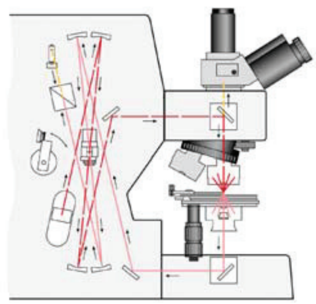
Transmission Analysis



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Objectives

Issues affecting Transmission

- *Diffraction*
- *Refraction*
- *Reflection*
- *Scatter*

Sample preparation to optimize data quality

Proper use of sampling accessories

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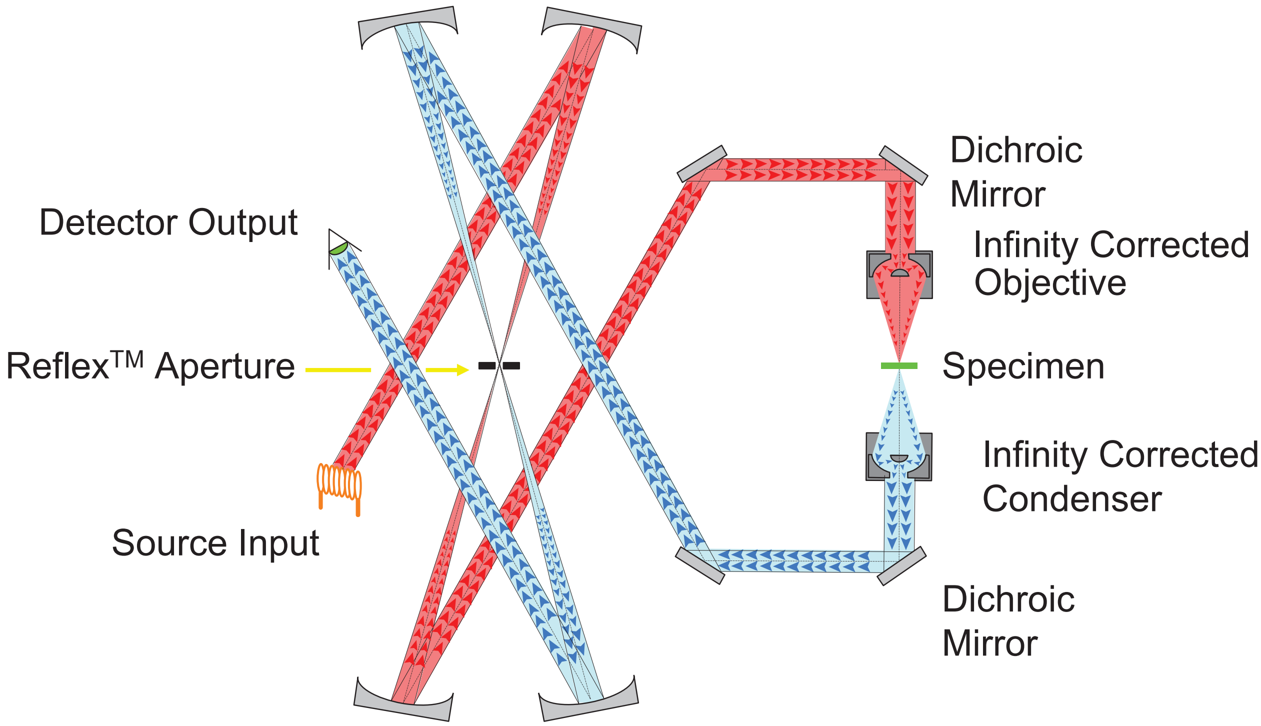
During this presentation the analyst will learn about issues that affect the quality of Transmission data, proper use of sampling accessories, and proper sample preparation techniques to minimize sampling errors.

IR Transmission Path Diagram



Transmission Challenges

- **Fundamental Optical Factors Affecting FT-IR Microscopy**
  - **1. Diffraction**
  - **2. Refraction**
  - **3. Reflection**
  - **4. Scatter**
- **Sample preparation and optical corrections can address all of these factors**
  - **Dual apertures minimize diffraction problems**
  - **Compensator rings address refraction problems**
  - **Sample preparation can minimize sample reflection**
  - **Baseline corrections can be applied for scatter problems**

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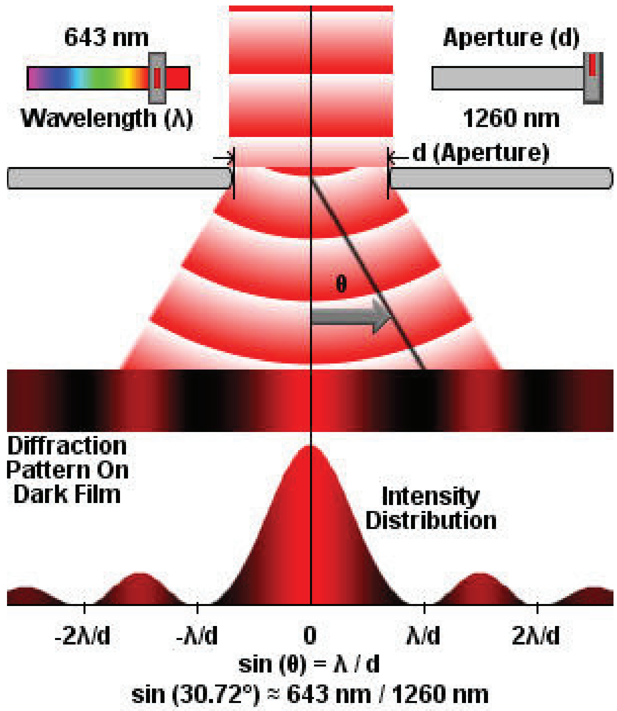
Transmission analysis is the most difficult analysis technique because of the added instrumental complexity with the condenser, sample preparation details, and light interactions with the sample that can reduce the quality of infrared spectral results.

All of the above interactions result in baseline shifts due to spectral contributions from the matrix surrounding the sample area of interest. Many of these optical effects can be mitigated through sampling techniques and instrumental features discussed in detail during this presentation.

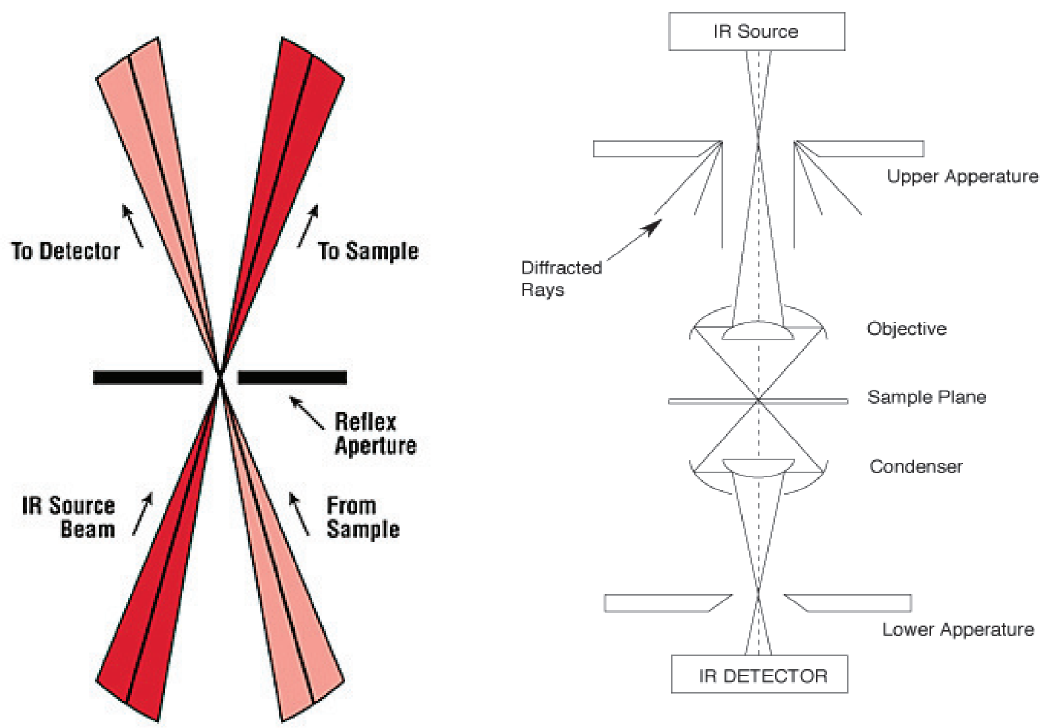
Diffraction



http://micro.magnet.fsu.edu/primer/java/diffraction/basicdiffraction/index.html

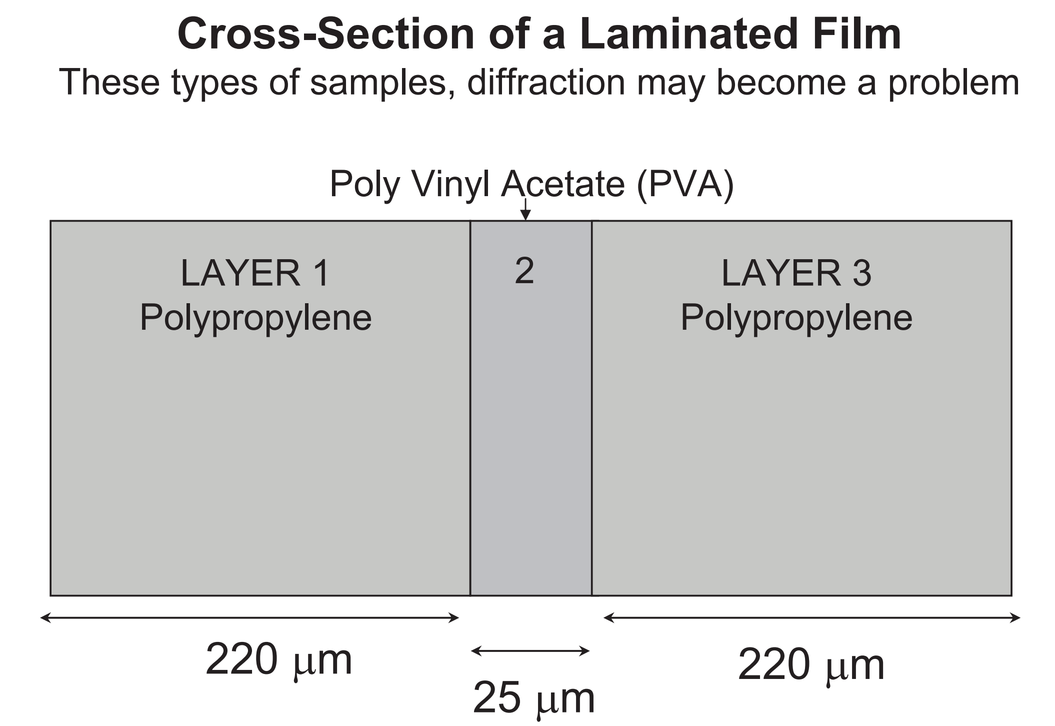
Diffraction is a frequency dependent phenomenon. Longer wavelengths of light, especially with smaller apertures set to less than 50 µm are affected first. These effects are revealed as excessive noise, loss of band shape, and perhaps spectral peaks from the surrounding matrix, which is probably the most detrimental effect, since it can lead to inaccurate conclusions of sample identity.

Dual Apertures



The Continuμm and other high quality infrared research microscopes remove diffracted energy by employing a second aperture to block this energy from the detector.

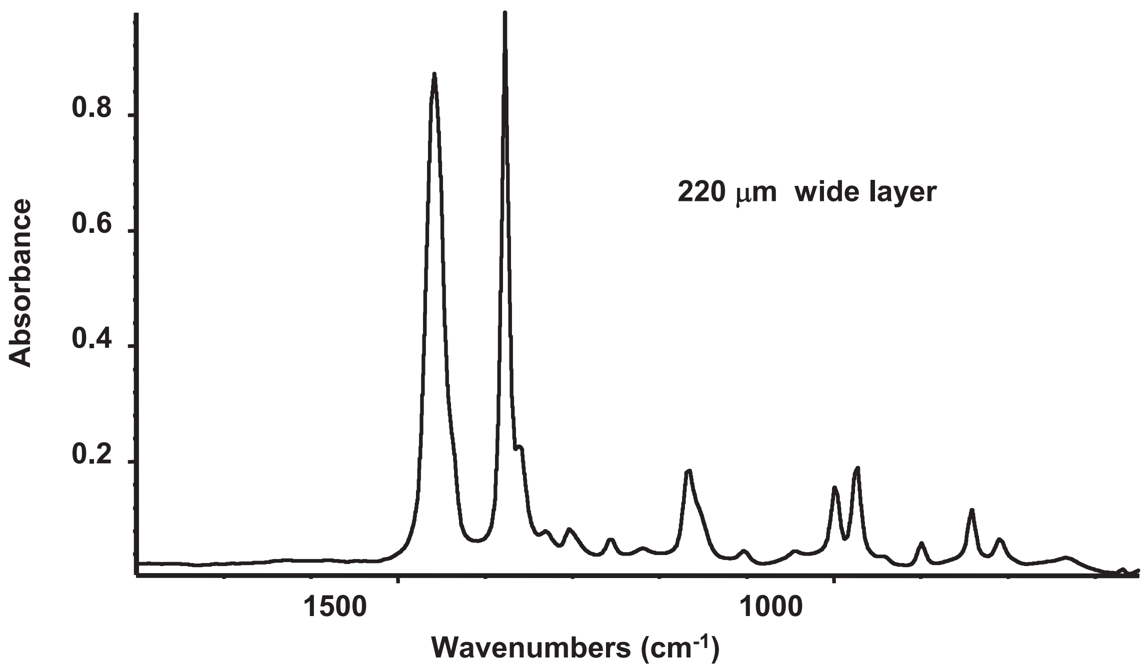
Multi-layer Films



Refraction is caused by the change in light direction due to changes in the refractive index between dissimilar layers. Though advantageous in light microscopy, infrared analysis can be adversely affected by this phenomenon.

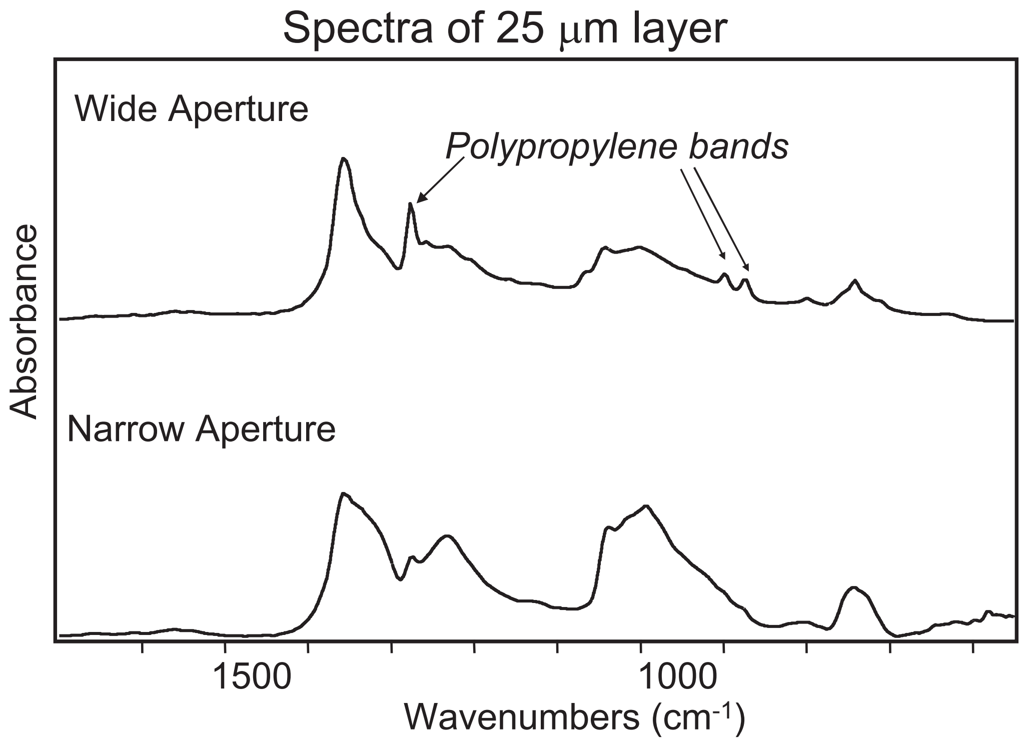
In this example, the outer layers are comprised of Polypropylene while the inner layer is Poly Vinyl Acetate (PVA).

Outer Layer - Polypropylene



This layer is very large in regard to infrared. Spectral results are clean and have a high signal to noise ratio.

Middle Layer - PVA



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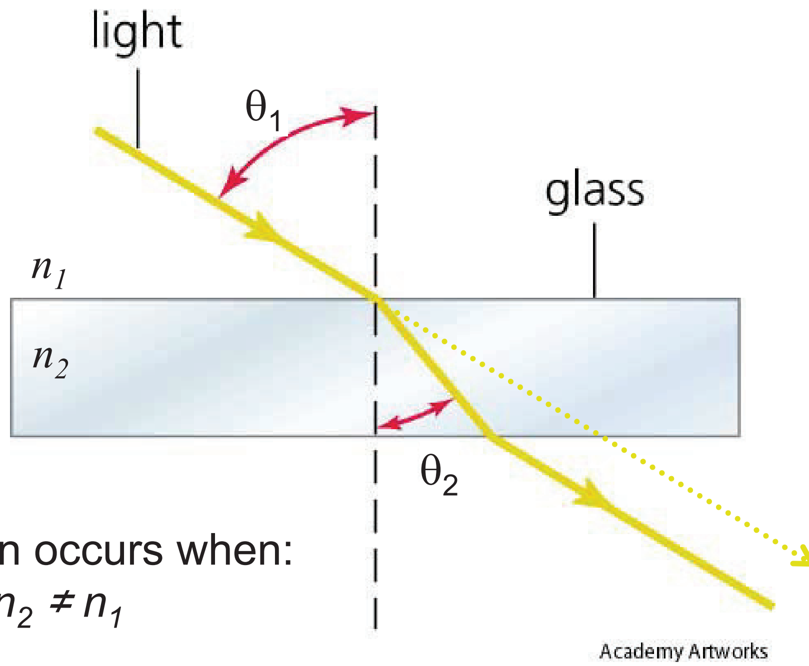
These are offset spectra of the middle layer with the aperture at a narrow setting. The top spectrum shows polypropylene bands. This can be corrected by closing the aperture further around the inner layer. Remember, longer wavelengths are more susceptible to diffraction.

Visually, the apertures are positioned just inside the edge of interest, but the longer wavelength infrared energy may pick up unwanted interactions from the adjoining layers.

Perhaps flattening this sample will allow all the sample layers to become effectively wider, allowing for larger apertures and more energy to pass to the detector. However, this may cause delamination or other sample morphology problems.

Refraction



Refraction occurs when:
$n_2 \neq n_1$

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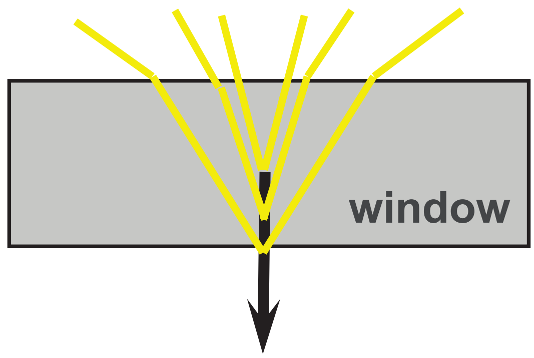
Indices of refraction are always equal to or greater than 1; for air, n=1.00029; for water, n=1.33. If the original medium is different from the refracting medium (e.g. n_2 greater than n_1), there will be a change in direction of the incident beam through the medium. This causes sample focus problems and spectral noise issues.

Window Effects

- Spherical Aberration is produced when the sample is placed on, or between, infrared transparent windows
- A vertical separation of focal point due to increased refraction effect on high incident rays (from outer edges of IR beam)

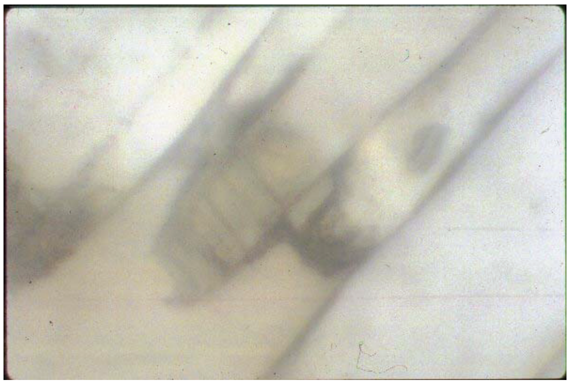


Spherical aberration is caused by the rays at high incident angles not coming to the same focal point as the low angle rays

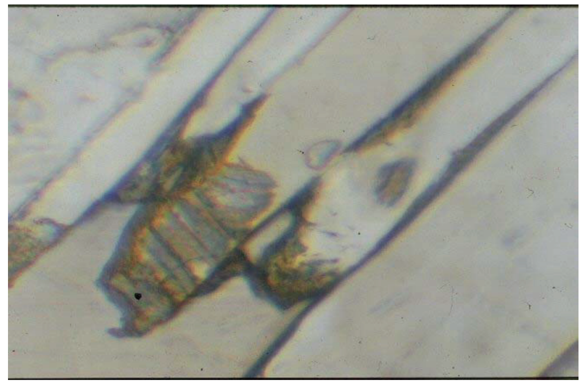
All quality microscopes allow for the correction of support windows or coverslips that are normally placed over the specimen. The Continuμm refractive lenses are normally corrected (0.17) for coverslips, while the reflective lenses are variable--0, 0.17, 1, 2, and 3 mm windows. These values assume the windows have a refractive index of about 1.5, including glass, NaCl, KBr, and BaF_2.

Spherical Aberration Correction

Images of Multi Layered Polymer



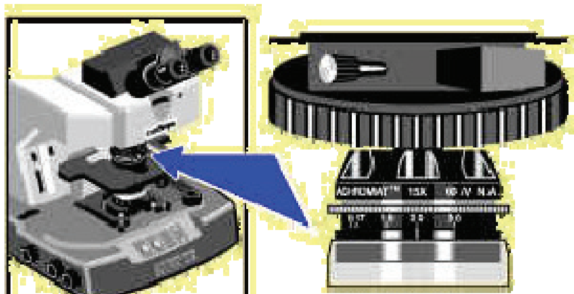
Before Reflachromat Correction



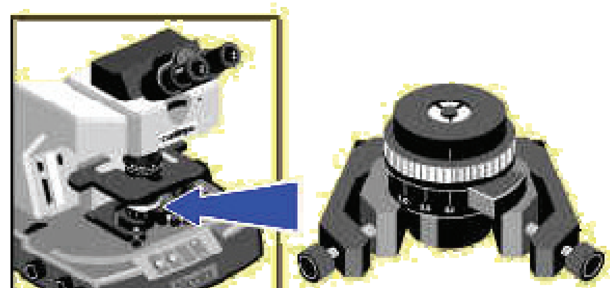
After Reflachromat Correction

Image quality as well as infrared spectral quality are improved with proper adjustment of the compensation ring. The compensation adjustment rings are found on both the objective and condenser of the Continuμm microscope. Typically, the value will be chosen based to correlate with the thickness of the NaCl, BaF_2, or KBr window. However, if the optical window has a refractive index different than these windows (all which have an n of about 1.5), then it will be necessary to calculate the correction.

Use of Compensation



Compensation calculation:
(n of salt plate / 1.49) X thickness of plate



* Use 0.17 for cover slips and a compensation of 1.0 for Diamond

Numbers on optics assume windows have a refractive index (n) of 1.49 (NaCl) because most salts are in this range

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Compensation allows the infrared and visible light to focus sharply on the sample even though the windows diffract light. The numbers of 1, 2 and 3 stand for thickness in mm of the window material assuming a refractive index of NaCl (1.49). Most commonly used windows have a refractive index very near that of NaCl. (BaF_2 = 1.42, CaF_2 = 1.4, KBr = 1.52). Set the compensation based on thickness. If using a window with a higher refractive index (AgCl = 1.98, ZnSe = 2.4) then you should calculate compensation.

To calculate what compensation is needed use the following equation:

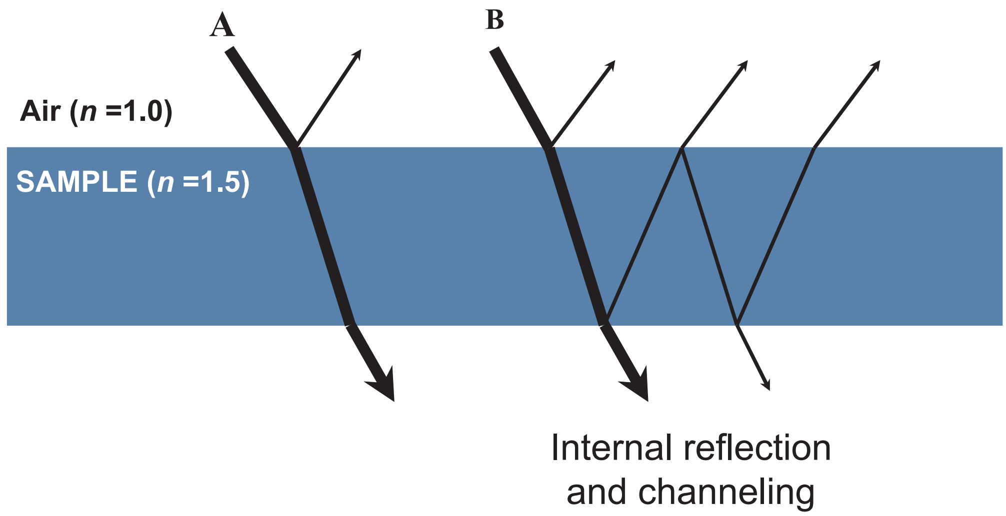
(refractive index of salt plate / 1.49) X window thickness = Compensation #

For example, 1mm ZnSe salt plates:

$$(2.4 / 1.49) \text{ X } 1.0\text{mm} = 1.6$$

An exception to this rule is using diamond compression cells. The thickness of the diamond crystal and refractive index have already been calculated. Always set the compensation to 1.0.

Interference in a Thin Film

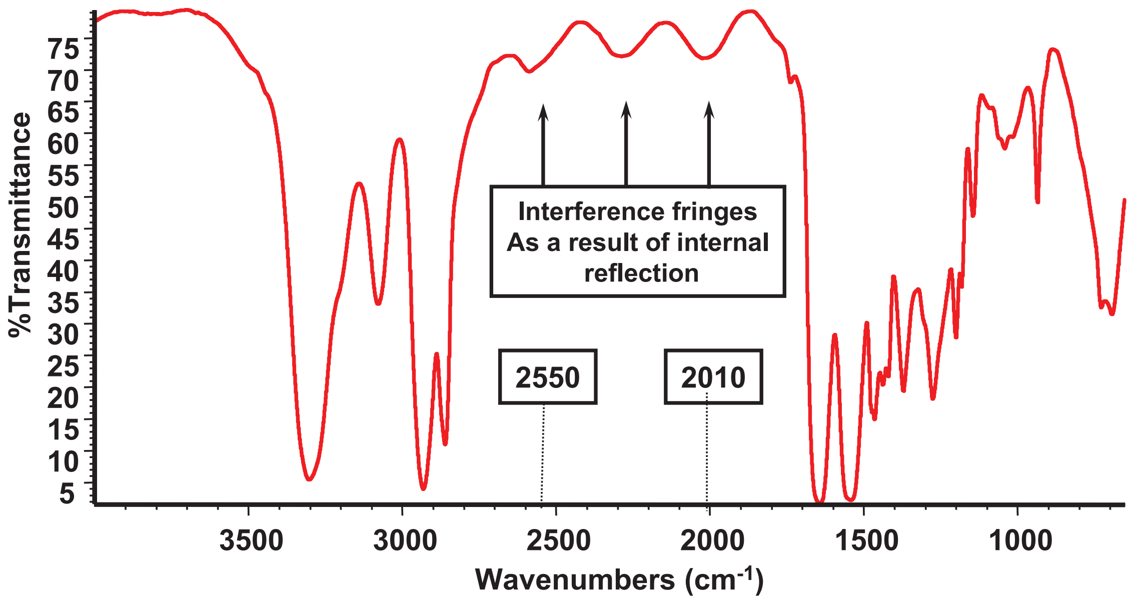


- Try to minimize the difference in refractive index between sampling mediums.

Infringement, internal reflection, and channeling are all terms that are used to describe energy that passes through the sample more than once. This is typically not desired, but can be used to calculate thickness.

In most instances, try to mitigate the channeling by reducing the differences in refractive indexes at the interface of the sample and air, or the sample and the support window.

Interference Fringing - Nylon



$$t(mm) = N/2 \times \{10/[(W_h - W_l)(\eta^2 - Sin^2\theta)^{1/2}]$$

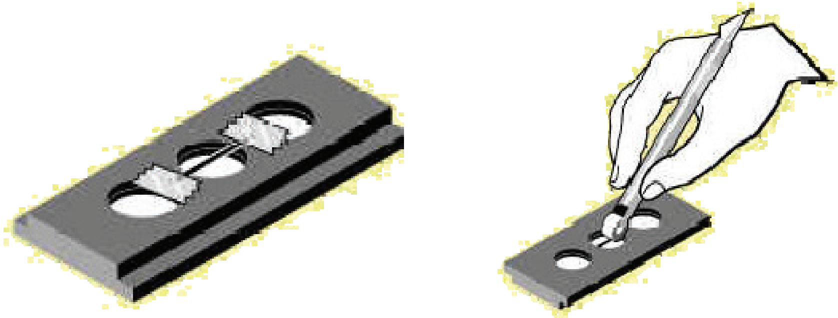
$$t(mm) = 2/2 \times \{10/[540(1.386)]\} \ = \ 0.0257 \text{ mm}$$

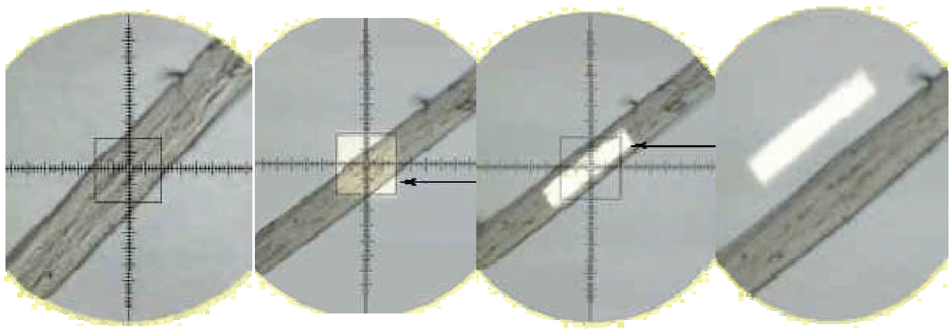
Often an interference fringe is observed in the transmission spectrum of a thin film such as a flattened nylon fiber.

The interference fringe is both FRIEND and FOE. As a friend, the interference fringe can be used to measure the thickness of a film. The angle of incidence for the 15x Reflachromat™ can be considered to be 35 degrees. For this example, the refractive index used for the nylon was 1.5.

As a foe, the interference fringe introduces new bands and alters the relative band intensities. The presence of interference fringes can degrade qualitative and quantitative measurements. If fringes are present, library search scores will be lower and false matches may occur. If fringe patterns are observed in spectral regions where no absorption occurs, then expect the fringe pattern to continue throughout the full spectral range.

Fibers





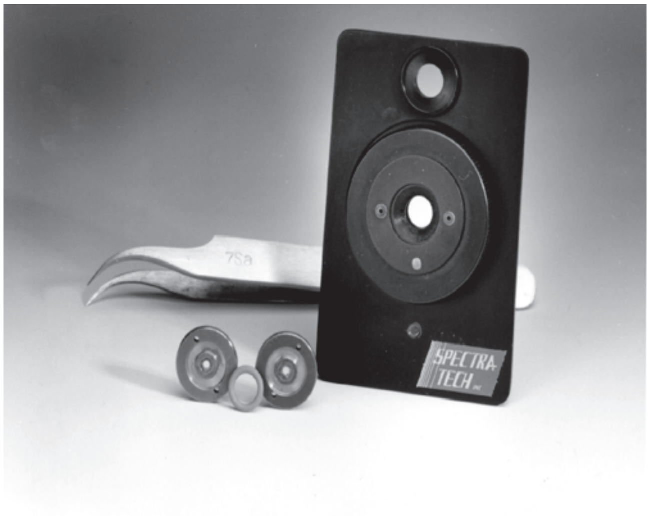
Fiber analysis is common to many different industries and may be accomplished by several different preparation techniques. In the sampling approach shown above, the fiber is taped to a sampling slide (included with the Continuμm sampling accessories) and then flattened using a roller knife. Many analysts, particularly in the field of forensics, find that fibers long enough to use in this sample preparation approach typically exist only in television crime labs. For fibers that are of sufficient length to use this preparation method, an additional disadvantage exists in that the sample may be stretched, contaminated or broken during the sample preparation.

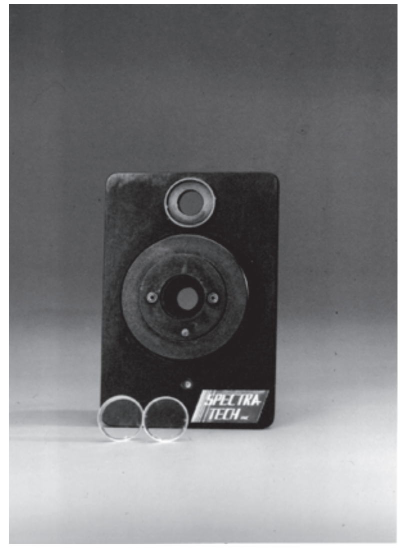
All of the previously mentioned transmission analysis problems may be observed in this type of analysis.

Alternatively, the fiber may be placed on a clean glass slide, rolled flat (using the roller knife provided in the sampling kit), and then taking a short section of the fiber for analysis. This section can be placed upon an infrared transparent window. The window serves both as a sampling support as well as a focusing spot. The same disadvantages exist with this technique that are mentioned above.

A third method is to place a short section of the fiber into a compression cell and using the cell to flatten the sample. This method offering will be discussed in more detail in a few moments.

Micro Compression Cells- Reduce Interference





Diamond Micro Compression Cell
Very useful accessory

Standard Micro Compression Cell
with standard 13x2 mm Windows

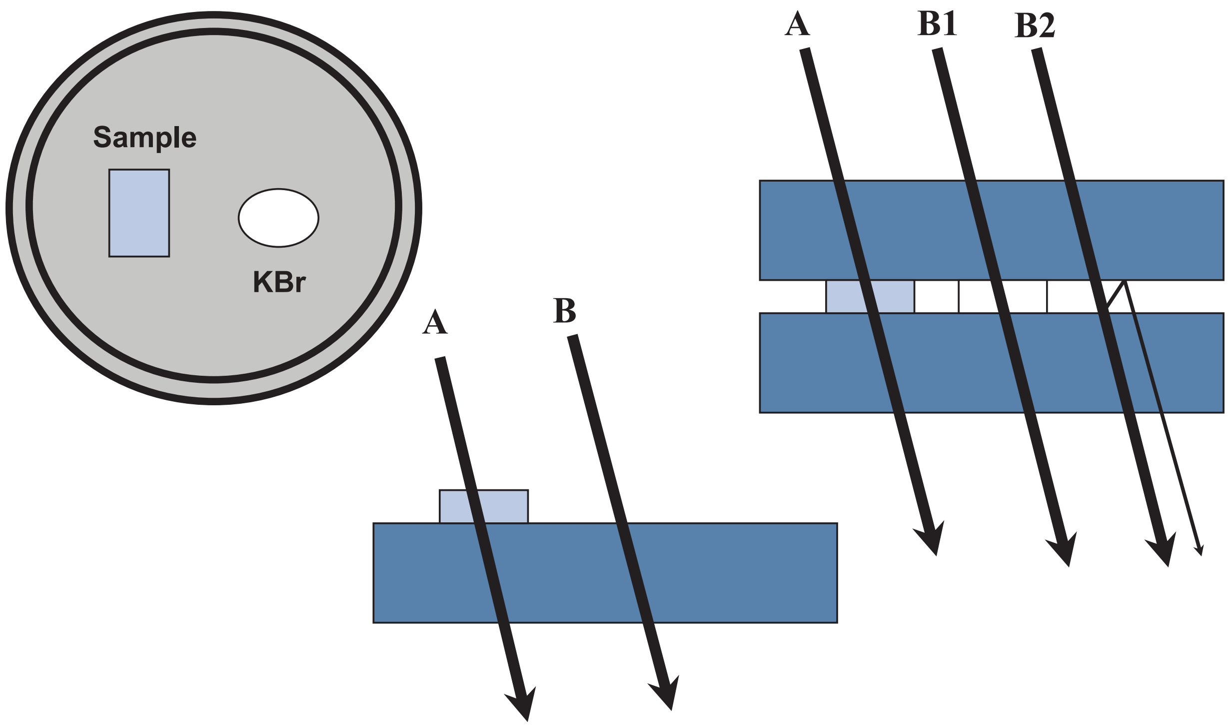
The micro compression cell is a sample holder used to flatten and thin the sample for transmission analysis by FT-IR microscopy.

The cell consists of a metal holder with a screw assembly that uses two IR transmitting windows. The sample is placed between the two windows, and the screw cap is tightened down, flattening and thinning the sample without undesirable window rotation.

13 mm Salt windows can be used with soft, elastic samples. For harder samples, such as rigid polymers and minerals, diamond windows are recommended.

The micro compression diamond cell uses Type IIA diamond windows, having a 1.8 mm diameter-free working area.

Micro Compression Cell



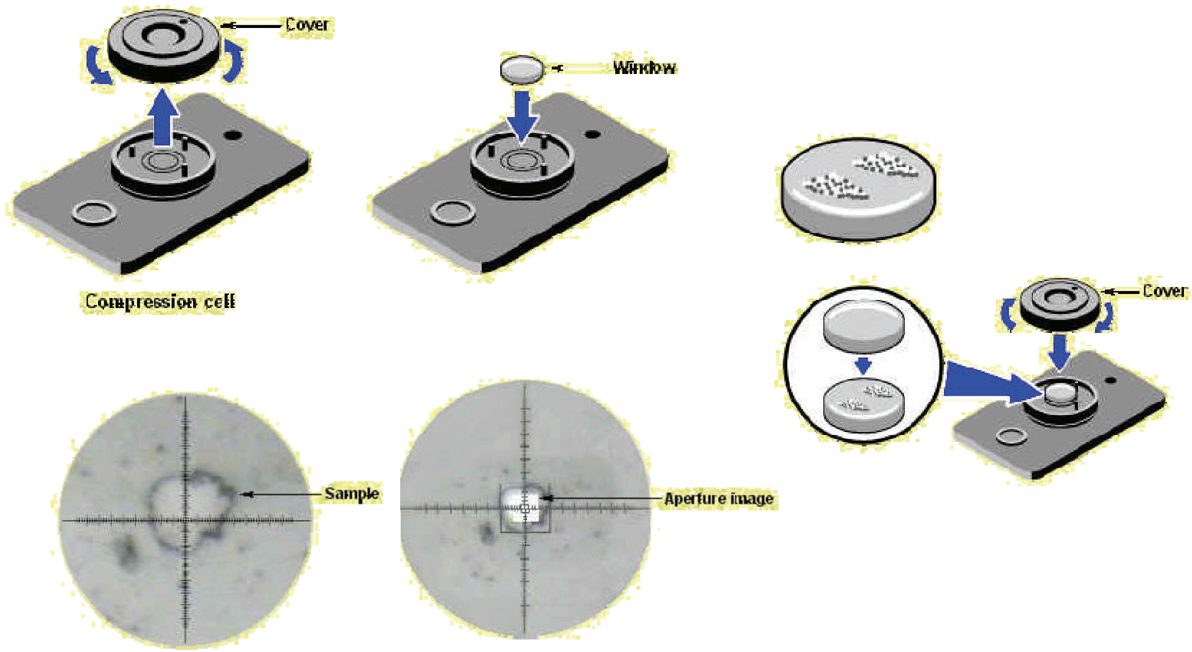
Mounting fibers or other samples in the micro compression cell can eliminate interference fringing by reducing or eliminating internal surface reflections as well as helping eliminate diffraction effects by providing a larger sampling area to focus on.

With this technique, the sample along with a single KBr crystal alongside is placed in the micro compression cell. The micro compression cell is then tightened so that the sample contacts both the top and bottom windows. Internal reflection is reduced because the refractive index of the infrared window is closer to the sample's refractive index than that of air.

The background is collected through the KBr crystal (B1) because the sample prevents the two windows from contacting each other, collecting a background in an area adjacent to the sample (B2) would result in an interference fringe. This fringe, generated by the air gap between the two windows, is in the background rather than in the sample spectrum. However, the sample-to-background ratio results in a fringe in the final spectrum. The KBr reference eliminates the background fringe when KBr windows are used.

We can also remove the top window and run open faced to prevent fringing in our background as well.

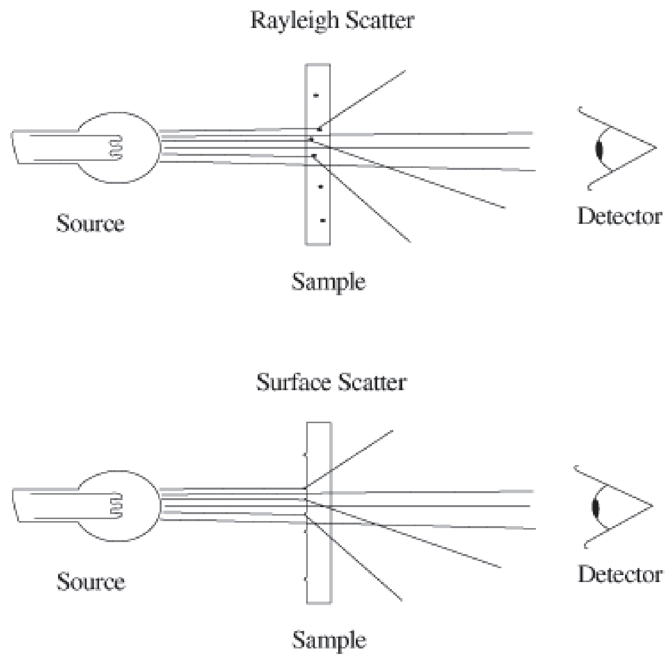
Compression Cell Setup



Here is a visual tour of sample prep on a compression cell. Use only enough sample and KBr to effectively conduct the experiment. Too much material may cause the window material to flex and break.

Micro compression cells can be used with a variety of windows. The idea is to support not only the sample, but the background media as well.

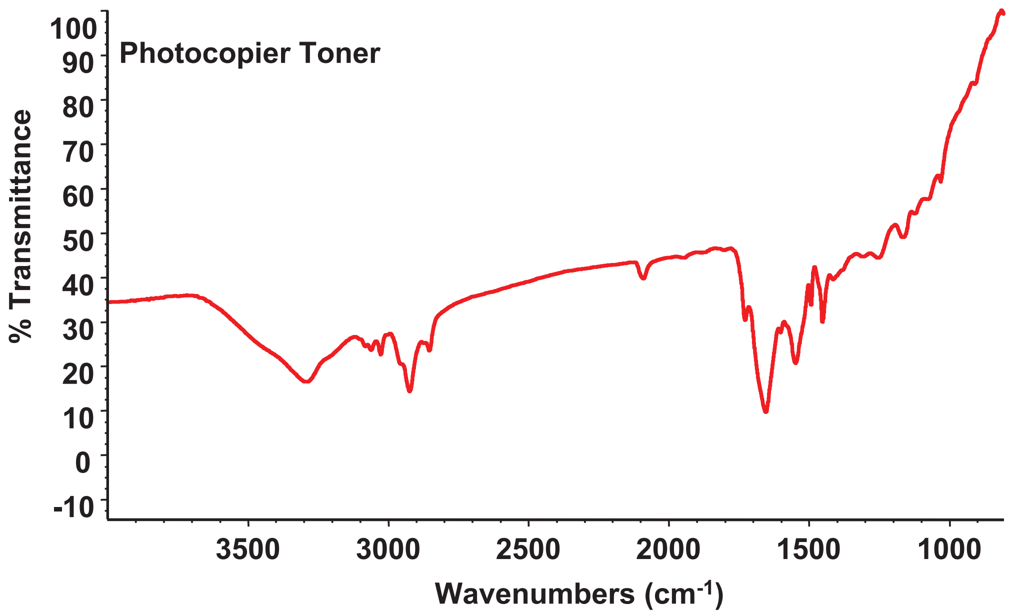
Scatter



Scattering is due to light reflecting off of the surfaces of the windows or sample particles and being directed outside of the collection cone of the condenser. Typically, scattering is observed as an elevated or sloping baseline in absorbance spectra.

This type of baseline artifact can have detrimental results on search functions. More important is the loss of signal this represents.

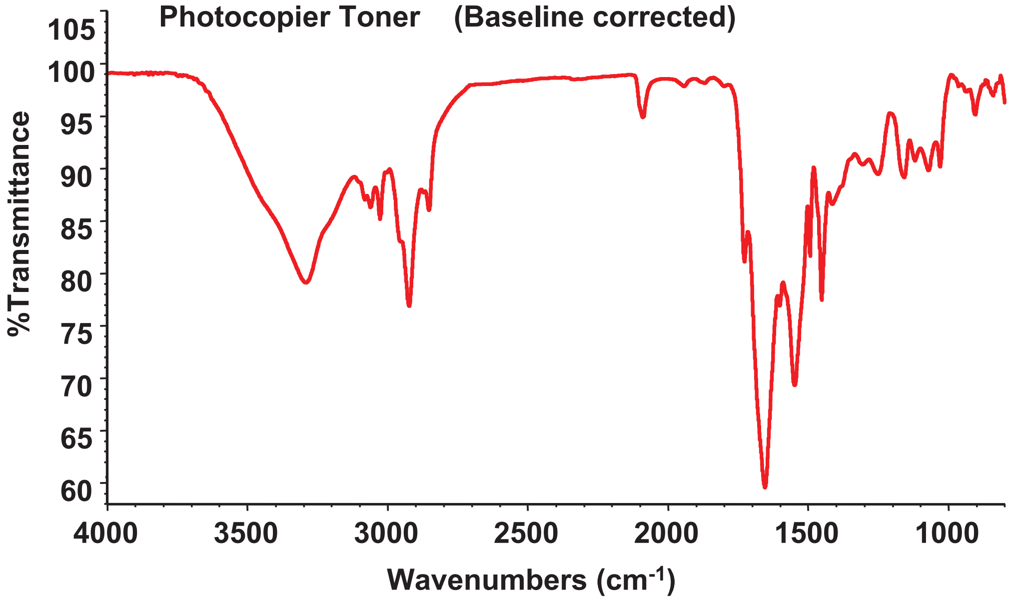
Scatter Experiment



Photocopier Toner Prepared in a Micro Compression Cell

The resulting spectra shows the baseline shift due to scattering.

Scatter Experiment



Spectrum after Baseline Correction

Baseline correction allows the analyst to straighten the baseline and return it to the theoretical optimum. Consider however, that some data manipulation may not be reversible, as in the case of automatic baseline correction. It may be desirable to save the spectrum to disk prior to manipulation.

Summary of Transmission

- Best control of sample analysis
- Standard Infrared technique
- Sweat equity required
- More complicated system setup

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Summary

Issues affecting Transmission

- *Diffraction*
- *Refraction*
- *Reflection*
- *Scatter*

Sample preparation to optimize data quality

Proper use of sampling accessories

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