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For similar reasons, DMT recommends that you do not install or run other software on the dedicated instrument computer. Although the installation of some software may be unavoidable, it is particularly important not to run other software while the computer is acquiring data.

CONTENTS

Figures

Introduction

The WIBS-NEO Toolkit is a software interface for the WIBS-NEO Instrument. It is a useful tool for further loading, processing, visualization, and inspection of data generated by the WIBS-NEO.

Functionality includes loading single and multiple raw data files containing relevant data such as particle-by-particle fluorescence, size, and asymmetry factor. The software converts particle-by-particle data into time-resolved particle concentrations and size distributions, as well as allowing the user to plot the data.

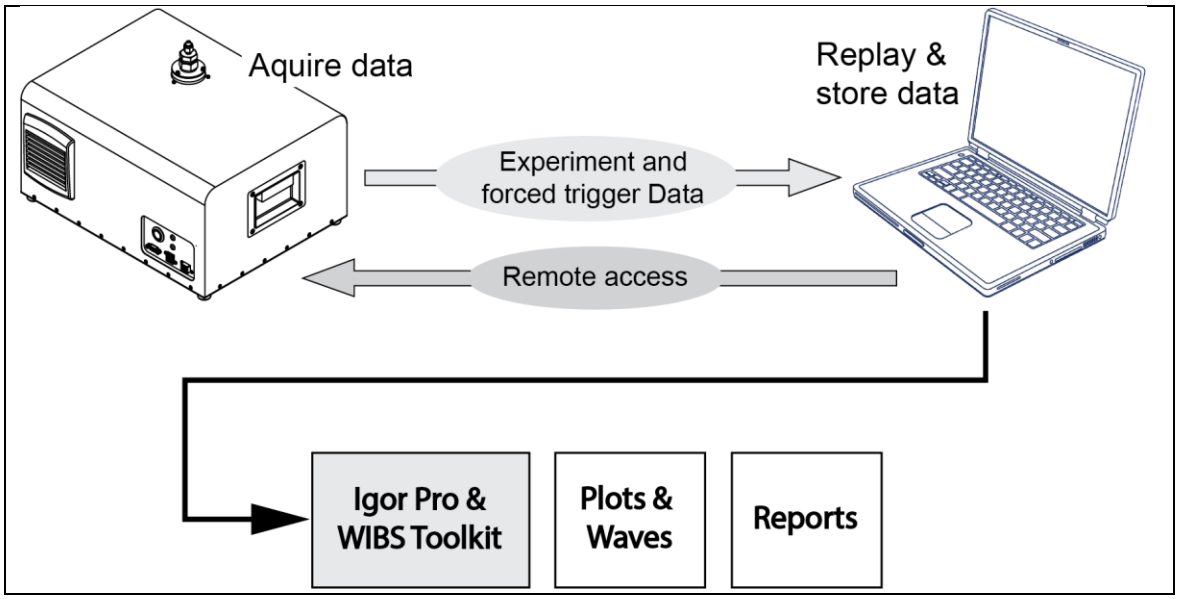

Figure 1: Flow diagram for WIBS-NEO data acquisition and evaluation

The Toolkit is not intended to be a substitute for a detailed analysis of all data available for any given project, but rather it serves as a conduit for creating the more commonly used output parameters that can be used for data analysis. Droplet Measurement Technologies intends the toolkit to evolve as the research community develops new ways of evaluating WIBS-NEO data. This Toolkit could include user contributions for improvement, and functionality as a part of its evolution in the field. If users discover bugs, have requests for new features, or would like to provide general feedback they should contact DMT, as the Toolkit is being developed and improved based on continual feedback.

1.1 Development Platform

The Toolkit was developed using Igor Pro (www.wavemetrics.com). Some familiarity with the Igor Pro environment, and programming language will aid in the use of the Toolkit, but a deep knowledge of Igor Pro is not necessary.
This manual will adopt some terminology used in Igor Pro, including referring to data arrays as “waves” and occasionally referring to data folders, packed experiment files, templates and other Igor Pro terms. Users interested in learning more about Igor Pro are encouraged to visit the Wavemetrics website, examine the Igor Pro manual and explore the environment using many of the widely available Igor Pro tutorials.

1.2 Toolkit Version Information

Version 1.0 is the first publicly released WIBS-NEO software from DMT.

Getting started

The WIBS-NEO toolkit requires a copy of Igor Pro, which can be downloaded at: https://www.wavemetrics.com.

An Igor Pro license and serial number is provided with each instrument.

NOTE: We recommend data analysis not be performed using the NEO onboard computer. Igor Pro, the WIBS-NEO Toolkit, and performance of data analysis should be tasked to a dedicated computer workstation. Very large files can become truncated and/or corrupted unless the drive where the files are being saved has the appropriate amount of storage space.

1.3 WIBS-NEO Toolkit Installation Instructions

The Toolkit software contains two files: **Packed experiment file .pxt**, and **Procedure file .ipf**. These files need to be placed in the proper folders, for the Toolkit to function properly (See steps below).

1. From the Igor Help menu, Choose **"Show Igor Pro 7 User Files"**, a file explorer window with the appropriate Igor folders should appear.
2. Place the **WIBS Toolkit v1.33.ipf** (or a shortcut to it) in the **"User Procedures"** folder.

The Toolkit also requires two additional installations:

- HDF5 Tools in Igor Pro (see Section 2.2)
- Screensizer in Igor Pro (see Section 2.3)

1.4 HDF5 Tools Installation Instructions

The HDF5 tools application is used as a bridge between the WIBS-NEO output and the Toolkit. It allows the program to directly read the HDF5 file output.

The HDF5 tools application can be installed easily using the following steps:

1. From the Igor Help menu, choose **"Show Igor Pro 7 User Files."**
2. From the Igor Help menu, choose **"Show Igor Pro 7 Folder."**
3. Copy the file: **Igor Pro 7 Folder/More Extensions\File Loaders\HDF5.xop**.
    - Paste into **"Igor Pro 7 User Files/Igor Extensions."**
4. Copy the file: **Igor Pro Folder/WaveMetrics Procedures\File Input Output\HDF5 Browser.ipf**.
    - Paste into **"Igor Pro7 User Files/Igor Procedures"** folder.
5. **Restart Igor Pro**. HDF5 should be ready to use.

NOTE: If you are using Igor64, the 64-bit version of Igor, the program will need to make sure there is an HDF5 Browser shortcut in the "Igor extensions (64-bit)" folder.

1.5 Screensizer Installation Instructions

You can find the current screensizer application on the Igor Exchange website. Screensizer allows experimental data to be displayed on most monitors.
To download the most recent version of Screensizer:

1. Go to http://www.igorexchange.com/project/ScreenSizer.
2. Locate and Download Screensizer-Igor.6.20.x-2.5.zip. Unzip the file.
3. Locate files: ScreenSizer.ipf and ScreenSizerHelp.ihf.
4. From the Igor Help Menu, choose **"Show Igor Pro User Files/ User Procedures."** Copy the **ScreenSizer.ipf** to the folder **"User Procedures."** Copy **ScreenSizerHelp.ihf** to folder **"Igor Help Files."**

1.6 Checking for correct installation

The **Igor Pro 7 User Files** folder should contain the following:

- The **User Procedures** folder should contain the **ScreenSizer** and **WIBS_Toolkit_v1.33** files.
- The **Igor Procedures, Igor Extensions and/or Igor Extensions (64-bit)** folders should all contain the **HDF5 Browser-Shortcut.**
- The **Igor Help Files** folder should contain the **ScreenSizerHelp** file.

When the above looks correct, open the WIBS_Toolkit from either the .pxt, or by going to the Windows Start menu and opening the program from there. The Toolkit program main window should appear (Figure 2).

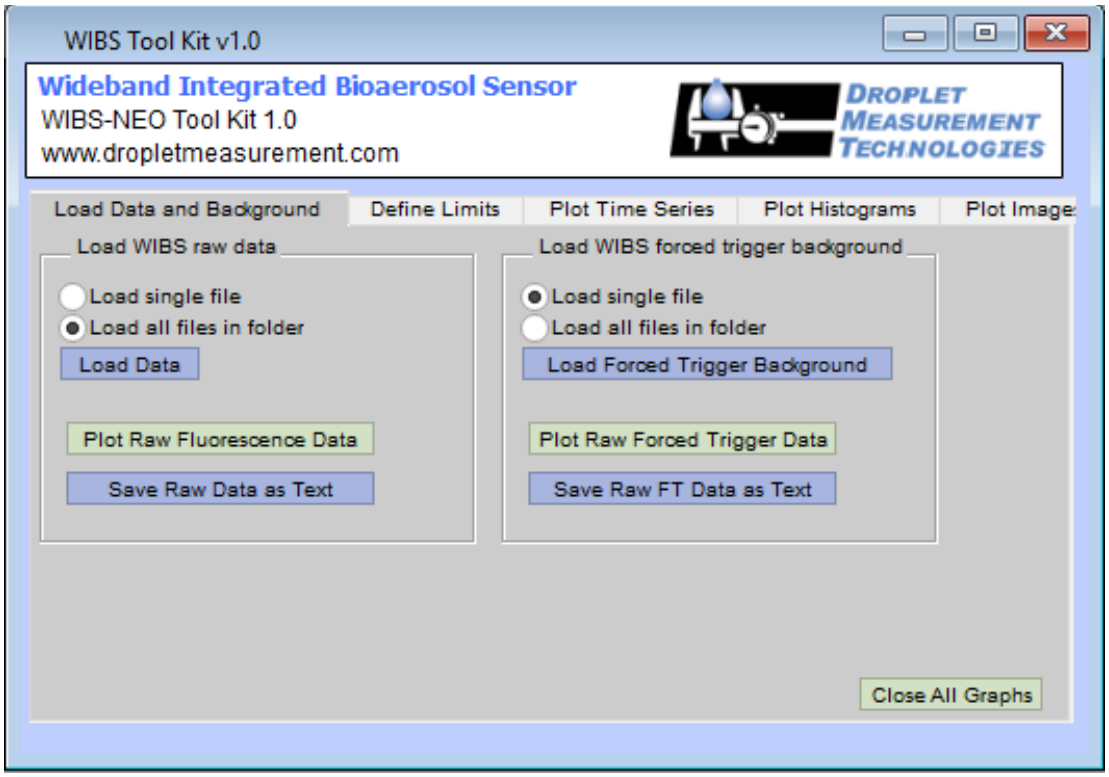

Figure 2: Load Data Tab

WIBS-NEO Toolkit and its Components

The WIBS-NEO Toolkit uses the Igor Pro platform. The software includes components that allow for advanced particle analysis, classification of particles, data processing, plotting, and data management.

1.7 Overview

- The toolkit loads HDF5 files
- Raw data is saved with all background noise
- Default limit for fluorescence classification is the Forced trigger average ± 3 standard deviations. The user should choose detection limits that best suit their data measurement conditions.

1.8 Basic Operation

The typical process for converting raw files into fluorescent particle concentrations involves the following steps:

- Loading the desired raw data into a user selected data folder.
- Subtracting the Forced Trigger background from raw data.
- Processing the background subtracted data into time-resolved outputs such as fluorescent particle concentrations.
- Generating standard output plots of the concentrations in the experiment.

1.9 Advanced Analysis

More advanced analysis can be performed on the data, including calculation of size distributions for different particle classifications, and examination of the raw fluorescent signals. At any point, users familiar with the Igor Pro environment can access data through the Data > Data Browser and generate their own plots or create their own functions for producing output data.

1.10 Data Structure

The WIBS-NEO toolkit is designed for analysis of data within a specific data folder. This is usually referred to as the "**Active Data Folder**". Particle data and forced trigger data waves will be loaded into separate data folders – (**NEO and NEO-FT** respectively). Within the **NEO** folder, the data is split into "**Monitoring Data**" and "**Particle Data**". The "**Monitoring data**" folder contains all of the housekeeping parameters such as flow rates, temperatures, and particle concentrations reported every second. Descriptions of these waves can be found in the operator manual.

1.11 File Size

Users should be careful to avoid loading too much raw data into any single experiment, especially from longer measurement periods. Memory limitations will depend on the specifications of the computer used in the data analysis.

1.12 File Loading

Raw data files and forced trigger files are loaded in the same manner. The toolkit provides options for loading all files in a single directory or for loading a single file within a directory. In all cases, data can be saved as a text file by selecting the button on the main Toolkit screen.

1.13 How the Toolkit Loads Files

The toolkit will automatically load the raw data, interpret the timestamp on the file, and combine the raw data into large concatenated waves. The raw data waves are loaded into the **Active data folder** and can be accessed through the data browser or command window. All waves have the same length, which is equal to the total number of particles in the combined files. The user may also view the raw data using the **HDF5** browser utility in IGOR Pro. This HDF5 browser is accessed by typing: **Data > Load Waves > New HDF5 browser**. This brings up a new window where you can open **HDF5** files for viewing. In addition, it is possible to use the **HDF5 browser** to export waves into another graphing software such as Microsoft Excel.

1.14 Loading and Processing Forced Trigger Background Data

Once Forced Trigger data has been loaded, the user should navigate to the “Define Limits” tab (Figure 3). Select the “**Average Forced Trigger Background”** button to automatically populate the average and standard deviation fields for each of the three emission channels.

The user can adjust the upper and lower limits of fluorescence detection by manually entering the value or using the up-and-down arrows. The upper limit by default is 1.0e+8. Selecting the “**Define Particle Types Using Limits**” will show feedback on whether a particle is fluorescent based on the user-defined limits. Particle types must be defined before any plots can be made.

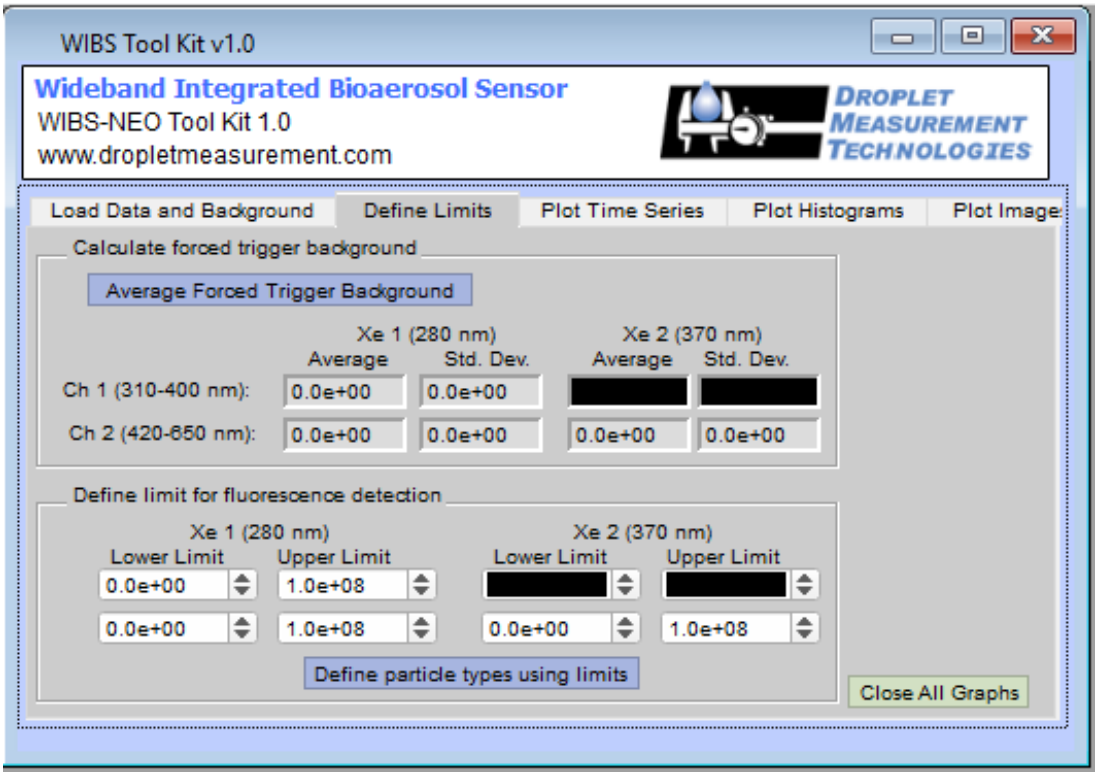

Figure 3: Define Limits Tab

1.15 Waves Created During File Loading

The file loading operation creates the waves listed below.

Monitoring Data:

BOARD_1	Sample_Temperature
Conc_Excited_cm3	Sample_VolumetricFlowRate
Conc_Total_cm3	Sheath_MassFlow
H12310_Temperature	Sheath_PSIA
Max_Transit_Time_counts	Sheath_SetPoint
Min_Transit_Time_counts	Sheath_Temperature
Num_Discarded_Particles	Sheath_VolumetricFlowRate
Num_Oversize_rejects	Temperature
RH	Total_Particle_Count
SYS_V	Valid_Particle_Count
Sample_MassFlow	XE1_Power
Sample_PSIA	XE2_Power
Sample_SetPoint	Timewave

Table 1: Monitoring Data waves

Particle Data:

Asphericity	NF_Shape_3
Density_g_cm3	NF_Sizer_Relative_Peak
EP_Overflow_flag	NF_Sizer_Transit_Time_nsec
Flag_Excited	Size_um
Mass_ug	Xe1_FluorPeak
NF_Shape_0	Xe2_FluorPeak
NF_Shape_1	Timewave
NF_Shape_2	MaskParticleTypes

Table 2: Particle Data Waves

Data Processing

Data processing functions classify particles based on several user-defined criteria and calculate particle concentrations for different classifications.

1.16 Fluorescence Classification

Particles are classified into one of 13 categories based on measured intensities in the three fluorescence channels. Plots can be filtered by fluorescence channel by selecting and deselecting the options at the top of each graph.

Name	Fluorescence must be above background for:
All	All particles
Excited	Particles excited by the flash lamp
Fluorescent	Fluorescent particle detected in any channel
FL1	Fluorescent particles detected in channel FL1 (excitation at 280 nm, emission 310-400nm)
FL2	Fluorescent particles detected in channel FL2 (excitation at 280nm, emission 420-650nm)
FL3	Fluorescent particles detected in channel FL2 (excitation at 370nm, emission 420-650nm)
A	Fluorescent particle detected in channel FL1 only
B	Fluorescent particle detected in channel FL2 only
C	Fluorescent particle detected in channel FL3 only
AB	Fluorescent particles detected in channels FL1 and FL2 only
AC	Fluorescent particles detected in channels FL1 and FL3 only
BC	Fluorescent particles detected in channels FL2 and FL3 only
ABC	Fluorescent particles detected channels FL1, FL2, and FL3

Table 3: Classification of particles

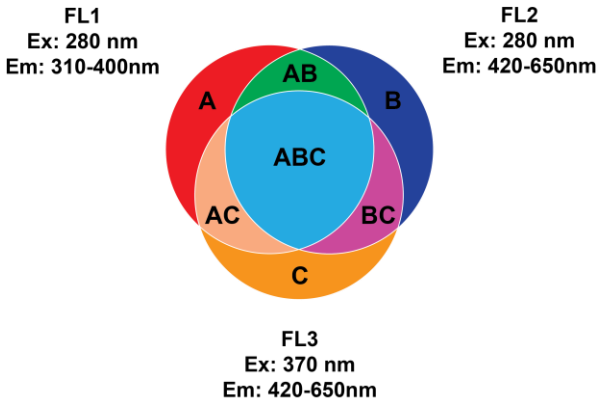

Figure 4: Particle Definitions, Savage et al., 2017

1.17 Thresholds used in Classification

A particle falls into one of the fluorescence categories if it exhibits fluorescence greater than the user defined threshold value for that channel. Thresholds can be a fixed value, or the user can determine thresholds based on the forced trigger data (recommended).

1.18 Reprocessing Data

Data can be re-processed at any time. Users should be aware that reprocessing overwrites any previously processed data outputs. If users wish to keep data with different processing parameters (different averaging intervals, etc), they either need to save the outputs before reprocessing, or copy the data file into a new data folder and load it separately (e.g. ambient-3sec and ambient-5sec).

Plot Time-series

The **"Plot time-series"** tab (*Figure 5*) allows the user to create averaged time-series plots of the following parameters:

- **Fluorescence Peak**
- **Diameter**
- **Concentration**
- **Surface Area**
- **Mass**
- **Asphericity**
- **Excited and Fluorescent Fractions**

The user can choose which of the 13 fluorescence channels are displayed on the time-series plots by checking the boxes at the top of the plot once it appears. By default, all possible options are checked.

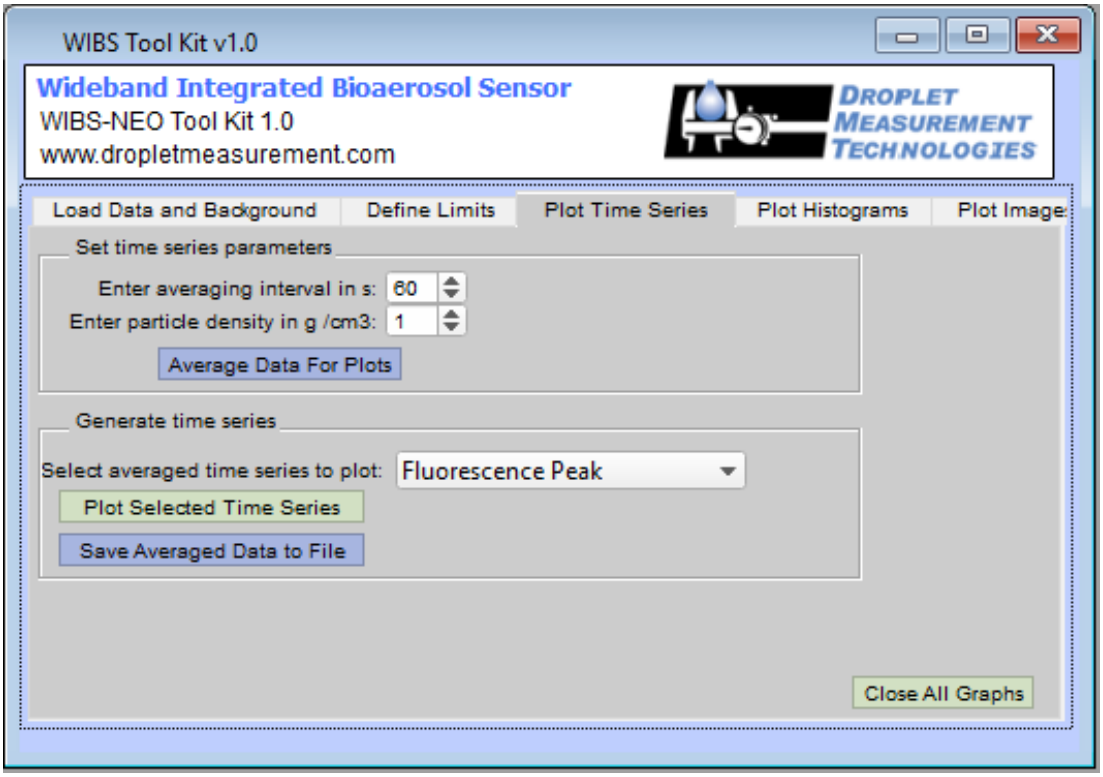

Figure 5 Plot Time-series tab

1.19 Setting Time-Series Parameters

To generate time-series plots, the user must define the averaging interval in seconds and assumed particle density in g/cm³. By default these are set to 60 and 1, respectively. Please note that the minimum interval for time averages is currently 2 seconds. Once the parameters are set, the **"Average Data for Plots"** button needs to be selected before generating time-series plots.

Time-series data can be exported as a .txt or .csv file by selecting the **"Save Averaged Data to File"** button.

Plot Histograms

The **"Plot Histograms"** tab will produce histograms with the following defined parameters:

- **Particle Concentration vs Diameter**
- **Fluorescence vs Diameter**
- **Particle Concentration vs Fluorescence**

Parameters:

- The start time and end time will automatically default to include all data loaded into the toolkit. The user can manually define the time period if only a portion of the data is to be analyzed.
- The user defines the lower and upper limit and size bin increment to be visualized on the histogram graph.
- The user defines the lower and upper limit and fluorescence peak to be visualized on the "Particle Concentration vs. Fluorescence" histogram only. Please note that the values used here are only used for visualization of the data and will not impact the fluorescence threshold values defined on the **Load Data** and **Background** Tabs.

The user must first check the histogram parameters and hit **"Calculate Histogram Data"** before attempting to plot or save the data to a file.

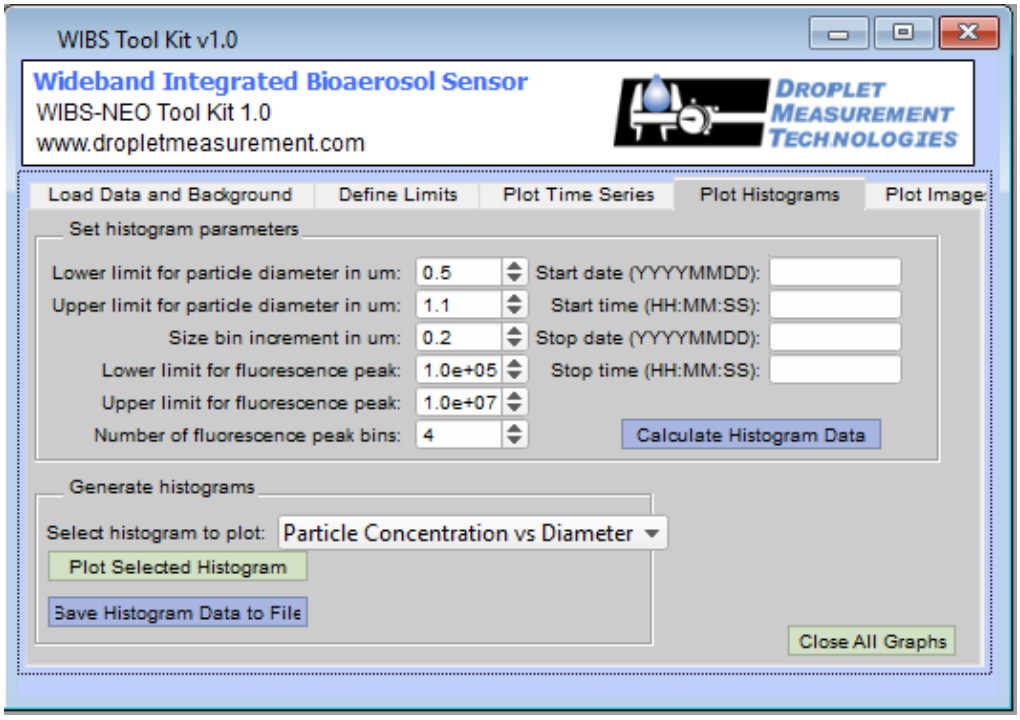

Figure 6: Plot Histograms Tab

Plot Images

The **"Plot Images"** tab produces 3D plots of particle size time series that are colored by either number (N), Mass (M) or volume (S) concentrations.

Parameters:

- **Lower and upper limit in um –** Sets the particle size range to be plotted.
- **Number of size bins –** Resolution of the specified size range on the plot.
- **Time Interval –** The averaging interval for the time-series data.

When the calculations are complete and the plot is produced, the user can then choose to view the Number, Mass, or Volume size distribution using the drop-down menu on the upper right-hand side of the graph.

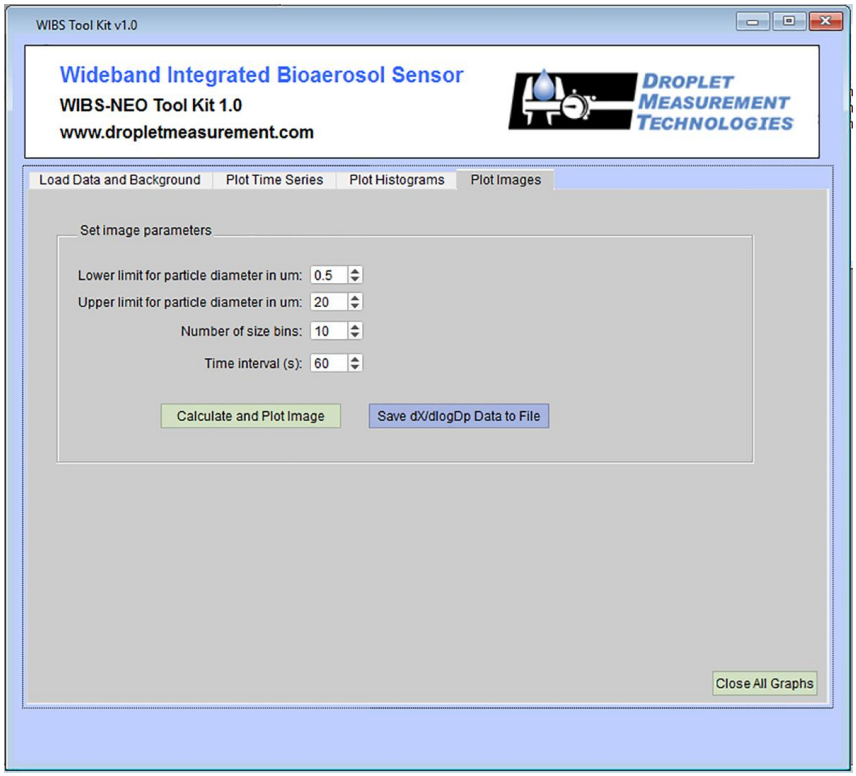

Figure 7: Plot Images Tab

Additional Information

1.20 Tips for WIBS-neo Data Collection

When collecting data, try to include forced trigger periods at regular intervals during measurement periods. The WIBS-NEO software will automatically store all forced trigger data in a separate folder designated as “FT”

Glossary

A

AF: Asymmetry Factor, calculated by the acquisition software.

F

FL1_280: Raw fluorescence intensity (A/D counts) for the FL1 detector (~310- 400 nm) from the 280 nm excitation.

FL2_280: Raw fluorescence intensity (A/D counts) for the FL2 detector (~420- 650 nm) from the 280 nm excitation.

FT: Instrument mode flag (Forced Trigger mode)

P

Pwr_280: Power recorded for the 280 nm excitation.

Pwr_370: Power recorded for the 370 nm excitation.

S

Size: Size in micrometers based on the internal calibration settings.

T

TPCT2: Detected T2 particle counts (see manual).

W

wibs_datetime: Time stamp for each particle in seconds from midnight 01/01/1904.