

AU-Advanced Operating System Beta User Manual

**Center to Advance
Molecular Interaction Science**



Biomedical, Inc.

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Chapter 1

Introduction & Manual Conventions

1.1 What is in This Manual?

1.2 Technical Terms

1.3 Support

1.4 System Requirements

1.5 Software Installation

Chapter 2

Hardware Description

The Aviv AU system (see figure 2.1) consists of several different optical instruments and an upgrade to the Beckman-Coulter Optima XL Ultracentrifuge.



Figure 2.1: Aviv AU System Components

2.1 Aviv AU Upgrade

The upgrade package includes physical modification of the centrifuge and the following equipment:

- Data acquisition computer.

- AU-Advanced Operating System.
- Serial cable for stepper motor communication.
- 100-pin high density shielded cable.
- 4 BNC signal cables.
- Aviv System Box.
- Aviv system cable.
- Aviv Distribution Box and electrical harness.
- Type 2 null modem cable for centrifuge communication.
- High speed commercial data acquisition board.
- Commercial analog/digital i/o board with counter/timers.
- FDS installation bracket.
- Calibration cell.

2.1.1 Data Acquisition and Control Hardware

The data acquisition computer is used to monitor and control the centrifuge and simultaneously control and acquire data from several optical instruments. The two commercial PCI cards in the data acquisition computer are used for data acquisition and system control. The PCI card with 5 BNC connectors is the high speed data acquisition board used to acquire signals from the optical detectors and the centrifuge's rotor timing pulse. The second PCI card in the data acquisition computer (the board with the 100-pin type-2 SCSI connector) is a general purpose analog/digital i/o board with two timer/counters. This board is used to monitor lower frequency signals including the FDS laser temperature, and the centrifuge vacuum pressure. It is also used to control the signal gains: both the detector PMT high voltage control and PGA level. In addition to the analog functions this board also monitors all of the digital input signals from the Aviv AU system.

2.1.2 Aviv AU System Box

The Aviv System Box contains electronics for signal conditioning and timing. The two lights on the front of the box indicate the state of the FDS laser. The yellow light indicates that the laser is powered correctly. If this light does not light when the system box is powered on call Aviv Biomedical, Inc. The second light indicates when light is present from the FDS laser. It will turn blue whenever the beam is present. The electronics are designed so that if the AU control computer is on and the system box is powered the laser will not come on at atmospheric pressure. If for some reason the blue light on the System Box is lit at atmospheric pressure when the FDS is installed in the centrifuge then be very careful looking in the vacuum chamber because the laser light is very bright and can damage your eyes. If this condition occurs, start up the AOS software immediately to correct the condition, and notify Aviv Biomedical Inc.

2.1.3 Signal Distribution Box

The Aviv Signal Distribution Box is installed inside the centrifuge cabinet and so is not visible to the user. This box distributes control, monitoring and data signals to, or from, the optical systems; controls the communication chain to the stepper motors installed in each of the optical instruments; and provides buffering of the signals.

2.1.4 Automated Functions

System Power Detection

The hardware provides two signals to the AOS so that the software can determine the power status of both the Aviv System Box and the centrifuge. If the AOS senses that either the System Box or the centrifuge is not powered on, it will inform the user. When the power is turned on for either system the AOS will re-initialize the appropriate system.

Detector Auto Sensing

The Aviv AU AOS is configured to auto sense which AU optical systems are installed in the centrifuge. After determining which systems are available, the operating system configures those options which are available to the user.

Stepper Motor Initialization

When either the power is cycled on the Aviv System Box or the FDS instrument is removed or reinstalled in the centrifuge, the AOS will re-establish communications with the chain of stepper motors in the AU system and re-initialize their settings.

FDS Laser Control

If the FDS instrument is installed, the AOS determines when it is safe to turn on the laser. When the vacuum pressure has reached 50 microns the laser is enabled. It takes approximately 1 minute for the laser to warm up and turn the light on, but the laser will not be fully ready until the light level is stable (this takes less than 5 minutes). The FDS laser remains on unless the AOS detects an increase in the vacuum pressure, the system is stopped, or the system is idle for more than 30 minutes. If the laser has been shut down because the AOS was idle for longer than 30 minutes the laser will be restarted as soon as any action is performed in the software. The status of the FDS laser is shown in the **Machine Settings** panel in the main AOS window in the **Optical System Status** box on the right side of the panel.

2.1.5 Power Up / Power Down Sequence

The start up and shut down sequences are itemized in Appendix A.

Power Up

We recommend that the System Box not be turned on without first starting the AU control computer. It is not necessary to start the AOS software. Other than having the controlling computer turned on, there is no required order for starting the hardware and software. The only precaution is that if the blue light on the System Box is lit, do not start the centrifuge vacuum pump because this could result in damage to the FDS internal electronics or detector. If the System Box blue light is on and the control computer has been started, start the AOS before starting the vacuum pump. The AOS software will not allow the laser to operate unless the vacuum pressure is below 50 microns. If this condition persists contact Aviv Biomedical, Inc. for support.

Power Down

We recommend that if you are going to vent the vacuum chamber to first turn off the power to the System Box. This is not required as long as the AOS is running as it will not let the laser operate at pressures above 50 microns.

2.2 Aviv AU Optical Detectors

2.2.1 Fluorescence Detection System

Included Equipment

- FDS Optics.
- Storage bracket.
- Dust cover.
- Installation screw driver.
- Storage case.

Description of Optics

The Aviv AU Fluorescence Detector System (FDS), shown in figure 2.2, is a new instrument concept for the Optima XL Ultracentrifuge. The FDS installs inside the centrifuge on a bracket protruding through the thermal shroud. The light source, detector and optics are all contained within the FDS instrument case. The FDS light source is a compact 15mW solid state laser. The laser emits cyan colored light at 488nm +/- nm. The light from the laser is filtered and focused to a small spot with an objective lens. The small spot is then imaged onto the target sample in the rotor with a series of lenses and mirrors in a confocal microscope configuration. The focusing of the spot onto the target affects the radial resolution of the instrument as the spot size determines the sharpness of any radial boundaries. This focus can be changed from software and after being set the system automatically re-initializes the focus position whenever the power is cycled or the AOS software is restarted. For more information on calibration and focusing the FDS instrument see Chapter 6. The light focused on the target

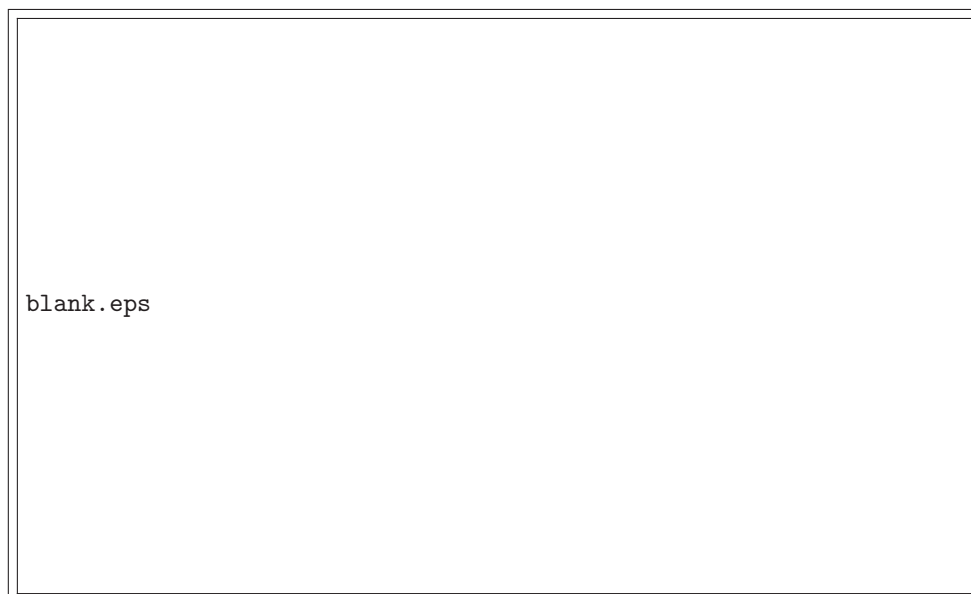


Figure 2.2: Aviv AU Fluorescence Detection System

causes the sample to fluoresce and the emitted light travels back up through the optics to the FDS detector. The volume of fluorescing sample that can make it to the detector is determined by a pinhole in front of the detector. The FDS detector is a compact photomultiplier tube (PMT) contained in the FDS optics box. The sensitivity of the PMT is determined by the high voltage control signal set in the AOS. This control voltage is referred to as the FDS gain. For information on setting the FDS gains see Chapter 8.

Installation and Handling of Optics

To install the FDS instrument remove it from its storage case, then remove it from its storage base plate by unscrewing the two large thumb screws on the mounting bracket. Make sure that the dust cover, located on the bottom side of the optics box, is not installed. The dust cover can be removed even after the FDS is installed in the centrifuge. To remove the dust cover simply pull the white tab, visible at the front of the optics box, until the dust cover completely separates from the optics box. The dust cover should be placed back in the FDS storage case for safe keeping. Never leave the dust cover partially installed or it could fall off during operation. After the dust cover has been removed, be careful not to touch the focusing lens which will be visible.

Take the FDS and seat it onto the 44-pin subminiature D-connector attached to the FDS bracket in the centrifuge vacuum chamber. To do so, it is best not to press down on the actual box holding the optics but rather on the mating bracket which has the two large securing screws. Be careful not to press on

the heat sink which protrudes from the front of the optics box because this could affect the alignment of the laser. After the instrument is seated on the connector use the large screw driver provided to tighten the securing screws. You may have to gently slide the optics away from the thermal shroud with one hand to be able to access the second securing screw.

Alignment & Collimation

The alignment and collimation of the laser and optics was carefully done when the optics were assembled and should not need adjustment if the optics are treated with care. If the optics are dropped or roughly handled and you suspect the laser is no longer aligned please contact Aviv Biomedical, Inc. for instructions on servicing the optics. It is advised that if the FDS is not in use it should be stored either on its bracket in the chamber or on its storage bracket and placed in its protective case.

Focusing

Focusing of the FDS system is done through the AOS and is described in detail in Chapter 6.

Calibration

The FDS system is used to calibrate the angular location of channels as well as the radial position of the instrument.

Operation and Temperature Constraints

Because the AU-FDS instrument is mounted to a moving slide inside the vacuum chamber the only means available to cool the laser is radiation. The laser is mounted to a heat sink, anodized black for better heat transfer, which protrudes from the FDS optics box through its front surface at the left side of the instrument. The maximum operating temperature of the laser is 40C. The efficiency of the radiative cooling of the laser depends on the temperature of the centrifuge's thermal shroud. The operating time of the FDS instrument may depend on the temperature chosen for the current experimental run. Temperatures up to 25C can be run indefinitely long, however, higher temperatures will result in limited experiment run times (see table ?? in Appendix F).

2.2.2 AU Rapid Scan Absorbance Instrument

Available Spring of 2005

2.2.3 AU Interference Instrument

Coming Soon

2.2.4 AU Schlieren Instrument

Coming Soon

2.2.5 AU Turbidity Instrument

Coming Soon

2.3 Aviv AU Accessories

There are several accessories available for use with your Aviv AU system.

- Cell Alignment Tool
- Loadable Calibration Cell.
- Fixed Calibration Cell.
- SedVel60k 2-channel Cells.
- Stafford 2-channel Synthetic Boundary Cells.
- Cell washer.

Chapter 3

Introducing the AU-Advanced Operating System

The AU-AOS Operating system is an efficient object-oriented multithreaded operating system which allows control and monitoring of the *Beckman Coulter Optima XLA/XLI Ultracentrifuge* and simultaneous data acquisition from up to three independent sources at frequencies up to 20 MHz. The system allows for user login at different privilege levels and has been designed to allow for future FDA validation. The AU-AOS software was designed with ease of use in mind. Almost all of the hardware setup requirements, including focusing, radial calibration, angular position of sample channels, gain control, and intensity calibration, have been automated. A user can set up and run an experiment manually or by using a setup wizard which walks the user through each step. Once an experimental method has been created, a user can re-run the same experiment at a later date simply by selecting that setup and pressing the **START** button. In addition to hardware control and data acquisition, the AU-AOS system gives the user a new way of managing data files through a file database utility. AU-AOS data files are compatible with existing analysis programs including: SedFit (ref here), SedAnal (ref here).

Figure 3.1 shows the default view of the AU-Advanced Operating System (AU-AOS), it consists of several parts: the Main Menu, the Button Tool Bar, the Machine Settings panel, the Data Graph Window, and the Machine Status bar.

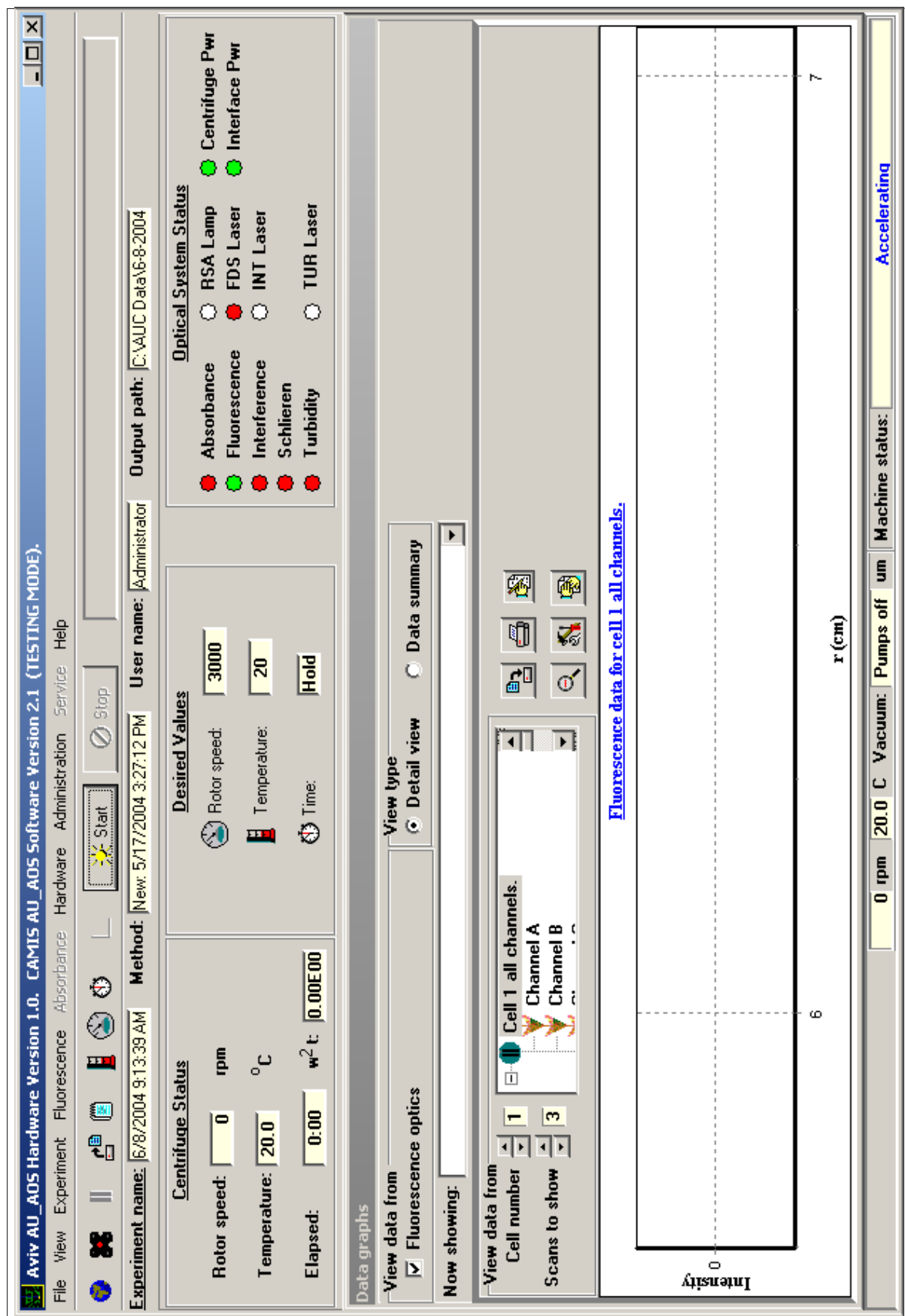


Figure 3.1: AU-AOS Main Program Window

3.1 Main Menu

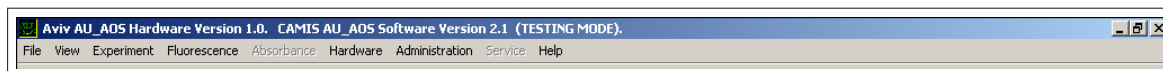


Figure 3.2: File Menu

3.1.1 File

User login Used to change current user and set access level.

User Account Management Allows individual users to view and modify their account preferences, data file path, password, and user information.

Exit Exits AU-AOS.

3.1.2 View

Machine settings display When checked makes *Machine Settings Panel* visible.

Experiment status display When checked makes *Experiment Status Panel* visible.

Data graphs Used to change location of data graph window.

Cell/sample notes Opens form to add cell/sample notes. This can also be accessed in *Cell Setup* form.

All Notes Used to view general system notes generated automatically.

Run log Used to view general experiment notes generated automatically.

Error log Used to view notes generated automatically when an error occurs in the software or hardware.

Error display visible Determines whether Error Log window is automatically displayed when an error occurs.

View previous experiments Used to view list of previous experiments by *Name*, *Date* and *Data Path*.

Display update interval Used to modify the interval between AOS updates and centrifuge communication.

Version information Displays version information.

Color Table Allows user to select new color table for data visualization.

3.1.3 Experiment

Experiment setup wizard Opens *Experiment Wizard* window that allows the user to run an automated tool that walks the user through all of the steps required to setup an experimental protocol or to select individual setup functions.

Set file output path Used to change location of data storage; only accessible to *Administrator* and *User* accounts.

Select rotor and cells setup Used to access the *Rotor Setup* form. See Chapter 5 for detailed description of how to use this form.

Select experimental method Used to access the *Experimental Method* form to select/modify experimental methods. See Chapter 9 for a detailed description of how to use this form.

Keep detailed experiment log When this option is checked the software will automatically write notes to the *Run Log*.

Set experiment information Used to view and change *Experiment Title* and *Data Path* if account level is *User* or *Admin* and *Method*.

Add cell/sample note Used to add notes at cell and channel level.

Add experiment/general note Notes entered into this form will be saved and can be viewed in *All Notes* form.

3.1.4 Fluorescence

The Fluorescence menu has tools related to the AU-FDS instrument.

Select cells to scan and add notes... Used to select cells to be scanned during the experiment. User can also add channel notes here too.

Set gains... Used to open *Set Gains* window. Chapter 8 contains instructions on how to use this form to manually set gains. Option will be greyed out if rotor is not spinning.

Set radial increment... Used to select radial increment value for all cells for use in the current experiment. This can also be done in the *Cell Setup* form described in Chapter 7.

Intensity Calibration Used to access *Intensity Calibration* tool for generating intensity normalization data. Operator accounts may not access this.

Fluorescence focusing Used to access focusing tool. Operator accounts may not access this.

3.1.5 Absorbance

The Absorbance menu has tools related to the AU-RSA instrument. This option is greyed out (disabled) if the RSA instrument is not installed.

3.1.6 Hardware

Setup rotor Used to access *Rotor Setup* form.

Rotor speed Used to *Set Rotor Speed*.

Temperature Used to *Set Temperature*.

Run time Used to *Set Run Time* of centrifuge.

Force Magnet Angle Causes system to reacquire the Magnet Angle.

3.1.7 Administration

The following options can only be viewed by *Administrator* accounts:

Account Managements Used to view and modify accounts.

Log status messages When checked causes status messages to be written to the run log.

Purge error log Clears records from the automatically generated error log.

Database utilities Used to *Check Database Entries*, *Compact Database* and *Fix Database Links*.

Simulation parameters Used to access simulation parameters.

Versions Displays current hardware and software versions.

Options... Used to set/view AOS options: *Login Required*, *Optical Systems Available*, *Transform Properties*, *Hardware Information*, and *A/D Input Signals*.

3.1.8 Service

This menu has items used by the service technician when the system is installed or serviced.

3.1.9 Help

Help topics Used to open the *Help Menu*.

Machine database view Used to view relationships between AU-AOS database tables.

About Displays the version number of the software.



Figure 3.3: Button Toolbar

3.2 Button Toolbar

The button toolbar shown in figure 3.3 has the following functions.

Wizard Represented by a picture of the globe. Used to open the *Experiment Wizard* which takes the user step by step through the tasks needed to perform an experiment.

Rotor Setup Represented by a picture of a 4-hole rotor is used to access the *Rotor Setup* form.

Cell Setup Represented by a picture of a 2-channel velocity cell is used to access the *Cell Setup* form. The button picture will show two bars if the system is not ready to set up the cells.

Experiment Setup Represented by a picture of an arrow pointing from a computer to a piece of paper. Used to access the *Experiment Setup* form.

Experiment Information Represented by a picture of a ringed notebook. Used to access the *Experiment Information* form, which shows the current experiment information. It also has buttons for changing the current data path (not the default path), method selected, and experiment name.

Desired Temperature Represented by a picture of a thermometer. Used to change the desired centrifuge temperature. This button will be disabled (grayed out) if the centrifuge is not started.

Desired Rotor Speed Represented by a picture of a speedometer. Used to change the desired rotor speed. This button will be disabled (grayed out) if the centrifuge is not started.

Desired Run Time Represented by a picture of a clock. Used to change the desired run time of the centrifuge. This button will be disabled (grayed out) if the centrifuge is not started.

Single Scan Represented by a picture of a graph. Used to take a single scan with all of the current settings. This button will be disabled (grayed out) if the centrifuge is not started.

3.3 Machine Settings Panel

Figure 3.4 shows the *Machine Settings* panel. The current *Experiment Name*, *Experiment Method*, *User Name*, and *Data Path* are shown at the top of the panel. The body of the panel shows three additional panels: *Centrifuge Status*,

Desired Settings, and *Optical System Status*. The *Centrifuge Status* panel shows the current state of the centrifuge. This panel should be consistent with the left side of the display on the centrifuge. The rotor speed is displayed to greater precision than on the centrifuge. This panel is updated at the rate set in the update interval (see the *View* menu), and so could lag the values shown on the centrifuge.

The *Desired Settings* shows the settings desired settings set by the user. These values are the same as those shown on the right side of the centrifuge display.

The *Optical System Status* panel contains several colored circles, which will be referred to as “lights”, to show the status of the optical systems and key machine parameters. The column of lights on the left of this panel is used to show which optical systems are currently installed: white indicates the system is not installed, yellow indicates the system is initializing, green indicates the system is present and configured correctly, red indicates an error has been detected with the system. The second column has a series of lights which show the status of the light source for the detector system to its left. White indicates that the system is not presently installed, yellow indicates the light source is warming up or initializing, green indicates the source is on and running stably, red indicates there was an error with the light source. The last column shows lights reflecting the status of key system parameters: centrifuge power, AU System Box power, and Magnet Angle status. The two power lights are red if the power is off and green if the power is on. The Magnet Angle light shows whether or not the Magnet Angle has been acquired for the current rotor speed. White indicates the Magnet Angle has not been determined for the current speed, yellow indicates the Magnet Angle determination is in process, green indicates the Magnet Angle has successfully be determined, and red indicates the Magnet angle has failed for some reason, see Appendix H.

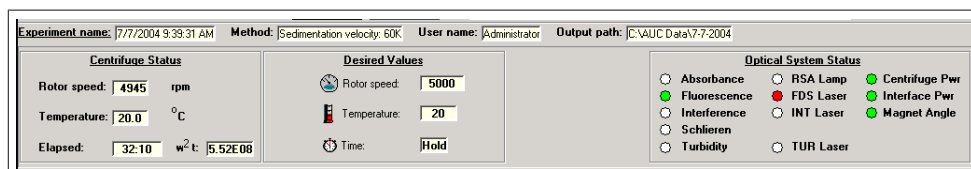


Figure 3.4: Machine Settings Panel

3.4 Data Display Window

The *Data Display Window* shows the data from scans of the optical systems. See Chapter 10 for more information.

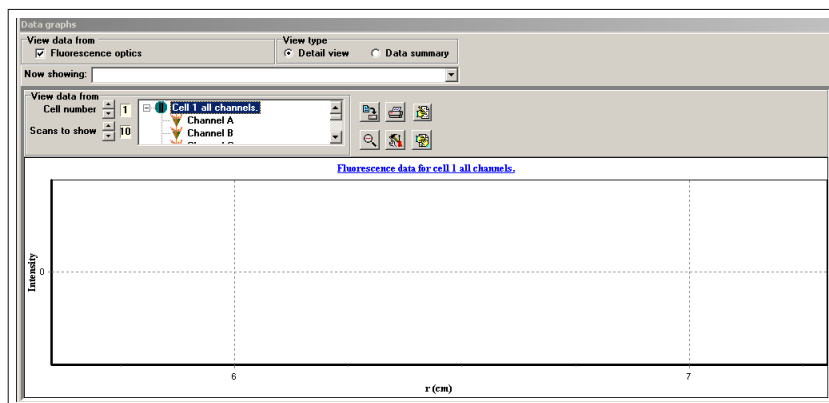


Figure 3.5: Data Display Window



Figure 3.6: Machine Status Bar

3.5 Machine Status Bar

The *Machine Status Bar* is shown at the bottom of the main **AOS** window. This status bar shows messages to the user (in the text box at the left), the rotor speed, the vacuum pressure, and the machine status.

Chapter 4

Getting Started

To start the AU-AOS either select the program from the Start menu or double click on the program icon. If the software is installed on a computer not containing the PCI Analog/Digital I/O boards, which come with the AU system upgrade, then the software will run in simulation mode. In this mode almost all of the system features are simulated, which allows users to install the AU-AOS on any system meeting the requirements in Section 1.4 for training purposes.

If the AU-AOS is being run from the computer with the AU hardware installed then shortly after the program starts it checks the power state of both the Aviv AU System Box and the Beckman Optima XL-A/XL-I centrifuge and warns the user if either system is not on. It is not necessary to exit the software if either hardware system was not powered up at the time the software was started. If a power warning occurs the user can simply turn on the power to either the Aviv System Box or the centrifuge and the AOS will automatically sense the system and initialize the hardware.

When the software is started for the first time the Rotor Setup form is automatically opened and the user is forced to select the current rotor configuration before proceeding. See Chapter 5 for details on how to modify the rotor setup.

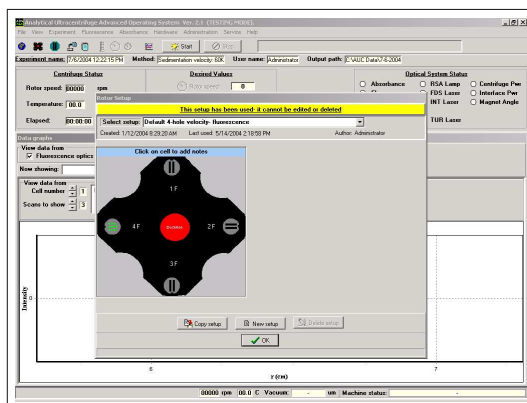


Figure 4.1: Opening Screen

4.1 Account Management & Login

The AU-AOS has many different configuration options which can be selected by the system administrator (see Section 13.1). One option is the *Login Required* option. The AOS can be configured so that the system will not run unless a valid user logs in at the time the program is started. If this option is selected the user has three attempts to enter a valid username and password before the program will automatically terminate.

After logging in to the AOS, users can modify their account settings by selecting *User Account Management* from the **File** menu. The user account management tool, shown in figure 4.2, allows the user to change their password, identity (first & last name), and their default data path depending on their access level.

The button bar at the bottom of figure 4.2 is used to post any changes to the AOS database. To post changes to the user account click the button on the left (labelled with a check mark). If a mistake is made the changes can be cancelled by clicking on the middle button (labelled with an X). If the user made changes but wishes to revert back to the original settings, before posting the changes to the database, they can click on the button on the right with the curved arrow.



Figure 4.2: File Menu

The default Data File Path can be changed by either entering the file path directly into the appropriate edit box, or by browsing the file system by either double clicking the field, or by selecting the field and then clicking the button with the ellipsis to the right of the field.

The operating system has three levels of user access: *Operator*, *User*, *Admin-*

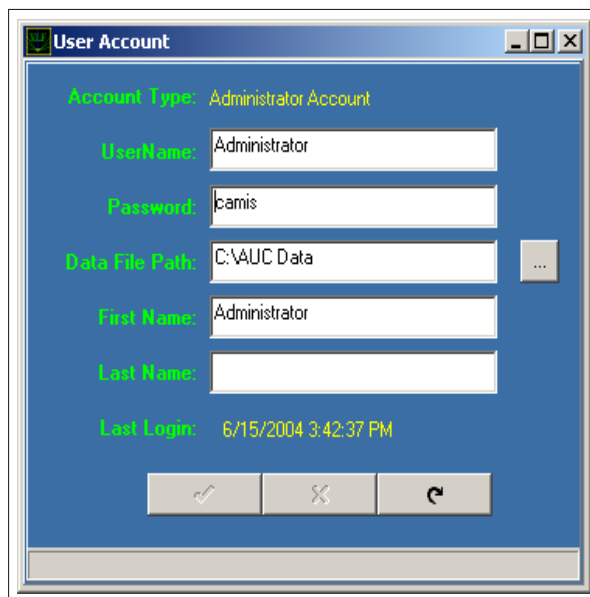


Figure 4.3: User Account Management Tool

istrator. *Operator* accounts are the most restricted type of account. *Operators* can control the centrifuge and run pre-defined experimental methods with pre-defined rotor setups. The data from *Operator* accounts get placed into the system default data directory. If the system is not setup for *Require Login* then the system starts with the *Administrator* account logged in to the system. The user can be changed after login whether or not the *Require Login* option is selected. If the system is configured to *Require Login* then at startup a login window will appear.

After logging into the system the user can manage their own account by selecting **Account Management** from the **File** menu. After the AOS has been started, user identity can be changed by selecting the **User Login** from the **File** menu.

Operator Accounts

With the *Operator* level of permission the centrifuge can be operated, existing experimental methods can be run, and data can viewed. *Operator* accounts must store the data they generate in the system default data directory that was created at time of installation or set by the system *Administrator*. *Operators* can change their passwords through the **User Account Management** tool in the **File** menu.

The screenshot shows a software window titled "Machine Settings Display". At the top, it displays experimental metadata: "Experiment name: 7/6/2004 2:00:58 PM", "Method: Sedimentation velocity: 60K", "User name: Administrator", and "Output path: C:\AUC Data\7-6-2004". The main area is divided into three panels. The "Centrifuge Status" panel on the left shows "Rotor speed: 0 rpm" and "Temperature: 20.0 °C". The "Desired Values" panel in the center shows "Rotor speed: 0" (with a motor icon), "Temperature: 20" (with a thermometer icon), and "Time: Hold" (with a clock icon). The "Optical System Status" panel on the right lists various systems with status indicators (colored circles): Absorbance (white), Fluorescence (green), Interference (white), Schlieren (white), Turbidity (white), RSA Lamp (white), FDS Laser (red), INT Laser (white), TUR Laser (white), Centrifuge Pwr (green), Interface Pwr (green), and Magnet Angle (white).

Figure 4.4: Machine Settings Display

User Accounts

User permission level lets the user create new experimental methods and rotor setups in addition to running the centrifuge. Unlike *Operator* accounts, *User* accounts are permitted to change their default data location through the **User Account Management** menu item. They may also override their default data location for any experiment by selecting the **Select file output path** from the **Experiment** menu or by selecting the **Experiment Information** button in the **Experiment Wizard** form.

Administrator Account

There is only one *Administrator* account for the AU-AOS. The *Administrator* has virtually unrestricted privileges. The *Administrator* has the ability to create or delete user accounts by opening the **Account Management Tool** from the **Administration** menu. see Section 13.2.

4.2 Controlling and Monitoring the Centrifuge

The AU-AOS software is fully capable of running and monitoring the Optima XL-A/XL-I centrifuge. The software can start the vacuum pumps, spin the rotor, change the chamber temperature, and modify the run time. It monitors all system parameters including pressure of vacuum chamber, temperature, rotor speed, $\omega^2 t$, and system power.

Figure 4.3 shows the **Machine Settings** panel, visible by default when the AOS is first started. This panel contains three panels: **Centrifuge Status**, **Desired Values**, and **Optical System Status**. The **Centrifuge Status** and **Desired Values** boxes have been designed to look very similar to the XL-A/XL-I centrifuge display. The **Centrifuge Status** box shows the current status of the centrifuge. The **Desired Values** box shows the current desired settings. The **Optical System Status** box contains a series of colored circles, referred to as “lights”. The lights in the first column show which Aviv AU optical systems are currently installed in the centrifuge. White indicates the system is not installed, yellow indicates the system is initializing, green indicates the system is present and configured correctly, red indicates an error has been detected with the system. The second set of lights shows the state of the optical systems’ light sources. White indicates that the system is not presently installed, yellow

indicates the light source is warming up or initializing, green indicates the source is on and running stably, red indicates there was an error with the light source. The last column shows lights reflecting the status of key system parameters: centrifuge power, AU System Box power, and Magnet Angle status. The two power lights are red if the power is off and green if the power is on. The Magnet Angle light shows whether or not the Magnet Angle has been acquired for the current rotor speed. White indicates the Magnet Angle has not been determined for the current speed, yellow indicates the Magnet Angle determination is in process, green indicates the Magnet Angle has successfully been determined, and red indicates the Magnet angle has failed for some reason (see Appendix H).

For real-time control of the desired temperature, rotor speed, or run time the user can either click the appropriate buttons in the **Desired Values** box, select **Rotor Speed**, **Temperature**, or **Run Time** from the **Hardware** menu, or change the values from the centrifuge control panel. After changing any of these settings there could be a short delay before the **Centrifuge Status** is updated due to the software update interval (see Section ??).

In addition to displaying the current status of the centrifuge in the **Centrifuge Status** box of the **Machine Settings** panel the status parameters are also shown in the **Machine Status Bar** at the bottom of figure 3.1. This status bar is visible whenever the main program window is visible and shows the current rotor speed, temperature, vacuum pressure, and machine status. The machine status will display one of the following messages to indicate the current operational status of the machine.

Initializing Centrifuge and AOS in startup cycle.

Accelerating Rotor is accelerating.

Decelerating Rotor is decelerating.

Running Centrifuge pumped down and rotor spinning at stable speed.

Additionally, the **Machine Status Bar** has a message box on the left which displays other system information to the user.

After starting the AU-AOS software, communication is established between the operating system and the centrifuge. To keep the settings in the software and on the centrifuge consistent, the AOS periodically requests the current settings from the centrifuge.

4.3 Starting the Machine

The centrifuge can be started from the AOS or by pressing either the **Vacuum** or **Start** buttons on the centrifuge control panel. The **Start** button initiates a faster pump-down than simply pressing the **Vacuum** button because it will start the diffusion pump after the roughing pump reaches a vacuum pressure of about 150 microns. Because the AOS periodically communicates with the centrifuge, any change in its state will be automatically sensed and updated in the software. The centrifuge can be started from the AOS by clicking the **Start** button in the button toolbar. When the AOS **Start** button is pressed the *Start*



Figure 4.5: Start Form

form appears (see figure 4.4). The two check boxes in this form allow the user to either start just the centrifuge or both the centrifuge and the experimental protocol. If the user has already set up the experiment then this quick start method is very convenient. In either case the centrifuge will be started with a fast pump-down; the current speed is set to 0 rpm.

4.3.1 Required Equipment

In order to use the AU-AOS system you must have a Calibration Cell (see Section E.3) installed and have selected a rotor setup correctly describing the current cell configuration in the rotor. The calibration cell is needed so that the system can automatically determine the location of all of the cell channels, perform radial calibration of the fluorescence instrument, focus the fluorescence optics, automatic gain control (if selected), and intensity calibration.

4.3.2 Automated Functions

Once started the AOS handles several system functions automatically.

Communication

The AOS communicates periodically with the centrifuge to request the current status. The interval of this communication is set by default to 2.5 seconds. This update interval can be changed from the *Display update interval* item of the *View* menu. Faster update intervals will impact the AOS performance and could result in slower experimental scan times. Longer update intervals may result in the AOS status panels lagging the actual status of the centrifuge.

Instrument Configuration

The instruments installed notify the AOS that they are present and the software updates the tools available automatically.

Stepper Motor Initialization

The stepping motors which control the radial position of each optical system, focusing, wavelength selection are automatically initialized.

Magnet Angle

The Magnet Angle is a key parameter of the AOS. It determines where samples are located with respect to the rotor timing pulse, see section 6.4.

Fluorescence Detection System

If the AU-FDS instrument is installed then after the centrifuge is started the operating system will determine when it is safe to turn on the laser. The AU-FDS laser can only be turned on when the vacuum pressure is below 50 microns, the rotor is spinning and the laser head temperature is below the maximum operating temperature of the laser. This means that it is not possible to turn on the laser while at atmospheric pressure (see fluorescence laser alignment and focusing). The status of the fluorescence laser is reported in the status panel at the bottom left of the main program window. Once the laser has been turned on, it will remain on as long as the above conditions are true and the user is actively using the system. If the system has been idle for longer than 30 minutes the fluorescence laser will be shut down until the user performs some action. The action could be practically any software function including the automated start of a pending experiment.

4.4 Quick Scan

The button with the graph icon in the button tool bar of the main AOS window is used for taking a single scan of the cells with the current settings. This option is only enabled if the machine is running and the rotor is spinning. The user may use this feature to take a quick scan of the samples in the rotor to verify that the gain settings, data angles, and radial cell boundaries are acceptable before beginning an experiment.

4.5 Customizing the Main Window

The *Experiment View Panel* has several options which affect how the AOS looks.

4.5.1 Machine Settings Display

Checking the *Machine Settings Display* causes the *Machine Settings* panel to be visible, see figure 4.3. The *Machine Settings* panel is visible by default when the AOS is first started and when the machine is running.

4.5.2 Experiment Status Display

When an experiment is started the *Machine Settings* panel is hidden and the *Experiment Status* panel is made visible. It is possible to hide or show this panel by checking or un-checking the option.

4.5.3 Data Graphs

The data graphs window can be hidden or shown docked or un-docked by checking the appropriate option under the *Data Graphs* menu item of the *View* menu.

4.5.4 Cell/sample notes

Selecting this option opens a window with tools for viewing or adding cell and channel notes.

4.5.5 All Notes

Selecting this option opens a window showing all AOS notes.

4.5.6 Run log

Selecting this option opens the *Run log* window. The run log contains system messages showing when key events occur.

4.5.7 Error log

Selecting this option opens the *Error log* window. The error log shows the errors that have occurred. It is possible to view errors by experiment, including past experiments, or for all experiments.

4.5.8 Error display visible

By selecting the *Error display visible* option the AOS will automatically open the *Error log* window whenever an error occurs.

4.5.9 View previous experiments

This option shows all information about all the previously run experiments; experiment name, date run, and data location.

4.5.10 Display update interval

Selecting this option allows the user to change the update interval for the AOS software. The default is 2.5 seconds. Faster update intervals may impact the performance of the AOS and data scan times. Slower updates could affect the synchronization between the machine status displayed in the AOS and the actual machine status.

4.5.11 Version information

Shows the current version of the hardware and software.

4.5.12 Color Table

Selecting this option allows the user to select the color table used for data visualization.

4.6 Stopping the Machine & Quitting the Program

It is possible to stop the centrifuge from the centrifuge's control keypad. If this method is used the AOS will recognize this change of state and respond appropriately.

To stop the machine from the AOS, click the stop button located in the AOS button tool bar. Select the *Stop machine* check box and click *Ok*. A command is then sent to the centrifuge to stop the machine. The desired speed is left at its current value but if the rotor is spinning it will decelerate to a stop. After stopping the centrifuge stops and cools the diffusion pump. When the diffusion pump has cooled it is possible to bring the vacuum chamber back to atmospheric pressure by pressing the *Vacuum* button on the centrifuge control keypad. It is recommended that the AU System Box be powered off before venting the vacuum chamber. If you do not follow this suggestion make sure that the laser is not on (blue light lit on the front of the System Box) before venting the chamber.

If the AOS software is quit while the centrifuge is running the centrifuge will continue to run in whatever state it is in. It is recommended that the System Box power is shut off whenever the AOS software is quit. To quit the AOS either click the close window control in the upper right corner of the main AOS window or select *Exit* from the AOS *File* menu. When quitting the program the **Exit Options** window will appear, see figure ??.



Figure 4.6: Exit Options

By default all of the experiment information is saved if you click *Ok*. If you do not wish to keep the experiment information un-check the *Keep experiment information* check box before clicking *Ok*. It is possible to keep the experiment information but not either the run log or notes, simply un-check which item you wish to discard and then click the *Ok* button. To abort exiting the program click the *Cancel* button.

Chapter 5

Rotor Setup

The rotor setup is a critical part of operating the Aviv AU system. For the AOS to work correctly, the rotor type, cell types, and cell locations must be defined. Figure 5.1 shows the **Rotor Setup** form. This form can be accessed by clicking on the picture of a 4-hole rotor in the button toolbar or by selecting **Setup Rotor** from the **Hardware** menu. The **Rotor Setup** form allows users to view the currently selected rotor configuration, change the currently selected rotor configuration, create new rotor configurations, and delete rotor configurations.

The **Rotor Setup** form (figure 5.1) is divided into several parts: the rotor setup selection and information panel at the top of the form, a visual depiction of the current rotor setup on the left side of the form, rotor configuration tools on the right side of the form, and a panel at the bottom with button controls and a message bar. When the **Rotor Setup** form is first opened the currently selected rotor setup is displayed.

5.1 Selecting an Existing Rotor Setup

The **Rotor Setup** form has a colored header at the top of the form showing the status of the setup. Setups that have been previously used in an experiment have a yellow background and a message indicating that the setup has been used and may not be edited or deleted. This feature ensures that setup information from previously run experiments will remain for validation purposes. Setups that exist but have not been used in an experiment have a green header and additionally will show configuration tools on the right side of the form (see figure 5.2).

The current rotor setup is shown in the image on the left of the **Rotor Setup** form. The image shows the rotor and what cell types are loaded into each of the holes. The rotor hole index is shown next to each rotor hole. These indices correspond to the numbers which physically appear on the rotor. Beside the rotor hole index, a series of letter codes may appear to indicate which optical

Figure 5.1: Rotor Setup Form

systems will be used to scan the cells:

F = Fluorescence

A = Absorbance

I = Interference

S = Schelieren

The pull-down menu at the top of figure 5.1 labelled *Select Setup*: shows the name of the current rotor setup. Below the *Select Setup*: pull-down menu, information pertaining to the current experiment is displayed: creation date, last time used, and the author who created the setup.

The setup can be changed to another existing setup by selecting the desired setup from the list in the *Select Setup* pull-down menu. To do this, click on the arrow at the right side of the pull-down menu. A list of currently defined rotor setups will be displayed by title. To select a new setup scroll through the list and click on the name of the rotor to be loaded. When a new rotor setup is selected, status messages are displayed in the message panel, at the bottom of figure 5.1, to provide the user with feedback while the new setup loads from the AOS database.

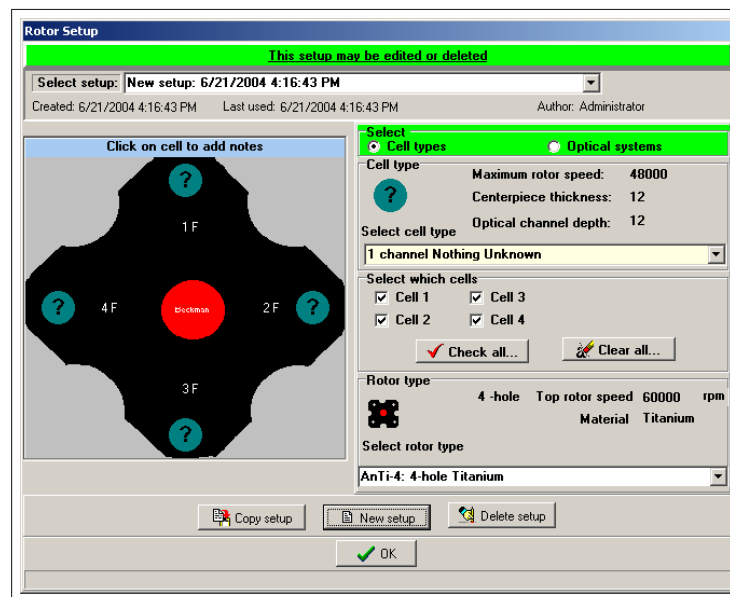


Figure 5.2: Creating a New Rotor Setup

5.2 Creating a New Rotor Setup

In order to create a new rotor setup the user must be either a *User* or the *Administrator*. A user can create a new setup either by copying the current setup (so that it can be edited) by clicking the **Copy Setup** button or by clicking the **New setup** button at the bottom of the form to create a new blank rotor setup. Clicking either of these buttons will produce a new, unused, rotor setup. The header at the top of the setup will change to a green background color indicating that this is a new setup and can be edited or deleted. A new panel containing all the controls to configure the rotor setup will appear to the right of the rotor picture.

5.2.1 Naming the New Setup

When a new rotor setup is created, the name displayed in the **Select setup** pull down menu at the top of the form is automatically generated from the date and time. The user should rename the setup by highlighting this name and entering more meaningful setup name.

5.2.2 Selecting Rotor Type

To create a new rotor setup, first select the type of rotor being used. At the bottom of the configuration tools is a panel labeled **Rotor Type**. This panel

contains all the information for the current rotor type and has a pull down-menu which allows the user to select a new rotor type from a list.

5.2.3 Selecting Cells

The radio buttons at the top of the configuration tools panel allow the user to have access to the two major configuration modes: **Cell types**, and **Optical systems**. If the **Cell types** button is selected the user can change the cell types or locations in the setup. The panel below the radio buttons shows information on the currently selected cell type. It has a picture of the cell and some information about the cell type: maximum rotor speed for cell, thickness, and optical channel depth. Below the cell type information is a pull-down menu showing the cell type. This menu allows the user to select the type of cell to put into the rotor setup. Below the cell type selection pull-down menu is a list of check boxes showing in which rotor holes the current cell type is installed. By checking or clearing the boxes, the user can change the location of this cell type in the rotor setup. Two buttons at the bottom of this panel allow the user to place the cell type in all rotor holes or remove the cell from all holes with a single click.

5.2.4 Selecting Optical Systems

The user can also change which optical systems are active for individual cells. Currently the only system available is the Fluorescence Detection System but in the future other systems will be added. To select which optical systems are active for the cells click on the Optical systems radio button at the top of the right panel. The controls on the right panel will change to show the Available optics panel. The pull-down menu allows the user to select optical systems and the cell selection check boxes allow the user to set the selected optical system to the desired cells as with the cell type selection process. The biggest difference between the Copy Setup and New Setup buttons is that the New Setup button allows the user to change rotor types. Below the cell type selection controls in the panel on the right are controls to change the rotor type. There is a picture of the current rotor type with some characteristics of the rotor and a pull-down menu to change rotor type.

5.2.5 Adding Cell Notes

To add notes about a given cell, left-click the picture of the cell. A new window will appear allowing the user to add notes concerning the cell at the cell level or channel level (see Section ??).

5.2.6 Finishing Up

After completing the new rotor setup the user can either close the **Rotor Setup** form which will retain the new setup as the current rotor setup or delete the

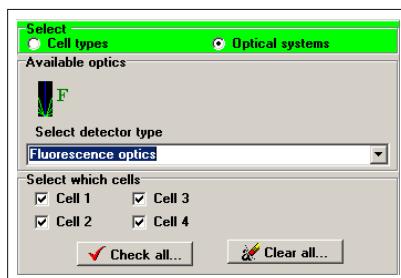


Figure 5.3: Optical System Selection

new setup by clicking the **Delete setup** button at the bottom of the form. Deleting the new setup causes all of the changes made for the new setup to be removed from the AOS database and the default rotor setup is loaded as the current rotor setup.

5.3 Deleting a Rotor Setup

Any rotor setup can be deleted by any user with access level of *User* or *Administrator* as long as the rotor setup has not been used in a previous experiment. To delete an unused setup simply select it from the **Rotor select** pull-down menu at the top of the form. After it has finished loading click the **Delete setup**. The rotor setup will be removed from the AOS database and the default rotor setup will be loaded as the current setup.

5.4 Example Rotor Setup

5.4.1 Example 1: 8-hole Rotor with Mixed Cells

To create a rotor setup for an 8 hole rotor with a 2-channel Charcoal-Epon Velocity cell in holes 1, 2, 5, 6, a counter weight in hole 4, a fluorescence calibration cell in hole 8 and 6 channel equilibrium cells in holes 3, and 7.

1. Click the *New setup* button.
2. If desired change the name of the setup by highlighting the name in the *Select setup* pull-down menu at the top of the form and typing a new name.
3. Select AnTi-8: 8-hole Titanium rotor from the *Select rotor type* pull-down menu.
4. Select 2-channel Charcoal-Epon Velocity from the *Select cell type* pull-down menu.
5. Check only boxes 1, 2, 5, 6 in the *Select cells* check box list.

6. Select 2-channel Aluminum Counterweight from the *Select cell* pull-down menu.
7. Check only box 4 in the *Select cells* check box list.
8. Select 10-channel Delrin Calibration from the *Select cell type* pull-down menu.
9. Check only box 8 in the *Select cells* check box list.
10. Select 6-channel Kel-F Equilibrium from the *Select cell type* pull-down menu.
11. Check boxes 3 and 7 in the *Select cells* check box list.
12. Cell setup is complete now we need to set which optical systems are available. Click the *Optical systems* radio button at the top of the right panel.
13. Select the optical detector system for each cell (currently only Fluorescence).
14. Select *Fluorescence optics* from the *Select detector type* pull-down menu.
15. Check all cell boxes to which this system applies (all boxes in this case).

This completes the new rotor setup. The new setup can either be saved, or deleted (which will cause the rotor setup to revert to the default rotor setup). By clicking *OK*, the rotor setup is saved to the AOS data base and this rotor setup is set as the current setup. In order to use the Aviv AU system the rotor setup must contain one cell of type 10 Channel Fluorescence Calibration. This cell is required to calibrate and control the data acquisition (see Section ??).

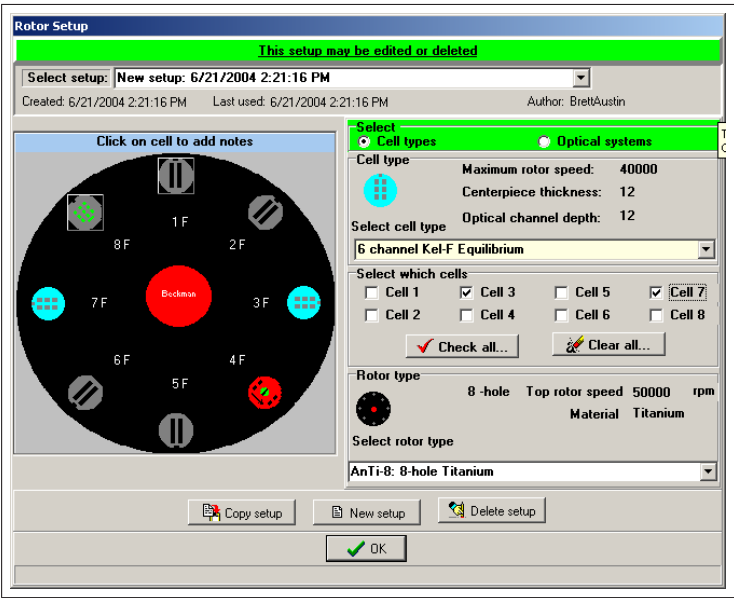


Figure 5.4: Creating an Example Rotor Setup

5.4.2 Example 2: 4-hole Equilibrium Setup

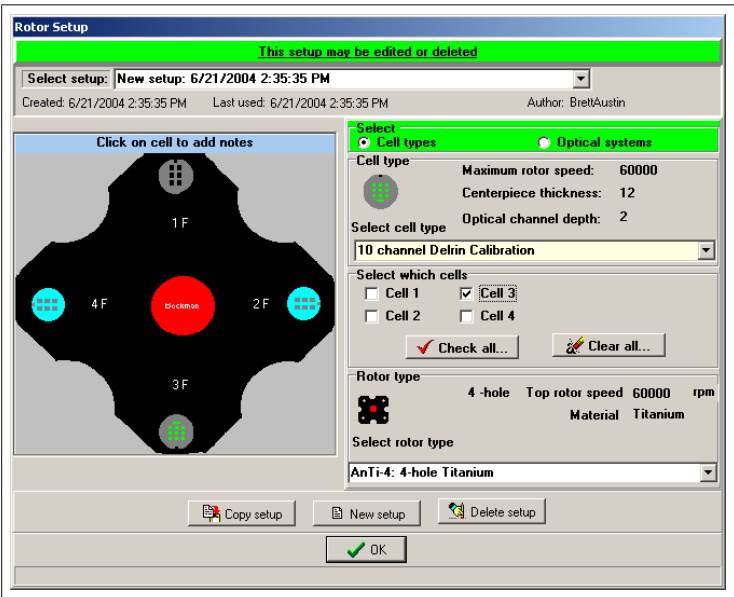


Figure 5.5: Creating an Example Rotor Setup

Chapter 6

System Calibration

6.1 Vacuum Calibration

The vacuum pressure is displayed in the status bar at the bottom of figure 3.1 on page 10. The vacuum pressure is a key parameter for the operation of the Aviv AU system. The AOS monitors the vacuum pressure from the centrifuge vacuum gauge and based on its state will allow various actions. The FDS laser will automatically turn on when the pressure drops below 50 microns. Experiments will not start until the pressure is below 50 microns. Also, the vacuum pressure is used to sense the power status of the centrifuge.

The vacuum pressure reported in the **AOS** is an estimate of the pressure which is derived from the centrifuge's vacuum gauge and is modelled as a geometric function. The coefficients of the geometric function can be modified to adjust the vacuum calibration. These parameters are accessible through the *Service* menu. It is not necessary for the vacuum pressure to track the reported pressure on the front of the centrifuge exactly but rather to match the pressure at a couple of key points. Because of its importance, the pressure at the 50 micron point needs to be calibrated so that the pressure reported by the **AOS** is close to the 50 micron point reported on the centrifuge but never below this value. Also, the vacuum pressure should read >1000 microns when the centrifuge is at atmospheric pressure.

6.2 AU Calibration Cell

The AU-FDS system requires the use of a special cell, supplied with the system upgrade, in order to operate the instrument. This calibration cell is used to calibrate the radial position of the detector and the angular location of each cell channel. It also provides automated focusing of the optics while under vacuum and a calibration standard for normalizing the intensity data.

The AU-Calibration cell is shown in figure 6.1. The figure shows the top of the cell (side closest to rotor center) at the top of the figure. Channel-A,

the rectangular channel, is referred to as the *calibration strip*. This calibration strip is precisely located so that its center is 5.85 cm from the center of the rotor and it is 1mm high (top to bottom). This strip is loaded with a sufficient concentration of fluorophor so that it can be easily detected (see table on page). The calibration strip is used for radial and angular calibration as well as focusing. The array of 9-1mm circular wells are used for generating an intensity calibration curve.

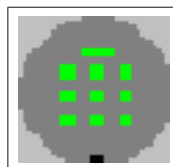


Figure 6.1: AU Calibration Cell

To perform calibration of the system, load the calibration cell into the rotor, and set up the rotor configuration (make sure the rotor is properly balanced), and start the rotor spinning at a speed less than 5000 rpm; it is important not to perform the radial calibration at high speeds due to the deformation of the rotor at high speeds (see Appendix ??) . When the vacuum pressure drops below 50 microns the FDS laser will automatically turn on. When the laser has warmed up and is operating at a stable light level, FDS laser light colored green in the Optical System Status panel, you can begin the calibration. To adjust the system focus or the radial calibration select the **Fluorescence focusing** item from the **Fluorescence** menu. To generate an intensity calibration curve select the **Intensity calibration** from the **Fluorescence** menu.

6.3 Fluorescence Focusing & Calibration Tool

The Fluorescence Focusing & Calibration Tool is shown in figure 6.2. The three panels at the top of the figure show the status of the FDS system and give the user some control over the instrument. The panel on the left contains slider bars which control the radial position of the instrument and the vertical position of the FDS focus motor. These sliders give the user the ability to actively move the instrument but also give feedback to the user when the instrument is being scanned. There are also pull down menus for selecting the step size used during scanning. These step sizes are only active locally while the focusing and calibration tool is active.

The center panel shows the currently active cell. When the form is first opened the default active cell is the calibration cell and the active channel is channel-A, the calibration strip. In almost every case these values will never be changed as ... , however, it is possible to change the cell and channel to scan other cells for comparison purposes. The bottom of the panel has a message window where results of focusing and calibration scans are displayed.

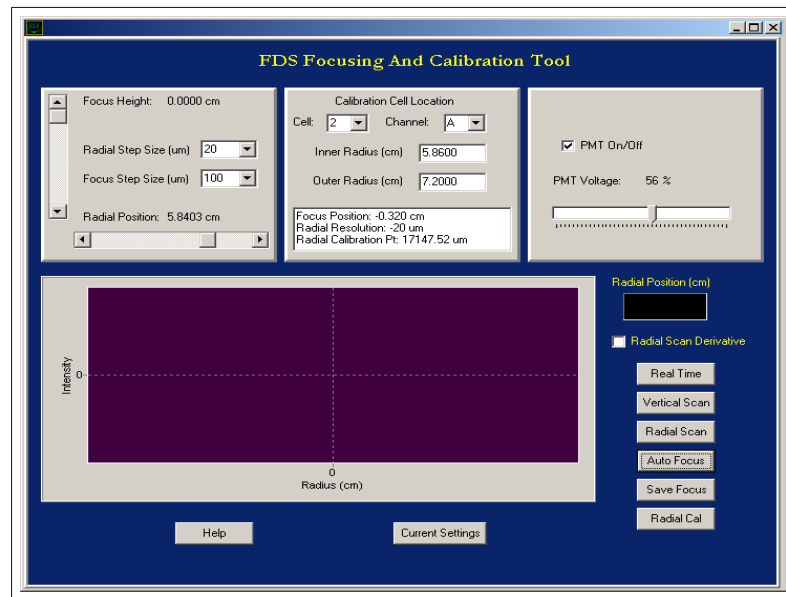


Figure 6.2: Radial Calibration

The right panel gives the user control of the FDS detector. The check box turns the detector on if checked and the slider bar adjusts the gain of the PMT. Make sure the PMT is on and at a gain to provide reasonable signal levels before doing any calibration of the system.

The chart window at the bottom of figure 6.2 is used to display the signals from the FDS instrument. The left axis shows the relative signal level and the bottom axis shows the position. The position can mean different things depending on the current mode of the system. It is possible to display real time data as the rotor spins under the detector (this is the default mode when the form is first opened). When this *Real Time* mode is selected, the bottom axis of the graph shows the angular position of the detector with the center of the current channel at the graph center. This mode can be selected by clicking on the **Real Time** button, located to the left of the graph window. Additionally, the average signal level of the data within the data angle is shown in the text box to the left of the graph with a resolution of 10 mV.

The other two modes are scanning modes. When either the vertical focusing motor or the radial positioning motor are moving, the graph's bottom axis displays the linear position of the motor in question and the text box to the right of the graph shows the current location of the moving motor. To vertically scan the focus motor click the **Vertical Scan** button to the right of the display graph; to radially scan the FDS detector click the **Radial Scan** button.

6.3.1 FDS Focusing

To achieve the highest possible radial resolution the FDS instrument must be aligned and focused correctly. The alignment of the optics is done before the FDS is delivered. If the instrument should need re-alignment it should be performed by a qualified service technician. The correct focus location is the point where the focal point of the exit lens of the FDS is located just below the top window of the cell (see Appendix ?? for cell description). At this point the signal level received by the FDS detector will be close to a maximum. Therefore, to obtain an estimate of the focus position we can position the instrument over the radial center of the calibration strip and then scan the focus motor through its entire range. The intensity will be peaked and the peak location is a rough estimate of the focus position. In most cases this focus estimate is adequate to do all experiments. To fine tune the focus position we would really like to select the vertical position of the focus motor which corresponds to the sharpest radial boundary at the bottom edge of the calibration strip. After performing a vertical scan to obtain an estimate of the focus position, click the **Auto Focus** button. The auto focus routine performs a series of radial scans at different vertical focus positions surrounding the estimated focus position.

After determining a new focus position, either by finding the estimated focus by performing a single vertical scan or by performing the full-blown auto focus, the new focus position must be saved. If the new focus is not saved before leaving the focus and calibration tool, the system focus reverts back to the original focus position. To save the new focus position click **Save Focus** to the right of the graph window. When the focusing and calibration tool window is closed the focus position is automatically set to the currently selected position.

The focus of the system should remain constant from run to run and so does not need to be redone each time an experiment is run. Instead it should be periodically checked, particularly if a different rotor or calibration cell is being used.

6.3.2 Radial Calibration

In order for the FDS instrument to function properly and produce accurate data it must be radially calibrated. This calibration is normally only needed at the time of installation of the optical system. If for some reason the optics are replaced or a different FDS unit is used in the centrifuge the radial calibration must be performed again.

The radial calibration provides a method for connecting the native coordinates of the radial stepping motor of the FDS instrument with the coordinate system fixed to the center of the centrifuge.

To perform a radial calibration:

1. Make sure the cell selected in the center panel is the calibration cell.
2. Make sure the PMT is turned on by checking the box in the right panel.
3. Set the PMT gain appropriately to produce an adequate signal level.

4. Click the **Radial Scan** button (do not perform radial calibrations above 5000rpm).

The radial stepping motor will then move to the bottom of the cell (displayed in the center panel) and start scanning until it reaches the top of the cell. When the scan is completed the data are displayed in the graph window (see figure ??). As shown in figure ?? the resulting intensity peak will have a boundary at both the top and bottom of the channel. The bottom boundary is produced by the physical edge of the calibration strip and the top boundary is likely caused by the meniscus since it is not possible to completely fill the channel (see Appendix ?? for instructions on loading the calibration cell). The best results for radial calibration are achieved by using small step sizes.

The signal from the bottom edge of the calibration strip is used to calibrate the system. The center of the slope in figure ?? is determined and this is assumed to be the physical edge of the calibration strip. Because the calibration strip is precisely machined the location of this strip edge is 5.9 cm from the center of the rotor, therefore, if we know the location of the motor in its native coordinates (micro steps from its zero position) we can calibrate the system so that we know the radial displacement from the rotor center for any motor position. When the radial scan is complete you can save the new calibration parameter by clicking the **Radial Cal** button. If the calibration is not saved the system reverts to the original calibration parameter when the focusing and calibration form is closed.

In addition to the radial calibration it is possible to get an estimate of the radial resolution of the system by looking at the width of the slope at the bottom edge of the radial scan of the calibration strip. When the radial scan is complete it will display the computed radial resolution (in microns) in the text box in the center panel at the top of the form. This resolution does not represent the resolution limit for the instrument but rather the computed resolution for the current scan settings. The radial step size and quality of focus can dramatically affect the computation of the resolution. To view the sharpness of this edge it is sometimes easier to look at the derivative of the radial scan data. To change the view to the derivative of the signal check the *Radial Scan Derivative* check box to the right of the graph. When looking at the derivative, the computed radial resolution is simply the width of the peak at the bottom edge of the calibration strip.

6.4 Angular Calibration

For the AU system to function correctly each detector system must know when a channel is within its view area. The angular position of all of the cells, and thus each channel, is fixed in the rotor coordinate system by the cell's position in the rotor setup. However, the rotor coordinate system spins in the fixed vacuum chamber coordinate system in which the AU optical detectors are fixed. Therefore, to connect these two coordinate systems we need a parameter which fixes the rotor position once per revolution. As discussed in Chapter ??, each rotor has a pair of small magnets attached to its base. These magnets are

embedded within the rotor overspeed disk. As the rotor spins these magnets are seen by the Hall sensor at the base of the vacuum chamber thus producing a pulse. This rotor timing pulse (RTP) gives us a means to relate the fixed vacuum chamber system to the rotating coordinate system fixed to the rotor. The magnet location on the rotor has some variation from rotor to rotor and will change if the rotor overspeed disk on a rotor has to be replaced. Therefore, it is not enough to know when the RTP occurred to locate the cell centers the uncertainty in the magnet position does not give the accuracy necessary to acquire data from the cell channels.

The FDS system can dynamically determine the precise location of the rotor magnet position with respect to the rotor coordinate system. It does this by determining the angular separation between the RTP and the center of the calibration strip. Whenever the rotor speed changes by more than 1000rpm this *Magnet Angle* is re-determined. *Magnet Angle* determination takes on the order of 5 seconds. If the *Magnet Angle* determination fails for some reason the system will not operate because the FDS would not be able to acquire data accurately. When an *Magnet Angle* failure occurs the **AOS** immediately shuts down the centrifuge. If this occurs there are a few likely things to check: make sure the calibration cell has the calibration strip at the top (closest to rotor center) and that it has not leaked, make sure there is sufficiently high concentration for the FDS to detect the calibration strip, and make sure the dust cover was removed from the FDS instrument case.

Another benefit of this dynamic determination of the angular location of the channels is that it also includes any individual system offsets and speed related electronics phase delays.

If the cell or channel center angles appear to be displaced from the center of the signal (as viewed in the cell setup tool) it may be that the calibration cell was not properly filled (or it may have leaked), the cells may not be aligned correctly in the rotor hole, or the database entry for the channel offset angle may be incorrect. The channel offset angle is the angle from the cell's center to the center of the channel. These values are set when the instrument was installed but can be modified if needed (see Chapter ??, *Service Utilities*).

A final note: the angular location of the cells in the rotor depend on the user accurately setting up the rotor from the *Rotor Setup* tool. If the rotor setup does not have a calibration cell or if it is located in the wrong hole the angular calibration will either fail or be inaccurate.

6.5 Intensity Calibration

In order to normalize the intensity data it is important to determine the equivalent concentration of the sample in solution. The calibration standard is 100% in solution as we use free dye, however, proteins that are tagged with fluorophors can stick to the walls and windows thus affecting the concentration of the fluorophor in solution. In order to normalize the data we have developed a tool for performing an intensity calibration. The **Intensity Calibration** tool

is available from the **Fluorescence** menu item in the main **AOS** menu.

Figure 6.3 shows the **Intensity Calibration** form. The panel at the top left of figure 6.3 shows information about the calibration cell. The center top panel shows the gain settings for the FDS PMT. The window at the bottom of the figure shows the calibration data.

To generate intensity calibration data click on the **Scan** button. The intensity calibration method will acquire intensity data from each channel in the calibration cell for a range of PMT gains. The scan takes a few seconds to complete. The data are displayed as a surface plot in the graph window as the scan proceeds.

The data can be saved by clicking the **Save Data** button. A chart editing window appears. The *Export* tab allows the user to save the data in a variety of formats. To save the data as an image select the desired format and click the **Save** button. To save the data as a text file click the *Data* tab. The data can be saved in a variety of formats including text file, Excel data file, HTML Table, and XML file. The data will be saved in three columns of the channel number, signal level, and PMT gain. Make sure that none of the check boxes on the right are selected, select the record delimiter from the pull down menu, and then click the **Save** button. The **Send** button allows the data to be emailed as an attachment.

To print the surface plot in the graph window click the button with the printer icon. A print window will open that allows you to select from any of your installed printers and adjust the print margins. To print the graph click the **Print** button.

The **Edit Chart** button provides access to tools for modifying the look and feel of the graph window.

The intensity calibration can be done prior to or after an experimental run for generating a data normalization curve. See Chapter 12 for information on how to utilize the calibration data for data analysis.

It is useful to use the intensity calibration tool to perform periodic checks on the calibration cell in order to determine the extent of photo-bleaching of the fluorescein.

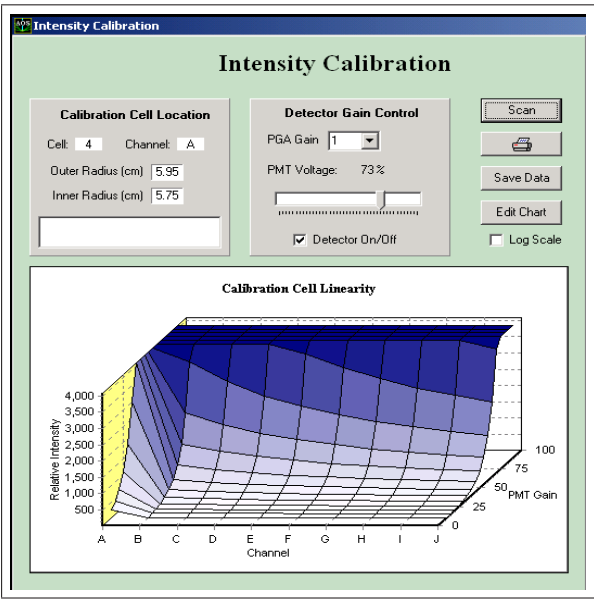


Figure 6.3: Intensity Calibration Tool

Chapter 7

Cell and Channel Setup

7.1 Channel Names

The AOS software uses a different naming scheme for cell channels than other centrifugation software. The naming scheme used is illustrated in figure 7.1 which shows the upward facing side of the AU-FDS calibration cell with the top of the cell towards the top of the figure. Channels are named by letter beginning with the channel closest to the center of the rotor (top of the cell). If there are multiple channels at a given distance from the center of the rotor, the channel farthest to the left (with the top of the cell up) is given the first letter. The example shown in figure 7.1 has a single rectangular channel named **A** at the top of the cell. The row of circular-shaped channels directly below this channel are located farther from the rotor center and the channel on the far left is given the name **B**, the center channel in that row is given the name **C** and finally the last channel in that row is given the name **D**. The same convention is used to name the remaining channels. The rationale for this naming scheme is as follows: the rotor of the Beckman Optima XL-A/XL-I spins clockwise and so channels on the left side cells are first to encounter AU optical detectors. Therefore, when viewing data at a fixed radial position, with angle increasing from left to right, the channels for a specific cell will be arranged from left to right in the display. The channel naming scheme for all cell types is shown in appendix E.

7.2 Channel Angles

The relative positions of cells in the rotor are fixed and determined by the angle between rotor hole positions. The center of each channel is determined by an offset from the cell center line, positive angles to the left and negative angles to the right. Each rotor has a small magnet attached to its bottom. There is a black-and-silver striped disc near the center of the rotor on the bottom. This disc is called the *Rotor Overspeed Disc*. In addition to providing the centrifuge with an optical means of checking the speed of the rotor it also has a small



Figure 7.1: Channel Naming Scheme

magnet embedded into the disc. The magnet has to be installed so that the falls between the scribe lines in the bottom of the rotor midway between rotor holes #1 and #4 (#1 and #8 for an 8-hole rotor). This magnet is sensed by a Hall sensor in the bottom of the centrifuge each time the rotor makes one revolution. The pulse from the Hall sensor, referred to as the rotor timing pulse (RTP), provides information about the rotor position at a specific time. The AU-FDS calibration cell provides a link between the fixed detector positions and the spinning rotor. By moving the detector out to the calibration strip (channel-A in figure ?? the FDS detector can find the angle between the strip center and the Hall sensor. This angle is referred to as the *Magnet Angle*. The *Magnet Angle* allows the FDS instrument to know the location of each channel. Because there are speed-dependent electronic delays in the system, the *Magnet Angle* is recalculated whenever the rotor speed changes by more than 1000 rpm. The *Magnet Angle* determination takes a couple of seconds during which time the user may not interact with the system; therefore, all of the menu items and buttons will be disabled during this process and a message in the status bar at the bottom of the main program window will provide information about what is happening. If the system can not determine the *Magnet Angle* the system will not function. If the AOS fails in three attempts to determine this angle the machine will automatically shut down and alert the user. If this happens refer to Appendix H.

The other important angle is the channel data angle. Each channel has an angle over which data will be acquired during a radial scan. For each radial po-

sition the signal value for a channel for one spin of the rotor is the average of the number of data points with the data angle surrounding the channel center. The default values are stored in the AOS database but can be changed for the current run in the **Cell Setup Tool**. To change the values permanently, by cell type, you can use the **Cell Characteristics Tool** located in the **Administration** menu.

7.3 Cell Setup Tool

If you click on the icon of a 2-channel centerpiece in the button tool bar or select the cell setup tool will open. This tool allows the user to manually set the boundaries and gain for each cell. It also allows the user to have real time control of various parameters so that they can examine the signals from the samples in the rotor.

It is very important to make sure that the cells are correctly set up before running an experiment. The detector gain levels and channel data angles should be checked. After the machine has been started, the rotor is spinning, and the FDS laser is on and stable, you can open the **Fluorescence Cell Setup** tool by clicking the icon in the tool bar which looks like a 2-sector velocity cell. The **Fluorescence Cell Setup** tool, shown in figure 7.2 will appear. At the top of the window there is a menu bar, below which is a panel showing the current rotor speed and buttons for saving current cell settings and exiting the form. Below this panel is another panel containing information about the current cell being viewed and the radial position of the current detector. Finally, there are panels for the current settings on the left side of the window and a panel for the data graphs on the right.

To start, first select the cell from the **Cell Number** selector, then select the channel you wish to examine from the *Cell Tree View* to the right. If the channel list is not visible click the + symbol next to the cell name or the cell name itself. The list of channels for the currently selected cell are displayed below the cell name. Select the channel you wish to look at by clicking the name of the channel or the + sign next to the channel name. After clicking on the channel a list of items appears below the channel name: **Gain Settings**, **Top & Bottom**, and **Sector Angles**. To view the signal from the current channel and set the gain level click on **Gain Settings**, and make sure the detector is on by checking the **Detector on** check box in the **Gain** box of the **Detector Settings** panel. With the detector on you should see the signal for the currently selected cell and channel displayed in the graphing window. The left axis of the display is the relative intensity of the signal and is simply the digital representation of the voltage produced by the detector. The A/D converter used is a 12-bit converter so the scale ranges from 0 to 4095. The bottom axis shows the part of the full rotation of the rotor which contains the current cell. The view should cover approximately 10 degrees. To change the viewing angles you can click the button at the bottom left labeled **Set display angles**, and a new form will appear that allows you to change the start angle and width of the view or to

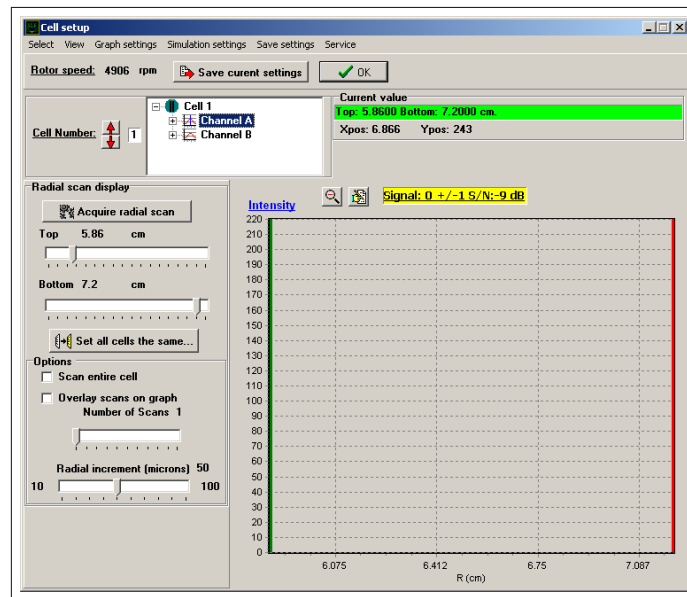


Figure 7.2: Fluorescence Cell Setup

check the box that allows the view to cover the full 360 degrees of the rotor. The full view is sometimes helpful to see the relative location of cells in the rotor. It is important to remember that the signals displayed in the graph while looking at either the **Gain Settings** or **Sector Angles** are for the current radial location of the detector. Above the graph on the right is a slider bar which allows the user to move the radial position of the detector. The current location is displayed to the right of this bar. In order to change the position of the detector simply click on the slider bar and drag it to the left to move the detector away from the center of the rotor and to the right to move it toward the center of the rotor. The desired position is shown to the right of the slider as you drag it. The stepper motor does not move the detector until you release the slider at the desired location. After releasing the slider an hourglass appears to indicate that the motor is moving; when it reaches its final position the cursor changes back to an arrow pointer.

Above the radial position slider bar is a set of coordinates which show the position of the cursor when in the graphing window. At the top of the graphing window in a yellow box is displayed the average signal and the signal-to-noise ratio.

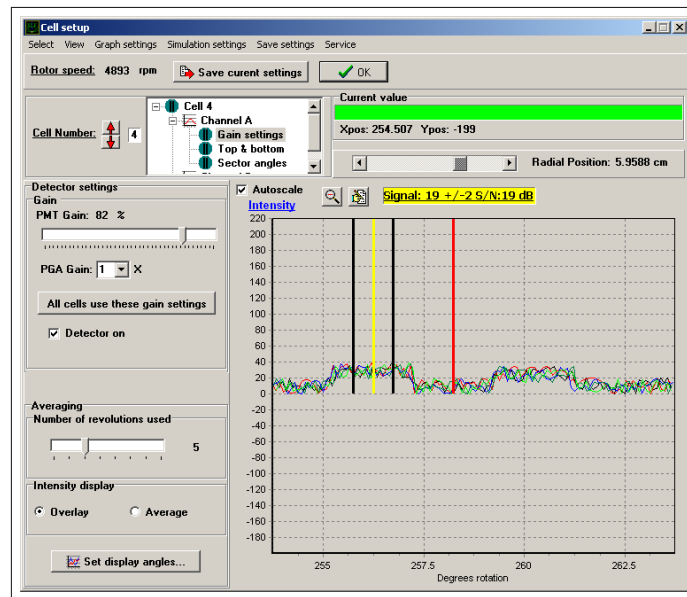


Figure 7.3: Sector Angles

7.3.1 Channel Boundaries

Angular Boundaries: Channel Data Angles

The data display in figure 7.3 shows a set of vertical bars in the graphing window. These bars show the center of the currently selected channel and angular region around which data will be acquired for any experimental scan (the data angle). To change the data angle for the current channel you can use the slider bar to the left of the graph. As you slide the control the data angle is changed; it is displayed above the slider and the two bars surrounding the channel center bar will move in or out. After changing the data angle you can save the value for this channel by clicking the **Save settings** button or by changing channels in the tree view control. When saving the settings you have the option of making the current settings the same for all channels or just for the current channel. These changes are only valid while the program is running. If you restart the program the channel data angles will revert back to the default values stored in the AOS database. To change the channel data angles permanently the *Administrator* can use the **Cell Characteristics Tool**.

Radial Boundaries: Channel Top & Bottom

If you select the **Top & Bottom** option from the cell/channel tree view in figure 7.2 then the options in the left hand panel will change to the **Radial Scan Display**. The two slider bars show the current top and bottom of the selected channel. The boundary closest to the rotor center is the top of the cell in the

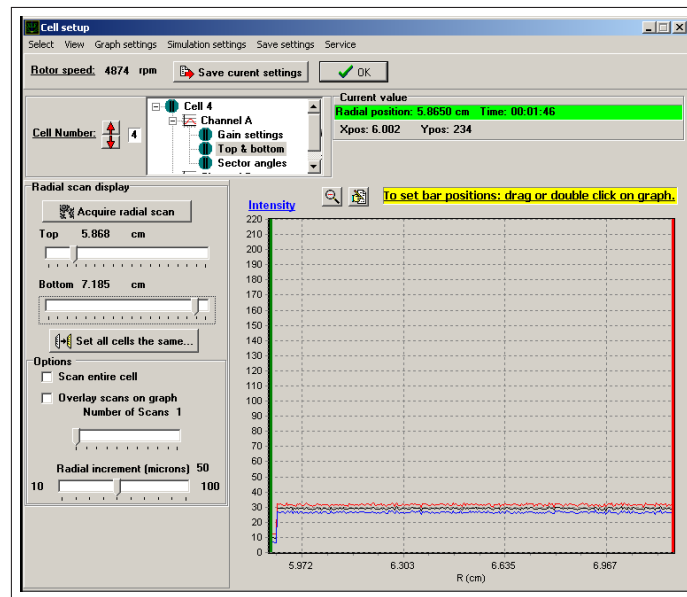


Figure 7.4: Radial Boundaries

artificial gravitational field produced by the spinning rotor and the boundary farthest from the rotor center is the bottom of the cell. As the rotor spins, particles in the channel will *fall* towards the bottom of the cell. The default values for the cell top and cell bottom are loaded from the AOS database when the program first starts. Use the slider bars to change these values.

The button above the slider bars allows the user to take a radial scan across the current channel. Clicking the button will initiate a scan. The current radial position of the detector, as well as the scan time, are displayed in the green bar above the data graph. When the scan is complete the data are displayed in the data graph. The center black colored line is the signal and the red and blue lines are the standard deviations. The green and red bars in the graph show the current top and bottom of the channel.

Scan options are shown on the bottom of the panel to the left of the graph window. It is possible to repeat the radial scan up to 100 times by changing the **Number of scans** slider bar, or indefinitely by checking the **Repeat scans** check box. The radial increment, in microns, is set with the slider bar at the bottom of the **Options** box. The **Scan entire cell** checkbox lets the scans override the top and bottom of the channel so that the radial scans cover the entire channel without modifying the current settings.

When scanning, the data displayed is an average of all the data within the channel's data angle (discussed above) and for several revolutions of the rotor. The number of revolutions to average over can vary between 1 and 10 and is adjustable with the

7.4 Cell and Channel Notes

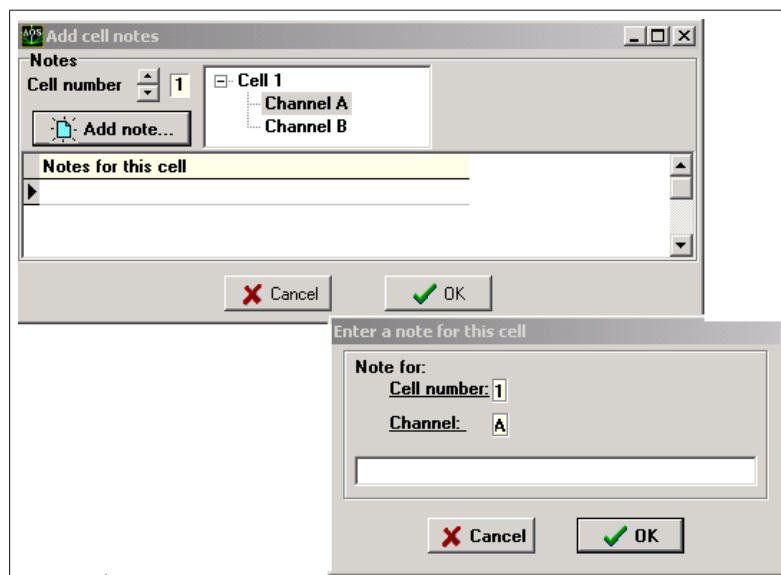


Figure 7.5: Cell and Channel Notes Window

To add cell or channel notes to the experiment run log click on the *Add cell/sample note* item from the *Experiment* menu. Figure 7.5

7.5 General Experiment Notes

Chapter 8

Gain Control

8.1 Gain Parameters

Both the AU-FDS and AU-RSA instruments use photomultiplier tubes (PMT) for their light sensitive detectors.

Photomultiplier tubes have very fast responses, high sensitivity and low noise. They have a photosensitive cathode which emits electrons when photons strike it. The photoelectrons emitted by the photocathode are directed toward the anode by a high voltage applied across the PMT. Along the way the photoelectrons strike a series of dynodes which then emit secondary electrons. The secondary electrons are also directed toward the anode striking any remaining dynodes producing even more secondary electrons. In this way the electrons initially produced at the photocathode are multiplied. The electron multiplication, or current gain, of the PMT is directly proportional to the supply voltage applied across the photocathode-anode pair. The PMT high voltage, and thus its gain, can be adjusted from the AOS using an analog control voltage ranging from 0V to 0.9V. Low light levels require higher PMT control voltages. Typical gains for a control voltage of 0.8V are on the order of 10^6 .

For low light levels the user may have increased the PMT to the highest level desired in order to maximize sensitivity and signal to noise ratio (S/N) but still the signal level doesn't cover the full 12-bit range of the AOS high speed analog to digital converters. This leads to a loss in signal resolution. In order to adjust the signal level so that it covers a wider dynamic range the Aviv Au system has high speed programmable gain amplifiers (PGA) for each of the signal channels. After selecting the desired PMT gain, changing the PGA level will cause the digitized signal to cover more of the 12-bit range. PGA levels include gains of 1, 2, 4, and 8. It is important to remember that changing the PGA level doesn't affect the S/N ratio but only improves the dynamic range and thus resolution of the signal.

The AOS software allows the user to set the PMT and PGA gains for each cell in the rotor. These gain parameters are used when scanning all of the

channels in the cell.

8.2 Manually Setting Gains

Figure 8.1 shows the the Fluorescence Cell Setup tool. This tool, initially discussed in Chapter 7, is used to manually set the gain levels for all of the cells in the current rotor setup. To use this tool the centrifuge must be at vacuum, the rotor must be spinning and the FDS laser must be at a stable light level.

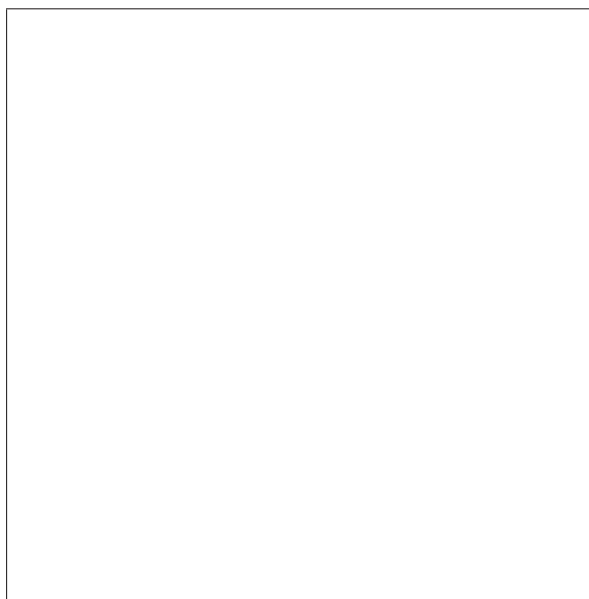


Figure 8.1: Fluorescence Cell Gains

In order to view or change the gain for a cell first select the cell from the **Cell Number** selector, then select the channel you wish to examine from the *Cell Tree View* to the right. If the channel list is not visible click the + symbol next to the cell name or the cell name itself. The list of channels for the currently selected cell are displayed below the cell name. Select the channel you wish to look at by clicking the name of the channel or the + sign next to the channel name. After clicking on the channel a list of items appears below the channel name; **Gain Settings**, **Top & Bottom**, and **Sector Angles**. To view the signal from the current channel and set the gain level click on **Gain Settings**. The signal from the current channel at the current radial location will appear in the graph window. In this view you will be looking at a fixed angular location of the rotor as it spins under the FDS instrument at a fixed radial position. It is important to make sure that the FDS detector is at the desired radial position, if the detector is outside the radial range of the current channel then you will likely see no signal. The radial position is shown to the right of the slider bar

above the graphics window. In order to change the position of the detector simply click on the slider bar and drag it to the left to move the detector away from the center of the rotor and to the right to move it toward the center of the rotor. The desired position is shown to the right of the slider as you drag it. The stepper motor does not move the detector until you release the slider at the desired location. After releasing the slider an hour glass appears to indicate that the motor is moving, when it reaches its final position the cursor changes back to an arrow pointer.

The panel to the left of figure 8.1 shows the controls to change gain levels and turn on/off the FDS detector. If you don't see any signal and you are sure you are at the correct radial position for the desired channel then check to make sure the FDS detector is turned on. To turn on the detector simply check the **Detector on** check box in the **Gain** box of the **Detector Settings** panel.

After confirming that the detector is on and you are at the correct radial position for the selected channel you may adjust the **PMT Gain** to optimize the (S/N) for this channel. The signal level plotted on the left axis of the graph is a relative scale, it runs from a minimum of 0 to a maximum of 4095 because of the 12-bit range of the A/D converters used to digitize the signals. Once the optimal PMT gain level has been determined you can adjust the PGA level to maximize the dynamic range of the signal. Be aware that any change in gain levels for this channel, if saved, will affect all other channels for the selected cell. It is important that you check the current gain settings for all the channels of the current cell to make sure that adequate S/N levels are achieved without saturating the signal. To save the gain levels for the current cell click on the **Save Current Settings** at the top of fluorescence cell setup window, see figure 8.1. If you have changed the gain levels and you change view, channel, or cell you will also be asked whether or not you would like to save the current settings. If you click the **No** button then the old settings are retained. It is also possible when saving gain settings to make the gain settings for all the cells use the same gains by clicking the **Save current gain settings for all cells**. This feature is useful when performing sedimentation velocity experiments because fewer distinct gains will produce faster scan times, see Chapter ??.

There are several locations in the AOS software where the photomultiplier gain and PGA level can be adjusted; Fluorescence Intensity Calibration, Fluorescence Focusing & Calibration. In all of these forms the PMT gain is changed locally while using the particular tool but doesn't affect the experimental gains of each cell.

8.2.1 Current Gain Settings

The current gain settings for each cell can be viewed without starting the machine or spinning the rotor. To view the settings select the *Set Gains* item from the *Fluorescence* menu.

Figure 8.2 shows the PMT gain and PGA level for each cell in tabular format. The first column shows the cell number (indexed from zero), the second column shows the PMT gain, and the last column shows the PGA level. The PMT

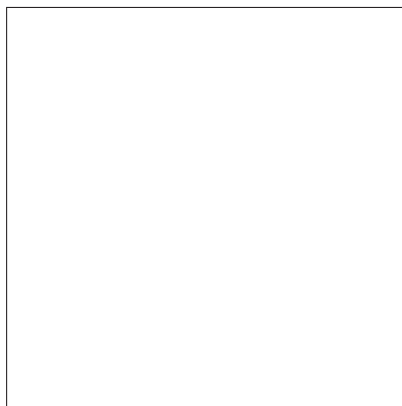


Figure 8.2: Current Fluorescence Cell Gains

gain is displayed as the number of bits out of 4096 bits. In other places in the software the gain is shown as percentage of the full range, $(\text{gain}/4096 \times 100)$. This tool is useful to check the current gain levels prior to beginning an experiment.

8.3 Automatic Gain Control

Automated optimization of the channel gains will be available in the next release of the **AOS**.

Chapter 9

Setting Up And Running An Experiment

9.1 Experimental Methods

Figure 9.1 shows the form used to create/modify experimental methods. This form is accessible from a number of places: clicking the experiment setup icon in the button tool bar of the main **AOS** window; selecting the *Select experimental method* item from the **Experiment** menu item from the main menu; and from the experiment wizard, see section 9.4.

The experiment setup form, like the rotor setup form, will not allow methods that have been previously used to be deleted or modified. The header at the top of the form contains a message indicating the status of the currently selected message and the background color will be yellow if the method has been used or green if it has not.

Experimental methods can be reused many times as they are simply templates for how the experiment will be performed. Details of individual experiments are saved in unique run logs, see .

The currently selected method is shown in the **Select method:** field at the top of the form. Under the method name field is a panel containing options and information for the current method. Below the options/information panel is a list of the method steps in the current experiment.

9.2 Selecting Existing Methods

The name of the currently selected experiment is displayed in the **Select method:** pull down menu at the top of figure 9.1. A new method can be selected from the pull down menu by clicking on the button with the down arrow to the right of the field and while holding the mouse button down scroll down to the desired method and then release the mouse button. After selecting a new method its

properties are loaded into the experimental setup form.

It is important to note that you can use methods that have been created by other users, you can use methods that have been previously used. These method setups are really a set of rules for how an experiment protocol will be carried out.

9.3 Creating New Methods

If you wish to create a new experiment you can click either the **Copy method** or **New method** buttons at the bottom of the form. Both buttons create a new unused method which can be edited or deleted. The new method is given an automatically generated name from the date and time of creation (displayed in the text field of the *Select method:* pull down menu at the top of the form. This name can be edited and given some more meaningful name. If the new name already exists the program will revert back to the automatic name.

9.3.1 Experiment Type

The check box labelled *Sedimentation velocity* tells the system that the experiment is not an equilibrium experiment. This affects how the FDS laser is operated and how gains are set by the automatic gain control (AGC), see .

9.3.2 Optical Systems to Use

The grid of check boxes under the heading *Optical systems to use* determines which optical systems will be used for this method. If an optical system is not currently installed then the option will be grayed out.

9.3.3 Starting Conditions

The starting conditions are shown under the heading *Prior to starting method*. *Fast pump down* when selected makes sure that the centrifuge's diffusion pump comes on for faster pumping; if the vacuum pressure is above XXX microns the desired rotor speed is set to 0rpm and the centrifuge is sent the *Start* command. If the *Fast pump down* option is not selected then the system only uses the roughing pump to evacuate the chamber and may take longer to achieve the necessary pressure (50 microns) to start an experiment.

Additionally the user add an extra delay after the centrifuge has come to the desired temperature before the experiment starts. The default delay after temperature is no delay. To change the delay simply click the time field below the *Prior to starting method* heading; a window with the delay time in time format will appear. To change the time click in the text field and use either the mouse or the cursor keys to highlight the value you want to change and then type the new value.

9.3.4 Stopping Conditions

The options under the heading *After method finishes* determine what happens when the current experimental protocol completes. If the *Stop centrifuge* option is selected then upon completion of the protocol the centrifuge is sent the *Stop* command; the rotor decelerates to zero speed and the diffusion pump begins to cool. After the diffusion pump has cooled the vacuum chamber stays at a rough vacuum waiting for the user to either restart the machine (from the AOS or the centrifuge panel) or to bring the vacuum chamber back up to atmospheric pressure by pressing the **Vacuum** button on the centrifuge front operating panel.

9.3.5 Method Information

The information about the creation date, time last used, author and method number are shown under the heading *Method Information*

9.3.6 Radial Increment

The default radial increment for each optical system is shown next to a button for each optical system. In order to change the default radial increment for any optical system simply click the button next to the increment value. After clicking the button a separate window will appear with a slider bar for changing the increment value. These default increments can not be changed once an experimental method has been used, however, the current radial increment can override the default at the time the protocol is run. That is you can select an experimental method with a default radial increment of $50\mu\text{m}$ and then before starting the experiment override the default setting it to say $10\mu\text{m}$. For information on overriding the radial increment see .

9.3.7 Revolutions to Average

The signal intensity at each radial position of an instrument is averaged over several revolutions of the rotor. The number of revolutions to include in this average can be between 1 and 10. The default number of revolutions to average is shown to the right of the default radial increments. To change the default number to average click the button labelled **Set default revs to average . . .**

9.3.8 Method Steps

The panel at the bottom of figure 9.1 shows a list of the steps in the current experimental method. Each step has the following items.

Step Number

The step number is just the index number of the current step.

Temperature

The temperature is the centrifuge temperature to be used for this step. To change the value click on the temperature text field; a new window will open with a vertical slider. Change the temperature to the desired new temperature and then click **Ok** to retain this value or **Cancel** to revert to the previous value.

Rotor Speed

The rotor speed is the speed to be used for this step. To change the value click on the rotor speed text field; a new window will open with a horizontal slider. Change the rotor speed by moving the slider bar to the desired speed and then click **Ok** to retain this value or **Cancel** to revert to the previous value.

Wait to Scan

Wait to Scan is the time period to wait before beginning this step of the current method. To change click the the time in the *Wait to scan* text field; a new window with a text entry box will appear. To change the wait time click in the text field and highlight the value you wish to change by using the mouse or keyboard arrow keys. When the desired items are highlighted you can change them by typing in a new value. The text is formatted as a time string (hh:mm:ss) and the window will not allow a string to be entered that is not a valid time.

Interval Between Scans

Interval between scans is the time period to wait before beginning the next scan of this step in the current method. For example, if there are 100 scans in the current step with an interval of 30 seconds then the **AOS** will wait 30 seconds after each scan before beginning the next scan in the method step. To change the Interval between scans, click the the time in the text field; a new window with a text entry box will appear. To change the interval time, click in the text field and highlight the value you wish to change by using the mouse or keyboard arrow keys. When the desired items are highlighted you can change them by typing in a new value. The text is formatted as a time string (hh:mm:ss) and the window will not allow a string to be entered that is not a valid time.

Repetitions

Repetitions is the number of scans to complete for the current method step. If the experiment has multiple gains then a single scan will consist of multiple physical movements of the FDS optics. For example: if a 4-hole rotor has a gain of 50% for cells 1, 2, and 3 but a gain of 75% for cell 4 then a single scan consists of the following actions. The FDS PMT gain is set to 50%, the FDS carriage is moved to the largest bottom position of the cells 1, 2, and 3 and then moves radially toward the rotor center until it reaches the top of those three cells which is closest to the rotor center. Next the the carriage is sent back out

to the radial position corresponding to the bottom of cell 4, the PMT gain is set to 75% and then the carriage is moved radially in to the position corresponding to the top of cell 4. This completes scan # 1. These steps are then repeated for each subsequent scan.

Appending Steps

Additional method steps can be appended to the experiment by clicking this button. The last step is copied and added to the end of the list of method steps. There is not a limit to the number of steps that can be added.

Inserting Steps

Additional method steps can be inserted between method steps by clicking this button. It inserts a step by making a copy of the of the current step and inserting it directly below the currently highlighted step (to select a step without opening editing windows click in the *Step number* column). There is not a limit to the number of steps that can be added.

Deleting Steps

To delete a method step simply highlight the step by clicking on the *Step number* and then click the *Delete step* button below the list of method steps.

9.3.9 Deleting Methods

If a method has never been used and you wish to delete it then simply click the **Delete method** button at the bottom of the select method form, see figure 9.1. The method will revert to the default method: *Sedimentation velocity 60K*.

9.4 Experiment Wizard

The *Experiment Wizard* is a tool that allows new users to run through an check list of items before starting an experiment. It can be accessed by clicking the globe icon on the far left of the button tool bar, figure 3.3 or by selecting *Experiment setup wizard ...* from the **Experiment** menu.

When the *Experiment Setup Wizard* is started the window shown in figure 9.2 appears. The button labelled **Wizard ...** at the top of the form takes the user through all of the steps in sequence. The steps to setting up an experiment are listed below the **Wizard ...** button and each has a button and a check box next to it. If the step has been performed (either by clicking the icon button to the left of the step or by automatically going through the list with the **Wizard ...** button then the check box is checked.

The list of steps shown in the Experiment Wizard is complete but not required for each experiment. For example if you have already logged in and

selected the correct rotor setup then the *User login done* and *Rotor setup has been selected* steps do not need to be performed.

A complete checklist of required steps to run an experiment is provided in Appendix ??.

9.5 Running the Experiment

After selecting the rotor setup, method, setting the gains for each cell, setting the radial increment and which cells to scan you are ready to start the experiment.

To start the experiment click the **Start** button at the top of the main **AOS** window. If the machine has not yet been started two items will appear; Start Machine, Start Experiment. If all the settings are correct the user can start both the machine and experiment together, this is particularly useful if the rotor setup, experimental method, gain settings, etc. are the same as the last experiment run. In this way the user can simply click the **OK** button and the **AOS** will start the machine first and when the machine is stable will start the experiment. If the machine is already running then the *Start Machine* option will not appear in the *Start* window.

When the experiment is started the *Machine Settings* panel is hidden (machine status is still available from the status bar at the bottom of the main window) and the *Experiment Status* panel becomes visible, see figure 9.3.

The experiment status panel is divided into several individual panels; *Experiment Status*, *FDS Status Panel*, *RSA Status Panel*. If an optical system is not being used for the current experiment then its status panel will not be visible in the experiment status panel.

9.5.1 Experiment Status Panel

The *Experiment Status* displays information about the current experimental method. It shows the total number of steps in this method, which step is currently being used, and the number of scans in the current step. It also shows the desired rotor speed, desired temperature, delay time before performing each step, and the interval to wait before performing each scan.

The two large text boxes are used to display messages to the user about the method status. For example if the method is waiting to start these message boxes will inform the user about the reason for the delay before starting.

The button with the arrow icon at the top left corner of the *Experiment Status* panel is used to override the *Wait for Temperature* option. This button can be pressed if the user decides that the temperature is close enough to the desired temperature to start the experiment. If the override wait for temperature button is pressed it doesn't affect the desired temperature setting on the centrifuge. For example, if the experiment had the wait for temperature selected and a desired temperature of 20C the experiment would normally wait until a stable temperature of 20C is achieved before starting the experiment. If the temperature has reached 20.2C and the user decides this is close enough to

start the experiment then they can press the temperature override button. The centrifuge will continue to bring the temperature down to the desired 20C but the experiment will not wait to begin scanning.

The button at the bottom left corner of the screen with the hand and paper icon allows the user to view the details of all of the steps in the current experiment. Clicking this button opens a window showing all of the steps.

9.5.2 Optical System Status Panels

Each optical system, when active for an experiment, scans independently. The progress of the scanning is shown in the optical system status panels in the experiment display panel. The progress bar at the bottom of each panel shows the current radial position of the detector and the text box above displays the exact radial position and scan time. In addition, the current cells being scanned and which gains are being used are displayed above this text box.

9.5.3 Data Display

While the experiment proceeds the data from each scan is displayed in the Data Graphs window after each scan is completed, see Chapter 10 for a detailed explanation of the data display options.

9.5.4 Interrupting the Experiment

If an experiment is started and it is discovered that the current settings are different from what is required you can either stop or pause and resume the experiment. To stop or pause the experiment click the **Stop** button at the top of the **AOS** main window. If the experiment is running then a window will pop up asking the user if they want to stop the experiment. To stop or pause make sure the check box next to *Stop Experiment* is checked and click *Ok*. A second window will appear with options to stop or pause.

Pausing

If the *Pause Experiment* button is clicked then the experiment is suspended, a message indicating an experiment is currently paused is displayed in the main window, the experiment status panel is hidden, and the machine status panel is made visible.

There are several settings which are independent from the experimental method; cell gains, cell boundaries, cell notes, step size, averaging. If an experiment is started and then it is determined that any of these settings is different from the desired values then the experiment can be paused and then resumed after correcting the problem. This is most useful early in the experiment. Note, you can not change the details of the current experimental protocol.

Resuming

To resume a paused experiment click the *Start* button on the main **AOS** window, a window will appear with a check box next to the option *Resume experiment*. To resume the experiment make sure the check box is checked and click the *Ok* button. A new window will appear with two options; Restart, Repeat. The Restart option will reset the experiment and start again from the beginning of the experimental method. The Repeat option resumes the current experiment at the beginning of the current method step.

Stopping

If the *Stop Experiment* button is clicked then the experimental protocol is terminated and the machine is left running with the current settings.

9.5.5 Completion of Experimental Protocol

When the experiment is finished a message is displayed in the message box at the top of the main **AOS** window indicating normal completion of the method. The centrifuge will either be stopped, setting the rotor speed to zero rpm, or left running depending on the stopping conditions set in the experiment setup.

Appendix ?? shows several tutorial style examples of experimental methods.

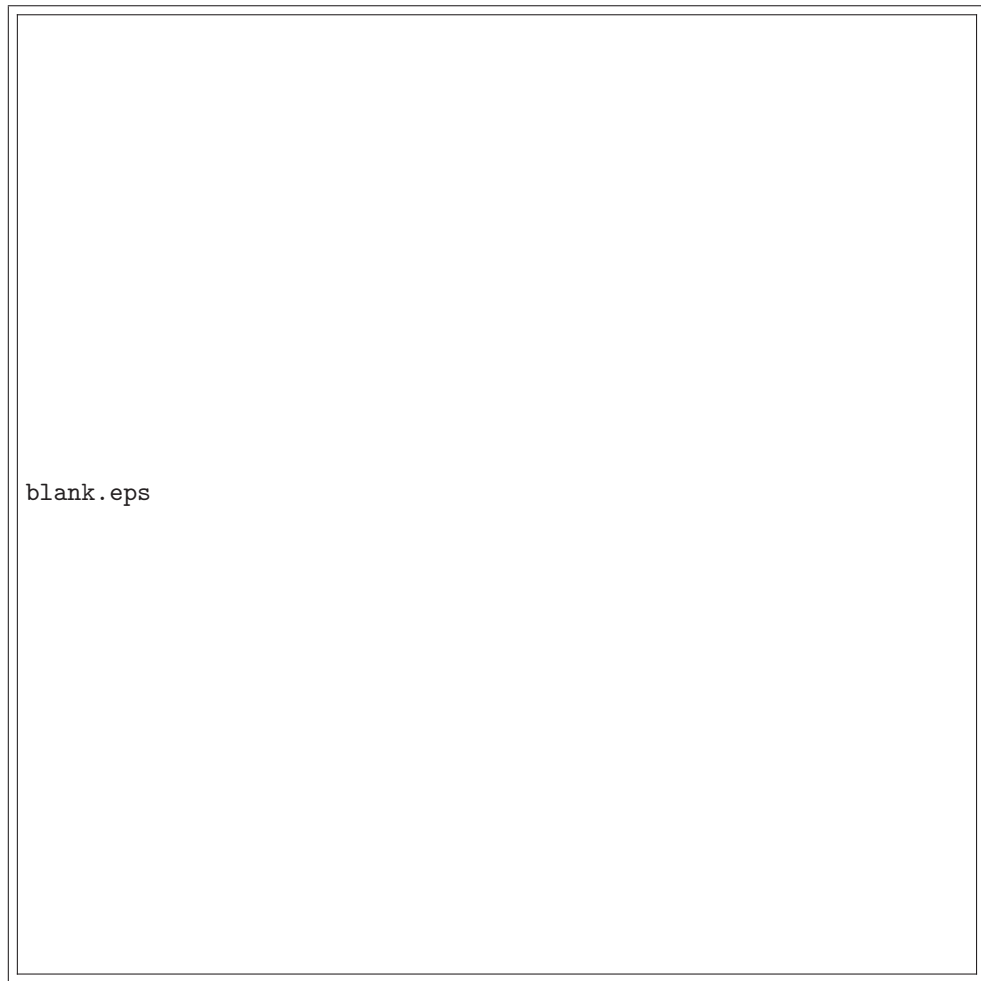


Figure 9.1: Method Setup Form

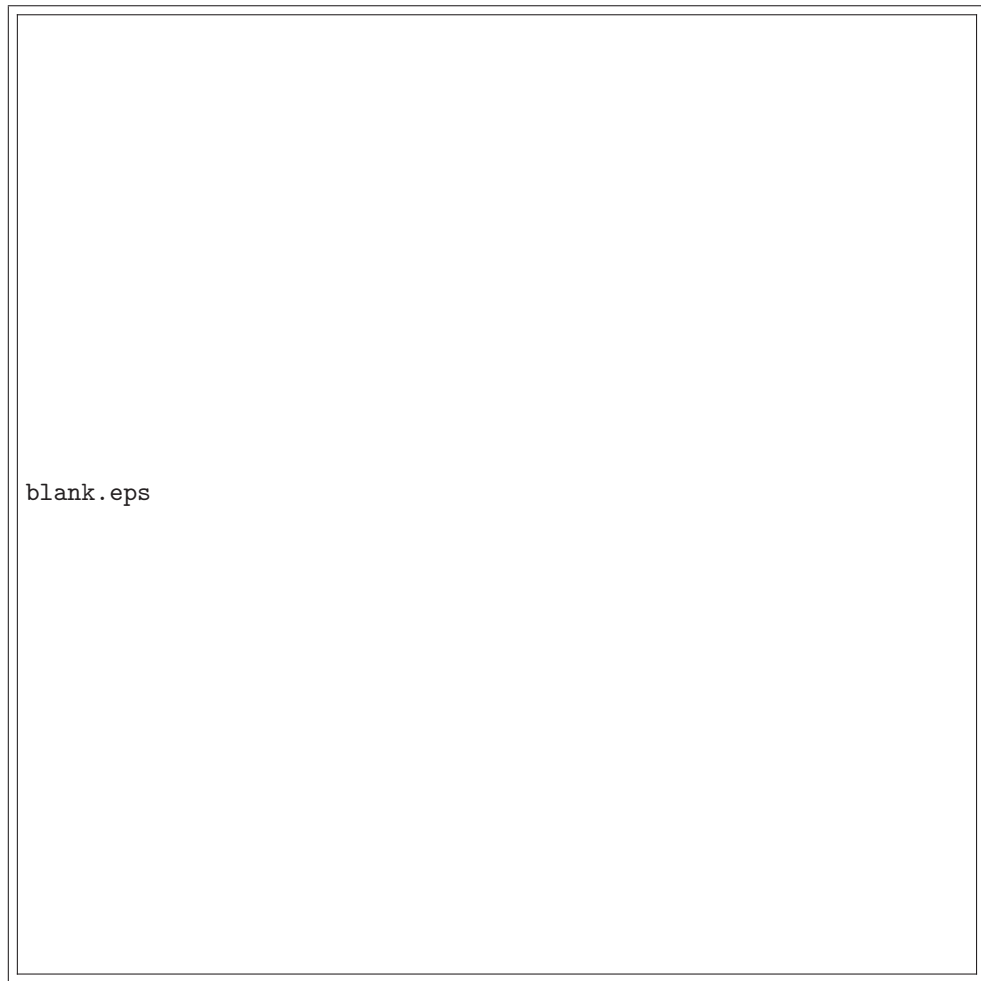


Figure 9.2: Experiment Setup Wizard

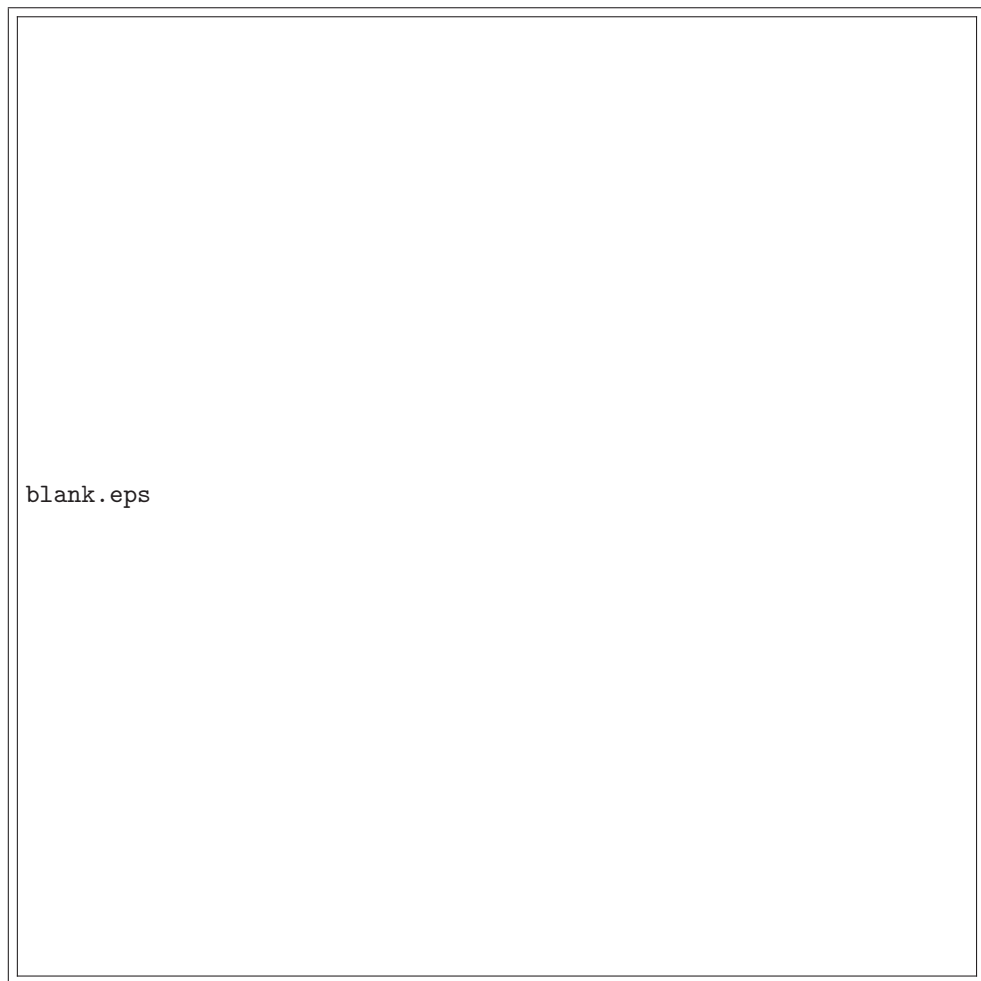


Figure 9.3: Experiment Status Panel

Chapter 10

Data Display

Scan data from experimental runs or individual scans are displayed in the **Data Graphs** window (see figure 10.1). The data are updated at the end of each scan or if the cell/channel selected is changed. The **Data Graphs** window can be docked to the main program window, un-docked and free floating, or hidden from view. To change the state of the Data Graphs window select one of the options from the View-¿Data Graphs menu item.



Figure 10.1: Data Graphs Window

The source of the data displayed in the data graphs can be selected at the top of the **Display Graphs** window. Only those optical systems which are being used for the current experiment are shown.

There are currently two different data views possible; detailed view or summary view.

10.1 Detailed Data View

The detailed view displays the data on a cell by cell basis. The tree view shows which cell is currently selected. If the heading is selected (eg. Cell 1 all channels), then the data from all channels for the current cell are displayed simultaneously with one color for each channel. To display the data from a single channel click on the desired channel in the tree view. When a single channel is displayed each scan is shown in a unique color. The number of scans to show in the chart is selected by the arrow keys next to the display of the number of scans to show. The maximum number of scans to show is currently 10. If the number of scans shown is set to 10 then the past 10 scans is shown. The number of scans shown can be changed during an experiment. To switch to a different cell use the arrow keys next to the Cell Number selected. The cell displayed can also be changed during at any time. The currently displayed cell/channel is displayed as the title of the chart.

The data can be inspected in more detail by zooming into the display. To zoom in, click the left mouse button in the graph window in the upper left location of the box you wish to zoom into and drag the cursor to the right and down while holding the mouse button down. A rubber band box will outline the area you will zoom to when the mouse button is released. After zooming into the graph if you wish to zoom back out to the complete graph left click anywhere in the graph, hold down the mouse button and move toward the left top of the graph and then release the button; it is not critical where in the graph you click if you are zooming out. There is also a zoom out button to the right of the cell channel tree view with the icon of a magnifying glass with a negative sign inside, see figure 10.2.

10.1.1 Changing Axis Scale

The button with the hammer/screw-driver/wrench tools shown in figure 10.2 is used to change the scaling of the y-axis of the data graphs. Clicking the button brings up a pull down menu. The menu includes items to allow changing the minimum and maximum values for the y-axis as well as an option for auto-scaling the graphs.

10.1.2 Printing Graphs

To print the graphs displayed in the chart window click the button with the printer icon in figure 10.2.

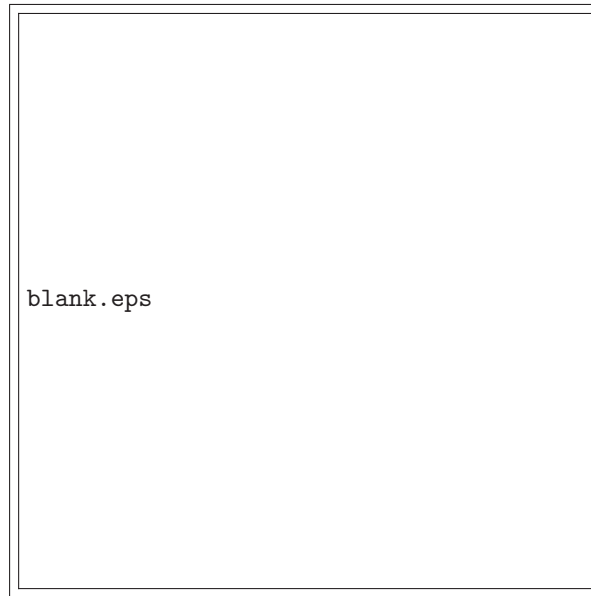


Figure 10.2: Chart Control Buttons

10.1.3 Saving Graphs to File

To save the displayed graph to a file click the top left icon in figure 10.2 showing a file, an arrow and a computer disk. Clicking this button brings up a standard file save dialog box allowing the user to select the name, location, and file type for the graphics file. Currently allowed file formats are bitmap (bmp), meta file (wmf) or enhanced meta file (wmf).

10.1.4 Hiding Graphs

10.1.5 Editing Chart Characteristics

The icon on the button in the upper right corner of figure 10.2 showing a hand writing on paper allows the user to edit many more chart characteristics including; customizing axes, printing the graph, viewing the raw data, etc.

10.2 Summary Data View

The summary view can be selected by clicking the *Data Summary* radio button at the top of figure 10.1. The summary view is a primitive display which is intended to give users a snap shot of the data from all channels in every cell simultaneously. As with the detailed view the data are updated at the end of each scan during an experiment run. The summary views are not configurable in any way.

Chapter 11

Data Files

11.1 File Names

11.2 Data Path

11.3 Format

```
6/22/20044:40:32 PM: Voltage: 1229 Gain: 1 Range: 4
F2 20.0 04966 0000191 1.8600E06 488 170
  5.8000  1.10424E+0001  2.41448E+0000
  5.8050  1.05152E+0001  2.71429E+0000
  5.8100  9.42424E+0000  2.40038E+0000
  5.8150  9.52121E+0000  3.11825E+0000
  5.8200  9.44848E+0000  2.65742E+0000
  5.8250  1.01576E+0001  1.99074E+0000
  5.8300  1.01758E+0001  2.42500E+0000
  5.8350  1.02727E+0001  2.84331E+0000
  5.8400  1.01152E+0001  2.59278E+0000
```

Figure 11.1: Sample Experiment Scan Data

6/22/20044:39:17 PM: Voltage: 3513 Gain: 1 Range: 4

F1 20.0 04978 0000116 1.4900E06 488 5

Channel	Intensity	Standard deviation
A	0010.2	.2
B	0010.1	.4
C	0010.1	.5
D	0010.0	.3
E	0010.2	.1
F	0009.9	.3
G	0009.8	.7
H	0010.0	.4
I	0009.9	.5
J	0009.6	.6

Figure 11.2: Sample Calibration Scan Data

Chapter 12

Data Analysis

12.1 Data Normalization & Intensity Calibration

Chapter 13

Administration Tools

13.1 Options

13.2 Account Management

Appendix A

Quick Start Checklist

A.1 Starting the Machine

- Is the calibration cell correctly installed in rotor?
- Has the correct rotor setup been selected?
- Is FDS instrument installed securely?
- Has the dust cover on the FDS optics been removed?
- Are the cables connected correctly?
- Is the AU system power on?
- Is the centrifuge power on?

A.2 Starting an Experiment

- Is the system focused?
- Has the system been calibrated?
- Have you selected the desired experimental method?
- Have you set the PMT gain for the cells?
- cells to scan
- step size
- cell notes
- root data directory

A.3 Turning Off the System

- Never vent the vacuum chamber with the AOS software off but the system power on, this could result in damage to the FDS electronics or PMT.
-
-
-

Appendix B

Overview of AUC Techniques

B.1 Sedimentation Velocity Analysis

B.2 Equilibrium Analysis

B.3 Absorbance Method

B.4 Fluorescence Method

Selectivity, sensitivity, speed.

B.5 Interference Measurements

B.6 Schlieren Measurements

B.7 Turbidity Measurements

B.8 Comparison of Methods

Determination of the sedimentation velocity can be made via fluorescence or absorbance techniques. The primary advantage of the AU-FDS fluorescence technique is that only the protein tagged with a fluorophor will be tracked by the instrument. With absorbance techniques all proteins in the sample will absorb equally well. Therefore, any contamination of the sample will make data analysis difficult. It is important to note that this also applies to fluorescence at 280nm, since all proteins will fluoresce equally at this wavelength.

The 488nm fluorescence detection system also has much higher radial resolution which results in a more accurate determination of the sedimentation velocity. In addition to the higher resolution the fluorescence detection system has a higher sensitivity thus allowing experiments with very low concentrations.

Somewhere talk about using FC43 so you can see bottom of cell and don't get window effect.

Appendix C

Fluorescent Materials

C.1 Fluorescent Labels

- Fluorescein Emission spectra is affected by pH
- Alexa Fluor This fluorophore has a narrow spectral bandwidth that can be useful in combination with other dyes for multiple labeling and for specificity in labeling. This dye is an important fluorescein substitute because it is significantly less sensitive to photobleaching but compatible with standard fluorescein optical filters. The fluorescence of this fluorophore is independent of pH
- Oregon Green

C.2 Naturally Fluorescing Materials

C.2.1 Green Fluorescent Protein

C.2.2 Aluminum

C.2.3 Epoxy

C.2.4 Delrin

Appendix D

Example Experiments

D.1 IgG labelled with Alexa Fluor

To illustrate how the AUF-OS operates we will go step by step through a sedimentation velocity experiment using two different fluorescent probes. The following information can also be found in detail throughout the rest of this manual.

D.1.1 Sample Preparation

The first sample is Molecular Probes goat anti-mouse IgG conjugate labeled with AlexaFluor488 dye (Catalog A11001) diluted into a standard buffer (0.1 M KCl, 0.01M Tris, pH 7.5). Ovalbumin was added at 0.1mg/ml to compete with the potential binding of IgG to the walls of the centerpiece. The final concentration of samples are

D.1.2 Cell Assembly

A standard Beckman analytical ultracentrifuge cell is assembled using quartz windows and SedVel60 graphite filled epoxy centerpiece. A reference of the sample buffer is not needed with the FDS so that both channels can be loaded with sample. The convention for loading the sample is with the top of the cell facing you and the loading holes facing up, the left sector is channel A and the right side sector is channel B. Channel A is loaded with 400ul of the mg/ml sample and channel B is loaded with 400ul of the mg/ml sample.

D.1.3 Loading the Rotor

A calibration cell must be loaded into the rotor however it does not matter what rotor position this cell is in. The calibration cell setup is described in Chapter?. The calibration cell and a counterbalance were loaded into rotor positions 2 and 4 respectively, of a four hole rotor. A counter balance is placed in cell 3 and our

cell is loaded into rotor position 1. All cells are aligned with the center of the rotor by using the Cell Alignment Tool.

D.1.4 Installing the Hardware

The rotor is set in the centrifuge on the center spindle and the optics are then seated on the bracket inside the centrifuge and secured by tightening the securing screw located near the bottom right side of the optical box. Close the cover to the centrifuge. Both the centrifuge and the Aviv AU System Box must be turned on and the rest of the system will be run from the computer.

D.1.5 Running the AOS Software

Open the Au-AOS and login to your account. For this experiment we will be logged in as ? .

Starting the Machine

Press the Start button located in the button toolbar. The *Start* form will come up - deselect the *start experiment* check box and press *OK*, this will start the centrifuge vacuum but not the rotor spinning. While you set up the experiment the system will automatically run through several functions that will determine when the laser can safely be turned on.

Calibration and Focusing

?

Selecting Rotor Setup

From the button toolbar select the picture of the 4-hole rotor to open up the *Rotor Setup Form*. The last used rotor setup will be displayed. Select *New Setup* from the bottom of this form and a new rotor set up form will be displayed. In the header a *Select SetUp* pull down menu will display the current date and time- you can rename the setup by highlighting it- for this experiment we will keep the default name. The *Cell Types* button is the default selection. From the pull down menu select *2 channel Charcoal Epon Velocity* and then check off the rotor position, one, where our cell is located from the *Select which cell* panel. Next from the pull down menu select *10 channel Delrin calibration* and check off cell position four. The counterbalances can be left alone. Selecting the *Optical System* check box will bring up the options for the optical systems available- the only option on this machine is fluorescence optics - uncheck cell 2 and 3 in the *Select which cells* option since we only want to scan cells 1 and 4. Left click on the picture of cell one and the *Add Cell Notes* window will appear. Select Channel A then the *Add Note* button and type AlexaFlour/ IgG ? mg/ml into the new Window and select *OK*, do the same for Channel B but change the concentration to / mg/ml. Select *OK* to exit the notes menu. Press *OK* at the

bottom of the *Rotor Set Up* form and when the window asks if you want to save the changes select *Yes*

Selecting the Experiment Setup

Select the *Select/Edit an Experimental Method* picture from the button toolbar. The *Experimental Setup Form* will display the last used method. From the bottom of the form press the *New Method* button. We will keep the default naming method of current date and time that is displayed in the pull down menu at the top of the form. The check boxes for **Sedimentation Velocity**, **Fast pump down** and **Fluorescence** will remain checked. Deselect **Wait for Temperature** and **Stop Centrifuge**. In the next panel for *Default Radial Increments* the default setting of 50um for the fluorescence optical system will be used and the *Default Revolutions to Average* setting will remain at 5 revolutions. The last parameters to change in this form are the **Method Steps**: For **Step Number** one, keep default Temp at 20C and change **Rotor Speed** to 60000 by selecting the *Rotor Speed* text field and moving the slider bar to 60000 then select *OK* to retain that value in the form. The *Wait to Scan* and *Interval between Scans* will remain at the default value of 0. Change the number of repetitions to 400 by selecting the *Repetitions* text field and type 400 into the box then select *OK* to save that value. The method is complete for this experiment, select *OK* from the bottom of the form to exit and you will be prompted to save the changes by selecting *Yes*

Setting Cell Gains

For this set up the rotor must be spinning. We have already started the machine so the vacuum should be below 50um and the laser light should be green indicating that the laser is ready. To start the rotor select the *Rotor Speed* picture from the button toolbar and set it to 3000 then press *OK* to start the rotor spinning at 3000. The **Cell Setup** form will be displayed and all cells that were selected to scan in the *Rotor Setup* form will be available to select the gain settings. Only cell one should be available for setting the gains. Under Cell 1, select Channel A by clicking on it and three items will appear below it; **Gain Settings** is the first option. Select *gain Settings* to view the signal from Channel A and a plot of the current intensities. A red line marks the center of the centerpiece, to the left will be the signal from Channel A. Check the radial position on the slider bar above the plot and if needed adjust the radial position so it is within the current channel. For channel A you should be able to see the signal at around 5.7cm. Move the slider bar on the *Detector Settings* to adjust the PMT gain to the optimal gain level for this channel to ?. Select Channel B and you will be asked if you want to save the gain settings from Channel A. Select *Yes* and make sure that these settings are okay for channel B. Cells can have different gain settings but both channels of one cell must be the same.

Setting Cell Top and Bottom

While still in **Cell Setup** form the next item to adjust after the *Gain Settings* is to set the *Top and Bottom* of the cell, the default is 5.6cm top and 7.3 cm bottom. To make sure the data falls within this range select *Acquire Radial Scan* and when the scan is complete you can adjust the top and bottom limits using the slide bars. The red and green bars on the graph show the current settings which we will not change.

Setting Channel Data Angle

This last option in the **Cell Setup** form is used to adjust the width of the scan which data will be collected from. Adjusting the *Sector Angle* will allow you to crop out some of the noise or to take in more data points depending on how the scan looks. This angle should be between the two walls of the channel while taking in as much of the data as possible(?). The red line indicates the center of the cell while the yellow line indicate the center of the area to be scanned. All data within the black lines will be included.

Setting Revolution to Average

Isn't this set someplace else too? After adjusting or checking the *Channel Data Angle* you can also check to make sure the revolution average that was set in the *Experimental Method* form provides enough signal intensity. Not sure what I'm looking at here.

Setting Radial Increment

this is in the experimental methods setup too?

Selecting Cells to Scan

The last item to check before starting the run is *Select Cells to scan and add Notes* under the **Fluorescence** menu. On the left side of the display window are check boxes of which cells to scan- uncheck all but Cell 1. On the right side of the window select **Channel A** and press the **Add Note** button to enter text for Channel A. In the space provided type IgG tagged AlexaFluor mg/ml, select *OK* and do the same for Channel B entering the text IgG tagged with AlexaFluor mg/ml 1:2 dilution. Select *OK* to exit this window.

Starting the Experiment

All the information needed to start the experiment has been entered. Go to the top of the AU-AOS page and press *Start*. Because the rotor is already spinning the only option is to **Start Experiment** by pressing *OK*. You now have the option to stop the rotor and start the experiment from 0 rpms. Select *Yes* and the rotor will automatically have a one minute delay and the speed will return to zero.

The **Machine Settings** panel will be replaced by the **Experiment Status** panel. You can monitor the scans and status of the machine in this mode.

Data Display

The **Data Graphs** panel will display each scan immediately after they are complete. Under the **View Data Mode** select one for the **Cell Number**. **Scans to Show** option refers only to the graphs being displayed. You can choose to overlay up to 10 graphs on the screen.

Data Files

Also under **Data Graphs** is a pull down menu **Now Showing** that displays the data file directory the current scans are being saved to. All scans are saved by the date, then the time of the start of the experiment, the scan number and rotor position. For fluorescence optics the extension is FI, so for cell one it will be .FI1

Description of Data

physically describe what data looks like- meniscus end of cell-

Data Normalization

description of calibration files and use to normalize intensity data what info we are looking at

D.1.6 Data Analysis

Fluorescence data can be analyzed with Sedfit by Peter Schuck and Sedanal by Walter Stafford.

Sedfit

SedAnal

Conclusions

D.2 Green Fluorescent Protein

short description of purpose for experiment of what's to follow

D.2.1 Sample Preparation

describe dilutions- what sample is - ovalbumin addition-

D.2.2 Cell Assembly

D.2.3 Loading the Rotor

how, alignment tool and why, calibration cell needed refer to cali cell

D.2.4 Installing the Hardware

rotor and optics setup- what order to turn on the power-

D.2.5 Running the AOS Software

Starting the Machine

Calibration and Focusing

Selecting Rotor Setup

Selecting the Experiment Setup

Setting Cell Gains

Setting Cell Top and Bottom

Setting Channel Data Angle

Setting Revolution to Average

Setting Radial Increment

Selecting Cells to Scan

Entering Cell/Channel Notes

Starting the Experiment

Data Display

Data Files

where and how structured

D.2.6 Data Analysis

Data Normalization

description of calibration files and use to normalize intensity data

Description of Data

physically describe what data looks like- meniscus end of cell- what info we are looking at

Sedfit

SedAnal

Conclusions

Appendix E

Cell Description & Types

Put description of XLA/XLI centerpieces here! (parts, assembly, etc). There are several different cell types which can be used in the Aviv AU system. These cells can be selected from the rotor setup form (see Chapter ??). The characteristics of each of these cells can be viewed and modified in the Define cell characteristics menu item under the Service menu (see Chapter ??). The most important cell type for the AU system is the 10 Channel Delrin Calibration cell. This cell is used by the AU system to establish a radial calibration of the instrument, focus the fluorescence optics, and actively determine both angular location and gain settings for the system. The calibration cell is shown in figure . The calibration cell has 10 channels; one rectangular channel located at the top of the cell and 9 circular channels located in an array below the top channel. The channel naming convention in the AOS is that channels are named with letters of the alphabet starting with the top most channel to the left. The reason for this naming convention is that the XL-A/XL-I direction of rotation of the rotor causes the left side of the cell to be seen first by the AU optics. Therefore when viewed with the fluorescence cell setup form chap the channel letters can be read left to right. Therefore, as shown in figure when viewing the topside of the cell with the top of the cell the top strip of the calibration cell is channel A, the first circular well

Velocity cells channel naming scheme, characteristics, etc. Equilibrium cells, channel naming scheme, characteristics, etc.

E.1 Cell Description and Assembly

Somewhere talk about using FC43 so you can see bottom of cell and don't get window effect.

Ovel bunum (sp?) to prevent protein sticking to walls windows.

E.2 Cell Parameters

E.3 AU Calibration Cell

E.4 2-Channel Velocity Cell

E.5 6-Channel Equilibrium Cell

E.6 8-Channel Short Column Cell

E.7 Aluminum Counterweight

E.8 Special Cell Types

E.9 Cell Parameter Editor/Viewer

Appendix F

System Specifications

F.1 Sensitivity

F.2 Resolution

F.3 Scan Times

F.4 FDS Laser Operation Times vs. Temperature

Appendix G

Unusual Signals

Somewhere talk about using FC43 so you can see bottom of cell and don't get window effect.

G.1 Meniscus Effects

G.2 Dye Leakage

G.3 Protein Sticking

G.3.1 Window Effects

G.3.2 Wall Effects

G.3.3 Elimination

G.4 Rotor Stretch

G.5 Shadowing by Window Holder

Appendix H

Trouble Shooting System Errors

H.1 Magnet Angle Failures

H.1.1 Failure to Detect

H.1.2 Not Located on Center of Calibration Strip

H.2 Rotor Timing Pulse Dropouts

H.3 Force Acquire Errors

H.4 Stepper Motor Problems

H.4.1 Position Verification Errors

H.4.2 Stepper Motor Communication Failure

H.5 Hardware Detection Failure

H.6 FDS Laser Errors

H.6.1 Overheating

H.6.2 Failure to Light

H.6.3 Light Level Unstable

H.7 FDS Signal Not Present

Index

calibration cell