Localisation of nanoparticles in whole cells using correlative cryo soft x-ray tomography and fluorescent microscopy

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Background incl. aims
In recent years nanoparticles have emerged as important players in modern medicine, with clinical applications ranging from contrast agents in imaging to carriers for drug and gene delivery into tumors. Nanoparticle surfaces can be easily functionalized to target specific disease sites, and their small size and specific material characteristics facilitate their delivery and detection. Current nanoparticles include metals and and other inorganic-based compounds, as well as polymer, lipid, or bioinspired nanoparticles. These are being developed to diagnose and treat a variety of different diseases ranging from cancer to inflammation. In order to assess both the efficacy and the effect of nanoparticle delivery on cells a number of questions arise such as; does the nanoparticle reach the cytoplasm and nucleus of cells where it might exert therapeutic effects on intracellular molecules?, what subcellular compartments does the nanoparticle enter following delivery to cells?, or does nanoparticle uptake influence cell structure? These questions can be addressed by examination of cell structure.

Methods
Cryo-soft X-ray tomography (cryo-SXT) was used to deliver 3D ultrastructural volumes of intact cells without chemical fixation or staining, to reveal nanoparticle uptake for nanomedicine. Initially, integrated cryo fluorescence was used to screen an entire EM grid to reveal the location of suitable cells for tomography. Low magnification/large field of view 2D x-ray mosaics were then acquired over large areas of the grid before acquiring a tilt series from ±60° on selected targets. Data from both modalities were then overlaid to provide the location of nanoparticles in the context of whole cell ultrastructure.

Results
Cryo-SXT volumes combined with fluorescent light images showing the 3D distribution of organic and inorganic nanoparticles ranging in diameter from around 50 nm to 200 nm, in the context of the cellular landscape and surrounding organelles.

Conclusion
Results prove the utility of lab-based cryo-SXT/FM for nanoparticle localisation in whole fully hydrated cells. The recent availability of the compact soft x-ray microscope will accelerate the further development of novel workflows and biological imaging applications that can benefit from this technique, including integration with electron microscopy.

Keywords:

cryo SXT/FM, correlative microscopy, nanomedicine

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