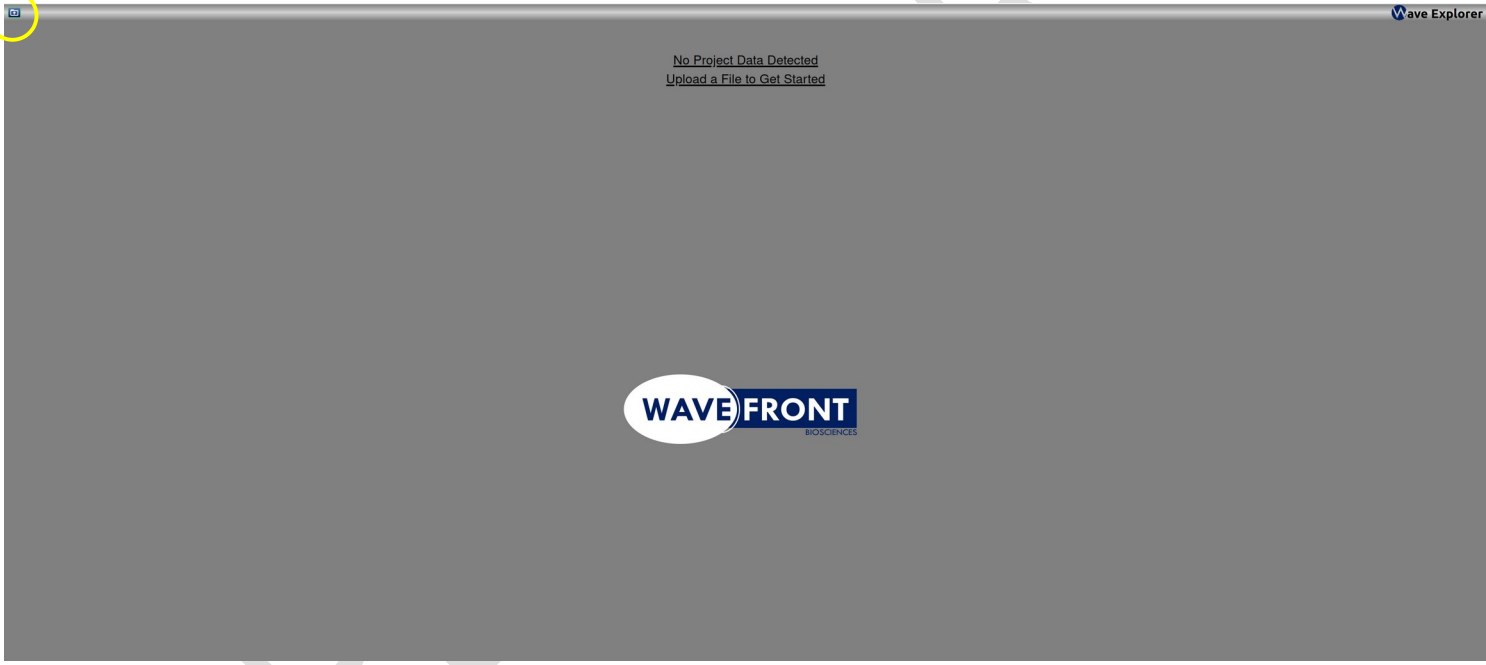
Web Wave Explorer – Quick Start Guide
Wavefront Biosciences LLC

A new web-based WaveExplorer application has been developed to allow users to analyze data that was captured from a Panoptic system. All you need is an internet browser and a saved .dat file that was created at the end of your experiment run. The following provides basic instructions on how to get the Web Wave Explorer app, and how to use it.

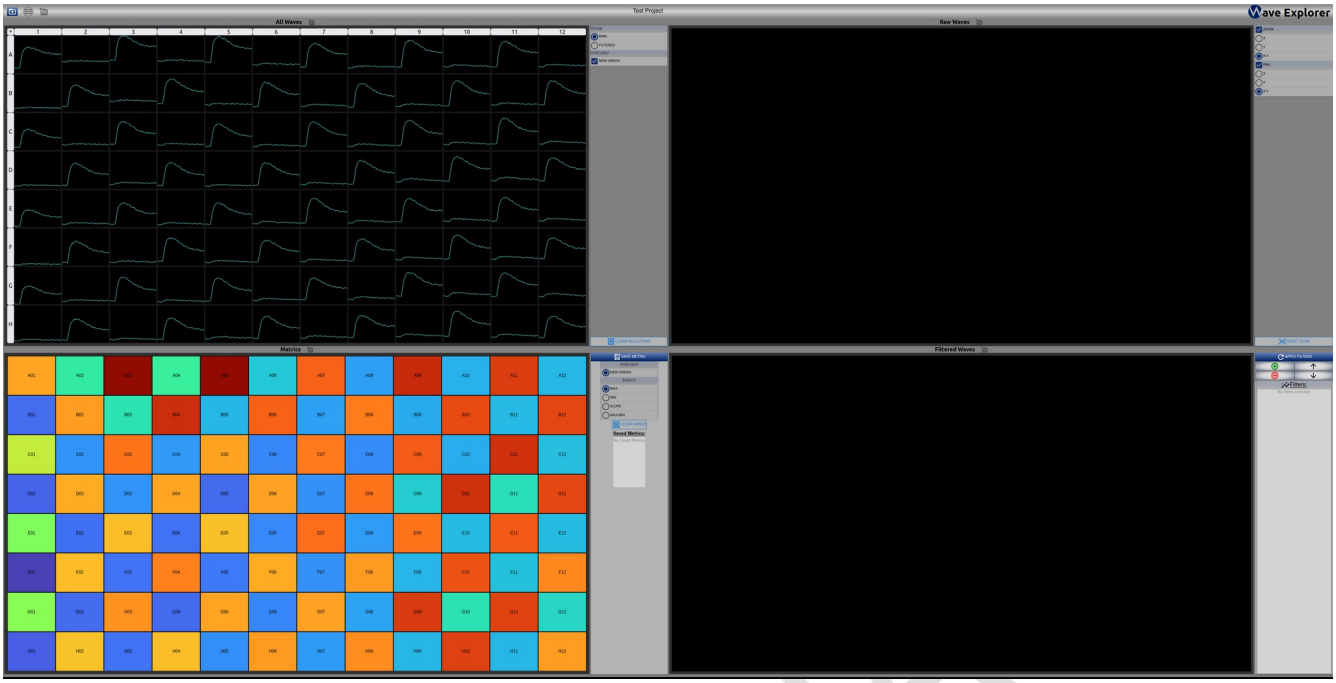
Launching the app

Go to www.wavefrontbio.com and click on the "Wave Explorer" menu option at the top of the page. The page shown below should appear.



Loading a ".dat" file

At the top left corner of the screen above is a small, folder icon. Click this icon to browse for a "*.dat" file on your system. After loading your *.dat file, your screeen should look something like that shown below.



Basic viewing of data

The operation of this app is very similar to the desktop application that is installed on the Panoptic computer. There are basically 4 quadrants, starting at the upper left and proceeding clockwise:

- All Data (upper left)
- Selected Data (upper right)
- Filtered Selected Data (lower right)
- Metrics (lower left)

Each quadrant has an associated control panel, shown at the right side of each quadrant. This control panel allows you to control the behavior of that quadrant.

All Data Control (upper left quadrant)

This presents all the wave graphs for the plate. You can toggle between viewing raw data and filtered data. Also, if your *.dat file had more than one indicator, you can control the visibility of the graphs for each indicator. Wells can be selected individually, by rubber-banding, or by using the row and/or column buttons. When a well is selected, an enlarged view of the graph for that well is shown both in the Selected Data and Filtered Data quadrants.

Selected Data Control (upper right quadrant)

This presents the raw data graphs for all the selected wells. You are allowed to zoom and pan the graphs in this control. To zoom, use the mouse wheel. To pan, click and drag. To reset the display to the default, click the “Reset Zoom” button at the bottom of the control panel.

Filtered Selected Data (lower right quadrant)

This presents the results of any/all filters that are set to be applied to the raw data. Filters can be added by clicking the “+” button at the top of the control panel. New filters are always added to the bottom of the filter list. Conversely, filters can be deleted by clicking on the “-” button. Note, in order to delete a filter, it must first be selected (click on it), followed by clicking the “-” button. Filters are applied to

the raw data from top to bottom. In order to change the order of the filters, you can use the "↑" and "↓" button to move a selected filter.

There are currently 7 filter types that can be added:

- Static Ratio
- Smoothing
- Control Subtraction
- Derivative
- Outlier Removal
- Flat Field Correction (a calibration file is needed here)
- Dynamic Ratio

Each filter has associated parameters. When a filter is added, it appears in the Filter list in the control panel. An example of this is shown below.

In the above figure, you can see that a Static Ratio filter is now in the filter list. Notice that on each side of the filter entry, there is a checkbox (on the left) and a pencil icon (on the right).

The checkbox is used to enable/disable the filter. A disabled filter will behave as if the filter was not in the list. This allows the user to quickly check the effects of a filter, by disabling it, hitting "Apply Filters", examining the curve, enabling it, hitting "Apply Filters", and noting the effect caused by the filter.

The pencil icon allows you to edit the specific parameters for the filter. If you click the pencil, a dialog will appear that allows you to set all parameters. Each filter type has a different set of needed parameters, so the dialogs for each filter are different.

To select a specific filter in the list, click near the center of the filter entry. The selected filter should now be highlighted. Once selected, you can move the filter up/down in the list by using the arrow buttons.

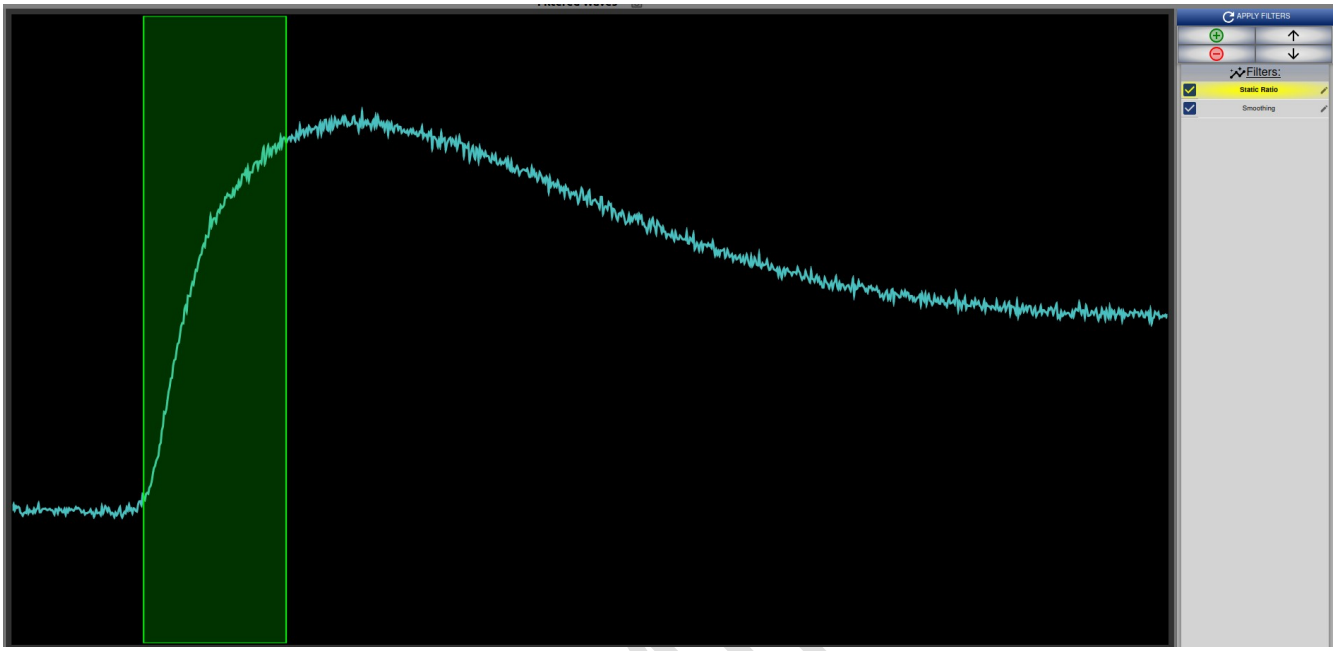
NOTE: The filter calculations can be slow, so they aren't automatically applied to the raw data. After filters are appropriately created and configured, click the "Apply Filters" button at the top of the control panel. This will re-calculate the filtered data which is then displayed in the graph.

Metrics (lower left quadrant)

This control presents metric heat maps using the filtered data. There are currently 4 metric types:

- Max
- Min
- Max-Min
- Slope

You can also select a data range over which the metric is calculated. This is accomplished by clicking and dragging a range on the filtered data plot. When a range is selected, the metric is calculated only on the data within the range. If no range is selected, the metric is calculated on the full data set.



In the figure above, you can see the highlighed range on the filtered curves. This will be the range used for calculating the metrics. The selected range can be cleared by clicking the “Clear Range” button in the control panel.

Because it is common for similar analyses to be used across experiments, it is possible to save a set of filters and metrics, and then reloaded with a new *.dat file. Thus you save a metric by clicking the “Save Metric” button at the top of the panel. This will result in the metric being added to the “Saved Metrics” list. Note that each saved metric can have a different selected range.

Once the desired set of filters and saved metrics have been created, these can be saved to file. This is done by clicking the menu icon at the top-left of the page.

Once this is clicked, a menu will appear which allows you to save the filters and metrics to a file. This file can later be reloaded. The menu button is circled in the image below.

Reports

Reports can be generated that contain the raw data, filtered data, and saved metrics. This can be done by clicking the menu button, and then selecting “Generate Report”. A dialog will appear that allows you to select what to include in the report.

Click “Download Report” for the report file to be saved. This file is a csv-formatted file with tab delimiters. It is compatible with Excel and other spreadsheet applications.

Screen Shots

Screen shots for each quadrant or the entire app can be generated. For the quadrants, click the camera icon located next to the quadrant title. For the entire app, click the camera icon that is next to the menu button at the top of the app.

Filter Descriptions

Static Ratio

This filter has a normalization effect on the data. It first calculates F for each well of an image, and repeats this for the first N images. And then it finds the average, for each well, across those N images, resulting in a unique F_0 value for each well, i.e. for a specific well, F_0 is the average of F across the first N wells. Finally, it calculates F/F_0 for each well's data.

Static Ratio = F/F_0

where

F = sum of pixel values for a well

F_0 = average of F values for the first N images for a well

Input Parameters:

N – the number of images to use for normalization. This is the first N images taken during the experiment.

This filter is used improve the ability to compare curves between wells.

Control Subtraction

This filter subtracts the average of a set of wells (e.g. the set specified of control wells) from the input data. For each image, $\frac{F}{F_0}$ is calculated for each of the designated control wells, and then averaged. This average value is then subtracted from the F value of each well.

Control Subtraction = $F - Avg\left(\frac{F}{F_0}\right)_{control\ wells}$

Input Parameters:

Selected Control Wells

This filter is used to make clear the difference between the control wells and all other wells. It is common to set up the control wells to have little or no change across the experiment, i.e. put nothing in these wells that would be indicative of anything across the experiment. This provides a "background" measurement from the control wells, which is then subtracted from all wells, including the control wells.

Dynamic Ratio

This filter allows for the direct comparison between two indicators from the same experiment. Hence, this filter can only be used on experiments that have more than one indicator. It simply calculates the ratio of one indicator over another. The first Indicator 1 data point for well A1 is divided by the first Indicator 2 data point for A1. This is repeated for each datapoint in a well data series, and for each well on the plate.

Dynamic Ratio = $\frac{F_1}{F_2}$

This filter is used to directly compare two indicators.

Smoothing

This filter implements a simple smoothing function. It is useful for smoothing data prior to other calculations. It is particularly useful before a derivative filter. The filter is implemented as a moving window of width W, in the form of classical low-pass filters. Here, the width is simply the number of images to average across. The greater the width, the smoother the result. Widths between 5 and 10 are typically used.

Input Parameters:

Window width

Derivative

This filter determines the slope between each set of adjacent points in a series. This results in a graph that represents the slope of the data across time. NOTE: although other filters do change the graphed data, the resulting graphs are usually similar in shape. With the derivative filter, the graph typically changes drastically. This happens because we are no longer plotting the static light values, but the rate of change in those light values across time. This is typically the last filter in a series of filters.

Outlier Removal

This filter searches the data for "outliers" and removes them. On occasion, you may get a bad reading or a bad image from the camera. This can result in a spike in the graph. If left in the data, it could make it difficult to analyze the data with other filters. This filter implements the well-known Hampel algorithm for outlier removal.

Input Parameters:

Half-Window (usually between 2 and 4)
Threshold (typically 3)

Flat-Field Correction

This is an experimental filter that is being tested to help reduce the effects of optical distortion. Due to the nature of most optics, the camera is more sensitive to light along the center axis of the lens. As you move away from the center axis, the sensitivity decreases. This results in a bias towards the center of the plate, i.e. even though the amount of light emitted from each well is the same, the wells near the center will have a higher measured value than those along the edges. The amount of sensitivity loss may be a result of many factors: optics, exposure time, excitation and emission wavelengths, amplifier gains, etc.

In order to use this filter, you need a calibration file that has been generated for your Panoptic. We are still in the process of developing a tool to generate this calibration file.