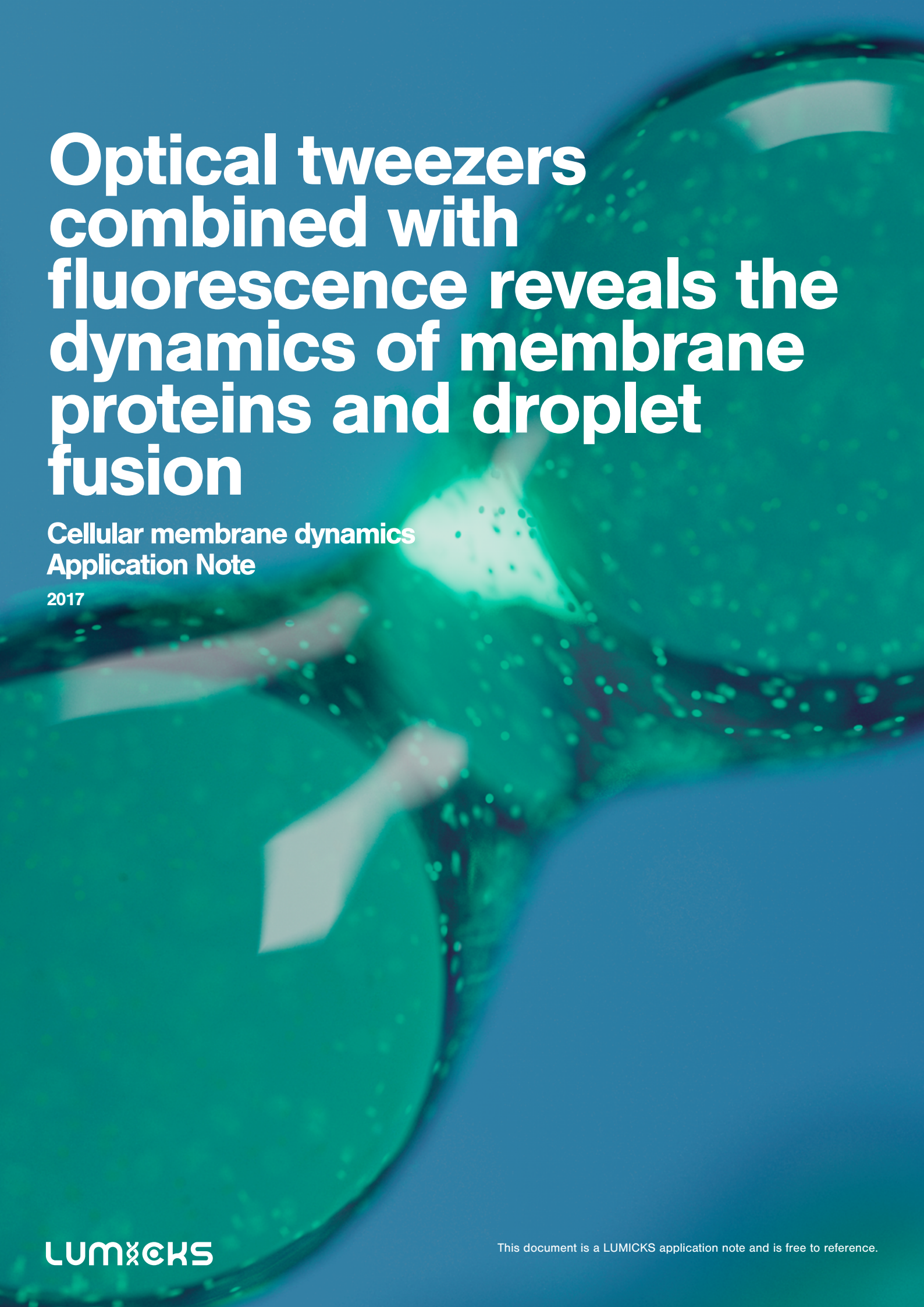
Optical tweezers combined with fluorescence reveals the dynamics of membrane proteins and droplet fusion

Cellular membrane dynamics Application Note

2017



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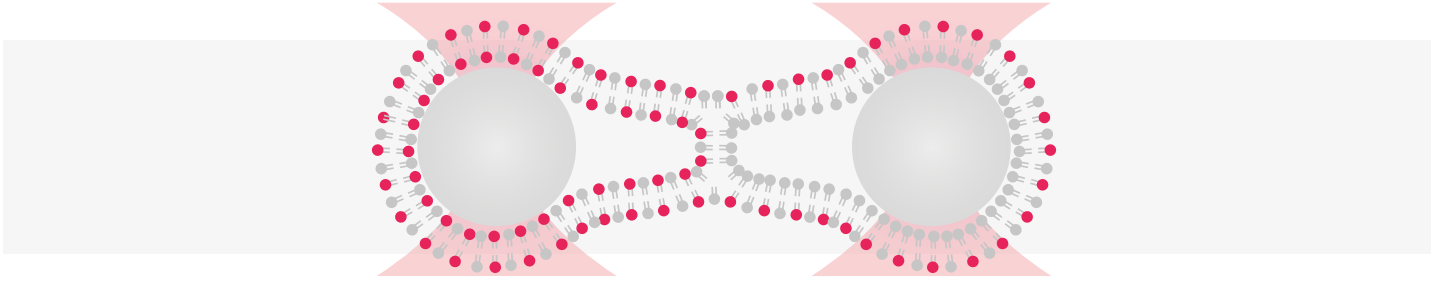
CELL MEMBRANE DYNAMICS
Application Note



1 A schematic representation of a membrane fusion experiment. Two optically-trapped beads are covered with a fused fluorescent lipid bilayer and brought in close proximity. By moving the beads apart, the fusion between the membranes can be observed by an increase in force. Concurrently, the membrane and stalk formation can be seen in the fluorescent signal.

Study membrane proteins and droplet fusion with force and fluorescence

Membrane fusion proteins play an essential role in a wide variety of cellular processes as they control

cell membrane dynamics and fusion. Correlative Tweezers – Fluorescence Microscopy enables direct quantification and visualization of membrane stalk formation and fusion events.

With the C-Trap's combination of optical tweezers and fluorescence microscopy it is possible to perform force-fluorescence membrane fusion experiments. Interactions between two lipid bilayer membranes can be visualized with confocal, widefield, or STED imaging and the kinetics can be tracked with high resolution.

These membrane interactions, such as protein-mediated membrane fusion, can be studied in high detailby correlating the liposomal lumen activity with the mechanical properties of membrane fusion.

The schematic illustration above shows a typical force-fluorescence membrane fusion experiment. Two optically trapped beads are covered with a fused fluorescent lipid bilayer and brought in close proximity. By moving the beads apart, the fusion between the membranes can be observed by an increase in force. Concurrently, the membrane and stalk formation can be seen in the fluorescent signal.

Figure 2 shows a proof-of-principle of correlated force and fluorescent measurements. Two beads covered with a phospholipid bilayer (the right contains fluorescently labeled lipids) are brought together in the presence Doc2b, **a membrane-interacting protein**, and separated sequentially after the initital contact. Upon separation of the beads, we can observe the formation of a micrometer-long membrane stalk in between. Moreover, our simultaneous force measurments showed that the formation of the stalk membrane is accompanied by high rupture forces (>600 pN), whereas low rupture forces (<25 pN) are measured if no membrane-interacting protein is present.

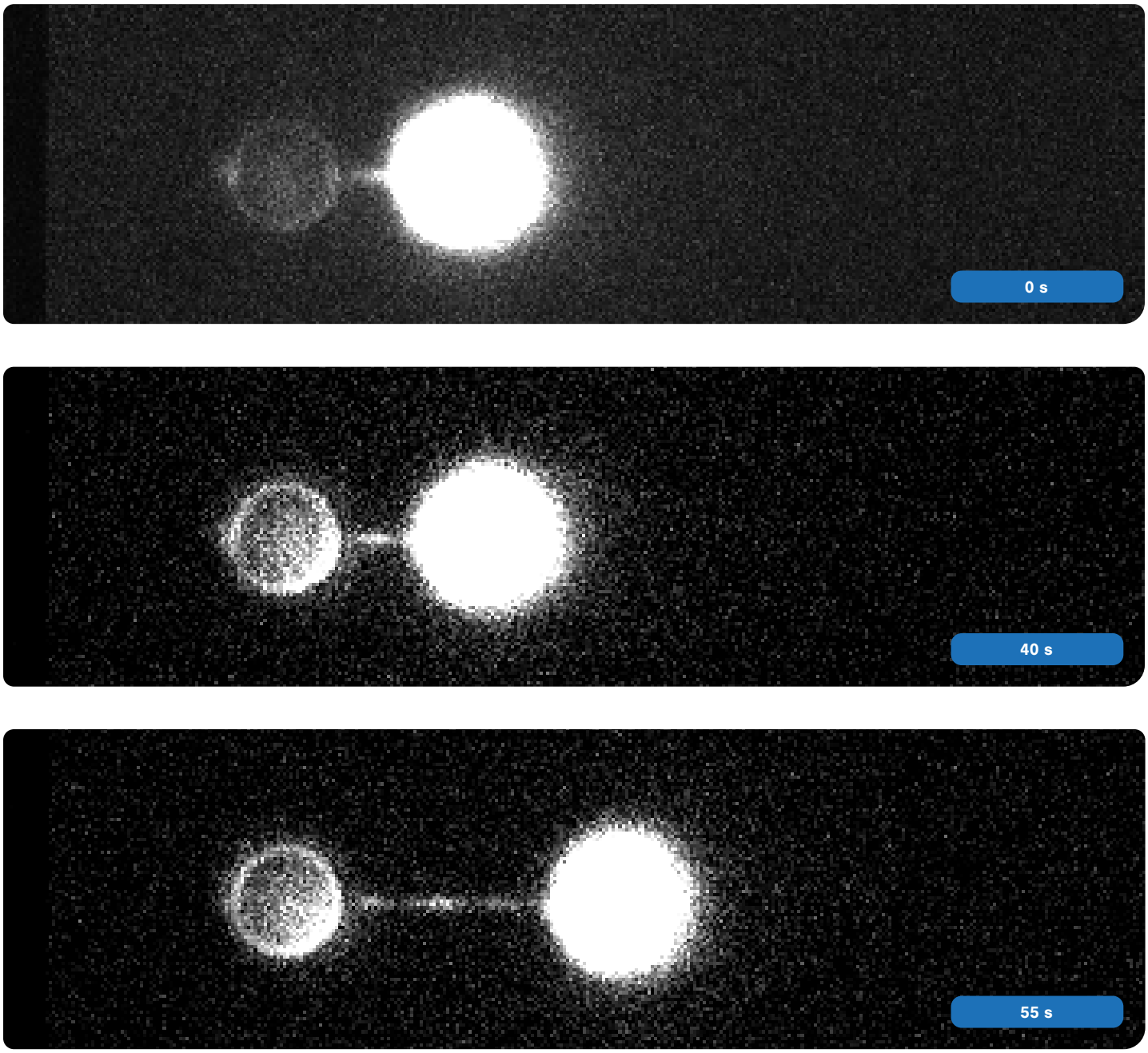
At the same time, confocal imaging of membrane fusion provides us with the ability to follow phospholipid mixing at video rate. The strong fluorescence increase in the unlabeled membrane and a concurrent decrease in the labeled membrane indicate that either hemifusion or full membrane fusion occurred [1].

User insights

Prof. Sarah Köster

Georg August University

"By imaging the mechanical properties and the dynamics of cells we can understand their function. With this instrument we've been able to start from scratch, go to 100% within a day and really focus on the biophysics and scientific problems."



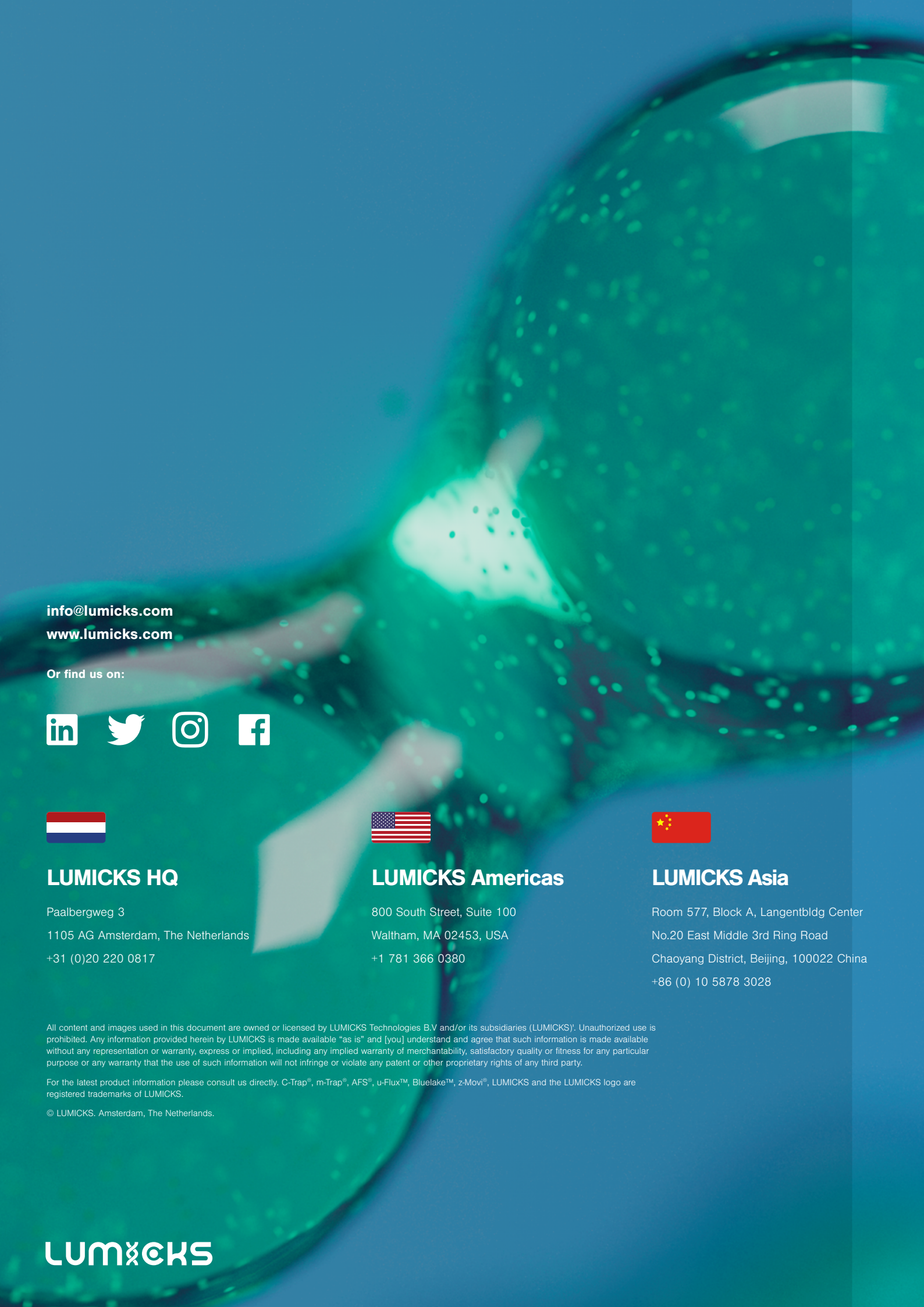
2 Fluorescent images of two membrane-coated beads at different time-points. As the beads are separated upon initial contact, the formation of a micrometer long stalk is observed. The increase in fluorescent signal of one bead and the concurrent decrease in fluorescent signal of the other bead indicates mixing of the phospholipids by the presence of a lumen, resulting from membrane fusion or hemifusion.

Read more:

[1] Brouwer et al. Nature Communications (2015)

Sample courtesy

Prof. Dr. Gijs Wuite and Prof. Dr. Erwin Peterman at the VU University Amsterdam



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