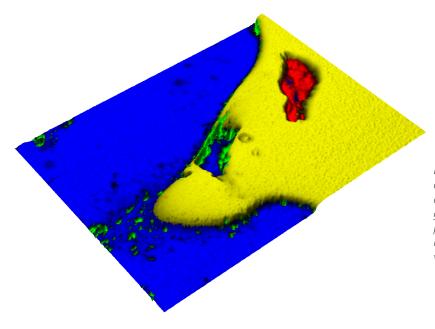# Low-frequency Raman spectroscopy

New technology for narrow-linewidth diode lasers facilitates pharmaceutical inspection.

Low frequency Raman image of the distribution of cysteine in a pharmaceutical emulsion of water and oil. Red; cysteine; blue: water; yellow: oil phase, green: excipients. Cysteine is a precursor in the food, pharmaceutical and personal-care industries and its polymorphic nature can be easily characterized with Raman spectroscopy. (Image: Courtesy of Witec)

The importance of Raman spectroscopy as an analytical tool is based on the ability to probe the unique vibrational and rotational modes of molecules in various materials. These phonon interactions induce material-characteristic frequency-shifts (Stoke-shifts) in the scattered light from the illumination laser. This fingerprint region is accessible with most commercial instruments and allows valuable information to be extracted about molecules such as aromatics, carbonates, sulphates, silicates, oxides and hydroxides within the 500-1500 cm^{-1} range and hydrogen interactions with carbon, nitrogen and oxygen at around 3000 cm^{-1}.

More recently there is also a lot of interest in accessing the low-frequency Raman spectroscopy region (<10 cm^{-1} to 200 cm^{-1}). Raman shifts in this frequency range give access to lattice vibrations of molecular crystals and have the potential to more directly probe intermolecular interactions in solid materials. The low-frequency Raman region probes the same low-energy vibrational and rotational modes of molecular structures as terahertz spectroscopy (300 GHz - 6 THz). The THz region of Raman spectra contains important structural information about the molecules or crystal lattices under investigation **(Fig 1).**

For example, being able to determine the structural form of the Active Pharmaceutical Ingredients (API) is a primary objective in the pharmaceutical industry during drug development, manufacturing and quality control. APIs exhibit polymorphism, which is characterized as having identical chemical compositions but different solid-state structures that may

affect the bioavailability and therapeutic index, and in turn could lead to compromised efficiency of any final drug product [2 – 3]. Specifically, low-frequency Raman spectroscopy provides an avenue to probe polymorphic structures of pharmaceutical systems before and after tableting [1].
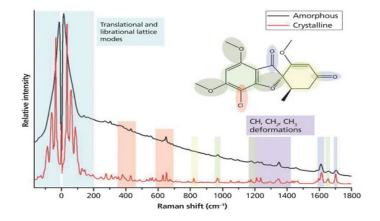

Figure 1. *Example of distinguishing between crystalline and amorphous griseofulvin through low-frequency Raman spectroscopy [1].*

Probing the low frequency Raman region has several advantages over existing techniques such as ease of collecting data (compared to traditional methods like X-ray powder diffraction and differential scanning calorimetry), collecting spectra that contain a lot of structural information as well as being able to discriminate crystalline forms.

The low-frequency Raman region for measurements of lattice phonons in pharmaceuticals has become more accessible in recent years with advances in precise optical filters and narrow linewidth lasers with a high level of frequency stability.

Therefore, low-frequency region investigations in pharmaceutical products has started to transition from the academic laboratory with customized laboratory set-ups to pharmaceutical labs and production lines. By equipping Raman microscopes with low-frequency Raman spectroscopy capability and performing multivariate analysis it has been possible to demonstrate mapping of the API distributions and crystal sizes in over-the-counter (OTC) pharmaceutical tablets [4].

Examples of other analytical applications which take advantage of probing low-energy vibrational and rotational modes through low-frequency Raman spectroscopy include; Polymer analysis (characterization of chemical composition, molecular structures, and chain orientation under mechanical deformation of polymeric material) [5], Semiconductor analysis (advanced semiconductor devices have strong signals in the low frequency region from folded acoustic and shear modes of multilayer super-lattice structures) [6] and Protein characterization.

Laser requirements for low-frequency Raman

In order to access the low-frequency Raman spectral range the notch filters used to separate out the Raman signal from the Rayleigh scattered light from the illumination wavelength needs to be very narrow-band and provide a high level of suppression. The Raman signal, which is a photon-phonon interaction, is inherently very weak, therefore a Rayleigh light suppression of over 60 dB is typically required to record useful Raman information. Filters meeting these requirements can be fabricated by recording holograms in Photo-thermo refractive (PTR) glass through exposure of the interference pattern from a UV laser. Such Volume Bragg Grating (VBG) elements can provide notch filters with FWHM of <1 cm⁻¹ and with a >60 dB cut-off at less than 5 cm⁻¹ from maximum [7] (**Fig 2**).

The spectral purity requirements on the laser source for low-frequency Raman are similar to the notch filter characteristics; the laser line has to be narrow and provide a side-mode suppression ratio (SMSR) of at least 60 dB at less than 5 cm⁻¹ from the main peak. The spectral linewidth of the laser sets a limit to the spectral resolution of the recorded Raman signal (i.e. how small of a difference in Stokes shift can be detected). However, the spectral resolution of a Raman spectrometer does not only depend on the laser source.

Also playing a role is the groove density of the diffraction grating, the spectrometer focal length and, in some cases, the pixel size of the detector. For most fixed-grating systems, the laser linewidth should be a few 10s of pm or less in order not to limit the spectral resolution of the system. Related to the linewidth parameter is the frequency stability, or spectral stability, of the laser. The laser line must be fixed in wavelength during recording of the spectrogram in order to preserve spectral resolution or fall out of the notch filter spectral range. Typically, the laser should not drift more than a few pm over time and over a temperature range of several °C. In addition, the laser line has to provide sufficient output power at a suitable wavelength for the specific material under investigation.
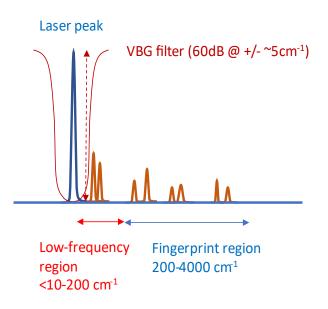

Figure 2: *Filter requirements for low frequency Raman. VBG filters are used due to the sharp cut-off, needed to access the low frequency region*

Narrow-line diode lasers

Laser sources suitable for Raman spectroscopy at 785 nm can be fabricated from AlGaAs-based semiconductor devices. Depending on the emitter size and geometry they can be designed to emit single-transversal mode beams (lower power) or multi-transversal mode beams (higher power).

Semiconductor lasers have a broad gain spectrum and typically have bandwidths of over 1 nm and with long spectral tails stretching several 10s of nm from the main peak.

They can be made to emit spectrally single-mode radiation by introducing distributed wavelength selective gratings (DBR/DFB) into the structure. DBR/DFB lasers at around 780 nm are available at up to a few 10s of mW output power. As an alternative for higher power levels, it is possible to turn the broad-band emission from a semiconductor laser into narrow-band by building an external cavity with a separate wavelength-selective cavity element. In this way the stimulated emission from the laser will be frequency-locked to the spectral distribution of the feed-back from the external cavity element. A conventional way of constructing such frequency-locked semiconductor laser is to build an external cavity using a Volume Bragg Grating element (VBG) (**Fig 3**).
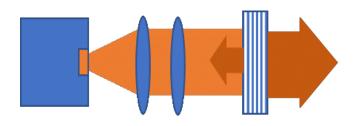

Figure 3. Typical laser design of a conventional wavelength locked diode laser with a partial- ly transmissive VBG. In order to fulfil the requirements on spectral purity for low-frequency Raman, an additional clean-up filter is required to suppress the ASE (Fig 4a).

The output beam from the 785 nm semiconductor emitter is collimated before going into the VBG element, which reflects a fraction of the light with a narrow spectral distribution back into the semiconductor. A draw-back for both DFB/DBR and conventional VBG frequency-locked laser devices is that a fair amount of broad-band Amplified Spontaneous Emission (ASE) from the semiconductor is still emitted from the laser device.

This limits the SMSR ratio to around 40-50 dB up to several nm's away from the main peak. In order for such lasers to be useful for Raman spectroscopy they have to be spectrally filtered with external clean-up filters. In the case of low-frequency Raman spectroscopy it is not enough to use standard dichroic filters with a typical bandgap of 1-2 nm, but necessary to use more narrow spectral filtering, typically by adding a second external VBG element. This additional VBG filter adds cost to the system and can be challenging to match spectrally to the specific output wavelength of the laser.

In order to overcome this draw-back, we present here an alternative and patent pending design for frequency-locking a semiconductor laser. Instead of using a partially transmissive VBG as in the conventional frequency locked diode laser, a highly reflective VBG element is used as the wavelength selective component in the external cavity. An intra-cavity polarizing element and a polarizing beam splitter are used to control the level of feed-back from the VBG to the emitter and the output coupling out of the cavity. In this way, only the stimulated emission is coupled out of the cavity and the broad-band non-stimulated emission is leaking out of the VBG element. The resulting spectral purity is similar to what is achieved with an external VBG clean-up filter, but with the use of only one single VBG element **(Fig 4).**

To ensure the performance of such a laser, especially in guaranteeing the wavelength stability and accuracy, all optical elements were assembled on a single temperature-controlled platform using Cobolt's proprietary HTCure™ technology with high-temperature cured adhesives to ensure robust and precis alignment of the cavity components as well as high level of thermo-mechanical stability and insensitivity to ambient conditions. In this way the wavelength stability and SMSR, can be maintained throughout the life of the laser and in varying ambient temperature conditions.
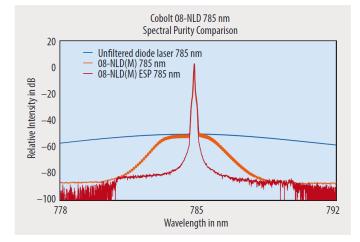

Figure 4: *The excellent spectral purity of the Cobolt 08-NLDM ESP 785 nm laser is shown by comparing its spectral peak (red) with a stan-dard frequency-locked diode laser with an external dichroic filter (orange) and with a standard frequency-locked diode laser without external filter (blue). The Cobolt 08-NLDM ESP 785 nm achieves > 60 dB SMSR at < 0.3 nm (or < 5 cm^{-1}) without any external filtering.*

Outlook

Being able to access the low-frequency region in Raman spectroscopy promises better insight into the detailed composition of pharmaceutical drugs and also stronger Raman signal levels. By combining low-frequency Raman spectroscopy with microscopy techniques and multi-variate analysis it is possible to determine the crystallinity levels and distribution of polymorphic Active Pharmaceutical Ingredients (APIs) in a drug. This information is very important in pharmaceutical manufacturing to ensure the thera-peutic quality of the final product. With the increased availability of precise narrow-bandwidth filters and stable narrow-linewidth laser sources with very high level of spectral purity, such as the 785 nm laser source pre-sented here, the use of low-frequency Raman spectroscopy for investigation of pharmaceutical products is starting to transition from research laboratory settings to production-lines in phar-maceutical industries.

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About the company

HÜBNER Photonics is committed to supplying high performance and innovative lasers that meet or exceed the market's expectations concerning quality, reliability and robustness. HÜBNER Photonics offers the full range of high performance Cobolt lasers, the CW tunable laser C-WAVE along and a full selection of C-FLEX laser combiners. Through continuous technology development, customer orientation and an ISO certified quality management system, HÜBNER Photonics has become a preferred supplier of lasers to major instrument manufacturers and leading research labs for cutting-edge applications in the areas of fluorescence microscopy, flow cytometry, Raman spectroscopy, metrology, holography, nanophotonics and quantum research. HÜBNER Photonics has manufacturing sites in Kassel, Germany and Stockholm, Sweden with direct sales and service offices in USA and UK.