INSTRUCTION MANUAL

DMF1000

Microprocessor Controlled Microforge

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070224

CONTENTS

ABOUT THIS MANUAL

The following symbols are used in this guide:

This symbol indicates a CAUTION. Cautions warn against actions that can cause damage to equipment. Please read these carefully.

This symbol indicates a WARNING. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and TIPS contain helpful information.

Fig. 1—DMF1000 System with optional foot switch

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 µm patch clamp pipettes to 100 µm 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 parameters can be stored 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 long working distance. The small filament can forge tips less than 0.5 µm, 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.

The complete **DMF1000-1** system includes both the microforge and matched microscope (WPI #**W30S-LED**); the **DMF1000-M** is the microforge only.

CAUTION: The Microforge Control Unit (power) and the heating filaments have been carefully matched to provide rapid filament response at optimum heat intensity. Use of either of these components with alternate power units or heating filaments may result in severe damage to any or all of these components.

Parts List

After unpacking, verify that there is no visible damage to the instrument. Verify that all items are included:

DMF1000-1 Complete Microforge, 110 V (includes microscope), or
DMF1000-2 Complete Microforge, 220 V (includes microscope):

- (1) **W30S-LED** Microscope (See **W30S-LED** Instruction Manual included for setup, assembly and operating instructions.)
- (1) **DMF1000** Microforge (See parts list below.)

DMF1000-M1 Microforge, 110 V (microscope not included), or
DMF1000-M2 Microforge, 220 V (microscope not included):

- (1) **DMF1000** Microforge Control Unit
- (1) DMF-1000 Start-Up Kit, including:
  - (1) **3354** 0.25” NPT Air Tank Fitting
  - (1) **4763** 0.25” OD Nylon Tubing, air input, 10’
  - (1) **5430-ALL** PicoNozzle Kit

- (1) **75040** Heating Filament Connecting Cable
- (1) **75050** Lucite and Glass Pipette Holder
- (1) **500883** Reticle, 1 mm/200 div. and 90º/18 div.
- (1) **300696** Filament Adjustment Assembly
- (1) **503513** Eyepiece with Linear Reticle
- (1) **800292** 40× Long Working Distance Objective
- (1) **MF200-H4** H4 Heating Filament
- (1) **DMF1000-H5** H5 Heating Filament

(1) **DMF1000** Instruction Manual

Fig. 2—The startup kit for the DMF1000

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 damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 31 of this manual. Please contact WPI Customer Service if any parts are missing at 941-371-1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. 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. For further details, please read the section entitled "Claims and Returns" on page 31 of this manual.

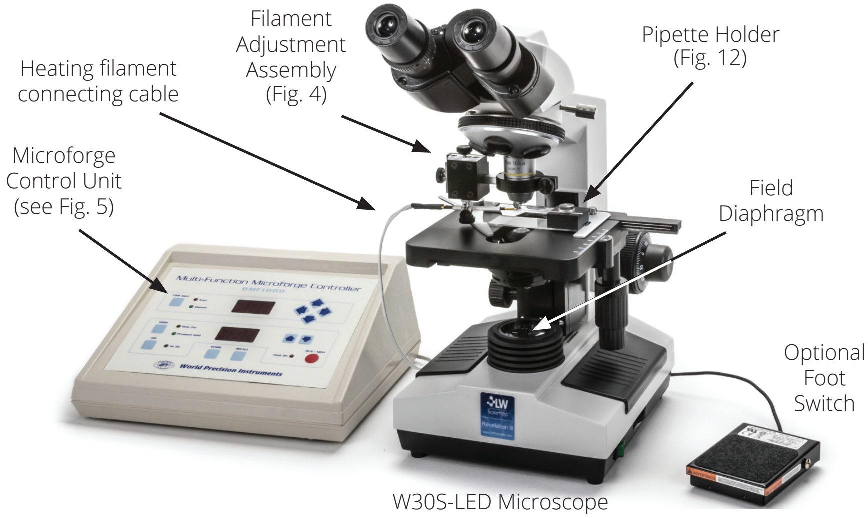


Fig. 3—DMF1000 System

INSTRUMENT DESCRIPTION

The complete **DMF1000-1** (110 V) and **DMF1000-2** (220 V) systems include both the Microforge and matched microscope (WPI Model **W30S-LED**). The **MF200–M1** (110 V) and **MF200-M2** (220 V) include the Microforge only.

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 linear eyepiece reticle is provided with this system for measuring pipette tip dimensions. Optional accessories (including a 25× LWD objective for the **W30S-LED** microscope) further expand the **DMF1000** system functionality.

Positioning and Focus

Finding and moving the pipette tip under the microscope objective is simple. 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 the heating filament, inserted into the Filament Adjustment Assembly, which is directly attached to the microscope's objective and (using the horizontal and vertical adjustment knobs of the assembly) 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 microscope stage. The X-Y-Z movements of the microscope stage adjustment controls its position relative to the heating filament. This design makes the positioning and microforging of pipettes extremely easy. The stage of the **W30S-LED** microscope has a high quality rail that ensures precise, smooth and stable control of the pipette's movement. The **DMF1000** system configuration eliminates the need and expense of an additional micromanipulator to control pipette movement.

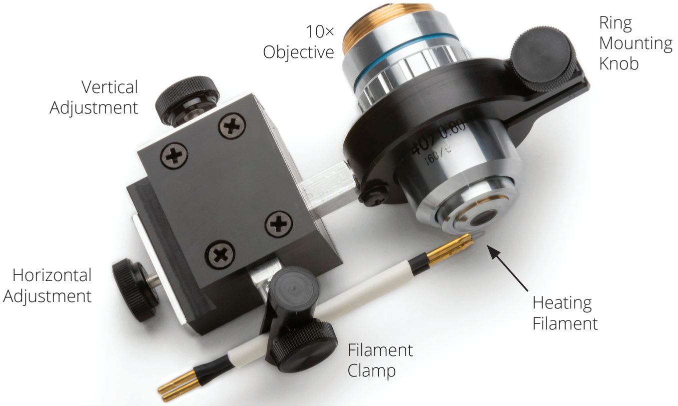
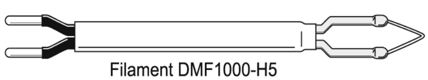
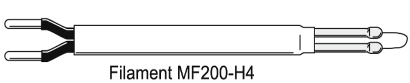


Fig. 4— Filament Adjustment Assembly

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 tip 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. The low thermal expansion characteristic of the filament ensures minimal displacement of the filament during heating. This feature takes much of the guesswork out of tip placement in relation to the filament. 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.
H4 	Small gauge: for polishing patch clamp pipettes.

CAUTION: Do NOT change the heating filament while the Heat Timer is running.

CAUTION: Since 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 heating filament. If, for example, the heat is set to 99%, the large filament should only be used in short bursts. For longer exposure times, lower heat settings should be used. *Do not use the standard 40× objective supplied with the microscope. (See "Appendix A: Microscope Objective Information (W30S-LED)" on page 28).*

Microscope

The microforge has been matched with WPI research grade microscope model **W30S-LED** to provide an uncomplicated and complete system with excellent performance. The Filament Adjustment Assembly supplied with the microforge has been designed to fit both the 40× LWD objective (included), the objective supplied with the **W30S-LED**, and the optional 25× LWD objective for the **W30S-LED** microscope along with the supplies 4× and 10× objectives. The Filament Adjustment Assembly fits most other microscopes with a focal length of 160 mm.

Control Unit

The **DMF1000** Control Unit (Fig. 5) 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.

Initialization

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

When any of the controller's buttons are pushed and released, a "beep" indicates the operation has begun. 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 flashes ten times. The audible alarm may be turned off by pressing the RUN button any time during the initialization period.

Optional Foot Switch

An optional foot switch (WPI **#MF200-FS**) is available for complex fire polishing. Use of the optional foot switch leaves the hands free to move the pipette and control the variable heat adjustment on the Control Unit.

Control Unit Functions

- **RUN/MEM** — This button executes all the functions and settings on the microforge. When in Auto or Manual mode, it turns on the heater and starts the timer. In the

Pressure mode, it turns on the air and start the timer. It also stores and recalls programs. The Heat On light illuminates when the heater starts to heat. If there is an open circuit in the heating filament system, the light does not illuminate. Press the button to start, or hold the button the change the timer setting.

- **TIME [sec]** — The unit has two timer modes of operation, Auto and Manual. In the manual mode the heat timer starts at zero and count up when the RUN/MEM button is pressed and held. After the desired heating time is established, push the TIME [sec] button to set the unit to Auto mode. The timer reading remains at the time established in Manual mode. When the RUN/MEM button is pressed, the unit starts, and the counter counts down from the high setting to zero, a long "beep" sounds, and then the unit shuts 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 reset the time up or down. This allows for minor adjustments to the time when in Auto mode. This control also sets air output time.

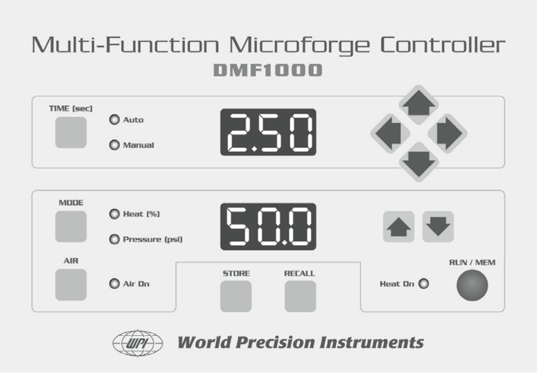


Fig. 5—Microforge Control Unit

- **MODE** — The unit has two modes of operation: Heat and Pressure. The Mode button switches the lower readout from heat reading in % to pressure reading in PSI. If left in Pressure mode, pressure injections can be made (see "Using DMF1000 as a Microinjector" on page 23) and the size of a pipette can be calibrated (see "Measuring Pipette Tip ID with the DMF1000" on page 24).
  - The **heat display** reads from 0 to 99.9%. The factory set maximum heat is hot. The filament glows cherry red when set at 99.9%. DO NOT use this setting for long periods of time. To prolong filament life, the best setting for heat is just hot enough to melt the glass. 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 (Fig. 5). The lower window displays the

current pressure in PSI when the unit is set to Pressure mode.

- **STORE** — When the store button is pressed, the lower display window displays 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 displays the contents of memory location 0. Use the arrows next to the heat display window to choose the desired memory location (0–9), then press Run/Mem to recall the parameters stored in the selected memory location.

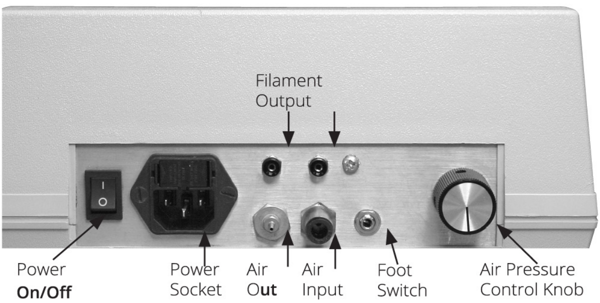


Fig. 6—DMF1000 rear panel

OPERATING INSTRUCTIONS

Mounting the Filament Adjustment Assembly to the Microscope

1. Attach the objective you want to use to an open location on the microscope turret.
2. Place a glass electrode on the slide and focus on it to gauge the working distance of the objective. (See "Appendix A: Microscope Objective Information (W30S-LED)" on page 28 for working distances for the **W30S-LED**).
3. Rotate the turret out and slide on the objective ring, leaving the set screw loose enough so it can be rotated on the objective.
4. Center the horizontal filament adjustment to the center of its travel. This is set so that a little over 1 cm of the square bar is visible (Fig. 7).

Fig. 7—Mounting the Filament Adjustment Assembly

5. Slide the filament into the filament clamp so that the tip of the filament wire is located near the center of the objective lens. Tighten the filament clamp so that the plastic section holding the filament can be rotated under tension.
6. Adjust the vertical adjustment knob (filament focus) so that the filament is closer (clockwise) to the objective lens than the working distance of the object is to the glass tip. (If the objective working distance is 5 mm, then set the filament distance to 2 mm.)
7. Rotate the microscope turret back to the normal working position.
8. Rotate the filament adjustment assembly to 90º and focus on the glass tip (Fig. 8).

Fig. 8—Objective in position

9. While looking at the glass tip, move the filament side to side until you see the shadow of the filament in the microscope. Stop and adjust the vertical adjustment knob (filament focus) so that the filament moves down (counter clockwise) and into focus. Adjust the horizontal adjustment knob (filament centering). The optimal distance to the glass tip depends on the forging application (Fig. 9).

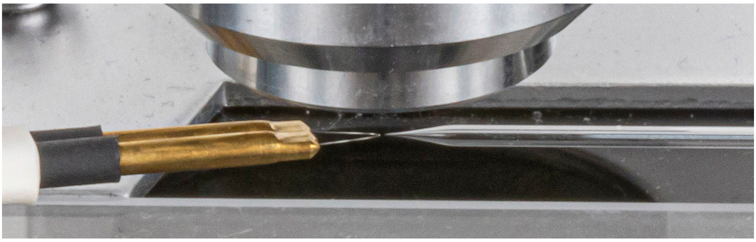


Fig. 9—Filament in position

NOTE: The filament clamp and base plate attached to the vertical filament adjustment can be angled slightly outward (toward the user) and the filament angled slightly inward. This angle facilitates better viewing of the filament under the microscope.

NOTE: Air is used to form patch clamp tips and in coating patch clamp tips. Fefer to pages 16–18 for these applications. Otherwise, steps 2 and 3 are not required.

2. Hook up the lab air or nitrogen to the AIR IN port on the back of the Control Unit.
3. Plug the polyurethane air output tubing into the pipette holder of choice. The nylon air input tubing (WPI #**4763**) supplied is 10′ long. A common 0.25″ NPT air tank fitting (WPI #**3354**) is also supplied.

Assembling the Pipette Holder

Two sets of micropipette holder assemblies are provided in the PicoNozzle Kit (WPI #**5430-ALL**). For fire polishing, coating and pressure forging, one holder should be assembled **without** the heavy metal handle (Fig. 12). For pipette tip diameter calibration and microinjection, the other holder should be assembled **with** the metal handle (Fig. 10).

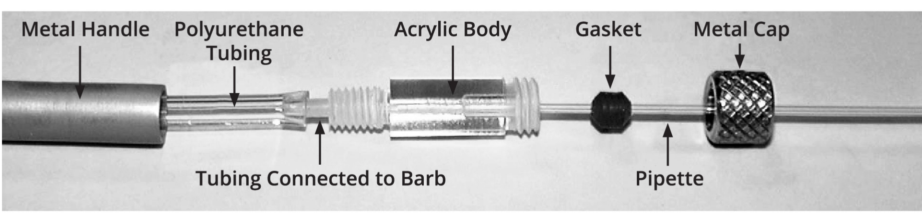


Fig. 10—Micropipette Holder Assemblies

Positioning the Micropipette Holder

Position the Pipette Holder on the microscope stage as if it were a slide. Orient the acrylic block of the pipette holder as shown in Fig. 11.

Fig. 11—Micropipette positioned on the microscope stage

Mounting the Heating Filament

1. Position the heating filament in the filament clamp and tighten the knob on the bottom of the filament holder.
2. 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.
3. Take the free end of each cable and insert each into one of the two Filament Output receptacles located on the side 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.

Basic Operations for Using the DMF1000

This section describes the final preparations and general instructions for using the **DMF1000.** Specific instructions are detailed for some of the **DMF1000** common uses in “Applications” on page 15.

NOTE: Remember that (because of microscope optics) any object seen through the microscope objective is a reverse image of the object and 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. See “Heating Filaments” on page 5.
3. Mount and connect the heating filament. See “Mounting the Heating Filament”

above.

4. Bring the filament into focus:
   a. 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 below the objective.
   b. Looking through the microscope, move the filament in the filament clamp until its shadow appears. Some vertical adjustment may also be required to bring the shadow into the field.
   c. Using the vertical adjustment knob, bring the filament into clearer view. With the horizontal adjustment knob, position the end loop of the filament to the far right side of the visual field.
5. Power up the **DMF1000** unit. To do this, connect the line cord to the power input jack on the Microforge Control Unit and the wall socket.
6. Set the power switch (located on the back of the Control Unit) to on. After initialization, the setting that had been stored in memory location 0 displays. The Heat and Timer displays illuminate, indicating that the unit is powered up.

   TIP: To turn the sound off, press the RUN/MEM button during the initialization period.

CAUTION: 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 heating filaments. If, for example, the heat is set to 99%, the larger filament should only be used in short bursts. For longer exposure times, lower heat settings should be used.

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7. Press the TIME button to set the unit in Manual mode. Pressing the RUN button sends current through the filament and turns on the Heat On lamp.
8. Set the Heat readout by pressing the Increase (up arrow)/Decrease (down arrow) buttons from setting 0–99.9%, varying the amount of power applied to the filament. An optional foot switch (WPI #**MF200-FS**) leaves the hands free to vary the filament heat intensity while positioning the pipette. Some microforging techniques require a two handed approach.
9. With the power on and unit set at Manual, depress the RUN button several times at various heat settings to see 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 expands farther.
10. Position the pipette:
    a. Position the pipette by first adjusting the stage of the microscope down and away from the objective, to provide sufficient room for mounting the pipette safely on the pipette holder.
    b. Place the pipette in the acrylic pipette holder (Fig. 12).

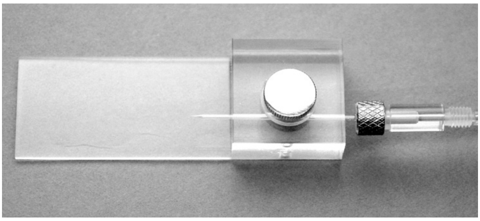


Fig. 12— Acrylic micropipette holder

c. Position the pipette using the horizontal adjustment on the microscope stage so that the pipette tip is slightly past the center of the objective.
d. Raise the stage until the filament is about 3 mm from the objective. (Fig. 11)
e. Slowly move the pipette back and forth, in and out, while looking through the microscope until the image of the pipette is observed.
f. Adjust the vertical position of the stage until the pipette is clearly visible and in focus.
g. Position the pipette tip in relation to the heating filament as required by the application.

11. Adjust the 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 (0–99.9%) provides a range of power. 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 necessary to operate the unit at high power with the Heat dial set at 100 if the system is used properly. This can cause the filament to burn out prematurely.

NOTE: Whenever changing filaments, turn off the power. When switching power levels, always set the Heat dial to 0.

Programming Procedure

1. Turn on the Control Unit by setting the power switch (located on the back of the unit) to the on position. The settings stored in memory location 0 display.

   The first time the unit is turned on:
   Both readouts display 000
   TIME selection is Auto
   MODE selection is Heat
   Air On light is off
   Heat On light is off
   Time display does 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 min-

utes).

- Pressing the left arrow moves the decimal point in the display to the left, and the maximum time for the counter is 99.9 seconds (1.66 minutes) before returning to 0 and starting over.
- Pressing the left arrow another time moves the decimal point further left and the maximum time for the counter is 9.99 seconds before returning to 0 and starting over.

2. Select a filament. The small filament is better suited for microforging pipettes 1 µm or less. The large filament should be used for larger pipette forging, forming and sizing.

2. Press the TIME button to select Manual mode.

3. Verify that the unit is 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 is off.

4. 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.

   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 needs to be the same distance from the filament.

5. Using the up and down arrow keys above the Heat On light, set the heat to a low setting between 30 and 50. ***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.

6. Press and hold down the RUN button to start the heating action. The time counter starts counting up from 0. Seen through the microscope, the pipette tip slowly starts 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, increase the heat a little or move the tip closer to the filament to start the melting action.

   NOTE: If the heat is set too high the tip will form too fast and may be not useable. Move the tip away from the filament or lower the heat to let the tip form slower so the heat can be turned off at the right time.

7. If the tip that was just formed is acceptable, push the TIME button to select Auto mode. The unit is now programmed and the time readout is set at the same duration to polish another tip of the same size and profile.
8. 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.
9. Press and release the RUN button. The heater turns on and the counter starts counting down to 0 and turns off the heater. The forming of the tip can be seen through the microscope. The newly formed tip is the same as the first tip.

   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

Choice of filaments, power and heat settings for each application vary with the use. If the desired result is achieved, the choice of parameters is acceptable. Always 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 varies depending on the tip bore, filament, power, heat settings and application. With the exception of the applications in which a glass bead is formed on the filament, 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” on page 21. It is not required for the other applications described in this manual, however, a glass bead on the filament may be used for other applications, if desired.

Fire Polishing Patch Clamp Pipette or Standard Pipette Tip

1. Choose and install the desired filament. (See “Heating Filaments” on page 5.)
2. Turn on the Control Unit power and press TIME to select 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 to provide excellent polishing results in most cases.

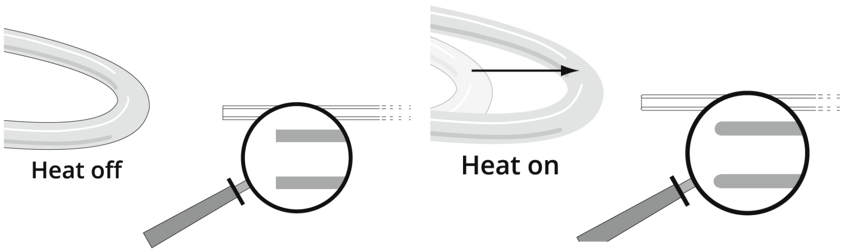


Fig. 13—Untreated tip (left)
Fig. 14—Fire polished tip (right)

NOTE: A red hot filament is unnecessary and undesirable and will decrease the life of the filament. It also heats 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.

3. 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. 13 & Fig. 14).
4. 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 is needed to reproduce an identical pipette. A minimal change in the shape of the filament typically yields good polishing results.
5. Press RUN and polish the pipette to the desired degree.
6. Press the TIME button to select Auto mode. Place an identical pipette to the one just polished in the tip holder. Position the tip in the same place as the first tip and press the RUN/MEM button. The counter starts counting down to zero, and the pipette is polished the same as the first.

Forming a Patch Clamp Pipette with Air

Another way to create a pipette that will seal with lower resistance is to increase the outside diameter of the tip end without changing the inside diameter opening size. This increases the seal surface area of the glass and creates 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.

1. Create a pipette with an inside diameter (ID) opening of about 0.5 µm, and fire polish the tip end. See “Fire Polishing Patch Clamp Pipette or Standard Pipette Tip” on page 15.
2. Select the large filament and form a bent up loop in the end (Fig. 14).

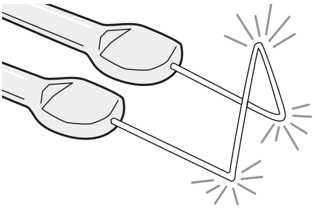


Fig. 15— Filament reformed into bent up loop

3. Mount the filament in the filament holder and position it.
4. Connect the polyurethane tubing to the air output line on the back of the Control Unit (Fig. 6). The other end of the polyurethane tubing must be connected to the barb of the acrylic body (Fig. 10). Be careful that the barb does not break when attaching or removing the tubing.
5. The metal handle must not be connected to the acrylic body. Before inserting the pipette into the acrylic body, place a gasket of the correct size in the metal cap.

   NOTE: The kit includes gaskets for pipette diameters of 1 mm (green), 1.2 mm (black), 1.5 mm (blue) and 1.65 mm (red).
6. Slide the pipette through the metal cap and gasket, then twist the metal cap to tighten it securely.
7. Mount the pipette on the stage mounted acrylic pipette holder and lock it in place with the locking screw (Fig. 15).

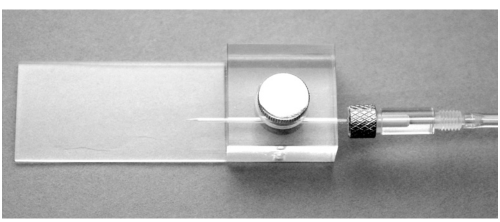


Fig. 16— Stage mounted acrylic pipette holder

8. 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 allows the pipette to be placed under the filament and still be in the field of view (Fig. 17).

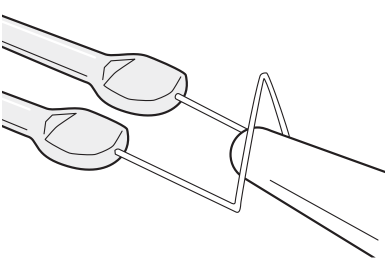
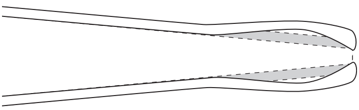


Fig. 17— Position pipette under the loop

9. Adjust the location of the pipette so that it is in the field of view and 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 inside diameter (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.



10. Press the MODE button to set the unit to Pressure mode. Set the pressure to 30 or 40 PSI.

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

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

13. Press the TIME button to select Manual mode.

14. Set the heat to 30 or 40%.

15. Press and hold the RUN button and observe the pipette end. A ball should start to form at the end of the pipette.

16. Release the RUN button when the desired tip is formed.

17. When the heat turns off, the air stays on for about five seconds. This delay is set at the factory and cannot be changed.

    TIP: 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 (Fig. 17). This procedure may be tried a few times before acceptable results are obtained.

Fig. 18—Properly formed tip

18. 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 the newly formed tip was. 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.

Coating a Single Channel Patch Clamp Pipette

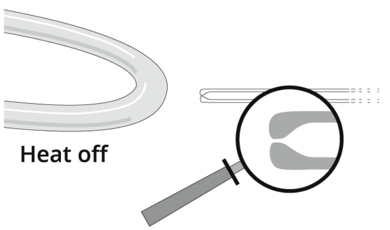
Coat the single channel patch clamp pipette with Sylgard 184 before polishing. Prior coating ensures that Sylgard does not seal the tip of the glass after fire polishing. (A simple and effective coating method has been described by Dr. Li. See "References" on page 29.)

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.

---

1. Assemble the pipette holder without the metal handle.
2. Briefly, 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.
3. 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.

   NOTE: Air is forced through the pipette at a pressure greater than 20PSI (for a 0.5 μm ID pipette) in order to prevent the Sylgard from entering the pipette tip during the coating process.
4. After mixing the Sylgard, press the MODE button to return the unit to Heat mode.
5. Press the AIR button to turn the air on.



nd remove it.

l applied, place the pipette tip over a heat gun

ready to be polished following the procedure
ee "Fire Polishing Patch Clamp Pipette or

TIP: Practice with a scrap pipette to determine the heat setting and curing procedure.

Tip Size Reduction

Tip size reduction creates a holding pipette by rounding the tip ends and reducing the length of the pipette tip (Fig. 19).

1. Choose and install the desired filament. See "Heating Filaments" on page 5.
2. Turn on the Control Unit power.

Fig. 19—Tip size reduction

3. Press the TIME button to select Manual mode.
4. Press the RUN button to see how hot the filament gets.
5. Increase the heat until the filament just starts to glow red.
6. Place the pipette to be reduced in the 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, because this is needed to reproduce an identical pipette.

7. 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.

   TIP: If the process is too slow, move the tip closer to the filament. (It is better to do this operation slowly in stages, in order to avoid making the tip too small.)

Fire Polishing Large Bore Pipettes

To fire polish large bore tips (100–200 µm), the **H5** filament can be shaped or reformed to be slightly larger than the pipette tip (Fig. 19.) This 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 reforming the filament, proceed to microforge as described in “Fire Polishing Patch Clamp Pipette or Standard Pipette Tip” on page 15.

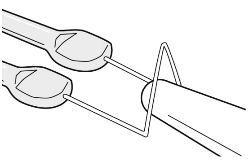


Fig. 20—Large bore pipette in reshaped filament

Tip Reduction of Large Bore Pipettes

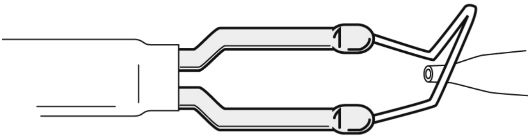


Fig. 21—Tip of large bore pipette in reshaped filament

The reformed H5 filament described in “Fire Polishing Large Bore Pipettes” can also be used for tip size reduction of large bore pipettes. To reshape the tip end and reduce its size, reform the filament so that the tip will fit inside the filament (Fig. 21), and then proceed to microforge as described in “Tip Size Reduction” on page 19.

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.

1. Mount the **H5** filament in the filament holder and bring the filament into focus. The filament does not need a glass bead melted to it.

2. Turn on the power to the Control Unit and press the TIME button to select Auto mode.
3. Verify that the heat display light is on.
4. Adjust the Heat readout from 30–50% depending on the outside diameter of the glass where the break point is to be made.

   NOTE: A 10 µm tip will break best at a heat setting around 50%.
5. Place the pipette to be broken in the acrylic pipette holder. Look at a point far away from the break point toward the tip end and watch the focus drift off a little. This loss of focus shows that the filament has moved the tip. Position the pipette tip so the filament is just touching the glass.
6. Set the time readout to three seconds.
7. Press the RUN button and the pipette should break off clean leaving the broken off part attached to the filament.

   NOTE: If the glass did not break off, the heat may be to low or the glass is not touching the filament. If the glass did break off but the tip is somewhat bent, the heat is set too high or the pipette is pressed to the filament too hard.
8. See “Fire Polishing Patch Clamp Pipette or Standard Pipette Tip” on page 15 to polish the broken tip.

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, using a two step process. First form a glass bead on the filament, and then sharpen the beveled edge of the pipette.

Step 1: Form Glass Bead on the Filament

First form a glass bead around the filament by coating the midpoint of the filament with a small amount of glass.

1. Press the TIME button to select Manual mode.
2. Position a small scrap pipette in the pipette holder.
3. Adjust the microscope stage until the pipette is in position to allow the tip to touch the filament during expansion.
4. Press the RUN button on and off to set the heat readout so the filament starts to glow red. Press the RUN button and coat the center of the filament with glass until a bead about twice the diameter of the filament is formed.
5. Release the RUN button. Remove the scrap pipette from the holder.

Step 2: Sharpen the Beveled Edge of the Pipette

1. Press the TIME button to select Manual mode.
2. Place the beveled pipette in the pipette holder. With the pipette tip far from the heat, press the RUN button on and off, and adjust the heat until the glass bead becomes molten (Fig. 21). Observe how far the filament has expanded.

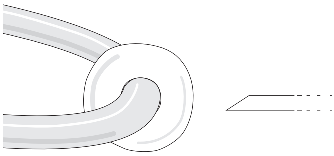


Fig. 22— Glass bead formed on filament

3. With heat off, move the tip close to the point that the filament expanded to (Fig. 23).

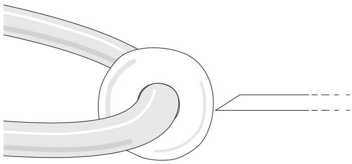


Fig. 23— Pipette tip close to glass bead

4. Press the RUN button. The filament expands, touching the tip of the beveled glass. As glass the 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. 24).

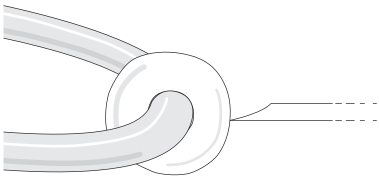


Fig. 24—Filament expands and contacts the tip

5. The resulting tip (Fig. 25) has a very sharp point for clean cell penetration.

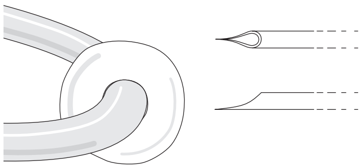


Fig. 25—Pipette has a sharp tip

Using DMF1000 as a Microinjector

DMF1000 can also be used as a pneumatic pico and femtoliter injector. It offers a digital pressure readout and memory features that outperforms dedicated injection units. In addition, the high speed solenoid used in the controller provides excellent volume resolution.

1. Turn on the power to the Control Unit. After a 30 second initialization period, the setting stored in memory location 0 displays.
2. The Heat/Pressure readouts display a value. Press the MODE button to select Pressure mode. The Pressure [PSI] light illuminates.
3. Adjust the pressure reading by turning the regulator knob on the back of the unit to the desired pressure.
4. Connect the polyurethane tubing to the air output line on the back of the Control Unit and the barb of the acrylic body (Fig. 26).
5. Carefully attach the metal handle to the acrylic body.

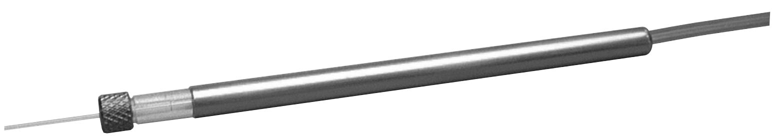


Fig. 26—Pipette with metal handle

6. Mount the metal handle onto a micromanipulator.
7. Slide the pipette through the metal cap and gasket and tighten the metal cap. Make sure the pipette is tight.

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.

NOTE: The gasket initially installed in the holder is for a 1 mm outside diameter (OD) glass capillary. Gaskets for 1.2, 1.5 and 1.65 mm glass capillaries are also supplied. When using other sizes of glass capillaries, change the gasket to the right size before installing the pipette.

8. Press the TIME button to select Manual mode. Time is displayed in seconds from zero up to a maximum of 360 (6 minutes).

   TIP: Press the left or right arrow next to the time display to move the decimal point to the left or right. When the timer is set to maximum time, the decimal point is not displayed. The up and down arrows next to the time display increase or decrease the time when in Auto mode, allowing for minor time adjustments.

9. In Manual mode, the RUN button turns on the output air line to the pipette and starts the timer. The sample that was loaded in the pipette is disbursed and may be stopped at any time by releasing the RUN button.

10. If the manual inject run was satisfactory, press the TIME button to select Auto mode. To make another injection, use the same pipette or one the same size and do not change the air pressure. Press and release the RUN button. The pressure turns on, and the counter counts down to 0. This injection time and pressure are the same as the injection time and pressure in the Manual mode.
11. 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. To recall the program at a later time, press RECALL and select the number under which it was stored. The program displays on the readouts. Since the air pressure is a manual setting, and the display shows the present pressure, the stored pressure reading blinks on the display ten times before the display begins to show the line pressure. Be sure to set the air pressure to stored number shown on the blinking display.

Notes for Microinjection

- 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.
- **DFM1000** does not have the balance pressure feature to reduce the capillary action. 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.
- Although **DMF1000** uses the fastest solenoid on the market, the system 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 40–80 ms range. To get the best accuracy for injection volume, set the injection time longer than 0.1 second.

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 (ID) based on the bubbling threshold pressure. By measuring the threshold of the bubbling pressure in methanol, the tip inner diameter can be precisely determined with the Laplace equation. For a pipette tip that has inner diameter less than 10 μm, the bubbling threshold method is the best non-destructive way to measure the inner diameter.

Measuring Procedure

1. Connect the polyurethane tubing of the calibration pipette holder to the **DMF1000.**
2. Mount the metal handle of the calibration pipette holder on to a micromanipulator.
3. 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.

4. 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 µm could push the pipette out of the holder if it is not secured tightly.

   NOTE: The gasket initially installed in the holder is for a 1 mm outside diameter (OD) glass capillary. Gaskets for 1.2, 1.5 and 1.65 mm glass capillaries are also supplied. When using other sizes of glass capillaries, change the gasket to the right size before installing the pipette.
5. Press the MODE button to select Pressure mode.
6. 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.
7. Press the AIR button to turn on the air.
8. Adjust the micromanipulator to move the pipette tip slowly into the methanol solution in the petri dish.
9. Air bubbles coming out of the pipette tip can be seen through the microscope. Adjust the regulator until the bubbles just stop coming out. The pressure reading on the **DFM1000** is the threshold pressure for the pipette.
10. Using Laplace's equation, the tip's inner diameter can be calculated from the threshold pressure.
    Laplace equation: $P = 4s/D$.

    P = threshold pressure

    s = surface tension of the gas/liquid interface

    D = 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 25ºC, s is equal to 3.22 PSI•µ.

    For water at 25ºC, **s** is equal to 10.44 PSI•µ.

Notes

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

- At least three publications detail the accuracy of the bubbling threshold pressure method. See "References" on page 29 (3, 4, 5). In Hagag's paper, a correction constant of 0.793 was introduced to the equation to correct the experimental error. There was a small mistake for the last equation. The numerator also has to be raised to the 1.01th power.

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

  In Bowman's paper, no correction constant was used. The calculated result is about 2–7% larger than that of scanning electron microscopy.

- Pipette tips larger than 10 µm inside diameter (ID) can be determined by measuring the outside diameter (OD) of the tip with a microscope that has a calibrated reticle in the eyepiece. Since the magnification of most microscope objectives is not necessarily the exact integer number as labeled on it, it needs to be calibrated with a precision stage micrometer. When a pipette tip is formed, the OD/ID ratio remains the same as that of the original tubing used. For example, if a 1.00 mm OD, 0.75 mm ID glass capillary is used to for making a microinjection pipette, and the OD of the pipette tip is measured to be 10 µm, then the ID of the pipette tip is 7.5 µm.

MAINTENANCE

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.

ACCESSORIES

Part Number	Description
75125-6	Replacement Acrylic Pipette Holder Tip for 5430-ALL, pkg of 6
500883	Optional Angular Reticle (19 mm)
500292	15X Eyepieces (pair)
500329	25× Long Working Distance objective (5 mm): fits most microscopes with a 160 mm Focal Length
MF200-FS	**MF200** optional foot switch

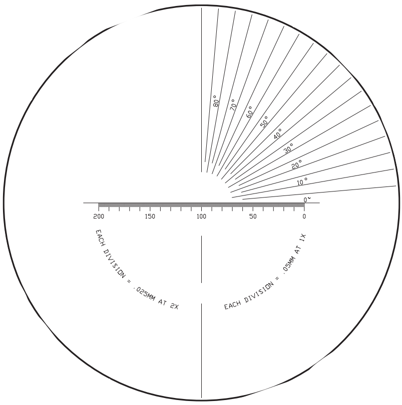


Fig. 27—Angular eyepiece reticle, for use in place of the Linear Reticle installed in 503513.

SPECIFICATIONS

DMF1000

POWER REQUIREMENTS ....100–240 VAC 50/60 Hz
FILAMENTS (2) H4, H5
POWER CONTROL ....Push button or Optional Foot Switch
TIMER RANGE ....0–6 minutes
TIMER RESOLUTION..10 ms
HEATING RANGE...0–99%
MAX. AIR INPUT ....100 PSI
MIN. AIR NEEDED..30 PSI (for forging)
AIR OPERATING RANGE....................................0.1 PSI
CONTROL UNIT DIMENSIONS ....25 x 20 x 12 cm (9.8 x 7.9 x 4.7”)
SHIPPING WEIGHT...3 kg (7 lb.)

Microscope

OBJECTIVE..40× Long Working Distance (3 mm)
EYEPIECE...10× (pair)
RETICLE ....(10× eyepiece for **W30S** only) Linear, 100 div/10: 0–90° angle at 5°/div. (Optional)
SHIPPING WEIGHT ....7.3 kg (16 lb.)

For **W30S-LED** microscope specifications, refer to the **W30S-LED** Instruction Manual.

APPENDIX A: MICROSCOPE OBJECTIVE INFORMATION (W30S-LED)

DIN Plan Achromat (160 mm) Information RMS THREAD

Magnification	N.A.	Approx. Field of View	Approx. Working Distance	Body Diameter	Approx. Depth of Focus
4×	0.10	4.5 mm	17 mm	22.9 mm	~90 µm
10×	0.25	1.8 mm	5 mm	23.0 mm	~15 µm
25× LWD	0.50	0.72 mm	5 mm	23.5 mm	~5 µm
40× *	0.65	0.45 mm	0.35 mm	23.0 mm	~20 µm
40× LWD	0.65	0.45 mm	3 mm	23.5 mm	~20 µm
100× (oil) *	1.25	0.18 mm	contact	NA	<1 µm

** Do not use with microforge — working distance is too small and damage may occur.*

APPENDIX B: EXCHANGING W30S-LED RETICLE

1. Locate the WPI reticle in the bag marked **500883**.
2. Remove the reticle retaining ring at the top of the inverted reticle eyepiece and remove the linear reticle

Fig. 28—Loosen the reticle ring.

NOTE: Do not loosen or open the larger section of the eyepiece.

Fig. 29—The reticle ring was removed.

3. Drop in the new angular reticle with the text side down and gently shake the eyepiece to center the reticle glass in the reticle seat built into the eyepiece.

Fig. 30—Insert the reticle with the text on the bottom side of the reticle.

4. You can check the text orientation by holding the eyepiece up to a ceiling light while keeping it inverted. Look through the front of the eyepiece to view the reticle. If the text is backwards then the glass needs to be remove inverted and inserted again.

5. Insert the retaining ring and tighten it. The glass may move off center, if it does then loosen the retaining ring move the glass by tapping the eyepiece with your finger. Then retighten the retainer.

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DECLARATION OF CONFORMITY

WORLD PRECISION INSTRUMENTS, LLC.
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e-mail wpi@wpiinc.com

DECLARATION OF CONFORMITY CE

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

as the manufacturer/distributor of the apparatus listed, declare under sole responsibility that the product(s):

DMF1000

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

Low Voltage Directive (Safety) 2014/35/EU:

- **EN 61010-1:2010+A1:2019**

EMC Directive 2014/30/EU:

- **EN IEC 61326-1:2021**
- **EN IEC 61326-2-3:2021**
- **EN IEC 61000-3-2:2019+A1:2021**
- **EN IEC 61000-3-3:2013+A2:2021**

Issued On: December 12, 2022

Cory Boyes / Director of Design and Development

Europe Representative
Mr Andrew Waldes
Managing Director
World Precision Instruments Germany GmbH,
Pfingstweide 16, 61169 Friedberg, Germany

F-QC-006 Rev D

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 30 days* 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 ten (10) days after receipt of shipment. Claims for lost shipments must be made within thirty (30) days of receipt of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim is settled. 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

Do not return any goods to us without obtaining prior approval and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. Goods accepted for restocking will be exchanged or credited to your WPI account. Goods returned which were ordered by customers in error are subject to a 25% restocking charge. Equipment which was built as a special order cannot be returned.

Repairs

Contact our Customer Service Department for assistance in the repair of apparatus. Do not return goods until instructions have been received. Returned items must be securely packed to prevent further damage in transit. The Customer is responsible for paying shipping expenses, including adequate insurance on all items returned for repairs. Identification of the item(s) by model number, name, as well as complete description of the difficulties experienced should be written on the repair purchase order and on a tag attached to the item.

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

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