Preface

About the Documentation

Documentation Overview

This preface will familiarize the user with the ThermoFluor® 384 system documentation set and explains how to use these documents.

Specifically, this preface includes the following sections:

- *Introduction to ThermoFluor® 384 System Documentation*
- *Scope of these Documentls*
- *Standard Conventions*
- *Typographical Conventions*
- *Special Terminology*

The following table identifies the appropriate section to read in order to learn about specific aspects of the documentation set.

To learn more about ...	Read the section....	See page
Overview of the ThermoFluor® 384 System documentation	Introduction to ThermoFluor® 384 System Documentation	1-2
Scope of these documents	Scope of these Documents	1-2
Standard conventions	Standard Conventions	1-6
Typographical conventions	Typographical Conventions	1-6
Special terminology	Special Terminology	1-7

Introduction to ThermoFluor® 384 System Documentation

The ThermoFluor® 384 System Documentation is a set of documents covering all aspects of the ThermoFluor® 384 system necessary to support executing applications. This information is organized into four separate documents.

- The first document, " ThermoFluor® Instrument Operation" described above provides basic information on how to install and use the ThermoFluor® 384 System.
- The second document, "ThermoFluor® Analysis Software," provides basic information on how to use the ThermoFluor® analysis software. This Software provides a powerful way of analyzing the data generated by the ThermoFluor® 384 System.
- The third document, "ThermoFluor® Applications," covers the use of the ThermoFluor® 384 instrument and Acquire version 3.0 analysis software for applications in general.
- The fourth document, " ThermoFluor® Tutorials," is a series of tutorials to help a new user learn how to perform common tasks. These tutorials are arranged in the order of increasing complexity. They are covered in detail during ThermoFluor® training.

The Scope of these Documents

These documents are designed, with 3-Dimensional Pharmaceutical's training class, to provide instructions and information needed to facilitate someone with background knowledge in screening to use the ThermoFluor® 384 system. They should enable the user to perform various tasks and develop an understanding of how the system works.

ThermoFluor® 384 System Instrument Operation

This document describes the basic construction and function of the ThermoFluor® 384 system. It also describes the installation, operation and maintenance of the ThermoFluor® 384 system. In addition it contains background information in the Appendix sections. You can use this manual when you need information on how to operate the system or to train new users of the system.

The following table outlines the information contained within the Instrument Operation section of the documentation and which chapter to refer to for more information.

To learn more about ...	Read ...
ThermoFluor® 384 System	Chapter One, Introduction
Instrument specifications	
Safety features	
Overall system construction	Chapter Two, Construction and Function
Windows™ NT workstation (PC)	
ThermoFluor® 384 instrument	
Plate processing system	
Universal power supply	

Continued from previous page.

To learn more about ...	Read ...
Safety instructions	Chapter Three, Installation and Calibration
Site preparation	
System installation	
Calibration of spots	
Acceptance test	
Safe operation	Chapter Four, Operation
Operating software and controls	
Total Control for Windows™	
ThermoFluor® 384 Acquire 3.0	
ThermoFluor® 384 System operation	
System cleaning	Instrument Operation Chapter Five, Maintenance
Focusing the camera	
Adding/Changing filters	
Replacing the light source	
Troubleshooting	
Repairs	
Total Control for Windows Manual	Appendix A
Hudson Group, Plate Crane Manual	Appendix B
Consumables List	Appendix C

ThermoFluor® Analysis Software

This document describes how to analyze ThermoFluor® 384 system data using the ThermoFluor® Analysis Software. It describes basic ThermoFluor theory, software navigation and data analysis. It also describes the data exporting, batch processing of data, and DB sheet data browsing. You can use this documentation when you need information on how to use the ThermoFluor® Analysis Software or to train new users of the system.

The following table outlines the information contained within the ThermoFluor® Analysis Software section of the documentation and which chapter to refer to for more information.

To learn more about ...	Read ...
Introduction to the ThermoFluor® analysis software	Chapter 1, Introduction
The ThermoFluor® analysis software interface	Chapter 2, Getting Started
Starting the application	
Opening an intensity file	
Automated data analysis	
Plate properties	

Continued from previous page.

To learn more about ...	Read ...
ThermoFluor ++ graphical user interface	Chapter 3, Navigating Analysis Results
Menu and toolbar options	
Plate view	
Bar chart view	
Plot view	
Layout table	
Results table	
Summary table	
Averaging replicates	
Modifying analysis settings	Chapter 4, Customizing Analysis
Specifying reference wells	
Excluding data points	
Manual data analysis	
Saving into a file	Chapter 5, Output Options
Uploading into the database	
Exporting into a text file	
Printing	
Copying views and tables	
Batch analysis	Chapter 6, Miscellaneous Options
Batch registration	
Connect	
Requery plate info	
Edge detection	Appendix A, Dropout and Edge Detection
Dropout detection	

ThermoFluor® Applications Guide

This document provides fundamental information for basic applications associated with the ThermoFluor® 384 system. It describes the basics of protein preparation and high thru-put screening (HTS). It also describes some general data analysis methods. You can use this document when you need information on these applications with regard to the ThermoFluor® 384 system or to train new users of the system.

The following table outlines the information contained within the ThermoFluor® Applications Guide of the documentation and which chapter to refer to for more information.

To learn more about ...	Read ...
Overview of ThermoFluor® applications guide	Chapter 1, Introduction
ThermoFluor® GLPs	
Basic theory behind ThermoFluor®	
Introduction to ThermoFluor® optimization	Chapter 2, Optimizing ThermoFluor® Conditions
Condition optimization	
Process optimization	
Positive Controls	
Secondary Confirmations	
Introduction to ThermoFluor® High Throughput Screening	Chapter 3, High Throughput Screening
ThermoFluor High Throughput Screening	
ThermoFluor® 384 assay setup	
High Throughput Screening	
Maintenance and Quality Assurance	
Introduction to data analysis	Chapter 4, General Data Analysis
Hit selection	
Hit qualification	
Hit re-testing	
Secondary assays	

ThermoFluor® Tutorials

This document provides procedures and exercises for common tasks associated with the ThermoFluor® 384 system. It includes tutorials on protein characterization, sample preparation and data collection. It also includes validation and optimization tutorials. You can use this manual when you need information on these common tasks with regard to the ThermoFluor® 384 system or to train new users of the system..

The following table identifies the common tasks described in the ThermoFluor® Tutorials of the documentation and which Tutorial to refer to for practice in performing a given task.

To learn more about ...	Read ...
Preparing the assay plate	Tutorial One, Initial Protein Screen
Setting up and running the assay plates	
Analyzing the assay plate data	
Viewing the analysis results	
Determining the best concentration, mode, and dye	

Continued from previous page.

To learn more about ...	Read ...
Preparing the assay plate	Tutorial Two, Buffer/Salt Screen
Setting up and running the assay plates	
Analyzing the assay plate data	
Viewing the analysis results	
Determining the best pH, buffer, and salt concentrations	
Preparing the assay plate	Tutorial Three, Uniform Plate Screen
Setting up and running the assay plates	
Analyzing the assay plate data	
Determining existence of irregularities or systematic errors.	
Preparing the assay plate	Tutorial Four, Compound Library Screen
Setting up and running the assay plates	
Analyzing the assay plate data	
Initial identification of potential hits.	
Select hits	Tutorial Five, Select and Qualify Hits
Qualify hits	
Preparing the uniform plates	Tutorial Six, Screening Libraries
Setting up and running the accuracy test	
Setting up and running the accuracy test	
Analyzing the assay plate data	
Verifying data quality	

Standard Conventions

To make the documentation easy to read, the manuals use standard guidelines, or conventions. In other words, formatting for specific items within the text will always be presented in the same way. For example, software dialog box names are printed in Tahoma font and all caps, such as the OUTPUT FILE dialog box.

Typographical Conventions

The documents use the following typographical conventions for the analysis and control software applications.

Item	Depicted as:	For example:
Menu name	Tahoma font, all caps, bold	The **FILE** menu
Toolbar name	Tahoma font, all caps, bold	The **PAGER** toolbar
Menu command	Tahoma font, initial cap, bold	The **Open** file command
Toolbar buttons	Tahoma font, initial cap, bold	The **Selection** button
Dialog box name	Tahoma font, all caps	Type the desired file name in the PRINT dialog box
Dialog box field name	Tahoma font, initial cap	Number Of Copies
Text entry	Courier (PCL6) font	Enter the file name `Batch.int`
Keyboard keys	Tahoma font, initial cap enclosed in < >	Press and hold the <Ctrl> and <Shift> keys

Special Terminology

The following table provides definitions for some of the terms used in conjunction with ThermoFluor®.

Term	Definition
Hit	A well on a screening plate that exhibits a signal strength that exceeds the Tm shift threshold set as a significant response for the run.
***.int/INT** filee	File created by the ThermoFluor® Acquire 3.0 application which contains integrated intensity values for wells in a plate.
***.psq/PSQ** file	File that contains raw camera exposure data. The ThermoFluor® Acquire 3.0 application creates ***.int** integration files from the ***.psq** files that correspond to the replicate exposures for a well.
***.tf/TF** file	The ThermoFluor® Analysis Software creates this file. It contains the fitted data along with other values that are calculated and/or downloaded from a data base for each well on a plate.

The table below lists common acronyms that are used in conjunction with ThermoFluor®.

Acronym	Definition
TCW	Total Control for Windows program developed by Hudson Control Group

Table of Contents

Chapter One

Introduction

Overview

This chapter will familiarize the user with the ThermoFluor® 384 system and its safety features.

Specifically, this chapter includes the following sections:

- *Introduction to the ThermoFluor®384 System*
- *Instrument Specifications*
- *Safety Features*

The following table identifies the appropriate section to read in order to learn how to perform a specific task.

To learn more about ...	Read the section....	See page
ThermoFluor® 384 system	Introduction to ThermoFluor® 384 System	1- 2
Instrument specifications	Instrument Specifications	1-4
Safety Features	Safety Features	1-5

Introduction to the ThermoFluor® 384 System

The ThermoFluor® 384 system performs miniaturized fluorescence based thermal shift assays for the high throughput screening of compound libraries. The ThermoFluor® screening strategy has several advantages over the more conventional HTS methodologies, the most important of which is that it relies on a general physical process common to most, if not all, drug targets - thermal unfolding of biomolecules and their ligand bound complexes. The ThermoFluor® instrument measures dye fluorescence in the presence of a protein. As the protein unfolds its hydrophobic parts are exposed which creates an environment that causes the dye to fluoresce. The more the protein unfolds the greater the intensity of the fluorescence.

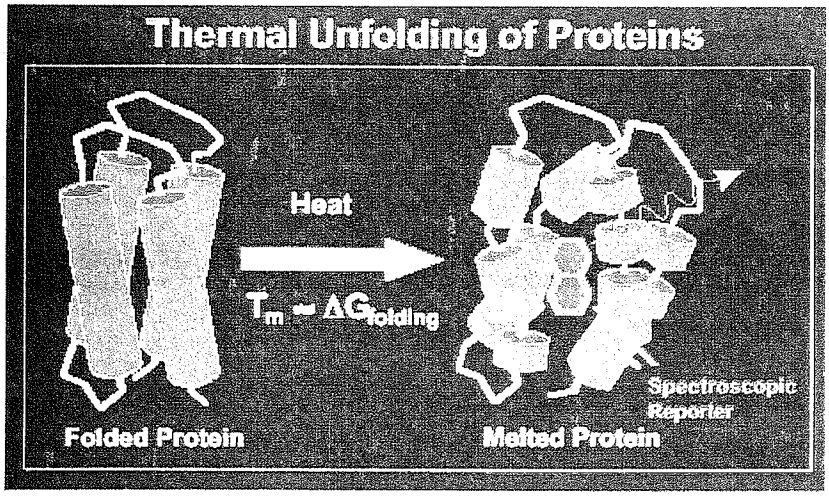


Figure 1- 1: The thermal unfolding of the protein allows the dye to enter a more hydrophobic environment and fluoresce.

In this manner the protein's melting transition can be measured. More importantly for the ThermoFluor® screening strategy the effects of ligand binding on the protein's melting transitions can be measured. See the Application Manual for more details on the theory behind ThermoFluor®.

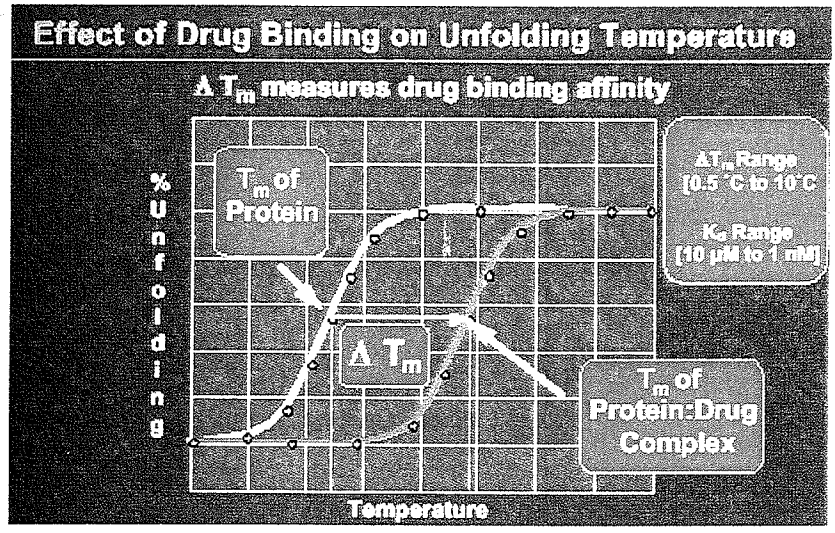


Figure 1- 2: Ligand binding shifts the unfolding temperature of the protein.

The ThermoFluor® 384 system provides a flexible and modular fluorescence assay system with an automated robotic microplate loading system to perform this function. The basic ThermoFluor® 384 system is capable of sequentially loading and unloading up to twenty (20) 384-well thermal cycler microplates. The ThermoFluor® 384 system works in conjunction with a personal computer running Total Control for Windows™ and 3-Dimensional Pharmaceuticals' ThermoFluor® Acquire 3.0 control software under Microsoft Windows™ NT.

The robotic microplate handler (PlateCrane™ by Hudson Control Group) moves sample plates from an input storage magazine to the imaging area of the instrument. As the microplate is removed from the input magazine a barcode reader reads the plate's bar code identification number. Once the microplate is in the imaging area the ThermoFluor® control software initiates a ThermoFluor® experiment on the microplate. When the experiment is completed, the robotic handler then retrieves the plate from the imaging area and places it into an output storage magazine. This process is repeated for up to 20 microplates in a single session. The PlateCrane™ robot and plate storage magazines (stacks) are optimized to handle a specified brand and model 384 well PCR plates.

Once the data have been collected the 3-Dimensional Pharmaceuticals' ThermoFluor® analysis software is used to analyze the data to determine melting temperatures and identify hits.

Instrument Specifications

This section gives the specifications of the ThermoFluor® 384 system and the conditions necessary for it to function properly.

Overall System Specifications

Height (H):		65"	(1575 mm)
Width (W):		24"	(610 mm)
Depth (D):		36"	(915 mm)
Weight:	Table Top	160 lbs.	(73 kg)
	Control Cabinet	300 lbs.	(136 kg)

Environmental Conditions

Storage Temperature	20° - 40° C
Storage Humidity:	20 - 80 %
Operation Temperature:	20° - 24° C
Operation Humidity:	30 - 50 % relative (non-condensing)

Power Supply Ratings

Line Voltage	110 VAC +/-10%
Frequency	60 Hz +/-10%
Power	15 A
	Properly grounded outlet

Windows™ NT workstation

Hewlett-Packard Vectra™ Pentium III, 300 MHz or better, 128 MB RAM, hard disk with 10.0 GB or better
SVGA monitor with 800 x 600 resolution
3 1/2" floppy disk drive
CD-ROM
250 MB Iomega ZIP™ Drive
2 available PCI slot (one for camera card and one for Rocketport™ serial expansion card)
Microsoft Windows™ NT 4.0 with Service Pack 6.0

Safety Features

The ThermoFluor® 384 system should be setup and run by 3-Dimensional Pharmaceuticals Inc. factory trained personnel only. If you are not factory trained, immediately contact 3-Dimensional Pharmaceuticals, Inc. by e-mail at engsupport@3dp.com to schedule training.

This Operating Manual contains information and procedures for the safe installation and operation of the ThermoFluor® 384 system. Before installing or running the ThermoFluor® 384 system, completely and thoroughly review the latest version of this document. If any instructions are unclear or unfamiliar to you, immediately contact 3-Dimensional Pharmaceuticals, Inc. by e-mail at engsupport@3dp.com for clarification and proper operation.

Abnormal instrument operation should be immediately noted and instrument use discontinued until examined by a representative from 3-Dimensional Pharmaceuticals, Inc.

As with all laboratory equipment, follow all standard operating procedures and policies. An operations and maintenance logbook should be maintained at the instrument's location.

Handle with Care

To avoid equipment damage handle components carefully. Handle the CCD Camera with particular care, as it is fragile.

Lift Properly

Use proper lifting techniques when lifting shipping containers and system components. Four (4) persons, positioned at each corner, should be used when lifting the system stand and the instrument base plate.

Electrical

Ensure the system's universal power supply is unplugged and that all power switches are in the "OFF" position before performing maintenance.

Use an ESD (electrostatic discharge) wrist grounding strap and discharge cable per manufacturer's instructions during all signal cable connections to avoid damage to the equipment by static electricity.

UV light

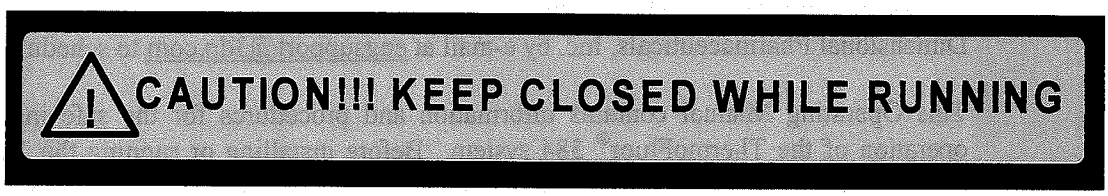
Avoid looking directly into UV light source as it may cause damage to the eye.

Labeling

Warning labels are located on the instrument to avoid potential dangerous situations to an operator or other lab personnel using the ThermoFluor® 384 instrument. Normal operational labels are provided to clarify function of the various indicator lights, switches and electrical connections used in normal operations. Each label, its location, and a brief description of the instrument operation associated with the label are listed below.

Light Tight Front Access Door Label

This label is located above the light tight compartment front access door of the ThermoFluor® 384 instrument. This door provides access to the light tight compartment for plate loading and unloading.



THE FRONT ACCESS DOOR IS NOT INTERLOCKED!!! Never open the front access door when the system active light is illuminated, during a sample run or when power is first applied to the instrument.

This door should only be open when plates are being loaded and unloaded from the

Plate Processing Area Warning Area Label

This label is located above the light tight compartment front access door of the ThermoFluor® 384 instrument. This door provides access to the light tight compartment for plate loading and unloading.

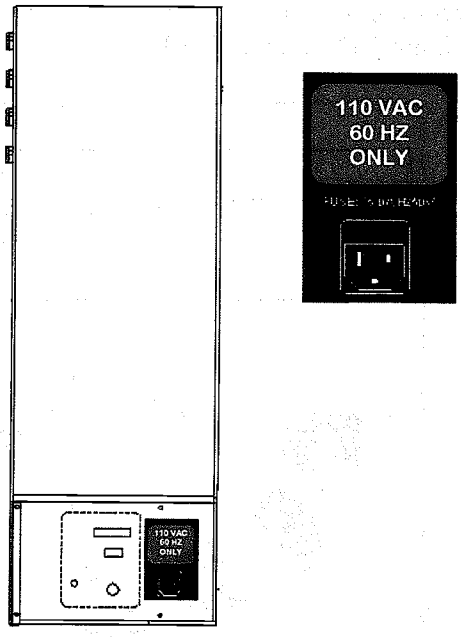
Never reach into the access door or place any items into the access door!!!

Normal operation should never require reaching through the access door into the light tight compartment. Such action is hazardous, and can result in injury or damage to the instrument.

Manufacturer's Serial Plate Label

This label in located on the right side of the ThermoFluor® 384 instrument. These connections should be made with no power applied to either the computer or the instrument. In addition there is labeling for the power entry module, "110 VAC, 60 Hz ONLY" &"Use 10A, 250 VAC Slo-Blo Fuses only!!"

Provide only 110 VAC, 60 Hz, single-phase electrical connection. Other operating voltages and frequencies may cause damage to the instrument and / or unexpected system performance.



A ground stud label is also provided to indicate the ground stud located on the side instrument panel.

PlateCrane™ Label

Labels are located on the side of the PowerCrane™ indicating its DB-9 serial control connection, AC power connection and manufacturer's serial number."

Bar Code Reader Label

Labels are located on the side of the Bar Code Reader indicating its DB-9 serial control connection and power connection.

Ground Labels

Ground labels are located throughout the instrument. These labels indicate a chassis ground connection point.

Electrical Warning Labels

Electrical warning labels are located on the back instrument panel and inside the instrument. There are no user serviceable parts in this panel, and opening this panel can expose the user to unsafe voltages.

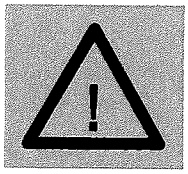
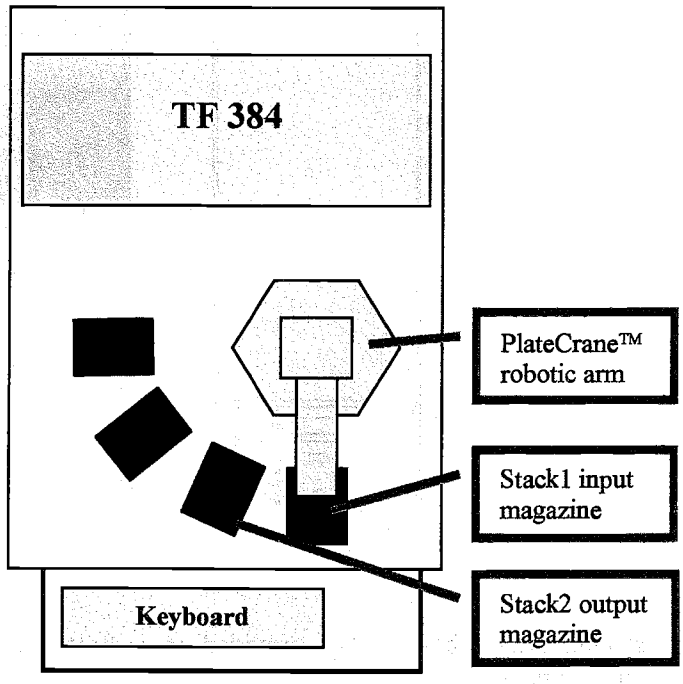
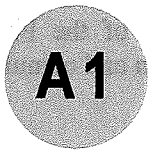


Plate Processing Labels

Several additional labels are best viewed from the top of the instrument. Specifically, plate orientation labels and input / output stack labels.



The plate orientation label locates well A1 on each plate placed in the instrument:



Connections

The ThermoFluor® 384 instrument CCD camera SCSI connection and controller serial connection should be made with no power applied to either the computer or the instrument. Similarly the plate processing PlateCrane™ and bar code reader cables should be connected with no power applied to either the computer or the instrument. The UPS inside the workcell must be turned off, even if no external power is applied.

Chapter Two

Construction and Function

Overview

This chapter will familiarize the user with the construction of the ThermoFluor® 384 system and the function of its major components.

Specifically this chapter will include the following sections:

- *Overall system construction*
- *Windows™ NT workstation (PC)*
- *ThermoFluor® 384 instrument construction*
- *Plate processing system construction*

The following table identifies the appropriate section to read in order to learn more about the construction of the ThermoFluor® 384 system.

To learn more about ...	Read the section....	See page
Overall system construction	Overall Construction	2-2
Windows™ NT workstation (PC)	Windows™NT Workstation	2-3
ThermoFluor® 384 instrument construction	ThermoFluor®384 Instrument	2-4
Plate processing system construction	Plate Processing System	2-9

Overall Construction

The ThermoFluor® 384 system is a flexible and modular fluorescence assay system with an automated plate processing system. The basic ThermoFluor® 384 system is capable of sequentially loading and unloading up to twenty (20) 384-well thermal cycler microplates. The ThermoFluor® 384 instrument works in conjunction with a Windows™ NT workstation running 3-Dimensional Pharmaceuticals' ThermoFluor® Acquire 3.0 control software to perform the fluorescence assay. The plate processing system has a PlateCrane™ robotic arm that moves sample plates from an input storage magazine to the imaging area of the instrument, after which the ThermoFluor® Acquire 3.0 control software program initiates a ThermoFluor® experiment on the microplate. When the experiment is completed, the Plate Crane retrieves the plate from the imaging area and places it into an output magazine. The various components or the system are powered by a universal power supply.

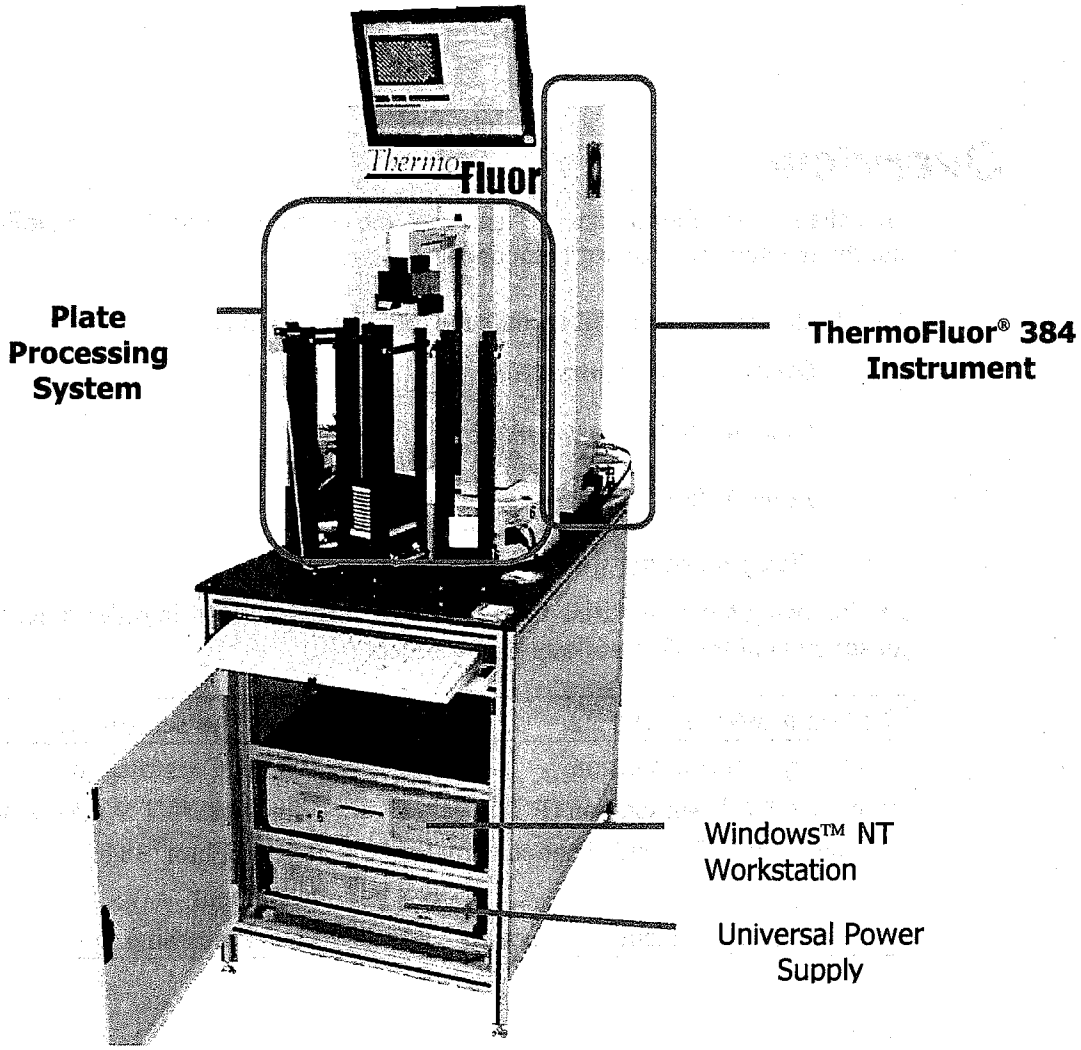


Figure 2- 1: The ThermoFluor® 384 system.

Windows™ NT Workstation

The Windows™ NT Workstation contains the software that controls the operation of the system and creates data files that are analyzed by the ThermoFluor® analysis software. The ThermoFluor® analysis software is usually installed on a separate analysis workstation. The ThermoFluor® analysis software will be discussed in the ThermoFluor® Analysis Software Manual. Total Control for Windows™ (TCW) operates the overall system and allows the running of multiple plates. It directly operates the plate processing system and it indirectly operates the ThermoFluor® 384 instrument through the ThermoFluor® Acquire 3.0 control software. The ThermoFluor® Acquire 3.0 control software interfaces with a CCD camera and an embedded controller in the ThermoFluor® 384 instrument. The ThermoFluor® Acquire 3.0 control software can be used to run a single plate without the use of TCW or the PlateCrane™ robotic arm. The software relationships are illustrated below.

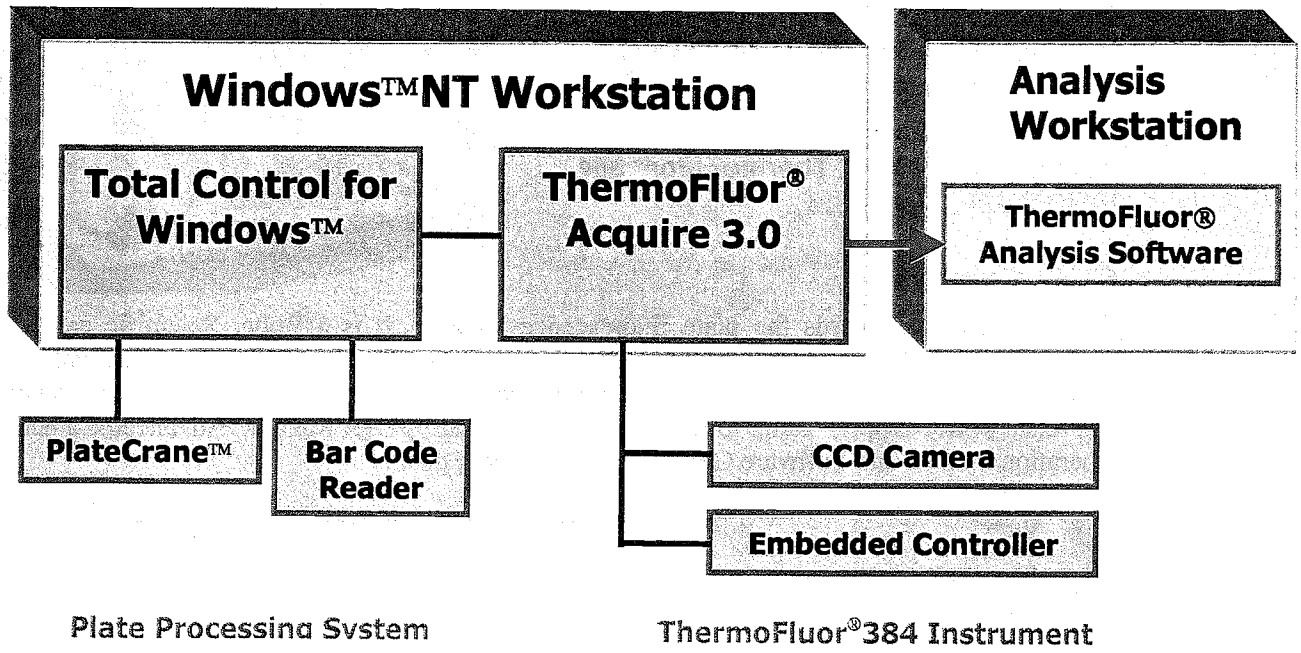


Figure 2- 2: Windows™ NT Workstation software applications.

Total Control for Windows™

This software controls the PlateCrane™ robotic arm movements and interfaces with the bar code reader. The method that is used to move the microplates from the input storage magazine to the imaging area then to the output magazine is programmed using this application. The program also interacts with the ThermoFluor® Acquire 3.0 control software to coordinate actions between the ThermoFluor® 384 instrument and the Plate Processing system. Refer to Appendix A for details.

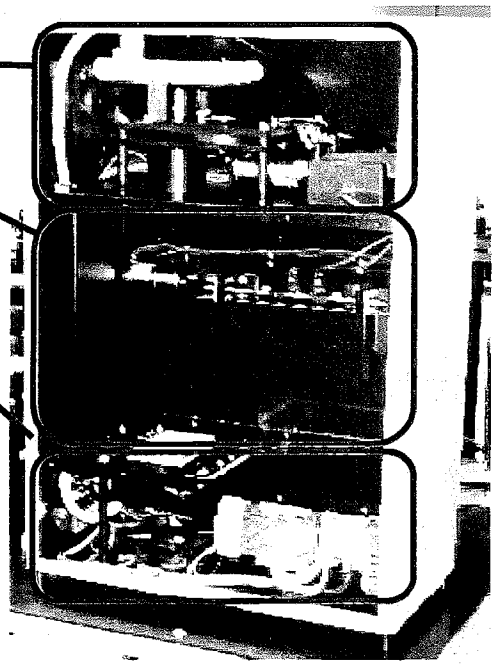
ThermoFluor® 384 Acquire 3.0

The ThermoFluor® Acquire 3.0 control software provides the user interface for the ThermoFluor® 384 system. This software allows the user to enter run parameters that are sent to the embedded controller that operates the instrument components. It also initiates the experiment and allows the user to designate where the resulting data will be saved. The details of this program and its user interface will be discussed in Chapter 4, Operation.

ThermoFluor® 384 Instrument

The ThermoFluor® 384 Instrument actually performs the fluorescence assays. It consists of three compartments. These are the:

- Optical compartment
- Light tight compartment
- Power and control compartment



Optical Compartment

The optical compartment contains the optical components that measure the fluorescence. These include the CCD camera, lens and filter wheel. This compartment is accessible by the user in order to change filters.

Figure 2- 3: Back of ThermoFluor® 384 Instrument.

CCD Camera

The CCD camera records the plate fluorescence images. It is a Roper Scientific camera model Sensys 0400. It has a half-inch mega pixel format. The CCD camera comes with its own power supply that is located in the optical compartment and plugged into the system Universal power supply. Refer to the Photometric's SenSys User Manual, Advanced Camera Operation Manual and Software Guide Manual for more details.

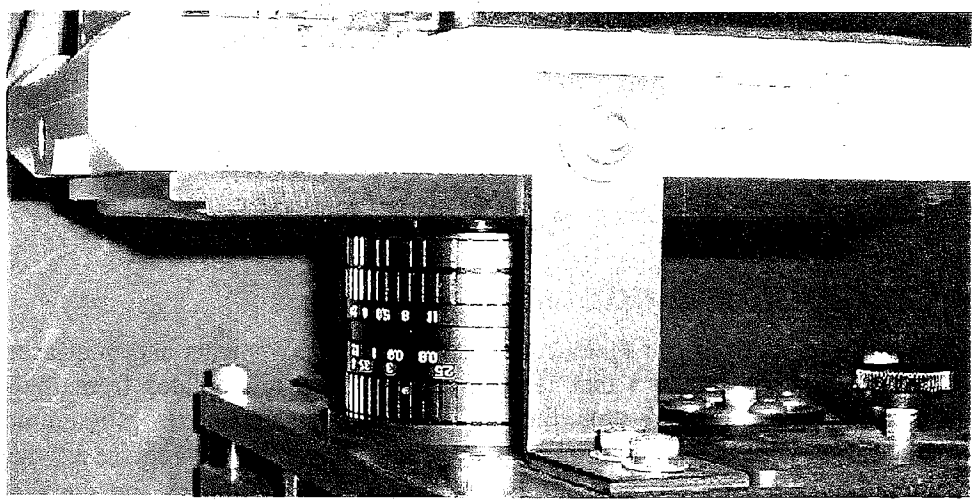


Figure 2- 4: CCD camera mounted in instrument.

CAUTION: The camera should never be connected or removed from a live power cable. This could damage the camera's electronic components. Verify the camera's power supply is switched OFF and unplugged from the Universal Power Supply before disconnecting its power cable.

Lens

The Lens focuses the image in the camera. It is a 25 mm F 0.95 C-mount lens with aperture and focus adjustment. **The aperture adjustment should always be set to wide open.** The focus adjustment will be discussed in Chapter 3, Installation and Calibration and Chapter 5, Maintenance.

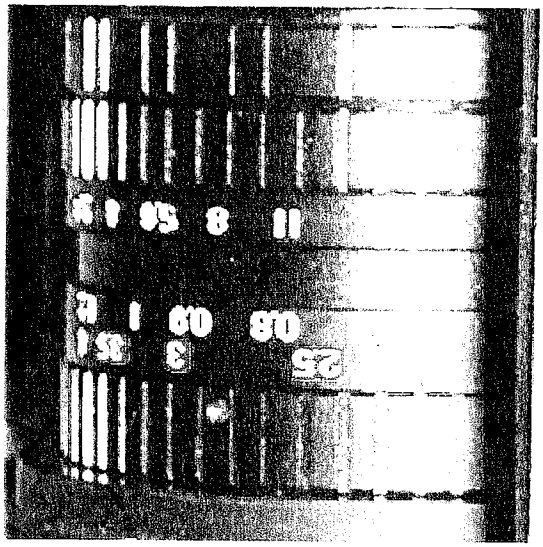


Figure 2- 5: 25 mm lens for CCD camera.

Filter Wheel

The filter wheel allows for various optical filters to be used in the instrument to provide the capability of measuring images from more specific regions of the UV spectrum. The filter wheel can hold 4 filters. The ThermoFluor® 384 instrument is supplied with a standard ThermoFluor filter in position 1. Oriel 50 mm by 4 mm filters are recommended. The embedded controller discussed below controls the positioning of the filter wheel.

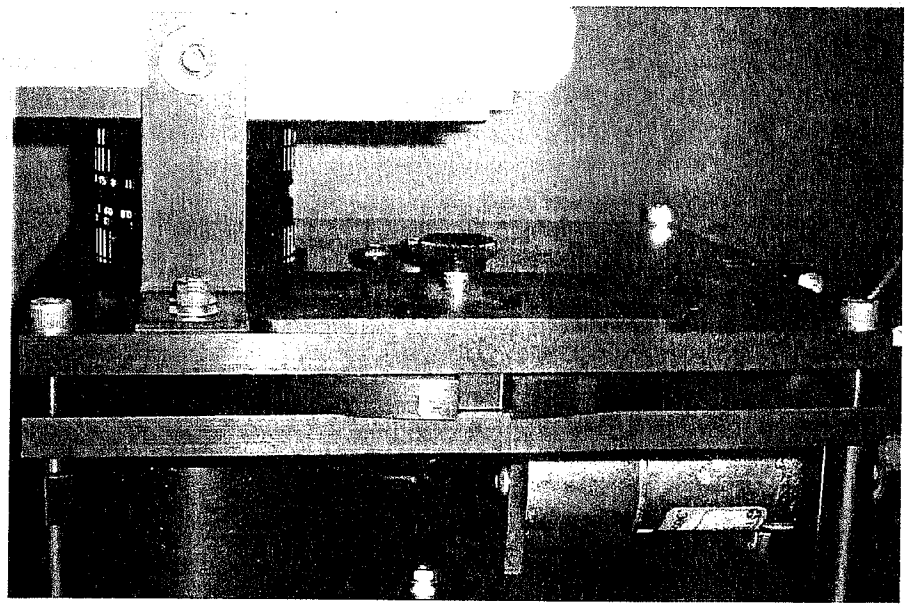


Figure 2- 6: Filter wheel in position 1.

Light Tight Compartment

The light tight compartment contains an UV light source and the thermal cycler's block that heats the microplates. A light tight access door opens and closes to allow the PlateCrane™ to place the microplates on the Thermal cycler's block and then remove them. Rear access to this compartment is limited to those trained in instrument maintenance.

UV Light Source

The UV light source provides a uniform, consistent source of UV light between 350 and 410 nm. The light source is turned on and off by the embedded controller. To ensure proper operation regular bulb service and replacement is necessary. See Chapter 5, Maintenance. Refer to the light source specifications for more details.

Caution! Do not look directly into UV light source.

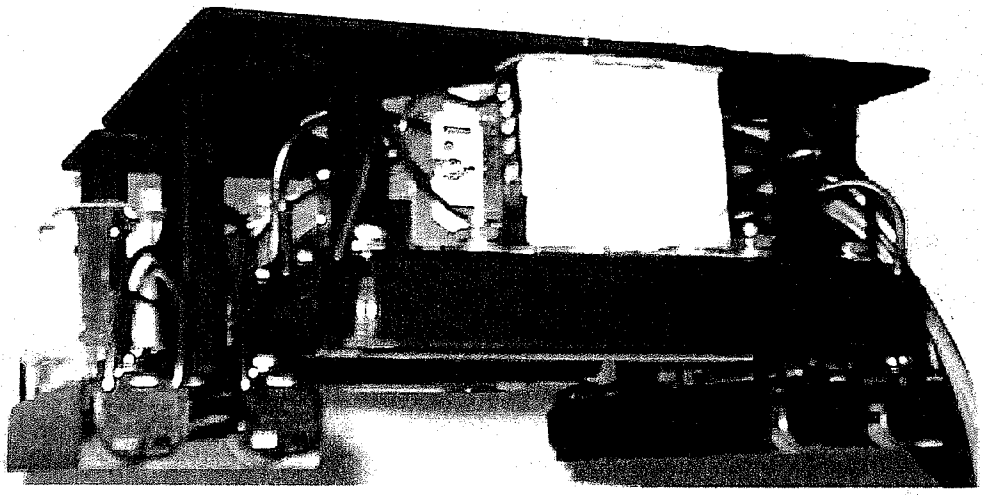


Figure 2- 7: UV lamps viewed as they appear in the instrument.

Thermal Cycler Block

The thermal cycler heats and cools the microplates to the prescribed temperatures. The thermal cycler block is designed to work with 384 well microplates to provide efficient and effective temperature control. See Thermal Cycler under Power and Control Compartment below.

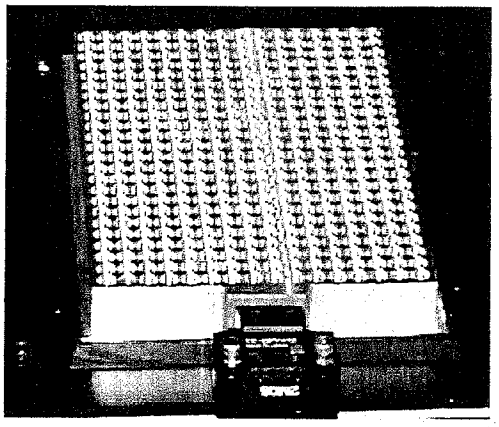


Figure 2- 8: The top of the Thermal Cycler blok.

Light Tight Door

The light tight door provides access to the top of the thermal cycler so the PlateCrane™ can place the microplates on the heater block. The door is opened and closed by the embedded controller. Its opening is programmed to work in conjunction with the PlateCrane™. **Caution!! Do not place hand(s) into the open door when running in batch mode as it could close and cause injury.**

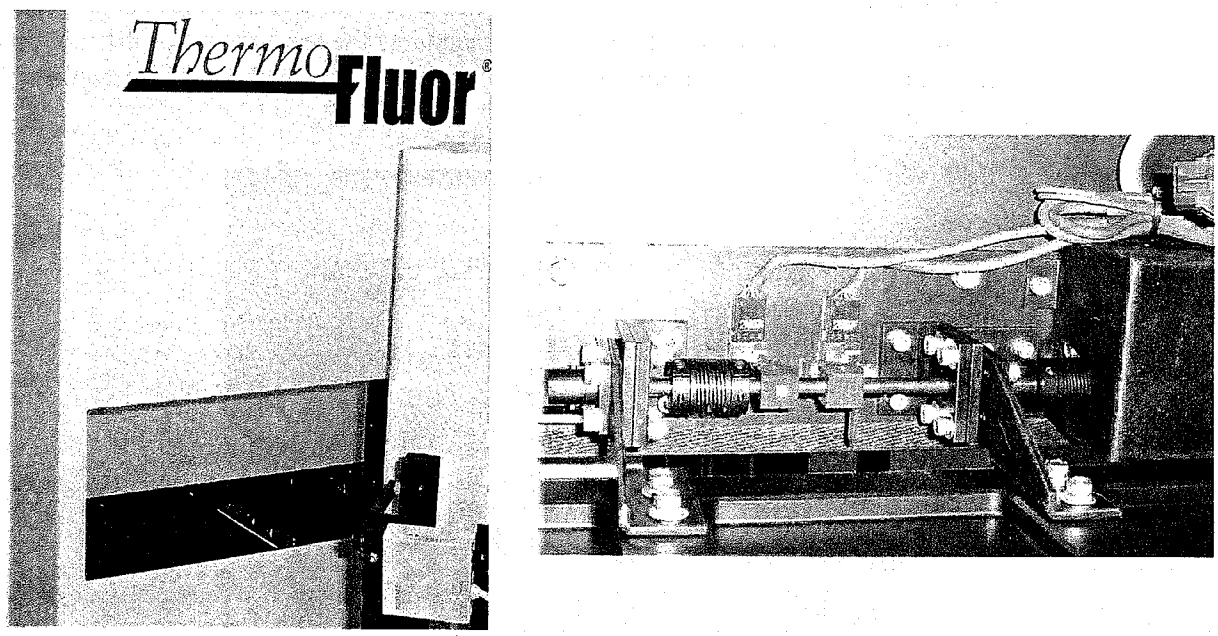


Figure 2- 9: The light tight door and it's drive motor.

Power and Control Compartment

The power and control compartment contains the rest of the thermal cycler assembly and the embedded controller. Because of the presence of high voltage components and the embedded controller, access to this compartment is limited to factory technicians.

Thermal Cycler

The thermal cycler assembly raises, lowers and maintains the temperature of the microplates as directed by the embedded controller. It is designed to work with a 384 microplate. The temperature is programmable from 4 to 99° C.

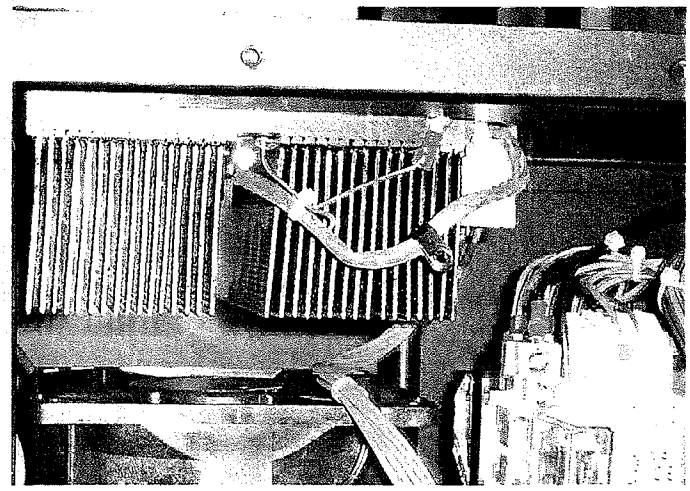


Figure 2- 10: The bottom of the Thermal Cycler and fan.

Embedded Controller

The embedded controller performs real time control functions for the ThermoFluor® 384 Instrument. It opens and closes the light tight door, turns the UV light source on and off, operates the filter wheel, and controls the temperature of the thermal cycler. The user interfaces with the controller through the ThermoFluor® 384 Acquire 3.0 software.

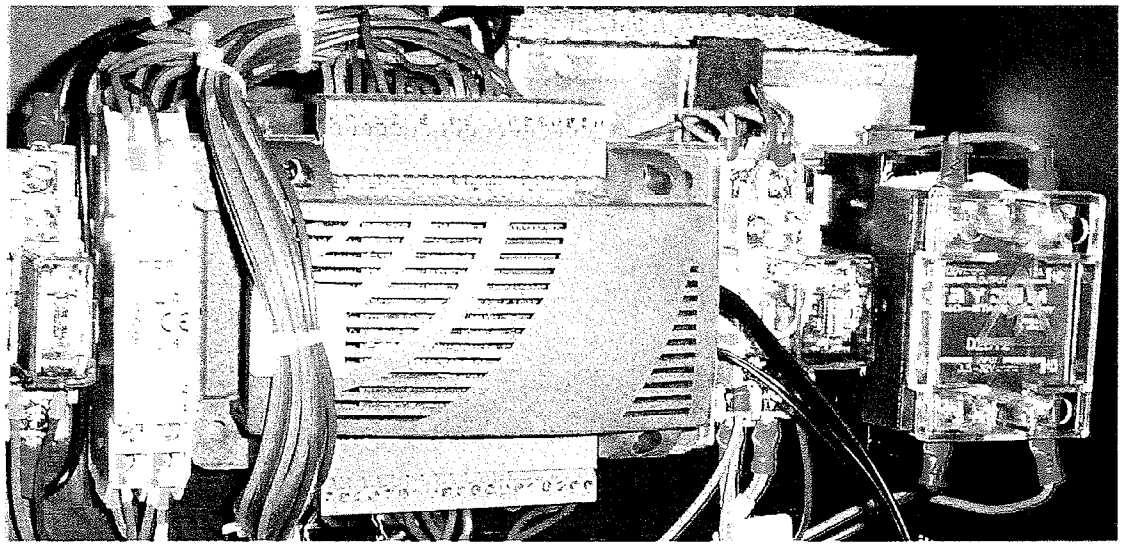


Figure 2- 11: The embedded controller performs real time functions.

Plate Processing System

The plate processing system consists of the input and output storage magazines, the PlateCrane™ robotic arm and the bar code reader. It is controlled by Total Control for Windows™. This system loads and unloads microplates for the ThermoFluor® 384 instrument.

Storage Magazines

The storage magazines are designed to hold up to 30 microplates in a stack for the PlateCrane™. The standard run is eight plates, which takes 24 hours in up/down mode. The magazines are held in position by magazine bases that are connected to the ThermoFluor® 384 system base plate. Four magazine positions are supplied with the system. Two are provided to hold plates for the ThermoFluor® 384 instrument and two magazines are provided to allow for the integration of a liquid handler. This allows for an input and an output stack for the ThermoFluor® 384 instrument.

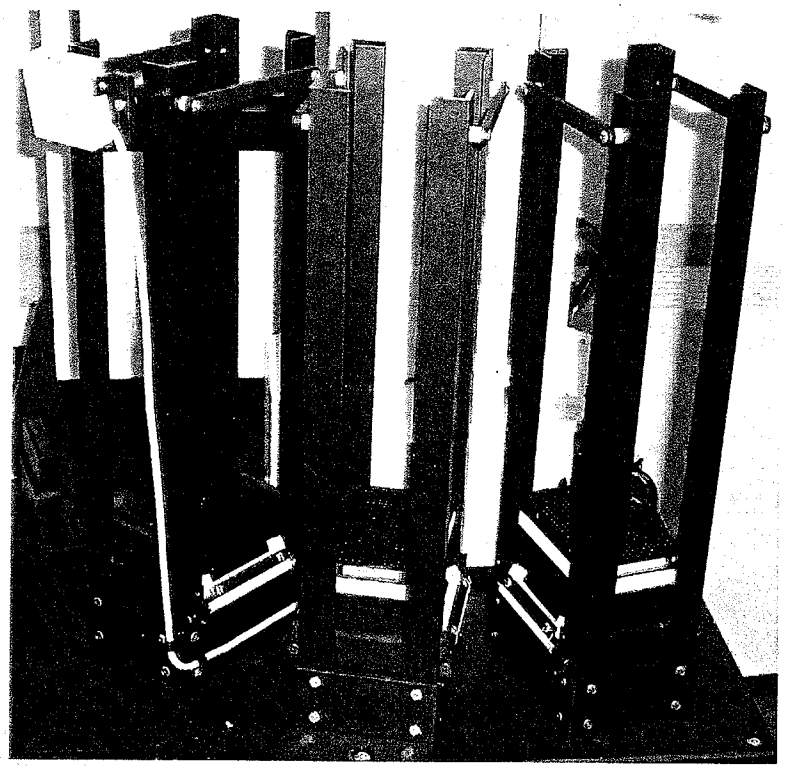


Figure 2- 12: Input, output and barcode reader magazines.

PlateCrane™

The PlateCrane™ robotic arm moves 384-well thermal cycler microplates from the input storage magazine to the thermal cycler block in the ThermoFluor® 384 instrument's light tight compartment. When the experiment is completed, the PlateCrane™ retrieves the plate and places it into the output magazine. This process is repeated for up to 30 microplates in each a single session. Alternatively, a lid can be used as a "topper" on the stack, if desired, to prevent sample contamination.

The PlateCrane™ has three degrees of motion. It rotates horizontally 345° about the R-axis, moves vertically19 inches along the Z-axis and the gripper rotates horizontally 350°about the P-axis. A stepper motor with encoder drives the movement in each axis. Each of these have a mechanical switch to determine the "Home" position. The R and Z-axis have hard stops. A serial cable from the rear of the PlateCrane™ connects (DB-9 connector) to a serial port on the Rocketport serial expansion card of the NT Workstation for communications. A rocker type power switch is located on the PlateCrane™ base. Power is ON when the switch is illuminated. See Appendix B for details about the PlateCrane™.

The PlateCrane™ gripper has two custom designed hands to securely grip the microplates. Two guides assist in centering the plate when it is picked up. A mechanical switch in the gripper indicates when the plate is located. The lip on the microplate activates this switch.

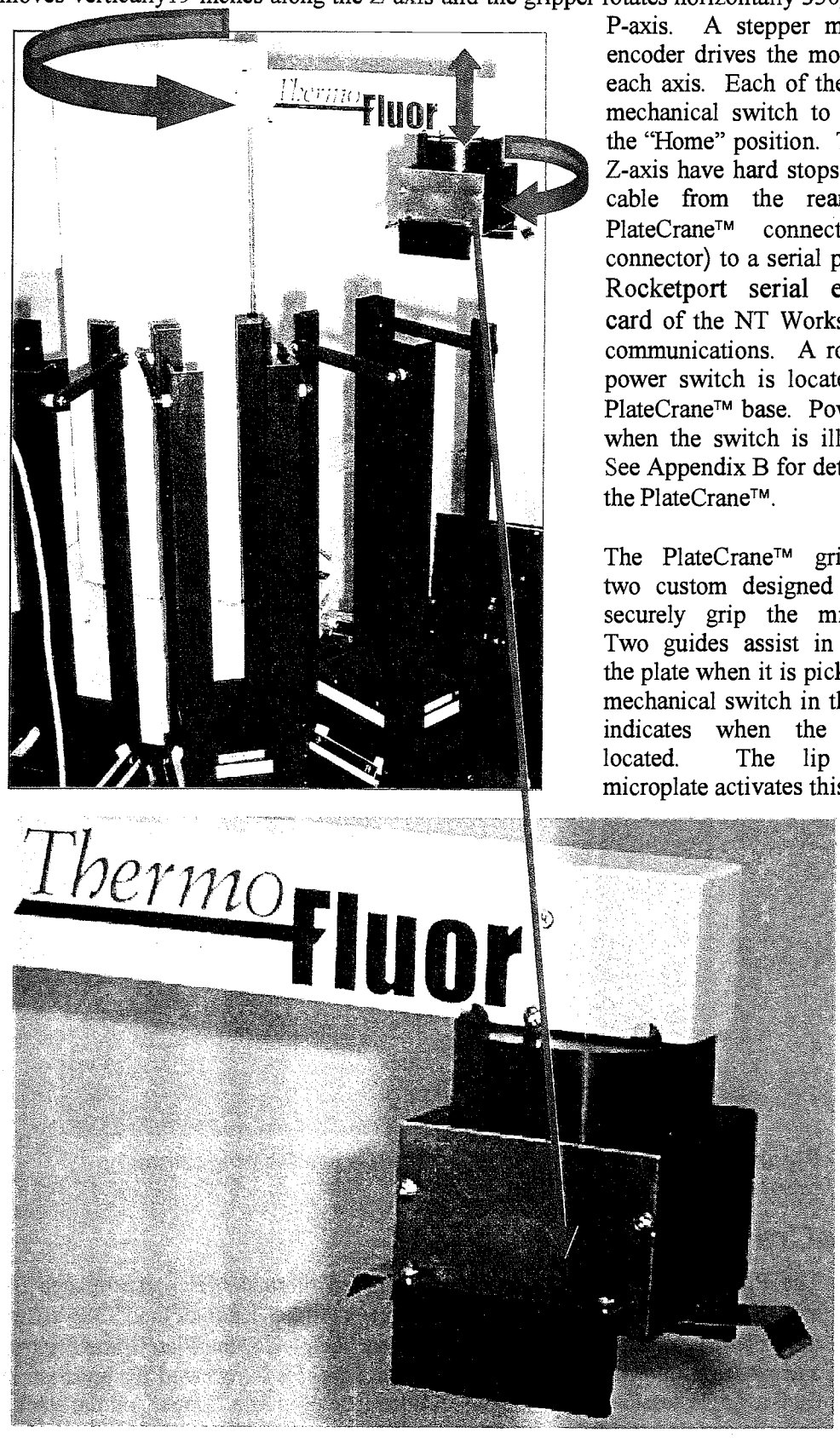


Figure 2- 13: The PlateCrane is the main component in plate processing.

Bar Code Reader

This is a standard Keyence BL 185 programmable bar code reader that reads 2 of 5 interleave. Each microplate has a bar code placed on it that identifies it to the system.

Figure 2- 14: 384 well microplate with barcode label.

This helps to ensure proper correspondence of plates to data. The barcode reader communicates with the NT Workstation though a serial cable connected to the control computer's Rockport expansion card

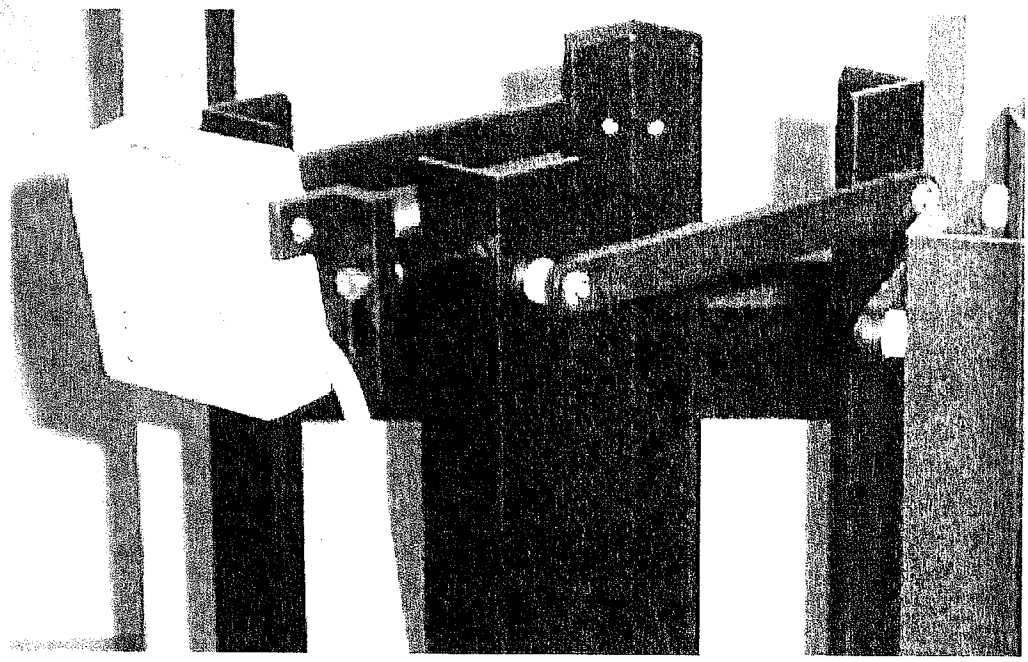


Figure 2- 15: Barcode reader records plate identification numbers.

Universal Power Supply

An APC Smart UPS universal power supply is used to provide stable uninterrupted power to the system. All system components should be plugged into this power supply to ensure proper operation. The power supply is then plugged into a standard 110-120 VAC outlet. The UPS is sized to provide 10 to 15 minutes of operating time. For continuous uninterrupted operation an emergency backup power source should be supplied. Refer to the APC Smart UPS Manual for additional details.

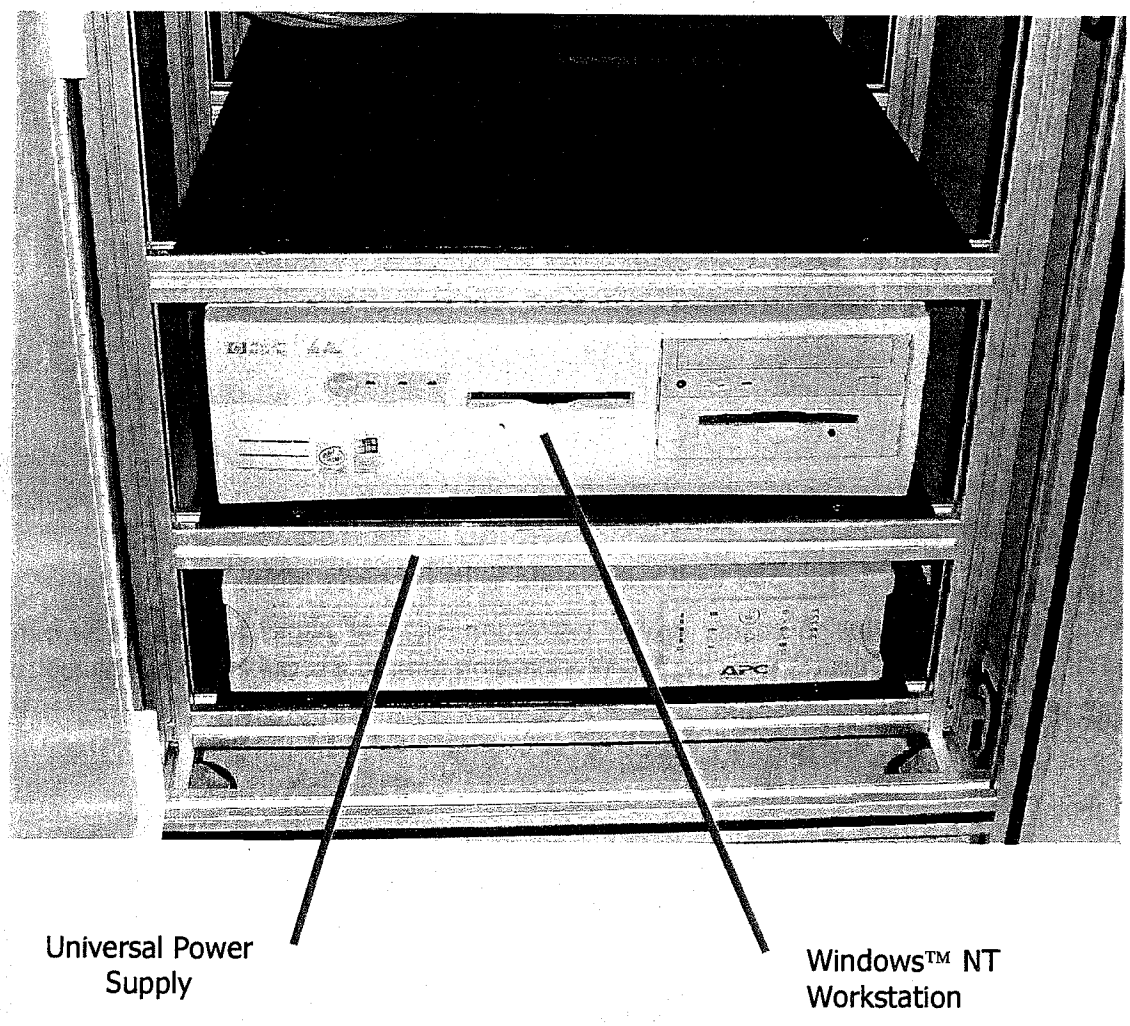


Figure 2- 16: Cabinet containing the universal power supply and Windows™ NT Workstation.

Chapter Three

Installation and Calibration

Overview

This chapter provides information and instructions for installing and calibrating the ThermoFluor® 384 system. It also describes how to perform an acceptance test for the system.

Specifically, this chapter includes the following sections:

- *Safety instructions*
- *Site preparation*
- *System installation*
- *Calibration of spots*
- *Acceptance test*

The following table identifies the appropriate section to read in order to learn more about a specific item.

To learn more about ...	Read the section....	See page
Safety instructions during installation.	Safety instructions	3- 5
Preparing a site for the system.	Site preparation	3- 3
Installing the system	System installation	3- 8
Calibrating the instrument.	Calibration of spots	3- 15
Performing an acceptance test for the system	Acceptance test	3- 19

Safety instructions

The ThermoFluor® 384 system should be set up and run by 3-Dimensional Pharmaceuticals Inc. factory trained personnel only. If you are not factory trained, immediately contact 3-Dimensional Pharmaceuticals, Inc. at 1-610-458-8959 to schedule training.

This Operating Manual contains information and procedures for the safe installation and operation of the ThermoFluor® 384 system. Before installing or running the ThermoFluor® 384 system, completely and thoroughly review the latest version of the Operating Manual. If any instructions are unclear or unfamiliar to you, immediately contact 3-Dimensional Pharmaceuticals, Inc. at 1-610-458-8959 for clarification and proper operation.

Please locate and identify all safety and informational labels on the instrument. If any labels are missing, smudged or damaged (see Chapter 1, Introduction) discontinue use and contact 3-Dimensional Pharmaceuticals, Inc. for replacements.

Please locate, identify and recognize all safety items on the instrument including covers and panels. If any items are missing, damaged or disabled in any way, discontinue use and contact 3-Dimensional Pharmaceuticals, Inc. for instrument repair.

Handle with Care

To avoid equipment damage handle components carefully. Handle the CCD Camera with particular care, as it is fragile.

Lift Properly

Use proper lifting techniques when lifting shipping containers and system components. Four (4) persons, positioned at each corner, should be used when lifting the system stand and the instrument base plate.

Electrical

Do not plug the universal power supply in until the installation procedure specifically instructs you to plug in the universal power supply. Then before plugging in the universal power supply verify that all power switches are in the "OFF" position. Verify that the wall outlet for the universal supply is 110 VAC, 60 Hz and properly grounded. Use a ESD (electrostatic discharge) wrist grounding strap and discharge cable per the manufacturer's instructions during all signal cable connections to avoid damage to the equipment by static electricity.

Site preparation

This section describes the site requirements for the ThermoFluor® 384 system to ensure proper function and access. It includes the recommended stand-alone placement and the alternate bench top placement

Overall Site Specifications

The ThermoFluor® 384 system should be located in a clean dry laboratory environment. The floor should be sufficiently level to provide for leveling the instrument front to back and sided to side with the supplied leveling feet. The ambient temperature should remain between 20° and 24° C. The relative humidity should be low enough to ensure a non-condensing environment. The system operates from a universal power supply that runs on 110 VAC, 60 Hz properly grounded outlet and should draw no more than 15 amps. The PC is equipped for connection to a network via a 10/100 Ethernet™ connector if desired.

Stand Alone Placement and Access

The footprint of the system is 24 inches wide by 36 inches deep. The system stands approximately 66 inches high. A minimum of 18 inches (24 inches recommended) should be provided on the side and back of the instrument to ensure adequate access for routine maintenance and operation. A minimum of 36 inches should be provided at the front of the instrument. See figure 3.1 below.

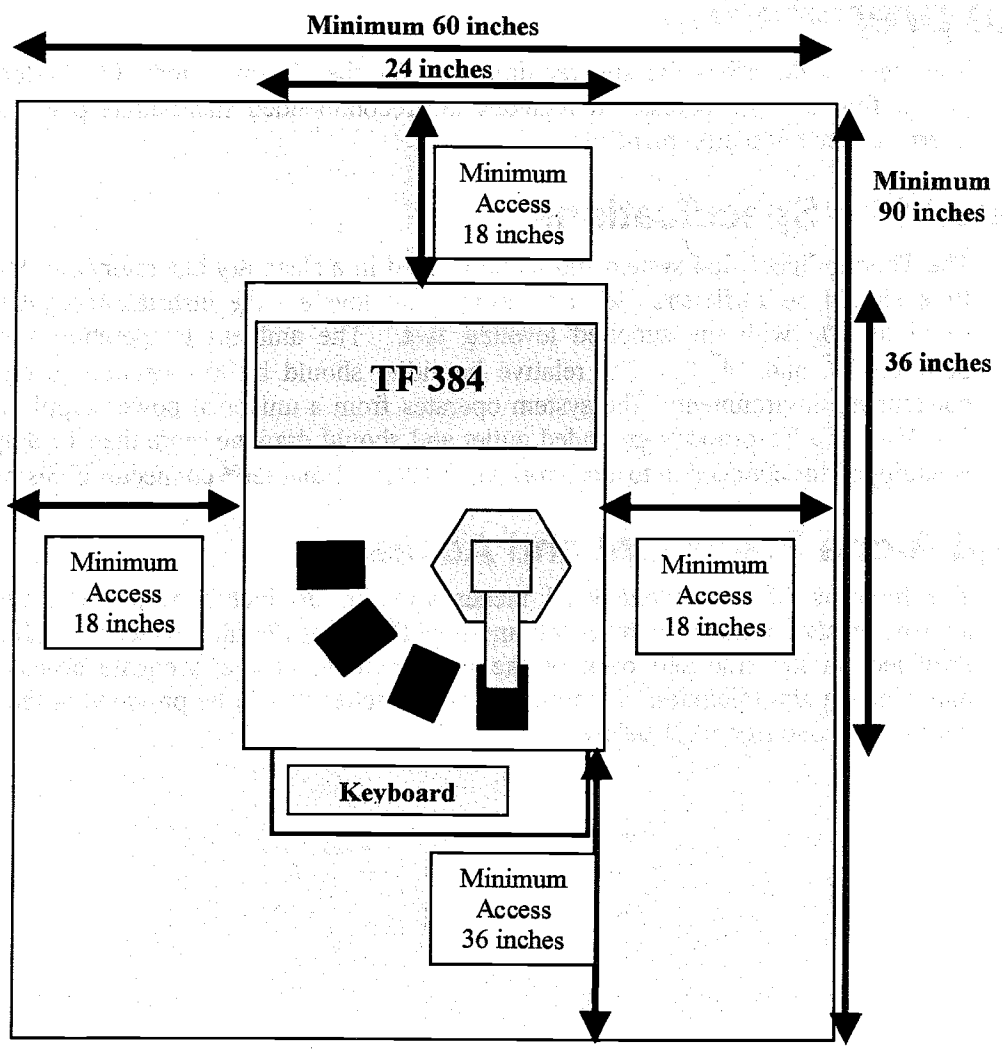


Figure 3- 1: ThermoFluor® 384 system, stand-alone placement and access

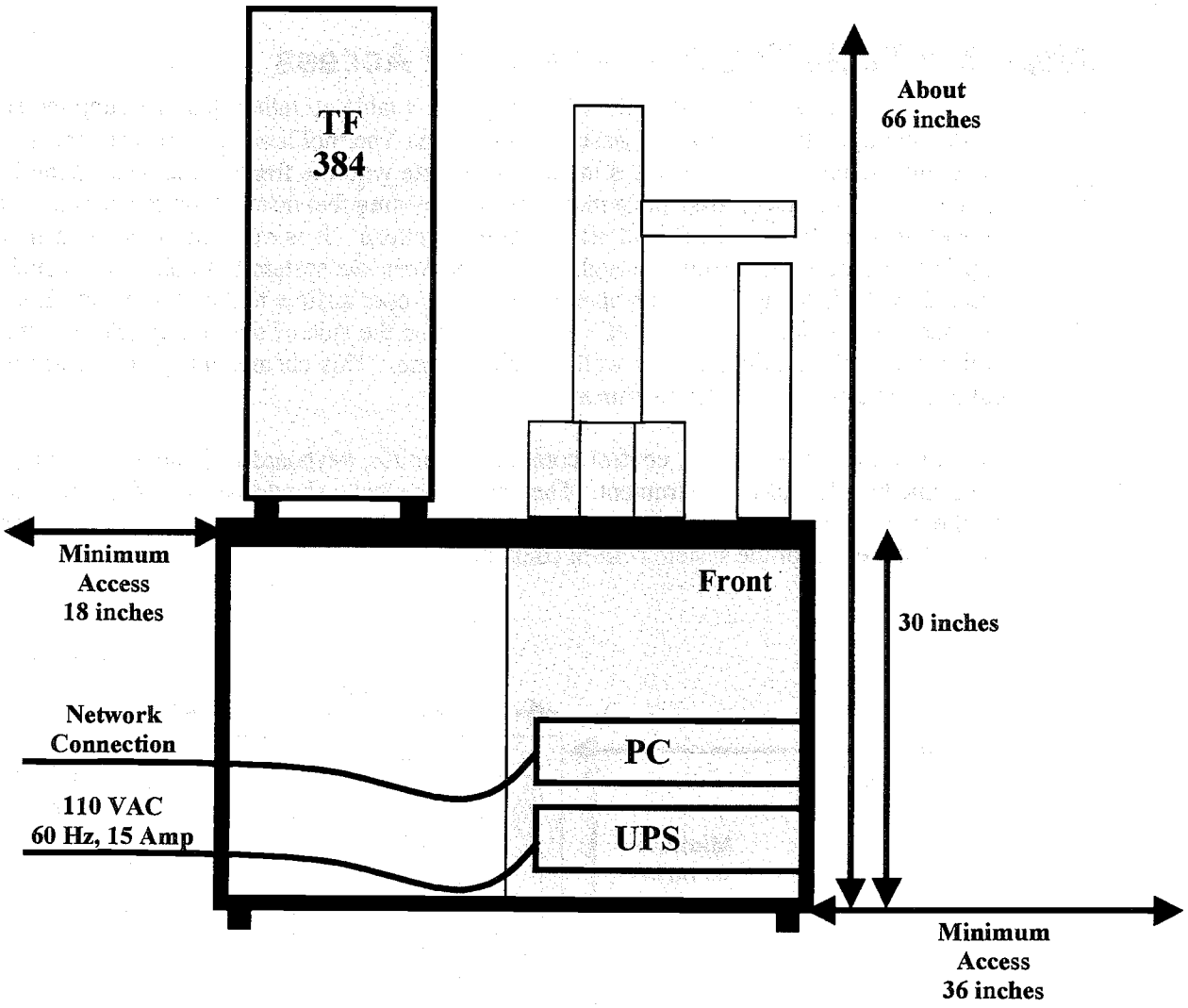


Figure 3- 2: ThermoFluor® 384 system, stand-alone placement side view.

The power receptacle should be located within four feet of the rear of the system. Optional network connection should also be located at the rear of the system.

Alternate Table Top Placement and Access

An alternative is to install the system base plate on a tabletop rather than the supplied stand. In this configuration the system base plate with the ThermoFluor® 384 instrument and the plate processing system attached is located on a table with the front of the instrument facing to the right. The system base plate must have the leveling feet installed on the corners and be leveled in both front to back and side-to-side directions. It is very important that nothing restricts or blocks the airflow through the gap between the system base plate and bench top created by the leveling feet. This opening ensures proper airflow to the instrument. It is also necessary to provide a minimum of 6 inches between the side of the instrument with the fan where the cabling exits and any wall or splash plate. This ensures adequate clearance for cabling and airflow from the instrument.

The universal power supply, control computer, monitor, keyboard and mouse are placed to the immediate left of the instrument. The power receptacle should be located within six feet of the rear of the universal power supply. Optional network connection should also be located at the rear of the system control computer.

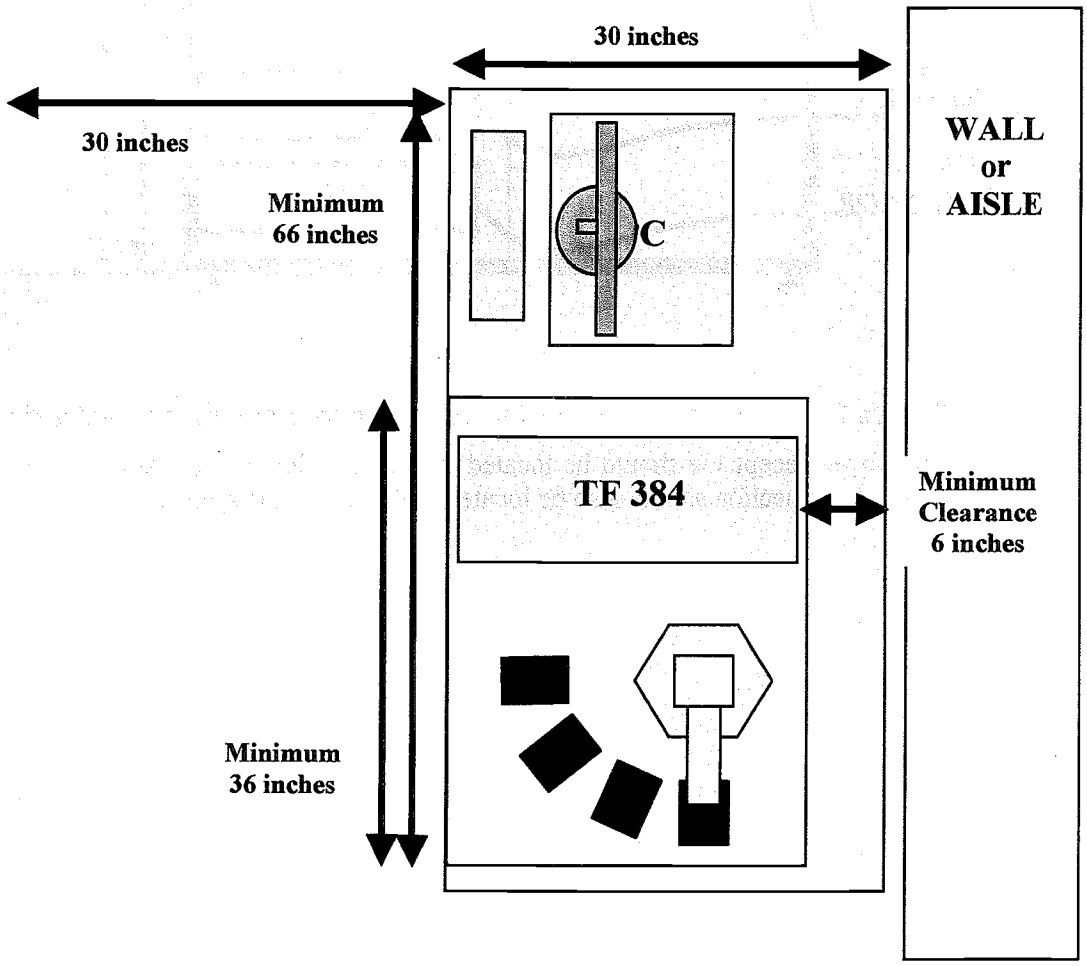


Figure 3- 3: ThermoFluor® 384 system, table top placement and access

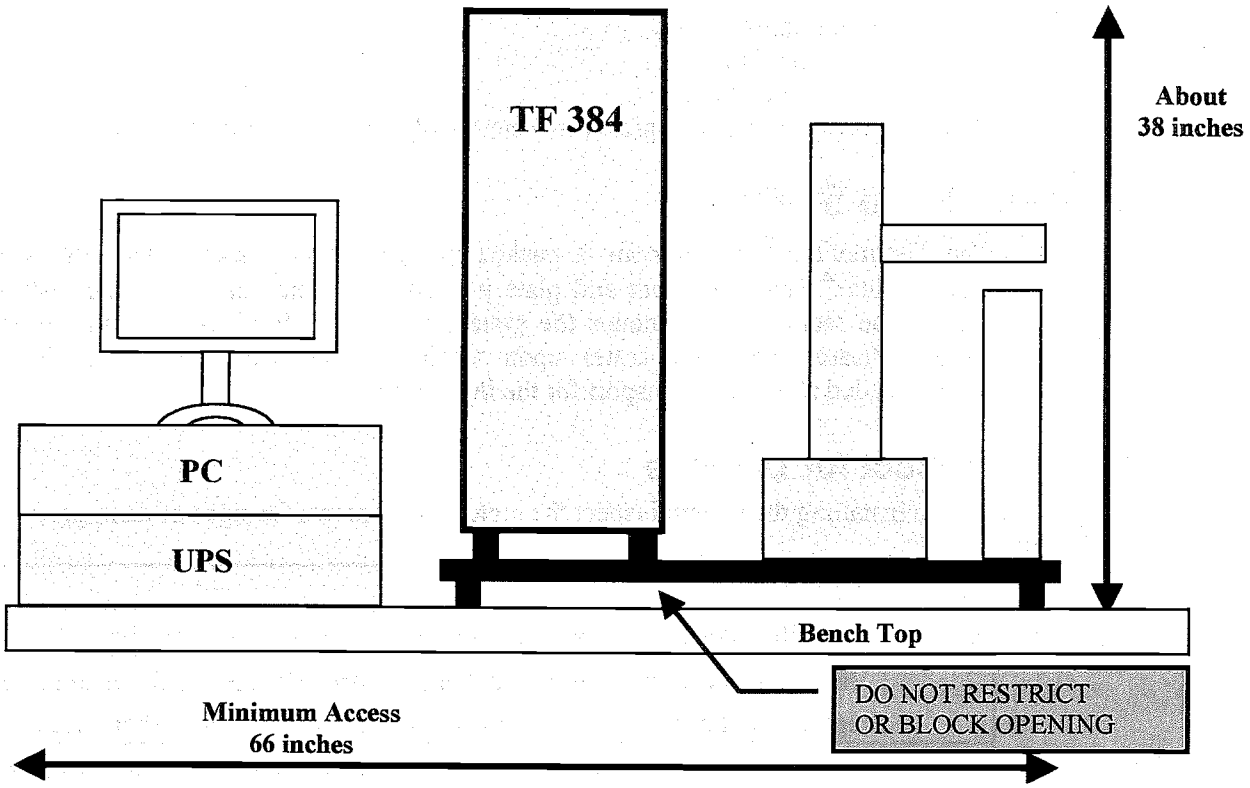


Figure 3- 4: ThermoFluor® 384 system, table top placement front view.

System installation

This section provides information and procedures for installing the ThermoFluor® 384 system. It includes unpacking and setting the system up. The system setup includes:

Power Up the System
System Initialization
Install the Filter(s)
Focus the Camera

When the installation is complete the system will be ready for operation.

Unpack the System

The ThermoFluor® 384 system is packed in two custom crates. One crate contains the ThermoFluor® 384 instrument and plate processing system attached to the instrument base plate. The second crate contains the system stands, NT Workstation, and universal power supply. Please save these crates upon receipt of the instrument! This is the factory recommended method of transport for the instrument

Inspect for Damage

Before installing the system inspect the crates and contents for signs of damage.

Step	Action
1	Set both crates on the floor with 4" x 4" support beams on the bottom.
2	Visually inspect for any external damage. Any damage should be immediately reported to the shipping company as well as 3-Dimensional Pharmaceuticals, Inc.
3	If any shock or temperature indicators are on the crate, locate and note their status.
4	Locate a row of Philips head screws along all four sides of the crates, approximately 5 inches from the floor. Black circles around each screw identify them.
5	Remove these screws from all four sides of each crate and store in a safe place.
6	Gently lift the top of the crates straight up, over and off the instrument. Caution, the contents are still attached to the bottom of the pallet.
7	Inspect the contents of the crates for any signs of damage.

Remove Contents from Pallets

Remove the contents of the crates from the pallets. Install leveling feet on the system stand and attach the instrument base plate to the system stand. Then place all packing materials back in the crates and store for future use.

Step	Action
1	Remove the computer, monitor, and universal power supply shipping boxes from the pallet and put them in safe location.
2	Remove the small shipping box secured to the system stand and pallet. Inside the box are cabling, manuals, tools and four (4) stainless steel leveling feet for the

	system stand. Remove the box and put it in safe location.
3	Unscrew the four bolts holding the system stand to the pallet. They are located on both the right and left side of the system stand. The bolts are captured in the pallet, so they will only drop half an inch or so from the pallet.
4	Prop up the front of the system stand with a 2" x 4" and install the front two (2) leveling feet in place of the bolts.
5	Gently remove the 2" x 4" and set the system stand down on the two front leveling feet.
6	Place the 2" x 4" under the back of the system stand and install the back two (2) leveling feet in place of the bolts.
7	Gently remove the 2" x 4" and set the system stand down on all four leveling feet.
8	Gently lift the system stand off the pallet and place it on the floor where the system is to be located.
9	Remove the small shipping box secured to the instrument base plate. Inside the box are four stainless steel bolts for attaching the instrument base plate to the system stand. Remove the box and put it in safe location.
10	Unscrew the four bolts holding the instrument base plate to the pallet. They are located on both the right and left side of the instrument base plate. The bolts are captured in the pallet, so they will only drop half an inch or so from the pallet.
11	Lift instrument base plate with the attached instrument and plate processing system onto the system stand. Four persons, positioned at each corner of the instrument, should lift and place the instrument onto the table. Proper lifting techniques should be strictly followed during this procedure.
	CAUTION!!! **Lifting the instrument base plate onto the system stand requires a minimum of four (4) persons.**
12	Bolt the instrument base plate to the system stand.
13	Place the magazines for the microplates onto their bases.
14	Remove the shipping material holding the PlateCrane™ robotic arm in place.
15	Open the top back panel and carefully remove the box labeled "FRAGILE – CCD Camera" and place in safe location.
16	Unpack computer, monitor, and universal power supply from their shipping boxes and place in safe location.
17	Place all packing materials on the pallets.
18	Replace the tops of the shipping crates onto the pallets and secure with the screws.
19	The system is now ready for setup.

Set up the System

After the system has been located at an appropriate site it can be set up. Start by positioning the system on the floor so that the plate processing magazines are at the front facing toward the operator. Adjust the system stand leveling feet so the robotic work area of the instrument base plate is level in both front to back and side-to-side directions. **A factory trained field engineer will install system components and connect the necessary cables and power cords.**

Power Up the System

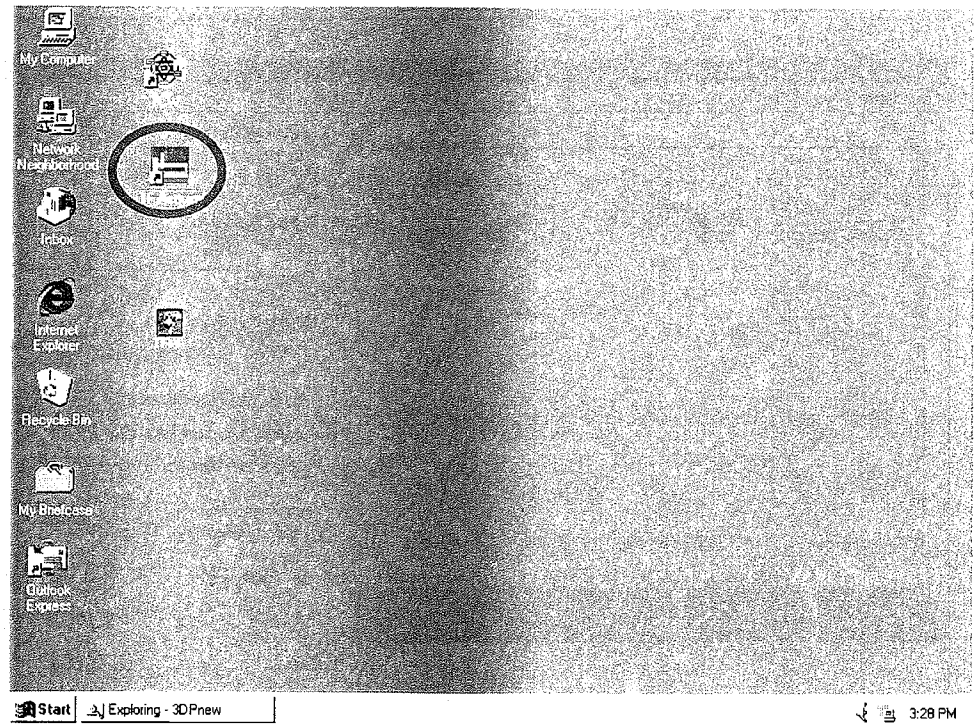
Use the procedure below to initially power up the instrument.

Step	Action
1	Verify that all power switches are in the "OFF" position.
2	Plug the universal power supply into the 110 VAC wall outlet.
3	Turn on the universal power supply.
4	Turn the PlateCrane™ power switch to the "ON" position. The PlateCrane™ grippers will open and close.
5	When the system initialization is complete, turn the computer power switch to the "ON" position.
6	Turn the instrument power switch to the "ON" position.
7	When the computer completes the Windows™ NT boot up, the control computer and system are ready for operation.

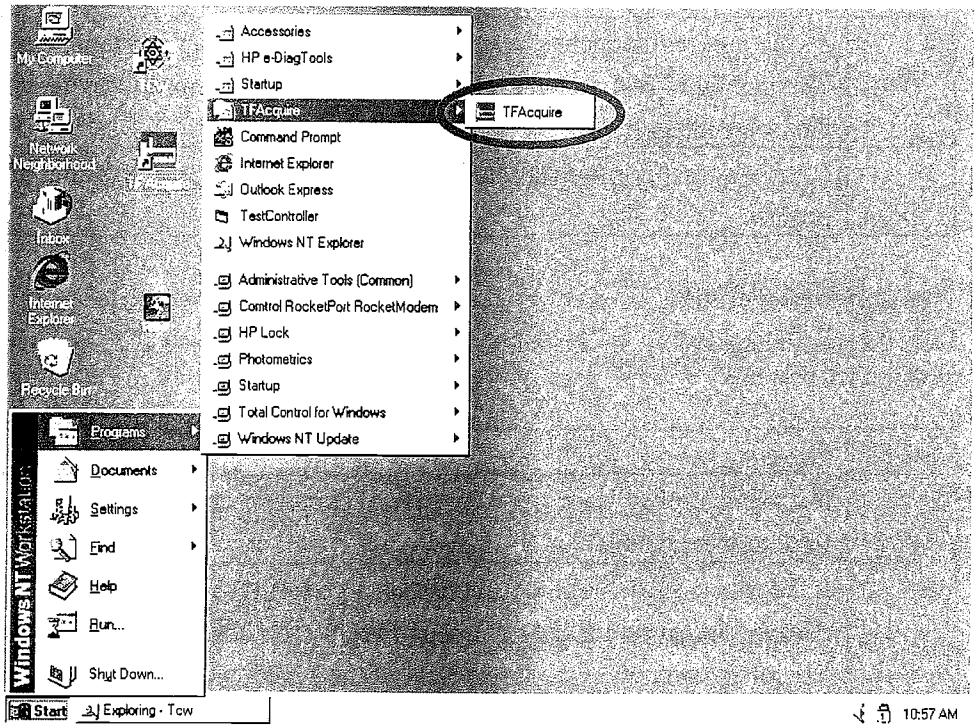
System Initialization

Once the ThermoFluor® 384 system is set up, powered up, and the control software installed, the system is ready for initialization and synchronization with the control computer. As the ThermoFluor® 384 Acquire 3.0 software launches, it searches for several available components in the ThermoFluor® 384 system. These include the Camera, thermo-cycler heater, and barcode reader. If any of the components fail to initialize an error message will be generated (see Troubleshooting in Chapter Five, Maintenance). Use the following procedure to initialize the system.

Step	Action
1	To run the system first boot up the NT workstation. A Startup screen similar to the following will appear:
2	Log on as specified by the System Administrator.

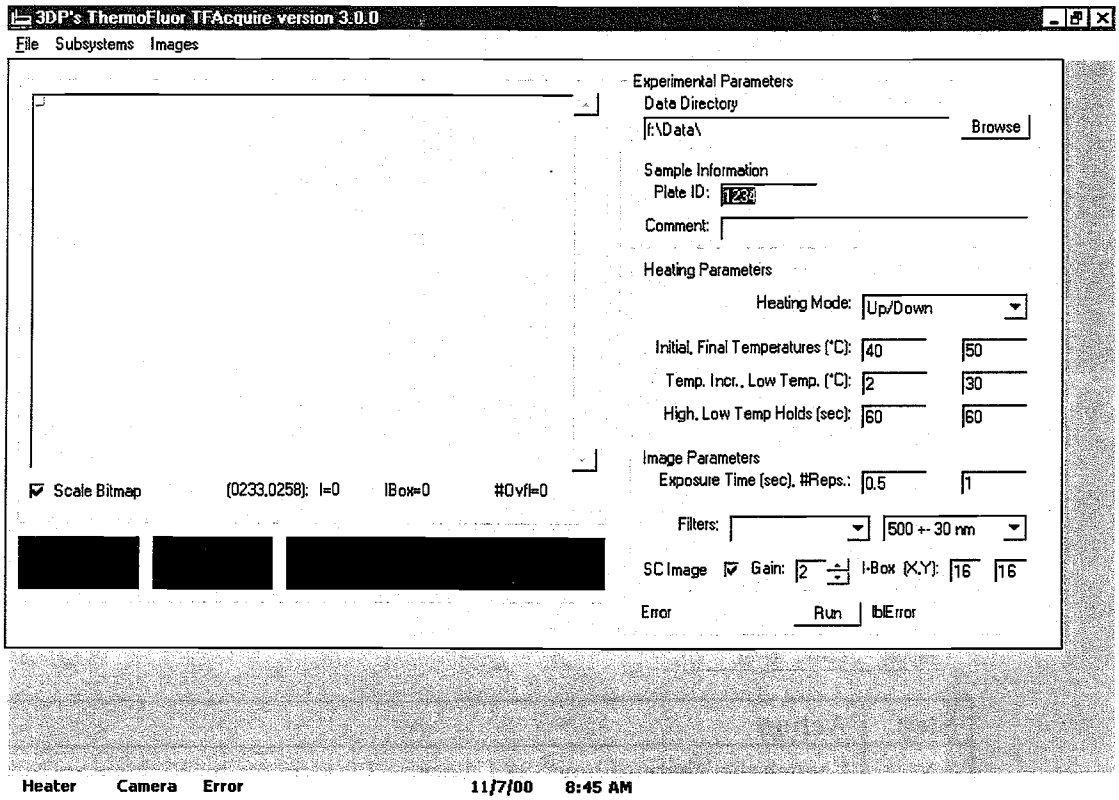


Step	Action
3A or	Launch the ThermoFluor® 384 Acquire 3.0 software by clicking on its icon. See above. The ThermoFluor® 384 Acquire 3.0 screen will appear.



3B	Launch the ThermoFluor® 384 Acquire 3.0 software by clicking the **Start** menu button then **Programs** then **TFAquire**. See above. The ThermoFluor® 384

	Acquire 3.0 screen will appear.



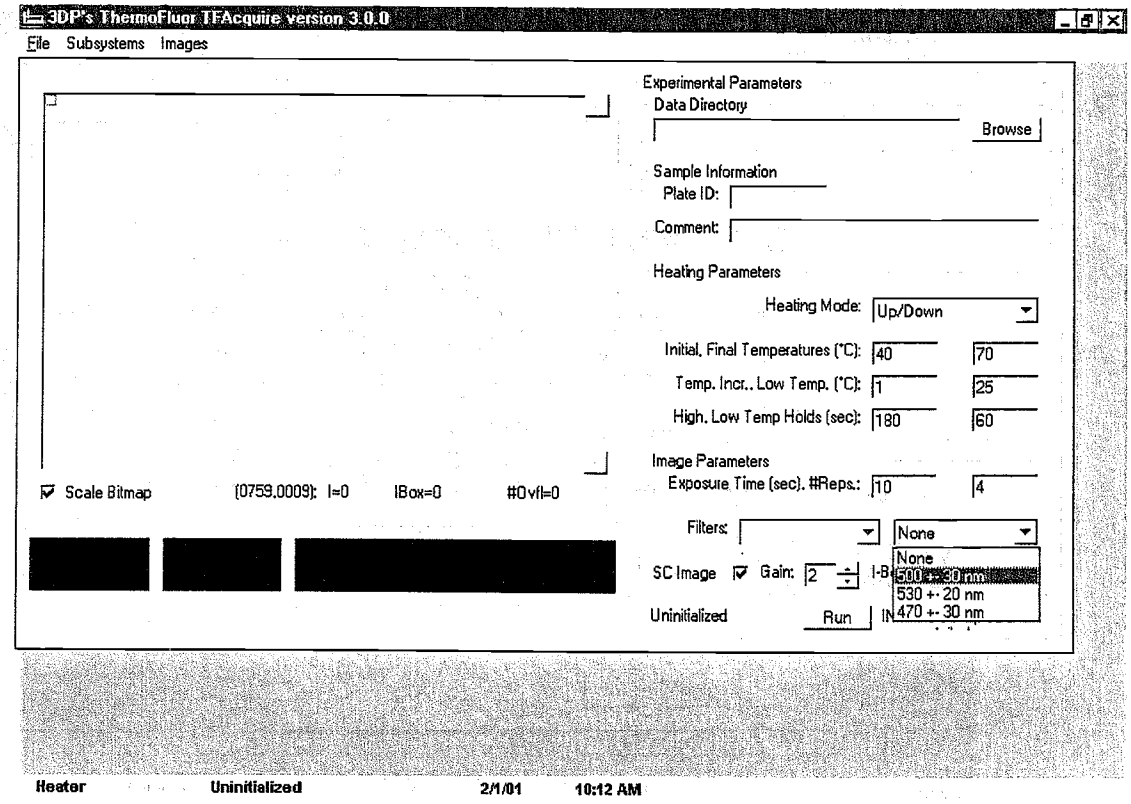
Step	Action
4	As the program launches, it searches for several available components inside the ThermoFluor® 384 system. There are indicator fields for the heater and camera at the lower left corner of the screen.
5	Upon successful initialization the system is ready for the remainder of the setup process.

Install the Filter

Use the procedure below to install the filter. The system software must be running to accomplish this task.

Step	Action
1	If it is not already open, open the optical compartment by removing the screws from the rear panel.
2	If the filter wheel is already in position 1 go to step 4. If it is not in position 1, click on the arrow for the right hand **Filters** drop down menu in the image parameters section at the lower right of the ThermoFluor® 384 Acquire 3.0 window. See Below.
3	Select **Position 1 (500 ± 30 nm)** from the drop down menu for the standard ThermoFluor filter supplied with the instrument. See figure below. The filter

	wheel will rotate to that position.



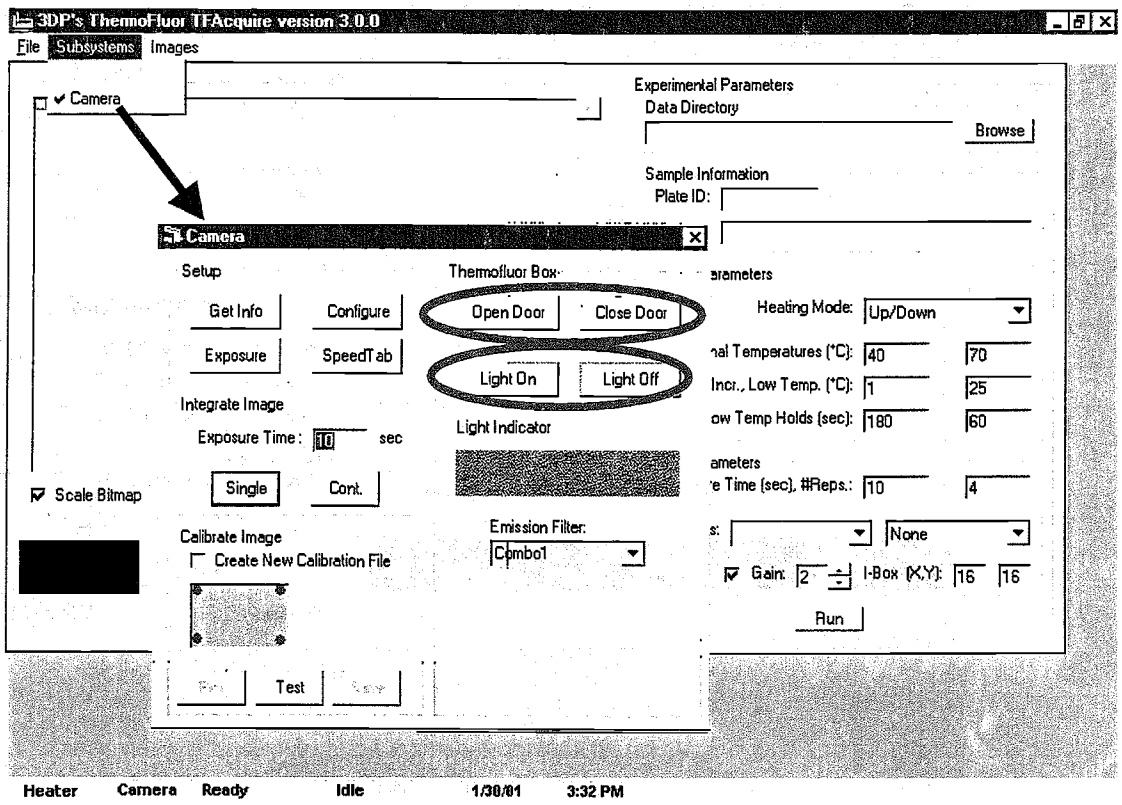
4	Locate the standard ThermoFluor filter supplied with the instrument and place it in the filter wheel at position 1 with the writing right side up.
	CAUTION **The filter is a sensitive optical item and should be handled by the edge with care**
5	Secure the filter wheel filter cover over the filter.
6	Close the optical compartment door and secure with screws.

Focus the Camera

Use the procedure below to focus the camera. The system software must be running to accomplish this task.

Step	Action
1	If it is not already open, open the optical compartment by removing the screws from the rear panel.
2	Place a white piece of paper with small print on the thermal cycler block. To do this manually open the light tight door as follows: From the Acquire 3.0 **SUBSYSTEM** menu select **Camera**. The CAMERA dialog box appears.

	Click the **Open Door** button. The light tight door opens

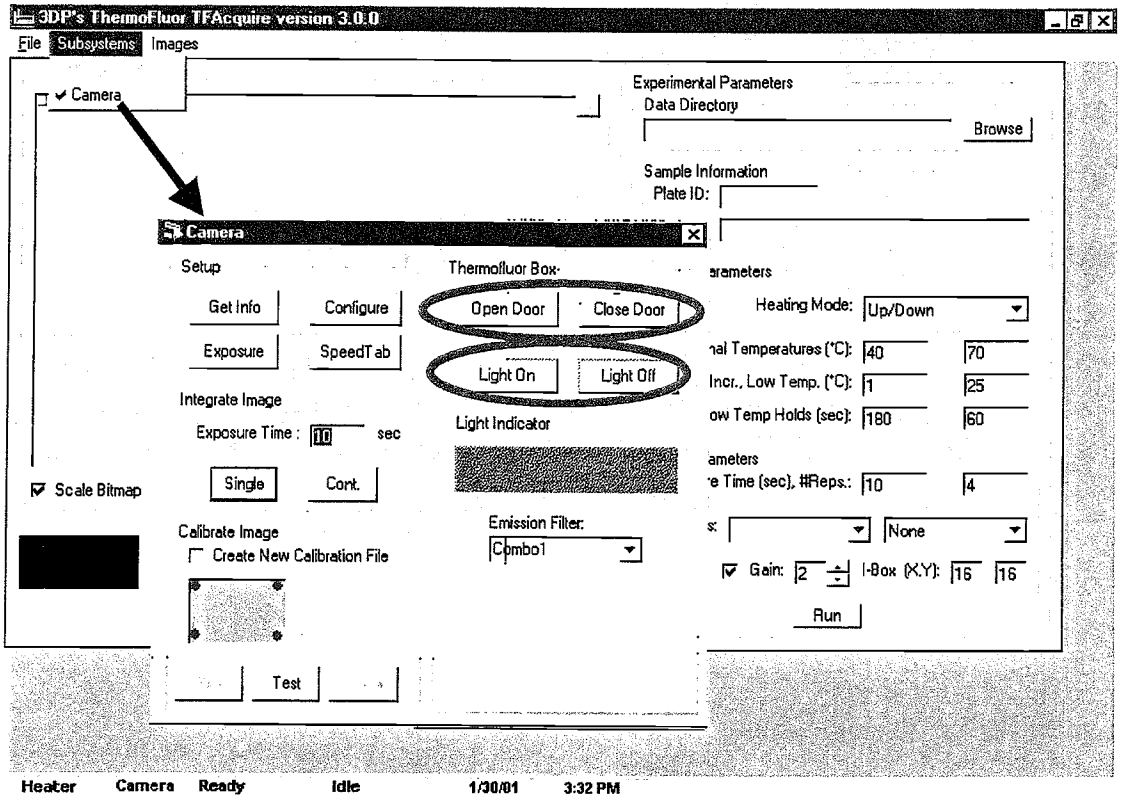


3	Once the piece of paper is placed on the thermal cycler block close the light tight door by clicking the **Close Door** button in the CAMERA dialog box.
4	From Acquire 3.0 CAMERA dialog box turn ON the UV light source by clicking the **Light On** button.
5	Enter an exposure time (one second) for the camera in the Integrate Image section of the CAMERA dialog box.
6	Click on the **Cont.** button to have the camera start taking a continuous series of images with the given exposure time.
7	From the rear of the instrument adjust the bottom adjustment ring on the lens until the image is sharp.
8	Close the optical compartment door and secure with screws.
9	Turn OFF the UV light source by clicking the **Light Off)** button.
10	Remove the white piece of paper from the thermal cycler block. To do this open the light tight door by clicking the **Open Door** button in the CAMERA dialog box as done above.
11	Once the piece of paper is removed from the thermal cycler block close the light tight door by clicking the **Close Door** button.

Calibration of Spots

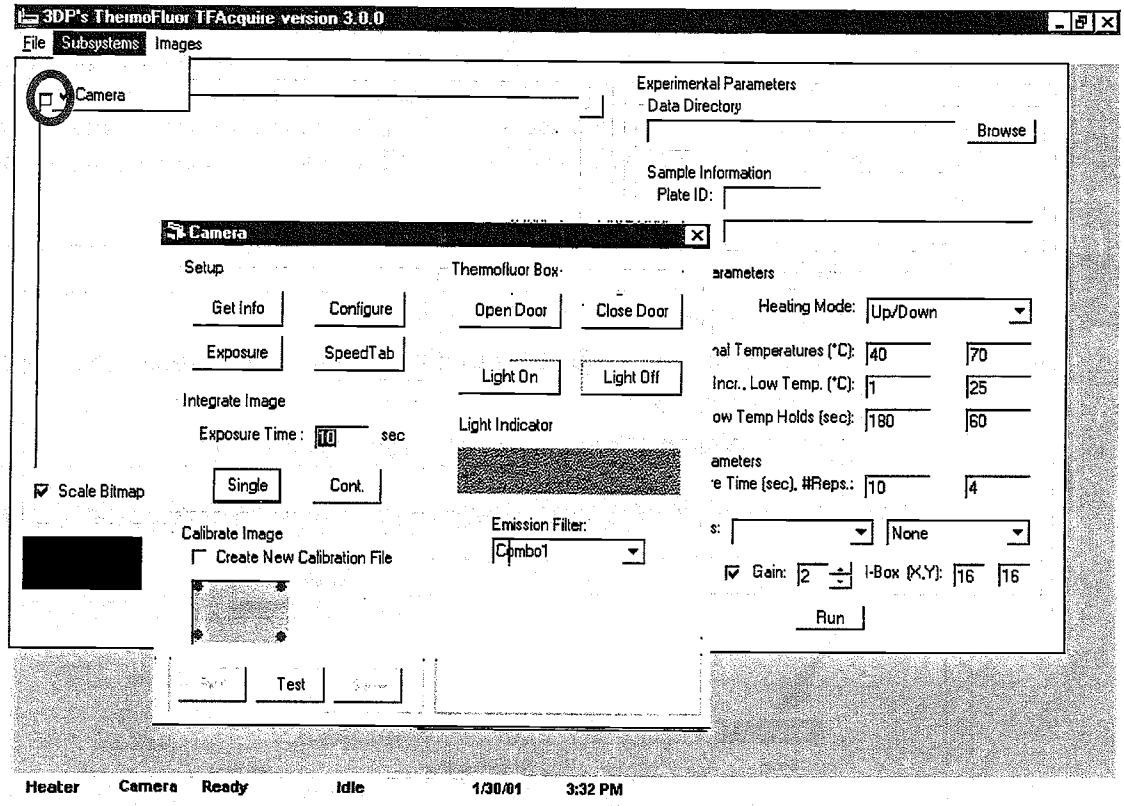
Before the system can properly measure microplates it must determine where the wells in the plates are located. The system software must be running to accomplish this task. First an image of a microplate with standard florescent beads in the wells is taken. Then the corner wells are identified for the Acquire 3.0 program. After this the program calculates the position of each of the 384 wells and scans the plate so the user can verify that the program has properly defined the well positions. Follow the procedure below to perform this calibration.

Step	Action
1	From Acquire 3.0 open the light tight door as follows: From the Acquire 3.0 **SUBSYSTEM** menu select **Camera**. The CAMERA dialog box appears. Click the **Open Door** button. The light tight door opens

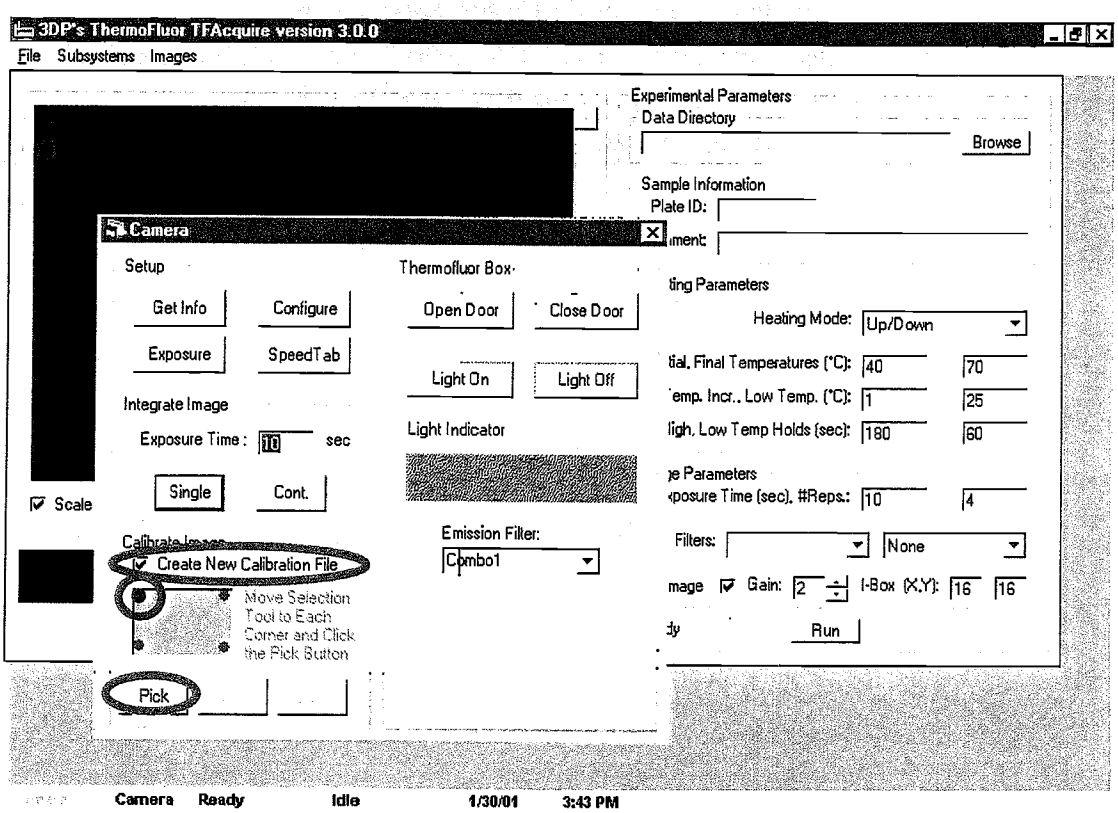


2	Place the microplate with standard florescent beads on the thermal cycler block.
3	Close the light tight door by clicking the **Close Door** button in the CAMERA dialog box, type CLOSE and press <Enter>.
4	Turn ON the UV light source by clicking the **Light On** button.
5	Enter an exposure time (ten seconds) for the camera in the Integrate Image section of the CAMERA dialog box.
6	Click on the **Single** button to have the camera take an image with the given exposure time.

7	Click on the Create New Calibration File box. A little green box [] appears in the window. This is the I-box.



8	Move the I box (the small green box in the image area) to the upper left well, A1, of the plate view section with the mouse by clicking, holding and dragging it.
9	Click Pick button in the Calibrate Image section of the Camera dialog box. The upper left red dot on the dialog box image should change to green.



10	Move the I box to the upper right corner, A24, of the plate view section with the mouse.
11	Click Pick button in the Calibrate Image section of the **Camera** dialog box. The upper right red dot on the dialog box image should change to green.
12	Move the I box to the lower right corner, P24, of the plate view section with the mouse.
13	Click Pick button in the Calibrate Image section of the **Camera** dialog box. The lower right red dot on the dialog box image should change to green.
14	Move the I box to the lower left corner, P1, of the plate view section with the mouse.
15	Click Pick button in the Calibrate Image section of the **Camera** dialog box. The lower left red dot on the dialog box image should change to green.
16	Click the **Test** button in the Calibrate Image section of the **Camera** dialog box. The I box will start to move across the screen covering all 384 well locations.
17	Visually verify that the I box remains centered on the wells as it moves. This confirms the program has correctly defined the position of each well.
18	Click **Save** to save this calibration. **NOTE:** As long as the camera or thermal cycler block are not moved this calibration should not have to be done again.
19	From Acquire 3.0 **CAMERA** dialog box manually turn OFF the UV light source by clicking the **Light Off** button.
20	Remove the microplate from the thermal cycler block. To do this open the light

	tight door by clicking the **Open Door** button.
21	Once microplate is removed from the thermal cycler block manually close the light tight door by clicking the **Close Door** button.
22	Properly store the microplate.

Acceptance Tests

This test is ran to validate the proper operation of the ThermoFluor® 384 instrument. The system and analysis software must be running to accomplish this task. For a procedure to prepare and run plates, and then analyze the data refer to Tutorial Seven in the Tutorial Manual.

The most fundamental measurement giving meaningful data using ThermoFluor® is the ΔT_m between a well containing compound and a reference well ($T_{m,+comp.} - T_{m,ref}$) . Since the T_m is independent of initial and final fluorescent intensities, validation of ThermoFluor® instruments should be based on the uniformity of T_m across the entire heating block.

Accuracy Test

To measure uniformity of T_m across a plate, uniform assay plates of bovine Carbonic Anhydrase II (Sigma, catalog #C2522, E.C. 4.2.1.1) should be made using the conditions listed below. These conditions were chosen to give a wide working range of T_m's, (~40°C – 70°C), while using an inexpensive protein and a procedure that can be accomplished relatively quickly (1 day or less).

Condition 1 (T_m ~ 40°C):	5 µl of 0.15 mg/ml Carbonic Anhydrase in 50 mM NaOAc, 100 mM NaCl, pH 4.4, 1 mM EDTA, 50 µM 1, 8 ANS, overlayed with 3 µl oil.
Condition 2 (T_m ~ 50°C):	5 µl of 0.15 mg/ml Carbonic Anhydrase in 50 mM NaOAc, 100 mM NaCl, pH 4.5, 50 µM $ZnCl_2$, 50 µM 1, 8 ANS, overlayed with 3 µl oil.
Condition 3 (T_m ~ 60°C):	5 µl of 0.15 mg/ml Carbonic Anhydrase in 25 mM NaPIPES, 100 mM NaCl, 1 mM EDTA, pH 7.0, 50 µM 1, 8 ANS, overlayed with 3 µl oil.
Condition 4 (T_m ~ 70°C):	5 µl of 0.15 mg/ml Carbonic Anhydrase in 25 mM NaPIPES, 100 mM NaCl, 50 µM $ZnCl_2$, pH 7.0, 1 mM Acetazolamide, 50 µM 1, 8 ANS, overlayed with 3 µl oil.

The ThermoFluor® Instrument parameters for measuring the thermal denaturation in this test should be:

Ramp Mode, heating 25-85°C, in 1°C increments.
High temperature equilibration for 0.25 min (15s).
Fluorescent intensity averaged over 4, 10s exposures.

Acceptance shall be achieved when deviation across a plate (measured as the standard deviation in the T_m of 384 wells) is < 0.2°C.

Uniform plates for the latter three conditions have a long shelf life and will give identical results after freeze-thaw (-80°C, 1 week).

These tests *can* also be run in step mode (0.1mg/ml protein is sufficient) but it will take longer. .

The lot number for the CAII that 3DP have is 100K9279. 100 mgs is $493.50 (at 0.15mg/ml, 5ul volume this comes to ~350ug/plate (including 20% excess); using the indicated conditions, each plate uses $1.72 of protein.

Precision Test

To measure the precision of the instrument as well as the accuracy a test is run as follows:

A total of three plates are run using the third set of conditions described for the accuracy tests above, on two consecutive days, with deviations in absolute T_m on the same instrument to be < 1.0°C.

A minimum of one plate will be run under the third set of conditions in step mode.

Chapter Four

Operation

Overview

This chapter will familiarize the user with the basic operation of the ThermoFluor® 384 system.

Specifically, this chapter includes the following sections:

- *Safe Operation*
- *Operating Software and Controls*
- *ThermoFluor® 384 Acquire 3.0*
- *ThermoFluor® 384 System Operation*

The following table identifies the appropriate section to read in order to learn how to properly operate the ThermoFluor® 384 system.

Safe Operation

The ThermoFluor® 384 system should be set up and run by 3-Dimensional Pharmaceuticals Inc. factory trained personnel only. If you are not factory trained, contact 3-Dimensional Pharmaceuticals, Inc. at 1-610-458-8959 to schedule training.

The Operating Manual contains up-to-date information on the safe installation and operation of the ThermoFluor® 384 system. Before installing or running the ThermoFluor® 384 system, completely and thoroughly review the latest version of the Operating Manual. If any instructions are unclear or unfamiliar to you, contact 3-Dimensional Pharmaceuticals, Inc. at 1-610-458-8959 for clarification and proper operation.

Please locate and identify all safety and informational labels on the instrument. If any labels are missing, smudged or damaged, discontinue use and contact 3-Dimensional Pharmaceuticals, Inc. for replacements.

Please locate and identify all safety items on the instrument including covers and panels. If any items are missing, damaged or disabled in any way discontinue use and contact 3-Dimensional Pharmaceuticals, Inc. for instrument repair.

Abnormal instrument operation should be immediately noted and instrument use discontinued until examined by a representative from 3-Dimensional Pharmaceuticals, Inc.

As with all laboratory equipment, follow all standard operating procedures and policies. An operations and maintenance logbook should be maintained at the instrument's location.

WARNING
Do not place hand into the open Light Tight Compartment door.
If it were to shut on the hand it could cause serious injury.

Remember, above all, safety first!!!

Operating Software and Controls

As mentioned in Chapter 2, Construction and Function, the Windows™ NT Workstation contains the software that controls the operation of the system. The Total Control for Windows™ (TCW) program coordinates the overall operation of the system while the ThermoFluor® Acquire 3.0 program provides the user interface to the ThermoFluor® 384 instrument. Total Control for Windows™ directly operates the plate processing system and it indirectly operates the ThermoFluor® 384 instrument through a DDE connection with the ThermoFluor® Acquire 3.0 control software. The ThermoFluor® Acquire 3.0 control software interfaces with the CCD camera and an embedded controller (Zworld™) in the ThermoFluor® 384 instrument that controls the filter wheel, UV light source, light tight compartment door, and the thermal cycler heater. This is illustrated below.

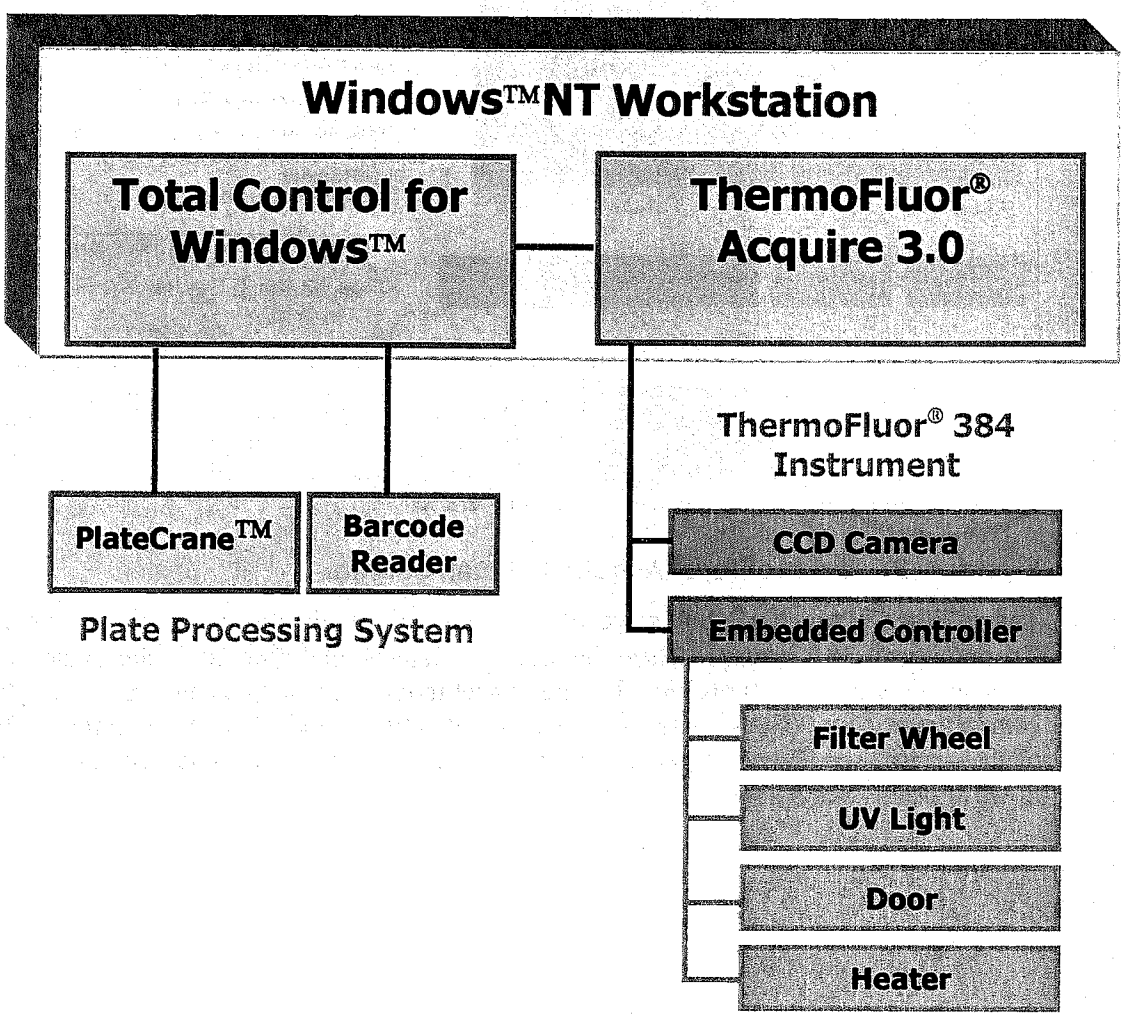


Figure 4- 1: Operating and control software applications.

System Initialization

After the system is powered up, the ThermoFluor® 384 Acquire 3.0 software is launched. The Acquire 3.0 user interface screen appears, see below.

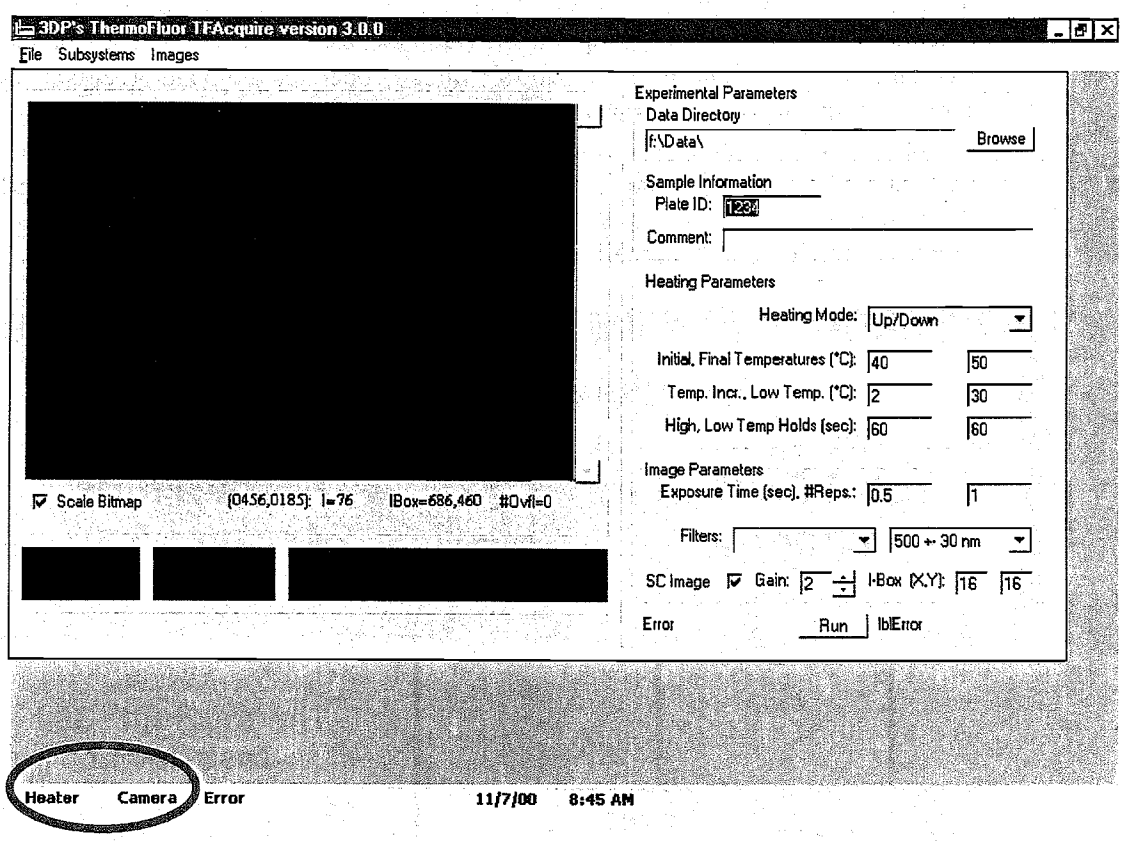


Figure 4- 2: ThermoFluor® Acquire 3.0 man screen.

ThermoFluor® Acquire 3.0 searches for several available components inside the ThermoFluor® 384 system. These include the Camera and thermal cycler heater. The embedded controller will position the filter wheel to its home position and open the light tight compartment door. The camera and heater initialization is indicated at the lower left bottom of the screen, see above. After this is done the TCW application is started and it's **MAIN** menu appears. See below.

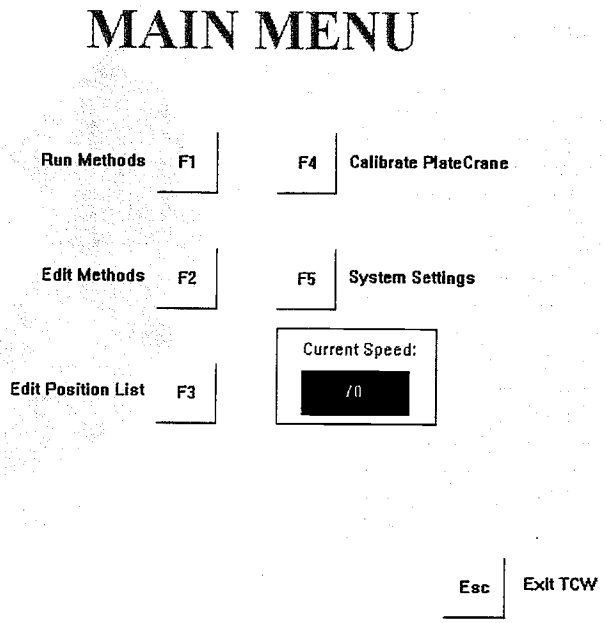


Figure 4- 3: Total Control for Windows™ (TCW) main menu screen.

Routine Analysis

Next the user enters experimental parameters. For a single plate these parameters can be entered using the ThermoFluor® Acquire 3.0 user interface screen since TCW is not needed. For a routine analysis of multiple plates TCW is used to set up the plate handling for the run and enter experimental parameters, which are relayed to the ThermoFluor® Acquire 3.0 program. The ThermoFluor® Acquire 3.0 program communicates the appropriate parameters to the embedded controller. If a filter wheel position is changed the embedded controller repositions the filter wheel immediately. The user then initiates the run by clicking the **Start Run** button on the TCW Current Method screen or the **RUN** button on the Acquire 3.0 interface screen.

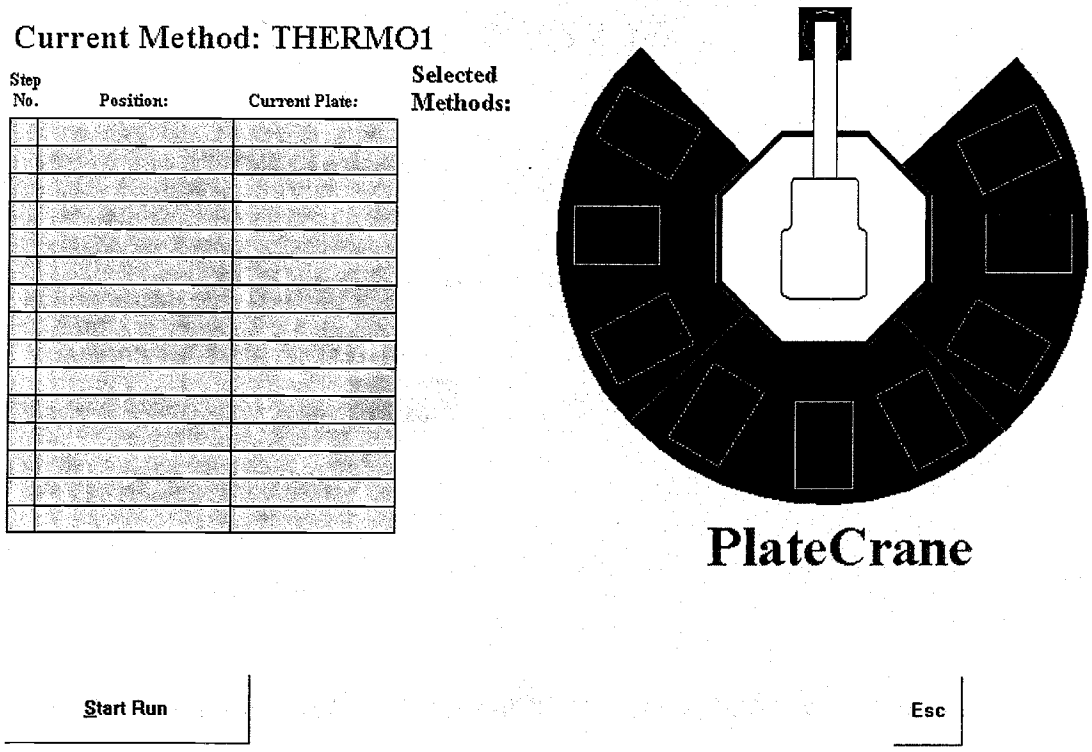


Figure 4- 4: The TCW Current Run screen.

PlateCrane™ Loads Plate

The TCW program then checks the status of the instrument through Acquire 3.0. Once this is done the PlateCrane™ goes to the input magazine, removes the top plate and loads it in the instrument. TCW then signals Acquire 3.0 that a plate has been placed in the light tight compartment. As the plate passes the barcode reader the barcode is read. This information is communicated to the Acquire 3.0 program. At the end of the run the Acquire 3.0 program stores it with the experimental data in the INT file in the directory designated when the experimental parameters are entered.

Heater Cycle

The Acquire 3.0 program then signals the embedded controller to begin the run. The embedded controller closes the door to the light tight compartment and begins heating depending upon which heating mode, Up/Down or Continuous Ramp mode, is selected.

Up/Down Mode

In the Up/Down mode the temperature is raised to a high temperature value, held there for a set time (High Temperature Hold Time) then lowered to the Low Temperature setting and held there. The plate is held at the low temperature for a set time (Low Temperature Hold Time) to allow equilibration and then while the camera records images. The embedded controller repeats this process for each temperature point in the selected range.

The embedded controller begins the first heating cycle (Up/Down mode) by signaling the heater to raise the temperature of the plate to the initially selected "High" temperature value. When the controller senses this temperature has been reached it signals the heater to maintain the "High" temperature and begins measuring the "High" temperature hold time. See below.

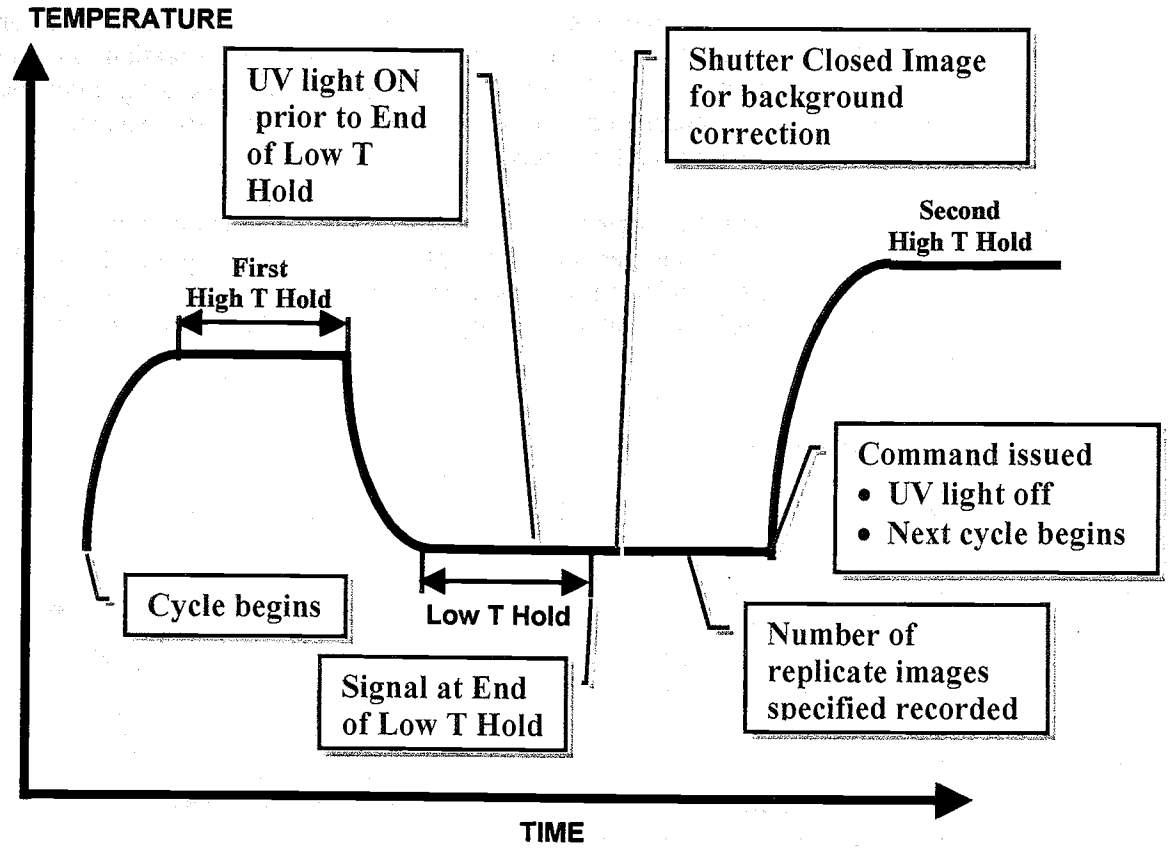


Figure 4- 5: Up/Down mode sequence of events.

When this time has elapsed the controller signals the heater to lower the temperature of the plate. When the controller senses this temperature has been reached it signals the heater to maintain the "Low" temperature and begins the "Low" temperature hold time. Prior to the end of the "Low" temperature hold the controller turns the UV light on so it can stabilize before images are taken. When the "Low" Temperature Hold time has elapsed the controller signals the Acquire 3.0 program.

During this time the Acquire 3.0 program polls the embedded controller for the signal that the low temperature hold time is complete. When it finds this signal the Acquire 3.0 program signals the camera to first acquire a "shutter closed image" (if selected) for background correction. It then directs the camera to take the number of replicate images (typically 4) with specified exposure times as indicated by the previously communicated experimental parameters. When these are completed the Acquire 3.0 program sends a command to the embedded controller. When the embedded controller receives the command it turns off the UV light and begins the next heater cycle at the next higher temperature increment. These cycles are continued until the cycle for the final temperature is completed.

Continuous Ramp Mode

In the Continuous Ramp mode the temperature is raised to a temperature value and held there for a set time (High Temperature Hold Time) to allow equilibration and then while the camera records images. Then the temperature is raised to the next temperature value and held there to allow equilibration and then while the camera takes shots. The embedded controller repeats this process for each temperature point in the selected range.

The embedded controller begins the first heating cycle (Continuous Ramp mode) by signaling the heater to raise the temperature of the plate to the initially selected "High" temperature value. When the controller senses this temperature has been reached it signals the heater to maintain the "High" temperature and begins measuring the "High" temperature hold time. See below.

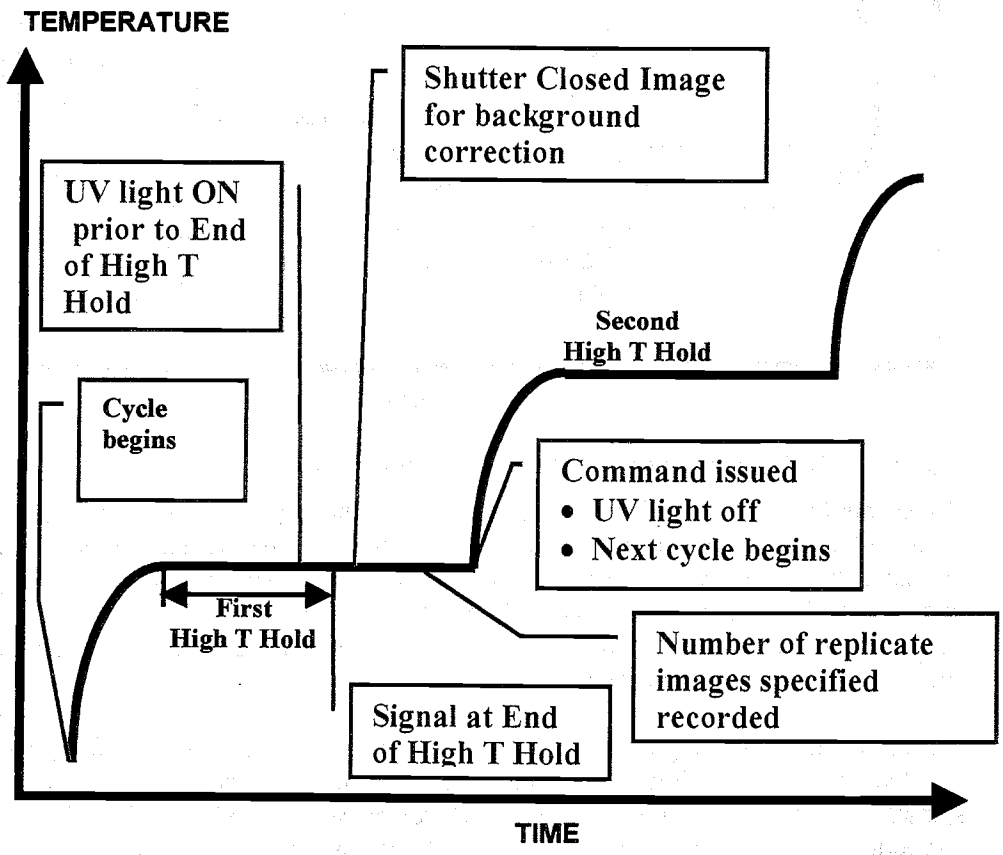


Figure 4- 6: Continuous Ramp mode sequence of events.

Prior to the end of the "high" temperature hold the controller turns the UV light on so it can stabilize before images are taken. When the "high" Temperature Hold time has elapsed the controller signals the Acquire 3.0 program.

During this time the Acquire 3.0 program polls the embedded controller for the signal. When it finds the signal the Acquire 3.0 program signals the camera to first acquire a "shutter closed image" (if selected) for background correction. It then directs the camera to take the number of replicate images (typically 4) with specified exposure times as indicated by the previously communicated experimental parameters. When these are completed the Acquire 3.0 program sends a command to the embedded controller. When the embedded controller receives the command it turns off the UV light and begins the next heater cycle at the next

higher temperature increment. These cycles are continued until the cycle for the final temperature is completed.

Plate Done

When the Final temperature cycle for the plate has been completed the embedded controller brings the heater block temperature back to 25°C (if it is not already there), opens the light tight compartment door, and indicates the plate is done. The Acquire 3.0 program recognizes the indication the plate done, stops polling the controller and communicates that the plate is done to the TCW program.

Complete Run

The TCW program then directs the PlateCrane™ to remove the plate from the heater block and place it in the output storage magazine. It then directs the PlateCrane™ to take the next plate in the input magazine and load it onto the heater block. As the plate passes the barcode reader it is read and this information is communicated to the Acquire 3.0 program that stores it with the experimental data. TCW then signals Acquire 3.0 that a plate has been placed in the light tight compartment and the Acquire 3.0 program signals the embedded controller to begin the measurements for the next plate. The embedded controller then repeats the above steps for this plate. This process is repeated until all the plates are measured.

Total Control for Windows™

The Hudson Control Group has developed the Total Control for Windows™ software. When Total Control for Windows™ (TCW) is started by double clicking the PlateCrane™ icon on the MS Windows™ desktop the TCW Main menu screen appears. See below. This screen provides access to the various functions of the program. In this section we discuss those functions that might be used during the operation of the ThermoFluor® 384 System.

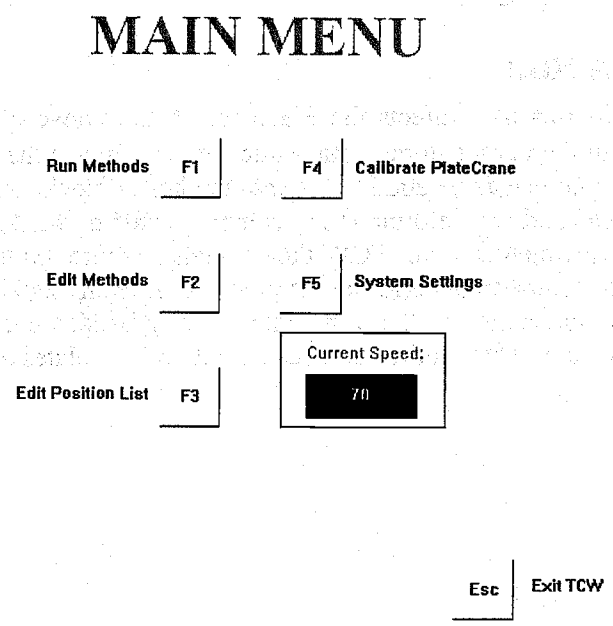


Figure 4- 7: Total Control for Windows™ (TCW) main menu screen.

If more details about this software are desired refer to Appendix A, Total Control for Windows™, for the TCW manual.

Run Methods

This selection is used to set up and start a run. The specific procedure for performing a run, including setup is discussed in the ThermoFluor® 384 System Operation section of this chapter. When Run Methods is selected the Current Method: screen appears.

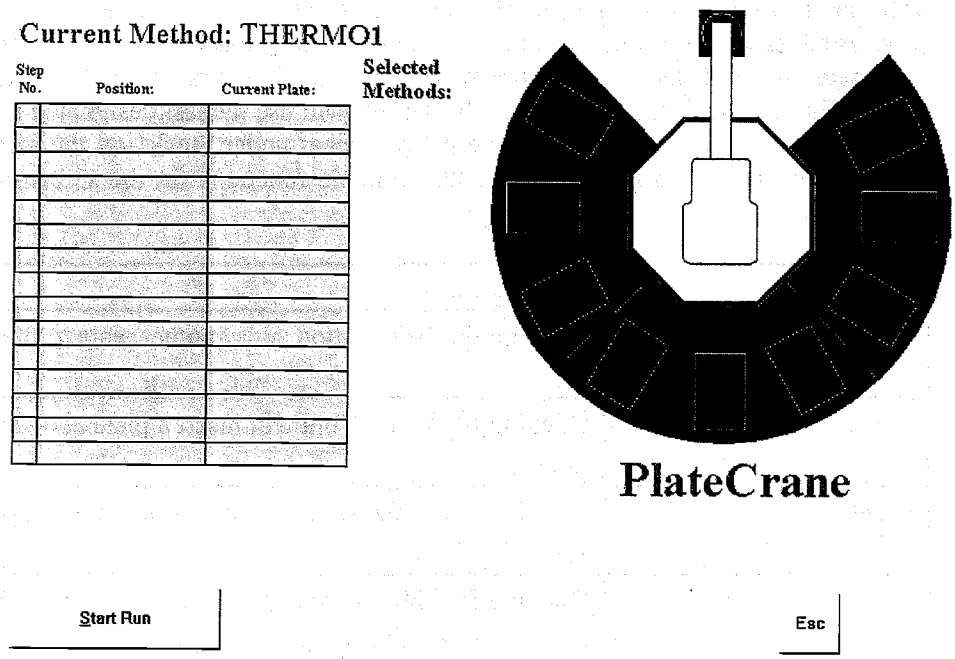


Figure 4- 8: The TCW Current Run screen.

The title of the run is displayed after the Current Run: screen title. In the case above the current run is THERMO1.

Clicking the Start Run button allows the user to select which method to run, create a method run list and access the ThermoFluor Plate Setup screen shown below:

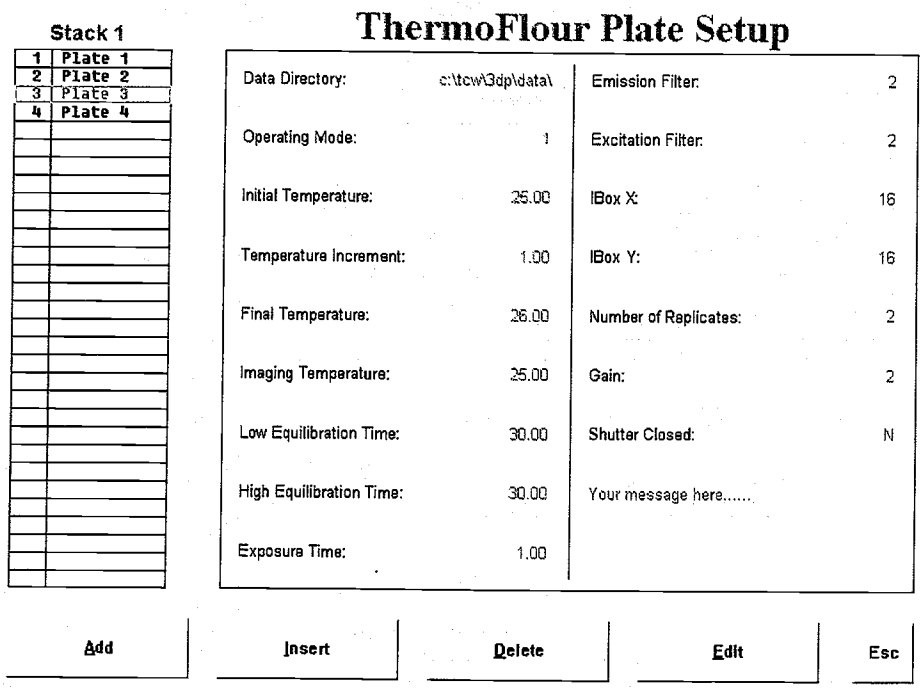


Figure 4- 9: Set up plate parameters from the TCW ThermoFluor Plate setup screen

This screen is used to specify which set of parameters will be used for each plate to be run.

The system will begin processing Plate 1 using the set of parameters entered for Plate 1. It will then proceed to Plate 2 using the set of parameters entered for Plate 2. It will continue processing each plate with its corresponding set of parameters until it comes to the last designated plate. If there are more plates in the stack in the magazine than designated on the ThermoFluor Plate Setup screen then the system will use the last designated set of parameters for the remaining plates. Click on the plate number under Stack1 on the screen to view the set of parameters for a given plate. The available functions for the screen are listed in the table below.

Function	Description
Add	Clicking the **Add** button adds another plate to the bottom of the list.
Insert	Clicking the **Insert** but will insert a plate above the plate selected.
Delete	Clicking the **Delete** button deletes the selected plate from the list.
Edit	Clicking the **Edit** button opens the ThermoFluor Setup Screen that is used for changing the parameters for the selected plate.
Esc	Clicking the **Esc** button Returns the user to the Current Method: screen.

To change the parameters for a plate select the Edit button. The ThermoFluor Setup Screen appears. See below.

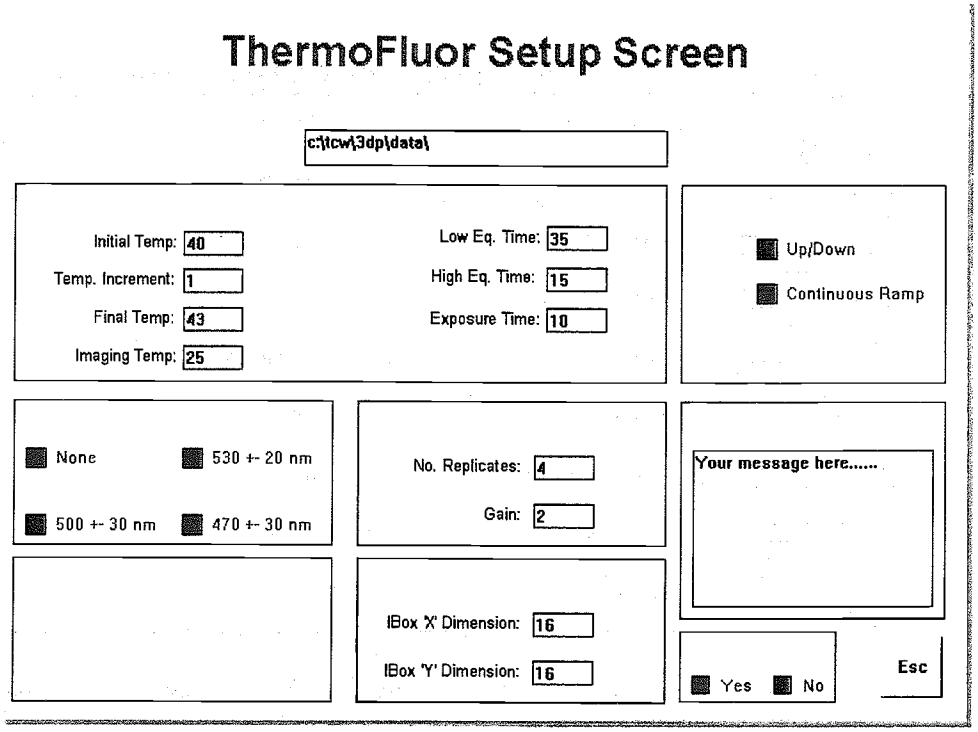


Figure 4- 10: Enter parameters for a plate on the ThermoFluor Setup screen.

This screen allows the user to:

1. Enter the path and directory for the data ***.psq** and ***.int** files to be stored. The ***.psq** files

are the raw camera data and the ***.int** files are integration files created by the Acquire 3.0 program. The folder in which these files are to be stored is designated here.

2. Enter the appropriate Imaging Profile Parameters. These fields are:

Field	Description
Initial Temp:	The starting temperature in °C for measurements. This is the first high temperature in the Up/Down mode or the start of the temperature ramp in the Continuous Ramp mode.
Temp. Increments:	The temperature difference between measurements in °C.
Final Temp:	The last temperature in °C for measurements. This is the last high temperature in the Up/Down mode or the end of the temperature ramp in the Continuous Ramp mode.
Imaging Temp:	The temperature to which the heater returns the plate and maintains in the Up/Down mode while images are taken. Also referred to as the low temperature.
Low Eq. Time:	The time the low temperature equilibration is held, in seconds, before exposures are taken in the Up/Down mode.
High Eq. Time:	The time the high temperature equilibration is held, in seconds, before returning to the low temperature in the Up/Down mode.
Exposure time:	Time each replicate shot of the camera is exposed, in seconds.

3. Select the desired operating mode. The options are:

Options	Description
Up/Down mode	Cycles the sample between successively higher temperature values and the low temperature value where images are taken. Designed for proteins that irreversibly unfold.
Continuous Ramp mode	Takes the sample from one temperature value to the next without lowering the temperature. Images are taken at each temperature value. Designed for proteins that easily refold.

Refer to Chapter 2, Optimizing ThermoFluor® Conditions, in the Application Manual for the appropriate use of the different modes.

4. Select the desired Emission Filter. The options are:

Options	Description
None	No filter is used.
500 ± 30 nm	This is the standard filter that comes with the ThermoFluor® 384 instrument.
530 ± 20 nm	For use in viewing higher wavelength fluorescence.
470 ± 30 nm	For use in viewing lower wavelength fluorescence.

5. Enter the appropriate System Parameters. These fields are:

Field	Description
No. Replicates:	The number of images of the well taken by the CCD camera following the shutter closed image during each cycle in the Up/Down mode.
Gain:	This represents the detection mode of the CCD camera: • 1 corresponds to High Signal to Noise Ratio. Use for bright plates • 2 corresponds to High Dynamic Range. Standard use. • 3 corresponds to High Sensitivity. Use for dim plates. The system is set at 2 during installation.

6. Enter the appropriate Integration Parameters. These values are usually set at installation. They determine the area to be integrated when the Acquire 3.0 programs create the INT files. These fields are:

Field	Description
IBox "X" Dimension:	The width of the integration field used for each well.
IBox "Y" Dimension:	The height of the integration field used for each well.

7. Enter User Comments, if any, in the text box provided.
8. Select rather the camera take a shutter closed image with each set of replicate images during data acquisition to use for background correction. Select YES or NO for shutter closed background correction images.

Edit Methods

This selection is used to set up a Method. It should not be used in the normal operation of the ThermoFluor® 384 system. If more details about this section of the software are desired refer to Appendix A, Total Control for Windows™, for the TCW manual.

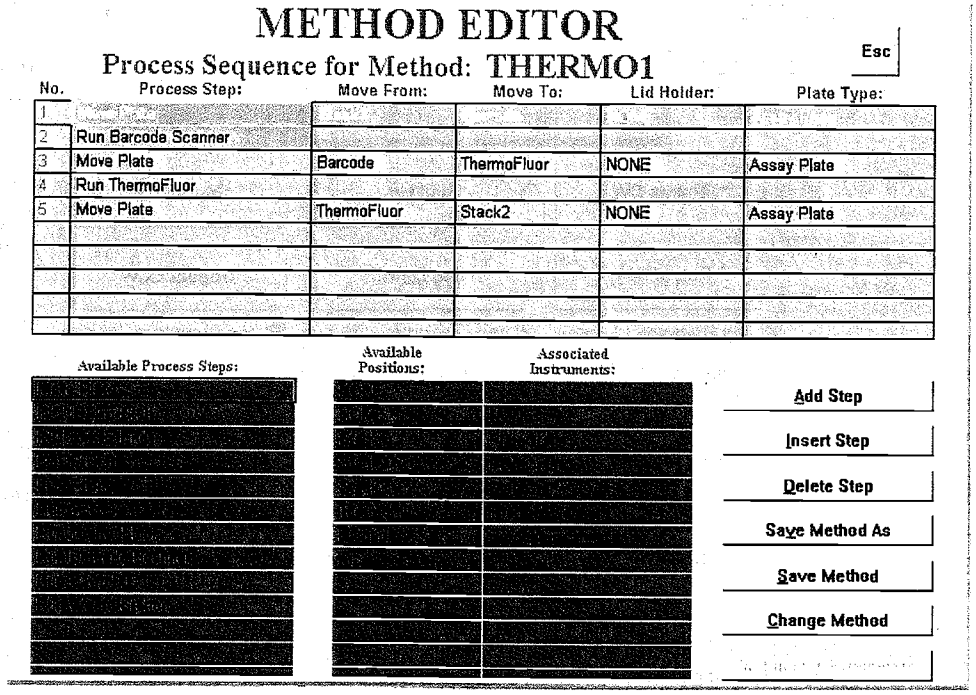


Figure 4- 11: The Method Editor screen is used to setup methods. It is not used during normal operation.

Edit Position List

This selection should not be used in the normal operation of the ThermoFluor® 384 system. If more details about this section of the software are desired refer to Appendix A, Total Control for Windows™, for the TCW manual.

Calibrate PlateCrane™

This selection opens the Calibration screen. See below. The various positions the PlateCrane™ moves to are set up from this screen. These positions should be established when the system is initially installed and should not have to be adjusted or changed during normal operation of the ThermoFluor® 384 system. It is possible that a crash of the system could cause the Stack Position to be altered or lost. This could affect the PlateCrane™'s ability to pick up plates. In this case the Stack Position would have to be re-taught (re-calibrated) to the PlateCrane™ arm. Refer to Appendix A, Total Control for Windows™, for the TCW manual to do this. To verify the Stack Position is correct refer to the Troubleshooting section of Chapter 5. Maintenance.

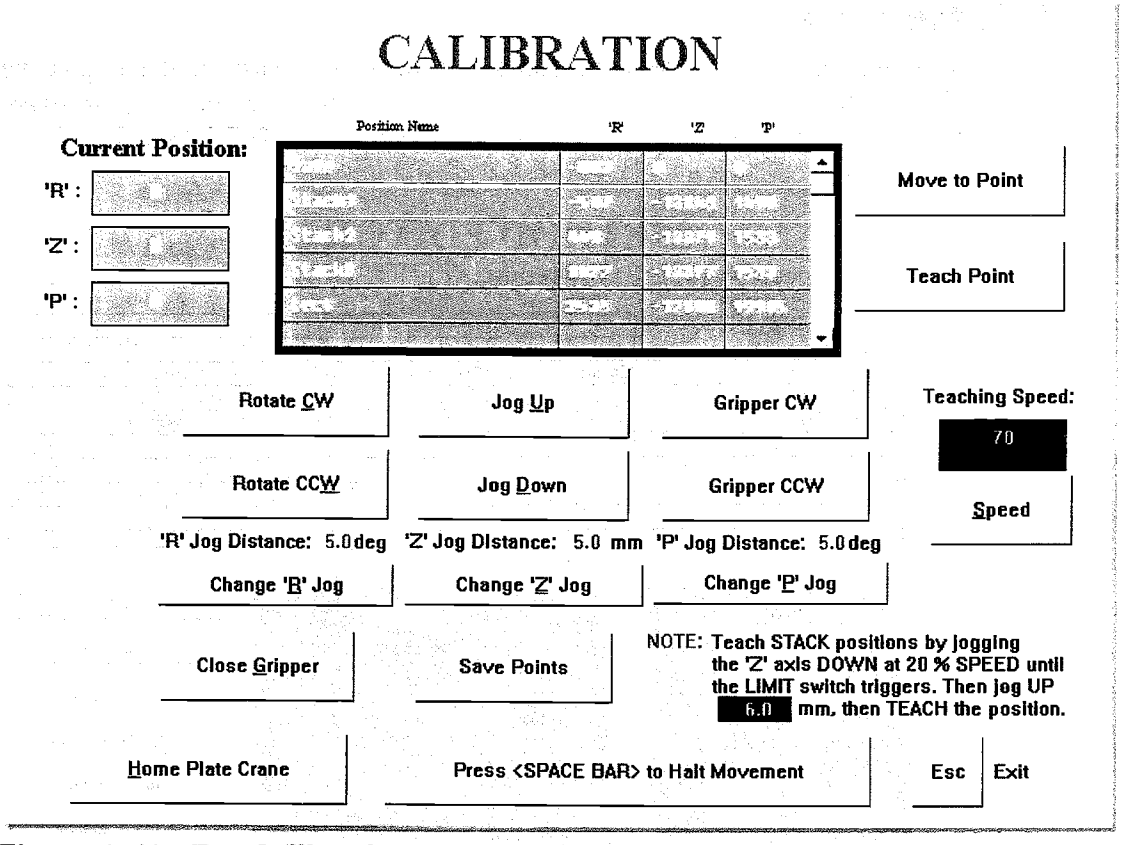


Figure 4- 12: The Calibration screen is used to establish the PlateCrane positions. This screen is not used during normal operation.

System Settings

This selection opens the PlateCrane System Setup screen. See below. The position of the assay plates relative to the PlateCrane™ arm grippers (plate position) is set up from this screen. These positions should be established when the system is initially installed and should not have to be adjusted or changed during normal operation of the ThermoFluor® 384 system. However, if new plates are used this position should be verified and may need to be reset. To verify or reset the plate position, refer to the Troubleshooting section of Chapter 5. Maintenance. If more details about this section of the software are desired refer to Appendix A, Total Control for Windows™, for the TCW manual.

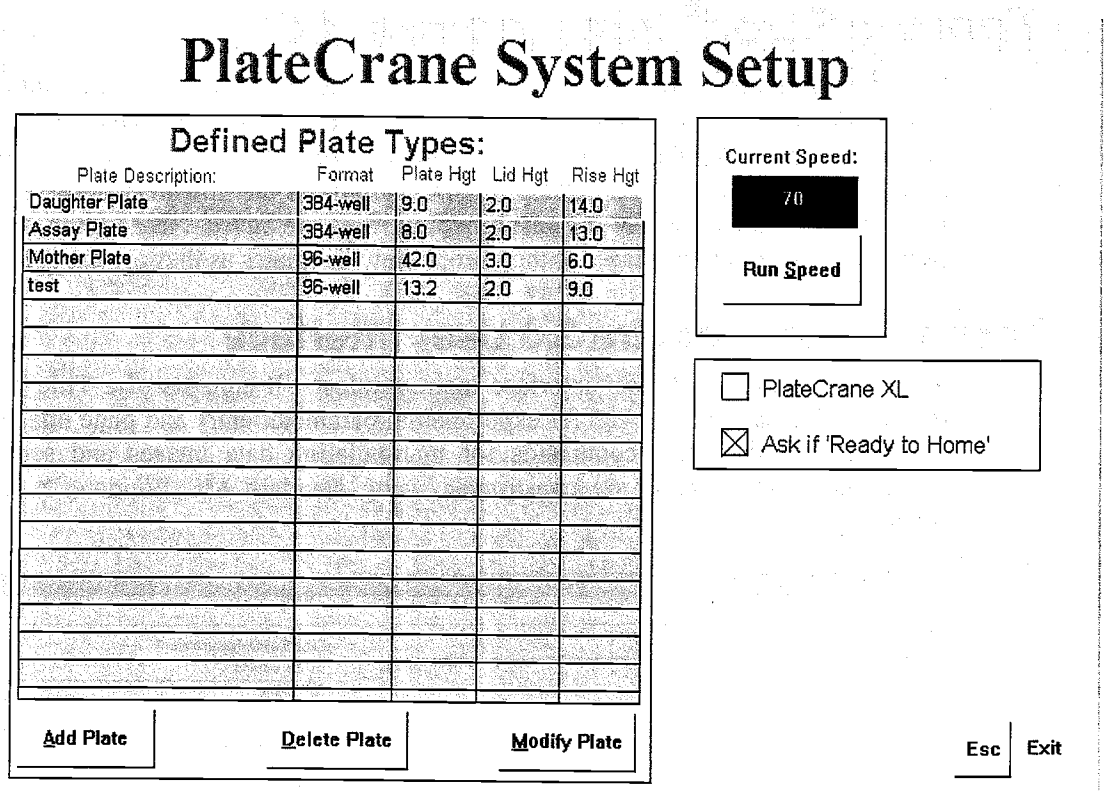


Figure 4- 13: The PlateCrane system setup screen is used to establish the position of the microplates. This screen is not used during normal operation.

Current Speed

This indicates the current speed of the PlateCrane robotic arm.

ThermoFluor® 384 Acquire 3.0

The ThermoFluor® Acquire 3.0 control software provides the user interface for the ThermoFluor® 384 instrument. It allows the user to enter run parameters that are sent to the embedded controller that operates the ThermoFluor® 384 instrument components. It also accepts parameters from the TCW program. It initiates the instruments operation and allows the user to designate where the resulting data files are saved. In addition, the Acquire 3.0 program provides an interface for the user to view the plates as they are exposed.

ThermoFluor® Acquire 3.0 User Interface

This is the Acquire 3.0 user interface main window. It consists of a Title bar, drop down menus, status bar and a screen for experimental parameter entry and plate image display. The menus access the basic commands for manipulating data images and setting subsystem parameters. The menus are constructed using standard MS Windows™ formatting and functions.

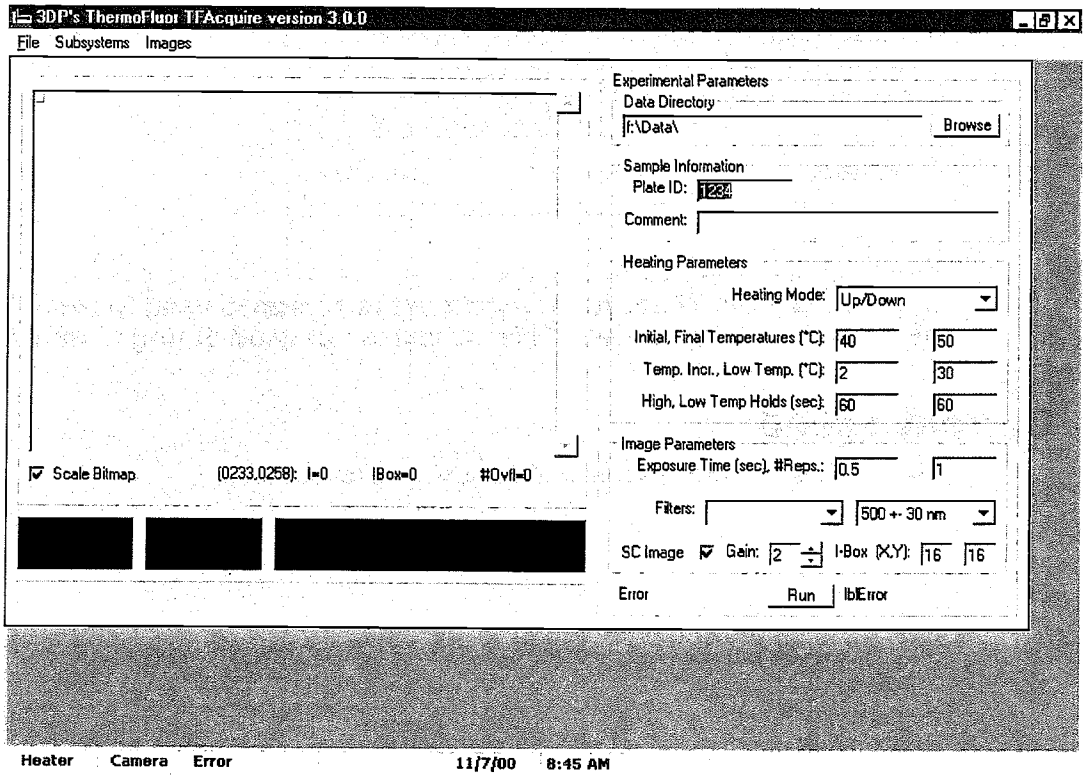


Figure 4- 14: ThermoFluor® Acquire 3.0 user interface.

Title Bar

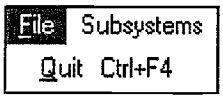
The title bar identifies the ThermoFluor® 384 Acquire 3.0 program window and contains standard Windows™ buttons for minimizing and maximizing the window, and closing the application.

Status Bar

The Status Bar is located at the bottom of the window. It gives the status of the instrument heater and camera. Bold letters for the heater or camera indicate they are operational. The Status Bar also displays system information and error messages. In addition it displays the current date and time.

File Menu

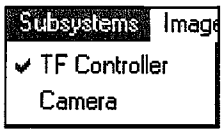
Contains the command for exiting the program.



Menu Selection	Function
Quit	Quit the application.

Subsystem Menu

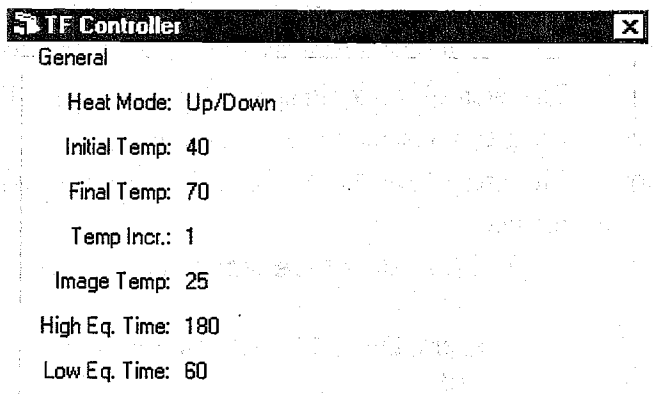
Contains the following commands:



Menu Selection	Function
TF Controller	Opens the TF CONTROLLER dialog box, which displays the current parameters of the embedded controller and the **Test Controller** button for accessing controller test functions.
Camera	Opens the CAMERA dialog box for making adjustments to the camera parameters. The CAMERA dialog box also contains some instrument functions.

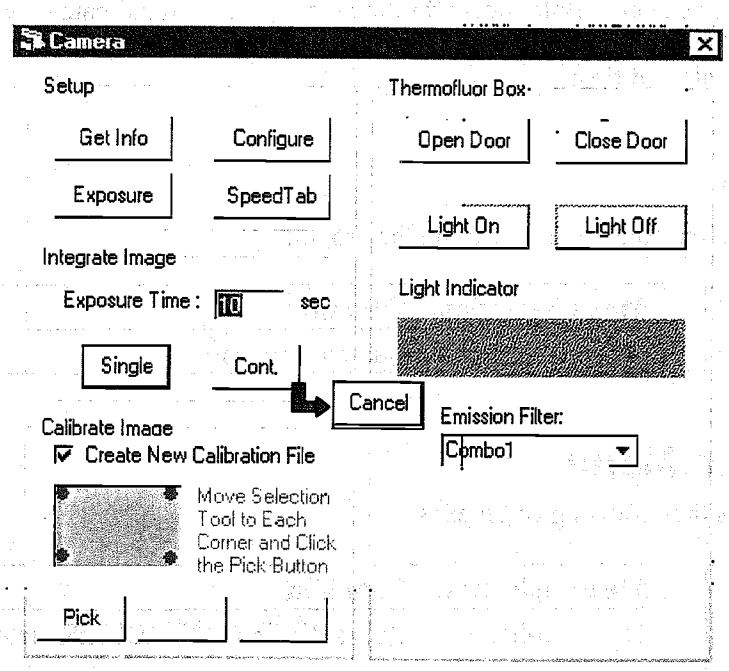
TF Controller pop up window

This window displays general information stored in the embedded controller for the current run.



Camera Dialog Box

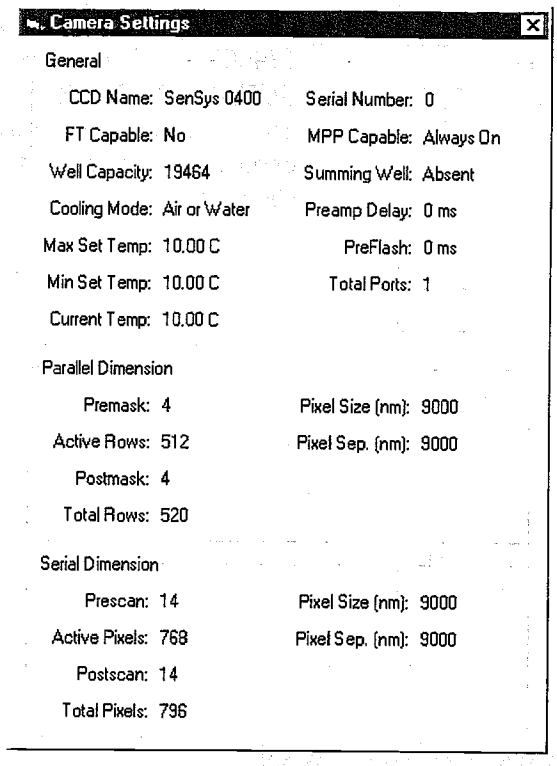
This dialog box provides a means of interacting with the camera and setting camera parameters.



Dialog Area	Description
Setup	Buttons allow access to camera information and camera setup entry dialog boxes.
Integrate Image	This area allows entry of the image exposure time and commands for the camera to take a exposures. • The **Single** button instructs the camera to take a single image that is displayed in the Image display area. • The **Cont**. button instructs the camera to take a continual series of images. • When the **Cont.** button is selected it changes to **Cancel** after the first image is taken. Click **Cancel** to stop taking images.
Calibrate Image	This area allows for image calibration. See Calibrate Spots in Chapter 3, Installation and Calibration.
ThermoFluor box	This area allows the user to operate instrument functions. These include: • **Open** and **Close Door** buttons open and close the light tight door. • **Light On** and **Light Off** buttons turn the UV lights on and off. • The Emission Filter drop down menu allows selection of the desired filter.

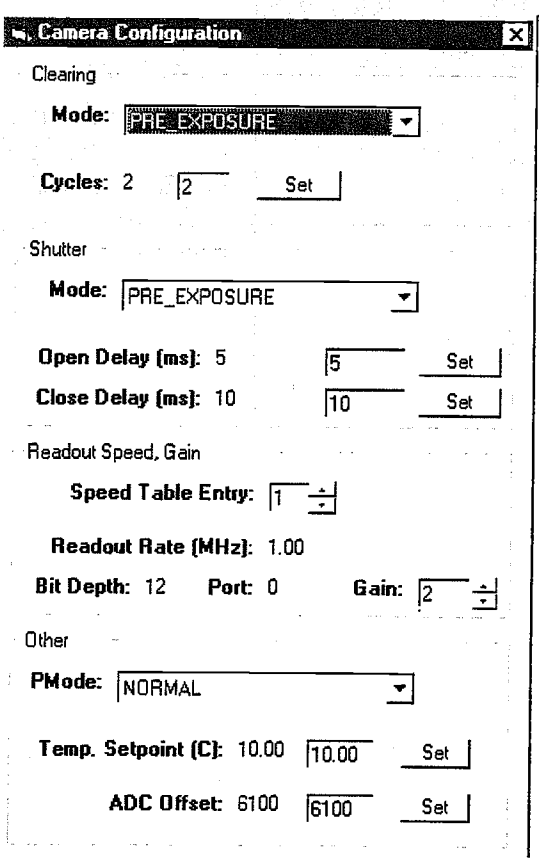
Setup Dialog Boxes

The buttons in the setup area of the CAMERA Dialog box opens several dialog boxes that allow camera configuration settings to be changed. **Only appropriately trained personnel should use the features in these dialog boxes for setup and maintenance of the system.**



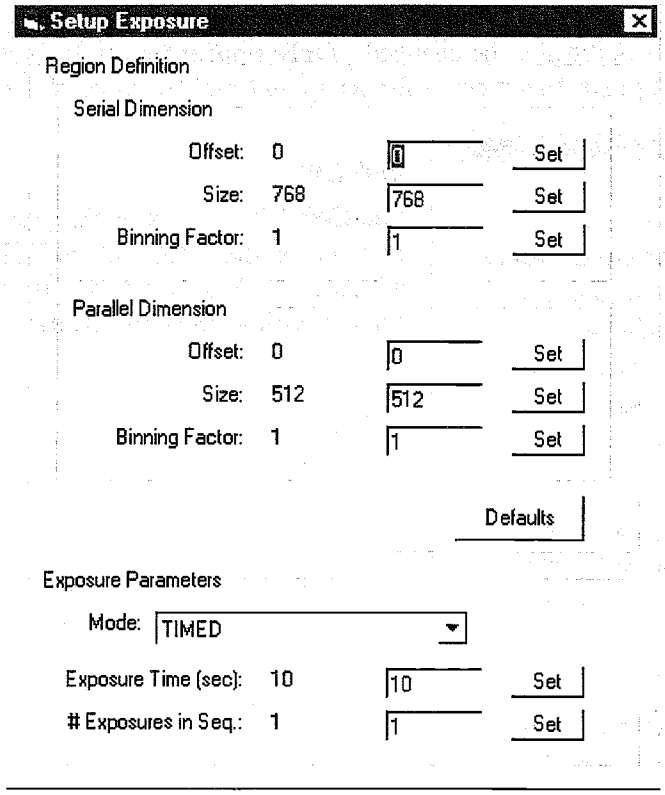
Get Info

The **Get Info** button on the CAMERA Dialog box opens the CAMERA SETTING pop-up window. This window displays various camera settings as shown below.



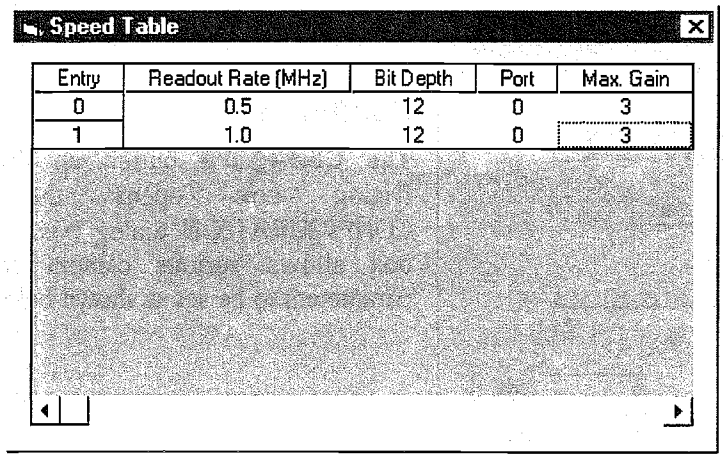
Configure

The **Configure** button on the CAMERA Dialog box opens the CAMERA CONFIGURATION dialog box. This dialog box allows various camera configuration parameters to be set as shown below.



Exposure

The **Exposure** button on the CAMERA Dialog box opens the SETUP EXPOSURE dialog box. This dialog box allows various exposure parameters to be set as shown below.

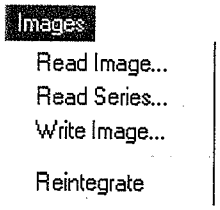


SpeedTab

The **SpeedTab** button on the CAMERA Dialog box opens the SPEED TABLE dialog box. This dialog box allows speed table parameters to be viewed and edited as shown below.

Images Menu

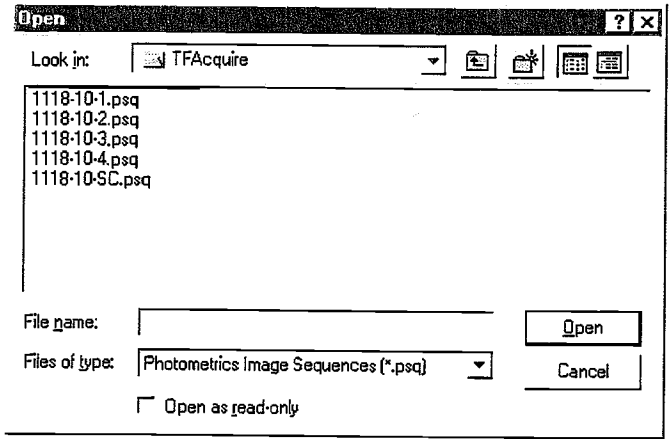
Contains the following commands for writing, reading and reintegrating raw camera data in the form of ***.psq** files. These are designed for offline use.



Menu Selection	Function
Read Image	Opens the OPEN dialog box for the selection of a ***.psq** file for retrieval. Used offline.
Read Series	Opens the SELECT IMAGE SERIES dialog box for the selection of a series of ***.psq** files corresponding to the data for a single plate at all temperatures. Used offline.
Write Image	Opens the SAVE AS dialog box designate which directory and what file name the current image should be saved to as a ***.psq** file.
Reintegrate	Used to reintegrate raw camera image files (***.psq**) offline. Used to recover plate data or process plate data from time-critical run modes. See the Recovering Plate Data Following System Disruption topic in the Troubleshooting section of Chapter 5, Maintenance.

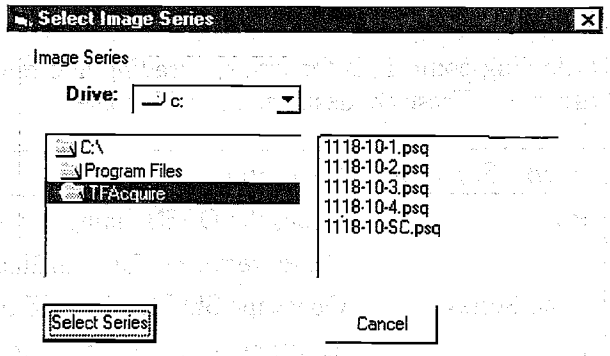
Read Image

This selection allows raw camera data in the form of a single ***.psq** to be viewed in the Image display area of the Acquire 3.0 interface. An Open dialog box with ***.psq** files already selected allows a directory and file to be selected.



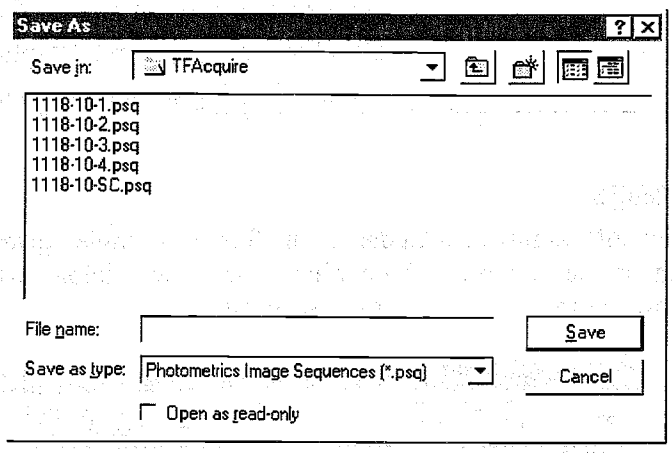
Read Series

This selection allows viewing of raw camera data in the form of a series ***.psq** files corresponding to the data for a single plate at all temperatures. The SELECT IMAGE SERIES dialog box opens to allow selection of a ***.psq** file from the series. By then clicking the **Select Series** button the series of ***.psq** files corresponding to the selected file are retrieved. This feature is also used is used in conjunction with the reintegrate feature. See the Recovering Plate Data Following System Disruption topic in the Troubleshooting section of Chapter 5, Maintenance.



Write Image

This selection allows the current image displayed in the Image Display area to be written to a file. The SAVE AS dialog box opens to allow the designation of the directory and naming of the ***.psq** file. By then clicking the **Save** button the image is saved as a ***.psq** file in the designated directory.



Experimental Parameters

This area of the Acquire 3.0 interface screen allows for the user to enter experimental parameters. These fields can be filled in directly by the user or the information can be relayed from the ThermoFluor Setup Screen in TCW.

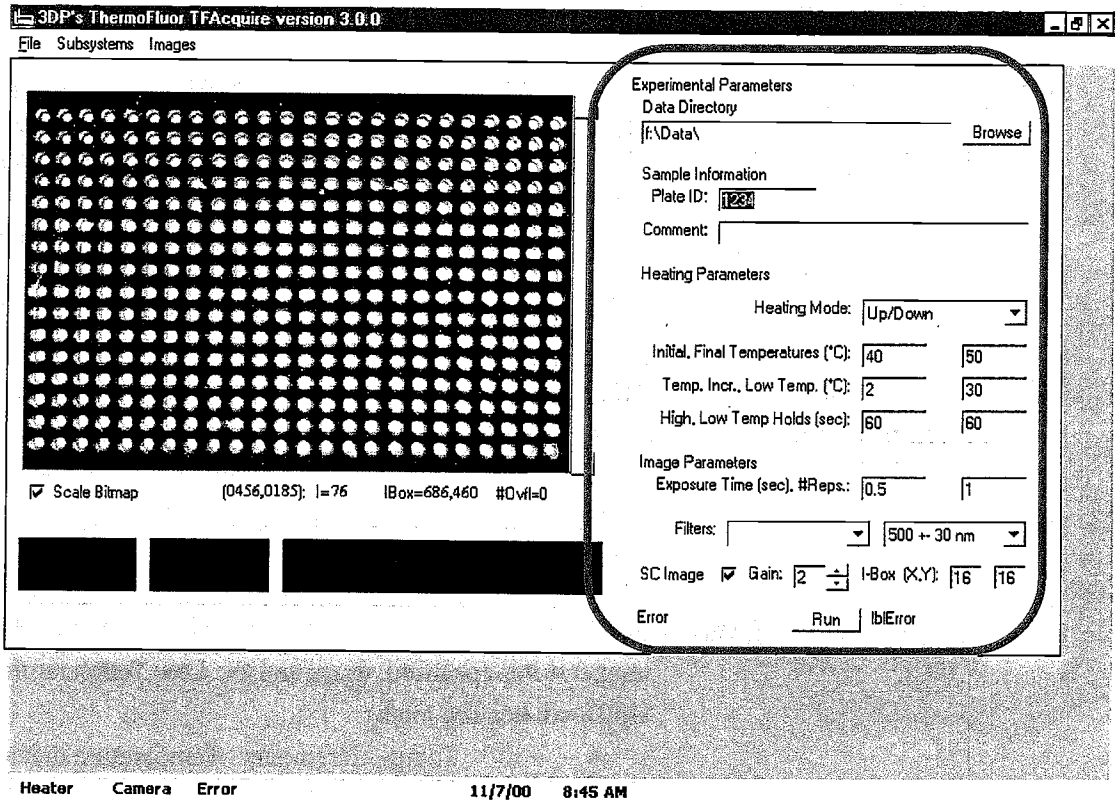
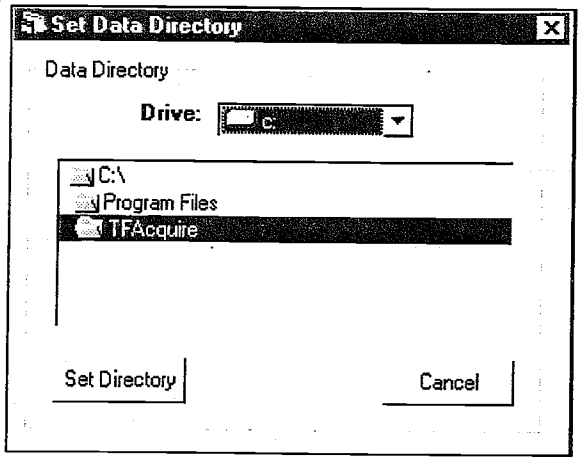


Figure 4- 15: Experimental Parameters area of Acquire 3.0 user interface.

Field(s)	Description
Data Directory	Enter the path and directory for the data ***.psq** and ***.int** files to be stored or click on browse to open the Set Directory dialog box to select the desired folder. See below



Field(s)	Description
Plate ID	The plate ID is an eight-digit number that correspond to the barcode number.
Comments	Enter User Comments in the text box.
Heating Mode	Specify the heating mode from the drop down menu. The modes are Up/Down or Continuous Ramp. • Up/Down Mode: Cycles the assay plate between successively higher temperature values and the low temperature value where images are taken. Designed for proteins that irreversibly unfold. • Continuous Ramp Mode: Takes the sample from one temperature value to the next without lowering the temperature. Images are taken at each temperature value. Designed for proteins that easily refold.
Initial, Final Temperature (°C)	This specifies the range of temperatures for the run. • Initial Temp: The starting temperature in °C for measurements (First high temperature in Up/Down mode). • Final Temp: The last temperature in °C for measurements (Last high temperature in Up/Down mode)
Temp. Incr Low Temp (°C):	Specify the temperature increments to be taken over the range of temperatures specified above and the Low Temperature for the Up/Down heating mode. • Temp. Increments: Temperature difference between measurements in °C • Low Temp: The low temperature in Up/Down mode that is held while images are taken. (Not used in ramp mode.)
High, Low Temp. Holds (sec)	Specify the High and Low Temperature Hold times for the heating cycle. • High Temperature hold: The high temperature equilibration or hold time in seconds for the cycle in Up/Down mode. • Low Temperature hold: The low temperature equilibration or hold time in seconds for the cycle in Up/Down mode.
Exposure time(sec). #Reps:	Specify the length of exposure for each image and the number of replicate images at each temperature. • Exposure time: Time each replicate image is exposed in seconds in Up/Down mode. • No. Reps: The number of images of the well taken during each cycle in the Up/Down mode. Using an average of replicate images may improve the overall signal to noise ratio.

Filter	Select the desired emission Filter from the drop down menu. The 500 nm is standard equipment with ThermoFluor® 384 instrument.
SC Image:	This is selected to designate a shutter closed image tobe taken for background subtraction.
Gain:	This represents the detection mode of the CCD camera: • 1 corresponds to High Signal to Noise Ratio. Use for bright plates • 2 corresponds to High Dynamic Range. Standard use. • 3 corresponds to High Sensitivity. Use for dim plates. The system is set at 2 during installation.
IBox "XY"	Specify the size of the integration area by designating IBox "X" and IBox "Y". • IBox "X"Dimension: The width of the integration field used for each well. • IBox "Y" Dimension: The height of the integration field used for each well.
Run button	Begins the run.

Image Display Area

This area of the screen displays the camera images. This allows the run to be monitored from the Acquire 3.0 interface screen. It will also display ***.psq** files read offline using the **Read Image** and **Read Series** selections of the **IMAGES** Menu.

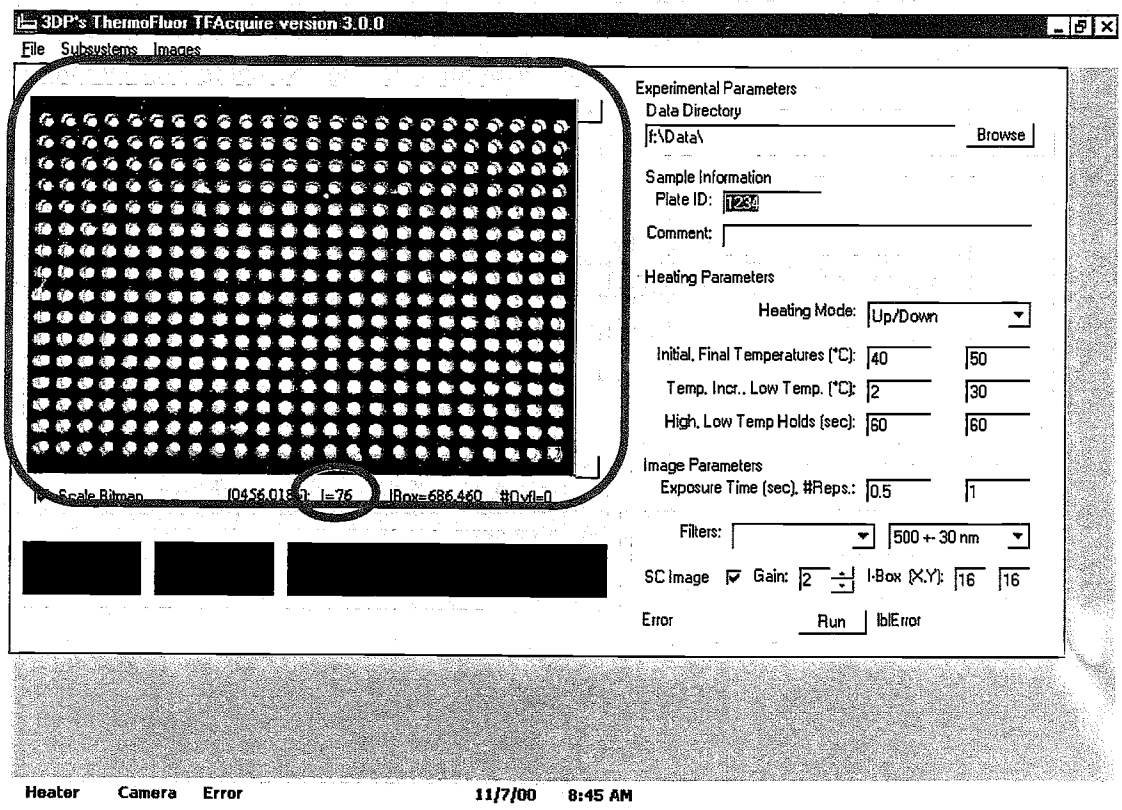


Figure 4- 16: Image Display area of the Acquire 3.0 user interface.

The intensity of an individual pixel can be viewed by placing the cursor over the particular well. The intensity reading is displayed as **I=** under the image. At the beginning of a run the intensity of some pixels can be checked to verify they do not saturate the camera. Moving the cursor over the wells does this by verifying that the intensities are less than 4096.

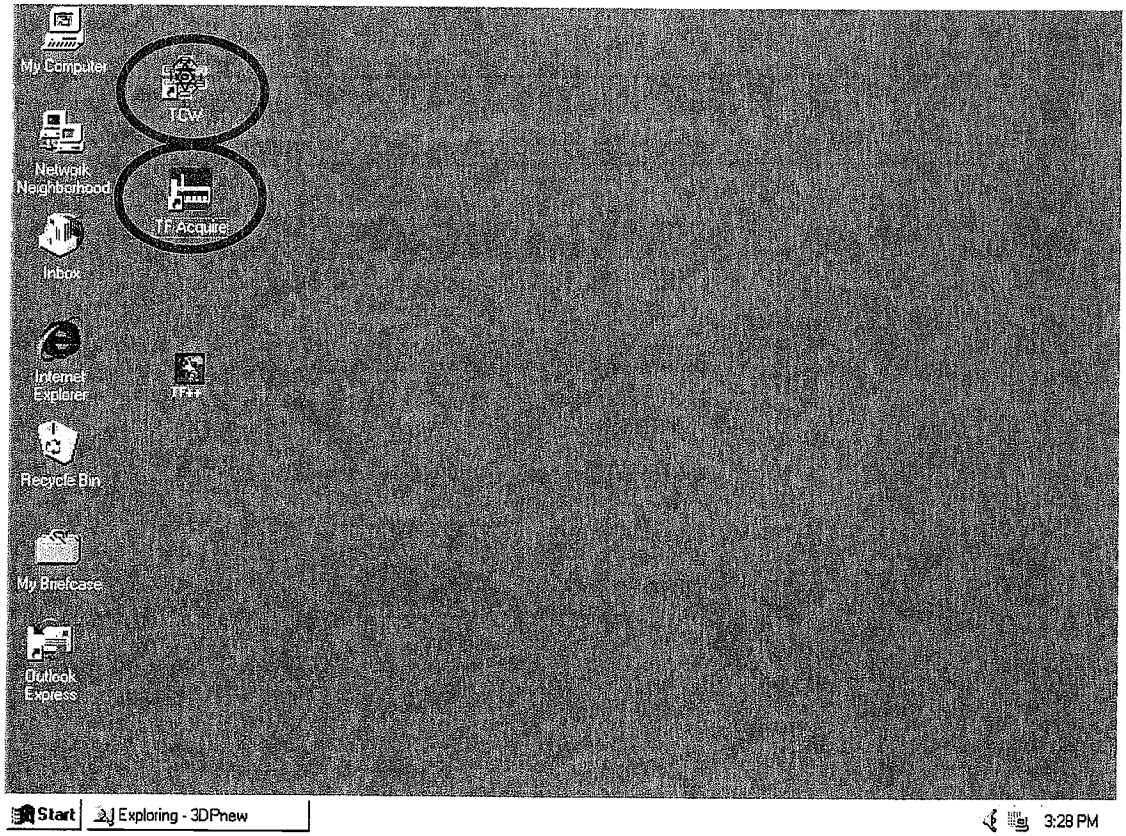
ThermoFluor® 384 System Operation

The ThermoFluor® 384 system should be turned on at least 20 minutes before a run to allow the system to warm up. After the run sheet is completed and the assay plates are placed in the input magazine the screening run is set up in Total Control For Windows™ (TCW). The plates are then run and data files written to a designated directory. The ThermoFluor® Analysis Software then analyzes these data files.

Typical Procedure for Performing a Screening Run

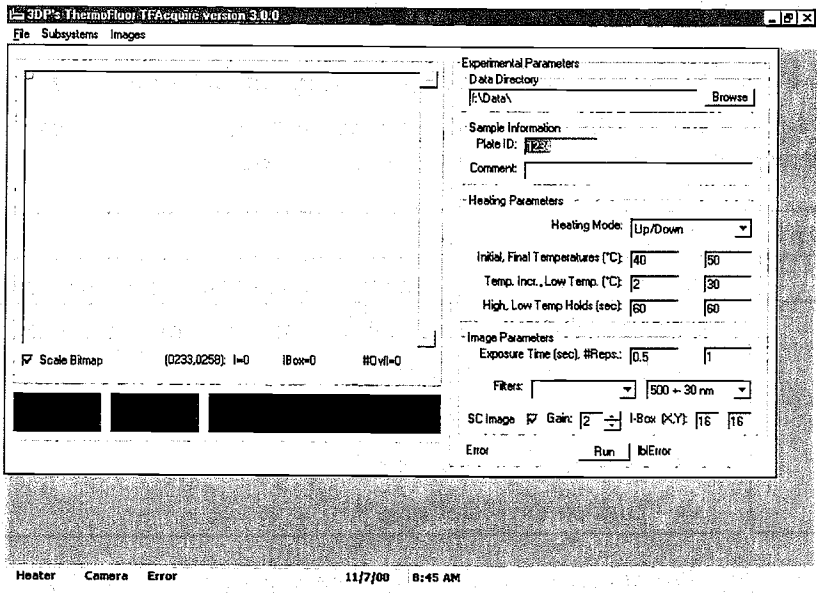
The following procedure represents the way a typical screening assay is conducted. This procedure uses TCW to set up a run of multiple plates.

Step	Action
1	Complete a screening run sheet or other documentation of the run.
2	Turn on the ThermoFluor® 384 System in the following order: • Universal power supply. • Boot-up the NT workstation. The startup screen appears, see below. • Turn on the instrument • Turn on the PlateCrane™.

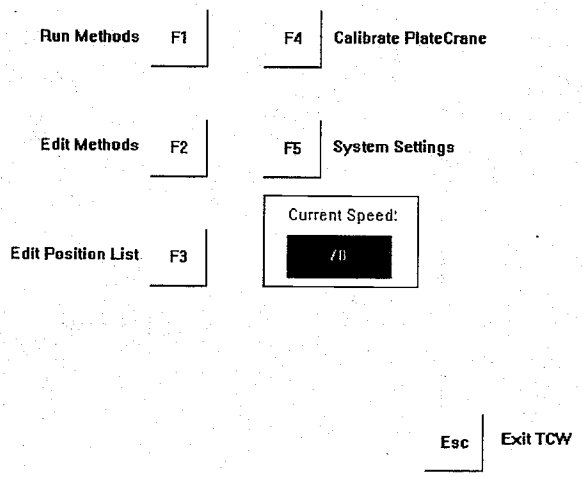


3	Place the assay plates in the input magazine of the ThermoFluor® 384 system.
4	Open the ThermoFluor® Acquire 3.0 application by double clicking the

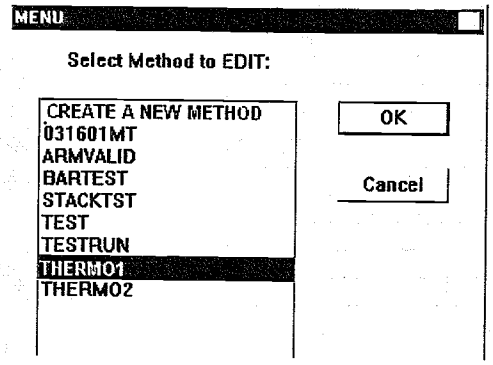
	ThermoFluor® Acquire 3.0 icon. • The ThermoFluor® 384 Acquire 3.0 program opens. • The instrument goes through an initialization. • Observe the screen to verify the heater and camera initialize properly. • Minimize the ThermoFluor® 384 Acquire 3.0 screen.



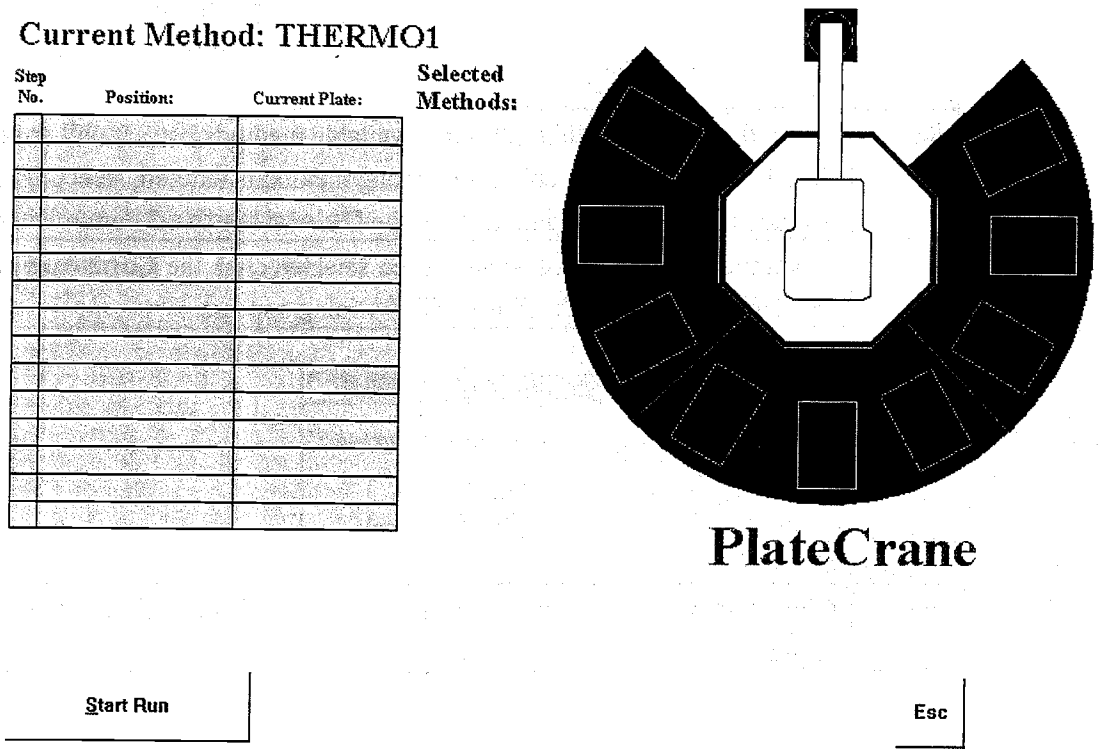
5	Open the Total Control for Windows™ (TCW) application by clicking on the **TCW** icon. The Main Menu screen for TCW opens.



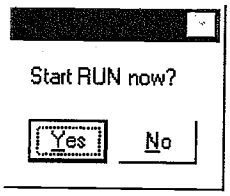
6	Access the THERMOFLUOR PLATE SETUP screen. This is done by:
6A	Clicking the **Run Methods** button or press the <**F1**> function key. The Menu dialog box appears



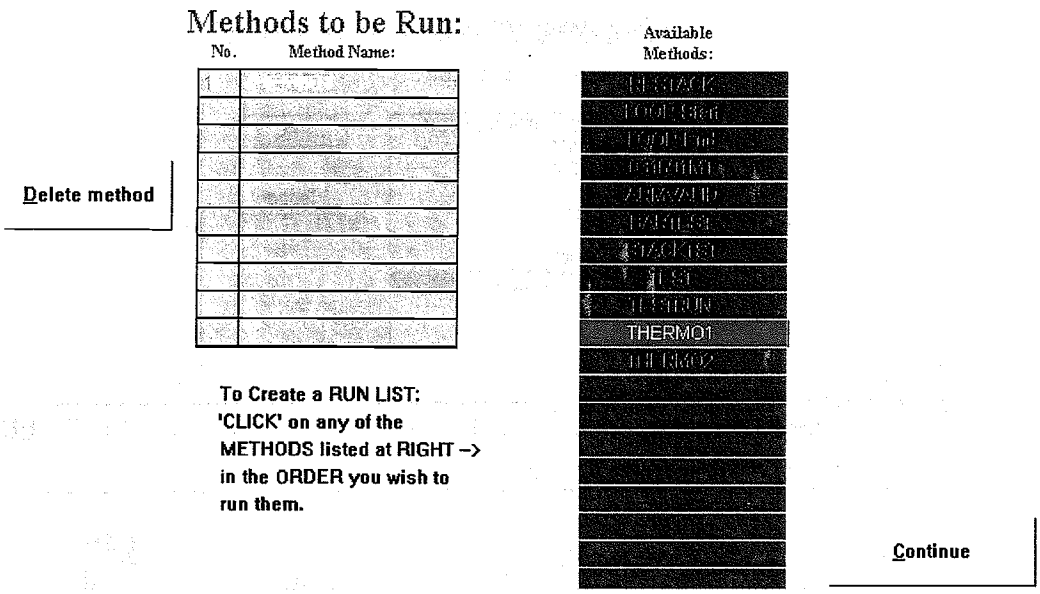
6B	Select the appropriate method from the list and click OK. The CURRENT METHOD screen appears.



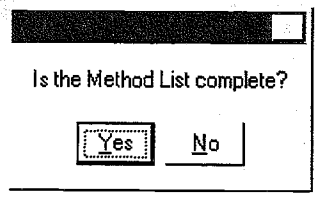
6C	Click the **Start Run** button. The START RUN dialog appears.



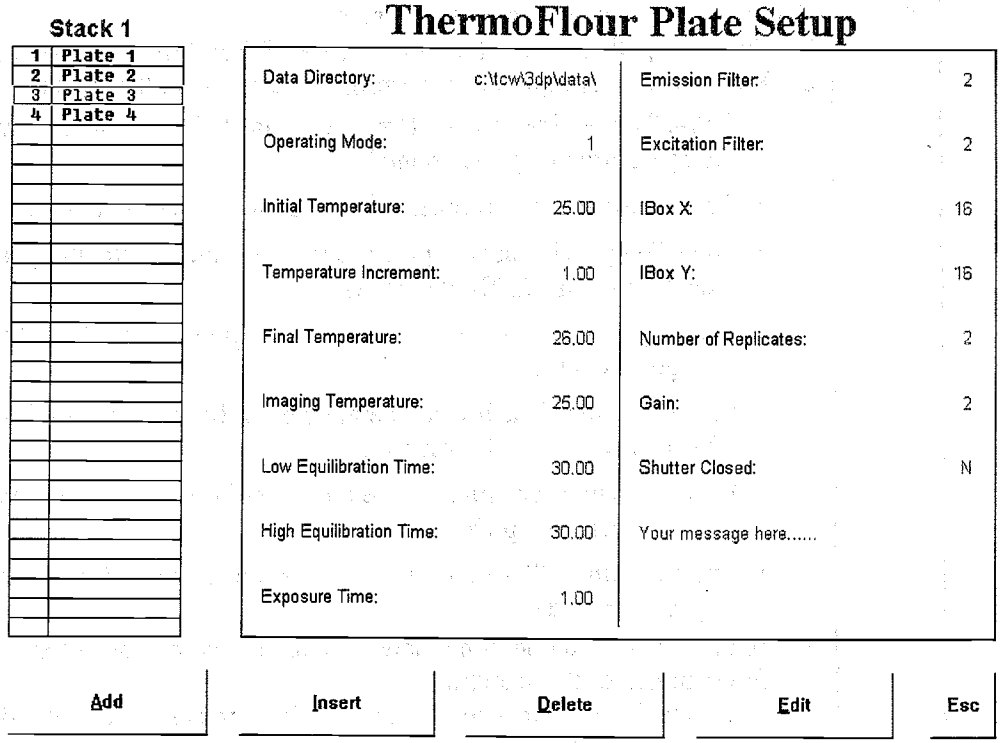
6D	Click **Yes** or press <Enter>. The CREATE METHOD RUN LIST screen appears.



6E	Select the desired method from the available methods list. It will appear under the Methods to be Run list. If the wrong method is entered, selecting it in the Methods to be Run list and clicking the **Delete Method** button will delete it.
6F	When the desired method(s) have been selected click the **Continue** button. The IS THE METHOD LIST COMPLETE? Dialog appears.

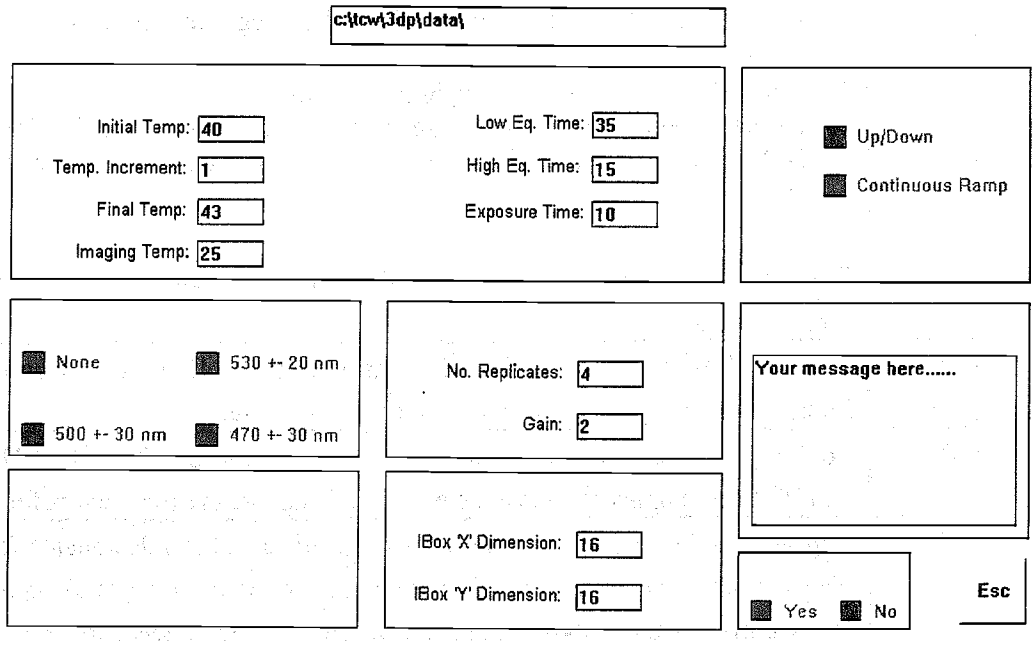


6G	Click **Yes** or press <Enter>. The THERMOFLUOR PLATE SETUP screen appears.



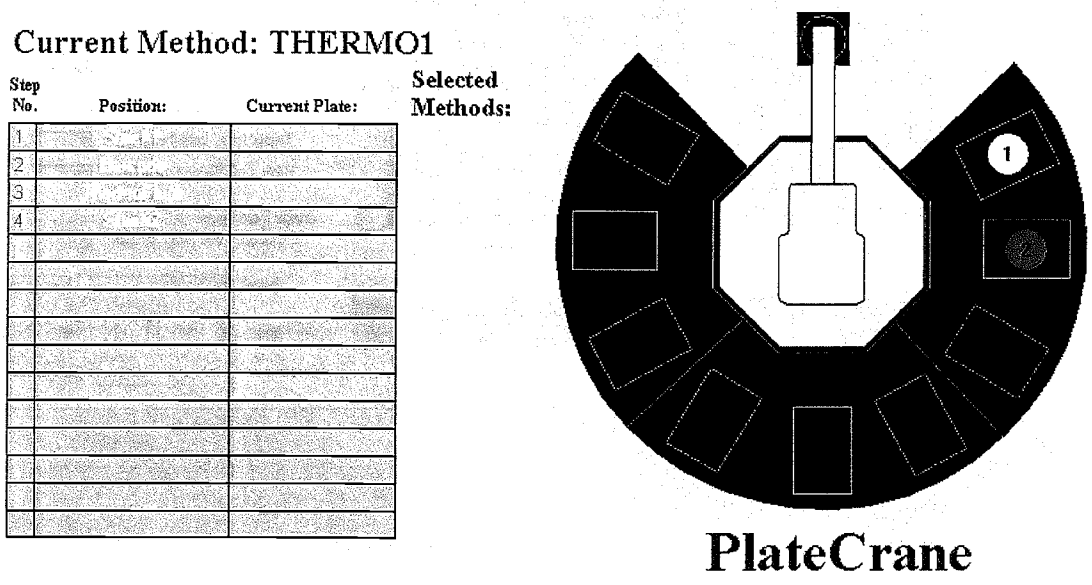
7	Designate the plates and plate parameters to be run in TCW. This is done by:
7A	Click the **Add** button to add Plate1 to the Stack1 list. If all the plates in the Stack1 magazine will use the default parameters go step 8.
7B	To change the parameters for Plate1 click the Edit Button. The THERMOFLUOR SETUP Screen appears.

ThermoFluor Setup Screen

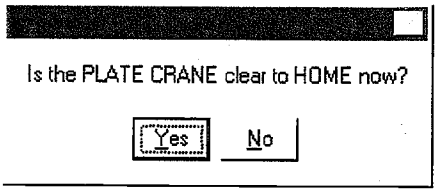


7C	Complete the ThermoFluor Setup Screen. 1. Enter the path and directory for the data PSQ and INT files to be stored. 2. Enter the appropriate Imaging Profile Parameters in the fields: • Initial Temp: The starting temperature in °C for measurements (First high temperature in Up/Down mode). • Temp. Increments: Temperature difference between measurements in °C • Final Temp: The last temperature in °C for measurements (Last high temperature in Up/Down mode) • Imaging Temp: The low temperature in Up/Down mode that is held while images are taken. • Low Eq. Time: The low temperature equilibration or hold time in seconds for the cycle in Up/Down mode. • High Eq. Time: The high temperature equilibration or hold time in seconds for the cycle in Up/Down mode. • Exposure time: Time each replicate image is exposed in seconds in Up/Down mode. 3. Select the desired operating mode by clicking on the corresponding box. Green indicates the selection. 4. Select the desired Emission Filter. The 500 nm is standard equipment with ThermoFluor® 384 instrument. 5. Enter the appropriate System Parameters in the fields: • No. Replicates: The number of images of the well recorded during each cycle in the Up/Down mode. • Gain: amplification value. This is usually set at installation. 1 can be used for particularly bright plates and 3 for particularly dim plates. 6. Enter the appropriate Integration Parameters in the fields. These values are usually set at installation. • IBox "X" Dimension: The width of the integration field used for each well. • IBox "Y" Dimension: The height of the integration field used for each well. 7. Enter User Comments, if any, in the text box. 8. Select whether the shutter is opened or closed. Select YES for closed and NO for Open. Green indicates the selection. Usually Closed at beginning of run. When complete press the <Esc> key. The THERMOFLUOR PLATE SETUP screen appears.
7D	Click the **Add** button to add Plate2 to the Stack1 list. If all the remaining plates in the Stack1 magazine will use the default parameters go step 8.
7E	To change the parameters for Plate2 click the Edit Button. The THERMOFLUOR SETUP Screen appears. Repeat step 7C to designate the parameters for Plate2.
7F	Repeat steps 7D and 7E until all the parameters have been designated for all the plates in the Stack1 magazine. If a plate needs to be inserted, clicking the **Insert** but will insert a plate above the plate selected. If a plate needs to be deleted,

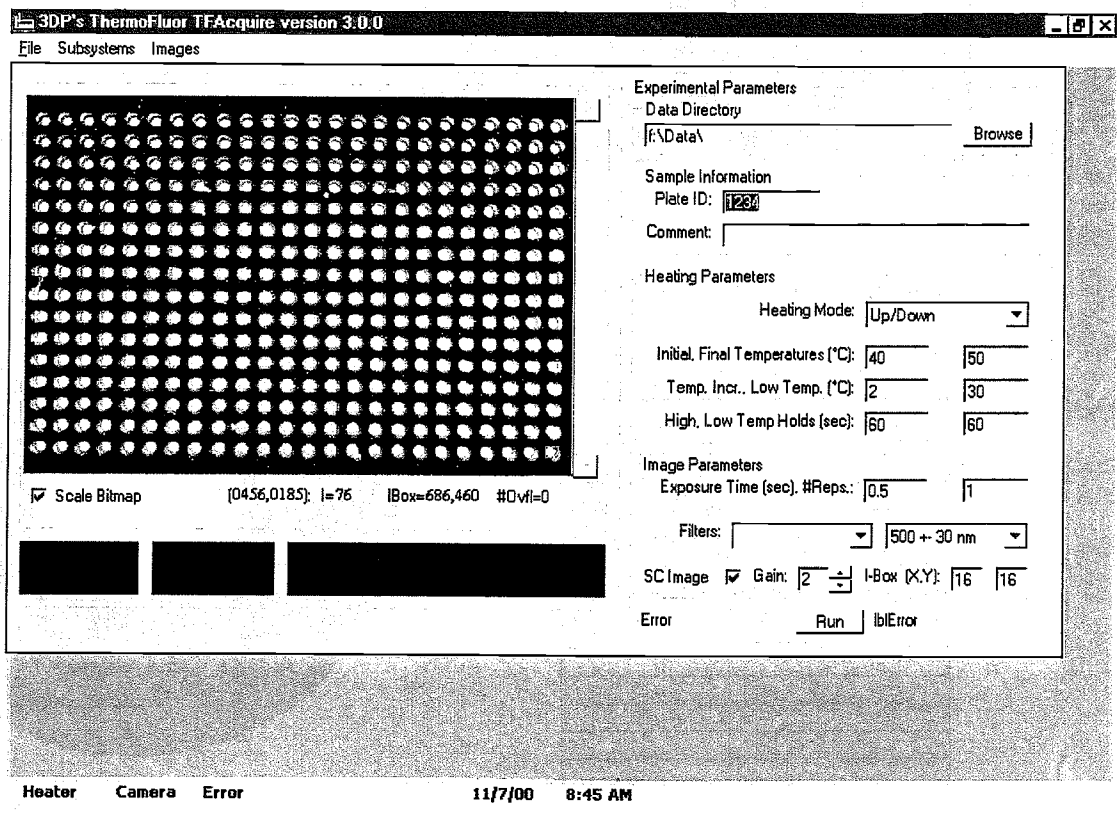
	clicking the **Delete** button deletes the selected plate from the list.
8	Start the run in TCW. To do this:
8A	Exit the THERMOFLUOR PLATE SETUP screen by pressing the <Esc> key. The CURRENT METHOD screen appears with the message "System is PAUSED".



8B	Press the <spacebar> to unpause the system. The IS THE PLATECRANE CLEAR TO HOME NOW? Dialog opens.



8C	Click **Yes** or press <Enter> to begin the run.
8D	The plates are run and data files are written to the designated data directory.
9	If it becomes necessary to terminate the run click the **Terminate Run** button.
10	The run can be monitored from the Acquire 3.0 interface screen. It will update the status bar and display the well exposures in the plate imaging display.

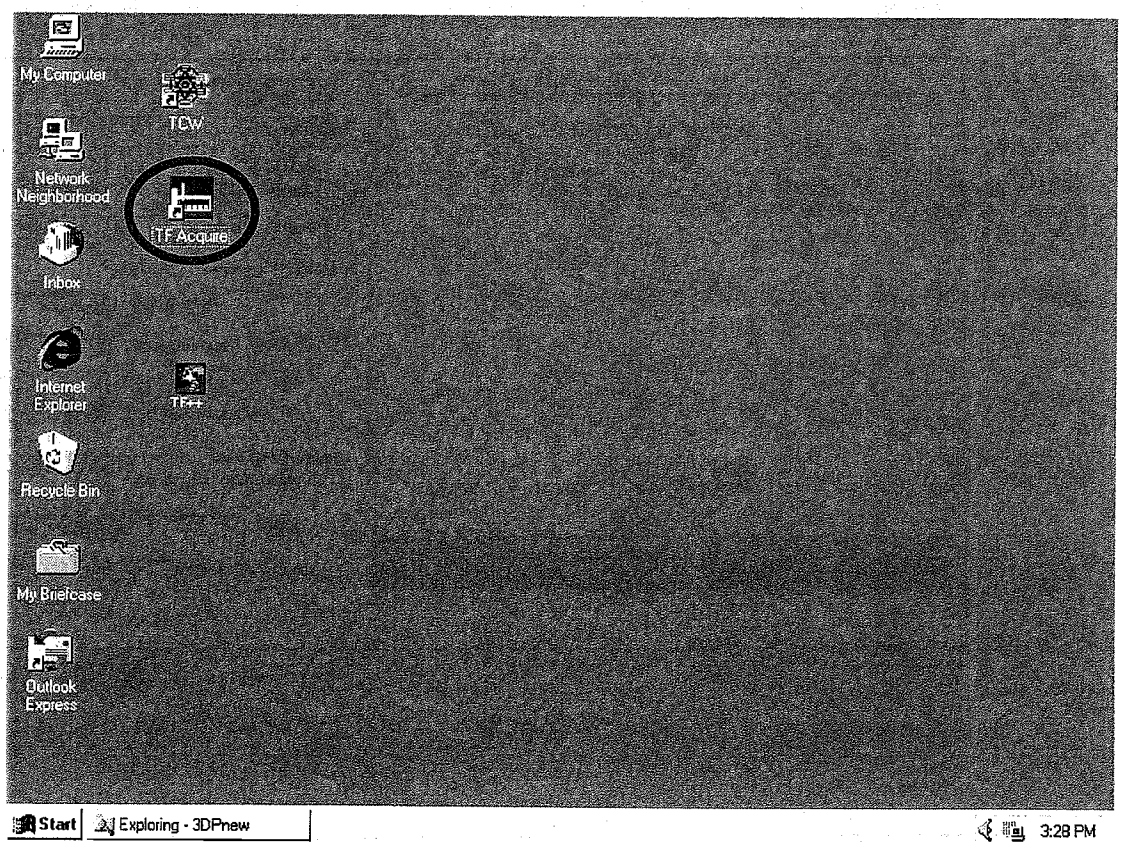


11	At the completion of the run remove the plates from the ThermoFluor® 384 system output magazine.
12	If the ThermoFluor® 384 system is not going to be used for an extended period of time shut down the system.
13	Analyze the data files using the ThermoFluor® Analysis software. See ThermoFluor® 384 Analysis Software Manual.

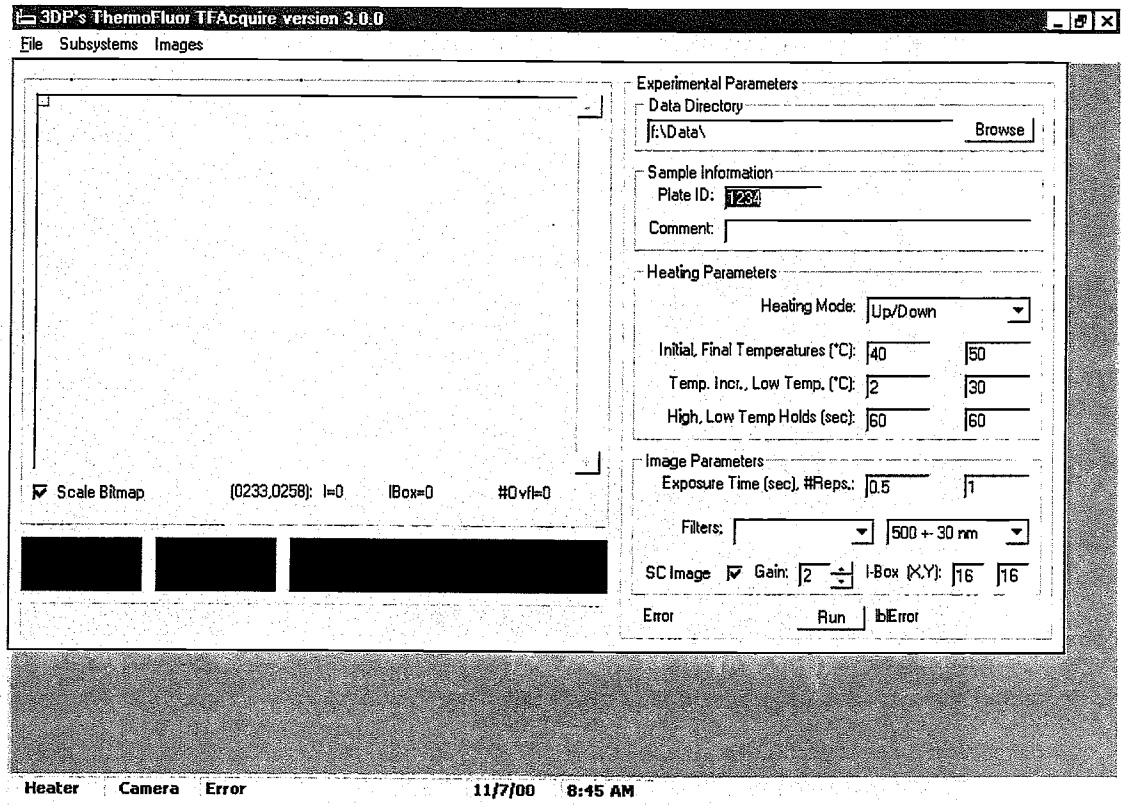
Performing a Single Plate Run with Acquire 3.0.

There may be times when it is necessary to run only a single plate and manually load it into the ThermoFluor® 384 instrument. This can be done from the Acquire 3.0 program directly. To do this follow the procedure below.

Step	Action
1	Complete the necessary run sheet.
2	Turn on the ThermoFluor® 384 System in the following order: • Universal power supply. • Boot-up the NT workstation. The startup screen appears, see below. • Turn on the instrument

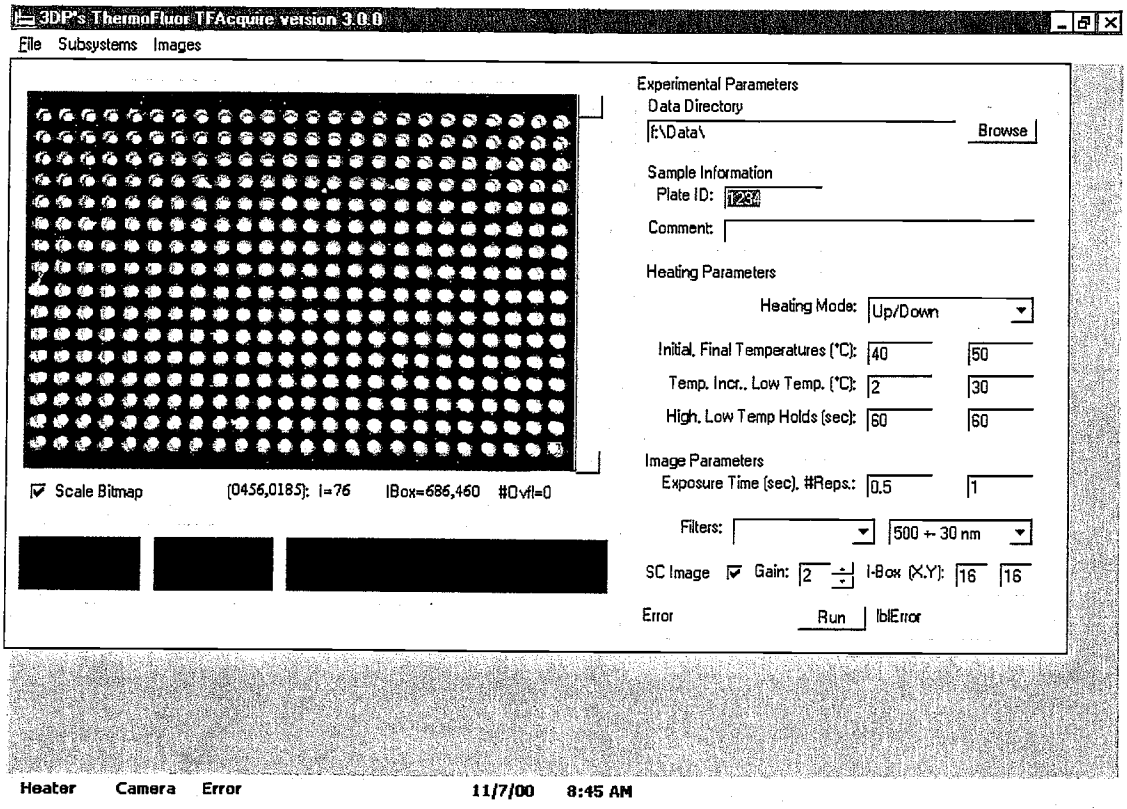


3	Open the ThermoFluor® Acquire 3.0 application by double clicking the ThermoFluor® Acquire 3.0 icon. • The ThermoFluor® 384 Acquire 3.0 program opens. • The instrument goes through an initialization. • Observe the screen to verify that the heater and camera initialize properly.
4	Place the assay plate on of the ThermoFluor® instrument heater block.



5	Enter the experimental parameters using the Acquire 3.0 interface screen. This is done by:
5A	Enter the path and directory for the data PSQ and INT files to be stored. By clicking on the **Browse** button the SET DATA DIRECTORY dialog box appears to select the desired folder.
5B	Enter the plate ID number. This is an eight-digit number that would correspond to the barcode number.
5C	Enter User Comments, if any, in the text box.
5D	Specify the heating mode from the drop down menu. The modes are Up/Down or Continuous Ramp.
5E	Enter the appropriate Heating Parameters in the fields: • Initial Temp: The starting temperature in °C for measurements (First high temperature in Up/Down mode). • Final Temp: The last temperature in °C for measurements (Last high temperature in Up/Down mode) • Temp. Increments: Temperature difference between measurements in °C • Low Temp: The low temperature in Up/Down mode that is held while images are taken. • High Temperature hold: The high temperature equilibration or hold time in seconds for the cycle in Up/Down mode. • Low Temperature hold: The low temperature equilibration or hold time in seconds for the cycle in Up/Down mode.

5F	Enter the appropriate Imaging Parameters in the fields: • Exposure time: Time each replicate image is exposed in seconds in Up/Down mode. • No. Reps: The number of images of the well recorded during each cycle in the Up/Down mode. • Select the desired emission Filter from the drop down menu. The 500 nm is standard equipment with ThermoFluor® 384 instrument. The remaining fields are usually set at installation. They are: • SC Image: This designates a closed shutter image be taken for background subtraction. • Gain: amplification value (2). This is usually set to two at installation and left. 1 can be used for particularly bright plates and 3 for particularly dim plates. • IBox "X" Dimension: The width of the integration field used for each well. • IBox "Y" Dimension: The height of the integration field used for each well.
6	Click the **Run** button to begin the run. The light tight door closes and the run begins. The plate is run and the data files are written to the designated data directory.
7	The run can be monitored from the Acquire 3.0 interface screen. It will update the status bar and display the well exposures in the plate imaging display area.



8	At the completion of the run the light tight door will open. Remove the plate from the ThermoFluor® instrument heater block.
9	If the ThermoFluor® 384 system is not going to be used for an extended period of time shut down the system.
10	Analyze the data files using the ThermoFluor® Analysis software. See ThermoFluor® 384 Analysis Software Manual.

Chapter Five

Maintenance

Overview

This chapter provides information and instructions for the routine maintenance of the ThermoFluor® 384 system.

Specifically, this chapter includes the following sections:

- *System Cleaning*
- *Camera Focusing*
- *Filter Changes*
- *Light Source changes*
- *Troubleshooting*
- *Repairs*

The following table identifies the appropriate section to read in order to learn how to perform specific maintenance tasks or troubleshooting.

To learn more about ...	Read the section....	See page
Cleaning the system	System Cleaning	5- 2
Focusing the camera	Camera Focusing	5-3
Changing filters	Filter Changes	5-5
Changing a light source	Light Source changes	5-7
Troubleshooting the system	Troubleshooting	5-8
Repairing the ThermoFluor® 384 system	Repairs	5-17

System Cleaning

The following cleaning procedures should be followed every three (3) months.

Internal Instrument Cleaning

Use the following procedure to clean the interior of the ThermoFluor® 384 instrument.

Step	Action
1	Shut down and disconnect power to the system.
2	Open the light tight compartment door and lock it in an open position.
3	Mix approximately 2 1/2 ounces of bleach with 1 gallon warm distilled water.
4	Dampen a cloth in the bleach solution.
5	Wipe all black anodized surfaces with a damp cloth.
6	Wipe any stainless steel components with the damp cloth.
7	Wipe and thoroughly dry all horizontal black anodized surfaces with a dry cloth.
8	Wipe and thoroughly dry the stainless steel components with a dry cloth.
9	Properly dispose of all clothes and cleaning materials when done.

External Instrument Cleaning

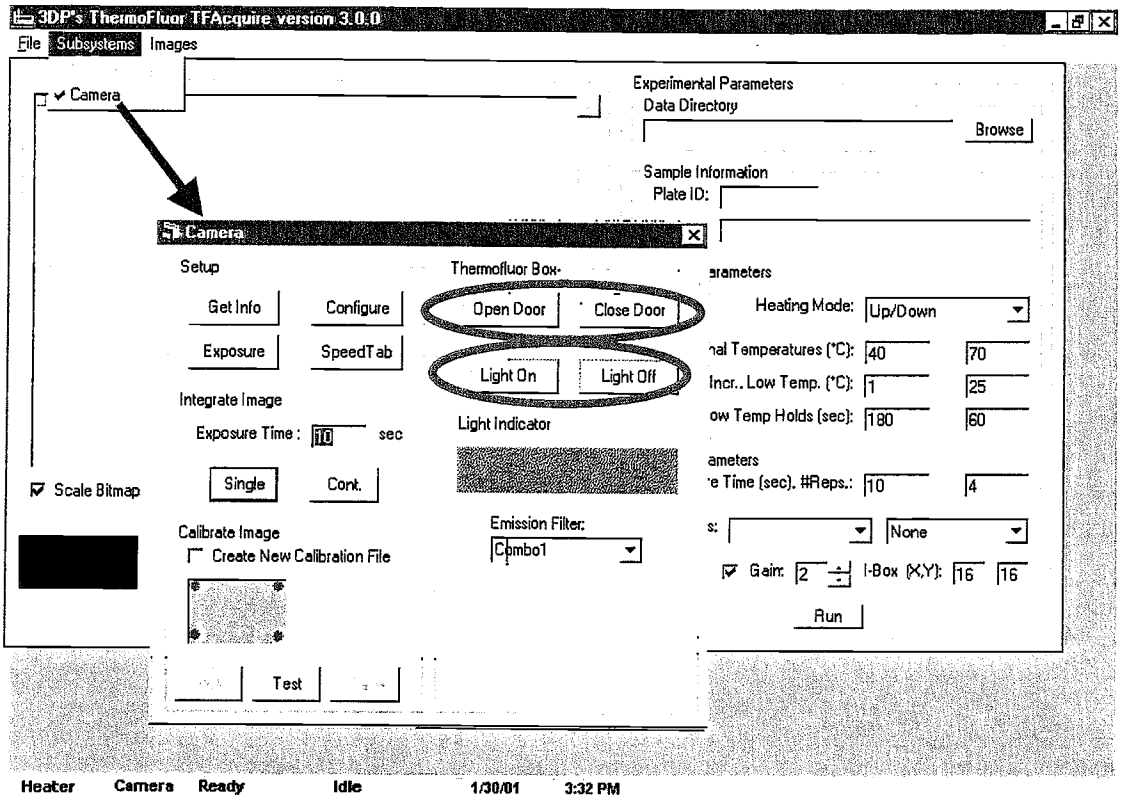
Follow the following procedure to clean the exterior of the ThermoFluor® 384 instrument.

Step	Action
1	Shut down and disconnect power to the instrument.
2	Dampen a cloth in glass cleaner solution.
3	Wipe all external surfaces on the instrument and around the plate processing system with the damp cloth. Avoid any connectors, plugs or cables.
4	Wipe all external surfaces on the instrument with a dry cloth.
5	Wipe all horizontal black anodized surfaces around the plate processing system with a damp cloth.
6	Wipe the stainless steel rods of the plate magazines with a damp cloth.
7	Wipe and thoroughly dry all horizontal black anodized surfaces around the plate processing system with a dry cloth.
8	Wipe and thoroughly dry the stainless steel rods of the plate magazines with a dry cloth.
5	Properly dispose of all clothes and cleaning materials when done.

Camera Focusing

Use the procedure below to focus the camera. The system software must be running to accomplish this task.

Step	Action
1	If it is not already open, open the optical compartment by removing the screws from the rear panel.
2	Place a white piece of paper with small print on the thermal cycler block. To do this manually open the light tight door as follows: From the Acquire 3.0 **SUBSYSTEM** menu select **Camera**. The CAMERA dialog box appears. Click the **Open Door** button. The light tight door opens



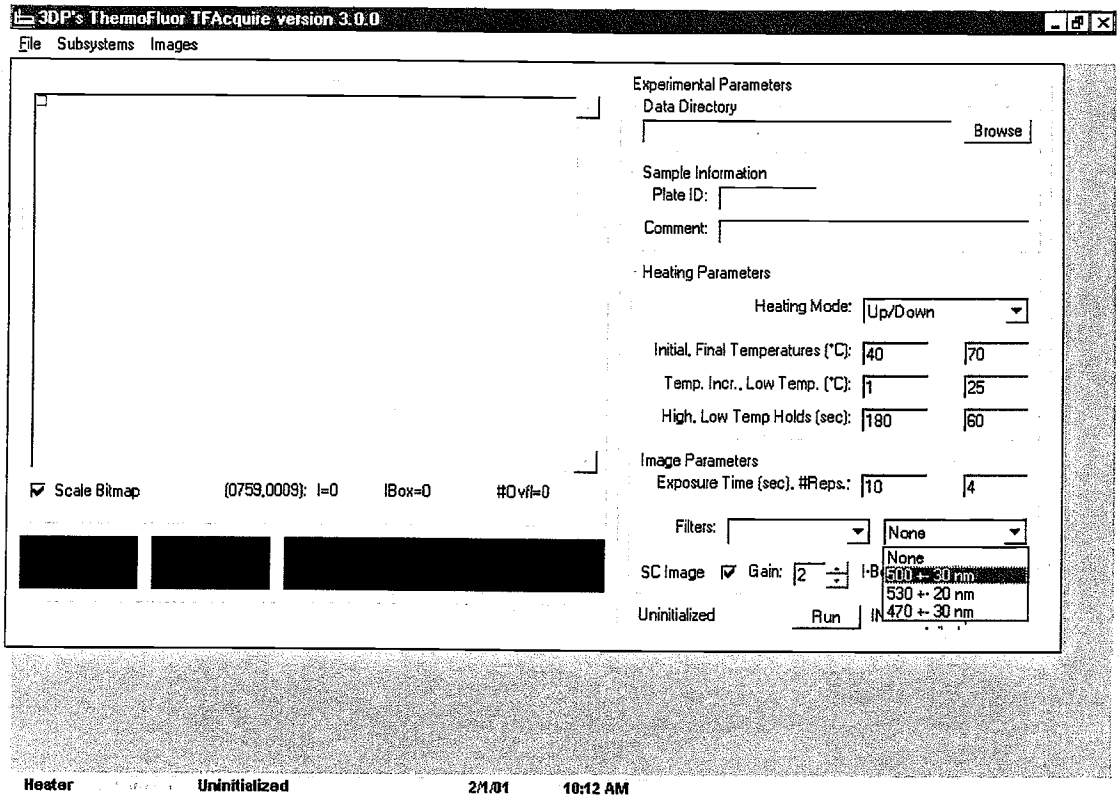
3	Once the piece of paper is placed on the thermal cycler block close the light tight door by clicking the **Close Door** button in the CAMERA dialog box.
4	From Acquire 3.0 CAMERA dialog box turn ON the UV light source by clicking the **Light On** button.
5	Enter an exposure time (one second) for the camera in the Integrate Image section of the CAMERA dialog box.
6	Click on the **Cont.** button to have the camera start taking a continuous series of images with the given exposure time.

7	From the rear of the instrument adjust the bottom adjustment ring on the lens until the image is sharp.
8	Close the optical compartment door and secure with screws.
9	Turn OFF the UV light source by clicking the **Light Off)** button.
10	Remove the white piece of paper from the thermal cycler block. To do this open the light tight door by clicking the **Open Door** button in the CAMERA dialog box as done above.
11	Once the piece of paper is removed from the thermal cycler block close the light tight door by clicking the **Close Door** button.

Filter Changes

Use the procedure below to change a filter. The system software must be running to accomplish this task.

Step	Action
1	Open the optical compartment by removing the screws from the rear panel.
2	If the filter wheel is already in the desired position go to step 4. If it is not in the desired position click on the arrow for the right hand **Filters** drop down menu in the images parameter section at the lower right of the ThermoFluor® 384 Acquire 3.0 window. See Below.



3	Select the desired position from the drop down menu by clicking that position on the listing. See the above figure. The filter wheel will rotate to that position.
4	Remove the filter wheel filter cover from over the filter.
5	Grasp the filter by the edge and remove from the filter wheel. Place in a safe position or holder.
	CAUTION!!! **Filters are sensitive optics and should be handled by the edge with care**
6	Locate the replacement filter, grasp it by the edge and place it in the filter wheel

	position with the writing right side up.
	CAUTION!!! **Filters are sensitive optics and should be handled by the edge with care**
7	Secure the filter wheel filter cover over the filter.
8	Close the optical compartment door and secure with screws.
9	Properly store the removed filter

Light Source Changes

Use the procedure below to change the light source.

1	Turn off power to the instrument.
2	Open the rear panel of the light tight compartment by removing the screws.
3	Disconnect the connectors to the UV light source.
4	Grasp the UV light fixture by the supports and pull backwards. The unit should slide back and drop down from its mounting pins.
5	Secure the filter wheel filter cover over the filter.
6	Align the fixture with the mounting pins and slide forward until it is secure.
	Reconnect the connectors to the UV light source.
8	Close the optical compartment door and secure with screws.
9	Turn on power to the instrument.

Troubleshooting

This section discusses some of the ThermoFluor® 384 system specific problems that might occur and their possible solutions. These problems include:

- Failure of a component to initialize.
- Recovering plate data following system disruption.
- Plate crane doesn't pick up plates.

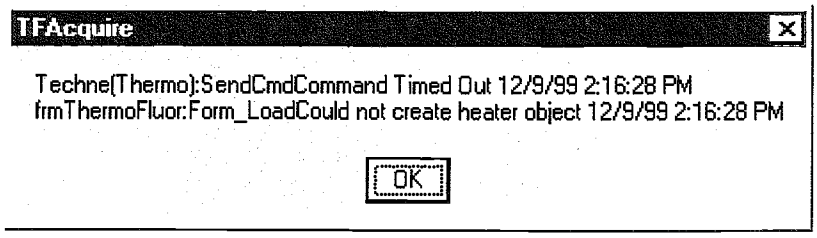
For additional information about problems with specific components of the system refer to their documentation that is supplied with the system.

Component Fails to Initialize

As the ThermoFluor® Acquire 3.0 program launches, it searches for the several available sub-systems inside the ThermoFluor® system. These are the CCD camera, the PlateCrane™, thermal cycler heater, and the bar code reader. There are indicator fields for the heater and camera at the lower left corner of the Acquire 3.0 screen. If any of these fail to initialize the system will not function properly.

Thermal Cycler Heater

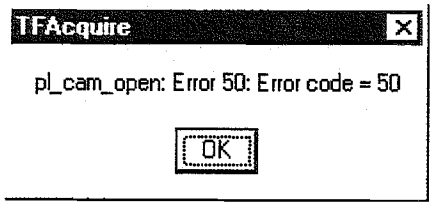
If the heater module fails to initialize, the following error message will appear:



If this message appears restart the ThermoFluor® Acquire 3.0 program. If this does not solve the problem contact 3-Dimensional Pharmaceuticals, Inc. at 1-610-458-8959 for technical support.

Camera

If the camera fails to initialize, the following error message will appear:



If this message appears follow the procedure below.

Step	Action
1	Shutdown the NT workstation
2	Turn power OFF to the ThermoFluor® 384 Instrument
3	Turn power ON for the ThermoFluor® 384 Instrument

4	Reboot the NT workstation.
5	Launch the ThermoFluor® Acquire 3.0 program.
6	Observe that the camera initializes in the indicator field at the lower left corner of the Acquire 3.0 screen.

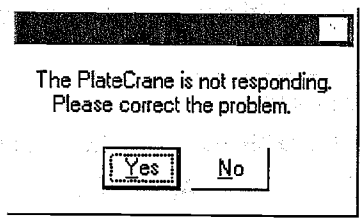
If this does not solve the problem contact 3-Dimensional Pharmaceuticals, Inc. at 1-610-458-8959 for technical support.

Barcode Reader

If the barcode reader fails to initialize there will be no beep when TCW is started. Also the red light will not come on when the PlateCrane moves a plate in front of the reader. If this happens verify there is power to the barcode reader. If there is then restart the system. If this does not solve the problem contact 3-Dimensional Pharmaceuticals, Inc. at 1-610-458-8959 for technical support.

PlateCrane™

If the PlateCrane™ fails to initialize, the following error message will appear:



If this happens verify there is power to the barcode reader. If there is then restart the system. If this does not solve the problem contact 3-Dimensional Pharmaceuticals, Inc. at 1-610-458-8959 for technical support. Contact 3-Dimensional Pharmaceuticals, Inc. at 1-610-458-8959 for technical support.

ThermoFluor® Instrument Recovery Upon an Error

If the ThermoFluor® 384 Instrument should fail because of an error follow the procedure below.

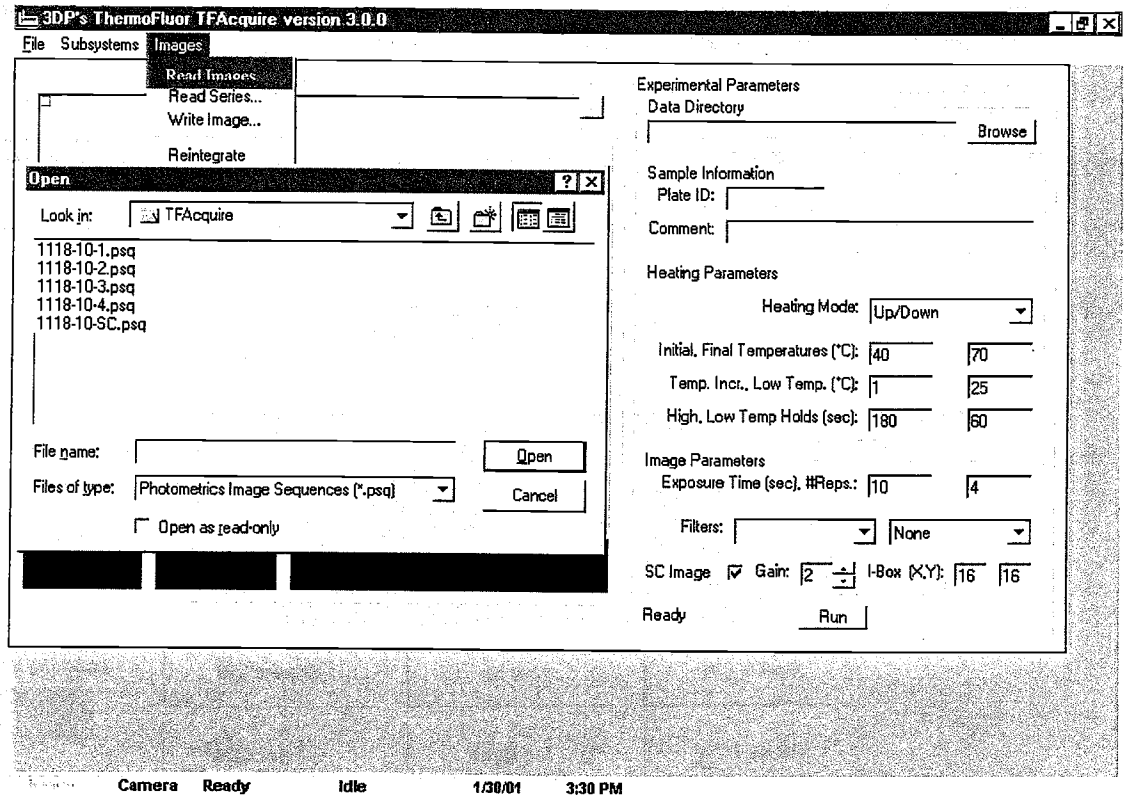
Step	Action
1	If the instrument fails, record the following information: • The exact error message, "word for word". • The state of the instrument. • Number of plates run. • Remaining number of plates. • If a plate was left in the instrument or not. • Is the door open or closed. • Is the UV lights on or off. • What is the PlateCrane™ arm position (rotary, vertical and gripper).
2	Additionally, record all of the steps that were taken in the instrument recovery process.
3	Prior to closing the program: A. Open the **FOLDER** named TFAquire on the C: drive. B. Copy the file named TFAquire.log. C. Paste it into a dated folder (MMDDYYTf#) in the designated "Test" folder in the ThermoFluor folder on the D: drive on the same unit with the date on it. If the Test folder does not exist, create one in the manner outlined. D. Rename the freshly pasted TFAquire.log to TFAquireMMDDYY.Log.
4	Close out of the program and restart it. This will reinitialize the instrument. (Often a camera issue will ensue, if this is the case, see step 6).
5	If this still does not work: A. Close the program B. Shut down the instrument and wait 30 sec C. Start the instrument, wait for it to finish its start up routine D. Then restart TFAquire.
6	If the instrument still does not function properly, repeat step 7. However, prior to restarting the instrument, reboot the computer. After a successful reboot, continue as normal: A. Restart the instrument B. Then restart TFAquire
7	Upon success or failure, notify others of the activity. Include the: • Specific TF unit • Error message that was recorded

	• Steps taken to address the error • Error log • Current state of the instrument.

Recovering Plate Data Following System Disruption

The **Reintegrate** command found under the **IMAGES** menu in the ThermoFluor® Acquire 3.0 application can be used to recover data for a plate if the system experienced a disruption after the initial images for a plate were taken but before TCW finished running the method for that plate. If the system is disrupted during image collection that data will be lost.

Step	Action
1	Open the ThermoFluor® Acquire 3.0 application.
2	Select **Read Images** from the **IMAGE** menu. The OPEN dialog box appears.



3	Select any image file (***.psq**) from the series of images files for the desired plate from the corresponding data directory using the OPEN dialog box. Press OPEN or double click the file listing. • The appropriate information is automatically entered in the Experimental Parameter panel of the ThermoFluor® Acquire 3.0 screen. • The first replicate of the first temperature increment for the plate is displayed in the Plateview panel. The first replicate image of each temperature increment can be viewed using the scroll bar.
4	Select **Reintegrate** from the **IMAGE** menu. A check will appear next to the

	command on the menu.
5	Click **Run** at the bottom of the **Experimental Parameter** panel of the ThermoFluor® Acquire 3.0 screen. The application runs the integration on the raw image data for the plate and writes the corresponding ***.int** files. These files can then be analyzed by the ThermoFluor® Analysis software application.

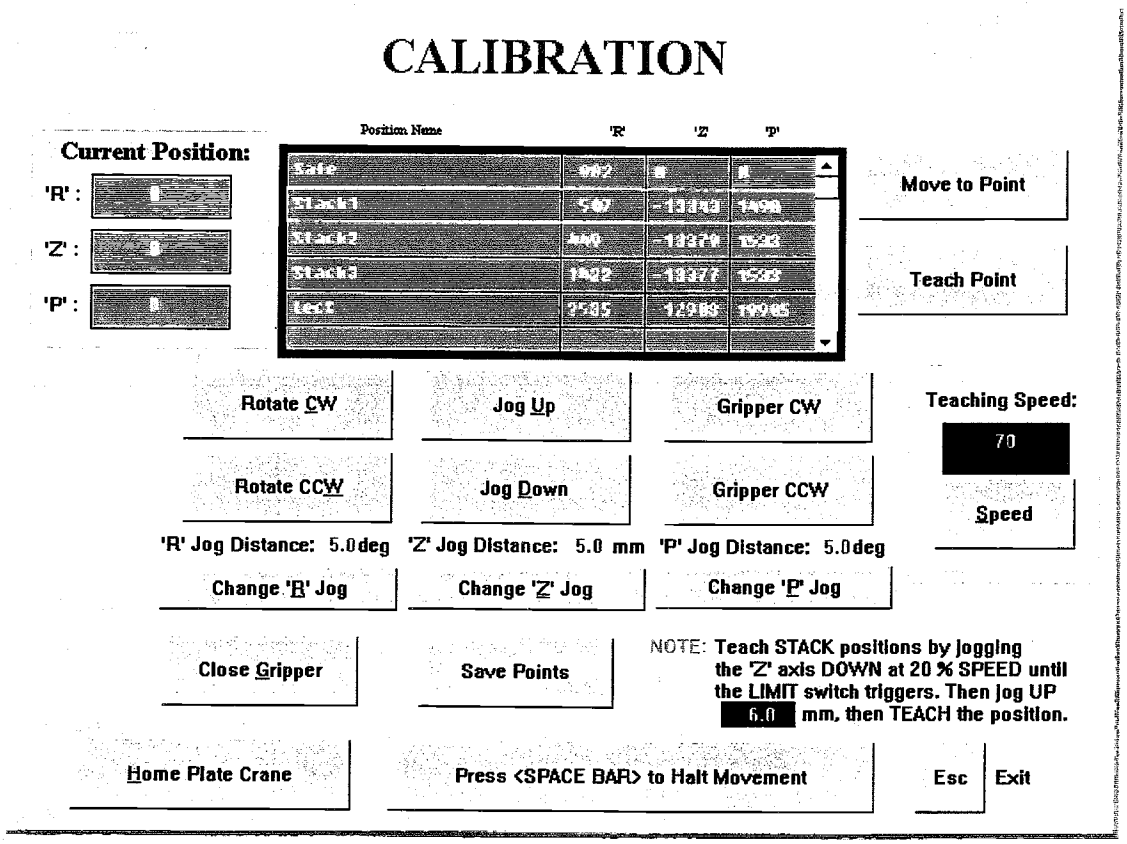
Plate Crane Doesn't Pick up Plates

This is an indication of a mechanical problem. Examine the PlateCrane™ arm and grippers for abnormalities. Also, if the system crashed, the stack (storage magazine) or plate position in the TCW program could have been altered.

Verify Stack Position

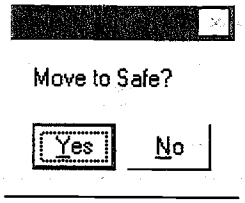
The stack position is set up by the technician when the system is initially installed. It should not have to be done again under normal operating conditions. To verify that the stack position in the TCW program is correct follow the procedure below.

Step	Action
1	Select the **Calibrate PlateCrane** (F4) button on the **MAIN** menu of TCW. The Calibration screen will appear.



2	Home the arm by selecting **Home Plate Crane** button. The plate arm will move to its home position.

3	Select the desired stack location on the Calibration screen. For example, Stack1.
4	Click the **Move to Point** button on the Calibration screen. .

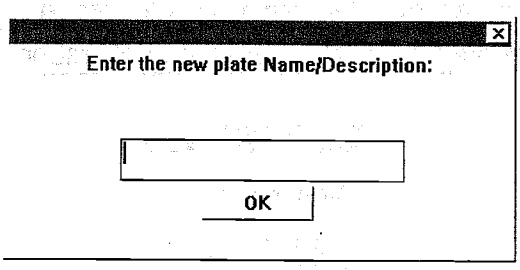


5	Select **Yes** in the MOVE TO SAFE? confirmation pop-up dialog box. The arm should move over the plate at the bottom of the desired stack.
6	Verify the grippers are positioned properly over the plate. (In a position to pick up the plate)
7	Click the **Close Gripper** button on the Calibration screen to verify they grip the plate.
8	Select the stack in question on the Calibration screen and click the **Move to Point** button.
9	Select **Yes** in the MOVE TO SAFE? confirmation pop-up dialog box. The arm should lift the plate and return it to the bottom of the magazine stack.
10	If this is done successfully there is no problem with the stack position calibration.
	Exit the Calibration screen by clicking the **Esc/Exit** button.
11	If the arm doesn't line up over the stack correctly refer to Appendix A, Total Control for Windows™, for the procedure to re-teach (recalibrate) this position to the arm.

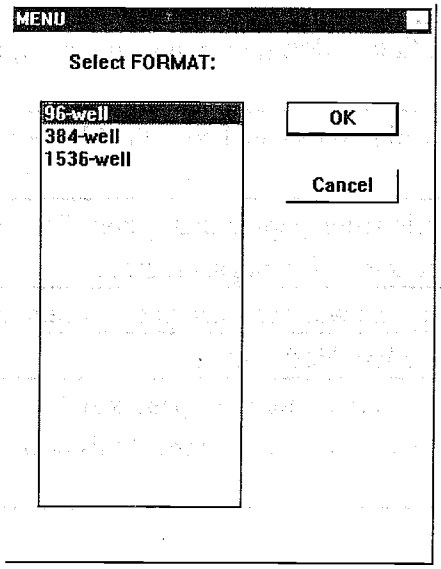
Verify or Reset Plate Position

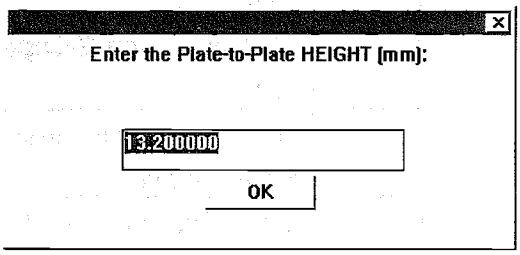
When the Plate processing system is first set up the position of the assay plates relative to the PlateCrane™ arm grippers (plate position) is set. When the type of plates are changed or possibly following a crash of the system the plate position may need to be verified or reset. To verify or reset information about plate position follow the procedure below.

Step	Action
1	Open the TCW program by double clicking its icon on the MS Windows™ desktop.
2	Click the **System Settings** (F6) button on the **MAIN** menu of TCW. The PlateCrane System Setup screen appears.
3	Click **Add** to add new plate dimensions.
4	Enter the plate name in the ENTER THE NEW PLATE NAME/DESCRIPTION pop-up dialog box. Click **OK**.

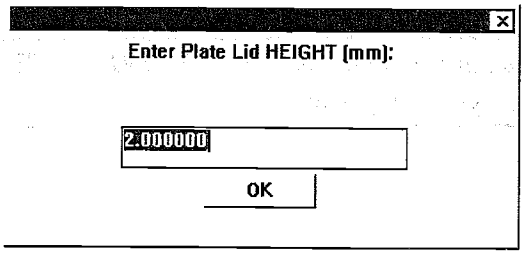


5	The MENU dialog box appears to enter plate specifications format. Default designations for 96, 384, and 1536 well plates are shown. Select the desired plate specifications format and click **OK**. The ENTER THE PLATE TO PLATE HEIGHT pop-up dialog box appears.

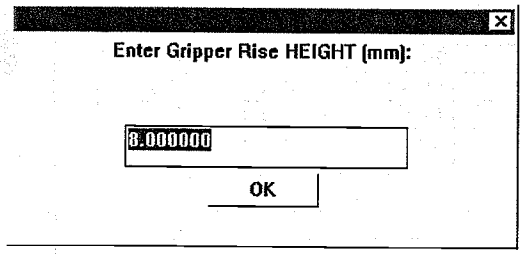




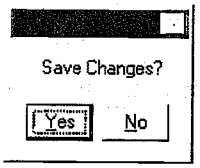
6	Enter or verify the plate height in the ENTER THE PLATE TO PLATE HEIGHT pop-up dialog box. Stack a minimum of 10 plates and measure their height in millimeters then divide the height by the number of plates to get the average height of the plates. Enter the average plate height value in mm and click **OK**.
	Caution **This is s very important parameter.** **The measurement should be done accurately.**
	The **Enter Plate Lid HEIGHT** pop-up dialog box appears.



7	If a lid is used enter or verify the plate lid height in mm (default assumes no lid) in **Enter Plate Lid HEIGHT** pop-up dialog box. Click **OK**. The **Enter Gripper Rise HEIGHT** pop-up dialog box appears.

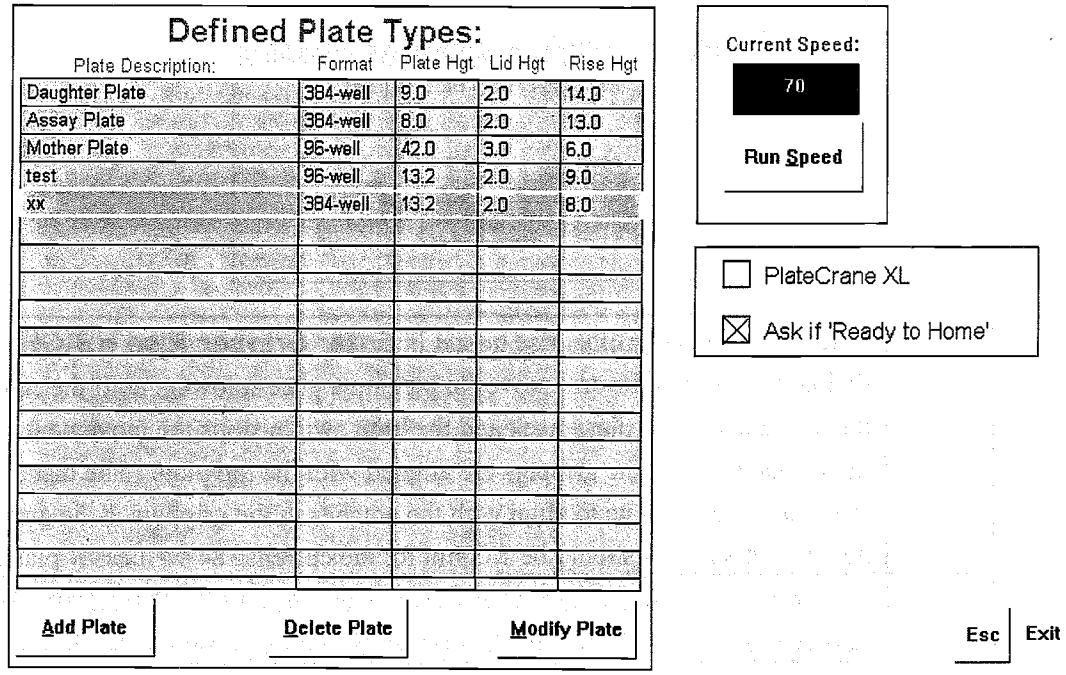


8	Enter or verify the gripper rise height in **Enter Gripper Rise HEIGHT** pop-up dialog box. When the arm moves to pick up a plate it lowers until the center of the arm touches the plate stack and the limit switch stops the movement. The arm then rises to where the grippers are aligned with the top plate. The distance the arm must rise for plate to align with the notches in the grippers is the gripper rise height. This height should be 2.0 mm for the current 384 well assay plate.
9	Following entries click the **Es/Exit** button to return to the PlateCrane System Setup screen. The SAVE CHANGES? confirmation pop-up dialog box appears.



10	Click **Yes** to save the changes. The new plate setup should be visible on the list on the PlateCrane System Setup screen.

PlateCrane System Setup



11	Press **Esc/Exit** button to return to the **MAIN** menu screen.

Repair

There are no user repairable items in this system. If you suspect instrument repair is required, please contact 3-Dimensional Pharmaceuticals, Inc. at 1-610-458-8959.

Appendix A

Total Control for Windows

Overview

This appendix contains detailed information about the control software Total Control for Windows used for the ThermoFluor® 384 system.

Specifically, this appendix includes the following sections:

- *Total Control for Windows™ Manual*

The following table identifies the appropriate section to read in order to learn more about a specific area.

To learn more about ...	Read the section....	See page
The Total Control for Windows control software	Total Control for Windows™ Manual	A-2

Appendix B

Hudson Group, Plate Crane Manual

Overview

This appendix contains the Hudson Group, PlateCrane™ Manual with detailed information about the Hudson Group, PlateCrane™ used in the ThermoFluor® 384 system.

Specifically, this appendix includes the following sections:

- *PlateCrane™ Manual*

The following table identifies the appropriate section to read in order to learn more about a specific area.

To learn more about ...	Read the section....	See page
The Hudson Group, PlateCrane™ Manual	PlateCrane™ Manual	B-2

Appendix C

Consumables List

Overview

This appendix contains a list of consumables that are used in the operation of the ThermoFluor® 384 system.

Specifically, this appendix includes the following sections:

- *Consumables List*

The following table identifies the appropriate section to read in order to learn more about a specific area.

To learn more about ...	Read the section....	See page
Recommended Consumables	Consumables List	2-2

Consumables List

Description	Manufacturer	Mgf. Tel. No.
Plate, PCR, 384 well, PP, blk	MJ Research, Inc.	1-888-735-8437
Neutral Disinfectant and Detergent Didecxyl dimethyl ammonium chloride Dimethyl benzyl ammonium chloride Water	Misco Products Company	1-800-548-4568
Glass Cleaner Isopropyl alcohol 2-Butoxyethanol Anionic surfactant Dye Water Ammonium hydroxide	Misco Products Company	1-800-548-4568
Distilled Water		
Bar code labels		