Supplementary Information "Mass Spectral Imaging of Glycophospholipids, Cholesterol and Glycophorin A in Model Cell Membranes"
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LB Film Construction

A schematic showing the process of LB film deposition on the prepared substrate is shown below in Figure 1.

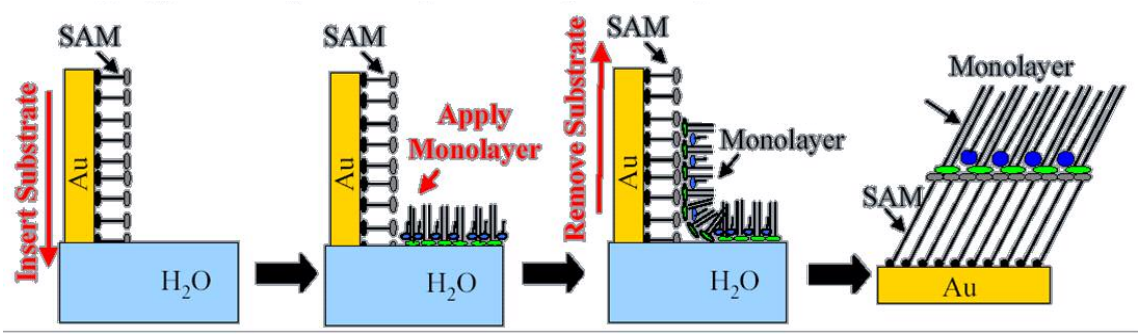

Figure 1. Schematic of the process of LB film depositon

Principal Component Analysis

The amount of variance captured by the principal component analysis model is shown in Figure 2.

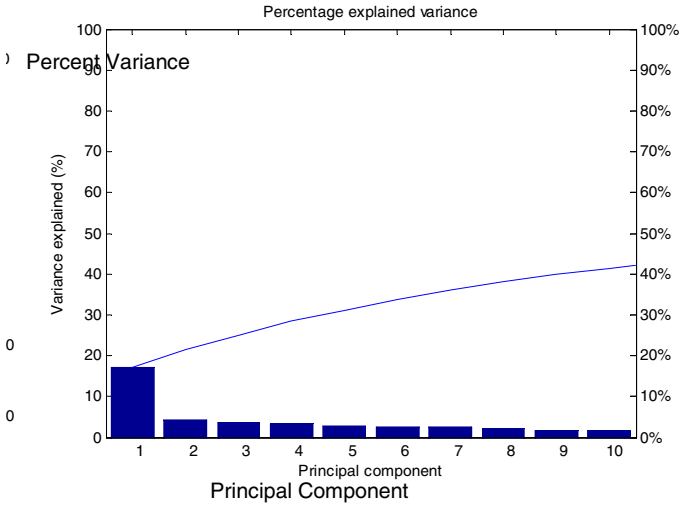

Figure 2. The amount of variance captured for each principal component

Principal component 1 captures the most variance with the variance decreasing with each successive principal component. The higher principal components do not contribute any more information to the structure of the film, this is due to the low amount of variance captured by these principal components.

DPPE / Cholesterol / Glycophorin A LB Film

The ToF-SIMS images of the inner leaflet membrane mimic are shown in Figure 3. These figures originate from a different LB film to the inner membrane mimic reported in the paper and serve as proof of the reproducibility of domain formation. The distribution of the dipalmitoyl tailgroup peak at *m/z* 551.5 exhibits clear heterogeneity. Moreover, the PE fragments at *m/z* 142.03 (not shown) and *m/z* 124.02 (not shown) and the cholesterol peaks at *m/z* 369.35 and *m/z* 385.35 (not shown) exhibit an identical pattern. This information suggests there is co-localisation of DPPE and cholesterol, with voids of signal throughout the image that are not occupied by these species.

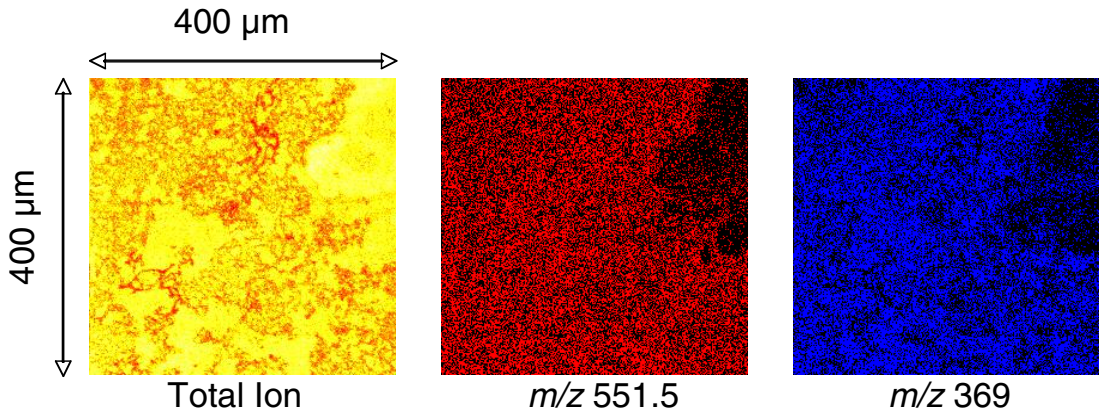

Figure 3. Mass spectral images of the DPPE / cholesterol / glycophorin A LB film (Total Ion = 0-354 counts, *m/z* 551.5 = 0-5 counts, *m/z* 369 = 0-12 counts).

ToF-SIMS images of the mass peaks identified by PCA to anti-correlate to the phospholipid/cholesterol domain are shown in Figure 4.

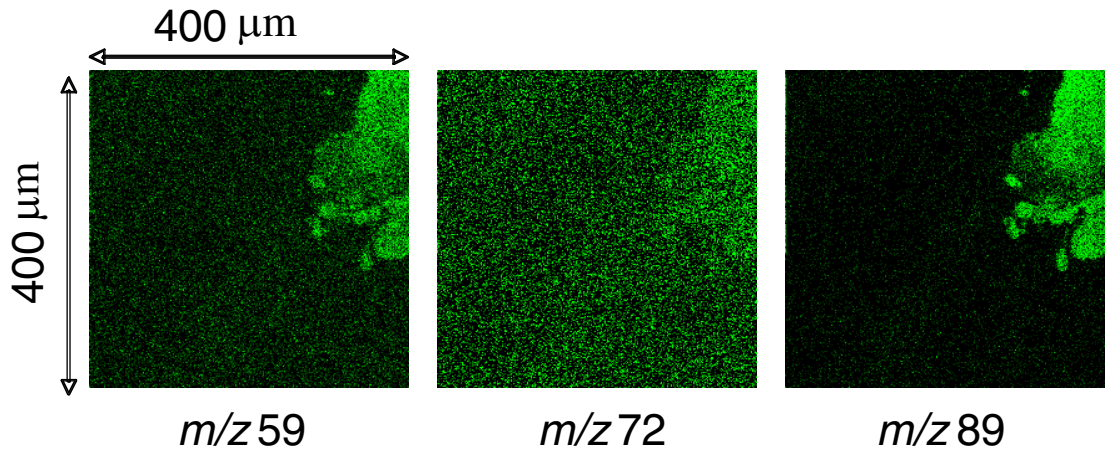

Figure 4. Mass spectral images of the DPPE / cholesterol / glycophorin A LB film (*m/z* 59 = 0-5 cts, *m/z* 72 = 0-2 cts, *m/z* 89 = 0-7 cts) .

The mass spectral image of *m/z* 197 (Au) is shown below (Figure 5). This consistency is found in other studies as well [1].

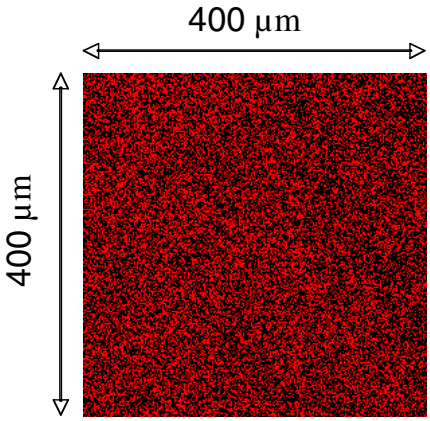

Figure 5. Au (*m/z* 197) mass spectral image 0-37 cts.

References

[1] McQuawCM, Zheng L, Ewing AG, Winograd N, *Langmuir* **2007**;23(10) 5645-5650