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CLIPR™ System

Chemiluminescence Imaging Plate Reader

C.L114

User Manual

Molecular Devices

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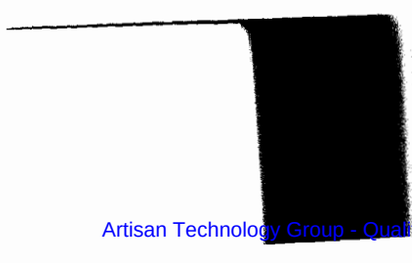
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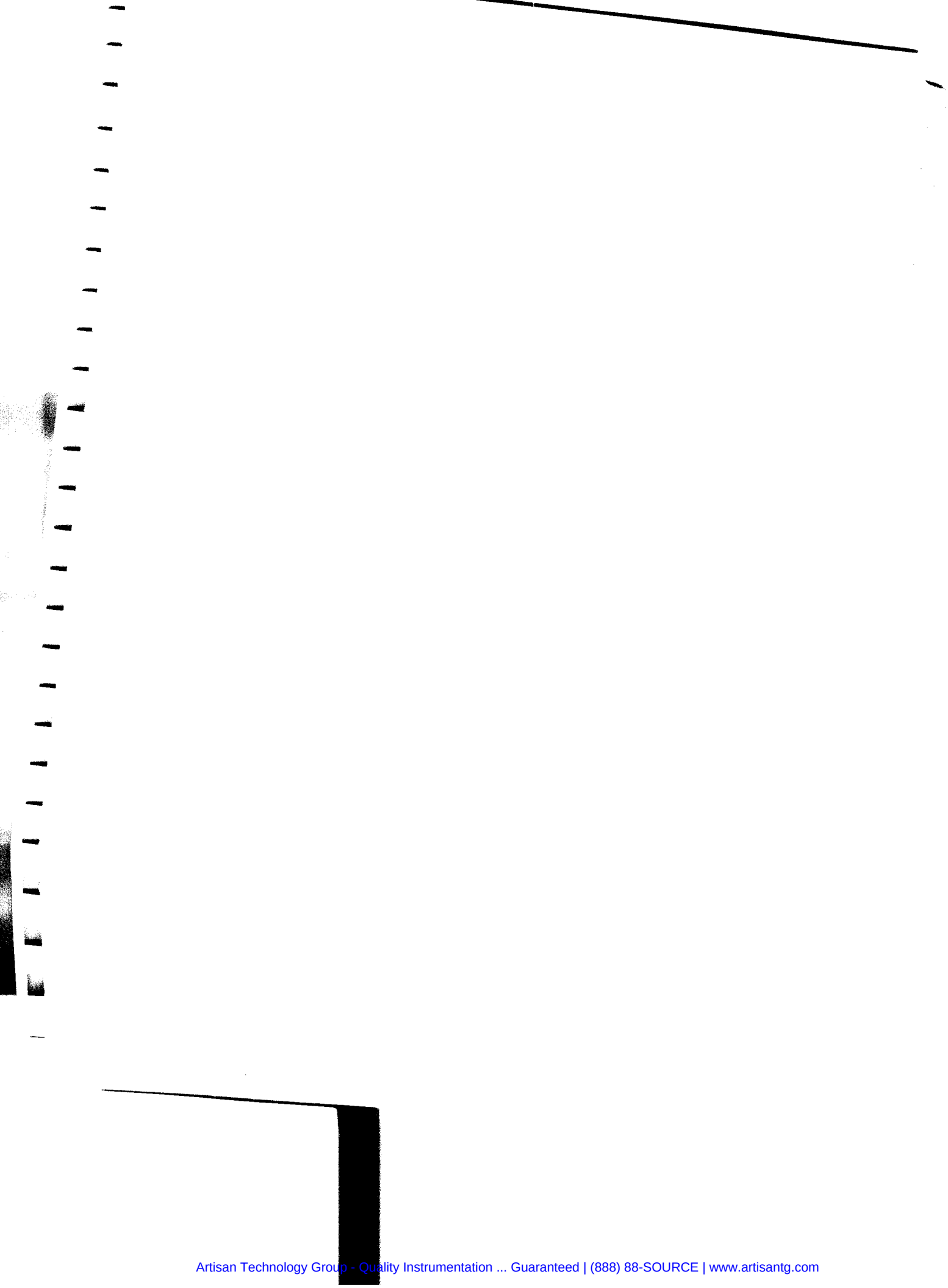
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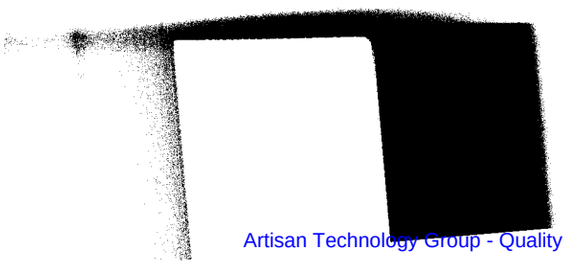


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Chapter 1– System Overview

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1.1 Introduction

The chemiluminescence imaging plate reader (CLIPR™) (see Figure 1-1) offers users a high-sensitivity, ultra-high throughput luminometer system that can simultaneously image all wells of microplates having up to 1536 wells. The CLIPR design efficiently performs chemiluminescent- and radiometric-based assays typically used in pharmaceutical drug-discovery research (e.g., reporter-gene and scintillation-proximity assays).

CLIPR incorporates a proprietary telecentric optical system, a cryogenically cooled, charge- coupled device (CCD) camera, a precision X, Y, Z plate- positioning stage and a computer with a Windows® NT operating system for efficient instrument control and data recording. Users can operate the system manually or integrated into a linear track robot, or use it as a

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stand-alone workstation when combined with the optional robotic plate handler.

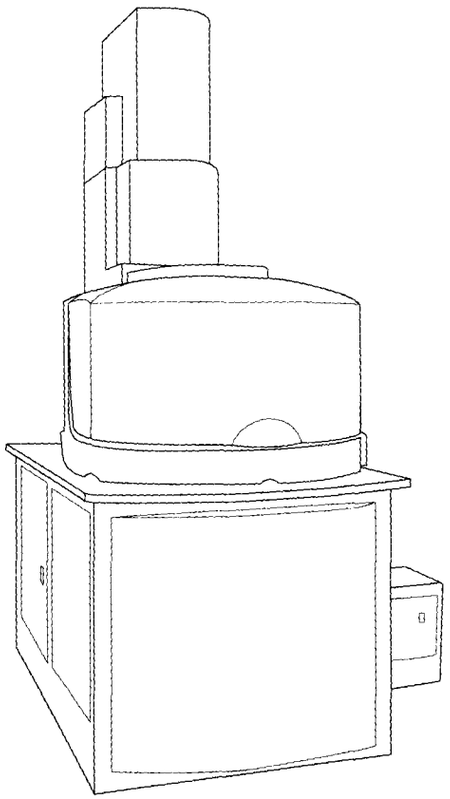


Figure 1-1 The complete CLIPR system with optional plate handler and stacker

1.2 System Description

CLIPR gathers photons of light emitting from microplate wells as a basic principle of operation and simultaneously measures the luminescence with a cryogenically cooled CCD camera. The wells contain cells, scintillants and/or reagents that emit light upon exposure to the test compound.

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The CLIPR system consists of the following components:

- Telecentric optical system
- Precision X, Y, Z plate-positioning stage
- Cryogenically cooled CCD camera
- Electronic controllers
- Light-tight system enclosure
- Computer, monitor and keyboard
- Customized software
- Optional robotic plate handler and stacker

The CLIPR system can accept any microplate having up to 1536 wells, as long as it conforms to the Society for Biomolecular Screening standards and maintains a 3:2 column to row aspect ratio. The system software controls the recording of the luminescence signal collected by the CCD camera and displays the luminescent data on the computer monitor for all wells (i.e., up to 1536.) The software can display both an image of the microplate and the numerical data for each well. It then stores the data in an ASCI II text-file format that can be imported by a spreadsheet program.

1.3 System Requirements

1.3.1 Electrical

The CLIPR system operates from a universal power supply, which allows either 100–120V or 200–240V electrical configuration. Three outlets will meet the system's electrical requirements: one for the CLIPR itself, one for the computer monitor and one for the refrigeration system. Molecular Devices recommends a 15- amp electrical service for 110 VAC applica-



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tions and an 8-amp service for 220 VAC applications. Mains supply voltage fluctuations should not exceed ±10% of the nominal voltage.

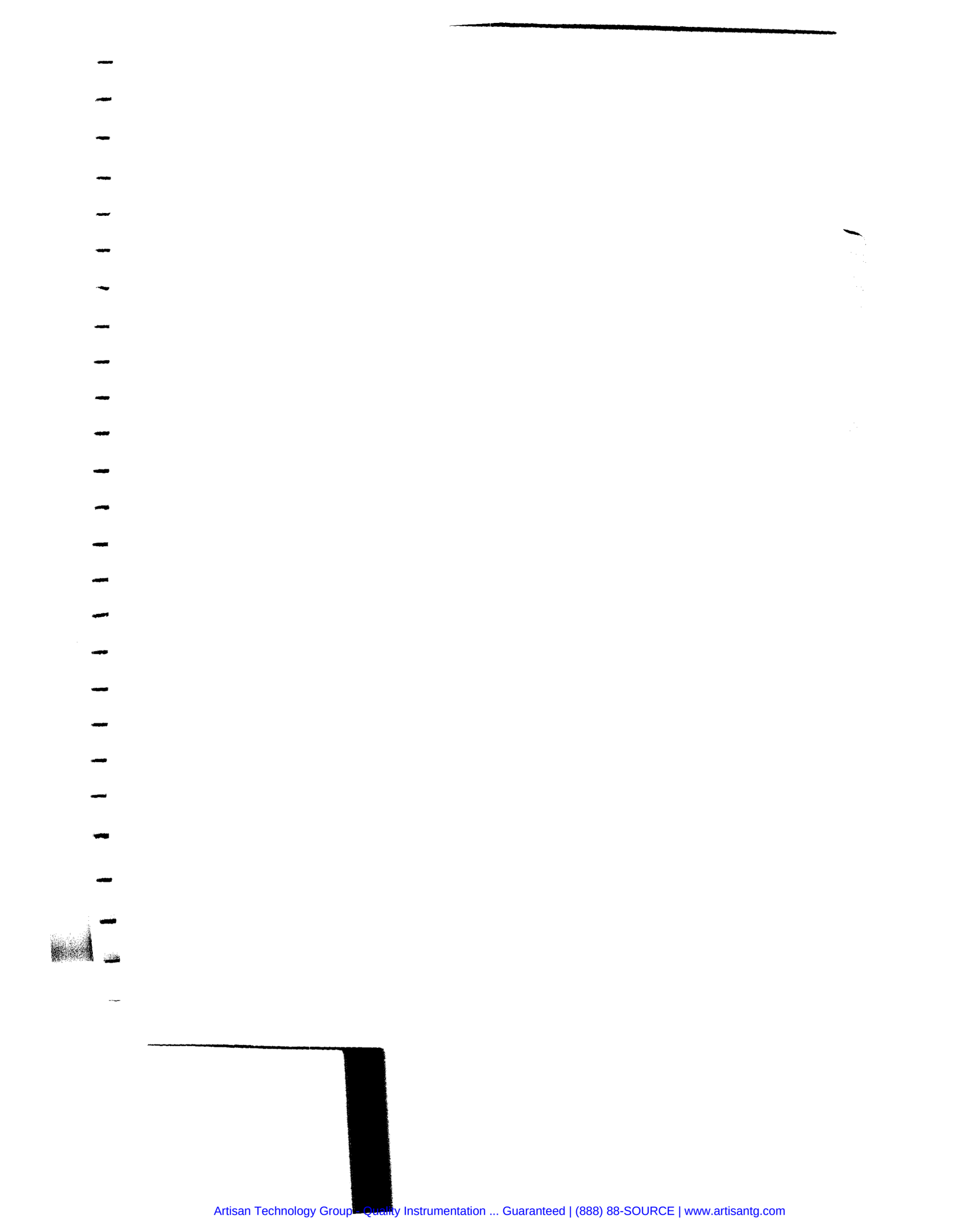
1.3.2 Physical

The CLIPR instrument dimensions are 38 inches long by 30 inches wide by 84 inches high. The base unit of the optional robotic plate handler and stacker measures 30 inches long and 30 inches wide. Their combined footprint measures 68 inches long by 30 inches wide. A minimum clearance of 6 inches at the rear of the instrument provides necessary access to the emergency stop button and power cord.

1.3.3 Environmental

The CLIPR is intended for indoor operation at an altitude of up to 2000 meters. The instrument requires an environment with room temperatures at or below 25°C and 50% relative noncondensing humidity for optimal operation.

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Chapter 2–Hardware Overview

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2.1 System Diagram

Figure 2-1 shows the hardware features of the CLIPR system.

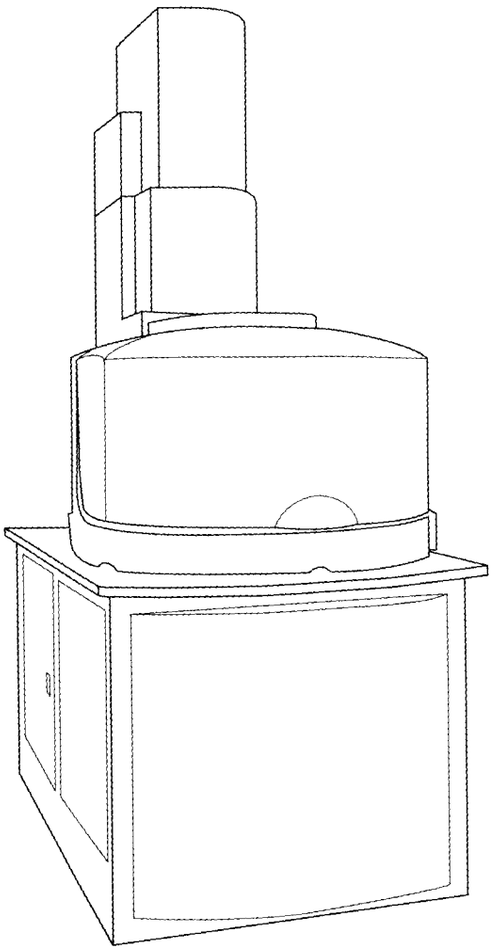


Figure 2-1 The CLIPR system with plate handler and stacker

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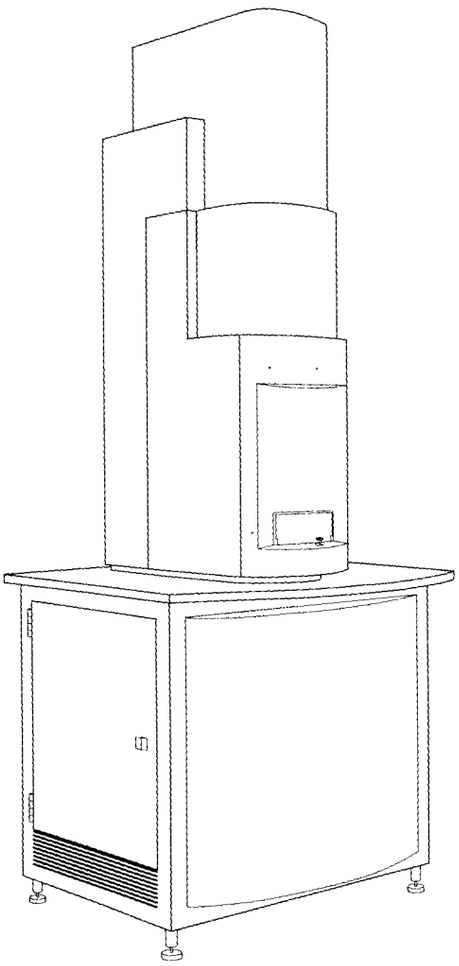


Figure 2-2 The CLIPR system alone

2.2 Telecentric Optical System

The telecentric optical system allows simultaneous imaging of all wells of the microplate. The sophisticated design of the system provides extremely high light-gathering efficiency and produces an image free of vignetting, chromatic and/or

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spherical aberration and distortion. The telecentric optical system incorporates an electronic shutter that modulates the CCD camera exposure time to produce the desired images. The system software provides shutter control during assay setup, and automatically adjusts the focus and magnification. The software also enables the user to manually adjust the focus and magnification. A click will sound as the shutter opens and closes during image exposure. The instrument tower completely encloses the optical system, which does not require customer maintenance or servicing.

2.3 X, Y, Z Plate-Positioning Stage

The X, Y, Z plate-positioning stage allows proper centering of the plate within the telecentric optical system and provides precise alignment and repeatable mapping between the wells and the CCD camera. The initial alignment for each plate type, which will depend on the number of wells, is made through the CLIPR system software and is subsequently automatically adjusted through software control.

An easily-accessible loading tray within the CLIPR instrument's plate-positioning stage allows plate loading via the stage door. During manual operation, the plate is loaded into the tray with the bottom of the plate pressed against the spring clips, allowing the spring tension to properly position the plate in the

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tray. The plate will seat squarely in the tray when its top edges are gently pressed downward (see Figure 2-3).

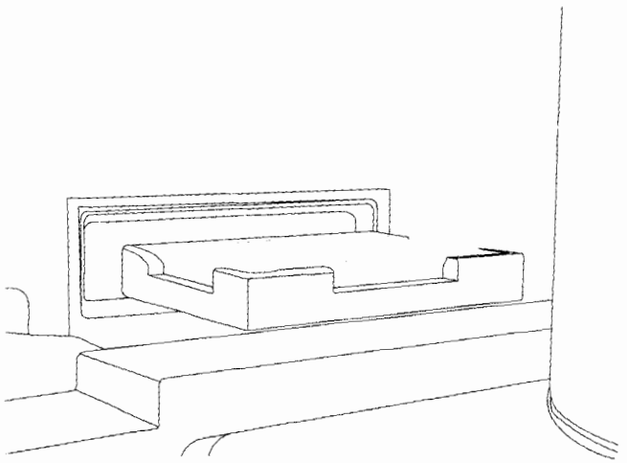


Figure 2-3 The X, Y, Z plate-positioning stage

Cautions: *When using a linear track robot, or the optional CLIPR plate handler and stacker, ensure that plates sit squarely in the loading tray in a flat position. Avoid contacting the glass surface above the plate-positioning stage.*

2.4 Cryogenically Cooled CCD Camera

The CCD camera resides at the top of the enclosed instrument tower and houses a 1024 x 1024-pixel CCD chip. The amount of light that reaches the CCD camera depends on the intensity of the assay emission and the exposure time. Users control the exposure time through the software's **Assay Setup** window. The CCD becomes light-saturated at approximately 65,000 relative light units. At that value, additional exposure time will not result in greater detected signal.

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The camera controller is rack-mounted in the base cabinet, and contains the electronics that operate the camera (Figure 2-4). A single switch on the front of the instrument supplies power to both the controller and the camera. The camera requires approximately 3 to 6 hours to reach its optimal operating temperature of -100°C. The system software displays the camera temperature in the bottom right corner of the **Status** window.

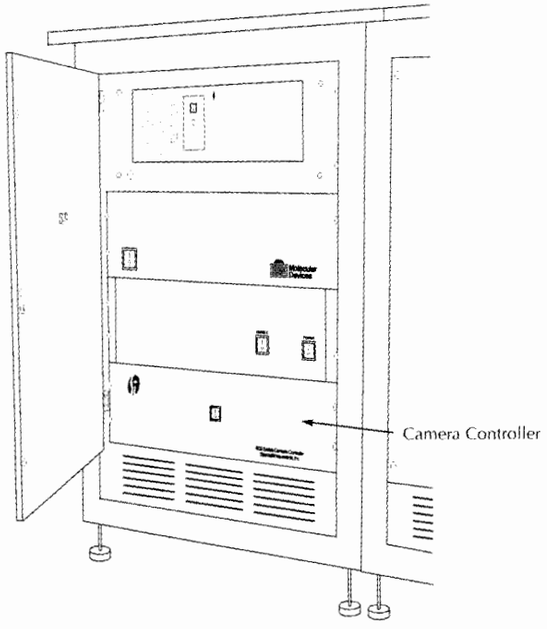


Figure 2-4 The camera controller

The Cryotiger® self-contained cooling system (see Figure 2-5) circulates refrigerant between the compressor and the CCD camera without the addition of liquid nitrogen. The Cryotiger

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has a dedicated AC power cord that can plug into a standard wall outlet, and a power switch located on the front panel.

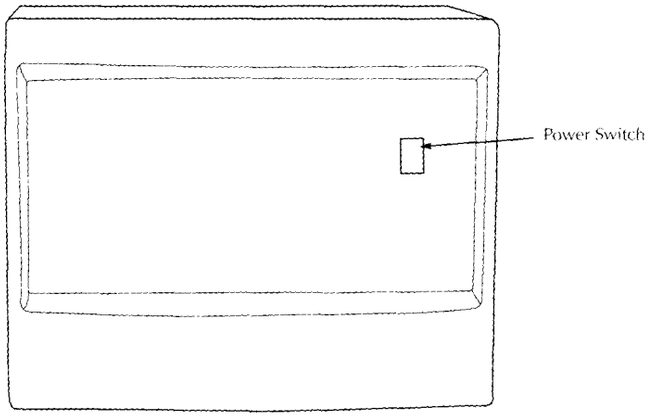


Figure 2-5 The Cryotiger cooling system

Note: *The design of the CCD camera and the Cryotiger cooling system allows them to run continuously.*

Caution: *Maintain the Cryotiger module in an upright position at all times. In the event the module is tilted 30° or more, allow the unit to remain in a level position for at least 4 hours before continued operation.*

Warnings: *Power off the Cryotiger cooling system whenever the CLIPR instrument or the camera controller is shut down for more than 30 minutes. Failure to turn off the cooling system will result in the camera temperature dropping to -120°C or lower, which may freeze the camera sealant and lead to a vacuum leak.*

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Never introduce high-intensity light to the CLIPR optical system via an open shutter (e.g., shining a flashlight into the plate-positioning stage during exposure).

2.5 Electronic Control Modules

The instrument base cabinet of the CLIPR system houses all of the control modules in a rack-mounted design that is easily accessible via a magnetically coupled door. The modules include the following components:

2.5.1 Computer

The control software of the CLIPR system runs in the Windows NT 4.0 environment on an Intel Pentium III processor-based computer. The hardware configuration includes the following features:

- 266MHz K6 Intel Pentium III Processor
- 64 MB RAM
- 4.5 GB hard-disc drive
- 1.4 MB floppy-disc drive
- Ethernet interface
- 17-inch color monitor
- Keyboard and mouse

Note: *The computer's power switch remains on at all times, even during periods when the CLIPR system is shut down. The computer and all other system controllers/components receive power through the interface chassis.*

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Cautions: *When shutting down the CLIPR system, turn off the computer via the* ***Shut Down*** *icon displayed in the* ***Start*** *window of the software.*

2.5.2 Motor Controller

The motor controller serves as the power amplifier and the system interface that drives the stepper motors operating the X, Y, Z plate-positioning stage, the stage door and the optical system focus mechanism.

Note: *The Power and Enable switches (see Figure 2-6) remain on at all times, even when the CLIPR system is shut down. The motor controller and all other system controllers/ components receive power through the interface chassis.*

2.5.3 Interface Chassis

A single controller provides integration of all electrical and electronic control functions. The Power switch on the interface chassis serves as the main power supply for the entire CLIPR system (see Figure 2-6).

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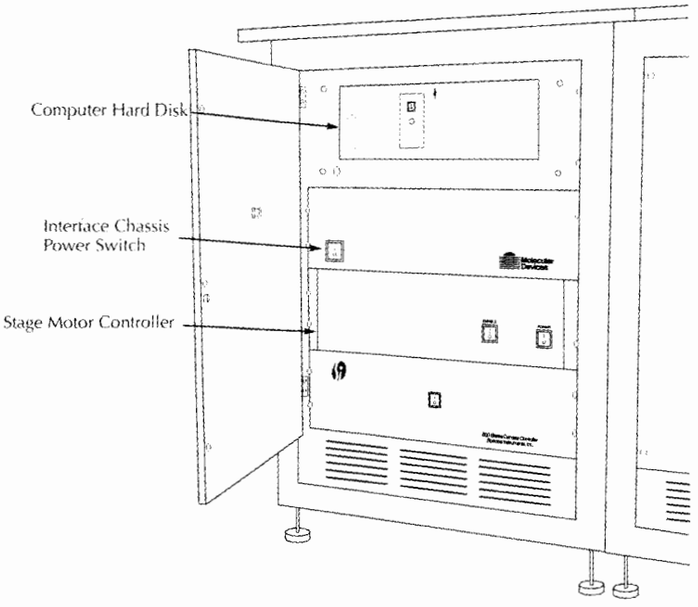


Figure 2-6 The CLIPR system controller

Note: *All other controller power switches (i.e., for the computer, motor controller and camera controller) remain in the ON position at all times, even when the CLIPR system is shut down. The interface chassis power switch controls power to all of these modules.*

2.5.4 Camera Controller

The camera controller contains the electronics enabling operation of the CCD camera.

Note: *The camera controller power switch remains on at all times, even when the CLIPR system is shut down. The camera*

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controller, and all other system controllers/components, receive power through the interface chassis.

2.6 Optional Robotic Plate Handler and Stacker

The robotic plate handler allows the CLIPR instrument to operate as a stand-alone workstation (see Figure 2-7). Capable of sequentially loading up to 80 plates in the CLIPR system, the handler retrieves and restacks the plates in their original order. When handling plates having lids, the plate handler removes the lids and places them on the lid storage platform, then subsequently returns the lids after CLIPR processing. A barcode reader enables the CLIPR system to identify individual plates by their barcode strips. The lower right corner of the system software's **Status** window indicates the barcode designation.

The entire plate-handler and stacker system resides in a light-tight enclosure. This enclosure allows loading of white plates into the stacker while protecting the plates from light and permitting their inherent phosphorescence to decay. This effec-

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tively minimizes background and maximizes the signal-to-noise ratio.

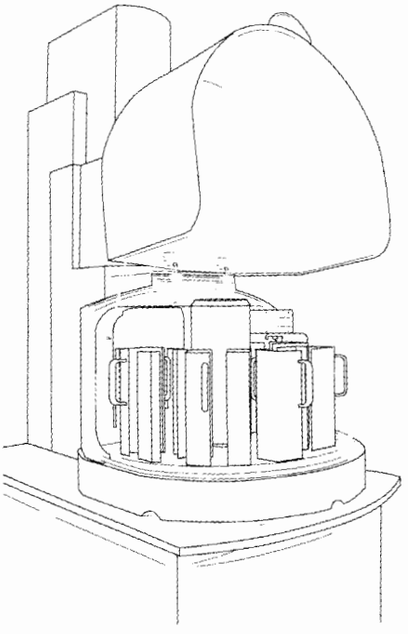


Figure 2-7 The robotic plate handler and stacker

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Chapter 3– Start-Up and Shutdown Procedures

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3.1 Start-Up Procedure

Ensure proper communication between the CLIPR system control software and hardware by precisely following the start-up and shutdown procedures documented in this chapter.

If needed at any time during operation, an Emergency stop button is located in the back of the cabinet (see Figure 3-1).

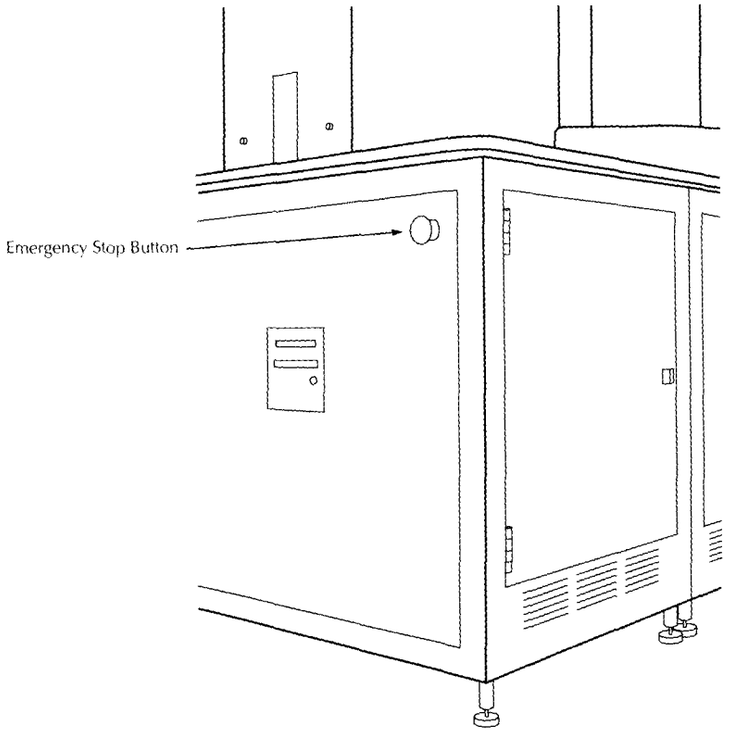


Figure 3-1 The back of the cabinet

Ensure that at least 6 inches of clearance is provided between the CLIPR and the wall to allow access to the Emergency stop button and power cord.

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1) Power the computer monitor by depressing the power switch on the front of the monitor (see Figure 3-2).

2) Power the camera controller by depressing the red rocker switch on the front of the controller to the ON position (see Figure 3-2).

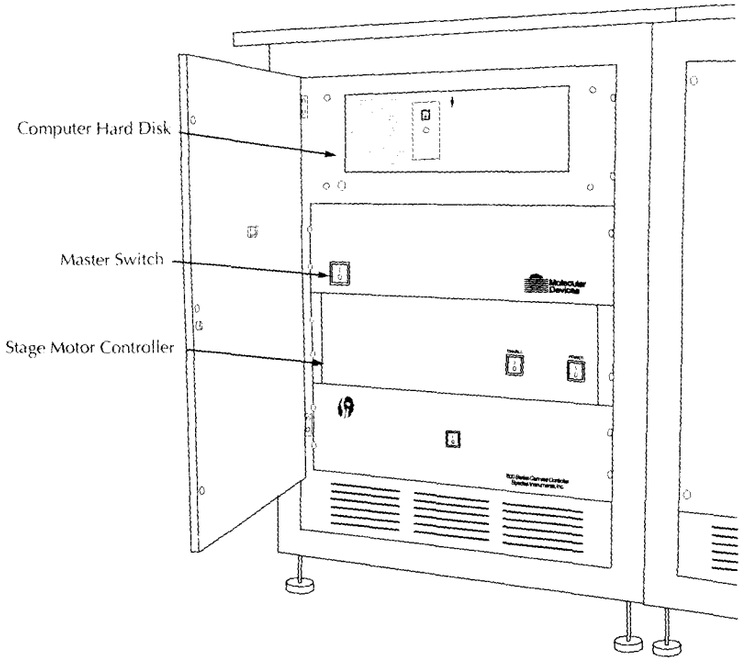


Figure 3-2 The side panel of the system cabinet and ON/OFF controls for the camera and computer

3) Power the motor controller by depressing the power rocker switch and moving the ENABLE switch to the ON position.

4) Power the CLIPR system and interface chassis by depressing the black rocker switch to the ON position.

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5) Power the cooling compressor of the Cryotiger unit by depressing the rocker switch on the front of the compressor to the ON position (see Figure 3-3).

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9-5-02
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800-635-5577
option 1
Tech Support

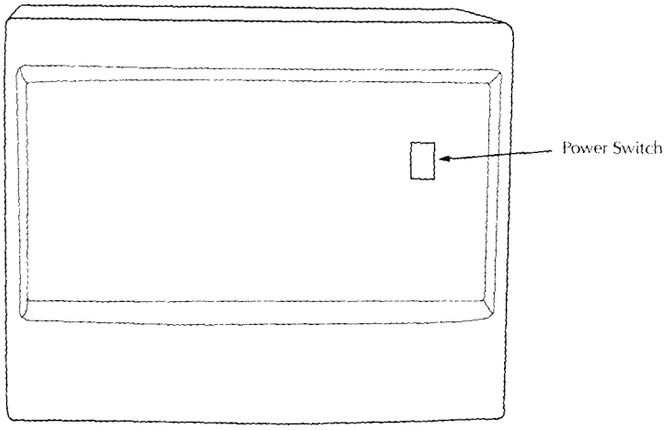


Figure 3-3 Cryotiger unit and ON/OFF control

Note: *After placing the camera and motor controller switches in the ON position, they remain in this position at all times. The black rocker switch on the interface chassis controls power to the CLIPR system, which in turn, supplies power to the camera controller, the computer and the motor controller.*

6) Once the computer completes its system-check routines, simultaneously press the CTRL/ALT/DEL keys to launch the Windows NT operating software. Enter the password when prompted.

Note: *Use "CLIPR" as the user id during initial installation.*

7) Launch the software by clicking on the CLIPR icon located on the Windows desktop.

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Note: *Users will momentarily hear the operation of the instrument motors during the software launch as the motors reset to their "home" positions. Four audible tones will sound from the camera controller as the system performs the initialization procedure.*

The CCD camera will require 3 to 6 hours to reach the correct operating temperature of -100°C from the ambient temperature of the room environment. The software status bar will display the camera temperature. If a temperature of -100°C is not reached within 3 to 6 hours, see the Troubleshooting guide.

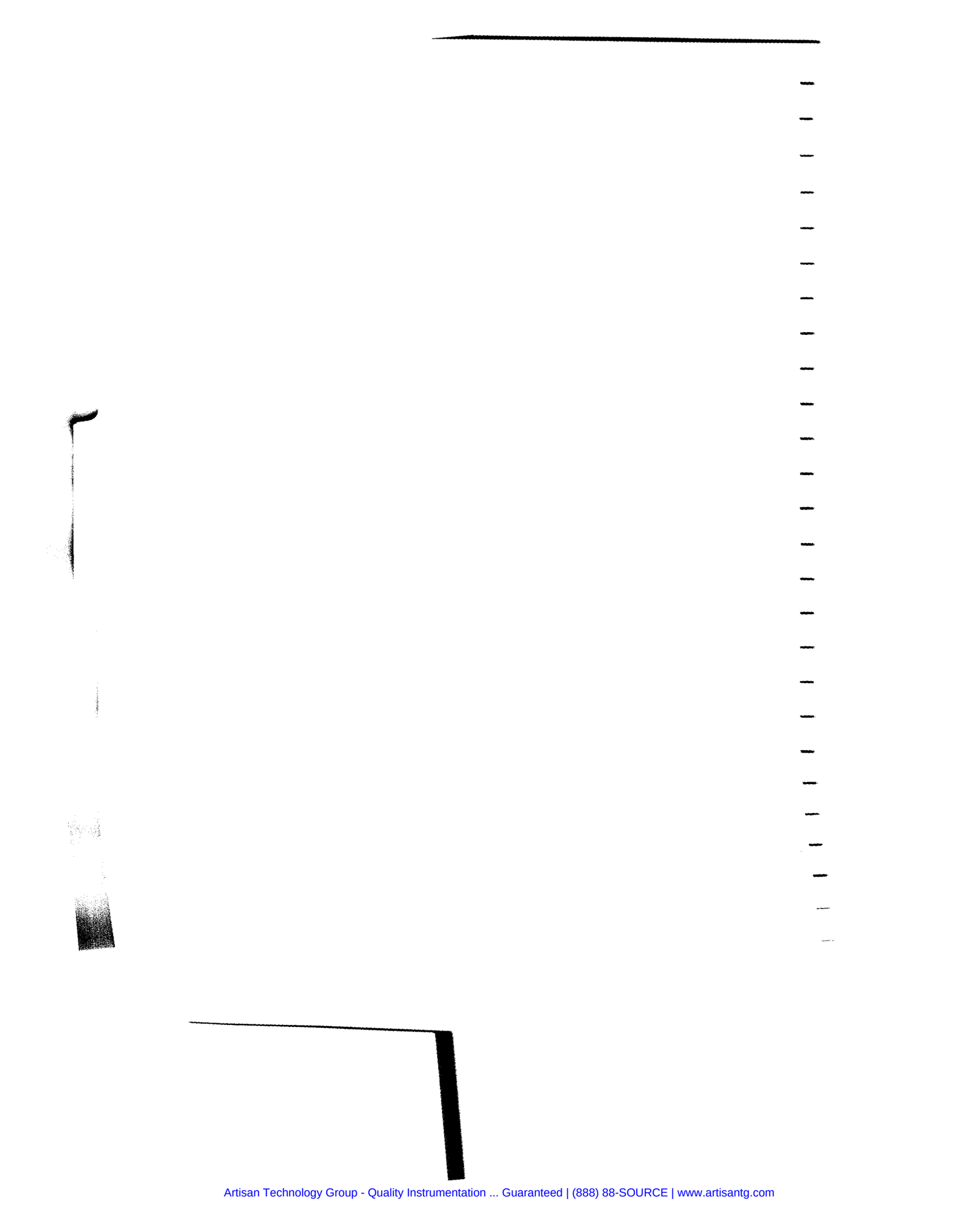
Caution: *Operating the camera at temperatures above -90°C may result in degraded images and poor sensitivity.*

3.2 Shutdown Procedure

1) Exit the CLIPR software by choosing **Exit** from the **File** menu.
2) Shut down the computer by following the prompt in the **Shutdown** menu located within the Windows **Start** bar.
3) Shut down the cooling compressor of the Cryotiger unit by depressing the rocker switch on the front of the compressor to the OFF position.
4) Shut down the instrument hardware by depressing the black rocker switch on the front of the interface chassis to the OFF position.

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Chapter 4– Preparing for an Assay

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4.1 Reporter-Gene Assay

The CLIPR Luciferase Assay Kit, commercially available through Molecular Devices, describes the setup for the luciferase reporter-gene assay. While other luciferase kits from other vendors have been used with CLIPR, the CLIPR rendition has been optimized for use with the CLIPR system and is thus the kit to which this entire section refers.

Luciferase derives from the North American firefly and provides a widely used enzyme for reporter-gene assays. Luciferase catalyzes the oxidation of a firefly-derived substrate known as luciferin to produce light. This produces an extremely efficient reaction with a quantum yield higher than any characterized bioluminescent reaction. The bright signal makes this a valuable enzyme in reporting promoter activity. Luminescence assays run most effectively in 1536-, 384- or 96-well white, opaque, flat-bottom, plates. With CLIPR, the signal can be read from the top, middle or bottom of the wells. Consequently, the cells are generally seeded as a cell suspension in growth medium for the CLIPR luciferase assay. Cells can be seeded in plates using a multichannel pipettor or a liquid dispensing system such as the Hydra.

Cell densities used in luminescence assays vary depending upon the cell type used and, in turn, the specific requirements of the cell type. Cell densities can range from 1,000 to 8,000 cells per well for 1536-well plates, 5,000 to 100,000 cells per well for 384-well plates and 10,000 to 200,000 cells per well for 96-well plates.

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For luciferase reporter-gene assays using the CLIPR Luciferase Assay Kit, users can seed cells either the day before or on the day of the experiment, depending upon the cell type and/or the effect of the test ligand. The basic steps of the CLIPR luciferase assay are:

1) Prepare and plate the cells.

2) Induce the luciferase expression by treating the cells with test compound and/or control reagents.

3) Add the reagent and incubate at room temperature for at least 10 minutes and up to 30 minutes.

4) Read using the CLIPR.

4.1.1 Preparation of the Cells

According to the following table, prepare a cell suspension in growth medium with at least 1% Fetal Bovine Serum.

Well plate format	Cells per ml	Cells µL per well
96 wells	$1.2 \times 10^5 - 2.2 \times 10^6$	90
384 wells	$2.5 \times 10^5 - 2.5 \times 10^6$	20
1536 wells	$5.0 \times 10^5 - 4.0 \times 10^6$	2

Note: *Aliquot 2 µL of cell suspension into each well for 1536-well plates, 20 µL per well for 384-well plates or 90 µL per well for 96-well plates.*

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4.1.2 Cell Treatment for Induction of Luciferase Expression

Add to the following compound and/or control reagents amounts to the cell plate for induction of the luciferase expression: 1 µL for 1536-well plates, 5 µL for 384-well plates or 10 µL for 96-well plates. Incubate the cell-compound plate for a predetermined period of time, typically from 4 hours to overnight in a 5% CO_2, 37°C incubator.

4.1.3 Reagent Preparation and Use Prior to Assay Reading by the CLIPR

Using a clean pipette, transfer 10 mL of CLIPR assay buffer (component B) into one vial of luciferase assay substrate (component A) and mix at room temperature.

Note: *It is important to ensure that the contents of the reagent kit are at room temperature well ahead of running the assay. Remove the reagents from storage and allow them to equilibrate to room temperature. Do NOT thaw the reagents in a water bath.*

Remove the cell plates from the incubator and allow them to equilibrate to room temperature. Add an equal volume of the reconstituted reagent to the volume in the wells (100 µL for 96-well plates, 25 µL for 384-well plates and 3 µL for 1536-well plates). For optimal signal, leave the plates in the dark at room temperature for a minimum of 10 minutes before reading by the CLIPR.

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4.1.4 Running the Assay on the CLIPR System

Proceed to Chapter 5 for details on how to set up the CLIPR to conduct the reporter-gene assay. Molecular Devices recommends integration times of 1 to 60 seconds.

4.2 Scintillation-Proximity Assay

As homogenous binding assays, scintillation-proximity assays (SPAs) employ a solid-phase material containing a scintillant. Examples of specific embodiments include multiwell plates manufactured to contain scintillant and small beads commonly known in the industry as SPA beads. Derivatized (e.g., with a selected receptor), the beads bind specific molecules (e.g., ligands for the selected receptors). When a radiolabeled molecule binds to a bead, it stimulates the scintillant in the bead to emit light. Unbound radioactivity does not produce a signal because the radioactive energy decays before it can reach the bead to produce a scintillation event.

As homogenous assays, SPAs eliminate the need to remove unbound radiolabeled molecules prior to detection of bound molecules. Consequently, SPAs are widely used in the areas of clinical research, drug screening and discovery. The basic steps of the SPA are:

1) Preparation of SPA beads.

2) Treatment with radiolabeled compound.

3) Measurement of scintillation events.

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4.2.1 Preparation of Scintillation-Proximity Beads

1) Maintain the cell line expressing the target of interest in culture.

2) Prepare membrane fragments from the cell line according to the respective standard procedure.

3) Incubate the membrane fragments with the SPA beads of choice for the assay.

4) Remove unbound membrane protein from the beads by centrifugation (700xg, 5 minutes).

5) Resuspend the pellet that contains the target of interest bound to the SPA beads in your standard assay buffer to generate an assay cocktail.

4.2.2 Treatment With Radiolabeled Compound

Add the required concentration of radioligand to the assay cocktail both in the presence and absence of various concentrations of unlabelled compound. Make this addition to a final volume of 100 µL in 96-well plates, 20 µL in 384-well plates and 3 µL in 1536-well plates. Greatest sensitivity with SPA is achieved using non-tissue culture treated white plates. Incubate the plates at room temperature in a dark adapted environment overnight to reach equilibrium.

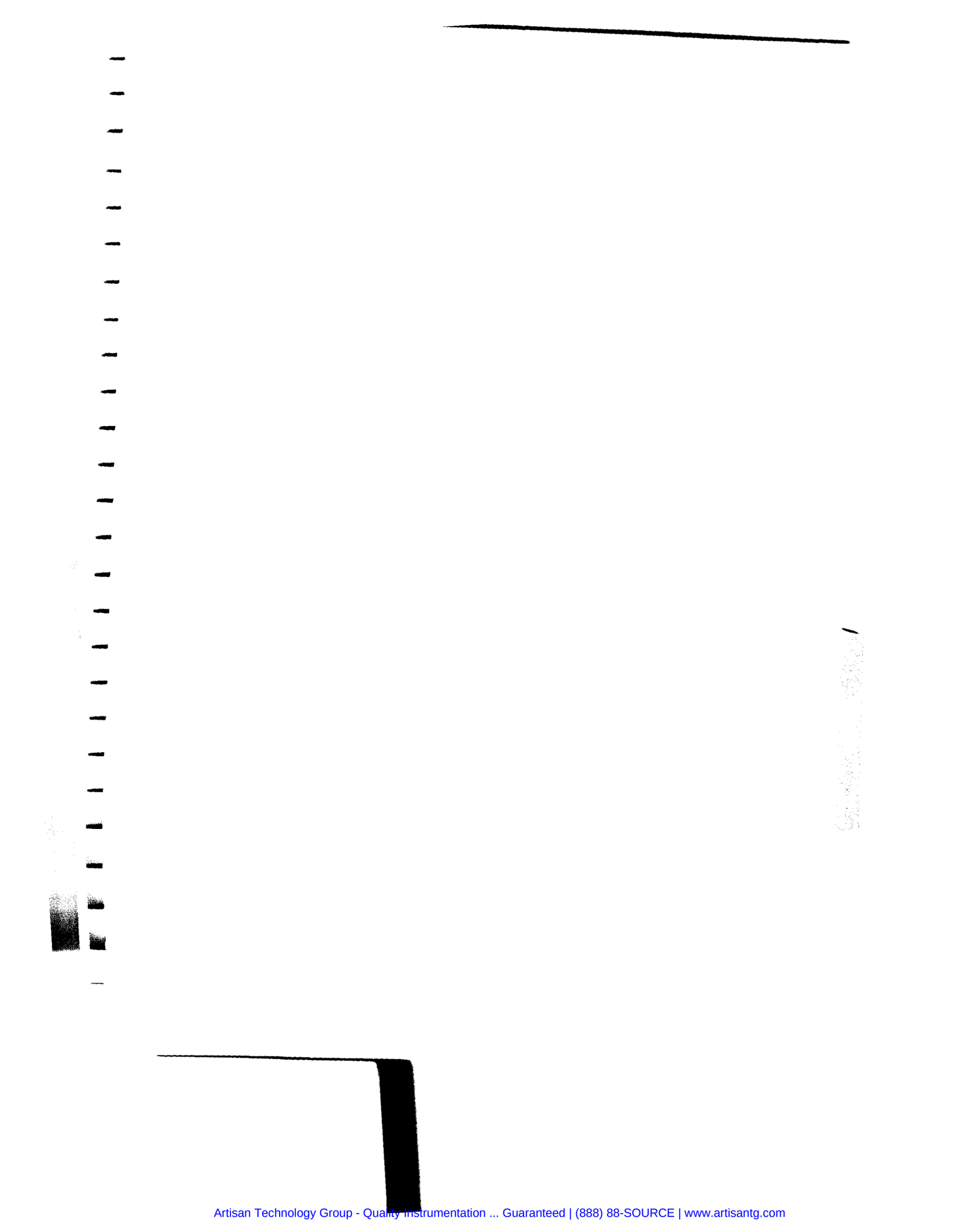
4.2.3 Measurement of Scintillation Events

Following the overnight incubation, transfer the plates to the CLIPR for measurement of the scintillation events. Typical

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exposure times for SPA assays are in the range of 2– 20 minutes per plate.

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Chapter 5– Software Overview

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5.1 Configuration Without Twister

To launch the CLIPR system, select the CLIPR icon on the software desktop or select **CLIPR** from the **Start** menu. Upon initial launch, users will hear three or more tones indicating camera initialization. See Chapter 7 for corrective troubleshooting if these tones do not sound. The main CLIPR system software window will appear upon successful launch (Figure 5-1).

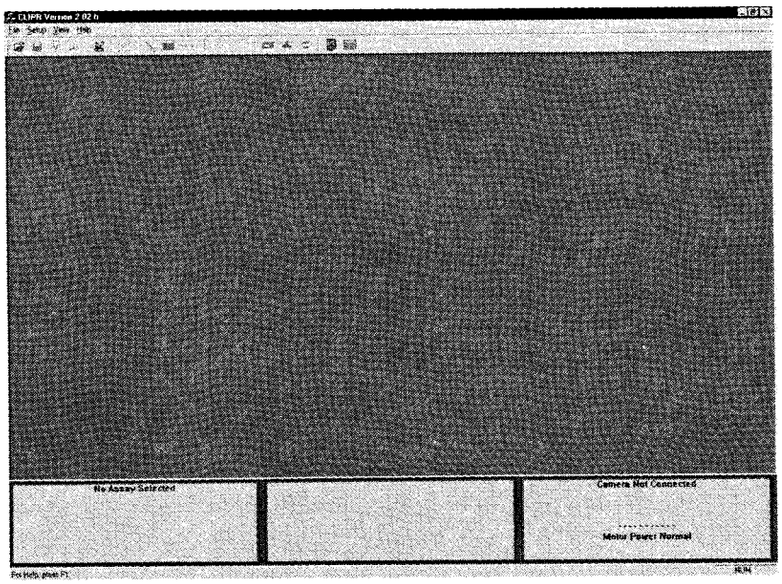


Figure 5-1 Main CLIPR system software window

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Users control all of the CLIPR operations through selections from either the menu bar or toolbar (see Figure 5-2). The toolbar offers shortcuts to the menu-bar items.

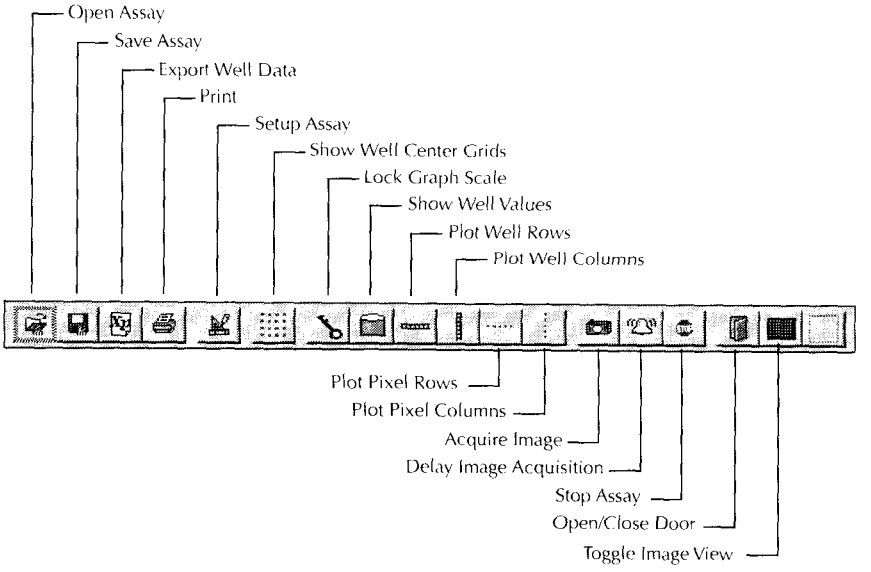


Figure 5-2 CLIPR system software toolbar

5.1.1 Plate Configuration

Plate alignment serves as the most important aspect in maintaining data accuracy. Although time consuming, the user performs the step only once for each plate; the system stores the settings for future assays. To display the plate

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configuration window, choose **Setup/Plates** from the menu bar (see Figure 5-3).

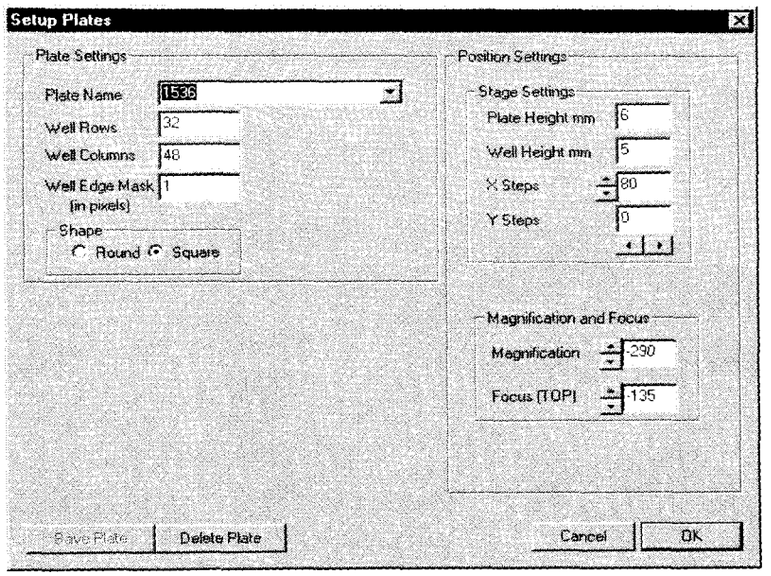


Figure 5-3 The **Setup Plates** window

Each plate type must have an individual plate definition recorded and stored. For example, a Greiner 1536 plate and a Corning 1536 plate might have slightly different characteristics necessitating independent definitions. A plate definition has the following components:

- **Plate Name**: The name by which the user refers to the plate.
- **Rows**: The number of horizontal rows in the plate.
- **Columns**: The number of vertical columns in the plate.
- **Well-Edge Mask**: The number of pixels from the edge of the well the user wishes to mask when computing the well averages (see Figure 5-4).

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- **Well Shape**: The user selection of either round or square well shapes. Note that round wells use a round shape to compute well averages. Conversely, square wells use a square shape.
- **Plate Height**: The height of the plate in millimeters (see Figure 5-5).
- **Well Height**: The height of the well in millimeters (see Figure 5-5).
- **X Steps**: Adjustment of the plate position from left to right.
- **Y Steps**: Adjustment of the plate position from front to back.
- **Magnification**: Adjustment of the image size.
- **Focus**: Adjustment of the image focus.

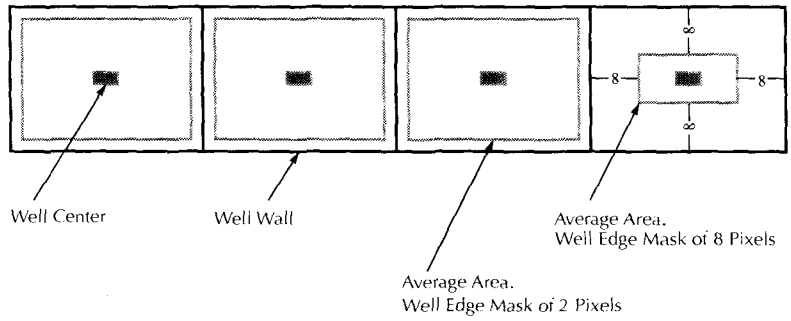


Figure 5-4 Well-edge masking

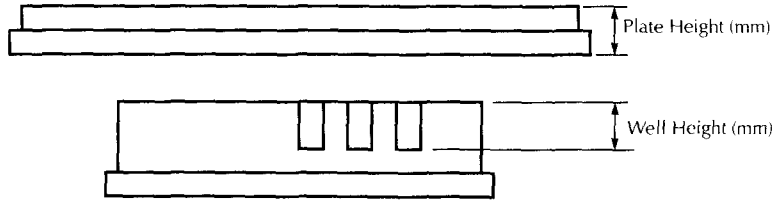


Figure 5-5 Plate height versus well height

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The CLIPR defaults to preset plate definitions that may require adjustment upon installation. The process begins with taking an image (refer to Section 5.1.3). Select the **Close Door 90%** option in the **Setup Assay** window, which allows the introduction of limited light. Then select the **Load Plate Manually** option. Depending upon the lighting conditions of the room, use a 1- to 10-second exposure time.

Load a white plate into the plate-positioning stage and take the image. If the image is overly dark, lengthen the exposure time. If the image is overly bright, adjust the grayscale slider bars to the right. Continue this process until the plate becomes visible in the image.

Select the **Grid** button on the toolbar  to display the plate grid (Figure 5-6).

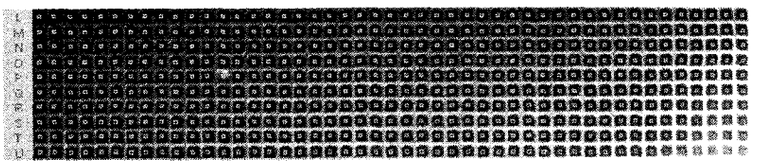


Figure 5-6 Plate image with center grids

The green squares that appear on the image represent the well centers. For perfect plate alignment with the well centers, ensure clear visibility of the wells on the image and a sharp focus. Adjust the focus parameter 10 to 20 steps at a time in either direction and retake the image until a sharp focus is achieved. The center of a plate equates to columns 24 and 25 and rows P and Q with a 1536-well plate. Move the wells

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toward the well-center grids by adjusting the X and Y step parameters.

Continue to retake the image until the center wells align over the center grids. If the left-edge well positions left of center, and the right-edge well positions right of center, decrease the magnification parameter by selecting the **UP** button in the **Setup Plate** window. Adjust this parameter 10 to 20 steps at a time. If the left- and right-edge wells both position inside center, increase magnification through the down arrow on the **Setup Plate** window (see Figure 5-7).

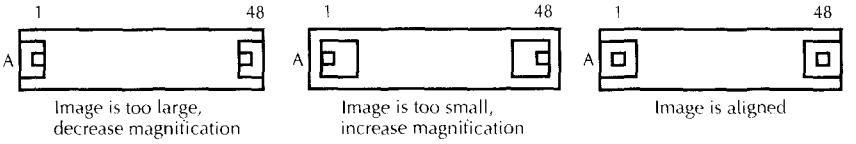


Figure 5-7 *Well-edge alignment*

Once the desired magnification has been achieved, the focus may require adjustment. Upon completion, all wells will have the green grid dots in their centers. With use of a 1536-well plate for the alignment, the 384- and 96-well plates will most likely use the same parameter values for the X and Y steps, magnification and focus. Select the plate in the **Setup Plates** window and modify the parameter values. Select **Save Plate** to complete the process. Verify the plate and well height parameters and confirm the alignment of each plate type by taking sample images.

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5.1.2 Preferences

Use the **Preferences** window to set options applicable throughout the application. The window allows you to adjust the following parameters (see Figure 5-8)

1) **AutoSave Directory Path**: Indicates the folder in which data files are stored during remote serial-interface use. The CLIPR will use the assay name and the date/time to create the data-file names.

2) **Assay Image Settings**: These settings determine the default state of the following options:
   - **Save Image**
   - **Subtract Dark**
   - **Filter Cosmic Rays**

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3) **Export Labels**: When selected, export files will contain well labels (1 = number of columns; A = number of rows). When not selected, the system will export only well data.

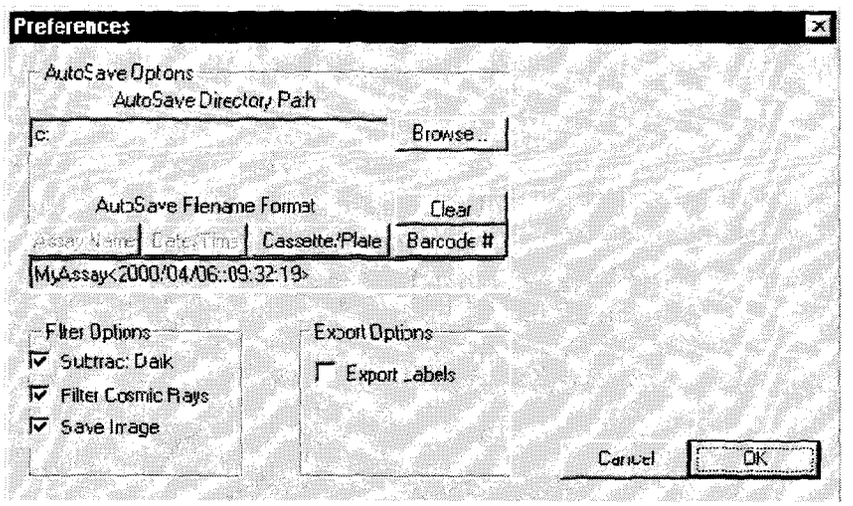


Figure 5-8 The **Preferences** window

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5.1.3 Setup Assay

To display the **Setup Assay** window, choose **Setup/Assay** from the menu bar (see Figure 5-9) or click the **Setup** button on the toolbar .

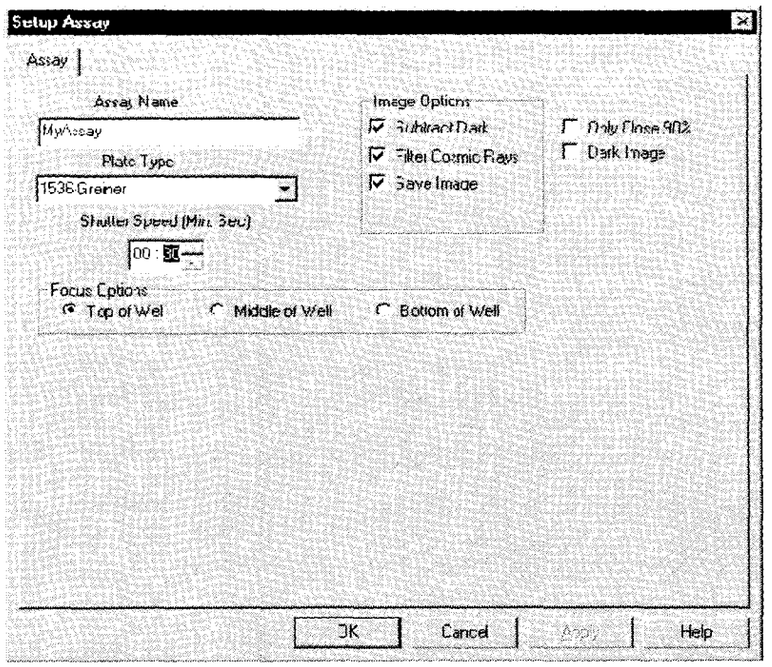


Figure 5-9 The **Setup Assay** window

An assay definition has the following components:

1) **Assay Name**: The name the user will use to refer to the assay.

2) **Plate Type**: The plate definition determined by the user.

3) **Shutter Speed**: The length of time the shutter will remain open.

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4) **Focus Options**: The portion of the well upon which the user wishes to focus (i.e., top, middle or bottom).

5) **Image Options**: Sets the quality of the image data and determines how they will be stored.

   - **Subtract Dark**: Subtracts background noise from image data.
   - **Filter Cosmic Rays**: Removes cosmic rays from the well-average areas.
   - **Save Image**: Saves image data to a 512K file along with the assay data. If not selected, the system saves only the assay data. The image remains viewable until the user closes the assay. When reopened, CLIPR generates and displays an image from the well- averages data.

6) **Only Close 90%**: Allows light into the system when taking an image. The plate-positioning stage door only partially closes—primarily used in plate alignment.

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5.1.4 Running the Assay

Load the plate into the plate-positioning stage as illustrated in Figure 5-10.

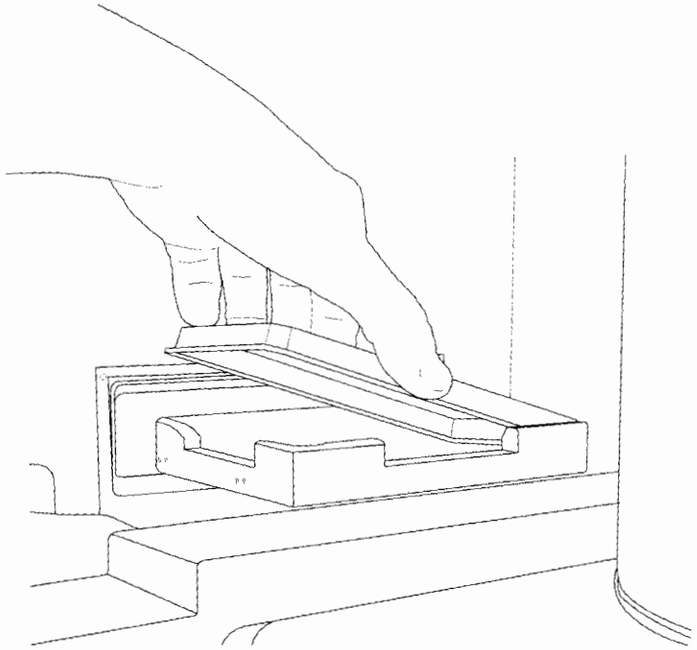


Figure 5-10 Inserting a plate in the positioning stage

Click the camera button or select **Run/Assay** from the menu bar. The stage will retract, the door will close and the CLIPR will take the image. Save the assay by clicking the **Save** button or selecting **File/Save** from the menu bar.

5.2 Configuration With Twister

To launch the CLIPR system, select the CLIPR icon on the software desktop or select **CLIPR** from the **Start** menu. Upon initial launch, users will hear three or more tones indicating camera initialization. (See Chapter 7 for corrective trouble-

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shooting if these tones do not sound.) The main CLIPR system software window will appear upon successful launch (see Figure 5-11). With software configured to include the robot handling system, the status bar will display four panels instead of three. The robot display resides on the far left of the panel.

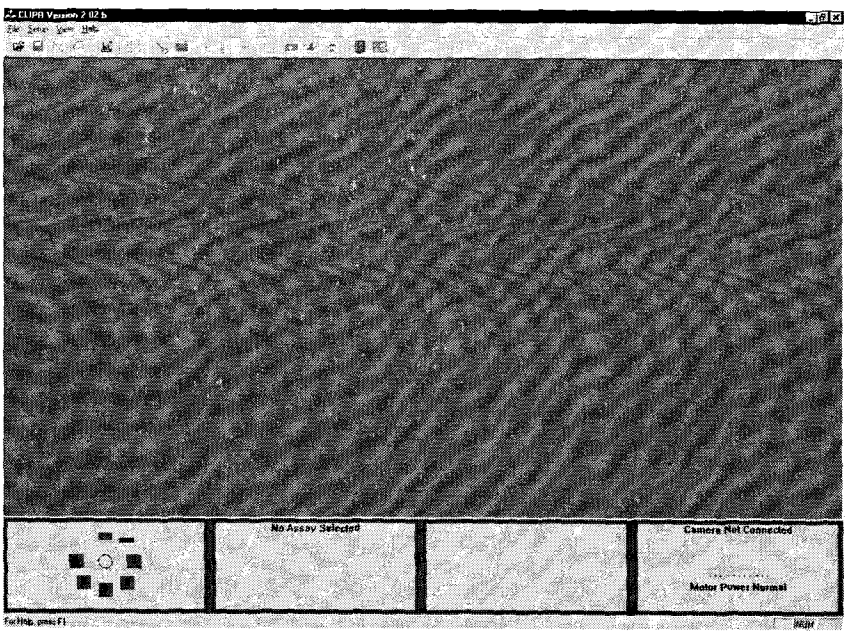


Figure 5-11 Main CLIPR system software window

Users control all of the CLIPR operations through selections from either the menu bar or toolbar displayed by the system

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software (see Figure 5-12). The toolbar offers shortcuts to the menu-bar items.

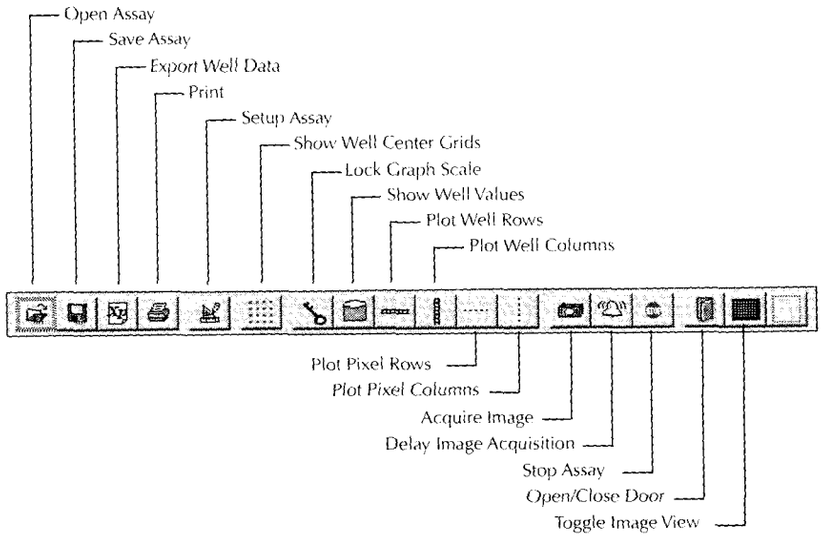


Figure 5-12 The CLIPR system software toolbar

5.2.1 Plate Configuration

Plate configuration serves as the most important aspect in maintaining data accuracy. Although time consuming, the user performs the step only once for each type of plate, and the system stores the settings for future assays. To display the

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plate-configuration window, select **Setup/Plates** from the menu bar (see Figure 5-13).

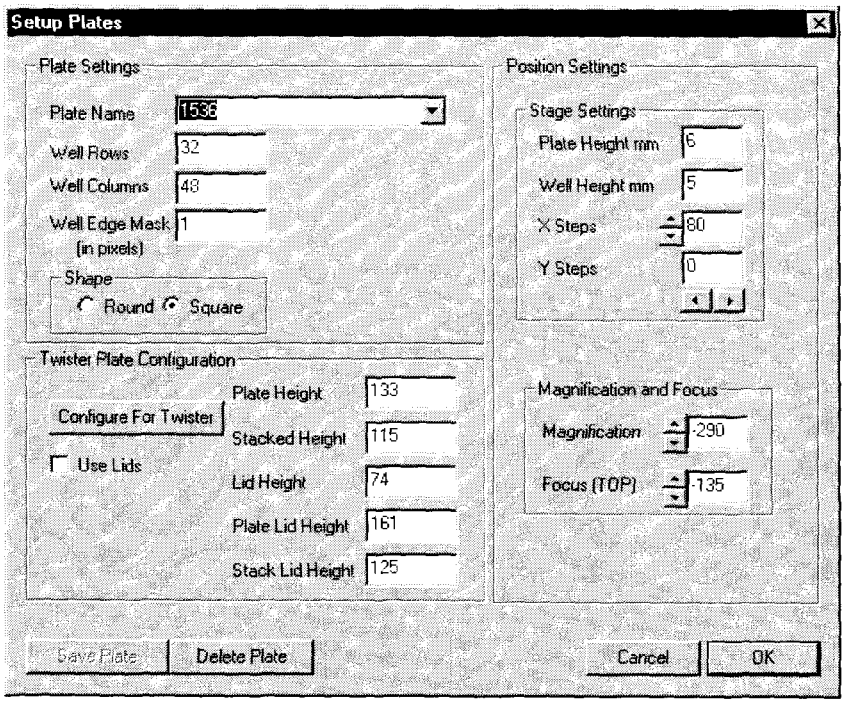


Figure 5-13 The **Setup Plates** window

Each plate type must have an individual plate definition recorded and stored. For example, a Greiner 1536 plate and a Corning 1536 plate might have slightly different characteristics necessitating independent definitions. A plate definition has the following components:

- **Plate Name**: The name by which the user refers to the plate.
- **Rows**: The number of horizontal rows in the plate.
- **Columns**: The number of vertical columns in the plate.

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- **Well-Edge Mask**: The number of pixels from the edge of the well the user wishes to mask when computing the well averages (see Figure 5-14).
- **Well Shape**: The user selection of either round or square well shapes. Note that round wells use a round shape to compute well averages. Conversely, square wells use a square shape.
- **Plate Height**: The height of the plate in millimeters (see Figure 5-15).
- **Well Height**: The height of the well in millimeters (see Figure 5-15).
- **X Steps**: Adjustment of the plate position from left to right.
- **Y Steps**: Adjustment of the plate position from front to back.
- **Magnification**: Adjustment of the image size.
- **Focus**: Adjustment of the image focus.

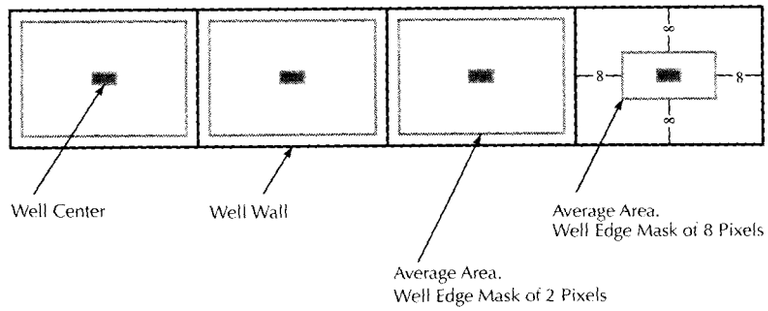


Figure 5-14 Well-edge masking

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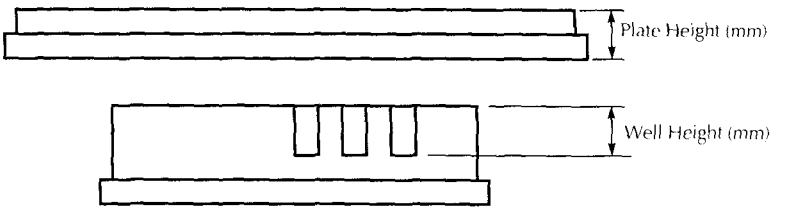


Figure 5-15 Plate height versus well height

The CLIPR defaults to preset plate definitions that may require adjustment upon installation. The process begins with taking an image (refer to Section 5.2.4). Select the **Close Door 90%** option on the **Setup Assay** window, which allows the introduction of limited light. Then select the **Load Plate Manually** option. Depending upon the lighting conditions of the room, use a 1- to 10-second exposure time. Load a white plate into the plate-positioning stage and take the image. Lengthen the exposure time if the image is overly dark. If the image is overly bright, adjust the grayscale slider bars to the right. Continue this process until the plate becomes visible in the image.

Select the **Grid** button on the toolbar  to display the plate grid (Figure 5-16).

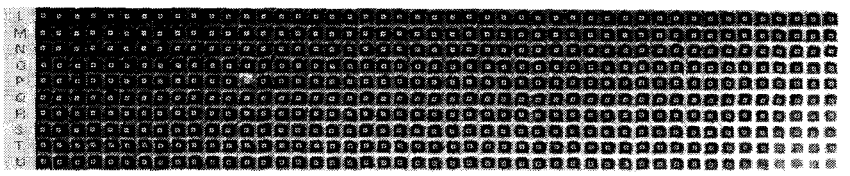


Figure 5-16 Plate image with center grids

The green squares that appear on the image represent the well centers. For perfect plate alignment with the well centers, ensure clear visibility of the wells on the image and a sharp

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focus. Adjust the focus parameter 10 to 20 steps at a time in either direction and retake the image until a sharp focus is achieved. The center of a plate equates to columns 24 and 25 and rows P and Q with a 1536-well plate. Move the wells toward the well-center grids by adjusting the X and Y step parameters.

Continue to retake the image until the center wells align over the center grids. If the left-edge well positions left of center, and the right-edge well positions right of center, decrease the magnification parameter by selecting the **UP** button on the **Setup Plate** window. Adjust this parameter 10 to 20 steps at a time. If the left- and right-edge wells both position inside center, increase magnification through the down arrow on the **Setup Plate** window (Figure 5-17).

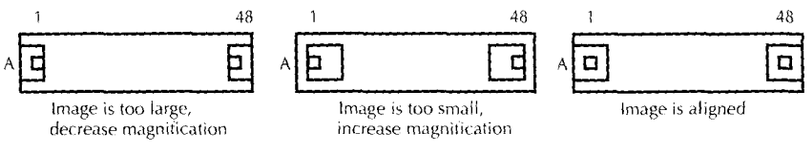


Figure 5-17 Well-edge alignment

Once achieving desired magnification, the focus may require adjustment. Upon completion, all wells will have the green grid dots in their centers. With use of a 1536-well plate for the alignment, the 384- and 96-well plates will most likely use the same parameter values for the X and Y steps, magnification and focus. Select the plate in the **Setup Plates** window and modify the parameter values. Select **Save Plate** to complete the process. Verify the plate and well height parameters and confirm the alignment of each plate type by taking sample images.

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5.2.2 Twister Plate Configuration

The Twister requires its own size information for the plate. The CLIPR software loads all parameters when the user clicks the **Configure For Twister** button and follows the subsequent prompts. Select the **Use Lids** option before clicking the **Configure for Twister** button if the Twister will be required to move lids as well as plates. The following list describes the Twister plate-configuration parameters for information only:

- **Plate Height**: The height of a plate in Twister steps.
- **Stacked Height**: The difference between the height of two stacked plates and 2X Plate height.
- **Lid Height**: The height of the lid used.
- **Plate Lid Height**: The combined height of a plate with lid.
- **Stacked Lid Height**: The height of a plate stacked on a lid.

When a user clicks the **Configure For Twister** button without the **Use Lids** option, the dialog shown in Figure 5-18 will appear.

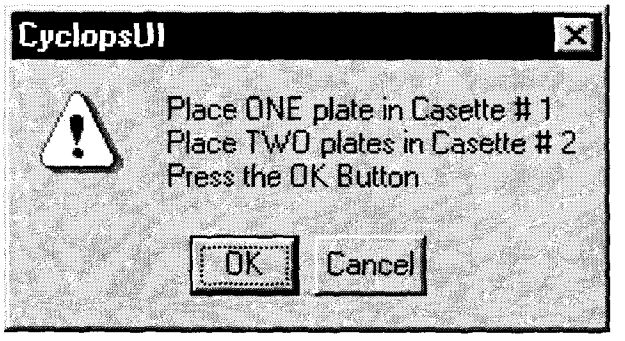


Figure 5-18 The **Configure for Twister** dialog when **Use Lids** is not selected

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When the user clicks the **Configure For Twister** button along with the **Use Lids** option, the dialog shown in Figure 5-19 will appear.

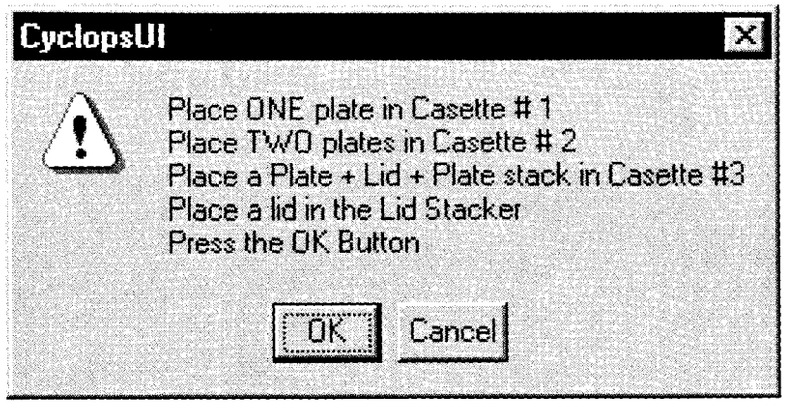


Figure 5-19 The **Configure for Twister** dialog when **Use Lids** is selected

If all instructions are followed properly, the CLIPR will compute all plate-size parameters.

5.2.3 Preferences

Use the **Preferences** window to set options applicable throughout the application. The window will display the following parameters (see Figure 5-20).

1) **AutoSave Directory Path**: This is the folder where data files are stored during multiplate assays using the robot handling system. Use the **Browse** button to select a path.

2) **AutoSave File-Name Format**: Use this file-name format to save data during a multiplate run. Select any or all of the following options in any order as long as an option other than **Assay Name** is chosen. This ensures a unique file name. Note

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that substitution of **Date/Time** will occur if a barcode fails to scan during an assay. Select the **Clear** button to clear the file-name format if the order of the options requires changing. A sample file name will appear in the box below the buttons as the user selects them.

- **Assay Name**: The name of the assay.
- **Date/Time**: The date and time the data was saved.
- **Cassette/Plate**: The robot cassette and plate number.
- **Barcode Number**: If scanning barcodes, this will contain the text equivalent of the barcode data.

3) **Assay Image Settings**: These settings determine the default state of the following options:

- **Save Image**
- **Subtract Dark**
- **Filter Cosmic Rays**

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- **Export Labels**: When selected, export files will contain well labels (1 = number of columns; A = number of rows). When not selected, the system will export only well data.

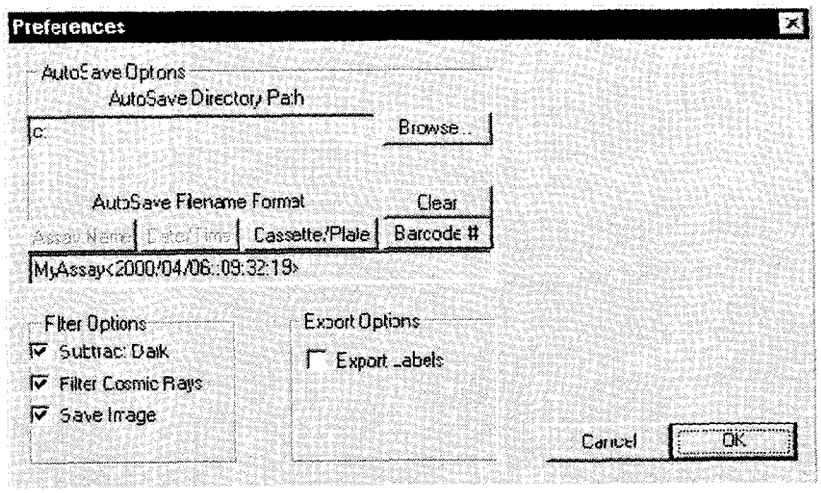


Figure 5-20 The **Preferences** window

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5.2.4 Setup Assay

To display the **Setup Assay** window (Figure 5-21), choose **Setup/Assay** from the menu bar or click the corresponding button on the toolbar .

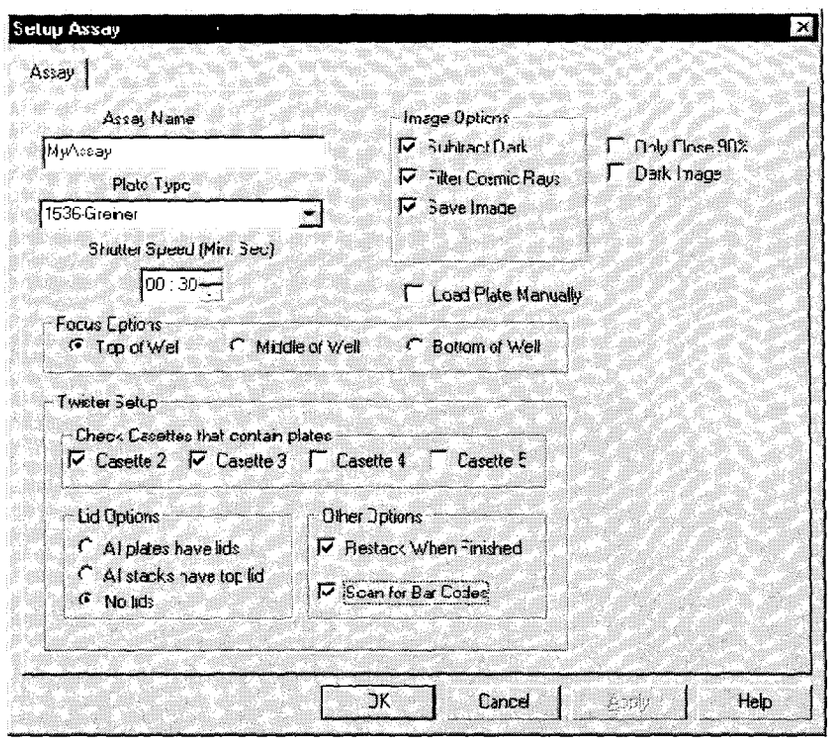


Figure 5-21 The **Setup Assay** window

An assay definition has the following components:

1) **Assay Name**: The name by which the user refers to the assay.

2) **Plate Type**: The plate definition determined by the user.

3) **Shutter Speed**: The length of time the user wishes the shutter to remain open.

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4) **Focus Options**: The portion of the well upon which the user wishes to focus (i.e., top, middle or bottom).

5) **Image Options**: The quality and storage of the image data.

- **Subtract Dark**: Subtracts background noise from image data.
- **Filter Cosmic Rays**: Removes cosmic rays from the well-average areas.
- **Save Image**: Saves image data to a 512K file along with the assay data. If not selected, the system saves only the assay data. The image remains viewable until the user closes the assay. When reopened, CLIPR generates and displays an image from the well-average data.

6) **Only Close 90%**: Allows light into the system when taking an image. The plate-positioning stage door only partially closes—primarily used in plate alignment.

7) **Twister Setup**:

- **Load Plate Manually**: For manual insertion of the plate into plate-positioning stage.
- **Check Cassettes**: The CLIPR will process all plates in each cassette selected.
- **Lid Options**: The user selects a lid configuration from the following options: (a) **All Plates Have Lids**, (b) **All Stacks Have A Top Lid** or (c) **No Lids**.

Note: *A lid option cannot be selected if the user has not configured the lids for the plate (see Setup Plates for information on configuring the plate for lids).*

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8) **Other Options**:

- **Restack When Finished**: Select this option to have all plates restacked in their original positions following completion of the assay.
- **Scan for Select Barcodes**: Select this option to have plates scanned for barcodes. Position the barcode on the topside edge of the plate, as shown in Figure 5-22.

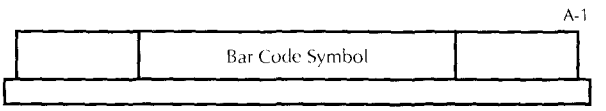


Figure 5-22 Proper location of the barcode symbol

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5.2.5 Running the Assay

Stack the plates in the Twister magazines. In order to properly orient plates in the instrument, the plates have to be stacked with well A1 on the side farther from the Twister arm.

Place the Twister magazine in the Twister platform (see Fig 5-23). Make sure the center hole of each magazine is fitted over the elevated disk on the platform, and the magazine is in place over the alignment peg (see Fig 5-24).

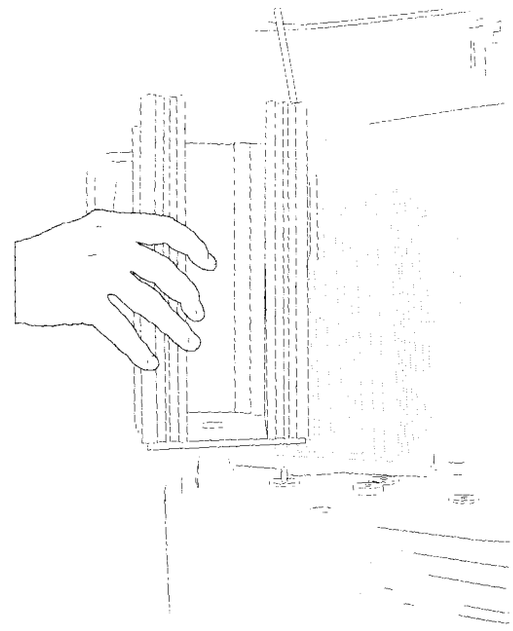


Figure 5-23 Placing a magazine in the Twister platform

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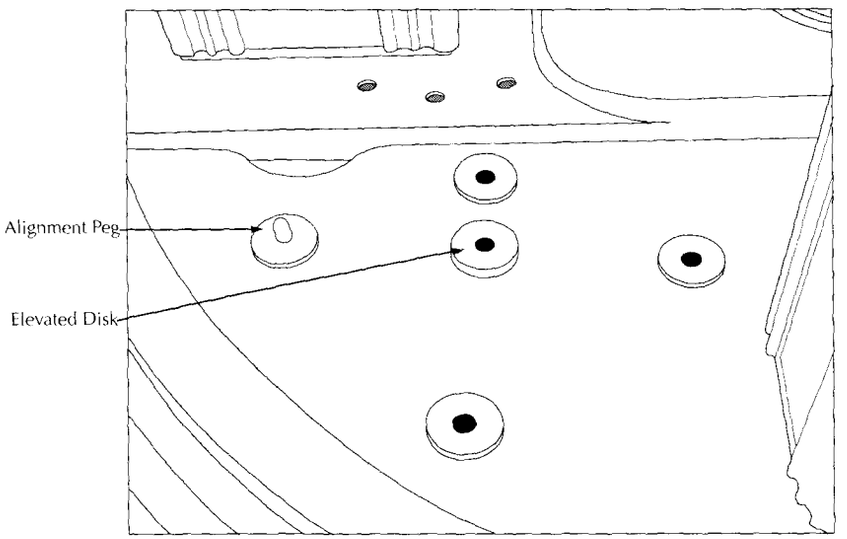


Figure 5-24 Aligning Twister magazines on the platform

Run the assay by clicking the camera button or choosing **Run/Assay** from the menu bar.

When performing a run with the Twister, the robot will move the plates into the plate-positioning stage and take the image automatically. The system will save the data files according to the **AutoSave** parameters specified in the **Preferences** window.

When performing a manual run, load the plate into the plate-positioning stage and click the **Camera** button or select **Run/Assay** from the menu bar. The stage will retract, the door will close and CLIPR will take the image. The manual load operation does not use the **AutoSave** feature, therefore the assay must be saved by clicking the **Save** button or choosing **File/Save** from the menu bar.

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5.2.6 Twister Setup

Molecular Devices preconfigures the Twister robot; however, final adjustments can be made at installation. If misalignment occurs between the Twister and the cassettes, calibrate the Twister by selecting **Setup/Twister** from the menu bar. This will launch the Twister Calibration Wizard (see Figure 5-25 through Figure 5-30).

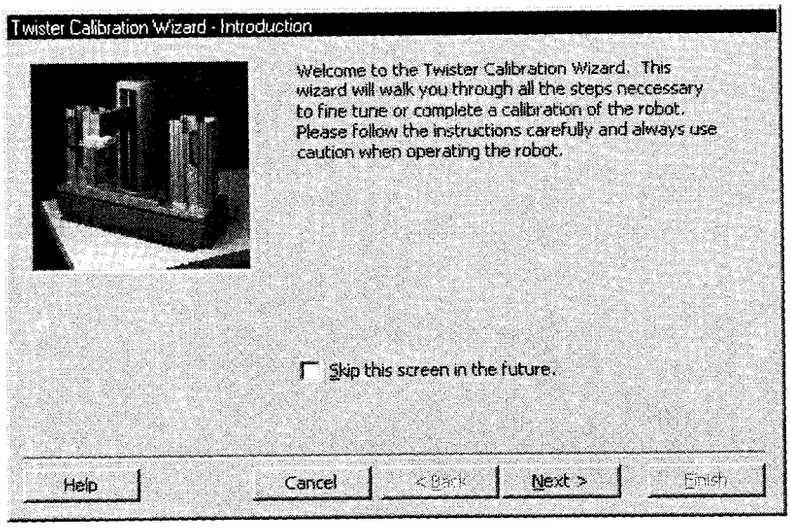


Figure 5-25 Initial **Introduction** screen of the Twister Calibration Wizard

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Select **Next** to begin the calibration.

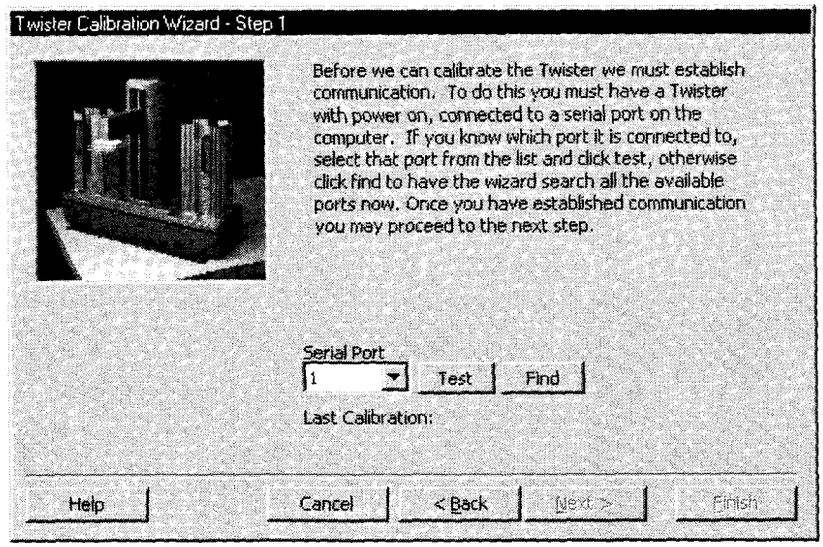


Figure 5-26 Twister Calibration Wizard – Step 1

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Select the **Test** button due to the Twister preconfiguration for Serial Port 1. Step 2 of the Twister Calibration Wizard will appear.

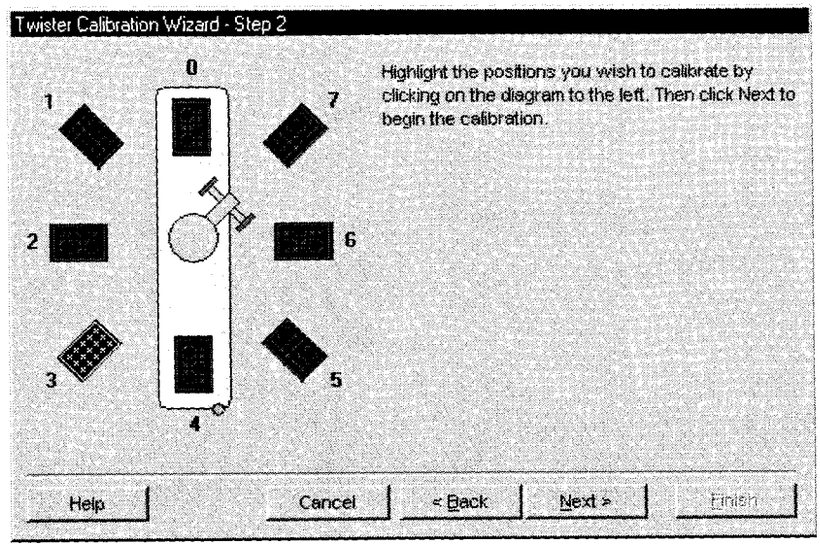


Figure 5-27 Twister Calibration Wizard – Step 2, part 1

Select the positions requiring calibration. All positions correlate to those used by the CLIPR system; however, note that the position numbers differ from those used for CLIPR operation. The CLIPR 1 through 5 cassettes correspond to the Twister Positions 4, 5, 6, 7 and 0. Position 2 represents the

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plate-positioning stage and Position 1 represents the lid stacker. Select the **Next** button.

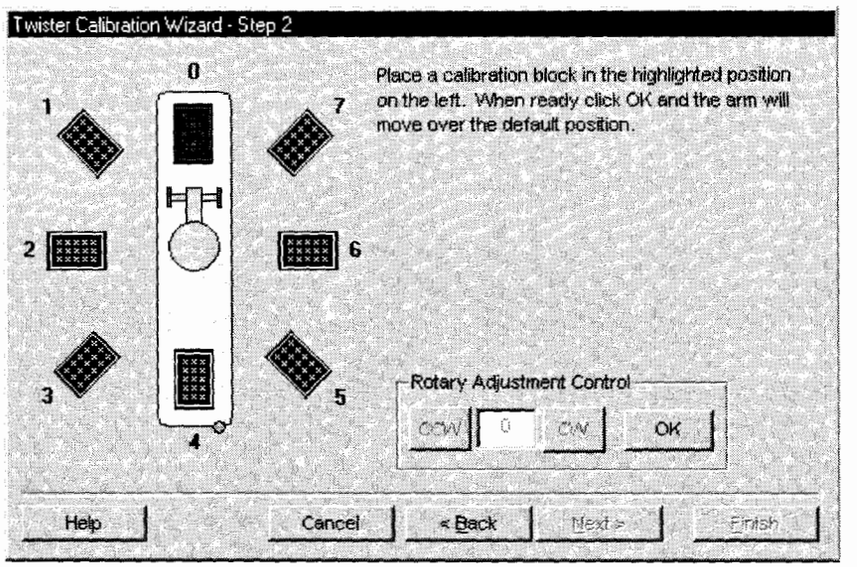


Figure 5-28 Twister Calibration Wizard – Step 2, part 2

Place the Twister calibration block in the position indicated in the Wizard window. The flat side of the block faces up, rather than the side with the notches. Select OK and the arm will move over the corresponding cassette. Select either the CCW (counter clockwise) or CW (clockwise) button to properly center the arm over the cassette. Position the left edge of the gripper 2 to 3 mm from the left (i.e., closed) edge of the cassette. Select **OK** and the arm will drop to the calibration block and then rise slightly. Readjust the arm if necessary until

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the gripper centers over the calibration block. Repeat this process for all selected positions.

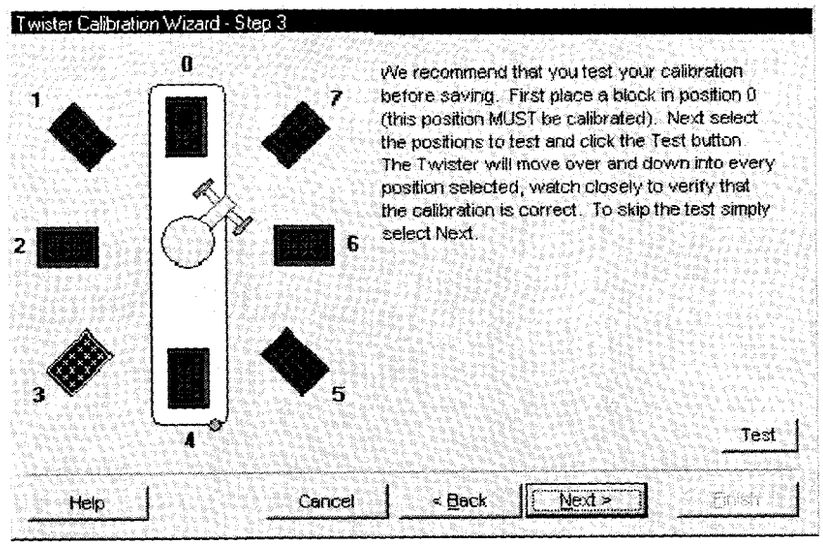


Figure 5-29 Twister Calibration Wizard – Step 3

To test calibration, select all positions shown in the Step 3 window (i.e., 1, 2, 4, 5, 6, 7 and 0). Place the calibration block in Position 0 with the flat side up and select the **Test** button.

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The Twister will move the calibration block into all selected positions. Select the **Next** button to complete the calibration.

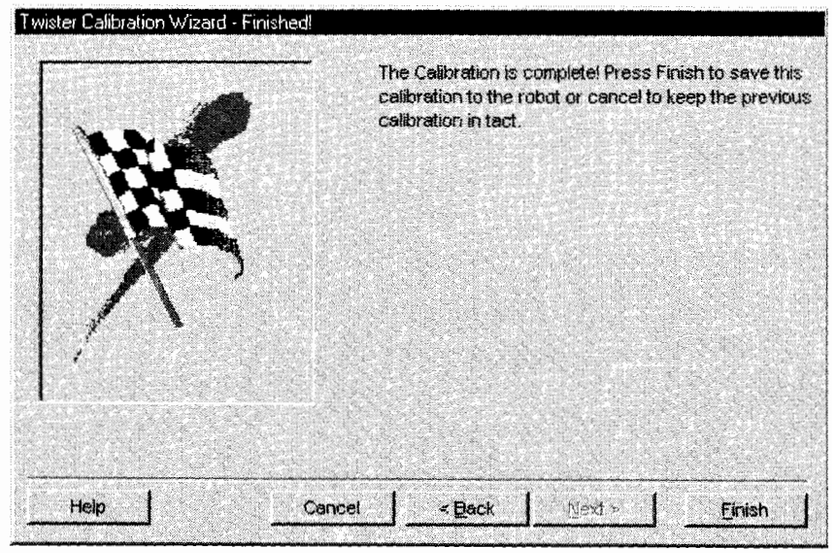


Figure 5-30 Final Twister Calibration Wizard window

If one or two positions require further calibration, repeat the entire process; however, select only the positions requiring further refinement. Then select all positions for the **Test** phase.

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Chapter 6– Maintenance

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6.1 Computer Backup

Although the computer provided with the CLIPR system has a large hard drive, the CLIPR image files are especially large (215K). Always back up the data files and remove them from the hard disk on a regular basis.

6.2 CCD Camera

Maintain the CCD camera vacuum below 0.025 Torr at -100°C, or 0.600 Torr at room temperature, as indicated in the right-hand corner of the software status bar. Contact Molecular Devices Service Department if the camera vacuum rises above these values.

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Chapter 7– Troubleshooting

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7.1 Start-Up

Symptom 1

The following error message appears on the computer screen: "Controller # (1 or 2), Motor # (1-4) Timed Out."

Solution

Verify that the green and yellow switches on the motor controller have illuminated. If not, a dialog box should appear requesting AC Power reset. Select the OK button and the switches should light. Contact Molecular Devices Technical Support if they do not.

Symptom 2

The following error message appears on the computer screen: "Camera Not Connected. Do you wish to continue?"

Solution

Exit the software, shut down the computer and power off the entire system via the main power switch. Turn the main power back on, and once the computer has completely booted, log in and restart the CLIPR user interface. Contact Molecular Devices Technical Support if the error message repeats.

Symptom 3

A loud, constant, high-pitched tone sounds following the camera connection.

Solution

The problem involves the CCD camera. Either the data cable has come loose or the Cryotiger has been cooling the camera without initialization of the controller,

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allowing the camera to become too cold. Turn off the camera controller — the bottom instrument inside the cabinet — to stop the alarm. Turn off the Cryotiger cooler and allow the camera to warm to room temperature for approximately 1 hour. Turn the camera controller back on. Check the lower right status window in the CLIPR software for the camera temperature. The temperature reading should indicate a minimum of 10°C. Exit and restart the CLIPR software to ensure a proper camera connection. An audible tone should sound three times. Turn the Cryotiger back on and allow the camera to cool back to –100°C for approximately 3 to 6 hours.

Symptom 4

The camera temperature does not reach -100˚C within 3 to 6 hours after powering up the system

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Solution

Check the back of the Cryotiger unit. The gauge should indicate a pressure between 245 and 280 psi. Call Technical Support for additional instructions.

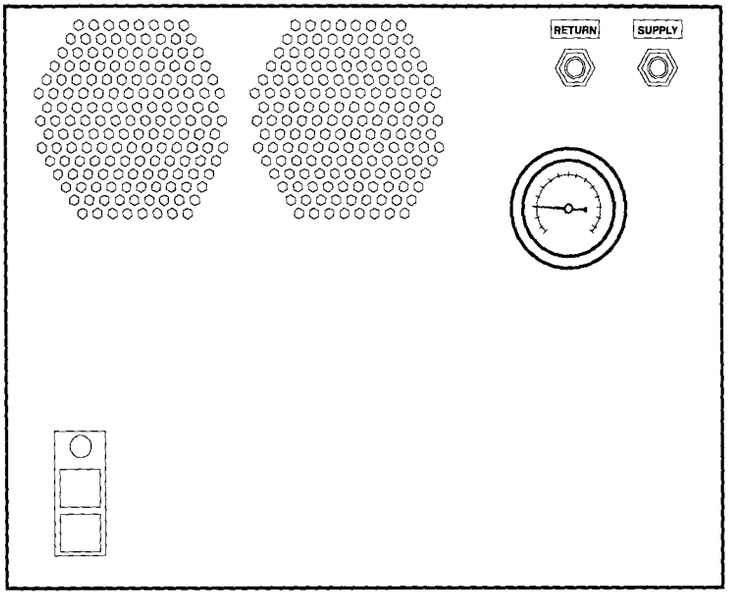


Figure 7-1 The back of the Cryotiger unit

7.2 Twister

Symptom 1

The arm stopped operating after an accidental bump.

Solution

Select the STOP button in the system software, restack all plates, create a new assay and run the operation again.

Note: *The system has saved all previously imaged plates, hence running only the remaining plates may suffice. If so, clear all previously imaged plates and run the remaining plates as a new assay. Change the* ***AutoSave*** *directory to a*

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*different folder if using only the Assay Name and Cassette #/ Plate # for your **AutoSave** name; otherwise, you may overwrite the existing plate data with the remaining plate data.*

Symptom 2

The Twister places the plates in the plate-positioning stage incorrectly.

Solution

Check the Twister alignment (see Section 5.1.5).

Symptom 3

The Twister collides with the cassettes.

Solution

Check the Twister alignment (see Section 5.1.5).

7.3 Image

Symptom 1

Previously saved assay reopened with a "blocky" image.

Solution

User did not select the **Save Image** option in the **Assay Setup** window; consequently, the system saved only the well-average data. The CLIPR generates an image from the well-average data in lieu of saved image data. Check the **Save Image** option to save a 512K image file as well as your assay data file. Then, upon reopening the assay, you will see the camera-generated image.

Symptom 2

The plate wells do not line up with the well-average squares or circles.

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Solution

Redefine your plate type (see Chapter 5).

Symptom 3

The CLIPR produces a dark, grainy gray image with no visible light.

Solution

Do not select the **Dark Image** option in the **Setup Assay** window. Use this option only for taking images without opening the camera shutter.

Symptom 4

All or some wells indicate a value of approximately 65,000.

Solution

Try a shorter exposure time to avoid too much signal. The maximum signal returned by the camera equates to 65,000.

Symptom 5

A large, bright fuzzy area appears at the bottom of the image that obscures the bottom of the plate.

Solution

Do not select the **Close 90%** option in the **Setup Assay** window. Use this option for plate alignment only to allow light into the system to enable visibility of an empty plate.

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7.4 Technical Assistance

For further technical assistance, contact Molecular Devices.

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Appendix A – CLIPR™ Technical Specifications

A.1 General Specifications

1) Ultra-high throughput luminometer system

2) Configurable for most commercially available 96-, 384-, 864-, and 1536-well microplates

3) Manual stand-alone workstation with optional robotic plate handler and stacker, or integrated to linear robot line

4) Doubly telecentric lens for simultaneous imaging of all microplate wells (patent pending)

5) Ultra-sensitive camera with self-contained cooling system

6) High precision x,y,z plate positioning stage

7) Light-tight enclosure

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8) Windows® NT-based control software

9) Dimensions:
Instrument: L27˜ x W34˜ x H88˜
Robotic plate handler/stacker: L27˜ x W34˜ x H54˜
Combined: L54˜ x W34˜ x H88˜

10) Power requirements:
110/120V: 4A 50–60Hz
220/240V: 2A 50–60Hz

A.2 Telecentric Optical System

1) 134 mm field of view

2) Anti-reflection coated optics

3) Background phosphorescence correction system

4) Electronic shutter

A.3 CCD Camera

1) Grade 1 back-thinned CCD detector

2) 1024 x 1024 pixels, 24 micron pixel size

3) Camera cooled to -100°C using self-contained compressed gas cooling system

A.4 Assay Performance

1) 0.1 fg luciferase in 1536-well plate detection sensitivity in 30 seconds integration time

2) Typical reporter gene assay time in 1536-well plate: 1–60 seconds

3) Typical SPA assay time in 1536-well plate: 1–10 minutes

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A.5 Optional Robotic Plate Handler

1) 80-plate capacity stacker/handler
2) Five removable plate cassettes
3) Laser barcode scanner
4) Light-tight enclosure
5) Cabinet assembly on casters with leveling feet
6) <30 second microplate cycle time from input stack to instrument and retrieve to output stack

A.6 Computer

1) APS 200 industrial chassis with 250W power supply
2) 266 MHz Pentium processor
3) 4.5 GB hard drive
4) 1.44 MB floppy drive
5) CD ROM drive
6) 64 MB RAM
7) Ethernet card
8) 19˜ high resolution color monitor

A.7 Software

1) Microsoft Windows® NT version 4.0
2) User-defined assay and plate setup files
3) Software-driven plate positioning, magnification and focus adjustment

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4) Individual well value averaging

5) Well average graphing

6) Pixel value graphing

7) Top, middle, and bottom well focus options

8) Dark background subtraction

9) Cosmic ray correction

10) Data export in tab-delimited ASCII format

11) Assay files 15 KB
Image files 505 KB

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