Outline

I. Coherent Control
   - Concepts of Coherent Control
   - Learning Loop: Pulse shaping, algorithms
   - Applications:
     - Control of 2-Photon-Absorption
     - Control of energy transfer

II. Single beam CARS
   - Nonlinear Raman spectroscopy
   - Shaped CARS
   - Multimodal microscopy
Light Scattering: Rayleigh / Raman
Raman spectroscopy

Important Raman spectral regions

Fingerprint-Region
Double
Triple
H-stretch

Highly delocalized modes

Sigma-Aldrich Database
Coherent Anti-Stokes Raman Scattering (CARS)

- Low scattering cross-section
- Fluorescence background
- Susceptibility $|\chi^{(3)}|^2$: Chemical selectivity
- Intensity $I^3$: fs-pulses, Signal only from focus $\rightarrow$ 3D-imaging
- Concentration $N^2$: Detection of majority species

$E_{\text{CARS}} = N \cdot \chi_{\text{CARS}}^{(3)} \cdot E_p \cdot E_s \cdot E_p$ 

$I_{\text{CARS}} \propto N^2 \cdot |\chi_{\text{CARS}}^{(3)}|^2 \cdot I_p \cdot I_s \cdot I_p'$
The nonlinear susceptibility $\chi^{(3)}$

Example for $\chi^{(3)}$

$$\chi^{(3)} \sim N \cdot \sum_{a,b} \left( \frac{\partial \sigma}{\partial \Omega} \right)_{ab} \frac{\left( \rho_{aa}^{(0)} - \rho_{bb}^{(0)} \right)}{\left( \omega_{ba} - \omega_p + \omega_S - i \gamma_{ba} \right)}$$

- area $\Rightarrow$ concentration
- position $\Rightarrow$ identification
- envelope $\Rightarrow$ temperature
CARS-Spectra of neat Polymers

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

Time-resolved CARS

- **Energy scheme**
  - Time delay $\tau$
  - Pump $P_P$
  - Stokes $P_S$
  - Four-wave-mixing signal

- **Pulse sequence**
  - Pump + Stokes
  - Probe
  - Time $\tau$

- **FWM transient**
  - $1/\tau_w$
  - $T_2$

**Equations:**
- Four-wave-mixing signal
  \[ I_{FWM} \sim \int dt \left| P^{(3)}(t) \right|^2 \]
- Third order nonlinear polarization
  \[ P^{(3)}_\tau(t) = E_P(t) \cdot \int_{-\infty}^{+\infty} E_L(t-\tau) E_S^*(t-\tau) \cdot R(t) dt \]
Time-resolved CARS

β-Carotene

Transient FFT spectrum

C=C
Major applications of CARS - today:

• Combustion
  → Temperature/concentration profiles in flames/engines

• Ultrafast Spectroscopy
  → Time-resolved changes of molecular structures

• Nonlinear Microscopy
  → Fast chemical imaging of bio/medical samples
Multiphoton Microscopy

2PFE

THG

CARS

\[ I_{\text{Signal}} \propto I_{\text{Exc}}^n \rightarrow 3D \text{ resolution} \]

\[ \rightarrow \text{Use ultrashort (fs) pulses:} \]

High peak intensity while low average power

Broad bandwidth for versatile excitation


Microscopic Chemical Imaging

- Scanning the laser focus
CARS 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
CARS-Spectra of neat Polymers

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

Poly(ethylene)

Poly(styrene)

Poly(methylmethacrylate)

Poly(ethyleneterephthalate)

CARS Microscopic Chemical Imaging

Ternary Polymer blend concentration map:

Lateral image of microscopic phase separation morphology

CARS Technological Challenges

+ Benchmark setup in literature
  - Detection of a single resonance: slow, problems with contrast in complex samples
  - Synchronization difficult

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

Multiplex CARS (MCARS)

Syncronized ps- and fs-laser:

- ps-Laser ($\omega_p, \omega_p'$) determines spectral resolution
- Broadband fs-Laser ($\omega_s$) for spectral coverage

- Rapid spectral acquisition
- Complex samples
- Synchronization
MCARS with only One Laser

Multiplex CARS (MCARS)

Syncronized ps- and fs-laser:

One laser broadband MCARS\(^{[1-3]}\):

Multiplex CARS

- Narrowband Pump (< 3 nm, better than 60 cm\(^{-1}\) spectral resolution)
- Broadband Stokes (> 300 nm, coverage up to 3500 cm\(^{-1}\))

Mouse brain tissue

HE stained samples

CARS microscopy

PCA

intensity at 2845 cm$^{-1}$

(a)

*b * *

100x100 μm

(b)

* * *

CARS microscopy

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

Samples: A. Pagenstecher Marburg
Fast tissue imaging with CARS: Mouse brain

Quantitative backward calculation of the sample components

Quantitative fitting \(\rightarrow\) Improved contrast \(\rightarrow\) CARS provides same information as HE stained reference !!

Samples: A. Pagenstecher Marburg

Biomedical Opt. Exp. 2 (2011) 2110
Simplify CARS even further…

One laser broadband MCARS:

Single-beam CARS\([1-3]\):

Nonlinear microscopy with shaped pulses

Single-beam CARS

- CARS with a single beam of fs-pulses
- Spectral width $\Delta \omega > \Omega_R$

$\Delta \omega = f / \Delta \tau_{\text{pulse}}$

Spectral width and pulse duration are directly related
Single-beam CARS: Need for short pulses

Important Raman spectral regions

- Fingerprint-Region
- Double
- Triple
- H-stretch

Relative Raman Intensity

Wavenumber / cm\(^{-1}\)

Highly delocalized modes

Sigma-Aldrich Database
All spectral components are provided by a single laser pulse
Control strategies

Tannor-Kosloff-Rice
JCP 85, 5805 (1986)

Brumer-Shapiro
CPL 126, 54 (1986)

Potential Energy

time delay: $\Delta t$

phase difference: $\Delta \phi = \phi_\omega - \phi_{3\omega}$
Control of Raman transitions

Two coherent Raman excitations → interfering pathways (like double slit)
Control of Raman transitions

Broadband spectrum, many colors
→ Many interfering pathways
Effect of phase shaping on CARS

CARS Signal:

\[ E_{CARS}(\omega) \propto \int E_{pr}(\omega - \Omega) \chi^{(3)}(\Omega) \int E^*_S(\omega' - \Omega) E_p(\omega') d\omega d\Omega \]

\[ E_{CARS}(\omega) \propto \int \left| E_{pr}(\omega - \Omega) \right| \left| E_S(\omega' - \Omega) \right| \left| E_p(\omega') \right| \chi^{(3)}(\Omega). \]

\[ \times \exp(i(\varphi(\omega') - \varphi(\omega' - \Omega) + \varphi(\omega - \Omega))) d\omega' d\Omega \]

Dependence on the phase
Modulation of phase:
time vs. frequency domain

- Transform limited pulse, no spectral discrimination

- Sine phase with period $\Omega_m$ creates subpulses spaced in time $\tau_m = \frac{2\pi}{\Omega_m}$
**Single-beam CARS with multipulses**

**Simple Femtosecond Pulse**

- $\nu_1$
- $\nu_2$
- $\nu_3$

**Femtosecond Pulse-Train**

- $\tilde{\nu}_1$
- $\tilde{\nu}_2$
- $\tilde{\nu}_3$

**Blurred CARS spectrum**

**Selective CARS excitation**

$\tau_m$
Single-beam CARS with Multipulses

Acetonitrile vibrational spectrum with < 30 cm⁻¹ resolution

Oscillations encode molecular vibrations
Principles of pulse shaping

Parameterization of excitation mechanism

Chirped

Impulsive

Phase-locked

Single-beam-CARS schemes

- Frequency domain
  - $\phi(\omega)$
  - $E_1, E_2, E_3, \ldots$
  - $\tau_m$

- Time domain
  - $t$

Step (c): $\phi(\omega) = \omega \tau + \sin(\omega)$
Truly time-resolved Single-beam CARS

**Multipulses**

- $E_1$, $E_2$, $E_3$, ... with indistinguishable roles: Pump, Stokes, probe
- Only one octave of wavenumbers

**Two-color double pulses**

- Defined roles: **Pump + Stokes** ($E_1$) and **probe** ($E_2$)

"Pump/Probe"-scheme with shaping from a single beam
Truly time-resolved Single-beam CARS

- Unambiguous data analysis, arbitrary wavenumber range
Raman Control of a Binary Mixture

- Combine **multipulse** sequence for selective excitation with **time-delayed probe pulse**
- Raman quantum control of molecular vibration!

Coherent Anti-Stokes Raman Scattering (CARS)

Dependencies of the signal at square law detection:

- **Susceptibility** $|\chi^{(3)}|^2$: Chemical selectivity
- **Intensity** $I^3$: Signal only from the focus, 3D-imaging
- **Concentration** $N^2$: Detection of majority species

Sensitivity?
CARS Microscopy

**Coherent Anti-Stokes Raman Scattering**

\[ E_{\text{CARS}} = N \cdot \chi_{\text{CARS}}^{(3)} \cdot E_p \cdot E_s \cdot E_{p'} \]

\[ I_{\text{CARS}} \propto N^2 \cdot |\chi_{\text{CARS}}^{(3)}|^2 \cdot |E_p| \cdot |E_s| \cdot |E_{p'}| \]

**CARS-Field: Coherent sum**

\[ E_{\text{CARS}} = \sum_{N} E_{\text{Mol, N}} \]

Detect Field: Linear in N!

\[ I_{\text{CARS}} \propto \left| \sum_{N} E_{\text{Mol, N}} \right|^2 \]
Interferometric / Heterodyne CARS

Interferometric Field detection - Mix CARS-Signal with Local oscillator:

\[ I_{\text{Det}} \propto \left| E_{\text{CARS}} + E_{\text{LO}} \right|^2 \propto I_{\text{CARS}} + I_{\text{LO}} + 2 \sqrt{I_{\text{LO}} I_{\text{CARS}}} \cdot \cos \Delta \phi_{\text{LO}} \]

- \( S^{(\text{Het})} \) scales linearly with \( N \): **Linearization**
- \( S^{(\text{Het})} \) is proportional to the square root of \( I_{\text{LO}} \): **Amplification**
- \( S^{(\text{Het})} \) is sensitive to \( \Delta \phi_{\text{LO}} \)
The **LO** is created from the blue spectral part (ND)

The **excitation part** of the spectrum is chopped for Lock-In detection

Results: Proof of Heterodyne Detection

Theory: \[ S^{(Het)} = 2 \sqrt{|I_{LO}|_{CARS}} \cos \Delta \phi_{LO} \]

Phase dependence: \( \Delta \phi_{LO} \) controlled by SLM

Intensity dependence

slope = 0.51 ± 0.01
Application to Microfluidic Detection

- Use CARS as detection scheme in a 100 μm capillary
- Further simplification: compact fiber laser

Pulse shaping for CARS control: Spectral focussing
Single-beam spectral focusing: time picture

\[ A(\Omega) = \int_{0}^{\infty} |E^*(\omega')| |E(\Omega + \omega') \exp(i \Delta \phi) \, d\omega' \]

\( \Delta \omega \)

\( \omega_{\text{Pump}} \)

\( \omega_{\text{Stokes}} \)

\( \rightarrow \) unspecific excitation!
Single-beam spectral focusing: time picture

\[ \Delta \omega \]

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

\[ \Delta \omega \rightarrow \text{increased specificity for imaging} \]
\[ \Delta \omega \rightarrow \text{decreased multiphoton photodamage} \]

Single-Beam fs-pulse shaping: Spectral Focusing

Focusing on transitions by controlling the excitation!

→ well suited for imaging
→ usually CH-stretching vibration \( \Delta \omega = 2845 \text{ cm}^{-1} \)
→ chemical map of lipid distribution

Contrast & increased signal

Transform-limited
- Concentration differences determine signal

Spectral Focusing
- Vibrational contrast achieved

Skin samples kindly provided by Prof. Schäkel from the department of dermatology at the Heidelberg University hospital

Time-delay Scan

**Concept**

![Diagram of Time-delay Scan concept](image1)

- **Probe delay scan**
  - Coherence build-up
  - Dephasing
  - Acetonitrile

**Time-dependent spectra**

![Graph of time-dependent spectra](image2)

- Suppression of fast decaying NRB
- Contrast based on coherence times

*JOSA B 33 (2016) 1482*
Single-beam-CARS schemes

**Multiplexing**

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

**b-scan**

*PCCP 10 (2008) 681*

**Spectral focusing**

Lim Group.

Review:

*Silberberg Annu. Rev. Phys. Chem. 79 (2009) 2009.60*
Multiplex CARS: Narrowband probing

Excitation scheme

\[ S(\omega) \propto |E_{\text{CARS},b}(\omega) + E_{\text{CARS},n}(\omega)|^2 = |E_{\text{CARS},b}(\omega)|^2 + |E_{\text{CARS},n}(\omega)|^2 + 2|E_{\text{CARS},b}(\omega)E_{\text{CARS},n}(\omega)|\cos\phi \]

→ Broadband probe provides a *Local oscillator*

Appl. Phys. Lett. 100 (2012) 071102
Multiplexing single-beam-CARS

\[ S(\omega) \propto |E_{CARS,b}(\omega) + E_{CARS,n}(\omega)|^2 = |E_{CARS,b}(\omega)|^2 + |E_{CARS,n}(\omega)|^2 + 2|E_{CARS,b}(\omega)E_{CARS,n}(\omega)|\cos \phi \]

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

DQSI:
Double quadrature spectral interferometry


Further modalities:
Heterodyne Multiplex CARS using phase gate

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 gives spontaneous Raman spectrum!
Single-beam-CARS and two-photon fluorescence
Measurements on acetonitrile and DCM

Increasing concentration of DCM decreases effectiveness of the DQSI operation.

Strong 2PEF decreases effectiveness of the DQSI operation.
Phase-dependence of the 2PEF

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

→ DQSI signal is overlaid by 2PEF
CARS and 2PEF

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

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

*J. Raman Spec. 44 (2013) 1379*
Outlook: Multimodal microscopy with shaped pulses

Contrast mechanisms: Nonlinear microscopy

Collagen

Fluorophores

Lipids

Opt. Express. 22 (2014) 28790
Simultaneous multimodal imaging

CH-resonance (lipids)  TPEF  SHG

Transform-limited probing region
- Highly increased multimodal signal
- Simultaneous acquisition together with resonant CARS

JOSA B **33** (2016) 1482
Multimodal Quantum Control Spectroscopy

Spontaneous Raman

Multiplex-CARS

Compensation of 2PEF

Selective multimodal imaging

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Single-beam CARS + Shaper

Compensation of 2PEF
*J. Raman Spec. 44* (2013) 1379

Selective imaging with CARS
*JOSA B 33* (2016) 1482

In addition:
- 2P Fluorescence
- SHG
- THG

Multiplex-CARS
*Appl. Phys. Lett.* 100 (2012) 071102

Spontaneous Raman