
PROTEAN[®] 2-D Spot Cutter
Automated Spot Excision System
with SpotOn[®] Software
Instruction Manual

Catalog Number
165-7005



Welcome

Dear Customer,

On behalf of Bio-Rad Laboratories and ARRM Bio-Tech Ltd., we would like to thank you for investing in the PROTEAN 2-D Spot Cutter System. This product was produced in collaboration between Bio-Rad and ARRM Bio-Tech Ltd.

One of the best ways to familiarize yourself with the capabilities of your new PROTEAN 2-D Spot Cutter System is to read this manual. In it, you will learn how to set up the system and operate all hardware and software components. After reading this manual, please keep it close to your system so that it can be conveniently referred to.

Your PROTEAN 2-D Spot Cutter System is protected by a comprehensive instrument warranty agreement. Please read this manual thoroughly, so that you fully understand the coverage provided and are aware of your rights and responsibilities. One of the responsibilities of system ownership is regular maintenance. Following the maintenance instructions provided with this manual will help to keep your system functioning optimally and will protect your investment. Please also keep in mind that Bio-Rad offers a range of comprehensive service agreements that can be tailored to meet your specific needs.

Bio-Rad Laboratories is dedicated to your total satisfaction and would be pleased to answer any questions or concerns that you may have.

How to Contact Bio-Rad Laboratories

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Section 1 Introduction

1.1 General Description

The PROTEAN 2-D Spot Cutter System is a precision piece of equipment for the accurate location, excision and loading into micrometer plates (MTP) of protein spots after separation on 2-D electrophoresis gels or PVDF membranes. The system has been designed to be both robust in construction and simple to operate.

1.2 Operation

Before using the system, please take a few minutes to read this manual. As delivered, the minimum requirement is to plug in the computer, load in the software and related peripherals and turn on the system, as instructed in this manual.

1.3 Caution

It is detrimental to the system to leave the lighting on for more than 10 minutes at a time as it may lead to permanent damage of sections of the system.

1.4 Moving The Spot Cutter

The System should be moved:

- In the upright position.
- By lifting and carrying from the base.
- By placing down in the upright position on a stable surface.

Important Note: The system must not be moved by lifting the system up by the camera arm.

1.5 Computer Recommendations

Processor:	300 MMX or greater
	2.1G Hard Drive or greater
RAM:	64 Mb or greater
Screen Resolution:	1024 x 768
Communication ports:	2 serial ports are required
	2 Mb Video RAM
Operating system:	Windows 95, 98, or NT

1.6 Cutting Excision Sizes

Tip Size:	Inside Diameter is 1 mm	
	Outside Diameter is 1.5 mm	
Maximum cutting area:	11.8 x 8.8 cm	
Gel Sizers and Frames provided:	Large Gel Sizer	11.8 x 8.8 cm
	Mini Gel Size:	9.5 x 7 cm

1.7 Power Setting

Power can either be 120 V or 240 V (50 Hz/60 Hz)

Verify that you are using the correct power voltage by inspecting the voltage setting indicator and the rating on the fuse installed in the system. (see Appendix C)

Section 2 Getting Started

2.1 Preliminary Instructions

1. Undo the two "L" Shaped brackets from the Systems X-Y Movement Bar (see Appendix A).
2. Undo the other "L" bracket from the back of the cutting head assembly.
3. Remove the electrical tape from the cutting tip.
4. Undo the zip tie from the back of the system.
5. Check that the voltage is set to the appropriate settings (see Appendix C). Regional operating and voltage requirements should be noted. The system voltage is turned from 120 V to 240 V with a small screwdriver turning the voltage indicator. The appropriate fuse must be used, the back panel has the fuse listings for the voltages. Both the main power and the voltage fuses should be checked with a fuse meter before operation to ensure the fuses are in working order.

2.2 Connector Cables

The software for this System has been programmed for x-y system movement communication on COM 2. The 25-pin male connector to 9-pin female connector cable is for the system to computer communication. The 25-pin connector is connected to the female connection on the system communications interface plate located at the rear of the system base box, see Appendix C. The 9-pin connector should be connected to COM 2 port.

The 9-pin female connector to round connector cable is for communication to camera. The 9-pin connector connects to a 9-pin COM port of the computer, and the round connector to the camera COM port of the camera communications interface plate located at the rear of the camera mounting arm. (see Appendix C.)

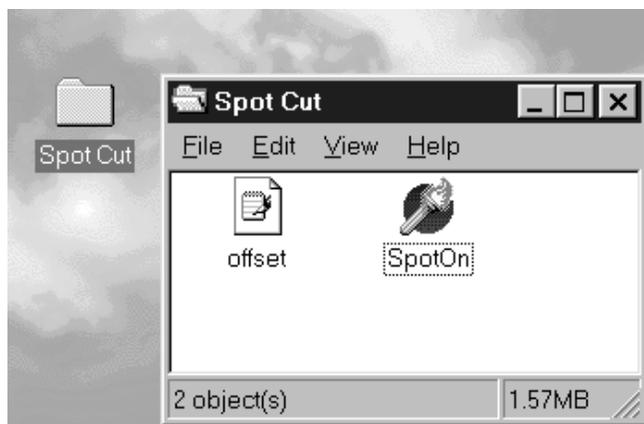
The camera power cord connects into the camera interface plate directly below the camera communications plug. (Refer to Appendix C)

The main power cord connects to the main power socket of the system (see Appendix C.)

2.3 Software Setup

Use the following steps to load the SpotOn software and the image acquisition software onto the computer.

1. Create a folder in the C: drive with the name **Spot Cut**. The spot cutter will look for the folder with this name to get its instructions for movement and orientation.
2. The SpotOn software and the offset file that contains calibration information are loaded into the **Spot Cut** folder.



3. The Kodak image acquisition software is loaded into the Program File folder of the computer. The Kodak software will self install in the Program Files folder.

2.4 Initial System Set Up

After connecting the system to the computer and loading the software, the system is ready for use. The first step will be to calibrate the x-y axis of the spot cutter to the image that is acquired by the camera. See Section 6 for calibration method.

2.5 Quick Guide to Spot Excision

The following is a quick guide to the operation of the spot cutter system. Details on individual steps and features are covered in the following sections of this manual.

1. Turn on the computer.
2. Turn on the Protean 2-D Spot Cutter System.
3. Double click on the SpotOn icon (the SpotOn Cutting System menu should now be displayed.)
4. The system must be reset to its home. This is done by pressing the start/pause button located on the system front panel. (See Appendix A) The excision head will now be homed to the back right corner of the system.
5. Load a liquid into each well of the microtiter plate. Water or other liquid helps with gel deposit, and it prepares the gel spot for downstream processing without drying out.
6. Place microtiter plate in position (refer to Section 5.4)
7. Place the cutting mat into the cutting stage area.

8. Cut the gel to the appropriate size with the cutting tool and place in the gel frame. For PVDF membrane, use the gel frame to center the membrane on the cutting stage.
9. Acquire an image of the target gel or membrane. See Section 3 for image acquisition instructions.
10. Once back in the SpotOn Cutting System program, open the image that was just acquired.
11. Select spots for cutting. Use the left mouse button to bring up the Zoom box, and use the right mouse button to select a position for cutting.
12. After you have selected the spots to be excised, start the cutting run.

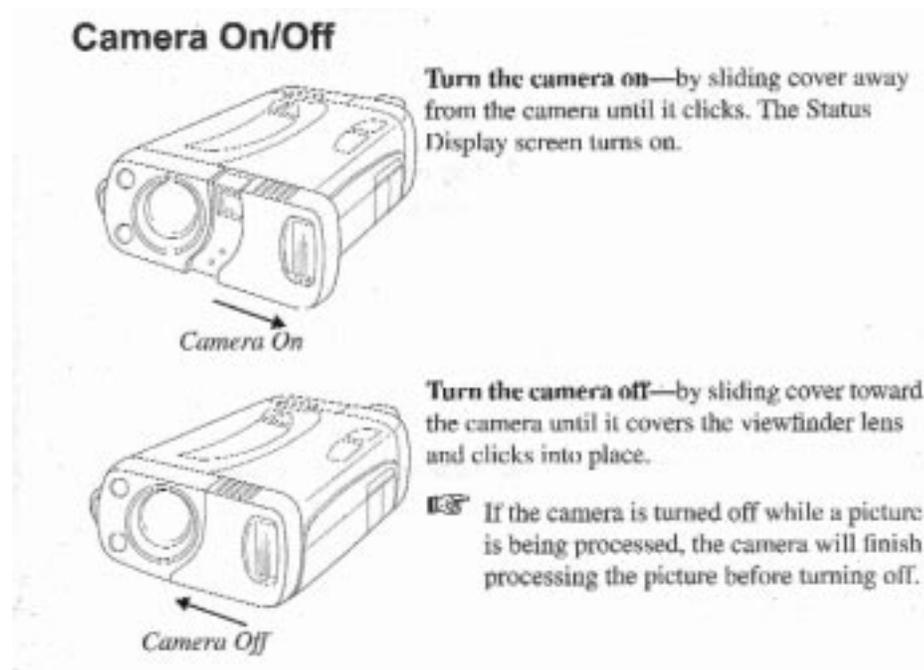
Section 3

How To Use Photo Enhancer and the Digital Camera

This section covers the steps for taking an image and saving the image file using the Photo Enhancer program. More specific instructions on using Photo Enhancer such as editing an image, are found in the "DC120 Zoom Digital Camera User's Guide" supplied with this manual.

3.1 Turning on the Digital Camera

The camera is turned on by opening the camera cover slide located at the bottom left hand corner of the camera. The cover is moved to the left to turn the camera on and to the right to turn the camera off.



3.2 Selecting the DC120 Digital Access (TWAIN Acquire) Window

In order to activate the lights, go to the menu item "Operation" in the SpotOn software. Choose, "Others". Check the "Light Select Enabled" option (a check mark shows up in the box next to the item). This allows the software to turn on the lights by the menu bar icon or during the process of setting up to take a picture.

Select **File** from the menu bar to display a drop down menu. Select Photo Enhancer. Alternatively, press the Photo Enhancer shortcut button located below the menu bar. (see Appendix D)

An information box will be displayed. Selecting "Yes" will move the system to parking position and switch the lights on. Selecting "No" will transfer to the image acquisition software without turning on the lights. This may be advantageous as you can turn on the lights with the red "pulse" button on the front of the instrument when taking a picture. Especially when an image is going to be optimized with the software, it is better to select NO to the lights, and turn them on with the pulse switch. Press and hold the pulse switch during the image acquisition process.

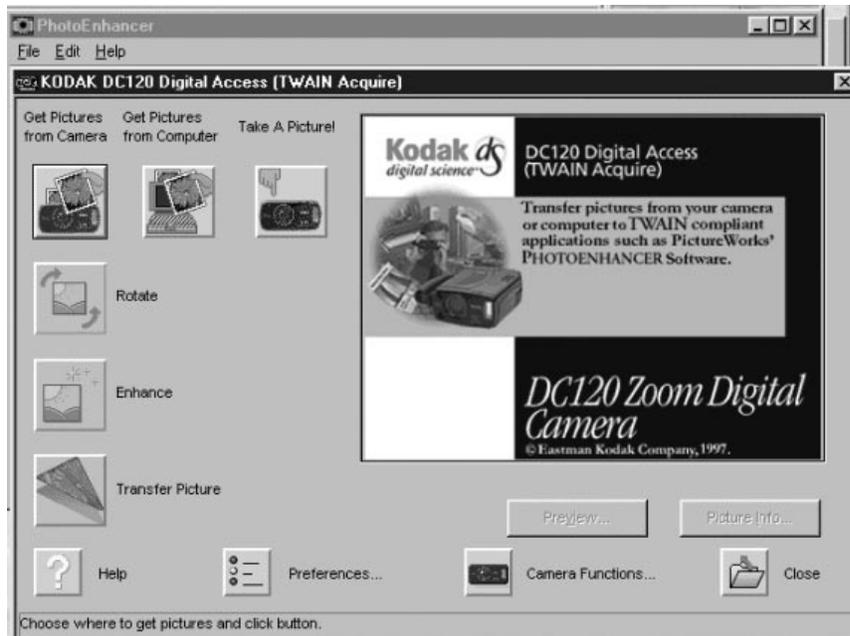
Important Note: do not leave the system lights on for extended periods of time. Leaving the lights on for **more than 10 minutes** may cause permanent damage to occur to sections of the system.

Another information box will be displayed providing instructions for use. Acknowledge by clicking on the OK button.

Select the File menu from the menu bar. Click on TWAIN Acquire or use shortcut key Ctrl+W. This will display the DC120 Digital Access (TWAIN Acquire) window.

3.3 Camera Functions

Once the DC120 Digital Access (TWAIN Acquire) window is displayed, click on the "Camera Functions" button to select camera settings. This will produce the Status LCD Function Set Up folder which contains the Focus and Flash settings.



In the Auto Focus panel, select Macro Mode. This will disable the auto focus on the camera. The AutoFocus is not recommended because it adjusts the focal length to each new image and changes the image area from that which was calibrated.

Suggested Flash settings:

For gels, turn the flash setting OFF.

For PVDF membrane and calibration templates, use FILL flash. The under lighting may or may not be needed for a FILL FLASH picture. The underlighting can be turned on and off through the SpotOn software. Adjustments to these guidelines may be made depending on ambient light conditions in the lab. No light may be needed in some cases.

In the Quality panel, select Better.

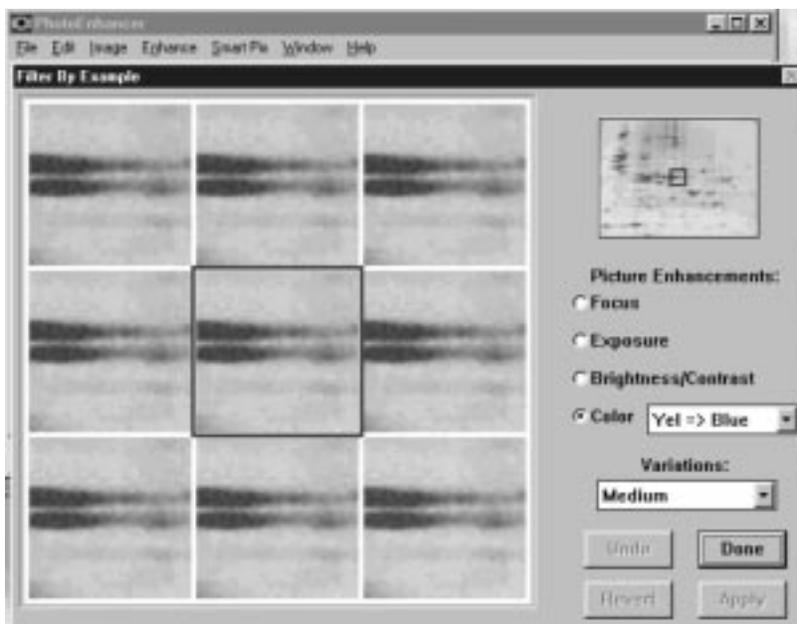
The "Update Camera" button in the Status LCD Function Set Up folder must be clicked to confirm your new selections. The software will hold the settings during multiple images in a normal workflow. The settings will need to be reset if the computer, camera, and spot cutter system are restarted.

3.4 Acquiring an Image

An image can now be acquired by clicking on the button labeled **Take a Picture**. Once the image has been taken, a small picture is displayed in the top right hand corner of the TWAIN ACQUIRE window. Once the image is acquired, the image can be enhanced for overhead lighting conditions by selecting the Enhance icon, and selecting **Fluorescent**. Evaluate the effects in the thumbnail picture, if the picture is worse, select Auto Lighting (Default) to change back to the original image.

Select the **Transfer Picture** button. The image is now being transferred to the Photo Enhancer screen. More enhancement functions are available in this screen. If more enhancement is needed and some time is going to be spent on this function, go back to the SpotOn software and select **Turn Lights Off**. This will allow work to be done without damage to the spot cutter or excessive drying out of the gel.

Now the image may be edited as required. The most useful menu is **Enhance**. Choose the **Enhance by Example** option. This will produce this screen:



In the small, full image in the upper right hand corner of this screen, move the blue box within the small image to an area that needs the most enhancement. Use the 3x3 example boxes to choose the best image for "Exposure" and "Brightness/Contrast". Choose Apply to set the new parameters to the entire image. When satisfied with the image enhancement, choose Done.

3.5 Saving an Image

To save the image, select **File, Save As**. The file must be saved as a Bit Map picture format. Click on the button in the **Save File as Type** list box. This displays a drop down list from which a (*.bmp) file should be selected. Select the directory and folder you want to save the image in. This should be on your C: drive as it needs to be accessible to the SpotOn software.

To select file name, click on the File Name text field, then the file name can be typed in. Make sure the name has a **.bmp** extension on the file name. Click on the "OK" button to save the image. Once the image has been saved, exit the Photo Enhancer software. This will return back to the SpotOn software that was running in the background.

3.6 Erasing Picture Memory

The DC120 camera has a memory chip that holds pictures as they are acquired. These files need to be periodically deleted from the camera. Erasing these pictures does not effect the saved images on the computer. The camera memory holds about 20 images.

To delete pictures, go to the Camera Functions screen and select the "Erase Pictures" option. This will bring up a screen with the file names of the pictures existing in the camera memory. Select the pictures to be deleted (usually all) by clicking on the first file name, hold the shift key, and click on the last file name. All the files should now be highlighted. Select "Add to List" which will produce a list in the right hand box of all the files that will be erased. Click "OK" to erase. A confirmation box will come up, click "OK". A processing bar will appear showing the status of the deleting files. The process will take ~5 minutes to delete 20 files. When it is finished, click on "Close" to return to the Camera Functions box.

Section 4 Selecting and Cutting Spots

4.1 Opening an Image File Format

To open an image file in the Spot On screen, select **File; Open Image File**.

Alternatively, this may be done by clicking on the Open Image File shortcut button located below the menu bar. (see Appendix D)

This will generate the Open window, which will display a list of files. Select the appropriate folder and open the image by either a double left mouse click on the file name, or a single left click on the file name followed by a click on the "Open" button.

The new project number window will be displayed. To move between text fields and enable typing of text, you must click on the required text field. Entering a project number and Gel/PVDF number is optional.

4.2 Using the Zoom Window

Left clicking on any position on the gel image will display the selected area in the zoom window at the top right hand corner of the screen. The position's x-y pixel coordinates will be displayed below this zoom window.

The zoom setting of the window can also be adjusted by using the + or - zoom setting shortcut buttons. (Refer to Section 7.2 for details.) The zoom window allows for a choice of whether selected spot numbers are displayed in the zoom window or not by selecting **View; Display with spot No.**

4.3 Selecting a Spot & MTP Location

A right mouse click on any position will select the spot at the cursor crosshair to be cut, and produce a pop-up menu with the following choices;

- a) **Select to next MTP well:** This will allocate the selected spot to be deposited in the MTP location immediately following the MTP position of the previous spot selected *i.e.* A2 after A1, B1 after A12 etc. If the spot is the first spot selected for the image file it will be allocated to A1.
- b) **Select to other MTP well:** This choice will display the MTP Location window. Any location can be selected for a particular spot, by
 - (i) Selecting the MTP position text field, then typing in desired location, or
 - (ii) Selecting the MTP position button (which will display a drop down list), then clicking on the desired location. The Scroll Bar may be used to move throughout the list. If you do not want to select this spot simply select the "Cancel" button. To confirm a spot you now click on the "Select" button.

A maximum of ten spots can be allocated to any one MTP position well.

The MTP well positioning layout scheme is detailed in Section 5.4.

Once selected, the spot will appear as a red circle containing a spot number.

4.4 Deleting Spots

Once a spot has been selected and allocated to a MTP well position, it may be deleted by either of the following methods;

- (i) Move the dotted line box within the MTP grid to the box containing the spot to be deleted by using the arrow keys on the keyboard. Right click on the MTP spot destination window located at the bottom right-hand corner of the screen. This will produce a pop-up menu, from which you should select **Delete** this spot.
- (ii) Select the **Window** menu from the menu bar then **Spot Location** from the drop down menu. The **Spot Location** window will now be displayed. Right click in the **Current State** column for the corresponding row of the spot number you wish to delete. This will produce a pop-up menu from which you click on delete to remove the spot. Alternatively, if a selected spot has been cancelled, you may follow the above steps but click on **Select** from the pop-up menu instead of **Delete**. Once the alteration has been made, click on the OK button on the spot location window to proceed.
- (iii) Select the **View** menu from the menu bar, then **Delete Spot** from the drop down menu. The **Delete Spot** window will now be displayed. Click on the spot number to be deleted in the larger text field. This spot number should now be displayed in the smaller text field. The spot can now be deleted by pressing the OK button. If you wish to leave the Delete Spot window without deleting a spot, simply click the Cancel button.

4.5 Making Cuts (Excisions)

Select the appropriate cutting tip action from the **Operation** menu, **Others**. A description of the options is provided in Section 8.4.

Hint: For best deposit of gels and membrane spots, add 150–175 µl of liquid (water, acetonitrile, etc.) to each microtiter plate well. This will provide the best condition to deposit spots effectively, and it will keep the spots from drying out in the microtiter wells.

Recommendations for cutting tip actions:

Acrylamide Gels: Use standard (default) method. For increased assurance of positive eject, select **Double Eject**.

PVDF Membrane: Use **Eject With Turn** in order to help eject the static membrane from the stainless steel tip.

Calibration: Use **Cut without Turn** in order to keep the cutting tip from marking the template.

A cutting run can be selected by either-

- (i) Selecting the Run shortcut button located below the menu bar.
- (ii) Selecting the Cutting menu, then selecting Run from the drop down menu.

You may not wish to cut all the spots selected during this cutting run. This can be achieved by putting the desired starting spot number and last spot number in the "Start from" and "To" fields of the Set-up Cut Spot window.

Note: An individual run must consist of a sequential subset of the total spots selected, *i.e.* you can not select to cut spots 4 & 6 on an individual run without spot 5 being cut. An individual cutting run cannot be out of order, *ie.* 4, 6, 5 is invalid, it would need to be 4, 5, 6.

Once the spot sequence to be cut has been selected, press the OK button of the Setup Cut Spot window.

When cutting is complete, the Cutting Complete box will be displayed, select OK to continue. It is advisable to check the completeness of the cutting run by visually verifying that each well has a gel spot in it. If there are any missing wells, it is likely that the gel spot is still in the gel. The cutter can very easily be directed to re-cut these spots. See next section on **Cut Previous Spot** methods.

4.6 Re-Cut Previous Spot

If there is the need to re-cut a previously selected spot, there are several ways to direct the spot cutter to re-cut a spot.

- a. The MTP grid area of the screen can be used to easily re-select the few spots that may not have been picked up from the gel. Move the dotted line box within the MTP grid to the box containing the spot to be deleted by using the arrow keys on the keyboard. (You may need to right click on the table to produce the dotted line box. Hit escape, then move the box with the arrow keys.) Right click on the MTP spot destination window located at the bottom right-hand corner of the screen. From the pop-up menu select Single Cut. The cutting arm will go to that spot location, perform the cut, and return to the appropriate MTP well location. This can be repeated for other individual spots to be re-cut.

- b. Select the **Window** menu, then **Spot Location** from the drop down menu. This will produce the **Spots Location** window, which displays details of all selected, deleted, and cut spots. The **Current State** of the particular spot may be changed from **Cut** to **Select** by right mouse clicking in the **Current State** column for the spot to be re-cut. This will produce a pop-up menu, select **Delete**. Right mouse click on the **Current State** column, choose **Select** which will change the spot back to the select state enabling it to be re-cut. This may be done for any number of spots. Once completed the spot may be cut using the same method as for any individual cutting run.
- c. Select the **Run** shortcut button, or choose **Cutting; Run**. Use the **Cut Previous Spot** selection which is found within the **Set-up Cut Spot** window. Cut Previous Spot is activated when a check appears in the check box. Enter the spot numbers to be re-cut. They must be in sequential order to be a valid cutting order, or one spot can be entered at a time.

Except for the case when several spots will be re-cut in sequence, the first re-cut method in this section should be used.

Auto Cut Previous Spot/Enabled

- (a) With Select Previous Spot enabled, if any previously cut spots are selected in the run to be cut, once "OK" has been pushed to commence the cutting run, and information box will be displayed individually in turn for each previously cut spot. In this box you may either select the "Yes" button to re-cut the spot or "No" button not to.
- (b) With Select Previous Spots disabled, selecting the "OK" button enables the operator to repeat the previous cut run. Additional spots can be selected and on clicking the "OK" button all previous selections, plus the additional spots will be cut.

Once all previously cut spots have been addressed, the program will automatically cut any new spots in the run.

This function allows for any combination of previously cut spots to be cut, ie. you may cut spots 1,3,5 without cutting 2 & 4, even though 2 & 4 are within the range of the selected spots to be cut (excised).

Auto Cut Previous Spots Disabled

With this function disabled, no previously cut spots will be re-cut.

Section 5 Working Surface

The working surface is shown in Appendix B and consists of two main areas, the cutting surface and the microtiter plate holder. In addition there is also a bubble level which may be used to ensure the cutting surface is level.

5.1 Excision Mats

The gel or membrane is cut on a custom designed PVC excision mat. Only use 1 mat for correct height.

Two PVC (Clear) excision mats are provided with the system. These mats may become scored by the cutting tip, stained by the gel stains, or become cracked with repeated use over

time. This is normal wear and replacement mats are available from Bio-Rad. Order catalog number 165-7021.

5.2 Cutting Surface

Gels and membranes should be cut on the excision mat to provide the correct backing for the excision tip. The overall surface dimensions are 178mm x 180mm, however, the recommended excision area size is 11.8 x 8.8 cm due to the area that can be imaged with the camera. Clean the cutting surface regularly with mild detergent and water. Clean water off the cutting platform and the base after use.

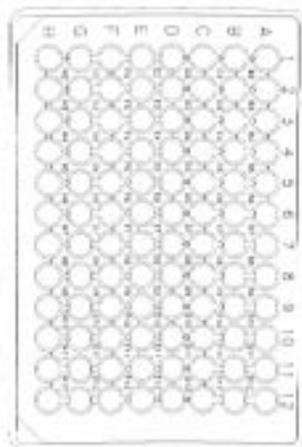
5.3 Gel Sizer and Frames for 2-D Gels

In order to facilitate cutting a larger gel down to the size that can be imaged on the spot cutter, two sizes of gel sizers and holding frames are provided with the system: a 11.8 x 8.8 cm Large Gel "quadrant size, and a 9.5 x 7 cm mini gel size.

5.4 Microtiter Plate (MTP) Holder

The MTP holder is to the immediate right of the cutting surface. To insert a microtiter plate (MTP) into the holder, hold the plate at a slight angle, push the corner of the microtiter plate corresponding to position H12 against the positioning pin located at the bottom left hand corner of the MTP holder. Once the positioning pin has been pushed in slightly, the microtiter plate can be pushed down into the holder. The positioning pin is spring loaded and will hold the plate in place.

As viewed on the surface of the Spot cutter the software allocates the microtiter plate well positions as follows -



The system accepts any standard microtiter plate. Polypropylene plates are recommended for protein processing. Corning and Grainer brand plates have been tested and work well in this system. Use "U" or "V" well bottoms for best recovery.

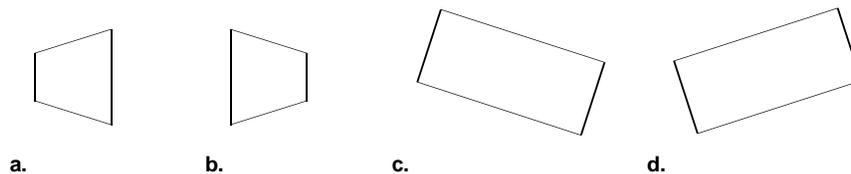
Section 6 Calibration

6.1 Camera Alignment

If the cutting head is not moving to the correct position as selected using the software, the system should be calibrated. To assess the alignment of the camera to the cutting stage, perform the follow steps using the calibration plate (the plastic plate with a rectangle):

1. Place the calibration plate on the cutting surface.
2. Take a photo of the calibration plate.
3. Save the photo.
4. Open the photo within the SpotOn program.

If the image of the calibration plate is not square, *i.e.* it appears to be generally shaped like any of the following pictures, a-d, the camera is out of alignment and requires Bio-Rad Service to arrange a service visit to align the camera.



If the rectangle is at right angles in the viewing screen and is accurate in its shape, but the system is not moving to the correct x,y position, then proceed with the following calibration routine. A copy of the System Serial Number is necessary for calibration and has been supplied upon delivery of the system.

6.2 Calibration Definitions

Pixel Position Y (PPY) & Pixel Position X (PPX): the scale/ratio between the image on the screen and the image in real life.

X and Y Offset: adjusts the distance between the position that the system actually moves to, and the position that the system should have moved to. Scale: 100 units in the numbering of X and Y Offset is equal to 1mm on the calibration plate. A four digit number is entered for both the X and Y offset values. For every 100 digits, the system is offset 1 mm on the cutting surface.

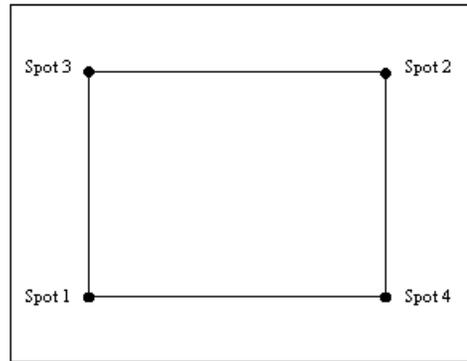
6.3 Calibration Procedure

1. Place the calibration plate or graph paper on the cutting surface
2. Take an image of the calibration plate or graph paper
3. Save the image
4. Open the image within the SpotOn software
5. In the **Operations** menu, select **Others**. A dialog box will appear and select **Cut Without Turn**. This will allow the cutting tip to go to the selected spot without cutting or marking the calibration template or graph paper.
6. Get a pad of paper to record coordinate locations during the calibration procedure. This will help in zeroing in on a coordinate location.

7. There are two parameters to adjust in the calibration for both x and y dimensions. The OFFSET is the distance the image area is from the 0,0 position for the system's x,y axis. Adjusting x and y offset moves the whole cutting area. This is defined in linear terms with 100 offset units equal to 1 mm on the cutting platform.

PPX and PPY are adjustments to the distance between two points in the cutting area. If the corners of the plate are too close or too far apart from each other to match the calibration template, the ppx or ppy is adjusted accordingly. The digits in the ppx and ppy do not relate to a linear dimension. Large movements of adjustment should be made with changes to the digits in the 3rd place (0.00X). Fine adjustments are generally made in the 4th place (0.000X). The process will require adjustments of the number value, and then checking for the result by a single "cut" without cutting the template.

The following diagram is an example for explanation purposes of the calibration routine. "Circles" are the positions that are selected on the SpotOn screen. Square spots show the position that the spot is actually "cut". The square spots are adjusted during calibration to "cut" the circles.



Circles indicate where the spot should be cut

Squares indicate the spot that is actually cut.

- The squares need to be adjusted to the same position as the circles in the examples.

Y Pixel and Offset Calibration (Y Offset and PPY)

1. Select spot #1 (see diagram), and select to next MTP Well.
2. Hint: Take note of where the 1st spot "y pixel" coordinate is on the monitor. This can be used to line up the 2nd spot y coordinate

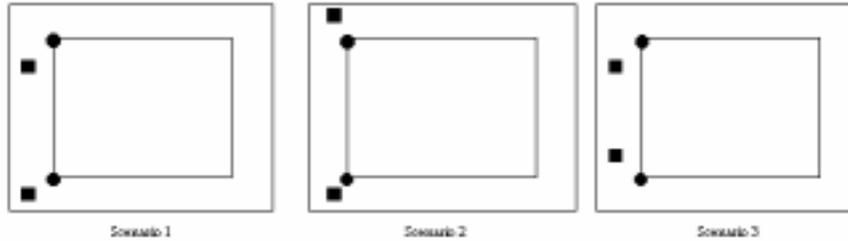
Hint: To get a more accurately cut spot, click on the Enable Arrow Keys shortcut button on the shortcut menu bar and use the arrow keys for fine cursor control.

3. Select spot #2 (see diagram).
4. Run the cutting run for spots #1 and 2.

Hint: Stop the cutting tip down on the calibration plate by clicking on any menu item as the tip comes down. (Do not select any of the options.) This will freeze the system and will provide a longer time to evaluate the location of the cutting tip.

Check to see which of the following scenarios is closest to how yours appeared.

5. Select Calibration from the Operation Menu, then select Manual Calibration.
6. Enter the Serial Number. Change the number as indicated by the needed movement for the cutting tip.



7. Scenario 1:

- (a) The Y Offset needs to be increased.
- (b) Once spot #1 is parallel to the bottom line, check to see if spot #2 is parallel to the top line. Increase or decrease PPY depending on whether the second spot is above or below the top line.
- (c) Once spot #2 is parallel with the top line, check to see if spot #1 is still parallel to the bottom line. If it is, go to the next step.

Scenario 2:

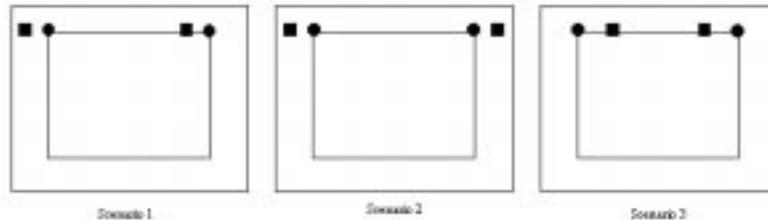
- (a) The PPY needs to be decreased.
- (b) Continue decreasing the PPY until it looks like Scenario 1.
- (c) Continue with Scenario 1

Scenario 3:

- (a) The PPY needs to be increased.
- (b) Continue increasing the PPY until spot #2 is approximately the same distance above the top line as spot #1 is above the bottom line.
- (c) The Y Offset will then need to be decreased.

X Pixel and Offset Calibration (X Offset and PPX)

1. Select spot #3" (see diagram), and select to next MTP well.
2. Run the cutting run with just spots #2 and 3.
3. Check to see which of the following figures is closest to how yours appeared.
4. Select Calibration from the Operation Menu, then select Manual Calibration.
5. Enter the Serial Number.



6. Scenario 1:
 - (a) The X Offset needs to be increased.
 - (b) Once spot #2 is parallel to the left hand line, check to see if spot #3 is parallel to the right hand line. Increase or decrease PPX depending on whether the third spot is left or right the right hand line.
 - (c) Once spot #3 is parallel with the right hand line, check to see if spot #2 is still parallel to the left hand line. If it is, go the "Adjustments" below.

Scenario 2:

- (a) The PPX needs to be decreased.
- (b) Continue decreasing the PPX until it looks roughly like Scenario 1.
- (c) Continue with Scenario 1

Scenario 3:

- (a) The PPX needs to be increased.
- (b) Continue increasing the PPX until spot #3 is approximately the same distance right of the right hand line as spot #2 is right of the left hand line.
- (c) The X Offset will then need to be decreased.

Adjustments

Once the calibration has been completed, run the cutting run with all of the spots. If these are all selected by the cutting tip in the right position, then select a 4th spot and run that. If any changes are needed, adjust accordingly.

Section 7 The Gel Cutting System Menu Bar

7.1 File Menu

Selecting the **File** menu will produce the following drop down menu.



Open Image File

Select this menu to open an image file, or click the Open Image File shortcut button located below the menu bar. When the Open window is displayed, an image file can be selected by doing the following:

- (i) Click on the file to be opened, then press the Open button to open the file, or
- (ii) Double click on the file name displayed in the file list window.

To leave the Open window without selecting a file, simply click on the Cancel button.

Save Image File

Select this menu or click the **Save Image File** shortcut button located below the menu bar to save changes to an image file. This will display the **Save Image File** window. Selecting the **Save In** field of this window allows you to select the drive and directory into which you want to save the picture file.

Selecting the **File Name** field of this window enables you to select a file name for new files or change the file name of existing files. If you change the file name of any existing file and save it, you will then have two copies of this image file, one with the new name and one with the old name. However, if changes were made to the file before saving, they will only be updated to the new file.

Once you have made your selection, you can either save by selecting the Save button or cancel your selection with the Cancel button.

When the Save button is selected, the image file will be saved and a **Save Project File** window will be displayed. This window is used to save the text details of the image file, *i.e.* the project number, spot selected, spot destination, locations, etc. It is recommended that you use the same file name as the image file.

To save the text file, press the Save button.

Photo Enhancer

The Photo Enhancer program is used for the operation of the Digital Camera. This program is accessed from the SpotOn System Menu Bar. Either:

- (i) Select the File menu, then select the **Photo Enhancer** menu from the drop down menu, or
- (ii) Press the **Photo Enhancer** shortcut button.

This will produce an information box. Press the Yes button to move the cutting head to the parking position and turn lights on if you wish to take a picture. Alternatively, select "No" and use the pulse button on the front of the system to turn lights on. Next, another information box will appear, press the OK button to continue. You should now be in the Photo Enhancer program.

Print

Select this menu, or pressing the **Print** shortcut key located below the menu bar to print an image file. The image must have a project number to be printed. If it does not, upon selecting the Print menu an information box will appear stating "No project number, do you want to enter new number?" Press Yes and enter a project number to proceed with printing.

Note: A Gel number is not needed to print.

The **Name** field of the Printer panel enables you to select a printer if more than one printer is connected to your computer.

Selecting the **Size** list box of the Paper panel displays a drop down list from which you can select the paper size to be used.

Selecting the **Source** list box of the Paper panel displays a drop down list from which you can select either manual or auto sheet feed.

The **Orientation** panel allows you to select either Portrait or Landscape page orientation.

Once the above settings are completed, press OK. This will display the **Print Selection** window.

This contains a number of options for printing:

- (a) The **Original Picture** selection will print the picture as it was when the image was last opened using **Open Image file**, *i.e.* no matter how many changes (new spots) have been made, the original opened image will be printed.
- (b) The **Amended Picture** selection prints the original image plus any amendments made to it since the picture file has been opened. It will print all spots cut and all spots selected, but not yet cut.
- (c) By selecting the **With Spots List** check box, the Digital Camera x-y coordinates for each spot are also given.

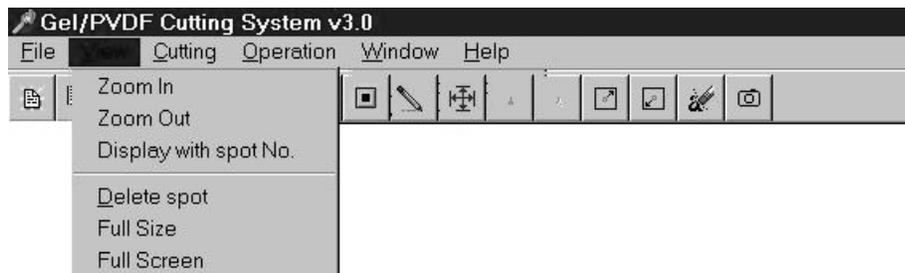
Once selections have been made, select the OK button to print picture, or Cancel if you no longer wish to print.

Exit

Select the **Exit** menu to leave the SpotOn System program.

7.2 View Menu

Selecting the View menu will produce the following drop down menu.



Zoom In

Selecting the **Zoom In** menu, zooms in on the picture displayed in the Zoom window at the top right hand corner of the screen. This can also be achieved by pressing the **Zoom In** short cut button located below the menu bar.

Zoom Out

Selecting the **Zoom Out** menu, zooms out on the picture displayed in the Zoom window at the top right hand corner of the screen. This can also be achieved by pressing the **Zoom Out** shortcut button located below the menu bar.

Display with Spot Number

By clicking on the **Display with Spot Number** menu, a check will appear next to it or disappear depending on its current state. With a check present, any location on the image file shown in the Zoom box will show a circle and spot number for selected spots in the Zoom box window. Without the check present in the menu, no spot numbers will be shown in the Zoom box.

Delete Spot

Selecting the **Delete Spot** menu produces the Delete Spot window. To delete a spot, click on the spot number to be deleted in the larger text field. This spot number should now be displayed in the smaller text field. The spot can now be deleted by pressing the OK button. If you wish to leave the Delete Spot window without deleting a spot simply click the Cancel button.

7.3 Cutting Menu

Selecting the Cutting menu displays the following drop down menu.



Run

A cutting run can be selected by either:

- (i) Selecting **Run** from the Cutting menu, or
- (ii) Selecting the **Run** shortcut button located below the menu bar.

A window will come up confirming the sequence of spots to be cut. You may not wish to cut all the spots selected during this cutting run. This can be achieved by putting the desired starting spot number and last spot number in the **Start from** and **To** fields respectively of the Set-up Cut Spot window.

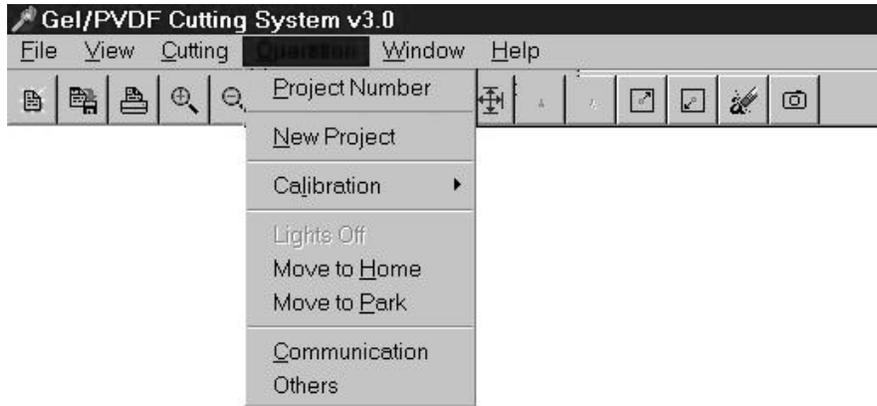
Note: An individual run must consist of a sequential subset of the total spots selected, *i.e.* you can not select to cut spots 4 & 6 on an individual run without spot 5 being cut. You also can not have an individual run cutting spots out of order, *i.e.* 4, 6, 5 is invalid, would need to be 4, 5, 6.

Once the spot sequence to be cut has been selected, press the OK button of the Setup Cut Spot window. When cutting is complete, the Cutting Complete window will be displayed, select OK to continue.

You may now either make more cuts and or spot selections, or exit the program.

7.4 Operation Menu

Selecting the Operation menu displays the following drop down menu.



Project Number

Select this menu to change or add a new **Project number** and or **Gel/PVDF number**.

New Project

Select this menu to change or add a new **Project number** and or **Gel/PVDF number**. Using this menu, any currently selected spots not yet cut will be lost.

Calibration

This function is used to calibrate the x, y movements of the cutting head. Alternatively you may select the **Manual calibration** shortcut buttons located below the menu bar (see Appendix D).

Upon selecting the calibration menu the **Serial Number** box will appear.

You must click on the **Enter Serial Number** text field and type in the serial number. (The serial number was provided when the system was delivered).

Light On/Light Off

Selecting this menu will either turn system lights on or off, depending on their current state.

- (a) Lights on can also be achieved by pressing the **Light On** shortcut button located below the menu bar. (see Appendix D)
- (b) Light off can also be achieved by pressing **Light Off** shortcut button located below the menu bar. (see Appendix D)
- (c) The lights may be turned on temporarily at any time by pushing and holding in the lights on push button located at the front of the system. See Appendix A.

Move to Home

Select this menu or press the **Move to Home Position** shortcut button located below the menu bar (see Appendix D) to move system cutting head to the home position. This is the location at the top left-hand corner of the working surface. This position may be used for changing cutting tips.

Move to Park

Select this menu or press the **Move to Park** position shortcut button located below the menu bar, (see Appendix D) to move the cutting head to the park position. This is located at the top right hand corner of the working surface. The cutting head should be in the park position before a picture is taken. On system initialization, the cutting head is automatically moved to the park position.

Communication

Select this menu to change the set-up of the communication ports for data transfer between computer and cutting system. The system will default select the COM 2 port. This can be changed to the COM 1 port in the communication panel. This selection will only be held for that set-up. The next time the system is turned on, it will default back to COM 2.

Others (Cutting Tip Control and Lights)

Selecting this menu produces the **Other Function** setup window. This contains several alternative methods of cutting head operation. By trying these methods under varying circumstances, you can work out which method will work best for your needs and under certain conditions.

The standard operation (nothing selected in Others) will direct cutting tip rotation as the spot is being cut. The cutting head ceases rotation before the spot is dispensed, the spot is then dispensed to the MTP well while the cutting head is down.

Recommendations:

- | | |
|------------------|--|
| Acrylamide Gels: | Use standard (default) method. For increased assurance of positive eject, select Double Eject . |
| PVDF Membrane: | Use Eject With Turn in order to help eject the static membrane from the stainless steel tip. |
| Calibration: | Use Cut without Turn in order to keep the cutting tip from marking the template. |

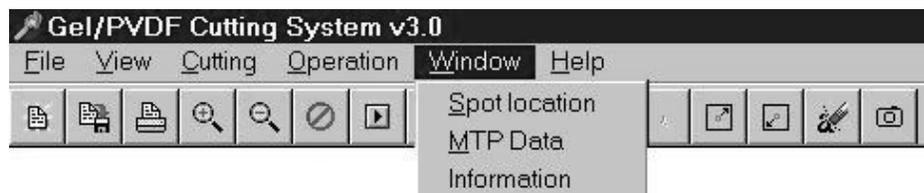
The difference between each of the six alternative methods from the standard method normally used by the system are the following:

- (a) Double Eject—same as standard operation except there is a double movement of the eject pin.
- (b) Eject With Turn—same as standard operation except the cutting tip is rotating during ejecting.
- (c) Eject with Cutting Head Up—same as standard operation except the cutting head does not move down while ejecting.
- (d) Lights Select Enabled/Disabled—allow the **lights on/off** shortcut buttons to be enabled or disabled.
- (e) Cut Longer—cutting tip will stay down longer on the cutting surface.
- (f) Cut without Turn -same as standard operation except the cutting tip does not rotate during the cut.

Note: Combinations of the above may be tried by the operator to suit different conditions.

7.5 Window Menu

Selecting the Window menu displays the following drop down menu.



Spot Location

Selecting this menu produces the **Spot Location** window. For each selected spot, this window displays:

- (i) The camera image x, y location coordinates.
- (ii) The MTP (microtiter plate well destination position).
- (iii) The current state of the spot, *i.e.* selected, cut or deleted.

The current state of a spot may be changed inside this window

Right clicking in the **Current State** column for the corresponding row of the spot number you wish to amend, will produce a pop-up menu from which you may select the desired change required. This may be used to implement the following;

- (i) Delete a selected spot.
- (ii) Select a cancelled or deleted spot.
- (iii) Reselect a cut spot to enable recutting.

Use the scroll bar to move up and down the list of spots, and then select a spot by clicking on the desired field.

To print the details of this window, press **Print**.

To leave this window, select the OK button.

MTP Data

Selecting this menu produces the Microtiter Plate (MTP) Location window. This window lists all MTP locations vertically. Each individual location has a row with space provided for up to 10 spots to be allocated to it. Any spot number assigned to a given MTP location will be given in the row of that location.

The scroll bar may be used to move within the list.

To print this screen, click the **Print** button.

To leave this window, select the OK button.

7.6 Help

Selecting the Help menu displays the following drop down menu.



This menu provides documented help topics and information about the system.

Section 8 Maintenance

8.1 General Upkeep and Cleaning

When the System is not in operation, use the dust cover supplied with the System. This will reduce the amount of dust and keratin that collects on the cutting surface.

For general cleaning of the system, wipe with a damp cloth. Ethanol or mild detergents may be used. Do not use any harsh solvents or cleaners.

8.2 Cutting Surface Lights

Location of Lights

The cutting surface lights are located directly beneath the cutting surface and consist of four 9W low temperature (4100 K) fluorescent tubes.

Replacing Surface Lights

Please see Appendix B when changing fluorescent tubes. The lighting front cover must be removed first by removing the two front cover screws. Once the cover has been removed, the required fluorescent tube(s) may be gently pulled out and then replaced by pushing the replacement tubes into position. After the tube(s) have been changed the front cover is reattached.

8.3 Fuses

The main power fuse is located on the system box interface plate, refer to Appendix C for exact location. At delivery, there is no voltage fuse installed. Verify regional voltage input and select the appropriate fuse and voltage setting. The fuse for 240 VAC operation is the 1.6A fuse. For 120 VAC operation a 3.15A fuse is used. Two of each of these fuses have been supplied with the System. Bio-Rad Sales and Service will make arrangements in the purchase agreement to install the System and set the correct voltage and fuse configuration.

The camera power fuse is located on the system box interface plate, refer to Appendix C for exact location. The fuse is a 2.5A fuse. One spare fuse has been supplied with the System.

Note: All other fuses are located internally in the system box. Service assistance should be obtained if these fuses need replacing.

8.4 Cutting Tips

Cutting Tip Cleaning

The cutting tip is made of high quality 303/304 stainless steel. It will retain its cutting ability through repeated use and should not need to be replaced even after 6 months of use. If the tip is not picking up gel pieces as efficiently as earlier runs, there may be gel material building up inside the tip. Either clean out the tip with water or alcohol and a wire, or replace the cutting tip with a new one. Daily maintenance includes washing the cutting tip with water or alcohol. Take the tip off the instrument and squirt water or alcohol through the tip. To remove the cutting tip, see the following section on "Changing Cutting Tips".

Ejection Pin

If the ejection pin is not recovering after pushing out through the cutting tip, a small amount of lubricant may be used in the mechanism. Take the cylindrical cutting assembly cap off and spray or drop silicon lubricant (like WD40) down the shaft of the ejection pin (where the spring is). Manually work the mechanism by pushing down on the top of the ejection pin. It may also help to take the cutting tip off and clean it out with the alcohol and wire method recommended above.

Changing Cutting Tips

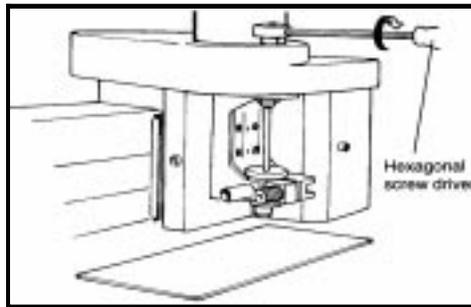
To change a cutting tip, move the cutting head to the home position. This can be done by, pressing the **Move to Home Position** shortcut button located on the menu bar. Once in the home position, use the two wrenches (spanners) supplied with the system. The customized wrench (spanner) is used to hold the cutting tip holder by fitting into the grooves located on the cutting tip holder. The 6mm open ended wrench (spanner) will then fit the top of the cutting tip. Turn the wrench (spanner) counter-clockwise to remove the tip. A new tip can then be inserted and turned clockwise to install. Use the same wrenches (spanners) to tighten the cutting tip. This does not have to be over-tightened, but snug.

Note: the top of the cutting tip is rectangular in shape. If the 6 mm wrench (spanner) does not appear to fit you will need to rotate the cutting tip 90° and then the wrench (spanner) should fit.

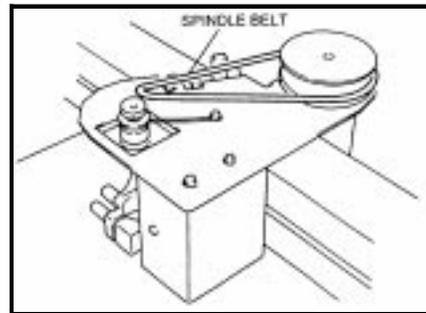
Adjusting Cutting Tip Height

1. Remove the spindle cover.
2. Loosen the spindle hexagonal screw as indicted in diagram 1.
3. Adjust the height by moving the shaft up or down as required.
4. Tighten the screw after adjusted.

If the cutting tip is not making contact with the cutting surface, the cutting tip needs to be lowered. If cutting results in the cutting surface being indented, the cutting tip needs to be raised.



1.

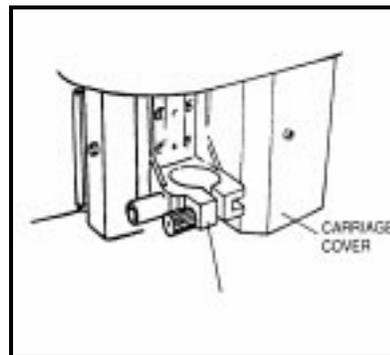


2.

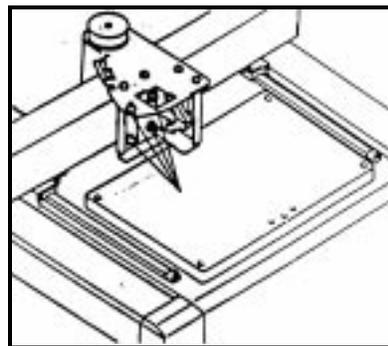
If the shaft will not go any further but the cutting tip still needs to be raised, the carriage needs to be adjusted. Use the following procedure to adjust the cutting carriage.

Adjusting the Cutting Carriage

1. Remove the spindle belt in diagram 2 (previous).
2. Unscrew the ground cable connecting the cutting head to the mounting plate.
3. Remove the spindle cutting assembly by removing the two mounting screws. See diagram 3.
4. Undo the two hexagonal screws holding the carriage cover, remove cover See diagram 3.
5. Loosen the 4 screws fixing the carriage. See diagram 4.
6. Adjust the height as required.
7. Tighten the 4 screws.
8. Place the carriage cover back into position.
9. Mount the cutting assembly using the two screws.
10. Replace the grounding cable, belt, and spindle cover.



3.



4.

8.5 Camera Cover Cap

The camera cover cap is attached to the camera cover by a single hinge. There is a screw located at the center of this hinge which can be tightened or loosened to adjust the tightness of the cap. The system will be delivered with the screw tightened so that the cover cap will remain in place during transportation. If you need to lift the cap to obtain access to the camera, you will need to loosen this screw first.

8.6 Technical Service

For technical assistance with the system including all hardware and software, contact your local Bio-Rad office, or in the US call 1-800-424-6723. All spare parts not listed in this document can be ordered by contacting your local Bio-Rad office.

For inquiries and requests regarding system repair or service, contact your local Bio-Rad office or distributor (in the U.S., call Technical Service at 1-800-424-6723). Please have the following details available:

1. Instrument model and catalog number.
2. Serial number (located on the back of the system).
3. Hardware and software version information.

8.7 PROTEAN 2-D Spot Cutter Warranty Information

This warranty statement may vary outside of the continental United States. Please contact your local Bio-Rad office for the exact terms of your warranty.

Bio-Rad Laboratories warrants to the customer that the PROTEAN 2-D Spot Cutter system (catalog number 165-7005) will be free from defects in material and workmanship and will meet all of the performance specifications for a period of one year from the date of shipment. This warranty covers all parts and components of the instrument except those such as gaskets, o-rings, batteries, and other items of normal wear that require frequent replacement. This warranty does not cover items broken or damaged, including glass or platinum electrodes, by improper handling or use.

If any defects should occur during this period, Bio-Rad Laboratories will either replace or repair the defective parts free of charge. For the exact terms of warranty, please see the Instrument Warranty Card.

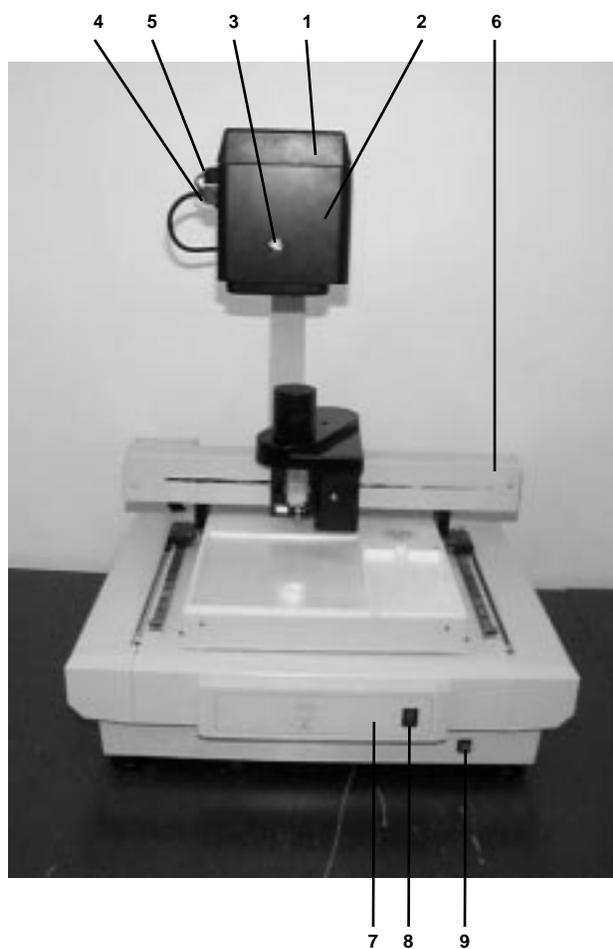
In the event that the system must be returned to the factory for repair under warranty, the instrument must be packed and returned in its original shipping container. If this is not available, contact Bio-Rad Technical Service for instructions.

Bio-Rad shall not be liable for any incidental, special or consequential loss, damage or expense, directly or indirectly arising from use of the PROTEAN 2-D Spot Cutter system. Bio-Rad makes no warranty whatsoever in regard to products or parts furnished by third parties, such being subject to the warranty of their respective manufacturers. Service under this warranty shall be requested by contacting your nearest Bio-Rad office.

This warranty does not extend to any instruments or parts thereof that have been subject to misuse, neglect, or accident, or that have been modified or serviced by anyone other than Bio-Rad or its representative, or that have been used in violation of Bio-Rad instructions. It also does not extend to instruments or parts thereof that have been used with fittings or other spare parts not authorized by Bio-Rad Laboratories, that are interfaced to inappropriate external devices, that have been exposed to inappropriate solvents, cleaning agents or samples. The warranty also does not cover instrument damage resulting from facility problems such as power surges.

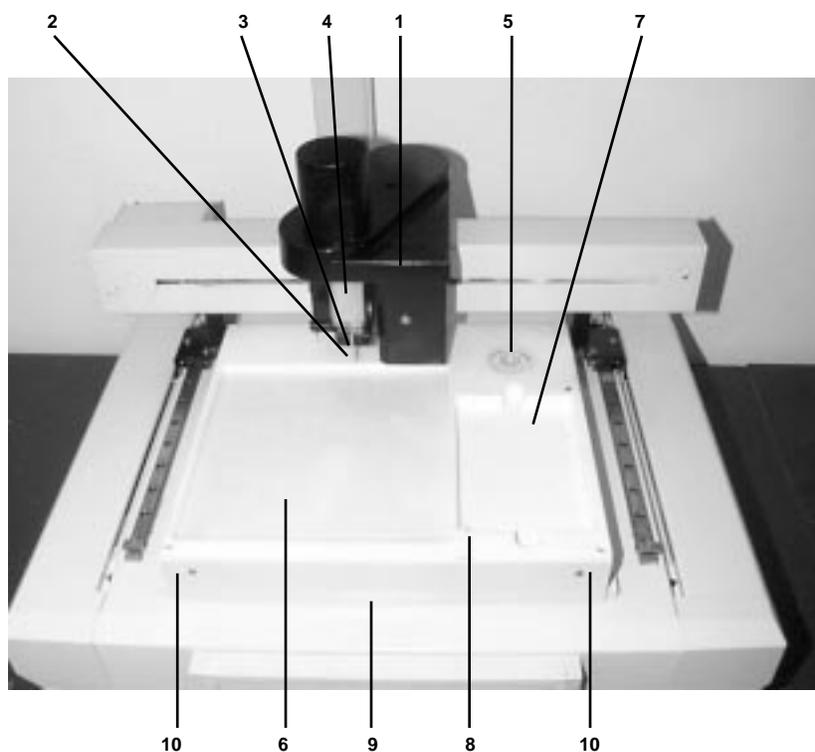
The foregoing obligations are in lieu of all other obligations and liabilities including negligence and all warranties of merchantability, fitness for a particular purpose otherwise expressed or implied in fact or by law, and state Bio-Rad's entire and exclusive liability and buyers exclusive remedy for any claims or damages in connection with the furnishing of goods or parts, their design, suitability for use installation and operation. Bio-Rad Laboratories will in no event be liable for any special, incidental or consequential damages whatsoever, and Bio-Rad's liability under no circumstances will exceed the contract price for the goods for which liability is claimed.

Appendix A System Box (Front)



1. Camera Cover Cap
2. Camera Cover
3. Camera Mounting Screw
4. Camera Communications Cable Plug
5. Camera power cable Plug
6. X-Y Movement Bar
7. Start/Pause Button
8. On/Off Switch
9. Light Push Button

Appendix B Working Surface



1. Cutting Mechanism Cover
2. Cutting Tip
3. Cutting Tip Holder
4. Cutting Head Shaft Cover
5. Bubble Level
6. Cutting Surface
7. Microtiter Plate Well
8. Microtiter Plate Positioning Pin
9. Light Front Cover Panel
10. Front Cover Panel Screws

Appendix C System Box (Back)

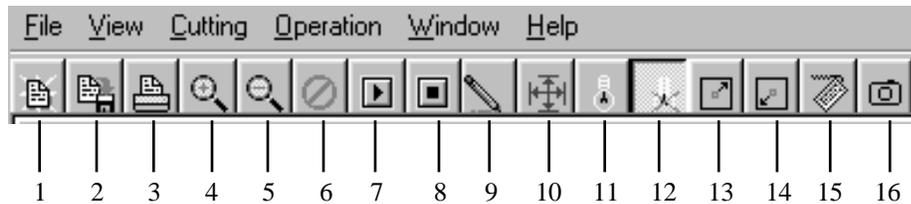


1. Voltage Selector
2. Main Power Plug
3. Fuse for Voltage Settings
4. System Communication Port
5. Camera Power Plug
6. Camera Communications Port
7. Camera Mounting Arm
8. Ground Plug

Appendix D

The Shortcut Button Menu Bar

The following figure shows the Shortcut Button Menu Bar.



Button 1 is the **Open Image File** shortcut button.

Button 2 is the **Save Image File** shortcut button

Button 3 is the **Print** shortcut button

Button 4 is the **Zoom In** shortcut button.

Button 5 is the **Zoom Out** shortcut button.

Button 6 is the **Stop Process** button. This button may be pressed at any time during a cutting run to stop the run.

Button 7 is the **Run** shortcut button.

Button 8 is the **Pause** button. This button may be pressed at any time during a cutting run to pause that run. Once a run has been paused to recommence, press the pause button once.

Button 9 is the **calibration** shortcut button.

Button 10 is not active in this version of the software.

Button 11 is the **Light On** shortcut button.

Button 12 is the **Light Off** shortcut button.

Button 13 is the **Move to Park Position** shortcut button.

Button 14 is the **Move to Home Position** shortcut button.

Button 15 is the **Enable Arrow Keys** shortcut button.

Button 16 is the **Photo Enhancer** shortcut button.

Glossary

Box	Refers to a dialog box. This is a box (window) which pops up onto the screen containing dialog to be read, and then requires a button to be pressed, e.g. "OK" or "Yes" to progress any further in the program.
Click	Unless otherwise specified, this refers to a single left button mouse click on the desired menu or item.
Drop Down List	Is a list of selections that appear below a list box when the list box is selected.
Drop Down Menu	Refers to a list of menus which appear below a specific menu when it has been selected.
Folder	When there is more than one screen that can be selected inside of a window, each screen is called a folder. Each folder contains different data concerned with the window's specific function.
List Box	A list box is a text field which contains a drop down list from which a selection can be made.
Panel	A panel is a segment inside a window or folder containing specific information. It usually contains at least one text field or list box.
Press	Unless otherwise specified, this refers to a single left button mouse click on the desired menu or item.
Select	Unless otherwise specified, this refers to a single left button mouse click on the desired menu or item.
Spot	A spot is a position within the image which has been selected to be cut.
Text Field	Is an area into which text can be entered.
Window	Is either - <ul style="list-style-type: none">(i) Similar to a box (dialog box) except it contains text fields and folders which can be manipulated, or(ii) A view window which contains a picture.

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Canada Ph. (905) 712-2771, Fx. (905) 712-2990 **China** Ph. 86-10-62051850/51, Fx. 86-10-62051876 **Denmark** Ph. 45 39 17 99 47, Fx. 45 39 27 16 98
Finland Ph. 358 (0)9 804 2200, Fx. 358 (0)9 804 1100 **France** Ph. 01 43 90 46 90, Fx. 01 46 71 24 67 **Germany** Ph. 089 318 84-0, Fx. 089 318 84-100
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