Absorbance Experiments

Absorbance spectra are a measure of how much light a sample absorbs. For most samples, absorbance relates linearly to the concentration of the substance. SpectraSuite calculates absorbance (A_λ) using the following equation.

$$A_\lambda = -\log_{10}\left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda}\right)$$

Where:

S_λ = Sample intensity at wavelength λ

D_λ = Dark intensity at wavelength λ

R_λ = Reference intensity at wavelength λ

Typical absorbance setup: The light source (far right) sends light via an input fiber into a cuvette in a cuvette holder (bottom center). The light interacts with the sample. The output fiber carries light from the sample to the spectrometer (top center) connected to the computer (far left).

Absorbance is also proportional to the concentration of the substance interacting with the light (this is known as Beer's Law). Common absorption applications include the quantification of chemical concentrations in aqueous or gaseous samples.

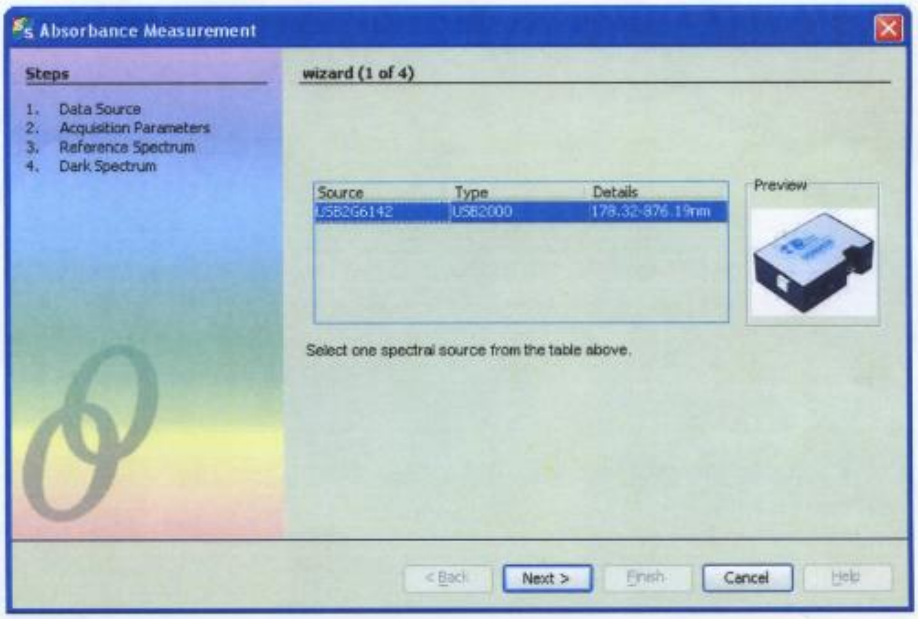
► ***Procedure***

Follow the steps below to take an absorbance measurement using SpectraSuite:

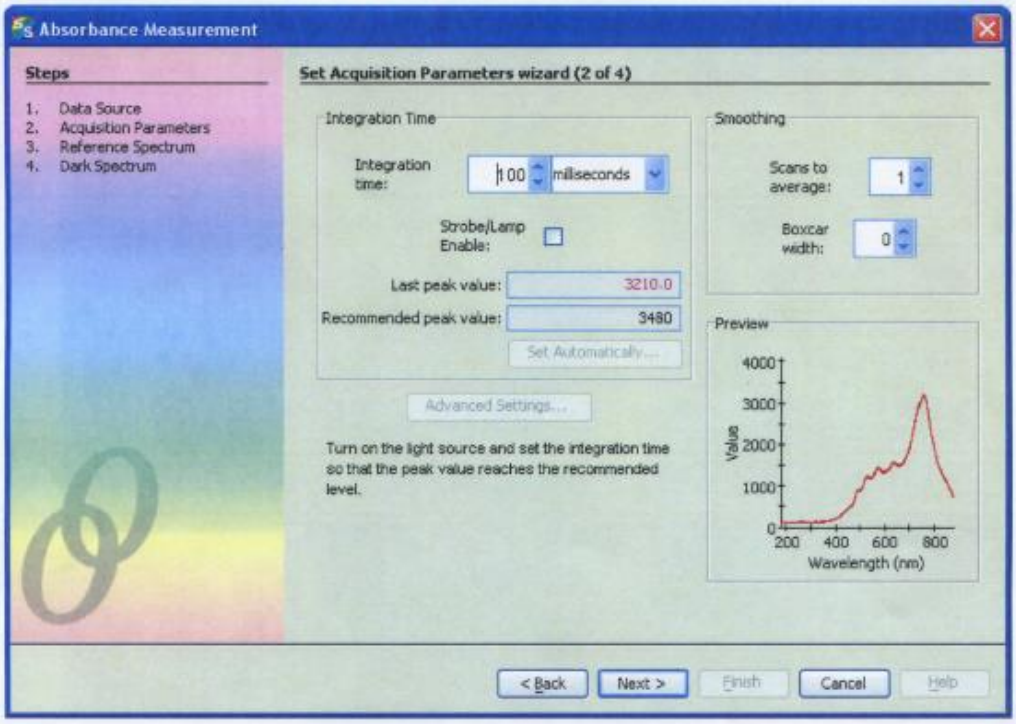
1. Place SpectraSuite in Scope mode by clicking the Scope (S) icon in the Experiment mode toolbar or selecting **Processing | Processing Mode | Scope** from the menu.

2. Ensure that the entire signal is on scale. The intensity of the reference signal's peak differs depending on the device being used. If necessary, adjust the integration time until the intensity is appropriate for your device.

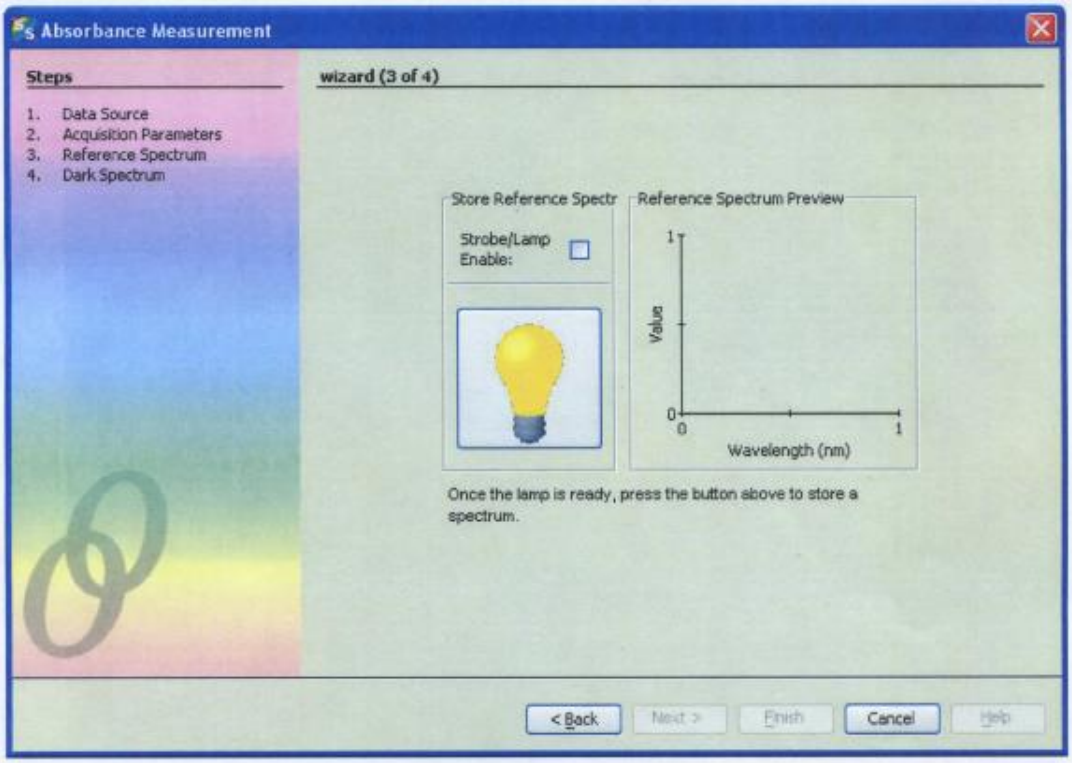
3. Select **File | New | Absorbance Measurement** from the menu or click A to start the Absorbance Measurement Wizard.



4. Select the source of your absorbance measurement and click Next >. The second page of the wizard appears.



5. Turn on your light source and set your acquisition parameters so that the peak value reaches the recommended level. Then click Next >. The third page of the wizard appears.



6. If you have not already done so, place a sample of the solvent into a cuvette to take a reference spectrum. You must take a reference spectrum before measuring absorbance.

Note

Do not put the sample itself in the path when taking a reference spectrum, only the solvent.

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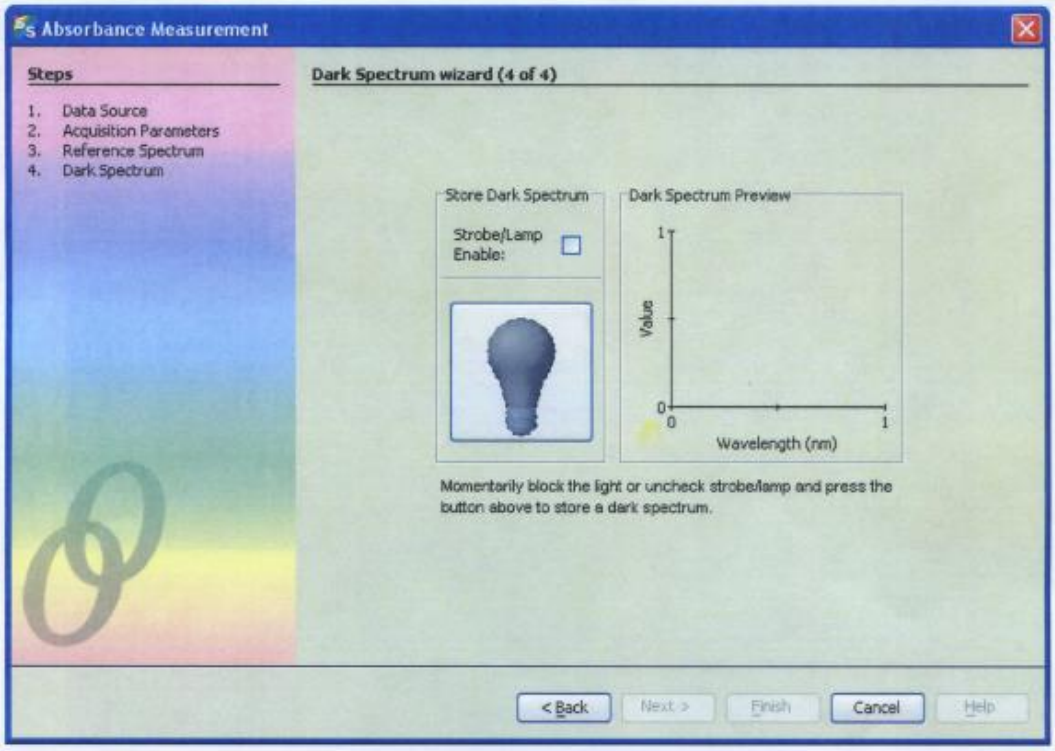
7. Click the Store Reference Spectrum ( ) icon on the screen. This command merely stores a reference spectrum in memory. You must click the Save Spectra ( ) icon on the toolbar or select **File | Save | Save Spectra Collection** from the menu bar to permanently save the reference spectrum to disk.

   Then click Next >. The fourth page of the wizard appears.



8. Block the light path to the spectrometer, uncheck the **Strobe/Lamp Enable** box, or turn the light source off. Then, take a dark spectrum by clicking . You must take a dark spectrum before measuring absorbance.

   This command merely stores a dark spectrum in memory. You must click the Save Spectra ( ) icon on the toolbar or select **File | Save | Save Spectra Collection** from the menu bar to permanently save the spectrum to disk.

Note

If possible, do not turn off the light source when taking a dark spectrum. If you must turn off your light source to store a dark spectrum, allow enough time for the lamp to warm up again before continuing your experiment. After the lamp warms up again, store a new reference.

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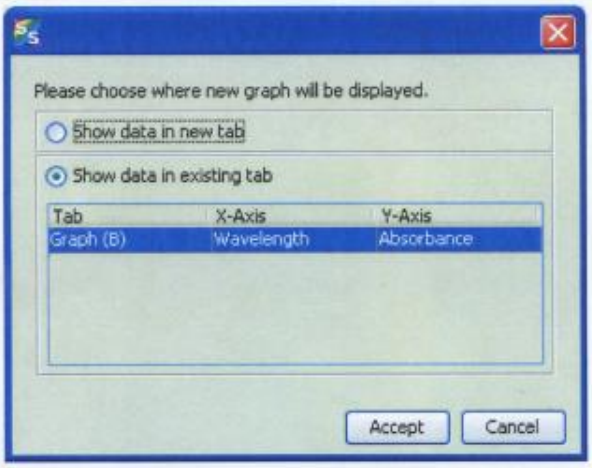
9. Put the sample in place and ensure that the light path is clear. Then, click Finish.

   If you have already taken one or more absorbance measurements, a dialog box appears asking you to specify whether to display the new data in a new graph, or on the existing graph.



Note the following changes on the screen:

- The experiment mode 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 sampling variable (integration time, averaging, smoothing, fiber size, etc.), you must store a new reference and dark spectrum.
