Using shaped femtosecond laser pulses for multimodal nonlinear spectroscopy

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1. Extending the shaping range:
   UV Shaping

2. Multimodal microscopy:
   Microscopy and coherent control

3. Coherent Control of Amplification of
   UV emission in a filament

Safed, Israel – 6. September 2012

Thanks to …

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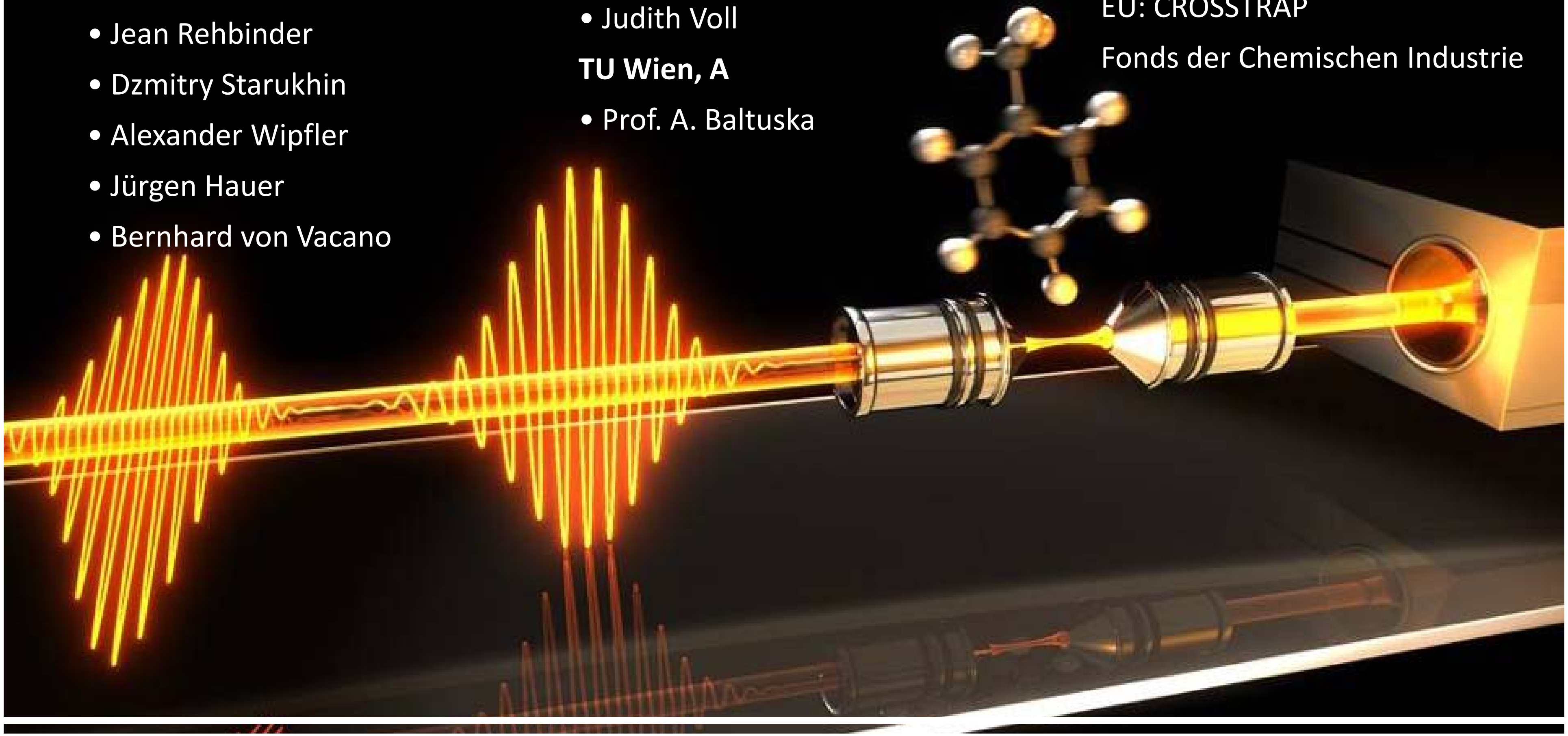
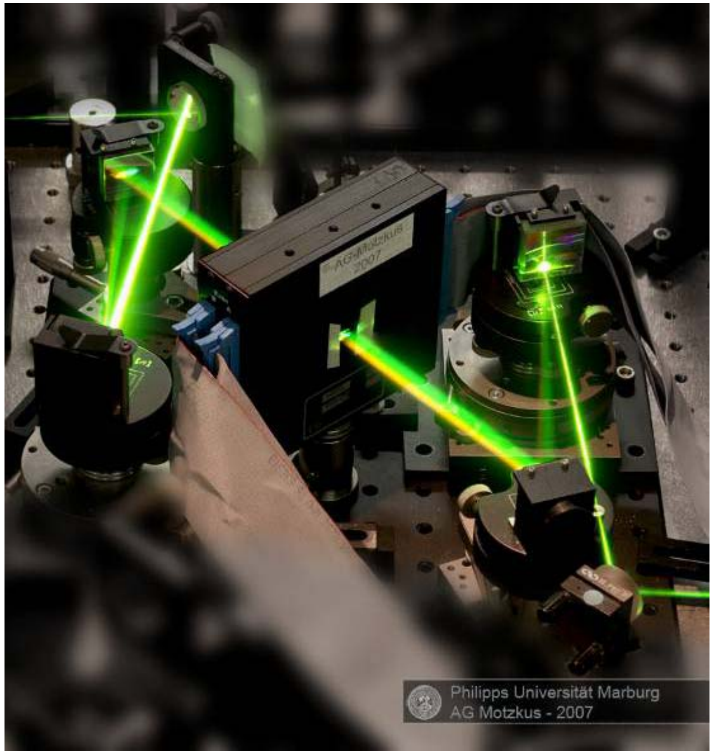
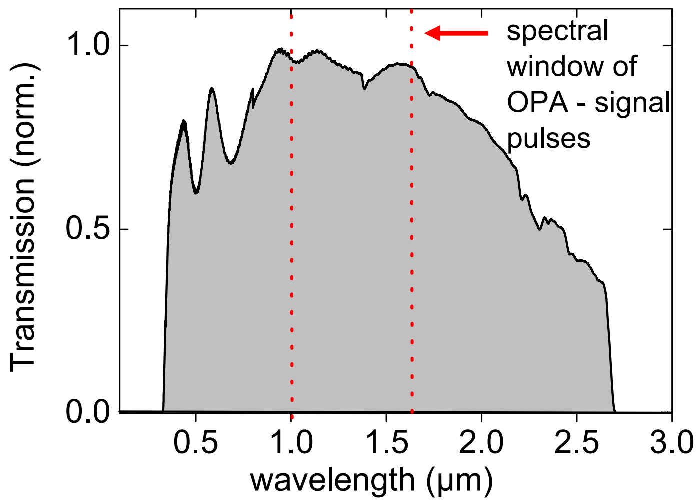


Spectral range of a liquid crystal mask

NIR / VIS: 4-f-setup



Transmission spectrum of liquid crystal mask CRI-256



→ No modulation of pulses in the UV and mid IR

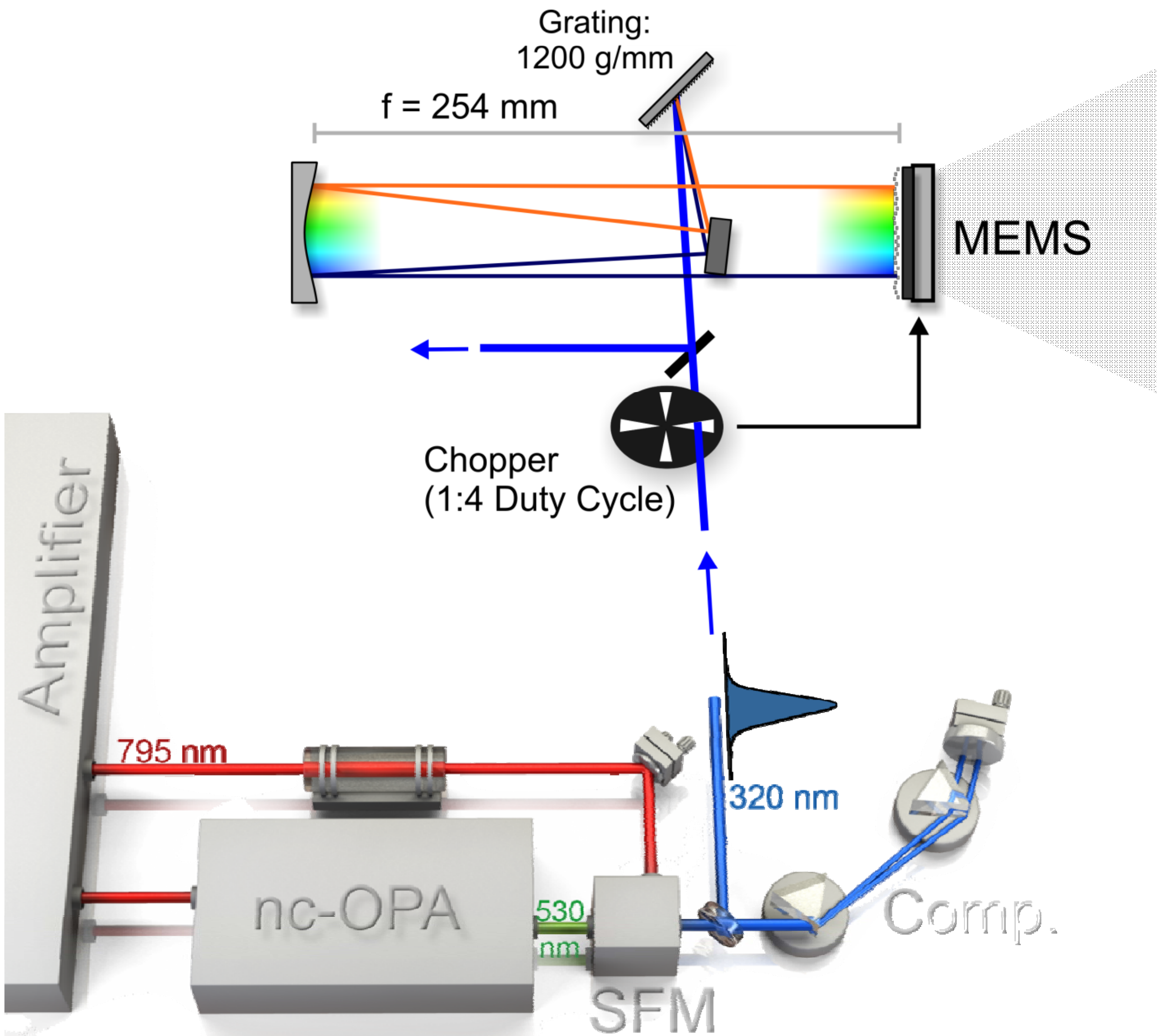
Shaping of UV fs pulses

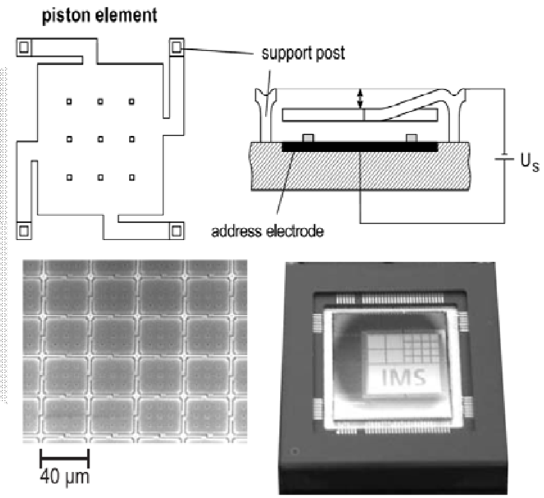
- **Indirect shaping by sum frequency mixing**:
  → appropriate crystals, complex setup, delicate overlap with reference pulses
  M. Hacker et al., JOSA B 18 (2001) 866
  H. Wang et al., IEEE J. Quantum Electron. 40 (2004) 937
  S. Shimizu et al. Opt. Exp. 13 (2005) 6345
  C. Schriever et al., Opt. Lett. 31 (2006) 543

- **Direct shaping:**
  adaptive mirror → Aluminum coating
  - micromirrors: high dynamical range, 2-D capability
    M. Hacker et al., Appl. Phys. B 76 (2003) 711
  - membrane based mirrors: low dynamical range, good for pulse compression

  AOM's: phase + amplitude, low efficiency, fused silica (> 180 nm)
  M.Roth et al, Appl. Phys. B 80 (2005) 441
  AOPDF ("DAZZLER") TeO_2, (200-500nm), low efficiency, p + a
  S. Coudreau et al. Opt. Lett. 31 (2006) 1899

UV pulse generation and shaping setup

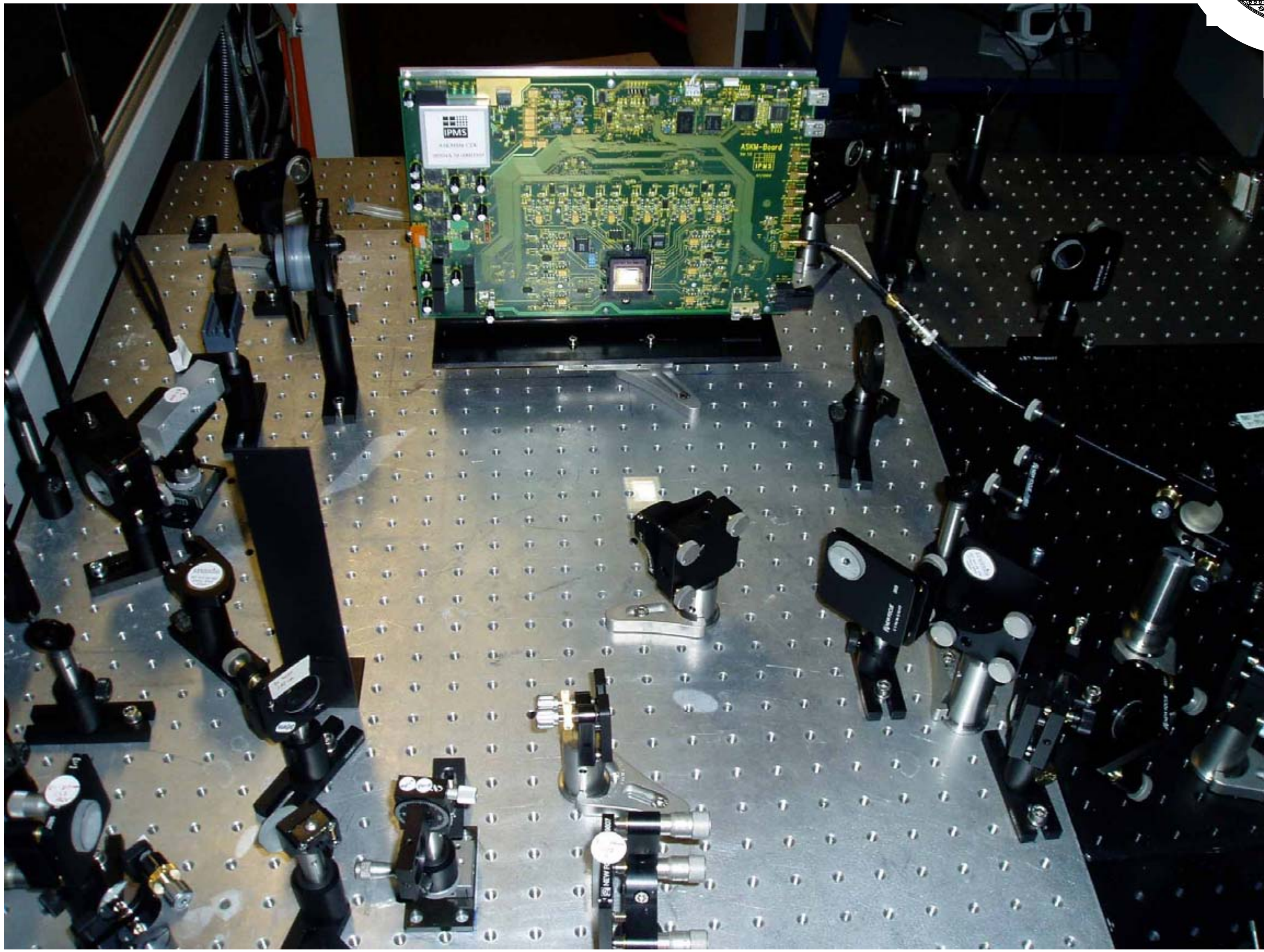




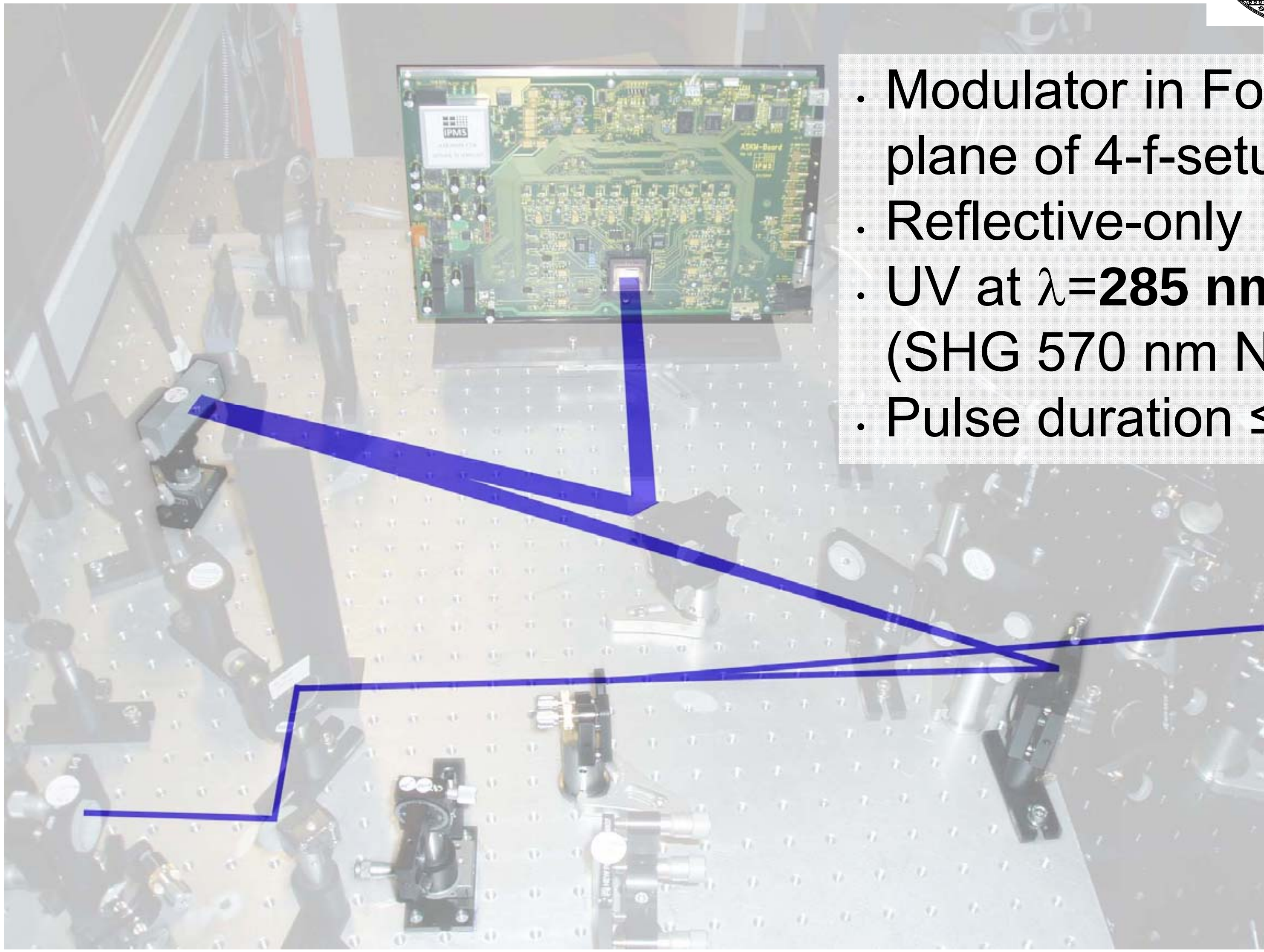
- 2D-MEMS micromirror array
- Direct UV shaping
- 240x200 pixels
- 8 bit analog deflection
- synchronized run enables 20 % duty cycle

*Appl. Phys. B **76** (2003) 711; JOSA B **26** (2009) 1538; Opt. Lett. **35** (2010) 1816*

A view into the Lab



Implementation in a 4-f-setup

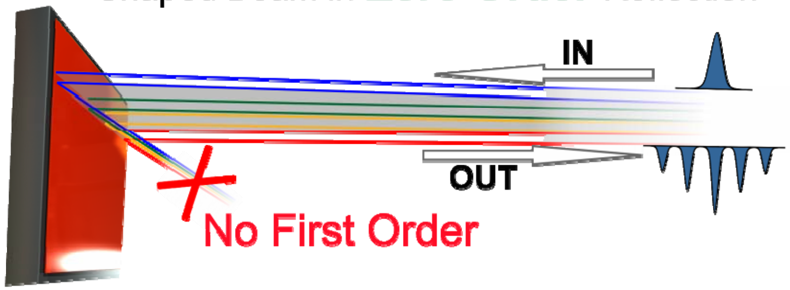


- Modulator in Fourier-plane of 4-f-setup
- Reflective-only
- UV at λ=**285 nm** (SHG 570 nm NOPA)
- Pulse duration ≤ **30 fs**

Principles of Diffractive Shaping

Conventional:

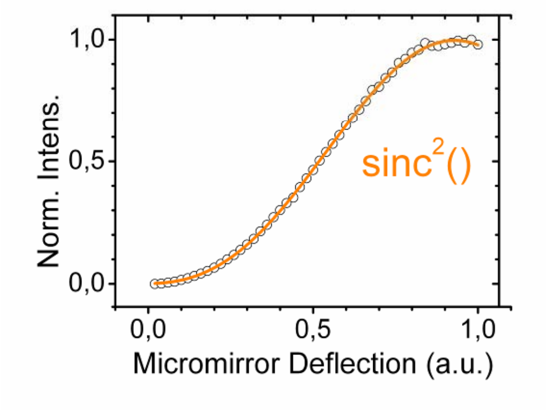
Shaped Beam in **Zero Order** Reflection

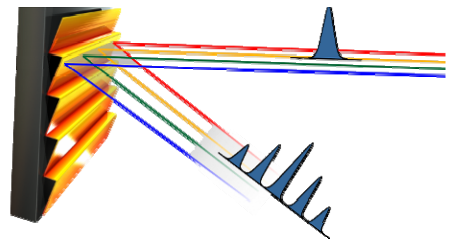


J. C. Vaughan et. al. OL 323 (30) 2005

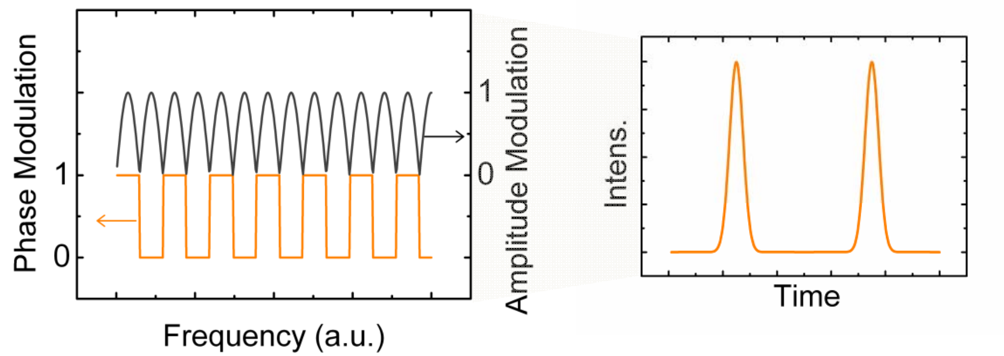
Principles of Diffractive Shaping

Amplitude shaping on
phase only modulator

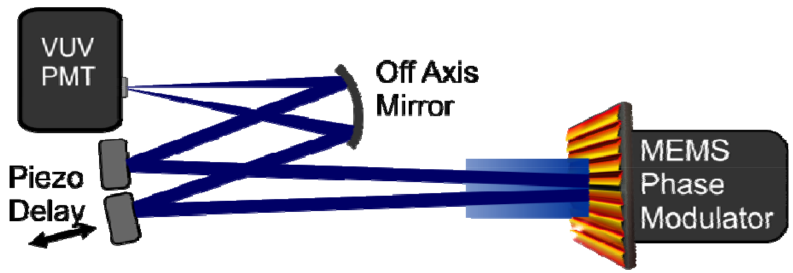




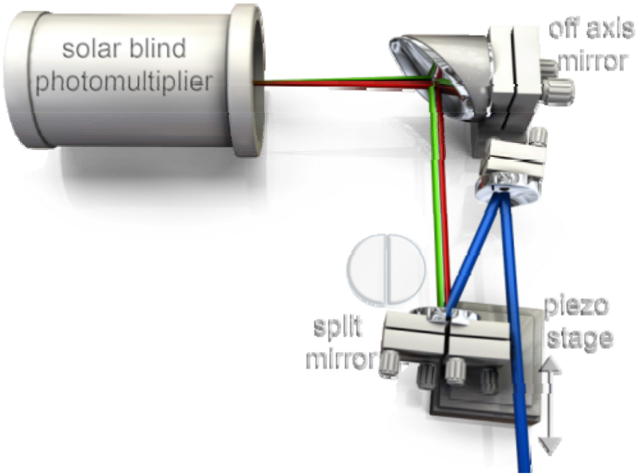
Full E-field modulation:
Double pulse generation on
phase only modulator

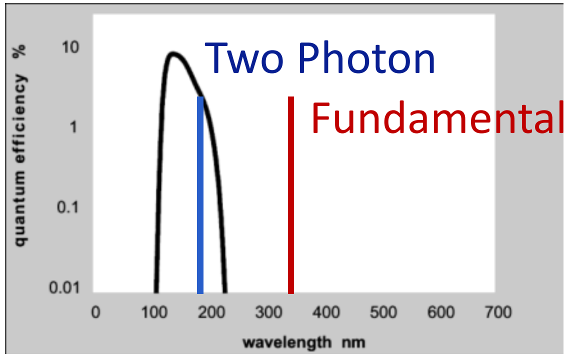


Simple Diagnostic by shaper
assisted cross correlation

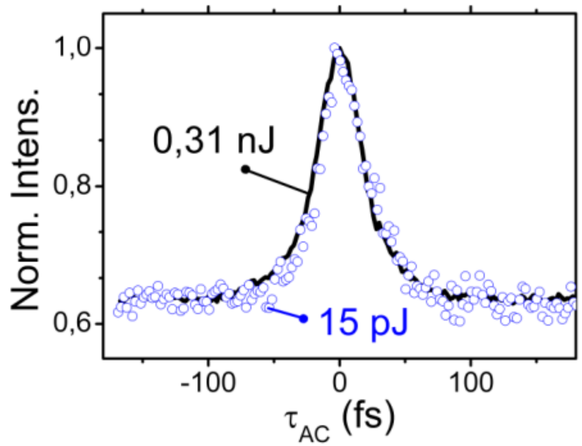


UV - Cross and Autocorrelations





Solar Blind Photomultiplier

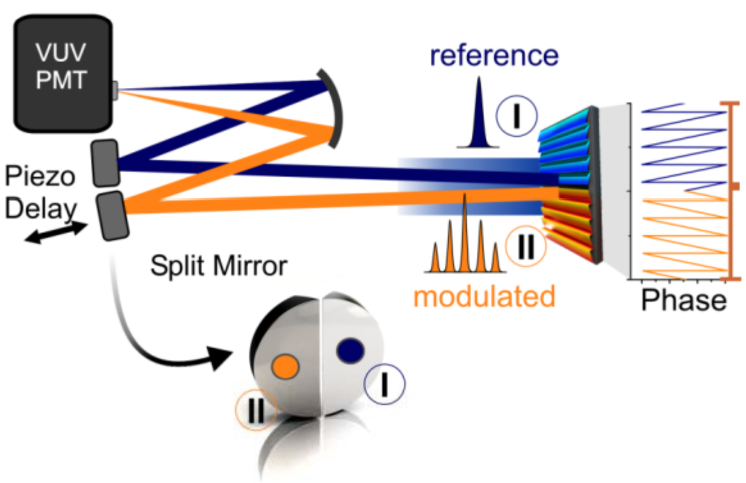


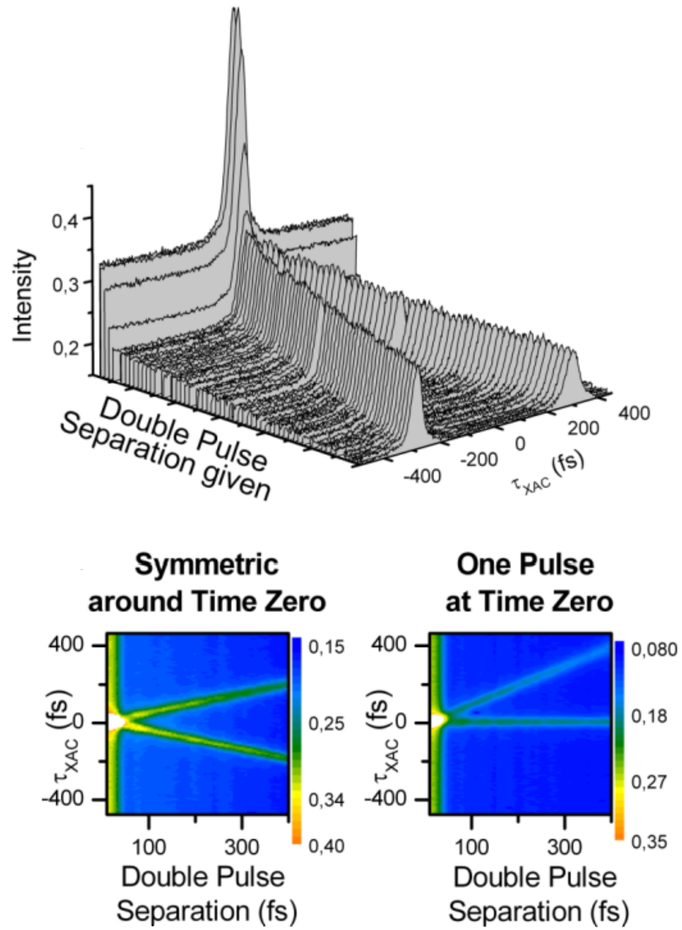
- UV autocorrelation by two photon photoemission in a solar blind photomultiplier

- High sensitivity

- Two photon response must be instantaneous

UV - Cross and Autocorrelations



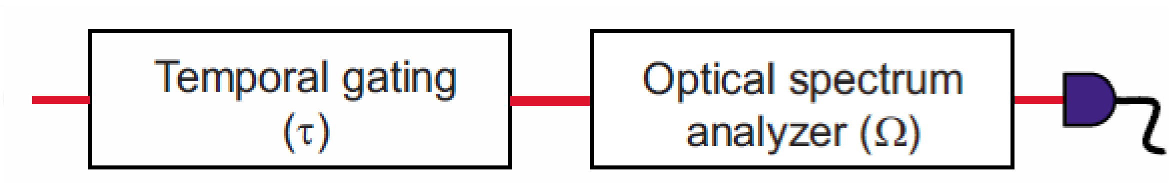


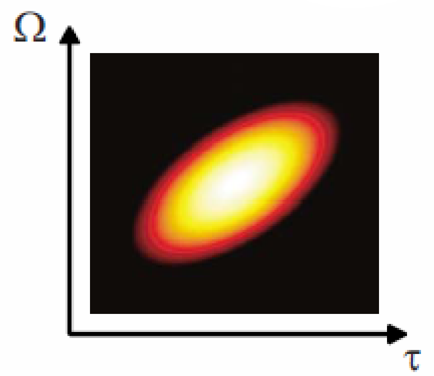
- Shaper assisted UV Cross Correlations
- Beam steering by diffractive shaping
- No external reference required

Opt. Lett. ***35*** *(2010) 1816*

Full phase characterization from Time Frequency Measurements

FROG

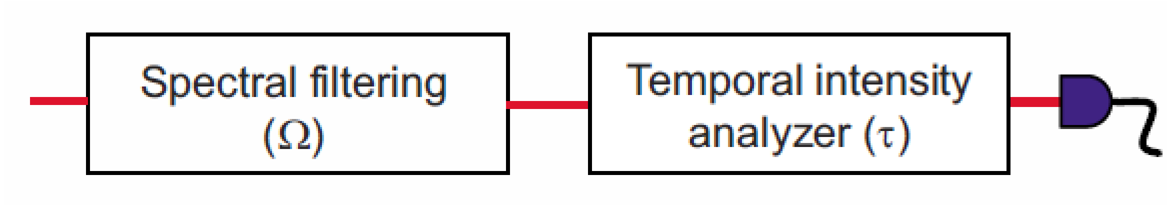


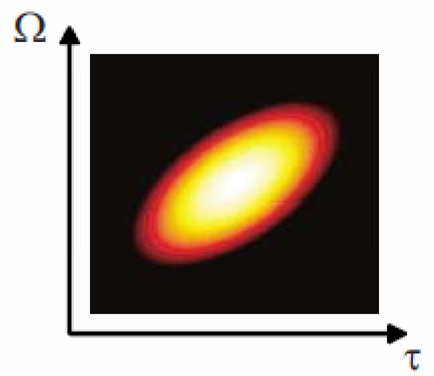


e.g. SHG
Autocorrelation

Resolve spectral distribution
for each delay

Sonograms



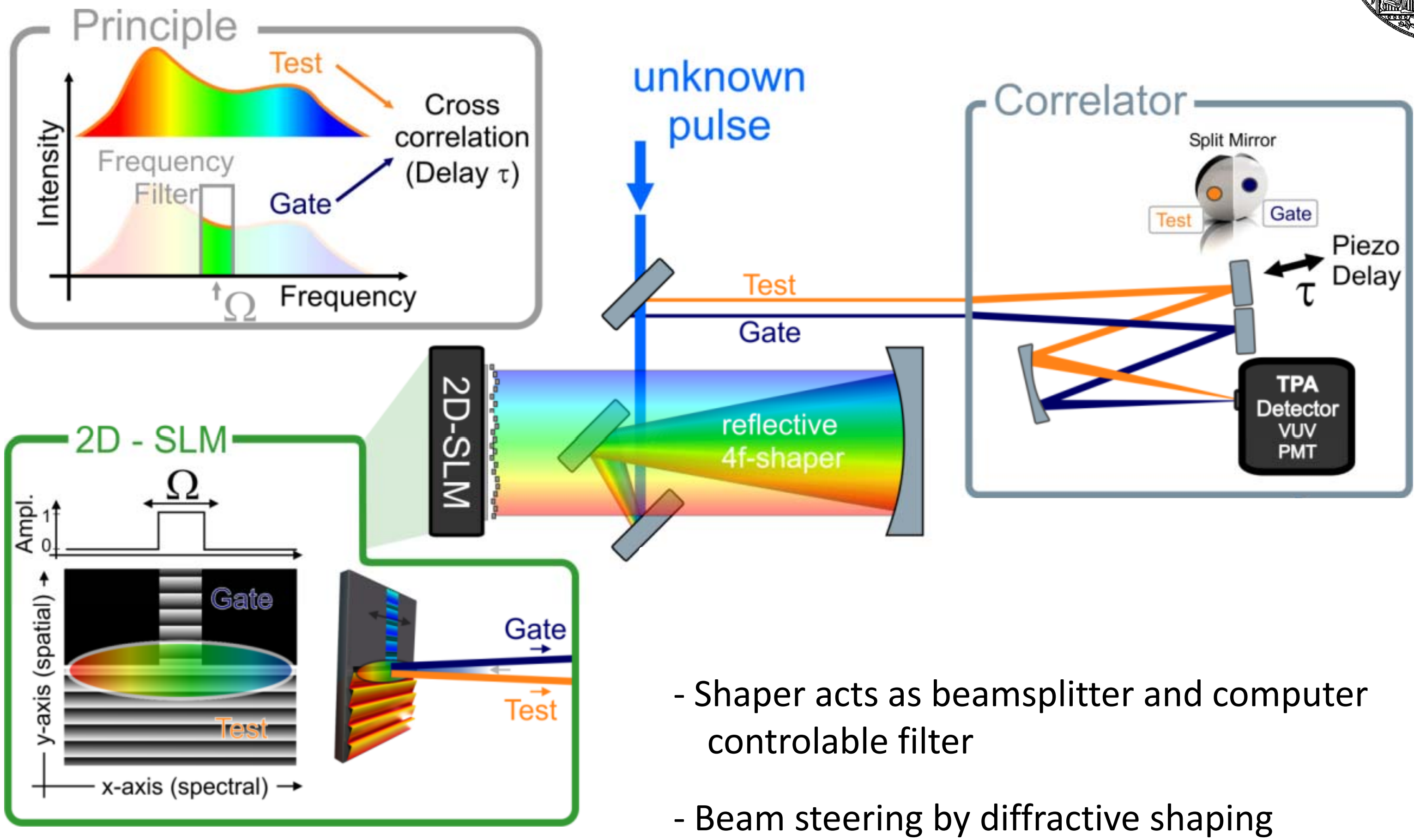


e.g. SHG
cross correlation

Resolve delay of each frequency

I. A. Walmsley and C. Dorrer ,Adv. in Opt. Phot. 1, 2009

Shaper assisted UV Sonogram Acquisition



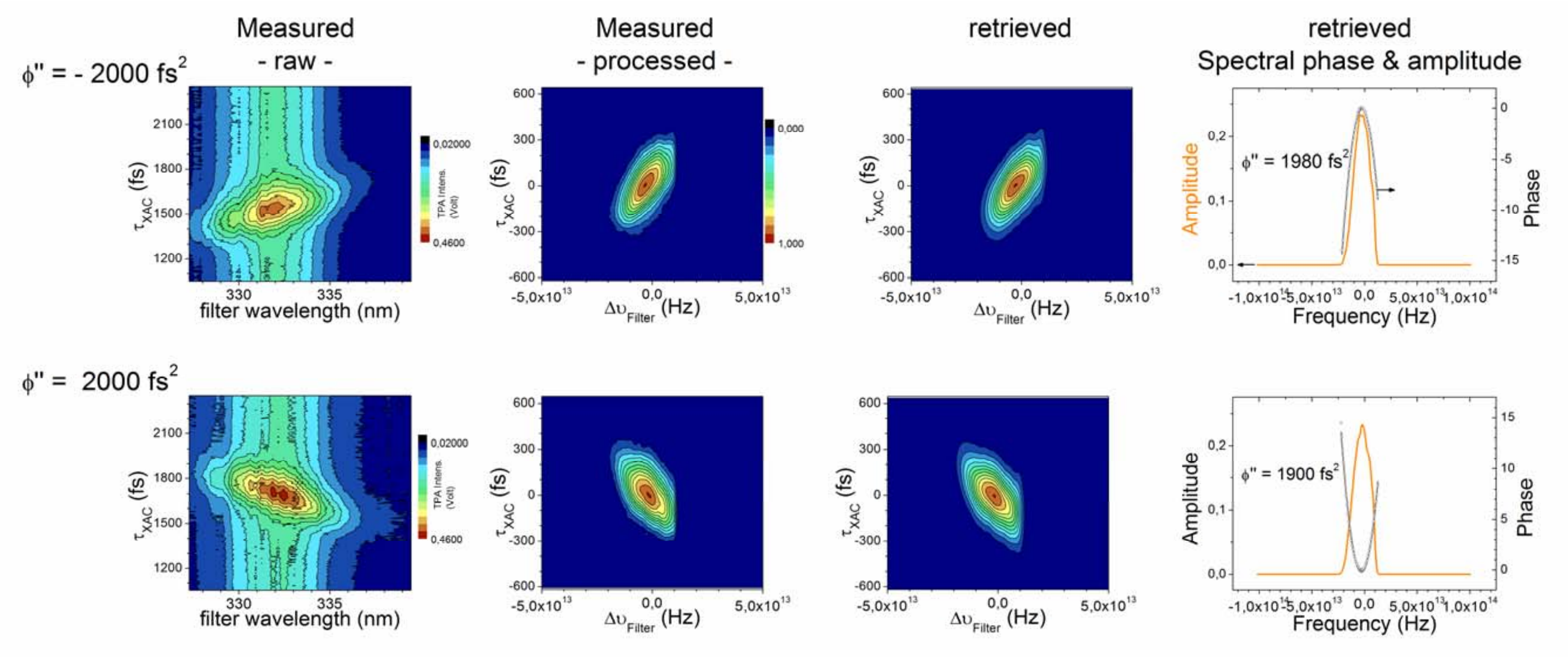
- Shaper acts as beamsplitter and computer controlable filter

- Beam steering by diffractive shaping

- Cross correlation by split mirror correlator

Opt. Lett., ***35*** *(2010) 3916*

Phase Retrievals from Sonograms

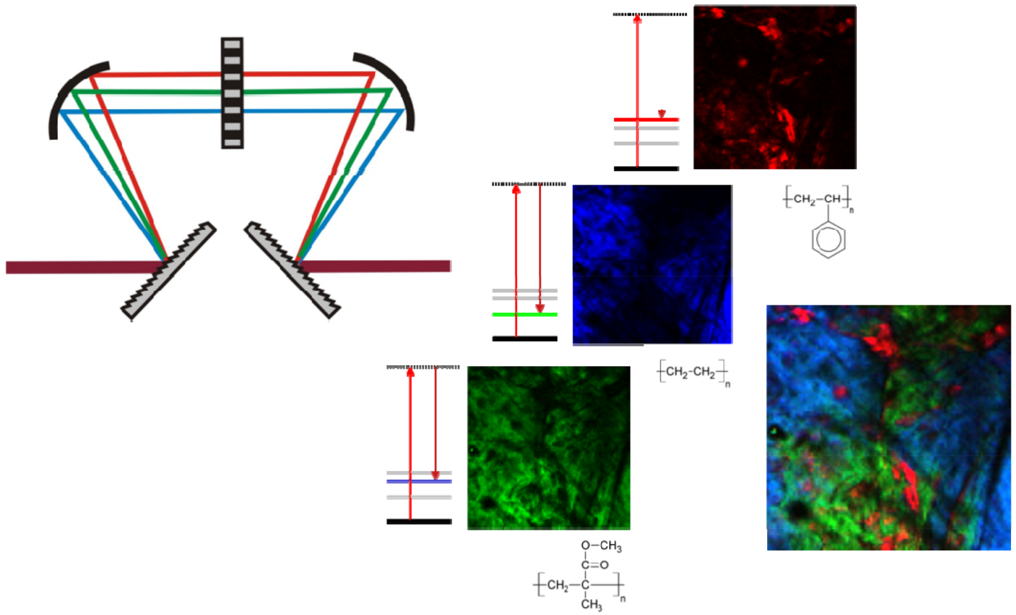


- Reconstruction of linear chirped pulses

- Quadratic phase is retrieved with good agreement

Opt. Lett. ***35*** *(2010) 3916*

Using shaped femtosecond laser pulses for multimodal nonlinear spectroscopy

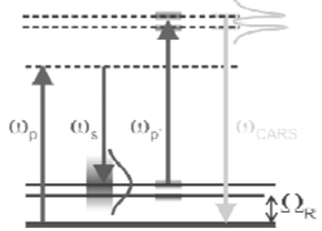


C. Pohling, J. Rehbinder, A. Wipfler, T. Buckup, and M. Motzkus

Ruprecht-Karls-Universität Heidelberg, Germany

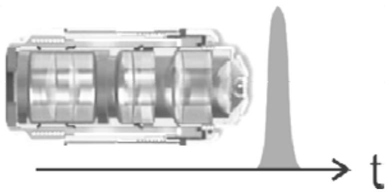
Safed, Israel – 6. September 2012

Outline

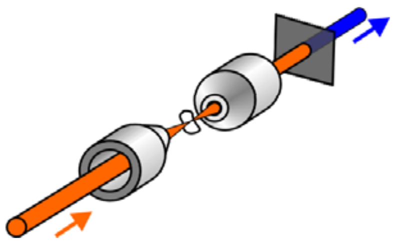


• Intro: Broadband CARS spectroscopy

• Hyperspectral data processing
using principal component analysis

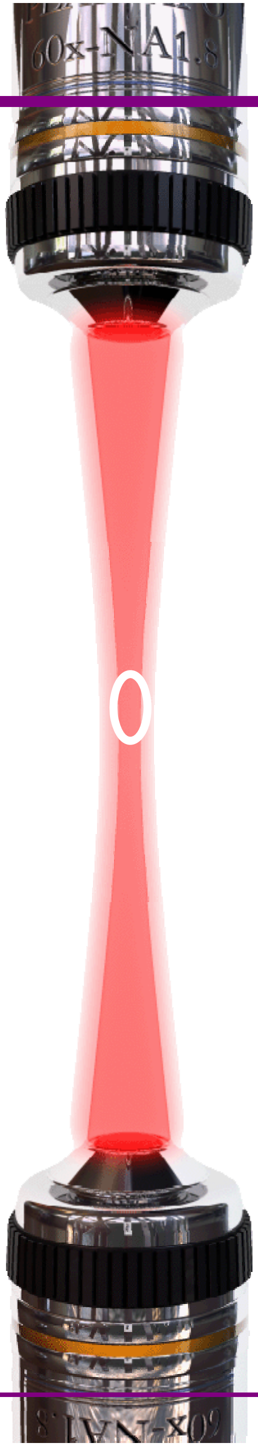


• Shaper-assisted Multiplex CARS



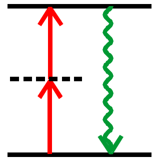
• Broadband single-beam CARS

• Summary and Outlook

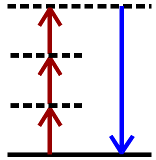


Multiphoton Microscopy

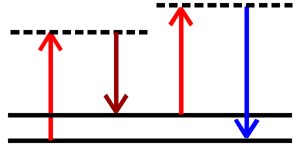
2PFE

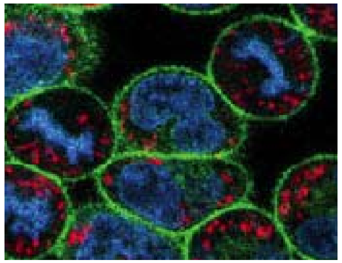


THG

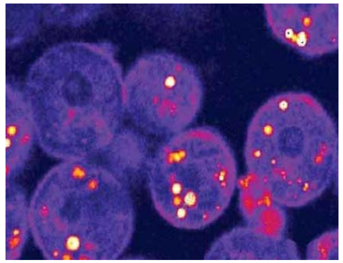


CARS

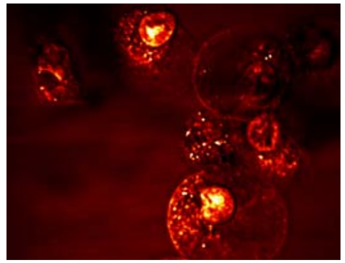




Zipfel et al., Nature Biotech. 21, 11, 1369 (2003).



Débarre et al., Nature Methods 3, 47 (2006)



Cheng et al., Biophys. J. 83, 502 (2002).

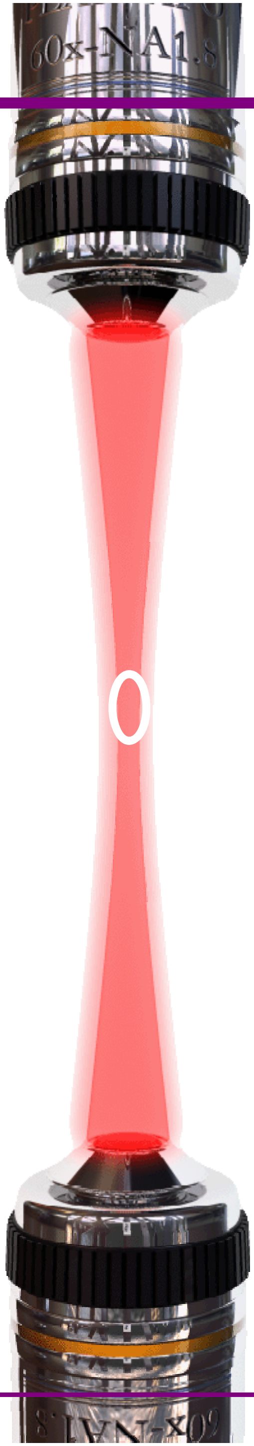
$I_{Signal} \propto I_{Exc}^{n}$

→ **3D resolution**

→ **Use ultrashort (fs) pulses:**

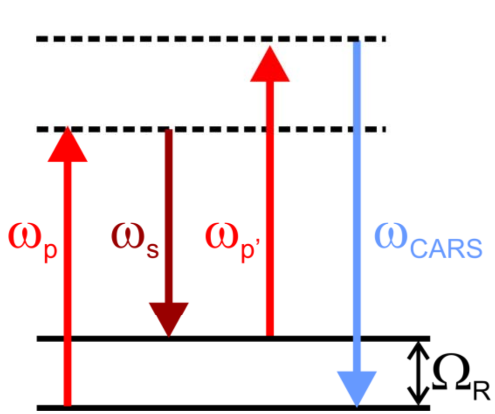
High peak intensity while low average power

Broad bandwidth for versatile excitation



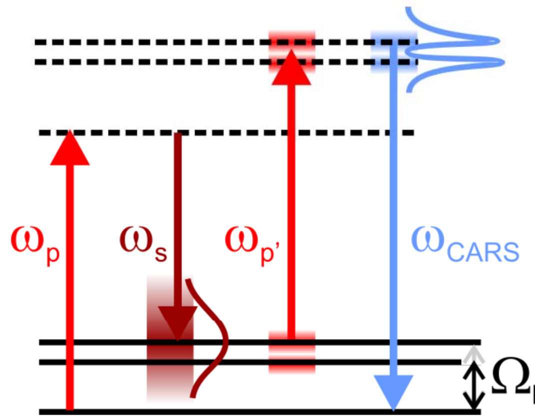
CARS Implementations

CARS



- Detection of a single resonance

Multiplex CARS (MCARS)

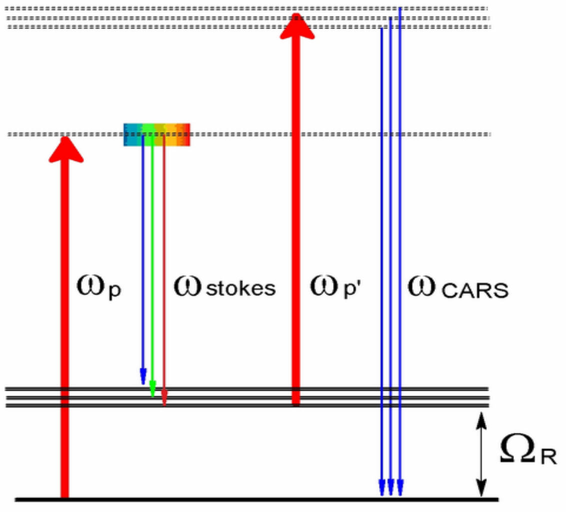


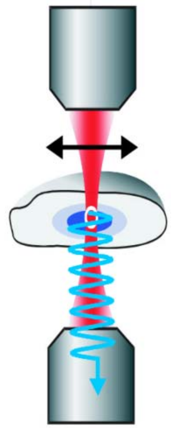
+ Very rapid acquisition of complete spectra

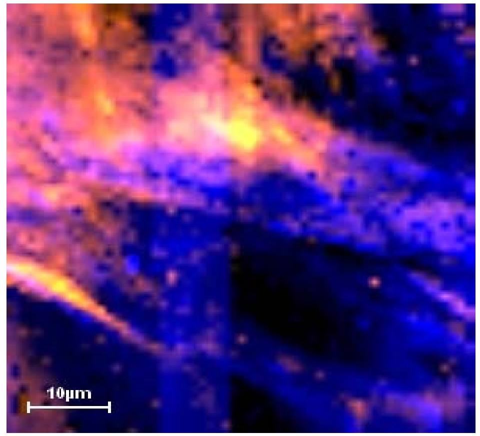
MCARS requirements:

- Narrowband laser (ω_p, $\omega_{p'}$) for high spectral resolution
- Synchronized broadband laser (ω_s) for spectral coverage

A short intro to Multiplex-CARS microscopy

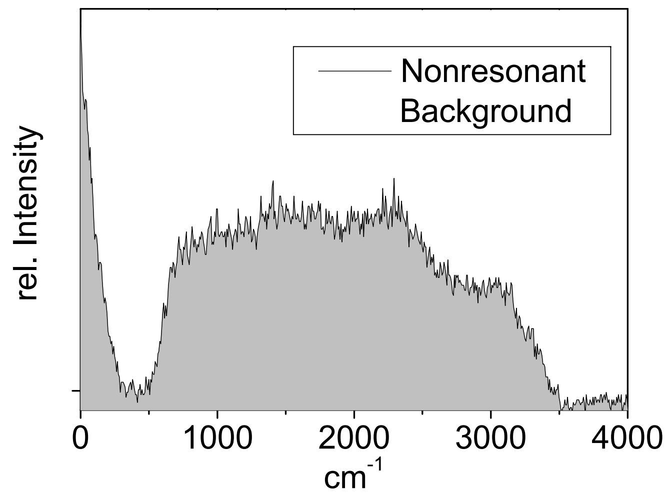






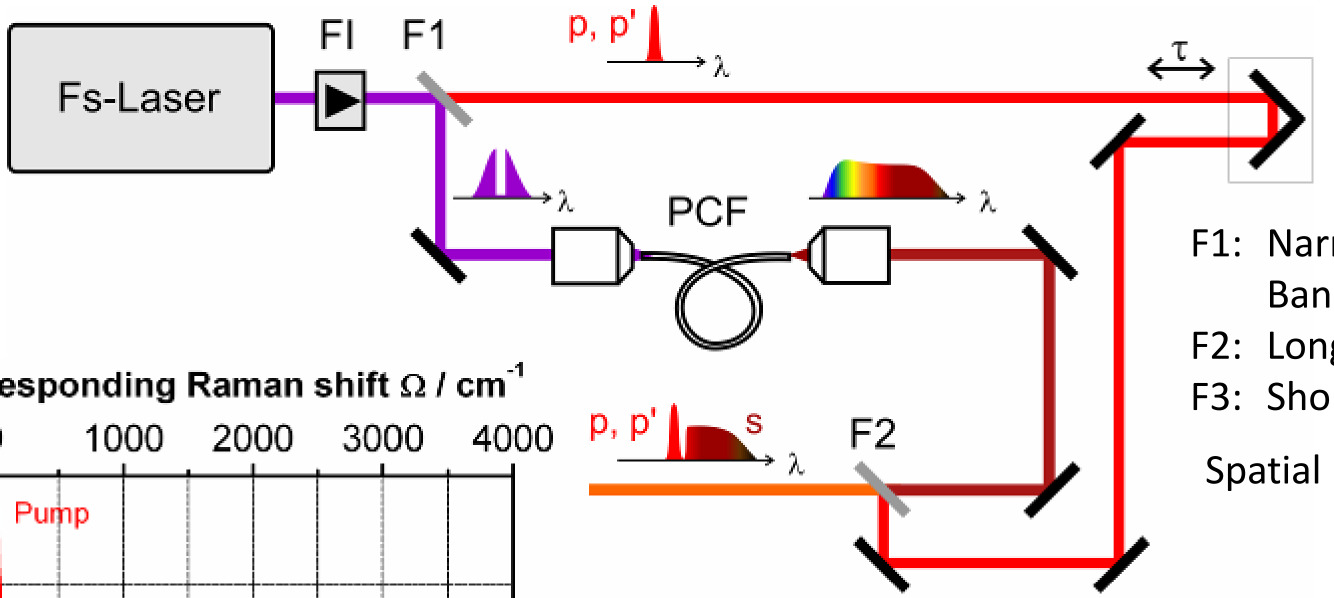
topological scan of paper

- Nonlinear 4-wave-mixing Raman-Process
- Free of 1$h\nu$-fluorescence
- Broadband excitation (3000cm^{-1}) of multiple vibrational modes



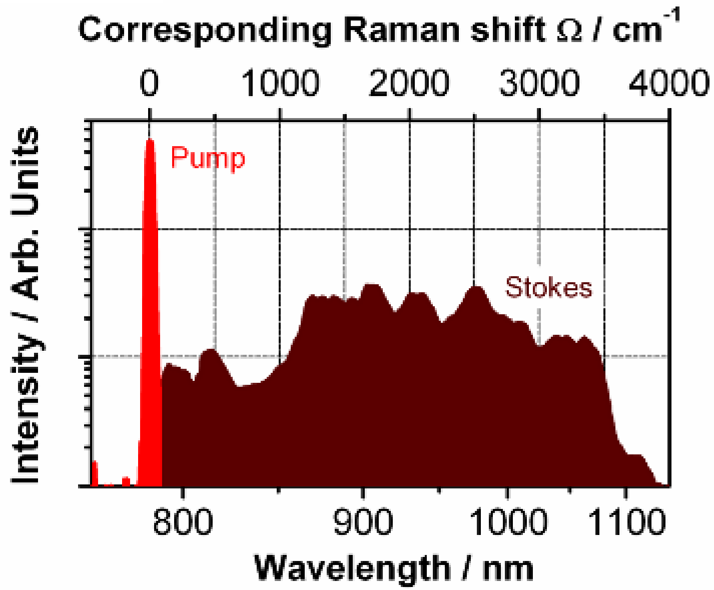
background signal of glass

Multiplex-CARS setup



F1: Narrow Bandpass filter
F2: Longpass filter
F3: Shortpass filter

Spatial Res.: 0.5 µm lateral, 2.6 µm axial



- **Narrowband Pump** (< 1 nm, better than 20 cm^{-1} spectral resolution)

: Use of interference filters which reflect rejected elements

- **Broadband Stokes** (> 300 nm, coverage up to 3500 cm^{-1})

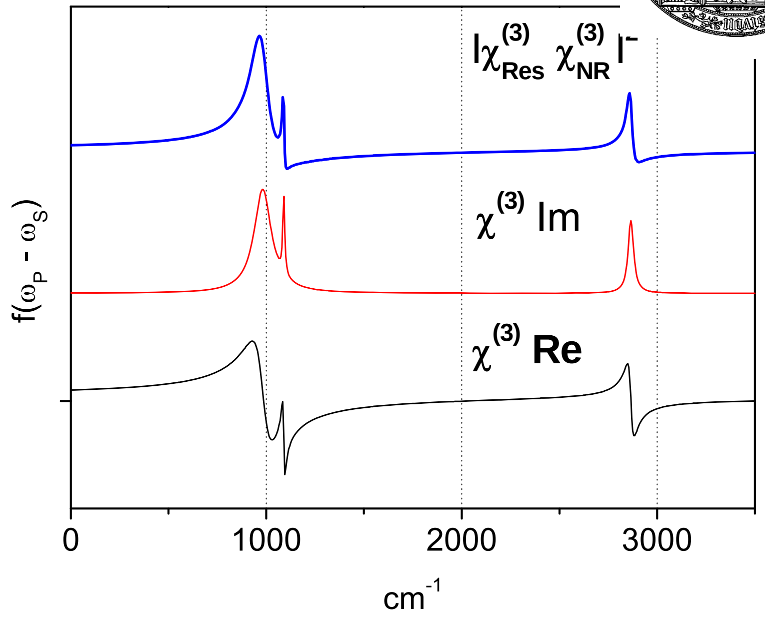
J. Raman Spectrosc. ***38*** *(2007) 916*

Complex spectra and dispersive line shape

$$E_{CARS} \propto \left(\chi^{(3)}\right) \cdot E_{pump} \cdot E_{Stokes} \cdot E_{probe}$$

$$\chi^{(3)}(\omega_{as}) = \chi_{NR} + \chi_R(\omega_{as})$$

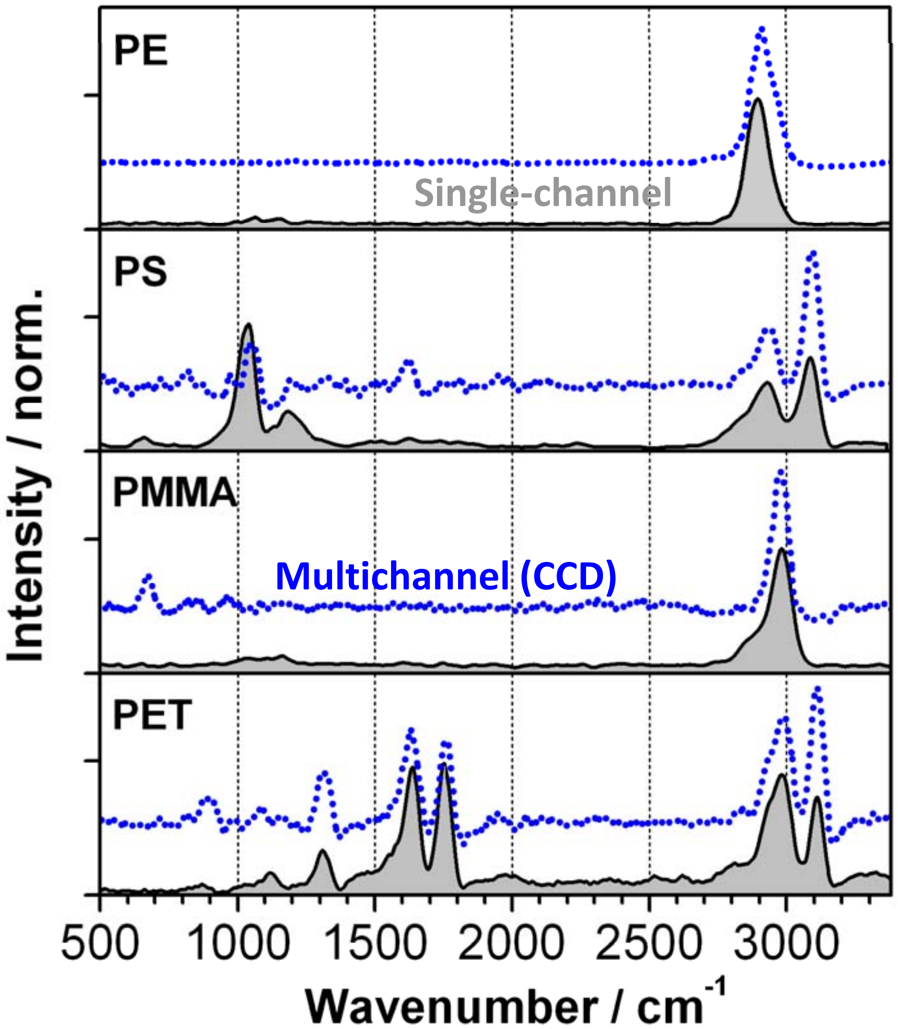
$$\chi_R(\omega_{as}) = \sum_j \frac{A_j}{\Omega_j - (\omega_p - \omega_s) + i\Gamma_j}$$

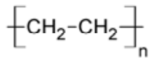


$$I_{CARS} \propto \left|E_{CARS}\right|^2 \propto \left|\chi_{NR}{}^{(3)}\right|^2 + 2 \cdot \mathrm{Re}\left(\chi_{NR}{}^{(3)} \cdot \chi_R{}^{(3)}(\omega_{as})\right) + \left|\chi_R{}^{(3)}(\omega_{as})^2\right|$$

- No direct comparison to spontaneous Raman spectra
- Intensity and spectral position influenced due to the coherence of the signals, fitting routines required

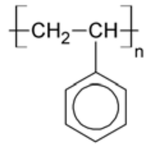
MCARS-Spectra of neat Polymers





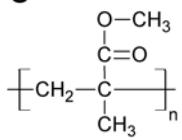
Poly(ethylen

PE



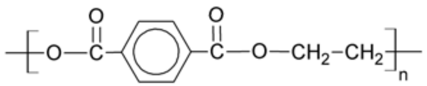
Poly(styrene

PS



Poly(methylmethacrylate)

PMMA



Poly(ethyleneterephthalate)

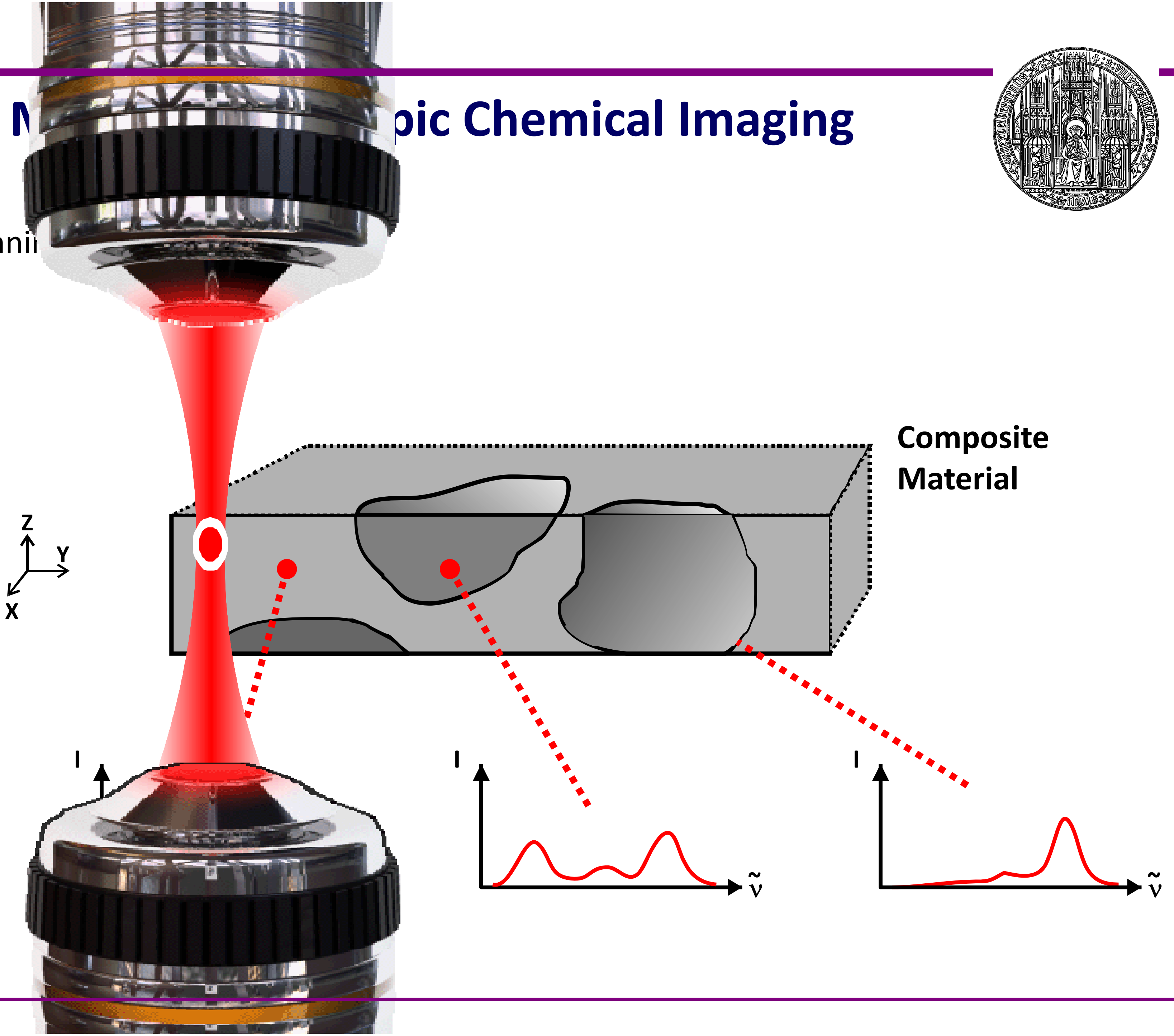
PET

CCD-Acquisition of >2000 cm^{-1} CARS spectrum in **~100 ms!**

J. Raman Spectrosc., **38**, 916 (2007).

M… pic Chemical Imaging

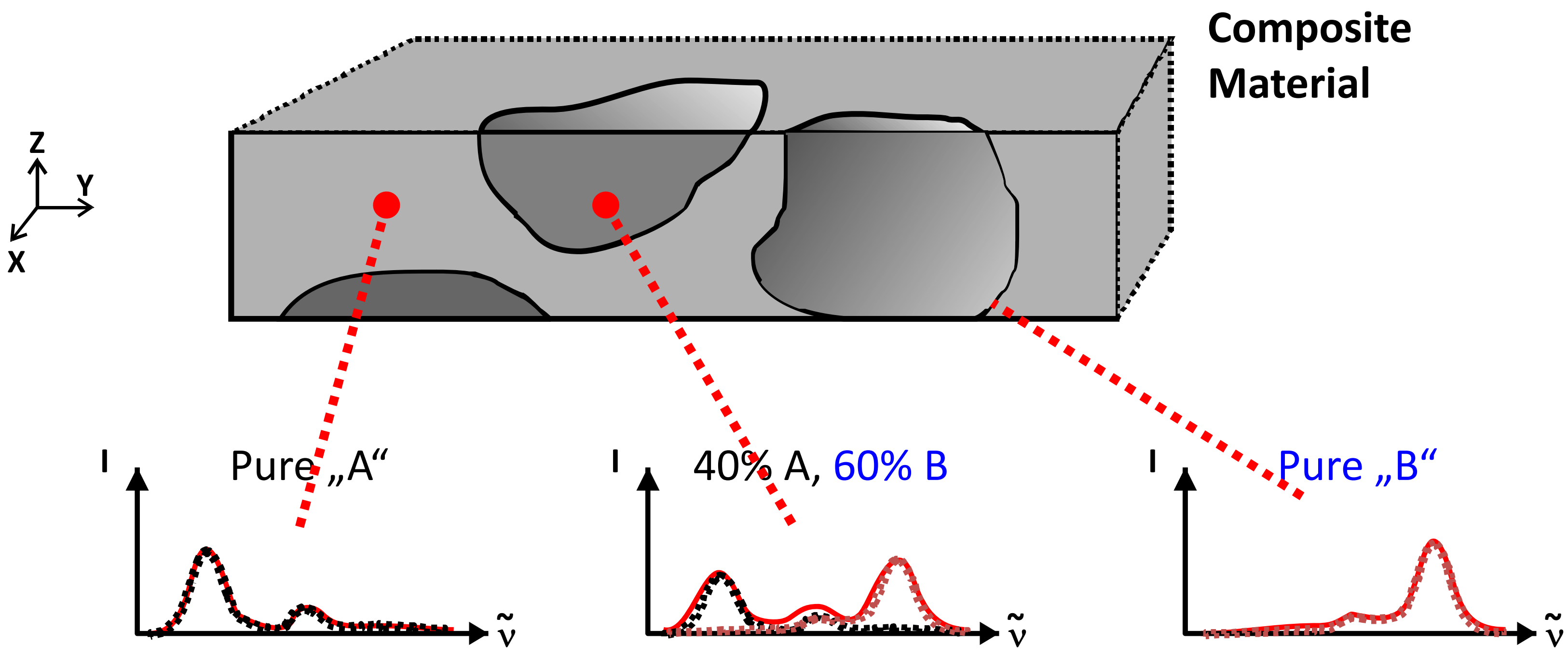
- Scanni…



MCARS Microscopic Chemical Imaging

- Scanning the laser focus
- „Hyperspectral data“: Spectrum for each spatial position in the sample

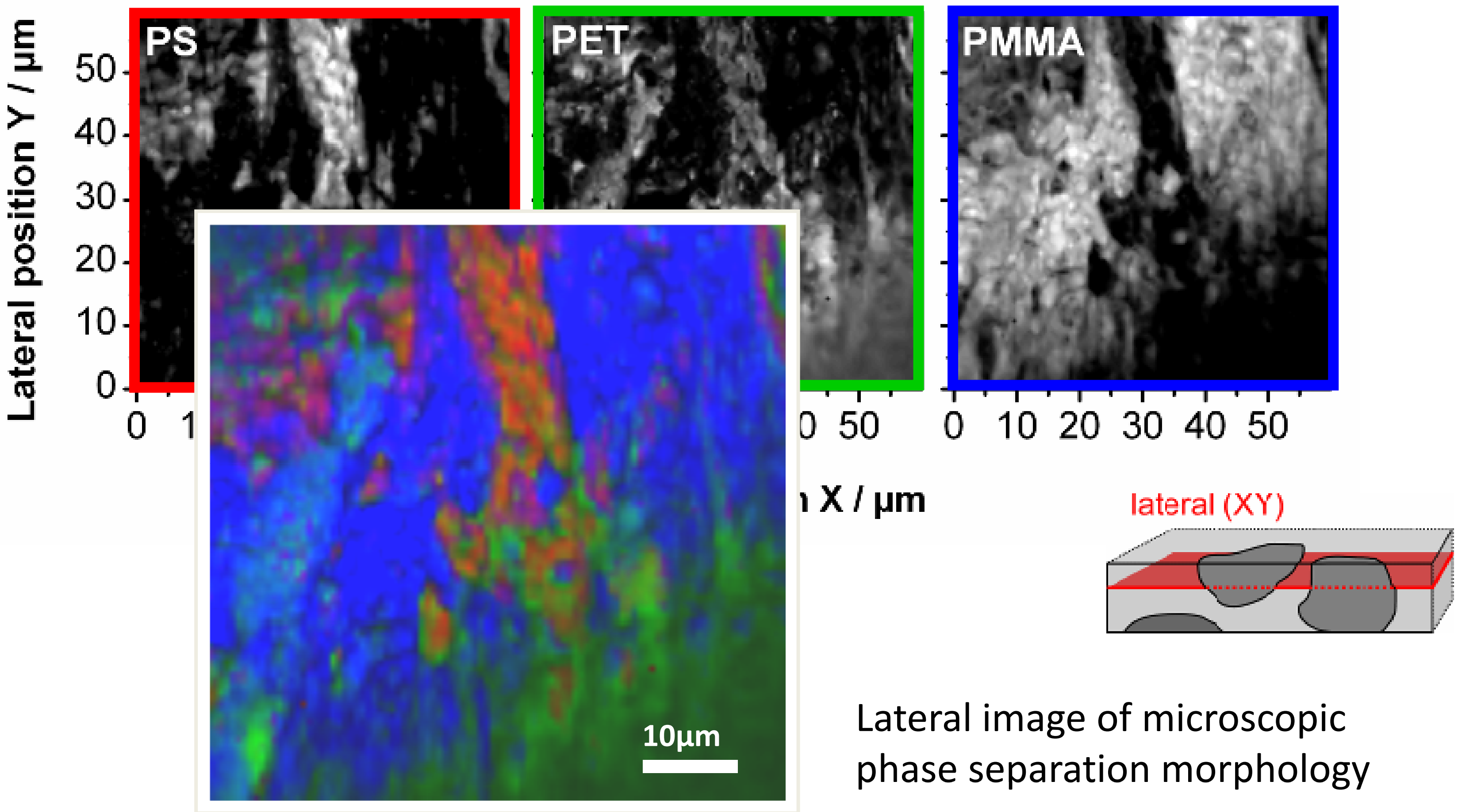
Decompose into chemical constituents



Composition from spectral fitting → Chemical maps

MCARS Microscopic Chemical Imaging

Ternary Polymer blend concentration map:

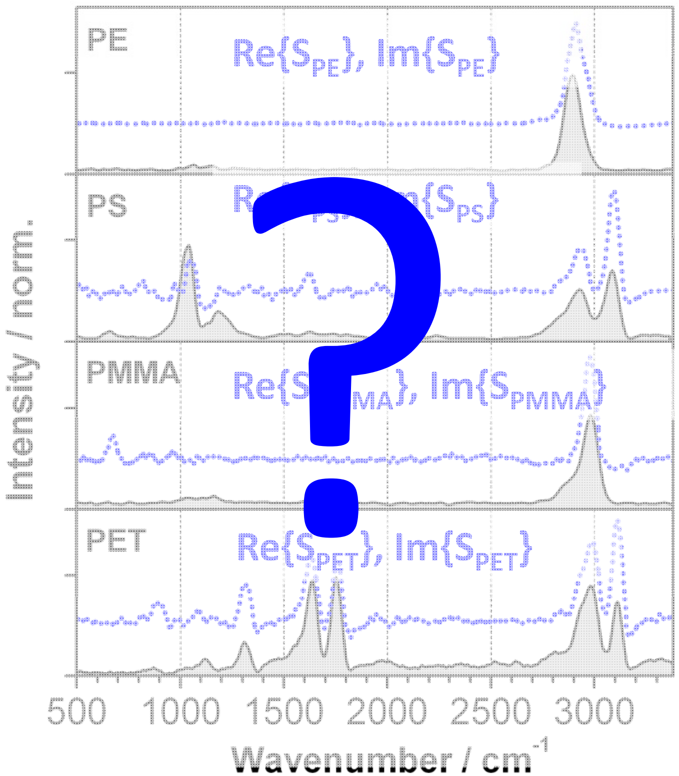


Lateral image of microscopic phase separation morphology

J. Raman Spectrosc., **38**, 916 (2007).

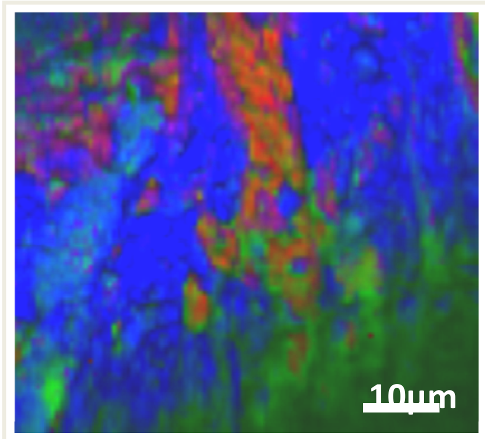
Lorentzian line shape fitting of pure spectra

- When the pure components are known, fitting of MCARS images can be performed (**Evolutionary Algorithm**)



Decomposition of a coherent sum:
Set of c_i

$$\left|S_{\mathrm{mix}}\left(\omega_{\mathrm{p}}+\Omega\right)\right|^{2}=\left|\sum_{i} c_{i} \times S_{i}\left(\omega_{\mathrm{p}}+\Omega\right)\right|^{2}$$



Polymer blend:

Red: PS
Green: PET
Blue: PMMA

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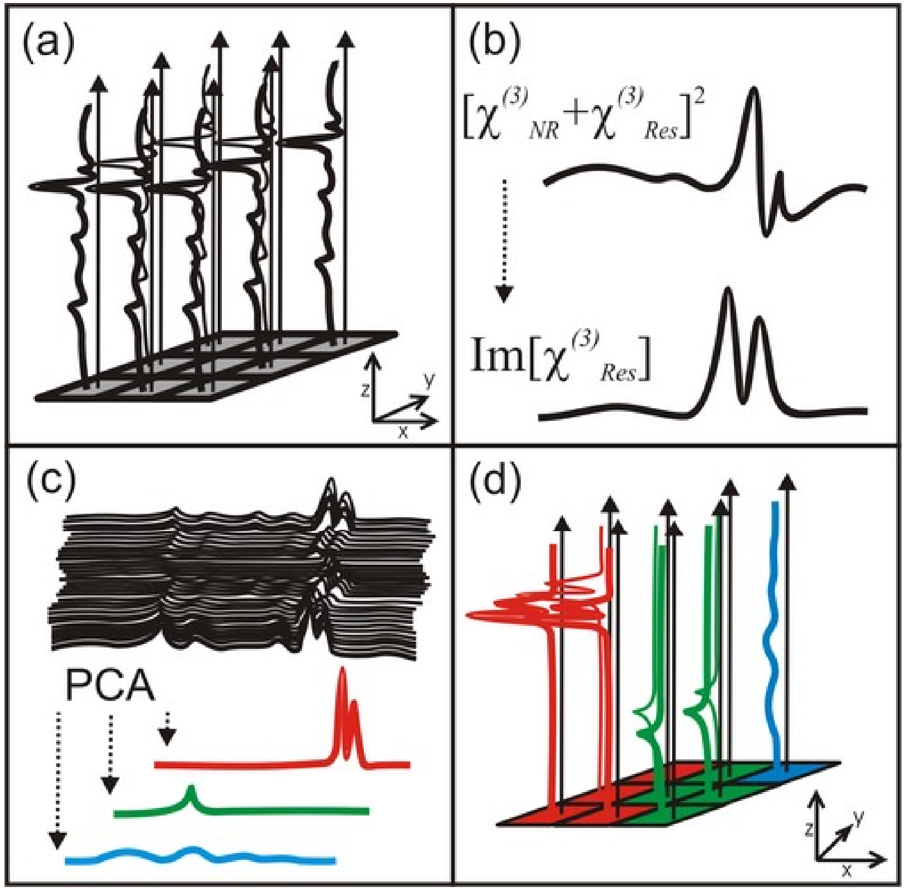
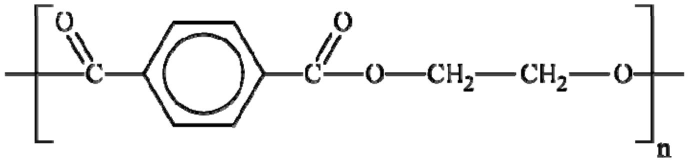
Imaging of unknown samples: 2-Step approach



- (a) measurement of hyperspectral data
- (b) imaginary part extraction
- (c) global fitting procedure, Principal Component analysis (PCA)
- (d) False colour map of spectral differences and spatial patterns within the sample

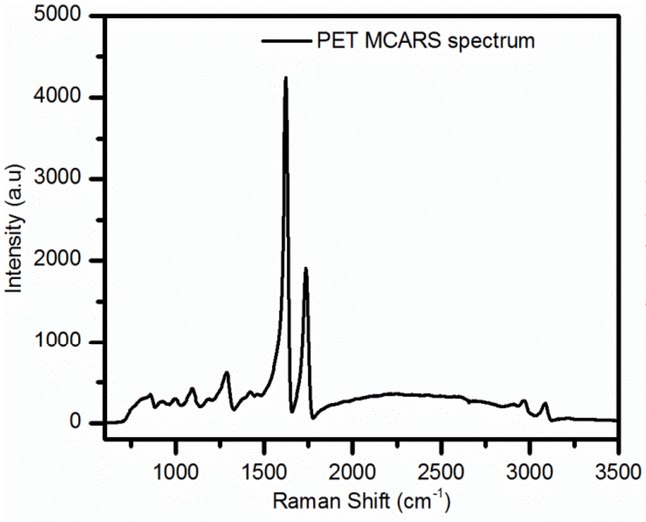
J. Biomed. Opt. ***16*** *(2011) 021105*

ME phase retrieval - PET

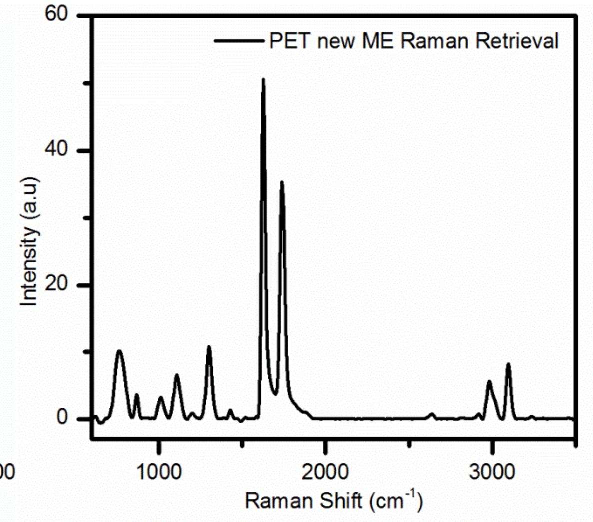


Poly(ethylene) terephthalate - PET

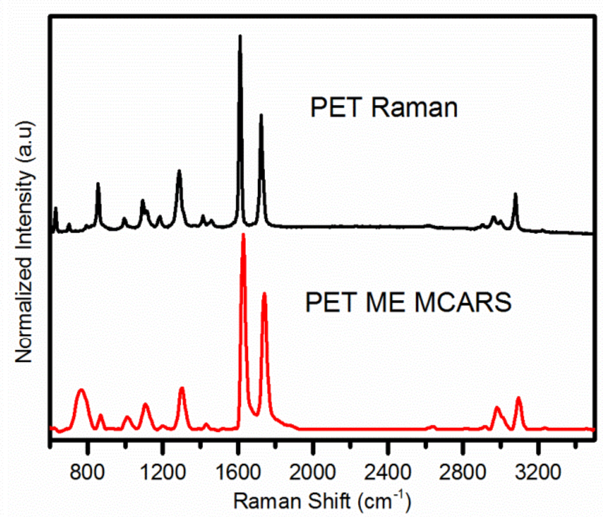
MCARS raw data



Processed Im[χ^3]



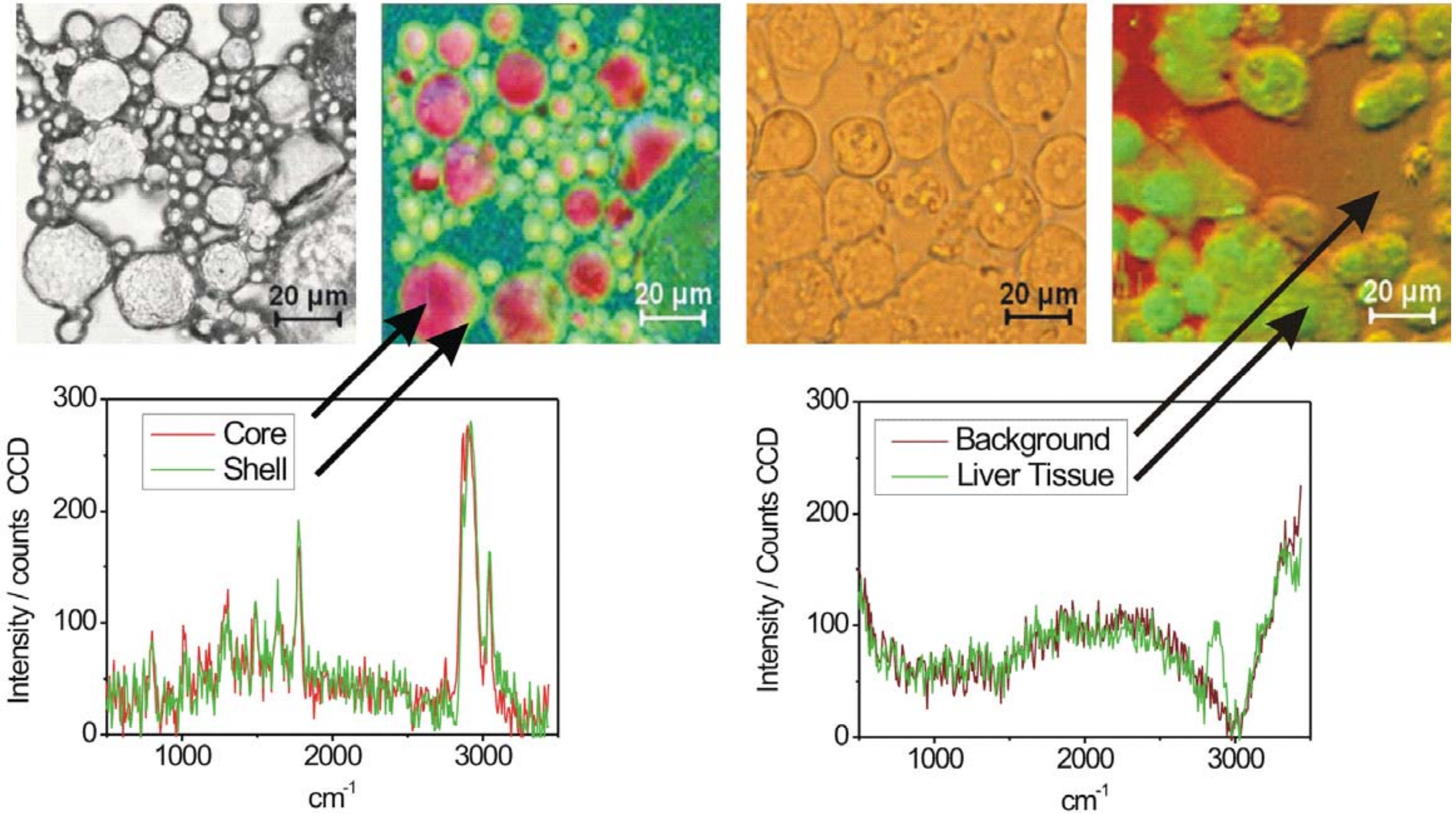
Raman Comparison



PCA: polymer and biological samples

polymer microcapsules* **liver cells****

brightfield MCARS & PCA brightfield MCARS & PCA



Bright image contrast by small spectral differences distributed over 3000 cm^{-1}

Not possible to achieve by plotting of single wavenumbers intensities

* A. Greiner, Polymer Chemistry, Marburg

** G. K. Ganjam, Dept. of Biology, Marburg

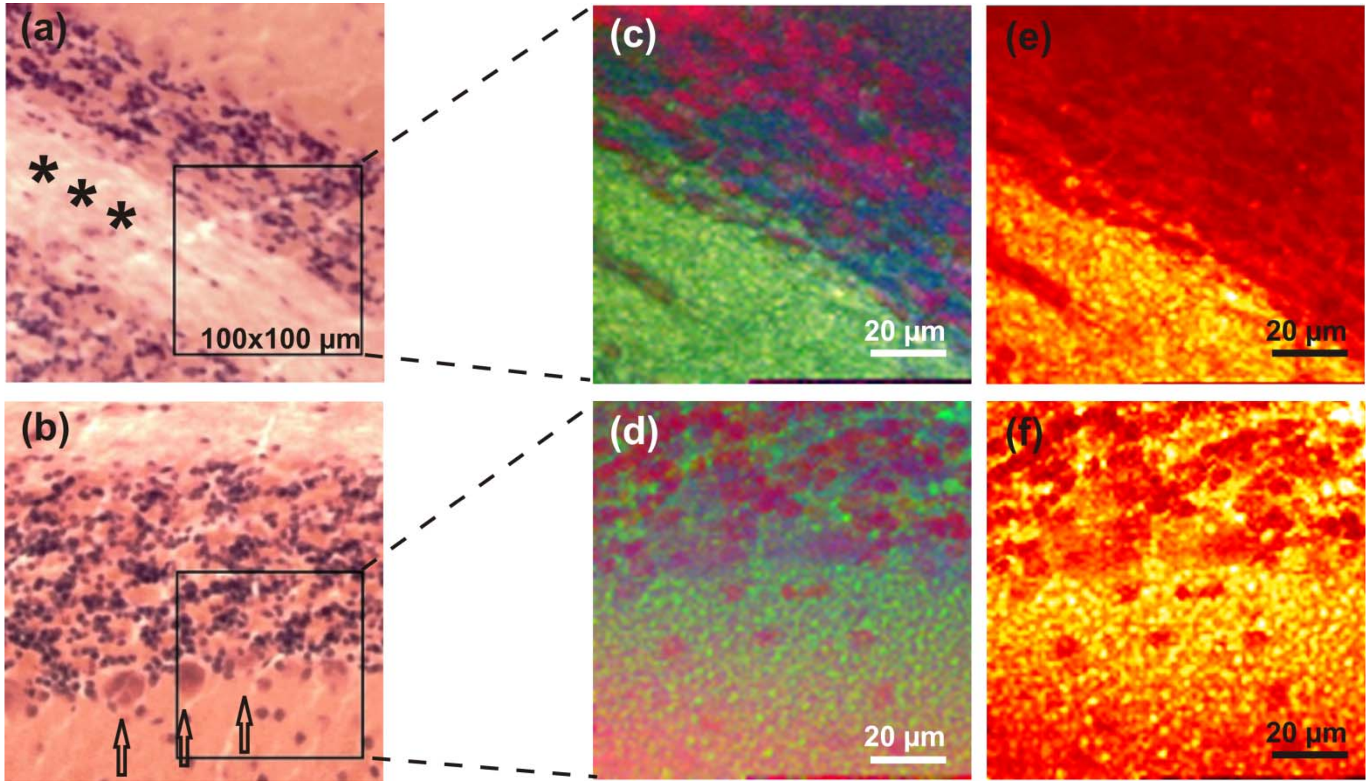
Mouse brain tissue

HE stained samples

CARS microscopy

PCA

intensity at 2845 cm^{-1}

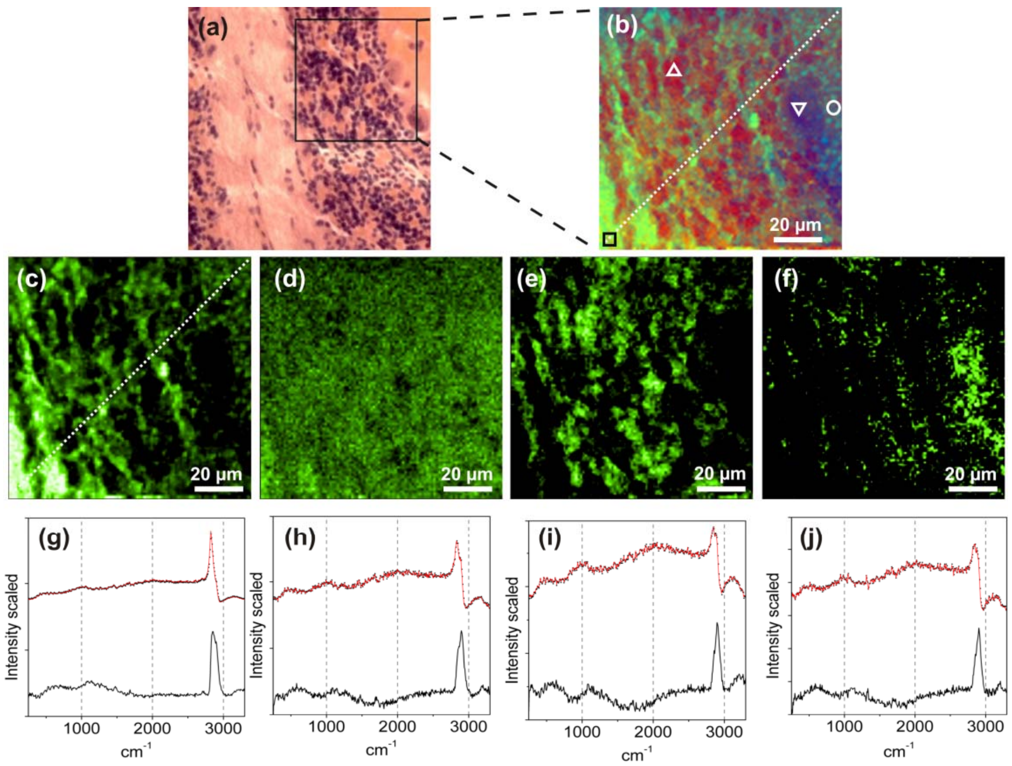


Purkinje cells (red)
grey matter (orange)
nuclei of granule cells (dark blue)
white matter (myelin, pink fiber bundles)

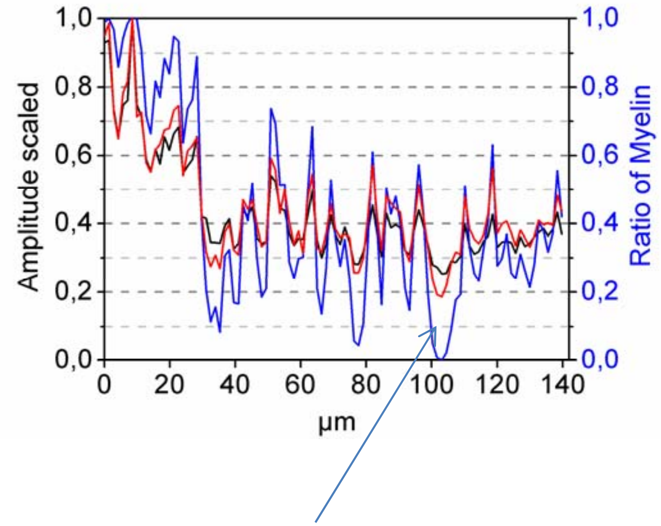
Samples: A. Pagenstecher Marburg

Mouse brain tissue

Quantitative backward calculation of the sample components



Lipid monitoring



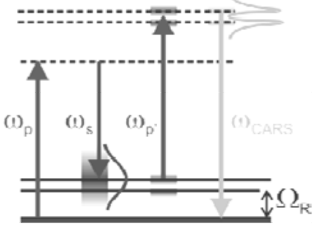
Quantitative fitting → Improved contrast →

CARS provides same information as HE stained reference !!

Samples: A. Pagenstecher Marburg

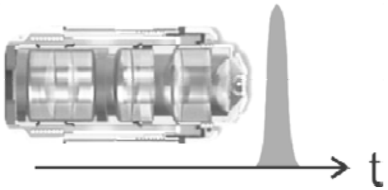
Biomedical Opt. Exp. ***2*** *(2011) 2110*

Outline

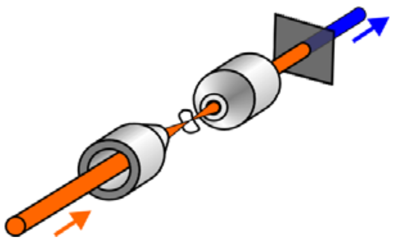


- Intro: Broadband CARS spectroscopy

- Hyperspectral data processing
  using principal component analysis



- Shaper-assisted Multiplex CARS

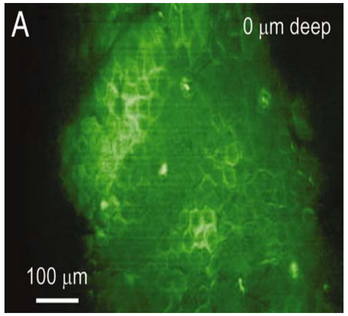


- Broadband single-beam CARS

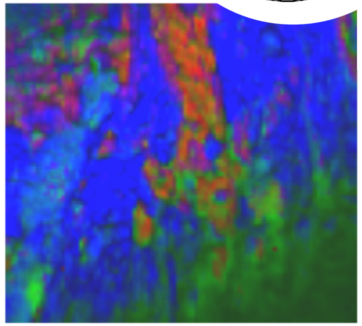
- Summary and Outlook

Multiplex-CARS and Picosecond CARS

Lipids in a mouse ear @ videorate



Chemical map of a polymer blend:
Red: PS
Green: PET
Blue: PMMA



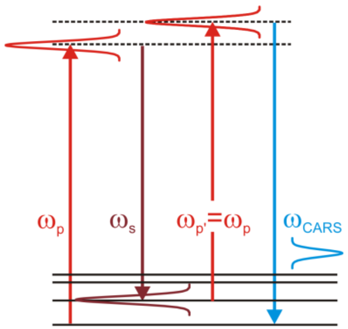
-Rapid acquisition of images

-Contrast based on a single mode

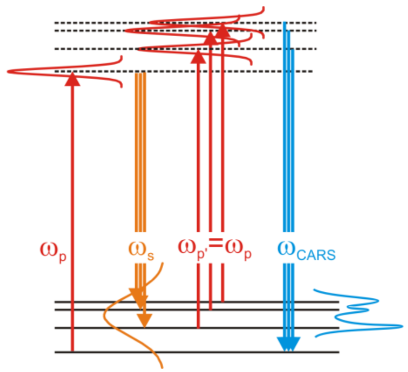
-Rapid acquisition of spectra

- CCD-detection limits imaging speed

ps-CARS



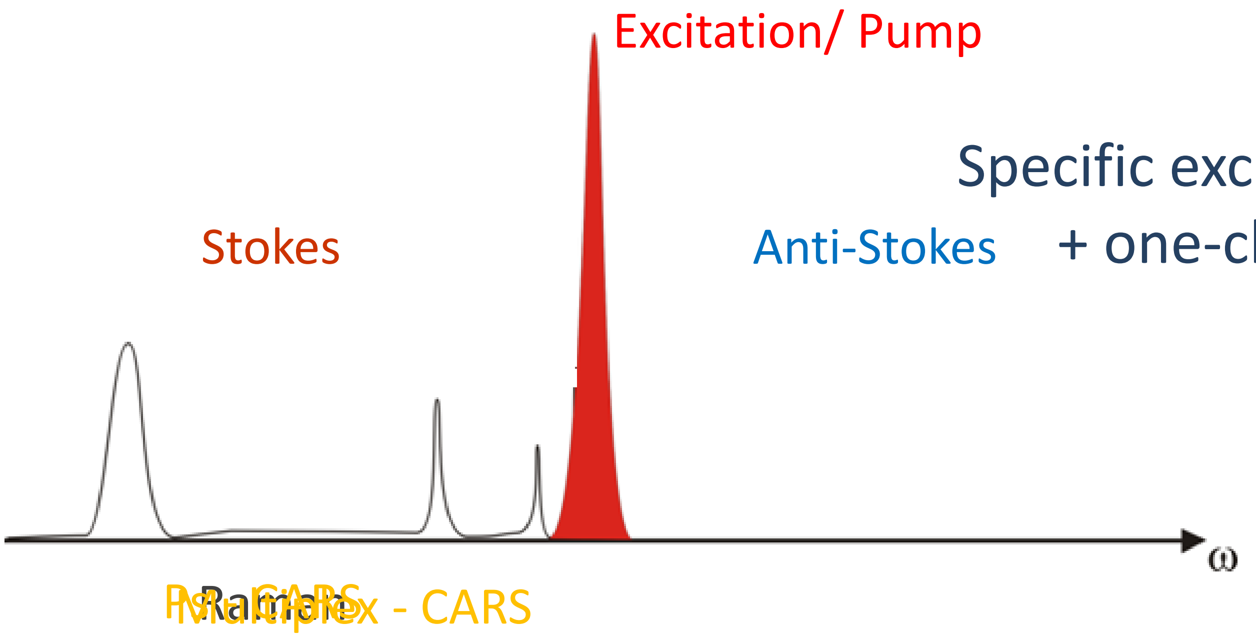
Multiplex CARS



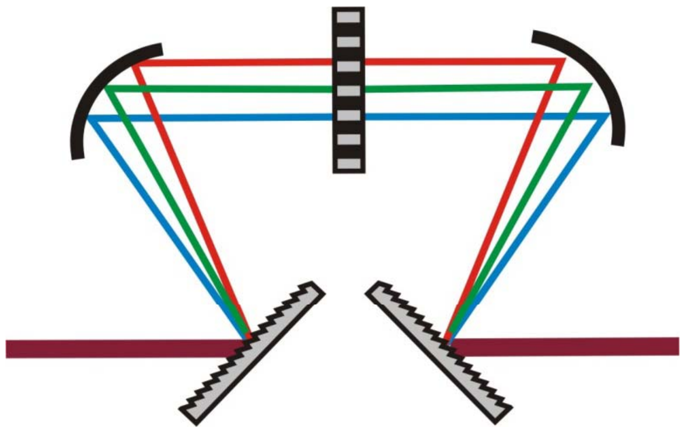
C.L. Evans et al. *PNAS* **102** (2005) 16807

B. von Vacano et al. *J.Raman Spectrosc.* **38** (2007) 91

Concept of Shaper-Assisted Multiplex (SAM)-CARS



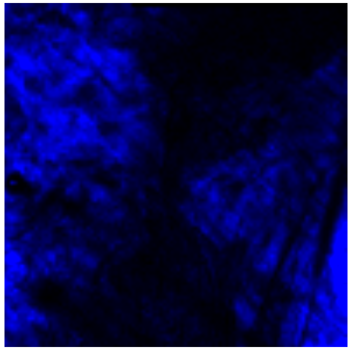
Specific excitation
+ one-channel detection

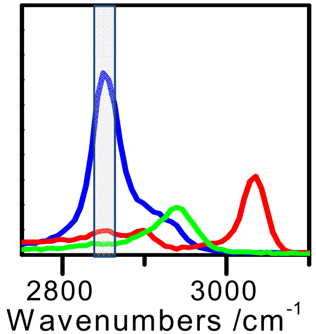


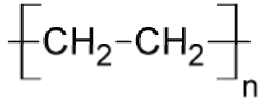
- Amplitude shaping
- 300-600 pixel
- Extinction >100 times

Shaper Assisted
Multiplex CARS

Blend imaging with the masks

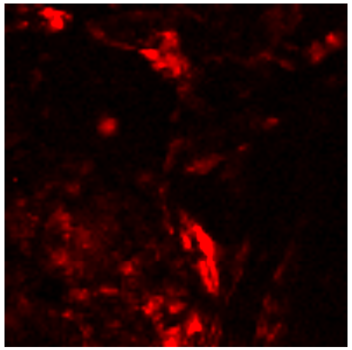


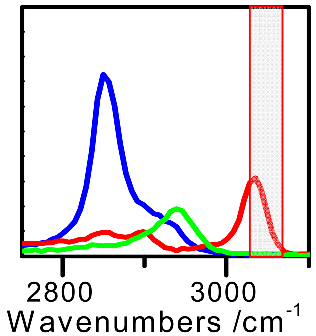


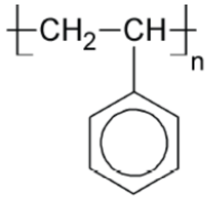


Poly(ethylene)

PE

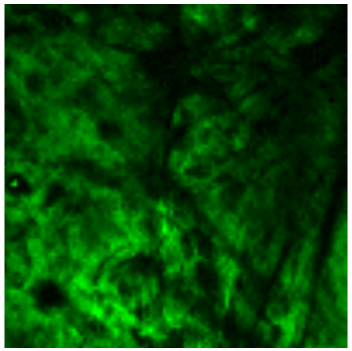


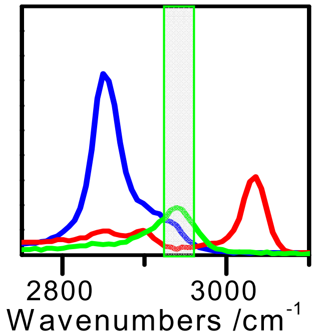


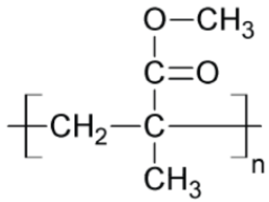


Poly(styrene)

PS



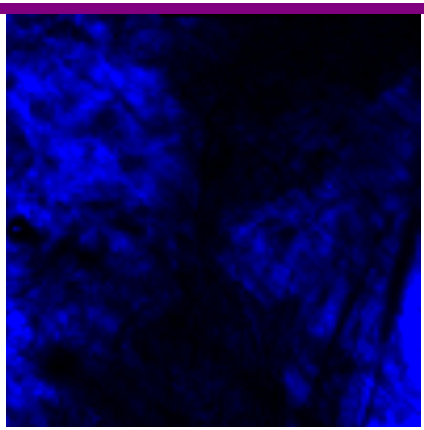




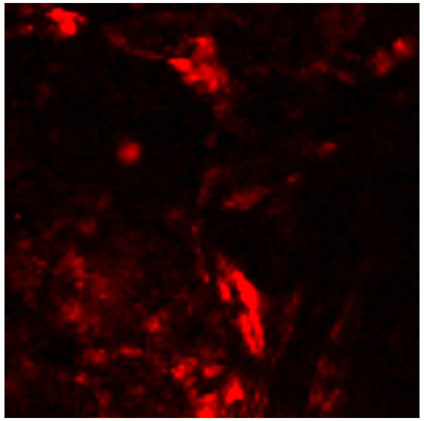
Poly(methylmethacrylate)

PMMA

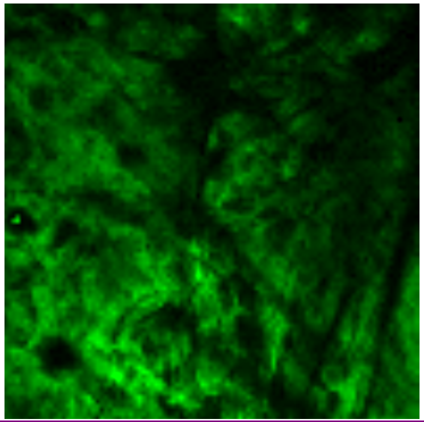
Resulting map



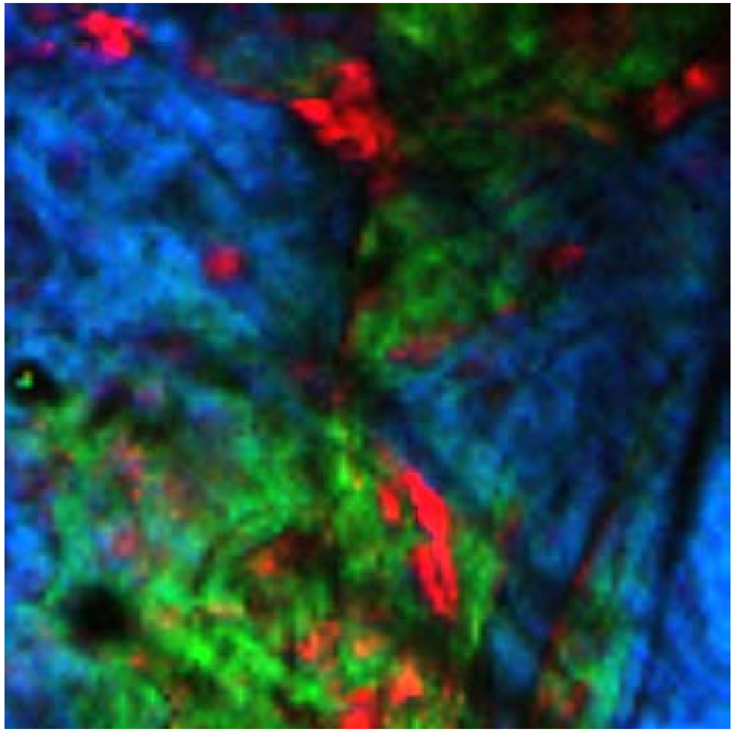
PE Mask



PS Mask



PMMA Mask

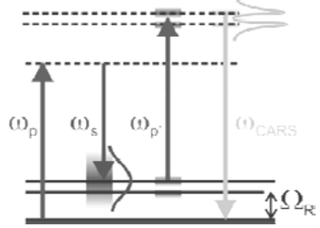


Shaper-assisted Multiplex CARS:

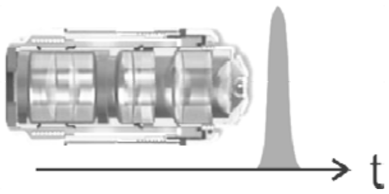
➔Multiplex information is transfered into single channel detection

Optics Letters ***35*** *(2010) 22*

Outline

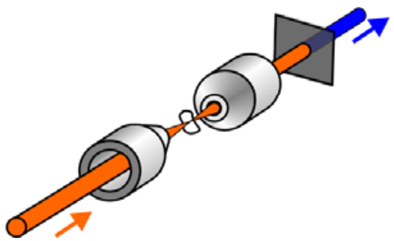


- Intro: Broadband CARS spectroscopy



- Hyperspectral data processing using principal component analysis

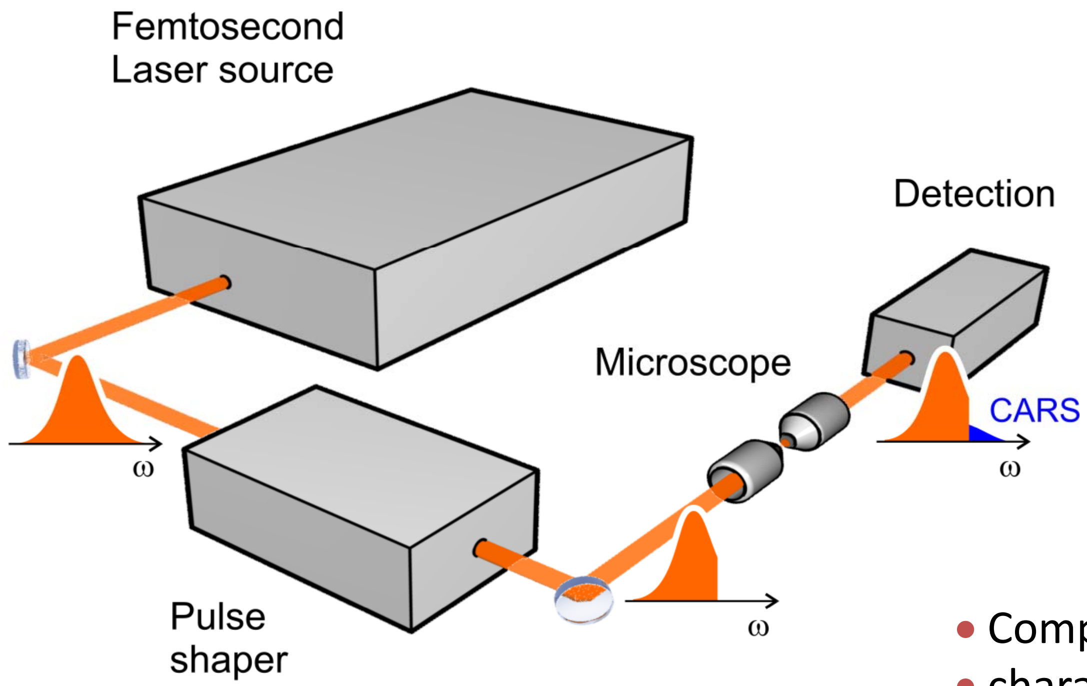
- Shaper-assisted Multiplex CARS



- Broadband single-beam CARS

- Summary and Outlook

Nonlinear microscopy with shaped pulses



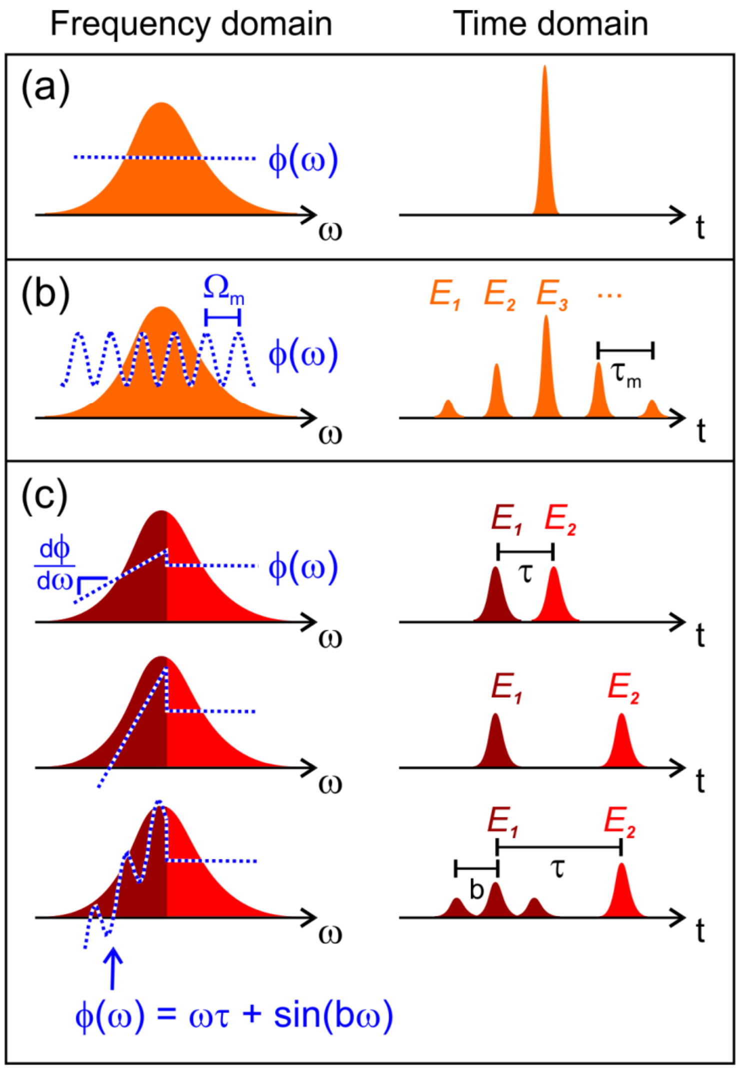
In addition:

- 2-Photon-Fluores.
- SHG
- THG

- Compression
- characterization
- CARS-generation:
  - Contrast enhancement
  - background supression
  - time-dependent processes
  - heterodyne amplification

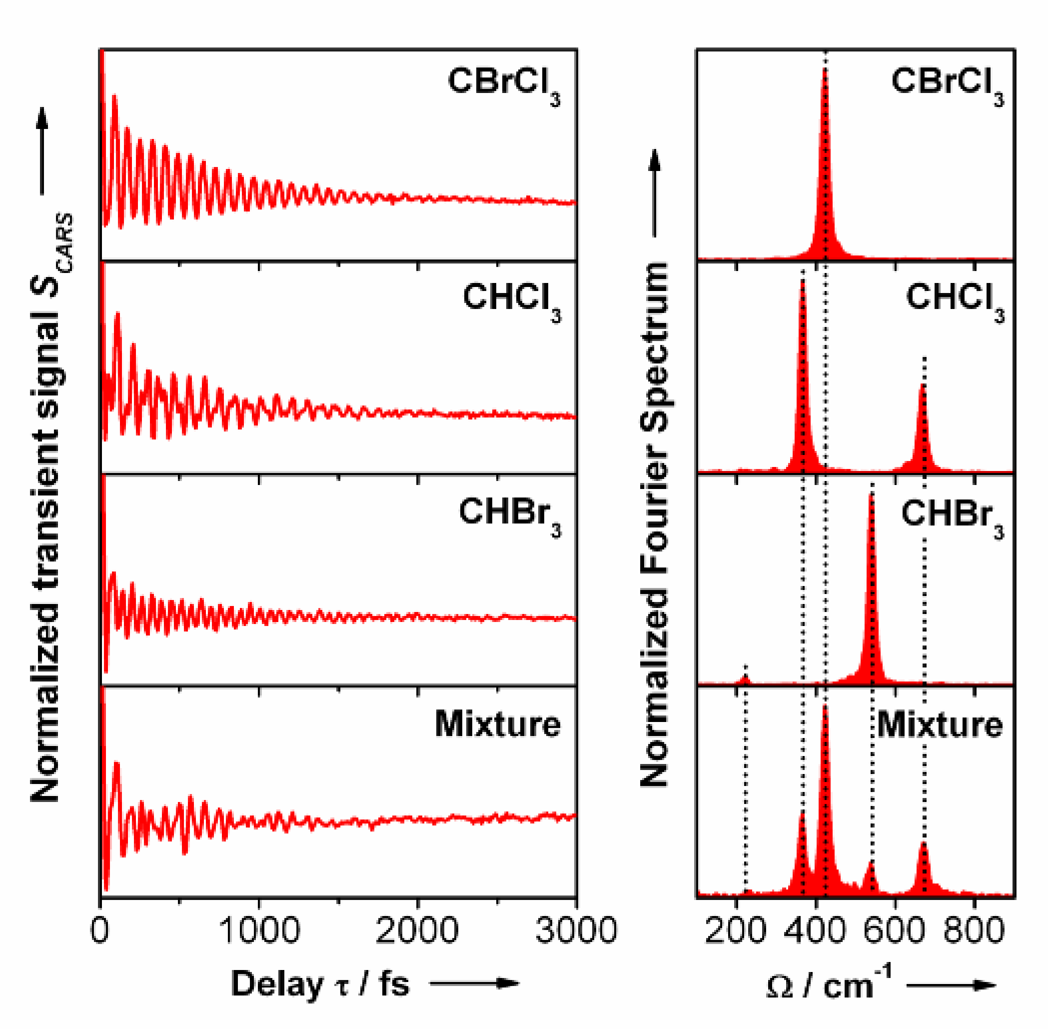
Phys. Chem. Chem. Phys. ***10*** *(2008) 681*

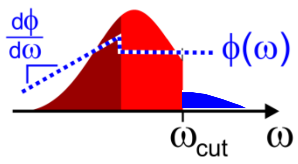
Time-resolved CARS and control

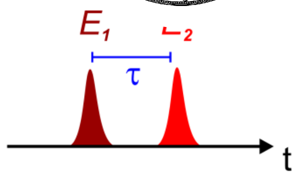


Opt. Lett. **31**, 413 (2006)
Optics Letters **31**, 2495 (2006)
Opt. Commun. **264**, 488 (2006)
J. Chem. Phys. **127**, 144514 (2007)
Appl. Phys. B **91**, 213 (2008)
Phys. Chem. Chem. Phys. **10**, 681 (2008)
J. Raman Spectrosc. **40**, 809 (2009)

Truly time-resolved Single-beam CARS



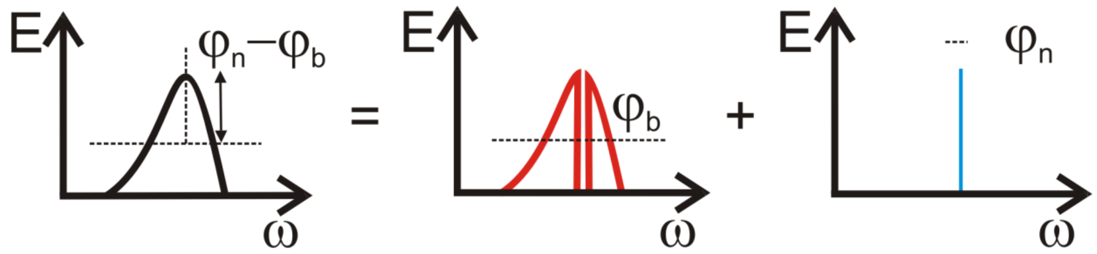




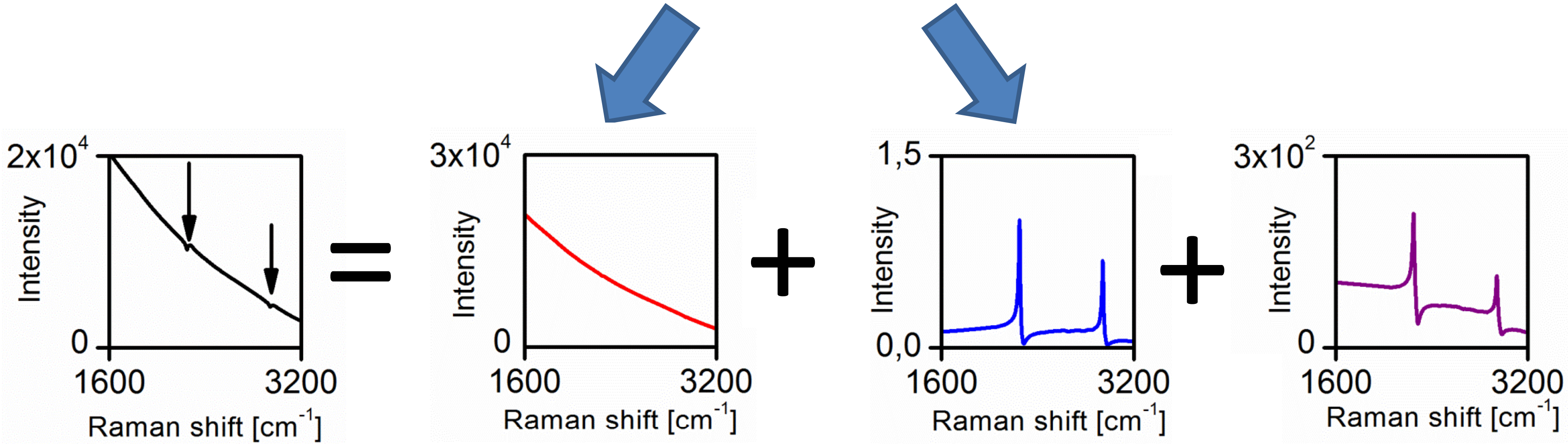
- **Unambiguous data analysis**, arbitrary wavenumber range

Phys. Chem. Chem. Phys. ***10*** *(2008) 681*

Multiplexing Single-Beam-CARS



Excitation scheme



$$S(\omega) \propto \left| E_{CARS,b}(\omega) + E_{CARS,n}(\omega) \right|^2 = \left| E_{CARS,b}(\omega) \right|^2 + \left| E_{CARS,n}(\omega) \right|^2 + 2\left| E_{CARS,b}(\omega) E_{CARS,n}(\omega) \right| \cos\varphi$$

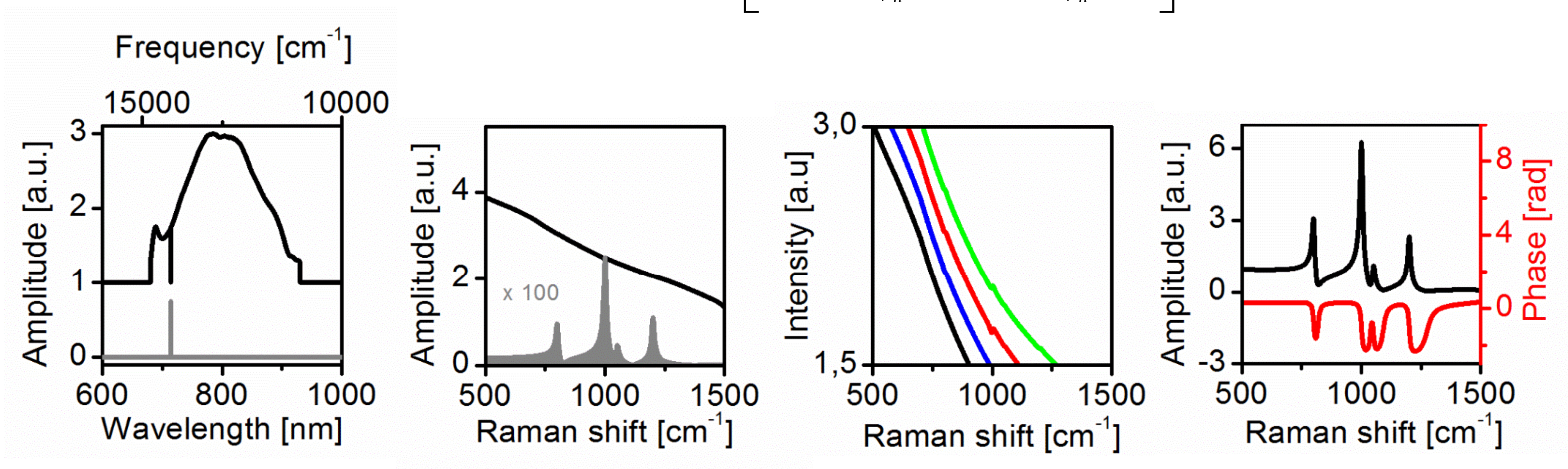
→Broadband probe provides a *Local oscillator (LO)*

O. Katz et al.,
Opt. Expr. ***18*** *(2010) 22693*

Multiplexing Single-Beam-CARS - Simulation

→ $$\left|E_{CARS,n}(\omega)\right| = \frac{1}{4\left|E_{LO}(\omega)\right|}\sqrt{\left[S(\omega)\big|_{\varphi_n=0} - S(\omega)\big|_{\varphi_n=\pi}\right]^2 + \left[S(\omega)\big|_{\varphi_n=\pi/2} - S(\omega)\big|_{\varphi_n=-\pi/2}\right]^2}$$

→ $$\varphi = \arctan\left[\frac{S(\omega)_{\varphi_n=\pi/2} - S(\omega)_{\varphi_n=-\pi/2}}{S(\omega)_{\varphi_n=0} - S(\omega)_{\varphi_n=\pi}}\right]$$

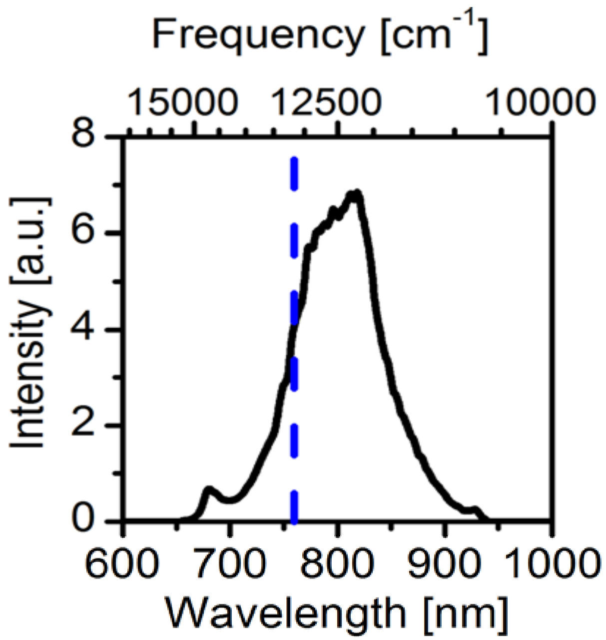


Excitation spectrum ***CARS electric fields*** ***CARS spectra*** ***Retrieved amplitude and phase***

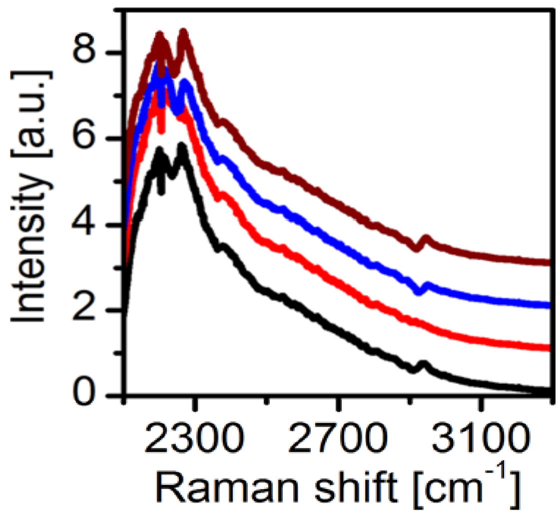
→Intrinsic LO enables detection of Multiplex signal

Multiplexing Single-Beam-CARS - Experiment

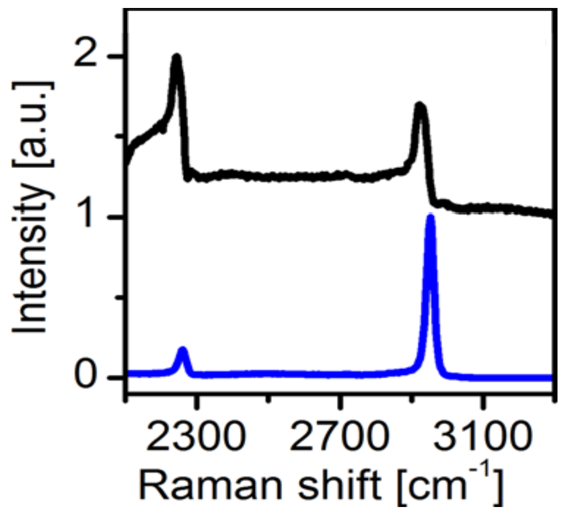
Measurement on acetonitrile:



Excitation spectrum



CARS spectra



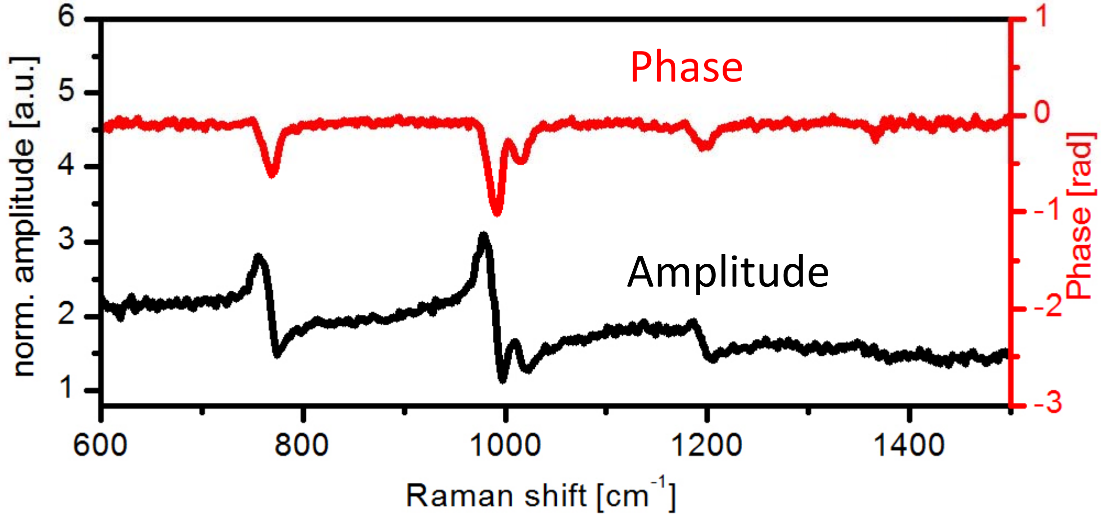
Retrieved Multiplex CARS spectrum

Blue: reference measurement with MCARS setup

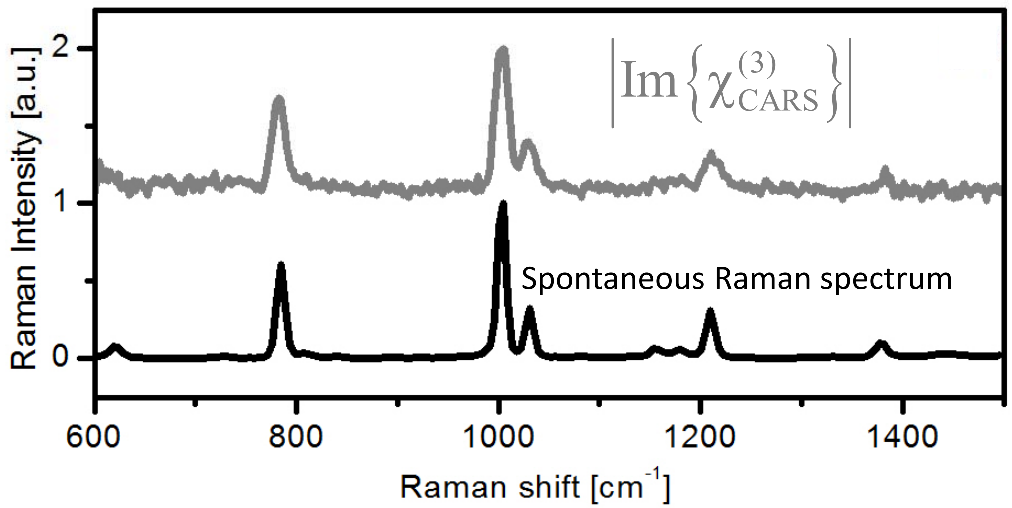
→ Agreement with Multiplex-CARS measurement

Appl. Phys. Lett. ***100*** *(2012) 071102*

Full characterization of the susceptibility



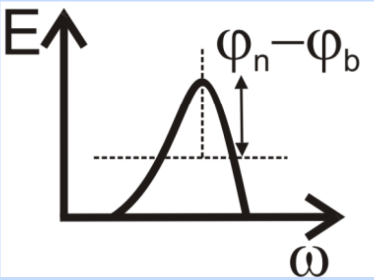
Amplitude and phase of the susceptibility can be extracted
→ **MCARS spectrum**



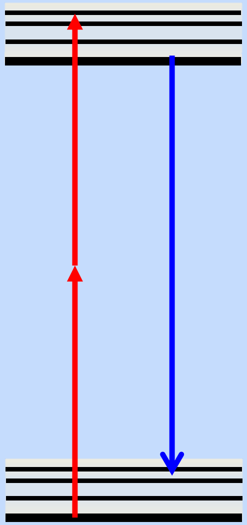
Imaginary part can easily be obtained
→ **Spontaneous Raman spectrum**

→ Single-beam-CARS and phase shaping delivers spontaneous Raman spectrum!

CARS and 2PEF

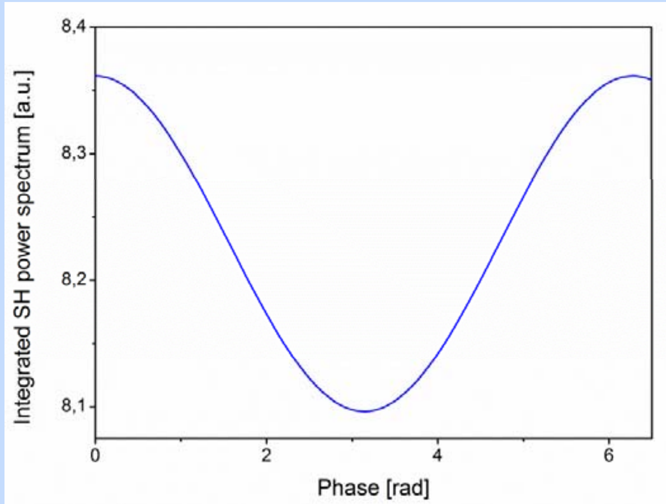


Excitation spectrum



TPA

$$S_{tot}^{(2)} \propto \int \left| \int E(\omega)E(\Omega-\omega)d\omega \right| d\Omega$$



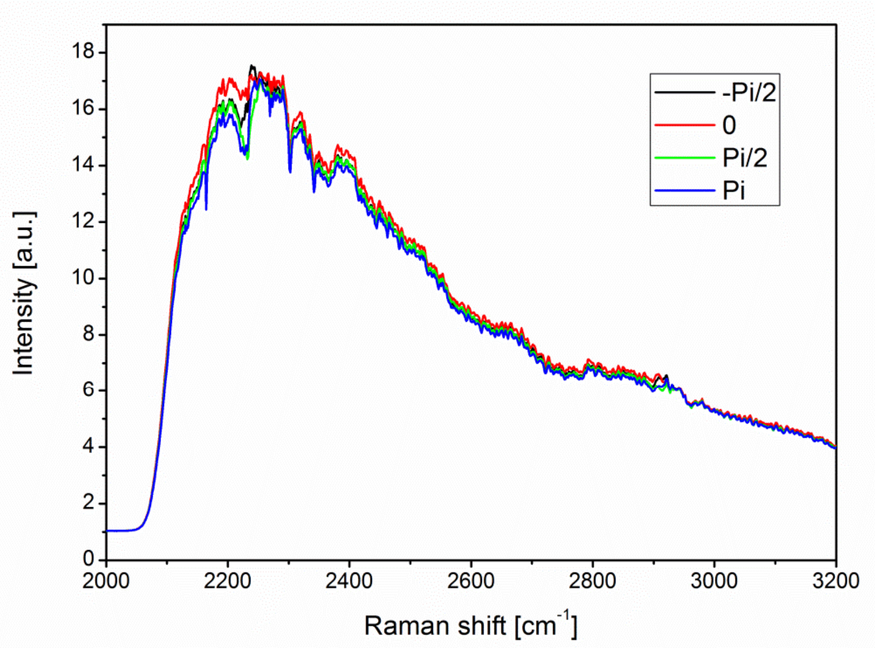
Integrated SH power spectrum

$$\left|E_{CARS,n}(\omega)\right| = \frac{1}{4\left|E_{CARS,b}(\omega)\right|}\sqrt{[S(\omega)|_{\varphi_n=0} - S(\omega)|_{\varphi_n=\pi}]^2 + [S(\omega)|_{\varphi_n=\pi/2} - S(\omega)|_{\varphi_n=-\pi/2}]^2}$$

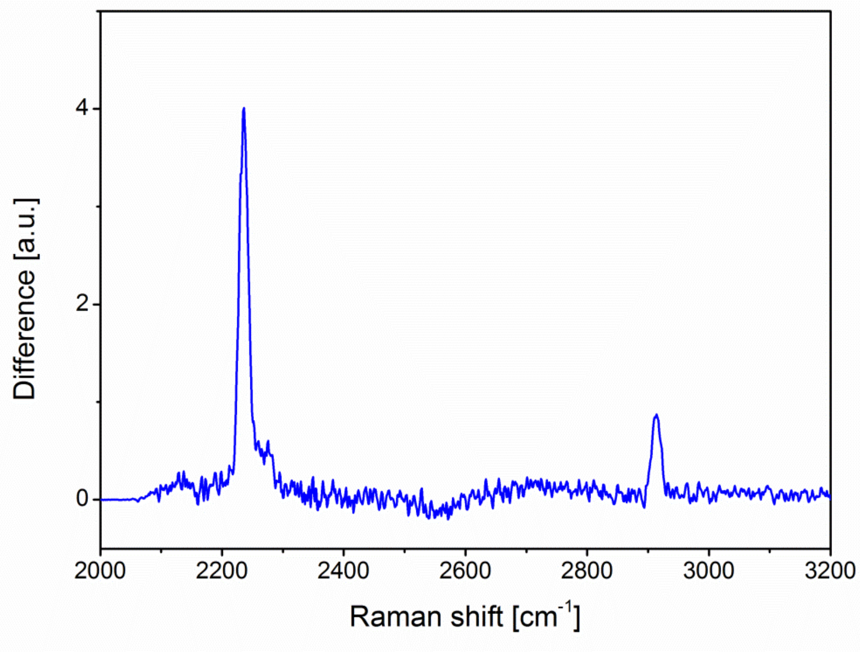
2PEF shows a sinusoidal dependence on the gate phase

CARS and 2PEF

CARS spectra of acetonitrile and DCM for four different phases of the gate.



Difference of spectra for $\pi/2$ and - $\pi/2$ → Raman spectrum!



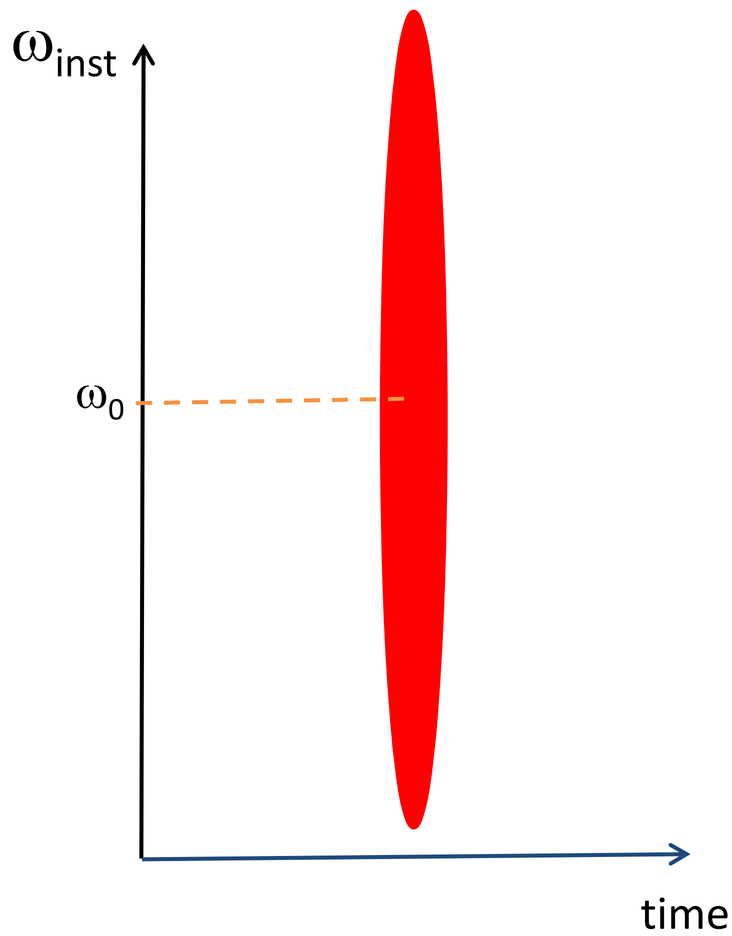
Selective pulses in Single-beam CARS : spectral focusing

Fourier limited pulse:
-Broadband and very short
-Very high peak power

-Efficient non-linear generation
-Potential multiphoton photodamage

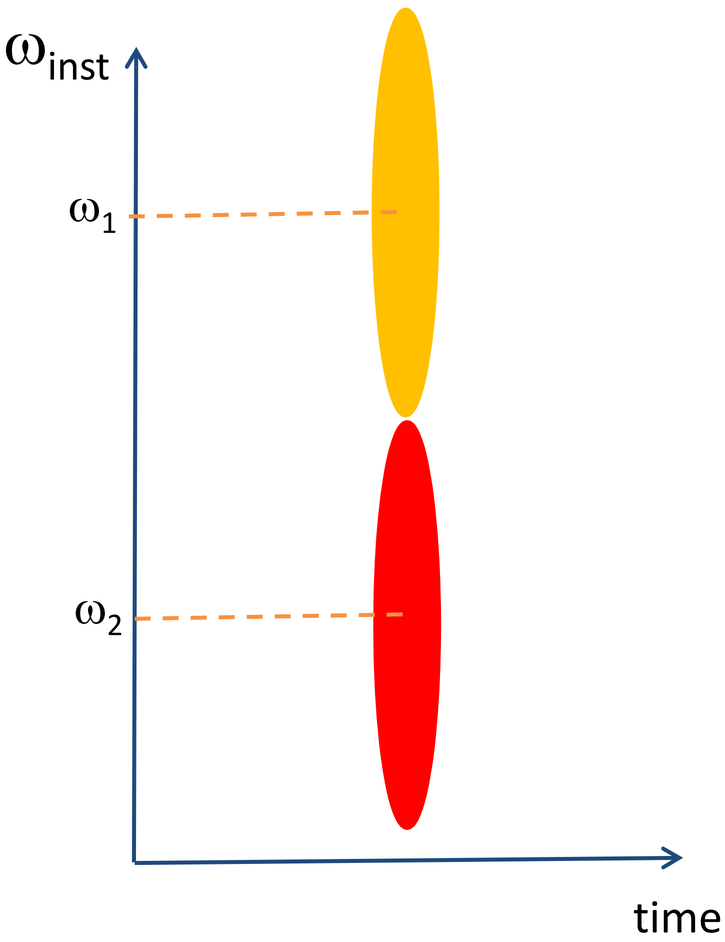
Selective pulse:
-Specific molecule excitation
-High efficiency

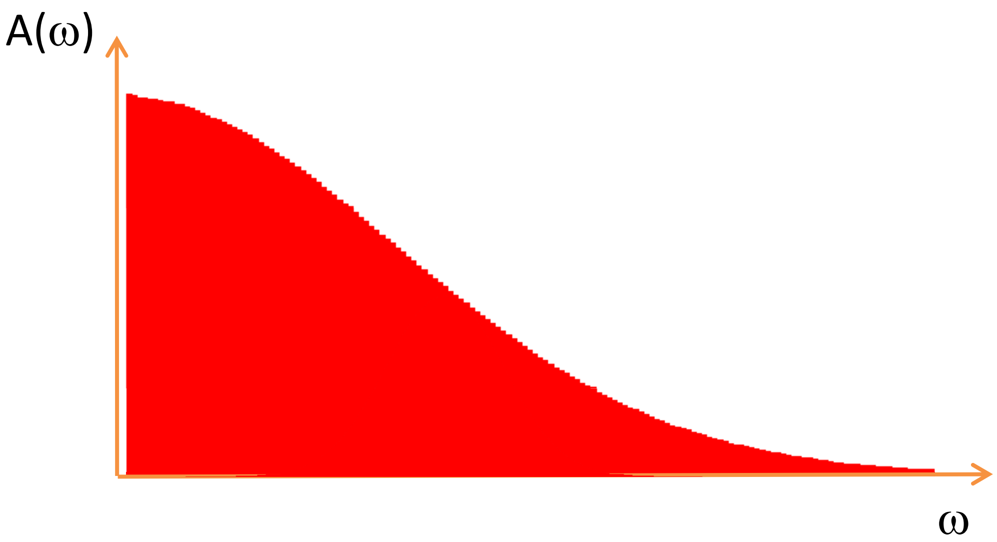
-Rapid imaging
-Reduced photodamage



→ Example : spectral focusing

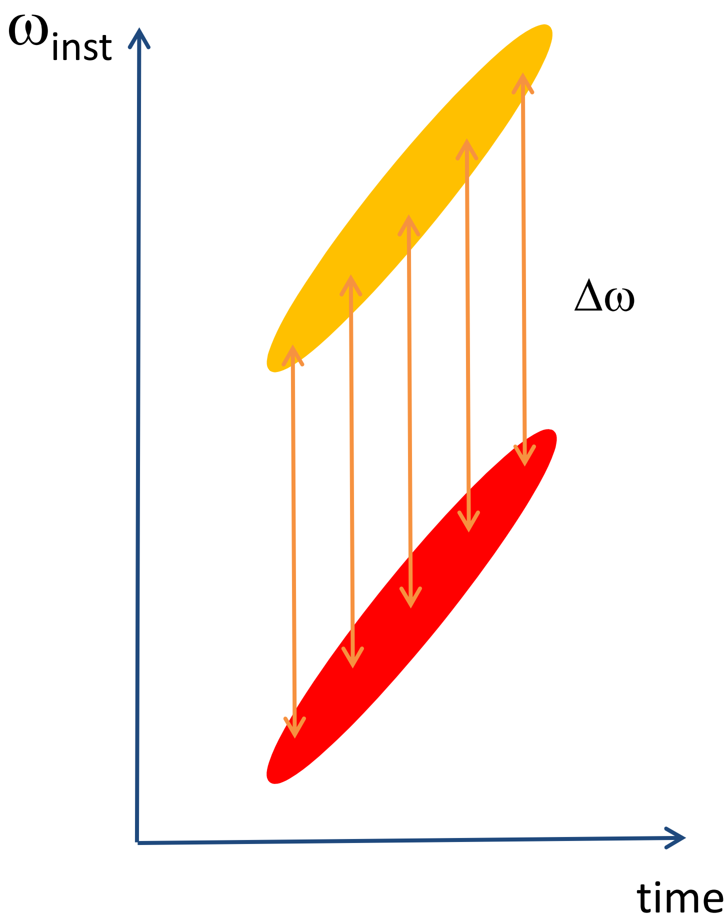
Single-beam spectral focusing: time picture





Naumov et al. *Appl. Phys. B* **77** (2003) 369
Hellerer et al. *Appl. Phys. Lett.* **85** (2004) 25

Single-beam spectral focusing: time picture



Shaped pulse:
-From fs to ps pulse duration
-Efficient excitation of one resonance

→

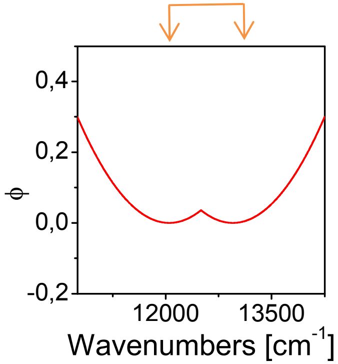
-Increased specificity for imaging
-decreased multiphoton photodamage

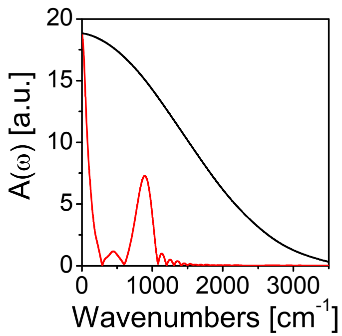
Control of the excitation

Spectral focusing

$$\varphi(\omega) = a(\omega-\omega_1)^2\,\theta(\omega-\omega_c) + a(\omega-\omega_2)^2\,\theta(\omega_c-\omega)$$

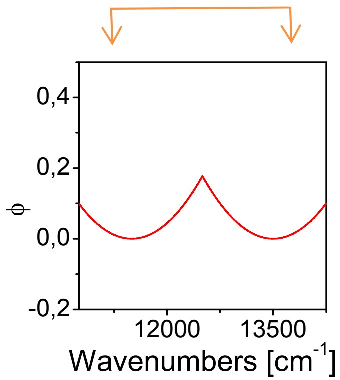
$\Delta\omega$=900 cm^{-1}

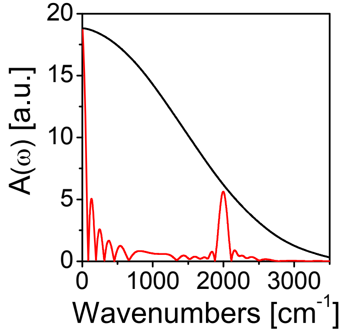




GDD=1000 fs^2

$\Delta\omega$=2000 cm^{-1}





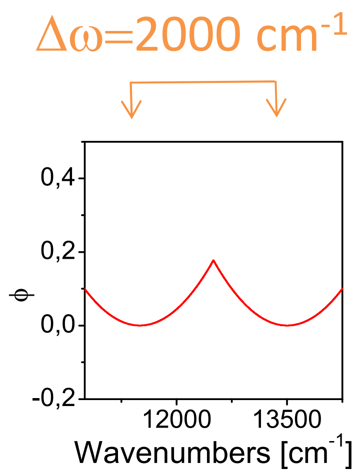
The distance between the central wavenumbers determines the excited mode

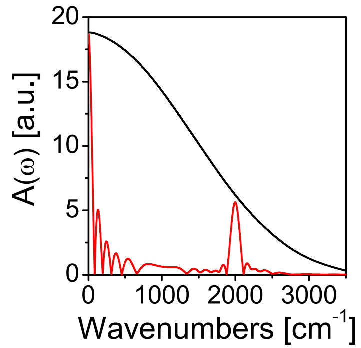
Chen et al. J. *Phys. Chem.* **114** (2010) 16871

Control of the excitation

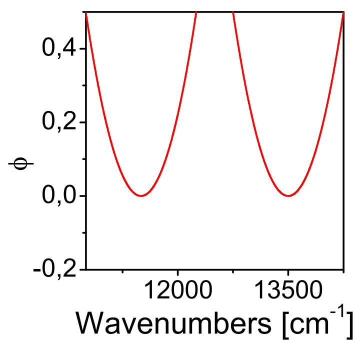
Spectral focusing

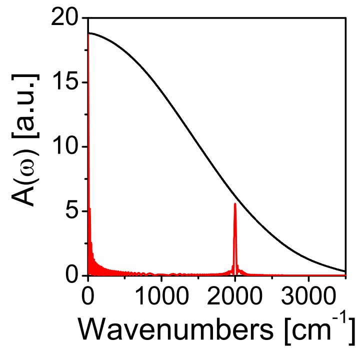
$$\varphi(\omega) = a(\omega-\omega_1)^2\,\theta(\omega-\omega_c) + a(\omega-\omega_2)^2\,\theta(\omega_c-\omega)$$





GDD=1000 fs^2





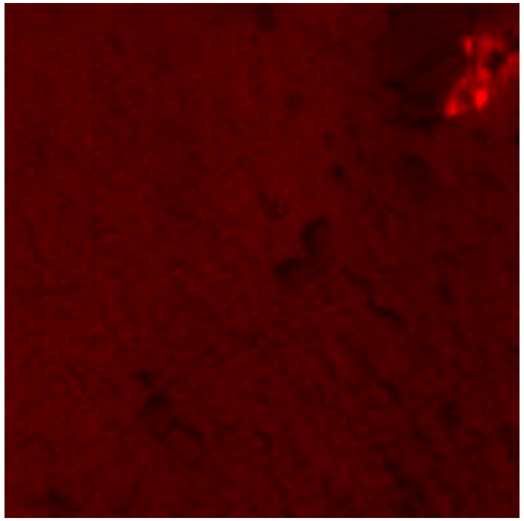
GDD=5000 fs^2

Amount of GDD determines the precision of the selection

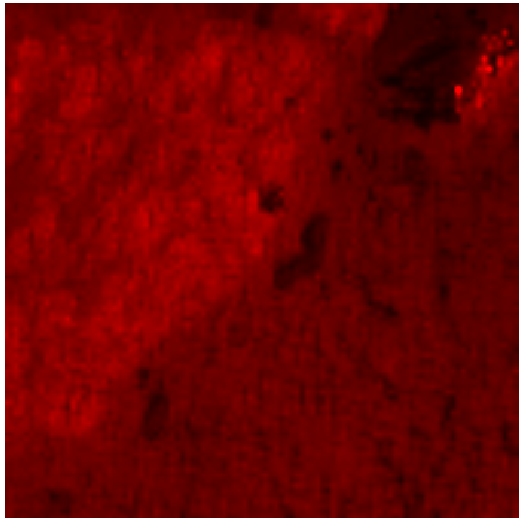
Chen et al. J. *Phys. Chem.* **114** (2010) 16871

Spectral focusing: summary

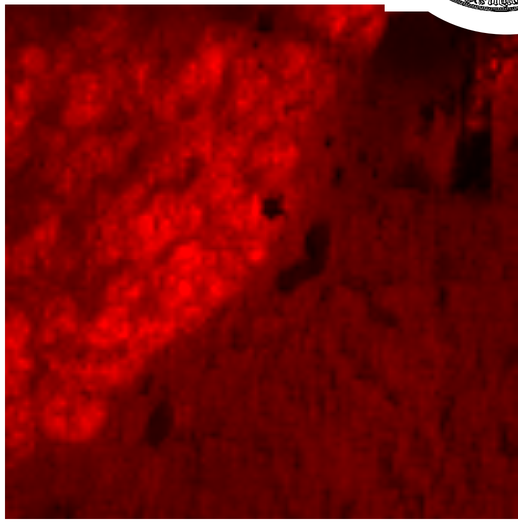
Transform limited



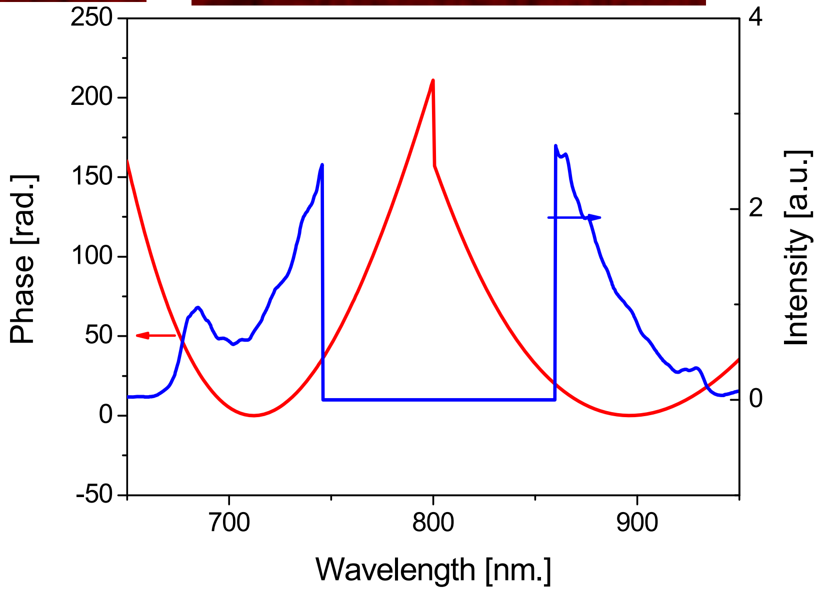
off resonance



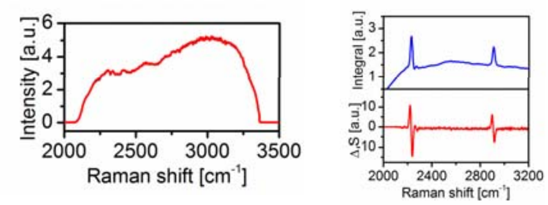
on resonance



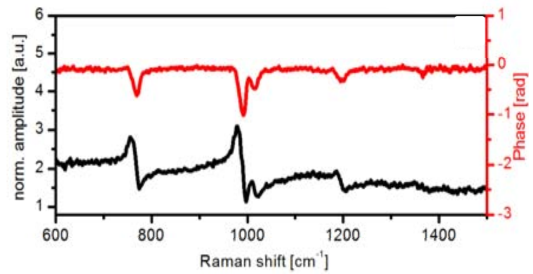
Single-frequency CARS with a Single-beam setup



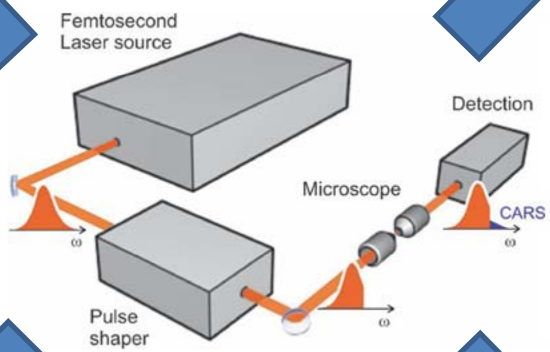
Single-beam CARS + Shaper

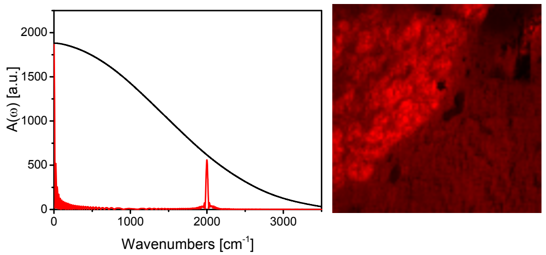


Compensation of 2PEF

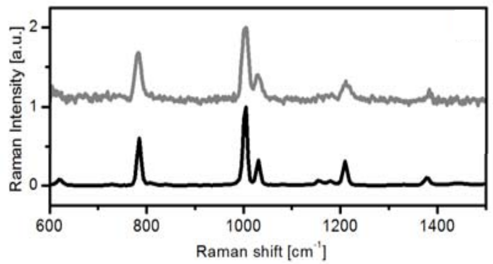


Multiplex-CARS





Selective imaging with CARS



Spontaneous Raman

Acknowledgement: BMBF MediCARS

Conclusions

- Hyperspectral data processing using PCA
  - Highly-sensitive chemo selective imaging in MCARS microscopy
  - No a priori knowledge required
  - Manual weighting of data, e.g. the designation of “pure spectra”
  - High sensitivity of the PCA method better than the experimental conditions

J. Biomed. Opt. ***16*** *(2011) 021105*

Biomedical Opt. Exp. ***2*** *(2011) 2110*

- Shaper-assisted Multiplex CARS
  - Tailored Stokes excitation for single-channel chemical mapping
  - Highly flexibel setup: From multi-pump to broadband multiplex CARS
  - Reduced photon-load

Optics Letters ***35*** *(2010) 22*

- Broadband single-beam CARS
  - Full Multiplex CARS spectrum (>3000 cm^{-1}) using one laser + shaper
  - CARS + Shaping => Spontaneous Raman,

Appl. Phys. Lett. ***100*** *(2012) 071102*
Opt. Lett. (2012) online