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Journal of Visualized Experiments, Senior Science Editor Nandita Singh 6th of July, 2019.

Manuscript entitled: "A quantitative fluorescent microscopy based single liposome assay for detecting the compositional inhomogeneity between individual liposomes". **Author list:** Rasmus Münter, Thomas Lars Andresen and Jannik Bruun Larsen

Dear Nandita Singh

Based on our previous communication we have now finalized the aforementioned manuscript and would like to submit it to *Journal of Visualized Experiments* for consideration as a full paper.

In this work we provide an extensive protocol describing a fluorescent based single liposome assay and its use for quantifying the compositional inhomogeneity between individual liposomes of the ensemble. We especially highlight critical steps as we describe the complete process going through liposome formulation, surface immobilization, fluorescent imaging and data analysis. Also, we suggest how the assay can be expanded to study other scientific questions. We premise the assay with a thorough discussion how single liposome studies has made an enormous impact and facilitated novel insights in a vast number of scientific areas, including membrane fusion, protein structure dynamics and drug delivery.

We trust that this work will meet with your interest, and look forward to hearing from you in due course.

Sincerely Yours, Jannik Bruun Larsen

Further details:

The manuscript contains approximately 5700 words in the main text and figure captions; it has 4 figures, in color, and 26 references. The material in this manuscript has not been published and is not currently under review for publication by another journal.

Referee suggestions:

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