

## Supplementary Data

# Aerosol–Cell Exposure System Applied to Semi-Adherent Cells for Aerosolization of Lung Surfactant and Nanoparticles Followed by High Quality RNA Extraction

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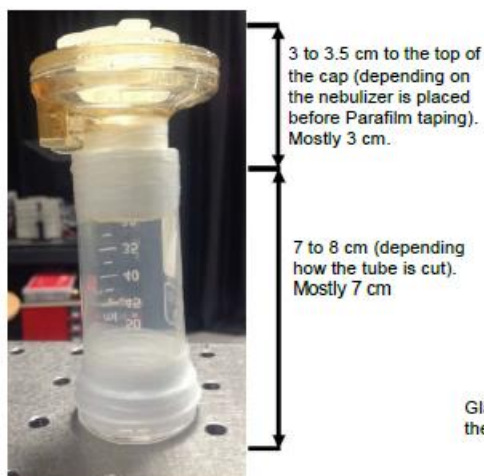
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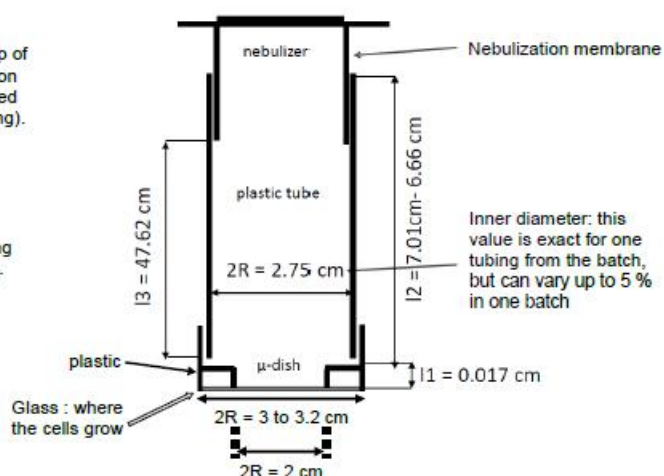
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A) Photograph of the nebulizer/tube construction



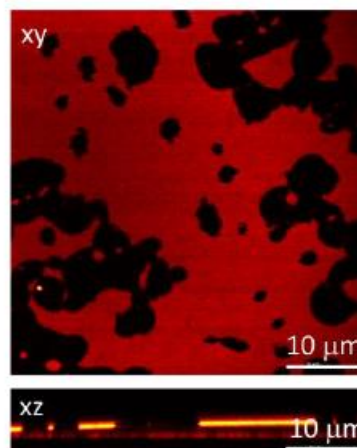
B) Schematics of the Nebulizer/tube/ $\mu$ -dish construction



C) Picture of the nebulizer/tube/ $\mu$ -dish setup on the microscope

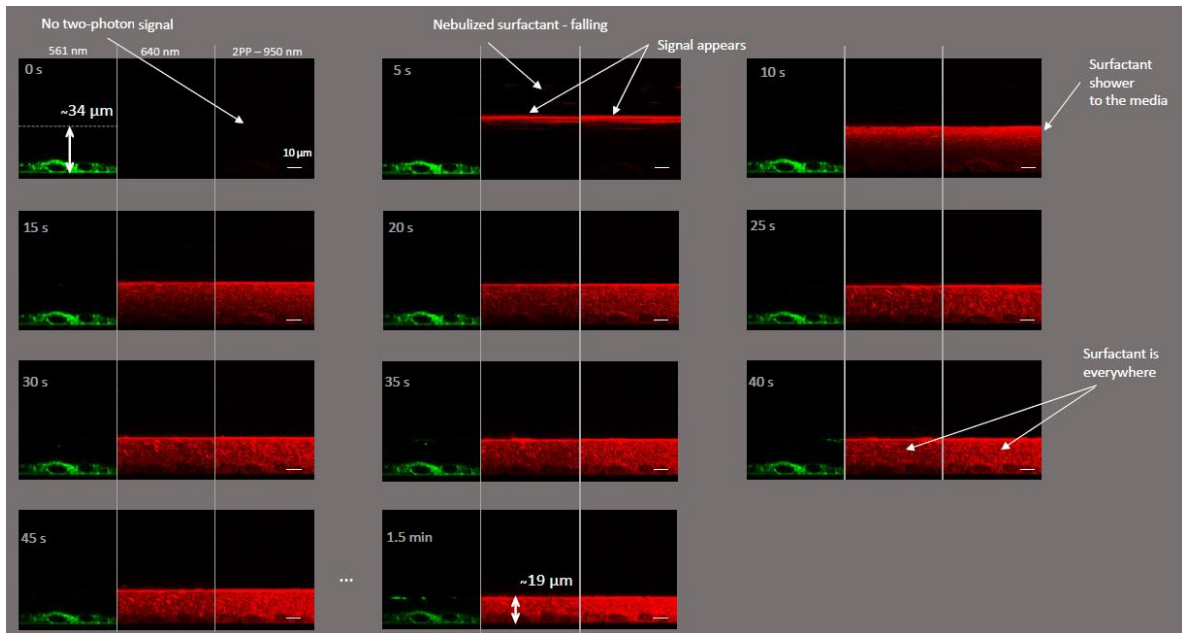


D) Nebulization of 0.5 monolayer of surfactant

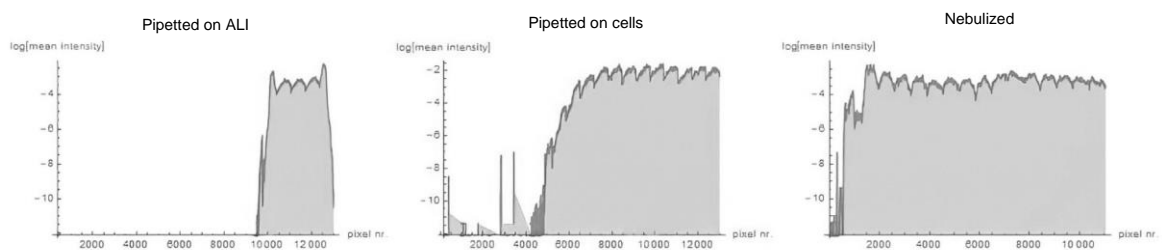


**Figure S1:** Nebulizer/tube/ $\mu$ -dish setup for microscopy.

The estimation of the fraction of nebulized surfactant which reaches the sample was performed by nebulizing 12  $\mu\text{g}$  of surfactant (100  $\mu\text{L}$  of 0.12  $\mu\text{g}/\text{mL}$  surfactant), resulting in approximately 0.5 monolayers of surfactant on the sample (red area on Supp Data 1D). Considering that a lipid monolayer has a surface area of 500  $\text{m}^2/\text{g}$  and the surface of the sample was 3.5  $\text{cm}^2$ , the mass of the 0.5 monolayer deposited on the sample was 0.3  $\mu\text{g}$ . This estimation was used to determine the amount of surfactant nebulized in other experiments as well.



**Figure S2:** Time series of nebulizing Curosurf-StarRed, fluorescence microscopy at 561 nm, 640, and 950 nm. Most of the material is nebulized in the first 30 s, and the system settles in a few minutes;



**Figure S3:** Intensity distribution of Curosurf-StarRed fluorescence, under different conditions