# **CARSCOPE** SERIES



Powerful and versatile tool for vibrational spectroscopy and chemically selective imaging:

- Label-free optical imaging
- Minimally invasive technique
- Non-photobleaching signal

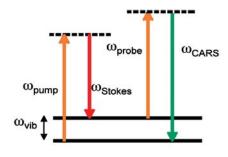
#### INTRODUCTION

Coherent anti-Stokes Raman scattering (CARS) spectroscopy primarily was used in chemistry, physics and related fields. It is sensitive to the same vibrational signatures of molecules as seen in Raman spectroscopy, typically the nuclear vibrations of chemical bonds. Unlike Raman spectroscopy, CARS employs multiple photons to address the molecular vibrations, and produces a signal in which the emitted waves are coherent with one another. As a result, CARS is orders of magnitude stronger than spontaneous Raman emission. CARS is a third-order nonlinear optical process involving three laser beams: a pump beam of frequency  $\omega_{\scriptscriptstyle pump}$  , a Stokes beam of frequency  $\omega_{\textit{Stokes}}$  and a probe beam at frequency  $\omega_{probe}$ . These beams interact with the sample and generate a coherent optical signal at the anti-Stokes frequency  $\omega_{CARS}$  =  $\omega_{pump}$  -  $\omega_{Stokes}$  +  $\omega_{probe}$  . The CARS signal

- 3D sample imaging capability
- NIR pump and Stokes wavelengths suitable for deep-tissue imaging
- Picosecond pulse duration good compromise between efficiency and spectral resolution

 $\omega_{CARS}$  is resonantly enhanced when the difference between the pump  $\omega_{pump}$  and Stokes  $\omega_{Stokes}$  frequencies matches a vibrational transition  $\omega_{vib}$  of the molecule.

Combining of coherent anti-Stokes Raman scattering (CARS) spectroscopy with the microscopy opens up unique method for chemical imaging. CARS microscopy permits vibrational imaging with high-sensitivity, high speed, and three-dimensional nearly diffraction limited spatial resolution.



### CARS Microspectrometer

#### FEATURES

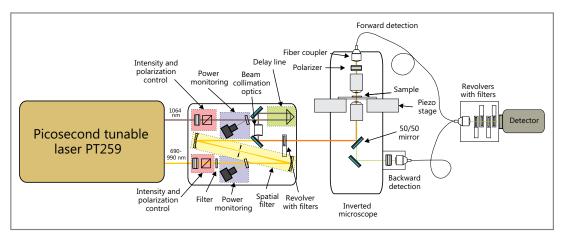
- Wide range of accessible vibrations: 740-4000 cm<sup>-1</sup>
- Minor fluorescence interference
- High spectral resolution and sensitivity
- Sub-wavelength spatial resolution
- F-CARS, E-CARS, P-CARS detection geometries
- Easy transformable to fluorescence, TPEF and SHG microscopes
- Up to 1300 μm excitation for TPEF
- Specially designed cost-effective picosecond tunable laser system

#### APPLICATIONS

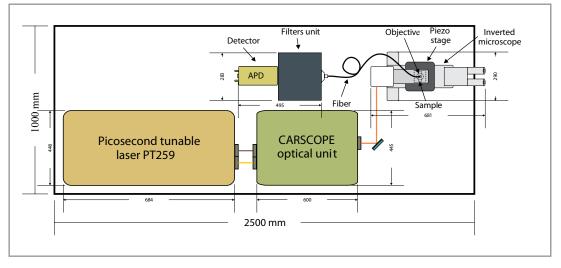
- Species selective spectroscopy and microscopy
- Multimodal nonlinear imaging
- Deep tissue in vivo imaging
- Long term live cell studies
- Non-destructive research for the biological and material sciences
- Your application is welcome...

## **BEKSPLA**

#### DESIGN OF CARS MICROSPECTROMETER



Optical-block diagram of microscpectrometer CARSCOPE with PT259 laser and piezo stage



Drawings of CARS microspectrometer with PT259 laser (all dimensions are in millimeters)

Main part of CARSCOPE is special design picosecond tunable wavelength laser PT200 series with up to 88 MHz pulse repetition rates. This laser has two output ports: one accesses tunable wavelength radiation from optical parametric generator (OPG) and another – fundamental laser radiation. In standard configuration of CARSCOPE NIR range radiation from OPG output is used as pump and probe beams; fundamental laser radiation is used as Stokes beam. Such solution enables deep tissue penetration and significantly reduces photobleaching problems.

Both beams used for sample excitation are combined in external unit. It enables incidence beams intensity control and monitoring, polarization control. All functions are automated and can be accessed from PC. On the output the unit both beams are perfectly spatially and temporally overlaped.

Microspectrometer is based on inverted microscope. One or several objectives optimized for near-IR region with oil or water immersion can be selected. Sample scanning is organized using either three-directional piezo stage or beams scanning head. There are two standard signal collection geometries – forward and backward (F-CARS, E-CARS). In E-CARS geometry the same objective is used for pump beam delivery to the sample and CARS signal collection. This configuration allows measurements of nontransparent samples. In F-CARS geometry another objective on opposite side of the sample is used. Due to fiber delivery of CARS signal from microscopy to detection unit, switching between configurations becomes very easy. Complementary information can be revealed, if measurements in both geometries are performed simultaneously.

Typically detection unit consist of filters set and photon counter. In a standard version avalanche photodiode (APD) sensitive in NIR range is used as a detector. It is possible to order additional detectors optimized for other spectral ranges. Another option is monochromator instead of filters unit. This solution is excellent for investigation of samples with multiple Raman signatures, where separation of two nearby located peaks is needed. Combined version of signal separation system is also available.

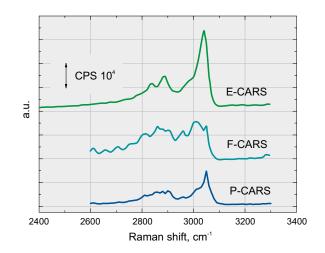
Picosecond pulse duration of excitation beams and versatile CARSCOPE design allows use this device for other nonlinear imaging techniques like second harmonic generation (SHG) or two photon excitation fluorescence (TPEF). In case, if PT259 series laser is used, UV range extension is available. This allows use system for linear fluorescence microscopy.



#### CARS MICROSPECTROMETER INCLUDES:

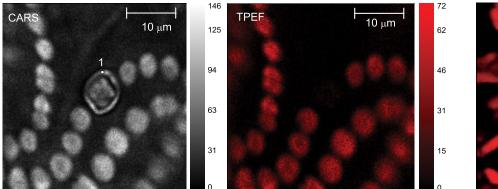
- Tunable wavelength picosecond laser PT25X series
- Excitation beams combining, control and monitoring unit
- Inverted microscope platform with forward and backward detection ports
- Three dimensional scanning system (piezo stage or scanning head)
- Signal separation unit (monochromator or set of filters)
- Signal detector and data acquisition unit
- Personal computer
- Control software

#### CARS SPECTRA EXAMPLE

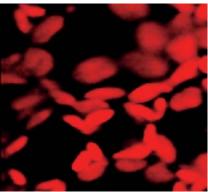


E-CARS, F-CARS, P-CARS spectra of a polystyrene bead (1.1  $\mu m$  in diameter)

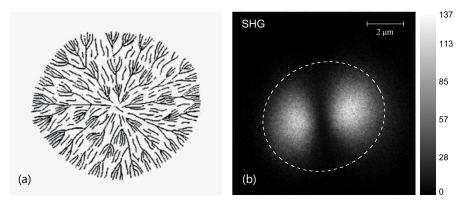
#### IMAGES OF BIOLOGICAL SAMPLES



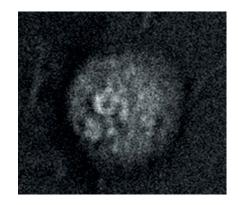
Nostoc commune. Images taken by CARS and TPEF techniques



CARS image of chloroplasts



Starch granule, sketch (a) and SHG images (b)



CARS image of lymphocyte

#### **SPECIFICATIONS**

VERSION	CARSCOPE-1M-PZ	
OPTICAL PARAMETERS		
Spectral range	740 – 4000 cm <sup>-1</sup>	
Pump/probe beams wavelength range	740 – 990 nm	
Stokes wavelength	1064 nm	
Spatial resolution	0.5 µm	
SCANNING SYSTEM		
Scanning method	Sample movement	
Scanning device	Three-dimensional piezo stage	
Maximum imaging speed <sup>1)</sup>	~40 sec	
Travel range	100 × 100 × 20 µm	
Resolution	1 nm	
PUMP LASER		
Model	РТ259-Н	
Pulse repetition rate <sup>2)</sup>	1 MHz	
Tuning range	700-990, 1150-2300 nm	
UV extension range (optional)	350-400 nm	
Output power <sup>3)</sup>	25 mW	
UV range output power 4)	1 mW	
Linewidth⁵	< 8 cm <sup>-1</sup>	
Typical pulse duration, ps <sup>3) 5)</sup>	~ 5 ps	
Typical time bandwidth product	< 0.8	
Typical beam diameter <sup>3) 6)</sup>	2 mm	
Typical beam divergence <sup>3) 7)</sup>	< 2 mrad	
M <sup>2</sup>	< 1.6	

#### **OPTIONS**

### FLUORESCENCE MICROSCOPY

(available only with PT295 laser)		
Excitation wavelength range	350 – 400 nm	
Detection wavelength range	450 – 700 nm	
TPEF MICROSCOPY		
Excitation wavelength range	700 – 1300 nm	
Detection wavelength range	450 – 900 nm	
SHG MICROSCOPY		
Excitation wavelength range	800 – 990, 1064 nm	

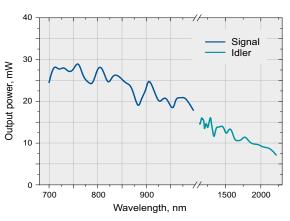
<sup>1)</sup> Measured 200 × 200 pixels.

- <sup>2)</sup> Inquire for custom pulse repetition rates.
- <sup>3)</sup> Measured at 800 nm.
- <sup>4)</sup> Measured at 400 nm.
- $^{\scriptscriptstyle 5)}\,$  Pulse duration can vary depending on wavelength and pump energy.
- <sup>6)</sup> Beam diameter at the FWHM level and can vary depending
- on the pump pulse energy.
  <sup>7)</sup> Full angle measured at the FWHM level.

#### PT200 SERIES PICOSECOND TUNABLE LASERS



PT200 series lasers integrate picosecond optical parametric oscillator and picosecond DPSS pump laser into single compact housing. Picosecond tunable lasers feature 690-2300 nm tuning range, 5 ps pulse duration, nearly Fourier transform-limited linewidth



Typical output of PT259 tunable laser

and up to 88 Mhz repetition rate. Megahertz pulse repetition rate is optimal for using photoncounting detection technique in numerous **nonlinear** spectroscopy and microscopy applications.

## **BEKSPLA**

EKSPLA 02300 Vilnius LITHUANIA

Ph.: +370 5 2649629 Savanoriu av. 231 Fax: +370 5 2641809 sales@ekspla.com www.ekspla.com

Find local distributor at www.ekspla.com



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