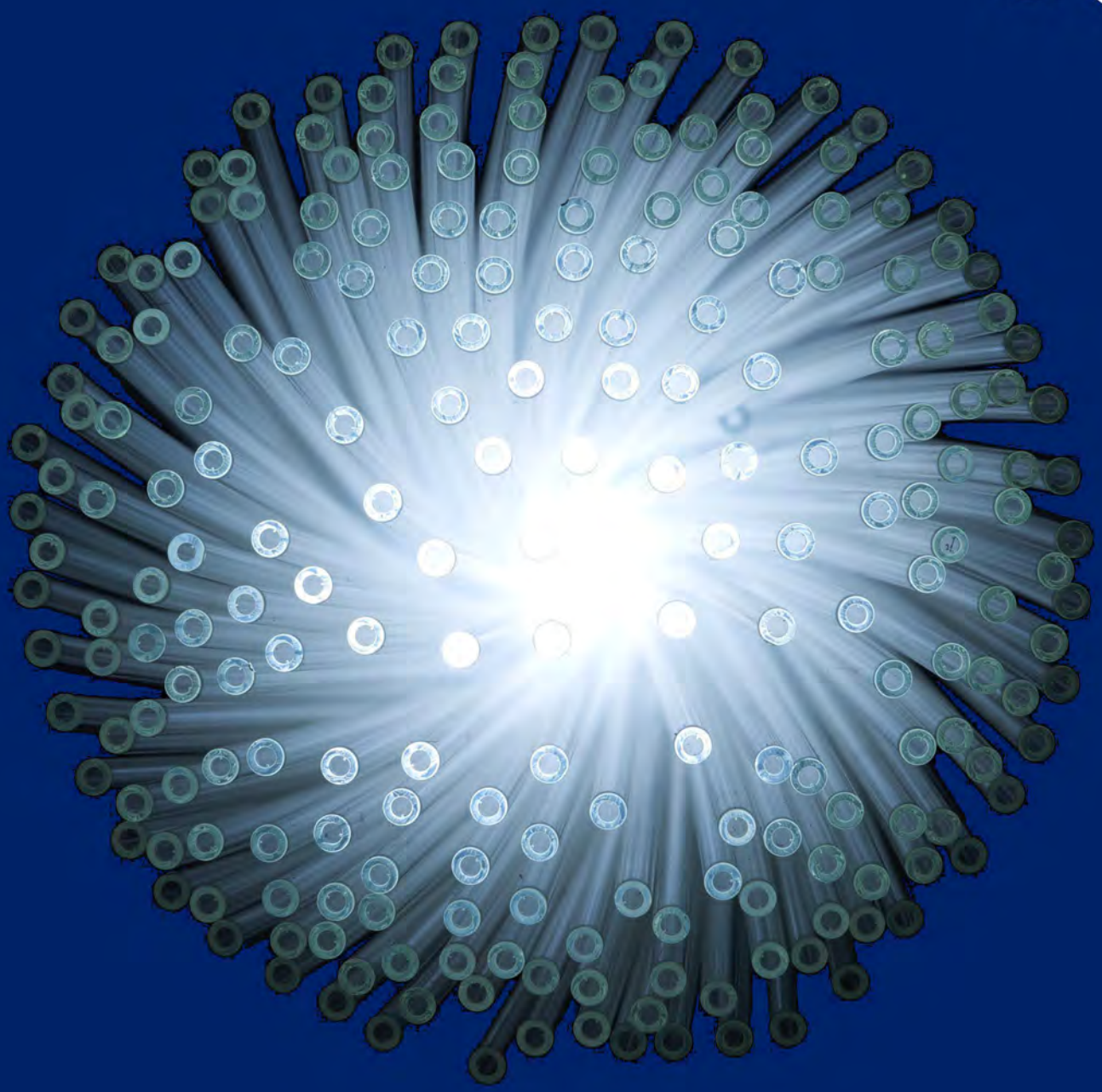

Pipette Cookbook 2025

P-97 & P-1000 Micropipette Pullers

(New: P-2000/G Quartz Injection Applications)

Rev. G

SUTTER INSTRUMENT

The Pipette Cookbook was written, compiled and created by Adair L. 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 be downloaded for free through the Sutter Instrument website. **https://www.sutter.com/PDFs/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 with her favorite Rolleiflex camera (Cuba, 2018)

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Meet Dale Flaming:
Founder of Sutter Instrument

Dale Flaming's journey from humble beginnings to becoming a pioneering force in scientific instrumentation is a testament to his innovation, perseverance, and passion for science. Spending his formative years in Kansas, Montana, and Bakersfield, California, Dale's early life was shaped by hard work, from picking crops in high school to repairing electronics at a local radio shop. These experiences ignited his curiosity and hands-on approach to problem-solving, traits that would later fuel his entrepreneurial spirit.

Dale earned a Bachelor of Science in Chemistry from Tabor College in Kansas, followed by teaching science classes in the children's clinic at The Menninger Foundation. His academic pursuits deepened with a Master's in neurophysiology from UC San Diego, where he worked at the prestigious Scripps Institute of Oceanography. But, it was during his tenure at UCSF in the early 1970s that Dale's groundbreaking innovation in micropipette technology began.

Faced with challenges obtaining reliable micropipettes for recording retinal nerve cell activity, Dale engineered a solution in 1974—developing a micropipette beveler, which marked the start of Sutter Instrument. His work didn't stop there. As an early adopter of computer technology, Dale used a Data General Nova, an early microcomputer, in his research and attended meetings of the legendary Homebrew Computer Club, laying the groundwork for even more advanced inventions. In 1977, Dale developed the P-77 micropipette puller, the first digitally-controlled puller on the market. The P-77 revolutionized the field by allowing precise, repeatable control over micropipette production, providing scientists with the reliable tools they needed for critical research. A few years later, the P-80PC was introduced as the first puller on the market to utilize a microprocessor.

From its modest beginnings in Dale's garage, Sutter Instrument has since grown into a global leader in scientific equipment. Now based in a 55,000-square-foot facility in Novato, California, Sutter employs over 70 talented individuals, many with neuroscience backgrounds. Under Dale's leadership, the company has continuously expanded its product offerings, empowering scientists worldwide in their research endeavors.

In 2007, in recognition of the team that helped build Sutter Instrument, Dale made the extraordinary decision to give back 30% of the company to his employees, solidifying his legacy of collaboration, innovation, and integrity.

Dale Flaming's vision and dedication have helped establish Sutter Instrument as a trusted partner in the scientific community, enabling groundbreaking research across the globe.

A History of Sutter Instrument Company

Sutter Instrument Company was founded in 1974 by Dale Flaming, a research scientist at the University of California, San Francisco (UCSF). While studying the neurophysiology of the retina, Dale encountered a significant challenge: the available tools to make micropipettes produced pipettes that were not sharp, thin, or consistent enough to support his experiments. To overcome this, Dale developed a pipette beveler. Expanding on this innovation, he then created the first electronically controlled pipette puller (the Brown-Flaming Puller) and published a paper detailing the technology.

In 1977, Sutter Instrument was incorporated to fulfill the growing demand for these tools. The company began manufacturing and selling bevelers and pipette pullers, eventually capable of producing pipette tips as small as 10 nanometers. To put this into perspective, a human hair is approximately 80 microns wide—about 800 times larger. The technology advanced to the point where pipette tips were smaller than the wavelength of visible light, showcasing groundbreaking precision in microfabrication.

Initially a modest operation, Sutter's growth was steady. By 1982, the company hired its first full-time employee and expanded from a garage operation into a larger commercial space in San Rafael, California. By 1989, the team had grown to nine employees, prompting the purchase of a 20,000-square-foot facility in Novato, California. As the company continued to grow, Sutter moved into increasingly larger facilities, ultimately settling into a 55,000-square-foot space to accommodate its expanding product lines and workforce.

Over the years, Sutter's portfolio diversified, including amplifiers, microscopes, micromanipulators, and imaging systems. The company also developed expertise in motor control and precision CNC machining, allowing for the production of high-quality optical and motion control products. Today, Sutter's on-site manufacturing facility is one of the finest precision machining operations in Northern California, combining state-of-the-art CNC technology with its decades of engineering expertise.

In 2008, Dale sold 30% of the company's stock to an Employee Stock Ownership Plan (ESOP), reflecting his commitment to the team that helped drive Sutter's success. The company is now partially employee-owned and employs over 70 staff, many with neuroscience and engineering backgrounds. Despite its growth from a garage-based operation to a leading precision instrument manufacturer, Sutter remains true to its roots, with Dale Flaming still actively leading the company as CEO.

Sutter Instrument Company continues to innovate and support researchers worldwide with precision tools for advancing scientific discovery.

TABLE OF CONTENTS

Introduction

The Sutter Instrument Pipette Cookbook is organized according to application and describes the required pipettes needed for that application. While I have attempted to cover a wide range of applications and types of pipettes, it is possible I have yet to include what you might be looking for. Additional applications can be added to new revisions of the cookbook, so 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, I provide the "ideal" combination of capillary glass, heating filament, and recommended parameter for your specific application. I 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, I have also provided information on the XenoWorks microinjection equipment recommended for these applications, the set-up of the microinjection rig, and the settings on the XenoWorks BRE Digital Injector that are recommended for that application.

The "ideal" filament and glass combination, and the associated recommended parameter settings in each Chapter have been established over the past 25 years, and are a result of extensive research, publications, and customer feedback. If you are unable to match the filament and glass combination that is provided for a specific application, or you are lacking the "recommended ingredients," please refer to Chapter 31, "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 size of glass (outer and inner diameters). Programs are listed in the Tables as Type A, B, C, D, & E, and each "Type" classification is explained at the beginning of that section.

It is important to keep in mind that what is provided in the General Look Up Tables might not be perfect for your application. So gradually adjusting the parameter settings to modify the taper length and tip size of the resulting pipette will most likely be required. Please refer to the "Cheat Sheet" on page 11.

The most common sources of difficulty in producing the right morphology of pipette can be attributed to the use of poor parameter settings. Just one "bad setting" in the program, like a parameter setting that is too high or too low, can lead to variability in tip size, taper length and resistance. It is possible that the not-so-ideal parameter settings were established in a somewhat haphazard manner by various well-intentioned researchers who are no longer in the lab. It is also conceivable that the program one is using was adopted from someone who had a different model of Sutter puller and/or a different combination of filament and glass.

Warning: Not being able to pull the proper pipettes can lead to feelings of heartache, insufficiency, self-doubt, disgust, anger, panic and hopelessness. Before succumbing to these feelings and giving up on your research, then submitting your letter of resignation and becoming a barista at your favorite coffee shop, please refer to page 11 to find a "General Guideline for Parameter Settings.". If you stay within the suggested range of values for each parameter while writing a program, it is less likely you will lose your way and become a barista. Disclaimer: Some of my best friends are baristas, so no disrespect to all you amazing baristas out there. There is no research without coffee!

Please be aware that using a program recommended by someone else, even one that is apparently vetted or in a publication, 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 the puller or burn out your filament. Information about the Ramp Test can be found on page 12.

If a program in this Cookbook results in a lot of variability, this might be a result of poor alignments or incorrect mechanical adjustments on your puller. Please refer to pages 13-18. You can also explore the mechanicals and alignments on your puller by watching the Sutter Instrument YouTube Channel webinar "Puller Maintenance & Imaging with Patch Clamp." The discussion on Puller Maintenance begins at minute 7:37 into the webinar. https://www.youtube.com/watch?v=I8nT3CPUoo0

If you find that the program provided and the suggested changes to the parameter settings do not produce the desired results, please refer to page 11, "General Guideline for Parameter Settings" and Chapter 31 discussing "Variability." If you think your puller might need a repair or refurbish, please contact Sutter.

The P-97, P-1000 and P-2000/G pullers featured in the Pipette Cookbook are used to create a wide range of pipette morphologies for various application. Pipette morphologies and Puller Programs for ALL applications provided in the Pipette Cookbook are solely intended for the P-97 and P-1000 Pipette Pullers. The new addition of the P-2000/G laser puller in this 2025 rev. of the Pipette Cookbook will include descriptions of pipettes and programs specifically used for microinjection applications requiring Quartz Glass. For electrophysiology applications, please contact Sutter Instrument.

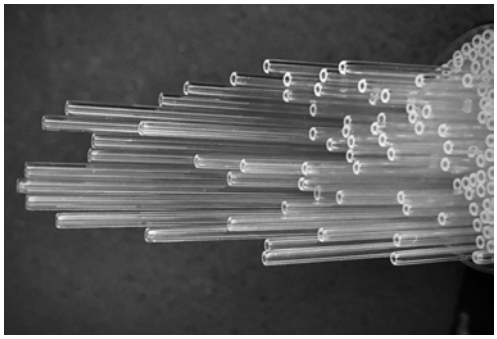
P-97 Pipette Puller

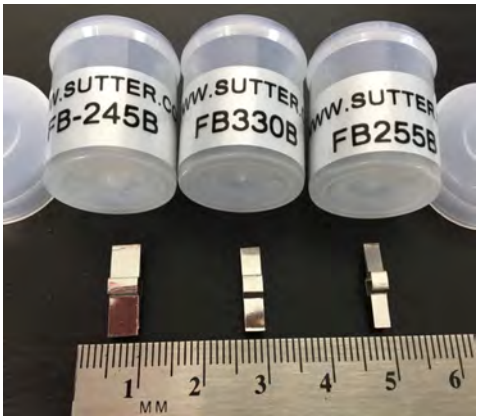
P-1000 Pipette Puller

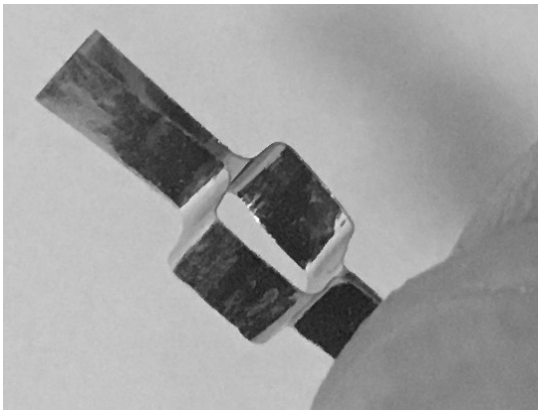
P-2000 Pipette Puller

ALL YOU NEED = THREE INGREDIENTS:

Capillary Glass + Heating Filament + Parameter Settings







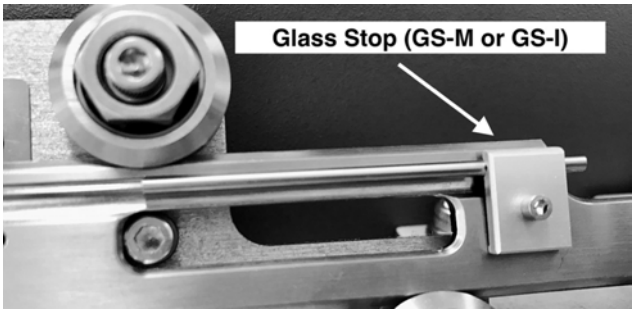
HEAT	PULL	VEL	DEL	PRESSURE
Ramp +10	60-90	80	90	200

And Maybe a Few Puller "Condiments"

Pipette Examining Tiles (PET)

Pipette Storage Box (BX-10)

Ceramic Tiles (CTS)



Glass Stop (GS-M or GS-I)

Fire Polishing Spacer (FPS)

CHAPTER 1 – Ingredients for Making a Pipette

Capillary Glass, Heating Filament, and Parameter Settings

CAPILLARY GLASS - Filamented and Non-Filamented

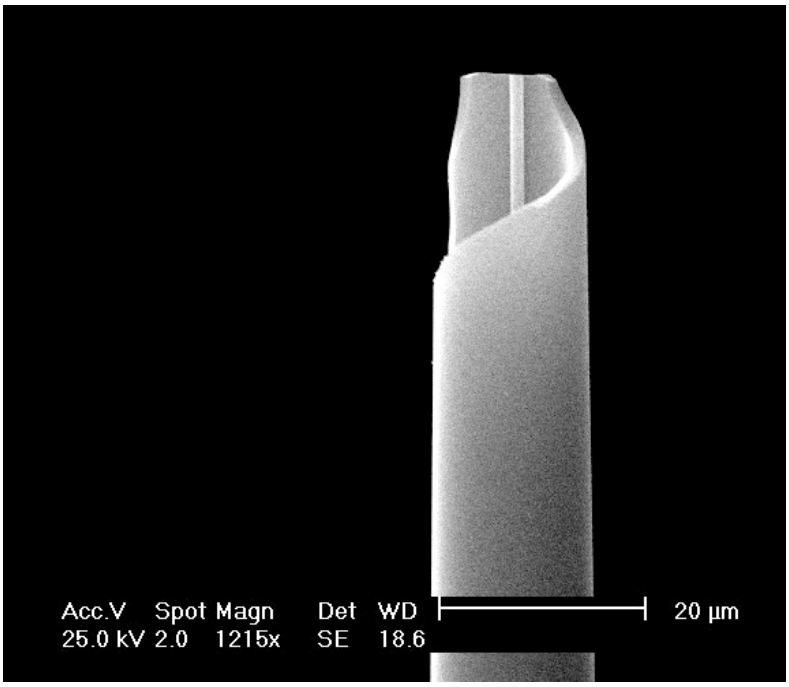
You have a choice of using “filamented” or “non-filamented” capillary glass. The "filament" in the glass refers to a ~120-160µm tiny solid rod of glass that is annealed to the inner wall of the lumen. This filament in the glass acts as a wick, creating capillary action, which allows for the easy back 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, and most researchers prefer to use filamented glass when making patch pipettes that have 1-3µm tips. The filamented in the patch pipette will not interfere with establishing a gigaohm seal and will help reduce the development of air bubbles in the pipette when it is being filled.

As your capillary glass is heated and pulled to create a micropipette, the inner diameter of the glass and the inner 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 less than 100 nanometers at the tip.

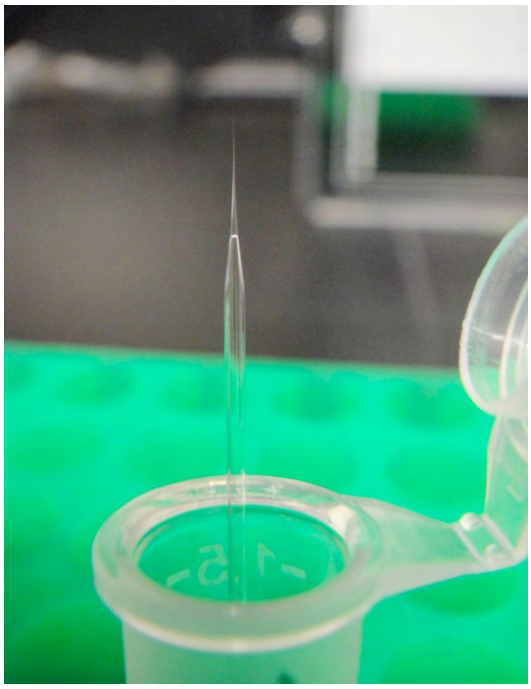
When backfilling a patch pipette, the tip and most of the taper will fill within 30-60 seconds. When backfilling a sharp and long microelectrode or a microinjection pipette, the tip and most of the taper will fill within 2-3 minutes.

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.



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 CRISPR injection pipette via capillary action using filamented thin-walled glass.

CAPILLARY GLASS - Borosilicate, Aluminosilicate & Quartz

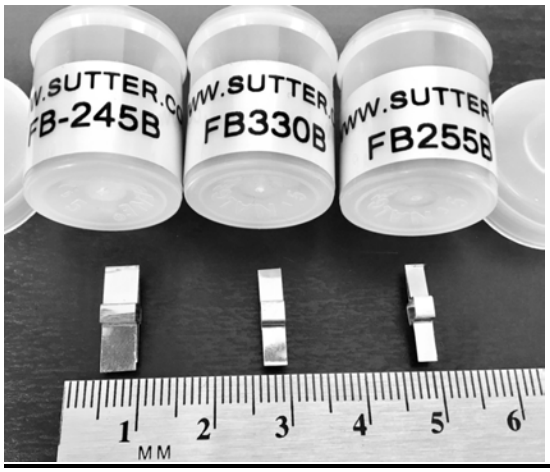
1. **BOROSILICATE**
   Borosilicate glass is most widely and commonly used for making micropipettes. This capillary glass is used for electrophysiology applications including patch clamp, intracellular and extracellular recording. Borosilicate glass is also the preferred glass to make pipettes for mouse pronuclear and cytoplasmic injection, aspiration and transfer, and holding pipettes.

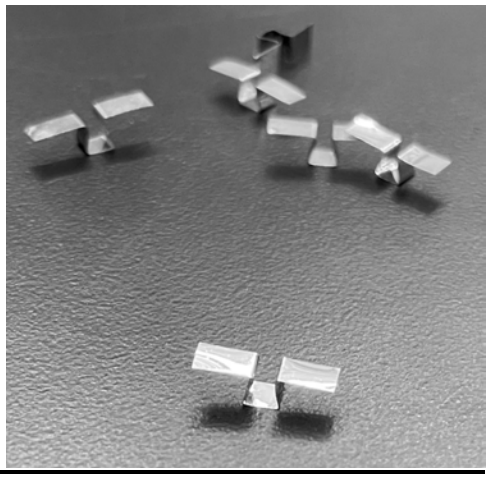
2. **ALUMINOSILICATE & QUARTZ – MICROINJECTION**
   While Borosilicate glass is quite strong and resilient, Aluminosilicate or Quartz glass is recommended for difficult conditions and for applications that require a harder and more durable glass. In many microinjection applications the pipette must penetrate through eggs with fibrous membranes or a hard chorion. Examples where Aluminosilicate or Quartz pipettes are preferred or required include Mosquito, Drosophila, Beetle, Moth, Butterfly Cephalopod, Fish, Ctenophore, and Limulus eggs. Aluminosilicate and Quartz Pipettes are also often required for larvae and adult insect injections. Aluminosilicate and Quartz glass have higher melting temperatures than Borosilicate glass. **The P-97 and P-1000 Pipette Pullers can pull Aluminosilicate glass, but they cannot generate enough heat to pull Quartz glass**. You must have a P-2000/G Sutter Laser Puller to pull Quartz. For more information on Aluminosilicate glass and required program settings, please see Chapter 21. For details on using the P-2000 laser puller and pulling Quartz microinjection pipettes, please refer to Chapters 22, 23 and 24.

3. **QUARTZ – ELECTROPHYSIOLOGY**
   When it comes to electrophysiology and single channel recording, there are some conditions where Quartz glass is required for creating low noise recording electrodes. Quartz glass has a lower dissipation factor and lower dielectric constant than Borosilicate glass, and therefore the patch pipettes pulled from Quartz glass create pipettes with significantly less noise. For more information on making quartz electrodes for low noise recordings, contact Sutter.
   https://ftp.rush.edu/users/molebio/RickLevis/Levis_Publications_CV/Levis_BiophysJ_1993.pdf
   https://www.sutter.com/AMPLIFIERS/amplifier.html

HEATING FILAMENTS – BOX or TROUGH

The “filament” in your puller is the heating element used to melt the glass. The filaments come in two basic shapes, box and trough. Box filaments will heat the glass evenly on all sides, cause the glass to collapse faster and when pulling a sharp electrode, it will pull a slender taper with a more narrow region behind the tip. When pulling a patch pipette, the box filament allows for shorter tapers, higher cone angles and lower resistances. The trough filament can pull longer tapers, but it is not often recommended for patch pipettes or microinjection. Our filaments come in various widths and inner diameters.
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 (see Chapter 2).





PARAMETER SETTINGS

The P-97 and P-1000 puller use the following parameters to heat and pull the glass.

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. You can introduce a lot of variability if the heat setting is not ideal. In addition, if you use a heat setting that is too high or too low, you risk burning out the filament or damaging the puller. Therefore, guessing at the heat setting is not recommended! To determine the proper and safe HEAT value to install, run a RAMP TEST (see Chapter 2).

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 28. 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 as it 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 alternative mode of cooling 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 300ms of cooling is initiated. The Delay value determines how long the glass is cooled before the hard pull is engaged. By cooling the glass while delaying the hard pull, one can determine how viscous the glass is when it is pulled. 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 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 will result in a shorter tapered pipette. The delay mode of cooling is most often used when pulling patch pipettes to deliver a longer duration of cooling to the glass which helps produce shorter tapered patch pipettes.

PRESSURE – This is the pressure of air used to cool the filament and the glass. The default Pressure setting is 500 units is equivalent to 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. Higher Pressure settings will provide greater cooling and shorten the taper. A decrease in Pressure will reduce the cooling and allow for longer and more gradual tapers.

Each application described in the Pipette Cookbook will offer a starting program where the heat, pull, velocity, time/delay and pressure settings are suggested. The manual included with your puller will also have a description of the parameter settings. Please watch the SutterInstrument YouTube Channel Webinar, "How to Make Better Pipettes," https://www.youtube.com/watch?v=_mr_Q4KNEjI which describes the parameters settings in more depth.

General Guideline for Parameter Settings

If you are in the midst of fine-tuning your 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 final morphology of pipette you are aiming for. The chart below shows the recommended range of parameters following: **Patch,** 3-5mm taper and 1-3µm tips, **Microinjection**, 6-9mm taper, 0.9 - 0.5µm tip (before trimming or beveling), **High MΩ**, 9-15mm taper, 0.5 to 0.06µm tip

PIPETTE TYPE	HEAT	PULL	VEL	TIME	DELAY	PRESSURE
PATCH	Ramp	0	18 - 65	200 - 250	1	500
MICROINJECTION	Ramp ±15	30 - 90	50 - 80	175 - 250	60 - 140	200 - 500
HIGH MΩ RECORDING	Ramp +5 to +20	70 - 150	70 – 110	200 - 250	40 - 90	400 - 500

CHEAT SHEET – Guide for adjusting the parameter settings

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

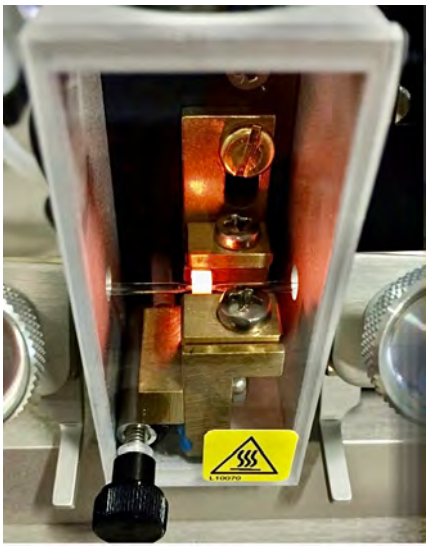
	INCREASE	DECREASE
HEAT		
Δ 5 units	Smaller Tip, Longer Taper, ↑ MΩ	Larger Tip, Shorter Taper, ↓ MΩ
PULL		
Δ 10 units	Smaller Tip, Longer Taper, ↑ MΩ	Larger Tip, Shorter Taper, ↓ MΩ
VELOCITY		
Δ 1 - 3 (patch) Δ 10 (sharps)	Smaller Tip, Longer Taper, ↑ MΩ	Larger Tip, Shorter Taper, ↓ MΩ
TIME (cooling)		
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

CHAPTER 2 - 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. Run a ramp test when using the puller for the first time**,** before writing or editing a program**,** after installing a new filament, or when changing the glass type or glass size.

How to Run a Ramp Test on a P-97

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



How to Run a Ramp Test on a P-1000

- Enter any program number <0-99>
- Touch the area below "RAMP"
- Install glass and press <PULL>

  OR
- Enter the Ramp Value if you have already run the ramp test with the same filament and glass
- Press <ENTER> to record Ramp Value in program

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

1. The current to the filament gradually increases, incrementing the HEAT.
2. Once the HEAT is high enough to soften the glass, the puller bars will drift apart slightly.
3. When the factory-set ramp velocity (trip-point) is reached, the HEAT is turned off.
4. The Ramp Test value will be shown on the display and there will be a slight hour-glass shaped reduction in the middle of the glass. The glass will not separate during a ramp test.

The **Ramp Test Value is the Efficient and Stable Heat Setting to Use for Your Program**. Additionally, the ramp test value is the reference heat for the P-1000 Safe Heat Mode. If the ramp test value is not updated, or the previous ramp value was for a larger or smaller filament, the Safe Heat Mode will not function properly.

Expected ramp test values

Filament #	Filament Dimensions	Expected Ramp Test Values	Maximum Heat
FT330B	3mm x 3mm TROUGH	~ 280 to 320*	Ramp + 25
FB255B	2.5mm x 2.5mm BOX	~ 480 to 540	Ramp + 40
FB330B	3.0mm x 3.0mm BOX	~ 550 to 650	Ramp + 50
FB245B	2.5mm x 4.5mm BOX	~ 750 to 880	Ramp + 100

* **Warning -** If the ramp test value for your trough filament is OVER 300 units, this could indicate that the filament shape is incorrect and inefficiently heating the glass. Please remove your filament and reshape it according to the instructions in Chapter 27.

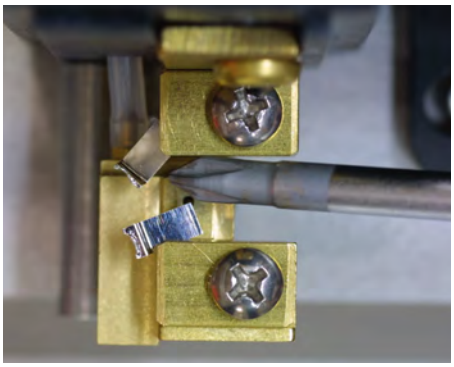
Caution - If your Heat setting is greater than 20 to 50 units above the ramp value, depending on the filament shape and size, you will risk burning out the filament.

CHAPTER 3 - INSTALLING A FILAMENT

Video instructions for installing a filament are available on the
Sutter Instrument YouTube Channel
https://www.youtube.com/watch?v=cCsJsIZlzLw

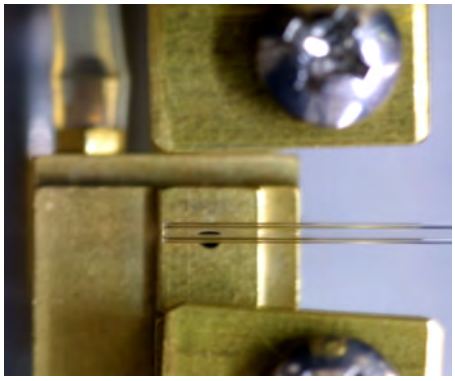
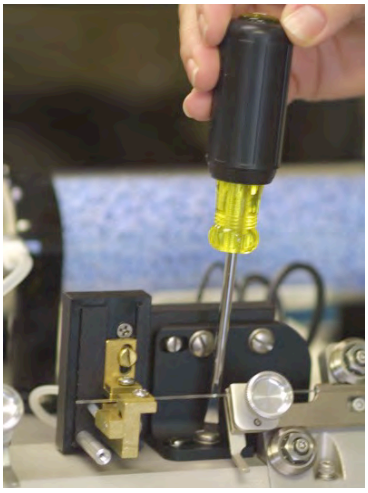
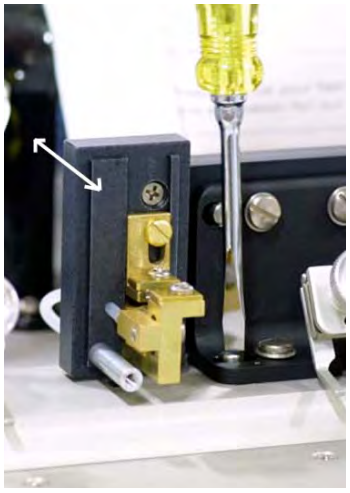
Installing a Filament - Step 1

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

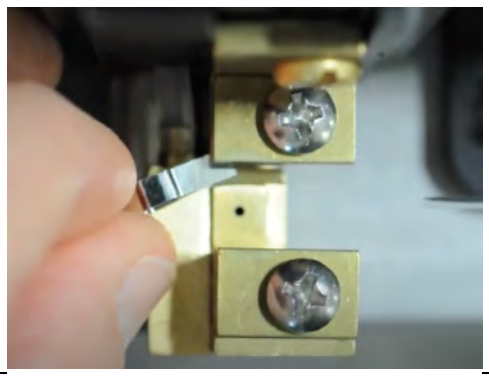
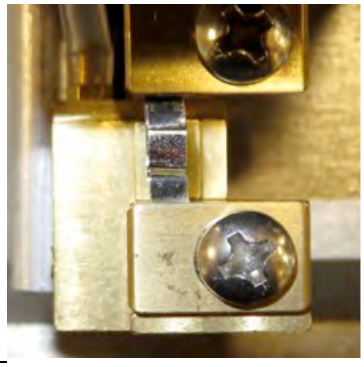
Installing a Filament - Step 2

Install a piece of glass in the right puller bar. Slide the glass to the left so it is positioned over the air jet and clamp the glass in place as shown below. If the glass is not centered over the air jet hole, use the eccentric locking and adjusting screws to place the glass exactly over the air jet.

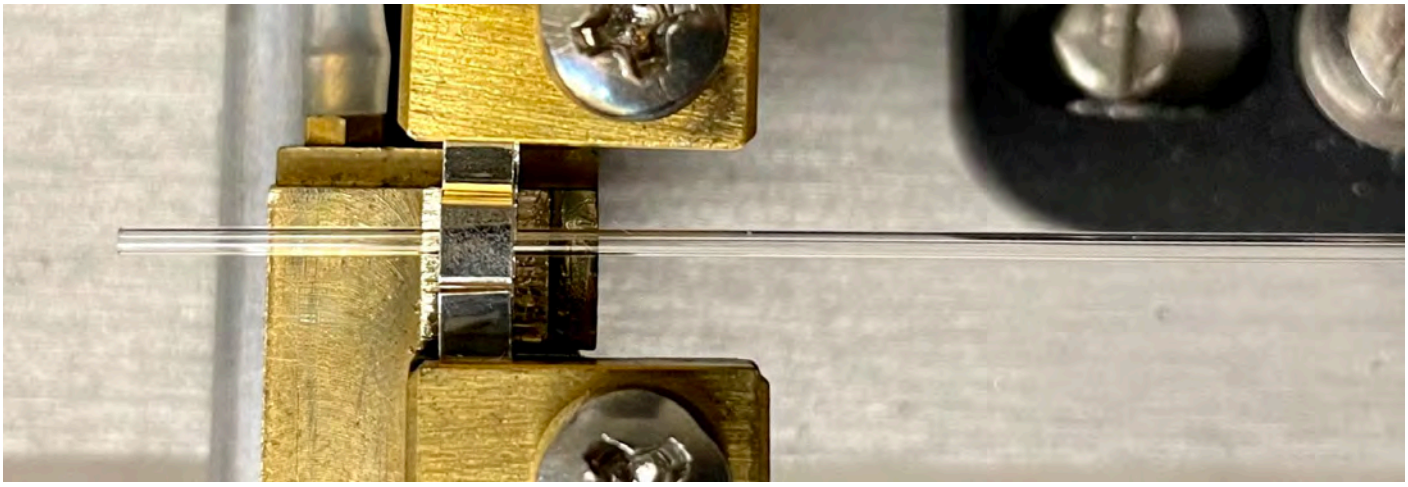
Installing a Filament - Step 3

Before installing a box or trough filament, you might need to correct the shape of the filament. You can use your fingers and tweezers to produce the proper shape. See Chapter 25, “Crazy Lab Lore,” for additional instructions on how to “re-square” your box filament if it is out of shape.

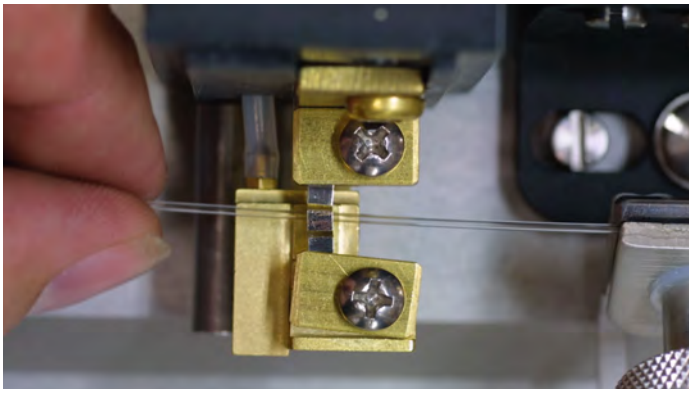
Installing a Filament - Step 4

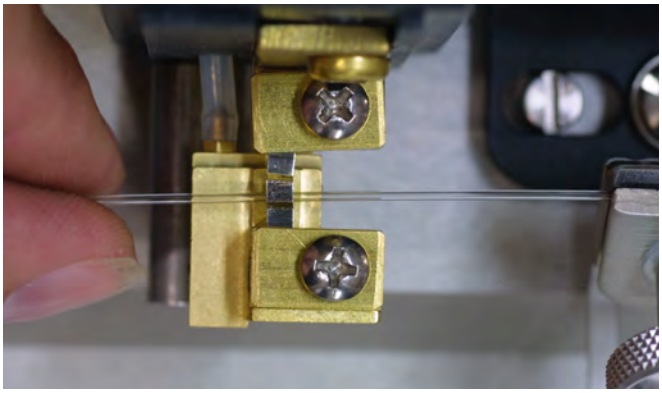
Install the filament so it is centered right to left over the air jet and the glass runs through the center of the filament. You can use your fingers to gently handle the filament to install it. This will not damage the filament and any oils from your hands will burn off when you run a ramp test.

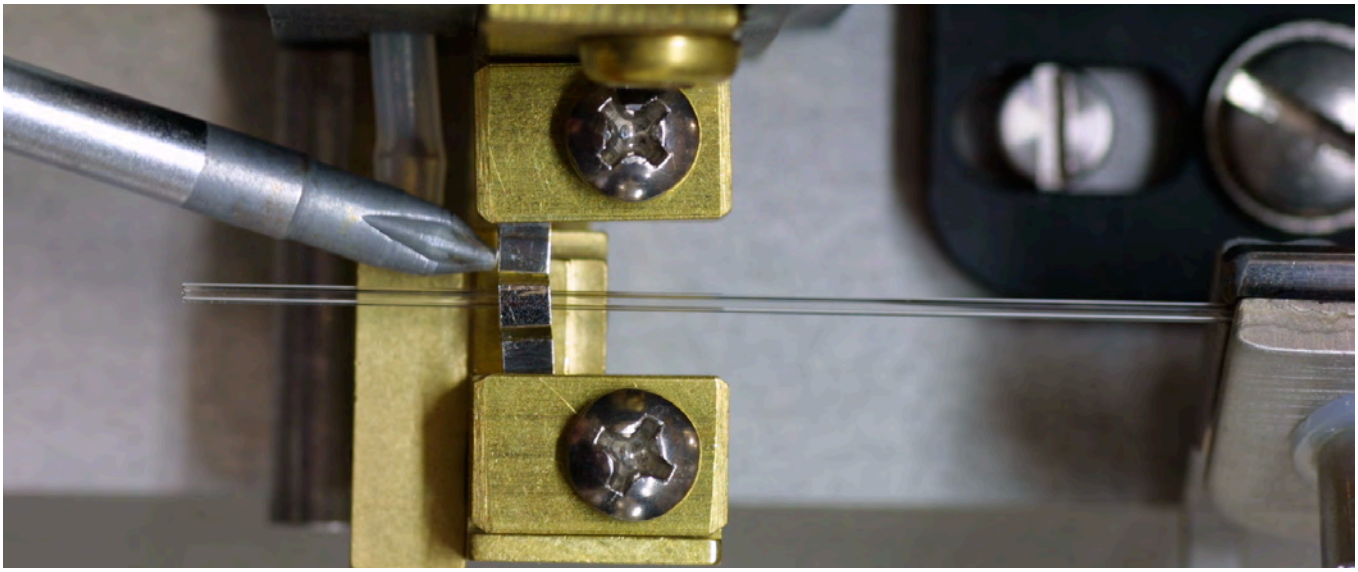


Installing a Filament - Step 5

Using the glass as a tool, hold the left end of the glass and push it front to back to gently position the filament so it is centered around the glass. Then use the tip of a screwdriver or tweezers to nudge the filament right/left to center it over the air jet (VERY IMPORTANT).







Installing a Filament - Step 6

The box filament needs to be installed so it is centered right to left over the air jet

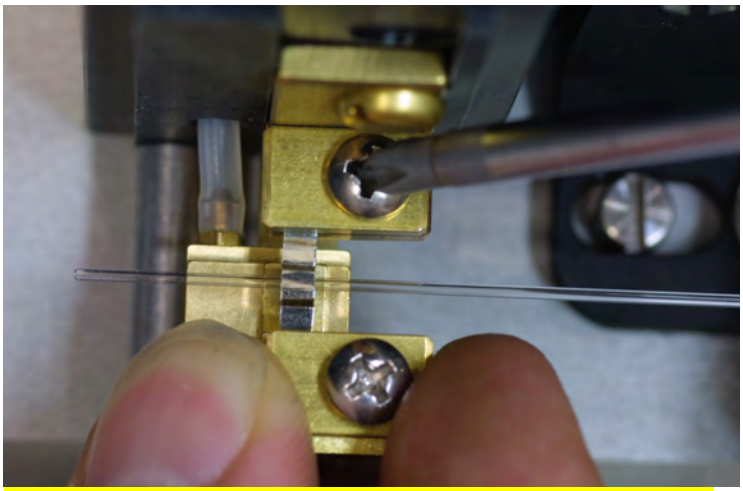
Box filament is not centered over air jet (BAD)

Box filament is centered over the air jet (GOOD)

If the filament is not perfectly centered over the air jet, a newly installed program or previously stable program will no longer be stable. Follow the instructions in Step 9 to ensure the filament is perfectly centered over the air jet.

Installing a Filament - Step 7

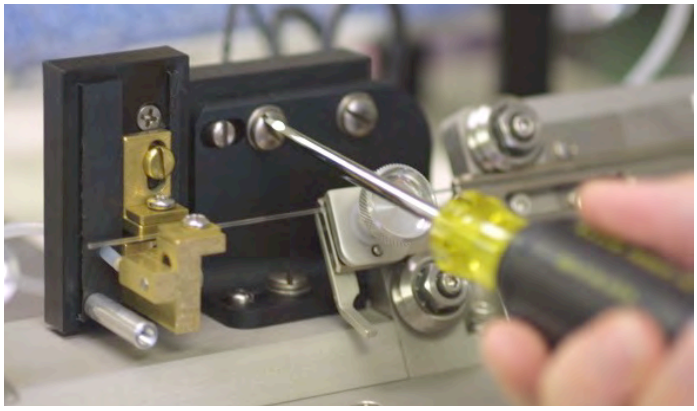
Hold the front filament clamp in place before tightening the rear clamp screw. This prevents the filament from moving when tightening the back screw. Then secure the inside left corner of the front filament clamp (use your fingernail) so the clamp does not pivot and distort the filament when tightening the front screw.


Hold front clamp in place as you tighten rear screw.


Firmly hold the inside left corner of clamp before tightening the screw to prevent clamp from twisting and distorting the filament.

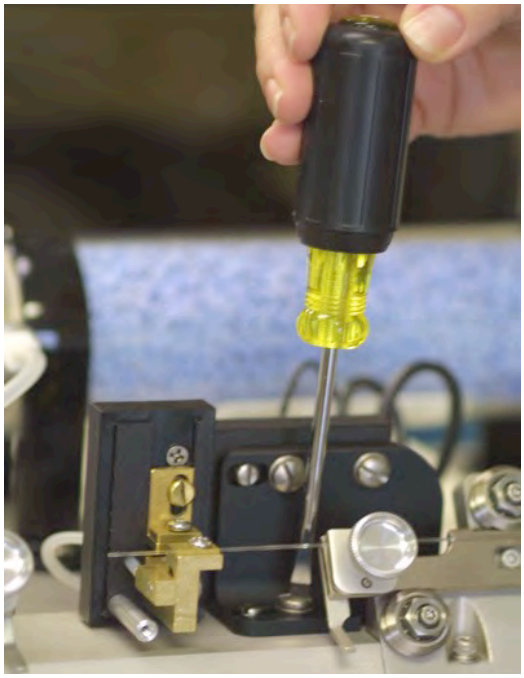
Installing a Filament - Step 8

ECCENTRIC ADJUSTMENTS. - With the glass in place, examine the alignment of the filament to determine if the glass is sitting too high or too low, or if it is sitting too far back or too far forward. For **LARGE** adjustments, reposition the filament. For **SMALL** adjustments, use the Eccentrics.



➔

Loosen top locking screw by a ¼ turn, then turn the top eccentric screw to adjust the **Up and Down alignment** of the filament to the glass, then tighten locking screw.



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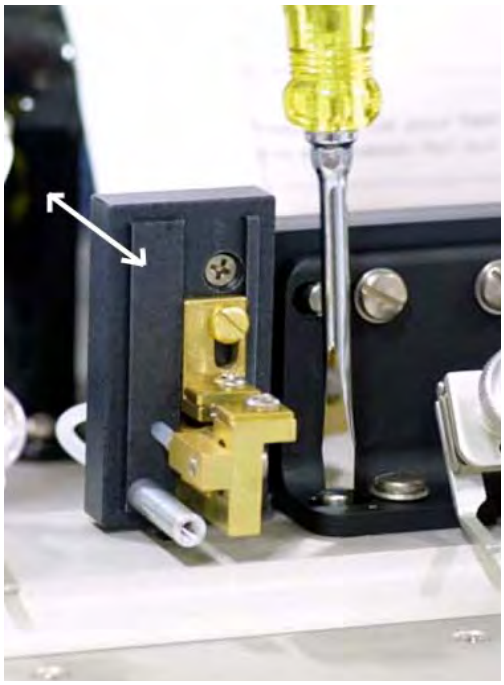
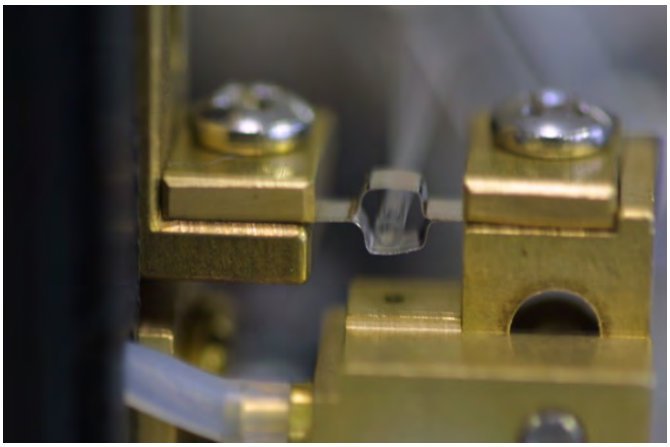


Loosen bottom locking screw, then turn the bottom eccentric to adjust the **Front to Back alignment** of the filament around the glass, then tighten locking screw.



GOOD…glass centered in the filament

Centering Filament Over Air Jet – THE MOST IMPORTANT STEP!

After installing a new filament, it is imperative to pull a pair of long tapered pipettes and compare the right and left tapers to see if they are identical in length. The is the best and only way to properly determine if there is an equal distribution of heat and cooling to the right and left sides of the filament and glass. The goal is to create an 8-12mm long taper (from shoulder to tip) to accentuate the balance of heat and cooling to determine if the right and left sides are "seeing" equal levels.

To prevent your favorite coveted patch programs from becoming unstable, you must check to see if the filament is perfectly centered over the air jet. This is **especially true for patch pipette programs!** If your filament is not properly centered over the air jet (right to left), this will make one pipette taper longer than the other, the right and left pipettes will have different resistances, and it will introduce instability in all patch pipette programs already installed in your puller. And now that barista job is looking more attractive!

Use the following settings to pull a long taper:

HEAT	PULL	VEL	TIME	PRESSURE
Ramp	60	60	250	500

If the pipette does not pull in one stage, add 20 units to the Heat. If the pipette taper is too long, over 10mm and/or is wispy with not distinct tip, reduce the heat by 20 units. Remove the pipettes keeping track of the right and left. Hold them side-by-side, lining up the shoulders of the tapers, and compare them to each other. If the right pipette taper is longer, this indicates that the filament is positioned too far to the right. In this case the right pipette "sees" more heat and less cooling, and the left pipette "sees" less heat and more cooling. To correct this imbalance, loosen the filament clamp screws and **gently nudge the filament 0.1 to 0.5mm in the direction that produced the shorter taper.**

If the **left pipette is shorter**, **shift the filament to the LEFT.** If the **right pipette is shorter**, **shift the filament to the RIGHT.** After making an adjustment, pull another pair of pipettes to check to see if the tapers are the same length. If they are not, continue to adjust the filament right to left until they are identical in length. It is not uncommon to have to adjust the filament several times until it is perfectly centered over the air jet and both pipette tapers are the same length.

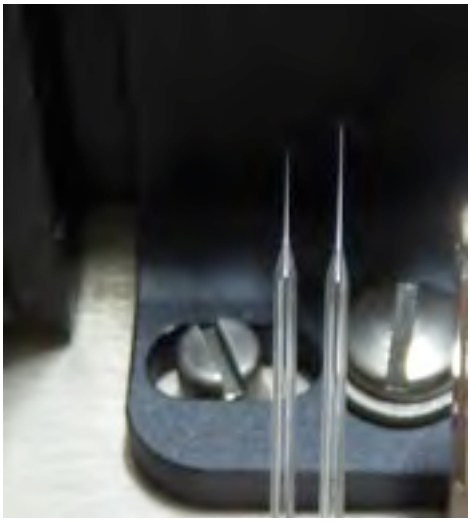
Box filament is not centered over air jet (BAD)

Box filament is centered over the air jet (GOOD)

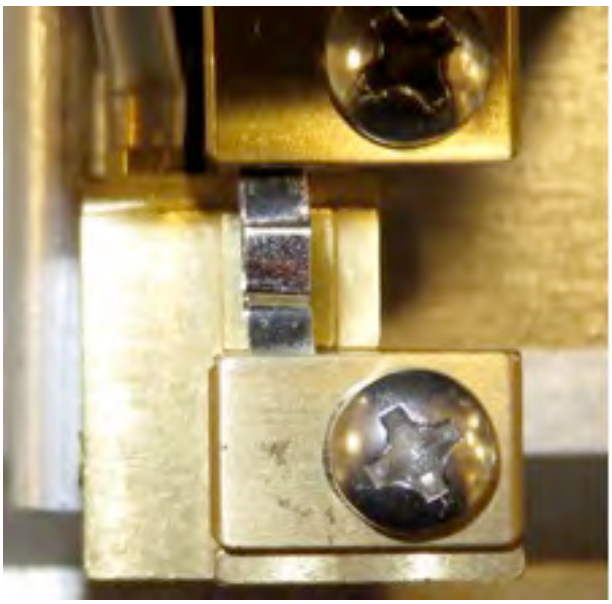


When the filament is installed too far to the left . . . . . the left taper will be longer.
MOVE the filament to the RIGHT.





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 tapers
will be the SAME LENGTH.
This is GOOD!

CHAPTER 4 - ELECTROPHYSIOLOGY

Extracellular & Intracellular Recording

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, 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

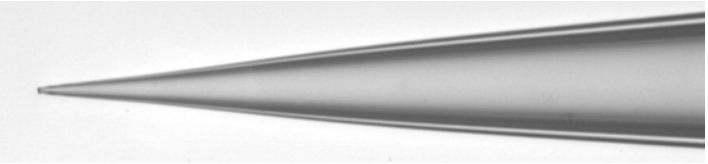
Intracellular Recording measures:

- 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

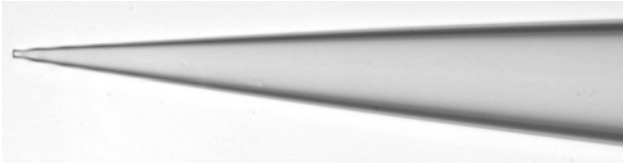
Extracellular Recording

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 1-3μm 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 Microelectrodes



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



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

Extracellular Microelectrodes - Recommended Program

Goal = 1 - 3µm tip ID, 1-10MΩ, 3-5mm Short or Gradual Taper

3.0mm Box (FB330B) Filament, 1.5 x 1.1 (BF150-110-10) Thin-Walled Glass
2.5mm Box (FB255B) Filament, 1.5 x 1.1 (BF150- 110-10) Thin-Walled Glass

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

3.0mm Box (FB330B) Filament, 1.5 x 0.86 (BF150-86-10) Thick-Walled Glass
2.5mm Box (FB255B) Filament, 1.5 x 0.86 (BF150-86-10) Thick-Walled Glass

Heat	Pull	Velocity	Delay	Pressure	Ideal # of Loops
Ramp	0	19-26	1	500	4-5

3.0mm Trough (FT330B) Filament, 1.5 x 1.1 (BF150-110-10) Thin-Walled Glass

Heat	Pull	Velocity	Time	Pressure	Ideal # of Loops
Ramp +10	0	90	150	500	3

3.0mm Trough (FT330B) Filament, 1.5 x 0.86 (BF150-86-10) Thick-Walled Glass

Heat	Pull	Velocity	Time	Pressure	Ideal # of 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 box or trough filament

* *For different filament and glass combinations, please refer to the "General Look Up Tables"*

Intracellular Recording Microelectrode Morphology

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

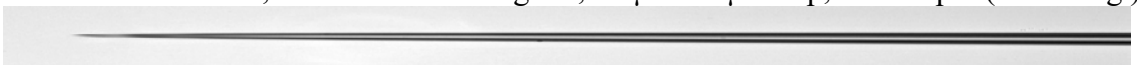
For sharp microelectrodes with 30 to100 MΩ resistances and higher, the tip size and the geometry of the taper are usually the key factors determining if an electrode can successfully impale a cell. Small tips and gradual, uniform tapers have an obvious advantage in terms of causing less damage when a 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 Programs

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 or 3.0mm Box Filament (FB255B or FB330B)

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

Heat	Pull	Velocity	Delay	Pressure
Ramp	65-125	75	90	500

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

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

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

Heat	Pull	Velocity	Delay	Pressure
Ramp	45-90	80	100-200	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**.*

Sutter Amplifier Systems: dPatch & IPA

The dPatch® Digital Patch Amplifier System combines an unmatched sampling rate of up to 5 MHz with noise performance that supports the quietest single-channel recordings and digital architecture for the highest signal fidelity and ultra-stable compensation circuitry. The dPatch represents the most advanced amplifier for electrophysiology on the market today. The IPA® family of Integrated Patch Amplifiers, enables efficient, low-noise whole-cell recordings. The IPA system, available with one (IPA) or two recording channels (Double IPA), combines state-of-the-art amplifier technology with fully integrated D/A and A/D conversion and a high speed USB interface. Acquisition, data management, and streamlined analysis are performed using the bundled SutterPatch® Data Acquisition and Analysis Software, built on the foundation of Igor Pro (WaveMetrics, Inc.). Contact Sutter for more details and to explore our bundled systems for building a complete electrophysiology rig.

https://www.sutter.com/AMPLIFIERS/amplifier.html

https://www.sutter.com/BUNDLES/electrophysiology.html

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.

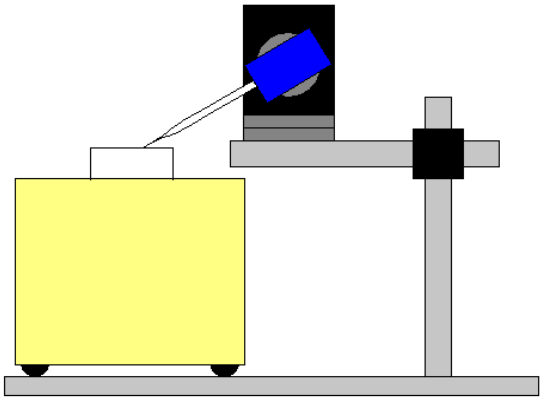
Avoid mounting any source of vibration on the setup. Take care to avoid stiff cables or tubes that can bring vibration into the setup.

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.

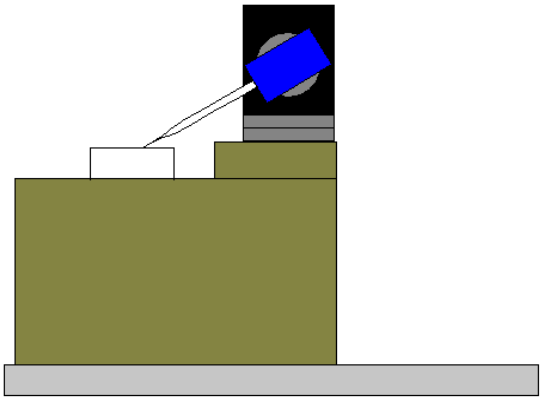
How do you make the connection from the tissue to the microelectrode stiff? Obviously we cannot expect the tissue to be rigid, but it should be constrained so that it does not move around easily. You will need to be especially attentive to this issue if the tissue is located in moving fluid. Each mechanical 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.

Micromanipulators Specifications for Intracellular Recording

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 the 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.

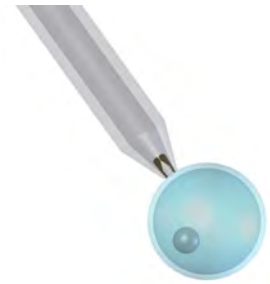
The micromanipulator is of little use without the microelectrode and amplifier. The microelectrode must be mounted close to the head stage of the amplifier so that the electrical connection is as short as possible. It is common practice to mount the headstage to the micromanipulator and then the microelectrode 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.
https://www.sutter.com/MICROMANIPULATION/index.html, https://www.sutter.com/AMPLIFIERS/amplifier.html

* *Thanks to Jack Belgum for his contribution to this section of the Pipette Cookbook. You are missed!* ***https://www.marinij.com/obituaries/jack-henrik-belgum/***

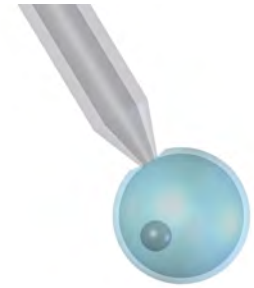
CHAPTER 5 - Patch Pipettes

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).

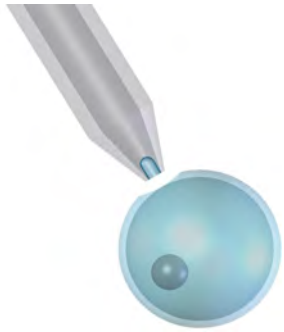
Cell-Attached Patch



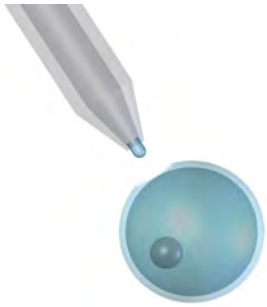
Whole Cell Patch



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.

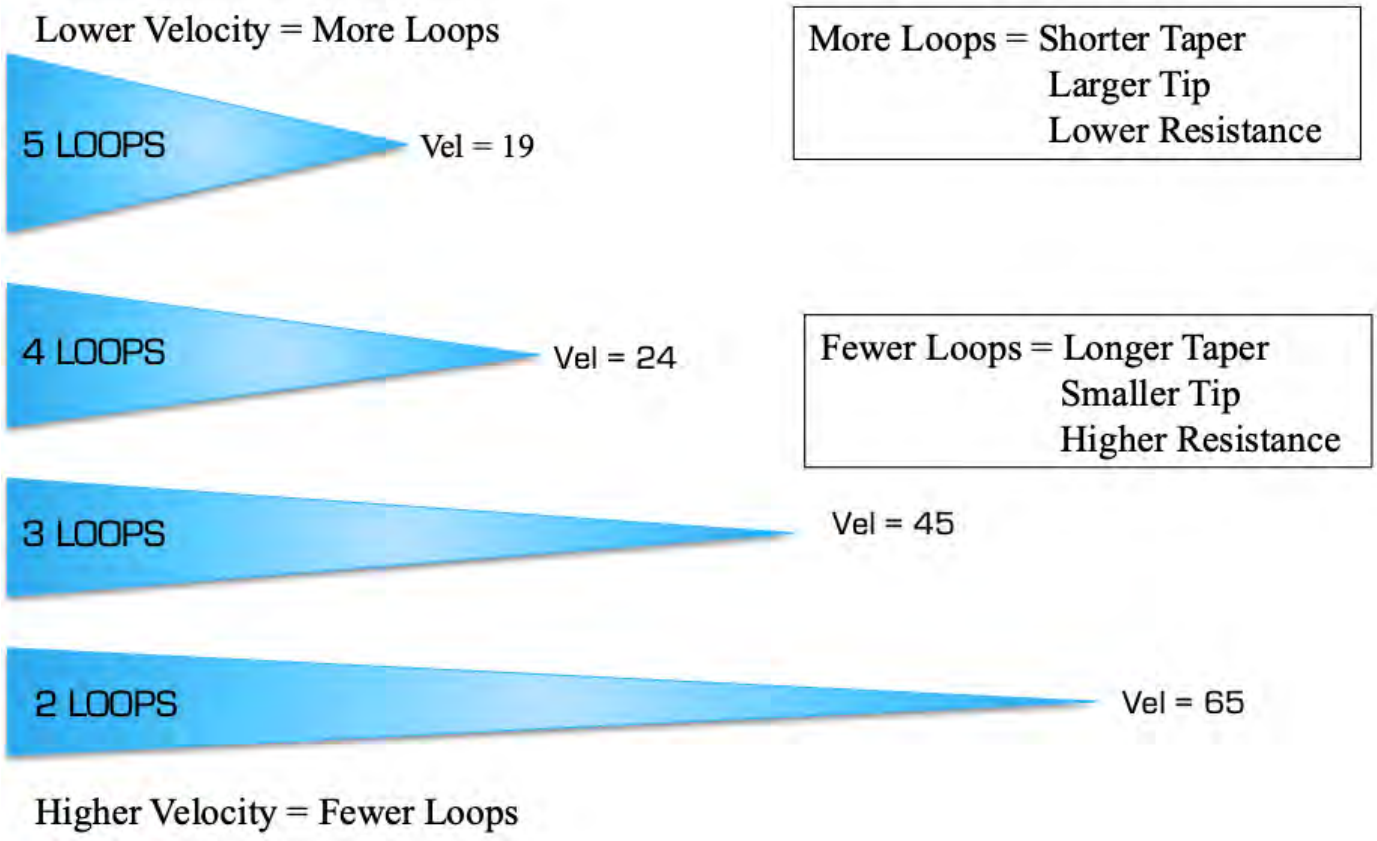
Looping

The programs provided for making patch pipettes traditionally start off as one-line programs where the puller will pull the glass in multiple stages (heating cycles) 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 the first heating cycle, and there are no values on line two, the puller will return back to line one, also known as "LOOPING", and read this first and only line over and over again until the glass separates.

The greater number of loops = shorter tapers, larger tips, and lower resistances.
The fewer number of loops = longer tapers, smaller tips, and higher resistances.

Whole cell patch applications 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 1.5mm x 0.86mm 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 (pg. 30).

Those working within a slice preparation often require and 1-2µm tip, a longer and more gradual 4-5mm taper, and a resistance between 3-6MΩ. In this case it is best to use **thin-walled glass 1.5mm x 1.10mm glass, a 2.5mm or 3.0mm box filament (FB255B, FB330B) and a program that allows 2-3 LOOPS** Thin-walled glass has a larger ID than thick-walled glass so when the glass is pulled to have a longer taper, which is most often required for slice recording, the larger lumen allows the glass tip to remain more open. You will want to aim for 2-3 loops to achieve this more gradual taper (pg. 31).



Throwback Thursday Images

Repairing a Puller in a Customer's Kitchen

Repairing a Puller at the Hair Salon

GEMMA, Marine Biological Labs, Woods Hole, MA

MBL 1984 Microbiology Course

Writing a Stable Patch Program: Mid-Point Velocity = Stable Velocity

To make a patch program with consistent results, you must find the mid-point velocity which is the recommended stable velocity to use in your program. To do this you must find the entire range of velocity settings that loop the number of times recommended the type of patch pipette specified below. After pulling a series of pipettes using the mid-point velocity and you find the resistance too high or too low, see pages 32 and 35 to tweak the settings and fine tune your patch program.

WHOLE CELL RECORDING (1 - 5MΩ) THICK WALLED GLASS

Use 1.5 x .86 glass and a 2.5 x 2.5 box filament, then install the following settings:

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

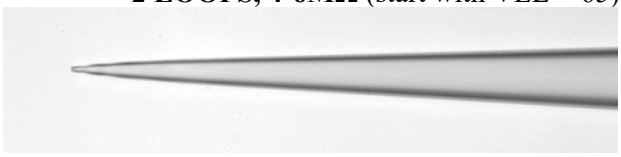
Using the above **one-line program**, change the velocity in ONE UNIT increments and pull a pipette at each value to find the entire range of velocity values which allows **5 loops**. Increase the velocity until the puller loops 4 times, then decrease the velocity until the puller loops 6 times. Do not look at the tips or measure the resistance of these pipettes. Resist the urge! Just pull and toss! You are only interested in finding the velocity vs looping results! Then use the mid-point velocity for 5 loops for your program.

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 (2 - 6MΩ) THIN WALLED GLASS

2 LOOPS, 4-6MΩ (start with VEL = 65)



3 LOOPS, 2-4MΩ (start with VEL = 45)

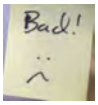
Use 1.5 x 1.1 glass and a 2.5 x 2.5 box filament, then install the following settings:

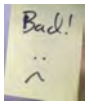
Heat	Pull	Velocity	Time	Pressure	Ideal # of Loops
Ramp	0.	**45 - 65**	250	500	**2 – 3 times**

Using the above **one-line program**, change the velocity in THREE UNIT increments and pull a pipette at each value to find the entire range of velocity values which allows **2 or 3 loops** (depending on your desired resistance). For 2 loops, increase the velocity until the puller loops 1 time, then decrease the velocity until the puller loops 3 times. Do not look at the tips or measure the resistance of these pipettes. Resist the urge! Just pull and toss! You are only interested in finding the velocity vs looping results.

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

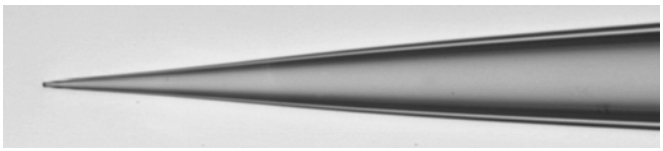
Threshold **(Mid-Point Velocity)** **Threshold**





Then use the mid-point velocity for 2 loops for your program...
or the mid-point for 3 loops depending on the desired resistance.

SUPERFICIAL SLICE RECORDING (5 - 10MΩ) THICK WALLED GLASS



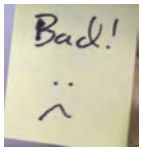
Use 1.5 x .86 glass and a 2.5 x 2.5 box filament, then install the following settings:

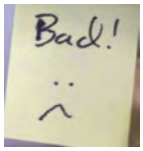
Heat	Pull	Velocity	Delay	Pressure	Loops
525	0	**26**	1	500	**4**

Using the above **one-line program**, change the velocity in TWO UNIT increments and pull a pipette at each value to find the entire range of velocity values which allows for **4 loops**. To find the entire range, increase the velocity in 2 unit increments until the puller loops three times and decrease the velocity until the puller loops five times. Do not look at the tips or measure the resistance of these pipettes. Just pull and toss! You are only interested in finding the velocity vs looping results.

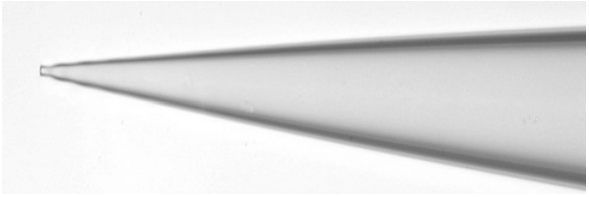
Velocity	**21**	**23**	**25**	**27**	**29**	**30**	**32**	**34**	36
Loops	**5**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	3

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





LARGE PATCH PIPETTE (3-5µm ID, 1-2MΩ) Whole Cell or Slice Recording



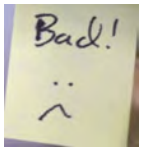
Use 1.65 x 1.20 or 2.0 x 1.56 glass and a 2.5 x 2.5 box filament, then install the following settings:

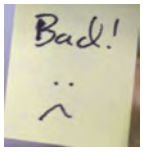
Heat	Pull	Velocity	Time	Pressure	Ideal # of Loops
Ramp	**0**	**45**	**250**	**500**	**3 - 4 times**

Using the above **one-line program,** change the velocity in THREE UNIT increments and pull a pipette at each value to find the entire range of velocity values which allows for **3 loops**. To find the entire range, increase the velocity in three-unit increments until the puller loops 3 times and decrease the velocity until the puller loops 5 times. Do not look at the tips or measure the resistance of these pipettes. Resist the urge! Just pull and toss! You are only interested in finding the velocity vs looping results.

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**





CHAPTER 6 - Fine Tuning the Patch Program

THICK WALLED WHOLE CELL PATCH & THIN WALLED SLICE RECORDING

The main goal here is to adjust the parameters to create a small change in tip resistance without changing the overall taper length. This can be a challenge since a shift in any parameter normally has an effect on both the taper length and tips size. To edit your program and isolate the change in resistance to changes in the tip size and not the taper length, it is best to **change the parameters on the last line of programming when the tip is forming.** So, if you have a one-line program that loops 5 times, and want a slightly higher or lower resistance, you will need to **write out the program into identical multiple lines, and then change the parameters on the last line.** There are some conditions where the velocity on the second to last line is increased or decreased, and these approaches are described below.

A. THICK WALLED WHOLE CELL PATCH PIPETTES – FINE TUNING

1. HIGHER RESISTANCE - SMALLER TIP but NOT A LONGER TAPER (4-6MΩ)

Increase the heat, pull and/or velocity on the last line when the tip is forming. Write out your program into five identical lines based on your one-line five-looping program you previously established using the mid-point velocity. **Then on the last line only**, increase the heat by 5-20 units, add a slight amount of pull, and/or increase the velocity by 1-4 units. As good researchers, we are to follow the laws of science by **changing one variable at a time**! This will also help avoid electrode chaos.

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	**19**	1	500
5	**≥ 530**	**+20 to 40**	**21 to 25**	1	500

For an even greater increase in the resistance (we are having fun now!), reduce the velocity on the 4th line by 2-3 units which will "save" some additional glass for line 5. "Saving" glass on second to last line of programming increases the volume of glass available for the final line when the tip is forming.

NOTE: If there is too great an increase in the heat or velocity, the glass will separate on Line 4 and will not read Line 5 to complete the pulling of the glass. If this happens, you will not get the desired results. Make finer adjustments in the heat and/or velocity to avoid this behavior.

2. HIGHER RESISTANCE - SMALLER TIP & LONGER TAPER (5-10MΩ)

Using your one-line program that loops 5 times, do not write out the program into multiple lines, just increase the velocity 5 units to allow the puller to loop one less time. So you will aim for 4 loops instead of 5 loops.

Heat	Pull	Velocity	Delay	Pressure	Loops
525	0	21➞ **25**	**1**	500	**4** (instead of 5)

Find the full range of velocities that allows 4 loops (instead of 5) by increasing and decreasing the velocity in three unit increments to reestablish a new mid-point velocity for 4 loops. This will create a slightly longer taper, a 1-1.5µm tip, and a higher resistance (5-10MΩ). Refer to the top of pg. 31 to review the mid-point velocity instructions.

3. LOWER RESISTANCE - LARGER TIP but NOT A SHORTER TAPER (1-2MΩ)

Decrease the heat and/or velocity on the last line when the tip is forming. Write out your program into five identical lines based on your one-line, five-looping program you previously established using the mid-point velocity. Then **on the least line only**, decrease the heat by 5-20 units and/or decrease the velocity by 1-3 units. As good researchers, we are to follow the laws of science by **changing one variable at a time**! This will also help avoid electrode chaos.

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	**23**	1	500
5	**≤ 520**	0	**19 to 20**	1	500

For an even greater decrease in the resistance, increase the velocity on the 4th line by three units which will "use up" some of the glass before line 5. "Using up" glass on second to last line of programming, increases the volume of glass available for the final line when the tip is forming.

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, you will not get the desired results. Make finer adjustments in the heat and/or velocity to avoid this behavior.

4. LOWER RESISTANCE - LARGER TIP & SHORTER TAPER (0.5-1.5MΩ)

Using your one-line program that loops 5 times, do not write out the program into multiple lines, just decrease the velocity in 1 unit increments to allow the puller to loop one more time. You will aim for 6 loops instead of 5.

Major Disclaimer: A program that loops 6 times using thick-walled glass is **at the threshold of being a stable program**. The glass volume is reduced with each loop and often there is not enough glass for the final heating cycle to form a good tip. As a result, the tip can become too fragile when it separates, creating a slightly angle tip or a crackled and splintered end. This 6-looping approach can sometimes work better with a 3mm box filament.

Heat	Pull	Velocity	Delay	Pressure	Loops
5250	21 ➞	**18**	1	500	**6** (instead of 5)

Find the full range of velocities that allows 6 loops (instead of 5) by decreasing the velocity in one unit increments to reestablish a new mid-point velocity for 6 loops. This will create a slightly shorter taper, a 2.5-3µm tip, and a lower resistance (0.5-1.5MΩ).

NOTE: You will most likely find that the range of velocity values that allows 6 loops is a narrow range of just 2 units. Therefore, there is no mid-point velocity. See top of pg. 30 for an example. As a result, you will need to test each value to see which might be most stable. This program will also be more vulnerable to the heat retention in the filament jaws, so as the jaws and temperature inside the humidity control chamber get warmer, the program will have the tendency to pull in 5 stages instead of 6. And looping 6 times instead of 5 will also cause the brass jaws to reach and retain higher temperatures. One way to possibly counter this, is to increase the "Air Time Before Pull" to 10-20 seconds to cool down the jaws and purge the warm air from inside the chamber. Another option might be to remove the humidity control chamber so there is less retention of heat. The temperature sensor and high heat warning on the P-1000 will help alert you if the jaws become too hot. If pulling with 6 or more heating cycles becomes a standard approach to pulling your electrodes, a high heat-resistant version of the filament block assembly is available. Contact Sutter Instrument for more details.

B. THIN WALLED SLICE PATCH PIPETTES – FINE TUNING

1. HIGHER RESISTANCE - SMALLER TIP but NOT A LONGER TAPER (4-6MΩ)

Increase the heat and/or velocity on the last line when the tip is forming. Write out your program into 2 identical lines based on your one-line two-looping program you previously established using the mid-point velocity. Then **on the last line only**, increase the heat by 10-20 units and/or increase the velocity by 10-20 units. As good researchers, we are to follow the laws of science by **changing one variable at a time**! This will also help avoid electrode chaos.

Line	Heat	Pull	Velocity	Time	Pressure
1	525	0	65	250	500
2	**≥ 535**	0	**75 to 85**	250	500

For a one-line program that loops 3 times, follow the same approach by writing the program out into three identical lines. Then **on the last line only,** increase the heat by 5-15 units and/or the velocity by 5-15 units.

Line	Heat	Pull	Velocity	Time	Pressure
1	525	0	45	250	500
2	525	0	45	250	500
3	**≥ 535**	0	**50 to 60**	250	500

2. HIGHER RESISTANCE - SMALLER TIP & LONGER TAPER (5-6MΩ)

Using your one-line program that loops 3 times, do not write out the program into multiple times, just increase the velocity 20 units to allow the puller to loop one less time. So you will aim for 2 loops instead of 3 loops.

Heat	Pull	Velocity	Delay	Pressure	Loops
525	0	45 → **65**	**1**	500	**2** (instead of 3)

Find the full range of velocities that allows 2 loops (instead of 3) by increasing and decreasing the velocity in 3 unit increments to reestablish a new mid-point velocity for 4 loops. This will create a slightly longer taper, a 1-1.5µm tip, and a higher resistance (5-10MΩ). Refer to the top of pg. 31 to review the mid-point velocity instructions.

3. LOWER RESISTANCE - LARGER TIP but NOT A SHORTER TAPER (1-2MΩ)

Decrease the heat and/or velocity on the last line when the tip is forming. Write out your program into two identical lines based on your one-line two-looping program you previously established using the mid-point velocity. Then **on the last line only**, decrease the heat by 10-20 units and/or decrease the velocity by 5-10 units. And good researchers, we are to follow the laws of science by **changing one variable at a time**! This will also help avoid electrode chaos.

Line	Heat	Pull	Velocity	Time	Pressure
1	525	0	65	250	500
2	**≤ 515**	0	**50 to 60**	250	500

For a one-line three-looping program, write out your program into three identical lines and **on the last line only**, decrease the heat on line 3 by 5-15 units and/or decrease the velocity by 5-10 units.

Line	Heat	Pull	Velocity	Time	Pressure
1	525	0	45	250	500
2	525	0	45	250	500
3	**≤ 515**	0	**35 to 40**	250	500

4. LOWER RESISTANCE - LARGER TIP & SHORTER TAPER (1-2MΩ)

Using your one-line program that loops 3 times, do not write out the program into multiple times, just increase the velocity 20 units to allow the puller to loop one less time. So you will aim for 2 loops instead of 3 loops.

Heat	Pull	Velocity	Delay	Pressure	Loops
525	0	65 ➞ **45**	**1**	500	**3** (instead of 2)

NOTE: If the heat or velocity 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, you will not get the desired results. Do not make such a large reduction in the heat and/or velocity to avoid this behavior. Refer to the top of pg. 31 to review the mid-point velocity instructions.

ANOTHER NOTE: The P-97 and P-1000 Pullers have a Fire-Polishing Mode. You can use the Fire-Polishing Spacer (FPS) to provide a slightly thicker glass tip. This will increase the surface area at the tip and help make a better giga seal. This is often employed when patching onto difficult cells, small (5-15µm) cells and mitochondria. See "Achieving the Impossible" Sutter Instrument Webinar (@ 26min onward) for details. **https://www.youtube.com/watch?v=gC4Ktmb6ndk**

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
- 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).
- "Single Channel Recording" 2nd edition 1995 by Sakmann & Neher

Advanced Research Training Courses for Electrophysiology and Other Research Applications

Marine Biological Lab (MBL), Woods Hole, MA

Advanced Research Training Courses
https://www.mbl.edu/education/advanced-research-training-courses

GRASS Foundation Fellowships
https://www.mbl.edu/research/funding-opportunities/grass-foundation

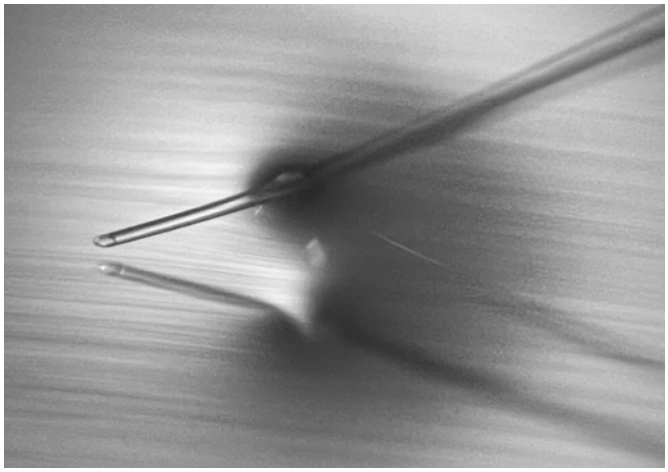
Whitman Center Fellowships
https://www.mbl.edu/research/funding-opportunities/whitman-center-fellowships

MBL Research Facilities
https://www.mbl.edu/research/resources-research-facilities

Cold Spring Harbor Lab, Cold Spring Harbor, NY

https://meetings.cshl.edu/courses

CHAPTER 7 – Beveling Pipettes



The BV-10 Beveler is an elegant and simple device for precision beveling of pipettes to achieve tips between 0.1 and 175µm. 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 greatly benefit from beveling as this creates a sharp clean cutting edge that promotes entry into cells and eggs with minimal damage. Beveling is often required for injections into eggs that have a fibrous membrane or hard chorion. Not only does this create an injection pipette that can last 100-500 consecutive injections (depending on the material of the glass), but it also simultaneously enhances the flow of materials through the needle. The pipettes used for the majority of microinjections applications described in the Pipette Cookbook often benefit from, or absolutely require, a beveled pipette. Examples include Mosquito, Medaka, Cephalopod, Squid, Shrimp, Beetle, Ctenophore, Starfish, Limulus, IVF, Nuclear Transfer, ES Cell injections to name a few! Beveled pipettes are also preferred for Larval injections, Adult Insect injections, Tardigrades, and Brain &
Spinal Cord injections.

Using the BV-10 Beveler with the 104D fine diamond abrasive plate (most often used to make tips between 0.5µm to 25µm) and beveling times between 5 and 60 seconds, will produce a sharp bevel pipette that will change your life. :-) Okay, maybe I'm overselling it, but the resulting pipettes will most definitely change what is possible for your protocol and will increase the yield of survival. And it will also reduce the incidence of clogged tips. Bevel angles can be established between 0-90 degrees, but most researchers prefer tips that are beveled between 18-45 degrees, where 25-30 degrees is most common. Bevel angles lower than 15 degrees become difficult to achieve due to the flexibility of the glass taper and insufficient pressure of the tip on the beveling surface. Angles over 50 degrees can cause the pipette tip to chip and fracture due to the high angled approach and less control of the higher pressure of the glass tip on the beveling surface.

Please watch the SutterInstrument YouTube Channel Webinar: "How to Make a Beveled Pipette"
to see how to assemble and set up the beveler, and how to bevel glass pipettes.
https://www.youtube.com/watch?v=aOFv4fDQ9FY

Beveling Plates

To bevel a pipette, you will need to select the beveling plate best suited for the tip size you want to create. There are four types to choose from, ranging from a course to extra fine diamond granule surfaces.

- 104C (Copper) = Coarse for 25 - 150µm tips
- 104D (Lavender) = Fine for 1- 25µm tips
- 104E (Gray) = Very Fine for 0.5 - 2µm tips
- 104F (Sage) = Extra Fine for <0.5µm tips

Glass Types:

- **Borosilicate –** Mouse Pronuclear, Cytoplasmic, CRISPR Injection, ICSI, Nuclear Transfer, ES Cell Injection, Aspiration, Transfer, Canulations, Brain & Spinal Cord Injections, Zebrafish, Xenopus, *C.elegans*, and mRNA & DNA injection into Mammalian Embryos

- **Aluminosilicate** – Mosquito, Drosophila, Medaka, Fly, Moth, Butterfly, Zebrafish

- **Quartz** – Mosquito (Aedes Aegypti, Albopictus, Culex, Anopheles, etc.), Fly (Drosophila, Stomoxys, Screw Worm, etc.) Moth, Butterfly, Beetle, Cricket, Tick, Sea Start, Cephalopod, Squid, Shrimp, Limulus Fish (Medaka, Salmon, Trout, etc.)

The Advantage of Aluminosilicate Glass is that it is 10X stronger than Borosilicate Glass and it can be pulled on the P-97 and P-1000 Sutter Pipette Pullers. If you do not have access to a P-2000/G laser puller and are therefore unable to use Quartz glass, Aluminosilicate is the next best thing. It will work for all the injection applications listed above for Quartz glass, but it is not quite as durable and will not maintain a sharp tip for as long as when using Quartz.

The Beauty of Quartz Glass is that it is very strong and makes for an extremely sharp and durable injection pipette. I have found that it is not necessary to dechorionated (using bleach to partially or completely remove the chorion) when using beveled quartz pipettes. And as a result of leaving the chorion intact, there is increased survival and higher yield of the genetically modified organism. The best Quartz glass to use is QF100-70-10 or QF100-60-10. Of the two, I have found that the thicker walled 1mm x 0.60mm Quartz glass is a bit more durable and can maintain a sharp tip for longer than the thin-walled Quartz. For example, my Aedes injection pipettes on average lasted through 300-500 injections, compared to 300-350 injections when using the thinner walled Quartz glass. Yet, in conditions where you want to minimize the insult to the membrane or chorion, it is best to use thin-walled glass where the OD of the glass is smaller. The P-2000/G Laser Puller is required to heat and pull Quartz glass. Information on the P-2000/G laser puller can be found in Chapters 22, 23 and 24.

Duration of Beveling

The duration of beveling is dependent on the tip size being beveled and the glass composition and wall thickness of the glass pipette. Smaller tips take 5-15 seconds, while large tips can take up to 2-5 minutes. Thin-walled glass takes less time to bevel than thick-walled glass. Quartz and Aluminosilicate glass often bevel much faster because these glass types are 10-20x harder than Borosilicate glass. For most difficult injection applications where the tip is penetrating through a fibrous membrane or chorion, Aluminosilicate and Quartz glass are preferred.

TIP SIZE	BEVELING DURATION	BEVELING PLATE
0.2µm – 0.5µm	2 – 4 seconds	104F
0.5µm – 2.0µm	5 – 15 seconds	104D
2µm – 5µm	15 – 30 seconds	104D
5µm – 25µm	30 – 45 seconds	104D
25µm – 50µm	45 – 90 seconds	104C
50µm – 150µm	1 – 2 minutes	104C
*150µm – 200µm	1 – 3 minutes	104C or Custom Plate

*Larger tip sizes cannot be beveled if the glass wall is too thick
125µm – 150µm OD is approximately the limit for thick-walled glass
175µm – 200µm OD is approximately the limit for thin-walled glass

Range of Taper Lengths that can be Beveled:

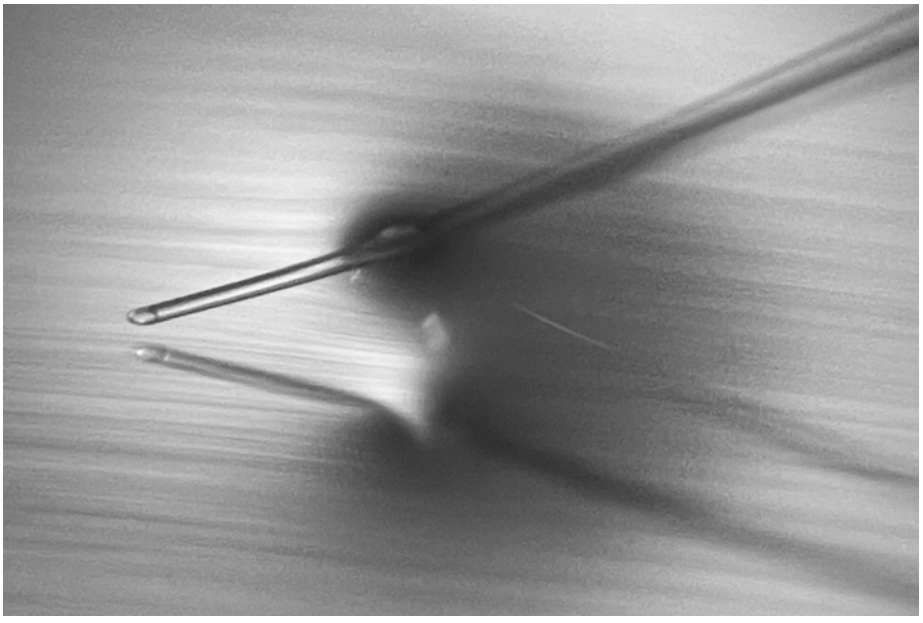
3mm – 4mm Taper = **Doable**, but difficult to control final tips size

5mm – 10mm Taper = **Ideal** range of taper lengths to bevel

10mm – 15mm Taper = **More Challenging** to bevel since the taper is more flexible at longer lengths.

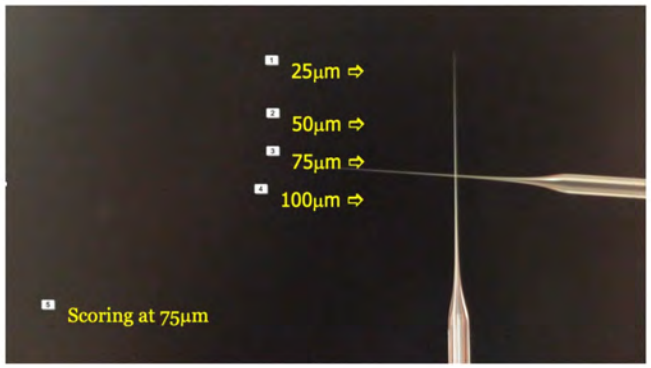
15mm and Longer = **Possibly Impossible** :-) since the taper is way too flexible

To bevel **longer tapers**, one can approach the tip to the beveling plate at a 10-20 degree higher angle (than the final bevel angle you are aiming for) and advance the pipette further into the plate to create sufficient pressure of the glass tip against the grinding plate so it can be beveled. This will help allow the pipette with a longer, more flexible taper to be beveled. Example: Approach at 40–45 degrees, advance the glass downward 2-3x more rotations of the fine manipulator dial to get final bevel angle of 30 degrees.

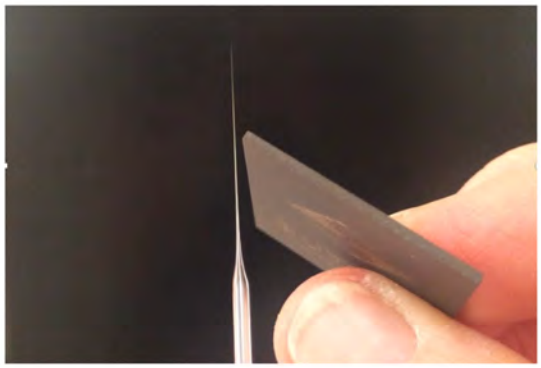


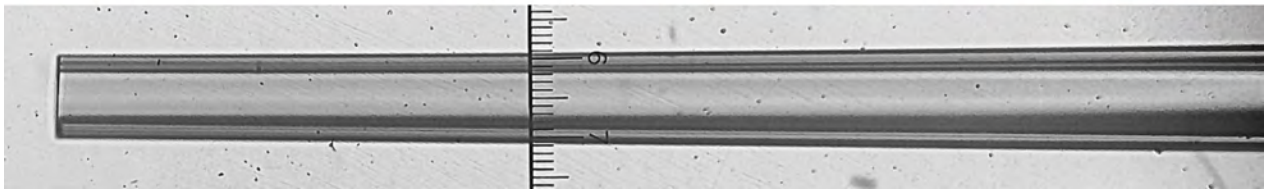
Scoring, Trimming and Beveling

Taper scored & trimmed using "Glass-on-Glass" technique to create a clean 25µm – 75µm tip.



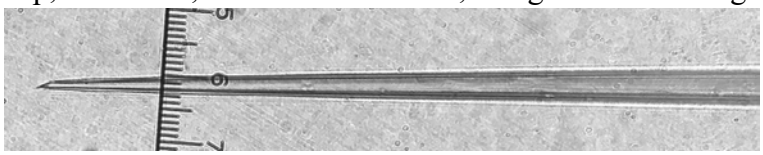
Taper scored & trimmed using the CTS Ceramic tile to create a clean 50-100µm tip.



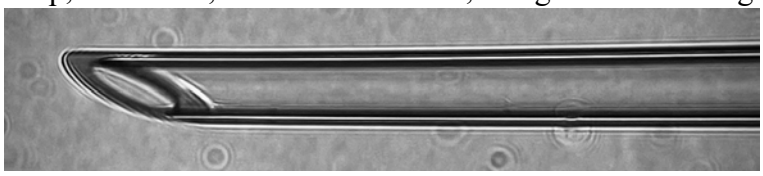


See Chapter 8 and "Achieving the Impossible" webinar (17:55min) on the Sutter Instrument YouTube Channel https://www.youtube.com/watch?v=gC4Ktmb6ndk

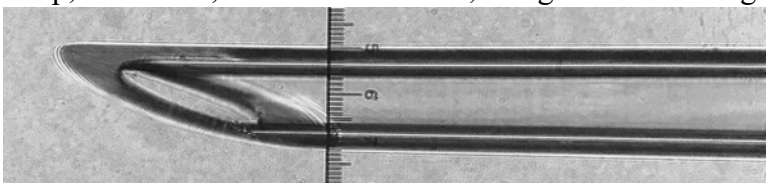
2µm tip, 25° Bevel, BF100-78-10 Glass, using 104D Beveling Plate



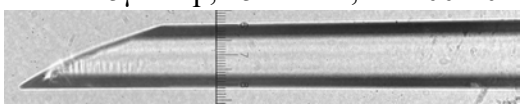
15µm tip, 30° Bevel, B100-50-10 Glass, using 104D Beveling Plate

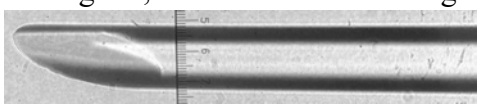


25µm tip, 20° Bevel, B150-86-10 Glass, using 104D Beveling Plate

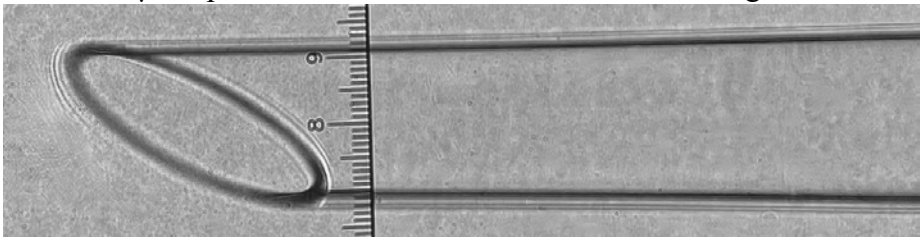


25µm tip, 25˚ Bevel, BR100-10 Solid rod glass, Microblade for cutting

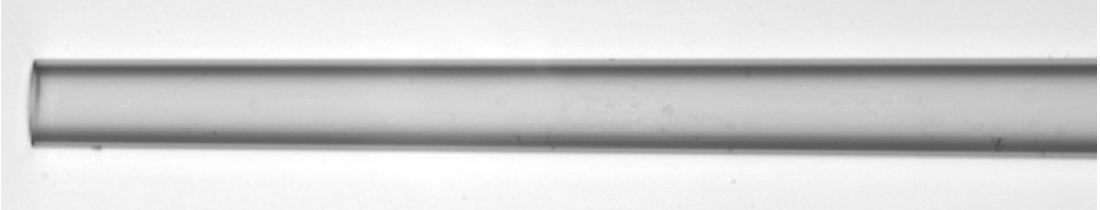




200µm tip, 35° Bevel, B200-156-10, 104C Beveling Plate



CHAPTER 8 – Making Large Pipette Tips (20µm - 200µm)



Scoring and Trimming Using the Ceramic Tile (CTS) or the "Glass-on-Glass" Technique

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 - 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 thin or thick-walled glass and the "Glass on Glass" technique or a Ceramic Tile to create a tip between 20-200µm and a clean 90-degree break.

The front of the ceramic tile is engraved with the Sutter logo. The front edges of the tile (side with the Sutter logo) are rough and are 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.

But the rule of thumb is:

¼ back from the tip, the ID is ~25µm,
½ way down the taper, the ID is ~50µm,
2/3rd down the taper, the ID is ~75µm
3-4mm above the shoulder of the taper, the ID is ~100µm

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:

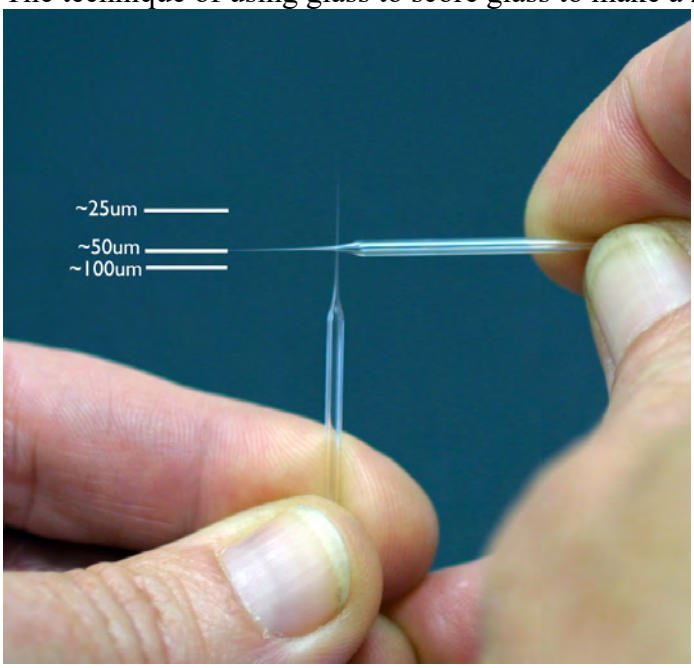
Example program using 1.5mm x 0.86mm OD/ID glass and a 3mm x 3mm box filament:

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

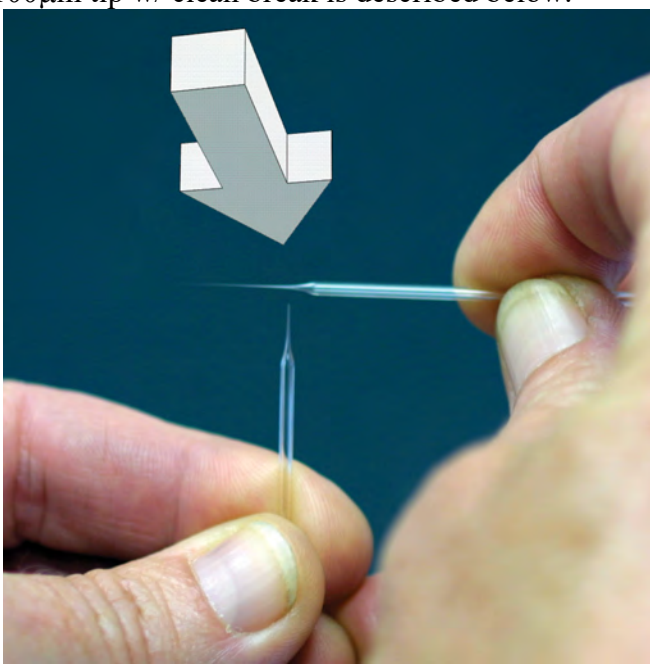
Remove the resulting long tapered pipette and hold it vertically against a dark background and tilt the glass so the light in the room illuminates the glass so the taper appears like a while line against the black background. Using a front edge of the ceramic tile or the glass-on-glass technique (both described in more detail below) to score the glass in a perpendicular fashion (45-65 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 little practice to find the right "touch" to be able to consistently break the glass cleanly at the proper location.

"Glass-on-Glass"

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

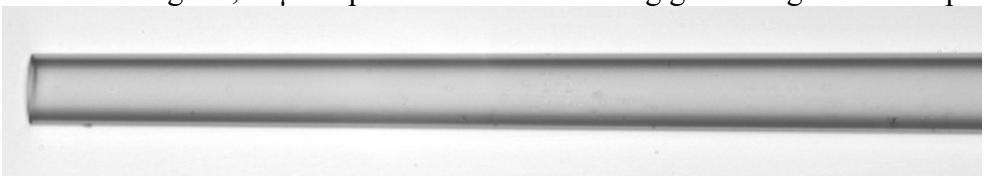


Step 1. *Score only, do not break!*



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

B100-75-10 glass, 25µm tip with clean break using glass-on-glass technique



To score the glass held in the left hand, use the thicker region (closer to shoulder of taper) of the pipette held in the right hand. It is helpful to hold the glass vertically against a dark background and tilt the glass so the light in the room illuminates the glass so the taper appears like a while line against the black background. A light contact of glass-on-glass, with a very slight 1mm horizontal movement of the scoring pipette (horizontal glass in right hand), is all that is required to score the glass. (*Step 1)* You do NOT want it to break when scoring. The breaking happens in step 2! When you rub one glass taper against the other, and when the tip is over 25µm, it will feel rough like sandpaper. At smaller sizes, you might not feel anything in the scoring pipette, but you will see the glass being scored vibrate or wiggle a bit as you score it. One to two motions sideways is sufficient. Many back and forth sawing motions will lead to a bad break! The ideal motion is like playing a note on the violin string and not playing a concerto! After scoring, move the same scoring pipette 1-2mm 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. If the glass breaks while scoring the taper, this will create an uneven or fractured break.

General Rule of Thumb:

¼th back from the tip = 25µm

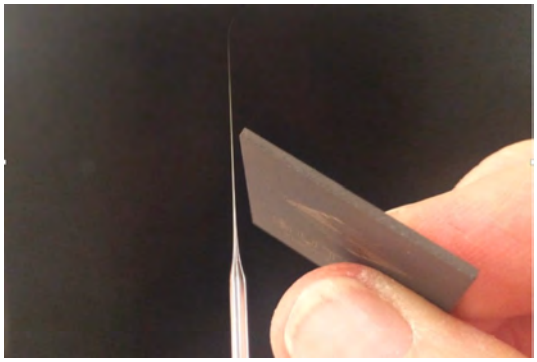
½ way down the taper = 50µm

2/3rd back from the tip = 75µm

3/4th back from the tip = 100-200µm

See "Achieving the Impossible" webinar (17:55min) on the Sutter Instrument YouTube Channel
https://www.youtube.com/watch?v=gC4Ktmb6ndk

Ceramic Tile (Item# CTS) – Used to make 50-200µm Clean Break



Score the glass with the front edge of the tile (logo side) at a 45-65-degree angle to the taper The front edge of the tile has a microscopic, serrated edge which will cut into the side wall of the glass creating a distress point. You will feel a few tiny knocks or bumps as the serrated edge of the tile scores the glass wall. All that is required is a small 0.5 to 1mm horizontal motion of the tile to create a distress point on the glass. Too much pressure or too long of a stroke, the taper it will break back and produce a splintered or fractured tip. Do not saw away at it. After scoring, move the tile 2-3mm above this location and slowly push the glass on the same side that you scored it. All you need is a gentle push. Think Tai Chi, not Tae Kwon Do. The trimmed piece of the glass taper will fly away (literally) and this will create a clean break at the tip. It's like a miracle! :-) Consider wearing protective eyewear and always score sideways or away from your face.

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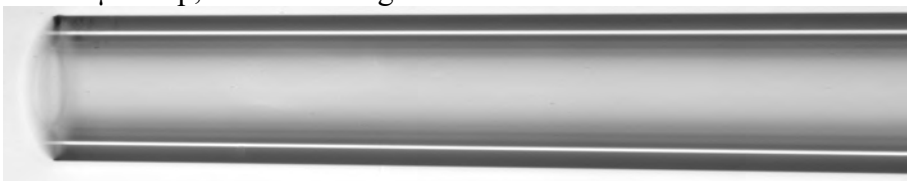
- 75µm Tip, thick-walled glass



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 stab the needle through the Kimwipe. For what has yet to be explained to me, this technique ends up producing a **25-35µm angled break** at the tip and about a 75% 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!

Stabbing through Kimwipe, Kleenex, or Paper Towel:

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

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

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

Trimming Techniques to Achieve Specific Tip Sizes

Tip Size	Glass	Method
1µm - 5µm	Sutter Glass (Thin or Thick Walled)	• Very gently touch pipette tip to a loose piece of Kimwipe a.k.a. "Tickling the Kimwipe" • Break on the edge of a cover slip in the dish on microscope stage • Use a Microforge
6µm - 15µm	Sutter Glass (Thin or Thick Walled) using a Microforge Aluminosilicate or Quartz Glass using modified "Glass-on-Glass" technique	• Use a Microforge – Contact Sutter for more information • Use Aluminosilicate Glass and perform a modified version of the Glass-on-Glass technique. Contact Sutter for additional instructions.
15µm - 50µm	Sutter Thin or Thick-Walled Glass	• Use Glass-on-Glass technique. • Use Ceramic Tile (CTS)
50µm - 100µm	Sutter Thin or Thick-Walled Glass	• Use Ceramic Tile (CTS) • Diamond Knife – Not advised! These are bulky and heavy for scoring The ceramic tile is more delicate and provides better physical feedback. • Glass-on-Glass technique might work, but the ceramic tile is best for wider/thicker tapers.
100µm - 200µm	Sutter Thin or Thick Walled Borosilicate or Aluminosilicate Glass	• Use one-sided manual pull technique on the P-97 or P-1000 puller. See "Achieving the Impossible" Sutter Instrument Webinar (17:55 onward) for instructions. • Use higher heat settings and no cooling to allow a larger ID to be further from the shoulder of the taper. Contact Sutter for further instructions.

CHAPTER 9 - Clogging Tips

The eventual clogging of pipettes during injection is inevitable, but one hopes to be able to do multiple injections before encountering this event. After many consecutive injections, the pipette can clog, and, at this stage one can clean 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 listed below:

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

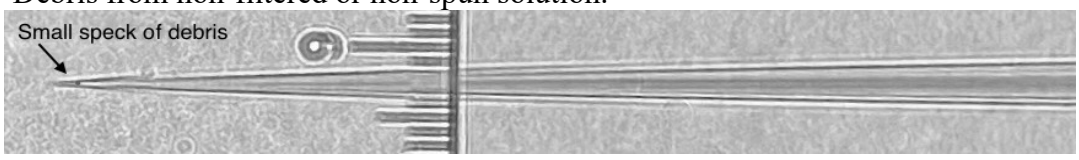
Unclogging or Clearing a Pipette

If the pipette clogs, it is best to use the [CLEAR] button on the injector to attempt to clean the tip of debris. On the XenoWorks BRE Digital Injector, [CLEAR] will increase the pressure to100psi to clean the tip. If there is a dark spec inside the pipette and right before the tip, it is clogged with a particle larger than the tip and, in this case, it is permanently clogged, and you will need to change out the injection needle. But, most often I find that the tip is clogged from the outside in, rather than from the inside out. In other words, the clog is due to debris (sticky lipids and proteins) stuck on the outside of the tip at the opening. In this case the clear or clean feature on your injector will most likely work. If it clears briefly or does not clear at all, and there is no black spec inside the glass near the tip, the clog is often due to sticky debris hanging onto the edge of the glass and not completely falling away. This is analogous to blowing your nose without a tissue! Since they have yet to invent miniature Kleenex to wipe debris off the tip, it is best to gently rub the tip against the surface of the holding pipette **while** you are holding down the clear button. This will help wipe the debris off the tip so it will not come back to haunt you. You can also attempt to gently break back the tip to remove a clogged, but this often renders the pipette too big and this will lyse the cells or eggs. If you establish a compensation pressure that allows for a slight "bleeding" or leaking of solution out the tip, this could extend the "life" of the pipette and help prevent it from clogging while you are injecting your cells.

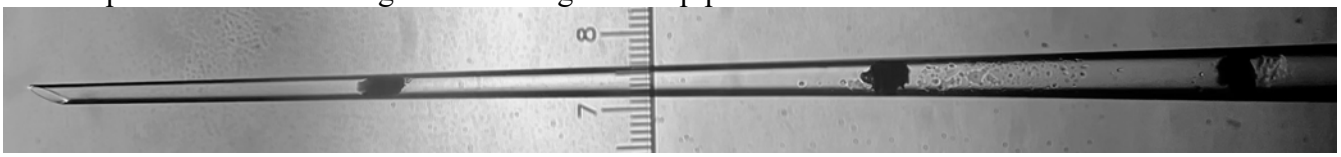
Clogging – Pipette tips can clog due to:

- 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 due to lipids and proteins from the membrane coating the tip.

Debris from non-filtered or non-spun solution.



Rubber particles from disintegrated rubber gasket in pipette holder



CHAPTER 10 – Bee-Stinger Needle

Bee-Stinger Morphology

A Bee-Stinger Needle is mainly recommended for *C.elegans* and other worm injections. This short stubby taper of a bee-stinger pipette makes for a more durable pipette and is most popular for worm injections. It can also be used for penetrating through fibrous membranes, cuticles and chorion as long as what is being injected can handle the larger hole created by the wider taper behind the tip. Due to the wider taper, this shape is not advised for injections where the pipette must advance far into the tissue or egg. This unique tip morphology can cause greater damage to the membrane, maybe killing what you are trying to keep alive.

Use a 2.5mm x 4.5mm Box filament (FB245B) 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 create bee-stinger pipette, so if you are encountering difficulty, please contact Sutter.
1.0mm x 0.78mm, 1.0mm x 0.58mm or 1.0mm x 0.50mm Borosilicate Glass

0.5µm Tip ID, 4 to 5mm Taper, 50-500µm final "bee stinger" short tapered tip

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.

Your Goal is 3 Loops!

Heat	Pull	Velocity	Time	Pressure
Ramp	100	10	250	500

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 ensure 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 bee-stinger morphology of pipette. But if this is what is installed in your puller, try a two line program like the following:

Line	Heat	Pull	Velocity	Time	Pressure
1	RAMP	0	30-60	250	500
2	**RAMP**	**70-100**	**20-40**	250	500

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 Needles - **Recommended for C. elegans and other worm injections**

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

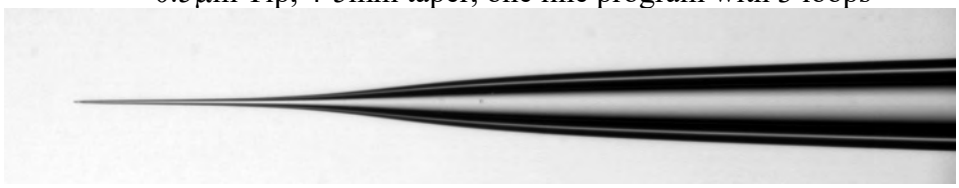
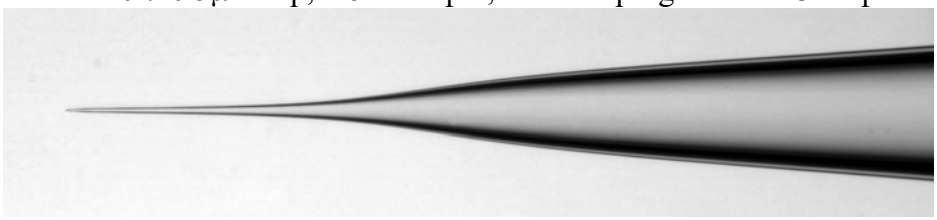
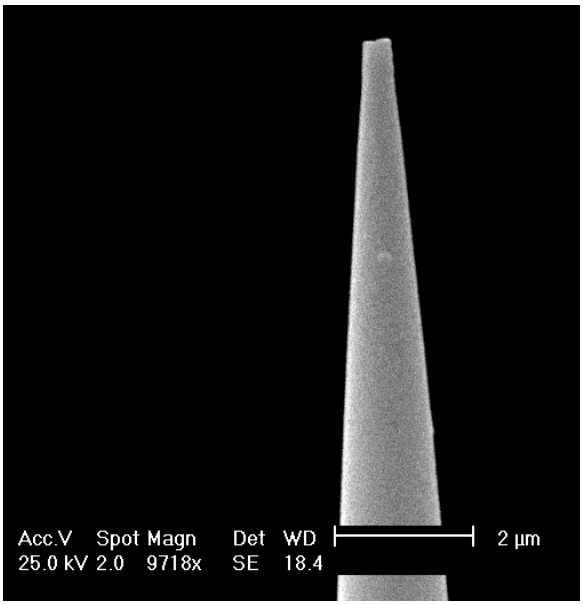


Image B **(40x mag.)** 1.0 x 0.5 thick-walled glass (BF100-50-10)
~0.5µm Tip, w/ a shorter taper than that shown above.
Three-line program with a reduced velocity on Line 3

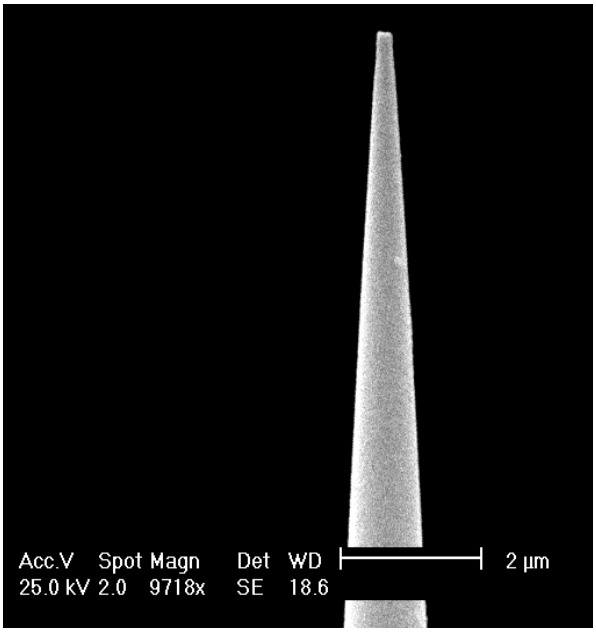
Image C **(100x mag.)** 1.0 x 0.78 thin-walled glass (BF100-78-10)
0.7-0.5µm Tip, 4-5mm taper, one-line program with 3 loops



THICK-WALLED INJECTION PIPETTE



THIN-WALLED INJECTION PIPETTE



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

CHAPTER 11 - *C.elegans* and Worm Injections

Using the P-97 or P-1000, the pipettes for this application can be made with thick-walled **Borosilicate glass (**BF100-58-10) or thin-walled **Aluminosilicate glass** (AF100-64-10). Even sharper needles can be achieved by beveling the tips using the Sutter BV-10 Beveler (Chapter 7). For a shorter and quick taper, you might want to try a "Bee Stinger Needle" for this application (Chapter 10).

Depending on your specific application and technique, pipettes can be used "as is," broken back, or beveled. For worm injection, the starting pipette will have a 0.5 to 0.7µm tip and this is too small of an opening to allow solution to come out of the tip. So your injections will stop there unless you make the tip opening a little larger. You will need to break back or "tapping off" the tip ever so slightly to "open it up". If you will be beveling the tip to make a sharp and more open injection needle, it is best to introduce 10-20 additional units of pull so your final tip starts off a little smaller.

HEAT	PULL	VEL	DEL	PRESSURE
Ramp	50 - 60	70 - 90	130 -170	500

Pipette Morphology

Preferred Morphology – Gradual Taper

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

Alternate Morphology – Stubby Taper∗

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

∗ See Chapter 10 for how to make a bee-stinger type pipette.

XenoWorks BRE Digital Injector and XW-225 used for Worm Injections

BRE Settings: Range Setting (1), Compensation pressure (10-20hPa),
Injection pressure (150-500hPa), Pulse Mode: Duration (0.20sec)

CHAPTER 12 - Adherent Cell Microinjection

The pipettes required for adherent cell injection are most commonly made using thick-walled Borosilicate glass with filament (BF100-58-10). This application requires a moderate 5-7mm taper and a tip that is just under 1µm. The tip and taper need to be fine enough not to lyse the cell, and the taper should not be so long that it becomes too flexible and not durable. To maintain good durability in the pipette, it is best to keep the taper on the shorter side (≤ 7mm taper) by using a 2.5mm x 2.5mm box filament. Using the P-97 or P-1000, the most durable and sharp needles for this application can be made with BF100-58-10.

Any time you are making needles with tip sizes under 1µm, filamented glass is used to allow for easy backloading of solution into the needle. The best ingredients to start with would be 1mm x 0.58mm filamented glass and a 2.5mm x 2.5mm box filament (FB255B). While a 3mm x 3mm box filament (FB330B) would also work well for pulling these types of pipettes, the more narrow 2.5mm box filament is preferred as it will heat a shorter width of glass and 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 Tables and use a Type B program for your glass and filament.

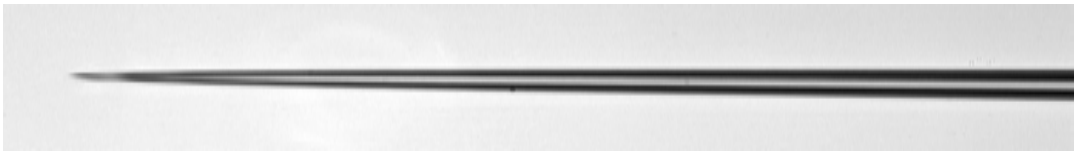
Pipette Morphology

For Adherent Cell microinjection, the pipettes can be used "as is," and not be beveled or broken back. Since you will be using them "as is," it is best to use a lower pull value so the tips do not become too small. Sometimes the tip breaks back a little as you are doing your injections since you are often coming up against the surface of the petri plate or coverslip. As you do the injections, the tips will sometimes penetrate through both membranes and press into the surface of the coverslip you touch down to find the cell surface, you can introduce 10-20 additional units of pull strength so the tip does not break off too big or become too large too fast.

Programs for a 2.5 x 2.5 box filament (FB255B)

THICK-WALLED GLASS - BF100-58-10 (1.0mm x 0.58mm)

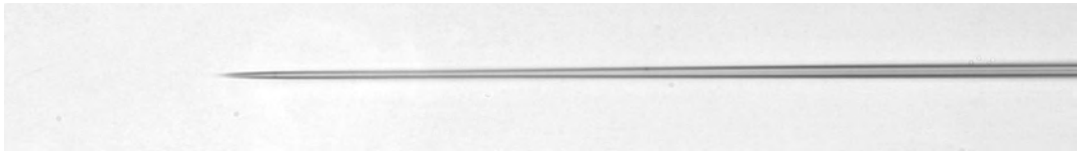
HEAT	PULL	VEL	DEL	PRESSURE
Ramp	50	80	130-170	500



0.6 - 0.9µm tip, 6 - 8mm taper (400x)

THIN-WALLED GLASS - BF100-78-10 (1.0mm x 0.78mm)

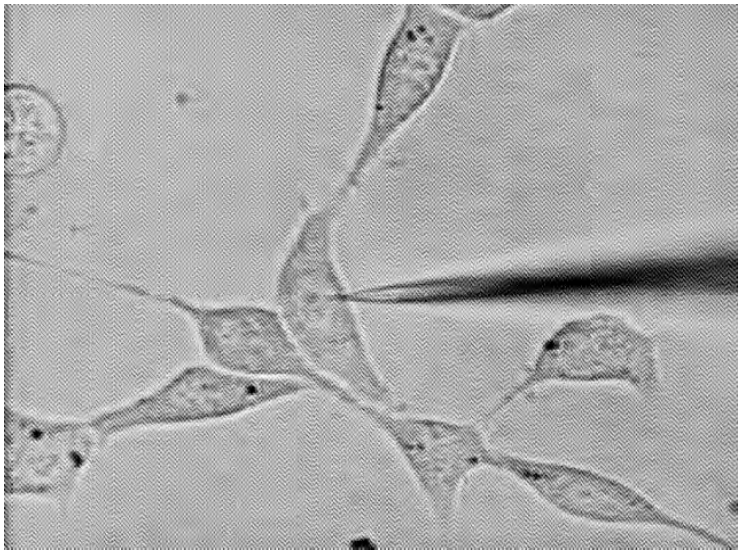
HEAT	PULL	VEL	DEL	PRESSURE
Ramp	60	70	120	200

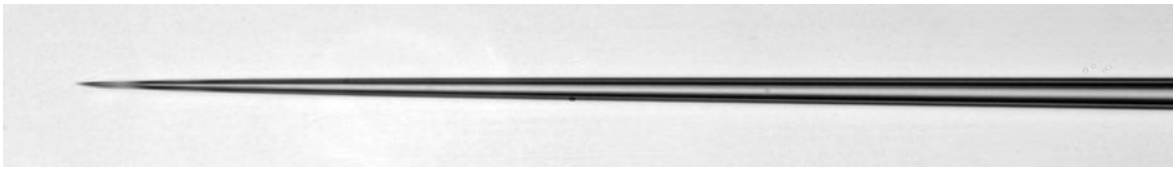


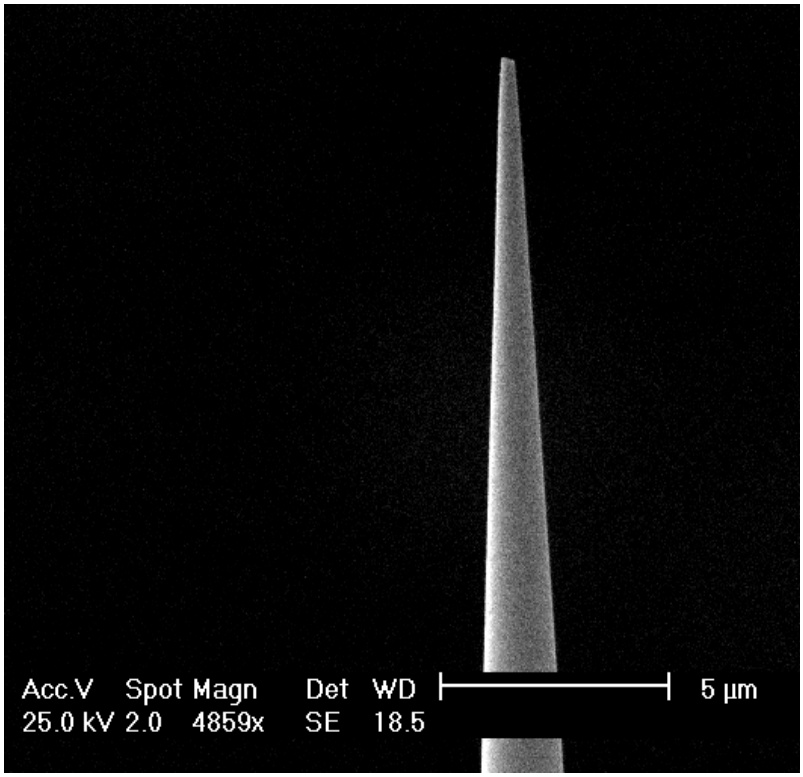
0.7 - 0.9µm tip, 5 - 7mm taper (400x)

- **Larger Tip & Shorter Taper** = Increase the Delay in 10-unit increments
- **Smaller Tip & Longer Taper** = Decrease the Delay in 10-unit increments
- **Reduce the Shoulder**, narrow taper behind tip = Increase Pull in 10-unit increments

Adherent Cell Microinjection – Pipette Images







Scanning Electron Microscopy Image of a
Microinjection Pipette for Adherent Cells
(~5,000x mag)

XenoWorks Digital Injector & Manipulator for Adherent Cell Microinjection

XENOWORKS DIGITAL INJECTOR SETTINGS:

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

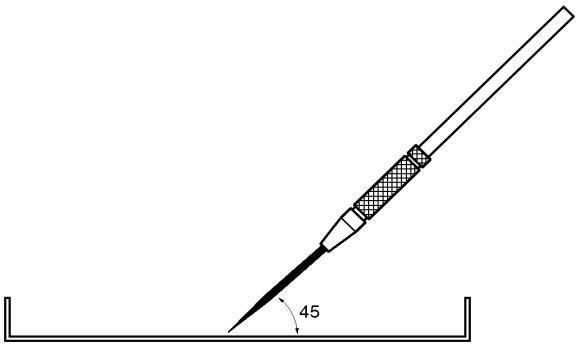
Recommended Approach……

PULSE MODE: Adherent cell = 200-1000 hPa
Pulse Width = 0.20 seconds

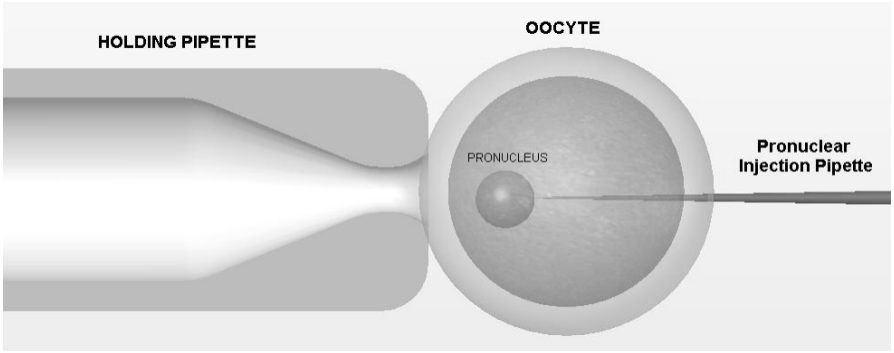
Alternate Approach……

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

XenoWorks Micromanipulator (XWMR) Position for Adherent Cell Injection



CHAPTER 13 –CRISPR, Pronuclear & Cytoplasmic (Mouse) Injection



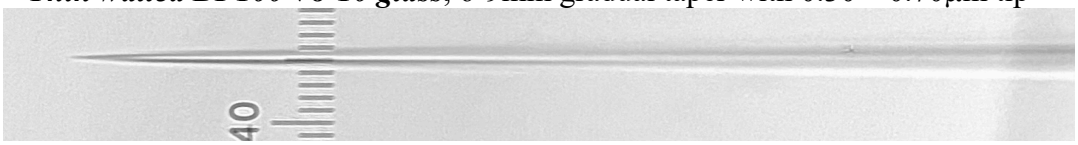
Microinjection into the pronucleus (Pronuclear Injection) is one of the most commonly used techniques for the production of transgenic animals, including the development of transgenic mice which are among the most useful research tools in the biological sciences. Pronuclear injection involves the mechanical 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 a Very Low Shoulder Behind Tip

Thin walled BF100-78-10 glass, 6-9mm gradual taper with 0.30 – 0.70µm tip



"Tapping Off" the Tip

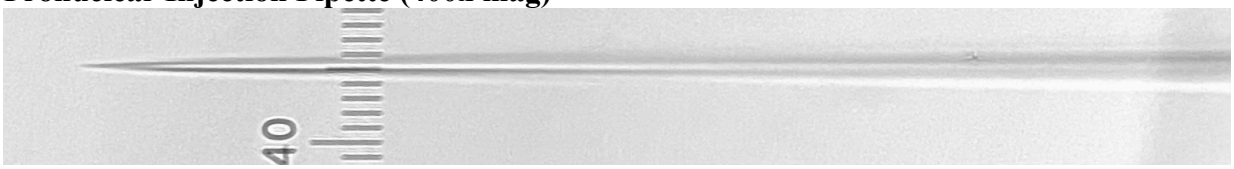
When the pipette is pulled, it will create a long gradual taper and a 0.30 – 0.70µm tip. This opening is too small to expel solution out the tip, therefore it is necessary that you break back, or "tapping off" the tip before using it to allow a good flow rate of mRNA and DNA out the needle. When the pipette is pulled correctly, **the final 3-5µm length of the pipette tip will look like a small black line or splinter** at 400X magnification. When you see no while light or gap between the two walls of the taper, then you know the pipette is under 1 micron. To make a slightly larger opening at the tip, you will gently rub the injection pipette against the smooth surface of the holding pipette. The goal is to break off a small portion of that final black splinter at the tip. If you can see an opening at the tip, you've broken it back too far! And your eggs might lyse. So this is a gentle technique and aggressively shoving the injection pipette against the tip of the holding pipette will not give you the desired results. The method of successfully breaking back the tip is influenced by how well you are able to control the outcome of the pipette morphology (mainly the taper length) and the level of patience and delicacy in tapping off the tip.

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. It requires a very gradual taper that is approximately 7-9mm 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. When the taper length that is too short (under 6mm), the tip size will become too big (over 1.5µm) as you rub it against the holding pipette to "open it up" and will cause the egg to lyse. And when the taper is too long (over 10mm), the pipette becomes to flexible to successfully tap off the tip on the holding pipette. This will make it difficult to reproducibly tap off the tip to the proper tip size, and in frustration you will probably end up forcibly stabbing the tip against the holding pipette and end up making a tip opening that is far too big.

Goal = 7-8mm taper, 0.3 to 0.5µm tip, gradual taper and no distinct shoulder behind the tip.

Pronuclear Injection Pipette (400x mag)



Recommended Programs using a box filament and thin-walled glass:

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

	Heat	Pull	Velocity	Delay	Pressure
Line 1	Ramp+15	90-110	70	90	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 and reduce the delay or time (cooling) in 5-unit increments.

Injection System Considerations

The need for a smaller or larger tip opening is not only determined by the DNA construct and dilution but 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.

BAC and YAC (bacterial and yeast artificial chromosome) DNA or mRNA can have large constructs, or be at higher concentrations, and often require a pipette with a shorter taper and larger tip. Often it is necessary to tap off the final tip even further or use beveled pipette tips to provide a larger bore so as to prevent clogging or shearing of the DNA.

BRE Digital Injector Settings – CRISPR, Pronuclear & Cytoplasmic Injection

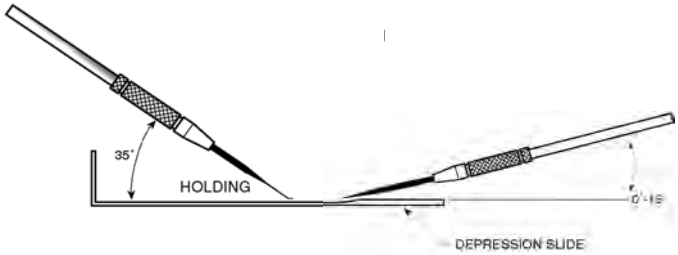
- **Range Setting: 1**
- **Compensation Pressure = 20 - 40hPa**
- **Injection Pressure: (Pulse or Continuous) = 500 - 1000hPa**
- **Holding Pressure Range (for egg) = - 40 to +40hPa**

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

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

To use the alternate method of Continuous Injection using the Compensation Pressure, increase compensation pressure until desired flow rate is created. For even higher pressures, switch to Range Setting 2 which will double available compensation pressure.

XenoWorks (XWMR & XWML) - Set Up for CRISPR, Pronuclear & Cytoplasmic Injection



Holding Pipette with 25-35 degree approach. Injection Pipette with 10-15- degree approach. A low angle for the injection pipette is ideal, and the degree of approach will depend on side walls of dish or depth of well.

CHAPTER 14 - Embryonic Stem Cell Transfer (ES Cell)

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 10-12mm 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 microinjector like the XenoWorks Digital or Hydraulic Injector, 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

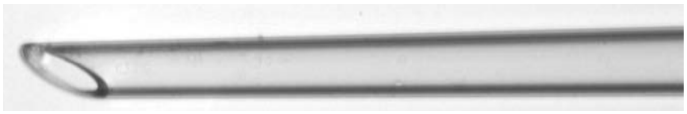
- If you find the *taper too long*, reduce the heat in 5-unit increments (but do not go below the ramp value) and then gradually increase the time in 10-unit increments.
- If you find the *taper too short*, reduce the time in 25-unit increments and then increase the pull in 10-unit increments.

Technical Notes:
The injection of ES cells into embryos requires a micromanipulation system similar to that used for pronuclear microinjection of DNA. Please see Chapter 28 which describe the XenoWorks Microinjection System Configurations.

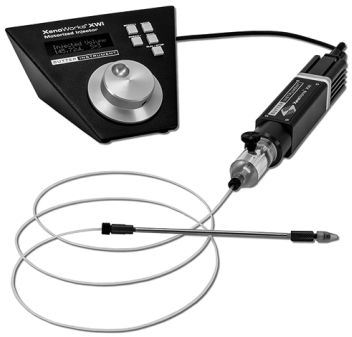
* *If you are using a puller where the filament cannot be changed, please find the filament type that is installed in your puller in the Borosilicate Glass General Look Up Tables and use the program designed for B100-75-10 glass.* ***Type E*** *programs are recommended for this application.*

Embryonic Stem Cell Pipette

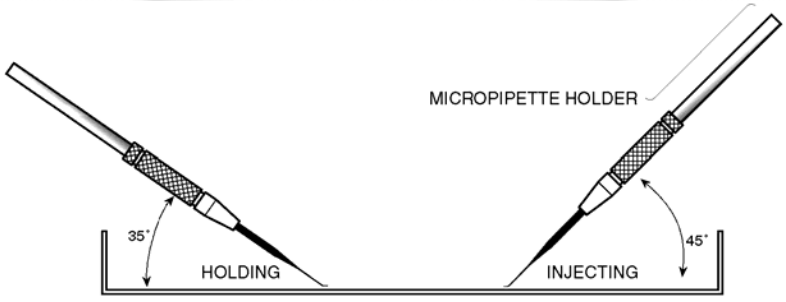
The pipette has been trimmed using a microforge to make an 18-23µm tip, and then beveled at 30-degrees on a BV-10 Beveler using a104D fine grinding plate. The pipette is then spiked using a microforge.



XenoWorks (XWI) Hydraulic or (BRE) Digital Injector for ES Cell Transfer



XenoWorks (XWMR & XWML) Set Up for ES Cell Transfer



Suggested set up for **suspended cells using angled pipettes** (CRISPR, ICSI, ES Cell, & NT)

CHAPTER 15 - 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, gradually 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 15 to 25μm. 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 PMM6 (See Sutter Instrument Catalog).

Nuclear Transfer – Recommended Program

These pipettes are made from 1mm x 0.75mm **(B100-75-10)** glass using a 2.5mm x 4.5mm **(FB245B)** or 3.0mm x 3.0mm **(FB330B)** Box filament. The following settings are a good starting point:

Heat	Pull	Velocity	Time	Pressure
Ramp + 30	0	60 - 150	0	200

If the *taper is too long*, reduce the heat in 10-unit increments, then use lower values for the velocity setting

If the *taper is too short*, increase the heat in 10-unit increments, then use a higher value for the velocity setting

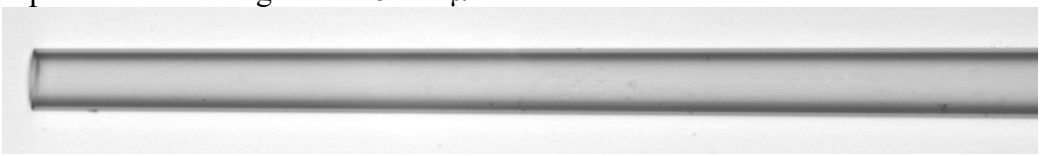
Additional Information on Embryonic Stem (ES) Cells, Nuclear Transfer (NT), and Cloning:

- The following PBS/Nova web site
  http://www.pbs.org/wgbh/nova/sciencenow/3209/04-clon-nf.html

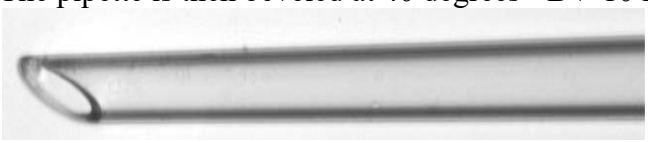
- Nuclear Transfer: Bringing in the Clones
  http://www.pnas.org/misc/classics4.shtml

Nuclear Transfer Pipettes

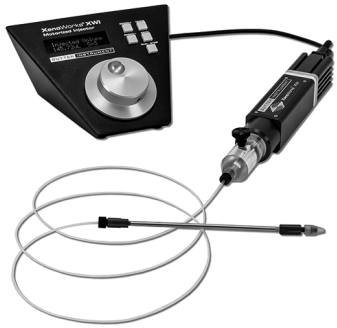
Pipette has been forged at ~15 - 21µm ID



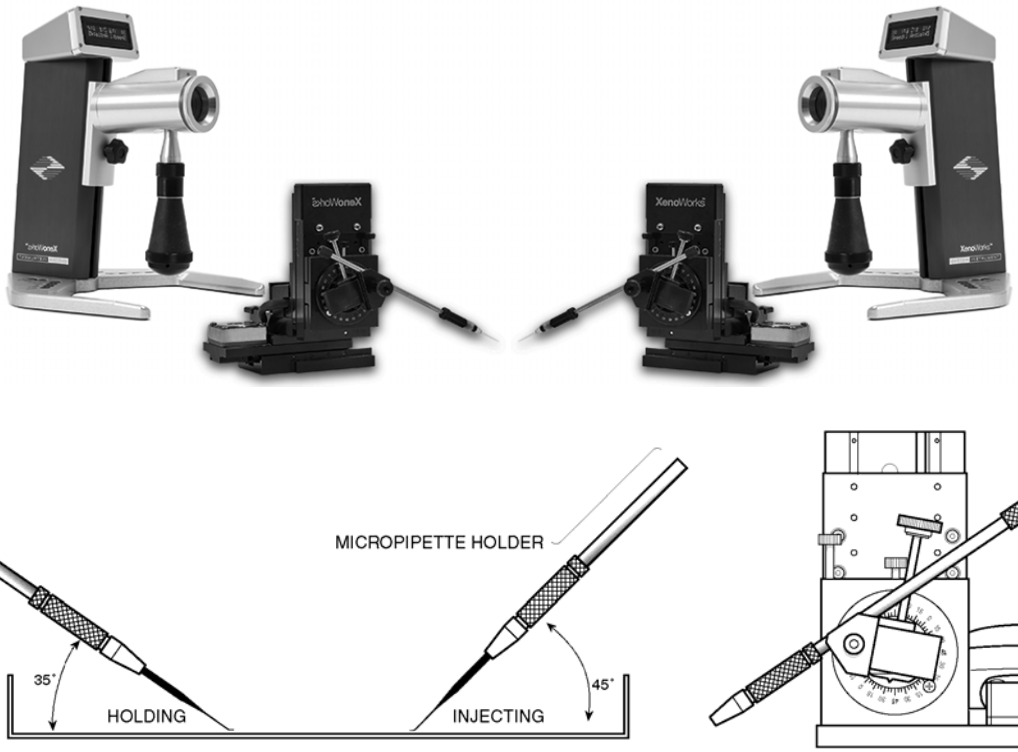
The pipette is then beveled at 40 degrees - BV-10 Beveler, 104D fine plate



XenoWorks Hydraulic Motorized Injector (XWI) for Nuclear Transfer



Xenoworks Micromanipulator (XWMR & XWML) Positions for Nuclear Transfer



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

CHAPTER 16 - ICSI (Intracytoplasmic Sperm Injection)

Intracytoplasmic Sperm Injection 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 28).

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, gradually 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 (Chapter 7). The pipette tip should be beveled back to the proper ID and beveled at the desired angle, which is usually between 25- 30 degrees. If required, creating a small spike on the pipette tip and bending the taper 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

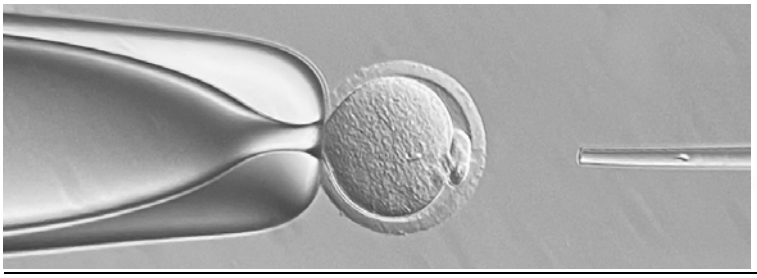
- If the *final taper is too long*, on line 2 reduce the heat in 5-unit increments and then gradually reduce the velocity in 10-unit increments.
- If the *final taper is too short*, on line 2 reduce the time in 25-unit increments and then increase the heat in 10-unit increments.

* *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.*

PIEZO-ASSISTED ICSI

The pipette has been pulled to have a long gradual taper where the final taper has parallel walls. It is then forged and cut at 4-5µm ID and 6-8µm OD using a microforge. This clean break and blunt end is required for Mouse Piezo ICSI.

ICSI Pipette used with PMM6 Primetech Piezo

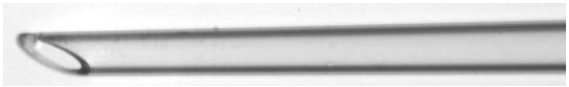


For proper delivery and resonance of the piezo vibration to the tip, the pipette is filled with Fluorinert or a small slug of mercury. For **Piezo ICSI, Fluorinert ™ FC-770** is the preferred pipette filling solution. Alternatively, 1-2µl of **Mercury** has been the past recommended solution to load into the final taper of the pipette, but many labs are not rated for biohazardous solutions. Sutter Part #FC-770.

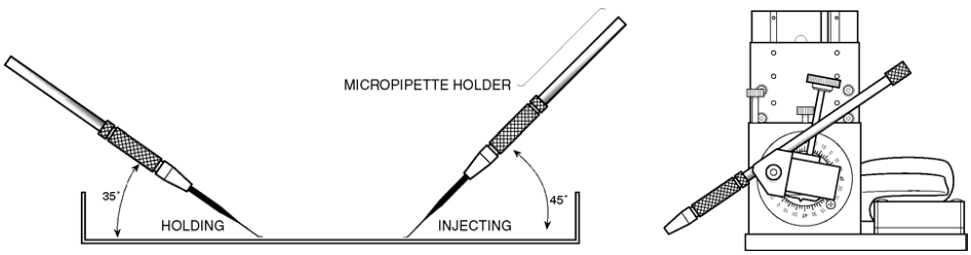
TRADITIONAL ICSI

If a beveled pipette is needed rather than a blunt Piezo ICSI pipette, it is created by first forging the pipette to make a 3-5µm tip and then beveling the tip with the Sutter BV-10 (Chapter 7). The pipette tip should be beveled at the desired angle, which is usually between 30 and 40 degrees. If required, a small spike on the pipette tip is made using a microforge.

When doing Traditional ICSI and not using a Piezo, the tip is beveled and/or spiked to successfully cut through the zona and plasma membranes.



XenoWorks Micromanipulator (XWMR & XWML) Positions for ICSI



Suggested set up for suspended cells using angled pipettes (CRISPR, ICSI, ES Cell, & NT)

CHAPTER 17 - Holding Pipettes

When performing techniques such as Mouse CRISPR, Pronuclear, ICSI, NT, & ES Cell injection, a 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 then fire-polished using a microforge to create a smooth surface to interface with the cell. Holding pipettes are made to have a fairly large outer diameter and small opening at the tip. This provides better support and reduces any possible distortion of the oocyte during the micromanipulation. Holding pipettes traditionally have an inner diameter of 5-15µm and an outer diameter between 90-125µm.

Holding Pipettes – Recommended Program

Holding pipettes are made from 1mm x 0.75mm **(B100-75-10)** capillary glass and a 2.5mm x 4.5mm **(FB245B)** box filament. A more narrow heating filament, like the 3mm or 2.5mm box filament, will not melt as wide a region of glass and the taper at 100µm ID will be closer to the shoulder of the pipette and the walls might not be as parallel. So the FB245B (4.5mm wide box filament) is the best filament to consider using.

Using FB245B filament, B100-75-10 glass, and the following settings:

Heat	Pull	Velocity	Time	Pressure
Ramp + 30	0	100-150	0	200

This will generate a long, even taper with parallel walls

- If the *taper is too long*, reduce the Velocity in 10-unit increments.
- If the *taper is too short*, increase the Velocity in 10-unit increments, and then increase the Heat in 10-unit increments, but do not exceed Ramp + 50

Additional Steps Needed to Fabricate a Holding Pipette

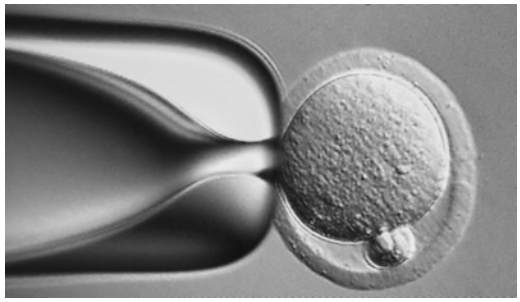
Breaking back the glass – The pipette will need to be broken back to have an inner diameter between 90 and 120µm. This will depend on the size of cell you intend to "hold" and the extent of fire polish you will need to provide to the tip. To break the glass back clean at tip sizes of 20µm and larger, 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 8 for instructions. A microforge can also be used, but when cutting glass with a forge at tip sizes over 25µm, 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.**

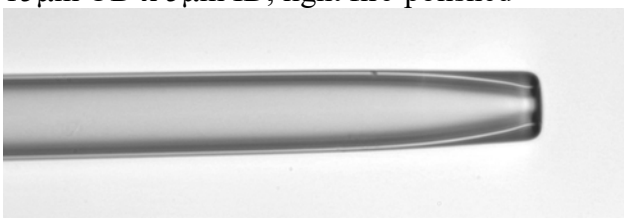
Bending the Pipette – Depending on your application and set up, it is sometimes necessary to create a 20 to 35 degree bend in the taper of the pipette. The bending of the pipette is most often needed when your cells are in a dish rather than on a slide and a specific incident angle is preferred. **A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

* *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.*

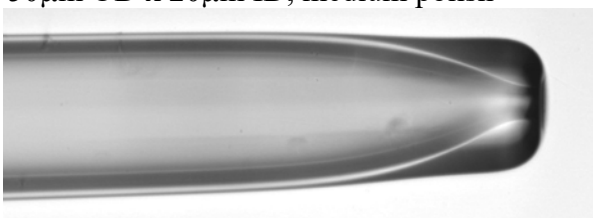
Holding Pipettes



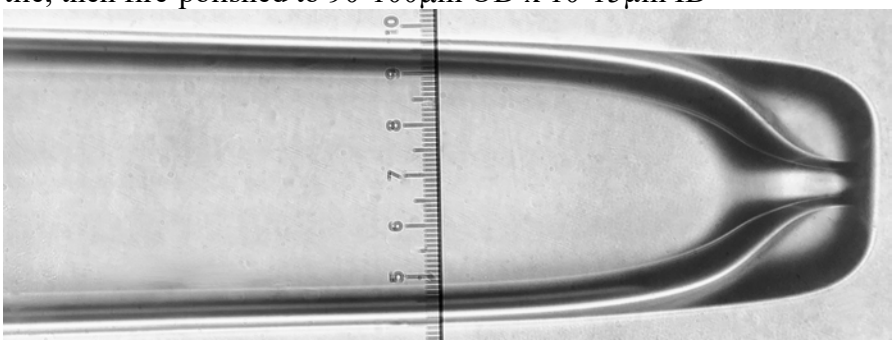
Small Holding Pipette (400x mag.)
Scored and cut using "Glass-on-Glass"
15µm OD x 5µm ID, light fire-polished



Medium Holding Pipette (400x mag.)
Scored with cut using the Ceramic Tile
50µm OD x 20µm ID, medium polish

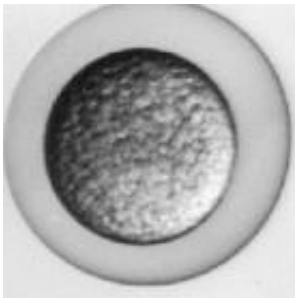


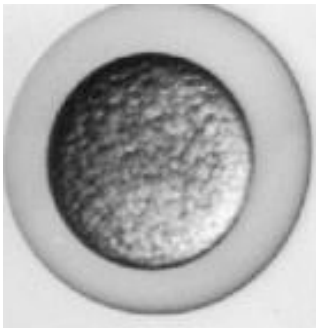
Large Holding Pipette (400x mag.)
Scored and cut with clean break at 100-120µm ID using the ceramic tile, then fire-polished to 90-100µm OD x 10-15µm ID



XenoWorks (XWI) Hydraulic Motorized or (BRE) Digital Injector for Holding

CHAPTER 18 - Zebrafish Embryo Injection





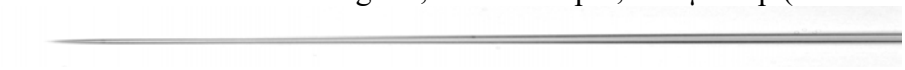
Pipette Morphology:

Zebrafish microinjection pipettes traditionally start off having a gradual 8-10mm 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 (4-10µm) at the end of the pipette. To make sure your pipette has a slender taper, it is best to use Pull settings between 40-70 units.

Use the settings provided below to make your microinjection needles. The expected tip size will be between 0.3 – 0.7µm 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

Thin-walled BF100-78-10 glass, 8-12mm taper, ~0.7µm tip (to be trimmed)



Thick-walled BF100-58-10 glass, 9-8mm taper, ~0.7µm tip (to be trimmed)

Trim with forceps to make 10-15µm tip and aim for an angled break

Beveled to have a sharp 12µm tip. Might last forever and never get dull :-)

Programs using 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	40-70	120	80-100	500

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

HEAT	PULL	VEL	DEL	PRESSURE
Ramp + 10	40-70	120	60-90	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

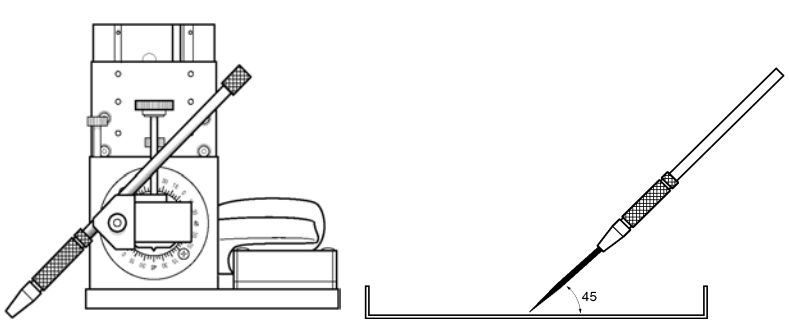
XENOWORKS DIGITAL INJECTOR SETTINGS:

- **RANGE SETTING: 1**
- **COMPENSATION PRESSURE = 5-10hPa**
- **INJECTION PRESSURE: (PULSE)**

Recommended Approach……
PULSE MODE: Zebrafish egg = 4,500hPa
Pulse Width = 0.04 seconds

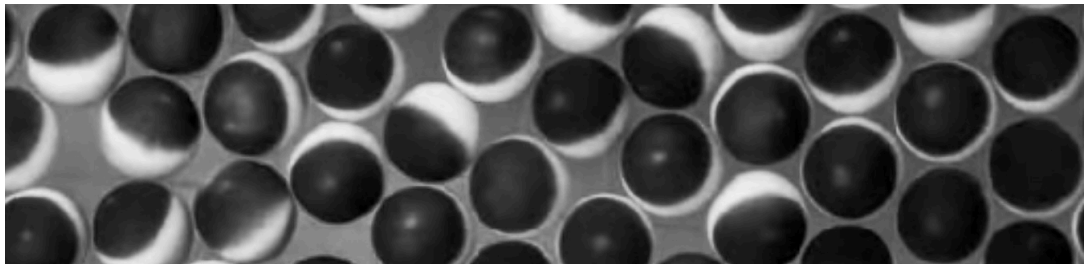
After you have back-filled the pipette and broken back the taper to create a 8µm to 12µm tip, the goal is to establish a pressure and duration that provides the proper injection volume. To calibrate the pressure and duration required for your pipette opening, back fill the pipette with the injection 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.

XW-225 SUTTER MICROMANIPULATOR POSITION for Zebrafish Embryo Injection



Zebrafish embryo approached at a 30-45 degree angle

CHAPTER 19 - 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 **(BF100-75-10)** or 1 x 0.58 **(BF100-58-10)** 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 8-10µm 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 8-10µm opening at the tip. A pipette tip that has a somewhat angled 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.

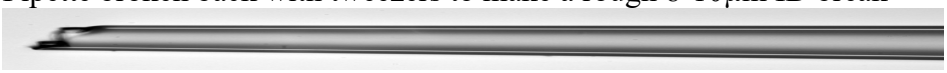
* *If you are using a puller where the filament cannot be changed, please find the filament type that is installed in your puller in the General Look Up Tables and use the program designed for the appropriate glass.* ***Type D*** *programs are recommended for this application.*

Xenopus Microinjection Pipettes, 8µm - 10µm Tips

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

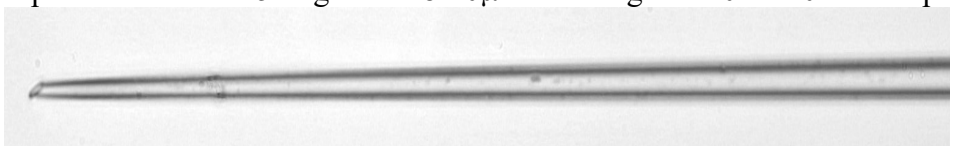
To make the proper tip opening, you must break off the final tip

Broken with Tweezers
Pipette broken back with tweezers to make a rough 8-10µm ID break

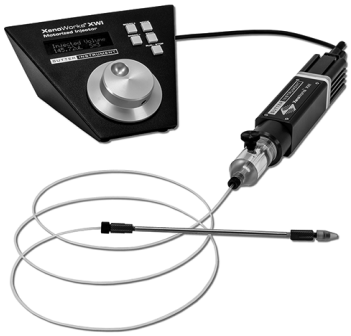


Broken by gently dragging tip on taught piece of parafilm or Kimwipe
8-10µm tip, thin or thick-walled glass

Beveled with the BV-10 Beveler
Pipette beveled at 45-degrees to 8-10µm ID using BV-10 w/ 104D fine plate



XenoWorks (XWI) Hydraulic or (BRE) Digital Microinjector for Xenopus Injection



XenoWorks BRE Digital Injector Settings

- **Range Setting: 1**
- **Compensation Pressure = 5-10 hPa**
- **Injection Pressure: (Pulse or Continuous) = 75-200 hPa**

Recommended Approach……
PULSE MODE: Pressure = 75-200 hPa
Pulse Width = 0.20 seconds

Alternate Approach……
CONTINUOUS MODE: Pressure = 75-200 hPa
Duration of injection determined by how
long the inject key or foot switch is held down.

*Alternate Method of Continuous Injection using High Compensation Pressures

CHAPTER 20 - Aspiration & Transfer Tips

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, ES Cell, Blastomere Biopsy procedures, and for Single Cell Mechanics.

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 final walls are 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.

For tips 20µ and larger, one can pull the glass out long, then score and break the taper according to the instructions in Chapter 8. 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.

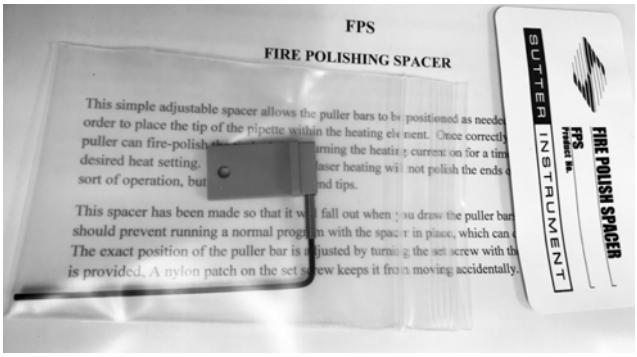
It is most ideal to have a wide 2.5mm x 4.5mm box filament (FB245B) to heat and pull the glass to create a long even taper with parallel walls. We recommend using thick/standard walled glass since this optimizes one's ability to make a clean break at the tip.

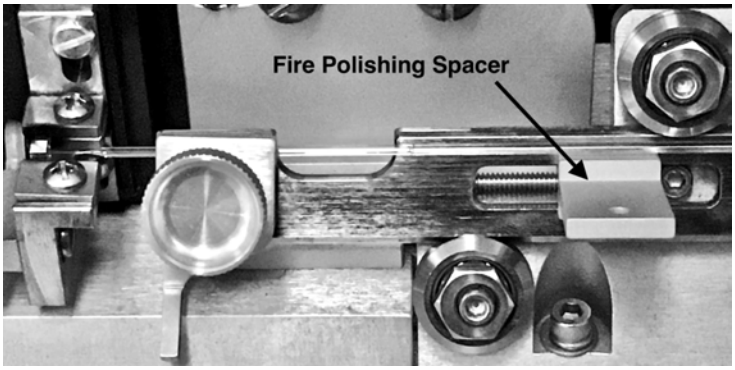
Please install the following parameter settings and follow the instructions in Chapter 8 to score and break the glass back to the proper tip size.

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

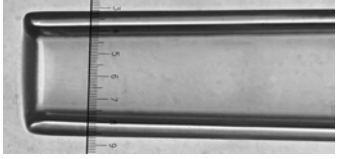
▼ 50µm Tip, thick-walled glass

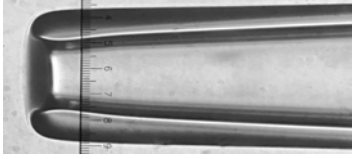
After scoring and trimming the glass, you can then use the Fire-Polishing Spacer (FPS) polish the tip if you need remove sharp edges and create a smooth surface. Polishing tips can be done on the P-97 and P-1000 pullers. The P-2000 cannot polish tips, but this spacer can be used to bend tips on a P-2000/G.





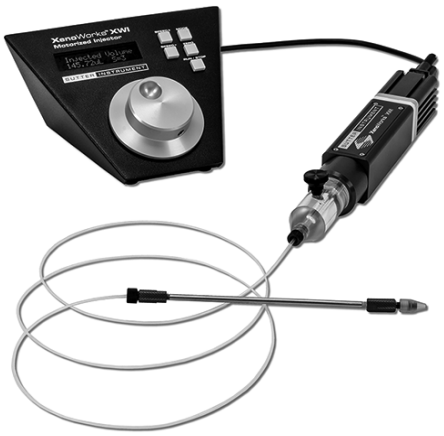
50µm Tips, thick-walled glass with light to medium fire-polish





For very long 1-2cm tapers and 75-200µm ID tips, use a one-sided manual pull described in the Sutter Instrument YouTube Webinar "Achieving the Impossible." https://www.youtube.com/watch?v=gC4Ktmb6ndk

XenoWorks Hydraulic Motorized Injector (XWI) for Aspiration & Transfer



Recommended Set-up
Oil or Water: Fill tubing with light mineral oil or water (oil preferred for finer control)
Pipette Morphology: Application dependent, contact Sutter Technical Support for advice

XenoWorks Digital Injector (BRE) for Aspiration & Transfer

Recommended Set-up
Range Setting: 1 or 2 (Setting 2 doubles the negative and positive pressure range)
Compensation: Use compensation dial on remote user interface to control aspiration
Pipette Morphology: Application dependent, contact Sutter Technical Support for advice

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

CHAPTER 21 - Aluminosilicate Glass

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. Aluminosilicate glass is often required to inject insect eggs, fish eggs, worms, and anything with a fibrous membrane, cuticle or chorion. The P-97 and P-1000 pullers can pull both Borosilicate and Aluminosilicate Glass. The P-2000/G can pull Borosilicate, Aluminosilicate and Quartz glass, Quartz being the absolute hardest. So, if you have a P-2000 puller, going straight to Quartz is recommended for performing injections through tough membranes.

While the original ratio of a borosilicate capillary's inner to outer diameter remains relatively unchanged over its total taper length, Aluminosilicate and Quartz 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. This is also true for Quartz glass.

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 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 three box filaments installed in your puller, you can pull this glass without changing the filament. If you have a filament less than 2.5mm wide or a trough filament, and you 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.

The following page provides custom programs design specifically for making pipettes using Aluminosilicate glass. To find the recommended program and parameter settings with which to get started, please follow steps 1-4 to find your starting settings.

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

   2.5mm x 2.5mm Box (FB255B)
   3mm x 3mm box (FB330B)
   2.5mm x 4.5mm Box (FB245B)

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
Aluminosilicate Glass: 1.5mm OD x 1.00mm ID	AF150-100-10 and A150-100-10

3) *Select your pipette "Type."*

   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 for Aspiration, to be scored & broken (see Chapter 20).

Aluminosilicate Glass General Look Up Tables

2.5mm Box Filament (FB255B)

Aluminosilicate Glass: 1.0 x 0.64, 1.2 x 0.87, 1.5 x 1.00 2.5mm Box Filament (FB255B)

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

3.0mm Box Filament (FB330B)

Aluminosilicate Glass: 1.0 x 0.64, 1.2 x 0.87, 1.5 x 1.00 3.0 mm Box Filament (FB330B)

Type	Heat	Pull	Vel	Time/Delay	Pressure	Loops
A	Ramp	0	30-40	250 time	500	3-4
B	Ramp + 5	60	70	200 time	400	1
C	Ramp + 5	90	80	60 delay	200	1
D	Ramp + 10	120	70	60 delay	400	1
E	Ramp + 30	0	150	0	100	1

4.5mm Box Filament (FB245B)

Aluminosilicate Glass: 1.0 x 0.64, 1.2 x 0.87, 1.5 x 1.00 4.5 mm Box Filament (FB245B)

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

3) 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.

4) 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.

5) 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.

6) Curved Tips – Aluminosilicate glass that is filamented (AF100-64-10 for example) will produce a slight curve (sort of like an eye lash) along the final taper and tip. Since non-filamented Aluminosilicate does not do this, I assume the glass is pulling toward the filament as it is cooling and forming the finale taper and tip. There is no way to avoid this when using filamented glass, not matter what changes you make to the parameter settings. Trust me, I have tried everything! Since most injections require filamented glass for successful backloading of the solution, this is something that has to be tolerated. I have found that this slight curve does not introduce added insult to the membrane or chorion and, not being inserted far into the egg, there is no added disruption to the cytoplasm. Borosilicate and Quartz do not exhibit this behavior. If you are needing perfectly straight tips and need harder glass, Quartz is the way to go.

7) Beveling Aluminosilicate Glass – If you are using Aluminosilicate Glass with a filament, there will be a final “eye lash” type curve along the end of the pipette. If the pipette tip is curved to one side or upward, it will be hard to bevel as it makes contact with the beveling plate. When the glass is just above the beveling plate, slightly loosen the glass clamp and rotate the glass so the tip points down toward the plate. This will make the outcome of beveling more reproducible.

Technical Data for Aluminosilicate & Quartz 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 Borosilicate. 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 %	Al_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	**SiO_2%**	**Li %**	**Na %**	**Al %**	**K %**	**Ca %**	**Fe %**
Quartz Heraeus HSQ 300	46.7	0.5	0.2	15	0.3	0.5	0.1

GLASS TYPE	Annealing Point	Softening Point
Borosilicate Pyrex Corning 7740	560 °C	821 °C
Aluminosilicate Schott 8252	725 °C	935 °C
Quartz Heraeus HSQ 300	1710 °C	1220 °C

Technical Data Sheet for 7740 Borosilicate Glass
https://www.corning.com/media/worldwide/csm/documents/fiches%20Verre%20pour%20poudre%207740.pdf

Technical Data Sheet for 8252 Aluminosilicate Glass
https://media.schott.com/api/public/content/48e4d860d8434f6d817379c5072bfc73?v=31b97bd9

Technical Data Sheet for HSQ 300 Heraeus Quartz Glass
https://www.heraeus-conamic.com/media/Media/Documents/Products_and_Solutions/SEMI/EN/Solids_HSQ300_330MF_EN.pdf

Quartz glass (Heraeus HSQ300) - Reminder! P-97 & 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 the higher temperatures required to melt quartz can be only achieved using the **Sutter P-2000/G Puller**.
https://www.sutter.com/MICROPIPETTE/p-2000.html

CHAPTER 22 – Quartz Glass for Microinjection (using P-2000 Puller)

Microinjection Pipettes

The classic sharp pipette application is an intracellular recording electrode or a microinjection pipette. In this section we will discuss in detail the use of the P-2000/G for making microinjection pipettes. Microinjection pipettes are drawn from many different types and sizes of capillary glass and are formed into a range of geometries and tips sizes. The choice of glass type and size is the first step in producing a usable injection needle. Borosilicate is the most commonly used glass for a wide variety of applications, but when one is injecting through tough fibrous membranes or an egg with a hard cuticle or chorion, Quartz (or fused silica) and, to a lesser degree, Aluminosilicate, are superior to Borosilicate in strength, stiffness and the ability to form a durable tip.

Resistance and Geometry

The tip size and the geometry of the taper in contact with the tissue are usually the key factors determining if an electrode can successfully impale a cell or egg. Small tips and gradual, uniform tapers have an obvious advantage in terms of causing less damage when a cell or egg is impaled. They also tend to produce a high electrical resistance, which can add noise and make current injection 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 membranes. When a microelectrode is advanced into the membrane, it can cause a local compression of the tissue. After the microelectrode stops, the membrane will gradually expand back to its original form, causing any cell or egg that was injected to be carried up the microelectrode along with the outer membrane. This effect is most pronounced when the micropipette has a rapid change in the OD of glass behind the final taper, especially when that portion of the microelectrode must enter the tissue. As a result, it is ONLY advisable to use electrodes with a dramatic inflection near the tip, like a bee-stinger pipette, when you are injecting worms, or when the tip is only superficially being advanced into the cell/egg.

Advantages using Quartz Glass

1) Higher Durability and Hardness – There has been an increased interest in fabricating microinjection pipettes from Quartz glass since it provides most durable needles, has the highest chemical durability, and reduced electrical conductivity (for electrophysiology and recording applications) when compared to Borosilicate and aluminosilicate glass.

2) Extremely fine tips – While the original ratio of a borosilicate capillary's inner to outer diameter remains fairly unchanged over the total taper length, Quartz 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) High Melting Temperatures – Compared to Borosilicate and Aluminosilicate glass, Quartz requires extremely high temperatures (~1650 °C) to soften the glass. The heating filaments in the P-97 and P-1000 pullers cannot generate enough heat to melt Quartz, and the platinum filament will evaporate if you try. A burned out filament in your P-97 or P-1000 puller will result in a sad scientist, a disappointed PI, and possibly a little panic. Make sure to only use the Sutter P-2000/G CO2 Laser-based puller when pulling Quartz glass.

4) Sensitivity to Parameter Settings – Since Quartz glass has a shorter range of workable temperatures, you will find that this glass is more sensitive to small adjustments in the parameter settings. Even small changes to the settings can result in large changes to the morphology of the pipette. Please see the following tables for recommended settings to start with.

Quartz Glass General Look Up Tables

Quartz Glass – Thin Walled QF100-70-10

Type	Heat	Fil	Vel	Delay	Pull	Heat on Time (sec)
Bee-Stinger 3-4mm Taper	600	3	25	145	200	4.5
5-6mm Taper	600	3	70	150	175	3.65
7-8mm Taper	700	4	60	140	175	3.50
9-10mm Taper	750-775	4	60	130	200	2.82
10-13mm Taper	825-850	5	150	128	100	3.04

Quartz Glass – Thick Walled QF100-60-10

Type	Heat	Fil	Vel	Delay	Pull	Heat on Time (sec)
Bee-Stinger 2-3mm Taper	550	2	25	145	200	4.7
5-6mm Taper	550-600	3	70	175	175-200	3.75
7-8mm Taper	725-750	4	60	150	175	3.60
9-10mm Taper	800-825	4	60	150	175	2.87
10-13mm Taper	850-875	5	60	128	175	3.09

Quartz Glass – Thick Walled QF100-50-10

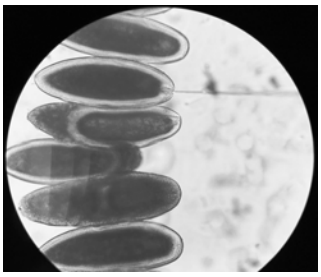
Type	Heat	Fil	Vel	Delay	Pull	Heat on Time (sec)
Bee-Stinger 2-3mm Taper	550	2	25	145	200	4.9
5-6mm Taper	600	3	70	175	175	3.8
7-8mm Taper	700	4	60	155	175	3.6
9-10mm Taper	750	4	60	130	200	2.9
10-13mm Taper	850	5	65	128	200	3.25

CHAPTER 23

Mosquito (Aedes Aegypti, Albopictus, Culex, Anopheles, etc.)

and

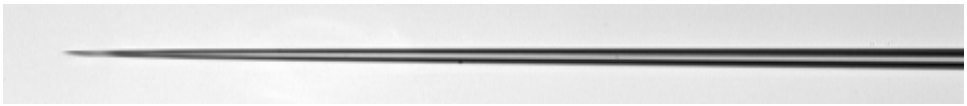
Fly (Drosophila, Stomoxys, Screw Worm, etc.)



The pipettes required for mosquito and fly egg injections are most commonly made using thin-walled Aluminosilicate or Quartz glass with a filament (AF100-64-10 or QF100-70-10). The P-97 and P-1000 can pull Aluminosilicate glass, but you must have a P-2000/G puller to heat and pull Quartz. Aluminosilicate and Quartz glass types are stronger and stiffer than Borosilicate glass and are both recommended in situations where the pipette is inserted through a chorion or tough fibrous membranes. Mosquito and Fly 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).

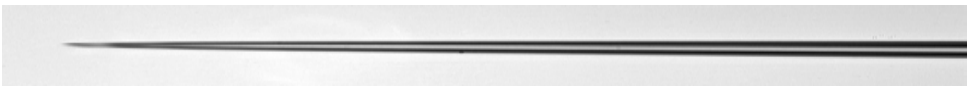
P-97/P-1000 Puller
Aluminosilicate Glass (AF100-64-10), 2.5mm Box Filament (FB255B)

HEAT	PULL	VEL	DEL	PRESSURE
Ramp + 5	70	70	60-90	300



P-2000/G
Quartz Glass (QF100-70-10 or QF100-60-10), Filament 4 or 5

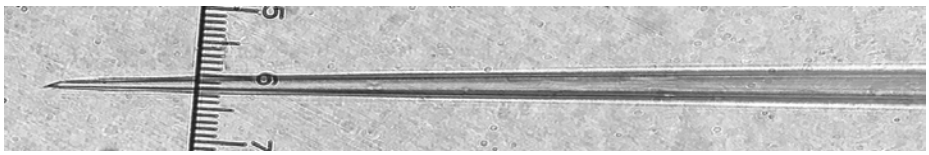
HEAT	FIL	VEL	DEL	PULL
825	5	60	150	175



Parameter Setting Adjustments

- Shorter Taper = Reduce Heat 10-unit increments
- Smaller Tip & Longer Taper = Increase Heat in 5-unit increments
- Reduce Shoulder behind tip for finer needle = Increase Heat in 10-unit increments

The Best Pipette is a Beveled Pipette! Especially if it is made of Quartz QF100-60-10 glass!



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

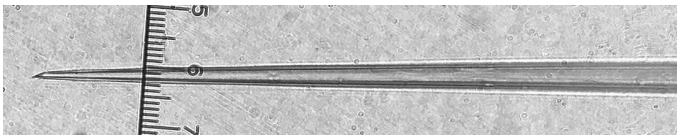
Using the P-2000 Laser-based puller and Quartz glass, the most durable pipettes for this application can be made. The chorion of the mosquito egg is exceptionally difficult to penetrate, and we have found that Quartz pipettes are the most durable and long lasting compared to the pipettes made with Borosilicate 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 to 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 1-3µm 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 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 hydraulic injector and create air bubbles in the air line. Once there is air in the oil-filled injection line of an oil – based 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.

XenoWorks (BRE) 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 3-7µm 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 µm beveled pipettes.

Primetech PMM4G Piezo Drill

If only mRNA and DNA injections are being performed, but cytoplasmic transfers (e.g. Wolbachia transfer) are not part of your protocol, it is not necessary to use a Piezo drill. But if a Piezo drill is part of the rig, it can be used to clean the pipette and allow one to use the injection pipette for a longer duration and much higher sequential series of injections 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.

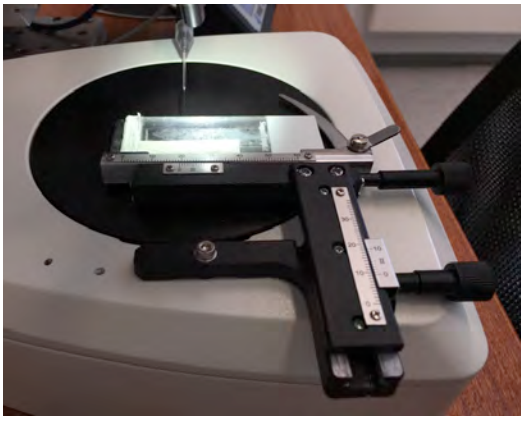
- Coating the Capillary Glass - GOOD
  Approximately 10ml of Sigmacote® was introduced inside the glass capillary, the glass was tilted end-to-end 4X to allow the solution to travel from one end to the other to coat the lumen of the glass and then the solution was blown out. The capillary glass was then 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.

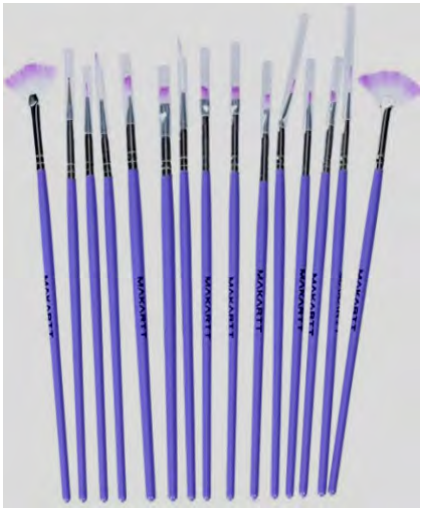
- Coating the Pipettes & Baking Dry - BAD
  After the pipettes were pulled, they were treated with Sigmacote® after they were pulled and bevelled was also tested, but upon baking dry the pipettes, the remaining nanoliters or picoliters of solution in the shaft of the pipette would travel to the tip and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

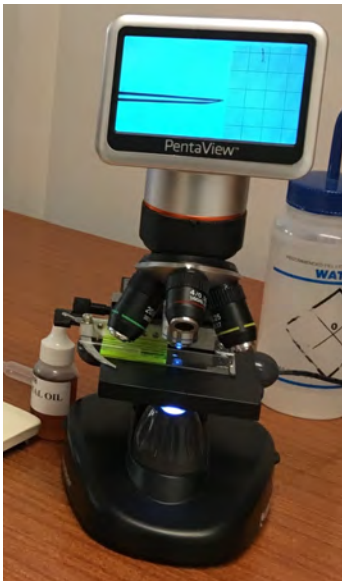
- Coating the Pipettes Right Before Use - GOOD
  The ID of the injection pipettes were also coating with Sigmacote® just after pulling and bevelling, and right before injecting, and this was found to be successful in making the pipettes less sticky. Approximately 0.5 microliters of Sigmacote® was front-loaded into the pipette and then expelled and then rinsed with water and then 100% ETOH. The tip was washed three times in each solution and allowed to air dry before use.

Additional Tools

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





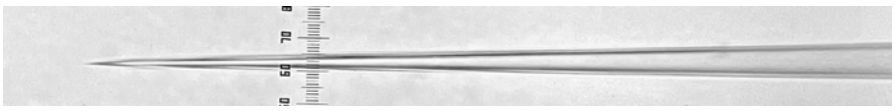


CHAPTER 24 – Microinjection Applications Requiring Quartz Pipettes

Mosquitoes, Cephalopods, Ctenophores, Tardigrades, Limulus,
Shrimp, Salmon, & Trout, Larvae, Juvenile & Adult Insects,
Ticks & Spiders

All of the above species have a fibrous membrane, cuticle, or chorion. Therefore, a Quartz microinjection pipette is usually required to penetrate the cell, egg, larvae or adult insect. Using the Sutter P-2000/G CO_2 laser-based puller and thin or thick-walled Quartz glass, sharp micropipettes were pulled to have a 9 to 11mm taper using the following range of settings:

HEAT	FIL	VEL	DEL	PULL
700-750	4	60	140	175



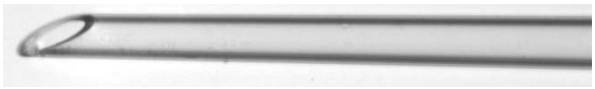
BV-10 Beveler

These pipettes were beveled using a 104D (fine) plate

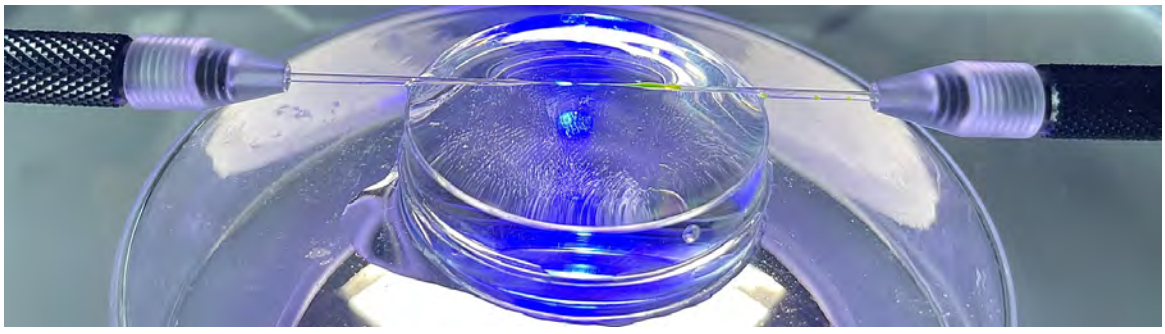
- 1-3µm tip = 5-15 seconds of beveling (1/4 turn of fine dial)
- 4-7µm tip = 15-30 seconds of beveling (1/4 turn of fine dial)
- 7-25µm tip = 30-60 seconds of beveling (after trimming)
- All pipette tapers need to be washed/cleaned with 75% ETOH after beveling.

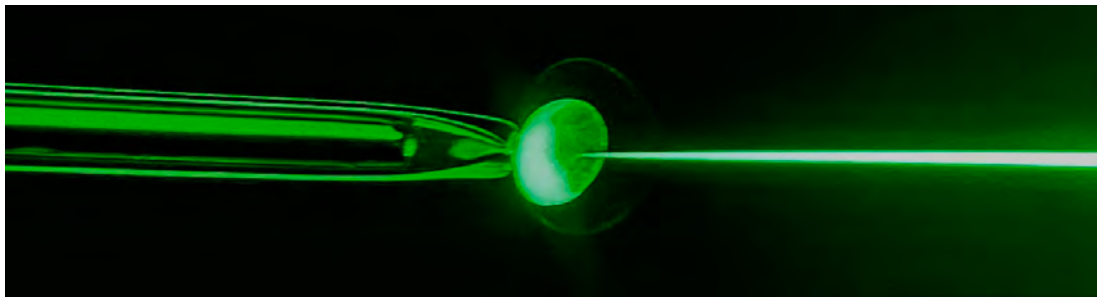
Mosquito, Cephalopod, Ctenophores, Tardigrades, Shrimp, Salmon, & Trout (1.5 - 3µm tip, 20° bevel)

Limulus, Larvae, & Adult Insects, Ticks & Spiders (15 - 25µm tip, 22° bevel)



Ctenophore Egg Injection: 100µm Holding Pipette (Borosilicate, B100-75-10), 3-5µm Beveled Injection Pipette (Quartz, QF100-70-10)

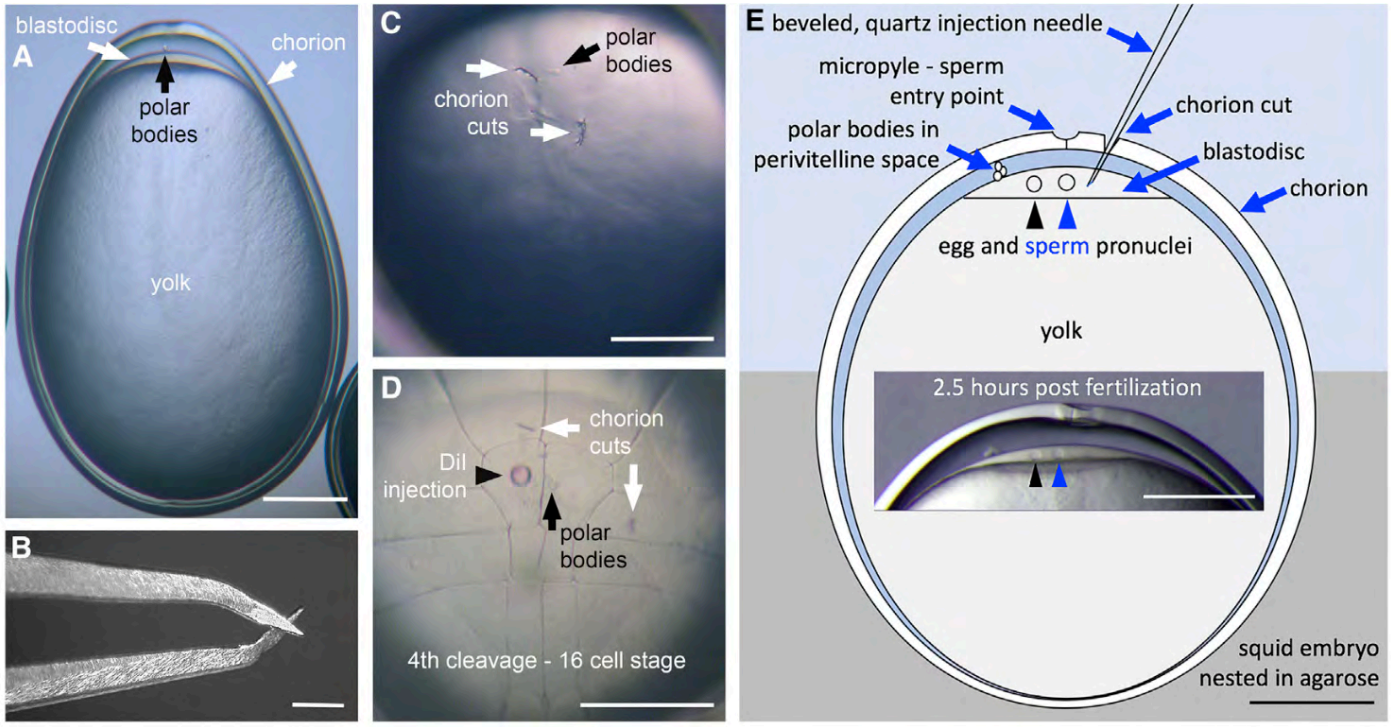


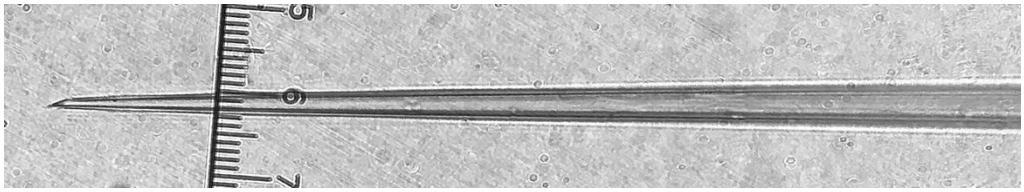


Protocol & Photo Courtesy: **Oscar Arenas Sabogal**
2021 Grass Fellow, 2023-2025 Grass Lab Associate Director

Cephalopod Injections

The main difference between squid species is the chorion strength, so in addition to using a beveled Quartz injection needle, some species like Doryteuthis require the physical cutting of the outer layer of the chorion.





Karen Crawford, Juan F. Diaz Quiroz, Kristen. M. Koenig, Namrata Ahuja, Caroline B. Albertin, Joshua J.C. Rosenthal

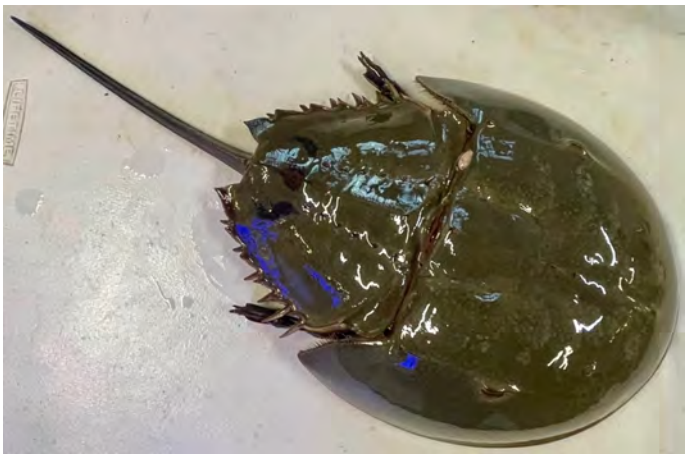
Highly Efficient Knockout of a Squid Pigmentation Gene
https://www.sciencedirect.com/science/article/pii/S0960982220309854

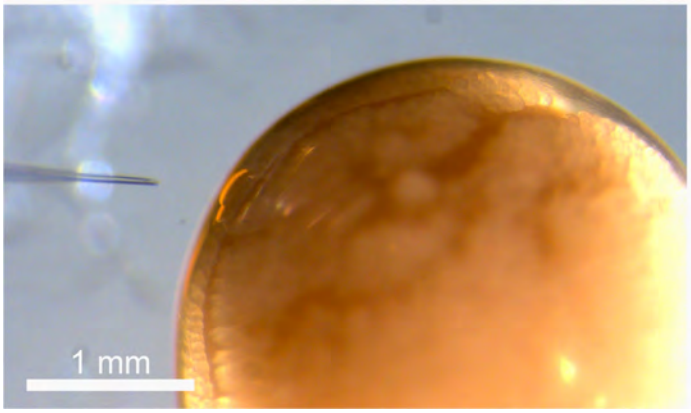
Scientists 'knock out' squid gene for the first time
https://news.uchicago.edu/story/scientists-knock-out-squid-gene-first-time

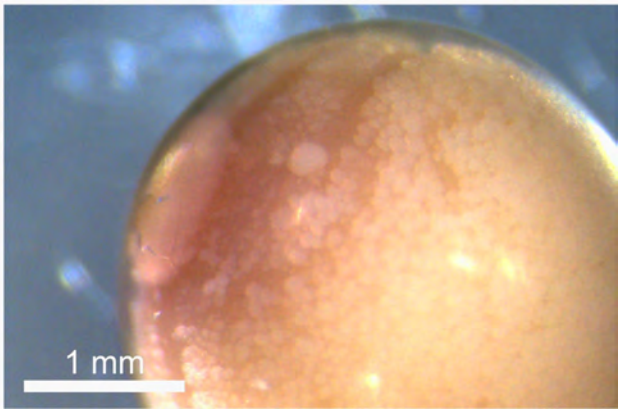
Creation of an albino squid line by CRISPR-Cas9 and its application for in vivo functional imaging of neural activity
https://www.sciencedirect.com/science/article/pii/S096098222300739X

Embryonic development of the camouflaging dwarf cuttlefish, *Sepia bandensis*
https://anatomypubs.onlinelibrary.wiley.com/doi/full/10.1002/dvdy.375

Limulus (Horseshoe Crab) Injection





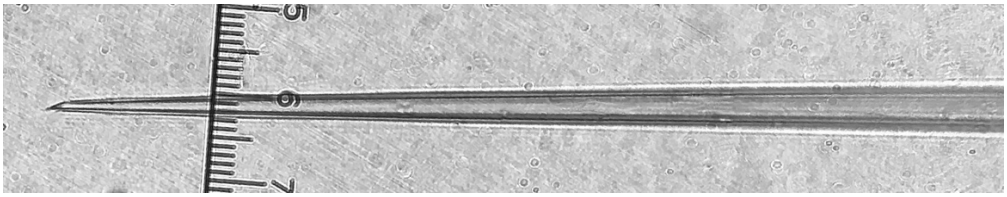


Guilherme Gainett, MBL GRASS FELLOW 2024
https://grassfoundation.org/people/guilherme-gainett/

Limulus embryos have an outer mucous layer which is first removed with forceps. The outer membrane, the chorion, is tough and elastic and is challenging to inject through. To solve this issue, a 27G needle or a disposable scalpel can be used to open a small hole into the outer chorion membrane, which will still leave a thin membrane intact under the chorion. Embryos can be held with forceps and injected under FASW through the chorion hole using a quartz needle (QF100-60-10), beveled at ~22º to have a 20-25µm tip opening.

Embryonic microinjections are performed using a Sutter XenoWorks Microinjection System: XenoWorks (XWMR) Micromanipulator and a XenoWorks (BRE) Digital Microinjector. Injection pressures ranged from 400-1200hPa. The range of internal pressures of the horseshoe crab can vary, so the compensation and injection pressures need to be adjusted accordingly. The injected volume (co-injected with phenol red) was empirically determined to produce a visible red volume inside or at the periphery of the yolk. Each embryo received approximately 160nL of injection mix.

HEAT	FIL	VEL	DEL	PULL
700-750	4	60	140	175



Sutter Equipment for Quartz Pipette Microinjection Applications:

Mosquitoes, Cephalopods, Ctenophores, Tardigrades, Limulus,
Shrimp, Salmon, Trout, Larvae, Juvenile &
Adult Insects, Ticks and Spiders.

Depending on the size of the egg and how it is needs to be secured, some of these applications require a holding pipette (Chapter 17) on the one side and an injection pipette on the other. The holding pipette does not need to be moved or repositioned as much as the injection needle, so an XW-225 manipulator with the ROE interface is sufficient. The injection pipette will need to have more fluidity and simultaneous motion for all three X, Y and Z axes to conduct accurate and efficient injections. Therefore, we recommend the XWMR XenoWorks manipulator with Joystick for the injection pipette.

XW-225 for Holding Pipette

XWMR for Injection Pipette

BRE Digital Injector
Please refer to page 95
"BRE Digital Injector Cheat Sheet"

P-2000/G
QF100-70-10 or QF100-60-10
Quartz Glass

CHAPTER 25 - CRAZY LAB LORE

Misconceptions, Old Wives' Tales & Superstitious Beliefs

Crazy Lab Lore #1: Never Touch the Filament.

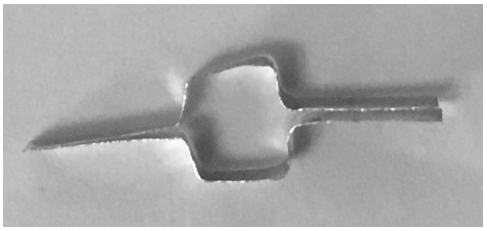
ANSWER = FALSE!

You CAN touch the filament when installing it, reshaping it or realigning it . . . . but obviously not while it is hot! It is best to avoid touching the filament with the glass since this can bend or dent the filament.

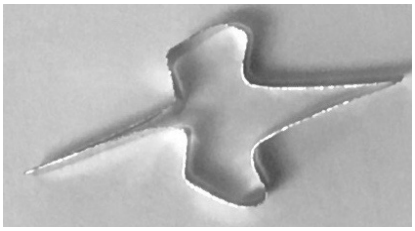
When handling the filament, you just need to be gentle. It is not necessary to use gloves, but be very careful not to smash or bend the filament while holding onto it. It does not matter if the oils from your fingers get onto the filament since any oils or debris from your fingers will simply burn off the filament the first time it heats up (when running a ramp test or when pulling a pipette). The oils or debris on the filament will burn off even before the glass softens. You might even see a tiny poof of smoke when this happens.

If the filament becomes smashed or dented with the glass running into it, the heating characteristics of the filament will change. And, on occasion, when replacing the filament and 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, it is okay to 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.

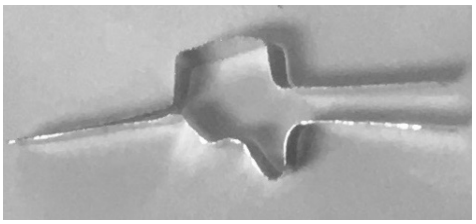
RESHAPING the FILAMENT



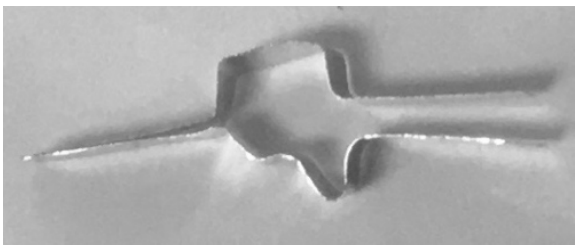
GOOD = Uniform Square

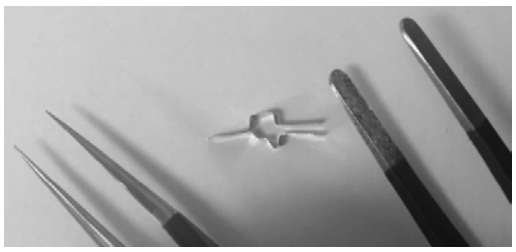


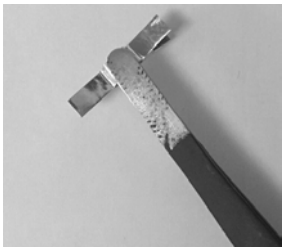
Squashed = Tall Rectangle



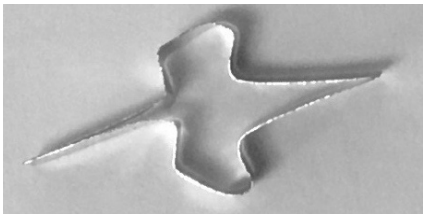
Smashed = Collision with glass

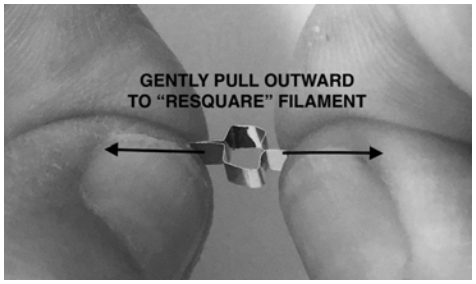


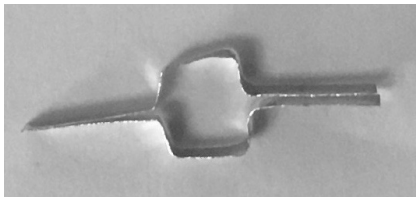




Use flat needle nosed forceps to flatten the top and/or bottom wall to "re-square" the filament.







Pull the wings of the filament outward to reshape it.

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 that the oils from one's fingers that are transfer to the glass will damage the filament or the puller. 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. So, oils on the glass are not of concern when using a filament-based puller like the P-97 and P-1000.

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.

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

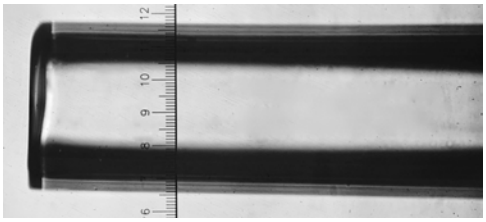
Crazy Lab Lore #3: You always need to wash the glass before you use it.

ANSWER = FALSE

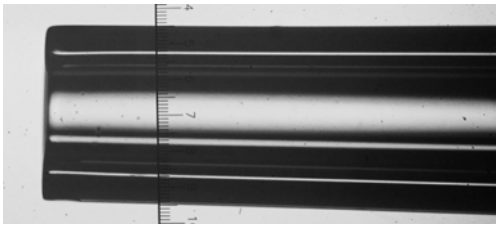
Sutter capillary glass is **pre-washed** and does not need to be washed in the lab unless you are requiring RNase free conditions. For more details on RNase and decontamination of the capillary glass, please research using Invitrogen RNaseZap or RNase Away solutions.

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

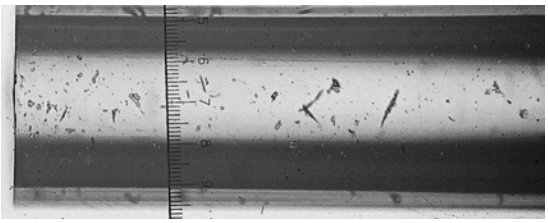


Sutter Thin-Walled Glass– cleaned

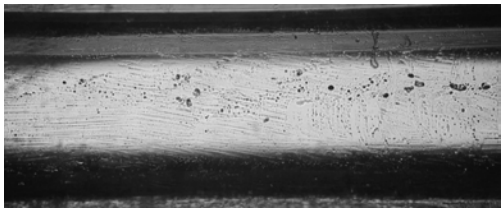


Sutter Thick-Walled Glass – cleaned

DIRTY GLASS



Glass from another supplier, unpolished with sharp edges & debris



Glass handled with oily fingers

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.

Crazy Lab Lore #4: Very strange things can end up inside the puller.

ANSWER = TRUE

In addition to broken glass, broken heating filaments, and one time a large screwdriver left inside a puller when someone attempted a repair; we discovered this perfectly preserved reptile. He looks a bit thirsty.

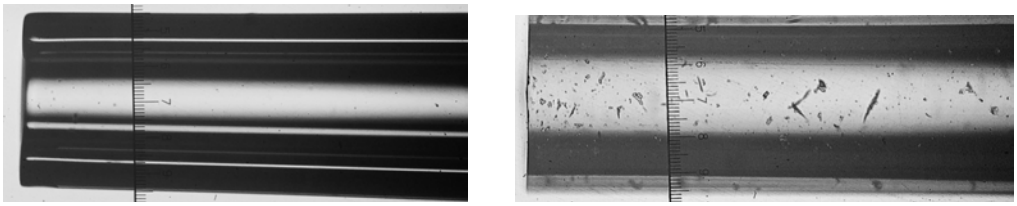
Crazy Lab Lore #4: You need to fire-polish the back ends of the glass by hand, one at a time, piece by piece, over a flame, in the lab & wasting your precious research time!

ANSWER = FALSE when using Sutter Glass

ANSWER = TRUE when using non-Sutter glass or when you want to punish the T.A. or the post-Doc!

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.

POLISHED ENDS **NON-POLISHED ENDS**



Sutter Glass with Smooth Polished Ends Capillary glass from another supplier

When the ends of the glass are not polished, the glass 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. When the back ends of the glass are not clean cut and polished, the rough sharp edges will generate small glass fragments which will potentially clog the pipette tip when backfilled with solution. In addition, the rough sharp edges of the glass will also damage the O-rings and gaskets inside the head stage or pipette holder.

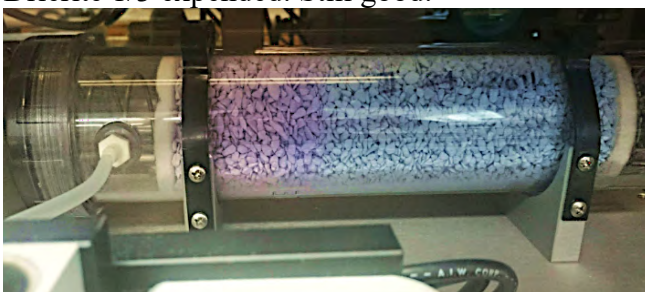
Crazy Lab Lore #5: It's okay if ALL the Drierite is Pink or Lavender

ANSWER = FALSE

The Drierite in the canister is 8 MESH Drierite with a Calcium Chloride indicator. The stones are blue when it is dry and turn pink when the Drierite becomes moist. The air used to cool the glass and filament is filtered through the Drierite canister to remove moisture. The cooling air should be dry since humidity will introduce variability to the rate of cooling, and this will affect 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. The dry air from the Drierite canister is 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 seasons or in humid environments, we recommend that you increase the "Air Time Before Pull" to 10 seconds to purge the humidity control chamber. For the P-97, go into the control settings and selecting functions 4 & 5 to change the Air Time before and after the pull. For the P-1000, go into the menu of the program and change the air time.

Drierite 1/3 expended. Still good.



Drierite completely expended. Time to replace it!

Instructions on how to replace the Drierite: https://www.youtube.com/watch?v=BLvYLLJmcnY

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

Crazy Lab Lore #6: You must turn on the puller and let it heat up before pulling.

ANSWER = FALSE

There are no internal or external components on the puller that need to heat up. But if you find that your program is more stable after pulling a few pipettes to get the jaws warmed up, this is probably due to the program being a little unstable. If you have a P-1000 Puller, you can use the Pre-Heat Mode to raise the heat to 70 °C and this will be maintained as you pull your electrodes. If you have a P-97, you can put a rubber band around the puller bars, holding them together, and with NO glass loaded, press pull. The heat will stay on for 1.5min and time out with an error message. This will bring your jaws up to 45-55°C and you can proceed from there, obviously removing the rubber band first! Remember, most instability of a Patch Program is due to the filament not being centered over your air jet, and not using the mid-point velocity. See pages 17 & 18, and pages 30-35. Contact Sutter for help troubleshooting.

Crazy Lab Lore #7: You need to let the puller cool down between pulls.

ANSWER = FALSE

If you find that your program is unstable after pulling a series of pipettes and as the jaws warmed up, this is due to the program being a bit unstable. Remember, most instability of a Patch Program is due to the filament not being centered over your air jet, and not using the mid-point velocity. See pages 17 & 18, and pages 30-35. If you are using a Heat setting near or at the Ramp Test Value, the Heat-On-Time at the end of pull is not over 25 seconds, and you are using a mid-point velocity, these factors should create enough stability in the program, even when the jaws heat up from 23°C to 70°C.

Crazy Lab Lore #8: I can see dark gray debris on the shoulder of my glass. My glass must be getting charred.

ANSWER = FALSE

When the filament heats up, microscopic particles of the platinum:iridium evaporate off of the surface of the filament. Since the glass is molten at this time of pulling, these black particles will get incorporated into the shoulder of the glass taper. As a result, the taper of your pipette will appear gray in color. This gray shoulder be more apparent when a new filament has been installed, when the heat settings are too high or too low, or when making patch pipettes since they are exposed to multiple stages of heating. The microscopic particles incorporated into the shoulder of the taper are inert and will not effect your experiment.

CHAPTER 26 - 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 Aluminum 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:* See Chapter 25 for instructions on reshaping your filament or replace the filament with a new one.
- **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, go to pages 17 & 18 and following the instructions. You will need to pull a long-tapered pipette with a and compare the taper length of the right and left pipettes. If by eye they are NOT EXACTLY identical in length, loosen the filament clamp screws and "nudge" the filament in the direction of the shorter pipette. Repeat this procedure until both pipette tapers are the same length. 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 (pg. 12) and adjust your heat values accordingly. If you are switching from a trough filament to a box filament (pg. 87), your new ramp value could increase two-fold.
- **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 ensure 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 or P-1000 Puller to Sutter to be refurbished! The electronics will be upgraded, worn parts will be replaced, and you will get an extended one year warranty. This is often recommended for pullers that are 10-15 years old.

“The 15 Questions”

When using the Sutter P-97 and 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 determine 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 and 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 require?

7) What OD and ID (outer and inner diameter) of glass are you using?

8) What is the Item number of the capillary glass?

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

10) Are you using a one-line program?

11) If you are using a one-line program, know how many times does the program loop.
    Please also check line 2 to make sure it is blank

12) If you are using 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. We need a top view and a horizontal view (from the left looking into the right) with and without with the glass in place. Send 4 pictures total.

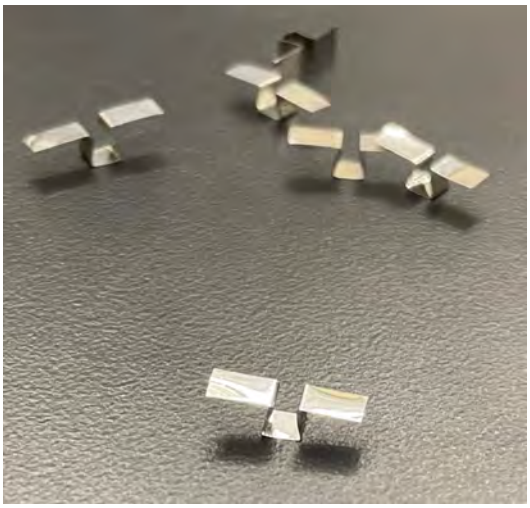
14) Push the puller bars all the way back (in the spring lock position) and to lift the cable off the black or silver pulley (wheels the cables are guided through). Spin each black or silver pulley and to see if it spins freely and multiple times around. Is there resistance or uneven friction?

15) There is also a pair of pulleys inside the puller and these too might need to be examined. Remove the front panel screws using a medium/large Phillips head screwdriver and drop down the front panel like a toaster oven door. The pulleys are mounted above the pull solenoid and below the base plate.

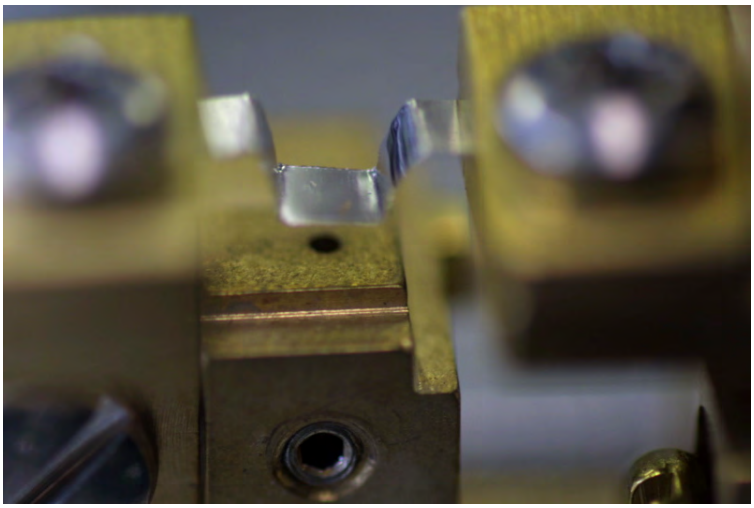
Providing a response to all questions will accelerate our ability to troubleshoot and resolve the problems you are encountering. If it is determined that the puller has an electronic and/or mechanical issue that cannot easily be addressed on-site, we recommend you return the puller to the Sutter Factory for a REPAIR or a REFURBISH. Contact ADAIR@SUTTER.COM and RMA@SUTTER.COM to arrange for a repair or refurbish.

CHAPTER 27 – TROUGH FILAMENT

Installing a 3mm x 3mm Trough Filament (FT330B)



GOOD SHAPE

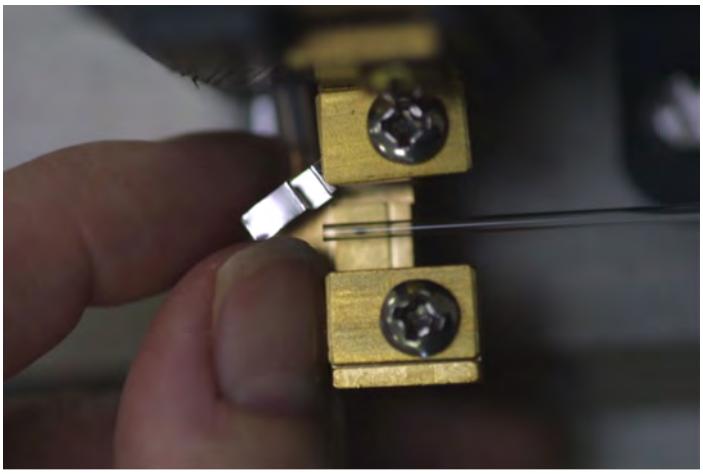


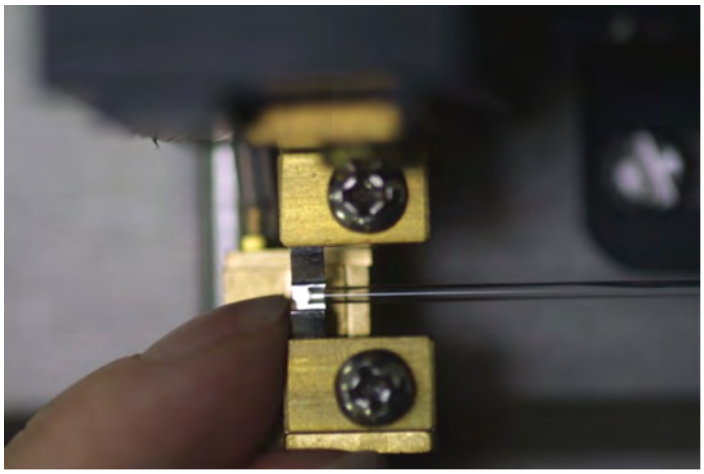
BAD SHAPE

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.

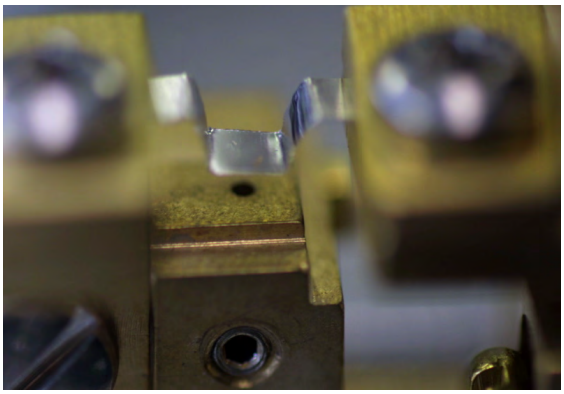
BAD filament shape: A "Bad" trough filament shape is when the walls are angled outward. In this situation the heat escapes out the top and will provide inefficient heating to the glass. This will cause the ramp values of the standard 3mm trough filament (FT330B) to climb over 300 units, and you will have a high probability of burning out your filament. If your ramp test values is above 320 units, it is best to remove the filament and angle the walls inward. 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 320 units for your heat.

Installing a TROUGH 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. When the filament is sitting 0.5 to 1mm in from the left edge of the brass jaws, it is usually well centered over the air jet.

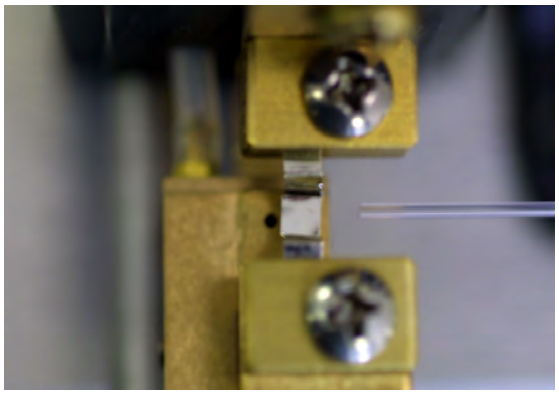




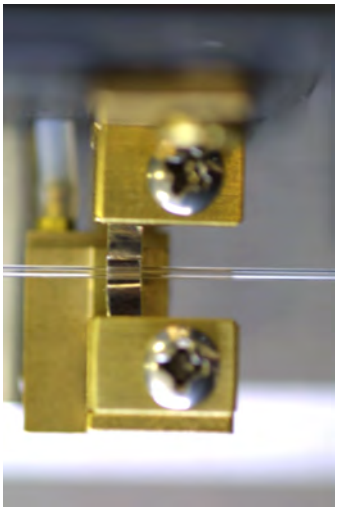
Trough Filament continued



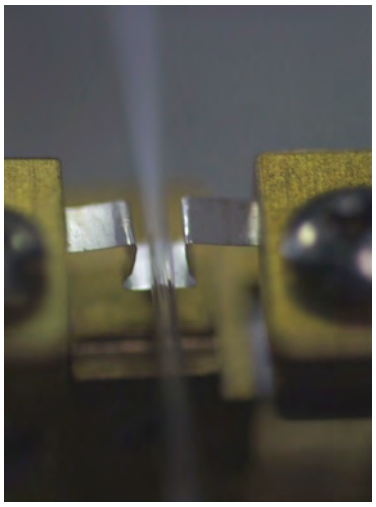
BAD SHAPE - Walls angled outward.



BAD ALIGNMENT – filament too far right



Good - Filament centered over Air Jet



Good - Filament centered around glass

Good - Glass sitting low and 0.5 to 1mm above the base of a Trough Filament

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.

STEP 2.
Slide down 3mm and pry up to adjust.

STEP 3.
Loosen bottom brass screw.

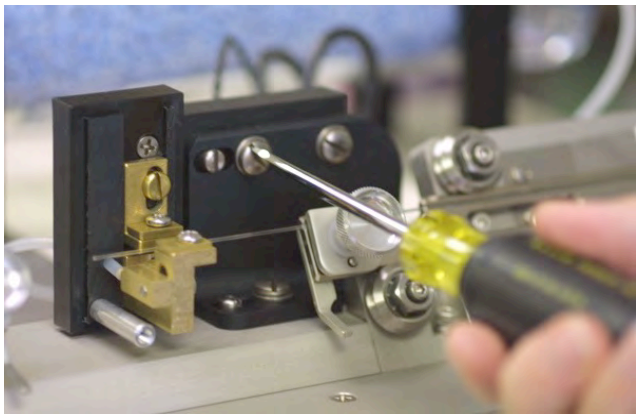
STEP 4.
Slide jaw down 3mm and pry up to adjust.

ECCENTRIC ADJUSTMENTS

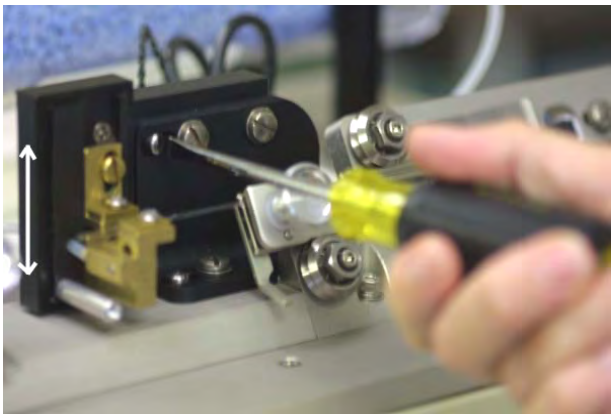
Large adjustments to Box or Trough Filament Position (more than 1 to 2mm)
If you find that the glass is not centered in the filament from top to bottom, it is best to make large adjustments by adjusting the brass jaws (pg. 89). If the filament is not centered in the filament front to back (the glass 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)



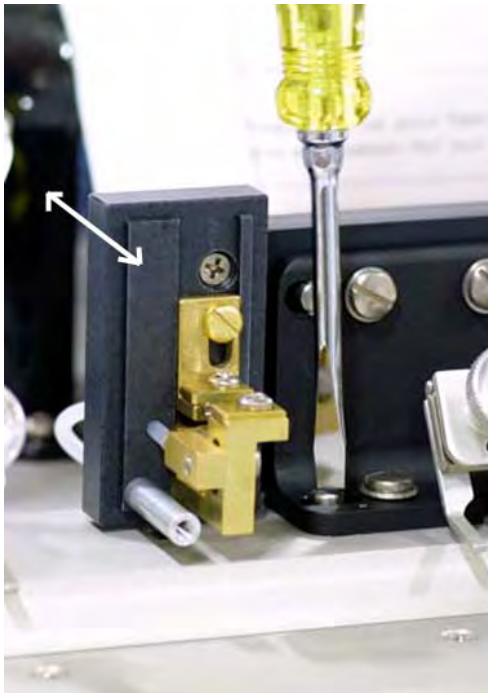
Loosen the locking screw



Turn Eccentric Screw

Horizontal Eccentric Adjustment (Moving Filament Forward and Back)

Loosen the locking screw



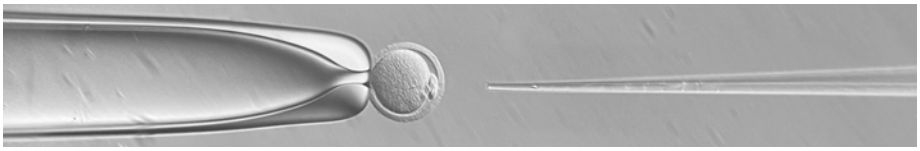
Turn Eccentric Screw

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

CHAPTER 28 - XenoWorks Microinjection System Configurations

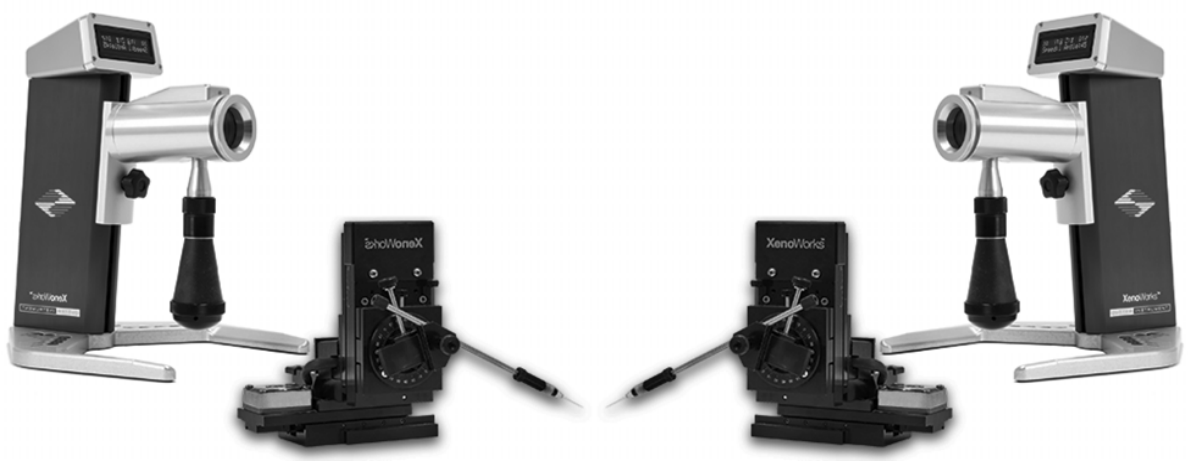
Using our 50 years of experience and expertise in motorized micropositioning, air pressure control and electro-mechanical design, we have created the XenoWorks™ Microinjection System. The XenoWorks System has been designed for a wide variety of applications for the manipulation of cells and embryonic tissues. By combining micromanipulators, microinjectors and microscope mounting adapters, the XenoWorks system can be configured for a wide variety of applications.

APPLICATIONS INCLUDE (but not limited to): CRISPR, Pronuclear Injection, ICSI & Piezo ICSI, ES Cell, Nuclear Transfer, Adherent Cell, Drosophila, *C.elegans*, Mosquito, Fly, Moth, Butterfly, Beetle, Zebrafish, Cephalopod, Squid, Medaka, Salmon, Trout, Ctenophore, Tardigrade, Sea Star, Limulus, Xenopus, Zebrafish Injection, Spider, Tick, Ant, Larvae and Adult Insect Injections.



Marine Biological Lab, Frontiers in Reproduction Course (FIRs), Woods Hole, MA

XenoWorks Micromanipulator – XWMR & XWML



Features include:

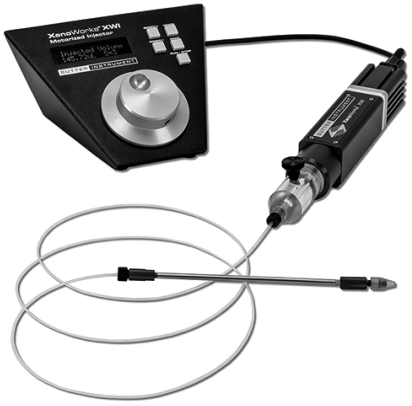
- Stepper Motor Control
- Joystick Interface
- 8 speeds, course to very fine
- 2 Work, 1 Home, Z-limit, X-limit memory positions
- Y-axis lockout for pure X-axis travel
- Adjustable Joystick (height and tension)
- Touch Declutch
- Set up routine
- Pulse Mode

XenoWorks BRE 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
- Compensation pressure
- Remote User Interface
- Clear Mode

XenoWorks XWI Motorized Hydraulic Injector



Features Include:

- Used for holding, injection, aspiration and transfer
- Course to fine control (4 speeds)
- Use with air, water or oil
- Programmable volume injections

Recommended Configurations for the XenoWorks Microinjection System

The configuration of the required 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.

CRISPR, Pronuclear & Cytoplasmic Injection

Suggested system configuration:

1 x XWMR - XenoWorks Micromanipulator (Right)
1 x XWML - XenoWorks Micromanipulator (Left)
1 x BRE - XenoWorks Digital Injector
2 x XenoWorks Microscope Adapters (microscope make/model)

Mosquito, Drosophila, Adherent Cell

Suggested system configuration:

1 x XWMR - Micromanipulator w/ Joystick (Right)
1 x BRE XenoWorks Digital Injector
1 x XenoWorks Microscope Adapter (specify microscope make/model)

Zebrafish, *C.elegans,* Xenopus, Cephalopod, Moth, Butterfly, Beetle

(For any large fish and insect eggs)

Suggested system configuration
1 x BRE XenoWorks Digital Injector
XW-225 – Micromanipulator w/ ROE
MT-81-DOV8 – Low profile gantry stand
Mag Feet & Metal Plate

Embryonic Stem Cell Transfer into Blastocysts

Suggested system configuration:

1 x XWMR - XenoWorks Micromanipulator (Right)
1 x XWML - XenoWorks Micromanipulator (Left)
1 x BRE - XenoWorks Digital Injector or 2 x XWi Hydraulic Injectors
2 x XenoWorks Microscope Adapters (specify microscope make/model)

ICSI or Piezo-Assisted ICSI

Suggested system configuration:

1 x XWMR - Micromanipulator (Right)
1 x XWML - Micromanipulator (Left)
2 x Hydraulic Injectors (contact Sutter)
2 x XenoWorks Microscope Adapters (specify microscope make/model)
1 x PMM6 Primetech Piezo (for Piezo ICSI)

Nuclear Transfer

Suggested system configuration:

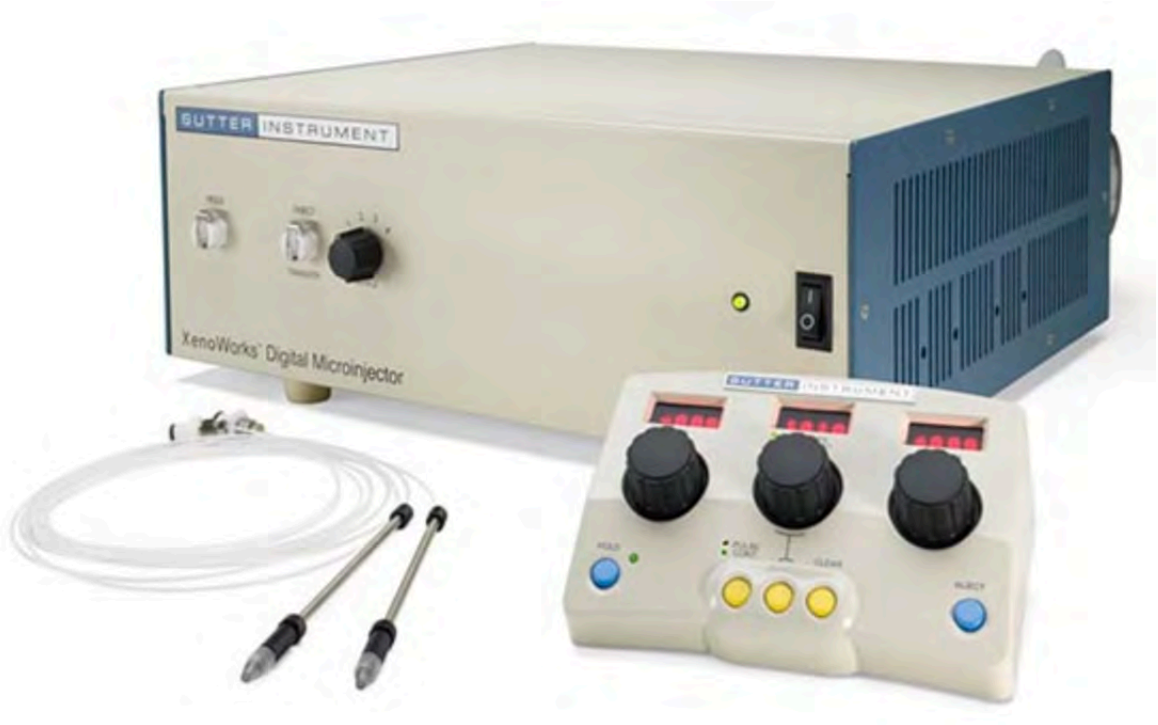
1 x XWMR - Micromanipulator (Right)
1 x XWML - Micromanipulator (Left)
2 x XWI – XenoWorks Hydraulic Injector
2 x XenoWorks Microscope Adapter (specify microscope make/model)

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

BRE Digital Injector Pressure Settings Cheat Sheet

Sutter Instrument XenoWorks Digital Injector (BRE) Pressure Settings

Application	Taper Length	Tip Size	Glass Size & Special Note	"Pipette Type" in Pipette Cookbook	Compensation Pressure (hPa)	Injection Duration (seconds)	Injection Pressure (hPa)	Injection Pressure (psi)
Adherent Cell (5-10µm Neurons)	7-8mm	0.1-0.3µm	Glass: BF100-78-10 Slightly larger tips are needed for injecting beads or quantum dots.	Type C	20 - 40	0.20	500-1000	7.5 - 15 psi
Adherent Cell (20-40µm Cells)	4-6mm	0.3-0.5µm	Glass: BF100-78-10 or BF100-58-10 Tip may be "tapped off" (broken back) once tip can not be cleared.	Type B	20 - 40	0.20	300-800	4.5 - 11.5 psi
Pronuclear & Cytoplasmic Injection	7-9mm	0.3-0.5µm	Glass : BF100-78-10 Tip may be "tapped off" (broken back) once tip can not be cleared.	Type C	20 - 40	0.20	400-1200	4.5 - 15 psi
C.Elegans	6-8mm	0.5-0.9µm	Glass : BF100-78-10 or BF100-58-10 Tip beveled or broken back to create a **1-3µm** opening	Type B	5 - 10	0.20	100-200	1.5 - 3.0 psi
Drosophila	6-8mm	0.5-0.9µm	Glass: BF100-78-10 Tip beveled or broken back to create a **1-3µm** opening	Type B	5 - 10	0.20	100-200	1.5 - 3.0 psi
Zebrafish	6-8mm	0.5-0.9µm	Glass: BF100-78-10 Tip beveled or broken back to create a 6-10**µm** opening	Borosilicate, Aluminosilicate or Quartz Glass Trim back and/or bevel	5 - 10	0.04	4500 - 5000	65 - 72.5 psi
Xenopus	10-15mm	0.5-0.9µm	Glass: BF100-78-10 Tip beveled or broken back to create a 3-**7µm** opening	Type D & E	5 - 10	0.2 -0.5	50-200	0.75 - 3.0 psi
Sea Urchin Eggs SQUID Mosquito Egg	7-9mm	0.3-0.5µm	Glass: AF100-64-10 (Aluminosilicate glass) Glass: QF100-70-10 (Quartz glass) Tip beveled or broken back to create a **0.5-2µm** opening	Aluminosilicate = Type C Quartz = P-2000 Puller then bevel BV-10 Beveler & 104C plate	10 - 20	0.2 -0.5	200-400	3.0 - 5.75 psi
Small Fish Egg (100-500µm)	8-10mm	0.3-0.5µm	Glass: AF100-64-10 (Aluminosilicate glass) Glass: QF100-70-10 (Quartz glass) Tip beveled or broken back to create a **0.5-2µm** opening	Aluminosilicate = Type C Quartz = P-2000 Puller then bevel BV-10 Beveler & 104C plate	10 - 20	0.40	500-1000 or 200-500 (if broken back)	7.5 - 15 psi or 3.0 - 7.5 psi
Large Fish Egg (500-1500µm)	8-10mm	0.3-0.5µm	Glass: AF100-64-10 (Aluminosilicate glass) Glass: QF100-70-10 (Quartz glass) Tip beveled or broken back to create a **0.5-2µm** opening	Aluminosilicate = Type C Quartz = P-2000 Puller then bevel BV-10 Beveler & 104C plate	10 - 20	0.40	500-1000 or 200-500 (if broken back)	7.5 - 15 psi or 3 - 7.5 psi



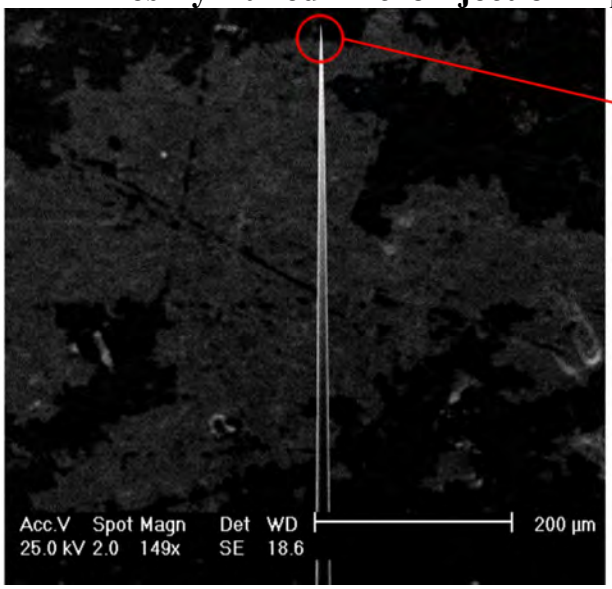
CHAPTER 29 - Pre-Pulled Microinjection Pipettes

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.

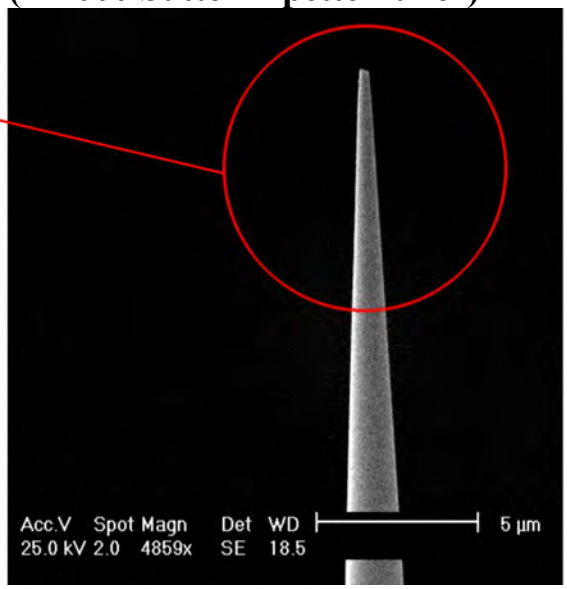
As a quality control measure, Sutter Instrument routinely performs scanning electron microscopy on pipettes pulled from each and every pipette puller we sell. During the past ten years we have, on occasion, been asked by customers to examine the pre-pulled pipettes they had purchased from a manufacturer. 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).

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

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

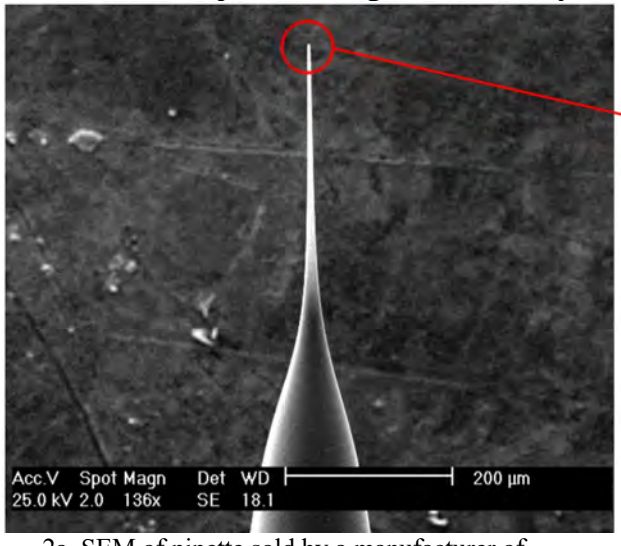


1a. SEM of freshly pulled pipette (~140x)

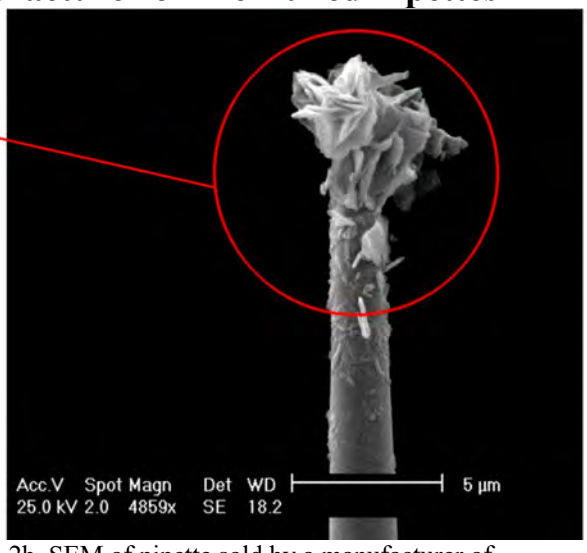


1b. SEM of freshly pulled pipette (~5,000x).

Microinjection Pipette Sold by a Manufacturer of Pre-Pulled Pipettes



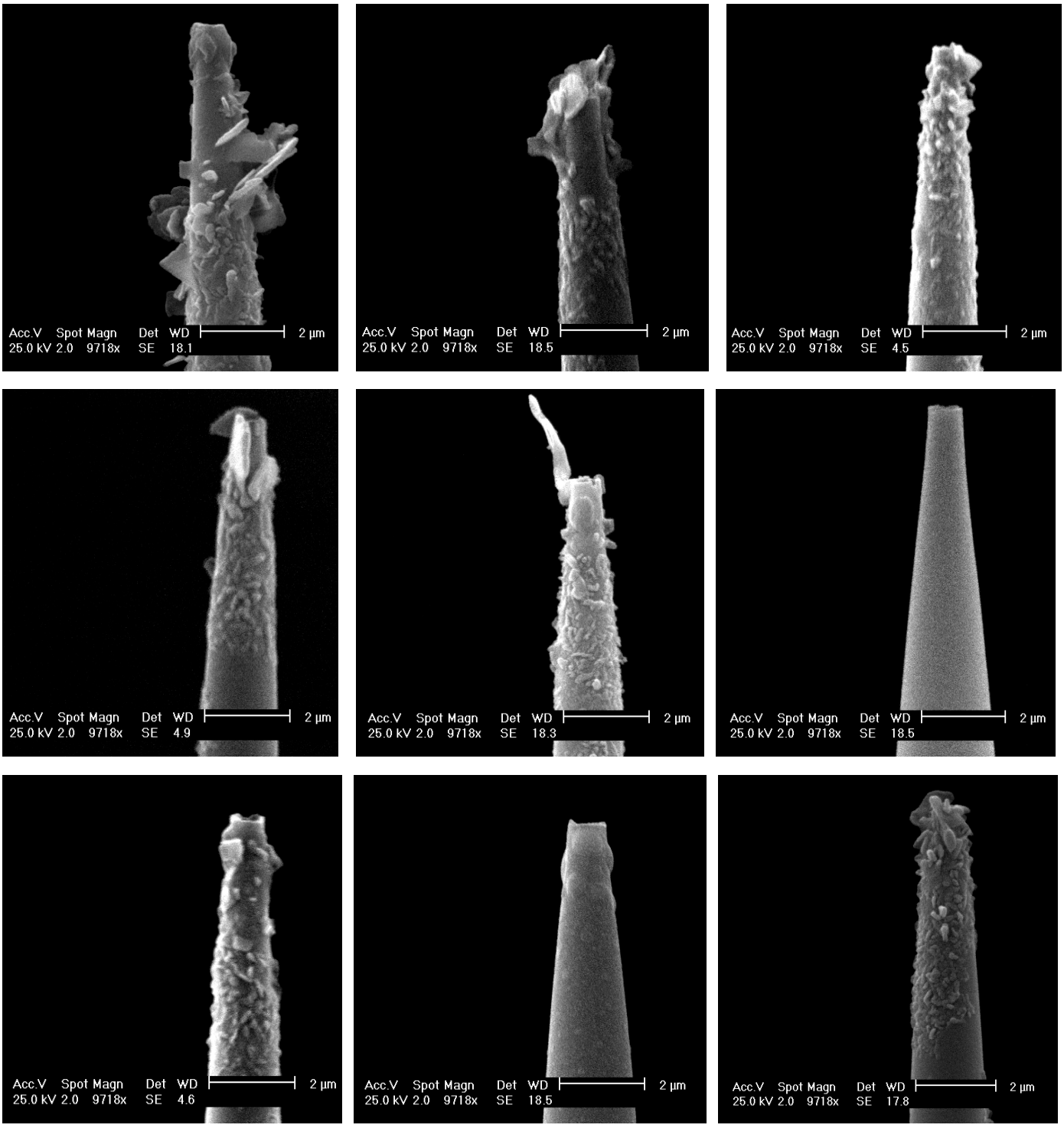
2a. SEM of pipette sold by a manufacturer of pre-pulled pipettes (~140x).



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.

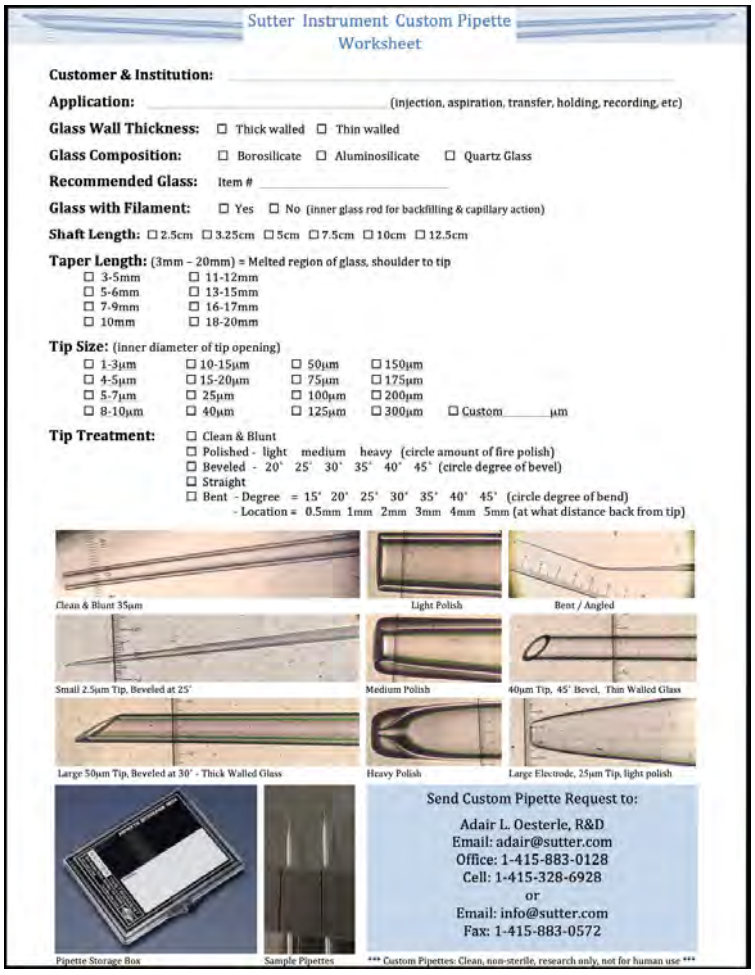


SEM analysis of microinjection pipettes sold by a manufacturer of pre-pulled pipettes (~10,000X mag).

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 performed to identify these artifacts.

Sutter Custom Pipettes

Custom Pipettes purchased through Sutter and hand made for each order and are intended for non-human research only. The pipettes are forged and/or beveled and then cleaned. The custom pipettes are then packaged in a dust free BX-10 Pipette Storage Box. The pipettes are not sterile, but the taper and tip have been triple washed in medical grade H2O and ETOH, then air dried.



Sutter Instrument Custom Pipette Worksheet

Customer & Institution: _____

Application: _____ (injection, aspiration, transfer, holding, recording, etc)

Glass Wall Thickness: ☐ Thick walled ☐ Thin walled

Glass Composition: ☐ Borosilicate ☐ Aluminosilicate ☐ Quartz Glass

Recommended Glass: Item # _____

Glass with Filament: ☐ Yes ☐ No (inner glass rod for backfilling & capillary action)

Shaft Length: ☐ 2.5cm ☐ 3.25cm ☐ 5cm ☐ 7.5cm ☐ 10cm ☐ 12.5cm

Taper Length: (3mm – 20mm) = Melted region of glass, shoulder to tip

☐ 3-5mm ☐ 11-12mm
☐ 5-6mm ☐ 13-15mm
☐ 7-9mm ☐ 16-17mm
☐ 10mm ☐ 18-20mm

Tip Size: (inner diameter of tip opening)

☐ 1-3µm ☐ 10-15µm ☐ 50µm ☐ 150µm
☐ 4-5µm ☐ 15-20µm ☐ 75µm ☐ 175µm
☐ 5-7µm ☐ 25µm ☐ 100µm ☐ 200µm
☐ 8-10µm ☐ 40µm ☐ 125µm ☐ 300µm ☐ Custom_____µm

Tip Treatment:

☐ Clean & Blunt
☐ Polished - light medium heavy (circle amount of fire polish)
☐ Beveled - 20° 25° 30° 35° 40° 45° (circle degree of bevel)
☐ Straight
☐ Bent - Degree = 15° 20° 25° 30° 35° 40° 45° (circle degree of bend)
- Location = 0.5mm 1mm 2mm 3mm 4mm 5mm (at what distance back from tip)

Clean & Blunt 35µm | Light Polish | Bent / Angled

Small 2.5µm Tip, Beveled at 25° | Medium Polish | 40µm Tip, 45° Bevel, Thin Walled Glass

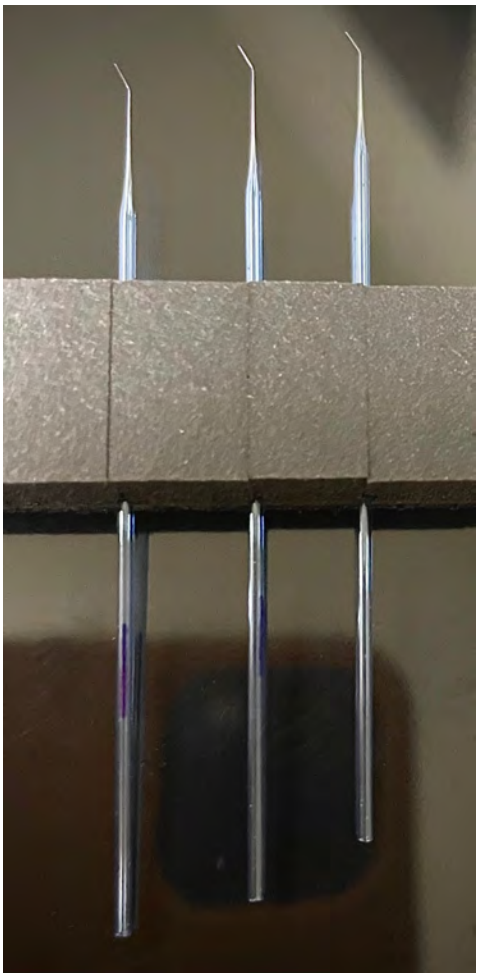
Large 50µm Tip, Beveled at 30° - Thick Walled Glass | Heavy Polish | Large Electrode, 25µm Tip, light polish

Pipette Storage Box | Sample Pipettes

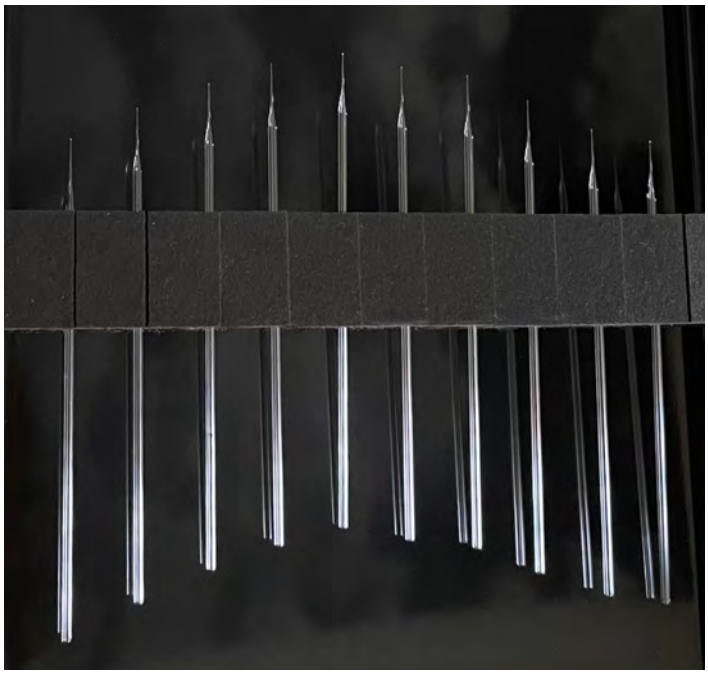
Send Custom Pipette Request to:

Adair L. Oesterle, R&D
Email: adair@sutter.com
Office: 1-415-883-0128
Cell: 1-415-328-6928
or
Email: info@sutter.com
Fax: 1-415-883-0572

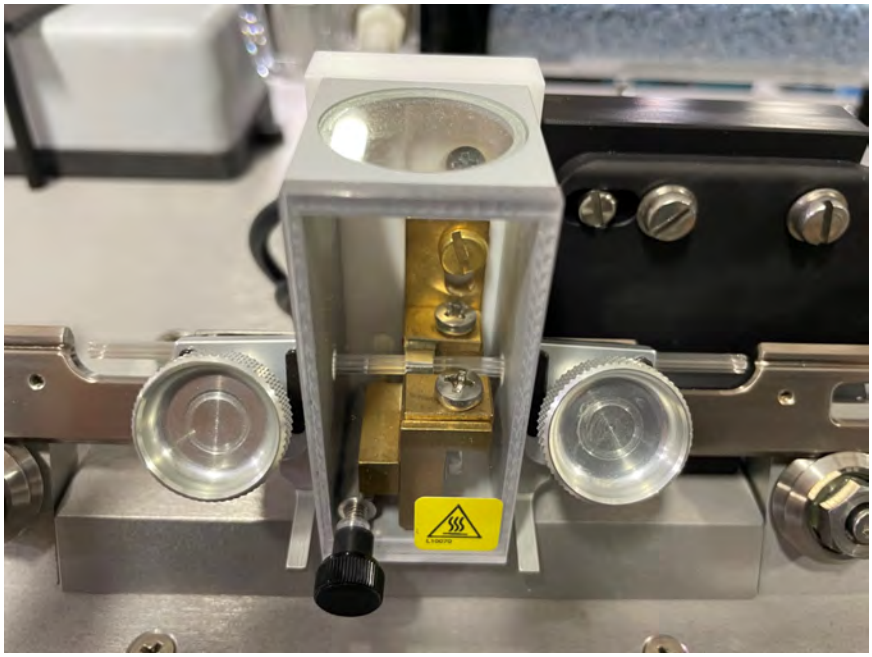
*** Custom Pipettes: Clean, non-sterile, research only, not for human use ***

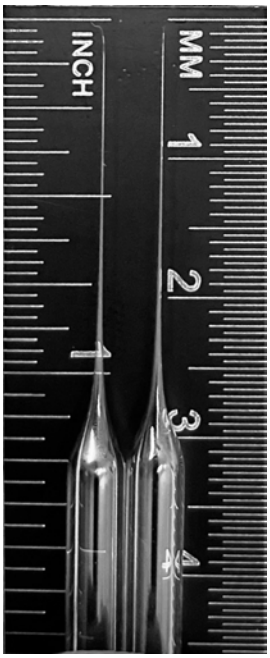


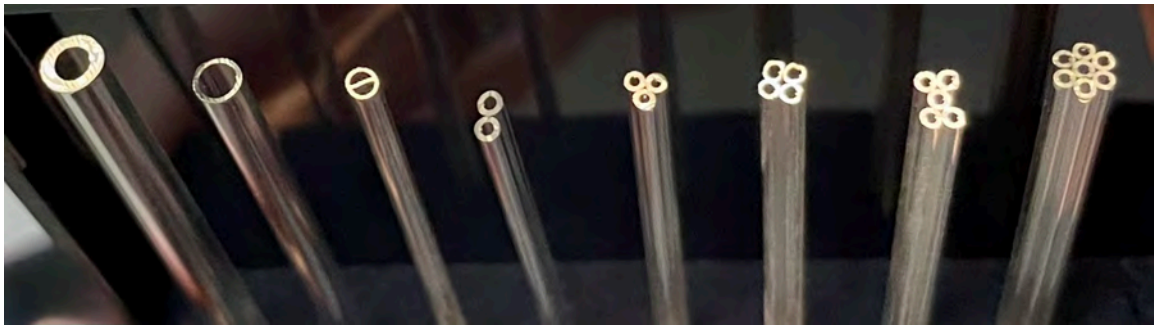
https://www.sutter.com/PDFs/Custom_Pipette_Worksheet.pdf



CHAPTER 30 – Multibarreled & Large Glass (≥ 2mm OD)

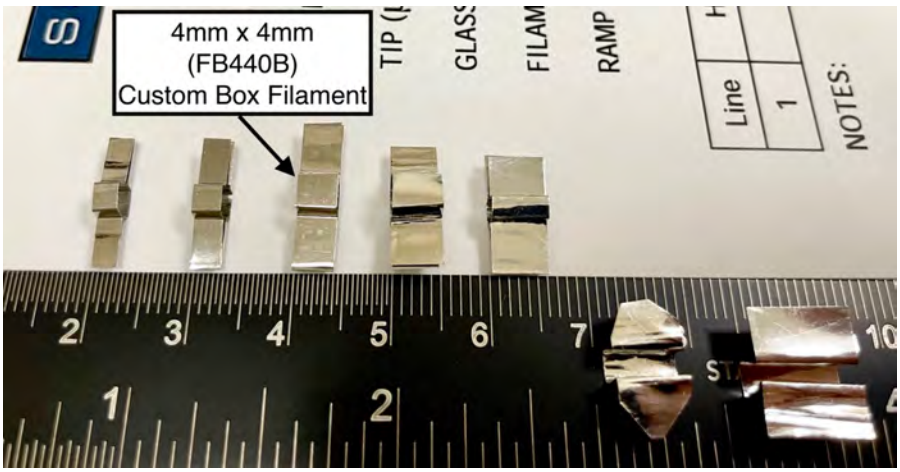






Custom Box Filament Required:
Very large capillary glass, where the cumulative outer diameter is greater than 2mm OD, like multibarreled or 3mm OD capillary glass, cannot be heated and pulled using the standard trough filament or box filaments. This is because the OD of the glass is similar to, or larger than, the ID of the heating filament. Therefore, a custom large box filament is required. If you do not already have a custom box filament for your larger or multibarreled glass, you may alter the shape of a pre-existing box filament, which often result in variable outcomes. If you choose to alter the shape a pre-existing box filament, ensure that there is at least 0.5mm clearance on all sides between the glass and the filament. It is best to request a custom-made box filament (4mm square ID and 4, 5 or 6mm wide) from Sutter.

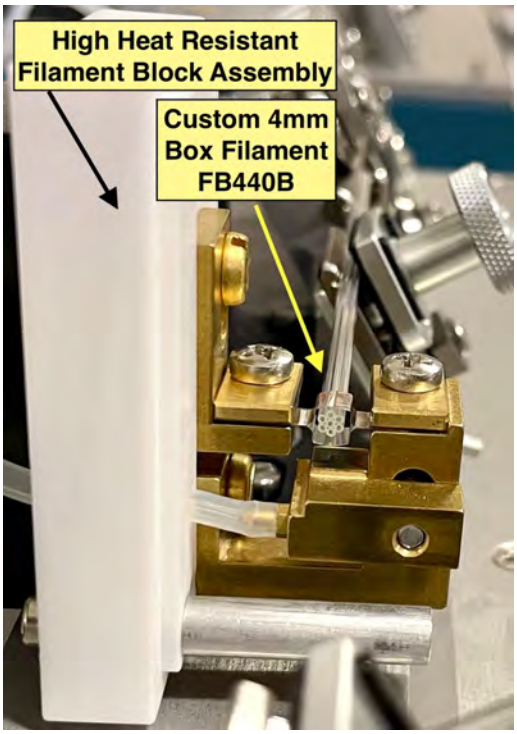
NOTE: If you are sharing the puller with other researchers or are still pulling standard sized capillary glass (1.0mm - 1.65mm OD), installing a custom large filament will completely alter the pulling behavior and change how the smaller glass is pulled. If you are the only one using the puller or all others also will only use larger glass, a 4mm square x 4mm wide (FB440B) custom box filament will work well with most dimensions of larger and multibarreled glass.

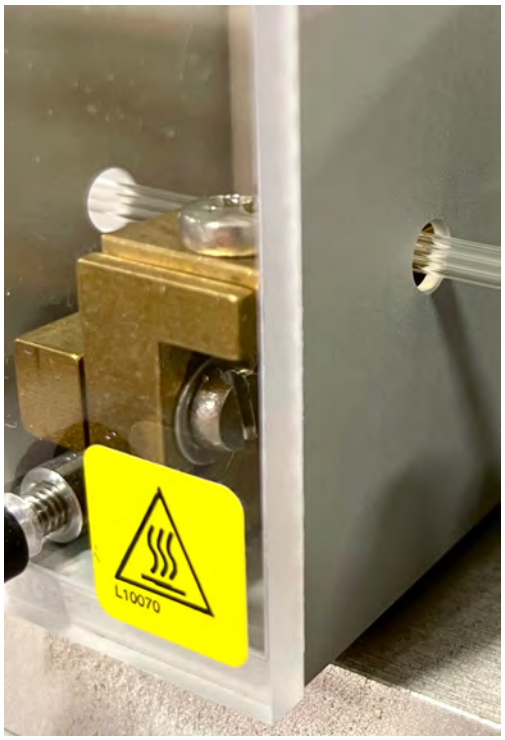


High Heat-Resistant Filament Block Assembly:

If you are using multibarreled glass and a larger custom box filament, the chances are the puller will have longer "heat on times" and will require much higher heat settings. In this case the brass jaws that retain the filament will retain quite a bit more heat. While the P-1000 puller has a temperature sensor on the bottom brass jaw to detect overheating, it is still possible the filament block assembly could be exposed to too much heat and get damaged. The P-97 does not have this level of protection, so with either P-97 or P-1000 puller, we recommend you outfit your puller with the High Heat-Resistant Filament Block Assembly. In addition, if the puller is used in industrial settings with standard sized glass where pipettes are being mass produced and ≥500 pipettes are pulled per day, we recommend the puller be upgraded with the custom Hight Heat Filament Block Assembly.

Humidity Control Chamber:
You will probably have to pull the glass with the humidity control chamber removed since the hole through which the glass is inserted might not allow the glass to clear without running into the chamber. One can purchase a second humidity control chamber from Sutter that has larger access holes drilled into the sides. You may also make the alteration yourself or have the machine shop or engineering department at your institution make the alteration for you.





Loading Larger & Multibarreled Glass:
Multibarreled glass is most easily loaded with one barrel of glass sitting in the puller bar grove, and the others sitting above or below it. In either case, the glass will be at a slight angle and cause the electrode clamp to tilt when tightening the thumbscrews. Depending on the clearance of the pin through the lower hole of the clamp, you might discover that the clamp gets caught on the pin. If you find this to be the case, you can drill or file the lower hole in the clamp at a similar angle so there is no longer any friction on the pin. Contact Sutter Technical Support to request custom clamps.

Parameter Settings for Pulling Large Outer Diameter and Multibarreled Glass:
These conditions are so specialized that is it best to contact Sutter to review your application, including the glass-filament combination, before embarking down this path. The Sutter Pullers were not designed for heating and pulling these larger filaments and glass sizes, but the capabilities and accuracy of the Sutter Pullers never ceases to amaze me.

CHAPTER 31 – Borosilicate Glass General Look Up Tables

The General Look Up Tables provide 120 programs organized by filament type. If you are using a 2.5mm x 2.5mm Box filament (FB255B), you will want to refer to the charts which provide the programs using this filament. First find the series of charts (6 charts for each filament) for the 2.5 box filament, then find the glass size (OD & ID) or item number of the glass you are using. 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 chart, the taper becomes longer and the tip becomes smaller. The longest taper and smallest tip would therefore be Type D, and Type E programs will pull extremely long tapers but with a wispy tip. These are intended to be trimmed or forged. Once you have located the chart of programs for your filament and glass combination, you select the program "**TYPE**" best suited for your application. 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 1-3µm 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 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 0.5 - 0.8µm 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 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. Please refer to Chapters specifically describing these pipette types for the best results.

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

1mm x 0.50mm or 1mm x 0.58mm (BF100-50-10, BF100-58-10, B100-50-10, B100-58-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	1	1mm thick walled	Line 1 Loops (4)	Ramp	0	30	1 delay	500
B	2	1mm thick walled	Line 1	Ramp+5	45	75	90 delay	500
C	3	1mm thick walled	Line 1	Ramp	55	75	80 delay	400
D	4	1mm thick walled	Line 1	Ramp+10	75	75	90 delay	400
E	5	1mm thick walled	Line 1	Ramp+30	0	120	0 time	200

1.0mm x 0.78mm or 1.0mm x 0.75mm (BF100-78-10, B100-75-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	6	1mm thin walled	Line 1 Loops (3)	Ramp	0	40	200 time	500
B	7	1mm thin walled	Line 1	Ramp	50	60	90 delay	200
C	8	1mm thin walled	Line 1	Ramp+5	80	70	80 delay	200
D	9	1mm thin walled	Line 1	Ramp+5	105	70	50 delay	200
E	10	1mm thin walled	Line 1	Ramp+30	0	120	0 time	200

1.2mm x 0.69mm Glass (BF120-69-10, B120-69-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	11	1.2mm thick walled	Line 1 Loops (4-5)	Ramp	0	20	250 time	500
B	12	1.2mm thick walled	Line 1	Ramp	45	80	200 delay	600
C	13	1.2mm thick walled	Line 1	Ramp	60	80	90 delay	500
D	14	1.2mm thick walled	Line 1	Ramp	80	80	70 delay	300
E	15	1.2mm thick walled	Line 1	Ramp+40	0	120	0 time	200

1.2mm x 0.94mm or 1.2mm x 0.90 (BF120-94-10, B120-90-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	16	1.2mm thin walled	Line 1 Loops (3)	Ramp	0	40	250 time	500
B	17	1.2mm thin walled	Line 1	Ramp	50	85	90 delay	450
C	18	1.2mm thin walled	Line 1	Ramp	85	90	70 delay	350
D	19	1.2mm thin walled	Line 1	Ramp+5	105	70	80 delay	300
E	20	1.2mm thin walled	Line 1	Ramp+40	0	80	0 time	200

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

1.5mm x 0.86mm (BF150-86-10, B150-86-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	21	1.5mm thick walled	Line 1 Loops (4-5)	Ramp	0	21	1 delay	600
B	22	1.5mm thick walled	Line 1	Ramp	35	70	200 delay	600
C	23	1.5mm thick walled	Line 1	Ramp	70	75	200 delay	500
D	24	1.5mm thick walled	Line 1	Ramp	85	85	100 delay	500
E	25	1.5mm thick walled	Line 1	Ramp+50	0	120	0 time	200

1.5mm x 1.10mm or 1.5mm x 1.17mm (BF150-110-10, B150-110-10, BF150-117-10, B150-117-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	26	1.5mm thin walled	Line 1 Loops (2-3)	Ramp	0	65	250 time	500
B	27	1.5mm thin walled	Line 1	Ramp	55	75	120 delay	400
C	28	1.5mm thin walled	Line 1	Ramp	85	75	80 delay	400
D	29	1.5mm thin walled	Line 1	Ramp+5	95	70	70 delay	250
E	30	1.5mm thin walled	Line 1	Ramp+50	0	120	0 time	200

* The above settings can also be used for BT150-10 Theta Glass

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

1mm x 0.50mm or 1mm x 0.58mm (BF100-50-10, BF100-58-10, B100-50-10, B100-58-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	31	1mm thick walled	Line 1 Loops (4)	Ramp+5	0	30	1 delay	500
B	32	1mm thick walled	Line 1	Ramp+5	35	75	130 delay	500
C	33	1mm thick walled	Line 1	Ramp	55	75	90 delay	500
D	34	1mm thick walled	Line 1	Ramp	100	75	250 time	500
E	35	1mm thick walled	Line 1	Ramp+25	0	120	0 time	200

1.0mm x 0.78mm or 1.0mm x 0.75mm (BF100-78-10, B100-75-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	36	1mm thin walled	Line 1 Loops (2-3)	Ramp+5	0	40	200 time	500
B	37	1mm thin walled	Line 1	Ramp+5	55	75	80 delay	300
C	38	1mm thin walled	Line 1	Ramp+5	90	80	80 delay	200
D	39	1mm thin walled	Line 1	Ramp+15	80	70	50 delay	200
E	40	1mm thin walled	Line 1	Ramp+35	0	100	0 time	200

1.2mm x 0.69mm Glass (BF120-69-10, B120-69-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	41	1.2mm thin walled	Line 1 Loops (4)	Ramp+5	0	25	1 delay	500
B	42	1.2mm thin walled	Line 1	Ramp+5	45	80	120 delay	500
C	43	1.2mm thin walled	Line 1	Ramp+5	55	80	90 delay	500
D	44	1.2mm thin walled	Line 1	Ramp+10	80	80	60 delay	500
E	45	1.2mm thin walled	Line 1	Ramp+35	0	120	0 time	200

1.2mm x 0.94mm or 1.2mm x 0.90 (BF120-94-10, B120-90-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	46	1.2mm thin walled	Line 1 Loops (2-3)	Ramp+5	0	35	200 time	500
B	47	1.2mm thin walled	Line 1	Ramp	55	85	110 delay	300
C	48	1.2mm thin walled	Line 1	Ramp+5	85	90	90 delay	300
D	49	1.2mm thin walled	Line 1	Ramp+10	85	90	90 delay	200
E	50	1.2mm thin walled	Line 1	Ramp+40	0	90	0 time	200

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

1.5mm x 0.86mm (BF150-86-10, B150-86-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	51	1.5mm thick walled	Line 1 Loops (4-5)	Ramp	0	25	1 delay	500
B	53	1.5mm thick walled	Line 1	Ramp	45	80	200 delay	600
C	53	1.5mm thick walled	Line 1	Ramp	45	80	150 delay	500
D	54	1.5mm thick walled	Line 1	Ramp	75	95	110 delay	500
E	55	1.5mm thick walled	Line 1	Ramp+25	0	120	0 time	200

1.5mm x 1.10mm or 1.5mm x 1.17mm (BF150-110-10, B150-110-10, BF150-117-10, B150-117-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	56	1.5mm thin walled	Line 1 Loops (2-3)	Ramp+5	0	65	250 time	500
B	57	1.5mm thin walled	Line 1	Ramp+5	55	90	120 delay	400
C	58	1.5mm thin walled	Line 1	Ramp+5	85	95	90 delay	400
D	59	1.5mm thin walled	Line 1	Ramp+5	95	80	60 delay	300
E	60	1.5mm thin walled	Line 1	Ramp+50	0	120	0 time	200

* The above settings can also be used for BT150-10 Theta Glass

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

1mm x 0.50mm or 1mm x 0.58mm (BF100-50-10, BF100-58-10, B100-50-10, B100-58-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	61	1mm thick walled	Line 1 Loops (4)	Ramp	0	25	250 time	500
B	62	1mm thick walled	Line 1	Ramp	0	30	250 time	500
			Line 2	Ramp-10	0	40	250 time	500
			Line 3	Ramp-10	25	55	250 time	500
C	63	1mm thick walled	Line 1	Ramp	0	50	150 delay	500
			Line 2	Ramp-10	35	60	150 delay	500
D	64	1mm thick walled	Line 1	Ramp-20	50	80	200 time	600
E	65	1mm thick walled	Line 1	Ramp+50	0	120	0 time	300

1.0mm x 0.78mm or 1.0mm x 0.75mm (BF100-78-10, B100-75-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	66	1mm thin walled	Line 1 Loops (3)	Ramp	0	25	250 time	500
B	67	1mm thin walled	Line 1	Ramp+5	0	50	200 time	500
			Line 2	Ramp+5	25	65	200 time	500
C	68	1mm thin walled	Line 1	Ramp	0	30	200 time	500
			Line 2	Ramp-10	80	60	200 time	500
D	69	1mm thin walled	Line 1	Ramp	45	85	200 time	500
E	70	1mm thin walled	Line 1	Ramp+50	0	120	0 time	300

1.2mm x 0.69mm Glass (BF120-69-10, B120-69-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	71	1.2mm thick walled	Line 1 Loops (4-5)	Ramp	0	20	250 time	500
B	72	1.2mm thick walled	Line 1	Ramp	0	40	250 time	500
			Line 2	Ramp-10	0	40	250 time	500
			Line 3	Ramp-10	65	50	250 time	500
C	73	1.2mm thick walled	Line 1	Ramp	0	50	250 time	600
			Line 2	Ramp-15	60	85	250 time	600
D	74	1.2mm thick walled	Line 1	Ramp	0	40	250 time	500
			Line 2	Ramp-10	60	85	250 time	500
E	75	1.2mm thick walled	Line 1	Ramp+25	0	120	0 time	300

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

1.2mm x 0.94mm. or 1.2mm x 0.90mm (BF120-94-10, B120-90-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	76	1.2mm thin walled	Line 1 Loops (3)	Ramp	0	40	200 time	500
B	77	1.2mm thin walled	Line 1	Ramp+5	0	50	200 time	500
			Line 2	Ramp+5	45	65	200 time	500
C	78	1.2mm thin walled	Line 1	Ramp	0	30	200 time	500
			Line 2	Ramp+10	80	60	200 time	500
D	79	1.2mm thin walled	Line 1	Ramp+5	85	85	250 time	500
E	80	1.2mm thin walled	Line 1	Ramp+50	0	120	0 time	300

1.5mm x 0.86mm (BF150-86-10, B150-86-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	81	1.5mm thick walled	Line 1 Loops (4-5)	Ramp+5	0	20-25	1 delay	500
B	82	1.5mm thick walled	Line 1	Ramp	0	35	1 delay	500
			Line 2	Ramp	0	35	1 delay	500
			Line 3	Ramp-65	35	55	110 delay	500
C	83	1.5mm thick walled	Line 1	Ramp	0	35	1 delay	500
		1.5mm thick walled	Line 2	Ramp	0	35	1 delay	500
		1.5mm thick walled	Line 3	Ramp-30	35	65	110 d	500
D	84		Line 1	Ramp-20	60	90	250 delay	600-700
E	85	1.5mm thick walled	Line 1	Ramp+15	0	120	0 time	500-600

1.5mm x 1.10mm or 1.5mm x 1.17mm (BF150-110-10, B150-110-10, BF150-117-10, B150-117-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	86	1.5mm thin walled	Line 1 Loops (3)	Ramp+5	0	65	250 time	500
B	87	1.5mm thin walled	Line 1	Ramp+5	0	50	250 time	500
			Line 2	Ramp+5	45	55	250 time	500
C	88	1.5mm thin walled	Line 1	Ramp	55	75	110 delay	600
D	89	1.5mm thin walled	Line 1	Ramp+10	55	75	110 delay	500
E	90	1.5mm thin walled	Line 1	Ramp+40	0	120	0 time	500

* The above settings can also be used for BT150-10 Theta Glass

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

1mm x 0.50mm or 1mm x 0.58mm (BF100-50-10, BF100-58-10, B100-50-10, B100-58-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	91	1mm thick walled	Line 1 Loops (4)	Ramp+15	0	50	150 time	500
B	92	1mm thick walled	Line 1	Ramp+15	0	20	200 time	400
			Line 2	Ramp+15	55	65	150 time	400
C	93	1mm thick walled	Line 1	Ramp+10	85	100	200 time	400
D	94	1mm thick walled	Line 1	Ramp+15	150	100	150 time	500
E	95	1mm thick walled	Line 1	Ramp+15	0	150	0 time	200

1.0mm x 0.78mm or 1.0mm x 0.75mm GLASS (BF100-78-10, B100-75-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	96	1mm thin walled	Line 1 Loops (3)	Ramp+15	0	70	150 time	500
B	97	1mm thin walled	Line 1	Ramp+10	45	100	175 time	300
C	98	1mm thin walled	Line 1	Ramp+15	55	100	175 time	200
D	99	1mm thin walled	Line 1	Ramp+15	50	100	150 time	300
E	100	1mm thin walled	Line 1	Ramp+15	0	120	0 time	200

1.2mm x 0.69mm Glass (BF120-69-10, B120-69-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	101	1.2mm thick walled	Line 1 Loops (4)	Ramp+15	0	45	150 time	500
B	102	1.2mm thick walled	Line 1	Ramp+10	0	20	200 time	400
			Line 2	Ramp+15	75	95	150 time	400
C	103	1.2mm thick walled	Line 1	Ramp+15	45	85	200 time	300
D	104	1.2mm thick walled	Line 1	Ramp+15	60	95	150 time	500
E	105	1.2mm thick walled	Line 1	Ramp+30	0	120	0 time	200

1.2mm x 0.94mm or 1.2mm x 0.90 (BF120-94-10, B120-90-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	106	1.2mm thin walled	Line 1 Loops (3)	Ramp	0	65	150 time	500
B	107	1.2mm thin walled	Line 1	Ramp+5	45	110	175 time	300
C	108	1.2mm thin walled	Line 1	Ramp	45	105	150 time	200
D	109	1.2mm thin walled	Line 1	Ramp+15	55	100	150 time	300
E	110	1.2mm thin walled	Line 1	Ramp+30	0	120	0 time	200

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

1.5mm x 0.86mm (BF150-86-10, B150-86-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	111	1.5mm thick walled	Line 1 Loops (4)	Ramp+10	0	55	150 time	500
B	112	1.5mm thick walled	Line 1	Ramp+10	0	30	200 time	200
			Line 2	Ramp+10	35	155	200 time	200
C	113	1.5mm thick walled	Line 1	Ramp+10	45	85	200 time	400
D	114	1.5mm thick walled	Line 1	Ramp+10	55	95	200 time	400
E	115	1.5mm thick walled	Line 1	Ramp+10	0	120	0 time	200

1.5mm x 1.10mm or 1.5mm x 1.17mm (BF150-110-10, B150-110-10, BF150-117-10, B150-117-10)

TYPE	PROG #	GLASS	LINE(S)	HEAT	PULL	VEL	TIME/DEL	PRESSURE
A	116	1.5mm thin walled	Line 1 Loops (3)	Ramp+15	0	90	150 time	300
B	117	1.5mm thin walled	Line 1	Ramp+5	45	120	175 time	200
C	118	1.5mm thin walled	Line 1	Ramp+10	45	120	175 time	200
D	119	1.5mm thin walled	Line 1	Ramp+10	45	120	150 time	200
E	120	1.5mm thin walled	Line 1	Ramp+30	0	120	0 time	200

* The above settings can also be used for BT150-10 Theta Glass

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