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Introduction

The Sutter Instrument Pipette Cookbook is organized according to application and describes the required pipette needed for that application. The types of micropipettes described in this cookbook include the following: Extracellular Recording; Intracellular Recording; and Patch Pipettes; Microinjection Needles, including Adherent Cell, *C.elegans, Drosophila*, Zebrafish, Bee-Stinger Needles; Pronuclear Injection; ICSI; ES Cell; Nuclear Transfer; Holding; Xenopus; and Large Pipettes with a 20-200µm tip. While we have attempted to cover a wide range of applications and types of pipettes, it is possible we have yet to include what you might be looking for. Additional applications will be added to the future revisions of this cookbook. If you have suggestions for what can be added, please do not hesitate to contact Sutter Instrument Company.

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

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

Introduction continued...

The most common sources of difficulty in producing the right kind of pipettes can be attributed to the use of poor parameter settings. Just one poorly adjusted parameter setting in the program, too high or too low, can lead to a lot of variability in tip size, taper length and resistance. These very high or low settings can be seen as "bad ingredients" in your recipe. For a general guideline on each parameter setting and the range of values we recommend you stay within, please refer to Chapter 12. If you stay within the suggested range for each parameter, it will be less likely that you will "get lost" or off track when trying to write a program or adjust your parameter settings. It is possible that the not-so-ideal parameter settings were established in a somewhat haphazard manner by various well-intentioned researchers who are possibly no longer in the lab. It is also conceivable that the program one is using had been adopted from someone who has a different model of Sutter puller and/or a different combination of filament and glass. Please be aware that simply using a program from someone else can often lead to undesirable tip sizes, and in worse cases, the unfortunate event of burning out your filament. If you have acquired your settings from someone outside of Sutter, it is best to run a ramp test to make sure the heat settings you have been advised to use will not damage or burn out your filament. Information about the Ramp Test can be found in Chapter 11.

If a recommended program in this Cookbook does not produce the proper pipette, this might be a result of a poor alignment or mechanical adjustment on your puller. It is important to make sure your puller is in good working order. Be sure to check that the filament is perfectly centered over the air jet, the air jet is set 2 to 3mm below the base of the filament, the glass capillary is properly positioned within the filament, the filament shape is correct, and that dirt and grime are not interfering with the movement of the puller bars. If you find that the program provided and the suggested changes to the parameter settings do not produce good results, please refer to the Chapters 12, "General Guideline for Parameter Settings" & Chapter 13, "Problems with Variability". If you feel your puller might be in need of a tune up or repair, please contact Sutter Instrument and inquire about having your puller refurbished.

SUTTER CAPILLARY GLASS

Standard Wall Borosilicate Tubing (WITH Filament) (2006 Price List)

Catalog Number	Outside Diameter	Inside Diameter	Overall Length	Pieces per Package	Price
BF100-50-10	1.00mm	0.50mm	10cm	225	$31
BF100-50-15	1.00mm	0.50mm	15cm	225	$46
BF100-58-10	1.00mm	0.58mm	10cm	250	$31
BF100-58-15	1.00mm	0.58mm	15cm	250	$42
BF120-60-10	1.20mm	0.60mm	10cm	225	$38
BF120-69-10	1.20mm	0.69mm	10cm	250	$34
BF120-69-15	1.20mm	0.69mm	15cm	250	$28
BF150-75-10	1.50mm	0.75mm	10cm	225	$38
BF150-86-7.5	1.50mm	0.86mm	7.5cm	250	$35
BF150-86-10	1.50mm	0.86mm	10cm	250	$35
BF150-86-15	1.50mm	0.86mm	15cm	250	$78
BF200-100-10	2.00mm	1.00mm	10cm	225	$78
BF200-116-10	2.00mm	1.16mm	10cm	250	$90
BF200-116-15	2.00mm	1.16mm	15cm	250	$95

Standard Wall Borosilicate Tubing (without Filament)

Catalog Number	Outside Diameter	Inside Diameter	Overall Length	Pieces per Package	Price
B100-50-10	1.00mm	0.50mm	10cm	225	$32
B100-50-15	1.00mm	0.50mm	15cm	225	$73
B100-58-10	1.00mm	0.58mm	10cm	250	$28
B100-58-15	1.00mm	0.58mm	15cm	250	$39
B120-69-10	1.20mm	0.69mm	10cm	250	$37
B120-69-15	1.20mm	0.69mm	15cm	250	$27
B150-86-7.5	1.50mm	0.86mm	7.5cm	250	$35
B150-86-10	1.50mm	0.86mm	10cm	250	$37
B150-86-15	1.50mm	0.86mm	15cm	250	$71
B200-116-10	2.00mm	1.16mm	10cm	250	$58
B200-116-15	2.00mm	1.16mm	15cm	250	$69

Thin Wall Borosilicate Tubing (WITH Filament)

Catalog Number	Outside Diameter	Inside Diameter	Overall Length	Pieces per Package	Price
BF100-78-10	1.00mm	0.78mm	10cm	250	$28
BF100-78-15	1.00mm	0.78mm	15cm	250	$45
BF120-94-10	1.20mm	0.94mm	10cm	250	$34
BF120-94-15	1.20mm	0.94mm	15cm	250	$48
BF150-110-7.5	1.50mm	1.10mm	7.5cm	250	$35
BF150-110-10	1.50mm	1.10mm	10cm	225	$34
BF150-117-10	1.50mm	1.17mm	10cm	250	$30
BF150-117-15	1.50mm	1.17mm	15cm	100	$33
BF200-156-10	2.00mm	1.56mm	10cm	250	$37
BF200-156-15	2.00mm	1.56mm	15cm	100	$39

Thin Wall Borosilicate Tubing (without Filament)

Catalog Number	Outside Diameter	Inside Diameter	Overall Length	Pieces per Package	Price
B100-75-10	1.00mm	0.75mm	10cm	225	$24
B100-75-15	1.00mm	0.75mm	15cm	225	$39
B120-90-10	1.20mm	0.90mm	10cm	225	$27
B150-110-7.5	1.50mm	1.10mm	7.5cm	225	$29
B150-110-10	1.50mm	1.10mm	10cm	225	$29

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

Filaments & Accessories

Box Filaments

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

FB215B 2.0mm square box filament, 1.5mm wide..... $ 15

FB220B 2.0mm square box filament, 2.0mm wide..... $ 15

FB230B 2.0mm square box filament, 3.0mm wide..... $ 15

FB255B 2.5mm square box filament, 2.5mm wide..... $ 15

FB245B 2.5mm square box filament, 4.5mm wide..... $ 15

FB315B 3.0mm square box filament, 1.5mm wide..... $ 15

FB320B 3.0mm square box filament, 2.0mm wide..... $ 15

FB330B 3.0mm square box filament, 3.0mm wide..... $ 15

Trough Filaments

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

FT315B 1.5mm wide trough filament..... $ 15

FT320B 2.0mm wide trough filament..... $ 15

FT330B 3.0mm wide trough filament..... $ 15

FT345B 4.5mm wide trough filament..... $ 15

Accessories

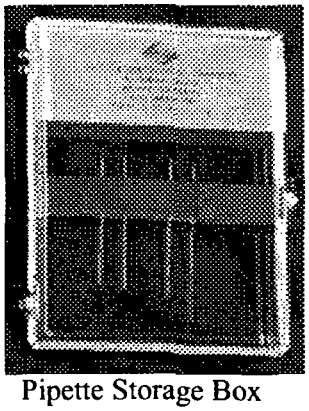
Custom Filament: Custom platinum/iridium filament..... $18

FPS Fire polishing spacer for P97, P87, and P2000 pullers..... $ 25

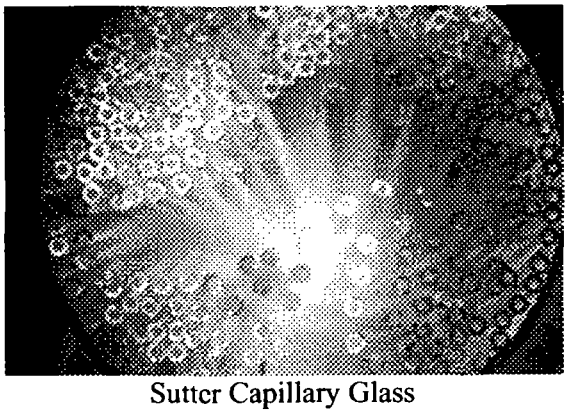
FS1875 Platinum/iridium sheet, 18mm x 75mm x 0.05mm (0.002in)..... $ 90

CTS Ceramic tile for scoring glass (large tips 20 to 200 microns)..... $ 15

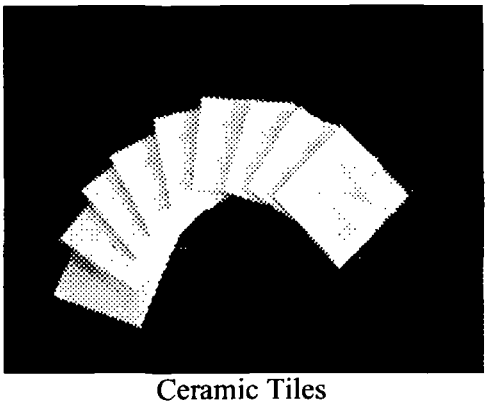
IMOXAB Instruction manual (specify product when ordering)..... $ 15



Pipette Storage Box
Item# BX10, $10.00



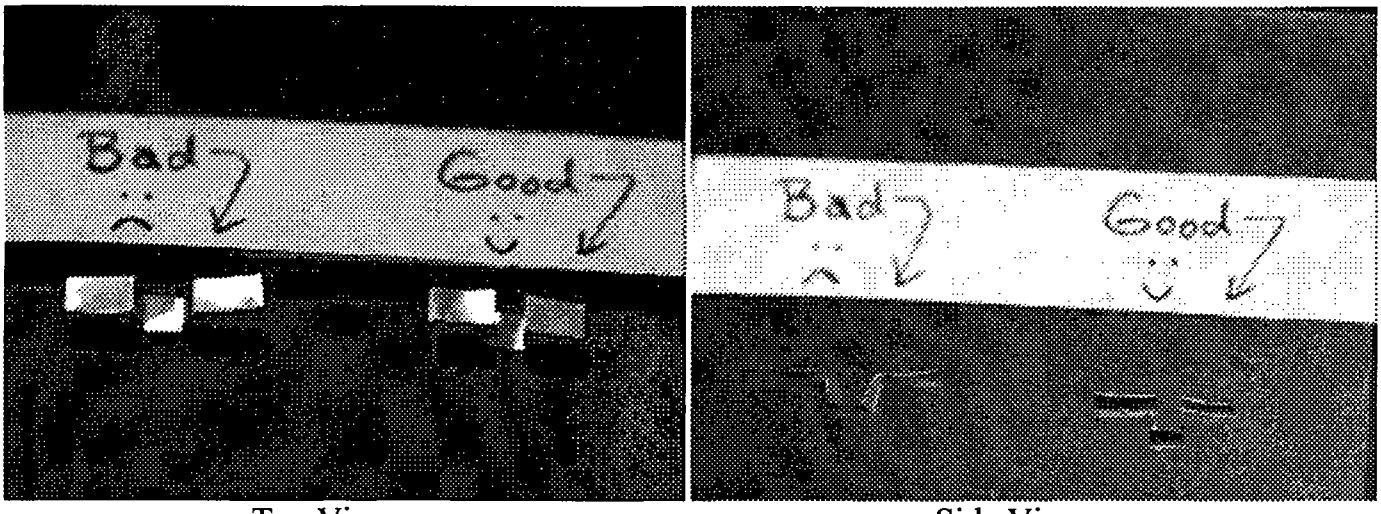
Sutter Capillary Glass
See Page 7 for Part Number and Price



Ceramic Tiles
Item # CTS, $15.00 each

Trough Filament Shape & Alignment

3mm Trough Filament (FT330B)



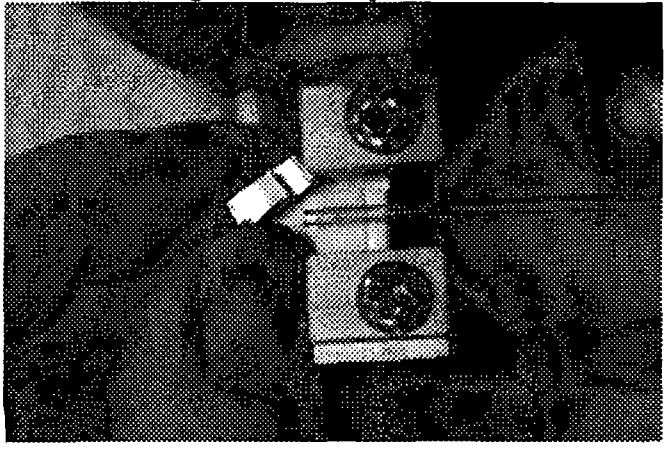
Top View — **BAD shape** **GOOD shape**

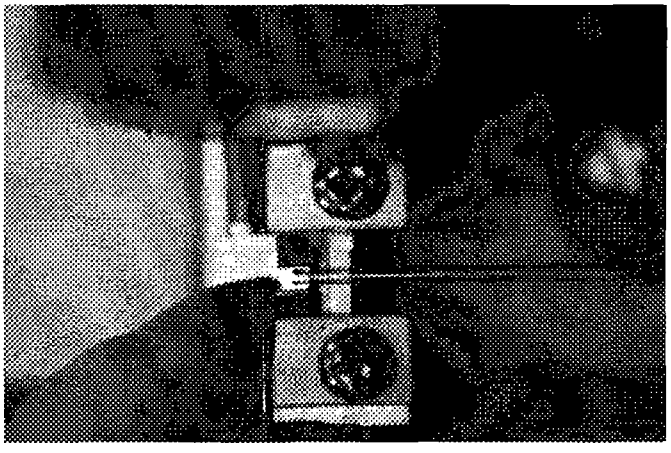
Side View — **BAD shape** **GOOD shape**

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

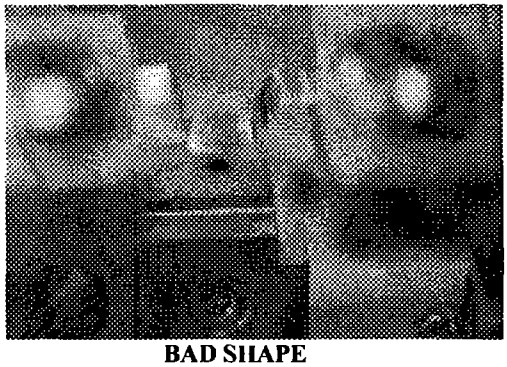
GOOD filament shape: A "Good" filament shape for the 3mm trough (FT330B) pictured above will provide efficient heating of the glass, a ramp value between 240 - 280 units, and a long filament life span of one to two years. The walls should angle inward at 80 degrees and there should be a 2mm opening at the top of the filament.

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

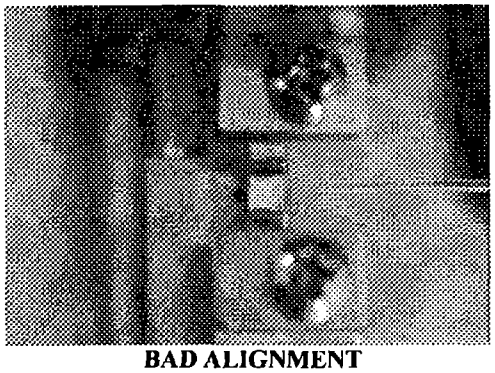




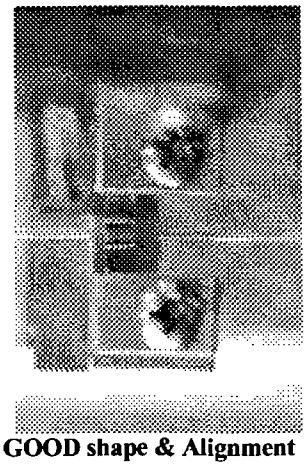
Trough Filament continued....



BAD SHAPE

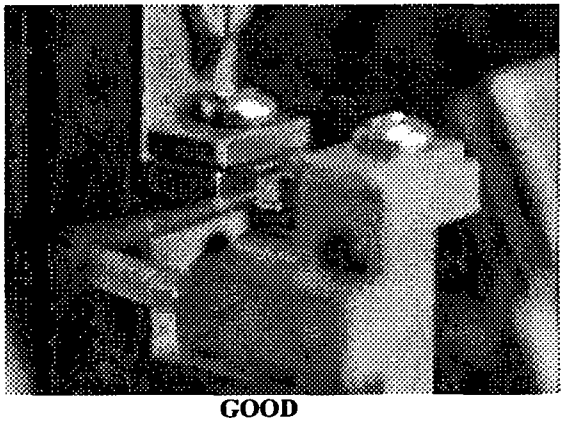


BAD ALIGNMENT



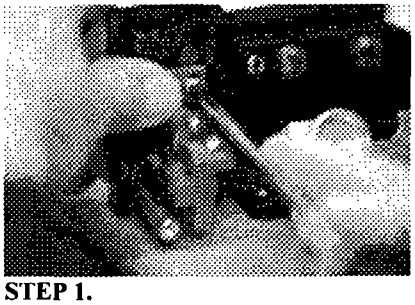
GOOD shape & Alignment

GOOD

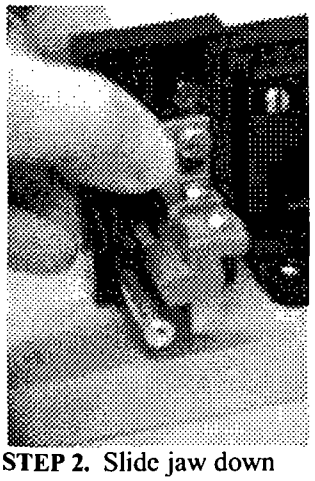


GOOD

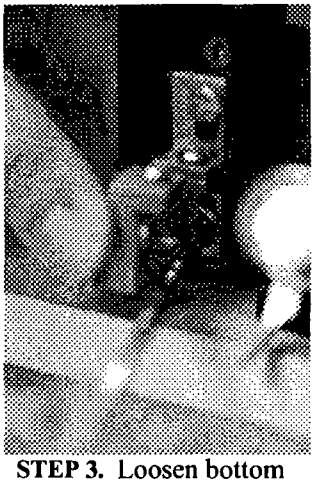
Changing from a Trough to a Box Filament: If you are changing from a trough filament to a box filament, you will need to change the position of the brass jaws. The trough sits higher than the box, so you will need to loosen the brass screws (one at a time) and slide each jaw **down** about 3mm.



STEP 1.
Loosen top brass screw then...



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

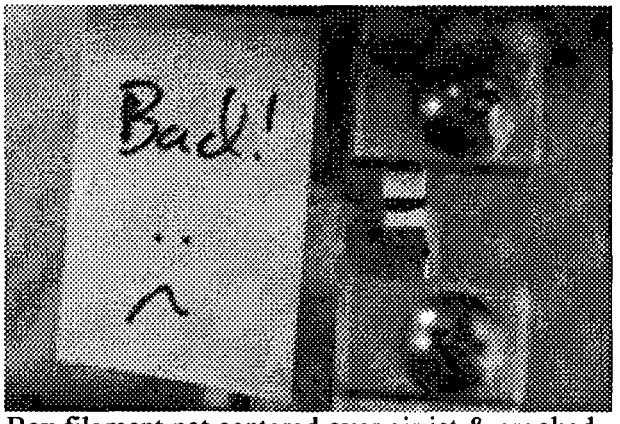


STEP 3. Loosen bottom brass screw and then...

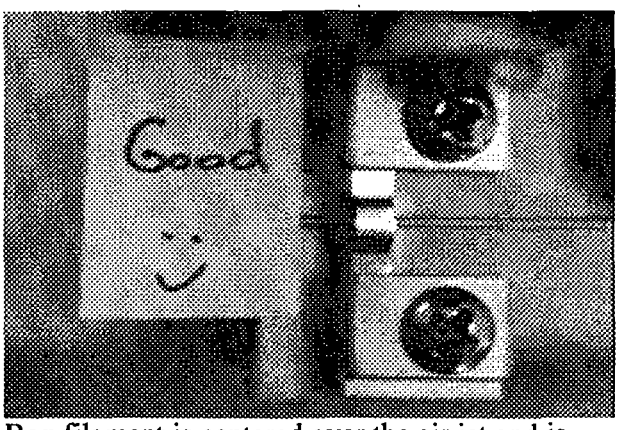
STEP 3. Use the screw driver to pry jaw down 3mm, then tighten the brass screw.

Installing the BOX filament...

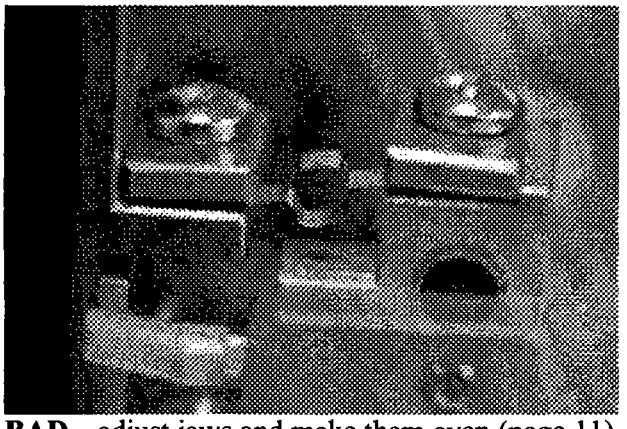
The box filament does not require shaping, but needs to be installed so it is centered right to left over the air jet and also centered around the glass. The glass should run through the middle of the filament.



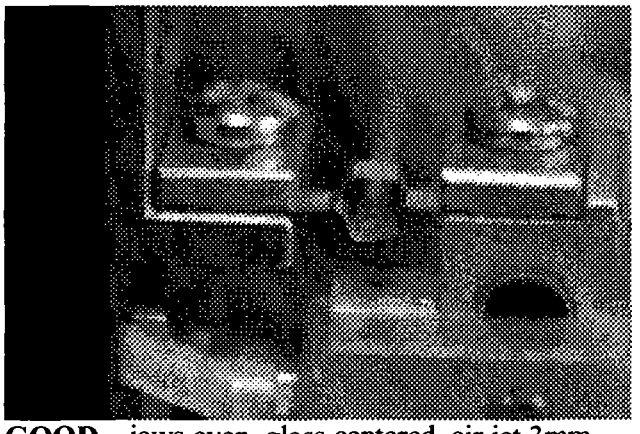
Box filament not centered over air jet & crooked
This is BAD!



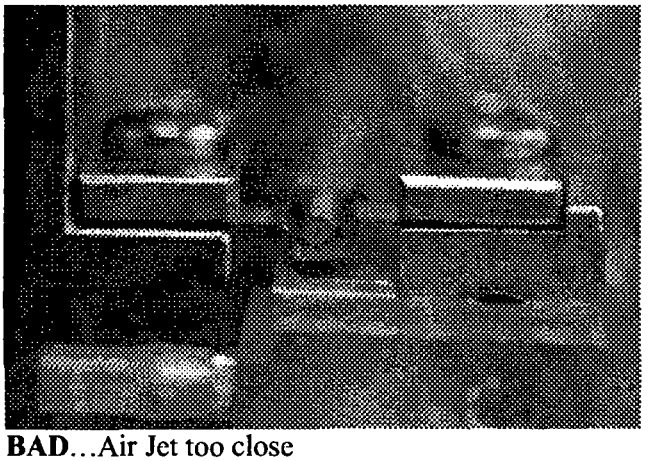
Box filament is centered over the air jet and is 1.0mm in from the left edge of the brass jaws. The filament is positioned so the glass runs through the center of the filament.
This is GOOD!



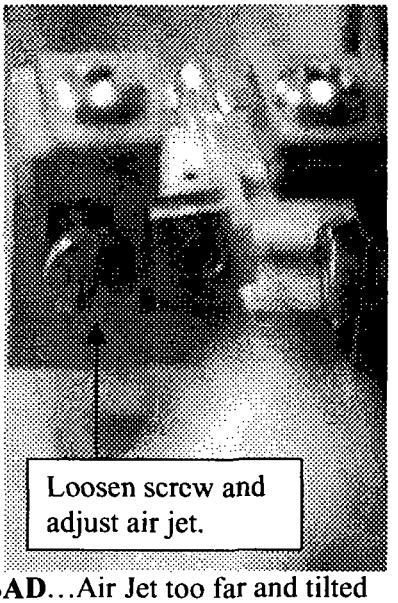
BAD...adjust jaws and make them even (page 11)



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



BAD...Air Jet too close



BAD...Air Jet too far and tilted

ECCENTRIC ADJUSTMENTS

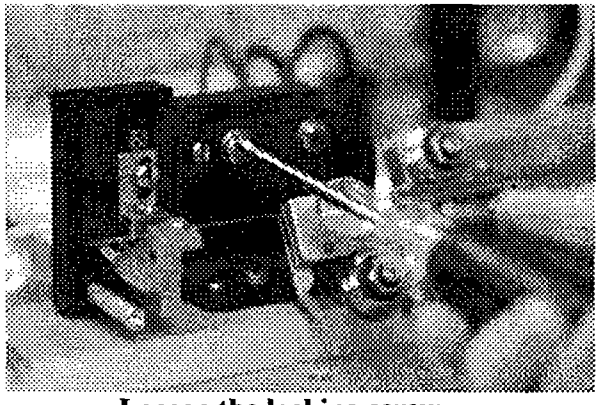
Large adjustments to Box or Trough Filament (more than 1-2mm)

If you find that the glass is not centered in the filament from top to bottom, it is best to make large adjustments by adjusting the brass jaws (as seen at the bottom of pg.10). 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.

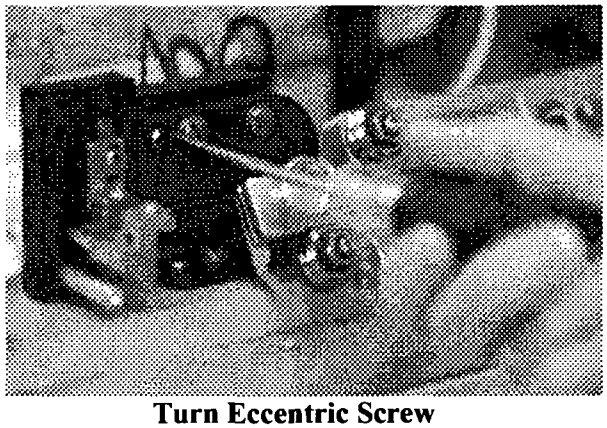
Eccentric Adjustments (less than 1-2mm)

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)

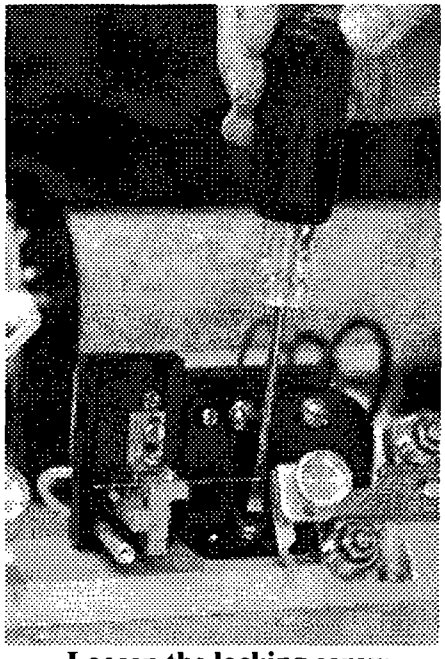


Loosen the locking screw

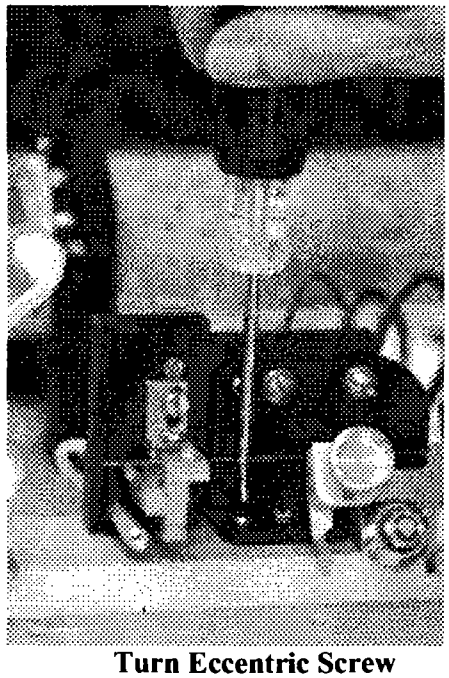


Turn Eccentric Screw

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 1

Electrophysiology Overview

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

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

Extracellular Recording measures:

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

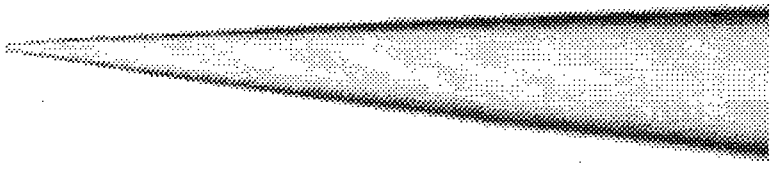
Intracellular Recording measures:

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

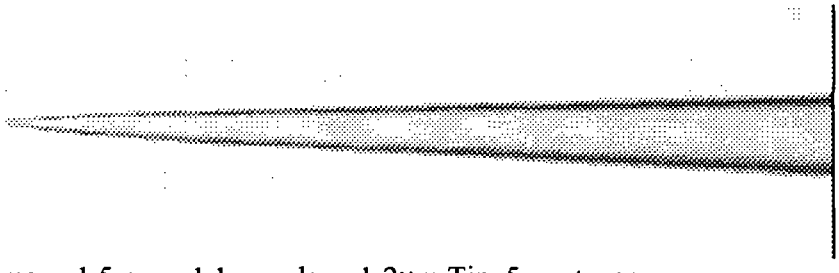
Extracellular Recording

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

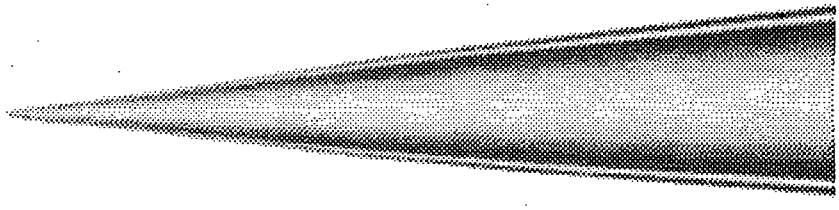
Extracellular Microelectrode Images



400X mag., 1.5mm x 1.1mm glass, 3.5µm Tip, 4mm taper
General Look Up Table - Prog #56



400X mag., 1.5mm x 1.1mm glass, 1-2µm Tip, 5mm taper
General Look Up Table - Prog #26



400X mag., 1.5mm x 0.86mm glass, 1-1.5µm Tip, 4mm taper
General Look Up Table - Prog #51

Extracellular Microelectrodes - Recommended Programs

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

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

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

- For a larger tip and lower resistance, decrease the velocity to allow four loops instead of three.
- For a smaller tip and higher resistance, increase the velocity to allow two loops instead of three.

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

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

- For a larger tip and lower resistance, decrease the velocity to allow five loops instead of four.
- For a smaller tip and higher resistance, increase the velocity to allow three loops instead of four.

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

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

- For a larger tip and lower resistance, decrease the velocity to allow four loops instead of three.
- For a smaller tip and higher resistance, increase the velocity to allow two loops instead of three.

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

Heat	Pull	Velocity	Delay	Pressure	Loops
Ramp + 5	0	20-25	1	500	4-5

- For a larger tip and lower resistance, decrease the velocity to allow five loops instead of four.
- For a smaller tip and higher resistance, increase the velocity to allow three loops instead of four.

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

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

* *For different filament and glass combinations, please refer to the "Look Up Table for Patch Pipettes" on Page 24 and increase the velocity to allow one more loop than what is indicated in the last column.*

Intracellular Recording

Resistance & Geometry

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

A wide range of microelectrodes is used for intracellular recordings. Microelectrodes are drawn from many different types and sizes of glass capillaries, and borosilicate is the most common glass used for intracellular recording. Quartz (or fused silica) and, to a lesser degree, aluminosilicate are superior to borosilicate in strength, stiffness and the ability to form a small tip, but are also somewhat more expensive. Aluminosilicate can be pulled using the P-97 Pipette Puller, 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 Look Up Table at the end of the Patch Pipette Section. 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 in the last column of the program.

High Resistance Microelectrodes, Sharp & Long

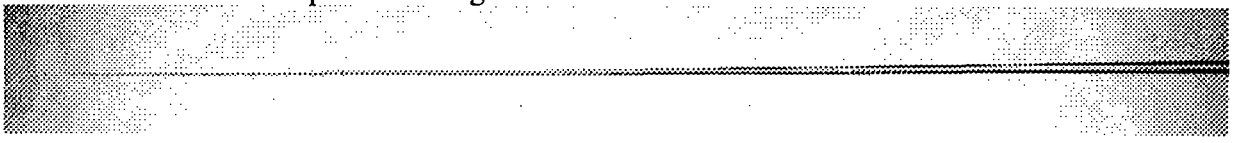
For sharp microelectrodes with 30 to100 MΩ resistances and higher, the tip size and the geometry of the taper are usually the key factors determining if an electrode can successfully impale a cell. Small tips and gradual, uniform tapers have an obvious advantage in terms of causing less damage when a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

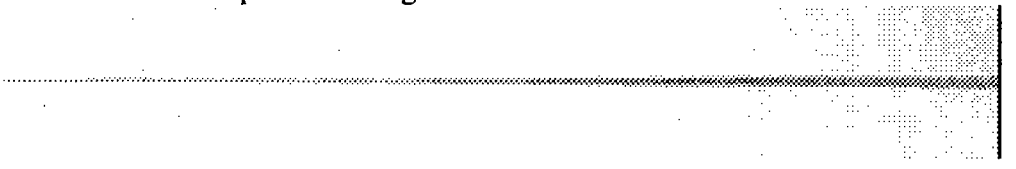
- *"Additional Concerns about IntracellularRecording" see pages 15-18.*

Intracellular Microelectrode Images

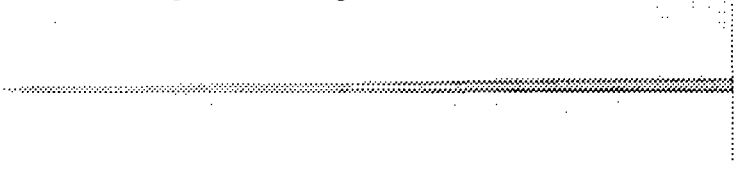
Standard or Thick Walled Glass
1.0mm x 0.5mm glass, 0.06µm Tip, 12mm taper **(400x mag.)**
▼ General Look Up Table - Prog #4



Standard or Thick Walled Glass
1.0mm x 0.50mm glass, 0.3µm-0.5µm Tip, 9mm taper **(400x mag.)**
▼ General Look Up Table - Prog #33



Thin Walled Glass
1.0 x 0.78mm glass, 0.5µm-0.7µm Tip, 7mm taper **(400x mag.)**
▼ General Look Up Table - Prog #37



Intracellular Microelectrodes - Recommended Programs

Goal = 0.7 to 0.06µm, 30 to100+MΩ, 7-12mm Long and Gradual Taper

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

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

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

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

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

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

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

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

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

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

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

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

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

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

- Programs using a **3mm Trough** (FT330B) Filament

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

Heat	Pull	Velocity	Time	Pressure
Ramp + 10	85-150	100	200	400

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

Heat	Pull	Velocity	Time	Pressure
Ramp + 15	45-75	85	200	300

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

Heat	Pull	Velocity	Time	Pressure
Ramp + 10	45-75	85	200	400

- 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 Table" in the Index and use* ***Type C or D*** *programs appropriate for your filament and glass combination.* ***Type D*** *programs will provide longer tapers, smaller tips, and higher resistances than* ***Type C.***

Additional Concerns about Intracellular Recording

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

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

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

Biology

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

You need to know what to expect when you run an electrode into the preparation. How will you know when you have made contact with the tissue? How will you know if you have got a penetration? Are there clues available that will help you get a superior yield? Can you tell where you 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 megohms. If the input resistance of the amplifier is not considerably greater than that of the recording electrode, the voltage measured at the amplifier will be reduced by a voltage drop across the resistance of the microelectrode. This is a simple voltage divider, and you need to understand electronics at this level. The ideal amplifier for intracellular recording would not source or sink any current when you are simply measuring voltage. Any current that does flow under these conditions will create a voltage drop across the microelectrode and also across the cell membrane, thus altering both the real and the measured membrane potentials. The amount of bias current that is tolerable depends on the electrode resistance and the target cells. A nanoamp of current (which is pretty massive for a bias current) produces one millivolt of voltage drop with a 1 megohm resistance. It is not difficult to design an amplifier that has a bias current of just a few picoamps, but the user should check bias current from time to time. It is a simple matter of looking at the

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

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

Microelectrode Resistance

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

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

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

The Micromanipulator and Other Mechanical Considerations

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

Mechanical Considerations

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

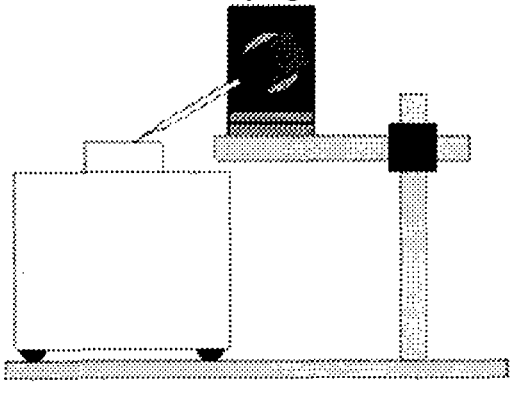
Once you have found your best possible location, you will want to build your setup on something that can isolate it from any vibration that does exist. There are many ways to do this. It is a common practice to use an air table or a similar commercial product. In many cases a heavy balance table or a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

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

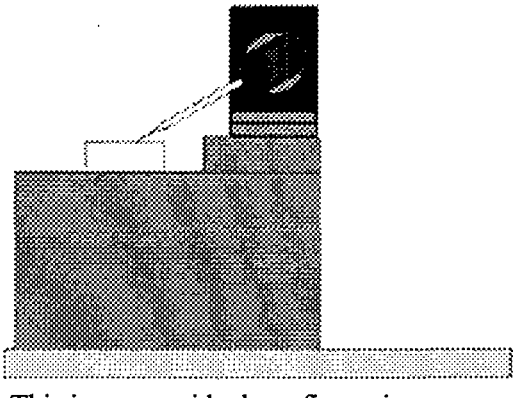
How do you make the connection from the tissue to the microelectrode stiff? Obviously we cannot expect the tissue to be rigid, but it should be constrained so that it does not move around easily. You will need to be especially attentive to this issue if the tissue is located in moving fluid. Each mechanical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

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

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



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



This is a more ideal configuration.

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

Micromanipulator

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

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

If you were driving a car, an odometer that had absolute accuracy to 1 meter would be of little advantage if your directions say 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 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.

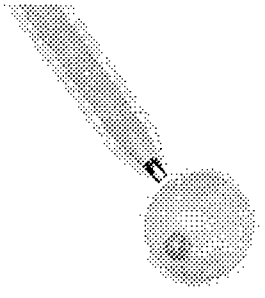
Although the distance and speed of movement might be well specified, what is seldom specified is what happens during the move. If the microelectrode is at all stiff, it is essential that movement is straight ahead without any lateral excursions. If you take a step move, does the tip of the microelectrode 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 the your glass so that it can be clamped in place securely. If the headstage cannot be mounted right on the manipulator, be careful to avoid a springy rod or a plastic material that moves with small temperature changes.

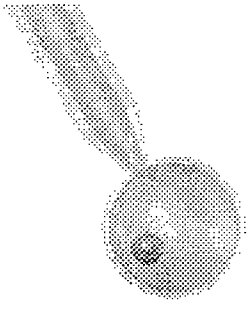
* *For Micromanipulators (MP-285, MP-225, MPC-200, and MP-265) and Micromanipulator Stands and Platforms (MT and MD Series), please see the Sutter Instrument Catalog.*

Patch Pipettes

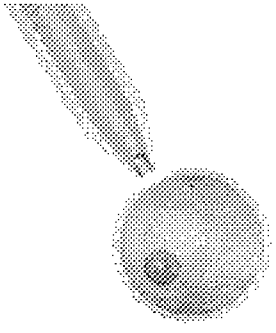
The patch clamp technique is used in electrophysiological research to study the electrical activity of neurons at the cellular level. The technique requires using a blunt pipette with a 3-4mm short taper and a 1-3μm tip to isolate a patch of membrane. In general, patch pipettes are used to electrically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



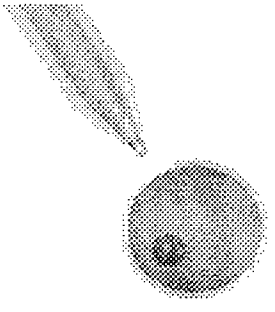
Cell-Attached Patch



Whole Cell Patch



Inside-Out Patch

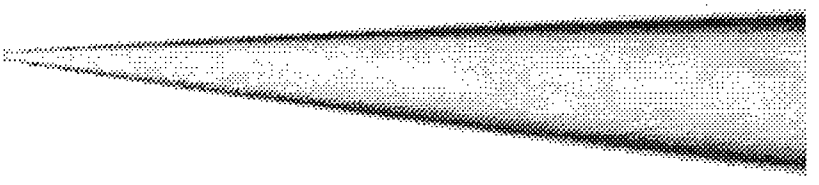


Outside-Out Patch

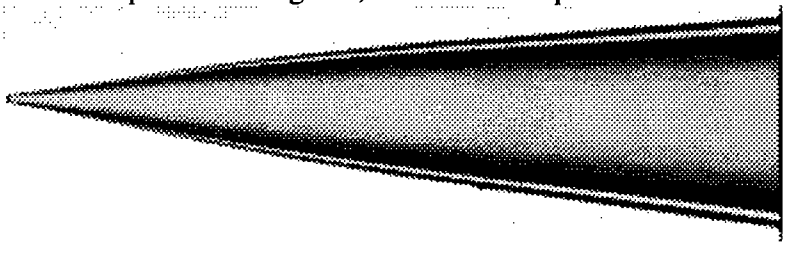
The patch technique is based on the electrical isolation of a small patch of membrane from the rest of the cell. To achieve this isolation, the patch pipette is placed against the cell membrane, and a slight suction or negative pressure is generated within the pipette. A tight seal is created between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

Patch Pipette Images

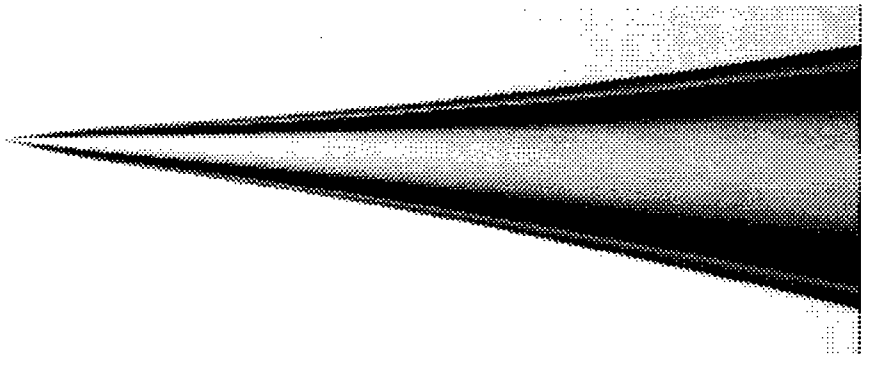
Thin Walled Glass (400X mag)
1.5mm x 1.1mm glass, 3.5µm Tip, 4mm taper
▼ General Look Up Table - Prog #56, two to three loops



Thick Walled Glass (400X mag)
1.5mm x 0.86mm glass, ~2µm Tip, 3-4mm taper
▼ General Look Up Table - Prog #51, four to five loops



Same image as above, but at 100x mag.
▼ **Thick Walled Glass**



Patch Pipettes - Recommended Programs

Whole cell patch applications often require a tip between 3-4µm and a resistance between 1-3 MΩ. To achieve this, it is best to use thin walled 1.5 x 1.1 glass with a 3mm trough filament (FT330B) or a 2.5mm x 2.5mm box filament (FB255B).

Those working within a slice preparation often require a slightly smaller tip of 1-2µm and slightly longer taper and/or need high resistances between 5-10 MΩ. In this case it is best to use standard or thick walled glass which include 1.5mm x .86mm, 1.2 x 0.69mm, and 1.0mm x 0.50mm glass and a trough or box filament.

If you are aiming for pipette resistance between 1-10 MΩ. and a tip size between 1-3 µm, any of the below programs should work well.

If you have a **3mm trough filament (FT330B)** installed in your puller, please choose the program below intended for 1.5 OD thin walled or thick-walled glass.

Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
1.5mm x 0.86mm	BF150-86-10	Ramp+10	0	55	150 t	500	4-5

Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
1.5mm x 1.10mm	BF150-110-10	Ramp+15	0	90	150 t	500	3

If you have a **2.5 x 2.5 box filament (FB255B)** installed in your puller, please choose the program below intended for 1.5 OD thin walled or thick-walled glass.

Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
1.5mm x .86mm	BF150-86-10	Ramp	0	25	1 (delay)	500	4-5

Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
1.5mm x 1.1mm	BF150-110-10	Ramp	0	40	250 t	500	3

If you have a **3.0 x 3.0 box filament (FB330B)** installed in your puller, please choose the program below intended for 1.5 OD thin walled or thick walled glass.

Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
1.5mm x .86mm	BF150-86-10	Ramp	0	25	1 (delay)	500	4-5

Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
1.5mm x 1.1mm	BF150-110-10	Ramp	0	40	250 t	500	3

You have a choice of using "filamented" or "non-filamented" capillary glass. Filamented capillaries have a small thin rod of glass adhered to the inner wall of the capillary to facilitate the capillary action of drawing solution to the tip of your pipette. This is absolutely required for microelectrodes which have a tip size less than 1 micron, but some researchers choose to use filamented glass even for patch pipettes with 1-3µm tips. The filament will not interfere with establishing a gigolm seal and might help reduce the development of air bubbles in the pipette when it is being filled.

* *If your filament and glass combinations are not found here, please refer to the "Patch Pipette Look Up Table" on Page 24 or refer to the "General Look Up Table" in the Index and install a* ***Type A*** *program designated for your filament and glass.*

How to Write a Stable Patch Pipette Program

Start with one of the suggested filament and glass combinations most suitable for your patch application and install the recommended program. To make a program with consistent results, you must find the stable velocity setting. To do this you must find the entire range of velocity settings that loop the number of times indicated on the table. For example, if you want to find a stable velocity setting for looping 4 times, pull a series of pipettes while increasing the velocity setting in 2-3 unit increments until the puller begins to loop 3 times. Then reduce the velocity setting in the same manner until the puller loops 5 times. Then by using the absolute mid-point of the velocity range, you will create the most stable and reproducible program. Here is an example for finding a stable velocity setting using 1.5 x .86 glass and a 3mm trough filament. Please start with the following ONE line program:

Heat	Pull	Velocity	Time	Pressure	Loops
Ramp + 5	0	30	250	500	4

After pulling a pipette with the above settings, write down the number of times it looped. Then pull a series of pipettes using all the same settings and only vary the velocity (up and down in 3 unit increments) to find the entire range which allows for **four loops**. To find the entire range, gradually increase the velocity in 3 unit increments until you loop three times and gradually decrease the velocity until you loop five times. So your chart of results might look like the following example:

Velocity	20	23	26	29	**32**	35	38	41	44
Loops	5	5	4	4	**4**	4	4	3	3

If these were your results, you would want to choose the midpoint velocity value of **32** for your velocity setting. The variation of heat retention in the brass jaws (which hold the filament) can sometimes introduce variability if you have an "unstable" velocity setting. So, by choosing the midpoint value, you will buffer these slight changes in heat, and your puller will loop the number of times you have intended. If you were to have randomly chosen a velocity value of 26, which in our example chart is on the threshold of values that allows for 4 loops, it is more likely that the puller would behave in an inconsistent fashion – it sometimes loops 4 times, and other times it loops 5 times. You can see that there is a bit of lee way on each side of this value of 32, so if you find your taper and tip are getting too long and small or too short and large during a series of consecutive pulls, you can test to see if the values of 34 to 37 are more stable.

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

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

A. To make a pipette with a **higher resistance and smaller tip (5-10MΩ, 1-2μ tip),** use 1.5 x .86 glass and try the following:

To create a *higher resistance with a slightly longer taper*, find the range of velocities that loops 4 times (instead of 5) by gradually increasing the velocity and choosing the middle value. For example, using a 2.5mm box filament and 1.5 x .86 glass and the following settings:

Heat	Pull	Velocity	Delay	Pressure	Loops
485	0	22	1	500	5

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

Heat	Pull	Velocity	Delay	Pressure	Loops
485	0	**30**	1	500	**4**

If you would like a **higher resistance & maintain the short taper**, write out a four-line program based on the one-line four-looping program you previously established. If your program was:

Heat	Pull	Velocity	Delay	Pressure	Loops
485	0	30	1	500	4

create a four-line program like the one below, where the velocity on the third line is reduced by five units, and a slight amount of pull and heat is added to the fourth line. Pull a pipette after each adjustment and make only one modification at a time.

Line	Heat	Pull	Velocity	Delay	Pressure
1	485	0	30	1	500
2	485	0	30	1	500
3	485	0	**25**	1	500
4	**+/- 485**	**10 – 40**	30	1	500

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

B. To make a pipette with a **lower resistance and a larger tip (1-5MΩ, 3-4μ tip)**, use a velocity setting which allows the puller to loop one or two more times (4 loops instead of 3 for thin walled glass, or 5 to 6 loops instead of 4 loops for thick walled glass). If your program was:

Heat	Pull	Velocity	Delay	Pressure	Loops
485	0	30	1	500	4

Find the range of velocities that allows 5 loops and install the mid-point value...

Heat	Pull	Velocity	Delay	Pressure	Loops
485	0	**25**	1	500	**5**

If the resulting tip morphology is too irregular (angled breaks or rough edges), write the one line program into multiple lines and reduce the heat on the last line only. If you are using thick walled glass, it might be best to switch to a thinner walled glass (1.5 x 1.1 instead of 1.5 x .86) to achieve 1-2MΩ lower resistance pipettes.

LOOK UP TABLE for Patch Pipette Programs, 1-10MΩ, 1-3µm Tip, with a 3-4mm short taper

Filament	Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
3mm Trough	1.5 x .86	BF150-86-10	Ramp+10	0	55	150 t	500	4-5
3mm Trough	1.5 x 1.1	BF150-110-10	Ramp+15	0	90	150 t	300	3
3mm Trough	1.2 x .69	BF120-69-10	Ramp+15	0	45	150 t	500	4
3mm Trough	1.2 x .94	BF120-94-10	Ramp	0	65	150 t	500	3
3mm Trough	1 x .50	BF100-50-10	Ramp+15	0	50	150 t	500	4
3mm Trough	1 x .78	BF100-75-10	Ramp+15	0	90	150 t	500	3

Filament	Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
2.5 x 2.5 box	1.5 x .86	BF150-86-10	Ramp	0	25	1 (delay)	500	4-5
2.5 x 2.5 box	1.5 x 1.1	BF150-110-10	Ramp	0	40	250 t	500	3
2.5 x 2.5 box	1.2 x .69	BF120-69-10	Ramp	0	20	250 t	500	4
2.5 x 2.5 box	1.2 x .94	BF120-94-10	Ramp	0	40	200 t	500	3
2.5 x 2.5 box	1 x .50	BF100-50-10	Ramp	0	30	250 t	500	4
2.5 x 2.5 box	1 x .78	BF100-78-10	Ramp	0	40	200 t	500	3

Filament	Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
3 x 3 box	1.5 x .86	BF150-86-10	Ramp+ 5	0	22	1 (delay)	500	4-5
3 x 3 box	1.5 x 1.1	BF150-110-10	Ramp	0	45	250 t	500	3
3 x 3 box	1.2 x .69	BF120-69-10	Ramp+ 5	0	25	250 t	500	4
3 x 3 box	1.2 x .94	BF120-94-10	Ramp+ 5	0	35	200 t	500	3
3 x 3 box	1 x .50	BF100-50-10	Ramp+ 5	0	30	250 t	500	4
3 x 3 box	1 x .78	BF100-78-10	Ramp+ 5	0	40	200 t	500	3

*The 2.5 x 4.5 box filament programs listed below are **not ideal for making short tapered patch pipettes** and are only recommended if you need a <u>much</u> longer taper.

Filament	Glass OD/ID	Glass Item #	Heat	Pull	Vel	Time/Del	Pressure	Loops
2.5 x 4.5 box	1.5 x .86	BF150-86-10	Ramp+ 5	0	23	1 (delay)	500	5-6
2.5 x 4.5 box	1.5 x 1.1	BF150-110-10	Ramp+ 5	0	45	250 t	500	3
2.5 x 4.5 box	1.2 x .69	BF120-69-10	Ramp	0	20	250 t	500	4-5
2.5 x 4.5 box	1.2 x .94	BF120-94-10	Ramp	0	40	200 t	500	3
2.5 x 4.5 box	1 x .50	BF100-50-10	Ramp	0	25	250 t	500	4
2.5 x 4.5 box	1 x .78	BF100-78-10	Ramp	0	25	250 t	500	3

References for Electrophysiology

For additional information about electrophysiology, it's history, and the various approaches, please refer to the following sources:

- Advanced Micropipette Techniques for Cell Physiology, K.T Brown, D.G. Flaming
- The Axon Guide – For Electrophysiology & Biophysics: Laboratory Techniques

  http://www.moleculardevices.com/pdfs/Axon_Guide.pdf
- Patch Clamping An Introductory Guide to Patch Clamp Recording, Areles Molleman
- The American College of Neuropsychopharmacology web site

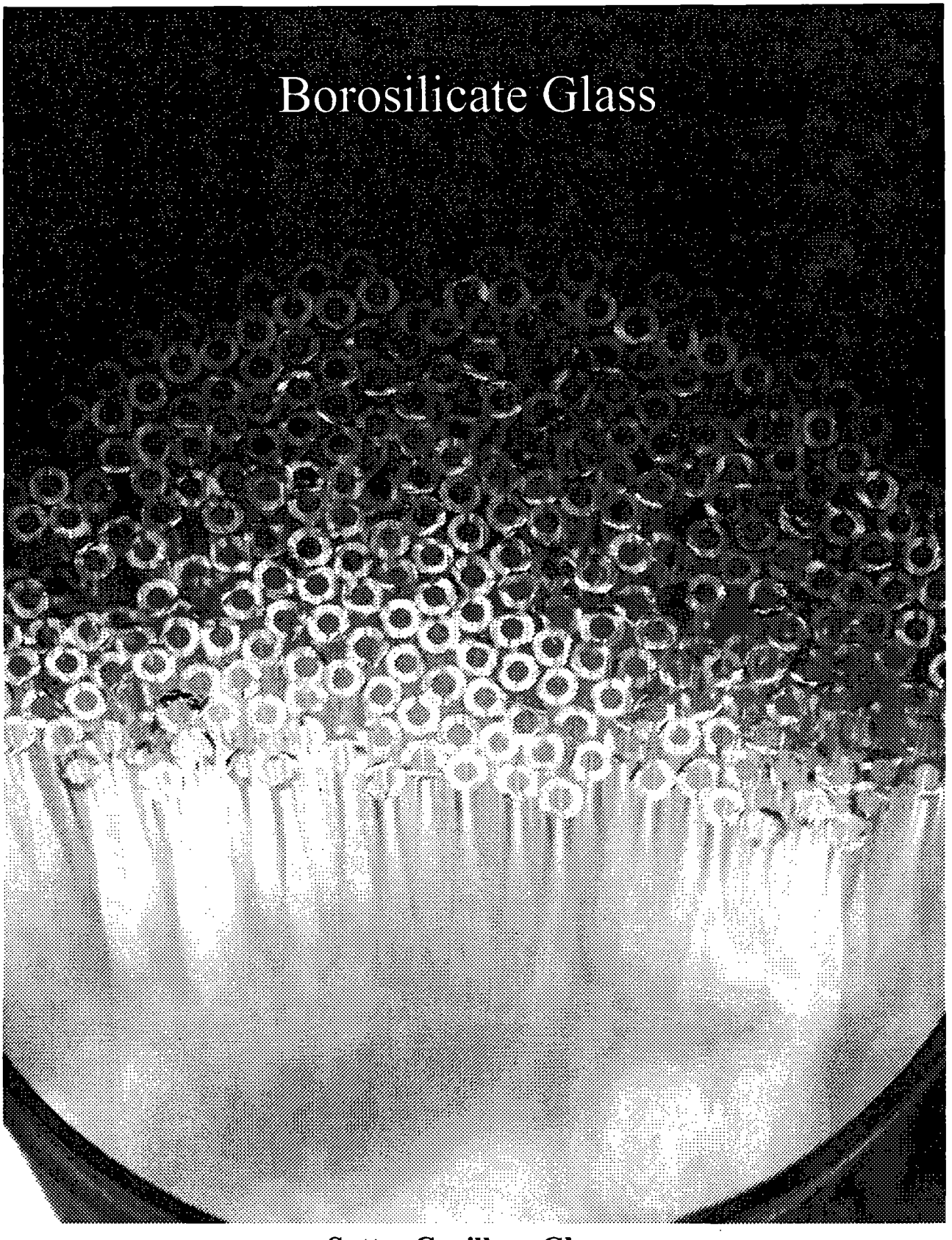
  http://www.acnp.org/g4/GN401000005/CH005.html
- Curtis, H.J. & Cole, K. S. Membrane action potentials from the squid giant axon. J. Cell. & Comp. Physiol. 15: 147-157, 1940
- Huxley AL and Hodgkin AF. Measurement of Current-Voltage Relations in the Membrane of the Giant Axon of Loligo. *Journal of Physiology* 1: 424-448, 1952(a).
- Neher E and Sakmann B. Noise analysis of drug induced voltage clamp currents in denervated frog muscle fibers. *J Physiol* 258: 705–729, 1976
- General descriptions about electrophysiology and various approaches: http://www.answers.com/topic/electrophysiology
- "Single Channel Recording" 2nd edition 1995 by Sakmann & Neher

Where to take a concentrated short course to learn electrophysiology techniques

Marine Biological Lab, Woods Hole, MA
http://www.mbl.edu//education/courses/summer/

Cold Spring Harbor Lab, Cold Spring Harbor, Long Island, NY
http://meetings.cshl.edu/courses.html

Mount Desert Island Labs
http://www.mdibl.org/courses/index.shtml



Sutter Capillary Glass

(See Page 7 for complete list and prices)

CHAPTER 2

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

The pipettes required for this application are most commonly made using standard or thick walled filamented glass; Sutter Part Number BF100-50-10, BF100-58-10 and BF120-69-10. These applications require a fairly short taper that is approximately 4-6mm in length and a tip that is just under 1µm. The tip and taper need to be fine enough to not cause damage, and the taper should not be so long that it becomes wispy and not durable. To maintain good durability in the pipette, and keep the taper on the shorter side, it is best to use 2.5mm x 2.5mm box filament. It is also best to use filamented glass any time you are making needles with tip sizes under 1µm. for easy loading of your solution into the needle. The best ingredients to start with would be the 1mm x 0.5mm filamented glass (BF100-50-10) and a 2.5mm x 2.5mm box filament (FB255B). Although a 3mm x 3mm box filament (FB330B) will also work well pulling these types of pipettes, the smaller and more narrow 2.5 box filament provides more efficient heating to the glass since the source of heat is in closer proximity to the glass. It is also easier to keep the taper from becoming too long and wispy using the smaller 2.5mm box filament. If you are using a puller where the filament cannot be changed, please find the filament type in the Look Up Table (pg. 29) and use the program designed for the glass available to you in your lab. The 2.5mm x 4.5mm box filament and 1.5mm OD glass are not ideal for this application. 1.5 x 0.86 glass will produce tapers that are too long and wispy and 1.5 x 1.1 glass might cause greater damage to the cell during the injection since the outer diameter of the taper increases. The following glass is recommended for this application: BF100-50-10, BF100-58-10, or BF12-69-10.

Programs for a 2.5 x 2.5 box filament (FB255B)

Using 1mm x 0.5mm glass (BF100-50-10) or (BF100-58-10) install the following settings:

Heat	Pull	Velocity	Delay	Pressure
Ramp+5	45	75	90	500

Using 1.2mm x 0.69mm glass (BF120-69-10) install the following settings:

Heat	Pull	Velocity	Delay	Pressure
Ramp+5	45	80	200	600

- If you find the *taper too long and the tip too small*, reduce the heat by 5 units and then gradually increase the delay in 10 unit increments.

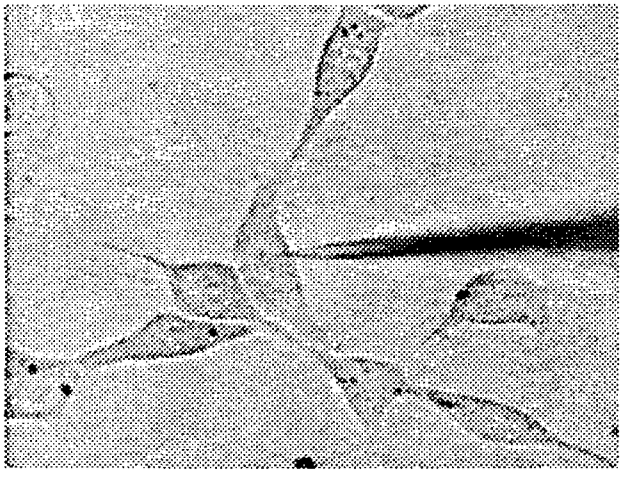
- If you find the *taper too short and the tip too large*, increase the pull in 5 unit increments (do not go beyond 80 units) and then begin to drop the delay in 5 unit increments.

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

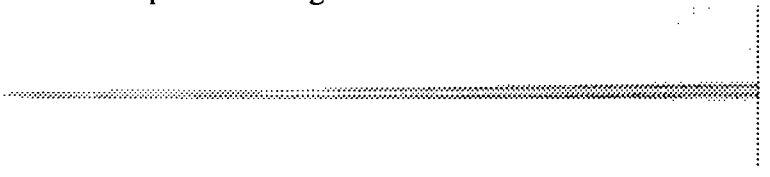
Using the P-97, the **most durable and sharp needles** for this application can be made with **Aluminosilicate glass**, AF100-68-10. Even sharper needles can be achieved by beveling the tips using the Sutter BV-10 beveler (Chapter 15). For a shorter and quick taper, you might want to try a "Bee Stinger Needle" for this application (Chapter 3).

* *For additional filament and glass combinations for this application, please refer to the "Look Up Table" on page 29 at the end of this Chapter or refer to the General Look Up Table in the Index and use a* ***Type B*** *program.*

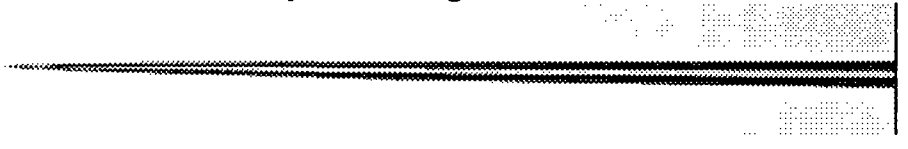
Adherent Cell, *C.elegans, Drosophila*, & Zebrafish microinjection



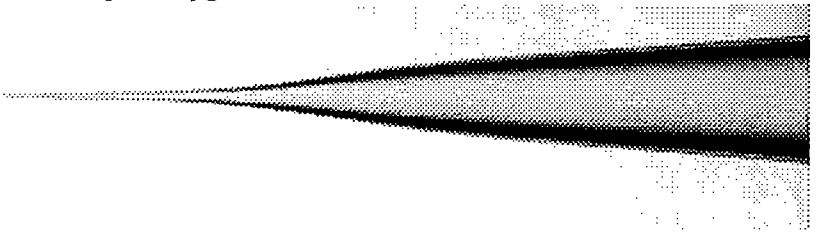
Thin Walled Glass (400x mag)
1.0mm x .78mm glass, 0.7 to 0.9µm Tip, 5-7mm taper
▼ General Look Up Table - Prog #37 or #38



Thick Walled Glass (400x mag)
1.0mm x 0.50mm glass, 0.6 to 0.9µm Tip, 6-8mm taper
▼ General Look Up Table - Prog #32 or #33



Bee-Stinger Pipette (short, 100x mag)
1.0mm x 0.50mm glass, 0.5µm Tip, 4-6mm taper
▼ See Chapter 3, pg 31-32 for instructions



LOOK UP TABLE for Adherent Cell, *C.elegans, Drosophila*, Zebrafish microinjection.
<1µm injection needle with a 4-6mm taper which can be used "as is", broken back, or beveled.

Filament	Glass OD/ID	Glass Item #	Line	Heat	Pull	Vel	Time/Del	Pressure
* 3mm Trough	1.5 x .86	BF150-86-10	1	Ramp+10	0	55	200 t	200
			2	Ramp+10	35	155	200 t	200
3mm Trough	1.5 x 1.1	BF150-110-10	1	Ramp+ 5	45	120	175 t	200
* 3mm Trough	1.2 x .69	BF120-69-10	1	Ramp+10	0	20	150 t	200
			2	Ramp+15	75	50	150 t	200
3mm Trough	1.2 x .94	BF120-94-10	1	Ramp+15	0	90	150 t	500
* 3mm Trough	1.0 x .50	BF100-50-10	1	Ramp+15	0	20	150 t	200
			2	Ramp+15	45	60	150 t	200
3mm Trough	1.0 x .75	BF100-75-10	1	Ramp+10	45	100	175 t	500

*** The 3mm trough filament and these programs are not the best choice (not ideal) for making this type of pipette!**

Filament	Glass OD/ID	Glass Item #	Line	Heat	Pull	Vel	Time/Del	Pressure
2.5 x 2.5 box	1.5 x .86	BF150-86-10	1	Ramp	35	70	250 (delay)	200
2.5 x 2.5 box	1.5 x 1.1	BF150-110-10	1	Ramp	55	75	120 (delay)	400
2.5 x 2.5 box	1.2 x .69	BF120-69-10	1	Ramp	45	80	200 (delay)	600
2.5 x 2.5 box	1.2 x .94	BF120-94-10	1	Ramp	50	85	90 (delay)	450
2.5 x 2.5 box	1.0 x .50	BF100-50-10	1	Ramp+ 5	45	75	90 (delay)	500
2.5 x 2.5 box	1.0 x .75	BF100-75-10	1	Ramp	50	80	90 (delay)	200

Filament	Glass OD/ID	Glass Item #	Line	Heat	Pull	Vel	Time/Del	Pressure
3 x 3 box	1.5 x .86	BF150-86-10	1	Ramp	45	80	200 (delay)	600
3 x 3 box	1.5 x 1.1	BF150-110-10	1	Ramp+ 5	55	90	120 (delay)	400
3 x 3 box	1.2 x .69	BF120-69-10	1	Ramp+ 5	45	80	120 (delay)	500
3 x 3 box	1.2 x .94	BF120-94-10	1	Ramp	55	85	110 (delay)	300
3 x 3 box	1.0 x .50	BF100-50-10	1	Ramp+ 5	35	75	130 (delay)	500
3 x 3 box	1.0 x .75	BF100-75-10	1	Ramp+ 5	55	75	80 (delay)	300

Filament	
2.5 x 4.5 box	**The 2.5 x 4.5 box filament, in combination with any dimension of glass, is not recommended for this type of needle or pipette morphology.**

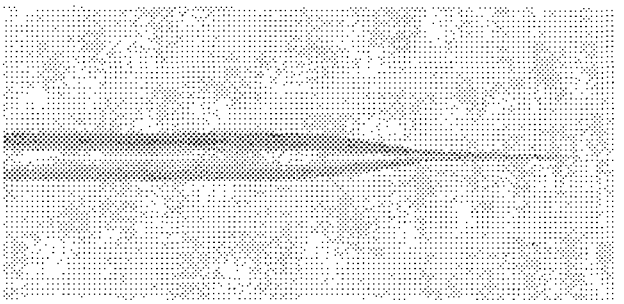
* *For even sharper needles, we recommend that you try using Aluminosilicate glass AF100-68-10 Please call Sutter Instrument for recommended parameter settings using Aluminosilicate glass. The programs listed above are identical to* ***Type B*** *programs on the General Look Up Table*

CHAPTER 3

Bee-Stinger Needle

~0.5µm Tip
~4 to 5mm Total Length
~50-500µm long "bee stinger" like projected tip

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



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

Heat	Pull	Velocity	Time	Pressure
Ramp	100	10	250	500

Your Goal is 3 Loops!

The velocity setting in this one line program is the determining factor which controls the number of times the program will loop. Three loops appears to work best and produce the most stable results. If the program does not run as expected (loops too many or too few times), please make small adjustments in 1-2 unit increments up or down in the velocity setting to insure that it loops three times. An increase in velocity will cause the program to loop fewer times, and a decrease in velocity will cause it to loop more times. If you need to fine-tune the final length of the "bee stinger" like projected tip, write the program out into three identical lines and make small adjustments to the velocity on the last line only (Image B, pg. 32) A slight reduction in the velocity will reduce the final taper and a slight increase in the velocity will lengthen the final taper.

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

SPECIAL NOTE: Although this morphology is sometimes considered good for adherent cell microinjection, and this might prove to be true with your protocol, sometimes a more gradually tapered pipette which is more durable and less brittle is a far superior morphology to aim for.

Bee-Stinger Needle

Image A **(40x mag.)**
1.0 x 0.5 thick walled glass (BF100-50-10)
~0.5µm Tip, 4-5mm taper
▾ One line program (pg. 31) with 3 loops

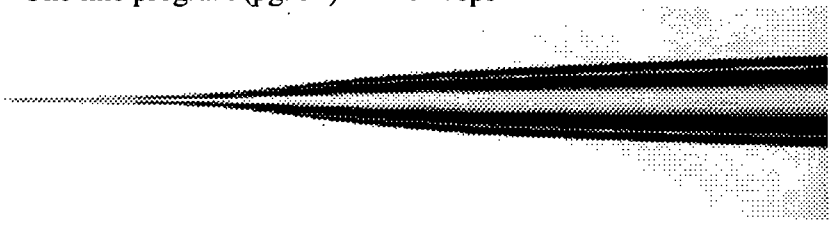


Image B **(40x mag.)**
1.0 x 0.5 thick walled glass (BF100-50-10)
~0.5µm Tip, 4-5mm taper
▾ Three line program w/ reduced velocity on line 3

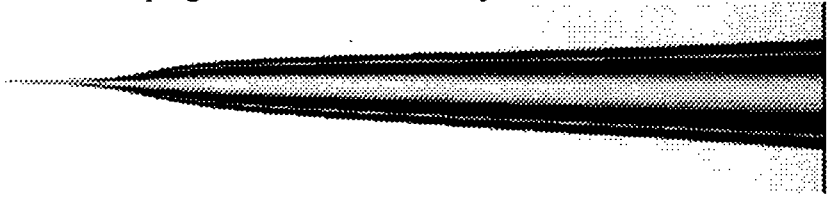
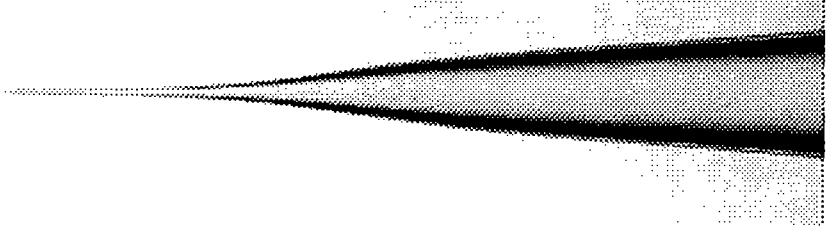


Image C **(100x mag.)**
1.0 x 0.75 thin walled glass (BF100-78-10)
0.7-0.5µm Tip, 4-5mm taper
▾ One line program (pg. 31) with 3 loops



CHAPTER 4

Pronuclear 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 0.9μm 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 being tapped off and broken back to "open it up", the ID of the resulting pipette should remain under 1μm (See images on pg. 34).

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

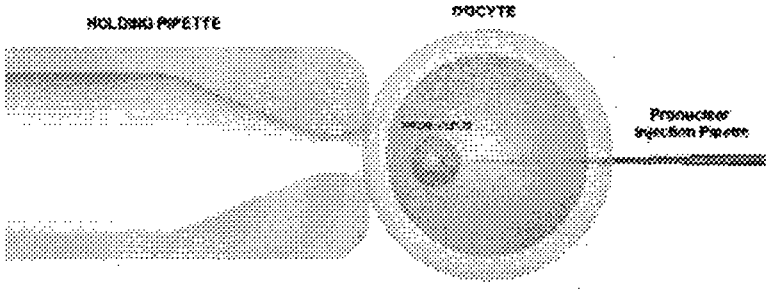
It appears that about half the population of microinjectionists doing pronuclear injection use the injection pipette "as is" and do not break back the tip until it becomes clogged and are unable to flush it out. The other half of microinjectionists are in the habit of breaking back, or tapping off the tip before using it to be able to have a good flow rate through the needle. While the technique being used is often determined by one's predecessor, it is also possible that the technique used is determined by how well one is able to control the outcome of the pipette morphology. Therefore, the technique of tapping off the tip is sometimes being done due to the inability to find proper parameter settings. The program settings provided below are intended to make a pipette that has a tip inner diameter and taper length appropriate for performing the injection with out tapping off or breaking back the tip. If you prefer to tap off the tip, please use a pull setting that is 10 to 30 units higher than what is suggested or use lower delay values.

Pipette Clogging

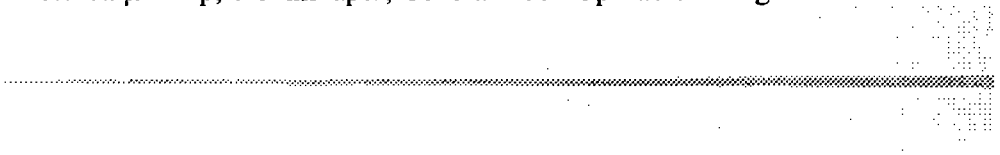
The eventual clogging of pipettes during pronuclear injection is inevitable, but one hopes to be able to do multiple injections before encountering this event. After a series of injections the pipette will clog, and, at this stage, one can clear out the pipette using the "clearing" or "flushing" pressure setting on the microinjector. When or if this fails, one can then tap off the tip to remove the clogged end 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 due to a number of factors which are listed below:

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

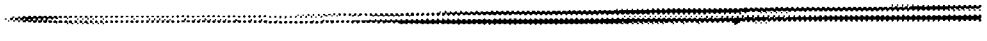
Pronuclear Injection



Pronuclear Injection Pipette (100x mag)
Thin walled 1.0mm x 0.78mm filamented glass,
▼ 0.7-0.9µm Tip, 6-8mm taper, General Look Up Table - Prog #38



Pronuclear Injection Pipette (same as above, but at 400x mag)
▼ Final taper is even and gradual with minimal "shoulder" behind the final tip



Pronuclear Injection – Recommended Programs

Goal = 6-8mm taper, 0.7 to 0.9µm tip, gradual taper and no distinct shoulder behind the tip.

Pronuclear injection pipettes are most commonly made using 1.0mm x 0.78mm thin walled filamented glass (BF100-78-10). This type of pipette is quite specific with very tight tolerances and requires a fairly gradual taper that is approximately 6-8mm long and a tip size just under 1µm. The tip and taper need to be fine enough to not cause excessive damage, therefore a gradual taper with a minimal "shoulder" behind the tip is desired.

A taper length that is too short (under 6mm) and a tip size that is too big (over 1µm) will cause too much damage. On the other hand, a taper that is too long (over 8mm) and a tip that is too small (under 0.7µm) will make it difficult to inject the dilution of DNA through the tip.

Listed below are the recommended programs using a 2.5mm x 2.5mm box filament (FB255B) or a 3mm x 3mm box filament (FB330B) and 1mm x 0.78mm filamented glass (BF100-78-10).

Program for **1.0 x 0.78 filamented glass** (BF100-78-10) & a **2.5 x 2.5 box filament** (FB255B). This is the most direct path to making good pronuclear injection pipette.

Heat	Pull	Velocity	Delay	Pressure
Ramp	60-90	70	80	200

Program for 1mm x 0.78mm OD/ID glass & **3 x 3 box filament** (FB330B)

Heat	Pull	Velocity	Delay	Pressure
Ramp+5	50-80	75	80	300

- If you find the *taper too long and the tip too small*, increase the delay in 5 unit increments and then gradually decrease the pull in 5 unit increments.
- If you find the *taper too short and the tip too large*, increase the pull in 5 unit increments (do not go beyond 110 units) and then begin to drop the delay in 5 unit increments.

Ranges to stay within:

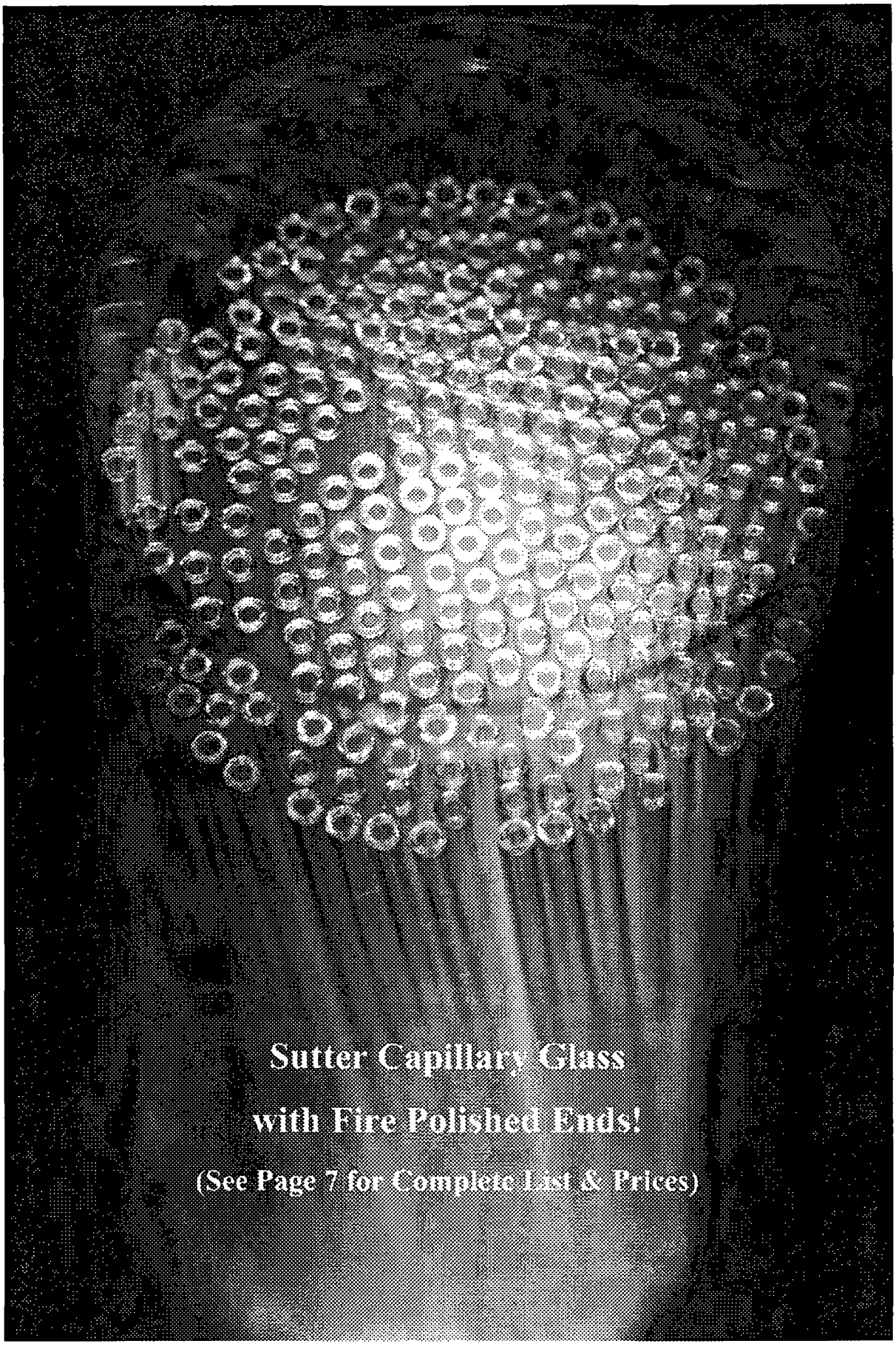
Heat = Ramp-5 to Ramp+10, Pull = 40-110, Velocity = 60-80
Delay = 60-100, Pressure = 200-400

Technical Notes:

For successful repair of the oocyte and incorporation of the DNA, a small amount of trauma to the plasma membrane is considered a key ingredient. As a result, using the pipette "as is" and then, after it has clogged, tapping it off to further open it up, can be seen as a good practice and a preferred technique.

The need for a smaller or larger tip size is often dependent on the dilution and size of your DNA. For example, the YAC (yeast artificial chromosome) DNA is very large and often requires a pipette with a shorter taper and larger tip. Often it is necessary to cut or tap off the final tip to provide a larger bore so as to prevent clogging or shearing of the DNA.

* *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 Table and use the program designed for the appropriate glass.* ***Type C*** *programs are recommended.*


Sutter Capillary Glass
with Fire Polished Ends!
(See Page 7 for Complete List & Prices)

CHAPTER 5

Embryonic Stem (ES) Cell Microinjection

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

Pipette Morphology

The ES cell injection needle has a long 8-12mm taper, where the taper at the tip is as parallel as possible, and the final tip has an internal diameter slightly larger than the ES cells (15-25µm inner diameter). Using a manual microinjector like the XenoWorks Analog Microinjector, (See Chapter 14), 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 15). 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

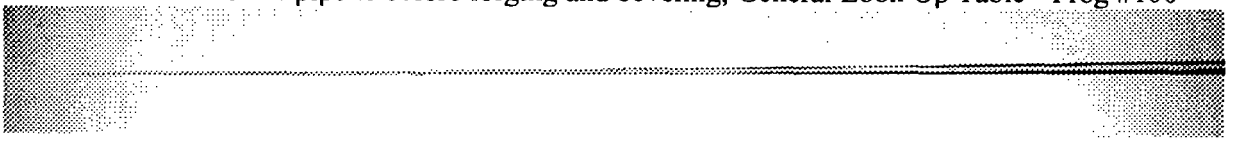
- If you find the *taper too long*, reduce the heat in 5 unit increments (but do not go below the ramp value) and then gradually increase the time in 10 unit increments.
- If you find the *taper too short*, reduce the time in 25 unit increments and then increase the pull in 10 unit increments.

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 14 which describes the XenoWorks Microinjection System.

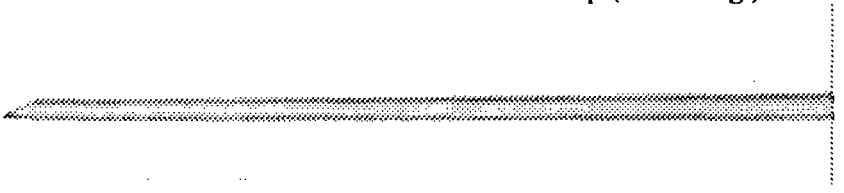
* *If you are using a puller where the filament cannot be changed, please find the filament type that is installed in your puller in the General Look Up Table and use the program designed for the appropriate glass.* ***Type D or E*** *programs are recommended for this application.*

Embryonic Stem (ES) Cell Injection

Example A – Small ES Cell Pipette (100xmag.)
1.0mm x 0.75mm glass is first pulled out long to have a 10-15mm taper
▼ Glass pipette before forging and beveling, General Look Up Table - Prog #100

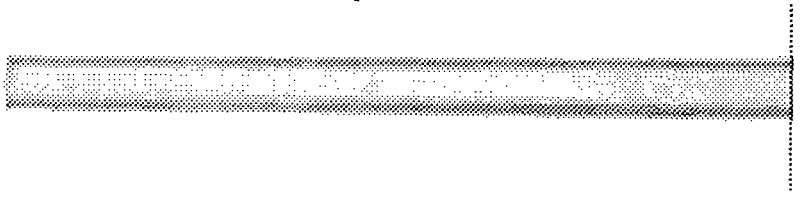


▼ **Forged and beveled** at a location 1 to 3mm back from tip **(400x mag.)**

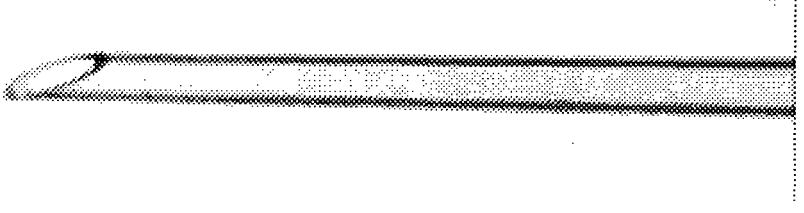


Example B – Large ES Cell Pipette (400x mag.)
1.0mm x 0.75mm glass has been scored and broken back using a ceramic tile (CTS)
23µm Tip, 8-10mm final taper, General Look Up Table-Prog #99, forged and beveled

▼ Forged 4 to 5mm back from the initial tip



▼ Same tip after it has been beveled (BV-10 Beveler with a course plate)



CHAPTER 6

ICSI (Intracytoplasmic Sperm Injection)

ICSI is an advanced form of in-vitro fertilization (IVF), which was traditionally performed in a test tube. ICSI pipettes are used to aspirate and inject a single sperm cell directly into a single oocyte in order to achieve fertilization. It has been declared one of the most important advancements in the research of reproductive medicine. In this technique the oocytes and sperm are placed on a slide and viewed under an inverted microscope. Mounted on the microscope stage is a system of two micromanipulators to which the micropipettes are attached. Commonly the holding pipette is positioned on the left to maintain the position of the ooctye, while the ICSI pipette is positioned on the right which is used to microinject the sperm into the egg. The movement of the pipettes are controlled by joysticks as demonstrated by the XenoWorks Microinjection System (Chapter 14).

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 (tail first) but not be so large as to allow the sperm to turn around inside the pipette. If the pipette inner diameter is too large, this can allow a successfully injected sperm to escape by swimming straight back out of the injected oocyte. A typical range for the ID of a pipette for this application is between 5-15µm.

Pipette Morphology

Thin-walled, unfilamented borosilicate capillary glass (B100-75-10) should be used with parameter settings which are sufficient to draw out a 10-15mm long, gently-tapering pipette. The resulting tip diameter is irrelevant since the pipette tip will be broken back and then beveled to create the appropriate tip size between 5 and 15µm.This process is performed by first clipping back the final tip to create a 3-10µm tip and then beveling the tip using the Sutter BV-10 Beveler (Chapter15). The pipette tip should be beveled back to a diameter appropriate to the sperm being injected and beveled at the desired angle, which is usually between 30 and 40 degrees. If required, creating a small spike on the pipette tip and bending the pipette is performed using a microforge.

ICSI – Recommended Program

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

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

- If you find the *taper too long*, reduce the heat in 5 unit increments 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 pressure in 50 unit increments.

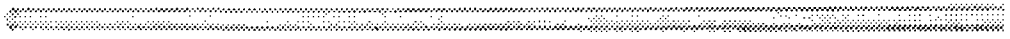
* *If you are using a puller where the filament cannot be changed, please find the filament type that is installed in your puller in the General Look Up Table and use the program designed for the appropriate glass.* ***Type D or E*** *programs are recommended for this application.*

ICSI (Intracytoplasmic Sperm Injection) Pipettes

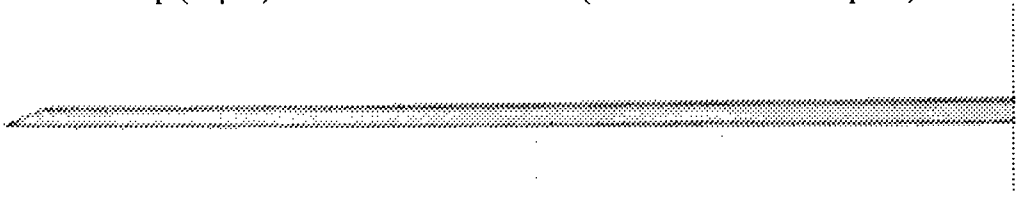
Small ICSI (400x mag)

This glass has been scored and broken back using a ceramic tile (CTS)

- This is approximately 2 to 3mm back from the initial tip

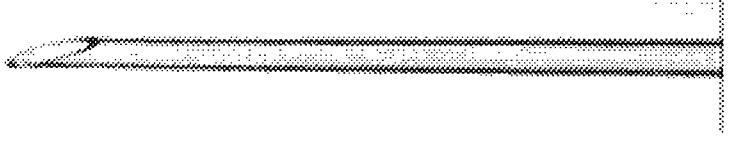


- Same tip (~7µm) after it has been beveled (BV-10 with a course plate)



Large ICSI (400x mag)

- Glass was broken further back to create a larger ID (~15µm tip) and then beveled (BV-10 Beveler with a course plate).



Technical Note:

Microinjection protocols for sperm injection, particularly those used for injecting mouse oocytes, sometimes call for micropipettes with a flat end that are used to first "core" through the zona pellucida and then through the oolemma using minute vibrations from a piezo device such as the PrimeTech PMM-150. The micropipettes used with a piezo drill require a clean 90-degree break at the tip (produced by a microforge), so a bevel or spike at the tip is not needed. The inner diameter of these pipettes must be carefully controlled for the pipette to be most effective. The pulled pipette is usually cut on a microforge to the appropriate diameter.

CHAPTER 7

Nuclear Transfer

Nuclear Transfer is one of the methods used for the cloning animals and involves removing the nucleus from a donor cell of an animal and then placing and 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 5-15µm.

Pipette Morphology

Thin-walled, unfilamented borosilicate capillary glass (B100-75-10) is recommended using parameter settings which are sufficient to draw out a 10-15mm long, gently-tapering pipette. The resulting tip diameter is irrelevant since the pipette tip will be broken back clean or it will be broken back and beveled to create a tip size between 5 to15µm. Breaking the glass back to have a clean blunt end is performed using a microforge. The resulting blunt needle is used in conjunction with a Piezo device like the Primetech PMM150-FU (See Sutter Instrument 2006 Catalog). If a beveled pipette is needed, rather than a blunt tip, it is created by first clipping back the pipette to make a 3-5µm tip and then beveling the tip with the Sutter BV-10 (Chapter 15). The pipette tip should be beveled back to a diameter appropriate to the sperm being injected and beveled at the desired angle, which is usually between 30 and 40 degrees. If required, creating a small spike on the pipette tip is performed using a microforge.

Nuclear Transfer – Recommended Program

These pipettes are usually made from 1mm x 0.75mm **(B100-75-10)** capillary glass. The 2.5 x 4.5 **(FB245B)** 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 + 30	30	120	200	200

- If you find the *taper too long*, reduce the heat in 10 unit increments and then gradually increase the pressure in 25 unit increments.
- If you find the *taper too short*, reduce the time in 25 unit increments and then increase the pull in 20 unit increments.

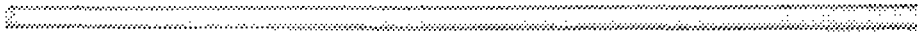
* *If you are using a puller where the filament cannot be changed, please find the filament type that is installed in your puller in the General Look Up Table and use the program designed for the appropriate glass.* ***Type E*** *programs are recommended for this application.*

Nuclear Transfer Images

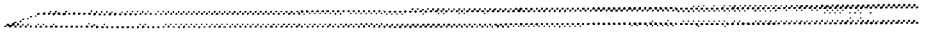
Small NT Pipette **(400x mag)**

This glass has been scored and broken back using a ceramic tile (CTS)

- This is 2 to 3mm back from the initial tip (~10µm)

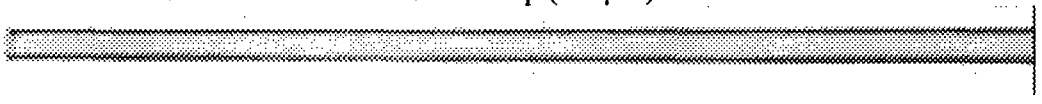


- Same tip after it has been beveled (BV-10 Beveler with a course plate)

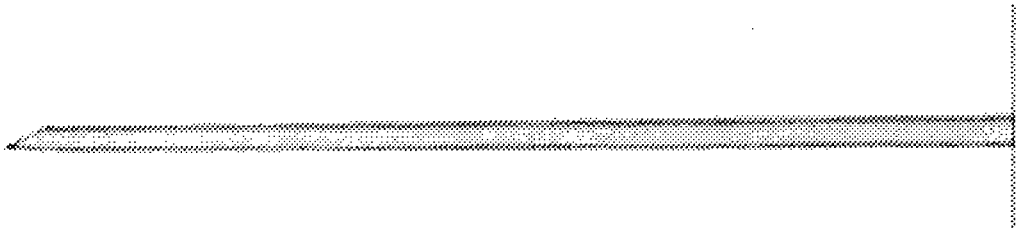


Large NT Pipette **(400x mag)**

- This is 2 to 3mm back from the initial tip (~15µm)



- Same tip after it has been beveled (BV-10 Beveler with a course plate)



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

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

CHAPTER 8

Holding Pipettes

When performing techniques such as pronuclear injection, ICSI, and ES Cell injection, the holding pipette is used to hold and immobilize the cell or blastocyst during the microinjection procedure. Holding pipettes have a clean 90-degree break at the tip which is fire-polished to create a smooth surface to interface with the cell. Holding pipettes are usually made to have a fairly large inner and outer diameter, which provides better support and reduces any possible distortion of the oocyte during the micromanipulation. Depending on the size of the oocyte, the extent of polish, and the thickness of the capillary glass used to make the pipette, holding pipettes traditionally have an inner diameter of 20- 25µm and an outer diameter between 75-125µm.

Holding Pipettes – Recommended Program

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

Heat	Pull	Velocity	Time	Pressure
Ramp + 30	0	140	200	200

This will generate a long, even taper with parallel walls

- If you find the *taper too long*, reduce the heat in 10 unit increments and then gradually increase the pressure in 25 unit increments.
- If you find the *taper too short*, reduce the time in 25 unit increments and then Increase the pull in 20 unit increments.

Additional Steps Needed to Fabricate your Holding Pipette

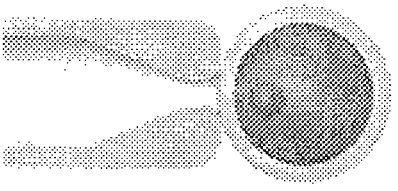
Breaking back the glass – The pipette will need to be broken back to have an inner diameter between 25 and 75 microns. This will depend on the size of cell you intend to "hold" and the extent of fire polish you will need to provide to the tip. To break the glass back clean at tip sizes of 20 microns and larger, it is best to use a diamond knife or ceramic tile to create the score on the glass at the location the glass needs to be broken. You might prefer a ceramic tile since it easier to use and it is light enough that one can feel the rough edge of the tile scratching the glass (Sutter Instrument Catalog number "CTS"). A microforge can also be used, but when breaking glass with a microforge at tip sizes over 25 microns, the glass tends to break at an angle or cause distortion and bending of the forge heating element.

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

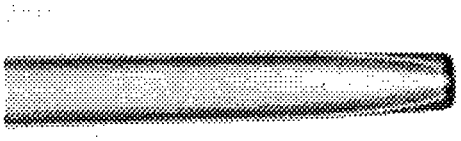
Bending the Pipette – Depending on your application and set up, it is sometimes necessary to create a 20 to 40 degree bend in the taper of the pipette. The bending of the pipette is most often needed when your cells are in a dish rather than on a slide and a specific incident angle is preferred. A microforge is required for this step.

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

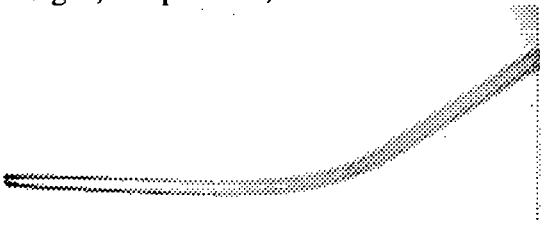
Holding Pipette Images



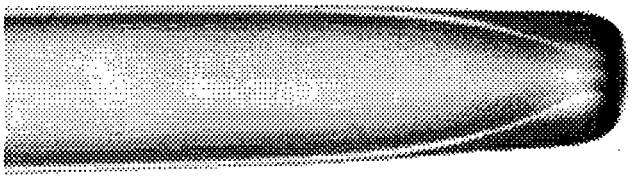
Small Holding Pipette (400x mag.)
5µm ID x 15 µm OD
- Forged & fire-polished



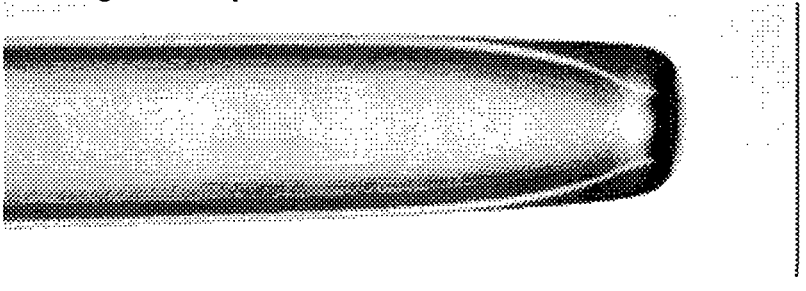
Small Holding Pipette (40x mag.)
5µm ID x 15 µm OD
- Forged, fire-polished, and bent



Large Holding Pipette (400x mag.)
15µm ID x 70 µm OD
- Forged & fire-polished



Large Holding Pipette (400x mag.)
25µm ID x 90 µm OD
- Forged & fire-polished



CHAPTER 9

Xenopus (frog egg) Microinjection

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

Xenopus Microinjection – Recommended Program

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

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

- If you find the *taper too long*, reduce the velocity in 10 unit increments and then gradually increase the pressure in 25 unit increments.
- If you find the *taper too short*, reduce the pressure in 25 unit increments and then increase the pull in 10 unit increments.

Breaking back the pipette to make a 5-15µm tip

These settings will create a 8-12 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 5-15µm opening at the tip. A pipette tip that has a somewhat rough, jagged break is ideal for cutting through the membrane of the Xenopus oocyte. Some folks will take the extra effort to bevel the pipette to make a clean, sharp, hypodermic-like needle. If you prefer an angled break to the tip, something between a clean bevel and a rough break, and you do not have a beveler, you might want to try the following "trick".

A Method to Breaking the Glass and get a Beveled/Angled 20-25µm tip

After pulling a long tapered pipette, stretch a piece of Kimwipe taut over a beaker and quickly and gently poke the needle through the Kimwipe. For what has yet to be explained to me, this technique ends up producing a 15-25µm angled break in the glass and about a 90% yield of good usable pipettes. If you use 1 or 2-ply tissue instead, you can sometimes get a 10-15µm angled break.

* *If you are performing intracellular recording you will need a pipette that is shorter and sharper. Please refer to the General Look Up Table, locate the chart with matches the type of filament in your puller and the glass you are using, and try* ***Type B or C programs*** *to make a sharp electrode.*

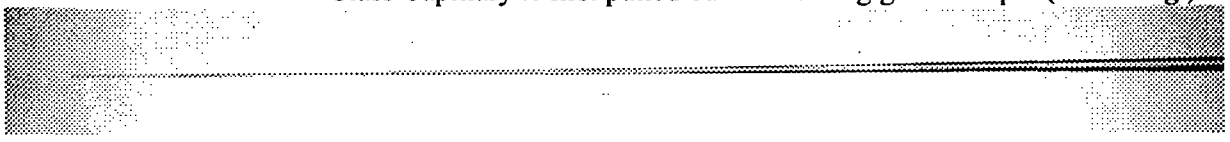
* *If you are using a puller where the filament cannot be changed, please find the filament type that is installed in your puller in the General Look Up Table and use the program designed for the appropriate glass.* ***Type D*** *programs are recommended for this application.*

Xenopus Microinjection Pipette Images, 3µm - 15µm Tips

Capillary Glass is first pulled out to have a long even taper.

* See Pg 45 for recommended glass, filament and parameter settings.

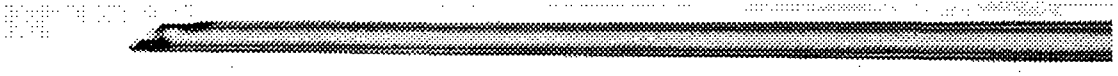
- Glass Capillary is first pulled out with a long gradual taper **(100x mag.)**



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

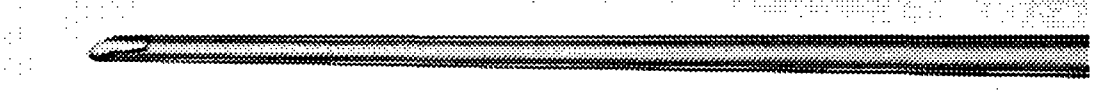
Broken with Tweezers

- This pipette has been broken back with tweezers to make a rough 5-10µm ID break **(400x mag)**.



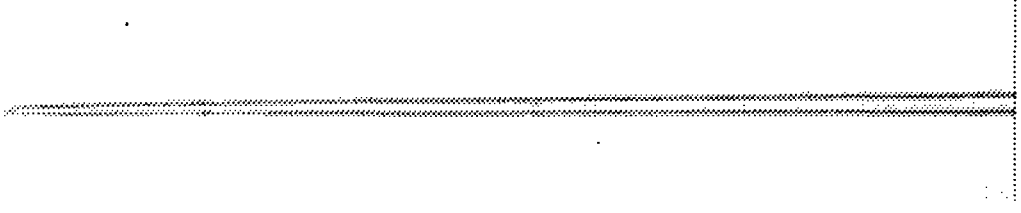
Broken with a glass coverslip

- 5-10µm Tip, thick walled glass, stabbed through 1 ply Kleenex **(400x mag.)**



Beveled with the BV-10 Beveler (Chapter 15)

- This pipette has been beveled at 45-degrees using the BV-10 Beveler, 3-5µm ID **(200x mag)**.



CHAPTER 10

Large Pipettes, 20µm to 200µm Tips....Using the Ceramic Tile (CTS)

Micropipettes with tip sizes over 20µm are often difficult to create without using a mechanical device to score and break back the glass. Microforges are often used to create tip sizes between 5 and 20µm, but once a larger tip is needed, the delicate filament on a Microforge is often too fragile to effectively break the glass back or produce a clean break. It is in these circumstances that we recommend using thick walled glass and the ceramic tile (Sutter Item # CTS, pg 50) to create a tip between 20-200µm and a clean 90-degree break.

The front of the ceramic tile (pg 50) is the surface with the Sutter logo. The front edges of the tile are rough and should be used for scoring the glass. You can use a microscope at 100 to 200 times magnification to examine the taper of the pipette and determine where the pipette needs to be scored to create a specific tip ID. Marking the glass with a Sharpie will give you a general target to aim for. You can also "blindly" score the glass by starting high and moving down the taper and use a little "trial and error" to find the proper location. You will find that the flexibility of the glass decreases as the diameter of the taper increases, and this tactile feedback can sometimes help one determine where the glass should be scored to achieve a specific tip size.

Using the P-97 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	140	100	200

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

Additional "Tricks" in making 15-75µm Tips

Clean Break

I have found that by simply bending over the finest aspect of the taper, by means of brushing and bending the glass over using my index finger, the glass will naturally break between **15-30µm** and will surprisingly have a clean break about 50-70% of the time (See top image, pg 49).

Angled Break

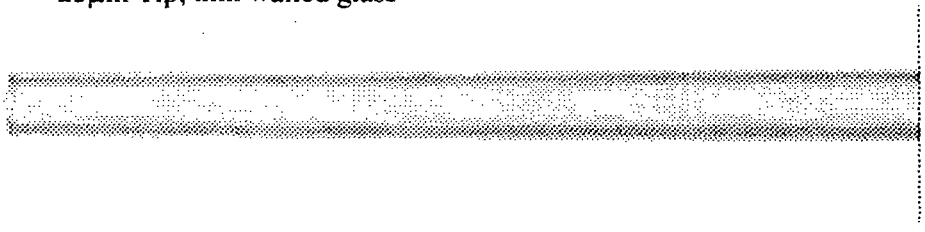
To create a beveled/angled semi-rough break, and a 20-25µm open tip, pull a long tapered pipette, stretch out a piece of Kimwipe taut over a beaker, and quickly and gently poke the needle through the Kimwipe. For what has yet to be explained to me, this technique ends up producing a **20-25µm angled break** in the glass and about a 90% yield of good usable pipettes. If you use 1 or 2-ply tissue, you can often get a **10-20µm angled break**, and if you use a paper towel, you can often get a **50-75µm angled break**. Additional paper products remain to be tested!
See page 49 for images.

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

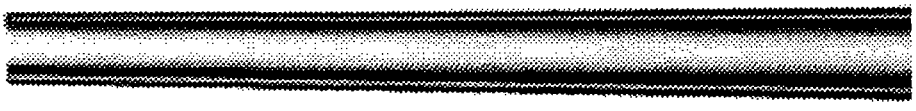
Large Pipette Images, 20µm - 200µm Tips
Using the Ceramic Tile

Glass is pulled out long and then scored and broken back to create a large clean break using the Ceramic Tile (CTS)

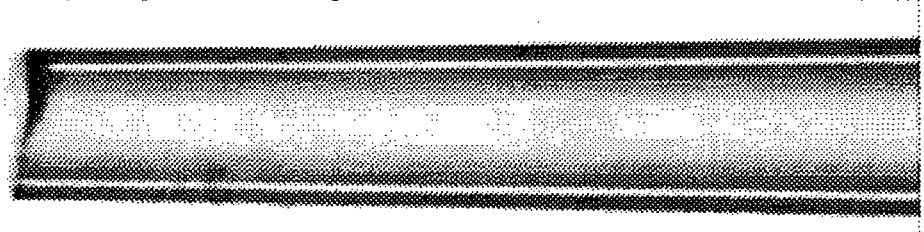
- 25µm Tip, thin walled glass



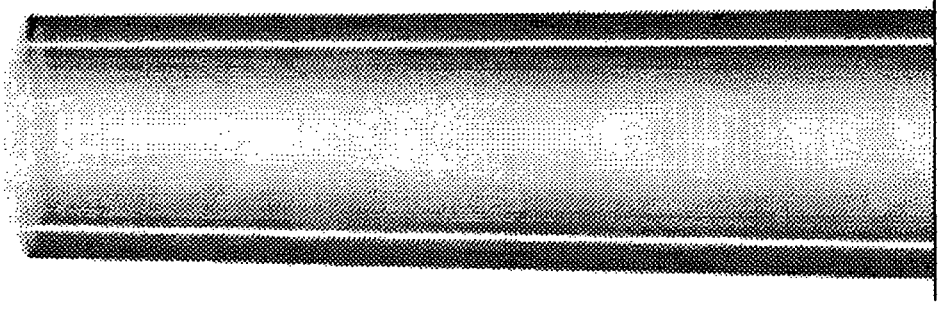
- 25µm Tip, thick walled glass



- 50µm Tip, thick walled glass



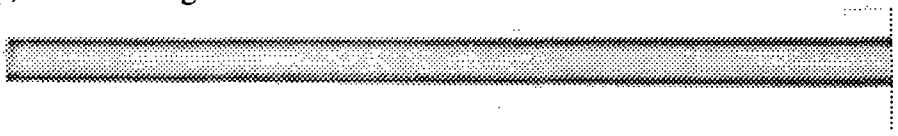
- 75µm Tip, thick walled glass



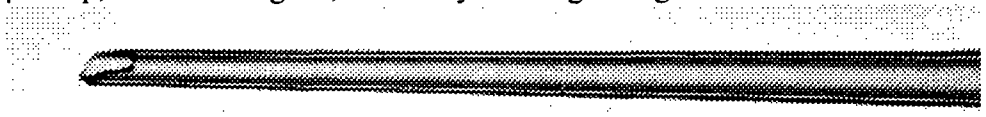
Large Pipette Images, 20μm - 75μm Tips

Glass has been pulled out long and bent over using index finger to find the natural breaking point along the glass taper.

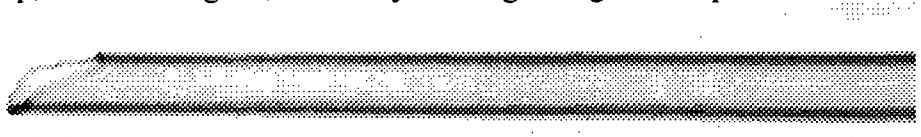
- 20μm Tip, thick walled glass



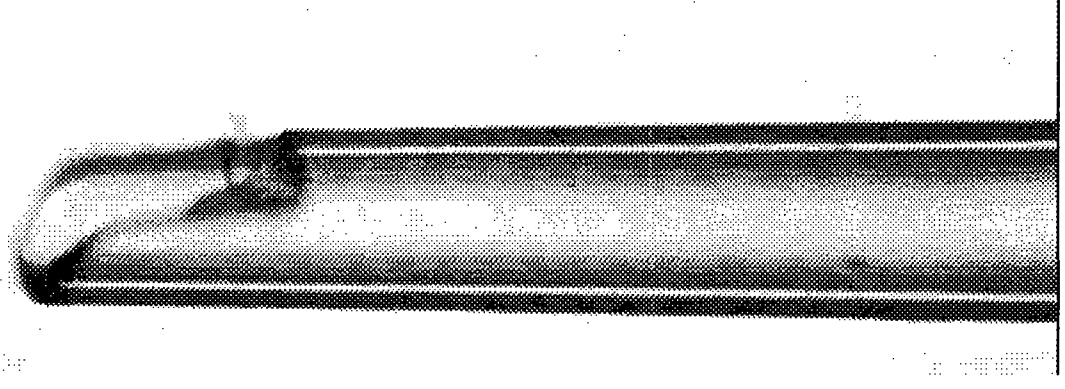
- 15μm Tip, thick walled glass, created by stabbing through Kleenex



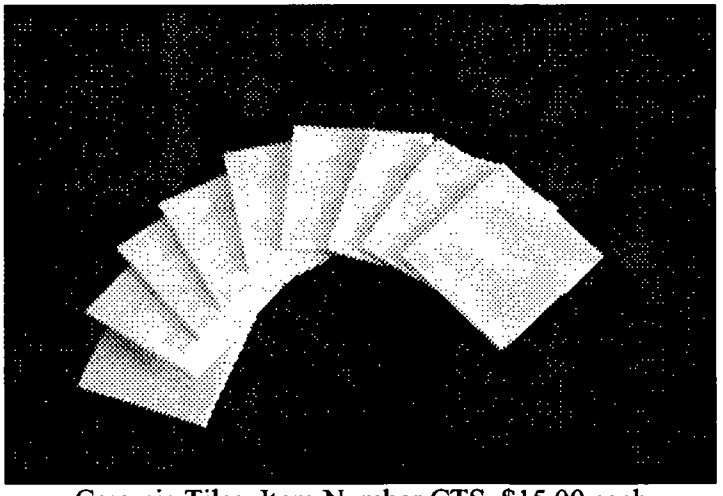
- 30μm Tip, thick walled glass, created by stabbing through Kimwipe



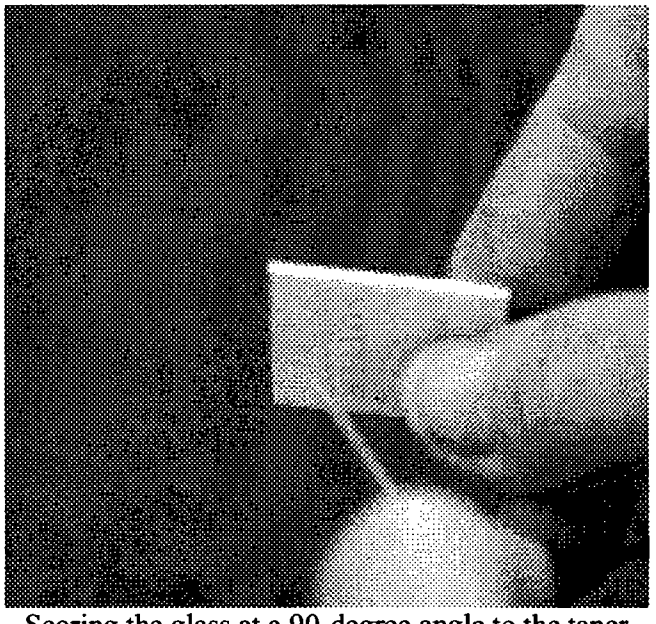
- 75μm Tip, thick walled glass, created by stabbing through Paper Towel



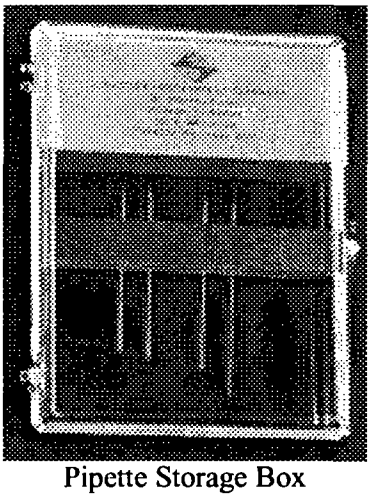
Ceramic Tile (CTS) Images



Ceramic Tiles, Item Number CTS, $15.00 each



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



Pipette Storage Box
Item # BX10, $10.00 each

CHAPTER 11

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 of glass you are using. The ramp test value for a box filament will traditionally be 1.5 to 2 times the value for a trough filament.

When to Run a Ramp Test

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

How to Run a Ramp Test

- Enter any program number <0-99> for a P-97 or <0-9> for a P-87
- Press clear <CLR> to enter the control functions
- Press <0> to not clear all parameter values
- Press <1> to run a RAMP TEST
- Install glass and press <PULL>

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

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

1. The puller increments the HEAT
2. As the HEAT output begins to soften the glass, the puller bars will move apart
3. The heat is then turned off when the factory-set Ramp Test velocity is reached
4. The Ramp Test value will be shown on the display

Expected ramp test values

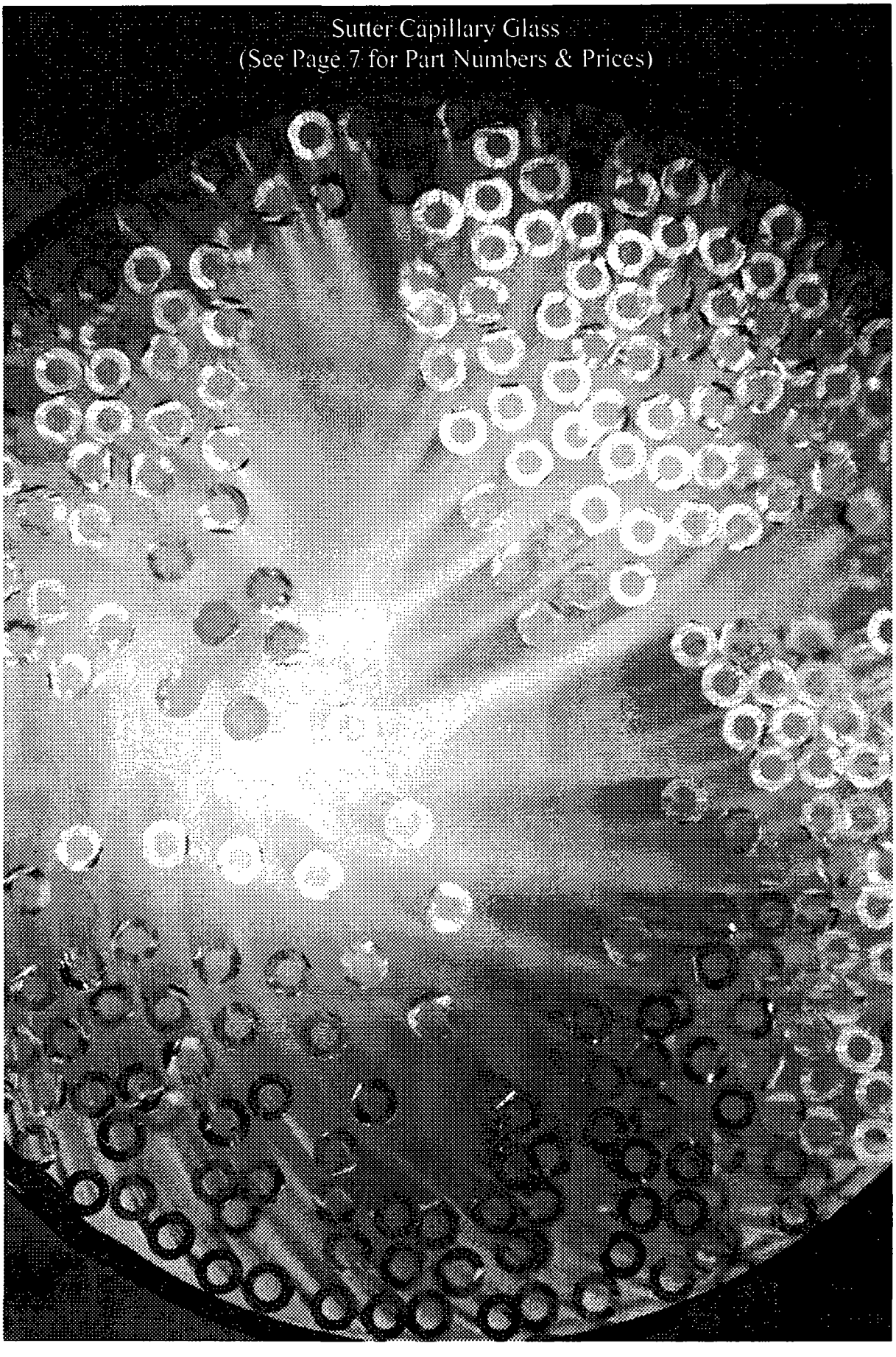
Filament #	Filament Dimensions	Expected Ramp Test Values	Maximum Heat
FT330B	3mm x 3mm TROUGH	230 – 280 **(see warning below)**	Ramp + 20
FB255B	2.5mm x 2.5mm BOX	425 – 475	Ramp + 30
FB330B	3.0mm x 3.0mm BOX	525 – 585	Ramp + 40
FB245B	2.5mm x 4.5mm BOX	750 – 850	Ramp + 75

* **Warning** - If the ramp test value for your trough filament is OVER 300 units, this is too high and indicates that the filament shape is poor and is inefficiently heating the glass. Please remove your filament and reshape it according to the instructions in the manual or on the Sutter web site.

Recommended Heat Settings for Each Filament Type

Filament	Recommended Heat Setting
3mm TROUGH	Ramp +15
2.5mm x 2.5mm BOX	Ramp + 5
3.0mm x 3.0mm BOX	Ramp
2.5mm x 4.5mm BOX	Ramp

* **Caution** - If your heat value is more than 20 to 75 units above the ramp test value, depending on the filament shape and size, (see above table), you will risk burning out the filament!



Sutter Capillary Glass
(See Page 7 for Part Numbers & Prices)

CHAPTER 12

General Guideline for the Parameter Settings

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

HEAT: Ramp – 5 to Ramp + 15.....For most applications!

PULL:
3-5mm taper and 1-3µm tips..... 0
6-8mm tapers and 0.9 to 0.5µm tips..... 30 - 70
9-15mm tapers and 0.5 to 0.06µm tips..... 70 - 150

VELOCITY:
3-5mm taper and 1-3µm tips..... 20 - 60 (with <0> pull)
6-8mm tapers and 0.9 to 0.5µm tips..... 50 - 80
9-15mm tapers and 0.5 to 0.06µm tips..... 70 - 100

TIME:
3-5mm taper and 1-3µm tips..... 150 for Trough, 250 for Box
6-8mm tapers and 0.9 to 0.5µm tips..... 150 for Trough, 250 for Box
9-15mm tapers and 0.5 to 0.06µm tips..... 150 for Trough, 250 for Box

DELAY:
3-5mm taper and 1-3µm tips..... 1 (with <0> pull)
6-8mm tapers and 0.9 to 0.5µm tips..... 60 - 110
9-15mm tapers and 0.5 to 0.06µm tips..... 40 - 90

PRESSURE:
Thin Walled Glass..... 200 - 500
Thick Walled Glass..... 300 - 600

PARAMETER	INCREASE	DECREASE
Heat	Longer Taper Higher Resistance	Shorter Taper Lower Resistance
Pull	Smaller Tips Longer Taper	Larger Tips Shorter Taper
Velocity	Smaller Tips	Larger Tips
Time (cooling time-ms)	Shorter Taper	Longer Taper
Pressure (air flow-psi)	Shorter Taper Lower Resistance	Longer Taper Higher Resistance

CHAPTER 13

Problems with Variability

Variability in the outcome of your 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 to see if they help pinpoint the possible mechanical source of variability.

- **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 the Drierite and bake out the moisture, or refill the canister with new Drierite.

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

- **Filament Shape and Alignment**: If you have just replaced your filament and are experiencing variability, please check the shape of the filament and the alignment of the glass within the filament. A box or trough filament should be centered over the air jet, and the air jet should be positioned 2 to 3mm below the base of the filament. A trough filament should be shaped such that its walls angle inward by 80-degrees to the base. The glass should run through the center of a box filament or through the bottom 1/3 of a trough filament. To make sure your filament is perfectly centered over the air jet, pull a pipette with a long taper and compare the taper length of the right and left pipettes. If they are not identical in length, loosen the filament clamp screws and "nudge" the filament in the direction of the shorter pipette. Repeat this procedure until both pipettes are the same length. Refer to pages 10-12 or 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. Our filaments are hand made and the thickness and width of the platinum may vary ever so slightly. This normal variation could represent a change of 30-50 units of required heat. If you are changing from a trough filament to a box filament (see page 11), your new ramp value could increase two-fold. Run a ramp test (Chapter 11) and adjust your heat values accordingly.

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

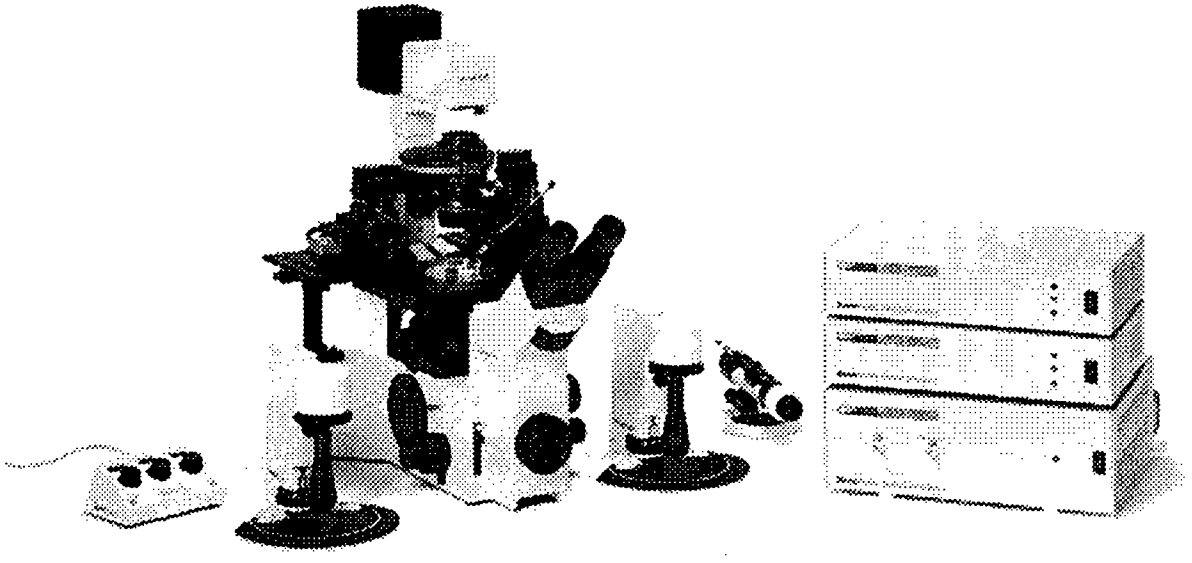
- **Cable Tension**: To check the tension, hold both puller bars together and depress/tap the cable between the bumper and the pulley with your forefinger. The cables should have about 1 to 2mm of slack and should not be taut. You should be able to push down slightly on each cable and hear the pull solenoid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

TRADE IN - If you have an older model Sutter Micropipette Puller (P-77, P-80, P80/PC, or P-87) you can ship us your old puller and get a credit towards a new P-97 Micropipette Puller. Contact Sutter for details.

REFURBISH – You can send your P-87 or P-97 to Sutter Instrument to be refurbished. The main board will be upgraded, all worn parts will be replaced, and you will get an extended 1-year warranty. Contact Sutter for details.

CHAPTER 14

XenoWorks Microinjection System



XenoWorks is a modular microinjection system designed to meet a wide variety of application needs. Our experience with precision motor control has culminated in this state-of-the-art system. The **XenoWorks Micromanipulator** uses a classic inverted joystick design for excellent ergonomics, intuitively linking the user with the micropipette. Combined with our smooth, responsive stepper motor drives, the XenoWorks Manipulator provides exceptional mechanical stability, sensitivity, and range of movement. The **XenoWorks Digital Microinjector** was designed for precision manipulation of cells via an easy-to-use interface. The Digital Microinjector employs a self-contained vacuum channel for gentle suspension cell-holding applications. A second channel is available for both high-pressure injection and gentle positive/negative pressure for transfer of embryonic stem cells. Both injection duration and pressure are easily selectable using rotary knobs on the remote interface. The internal low-speed compressor removes the need for an external pressure supply. The XenoWorks family also includes the **Analog Microinjector** for simple cell holding and transfer applications, which incorporates the same smooth response and ergonomic design of our other XenoWorks products.

The XenoWorks microinjection system has been designed to meet the needs of a wide variety of applications for the manipulation of cells and embryonic tissues. By combining micromanipulators, microinjectors and microscope mounting adapters, systems can be configured for a wide variety of applications including:

- Zygote pronuclear microinjection
- Embryonic stem cell blastocyst transfer
- Nuclear and cytoplasmic transfer
- Piezo impact microinjection
- Intracytoplasmic sperm injection
- Adherent cell microinjection

Applications

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

Pronuclear Injection

The microinjection of DNA into the pronucleus of a newly-fertilized mammalian egg is now a common and highly efficient method of creating transgenic offspring. Pronuclear injection was first described in the mouse, but now many different transgenic animals have been created in this way. Because the micropipette used for injection has an internal diameter typically less than one micron, relatively high pressure (>3000 hPa) is required to inject the DNA solution. Two micromanipulators are required, one to hold the zygote and one to inject the DNA. Gentle negative pressure is used on the holding side, while pulses of high pressure are used to inject 1–2 picoliters of DNA solution into the pronucleus. The XenoWorks Digital microinjector is ideal for this application, having simultaneous holding and high-pressure injecting capabilities.

Suggested system configuration:

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

Embryonic Stem Cell Transfer into Blastocysts

Animals, usually mice, can be engineered with a specific gene function reduced or knocked out altogether by introducing genetically altered embryonic stem cells into the cavity of a blastocyst so that the stem cells contribute to the embryo. The resulting live animal is a chimera of both genotypes. Subsequent selective interbreeding of manipulated animals results in pure-bred gene "knock-outs" or "knock-downs" and can be used for subsequent gene function studies. This operation requires two micromanipulators, one for holding the blastocyst and one for transferring the cells. Both holding and transfer functions require gentle positive and negative pressure for which the Digital microinjector is ideal. Alternatively, two Analog microinjectors may be substituted.

Suggested system configuration:

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

Intracytoplasmic Sperm Injection (ICSI)

ICSI can be employed for veterinary in-vitro fertilization during rare species preservation or for any veterinary assisted conception. ICSI may also be used as a gene transfer technique when sperm are co-injected with exogenous DNA (see also Piezo-assisted microinjection, below). Typically, two micromanipulators are used, one for oocyte holding and one for sperm aspiration and injection. Each micromanipulator grips a single micropipette holder with a microinjector attached. Since low positive and negative pressures are required for the delicate task of oocyte holding and sperm injection, an ICSI workstation should be configured with two Analog microinjectors.

Suggested system configuration:

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

Piezo-assisted ICSI

This relatively new technique can be employed for assisted conception in animals in which standard ICSI fails, such as mice. Piezo-assisted microinjection has been employed as a gene transfer method, where sperm are coated in exogenous DNA and injected into oocytes. The microinjection workstation required for this technique is very similar to standard ICSI, but with the addition of a piezo impact drive attached to the injecting micropipette holder. The device vibrates the injecting micropipette axially and drills its way into the oocyte. This method has been shown to increase success rates. Because the micropipette is vibrating at minute amplitude but high frequency, it is vital to use a mechanically stable micromanipulator, which will not vibrate in sympathy. The more stable the micromanipulator, the more efficient the energy transfer from the piezo impact drive to the micropipette tip. Some piezo-assisted microinjection protocols currently require a bead of mercury inside the injecting micropipette. Please note that mercury should not be used in conjunction with the Digital microinjector, though the Analog microinjector is ideal for this purpose.

Suggested system configuration:

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

Microinjection of Cultured, Adherent Cells

Cultured cell lines such as 3T3, CHO and HeLa can be microinjected while attached to a Petri dish. The procedure is best viewed through phase-contrast optics; a single micromanipulator and a single high-pressure microinjection channel are required. The tip of a sharp (inner diameter less than 1 micron) micropipette is brought down on top of a single cell and a pulse of high (100–1000 hPa) pressure applied. The cell membrane is ruptured and the cell can be seen to inflate slightly. Volumes injected are typically less than 5% of the cell volume. Success rates vary widely depending upon the type and volume of compound injected, the culture conditions and the cell line used. The high-pressure function of the Digital microinjector and the smooth, fine control of the micromanipulator are particularly well-suited to this application.

Suggested system configuration:

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

Somatic Cell Nuclear Transfer

The enucleation of an oocyte followed by the transplantation of a somatic cell is a method of producing genetically identical copies (clones) of the animal from which the donor cell was taken. Generally, two micromanipulators are required, one for holding the oocyte and one for the enucleating and injecting procedures. Each micromanipulator grips a single micropipette holder with a microinjector attached. Oocyte holding, enucleation and somatic cell transplantation require gentle, low positive and negative pressure, making the Analog microinjector ideal.

Suggested system configuration:

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

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

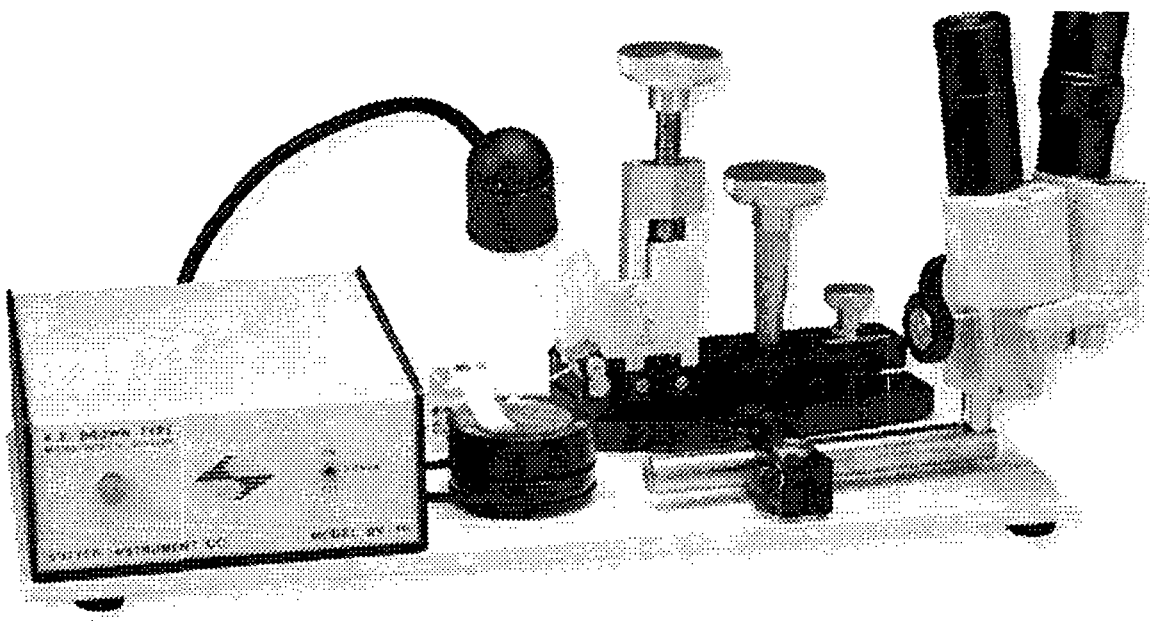
CHAPTER 15

BV-10 Beveler

Using the BV-10 Beveler is an elegant and simple approach for precision beveling between 0.1 and 75 microns. 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 is recommended for intracellular recording electrodes.

Microinjection needles also benefit from beveling by promoting entry into cells with minimal damage while simultaneously enhancing the flow of materials through the needle. Chapters 5, 6, and 7 describe the pipette morphologies needed for Embryonic Stem (ES) Cell Injection, Intracytoplasmic Sperm Injection (ICSI), and Nuclear Transfer (NT). The pipettes used for these applications more often than not need to be beveled at the tip. Using the BV-10 beveler (pictured below), and the 104C course diamond plate, with beveling times between 30 and 240 seconds, one can produce a sharp bevel at the desired angle - usually between 15 and 45 degrees.

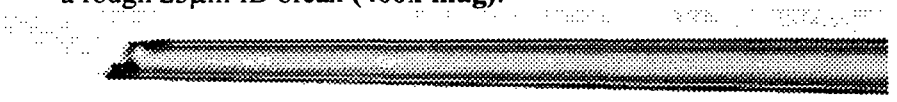


BV-10 Beveler

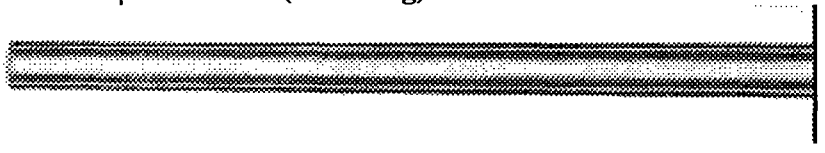
Images of Pipettes Before and After Beveling

BEFORE

- This pipette has been broken back with tweezers to make a rough 25µm ID break **(400x mag)**.

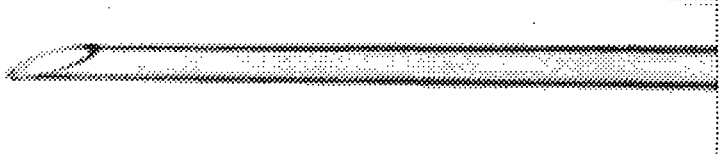


- This pipette has been broken back using the ceramic tile to make a clean 25µm ID break **(400x mag)**.

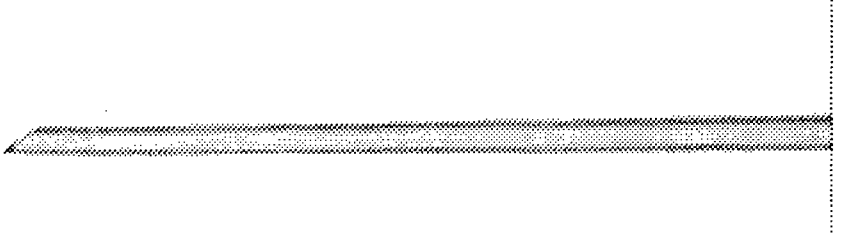


AFTER

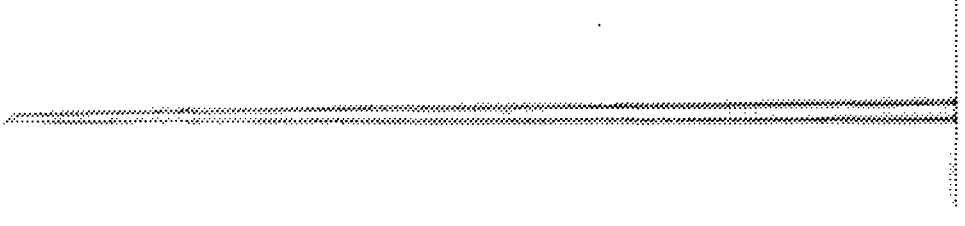
- This pipette has been beveled at 25-degrees using the BV-10 Beveler, 25µm ID **(400x mag)**.



- This pipette has been beveled at 35-degrees using the BV-10 Beveler, 15µm ID **(400x mag)**.



- This pipette has been beveled at 45-degrees using the BV-10 Beveler, 2-3µm ID **(400x mag)**.



INDEX

GENERAL LOOK UP TABLE

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

Type A
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 1-4 micron tip, and 1-10 MΩ of resistance. Type A programs are most likely best used in making pipettes for:
Extracellular recording and Patch clamp recording.

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

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

Type D
These programs are good for making pipettes with a long taper and a very small tip. One can expect to get a 8-13mm taper, a 0.5- 0.06µm tip, and 80 to100+ MΩ of resistance. Type D programs are most likely best used to make pipettes for: **Intracellular recording.**

Type E
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. Type E programs (and Type D if you prefer a slightly shorter taper length) are most likely best used to make pipettes for: **ES Cell, ICSI, Nuclear transfer, Holding, Xenopus injection, and large 20-200µm pipettes.** These pipettes require additional steps such as cutting, beveling and/or fire-polishing to create the final pipette.

The General Look Up Table and a Word of Advice!!!

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

Using the P-97 Cookbook with a P-87 or a P-80/PC

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

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

- If you have a **P-80/PC,** reduce the heat setting by 10 units. Since the Time Mode on the P-80/PC functions just like the Delay Mode on the P-97, please do the following If a Delay Value is provided in the P-97 cookbook program, you should install this same number as the "Time" Value on your P-80/PC. If a Time Value is provided for the P-97 cookbook program, please install a value between 50 to 110 for your "Time" Value on the P-80/PC.

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

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

GENERAL LOOK UP TABLE

3.0mm Trough Filament (FT330B)

Programs for 1mm x 0.5mm glass & 3mm trough filament								
TYPE	**PROG #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 1	B100-50-10	Line 1, Loops(4)	Ramp+15	0	50	150 t	500
B	Prog. # 2	BF100-50-10	Line 1	Ramp+15	0	20	200 t	400
			Line 2	Ramp+15	55	65	150 t	400
C	Prog. # 3	BF100-50-10	Line 1	Ramp+10	85	100	200 t	400
D	Prog. # 4	BF100-50-10	Line 1	Ramp+15	150	100	150 t	500
E	Prog. # 5	B100-50-10	Line 1	Ramp+15	30	150	100 t	200

3.0mm Trough Filament (FT330B)

Programs for 1mm x 0.75mm glass & 3mm trough filament								
TYPE	**PROG #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 6	B100-75-10	Line 1, Loops(3)	Ramp+15	0	90	150 t	500
B	Prog. # 7	BF100-78-10	Line 1	Ramp+10	45	100	175 t	300
C	Prog. # 8	BF100-78-10	Line 1	Ramp+15	55	100	175 t	200
D	Prog. # 9	BF100-78-10	Line 1	Ramp+15	50	100	150 t	300
E	Prog. # 10	B100-75-10	Line 1	Ramp+15	30	120	100 t	200

GENERAL LOOK UP TABLE

3.0mm Trough Filament (FT330B)

Programs for 1.2mm x 0.69mm OD/ID glass & 3mm Trough								
TYPE	**PROG #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 11	B120-69-10	Line 1, Loops(4)	Ramp+15	0	45	150 t	500
B	Prog. # 12	BF120-69-10	Line 1	Ramp+10	0	20	200 t	400
			Line 2	Ramp+15	75	95	150 t	400
C	Prog. # 13	BF120-69-10	Line 1	Ramp+15	45	85	200 t	300
D	Prog. # 14	BF120-69-10	Line 1	Ramp+15	60	95	150 t	500
E	Prog. # 15	B120-69-10	Line 1	Ramp+35	30	120	100 t	200

3.0mm Trough Filament (FT330B)

Programs for 1.2mm x 0.90mm OD/ID glass & 3mm Trough								
TYPE	**PROG #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 16	B120-90-10	Line 1, Loops(3)	Ramp	0	65	150 t	500
B	Prog. # 17	BF120-94-10	Line 1	Ramp+5	45	110	175 t	300
C	Prog. # 18	BF120-94-10	Line 1	Ramp	45	105	150 t	200
D	Prog # 19	BF120-94-10	Line 1	Ramp+15	55	100	150 t	300
E	Prog. # 20	B120-90-10	Line 1	Ramp+30	30	120	100 t	200

GENERAL LOOK UP TABLE

3.0mm Trough Filament (FT330B)

Programs for 1.5mm x 0.86mm OD/ID glass & 3mm Trough								
TYPE	**PROG #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 21	B150-86-10	Line 1, Loops(4)	Ramp+10	0	55	150 t	500
B	Prog. # 22	BF150-86-10	Line 1	Ramp+10	0	30	200 t	200
			Line 2	Ramp+10	35	155	200 t	200
C	Prog. #23	BF150-86-10	Line 1	Ramp+10	45	85	200 t	400
D	Prog. # 24	BF150-86-10	Line 1	Ramp+10	55	95	200 t	400
E	Prog. # 25	B150-86-10	Line 1	Ramp+60	30	120	100 t	200

3.0mm Trough Filament (FT330B)

Programs for 1.5mm x 1.10mm OD/ID glass & 3mm Trough								
TYPE	**PROG #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 26	B150-110-10	Line 1, Loops(3)	Ramp+15	0	90	150 t	300
B	Prog. # 27	BF150-117-10	Line 1	Ramp+5	45	120	175 t	200
C	Prog. # 28	BF150-117-10	Line 1	Ramp+10	45	120	175 t	200
D	Prog. # 29	BF150-117-10	Line 1	Ramp+10	45	120	150 t	200
E	Prog. # 30	B150-110-10	Line 1	Ramp+30	30	120	100 t	200

GENERAL LOOK UP TABLE

2.5mm x 2.5mm Box Filament (FB255B)

Programs for 1mm x 0.5mm OD/ID glass & 2.5 x 2.5 box filament								
TYPE	**PROG. #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 31	B100-50-10	Line 1, Loops(4)	Ramp	0	30	250 t	500
B	Prog. # 32	BF100-50-10	Line 1	Ramp+5	45	75	90 d	500
C	Prog. # 33	BF100-50-10	Line 1	Ramp	55	75	80 d	400
D	Prog. # 34	BF100-50-10	Line 1	Ramp+10	75	75	200t or 90d	400
E	Prog. # 35	B100-50-10	Line 1	Ramp+30	30	120	100 t	200

2.5mm x 2.5mm Box Filament (FB255B)

Programs for 1mm x 0.75mm OD/ID glass & 2.5 x 2.5 box filament								
TYPE	**PROG. #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 36	B100-75-10	Line 1, Loops(3)	Ramp	0	40	200 t	500
B	Prog. #37	BF100-78-10	Line 1	Ramp	50	60	90 d	200
C	Prog. # 38	BF100-78-10	Line 1	Ramp+5	80	70	80 d	200
D	Prog. # 39	BF100-78-10	Line 1	Ramp+5	105	70	50 d	200
E	Prog. # 40	B100-75-10	Line 1	Ramp+30	30	120	100 t	200

GENERAL LOOK UP TABLE

2.5mm x 2.5mm Box Filament (FB255B)

Programs for 1.2mm x 0.69mm OD/ID glass & 2.5 x 2.5 box filament								
TYPE	**PROG. #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 41	B120-69-10	Line 1, Loops(4-5)	Ramp	0	20	250 t	500
B	Prog. # 42	BF120-69-10	Line 1	Ramp	45	80	200 d	600
C	Prog. # 43	BF120-69-10	Line 1	Ramp	60	80	90 d	500
D	Prog. # 44	BF120-69-10	Line 1	Ramp	80	80	70 d	300
E	Prog. # 45	B120-69-10	Line 1	Ramp+40	0	120	150 t	200

2.5mm x 2.5mm Box Filament (FB255B)

Programs for 1.2mm x 0.94mm OD/ID glass & 2.5 x 2.5 box filament								
TYPE	**PROG. #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 46	B120-90-10	Line 1, Loops(3)	Ramp	0	40	200 t	500
B	Prog. # 47	BF120-94-10	Line 1	Ramp	50	85	90 d	450
C	Prog. # 48	BF120-94-10	Line 1	Ramp	85	90	70 d	350
D	Prog. # 49	BF120-94-10	Line 1	Ramp+5	105	70	80 d	300
E	Prog. # 50	B120-90-10	Line 1	Ramp+40	40	80	100 t	200

GENERAL LOOK UP TABLE

2.5mm x 2.5mm Box Filament (FB255B)

Programs for 1.5mm x 0.86mm OD/ID glass & 2.5 x 2.5 box filament								
TYPE	**PROG. #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 51	B150-86-10	Line 1, Loops(4-6)	Ramp	0	15-25	1 d	600
B	Prog. # 52	BF150-86-10	Line 1	Ramp	35	70	250 d	600
C	Prog. # 53	BF150-86-10	Line 1	Ramp	70	75	200 d	500
D	Prog. # 54	BF150-86-10	Line 1	Ramp	85	85	100 d	500
E	Prog. # 55	B150-86-10	Line 1	Ramp+50	30	120	100 t	200

2.5mm x 2.5mm Box Filament (FB255B)

Programs for 1.5mm x 1.10mm OD/ID glass & 2.5 x 2.5 box filament								
TYPE	**PROG. #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 56	B150-110-10	Line 1, Loops(3)	Ramp	0	35-45	250 t	500
B	Prog. # 57	BF150-117-10	Line 1	Ramp	55	75	120 d	400
C	Prog. # 58	BF150-117-10	Line 1	Ramp	85	75	80 d	400
D	Prog. # 59	BF150-117-10	Line 1	Ramp+5	95	70	70 d	250
E	Prog. # 60	B150-110-10	Line 1	Ramp+50	30	120	100 t	200

GENERAL LOOK UP TABLE

3.0mm x 3.0mm Box Filament (FB330B)

Programs for 1mm x 0.5mm OD/ID glass & 3 x 3 box filament								
TYPE	**PROG. #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 61	B100-50-10	Line 1, Loops(4)	Ramp+5	0	30	250t or 1d	500
B	Prog. # 62	BF100-50-10	Line 1	Ramp+5	35	75	130 d	500
C	Prog. # 63	BF100-50-10	Line 1	Ramp	55	75	90 d	500
D	Prog. # 64	BF100-50-10	Line 1	Ramp	100	75	250 t	500
E	Prog. # 65	B100-50-10	Line 1	Ramp+25	30	120	100 t	200

3.0mm x 3.0mm Box Filament (FB330B)

Programs for 1mm x 0.75mm OD/ID glass & 3 x 3 box filament								
TYPE	**PROG. #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 66	B100-75-10	Line 1, Loops(3)	Ramp+5	0	40	200 t	500
B	Prog. # 67	BF100-78-10	Line 1	Ramp+5	55	75	80 d	300
C	Prog. # 68	BF100-78-10	Line 1	Ramp+5	90	80	80 d	200
D	Prog. # 69	BF100-78-10	Line 1	Ramp+15	80	70	50 d	200
E	Prog. # 70	B100-75-10	Line 1	Ramp+35	0	100	100 t	200

GENERAL LOOK UP TABLE

3.0mm x 3.0mm Box Filament (FB330B)

Programs for 1.2mm x 0.69mm OD/ID glass & 3 x 3 box filament								
TYPE	**PROG. #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 71	B120-69-10	Line 1, Loops(4)	Ramp+5	0	25	250t or 1d	500
B	Prog. # 72	BF120-69-10	Line 1	Ramp+5	45	80	120 d	500
C	Prog. # 73	BF120-69-10	Line 1	Ramp+5	55	80	90 d	500
D	Prog. # 74	BF120-69-10	Line 1	Ramp+10	80	80	60 d	500
E	Prog. # 75	B120-69-10	Line 1	Ramp+35	0	120	150 t	200

3.0mm x 3.0mm Box Filament (FB330B)

Programs for 1.2mm x 0.90mm OD/ID glass & 3 x 3 box filament								
TYPE	**PROG. #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 76	B120-90-10	Line 1, Loops(3)	Ramp+5	0	35	200 t	500
B	Prog. # 77	BF120-94-10	Line 1	Ramp	55	85	110 d	300
C	Prog. # 78	BF120-94-10	Line 1	Ramp+5	85	90	90 d	300
D	Prog. # 79	BF120-94-10	Line 1	Ramp+10	85	90	90 d	200
E	Prog. # 80	B120-90-10	Line 1	Ramp+40	40	90	150 t	200

GENERAL LOOK UP TABLE

3.0mm x 3.0mm Box Filament (FB330B)

Programs for 1.5mm x 0.86mm OD/ID glass & 3 x 3 box filament								
TYPE	**PROG. #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 81	B150-86-10	Line 1, Loops(4-6)	Ramp	0	15-30	1 d	500
B	Prog. # 82	BF150-86-10	Line 1	Ramp	45	80	200 d	600
C	Prog. # 83	BF150-86-10	Line 1	Ramp	45	80	150 d	500
D	Prog. # 84	BF150-86-10	Line 1	Ramp	75	95	110 d	500
E	Prog. # 85	B150-86-10	Line 1	Ramp+25	30	120	100 t	200

3.0mm x 3.0mm Box Filament (FB330B)

Programs for 1.5mm x 1.10mm OD/ID glass & 3 x 3 box filament								
TYPE	**PROG. #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 86	B150-110-10	Line 1, Loops(3)	Ramp+5	0	45	250 t	500
B	Prog. # 87	BF150-117-10	Line 1	Ramp+5	55	90	120 d	400
C	Prog. # 88	BF150-117-10	Line 1	Ramp+5	85	95	90 d	400
D	Prog. # 89	BF150-117-10	Line 1	Ramp+5	95	80	60 d	300
E	Prog. # 90	B150-110-10	Line 1	Ramp+50	30	120	100 t	200

GENERAL LOOK UP TABLE

2.5mm x 4.5mm Box Filament (FB245B)

Programs for 1mm x 0.5mm glass & 2.5 x 4.5 box filament								
TYPE	**PROG #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 91	B100-50-10	Line 1, Loops(4)	Ramp	0	25	250 t	500
B	Prog. # 92	BF100-50-10	Line 1	Ramp	0	30	250 t	500
			Line 2	Ramp-10	0	40	250 t	500
			Line 3	Ramp-10	25	55	250 t	500
C	Prog. # 93	BF100-50-10	Line 1	Ramp	0	50	150 d	500
			Line 2	Ramp-10	35	60	150 d	500
D	Prog. # 94	BF100-50-10	Line 1	Ramp-20	50	80	200 t	600
E	Prog. # 95	B100-50-10	Line 1	Ramp+50	30	120	200 t	300

2.5mm x 4.5mm Box Filament (FB245B)

Programs for 1mm x 0.75mm OD/ID glass & 2.5 x 4.5 box filament								
TYPE	**PROG #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 96	B100-75-10	Line 1, Loops(3)	Ramp	0	25	250 t	500
B	Prog. # 97	BF100-78-10	Line 1	Ramp+5	0	50	200 t	500
			Line 2	Ramp+5	25	65	200 t	500
C	Prog. # 98	BF100-78-10	Line 1	Ramp	0	30	200 t	500
			Line 2	Ramp-10	80	60	200 t	500
D	Prog. # 99	BF100-78-10	Line 1	Ramp	45	85	200 t	500
E	Prog. # 100	B100-75-10	Line 1	Ramp+50	30	120	200 t	300

GENERAL LOOK UP TABLE

2.5mm x 4.5mm Box Filament (FB245B)

Programs for 1.2mm x 0.69mm OD/ID glass & 2.5 x4.5 box filament								
TYPE	**PROG #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 101	B120-69-10	Line 1, Loops(4-5)	Ramp	0	20	250 t	500
B	Prog. # 102	BF120-69-10	Line 1	Ramp	0	40	250 t	500
			Line 2	Ramp-10	0	40	250 t	500
			Line 3	Ramp-10	65	50	250 t	500
C	Prog. # 103	BF120-69-10	Line 1	Ramp	0	50	250 t	600
			Line 2	Ramp-15	60	85	250 t	600
D	Prog. # 104	BF120-69-10	Line 1	Ramp	0	40	250 t	500
			Line 2	Ramp-10	60	85	250 t	500
E	Prog. # 105	B120-69-10	Line 1	Ramp+25	30	120	200 t	300

2.5mm x 4.5mm Box Filament (FB245B)

Programs for 1.2mm x 0.90mm OD/ID glass & 2.5 x 4.5 box filament								
TYPE	**PROG #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 106	B120-90-10	Line 1, Loops(3)	Ramp	0	40	200 t	500
B	Prog. # 107	BF120-94-10	Line 1	Ramp+5	0	50	200 t	500
			Line 2	Ramp+5	45	65	200 t	500
C	Prog. # 108	BF120-94-10	Line 1	Ramp	0	30	200 t	500
			Line 2	Ramp+10	80	60	200 t	500
D	Prog # 109	BF120-94-10	Line 1	Ramp+5	85	85	250 t	500
E	Prog. # 110	B120-90-10	Line 1	Ramp+50	30	120	200 t	300

GENERAL LOOK UP TABLE

2.5mm x 4.5mm Box Filament (FB245B)

Programs for 1.5mm x 0.86mm OD/ID glass & 2.5 x 4.5 box filament								
TYPE	**PROG #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 111	B150-86-10	Line 1, Loops(4-5)	Ramp+5	0	20-25	1 d	500
B	Prog. # 112	BF150-86-10	Line 1	Ramp	0	35	1 d	500
			Line 2	Ramp	0	35	1 d	500
			Line 3	Ramp-65	35	55	110 d	500
C	Prog. # 113	BF150-86-10	Line 1	Ramp	0	35	1 d	500
			Line 2	Ramp	0	35	1 d	500
			Line 3	Ramp-30	35	65	110 d	500
D	Prog. # 114	BF150-86-10	Line 1	Ramp-20	60	90	250 d	600-700
E	Prog. # 115	B150-86-10	Line 1	Ramp+15	30	120	250 t	500-600

2.5mm x 4.5mm Box Filament (FB245B)

Programs for 1.5mm x 1.10mm OD/ID glass & 2.5 x 4.5 box filament								
TYPE	**PROG #**	**Glass Item Number**	**LINE(S)**	**HEAT**	**PULL**	**VEL**	**TIME/DEL**	**PRESSURE**
A	Prog. # 116	B150-110-10	Line 1, Loops(3)	Ramp+5	0	45	250 t	500
B	Prog. # 117	BF150-117-10	Line 1	Ramp+5	0	50	250 t	500
			Line 2	Ramp+5	45	55	250 t	500
C	Prog. # 118	BF150-117-10	Line 1	Ramp	55	75	110 d	600
D	Prog. # 119	BF150-117-10	Line 1	Ramp+10	55	75	110 d	500
E	Prog. # 120	B150-110-10	Line 1	Ramp+40	30	120	200 t	500

Notes

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