SINGER MSM MANUAL

SETTING UP AND OPERATING INSTRUCTIONS

SINGER INSTRUMENT CO. LTD
Roadwater, Watchet
Somerset, TA23 0RE,
England.
Tel : Int + 44 (0) 1984 640226 / 640325
Fax : Int + 44 (0) 1984 641166
Email: yeast@singerinst.co.uk

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SINGER MSM MANUAL

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DESCRIPTION

The ***SINGER MSM MANUAL*** is a complete centre for ascus dissection. It comprises a stage-mounted micromanipulator, and purpose designed microscope with a manually driven, detenting stage.

Dissection is carried out with the plate inverted. Limit stops to prevent over movement and tool breakage are factory set.

The ***SINGER MSM*** **microscope** has a built in light source with **substage condenser with iris, lamp intensity control and massive substage heatsink** to prevent temperature rise. The fine focus is operated by knobs positioned each side underneath the stage at bench height. **Coarse focus** is by the large knob at the top of the microscope spine. A **Stage Handle** controls the X (east-west) and Y (north-south) movements of the stage. **Detents** inform the operator of Matrix points in the Y axis while an **unique incrementing stop** controls stage movement in the X axis.

The ***SINGER MSM*** **micromanipulator** is clamped to right side of the microscope and is specially designed for ascus dissection. There are coarse controls for easy tool centring. A ring provides 15mm of movement in the vertical axis and a **co-axial, joystick** controls horizontal movements.

All the main controls are ergonomically positioned at bench top height for fatigue-free operation. Needles are supplied with your MSM and further supplies can be obtained by mail order.

See the description and illustrations in the front of this user handbook.

SINGER MSM MANUAL

SETTING UP

WARNING :

FAILURE TO FOLLOW INSTRUCTIONS MAY RESULT IN DAMAGE.

REMOVE THE TWO RED TRANSIT BRACKETS AND THEIR SCREWS WITH THE KEY PROVIDED. THERE IS ONE BRACKET AT THE LEFT SIDE OF THE MAIN MICROSCOPE STAGE AND ANOTHER AT THE LEFT REAR. REMOVE THE RED Z-AXIS TRANSIT BOLT FROM THE REAR RIGHT SIDE OF THE VERTICAL SPINE, A KEY IS PROVIDED. ENSURE THAT BOTH RED BRACKETS, SCREWS, AND RED BOLT ARE COMPLETELY REMOVED. DO NOT TURN THE COARSE FOCUS KNOB UNTIL THE BOLT IS OUT.

Electrical Connections

The IEC mains lead has been supplied with a bonded plug, connect this to the lead at the back of the ***MSM.*** **THIS INSTRUMENT MUST BE EARTHED (GROUNDED).**

Microscope Optics

Turn the microscope coarse focus knob **FULLY CLOCKWISE**, this raises the microscope head on its Z axis slide.

Fit the microscope head and insert the eyepieces. Screw the two objectives into opposite holes (more than two may cause obstruction problems).

For attaching a CCTV camera, see Appendix.

Micromanipulator

Slide the micromanipulator male dovetail tongue into the female slide on the right, lower side of the fixed stage, pushing it in as far as it will go. Lock with the grey handle. Note; the grey handle on the **MSM** can be rotationally realigned on its shaft by pulling the handle out against its spring, turning, and then releasing in the new position.

Needles

Place the toolholder on the bench with the plastic clamp small through-hole vertical and the Singer logo on the top. Using fine forceps, carefully remove a needle from the box and lower it into the hole in the plastic clamp. The needle should fall through until it touches the bench surface. Raise the needle about 3mm and then lock the needle in place by lightly turning the screw clockwise.

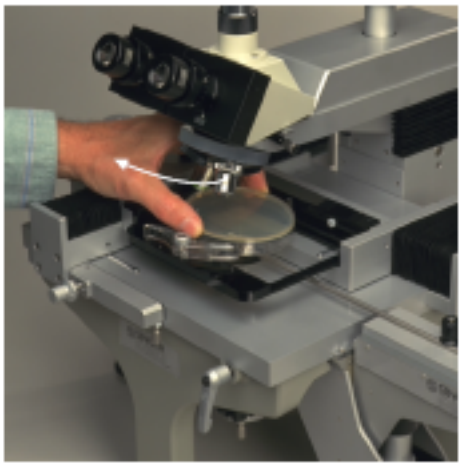
With the needle horizontal, slide the toolholder over the stage using the manipulator toolclamp "V" as a guide. When the microtool is in the centre of the field rotate the toolholder to bring the needle vertical and clamp with the black knurled knob.

Axial adjustments to the toolholder are made with the black knob on the end of the manipulator (*Fig 1*). Transverse adjustments are by loosening the small stainless knurled locking knob and swinging the top plate with the toolholder "V" in it from side to side. You can use the X4 objective for initial adjustment and then change to the X20 for final centring.

Make sure that the elliptical range of movement of the needle when moved by the micromanipulator joystick is central to the field of view.

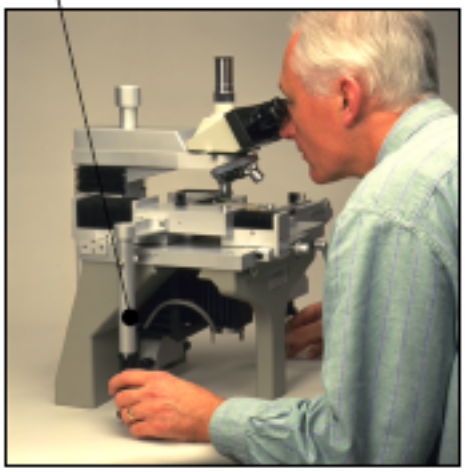
You are now ready to inoculate a Petri dish and do a dissection.

N.B. There is no need to remove the needle holder for needle replacement. Simply grip the broken needle with a pair of forceps, loosen the clamp screw and remove the needle. Lower a new needle into the holder, adjust for height (overhang) and tighten the locking screw.

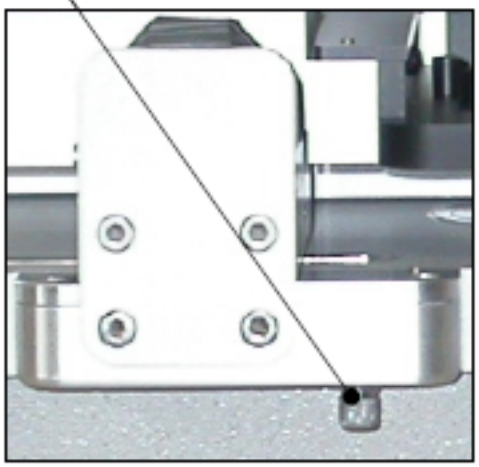


Twisting objective nosepeice to allow more room for positioning the Petri dish.

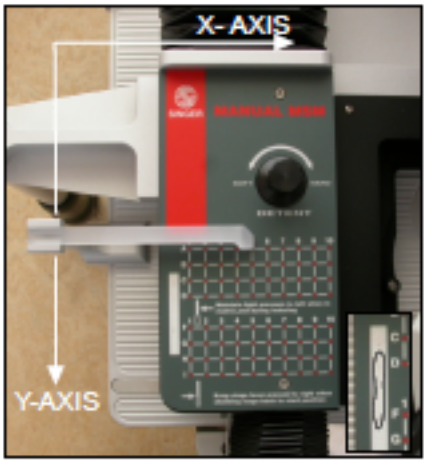
Fig: 1



In the matrix area keep any left/right pressure to a minimum so that you don't move diagonally instead of linearly.



Use knob 'L' to increase/decrease strength of X axis detents. This unit replaces the incremental 'shuttle' and is more intuitive and quicker to use.



The Index Pointer shows you where you are in both the 'Grid' and the 'Inoculum.' You may use a pencil to mark the position of your streak within the Inoculum area as a reminder.

Fig: 2 Petri Dish Layout

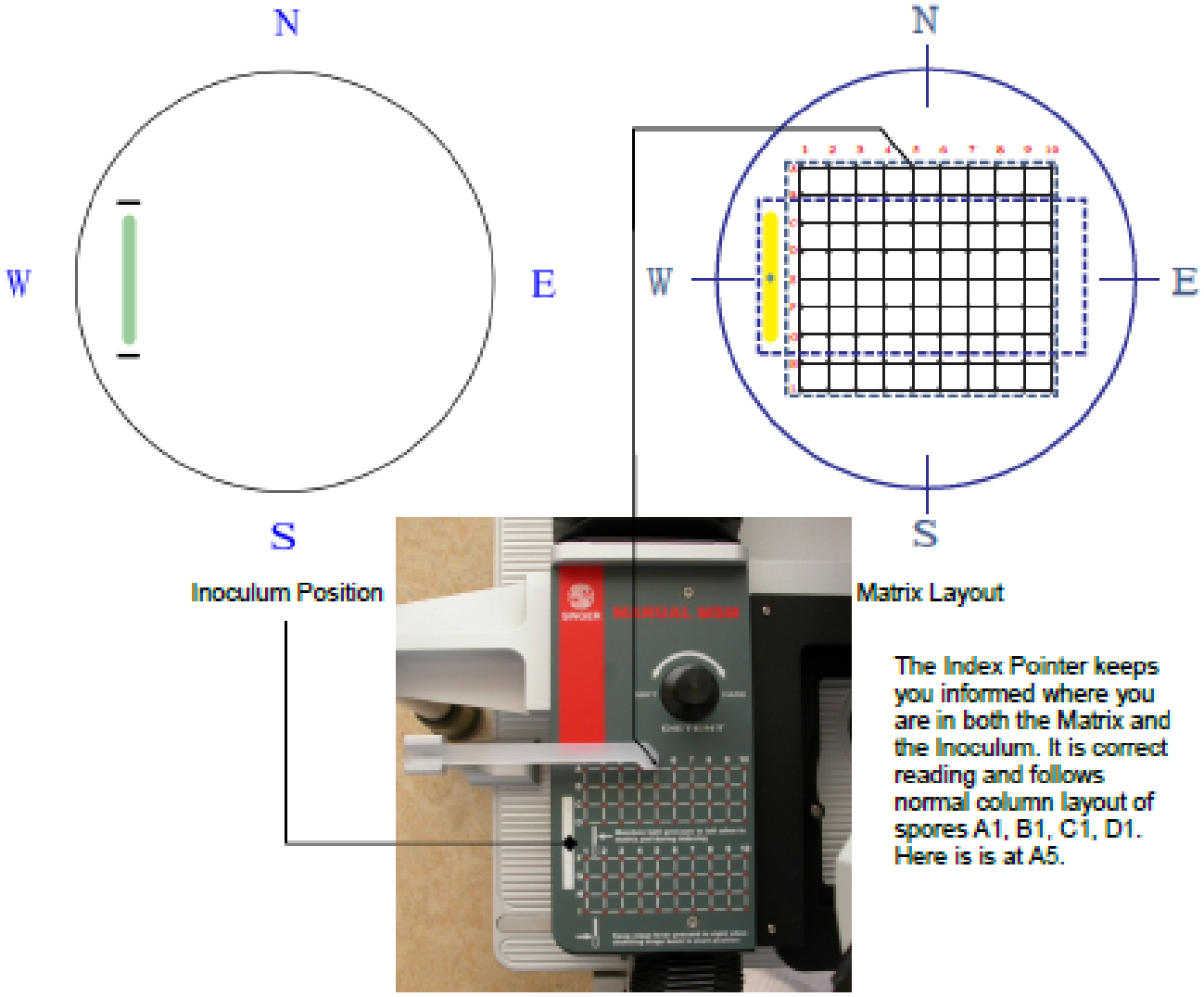
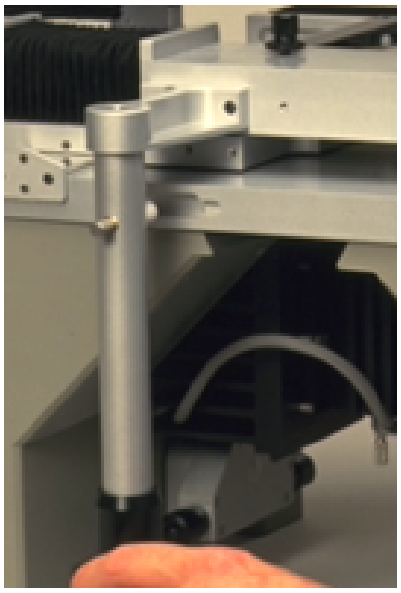
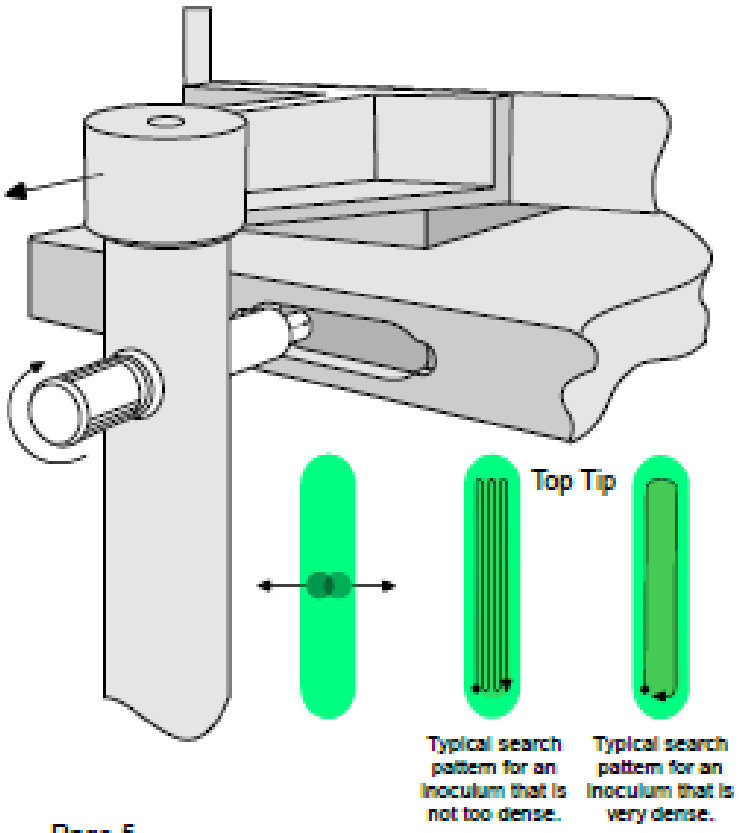


Fig: 3 Left edge of Stage Table





Petri Dish

Refer to *Fig.2*. This shows the plate layout for dissection. You should ensure that your dissecting plates have horizontal, flat and smooth agar with thickness not exceeding 6mm.

It is a good idea to photo copy *Fig.2*. and cut out a template to help you inoculate your dissecting plate in the correct place. Cut out the circular template and then cut along the red line (the cord), or a line parallel with that. Hold this template on the underside of your Petri dish and with a lab pen put two marks to show the ends of the green shaded inoculum area. It is very important to inoculate in the correct place.

You may wish to pre-cut the inoculum area along the red line so that the inoculum may be removed from the Petri dish after dissection. If required, this cut is made with a flamed spatula now.

Inoculate the plate with a single streak of a loop about 3mm in diameter. The density of this inoculum is important: too dense and you will have to 'dig' out the tetrads, too dilute and searching will be tedious.

If you have not done a digestion before, you may find the passage in the Appendix useful.

Place the Petri dish in the holder (to give more clearance, rotate the microscope nosepiece and if necessary raise the microscope overarm with the coarse drive), agar downwards, holding the spring-loaded arm back and rotate the dish so that the marks showing the ends of the inoculum are in line with the north-south movement of the stage. Let the spring-loaded arm rest on the dish rim. Make sure that the dish is properly located.

Switch on the lamp: you can regulate the lamp intensity with a knob on the Power Supply and by the substage iris.

Stage Movement

Your ***SINGER MSM MANUAL*** has been despatched from the factory with the stage movement limited to the inoculum area and column 1 on *Fig 2*

When the **Stage Handle** is fully to the right you will see that a **white nosepiece** some 9mm in diameter enters a slot in the left edge of the fixed stage (*Fig.3*). This nosepiece and slot restrict the movement of the stage in the Y axis between the blue dots NW1 and SW2 in *Fig.2*. When the stage is further to the left it has a fuller movement in the Y axis. You can move the stage along the Y axis by pulling the **Stage Handle** towards you and by pushing it away.

At no time should the **Stage Handle** be pulled down, rather, a slight upward pressure is preferred.

Maintain a slight pressure on the **Stage Handle** in an easterly direction as this will enable you to search the inoculum in a north-south direction in a straight line. The small, 6mm dia. knurled knob on the left of the **Stage Handle** trims the stage movement in the X axis in the inoculum area and allows you to return to the inoculum in the same position on the X axis.

If you move the table along the Y axis you will feel detented points which correspond to rows A,B,C,D,F,G,H,& I. You can increase and decrease the level of detent with knob 'D' in *Fig1* The stage should be encouraged to 'settle' at these detents by 'feeling' it in. Row E has been omitted as it left empty in the **Matrix** and also to assist inoculum search. The Y axis detent works both in the **Matrix** area and in the inoculum.

If you have placed the inoculum in the correct position you should be able, with a small movement of the stage via the **Stage Handle** (*Fig 1*), to move the streak into the field and focus on it. You may care to use the low power objective in the first instance. When you find the streak, change to the X20 objective, focus on the cells. Search up and down the Y axis, moving the stage with the **Stage Handle** and when you have a suitable tetrad in the centre of the field pick it up with the needle.

Picking up the Tetrad

Twist the micromanipulator vertical drive control **clockwise to raise** the needle towards the agar. Remember you **cannot lose the needle from the field if it is properly centred**, so just turn until it appears. You will first see the needle as an out of focus shadow, becoming gradually sharper as it approaches the agar surface. Carefully raise the needle, and as it touches the agar surface the liquid on the surface will suddenly "jump" up the sides of the needle and a black, circular meniscus will clearly be seen round the needle tip. **This sudden appearance of the meniscus is the vital clue to the plane of the agar surface** and can be perceived even when the needle tip is not in focus.

The diameter of the needle may be a surprise at first in looking very large. But its size and shape enable it to pick up anything from an ascospore to a zygote It is the surface tension of the liquid column formed between the needle and the agar that allows the picking and placing of microscopic structures.

Practice touching the agar surface so that the needle tip "skates " over the surface of the agar, just dipping into the liquid layer, when moved with the micromanipulator joystick. You can clean the needle tip by repeatedly dabbing it on a clean part of the agar, and more severely by digging it into the surface and stirring it around.

To pick up the tetrad with the needle, raise the needle until you see the meniscus, then with the micromanipulator joystick move the needle to the tetrad. Move the tetrad around a little, and then briskly turn the micromanipulator vertical drive anticlockwise, dropping the needle, and with any luck taking the tetrad with it. You may need to practice this a little until it becomes easy. Try using the outer edge of the needle and different places on the circumference. The secret is to keep on trying until it happens!

When you have your tetrad on the needle, **lower it away from the agar surface by three or four full turns of the micromanipulator black knob (in case the agar is not flat).**

Remember: **CLOCKWISE** moves the needle tip **TO** the agar.

Remember Clockwise to the Agar

Moving the Tetrad to the Matrix

You now need to transfer this tetrad to **Matrix point A1** for dissection.

If you gently move the **Stage Handle** to the west (left) the stage will come up against a stop which corresponds with column 1 in *Fig.2*.

Pull the **Stage Handle** gently towards you (south), keeping a slight pressure to the west so that the stage stays against the X axis stop, until you locate the stage at **Matrix point A1**. Make sure that the stage is held by the A1 detent and is not just up against the Y axis limit.

To deposit the tetrad on the agar surface by turning the micromanipulator vertical drive ring clockwise to raise the needle. **Be careful!** Look for the meniscus formed by the needle tip touching the agar. You may need to focus using the fine focus.

You can liberate the spores by shaking the ascus by oscillating the micromanipulator joystick, or by tapping the side of the micromanipulator near the ***Singer*** label with the end of the index finger. Make certain that for breaking up the tetrad the needle is just at the "meniscus" height: if it is pressing too strongly on the agar you may tear the surface and loose a spore. Be confident in this tapping. A sharp tap will cause the needle to oscillate and with luck the ascospores will fly apart. Sometimes this takes patience and persistence. Tapping the bench does not work with the ***MSM***. Tapping too violently may tear the agar with tedious consequences.

Dissect the tetrad, picking up the ascospores and depositing them at A1, B1, C1 & D1- the detents tell you where these **Matrix** points are. Remember to keep a slight pressure to the left (west) all the time.

When you have completed the dissection of the first tetrad, return to the inoculum and pick up a second one.

This second tetrad is dissected at F1, G1, H1 & I1.

Now return to the inoculum and pick up a third tetrad. Remember you can 'trim' the X axis position of the stage by the **Search Trim Screw** (*Fig 3*)-don't forget to move the needle two turns away from the agar (**anticlockwise**).

REMEMBER IN MATRIX MOVE NEEDLE TO AGAR...

THEN FOCUS.

Now move the stage back to **Matrix Point A1** and focus on the spore there. With gentle leftwards (west) pressure on the **Stage Handle**, rotate the **Knob 'L'** on the front of the stage (*Fig.1*) anticlockwise a quarter of a turn (45^0) until it hits a stop, and **then clockwise back to its original position**. Under pressure from the **Stage Handle,** the stage will move to the left 6mm. and column 2 will be in the field. This procedure requires a certain amount of "feel" and should be done with light pressure.

The third tetrad is dissected at A2, B2, C2 & D2 and so on

Continue dissecting and moving in this manner until all the **Matrix Points** have been filled.

Important: the **MSM** stage is very free moving and care must be taken to move the **Stage Handle** smoothly and gently so that the stage is brought to bear on the X axis incrementing stop without undue force.

Resetting the X Axis incrementing Stop

The X axis stop is comprised of a row of steel balls which are removed one at a time by the rotation of the knob 'L' (*Fig 1*). At the end of a dissection the balls must be 'shuttled' back to their starting position.

To do this move the **Stage Handle** gently as far to the right as it will go. The **white Stage Handle nosepiece** must be fully engaged in the slot in the edge of the microscope main table (*Fig 3*). With the knob 'L' rotated fully clockwise, push it fully in against its spring pressure and hold it in (NB you cannot push in knob 'L' unless the stage is in the far right position with the white nosepiece in the slot and knob 'L' is rotated clockwise).

Now, using the **Slider** 'S' (*Fig 1*) push the balls to the left past the plug until the slider will go no further. **Return the slider to its starting position** and let knob "L' come fully out. This traps the balls to the left of the knob and you are ready for the next dissection plate.

Warning: You **must** return the slider to the far right (east) position after 'shuttling' the balls back. Failure to do so may result in non operation of the incrementing stop and it may jam.

Dissecting *S.pombe*

The ***MSM Manual*** Handbook is written mainly with *S. cerevisiae* dissection in mind. *S. pombe* requires a "double dissection" technique, tetrads are isolated and left to mature so that their ascal sacks fall away. The spores are then separated.

The Petri dish holder on the MSM has been specially designed to allow accurate replacement of the dish to facilitate the above, but a few tips might be helpful.

The dish is inoculated as in the description for budding yeast, see page 6. Then, following the directions on page 6, the first tetrad is picked up and deposited at A1. A second tetrad is isolated and deposited at F1. The third tetrad is deposited in the next column at A2, the forth at F2 and so on until all the locations in rows A and F have been filled with tetrads.

It is very important that the tetrad at A1 is placed in the centre of the field with the detent properly located. This is essential for the accurate realignment of the plate.

Before the plate is removed for incubation, make a small mark with a lab pen on the rim of the dish next to the small. black plastic tapered pin against which the plate rests. Remove the plate and incubate.

Reset the incrementing stop to obtain column 1 as described on page 10.

Replacing the Dish

Carefully place the Petri dish back in the plate holder and rotate the dish so that the small mark you made previously is in line with the pin. Focus in the inoculum. Move the stage to Matrix point A1, **ensuring that it is properly detented**.

You should be able to see your tetrad . If not, ensure that you are focusing on the surface of the agar (you may need to raise the needle and touch it to establish this) and if necessary, rotate the Petri dish until the tetrad is dead centre of the field, as before.

You can now start dissecting the tetrads one by one. It is a good idea to make a note of each complete column of four so that you don't waste time going back to them.

NOTE: It is necessary to reset the incrementing stop (page 12) by shooting all the balls through every time, but you will find that with a little practice you will be able to move through the columns very quickly.

TROUBLE SHOOTING

Needle will not touch Agar in Inoculum Area.

If the agar is thin the length of the needle may not be sufficient to touch the agar surface before the tool-holder screw comes into contact with the underside of the Petri dish holder. This may manifest itself as an odd movement of the needle as a horizontal component is introduced during vertical movements. The remedy is to loosen the toolholder locking screw and, using forceps, pull the needle upwards towards the objective so as to achieve more 'overhang'. Re-tighten the screw.

X Axis Incrementing Stop jams.

If the slider 'S' is not returned fully to the right after shuttling back the balls, then the knob 'L' can jam because it has a ball stuck in it. The remedy is to push the slider 'S 'fully to the right and then tap sharply the top of the slider unit, at the same time as wiggling the knob. This should free the jammed ball. Refer to the diagram at the end of these instructions for the use of the special magnetic probe.

Knob 'L' jams in during or after ball shuttling.

Behind the Knob 'L' is a rotating plunger which, when rotated, transfers the balls via a shaped cavity from on side of the plunger to the other. If the plunger is pressed in with a ball still in the plunger (this can happen when the slider is not fully right during ball shuttling). The ball can be seen in the small gap between the slider unit and the microscope main table. The remedy is to unscrew the Search Trimming Screw five or six turns, and with the Knob 'L' pushed in, the Stage Handle is pushed firmly to the right when the ball should re-engage in the plunger and the Knob 'L' can be released. The Knob 'L' should be rotated fully clockwise and the top of the slider unit tapped to release the ball. Shuttling can then be undertaken.

INCREMENTING STOP

CARE AND MAINTENANCE

Cleaning

The ***SINGER MSM MANUAL*** is very well made and robust, but it is a scientific instrument. DUST is the enemy of the moving parts. Place the dust cover over the microscope unit and when not in use. There is no need to remove the micromanipulator.

Wipe surfaces with a soft cloth, moistened with soapy water if necessary. DO NOT USE SOLVENTS as they may damage the surface finish and will affect the plastic components

THE APPARATUS MUST BE EARTHED (GROUNDED).

Lamp Changing

A spare lamp is provided. To replace, unscrew the two knurled thumbscrews at the base of the lamp housing and withdraw the lampholder.

Pull off the old lamp and push on the new one. Replace the lamp holder. There is no need to centre the lamp.

If you have any technical problems that are not answered by this handbook please do not hesitate to contact us at the address given in the front of this book. We are always very happy to have suggestions for improvements from users.

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APPENDIX

Digesting the Sporulated Culture

It is a good idea to inspect the sporulated culture microscopically to ensure the presence of four spore asci and to assess the sporulation rate. You should be able to see the tetrads of *S. cervisciae* in their tetrahedral form.

S. pombe zygotic asci are classically banana shaped and appear a lot like peanuts in the shell. Four spores should be seen distinctly and good tetrads have a plump appearance.

S. cerevisciae

Prepare a 10% solution (in sterile water) of β Glucuronidase (or other suitable enzyme at suitable concentration) and pipette about 0.2ml into a small centrifuge tube. Pick up a pin - head size sample of the culture using a sterile toothpick or flamed loop and suspend it in the enzyme: It should appear slightly milky. Incubate the suspension for 10 - 20 minutes at 30^0C (depending on enzyme and yeast strain) so that the ascus is digested sufficiently to allow the spores to be liberated mechanically, but stopping short of random spores.

When digestion is optimal, carefully add sterile water to the top of the tube. This has the effect of stopping the digestion and washing the sticky results of it from the tetrads. Allow the solids to fall to the bottom of the tube, do not centrifuge, and then carefully aspirate the liquor from the top down to the original volume.

TIP: When optimally digested tetrads are microscopically inspected the ascospores appear to have clear, thick, black circumferences.

For *S. pombe*

Inoculate as described below, there is no need to digest as the ascospores liberate spontaneously on incubation.

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APPENDIX

Camera and Monitor

CCTV equipment is either supplied direct from ***Singer*** with the ***MSM***, or locally by Hitachi or Sony or their dealers. If you have specified CCTV, the microscope trinocular head will have a black, circular, dovetail fitting on the top which is part of the DIPS (**D**irect **I**mage **P**rojection **S**ystem) which fits the camera to the head.

If the Camera is supplied by ***Singer,*** then it will already be fitted with the DIPS adapter (a short, stainless steel cylinder containing the lenses). Enter its male dovetail into the black fitting on the trinocular head and tighten the black locking screw. The base of the camera with its four screw holes should face towards the rear of the main unit (if not the image sense will be reversed).

If the camera is supplied by others, screw the DIPS adapter into the camera first and then mount on the trinocular head as above. Later, it may be necessary to use the small hexagon key provided to centre the camera by adjusting the three small set screws on the black fitting.

The camera power supply has one power supply cable coming from it (this has a multi-pole plug on the end) with a second (BNC / BNC) cable held parallel to it with black spiral wrap plastic. Plug the power cable into the multi-pinned socket on the camera and the BNC plug into the coax socket on the camera. At one place on the cable pair, the black spiral wrap is around only one of the cables. Push this single cable part under the cleat on the left rear of the microscope to trap it. Allow the rest of the cable to run down the rear of the MSM and clip it under the clips provided.. Plug the BNC cable into the monitor “IN” socket. To neaten and support the cable at the camera end, secure the cable to the DIPS with one of the black cable ties provided.

Plug the camera power supply mains cable into the camera power supply.

If a camera is fitted, ensure that the trinocular head splitting prism slider (see **fig. 1**) is moved fully to the right. The camera should have its own handbook.

DON’T FORGET TO SWITCH OFF THE CAMERA SUPPLY AFTER USE.

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APPENDIX

Focusing of Camera and Monitor

When you switch on your ***MSM***, the image down the microscope and the picture on the monitor may not be in focus together. This is because the optical tube length is constant for the camera, but alters for the eyepieces when the inter-ocular distance is adjusted.

To achieve mutual focus, first adjust the inter-optical distance to suit you, and then sharply focus the image on the monitor with the microscope fine focus. Then, one at a time, adjust each of the diopter adjustment rings on the eyepieces to bring the image in the eyepieces into sharp focus.

If several people are using the ***MSM*** it is useful to make a note of the correct settings for their eyes.

Video Printer

When a Video printer is to be used then this is best connected between the power supply / junction box and the Monitor as the printer will control the monitor picture quality. Use the "Video Out" BNC socket on the printer to connect it to the "Video In " socket on the Monitor.

The Video printer is supplied with a user handbook. A BNC/BNC cable and a mains cable.