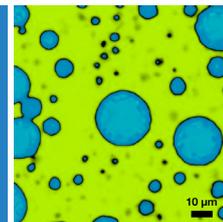


Improve your Raman System with Microscopy Options



Introduction

HORIBA Raman confocal microscopes use a variety of research grade upright, open space and inverted microscopes from different vendors, including Olympus and Nikon.

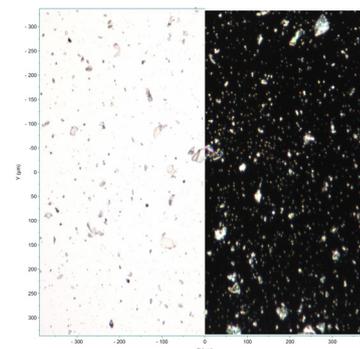
This technical note describes the available options and explains their typical use, assisting the selection of relevant options at the time of order or for future upgrade.

Customer Benefits

- Visualize any type of sample
- Find regions of interest quickly
- Quickly discriminate between points of interest and visualisation artifacts

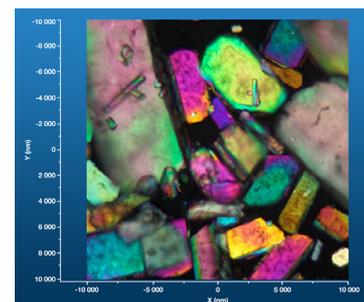
Basic Microscopy Options

Options	Episcopic (Reflected) Mode	Diascopic (Transmitted) Mode
Brightfield (BF)	<ul style="list-style-type: none"> • The samples are opaque, transparent, translucent generally, colored or stained, visible details, high contrast. 	<ul style="list-style-type: none"> • The samples are transparent, translucent generally, colored or stained, visible details. Sometimes the contrast of opaque powdered samples may be improved.
	<ul style="list-style-type: none"> • Illumination light is transmitted through the sample and the contrast is generated by the absorption or reflection of light in dense areas of the specimen. • The limitations of brightfield microscopy include low contrast for weakly absorbing samples and low resolution due to the blurry appearance of out-of-focus material. 	
Darkfield (DF)	<ul style="list-style-type: none"> • The samples are opaque, colored, or stained • Special DF objectives are needed with long working distance for compatibility with Raman collection. 	<ul style="list-style-type: none"> • The samples are transparent, translucent, colored, or stained. • Small aquatic organisms, bacteria, ovocytes, cells, tissues, particles or internal inclusions and pores in thin sections, specimens with topographical irregularities, such as etched metallographic or ceramics specimens, edges, grain boundaries, surface defects in internal structures. • DF condenser is needed, and standard objectives may be used.
	<ul style="list-style-type: none"> • The samples are often materials of low contrast, that have refractive indices very close in value to that of their surroundings and are difficult to image with conventional brightfield techniques. • The samples will appear bright against a dark background. • The main limitation of DF microscopy is the low light levels seen in the final image. The sample must be very strongly illuminated. 	
Polarization	<ul style="list-style-type: none"> • This option is using optical anisotropy to reveal the structure of the sample and to improve the contrast and quality of the final image. • Polarizer and analyzer are needed. • Types of samples: crystals, solid particles, structured samples (e.g. cellulose, collagen) 	



BF (left) and DF (right) episcopic images of teflon particles (10X)

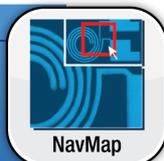
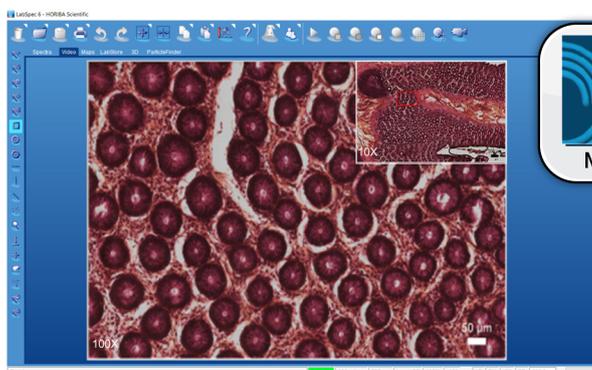
Interesting to highlight very fine details, micro and nano objects or diffusing interfaces (e.g.: microplastics)



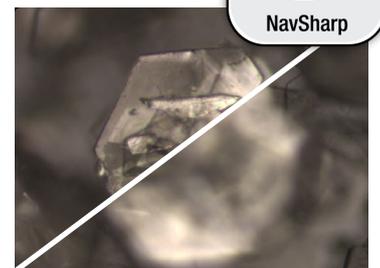
Extended polarized light microscope image

Microscopy Options for Easy Navigation and Topography

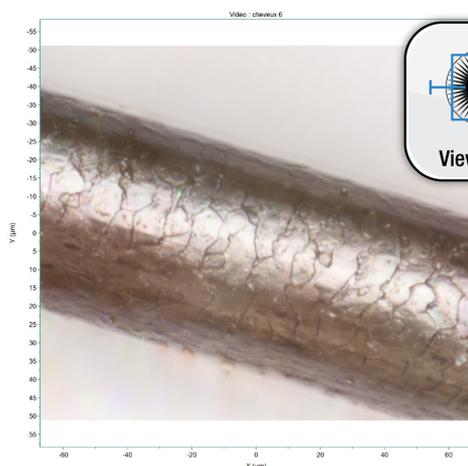
Options	Description
Video Mosaic	<p>Mosaic is a LabSpec application that allows easy acquisition of video montages.</p> <ul style="list-style-type: none"> • Compatible with XY motorized stage and all objectives. • Two operation modes: Manual selection and Presets. • Fully compatible with EasyNav™ Package, sample topography representation and all microscopy tools presented in this document. • Very useful for: large sample analysis, correlative microscopy on the same area on the sample.
EasyNav™ Package	<p>Three revolutionary applications, NavMap™, NavSharp™ and ViewSharp™. These three modules enable Raman users to navigate images while remaining in focus, to easily identify the region of interest, and obtain sharp, rapid Raman chemical images for a large variety of samples.</p> <ul style="list-style-type: none"> • Fully compatible with Mosaic. <p>NavMap™ is a video feature that shows the global sample and the zoomed region of interest within the sample, simultaneously, in real-time. This ensures straightforward navigation and identification of the region of interest.</p> <p>NavSharp™ technology delivers sharp and real-time navigation on a sample image with any topography. The surface focus is readjusted automatically with the use of an automated Z sample stage. NavSharp™ offers sharp path imaging, and optimum focus at all times, even on rough samples.</p> <p>Navigate your sharp image in real-time with NavSharp™ application.</p> <p>ViewSharp™ technology constructs an image in which all surfaces are in focus simultaneously, and creates a 3D topography image. ViewSharp™ guarantees the highest focal quality in Raman images, by using the recorded topography which corresponds to the best focus at any pixel of the image. There is no longer a need to do an automatic focus point by point, making 3D chemical Raman imaging faster than ever.</p> <p>Construct a 3D topography and acquire rapid, in-focus Raman images with ViewSharp™ application.</p>



Blood cells 10X mosaic and 100X within NavMap™ navigation interface



Calcite crystal with and without NavSharp™ autofocus (50X LWD)



ViewSharp™ Z-stacking 2D and 3D topographic view of a human hair (50X LWD)

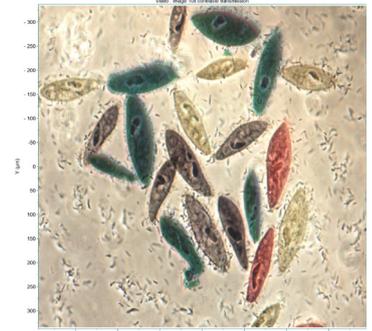
ViewSharp™/NavSharp™:
patent EP3351992B1

Microscopy Options for Biology

Options	Episcopic (Reflected) Mode	Diascopic (Transmitted) Mode
Differential Interference Contrast (DIC)	<ul style="list-style-type: none"> The samples are unstained transparent or non-transparent specimens (opaque substrates are more efficient). 	<ul style="list-style-type: none"> The samples are unstained transparent specimens fixed on transparent substrates.
	<ul style="list-style-type: none"> This option will improve the specimen and interface contrast by using polarized light. Images produced by a DIC microscope are relief-like and are generated by the gradient of optical paths for both high and low spatial frequencies present in the specimen. 	
Phase Contrast	<ul style="list-style-type: none"> Produces high-contrast images of transparent specimens such as living cells (usually in culture), microorganisms, thin tissue slices, lithographic patterns, fibers, latex dispersions, glass fragments, and subcellular particles (including nuclei and other organelles). No staining needed. Special phase objectives are needed. Available only in diascopic mode. 	
Epifluorescence	<ul style="list-style-type: none"> Epifluorescence option may be coupled to a Raman microscope allowing easy imaging of intense signals and co-localization studies with multi-colored labeling on the same sample. Available with several fluorescence filter cubes for several fluorophores within a single sample, e.g. DAPI, FITC, Cy3, TxRed, Cy5. Objectives are Raman compatible. Ideal for cells and bacteria analysis, as well as for all types of particles and contaminants. 	



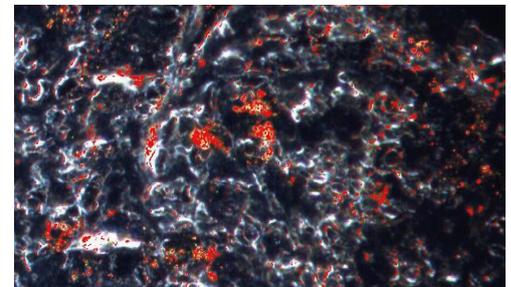
Daphnia 10X DIC diascopic illumination



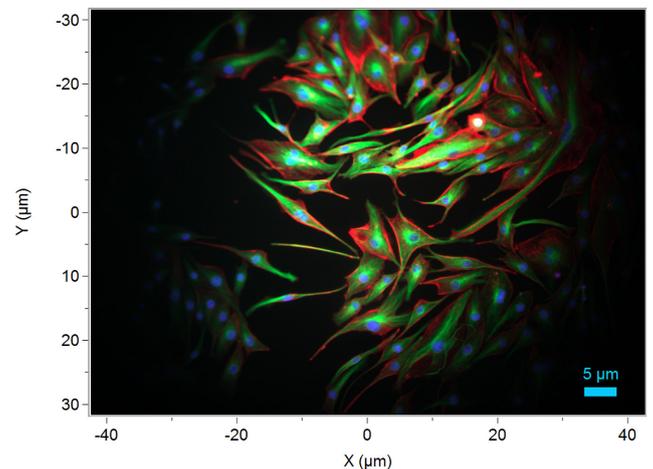
Paramecia cells 10X Phase Contrast diascopic illumination



Daphnia 10X phase contrast diascopic illumination

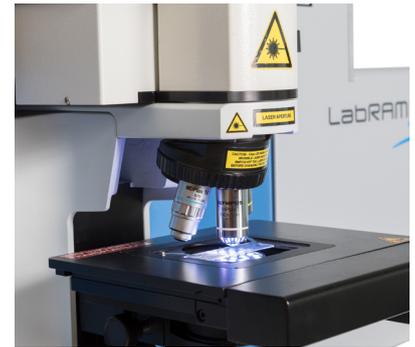


Hyperspectral gold nanoparticles (red) mapping in tissues 60X illuminated with EDF from CytoViva®

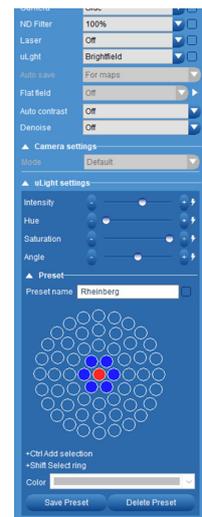


Bovine Pulmonary Artery Endothelial (BPAE) cells 40X epifluorescence

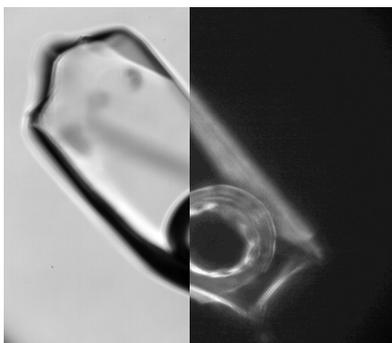
Options	Description
Microlight	<ul style="list-style-type: none"> The samples are transparent. Accessory based on LED technology to be used in diascopic mode for structured illumination. Simply positioned in place of the transmission condenser. Standard objectives for Raman measurements are used. Different images may be obtained: DF, Anomal Phase Contrast (simplified Phase Contrast), BF, Rheinberg (illumination that uses a colored Darkfield to illuminate the sample with two different colors of light).
CytoViva®	<p>CytoViva® is an integrated solution combining Enhanced Darkfield (EDF) and optical Hyperspectral Imaging (HSI).</p> <ul style="list-style-type: none"> The integrated system offers versatile modes of imaging and hyperspectral imaging that are very important for nanomaterials, nanotoxicology and life science applications. The Enhanced Darkfield (EDF) from CytoViva® enjoys much higher signal-to-noise than standard darkfield imaging. Samples are transparent or translucent, and typically they may be nanoparticles embedded in cells or tissues, nanoparticles in cosmetic products and polymers packaging. Hyperspectral Imaging (HSI) from CytoViva® adds a fast characterization capability to your Raman microscope with quick mapping and pre-identification of particles / nano-objects of interest. Using a white light source in transmission or reflection, or the enhanced dark field illuminator, it captures the scattering spectra of particles in a fraction of the time for further Raman chemical characterization. Particularly suitable for quick identification of SERS colloidal particles and drug nanovectors in early phase development applications. It helps to understand almost in real time the spectral modifications of nanoparticles due to environmental changes (pH, temperature, pressure...). Samples are usually nano-objects in a transparent or translucent medium.



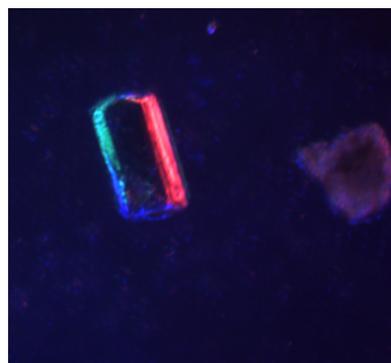
Microlight accessory mounted on LabRAM HR transmission illuminator



Microlight illumination UI in LabSpec 6



Automatic switch BF / DF diascopic illumination of a geologic solid inclusion with Microlight accessory (50X)



Rheinberg illumination of a salt crystal with Microlight accessory (10X)



Cytoviva® EDF and HSI mounted on XploRA Plus