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Molecular Order Probed by Polarization Resolved Four Wave Mixing Microscopy

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CARS images (2100 cm$^{-1}$); Multilamellar vesicles, two different preparations
Outline

- Molecular Order
- The contrast FWM/CARS
- Set up
- Applications
- Conclusion/Perspectives
Molecular Order

Example of order studies

Lipid membranes

Molecular Distribution
Molecular Order

Example of order studies

Lipid membranes

Molecular Distribution

Our approach

- No assumption on the sample’s symmetry
- 2D projection

Excitation light propagation direction

One molecule bond axis
Or
One molecule axis

\( f(\phi) \) Angular distribution

\( E \) Excitation field

Excitation light propagation direction
FWM/CARS polarisation responses are characteristic of:

- Molecular distribution orientation
- Molecular distribution symmetry

\[
f_{\text{FWM}} (\phi) = p_0 + p_2 \cos(2\phi) + q_2 \sin(2\phi) + p_4 \cos(4\phi) + q_4 \sin(4\phi)
\]

\( (O_2, \phi_2) \)

\( (O_4, \phi_4) \)
Contrast: CARS/ FWM

\[ P_i^1 = \chi_{ij}^{(1)} E_j \]

\[ P_i^2 = \chi_{ijk}^{(2)} E_j E_k \]

\[ P_i^3 = \chi_{ijkl}^{(3)} E_j E_k E_l \]
**Contrast: CARS/ FWM**

Resonant contribution \( \chi^{(3)}_R \)

- \( |e\rangle \) ———
- \( |v\rangle \) ———
- \( |g\rangle \)

Vibrational origin

Nonresonant contribution \( \chi^{(3)}_{NR} \)

- \( |e\rangle \) ———
- \( |v\rangle \) ———
- \( |g\rangle \)

Electronic response of the medium

\( \omega_p, \omega_v, \omega_s, \omega_{as} \)

\( \Omega_R \)
Polarization-resolved measurements

Typically:
1 image/s for 1 polarization angle
32 a angles (0-180°)
lateral resolution: ~300 nm

Brustlein et al. JBO 2011
From polar stack to orders images

\[ I_{I}^{axis} = \left| \sum_{JKL} \chi_{ijkl}^{(3)} \cdot E_{PJ}^{\omega} (\omega) \cdot E_{PK}^{\omega} (\omega) \cdot E_{SL}^{\omega} (\omega) \right|^2 \]

\[ I_{I}^{\omega as} = \sum A_n \cos(n\alpha) + \sum B_n \sin(n\alpha) \]

Summetry orders of the distribution function \( f(f) \)

\[ \chi_{ijkl}^{(3)} = \int R_{\phi} (\chi_{ijkl}) f(\phi) d\phi \]
Illustration on crystalline system

Pure Cubic System

White light image of NaCl crystals

4th order symmetry expected
The O₂ and O₄ signal are independant of the cristal orientation.
Model biological membrane
Multilamellar vesicles (MLV)
Chain-perdeuterated dipalmitoylphosphatidylcholine (DPPC-d62)

CARS spectrum

C-D
Non resonant contribution
Multilamellar vesicles (MLV-DPPC-d62)

Resonant case (2100 cm\(^{-1}\))

Non resonant case (2300 cm\(^{-1}\))

Orientation of order 2 contribution (\(f_2\))

Contribution of the C-D bond

Expected predominant contribution of the C-C backbone

FZ Bioud et al., in preparation
Multilamellar vesicles (MLV-DPPC-d62)

Resonant case (2100 cm\(^{-1}\))

Non resonant case (2300 cm\(^{-1}\))

Order 2 comparison

\[ <O_{2R}> = 0.26 \]
\[ <O_{2NR}> = 0.13 \]

Order 4 comparison

\[ <O_{4R}> = 0.1 \]
\[ <O_{4NR}> = 0.08 \]

Higher \(O_2\) is a signature of higher order
Multilamellar vesicles (MLV-DPPC-d62) + Cholesterol 5%

The presence of cholesterol (5%) increases the order with a higher $O_2$.

Resonant case (2100 cm$^{-1}$)

- $<O_{2R}> = 0.26$
- $<O_{4R}> = 0.16$
- $<O_{2}> = 0.3$
- $<O_{4}> = 0.13$

Non resonant case (2300 cm$^{-1}$)

- $<O_{2}> = 0.19$
- $<O_{4}> = 0.08$
- $<O_{2}> = 0.1$
- $<O_{4}> = 0.3$
Physical interpretation

O2 is related to the statistical disorder within a given distribution.
O4 brings additional insight into the shape of the distribution.
Conclusion

Tool to probe high symmetries molecular configurations (second and fourth order)

The obtained function is model free, no assumption on the angular distribution of the sample.
Perspectives

Apply the approach to complex media

CARS image at 2845 cm\(^{-1}\); Myelin from spuinal cord

Multimodal imaging

Composite image CARS /SHG/TPEF; Human skin lesion
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Thank you for your attention

Mosaic Team

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Summary

Label free tool for order quantification in MLV with no assumption on angular distribution
How is a 3D distribution projected in the sample plane when it is read-out by a field in (X,Y) in FWM? Ex: a cone...
FWD and FWM

Sample: 41C File: ech1_2D006.itx-PolarIncidente: 0° z=8

A0 seuil 0

x in microns
y in microns

x 10^4

x 10^4
Stack of images. One image per incident polarization.

Conclusion

Weaker symmetry signature comparing to crystals.
CARS image (2845 cm⁻¹); Myelin from spinal cord
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