

ISA-220 Integrating Sphere Accessory for the Evolution 220 UV-Visible Spectrophotometer

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Introduction

The ISA-220 is an Integrating Sphere Accessory (ISA) that fits into the sample compartment of the Evolution™ 220 Spectrophotometer. The ISA-220 is also fully compatible with the Evolution 260Bio Spectrophotometer. Installation of an ISA-220 in an Evolution 201 is possible, but is not recommended because its performance is severely limited at the Evolution 201's fixed 1nm spectral bandwidth.

The ISA expands the capabilities of the Evolution 220 instrument to include the measurement of near-normal diffuse reflectance, and transmittance of scattering materials and turbid solutions over the 200 nm – 1100 nm spectrum. The ISA-220 is equipped with its own 10mm x 10mm silicon photodiode detector and detector preamplifier board. A DB25F-DB25M cable connects the detector pre-amplifier to the instrument, providing both electrical power and data signal transmission.

The ISA-220 is fitted to a standard Evolution kinematic base and installs directly in the path of the sample beam. This means that there are no optical components to align upon installation. A silver thumb-screw on the front of the sphere assembly connects the sphere to the kinematic base. If samples are to be measured in reflectance mode, the sphere is positioned at the right side of the base. If samples are to be measured in transmittance mode, the sphere is positioned at the left side of the base.

Reflectance and transmittance measurements are recorded using INSIGHT software in the same manner as during other spectroscopy applications.

Unpacking and Inspection

Your ISA-220 accessory was thoroughly inspected before shipping and should be ready to operate after completing the set-up instructions. The accessory is packaged and shipped in a reinforced shipping container. Carefully check the components after unpacking for any damage that may have occurred during shipping. If there is any such damage, file a claim immediately with the freight carrier and contact the Thermo Scientific Customer Service Department at

1-800-532-4752 or by email at us.techsupport.analyze@thermofisher.com

The following standard components are included with your ISA-220 Accessory:

Accessory kit consisting of

- The CD with this manual on it
- 25 pin cable
- 0° plate for mounting Spectralon disc during transmittance measurements
- 1A mesh filter
- Uncalibrated 1" Spectralon diffuse reflectance standard

Assembled ISA-220 accessory consisting of:

- Evolution kinematic mount baseplate with T-handle
- Integrating sphere with attached detector and pre-amp board
- Base rail with spring loaded reflectance sample clamp (attached)
- 8° wedge (attached)

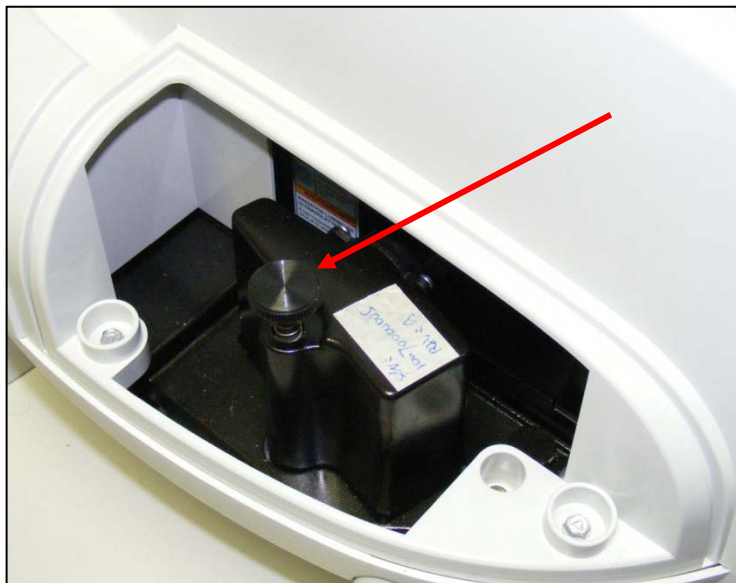
Installation

The Evolution 220 spectrophotometer must be turned off during installation/removal of the accessory.

Complete the steps below in order. Photographs are included to help to identify the components named.

Step 1

1. Ensure that the spectrophotometer is powered off.
2. Pull up on the detector chamber cover to remove it. Set it aside carefully.
3. Turn the thumb screw on top of the sample detector counterclockwise until it spins freely.
4. Grasp the detector module and lift straight up to remove the detector from the instrument. Store the sample detector module in a safe place. You will need to re-install it to use the instrument without the ISA-220.



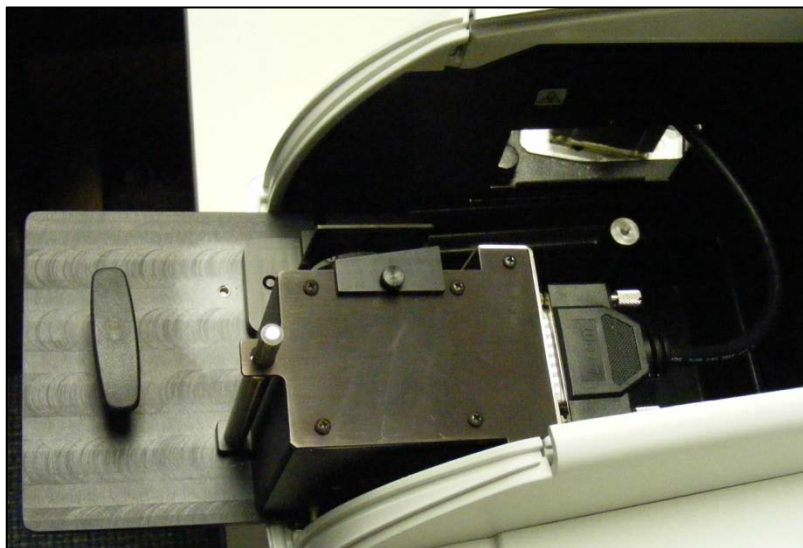
Step 2

1. Open the sample compartment sliding lid.
2. Remove the sample compartment front panel and set it aside.
3. Remove the standard sample holder baseplate and store it in a safe place. You will need to re-install it to use the instrument without the ISA-220.
4. There is a hatch cover on the left wall of the sample compartment, between the sample compartment and the detector compartment. Loosen the brass thumb screw that secures this cover and remove the cover. Store the cover with the detector module.



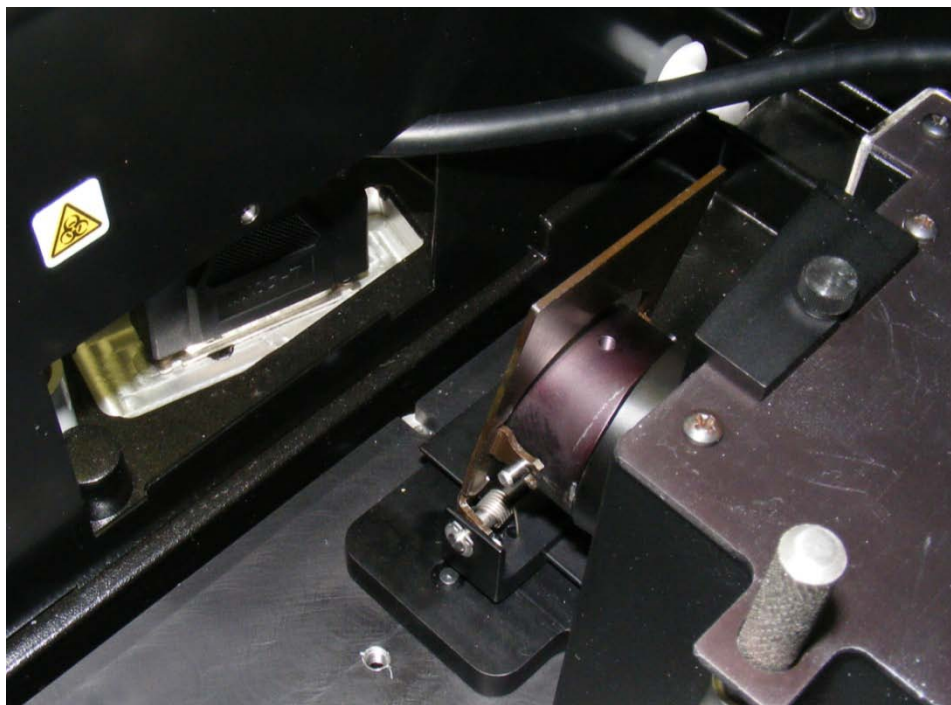
Step 3

1. Remove the ISA-220 from its packaging.
2. Remove the tape and the white cloths from the windows of the sphere.
3. Attach the DB25 cable to the socket at the back of the sphere. Use the screws to attach it securely.
4. Rest the base-plate of the sphere on the front edge of the sample compartment. Pass the cable through the hatch in the left wall of the sample compartment and plug it into the socket where you removed the standard detector in step 1.



5. Grasp the ISA-220 by the T-handle, lift it slightly, and move it towards the back of the sample compartment until you can locate the two holes in the base-plate on the two mounting pins in the sample compartment. Lower the front of the baseplate and push downward until it snaps into place behind the kinematic mount.

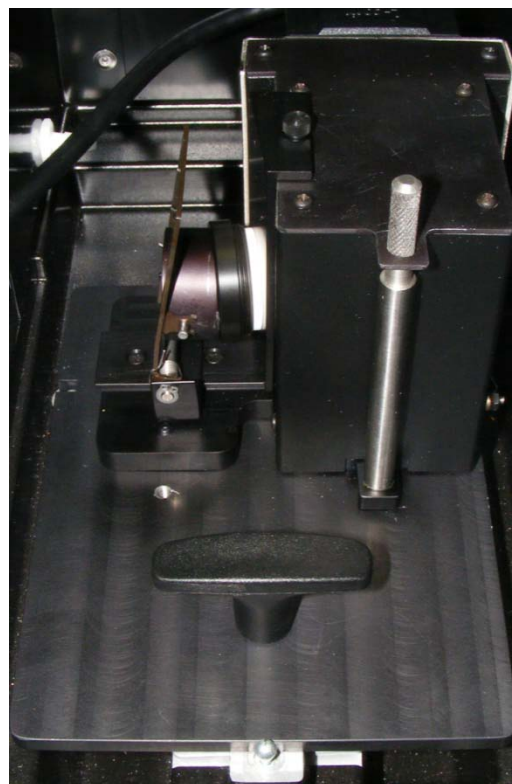
Note: The connector cable is only just long enough. You may find it useful to have a helper hold the connector in place in the detector compartment during the first installation. Once the cable has adjusted from its installed shape subsequent installations will be easier.



Step 4

1. Verify that the cable is properly attached to the sockets at both ends.
2. Replace the detector chamber cover.
3. Unpack the 1A mesh filter that shipped with the accessory. Open the reference compartment of the spectrophotometer. Lift out the cell carrier and place the 1A mesh filter in position. Replace the cell carrier and close the compartment.
4. Unpack the Spectralon disc that shipped with the accessory. The disk is mounted in a black plastic holder that protects it when not in use. Unscrew the two halves of the holder to reveal the surface of the Spectralon. Do not touch the Spectralon with your bare hand or allow it to come into contact with chemicals or surfaces other than the components of the ISA-220.
5. Position the Spectralon disc under the clamp at the reflectance port.
6. Replace the sample compartment front panel and close the lid.
7. Power up the instrument and wait about 4 to 6 minutes for it to complete its boot sequence.

Your ISA-220 is now installed and ready to use.



Operating in Reflectance Mode

The ISA-220 is packaged in the correct configuration for operation in reflectance mode. The Evolution 220 spectrophotometer has a focused beam with the focal point at the center of the sample compartment. In order to place the sample at the focal point of the beam in reflectance mode, the box containing the sphere is placed on the right side of the base-plate as shown in the figure.

An 8° wedge plate is included with the ISA-220 accessory. This plate serves to reduce the size of the reference port aperture to an area only very slightly larger than the spectrophotometer light beam, and also to position your sample with its surface at 8° off normal. This causes the specular portion of the reflectance to be trapped inside the sphere. Use the 8° wedge if you wish to record measurements that could be described as

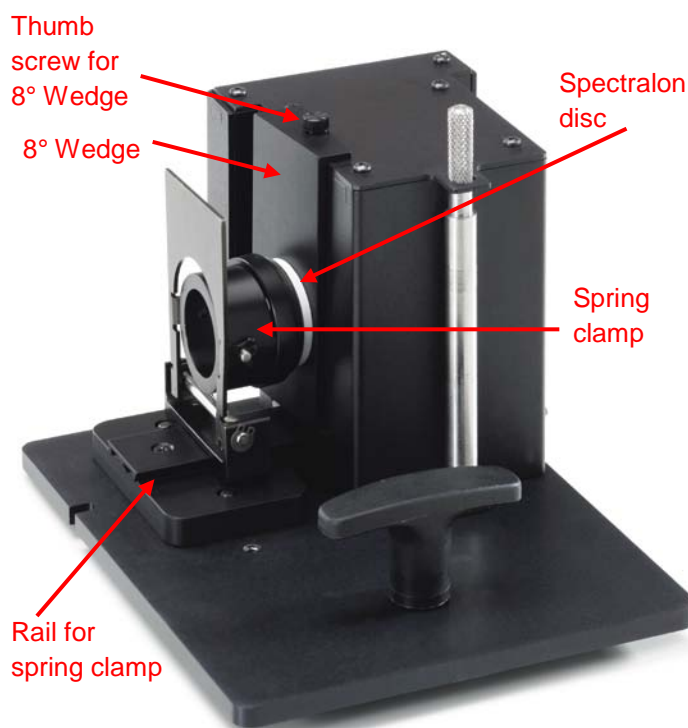
- total reflectance
- specular included
- SPIN.

If you remove the 8° wedge and clamp your reflectance sample directly against the wall of the sphere over the reflectance port, the beam will strike the sample at exactly 90° to the plane of the surface (i.e. normal to the surface). In this configuration, the specular portion of the reflectance will not be trapped inside the sphere, but will exit through the transmittance port on the right side of the sphere. Use this configuration for measurements described as

- diffuse only
- specular excluded
- SPEX.

The clamp for holding reflectance samples in place is spring-loaded. Its base has a rail along which the spring-loaded portion slides, both to accommodate samples of varying thickness and to allow you to alter the amount of pressure exerted by the clamp on the sample. The circular part of the clamp can be rotated through 180°. One side is angled to match the angle of the 8° wedge, the other is parallel to the wall of the sphere assembly and is used for clamping samples in *diffuse only* experiments.

Typically, you would use the included Spectralon disc to record the reference spectrum or blank measurement. The figure above shows the ISA-220 configured for *total reflectance* measurements with the Spectralon disc in place at the reflectance port.



Measuring powders

The optional powder cell holder, PN 222-269600, is sized to fit perfectly in the reflectance clamp (see figure). Record your reference measurement using the cell with the Spectralon disc inside. Unscrew the two parts of the other powder cell and place your powder on top of the quartz window, then replace the steel plug and spring and re-assemble the two halves of the case. Best results for powders are obtained with samples that are finely ground, tightly compressed and at least 3mm deep.

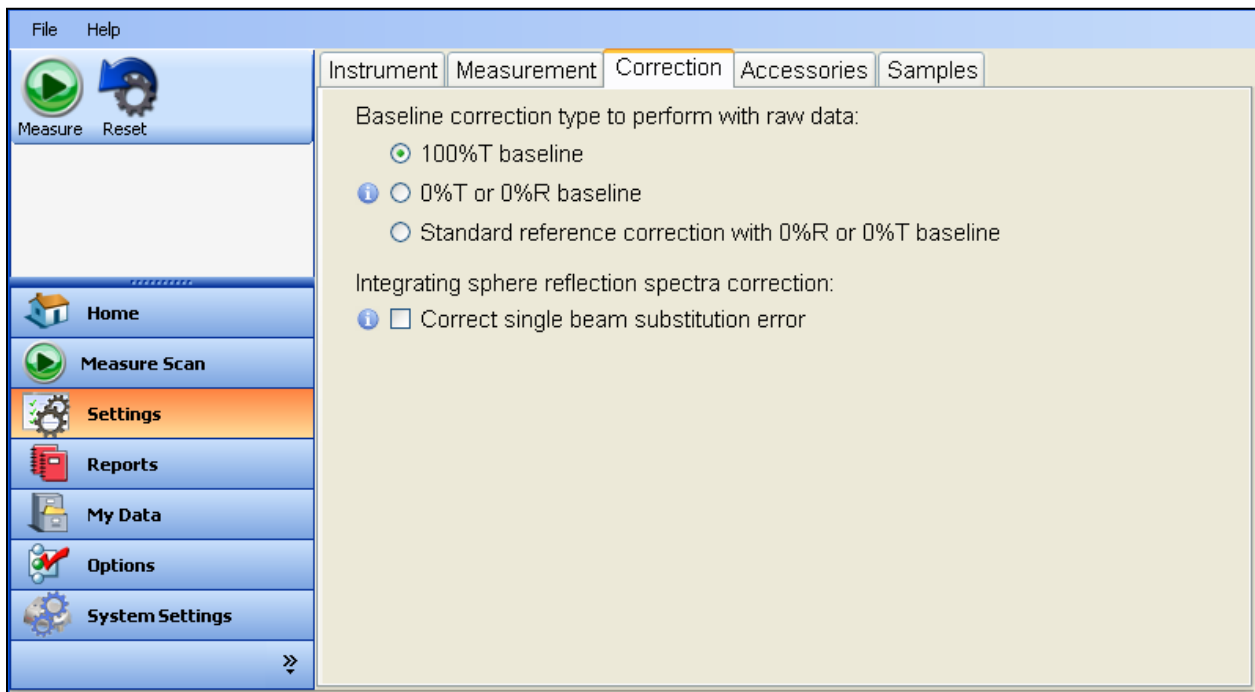


Setting up the Baseline

Decide what kind of baseline you want to use. Go to the **Correction** tab of the settings screen (see figure below) and select one of the three baseline types. Note that from the point of view of the instrument, %T and %R are the same thing.

100%T Baseline

This is the most common baseline. Typically, the included Spectralon disc is used as the reference material. This instrument records the energy at each measurement wavelength that represents *complete* reflectance, and then expresses the energy it sees for a sample measured at this wavelength as a percentage of the baseline value.



0%T or 0%R baseline

All integrating spheres exhibit a small, non-zero signal when there is nothing covering the reflectance port. This is due to scatter in the beam caused by a combination of dust in the air and imperfections in the mirrors in the optical system. Selecting this option means that you run both a 100% and a 0% baseline. The 0% baseline is recorded with the reflectance port open. The instrument subtracts the 0% baseline signal at each wavelength from both the 100% baseline and the sample reading before calculating %R. For high reflectance and comparison work, a 0% baseline may not be necessary. For low reflectance samples and exact measurements this type of baseline is the preferred choice.

Standard reference correction with 0%R or 0%T baseline

In this option, you use a calibrated standard to collect the 100% baseline and then collect a 0% baseline as described above. If you select this option, you will be prompted to upload a calibration file with the certified reflectance values at each wavelength. Calibrated reflectance standards available from Thermo Scientific are certified in 1nm intervals from 250nm to 1100nm. In this option, rather than taking the energy reading at each wavelength as being 100%, the instrument uses the value in the calibration file. This is the choice that will yield the most accurate reflectance results.

Integrating Sphere Reflection Spectra Correction

Results from a single beam sphere such as the ISA-220 can be skewed by *single beam substitution error*. This phenomenon results from the difference in average reflectance of the sphere wall caused by placing a relatively darker material at the sample port during the sample measurement than during the baseline, when the port is covered by Spectralon. The small size of the reflectance port in the ISA-220 minimizes this effect, but it can be corrected for by use of a look-up table. The procedure is described in the Labsphere library, see <http://www.labsphere.com/data/userFiles/Quantitation of Single Beam Substitution Correction.pdf> . Check the box on this window to implement the mathematical correction.

Measuring Your Sample

After setting up your baseline type, collect the baseline/blank and measure your sample using INSIGHT software. INSIGHT features an application focused beam geometry (AFBG) (selectable in the Bandwidth drop-down menu of the Instrument tab in Settings) called *Materials* which is a large aperture, high energy throughput setting designed specifically for use with the ISA-220. The spectral bandwidth of this setting is ~15nm, so it will not allow you to resolve sharp or closely spaced peaks, but the *Materials* AFBG does yield outstanding sensitivity, especially in low transmittance/reflectance samples. The *fiber* and *2nm* AFBG settings are also suitable for use with the ISA-220, but the lower energy throughput associated with these settings means that longer integration times are necessary to control noise and the maximum dynamic range of the system is lower.

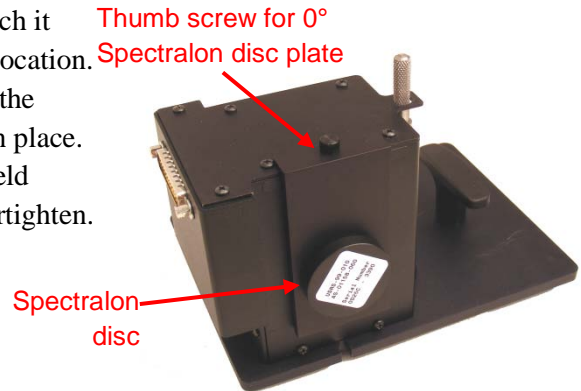
Operating in Transmittance Mode

Converting the ISA-200 from Reflectance to Transmittance Mode

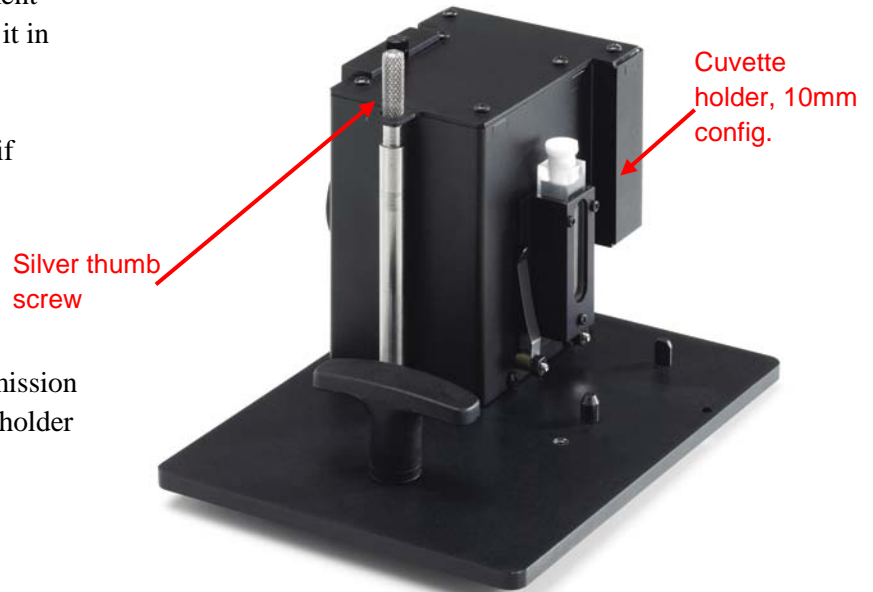
1. Power down the instrument. Accidental disconnection of the cable between the ISA-220 and the instrument during this process could seriously damage the detector pre-amplifier.



2. Loosen the black thumb screw that holds it, and remove the 8° wedge.
3. Lift up on the spring-clamp and rail assembly to detach it from the locating pins. Store the assembly in a safe location.
4. Install the 0° Spectralon disc plate on the left side of the sphere and tighten the black thumb screw to lock it in place.
5. Screw the Spectralon disc into the holder until it is held firmly in place over the reflectance port. Do not overtighten.

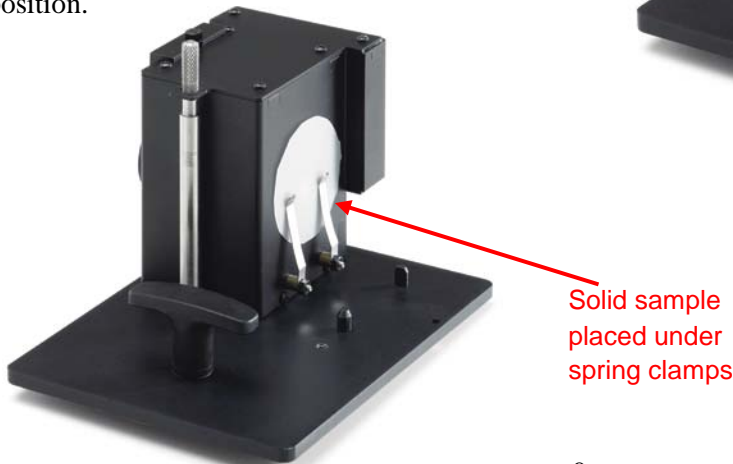


6. Loosen the silver thumb screw at the front of the sphere case until its threads come clear of the base and lift the sphere up off the locating pins.
7. Position the sphere on the locating pins at the left side of the sample compartment and tighten the thumb screw to hold it in place.
8. Install the cuvette holder (optional accessory) at the transmittance port if desired.



Positioning Solid Samples

Spring-steel clips on either side of the transmission port hold either solid samples or the cuvette holder in position.



The Cuvette Holder (PN222-269500)

The cuvette holder comes assembled for use with 10mm cuvettes. It can also be used with longer path cuvettes up to 50mm pathlength by installing the three stand-off bars that ship with this optional accessory. Simply remove the three screws that attach the back-plate to the body of the cell holder, screw in the stand-offs, then re-attach the back-plate to the stand-offs. The lower stand-off sits flush with the bottom of the body of the cell holder so that cuvettes of any supported length rest on a level surface.



Measuring Your Sample

After setting up your baseline type, collect the baseline/blank and measure your sample using INSIGHT software. INSIGHT features an AFBG (selectable in the Bandwidth drop-down menu of the Instrument tab in Settings) called *Materials* which is a large aperture, high energy throughput setting designed specifically for use with the ISA-220. Spectral bandwidth in this setting is ~15nm, so it will not allow you to resolve sharp or closely spaced peaks, but the *Materials* AFBG does yield outstanding sensitivity, especially in low transmittance/reflectance samples. The *fiber* and *2nm* AFBG settings are also suitable for use with the ISA-220, but the lower energy throughput associated with these settings means that longer integration times are necessary to control noise and the maximum dynamic range of the system is lower. Some experimentation may be necessary to determine the optimum AFBG for your sample. In general, the more opaque your sample is the more energy you want in the beam.

Converting the ISA-200 from Transmittance to Reflectance Mode

- 1. Power down the instrument. Accidental disconnection of the cable between the SA and the instrument during this process could seriously damage the detector pre-amplifier.**
2. Remove any cuvette or other sample holders from the transmittance port.
3. Remove the Spectralon disc from the fitting on at the reflectance port and remove the fitting also.
4. Loosen the silver thumb screw at the front of the sphere case until its threads come clear of the base and lift the sphere up off the locating pins.
5. Position the sphere on the locating pins at the right side of the sample compartment and tighten the thumb screw to hold it in place.
6. Slide the spring clamp onto the rail (if it was separated) and position the rail base on the locating pins at the left side of the sample compartment.
7. Install the 8° wedge over the reflectance port (if you intend to use it) and tighten the thumb screw that hold it in place. Note: There is a small plastic spring pin on the lower edge of the 8° wedge. This pin must sit on top of the rail, not at the side of the rail, as the latter position will cause the port in the wedge to be misaligned.



Initial Performance Qualification Testing

Thermo Fisher Scientific recommends that you perform these tests upon initial installation of the accessory in order to verify that it is properly installed and functioning correctly. There are no optical components that could go out of alignment during removal and re-installation, so once proper function has been verified there is no reason to expect that the accessory will not perform properly in subsequent installations.

Perform all tests in reflectance mode with the accessory configured as described in step 4, above.

Enter the Scan application by selecting it from the Home screen.

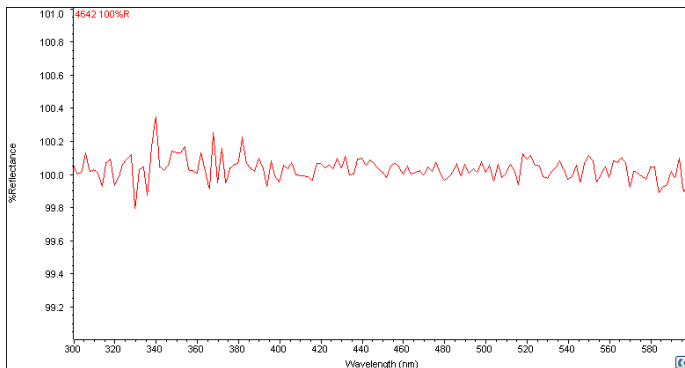
Set up a scan method with parameters as shown here.

Click Measure to record a baseline

Instrument	
Data mode:	% Reflectance
Factor:	0.1
Smooth:	None
Derivative:	None
Start wavelength:	600.00 nm
End wavelength:	300.00 nm
Bandwidth:	2 nm
Integration time:	0.30 sec
Data interval:	2.00 nm
Scan speed:	400.00 nm/min

100%R noise test

Leave the Spectralon disk used for the baseline in place and measure it as a sample. The spectrum should be a line along 100%R with a peak to peak noise level of less than 1.0%. See figure.

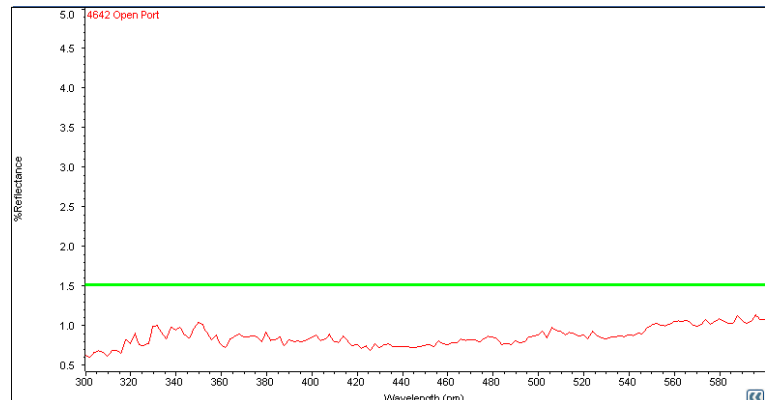


Open port test

Remove the Spectralon disc and slide the clamp up against the side of the sphere so that it sits flush with the outside of the sphere. With nothing over the reflectance port, the light beam from the monochromator exits the sphere and a near zero reading results.

Record a scan. There is always some stray light inside a sphere due to reflection of the beam from dust particles in the air. This should be less than 1.5%R (see figure), and will generally be significantly less.

If the accessory is not mounted in the sample compartment correctly, the beam, rather than going out through the reflectance port will reflect off the wall near the port and the signal will be significantly higher. If the signal is more than 1.5% in the defined wavelength range, remove and re-seat the accessory, then re-collect a baseline and repeat this test *before* calling technical service.



Appendix A Accessory Specifications

Parameter	Specification
Operation	
Spectral Range	220nm – 1100nm
Maximum transmittance sample size	60mm high x 60mm wide x 50mm thick
Maximum reflectance sample size	60mm high x 60mm wide x 40mm thick
Integrating Sphere	
Diameter	50mm
Reflective surface	Spectralon®
Detector	10mm x 10mm Si photodiode

Appendix B Typical Spectralon Reflectance Values

Wavelength	Spectralon 8° Hemispherical Reflectance
250	.973
300	.984
400	.991
500	.991
600	.992
700	.992
800	.991
900	.991
1000	.993
1100	.993