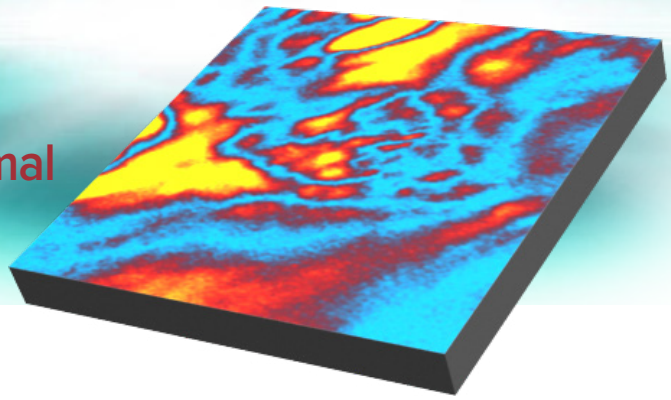
mIRage™ IR microscope

Sub-micron IR spectroscopy and imaging with photothermal IR spectroscopy



The Mirage™ IR microscope is an innovative new IR microscope uniquely providing submicron IR spectroscopy and imaging across a wide variety of industrial applications.

Using a proprietary Anasys technique based upon photothermal IR spectroscopy, Mirage breaks the diffraction limit and bridges the gap between conventional IR microspectroscopy and nanoscale IR spectroscopy.

The Mirage IR Microscope is a major breakthrough for industrial users of IR spectroscopy by solving two of the biggest problems facing the field of IR microscopy:

- Achieving submicron IR spatial resolution (an improvement of over 10X) without the need for contact-based ATR accessories.
- Measurement of thick samples in reflection mode, providing transmission quality IR spectra that correlate to industry standard IR databases.

Additionally, the Mirage IR microscope allows for these new unique capabilities with fast, easy measurement of samples using a new optical, non-contact based technique which utilizes established IR technology.

Mirage achieves sub-micron spatial resolution IR imaging and spectroscopy with photothermal IR spectroscopy (PTIR)

New non-contact reflection mode significantly simplifies sample preparation and improves data turnaround time

PTIR can easily and reliably measure a wide array of sample types, including biological materials, polymers, pharmaceutical samples and more

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Photothermal infrared (IR) spectroscopy

The photothermal infrared spectroscopy (PTIR) technology used on the Mirage IR microscope is a result of over a decade of expertise in photothermal physics that Anasys Instruments and its collaborators have built up since starting research on the AFM-based nanoscale IR spectroscopy platform.

Submicron spatial resolution IR microscopy

PTIR overcomes the IR diffraction limit by combining visible light with a mid-IR pulsed, tunable laser that heats the sample. When the IR laser is at a wavelength that excites a molecular vibration in the sample, absorption occurs, thereby creating photothermal effects. A visible probe laser, focused to 0.5 µm spot size, measures the photothermal response via the scattered light, as shown in figure 1.

The IR pump laser can be tuned through the entire fingerprint region in one second or less, to obtain an IR spectrum.

Transmission FT-IR quality in reflection mode

Due to its unique operating principle, PTIR can be used in both transmission and reflection mode. However, its primary method of operation is in reflection mode, which eliminates several longstanding limitations for IR microscopy. This provides substantial benefits for the IR community, including minimizing sample preparation and enabling submicron spectroscopy. PTIR has consistently shown transmission quality spectra in reflection mode across a wide range of sample types. The submicron resolution is demonstrated in figure 2, showing reflection mode spectra on a multilayer packaging film measured 0.5 µm apart with highly differentiated chemical fingerprints indicating different materials.

Correlates to bulk FT-IR databases

PTIR measurements of common polymeric materials have shown excellent correlation with bulk FT-IR spectra. Figure 3 shows excellent correlation for polystyrene (PS), polyethylene terephthalate (PET) and polymethyl methacrylate (PMMA) with high correlation to spectra from the KnowItAll® database.

PTIR measurements shown in figure 3 were made on samples of over 20 µm thickness in reflection mode, yet the strongest bands show no evidence of saturation. This is due to the reflected signal sampling only the top couple microns of the sample, making the depth of penetration comparable to what is achieved using ATR accessories, but without the optical band-shape distortions present in many ATR spectra.

Ease of use and minimizing sample preparation

PTIR is an optical, non-contact based approach, making it fast and easy to use, while maintaining transmission quality spectra. In addition its high quality spectra in

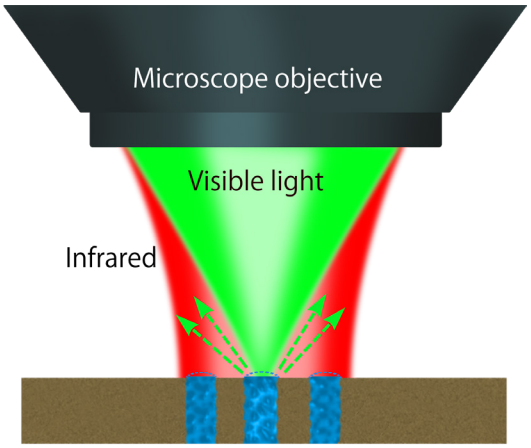

Figure 1: A pulsed tunable, IR source is focused on sample. Absorbed IR light causes sample to heat up, creating a photothermal response in the sample. A visible laser probe measures the photothermal response due to IR absorption

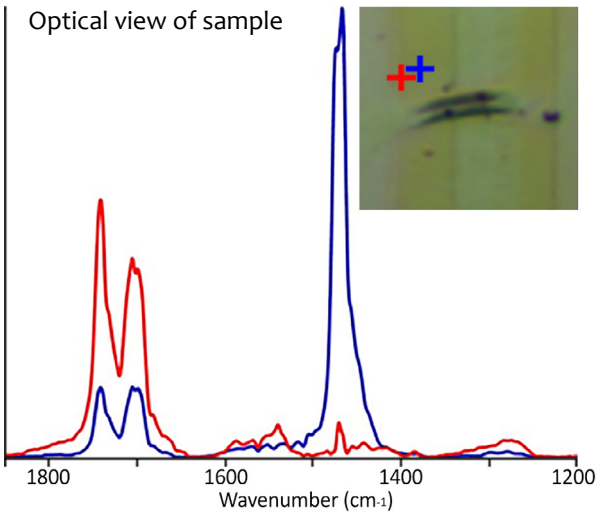

Figure 2: Spectra 0.5µm spacing showing different polymer materials in a multilayer film

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reflection mode enables IR measurement on thick samples and eliminates the need for thin samples in many sample types. This leads to dramatically easier sample preparation, improved ease of use and faster turnaround times.

Next generation infrared spectroscopy

PTIR eliminates several longstanding limitations for IR microscopy enabling submicron IR spectroscopy and minimizing sample preparation. PTIR is a unique technique that provides a huge step forward for the IR spectroscopy community.

Life Science

Hyperspectral imaging of mouse bone

Mice are a common model for biological studies, due to their genetic and physiological similarities to humans. Despite systems process differences between humans and mice, density and fracture studies in mouse bone have shown to be an insightful method to exploring these phenomena exhibited similarly in humans.

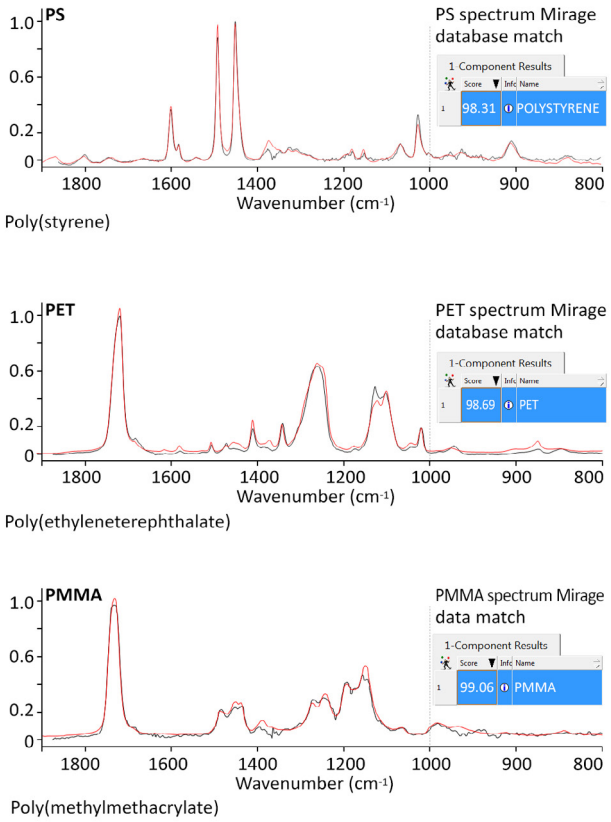

Figure 3: Three different spectra from Mirage searched against the database with high matches for PS (Top) PET (middle) and PMMA (bottom)

Using conventional FT-IR spectroscopy, bone density studies are limited to spatial resolutions on the order of multiple microns for each spectra. This can be a potential obstacle when trying to obtain more information into local variable composition. However, using PTIR, mouse bone mineral density concentration and dispersion can be closely and easily analyzed.

Mouse knee joint samples were analyzed by Mirage to acquire information on mineral distribution within the bone, as shown in figure 4. 25 x 25 µm Hyperspectral image scans were acquired of one section at 1047 cm^{-1} (a common absorption band for PO_4) and 1660 cm^{-1} (amide I). The images were acquired by scanning the image with 1 nm spacing, taking ~1 second for each full spectra. Looking at the hyperspectral images, it is evident that mineral and protein concentrations within bone are much more dispersed than observed in the conventional FTIR spectral image. Furthermore, when comparing spectra of conventional FTIR spectroscopy of mouse bone to a single pixel spectra using PTIR, they are actually quite similar, with even more local information provided. This example shows the ability of Mirage to unambiguously identify a mineral to matrix ratio with spatial resolutions of 0.5 µm.

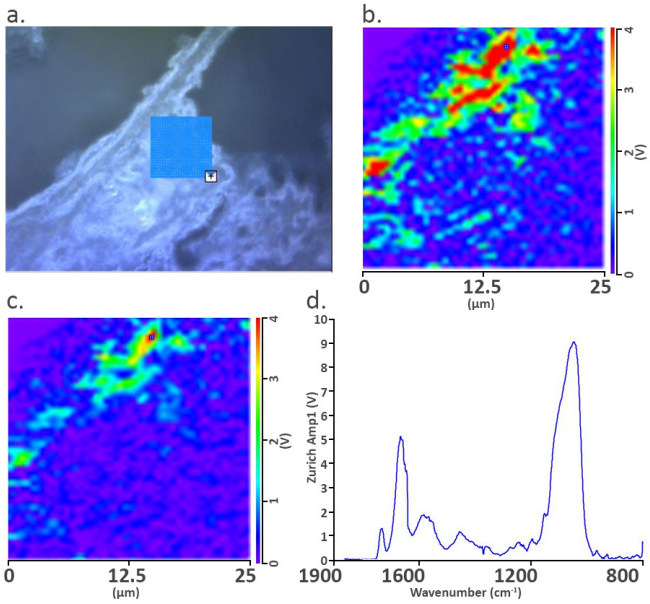

Figure 4: A. Hyperspectral array location of mouse bone. HYPERspectral images and taken at B. 1047 cm^{-1} and C. 1660 cm^{-1} showing mineral and protein distribution, respectively. D. Corresponding spectra taken from the inner bone, showing a higher absorption for phosphate. Data courtesy of Prof. Nancy Pleshko, Dr. Mugdha Padalkar, and Jessica M. Falcon, Temple University

Pharmaceutical

PLGA blend dispersion analysis

Drug-polymer blend studies are integral for the understanding of efficient drug delivery systems and drug efficacy within the system. PLGA, a polymer used in many drugs, is recognized for its biocompatibility and degradation rates, enabling faster uptake of the active components of the drug. Conventional FT-IR spectroscopy has been unable to study these blends with unambiguous characterization, as most spectra sizes are limited to a few microns in size, due to diffraction limits. Photothermal IR spectroscopy, on the other hand, is able to provide consistent chemical characterization of samples with an average spectra size of 500 nm.

Dexamethasone, a common anti-inflammatory, was studied with Mirage to analyze its concentrations when blended with PLGA. A hyperspectral image collected at 1760 cm^{-1}, consistent with the anhydride group of PLGA, shows the distribution of PLGA in a 40 x 40 µm section of the film. Within the image, a .5 x .5 µm area spectra was collected in an area that had strong absorption, as seen in figure 5, in order to confirm PLGA. A hyperspectral image was collected on the same area at 1666 cm^{-1}, characteristic of the phenyl group in dexamethasone. A .5 x .5 µm spot in a strongly absorbing region showed spectra consistent with the presence of aromatic ring-like carbon structures in dexamethasone. The clear discrimination of PLGA/dexamethasone dispersions shows the capability of photothermal IR spectroscopy for highly resolved analysis of drug-polymer dispersion studies.

Polymers

Sub-µm imaging of multilayer packaging

Mirage provides hyperspectral capability to quickly identify the spectra and chemical composition over a wide region. Figure 6 highlights an example of hyperspectral imaging with Mirage. The left image shows a hyperspectral IR image at 1730 cm^{-1} of a multilayer film cross section. Within the software the wavelength distribution visualized in the hyperspectral image can be changed by selecting different wavenumber points (denoted by the blue lines) in the spectra. The middle image shows the hyperspectral image at 1545 cm^{-1}, showing the polyamide layers within the multilayer film. This image is displayed by simply selecting this peak with the blue line in software. The top center hyperspectral image highlights the IR absorbance at 1470 cm^{-1}, showing the layers within the multilayer film that contain polyethylene chains.

Conclusion

This note shows how photothermal IR spectroscopy can be used on a wide variety of sample types with ease of use and efficiency. Hyperspectral imaging with 500 nm spatial resolutions revolutionizes the field of IR spectroscopy, and enables researchers to chemically characterize their samples in a way previously unachievable.

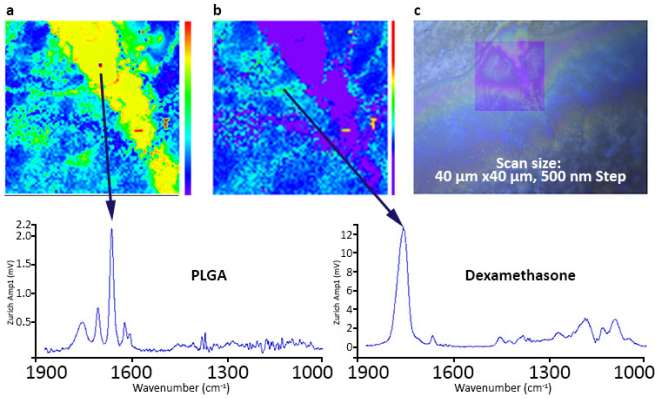

Figure 5: HYPERspectral images and corresponding spectra taken at (a) 1760 cm^{-1} showing PLGA distribution, and (b) at 1666 cm^{-1} showing dexamethasone. (c) Optical view of PLGA/dexamethasone blend, with a 40 x 40 µm image selected for simultaneous HYPERspectral measurements. Data courtesy of [??]

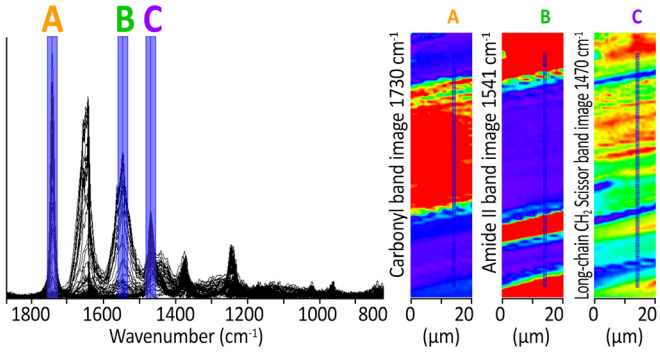

Figure 6: A block face of a multilayer polymer film sample with seven layers. HYPERspectral images of 20 x 85 µm size were taken at a rate of 1 sec/spectrasize, with 1 µm spacing. The spectra shows the corresponding spectra and images of absorption of carbonyl, amide II, and CH stretching bands of the film components, respectively. Data courtesy of G. Meyers, M. Rickard Dow Chemical Company

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