Raman imaging of lipid rafts in an artificial monolayer membrane
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Raman scattering microscopy has been utilized for visualizing molecular distribution of a specimen. Our developed slit-scanning Raman microscopy achieves more than 100 times faster imaging speed than that of conventional point-scanning Raman microscopy, which allows us to observe biological samples at high spacio-temporal resolution [1].

Here, we utilized slit-scanning Raman microscopy to observe lipid rafts in an artificial monolayer membrane [2]. Lipid raft is a micro-domain in bio-membranes, formed by sphingomyelin (SM) and cholesterol (chol). It has been thought to work for various biological functions, such as membrane signaling and protein trafficking. For the analysis of lipid rafts, artificial ternary membrane of SM/phosphatidylcholine(PC)/chol has been widely used as a model. Phase separation of this membrane can be visualized by fluorescently-labeled lipids, however, observation of SM inside of the domain has been challenging, because the steric effect of bulky fluorophore disturbs the entry of labeled lipids into tightly packed micro-domain. We directly observed the distribution of SM inside of micro-domain by using Raman microscopy. For the specific observation of SM, we synthesized SM analogue modified with diyne tag (Fig. A). Diyne moiety shows characteristic Raman peak at silent region of biomolecules (Fig. B), and it keeps the original property of SM to be incorporated into micro-domain due to its tiny chemical structure. Raman image of diyne-SM in ternary membrane monolayer visualized micro-domain (Fig. C). We found out that the diyne-SM was enriched in central area compared with the peripheral area of the domain. Raman imaging of lipid membrane, achieved here with single lipid-layer sensitivity, high spatial-resolution and hyper-spectral imaging capability, will widely contribute to lipid membrane research.

Figure (A) Chemical structures and (B) Raman spectra of diyne-SM, DOPC and cholesterol. (C) Raman image of diyne-SM/DOPC/chol ternary monolayer at 2263 cm<sup>-1</sup>. 344x190 pixels.