Coherent anti-Stokes Raman scattering (CARS) spectroscopy and imaging of biological molecules on graphene layers: advantages and disadvantages.

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OUTLINE

• CARS (Coherent anti-Stokes Raman Scattering ) method and technique
• CARS of carbon nanostructures
• CARS of biological molecules
• SECARS (Surface Enhanced Coherent anti-Stokes Raman Scattering and GECARS (Graphene Enhanced Coherent anti-Stokes Raman Scattering )
• Probable mechanisms of GECARS
• Advantages and disadvantages of CARS and GECARS
• CARS and GECARS: imaging and perspectives
The main question - could graphene replace a metallic substrate in Surface Enhanced Spectroscopy – *in special cases* - yes, *in general* - no.
What is surface enhanced spectroscopy

• Any spectroscopic technique, in which for increase of output signal, a substrate of metal or non-metal type (e.g. graphene) is used, could be named as surface enhanced spectroscopy (SES).

• Types of substrates for SES - metal particles, rough metal surface, periodic metal surfaces, semi-conducting surfaces or particles, quantum dots, graphene, etc.
The main goal of our work-

1. to get enhancement of optical signals in SERS, CARS from molecules adsorbed on the graphene layers and carbon nanotubes

2. to create new SERS platforms on the basis of carbon-like nanostructures and gold particles

3. SERS or GERS (Graphene Enhanced Raman Scattering)

4. GECARS (Graphene Enhanced Coherent anti- Stokes Raman Scattering) or CARS (Coherent anti- Stokes Raman Scattering)
Basis for enhancement of optical signals from molecules near the surface

The basis is an enhancement of optical signals from molecules and/or cells adsorbed on the nanostructured metal surface. The first conditions for optical signal enhancement on the border of 2 medium (1 – molecule or cell, 2 - nanoparticle) is the followings:

\[ \varepsilon_2' \left( \bar{\omega} \right) < 0, \quad \varepsilon_1' \left( \bar{\omega} \right) < 0 \quad \text{for} \quad \bar{\omega}_1 < \bar{\omega} < \bar{\omega}_2, \quad \frac{\left| \varepsilon_2' \left( \bar{\omega} \right) \right|}{\varepsilon_2'' \left( \bar{\omega} \right)} >> 1 \]

The second condition is an increase the polarizability of the molecules (cell components) induced by nanoparticles or surface.

1. V.Kosobukin, Collective phenomena in enhancement of electrical field near metal surface, Seria fizicheskaya,. 1985, 49(6): 1111–1120
History of selected CARS publications

2. И. Ребане, В. Хижняков Интерференция резонансного и нерезонансного рассеяния света в примесных кристаллах. Известия академии наук Эстонской ССР, Том 29, Физика Математика, 1980, №2, 165-173
Raman, SERS, CARS та SECARS

Raman → SERS

CARS → SECARS – Surface Enhanced Coherent Anti-Stokes Raman scattering
AFM - images of gold surface of home-made SEIRA substrate and Klarite SERS substrate
Dependence of enhancement factor on the type of metal.
Factors which influences the enhancement in SES spectroscopy

- Type and morphology of metal or dielectric substrate, position of plasmon or phonon resonances or “size-structure” resonance

- Geometry of the experiment

- Type of interaction between molecules and substrates, composition of the adsorbed molecules
Method SERS, CARS could be efficiently used:

- for determination of type of molecules and impurity in the probe of extremely small amount
- for analysis of conformation forms of biological macromolecules and their interactions
- for enhanced and contrast imaging of cells in vivo and in vitro
CARS method

CARS (coherent anti-Stokes Raman scattering) – CARS phenomenon is based on nonlinear interaction of two incoming optical fields $\omega_p$ (pump) and $\omega_S$ (Stokes) of material, which results in generation of the blue-shifted anti-Stokes light with frequency $\omega_{AS}=2\omega_p-\omega_S$. The $\omega_{AS}$ field takes place when the frequency difference $2\omega_p-\omega_S$ coincides with the frequency of molecular vibrations of the studied material. Thus, tuning $\omega_p$ while keeping $\omega_S$ constant and detecting anti-Stokes light intensity we could obtain CARS spectra containing information about the vibrational spectrum of the material.
The experimental setup is based on a home-made CARS microscope with compact laser source (EKSPLA Ltd.). The laser consists of a picosecond (6 ps) frequency doubled Nd:YVO4 pump laser with optical parametric generator (OPG) with turning range from 690 to 2300 nm. For CARS implementation, the OPG radiation was coupled with a fundamental laser radiation (1064 nm) used as pump and Stokes excitation beams respectively. Such mixing provides probing within the 700 – 4500 cm\(^{-1}\) range of vibration frequencies. Both Stokes and pump beams were collinearly combined and directed to an inverted microscope (Olympus IX71). A spatial filter was used to improve the beam profile before directing into the microscope. The excitation light was focused on the sample with an oil-immersion objective (Olympus, Plan Apochrom., 60X, NA 1.42). In the forward detection scheme the CARS-light was collected by another objective with NA 0.4. Long-pass and short-pass filters were used as a blocking tool for spectral separation of the CARS signal. CARS radiation was detected using the avalanche photodiode (SPCM-AQRH-14, Perkin Elmer) connected to a multifunctional board PCI 7833R (National Instruments Ltd).
## Operating CARS frequencies

<table>
<thead>
<tr>
<th>CARS registration range, cm⁻¹</th>
<th>“Stokes”, nm</th>
<th>“Pump”, nm</th>
<th>Anti-Stokes (or CARS), nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1200-1700</td>
<td>1064</td>
<td>940-900</td>
<td>850-780</td>
</tr>
<tr>
<td>2500-3500</td>
<td>1064</td>
<td>840-775</td>
<td>690-610</td>
</tr>
</tbody>
</table>
CARS application

- Multiphoton imaging;
- CARS spectroscopy;
- Monitoring of temperature of hot gases and flame.
CARS imaging of cells and their components

(Macromolecular profiling of apoptosis via a multiplex biophotonic platform  Aliaksandr V. Kachynski and Paras N. Prasad)
Surface enhanced CARS (SECARS)

Surface Enhanced Coherent Anti-Stokes Raman Scattering on Nanostructured Gold Surfaces

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Enhancement ~ $10^5$
GECARS- graphene enhanced coherent anti-Stokes Raman Scattering

GECARS spectrum of Thy/GO, Thy/MWCNT seems to be similar to SECARS and it could be named as graphene enhanced CARS (GECARS) analogously to graphene-enhanced Raman scattering (GERS) technique in which graphene can be used as a substrate for SERS of adsorbed molecules [Xu W., Mao N., Zhang J: Graphene: A Platform for Surface-Enhanced Raman Spectroscopy. Nano Micro Small 8 2013, 9: 1206–1224].

CARS of nanocarbons- graphite, MWCNT, GNP, graphene flakes, graphene layers
CARS and SEM Imaging of Graphene Nanoplatelets

[Images of CARS and SEM images of graphene nanoplatelets]
Microscopic imaging of carbon nanotubes
Graphene oxide
Microscopic imaging of carbon nanotubes

SEM

CARS

TEM
Raman spectrum of HOPG (1) and monolayer graphene on Cu (3) at $\lambda_{ex}=633$ nm; CARS spectrum of HOPG(2)
CARS and Raman spectra of grapnene nanoplatelets (GNP-1) and graphene oxide (GO-2)

D-mode

G-mode

CARS signal, 10^3 CPS

Raman shift, cm\(^{-1}\)

graphene oxide
Raman (1) and CARS (2) at $\lambda_{\text{ex}}=785$ nm spectra of MWCNT
CARS and Raman spectra of graphene nanoplatelets (GNP)
<table>
<thead>
<tr>
<th>Assignment</th>
<th>GNP, cm⁻¹</th>
<th>GO, cm⁻¹</th>
<th>MWCNT, cm⁻¹</th>
<th>HOPG, cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-mode</td>
<td>1307</td>
<td>1312</td>
<td>1314</td>
<td>Not detected</td>
</tr>
<tr>
<td>G-mode</td>
<td>1582</td>
<td>1595</td>
<td>1589</td>
<td>1580</td>
</tr>
<tr>
<td>D′</td>
<td>1605</td>
<td>Not detected</td>
<td>1611</td>
<td>Not detected</td>
</tr>
<tr>
<td>G′ mode (2D)</td>
<td>2595</td>
<td>2616</td>
<td>2615</td>
<td>2684</td>
</tr>
<tr>
<td>D+ D′ (or D+G)</td>
<td>2902</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
</tbody>
</table>
### CARS bands of the different carbon materials

<table>
<thead>
<tr>
<th>Assignment</th>
<th>GNP, cm(^{-1})</th>
<th>GO, cm(^{-1})</th>
<th>MWCNT, cm(^{-1})</th>
<th>HOPG, cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>1300</td>
<td>1306</td>
<td>1310</td>
<td>Not detected</td>
</tr>
<tr>
<td>new band</td>
<td>Not detected</td>
<td>1419</td>
<td>1421</td>
<td>Not detected</td>
</tr>
<tr>
<td>new band</td>
<td>1500</td>
<td>1516</td>
<td>1527</td>
<td>Not detected</td>
</tr>
<tr>
<td>G</td>
<td>1555</td>
<td>1584</td>
<td>1590</td>
<td>1587</td>
</tr>
<tr>
<td>D’</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not measured</td>
<td>Not measured</td>
</tr>
<tr>
<td>2D (G’)</td>
<td>Not detected</td>
<td>Not measured</td>
<td>Not measured</td>
<td>Not measured</td>
</tr>
<tr>
<td>D+D(_1)</td>
<td>2460</td>
<td>Not measured</td>
<td>Not measured</td>
<td>Not measured</td>
</tr>
<tr>
<td>2G(_{\text{CARS}})</td>
<td>2960</td>
<td>Not measured</td>
<td>Not measured</td>
<td>Not measured</td>
</tr>
</tbody>
</table>
I a) The laser photon excites an electron with wave vector $k_e$ in the conduction zone and a hole with the wave vector $k_h$ in the valence zone (the wave vectors are calculated in point Γ, transition 1-2). This process could be named resonant Raman in contrast to non-resonant Raman, where the virtual states of the electron and the hole are excited.

b) The electron comes to the other equivalent well and emits a phonon of i-TO branch of the dispersion curve (transition 2→3).

c) The electron returns to the state with approximately the same wave vector ($\approx k_e$) but its energy decreased by the energy of emitted phonon. As result another phonon is emitted (transition 3→4).

d) The electron recombines with the hole (transition 4→1) and emits the Stocks Raman photon. Such process leads to a double electron-phonon resonance.

II Transitions occur simultaneously with the electron in the conductance zone (electron goes through the states 1-2-3-4-1). The hole goes through equivalent states in the valence zone.

III The electron goes through the states 1-2, 2-3 and transfers to the second well. The hole goes through the states in the valence zone simultaneously. Recombination of the electron and the hole occurs afterwards.

The transition 3→4 for D-band, which involves the structural defects, is a phonon-less. The sequence of transitions for D' and 2D' bands is the same, but the processes occur within one well.

In the case of G-band a laser photon excites an electron with the wave vector $k_e$ in the conductance zone and a hole with the wave vector $k_h$ in the valence zone (the value are calculated in point Γ). The electron emits a phonon with the wave vector $q=0$ and recombines with a hole.
CARS of thymine on graphene oxide and MWCNT
CARS spectra of thymine and thymine-graphene oxide

![CARS spectra of thymine and thymine-graphene oxide](image)

C₅H₆N₂O₂
CARS imaging of thymine and thymine-graphene oxide
CARS spectra of thymine and thymine - MWCNT

![CARS spectra graph]

- 1- thymine
- 2- thymine+MWCNT

CARS signal, $10^3$ CPS

Raman shift, cm$^{-1}$
CARS imaging of thymine and thymine on carbon nanotubes

Thymine-nanotubes

Thymine

1355 cm⁻¹

20 μm

1355 cm⁻¹

20 μm
One, double graphene layers in CARS
One graphene layer with thymine

Graphene Thy 1 layer

CARS signal, $10^3$ CPS

wavenumber, cm$^{-1}$

Graphene Thy 1 layer

CARS signal, $10^3$ CPS

wavenumber, cm$^{-1}$

One graphene layer with thymine

$3.0 \, \mu m$
Enhancement in CARS

• To determine the enhancement factor of the CARS signal for Thy/GO complex relative to Thy, the filling factor and the conditions of the CARS experiment should be evaluated.

• In CARS experiments, the radiation outcomes from the space volume of \(\sim 1 \,\mu m^3\). Such volume can contain \(\sim 10^9\) molecules of Thy (without graphene). When GO is added to Thy, in accord with our estimation, the number of Thy molecules within the mentioned volume is \(\sim 10^8\).

• Taking in account these assumptions and the difference between the intensity of the CARS signal for Thy/GO complex and Thy (\(\sim 10^4\)), we could obtain the CARS enhancement factor equal to \(\sim \cdot 10^5\). The enhancement obviously arises from those molecules of Thy which are in close proximity to the surface of GO. The number of such Thy molecules is really lower than the whole number of the molecules in the volume.
Mechanism of CARS Enhancement

The enhancement effect could have several reasons:

a) the resonant interaction of exciting light with electronic states of the carbon nanostructures;

b) the so-called chemical mechanism, which involves charge transfer between the molecule and the carbon nanostructure, as well as the increase of the dipole moment in the molecule;

c) the increase the local electromagnetic field at the edges of graphene nanosheets and MWCNT
Possible mechanism of GERS effect

- Local field enhancement in the near-field zone of the finite-length metallic SWCNTs.


Possible GERS mechanism (HOMO-LUMO)- literature data

\[ h\omega_0 = \text{LUMO} - \text{HOMO} \quad \text{OR} \]
\[ h\omega_0 = \text{LUMO} - \text{HOMO} + h\omega_q \]

\[ E_F = \text{HOMO} \pm h\omega_q \quad \text{OR} \quad E_F = \text{LUMO} \pm h\omega_q \]

\[ h\omega_0 = E_F - \text{HOMO} \quad \text{OR} \quad h\omega_0 = E_F - \text{HOMO} + h\omega_q \]

\[ h\omega_0 = \text{LUMO} - E_F \quad \text{OR} \quad h\omega_0 = \text{LUMO} - E_F - h\omega_q \]

in which \( h\omega_q \) is the incident photon energy, \( h\omega_q \) is the phonon energy, and \( E_F \) is the graphene Fermi level.

Figure 3. Influence of the molecular structure on GERS. (a) The first row shows the molecular structures of TCTA, TTP, and sp2-NPB molecules from left to right with labels above their corresponding Raman spectra with their symmetries labeled above. The second row shows the Dirac cone of graphene, the HOMO/LUMO energy levels of TCTA, TTP, and sp2-NPB. (b−d) Raman spectra of 5 Å (b) TTP, (c) TCTA, and (d) sp2-NPB, on graphene (red line) and on a blank SiO\(_2\)/Si substrate (black line) with the excitation laser wavelengths of 633 nm. The "*" marked peaks in (b−d) are the G-band from graphene. Other peaks marked by the numbers come from the corresponding molecules.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Structure</th>
<th>Symm.</th>
<th>HOMO/LUMO (eV)</th>
<th>$\Delta E_{HF}/\Delta E_{LF}$ (eV)</th>
<th>$d_0$ (Å)</th>
<th>$E_{em}$ (eV/atom)</th>
<th>$\alpha_{ph}$ (cm$^{-1}$)</th>
<th>EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuPc$^{[39]}$</td>
<td>D$_{4h}$</td>
<td>-5.2/-3.5</td>
<td>-0.6/1.1</td>
<td>3.24</td>
<td>-0.06</td>
<td>1048 (532)$^a$&lt;br&gt;1538&lt;br&gt;1451</td>
<td>37.5&lt;br&gt;11.6&lt;br&gt;24.3</td>
<td>1530 (633)&lt;br&gt;1451</td>
</tr>
<tr>
<td>ZnPc$^{[30]}$</td>
<td>D$_{4h}$</td>
<td>-5.2/-3.8</td>
<td>-0.6/0.8</td>
<td>3.22</td>
<td>-0.06</td>
<td>1448 (532)&lt;br&gt;1508</td>
<td>4.1&lt;br&gt;11.9</td>
<td>1508 (633)&lt;br&gt;1448</td>
</tr>
<tr>
<td>F$_4$CuPd$^{[31]}$</td>
<td>D$_{4h}$</td>
<td>-6.3/-4.8</td>
<td>-1.7/-0.2</td>
<td>3.19</td>
<td>-0.07</td>
<td>1540 (532)&lt;br&gt;1461</td>
<td>4.2&lt;br&gt;2.7</td>
<td>1540 (633)&lt;br&gt;1461</td>
</tr>
<tr>
<td>PTCDA$^{[36]}$</td>
<td>D$_{2h}$</td>
<td>-6.8/-4.7</td>
<td>-2.2/-0.1</td>
<td>3.17</td>
<td>-0.07</td>
<td>1532 (532)&lt;br&gt;1455</td>
<td>5.8&lt;br&gt;3.9</td>
<td>1531 (633)&lt;br&gt;1454</td>
</tr>
<tr>
<td>TTP$^{[38]}$</td>
<td>D$_{2h}$</td>
<td>-5.6/-2.3</td>
<td>-1.0/2.3</td>
<td>3.24</td>
<td>-0.07</td>
<td>1450 (633)</td>
<td>23.3</td>
<td>1449 (633)</td>
</tr>
<tr>
<td>TCTA$^{[39]}$</td>
<td>C$_3$</td>
<td>-5.7/-2.4</td>
<td>-1.1/2.2</td>
<td>4.34</td>
<td>-0.04</td>
<td>1340 (532)&lt;br&gt;1455</td>
<td>1.3&lt;br&gt;0.9</td>
<td>1449 (633)</td>
</tr>
<tr>
<td>Sp2-NPB$^{[40]}$</td>
<td>S$_d$</td>
<td>-5.5/-2.4</td>
<td>-0.9/2.2</td>
<td>4.36</td>
<td>-0.04</td>
<td>1503 (532)&lt;br&gt;1450</td>
<td>5.3&lt;br&gt;1.7</td>
<td>1447 (633)</td>
</tr>
<tr>
<td>Sp2-TPD$^{[42]}$</td>
<td>S$_d$</td>
<td>-5.5/-2.3</td>
<td>-0.9/2.3</td>
<td>4.27</td>
<td>-0.03</td>
<td>1450 (532)&lt;br&gt;1290</td>
<td>0.9&lt;br&gt;2.3</td>
<td>1446 (633)</td>
</tr>
</tbody>
</table>
Advantages and disadvantages of surface enhanced spectroscopy:

Advantages:

- Enhancement of intensity of some bands sometimes 10-100 (SEIRA) and up to $10^{10}$ (SERS);
- Achievement of much-improved observation of the bands attributable to using a gold substrate (for example an application of SEIRA for nucleic acids gives a possibility to reveal additional structural features of tumor nucleic acids in the 1100-600 cm$^{-1}$ region with enhancement factor of 3-5);
- Reflected radiation is preferentially polarized due to experimental geometry;
- Determination of molecular orientation in orientated molecules (SFG);
- It is possible to do in situ study of electrochemical dynamics on metal electrodes. High sensitivity of the method. The lowest detection limit, which could be reached, is 10pg/cm$^2$ (for p-nitrobenzoic acid) in SEIRA and single molecules (DNA) in SERS.
Disadvantages (miss beside 1) in the case of graphene as substrate):

1. Possible decrease of the intensities of the bands or even their absence due to surface selection rules;
2. In some cases, interaction of adsorbed molecules with metal surface could change IR and RS spectra, shift of the bands about 15-20 cm$^{-1}$, widening the bands, intensity decrease;
3. Spectra with great enhancement (10$^{10}$ and more) could be not reproducible, big enhancement lead to fault in spectra recognition; No in the case of GERS.
4. Conformation changes in polymers under interaction with metal nanoparticles.
• Could we create sub-diffraction resolution on the basis of graphene-type structures in CARS?
Conclusions

• CARS is better to use for imaging of the samples, including biological molecules, SERS is better to apply for identification of type of materials, including extremely small amount and single layers.

• CARS and GECARS could be effectively used for spectroscopic study of biologic molecules and their interaction with other molecules and particles, especially in the case of protonation-deprotonation processes.

• CARS (GECARS) has potential as method for sub-diffraction limit.
My co-workers

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- Tommi Kaplas, Yuriy Svirko - Finland
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