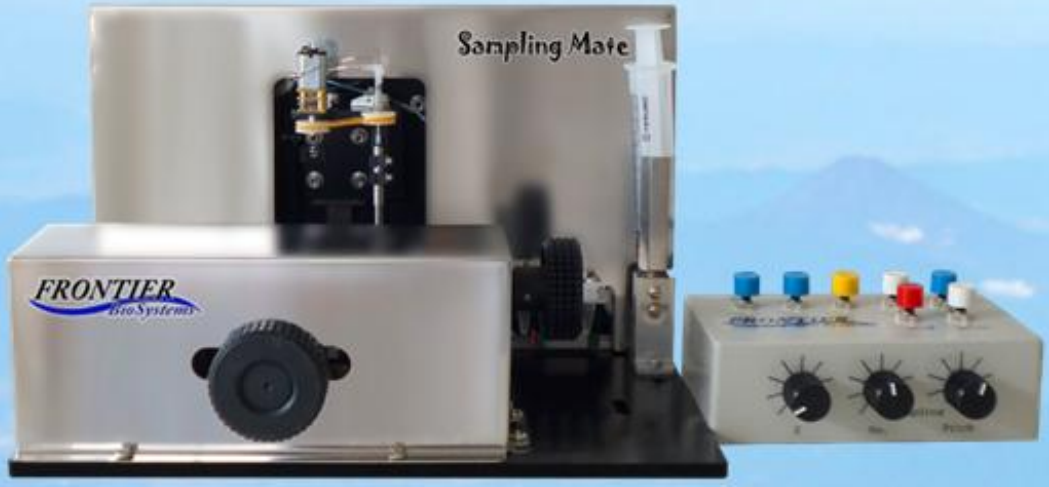
FRONTIER BioSystems

# Micro-Biopsy System

## Sampling Mate




Sampling Mate is an automated micro-biopsy system. It is mounted on a digital microscope. It can be configured on any inverted microscope as well. Sampling Mate can collect many microdissections from various biological and medical specimens automatically. As the sampling process is fully automated, it is not necessary to adjust the sampling parameters. You move the target point to the marker position on a microscope image. Then just push the sampling button. A hollow punching needle goes down to collect a microdissection from the target position by punching. The microdissection is recovered in a collection tube automatically. Then the hollow punching needle is washed for the next sampling. The collected microdissections can be used for spatial analysis of gene and/or protein expressions in tissues

**Frontier BioSystems, Inc.**
Hachioji, Tokyo, Japan

# Micro-Biopsy System

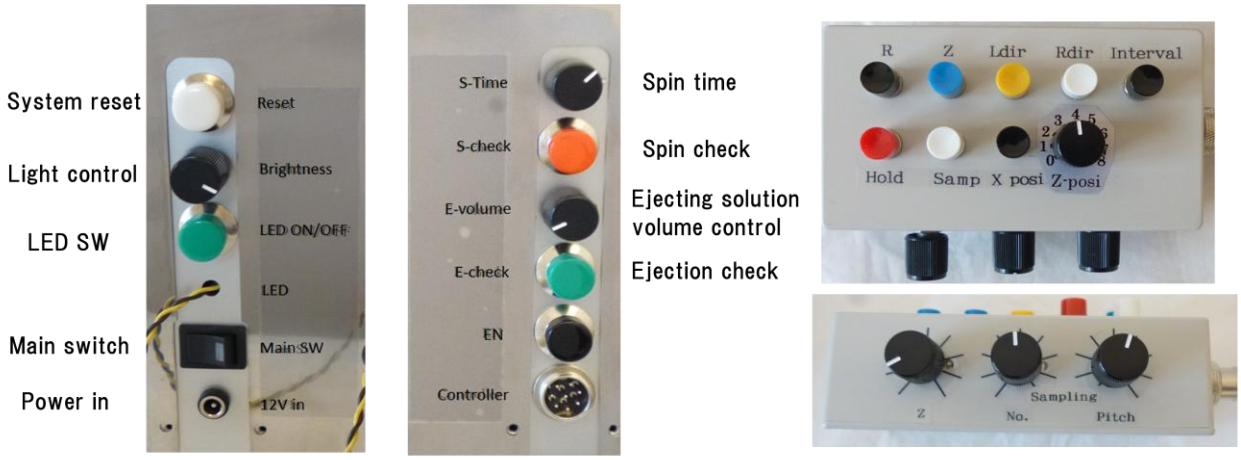
## (Sampling Mate)

# User Manual

## Table of contents

# 1. Setup of Sampling Mate system

## 1.1 Explanation of buttons and knobs




Left side panel  Right side panel  Hand held controller

**Figure 1 Side panel and hand-held controller**

### 1.1.1 Knobs and buttons on the left-side panel

**Reset**: Resetting of the system. The initialization of the system starts by pushing **Reset** button.

**Brightness**: LED ring light intensity adjustment (which is used when the system is mounted on an inverse microscope)

**LED ON/OFF**: not used

**Main SW**: Main switch

### 1.1.2 Knobs and buttons on the right-side panel

**S-Time**: Spin time adjustment

**S-check**: This is used for checking the spin rotation of a hollow punching needle.

**E-volume**: Adjusting the ejection volume of the solution

**E-check**: Ejecting solution volume check

**Controller**: To hand-held controller

### 1.1.3 Upper-side of hand-held controller

**Hold**: A hollow punching needle goes down to a position close to a specimen surface by its first push. The hollow punching needle goes back to the waiting position by the second push.

**Samp**: Start the sampling of a micro-dissection and its recovery in a collection tube.

The sampling process is as follows.

1) A hollow punching needle goes down to touch a specimen. Then it goes down by an extra amount determined with **Z-posi dial**.
2) The hollow punching needle spins to produce a micro-dissection.
3) The micro-dissection is aspirated a little bit to be held in the hollow punching needle.
4) The hollow punching needle goes up and a collection tube on the collection tube tray moves to the position below the hollow punching needle.
5) The tip of the hollow punching needle goes in the collection tube to eject the micro-dissection in the collection tube together with a solution.
6) The hollow punching needle goes up to the waiting position.
7) The collection tube tray moves so as to place a cleaning tube with a cleaning pad beneath the hollow punching needle.
8) The hollow punching needle goes down to stick in the cleaning pad and then spins over ejecting a cleaning solution.
9) The hollow punching needle and the collection tube tray go back to the waiting positions, respectively.

**X-posi**: Move the sample holder along X-axis with a motor.

This is used for correcting the X-position which is frequently shifted from the initial position when the motor is energized. It can move the sample holder with a 5 $\mu$m pitch

**Z-posi**: This is used to determine the pushing power of the hollow punching needle against a specimen. One division of the rotating dial corresponds to 60 $\mu$m extra descent.

**R**: Reset the collection tube tray. A micro-dissection can be recovered in the first PCR tube of 8-strips after the reset.

**Z**: Control a hollow punching needle position for ejecting a micro-dissection into a collection tube. The control process is as follows:

1) Push **Z-button**,
2) the hollow punching needle goes down to the position determined by **Z-dial,**
3) by rotating **Z-dial**, you can change the position.
4) Push **Z-button** again to end the position change.

**Ldir**: This is used to determine the direction of the specimen holder for continuous sampling.

The specimen tray moves in the right direction at first after pushing the button. Then it moves in the left direction. The marker position is frequently

shifted from the target position which should be adjusted with **X-posi button**. This is to avoid the error due to screw Play.

**Rdir**: This is used to determine the direction of the specimen holder for continuous sampling.

The specimen tray moves in the left direction at first after pushing the button. Then it moves in the right direction. The marker position is frequently shifted from the target position which should be adjusted with **X-posi button**. This is to avoid the error due to screw Play.

**Interval**: This is used to check the sampling interval for continuous sampling. After pushing **Interval-button**, the sample holder moves to the left side (or right side) by pushing **LdiR-button** (or **RdiR-button**). The moving amount is determined by **Sampling-Pitch dial**.

### 1.1.4 Front side of the hand-held controller

**Z**: This determines the hollow punching needle position for recovering a micro-dissection in a collection tube.

**Sampling No**: This is used to select the sampling number for continuous sampling. The sampling number is one for the first two dial positions. Then it goes up one by one.

**Sampling Pitch**: This is used to select the sampling interval for continuous sampling. The interval for the first dial position is 0.05mm. Then it increases to 0.1mm, 0.15 ㎜, 0. 2 ㎜,0.25 ㎜, 0.3 ㎜, 0.4 ㎜, and 0.5 ㎜ by rotating Sampling-Pitch dial.)

## 1.2 Setting up Sampling Mate

1) Mounting Sampling Mate on a digital microscope unit

Carefully take Sampling Mate and a digital microscope unit out of the boxes. After removing the packing materials, put Sampling Mate on the digital microscope so that the two guide pins go into the two holes in the base plate of Sampling Mate. Fix it on the digital microscope with screws.

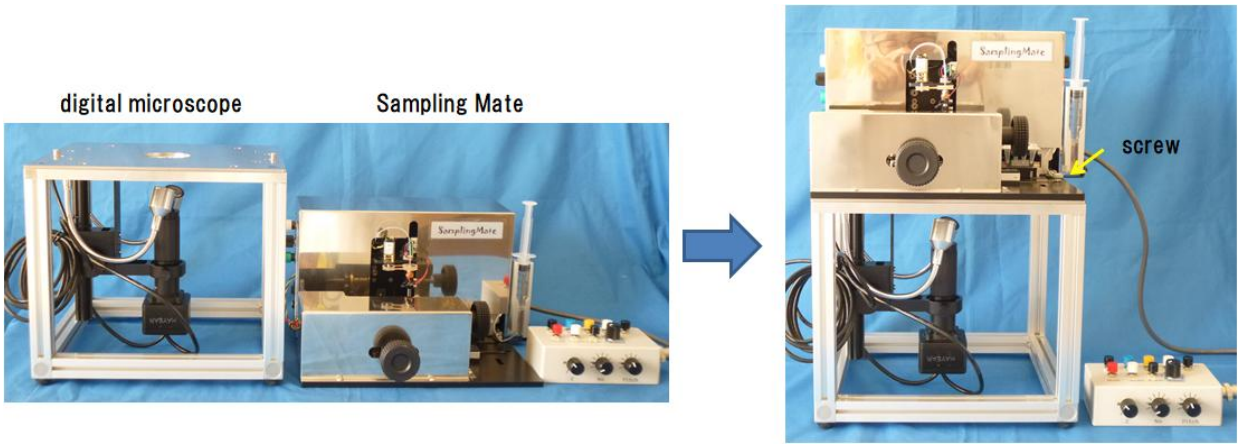


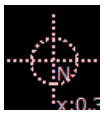

Figure 2 Place Sampling Mateon the digital microscope unit

2) Install the software on your computer. Copy Cell Point Marker program and set up S-Eye program on your computer.

3) Connect the USB of the digital microscope to your computer. Then turn on the irradiation light.

4) Just click Cell Point Marker. Then you will see a black image with a tub as shown in figure 3. Click **ON-tub** to get a microscope image.


Figure 3 The upper tub of Cell Point Marker program

The image magnification can be changed with the indication scale. The needle marker  is shown in the image. It is changed to  by clicking it with a mouse. Now you can move the needle marker to anywhere you like by dragging it with the mouse. So place the marker on the position where the hollow punching needle comes down in the image.

5) By turning on **Main SW**, the system initialization starts. After the initialization, the motors are not energized. You can move the moving arm, the collection tube tray, and the sample holder manually.

6) Adjust the digital microscope lens to focus on the silicon rubber on a dish placed in the sample holder.

7) Push **Hold-button** on the hand-held controller. A hollow punching needle goes down to stop above the silicon rubber sheet. As the tip of the needle can be observed in the microscope image, move the needle marker to the center of the hollow punching needle.

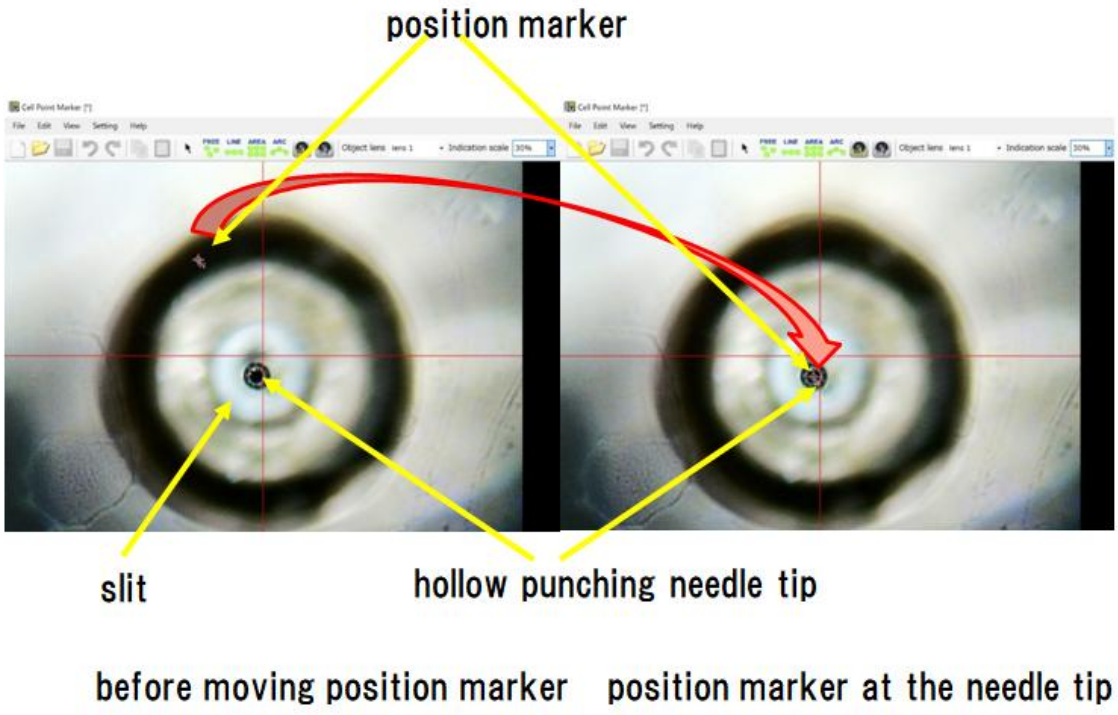



Figure 4 Microscope images of a hollow punching needle

8) Supply a buffer solution in the additional tank which is connected to the solution reservoir with a communicating tube. There is a small hole on the wall of the additional tank. After putting 4ml solution in the additional tank, close the hole with your finger and then push the syringe shaft to remove air in the solution reservoir. Then the solution levels in the solution reservoir and the additional tank become the same.

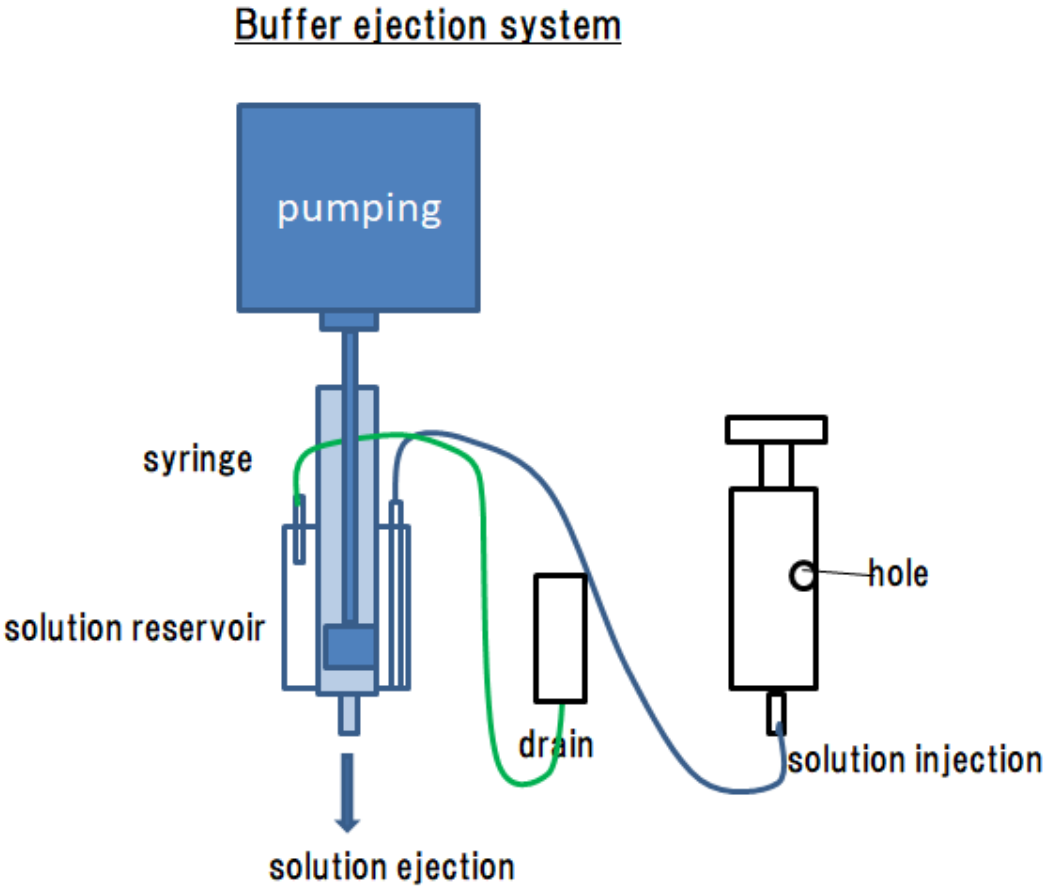



Figure 5　Supply an ejection solution

### 1.3 Preparation for micro-dissection collection

1） Put a piece of silicon rubber sheet of 50 μm on a dish. Then place a specimen to fix on it. The dish is placed on a sample holder as follows.

① Move the collection tube tray to the left side.

② Loosen the screw to fix the dish on the sample holder and move the sample holder to the right side.

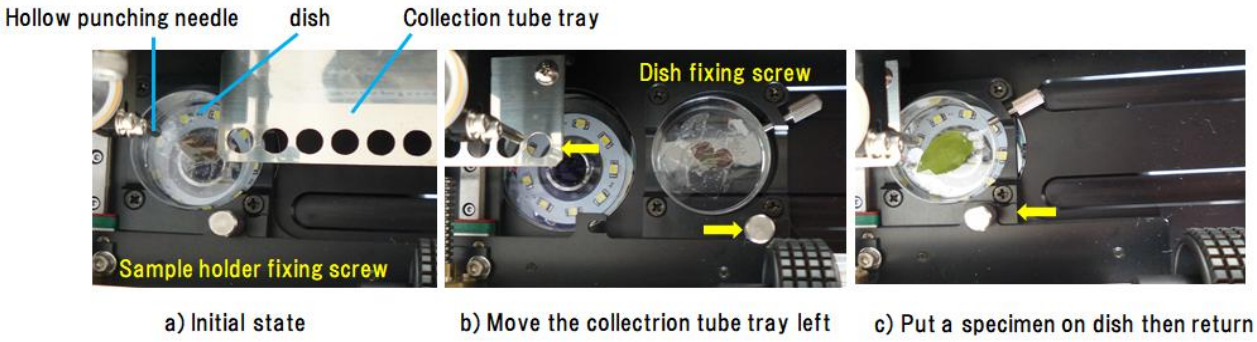



Figure 6. How to place a dish in the sample holder

③ Put a specimen on the dish. Then put it on the sample holder. Confirm that

the dish contacts tightly with the base plate of the system.

④ Return the sample holder to the left side and fix it with a screw. The bottom of the dish should be touched by the quartz plate fixed in the base plate. (The way of using a glass slide instead of a dish is described in 1.12)

⑤ Push R-button to return the collection tube tray to the waiting position. (don't forget to return the collection tube tray to avoid trouble crashing a hollow punching needle with the collection tube tray)

2) Open the view-tub and click the center cross line to show a cross line in the microscope image.

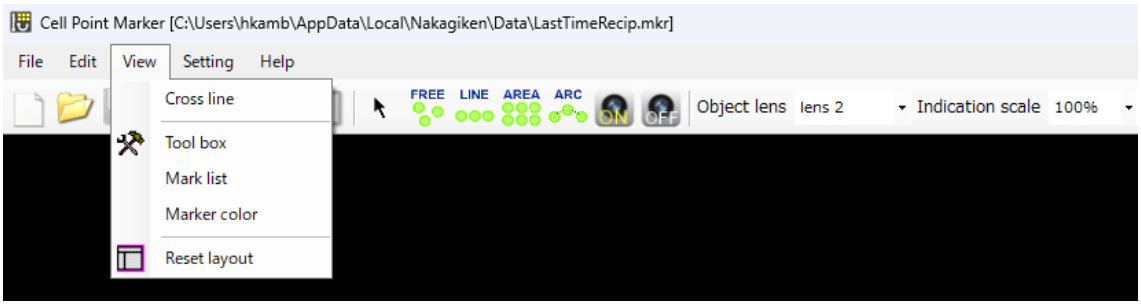



Figure 7. Center cross line

You can skip the items from No.3 to No.6 because they were finished before shipping.

3) Watch any point you like in a microscope image over moving the sample holder manually. If the point moves in parallel to the cross line, it is OK. Otherwise, it is necessary to make the horizontal line of the camera in parallel to X-axis along which the sample holder moves by rotating the camera manually.

4) Put a hollow punching needle unit in the hole at the moving arm and fix it with a screw. Connect the sensing wire to its connector. Lift the pulley a little to confirm the light is on.

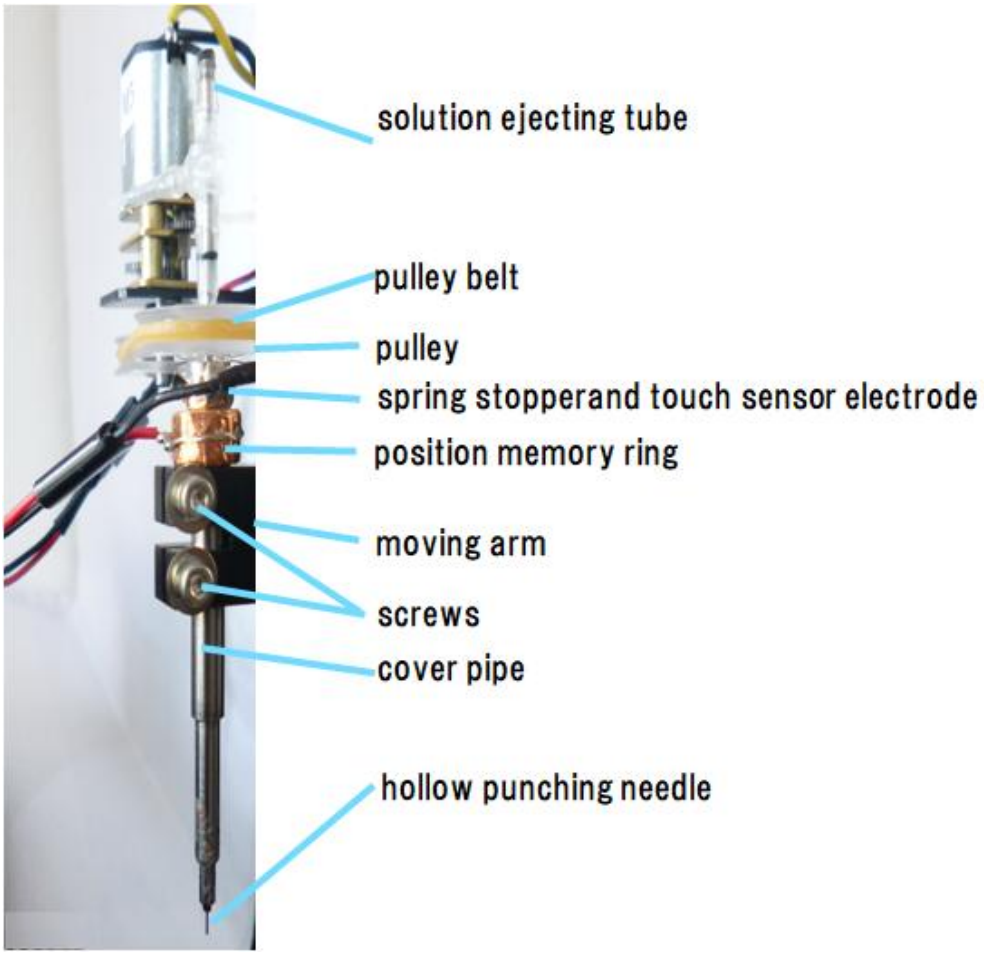


Figure 8 Hollow punching needle unit

5) Put a pulley belt on the pulleys
6) Connect a solution ejection tube to the main shaft pipe.

---

7) Check spin time by pushing **S-check button**. For changing spin time, turn **S-time knob**. Usually, the spin time making 1/4 spin is used.
8) Check the amount of solution used for ejecting a micro-dissection by pushing **E-check button**. The ejection amount of buffer solution can be changed by turning **E-volume knob**.
9) Put one tube with a cleaning pad and a PCR tube 8-strips on the collection tube tray. If you want to check the amount of ejecting solution, place a white sheet instead of the 8-strips tubes on the collection tube tray. Then press **Z-button** to check the needle position for ejecting solution. Move the hollow punching needle tip just above the white paper. When you use the PCR tube 8-srips, the position of a hollow punching needle tip should be in the middle point of the tube.

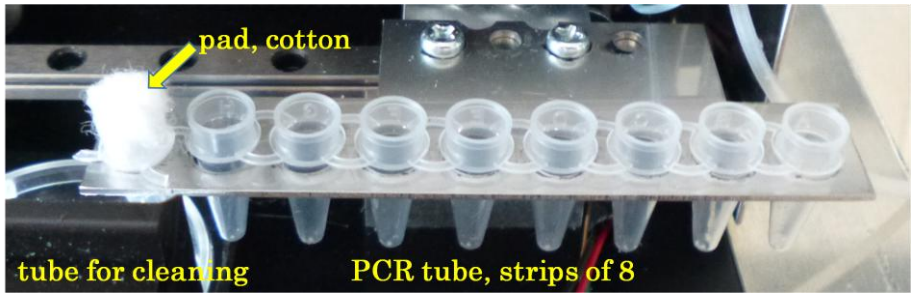


Figure 9 cleaning tube and PCR tube 8-strips

10) Sampling test: Turn **Z-posi knob** to place the dial at No.1 position which gives the lowest pushing power against a specimen. Press **Samp-button**. When you can't get a micro-dissection, increase the spin time or turn **Z-posi dial** to the right for getting a large descent value.

11) End of the preparation

## 1.4 Sampling operation

### 1.4.1 One-time sampling

1) Put a specimen on a dish covered with a silicon rubber sheet and place it on the sample holder.
   Put a PCR tube 8-strips on the collection tube tray.
   Then press **R-button** for recovering the first micro-dissection in the first tube.
   Press **Z-button** to check the position of the hollow punching needle tip during the recovery.
2) Move the sample holder to place the target position on the needle marker.
3) Confirm that **No knob** is in the first position.
4) Push **Hold-button** to check whether the target position is still on the needle marker.
   Yes ➔ Push **Samp-button** for sampling.
   No ➔ go to 5).
5) Push **LdiR-button**.
6) Push **X-posi** button to move the sample holder to place the target position on the needle marker.
7) Press **Samp-button** for sampling.

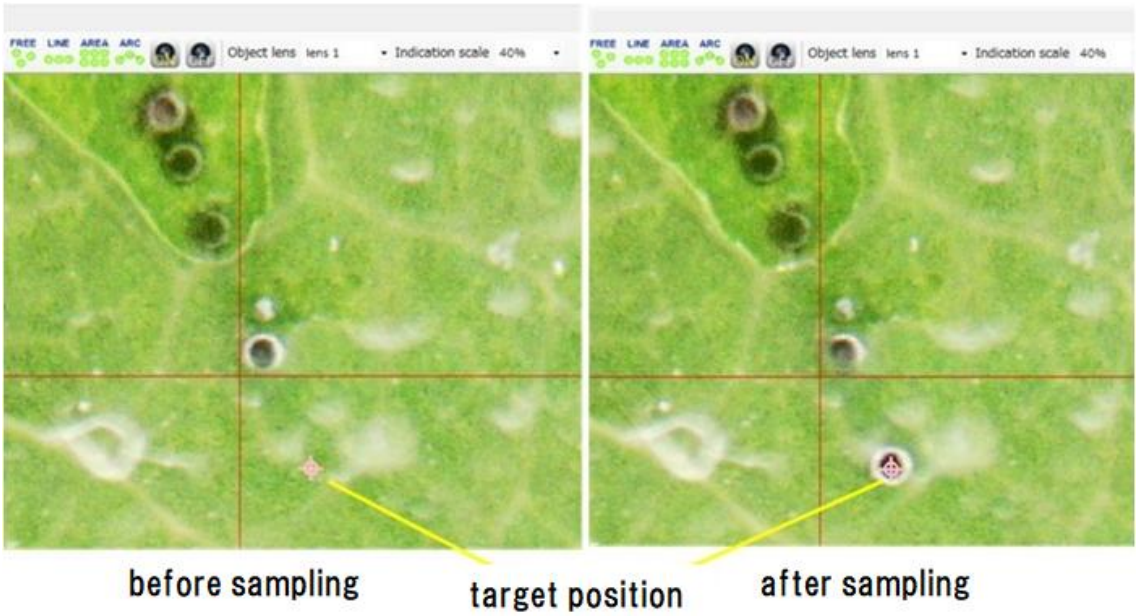



Figure 10 specimen image before punching and after punching

### 1.4.2 Continuous sampling along X-axis

1) Place a dish with a specimen on the sample holder.

The target positions should be on one line. The dish is rotated so that the line is placed parallel to X-axis.

Put a PCR tube 8-scrips on the collection tube tray.

Push **R-button** to reset the collection tube tray position.

2) Move the first target position on the needle marker manually.

3) Select the sampling interval and the sampling times by rotating **Pitch-knob** and **No-knob** on the front side of the hand-held controller.

**Pitch dial**: 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.4, and 0.5mm from the left side.

**No dial**: 1, 1, 2, 3, 4, 5, 6, 7, and 8 from the left side.

4) You can check the sampling positions by the following process.

① Push **Interval-button**

② Select the moving direction with **Ldir** or **RdiR-button**.

The sample holder moves along X-axis in that direction.

5) Continuous sampling

① Select the moving direction with **Ldir-button** or **RdiR-button**. When the target position shifts from the needle marker, correct the position by using **X-posi button**.

② By pushing **X-posi button**, you can move the sample holder by 5 $\mu$m per step. If the direction is wrong, press the other direction button and then push **X-posi button**. After the correction press the original direction button.

③ Press **Samp-button.**

## 1.5 Changing hollow punching needle

A hollow punching needle can be normally used 100 times for sampling micro-dissections before the cutting edge becomes dull. The dull cutting edge frequently fails to separate a micro-dissection from the specimen. It should be replaced with a new one or sharpened. The replacement is carried out after removing the hollow punching needle unit from the moving arm as follows.

(1) Removing a hollow punching needle unit from the moving arm: A hollow punching needle unit is fixed with a screw to the moving arm. First, you unscrew, then disconnect the sensing wirer, then take off the solution ejecting tube and the pulley belt.

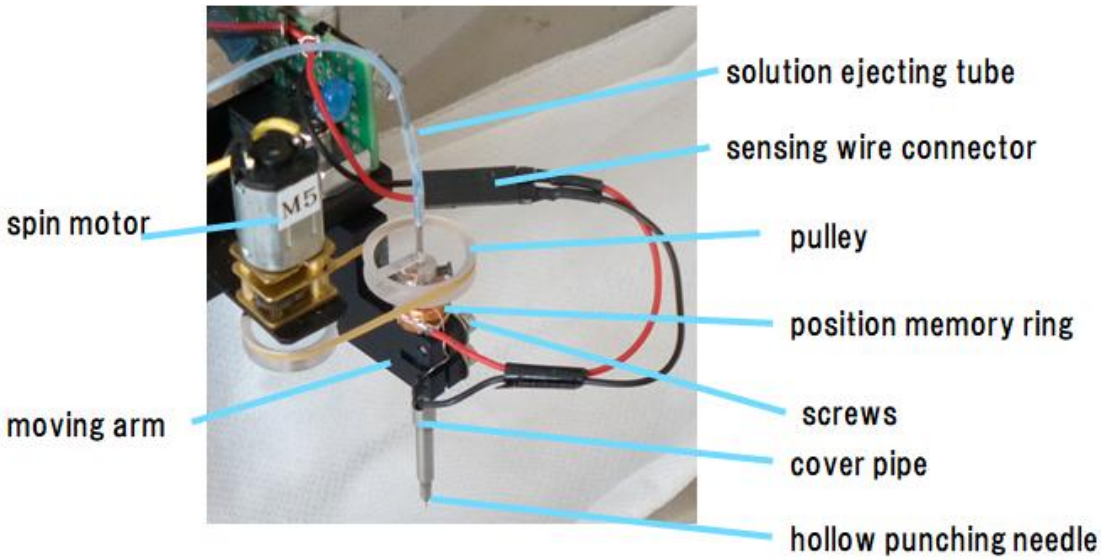



Figure 11 Removing a collection needle unit from the arm

(2) Take a hollow punching needle out: A hollow punching needle unit consists of a main shaft pipe, a cover pipe, a slit pipe, and a coil spring as shown in Figure 12. A hollow punching needle is pushed into the main shaft pipe and fixed. The hollow punching needle tip protrudes by 2mm from the slit pipe. You push the slit pipe a little bit to the main shaft pipe to make the protruding part long. Then you hold the protruding part of the hollow punching needle tightly with a pin vise or tweezers. You push the tweezers with your thumb to take the hollow punching needle out from the slit pipe (Figure 13). Be careful not to slide the tweezers on the hollow punching needle, which gives heavy damage to the needle tip. As the back end of the hollow punching needle wedges into the main shaft pipe, the hollow punching needle is tightly connected to the main shaft

pipe. However, if the wedged part is separated from the main shaft pipe, the hollow punching needle can be easily taken out from the slit pipe

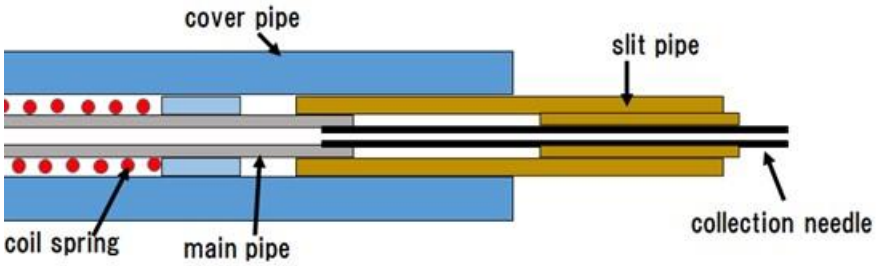



Figure12 Structure of a hollow punching needle

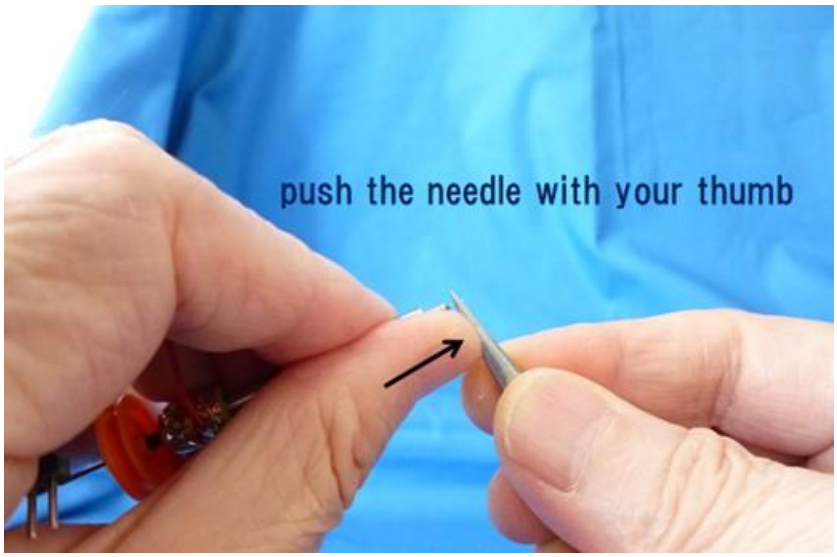



Figure 13 Way of taking a hollow punching needle out of the hollow punching needle unit

(3) Cleaning of a main shaft pipe: It is recommended to flash the inside of a main shaft pipe with a washing buffer solution because there may be dust inside the pipe. You put the cleaning nozzle of a cleaning syringe into the main shaft pipe. Then push the cleaning buffer into the main shaft pipe to wash out the dust.

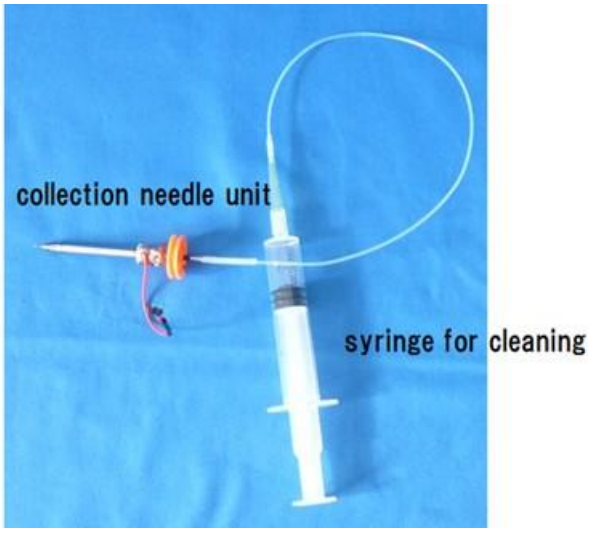


Figure 14　Cleaning of a main shaft pipe

(4)　Mounting a new hollow punching needle to a hollow punching needle unit: You put the end of a new hollow punching needle in the slit pipe with your hand. Then, you pinch the hollow punching needle with tweezers tightly and push it into the main shaft pipe. You support the other side of the main shaft pipe with your hand during you push the hollow punching needle into the main shaft pipe (Figure 15). You pick it out with tweezers to check whether the hollow punching needle is fixed by wedging it into the main shaft pipe. You pick the slit pipe out a little bit so that the hollow punching needle protrudes from the slit pipe by 1.5～2mm.

After mounting the hollow punching needle on the hollow punching needle unit, you wash out the hollow punching needle unit again.

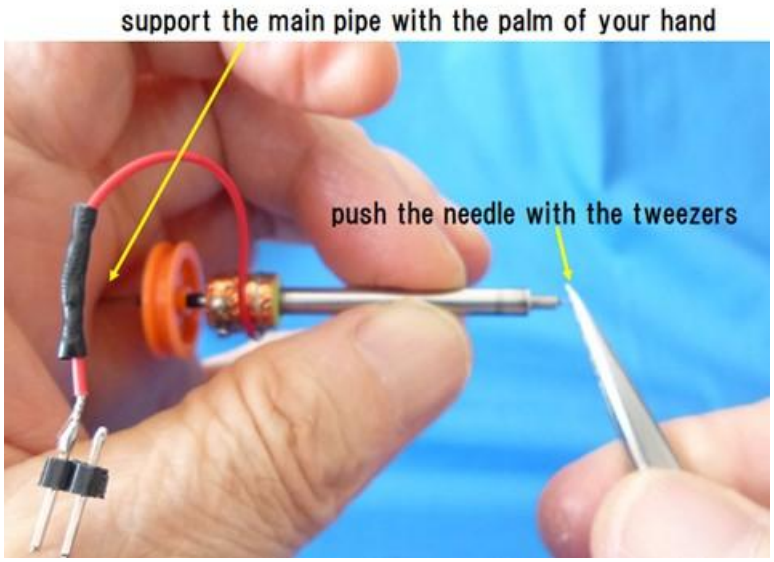


Figure 15　Mounting a hollow punching needle on the hollow punching needle unit

(5) Put the hollow punching needle unit back into the moving arm: Be careful not to hit the hollow punching needle tip with the moving arm during mounting it back to the arm. You fix the hollow punching needle unit with a screw, connect the sensing wire to the connector, and put the pulley belt and the solution ejecting tube back.

(6) Lift the pulley a little bit to check the white light on.

## 1.6 Ejection buffer solution level

The ejection buffer solution level can be checked by irradiating the inside of the cover.

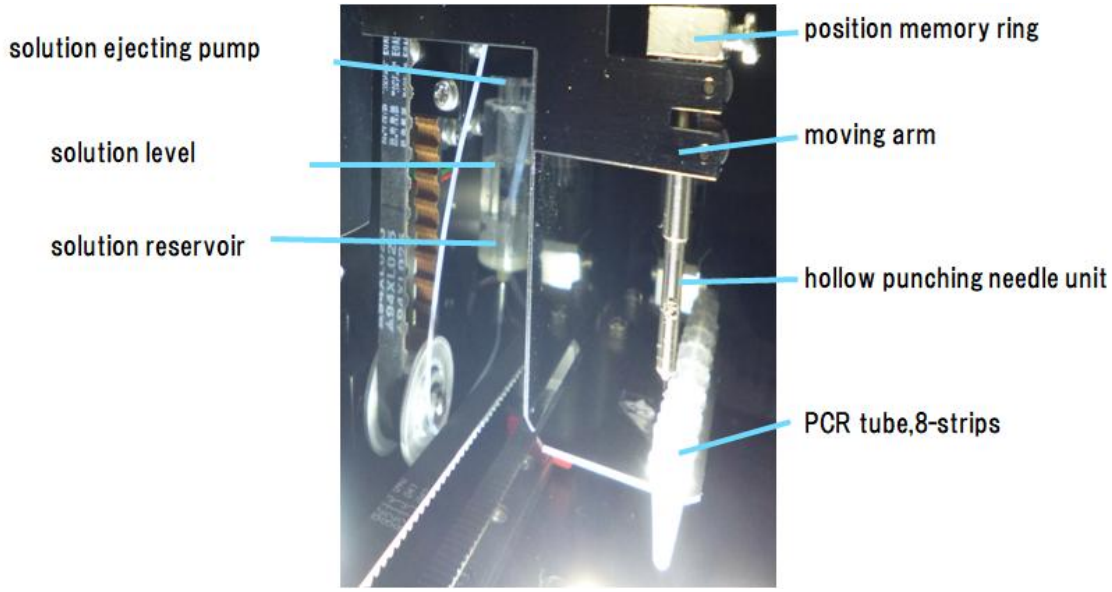



Figure 16 Ejection buffer solution level

## 1.7 Adjusting hollow punching needle position for recovery

A micro-dissection can be recovered in a tube or on a sheet by changing the needle position in the recovery process as follows.

1) Push **Z-button**.
2) Rotate **Z knob dial** to change the needle position
3) Push **Z-button** again to stop the needle position change.

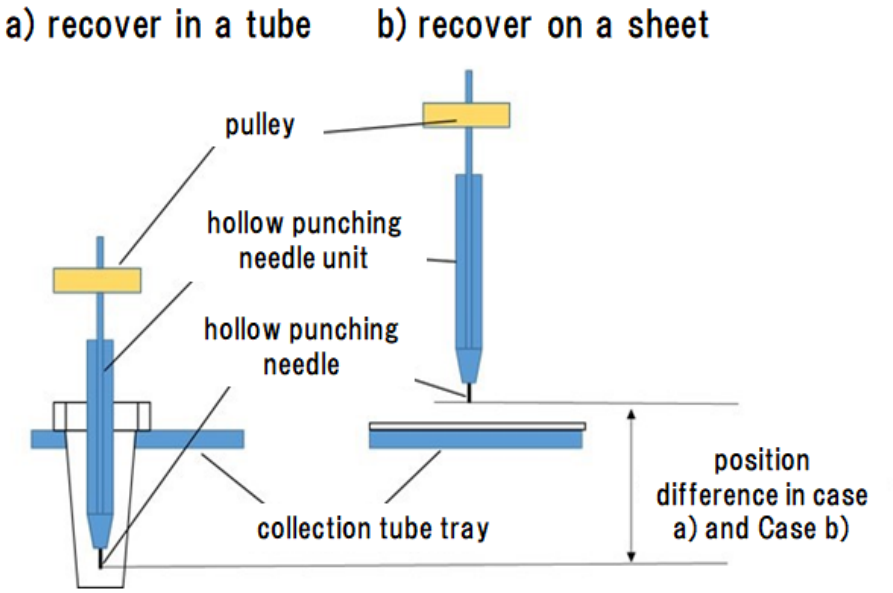



Figure 17 positions for the recovery of a micro-dissection

### 1.8 Cleaning of hollow punching needle

The first bottle with a cleaning pad on the collection tube tray is used for cleaning a hollow punching needle. A piece of cotton cloth is used as a cleaning pad. In the cleaning process, a hollow punching needle goes down to stick in the pad. Then it spins over ejecting a buffer solution to clean up its tip. To avoid contamination, the needle sticking position moves with the position of the collection tube used for the recovery.

### 1.9 Position reset of collection tube tray

The sampled micro-dissections are recovered in order in tubes in 8-strips. A recovery process can be reset to recover a micro-dissection in the first tube of 8-scrips by pushing **R-button** on the hand-held controller.

### 1.10. System reset

Push **Reset button** on the left side panel to start initialization.

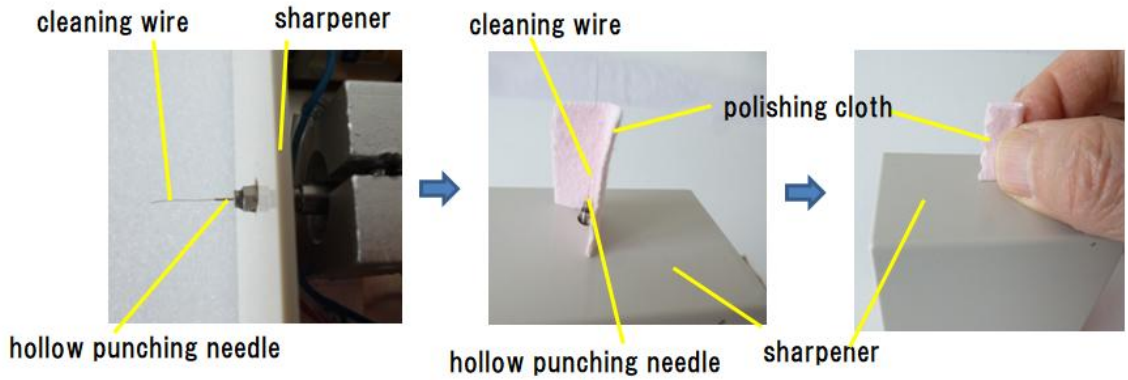
### 1.11 Sharpening hollow punching needle (option)

A hollow punching needle can be sharpened with a sharpening tool as follows.

1) Push a hollow punching needle in a sharpening tool as shown in Figure 14-a.
2) Put a piece of cleaning wire into the hollow punching needle while 5-10mm of the wire remains outside,
3) Cover the hollow punching needle tip with a piece of polishing cloth and pinch the tip with your fingers. Be careful not to bend the cleaning wire. (Figure 14-c)
4) Turn on the motor switch to rotate the hollow punching needle for 60sec.

5) Observe the tip with a handy microscope to confirm the width of the tip is smaller than 8 $\mu$ m.

6) Take it out with a pin vise from the tool.

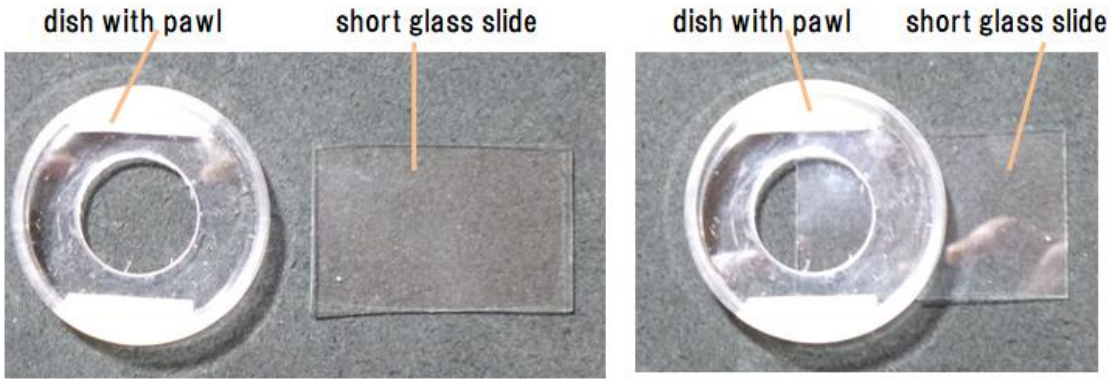



(a) (b) (c)

Figure 18 Sharpening a hollow punching needle

### 1.12 Using dish with pawl holding glass slide instead of regular dish

As the sample holder can hold a dish but not a glass slide, you have to use a dish with pawl (Figure 19). It can hold a glass slide under the dish. At first, you set the dish with pawl on the sample holder. You can insert a glass slide under the dish to be held with the pawl. The glass-slide length should be short enough to be rotated with the dish.




(a) before insertion (b) inserting short glass slide between pawl

Figure 19 Using dish with pawl to hold a glass slide

## 2. System configuration and specification

### 2.1 Overview of system configuration and operation

### 2.1.1 System configuration

Sampling Mate consists of the main device body (including the mechanical and electronic system), a hand-held controller, and a power supply unit.

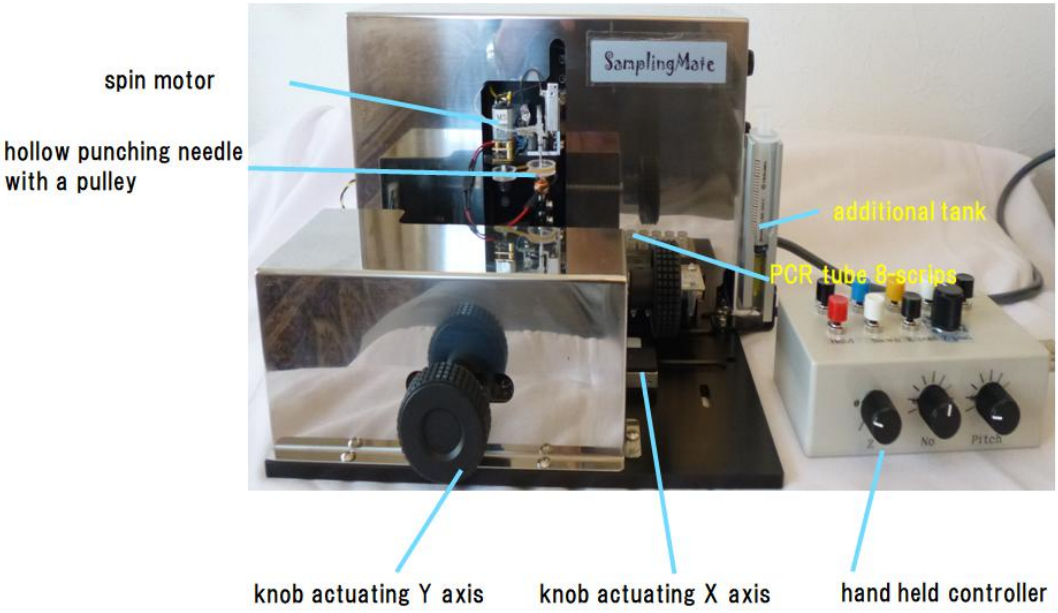



Figure 19 Outlook of Sampling Mate

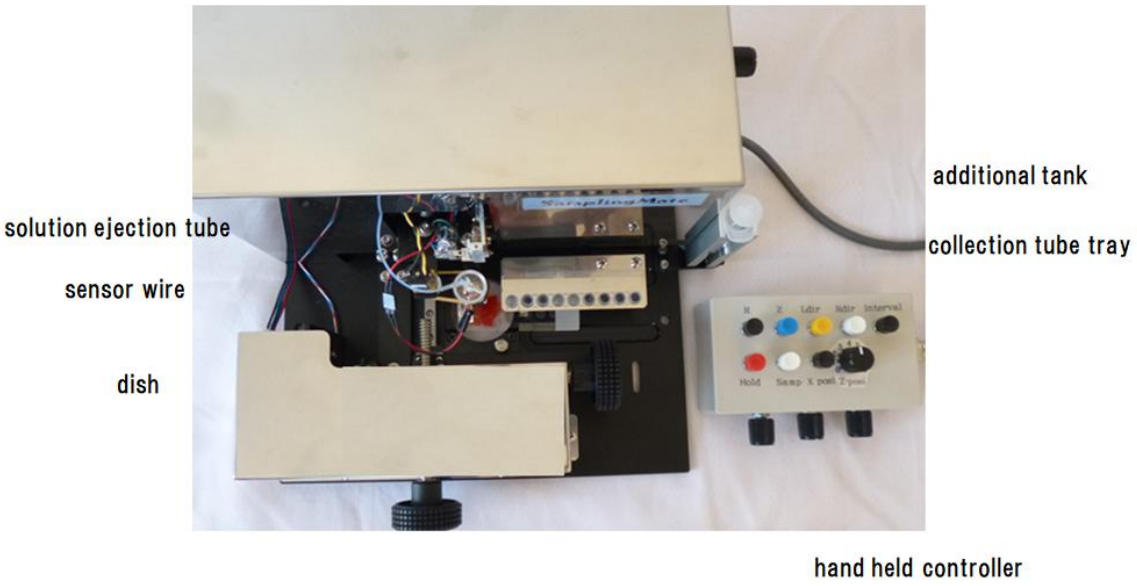



Figure 20 Top view of Sampling Mate

The configuration of the mechanical part is shown in figures 21-22. Most of the parts are fixed on the screen board while the collection tube tray unit and the sample holder unit are fixed on the baseboard. There are four actuators to

operate the moving arm with a hollow punching needle, the buffer solution ejecting unit, the sample holder unit, and the collection tube tray unit.

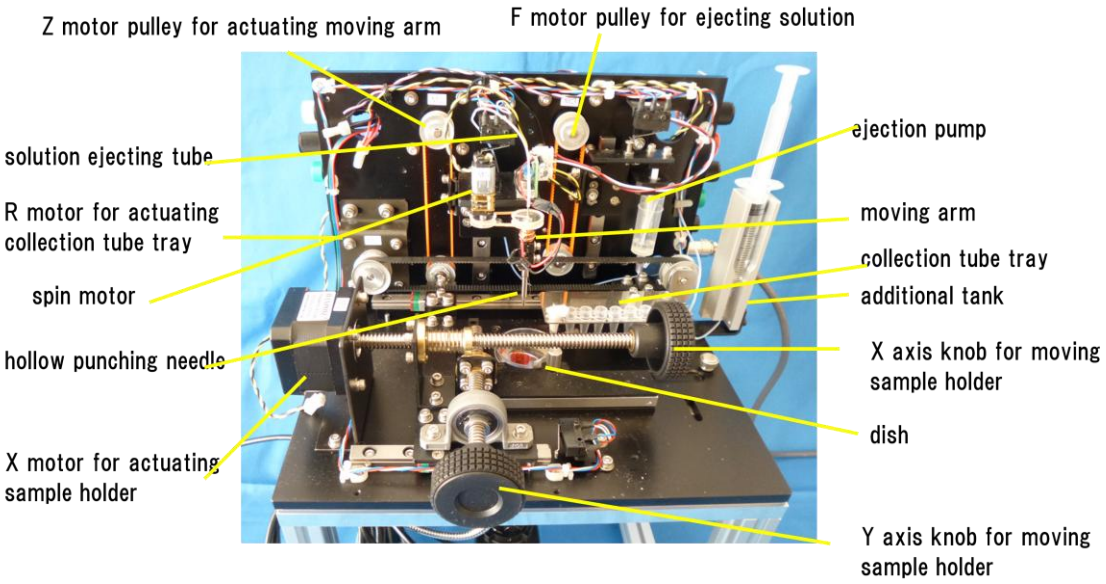



Figure 21　Inside of Sampling Mate

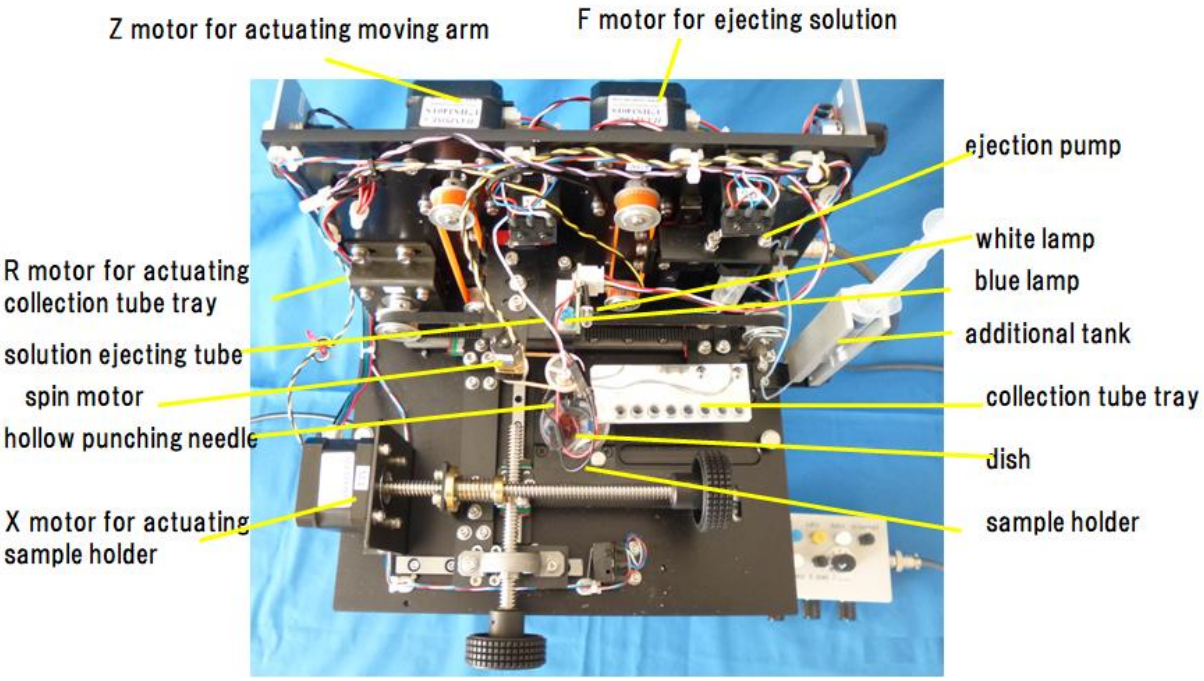



Figure 22 Top view of the inside

The positions of a hollow punching needle tip (indicated as ⇩) and the time course are shown in Figure 23. The vertical level shows the tip position at various

operations. The horizontal level shows the time course with the operations. The mark ▼ shows the timing to push **Samp- button**.

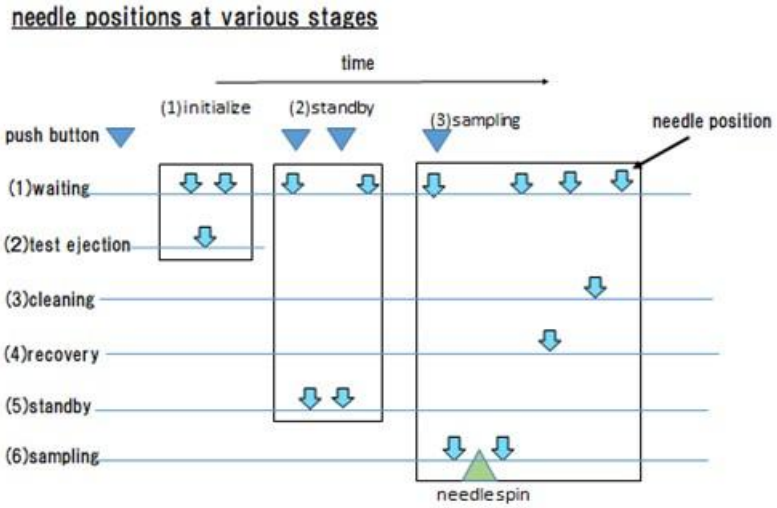



Figure 23 Time course of a hollow punching needle tip in various operations

In the case of sampling, the movement of a hollow punching needle tip after pushing **Samp-button** is as follows.

1) the moving arm with a hollow punching needle tip goes down to hit a specimen and stops for a while.
2) It goes down again for the extra distance determined with the **Z-posi dial**. As there is a coil spring between the cover pipe(fixed to the moving arm) and the main shaft pipe fixing a hollow punching needle, the extra descent gives a strong push of the hollow punching needle against the specimen.
3) After the extra descent, the hollow punching needle spins to cut the specimen. A micro-dissection is produced and held in the hollow punching needle tip.
4) The moving arm with the hollow punching needle goes up and the collection tube tray moves to place a recovery tube beneath the hollow punching needle.
5) The moving arm goes down until the hollow punching needle tip is placed in the collection tube. Then a buffer solution is ejected to push the micro-dissection out from the tip to the collection tube.
6) The moving arm goes up and the collection tube tray moves so as that the cleaning tube with a cleaning pad is under the hollow punching needle.
7) The moving arm goes down until sticking in the pad with the needle tip. A buffer solution is ejected and the hollow punching needle spins to clean up the needle tip.

8)The moving arm with the hollow punching needle goes up to the waiting position and the collection tube tray also returns to the waiting position.

### 2.1.2 Electric control unit

Arduino Mega 256 is used as the operating computer. A CNC shield to control stepping motors and an extra panel for controlling additional parts are attached to the computer panel. An external power of 12V is supplied to the Mega board, the CNC shield, and the DC-DC converter (12V=>5V).

In the system, a new operating command is given by pushing a command button. Sometimes it takes a little bit long time to start the command. If you stop to push the button before the acceptance of the new operating command, nothing occurs. As the acceptance of the command is informed by lighting the blue LED lamp (the first lamp), continue to push the button until you see the blue LED lamp on. Besides, there is a white LED lamp (the second lamp) which informs you that the hollow punching needle touches something and the moving arm has stopped.

### 2.1.3 Mechanical unit

The mechanical unit consists of a moving arm with a hollow punching needle actuated along Z-axis with a stepping motor, a collection tube tray actuated with a stepping motor along X-direction, a solution ejecting system of a syringe actuated with a stepping motor as well. A sample holder attaches to an XY stage, X-axis of which is operated with a stepping motor as well as manually. The Z- axis actuator goes up first to touch a limiter. Then it goes down to the final destination. The use of the limiter increases the reliability of the position control for a hollow punching needle as well as the collection tube tray. If you have trouble with the limiters, you will lose the normal operation of the system.

The collection tube tray has 9 holes. The first one is used for cleaning a hollow punching needle after recovering a micro-dissection. You put a piece of cotton as a cleaning pad in a tube and put it in the first hole. In the cleaning operation, a hollow punching needle comes down to stick in the cotton pad. The sticking position of a hollow punching needle shifts by 0.3mm with the recovery position change. It is recommended to change the cotton pad when you put a new PCR tube, 8 strips.

## 2.2 Overview of digital microscope

A digital microscope unit consists of a 5M CMOS camera, a microscope lens, two irradiation lamps, and a microscope stand. The position of the camera is fixed at

the optimum position for observing a specimen placed on a dish. The image focusing is carried out by rotating the lens. The positions and the directions of the two irradiation lamps can be changed with your hand. These two lamps illuminate a specimen from below. The horizontal line of the camera should coincide with X-axis direction of the XY stage. If necessary, you can adjust the horizontal axis of the image by rotating the camera.

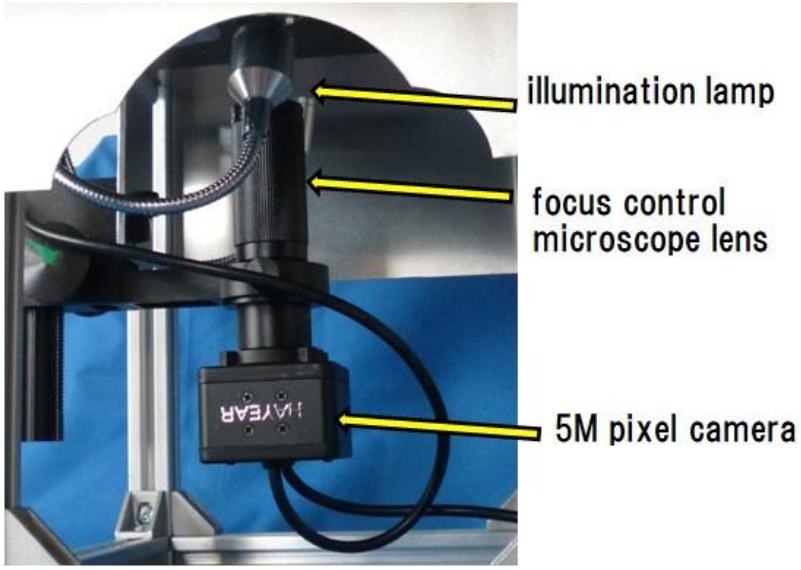



Figure 24 digital microscope

You can estimate the distance between two positions in a microscope image by checking their position coordinates in the image. Because the mouse position coordinate is displayed in the tab at the bottom of the screen. The length per pixel was calibrated with a scale standard in advance. (see figure 26)

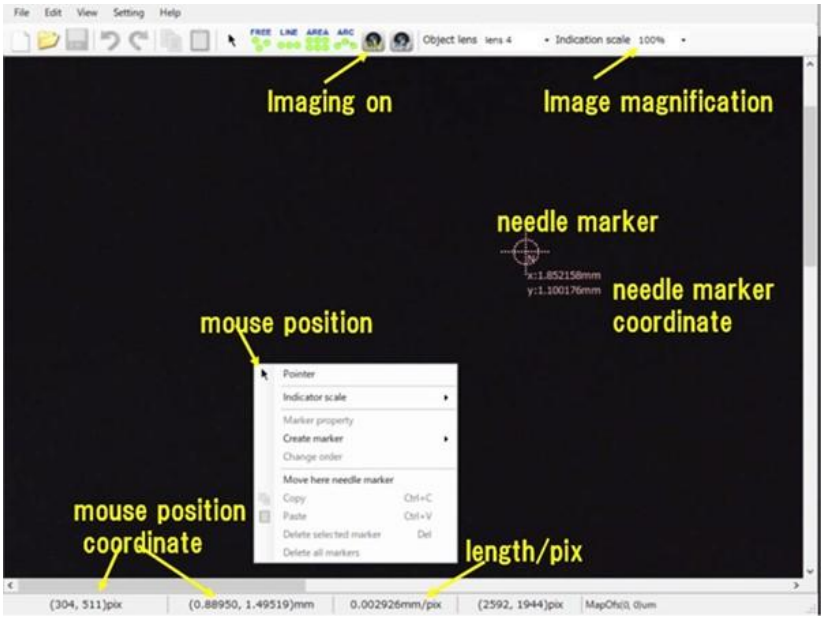



Figure 25 Screen image of Cell point marker

A microscope image magnification can be controlled with the image magnification tab. Firstly it will be better to use 50% to observe a whole image.

The needle marker is expressed with a dotted line by clicking it with the mouse. Then it can be drugged with the mouse to the other position. You can move the needle marker by right-clicking the position, where a message table pops up as shown in figure 25. Then you just click “move here needle marker” to move the needle marker to the point where you want.

If you change the microscope to the other one, you have to do the calibration of the length /pixel with a scale standard again. Then you click the setting tab to put the new length/pixel ratio. (see figure 26)

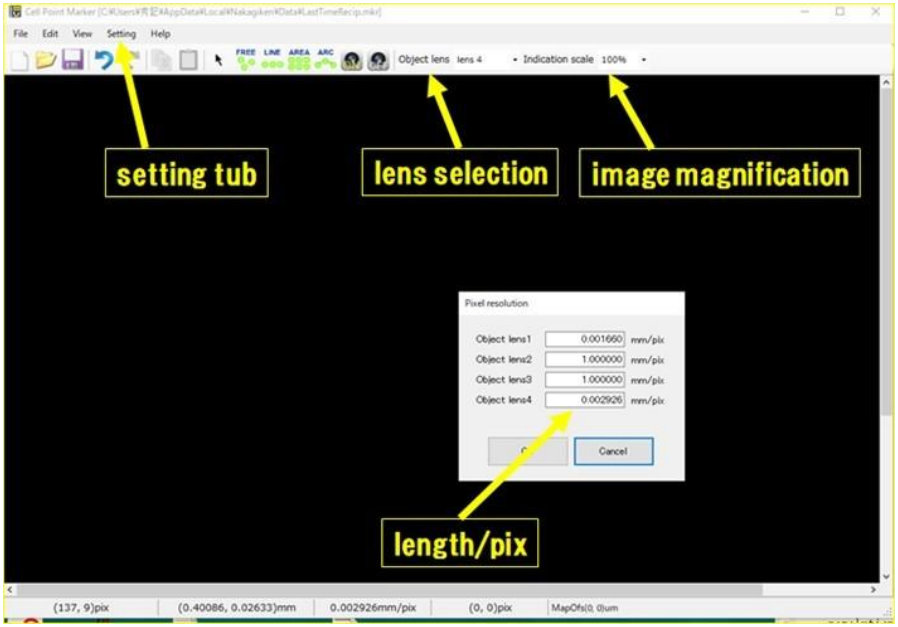



Figure 26 Optimization of length/pix

## 2.3 Size and weight and others

Size of Sampling Mate:300 mm X230 mm X160 mm(WXDXH)
(placed on digital microscope 300 mm X230 mm X380 mm(WXDXH))

weight : 4.8kg(together with digital microscope unit : 9,4kg)

sample holder: 40$\phi$ dish

collection tube tray: PCR tubes, 8-scrips

actuation system for a hollow punching needle(Z direction actuator controlled by a stepping motor):stroke 52 mm with a spring mechanism to avoid the needle crash

XY position blur: about 10$\mu$m

Spinning mechanism of a hollow punching needle for easy sampling of a specimen

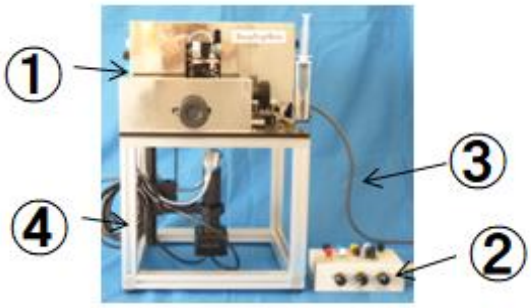
Automatic continuous sampling of up to 8 samples with an interval of 0.05、0.1、0.15、0.2、0.25、0.3、0.4、0.5、mm

Micro-dissection recovery method：ejection with a buffer solution of 2-10μl

Specimen image observation： digital microscope

## 2.4 Devise configuration and Accessories

1. Device main body
2. Hand held controller
3. Connection cable
4. Digital microscope unit
5. 12V power supply
6. LED ring light
7. Hollow punching needle(10)
8. Cleaning wire(5)
9. Injector for cleaning main shaft pipe
10. Handy microscope
11. Scale standard
12. Tools (tweezers, pin vise, driver set, wrench set)
13. Parts (pulley belt, 50μm silicon rubber sheet, silicon tube, dish, dish with pawl, PCR tube 8-strips)
14. Software CD
15. Handy light and ball point pen
16. User manual



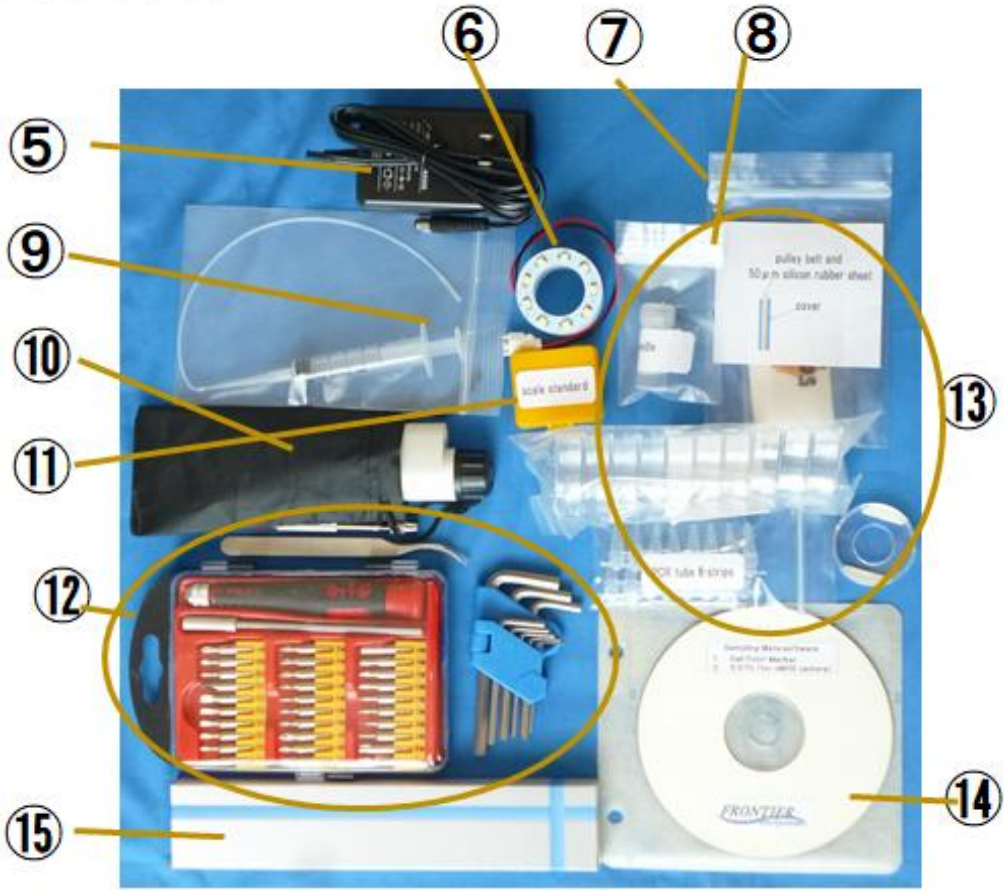




Figure 27 Accessories

## 2.5 Option

1) Computer
2) Needle sharpener
3) Laser

# 3. Mounting Sampling Mate on inverse microscope

The base plate of Sampling Mate consists of two plates. The lower plate is fixed to the inverse microscope with screws to place the center of the view window on the objective lens. If necessary, you can make new holes on the lower plate for fixing the lower plate with screws on the inverse microscope.

## 3.1 Mounting procedure

1) Place a ring light on the objective lens.
2) Fix the lower base plate of Sampling Mate on the sample plate of the inverse microscope. The place Sampling Mate on the lower base plate and fix it with screws.
3) Connect the lead wire of the ring light with the connector.
4) Turn on **Main SW**. Then the initialization starts.
5) Push **LED SW button** and turn **Light control knob**.
6) Turn on a microscope camera to see a microscope image on a computer.
7) The moving arm can be lowered manually to observe the hollow punching needle. It should be around the center of the microscope image.
8) Place a trial sample on a dish and put it on the sample holder.
9) As the horizontal line of a microscope image should be in parallel to the moving line along X-axis, the camera axis is adjusted as described in section 3.2.

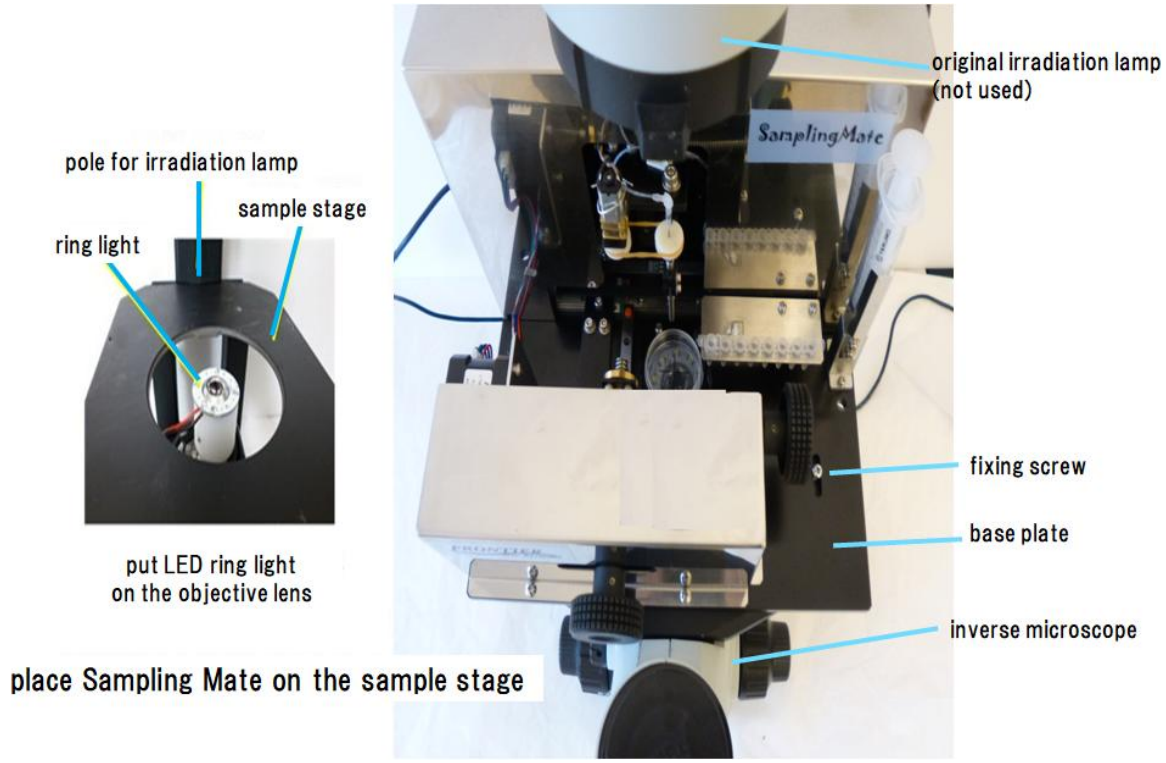



Figure 28 Placing Sampling Mate on an inverse microscope

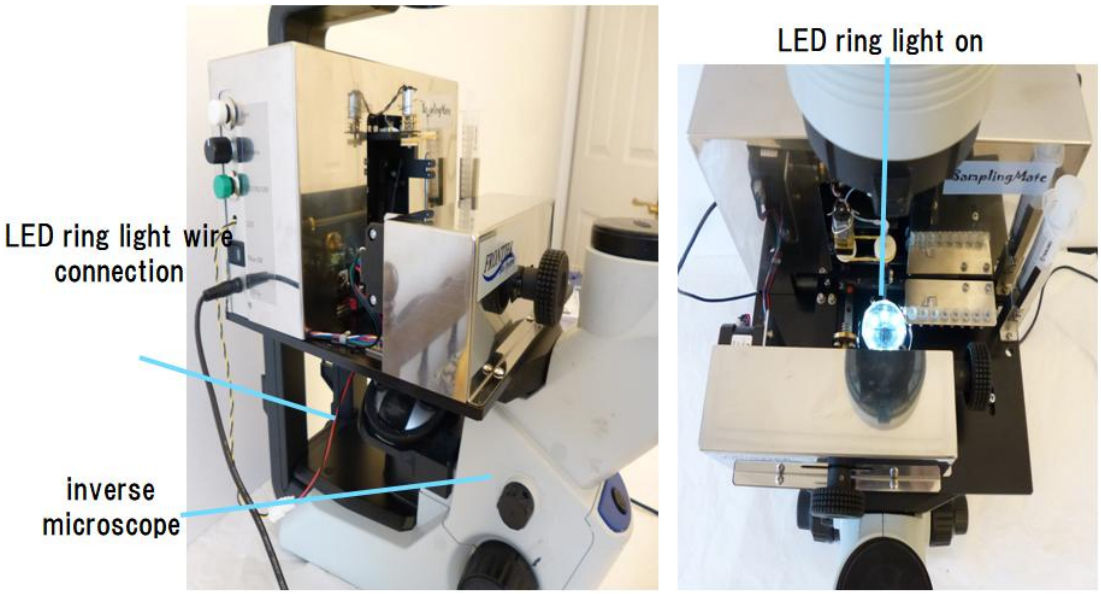



Figure 29 Sampling Mate mounted on an inverse microscope

### 3.2 Align the horizontal camera line with X-axis of Sampling Mate

As the sample holder moves along X-axis in the continuous sampling mode, the horizontal axis of a microscope image should be aligned with X-axis and the ratio of image length/pixel is optimized as follows.

1) Observe a microscope image on Cell Point Marker software.
2) Open the view-tub and click Cross line to display a cross line on the image.
3) Place a scale standard for a microscope on a dish.
4) Determine the marker point in the image and move it on the horizontal line by controlling the Y-axis knob manually.
5) Move the sample holder along X-axis by turning the X-axis knob manually.
6) Rotate the camera so that the marker position moves along the horizontal line in an image.
7) Fix the camera position.
8) Rotate the scale standard to be parallel to the horizontal line.
9) Check the 2mm distance of the scale in pixels by placing the mouse at the checking positions. Calculate the ratio of real distance /pixel.
10) Open the **setting-tub** and click pixel resolution. An image as shown in Figure 30 appears. Then put the calculated value in the scale tab. The value is dependent on the magnification of the microscope lens. It is possible to register 4 values on the scale tub corresponding to 4 different lenses.

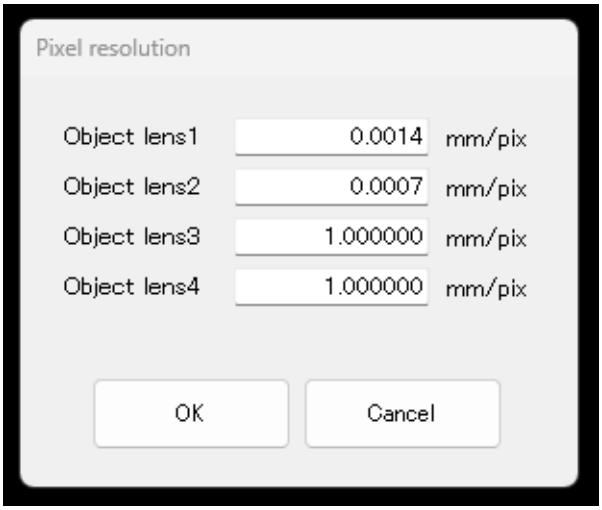



Figure 30 Put the optimum value of length(mm) / pixel

## 4 Troubleshooting

1) Clogged hollow punching needle: Remove the solution ejection tube and put a cleaning wire in the main shaft pipe to push out the clogged material. After pulling out the cleaning wire, connect the ejection tube to the main shaft tube. Then wash the inside of the main shaft pipe by ejecting a buffer solution.

Clogging frequently occurs when the tip of the hollow punching needle is damaged. The damage can be recovered by sharpening with the sharpening tool.

2) System malfunction：push **Reset-button**

3) Recovery error: Although a micro-dissection is produced, it is not observed in the recovery tube. The phenomena are frequently observed by using a damaged hollow punching needle. A part of the micro-dissection sticks to the hollow punching needle. It is scattered around or attached to the outer surface of the needle while ejecting buffer solution. The hollow punching needle should be replaced with a new one or sharpened with a sharpening tool. Usually, a hollow punching needle can be used for sampling about 100 times.

FrontierBioSystems Inc.
e-mail:home@frontierbiosystems.com
phone: +81-42-635-4323