SINGER MSM SYSTEM

SERIES 200

- ☐ **Designed for Yeast**
- ☐ **Integrated Workstation**
- ☐ **Appropriate Automation**
- ☐ **Flexible Program**
- ☐ **Microprocessor Control**
- ☐ **Needles Supplied**
- ☐ **Easy to Use**

A Complete System

The Singer MSM System series 200 is a complete, state-of-the-art workstation for micromanipulation in yeast genetics. The MSM revolutionises tetrad dissection, pedigree analysis, cell and zygote isolation and cell progression and mutant studies by automating many of the repetitive aspects of the necessary procedures. Every detail of the Singer MSM System series 200 has been designed and built for yeast research. The workstation includes a high quality **Integral Microscope** with a specially developed **Motor Driven Stage** and purpose designed **Micromanipulator.**

Design for Yeast

Close co-operation with major yeast laboratories and feedback from original MSM System users have enabled us to refine the Series 200 into an instrument that not only is easy for the novice to use, but one that supports and makes possible many advanced research techniques.

Appropriate Automation

The heart of the Singer MSM System series 200 is the microprocessor controller which, with its built in program for yeast, operates the motor driven microscope stage semi automatically. The controller is housed in a new, neater computer console which can be placed on the bench or on a shelf. A separate, small, stage joystick unit (again, new to the Series 200) connects to the console and this operates the stage functions and the remote electronic focusing.

The LCD display prompts users to choose matrix size, inoculum search rate and other values which are then retained in memory. The automatic operating system then handles stage movement in tetrad location, placing of ascospores on the matrix and movements in other procedures. The display keeps the user informed of exact stage position and operating mode.

The microprocessor always "remembers" the last place that a spore was deposited on the matrix and the last place visited in the inoculum. This means no more missed rows, very neat results and returning to matrix points after incubation is simple. Software "stops" prevent needle breakage.

Singer Instruments...

...a responsibility to science

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Singer MSM System series 200

Microscope and special Petri dish holder

Microscope overarm raised

Integrated Workstation

With a resolution of 4 microns, repeatability of 2 microns, and a movement of 115 mm x 100 mm, the electric motor driven stage scans Petri dishes without turning.

Standard equipment includes specially made Singer X15 widefield eyepieces, X4 and X20 XLWD objectives, halogen light source with intensity control and in-stage condenser with iris diaphragm and filter tray. Illumination is via a special window in the sub-stage which reduces airborne contamination.

Details that Count

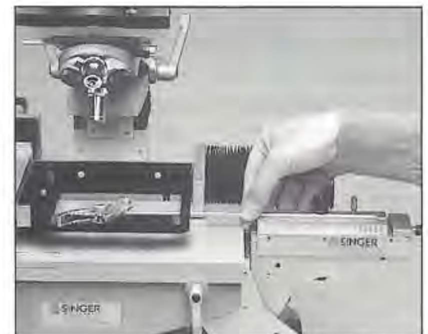
The MSM System integral trinocular microscope has electronic remote control fine focusing from the joystick unit and hinged overarm to clear Petri dishes with ease. This, together with the specially designed inverted Petri dish holder means that tool breakage is virtually eliminated. The precision holder accepts standard Petri dishes of all makes.

Convenient and comfortable pendant joystick operation

The MSM Micromanipulator is locked by a single handle to either side of the massive sub-stage for right or left hand operation. Direct mounting to the microscope eliminates vibration. The MSM System may be used on an ordinary bench.

Horizontal movements of the needle are controlled by a pendant joystick. A coaxial ring operates a fine, counterbalanced vertical drive and all movements can be carried out with the hand resting comfortably on the bench surface. Coarse controls are equally convenient. Great attention has been paid to the reduction factor, robustness and type and range of movements of the MSM Micromanipulator to make it particularly suitable for yeast and easy to use.

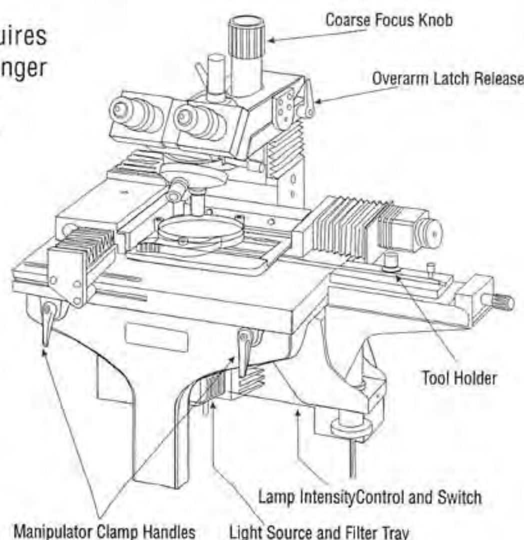
Manipulator mounting from left



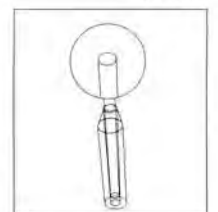
Toolholder

Needles Supplied

Successful micromanipulation requires good needles - often a problem.The Singer MSM System comes complete with toolholder and two packs of specially developed, factory made needles. Once the toolholder has been centered, needles may be replaced without toolholder resetting. This takes only a few seconds. Replacement needles are readily available.



Needle Design



Needles are of special construction: the light transmitting, fibre core with flat working tip is supported by a thicker, taper ended glass envelope. This cylindrical envelope gives rigidity to the needle, permits easy handling and placement in the holder. It also means the "overhang" is big allowing the use of thin agar if required.

Singer MSM System series 200

Control console and Joystick.

Flexible Program

The MSM System series 200 comes with a factory set routine ready to use, but all of the operating parameters can be changed easily by the operator from a simple menu. Matrix size, for instance, can be as small as 1 mm, or as large as 9 mm. The former is used for mutant scanning, while the latter supports dissection at the same pitch as a 96 well microtiter plate.

Alphanumeric or numerical co-ordinates may be keyed in for automatic recall of any matrix point. This recall ability is used for time lapse studies where cells can be located and "photographed". The Petri dish can be removed from the MSM, incubated and accurately replaced in the properly designed inverted dish holder.

By keying in the appropriate co-ordinates, the cells of interest can be photographed again and so a series of time-lapse images can be recorded. A video printer is used for this work, or the images can be downloaded to a computer.

All controls are at benchtop height

Data and Imaging

The MSM System series 200 has provision for an RS-232 (RS-422) communication port and software which can down load positional information direct to a PC or Mac. Optional accessories include a CCD CCTV Camera and monitor, and video printer. The camera is fitted to the microscope head with new Singer DIPS (direct image projection system) adapter which improves picture quality and optimises monitor magnification. Over 75% of the MSM's that we supply are fitted with CCTV, as it is invaluable for teaching and for cell progression studies.

Singer MSM System series 200 with full C.C.T.V. and Video Printer.

The Singer MSM System is in use in many of the world's leading yeast laboratories where it has become the instrument of choice for both experienced researchers and those new to the field. The MSM System's operational routine and the dissecting needles are equally good for *Saccharomyces cerevisiae* and *Schizo–saccharomyces pombe*.

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For further details please contact Carl Singer.

SPECIFICATIONS

MAIN UNIT	Dims	L: 400mm W: 425mm H: 500mm
	Weight	24 Kg.
	Finish	Epoxy paint and clearcoat matt Grey
Microscope	Head	Trinocular with camera port.
	Spec.	Width and focus adjustment to both oculars.
	Coarse focus	Manual, 35mm
	Fine Focus	.75mm by remote control electronic servo.
	Overarm travel	60mm vertical (200) Hydraulically damped.
	Objectives	X20 XLWD, X3.2, flatfield, parfocal.
	Eyepieces	X15, wideangle.
	Illumination	20 Halogen, close coupled with massive heatsink.
	Condenser	LWD with stage optical window, iris and filter tray.
	Lamp PSU	Switch mode with intensity control and on/off switch
Stage	Max movement	115mm x 100mm
	Resolution	4 microns.
	Bearings	High accuracy, rolling, on hardened and nitrited rollways.
	Drive	Precision s/steel leadscrew with anti - backlash nuts.
	Motors	400 steps/rev. bipolar, 375mA/phase, 24v.
	Plate Holder	High accuracy for Petri dish, inverted. Takes all makes.
MANIPULATOR	Dims.	L. 80mm W.50mm H.230mm (Ex. stage).
	Weight	1.5 Kg.
	Controls coarse	Z-15mm. Y-10mm.
	Controls fine	Z-15mm, X/Y elliptical, 600 x 250 microns.
	Bearings	Hardened, nitrided and lapped rollways.
TOOLHOLDER	Material	Stainless steel shaft 4.75mm dia. x 285mm long.
	Clamp	Clear moulded plastic, screw fixing
NEEDLES	Core	Optical fibre, 40-50 micron dia.
	Tip	Cleaved tip, flat and normal to axis.
	Length	21mm overall
	Envelope	Tapered capillary glass, 1.25mm dia.
CONSOLE	Enclosure	Steel case with alloy end castings and side extrusions.
	Spec.	IEC 297-1 and DIN 41494
	Finish	Two tone grey matt finish.
	Dims.	3U 10.5" : Width 314mm, Depth 322mm, Ht. 162mm.
	Keyboard	18 key, matrix, alphanumeric.
	Weight	7.75 Kg.
	Display	LED Backlit, high contrast supertwist, 2 x 20 character LCD.
	Supply	240, 220 or 115 V (specify which). 100W
	Protection	Fused filter approvals: SEV, VDE, UL, CSA, SEMKO.
	Outputs	1, 2 , 3 IEC mains to lamp supply, 25 way to Main Unit, RT Video.
	Outputs	4, 5 , 6 15 Way to Joystick unit, 12 V for Camera, RS-232 port.
	PSU	Switch Mode UL, CSA, BABT BS6310 approved.
	Filter / EMI	VDE Curve A
	Microprocessor	Enhanced 6502 CPU.
	Memory ROM	16K Eprom. Factory data installed.
	Memory RAM	10Yr. Lithium battery-backed for user data.
JOYSTICK UNIT	Enclosure	High impact plastic. Off white matt finish.
	Dims.	L: 190mm, W: 138mm, H: 45mm.
	Focus	Single turn, precision servo.
	Joystick	Self - centring, analogue, 2 axis.
	Keyswitches	Keyboard type, long life
PROGRAM	Language	Forth and assembler.
Matrix	Range	1 - 9mm.
	Max. points	9801 (in 1mm grid) 99 points each axis.
Search	Range	4 - 999 microns.
Nudge	Range	4 - 99 microns.
Drive	Speeds	3
Limits	Ram	2 per quadrant (total 8).
	Rom	4 (factory set).
Datum	Type	Optically derived, auto on start & at user command.

CONTENTS

1 **Introduction.**

2 **Setting Up**

3 **Getting Started Quickly** (fast dissection)

4 **Other Techniques** (mutant screening, pedigrees etc.)

5 **Custom Plate Layout** (factory and other settings)

6 **Troubleshooting**

7 **Appendices** (A-definitions, B-flowchart, C-comms settings)

8 **How to use the Interactive CD**

9 **Zapper Fitting Instructions**

This user handbook has been written to enable the new *MSM System* user to set up and operate the instrument with the least amount of time and effort.

Follow the Setting Up instructions carefully and then Getting Started Quickly will guide you through very easy ascus dissection using the factory, pre-programmed settings.

You should be able to set up the MSM and dissect your first tetrad in about three hours.

The other sections of the handbook can be read later.

1. INTRODUCTION

The ***SINGER MSM SYSTEM*** is a complete centre for ascus dissection. It comprises a stage-mounted micromanipulator, a purpose designed microscope with motor driven stage, and control console housing the microprocessor and other electronics.

Dissection is carried out with the plate inverted. Circular and rectangular dishes may be used up to 115mm X 100mm maximum size. Limit stops to prevent over movement and tool breakage can be pre-selected by the operator.

A joystick controls the X and Y movements of the stage. A sixteen key alphanumeric keyboard addresses the microprocessor. Messages and positional information are displayed on a back lit supertwist liquid crystal display (LCD). Many combinations of step sizes and operational modes are user programmable and are selected on prompt from the LCD.

The microscope has a built in light source with massive sub-stage heatsink to prevent temperature rise. The fine focus is operated electronically from the console, as is the lamp intensity. The microscope overarm is hinged at the rear and can be raised and latched in position for easy plate changing.

The ***SINGER MSM SYSTEM*** micromanipulator can be clamped to either side of the microscope and the console placed on the other. The micromanipulator is specially designed for ascus dissection. There are coarse controls for easy tool centring. A ring drives 15mm in the vertical axis and a coaxial, pendant joystick controls horizontal movements.

All the main controls are ergonomically positioned at bench top height for fatigue free operation.

2. SETTING UP

Please read the following directions carefully.

2.1 Positioning

The ***MSM System*** is very heavy and robust and is resistant to vibration, but it should not be put next to a source of strong vibration like a refrigerator. It should be placed on a sturdy bench but a special anti-vibration table is unnecessary. It does not need to be placed in a hood. Do not place in direct sunlight. An adjustable stool is a good idea.

2.2 Transit Brackets and Transit Bolt.

There are two red transit brackets and a red transit bolt which must be removed from the main unit before operating the instrument. Each of the brackets has three screws. Completely remove these with the key provided, do not put the screws back in the holes. Remove the transit bolt with the key provided for it. Note: this bolt supports the weight of the microscope overarm and it may be necessary to turn the microscope coarse focus knob slightly one way or the other to take the weight off this bolt so it can be unscrewed easily and removed.

FAILURE TO REMOVE THESE MAY RESULT IN DAMAGE TO THE INSTRUMENT.

2.3 Console and Electrical Connections

With the exception of the CCTV Monitor (where supplied) all power supply and signal connections are made at the rear of the ***MSM*** Console.

Place the Console to one side of the Main (microscope) Unit. There are two cables that connect the Main Unit and the Console; a grey double ended cable and a black power supply cable. Ensure that they are pushed right home and that the screws are tight. See **fig 2.3**.

A suitable power cable with a bonded plug is supplied to connect the Console to the mains supply in most countries. If the mains cable does not have a plug on it then fit a suitable one. The yellow/green wire is earth/ground.

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Fig 2.3

CONSOLE REAR PANEL

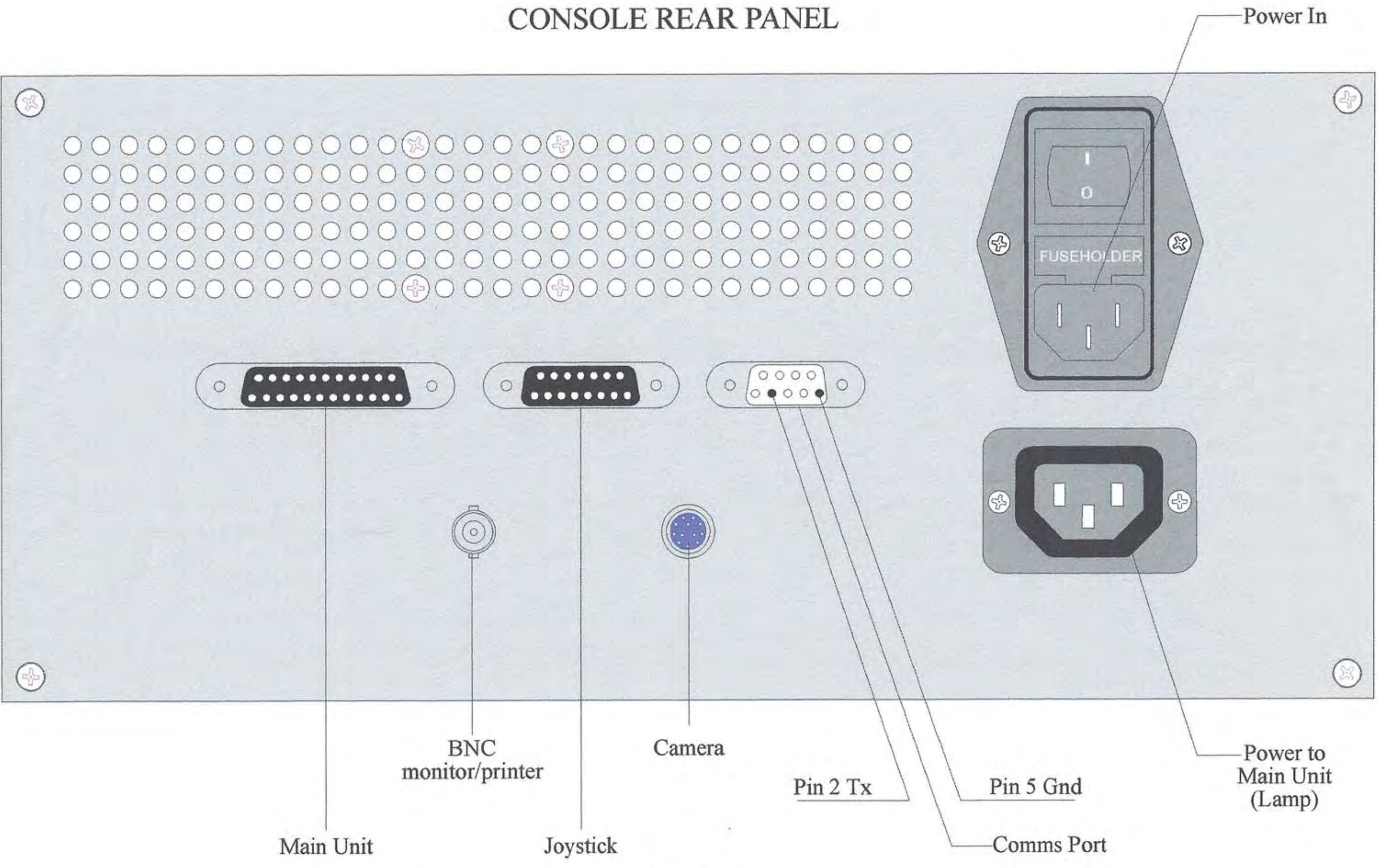

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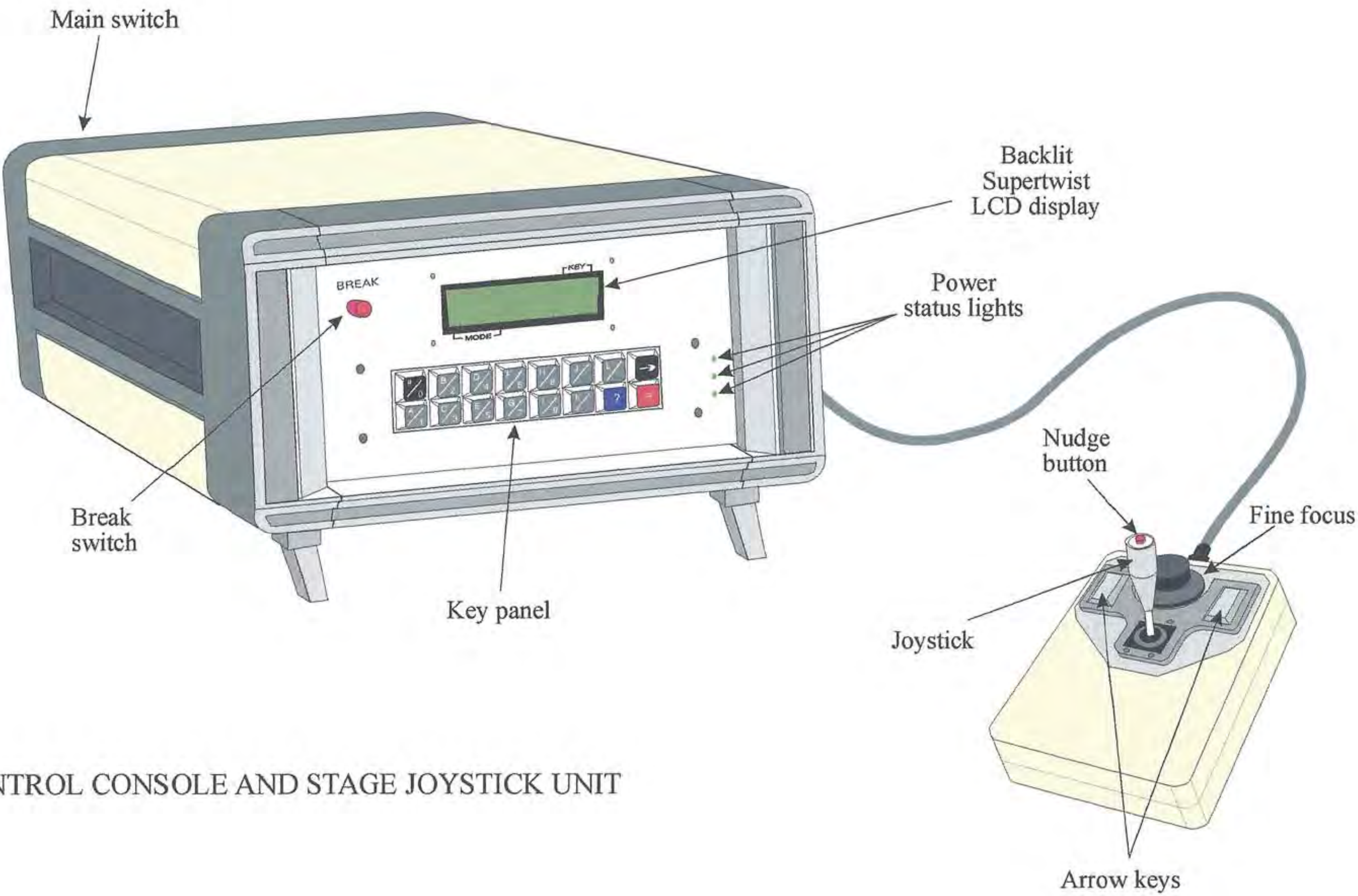
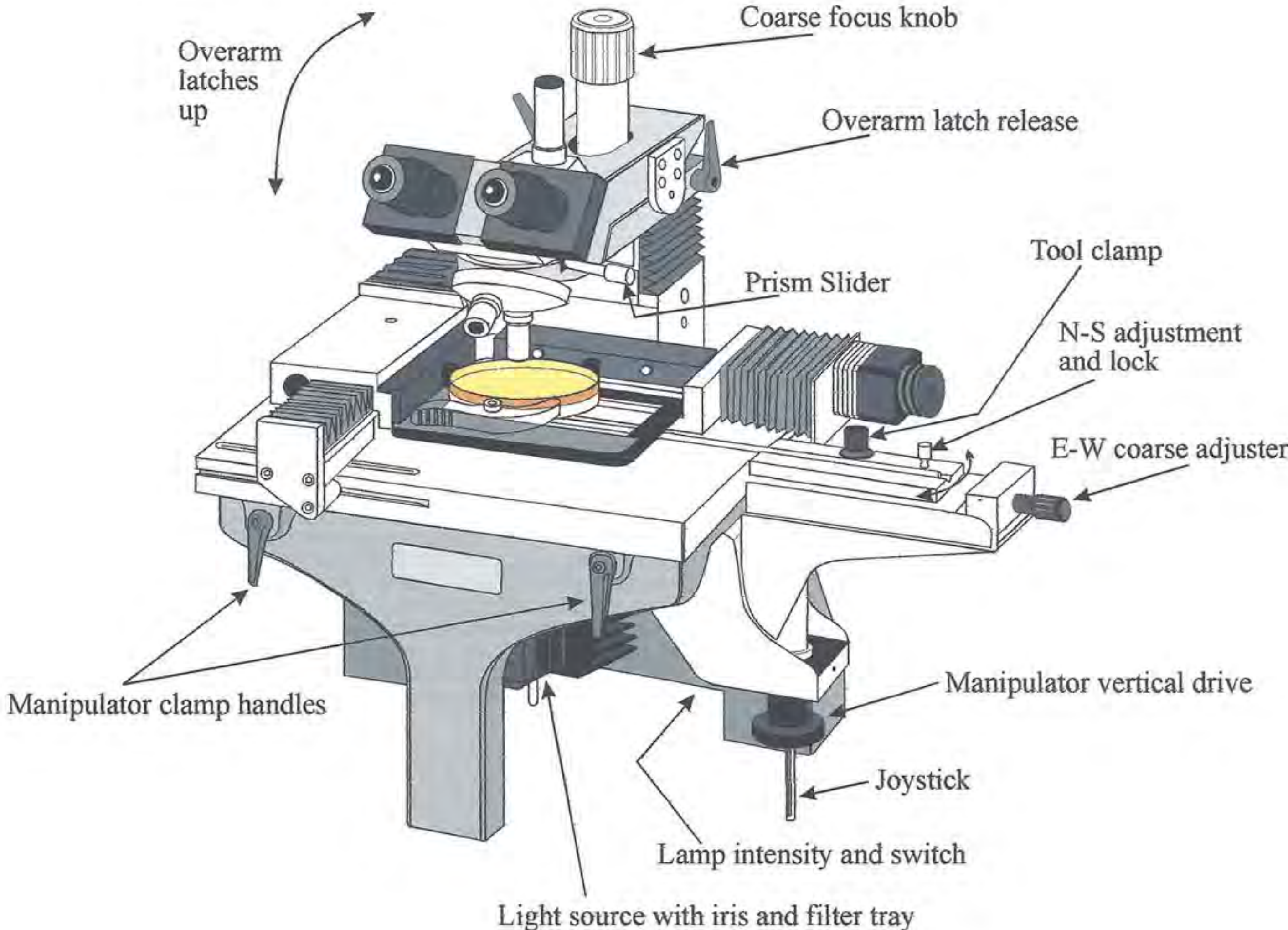
Fig 2.4



MSM CONTROL CONSOLE AND STAGE JOYSTICK UNIT

Page 5



MSM MAIN UNIT AND MICROMANIPULATOR

THIS INSTRUMENT MUST BE EARTHED. If the plug has a fuse, its value should be 2 Amps **DO NOT PLUG IN AND SWITCH ON YET.**

WARNING !! never attempt to run the console without it being connected to the microscope unit or with any of the plugs at the rear of the MSM microscope unit unplugged. Damage to the motor drive circuit may result

NEVER obstruct the ventilation slots on the bottom and rear of the console. Place it on a flat surface. Do NOT place anything on or against either unit (apart from CCTV Monitor). Do NOT operate the console in direct sunlight. SWITCH OFF before covering with dust cover.

Familiarise yourself with the Console and Stage Joystick controls by referring to **fig 2.4**

2.4 Joystick Unit

Plug this in at the rear of the Console. Position the joystick conveniently so that you are able to operate it with one hand and the micromanipulator with the other.

2.5 Microscope Optics (fig 2.5)

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

Fit the trinocular head and insert the eyepieces. Now hinge up the overarm, holding it by the rotating turret, until it locks into its raised position. Screw two objectives into opposite holes (more than two may cause obstruction problems).

Test the operation of the overarm latch mechanism by depressing either of the grey latch handles. The arm will drop down into the operating position.

ALTHOUGH THE ARM IS HYDRAULICALLY DAMPED IT IS BETTER TO SUPPORT THE ARM AS IT DROPS.

2.6 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 (Direct Image Projection System) which fits the camera to the head.

If the Camera is supplied by ***Singer,*** then it will be fitted with the DIPS adapter (a short, stainless steel cylinder containing the lenses). Enter the male dovetail into the black fitting on the trinocular head and tighten the 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 and then mount on the trinocular head as above. It may be necessary to use the small hexagon key provided to align the camera by adjusting the three small set screws on the black fitting.

Plug the camera cable into the multi-pinned socket on the camera. With ***Singer*** supplied cables, the camera plug end of the cable is wrapped with black spiral plastic to protect it. With cables from third parties make sure that the male plug is attached to the camera and wrap the camera cable with the spiral wrap provided. Push the cable under the cleat on the microscope overarm leaving sufficient cable to run smoothly down the back of the camera/dips assembly. Secure the cable to the DIPS with one of the black cable ties provided.

Push the cable under the clips at the back of the main unit. A small black plastic blanking plug is supplied for the spare socket on top of the camera.

Plug the female end of the camera cable into the socket at the back of the console **fig 2.3**. Connect the console to the monitor with the BNC/BNC cable. The monitor can be placed on top of the console but fit the self-adhesive pads supplied on the feet to prevent slippage.

If a camera is fitted, ensure that the trinocular head splitting prism slider (see **fig. 2.5**) is moved fully to the right. The camera should have its own handbook.

2.7 Focusing of Camera and Monitor

When you switch on your ***MSM*** later, 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.

2.8 Video Printer

When a Video printer is to be used then this is best connected between the Console 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.

2.9 RS232 Communications Port

The comms port is a standard 9 pin D socket. Pin assignments are shown in appendix C.

2.10 Micromanipulator

Slide the micromanipulator male dovetail tongue into the female slide on the appropriate lower side of the fixed stage, pushing it in as far as it will go. See the illustration on page 2 of the MSM brochure in the front of this handbook.

Lock with the grey handle. Note; all the grey handles on the ***MSM*** can be rotationally realigned on their shafts by pulling the handle out against its spring, turning, and then releasing in the new position.

Place a glass microtool in the ***MSM*** toolholder with sufficient projecting to reach the agar surface, and lock in place with the screw.With the glass needle horizontal, slide the toolholder over the stage, using the manipulator tool clamp "V" as a guide. When the microtool is in the centre of the field rotate the toolholder to bring the glass microtool vertical and clamp with the black plastic knob.

Axial adjustments to the toolholder are made with the black knob on the end of the manipulator. Transverse adjustments are by loosening the small knurled locking knob and swinging the top plate with the toolholder "V" in it from side to side with the small vertical knob.

To act as a reminder when you start dissecting, you may like to pin up the two "REMEMBER" illustrations which you will find in the back of this handbook.

To centre the glass needle you will need to switch on your ***MSM System***. Refer to the next section **Getting Started Quickly.**

3. Getting Started Quickly

The *MSM* may seem a little complicated, but the controls are simple, logical and intuitive.

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.

WARNING.... THE STAGE MOVEMENT OF THE ***MSM*** IS VERY POWERFUL. ENSURE THAT NOTHING IS TRAPPED BY THE MOVING STAGE. DO NOT TRY TO STOP THE STAGE BY HAND. PRESSING 'BREAK' STOPS THE STAGE IMMEDIATELY.

3.1 Flowchart and Switching On

Set up the ***MSM*** as described earlier in this handbook. Refer to the **Main Flowchart** in Appendix B. You will be able to follow the program progress by comparing the messages on the LCD display with those windows in the flowchart.

Switch on the ***MSM*** (the main switch is at the back of the Console) so that the microscope light works (the LCD window will display a scrolling message, stop at screen 3 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 condenser glass window in the stage. You may wish to practice with no dish loaded, if you do, go to paragraph 3.3. and refer to the plate layout **fig. 3.3.**

If you are familiar already with digesting your tetrads, please skip through the next few paragraphs, but please pay attention to the comments on suspension density and dish inoculation.

3.2 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. cervisiae* 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.

3.2.1 For *S. cerevisiae*

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°C (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.

3.2.2 For *S. pombe*

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

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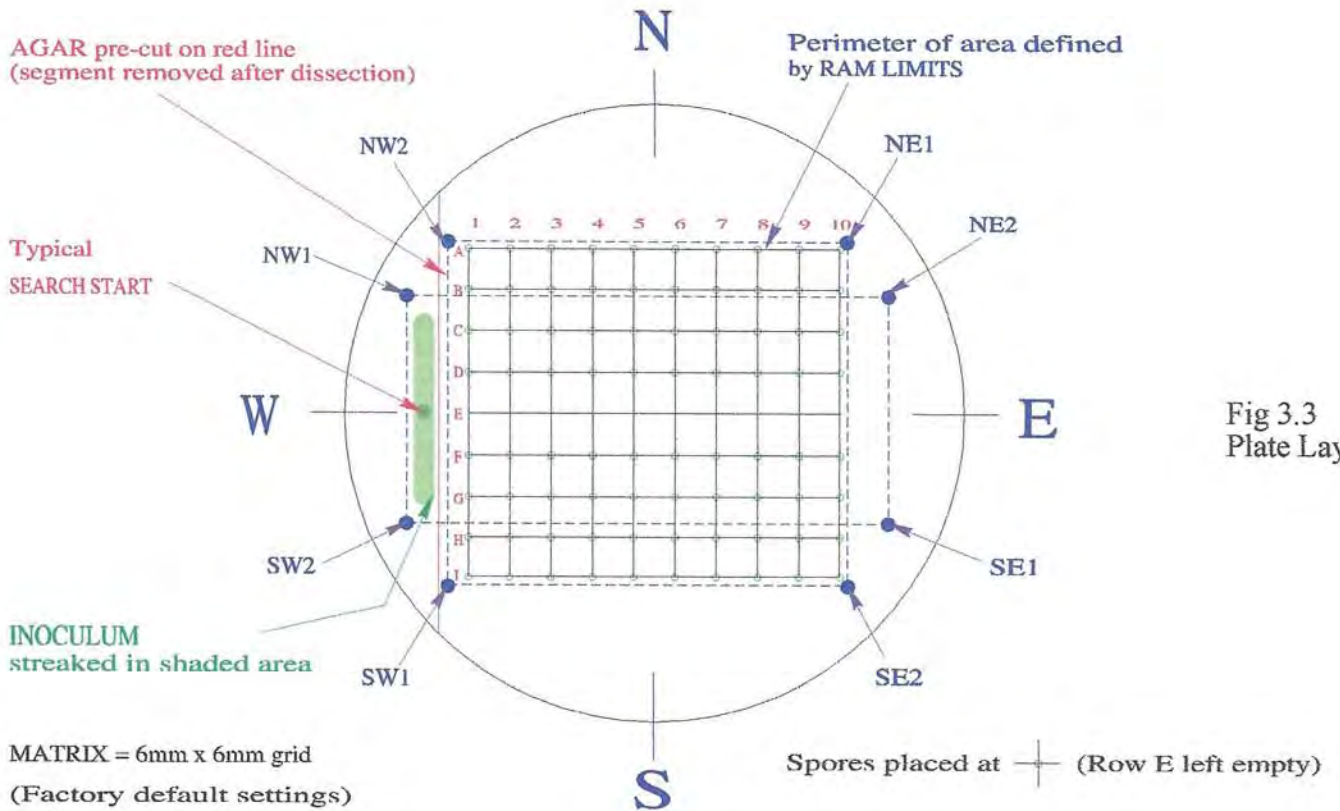


Fig 3.3
Plate Layout

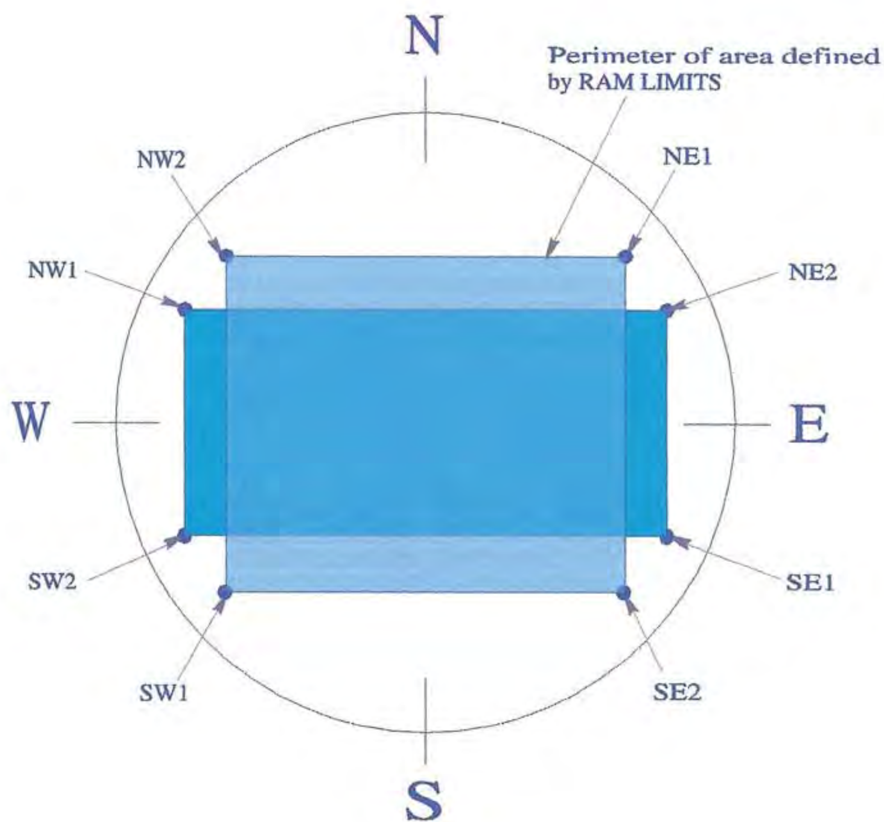


Fig 3.3.1
Limits

3.3 Inoculating the Dish (dry agar is best for dissection).

The *MSM* is factory programmed to reproduce the Petri dish layout shown in **fig.3.3** This design produces cloned 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). Mark the bottom of your Petri dish with a lab pen with a small mark at each end of where the inoculum should be. Inoculate the dish with your digested sample using a flamed 3mm. loop: a single, straight , light stroke is best.

3.4 Loading the Petri Dish

The LCD will show screen 3 (see flowchart) .

Key →

Remember, there is one → key on the console and both of the grey keys on the joystick unit are → keys . The stage will move and do a "double shuffle" or Datum move when the Petri dish is nominally centred in the field. The LCD will show screen 5.

Raise the overarm of the microscope by lifting it up until it latches firmly in position. Place your Petri dish, inverted, in the plate holder after holding back the sprung arm with your left thumb. Release the arm so that it traps the Petri dish into the top left corner of the plate holder and pushes it against the two tapered black pegs. Your inoculum marks should be on the left and lie north-south.

Carefully lower the overarm after depressing one of the latch levers. Make sure that the objective does not hit the Petri dish.

Key →

The stage will move so that the inoculum area is in the field automatically (screen 6).

Key →

This takes you into the **SEARCH** mode (screen 7).

3.5 Searching the Inoculum

Focus the microscope with the coarse focus knob. If the inoculum is in the correct place you should see it. Try the fine focus (black knob on the stage joystick unit). Move the stage joystick : it makes the stage move in "**Search Steps**" of 300μ in all four directions.

TIP: 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 to, or aspirating it from, the suspension.

Search the inoculum north-south for a tetrad. When you see one you can move it to the centre of the field by **first** holding down the small button on the top of the joystick and **at the same time** moving the joystick in the desired direction. The stage will make a much smaller, 25μ "**Nudge Step**", which repeats for as long as you hold the joystick over **with the button depressed.**

3.6 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 four or five full turns of the micromanipulator black knob (in case the agar is not flat).**

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

3.7 Moving the Tetrad to the Matrix

Refer to **fig 3.3.** To move the tetrad to the **Matrix** (grid)

Key → key.

The stage will go automatically to the top left position - A1 (The stage does not go to A1 automatically each time, but rather to the **place it was in LAST before the *MSM* was switched off**). You will get LCD screen 8.

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.

Dropping the tetrad 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.

3.8 Dissecting the tetrad

3.8.1 *S. cerevisiae*

Raise the needle to the "miniscus" level and then move it sideways to touch the tetrad. 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.

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.

If you wish to be able to replace the Petri dish accurately after incubation is very important to place the ascospore at A1 in the centre of the field.

You can move the stage up and down the Matrix (grid) column, row A through row I, by displacing the stage joystick in a north-south direction. Each movement of the joystick will move one Matrix square of 6mm. The stage position will be shown on the console LCD. Dissect the ascospores, placing them at A1, B1, C1 and D1. **Don't forget to drop the needle away from the agar every time you move from point to point!** (clockwise is to the agar).

After you have dropped the last ascospore of the four, **lower the needle** and move the stage back to the top row, A.

To return to the inoculum:

Key →

The stage will return automatically to **the exact place in the inoculum from where you picked up the ascus.** The LCD will show screen 7. Search for another tetrad by moving the stage with the stage joystick as before. Don't forget the "Nudge" button on the top of the stick. Pick up another ascus.

Remember : Lower the needle before moving the stage.

Key →

The stage will move back to the last Matrix point that it was at-in this instance, A1. Focus on the spore already at A1. By displacing the stage joystick east-west, move the stage to Matrix position A2. Dissect this tetrad A2, B2, C2 and D2. Continue in this fashion, toggling from the inoculum to the Matrix by pressing the → key, searching the inoculum, and moving across column by column and up and down the rows of the Matrix with the stage joystick. The first session of tetrad dissection may take some time as you will be slow to begin with. After six to ten tetrads you may have had enough. If so, go to **para 3.10** and follow out the directions for taking your Petri dish out. Build your performance up over a number of sittings, aiming at completing a plate of 20 tetrads in 20 - 30 minutes.

3.8.2 *S. pombe*

Pombe tetrads are slightly more "sticky" than those of *S. cerevisiae,* but may be picked up with facility as can the spores.

The method for finding and isolating pombe tetrads is as above, but as the asci are intact and cannot immediately be dissected, one is placed at the top of each column in row A and each column in row F (20 tetrads in all, but you can do fewer to start with).

The Petri dish is then marked, **para 3.10,** removed and incubated for anything from 1 to 6 hours so that the ascospores liberate from the sack, but do not divide.

The dish is then replaced on the stage and each tetrad dissected as above. This may need several sessions of incubation and dissection as rates vary. Another way of dissecting pombe asci is described in **para 3.13.**

3.9 Reaching the Lower half of the Dish-Keying Matrix Points

When you have completed the top half of the dish by placing and dissecting ten tetrads, and before you return to the inoculum, key F1 on the Console keyboard followed by =.

The stage will go automatically to point F1.

Key →

The stage returns to the inoculum. Search for and pick up a tetrad.

Key →

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 will have realised that by using the method described above you can reach any Matrix Point by keying in its co-ordinates and then keying =.

3.10 Removing your Petri dish

When you come to the end of the last column, it is good "housekeeping" to key A1 and then =.

This returns the stage to Matrix point A1, so that the next Petri dish is started at A1 automatically.

It is essential if you wish to replace the Petri dish in the same orientation to mark the rotational position of the dish. **Make a mark with a fine lab pen on the edge of the dish near the rearmost round, black plastic peg against which the Petri dish rests.**

Key → [to return to the inoculum].

Key **?** [(this will display a menu on the LCD].

Key # [the Datum key].

The stage will drive to its central position and complete the "shimmy" (Datum) and stop.

Now remove the Petri dish after raising the microscope overarm.

If you want to switch off now read **para 3.12**

If you wish to dissect another dish, put in the plate holder now.

Key → [to cancel the menu]

Key → [to go back to the inoculum.]

3.11 Replacing a Petri Dish Accurately

Place the Petri dish in the holder as described in **para 3.3**, rotating it until the pen mark is opposite the black peg. By pressing the → key repeatedly (and perhaps using the joystick) move the stage to MATRIX position A1. You should see your spore or tetrad that you placed at A1 in the field. If you do not, rotate the dish a little to centre the object. Make sure that you are in the correct focal plane: you might need to focus on the inoculum, or bring the needle up to touch the agar surface and focus on it to find the surface of the agar and hence the object.

Once the object or mark at A1 is centred all other MATRIX points will be in register.

3.12 Switching off

If you want to switch the ***MSM*** off, key "E" from in Menu and you will get the message *"switch off now"* on the LCD. Switch off the ***MSM.***

All of the data - the Matrix size, the Search Step, the inoculum position and the like, can be changed to suit your own layout.

3.13 Another Method for Pombe

Because the ***MSM*** always "remembers" the last inoculum position during dissection, another technique is possible for pombe dissection.

The dish is inoculated and incubated. The dish is placed in the ***MSM*** and the inoculum searched in the usual way. It is possible to recognise groups of four spores which have liberated from a single ascus as often the discarded sack can be seen lying round the spores. The spores are picked up on the needle and transferred to the MATRIX.

It is not always easy (although desirable) to pick up all four spores at once, but the ***MSM*** memory function ensures that the stage returns to the same place in the inoculum to retrieve any spores left behind on the first visit.

With this technique the concentration of the inoculum must be controlled carefully so that tetrads can be left to mature in the inoculum without fear of contamination by neighbours and far enough apart so that they can be picked up on the needle without a lot of clearing of unwanted cells.

4 OTHER TECHNIQUES

The skills learnt 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 co-ordinates on a Petri dish . If two or more structures are picked up on the needle they will 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 1mm x 1mm grid giving 3600 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.

All of the data - the Matrix size, the Search Step, the inoculum position and the like, can be changed to suit your own layout. To do this, read Custom Plate Layout. Your ***SINGER MSM SYSTEM*** is a very powerful tool - explore its potential thoroughly.

4.1 Mutant Screening and Cell Progression Studies

By changing the matrix step size to 1 mm, a plate that has been inoculated all over with, say, a mutant strain, may be searched in 1mm X 1mm "windows". These windows are given co-ordinates automatically by the MSM program: these are displayed on the LCD. When a window containing an interesting subject is located, a video print (or digital image record) is taken of the window and the co-ordinates written on the print, or downloaded via RS 232 to the image storing computer.

The plate is marked as in para 3.10, incubated, and replaced as in para 3.11.

The previously recorded co-ordinates are keyed in as described in para 3.9 and the subjects can be re-recorded. By this method an elapsed time sequence can be built up showing cell progression. **Important: See para 4.2 :all-numerical co-ordinates. To change the program variables of Matrix Step and Nudge Step to values of 1mm and 4μ respectively, follow the directions in para 5.5.1.**

4.2 All-numerical co-ordinates

As the Console keyboard (**fig 2.4**) has a restricted range (A - L) of alphabetical keys, the **MSM** is programmed to display and accept, all-numerical co-ordinates when the alphabetical range is exceeded in the Y (N/S) axis. Obviously, a procedure such as that described in 4.1, involving a 1mm X 1mm Matrix will require more addresses than the alpha-numerical keyboard can address.

When, in Matrix Mode, the stage is moved by the joystick to a position that exceeds the Y axis alphabetical capability, the display adopts an all-numerical system.

In other words, what would be M5 will be displayed as NS: 13 EW: 5.

To avoid confusion, once the display has adopted the all-numerical mode, it will not revert to alpha-numerical until an alpha-numerical value is KEYED in (as described in 3.9).

4.2.1 Keying all-numerical co-ordinates

In 4.2, it is explained that the all-numerical system is adopted when the stage is driven to an address, the co-ordinates of which exceed the alphanumeric range of the keyboard.

But in mutant screening (see 4.1), not only will the stage be moved to such addresses by the joystick, but will need to return to those addresses repeatedly by keying in their all-numerical locations.

In order that the keyboard recognises such all-numerical input, a different protocol has to be adopted. This is:

 # n_1 = n_2 = **Where n_1 and n_2 are the NS and EW ordinates.**

Example: You wish to move the stage to NS: 22 EW: 30

Key # 22 = 30 =

The stage will move to those co-ordinates and they will show on the LCD.

4.2.2 Co-ordinate download via RS 232

If your ***Singer MSM System*** console is connected via the (RS 232) comms port to a P.C. or Mac running some form of communications software (e.g. Windows Terminal), the co-ordinates displayed on the Console LCD can automatically be downloaded by keying =.

See **fig 2.3** for D connector pin outs and appendix C for software settings.

If you intend to use this facility a lot, one of the keys of the stage joystick unit can be reconfigured to act as an = key, rather than →. Take out the four screws attaching the lower part of the stage joystick enclosure and pull off the cover.

On the PCB you will see a clearly marked removable link. Change this from "arrow" to "equals".

The left grey key of the stage joystick unit will now act as an = key.

Don't forget to tell other users that you have done this.

5. CUSTOM PLATE LAYOUT

5.1 Variables

The ***MSM*** program is very flexible and easily configured by the user. No special skill is needed and all that is required is to enter a new set of variables. The ***MSM*** is factory configured with the following default variables which produce the layout shown in **fig.3.3:**

Name	**Default**	**Range**	**Multiple**
Matrix Step	6mm x 6mm	1 - 9mm	1 mm
Search Step	300 microns	1-999 microns	4 microns
Nudge Step	24 microns	1-99 microns	4 microns
A1 Position	23mm N/S, -27mm E/W	Anywhere within Limits	1 mm
Search Start	0 mm N/S, -34mm E/W	Anywhere within Limits	1 mm
Limits NE 1	24mm N/S, 27mm E/W	Anywhere within movement	"
Limits NE 2	16mm N/S, 34mm E/W	" " "	"
Limits SE 1	-17mm N/S, 34mm E/W	" " "	"
Limits SE 2	-27mm N/S, 27mm E/W	" " "	"
Limits SW 1	-27mm N/S, -30mm E/W	" " "	"
Limits SW 2	-17mm N/S, -36mm E/W	" " "	"
Limits NW 1	13mm N/S, -36mm E/W	" " "	"
Limits NW 2	24mm N/S, -30mm E/W	" " "	"

Table 1

5.2 Reloading Factory Settings

The factory settings shown above are permanently stored in the ***MSM*** memory. If you need to reload them, switch the ***MSM*** on, press the → key until you get LCD screen 4. Then key = IMMEDIATELY followed by #. The factory settings will be reloaded and the stage will move to the SET SEARCH START position, LCD screen 6.

5.3 Definitions

In order to explain fully the method of plate layout, we have used various definitions to describe the geometry of the layout and the operation of the *MSM*. Definitions is given in Appendix A.

5.4 Other Plate layouts

If you do not want to use the factory layout, then you can change all or part of it by keying in new values or accepting existing ones. Refer to **fig 3.3.** We can check, and alter if we wish, all of the variables that constitute the design in **3.3.** The list of variables for the design is shown in table 1.

5.5 Checking and Changing Variables (refer to flowchart)

If the *MSM* is switched off, switch it on and at screen 3

Key → repeatedly

until you get LCD screen 5 :DATA ENTRY.

If the *MSM* is on, press the BREAK button on the front of the Console and this will restart the system. At screen 3

Key → repeatedly

until you see LCD screen 5: DATA ENTRY.

Key =

This will access screen 5.1: LIMITS.

5.5.1 Grid Step

At this stage, we are not going to concern ourselves with LIMITS.

Key → [to go to screen 5.1.1 MATRIX STEP].

You will see that the screen displays the factory setting of NS 6mm EW 6mm.

Key = [The cursor will flash over the NS 6.]

Key 3. [NS 6 will change to NS 3 and the cursor will flash over EW 6]

Key 4. [EW 6 will change to EW 4]

You have now changed the Matrix Step variables to recreate a 3mm by 4mm Matrix (grid) instead of the factory 6mm by 6mm Matrix.

If you wish, you can repeat the process with the Matrix Step at screen 5.1.1 and change the axes to anything within the range.

5.5.2 Search Step

When you are satisfied with the settings in screen 5.1.1,

Key →. [screen 5.1.2, "SEARCH STEP 300 μm" will show]

Key = [300 will change to 0, and the cursor will flash]

Key 200

Key = [SEARCH STEP 200 μm will display]

Again, you can repeat the process as many times as you like while screen 5.1.2 is up.

You may have noticed that the → and = keys play a major part in checking and changing the settings in the ***MSM*** program.

The general rule is: Key → to accept the setting and go to the next screen and key = to change something.

5.5.3 Nudge Step

Key → [to obtain screen 5.1.3. You will see NUDGE STEP 300µm]

Key = [300 will become 0 and the cursor will flash]

You can now key in any value you wish in the range, followed by =.

WARNING: The Nudge Step setting is unusual in that it can be set to 0. If this is the case the Nudge function will appear not to work.

5.5.4 Matrix A1

Key → [You will get screen 5.1.4, MATRIX A1]

The position of the Matrix on the Petri dish is dictated by the position of A1. This position is relative to the nominal centre of the dish, 0,0. The factory setting for A1 is 23mm N/S, -27mm E/W. Note that the N/S axis is stated first.

Key = [The stage will move to the stated position, 23, -27]

Screen 5.1.4.1 SET A1 is displayed. You will notice that DRIVE is shown in the Mode bracket on the LCD.

By displacing the stage joystick you can "drive" the stage around. Every time the joystick is allowed to return to its resting position the position of the stage is

updated on the LCD. This positional information is digital in nature and is in increments of 1 mm. When the desired, new position of A1 is reached,

Key = [and this new position will overwrite the old position in memory]

If you decide not to change the position of A1

Key → [the original settings will be retained and the LCD will revert to screen 5.1.4]

5.5.5 Set Search Start

Key → [the stage will move and the LCD will show screen 6].

The program has completed the Data Setting Loop and has rejoined the main spine of the flow chart.

You will notice that DRIVE is displayed in the LCD Mode bracket, together with the co-ordinates of the current position. Set Search Start is a screen through which the main spine of the program always passes. The logic behind this is that that the operator is always offered a chance to start the inoculum search in a new place in the event of the streak being imprecisely positioned.

Displace the joystick. The stage can be driven around in all directions and at three different speeds depending on the degree of displacement of the joystick. Every time the joystick is centralised and the stage stops the current position is displayed.

To reset the point of search start, Key = when you have driven the stage to the new position which can be anywhere on the plate inside limits. This will overwrite the old position in the memory.

To go back to, and/or retain the existing position,

Key → [you will get screen 7, ready to search the inoculum].

All of the first set of variables have now been reset. In practice a new plate layout is best designed on squared paper. This can then be stuck to

the base of a Petri dish which can be loaded on the stage. All of the new variables can be entered and marks on the paper can be used in conjunction with the low power objective to set the position of A1 and Search Start.

5.6 Limits

RAM LIMITS (so called because they may be operator set) are to prevent the plate from moving outside the optical axis and breaking the needle.

Refer to **fig 3.3.** The points NE1, NE2, SE1 and SE2 etc. are the limits that have been set for the factory design. In **fig 3.3.1** the limits are shown without the rest of the design and things are clearer. You will see that joining the points forms two, overlapping rectangles. The stage can travel anywhere within the shaded area, but not outside it.

A Limit Point can be set anywhere in its respective quadrant. The Limit Points always join in the same order to produce the two rectangles i.e. NE1, SE2, SW1 & NW2 form one rectangle and the remainder form the other.

5.6.1 Reviewing Limits (figs 3.3 & 3.3.1)

IMPORTANT: Do not key = unless instructed to do so.

Consult the Flow chart in the Appendix. By keying = at screen 5, obtain screen 5.1 LIMITS.

Key = [this will enable you to access the Limits Loop].

The screen will show: LIMIT NE1 and the NS and EW co-ordinates will be shown.

Key → [The screen will show the next limit, NE2 and its co-ordinates.

Keying → repeatedly will show all the current limits in CLOCKWISE ORDER i.e. NE1, NE2, SE1, SE2, SW1, SW2, NW1, NW2.

Key → [this takes you back to screen 5.1]

Now you have reviewed all the current RAM Limits as in Table 1.

5.6.2 Changing Limits

If you have a new plate design as described in 5.4.5, it will probably require new Limits to be set. The changing of limits is easily achieved by DRIVING the stage, with the joystick to the new Limit positions in turn and entering those positions by Keying =. These will overwrite the old Limit points.

5.6.3 Limit Rules

There are three golden rules relating to the setting of limits:

1. **There must be TWO Limits set in each quadrant.**

2. **Limits must be set in clockwise order.**

3. **The optical axis must be in the correct quadrant when setting that quadrant's limits.**

5.6.4 Driving and Entering

Obtain screen 5.1 as above and then Key = to get LIMIT NE1.

Displace the Joystick in a NE direction. The stage will move so that the NE quadrant of the dish will be about the optical axis. Every time the stage is stopped, the new co-ordinates are displayed.

Note: A low tone indicates acceptance of the point, a high tone indicates an incorrect quadrant.

Drive the stage in this fashion until your new NE1 Limit point is in the centre of the field.

Key =

You have now redefined LIMIT NE1 and the screen will show LIMIT NE2. Using the joystick, move the stage to the position of your new NE2 LIMIT, then:

Key =

Note: some plate designs can accept a single Limits rectangle, although you MUST set two points per quadrant they CAN be in the same place.

LIMIT SE1 will now be displayed on the LCD

Displace the joystick and move the stage to the SE Quadrant. Set two limits in this Quadrant.

Do the same for Quadrants SW and NW

Warning: If you do not set TWO LIMIT points per Quadrant, the stage will not move into that quadrant.

All the main functions have now been covered . By studying the main flowchart and, more importantly, using your ***SINGER MSM SYSTEM Series 200***, you will very quickly learn to use all the controls and come to understand the operating method and philosophy.

6. TROUBLESHOOTING

From time to time you may find that for some reason the ***MSM*** will not operate as you would expect and the remedy is obscure. The most likely cause is a settings change.

The ***MSM*** program is well tried and tested, and very logical. Problems usually arise after a change of DATA or LIMITS, when the operator is trying something new or when someone else has changed the program.

Below are some examples of things that can happen and the explanation for them.

Remember: You can always reload factory settings quickly and easily: see para 5.2.

6.1 Joystick Does not Move Stage

6.1.1 In Search or Matrix Mode,

Displacing the joystick does not move the stage.

You hear a high tone: stage is at a LIMIT, see A3.

If you hear no tone, and in MATRIX the Screen co-ordinates change, but the stage does not move:

1. Check that all electrical cables and plugs are properly connected.
2. Check values in DATA of MATRIX STEP- are they set at ZERO? If they are, change them! See 5.4

In SEARCH, no change will occur to co-ordinate display, but stage does not move:- check 1 and 2 as above.

6.1.2 Nudge Does Not Work (very common)

If in SEARCH or MATRIX, NUDGE does not work, check NUDGE value in DATA. Is it Zero? if so, reset with desired value. See 5.4

6.2 Stage Will Not Move at screen 7

On keying → at Screen 7, a high tone is heard, a co-ordinate is displayed, but the stage does not move.

Have LIMITS or other DATA been changed? If so, the last left MATRIX co-ordinate in RAM is now outside LIMITS because of changed MATRIX STEP or new LIMITS.

Key A1= [and the stage will go there.]

6.3 Errors in Keying Co-ordinates

Example: You wish to make the stage go to L12 in MATRIX MODE.

By mistake you key L2

Remedy, either:

Key =

The stage will go to L2 then re-key L12= and the stage will go to L12. Or better

Key ? [This will access Screen 7.1 "MENU"]

Key→ ["cancel"]

You will get the original co-ordinate displayed on Screen 8. Key correct co-ordinates.

6.4 Continuous Drive after Screen 5.

On warm start, → is pressed at Screen 5 and the stage continuously until it reaches a ROM LIMIT at the extreme end of travel. A high tone is emitted.

Remedy: It is likely that the joystick was not in the central, resting position during DATUM SEARCH.

Press BREAK and restart, ensuring that the joystick is properly centred.

6.5 Needle will not touch Agar in Inoculum

When the stage is driven to the extreme right to give access to the inoculum in the factory set layout (**fig 3.3**), the needle locking screw of the toolholder may hit the underside of the black plastic plateholder preventing the needle tip from touching the agar.

Remove the Petri dish.

Key ?

Key #

The stage will DATUM, centralising it. Using a pair of forceps to grip the needle pipette, undo the locking screw and raise the needle 3 mm or so. Tighten the locking screw.

Key → [the stage will move to the inoculum]

Key → [continue with search. The needle should now reach the agar surface].

6.6 Dissecting Problems

6.6.1 Needle will not pick up

There are a number of possible causes for this.

1. **The agar is too wet**. Remedy: Dry the plates.
2. **Too much liquid in inoculum**. Remedy: Use smaller/finer loop.
3. **Needle dirty**. Remedy: Rub vigorously on agar and if that fails, clean in ultrasonic bath using water only.
4. **Needle faulty**. Remedy: Replace.
5. **Lack of speed**. Remedy: Move the tetrad around a little and then very quickly lower the needle.
6. **Lack of practice**. Remedy: Try and try again.Have faith.

6.6 Other Faults

Please do not hesitate to contact ***Singer Instruments*** if you have a problem with your ***MSM System***. Telephone, Fax numbers and our e-mail address are given at the front of this handbook. Before you contact us, please run through the various checks above and make sure that someone else has not altered the settings of you instrument. Remember, you can always re-enter the factory settings very easily, see para 5.2. If you do telephone us, it is useful if you use a 'phone next to the ***MSM*** so that we can ask you to operate it to do some tests.

6.6 Care and Maintenance

The ***SINGER MSM SYSTEM*** 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 both the microscope unit and the console when not in use. There is no need to remove the micromanipulator.

Ensure that the ventilation holes in the console are not obstructed and it sits on a flat surface.

DO NOT spill liquids over the joystick unit or the keyboard.

DO NOT place anything on or against either unit (the monitor on the console is OK)

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

APPENDIX A : Definitions

For the sake of clarity we define below some of the general and specific terms used in this handbook.

A.1 Datum (fig A1)

This is the point on both the stage X and Y axes from which the microprocessor calculates all positions of the stage. This point is the nominal centre of a Petri dish mounted in the stage dish holder. The ***MSM*** always "DATUMS" on start up, and the user can re-DATUM at any time during operation via "MENU" screen 7.1. The DATUM is regarded as zero by the microprocessor for calculation purposes; all positions NORTH and EAST of DATUM are positive and all positions SOUTH and WEST of DATUM are negative.

In certain operating MODES, the LCD display gives indication of the position of the field centre in relation to DATUM. This is shown in millimetres as either a positive or negative number on both the N.S. and E.W. axes. These values are always to the nearest whole millimetre, rounded towards zero. Although the resolution of the ***MSM*** stage is 4μ, these co-ordinates are to the nearest millimetre and are for indication purposes only and not for measurement.

A.2 Drive Mode

DRIVE MODE may be selected using the keyboard. In this MODE the stage may be driven, within LIMITS, continuously and freely with the joystick. The speed of the stage is proportional to the joystick displacement and is at three different rates. The stage will move both axially and diagonally.

In DRIVE MODE the NUDGE BUTTON is disabled

Fig 1A

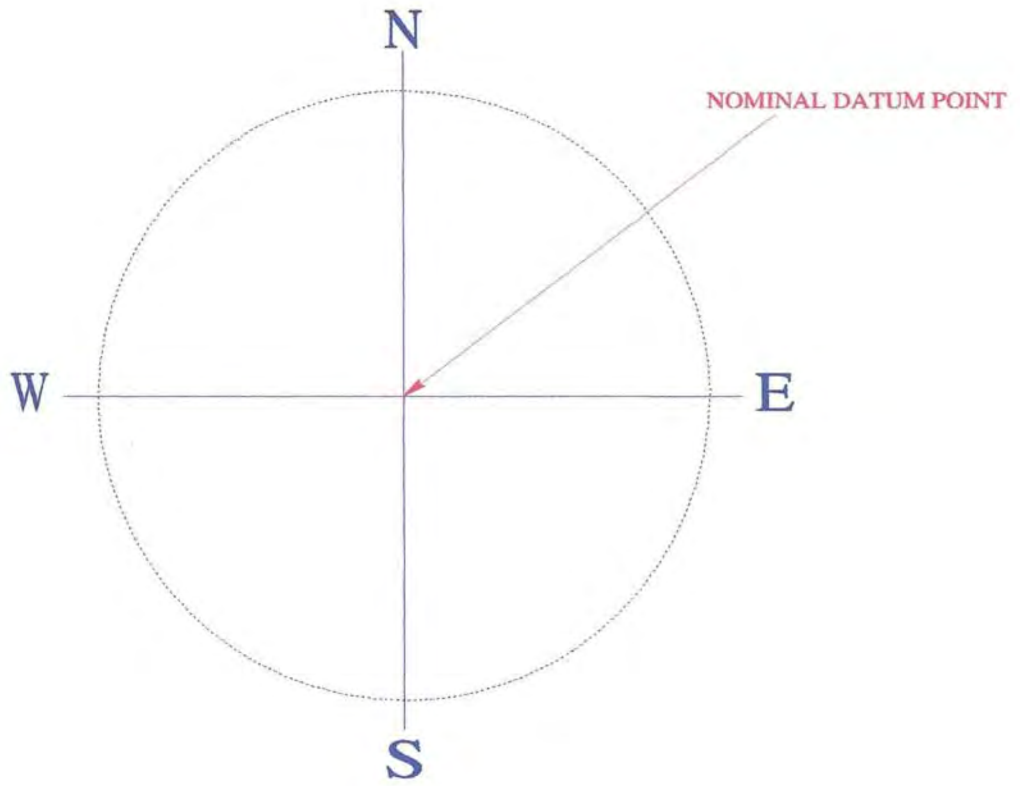
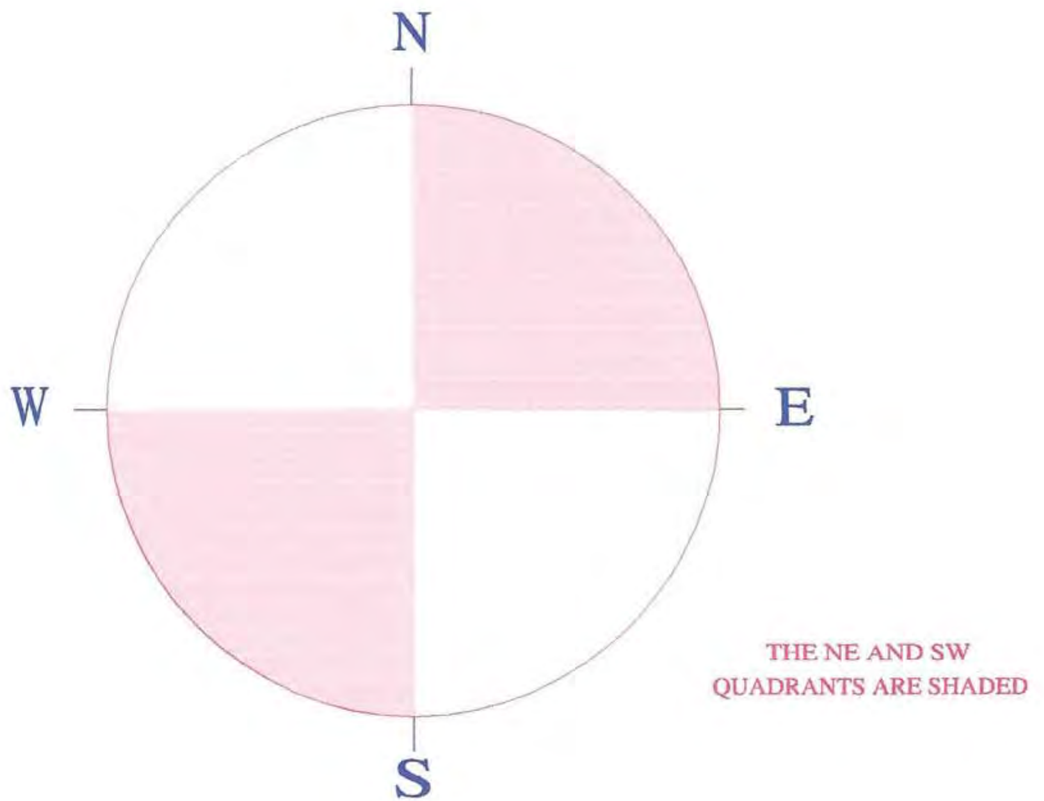


Fig A7



A.3 LIMITS

A.3.1 ROM Limits

There are mechanical stops to prevent the stage from moving too far. Before the stage can be driven to these extremes, software LIMITS come into operation to halt the movement of the stage. These LIMITS are factory set and are embedded in the ROM. If the stage comes up against these ROM LIMITS it will stop and a high pitched tone will be sounded from the console.

A.3.2 RAM Limits

To restrict the movement of the stage within the area of a given plate, the ***MSM*** is equipped with a further set of LIMITS which are retained in the RAM and may be operator set. There are two RAM LIMITS in each QUADRANT. LIMITS are derived from DATUM.

ROM LIMITS are permanently set at the factory.

RAM LIMITS are battery backed and user changeable

The SINGER ***MSM*** arrives from the factory with RAM LIMITS preset for a Petri dish, see **fig 3.3.**

A.4 Matrix

Once tetrads are located and picked up on the tip of the microtool controlled by the micromanipulator, they are deposited on an area in the plate and subsequently dissected and deposited in groups of four. These groups or columns of four form an orthogonal pattern which we will refer to as the MATRIX.

A.4.1 Matrix Step

The pitch of the grid forming the MATRIX is variable in the ***MSM***. Values may be set by the operator in the range 1-9mm., in multiples of 1mm. This pitch , or MATRIX STEP, may be different in the NS axis from the EW axis. The ***MSM*** has a factory set MATRIX STEP of 6mm in both axes.

A.4.2 Matrix Mode

This is the term given to that mode of operation of the ***MSM*** when the tetrads are being deposited on the MATRIX and being dissected into groups of four. Each time the console joystick is displaced the stage will move one MATRIX STEP.

MATRIX co-ordinates may be entered on the keyboard and the stage will automatically move to that point on keying =.

A.4.3 Matrix Co-ordinates

So as to be able to identify MATRIX points, the ***MSM*** uses and recognises a system of co-ordinates. This system is primarily alphanumeric. The NS axis of the MATRIX is alphabetical, with A to the North. The EW axis is numerical with 1 to the West. So the NW corner of the MATRIX is A1 (Just like a Microtiter plate). The position of A1 is important, as all other Matrix points are calculated from A1. There can be no MATRIX points to the North or West of A1. A1 can be user set anywhere in the stage movement within LIMITS.

In MATRIX MODE the LCD displays the current MATRIX co-ordinate.

The keyboard has a full set of numerical keys which double as alphabetical keys in the range A through L. This limits the number of alphabetical co-ordinates on the NS axis to 12. However, if this proves to be too few, the NS axis can be programmed to change to all-numerical values.

The LCD has the capacity of two characters for each axis and there are, therefore, up to 9801 recallable MATRIX points. See also para 4.2.

A.5 Mode

In different MODES, joystick displacement will move the stage in different ways, and different controls will be enabled and disabled. For instance; in MATRIX MODE one movement of the joystick will move the stage, say, in a 5mm. STEP. The same joystick movement in SEARCH MODE may move it in a 300 micron STEP.

The NUDGE BUTTON is disabled in DRIVE but enabled in SEARCH and MATRIX.

A.6 Nudge & Nudge Step

In SEARCH and MATRIX MODES the stage is moved in relatively large steps on displacing the joystick. To allow for smaller adjustments to the stage position, NUDGE STEPS are used. The NUDGE is activated by holding down the red NUDGE button on the top of the joystick and then displacing the joystick. The stage will make a much smaller NUDGE STEP in the required direction. The NUDGE button must be depressed before joystick displacement and if the NUDGE is held on it will repeat every half second. The stage will move diagonally as well as axially.

NUDGE STEPS may be user set in the range 4-99 microns, in multiples of 4 microns. Factory setting is 24 microns.

Using NUDGE alters the SEARCH position, it does not change MATRIX points.

A.7 Quadrant (See fig A.7)

With a circular plate the four quarters of the plate are referred to as QUADRANTS.

A.8 Search Mode & Search Step

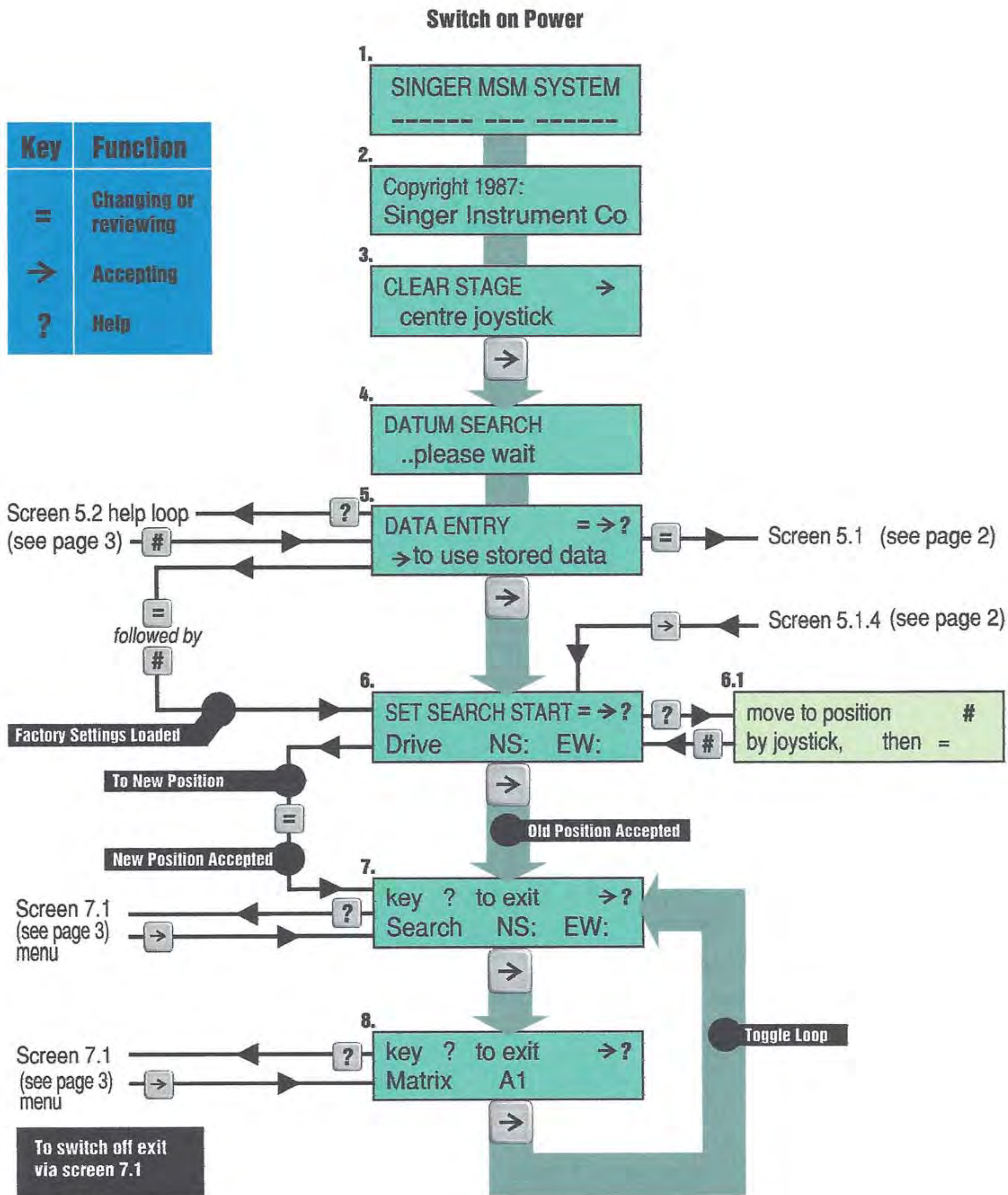
SEARCH is that mode of operation when the inoculum is being searched for tetrads. When the Stage Joystick is displaced the stage makes a single STEP either N, S, E, or W. Test have shown that it is more comfortable for the operator to SEARCH the inoculum in a series of quick steps, rather than in a continuous movement.

A.9 Search Start

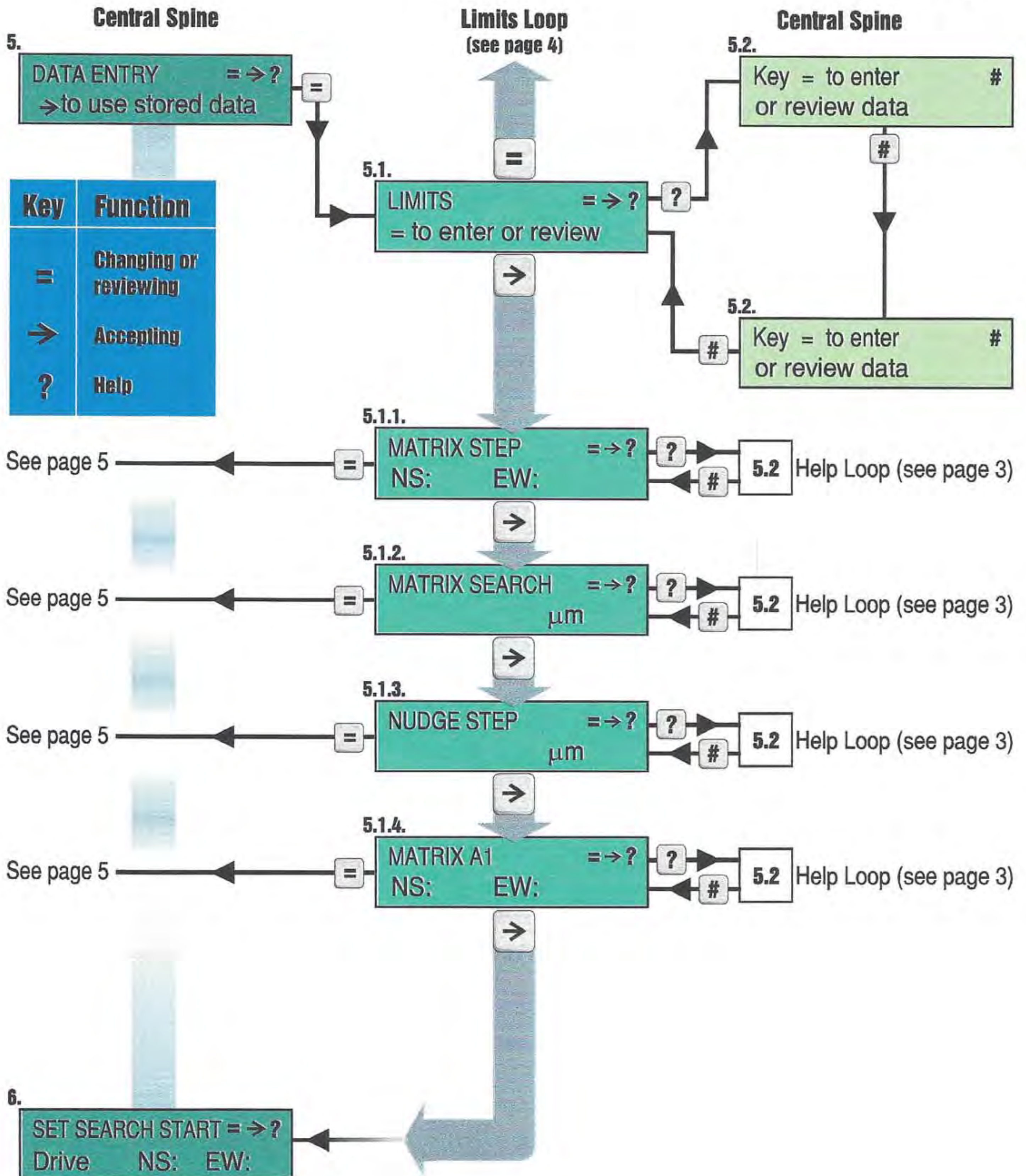
For practical purposes it is assumed that the inoculum will always be spread on the plate in the same area. Within that area, a point is chosen to which the stage will automatically go at the beginning of the SEARCH MODE . This point is SEARCH START. Again, an example value has been factory set but this may be changed easily by the user as SEARCH START values are held in the RAM. When the SEARCH START screen is displayed, the co-ordinates of the position are displayed on the LCD.

SINGER MSM SYSTEM SERIES 200

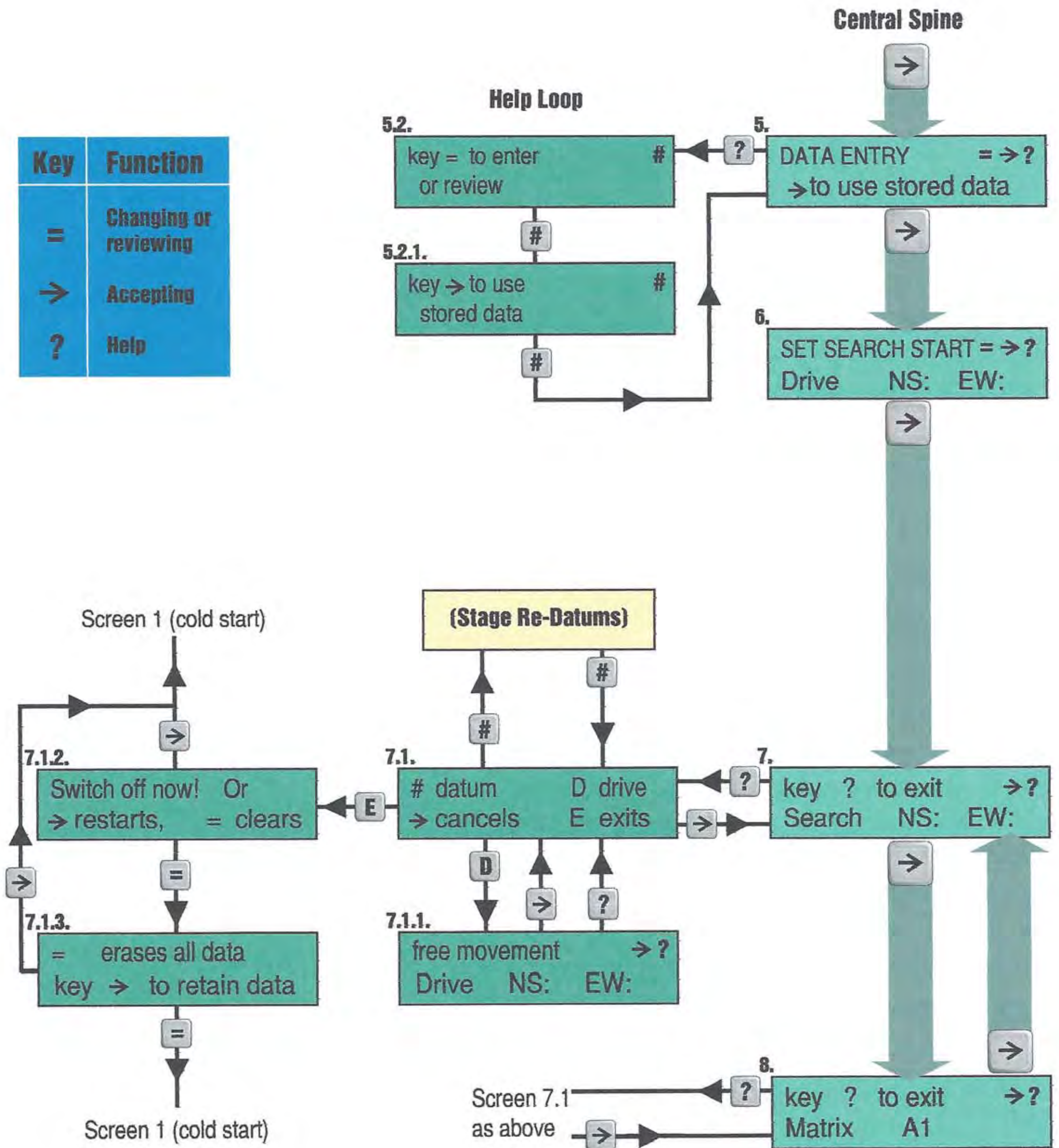
MAIN FLOWCHART
Central Spine



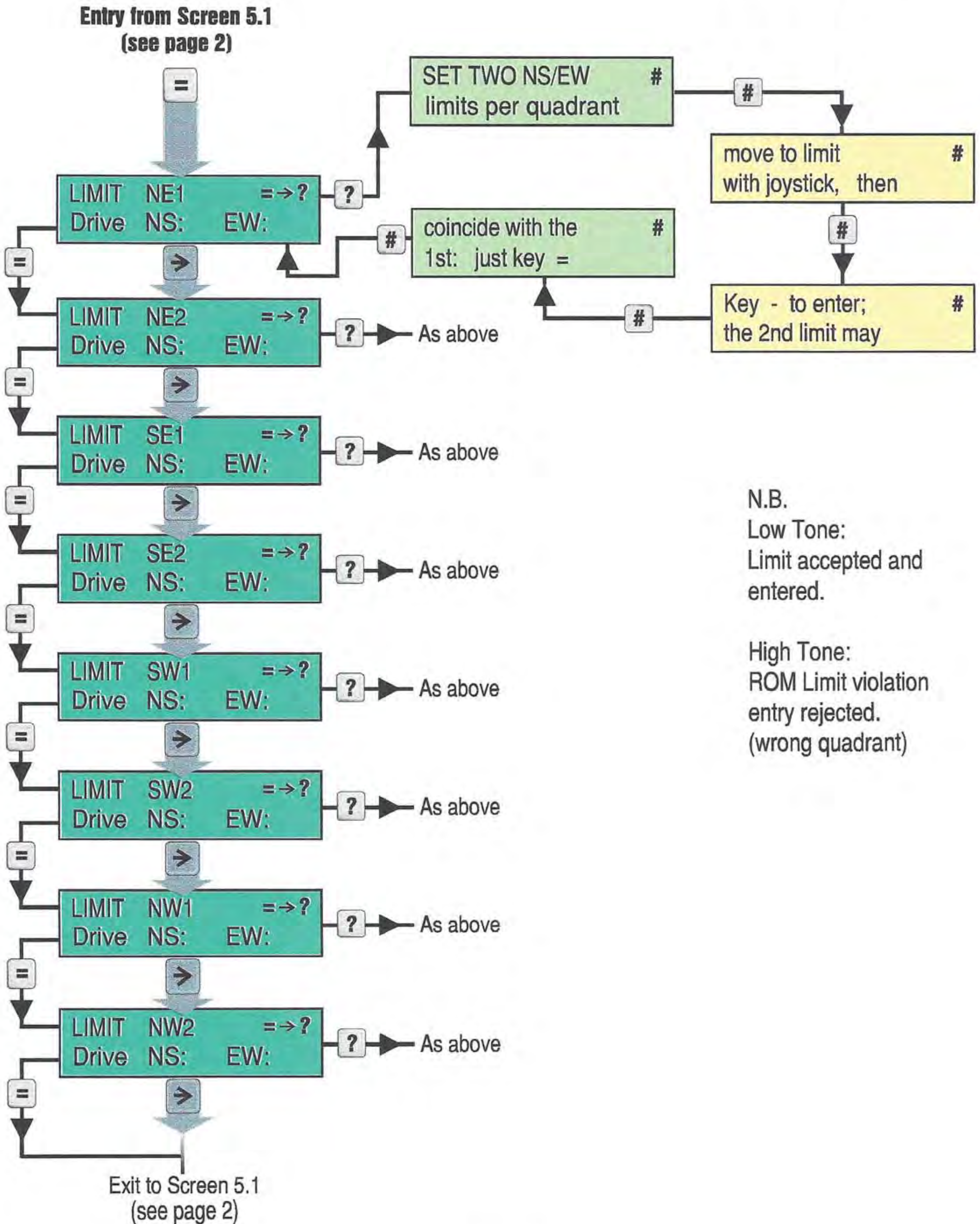
MAIN FLOWCHART
Data Setting Loop



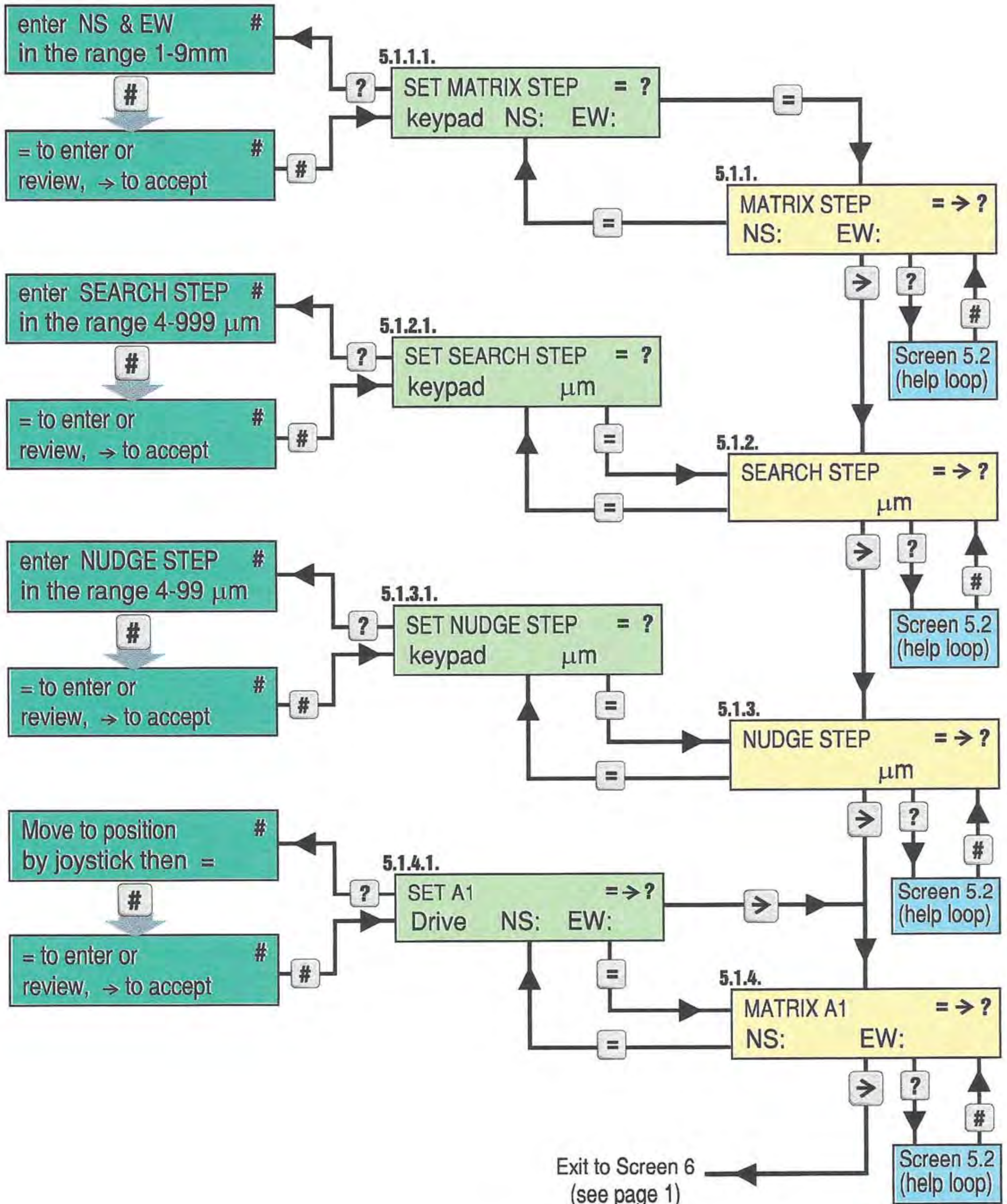
MAIN FLOWCHART



MAIN FLOWCHART
Limits Loop



MAIN FLOWCHART
Data Reset



APPENDIX C: Comms Software Settings

C.1 Hardware

The serial link comprises the "transmit" and "earth" wires of a typical, 3-wire implementation of the RS232 serial interface.

The ***MSM*** Console rear panel **(fig 2.3)** is fitted with a standard 9-way female D connector.

Tx is Pin 2 of the D connector and **signal ground is Pin 5.**

C.1.1 P.C. Connections

For connection to a PC, a standard 9 -way, pin-to-pin, male to female extension lead is used. For a 25-way D socket, a 9-25 way adapter may be used. This crosses over Pins 2 and 3 and connects Pin 5 on the 9-way D to Pin 7 on the 25-way D.

C.1.2 Mac Connections

Via suitable cables, Pin 2 of the ***MSM*** 9-way D is connected to Pin 5 of the MAC DIN-8 connector. Pin 5, ground, on the 9-way is connected to Pins 8 and 4 of the DIN-8.

C.2 Software

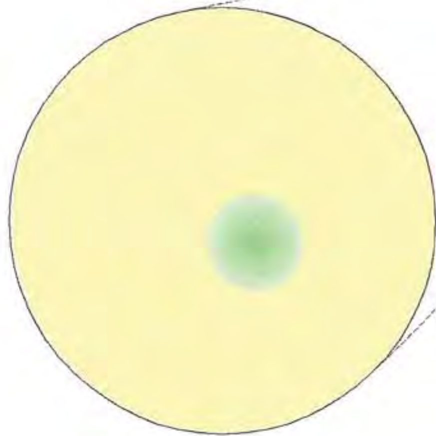
Settings for software are: **1200 Baud, 7 bits, 2 stop bits, Handshake: None, Parity: None.**

If simulated handshaking is required, this can be provided by hardwiring on the 25-way D connector. Link pins 7 and 8 (CTS and RTS) and separately link pins 4,1 and 6 (DTR, CD and DSR).

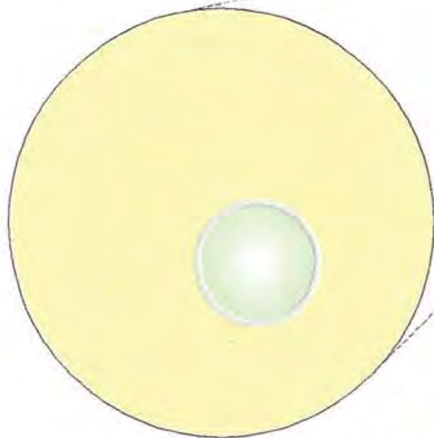
SINGER MSM SYSTEM SERIES 200

REMEMBER

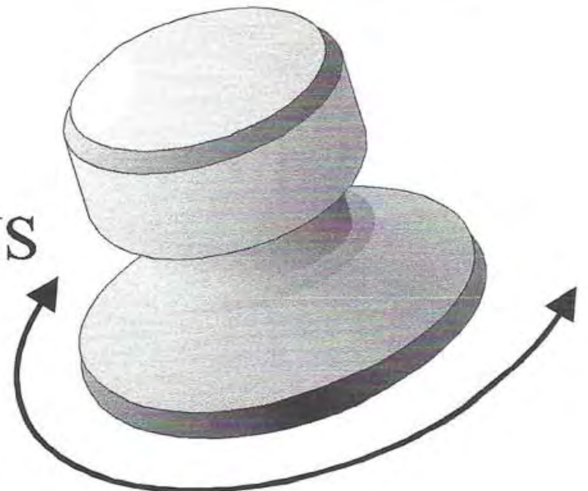
IN MATRIX.
MOVE NEEDLE
TO
THE AGAR



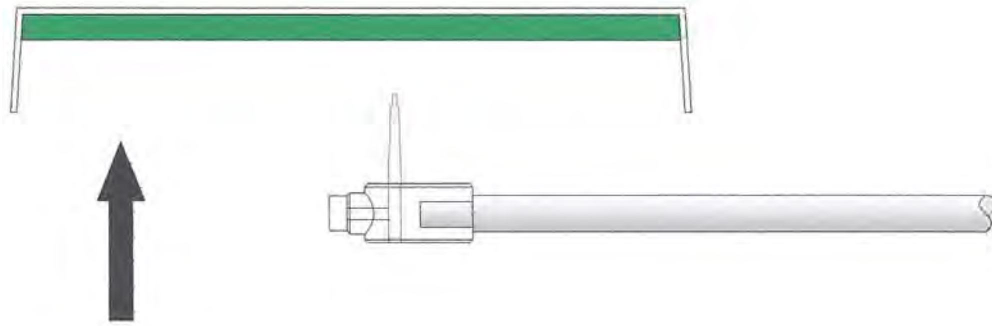
UNTIL
MENISCUS
APPEARS



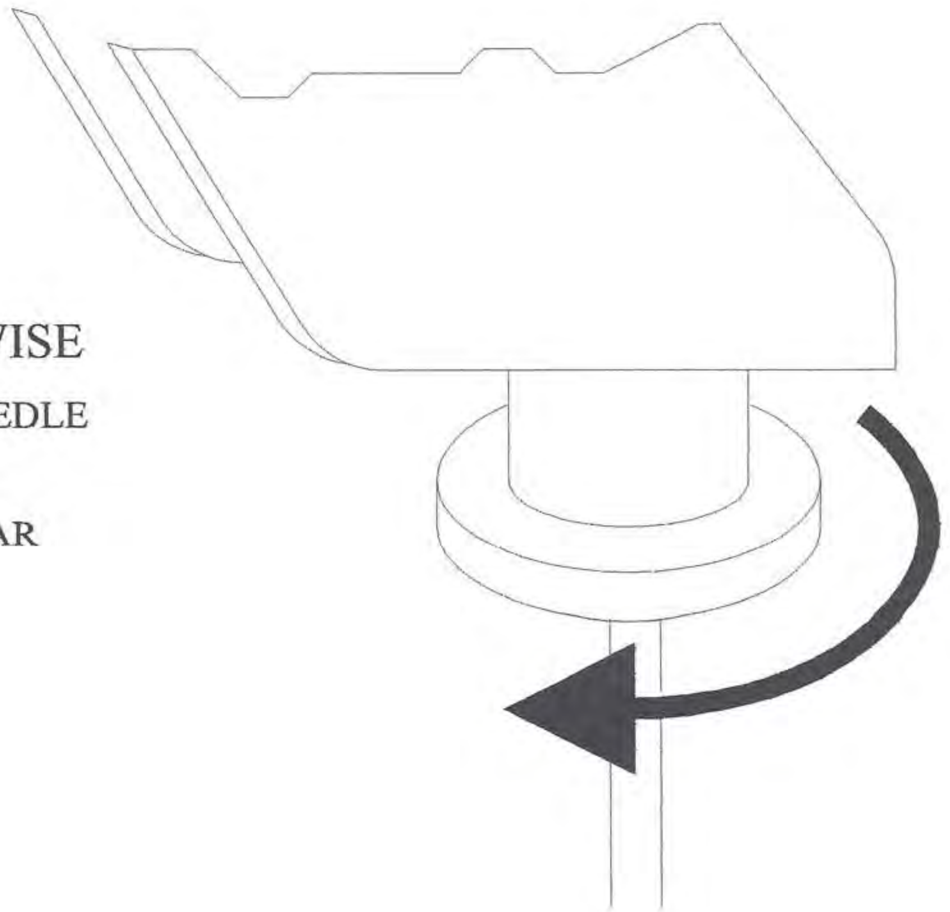
THEN FOCUS



SINGER MSM SYSTEM SERIES 200



REMEMBER



CLOCKWISE

MOVES NEEDLE

TO

THE AGAR

How to use the Singer MSM interactive CD

Although this disk will run on a fast 486 PC without a sound card, for best results it should be run on a Multimedia capable **Pentium™** with at least 16Mb Ram and a **SoundBlaster™** compatible sound card. The CD is produced in standard **VGA** (640 x 480) **High-Color** ;16-Bit, thousands of colours. If you are using **Super VGA** resolution or higher it will still run, but the image will not be full screen. Likewise, if your display is set to only 256 colours it will still work but will not do justice to the images. "Computer Nerds" will not need any instructions for adjusting these settings if they so wish. Lesser mortals should search **Windows Help** on Display Settings.

If you want to see the video clips (very important), you will need to have the appropriate version of **Quicktime for Windows™** installed on your computer. Many people already have this, however, if you don't we have included it on the disk for your convenience. If you are not sure if it's installed, run the disk anyway: it will run until it needs to show a video clip, then if Quicktime is not present it will pop up a message saying that it "cannot load external movie driver". You can then either continue without the video, or exit the program and install Quicktime from the CD (see note below for your operating system).

Apart from the optional Quicktime installation the **Singer MSM interactive CD** will **not** install any files or modify any system or configuration files on your computer.

Running the CD and Installing **Quicktime for Windows™**

Close all other applications that are running. If you have more than 16Mb of Ram, this is not necessary but it will give better performance. Put the disk in your CD Drive and close the tray.

Windows 95/98 or **NT (V3.51 or later)**

If you are running **Windows 95/98** or **NT (V3.51 or later)** and your CD Drive is set to **AutoPlay** then you will see a Blue Welcome Screen. If your CD AutoPlay is disabled and you do not see this screen then go to <**Start**> then <**Run**> choose <**Browse**> (Select your CD Drive) <**OK**> then **Double-Click on Cdsetup.exe** then <**OK**> the CD will run and the Blue Screen will appear. Alternatively, you can use **Windows Explorer** (or **Program Manager in NT3.51)** and Double-Click on **Cdsetup.exe.**

The Blue Screen will remind you that you need Quicktime to view the video clips and it gives you the opportunity to quit the program and install it from the disk. To do this use **Windows Explorer** to find the **Qtime** Folder on the CD, open it and double-Click on **Qt32.exe** and follow the on screen instructions. When it is installed double-click on **Singwin.exe or Cdsetup.exe** to restart the CD.

Windows 3.x or Windows for Workgroups

If you are running **Windows 3.x or Windows for Workgroups** you will need to go to **Program Manager / File / Run** then **Browse** the CD drive for **Cdsetup.exe** or **Singw16.exe** then click <**OK**>. If you need to install Quicktime select **Qt16.exe**

Please note: **Singwin.exe** and **QT32.exe** are true 32-Bit programs and will not run properly even if you have **win32S** installed. **Use only the 16 bit versions**.

Singer MSM Micro-Zapper Fitting Instructions

The Singer MSM Micro-Zapper consists of three main parts:

1.The Actuator with connecting lead and plug.
2.The Control Box and connected Pneumatic Pad.
3.The universal Power Supply and Mains cable.

The Micro-Zapper is fitted last after all other assembly and adjustment. Make sure that he toolholder and needle are in place, with the needle properly vertical, centred and focused. Make sure that the black toolholder clamp knob is tight.

1.

1a
Slide the Actuator head on to the toolholder as shown.

1b
Tighten the screws with the key provided. Make sure that the body of the Actuator is horizontal.

IMPORTANT:
The Actuator must fit into the gap between the Micromanipulator and the Microscope main table. It MUST NOT touch the Micromanipulator or overhang the edge of the Microscope main table, or it will not work properly.

Check that fitting the Actuator has not moved the needle tip, if it has, re-adjust the needle and the Actuator. Pass the Actuator lead below the motor to the back of the MSM.

2.

2a
Enter the angled foot on the Control Box into the top of the slotted Main Unit rear leg as shown.

2b
Push the Control Box downwards so that it jams in the slot.

2c)
Position the Pneumatic pad in a convenient location: under the heel of the micromanipulator hand often works well.

2d
Plug the Actuator lead into the Control Box.

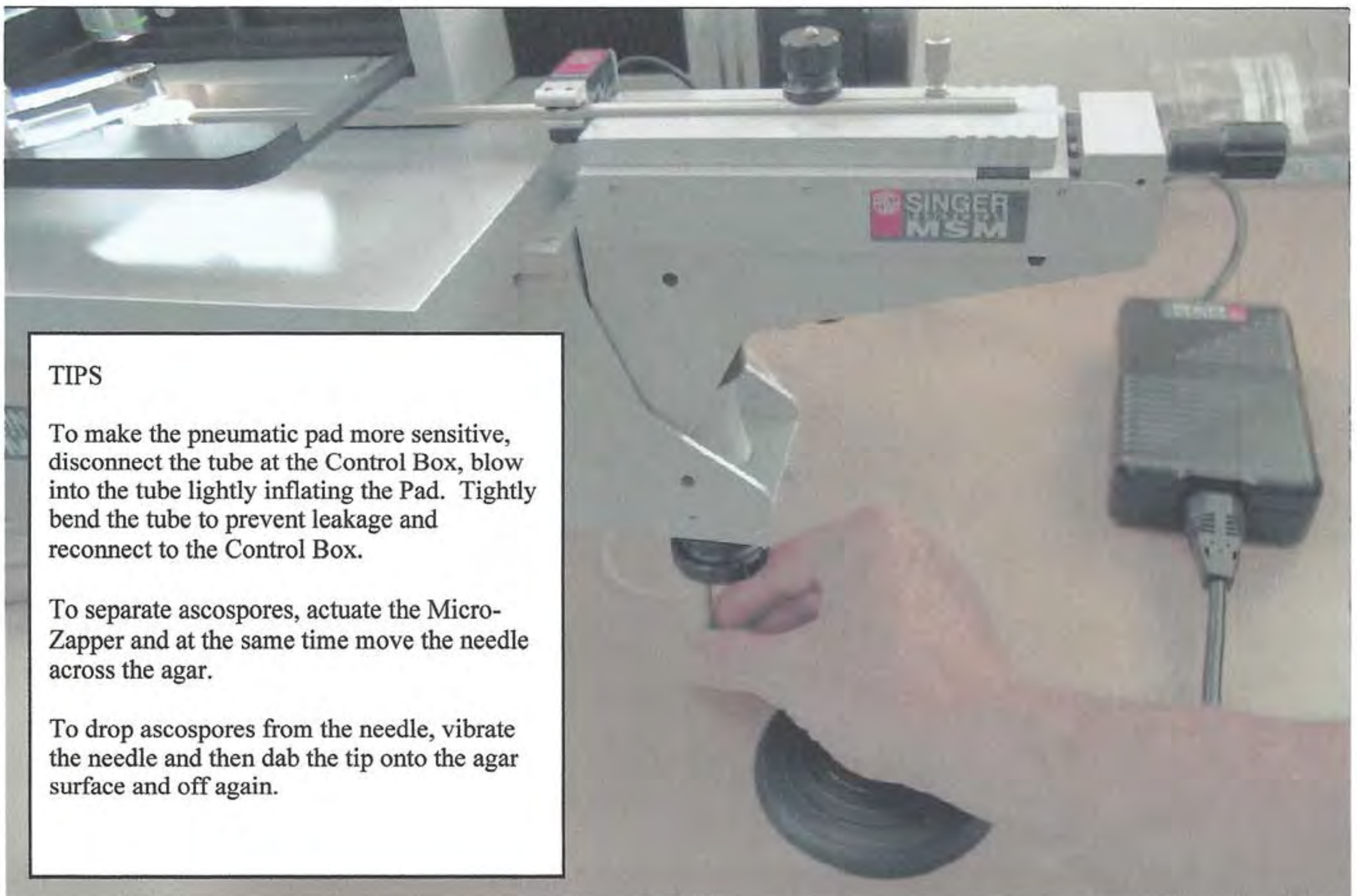
3.

3a
Position the Power Supply on the bench at the rear of the MSM, plug in the Mains Lead.

3b
Plug the Power Supply into the Control Box.

TEST

Ensure that the power is switched on and press down on the Pneumatic Pad. The Actuator should continuously oscillate at about 10Hz. The Micro-Zapper is ready to use.



TIPS

To make the pneumatic pad more sensitive, disconnect the tube at the Control Box, blow into the tube lightly inflating the Pad. Tightly bend the tube to prevent leakage and reconnect to the Control Box.

To separate ascospores, actuate the Micro-Zapper and at the same time move the needle across the agar.

To drop ascospores from the needle, vibrate the needle and then dab the tip onto the agar surface and off again.