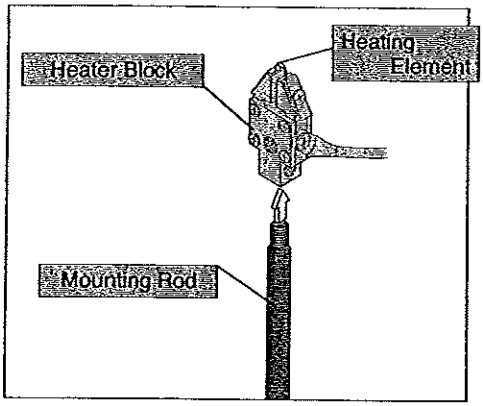
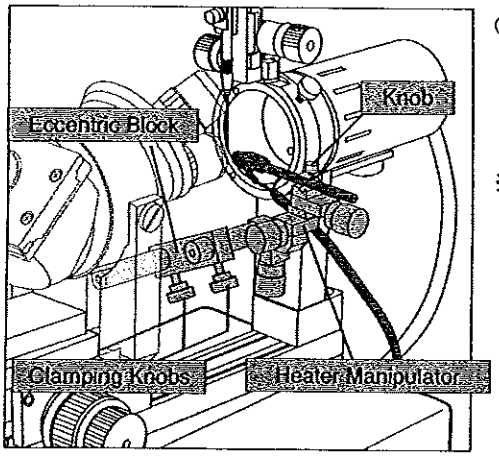
(3) To Mount the Heater

The heater can be mounted either in an upward position or sideways position. This chapter describes "mounting the heater in a sideways position". The heater mounting position varies depending on the intended use. For details, refer to "6. Functions – (2) Repositioning the Heater".



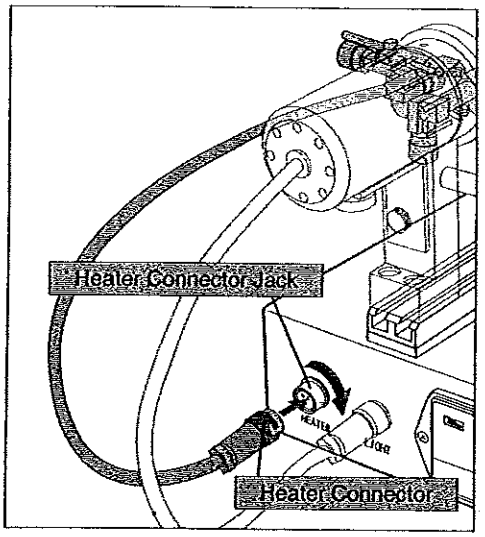
①Change the connecting position of the mounting rod to the underside of the heater block.

※ The heater comes factory-configured to the sideways mounting position and thus there is no need to reassemble the heater block and mounting rod.



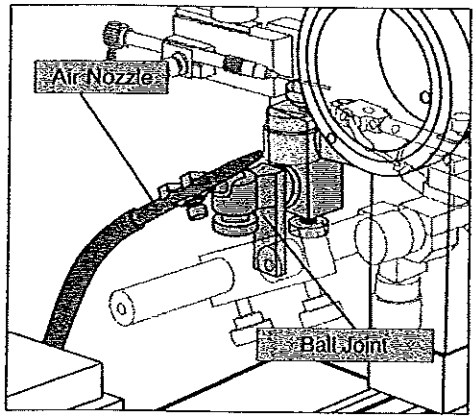
②Set the mounting rod to the heater manipulator and fasten it securely with the knob. Loosen the clamping knob and adjust the position of the eccentric block so as to place the heater in the center of the microscopic field of view, and then tighten the clamping knobs securely.

※ The clamping knobs are securely fastened to prevent loosening during transit. If they are too tightly fastened to loosen by hand, insert the supplied Allen wrench into a hole in the clamping knob to loosen it.



③Put the heater connector, with its slotted part facing upward, into the heater connector jack marked "HEATER" on the back panel. While carefully pressing the cover of the heater connector, turn it clockwise to lock it firmly.

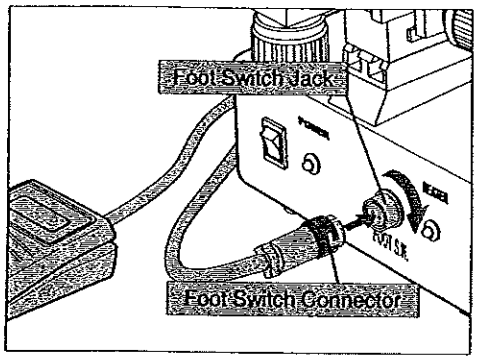
(4) To Attach the Air Nozzle



Attach the air nozzle to the ball joint as necessary.

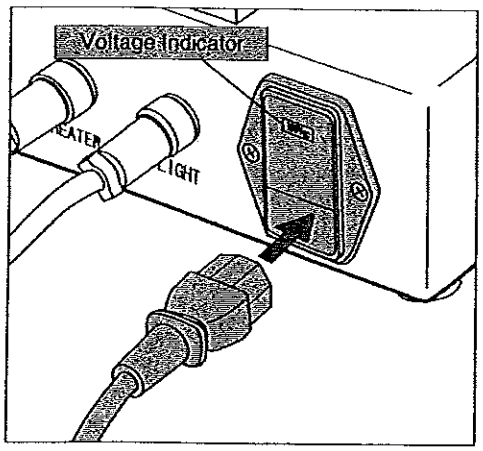
※To adjust the position of the air nozzle, insert the supplied Allen Wrench into the clamping hole in the clamping knob to loosen it.

(5) To Connect the Foot Switch



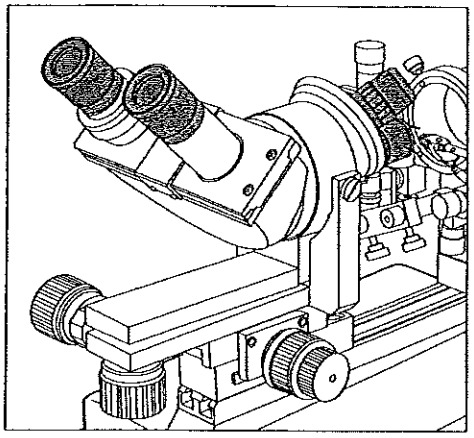
Put the foot switch connector, with its slotted part facing upward, into the foot switch jack marked "FOOT S.W." on the front panel. While carefully pressing the cover of the foot switch connector, turn it clockwise to lock it firmly.

(6) To Plug In the Power Cord



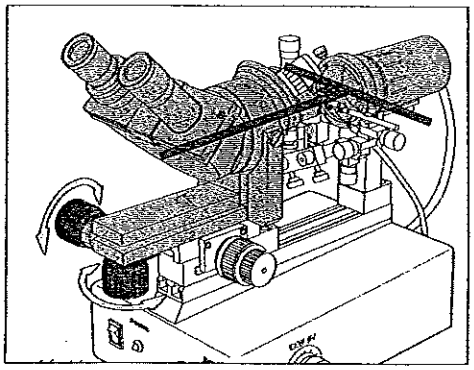
Before plugging in the power cord, be sure to check the voltage indicated at the top. If the indicated voltage does not agree with the working voltage, switch the input voltage properly before plugging in the power cord. To switch the input voltage, refer to "7. Maintenance – (4) To Change the Working Voltage".

(7) Assembly of the Microscope



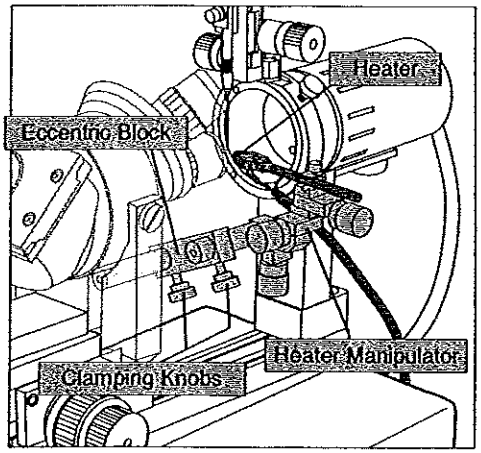
Put the eyepieces into the binocular tubes and screw the objectives into the revolving nosepiece. After the microscope is assembled, manipulate the swing adjustment knob and the tilt adjustment knob to allow the microscope to face straight.

(8) Adjustment of the Microscope unit



Turn the swing adjustment knob and the tilt adjustment knob to put the microscope unit in a position perpendicular to the illuminator. Setting the adjustment knobs to the midpoint of the respective working ranges allows the microscope unit to be positioned perpendicular to the illuminator.

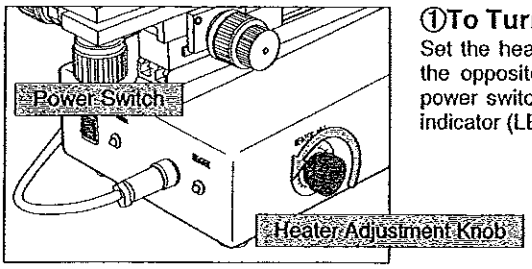
(9) Adjustment of the Heater unit



Adjust the heater (mounted in the procedure (3)) to sit right at the center of the objectives. In this procedure, installing the heater manipulator in a horizontal position allows the heater to move in each axis direction only in the microscopic field of view making it easy to adjust the heater position.

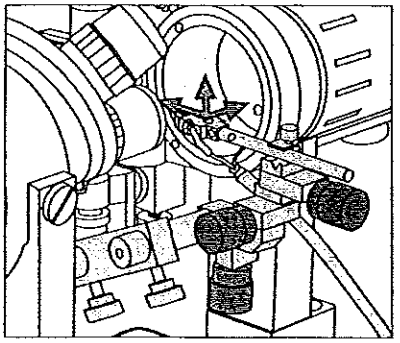
5. Operation

(1) To Set the Heater and Micropipette



①To Turn the Power Switch On

Set the heater adjustment knob and the light adjustment knob on the opposite side to the minimum (0) respectively, and turn the power switch on. When the power switch is turned on, the power indicator (LED) lamp lights up.

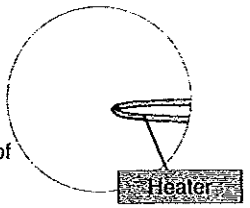


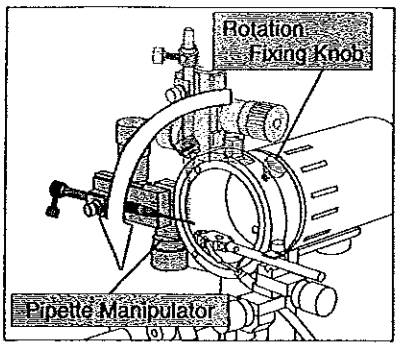
②To Put the Heater into Focus

Set the objectives of the microscope to a low magnification (5x) and turn the light adjustment knob to adjust it to an appropriate brightness.

Operating the heater manipulator in three axes, bring the heater into focus in the center of the field of view.

Conceptual illustration of microscopic field of view

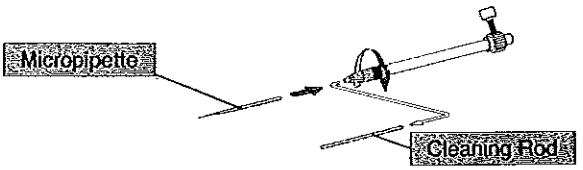


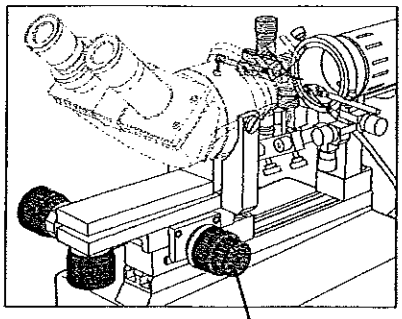


③To Set a Micropipette

Remove the cleaning rod and set a micropipette in the pipette holder.

Loosen the rotation fixing knob and set the pipette manipulator sideways, and then attach the pipette holder.



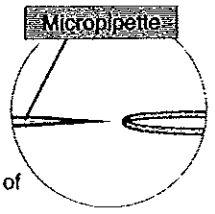


④To Put the Micropipette into Focus

By referring to the heater position (focused in the procedure②), operate the pipette manipulator to bring the tip of a micropipette to a position near the heater. Manipulate the focus adjustment knob to bring the micropipette into focus.

With the micropipette in view in the microscopic field of view, manipulate the swing adjustment knob and the tilt adjustment knob to bring the micropipette in the center of the field of view. Be careful not to cause damage to the micropipette by contact with the heater.

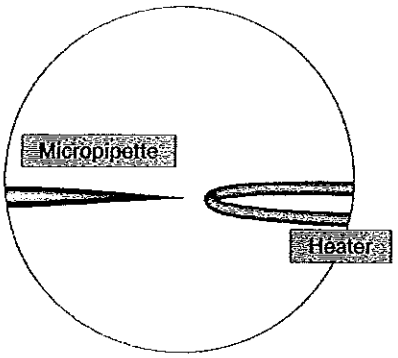
Conceptual illustration of microscopic field of view



(2) To Form a Glass Bead

Before fabricating the micropipette, allow a glass bead to form on the heater.
Forming a glass bead on the heater permits keeping the amount of heat generated by the heater under stable control and permits the fabrication of a thin micropipette by making use of the surface tension of glass.

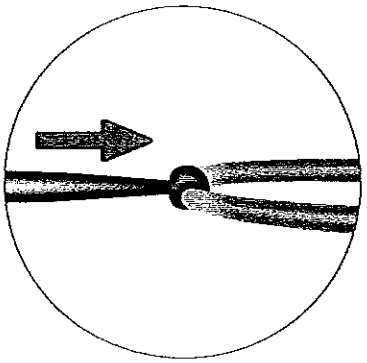
Conceptual illustration of microscopic field of view



①To Set the Heater and Micropipette

By referring to P.12 (1) Setting the Heater and Micropipette, bring the heater and the micropipette in the microscopic field of view.

※In this procedure, use a thin-tipped micropipette.

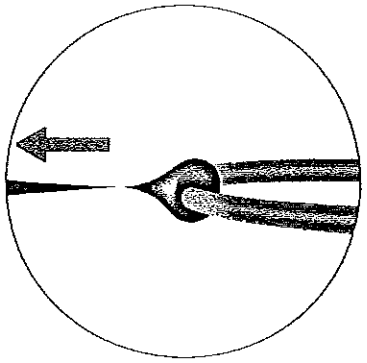


②To Melt the Micropipette on the Heater

Set the heater adjustment knob at a temperature a little higher and depress the foot switch to allow the heater to generate heat.
Operating the pipette manipulator in the X-axis, bring the micropipette into contact with the heater to melt the tip of the micropipette by heat.

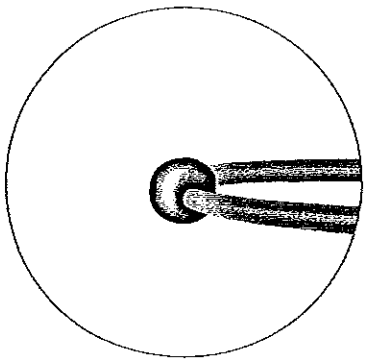
CAUTION!

Too high a temperature will cause the heater to glow white and burn out. To prevent it, adjust the heater adjustment knob at a temperature just a little higher than the temperature that will cause the micropipette to melt.



③To Leave a Glass Bead on the Heater

With the foot switch being depressed, turn the pipette manipulator in the X-axis to release the micropipette and allow a glass bead to be left on the heater. If no glass bead is left on the heater successfully, with the foot switch kept depressed, operate the pipette manipulator in the X-axis to allow the micropipette to contact or release it alternately.



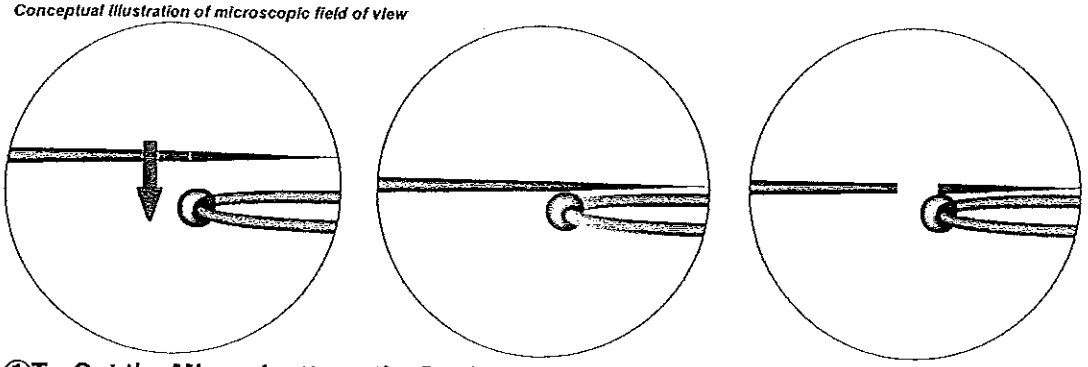
④To Heat the Glass to Make it Spherical

With the heater adjustment knob set at a temperature a little low, depress the foot switch to cause the glass left on the heater to melt and make it spherical.
An out-of-round glass bead, when processed, makes it difficult to conduct heat evenly.

(3) To Produce a Holding Pipette

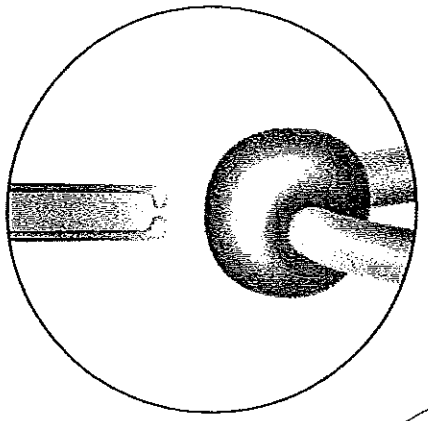
Holding pipettes are used to hold floating egg cells or oocytes. The tip of a micropipette is cut according to the size of a cell to hold, and the cut surface is smoothed out by fire-polishing. Forming an angle on the tip of a micropipette as necessary will enable the angled micropipette to be brought in the microscopic field of view horizontally.

Conceptual illustration of microscopic field of view



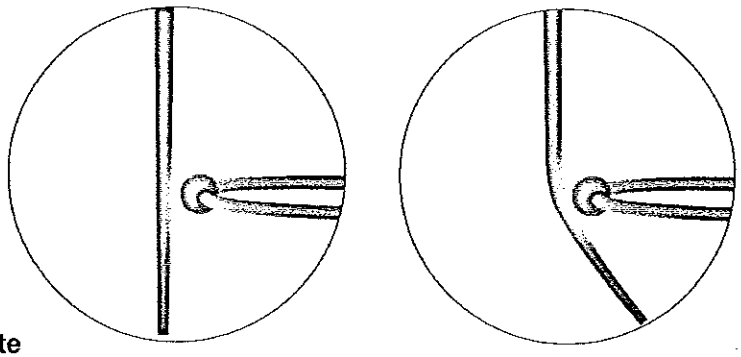
①To Cut the Micropipette at the Desired Thickness

Bring the heater and the micropipette in the microscopic field of view by referring to P.12 (1) Setting the Heater and Micropipette. Manipulate the swing adjustment knob, tilt adjustment knob, and pipette manipulator to bring the micropipette over the glass bead at the desired thickness to be cut. To cut the micropipette, bring the micropipette into contact with the glass bead and set the heater adjustment knob to "0". In this state, while depressing the foot switch, increase the heater output gradually to allow the micropipette to melt into the glass bead. By releasing the foot switch immediately after the micropipette melting into the glass bead, the tip of the micropipette will adhere onto the glass bead allowing it to be cut, as shown in the illustration.



②To Fire-polish the Micropipette

To fire-polish the tip of a micropipette, move the cut tip of the micropipette to a position parallel to the glass bead. Manipulate the heater adjustment knob to increase the output to the extent that the glass bead turns orange, and depress the foot switch to melt the tip of the micropipette and fire-polish it. By keeping it heated, the inside diameter of the micropipette tip will become smaller allowing you to fabricate it to the desired shape.



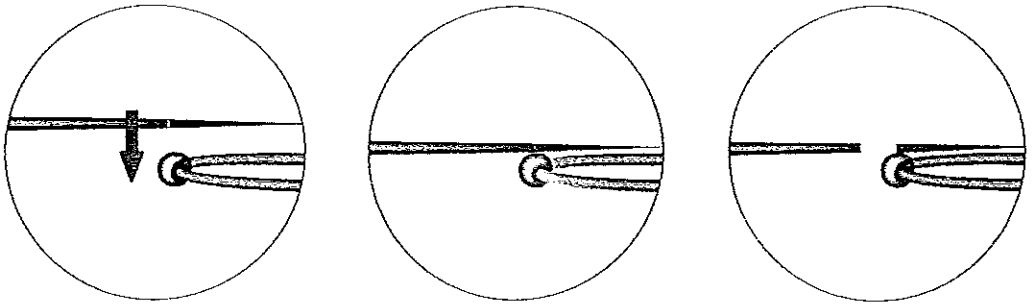
③To Bend the Micropipette

Allow the micropipette to be brought in the microscopic field of view from above by referring to P.12 (1) Setting the Heater and Micropipette ③Setting a micropipette. Set the micropipette in a position where it does not make contact with the glass bead. Manipulate the heater adjustment knob to increase the heater output to the extent that the glass bead turns orange, and depress the foot switch to heat the micropipette on the lateral side and bend it. To bend the micropipette, melt the micropipette on the side that faces the heater by exposing it to radiant heat generated by the heater to allow the micropipette to bend by surface tension of the glass capillary. If the heater output is too high or the micropipette gets thin, the heat will spread over the micropipette inside and out, keeping the micropipette from bending toward the glass bead, or the micropipette may be exposed to the airflow of heat, keeping it from bending toward the glass bead.

(4) To Produce an Injection Pipette

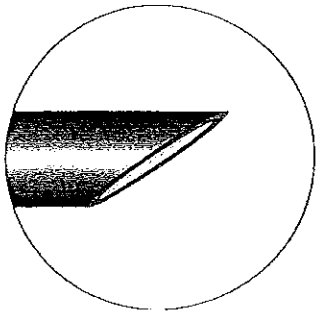
Injection pipettes are used to inject ES cells, sperms, etc. into cells such as oocytes. To allow an injection pipette to inject ES cells, sperms, etc. into elastic cell membranes, a micropipette already sharpened like a bamboo spear by a separately available micropipette grinder needs to be further sharpened. Forming an angle on the tip of a micropipette as necessary will enable the angled micropipette to be brought in the microscopic field of view horizontally.

Conceptual illustration of microscopic field of view



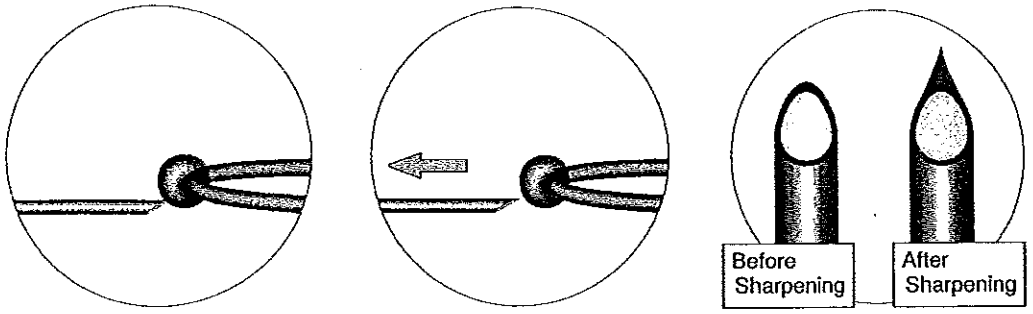
①To Cut the Micropipette at the Desired Thickness

Bring the heater and the micropipette in the microscopic field of view by referring to P.12 (1) Setting the Heater and Micropipette. Manipulate the swing adjustment knob, tilt adjustment knob, and pipette manipulator to bring the micropipette over the glass bead at the desired thickness to be cut. To cut the micropipette, bring the micropipette into contact with the glass bead and set the heater adjustment knob to "0". In this state, while depressing the foot switch, increase the heater output gradually to allow the micropipette to melt into the glass bead. By releasing the foot switch immediately after the micropipette melting into the glass bead, the tip of the micropipette will adhere onto the glass bead allowing it to be cut, as shown in the illustration.



②To Sharpen the Micropipette Tip to Shape like a Bamboo Spear

Using a separately available micropipette grinder Model EG-400/EG-44, grind the tip of the micropipette to shape like a bamboo spear.
For details on grinding a micropipette, refer to the instruction manual of a micropipette grinder to be used.



③To Sharpen the Already Bamboo Spear-shaped Tip Even Sharper

Sharpen the already bamboo spear-shaped micropipette tip even sharper to shape like a horn.
With the bamboo spear-shaped tip facing upward, set the micropipette at 45 degrees diagonally downward left to the glass bead. With the heater output adjusted to the minimum required to melt a glass capillary, depress the foot switch. When the bamboo spear-shaped tip begins to melt into the glass bead, with the foot switch kept depressed, manipulate the swing adjustment knob to release the micropipette from the glass bead. If the heater output is too high, the micropipette tip will melt too much, thus making the procedure difficult. Exercise added care to maintain the most appropriate heater output.

④To Bend the Micropipette

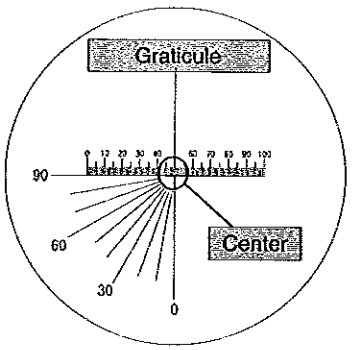
Refer to P.14 (3) Producing a Holding Pipette ③Bending the micropipette.

6. Functions

(1) How to Use the Pipette Manipulator Rotation Mechanism

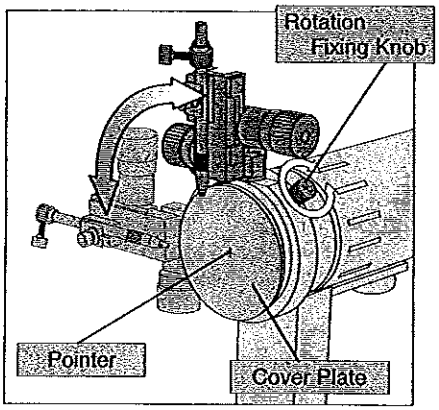
By aligning the pointer on the illuminator with the center of the microscopic field of view, the micropipette can be brought in the field of view in a lateral direction as well as in a vertical direction without causing the micropipette tip to go out of the field of view. In cutting and bending, a micropipette often needs to be put in a lateral position or in a vertical position. Use the following procedure as necessary.

Conceptual illustration of microscopic field of view



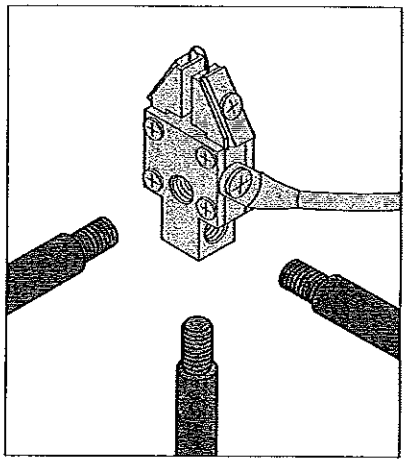
(1) Without setting a micropipette, focus the microscope on the cover plate of the illuminator.
(2) Manipulate the swing adjustment knob and the tilt adjustment knob to align the pointer in the center of the cover plate with the center (50 on the graticule) of the microscopic field of view.
(3) Manipulate the focus adjustment knob to bring the microscope unit back into position. Then, set a micropipette and bring it in the field of view to focus on it.

※Please note that manipulating the swing adjustment knob and the tilt adjustment knob for setting a micropipette will cause the rotational axis of a micropipette to go out of alignment and be moved off center in the microscopic field of view by the amount the micropipette was moved.



(4) By loosening the rotation fixing knob to allow the pipette manipulator to rotate freely, the tip of the micropipette can be rotated with the center of the microscopic field of view as its axis without causing the micropipette to go out of the field of view.

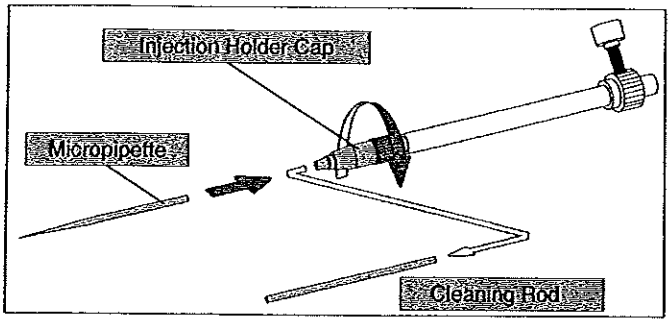
(2) To Reposition the Heater



The heater unit permits repositioning other than the factory-set position.
To reposition the heater, remove the mounting rod and reattach it through the most desired mounting hole. Depending on the heater condition, it can be situated at a low position, which may disable the heater from being brought in the field of view. In that case, allow the mounting block of the heater manipulator to tilt upwardly.

(3) How to Use the Pipette Holder

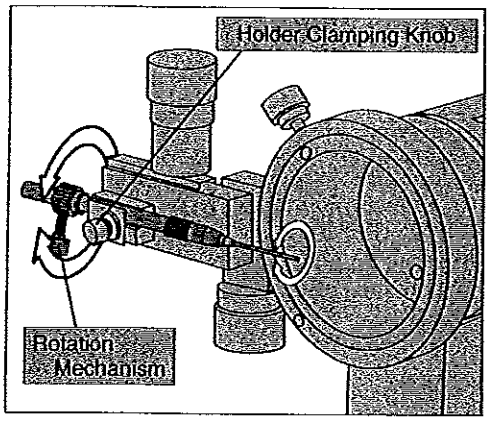
The pipette holder that comes with this instrument incorporates the rotation mechanism which permits changing the orientation of a bamboo spear-shaped micropipette. The supplied pipette holder is intended specifically for use with ϕ1mm micropipettes. To use a micropipette of different outer diameter, a suitable pipette holder is separately required. As the supplied pipette holder is similar to injection holders manufactured and sold by Narishige, you can use one of such injection holders you have on hand in order to use a micropipette other than ϕ1mm.



①To Set a Micropipette

Before setting a micropipette, remove the factory-installed cleaning rod by loosening the injection holder cap. To fix a micropipette, tighten the injection holder cap securely.

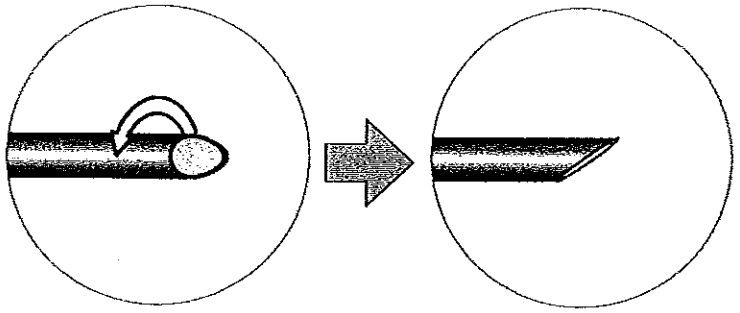
※Keep the cleaning rod in a safe place. The cleaning rod is used to push out pieces of broken glass capillaries clogged in the pipette holder.



②How to Use the Pipette Rotation Mechanism

By making use of the rotation mechanism incorporated in the pipette holder, the pipette holder can be rotated concurrently with the micropipette. With the holder clamping knob tightened to the extent that the pipette holder won't wobble, turning the rotation mechanism will permit changing the orientation of a bamboo spear-shaped micropipette toward the desired direction.

Conceptual illustration of microscopic field of view



③To Clean the Pipette Holder

In the event that the pipette holder is clogged with pieces of broken glass capillaries, put the supplied cleaning rod into the front end of the pipette holder to push the broken pieces of glass out of the back end.