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and
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Optimized coherent Raman scattering microscopy with a novel tunable dual wavelength lightsource

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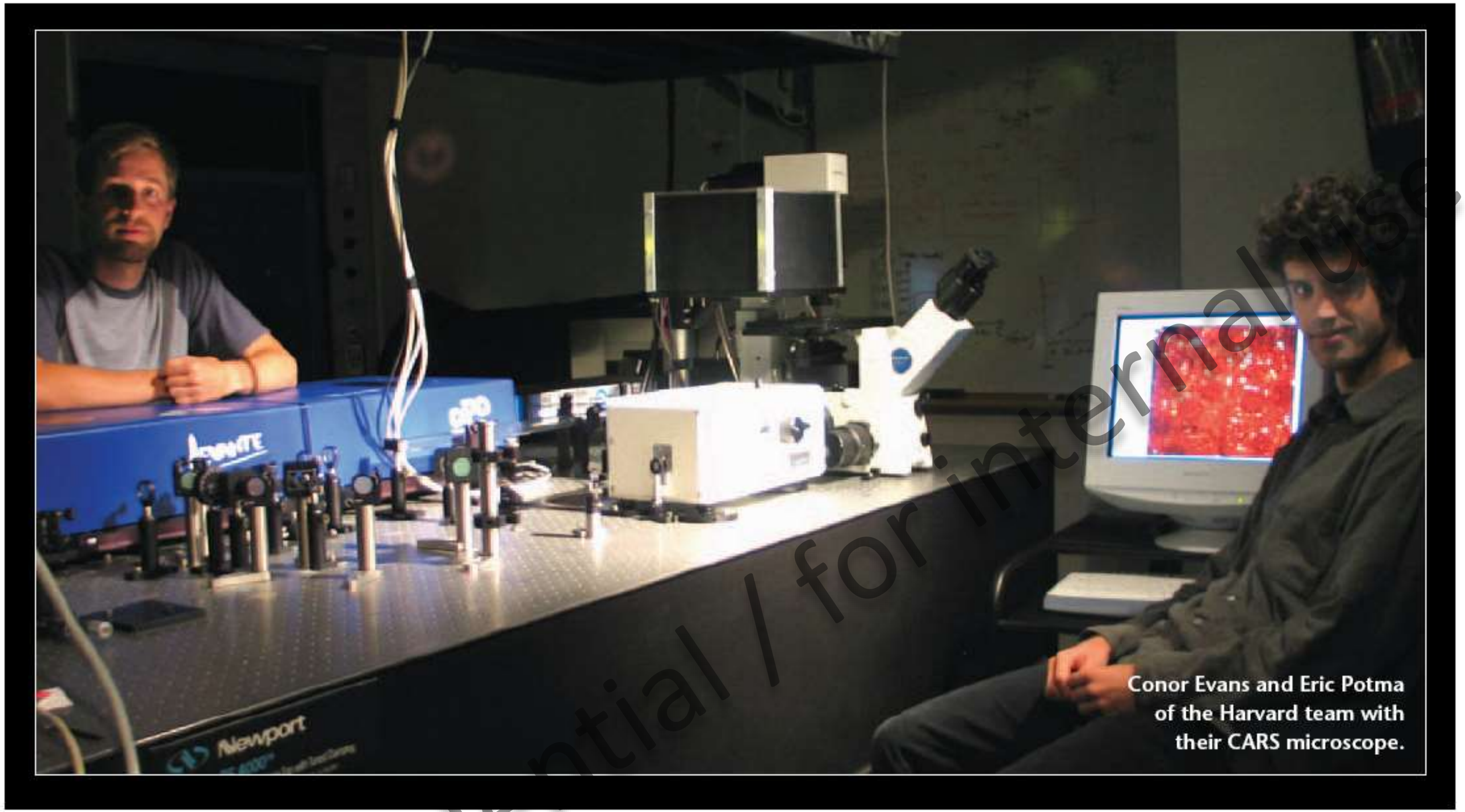
Outline

- OPO based picosecond CRS light sources
- Comparison: fiber vs solid state pumped systems
- Conclusion

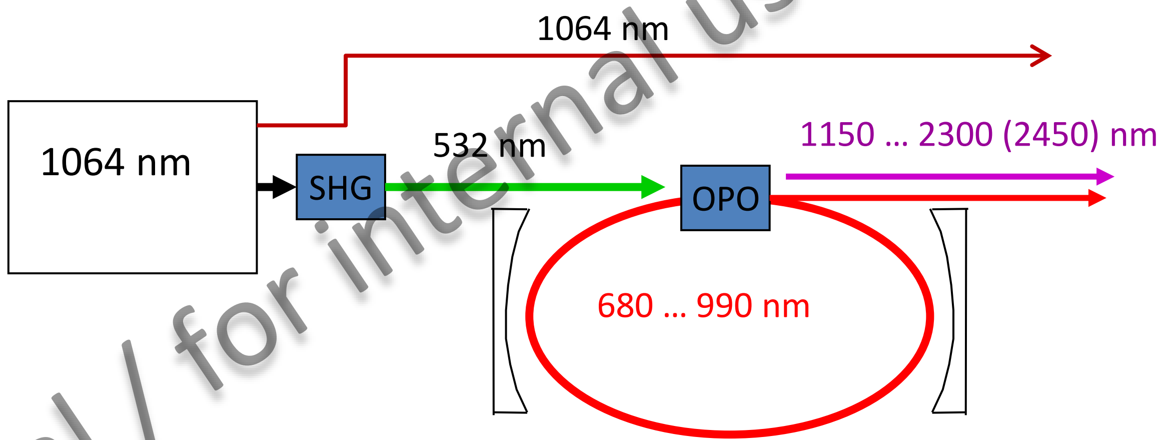
OPOs for CRS microscopy coming of age



- First CARS-OPO in 2004:
  Levante: 1064 nm pumped, intracavity doubled
- Second Generation in 2006:
  Levante Emerald: 532 nm pumped

green pumped ps-OPO

- Pump laser: mode locked 1 µm laser
- OPO pumped with SHG



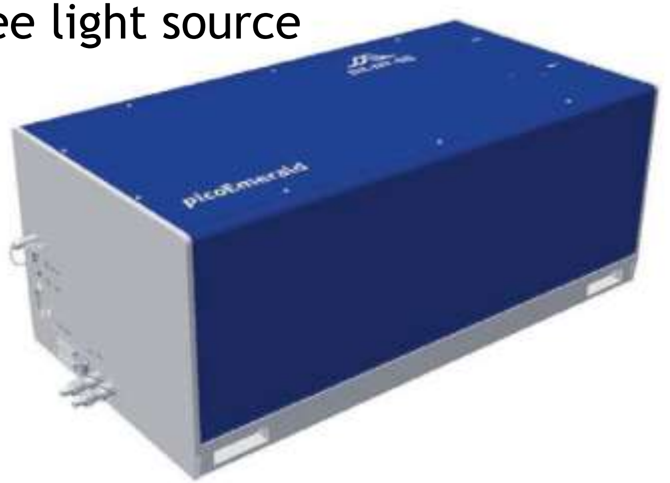
- Pulse lengths Nd:YVO: 7 ps @ 1064 nm (CRS Stokes)
  5.5 ps @ OPO Signal (CRS-Pump)
- ~ 1 W output @ 1064 nm and 1 W @ OPO-Signal

Design based on: Tukker et al, Optics Communications 154(1998).83–86

Motivation for this work

How to get better CRS images (Signal to noise) and not compromising on the spectral resolution?

- We believe in picosecond light sources based on OPOs due to their reliability and low noise characteristics
- It would help to get pulses shorter: 10 cm^{-1} and 1 - 2 ps ideal, but keeping the low noise
  → video rate SRS-imaging
- Ease of use: we want to have a one-box hands free light source



Starting point for developmet: Integrated OPO *pico*Emerald™

a one-box turnkey system for CRS, integrating pump laser and OPO

- Compact and sealed cavity
- Automatic overlap in space and time for CRS- Pump and Stokes
- Independent power setting for
  1064 nm (Stokes) beam
  and OPO-Signal (Pump)
- Integrated modulator (EOM) for the
  1064 nm- beam (10 or 20 MHz, fixed)

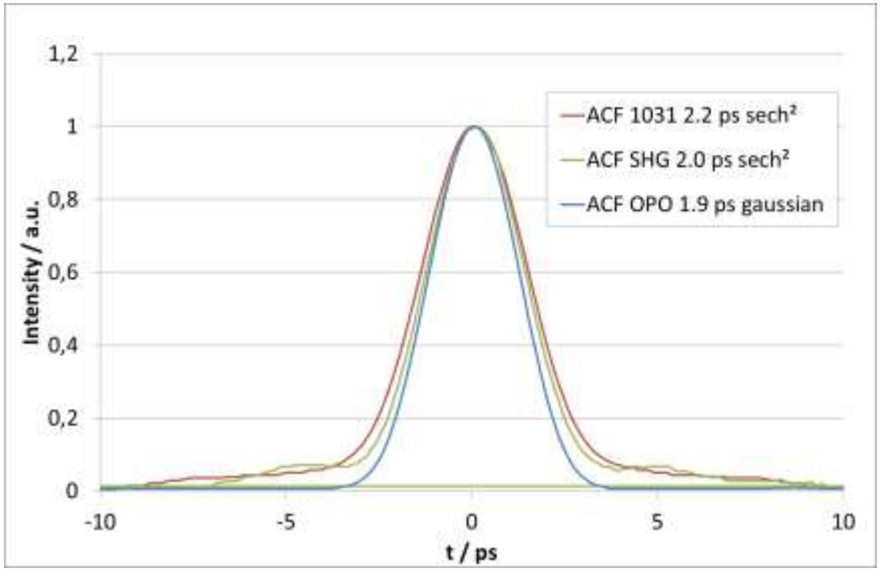
fiber based pump laser

integrating fiber laser into the picoEmerald

- Modelocked ps fiber laser: Yb-based MOPA design (aeroPULSE, NKT Photonics)
- 10 W output power
- λ = 1031 nm ; $\Delta\lambda$ = 1 nm (10 cm^{-1})

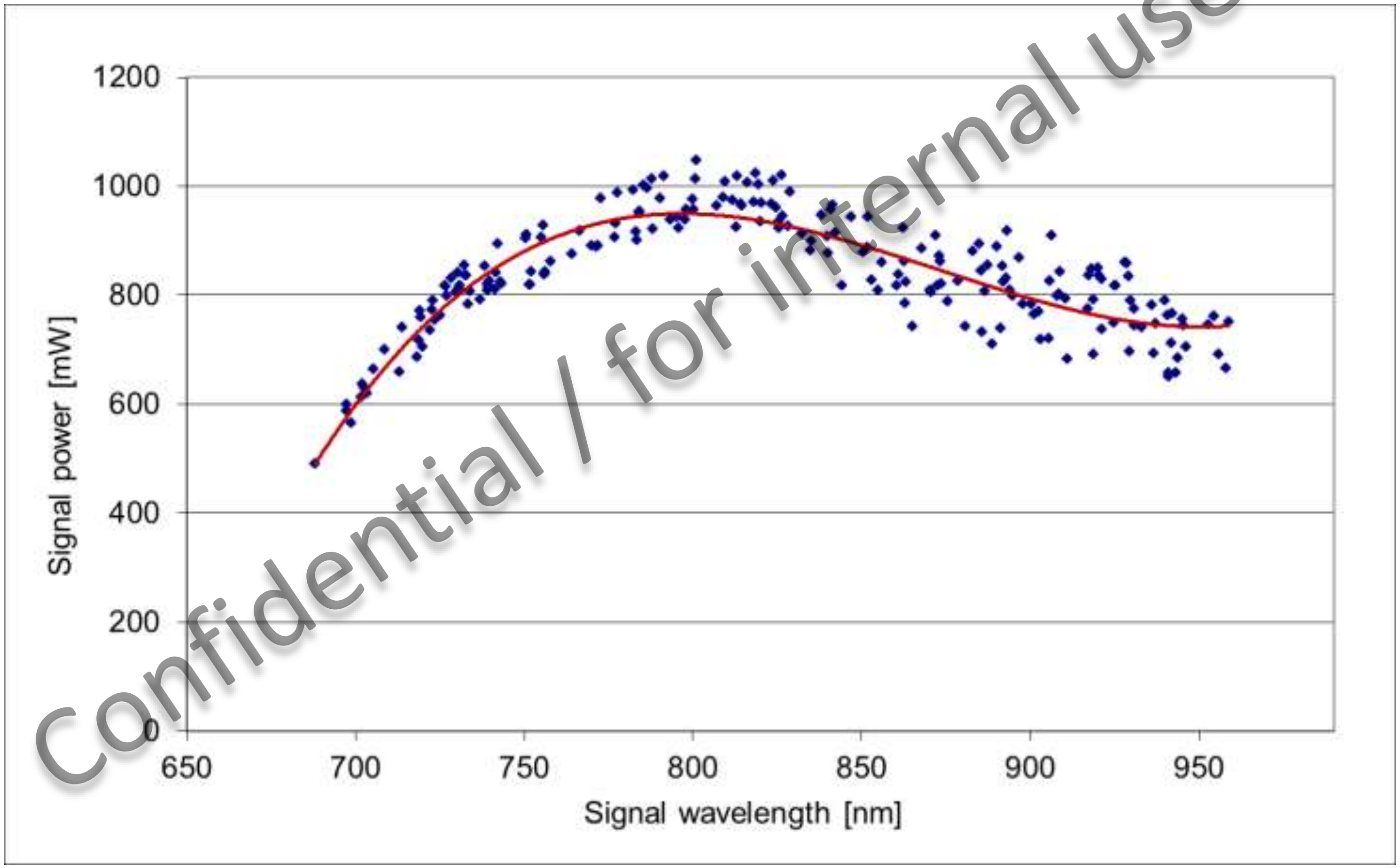
- Pulse compression of fiber laser 2.2 ps
- OPO-Signal @ 800 nm: 1.9 ps

- 10 cm^{-1} bandwidth of pump and OPO



Tuning and output power

- Test of repeatability and reliability: Random tuning routine
- Tuning: 690 – 960 nm → 725 – 4800 cm^{-1}



Fiber laser and noise

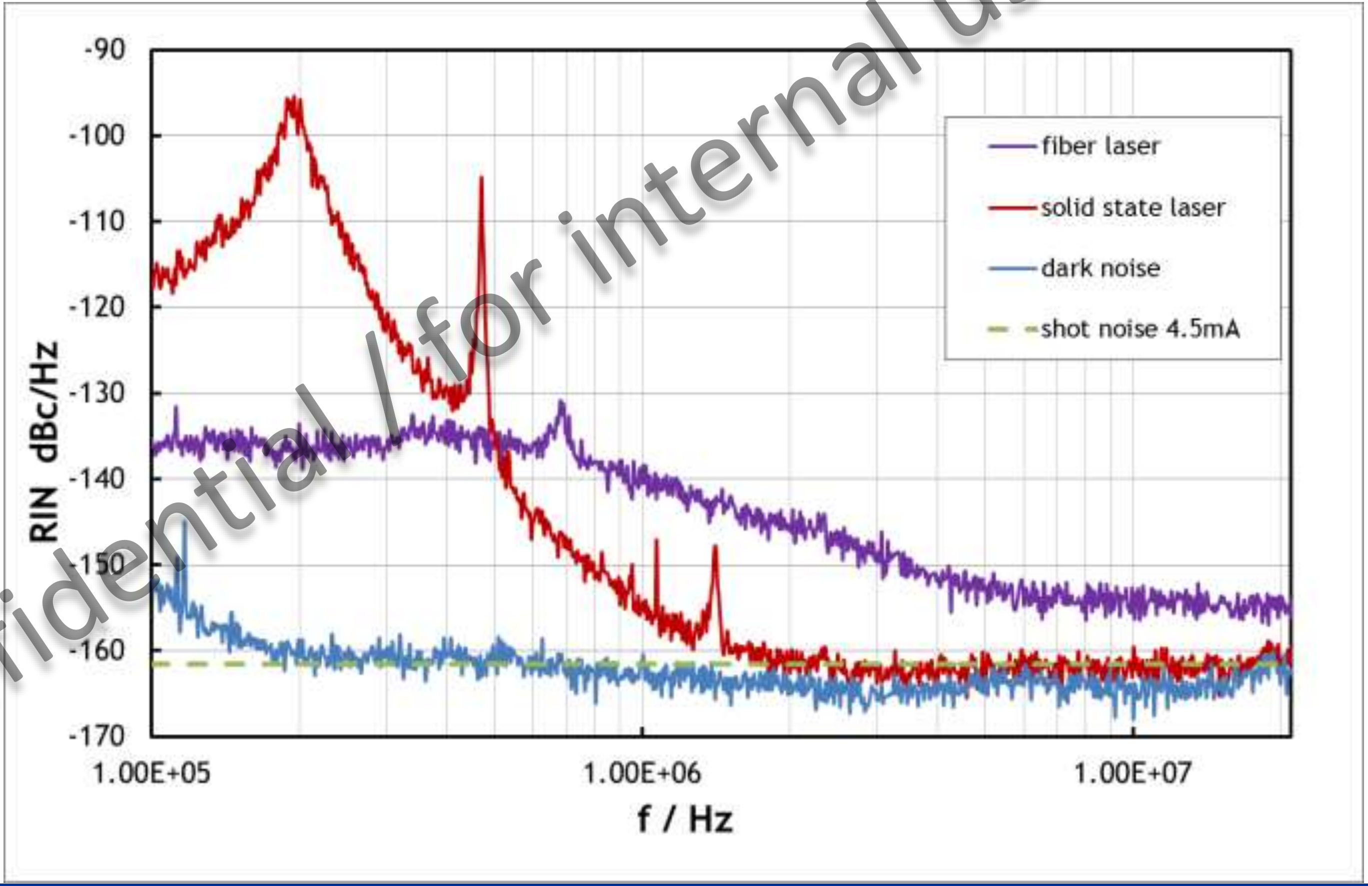
- Intrinsically higher noise of fiber lasers (due to MOPA design) than solid state Laser with the same power levels.
  How strong is the effect?
- Is the system suitable for video-rate SRS-imaging?

- Setup of RIN noise measurement

Si-PD 1 ns rise time
Thorlabs DET10A

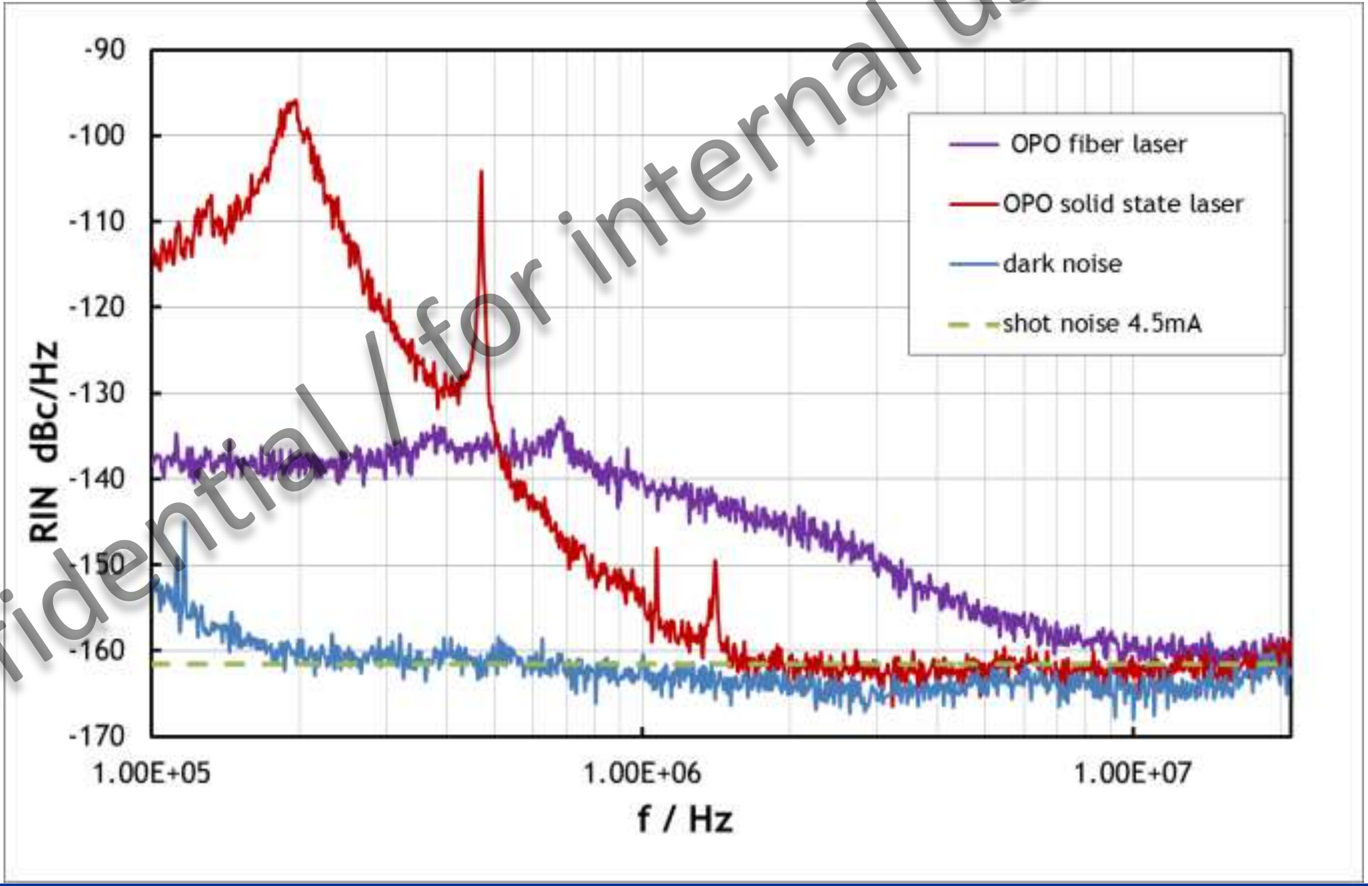
RIN: laser noise @ 1 µm

	1 MHz	10 MHz	20 MHz
Solid state laser	-155 dBc	-162 dBc **SNL**	-162 dBc **SNL**
Fiber laser	-140 dBc	-153 dBc	-154 dBc



RIN: OPO noise @ 810 nm

	1 MHz	10 MHz	20 MHz
Solid state laser	-155 dBc	-162 dBc **SNL**	-162 dBc **SNL**
Fiber laser	-140 dBc	-160 dBc	-162 dBc **SNL**



Comparative setup for CARS and SRS signal strength

System pulse lengths

solid state laser	7 ps
OPO @ 817 nm	5.5 ps
Fiber-Laser	2.2 ps
OPO @ 798 nm	1.9 ps

- Setup at Leica Microsystems / Mannheim
- picoEmerald and picoEmerald S coupled into a Leica SP8

CRS signal dependence on laser pulse duration

$E_{p/S}$	... peak amplitude of pump/Stokes pulse
τ	... pulse duration of pump and Stokes pulses
f_{rep}	... pulse repetition frequency
$P_{p/S} \propto \left\|E_{p/S}\right\|^2 \tau f_{rep}$	... average power of pump/Stokes pulse

Simplifying approximation: $\tau >> T_2$ (T_2 .... vibrational dephasing time)

	CARS	SRL
induced third-order polarization ($p^{(3)}$)	$p_{CARS}^{(3)} \propto \left(X_{NR}^{(3)} + X_R^{(3)}\right) E_p^2 E_S$	$p_{SRL}^{(3)} \propto \left(2X_{NR}^{(3)} + X_R^{(3)}\right) E_p \|E_S\|^2$
number of signal photons (S)	$S_{CARS} \propto \left\|X_{NR}^{(3)} + X_R^{(3)}\right\|^2 \frac{P_p^2 P_S}{(\tau f_{rep})^2}$	$S_{SRL} \propto Im\left[X_R^{(3)}\right] \frac{P_p P_S}{\tau f_{rep}}$

[adapted from Ozeki et al. Optics Express 17 (2009) 3651]

CARS: $S_{CARS} \propto \frac{1}{\tau^2}$

➔ **~9 x enhancement**

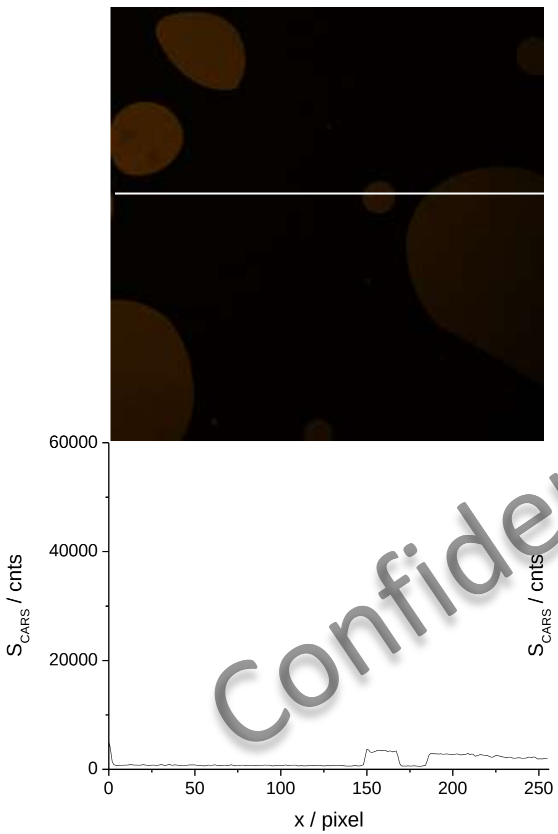
SRL: $S_{SRL} \propto \frac{1}{\tau}$

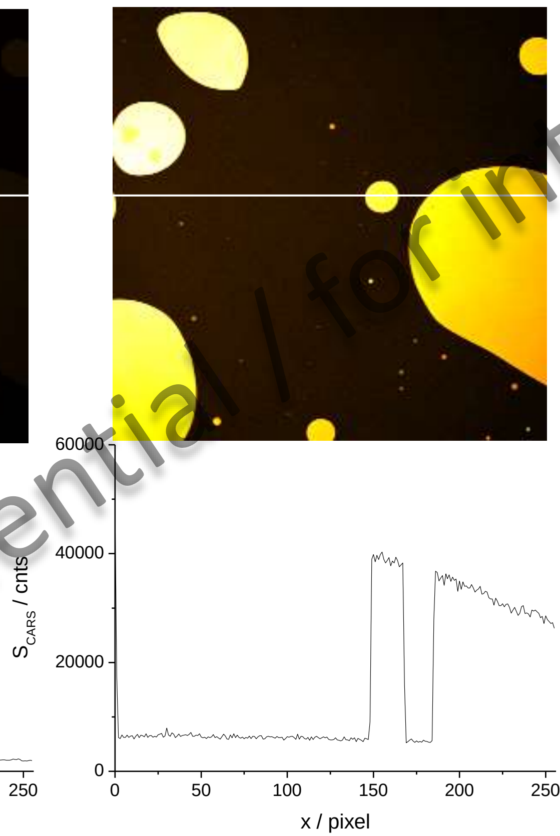
➔ **~3 x enhancement**

Enhancement factor in the CARS signal

- dodecane in water probed at 2855 cm^{-1}
- same average powers of pump and Stokes pulses: 36 mW
- picoEmerald: $\tau = 5.5\ ps$
- picoEmerald-S: $\tau = 1.9\ ps$

$S_{CARS}(\tau = 5.5\ ps)$ $S_{CARS}(\tau = 1.9\ ps)$

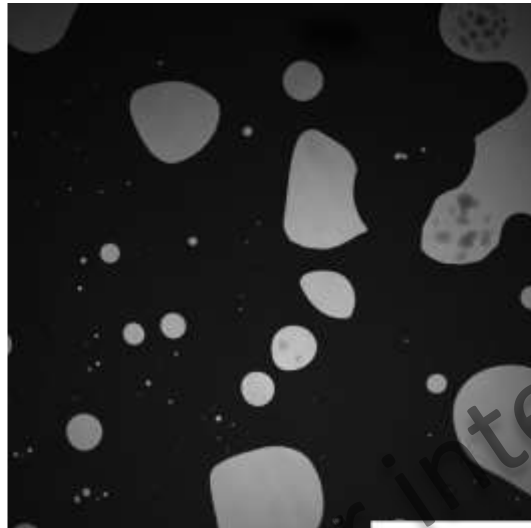




measured signal enhancement factor

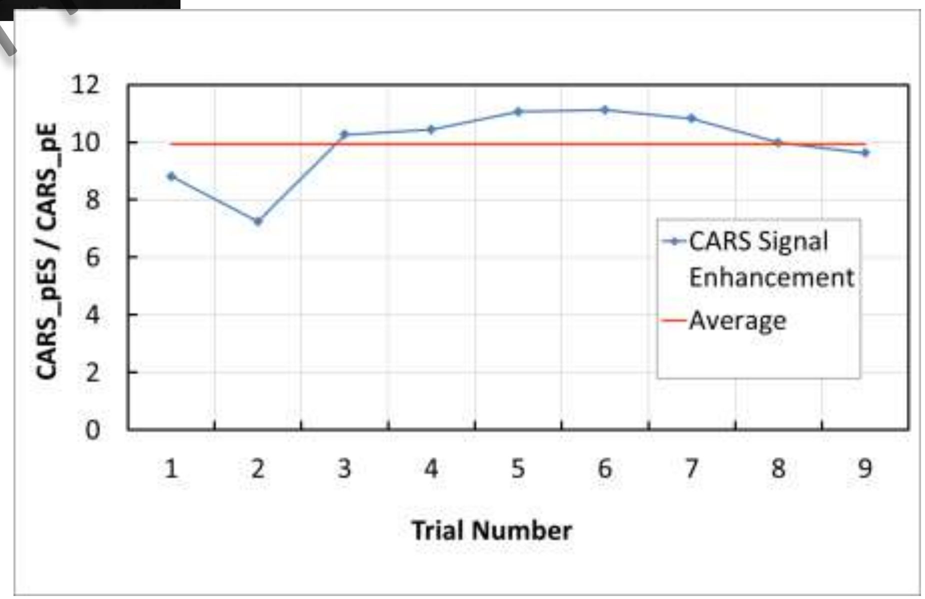
$$\frac{S_{CARS}(\tau = 1.9\ ps)}{S_{CARS}(\tau = 5.5\ ps)} = \mathbf{9.9 \pm 1.2}$$

Enhancement factor in the CARS signal



- Dodecane – water emulsion 2855 cm-1 (36 mW in pump and in stokes)

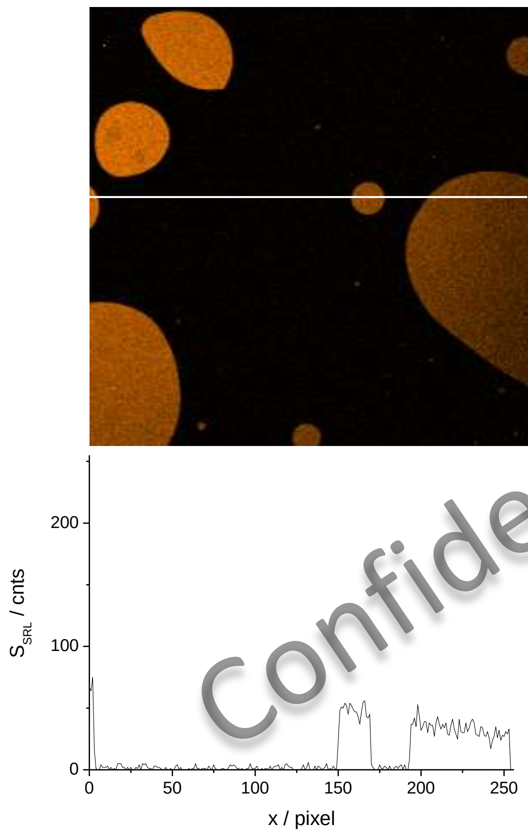
- 10 x Signal enhancement measured at 3198 cm-1; 3249 cm-1 and 2855cm-1 for different power levels



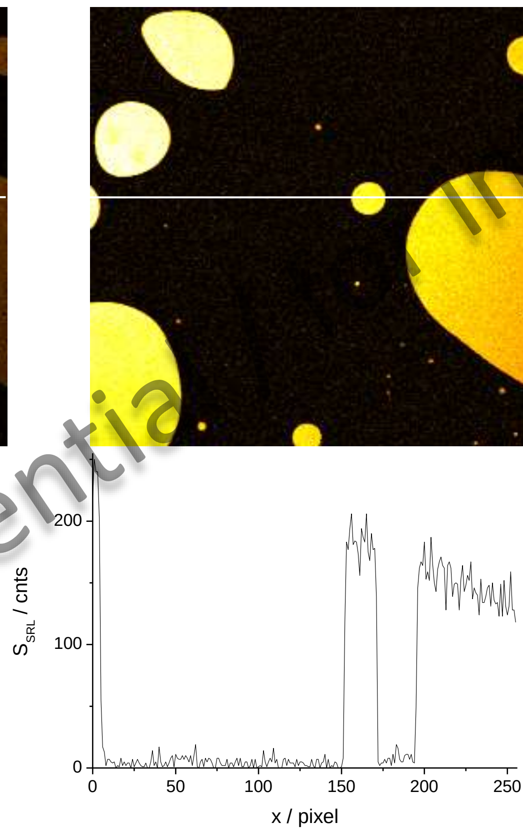
Enhancement factor in the SRS signal

- dodecane in water probed at 2859 cm^{-1}
- same average powers of pump and Stokes pulses: 27 mW
- picoEmerald: $\tau = 5.5\ ps$
- picoEmerald-S: $\tau = 1.9\ ps$

$S_{SRL}(\tau = 5.5\ ps)$



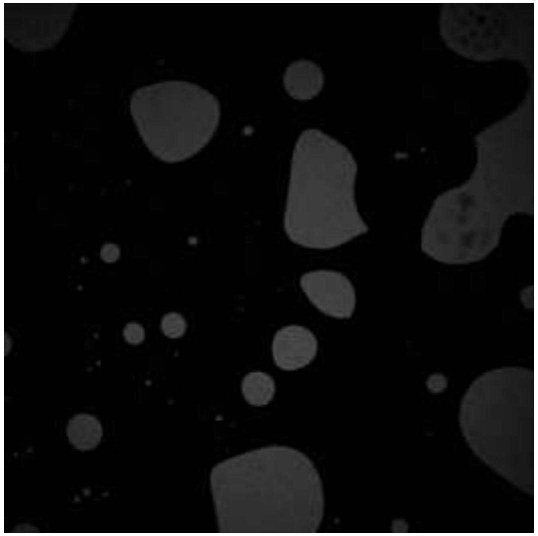
$S_{SRL}(\tau = 1.9\ ps)$

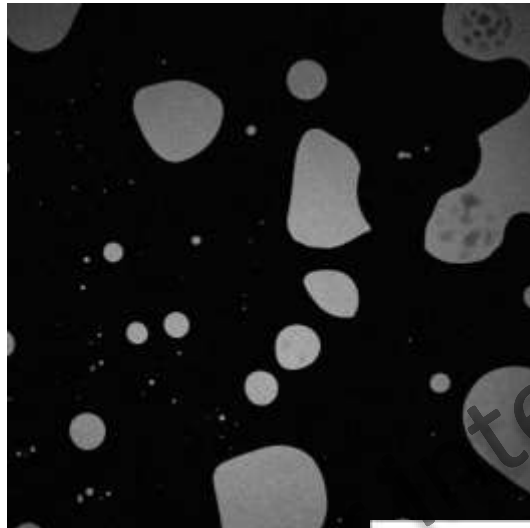


measured signal enhancement factor

$$\frac{S_{SRL}(\tau = 1.9\ ps)}{S_{SRL}(\tau = 5.5\ ps)} = \mathbf{2.5 \pm 0.4}$$

Enhancement factor in the SRS signal

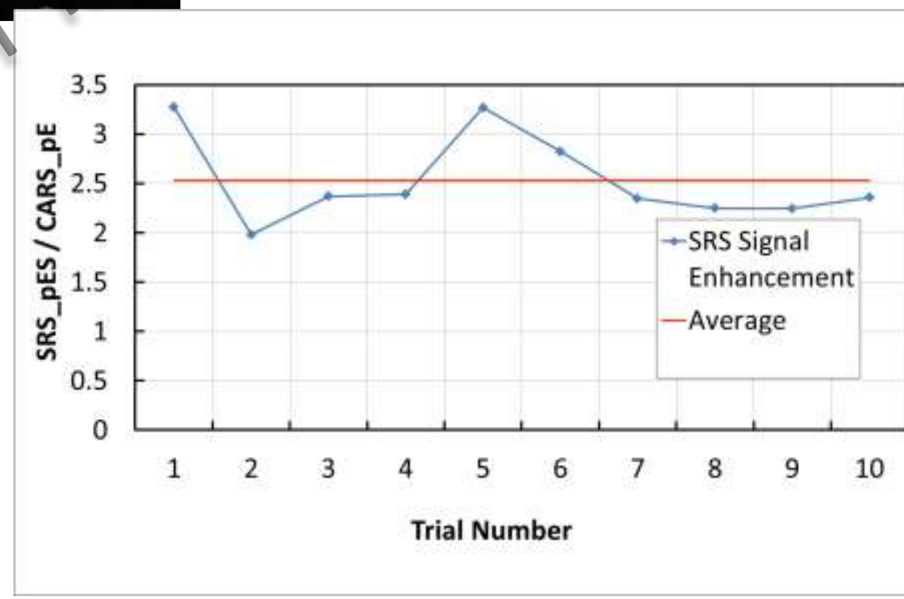




Modulation frequency of the stokes beam:20MHz

- Dodecane – water emulsion 2855 cm-1 (36 mW in pump and in stokes)

- 2.5 x Signal enhancement measured at 3198 cm-1; 3249 cm-1 and 2855cm-1 for different power levels



Detector- and Lock-In Amplifier unit for video rate SRS

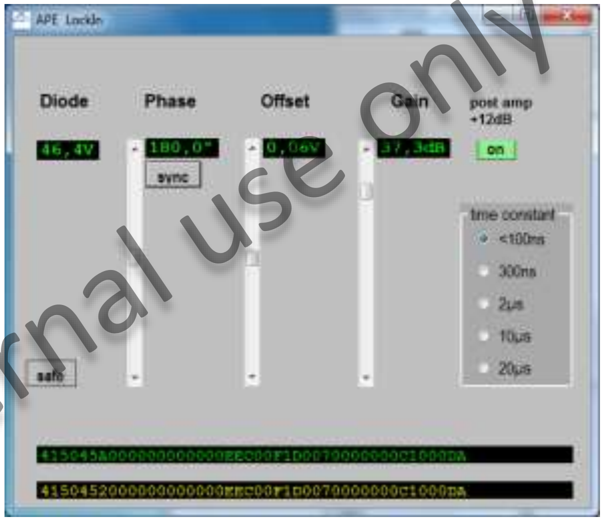
- Integrated large area Si-photo diode
- Max. Signal slope - 500 mV / µW

- sensitivity limits for 50 mW shot noise limited laser power on photo diode (Signal amplitude equivalent to rms noise):

$\Delta I / I = 5$ E-6 for 100 ns integration time

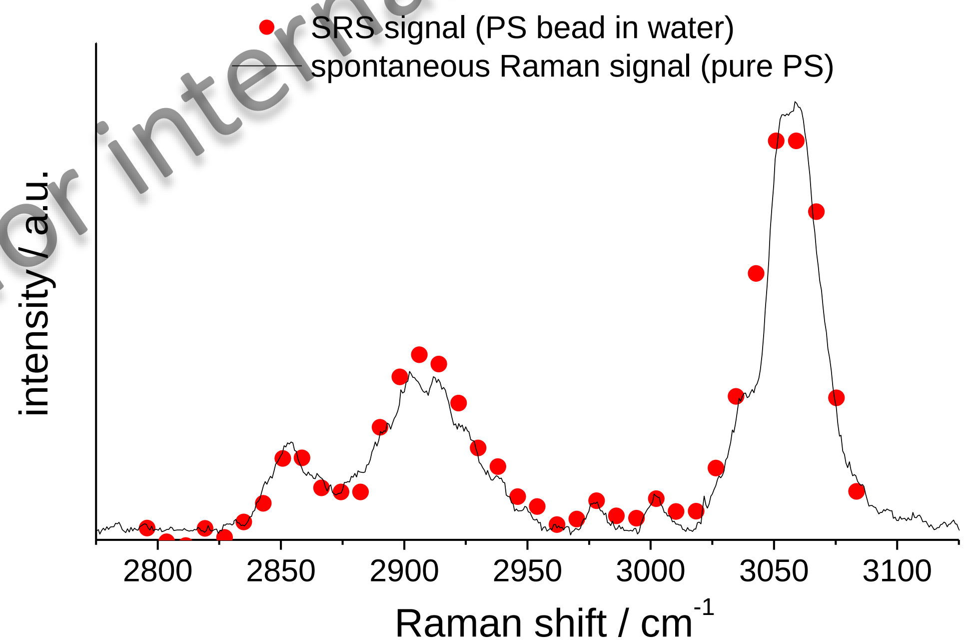
$\Delta I / I = 1.5$ E-6 for 2 µs integration time

$\Delta I / I = 5$ E-7 for 20 µs integration time



Demonstration of multi-spectral SRS imaging in CH-stretching region of PS beads (1 µm ∅) in water

- Sweep of signal wavelength from 782.0 nm to 800.0 nm in 0.5 nm steps (37 frames)
- Typical tuning time between wavelengths points: 5 s
  → less than 5 minutes for taking this movie

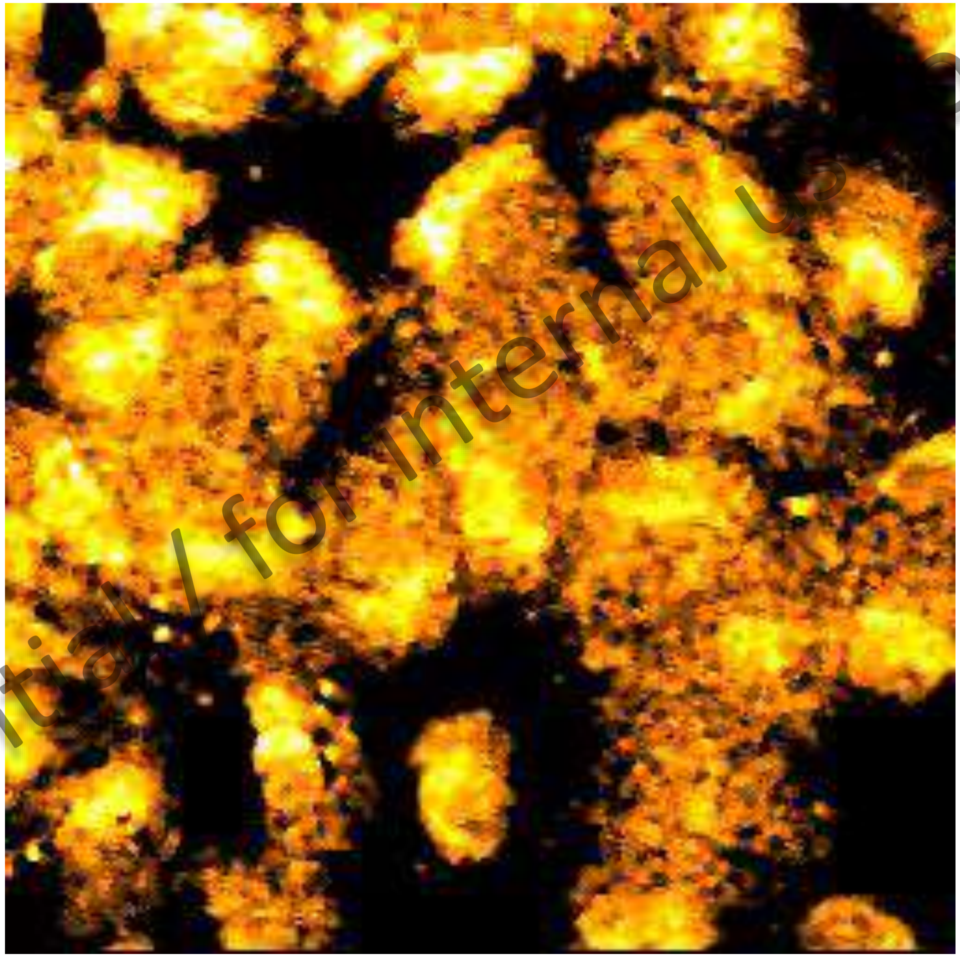


P_{Pump} = 12 mW,
P_{Stokes} = 20 mW

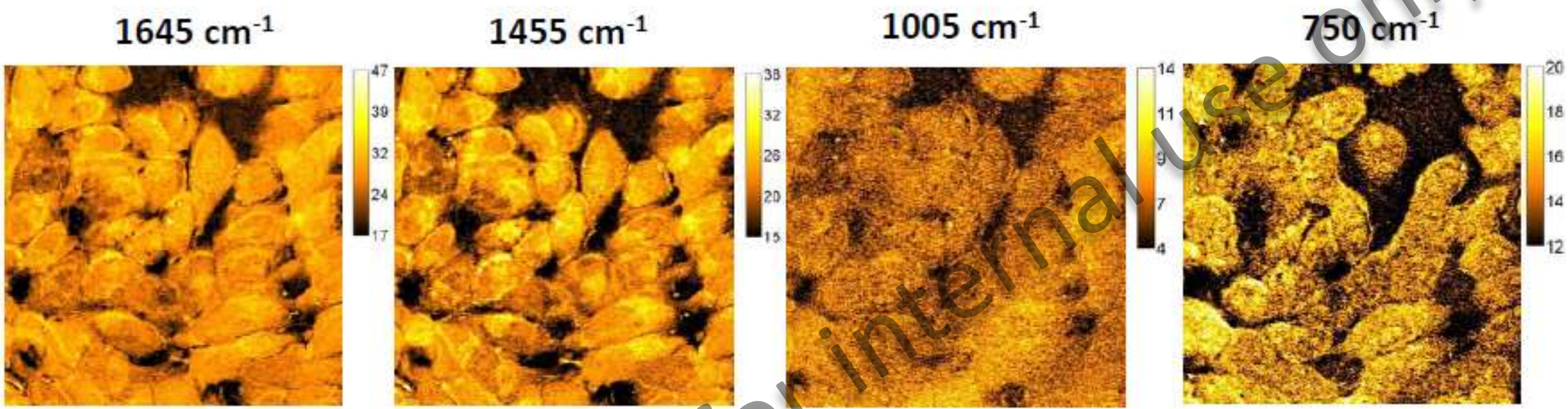
Fast scanning of living HeLa cells

2939.9 cm-1 ,
50 mW Signal / 200 mW IR

28 Frames / sec
256*256 Pixel
Pixel dwell time: 120 ns



Fast scanning of living HeLa cells in the fingerprint region



50mW Signal
200 mW IR
1000Hz
1024 Pixels
10 Frames average
Pixel dwell time: 980 ns

100mW Signal
200 mW IR
1000Hz
1024 Pixels
10 Frames average
Pixel dwell time: 980 ns

Summary and Conclusions

- Compressed fiber laser used as OPO pump source
- 2 ps pulses with 10 cm^{-1} bandwidth, ideal for ps-CRS-imaging
- **2.5x higher Signal in SRS; 10x higher Signal in CARS** compared with standard solid state pumped OPO system
- 2 ps: already a good pulse length for MPE fluorescence and SHG imaging
  - Good for multi modal imaging
- Extremely low noise system, same level as solid state pump
- Sensitive lock-in amplifier and detector unit easy to integrate in every standard microscope