**Supplemental Information**

Cholesterol enhances the negative impact of vaping additives on lung surfactant model systems

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**Brewster Angle Microscopy (BAM)**

Brewster angle microscopy is a well characterized tool for visualization of films on the surface of a subphase. It works by directing plane polarized light towards the air-water interface at the so-called Brewster angle (~53.1º for water). At this angle, the incident light impacting the subphase will largely be refracted [1,2]. There is a change in the refractive index at the air-water interface by the addition of a lipid film. This results in reflection off the surface into a camera for imaging. Using this technique, qualitative information regarding lateral organization, phase separation, and domain formation can all be directly observed. The intensity of the reflected light is also captured providing information in the z-axis that is used to generate 3D images.

Shape

Description automatically generated with medium confidence

Figure S1: Principle of Brewster angle microscopy, comparing refractive index between two phases [3]

**Brewster Angle Microscopy Images**

BAM images of pure subphase will appear as dark grey as light is predominantly refracted (Figure S2 left). Lipid domains show up as light grey clusters (Figure S2 right) with the brightness corresponding to their height. These domains are often contrasted by a dimmer background of LE phase (yellow arrow, right image).

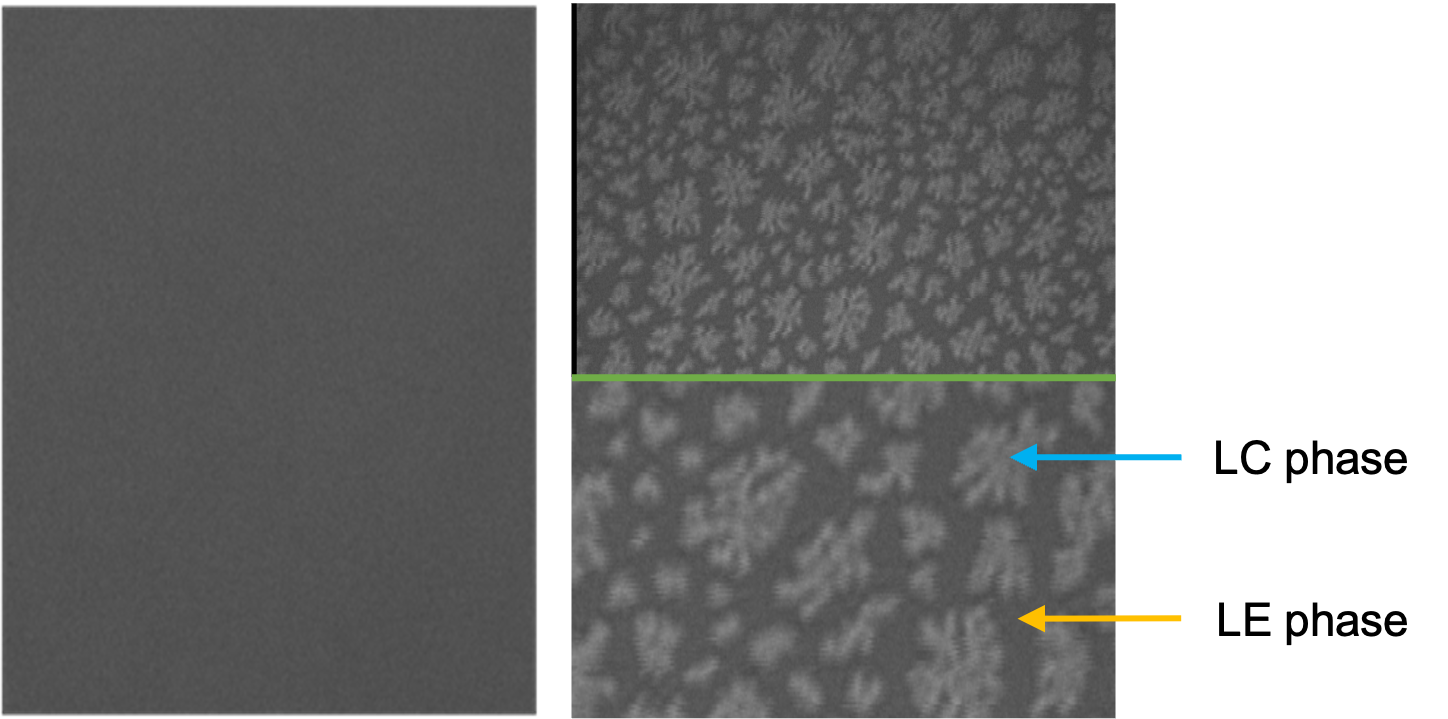


Figure S2: Brewster Angle microscopy images of subphase (left) and lipid film (right) showing the LC phase domains (blue arrow) and LE phase (yellow arrow). A 4x magnification of the lipid film is shown below the green line.

**Domain Thickness**

Domain thickness can be calculated using reflectivity data from the BAM images and the refractive indices of the film and subphase [4,5].

Signal intensity is given in gray level, which is first converted into a reflected light intensity (Rp) This is done by generating a calibration curve of the reflectivity vs the gray level using a BAM image of a clean subphase.

The refractive index of the subphase (n2) was calculated using measurements of the reflectivity across angles 52-56° and equation 1. The refractive index of the monolayer was estimated by plotting reflectivity against the calculated refractive as described by equation 2. The minimum value of the parabola was used as the refractive index of the monolayer (n).

Equation 1:

Equation 2:

Domain thickness was then calculated from the reflectivity provided by BAM images and previously found reflective indices using equation 3. Using values of n1 =1.000, n = 1.478, and n2 = 1.330.

Equation 3:

**References**

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