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Dear Benjamin Werth,

Enclosed, please find our invited submission titled "*Direct Stochastic Optical Reconstruction Microscopy of Extracellular Vesicles in Three Dimensions*" for consideration in *the Journal of Visualized Experiments*.

Extracellular vesicles (EVs) or exosomes are intimately involved in gene regulation and cell signaling and thus play an integral role in organismal homeostasis. The visualization and biophysical characterizations of EVs have become a popular area of research; however, due to their small size falling below the diffraction limit, visualization of EVs has proven difficult.

Here, we present our workflow on the resolving of individual EVs through super-resolution imaging, namely direct stochastic optical reconstruction microscopy (dSTORM). This approach can be used to visualize single EV morphology in all three dimensions. It yields the most physiologically relevant visualizations yet, by avoiding dehydration and freeze-fracture steps, high-speed centrifugation, and stringent fixation.

In conclusion, we propose that dSTORM be considered a reproducible and scalable method for the characterization of EVs.

We thank you very much for your time and consideration.

Sincerely,

Dirk Dittmer

Dirk Dittmer, Ph.D.