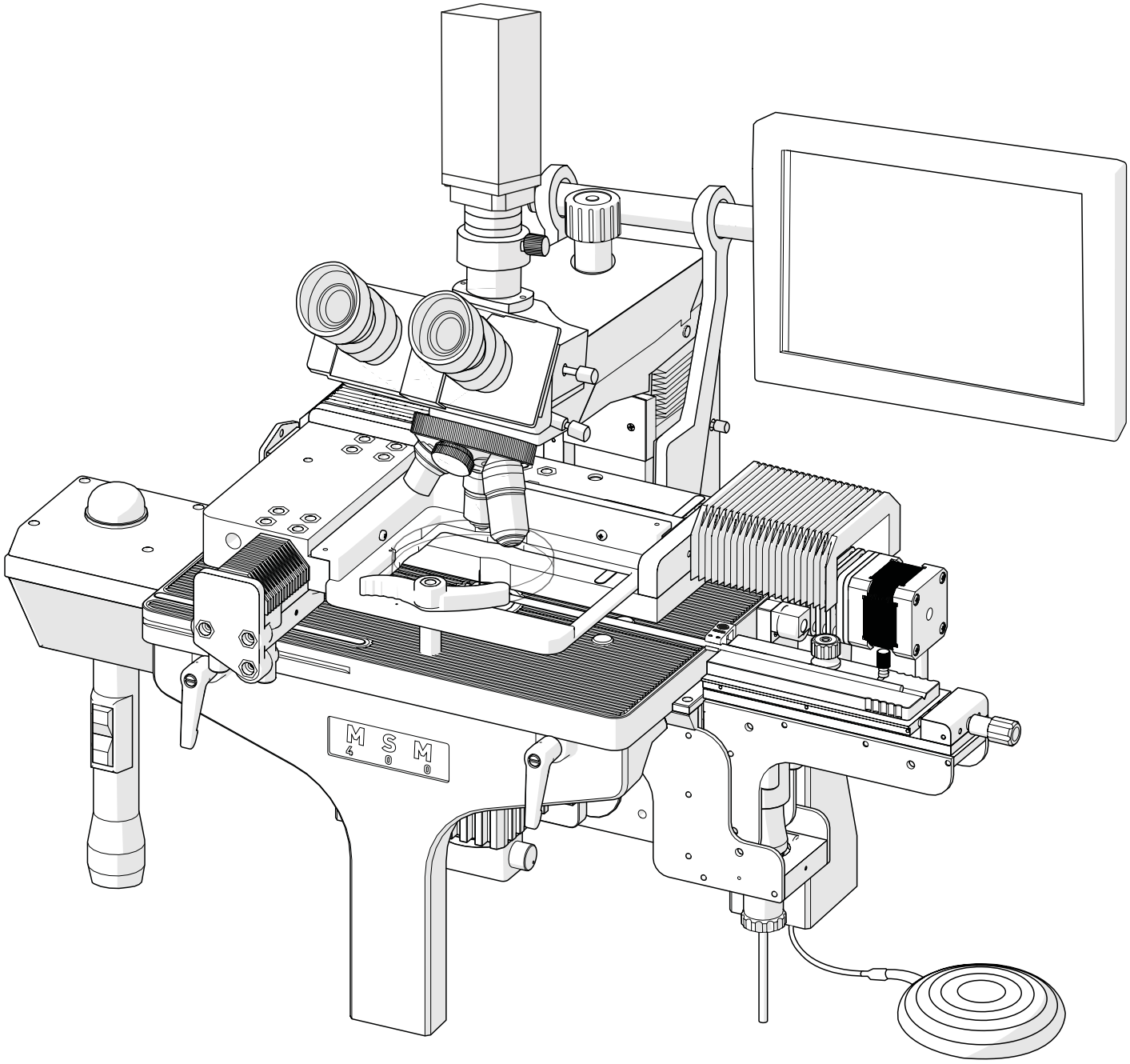
SINGER INSTRUMENTS

A RESPONSIBILITY TO SCIENCE!

MSM™ 400

USER GUIDE v2.8



BEFORE YOU START!

- Needle are sharp! Take care when handling dissection needles as they are very sharp.

- Ensure light path lever is open. If the Light Path Lever is closed you will not be able to see the plate. See step 6 on page 12.

- Remove plate and tool holder before moving the MSM. To avoid equipment damage, ensure that all consumables and accessories are removed before moving the MSM 400.

- If possible, keep your packaging. You may need to move your MSM 400 in the future to another lab or return it to Singer HQ. For this you will need to keep the original box, packing foam and red transit bolts — they will be expensive to source in the future!

- Ensure power is switched off at the mains while assembling. Do not insert the power cables into the MSM while the mains power is on.

- Do not remove the clamp screw. See step 5 on page 12.

MSM 400™

INTRODUCTION

The MSM 400™ is a computer-controlled, motorised microscope platform for the dissection and documentation of yeast and fungal cells and spores. The small footprint means that there is room for an MSM 400 in every lab and multiple users can quickly perform, document and store their dissections, ageing studies and screens.

USER GUIDE

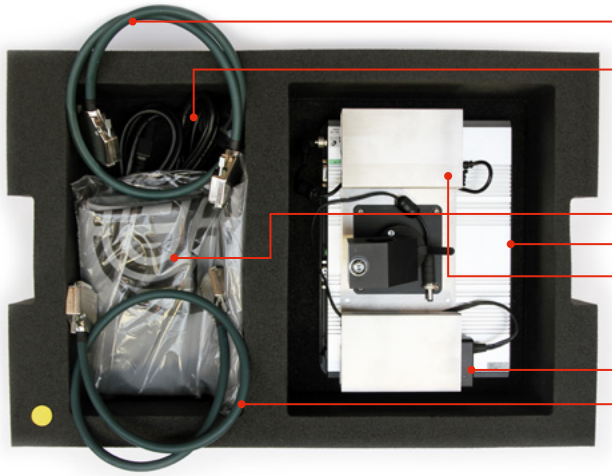
Follow these instructions alongside the on screen instructions to get the most out of the MSM 400™ dissection workstation. This guide outlines basic operation of the MSM 400™ as well useful dissection techniques. Read through this guide and you'll be ready for the exciting world of tetrad dissection.

DISCLAIMER

At Singer Instruments, we are constantly seeking to improve our products and adapt them to the requirements of modern research techniques and testing methods. This involves modification to the mechanical structure and optical design of our instruments. Therefore, all descriptions and illustrations in this user guide, including all specifications are subject to change without notice.

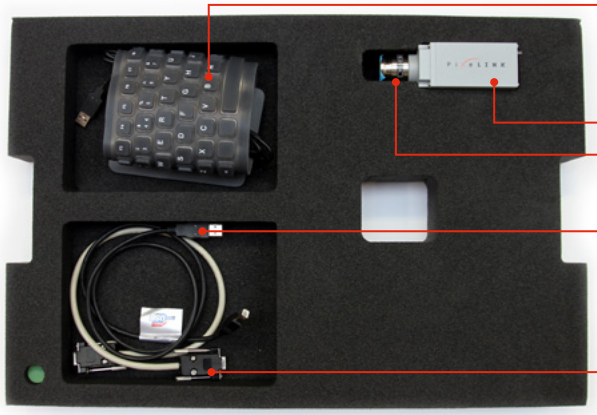
OUT OF THE BOX

Find out what comes with the MSM 400. We'll take you through the steps involved in unboxing and assembling ready for dissection.



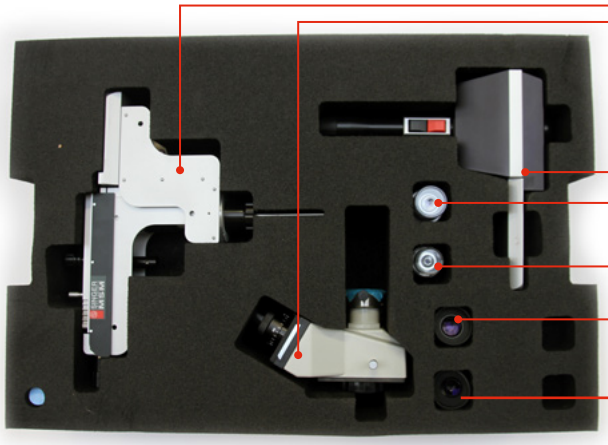
YELLOW TRAY

- 25 Way D Connector Cable
- Mains Power Cable x2
- Dust Cover
- MCI Touch-screen
- MCI Power Supply
- MSM Power Supply
- 15 Way D Connector Cable



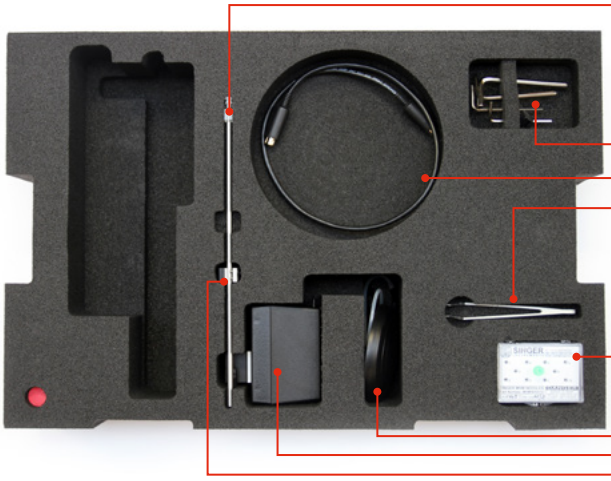
GREEN TRAY

- Flexible Keyboard
- CMOS Camera
- DIPS Adaptor
- Camera USB A-B Cable
- Serial Cable



BLUE TRAY

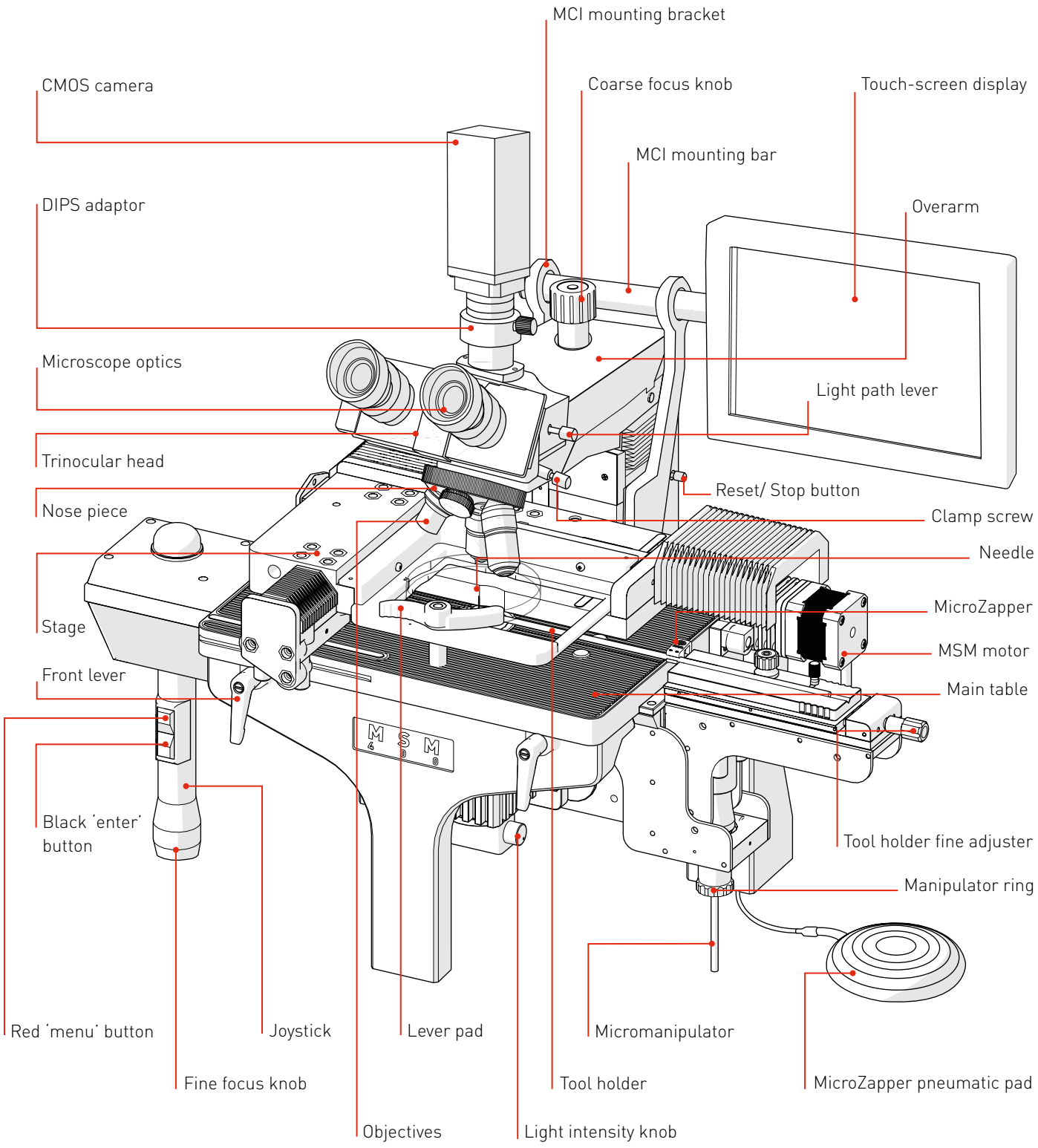
- Micromanipulator
- Trinocular Head
- Joystick
- Objective x4
- Objective x20
- 2x Eyepieces



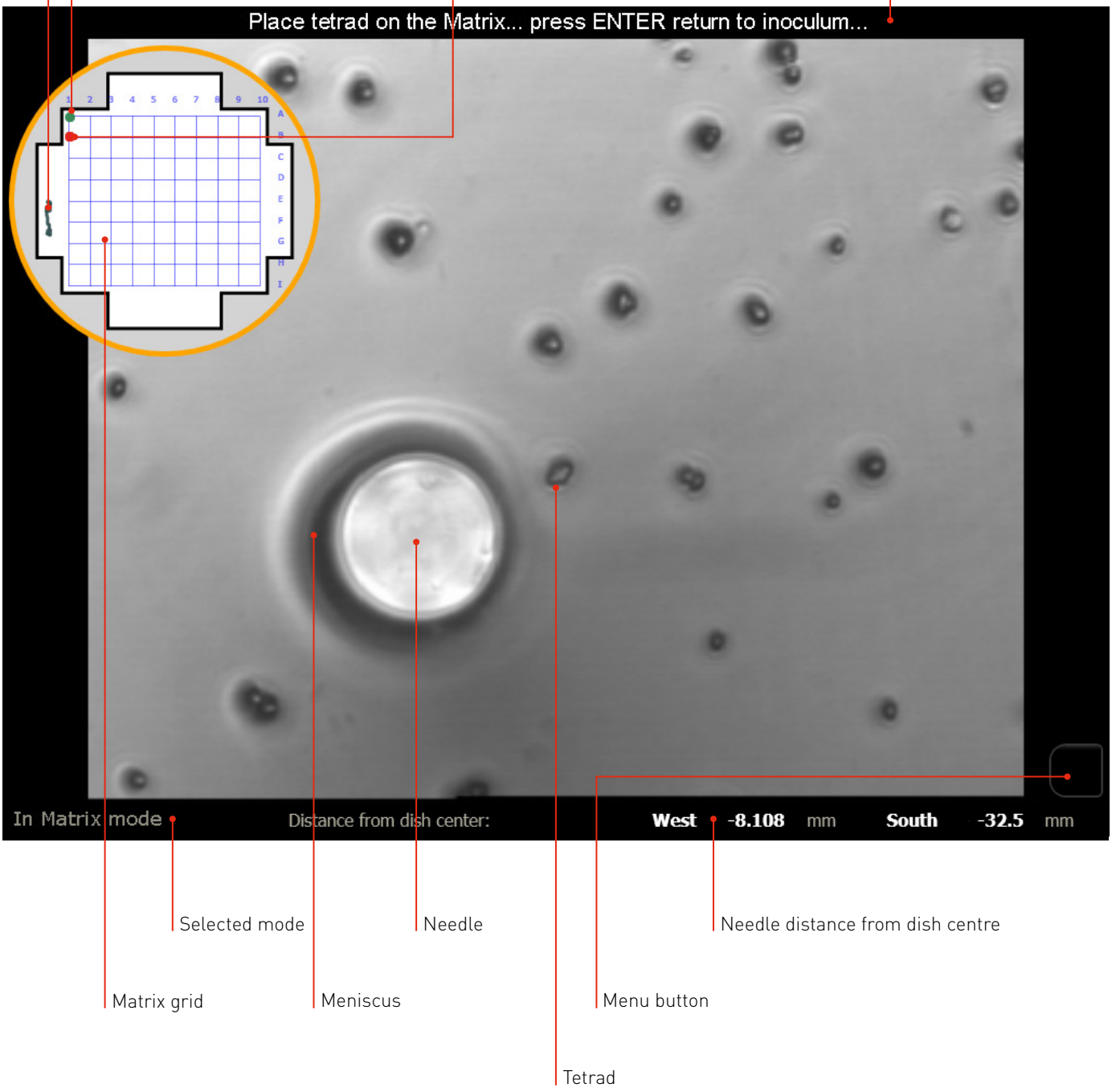
RED TRAY

- Tool Holder
- MSM Tools
- MicroZapper Power Supply Cable
- Forceps
- Needles
- MicroZapper Pneumatic Pad
- MicroZapper Module
- MicroZapper Body

MSM 400 WITH ALL OPTIONAL EXTRAS

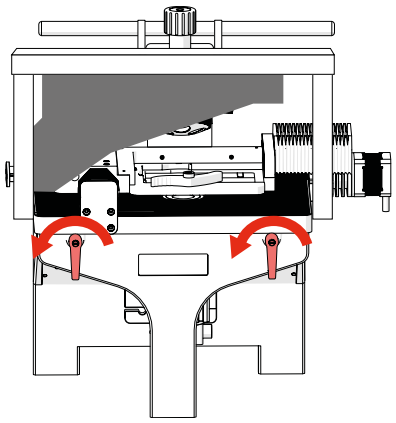


MSM 400 SOFTWARE



SETTING UP

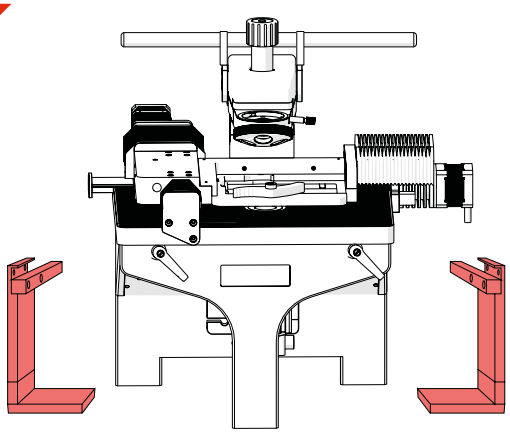
1



BOX 1

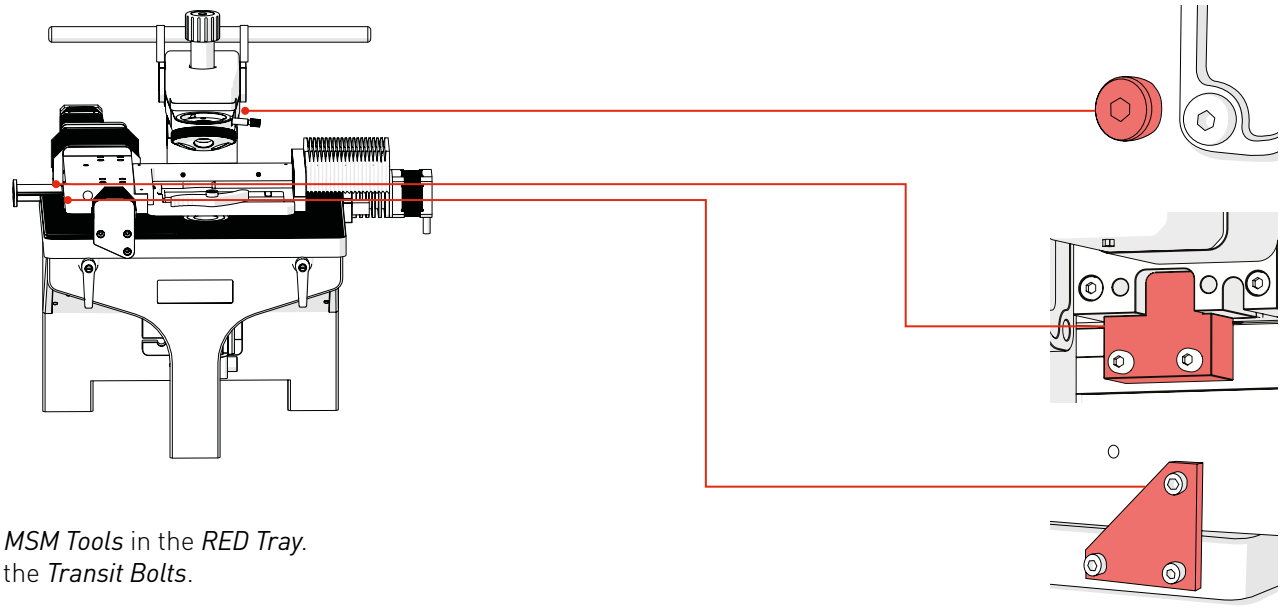
- Open and remove packaging, and lift out carefully. CAUTION: Heavy. May require two people.
- Loosen the *Front Levers*.
- NOTE: We recommend keeping your packaging.

2



- Remove the *Transit Handles (see diagram on top of transit table).*

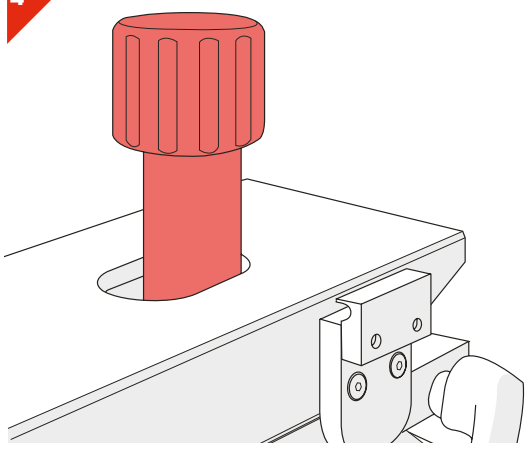
3



BOX 2

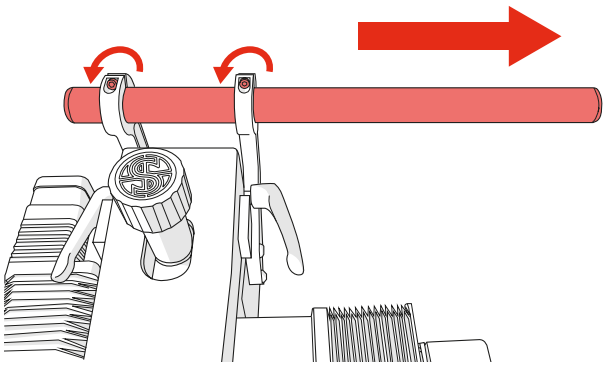
- Find the *MSM Tools* in the *RED Tray*.
- Remove the *Transit Bolts*.

4



- Turn *Coarse Focus Knob* to raise the *Overarm* all the way up.

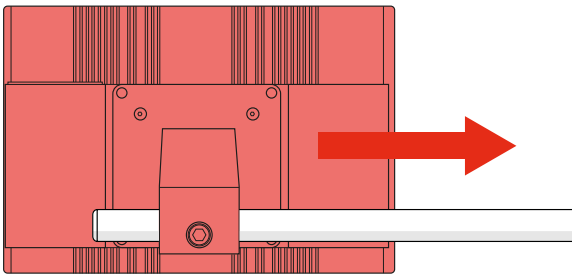
5



- Slacken the *MCI Mounting Bracket Screws* and adjust the *MCI Mounting Bar* as shown.

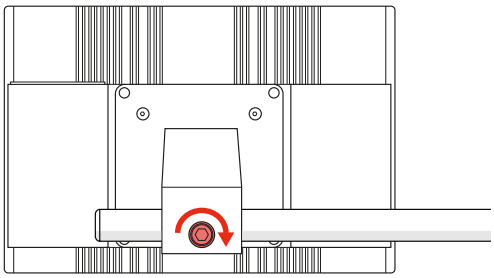
MCI CONNECTIONS

1



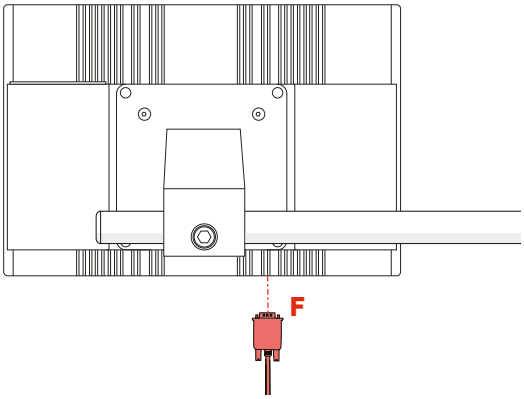
- Slide the *MCI Touch-screen* onto the *MCI Mounting Bar.*

2



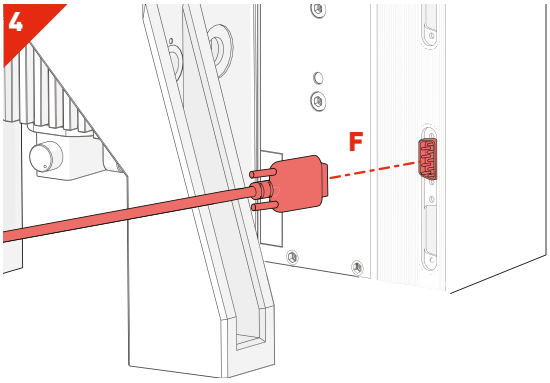
- Secure the *MCI Touch-screen* using the *MSM Tools*.

3



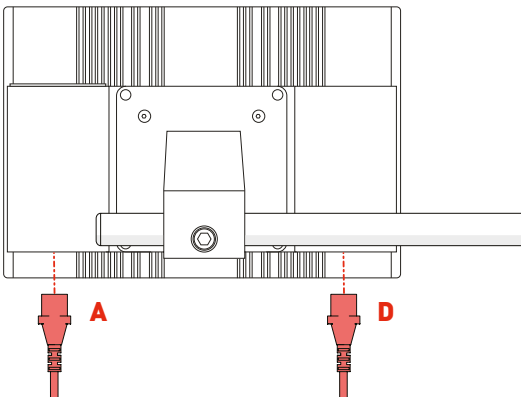
- Connect the *Serial Cable* to the serial port on the *MCI Touch-screen (see connection F on page 17).*

4



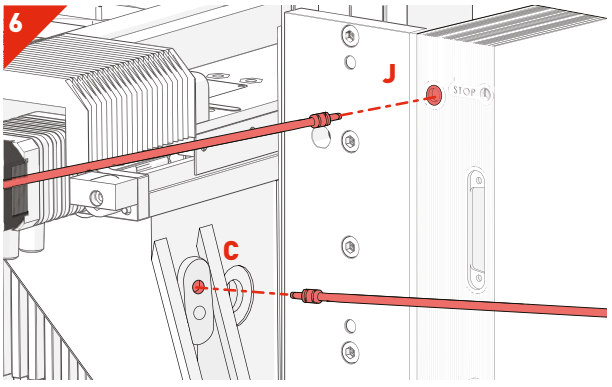
- Connect the other end of the *Serial Cable* to the serial port on the *MSM 400 (see connection F on page 17).*

5



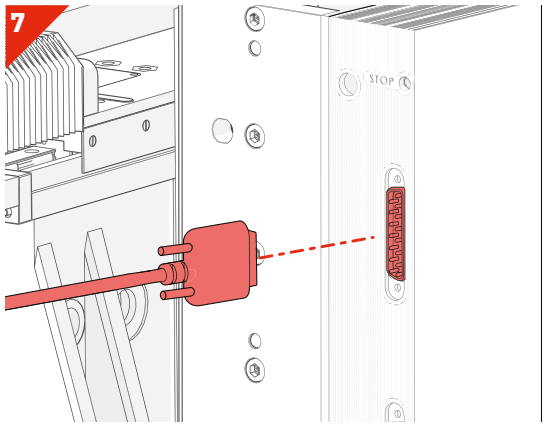
- Insert the two *Mains Powers Cables* into the *Power Supplies* attached to the *MCI (see connections A & D on page 17).*
- WARNING: ensure power is switched off at the mains.

6

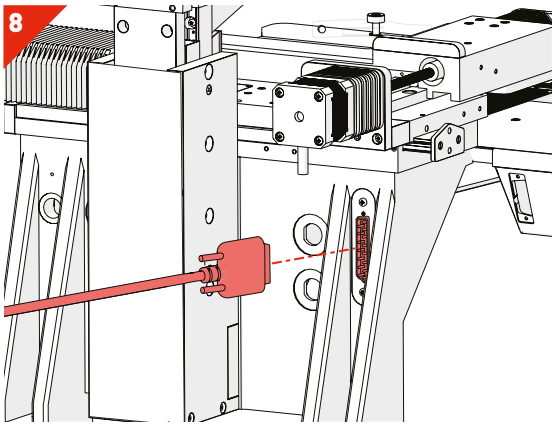


- Connect the *MSM Power Cable* into the *MSM 400 (see connection C on page 17).*
- Connect the *BakPak Power Cable* into the *MSM 400 (see connection J on page 17).*

MCI CONNECTIONS

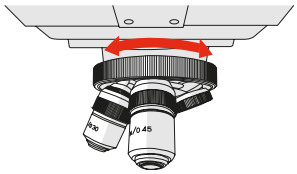


· Connect the *25 way connector cable* to the back of the *MSM 400* as shown *(see connection K on page 17)*.



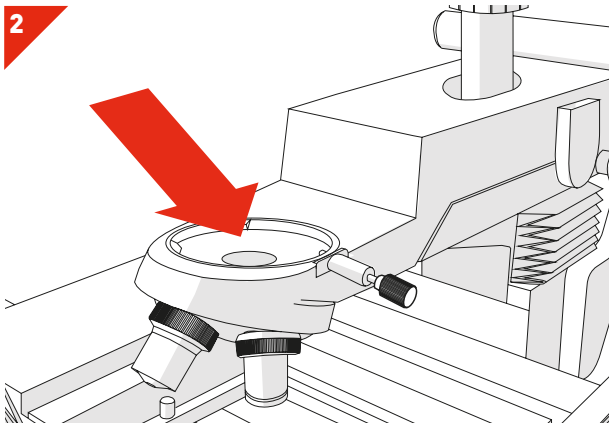
· Connect the other end of the *25 way connector cable* to the back of the *MSM 400* as shown *(see connection K on page 17)*.

1



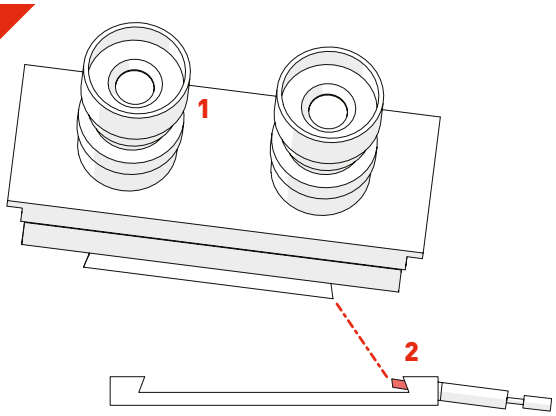
· Choose any hole on the *Nosepiece*, remove dustcap and screw *x20* and *x4 Objectives* into place.

2



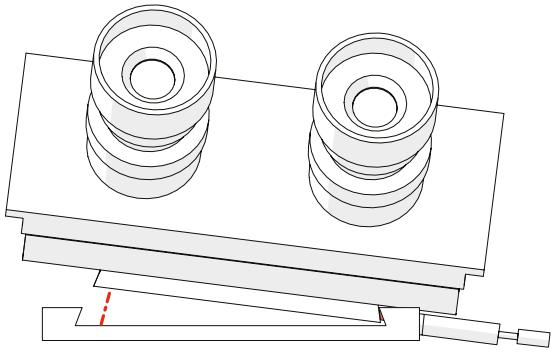
· The *Trinocular Head* attaches to the MSM 400 above the *Objectives*..

3



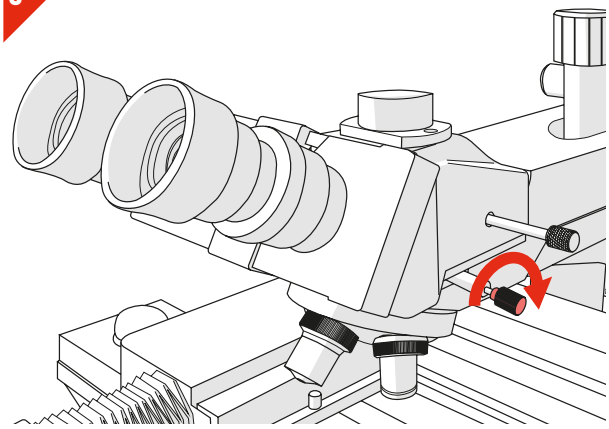
· Insert the *Eye Cups* into the *Trinocular Head* (1).
· Angle the *Trinocular Head* and lower it to depress the *Spring Catch* (2).

4



· Lower the left side of the *Trinocular Head* into the *Clamp*.

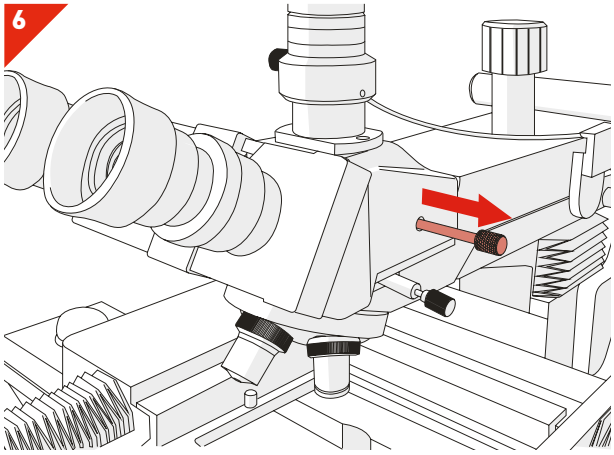
5



· Tighten the *Clamp Screw*. This will lock the dovetail mechanism, securing the Trinocular Head.

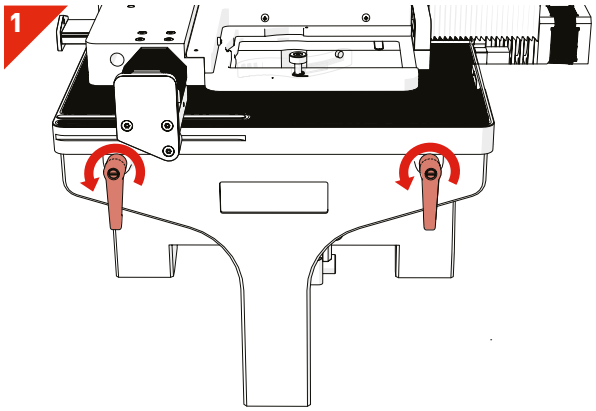
WARNING: Do not remove the *Clamp Screw*.

6

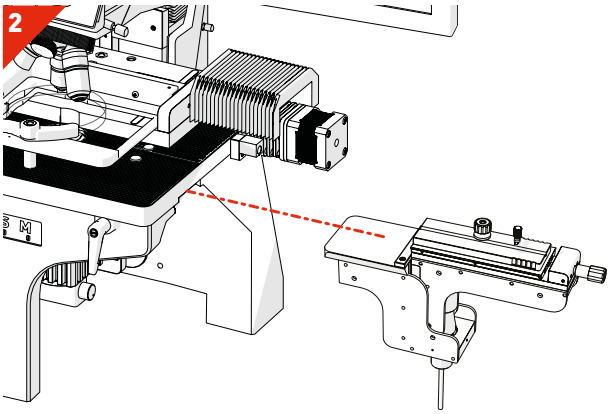


· Open the light path by fully extending the *Light Path Lever*.

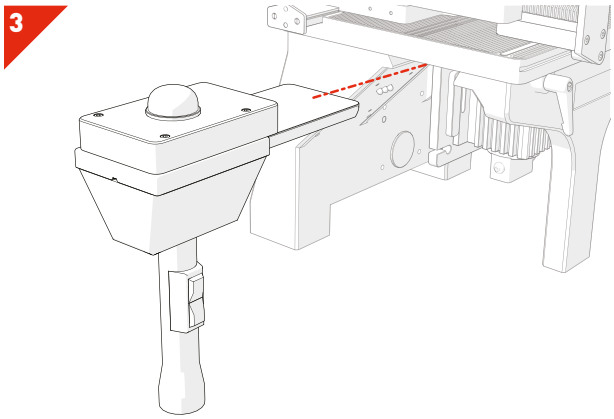
MICROMANIPULATOR & JOYSTICK



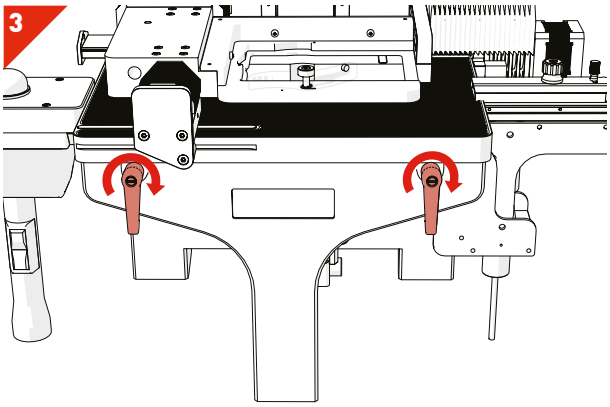
· Ensure the *Front Levers* are still loose.



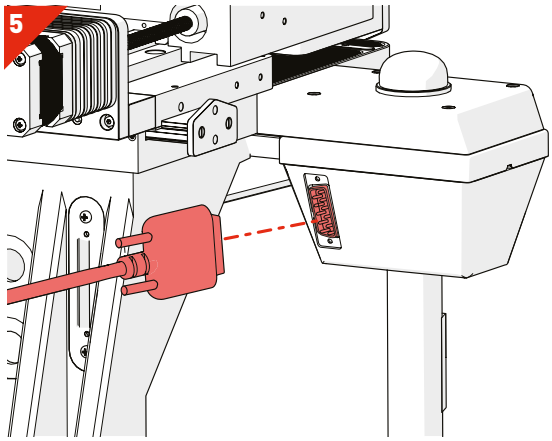
· Slot the *Micromanipulator* into the grooves on the right of the *Main Table*.



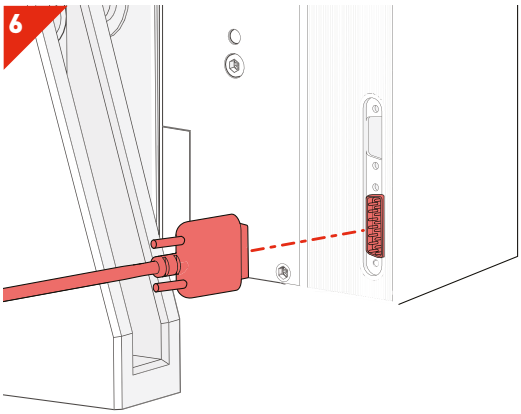
· Slot the *Joystick* into the grooves on the left of the *Main Table*.



· Tighten the *Front Levers.*
· WARNING: Ensure levers point down. If they point up the motor can crash.

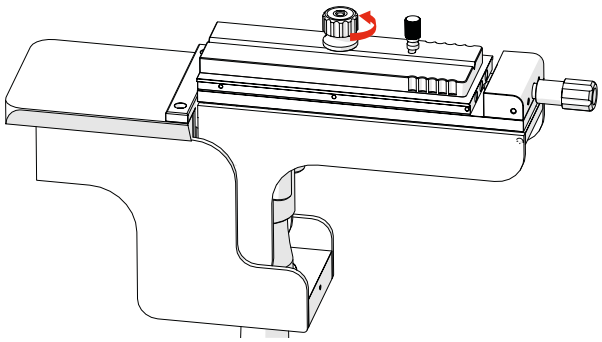


· Connect the *15 way connector cable* to the back of the *Joystick (see connection L on page 17)*.



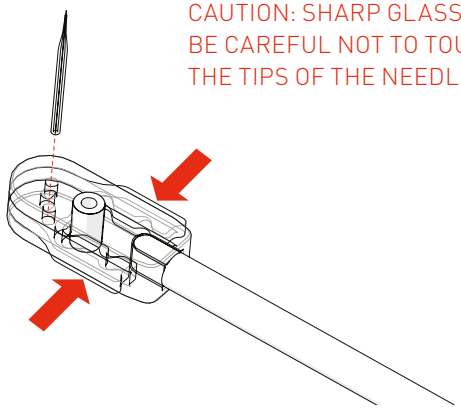
· Connect the other end of the *15 way connector cable* to the back of the *MSM 400 (see connection L on page 17)*.

1



· Loosen the Micromanipulator *Securing Knob* ready for the toolholder.

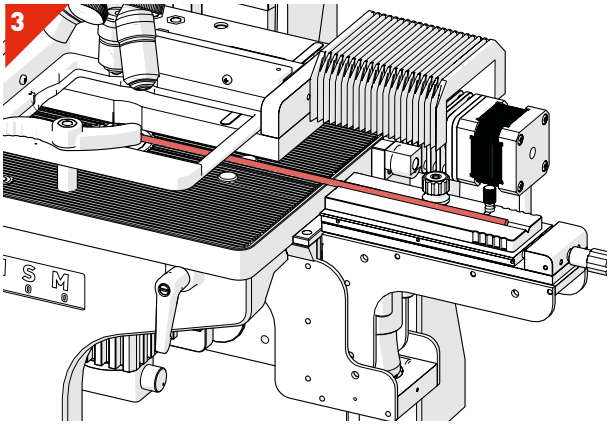
2



CAUTION: SHARP GLASS!
BE CAREFUL NOT TO TOUCH
THE TIPS OF THE NEEDLES.

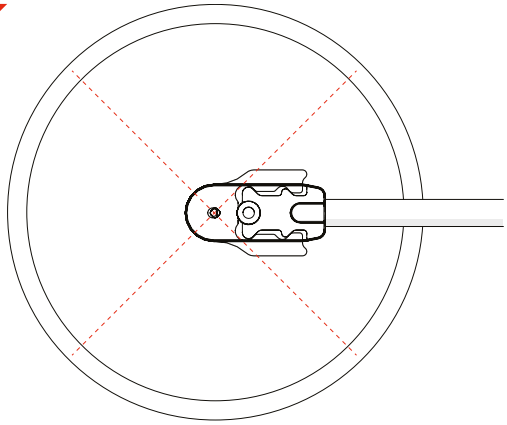
· Squeeze the tabs of the *Toolholder* and, using the *Tweezers*, carefully insert the *Needle* and let go of the tabs. The bottom of the needle should be flush with the Toolholder.

3



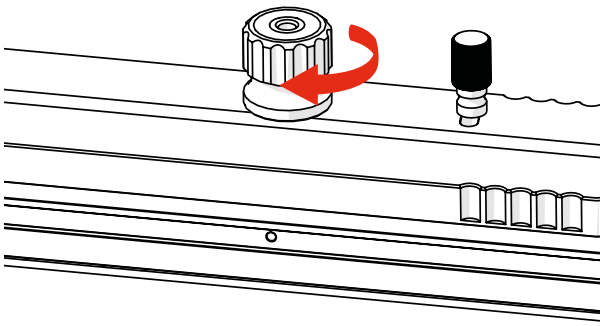
· Very carefully slide the *Toolholder* into the groove on top of the *Micromanipulator* with the needle pointing upwards.

4



· Try and align the needle with the centre of the light path. We'll fine tune this on page 19.

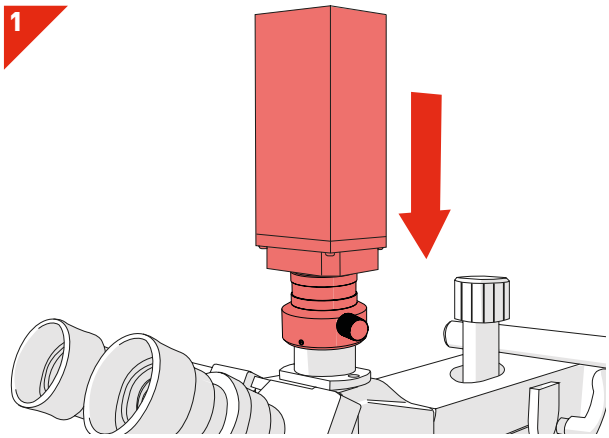
5



· Tighten the Micromanipulator *Securing Knob* to lock the toolholder in place.

OPTIONAL EXTRAS

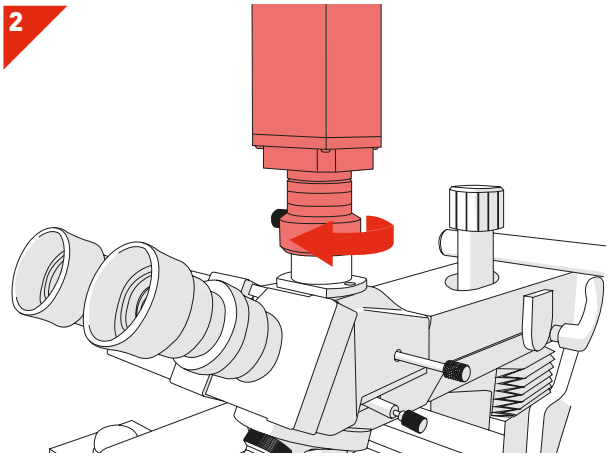
1



CMOS CAMERA

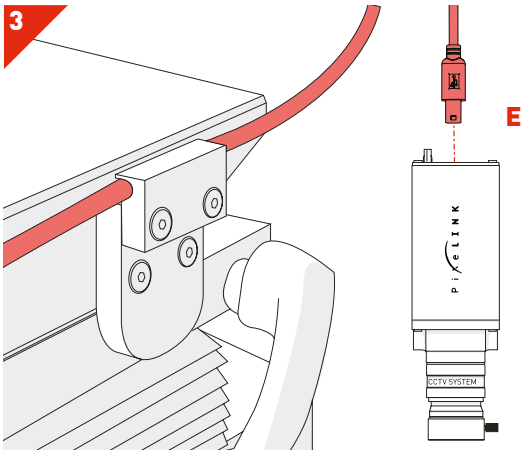
- Insert *CMOS Camera* with *DIPS Adaptor* into the *Trinocular Head*. If the camera is not already attached to the DIPS Adaptor, attach it and secure with the DIPS locking screw.

2



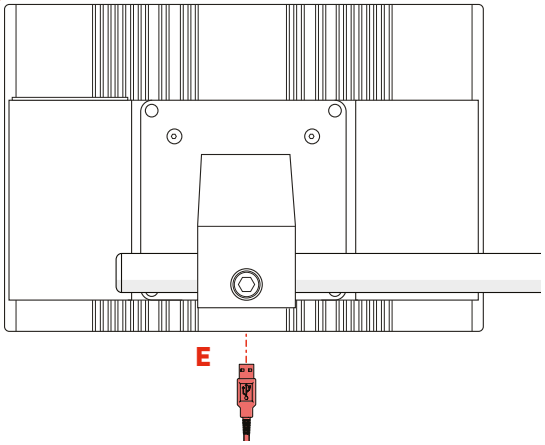
- Screw in the *CMOS Camera* to secure.
- NOTE: Ensure the camera's serial label is at the back, otherwise the image will be upside down!

3

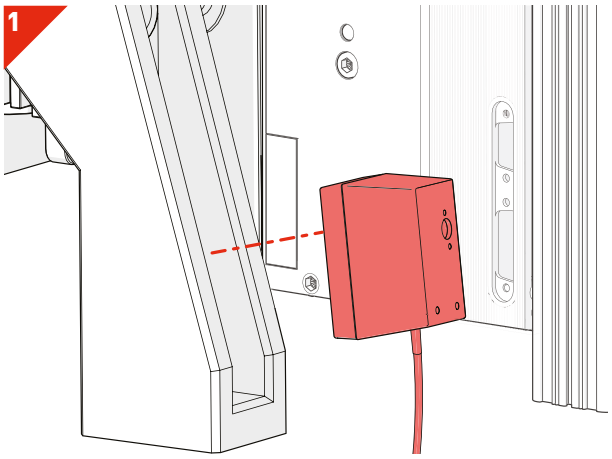


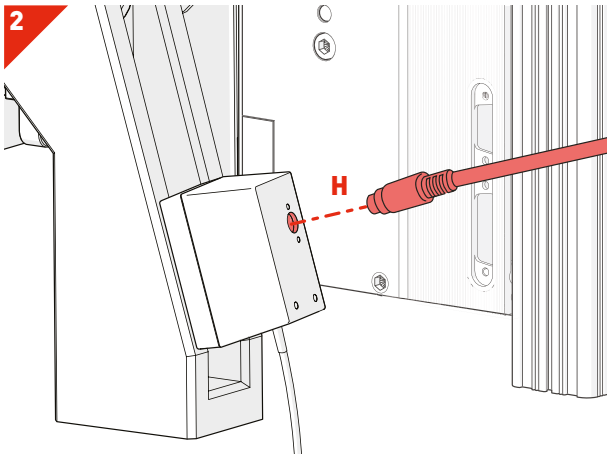
- Connect the *USB A-B Cable* into the top of the *CMOS Camera*.
- Slide the cable slack under the *Cable Cleat*.

4



- Connect the other end of the *USB A-B Cable* into the USB port on the *MCI Touch Screen (see connection E on page 17)*.

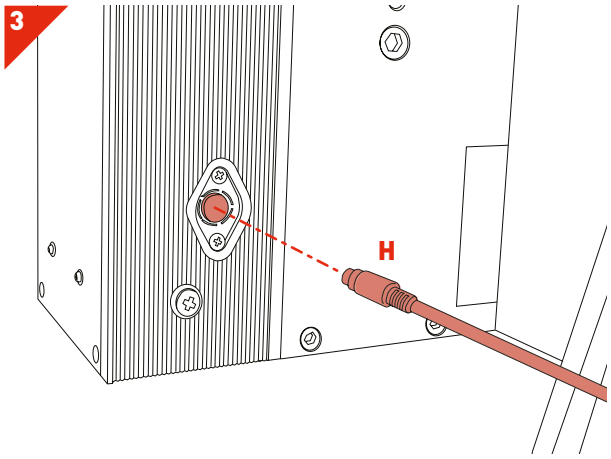


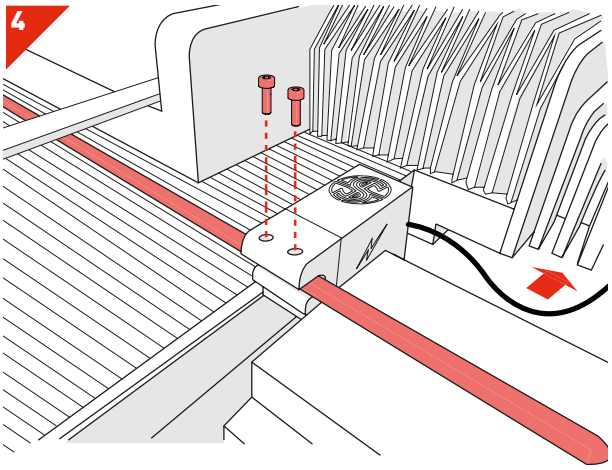


MICROZAPPER

· Slot the *MicroZapper Module* into the right rear leg of the *MSM*.

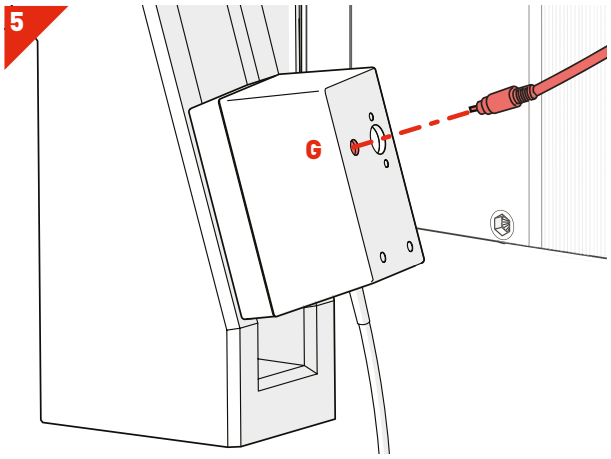
· Plug the *MicroZapper Power Supply Cable* into the back of the *MicroZapper Module (see conntection H on page 17).*

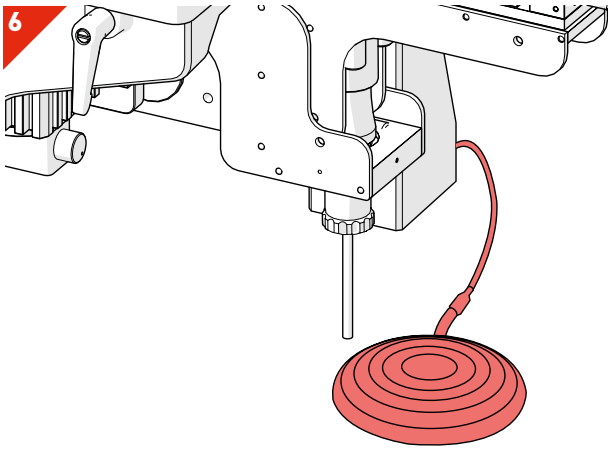




· Plug the other end of the *MicroZapper Power Supply Cable* into the *MSM 400.*

· Attach the *MicroZapper Body* to the *Tool Holder* in between the micromanipulator and the main table.
· Fix into position with the *Microzapper Screws.* Pass the *MicroZapper Body Cable* under the *MSM Motor.*



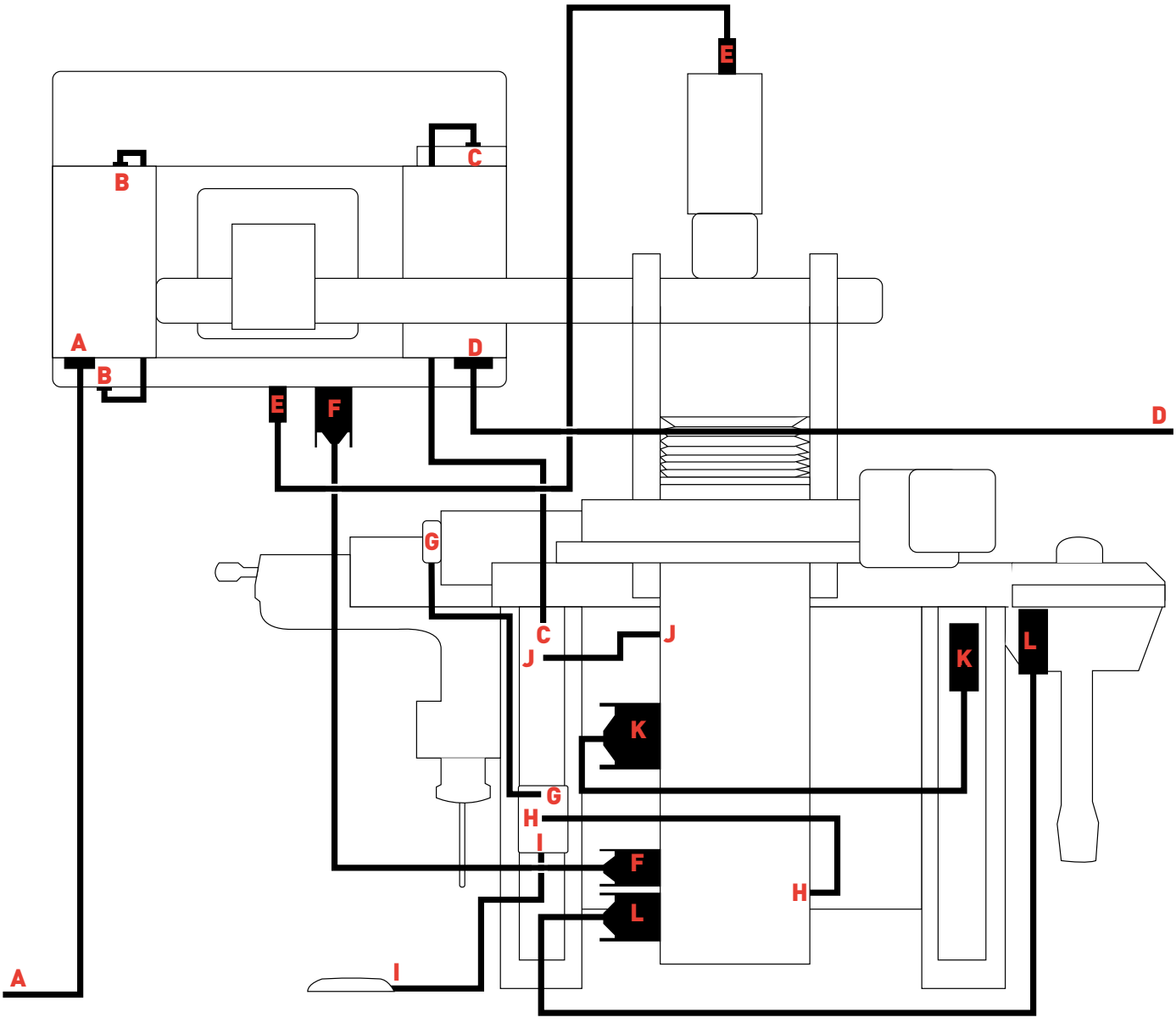


· Plug the *MicroZapper Body Cable* into the back of the *MicroZapper Module (see connection G on page 17).*

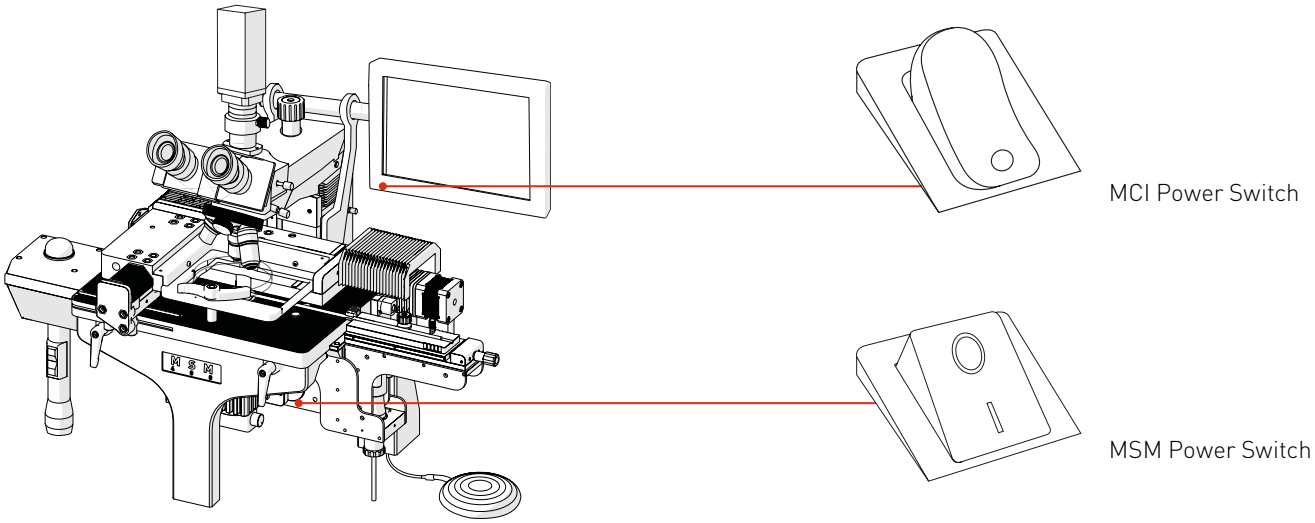
· Position the *MicroZapper Pneumatic Pad* under the *Micromanipulator* to allow ease of use during dissection.

CONNECTION POINTS

Schematic for all main connections.



A - Mains power cable (country specific)
B - MCI power supply - power 12vdc
C - MSM power supply - power 24vdc
D - Mains power cable (country specific)
E - Camera USB A-B cable
F - Serial cable
G - MicroZapper body cable
H - MicroZapper power supply cable
I - Air tube for pneumatic pad
J - Bakpak power cable
K - 25 way D connector cable
L - 15 way D connector cable



POWERING UP

- Switch on *Mains power sockets*.
- Set the red *MSM power switch* to the *ON* position.
- Turn on the *MCI* via the switch at the bottom left of the screen.

POWERING DOWN

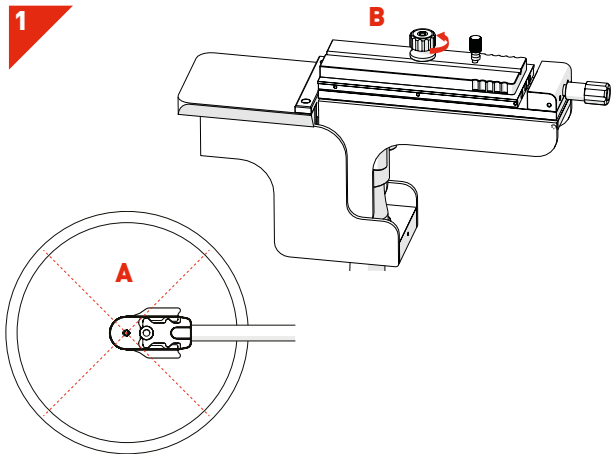
- Select *Shut Down* from the protocol menu.
- The MCI will turn off automatically *(may take a few moments)*.
- Set the red *MSM power switch* to the *OFF* position.
- Switch off *Mains power sockets*.

YOU'RE READY TO GO!

Now refer to your *MSM 400 User Guide* to get the most out of your MSM 400.

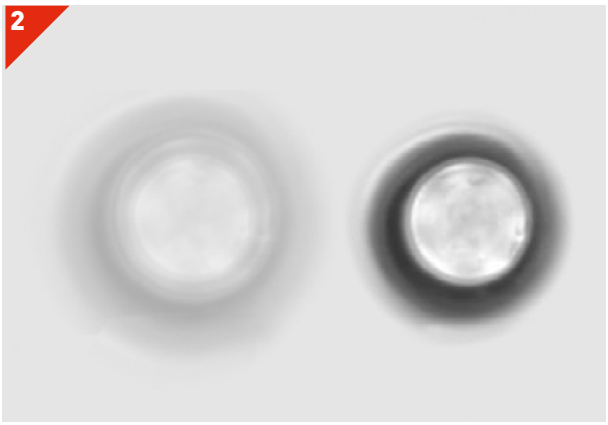
CENTRE NEEDLE

1



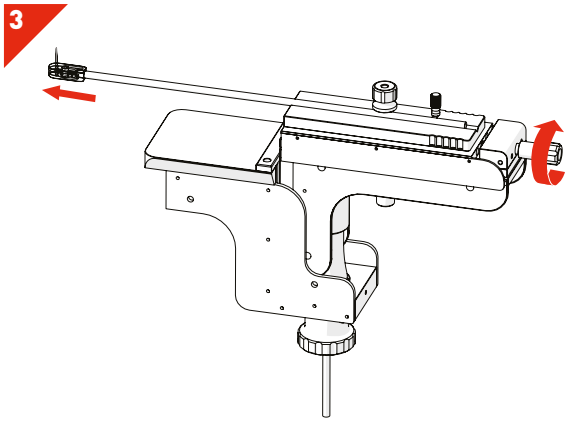
- Ensure the *Toolholder* is roughly in the centre of the light path (A). To adjust the position loosen the *Securing Knob* and slide the *Toolholder* into the correct position (B). Re-tighten when complete.

2



- Turn on the MSM 400 and focus the *Needle* using the instruction on page 34.

3

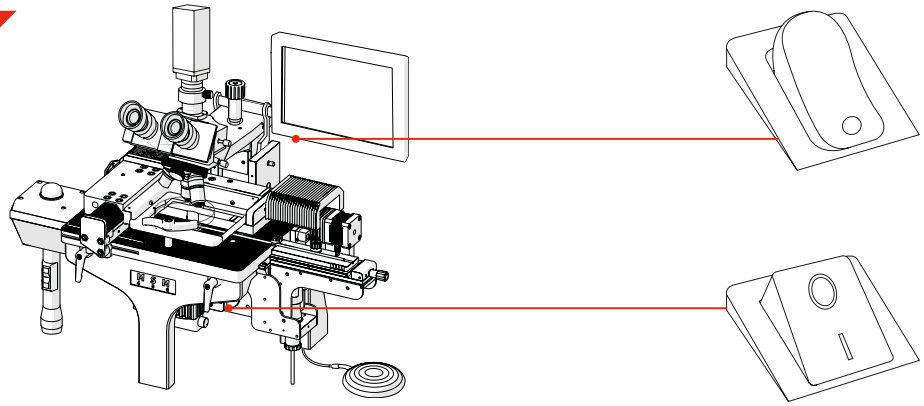


- Use the *Fine Adjustment Knob* to move the needle into the centre of your field of view.
- Clockwise will move the needle left and anti-clockwise will move the needle right.
- NOTE: Centring the needle is a crucial step for optimal tetrad dissection.

USING YOUR MSM 400

Your MSM 400 is all set up, let's take a look at how to use it!

1

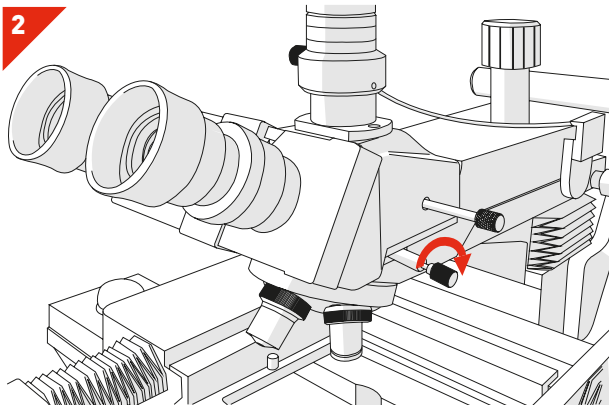


POWERING UP

· Ensure the *MSM 400* and *MCI Power Cords* are plugged in.

· Turn on the *MSM 400 power switch* under the stage and the *MCI power switch* at the bottom of the monitor.

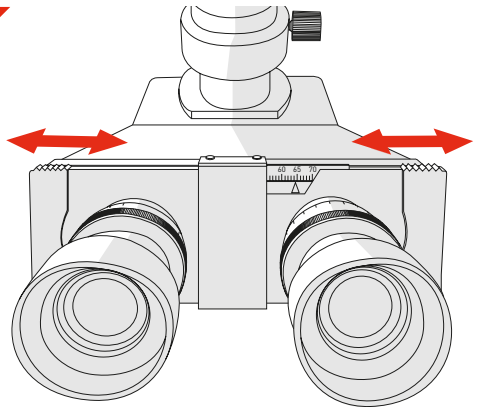
2



SECURING TRINOCULAR HEAD

· To ensure the *Trinocular Head* is secured, tighten the *Securing Knob* in a clockwise direction.

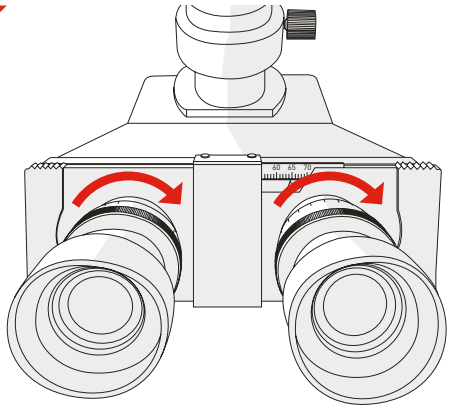
3



ADJUSTING EYEPIECES

· Slide the *Eyepieces* to a comfortable position using the grips on the *Trinocular Head*.

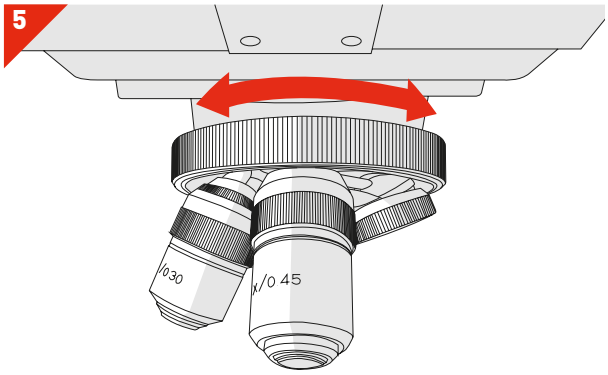
4



FOCUSSING EYEPIECES

· Rotate the *Eyepieces* to focus the scope.

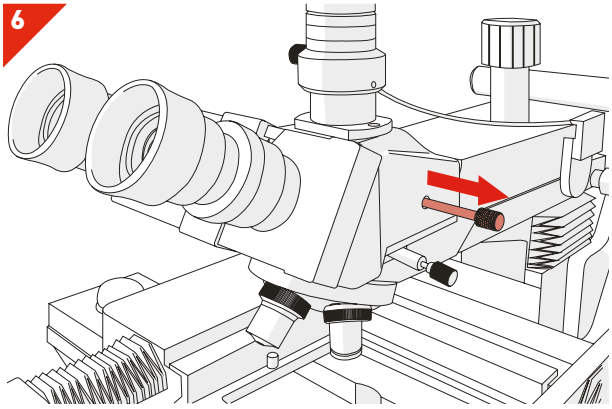
5



CHANGING OBJECTIVES

· The *Nosepiece* houses up to four *Objectives* and can be rotated 360° in both directions for easy selection.

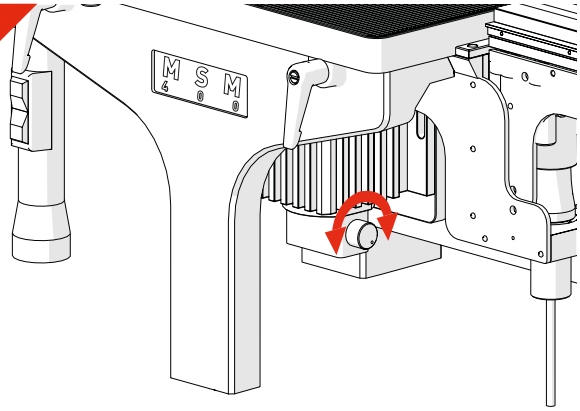
6



OPENING LIGHT PATH

· To open the light path to the *CMOS Camera*, fully extend the *Light Path Lever*.

7

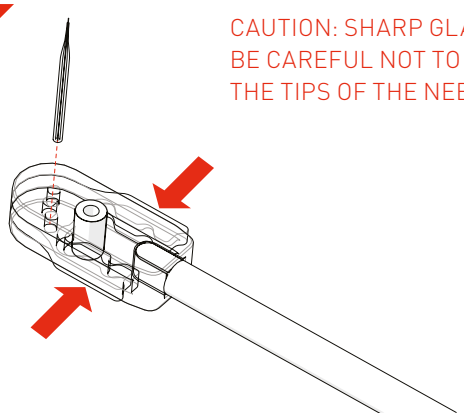


ADJUSTING LIGHT INTENSITY

· Rotate the *Light Intensity Knob* to alter the lamp brightness.

8

CAUTION: SHARP GLASS!
BE CAREFUL NOT TO TOUCH
THE TIPS OF THE NEEDLES.

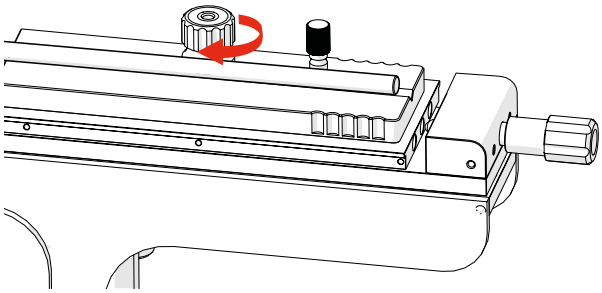


INSERTING NEEDLE

· Squeeze the tabs of the *Tool Holder* and insert the *Needle* with the tip protruding .

· Let go of the tabs of the *Tool Holder* to secure the *Needle*.

9

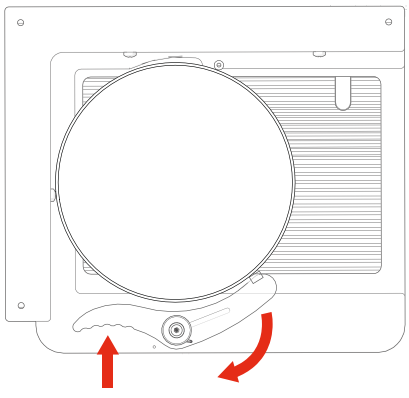


ATTACHING TOOL HOLDER

· Slot the *Tool Holder* into the groove on top of the *Micromanipulator*.

· Ensure the *Needle* is pointing straight up then secure by tightening the Securing Knob in a clockwise direction.

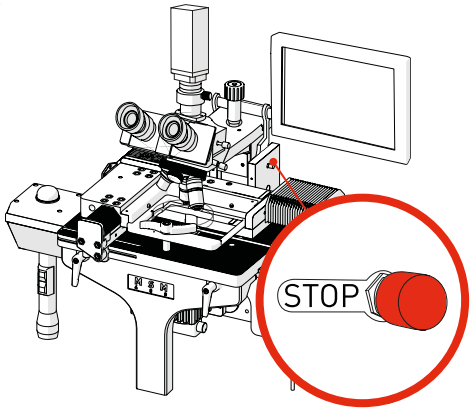
10



INSERTING PETRI DISH

· Petri dish mount

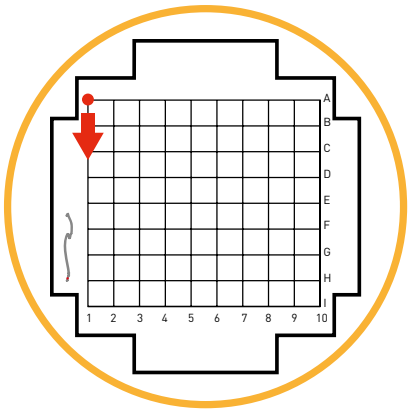
11

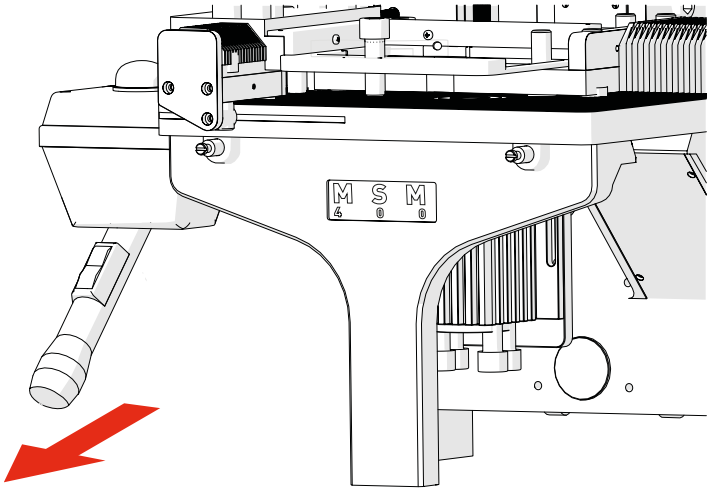


RESET/ STOP BUTTON

· Resets PIC controller and stops motors

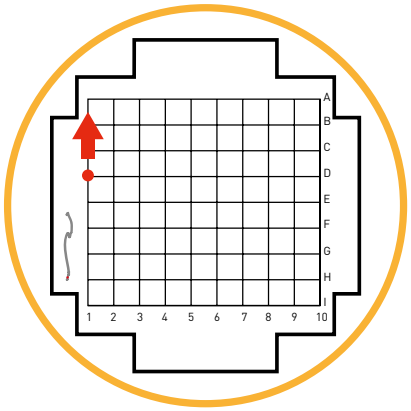
1

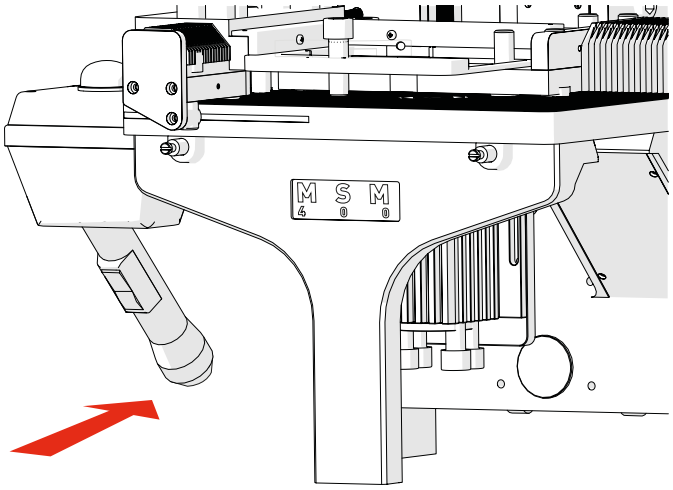




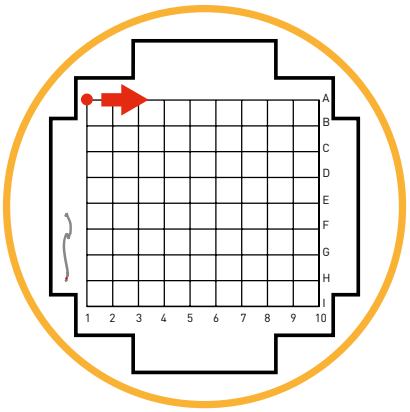
JOYSTICK MOVEMENT

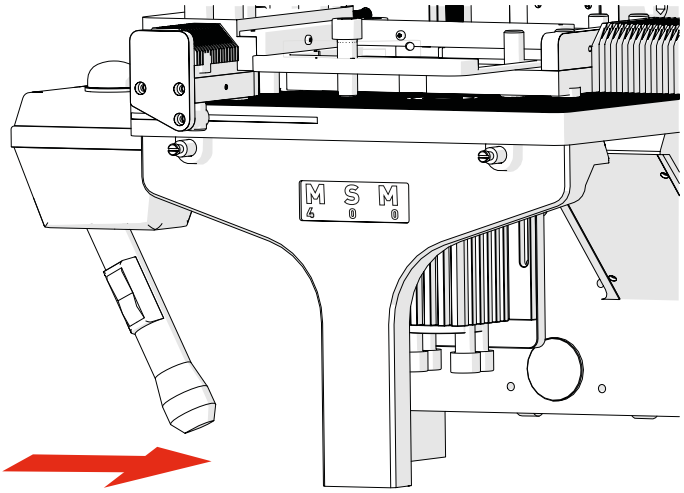
· Pulling the *Joystick* towards you will move you down one row on the *Matrix Grid.*





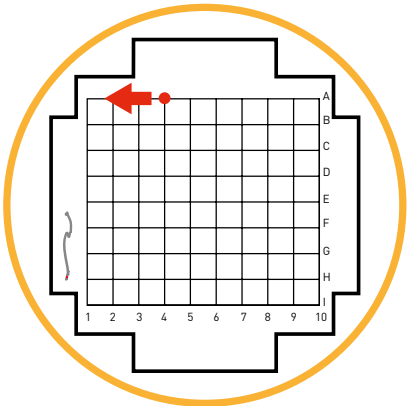
· Pushing the *Joystick* away from you will move you up one row on the *Matrix Grid.*

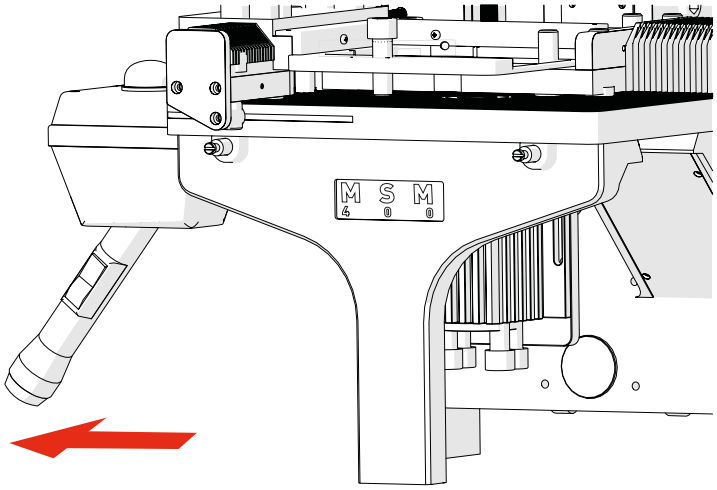




· Pushing the *Joystick* right will move you one column to the right on the *Matrix Grid.*

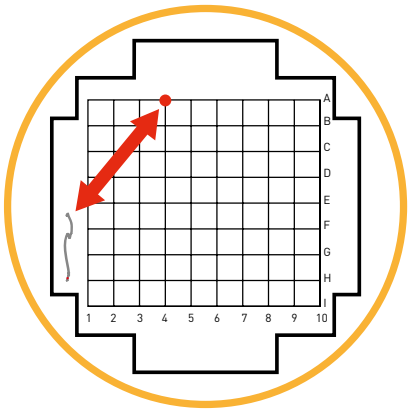
JOYSTICK

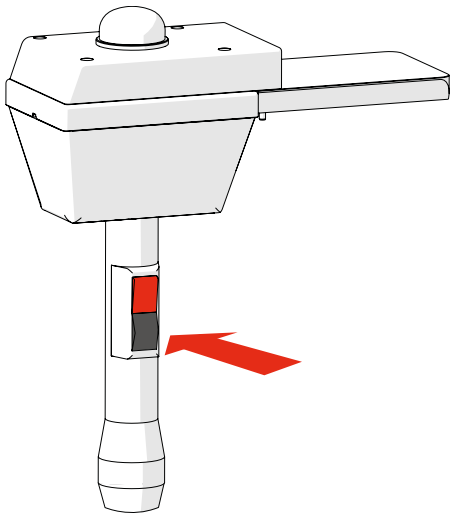




· Pushing the *Joystick* left will move you one column to the left on the *Matrix Grid.*

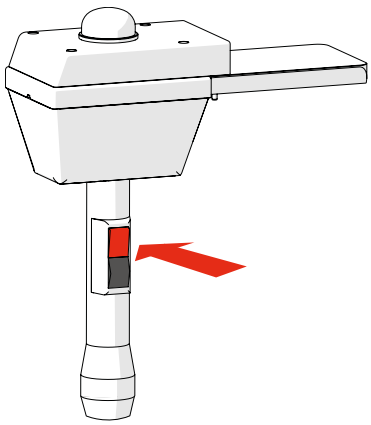
2





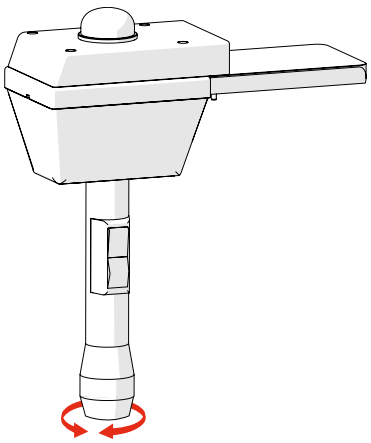
JOYSTICK BUTTONS

· Pressing the *Black Button* with move you between the *Inoculum* and *current cell position*



· Pressing the *Red Button* will bring up the *MSM Menu.*

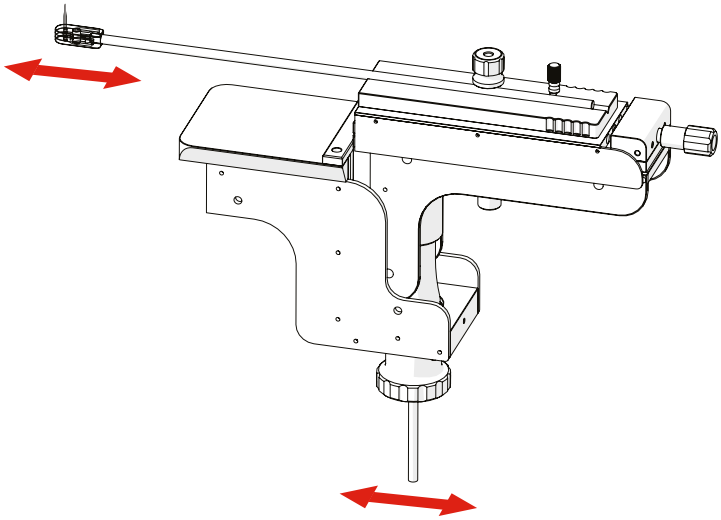
3



FINE FOCUS

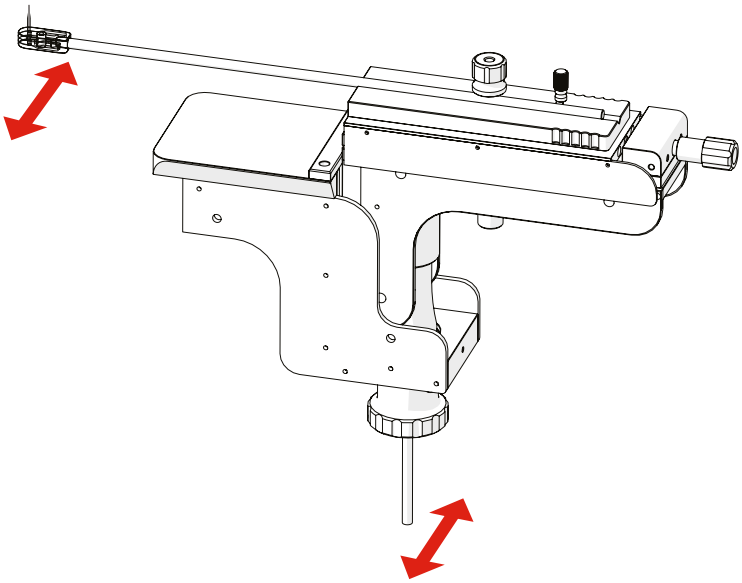
· Twist the *Fine Focus Knob* at the bottom of the *Joystick* to adjust the focus.

1



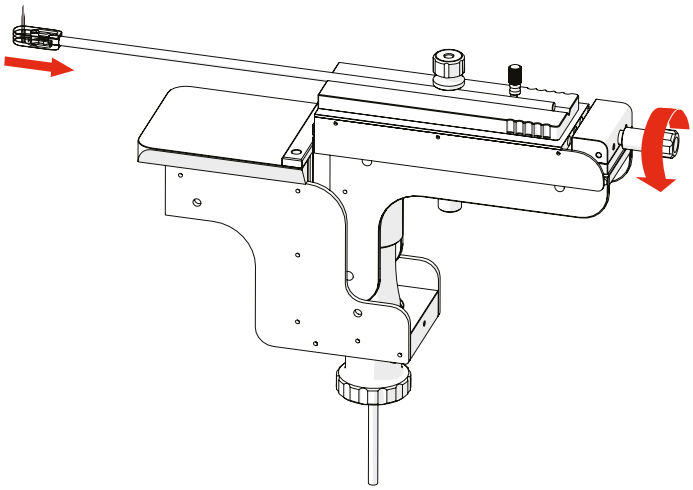
X & Y AXIS ADJUSTMENT

· Move the *Micromanipulator* from side to side to move the *Needle* along the *X Axis*.



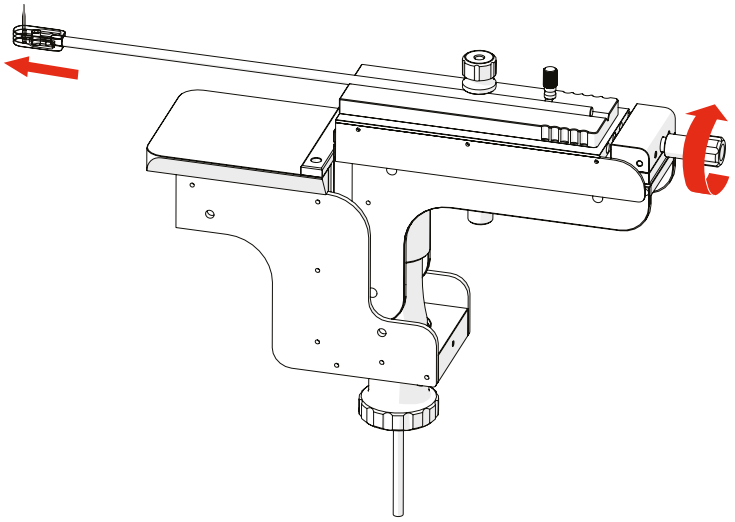
· Move the *Micromanipulator* from back and forth to move the *Needle* along the *Y Axis*.

2



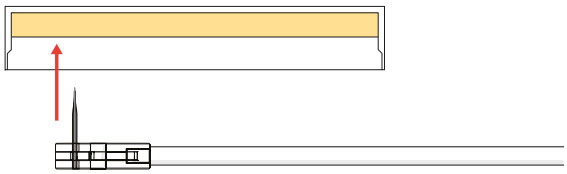
X AXIS FINE ADJUSTMENT

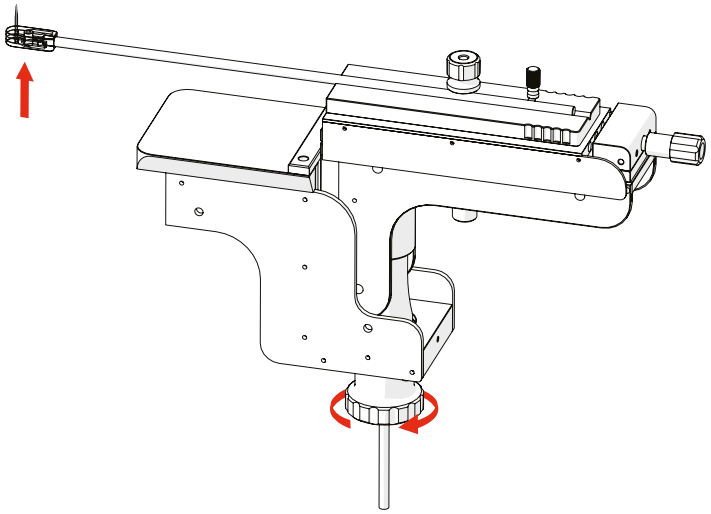
· Fine adjustments to the *Needle's Y Axis Position* can be made by turning the *Fine Adjustment Knob*. To move the *Needle* to the right, turn anti-clockwise.



· To move the *Needle* to the left, turn *Fine Adjustment Knob* clockwise.

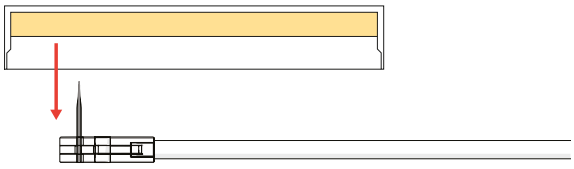
3

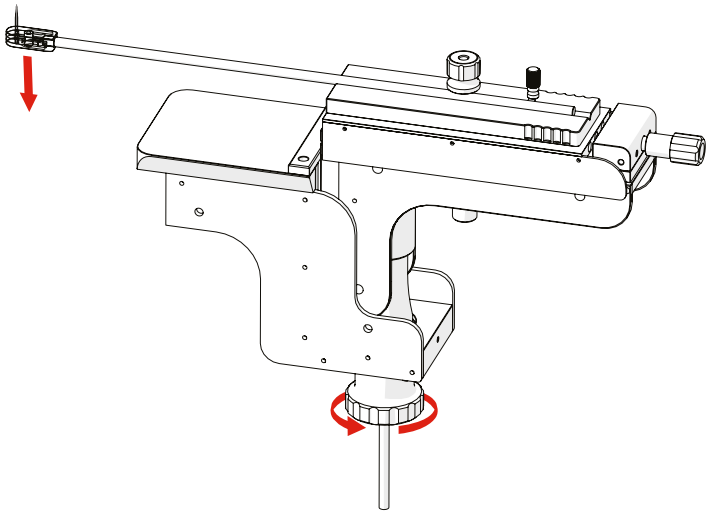




Z AXIS ADJUSTMENT

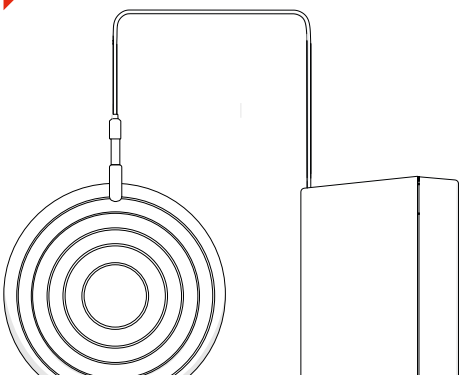
· Rotate the *Manipulator Ring* clockwise to bring the *Needle* up to the *Agar*.



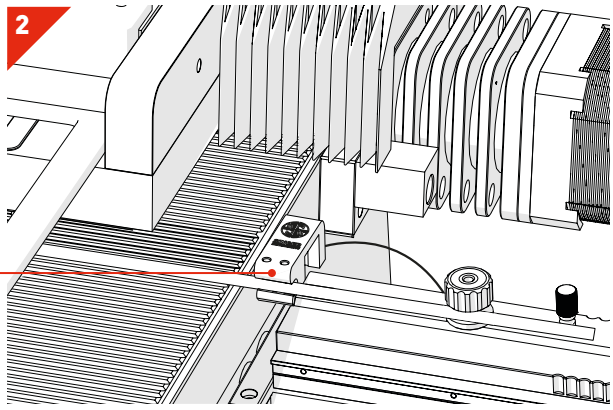


· Rotate the *Manipulator Ring* anti-clockwise to bring the *Needle* away from the *Agar*.

1



2



· Pressing the pad vibrates the MicroZapper body.

USING THE MICROZAPPER

When in the *Matrix*, users will note that the MSM 400 knows where it is and will now move in 6mm steps from column to column and from row to row. In the event of loosing a spore from the camera's field of view, look down the *Microscope* to find it. The *x15 Wide-Field Eyepieces* allow a greater field of view. If however, you cannot reach the errant spore then there are ways to retrieve it without shifting the needle setup. If you pull down on the *Joystick* (left hand) and move it, you will find that it will nudge much like it did in the *Inoculum*. Simply nudge the lost spore to the centre of the field and drag it back or pick it up. To return to 6mm centres you only need to move to the next row and return and the MSM 400 will revert to the *Matrix Proper*. You will also find that you can rest your hand on and press the *Pneumatic Pad* with your wrist as you manipulate the *Needle*. This is useful particularly where you have two spores that do not wish to part. The agitation of the *Needle Tip* while in contact with the agar can cause bubbles on a wet surface. On a particularly dry surface the *Needle* can tear the agar so short bursts are recommended and a very light contact of the needle.

I DON'T HAVE A MICROZAPPER!

Oh dear, you will have to be more careful with your digestion without one. Tapping the side of the toolholder may seem like an inviting alternative but you will probably cause damage to your needle. Banging the benchtop with a fist is definitely not a good idea, not very elegant and may annoy other lab users. The *MicroZapper* is retro fittable so you can always buy one later.

DIGESTION AND INOCULATION

S. CEREVISIAE

This series of steps acts as a reminder to those who have experienced the difficulties of successful tetrad dissection. It also serves as a teaching aid to those who have never dissected before.

S. POMBE

If you are using Pombe then digestion isn't necessary as spontaneous lysing occurs. However, similar sporulation checks and volume (pin head) is used in the suspension.

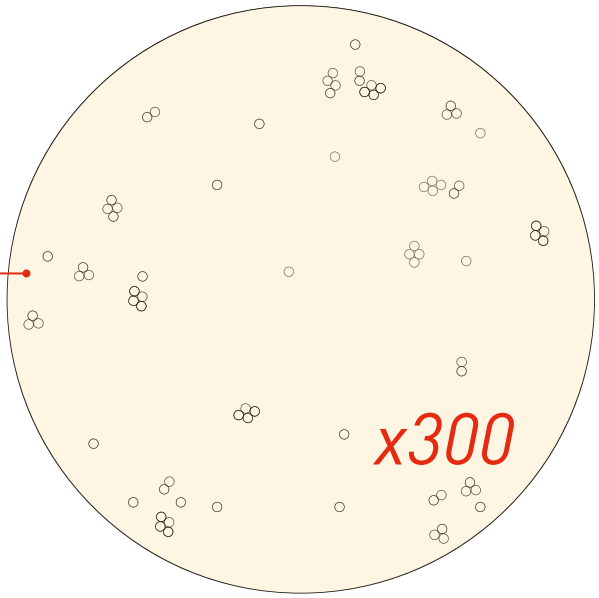
TOP TIP

At the Cold Spring Harbor yeast course Beverly Errede (course instructor) counts viable tetrads under the microscope and prepares several Ependorff tubes to digest at 5 mins, 10 mins, 15 minutes and 20 minutes. Then makes a selection based on cruxiformed tetrads (collapsed) that do not easily fall apart when moved. If the inoculum is too dense then sterile water is added to dilute the suspension.

TEN STEPS TO PERFECT DIGESTION

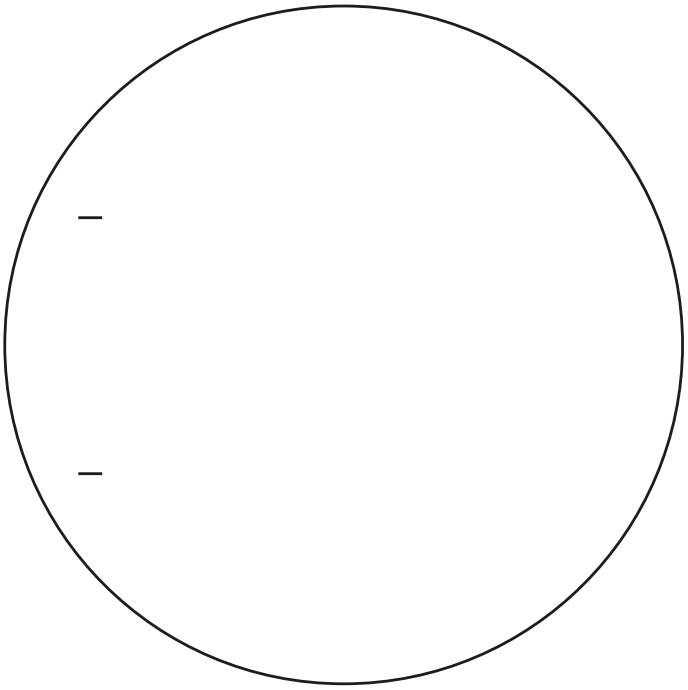
1. Inspect the sporulation culture microscopically to ensure the presence of four spore asci and to assess the sporulation rate.
2. Use a flamed loop or sterile toothpick to take a pinhead quantity of sporulated culture.
3. Suspend the sporulated culture in 50-100µl of prepared enzyme solution, e.g. 10µl enzyme to 100µl water. Incubate for 10-20 minutes depending on strain.
4. Fill Ependorff will ice cold water (1ml+)
5. Leave to settle for 5 minutes. Do not agitate.
6. Aspirate back to the original volume. By leaving the solution unspun, sediments float to the surface and solids sink to the bottom.
7. Lightly re-suspend cells by hand.
8. Mark a plate with a lab pen, flame a 3mm loop then dab it onto an unused area of your plate to cool it.
9. Insert the flamed loop into your suspension.
10. With a single and slight stroke, inoculate the dish.

This would be an ideal inoculum as it is not too dense.

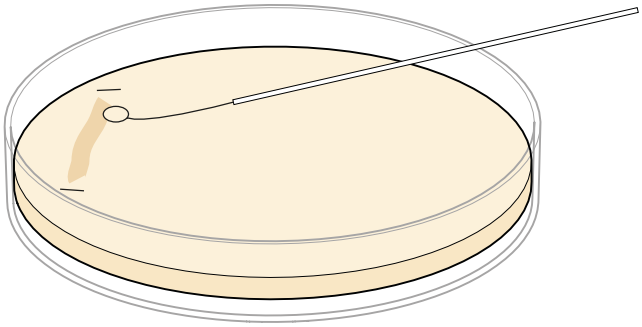


MARKING THE DISH

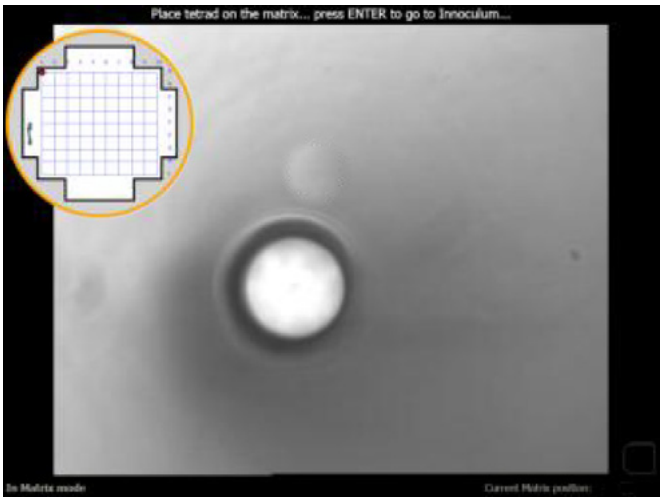
There are two ways to mark the position of the inoculum. One is to place the plate over the template below and the other way is to place it against the screen and mark the positions with a lab pen.

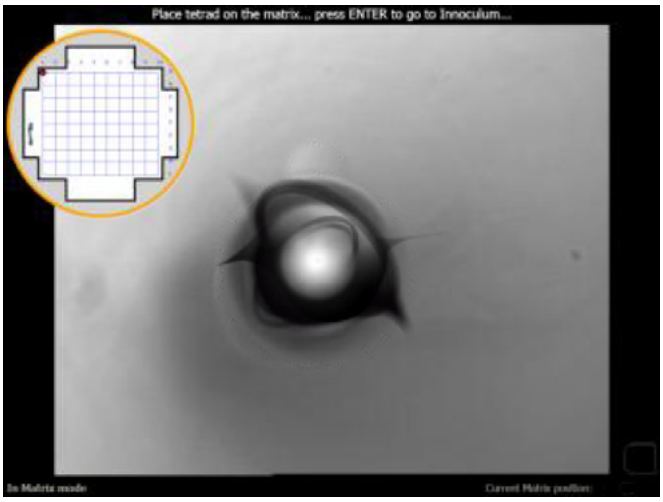


With a *3mm Flamed Loop*, inoculate the dish between the two marks as shown below.



More accurate positioning takes place at position *1A*. Plunge the *Needle Tip* into the agar and make a big hole. The dish datums at *1A* so this mark will be seen at this point even if it is out of focus. All you will need to do is rotate the dish until the mark is in the centre of the field. Leaving a spore to the side of the hole is fine.

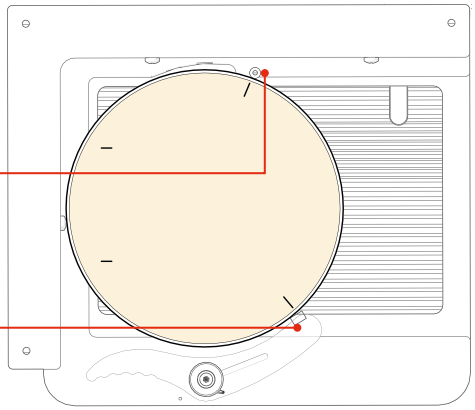




When taking the dish out of the MSM 400 it is useful to have some way of orientating the plate. Marking it either at the *Rear Post* or the *Lever Pad* will help to position the dish when returning it.

Rear post

Lever pad



· On switching on the MSM 400 the display will present you with a *Splash Screen* that lets you know that the program is loading.

· Select *OK* to continue.

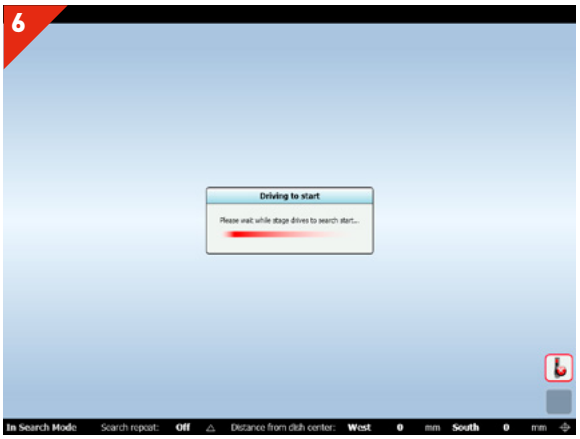
· Software is loading

· Select desired protocol from the following:
Tetrad Dissection
Aging
Screening/Progression

· Select *Use current Setup (Singer).*

· Follow the on-screen instructions to; raise overarm, lower needle, load dish and lower overarm.

· Select *OK*.



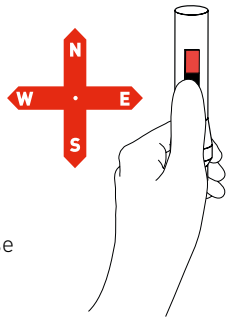
· Please wait.

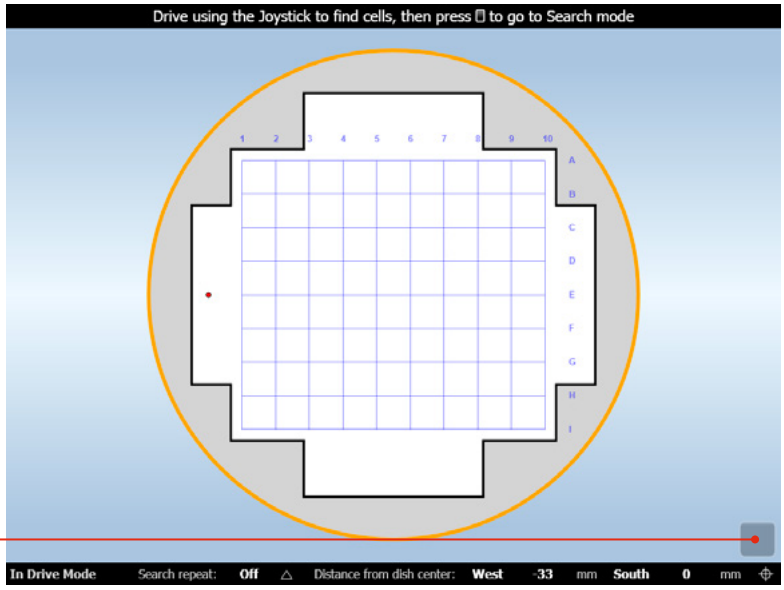
1

· You are now presented with the *Matrix*. Drive to your inoculum position ready to search.

· Move north, south, east, west as well as 360°.

· This button opens the *Menu Bar*. All menu selections must be made using the touchscreen, as the joystick will not navigate these commands or selections.

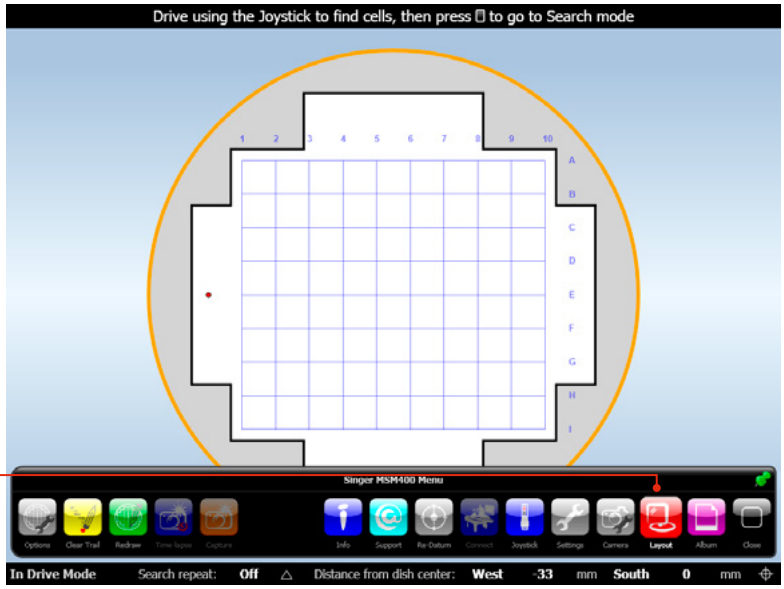




2

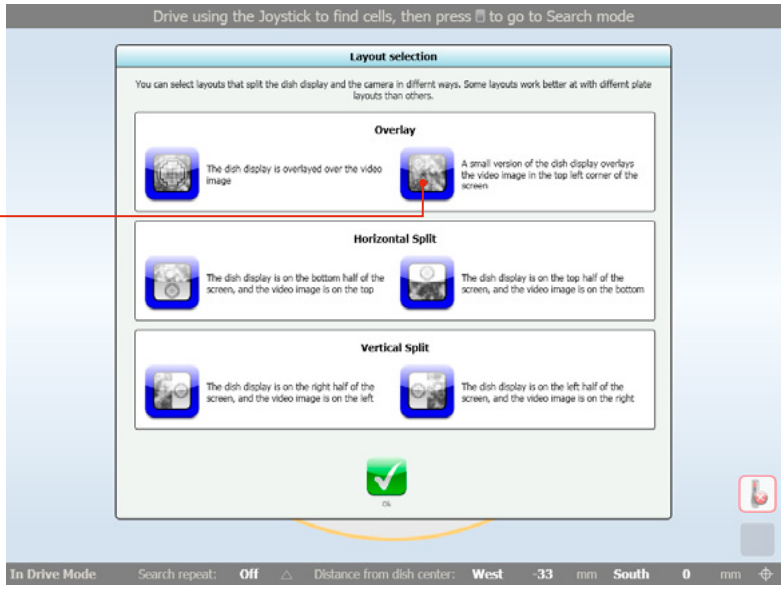
· When you have found the *Inoculum*, accept position by clicking the *Black Button* on the Joystick.

· Select *Layout* to change the layout.



3

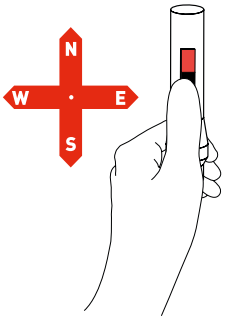
· Choose a layout from the *Layout Selection.* In this user guide we will be using the second *Overlay* option.

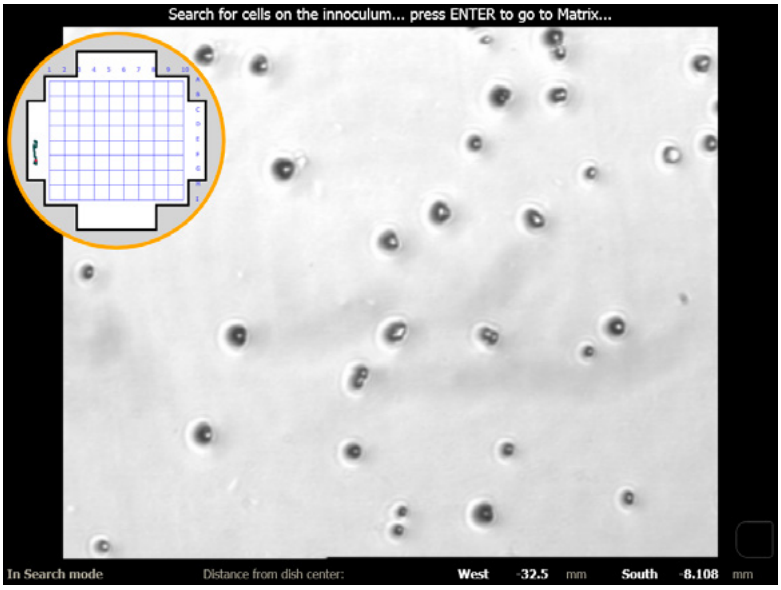


4

· You can step in any direction within the *Inoculum*.

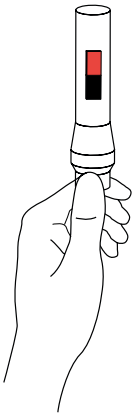
· Move North, South, East, West as well as 360°.

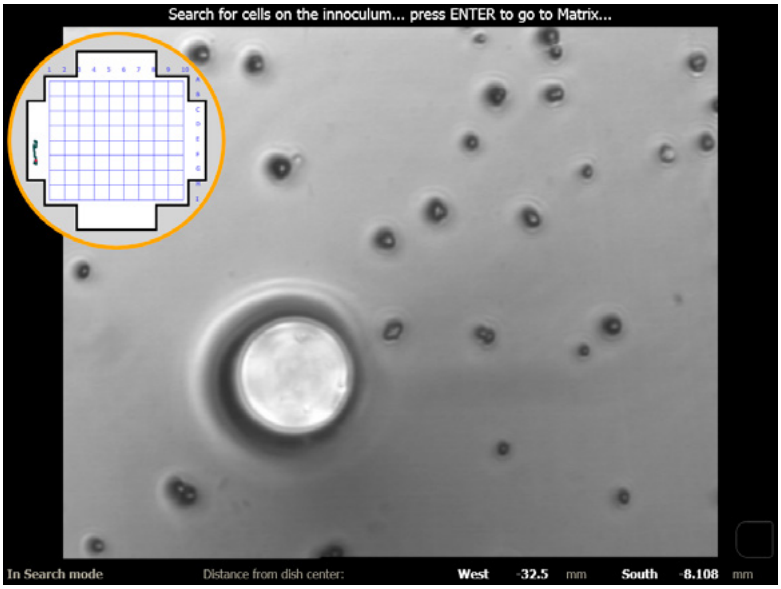




5

· Use the *Fine Focus Knob* to adjust focus.





FOCUSSING THE NEEDLE

It is good practise to move (step) to the side of the *Inoculum*, bring the *Needle* up to the surface, plunge the *Needle Tip* into the sterile agar and move the tip rigorously in to remove contaminates (don't worry you will not break the needle). While there, you should practice dabbing the *Needle* on the surface of the agar by turning the *Manipulator Ring* counter-clockwise and clockwise about a quarter of a turn. Also you should alter the focus so that you can experience watching for the *Meniscus* when out of focus.

The reason you should do this is because when you move to a position on the agar where there is nothing there (blank agar) you need to now where the surface is. By bringing the *Needle* up and watching for the *Meniscus* you will see it move the surface creating an out of focus ring (a bit like a pebble dropping into water). Then all you need to do is focus on the *Needle Tip* which will now be in contact with the agar. When you have successfully identified what is meant by the *Needle's Meniscus*, lower the *Needle* and return to the original position and search for a tetrad.

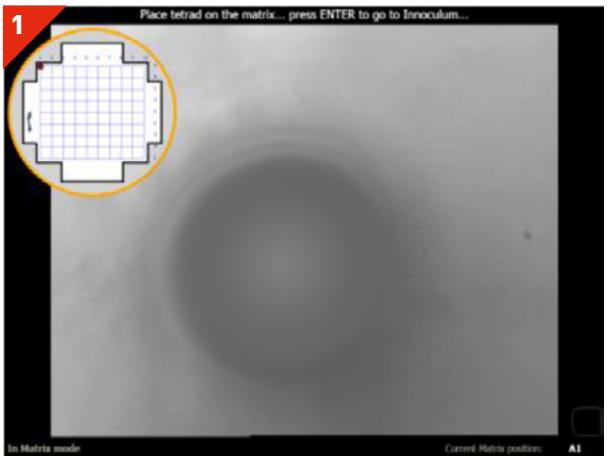
Seeing the formation of the meniscus around the needle top confirms that it is on the surface.

· *In focus, not touching*

· *Not in focus, touching*

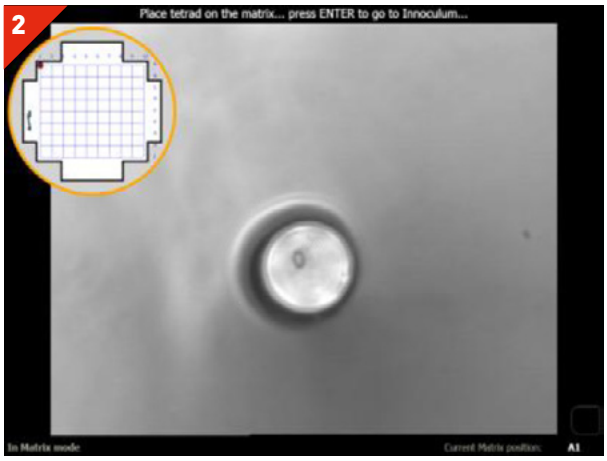
· *In focus, touching*

IMPORTANT
It is important to pour flat plates for dissection purposes. However, some unevenness may exist across the surface especially at the edges where the agar meets the sides of the dish. Therefore, never presume that the needle will be away from the surface when the stage moves and turn the ring counter clockwise (away from the agar) by at least a full turn.



· Here the *Needle* is being brought to the surface and watched carefully until the *Meniscus* is seen.

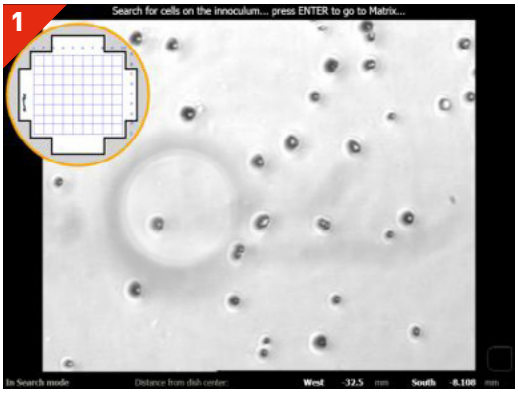
· Focus after the *Needle* has touched the agar surface.



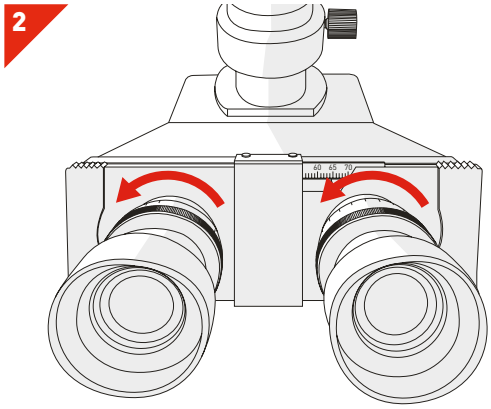
· Your *Needle*, and by inference the surface of the agar, are now in focus.

OPTIMISING THE OPTICS

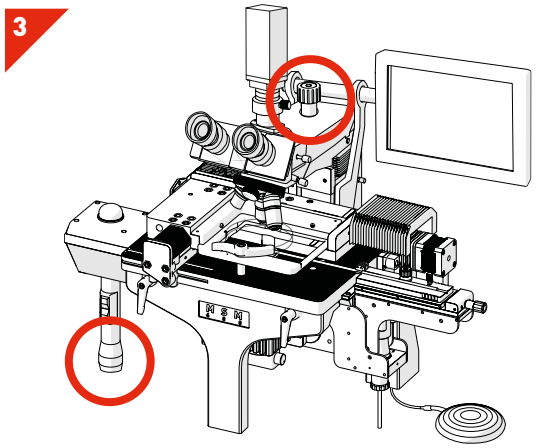
WITH A CAMERA



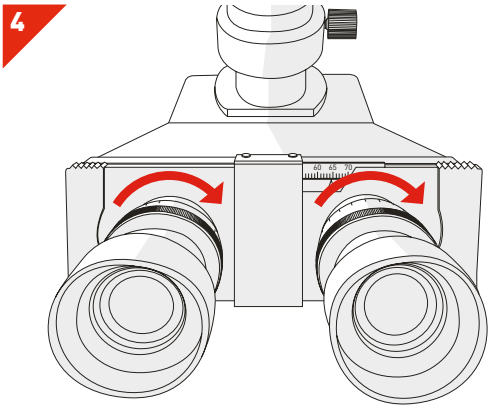
· Find some cells in the inoculum.



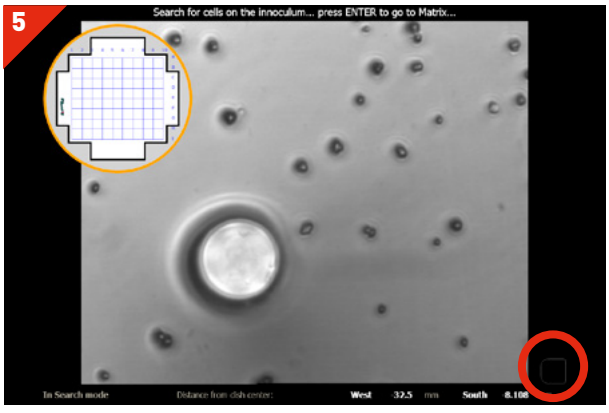
· Set both eyepiece's focus to zero.



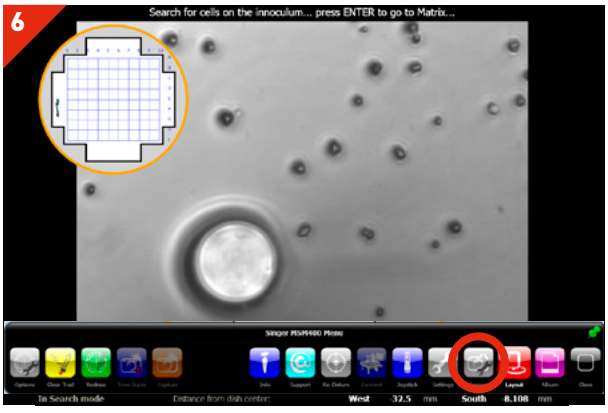
· Use a combination of fine and coarse to focus on-screen. See p.34 for focussing help.



· Focus eyepieces one by one to match on-screen focus.



· Select the MSM Options tab.



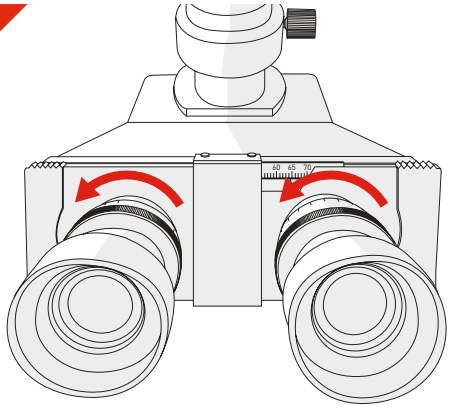
· Using the camera options, adjust the camera's light intensity to match the intensity through the eyepieces.

WITHOUT A CAMERA

1

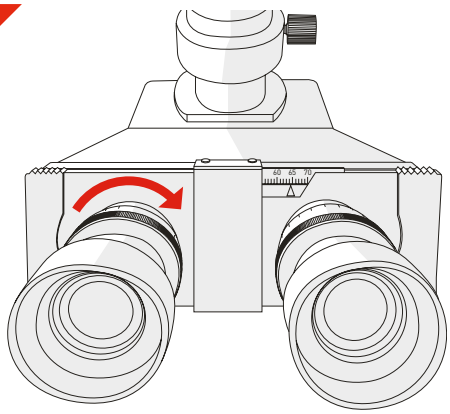
· Find some cells in the inoculum.

2



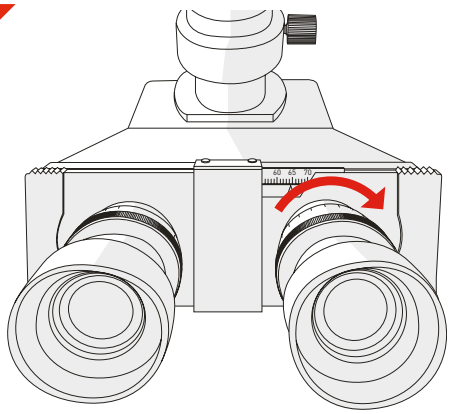
· Set both eyepiece's focus to zero.

3



· Adjust your dominant eye's eyepiece first and bring into focus.

4



· Now adjust the other eyepiece until optimum focus is achieved.

BRING ON THE TETRADS!

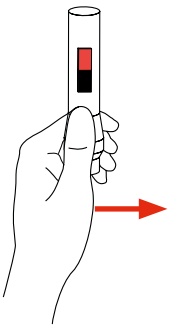
You should be familiar with using the MSM 400 now. Let's dissect some tetrads!

We also have a great video demonstration of tetrad dissection with the MSM 400 here:

singerinstruments.com/resource/how-to-dissect-a-tetrad/

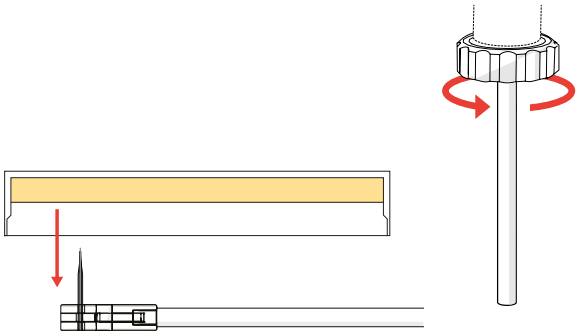
PICKING UP THE TETRAD

1

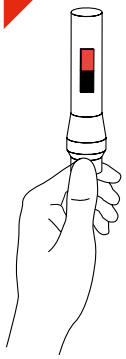


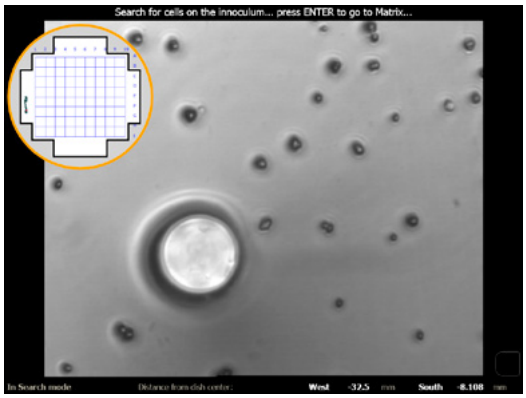
· Step - 300 micron movement.

· *Needle* away from agar surface.



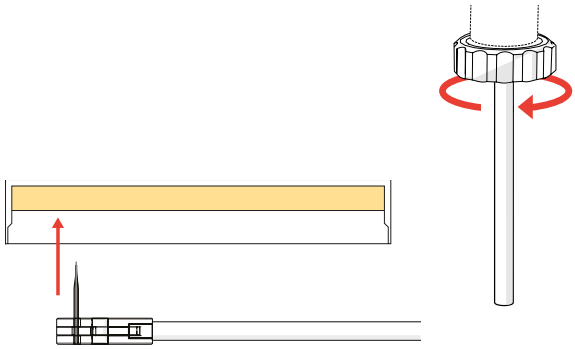
2



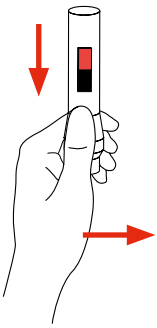


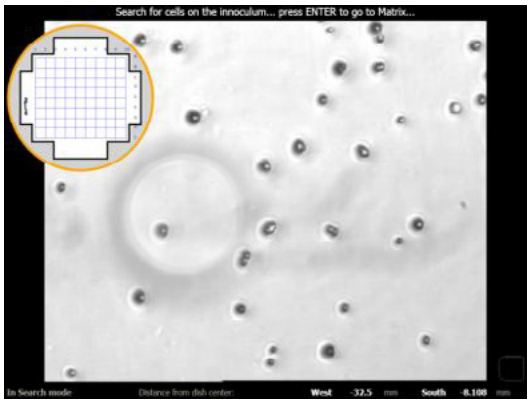
· Fine focus control.

· *Needle* towards agar surface.



3

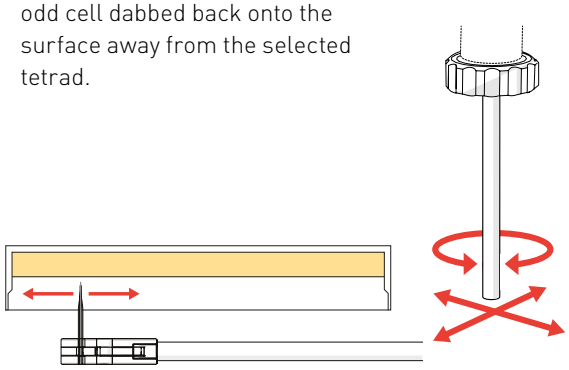




· Pull down *Joystick* for 24 micron movement.

· Nudge the *Joystick*.

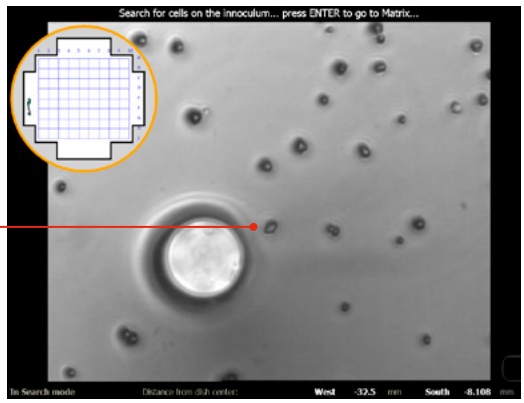
· The area can be cleared and the odd cell dabbed back onto the surface away from the selected tetrad.



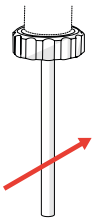
PICKING UP THE TETRAD

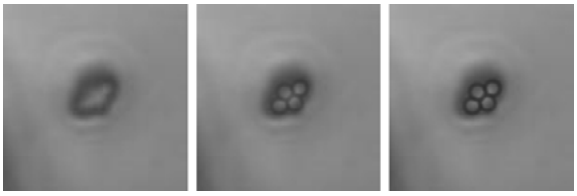
4

Tetrad



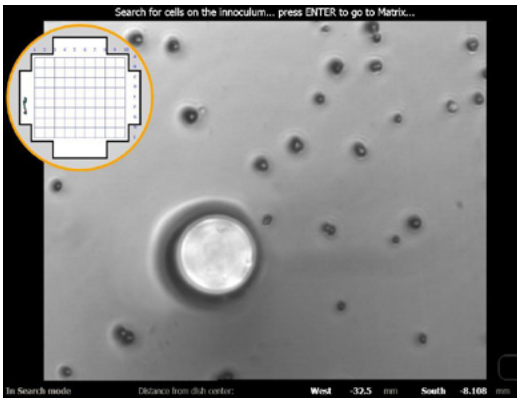
· Move towards the *Tetrad* while still on the surface.

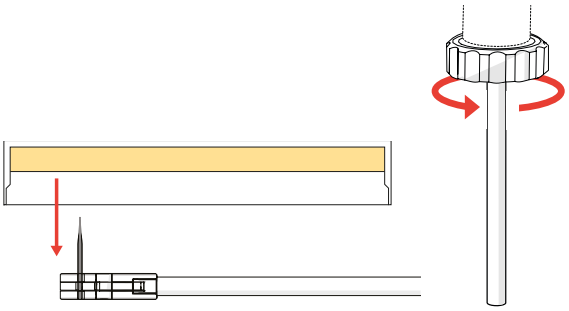




· Moving the tetrad in the agar not only prepares it to be picked up but also washes it, clearing away the ascus sack and presenting the spores with clear dark outlines. This will help you identify the four spore asci in it's cruxiform shape.

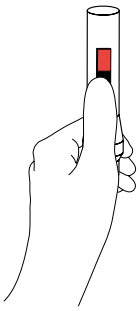
5

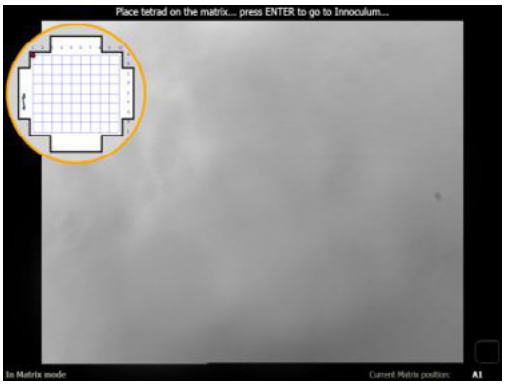




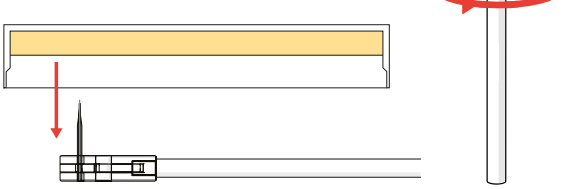
· When picking a tetrad off the agar surface you will find that the needle has a 'sweet spot' and the asci always picks up best from that position. You will also find that successful pickup is down to confidence and the rate that the needle leaves the surface. Sometimes quicker is better and sometimes smooth action and pace can do it. Keep practising until you find the perfect technique.

6





· *Needle* away from surface with a complete counter-clockwise turn incase the agar surface is not flat.



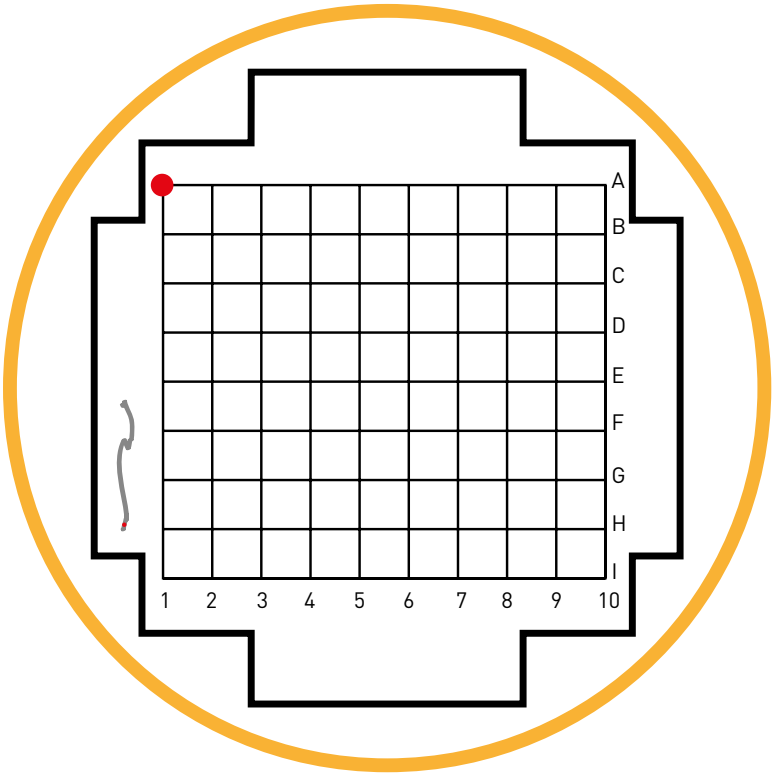
· Press the *Black Enter Button* to move to the *Matrix*.

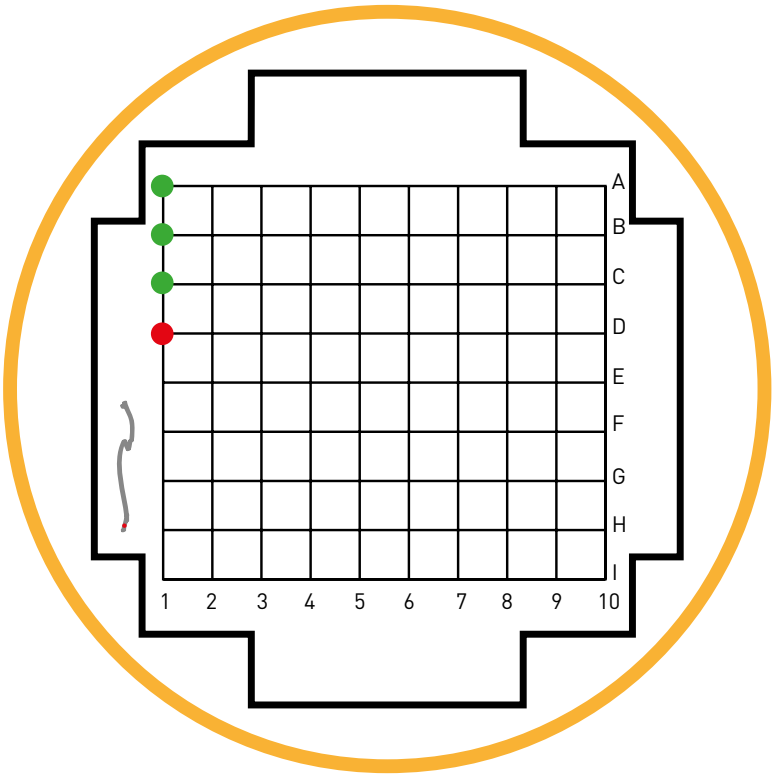
· You will now move to an area of the agar that has nothing on the surface to show where it is.

PLACING TETRAD IN THE MATRIX

- You will be placing the tetrad at *A1* which is also the datum position for the whole plate. This is very important because this position will need to be marked so that if the plate is removed and replaced you can continue to dissect. It is also useful should you wish to review a completed plate. See Section 3 *Marking the Dish (page 30)*.

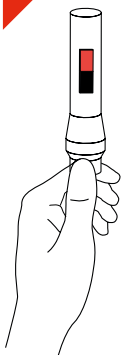
- With the Singer setup shown below you will be dissecting in columns downwards from starting at column 1 row A. Placing the individual spores at 1A, 1B, 1C and 1D.

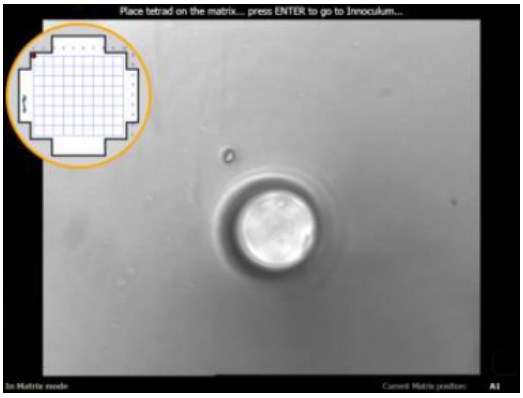


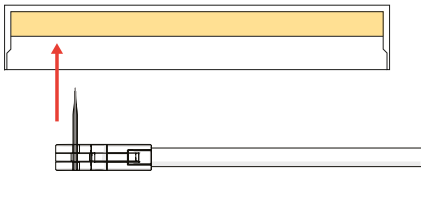


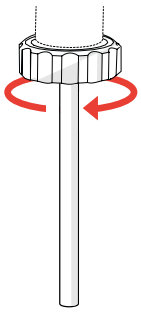
DISSECTING THE TETRAD

1



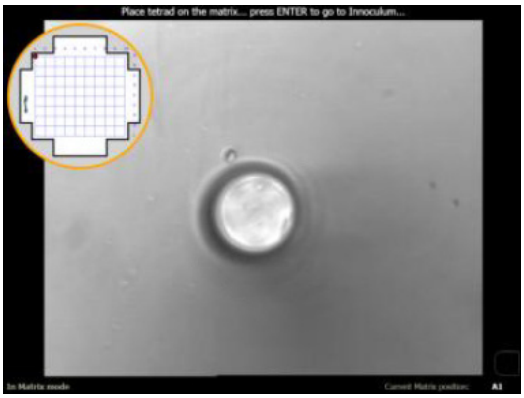


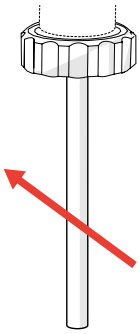


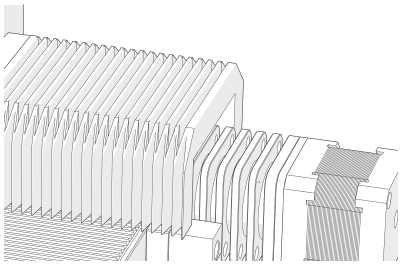


· Use the *Fine Focus Knob* to adjust focus. Remember to watch for the *Meniscus* first.

2



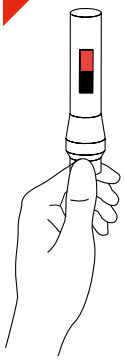


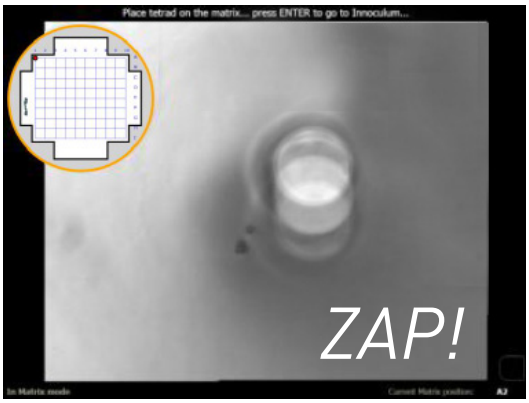


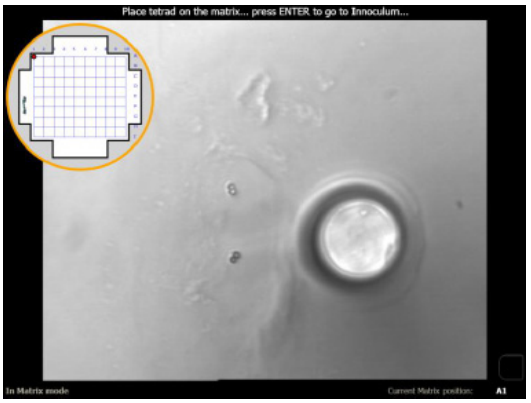
· After dropping the *Tetrad*, move towards it to separate the spores.

· Now press the *MicroZapper Pad* to activate the *MicroZapper.*

3

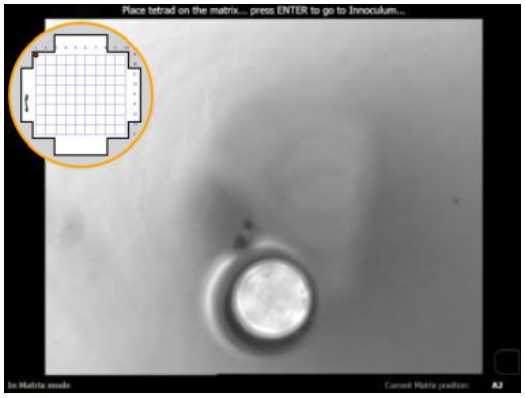




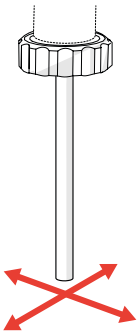


· Zapping the *Tetrad* can separate one from three or in pairs or even single spores. Whatever the case,try and leave one here and take the others to the next position *2A*.

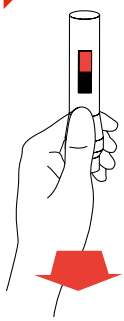
4

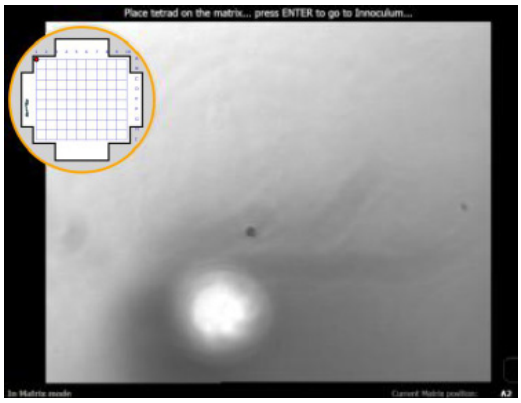


· On separating the *Tetrad* move *Needle* to pick up three spores and leave one at *1A*.

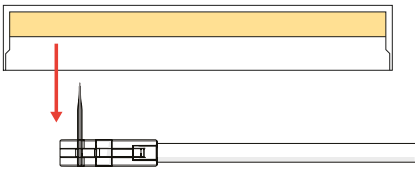


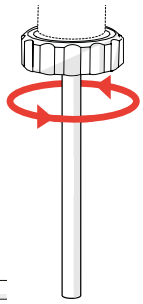
5





· Move the *Needle* away from surface with a complete counter-clockwise turn incase the agar surface is not flat.

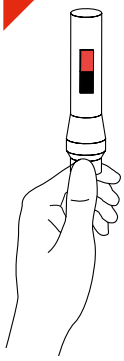


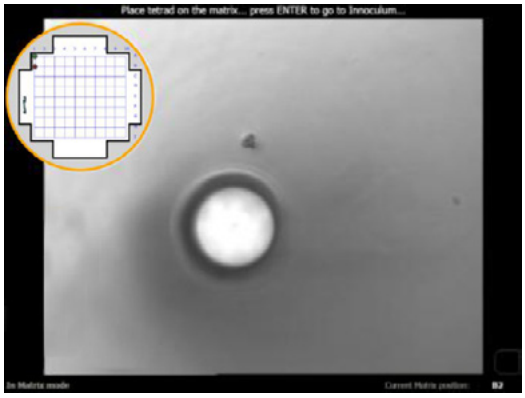


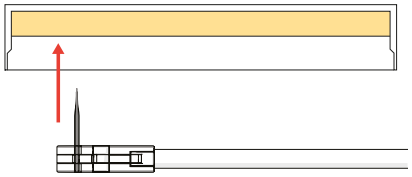
· After moving the *Needle* away from the surface of the agar, pull the *Joystick* towards you once to move to position *2A*.

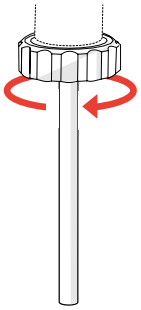
DISSECTION OVERVIEW

1



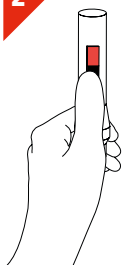




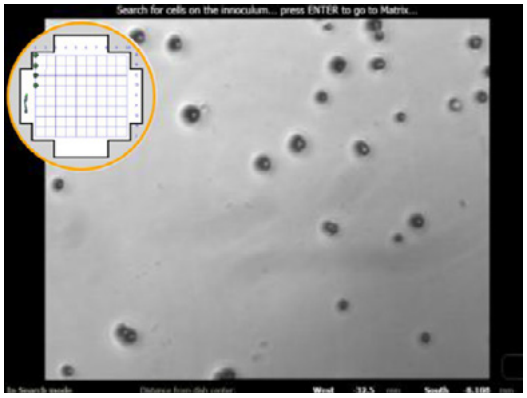


· Separating the three spores is much the same as separating the four spore asci. You may use the *MicroZapper* to separate them, pick up two and move to position *3A*, separate and pick up one spore move to *4A* and place it there. The technique is virtually the same and very quick. It is also good practice to move back up the column, refocusing at each grid point to check for a *Spore*. This ensures that you haven't mistaken a bubble in the agar as a spore. It also serves to put you back to *1A*. At this position you will mark the agar so that you may find this datum position after plate removal and incubation or a break in tetrad dissection. This is done by first moving the *Spore* to an area away from the centre, plunging the *Needle* into the agar and jiggling it a bit creating a hole. Don't worry that you will break the needle, you won't.

2



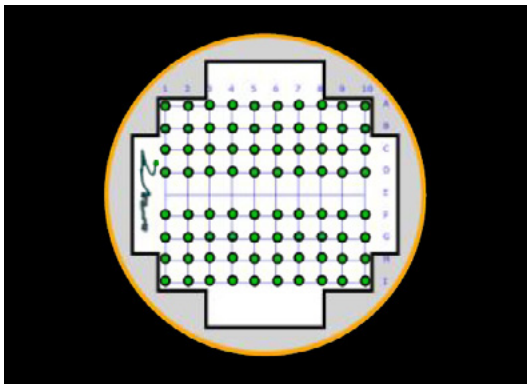
· Press *Black Button* to return to *Inoculum*



· The stage will return to the last position always. You can continue the search for tetrads using the *Joystick* as before. Being able to return to the same position in the inoculum serves two useful purposes; one is from a point you have already searched and two if you have over digested then you may return to pick up a dropped spore without ruining the experiment.

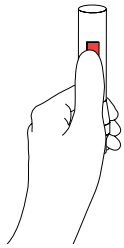
· You may also use the nudge facility in the *Matrix* if a spore moves outside the ellipse of the dissecting needle. Step, Nudge and Matrix parameters can be changed from the *Menu Bar*.

3



· The authogonal array at 6mm centres will allow you to dissect 20 tetrads. There is no need to measure distances as the MSM 400 automatically uses this grid setup when in the Matrix. On moving to the *Inoculum* it reverts to 300 micron steps and 24 micron nudge movements automatically. The appropriate automation allows users to concentrate on selecting good asci.

4



· Pressing the *Red Button* will bring up the *Main Menu* which allows you to dissect another dish or quit.

SOFTWARE PROTOCOLS

Let's take a look at the other protocols found on the software.

SELECTING AGEING PROGRAM AND PROTOCOL

1

· On the *Protocol Menu,* select *Ageing*.

2

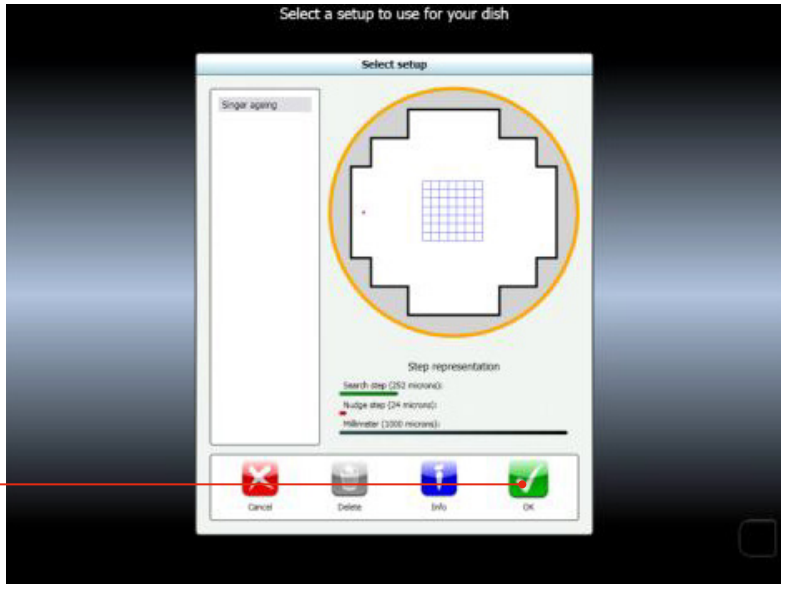
· Here you can select the *Singer Setup* to work in the default setup.

· Select *Choose Setup* to work in a different setup.

· To create a new setup, select *Create New Setup.* New setups are constructed by first selecting then modifying the *Singer Setup.*

3

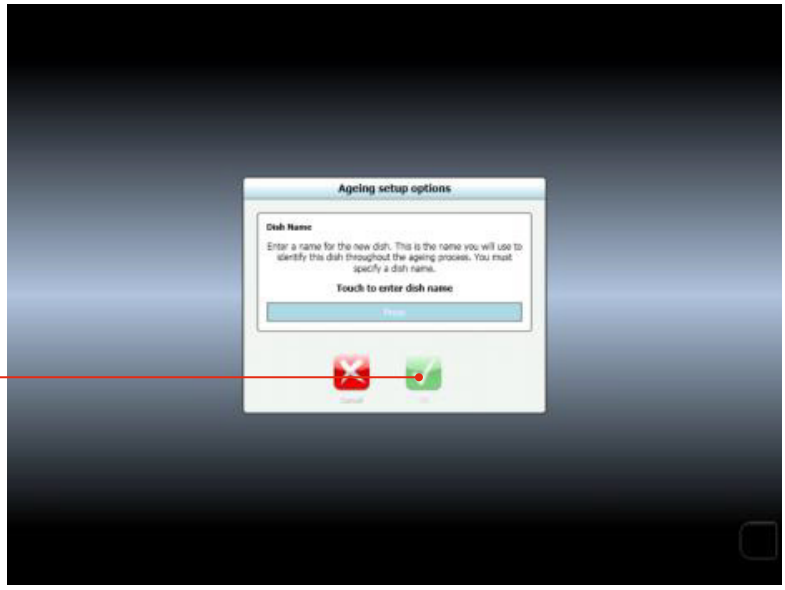
· If you have selected *Choose Setup*, pick your desired setup and press *OK*.



AGEING NAMING SETUP

4

· Select *OK* to enter a name for your dish.



5

· Use the *Touch Screen Keypad* to enter the dish name.

6

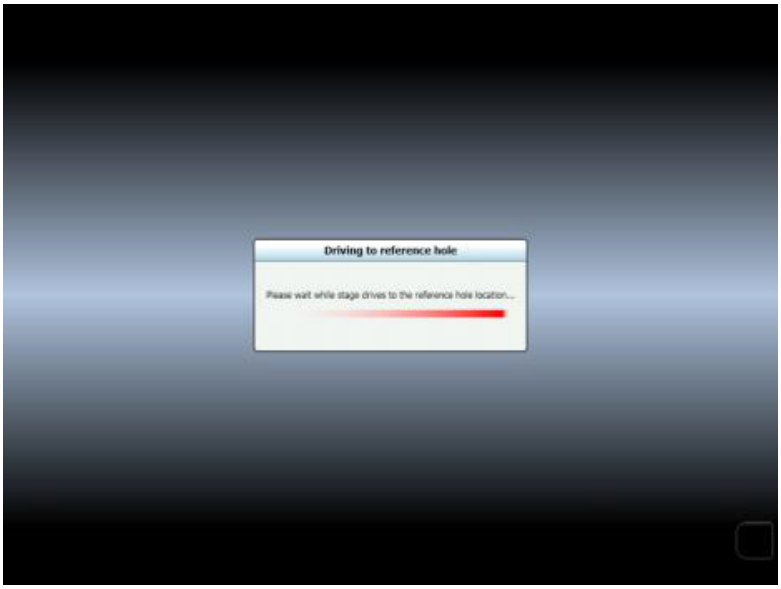
· Once a name has been entered, select *OK*.

7

· At this point you will be prompted to raise the *Overarm* to allow plenty of room to locate your dissection plate.
Once raised, select *OK*.

8

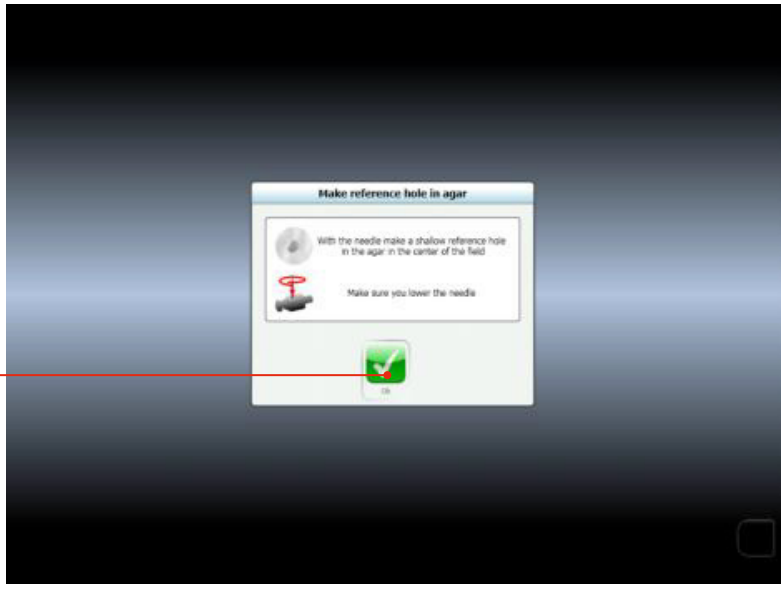
· The stage will automatically move to a position where a mark can be made in the agar to easily re-position the plate after incubation.



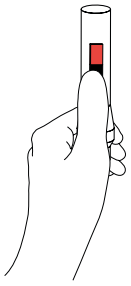
9

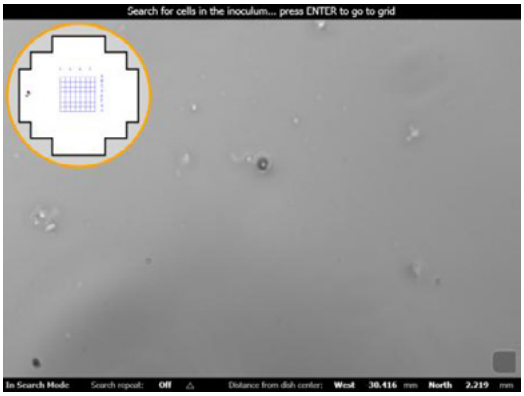
· Marking the agar at this position (datum) will allow very accurate replacement of the dish after a suitable incubation period. Even if the mark is out of focus the dish may be rotated and the shadow of the hole will be visible. It also serves to help find the surface of the agar when the hole is brought into focus.

· Mark the agar as described on *page 30*, then select *OK*.



10

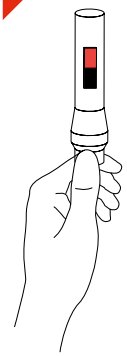


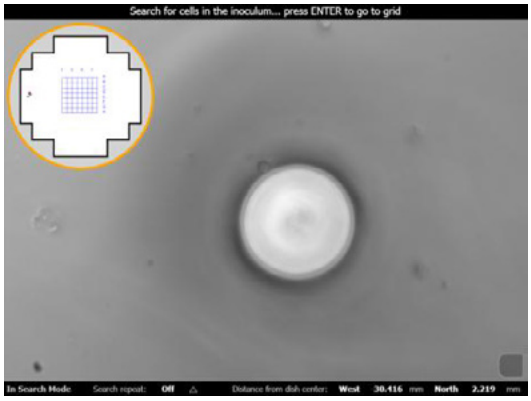


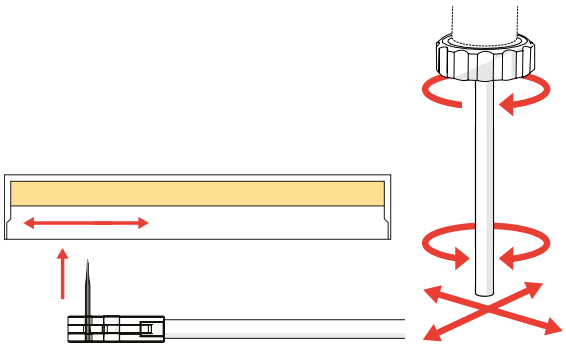
· Use the *Joystick* to find the *Inoculum.*

· Once found, accept position by clicking the *Black Button.*

11





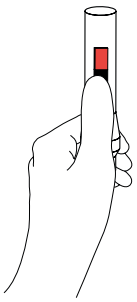


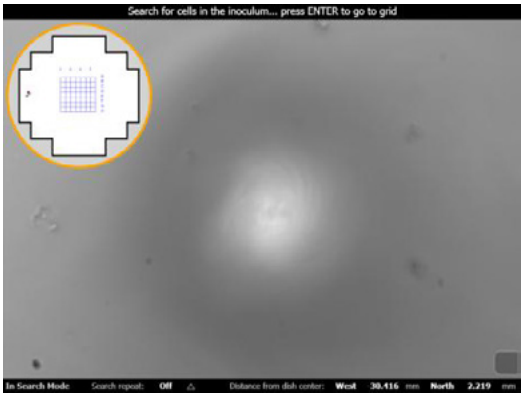
· Bring *Needle* to the surface of the agar.

· Focus, then move the *Needle* towards the cell.

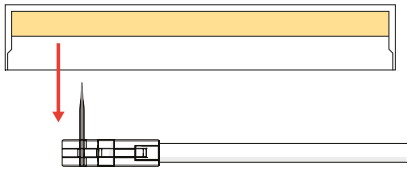
Please note that what is shown is for the purposes of procedure only. The cells in these photos don't necessarily represent the cells you will be looking for.

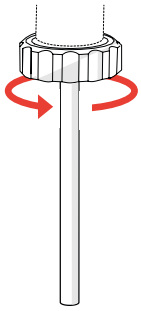
12





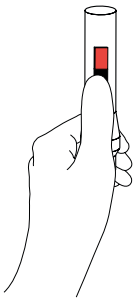
· Make sure that the *Needle* is well away from the agar surface before moving to the *Matrix*.

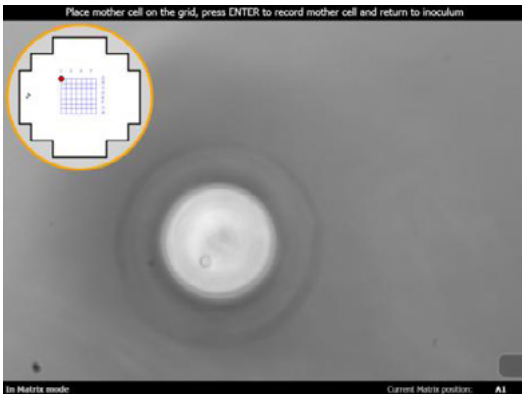




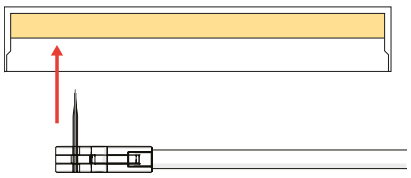
· Once you have found the *Cell*, raise the *Needle* from the surface and use the *Black Button* to move from the *Inoculum* to the *Matrix*.

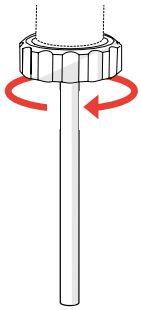
13





· Make sure that the *Needle* is well away from the agar surface before moving to the *Matrix*.

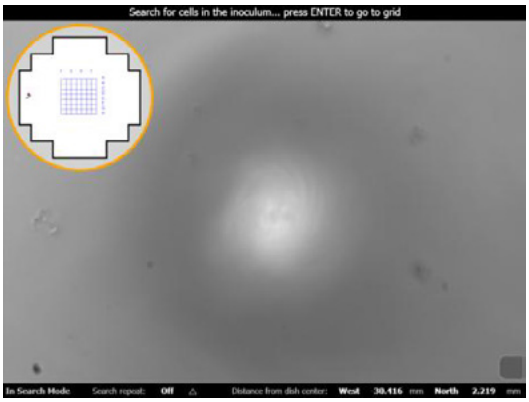


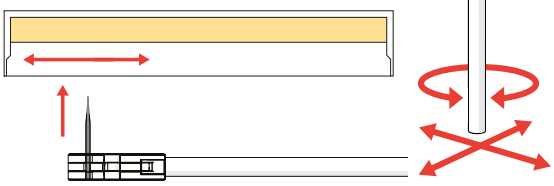


· Move the *Needle* to the surface and place the *Cell* at position *1A* on the grid.

· Move the *Needle* away from the surface and use the *Black Button* to record cell and return to the *Inoculum*

14



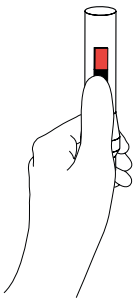


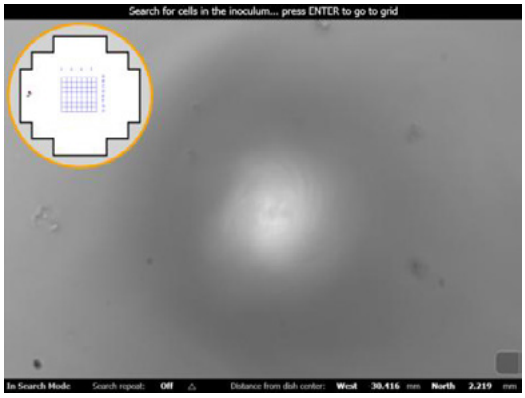
· Search for cells in the *Inoculum*, focus then move towards the *Cell*.

REMINDER:
1. Needle away from agar.
2. Then move.
3. Needle towards agar to find surface.

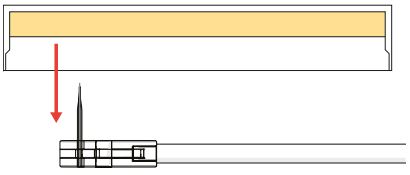
PLACING CELL IN MATRIX

15



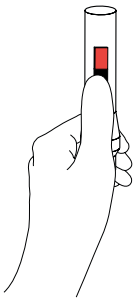


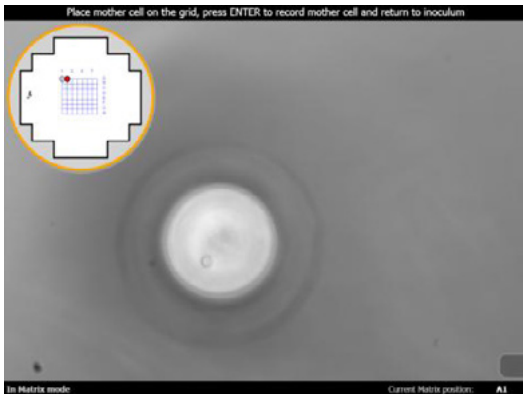
· Make sure that the *Needle* is well away from the agar surface before moving to the *Matrix*.



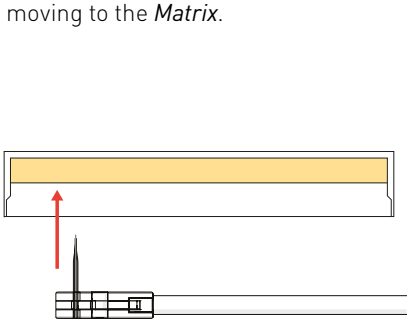
· Once you have found the next *Cell*, raise the *Needle* from the surface and use the *Black Button* to move from the *Inoculum* to the *Matrix.*

16





· Make sure that the *Needle* is well away from the agar surface before moving to the *Matrix*.



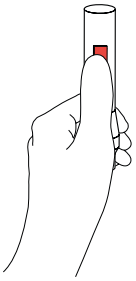
· Move the *Needle* to the surface and place the *Cell* at position *2A* on the grid.

· Move the *Needle* away from the surface and use the *Black Button* to record cell and return to the *Inoculum*

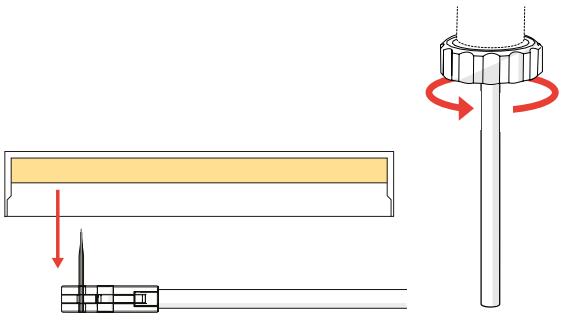
17

· Continue placing cells in the *Matrix*. The matrix is 8 x 8 so 64 cells can be placed there for incubation.

· When you have finished placing cells, use the *Red Button* to bring up the menu and select *Done*.

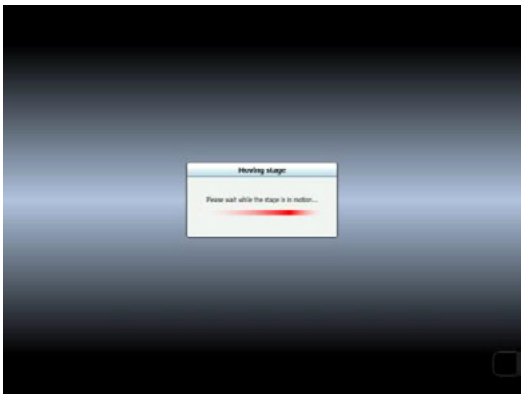


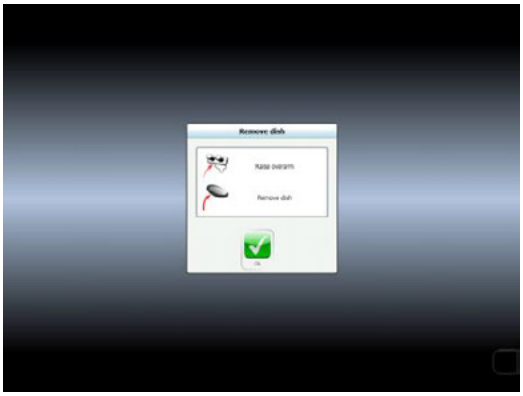
1



· Move the *Needle* away from the surface and select *OK*.

2





· Once the stage has stopped moving, raise the *Overarm* and remove the *Dish*.

· Select *OK* and the MSM 400 will close down.

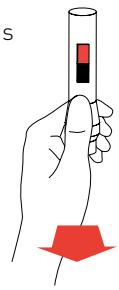
REPLACING THE DISH AFTER SUITABLE INCUBATION

You will first be asked to select your dish (this is the name that you gave this particular experiment) you will do this by selecting the *Review Existing Dish* button. By rotating the dish while looking through the microscope the mark (hole) made in the agar at position *1A* will be evident. Place the mark in the centre of your view, focus, adjust and then press *Enter*.

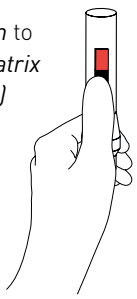
NAVIGATION

You will be at the first position in the ageing matrix. By pulling back on the *Joystick* you will go to the second position and so on and so forth in your grid array. This can be done relatively quickly so you can check the cells for removable buds. At this stage you will be removing the mother cell and leaving the daughter cell behind as a virgin mother cell. You will be prompted to accept that this first dump (deposit) should not be counted. Accept this proposition and you will return to that matrix position. You will note that there is plenty of room at the matrix and at the dump area.

· Pull the *Joystick* towards you to move to the next *Matrix Position.*

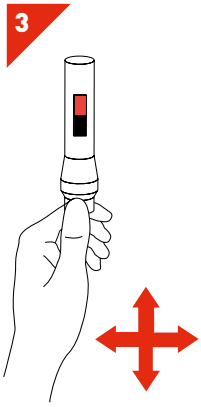


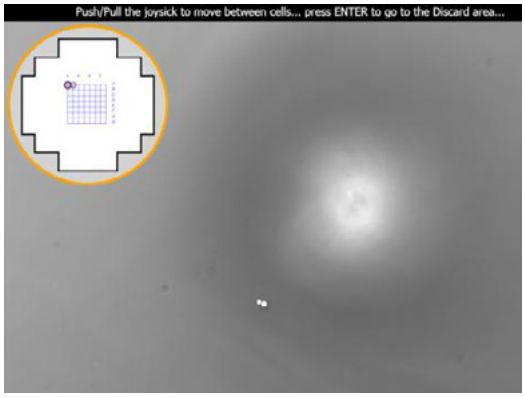
· Press the *Black Button* to toggle between the *Matrix* and the *Deposit (dump) Area.*

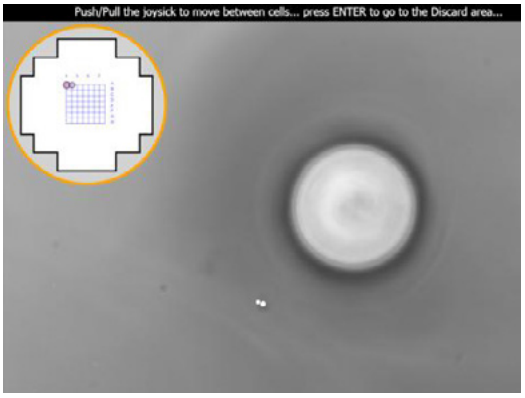


· Push the *Joystick* away from you to move back to previous *Matrix Positions.*

ENDING PROGRAM

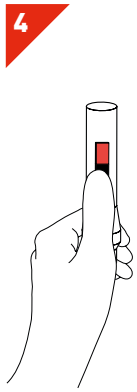


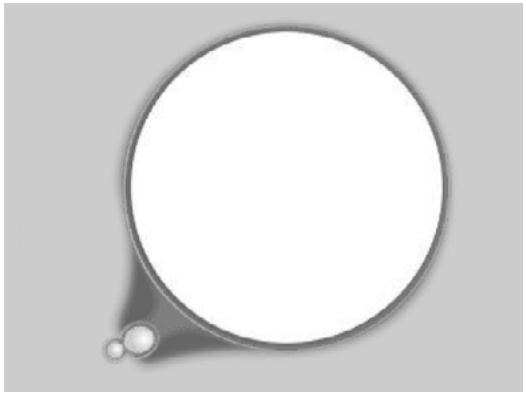


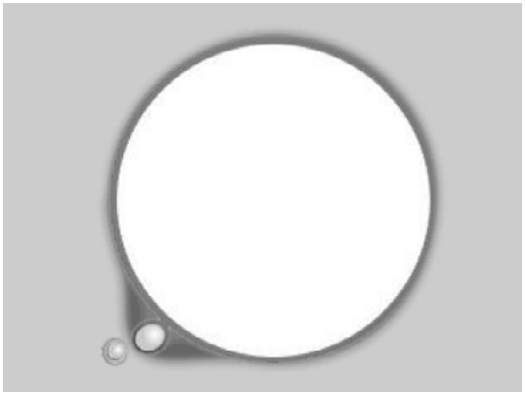


· Move the *Joystick* to move between cells.

· After seeing the *Bud*, focus the Needle.



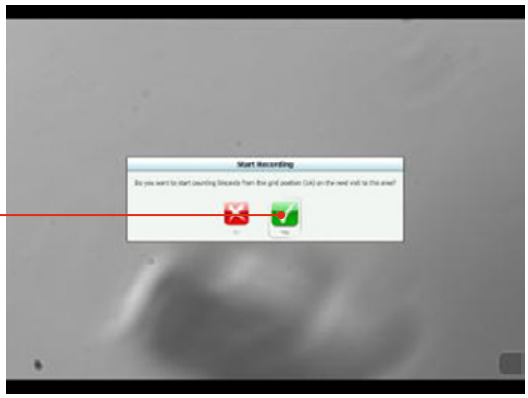




· The lightest of touches on the surface of the agar and waiting for the *Mother Cell* to be attracted to the *Needle* removes unwanted mechanical shock.

· Press the *Black Button* to move to the *Deposit Area*.

· Select *OK* on screen to discard *Mother Cell*.



· Remember that you pull back on the *Joystick* to move to the next position on the *Matrix*. You can continue to search the matrix for *Budded Cells*. Repeat the above until all the mother cells have been removed. You will now have virgin mother cells in each position. You may wish to incubate the dish before continuing. After incubation you will be removing the daughter cell and the MSM 400 will note the count from each position and keep an accurate record of it for your replicative lifespan analysis. Some researchers will wait until a second bud starts to form to ensure the first bud has separated. Sometimes the bud will separate with a clear gap which obviously makes removal much easier. Please note that the techniques shown above may vary from lab to lab. Some researchers will allow as many as four buds to develop but sometimes this makes it difficult to discern which one is the mother cell. It is therefore desirable to discover which technique works best for you.

VIEWING EXISTING DISH

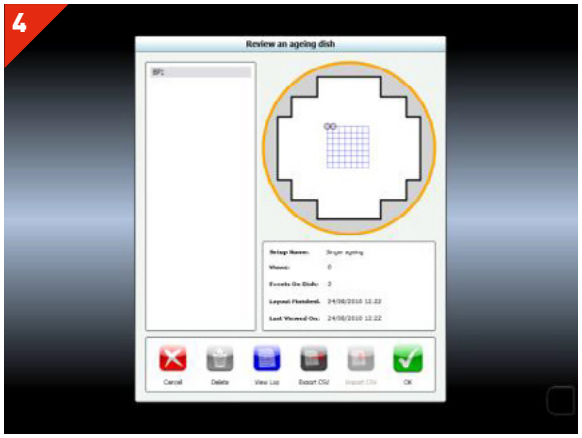
1

· After a suitable incubation period, the *Dish* may be returned to the *MSM 400 Plate-holder* and roughly put into the correct position via lab pen marks.

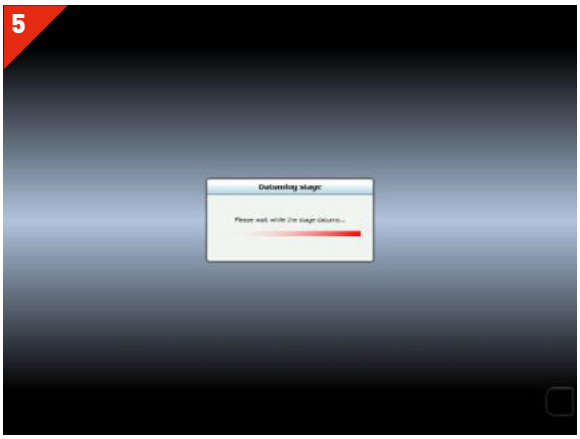
· You will now be selecting the protocol for reviewing your placed cells. Here you will be looking for a single daughter cell (bud) that has developed and has separated from the mother cell. This daughter cell will be your 'Virgin' Mother cell. As it is a new cell, it's clock has returned to zero. This will be the mother cell for age experiments. Do not use the original cell for this purpose because it may have already produced a daughter cell.

· On the *Protocol Menu* select *Ageing*.

· Select *Review Existing Dish.*



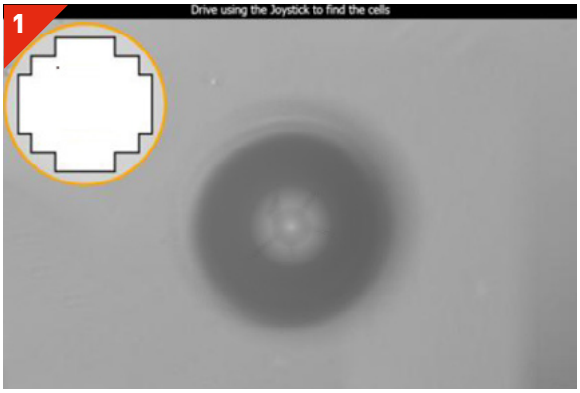
· Choose your desired dish and select *OK*.



· Please wait.

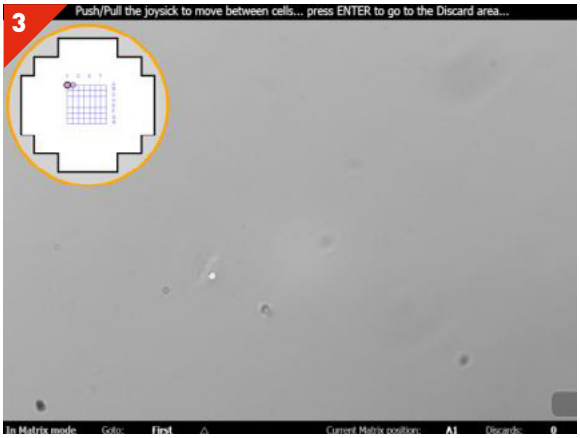
· Follow on-screen instructions to; raise overarm, lower needle, load dish and lower overarm.
· Then select *OK*.

MOTHER/DAUGHTER SEPARATION

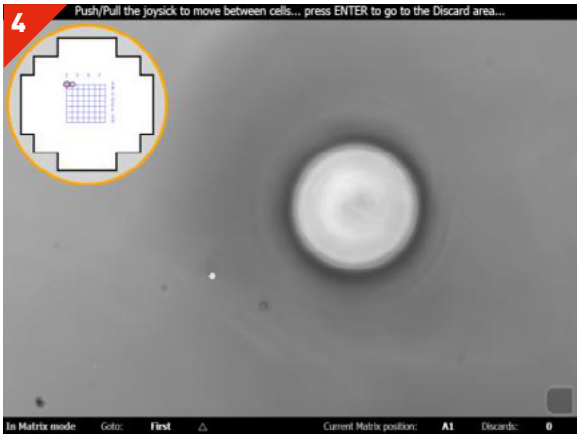


· Turn the *Dish* to centre the reference mark.

· Select *OK* and the stage will begin to adjust it's location.



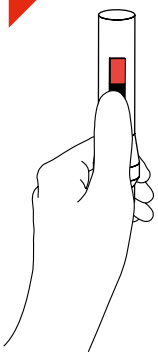
· Push/pull the *Joystick* to move between the cells.

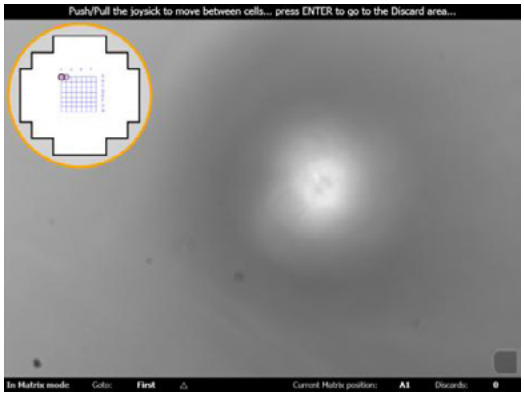


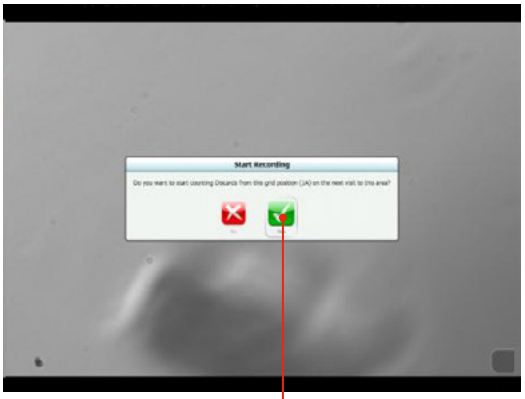
· Bring the *Needle* to the surface and focus.

The mother cell has a daughter cell that is clearly seen on this screen capture. However, a much clearer image can be had by looking through the eyepieces. It is important to know that the cells have undergone cytokineses and the cells have separated. A sure sign of completion is that the mother cell is starting to produce another bud. Whatever the technique it is important to determine which cell is the mother and which is the daughter. Generally the daughter is smaller and can even have separated and helpfully moved some distance from the mother. Whatever the situation it is important to remove the *Mother Cell* at this stage so as to leave a *Virgin Cell*. This must be done with some caution so as to reduce mechanical shock to the *Virgin* and new Mother Cell. Subsequent daughter removal should be made with the same caution. The MSM 400 ageing protocol will count the number of times the daughter is removed at each matrix position regardless of the number of times the dish is removed and replaced after further incubation.

5



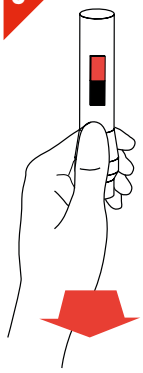


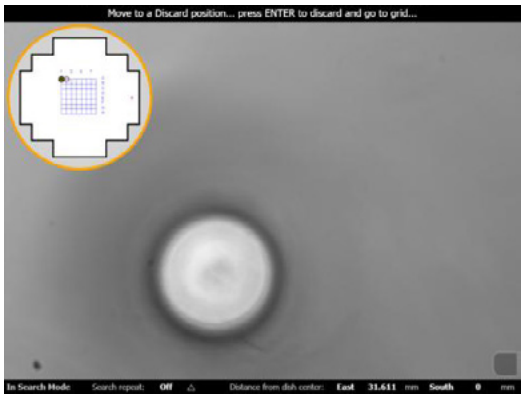


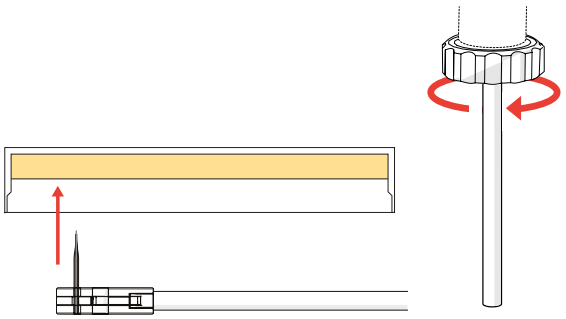
· Push/pull the *Joystick* to move between cells. Press the *Black Button* to go to the *Discard Area*.

· Press *Yes* to start recording.

6



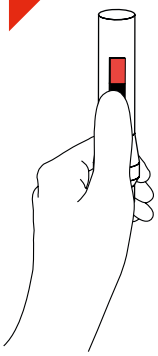


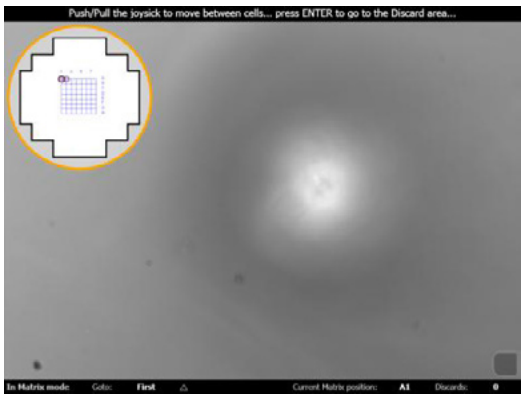


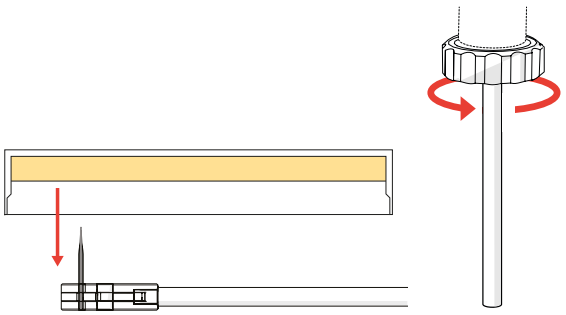
· Use the *Joystick* to move to a *Discard Position*.

· Bring the *Needle* to the surface.

7







· Move the *Needle* away from the surface.

· Press the *Black Button* to discard and go back to the *Grid*.

MOTHER/DAUGHTER SEPARATION

At this stage it is just a case of continuing to review each matrix position and remove each mother cell. After a second incubation period the virgin daughter cells (now the mother cells) will be producing daughter buds. The named dish will have retained information on each matrix position and where you have indicated that the count shall start, it will automatically keep a record of each daughter cell that is removed and discarded.

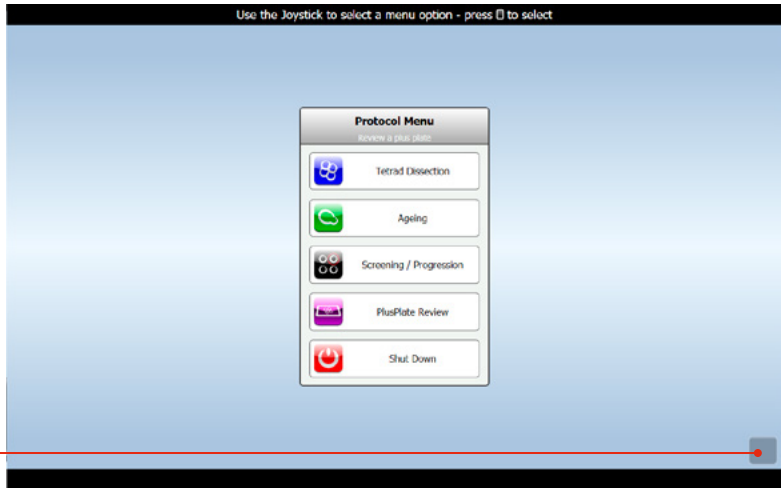
Many highly skilled researchers have developed the technique of removing several daughter cells from the mother cell using other dissecting instruments. However, they still have to keep count of each discard and at the same time remember and record each position manually. The Singer MSM 400 allows any user the ability to accurately perform age studies by simplifying the process and automatically recording discards. This data can then be collected and downloaded to a USB memory stick or via RS232 connection to another computer. To a seasoned researcher this method may at first seem slow. However, when presented with the ease that this powerful program performs the task with a full matrix, the advantages become obvious. Finally, the MSM 400 ageing program, removes the need to remember position, count discards and record all cell divisions. This will allow several dishes to be completed with minimum stress to the user. Within a relatively short time, researchers new to ageing studies will be able to dramatically increase their speed at removing and discarding daughter cells and maintain very accurate results on a par with experts.

SETTINGS MENU

The MSM 400 is jam packed with settings so let's quickly cover the basics.

1

· To access the MSM menu tab, press the *Menu Button*.

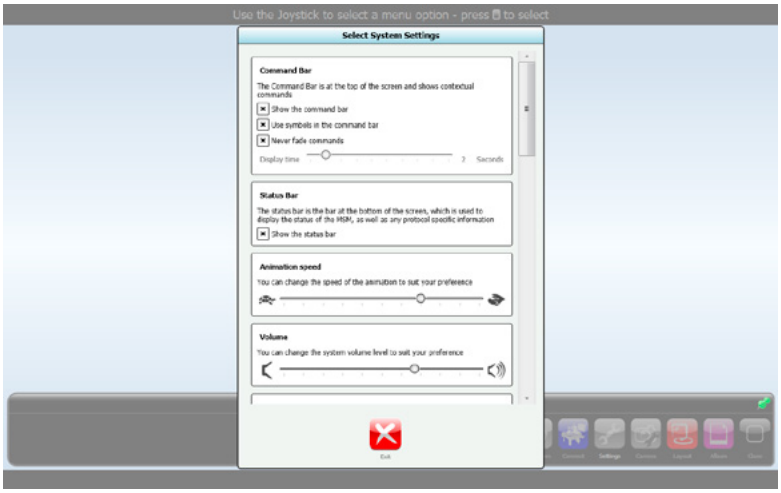


2

· To access general settings, click on the *Settings Button*.

3

· Here you can edit general software options for the MSM 400.



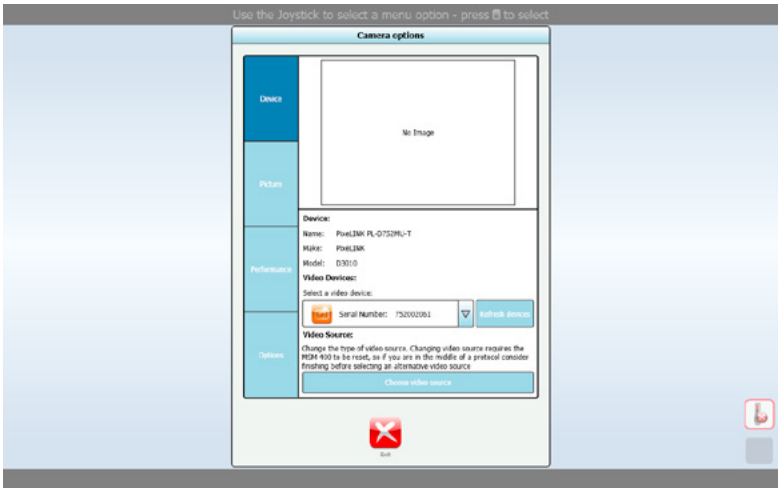
MSM MENU - CAMERA SETTINGS

1

· To access the camera setting, click on the *Camera Button*.

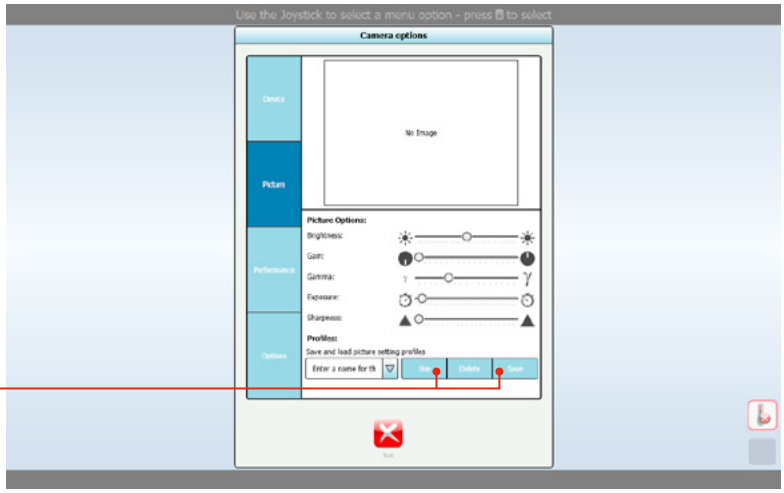
2

· On the device tab you can view the camera information.



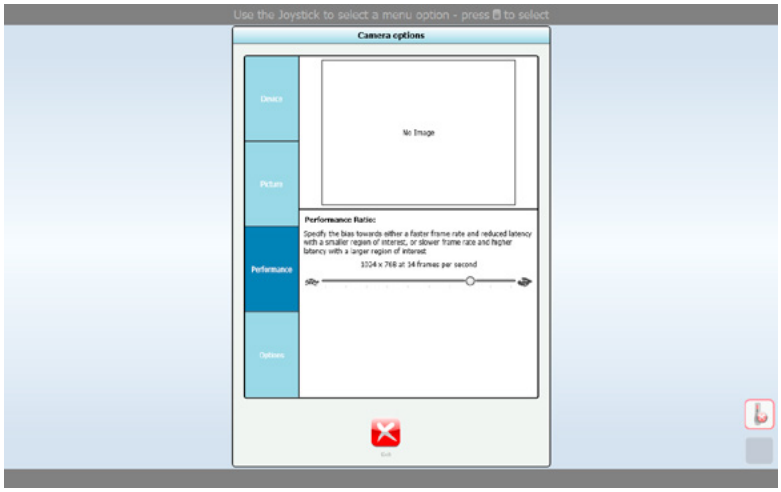
3

· On the picture tab you can edit camera settings and save them as a preset to load in the future.
· Once you have the settings you want click *Use* to apply these settings and *Save* to save a preset.



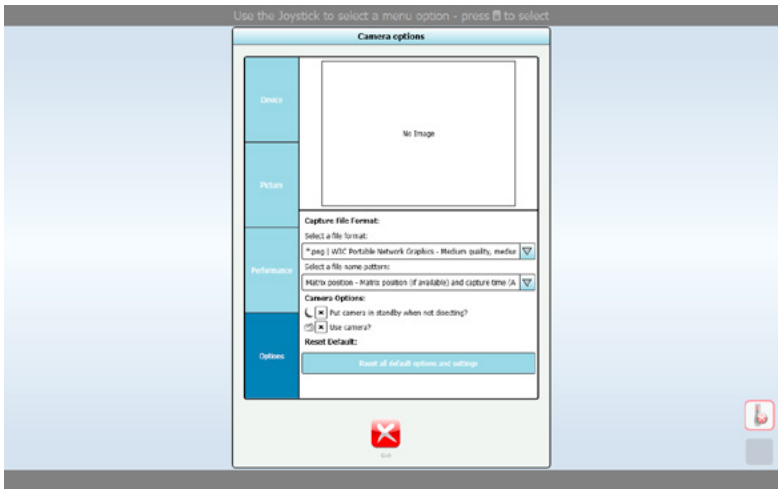
4

· On the performance tab you can edit the camera frame rate.



5

· On the options tab you can edit some general camera settings.



MSM MENU - CAPTURED IMAGE ALBUM

1

· To view your captured images, click the *Album Button*.

2

· Here you can manage your images and videos.

GO FORTH & DISSECT!

NOTES

NOTES

NOTES

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