Application Note

Mass Density as Cross Sectional-Related Value for Enhanced Deep Imaging Analyses Outcomes: the Impact of Precise Sample Selection.

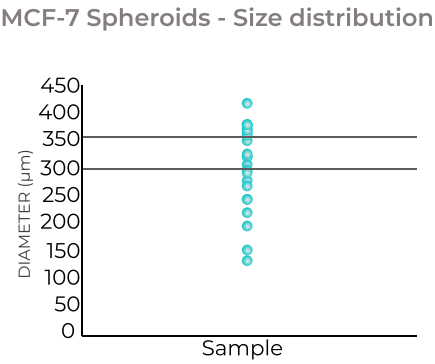
In the broad field of 3D cell culture, the utilization of confocal imaging analyses is key for unravelling the complexities inherent in these dynamic systems. Sophisticated imaging techniques offer unparalleled insights into the spatial organization and molecular dynamics of such models, driving discovery to the highest levels.
However, these benefits come at the price of being expensive, in both technologies involved and routinary procedures, as well as requiring significant expertise to be executed effectively. Moreover, the procedures involved are often intricate, adding layers of complexity increasing variability to already challenging and notably heterogeneous 3D cell culture. The high risk of investing resources, money, time and effort in investigating non-representative samples, specifically for a very low number (2/3 organoids), is still a crucial factor from which depends the entire outcome the experiment.
Given these challenges, the selection of truly reliable samples emerges as a critical priority. By ensuring the representativeness of samples, researchers can mitigate the limitations associated with deep imaging analyses, optimizing resource allocation and accelerating discoveries in the dynamic field of 3D cell culture research.

Challenges in Sample Selection

Traditionally, sample selection has been fraught with challenges. Common methods, such as size-based selection or operator discretion, often fall short of capturing the true complexity of 3D cell culture systems. Size-based selection neglects the intricate spatial organization within the sample, while operator discretion introduces variability and uncertainty into the process

Case Study Rationale: the Bias Behind Size-Based Selection

In scenarios where a researcher has optimized the production and growth protocols for the 3D cell culture under study, altready difficult itslef, the diameter distribution obtained through brightfield imaging often serves as the primary and unique quantitative analysis available to define population heterogeneity.



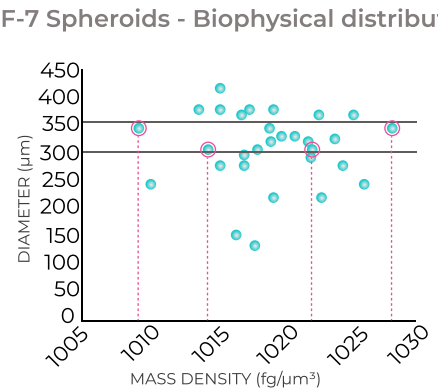
Diameter distribution of a batch of brest cancer spheroids population produced from the MCF7 cell line

Suppose the researcher's goal is to select the most representative spheroid from the population for further detailed investigation via confocal imaging. Given the wide size distribution observed, it would be reasonable to consider the range between 260 and 370 µm as the most representative. Experienced operators might even discern the presence of two subpopulations around 300 and 350 µm, which is an important consideration.

However, an intriguing question arises: would this range truly represent a three-dimensional object?

The impact of Mass Density: Towards the Perfect Footprint for Sample Selection

With the W8 system, we've brought in the concept of Mass Density to lead this revolution, striking the perfect balance between simplicity and efficiency. This metric, strongly correlated with cross-sectional information, offers researchers a quantitative measure of sample complexity and organization. Furthermore, when paired with size measurements, it enables the derivation of the ideal biophysical footprint and heterogeneity of the population. By considering both size and mass density, researchers can identify samples that truly represent their 3D cell culture systems, ensuring reliable and reproducible results.

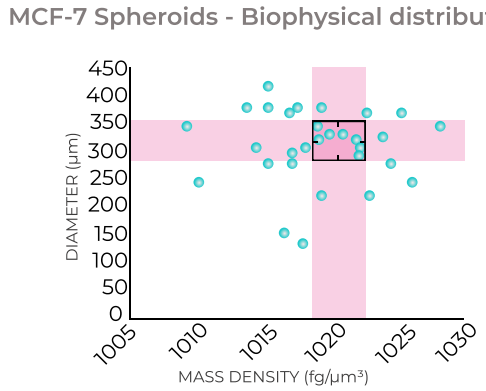


Mass Density over Diameter distribution of the same batch of brest cancer spheroids population produced from the MCF7 cell line

The upgraded quantitative perspective, transitioning from a single metric (diameter) to a multi-metric (Mass Density / Diameter) distribution, reveals to researchers an enhanced awareness of population heterogeneity.
Even with the most detailed diameter analyses revealing two subpopulations, spheroids exhibit significant diversity in compaction, which would significantly influence the outcome of deep imaging. Specifically, samples in the narrower interval of 300 µm display a noticeable Mass Density variation ranging between 1014 and 1022 fg/µm³. A more extensive cross-sectional heterogeneity is evident for samples around the 350 µm range, with a Mass Density variation spanning from 1008 to 1027 fg/µm³.

Eyes On The Target: The Sampling Function

Operators still require gentle sample handling to minimize shear stress on the analyzed spheroid/organoid. The W8 system was designed with this in mind, incorporating a specialized software-fluidic network combination. This setup enables researchers to easily select the range of interest based on Mass Density or Size and effortlessly collect the sampled samples in a separate reservoir.



Biophysical sampling of the MCF7 spheroids subpopulation of interest based on Mass Density and/or Size

From now on, the biophysical-based sample selection will emerge as a routine tool in research laboratories, initiating a beneficial chain reaction that conserves time, resources, and finances. This approach empowers researchers to thoroughly analyze their desired samples, maximizing the critical outcomes of confocal imaging.

Conclusion

In conclusion, the marriage of advanced imaging and analysis techniques with innovative metrics such as Mass Density Value represents a paradigm shift in sample selection for 3D cell culture research. By providing researchers with a reliable and objective method for identifying representative samples, this technology promises to enhance the robustness and reproducibility of research outcomes, ultimately driving discovery forward into new frontiers.

CASE STUDY

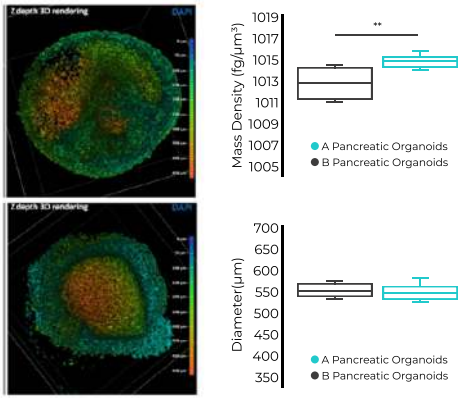
The impact of Mass Density Selection for deep imaging analyeses hiPSC-derived pancreatic organoids

Introduction

Differentiated pancreatic organoids represent an important tool for both in vitro studies and potential cell replacement therapy for the treatment of diabetes. Predicting the optimal functionality of a pancreatic organoid using a non-invasive technology will allow for a pre-selection of the best-fit organoids to be used in laboratory, pre-clinical, and clinical research. The unique biophysical properties of hiPSC-derived pancreatic organoids mean they can be used for predictive screening when quantified against benchmark methodologies that verify their physiological function.

Method

The biophysical characterization was performed using the W8 system as a label-free technique combined with specific sampling. Two different differentiation protocols for hiPSC-derived pancreatic organoids (named A and B) were applied and initial results obtained with the the W8 system compared with the classical biomolecular methodologies for pancreatic function, such as analysis of pancreatic gene markers, and protein expression. Organoids were then unified by size, but selected by Mass Density diversities, for a fine sample standardization to better highlight biophysical differences. This allowed us to standardize samples derived from both protocols to undertake and improve their comparative analysis.



Deep imaging analysis of hiPSC-derived pancreatic orgnoids of similar size bud different mass density highlights a direct correlation betheen compaction values and cavities volume.

Results and Conclusion:

Variations in mass density, obtained on comparable-size organoids, demonstrated, tush correlations between biophysical and functional properties that allowed for direct comparison of biophysical characteristics and active pancreatic function showing interesting trends correlation also with insulin, ZnT8 genes expression, C-Peptide.
These results demonstrate that differentiated pancreatic organoids can be readily pre-screened and monitored using non-invasive techniques, which preserves the integrity of the organoids allowing for their future use, both in vitro and in vivo.