



## Micro-ATR Identification of Forensic Materials Using the $\mu$ MAX™ Microscope Accessory

Gabor J. Kemeny  
PIKE Technologies, Inc.

Positive identification of microscopic amounts of materials, especially when they are in a mixture, is a challenging task for infrared spectroscopy. Individual crystals are easily observable in a visual microscope and can be measured in reflectance or transmittance using an infrared microscope. The infrared reflectance spectra of pure materials, however, are never as high quality as those of a KBr pellet, and the infrared transmission of most crystals is still too thick to obtain good transmission spectrum, no matter how small a crystal is chosen. Micro-ATR has been used for many purposes as diverse as tracking chemical treatment of natural fibers (1), monitoring combinatorial chemistry (2), or identifying microbial colonies (3). Micro ATR techniques provide very good quality spectra even from the smallest crystal and should be considered the technique of choice when very limited amount of sample is available, such as in many forensic applications (4,5).

### Experimental Conditions

A PIKE Technologies  $\mu$ MAX™ sample compartment mounted microscope accessory was used with an FT-IR spectrometer equipped with a DTGS or an MCT detector. The  $\mu$ MAX Ge-ATR objective (Figure 1) is magnetically attached to the Schwarzschild objective of the microscope. The ATR objective easily rotates out of the infrared beam on its pivot mount to allow visual observation of the sample. Microscopic amounts of different forensic materials were placed on a KBr window, typically used for transmission measurements. For visualizing the small crystals, the field can be viewed using either illumination from the top, or through the KBr window. After the crystal is moved into the center of the crosshair, the ATR crystal is rotated in place and the sample stage is raised to make contact with the crystal. In order to obtain optimum contact pressure, the FT-IR instrument was put into monitor mode and the effect of the contact between the sample and the ATR crystal was clearly observable.

In practice, we found small KBr windows to be very useful substrates for the micro-ATR measurement because the 100- $\mu$ m tip of the ATR crystal is best protected. As the contact is made between the sample and the Ge tip, it is possible to press the ATR crystal too strongly into a hard surface, damaging the tip, such as with a gold coated steel or glass microscope plate. KBr is a relatively soft material; thus, if the force applied is more than necessary to compress the sample, only a conical dimple will be formed on the KBr surface, without damaging the Ge tip. The location of such

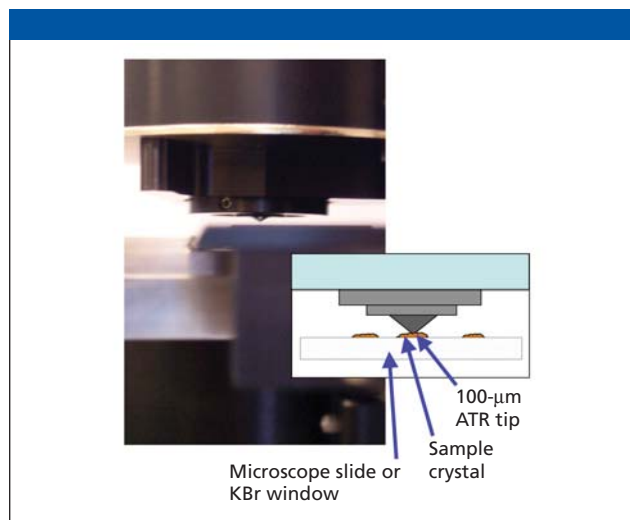


Figure 1:  $\mu$ Max Infrared Microscope with Ge-ATR objective.

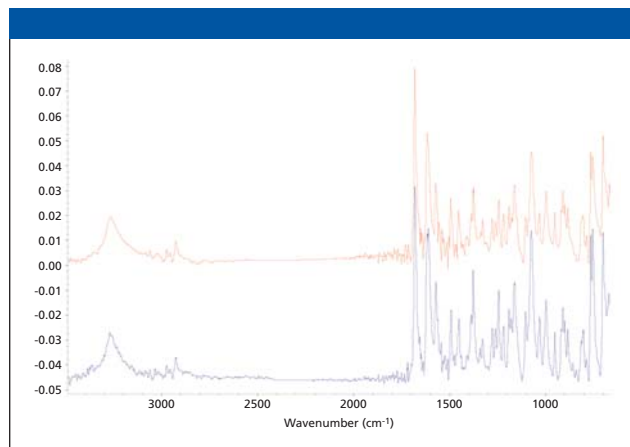
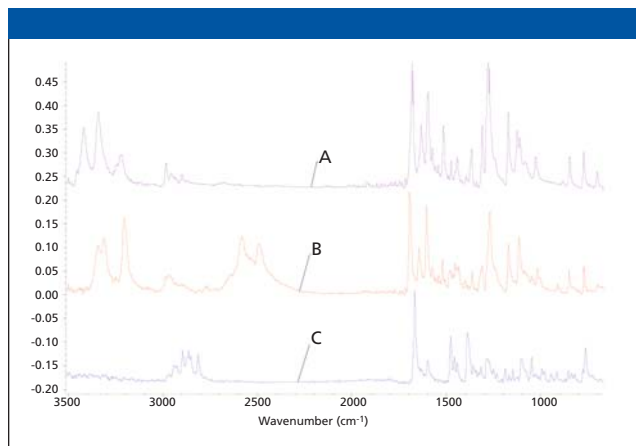


Figure 2: Micro-ATR Spectra of warfarin, with MCT detector (red trace) and DTGS detector (blue trace). No ATR correction was applied.

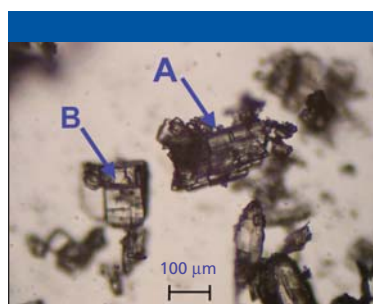
a small impression after the measurement validates how well the visual and infrared foci of the microscope overlap.

### Results

Due to the coupling and other optical losses, the throughput of a micro-ATR is less than the throughput of the microscope in transmittance, which could be up to 20% of the open beam signal without the microscope. Considering that the ATR crystal is only sensing a 100  $\mu$ m diameter sample area, the comparison is more favorable. Given the small signal, it is logical to test whether a liquid nitrogen cooled



**Figure 3:** Micro-ATR spectra of forensic samples: A – Benzocaine, B – Procaine, C – Strychnine.



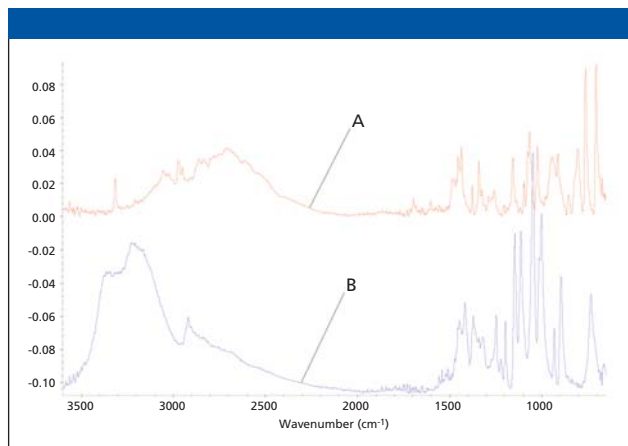
**Figure 4:** Visual identification of (a) inositol and (b) pseudoephedrine from their powder mixture.

detector would be necessary. Figure 2 shows the spectra of warfarin measured with both the DTGS and MCT detectors. One-minute scans with  $4\text{ cm}^{-1}$  resolution resulted in very good quality spectra with both detectors. Between  $2050\text{ cm}^{-1}$  and  $2250\text{ cm}^{-1}$ , the MCT detector root-mean-square (RMS) noise was 71 micro-absorbance, whereas the TGS was somewhat higher, 223  $\mu\text{Abs}$ .

Forensic samples of pure reference chemicals, ranging from a single crystal to a single particle, were analyzed using 1 min scan times with the DTGS detector and  $4\text{ cm}^{-1}$  resolution. Figure 3 shows three examples to demonstrate the quality of the spectra obtained. For identification purposes, the spectra were searched using the PIKE Technologies ATR Spectral Data Base of Pharmaceuticals, Drugs and Antibiotics containing 1338 spectra (6).

Assuming that a  $100\text{ }\mu\text{m}$  cube size crystal is sufficient for the analysis, the practical measurement can be done on a  $1.5\text{ }\mu\text{g}$  sample particle (assuming a  $1.5\text{ g/cm}^3$  density). The penetration of infrared light using a Ge ATR is only approximately  $0.5\text{ }\mu\text{m}$ . This means that a full coverage of the ATR tip can be achieved by  $7.5\text{ ng}$  material. Considering the very good signal-to-noise of the infrared spectra obtained with all of the materials, the detection limit could even be a fraction of the  $7.5\text{ ng}$ , although this would assume that the crystal is uniformly thin and achieves a good contact with the  $100\text{ }\mu\text{m}$  tip.

Mixtures of crystalline materials can be identified from their macro spectra by subtracting one of the components, if the components are known. Identifying the individual crystals, however, is far superior, because the pure component spectrum does not have to be known. Often the mixtures of crystalline materials can be visually identified using the visual



**Figure 5:** Micro-ATR spectra of single crystals isolated from powder mixture: A – Pseudoephedrine, B – Inositol

microscope function, then placing them on the crosshair and targeting the individual crystals by the micro-ATR. Figure 4 shows inositol with long thin needle aggregate structure. Pseudoephedrine has very different broader rhombic crystals, clearly different from the inositol and was easily identified by the micro-ATR method (Figure 5).

### Conclusions

Single-reflection micro-ATR is a powerful sampling technique allowing the use of microscopic amounts of sample materials. Sample crystals and particles, as small as  $100\text{ }\mu\text{m}$  or less, can be visualized and are identified easily. The sample compartment mounted  $\mu\text{MAX}$  Infrared Microscope allows the use of the standard DTGS detector of the FTIR instrument, providing excellent micro-ATR spectral data. Using dedicated ATR spectral databases, proper identification of unknown forensic materials is relatively straightforward.

### References

- (1) B. Singh, M. Gupta, A. Verma, O.S. Tyagi, *Polymer Int.* **49**(11), 1444–1451 (2002).
- (2) W.L. Fitch, "Analytical methods for quality control of combinatorial libraries," in *Molecular Diversity* (Springer Verlag, New York, 1998).
- (3) F. Orsini, D. Ami, A.M. Villa, G. Sala, M.G. Fellotti, S.M. Doglia, *J. Microbiol. Methods* **42**(1), 17–27 (2000).
- (4) J.A. Reffner, *Am. Lab.*, June 2000.
- (5) E.G. Bartick, "Application of Vibrational Spectroscopy in Criminal Forensic Analysis," in *Handbook of Vibrational Spectroscopy* vol. 4, eds. J.M. Chalmers, P.R. Griffiths, pp 2993–3004 (2002).
- (6) Product Data Sheet - ATR Spectral Data Bases – [www.piketech.com](http://www.piketech.com).

### PIKE Technologies, Inc.

2901 Commerce Park Drive  
Madison WI 53719  
Tel. (608) 274-2721, Fax (608) 274-0103  
[www.piketech.com](http://www.piketech.com), [sales@piketech.com](mailto:sales@piketech.com)