

PC Controls

- 1 Turn on the power at the 'Power Block' by switching the RED switch in the 'ON' position.

The 'Power Block' should be located on the lab bench behind the main unit and connected to mains power via the lead supplied.



SWITCH ON



2

A Special program has been written by Singer to utilise the function of the three buttons on the front of the Cassiopeia. The special instructions are held on a flash memory chip.

Press the RUN MSM button

Note: this may sometimes have to be pressed once to power-up and once more to initiate the MSM program.

The above shows the function of all buttons that are of importance to the MSM System Series 300. All other buttons (not highlighted) are for the Cassiopeia in its stand-alone mode. The screen is also TOUCH sensitive.



ON/OFF

This is the On/Off button for the Cassiopeia only. Unlike the 'Run MSM' button this only energizes the PC, it does not start the program.



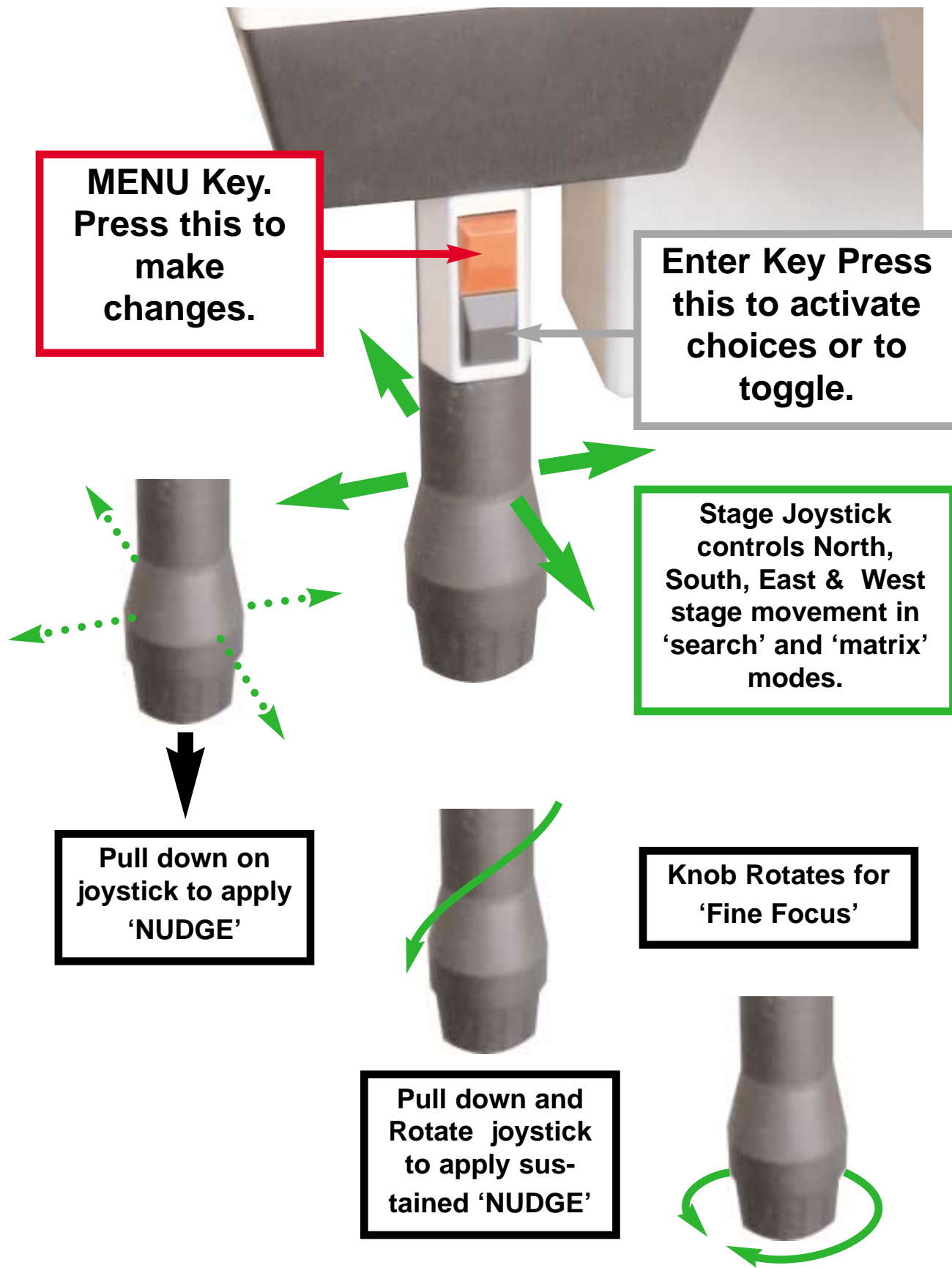
This is the RE-BOOT button. It is used to clear a stalled interface and for trouble-shooting. Use this as a last resort not a short cut to enable factory settings.



This is the Stylus that is supplied by Cassio for touch-screen operations. Some operations can be made with the operators little finger-nail, but not all.

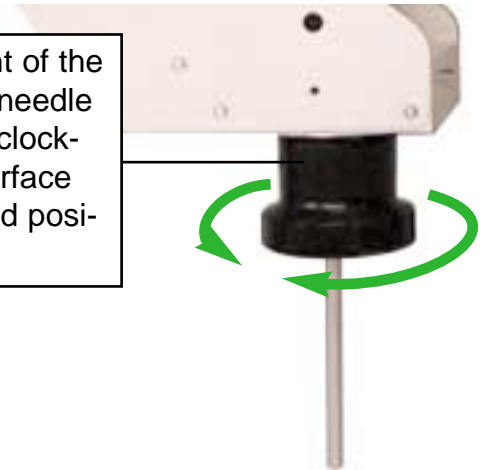


Stage Joystick Controls



The Micromanipulator Controls

This 'Ring' controls the vertical movement of the needle tip. Turning clockwise moves the needle towards the surface of the agar, counter-clockwise moves the needle away from the surface of the agar. The dish is held in an inverted position with the needle underneath.



This 'Joystick' controls the horizontal movement of the needle tip. Having lightly touched the agar with the needle tip the operator can now make the needle 'skate' across the surface in the same plane. The edge of the needle can now be positioned to touch the tetrad and cause it to be attracted to the needle tip.



Operators will find the controls on the micromanipulator extremely intuitive to use. The controls fall easily to hand at bench top height. The hand is supported at all times allowing extended manipulation without fatigue.



This is the 'Coarse Focus' knob.



On first use ensure there is clearance between the longer objective and the dish.



Raising the overarm is done by manually lifting the overarm until it locks. This can be done single handed.



Lowering the overarm is carried out by first supporting the overarm with one hand and pulling the overarm latch-handle towards you with the other. Gently lower the arm until it stops.



Eyepiece focusing can be made by turning the 'knurled' ring (not the rubber cup).

Eye width adjust is made by pulling or pushing one of the linear eyepiece tracks. Standard distance is 63 or at dot.



Start Sequence

This section deals with how to start the MSM. You will have now ensured all plugs and leads are correctly installed and the Pocket PC in its cradle.

Switch on the Red switch on the Power-block. The microscope lamp will light.

Press the RUN MSM button. You will see a small box in the centre of the screen momentarily. At this point the Pocket PC is communicating with the MSM and testing the link.



This is the next screen you will see when the Pocket PC has successfully interfaced with the MSM.

Follow the instructions on the screen. Make sure that the stage can move without obstruction.

Press Enter or Tap OK.

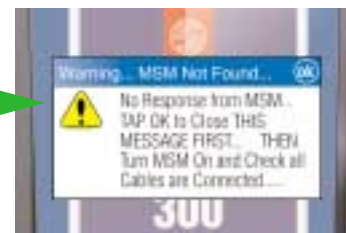
You will now get a series of screens in rapid succession until you are asked to select one of two options.



Press RUN MSM button



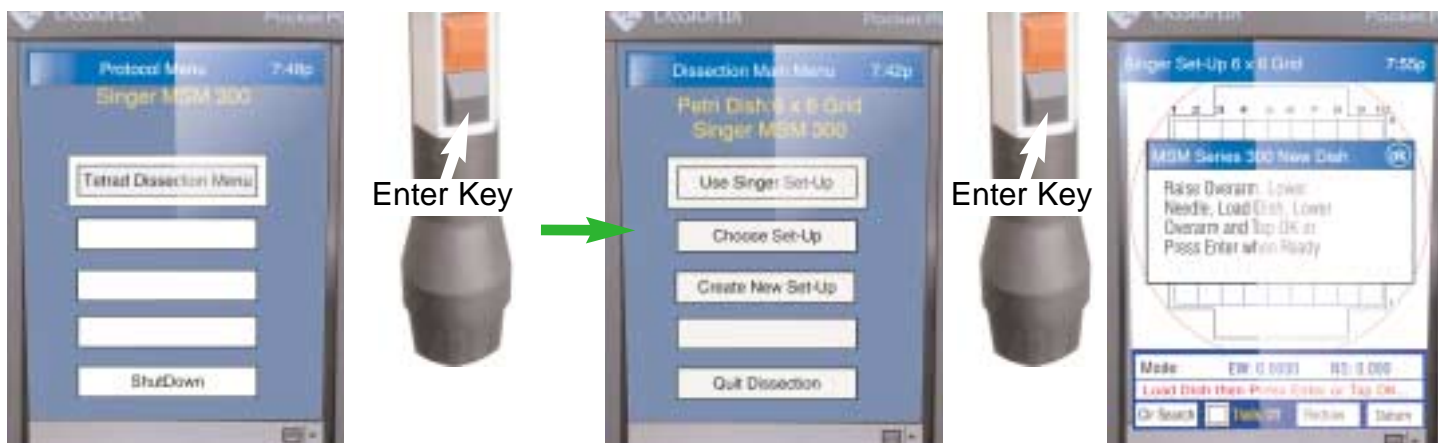
FAULT



If the above screen comes up, read and close the message box. Then Switch off the MSM at the Power-Block. Reboot the Pocket PC by pushing the stylus into the small hole on the back of the PC. If this fails to clear the problem try removing the Pocket PC from the cradle. Turn everything off. Replace the Pocket PC and turn on the power again. See if this has rectified the connection problem. Contact us if it does not.

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If you follow the screen instructions you will have a Petri-dish (see page 16) loaded and you will see the screen on the right. The stage will have moved to the inoculum area. Use the Stage Joystick to find the inoculum and focus on it using the coarse and fine focus.



If you bring the needle to the surface of the agar you can focus easily. Drop the needle away from the agar. Now Drive to the inoculum streak and re-focus.

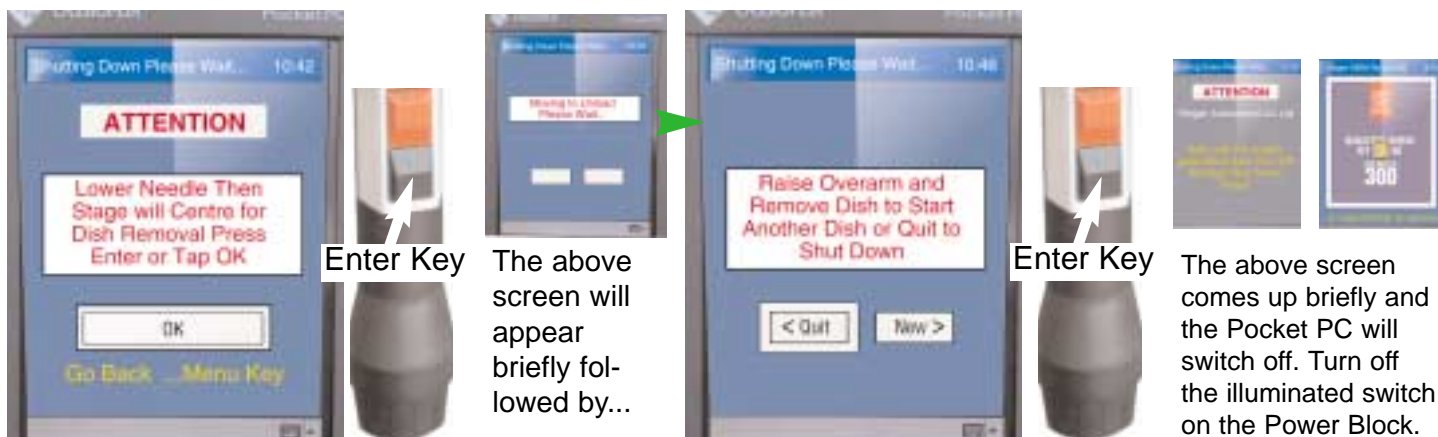
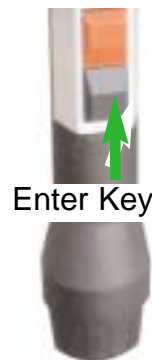
You can now proceed to pick up and dissect a tetrad. Please turn to page 20 for the best technique and to page 18 to move to the Matrix (Grid).

Quit Sequence

You may decide to quit from any position (Matrix or Search). The procedure is much the same and is very simple. Here are a series of screens and commands that will allow you to QUIT properly. Press the Menu Key.



From the Matrix the program anticipates that you wish to quit or start a new dish. To do so press the Enter Key. If, however, you try to quit from the Search position then the program anticipates that you wish to Go To Matrix Position then you must select Done...New Dish or Quit



The above screen will appear briefly followed by...

The above screen comes up briefly and the Pocket PC will switch off. Turn off the illuminated switch on the Power Block.

Loading the Petri Dish

1. Raise the overarm of the microscope.

Note 10. This is convenient as it allows a lot of room when loading the Petri dish and minimises needle breakage.

2. Place the Petri dish, inverted, in the plate holder with inoculum marks on the left and lying north-south Fig. 3.

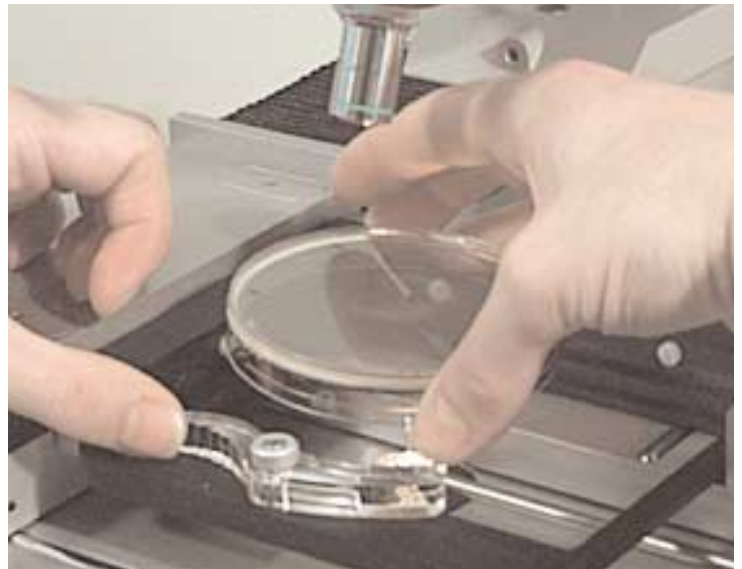


Fig. 3

3. Carefully lower the microscope overarm and move the stage to the streak.

Note 11. The Singer MSM System automatically goes to the streak if it is placed in the correct location.

Useful Tip

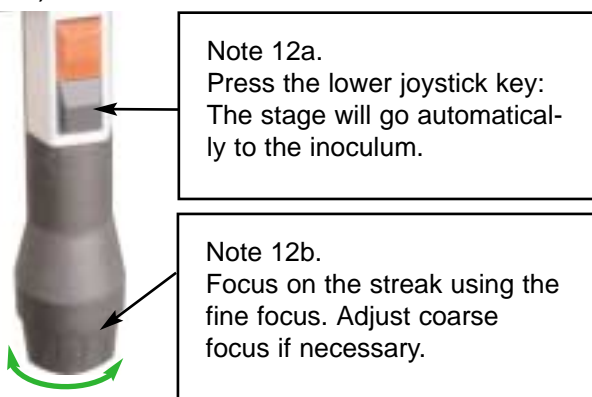
When in SEARCH mode ($250\mu m$) it is easy to take several steps to the outside of the inoculum streak (plate edge side) and plunge the needle into the agar. Here you will be able to vigorously move the manipulator joystick to clean the tip of the needle. The agar is by nature sterile and the needle strong enough not to break. Step back to the streak and continue.

Searching the Inoculum

1. Search the inoculum (fig. 4) north-south for a tetrad (See note 12a&b).



Fig. 4



Note 13a. Move the stage by displacing the joystick: It will move in all directions in a series of quick, small Search Steps ($250\mu m$). Pulling down gently on the joystick while traversing will engage 'Nudge' this will move in ($24\mu m$) steps for finer movements.

2. Centre the tetrad (fig. 5) in the field (See note 13a&b).

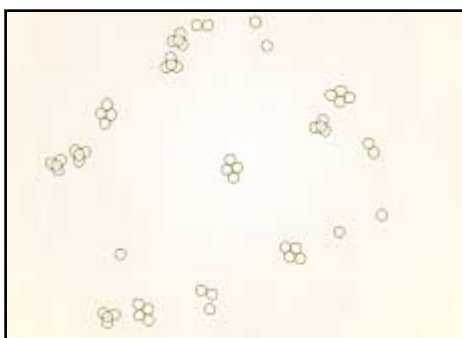
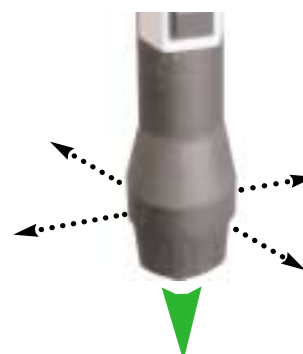


Fig. 5



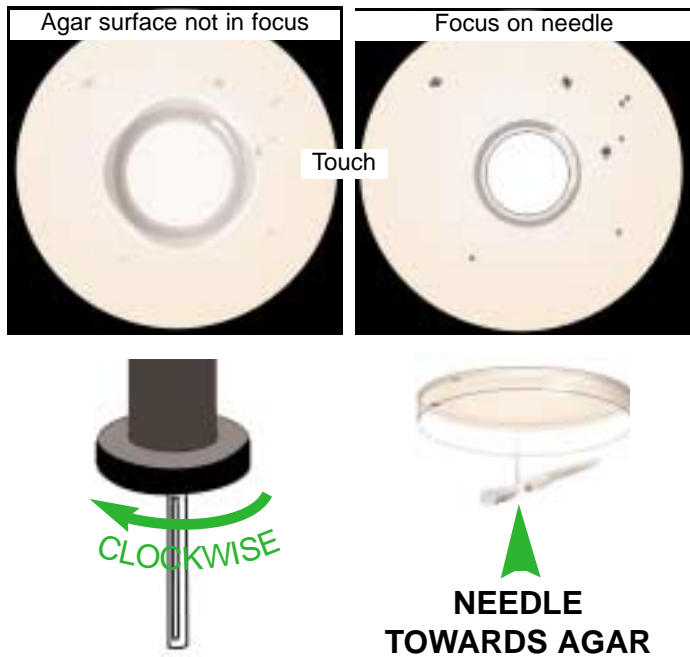
Note 13b. Smaller (Nudge) movements may be made by first (gently) pulling down the joystick and at the same time moving the joystick in the desired direction. "Walk" the tetrad to the centre of the field.



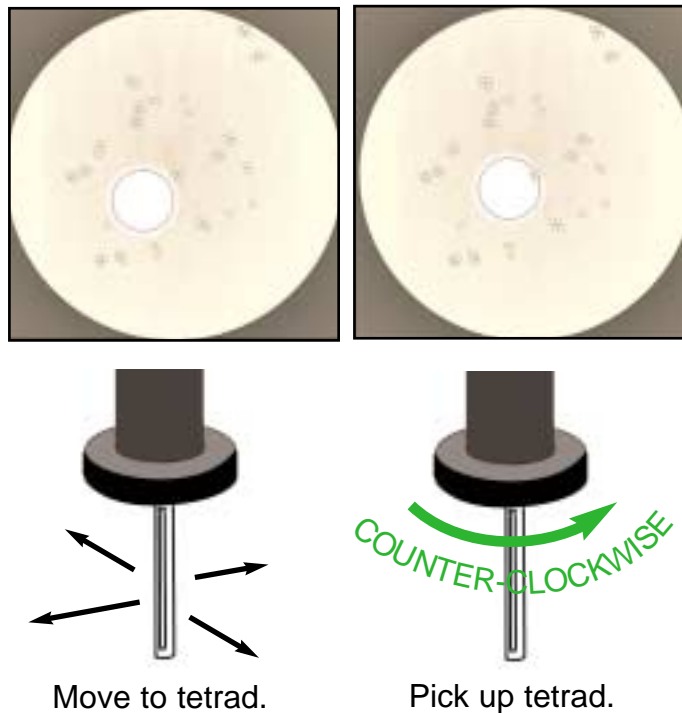
Note 14.
 Rotate the micromanipulator vertical drive control clockwise to raise the needle toward the agar. Remember, you cannot lose the needle from the field if it is properly centred, so just turn until it appears. You will first see the needle as an out of focus shadow, becoming gradually sharper as it approaches the agar surface.

Pick up the tetrad on the needle (See notes 14, 14a, 14b, 14c and 14d).

Note 14a.
 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 be seen clearly around 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.



Note 14b.
 To pick up the tetrad, raise the needle until you see the meniscus, and then with the micromanipulator joystick move the needle to the tetrad.
 The tetrad will be attracted to the edge of the needle.
 Place thumb and index finger on the vertical control ring of the micromanipulator and make a quick COUNTER-CLOCKWISE turn. This should pick up the tetrad. If it fails to pick it up try again until it does. Tetrads can be sticky and resist being moved at first. Sometimes the dissecting plate can be too wet to begin with. However, a few minutes at room temperature will slowly dry the plate enough for perfect dissection.



IMPORTANT

Note 14c. Turn the vertical control ring at least three more movements counter-clockwise so that the needle may clear the agar as the stage moves to the first position on the matrix.

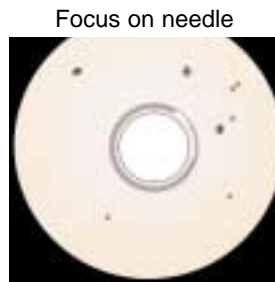
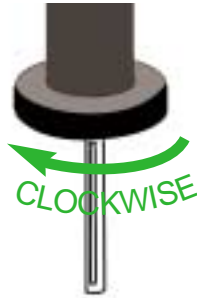
Move the Tetrad to the Matrix

Move the stage to the matrix (See note 15). See the next page to see Matrix Screen layout.



Note 15.
Press lower key on the control joystick. The stage will go automatically to the top left position-A1.

Drop the tetrad at A1 (see Note 16a & b).



Watch for the meniscus formed by the needle to ensure it has touched the surface of the agar.

16a.
Rotate the micromanipulator control clockwise to raise the needle to the agar. Be careful: Look for the meniscus! Focus the microscope with the fine focus knob so that the meniscus is as sharp as possible. You may be able to see the tetrad on the tip of the needle.

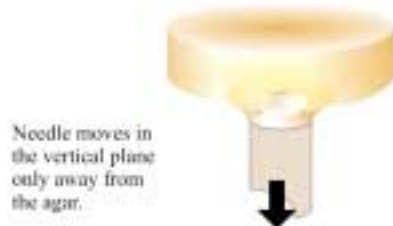
Useful Tip

A1 is the datum position for the whole plate and is therefore a very useful coordinate. By pressing the tip of the needle into agar to make a dark hole it can be found again very easily when the plate is returned after incubation. Even if the surface of the agar is out of focus users will find the mark when rotating the dish. Of course the first spore will have to be placed outside this mark at A1.

TECHNIQUE



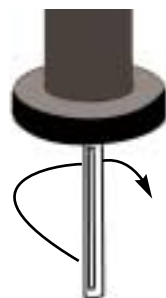
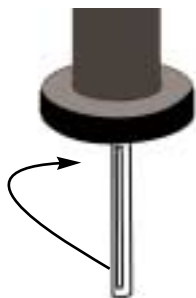
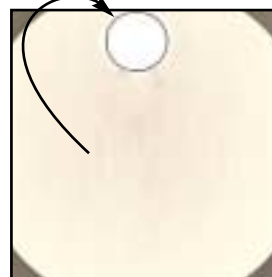
Needle moves in the vertical plane only towards the agar.



Needle moves in the vertical plane only away from the agar.

16b.
Dropping the ascus is the reverse of picking it up: You need to "dab" the needle tip on the agar until the tetrad comes off. After each "dab" it is a good idea to move the needle to one side, as this may reveal that it has been dropped.

Separate the spores (see Note 16c & d).



16c.
Break the ascus by touching the tetrad with the needle edge and pressing the pneumatic pad of the Singer Micro-Zapper. Alternatively tapping the far end of the micromanipulator on the side with the index finger. Make certain that for breaking up the tetrad the needle is just at the "meniscus" stage: If it is pressing too strongly on the agar, you may tear it and lose a spore.

Moving the needle while still in contact with the surface of the agar. The needle will 'skate' across the wet surface and will attract the spores when close enough. Turn ring counter-clockwise to pick up spores.

16d.
When you have broken the ascus apart, pick up any combination of spores using the same technique as for the tetrads. Of course, you want to leave one on A1.

THE MATRIX - Take tetrad to the first position on the Matrix (see Note 17).

To move to the next row pull towards you once.

You will notice that the movement in the matrix mode is different from the movement in the search mode.

In the matrix the factory settings are 6mm centres in both axes (this can be customised to 1mm centres for special research).

**Note 17.**

Dissect the ascospores placing them at A 1, B 1, C 1, and D 1. Do not forget to drop the needle away from the agar every time you move from point to point!

You can move the stage up and down the matrix (grid) column, row A through row 1, by displacing the stage joystick in a north-south direction. The stage position will be shown on the Pocket PC screen.

Return to the inoculum and search for another tetrad (see Note 18).

Push once to return to the inoculum

In search mode the movements are 250 microns in both axis and 24 microns in nudge mode (this can also be modified to suit the individual). You can clear 'search trails here.

**Note 18.**

After you have dropped the last ascospore of the four, move the stage back to the top row, A, and lower the needle. The "Black" or lower key on the joystick unit is like the "Enter" key on a computer. Pressing causes the stage to "Toggle" back and forth between the inoculum and the matrix. Do not forget to lower the needle away from the agar each time before pressing this key or you will lose the tetrad. On pressing the 'Enter' key The stage will return automatically to the exact place in the inoculum from where you picked up the ascus. Search for another tetrad by moving the stage with the stage joystick as before. Do not forget the "nudge" option by gently pulling down on the joystick. Pick up another tetrad. Lower the needle. 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.

TIP

It is good practice after dissecting and placing the four spores to return to each matrix position and check that the spores are there and that what you thought was a spore was not just a bubble in the agar. Also returning to A1 means you can toggle from it to the inoculum and from the inoculum to A1. It is then easy to focus on the agar in this position and move to A2 (push joystick to the right) and be almost in focus for the next dissection.

Returning to A1 also facilitates marking the dish so that it can be replaced accurately (even when out of focus). A1 is a datum point for the whole dish and a mark in the agar can always be found. Simply move the spore to one side and plunge the needle into the agar and make a nice dark hole. When removing the dish prior to completion, further lab-pen marks are advised to roughly locate the dish rotationally prior to moving the stage to A1 and locating the mark in the agar. The post at the back edge of the dish is an ideal location point for a (Λ) pen mark.



Dissecting at A2 B2, C2, and D2 (see Note 19).

Push once to return to the last position in the matrix.

Turning 'trails' on allows user to see where spores have been placed on the Matrix (Grid).



Note 19. Press the lower joystick key; the stage will go back to A1.

Focus on the spore at A1 and then, using the joystick, move to A2.

Dissect this tetrad at A2, B2, C2, and D2.



Press joystick to the right to go to row "A" column "2". Dissect tetrad at A2 and place spores at B2, C2 and D2.



Turning on the Trails option Using the stylus (provided) click in the checkbox provided for this facility. Trails allows you to see where you have been in both the 'matrix' and the 'inoculum.'

Repeat the process for 20 tetrads (see Note 20).



Note 20. When you have completed the top half of the dish by placing and dissecting ten tetrads, you will need to go to position F1.

Using the joystick move to position F1. Now press the lower "Black" key on the joystick to return to the inoculum. Pick up a tetrad, key the lower joystick key, and the stage will return to F1. Dissect the tetrad F1, G1, H1, and I1. Carry on until you finish the bottom of the Petri dish.

You can step to the matrix position F1 by using the joystick.

Another and much quicker way to move to F1 is as follows: Having returned to the inoculum to pick up another tetrad, press the 'red' menu key (wait) at menu select 'Go to Matrix Position.' Press the lower 'black' key and you will return to the matrix. Using the joystick you can quickly go to F1 or any position without having to wait for the stage movement. Press the lower 'black' key again and you can carry on dissecting. See section 'Review Dish' page 24 for further information on this useful feature.

DISSECTION MATRIX MENU

Selecting, Changing Mode or Display



By pressing the 'Red' menu button while in the Matrix you will bring up the Matrix Menu screen. There you will find 5 options.



At any time, the user may go to the Matrix selection menu from the matrix position by pressing the 'red' menu key.

The matrix menu offers a number of options and there is a help check-box to clarify any selection.



Shown here is always the default position and anticipates that the user wishes to quit or work on another dish. This selection box is outlined by a frame denoting that it can be activated by keying 'Enter.'



Selection is quite straight forward and requires the 'Enter' key to activate. See Quit Sequence on page 15.

The user can opt to cancel the 'Selection Menu' and go back to the matrix by pressing the red 'Menu' key. This can be done at any selection position.



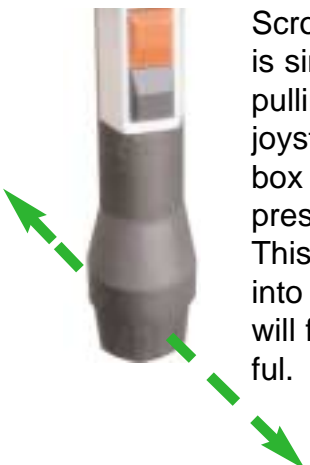
Using the joystick for menu selection is very convenient. By pulling or pushing the joystick the options can be selected up or down in succession.

Pushing forward or pulling back on the joystick will scroll to the next selection option. Alternatively, touching the display with stylus or fingernail will immediately activate the selection.



This function will allow users to retrace the route taken when a tetrad has been accidentally lost in the agar while moving from the inoculum to the matrix.

The MSM 'remembers' the route taken and it is simply a matter of pushing the joystick away from you while looking.



Scrolling to 'Review Dish' is simply a matter of pulling or pushing the joystick until the selection box appears. Select by pressing the 'Return' key. This will put the MSM into a mode that users will find increasingly useful.



This mode allows users to return to the matrix and go to any grid reference point. It facilitates very quick movement about the matrix and only when 'Enter' key is pressed will the stage move to the selected position. This is a repeatable action. Press 'Enter' again to go to another position or 'Menu' key to quit.

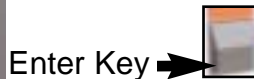
DISSECTION MATRIX MENU



This returns the user to the dissection menu, where changes can be made to the way the MSM operates. Select this option by pressing the enter key.



This will bring up the Dissection Main Menu



The help check box will guide you through each selection when activated.



Enter Key →

Pulling back on the joystick or touching the display in the required box will take you to the next selection option.



Successive scrolling will bring up help screens for each highlighted selection.



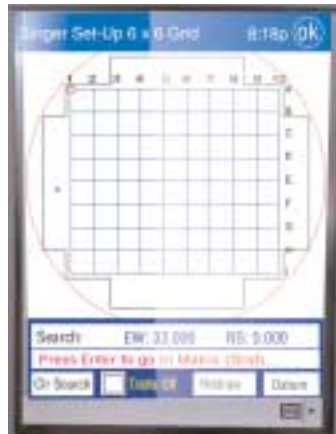
This is a useful selection when a tetrad has accidentally been scraped off the tip of the needle during transfer. The instrument will 'automatically' retrace the furrow accidentally left in the agar. Users may visually scan to the point where the needle made first contact with the agar. This is usually the place where lost tetrad can be found.



SEARCH MENU



By pressing the 'Red' menu button while in Search you will bring up the Search Menu screen. There you will find 5 options.



At any time, the user may go to the Matrix selection menu from the matrix position by pressing the 'red' menu key.

The matrix menu offers a number of options and there is a help check-box to clarify any selection.



The default on this menu page is 'Go To Matrix Position'. Pressing the 'Enter Key' enables Toggle up and down the selection boxes by pulling or pushing the control joystick.

Pulling back on the joystick or touching the display in the required box will take you to the next selection option.



This allows you to change step and nudge distance and speed.



This will allow you to put your inoculum where you want it.



Enter Key

Using the joystick for menu selection is the same for this menu as for the Matrix Menu. Here 'Drive Mode' is highlighted. Activate the 'Show Help' for instructions.

Drive mode is a useful selection for going anywhere on the dish without grids or restrictions.



Pulling down on the Control Joystick will make the stage move faster in this mode. Pulling down and twisting the Control Joystick clockwise will lock it into fast operation. Good for scanning sporulated dishes to ensure tetrads are developed in tetrahedral form.



Enter Key



Making quick changes to basic setup' is made very simple. Press Enter from this selection position and another menu box will open.



This mode allows users to change settings for the Nudge Step (distance in microns in a series of movements), Search Step (distance in microns in a single movement) and the speed at which these two operations may be set.

TIP Try using 24 microns Nudge and 750 Microns Step at Fast for a very non-dense inoculum.

Review Dish Mode

This is a separate section that deals with this very useful mode

The review mode is the program used for looking at specific areas on a completed dish. After incubation it is useful to be able to return the dish to the MSM and go to various Matrix (Grid) references to look at spore development. It is also useful during dissection and after completing the top half of the plate to quickly move from the last position D10 to the first position at the bottom half at F1. This can only be done from the Matrix position.



Pressing the Menu (Red) Key while in the Matrix will open the Dissection Matrix Menu.



This will bring up the Matrix Menu. Scroll or Press Enter to Quit.



Pulling the Control Joystick towards you twice will highlight Review Dish.



Selection will take you to the Matrix (Grid) showing the last position you were at on the Matrix.



Moving the Control Joystick will move the blue target mark to any desired coordinate very quickly.



The stage does not move when the screen is in 'Review Dish' mode. To make the stage move to the selected target the enter key needs to be pressed. The stage will now move to the desired position. The display will confirm your selection and you will be able to view the spore. Pressing the Enter Key again will not take you to the inoculum as it does in Dissection Mode. It returns you to 'Review Dish' mode again so that you may quickly go to another position on the Matrix (Grid). Pressing the Enter Key will activate the stage again. Continue to Review the Dish in this fashion. When you wish to leave the Review Dish mode press the Menu Key (Red) and you will return to the Matrix Menu.



The User Set-Ups selection bar displays all pre-configured preferences. Any researcher can design their own Matrix size, number of co-ordinates and inoculum position and record it for instant access. Their set-up can also be established as the default version or selectable.

Continuing Development and Support

Singer Instruments continue to responding to customer requirements in the field of yeast manipulation. The Program above has been written allowing users to store and retrieve their own 'named' preferences for ascus dissection. This would mean that when 'Tom' wants to use his specified setup he will be able to go to a mode that has the name 'Tom' and he will select that instead of the factory settings. 'Amy' might prefer to put her inoculum in a different position than 'Tom' and when she goes to use the instrument she may also select her customised setup. These programs will be available in the next few months and users will be contacted when the downloads are ready to ship.

Other important software developments for inclusion on the Singer MSM System Series 300 is the addition of pre-designed programs for Screening and Ageing. You will have no doubt noticed two boxes in the Protocol Menu at Start-Up that are greyed-out that have these nomenclature. These are in readiness for the downloads that will activate them namely: Screening and Ageing. Singer is presently developing this software included on a 'Replacement' Flash card that customers may order separately. Please go to our URL <http://www.singerinst.co.uk> and look under MSM System series 300 New Developments.

Singer Instruments has a customer support that is second to none. Most of the reported problems encountered are overcome by a short telephone call. Over 95% are user error and require very little assistance to put right. Many improvements have been made to address the problems accidentally occurring. If they do, then simple 'resets' return the program to 'factory default settings' when things go very wrong for the user. We have an email address so that problems can have a 24 hour 7 days a week receipt. Detailed answers will be emailed by the end of the next working day with attached images where necessary. The Singer MSM System has been designed for optimum performance over an extended period. As such great care has gone into ensuring trouble-free operation. We are confident that any operation failures can be corrected with the minimum of down-time to our customers. Singer Instruments reserve the right to change specifications without prior notification.

NEEDLE ORDERING

Needles can be ordered in packs of 30 needles. Please see our website at www.singerinst.co.uk or order direct by telephone: +44 (0)1984 640226 or Email yeast@singerinst.co.uk.

CUSTOM CONFIGURATIONS

Other Screen Controls

This section deals with changing settings on the Pocket PC.

Changing settings is not as complicated as it first seems. On selecting 'Creating a New Set-Up' you will be presented with a comprehensive guide and a series of drop-down selection boxes. After selecting the various changes the program asks you to give it a name. Give it any other name but the Singer (factory settings) name. You may set this personal setup as the instrument's default setting if you are, say, the sole user or leave the Singer (factory) setting as the default so that other users may choose this or their own setup. First, select 'Create New Set-Up' from the menu.

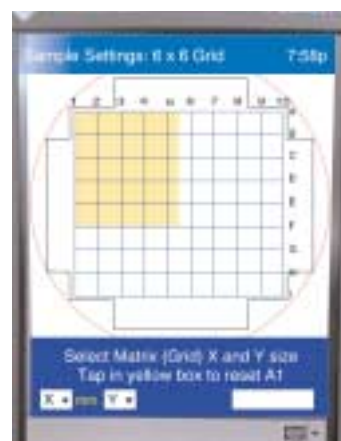


This is the comprehensive guide to the selection process.



Choosing the format i.e. Petri Dish or Slide. Here Petri Dish has been chosen.

You will see the last or default setup which in this case is 10 columns by 9 rows (Row E is left empty). This gives 20 positions on the top half and 20 on the lower half. Use the X and Y box for changing the number of columns and rows you require.



Here you will have to use the stylus provided. When you select the first box marked 'X' a pop-up box will appear with numbers from 1-20.

You may wish to leave position A1 as the default in which case you may just select 'Continue.'



You can also change the position of A1 by using the Stylus and touching anywhere in the yellow box. until you get the layout you desire then select 'Continue.'



This selection grid is again self explanatory. You can choose where you require your streak position.



press 'Continue' or use the 'Enter' key to move to the next menu.

See page 23 for Search and Nudge Step changes.

After selecting the revised search position and changing nudge and search-step distances it will be necessary to give the setup a name. Giving it your own name distinguishes this new setup as being yours. Selection is made using the touch screen qwerty keyboard. Press save setup and you can begin using this for dissection.

CUSTOM CONFIGURATIONS

Slide Set-Up Changes



Select 'Create New Set-Up.'



Press Enter Key to continue.



Select Standard Slide in Singer Holder.



You will then be present with this Factory Set-Up. Changing this is the same procedure as the previous section. See page 31.

NOTE
You must have a Singer Holder to use this slide facility.

If you wish to use the Singer Factory Default Set-up for slides then go to Choose Set-up and select the 'Slide 4X4' default. The default set-up uses 4mm x 4mm centres allowing 60 spores to be placed on a 4 x 15 Grid.

Alternative Techniques

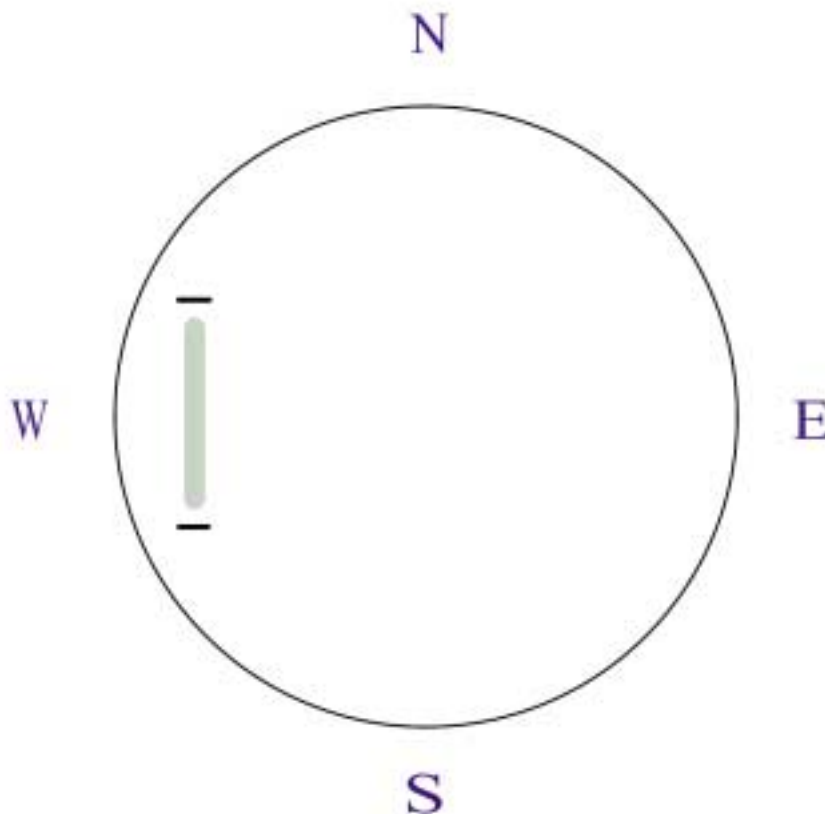
Manipulating and Dissecting Other Structures

The skills learned during ascus dissection are directly applicable to other micromanipulation techniques. As well as spores, vegetative cells, zygotes, and other structures can be isolated and moved to recordable coordinates on a Petri dish. If two or more structures are picked up on the needle, they will automatically come together and can be deposited, touching, on the agar: This can be used for spore-spore or spore-cell pairing. The matrix can be changed to a 1-mm x 1-mm grid giving 360 addressable and, moreover, accurately recoverable locations on a Petri dish. Each generation can be assigned to an address for pedigree analysis or the plate can be scanned for mutants: The Petri dish holder is very precise and so the dish can be removed, incubated, replaced, and addresses can be revisited to record cell progression.

Other Yeast(s)

The techniques described herein can just as easily be used for other yeasts such as *Schizosaccharomyces pombe*. *S. pombe* tetrads appear completely different from those of *S. cerevisiae* in that they can look like bananas or peanuts in the shell.

Sporulation techniques are different, and conveniently with this yeast, the ascospores liberate spontaneously from the ascus on incubation. *S. pombe* tetrads are slightly more "sticky" than those of *S. cerevisiae*, but may be picked up with facility as can the spores.



This is a useful template for the marking of a Petri dish with a lab pen. Invert your dish and place it over the template. Place two marks, as shown, for the correct position of the inoculum streak. This is correct for the factory setup.

SERVICE AND REPAIR

The Singer MSM Series yeast dissecting station has a one year comprehensive guarantee. In most cases many problems or faults are simply overcome by telephone or email communication.

Service contracts can be agreed and our qualified staff will undertake on-site minor services if visiting the country and a neighbouring state (Singer Instruments frequently visit the USA).

Major services require a return to the factory for more extensive work with specialised test equipment. The relevant boxes and return labels will be sent for the safe return of the instrument along with instructions for packing.

For a Service Quotation please contact us by any of these methods...

Telephone: +44 (0)1984 640226 or Email: yeast@singerinst.co.uk Fax: +44 (0)1984 641166

Singer Instrument Company Limited, Roadwater, Watchet, Somerset TA23 0RE England

Singer Instruments reserve the right to change specifications without prior notification.

Singer Instruments...

...a responsibility to science

Removal and Replacement of Flash-Card

The Singer MSM Program is held on a Flash-Card located in the top rear of the Cassiopeia. replacement and upgrading is a simple matter of opening the cover, removal of existing card and replacing with the upgrade card. Initialisation is automatic on reboot.



Location of the Compact Flash-Card. It may be necessary to remove the PC from the cradle



Remove the stylus and unclip the top. It will remove so be careful that it does not get misplaced. Pushing the tag down ejects the card



With the old card removed locate replacement.



This is the Flash-Card with the update.



Note the way the Flash-Card enters the PC.



Push the Flash-Card all the way in and replace the lid.

DIGESTING SPORULATION AND INOCULATING DISH



Fig. 1

Note 1.

The MSM System comes factory configured and ready to use. It is a good idea to read the handbook thoroughly, but this can be left until later if you wish. Set up the MSM as described in the handbook. Switch on the MSM so that the microscope light works (the Pocket PC screen will display a connection message and a tone will sound). Familiarise yourself with the micromanipulator controls. Ensure that the needle is vertical and centred in the field. Lower it until the holder is just above the glass window in the stage. High-specification needles made by the company are supplied with the instrument, and are available for resupply (part no.MSM/N/SO/10). The needles, comprising a pipette-mounted glass fibre cleaved flat at one end, also function as a light conductor for specimen illustration.

Note 2.

It is important to prepare high-quality dissection plates. Autoclave all components together for 15 min at 120°C and 1 bar. For the clearest medium, autoclave the components separately. Allow the mixture to cool slightly before pouring 15-mL into each Petri dish with the dishes on a horizontal surface. Dissection plates should be dried at room temperature for 2-3 hours or it may be difficult to pick up the tetrads, if necessary dry the plates in a laminar flow cabinet.

Note 3.

Enzyme may be diluted 1:5 to 1:40, depending on the enzyme, with sterile water and stored for weeks in the refrigerator.

Note 4.

You should be able to see the tetrads in their tetrahedral form.

Materials

1. Singer MSM System series 300 (Watchet, Somerset, UK), with dissecting needles (see Note 1 and Fig. 1).
2. Sporulated culture.
3. YPD dissection, Petri plates: 10 g yeast extract, 20 g peptone, 20 g glucose, 20 g agar, 1000 mL distilled water (Difco, Detroit, MI) (see Note 2).
4. 3-mm loop.
5. Enzyme: (*beta glucuronidase*, zymolyase or lyticase (Sigma, St. Louis, MO) (see Note 3).

3. Methods: *S. Cerevisiae*

Prepare dissection plates (see Note 2).



Digesting the Sporulated Culture

1. Inspect the Sporulated culture microscopically to ensure the presence of four spore asci and to assess the sporulation rate (see Note 4).
2. Prepare enzyme and pipette about 0.2-mL into a small centrifuge tube.



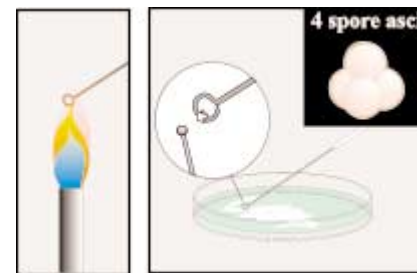
DIGESTING SPORULATION AND INOCULATING DISH

Getting Started Quickly

3. Pick up a pinhead size sample of the culture using a sterile toothpick or flamed loop and suspend it in the enzyme (see Note 5).

Note 5.

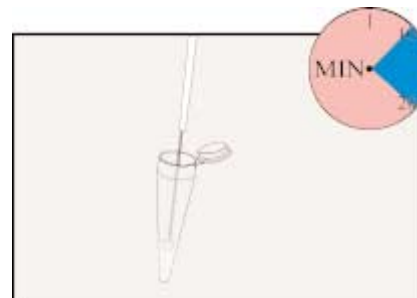
It should appear very slightly milky.



4. Incubate the suspension for 10-20 min at room temperature (see Note 6).

Note 6.

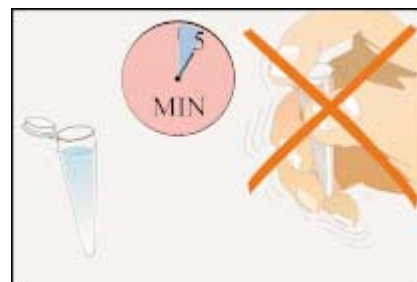
A proper digestion is vital for easy dissection. Inspect the digesting asci on a slide under 400X magnification. Properly digested tetrads have the four spores in a cruciform shape in a single plane. The spores should be easy to see and each has a strong, black outline. The best way to assess a sample is by a trial dissection. The asci should stay together for streaking, but break easily on dissection. There should be minimal signs of random spores.



5. Carefully add sterile water to the top of the tube. Allow the solids to fall to the bottom of the tube. Using cold water in this quantity stops the enzymatic action.



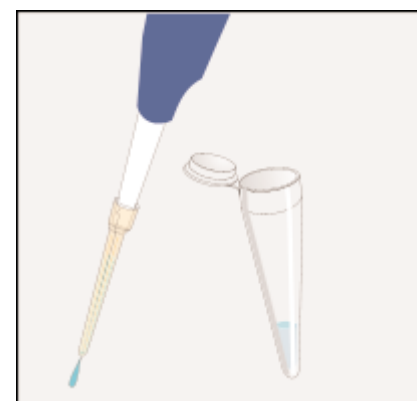
Do not agitate. Leave for approximately 5 minutes for solids to settle...



...and then carefully aspirate the liquor from the top down to the original volume (See note 7).

Note 7.

The concentration of the inoculum is important: Too dense and you will have to "dig" out the tetrads, too dilute and you may have trouble finding a tetrad in a poorly sporulated strain. Remember, you can always adjust the concentration by adding water or aspirating it from the suspension.



Inoculating the Dish

1. Mark the bottom of the Petri dish with a lab pen (fig.1) with a small mark at each end of where the inoculum should be (see Note 8).



Figure 1.

Note 8.

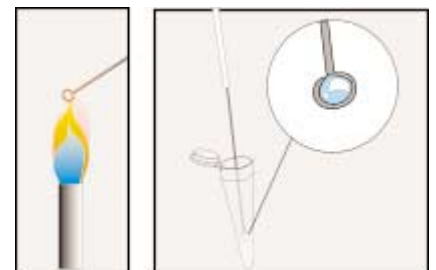
The MSM is factory programmed to reproduce the Petri dish layout shown in Fig. 2. This design produces colonies at a convenient spacing for replica plating and makes use of the whole of the Petri dish. Note that the area on the dish where the inoculum should be spread is shown hatched on the left (this segment may be pre-cut with a flamed spatula so that the whole of the inoculum can be removed after dissection).

A full sized plate marking reference can be found on page 27.

2. Agitate the suspension.



3. With a flamed loop (cooled on edge of agar) put loop into the suspension and check to see the loop is wetted (liquid is present across the loop).



Note 9. A single, light stroke is best. Try not to disrupt the agar surface.

4. Inoculate the dish with the digested sample.

