The impact of non-equilibrium conditions in lung surfactant: structure and composition gradients in multilamellar films

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SUPPLEMENTARY INFORMATION

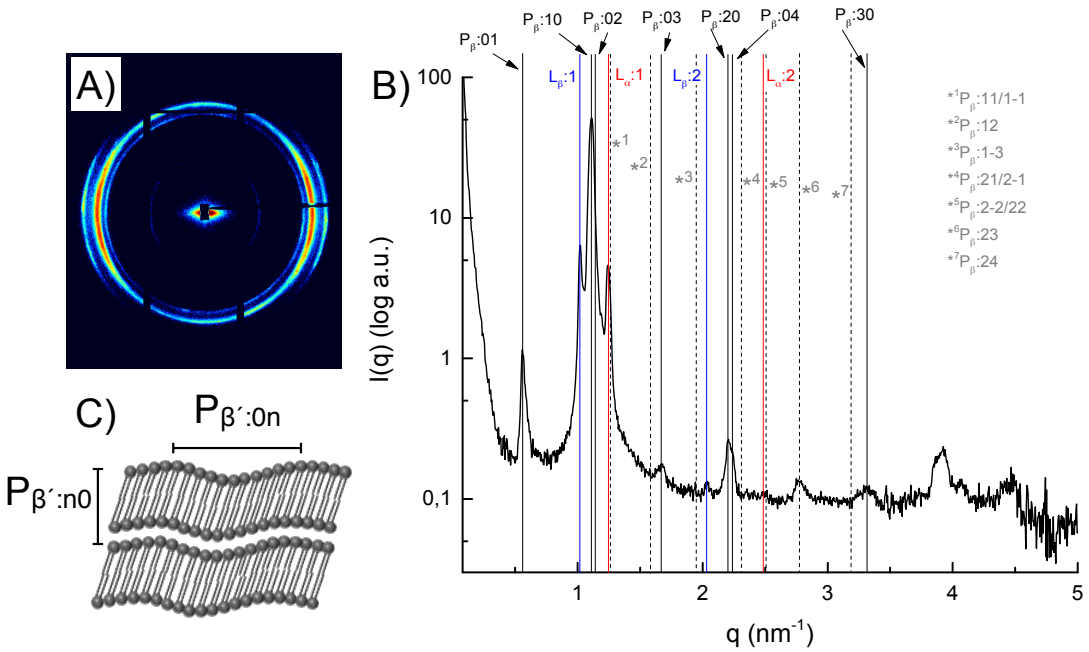

FIG. S1: A) 2D SAXS of the clinical lung surfactant extract close to the interface showing the alignment of the Bragg peaks and thus indicating unidimensional structures B) Peak assignment of the lamellar phases present in the co-existing region of film formed from the clinical lung surfactant extract with the hypothesis that a ripple phase is present. Peaks corresponding to the planar lamellar gel phase (L_β) are marked with blue lines (−) and peaks corresponding to the lamellar liquid crystalline phase (L_α) are marked with red lines (−). Peaks corresponding to the P_β phase are marked with black lines. Solid black lines (–) and dashed black lines (− −) indicate peaks originating respectively from interference between scattered waves from P_β:0n and P_β:n0, according to fit. (e.g. P_β:10 corresponds to the first reflection of the lamellar repeat distance and P_β:01 corresponds to the first reflection of the ripple distance) C) Cartoon of the P_β phase.

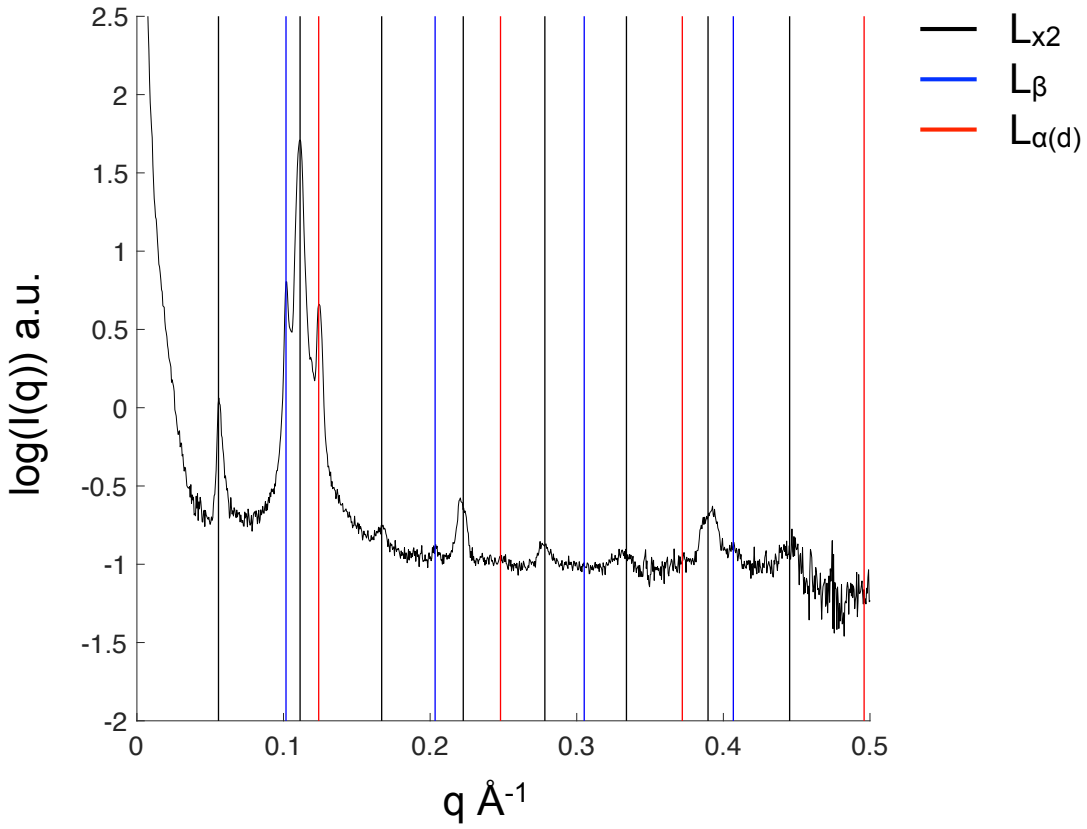

FIG. S2: Peak assignment of the lamellar phases present in the co-existing region of film formed from the clinical lung surfactant extract with the hypothesis that a double-spaced lamellar phase is present. Peaks corresponding to the planar lamellar gel phase (L_β) are marked with blue lines (–) and peaks corresponding to the lamellar liquid crystalline phase (L_α) are marked with red lines (–). Peaks corresponding to the double-spaced lamellar phase $L_{\times 2}$ are marked with black lines (–).

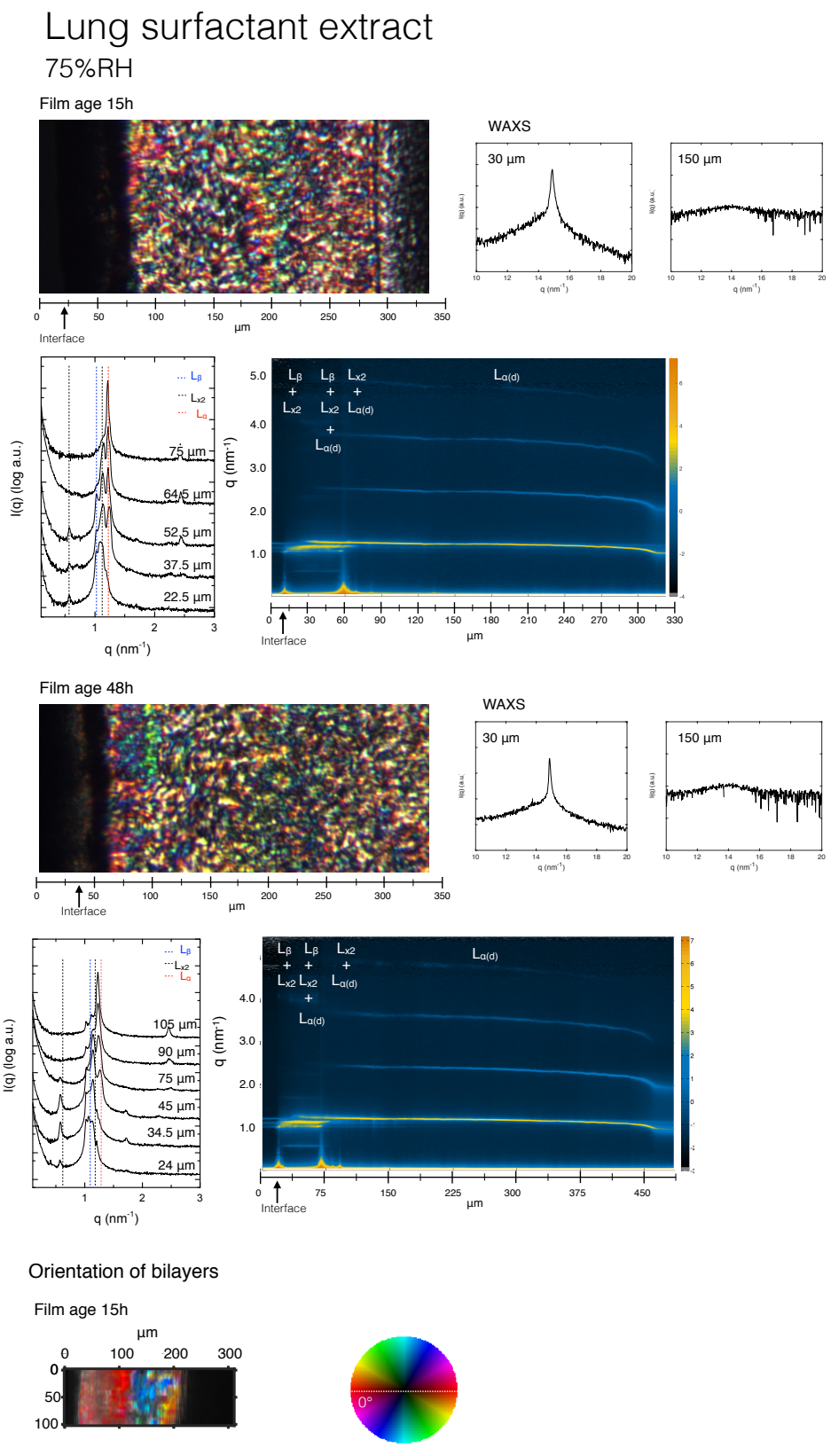

FIG. S3: SAXS maps of the clinical lung surfactant extract at 75%RH at film age of 15 and 48h with corresponding cross-polarised images. Stepwise 1D spectra of the interface are shown to clarify the phases present (planar lamellar gel (L_β), double-spaced lamellar phase ($L_{\times 2}$) and liquid crystalline (L_α) phase) at specific points from the interface. WAXS spectra at specific points in the film are displayed next to the microscopy images showing a peak (4.1 Å) and indicate solid acyl chains close to the interface. Orientation map of the film at 15h is shown at the bottom showing scattering at 0° (red) close to the interface signifying bilayers oriented parallel with the interface.

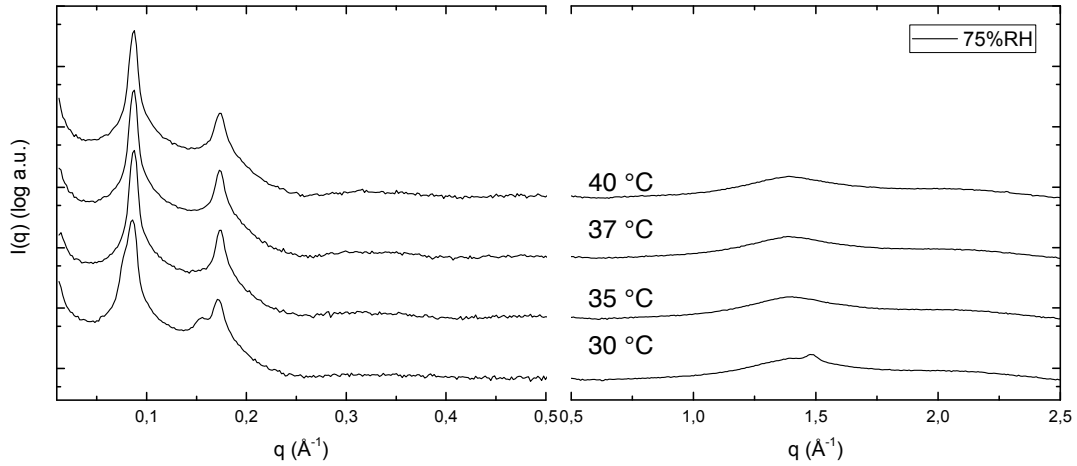

FIG. S4: SAXS and WAXS of bulk samples of clinical lung surfactant extract equilibrated at 75 %RH between 30 and 40 °C.

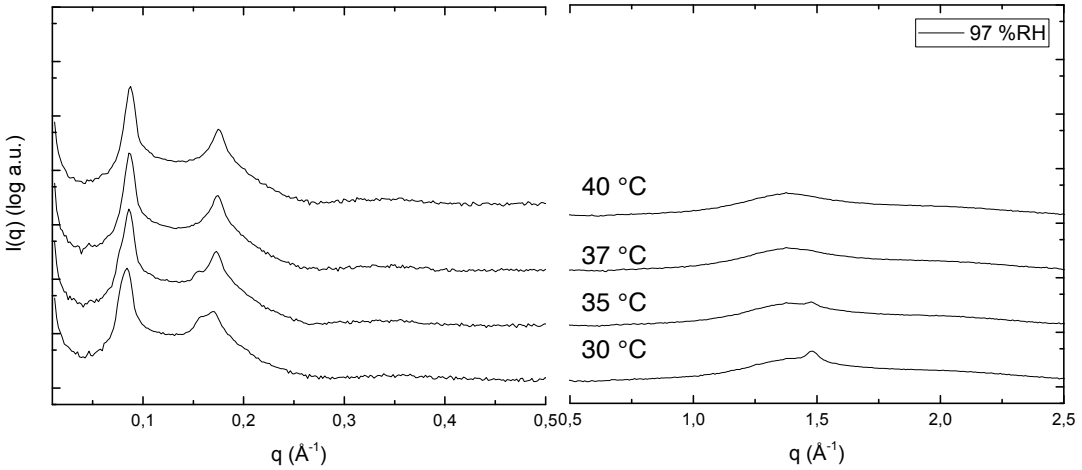

FIG. S5: SAXS and WAXS of bulk samples of clinical lung surfactant extract equilibrated at 97 %RH between 30 and 40 °C

Table S1. Lamellar repeat distance (d) in nm in the bulk samples of the clinical lung surfactant extract shown in Figure S3 and S4 equilibrated at 75 and 97%RH and at 30 – 40 °C.

	30 °C	35 °C	37 °C	40 °C
RH 75%	7.31	7.31	7.31	7.15
RH 97%	7.39	7.31	7.31	7.15

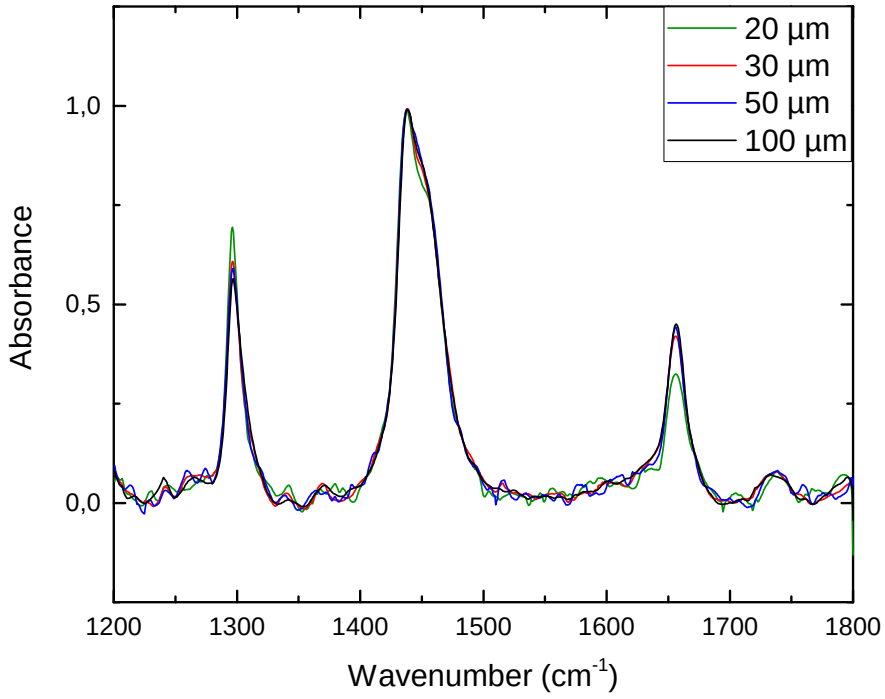

FIG. S6: Normalized Raman spectra recorded at intervals from the interface. Each spectra is renormalized by the 1440 cm^{-1} peak height from the CH_2 scissoring deformation. This allows to compare peak heights at $\sim$1655 cm^{-1} from the C=C stretching. This comparison shows a lower intensity from the unsaturated acyl chains in the vicinity of the air/liquid interface (green).

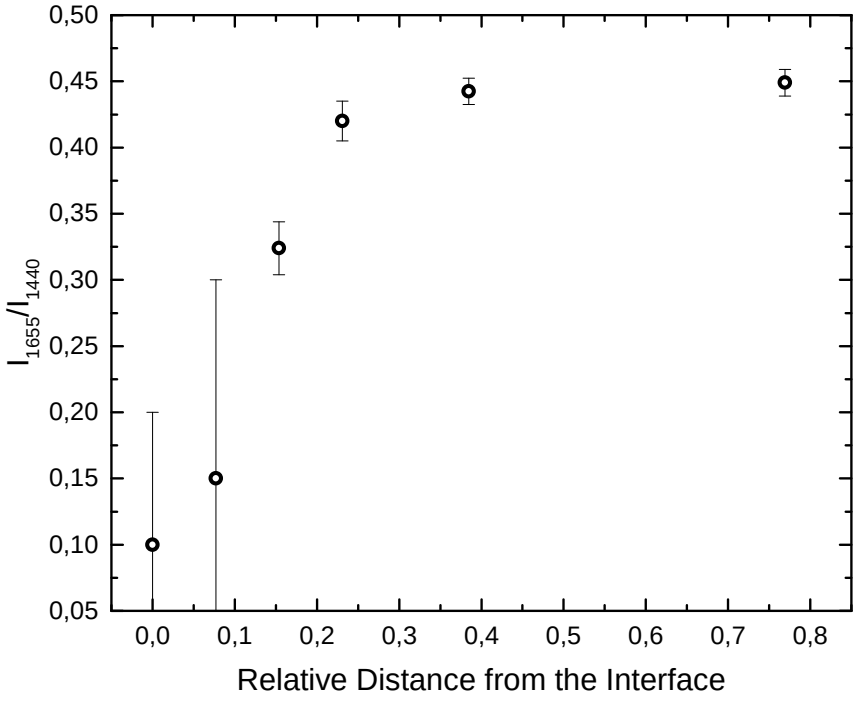

FIG. S7: Ratio between the peak height of the 1440 cm^{-1} peak from the CH_2 scissoring deformation and the 1655 cm^{-1} peak from the C=C stretching. The peak at 1655 cm-1 measured at the interface (0 μm) and 10 μm from the interface is within the noise level, which explains the large error bar displayed for these two data points.

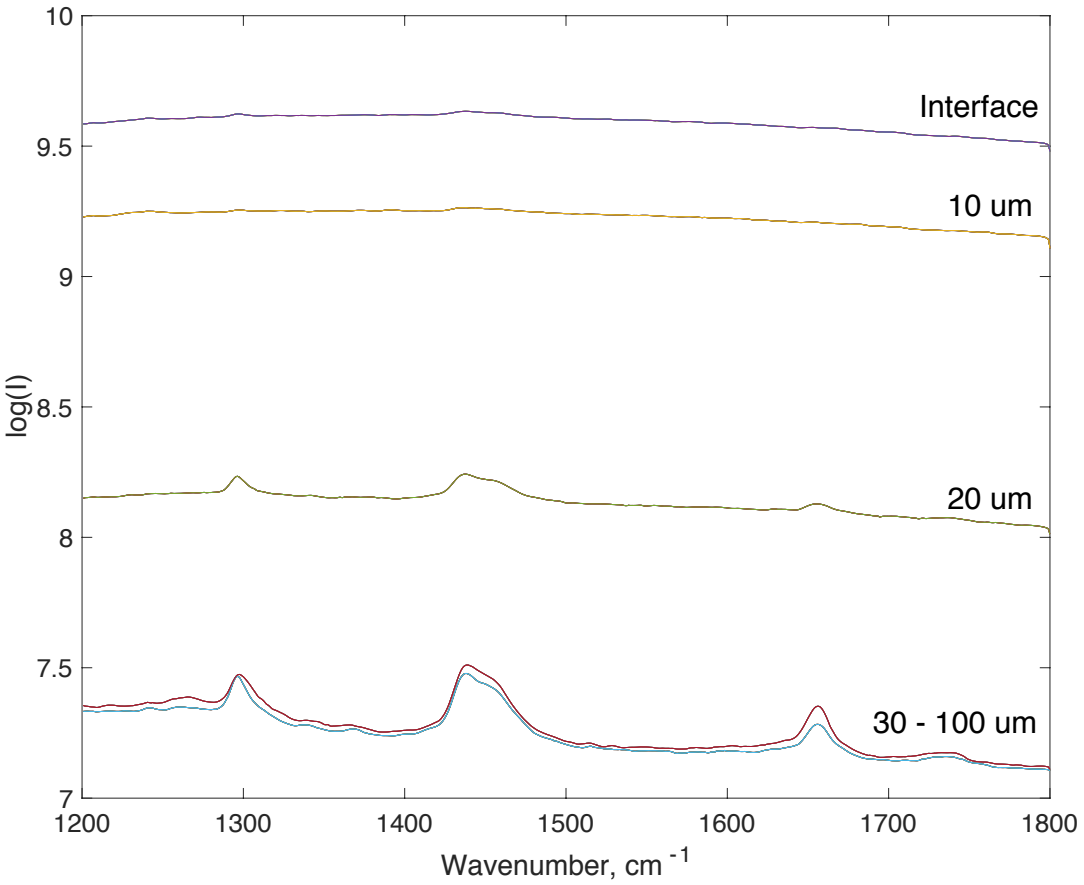

FIG. S8: Raman spectra recorded at intervals from the interface between 1200-1800 cm^{-1} showing a higher fluorescent background close to the interface, here interpreted as originating from a higher concentration of proteins closer to the interface.

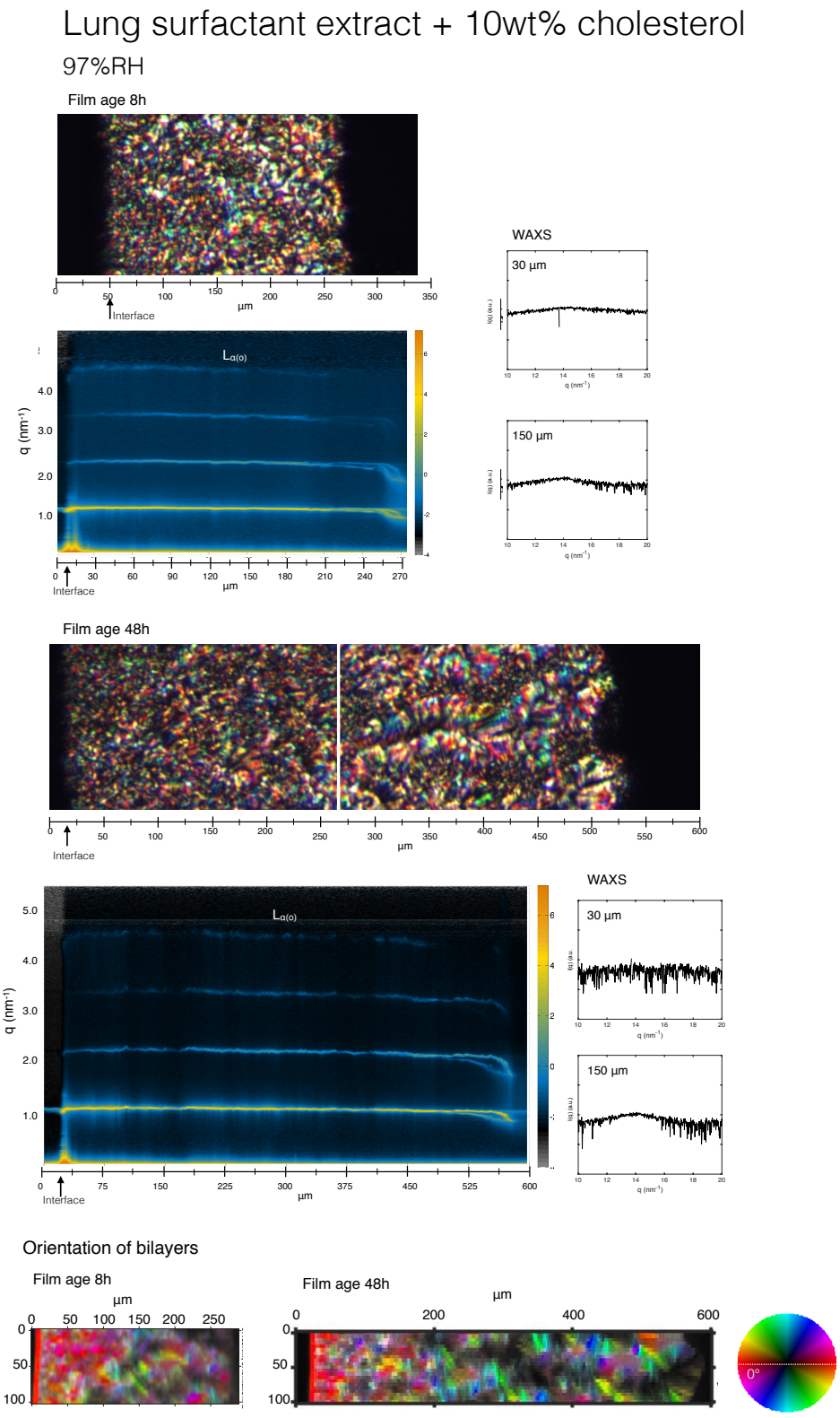

FIG. S9: SAXS maps of the clinical lung surfactant extract + 10wt% cholesterol at 97%RH at film age of 8 and 48h with corresponding cross-polarised images showing one ordered liquid lamellar phase throughout the entire film. WAXS spectra at specific points in the film are displayed next to the maps showing the absence of the peak signifying solid acyl chains. Orientation maps of the film at 8 and 48h are shown at the bottom showing a mixed orientation of the bilayers through the film.

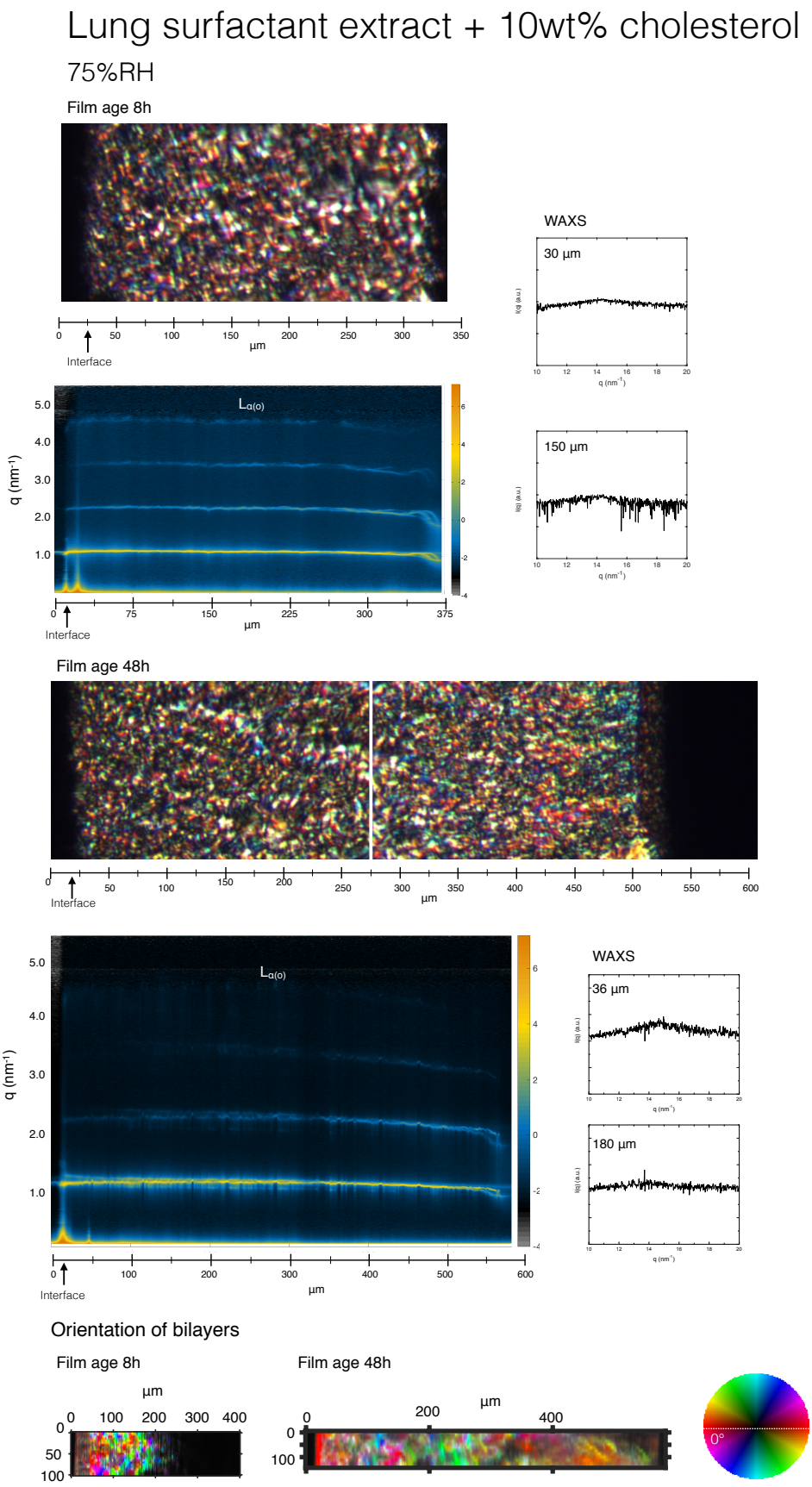

FIG. S10: SAXS maps of the clinical lung surfactant extract + 10wt% cholesterol at 75%RH at film age of 8 and 48h with corresponding cross-polarised images showing one ordered liquid lamellar phase throughout the entire film. WAXS spectra at specific points in the film are displayed next to the maps showing the absence of the peak signifying solid acyl chains. Orientation maps of the film at 8 and 48h are shown at the bottom showing a mixed orientation of the bilayers through the film.

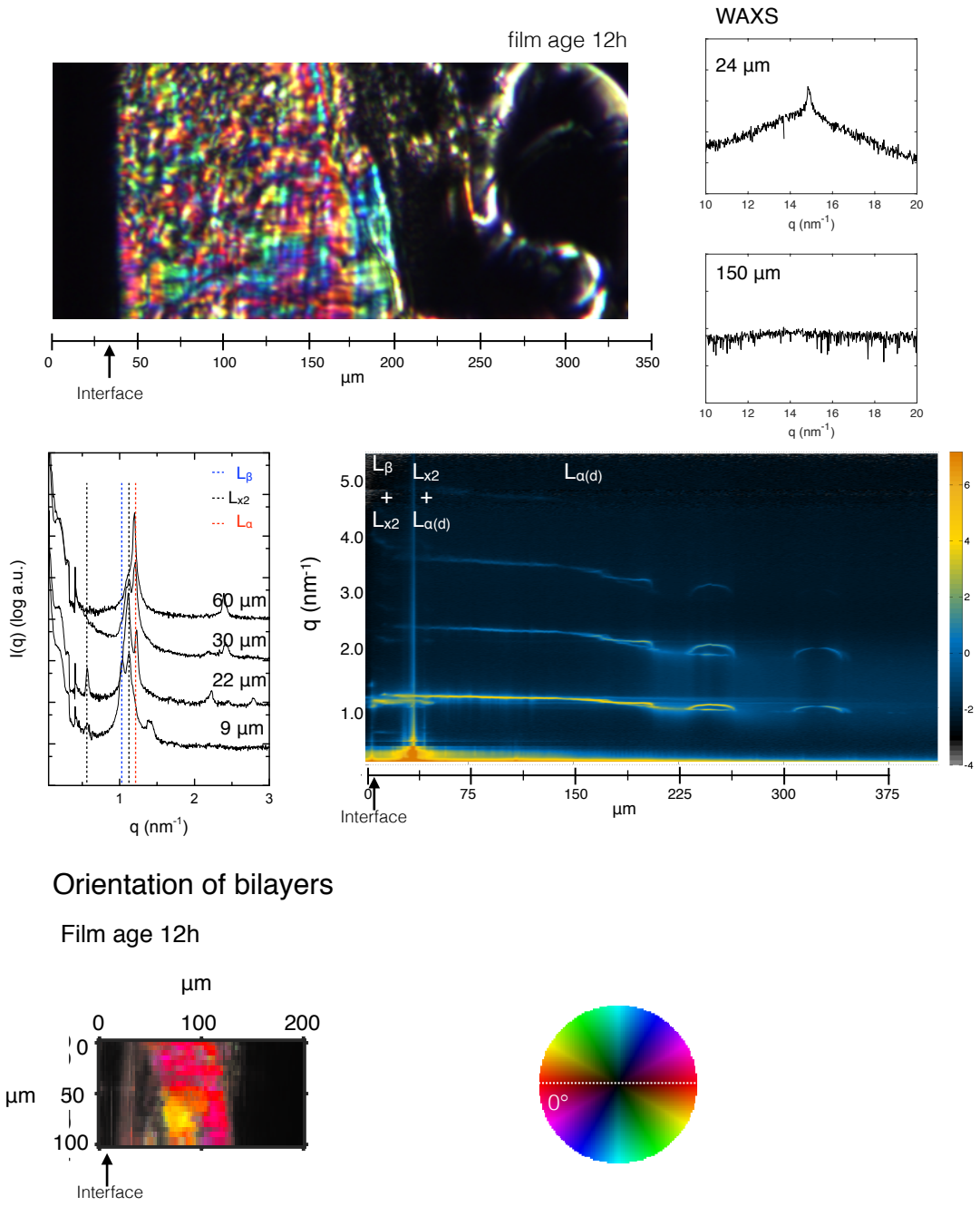

FIG. S11: SAXS map of the clinical lung surfactant extract with 4 mM CaCl2 at 97%RH at film age of 12h with corresponding cross-polarised image. Stepwise 1D spectra of the interface are shown to clarify the phases present (planar lamellar gel (L_β), double-spaced lamellar phase ($L_{\times 2}$) and liquid crystalline (L_α) phase) at specific points from the interface. WAXS spectra at specific points in the film are displayed next to the microscopy images showing a peak (4.1 Å) signifying solid acyl chains close to the interface. Orientation map of the film at 12h is shown at the bottom showing scattering at 0° (red) signifying bilayers oriented parallel with the interface.

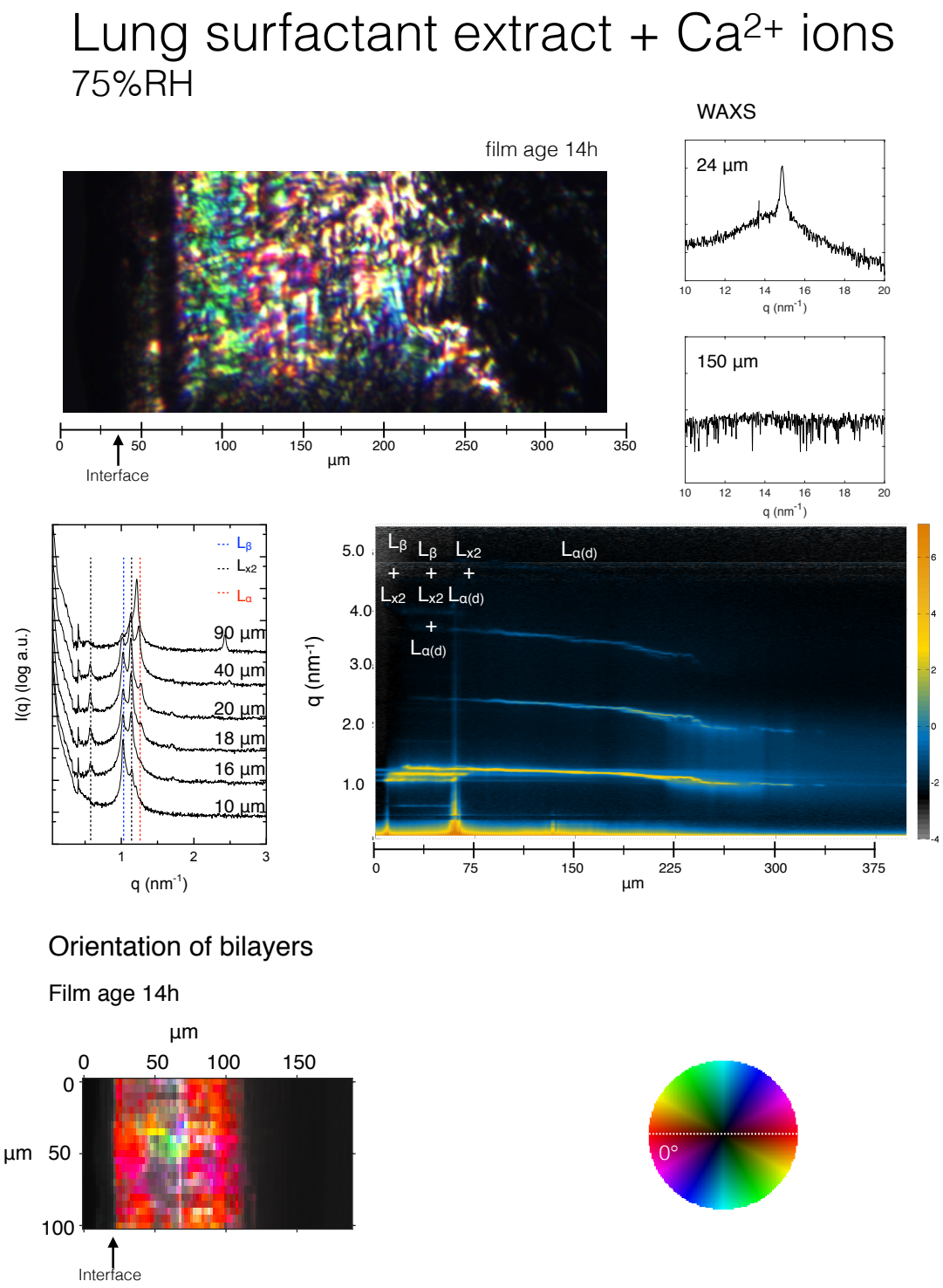

FIG. S12: SAXS map of the clinical lung surfactant extract with 4 mM CaCl2 at 75%RH at film age of 14h with corresponding cross-polarised image. Stepwise 1D spectra of the interface are shown to clarify the phases present (planar lamellar gel (L_β), double-spaced lamellar phase ($L_{\times 2}$) and liquid crystalline (L_α) phase) at specific points from the interface. WAXS spectra at specific points in the film are displayed next to the microscopy image showing a peak (4.1 Å) signifying solid acyl chains close to the interface. Orientation map of the film at 14h is shown at the bottom showing scattering at 0° (red) close to the interface signifying bilayers oriented parallel with the interface.

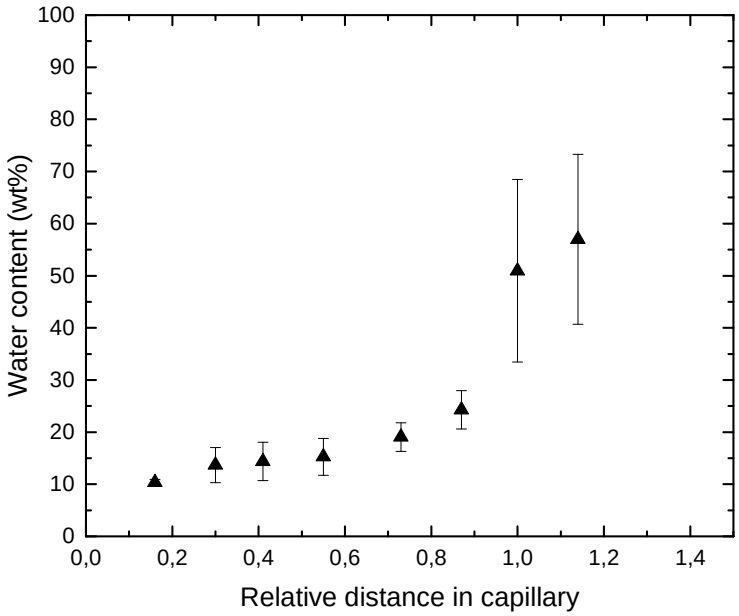

FIG. S13: Water gradient of lung surfactant extract at 75%RH with the distance in the capillary normalised by the total thickness of the film.

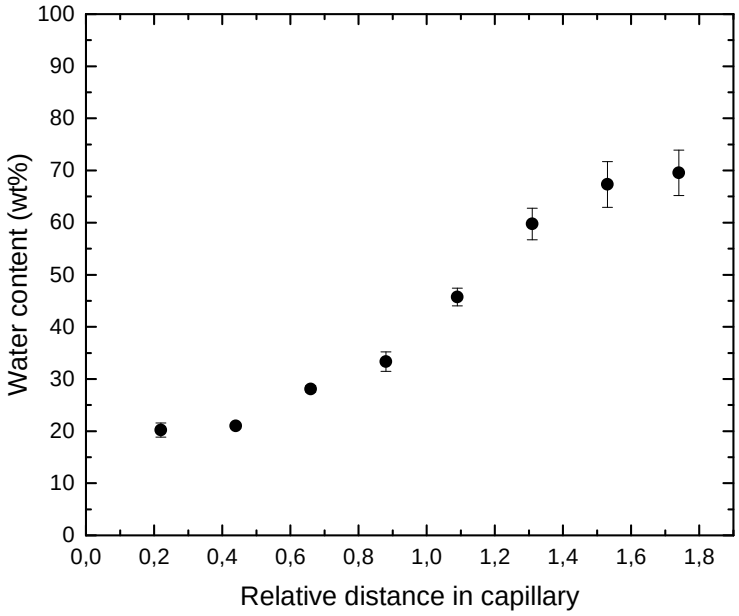

FIG. S14: Water gradient of Lung surfactant extract at 97%RH with the distance in the capillary normalised by the total thickness of the film.

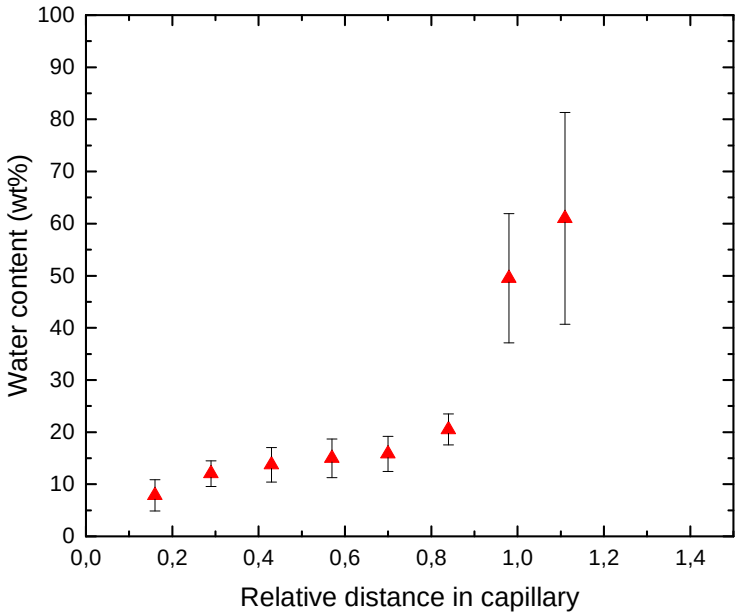

FIG. S15: Water gradient of lung surfactant extract + 10wt% cholesterol at 75%RH with the distance in the capillary normalised by the total thickness of the film.

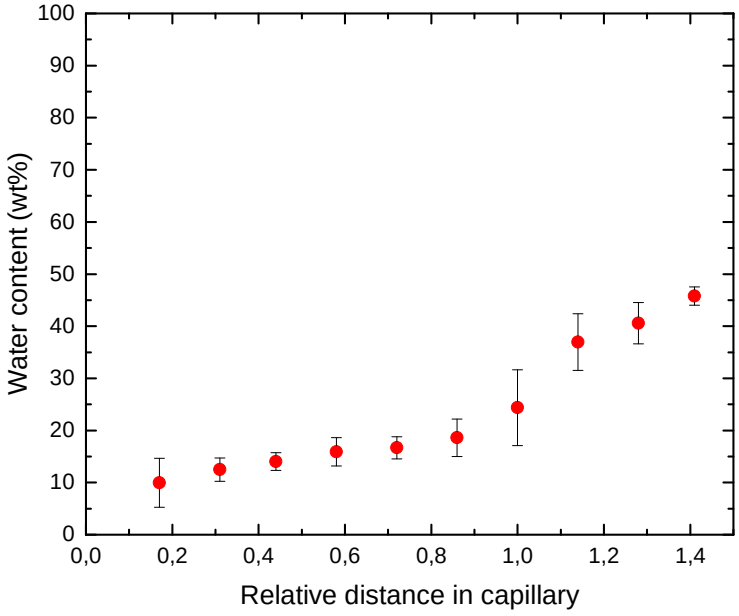

FIG. S16: Water gradient of lung surfactant extract + 10wt% cholesterol at 97%RH with the distance in the capillary normalised by the total thickness of the film.

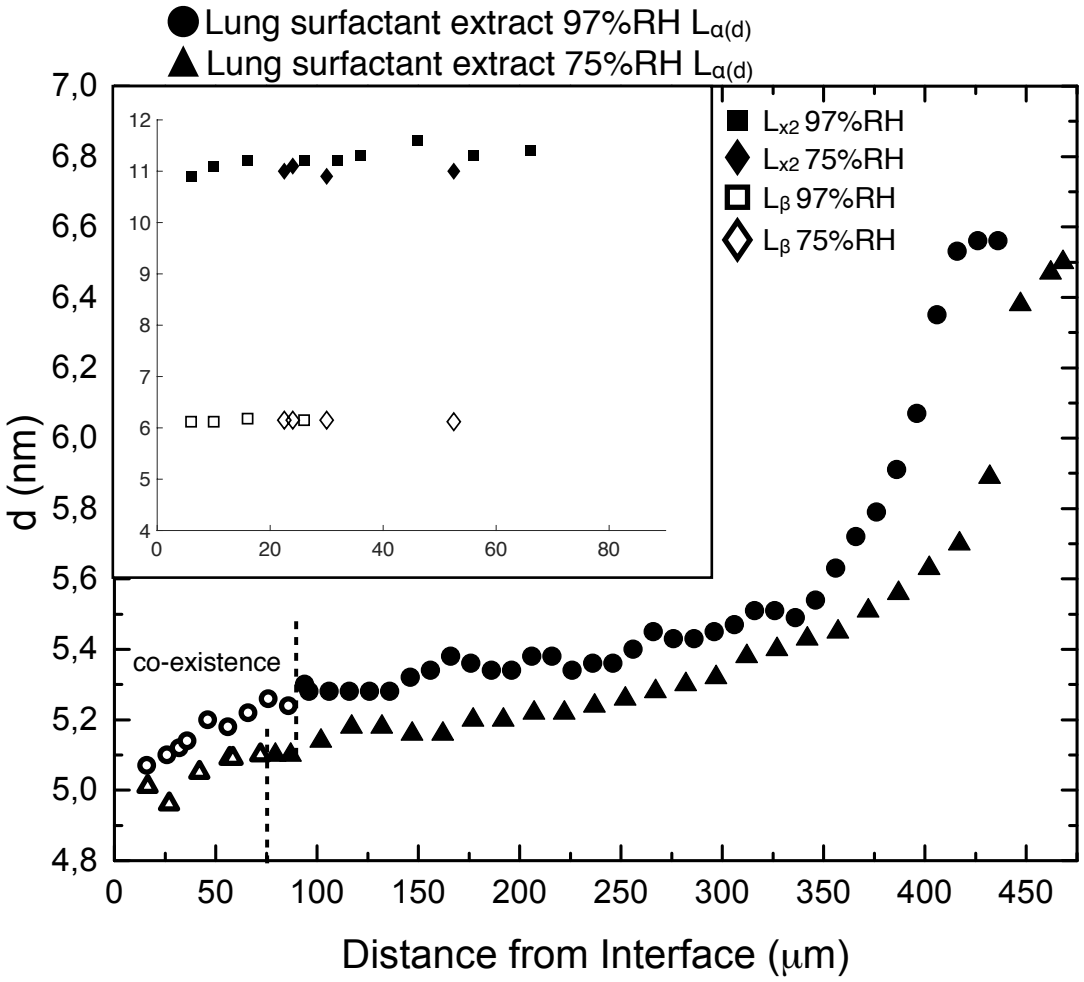

FIG. S17: Repeat distance (d) of the disordered lamellar liquid crystalline phase ($L_{\alpha(d)}$) in the interfacial film of clinical lung surfactant extract formed at 97 and 75 %RH. In the insert d from the two lamellar gel phases (planar gel phase, double-spaced lamellar phase ($L_{\times 2}$), P_β co-existing with the $L_{\alpha(d)}$ phase close to the interface (co-existence region marked with dotted line for each film) are shown.

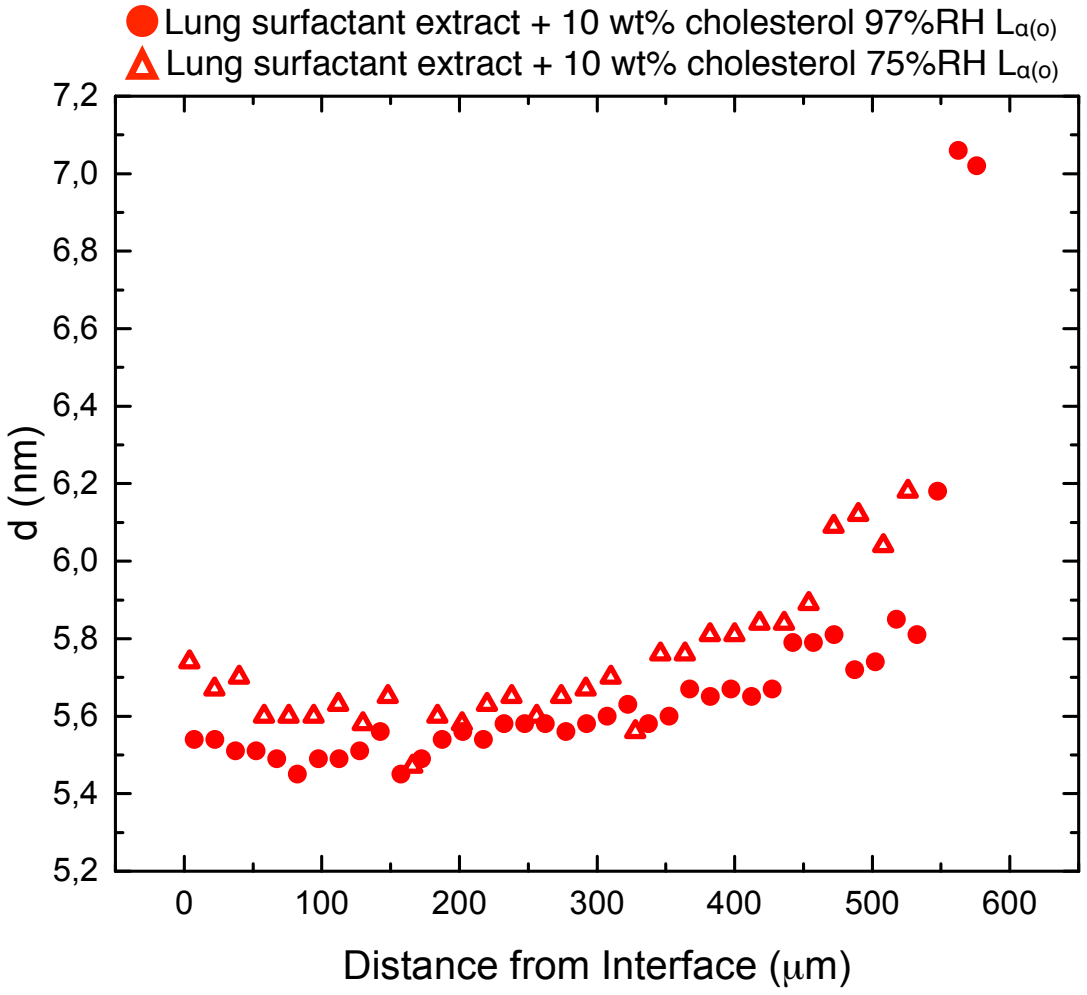

FIG. S18: Repeat distance (d) of the ordered lamellar liquid crystalline phase ($L_{\alpha(o)}$) in the interfacial film of lung surfactant extract + 10 wt% cholesterol formed at 97 and 75 %RH.

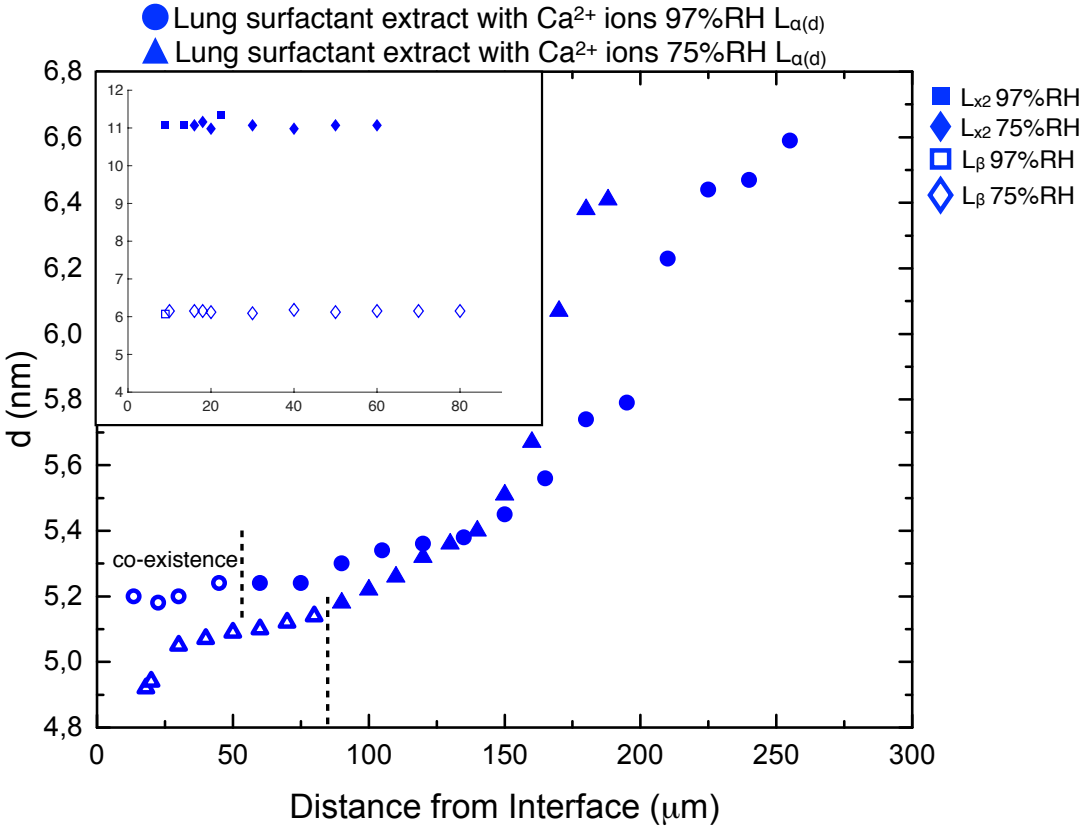

FIG. S19: Repeat distance (d) of the disordered lamellar liquid crystalline phase ($L_{\alpha(d)}$) in the interfacial film of clinical lung surfactant extract with Ca2+ ions formed at 97 and 75 %RH. In the insert d from the two lamellar gel phases (planar gel phase, L_{β} and double-spaced lamellar phase ($L_{\times 2}$) co-existing with the $L_{\alpha(d)}$ phase close to the interface (co-existence region marked with dotted line for each film) are shown.