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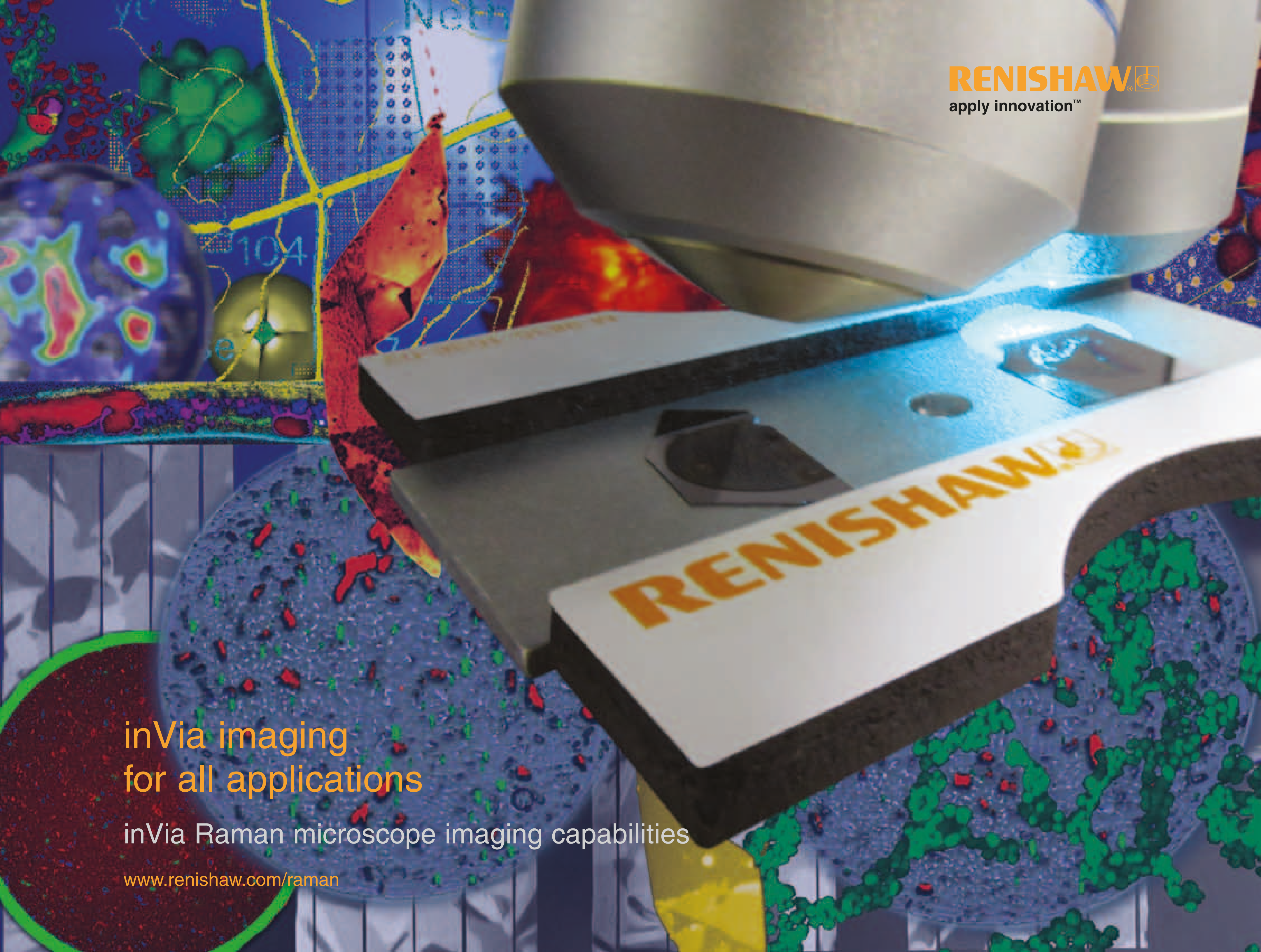


inVia imaging for all applications

inVia Raman microscope imaging capabilities

www.renishaw.com/raman

See the whole picture

From points to areas

You can make manual measurements of points on your sample, but the true power of Renishaw's inVia Raman microscope and its Windows-based Raman Environment (WiRE™) software is revealed when you use its mapping and imaging capabilities.

Rather than just viewing discrete spectra, you can create information-rich maps and images that fully illustrate your most complex samples.

You may have a wide range of sample types, sample sizes, and resolution requirements, but inVia can handle them all. With not just one technology but three—point mapping, StreamLine™ Plus, and True Raman Imaging™—you know you can get the job done.

Easy data collection

Renishaw's WiRE™ software makes collecting data easy.

You view a video image of the sample and define on it the region to analyse as a series of points, a line, or a rectangle. The region is superimposed on the white light image, with the sampling points indicated by a grid.

As the experiment progresses, you can monitor the most recently acquired spectrum, and an estimate of the time until completion while continuing to use WiRE and other applications.

Creating images from data

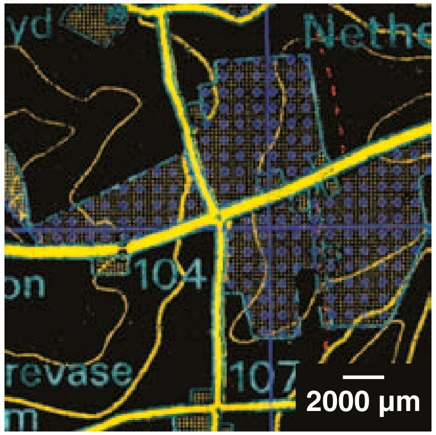
Once inVia has acquired your data, you can reveal the images you need with Renishaw's powerful WiRE™ software.

Which of the powerful image generation methods you use will depend on the details of your samples and the information you require. If your samples are relatively simple, with a few species with distinct Raman bands, then images of the intensity of the Raman signal—taken at some carefully chosen wavelengths—will reveal the distribution of the species.

More complicated samples require more complex multivariate methods, such as principal component analysis (PCA). These methods analyse all of the spectral data and can extract information when simpler methods fail. They are particularly useful when different species have spectra with overlapping Raman bands. A good example of this is in the analysis of pharmaceutical tablets, which have a large number of species (active ingredients, fillers, binders, etc.), each with a relatively complicated spectrum.

If you are interested in the stresses within your samples then detailed analysis of the Raman bands by curve fitting will be appropriate; images illustrating the level of stress can then be generated from parameters such as the change in position of Raman bands.

inVia Raman microscope.



StreamLine Plus Raman image of a section of a UK Ordnance Survey Landranger Map.

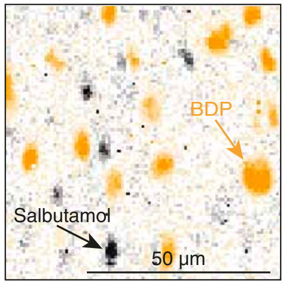
The image illustrates how StreamLine Plus can be used to create a Raman image of the inks used in printed materials.

Point mapping

Point mapping involves acquiring spectra sequentially from a series of positions on the sample, using a software-controlled motorised stage to move the sample between each spectral acquisition.

Point mapping is the traditional method of mapping samples, and is the most basic form of mapping available on an inVia.

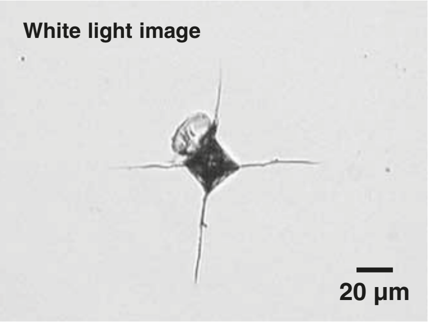
The system is motorised in the vertical axis too, and can automatically maintain optimum focus during analysis of samples with uneven surfaces. It also enables Raman depth slicing, a technique which involves acquiring a map of a vertical slice through a transparent sample. This technique is especially useful for studies of layered materials such as polymer laminates, where it can provide information previously only accessible by mechanical sectioning.

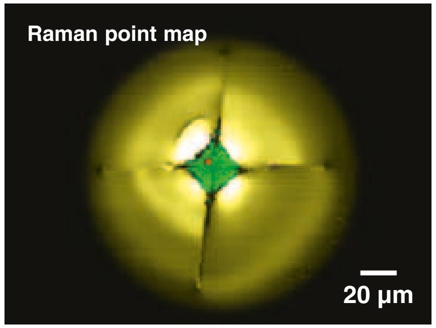


Raman point map of material deposited from an asthma inhaler, showing the distribution of salbutamol bronchodilator and beclomethasone dipropionate (BDP) corticosteroid.

Data courtesy of D F Steele, University of Bath, UK.

Reference
Steele, DF; Young, PM; Price, R; Smith, T; Edge, S; Lewis, D. The Potential Use of Raman Mapping To Investigate In Vitro Deposition of Combination Pressurized Metered-Dose Inhalers. *AAPS J.*, **2004**, *6(4)*, article 32.





White light image and Raman point map of a region of silicon wafer indented with a diamond tip at high temperature and pressure.

The microscope white light image shows the location and appearance of the indentation.

The Raman map reveals changes in structure, composition, and stress. At the indentation site, crystalline silicon IV has been transformed to amorphous silicon (green), and small spots of silicon carbide (red) have formed. Compressive (light yellow) and tensile (dark yellow) stresses are also revealed, as is the absence of stress along the cracks (black).

Analysis of how crystalline silicon behaves under these conditions helps researchers understand its mechanical properties and aids in the quest for higher performing electronic devices.

Data courtesy of G K Banini, University of Cambridge, UK.

StreamLine™ Plus imaging

StreamLine Plus is inVia's ultra-fast imaging technique and is the spatial equivalent of Renishaw's patented SynchroScan™ method for continuous extended scanning. It produces a seamless rapid data stream. The result: images are delivered to you unbelievably quickly.

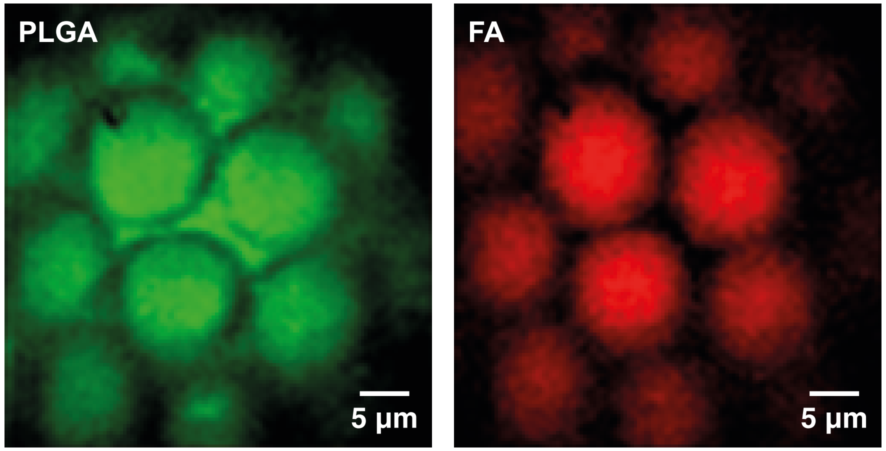
Whether your samples are large or small, whether they require rapid surveying or close scrutiny of a tiny area, StreamLine Plus has it covered.

With StreamLine Plus you can image as large an area as the travel of your motorised microscope stage allows; you are not limited to the field-of-view of the microscope. You can choose the best resolution for your measurements, from sub-micrometre to millimetre ranges, with the confidence that StreamLine Plus's scanning method can give full coverage of the imaged region. And with StreamLine Plus's scanning method there is no tiling of many smaller images, just the smooth production of one large artefact-free image.

If you need better than sub-micrometer spatial resolution, StreamLine Plus's high resolution (StreamLineHR™) option rapidly generates Raman maps with pixel sizes down to as low as 250 nm. This enables you to apply the analytical power of Raman spectroscopy to many challenging nanotechnology structures.

Whether you want to open up your research avenues, improve your products, or increase your understanding of your samples, there is a StreamLine method to help you. Read on to see some examples of how our users employ StreamLine. How would you use it?

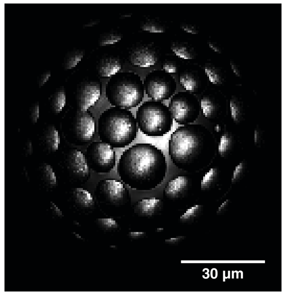
StreamLining biochemical research



StreamLine Plus Raman images of a biodegradable microsphere drug carrier synthesised from poly(DL-lactic-co-glycolic acid) (PLGA) and fusidic acid (FA) antibiotic.

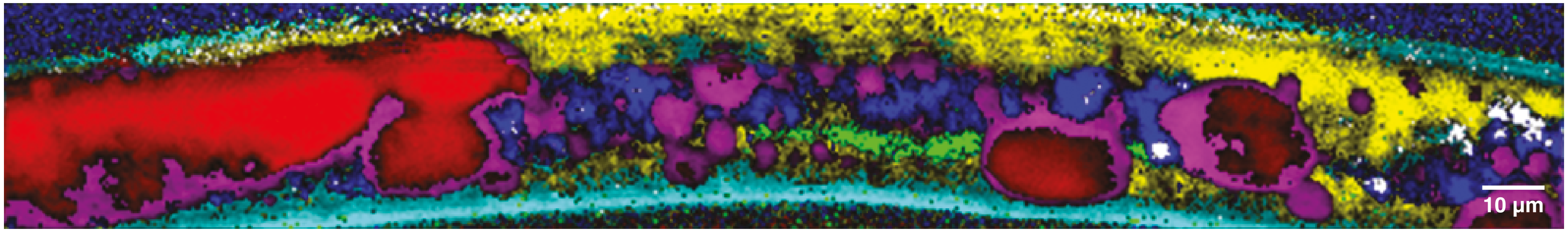
The StreamLine images show that FA (red) is predominantly found in the protruding domains, while the PLGA (green) is uniformly distributed.

Understanding drug carrier formation and drug release efficiency are of paramount importance in optimising the design of new targeted drug delivery systems.



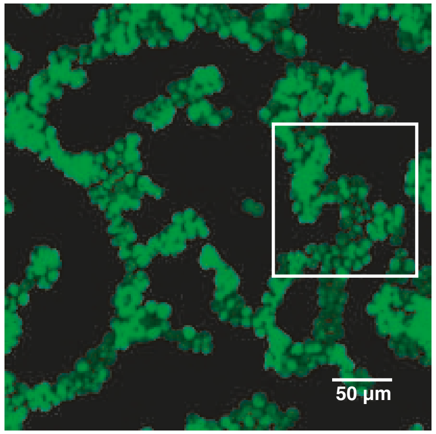
A confocal laser scanning microscopy (CLSM) image of an FA-PLGA microsphere. This image clearly shows the protrusions on the surface that form during the fabrication process.

Samples and CLSM image courtesy of the Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, B.C., Canada.

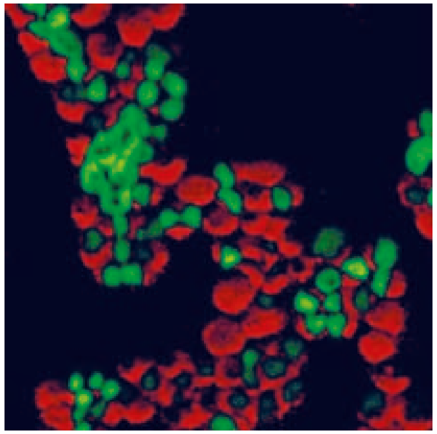


StreamLineHR image of mid-section of a nematode (*Steinernema kraussei*) showing distribution of cell types within the worm at 0.5 µm spatial resolution. Regions were identified as rich in lipids (magenta), pure lipids (red), collagen rich (cyan), and muscular (yellow).

StreamLine Raman imaging has the potential to be a major analytical tool for assessing phenotypic changes in model organisms, such as nematodes, and could play a key role in the search for cures for human diseases, such as HIV and Alzheimer's Disease.



StreamLine Plus Raman image of air-dried *Chlamydomonas reinhardtii* algae, showing distribution and strength of carotenoid Raman bands.



Zoomed region (indicated by white box on image to the left), showing algae with strong carotenoid signals (green), and algae with weak/absent carotenoid signals (red). Such information sheds light on their efficiency for capturing the energy of sunlight.

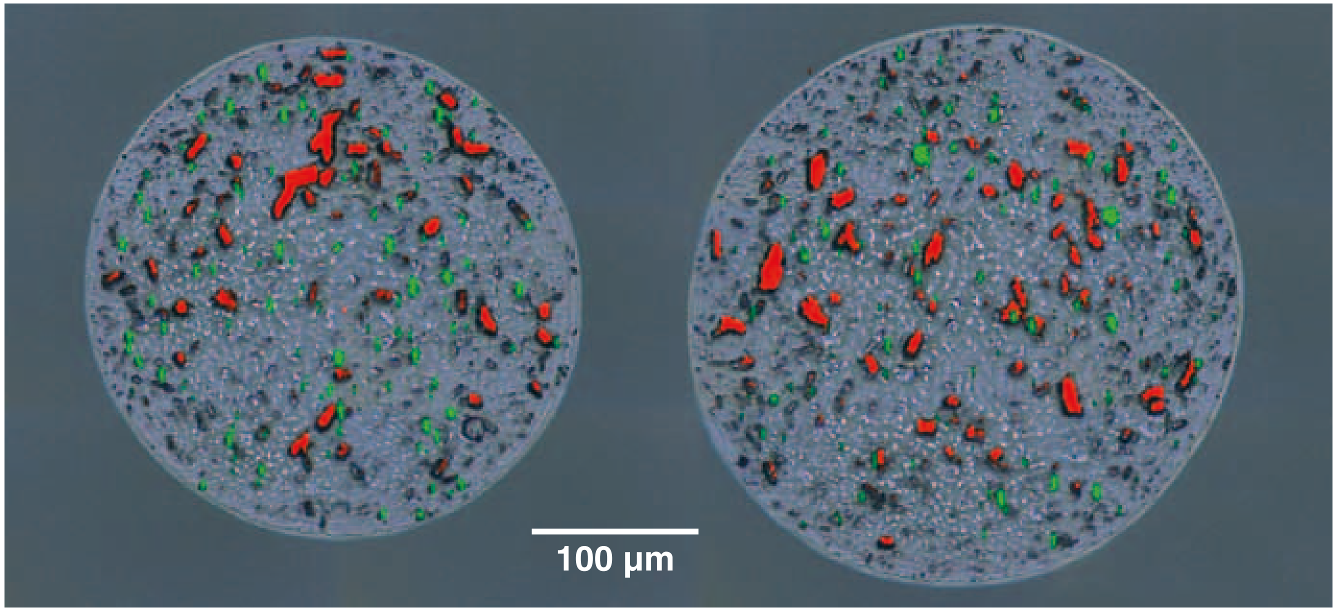
Algae data courtesy of Prof R Goodacre and J Pittman, Manchester Interdisciplinary Biocentre, School of Chemistry, University of Manchester, UK.

"The new StreamLine Plus mapping capability has opened new avenues for our research due to the ability to obtain high quality spectra from a large area in a short time and due to the line scan, with minimal sample damage. The mapping works equally well on biological cells and inorganic surfaces and really takes SERS and Raman mapping to the next level."

Prof. Duncan Graham,
Department of Pure and Applied Chemistry,
University of Strathclyde, Scotland.

StreamLining pharmaceutical formulations

Proprietary anti-allergen nasal spray product used as a model for droplet studies.



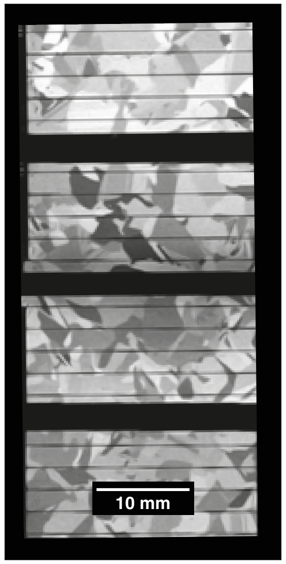
StreamLine Plus images of dried droplet deposits from a proprietary nasal spray showing the distribution of the drug (fluticasone propionate) in green and an excipient (microcrystalline cellulose (MCC)) in red.

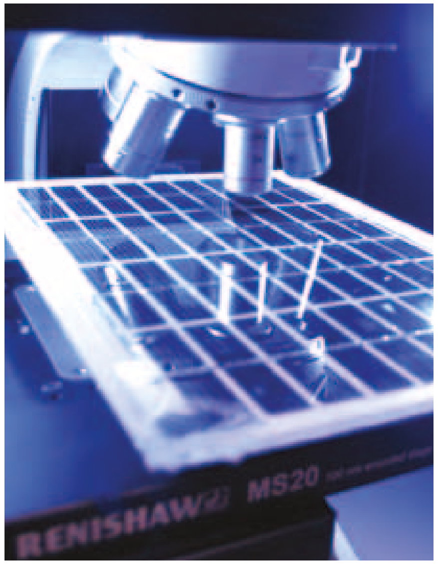
Understanding how the relative amounts of active ingredients and excipients delivered in sprayed droplets relate to the proportions in the bulk product is crucial for optimising drug formulation.

In addition to this semi-quantitative information, high resolution StreamLine images can reveal detailed structural and chemical information, including the chemical purity of particles, co-crystals, and agglomerates.

StreamLine is also ideal for confirming co-location of specific components in preparations where an excipient, such as lactose, is used to 'carry' a drug to the treatment site (e.g. the lungs).

StreamLining semiconductor products





StreamLine Plus Raman image of crystal domains in a polycrystalline photovoltaic (PV) cell.

The size and shape of these domains is used to study cell efficiency.

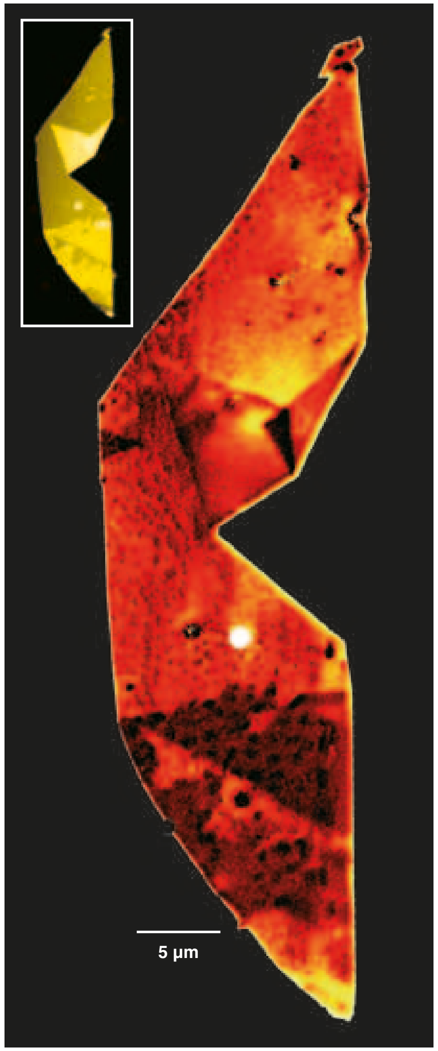
This Raman image highlights StreamLine's ability to image large areas, and was acquired from a commercial PV module through the encapsulation layer.

PV production facility at PRAMAC Swiss SA.

"The use of the Renishaw Raman microscope in PRAMAC Swiss SA has given a fundamental contribution in terms of performance growth, both at initial production steps and on the final product."

Dr. Nadia Galimberti,
head of Quality Control,
PRAMAC Swiss SA,
the largest solar panel production facility in Switzerland.

StreamLining carbon materials



StreamLineHR images of graphene. The main image (G-band position) reveals sub-layer graphene growth defects, while the inset image (G-band area) illustrates variation in thickness.

Growth defects in graphene produce subtle shifts in the position of the G-band, so require high spectral and high spatial resolution Raman data to produce images such as the one shown here.

StreamLineHR employs Renishaw's High Speed Encoded Stage (HSES), which delivers 100 nm repeatability, with the highest achievable spatial resolution reached by oversampling. This limit is approximately 250 nm using a green laser.

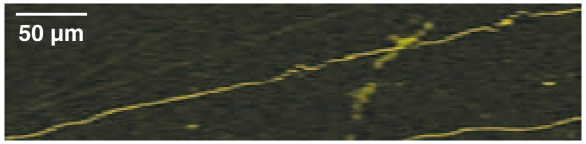
inVia's optimised imaging methods detect even the subtlest spectral changes, and in combination with the HSES, enable StreamLineHR to deliver the highest quality data in the shortest possible total time.

Imaging even the most challenging samples at very high resolution becomes a breeze with StreamLineHR, and all without the need for a Piezoelectric stage!

Sample fabricated at the Centre for Graphene Science, University of Exeter, UK.

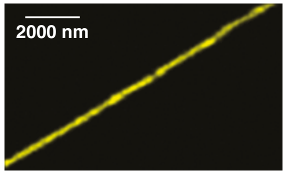
"I have a project on ultra-long (up to centimeters) carbon nanotubes. In order to characterize the local structures along the whole length, it is better to do Raman imaging in large area (> mm × mm) with fine spatial resolution (< 1 µm) within reasonable time. Renishaw's StreamLine Plus Raman can scan samples line-by-line without compromising the resolution. This operation mode can save time significantly, and thus meet my needs."

Dr. Zheng Lianxi, Division of Engineering Mechanics, Nanyang Technological University, Singapore.



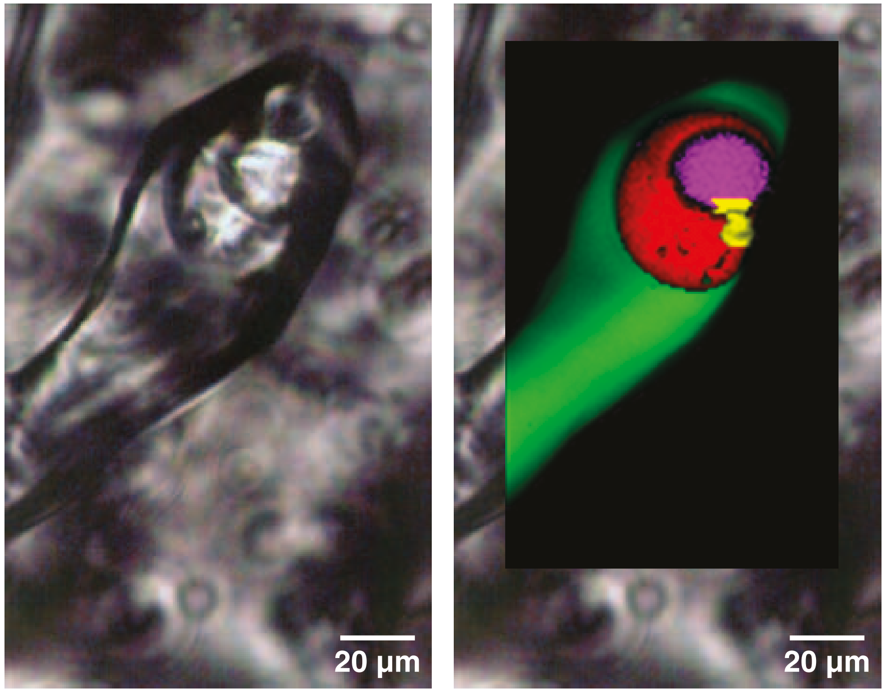
Large area (500 µm in X) StreamLine Plus image showing very long continuous carbon nanotube (CNT). Y resolution is 500 nm, X resolution is 5 µm.

High spatial (100 nm step in X and Y) resolution StreamLineHR image of single CNT (< 300 nm profile generated).



Data courtesy of Dr. Z Lianxi, Division of Engineering Mechanics, Nanyang Technological University, Singapore.

StreamLining mineral samples



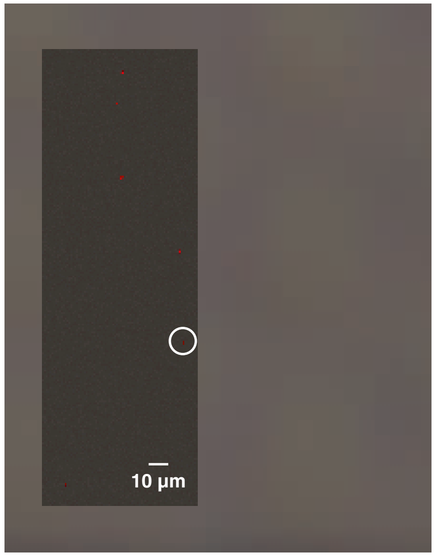
Left: white light image of fluid inclusion contained within quartz.

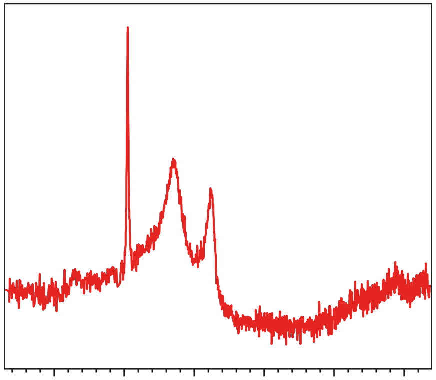
Right: StreamLineHR Raman image of the inclusion overlaid on the white light image. The StreamLineHR image reveals the presence and location of gaseous CO_2/H_2S (purple), liquid CO_2 (red), an aqueous phase (green), and solid sulfur (yellow).

StreamLine imaging of these inclusions helps researchers identify the fluids present at the time of mineral formation and better understand the origin of the earth's crust.

For background information about characterisation, see reference:
Beny, C; Guilhaumou, N; Touray, J-C. *Chemical Geology,* **1982**, *1-2*, 113.

StreamLining contaminant analysis





Top: StreamLine imaged area overlaid on microscope white light image.

Bottom: Raman spectrum of contaminant particle.

Contaminant particles that cannot be found from the white light image were easily pinpointed using StreamLine's rapid survey 'slalom' mode. This mode ensures complete coverage of the imaged area, enabling location of tiny contaminants that could be missed by other Raman mapping techniques.

Particle positions were recorded in inVia's WiRE software. The particles were then revisited and identified from their Raman spectra.

The contaminant particle circled above was identified as amorphous carbon with either phosphate or sulfate groups also present.

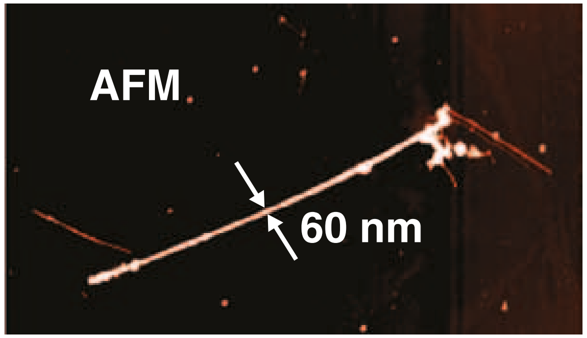
Combined Raman/SPM imaging

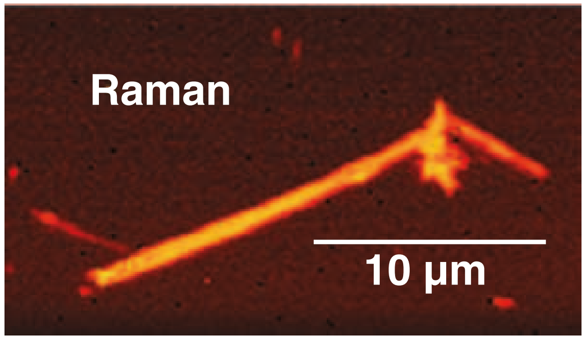
While Raman spectroscopy is well-suited to the analysis of micrometer-sized features and is ideal for many users, sometimes you need the higher resolutions attainable by scanning probe microscopies (SPM), such as atomic force microscopy (AFM) and near-field scanning optical microscopy (NSOM). Renishaw's innovative SPM interfaces make inVia the instrument of choice for a wide range of SPM manufacturers who wish to offer seamless Raman integration for the most demanding applications.

The spatial resolution of Raman systems employing normal optical microscopes is diffraction-limited to approximately half the wavelength of the incident laser light, because both the illuminating laser light and the Raman scattered light are collected in the optical far-field (i.e. many wavelengths of light away from the scattering material).

Renishaw has developed high optical efficiency interfaces that directly couple the inVia microscope to a wide variety of scanning probe microscopes from different manufacturers, including Bruker, NT-MDT, and Nanonics Imaging Ltd. Now you can augment high spatial resolution imaging techniques, such as AFM, with the chemical analysis power of Raman spectroscopy.

inVia also supports the new optical techniques of tip-enhanced Raman spectroscopy (TERS) and near-field optical microscopy (NSOM/SNOM); these offer you the potential of Raman spectroscopic data at unparalleled spatial resolutions.





Simultaneously collected AFM and Raman images of a 60 nm diameter silicon nano-wire.

Sample courtesy of Prof M Kuball, University of Bristol, UK, and Prof J Redwing, Penn State University, USA.

"Direct optical coupling of NTEGRA-Spectra with inVia system provides reliability and stability for the challenging research with TERS. This, combined with the relative ease of use of inVia system, promotes our confidence in performing these experiments. We are getting new data with TERS now...actually we can resolve 14 nm."

Prof. Sergei Kazarian,
Chemical Engineering and Chemical Technology,
Imperial College London, UK.

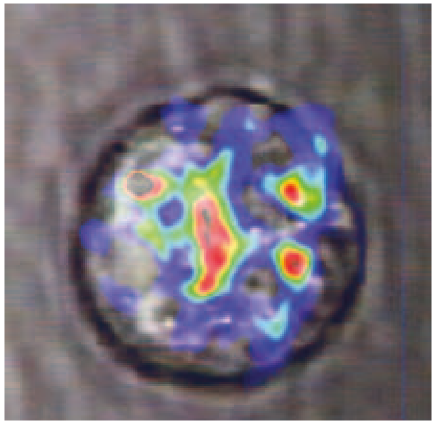
True Raman Imaging™

True Raman Imaging™ technology enables the rapid determination of the spatial distribution of chemical species. It is ideal for surveying large sample areas, and for studying the spatial variations of samples that change rapidly with time. True Raman Imaging™ directly images Raman-scattered or photoluminescent (PL) light to reveal chemical distribution.

This patented technique is radically different to traditional point imaging techniques, as it does not use a diffraction grating to produce a series of spectra, but instead uses tunable filters to directly image the Raman band of interest, capturing the data in one exposure.

The laser beam is expanded to illuminate a disc-shaped area on the sample and the resulting Raman scattered light filtered and focused to produce an image directly on the detector. The size of the illuminated disc is inversely proportional to the magnification of the objective lens: it is typically 50 µm diameter for a 50× objective.

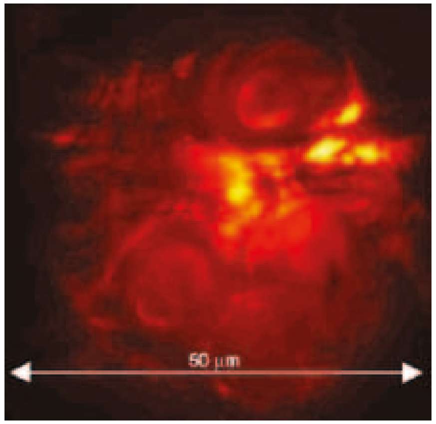
The key advantage of True Raman Imaging™ is its speed, enabling you to rapidly survey large areas.



Composite white light and True Raman Image showing the distribution of an anti-cancer drug within a cancerous cell (~20 µm diameter). The image has been processed using SwRI software to enhance the Raman signal.

Data courtesy of L Jian, Southwest Research Institute (SwRI), San Antonio, Texas, USA.

Reference
Jian, L; Weitman, SD; Miller, MA; Moore, RV; Bovik, AC. Direct Raman Imaging Techniques for Study of the Subcellular Distribution of a Drug. *Applied Optics*, **2002**, *41(28)*, 6006.



A True Raman Image of a cosmetic hand cream reveals the distribution of the oil component.

Renishaw plc

Spectroscopy Products Division
Old Town, Wotton-under-Edge,
Gloucestershire GL12 7DW
United Kingdom

T +44 1453 524524
F +44 1453 523901
E raman@renishaw.com

www.renishaw.com

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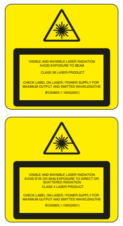
Safety features



- Class 3B laser product in standard configurations
- Optional Class 1 laser safe enclosure
- Deep UV use requires Class 4 laser safety precautions
- Fully enclosed laser paths for multiple lasers
- Fully interlocked with interlock self-test features

About Renishaw

Renishaw is an established world leader in engineering technologies, with a strong history of innovation in product development and manufacturing. Since its formation in 1973, the company has supplied leading edge products that increase process productivity, improve product quality, and deliver cost effective automation solutions.

A worldwide network of subsidiary companies and distributors provides exceptional service and support for its customers.

Products include:

- Dental CAD/CAM scanning and milling systems
- Encoder systems for high accuracy linear, angle and rotary position feedback
- Laser and ballbar systems for performance measurement and calibration of machines
- Medical devices for neurosurgical applications
- Probe systems and software for job set-up, tool setting and inspection on machine tools
- Sensor systems and software for measurement on co-ordinate measuring machines

inVia imaging for all applications

You may have a wide range of sample types, sample sizes, and resolution requirements, but inVia can handle them all. With not just one technology but three—point mapping, StreamLine™ Plus, and True Raman Imaging™—you know you can get the job done.

Contact your local Renishaw supplier for further information about inVia, or to discuss any special requirements you may have.

www.renishaw.com

inVia has been manufactured under the controls established by a Bureau Veritas Certification approved management system that conforms with ISO 9001:2000.

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