ADVANCING LIPID NANOPARTICLE CHARACTERIZATION WITH SUPER RESOLUTION IMAGING

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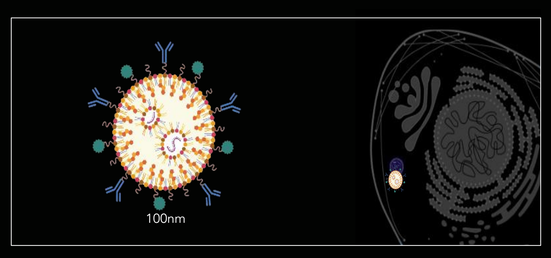
BACKGROUND

Lipid nanoparticles (LNPs) have emerged as an efficient vehicle for delivery of nucleic acid therapeutics, cytotoxic chemotherapy agents and antibiotics. Most LNPs for nucleic acid delivery contain phospholipid, ionizable lipid, PEGylated lipid and cholesterol which encapsulate the nucleic acids during formulation to make a spherical particle with a ~100 nm diameter (Figure 1).
Once in the body, an undesirable behaviour of LNPs is their propensity to accumulate in the liver. Targeting the particles to specific organs or tissues by adding molecules such as antibodies and ligands to the LNP surface is an active area of development.



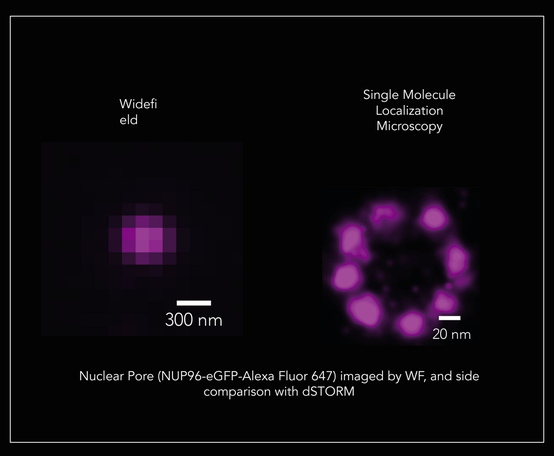


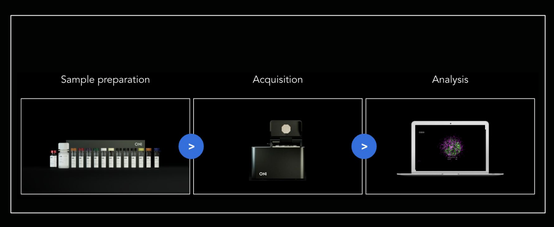
Figure 1: Single molecule localization microscopy (SMLM) provides higher resolution compared to diffraction limited widefield image. U2OS cells with nuclear pore subunit NUP96 endogenously tagged with GFP were labelled with anti-GFP antibody conjugated to Alexa Fluor 647 and imaged in widefield mode and subsequently in SMLM mode. SMLM localizations are fit from individual photon peaks and the dots represent individual localizations. Single molecule localization microscopy has the power to break the diffraction limit of light and resolve structures that are smaller than 100 nm in diameter all the way down to 20 nm.

Super-resolution microscopy brings new information for LNP research:

- Identify and characterize LNP populations - morphology and size
- Visualize single LNPs with 20 nm resolution
- Measure positivity, abundance and spatial distribution of targeting ligands
- Measure loaded fraction of RNA or DNA
- Image LNPs in living cells, track and measure their uptake
- Localize LNPs in fixed cells and determine their trafficking into organelles

METHODS & RESULTS

ONI offers the complete workflow for LNP capture, detection and quantification with our benchtop super-resolution microscope, the Nanoimager, accompanying reagent kit, LNP Profiler, and CODI software tools.



LNP PROFILER KIT

Application Kit™: LNP Profiler includes a microfluidic chip and reagents for capture of LNPs onto a glass surface as well as reagents for staining and imaging. It provides flexibility to suit different LNP samples: with the option of choosing between PEG, biotin or user-defined capture. ONI's pan-LNP stain and targeting ligand detection facilitates super-resolution imaging of the LNP itself and the targeting ligand. Finally, the RNA stain reveals the fraction of LNPs that are loaded with RNA. With the ability to image up to three markers at once, the LNP Profiler Kit simplifies identification and quantification of LNPs and their biomarkers.

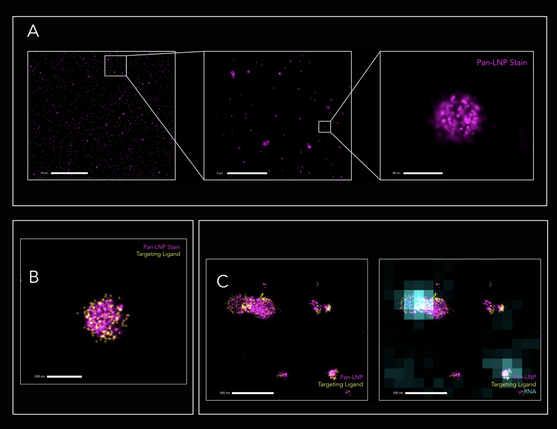


Figure 2. Visualization of LNP morphology, targeting ligand spatial distribution and RNA loading. PEGylated LNPs containing mRNA and functionalized with a targeting ligands were immobilized onto a glass surface using an anti-PEG antibody. Captured LNPs were then labelled with Pan-LNP, a targeting ligand and RNA staining reagents and imaged on a Nanoimager. A) With the Pan-LNP stain, thousands of LNPs are imaged per field of view with each LNP in super-resolution. B) The abundance and spatial distribution of the targeting ligand C) RNA positivity is revealed using the RNA stain

Figure 3. Individual LNPs can be accurately assessed for their size. A) The ONI platform facilitates direct measurement of the size of LNPs based on calculating the diameter of the localization cluster. B) Tens of thousands of individual LNPs can be analyzed per lane, revealing heterogeneity in the sample. Small differences in sizes can be detected without bias being introduced by large or small LNPs. LNP samples provided by Acuitas.

NANOIMAGER

The Nanoimager is a benchtop microscope, with a range of imaging modalities, e.g. dSTORM, DNA-PAINT, Single Particle Tracking, smFRET. This means researchers are able to directly characterize the LNPs as described previously but can also study their function by following their uptake into cells and individual organelles such as the endosome or lysosome in super-resolution. Examples shown here are with extracellular vesicles (EVs).

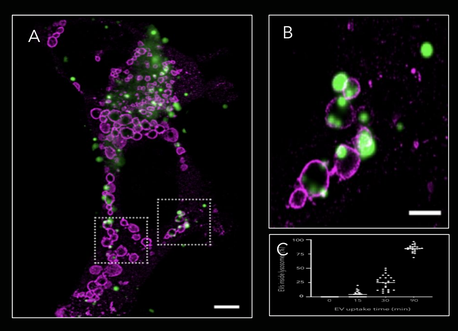


Figure 4. Individual extracellular vesicles can be detected in lysosomes on the Nanoimager. A) and B) Merged two-color images with dSTORM of the lysosomes (LAMP-1) (magenta) and TIRF of GFP-EVs (green) in human U2OS cells. Scale bars: 5 µm (left), and 2 µm (right). B) Proportion of GFP-EVs inside lysosomes per individual cell, over time. Almost all internalized GFP-EVs are found inside lysosomes after 90 min.

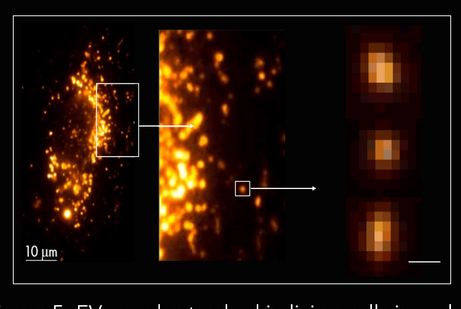


Figure 5. EVs can be tracked in living cells in real time on the Nanoimager. Tracking of EVs labeled with NHS-AF488 within a human umbilical vein endothelial cell to assess EV uptake. Sample prepared by Prof. Christopher Gregory, Edinburgh, UK.

CODI - AUTOLNP

AutoLNP on CODI is ONI's software platform that enables automated data acquisition and analysis so that novice users can get super-resolved LNP data within 30 mins with very little hands-on time. It provides nanoscale resolution visualization of single LNPs and their biomarkers. The platform offers analysis tools tailored to the LNP Profiler workflow, including correction of spatial drift, filtering of individual localizations, and cluster-based single molecule analysis. Researchers can extract quantitative data on LNP populations, such as targeting ligand and RNA positivity per sample, LNP size distribution, marker density per LNP, and LNP morphology parameters.

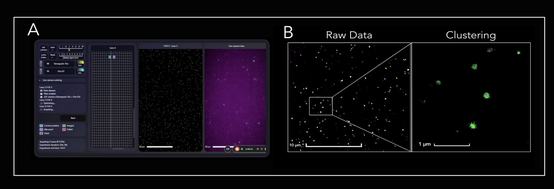


Figure 6. AutoLNP provides automated data acquisition and analysis. A) Once the user has loaded the microfluidic chip onto the Nanoimager AutoLNP takes care of the rest. First it finds focus, loads the acquisition settings then automatically acquires multiple fields of view per sample before moving to the next lane and continuing the acquisition. Currently, AutoLNP will acquire four samples before the user needs to load the next chip onto the Nanoimager. B) The CODI analysis tools localize the data as it comes off the camera and calculate clusters which correspond to the LNPs. Once clustering has been performed co-localization with other channels is performed to extract marker positivity, abundance and size. Finally, a report is generated with a visually representation of all the measurements.

APLO FLOW

Aplo Flow is ONI's automation solution for sample preparation. Default settings are provided that are specifically tailored to ONI reagent kits so that hands-on time and variability is kept to a minimum. Aplo Flow streamlines super-resolution microscopy, enhancing both speed and efficiency. It can prepare 12 samples for imaging in only 4.5 hours, reducing your hands-on time by up to 85% per sample.

CONCLUSIONS

A super-resolution based end-to-end workflow allows LNP researchers to better understand LNP formulations and their heterogeneity. The combination of LNP Profiler, AploFlow, Nanoimager and AutoLNP will simplify the workflow so that anyone can generate stunning super-resolution data and acquire previously inaccessible information on their benchtop.

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