

Absorbance Experiments

Absorbance spectra are a measure of how much light a sample absorbs. For most samples, absorbance relates to concentration via Beer's Law:


$$A_{\lambda} = \epsilon_{\lambda} c l$$

A_{λ} = Absorbance at wavelength λ
 ϵ_{λ} = Molar absorptivity at wavelength λ
 c = Concentration of the sample
 l = Path length of the sample

$$R_{\lambda} = \text{Reference intensity at wavelength } \lambda$$



1. Place SpectraSuite in Scope mode by clicking the Scope () icon in the Experiment mode toolbar or selecting Processing | Processing Mode | Scope from the menu.

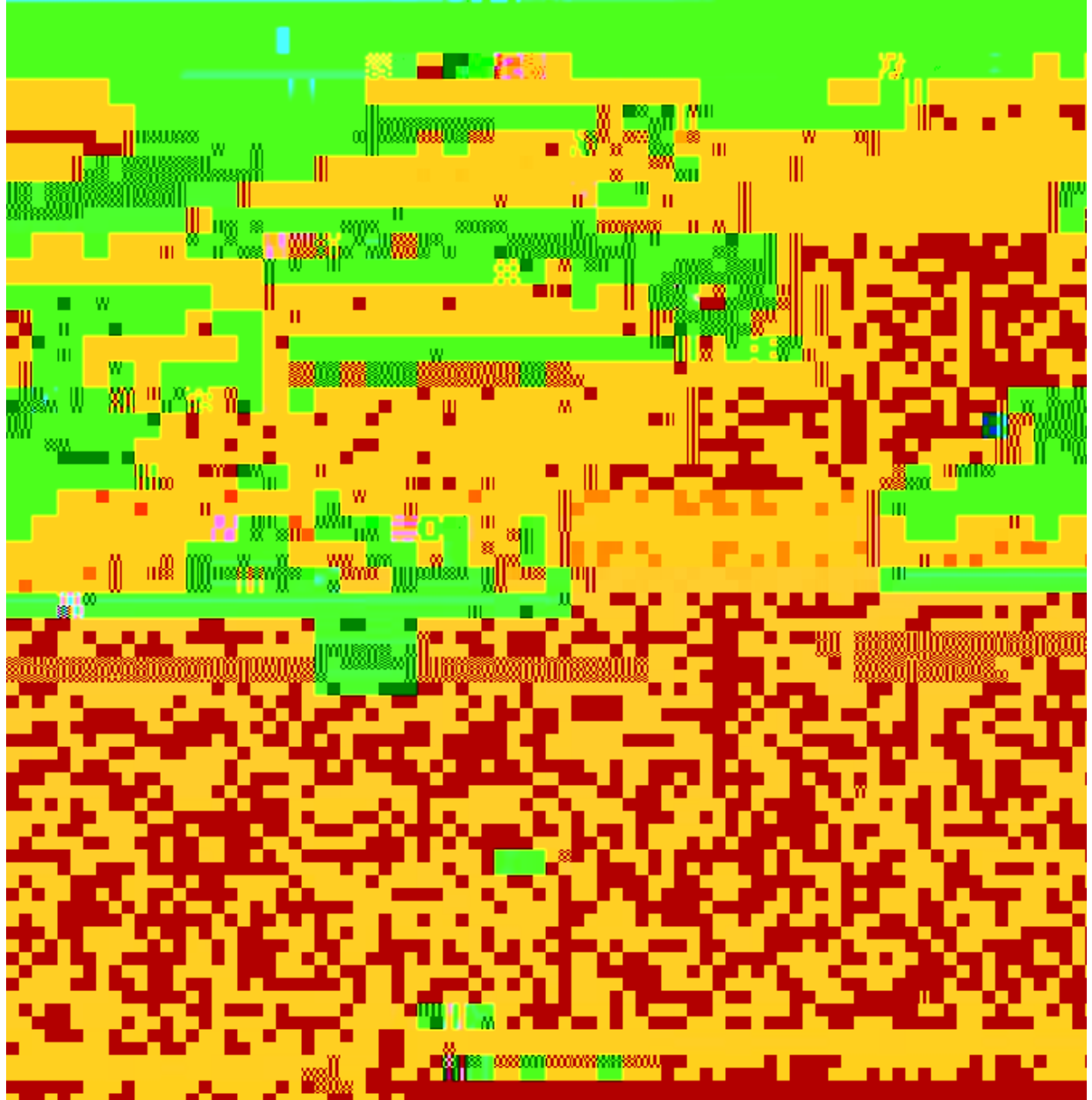
1. Place SpectraSuite in Scope mode by clicking the Scope () icon in the Experiment mode toolbar or selecting Processing | Processing Mode | Scope from the menu.

2. Ensure that the entire signal is on scale. The intensity of the reference







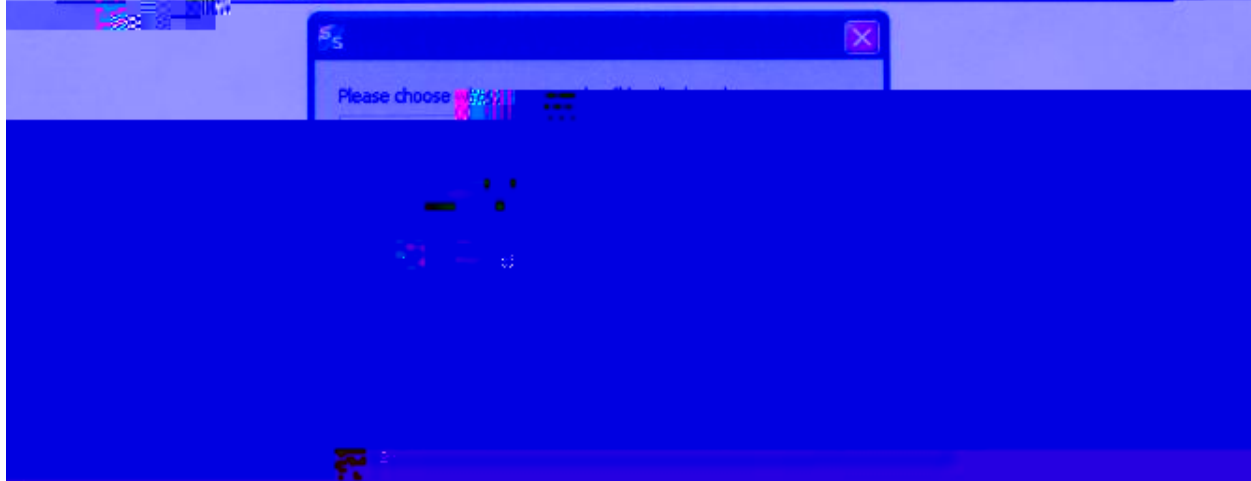


The screenshot displays the 'Set Acquisition Parameters wizard (2 of 4)' in the Ocean Optics software. The interface is divided into several sections:

- Steps:** A list of four steps: 1. Data Source, 2. Acquisition Parameters (highlighted in blue), 3. Integration Spectrum, and 4. Plot Spectrum.
- Integration Time:** A section with a '100 milliseconds' dropdown menu, an 'Enable' checkbox, and a 'Peak peak value' of 3210.0. Below this is a 'Set Automatically' button and an 'Advanced Settings' link.
- Smoothing:** A section with a 'Saves to average' dropdown set to '1' and a 'width' dropdown set to '0'.
- Graphs:** A 'Preview' window on the right shows a spectral plot with a peak at approximately 3210.0. The y-axis is labeled 'Intensity' and ranges from 0 to 4000. The x-axis is labeled 'Wavelength (nm)' and ranges from 300 to 400. A 'Zoom' button is located above the graph.
- Instructions:** A text box at the bottom of the wizard states: 'Turn on the light source and set the integration time so that the peak value reaches the recommended level.' Below this is a 'Help' link.
- Navigation:** At the bottom of the wizard are icons for back, forward, and cancel.
- Bottom Panel:** A 'Data' panel at the bottom of the software window shows a 'Data' table with columns for 'Wavelength (nm)' and 'Intensity'. The table is currently empty.








The units listed in the Data Sources and Data Views panes changes to **Absorbance Mode**.

- The units listed on the Graph pane changes to **Absorbance (OD)**.

10. To permanently save the spectrum to disk, click the Save Spectra () icon on the toolbar.

Note

If you change any of the parameters (e.g., averaging, smoothing, fiber size, etc.) you must store a **new reference and blank spectra**.