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## How To Characterize Individual Nano-Size Liposomes With Simple Self-Calibrating Fluorescence Microscopy

Kim I. Mortensen\*<sup>1</sup>, Chiara Tassone<sup>1</sup>, Nicky Ehrlich<sup>1</sup>, Thomas L. Andresen<sup>1</sup>,  
and Henrik Flyvbjerg<sup>1</sup>

<sup>1</sup> *Department of Micro- and Nanotechnology, Technical University of Denmark,  
Kongens Lyngby, DK-2800, Denmark.*

\*E-mail: [kim.mortensen@nanotech.dtu.dk](mailto:kim.mortensen@nanotech.dtu.dk)

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Nano-size lipid vesicles are used extensively at the interface between nanotechnology and biology, e.g. as containers for chemical reactions at minute concentrations and vehicles for targeted delivery of pharmaceuticals. Typically, vesicle samples are heterogeneous as regards vesicle size and structural properties (Fig. 1). Consequently, vesicles must be characterized individually to ensure correct interpretation of experimental results. Here [1] we do that using dual-color fluorescence labeling of vesicles—of their lipid bilayers and lumens, respectively (Fig. 1). A vesicle then images as two spots, one in each color channel. A simple image analysis determines the total intensity and width of each spot. These four data all depend on the vesicle radius in a simple manner for vesicles that are spherical, unilamellar, and optimal encapsulators of molecular cargo. This permits identification of such ideal vesicles. They in turn enable calibration of the dual-color fluorescence microscopy images they appear in. Since this calibration is not a separate experiment but an analysis of images of vesicles to be characterized, it eliminates the potential source of error that a separate calibration experiment would have been. Non-ideal vesicles in the same images were characterized by how their four data violate the calibrated relationship established for ideal vesicles. In this way, our method yields size, shape, lamellarity, and encapsulation efficiency of each imaged vesicle. Applying this procedure to extruded samples of vesicles, we found that, contrary to common assumptions, only a fraction of vesicles are ideal.

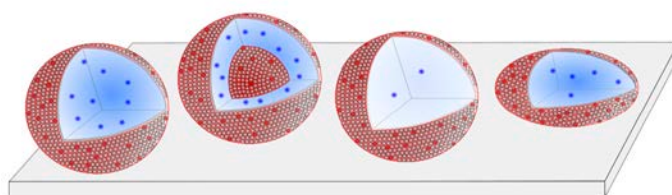


Figure 1: Fluorescence microscopy allows characterization of individual, dual-color labeled (red, lipid bilayer; blue, lumen) vesicles with regards to size, shape, lamellarity, and encapsulation efficiency.

[1] K.I. Mortensen, C. Tassone, N. Ehrlich, T.L. Andresen, and H. Flyvbjerg, *Nano Letters* (2018); doi: 10.1021/acs.nanolett.7b05312