

# Micro-Biopsy System

Sampling Junior-T



Sampling Junior-T is an automated microdissection system. It is mounted on a digital microscope. It can be configured on any inverted microscope as well. Sampling Junior-T 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 the microscope image. Then just push the sampling button. A hollow collection needle goes down to collect a microdissection from the target position by punching with the hollow collection needle. The microdissection is recovered in a recovery tube automatically. Then the collection needle is washed for the next sampling. The collected microdissections can be used for space analysis of gene and/or protein expressions in tissues.

# **Micro-Biopsy System**

**(Sampling Junior-T)**

## **User Manual**

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## 1 Easy setup of Sampling Junior-T

### 1. 1 mounting the system on a microscope

Carefully take Sampling Junior-T and a digital microscope out from the boxes. After removing packing materials, put Sampling Junior on the digital microscope along two side rails



Figure 1-1 Mounting Sampling Junior on a digital microscope

### 1. 2 software launch

- 1) Install the software for the camera operation and microscope image display. As to Cell point marker software, you just copy the program on your computer. Turn off the other camera software.
- 2) connect digital microscope USB to computer and turn on the irradiation light of the microscope.
- 3) Just click Cell Point Marker. Then you will see a black image with a tub as shown in figure 1.2. Click ON tub to get a microscope image.



Figure1-2 Upper tub of Cell Point Marker

4) You will see X character which is stapled on the glass plate below the collection needle. You move Sampling Junior-T so as that you see the character X almost at the center of the image.

Put the mouse on the center of the character X and do right click to get the image shown in figure 1-3. Then click “Move here needle marker” tub to place the needle marker at the center of character X. (see figure 1-4)

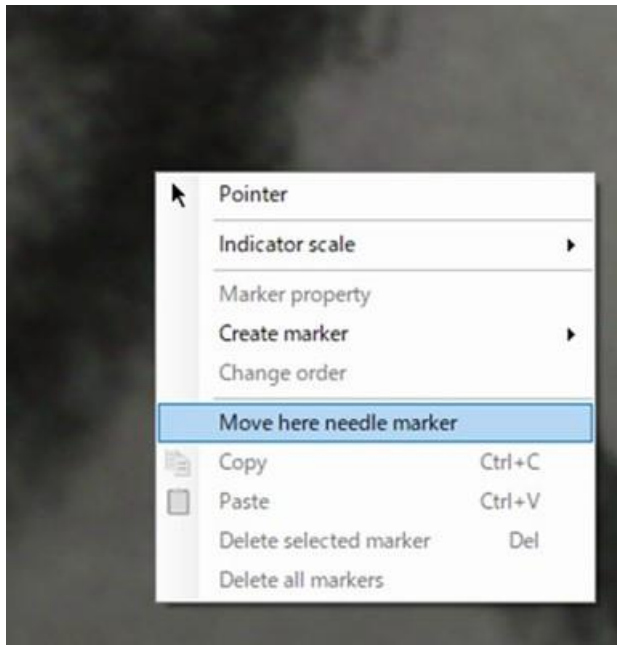


Figure 1-3 given by right click



Figure 1-4 move marker at the center of Character X

- 5) You fix Sampling Junior-T with screws at the both sides of the XY stage for specimen holder. Two of the screws attached on the XY stage should be used for fixing Sampling Junior on the digital microscope.
- 6) Remove the sheet attached on the glass plate.
- 7) The space resolution of the digital microscope is shown in figure 1-4. It may be enough for the microdissection collection works.

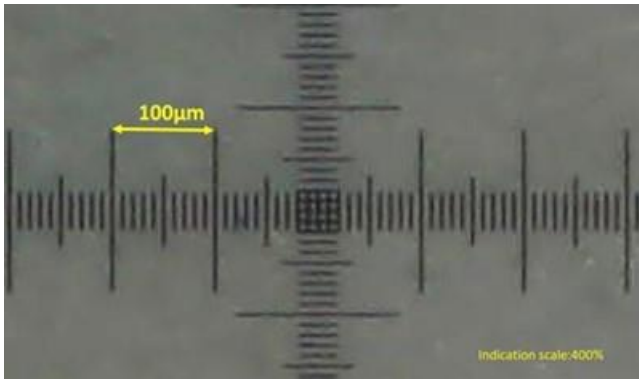


Figure 1-5an image of a scale

### 1. 3 Preparation for the operation check

- 1) You put buffer solution in the outer reservoir. Then push shaft to inject the buffer solution into the solution tank over covering the hole at the 4ml position with your finger. A conceptual diagram is shown in figure 1-6. The buffer solution goes into the solution tank from the bottom side and goes up to flow out from the upper side.

#### Buffer ejection system

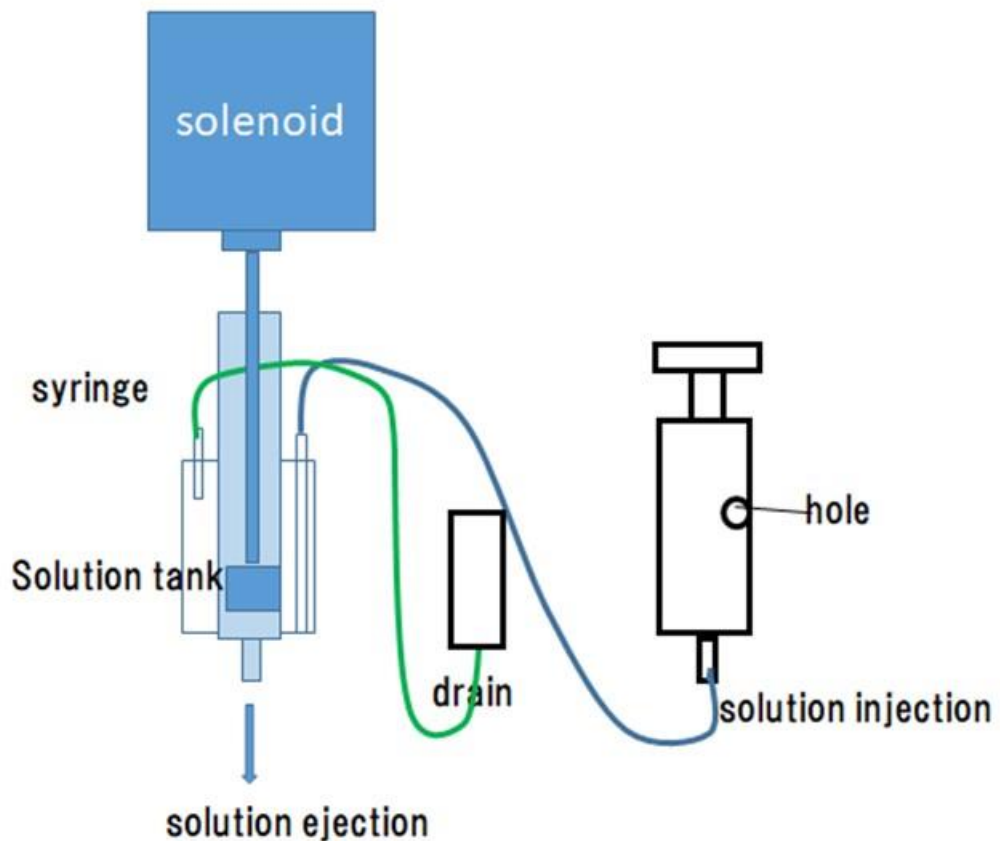


Figure 1-6 Conceptual diagram of the solution supply system



- 2) After confirming the solution flow out from the drain, stop to push the syringe shaft and remove your finger from the hole. ( Be sure that the bottom of the syringe shaft is placed at the upper side of the hole.)
- 3) Put a piece of cleaning pad such as cotton in a cleaning tube and place it at the head of the recovery plate.
- 4) Then PCR tubes, strips of 8, are placed on the recovery plate.

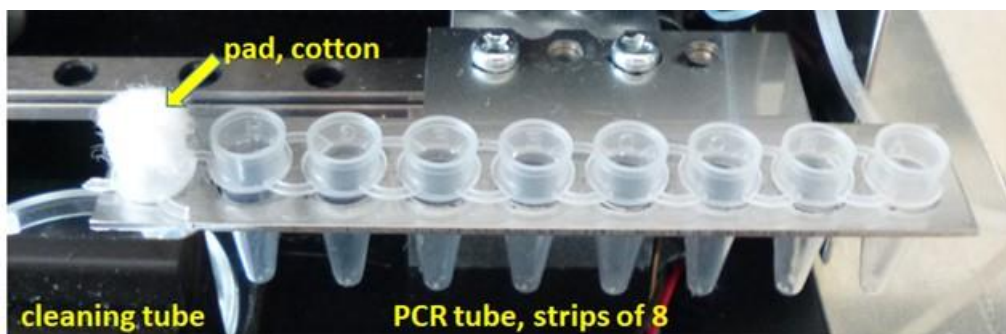


Figure 1-7 Tubes placed on the recovery plate

- 5) Put a dish on the sample holder and fix it with a screw so as that the bottom of the dish is in close contact with the glass window. When you use a dish which can't be placed in the holder from the upper side, you remove the holder from the XY stage to put it on the dish placed on the glass window. After fixing the holder to the XY stage, you fix the dish with the screw as the bottom of the dish is in close contact with the window, (figure 1-8)



Figure 1-8 placing a dish in the sample holder with a screw so as that the bottom is in close contact with the glass window

#### 1.4 System operation check

##### 1) Confirm the followings as to the collection needle holder

- (1) the needle holder is fixed to the moving arm with a screw tightly
- (2) two pulleys are connected with a pulley belt
- (3) the tube from the solution ejecting system is connected with the

main pipe.

- (4) The sensor electrode is connected to the monitoring connector

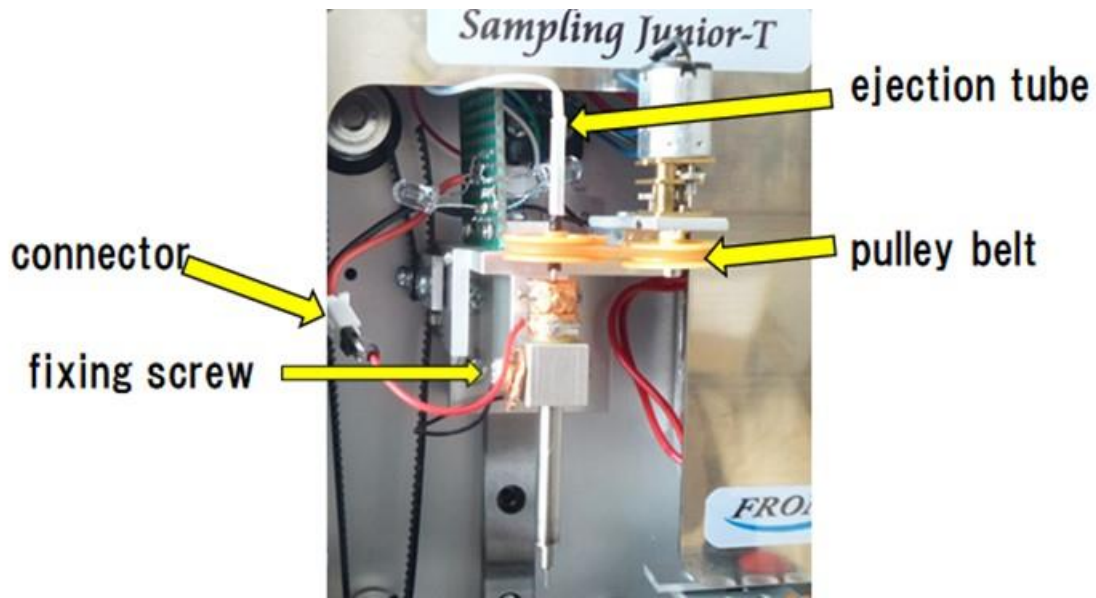


Figure 1-9 collection needle holder

2) Connect the electric power cable and power on. The initialization starts. Confirm that the right lamp is off.

3) After setting the sampling dial to the standby position, push the start button. The collection needle tip goes down to the standby position just 0.1mm above the dish.



Figure 1-10 Sampling controller

- 4) Check the needle tip figure on a microscope image
- 5) Move the needle marker to the center of the collection needle image by right click of mouse and “move here needle marker “ tub.
- 6) Push the start button again. Then the collection needle holder goes back to the waiting position.

#### 1.5 Needle position adjustment for recovering a microdissection

Usually a microdissection is recovered in a tube. However, sometimes it is convenient to recover it on a flat sheet for checking the sampling capability. In these two cases, the optimum collection needle positions for the recovery are different. So the collection needle position in the recovery process can be changed with changing the Z\_posi dial. The green arrow on the Z\_posi dial is good for recovering a microdissection on a flat sheet. The red arrow position on Z\_posi dial is good for recovering a microdissection in a tube.

The collection needle position during a recovery process can be set as follows.

- 1) Push Zcheck dial on the right side panel. Then the collection needle goes down to the recovery position.
- 2) When you want to change the position, You rotate the Zposi dial. As the collection needle position change, you can set the position as you like.
- 3) When you push the Zcheck button, the setting is over. (Or you can end the position setting by put it as it is for a while.)

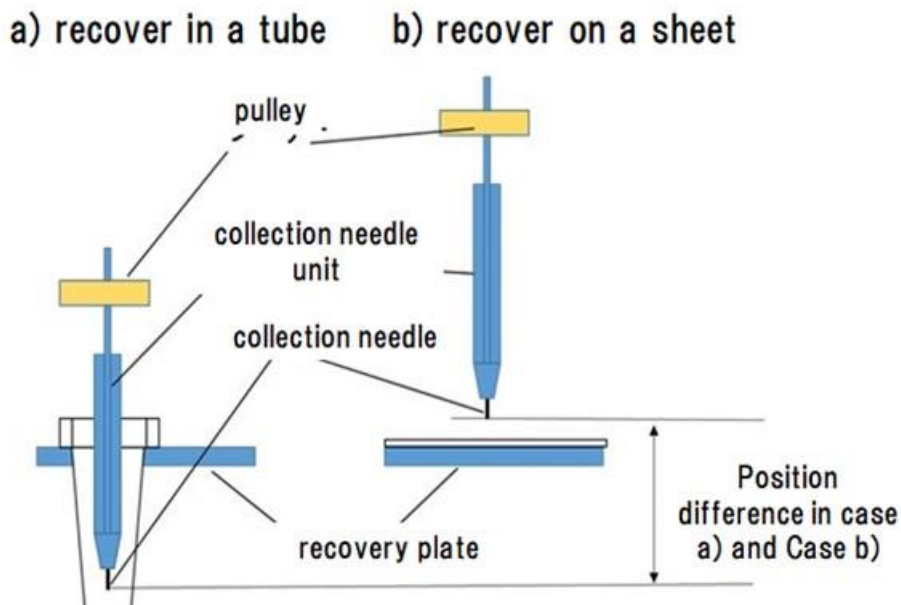


Figure 1-11 Adjustment of the optimum collection needle position for recovering a microdissection



## 1. 6 Fixing a plastic dish with a sample to the sample holder

A piece of 50 micron silicon gum sheet is put on a plastic dish. Then tape a sliced specimen on the silicon gum sheet. Now the plastic dish with a specimen is placed in the sample holder.

- 1) When the plastic dish is small enough to be put in the holder from upper side, just put it in the sample holder and fix it with a screw. The bottom of the plastic dish should be in close contact with the glass window.
- 2) When the plastic dish could not be put in the holder from upper side while the upper side of the dish fit to the holder, you can put it in the sample holder as follows.
  - (1) remove the sample holder from the XY stage.
  - (2) put the plastic dish on the glass window followed by putting the sample holder on the dish.
  - (3) fixing the sample holder to the XY stage and then fix the dish to the sample holder with a screw so as to be close contact with the glass window.

The use of glass dish is not recommended.

===Now the end of the preparation===

## 1. 7 Microdissection collection by punching

- 1) Set the sampling dial to 1. The dial position of 0 or 2 is also frequently used for collecting microdissections from a thin specimen. The dial position of 0 is where the collection needle tip just touched to the specimen. By increasing the number of dial position, the arm supporting the collection needle holder goes down further. One dial position increase corresponds to about 0.1mm down of the arm. As there is a coil spring between the arm and the main pipe holding the collection needle, the needle tip push the specimen with more strong force. The big dial number position is used for getting a microdissection from a thick specimen.
- 2) Determine the position from where you want to get a microdissection by observing a microscope image. The move the point to the marker position by moving XY stage manually.
- 3) Push the start button.

- (1) The collection needle goes down until touching a specimen. Then the collection needle goes down further a little bit by the amount determined with the sampling dial.
- (2) After the descent, the collection needle rotates as much as specified by the spin dial.(the recommended rotation amount is 1/4as indicated with a green tag on the spin dial.)
- (3) The collection needle goes up to return to the waiting position. The recovery plate moves to place recovery tube under the collection needle. Then the collection needle comes down to push out a microdissection together with a solution ejection into the tube. After the recovery of the microdissection, the collection needle goes up and the recovery plate moves to place the cleaning tube under the collection needle.
- (4) The collection needle comes down to hit the pad in the cleaning tube then rotate for a while to clean up the collection needle tip over ejecting a solution.
- (5) After the cleaning process, the collection needle goes back to the waiting position.

See figure 2-2.

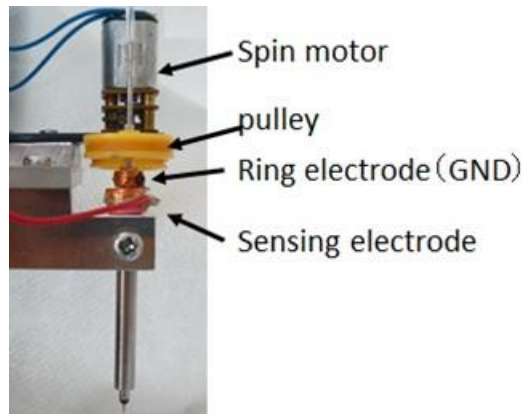
- 4) When you want to get more microdissections, you repeat the process 2) and 3).
- 5) If you want to recover a new microdissection in the first tube, you push R\_rest button on the left side panel.

## 1. 8 Notes

- 1) If you want to use a camera software included in your computer instead of Cell point marker software, you do the followings.
  - (1) the camera function on
  - (2) click the camera icon to start the application. You will see the microscope image.
  - (3) put the computer mouse on the position where the collection needle comes down. Left click to make a square marker on the spot. It can be use as the needle position marker.
- 2) Troubles due to contact failure of the needle touch sensor
 

The needle touch sensor consists of a ring electrode and a sensor electrode as shown in figure 1-12. The ring electrode is fixed to the main shaft and is grounded through the main shaft, a coil spring and the moving arm. When the tip of a collection needle goes down to touch a specimen, the ring electrode is lifted up relative to the sensor electrode. Then a control voltage is applied on the sensor electrode to light a monitor lamp on the right side.

As the monitor lamp is off usually, you will notice troubles with the collection needle holder by observing the lamp.



The ring electrode is fixed with a screw to the spindle pipe of the sampling needle unit. As the spindle contacts to GND, The sensing electrode is at GND and the indication light is off. When the contact of the two electrodes is not good, the indication light is on or blinking. Then you change the position of the ring electrode to realize the perfect contact..

Figure 1-12 contact of the ring electrode and the sensing electrode in a collection needle

There is a coil spring in a collection needle holder. As the coil spring usually forces the ring electrode to contact to the sensor electrode, the sensor electrode is grounded and can't be applied with a voltage. When the contact is not perfect, you will have troubles. You will observe the lamp lighting or flashing. In that case, a sampling is not operated properly. Because the control system think that the collection needle is touching to something.

- 3) The movement of the collection needle is normal. However, a microdissection can't be obtained. This is mostly due to the collection needle, the tip of which is not sharp enough to get a microdissection. It is necessary for you to do the followings to overcome the trouble.
  - 1) Polish the collection needle tip or exchange the collection needle with a new one.
  - 2) Try again to get a microdissection without changing the target position. Just push the sampling button again. Sometimes you will success to get a microdissection.
  - 3) Change the sampling dial to a higher level and push the sampling button.

## 2. System configuration and specifications

The sampling Junior can be operated with a digital microscope (option) or with any inverted microscope with a digital camera.

### 2.1 Overview of overall system configuration and operation

2.1.1 System configuration: The configuration of the mechanical part can be seen in figure 2-1. It consists of the Z axis actuator to move the arm with a collection needle, the recovery plate actuator, the buffer solution ejecting system with a solenoid, and XY table to move sample holder.

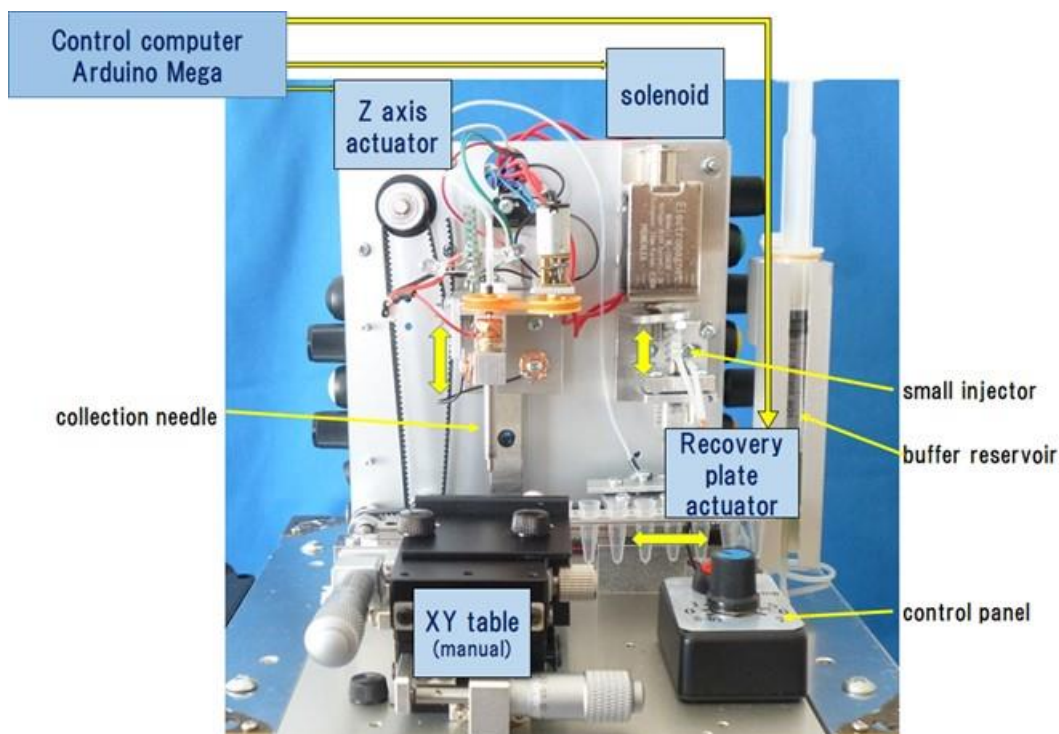




Figure 2-1 Configuration of the moving part

The position of a collection needle tip (indicated as  and its time change are shown in Figure 2-2. The vertical level shows the tip position at various operations. The horizontal level shows time change in various operations. The mark  shows a timing to push the sampling button.

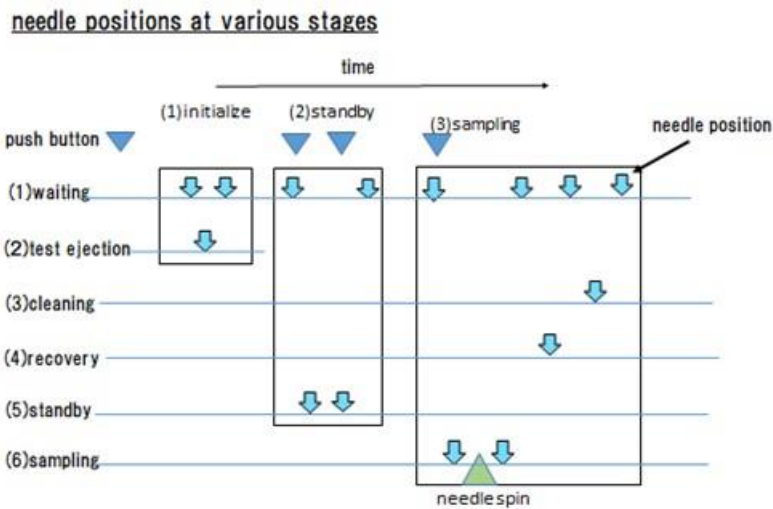


Figure 2-2 Time course of a collection needle tip in various operation

In the case of a sampling, the movement of a collection needle tip after pushing the start button is as follows.

- 1) the arm with the collection needle tip goes down to a specimen and stops for a while.
- 2) It goes down again for the distance determined with the sampling dial. As there is a coil spring between the arm and the collection needle tip, a long going down distance push the collection needle strongly against the specimen.
- 3) After stopping the going down movement, the collection needle rotate to cut the specimen. A microdissection is kept in the collection needle tip.
- 4) The arm with the collection needle goes up and the recovery plate moves to place a recovery tube beneath the collection needle.
- 5) The arm with the collection needle goes down until the collection needle tips in the recovery tube. Then a buffer solution is ejected to flow the microdissection out of the tip to the tube.
- 6) The arm goes up and the recovery plate moves so as that the cleaning tube with a cleaning pad is under the collection needle.
- 7) The arm goes down until hitting the pad with the needle tip. A buffer solution is ejected and the collection needle rotates to clean up the needle tip.
- 8) The arm with the collection needle goes up to the waiting position and the recovery plate also returns to the waiting position.



## 2.1.2 Various parameters for sampling and recovery

Besides the additional going down of the arm determined by the sampling dial for cutting a specimen, there are several parameters related to the sampling and recovery. They include the amounts of rotation, solution ejection, and the collection needle position during the recovery of a microdissection. They can be controlled with buttons on the side panels.

(1) Sampling operation can be controlled with a control panel shown in figure 1-10. Usually the sampling dial is placed at 0~2 position. For a thick specimen, it will be set at over 3.

(2) side panels: Reset sequence of actions, control of irradiation power of ring LED, reset of the recovery plate position, the rotation amount of the collection needle, the solution ejection amount control, and the position control during a recovery can be carried out with buttons on the panels as follows.

### Left panel

- 1) Reset: reset sequence of actions
- 2) laser: laser illumination on/off; now it is not used.
- 3) R\_reset: The next recovery tube should be the first one on the recovery plate.
- 4) LED: ring LED irradiation control. This is used when you use your own microscope together with the ring LED device.
- 5) Main SW: Main switch
- 6) 12V: 12V in

### 右側板

1) S-Time: Spin time control with the dial. The rotating amount of a collection needle can be controlled with the dial. A quarter rotation is adequate in most cases. A green mark is placed at the position.

- 2) S-Check: Push to check the rotation of a collection needle.
- 3) Ejec-Vol: The control of a solution amount ejected during recovery.
- 4) E-Check: push to check the ejection amount
- 5) Z-Posi: control the collection needle tip position during recovery.
- 6) Z-Check: check the position of the collection needle tip during recovery



Figure 2-3 left & right side panels

There are two factors to cut a specimen with a collection needle. One is the force to press the collection needle tip to a specimen which is controlled with a coil spring and an additional going down of the arm determined by the sampling dial. Another is the amount of the collection needle rotation. A quarter rotation is recommended and a green mark is posted on the dial. A rotation shake should be avoided. (Usually it is smaller than 10 microns)

An obtained microdissection is recovered in a tube by ejecting a solution through the collection needle. The ejection is carried out by pushing a syringe with a solenoid. The amount of the ejected solution is controlled by changing the pushing period of the solenoid with the Ejec\_Vol dial. The buffer solution is supplied from the outer reservoir into the syringe as shown in figure 1-6. You can check the ejection by pushing E-check button or pushing button on the roof.

Microdissections are usually recovered in PCR tubes , trips of 8. The collection needle tip goes down to the middle position of the tube during the recovery. Sometimes we need to recover microdissections on a flat paper or a shallow tube. The position of the collection needle during the recovery have to be changed. It can be done with the Z-posi dial.

At first you push Z-check. Then the collection needle goes down at the predetermined position. You change the Z-posi dial to change the collection needle position. After determining the position, you push Z-check button again. (see 1.5)

## 2.2 System specification

Sampling Junior is composed of one including the electric control unit and the mechanical unit.

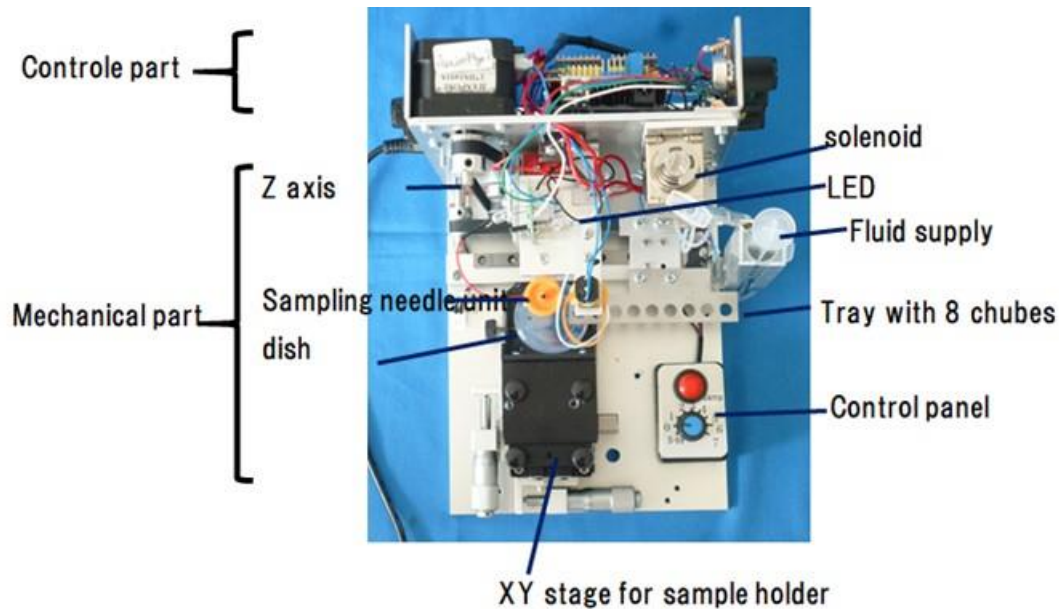


Figure 2-4 A system view from upside

### 2.2.1 Electric control unit

Arduino Mega 256 is used as the control computer. A CNC shield to control stepping motors and an extra panel for control additional parts are attached to the control computer. A photo of the control unit and a wiring diagram of the circuit are shown in figure 2-5. An external power 12V is supplied to the Mega board , the CNC shield and DC-DC converter (12V=>5V).

In the system, a new control instruction is supplied by pushing a button. Sometimes it takes a little bit long time to accept the instruction. If you stop to push the button before the acceptance of the new control instruction, nothing occurs. The acceptance is informed by lighting the right side LED lamp (the first lamp). In addition there is another LED lamp (the second lamp) which inform you that the ring electrode is lifted from the original position and the Z axis movement has stopped because the ring electrode is lifted a little bit from the original position. The second lamp changes its color by time to be distinguished from the first lamp.

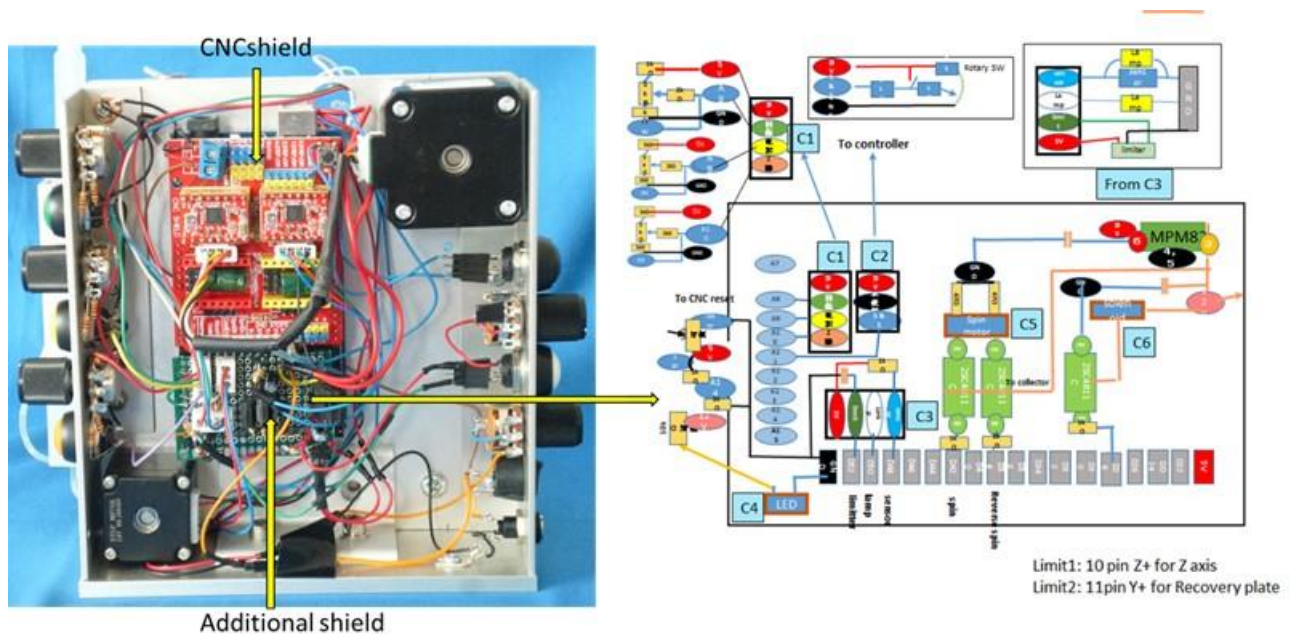


Figure 2-5 Control unit consists of Arduino Mega board coupled with a CNC and an additional small board.

### 2.2.2 Mechanical unit

The mechanical unit consists of a moving arm with a collection needle actuated with a stepping motor, a recovery plate actuated with a stepping motor along X direction, a solution ejecting system of a syringe actuated with a solenoid, and a sample holder attached to XY stage operated manually. The z axis actuator goes up firstly to touch a limiter. Then it goes down to the final destination, which increase the reliability of the position control for a collection needle. It is the same as to the recovery plate. If you have a trouble with the limiters, you will lose the normal movement of the system.

The recovery plate has 9 holes. The first one is used for cleaning a collection needle after recovering a microdissection. You put a piece of cotton as a cleaning pad in a tube and put it at the first hole. In the cleaning operation, a collection needle comes down to hit the cotton pad. The position of the hitting with a collection 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, trips of 8.

### 2.2.3 digital microscope

A digital microscope consists of a 5M CMOS camera, a microscope lens, and two irradiation lamps. The position of the camera is fixed at the optimum position for observing specimen place on a dish placed in the sample holder. The focusing is carried out by rotating the lens. The positions and the directions of the two irradiation lamp can be changed with your hand. These two lamps irradiate a

specimen from below. The vertical line of the camera should be coincided with the X axis direction of the XY stage. If necessary, you can adjust the horizontal axis of the image by rotating camera.

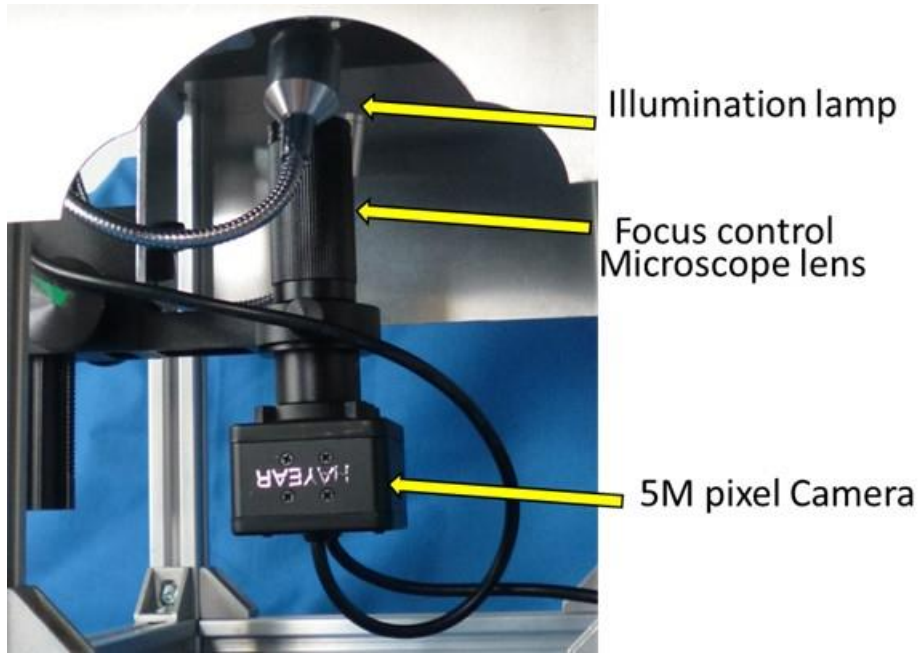


Figure 2-6 digital microscope

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 2-7)

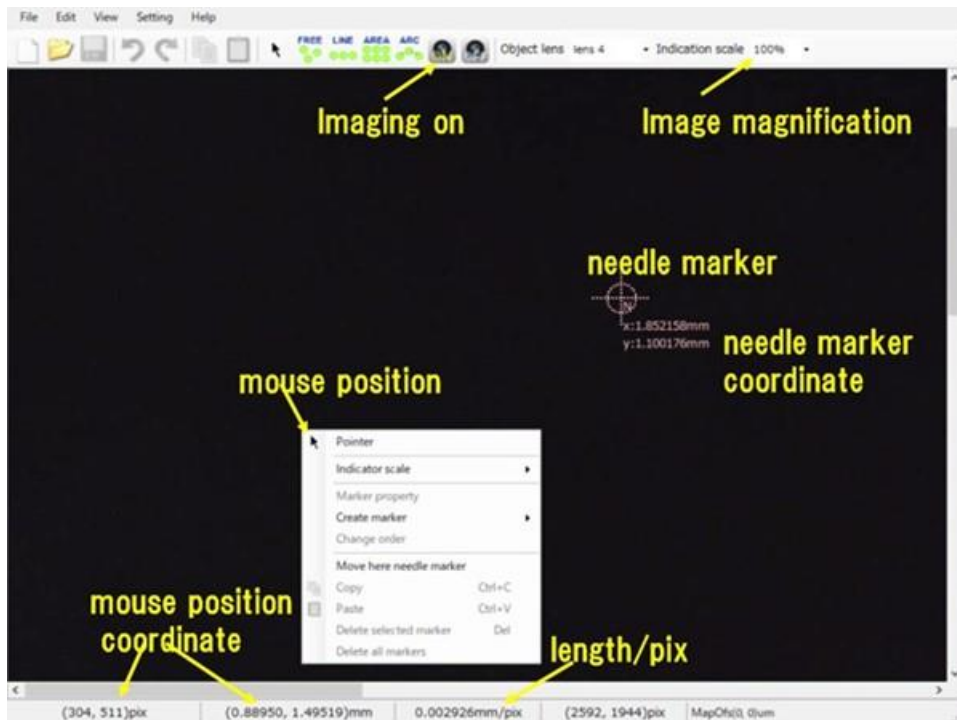


Figure 2-7 Screen image of Cell point marker



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.

Needle marker is expressed with a dotted line by clicking it with the mouse. Then it can be dragged with the mouse to the other position. You can move the needle marker by right clicking the position, where announcement appears as shown in figure 2-7. Then you just click “move here position marker” to move the position marker at 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 ratio. (see figure 2-8)

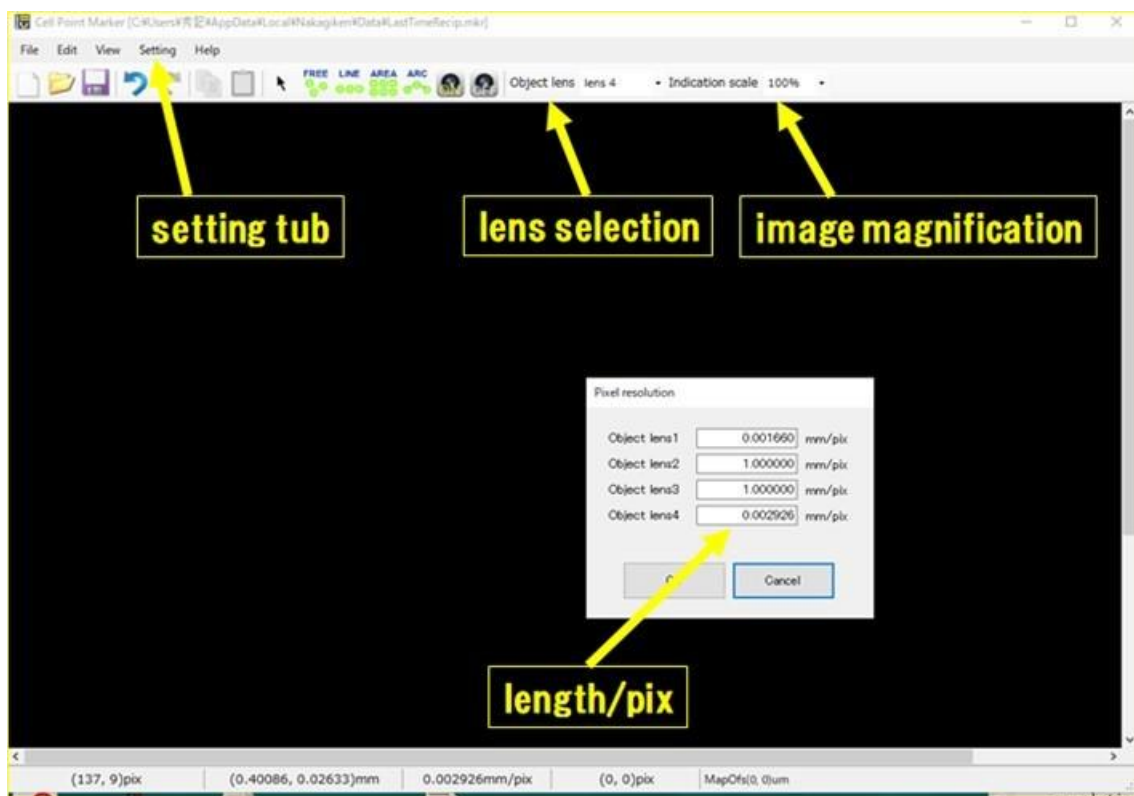


Figure 2-8 optimization of length/pix

#### 2.2.4 size and weight

The weights of Sampling Junior and the digital microscope are 2.6 kg and 2.4kg, respectively. Their sizes (D x W x H) are 210x180x157 and 240x212 x245(mm), respectively.

## 2.3 Accessories

- (1) 12V power supply (1)
- (2) Handy microscope (1)
- (3) collection needle (10)
- (4) cleaning wire (5)
- (5) LED ring light (1)
- (6) tools (1)
  - ( tweezers (1), pin vise (1), driver set (1), wrench set (1) )
- (7) accessories (1)
  - ( pulley belt (5), 50  $\mu$  m silicon rubber sheet (1), silicon tube (2), dish (2), injector for cleaning the main shaft of a collection needle unit (1) )
- (8) software CD (1)
- (9) ball point pen (2)
- (10) Handy light (2)
- (11) User manual (1)
- (12) Polishing tool (1)



- (1) power supply
- (2) handy microscope
- (3) collection needle
- (4) cleaning wire
- (5) LED ring light
- (6) tools(6-1 tweezers, 6-2 pin vise, 6-3 driver set, 6-4 wrench set)
- (7) accessories (7-1 pulley belt, 7-2 silicon rubber sheet(50  $\mu$  m), 7-3 silicon tube, 7-4 dish, 7-5 syringe for cleaning a main shaft pipe)
- (8) software CD for microscope image
- (9) ball point pen
- (10) handy light

Figure 2-9 Accessories except for a the user manual and the polishing tool



Figure 2-10 Polishing tool

## 2.4 Replacing the collection needle

A collection needle can be used normally for sampling microdissections 100 times before the cutting edge becomes dull with Sampling Junior. The dull cutting edge frequently fails to separate a microdissection from the specimen. It should be replaced with a new one or polished.

The replacement of a collection needle is carried out after removing the collection needle unit from the arm as follows.

(1) Removing a collection needle unit from the arm: A collection needle unit is fixed with a screw to the arm. At first you unscrew, then disconnect the connector, take off the ejection tube and pulley belt.

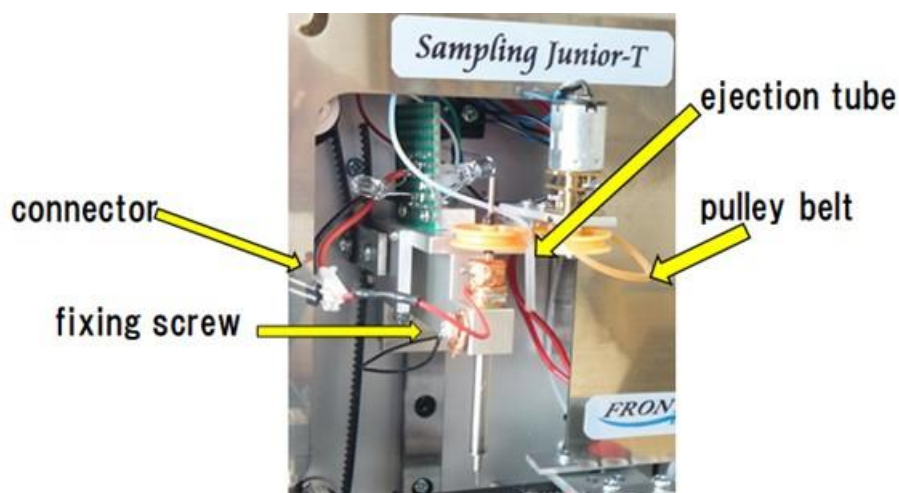


Figure 2-11 Removing a collection needle unit from the arm

(2) Take a collection needle out of the collection needle unit: A collection needle unit consists of a main shaft pipe, a cover pipe, a slit pipe and a coil spring as shown in figure 2-12. A collection needle is pressed into the main shaft pipe and fixed. The tip of a collection needle normally 2mm protrudes from the slit pipe. You push the slit pipe a little bit into the main shaft pipe to make the protruding part long. Then you hold the protruding part of the collection needle part tightly with tweezers. You push the tweezers with your thumb to remove the collection needle from the collection needle unit. Be careful not to slide the tweezers on the collection needle, which gives heavy damage on the needle tip. As the end of the collection needle is digging in the main shaft pipe, the collection needle is tightly connected to the main shaft pipe. However, if the digging is eliminated, the collection needle can be easily taken out of the collection needle unit.

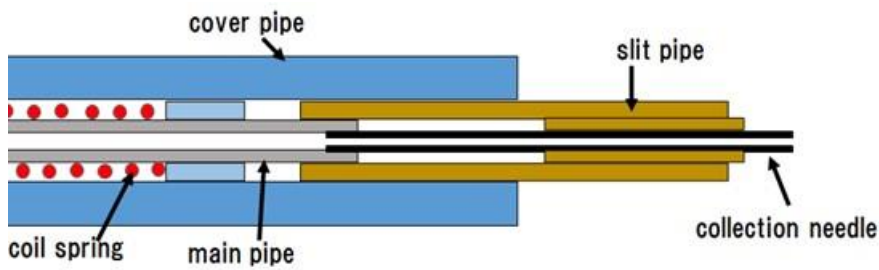


Figure 2-12 Structure of a collection needle



Figure 2-13 the way of taking a collection needle out of the collection needle unit

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



Figure 2-14 Cleaning of a main shaft pipe

- (4) Putting a new collection needle to a collection needle unit: You put the end of a new collection needle in the slit pipe with your hand. After pushing it to touch the edge of the main shaft pipe, you pinch the collection needle with tweezers tightly. Then you push it into the main shaft pipe with tweezers. You support the other side of the main shaft pipe with your hand during you push the collection needle into the main shaft pipe. You check if the collection needle is fixed by digging into the main shaft pipe by picking it out with the tweezers. You pick the slit pipe out a little bit so as that the collection needle 2mm protrudes from the slit pipe.

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

support the main pipe with the palm of your hand

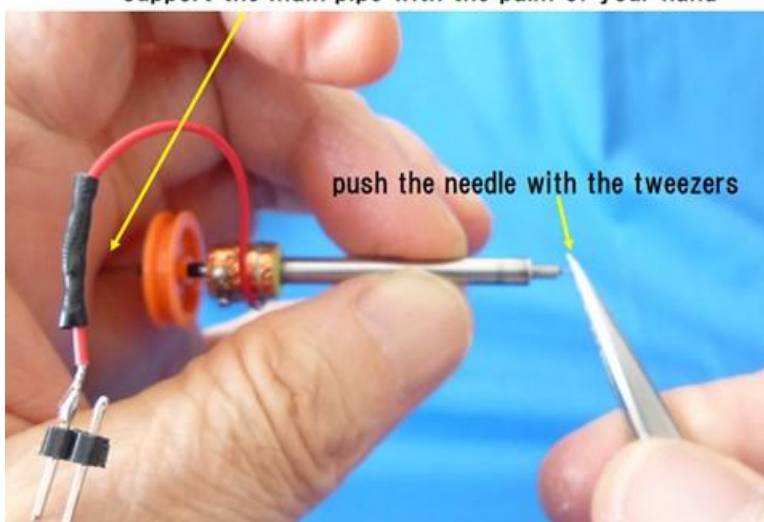


Figure 2-15 Mounting a collection needle on the collection needle unit

- (5) Put the collection needle unit back to the arm: Be careful not to hit the collection needle tip with the arm during its mounting on the arm. You fix the collection needle unit with a screw, connect the sensor lead to the connector, and put the pulley as well as the ejection tube back. Then you push the pushing rod on the cover roof for ejecting buffer solution as an ejection test.



pushing rod for ejecting solution manually

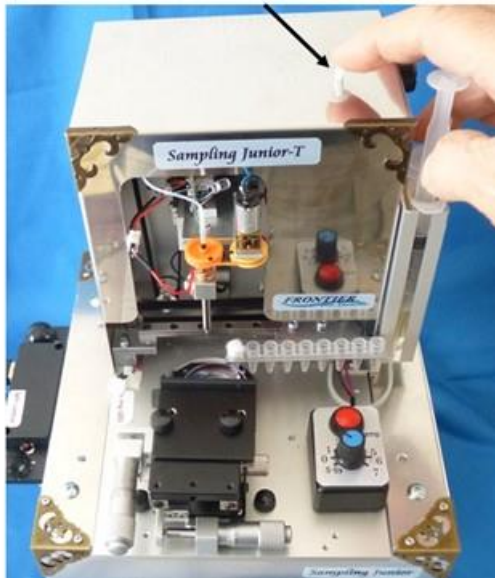


Figure 2-16 Ejection test with a pushing rod equipped on the cover roof

2.5 Sharpening a collection needle edge: A collection needle can be recovered repeatedly by sharpening its edge with a needle edge sharpener (option). It is carried out as follows.

(1) put a collection needle in the rotating shaft pipe of the needle edge sharpener with tweezers tightly.

(2) put a cleaning wire into the collection needle from the needle edge by more than 10mm. It is possible to put the wire from the reverse side of the needle edge sharpener. (see figure 2-17)



Figure 2-17 Setting for sharpening the edge of a collection needle

(3) Cover a abrasive cloth on the tip of a collection needle. Then sandwich the abrasive cloth covered tip with your fingers. Don't vent the cleaning wire.

(4) Turn on the rotation motor to polishing the tip with the abrasive cloth for about 30 sec.

(5) Check the shape of the tip with a handy microscope. If the shape of the tip is smooth and good, you can use the collection needle as a sampling needle. If it

seemed an irregular tip, you touch the tip with a piece of sand paper (#2000) or a whetstone (#3000) over rotating the tip for 2 sec. Then polish it again with the abrasive cloth.

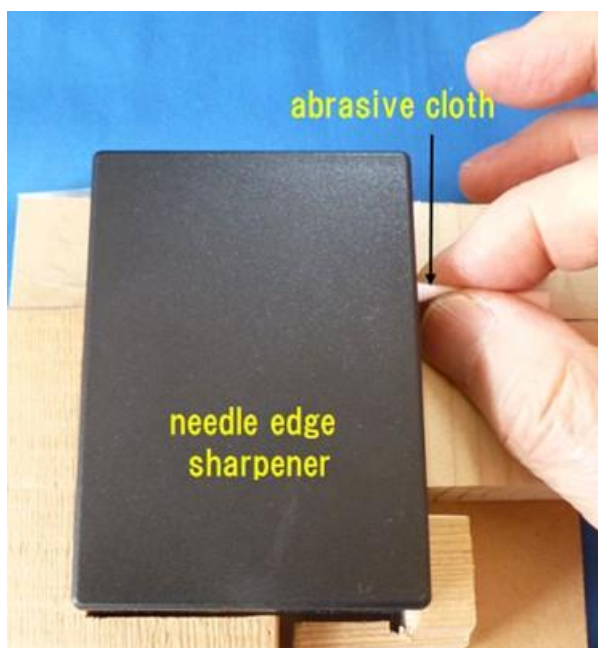


Figure 2-18 Polishing the edge of a collection needle with a piece of abrasive cloth