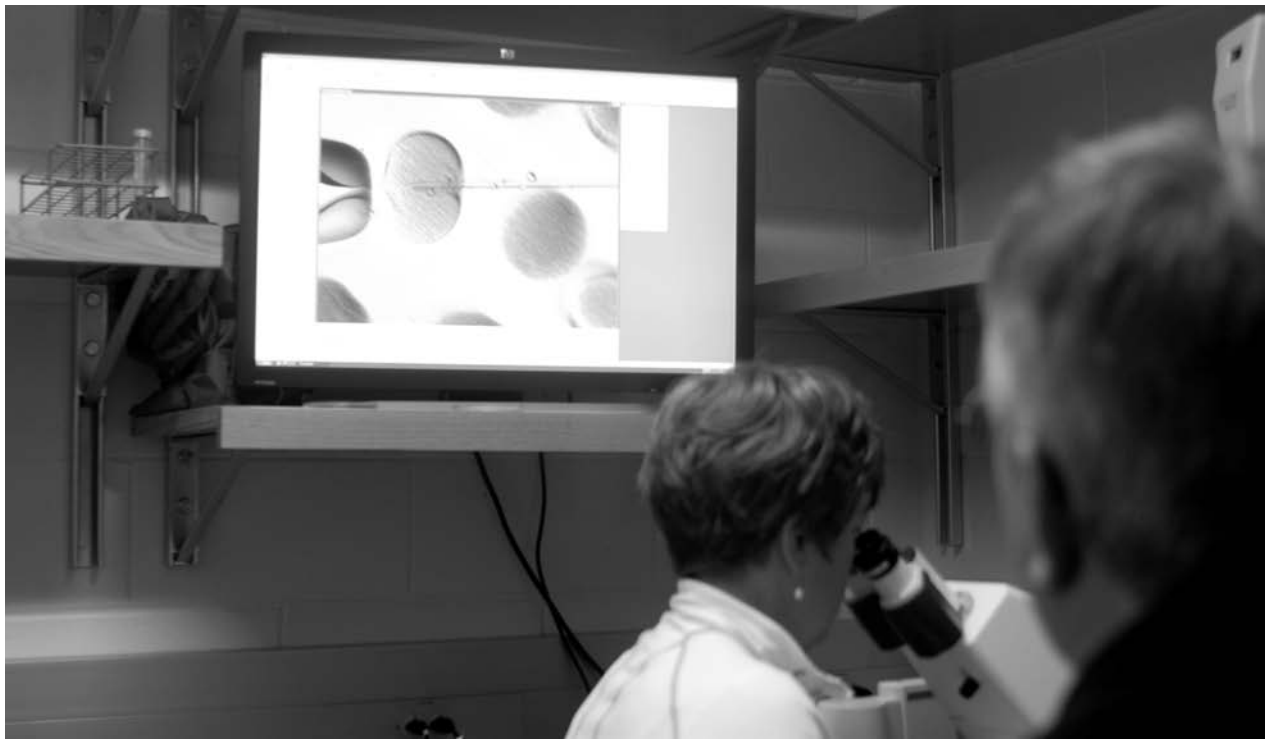
Back-filling microelectrode via capillary action using BF100-78-10 filamented glass

CHAPTER 18

XenoWorks Microinjection System

Using our 30 years of experience and expertise in motorized micropositioning, air pressure control and electro-mechanical design, we have created the XenoWorks™ Microinjection System. The XenoWorks System has been designed to meet the needs of a wide variety of applications for the manipulation of cells and embryonic tissues. By combining micromanipulators, microinjectors and microscope mounting adapters, the XenoWorks system can be configured for a wide variety of applications.

APPLICATIONS: Pronuclear Injection, Adherent Cell Injection, ICSI - Intracytoplasmic Sperm inj., ES Cell - Embryonic Stem Cell inj., NT- Nuclear Transfer, *C.elegans* microinjection Drosophila microinjection, and Zebrafish microinjection.



Marine Bio. Labs, Stem Cell Course, Woods Hole, MA Photo courtesy of David McFarland

XenoWorks Micromanipulator

Features include:

- Stepper Motor Control
- Six speeds, course to very fine
- Work, Home and Z-limit memory positions
- Adjustable Joystick (height and tension)
- Y-axis lockout for pure X-axis travel
- Set up routine
- Declutch

XenoWorks Digital Injector

Features Include:

- Built-in compressor – very quiet
- Two pressure ports – "Hold" & Inject/Transfer
- 80 psi (max) of injection pressure
- Two injection modes: Continuous or Pulse
- Remote keypad

XenoWorks Analog Injector

Features Include:

- Course and fine control
- Use with air, water or oil
- Interchangeable syringes to modify sensitivity
- Used for holding, transfer, and low pressure injections.

Recommended Configurations for the XenoWorks Microinjection System

The configuration of any XenoWorks microinjection system will depend upon the application for which it will be used. Listed below are some of the more commonly-used applications and recommended configurations.

Pronuclear Injection
Suggested system configuration:

1 x Micromanipulator (Right), Item # BRMR
1 x Micromanipulator (Left), Item # BRML
1 x Digital Microinjector, Item # BRE110/BRE220
2 x Microscope Adapters

Embryonic Stem Cell Transfer into Blastocysts
Suggested system configuration:

1 x Micromanipulator (Right), Item # BRMR
1 x Micromanipulator (Left), Item # BRML
1 x Digital Microinjector, Item # BRE110/BRE220 or 2 x Analog Microinjectors BRI
2 x Microscope Adapters

Intracytoplasmic Sperm Injection (ICSI)
Suggested system configuration:

1 x Micromanipulator (Right), Item # BRMR
1 x Micromanipulator (Left), Item # BRML
2 x Analog Microinjectors, Item # BRI
2 x Microscope Adapters

Piezo-assisted ICSI
Suggested system configuration:

1 x Micromanipulator (Right), Item # BRMR
1 x Micromanipulator (Left), Item # BRML
2 x Analog Microinjector, Item # BRI
2 x Microscope Adapter

Adherent Cell, Drosophila, Zebrafish, and *C.elegans* Microinjection
Suggested system configuration:

1 x Micromanipulator (Right), Item # BRMR
1 x Digital Microinjector, Item # BRE110/BRE220
1 x Microscope Adapter

Nuclear Transfer
Suggested system configuration:

1 x Micromanipulator (Right), Item # BRMR
1 x Micromanipulator (Left), Item # BRML
2 x Analog Microinjector, Item # BRI
2 x Microscope Adapter

For other microinjection applications, please contact Sutter Instrument for advice with these and any other microinjection needs.

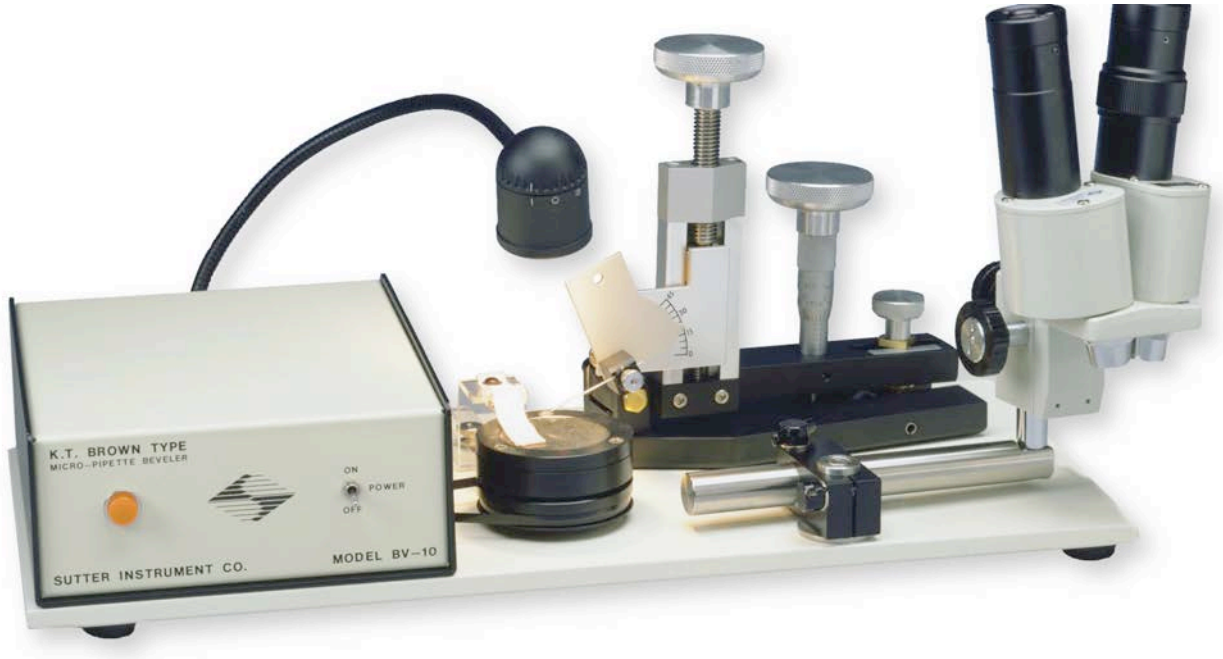
CHAPTER 19

BV-10 Beveler

The BV-10 Beveler is an elegant and simple approach for precision beveling of pipettes to achieve tips between 0.1 and 75 µm The unique abrasive plate drive system is vibration free for greater control of the beveling process. Beveling can be accomplished very rapidly and produces consistent tip diameters using the techniques described by Brown and Flaming, Science, August 1974, Vol. 185.

Intracellular recording electrodes can benefit from beveling because of a reduction in tip diameter, by creation of a sharp point on the electrode and also because of a lowered electrical resistance due to a larger cross section of the inner diameter of the glass. This greatly facilitates penetrating and holding very small or difficult cells. The 104F diamond abrasive plate and a beveling time between 5 and 20 seconds are recommended for intracellular recording electrodes.

Microinjection needles also benefit from beveling by promoting entry into cells with minimal damage while simultaneously enhancing the flow of materials through the needle. Chapters 5, 6, and 7 describe the pipette morphologies needed for Embryonic Stem (ES) Cell Injection, Intracytoplasmic Sperm Injection (ICSI), and Nuclear Transfer (NT). The pipettes used for these applications most often require a beveled pipette. Using the BV-10 Beveler (pictured below), and the 104D fine diamond plate, with beveling times between 30 and 240 seconds, one can produce a sharp bevel at the desired angle - usually between 15 and 45 degrees.



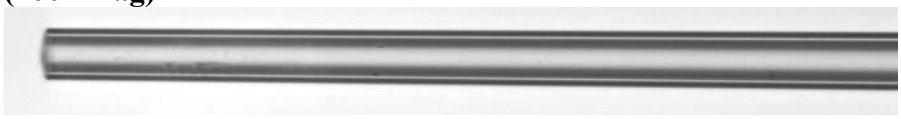
BV-10 Beveler

Images of Pipettes Before and After Beveling

BEFORE

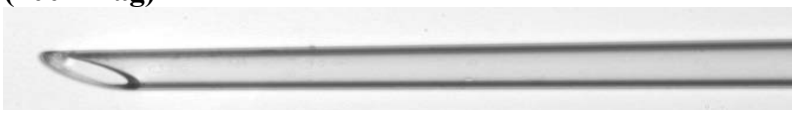
Pipette broken back with tweezers to make a rough 25µm ID break
(400x mag)

Pipette broken back using a ceramic tile to make a clean 25µm ID break
(400x mag)

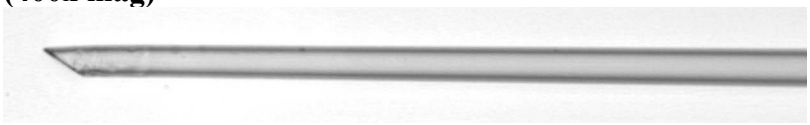


AFTER

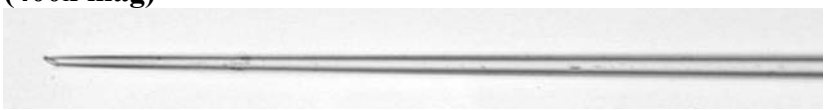
Pipette beveled at 25-degrees using BV-10 Beveler, 25µm ID
(400x mag)



Pipette beveled at 35-degrees using BV-10 Beveler, 15µm ID
(400x mag)



Pipette beveled at 45-degrees using BV-10 Beveler, 2-3µm ID
(400x mag)



GENERAL LOOK UP TABLES

The General Look Up Tables provide 120 programs organized by filament type. For example, if you are using a 3mm Trough filament (FT330B), you will want to refer to the charts which provide the programs using this filament. The filament size is located at the upper left corner of each chart. First find the series of charts (6 charts for each filament) and then find the outer and inner diameter or part number of the glass you are using. Once you have located the chart of programs for your filament and glass combination, you will then need to determine which program "**TYPE**" is designed for your application. Each table provides 5 program types; **Type A, B, C, D, and E**. The most basic way to think of these program types is that Type A programs will provide the shortest taper and largest tip, and as you move down the list, the taper becomes longer and the tip becomes smaller. The longest taper and smallest tip would therefore be Type D and E programs. Below is a general description of the morphology and application for each program "Type".

Type A – Patch & Extracellular recording, 1 to 10MΩ
These programs are good for making pipettes with a short taper, a large tip and a low resistance. One can expect to get a 3-5mm taper, a 1-3 micron tip, and 1-10 MΩ of resistance.

Type B – Adherent Cell, *C.elegans,* Drosophila, & Zebrafish, 10 to 30MΩ
These programs are good for making pipettes with a short to medium length taper, a tip just under 1μm and a low resistance. One can expect to get a 5-7mm taper, a 0.9- 0.7μm micron tip, and 10-30 MΩ of resistance.

Type C – Pronuclear Injection and Intracellular recording, 40 to 80MΩ
These programs are good for making pipettes with a medium length taper, and a small sharp tip. One can expect to get a 7-9mm taper, a 0.8 - 0.5μm tip, and 40-80 MΩ of resistance. These programs are also used to make injection pipettes for small cells or eggs which require breaking back the tip**.**

Type D – Intracellular recording, >100 MΩ
These programs are good for making pipettes with a long taper and a very small tip. One can expect to get an 8-13mm taper, a 0.5- 0.06μm tip, and 80 to100+ MΩ of resistance.

Type E – ES Cell, ICSI, ES Cell, Nuclear Transfer, Holding, Xenopus, and 20-200μm Tips
These programs are good for making pipettes with an extremely long 10-15mm taper and a very small wispy tip which is then intended to be broken back to create a 5 to100+μm tip. These pipettes require additional steps such as cutting, beveling and/or fire-polishing to create the final pipette. On the **P-1000 Puller,** select a Type D program in the cookbook menu for these applications. For longer tapers, increase the velocity to 150 units and reduce the pressure to 100 units.

GENERAL LOOK UP TABLES: 3.0mm x 3.0mm Trough Filament (FT330B)

1mm x 0.5mm Glass (B100-50-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 1	B100-50-10	Line 1 Loops (4)	Ramp+15	0	50	150 t	500
B	Prog. # 2	BF100-50-10	Line 1	Ramp+15	0	20	200 t	400
			Line 2	Ramp+15	55	65	150 t	400
C	Prog. # 3	BF100-50-10	Line 1	Ramp+10	85	100	200 t	400
D	Prog. # 4	BF100-50-10	Line 1	Ramp+15	150	100	150 t	500
E	Prog. # 5	B100-50-10	Line 1	Ramp+15	30	150	100 t	200

1.0mm x 0.75mm Glass (B100-75-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 6	B100-75-10	Line 1 Loops (3)	Ramp+15	0	90	150 t	500
B	Prog. # 7	BF100-78-10	Line 1	Ramp+10	45	100	175 t	300
C	Prog. # 8	BF100-78-10	Line 1	Ramp+15	55	100	175 t	200
D	Prog. # 9	BF100-78-10	Line 1	Ramp+15	50	100	150 t	300
E	Prog. # 10	B100-75-10	Line 1	Ramp+15	30	120	100 t	200

1.2mm x 0.69mm Glass (B120-69-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 11	B120-69-10	Line 1 Loops (4)	Ramp+15	0	45	150 t	500
B	Prog. # 12	BF120-69-10	Line 1	Ramp+10	0	20	200 t	400
			Line 2	Ramp+15	75	95	150 t	400
C	Prog. # 13	BF120-69-10	Line 1	Ramp+15	45	85	200 t	300
D	Prog. # 14	BF120-69-10	Line 1	Ramp+15	60	95	150 t	500
E	Prog. # 15	B120-69-10	Line 1	Ramp+30	30	120	100 t	200

1.2mm x 0.90mm Glass (B120-90-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 16	B120-90-10	Line 1 Loops (3)	Ramp	0	65	150 t	500
B	Prog. # 17	BF120-94-10	Line 1	Ramp+5	45	110	175 t	300
C	Prog. # 18	BF120-94-10	Line 1	Ramp	45	105	150 t	200
D	Prog # 19	BF120-94-10	Line 1	Ramp+15	55	100	150 t	300
E	Prog. # 20	B120-90-10	Line 1	Ramp+30	30	120	100 t	200

GENERAL LOOK UP TABLES: 3.0mm x 3.0mm Trough Filament (FT330B)

1.5mm x 0.86mm Glass (B150-86-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 21	B150-86-10	Line 1 Loops (4)	Ramp+10	0	55	150 t	500
B	Prog. # 22	BF150-86-10	Line 1	Ramp+10	0	30	200 t	200
			Line 2	Ramp+10	35	155	200 t	200
C	Prog. #23	BF150-86-10	Line 1	Ramp+10	45	85	200 t	400
D	Prog. # 24	BF150-86-10	Line 1	Ramp+10	55	95	200 t	400
E	Prog. # 25	B150-86-10	Line 1	Ramp+10	30	120	100 t	200

1.5mm x 1.10mm Glass (B150-110-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 26	B150-110-10	Line 1 Loops (3)	Ramp+15	0	90	150 t	300
B	Prog. # 27	BF150-110-10	Line 1	Ramp+5	45	120	175 t	200
C	Prog. # 28	BF150-110-10	Line 1	Ramp+10	45	120	175 t	200
D	Prog. # 29	BF150-110-10	Line 1	Ramp+10	45	120	150 t	200
E	Prog. # 30	B150-110-10	Line 1	Ramp+30	30	120	100 t	200

GENERAL LOOK UP TABLES: 2.5mm x 2.5mm Box Filament (FB255B)

1.0mm x 0.50mm Glass (B100-50-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 31	B100-50-10	Line 1 Loops (4)	Ramp	0	30	250 t	500
B	Prog. # 32	BF100-50-10	Line 1	Ramp+5	45	75	90 d	500
C	Prog. # 33	BF100-50-10	Line 1	Ramp	55	75	80 d	400
D	Prog. # 34	BF100-50-10	Line 1	Ramp+10	75	75	200t or 90d	400
E	Prog. # 35	B100-50-10	Line 1	Ramp+30	30	120	100 t	200

1.0mm x 0.75mm Glass (B100-75-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 36	B100-75-10	Line 1 Loops (3)	Ramp	0	40	200 t	500
B	Prog. # 37	BF100-78-10	Line 1	Ramp	50	60	90 d	200
C	Prog. # 38	BF100-78-10	Line 1	Ramp+5	80	70	80 d	200
D	Prog. # 39	BF100-78-10	Line 1	Ramp+5	105	70	50 d	200
E	Prog. # 40	B100-75-10	Line 1	Ramp+30	30	120	100 t	200

1.2mm x 0.69mm Glass (B120-69-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 41	B120-69-10	Line 1 Loops (4-5)	Ramp	0	20	250 t	500
B	Prog. # 42	BF120-69-10	Line 1	Ramp	45	80	200 d	600
C	Prog. # 43	BF120-69-10	Line 1	Ramp	60	80	90 d	500
D	Prog. # 44	BF120-69-10	Line 1	Ramp	80	80	70 d	300
E	Prog. # 45	B120-69-10	Line 1	Ramp+40	0	120	150 t	200

1.2mm x 0.90mm Glass (B120-90-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 46	B120-90-10	Line 1 Loops (3)	Ramp	0	40	200 t	500
B	Prog. # 47	BF120-94-10	Line 1	Ramp	50	85	90 d	450
C	Prog. # 48	BF120-94-10	Line 1	Ramp	85	90	70 d	350
D	Prog. # 49	BF120-94-10	Line 1	Ramp+5	105	70	80 d	300
E	Prog. # 50	B120-90-10	Line 1	Ramp+40	40	80	100 t	200

GENERAL LOOK UP TABLES: 2.5mm x 2.5mm Box Filament (FB255B)

1.5mm x 0.86mm Glass (B150-86-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 51	B150-86-10	Line 1 Loops (4-5)	Ramp	0	21	1 d	600
B	Prog. # 52	BF150-86-10	Line 1	Ramp	35	70	200 d	600
C	Prog. # 53	BF150-86-10	Line 1	Ramp	70	75	200 d	500
D	Prog. # 54	BF150-86-10	Line 1	Ramp	85	85	100 d	500
E	Prog. # 55	B150-86-10	Line 1	Ramp+50	30	120	100 t	200

1.5mm x 1.10mm Glass (B150-110-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 56	B150-110-10	Line 1 Loops (2-3)	Ramp	0	65	250 t	500
B	Prog. # 57	BF150-110-10	Line 1	Ramp	55	75	120 d	400
C	Prog. # 58	BF150-110-10	Line 1	Ramp	85	75	80 d	400
D	Prog. # 59	BF150-110-10	Line 1	Ramp+5	95	70	70 d	250
E	Prog. # 60	B150-110-10	Line 1	Ramp+50	30	120	100 t	200

GENERAL LOOK UP TABLES: 3.0 mm x 3.0 mm Box Filament (FB330B)

1.0mm x 0.50mm Glass (B100-50-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 61	B100-50-10	Line 1 Loops (4)	Ramp+5	0	30	250t or 1d	500
B	Prog. # 62	BF100-50-10	Line 1	Ramp+5	35	75	130 d	500
C	Prog. # 63	BF100-50-10	Line 1	Ramp	55	75	90 d	500
D	Prog. # 64	BF100-50-10	Line 1	Ramp	100	75	250 t	500
E	Prog. # 65	B100-50-10	Line 1	Ramp+25	30	120	100 t	200

1.0mm x 0.75mm Glass (B100-75-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 66	B100-75-10	Line 1 Loops (2-3)	Ramp+5	0	40	200 t	500
B	Prog. # 67	BF100-78-10	Line 1	Ramp+5	55	75	80 d	300
C	Prog. # 68	BF100-78-10	Line 1	Ramp+5	90	80	80 d	200
D	Prog. # 69	BF100-78-10	Line 1	Ramp+15	80	70	50 d	200
E	Prog. # 70	B100-75-10	Line 1	Ramp+35	0	100	100 t	200

1.2mm x 0.69mm Glass (B120-69-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 71	B120-69-10	Line 1 Loops (4)	Ramp+5	0	25	250t or 1d	500
B	Prog. # 72	BF120-69-10	Line 1	Ramp+5	45	80	120 d	500
C	Prog. # 73	BF120-69-10	Line 1	Ramp+5	55	80	90 d	500
D	Prog. # 74	BF120-69-10	Line 1	Ramp+10	80	80	60 d	500
E	Prog. # 75	B120-69-10	Line 1	Ramp+35	0	120	150 t	200

1.2mm x 0.90mm Glass (B120-90-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 76	B120-90-10	Line 1 Loops (2-3)	Ramp+5	0	35	200 t	500
B	Prog. # 77	BF120-94-10	Line 1	Ramp	55	85	110 d	300
C	Prog. # 78	BF120-94-10	Line 1	Ramp+5	85	90	90 d	300
D	Prog. # 79	BF120-94-10	Line 1	Ramp+10	85	90	90 d	200
E	Prog. # 80	B120-90-10	Line 1	Ramp+40	40	90	150 t	200

GENERAL LOOK UP TABLES: 3.0 mm x 3.0 mm Box Filament (FB330B)

1.5mm x 0.86mm Glass (B150-86-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 81	B150-86-10	Line 1 Loops (4-5)	Ramp	0	25	1 d	500
B	Prog. # 82	BF150-86-10	Line 1	Ramp	45	80	200 d	600
C	Prog. # 83	BF150-86-10	Line 1	Ramp	45	80	150 d	500
D	Prog. # 84	BF150-86-10	Line 1	Ramp	75	95	110 d	500
E	Prog. # 85	B150-86-10	Line 1	Ramp+25	30	120	100 t	200

1.5mm x 1.10mm Glass (B150-110-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 86	B150-110-10	Line 1 Loops (2-3)	Ramp+5	0	65	250 t	500
B	Prog. # 87	BF150-110-10	Line 1	Ramp+5	55	90	120 d	400
C	Prog. # 88	BF150-110-10	Line 1	Ramp+5	85	95	90 d	400
D	Prog. # 89	BF150-110-10	Line 1	Ramp+5	95	80	60 d	300
E	Prog. # 90	B150-110-10	Line 1	Ramp+50	30	120	100 t	200

GENERAL LOOK UP TABLES: 2.5 mm x 4.5 mm Box Filament (FB245B)

1mm x 0.5mm Glass (B100-50-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 91	B100-50-10	Line 1 Loops (4)	Ramp	0	25	250 t	500
B	Prog. # 92	BF100-50-10	Line 1	Ramp	0	30	250 t	500
			Line 2	Ramp-10	0	40	250 t	500
			Line 3	Ramp-10	25	55	250 t	500
C	Prog. # 93	BF100-50-10	Line 1	Ramp	0	50	150 d	500
			Line 2	Ramp-10	35	60	150 d	500
D	Prog. # 94	BF100-50-10	Line 1	Ramp-20	50	80	200 t	600
E	Prog. # 95	B100-50-10	Line 1	Ramp+50	30	120	200 t	300

1mm x 0.75mm Glass (B100-75-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 96	B100-75-10	Line 1 Loops (3)	Ramp	0	25	250 t	500
B	Prog. # 97	BF100-78-10	Line 1	Ramp+5	0	50	200 t	500
			Line 2	Ramp+5	25	65	200 t	500
C	Prog. # 98	BF100-78-10	Line 1	Ramp	0	30	200 t	500
			Line 2	Ramp-10	80	60	200 t	500
D	Prog. # 99	BF100-78-10	Line 1	Ramp	45	85	200 t	500
E	Prog. # 100	B100-75-10	Line 1	Ramp+50	30	120	200 t	300

1.2mm x 0.69mm Glass (B120-69-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 101	B120-69-10	Line 1 Loops (4-5)	Ramp	0	20	250 t	500
B	Prog. # 102	BF120-69-10	Line 1	Ramp	0	40	250 t	500
			Line 2	Ramp-10	0	40	250 t	500
			Line 3	Ramp-10	65	50	250 t	500
C	Prog. # 103	BF120-69-10	Line 1	Ramp	0	50	250 t	600
			Line 2	Ramp-15	60	85	250 t	600
D	Prog. # 104	BF120-69-10	Line 1	Ramp	0	40	250 t	500
			Line 2	Ramp-10	60	85	250 t	500
E	Prog. # 105	B120-69-10	Line 1	Ramp+25	30	120	200 t	300

GENERAL LOOK UP TABLES: 2.5 mm x 4.5 mm Box Filament (FB245B)

1.2mm x 0.90mm Glass (B120-90-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 106	B120-90-10	Line 1 Loops (3)	Ramp	0	40	200 t	500
B	Prog. # 107	BF120-94-10	Line 1	Ramp+5	0	50	200 t	500
			Line 2	Ramp+5	45	65	200 t	500
C	Prog. # 108	BF120-94-10	Line 1	Ramp	0	30	200 t	500
			Line 2	Ramp+10	80	60	200 t	500
D	Prog # 109	BF120-94-10	Line 1	Ramp+5	85	85	250 t	500
E	Prog. # 110	B120-90-10	Line 1	Ramp+50	30	120	200 t	300

1.5mm x 0.86mm Glass (B150-86-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 111	B150-86-10	Line 1 Loops (4-5)	Ramp+5	0	20-25	1 d	500
B	Prog. # 112	BF150-86-10	Line 1	Ramp	0	35	1 d	500
			Line 2	Ramp	0	35	1 d	500
			Line 3	Ramp-65	35	55	110 d	500
C	Prog. # 113	BF150-86-10	Line 1	Ramp	0	35	1 d	500
			Line 2	Ramp	0	35	1 d	500
			Line 3	Ramp-30	35	65	110 d	500
D	Prog. # 114	BF150-86-10	Line 1	Ramp-20	60	90	250 d	600-700
E	Prog. # 115	B150-86-10	Line 1	Ramp+15	30	120	250 t	500-600

1.5mm x 1.10mm Glass (B150-110-10)

TYPE	PROG #	Glass Item Number	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	Prog. # 116	B150-110-10	Line 1 Loops (3)	Ramp+5	0	65	250 t	500
B	Prog. # 117	BF150-110-10	Line 1	Ramp+5	0	50	250 t	500
			Line 2	Ramp+5	45	55	250 t	500
C	Prog. # 118	BF150-110-10	Line 1	Ramp	55	75	110 d	600
D	Prog. # 119	BF150-110-10	Line 1	Ramp+10	55	75	110 d	500
E	Prog. # 120	B150-110-10	Line 1	Ramp+40	30	120	200 t	500

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