DMF1000

Microprocessor-controlled microforge

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INSTRUCTION MANUAL

Serial No._____

070507

World Precision Instruments

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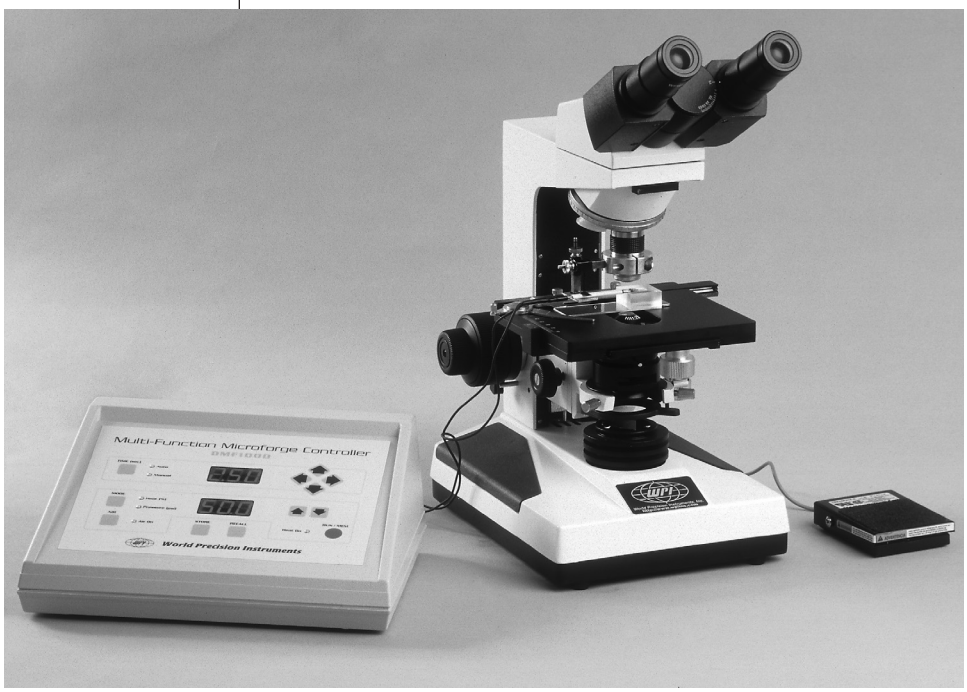
Introduction

The **DMF1000** is an advanced microprocessor-controlled microforge for fabricating a variety of glass pipettes and other related tools, including those for *in vitro* fertilization. It can reliably duplicate a large variety of fabrication procedures such as carbon fiber electrode sealing, contact stretching to sharpen large bore pipettes, and tip reduction of both small and large bore pipettes. IVF pipette tip ends can be bent and various tip ends can be formed using glass rod. All sizes can be fire polished from 0.5 micron patch clamp pipettes to 100 micron large bore pipettes. The built-in air pressure regulator is used for the forming of patch clamp pipettes, calibrating pipette tip size, and performing pressure injections. After a particular procedure is perfected, the user can save the parameters so that the conditions can be precisely duplicated. This permits both consistent fire polishing and precise pressure injection. Up to ten different programs can be stored and recalled for use at a later time.

In addition to the digital microforge controller, the DMF1000 system also comes with a microscope, reticle, and a 40× objective with a 3 mm working distance. The small filament can forge tips less than 0.5 microns, and the large filament makes it easy to forge and break large bore pipette tips. An injection tip holder is included for calibrating pipette tip size and performing pressure injection.

Figure 1 – *DMF1000 with optional foot switch*



The complete **DMF1000-1** system includes both the microforge and matched microscope (WPI Model **H602**); the **DMF1000-M** includes the microforge only.

Unpacking

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested.

Returns: Do not return any goods to WPI without obtaining prior approval and instructions from our Service Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100 mm (four inches) of shock absorbing material. Please read the section entitled "Claims and Returns" on the Warranty page of this manual.

Parts List

Systems **DMF1000-1** or **DMF1000-2** (complete systems):

H602 Microscope (see H602 Instruction Manual included for set-up, assembly and operating instructions)

Microforge (see parts list below for DMF1000-M1/M2 Microforge)

DMF1000-M1 or **DMF1000-M2** Microforge (microscope not included):

DMF1000 Microforge Control Unit

Man-DMF1000 DMF1000 Instruction Manual

DMF-1000 Start-Up Kit:

800292	40× long-working distance objective
75050	Acrylic and glass pipette holder, stage mounted
75030	Filament Adjustment Assembly for 40× and optional 25×LWD objective
75070	Filament Adjustment Assembly for 10× objective
75040	Heating filament-connecting cable
5430-ALL	PicoNozzle Kit
800003	3/16 hex wrench
MF200-H4	H4 Heating Filament
DMF1000-H5	H5 Heating Filament
500883	Eyepiece Reticule
200027	O-ring 20 mm OD
4763	0.25" OD nylon tubing, air input, 10 ft.
3354	1/4" NPT air tank fitting

Optics

The DMF1000 includes a 40× long-working distance (LWD) objective. This LWD objective is one of the most powerful currently available on any commercially produced microforge. Its 40× magnification is essential when polishing pipettes as small as half a micron (0.5 µm) in diameter. A specially designed eyepiece reticle (supplied) fits the eyepiece of the microscope, enabling the user to measure pipette tip dimensions and angle of bend (if any). Additional optional accessories, such as a 25× LWD objective, can expand the DMF1000 system functionality.

Positioning and Focus

Finding and moving the pipette tip under the microscope objective is easily accomplished. With a conventional microforge, it is difficult and time-consuming to position both the heating filament and pipette in the viewing area using independent micromanipulators. A unique feature of the DMF1000 is that the heating filament, inserted into the Filament Adjustment Assembly, is directly attached to the microscope's objective. Thus, using the horizontal and vertical adjustment knobs of that assembly, the filament can be easily maneuvered to any position within the viewing area. Once the correct focus is obtained, the filament will remain fixed and within focus and attention can be turned towards positioning the pipette that rests on the block on the microscope stage.

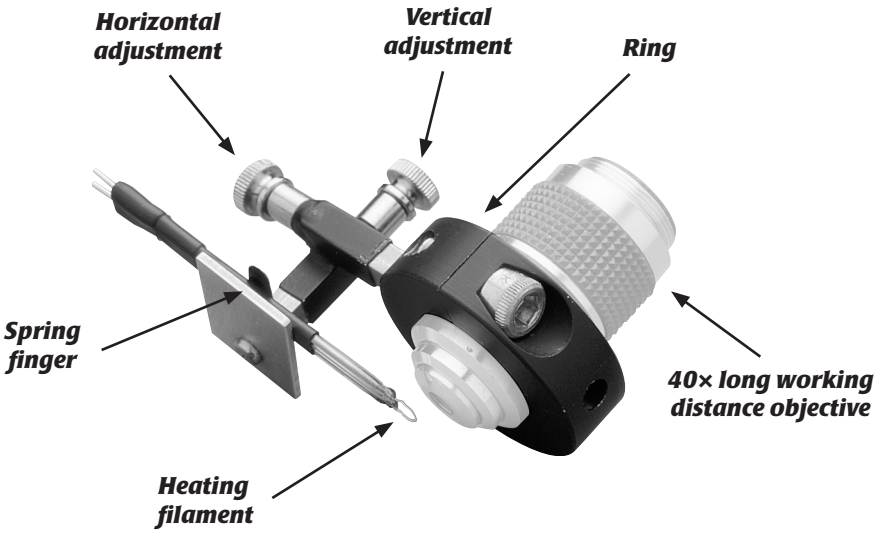


Figure 2 – *Filament adjustment assembly*

The X-Y-Z movements of the microscope stage control the pipette's position relative to the heating filament. This design makes the positioning and microforging of pipettes extremely easy. The stage of the **H602** microscope (included in the full system configuration) has a high quality rail that ensures precise, smooth and stable control of the pipette movement. The DMF1000 eliminates the need and expense of an additional micromanipulator to control pipette movement.

WARNING: Do not change filaments while the Heat timer is running.

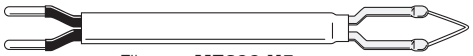
Heating Filaments

Low heat capacity and low thermal expansion of the filaments are key design features of the DMF1000 microforge. The low heat capacity of the filament allows it to reach fire-polishing temperatures without excessive heat. This permits the pipette to be brought close to the filament during polishing without fear of collapsing the pipette tip, and eliminates the need for an auxiliary air-cooling system. Furthermore, the low thermal expansion characteristic of the filament ensures minimal displacement of the filament during heating. This takes much of the guesswork out of tip placement in relation to the filament.

Table I

Two functionally distinct heating filaments are provided to meet diverse application needs:

Filament	Application
H5—Large Gauge: This filament can be formed into a U, bent upward (reformed) for fabrication of pipettes and air forming of patch pipettes. Reforming the filament can result in a greater heated surface area to present to the pipette tip. ***Warning: Since the working distance of the 40× LWD objective is only 3 mm, the objective lens may be damaged by prolonged exposure to the heat produced by the H5 filament. If, for example, the heat is set to 99%, the H5 filament should not be used for more than 30 seconds at a time. For longer exposure times, lower heat settings should be used and vice versa.***	
H4	**H4**—Small Gauge: For polishing patch clamp pipettes.



Filament **MF200-H5**

Filament **MF200-H4**

CAUTION: Lens cracks resulting from use of the H5 filament with the 40× objective are not covered by the warranty.

Microscope

The microforge has been matched with WPI's research-grade microscope model H602 to provide a simple and complete system with excellent performance. Two Filament Adjustment Assemblies are supplied with the microforge. One fits both the 40× LWD objective (included) and the optional 25× LWD objective. A second Filament Adjustment Assembly for the 10× objective of the H602 microscope is also included. The 40× and 25× objective with Filament Adjustment Assembly will fit most other microscopes with a focal length of 160 mm. The additional Filament Adjustment Assembly for the 10× objective is, however, designed specifically to fit the model H602 10× objective.

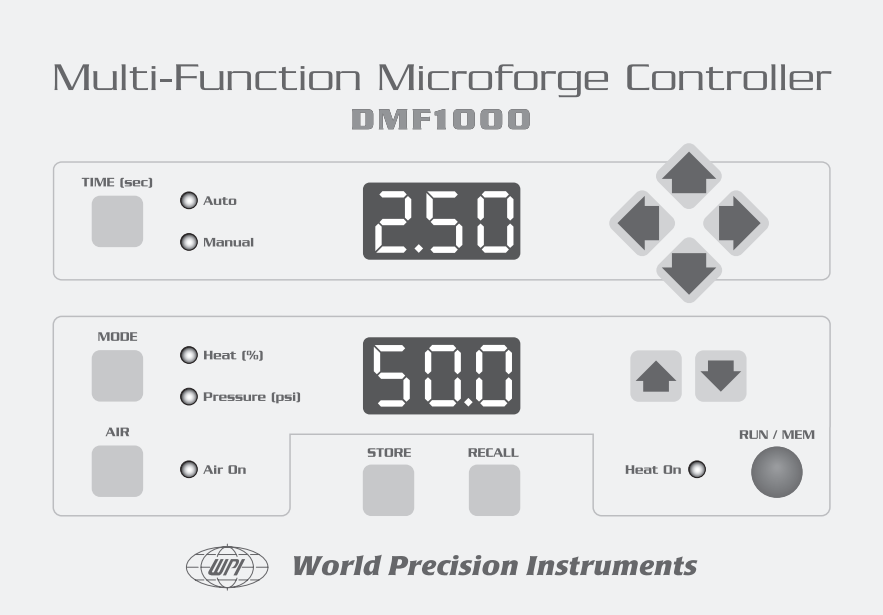


Figure 3a – *Control panel*

Control Unit Functions

The DMF1000 Control Unit supplies power to the heating filament. The Control Unit's output power is electrically stable and reproducible; fluctuations in the mains voltage input will not affect the output to the filament. This ensures the same polishing results day-to-day at the same settings. The unit senses which filament is used and automatically adjusts the heat output.

After initialization, the settings stored in memory location 0 will be displayed, and the heat and timer displays will light, indicating that the unit is powered up.

When any of the controller's buttons is pushed and released, a "beep" indicates the operation has started. If a button is pushed and a function cannot start, four "beeps" indicate that a button was pushed in error. If the filament breaks or burns out, there will be four "beeps" and

the heat reading will flash ten times, signaling the operator to check the filament. The audible alarm may be turned off by pressing the RUN button any time during the initialization period.

An optional foot switch (WPI **#13142**) will aid in performing complex fire-polishing, freeing one hand to move the pipette while the other hand controls settings and adjustments on the Control Unit.

RUN / MEM — This button will be used to execute all the functions and settings on the microforge. When in auto or manual mode it will turn on the heater and start the timer running. In the pressure mode, it will turn on the air and start the timer running. It is also used to execute storing and recalling programs. The Heat On light will light when the heater starts to heat. If there is an open circuit in the heating filament system, the light will not light.

TIME [sec] — The unit has two timer modes of operation, Auto and Manual. In the manual mode the heat timer will start at zero and count up when the RUN/MEM button is pushed. After the desired heating time has been established the TIME [sec] button can be pushed to set the unit to Auto mode. The timer reading will remain at the time was that established in manual mode. When the RUN/MEM button is pressed the counter will count down from the high setting to zero, a long "beep" will sound, and then the unit will shut off.

The **timer display** shows seconds from zero up to a maximum of 360 (six minutes). The left arrow ← next to the timer readout moves the decimal point to the left and the right arrow → moves it to the right. The up ↑ and down ↓ arrows will reset the time up or down. This allows the user to make minor adjustments to the time when in auto mode. This control will also set air output time.

MODE — The unit has two modes of operation: Heat and Pressure. The mode button will switch the lower readout from heat reading in % to pressure reading in psi. If left in pressure mode, pressure injections can be made and the size of a pipette can be calibrated.

The **heat display** reads from zero to 99.9 %. The factory set maximum heat is very hot. The filament will glow a cherry red when set at 99.9 % and it is advised to not use this setting for prolonged periods of time. The best setting for heat is just hot enough to melt the glass, thus prolonging the filament's life. *In most cases, microforging is accomplished without seeing the filament change color.*

The **air pressure display** can be adjusted from 0 to 60 psi with the control knob found on the back of the unit. The lower window will display the current pressure in psi when the unit is set to pressure mode.

STORE — When the STORE button is pressed, the lower display window will display the memory number. Use the arrow buttons next to the window to choose the desired memory

location (0 through 9). Press the RUN/MEM button to store the current parameters to that memory location. It is a good idea to reserve memory 0 for the last program used since this is the memory location selected when the unit is first turned on.

RECALL — When the RECALL button is pressed, the upper window will display the contents of memory location 0. Use the arrows next to the heat display window to choose the desired memory location (0 through 9), then press RUN/MEM to recall the parameters stored in the selected memory location.

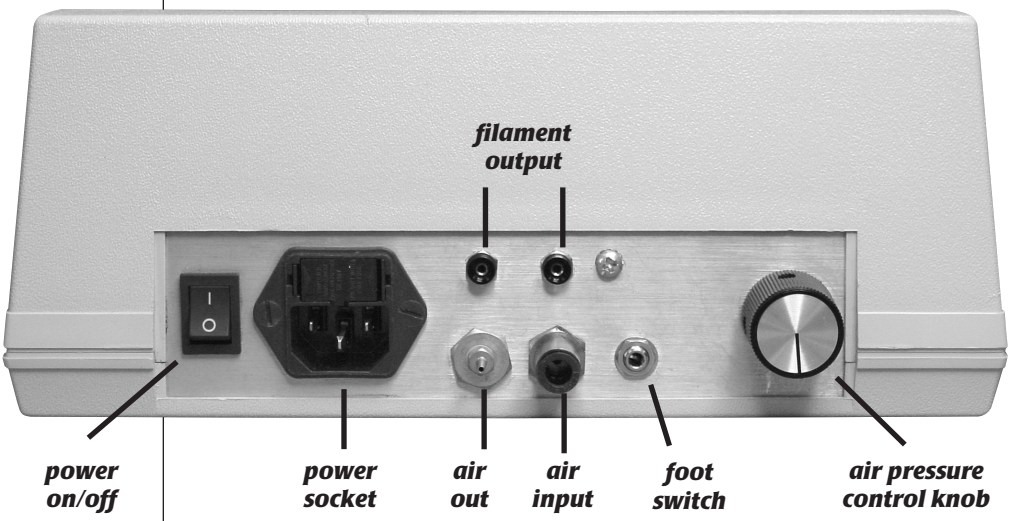


Figure 3b —
DMF1000 rear panel

Assembly

Mounting Filament Adjustment Assembly to Microscope

1. Mount the objective of choice to an available position on the microscope.
2. Lower the microscope stage down as far it will go.
3. Using the 3/16 hex wrench provided, loosen the screw on the Filament Adjustment Assembly to open the ring so that it will fit comfortably over the objective.
4. Swing the objective to an outside position (*i.e.,* not pointed directly down). Mount the Filament Adjustment Assembly onto the objective by carefully placing the ring around the objective and then sliding it up until it stops.
5. While maintaining the Filament Adjustment Assembly in position on the objective, slowly swing the objective down into the viewing position. Once in place, position the horizontal adjustment slider to the left of the microscope objective and parallel to the long edge of the microscope stage. With the 3/16 hex wrench, tighten the holding screw on the ring thereby securing the Filament Adjustment Assembly to the objective.

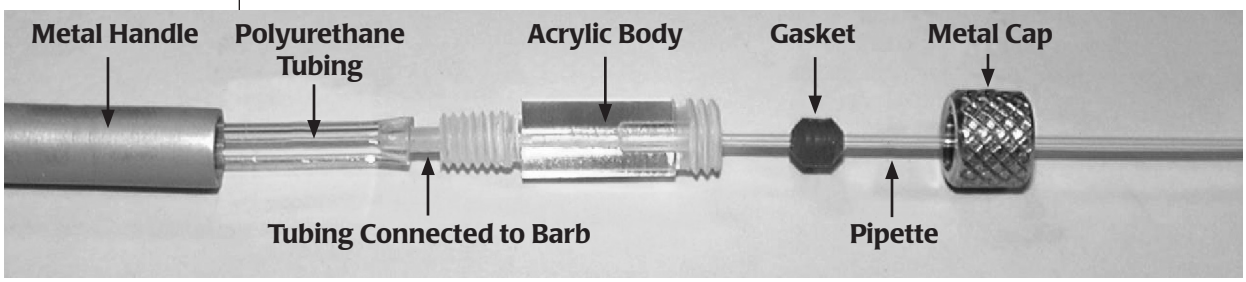
NOTE: The spring finger and base plate attached to the vertical filament adjustment is angled slightly inward. ***This is normal. Do not attempt to straighten it.*** This angle facilitates viewing of the filament under the microscope.

6. Hook up lab air or nitrogen to "Air In" on back of unit. Plug polyurethane air output tubing into the pipette holder of choice. The nylon air input tubing (#**4763**) supplied is 10 feet long. A common 1/4-in. NPT air tank fitting (#**3354**)is also supplied.

Assemble Pipette Holder

Two sets of micropipette holder assemblies are provided in the PicoNozzle Kit (#**5430-ALL**). For fire polishing, coating and pressure forging, one holder should be assembled ***without*** the metal handle. For pipette tip diameter calibration and microinjection, the other holder should be assembled ***with*** metal handle (see **Fig. 4.1**).

Figure 4.1 — *Micropipette Holder Assemblies*



Reticle

The 10× reticle is installed in one of the **H602** microscope eyepieces. No assembly is required.

Use of the Eyepiece reticle

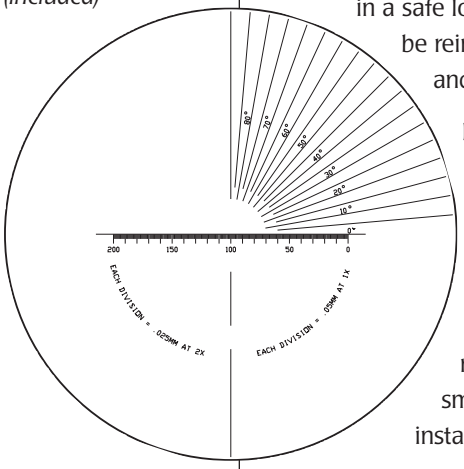
This reticle is designed to be used with a stage micrometer for calibration. Measuring the **H602T** with 25× and 40× objectives (3mm working distance) off a known value stage micrometer to the 200 unit linear scale in the reticle:

25× objective, 10× eyepiece, 200 units = 390 microns, 1 unit ≈ 1.95 microns.
40× objective, 10× eyepiece, 200 units = 250 microns, 1 unit ≈ 1.25 microns.

Removal of reticle (if desired):

Remove the eyepiece and place it on a desktop so that the smaller open tube is facing upwards. Carefully unscrew the barrel (the part that fits into the microscope) and remove that section. *Be careful not to disturb the lenses in the large cylinder below.* Take the smaller barrel section and gently push on the glass reticle from the threaded end of the tube, so as to remove the retainer and the reticle from the back. Do not use excessive force. Replace the smaller barrel part into the eyepiece without disturbing the lenses below. Store the reticle and in a safe location so it may be reinstalled at a later date. The retaining ring should be reinserted into this eyepiece to prevent loss; this part is *unique to this eyepiece* and will not fit the other eyepiece.

Figure 4.2 – *Eyepiece reticle (included)*



Reassembly:

Clean the eyepiece, reticle and retaining ring (glass cleaner and air is sufficient). Lower the reticle into the eyepiece small barrel (do not disassemble the eyepiece) so that the reticle is centered on the small shelf in the smaller barrel. Before inserting the retaining ring, hold the eyepiece overhead (so the reticle doesn't fall out) and look up into it to see if the reticle image may be read correctly. If so, place the retaining ring into the small barrel so that the knurling (small cuts) are the last part of the ring to be installed. Push the retainer down until it contacts the reticle and holds it flat.

Stage Mounted Micropipette Holder

Position the stage-mounted acrylic pipette holder on the microscope stage just as if it were a slide. Orient the acrylic block of the pipette holder (see **Fig. 7b**).

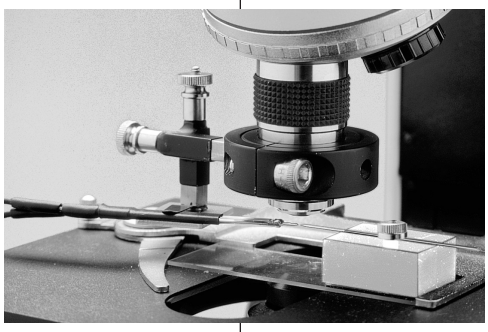
Operating Instructions

NOTE: Any object seen through the microscope objective will appear reversed in orientation. For example, the heating filament (attached to the left side of the objective) will appear through the microscope as coming from the right.

1. Turn on the power to the microscope.
2. Choose the desired filament (large or small).
3. Mount and connect the heating filament. Raise the spring finger on the filament holder with one hand, and gently slide the heating filament under the spring with the other hand. Attach both of the microforge connecting cables to the filament by fitting the socket end of each cable into the filament plugs. The cables are interchangeable and can be used for either plug. Take the free end of each cable and insert each into one of the two Filament Output receptacles on the rear of the Microforge Control Unit. Again, it does not matter which cable is connected to each receptacle. The connecting cable wires from the microforge control unit are not polarized, so reversing these cables will do no harm.

Warning: Since the working distance of the 40× LWD objective is only 3 mm, the objective lens may be damaged by prolonged exposure to the heat produced by the H5 filament. If, for example, the heat is set to 99%, the H5 filament should not be used for more than 30 seconds at a time. For longer exposure times, lower heat settings should be used and vice versa. Lens cracks resulting from use of the H5 filament with the 40× objective are not covered by the warranty.

Figure 5



4. Bring the filament into focus: By eye, without using the microscope, adjust the position of the filament by moving it in or out and side to side until the filament wire is centered approximately 3 mm (for 40× objective) below the objective. Looking through the microscope, move the filament in the spring finger until its shadow appears. Some vertical adjustment may also be required to bring the shadow into the field. Then, using the Vertical Adjustment, bring the filament into clearer view. With the Horizontal Adjustment, position the end loop of the filament to the far right side of the visual field.
5. Power up the unit: Connect the line cord to the power input jack on the microforge control unit and then into a properly grounded mains power outlet.
6. Switch the power switch on the back of the unit on. After initialization, the setting that had been stored in memory location 0 will be displayed. If the sound is

not wanted it may be turned off by pressing the RUN/MEM button during the initialization period. The Heat and Timer displays will light up, indicating that the unit is powered up. Press the TIME button to set the unit in Manual mode. Pressing the RUN push-button switch sends current through the filament and turns on the Heat On lamp. Set the Heat readout by pressing the Increase (up arrow) / Decrease (down arrow) buttons from setting 0 to 99.9 % (an arbitrary numbering scale), varying the amount of power applied to the filament. An optional foot switch (WPI **#13142**) allows the operator to use both hands to vary the filament heat intensity while positioning the pipette. Some microforging techniques will require this two-handed approach.

7. With the power on and unit set at Manual, depress the RUN button several times at various heat settings to visualize the expansion of the filament loop and determine approximately where the pipette should be positioned in relation to it. With a higher heat setting the filament will expand out farther. To mount a pipette, first adjust the stage of the microscope down and away from the objective, thereby providing sufficient room for mounting the pipette safely on the pipette holder. Place the pipette in the acrylic pipette holder (**Fig. 7b**). Using the horizontal adjustment on the microscope stage, position the pipette so that the tip is slightly past the center of the objective. Raise the stage until the filament is about 3 mm from the objective. Slowly move the pipette back and forth, in and out, while looking through the microscope until the image of the pipette is observed. Adjust the vertical position of the stage until the pipette is clearly visible and in focus. Position the pipette tip in relation to the heating filament as required by the application.

8. Filament Power and Heat Readout. The filament is auto sensed by the unit so no settings need be changed when changing filament types. The Heat readout (0 to 99.9%) provides the user with a range of power up to the maximum as determined by the factory settings. Always begin with the readout at the low end of the range and increase the heat only as necessary and by small increments. The lowest power and heat setting that can be used to accomplish a task should be used. Higher heat than necessary may shorten filament life as well as increase the possibility of overheating the pipette tip.

CAUTION: It is not advisable to operate the unit at high power with the Heat set at 99.9%. This can cause the filaments to prematurely burn out.

NOTE: When changing filaments, the power need not be turned off. ***However, the Heat timer should not be running. The filament can be damaged if changed when the heat timer is running.***

Programming Procedure

1. To turn the unit on, switch the on /off switch located on the back of the unit to the on position. The settings stored in memory location 0 will be displayed.

2. The first time the unit is turned on both readouts will display 000. The time selection will be in Auto. The mode selection will be in Heat. The Air On light will be off. The Heat On light will be off. The time display will not show a decimal point. With the timer set in this position the maximum amount of time that the unit can run is 360 seconds (6 minutes). Pressing the left arrow will move the decimal point in the display to the left, so that the maximum time that the counter will read will be 99.9seconds (1.66) minutes before returning to 0 and starting over. Pressing the left arrow another time will move the decimal point further left and the maximum time the counter will be 9.99 seconds before returning to 0 and starting over.

   A. Select the proper filament for the job. The small filament is better suited for microforging pipettes one micron and under. The large filament should be used for larger pipette forging, forming and sizing.

   B. Press the TIME button and select Manual.

   C. The unit should be in the Heat mode. If the pressure light is on, press the MODE button to switch the unit to Heat mode. If the Air On light is on, press the AIR button to turn the light out. The Heat On light will be off.

   D. Mount the pipette in its holder and position it focused in the field of view close to the filament, far enough so the filament's expansion will not allow it to touch the tip. Using the reticle, measure the distance between the tip and the filament. In order to get reproducible results with a tip of the same size, the new tip will need to be the same distance from the filament.

   E. Using the up and down keys above the Heat On light, set the heat to a low setting between 30 and 50 to start with. Better control of the forging operation is accomplished by setting the heat low and letting the heater run longer. This allows the operator to see the tip forming slowly and to stop the heating action at exactly the right time.

   F. Pressing and holding down the RUN button will start the heating action. The time counter will also start counting up from 0. Seen through the microscope, the pipette tip will slowly start to form a smooth surface. When the tip is formed, release the RUN button to stop the heater and its timer. If there wasn't enough heat to melt the glass, no harm done. Increasing

the heat a little or moving the tip a little closer to the filament will start the melting action. However, if the heat is set too high the tip will form too fast and may be not useable. Moving the tip away from the filament or lowering the heat a little will let the tip form slower so the heat can be turned off at the right time. *The best pipette tip is formed using a lower heat setting and running the heater longer.*

G. If the tip that was just formed is acceptable, push the TIME button and select Auto operation. The unit is now programmed and the time readout will be set at the right time to polish another tip of the same size and profile.

H. Load another pipette the same size and profile, focused in the field of view. Position the new pipette at the same distance from the filament as the programmed pipette.

I. Press and release the RUN button. The heater will turn on and the counter will start counting down to 0 and turn off the heater. The forming of the tip can be seen through the microscope. The newly formed tip will be the same as the first tip. If the forming of the tip appears to be going too far, a second push of the RUN button will stop the heating action. If this happens, the tip may have been placed closer to the filament than was the programmed tip.

Applications

There will be user variation in the choice of filaments, power and heat settings for every application. If the desired result is achieved, the choice of parameters should be acceptable. In general, it is always desirable to use the least amount of heat possible in order to prolong the life of the filaments.

The distance that should be maintained between the filament and the pipette tip during microforging will vary depending on the tip bore, the filament, the power, the heat settings and the application. With the exception of the applications in which a glass bead is formed on the filament and the tip is resized by breaking, the tip should not come in contact with the filament. In general, it is best to begin with the tip at a safe distance from the filament and move toward it as necessary.

The formation of a glass bead on the filament is required for certain applications (see "Microforging Beveled Injection Pipettes"). It is not required for the other applications described in this manual. However, depending on the preferences of the user, a glass bead on the filament can be used for these applications as well.

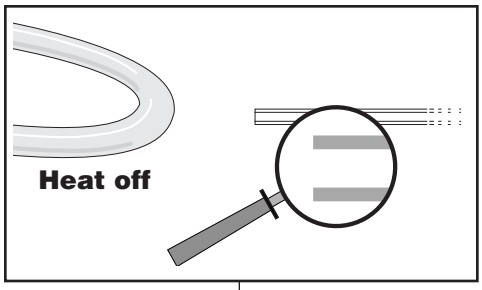


Figure 6a – *Untreated tip*

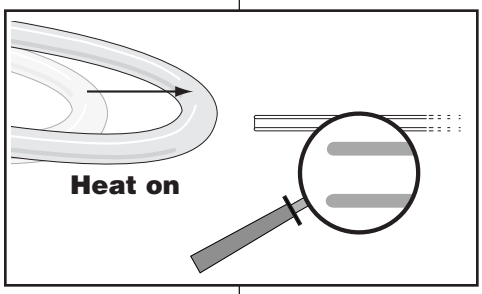


Figure 6b – *Fire polished tip*

1. Fire Polishing a Patch Clamp Pipette

a. Choose and install the desired filament (see **Table I**).

b. Turn on the power to the Control Unit and press TIME to set Manual mode. Press the RUN button and adjust the Heat readout from low to high with the arrows while observing the expansion of the filament under the microscope. A slight movement of the filament indicates that it has sufficient heat and will provide excellent polishing results in most cases. A red-hot filament is unnecessary and undesirable and will decrease the life of the filament. It will also heat the tip too quickly, making it difficult to control the degree of polishing. In addition, a red-hot large filament could permanently damage the 40× objective.

c. Place the pipette to be polished in the acrylic pipette holder. Adjust the microscope stage until the pipette is in position with sufficient distance to account for filament expansion (**Fig. 6a**).

d. Press RUN/MEM and observe the expansion movement of the filament. Determine the appropriate Heat setting and then fine-tune the position of the tip. Note the distance between the pipette and the filament; this will be needed to reproduce an identical pipette. A minimal change in the shape of the tip will result typically yield good polishing results (**Fig. 6b**).

e. Press RUN and polish the pipette to the degree needed.

f. Press the TIME button and select the Auto mode. Place in the tip holder an identical pipette to the one just polished. Position the tip to the same place as the first tip and press the RUN/MEM button. The counter will start counting down to zero and stop, and the pipette will be polished the same amount as the first.

2. Forming a Patch Clamp Pipette with Air

Another way to create a pipette that will seal with lower resistance is to increase the OD of the tip end without changing the ID opening size. This will increase the seal surface area of the glass and create a better seal for the patch pipette.

WARNING: Always wear safety glasses during this procedure. Never point the pipette at anyone. The pipette can be forcefully shot out of its holder if not tightly secured.

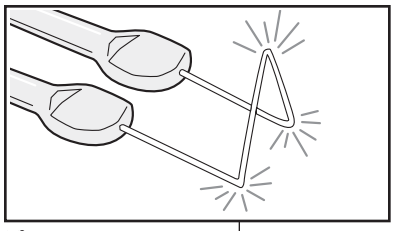


Figure 7a

a. Create a pipette with an ID opening of about one half micron. See application number 1, and fire polish the tip end.

b. Select the large filament and form a bent-up loop in the end (see **Fig. 7a**). Mount the filament in the filament holder and position it.

c. Connect the polyurethane tubing to the air output line on the back of the Control Unit (see **Fig. 3b**). The other end of the polyurethane tubing must be connected to the barb of the acrylic body (see **Fig. 4.1**). Be careful that the barbdoes not break when attaching or removing the tubing. ***The metal handle must not be connected to the acrylic body.*** Before inserting the pipette into the acrylic body, a gasket of the correct size must be placed in the metal cap. The kit includes gaskets for pipette diamets of 1mm (green), 1.2mm (black), 1.5mm (blue), and 1.65mm (red). Slide the pipette through the metal cap and gasket, then twist the metal cap to tighten it securely.

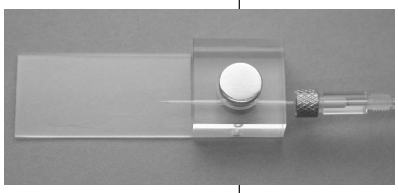


Figure 7b

d. Mount the pipette on the stage-mounted acrylic pipette holder and lock in place with the locking screw (see **Fig. 7b**).

e. Position the filament so the top of the looped end is in the field of view. Using the reticle, note the location of the *inside edge* of the filament. Move the filament, looped end up, closer to the objective until the looped end is out of view. This will allow the pipette to be placed under the filament and still be in the field of view (see **Fig. 7c**).

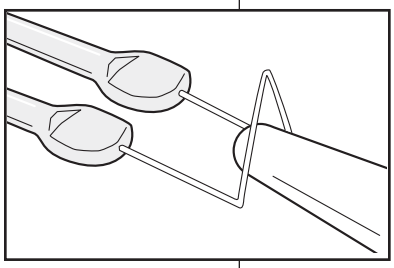


Figure 7c

f. Adjust the location of the pipette so that it is in the field of view and positioned so the tip end is centered just past the inside edge of the filament. The placement of the pipette is important. If the tip end is too close to the filament its ID size will be reduced a little when the tip is formed. If the tip end is too far away from the filament the end will not form properly.

g. Press the mode button to switch the unit to pressure mode. Set the pressure to 30 or 40 psi.

h. Press the MODE button to return the unit to Heat mode

i. Press the AIR button to turn the air on.

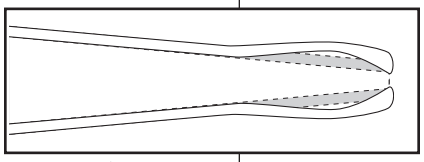


Figure 7d

j. Set the unit in Manual mode by pressing the TIME button, and set the heat to 30 or 40. Press and hold down the RUN button and observe the pipette end. A ball should start to form at the end of the pipette. Release the RUN button when the desired tip is formed. When the heat turns off, the air will stay on for about five seconds; this delay is set at the factory and cannot

be changed. (If the ball is too far back from the tip, the pipette can be salvaged by releasing the RUN button and resetting the tip a little closer to the filament's inside edge.) Wait until the air turns off, and then turn the air back on by pressing the AIR button on the instrument panel. Hold the RUN button down until the tip forms properly (see **Fig. 7d**). This procedure may be tried a few times before acceptable results are obtained.

k. To form another tip the same size, place the unit in Auto mode and position the new pipette in the same place in relation to the filament as was the newly formed tip. Press the Air button to turn the air on. Press and release the RUN button to start the timer counting down until the pipette is formed.

3: Coating a Single-Channel Patch Clamp Pipette

WARNING: Always wear safety glasses during this procedure. Never point the pipette at anyone or at your eyes. The pipette can be forcefully shot out of its holder if not tightly secured.

The user should coat the single-channel patch clamp pipette with Sylgard 184 (WPI **#SYLG184**) before fire polishing. Prior coating ensures that Sylgard does not seal the tip of the glass after fire polishing. A very simple and effective coating method has been described by Dr. Li *(2)*.

a. Fit the pipette into the acrylic body and connect the polyurethane tubing to the barb of the acrylic body and the air output line on the back of the Control Unit. The pipette holder should be assembled ***without*** the metal handle.

b. Press the MODE button to set to Pressure mode and use the air knob on the back of the unit to set the pressure slightly above 20 psi. Air is forced through the pipette at a pressure greater than 20 psi (for a 0.5 ID pipette) in order to prevent the Sylgard from entering the pipette tip during the coating process.

c. After mixing the Sylgard, press the MODE button to return the unit to Heat mode.

d. Press the Air button to turn the air on.

e. Dip the pipette tip into the coating and remove it.

f. With the low pressure air supply still being applied, place the pipette tip over a heat gun for two seconds to cure the Sylgard.

The pipette is now coated and the tip is ready to be polished following the procedure for the whole cell patch clamp pipette as described earlier in this manual. Practice with a scrap pipette may be useful in determining the heat setting and curing procedure.

4. Fire Polishing a Standard Pipette Tip

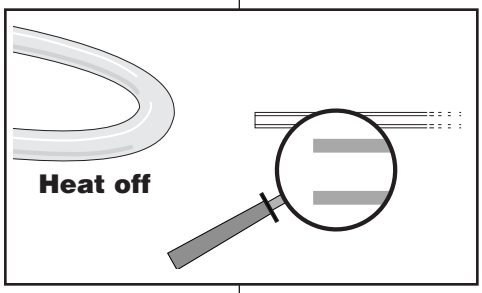


Figure 8a – *Untreated tip*

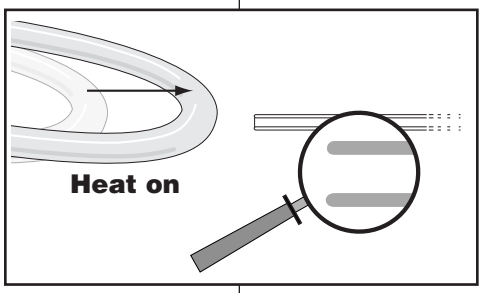


Figure 8b – *Fire polished tip*

a. Choose and install the desired filament (**Table I**).

b. Turn on the power to the Control Unit and press TIME to select Manual mode. Hold down the RUN button and adjust the Heat readout from low to high while observing the expansion of the filament under the microscope. A slight movement of the filament indicates that it has sufficient heat and will provide excellent polishing results in most cases. A red-hot filament is unnecessary and will decrease the life of the filament. A filament that is too hot will heat the pipette tip too quickly and the operator will be unable to control the degree of polishing.

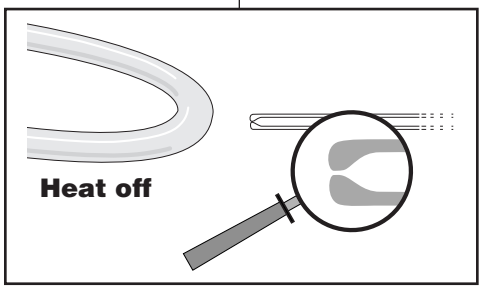
c. Place the pipette to be polished in the acrylic pipette holder. Adjust the microscope stage until the pipette is in position with sufficient distance to account for filament expansion (**Fig. 8a**).

d. Press RUN and observe the expansion movement of the filament. Determine the appropriate Heat setting and then fine-tune the position of the tip. Note the distance between the pipette and the filament; this will be needed to reproduce an identical pipette. A minimal change in the shape of the tip will typically yield good polishing results (**Fig. 8b**).

e. Press RUN and polish the pipette to the degree needed.

f. Press the TIME button and select the Auto mode. Place an identical pipette to the one that was just polished in the tip holder. Position the tip to the same place as the first tip and press the RUN button. The counter will start counting down to zero and stop, and the pipette will be polished the same as the first.

Figure 9 – *Tip size reduction*



5. Tip Size Reduction

Tip size reduction creates a holding pipette by rounding the tip ends and reducing the length of the pipette tip.

a. Choose and install the desired filament (see **Table I**). Select the timer to Manual. Press the RUN button and to see how hot the filament gets Raise the heat until the filament just starts to glow red.

b. Place the pipette to be reduced in the acrylic pipette holder. Adjust the microscope stage until the pipette is in position with sufficient

distance to account for filament expansion. Note the distance between the pipette and the filament; this will be needed to reproduce an identical pipette.

c. Turn on the heat by pressing the RUN button and observe the melting of the tip. Maintain the heat until the desired opening size is obtained (see **Fig. 9**). If the process is too slow, move the tip closer to the filament. (It is better to do this operation very slowly, in stages, to avoid making the tip too small.)

d. Press the TIME button and select the Auto mode. Place an identical pipette to the one that was just polished in the tip holder. Position the tip to the same place as the first tip and press the RUN button. The counter will start counting down to zero and stop, and this pipette's size will be reduced the same amount as was the first tip.

6. Fire Polishing Large Bore Pipettes

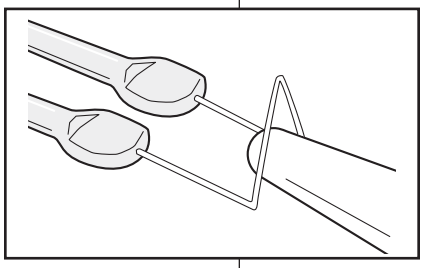


Figure 10

To fire polish large bore tips, the **H5** filament can be shaped or re-formed to be slightly larger than the pipette tip (**Fig. 10**). This effectively provides an increase in the heated surface area presented to the tip with a resulting increase in the heat directed to the large bore tip. This is necessary to melt the thicker glass of a large bore pipette. Larger bore tips generally require the use of the 10× objective. Under some circumstance, it may be possible to use the 25× (LWD) objective. After re-forming the filament, proceed to microforge as described in the section entitled "Fire Polishing a Pipette Tip".

7. Tip Reduction of Large Bore Pipettes

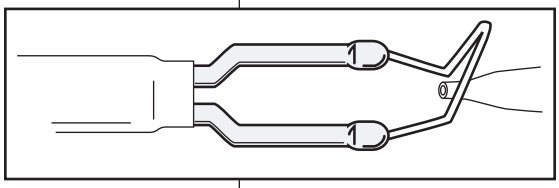


Figure 11

The re-formed **H5** filament can also be used for tip size reduction of large bore pipettes. To reshape the tip end and reduce its size, re-form the filament so that the tip will fit inside the filament and then proceed to microforge as described in the section entitled "Tip Size Reduction" (**Fig. 11**).

8. Breaking and Microforging, Injection Pipettes

A variety of pipette configurations, including those for *in vitro* fertilization and patch pipettes, are hard to pull reproducibly in quantity. Pulling a number of pipettes a little longer than the general size and breaking them using the DMF1000 can be much faster and more exact. All pipette breaking, sealed end probes and other tools are formed using the **H5** filament.

a. Mount the **H5** filament in the filament holder and bring the filament into focus. To do this procedure the filament need not have a glass bead melted to it.

b. Turn on the power to the Control Unit and select Auto time. Check that the heat display light is on.

c. Adjust the Heat readout from 30% to 50% depending on the outside diameter of the glass where the break point is to be made. **Note:** A ten-micron tip will break best at a heat setting around 50%.

d. Place the pipette to be broken in the acrylic pipette holder. Looking at a point far away from the break point toward the tip end and seeing the focus drift off a little reveals this. This loss of focus shows that the filament has moved the tip. Position the pipette tip so the filament is just touching the glass.

e. Set the time readout to read three seconds.

f. Press RUN and the pipette should break off clean, leaving the broken off part attached to the filament. If the glass did not break off one or two things could be wrong: the heat may need to be set a little higher or the glass was not touching the filament. If the glass did break off but the tip is somewhat bent, the heat was set too high or the pipette was pressed to the filament too hard.

g. Refer to Section 3 above to polish the broken tip.

9. Microforging Beveled Injection Pipettes

Frequently, a beveled large bore pipette is not sharp enough to penetrate a cell without causing damage to the surrounding area. With the DMF1000 and the H5 heating filament, a sharp point can be formed on a beveled tip to assist in the penetration of the cell with minimal damage.

Step 1: Glass bead formation

For this application, a glass bead is first formed around the filament. This is done by coating the midpoint of the filament with a small amount of glass (**Fig. 12a**).

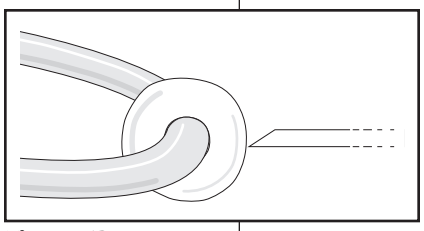


Figure 12a

a. Select the Manual mode by pressing the TIME button.

b. Position a small scrap pipette in the acrylic pipette holder.

c. Adjust the microscope stage until the pipette is in position to allow the tip to touch the filament during expansion.

d. Press RUN on and off to set the heat readout so the filament starts to glow red. Press RUN and coat the center of the filament with glass until a bead covers the diameter of the filament.

e. Release the RUN button. Remove the scrap pipette from the holder.

Step 2: Sharpening the beveled edge

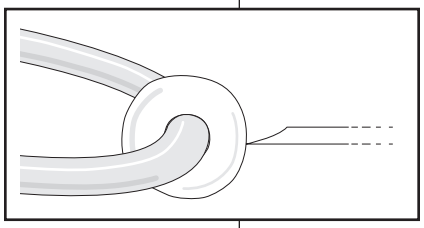


Figure 12b

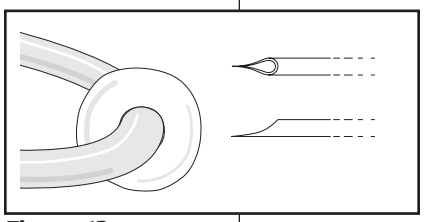


Figure 12c

a. Press the TIME button to select Manual mode.

b. Place the beveled pipette in the acrylic pipette holder. With the pipette tip far from the heat, press RUN/MEM on and off, adjusting the heat until the glass bead becomes molten. Observe how far the filament has expanded.

c. With heat off, move the tip very close to the point that the filament expanded to.

d. Press the Run button. The filament will expand, touching the tip of the beveled glass. As the glass bead becomes molten and the beveled tip makes contact with the bead, quickly pull the tip away and simultaneously release the Run button to turn off the heat (**Fig. 12b**).

e. The resulting tip has a very sharp point for clean cell penetration (**Fig. 12c**).

10. Using DMF1000 as a Microinjector

DMF1000 can also be used as a very good pneumatic pico and femto-liter injector. It offers a digital pressure readout and memory features that can out-perform dedicated injection units. In addition, the high-speed solenoid used in the controller provides a better volume resolution than most dedicated injection instruments.

Procedure for microinjection:

WARNING: Always wear safety glasses during this procedure. Never point the pipette at anyone. The pipette can be forcefully shot out of its holder if not tightly secured.

a. To turn the unit on, switch the on /off switch located on the back of the unit to the on position. After a 30-second initialization period, the setting stored in memory location 0 will be displayed.

b. The heat/pressure readouts will display some value. The MODE button toggles the displays between Heat or Pressure readings. Press the MODE button so the Pressure [psi] light is on and the unit is in pressure mode.

c. Adjust the pressure reading by turning the regulator knob on the back of the unit to the desired pressure.

d. Connect the polyurethane tubing to the air output line on the back of the Control Unit and the barb of the acrylic body (see **Fig. 4.1**). The metal handle ***must*** be carefully attached to the acrylic body. Mount the metal handle onto a micromanpulator.

Figure 13

e. Slide the pipette through the metal cap and gasket and tighten the metal cap. Make sure the pipette is tight, or the pressure may shoot the pipette out. ***Never point the pipette toward yourself or another person when it is pressurized.*** The gasket initially installed in the holder is for a 1 mm OD glass capillary. Gaskets for 1.2, 1.5 and 1.65 mm glass capillaries are also supplied in a plastic bag. When using other sizes of glass capillaries, change the gasket to the right size before installing the pipette.

f. Press the TIME button and select Manual to set up the timer. Time is displayed in seconds from zero up to a maximum of 360 (six minutes). Pressing the left arrow ← next to the time display will move the decimal point to the left and the right arrow → will move it to the right. When the timer is set to maximum time, the decimal point is not on. The up ↑ and down ↓ arrows next to the time display will increase or decrease the time when in Auto mode, allowing the user to make minor adjustments to the time. With the time set to Manual, the RUN button will turn on the output air line to the pipette and start the timer. The sample that was loaded in the pipette will now be disbursed and may be stopped at any time by releasing the RUN button.

g. If the manual inject run was satisfactory the TIME button may be pushed now to switch the unit into auto run mode. If the same pipette or one the same size is used to make another injection and the air pressure was not changed, another injection can be made by pressing and releasing the RUN button. The pressure will be turned on and the counter will count down to 0 and turn off. This injection time and pressure will be the same as the injection time and pressure in the manual mode.

h. To save this program, press the STORE button and select a memory location from 1 to 9 by pressing the arrow buttons ⇧ ⇩ next to the heat/pressure display, then press RUN. The program may be recalled at a later time by pressing RECALL and selecting the number under which it was stored. The program will be displayed on the readouts. Since the air pressure is a manual setting and the display shows the present pressure, the stored pressure reading will blink on the display ten times before the display begins to show the line pressure. Take note of the blinking pressure number, then set the present air pressure to that stored number.

Notes for microinjection:

a. It has been established in literature that injection volume is linearly proportional to the pressure and time duration. By changing the injection pressure or time duration, different volume of injection can be achieved. Injection volume can be calibrated by injecting the liquid into a petri dish that contains hydrated mineral oil and measuring the liquid drop size to calculate the volume.

b. DFM1000 does not have the balance pressure feature to reduce the capillary action. On the other hand, most balance pressure features on the dedicated injectors do not work properly any way. When the liquid meniscus is in the chunk section of the pipette, the capillary pressure is too small to balance with any precision regulator on the market. When the liquid meniscus is in the taper section of the pipette, the capillary pressure is always changing as the liquid/air interface moves. The best way to reduce the effect of capillary action is to remove it completely by silanizing the pipette with silane to form a hydrophobic surface.

c. Although DMF1000 uses the fastest solenoid on the market, the system still has a finite response time. The system capacity and the restriction of the solenoid orifice limit the rising phase of the injection pulse to the 40 to 80 ms range. To get the best accuracy for injection volume, set the injection time longer than 0.1 second.

11. Measuring pipette tip ID with the DMF1000

With a precision pressure regulator and a precision digital monometer, the DMF1000 can be used to measure or calibrate the micropipette inner diameter based on the bubbling threshold pressure. By measuring the threshold of the bubbling pressure in the methanol, the tip inner diameter can be precisely determined with the Laplace equation. For pipette tip that has inner diameter less than 10 micron, the bubbling threshold method is the best non-destructive way to measure the ID.

Measuring procedure:

a. Connect the polyurethane tubing of the calibration pipette holder to the DMF1000. Mount the metal handle of the calibration pipette holder on to a micromanipulator.

b. Place a small container filled with methanol under a stereo microscope.

WARNING: Always wear safety glasses during this procedure. Never point the pipette at anyone. The pipette can be forcefully shot out of its holder if not tightly secured.

c. Slide the pipette through the metal cap and gasket and tighten the metal cap. Make sure the pipette is tight. The higher pressure needed to measure pipettes under

0.5 microns could push the pipette out of the holder if it is not secured tightly. The gasket installed in the holder is for 1 mm OD glass capillary tubing. Gaskets for 1.2, 1.5 and 1.65 mm glass capillaries are also supplied in a plastic bag. Make sure the gasket is the right size before installing the pipette.

d. Press the Mode button on the DFM1000 controller to select the pressure mode. Adjusting the regulator in the back of the instrument so that the output pressure is slightly higher than the predicted threshold pressure of the tip. Press the AIR button to turn on the air.

e. Adjust the micromanipulator to move the pipette tip slowly into the methanol solution in the petri dish.

f. Through the microscope, you should see air bubbles coming out of the pipette tip. Adjusting the regulator until the bubbles just stop coming out. The pressure reading on the DFM1000 is the threshold pressure for the pipette.

g. Using Laplace's equation, the tip inner diameter can be calculated from the threshold pressure. Laplace equation: **P=4s/D**. Where **P** is the threshold pressure, **s** is the surface tension of the gas/liquid interface and **D** is the inner diameter of the pipette. If pressure **P** is measured in PSI, and inner diameter **D** is measured in microns, then, for 100% methanol at 25˚C, **s** is equal to 3.22 (psi•µ). For water at 25˚C, **s** is equal to 10.44 (psi•µ).

Special notes:

a. Always use fresh methanol for the calibration. Water vapor in the air is slowly absorbed by the methanol. This changes the surface tension of the methanol and will affect the accuracy of the measurement.

b. There are at least three publications that detail the accuracy of the bubbling threshold pressure method (see reference 3, 4, 5). In Hagag's paper, a correction constant of 0.793 was introduced to the equation to correct the experimental error and there was a small mistake for the last equation, the numerator also has to be raised to the 1.01th power.

$$D = \frac{0.793 \cdot 4 \cdot S^{1.01}}{P^{1.01}}$$

c. In Bowman's paper, no correction constant was used. The calculated result is about 2-7% larger than that of scanning electron microscopy. It is up to the user to decide which equation to use.

d. Pipette tips larger than 10 micron ID can be determined by measuring the OD of the tip with a microscope that has a calibrated reticle in the eyepiece. Since the magnification of most microscope objectives are is not necessarily the exact integer number as labeled on it, it needs to be calibrated with a precision stage micrometer. It is well established that when a pipette tip is formed, the OD/ID ratio will remain the same as that of the original tubing used; *e.g.*, a 1.00 mm OD, 0.75mm ID glass capillary is used to for making a microinjection pipette. If the OD of the pipette tip is measured to be 10 micron, then the ID of the pipette tip will be 7.5 micron.

Instrument Maintenance and Storage

The requirements for the maintenance and storage of the DMF1000 are minimal. Care should be taken to protect the filaments. Store them in their original container when not in use. In general, it is advisable to keep the DMF1000 in an area with minimal dust and particulates as would be appropriate for any microscope or similar apparatus.

DMF1000 Specifications

POWER REQUIREMENTS	100-240 VAC 50/60 Hz
FILAMENTS (2)	H4, H5
POWER CONTROL	Pushbutton or Optional Foot Switch
TIMER RANGE	0-6 minutes
TIMER RESOLUTION	10 msec
HEATING RANGE	0-99%
MAX. AIR INPUT	100 psi
MIN. AIR NEEDED	30 psi (for forging)
AIR OPERATING RANGE	0 - 60 psi (for injection)
PRESSURE RESOLUTION	0.1 psi
DIMENSIONS (Control Unit)	25 × 20 × 12 cm (9.8 × 7.9 × 4.7 in.)
SHIPPING WEIGHT	3 kg (7 lb)
MICROSCOPE	See Model **H602** Instruction Manual
OBJECTIVE	40× Long-Working Distance (3 mm)
EYEPIECE	10× (pair)
RETICLE	(10× eyepiece for H602 only) 1.25 Fm/div. (at 40×): 0-90˚ Angle at 5˚/div.
SHIPPING WEIGHT	7.3 kg (16 lb)
ACCESSORIES	
FILAMENT ASSEMBLY 1	Mounts on 40× & 25× Long-Working Distance Objectives
FILAMENT ASSEMBLY 2	Mounts on 10× & 4× Objectives
GLASS HOLDER	Mounts on microscope stage

Optional Accessories

Part Number	Description
500292	15× Eyepieces (pair) for H600 Series Microscopes (no reticle available to fit these eyepieces)
13142	Optional foot switch
500329	25× Long-Working Distance objective (5 mm): fits most microscopes with a 160 mm focal length

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2. M. Li, J. D. McCann, M. J. Welsh. *American Journal of Physiology*, **259**, pp C295-C301. (1990)
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4. D. K. Martin, D. I. Cook. "A direct-reading device for measurement of patch-clamp micropipette tip diameters". *Pflugers Arch.* 417, pp255-258. (1990)
5. C. L. Bowman, A. M. Ruknudin. "Quantifying the Geometry of Micropipets". *Cell Biochemistry and Biophysics,* vol. 31, pp185-206. (1999)

Warranty

WPI (World Precision Instruments, Inc.) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of one year* from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

Claims and Returns

• Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within 10 days after receipt of shipment. Claims for lost shipments must be made within 30 days of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim settles. In some instances, photographic documentation may be required. Some items are time sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container.

• WPI cannot be held responsible for items damaged in shipment en route to us. Please enclose merchandise in its original shipping container to avoid damage from handling. We recommend that you insure merchandise when shipping. The customer is responsible for paying shipping expenses including adequate insurance on all items returned.

• Do not return any goods to WPI without obtaining prior approval and instructions (RMA#) from our returns department. Goods returned unauthorized or by collect freight may be refused. The RMA# must be clearly displayed on the outside of the box, or the package will not be accepted. Please contact the RMA department for a request form.

• Goods returned for repair must be reasonably clean and free of hazardous materials.

• A handling fee is charged for goods returned for exchange or credit. This fee may add up to 25% of the sale price depending on the condition of the item. Goods ordered in error are also subject to the handling fee.

• Equipment which was built as a special order cannot be returned.

• Always refer to the RMA# when contacting WPI to obtain a status of your returned item.

• For any other issues regarding a claim or return, please contact the RMA department

** Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.*

Warning: This equipment is not designed or intended for use on humans.

World Precision Instruments, Inc.
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DECLARATION OF CONFORMITY

We: World Precision Instruments, Inc.
175 Sarasota Center Boulevard
Sarasota FL 34240-9258
USA

as the manufacturer of the apparatus listed, declare under sole responsibility that the product:

Title: DMF1000 Multi-Function Microforge Controller

to which this declaration relates is in conformity with the following standards or other normative documents:

Safety: EN 61010-1:2001
EMC: EN 61326:1997
EN 61000-4-2:1995
EN 61000-4-3:1996
EN 61000-4-4:1995
EN 61000-4-5:1995
EN 61000-4-6:1996
EN 61000-4-11:1994

and therefore conforms with the protection requirements of Council Directive 89/336/EEC relating to electromagnetic compatibility and Council Directive 73/23/EEC relating to safety requirements.

Issued on: July 6, 2007

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