Two-color resonant CARS microscopy for an interfacial dip-free detection

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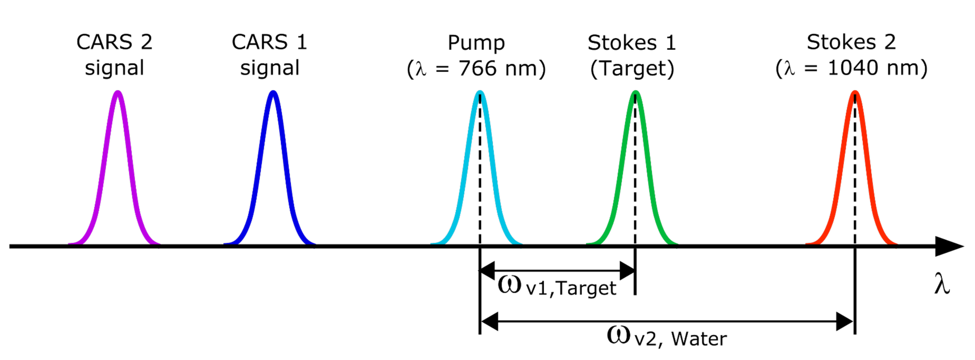
Introduction

Coherent anti-Stokes Raman scattering (CARS) microscopy is a label-free imaging technique that can visualize molecular vibrational modes in biomolecular specimens. It has been successfully utilized to visualize the lipid structures in skin, retina, and brain tissues. However, despite of many advantages in CARS imaging, it intrinsically contains an inherent non-resonant background as a result of far off-resonance transitions, reducing the contrast of CARS signal. To date, a variety of CARS techniques have been suggested to remove the non-resonant background possibly caused by refractive index mismatch. For examples, epi-CARS microscopy which detects the CARS signal in the backward direction completely eliminates the non-resonant signal from the solvent in the focal volume, particularly sensitive to smaller objects than optical wavelength. Polarization CARS can only select the resonant contribution from the CARS signal by rejecting a non-resonant background with a different polarization property through an analyzer. Time-resolved CARS completely suppresses the non-resonant background by inducing a time delay between the excitation and probe pulse with a femtosecond laser. Other techniques include interferometric CARS, frequency modulated CARS, and spatial phase control CARS.

In a line with those efforts, we report the two-color resonant CARS microscopy that can concurrently eliminate the interfacial dip as well as the non-resonant background. To do this, we **simultaneously obtain each CARS signal at two different resonant modes**, one is **for the particular vibrational mode of target objects** and the **other for the O-H stretching vibration in the medium of water**, by tuning two Stokes beams to find the vibrational mode frequencies with a single pump beam. By subtracting the resonant and non-resonant signals obtained from the water molecules to the CARS signal from the target objects with non-resonant background correction factors, **the resonant CARS signal could be purely obtained** with an elimination of interfacial dip at the interface and also its loss is minimized up to >50%.

Experimental Methods

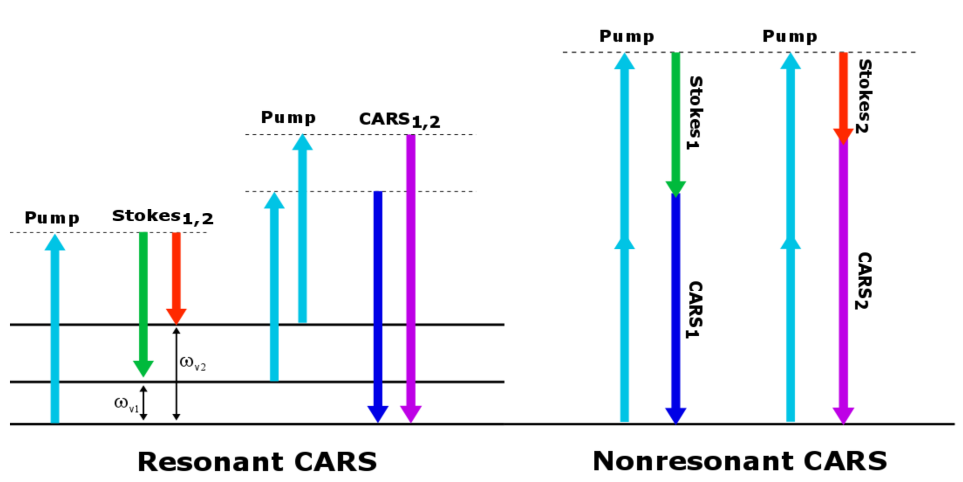
· Energy diagram of two-color resonant Coherent anti-Stokes Scattering (CARS)



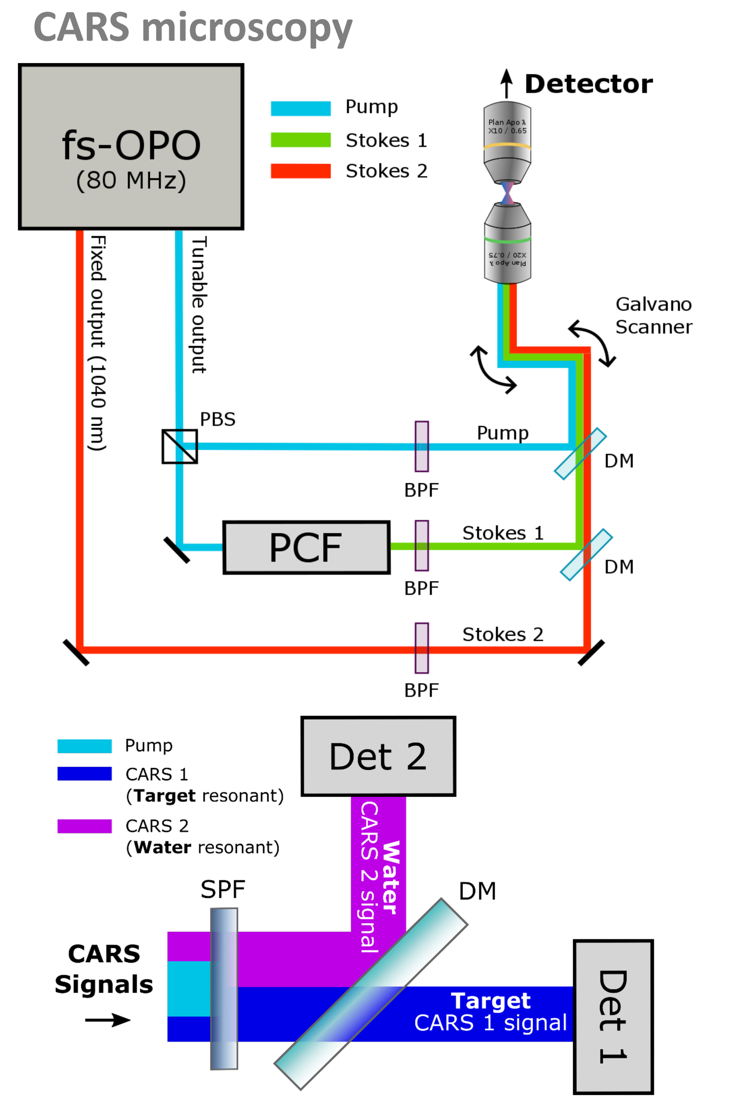
Phase matching condition of CARS

$\Delta\omega_{v1} = \omega_{CARS1} = 2\omega_{pump} - \omega_{Stokes1}$

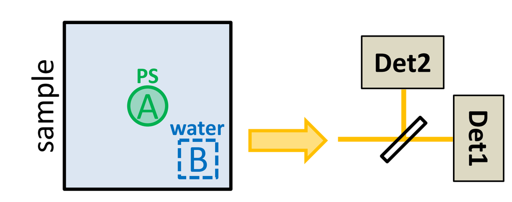
$\Delta\omega_{v2} = \omega_{CARS2} = 2\omega_{pump} - \omega_{Stokes2}$



· Experimental scheme of three-beam CARS microscopy



· Comparison of on-resonance and off-resonance



*** Assumptions**

1. Det1 = Det2 (→Detectivity of D1 and D2 are the same.)
2. $\chi_{NR}^{PS}(-\omega_{CARS1};\omega_p,\omega_p,-\omega_{s1}) = \chi_{NR}^{W}(-\omega_{CARS2};\omega_p,\omega_p,-\omega_{s2})$
   → Non-resonant third-order susceptibilities of pump & Stokes1 and pump & Stokes2 are the same.
3. The pump, Stokes1, and Stokes2 intensities are the same.

① Water **on-resonance** $w_{PS} = w_p - w_{s1}, w_W = w_p - w_{s2}$

①-1. **Region B** : only water region $(\chi_{CARS}^{W} = \chi_{NR}^{W} + \chi_{R}^{W})$

$I_{CARS1}(Det1) = \left|\chi_{NR}^{W}\right|^2 \cdot I_p^2 I_{s1}$ $\quad I_{CARS2}(Det2) = \left|\chi_{NR}^{W} + \chi_{R}^{W}\right|^2 \cdot I_p^2 I_{s2}$

$$\alpha^{on-res} = \frac{I_{CARS1}(Det1)}{I_{CARS2}(Det2)} = \left[\frac{\left|\chi_{NR}^{W}\right|^2}{\left|\chi_{NR}^{W} + \chi_{R}^{W}\right|^2}\right] \cdot \frac{I_{s1}}{I_{s2}} = \beta \cdot \frac{I_{s1}}{I_{s2}}$$

①-2. **Region A** : only PS region $(\chi_{CARS}^{PS} = \chi_{NR}^{PS} + \chi_{R}^{PS})$

$I_{CARS1}(Det1) = \left|\chi_{NR}^{PS} + \chi_{R}^{PS}\right|^2 \cdot I_p^2 I_{s1}$ $\quad I_{CARS2}(Det2) = \left|\chi_{NR}^{PS}\right|^2 \cdot I_p^2 I_{s2}$

$$\Delta I_{region-A}^{on-res} = I_{CARS1}(Det1) - \alpha^{on-res} \cdot I_{CARS2}(Det2) = \left|\chi_{NR}^{PS} + \chi_{R}^{PS}\right|^2 \cdot I_p^2 I_{s1}\left[1 - \beta \cdot \frac{\left|\chi_{NR}^{PS}\right|^2}{\left|\chi_{NR}^{PS} + \chi_{R}^{PS}\right|^2}\right]$$

② Water **off-resonance** $w_{PS} = w_p - w_{s1}, w_W \neq w_p - w_{s2}$

②-1. **Region B** : only water region $(\chi_{CARS}^{W} = \chi_{NR}^{W} + \chi_{R}^{W})$

$I_{CARS1}(Det1) = \left|\chi_{NR}^{W}\right|^2 \cdot I_p^2 I_{s1}$ $\quad I_{CARS2}(Det2) = \left|\chi_{NR}^{W}\right|^2 \cdot I_p^2 I_{s2}$

$$\alpha^{off-res} = \frac{I_{CARS1}(Det1)}{I_{CARS2}(Det2)} = \beta' \cdot \frac{I_{s1}}{I_{s2}} \qquad \beta' = 1$$

②-2. **Region A** : only PS region $(\chi_{CARS}^{PS} = \chi_{NR}^{PS} + \chi_{R}^{PS})$

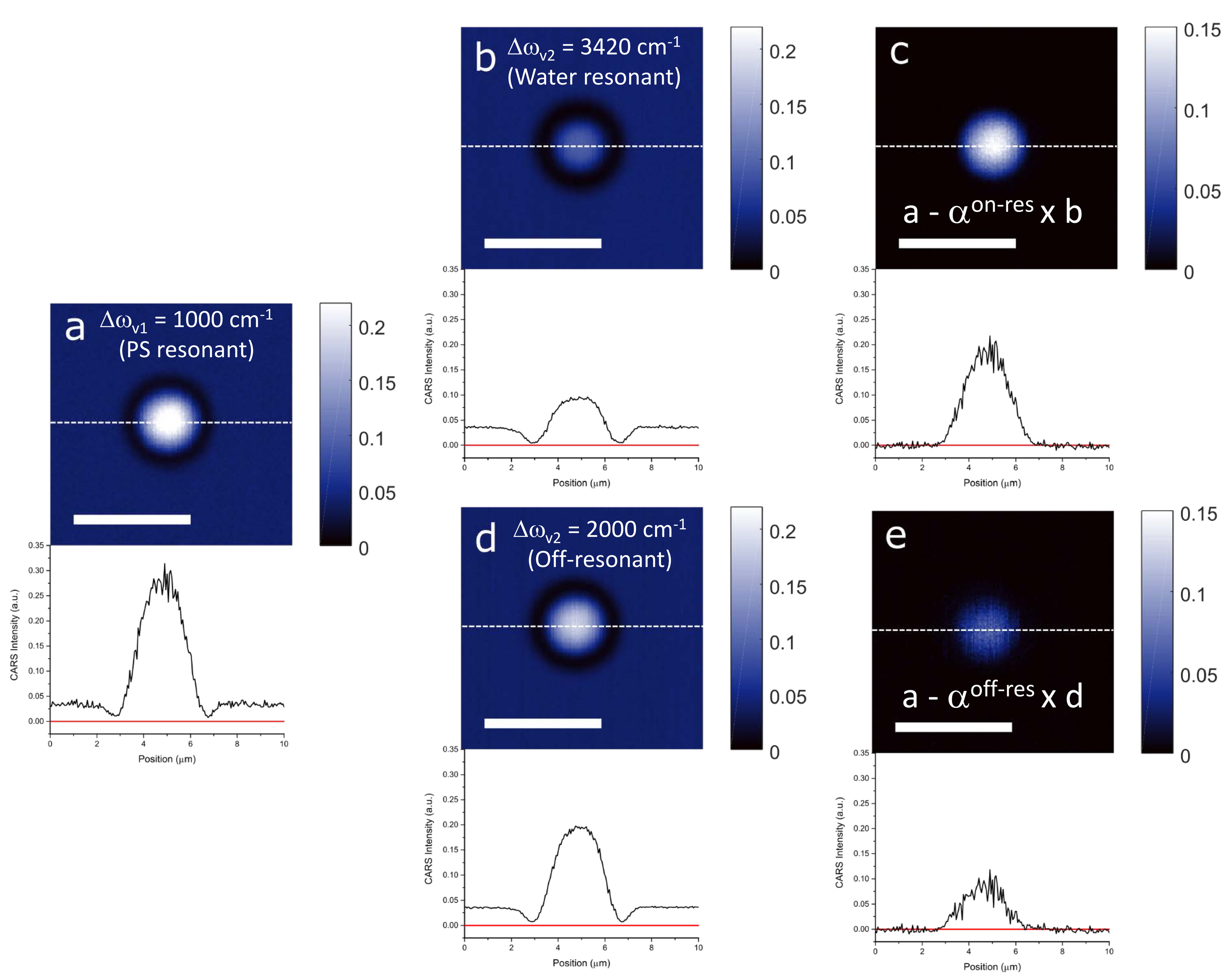
$I_{CARS1}(Det1) = \left|\chi_{NR}^{PS} + \chi_{R}^{PS}\right|^2 \cdot I_p^2 I_{s1}$ $\quad I_{CARS2}(Det2) = \left|\chi_{NR}^{PS}\right|^2 \cdot I_p^2 I_{s2}$

$$\Delta I_{region-A}^{off-res} = I_{CARS1}(Det1) - \alpha^{off-res} \cdot I_{CARS2}(Det2) = \left|\chi_{NR}^{PS} + \chi_{R}^{PS}\right|^2 \cdot I_p^2 I_{s1}\left[1 - \beta' \cdot \frac{\left|\chi_{NR}^{PS}\right|^2}{\left|\chi_{NR}^{PS} + \chi_{R}^{PS}\right|^2}\right]$$

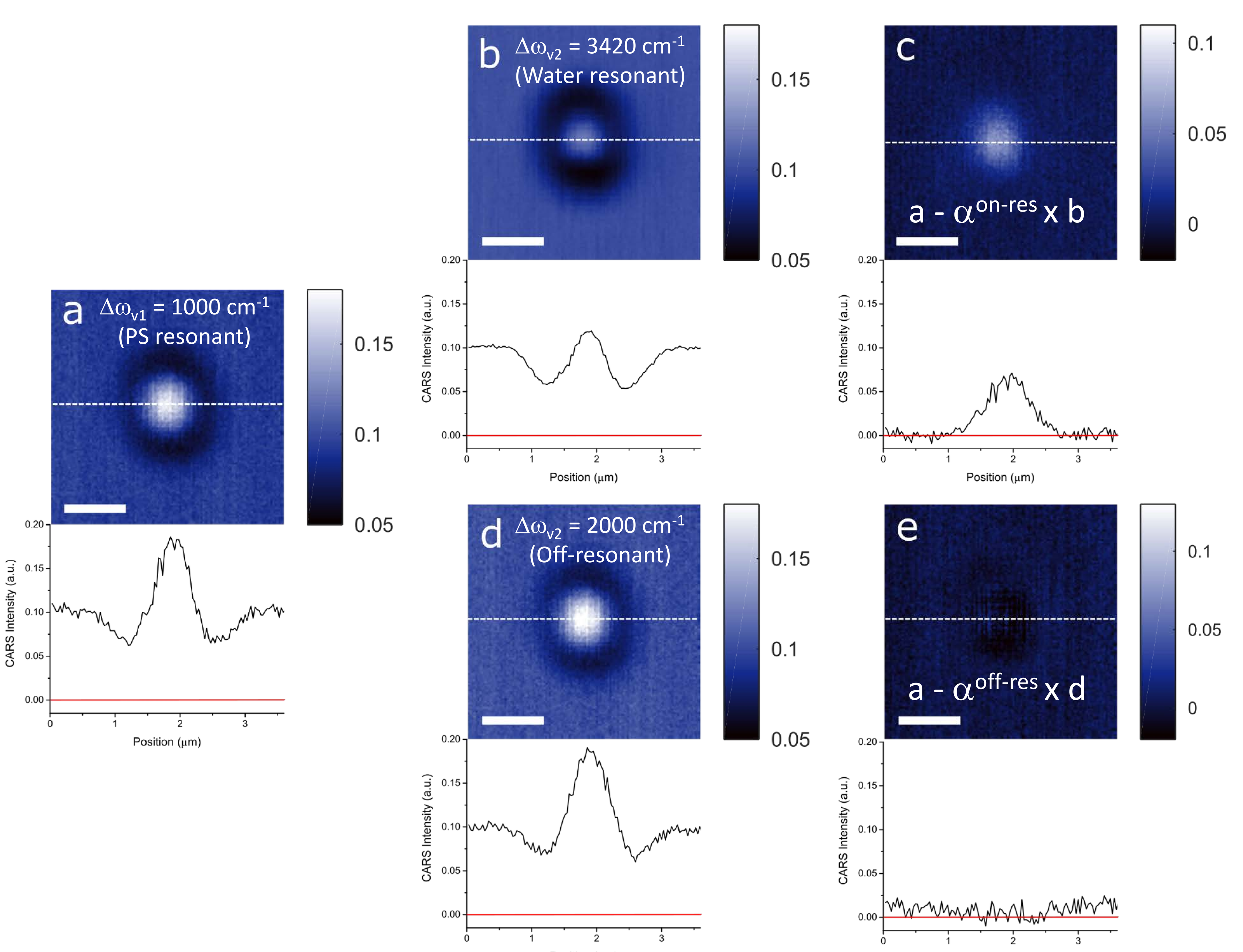
$\Delta I_{region-A}^{on-res} > \Delta I_{region-A}^{off-res}$ → less subtracted by a factor of β
→ Our method has a relatively higher S/N ratio than off-resonance subtraction methods

Experimental Results

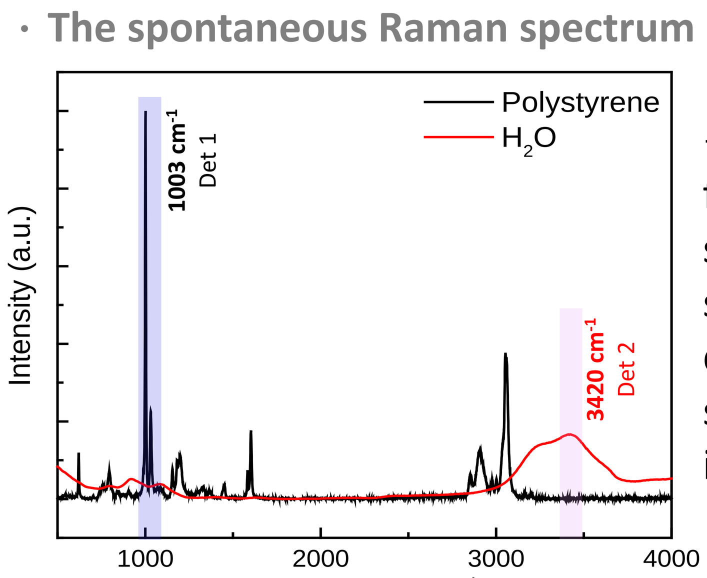
· 3 µm polystyrene beads CARS image and profile



· 1 µm polystyrene beads CARS image and profile

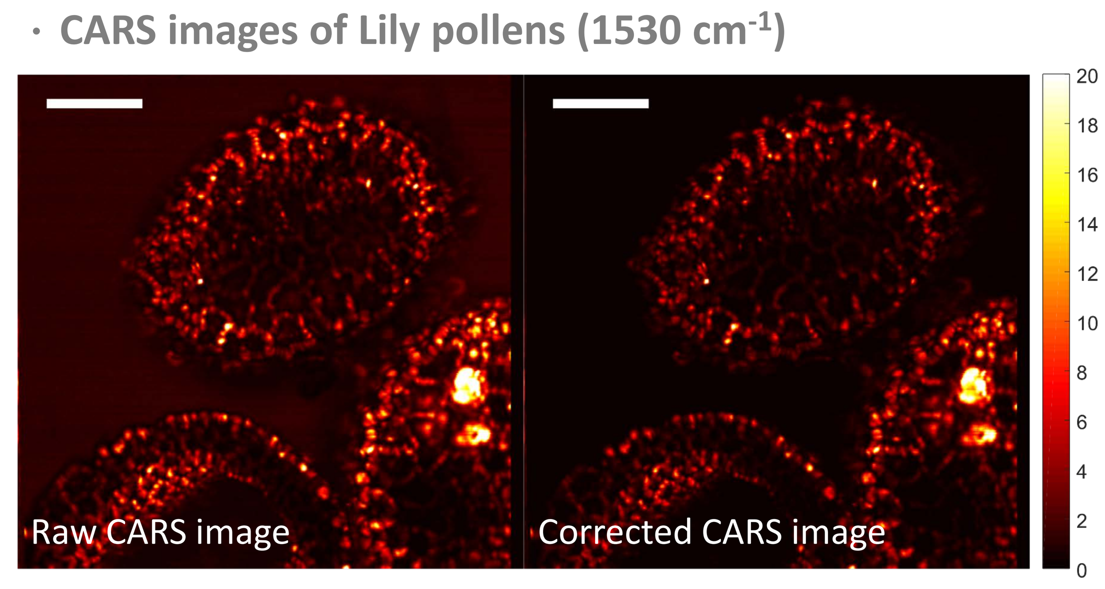


· The spontaneous Raman spectrum

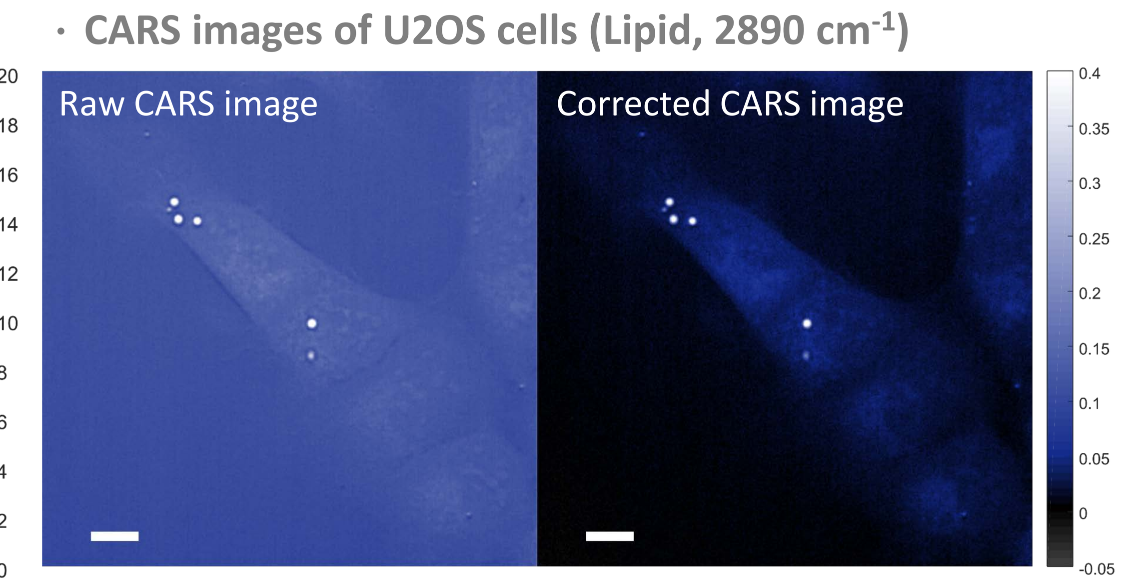


→ The resonant CARS signals from each target molecules in the sample (polystyrene) and the surrounding medium (water) divided by the dichroic mirror are seperately detected by two PMTs in simultaneously.

· CARS images of Lily pollens (1530 cm-1)



· CARS images of U2OS cells (Lipid, 2890 cm-1)



References

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