Wideband Integrated Bioaerosol Spectrometer (WIBS-4A) Operator Manual

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2545 Central Avenue
Boulder, CO 80301-5727 USA

2545 CENTRAL AVENUE
BOULDER, COLORADO, USA 80301-5727
TEL: +1 (303) 440-5576
FAX: +1 (303) 440-1965
WWW.DROPLETMEASUREMENT.COM

Trademark Information

Warranty

The seller warrants that the equipment supplied will be free from defects in material and workmanship for a period of one year from the confirmed date of purchase of the original buyer. Service procedures and repairs are warrantied for 90 days. The equipment owner will pay for shipping to DMT, while DMT covers the return shipping expense.

Consumable components, such as tubing, filters, pump diaphragms, and Nafion humidifiers and dehumidifiers are not covered by this warranty.

Software License

DMT licenses WIBS software only upon the condition that you accept all of the terms contained in this license agreement.

This software is provided by DMT “as is” and any express or implied warranties, including, but not limited to, the implied warranties of merchantability and fitness for a particular purpose are disclaimed. Under no circumstances and under no legal theory, whether in tort, contract, or otherwise, shall DMT or its developers be liable for any direct, indirect, incidental, special, exemplary, or consequential damages (including damages for work stoppage; computer failure or malfunction; loss of goodwill; loss of use, data or profits; or for any and all other damages and losses).

Some states do not allow the limitation or exclusion of implied warranties and you may be entitled to additional rights in those states.

Laser Safety

The WIBS-4A is a Class 1 Laser Product. It features a 635 nm, 12 mW laser.

An identification label is located on the rear panel of the WIBS.

CAUTION – Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

CONTENTS

Figures

1.0 Introduction

The Wideband Integrated Bioaerosol Spectrometer (WIBS) provides highly sensitive measurements of mold and other bioaerosols. The instrument uses a UV xenon source to excite fluorescence in individual particles. Unlike UV lasers, the UV xenon source allows for the precise selection of particular UV wavebands. On the WIBS, these wavebands have been selected to optimize detection of the common bioaerosol components tryptophan and nicotinamide adenine dinucleotide (NADH). The xenon source is also far less expensive than a UV laser, making the WIBS-4A a cost-effective alternative to other bioaerosol measurement instruments.

The WIBS was originally developed by the University of Hertfordshire and is licensed to and manufactured by Droplet Measurement Technologies.

Figure 1: The WIBS-4A

This manual describes the WIBS-4A.

2.0 Unpacking

Unpack the WIBS-4A and ensure all the components are present. Shipped with your instrument are the following items:

- Laptop
- Power supply
- Zero filter and inlet adapter
- USB storage device
- This document, the *Wideband Integrated Aerosol Spectrometer Operator Manual*

3.0 Overview of Operation

3.1 Design

The single-particle fluorescence sensor, WIBS-4A, employs a central optical chamber, shown in Figure 2, around which are arranged the following components:

- A continuous-wave 635nm diode laser used in the detection of particles and the determination of particle size
- A forward-scattering quadrant photomultiplier tube (PMT) used in the determination of particle size and shape
- Two pulsed xenon UV sources emitting at different wavebands
- Two fluorescence detection channels, FL1 and FL2, detecting intrinsic particle fluorescence across two wavebands.

Thus, for each particle, a 2x2 excitation-emission matrix is recorded along with an estimate of particle size and particle shape. The excitation and emission bands are selected to optimize detection of biological particles containing tryptophan and NADH.

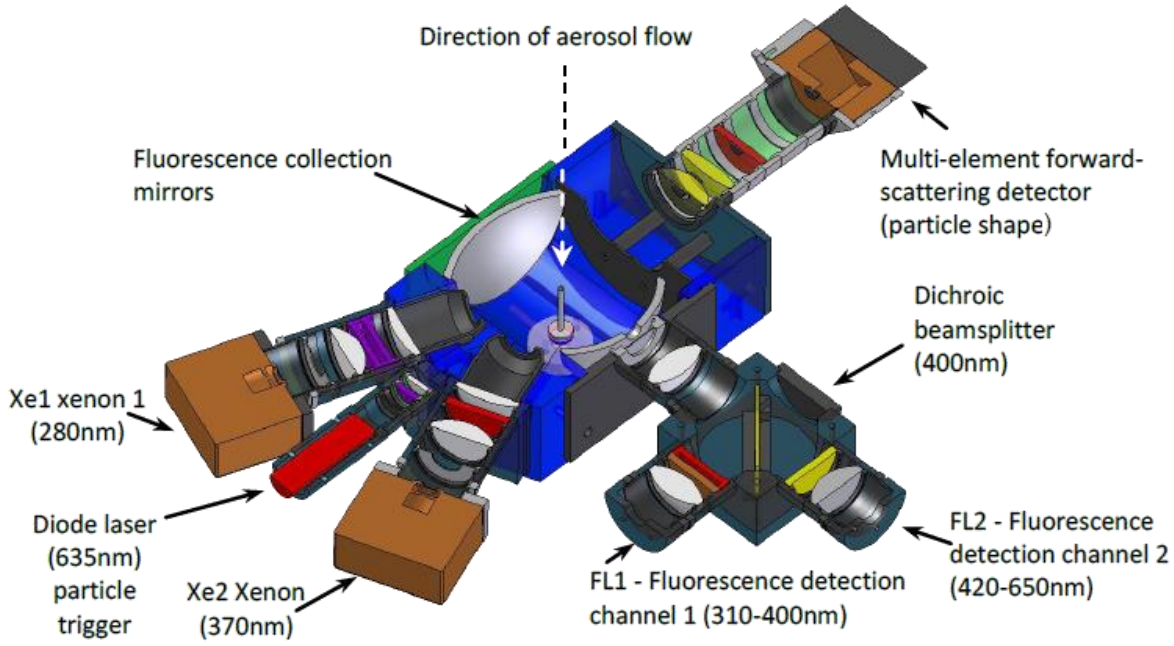


Figure 2: The WIBS-4A Central Optical Chamber

In operation, aerosol is drawn from the ambient atmosphere via a laminar-flow delivery system. This system renders suspended particles in essentially single file as they traverse the focused laser beam. The total aerosol flow is approximately 2.5 l/min, of which approximately 2.2 l/min is filtered before being re-introduced to form a sheath flow. The sheath flow confines the remaining sample flow as well as a small bleed flow that continually purges the optical chamber of any fugitive particles. The intersection of this aerosol sample flow and laser beam defines the scattering volume, a circular disc approximately 0.7 mm diameter and 130 µm depth.

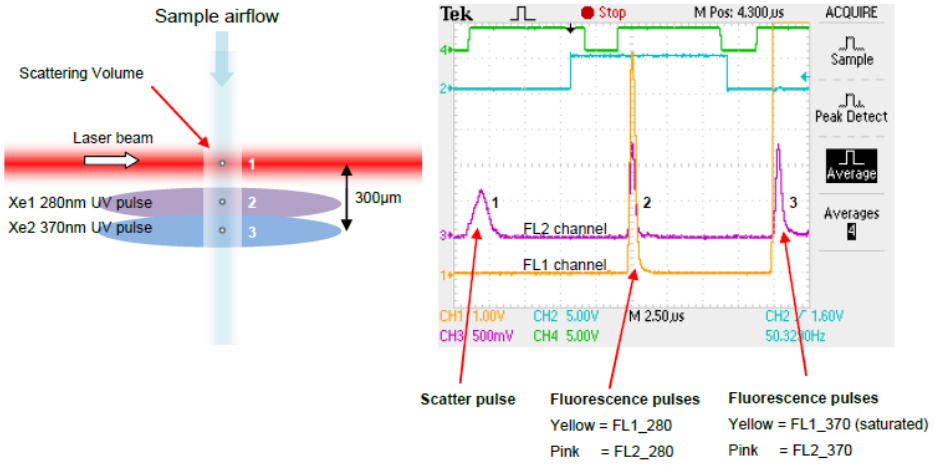


Figure 3: The WIBS-4A Measurement Cycle

Each particle entering the scattering volume (position 1 in Figure 3) scatters light in all directions. The side-scattered light is collected by the two high numerical-aperture chamber mirrors and passes through an aperture in one of the mirrors, then through a dichroic beam-splitter, and onto the FL2 channel PMT. This produces an electrical scatter pulse, as shown in the oscilloscope plot in the right of Figure 3. (Note that light scattered in the forward direction falls onto a separate Quadrant PMT used to assess particle shape; see Appendix B).

The magnitude of this pulse is compared to a pre-set threshold voltage, equivalent to a particle of approximately 0.5 µm in size. If the pulse size exceeds the threshold, the first xenon flash-tube, Xe1, is fired some 10µs later when the particle has travelled down to position 2 in Figure 3. This flash-tube is optically filtered to a narrow band centered on 280nm (see Appendix E). The 280nm band is tuned to excite the specific target biological molecule tryptophan that may be present within the particle. The resulting fluorescent emission from the particle is collected by the two chamber mirrors and delivered through the mirror aperture onto the dichroic beam-splitter. Light of wavelength less than 400nm is reflected and passes through an additional bandpass filter[1] to improve out-of-band blocking. The filtered light then falls onto the photocathode of the FL1 detector. Light being transmitted by the dichroic beam-splitter also passes through additional filters to improve out-of-band blocking, before falling onto the FL2 detector. Thus, simultaneous electrical pulses are recorded by the FL1 and FL2 detectors, proportional to the magnitude of fluorescence in the 310-400nm and 420-650nm bands, respectively. (See oscilloscope plot in Figure 3).

The FL1 detector channel thus records the intensity of fluorescence corresponding to the peak emission band of tryptophan, and the FL2 detector channel corresponding to the emission band of the biofluorophore NADH.

Approximately 10µs after the firing of the Xe1 xenon, the particle will have moved down further to position 3 in Fig.2. The Xe2 xenon flashlamp is then fired, again illuminating the particle with an intense UV pulse. The Xe2 flashlamp is optically filtered to emit a UV band that corresponds to the peak excitation wavelength of the biofluorophore NADH. Again, the fluorescence from the particle is collected by the chamber mirrors before being split by the dichroic beam-splitter to the FL1 and FL2 detection bands, resulting in further electrical pulses on the FL1[2] and FL2 channels, respectively (see Figure 3).

[1] Further details of the optical filters used in WIBS4 are given in Appendix E.

[2] Note the saturation of the FL1_370 pulse. This is because the Xe2 UV pulse (370nm) lies in the detection band of FL1 (310-400nm) and therefore the detector receives not only fluorescence light from the particle, but also elastically scattered UV at 370nm. The elastically scattered light is typically 3 orders of magnitude larger than the fluorescence and therefore temporarily saturates the detector. The FL1_370 measurement is therefore ignored.

The magnitudes of the scatter pulse and the three useable fluorescence pulses are measured and stored by the WIBS-4A electronics, along with other useful parameters. The whole measurement cycle for the particle takes approximately 25µs, during which time the particle moves approximately 300µm downwards.

After particle measurement, the xenons are recharged for a period of ~5ms, during which time any particles passing through the scattering volume will be counted but no fluorescence measurements will be recorded. The xenons are capable of firing at a maximum rate of approximately 125/s. Thus, all particles may be measured for particle concentrations up to ~2x104 particles/liter.

3.2 Particle Detection Thresholds T1 and T2

The WIBS-4A has two user-selectable particle detection Thresholds, T1 and T2. T1 is always less than or equal to T2. The threshold settings can be accessed from the WIBS software (see Figure 4).

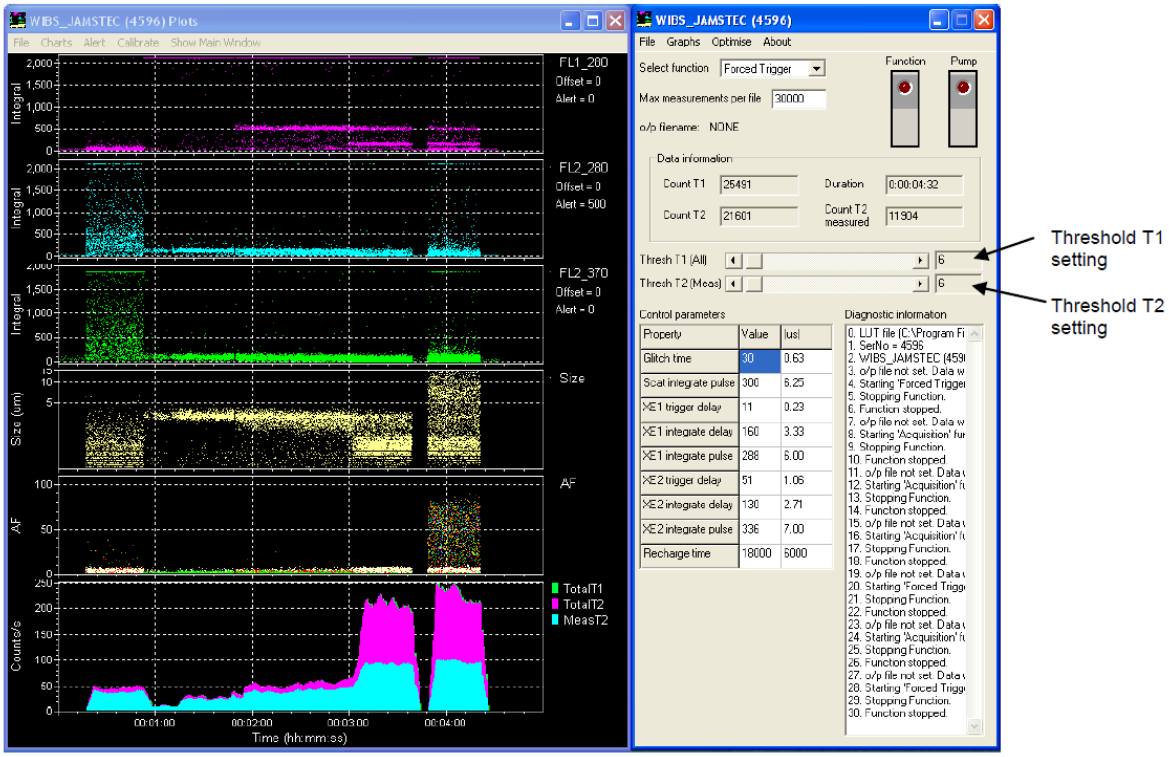


Figure 4: Screen Display Showing Location of T1 and T2 Threshold Settings

Each Threshold can be set by the user via the laptop screen display and can have a value up to 256.

3.2.1 T1 Threshold

The T1 threshold is the Particle Detect threshold. When the particle passes through the laser beam and scatters light to the FL2 channel detector, the signal voltage pulse produced by the detector is compared to the T1 threshold and if greater than T1, the particle will be counted. This occurs for particles greater than about 0.5µm in diameter.

The normal minimum setting for the T1 threshold is just above the level at which electrical noise can cross the threshold and trigger the instrument. Normally this occurs for a T1 value of about 6. The method for testing this is to gradually reduce the T1 level with the WIBS-4A pump OFF. No particles should be registered as counted, but eventually the threshold will drop into electrical noise and "false" particles will be counted. The T1 threshold should then be raised 2 steps above this noise floor. In very electrically noisy environments, such as those with heavy machinery, T1 may need to be raised higher to avoid triggering on noise.

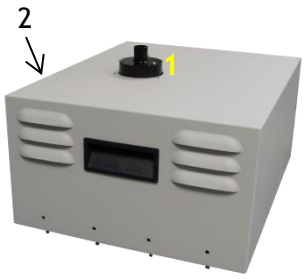
3.2.2 T2 Threshold

The WIBS-4A also has a second trigger, **Threshold T2.** This can be set equal to or higher than T1, and a full particle measurement will only occur if T2 is crossed (as well as T1). For example, if T1 = 6 and T2 = 15, then all of the small particles will be counted but only the larger particles will be measured in full.

The T2 threshold was implemented to allow users to focus on particles in larger size ranges. Earlier versions of the instrument did not have a T2 threshold. In these instruments, as soon as the T1 threshold was crossed and a particle detected, the full measurement cycle would take place at up to the maximum of ~125 times per second. In many applications this would be perfectly acceptable. However, if the user was interested in, say, particles of 5 -10 µm in diameter within an ambient aerosol that contained a far higher population of 1-2 µm particles, then statistically far more of the (unwanted) smaller particles would be measured.

3.3 Components

The main components of the WIBS-4A are shown below.



1	Aerosol inlet
2	USB and power connectors, air sample outflow, LED indicators
3	Oversize particle trap
4	Pump
5	Main optical chamber
6	Xenon sources
7	Fluorescence detection channels
8	Quadrant PMT, particle “shape” detector
9	Power distribution Board
10	Analog acquisition and digitization board

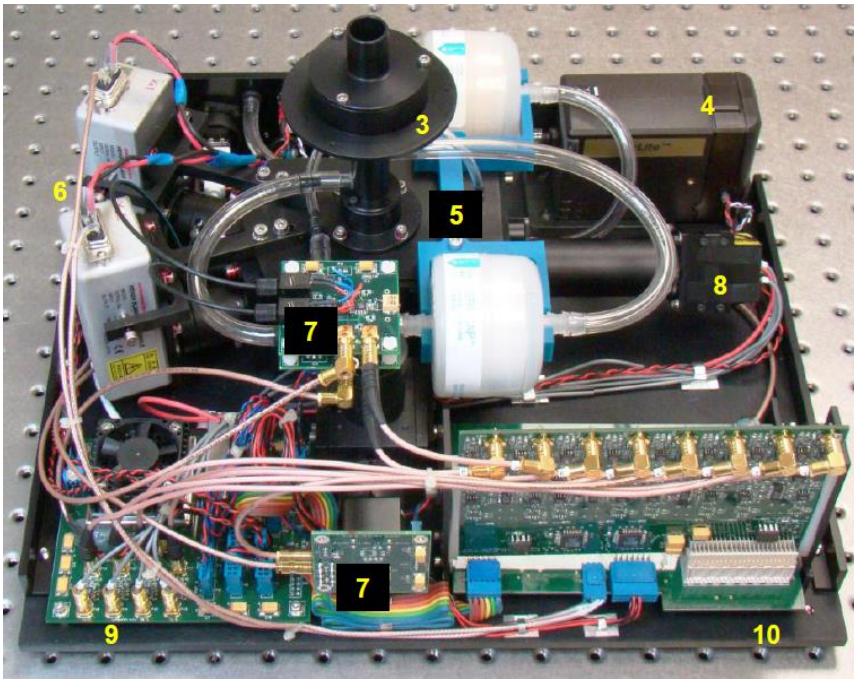


Figure 5: WIBS-4A Components

3.4 Aerosol Sampling System

3.4.1 System Diagram

Figure 6 shows a section view of the aerosol flow though the WIBS-4A.

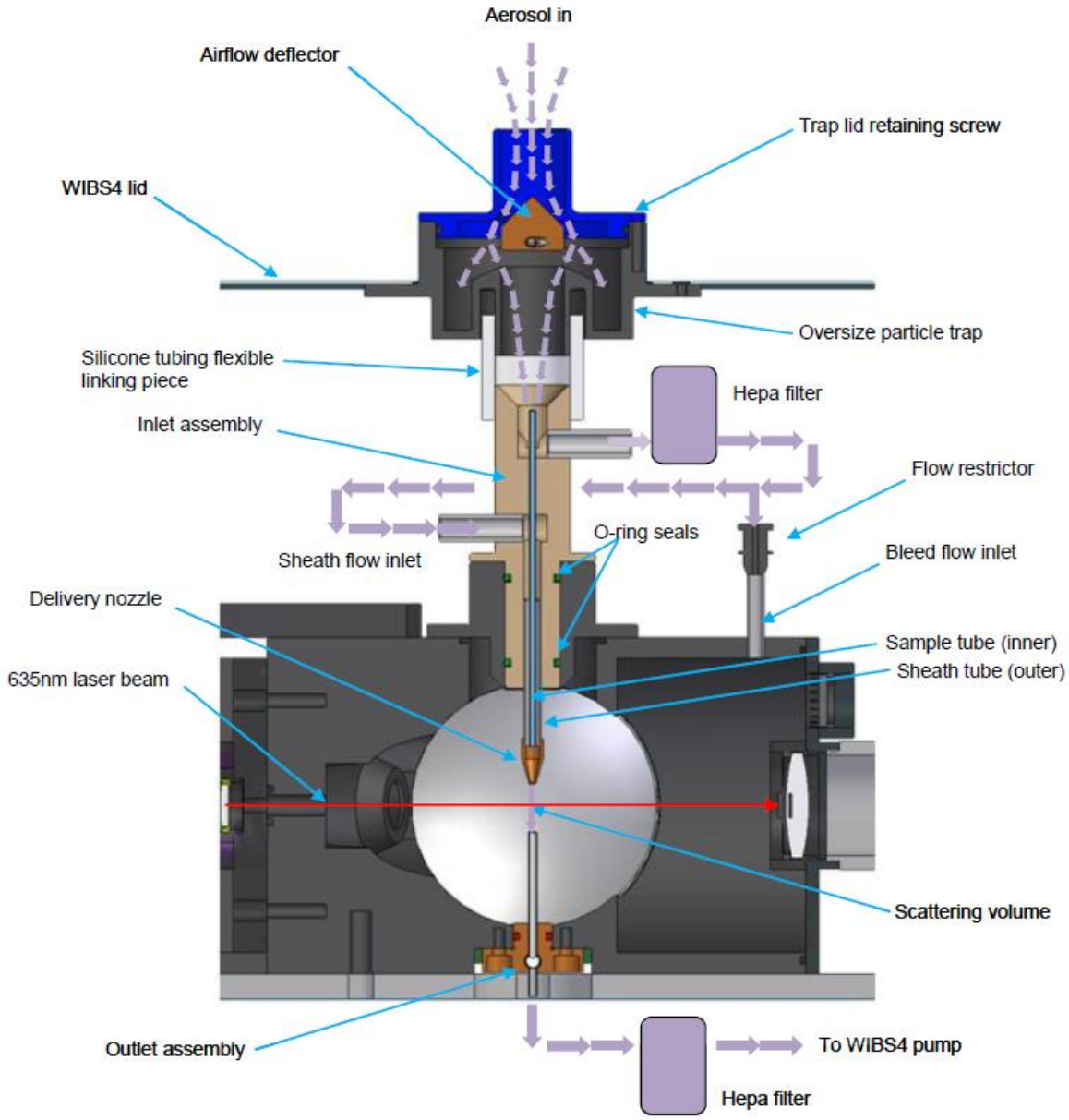


Figure 6: WIBS-4A Aerosol Flow

3.4.2 Aerosol Flow

Once the aerosol has entered the Inlet assembly, the majority of the flow (approx. 90%) is directed through a HEPA filter and then returned as both a sheath flow (to surround the sample flow with particle-free air) and a small bleed flow of clean air into the chamber.

The sheath flow has the effect of constraining the sample flow as the two flows pass through the tapered delivery nozzle, as illustrated in Figure 7 below.

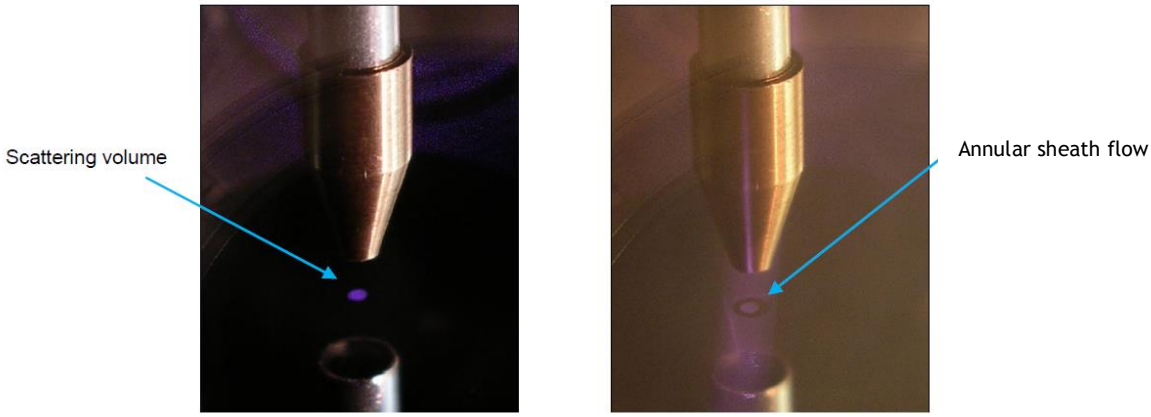


Figure 7: Smoke Visualization of Scattering Volume and its Surrounding Clean Sheath Flow

In WIBS-4A, the set total aerosol flow rate is approximately 2.5 liters/min, and the sample flow rate ~300 ml/min. (see Appendix D for exact delivery settings). The scattering volume, defined by the intersection between the laser beam and the sample airflow column, is approximately 0.7mm diameter and 130µm deep. The flow velocity at this point is typically ~12 m/s.

The full aerosol flow (sample + sheath + bleed) is drawn out of the chamber by the WIBS-4A sampling pump via a HEPA filter. The outflow of the pump is delivered to a vent port adjacent to the LED status lights on the WIBS-4A connector panel.

3.4.3 Oversize Particle Trap (OPT) and OPT Removal

At the top of the WIBS-4A chamber the aerosol is first drawn through an Oversize Particle Trap (OPT). The OPT device serves to remove very large particles within the airflow and therefore prevent possible blockage of the delivery nozzle, which has a 1.2mm exit diameter. The OPT contains a simple airflow deflector around which the airflow is forced.

If the WIBS-4A is being operated in an environment where extremely large particles (such as organic fibers of mm length) are unlikely, then the airflow deflector is not necessary and should be removed. This is done by unscrewing the three retaining screws holding the OPT lid, as in Figure 8 below, then removing the deflector and replacing the lid.

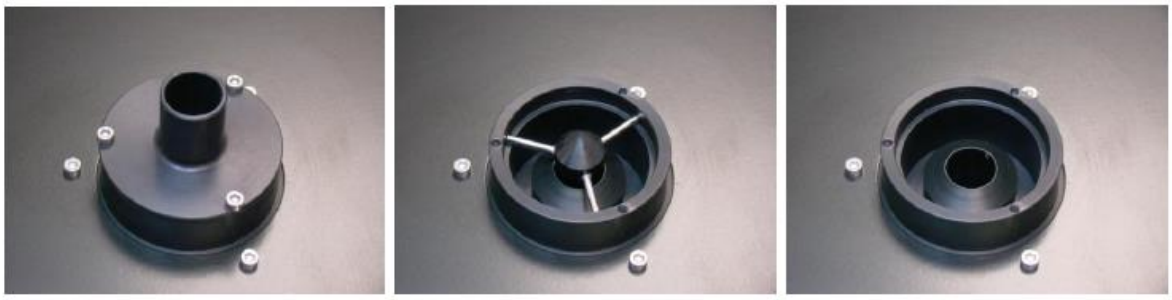


Figure 8: Oversize Particle Trap (left) and Removal of OPT

The OPT is connected to the main WIBS-4A inlet assembly by a short length of flexible silicone tubing. See Figure 6. This is to prevent damage to the WIBS-4A chamber should the OPT receive a severe shock.

4.0 Quick-Start Navigation

To run the program, first make sure the WIBS instrument is switched on and its USB link connected. Then click on the WIBS shortcut that should be present in the **Start Menu > All Programs** list.

When the program is started, a window will briefly appear showing progress while the WIBS firmware is being programmed. This only happens once each time the instrument power is switched on or the USB cable is connected. If either the power is cut or the USB cable is disconnected, the software must be restarted in order to reprogram the firmware. Do not disconnect the USB cable or switch of the power to the instrument without first closing the program.

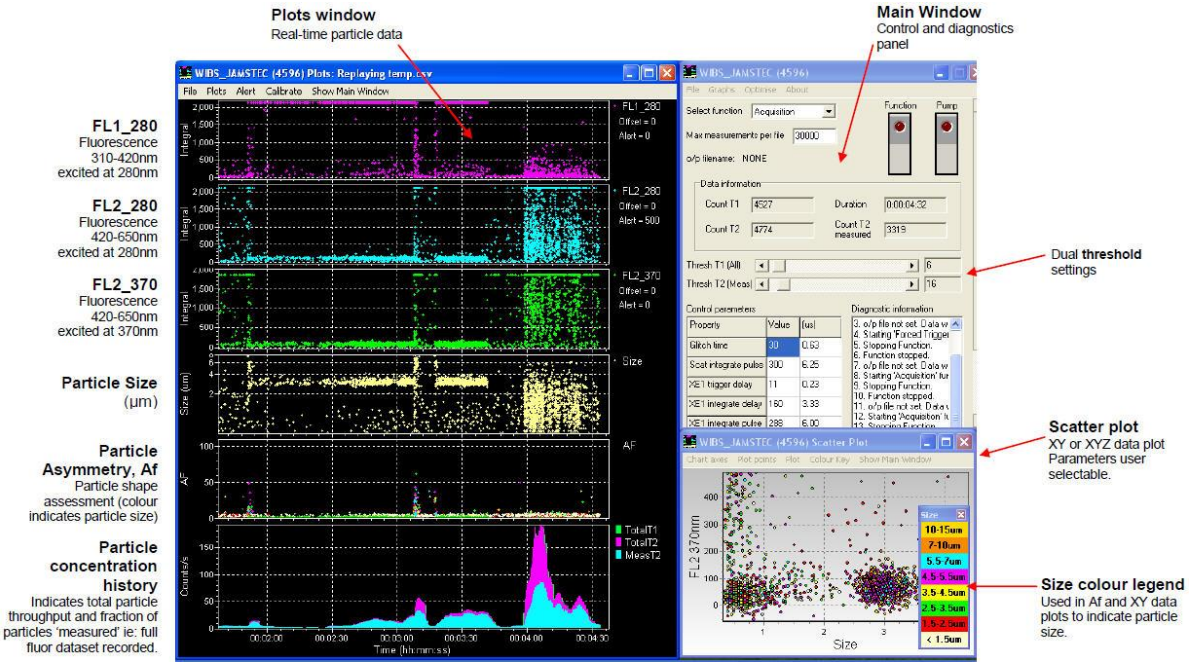


Figure 9: WIBS-4A User Interface

Once the program is running, do the following:

1. Set a data output file. See **Main Window > File menu > Set o/p file**.
2. Select the operation mode, "Forced Trigger" or "Acquisition," using the **Select Function** drop-down list. "Acquisition" mode is the default. See the following section for additional information.
3. Switch the pump on by clicking on the upper area of the **Pump** switch.
4. Start the Forced Trigger / Acquisition function by clicking on the upper area of the **Function** switch.

To stop data acquisition, click on the lower area of the **Function** switch. Stop the pump by clicking on the lower area of the **Pump** switch.

For more details on the user interface, see the following section.

5.0 WIBS Software

The WIBS software consists of several windows:

- The main window (upper right)
- The plots window (left)
- The scatter plots window (lower right)
- The frequency plot (see Figure 13)

5.1 Main Window

Figure 10 shows the WIBS-4A main window.

5.1.1 User Controls and Displays

Select Function is a control that determines the WIBS mode, as follows:

"Acquisition" is the normal mode of the instrument. In this mode, when the pump is on, particle size, shape, and fluorescence data are recorded.

In "Force Trigger" mode, the two Xenons are triggered periodically at approximately 1 second intervals. This allows background data to be recorded for the three fluorescence channels in the absence of a particle. The background is due to second-order effects such as filter fluorescence, filter breakthrough, and molecular fluorescence in the scattering volume.

The **Function** switch starts and stops the selected Function (Acquisition or Forced Trigger).

The **Pump** switch allows the user to turn the WIBS-4A internal-air sampling pump on or off.

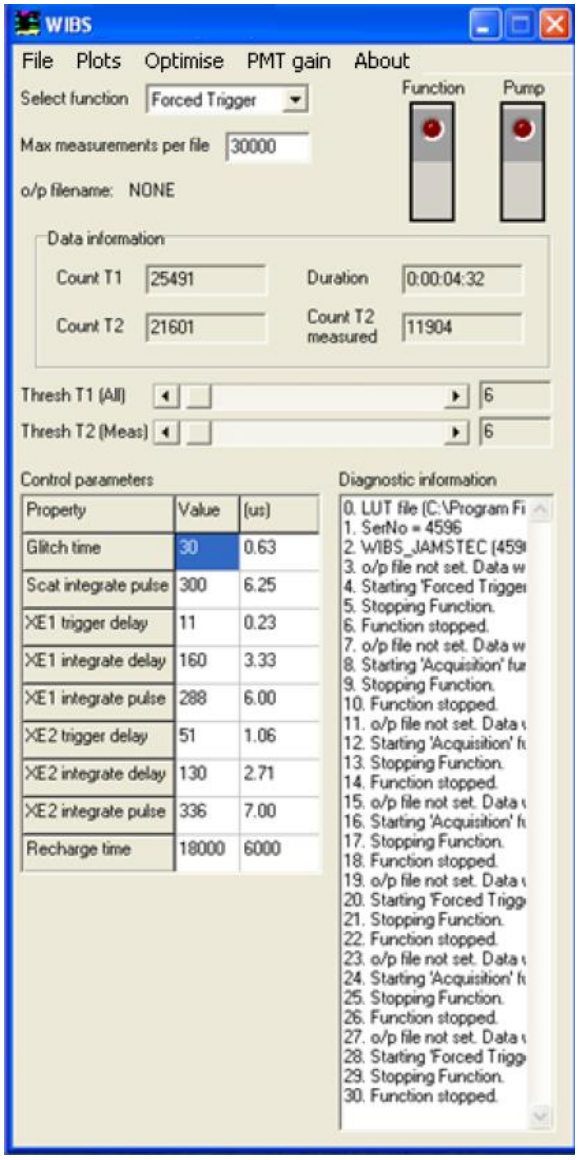


Figure 10: WIBS-4A Main Window

Max Measurements per file sets the maximum number of particle measurements a single output file can contain. A new output file with an incremented index is automatically generated when the current output file is full. (This feature helps minimize data loss in events such as a power failure.)

O/p filename displays name of user-specified output file.

Data Information

Count T1 displays a running total of all Threshold 1 particle counts recorded in the output data file (see element TPCT1 in section on data output file. Total Particle Count T1 (TPCT1) is the total number of particles producing a signal above Threshold 1 that may pass through the scattering volume each time the xenons are recharging (approximately 5 ms in duration). The number includes Count T2 below.

Count T2 displays a running total of all Threshold 2 particle counts recorded in the output data file. The Total Particle Count T2, TPCT2, is the total number of particles producing a signal above Threshold 2 that may pass through the scattering volume each time the xenons are recharging (approximately 5ms in duration). The number includes the count of measured particles.

Count T2 measured displays a running total of all measured particles (i.e., particles with the full data set of size, shape, and fluorescence).

Duration displays the time in d:hh:mm:ss format since the **Function** switch has been started.

Thresh T1 is a slider control that adjusts the particle *detection* and *counting* trigger threshold of the instrument. In acquisition mode, this should be reduced until instrument starts triggering on noise, then raised 2 steps above the noise level. Normally the setting is 6, corresponding to a particle of ~0.5µm in size.[3] All particles crossing the T1 threshold will be counted.

Thresh T2 is a slider control that adjusts the particle *measurement* trigger threshold of the instrument. This threshold can only be set greater than or equal to Thresh T1. It can be raised above Thresh T1 if desired to allow the instrument to measure (i.e. full size, shape, and fluorescence data) only larger particles, while still counting the smaller particles that produce signals above Thresh T1. The T2 threshold can be raised to a maximum rate of ~125 particles/second.

Control Parameters

These parameters control the timing of the sequence of events that occur each time a particle triggers the instrument (i.e., xenon flash timing). It is possible to alter these by clicking on the appropriate parameter in the **Value** column and entering a number. The number corresponds to a number of clock counts. It is translated into microseconds in the adjacent column in the Control Parameters box. If a change is made, the acquisition / forced trigger session must be stopped and restarted to allow the change to be applied to the instrument. The users should not change these settings without consulting DMT staff.

[3] If the setting is reduced, the system will begin to trigger on noise pulses. In an environment of high electrical noise, the threshold may need to be raised to a point where, with the Pump off, no triggering of the xenons occurs.

Diagnostic information

This displays information about the current status of the instrument.

5.1.2 Menus

5.1.2.1 File Menu

File > Set o/p file sets file to record data to. An index number will be appended to this file. Every time a function is started, a new file will be created with an incremented index. A new incremented file will also be created when the measured data count reaches the number shown in the 'Max Measurements per file' text box (30,000), or when approx. 3 hours has passed since the last file was created.

File > Save settings on exit
If this menu item is checked, most of the software settings including instrument control parameters and software windows appearance will be saved when the program is closed.

File > Exit closes the program.

5.1.2.2 Plots Menu

Displays the main plots window.

5.1.2.3 Optimize Menu

This is a diagnostic menu that the development staff use for internal instrument settings. It is not normally available to end-users, and may not be visible in some software versions.

5.1.2.4 PMT Gain Menu

This function is disabled on the DMT WIBS-4A.

5.2 Plots Window

The Plots Window (Figure 11) can display real-time rolling plots of all the data available from particle measurements.

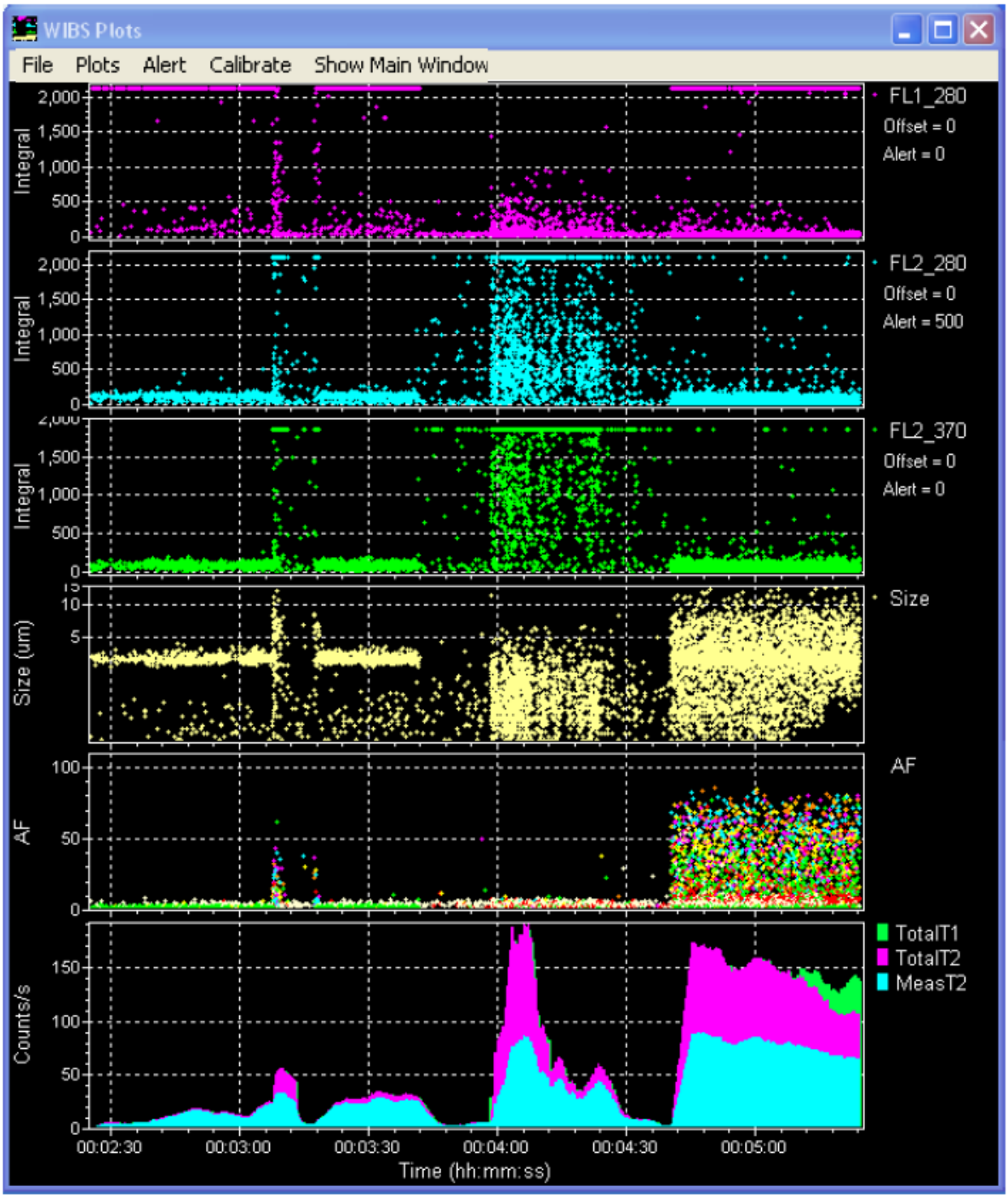


Figure 11: WIBS-4A Plots Window

The data types to be displayed are selected from the Plots menu. There are two general classes of Plots:

- *Particle Data Plots:* Normal plots used to display particle measurement data (fluorescence, size and shape)

- *Diagnostic Plots:* Secondary instrument data, useful for diagnosing instrument performance

5.2.1 Particle Data Plots

Particle Data plots are shown in Figure 11. There are six data plots:

- *FL1_280*: 310-400nm fluorescence detected by FL1 detector with Xe1 280nm excitation
- *FL2_280:* 420-650nm fluorescence detected by FL2 detector with Xe1 280nm excitation
- *FL2_370:* 420-650nm fluorescence detected by FL2 detector with Xe2 370nm excitation
- *Size:* Particle size in µm based on FL2 scattered light magnitude.
- *AF:* Asymmetry Factor; a measure of particle asphericity based on the azimuthal intensity variation of scattered light intensity detected by the four elements of the Quadrant detector
- *Particle rate in counts/sec*: rate plots of **Count T2 measured**, **Count T2** and **Count T1**, described above. The plotted rates are averaged over the preceding ~5s period.

5.2.2 Diagnostic Plots

The WIBS-4A can display the following diagnostic plots:

- *Pwr_280:* Magnitude of 280nm flash, a measure of xenon output stability.
- *Pwr_370:* Magnitude of 370nm flash, a measure xenon output stability.
- *FL2 SctPk:* Scattered 635nm laser light detected on FL2 detector and measured by peak detection. (Parameter used in determination of particle size).
- *FL2 SctInt:* Scattered 635nm laser light detected on FL2 detector and measured by integration. (Parameter not currently used).
- *TOF:* Time-of-flight of particle through 635nm laser beam. There is a minimum allowable TOF, below which WIBS-4A electronics ignores signals assuming them to be due to noise. There is a maximum limit of TOF indicated by a yellow dotted line in the plot. This limit is the time in the measurement cycle at which the fluorescence measurement begins. Scattered 635nm light at this time will affect fluorescence measurements. Therefore measurements which carry the maximum TOF values should be treated with caution. Such measurements are tagged in the data output file. See element FT in section on data output file. TOF values longer than the maximum limit are recorded as the limit value.
- *TPC (Total Particle Counts):* See elements TPCT1 and TPCT2 in section on data output file.

5.2.3 Menus

The **Plots** window offers the user the following

5.2.3.1 File Menu

File > Save: Save data currently recorded in plots.

File > Save Visible: Similar to "Save," but only saves data within the time range actually visible on the plots.

File > Load: Load previously saved data.

File > Replay: Play back previously saved data. Data will appear progressively in plots as it did when originally recorded. A replay can be paused or aborted using appropriate options in the File menu.

File > Clear on load: If this is checked, any existing data in the plots will be cleared before new data is loaded.

File > Recalc AF and Size on load: The Size, AF and Particle rate plots are calculated from the data in other plots. If this menu item is checked, these plots are re-calculated rather than taken from the file being loaded.

File > Trim lead time: Any newly loaded or newly acquired data is plotted after the latest plot time on the plots rather than starting from 0. If this menu item is checked, any lead time in a file to be loaded (i.e. time before the first particle measurement in the file) is trimmed avoiding potentially long gaps in the displayed data.

5.2.3.2 Plots Menu

Plots > Clear: Clear data from all plots.

Plots > Export: Save plot images as windows meta files.

Plots > Invert colors: Alter the plot colors such that the background is white rather than black. (Useful for printing.)

Plots > Show FT points: If this is checked, Forced Trigger data will be shown on the plots along with the particle data.

Plots > Point size: Increase or decrease the size of plotted points.

Plots > Auto set offsets: Automatically adjust offsets of fluorescence plots. This function is not normally used, but can be used while sampling non-fluorescing particles to offset the background level to 0. The offset for each plot is displayed to the right of the plot. Offsets can be adjusted manually by clicking the offset indicator and entering a new value in the window that appears.

Plots > Scatter plot: Display the scatter plot.

Plots > Frequency plot: Display the frequency plot.

Plots > Pwr_280 (and all other colored menu items): Show or hide the relevant plot. Visible plots can also be hidden by right-clicking in the plot area of the plot.

5.2.3.3 Alert Menu

Enable or disable alert mode. If this is enabled, a visible and audible warning will occur if one or more of the five channels: FL2 SctPk, FL2 SctInt, FL1_280, FL2_280 or FL2_370 goes above the alert threshold set for that channel. Alert thresholds can be set by clicking on the alert level indicator adjacent to the plot and entering a new level in the window that appears.

5.2.3.4 Calibrate

Not normally used, but can be used with calibration particles (PSL spheres or similar) to calibrate the particle Size and AF calculations made by the software.

To calibrate Asymmetry Factor calculations

Record data for some preferably monodisperse PSL spheres of size typically ~ 3µm. About 500-1000 measurements will suffice for the calibration. In the Plots window, make sure the Scat_EL plot is displayed and adjust the time axis and scroll the plot so that all the recorded measurements are visible. Then click on the **Calibrate** menu and select the AF option. Appropriate normalization factors will be calculated for Scat_EL channels 2, 3 and 4. These factors will be displayed in the Diagnostic info. textbox in the main Control Window. The new normalization factors will not come into effect until the next time an Acquisition function is started. To save the new factors for future use, click on the **Save settings on exit** option in the **File** menu of the main control window before closing the program.

5.2.3.5 Show Main Window

Brings the main program window to the foreground if it is hidden.

5.3 Scatter Plot

The Scatter Plot Window () displays a scatter plot of particle data. The X and Y (and Z if 3D option selected) axis parameters are user-selectable by right-clicking on any area in the plot to display a selection menu of available parameters for each axis of the scatter plot.

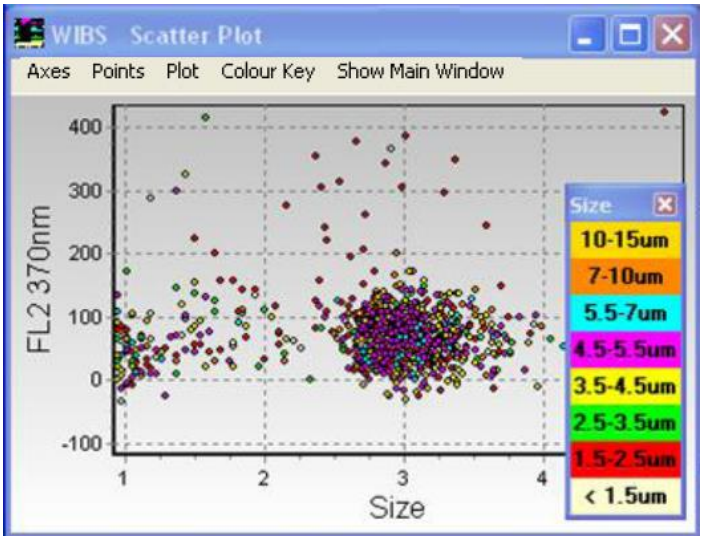


Figure 12: WIBS-4A Scatter Plot

5.3.1 Menus

5.3.1.1 Axes Menu

Use this menu to select 2D or 3D plot display.

5.3.1.2 Points Menu

Points menu > Point size increases or decreases the size of plotted points.

Points menu > Point colors allows the user to select the desired color for plotted points.

Only points plotted after the selection is made are affected. The **Color by Size** option will color data points according to the legend used with the AF Plot in the Main Plots window. The **Color by AF** option will color data points according to the calculated particle AF. A legend for the colors is shown when clicking on the **Color Key** menu. The **Color Timer Interval** option allows the plot point colors to change periodically so that new points can be distinguished from older ones.

Points menu > Max no. of points sets the maximum number of point the plot can display. If too many points are visible, the plot can slow the running of the program. A limit is therefore necessary on the amount of data that can be displayed.

Plot menu > Copy to clipboard copies the displayed plot to the Windows clipboard.

Plot menu > Clear clears all data in the plot.

Plot menu > Change colors (3D mode only) opens a window allowing the display colors of the 3D plot to be altered.

Plot menu > Toggle projection (3D mode only) switches the 3D plot between orthographic mode and 3D perspective mode.

5.3.1.3 Color Key Menu

This item shows the color key. The color key will automatically display color-coding for Size or AF depending on what is being displayed in the scatter plot.

5.3.1.4 Show Main Window Menu

This brings the main program window to the foreground if it is hidden.

5.3.2 Other functions, 2D and 3D modes

Right click on any area in the plot to display a menu allowing selection of desired parameter for each axis of the scatter plot.

When double-clicking in the plot's plotting area, a pop-up menu appears indicating whether or not a filter of FL1/FL2 is being applied. If the FL1 280nm/FL2 280nm axis is being displayed, the FL1 280nm / FL2 280nm values can vary significantly because of noise when one of both of the data values is small. The filter removes the data where noise on either channel would have affected the FL1 280nm / FL2 280nm value significantly. Other plots are not affected.

5.3.2.1 2D Mode Only

To zoom in on an area in the plot, use the mouse (left button) to draw a box from top left to bottom right around the area of interest. To un-zoom, draw a box from bottom right to top left anywhere in the plot area of the plot.
To pan around the plot, hold the right button of the mouse down while over the plot area of the plot and drag the plot area up, down, left or right. When clicking on the X or Y axis a menu appears allowing the axis setting to be toggled between normal and logarithmic.

5.3.2.2 3D Mode Only

By dragging the mouse across the 3D plot while holding down the left mouse button the plot can be rotated to any desired viewing angle.

5.4 Frequency Plot

This plot (Figure 13) displays histogram plots for most of the data parameters.

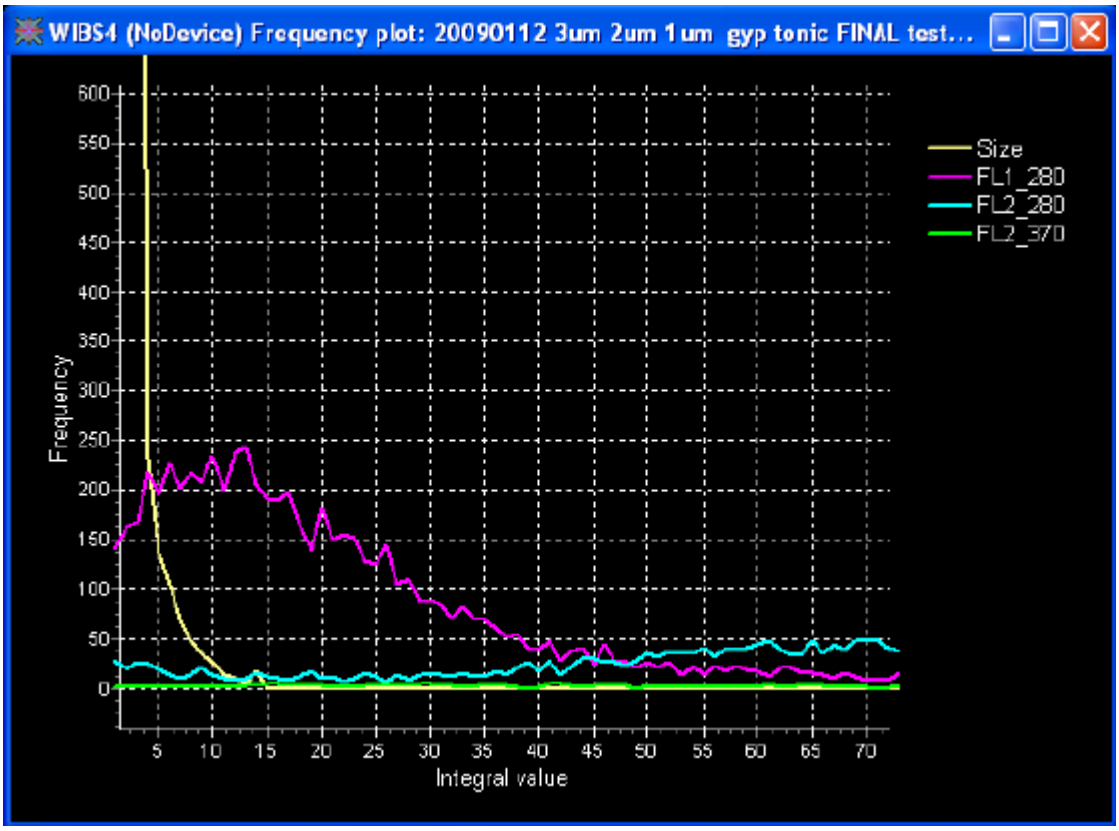


Figure 13: WIBS-4A Frequency Plot

The different parameters can be shown or hidden by typing the appropriate number or letter indicated by the text box at the bottom right of the plot. This text box can be hidden from view by clicking on it. It can be made to reappear by clicking on the plot legend.

5.4.1 Y axis

The Y axis is set to automatically scale to suit the data presented and allows no adjustment.

5.4.2 X axis

Min and max values can be adjusted by clicking on the axis and typing in new max and min values in the window that appears. A log axis option can also be selected in this window.

The X axis bin size can be altered by right-clicking on the plot and selecting the appropriate pop-up menu that appears. The plot can also be cleared of data using this pop-up menu.

6.0 Maintenance

DANGER

The laser used in the WIBS is a CLASS 1 Laser

The standard laser operates at 635 nm and 12 mW. Do not open the WIBS cell unless the laser is turned off.

Caution

The use of controls, adjustments, or procedures other than those specified in this document may result in hazardous radiation exposure. In particular, reflections caused by placing an object in the laser beam path can cause skin burns and/or blindness.

6.1 Zero-Count Check

A zero-count check should be conducted weekly or if issues are suspected. To conduct a zero check, place the zero-count filter and inlet adapter on the inlet. Run the instrument in Acquisition mode with the pump on for a minimum of five minutes. Particle counts should be less than 1 per liter after five minutes of operation with a filter in place. Remove the filter and adapter to resume normal operation.

Users should also check the flow on a monthly basis. To check the flow, place a flow meter on the inlet. Run the WIBS in Acquisition mode with the pump on. The flow reading should be approximately 2.5 l/min.

6.2 Cleaning the Inlet Assembly

In the unlikely event that the aerosol inlet becomes blocked, it may be carefully removed for cleaning as described below.

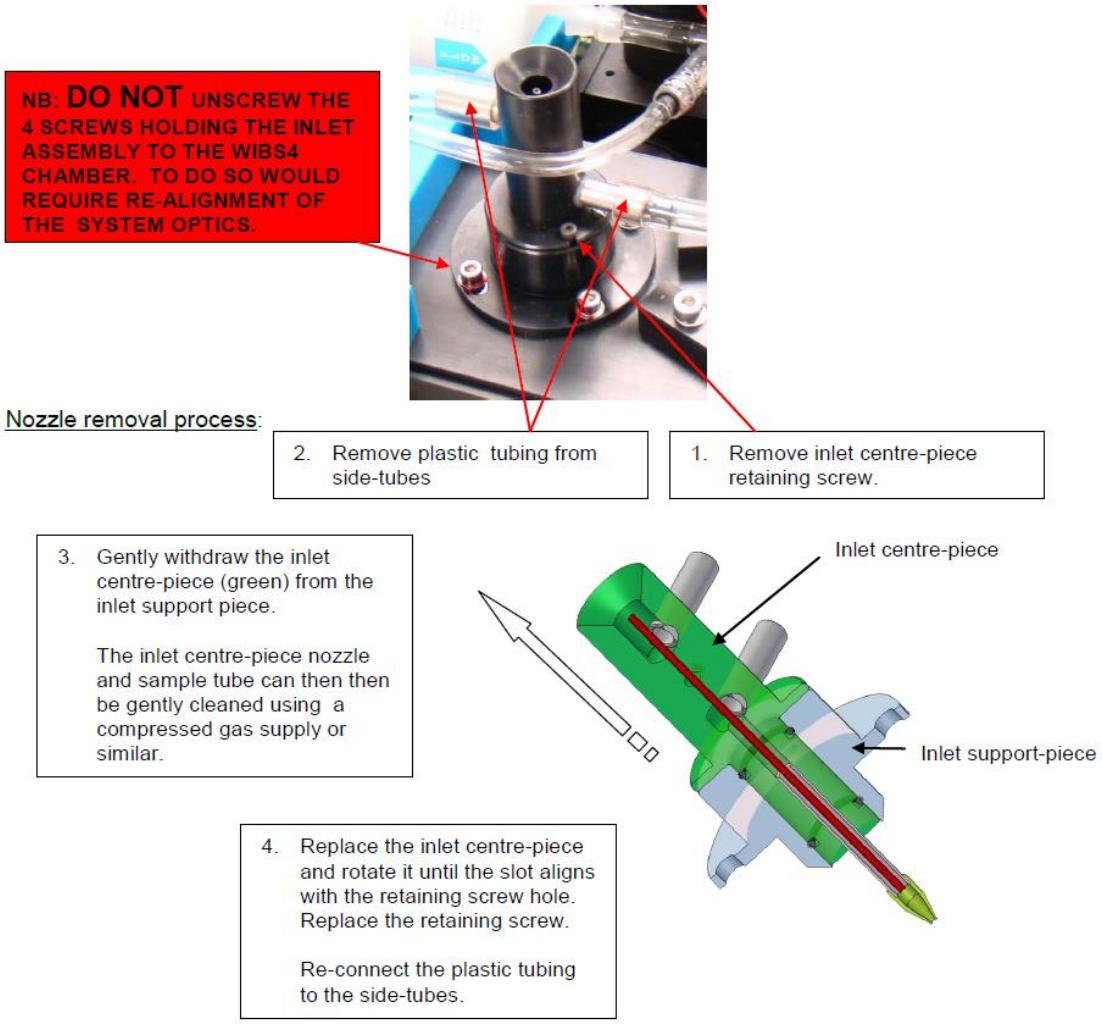


Figure 14: Cleaning the Inlet Assembly

Appendix A: Specifications

General Specifications

Measured Parameters	• Single-particle light scattering • Single-particle fluorescence (three separate measurements) • Particle Size • Particle Asymmetry Factor (AF)
Derived Parameters	Particle Concentration
Particle Size Range	0.5 to 15 µm
Maximum Concentration	~2 x 104 particles/L for full measurement (maximum is higher for particle counts only)
Fluorescence Excitation	Dual wavelength, 280 and 370 nm
Fluorescence Emission	Dual wavelength, 310-400 nm and 420 - 650 nm
Flow Rate	• Sample flow: 0.3 L/min • Sheath flow: 2.2 L/min
Rear Panel Features	• Power connection • ON/OFF LED • Pump status LED • Particle detection LED • Air sample outflow • USB connector • Laser 635 nm diode laser
Laser	635 nm diode laser, 12 mW
Pump	Diaphragm pump
Power Requirements	150 W, 90 - 230 VAC, 24 VDC

Physical Specifications

Weight	13.6 kg
Dimensions	11.9” W x 15.1” L x 6.75” H / 30.4 cm W x 38.2 cm L x 17.1 cm H Inlet adds an extra 1.5”/3.8 cm in height

Appendix B: Particle Size and Shape Determinations

Particle Size

Like most optical particle counters (OPCs), WIBS-4A uses a particle size calibration based on a theoretical curve that assumes the particles are spherical and of a specified refractive index (Mie theory). In the case of WIBS-4A, the calibration curve is based on aerosols of standard monodisperse polystyrene latex (PSL) microspheres. The refractive index of these spheres is quoted as 1.58 +/-0.2.

Since this calibration curve is based on PSL spheres, the reported size should be taken only as an estimate when measuring spherical particles of different refractive index (e.g., water droplets) or non-spherical solid particles.

The WIBS-4A Particle Size calibration curve for the normal setting (0.5µm – 15µm) is shown in Figure 15. The theoretical Mie curve (blue) is overlaid by a second-order polynomial (black line) that is used as the calibration function. The red squares show the WIBS-4A response to aerosols of PSL spheres of quoted sizes.

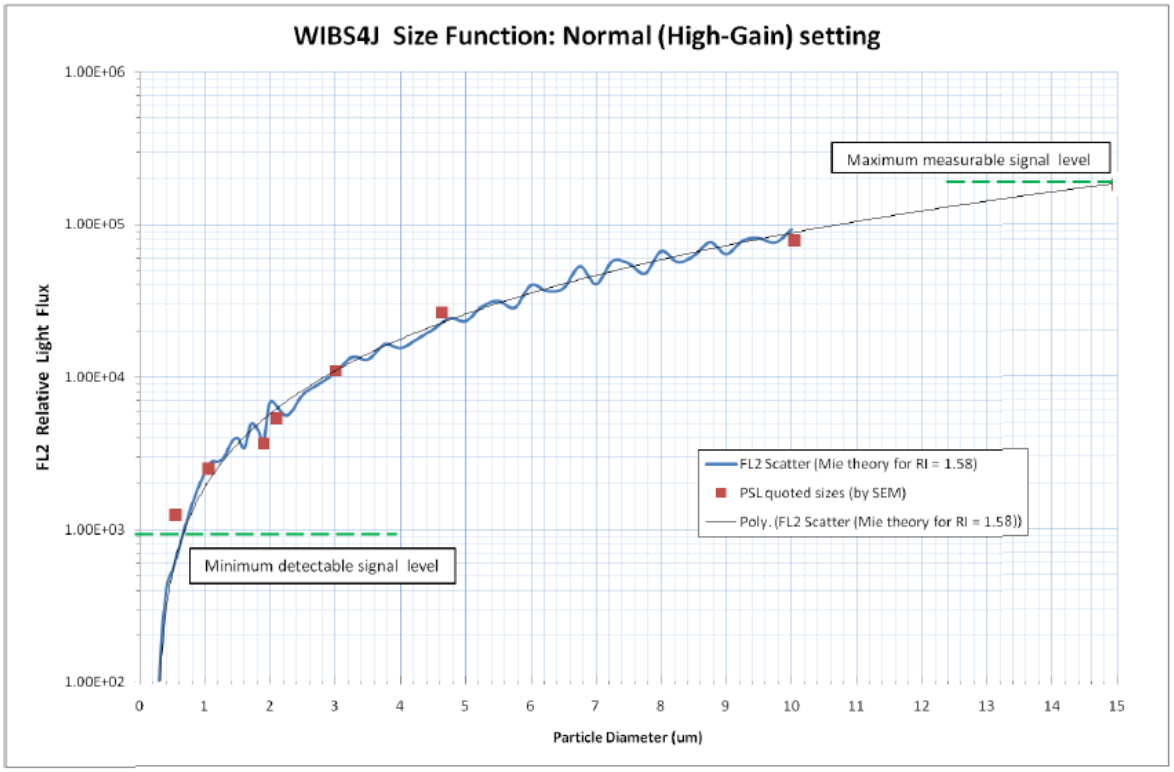


Figure 15: WIBS-4A Size Calibration Curve

Particle Shape

WIBS-4A incorporates an analysis of the forward scattered light captured by the Quadrant PMT to determine an index of particle shape, or more correctly, scattering asymmetry. This approach has been exploited by UH in a number of aerosol analysis instruments (e.g., references 8-12). The process used in WIBS-4A is illustrated in Fig. 9 below.

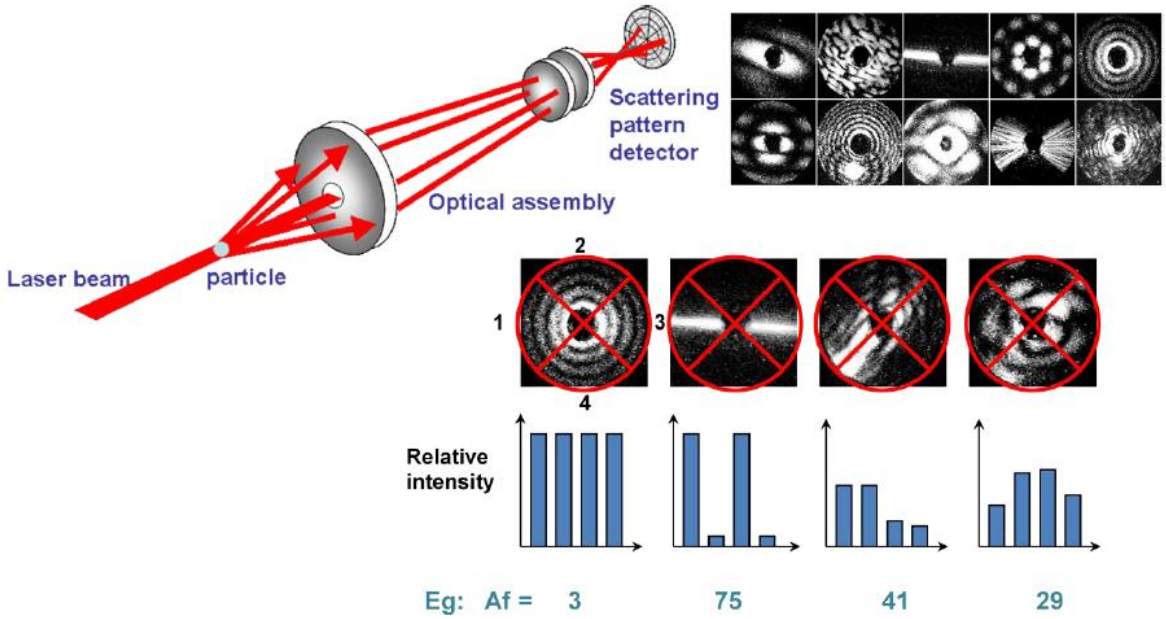


Figure 16: Schematic diagram showing derivation of a particle Asymmetry Factor, AF, by measurement of the azimuthal variation in forward scattering from the particle.

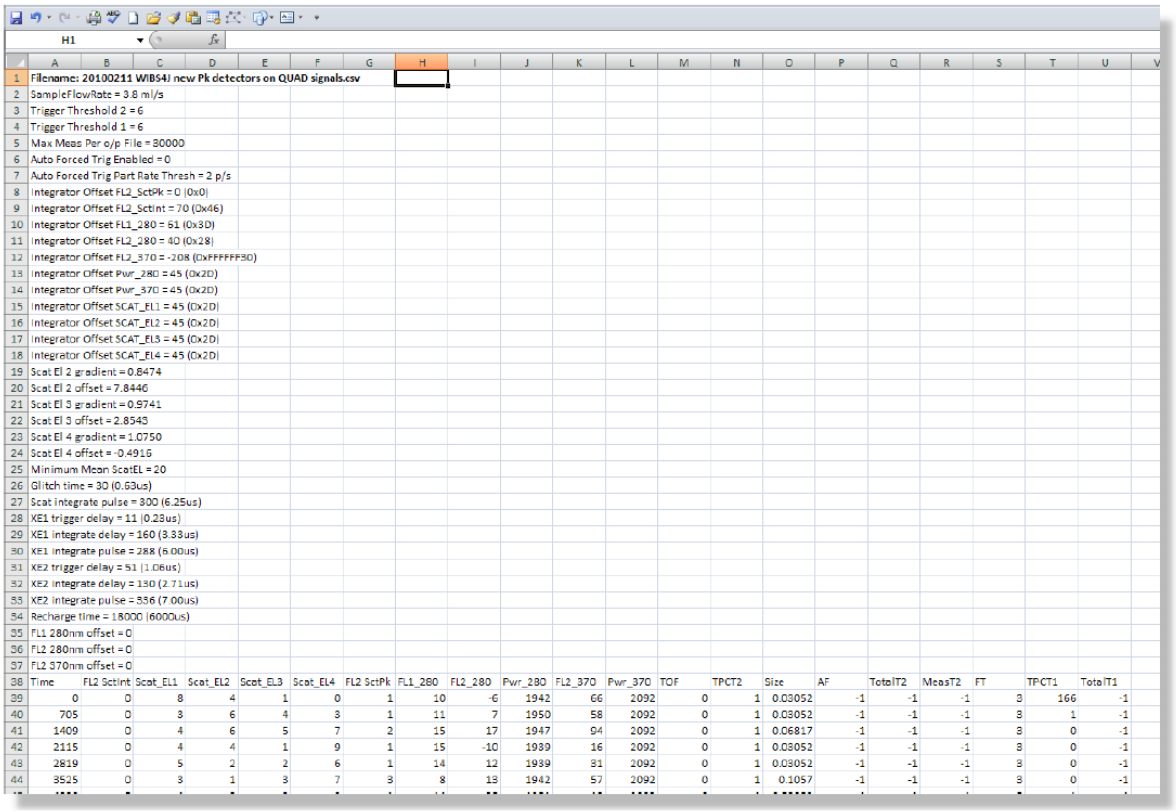
In Figure 16, examples of typical forward scattering patterns produced by particles of differing shape are shown in the top-right. These images were recorded using a high resolution intensified CCD camera. In WIBS-4A, a much simpler detector configuration is used, based on the quadrant PMT. This is for reasons of both cost and speed of response. The figure illustrates how the quadrant PMT would respond to particles of different shape – from left to right a droplet (spherical), fiber, flake, and irregular cubic particle. WIBS-4A records the scatter intensity values received by each quadrant and determines the root-mean-square variation around the mean value to yield an Asymmetry Factor, AF, such that a perfect sphere would correspond to AF = 0, and a high aspect ratio fiber to an AF approaching 100. In reality, electronic and optical noise results in spherical particles having measured AF values ~2 to 6, rather than zero. ***Note**: For particles less than ~1.5µm, the AF value is inevitably increased as the signal-to-noise in the quadrant PMT outputs becomes very low for such small particle sizes.*

All AF data are provided in real-time to the user, but the 4 quadrant values are stored independently for later analysis by another method if preferred.

Appendix C: Output Files

File Format

The data output file is a text file with comma (csv) or tab (txt) separated variables depending on the selection made when saving. (It can be easily be opened in Excel as shown in the example below). The file has a number of header lines containing instrument configuration details and software at the time the file was written. There is also a line showing the time (time set on host computer) at which the file was begun. The data from each measured particle occupies one row in the file as shown below. These are described more fully below.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	Filename: 20100211 WIBS4J new Pk detectors on QUAD signals.csv																				
2	SampleFlowRate = 3.8 ml/s																				
3	Trigger Threshold 2 = 6																				
4	Trigger Threshold 1 = 6																				
5	Max Meas Per o/p File = 30000																				
6	Auto Forced Trig Enabled = 0																				
7	Auto Forced Trig Part Rate Thresh = 2 p/s																				
8	Integrator Offset FL2_SctPk = 0 (0x0)																				
9	Integrator Offset FL2_SctInt = 70 (0x46)																				
10	Integrator Offset FL1_280 = 61 (0x3D)																				
11	Integrator Offset FL2_280 = 40 (0x28)																				
12	Integrator Offset FL2_370 = -208 (0xFFFFFF30)																				
13	Integrator Offset Pwr_280 = 45 (0x2D)																				
14	Integrator Offset Pwr_370 = 45 (0x2D)																				
15	Integrator Offset SCAT_EL1 = 45 (0x2D)																				
16	Integrator Offset SCAT_EL2 = 45 (0x2D)																				
17	Integrator Offset SCAT_EL3 = 45 (0x2D)																				
18	Integrator Offset SCAT_EL4 = 45 (0x2D)																				
19	Scat El 2 gradient = 0.8474																				
20	Scat El 2 offset = 7.8446																				
21	Scat El 3 gradient = 0.9741																				
22	Scat El 3 offset = 2.8543																				
23	Scat El 4 gradient = 1.0750																				
24	Scat El 4 offset = -0.4916																				
25	Minimum Mean ScatEL = 20																				
26	Glitch time = 30 (0.63us)																				
27	Scat integrate pulse = 300 (6.25us)																				
28	XE1 trigger delay = 11 (0.23us)																				
29	XE1 integrate delay = 160 (3.33us)																				
30	XE1 integrate pulse = 288 (6.00us)																				
31	XE2 trigger delay = 51 (1.06us)																				
32	XE2 integrate delay = 130 (2.71us)																				
33	XE2 integrate pulse = 336 (7.00us)																				
34	Recharge time = 18000 (6000us)																				
35	FL1 280nm offset = 0																				
36	FL2 280nm offset = 0																				
37	FL2 370nm offset = 0																				
38	Time	FL2 SctInt	Scat_EL1	Scat_EL2	Scat_EL3	Scat_EL4	FL2 SctPk	FL1_280	FL2_280	Pwr_280	FL2_370	Pwr_370	TOF	TPCT2	Size	AF	TotalT2	MeasT2	FT	TPCT1	TotalT1
39	0	0	8	4	1	0	1	10	-6	1942	66	2092	0	1	0.03052	-1	-1	-1	3	166	-1
40	705	0	3	6	4	3	1	11	7	1950	58	2092	0	1	0.03052	-1	-1	-1	3	1	-1
41	1409	0	4	6	5	7	2	15	17	1947	94	2092	0	1	0.06817	-1	-1	-1	3	0	-1
42	2115	0	4	4	1	9	1	15	-10	1939	16	2092	0	1	0.03052	-1	-1	-1	3	0	-1
43	2819	0	5	2	2	6	1	14	12	1939	81	2092	0	1	0.03052	-1	-1	-1	3	0	-1
44	3525	0	3	1	3	7	3	8	13	1942	57	2092	0	1	0.1057	-1	-1	-1	3	0	-1

Output Channels

In addition to the three fluorescence values FL1_280, FL2_280 and FL2_370 recorded from each particle, and the FL2 scatter signal used for particle size determination, the WIBS-4A also records the four outputs from the forward-scatter Quadrant PMT used to assess particle shape.

The WIBS-4A also records the relative power of each xenon flash from the Xe1 and Xe2 xenons. The manufacturer's quoted pulse-to-pulse power variability is only +/- 3%, but the xenon output will decrease slightly in power over time. The Xe1_Pwr and Xe2_Pwr measurements allow correction to be made for this in the measured particle fluorescence values. Note that the xenons have a quoted minimum life-span before the power drops to 50% of at least 10^9 pulses. This corresponds to approximately one year of continuous 24/7 operation at typical aerosol concentrations. Replacement xenon units can be fitted when necessary.

The particle data also includes the Particle time-of-flight (ToF) through the laser beam, a useful diagnostic to help eliminate particle coincidence events where a second particle may enter the laser beam before the first particle has left it. The particle Arrival Time at the scattering volume is also recorded to allow particle concentration assessment and the generation of the Particle Concentration History Plot in the display.

Table 1 below summarizes the data that are recorded for each particle measured. Definitions for all the output channels appear after this table.

Channel	***Description***	***Purpose***
Xe1_Pwr	Relative energy of Xe1 flash	Monitor Xe1 UV pulse energy
Xe2_Pwr	Relative energy of Xe2 flash	Monitor Xe2 UV pulse energy
Fwd Scatter	Four signals from Quadrant- PMT : • Scatter ch1 • Scatter ch2 • Scatter ch3 • Scatter ch4	Used to assess particle shape
FL2 scatter	Elastically scattered 635nm light onto FL2 channel	Used in particle size determination
FL2 scatter	Fluorescence (~310 - 400nm) with 280nm excitation	Intrinsic fluorescence
FL2_280	Fluorescence (~420 - 650nm) with 280nm excitation	
FL2_370	Fluorescence (~420 - 650nm) with 370nm excitation	
TOF	Particle time-of-flight through laser beam	Eliminating particle coincidence events
Time stamp	Arrival time of particle at scattering volume	Determining small-scale aerosol concentration variations

Table 1: Data Recorded for Each Measured Particle

Channel Definitions

Data are arbitrary units unless otherwise stated.

Time: Count in milliseconds since start time of file.

FL2 SctInt: Scattered 635nm laser light detected on FL2 detector and measured by integration.

Scat_EL1: Scattered 635nm laser light detected on quadrant 1 of forward scatter detector.

Scat_EL2: Scattered 635nm laser light detected on quadrant 2 of forward scatter detector.

Scat_EL3: Scattered 635nm laser light detected on quadrant 3 of forward scatter detector.

Scat_EL4: Scattered 635nm laser light detected on quadrant 4 of forward scatter detector.

FL2 SctPk: Scattered 635nm laser light detected on FL2 detector and measured by peak detection.

FL1_280: Fluorescence detected by FL1 detector after particle irradiation by 280nm light.

FL2_280: Fluorescence detected by FL2 detector after particle irradiation by 280nm light.

Pwr_280: Magnitude of 280nm flash.

FL2_370: Fluorescence detected by FL2 detector after particle irradiation by 370nm light.

Pwr_370: Magnitude of 370nm flash.

TOF: Time-of-flight of particle through 635nm laser beam. The WIBS electronics ignores events with TOF less than a preset value (typically 0.25µs) as these are deemed to be either noise spikes or very small particles < ~0.5µm in size. It also ignores events with TOF > ~10µs, as these elongated times of flight are principally due to particle coincidence events where a particle enters the beam before the preceding particle has left the beam.

Total TPCT2: The total number of particles producing a signal above Threshold 2 that may pass through the scattering volume each time the xenons are recharging (approx. 5ms in duration). The number includes the count of measured particles.

Size: An interpretation of particle size in µm based on the amount of scattered 635nm laser light detected by the FL2 detector according to the calibration function given in Appendix B.

AF Asymmetry Factor: A calculation of particle asphericity based on the balance of scattered 635nm laser light detected by the four elements of the Quadrant forward scatter detector.

TotalT2: Rolling average frequency of TPCT2.

MeasT2: Rolling average frequency (averaged over the preceding ~5s) of the number of particles measured (i.e., full dataset of fluorescence, size, and asymmetry is acquired from particle).

FT: The time of the measurement:

- Bit 0: Forced Trigger mode (1), Acquisition mode (0).
- Bit 1: FL2 PMT HIGH-GAIN (1), LOW-GAIN (0); note low-gain is disabled on the DMT WIBS-4A.
- Bit 2: FIFO full (1), not full (0).
- Bit 3: TOF upper limit exceeded (1), not exceeded (0)

TPCT1: The total number of particles producing a signal above Threshold 1 that may pass through the scattering volume since the time of previous particle 'measurement'. This time includes the time the xenons are recharging (approx. 5ms in duration). The number includes the count of particles, measured or not, that produce a signal above Threshold 2 in this time.

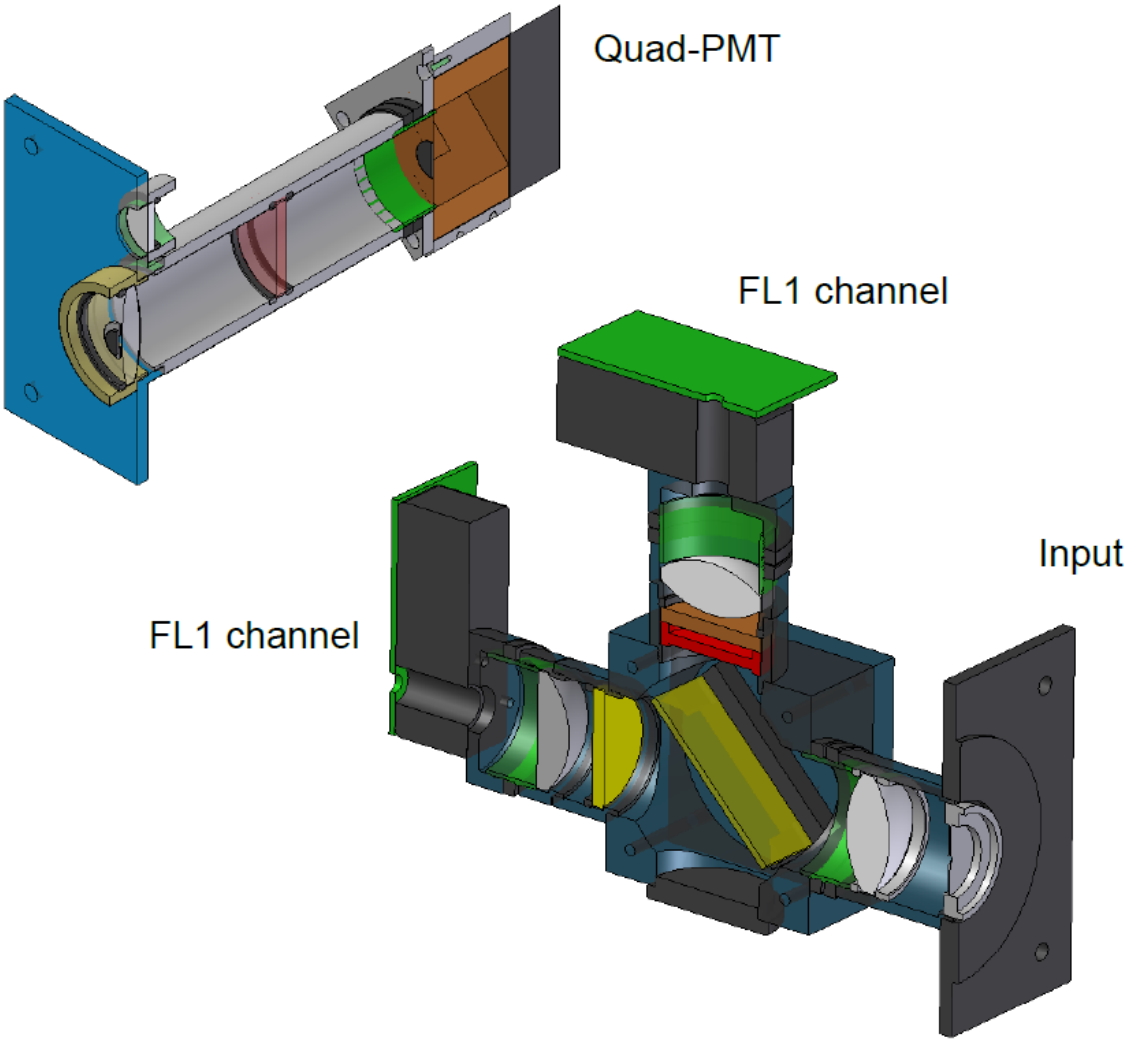
TotalT1: Rolling average frequency of TPCT1.

If a data file is created using the **Save** option in the **File** menu of the main **Plots** window, its structure is identical to that described above apart from the absence of the **Start of file** time indication.

Appendix D: WIBS-4A Delivery Settings

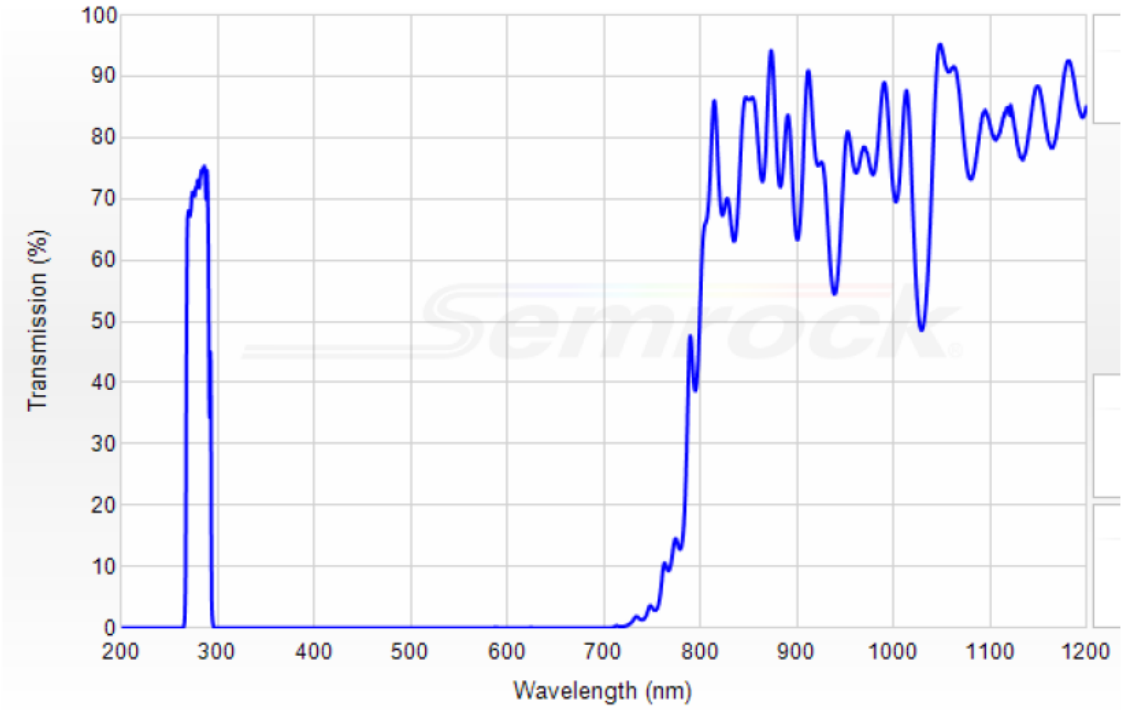
Parameter		Value	µs	Comments
Settings	Glitch time			
	Scat Integrate Pulse			
	Xe1 Trig Delay			
	Xe1 Integ Delay			
	Xe1 Integ Pulse			
	Xe2 Trig Delay			
	Xe2 Integ delay			
	Xe2 Integ Pulse			
	Recharge time			
		High Gain	Low Gain	
PMT values	FL1 PMT (V)			
	FL2 PMT (V)			
Flow-rates	Total Flow-rate at inlet (ml/min)			
	Sample Flow-rate (ml/min)			
FL2 aperture	(dia. mm)			
		High Gain	Low Gain	
Forced trigger Backgrounds Estimated from screen display	FL1_280			
	FL2_280			
	FL2_370			
Xenon Pwr readings(a.u.)	280nm			
	370nm			
Laser power at end of chamber				
Trigger Thresholds	T1			
	T2 (where dual trigger			
Hardware offsets				See data files for integrator offsets.

Appendix E: WIBS 4 Optical Filters



Xe1 Filters

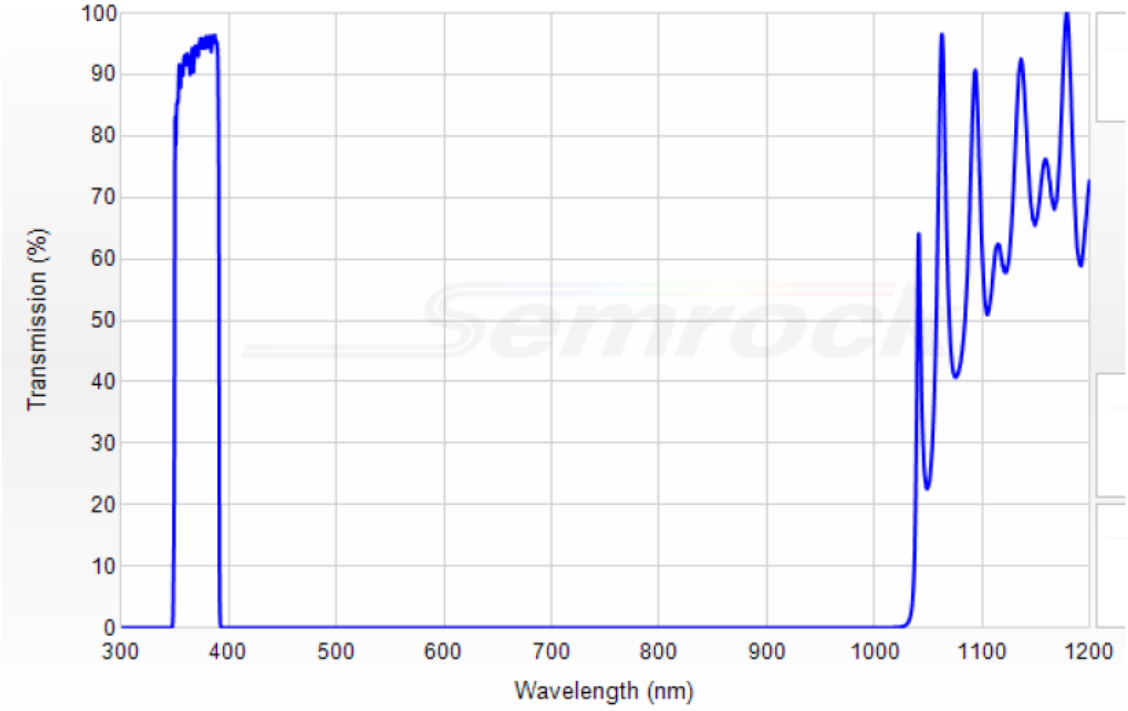
FF01-280/20-25



To deliver the 280nm UV pulse from Xenon 1, WIBS-4A uses two UV bandpass filters from Semrock Inc., type FF01-280/20-25. The transmission curve for a single filter is shown above. Two filters are used in series to improve out-of-band blocking and reduce the levels of infra-red transmission (where the xenon spectrum is still strong).

Xe2 Filters

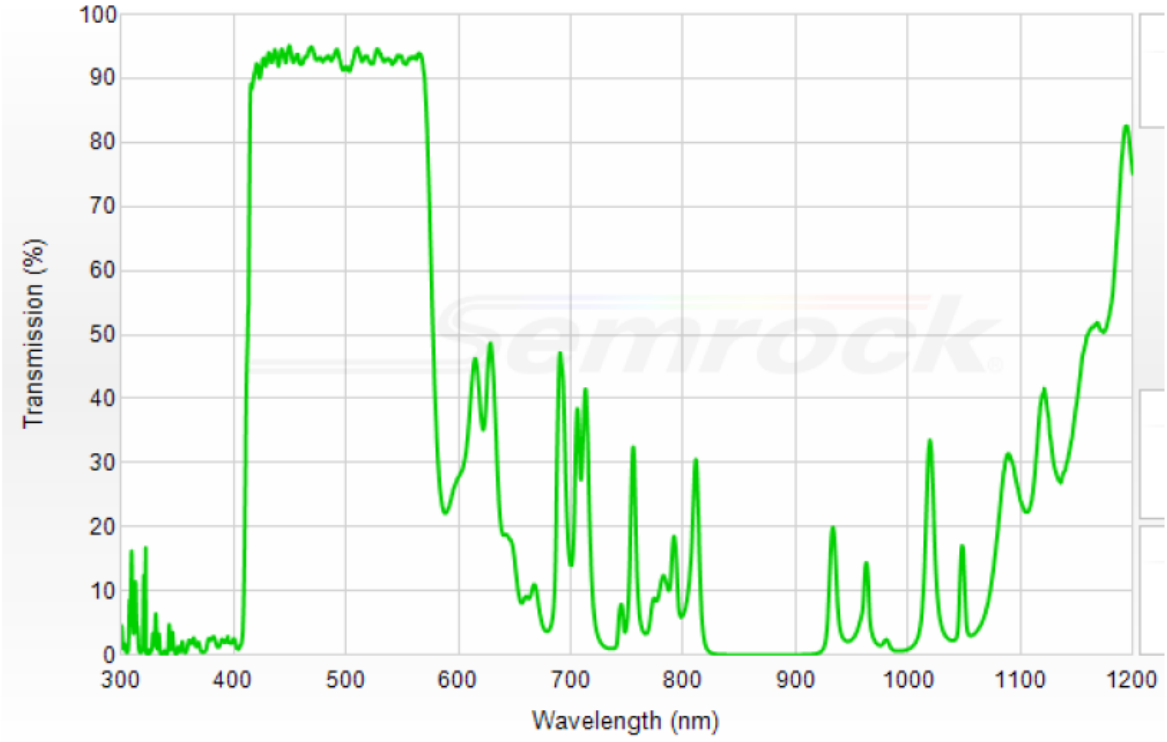
FF01-370/36-25



To deliver the 370nm UV pulse from Xenon 2, WIBS-4A uses a UV bandpass filters from Semrock Inc., type FF01-370/36-25. The transmission curve for a single filter is shown above.

Dichroic Beamsplitter

FF409-Di02-25x36

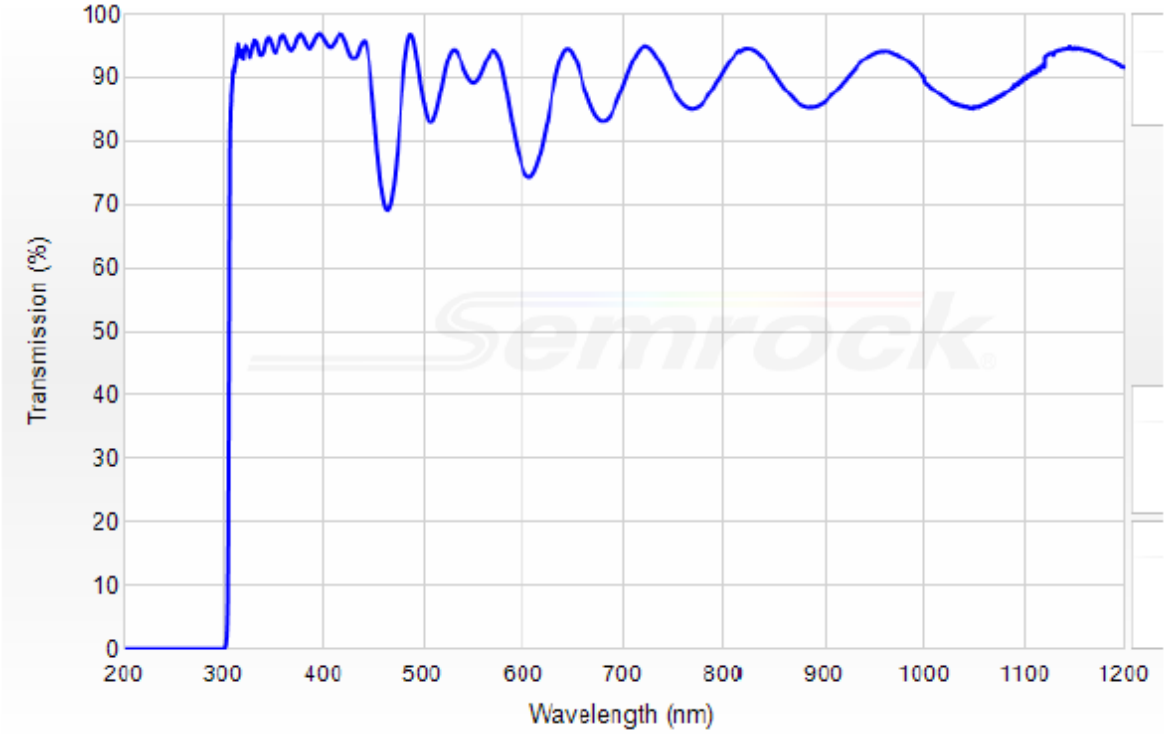


The dichroic beamsplitters helps separates the fluorescent light from the particle into two broad bands: 300-400nm (approx.) light is reflected to the FL1 channel and 400-650nm (approx.) light is transmitted to the FL2 channel.

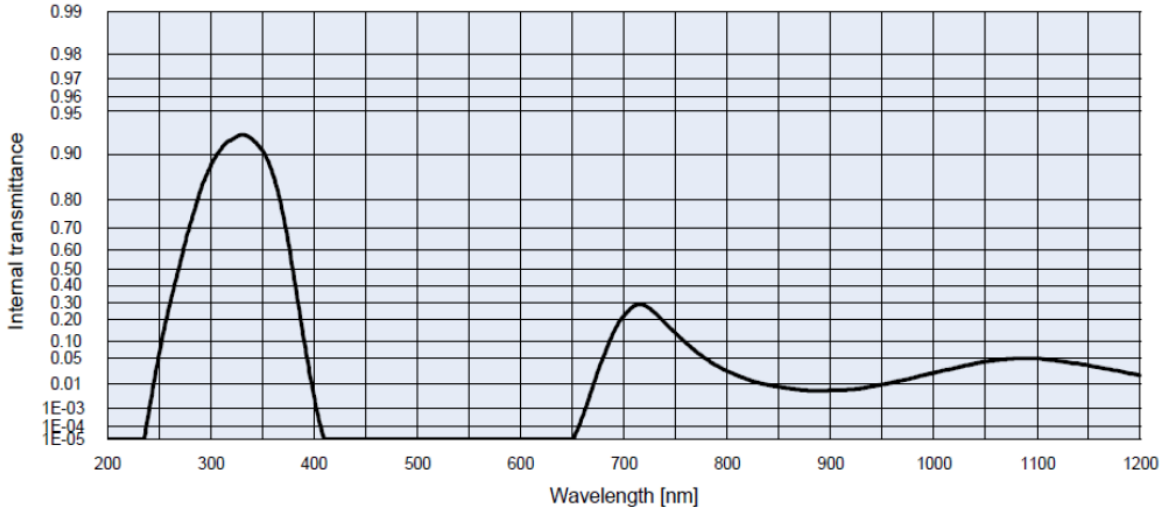
However, the dichroic is not perfect and some of the 300-400nm light is transmitted and some of the 400-650nm light is reflected. This is why WIBS-4A uses additional filters in the FL1 and FL2 channels to reduce this unwanted light.

FL1 Channel

FF01-300/25

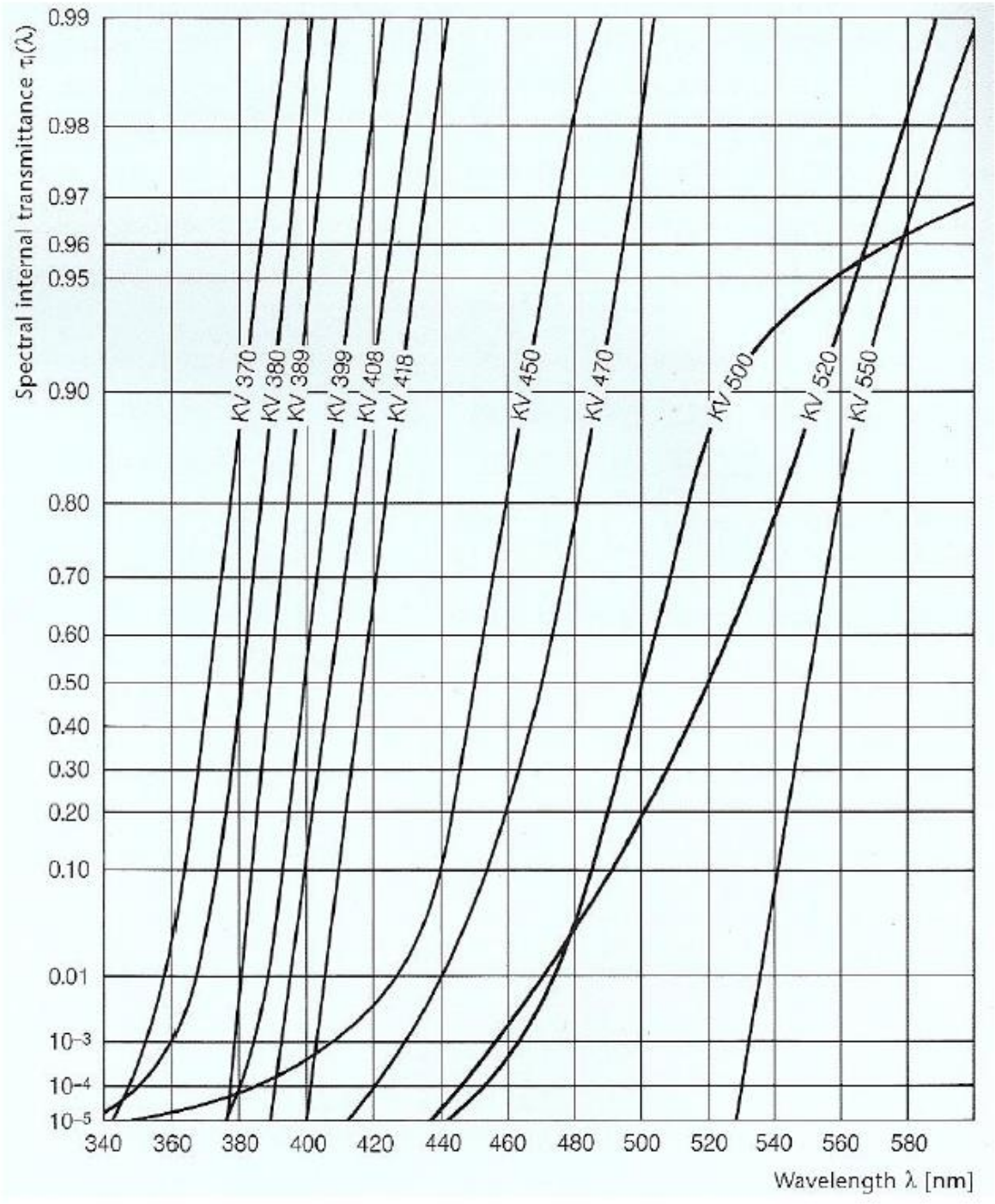


DUG11



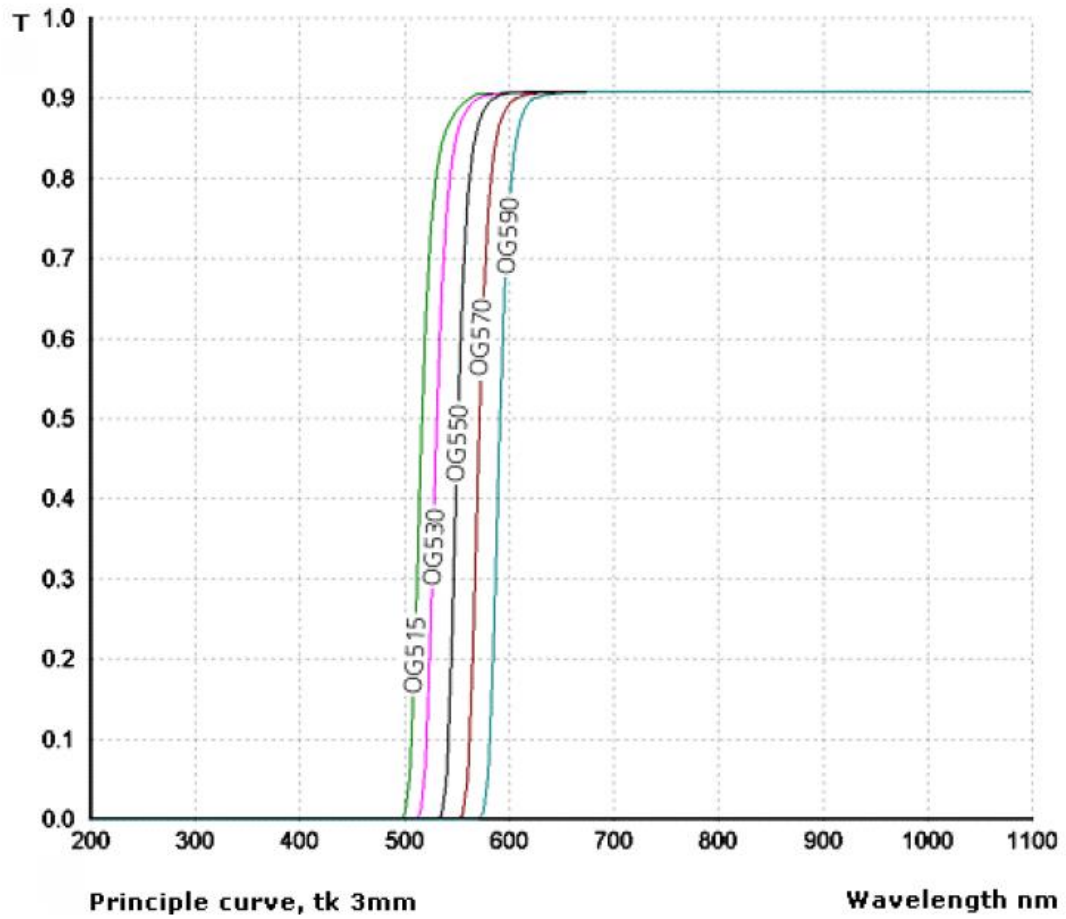
FL2 Channel

KV418



Quad-PMT Channel

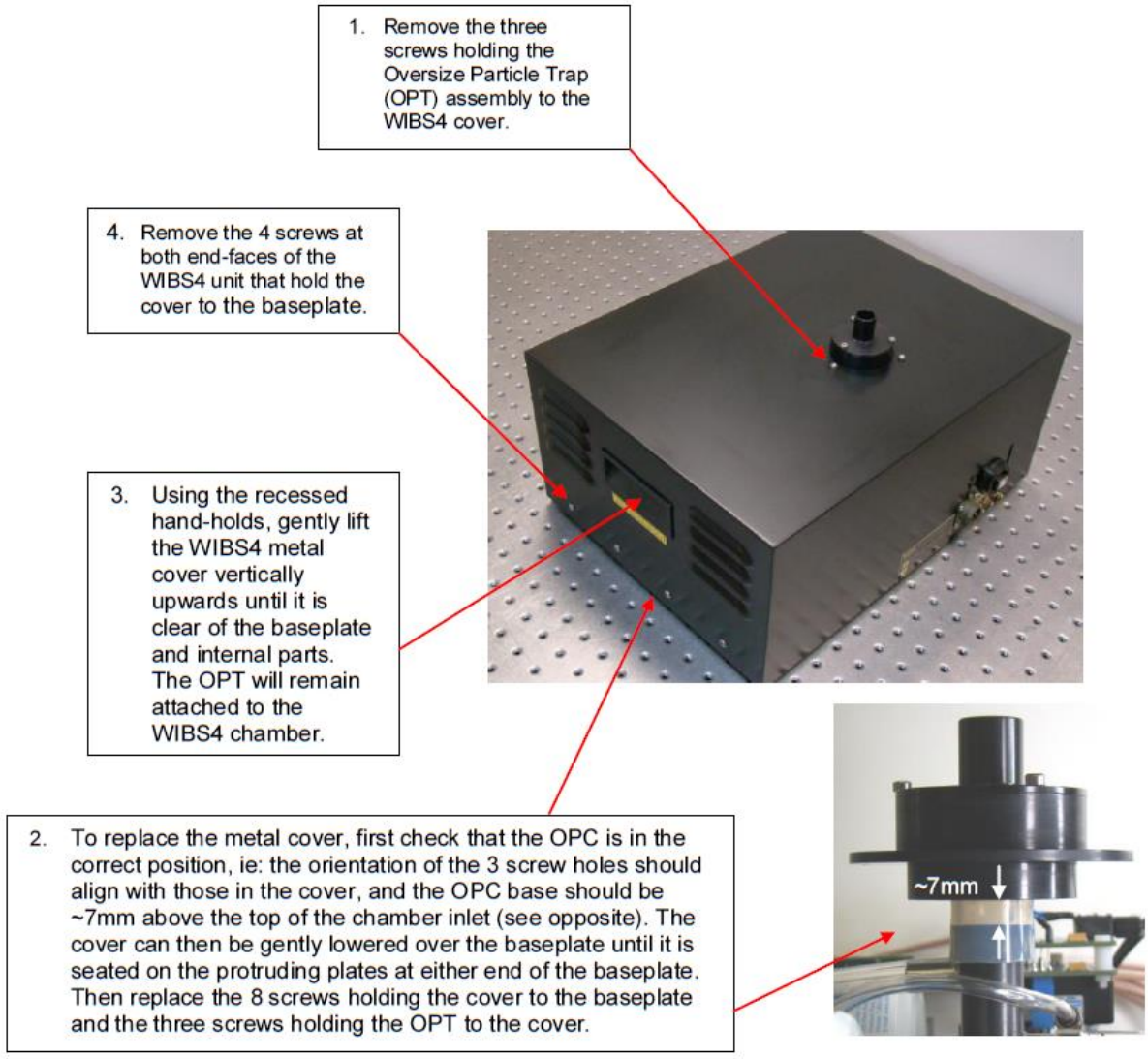
OG515



The Quadrant PMT channel uses a single OG-515 long pass filter to significantly reduce the level of UV light reaching the PMT when the Xe1 and Xe2 xenons fire. It does allow the forward scattered light from the particle (635nm) to reach the PMT and be used in determination of the particle Asymmetry Factor.

Appendix F: Removing WIBS-4A Cover for Access

If for any reason access to the internal parts of the WIBS-4A is required, the WIBS-4A metal cover can be removed as described below. Note that while the WIBS-4A cover looks different in this diagram, the removal process is the same.



Appendix G: References

[1] A single-particle multichannel bioaerosol fluorescence sensor, Kaye P.H., Stanley W.R., and Hirst E. Optics Express 13, 10, 3583-3593 (2005).

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Appendix H: Revisions to the Manual

Rev. Date	Rev. No.	Summary	Section
11-5-12	A	Revised University of Hertfordshire manual	Throughout
5-29-13	A-1	Changed reference to Section 4 to Appendix B	Appendix C
8-14-13	A-2	Corrected second fluorescence wavelength (360 nm to 370 nm)	Appendix A