

Industrial Diode Lasers

High-resolution Raman microscopy

Bernhard Wondra, Harald Rossmeier and Thomas Hellerer
TOPTICA Photonics AG

Raman microscopy faces a bright future

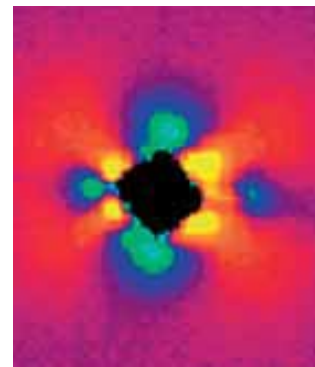
Modern quality assurance benefits from optical analysis methods. Fast, accurate, non-destructive and remote are just some of the advantages light-based techniques can offer. Raman microscopy is a rather new optical inspection procedure. It extracts a sample's intrinsic chemical information without the need for time-consuming sample preparation or labeling. Various markets already base their quality assurance on it:

- Semiconductor industry analyzes surfaces (picture 1), crystal structures or local doping of semiconductors.
- Material science identifies organic compounds in liquids (picture 2) or synthetics, analyzes strongly corrosive liquids and monitors chemical processes, e.g. the curing of glue.
- Pharmaceuticals screens the components of drugs (pictures 3 and 4) in a high-throughput process. Most active pharmaceuticals are polymorphic. The polymorphs often differ in their physical and/or chemical characteristics (stability, dissolution rates etc.) and can be distinguished by their specific Raman spectra.
- At coating production, the quality of ceramic layers or optical coatings can be evaluated by Raman analysis of microstructures.
- A recent application for Raman scattering in medical R&D is the detection of tooth decay. Infected regions within the dentin beneath the surface of the tooth, which are hard to detect by traditional means, can be located by spectroscopic techniques.

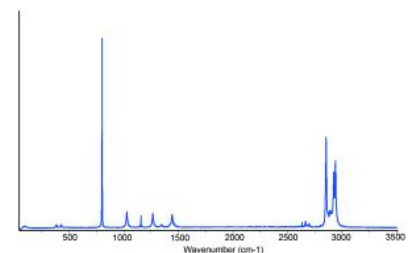
Light and matter interaction

The Raman effect is only one of several ways light and matter can interact. Whenever light respectively a photon hits a molecule or a lattice, several processes can occur:

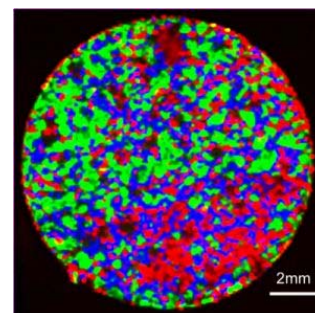
- The photon's energy matches the real state of a molecule and



Picture 1: Stress in silicon.
Courtesy of WITec



Picture 2: Raman spectrum of cyclohexane. Courtesy of HORIBA Jobin Yvon



Picture 3: Raman mapping of whole pharmaceutical tablet, imaged in 10 minutes. Courtesy of HORIBA Jobin Yvon

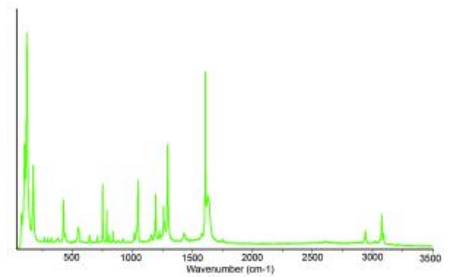
is absorbed, while the molecule is excited into an electronic state. This molecule relaxes to the ground state by non-radiative processes plus a radiative process (fluorescence). Compared to the incident light, the fluorescence wavelength is red-shifted (Stokes shift).

- The photon's energy matches the real state of a molecule and is absorbed, while the molecule is excited into a vibrational state. This molecule relaxes to the ground state by non-radiative processes only. IR-spectroscopy probes such interaction to analyze specimen.
- The photon's energy doesn't match the real state of a molecule and is scattered elastically (Rayleigh or Mie scattering), i.e. the photon's energy before and after scattering is identical. This phenomenon can be considered as an absorption of the photon plus a transfer of the molecule into a virtual intermediate state, followed by an immediate relaxation into ground state and immediate re-emission of the photon.
- The photon's energy doesn't match the real state of a molecule and is scattered inelastically (Raman scattering, picture 5). The molecule is excited to a virtual intermediate state, but relaxes to a vibrational state above the ground state. Consequently, the scattered photon's energy is lower than the incident photon's energy and the referring wavelength is red-shifted (Stokes shift). However, if an incident photon hits a molecule already in vibrational state, it is excited to a virtual intermediate state and can relax to its ground state. Therefore, the scattered photon's energy is higher than the incident photon's energy and the referring wavelength is blue-shifted (Anti-Stokes shift).

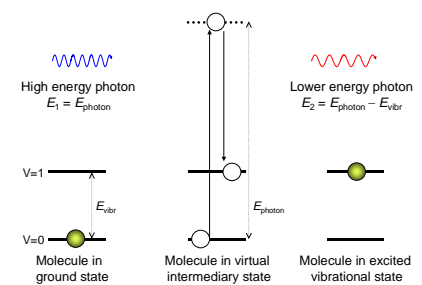
Raman scattering is exploited to analyze all kinds of matter as the Stokes shift depends on the vibrational state of a molecule or lattice. This application note describes only Raman scattering and its usage whilst other interactions (fluorescence and IR spectroscopy) are subject to further application notes.

Stokes and Anti-Stokes

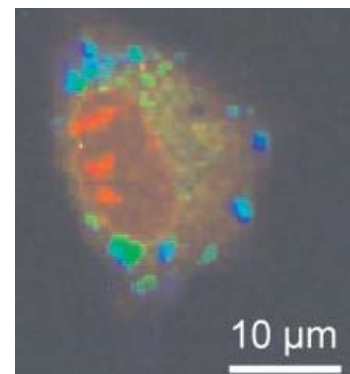
Stokes shift and Anti-Stokes shift are symmetrical to each other. Both of them are determined by the difference of energy between ground state and vibrational state. However, the probabilities of red-shifted photons and blue-shifted photons are different. At ambient temperature, the Anti-Stokes process occurs less often than its Stokes counterpart, simply according to the Boltzmann's rule. Changing to higher temperatures, the probability for blue-shifted photons increases. Thus the temperature of a specimen can be determined by the ratio of Anti-Stokes to Stokes intensity. Most Raman techniques only examine the red-shifted photons while ignoring all blue-shifted and elastically scattered ones.



Picture 4: Raman spectrum of ASS (Acetyl-salicylic-acid).
Courtesy of HORIBA Jobin Yvon



Picture 5: Theory of the Raman effect:



Picture 6: Epithelial rat cell and its components mitochondria (blue), endoplasmic reticulum (green) and nucleoli (red).
Courtesy of WITec.

Raman spectrum

The Stokes shift depends only on the vibrational state of a molecule and not on the wavelength of the incident light. Each molecule provides a characteristic pattern of Stokes shifts, determined by the molecule's bonding structure. A chart containing all Stokes shifts is called Raman spectrum (e.g. picture 4).

Identification of matter by its Raman spectrum

Every substance shows a unique Raman spectrum like a fingerprint: All Stokes shift lines can be addressed to its structure and individual bonds, for instance C-H or C-H₂ bonds for organic compounds, intermetallic bonds for alloys, etc..

For this reason material science exploits this technology: All constituents in a composition can be identified by their individual Raman spectra. One challenge remains: The Raman signal intensity is relatively weak compared to the laser beam intensity or potential fluorescence signals. Separation of the Raman signal is crucial for the acquisition of a perfect Raman spectrum.

Raman spectroscopy and Raman microscopy

Raman spectroscopy provides qualitative and quantitative information as to a specimen's constituents ("chemical contrast") across the examined area. Valuable statistical data is obtained by this method, but detailed insight into the spatial distribution of the constituents is not achieved.

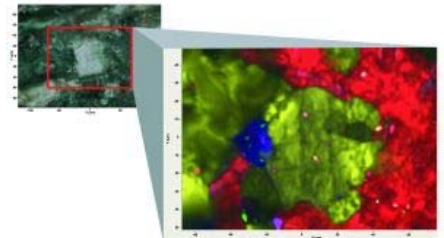
Many researchers, however urgently need that spatially resolved information: A contamination's location can have a high impact on the functionality of a semiconductor device. The exact position of components inside a human cell (pictures 6) is important to know for biologists. Similarly, geologists want to know the spatial distribution of constituents in a geological sample (picture 7).

Modern high-resolution Raman microscopes (pictures 8 and 9) offer that added value: A laser beam is focused on a tiny spot of the specimen. The Raman signal is separated from the elastically scattered photons by a notch filter, then directed at an optical grating and a subsequent CCD detector (picture 10). When reading out the CCD data, a high resolved Raman spectrum is acquired for one focal spot. By scanning rapidly across the specimen and merging of all data, an entire Raman image with high spatial resolution is generated.

Professional software turns the recorded data into useful charts, for instance color-coded charts to indicate various proteins of interest in an organic cell (pictures 6).

What laser suits best a Raman microscope?

There are several requirements concerning an optimum laser



Picture 7: Raman image of a geological sample obtained using SWIFT™ mapping mode. Courtesy of HORIBA Jobin Yvon



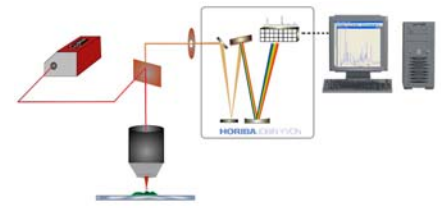
Picture 8: Modern analytical confocal Raman microscope the LabRAM ARAMIS. Courtesy of HORIBA Jobin Yvon



Picture 9: Modern Confocal Raman Imaging Microscope WITec alpha300 R. Courtesy of WITec.

system for integration into a modern Raman microscope:

- High output power. The (weak) Raman signal is proportional to the laser intensity and inversely proportional to the fourth power of the laser's wavelength ($S_{\text{Raman}} \propto 1/\lambda^4$). A rule of thumb states 10^{-6} efficiency in the near-infrared range (Raman signal to laser intensity).
- Transversal single mode beam. To analyze specimen with best spatial resolution a high quality beam with Gaussian beam profile (TEM_{00}) is necessary.
- Extreme narrow linewidth. For reliable assignment of a Raman pattern to a chemical substance the laser of choice has to provide a narrow emission band. A broad linewidth causes blurred patterns due to emission overlapping.
- High Amplified Spontaneous Emission (ASE) suppression. ASE is shifted in wavelength from the laser main excitation peak and can obscure the weak Raman signal. If the laser does not provide sufficient suppression, expensive filters need to be installed into the excitation beam path to eliminate unwanted ASE.
- Stable operation during the entire acquisition period. Any drift in power or wavelength (respectively laser frequency) results in a drift of the Raman signal.



Picture 10: Function sketch of Raman spectroscopy. Courtesy of HORIBA Jobin Yvon



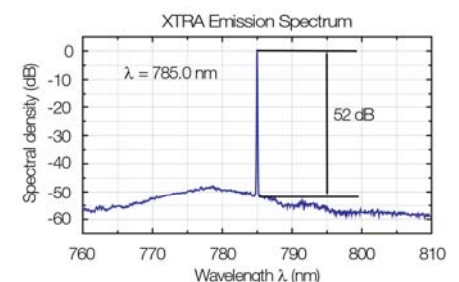
Picture 11: Diode laser system XTRA

The relation between Raman signal intensity and excitation wavelength seems to demand usage of short wavelengths. Contrary to this, visible or UV light has the inherent disadvantage of inducing fluorescence in many organic compounds. As the fluorescence signal is many orders of magnitude higher than the Raman signal, a weak fluorescence background suffices to obscure a Raman spectrum completely. In practice, short wavelengths (blue, violet and UV) are used for non-fluorescent samples (picture 1), such as inorganic pigments or catalysts. However, for the investigation of new or unknown petrochemicals, pharmaceuticals or biomaterials, where fluorescence may occur, one generally prefers infrared lasers.

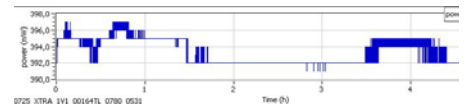
Moreover, many confocal Raman microscopes take advantage of optical fibers to decouple the laser from the microscope. In such case the laser can be installed at any place. To maintain high laser power (at the end of fiber) and best beam quality simultaneously, single-mode lasers and single-mode fibers are essential. This excludes usage of multimode or low power lasers.

Addressing manifold requirements

TOPTICA started production of narrow-linewidth TEM_{00} lasers in 1995 and designed the OEM laser module XTRA in 2000 (Picture 11) especially for Raman applications. For high-end microscopes the matured XTRA provides a great performance due to several



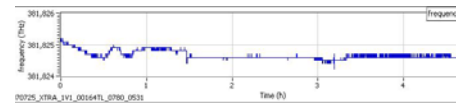
Picture 12: XTRA laser spectrum: more than 40 dB ASE suppression (incoherent background light)



Picture 13: XTRA power stability during acceptance test (drift < ± 1% within 4 h)

reasons:

- 300 mW output power at 785 nm. Such power level is well suited to generate sufficient signal-to-noise ratio at the CCD detector.
- Transversal single mode beam profile (TEM_{00} , $M^2 < 1.5$) plus circular beam shape. A focus diameter of 1 micron can be achieved with appropriate optics in order to achieve best spatial resolution of your specimen.
- Extreme narrow linewidth (< 10 MHz) due to its grating-stabilized external cavity design. This single-frequency emission enables most accurate Raman patterns to identify substances under test.
- High ASE suppression (more than 40 dB, picture 12) to enable usage of affordable notch filters.
- Ultra-stable laser diode driver for smooth and reliable laser operation. The power drift (picture 13) of less than 5% over 100 hours and the frequency drift (resp. wavelength drift) below 1 cm^{-1} over 100 hours (picture 14) clearly indicate the XTRA stability. Contrary to gas lasers, diode laser systems do not generate vibration and subsequent beam walks.



Picture 14: XTRA frequency stability during acceptance test (drift < 1 GHz within 4 h)



Picture 15: Patented fiber coupler FiberDock

The XTRA emits a wavelength of 785 nm, an excitation line well established in Raman microscopy, which avoids fluorescence problems. Furthermore, this wavelength matches the spectral sensitivity of silicon-based CCD detectors: A spectrum up to relative wavenumbers of 3500 cm^{-1} can be read using off-the-shelf CCD detectors.

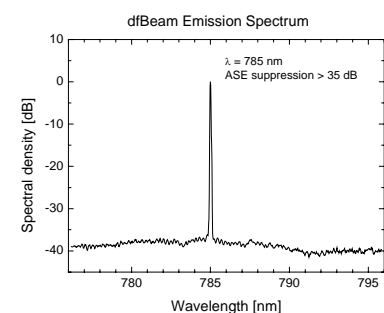
Optional single-mode fiber coupling is available: Our proprietary FiberDock (picture 15) matches the XTRA perfectly and is insensitive to thermal drifts. Both standard single-mode (SM) fibers and polarization-maintaining (PM-SM) fibers can be used. An output power of more than 200 mW is obtained after a polarization maintaining single-mode fiber, which forms a perfect Gaussian beam.

For constant operation, any optical feedback from the Raman system or a fiber facet into the XTRA laser chip is avoided by an integrated optical isolator (35 dB isolation), maintaining highest power stability.

OEM customers expect full computer control over a laser system. The XTRA can be controlled via its RS-232 interface and a standard PC. Switching the laser on and off, changing the power and reading out all relevant parameters (e.g. operation current or operation hours) are thus performed remotely.



Picture 16 OEM laser system dfBeam 785



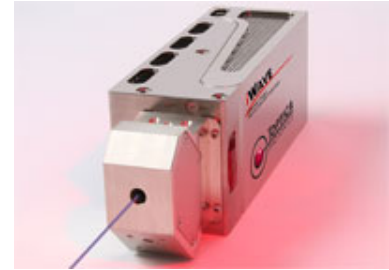
Picture 17: ASE suppression of dfBeam: > 35 dB

On special request, the XTRA is also available at 730 nm, a wavelength used for instance in detection of humidity in the upper skin region. A new version at 650 nm is planned to be released in 2008.

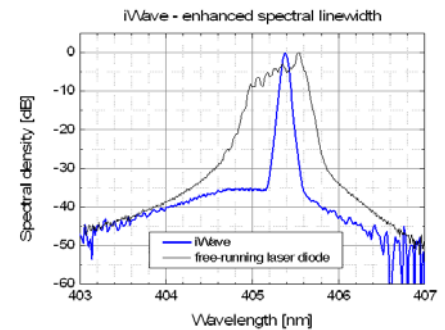
Some customers do not require the high output power of 300 mW, but are satisfied with 120 mW transversal single mode, maintaining high power stability and frequency stability. For that particular demand the dfBeam (picture 16) represents an alternative solution. Based on DFB diodes, a TEM₀₀ laser beam with extreme narrow linewidth (< 0.0004 cm⁻¹) is provided. Mode-hops are avoided due to the intrinsic grating structure in the semiconductor laser chip itself. Even vibrations cannot disturb the extreme narrow linewidth emission. Amplified spontaneous emission (ASE) is suppressed by 35 dB (picture 17).

Raman customers who only examine non-fluorescent specimen prefer blue-violet wavelengths due to the higher Raman efficiency. Until recently, there was only the choice between inconvenient gas lasers with all their well-known drawbacks (vibration, water cooling, mediocre lifetime) and grating-stabilized diode laser systems with a maximum output power of 13 mW. Since June 2007, the new iWave laser module (picture 18) represents a suitable OEM module with sufficient output power (50 mW) at a reduced linewidth (5 cm⁻¹, picture 19). Various Raman examinations do not require ultra-narrow linewidth, but can be performed with a linewidth of very few wavenumbers.

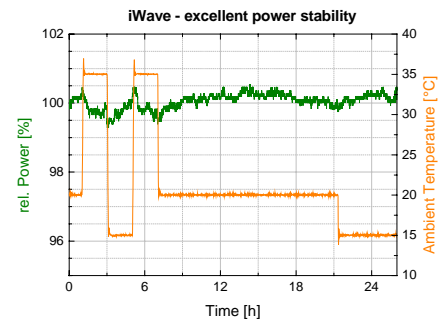
Identical to XTRA and dfBeam, the iWave emits a TEM₀₀ beam to achieve a spatial resolution of one micron with appropriate focusing optics. High attention was paid to power stability (picture 20), even under ambient temperature drifts, in order to guarantee Raman images with best reproducibility and reliability. Further wavelengths (e.g. 375 nm and 442 nm) are planned to be launched in 2008.



Picture 18: iWave 405 nm OEM diode laser system



Picture 19: iWave 405 linewidth measurement of (< 5 cm⁻¹)



Picture 20: iWave power stability during 24 h temperature cycle (power drift < ±0.5 % within 24 h)