VISUALISING INTRACELLULAR UPTAKE USING STRUCTURED ILLUMINATION MICROSCOPY

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As a rapid, flexible super-resolution imaging technique requiring relatively modest illumination intensities, structured illumination microscopy (SIM) is well suited to live cell imaging experiments. We have used 2D SIM with attenuation of out of focus light [1] to visualise the process of intracellular uptake of exosomes and synthetic virus-like particles in real time.

Exosomes are small (30 – 100 nm) vesicles that transfer biologically active molecules, such as proteins and RNA, between cells. They hold great promise as a delivery mechanism for molecules used to treat a wide range of diseases, however limitations to our understanding about uptake efficiency and specificity limits their therapeutic use.

Virus-like capsules can be created from self-assembling peptides and offer a means of gene delivery and silencing in mammalian cells. Such capsules can also be engineered with antimicrobial properties [2].

In this presentation I will describe our experimental approach to time-lapse super-resolution imaging of particle uptake and give results for these two different delivery systems.

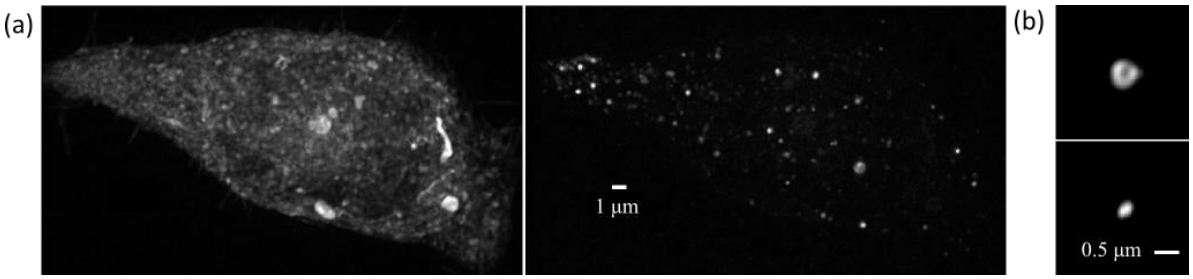

Figure 1. (a) Maximum intensity projection of a HEK293 cell labelled with CellMask deep red (left) containing DiO labelled exosomes (right). (b) A single peptide capsule (upper panel) loaded with siRNA (lower panel) within a HeLa cell (unlabelled).

[1] K. O'Holleran & M. Shaw, "Optimized approaches for optical sectioning and resolution enhancement in 2D structured illumination microscopy", *Biomed. Opt. Express*, **5** (2014)

[2] V. Castelletto et al., Structurally plastic peptide capsules for synthetic antimicrobial viruses, *Chem. Sci.*, **3** (2016).