

## Working Principles

AFM-IR works by using an AFM tip to locally detect a sample's thermal expansion resulting from absorption of infrared radiation at the tip location. In this way, a spatial resolution on the scale of the AFM tip radius is achieved. In AFM-IR measurements, a pulsed infrared laser beam is focused onto a region of a sample in the proximity of the AFM tip. Absorption of the infrared beam causes an instantaneous thermal expansion of the sample, which induces a transient force on the AFM cantilever that drives the cantilever into oscillation. The cantilever oscillation could be over a mixture of multiple oscillatory eigenmodes in conventional AFM-IR mode, or a certain eigenmode could be selectively enhanced in Resonance-Enhanced or Tapping AFM-IR modes.

AFM-IR spectra are obtained by measuring the AFM cantilever oscillation amplitude as a function of wavelength while maintaining the AFM tip at stationary xy positions on the sample. AFM-IR maps that show the distribution of chemical species across a sample are created by scanning the sample while illuminating it with fixed wavelengths. With the improvement of measurement speed, hyperspectral imaging (in which full AFM-IR spectra are measured on a dense grid of points) has become more popular, and it provides richer information about molecular species and their distributions in the sample.

Experimentally, AFM-IR requires a pulsed laser source together with optics for beam steering, focusing, and polarization control. To maintain the laser alignment to the tip, a sample xy scanner is required. With a small temperature rise (<10 K with OPOs; <1 K with QCLs) and a typical thermal expansion coefficient ( $10^{-6}$  to  $10^{-4}$ ), the resulting photothermal expansion is on the scale of several to tens of picometers for a polymer sample with a thickness of hundreds of nanometers. The rapid sample thermal expansion changes the instantaneous equilibrium position of the cantilever, converting the thermal expansion on the scale of tens of picometers into cantilever oscillations on the nanometer scale. With the improvement of sensitivity, the AFM-IR technique is now capable of detecting a single monolayer sample with a thermal expansion orders of magnitude lower.

The physics of how AFM-IR works is straightforward and well-understood. The overall AFM-IR signal intensity due to the infrared absorption by the sample at the tip location is expressed as:<sup>4,5</sup>

$$S_{\text{AFM-IR}} \propto H_{\text{AFM}} H_{\text{exp}} I_{\text{inc}}(\lambda) \alpha(\lambda)$$

---

EQUATION 1.

where  $H_{\text{AFM}}$  and  $H_{\text{exp}}$  are contributions from the AFM cantilever oscillation dynamics and the sample thermal expansion coefficient,  $I_{\text{inc}}(\lambda)$  is the laser power at the tip, and  $\alpha(\lambda) = 4\pi\kappa(\lambda)/\lambda$  is the sample absorption coefficient at the wavelength  $\lambda$  with  $\kappa(\lambda)$  as the imaginary part of sample refractive index.

From the above equation, while the AFM-IR signal intensity does depend on other material properties of the sample, such as thermal expansion coefficient and mechanical stiffness, these properties remain constant at a specific point on the sample, so they do not affect the relative peak intensities or peak shapes. Therefore, the AFM-IR signal measured as the oscillation amplitude of the cantilever at a fixed point ( $X_0, Y_0$ ) on a sample is directly proportional to the absorption coefficient:

$$S_{\text{AFM-IR}}(X_0, Y_0) \propto \alpha(\lambda, X_0, Y_0)$$

---

EQUATION 2.

and the AFM-IR spectrum directly correlates to the bulk FTIR spectra collected in transmission mode. This lays the foundation for the use of AFM-IR as a nanoscale chemical identification technique, as the AFM-IR spectra can be digitally searched against commercial databases of FTIR spectra and otherwise identified like any typical transmission FTIR spectrum.

The AFM probe is a crucial element to obtain quality AFM-IR data. For earlier nanoIR systems with a bottom-up illumination, no infrared beam shines directly on the cantilever, which allows for little restriction on the probe selection, enabling the use of common silicon or silicon nitride probes. As the technique has become more advanced, Bruker has developed a family of probes optimized for different AFM-IR applications. To have the best AFM-IR signal, metal-coated (usually gold) probes in certain geometries are used. By reflecting most of the incoming illumination, the metal coating helps to minimize absorption of the infrared beam by the cantilever arm. This results in purer AFM-IR signal from the sample. The metal coating also helps to enhance the AFM-IR signal by increasing the electric field of the infrared illumination near the sharp AFM tip.

The CnIR-B probe is selected for both Resonance-Enhanced and Surface Sensitive modes. It is a soft probe with a cantilever length of 450  $\mu\text{m}$  and a spring constant of 0.2 N/m, which provides a good balance between sensitivity and stability. In Resonance-Enhanced mode, the probe is driven at one of the contact resonance frequencies. For spectroscopic measurement in this mode, the signal is typically detected at the second resonance near 180 kHz, providing a high signal-to-noise ratio. For Surface Sensitive mode, the probe is typically driven at a higher resonance above 1 MHz and detected at the resonance near 180 kHz.

The TnIR-A probe is selected for use in both Resonance-Enhanced and Tapping AFM-IR modes. The semi-rigid probe has a cantilever length of 225  $\mu\text{m}$  and a spring constant of 3 N/m. In Resonance-Enhanced mode, the probe operates at the contact resonance frequency near 300 kHz, and the AFM-IR signal is recorded at this frequency. In Tapping AFM-IR, the probe is driven at the first resonance frequency of 60 kHz and detected at the second resonance frequency of 380 kHz.

The TnIR-D probe is selected specifically for Tapping AFM-IR mode. It is a rigid probe with a cantilever length of 125  $\mu\text{m}$  and a high spring constant of 40 N/m. In Tapping AFM-IR mode, the probe is typically driven at the second resonance near 1.6 MHz and detected at the first resonance near 250 kHz. The high sensitivity and high spatial resolution of AFM-IR measurements that can be achieved with the TnIR-D probe make it an attractive option for a wide range of applications.

## Understanding Photothermal AFM-IR Spectroscopy

The correlation between AFM-IR spectra and far-field bulk FTIR spectra is well understood and has been demonstrated over the past ten years through numerous publications. This correlation establishes AFM-IR as a reliable and powerful nanoscale chemical identification technique, and has driven the adoption of AFM-IR within the analytical chemistry community and beyond. Despite this correlation, various experimental factors could affect the relative intensities of IR absorption bands and peak ratios in the AFM-IR spectra. To correctly analyze and interpret AFM-IR data, it is critical to have a good understanding of these impacting factors.

### Impact of the IR Laser

#### Variations Caused by the Laser Beam

Several laser beam-based factors could impact the measured AFM-IR spectra, including attenuation, pointing, and collimation.

#### Attenuation

As shown earlier in Equation 1, the AFM-IR signal is proportional to the laser power  $I_{inc}(\lambda)$  and the sample absorption coefficient  $\alpha(\lambda)$ , thus normalization of the signal by the laser power would give the absorption spectrum that directly correlates to the FTIR spectrum in transmission mode. Thus for each AFM-IR experiment, a laser background file is recorded first by measuring the laser power as a function of wavelength using a power meter. Then, the AFM-IR signal is normalized by the laser power at each step in the spectral range. This method has been validated in a large number of application examples, but can be insufficient in some cases.

During AFM-IR measurements, the laser power is typically attenuated using a set of mesh filters. The attenuation percentage of the mesh filters is calibrated at a fixed wavenumber (for example, 1600  $\text{cm}^{-1}$ ) but actually changes as a function of wavelength. The deviation from the calibrated attenuation percentage becomes more pronounced at lower laser power (<5%), thus impacting peak ratios of the AFM-IR spectrum, since the signal is usually normalized to unattenuated laser power (100%). There are two compensation options to account for this deviation:

1. The laser background can be recorded at the same percentage as in the AFM-IR spectrum; however, this background will have a lower signal-to-noise ratio than the laser power curve collected at 100%.
2. One or multiple polarizers can be used to attenuate the laser power. Though this corrects the attenuation fluctuation, it could introduce beam distortion due to the nonideal parallelism of the polarizers.

### Pointing and Collimation

Variations in the laser beam pointing and collimation can cause fluctuations in the AFM-IR signal and affect peak ratios of the infrared absorption bands. Variations in beam pointing can be corrected by a set of two fast steering mirrors in the beam path. The mirror positions are calibrated for several selected wavelengths, then extrapolated for other wavelengths in the spectral range. The latest model of QCL laser from Daylight includes a “zero-pointing” option that reduces fluctuations in beam pointing from wavelength to wavelength, improving the accuracy of the AFM-IR signal. Variations in beam collimation can be corrected by automatically adjusting the focusing distance of an off-axis parabolic (OAP) mirror to the AFM tip, so that maximum signal is obtained for each wavelength in the spectral range.

### QCL Chip Transition

For a spectral range of 800–1800  $\text{cm}^{-1}$  covered by four QCL chips, each of the three chip transitions could lead to artifacts in the AFM-IR spectrum. The artifacts may show up as jumps or dips in the spectrum at wavenumbers corresponding to the chip transitions. They are usually caused by sudden changes in beam pointing, focusing, or laser output power across the chip transitions. The latest “zero-pointing” model can help alleviate the impact of chip transitions, and is included standard in Bruker systems. When picking QCL chips, it is recommended to avoid chip transitions in the middle of important absorption bands. For example, in biological studies, it is desirable not to have chip transitions within the amide I or II bands, around 1660 and 1540  $\text{cm}^{-1}$  respectively. Finally, artifacts in the spectra due to chip transitions are well understood and can be analyzed and corrected in post processing in the nanoIR/IcoIR software using mathematical methods.

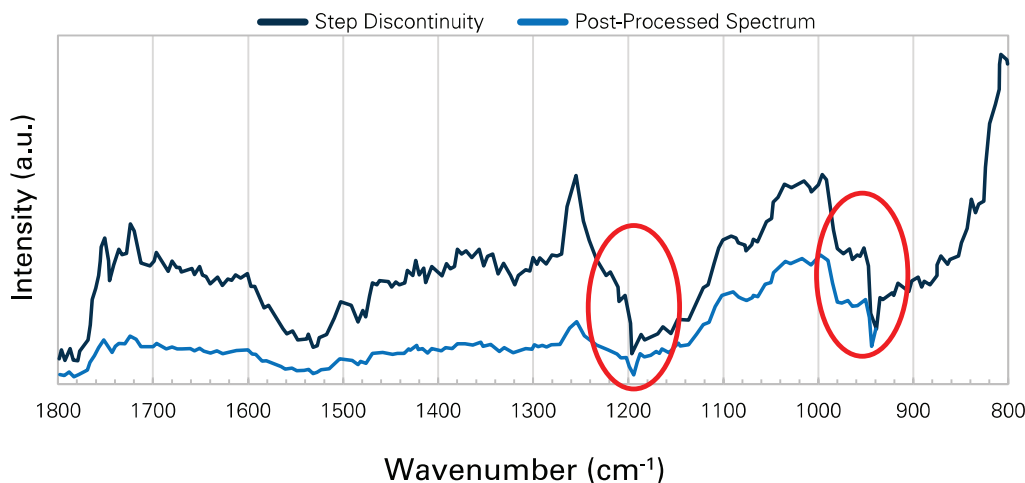


FIGURE 5. Comparison of AFM-IR spectra before and after correction of QCL chip transitions.