A HCS platform for systematic analysis of 3D spheroids at subcellular resolution

Margaritha M. Mysior & Jeremy C. Simpson
Cell Screening Laboratory, School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland

1. Background

- Compartmentalisation across the endomembrane system ensures spatial distribution of proteins, lipids and carbohydrates, and increases biochemical efficiency
- Exchange of material via trafficking pathways between compartments requires a high degree of molecular control

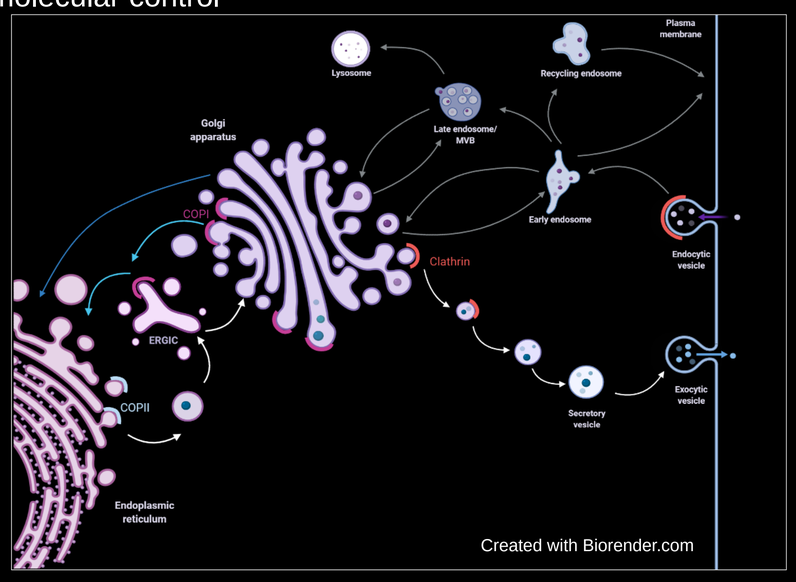


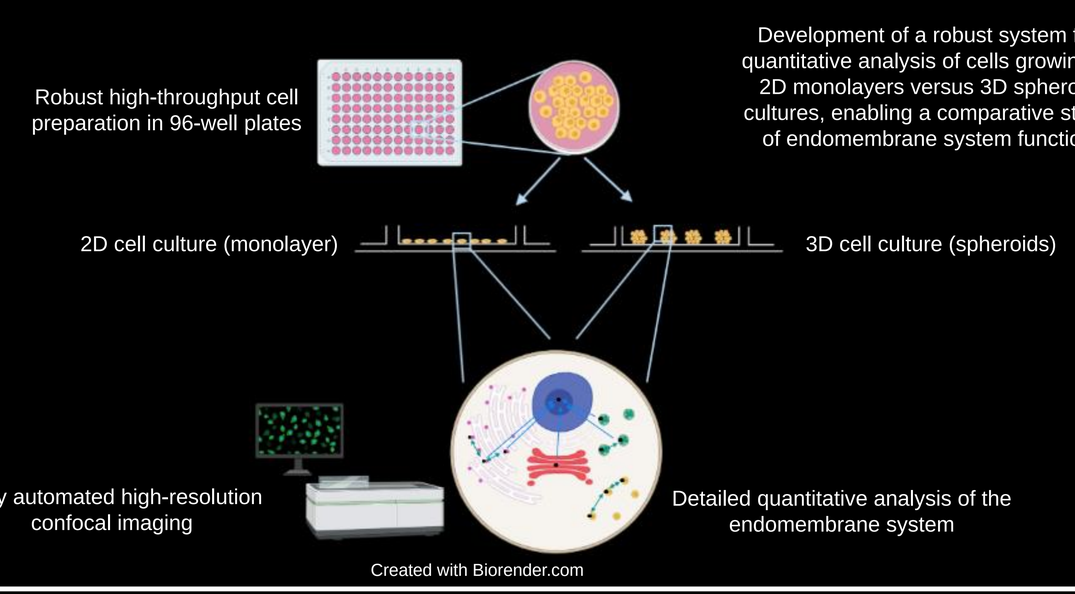
Fig 1: **The endomembrane system in eukaryotic cells.**

The endomembrane system with its organelles. Trafficking pathways are indicated by the coloured arrows.

2. Objectives

Using high-content screening (HCS) analysis, the aim of this study is to investigate and compare the organisation of the endomembrane system in cells growing as 3D spheroids versus traditional 2D monolayers

3. Methodology



4. Results

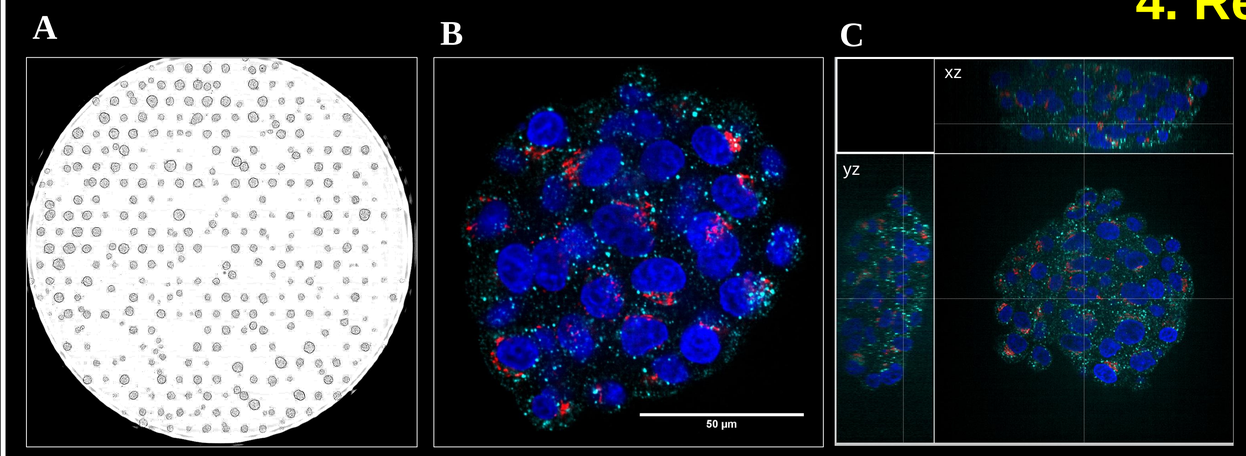


Fig 2: **HeLa Kyoto cell spheroids grown in a customised micropatterned 96-well plate.**

HeLa Kyoto cells grown for three days on customised disc micropatterns coated with fibronectin. (A) Brightfield overview of an entire well within a 96-well plate. (B) Image of a single spheroid. (C) XYZ views of a single spheroid.

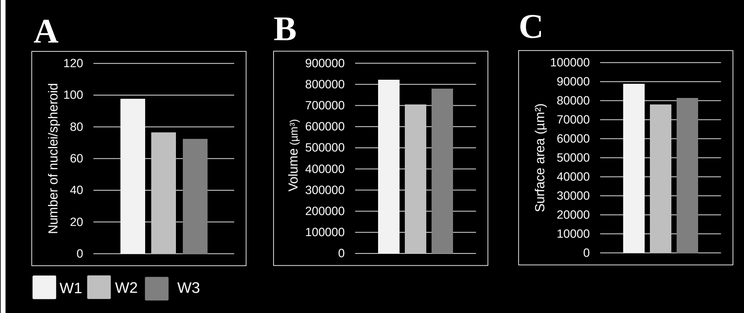


Fig 3: **Morphological properties of spheroids growing on a micropatterned plate.**

(A) Mean number of nuclei per spheroid for three wells. (B) Mean volume of a spheroid for three wells. (C) Mean surface area of a spheroid for three wells.

- Micropatterning allows spheroids to be grown in a highly reproducible manner
- Automated measurement of spheroid-level characteristics achieved

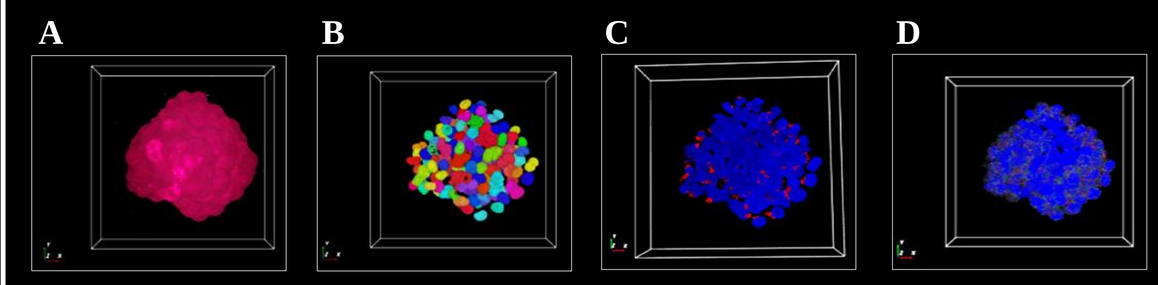


Fig 4: **Volumetric analysis of spheroids.**

(A) Volumetric segmented spheroid. (B) Volumetric segmented nuclei. (C) Volumetric segmented Golgi (red). (D) Volumetric segmented early endosomes (grey).

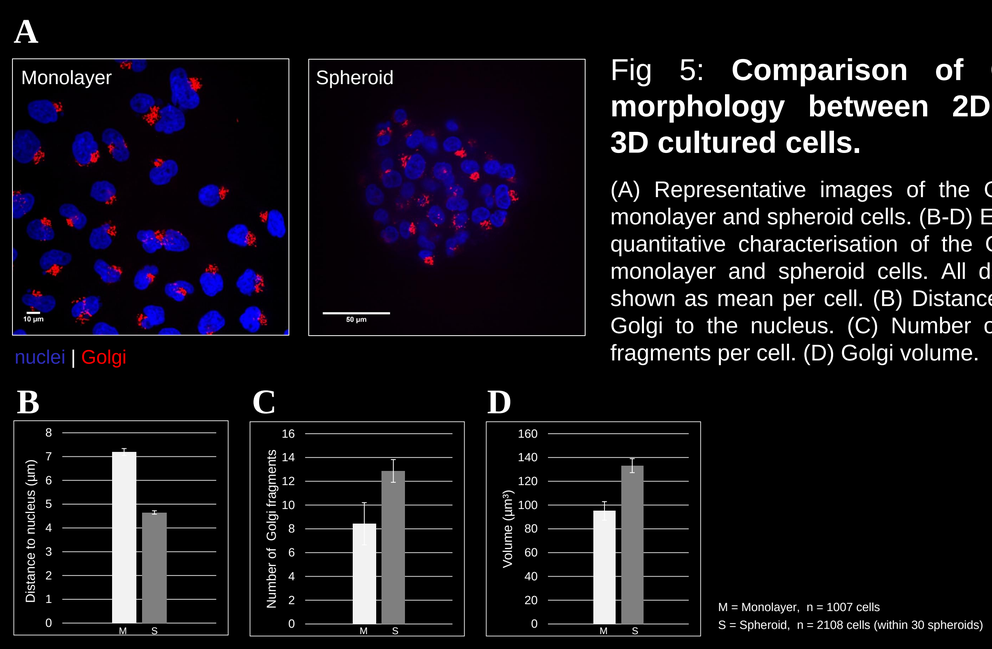


Fig 5: **Comparison of Golgi morphology between 2D and 3D cultured cells.**

(A) Representative images of the Golgi in monolayer and spheroid cells. (B-D) Example quantitative characterisation of the Golgi in monolayer and spheroid cells. All data are shown as mean per cell. (B) Distance of the Golgi to the nucleus. (C) Number of Golgi fragments per cell. (D) Golgi volume.

M = Monolayer, n = 1007 cells
S = Spheroid, n = 2108 cells (within 30 spheroids)

M = Monolayer, n = 648 cells
S = Spheroid, n = 1886 cells (within 30 spheroids)

- Automated measurement of organelle features in cells growing as spheroids
- Results suggest that there are differences in subcellular organisation in 2D versus 3D grown cells

5. Summary

- Disc micropatterned plates allow the growth of small uniform spheroids
- High-resolution images of the compartments of the endomembrane system can be obtained from small uniform spheroids using fully automated HCS microscopy
- Volumetric and morphological analysis of compartments of the endomembrane system are possible on a single cell and whole spheroid basis
- Quantitative comparison between monolayer and spheroid cells possible

6. Acknowledgements & References

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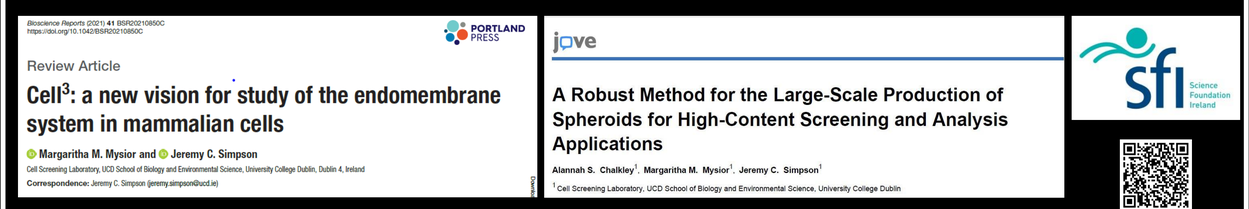
More information can be found here:



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