

Drug discovery screening in scalable organotypic 3D models



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Introduction

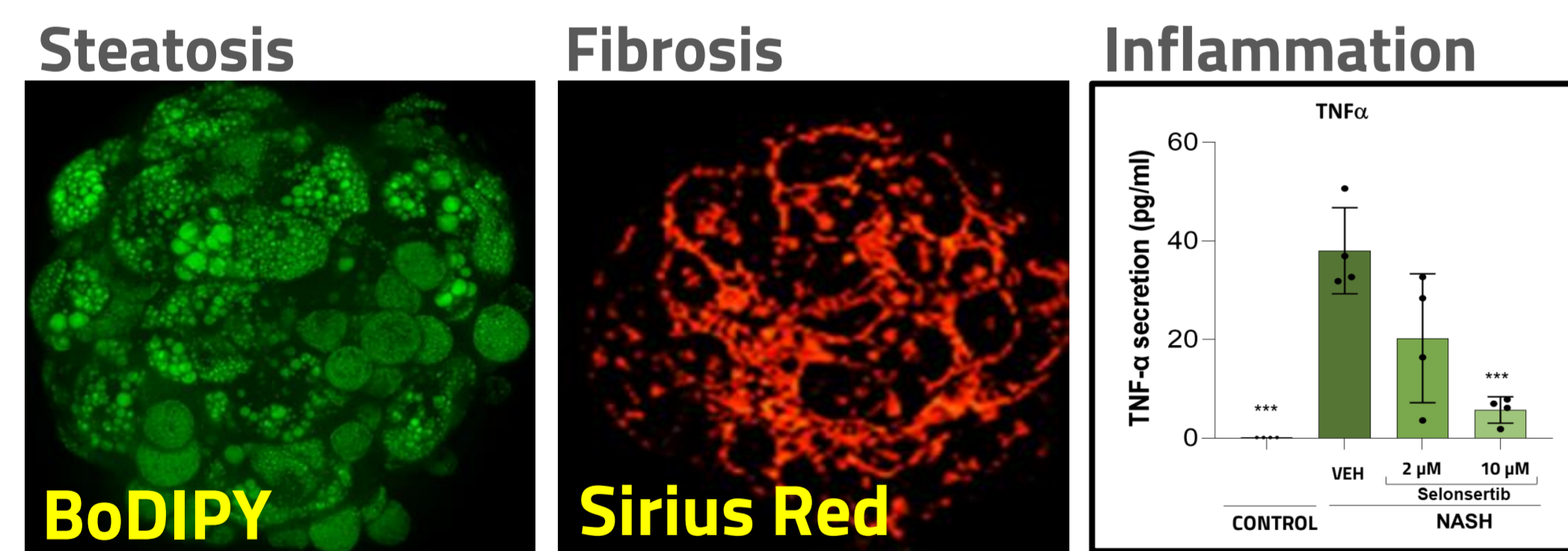
The cyto-architecture of human tissue, including the spatial organization and interactions of native cell types, is essential for normal organ function and viability. Therefore, it makes sense to study the development, prevention, and reversal of human disease in the context of heterotypic tissue, rather than in isolated cell populations. It also stands to reason that the inclusion of organotypic *in vitro* models in pharmaceutical drug discovery programs could increase the probability of identifying translational disease-modifying therapeutics.

Here we describe the workflow and key elements of a novel screening approach and present an example of a pilot study utilizing a 3D organotypic disease model. The results from this study emphasize the importance of assessing drug efficacy in a heterotypic model (one that is representative of human tissue), as well as the added value of multiparametric assays with single cell resolution.

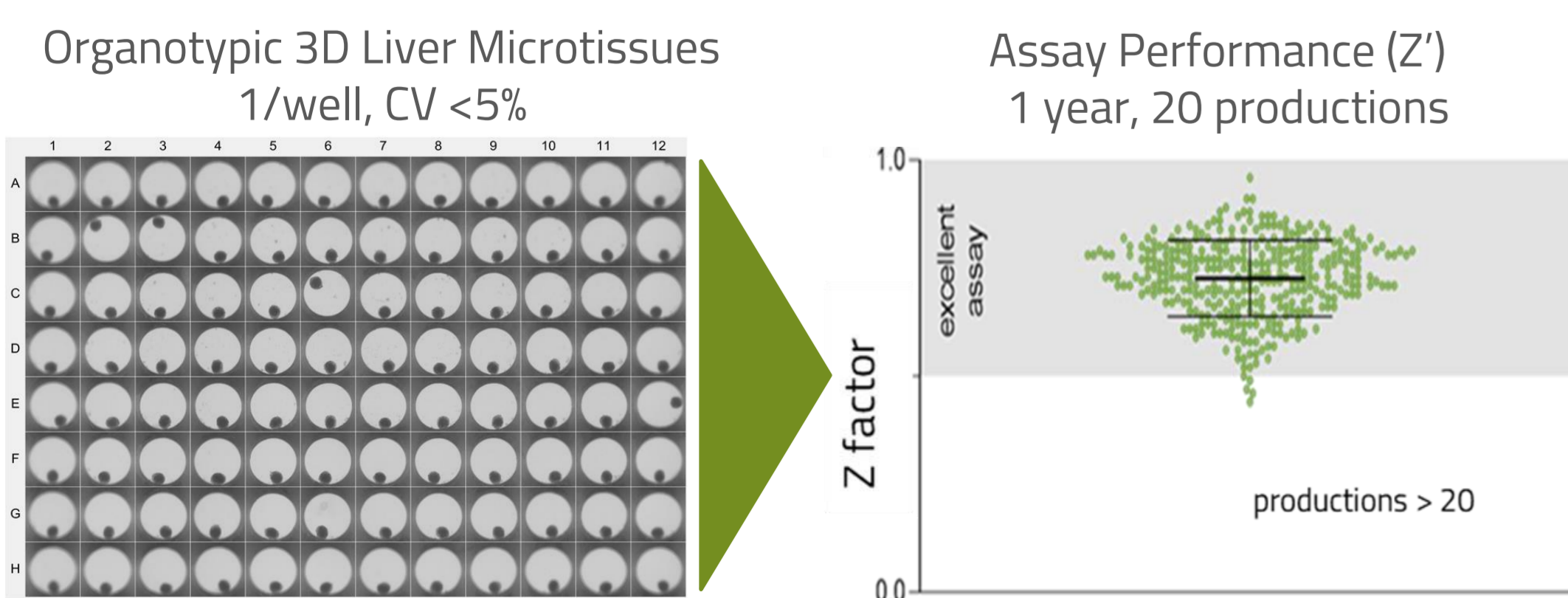
Scalable 3D Models

Organotypic 3D cell models have been shown to be more translational *in vitro* drug discovery tools than their 2D cell line counterparts. However, for 3D models to be used effectively in a compound screening environment the model must be scalable, reproducible and uniform in size and cellular composition. Microtissues reagggregated from dispersed human tissues meet those criteria.

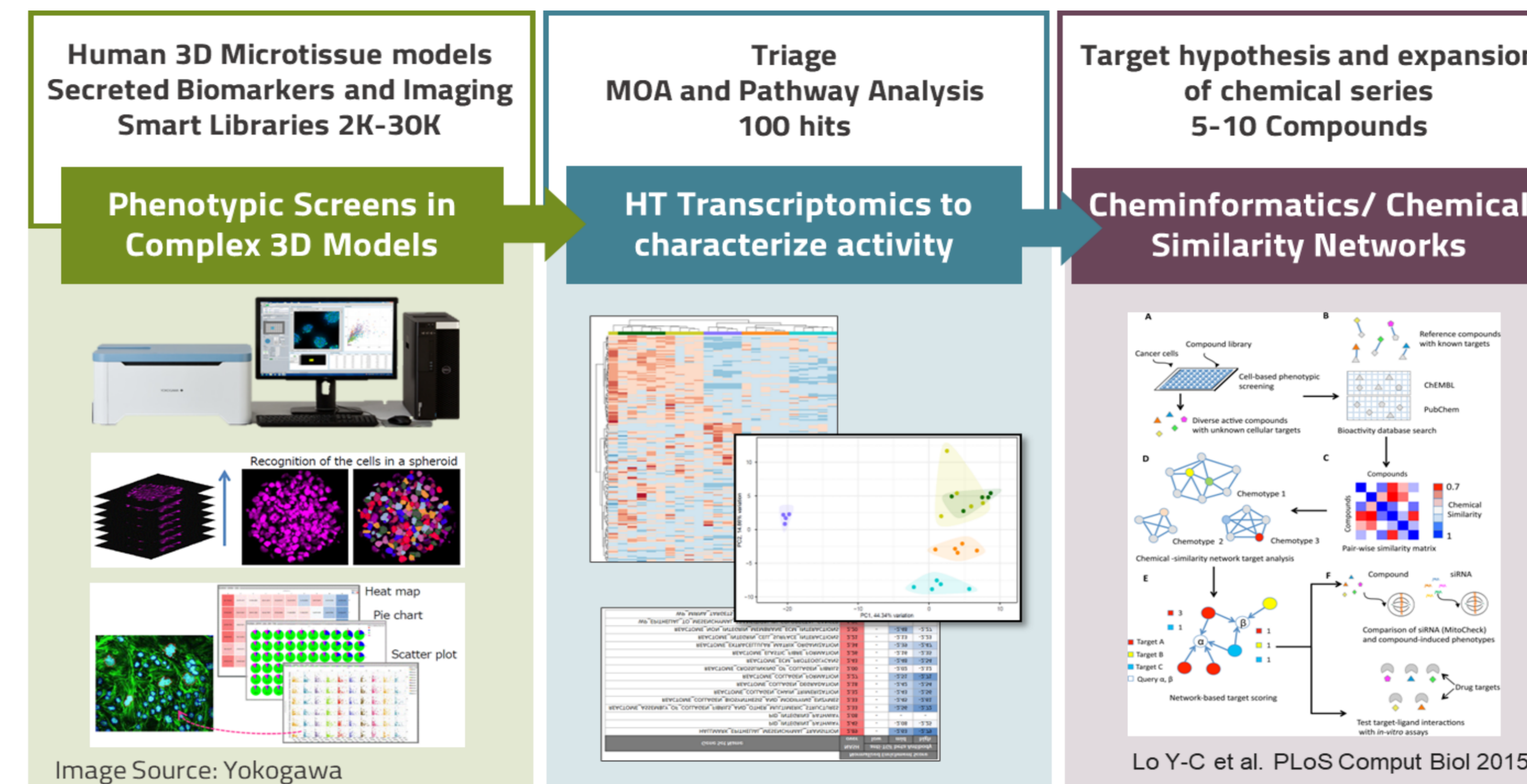
Organotypic Human Liver Disease Models exhibit the hallmarks of NAFLD and NASH



Standardized 3D Models and Assays Yield Screen-quality Assay Performance



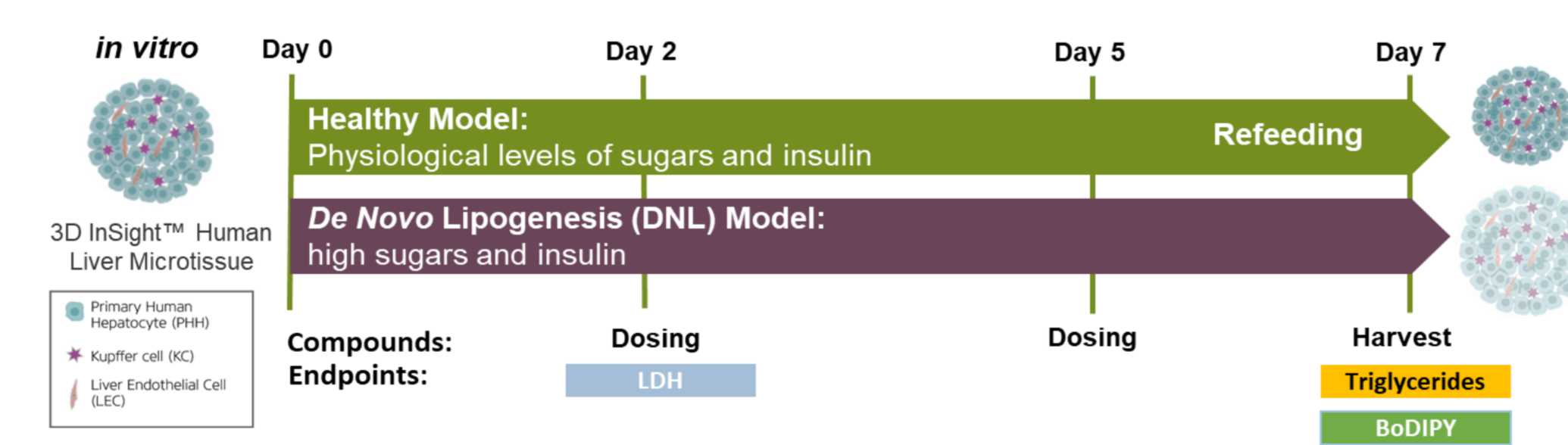
Proposed Workflow for Drug Discovery Screens in 3D Models



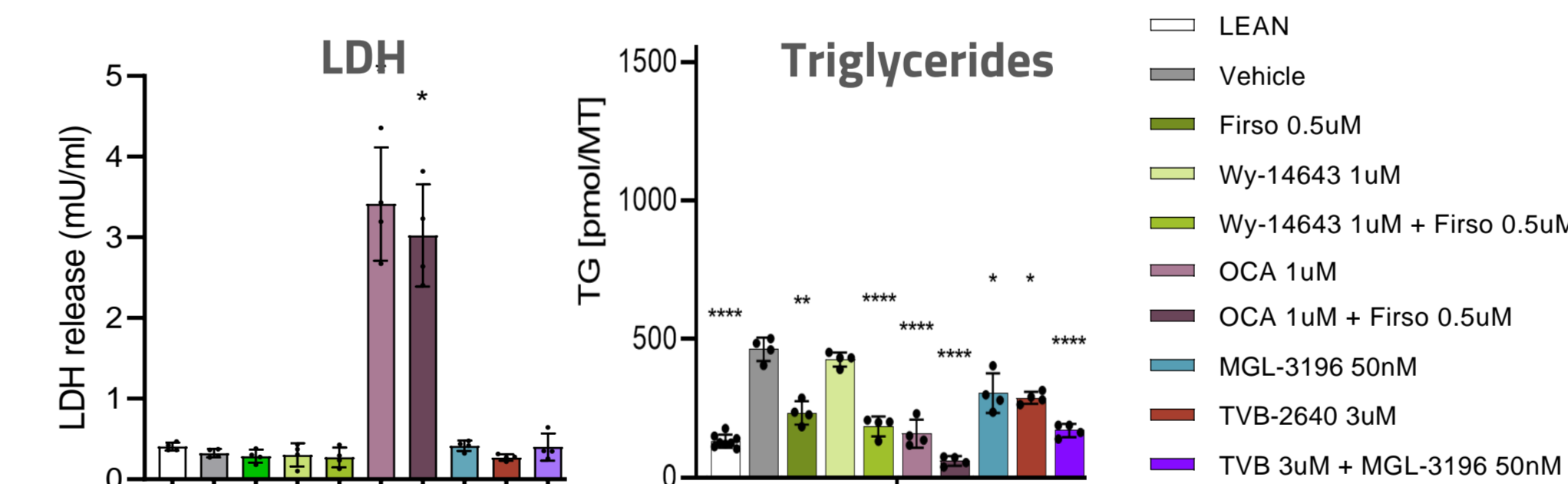
Phenomics Maximizes Information Yield from 3D Screens

Combining physiologically-relevant 3D models with a multiparametric readout maximizes the information gained from 3D screens and enables the early stratification of hits by mechanism of action, similarity to known compounds, efficacy, and safety. Utilization of assay endpoints directly related to clinical biomarkers provides additional translational value. In a pilot study, single and combinatorial treatments comprised of clinical compounds currently in phase 2/3 trials were evaluated in a 3D Insight™ model of NAFLD. Using the resulting multidimensional dataset, new insights regarding efficacy, safety, and phenomic similarity of the treatments were revealed.

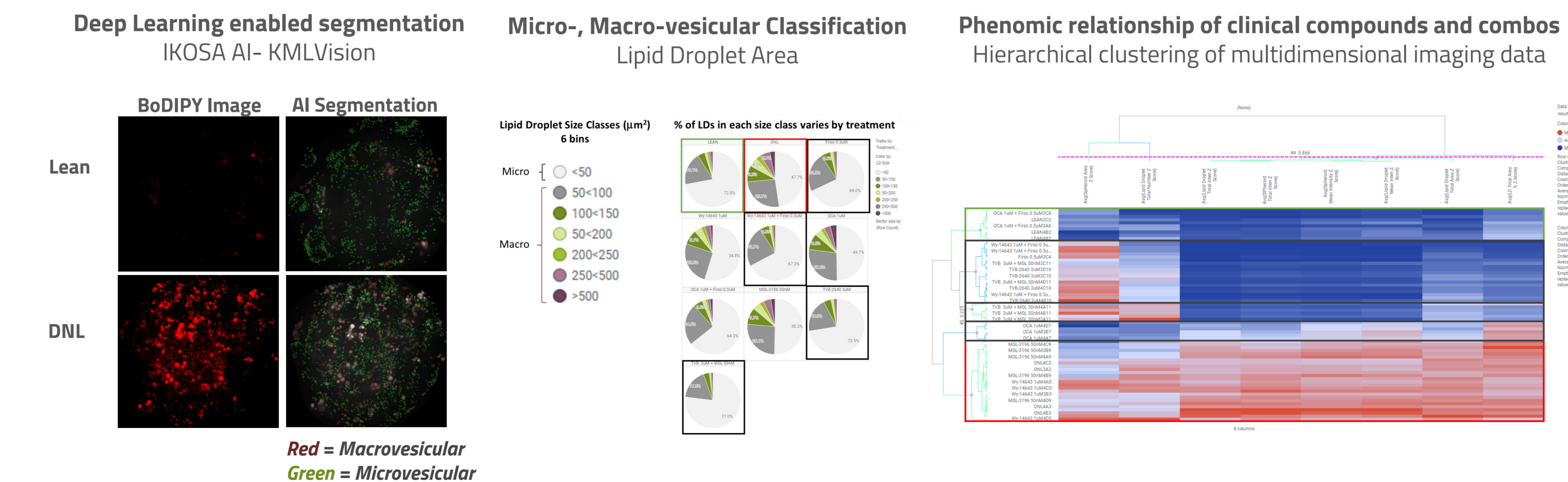
NAFLD Assay Workflow



Biochemical Endpoints



Phenomics- multidimensional analysis of fluorescent images



"Smart" Compound Collections

Screens conducted in complex cell models require suitably sized and carefully curated chemical libraries. Small annotated commercial collections can serve as the initial building blocks of such a collection. Cheminformatic mining of internal and public HTS databanks can further enhance and refine a "Smart" compound collection.

A "Smart" Chemical Library ~10,000 cpds

- Annotated compounds
- Structurally diversity
- Biological diversity
- Chemically tractable

Building Block Collections

- FDA Approved Drugs
- LoPAC
- Known Bioactives
- Diversity Collections

Cheminformatics Databases

- PubChem
- ChEMBL
- DrugBank

Advanced 3D HCI Platforms

A 3D-Compatible HCI Platform

- Automated confocal with laser light source and dual spinning disk
- Large format sCMOS camera
- 10X, 20X, 40X; LWD and high NA objectives
- Targeted acquisition for efficient object localization
- 3D image analysis
- Deep Learning algorithms



Summary

Even though recent science advises otherwise, drug discovery teams continue to select 2D cell culture models over more physiologically relevant 3D models for compound screening. Here, we show that a reasonable balance between physiological relevance and throughput can be achieved by choosing organotypic models that more readily lend themselves to scaling, e.g. spheroid microtissues models. Implementation of standardized 3D model production also improves well-to-well and batch-to-batch reproducibility of complex models.

We also highlight recent advances in high content imaging such as the commercialization of medium-throughput, high-resolution confocal instruments and AI-assisted image analysis solutions. Combined, these platforms, have made information-rich phenotypic screens in organotypic 3D models a reality.

Even still, screens conducted in 3D models may be limited in scale due to the associated costs (both labor and materials). This highlights the need for small "Smart" screening libraries and more sophisticated strategies, such as the application of phenomics and advanced cheminformatics, to generate novel leads and convert them into *bone fide* translational clinical candidates.