

## Setup of a Micromanipulation System for ICSI -2

This second issue of Setup of a Micromanipulation System for ICSI is about techniques of pipette adjustment under a microscope.

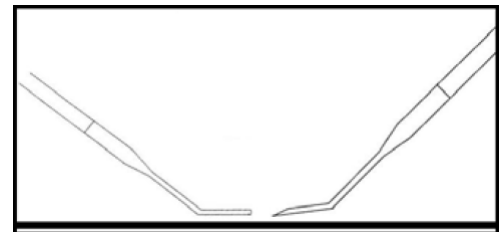
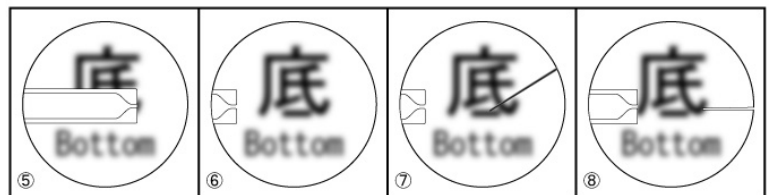
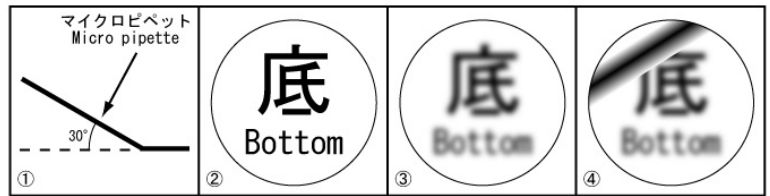
### Secure Method for Arranging Pipettes under a Microscope

Basically you use only Y-axis movement of coarse manipulators and a focus knob of microscope to find and arrange pipettes in the field of view of microscope.

If the Z-axis of manipulators is used carelessly, you can hit a pipette on the microscope stage at any time, breaking the pipette.

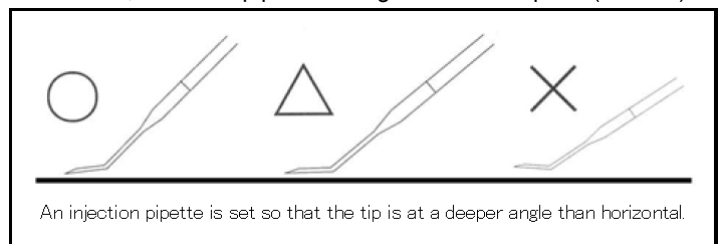
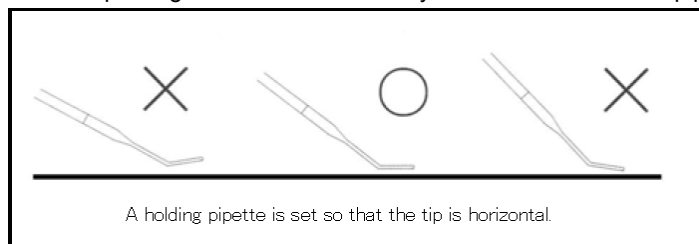
Therefore, use the Z-axis after locating the two pipettes in the field of view and when only evening their vertical levels.

After completing the adjustments of the pipettes, a petri dish is put on the microscope stage. An objective lens of 10x or lower is used during this adjustment.



**(6) Complete. Horizontal image.**

- (1) Start with a holding pipette that is larger and stronger than an injection pipette. The pipette tip is bent about 30 degrees (See Web News No.3). Please confirm that the injection holder is set about 30 degrees (See ① in the above drawings). When adjusting the angle of an injection holder, please use the tilt knob of the universal joint.
- (2) Get the stage surface into focus (See ②) and move the focus a little higher (See ③). Use a coarse manipulator to bring the holding pipette briefly to the optical axis before looking into the microscope. Bring the pipette tip to the opposite side across the optical axis.
- (3) Move the Y-axis of the coarse manipulator. When the pipette is brought into the optical axis, a shadow of the pipette can be seen in the field of view. In case the pipette is found angled (See ④), rotate the knob (HIR) of the injection holder so as to bring it to the right angle. In case you do not find a shadow of the pipette, move the focus of the microscope and repeat the procedure. Finally, bring the pipette into focus using the focus knob of the microscope (See ⑤). This is for holding pipettes.
- (4) Move the holding pipette to the left boundary of the field of view using the X-axis of the coarse manipulator (See ⑥). If the holding pipette is close to the microscope stage, move the pipette upward and bring it into focus. An injection pipette is bent 30 degrees and is used to damage a sperm's tail so as to immobilize a sperm. For the immobilizing procedure, set an injection pipette at a slightly deeper angle than horizontal. As with the holding pipette, there is no need to look into the microscope at this point. However, bring the injection pipette close to the tip of the holding pipette.
- (5) Look into the microscope and move the Y-axis of the coarse manipulator to find a shadow of the injection pipette (See ⑦). In case the shadow of the injection pipette is not found, advance the injection pipette using the X-axis of the coarse manipulator. Move the Y-axis of the coarse manipulator to repeat the process. If this does not help, change the focus level and repeat the procedure again. After locating the injection pipette, bring it to a right angle using HIR and bring the pipette tip into focus.
- (6) Check the vertical position between the holding and injection pipettes using the focus of the microscope. If the injection pipette is found in a higher position than the holding pipette, slightly move the focus down and then move the injection pipette down to get it into focus. Repeat this procedure until you get the injection pipette even with the holding pipette. In this way, hitting a pipette on the microscope stage is eliminated. When you have found the two pipettes in focus, the initial pipette arrangement is complete (See ⑧).



- (7) Move the two pipettes upward using the Z-axis of the coarse manipulators and place a petri dish on the microscope stage. Go back to (6) and start again with the holding pipette so as to get the two pipettes to reach the bottom of the petri dish.

**If you have any questions or need further information, please contact us.**