

# THREE-DIMENSIONAL CARS IMAGING OF MEMBRANE DISRUPTION AND REPAIR USING MULTI-FOCUS CARS MICROSCOPE

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Laser assisted modification, dissection, and trapping of a single cell and its organelles are effective techniques for analyzing characteristics of the cell because of their ability to make noncontact and direct access to intracellular organelles. Manipulation of cells by these techniques has been studied using several imaging methods, such as electron microscopy and fluorescence microscopy. These techniques, however, require fixation or staining with fluorophores, and it is difficult to visualize intracellular molecules in a living cells. In the present study, we demonstrated high-speed three-dimensional imaging of laser manipulated living cells by the combination of a multi-focus CARS microscope and laser-induced ablation technique. We also visualized lipid distribution after laser-induced disruption of the plasma membrane of a HeLa cell.

Figure 1 shows three-dimensional CARS images of a living HeLa cell whose plasma membrane was disrupted by near-infrared pulsed laser irradiation. The laser power and irradiation time used for ablation were 133 mW and 0.1 s, respectively. The image acquisition time was 7.5 s/image (30 slices). The observed molecular vibration was CH<sub>2</sub> stretching vibration

at 2840 cm<sup>-1</sup>. The plasma membrane of the HeLa cell was ablated and disrupted by laser irradiation, and the disrupted site was immediately repaired. After repair, higher CARS signal region was obtained at the disrupted site, indicating that the molecular density of lipids was increased after the repair. This was because of lipid vesicle resealing of the disrupted site. A high-speed three-dimensional CARS imaging method is useful because the lipid distribution is dynamically changed in a few tens of seconds due to the progression of the resealing.

[1] P. McNeil and T. Kirchhausen, *Mol. Cell Biol.* **6**, 499 (2005).

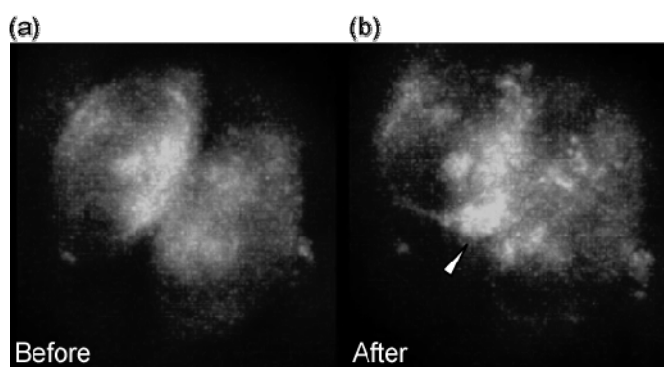


Fig. 1 Three-dimensional CARS images of living HeLa cells (a) before and (b) after ablation. The arrow indicates the resealed site.