

Evaluation of the bioactivity of compounds using Cell Painting assay and alternative High-Content assays



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Introduction

Cell Painting assay

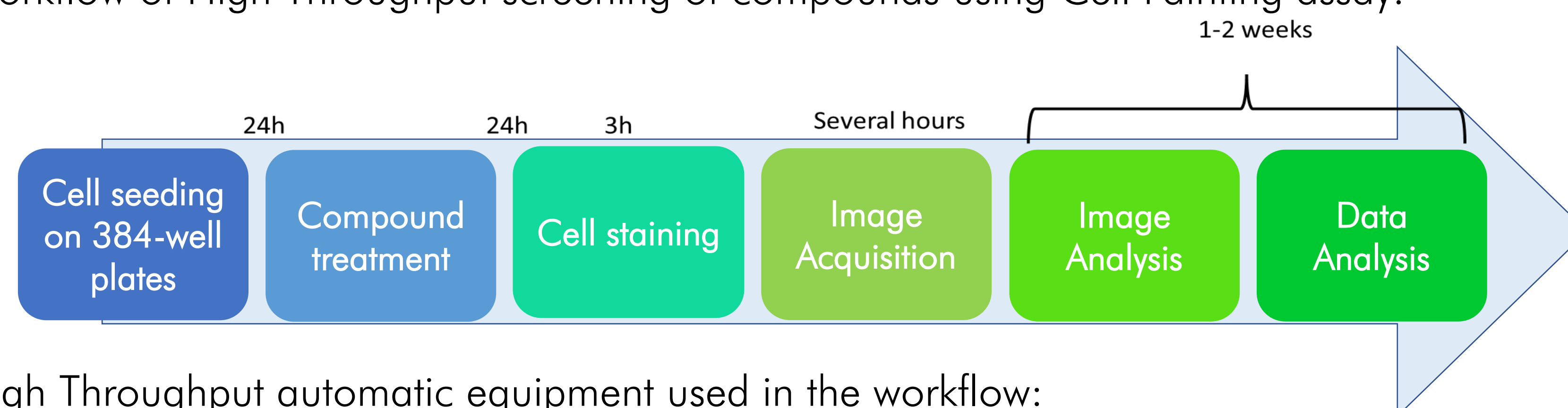
- Cell Painting assay is a popular method of High-Content screening, that allows visualising changes in the cell morphology caused by any perturbation.
- This assay employs 6 fluorescent dyes to stain cell components and compartments, generating images.
- The acquired images are used to define the phenotypic profile of cells through multi-parametric analysis.
- Cell Painting assay enables the prediction of the mode of action (MOA) of compounds and early detection of their subtle cytotoxicity.
- Our Centre aims to optimise the Cell Painting protocol for High-Content screening of libraries of compounds across various cell lines.

Other High-Content phenotypic assays

- Although Cell Painting offers several advantages, improvements can be made by replacing the dye selection and utilizing different sets of image parameters to create more diverse and detailed morphological profiles.
- Our current research aims to identify other High-Content phenotypic assays to the 6 dyes used in the standard Cell Painting protocol. This will enable the staining of unstained compartments and employ additional approaches to confirm the bioactivity of studied compounds.

Methods

- Workflow of High Throughput screening of compounds using Cell Painting assay:



- High Throughput automatic equipment used in the workflow:



- Software used to perform Image Analysis and extraction of features:

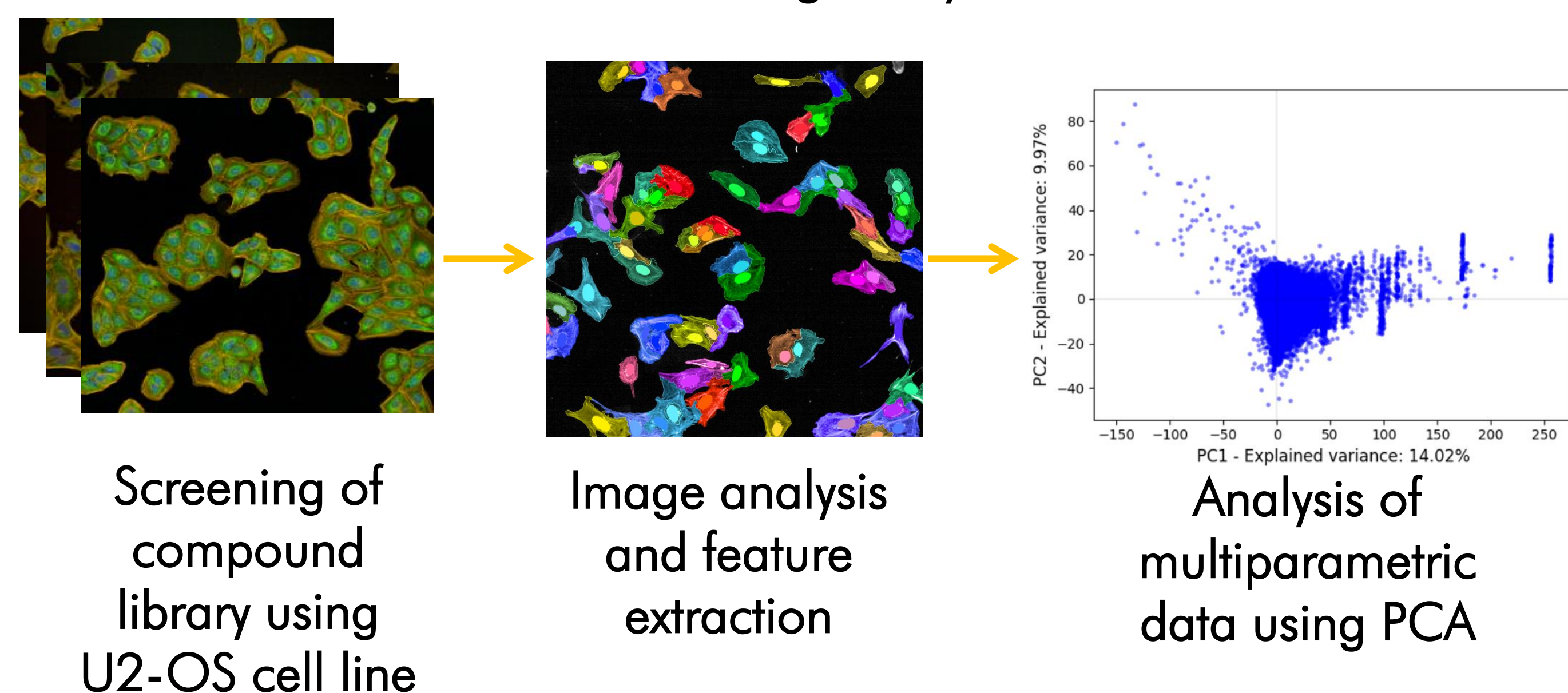


- Development of proprietary software:

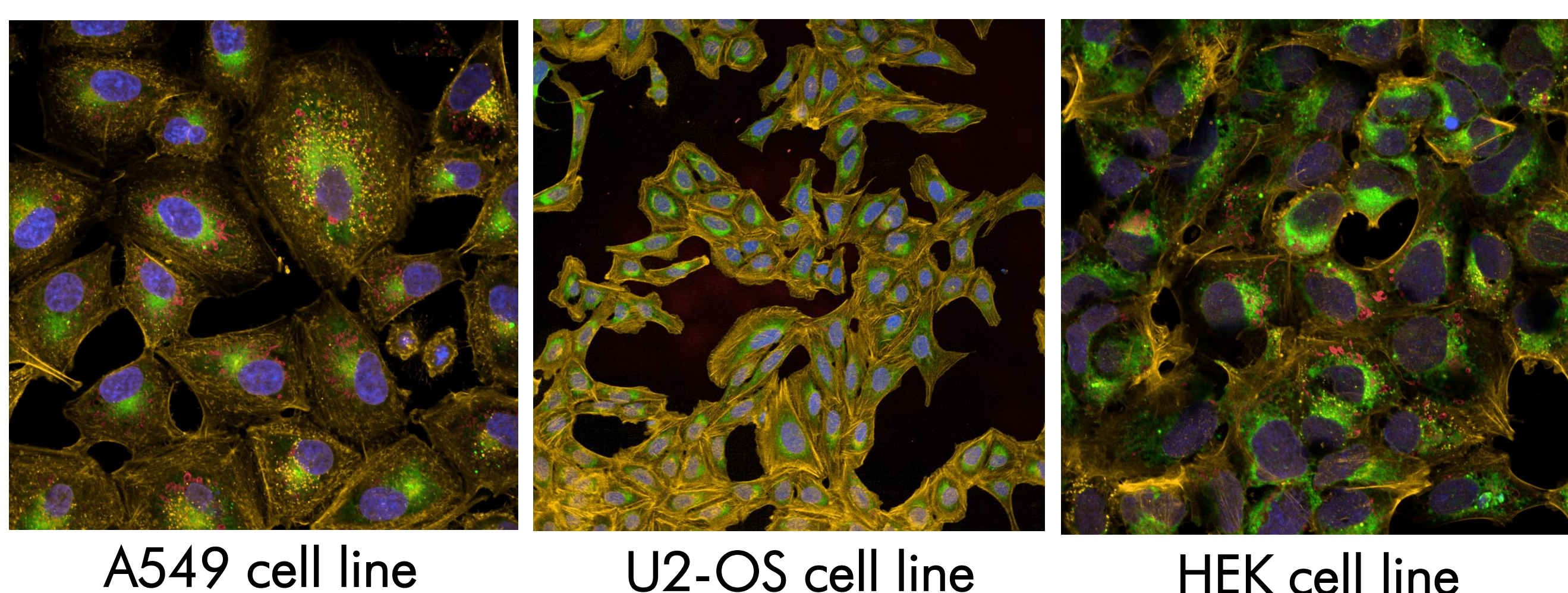


Results

Cell Painting assay

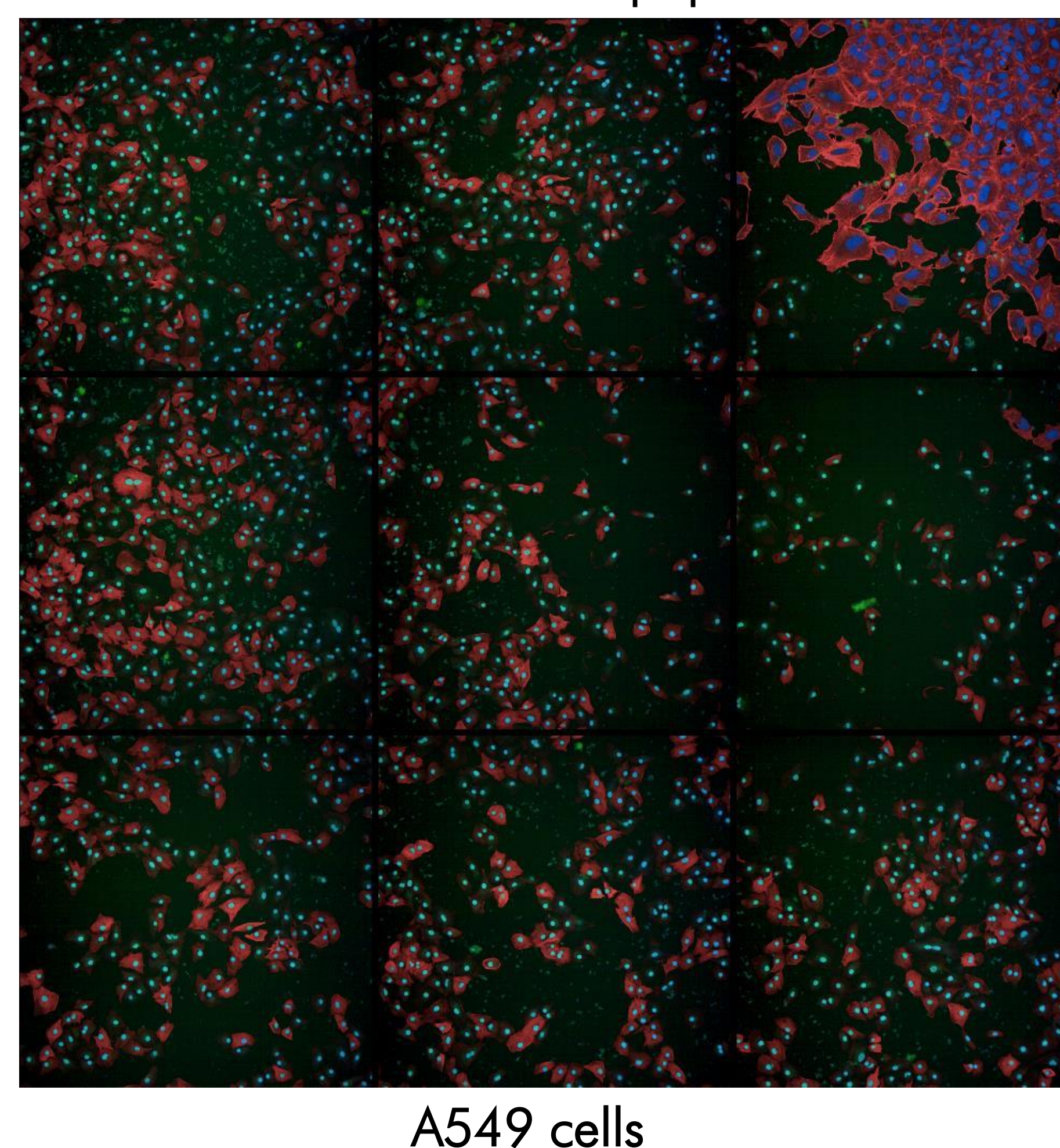


Cell Painting assay for different cell lines:



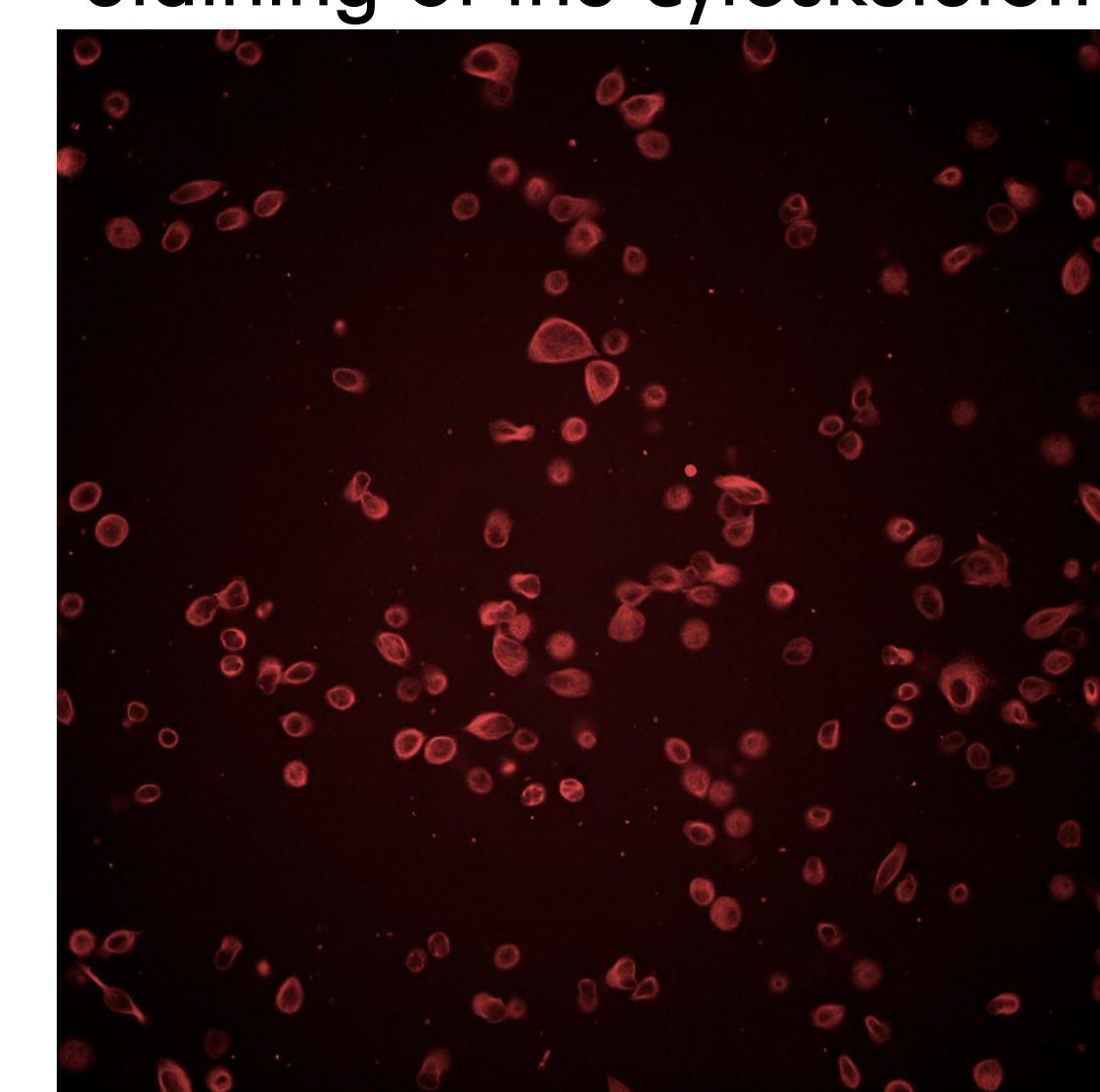
Other High-Content assays

Visualization of apoptosis



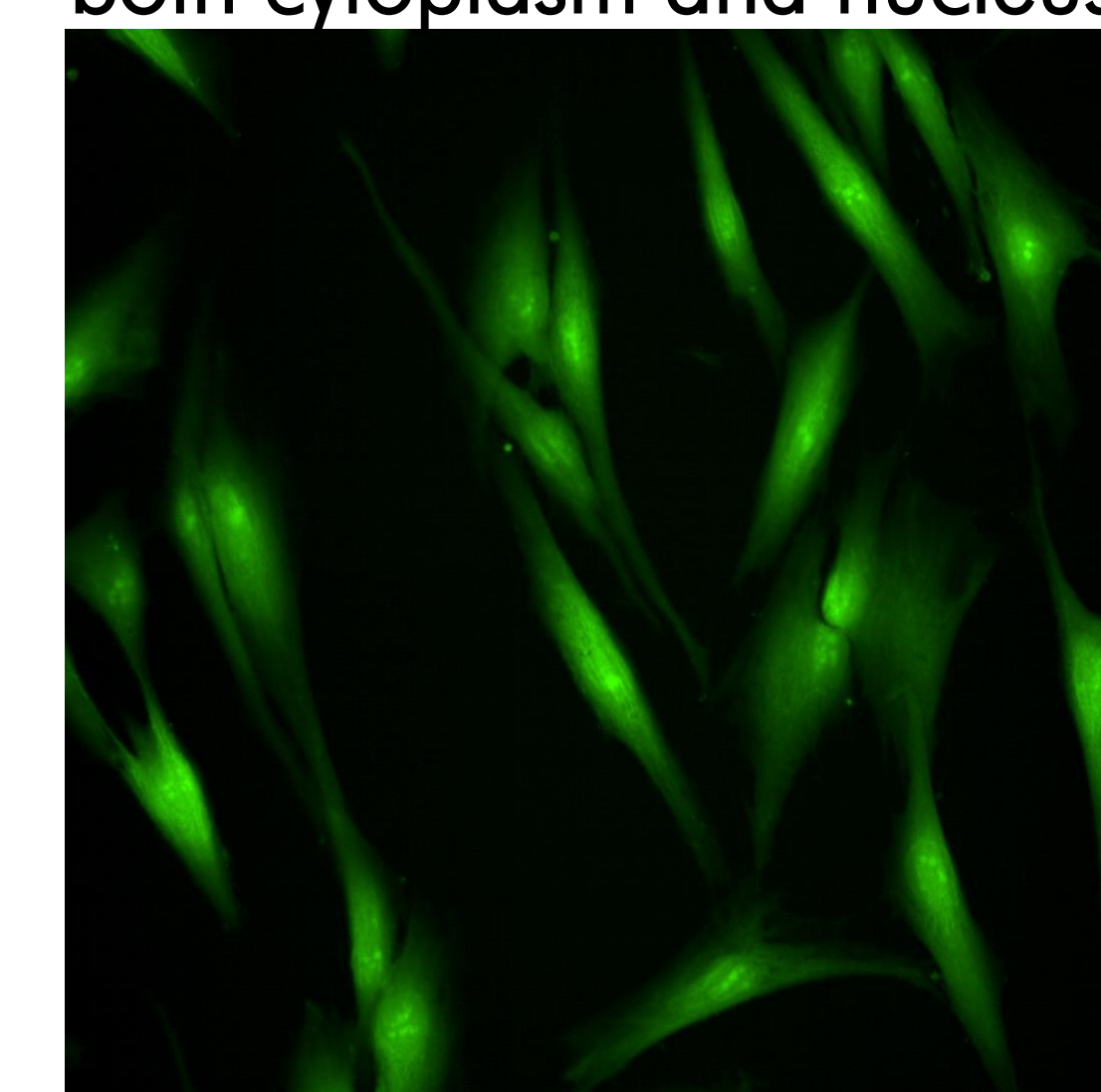
A549 cells

Staining of the cytoskeleton



PC3 cells

Compartmentalization of both cytoplasm and nucleus



DM2 cells

Conclusions

- Cell Painting is a powerful tool in drug discovery – it allows for multi-parametric and detailed/unique quantitative description of cell states induced by chemical, genetic or other perturbations.
- Cell Painting allows to understand deeper the impact of treatment on the cell phenotype and acquire information on potential MOA to facilitate further steps in drug discovery.
- Finding other High-Content approaches to Cell Painting assay allows to expand the knowledge about the bioactivity and potential cytotoxicity of compounds.
- Identification of other dyes may improve the recognition of a larger diversity of mechanisms which take place in the cell and enable additional staining of particular molecular targets of interest.

References

- M.A. Bray et al. (2016), Nat. Protoc. 11, 1757-1774.

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