A high-content method for the quantitative study of nanoparticle uptake and trafficking in 3D spheroids

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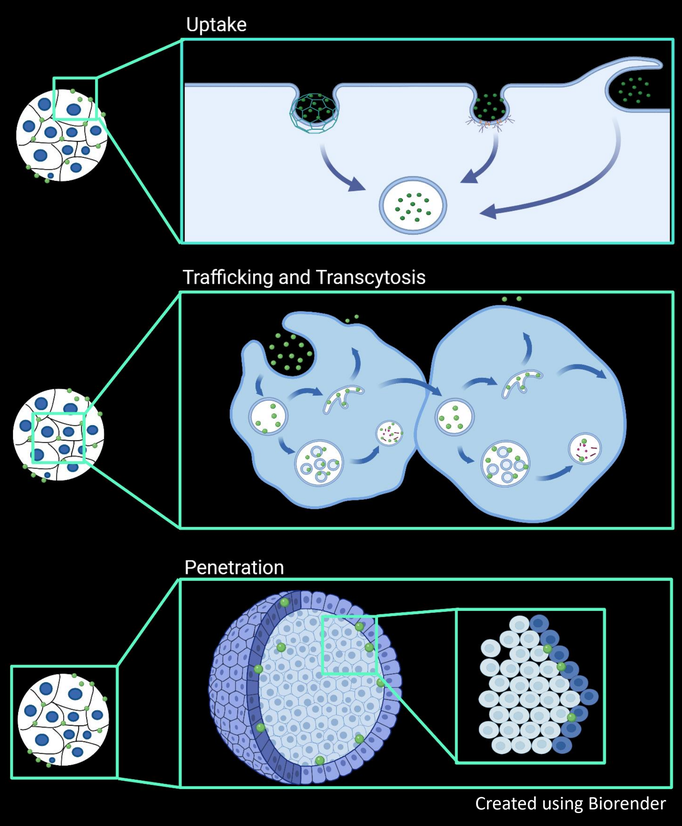
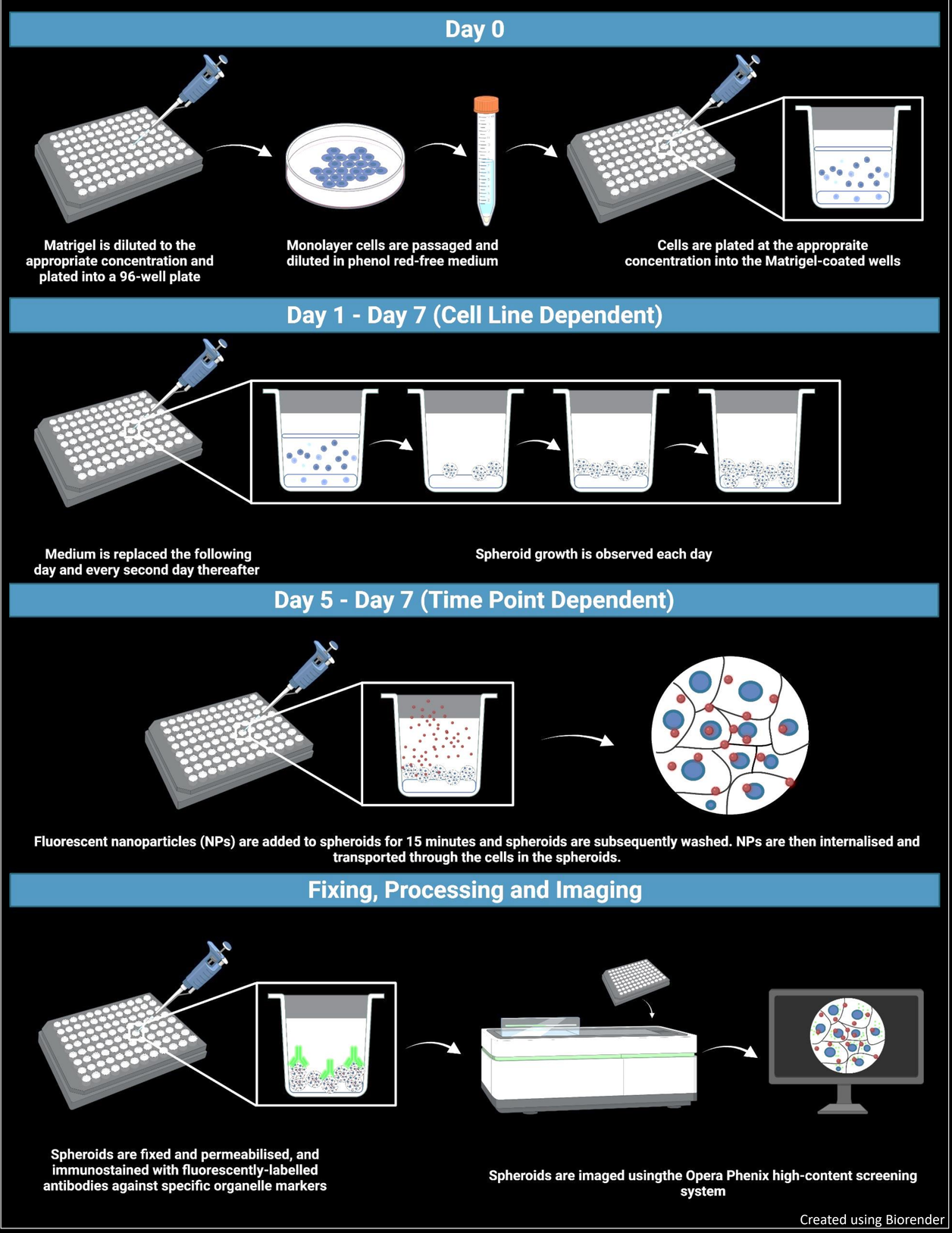
Introduction

- The attraction of using nanoparticles (NPs) in drug delivery comes from their ability to directly target diseased tissue, and their potential for improved efficacy and safety in comparison to conventional drugs used alone
- Little mechanistic knowledge of NP uptake, trafficking and transcytosis exists
- The majority of studies assessing NP uptake and transport have been performed in cells growing as 2-dimensional (2D) monolayers
- Multicellular tumour spheroids provide a more physiologically relevant model to assess NP transport and their potential as drug delivery vehicles



Methodology



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Cell Screening Team

Results

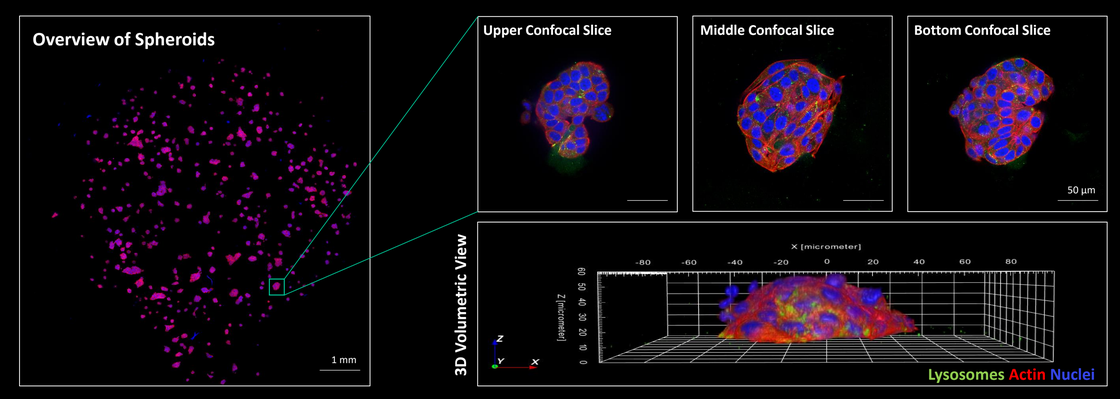

Figure 1: Fully automated HCS confocal imaging of H358 spheroids. Panels show a single well overview, as well as three example confocal slices and the volumetric reconstruction of one spheroid. Nuclei stained with Hoechst 33342 are shown in blue, actin stained with fluorescently-conjugated phalloidin in red, and lysosomes immunostained for LAMP1 in green

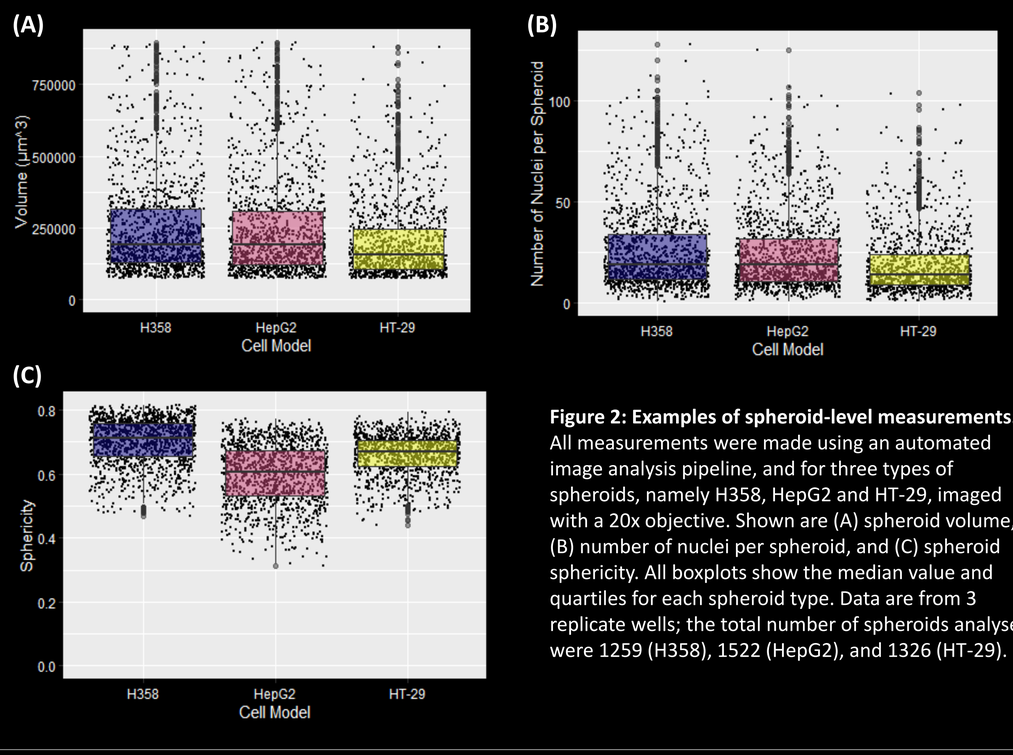

Figure 2: Examples of spheroid-level measurements. All measurements were made using an automated image analysis pipeline, and for three types of spheroids, namely H358, HepG2 and HT-29, imaged with a 20x objective. Shown are (A) spheroid volume, (B) number of nuclei per spheroid, and (C) spheroid sphericity. All boxplots show the median value and quartiles for each spheroid type. Data are from 3 replicate wells; the total number of spheroids analysed were 1259 (H358), 1522 (HepG2), and 1326 (HT-29).

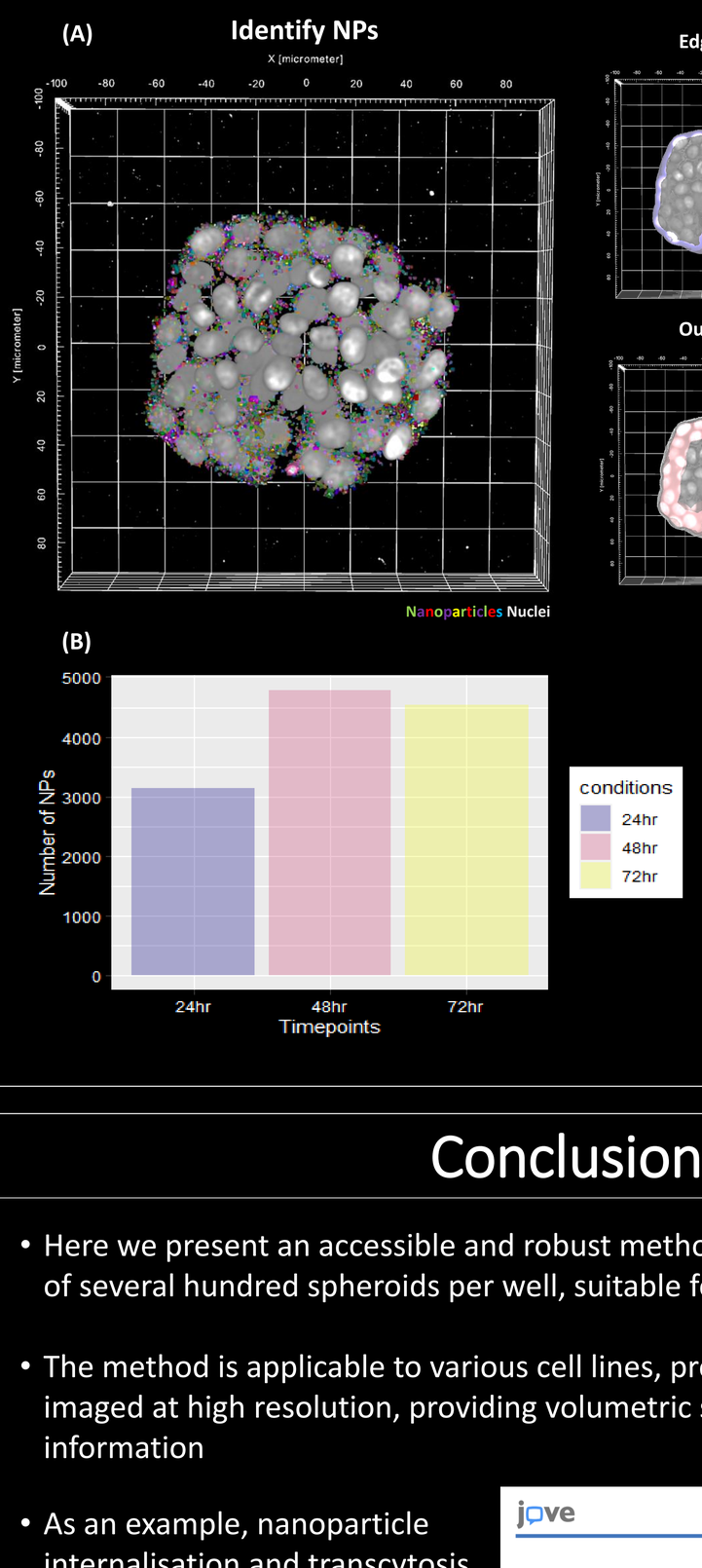

Figure 3: Examples of key steps in image analysis and quantification of NP internalisation. (A) NPs are identified in a volumetric manner by detection of the fluorescently labelled NPs. Spheroids can be segmented into various layers in order to study the distribution and penetration of NPs over time. (B) The number of NPs associated with cells in each spheroid can be subsequently calculated over time. Data are from 3 replicate wells per time point; the total number of spheroids analysed were 410.

Conclusions

- Here we present an accessible and robust method for the large-scale production of several hundred spheroids per well, suitable for HCS and HCA applications
- The method is applicable to various cell lines, producing spheroids that can be imaged at high resolution, providing volumetric spheroid, cell and subcellular information
- As an example, nanoparticle internalisation and transcytosis can be measured, providing information about efficacy of drug delivery and penetrance

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A Robust Method for the Large-Scale Production of Spheroids for High-Content Screening and Analysis Applications

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