ProteomeWorks™ Spot Cutter System with PDQuest Basic Excision Software

Instruction Manual

Catalog Number
165-7009

BIO·RAD

Safety Information

Caution Symbol

Read the manual before using the ProteomeWorks Spot Cutter. For technical assistance, contact your local Bio-Rad or, in the U.S., call technical services at 1-800-4BIORAD (1-800-424-6723).

Definition of Symbols

Caution (refer to instruction manual).

Notice

This instrument is intended for laboratory use only.

This product conforms to the "Class A" standards for electromagnetic emissions intended for laboratory equipment applications. It is possible that emissions from this product may interfere with some sensitive appliances when placed nearby or in the same circuit as those appliances. The user should be aware of this potential and take appropriate measures to avoid interference.

This Bio-Rad instrument is designed and certified to meet EN-61010 safety standards. Operation of this product is subject to the condition that no harmful radio interference is caused and that any interference must be accepted.

EN-61010 certified products are safe to use when operated in accordance with the instruction manual. This instrument should not be modified in any way. Alteration of this instrument will:

• Void the manufacturer's warranty.

• Void the EN-61010 safety certification.

• Create a potential safety hazard.

Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than those for which it is intended, or by modifications of the instrument not performed by Bio-Rad or an authorized agent.

Table of Contents

Section 1
Introduction

1.1 General Description

The ProteomeWorks Spot Cutter System is a precision instrument for the accurate location, excision and loading into microtiter 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 Components Checklist

The ProteomeWorks Spot Cutter System is shipped in two boxes under the catalog order number 165-7009. The spot cutter instrument and accessories box is catalog number 165-7040 and the Camera System box is catalog number 165-7029.

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 to 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 and level surface.

Note: DO NOT move the system 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 port	1 serial port is required
	Add PCI Interface card (included with spot cutter)
	2 Mb Video RAM
Operating system	Windows 98, NT, or 2000

1.6 Cutting Excision Sizes

Nominal Tip Size:

1.5 mm internal diameter:	Supplied with spot cutter
1.0 mm internal diameter:	Supplied with spot cutter

1.7 Power Setting

Nominal input voltage range from 100 V to 240 V (50 Hz/60 Hz)

Section 2
Getting Started

2.1 Software Description

The ProteomeWorks Spot Cutter is shipped with PDQuest Basic Excision software for selecting and then cutting spots from 2-D gels and blots. PDQuest software runs the spot cutter in two modes. The Basic Excision Tool is included with the spot cutter and provides the capability for imaging, calibrating, visually selecting spots to cut, cutting them, and tracking the microtiter plate well they were deposited in. With a separate purchase of PDQuest image analysis software, additional functionality is available. The full license provides the ability to use analysis sets to run spot excision, and all spot number tracking is within the PDQuest matchset database. The functions provided in this version of PDQuest allow for image acquisition, calibration, manual spot selection, cutter operation, and data tracking. The excised gel spots are deposited into a microtiter plate and are ready for mass spec identification, sequencing, amino acid composition, or any other individual protein analysis.
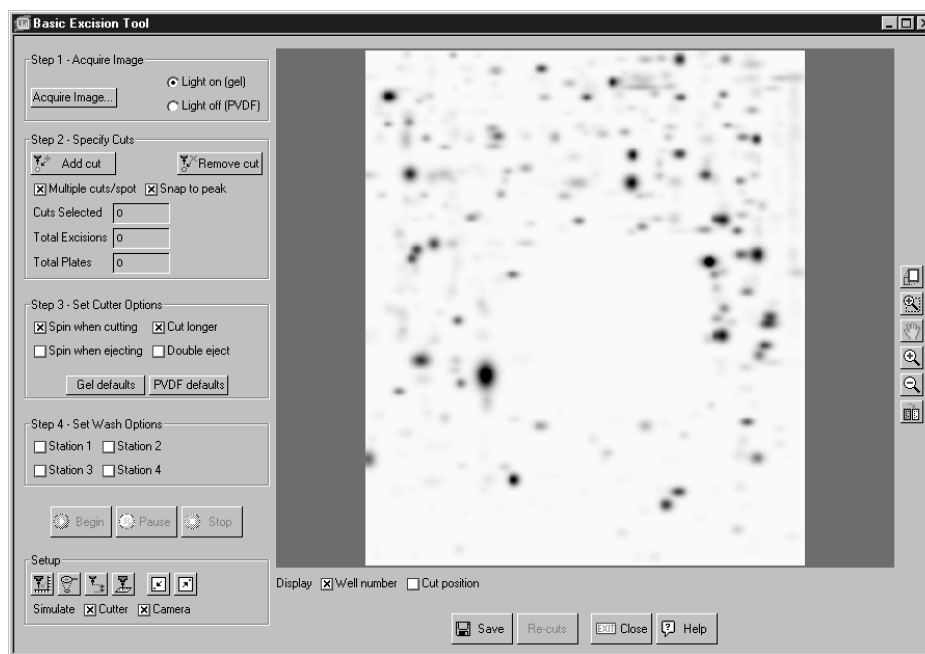
Figure 1. Example PDQuest Basic Excision software screen.

The Discovery Series CD contains two separate software components that work together to operate the ProteomeWorks Spot Cutter: PDQuest operating software, and RS Config camera driver software. Spot Cutter operation requires that all components be installed properly. After installing the software, the Spot Cutter will be operated using PDQuest exclusively. The other software component serves only to support the use of PDQuest with the Spot Cutter.

PDQuest and the supporting software are compatible with Windows 98, Windows NT 4.0, and Windows 2000.

2.2 Installing Camera Driver (PVCAM 2.5.8) and the PCI Card

The following procedure applies to new installations of PDQuest version 7.0 and higher, and the ProteomeWorks Spot Cutter. This is for installations on a system where no other Roper Scientific software exists.

** Important Note: install the <u>camera driver software</u> BEFORE physically installing the PCI card hardware in the computer.

Installation Procedure

1. Insert The Discovery Series / PDQuest install CD in the CD-ROM. The disk will start up and show the "Discovery Series" Window.

2. Select "Explore CD".

3. Select PDQUEST folder.

4. Select CoolSNAP folder
    (Readme file has detailed instructions for installing PVCam Setup)

5. Select PVCamSetup.

    Follow on screen instructions to set up the camera software.

    Select standard installation

    Use the default destination folder location C\: Program Files\Roper Scientific\PVCam

6. When software installation is complete, select "No. I will restart my computer later," remove CD, click finish, and shut down computer.

7. Install the PCI Card in an open PCI slot on the computer mother-board and connect the camera cable.

8. Reboot computer and turn camera on.

9. Run "RSConfig" program (Start\Programs\RoperScientific\RSConfig.) to confirm that camera name is entered. Do not rename the camera in the RSConfig program, leave the default name "Camera 1".

10. Run PVCam test to confirm that camera is functional. Make sure that the camera is on and connected to the PCI Card.

    Start\Program|Roper Scientific\PVCam test.

11. Click on collect image to acquire an image and verify camera functionality.

12. When completed, install PDQuest software as described in the PDQuest manual.

Troubleshooting Camera Driver and PCI Card Installation

The camera driver for the ProteomeWorks spot cutter camera exists on the install disk for the Discovery Series software. Beginning with PDQuest version 7.0, the camera driver software has changed to a new version (version 2.5.8). The installation procedure for new installations has changed significantly from the earlier version of the camera driver (which existed on the PDQuest 6.2.1 version). The new procedure has been included with the spot cutter manual 4006196 beginning with Revision E.

If an existing computer running a ProteomeWorks Spot Cutter is to be upgraded to PDQuest version 7.0 or higher, only the new version of PDQuest needs to be loaded. This will overwrite the existing version of PDQuest, but the existing camera driver will work with PDQuest 7.0 and higher. There is no need to load the camera driver that exists on the install disk with PDQuest 7.0 or higher.

If you have one of these cases:

- An older PVCAM driver needs to be uninstalled, and the new driver re-installed.

- Communication error with the camera after new install (error code 33 is the usual communication error).

- If the PCI card was installed before the camera driver software in a new installation.

Then, use the following procedure to un-install and re-install the camera software. You will need to UNINSTALL the old driver and PCI card completely from the system. Follow the steps below to ensure a clean installation.

✔ Shut down the computer

✔ Remove PCI card from the slot and reboot computer.

✔ Use the Windows "Add/Remove Programs" feature (Start\Settings\Control Panel) to uninstall the Roper Scientific HCK. Say "Yes" to delete all files.

✔ The "Add/Remove Programs" feature will not remove all files. You need to also manually remove the following files. Use the Windows "Search/Find" feature (Start\Find\Files or Folders) to locate the files. Search the entire computer. If you find any of the following files, delete them before installing PVCAM 2.5.8.

Windows 98	Windows NT and 2000
1. photopci.vxd	1. pmpcint.sys
2. pvcam.ini	
3. pvcam.inf	
4. pvcam32.dll	

✔ Once the PCI Card and the appropriate files have been removed go back to step 1 for installing the new PVCAM software.

2.3 Installing PDQuest Software

All Windows Versions

1. When the computer is booted with the Discovery Series CD inserted into the drive, the "Autorun" feature within Windows should automatically display the Discovery Series CD screen. If this does not occur, please use the "Start" menu to select "Run". Click on the "Browse" button on the dialog box. This will bring up a standard "Open" file dialog. Choose the CD-ROM drive, select the "Setup" application, and then click on "OK" to close the "Open" file dialog. Finally click on "OK" to run the "Setup" application.

2. While viewing the Discovery Series CD screen, click on the "Install Software" button to display a group of buttons to install the individual programs within the Discovery Series.

3. Click on the "Install PDQuest" button to run the installation program for PDQuest . This application will guide you through the installation of the PDQuest, simply follow the directions throughout the process.

2.4 Spot Cutter Setup

*   Remove the Spot Cutter instrument and accessories from the shipping container. **Do not lift the instrument by the camera arm**.

*   Remove the metal packing bars from the feet, place the instrument on a flat surface and level the instrument using the leveling feet.

*   Carefully remove the cutting tip protector and tape.

*   Remove the two "L" Shaped brackets from the system X, Y arm, there is one bracket located at each end of the X, Y arm.

*   Move the X, Y arm forward to access and remove the third L bracket located on the back of the X, Y arm and the cutting head assembly.

2.5 System Description
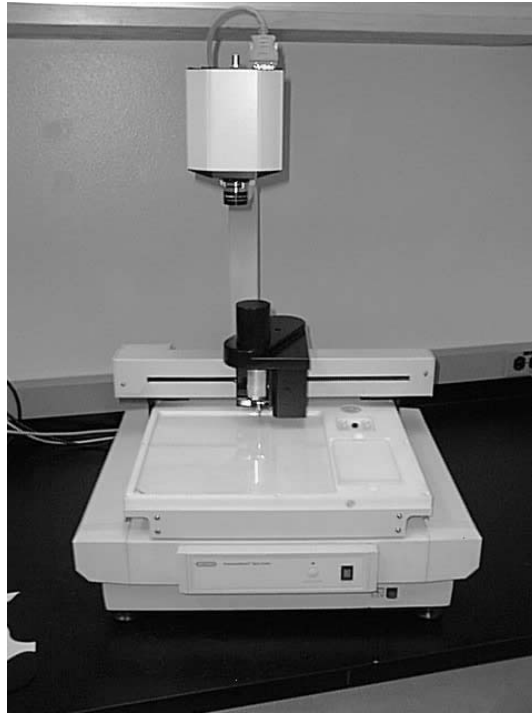
Fig. 2. The ProteomeWorks Spot Cutter.

*   The Spot Cutter
    Bench footprint of 50 x 50 cm with a curving camera support arm holding a camera over the platform of the work surface. Under platform lights used for gel imaging, an x,y arm supporting the cutting head mechanism, the gel or PVDF cutting area, a microtiter plate holding area, wash well area containing 4 wash tubes.

*   The Camera
    Cooled CCD camera, 1392x1040 imaging pixels, 12 bit ADC

*   Working Surface

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

• Cutting Surface

The cutting platform dimensions are 25 x 25 cm. The cutting tip can access an area of 22 cm x 20.5 cm within this 25 x 25 cm area. The cutting tip range will cover the protein resolving area of any commercially available gel type. The extra platform area was designed to accommodate the entire gel without the need for cutting the gel to fit the cutting platform. The diagram below shows the orientation of the cutting area within the platform. Gels should be positioned against the right wall of the cutting platform, next to the microtiter plate holder, and centered vertically in the platform.

Figure 3. Cutting Platform and Cutting Area.

• Cutting Mats

Two cutting mats are provided with the system. The cutting mats provide for a flexible surface beneath the gel to allow for complete excision of the gel spots. The 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 165-7019. To extend the life of the cutting mats, and to make gel handling easier, disposable Gel Cutting Sheets are available, catalog number 165-5018.

• Gel Cutting Sheets, catalog number 165-7018. Gel Cutting Sheets are thin plastic sheets that are positioned between the cutting mat and the gel in the cutting area. This prevents scoring by the cutting tip and extends the life of the cutting mat. The Gel Cutting Sheets can also be used to lift gels out of storage trays to minimize damage to gels from excessive handling. (If a Gel Cutting Sheet will be used during gel excision make sure that the cutting tip height is adjusted accordingly. See Section 3.5 "Set Cutting Tip Height".)

- Gel-holding bars

  Two custom designed stainless steel Gel-holding Bars are included in the accessory box with the spot cutter. These gel holding bars are used to immobilize the Coomassie stained PVDF membrane during the X, Y calibration procedure, as well as for general gel excision processing. While the cutting tip does not impart any orthogonal force to the gel, correct placement of these Gel-holding bars on the gel prevents any gel movement during the gel excision process.

  Position the bars resting on the outer edge of the gel or membrane. It is best to position them to the left side and bottom of the gel so they are out of the cutting tip travel path.

2.6 Camera Mount Installation

- Remove the camera mount and mounting screws from the Camera System box (catalog number 165-7029).

- The camera mount is bolted to the end of the camera arm. Position the camera mount on the camera arm of the spot cutter and use the 6 mm hex wrench from the accessories box to secure the camera mount onto the camera arm with the large hex screw.

- Tighten the screw to snug, but do not overtighten. Some mobility is needed for the camera alignment procedure.

Fig. 4. Camera mount.

2.7 Camera Installation

- Remove the camera from the Camera System box and remove the blue protective film from the front of the camera.

- Remove the 8 mm lens from the Camera System box.

- Use the red handled 2 mm hex driver in the accessories box to tighten the 2 mm hex screw in the silver locking collar of the lens (if it is loose) before mounting it on the camera.

- Screw the lens into the C-mount fitting on the camera until snug.

- Attach the camera to the camera arm by placing the camera against the ridge of the camera mount. Secure the camera to the mount with the camera screw through the slot in the camera mount.

2.8 Spot Cutter Setup

• Connect the PC cable. Connect the 9-pin connector to the Com 1 serial port of the computer and the 25 pin connector to the system communications interface located in the back panel of the Spot Cutter.

• Connect the AC power cord to the Spot Cutter instrument.

• Connect the camera power and communications cable to the PCI interface port on the PC and the connector on the camera top. Secure the cable through the clips on the back of the camera arm.

2.9 Power "ON' Startup Sequence

It is important for the correct sequence of devices to be powered on. The computer must be turned on and fully booted up before the spot cutter is turned on. If the spot cutter is on first, or if the computer is re-booted while the spot cutter is on, several commands will not be communicated. The symptoms are: lights not turning on, and cutting tip not spinning with cut.

When powering up the spot cutter system (including computer) use the following sequence:

1. Turn on the computer and let it boot up fully to its operating system.

2. Do not open PDQuest yet.

3. Turn on the spot cutter (switch the front black I/O switch to I) and push the yellow "Start/Pause" button. Let the spot cutter run through its home position routine until it returns to the back right position (park position).

4. Turn on the camera with the black rocker switch on top of the camera. The green light on the top indicates the power is on.

5. Open PDQuest and go to either Basic Excision Tool, or Excision Gel Selection.

Fig. 5. Rear panel connections.

Light toggle switch

Socket for UV light plug

Mains power connection

25 pin serial connection

2.10 Software Features

To open Basic Excision Tool:

- Double click on the PDQuest icon in the desktop.

- Select the menu item "Identification", then the drop down menu item "Basic Excision Tool".
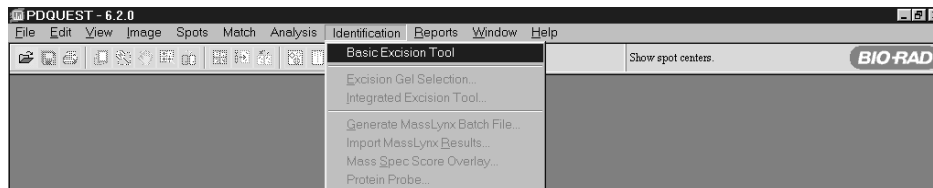

Fig. 6. Basic Excision Tool menu selection.

Select Basic Excision Tool from the Identification menu. The software will connect with the spot cutter platform and camera, and the Basic Excision Tool dialog box will open.

The Basic Excision Tool includes a window for viewing the spot cutter camera image and a control panel for running the spot cutter.

Before you can use the spot cutter for the first time, you must focus the camera and adjust the cutting tip height. You must also adjust the spot cutter settings and calibrate the instrument.

The control panel is designed to guide you through the spot cutting procedure. Click on the link for more information about each step.

- Step 1. Acquire Image. In this step, you capture an image of your gel or membrane using the spot cutter camera.

- Step 2. Specify Cuts. In this step, you identify the spots in the image that you want to cut.

- Step 3. Select the Cut Run Options. These options can be adjusted for each cut run.

- Step 4. Set Wash Options. Select the wash stations to use after each cut by clicking on one or more checkboxes.

Performing a Cut Run

After you have identified all the cuts you want to make and selected the appropriate options, click on the Begin button. You will be prompted to place a microplate in the plate holder.

As each cut is being made, it will be highlighted on the screen. After each cut has been made, the cut circle appears in white on the screen.

Click on Pause to pause the cut run. Note that the cutter will pause only after the most recent cut has been deposited in a well.

Click on Stop to stop the cut run. The cutter will stop only after the most recent cut has been deposited in a well. Any remaining cut requests will remain selected in the image.

When all cuts are complete, the cutting tip will return to home position and you will receive an alert message on the screen. At this point, you can review the cut deposits in the microtiter plate. If all the wells are filled, you can proceed with saving the image.

Recutting Spots

If the cutting tip has not picked up a spot, click on the Recuts button below the image window. In the pop-up box, enter the numbers of your recut requests (microplate number, well number), separated by semicolons. Click on OK to close the recut request dialog, then click on Begin to perform the recuts.

Acquiring the Image

Before acquiring an image using the spot cutter camera, select the Light On option if you are imaging a gel, or Light Off if you are imaging a PVDF membrane. (The platform light should be off for PVDFs, because the ambient light will produce a better image.)

Click on Acquire Image. The camera will take a single image of the gel or membrane on the platform, and the image will appear in the window.

Zoom and Transform

On the right hand side of the screen are the zoom controls. The first icon on the top is used to return the image to full view. The second icon down is used to magnify a region specified by click-dragging a box around the region. The third icon down (the drag hand) grabs the image to move to different locations within the image. The fourth icon down zooms in centered on the placement of the icon, the fifth zooms out in the same way. The transform icon is the last in the column of icons, and is used to adjust the brightness and contrast of the image.

Specifying Cuts

In this step, you will click on spots in the image to identify which spots to cut.

When clicking on spots in the image, select the Snap to Peak checkbox to automatically center the cut circle on the spot peak (calculated from the spot intensity in the image). You can also use the Zoom Box tool included in the Basic Excision Tool to magnify each spot.

To make a single cut in a spot, make sure the Multiple Cuts/Spot checkbox is unchecked, then click on the Add Cut button {insert add cut.gif} and click on the spot in the image that you want to cut.

If this is the first cut circle marked on the image, the Enter Plate Information dialog box will open, and you will be prompted to enter a name for the microplate and identify the first well you want to load. If you specify a starting well other than the default of A1, the skipped wells will be noted in the dialog box. Click on Done to make your selections.

A green circle will appear on the image, indicating the spot to be cut. Note that the circle is the size of the actual cutting tip (1.0 mm or 1.5 mm, as specified in the Spot Cutter Settings dialog box).

Continue clicking on spots in the image with Add Cut selected to make additional cuts. The number of cuts will appear in the Cuts Selected field.

If you specify more cuts than there are wells in the microplate, you will be prompted to name a new plate and starting well for subsequent cuts.

Multiple Cuts of the Same Spot

To place multiple cuts of the same spot in the same microtiter plate well, make sure the Multiple Cuts/Spot checkbox is checked, click on the Add Cut button, then drag a box over the spot. Multiple cutting circles will appear grouped inside a red box. All these cuts will be placed in the same well.
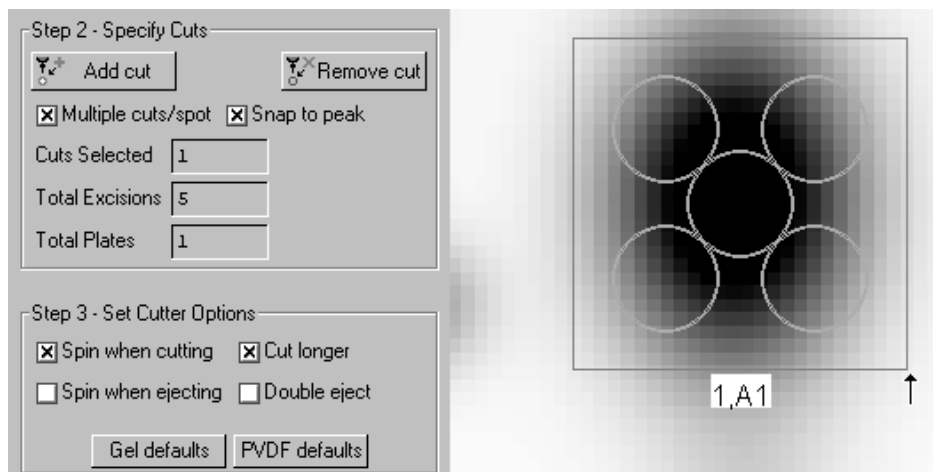
Note: If the box you create contains too few cutting circles, simply drag over the area again to create a bigger box.

To place multiple cuts of the same spot in different wells, make sure the Multiple Cuts/Spot checkbox is unchecked, then click on several locations inside the spot to create individual cuts of the same spot. Drag these circles so that they touch but do not overlap.

Note: Overlapping circles will result in incomplete cuts that may not be fully extracted by the spot cutter.

To move multiple circles inside a box, with Add Cut selected, position your cursor inside the center circle in the box. The appearance of the cursor will change to a multidirectional arrow. Drag the box to a new location and release the mouse button.

Moving a Cut Circle

To move a cut circle, make sure that Add Cut is selected, then position your cursor inside the cut circle. The appearance of the cursor will change to a multidirectional arrow. Drag the circle to a new location and release the mouse button.

Removing Cuts

To remove a cut circle from the image, click on the Remove Cut button, then click on the cut circle, or position your cursor on the spot and right-click. You will be asked to confirm the deletion. To remove multiple cut circles, click on the Remove Cut button, then click on the cut circles, or drag a box around the circles.

Display Options

Click on the Well Number checkbox (below the image window) to display the well number associated with each cut request.

Click on the Cut Position checkbox to display the position of the cut (in millimeters) on the gel.

Cut Run Options

Before beginning a cut run using the Basic Excision Tool, you must select the cutter options for that run.

If Spin When Cutting is selected, the cutting tip will spin as it cuts.

Spin When Ejecting will cause the cutting tip to spin as it ejects the cut into a microplate well.

Cut Longer will cause the cutting tip to hold in the "down" position longer when making a cut.

Double Eject will cause the cutting tip to emit two blasts of air when ejecting a cut into a microtiter plate well.

The Gel Defaults button automatically selects the most useful options when cutting gels. The PVDF Defaults button automatically selects the most useful options when cutting PVDF membranes.

Focus Cutter Camera

The Focus Cutter Camera dialog box controls the camera exposure setting. Focusing and aligning the camera are described in Section 3 of this manual.

Click on the Focus Cutter Camera button to open the dialog. The platform light on the spot cutter will turn on and the camera will focus on a small region at the center of the gel or membrane and begin taking continuous exposures. The exposures will appear in the Focus window at the rate of about 2 per second.

At the top of the dialog, click on Auto-Expose to reset the exposure time of the camera.

The Time field shows the time of each exposure in milliseconds. The Ceiling field shows the highest pixel value in the focused region. The Levels field shows the levels of gray scale in the image. All of these settings are automatically determined by the software, based on the position and aperture of the camera lens.

Manually adjust the camera lens and position as described in the hardware manual to optimize the image. When you are satisfied with the image and the levels of gray scale, click on Stop to close the Focus dialog.

Set Cutting Tip Height

Click on the Set Cutting Tip Height button to adjust the distance the cutting tip drops down to cut a spot. The adjustment dialog box will open, and the cutting tip will move to the center of the gel.

Click on Lower Tip to drop the cutting tip to its lowest position. In this position, manually adjust the cutting tip height as described in Section 3.5 until it presses lightly against the surface of the cutting mat.

Raise Tip raises the cutting tip. Test Cut performs a test cut in the center of the gel. Click on Done to accept the settings and return the cutting tip to home position.

The cutting tip height only needs to be set once, unless the spot cutter gets out of alignment.

Spot Cutter Settings

Click on the Spot Cutter Settings button {insert settings.gif} to open the settings dialog box for the cutter.

In the dialog box, select the communications port to the spot cutter by clicking on COM 1 or COM 2.

Specify the microtiter plate size you are using by clicking on 96 wells or 384 wells.

Specify the diameter of the cutting tip by selecting 1.0 mm or 1.5 mm. Note that the cut request circles that you make on the image reflect the actual size of the cutting tip, and will change based on the tip size selection.

Specify the order in which you want to the load the wells on the microplate by selecting A1, A2, A3, … or A1, B1, C1, …

Crop Left Margin repositions the image in the Basic Excision Tool window to the left. Enter a value (in screen pixels) in the field to reposition the image. Note that you must click on Done to close the settings dialog and then acquire a new image for this change to take effect.

Well A1 position

The fields and buttons in this section are used to calibrate the position of the microplate for proper loading of samples. The number fields contain the X and Y coordinates (in millimeters) of well A1 on a plate in the spot cutter plate holder. Once you have set the position of this well, the cutter robot will automatically "know" the positions of the remaining wells.

To set the well A1 position, position your cursor over the number in the X or Y coordinate field and press Enter on your keyboard twice. The tip will move to that position without attempting to lower into the well. If the tip is not centered directly over the well A1 on the microplate, you will need to adjust the numbers in each field.

You can either type a new value directly in each field, or (for small adjustments) use the arrow buttons to adjust the tip by fractions of a millimeter. The cutting tip will move as you either enter new numbers or click on the buttons.

Note that the arrow buttons are oriented as if you were facing the front of the spot cutter; also, if you are facing the cutter, higher numbers in the first field will move the cutting tip to the right, while higher numbers in the second field will move the cutting tip toward the back of the instrument.

When you are satisfied with the position of the cutting tip, click on Test to lower the tip into the well. The tip should be centered inside the well.

Park returns to the tip to its home position.

Wash Station 1 position

Calibrate the position of Wash Station 1 on the cutting platform by following the steps described above for Well A1. Once you have set the position of station 1, the remaining wash station positions are automatically calibrated.

Spot Cutter Calibration

Before you can begin excising spots, you must calibrate the spot cutter. This procedure requires Spot Cutter Gel Sheets and a coomassie blue stained PVDF membrane.

Note: Before calibration, you should focus the cutter camera and align the wash station position. You should make sure the spot cutter camera image is properly centered using the Crop Left Margin setting.

Click on the Calibrate Cutter button. A series of dialog boxes will guide you through the calibration procedure. At any point in the procedure you can move back to repeat a step.

Note: Calibration creates several parameter and image files in the Install.prm directory in the Discovery Series directory on your hard drive. These files should not be moved, deleted or altered, or calibration will be affected.

When calibration is complete, you can proceed with normal use of the spot cutter. Once the cutter is calibrated, you do not have to repeat the procedure during normal day-to-day use. You may have to recalibrate if the instrument is moved or if the camera is moved.

Other Spot Cutter Controls

Click on the appropriate button to move the cutting tip to the front left corner of the spot cutter or the back right corner.

You can simulate the action of the cutter and its camera by clicking on the Simulate Cutter and Simulate Camera checkboxes. These checkboxes will cut off communication with the cutter and camera if selected. In simulation mode, you will be prompted to open a file image of a gel in place of the camera cutter image.

Section 3
Calibration

3.1 Camera Calibration: Lens Aperture Setting

The aperture setting on the lens is a black ring on the lens with a small black thumbscrew attached. There are aperture settings marked on two sides of the lens. The white mark on the ring indicates the aperture setting. F-stop setting 1.4 is maximum open for the lens; f-stop 11-16 is the maximum closed for the lens (16 is not marked but is past 11). See Figure 7 for open and closed aperture settings. To adjust the aperture setting, loosen the thumbscrew, move the ring to the desired setting and gently tighten the thumbscrew. Tightening the thumbscrew is optional; the aperture setting does not drift but can be accidentally changed if it is touched.
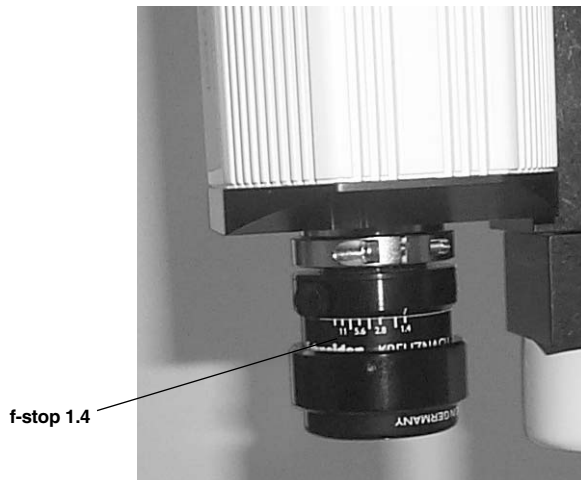

f-stop 1.4

f-stop 16

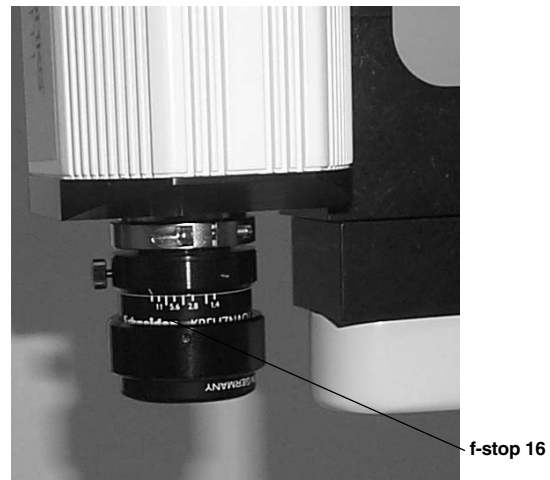**Fig. 7. Aperture at f-stop 1.4 for UV light mode.**   **Fig. 7a. Aperture at f-stop 16 for white light mode.**

3.2 Camera Calibration: Focus

The lens on the camera should be locked in place once it is focused. To accomplish this, the lens has a silver lock collar that is tightened after focusing is completed. Focus using the following steps.

- Loosen the 2mm hex screw in the silver lock collar of the lens with the red handled 2 mm hex driver.

- Loosen the black aperture thumbscrew

- Set the aperture to the open setting of f-stop 1.4

- On the back portion of the spot cutter instrument, behind the cutting platform, is a black toggle switch. This toggle switches the power to the white lights on or off. It is not the mains power switch. Switch the toggle to the lights off position. Check that the lights are off by pushing the red "Lights on" button on the lower front right side of the spot cutter. If the platform lights come on, switch the toggle switch to the other position.

- Take the cutting mat, and any other materials, off the cutting platform.

- Click the "Focus Cutter Camera" icon from the Basic Excision Tool window.

- Click the "Auto-expose" button. The software will reset the exposure time of the images. Click "OK" when "Auto-expose completed". Do not click "Stop".

- Put the cutting mat and the Bio-Rad Focusing Target or the Calibration Template on the cutting mat for focusing.

- You will see a zoomed in portion of the center of the platform in continuous acquire mode in the window.

- Center the Bio-Rad Focusing Target so that you can see the fine lines that are close together. Focusing the lens will resolve these lines from one another and sharply focus on the lines.

- To focus, physically turn the lens and watch both the image in the window and the "Focus levels" box. The image should resolve the lines and bring the image into sharp focus. The "Focus levels" box is a gauge of the gray scale across the image. When the focus is at the best focus, the "Focus levels" number should reach a bottom plateau. This is meant as a guide rather than a definite number point.

- When the lens is focused, hold the lens in place with one hand and tighten the 2 mm hex screw in the silver lock collar. This will hold the lens in the focus position.

- Click "Stop" on the "Focus cutter camera" window.

- If you are proceeding to Camera Alignment, you can keep the light setting at the same "lights off" position for the camera alignment steps.

3.3 Camera Alignment—Rotation & Centering

(Tools needed: one 6 mm and one 3/16 inch mm hex driver to loosen screw in camera mount)

3.3.1 Rotation

- Place the Calibration Template on the cutting mat in the cutting platform. If the Focus Cutter Camera window is already acquiring images, you do not need to close and reopen.

If it is not already on, click on the Focus Cutter Camera icon in the Basic Excision Tool screen. If the image is over exposed, check the light and aperture settings. If the settings are correct, take the cutting mat off the platform, click on "Auto-expose" and then click "OK" when "Auto-expose completed". Place the cutting mat and Calibration Template back on the cutting platform and proceed.

- If the red and black grid lines are parallel, proceed to the centering procedure. If the red and black grid lines are not parallel, proceed to the next step.

- Loosen the bottom camera mount screw slightly to be able to rotate the camera.

- Move the camera until the black and red lines are as parallel as possible.

- Tighten the bottom mounting screw while holding the camera in the correct position. The lines should remain parallel.

3.3.2 Centering

- Replace the Calibration Template with the Camera Target Template (contains three parallel line).

- Click "Focus Cutter Camera" button. The camera image shows three parallel lines that run across the cutting platform parallel to the X arm. The center horizontal red line must be lined up with the center line in the camera target.

- Loosen the set screw that slides the bracket of the camera mount to allow for proper camera alignment.

- Slide the camera mount until the two center lines overlap.

- Tighten the set screw while maintaining the camera position.

- Click the "Stop" button to stop the focus mode.

3.3.3 Switch to White Light Imaging

When changing back to using a white light illuminated image, which is suggested for the X crop step and regular gel imaging, use the following steps to insure the best image quality.

- Switch the light toggle switch to the "lights on" position. Test the lights by pushing the red "Lights on" button on the lower front right side of the spot cutter. If the platform lights do not come on, switch the toggle switch to the other position.

- Change the aperture setting to f-stop 11–16.

- Take the cutting mat, and any other materials, off the cutting platform.

- Click the "Focus Cutter Camera" icon from the Basic Excision Tool window.

- Click the "Auto-expose" button. The software will reset the exposure time of the images. Click "OK" when "Auto-expose completed".

- If the cutter has not been calibrated yet, click on the "Calibrate Cutter" icon. Perform Step 1 of the calibration. This step contributes to setting the optimum light balance for acquiring an image on the spot cutter. When it goes to Step 2, click "Cancel" unless you are ready to proceed with calibration. The information for Step 1 has been saved in the calibration file.

- Put the cutting mat and the next template on the cutting mat. If cutting a gel, remember to use the Gel Cutting Sheets to protect the cutting mat.

3.4 Camera Alignment—X Crop

- Place calibration template on cutting platform and click "Acquire Image" button.

- Evaluate the image and determine proper cropping. Look for a black border on the left and right sides of the image.

- To change the image positioning, click "Spot Cutter Settings" button. In the "Spot Cutter Settings" window the value for the "Crop left margin" can be changed. Change the setting by ~20 units to reposition the image. An increase in unit value moves the image to the left, a decrease in the unit value moves the image to the right.

- Click the "Done" button in the "Spot Cutter Settings" window each time a new value is entered before acquiring a new image.

- Acquire Image after each change. Continue until the image is centered, *i.e.*, a black border is visible on the left and right sides of image.

3.5 Setting Cutting Tip Height

- Place the cutting mat and protective gel sheet on the cutting platform.

- Click the "Set Cutting Tip Height" icon in the Set up area of the "Basic Excision Tool" dialog box to move the cutting head to the middle of the cutting platform and the "set cutting height" dialog box appears on the screen.

- From the "Set Cutting Tip Height" dialog box, use the "lower head" command to assess how far down the cutting tip goes. The cutting tip should lightly push down onto the protective gel sheet. If the cutting tip is too high off the cutting mat or too far down into the cutting mat adjust the cutting tip height.

- The adjustment is performed while the cutting head is in the Set Cutting Tip Height location, *i.e.*, in the center of the platform. This allows for quick testing of the setting.

✔ Adjusting Cutting Tip Height
Click on the "Set Cutting Tip Height" icon to move the cutting head to the middle of the cutting platform. From the "Set Cutting Tip Height" icon, use the "Head Down" command to assess how far down the cutting tip goes. Make sure a cutting mat and gel cutting sheet are included to adjust the height correctly. If it is visibly off the cutting surface, adjust the tip down as described next. The adjustment can be performed with the instrument "on" and in place in the Set Cutting Tip Height location. Steps for Adjusting Cutting Tip Height. Cutting tip should be in "Head Down" position.

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

When the tip depth looks correct, use the "Test Cut" function to test the cutting depth. A PVDF membrane on the platform works well for this test. The membrane needs to be wetted as described for best cutting capability.

- To test for correct cutting tip height make sure the cutting tip is in the raised position, click on "Raise Head".

- Place a scrap piece of wetted PVDF membrane under the cutting tip. Click the "Test Cut" function to test the cutting depth. The membrane should be cut through and the piece picked up in the cutting tip.

3.6 Calibration of the Microtiter Plate (MTP)

3.6.1 Calibration of MTP A1 Position

The microtiter plate holder is to the immediate right of the cutting surface. 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.

Note: 384 well plates and 96 well plates are oriented with the A1 in the position top right position.
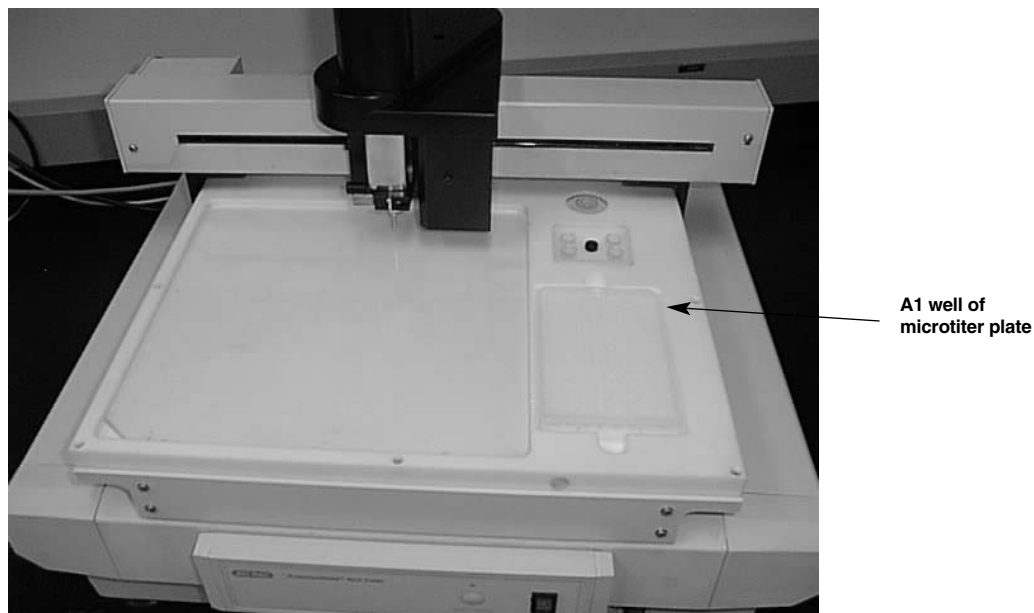

A1 well of microtiter plate

Fig. 8. A1 position for microtiter plates on spot cutter.

- 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 and push the microtiter plate down into the holder.

- Click "spot cutter settings" icon in setup area of the "Basic Excision Tool" dialog box.

- Select microtiter plate size, 96 well or 384 well. Different calibration settings are needed for each plate type. The software will save the last used calibration setting.

- To calibrate the microtiter plate position, place the cursor behind the value shown in either the x or y position box under "Well A1 position" and press the Enter key on the keyboard (the A1 position is in the upper right corner of the holder). This will move the cutting tip to the A1 position of the microtiter plate without lowering the cutting tip into the well, to prevent potential damage to the cutting tip in cases of severe misalignment.

- Visibly assess where the cutting tip is in relation to the A1 well. The number values in the boxes represent the x, y coordinates on the cutting platform (1 unit = 1 mm). Change the x, y coordinates to position the cutting tip to the center of well A1. When entering a new value, press return to move the cutting tip to the adjusted coordinates. To move the cutting tip towards the front of the platform, decrease the Y value; to move towards the back of the platform, increase the Y value. In order to move the cutting tip to the right, increase the X value; to move to the left, decrease the X value. See the Figure below for a description of the calibration movements.

- When the cutting tip is centered over the well, click the "Test" button to assess the clearance into the microtiter plate well. Once the tip is set to the A1 well, the interwell dimensions are already in the software, so the tip is calibrated for the entire plate. Press the "Done" button to save the calibration changes.
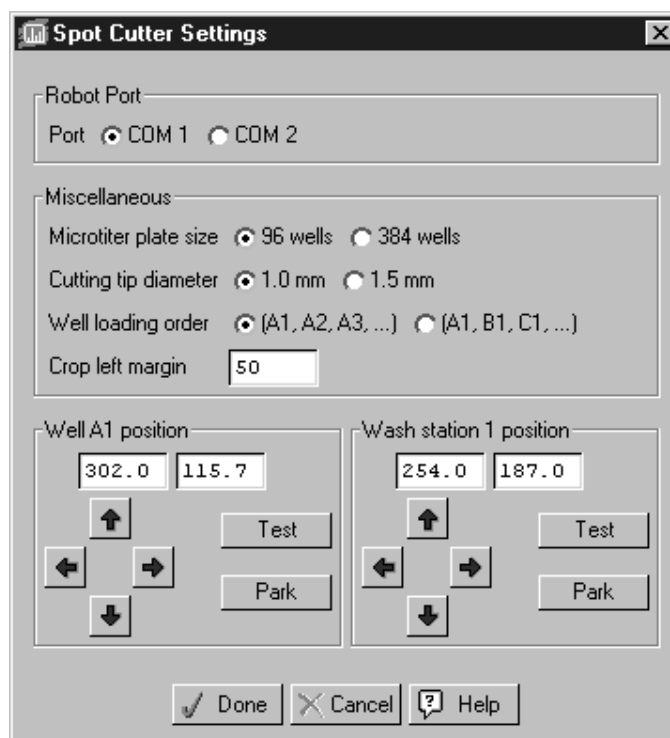

Figure 9. Calibration of Microtiter Plates.

3.6.2 Assignment of Microtiter Plate Deposit Sequence

There are two different deposit sequence modes into the microtiter plate, 1) deposit sequence A1, B1, C1, D1, etc. is indicated as "by number" or, 2) A1, A2, A3, A4, etc. is indicated as "by letter". The last used mode will be saved for the next operation.

- Click "spot cutter settings" icon in setup area of the "Basic Excision Tool" dialog box.

- Select the desired deposit sequence.

  This deposit sequence A1, B1, C1, D1, etc. is designed to accommodate partial plate filling in preparation for digestion in the Micromass MassPrep robot. This instrument uses 4 liquid delivery pipettes designed to work down the plate in sets of 4 from A1-D1, then E1-H1, A2-D2, etc. The PDQuest Spot Cutter software automatically assigns the spot numbers to the well locations, so there is no possibility of error in tracking the location in the microtiter plate.

3.7 Calibration of the Wash Well Position

The wash wells are an optional item in the sequence of spot cutting. If they are checked in the software screen for use, the spot cutter will cut the target, move to the microtiter plate, deposit, then go to the designated wash tube, lower, spin, eject, and raise up again, then move to the next wash station or back to the target. This option provides additional methods to prevent any sample contamination.

The wells are designed to accommodate a 1.5 ml capless micro test tube, such as the Bio-Rad catalog number 223-9500.

- Click "spot cutter settings" icon in setup area of the "Basic Excision Tool" dialog box.

- To calibrate the wash well position, place the cursor behind the value shown in either the x or y position box under "wash well position" and press the Enter key on the keyboard. This will move the cutting tip to the first wash well position without lowering the cutting tip into the well, to prevent potential bending of the cutting tip in cases of severe misalignment.

- Visibly assess where the cutting tip is in relation to the first wash well (the first wash well is the top left of the 4 wash wells). The number values in the boxes represent the x, y coordinates on the cutting platform (1 unit = 1 mm). Change the x, y coordinates to position the cutting tip to the center of well A1. When entering a new value, press return to move the cutting tip to the adjusted coordinates. To move the cutting tip towards the front of the platform, decrease the Y value; to move towards the back of the platform, increase the Y value. In order to move the cutting tip to the right, increase the X value; to move to the left, decrease the X value. The up/down and right/left arrow keys are used for fine adjustments of the x, y coordinates (one click = 0.1 mm)

- When the cutting tip is centered over the well, click the "Test" button to assess the clearance into the wash well. Once the tip is set to the first wash well, the interwell dimensions are already in the software, so the tip is calibrated for all four wash wells. Press the "Done" button to save the calibration changes.

3.8 X, Y Calibration of Cutting Platform

The interaction between the software, the computer, the camera and the spot cutter requires an initial calibration to bring these parts into alignment. The calibration process is designed to be very software driven with the user loading templates and confirming to the software that a step is ready. Then the software completes the step, and at the end the system is calibrated and ready for use. The calibration process should be run at initial setup, if the instrument is moved, or if there is significant changes in the accuracy over time. The instrument has not exhibited any drift in settings, so normal use should maintain system accuracy for over 6 months to 1 year.

To calibrate the x, y coordinates a 24 x 24 cm Coomassie stained PVDF membrane is needed.

3.8.1 Prepare Coomassie Stained PVDF Membrane

• Cut a piece of PVDF membrane to 24 x 24 cm from a roll, Bio-Rad catalog number 162-0177. (PVDF is preferred over nitrocellulose for calibration because it is not prone to wrinkling and shrinkage as is nitrocellulose)

• Wet the PVDF in 100% methanol in a tray, large enough for the PVDF to lay flat. Allow the membrane to lightly shake or rock for ~2 minutes.

• Add water until it is approximately a 50% methanol solution and shake gently for approximately 5 minutes.

• Remove the 50% methanol solution, and add undiluted Coomassie R-250 solution, Bio-Rad catalog number161-0436, to cover the membrane. Continue gentle shaking and turn the membrane over a few times. Allow the membrane to stain to a uniform medium to dark blue color.

• Remove the Coomassie solution and wash with water until excess Coomassie stain is removed. (**Do not use destain solution**).

• Pour off the wash water and add fresh water to keep the membrane completely wetted. Save membrane for appropriate step in the calibration procedure.

3.8.2 Calibration of X, Y Coordinates

• From the "Basic Excision Tool" dialog box in PDQuest Spot Cutter software, select the "Calibrate Spot Cutter" icon.

• Follow the onscreen directions for the calibration procedure.

• Before confirming the start of the calibration step using the PVDF membrane, put the cutting mat on the platform, add a Gel Cutting Sheet and place the Coomassie stained PVDF membrane on top of the gel cutting sheet. Make sure that all pieces are lying flat and are completely seated inside the cutting platform (no bubbles or high spots). Place the stainless steel Gel Holding Bars on the top and bottom of the PVDF membrane. The bars should be pushed against the platform edge. Misplacement of the Gel Holding Bars will interfere with the cutting tip during calibration and can lead to damage to the cutting tip.

• Cover the PVDF with water by filling the cutting platform area with water to completely cover the membrane. The water level should be a pool but not to the top of the cutting platform edge.

• Fill a disposable Wash Tube with water and place it in the #1 well of the Wash Station in the platform. The cutting tip will clean itself after each cut of the PVDF membrane in this one wash tube. This is important for most efficient cutting.

- Press the onscreen start key to begin the calibration process.

- The Spot Cutter will proceed to cut 25 spots in an array of 5 rows with 5 spots per row.

- Once all 25 spots are cut, the system will automatically take an image of the PVDF membrane with the 25 cut spots and calibrate itself.

- A dialog box will appear stating that the calibration is successfully completed. The system is now ready for gel spot cutting. Press OK and the process is complete.

The calibration will remain in place for many months of routine spot cutting. However, if there is excessive vibration in the lab, or on the lab bench, from instruments other than the spot cutter, or if the spot cutter and/or camera is moved or bumped, evaluate the accuracy of the cutting. If it is unacceptable, repeat the calibration procedure.

Note: If Calibration fails.

Calibration will fail if not all 25 spots are cut. Uncut spots are due to either insufficient water on the PVDF membrane, or the cutting tip not cutting deeply enough. Evaluate whether more water was needed and/or cutting tip adjustment is needed. If many of the holes did not have score marks or were barely scored, adjust the cutting tip depth to slightly down from the present position. See Section 3.5 for cutting tip height adjustment. Then restart the calibration procedure. Follow the onscreen directions and note that you can skip the steps leading up to the PVDF cutting step. If the holes have still not been cut completely, call Bio-Rad technical service for troubleshooting.

Section 4
Spot Excision

4.1 Gel and Blot Preparation

- Storage of gels can be in any type of container or plastic that keeps the gel protected and hydrated until they are needed for spot cutting. Before cutting the gel, it is best to equilibrate the gel in water. This will avoid any swelling that may occur from water being added to the gel on the cutting platform. 1–2 hours to overnight is sufficient to equilibrate the gel. When equilibrating, bring the gel to room temperature if they have been previously stored in the refrigerator. This minimizes the chances of swelling during cutting.

4.2 Transfer of Gel or Blot to Cutting Platform

- Place the excision mat and gel cutting sheet on the cutting platform of the Spot Cutter.

- Place the gel or membrane on the gel cutting sheet. (The gel cutting sheet can be used to transfer the gel from the storage container to the cutting platform.) Position the gel or membrane against the right side of the cutting platform to maximize the cutting range.

- Position the Gel-holding Bars on the edges of the gel or membrane partly resting on the cutting mat and partly resting on the edge of the gel.

- Add a sufficient amount of water to the surface of the gel to form a shallow pool (do not fill to the top of the platform). For optimum gel cut pick up rate, make sure there is a sufficient amount of water during the excision process.

- If the spot pickup rate is less than 95% of spots picked up first time, try less water on the gel. Reduce the amount of water added to a film of water on the gel - enough to keep the gel hydrated, but less than a "pool" of water. This may improve the cutting pickup rate. For more information on improving the pickup rate, see the Troubleshooting section.

4.3 Set-up PDQuest Basic Excision Tool Software

The PDQuest software contains the Basic Excision Tool module the Basic Excision Tool manages all the operations of the spot cutter including, image acquisition, calibration, manual spot selection, cutter operation and data tracking. (A detailed description of the fully integrated features with PDQuest image analysis software is discussed in the PDQuest user's manual.)

Open Basic Excision Tool:
- Double click on the PDQuest icon in the desktop.

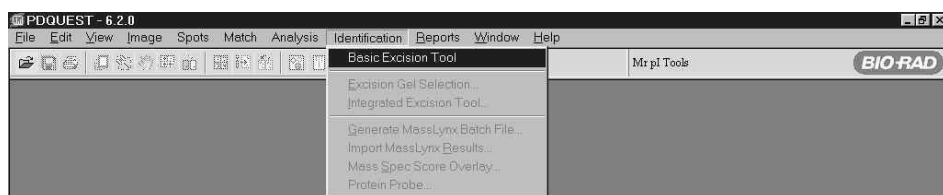- Select the menu item "Identification", then the drop down menu item "Basic Excision Tool".

Fig. 10. Basic Excision Tool menu selection.

- **Step 1: Acquire Image**

    There are two default options for acquiring an image, Light ON, for gels; and Light OFF for PVDF. For image acquisition of a gel the platform lights are on. For image acquisition of PVDF the platform lights remain off since ambient light produces a better image for PVDF.

- **Step 2: Specify cuts**

    This step activates the vertical arrow cursor tool for adding and removing cuts, allows for the selection of multiple cuts, and has the snap to peak feature.

    Adding a single cut.

    To add a single cut move the vertical arrow cursor to the center of the selected spot and left click to designate a spot to be cut. Using the zoom feature facilitates this process. First, zoom into the specified area, then activate the cursor tool. Once a spot is added, the add spot cursor tool changes to a 4-way arrow when placed over a selected spot. Click and move the cursor to re-position the spot. Each selected spot will be annotated with the microtiter plate well designated, and the x, y coordinates of the cutting platform, if selected.

    Snap to peak

    Snap to peak activates a pixel peak finding function within the software. This centers the cutting tip placement on the darkest pixel area of the spot regardless of exactly where on the spot the cursor is clicked, which may reflect the densest portion of the protein spot.

    Microtiter plate well number

    When the first spot selection is made, a dialogue box appears that asks for the starting well number. The default is A1, but allows any starting well number to be entered. This function allows a single microtiter plate to be more efficiently filled in order to optimize downstream digestion robot use.

    Adding multiple cuts.

    Multiple cuts can be selected for a single spot in two different ways. When the multiple cut box is activated, the add cut tool can be used click-drag a box which defines a number of cuts within the box. Click and drag the cursor over the spot to be cut. The software calculates how many cuts can be made in the boxed area and marks each cut with a green circle. All the cuts made with a click-drag are assigned to a single microtiter plate well. This allows the user to harvest a large amount of protein from a single spot and contain it in a single well.

    If multiple cuts from a single spot are desired in different microtiter plate wells, individually select the cuts to default them to the next microtiter plate well. The multiple cut box is not activated in this case, so that several individual cuts can be positioned within a single spot. Each cut from the multiple cuts thus selected for a single spot are deposited into separate microtiter plate wells.

24

Removing a cut (or multiple cuts from the boxed area)

Activate the "Remove cut" icon, place the cursor over a selected cut and click. The remaining cuts will not be re-numbered, but the next cut added is designated to the open microtiter plate well. When removing multiple cuts within a boxed area simultaneously, place the remove cut icon directly over the center cut and click.

Cuts Selected, Total Excisions, Total Plates

These headers provide feedback on the items listed.

Cuts selected denotes the number of cuts selected. Multiple cuts within a boxed area of multiple cuts is counted as a single cut. In other words, cuts selected also refers to the number of microtiter plate wells that will be filled.

Total excisions tracks the number of cuts made as the run progresses. This number should equal the number of cuts selected when the run is completed.

Total plates counts the number of microtiter plates filled.

- **Step 3 - Set Cut Options**

  The spot cutter has several cut and eject options. Any, or all four options can be selected for cutting and ejecting of cut spots. The recommended defaults for Gels and PVDF membranes can be selected by clicking on the appropriate "default" button. It is left to the user to customize for specific applications.

  The most effective cutting method is to "cut with turn". Optimum setting for ejecting the gel spot is either single or double eject. Single eject is effective in most situations. For PVDF membrane cuts, use the eject with turn to help spin the PVDF spot off the stainless steel tip. For both gels and PVDF membranes it is advisable to keep water on the sample during cutting, and to add liquid to the microtiter plate wells.

- **Step 4 - Set Wash Options**

  This feature allows the selection of up to four optional wash well stations for the cutting tip. The wash stations are selected by clicking a check in the box next to the station. The orientation of the tubes is as it is listed on the software screen. Once the cutting tip has deposited a cut into a microtiter plate well, it will then move to the selected wash well(s). The cutting tip lowers into the cleaning solution of the wash tube(s), spins and ejects to clean the tip, and proceeds to the next cut.

Well number and cut position

Immediately below the image display area the well number and cut position spot annotation can be activated. Each selected spot on the display can be annotated with the microtiter plate well number and or the x, y coordinates of the cut position.

Begin, Pause and Stop Icons

Press the "Begin" icon to start the cutting process after the spots have been selected.

Press the "Pause" icon to pause the run after the cutting tip deposits the cut in progress in its respective microtiter plate well. The system will pause until the "Resume" button in the pause dialog box is clicked.

The "Stop" button will cancel the run after the cutting tip deposits the cut in progress in its respective microtiter plate well. The screen will give an opportunity to confirm cancel before actually canceling the run.

4.4 Starting the Spot Excision Process

✔ Press the "Begin" icon to start cutting the selected spots on the gel or membrane.

✔ When the spot excision run is completed, evaluate if any cuts were missed. If so, click the "re-cut" icon to enter the microtiter plate well numbers of the missed cuts, and click the "begin" icon, to start cutting the missed cuts.

✔ The Save option allows the gel image with spot numbers to be saved, as well as an excel formatted table listing the spot number, image coordinate location, and microtiter plate well number.

✔ Click on the "close" icon to close the Basic Excision Tool screen. PDQuest remains open.

Section 5
Maintenance and Adjustments

✔ When the System is not in operation, cover the instrument with the dust cover provided. This prevents dust from collecting on the instrument.

✔ 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.

✔ Replacing cutting platform lights.
The cutting surface lights are located directly beneath the cutting surface and consist of four 24W fluorescent tubes. Appendix B has the diagrams showing the location of the fluorescent bulbs and the location of covers that need to be removed. The lighting front cover must be removed first by removing the four front cover screws and the center platform screw. Once the cover has been removed, the required fluorescent tube(s) are gently pulled out and then replaced by pushing the replacement tubes into position. After the tube(s) are changed the front cover is re-placed.

✔ Fuses
The main power fuse is located on the system box interface plate, refer to Appendix C for exact location. The fuse for operation is a 3.15A fuse. Two of these fuses have been supplied with the System.

✔ Cutting Tip Cleaning
The cutting tip is made of high quality 303/304 stainless steel. If properly maintained, the cutting tip will retain its cutting ability through repeated use. Replacement is needed if the cutting tip has been deformed or if the spot pick-up rate is unsatisfactory even after cleaning and cutting tip height adjustment.

If the pick-up rate is unsatisfactory, 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".

✔ Changing Cutting Tips
Click the "Set Cutting Tip Height" icon to move the cutting head to the middle of the cutting platform, or by turning off the spot cutter and pushing the cutting head to the empty cutting platform or empty microtiter plate area. Use the two wrenches (spanners) supplied with the system. The customized wrench is used to hold the cutting tip holder by fitting into the grooves located on the cutting tip holder. The open ended wrench will then fit the top of the cutting tip. Turn the wrench clockwise to remove the tip. A new tip can then be inserted and turned counter-clockwise to install. Use the same wrenches to tighten the cutting tip. **Do not** over-tighten.

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

✔   Adjusting Cutting Tip Height

Click on the "Set Cutting Tip Height" icon to move the cutting head to the middle of the cutting platform. From the "Set Cutting Tip Height" icon, use the "Head Down" command to assess how far down the cutting tip goes. Make sure a cutting mat and gel cutting sheet are included to adjust the height correctly. If it is visibly off the cutting surface, adjust the tip down as described next. The adjustment can be performed with the instrument "on" and in place in the Set Cutting Tip Height location. Steps for Adjusting Cutting Tip Height. Cutting tip should be in "Head Down" position.

1. Loosen the spindle hexagonal screw as indicted in diagram 1.

2. Adjust the height by moving the shaft up or down as required.

3. Tighten the screw after adjusted.

When the tip depth looks correct, use the "Test Cut" function to test the cutting depth. A PVDF membrane on the platform works well for this test. The membrane needs to be wetted as described for best cutting capability.

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

✔ Adjusting the Cutting Carriage

1. Remove the black Cutting Head Cover.

2. Remove the spindle belt, as in diagram 2.

3. Unscrew the ground cable connecting the cutting head to the mounting plate.

4. Remove the spindle cutting assembly by removing the two mounting screws. See diagram 3.

5. Undo the two hexagonal screws holding the carriage cover, remove cover. See diagram 3.

6. Loosen the 4 screws fixing the carriage. See diagram 4.

7. Adjust the height as required.

8. Tighten the 4 screws.

9. Place the carriage cover back into position.

10. Mount the cutting assembly using the two screws.

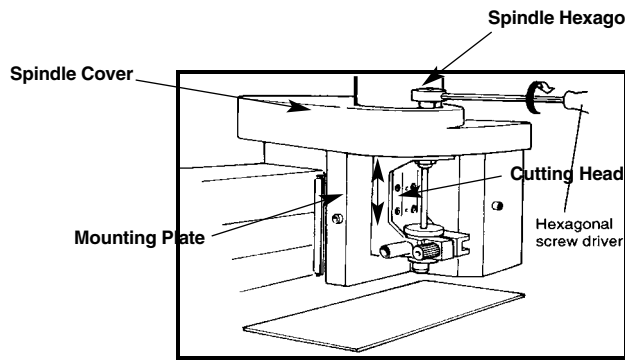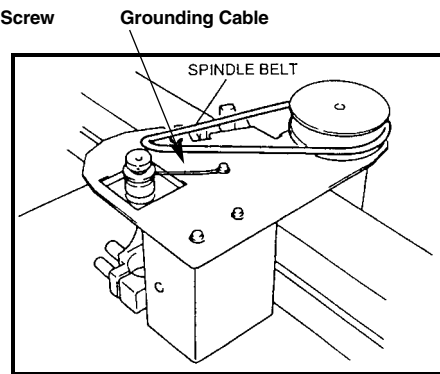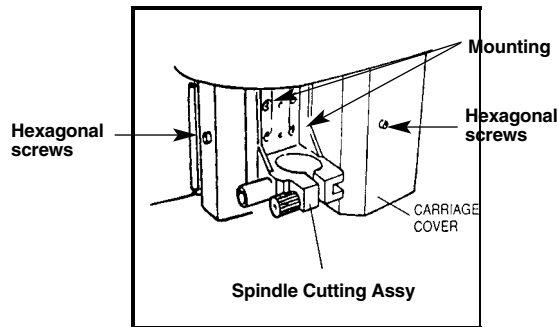11. Replace the grounding cable, belt, and spindle cover.

Spindle Hexagonal Screw   **Grounding Cable**

Spindle Cover

Cutting Head

Mounting Plate

Hexagonal
screw driver

SPINDLE BELT

Diagram 1   **Diagram 2**

Mounting

**Hexagonal
screws**

**Hexagonal
screws**

CARRIAGE
COVER

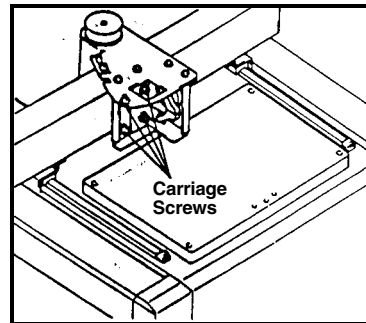**Carriage
Screws**

Spindle Cutting Assy

Diagram 3   **Diagram 4**

✔ 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.

Section 6
Troubleshooting

Communication Error

If the computer cannot make communication connection with the spot cutter or the camera, an error message will appear reading, "Cutter and/or camera not responding. Use 'simulation' to diagnose communication error. Would you like simulation?"

This means that one or the other (or possibly both) devices have not made communication with the computer. If either part fails, this message will appear. Answer "Yes" to this question and then use the "Simulate" check boxes to determine which device (spot cutter or camera) is not communicating.
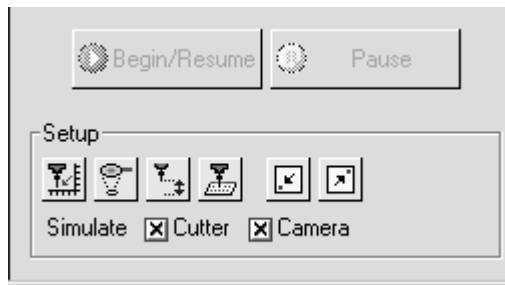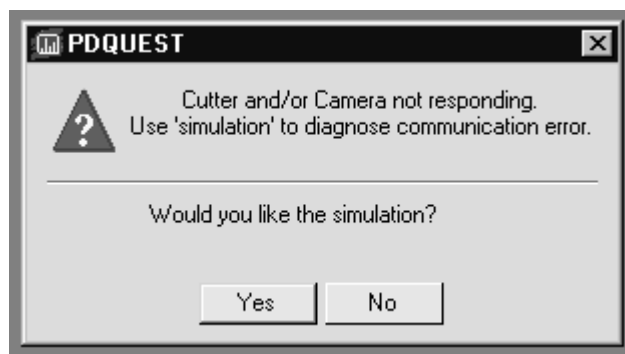
Click on the X in the "Simulate Cutter" box. If it de-selects the box, then the computer has made connection. If it comes back with the message: "Unable to initialize cutter. Please make sure it is on and connected." Then the spot cutter is not making connection with the computer.

Then, click on the "Simulate Camera" box. If it de-selects the box, then the computer has made connection. If it comes back with the message: "Unable to initialize cutter. Please make sure it is on and connected." Then the camera is not making connection with the computer.

Error code 53 is also an indication that the Camera is not connected or the drivers have not been installed.

Things to check if the Spot Cutter is not communicating:

- Check for Spot Cutter power on. The front panel black I/O switch should be switched to "I".
- Check that Pause/restart button was pushed to reset the spot cutter
- Spot cutter must be started after the computer has booted up.
- Serial cable securely attached

Things to try:

1. Close PDQuest. Turn off power to the spot cutter, then turn on spot cutter and press Pause/restart button to initialize. Open PDQuest, go to Identification, Basic Excision Tool, and look for communication connection.

2. Keep PDQuest open. Turn off power to the spot cutter, then turn on spot cutter and press Pause/restart button to initialize. Click on the "Simulate Cutter" box to deselect. If it deselects the X, then it has made connection.

3. Turn off Spot Cutter, close PDQuest, and Shut Down computer (do not just restart – but do a complete shutdown). Turn on computer, and let it boot all the way to Windows or Mac OS. Turn on the spot cutter, then press Pause/restart button to initialize. Open PDQuest, go to Identification, Basic Excision Tool, and look for communication connection.

Things to check if the Camera is not communicating:

• Camera not turned on

• Camera cable not plugged into the computer or the camera

• PCI card not properly installed, or camera name not entered in PCI setup. If you suspect that the PCI card is the problem, go to Section 2.2 of this manual for the software set up section to review the procedure for installing the camera software and drivers.

Poor image quality – shadows or overexposed.

Re-set the exposure time and light level. Use the following steps for white light imaging.

• Switch the light toggle switch (on the back of the ballast box of the spot cutter) to the "White lights on" position. Test the lights by pushing the red "Lights on" button on the lower front right side of the spot cutter. If the platform lights do not come on, switch the toggle switch to the other position.

• Change the aperture setting to f-stop 11–16.

• Take the cutting mat, and any other materials, off the cutting platform.

• Click the "Focus Cutter Camera" icon from the Basic Excision Tool window.

• Click the "Auto-expose" button. The software will reset the exposure time of the images. Click "OK" when "Auto-expose completed".

• If the cutter has not been calibrated yet, click on the "Calibrate Cutter" icon. Perform Step 1 of the calibration. This step contributes to setting the optimum light balance for acquiring an image on the spot cutter. When it goes to Step 2, click "Cancel" unless you are ready to proceed with calibration. The information for Step 1 has been saved in the calibration file.

Lights not turning on during image acquisition

• Switch the light toggle switch (on the back of the ballast box of the spot cutter) to the "lights on" position. Test the lights by pushing the red "Lights on" button on the lower front right side of the spot cutter. If the platform lights do not come on, switch the toggle switch to the other position.

Lights not turning on during image acquisition and cutting tip not turning during cut

- The spot cutter and computer "power on" sequence were done in the incorrect order. The computer needs to be on and fully booted up before turning on the spot cutter. Use the following steps to return the spot cutter to full communication mode.

- Close PDQuest (whichever spot cutter screen you are in). Turn off the power on the spot cutter (switch the front black I/O switch to O). Wait a few seconds. Turn on the spot cutter (switch the front black I/O switch to I) and push the yellow "Start/Pause" button. Let the spot cutter run through its home position routine until it returns to the back right position (park position).

- Open PDQuest and go to either Basic Excision Tool, or Excision Gel Selection. Full communication to all commands should be restored now.

Spot Cutting and Pickup

In the best conditions, the mechanism of cutting is very reproducible. Normal operation produces cutting and pickup efficiency at >95% of the spots cut. For any spots not picked up on the first cutting pass, the software allows recutting of specific spots based on microtiter plate well number. The second cutting pass generally picks up any spots not picked up the first time.

Spot Cut Pick Up Rate

Acrylamide gels are effectively cut with the spot cutter when they are laying on the cutting mat or the Gel Sheet. It is also effective at cutting gels which are bonded to a plastic backing. Varied results have been obtained using gels bonded to glass plates.

If the pickup rate is less than 95 spots out of 100 cuts, please review the wetting process, the cutting tip depth and cleanliness, and the level of the instrument. These are the most common sources of poor pickup rates.

a. The wetting instructions are listed in the Gel Preparation section before this section.

b. Cutting tip depth should be adjusted so that the cutting tip is going just below the gel and scoring into the Gel Sheet below the gel. If the tip is not going down far enough or is going down too far, adjust the cutting tip height as described in Section 3.5 "Setting Cutting Tip Height".

c. The cutting tip should be cleaned daily after spot cutting. This consists of taking the cutting tip off the assembly and rinsing with water, as from a squirt bottle. If the pickup rate on spots is decreased or less than 95% of cut spots, a more aggressive cleaning can be used. Prepare a detergent solution. Soak the tip for a few minutes to overnight. Scrape the inside of the tip with a metal probe (a straightened paper clip works well) to dislodge any acrylamide that may have stuck to the inside of the tip.

d. Using the Wash Wells. The wash wells may help improve the pickup rate.

e. The bubble level on the instrument is used to assess the level of the cutting platform. If the instrument is off level, one portion of the gel may dry out more quickly than another and can contribute to poor cutting and pickup efficiency.

If all the settings seem to be correct, contact Bio-Rad for further troubleshooting.

In depositing spots to the microtiter plate, it is important to prepare the plates with liquid in the wells. While deposit of the gel pieces does not depend on liquid in the wells, the reproducibility of accurate deposits is improved with this condition. The liquid can be the solution used as the first step in the digestion process, or any other liquid used in typical lab conditions. The stainless steel cutting tip and polypropylene microtiter plates are compatible with a wide variety of chemicals.

Technical Service

For technical assistance with the system including all hardware and software, contact your local Bio-Rad office. 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. 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.

ProteomeWorks 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 ProteomeWorks Spot Cutter

System (catalog number 165-7009) 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 ProteomeWorks 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)
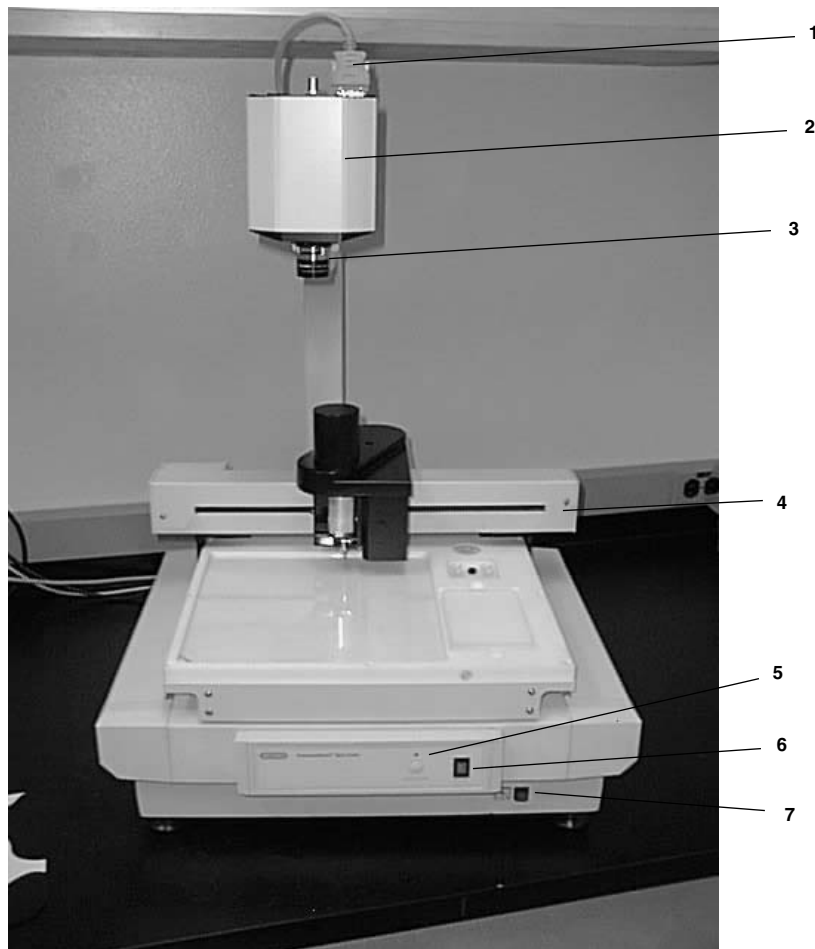
1. Camera Communication Cable

2. Camera

3. Camera Lens

4. X-Y Movement Bar

5. Start/Pause Button

6. On/Off Switch

7. Light Push Button

Appendix B

Working Surface
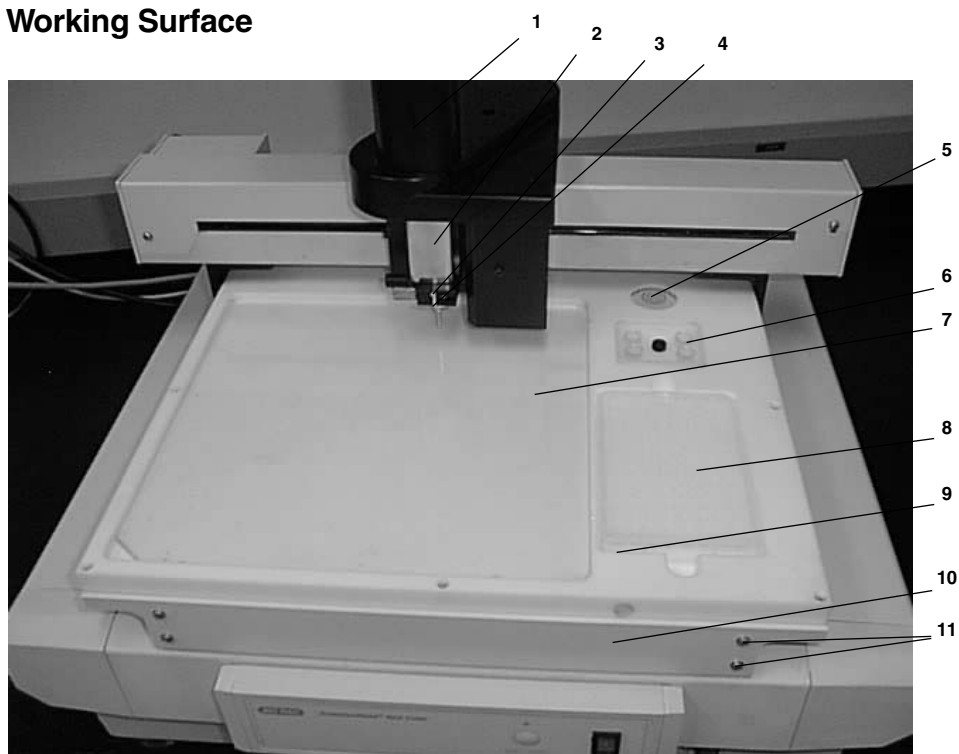
1. Cutting Mechanism Cover
2. Cutting Head Shaft Cover
3. Cutting Tip
4. Cutting Tip Holder
5. Bubble Level
6. Wash Well Station, positions 1–4
7. Cutting Surface
8. Microtiter Plate Well holder
9. Microtiter Plate Positioning Pin
10. Light Front Cover Panel
11. Front Cover Panel Screws

Appendix C

System Box (Back)
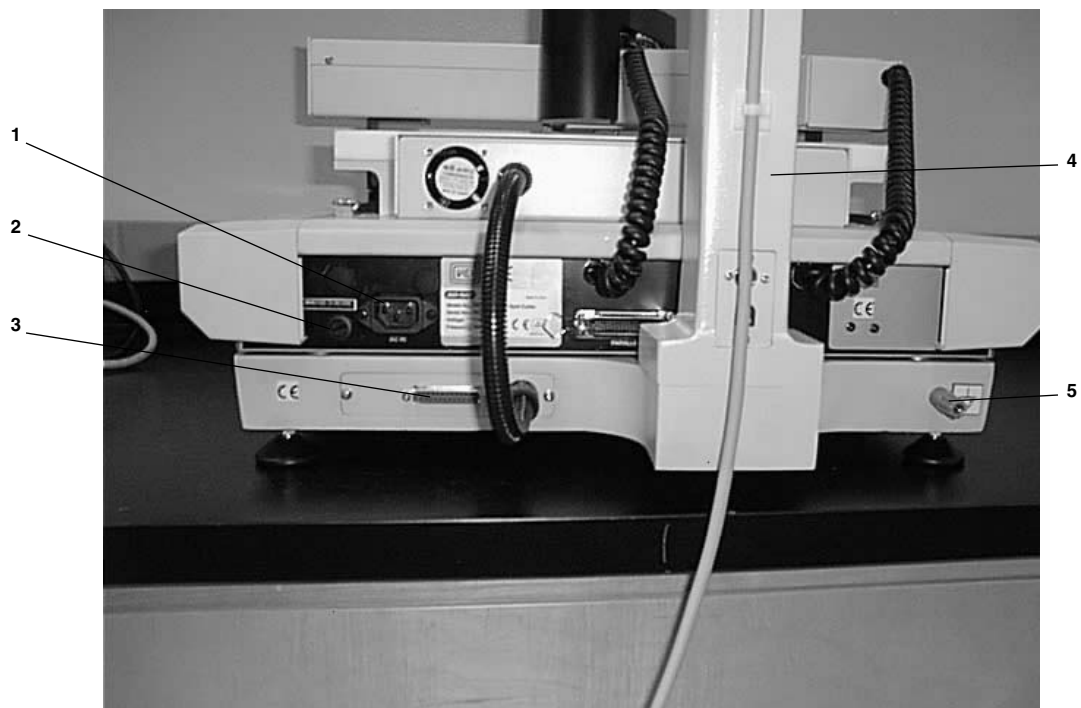
1. Main Power Plug

2. Fuse for Voltage Settings

3. System Communication Port

4. Camera Mounting Arm

5. Ground Plug

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   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 -

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.

Systems Record

Serial Number ————————————————————

Date of Purchase ————————————————————

Purchase Order
Number ————————————————————

Notes

————————————————————————————————

————————————————————————————————

————————————————————————————————

————————————————————————————————

————————————————————————————————

————————————————————————————————

————————————————————————————————

————————————————————————————————

————————————————————————————————

————————————————————————————————

Service Record

Date   Description of Problem   Name   Signature

BIO RAD

**Bio-Rad
Laboratories, Inc.**