

# Micro-Biopsy System

## FBS-1000

-- User Manual --



**FBS-1000 is a fully automated microdissection collection system for scientific research. It can be configured on any inverted microscope. FBS-1000 can collect many microdissections from various biological as well as medical specimens repeatedly and automatically over observing the microscope images. The collected microdissections can be used for analyzing site specific gene and protein expressions to understand the biological characteristics of specimens. (Research use only.)**

# Micro-biopsy system (FBS-1000)

## User Manual

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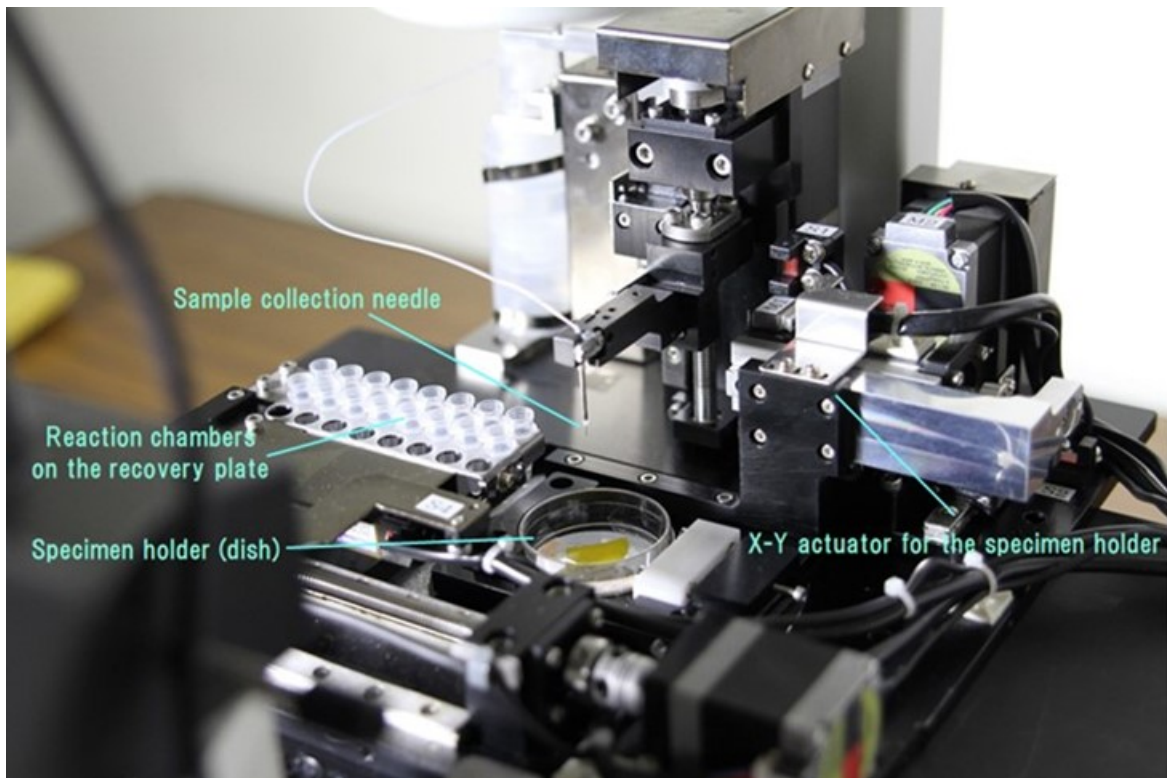
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# 1. Outline and specification of FBS-1000

## 1.1. Outline of FBS-1000

FBS-1000 consists of a mechanical unit, a control circuit unit, CPU, and a microscope camera. The mechanical unit is composed of a Z axis actuator to move a collection needle, an X-Y actuator to move a specimen holder and an X-Y actuator to move a recovery plate to recover a microdissection into a reaction chamber on the recovery plate. They are fixed on a base plate which is set on an inverted microscope so as that the collection needle is placed on the lens axis of the microscope.

A microscope image can be captured with a 5M pixel microscope camera mounted on the microscope. The image can be monitored on the PC display. We put position markers on the spots where we want to take microdissections from. The coordinates of the spots are transformed to the X-Y actuator coordinates, which enables to move the specimen holder for the spots to be under the collection needle by turns. A microdissection is produced from the specimen by punching it with the collection needle. The microdissection is ejected from the needle with solution into a reaction chamber on the recovery plate. All the processes are carried out automatically.



## 1.2 Specifications

- FBS-1000 includes a mechanical unit having three actuators, a control unit, a CMOS microscope camera (option), and a computer (option, should be operated by win10)
- Microdissections are automatically collected and recovered in reaction chambers by determining the target points over watching microscope images.
- The collection of a microdissection is carried out repeatedly in a short time by using a hollow punching needle of stainless steel (collection needle) which is washed before every collection automatically.
- Two types of collection needles can be used. Their inner diameters are 0.1mm and 0.18mm, respectively.
- The time required for one collection is about 8sec. (8sec/collection)
- The positioning accuracy for collection points on a specimen is about 0.01mm.
- Weight : 4.8kg (mechanical unit), 3.2kg (control unit)
- Size (H x W x D mm) : 130 x 245 x 245 (mechanical unit), 235 x 105 x 249 (control unit)

## 1.3 Accessories

Collection needle 1(0.1mm i.d.) : 3 pieces

Collection needle 2(0.18mm i.d.) : 3 pieces

Collection needle holders for needle 1 and 2 : one unit for each (total 2 units)

Tools: pin vise(1piece), hex wrench (10 pieces)

Dish : 3 pieces

## 1.4 Tubs and buttons appearing in PC display

Fig. 1-2 appears on the PC display by turn on the system. We can put position markers at any spot on the display by putting the cursor at the spot coupled with left clicking of the mouse after pressing the arrow button followed by pushing free button. How to put position marker at the spots to be collected will be precisely stated in the section 4.6. By pushing the camera button, a microscope image appears on the display. Usually lens 1 is selected. The calibration of the coordinate transform matrix from the microscope coordinates to the coordinates of X-Y actuator for the specimen holder is carried out by using a microscope image obtained with lens 1. If you want to use the other objective lens, you have to make the transformation matrix with the lens prior to the collection operation. A center cross line is frequently convenient for the calibration, which appears by pushing the view tub.

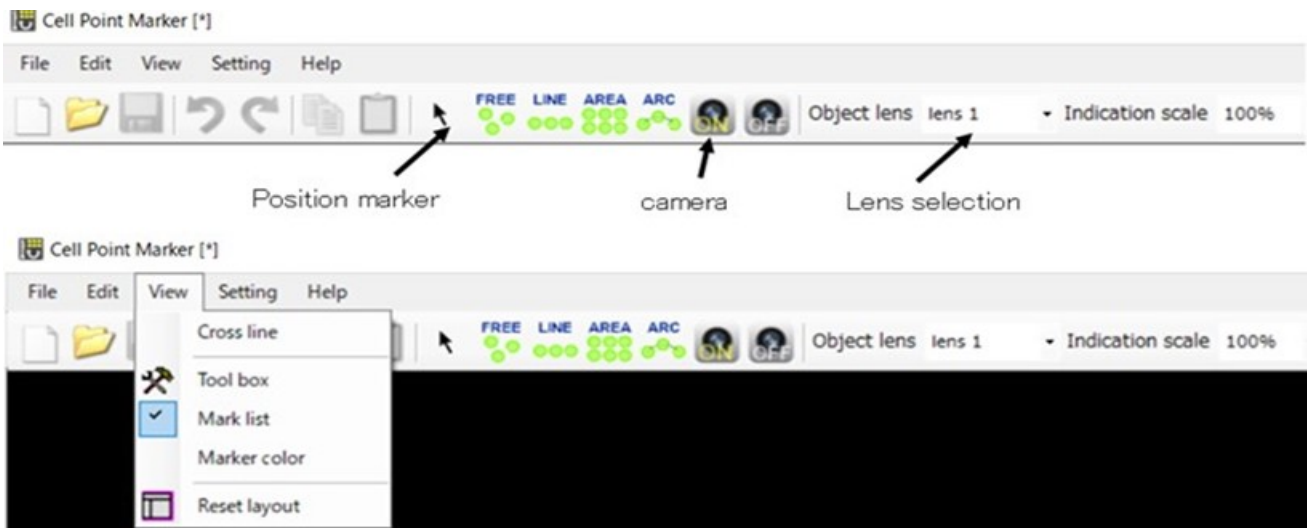


fig. 1-2

A control panel appears together with the image display when the operation software started. Four tabs, “Observation”, “Sampling”, “Setting”, and “Calibration” are on the top of the control panel. Initially, the “Calibration” tab is opened. There are many buttons on the tab as explained in figure 1-3. The tab



is used to select COM port for Bluetooth.

Initialize: initialization of the mechanical unit  
Light: illumination ring light attached on the objective lens

XY: X and Y coordinate control of the specimen holder  
The moving speed can be changed from “setting” tub.

Z: Z coordinate control of a collection needle.  
The coordinate of the needle tip is displayed on the window/  
The moving speed can be changed from “setting” tub.  
The lower limit of the collection needle can be set by pushing “Set to Lower Limit” button at the value displayed on the window.  
The finger button is used to get a micro-dissection from the marked spot on the specimen figure and recover it into the recovery bottle marked yellow..

The recovery bottles are displayed.  
The recovering is carried out into the bottle marked yellow

**Move:** move the recovering plate so as to put the yellow bottle to be under the collection needle.  
**Return:** return the recovering plate to the original position.  
**Solution:** eject solution from the collection needle.  
**Sample position:** draw out the specimen holder so as to put a dish with a specimen on the specimen holder easily.  
**Center:** return the specimen holder to the original position (center position).

fig.1-3

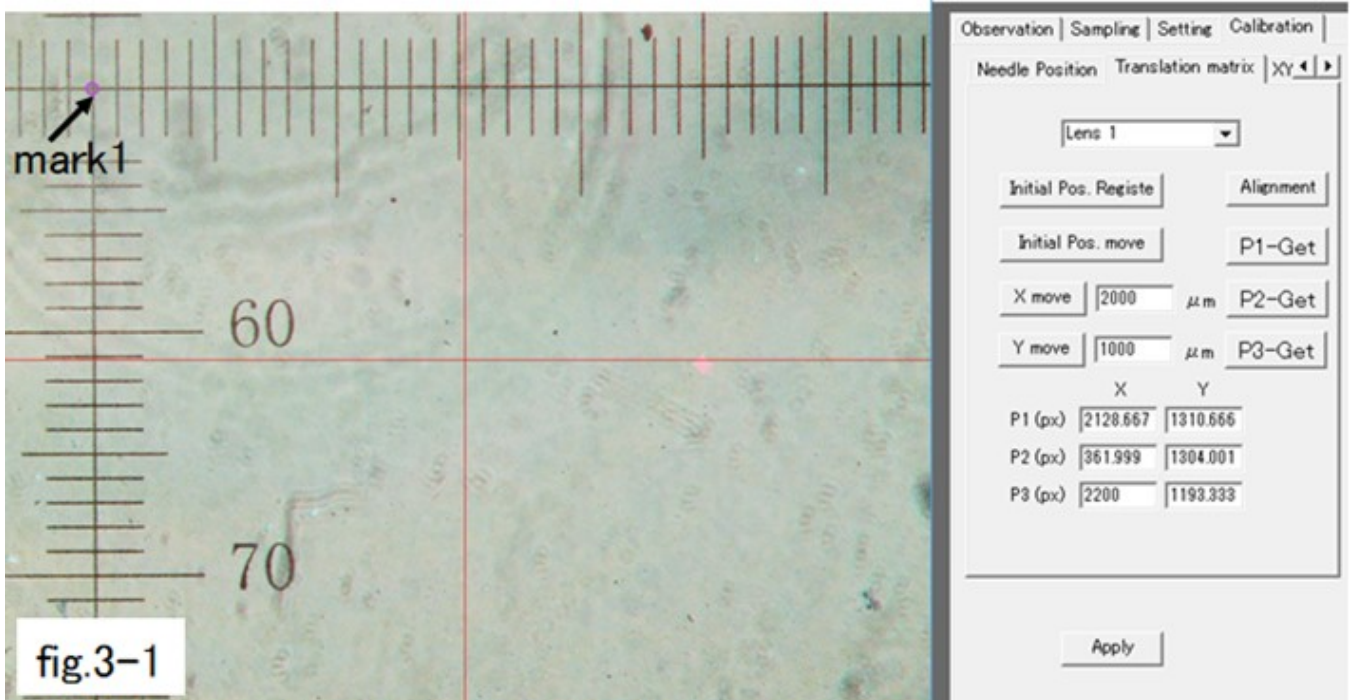
## **2. Mounting the mechanical unit on an inverted microscope**

The base plate of the mechanical unit consists of two plates. The lower plate is fixed on the sample placing plate of the inverted microscope by using screws being used for fixing a sample tray. Then the upper plate is overlaid on the lower plate to be fixed with screws. A microscope camera is mounted on the inverted microscope as well. A specimen image is obtained with the camera to be displayed on PC. The vertical line of the camera should be adjusted to be parallel with the X axis of the X-Y actuator for the specimen holder.

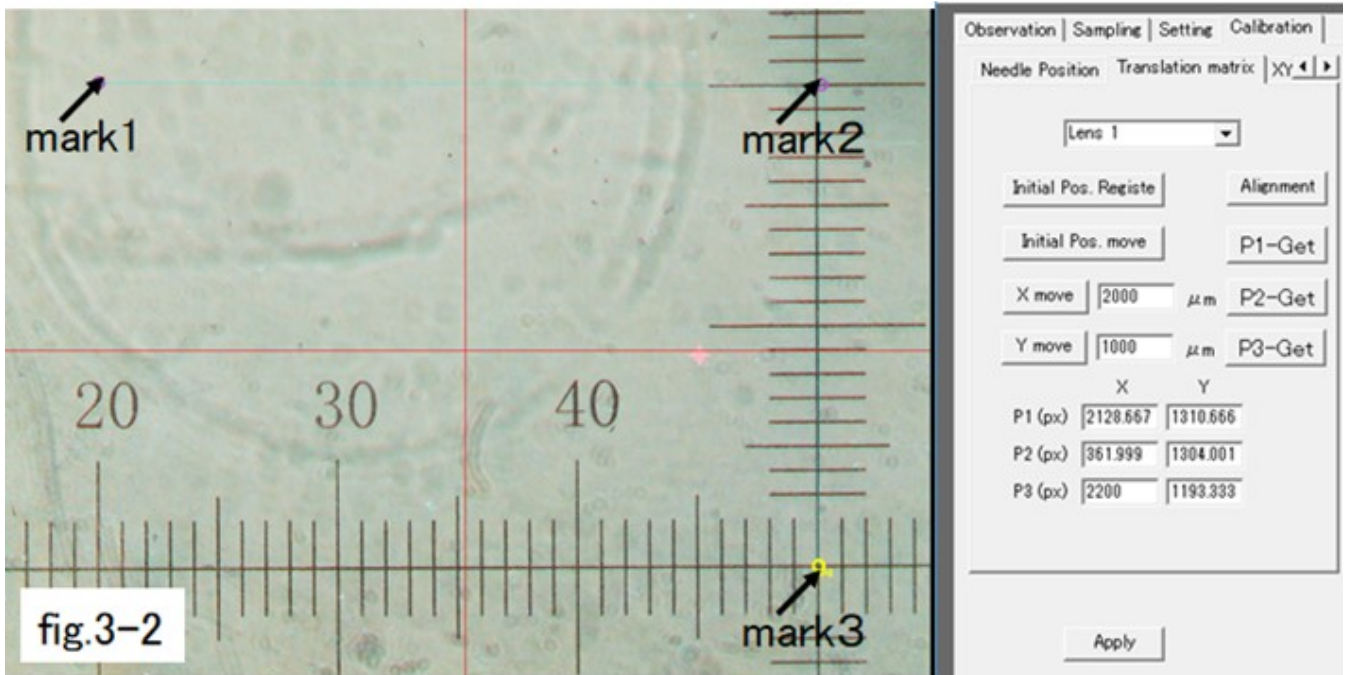
- 1) Push the initialize button.
- 2) Select lens 1 and indication scale of 40%.
- 3) Put a small mark on a dish surface and place it on the specimen holder.
- 4) Click “view” tub and push the cross line button. Move the specimen holder so as that the mark is on the cross line.
- 5) Move the specimen holder along X axis to check whether the Y coordinate of the mark is still on the cross line. If it is shifted from the cross line, rotate the camera. Repeat this processes until the mark stays on the cross line after moving the specimen holder along X axis.

## **3. Calibration of the coordinate transformation matrix**

- 1) Open “Calibration” tub. Push “Translation matrix” button and confirm that lens 1 is indicated in the window.
- 2) Push “arrow” button followed by pushing “free” button. Now you can create position markers on the display.
- 3) Any spot in an image can be used as a marker for the calibration. Here, the cross point of a scale (which is placed on the dish for the calibration) is used as the marker spot. Move the cross point (marker spot) to the upper left of the display. Put a position marker at the cross point. This is the mark 1. (fig.3-1).



- 4) The mark 1 is the initial position and push “initial pos. register” button.
- 5) Push “Initial Pos. move” button followed by putting 3000 in the X move window and then pushing “X move” button. Then the specimen holder moves along X axis by 3mm.
- 6) Put a position marker (mark 2) at the cross point (marker spot).
- 7) Putting 2000 at the Y move window followed by pushing “Y move” button. Then the specimen holder moves along Y axis by 2mm.
- 8) Put a position marker (mark 3) at the cross point (marker spot). (fig. 3-2)
- 9) Register these three mark positions as the reference positions for the calibration by pushing buttons of P1-get, P2-get, and P3-get by turns.
- 10) Push Apply button at the bottom to complete the calibration.
- 11) Return to “Observation” tab.



The calibration should be done for each lens because the transform matrix depends on the lens magnification.

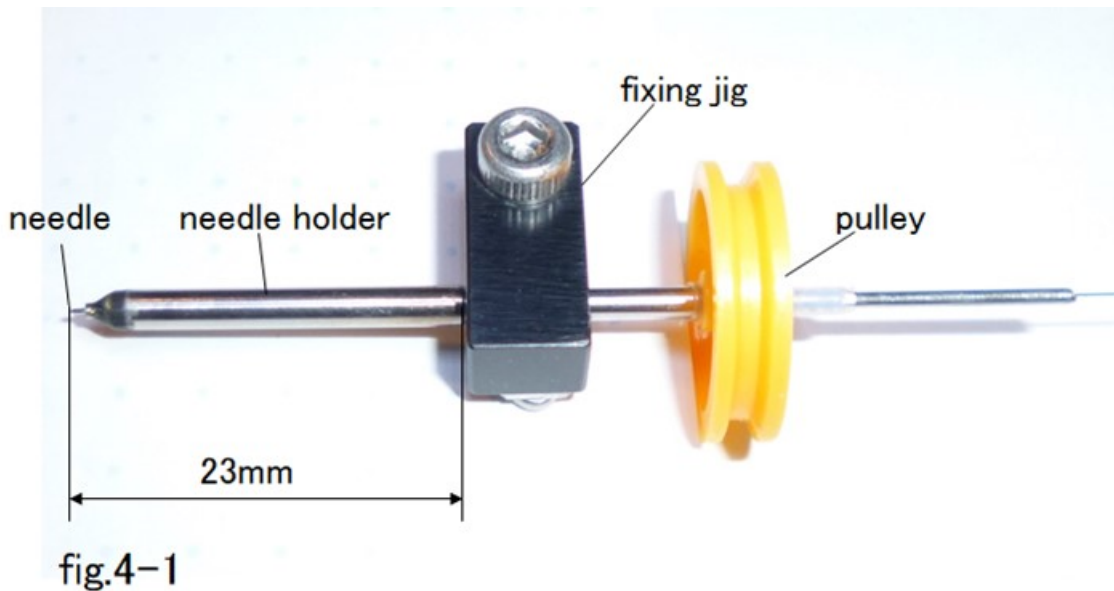
## 4. Collection of microdissections

Turn on the control unit and CPU. As figures 1-2 and 1-3 appear, push the camera and the light buttons. Then push “initialize” button to initialize the mechanical unit.



### 4.1 Collection needle

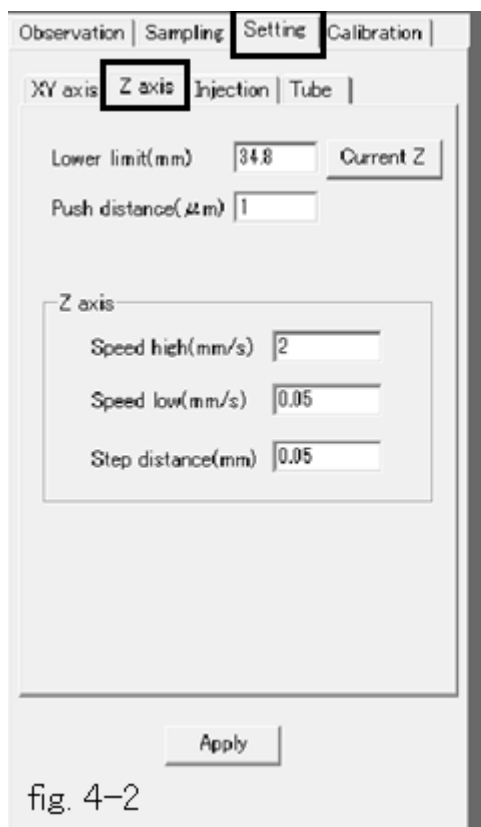
A collection needle is made of stainless pipe with sharpened edges. It is held in the needle holder. The needle holder with the collection needle is attached to a fixing jig for fixing the needle holder on the moving arm operated by the Z axis actuator. The distance between the tip of the collection needle and the fixing jig should be 23mm. The needle holder in figure 4-1 is an example of a spinning needle holder with a pulley.





#### 4.2 Determination of the lower limit for a needle position and placing a needle marker where a microdissection is collected.

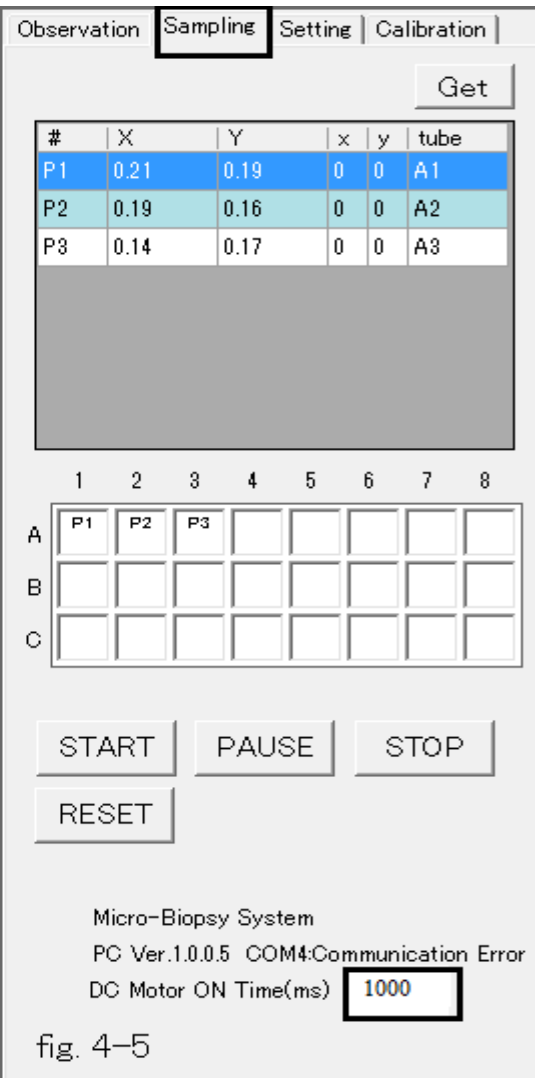
- 1) A dish or a slide glass with a specimen is placed on the specimen holder. It will be done easily by pushing “sampling position” button on “Observation” tub to draw out the specimen holder. After loading the dish, you push “center” button to return the specimen holder to the center position on the objective lens.
- 2) The focus point of the inverted microscope is adjusted to the bottom of the specimen.
- 3) Remove the dish and move down the collection needle so as to place the needle tip at the focus point by operating the Z axis actuator.
- 4) Move down the needle tip by 0.2mm further and push “set to lower limit” button. (Instead of using “set to lower limit” button, you can use “Setting” tub. You open “Setting” tub followed by opening “Z axis” tub. Put the lower limit value on the lower limit window. Then push “Apply” button.) (fig. 4-2)
- 5) After setting the lower limit, push  to pull up the collection needle to the standby position.
- 6) Push  button to pull down the collection needle to the lower limit and confirm the center position of the collection needle is unchanged. This needle position confirmation process should be repeated.
- 7) Put the needle marker on the center of the needle tip image. The needle marker position can be moved by left cricking the needle marker followed by dragging it to the position you want.



### 4.3 Determination of the lower limit for the needle tip in recovering a microdissection into a reaction chamber on a recovery plate.

Open “Observation” tub and select “A1” button. The collection needle is at the standby position. At first you should confirm that the needle tip does not touch chambers on the recovery plate.

- 1) Push “move” button to move the recovery chamber “A1” to the position under the needle.
- 2) Pull down the needle tip to the position 5mm above the bottom of the recovery chamber.
- 3) Click “Setting” tub followed by pushing “Tube” button. Then push “Current Z” button.
- 4) Push “Apply” button. Then return to “Observation” tub.
- 5) Pull up the needle to the standby position.
- 6) Push “Return” button to return the recovery plate to the standby position.



#### 4.4. Control of solution ejecting from a needle

The ejection of solution is controlled by the ejection time.

- 1) Click "Setting" tab followed by pushing "Injection" button. (fig,4-4)
- 2) Confirm that the check mark is on "Enabled" window.
- 3) Put 0.1 on the "Time" window. If you want more solution you can put a larger value.
- 4) Push "Apply" button. Return to "Observation" tab followed by confirming the ejected solution amount by pushing "Solution" button.

#### 4.5. Rotating time of the needle.



Open "Sampling" tab. Put 1000(ms) on the "DC Motor ON Time" window. The collection needle will rotate for 1000ms after arriving at the lower limit position during collection processes and then return to the standby position.


## 4.6 Collection of microdissections

There are four possible modes for collecting microdissections.

### 4.6.1. Easy collection of microdissections on the “Observation” tub.


“Observation” tub is used to observe a specimen for determining the way for collection. However, easy collection is possible on the tub.

- 1) Push the position marker button  followed by pushing “free” button. (Now you can place a position marker at any position you want.)
- 2) Put a position marker at the spot you want to get a microdissection.
- 3) Press the collection button .
- 4) The collection of a microdissection from the marked spot is carried out followed by recovering it into a recovery chamber marked yellow color.

As the possible recovery chamber is shifted to the next one, the automated collection can be repeated by putting the position marker followed by pushing the collection button  one by one.


### 4.6.2. Random collection of microdissections.

In this mode, you open “Observation” or “Sampling” tub. Firstly, select all the spots where microdissections are collected from. Then the automated collection of all the microdissections is carried out.

- 1) Push the position marker button  followed by pushing “free” button.
- 2) Put position markers at any spot you want. If you want to delete a placed position marker, you do left click on the marker to change the marker color to yellow and then push the delete button.
- 3) If you want to shift the position of a marker, you do left click on the marker and then drag the marker to the spot you want to place it.
- 4) Click “Sampling” tub.
- 5) Push “Get” button to transfer the coordinate information on the spots to CPU.
- 6) Push “Start” button for collecting microdissections automatically followed by recovering then into the recovery chambers one by one.
- 7) The collection and the recovery is carried out automatically. After collecting all the microdissections, the collection needle and the recovery plate return to the standby positions.


### 4.6.3. Collection of microdissections along a line

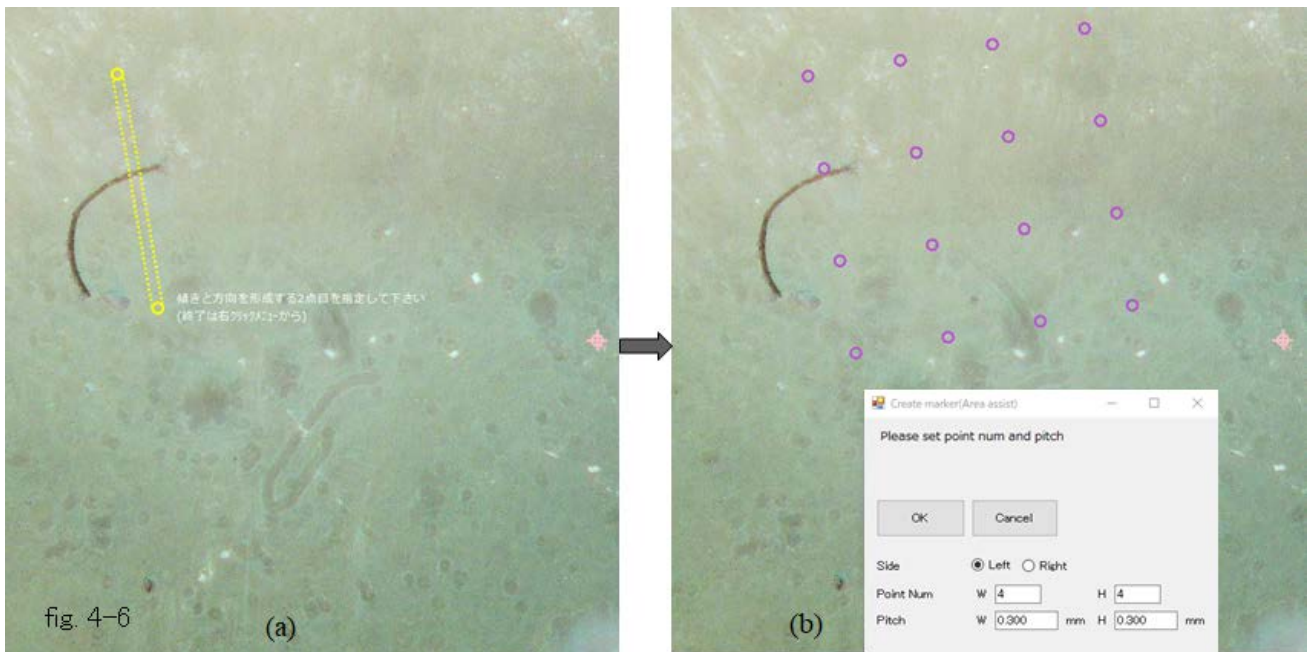
Open “Observation” or “Sampling” tube. Place the first position marker on the spot from where a microdissection is collected. Place the second position marker to determine the slope of the line along which the collection spots are selected.

- 1) Push the position marker button  followed by pushing “line” button.
- 2) Put the first marker by left click of the mouse on the spot from which you want to collect a microdissection.
- 3) Put the second marker to determine the slope of the collection line. Then a window open for determining the number of microdissections and their pitch.
- 4) Put the numbers of the microdissections and their pitch.
- 5) Push “OK” button. Then the position markers are displayed at the determined spots.
- 6) If you want to shift a marker position, make a left click on the position marker. Then the color of the position marker changed to yellow. You can drag the position marker to the desired position.
- 7) Click “Sampling” tub for getting the coordinate information of the marked spots by pushing “Get” button.
- 8) Push “Start” button to start the collection followed by getting the microdissections in recovery chambers.

### 4.6.4. Collection of microdissections from an area determined with a matrix


The collection of microdissections from the spots in an area determined with a matrix is carried out. The determination of the matrix positions and shapes are determined on “Observation” or “Sampling” tub.

- 1) Push the position marker button  followed by pushing “area” button.
- 2) Put the first and the second position markers by clicking the mouse to make a slope along which the matrix is produced.
- 3) A window appears to determine the numbers of spots along W (width) and H (height) as well as their pitches along W and H. Put the numbers of the collection spots and their pitches.
- 4) Push “OK” button.
- 5) Click “Sampling” tub and push “Get” button to import the coordinate information into PC.
- 6) Push “START” button to start the collection and recovery of microdissections.



#### 4.6.5. Collection of microdissections along a circle

The collection of many microdissections is carried out along a circle at a determined pitch.

- 1) Push the position marker button  followed by pushing “circle” button.
- 2) Put the first and the second markers on a specimen image by left clicking of the mouse.
- 3) Determine the radius of the circle by moving the mouse.
- 4) Windows appear for setting the number and the pitch of the collection spots.
- 5) Put the number of the collection spots and their pitch.
- 6) Push “OK” button.
- 7) Click “Sampling” tab and push “Get” button to import the coordinate information into PC.
- 8) Push “START” button to start the collection and recovery of microdissections.

## 5. Replacing the collection needle

Two types of collection needle and collection needle holders are prepared. The first one is 8mm long and the inner and outer diameters are 0.1mm and 0.35mm, respectively.

The second one is also 8mm long and its inner and outer diameters are 0.18mm and 0.39mm, respectively. They only fit to their own needle holders.

The needle replacing procedure is as follows.

1) A needle holder with a collection needle is detached from the arm movable along Z axis.

2) Grab the collection needle with a pin vise to pull it out from the needle holder.

Don't use a pair of tweezers to remove the collection needle.


3) Grab a new collection needle with a pin vise to push it into the needle holder.

Push the needle into the needle holder until it stops.

4) Put the needle holder back to the jig and control the jig position so as that the distance between the tip of the needle and the jig is 23mm.

5) Attach the jig together with the needle holder to the arm. Put back the tube connector to the needle holder for supplying solution to the needle.

6) Click "Observation" tab and push "solution" button to confirm the discharge of solution.

7) Push  button to move the needle to the lower limit. Check the position of the center of the needle tip to be at the needle marker. If the needle marker is not at the center of the needle tip, click the needle marker to drag onto the center of the needle tip image.

## 6. Miscellaneous

The present system uses Blue Tooth to communicate the control unit with PC. If the blue tooth signal is shielded by something or noises, please use an RS 232 Serial Cable.

All the moving parts are covered to avoid accidents. Don't remove them. Be careful not to drop small staffs such as screws, which causes malfunction.

Please remove solution in a collection needle when you finished your collection work. While a needle is made of stainless steel, it may be rusted.

Sometimes you may have a trouble not to recover a microdissection in a reaction chamber. It sometimes occurs for a thick specimen. Remove the tube connector from the needle holder, and then insert a cleaning wire into the needle holder. Push out the microdissection clogged in the needle tip with the cleaning wire.