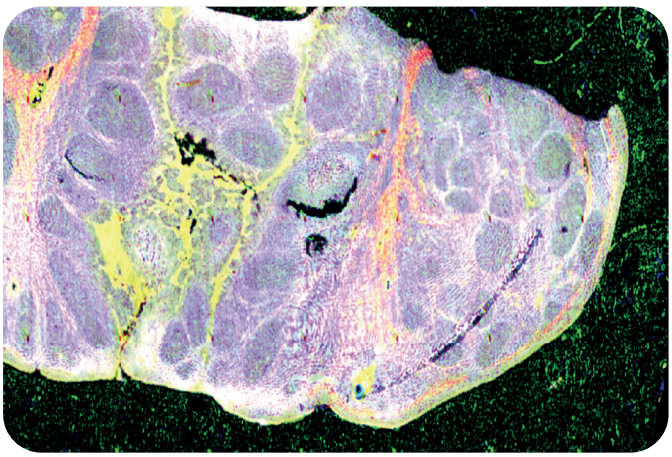

Application Note AN MIC418

Biological Tissue Analysis by Infrared Laser Imaging (QCL)

FT-IR in Tissue Imaging: a Stalemate

IR spectroscopy has been used for studying biological tissue for some time. It permits insight into the biochemical composition of tissues from plants, animals and humans without the use of staining agents. The most exciting finding is the capability to differentiate diseased from healthy tissue based on the IR spectroscopic data.

Thus, the IR technique has the potential to serve as an diagnostic tool, although its use has been limited to research purposes.[1] One reason for this is the high amount of time required to obtain meaningful spectral information, even when using ultra-fast focal-plane array detectors.

A New Player Enters: IR Laser Imaging

Today, new tools like IR Laser Imaging have the potential to challenge this stalemate. An IR laser offers much higher power density than a traditional MIR source. As a result, excellent IR spectral data can be collected at a fraction of the time needed for FT-IR image acquisition.

With the launch of HYPERION II, IR Laser Imaging takes the next step. Bruker's patented spatial coherence reduction finally enables the acquisition of artifact-free, impressive IR images, placing users in the perfect starting position for significant progress in the field of tissue research.

Keywords	Instrumentation and Software
FT-IR microscopy	HYPERION II IR Microscope
FT-IR imaging	LUMOS II FT-IR Imaging Microscope
FPA imaging	Infrared Laser Imaging Module (ILIM)
Infrared Laser Imaging	OPUS Software

What Users Must Know About IR Laser Imaging:

IR Laser Imaging supports multiple image aquistion modes in the MIR fingerprint region (1800-950 cm^{-1}).

IR live imaging permits real-time chemical imaging at video frame-rates to find regions of interest or follow reactions.

Imaging at **discrete wavenumbers** focuses on specific wavenumbers instead of full spectra and allows to speed up the aquisition process significantly.

In a **spectral sweep scan** a spectral range is selected and spectra are generated by a continuous sweep of the laser. The resulting spectra are equal to FT-IR.[2]

With the **discrete scan** a selected spectral range is recorded by stepwise tuning of the laser, resulting in higher wavenumber accuracy but longer acquisition time.

FT-IR vs Infrared Laser Lmaging: Tissue Samples

In the following we compare the fastest FT-IR imaging microscope (LUMOS II) with the HYPERION II infrared laser imaging microscope for the analysis of tissue samples.

	LUMOS II	HYPERION II
Area	14.7 x 5.9 mm	
Pixel size	5 µm	4.9 µm
Spectral resolution	4 cm^{-1}	4 cm^{-1}
Spectral range	4000 – 750 cm^{-1}	1800 – 950 cm^{-1}
Time required	113 min	8 minutes

Infrared laser imaging with the HYPERION II is more than 14 times faster than a comparable FPA FT-IR measurement with the LUMOS II. However, by using an infrared laser (QCL) only the MIR fingerprint region is acquired. For the HYPERION II this is no problem, as it also features FT-IR microscopy to access the full spectral range if needed.

Figure 1 shows another milestone in imaging technology. The adaptive K-means clustering of the OPUS software was used to autonomously create an IR chemical image from raw data. It automatically analyzes the distribution of components based on spectral variations and evaluates IR images extremely fast.

Even large samples, such as the tonsil tissue microtome section with >3 million spectra, shown in Figure 1, are analyzed in a few minutes, which includes the evaluation of the spectral data.

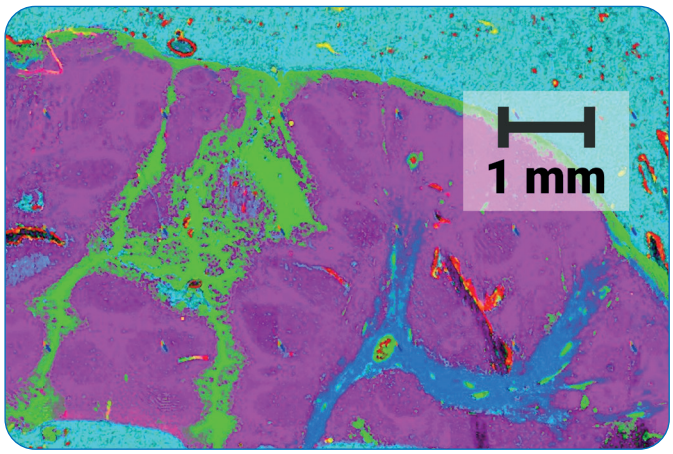

Figure 1: Autonomous evaluation of chemical image by adaptive K-means clustering. Distribution of components was based on spectral differences of 3.6 million spectra.

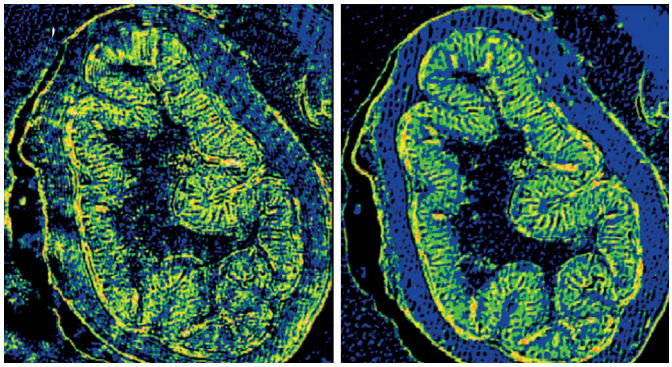

Figure 2: Effect of Bruker's patented spatial coherence reduction on on the quality of IR images acquired with QCL imaging: Instead of unwanted artifacts, fringes and speckles (left) we get a pristine IR image (right) without applying post-processing.[3]

The Game-Changer: Spatial Coherence Reduction

Figure 2 shows the impact of our patented hardware spatial coherence reduction when acquiring IR laser images. On the left, you can see the influence of the laser beam's spatial coherence on the imaging result. The images look blurry and show fringes and speckles. On the right, the coherence suppression technology eliminates spatial coherence phenomena at the source and records amazing, clear images without any post-processing.

The Benefit of Single Wavelength Imaging

The combination of a QCL with an IR camera enables the HYPERION II to collect detailed chemical images at discrete wavenumbers with incredible speed. When the composition of a sample is known , the user can choose to collect only relevant wavenumbers.

For the chosen example, an RGB image was created from three wavenumbers, which reduced the total measurement time to only 1 minute, further increasing the speed advantage to over two orders of magnitude.

The Result: Amazing Images

Laser infrared imaging is a great tool for life science and especially tissue analysis. Paired with the FT-IR capabilities of the HYPERION II it offers a comprehensive solution without limits that advances biological tissue imaging to the next stage.

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Bruker Scientific LLC
Billerica, MA · USA
Phone +1 (978) 439-9899
info.bopt.us@bruker.com

Bruker Optics GmbH & Co. KG
Ettlingen · Germany
Phone +49 (7243) 504-2000
info.bopt.de@bruker.com

Bruker Shanghai Ltd.
Shanghai · China
Tel.: +86 21 51720-890
info.bopt.cn@bruker.com

www.bruker.com/optics