White Paper

AFBR-S20xxx

Absorbance, Transmission, and Reflection Spectroscopy

Introduction

Spectrometry involves the measurement of the intensity of light, and it is important to ensure that the spectrometer provides accurate results. This white paper explains how to perform absorbance, transmission, and reflection measurements using Broadcom® spectrometers and the Waves software.

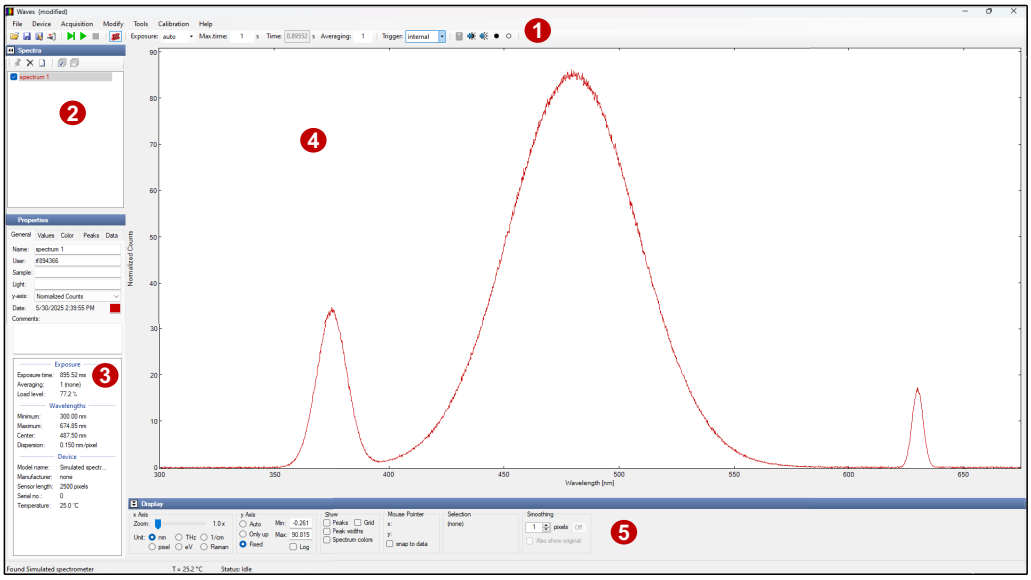
Absorbance, transmission, and reflection spectroscopy is a commonly used measurement technique in analytical chemistry. In these measurement principles, light is directed from a broadband light source onto a sample. The spectrometer detects the absorbed or transmitted light of the sample. In the case of absorbance, the signal relates to the sample concentration and provides significant information about its chemical composition and physical properties.

Waves Graphical User Interface

Waves is free-of-charge software provided by Broadcom to operate Broadcom's miniature spectrometers (you can download it at https://www.broadcom.com/products/optical-sensors/spectrometers). Waves provides a graphical user interface (GUI) with all the features needed to evaluate Qseries spectrometers.

Figure 1 provides an overview of the main areas of the GUI.

Figure 1: Waves GUI



1. Exposure toolbar
2. Spectra list
3. Properties panel
4. Spectrum window
5. Display parameters

How to Perform Absorbance and Transmission Measurements

To perform absorbance or transmission measurements, you need the following equipment at a minimum.

Light Source

For most applications that use this type of measurement technique, a broadband light source with a wide spectral range is a good choice. Alternatively, light sources with a smaller bandwidth can be used (for example a tungsten-halogen lamp).

Sample

Depending on your substance and its physical property (gas, fluid, solid), a suitable sample holder or sample chamber is needed. Determine what sample containment is the most suitable, and make sure that you select the correct material. For example, you cannot use a plastic cuvette for measuring UV signals because it is not transparent for ultraviolet light.

Spectrometer

Absorbance and transmission bands are found in many wavelengths from UV over VIS to NIR regions. Therefore, all Qseries spectrometers are a good choice. The spectrometer measures the intensity of the light that passes through the sample.

Fiber

Depending on your optical setup, you can work with an SMA 905 multimode fiber to guide the light between the emitter and the sample and between the sample and the spectrometer. Additional optics are another option to collimate the light beams properly through samples or to the spectrometer to perform accurate and reliable measurements.

Data Processing

You can easily transfer the measured data to a PC using a USB cable. The Broadcom Waves spectroscopy software helps analyze the spectra. For industrial integration, you can also work with the other serial interfaces (UART and SPI) in combination with the Broadcom software development kit.

Measurement Setup

This section shows a schematic and a picture of a typical setup for absorbance measurements.

Figure 2: Schematic of a Typical Absorbance Measurement Setup

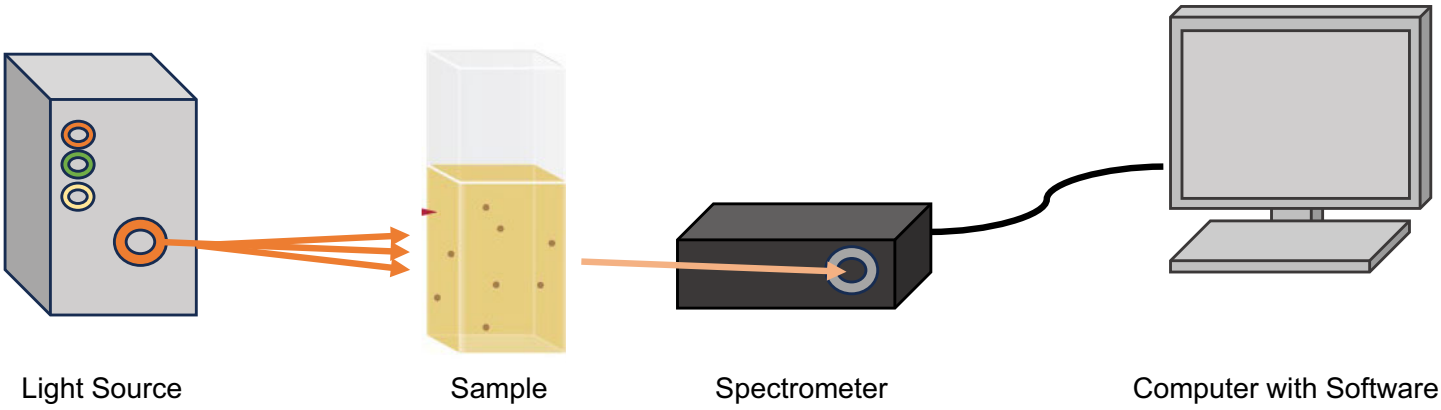
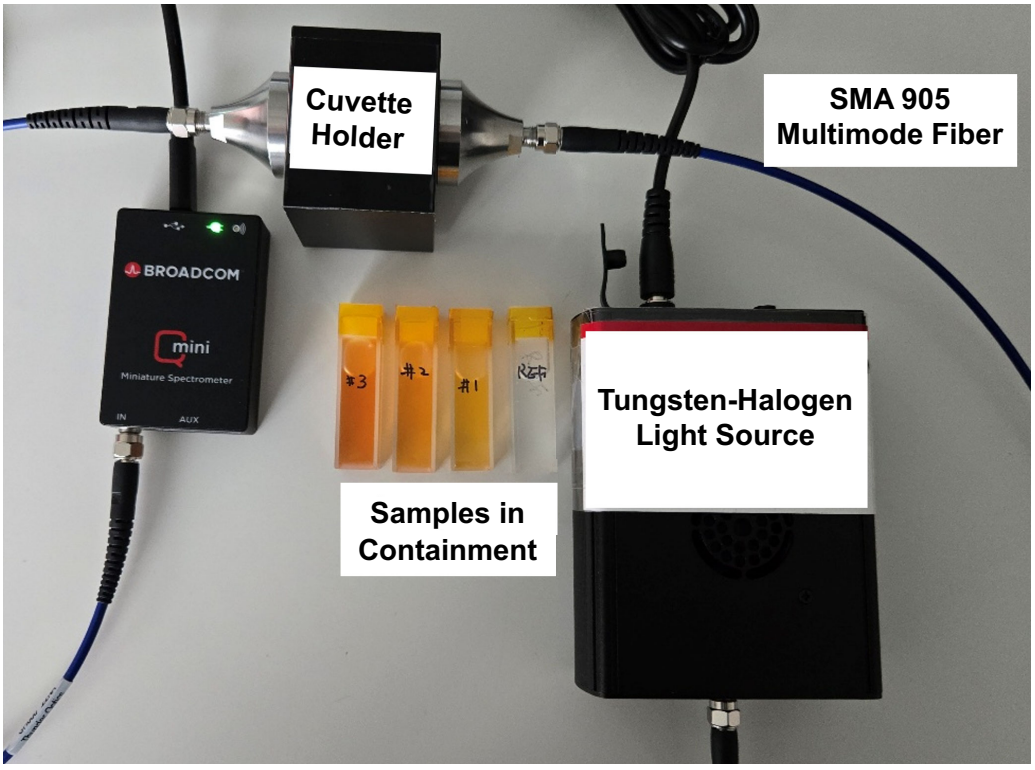


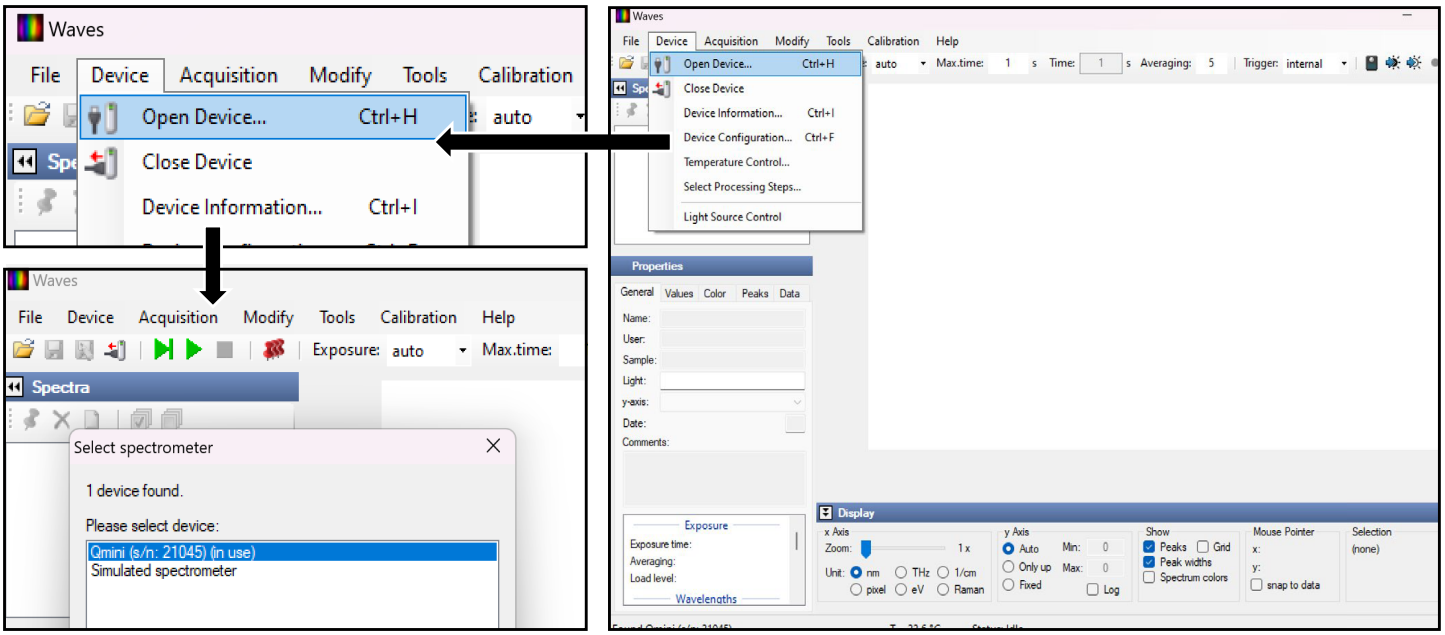
Figure 3: Picture of a Typical Absorbance Measurement Setup



Performing Measurements in the Waves Spectroscopy Software

This section describes the steps to connect the spectrometer to your PC and acquire spectra using the Waves software. Once your measurement setup is properly assembled and the spectrometer is connected to your PC, open the Broadcom Waves software and establish a connection to your spectrometer.

To establish a connection, select **Device > Open Device** and choose your spectrometer.



Step 1. Find the Exposure Time

Before acquiring spectra, you must set the exposure time for the sensors.

Waves allows two exposure modi:

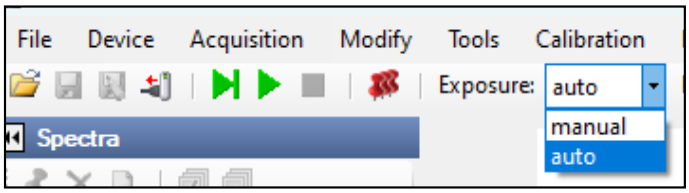
- Auto: The spectrometer chooses the exposure time such that the sensor load is approximately 70%.
- Manual: You can select the desired exposure time.

Unless there is requirement on the exposure time set by your application needs, Broadcom recommends acquiring one spectrum in auto mode to let the spectrometer find the ideal exposure time.

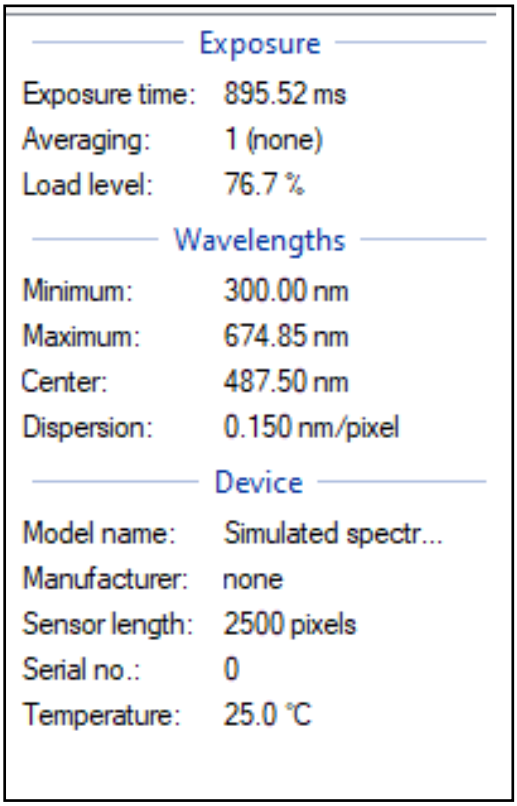
NOTE: Ensure that the light intensity during this measurement corresponds to the maximum intensity during all further evaluations. For example, for absorption measurements, use a dummy reference sample with no absorption (for example, an empty cuvette).

The spectrometer will automatically determine the exposure time.

NOTE: Once the exposure time is set automatically, change to manual exposure. The spectrometer will keep the initially determined exposure time for all future measurements.

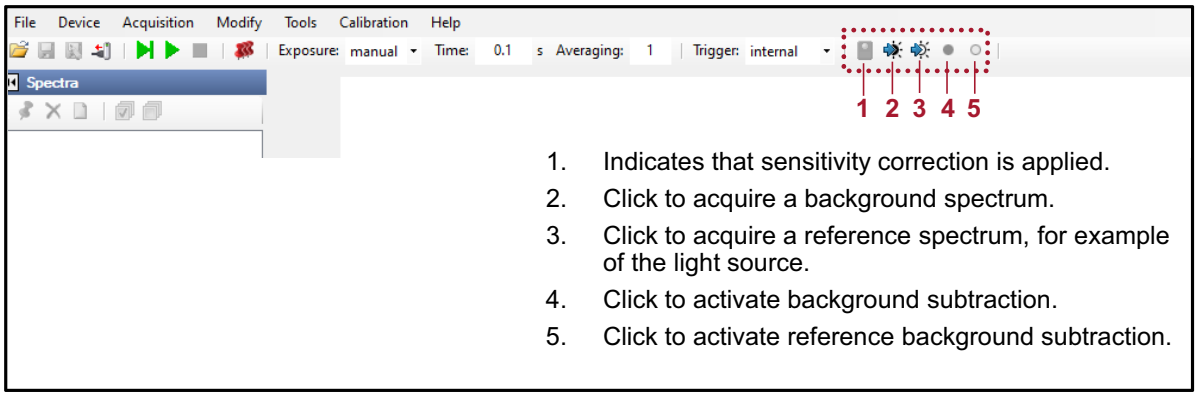


The exposure time and the sensor load level are displayed in the Properties panel of the Waves GUI and are saved as metadata for each spectrum.



Once you have properly set the exposure time, Broadcom recommends that you acquire a background and reference spectrum to calibrate the system. Both calibration steps are implemented in the Exposure toolbar of the Waves GUI.

Figure 4: Predefined Functions for Background and Reference Spectra Calibration in the Waves GUI



1. Indicates that sensitivity correction is applied.
2. Click to acquire a background spectrum.
3. Click to acquire a reference spectrum, for example of the light source.
4. Click to activate background subtraction.
5. Click to activate reference background subtraction.

Step 2. Acquire the Background Spectrum

To take a background spectrum, turn off the light source, keep the adjusted exposure time, and click **Take Background Spectrum** ([ ]) from the Exposure toolbar (#2 in Figure 4). To activate background spectrum subtraction for future measurements, click the **Use background spectrum** icon (#4). The icon changes from a black disc to a black disc with rays, indicating that background spectrum subtraction is activated ( ). If a spectrum is later taken, the background spectrum is automatically subtracted from the measured spectrum, eliminating unwanted background signals.

Step 3. Acquire the Reference Spectrum

Acquire a reference spectrum for either reflection spectroscopy or transmission and absorption spectroscopy.

Reflection Spectroscopy

To take a reference spectrum for reflection spectroscopy, a white reference sample is required to calibrate the setup for 100% reflection. However, all real-world reference samples have a reflection of less than 100% and an uneven spectral distribution. Therefore, for accurate measurements, a reference spectrum must be supplied for the reference sample. To take a reference spectrum, place the white reference sample where the measured sample will be later placed. Then, keep the adjusted exposure time and temporal averaging, and click **Take Reference Spectrum** ([ ], #3 in Figure 4). In the dialog window that follows, specify a data file that includes a calibrated spectrum of your reference sample. After selecting **Use Reference Spectrum** (, #5 in Figure 4), measure the reflectivity spectra.

Transmission and Absorption Spectroscopy

To take a reference spectrum for transmission or absorption spectroscopy, turn on the light source again, remove the sample or replace it by a reference sample, keep the adjusted exposure time and temporal averaging, and click **Take Reference Spectrum** ([ ], #3 in Figure 4). After selecting **Use Reference Spectrum** (, #5 in Figure 4), take spectra of the sample for measuring absorption or transmission. If the light source does not cover the full range of the spectrometer, the calculated spectrum might show large amounts of noise outside the wavelength range of the light source. This is normal and reflects the fact that the measurement has a large uncertainty at these wavelengths.

These measurements are quite sensitive to changes in the sample illumination. If in doubt, take another reference spectrum to ensure that it is still valid. Accurate calibration and sample preparation are essential for measurement performance.

The following table provides a brief overview of the typical preparation steps for reflection, transmission, and absorption spectrum measurements.

Table 1: Summary of Calibration Steps to Prepare the Setup for Measurements

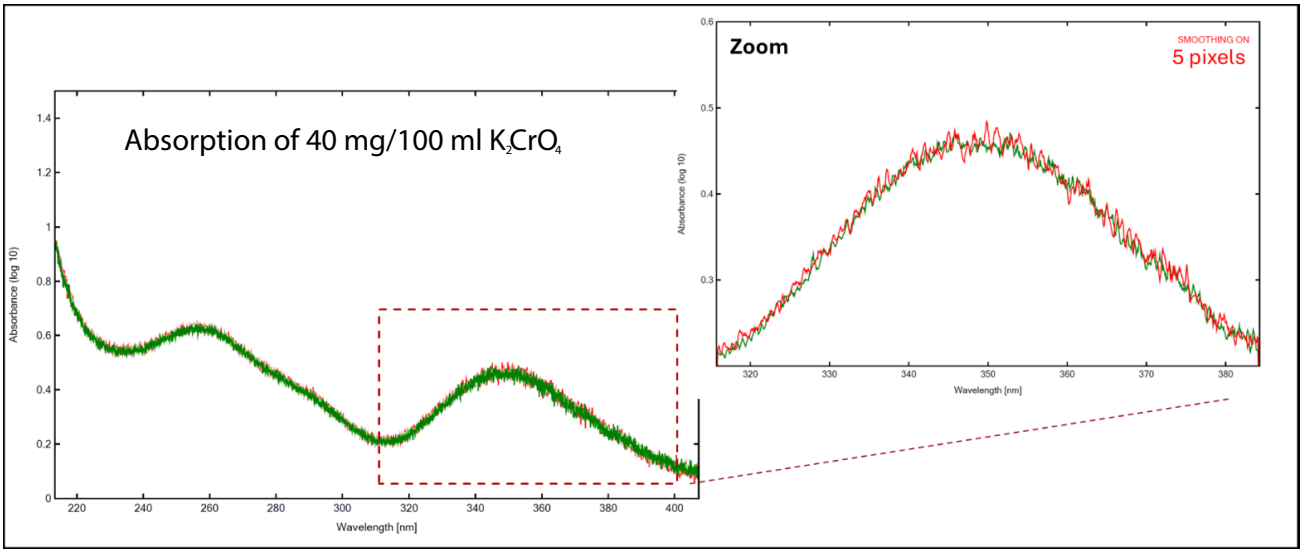
Step	Measurement Type	Exposure	Applied Corrections	Notes
1.	Exposure time	Auto	■ None	■ Light source **on**.
2.	Background spectrum for subtraction	Manual	■ Sensitivity correction	■ Light source **off**. ■ To apply the sensitivity correction, select **Device > Select Processing Steps**.
3.	Reference spectrum	Manual	■ Sensitivity correction ■ Background spectrum	■ Activate background subtraction (measured in Step 2). ■ Light source **on**. **NOTE:** The setup must be identical to the final setup. A dummy sample must be inserted to also correct for the absorbance of the probe holder and the like.
Start the absorbance measurement.		Manual	■ Sensitivity correction ■ Background spectrum ■ Reference spectrum subtraction	■ Activate reference spectrum subtraction (measured in Step 3). ■ Light source **on**.

Providing high reproducibility between setups in the preceding steps is crucial.

Using an optimized exposure time and adequate background and reference spectrum calibration, nonuniformities between setups (for example, intensity differences of the light sources and the quality of the light coupling into the spectrometer) can be corrected, and very high uniformity between measurement setups can be achieved.

Figure 5 shows an absorption measurement of a 40 mg/100 ml K_2CrO_4 using two Broadcom miniature spectrometers with different entrance slit settings (20 mm and 100 µm) to simulate variations in the uniformity of the light source and the quality of the light coupling into the spectrometer. Excellent reproducibility between the measurements of the two miniature spectrometers with different hardware settings is obtained by properly calibrating the measurement setup as previously described in Step 1, 2, and 3.

Figure 5: Absorption Spectrum of 40 mg/100 ml of Potassium Dichromate

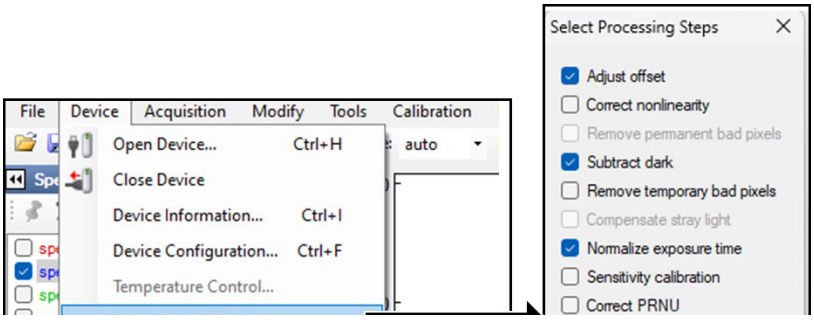


Additional Tips and Hacks

This section provides additional guidance on the basic features of the Waves software.

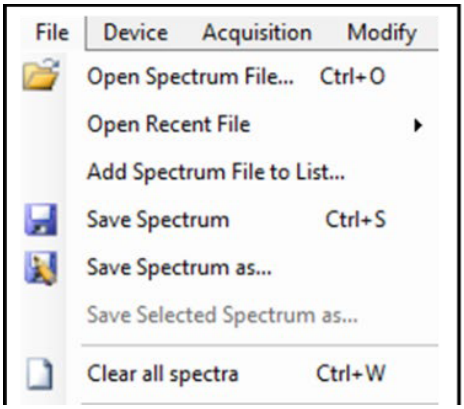
Calibrate the Sensitivity

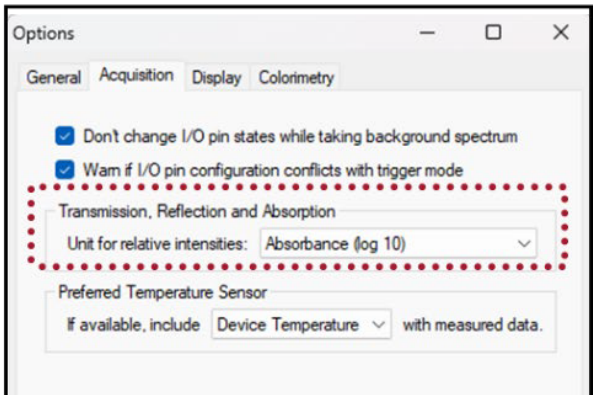
All Broadcom miniature spectrometers are calibrated for nonlinearity coefficients, offset, and sensitivity. The calibration data is stored in the EEPROM. You can turn on/off these calibrations under **Device > Select Processing Steps** in the Exposure toolbar.



Change the Y-Axis Scale

Select **File > Options > Acquisition > Unit for relative intensities**.





The transmittance is usually written as a percentage.

The spectral absorbance is sometimes defined as a base 10 logarithm and sometimes as a natural (base e) logarithm. In the Waves software, both options are available.

Save the Spectrum

In the default settings, each acquisition overwrites the previously acquired spectrum. To keep all spectra, activate “Keep all new spectra” ( ). All acquired spectra will now be visible in the Spectra list. To select or unselect individual spectra to be displayed in the Spectra window, select the box.

To save the acquired spectra, select **File > Save Spectrum**. The standard format in which the spectra are saved is a *.spz file. The format is a tab-delimited ASCII file and can be imported like a *.txt file to many common software tools such as MS Excel and Python.

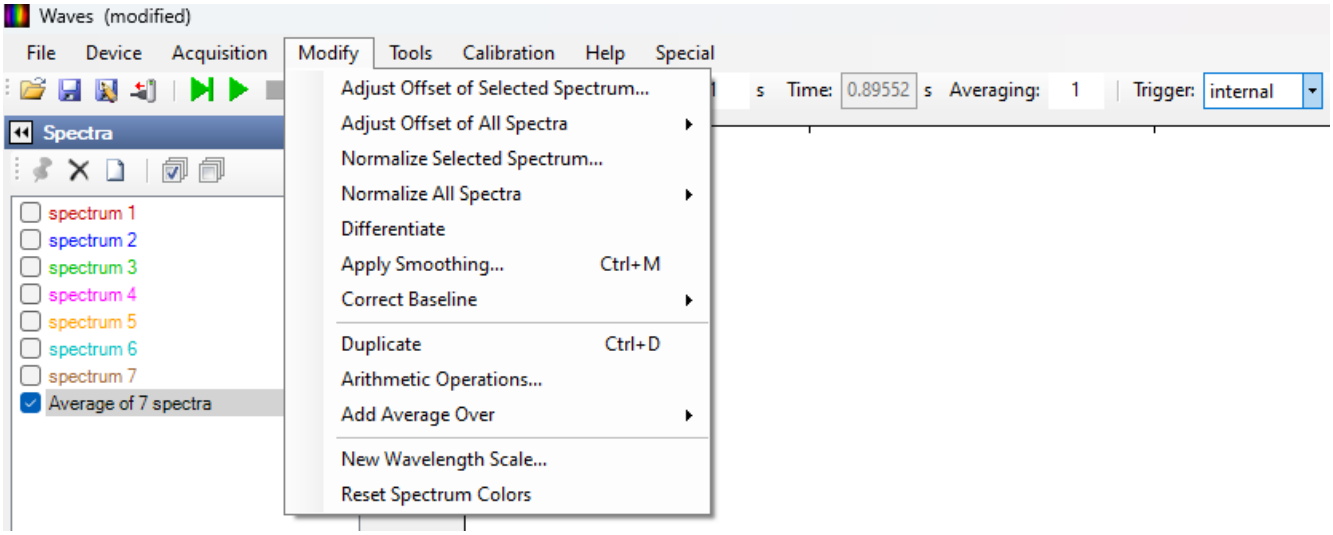
Compare with Various Spectra

To compare your latest spectra with reference data or older data, you can import spectra using the import function of Waves (**File > Add Spectrum to File to List**).

Manipulate/Analyze Multiple Spectra

In the Modify menu of the Exposure toolbar, the Waves GUI provides you with the basic features to work with the acquired spectra:

- Normalization
- Smoothing
- Arithmetic operations
- Averaging



If you need any help, contact Broadcom at support.spectrometer@broadcom.com.