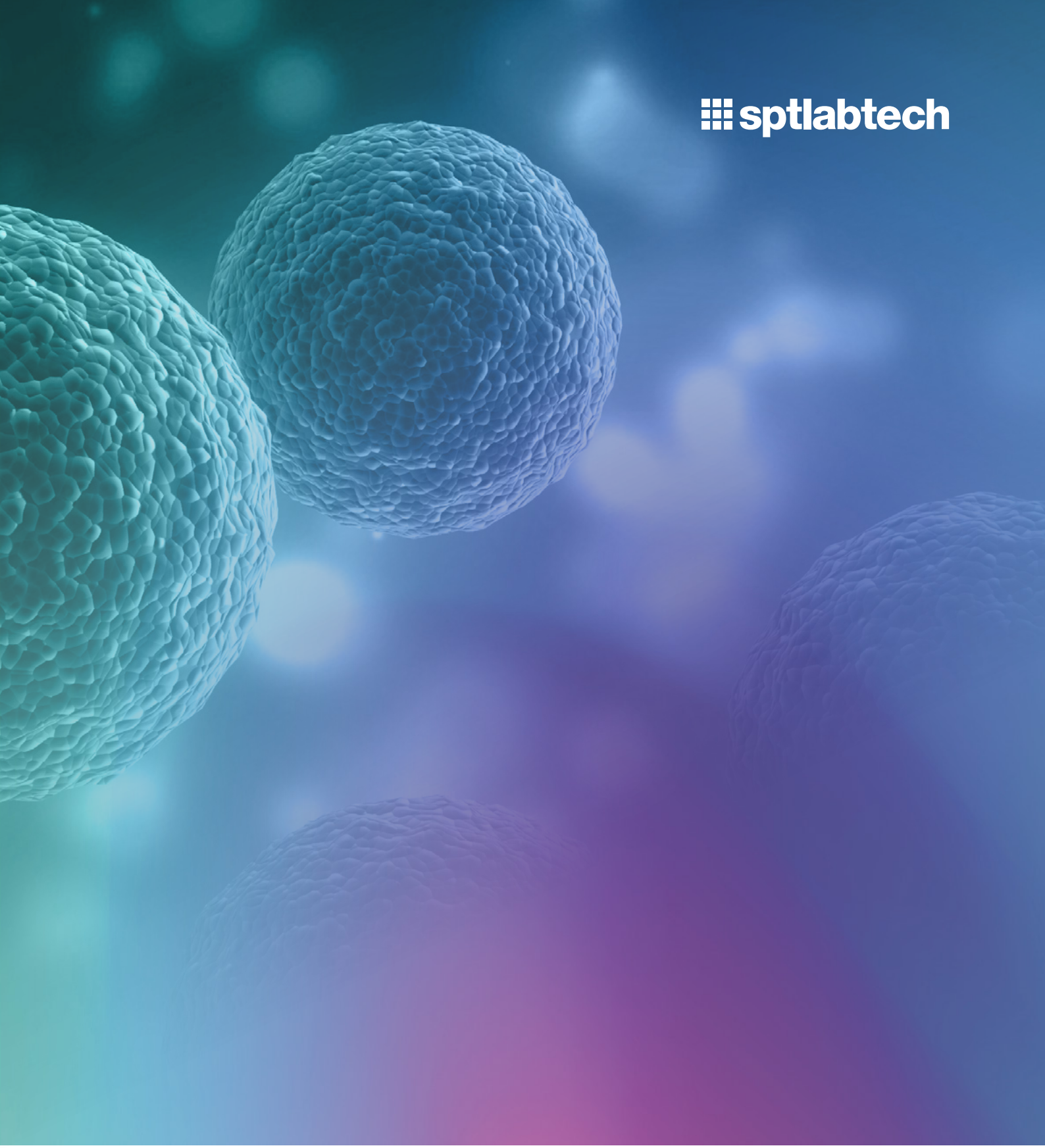
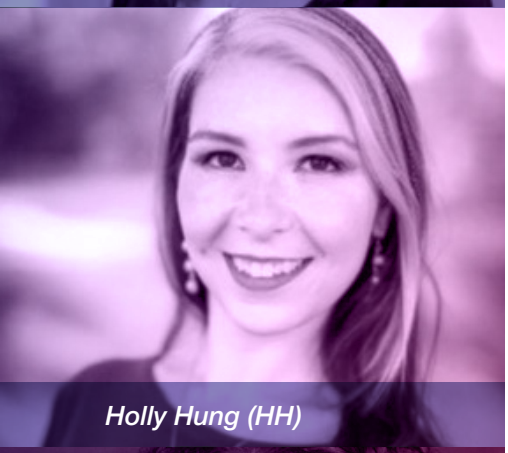



# Takeaways from SPT Labtech **3D Cell Culture Focus Group** at SLAS 2021

*Anne Hammerstein (AFH)*



*Holly Hung (HH)*

*Hilary Sherman (HS)*

## Focus group leaders:

**Anne Hammerstein (AFH)**
Product Manager at SPT Labtech - Moderator

**Hilary Sherman (HS)**
Senior Application Scientist at Corning - Presenter

**Holly Hung (HH)**
Field Application Scientist at SPT Labtech - Co-Presenter

## Introduction

Organoids and 3D cell cultures are a promising tool for research and development, as well as personalized medicine applications. However, we need more automated and high throughput methods to realize their full potential. Achieving automation for handling organoids has been historically challenging for several reasons, including a limited supply of cells to support these models, prohibitive liquid handling dead volumes, difficulties in manipulating viscous basement membrane extracts, and setting up highly reproducible 3D cell cultures.

SPT Labtech, in collaboration with Corning, organized and moderated a focus group at SLAS 2021 digital examining pain points, barriers and opportunities in working with 3D cell culture. Our goal for the session was to explore participants' experiences working in this area to improve understanding and inform future developments. We also wanted to allow a platform for delegates to share knowledge and help them optimize their own 3D cell culture approaches.

## Case study

The focus group began with a short presentation by Hilary Sherman, Senior Application Scientist at Corning Life Sciences. Hilary explained how SPT Labtech's dragonfly® discovery had been used to accurately and evenly dispense small (3 µL) droplets of human intestinal organoids mixed with Corning® Matrigel® matrix for organoid culture. These half dome cultures were assayed by high content imaging for physiological changes following forskolin-induced swelling (FIS), an in vitro assay for assessing drug response in cystic fibrosis patients.

Following the presentation, we opened the floor to participants to share their insights on three crucial areas - the pain points of working with 3D cells, their needs in cell culture optimization and trade-offs when automating elements of the process.

On the following pages we summarize key takeaways and insights from the discussion and share high level snapshots from our pre-focus group survey.

## What are your current pain points when it comes to setting up 3D cultures?

### Organoid disaggregation and scaling throughput

The process of disaggregation and then re-seeding into 3D cultures is a known challenge when working with organoids, shared by several focus group participants. It seems likely that the fragility of the organoids is not compatible with automated cell plating.

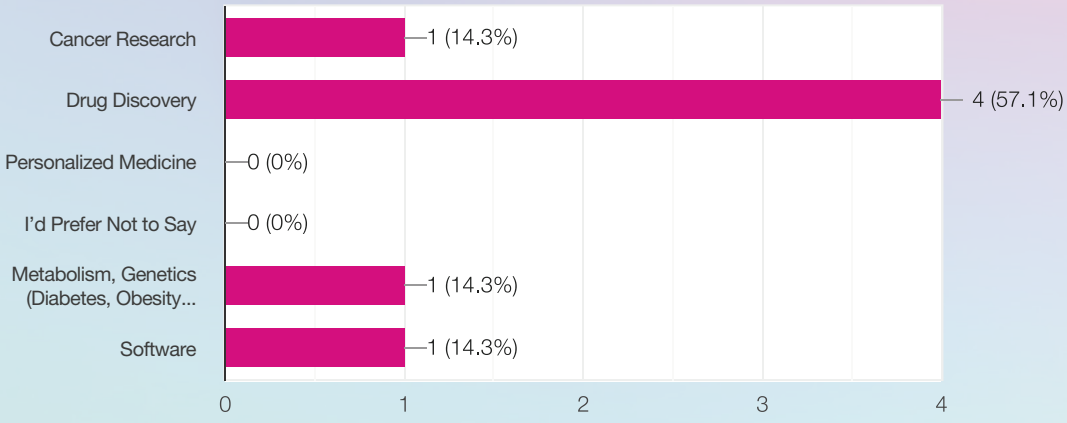
As confirmed by our survey responses, there is a strong interest in increasing the throughput of 3D cell culture applications. One obvious solution to achieving higher throughput is through assay miniaturization. In this context, we discussed the speed at which it would be possible to fill a 1536-well plate and noted that a non-contact dispenser such as dragonfly discovery achieves a faster rate than allowed by contact pipetting. HH described how a single syringe dispense could fill a 1536 well plate in around eight minutes while minimizing dead volumes to just 30 uL per channel using the dragonfly discovery dispenser. While it is possible to achieve even faster dispense times using multiple dispense channels, the additional dead volume required may only be justified in the highest throughput scenarios.

### Maintaining cool temperatures

Keeping material cool when working with Matrigel embedded 3D cell cultures was noted as a concern. SPT Labtech's positive displacement technology enables easier dispensing of viscous solutions, but maintaining tight temperature control is nevertheless essential.

As in the case study example, SPT Labtech and Corning had addressed this issue by pre-chilling dispense syringes and using passive chill blocks to keep reagents cold on the reservoir tray. AFH discussed a prototype in development with SPT Labtech that could improve the chilling functionality using a 'wine cooler' style design that wraps around the syringe body and helps maintain a cool reagent temperature between the reagent aspiration and dispensing steps. Maintaining a cool temperature becomes more problematic when needing to process multiple plates. While using a cold room environment may be viable when envisaging a very high-throughput setup, this does pose its own difficulties. Firstly, these conditions are uncomfortable for the operator and therefore often avoided. Secondly, taking instruments in and out of cold room conditions is not recommended as it risks damage to parts through condensation. A combination of passive cooling elements, rapid dispense times and positive displacement technology offers the best chance of achieving accurate and reproducible dispensing of temperature-sensitive materials like Matrigel.

What best describes your primary area of research or development?



## What kind of throughput would be desirable for your 3D assays? Do you have to compromise on model strength for throughput/cost considerations?

This question initiated a thought-provoking discussion about how the acceptable trade-offs and compromises were, to a great extent, dependent on the application.

When using organoids for imaging and drug screening purposes, any variation in size and the number of organoids per droplet is not perhaps the most critical issue, as high content analysis can normalize assay readouts to the organoid number. For this application, the droplets' consistency and the location are more important to ensure rapid and accurate imaging.

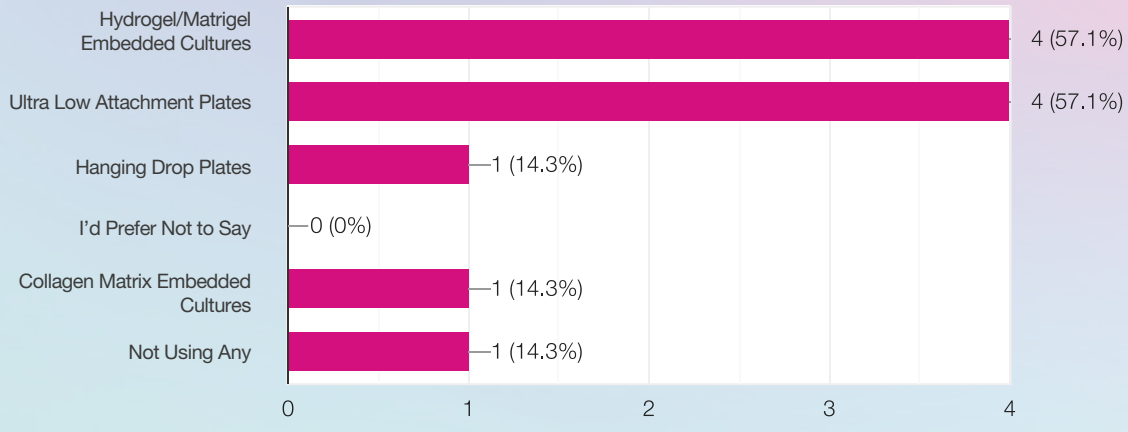
On the other hand, when working with an ATP-luminescence readout, consistency, number, and relative size of organoids are critical factors, and automation to enable standardization is needed.

When setting up 3D cultures, a certain level of variability is expected, in part due to the heterogeneity of the biological sample. However, while acknowledging this biological heterogeneity, it is essential not to add further technical variance that could mask genuine differences between, for example, treatment and control wells.

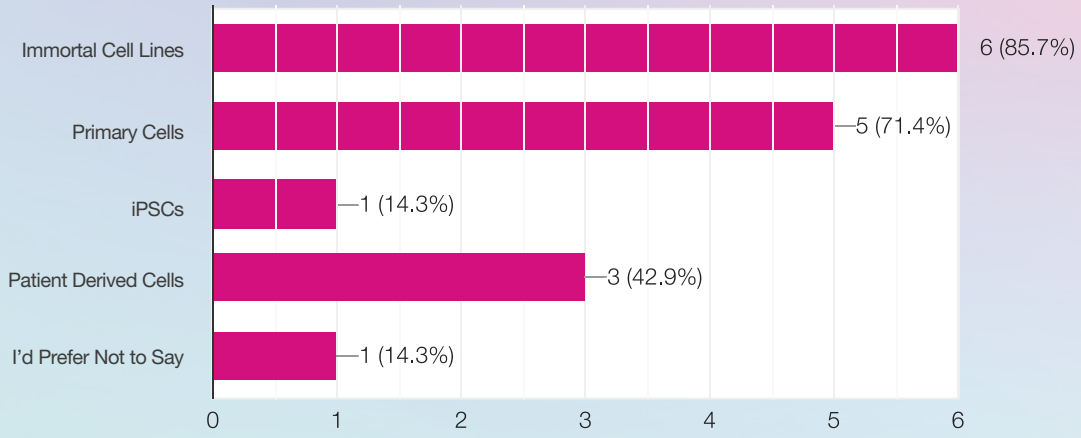
Using positive displacement dispensing allows robust control over the volumes to minimize technical variance. As was noted in the previous discussion regarding the maintenance of a cool temperature, our case study revealed that plate variability (as measure by %CV) would typically increase after dispensing two plates. The new syringe chiller should mitigate this effect by providing passive cooling of the Matrigel reagent for more extended periods.

A question arose regarding best practice to automate the dispense of multiple cell lines to test against a panel of drugs. Two different approaches could be used, depending on the number of cell lines and drugs involved in the screen. This could be achieved for small numbers of cells and drugs by laying out different cell lines in different plate sections and dosing each cell line with drugs according to a custom layout. For larger numbers of cell lines, it would be preferable to assign each cell line its own plate and to apply a standardized drug/dose layout (either through acoustic dispensing or by stamping from diluted, assay ready plates).

What 3D cell formats does your laboratory currently utilize?



What source of cells do you work with?




## Are there any other liquid handling pain points in your laboratory?

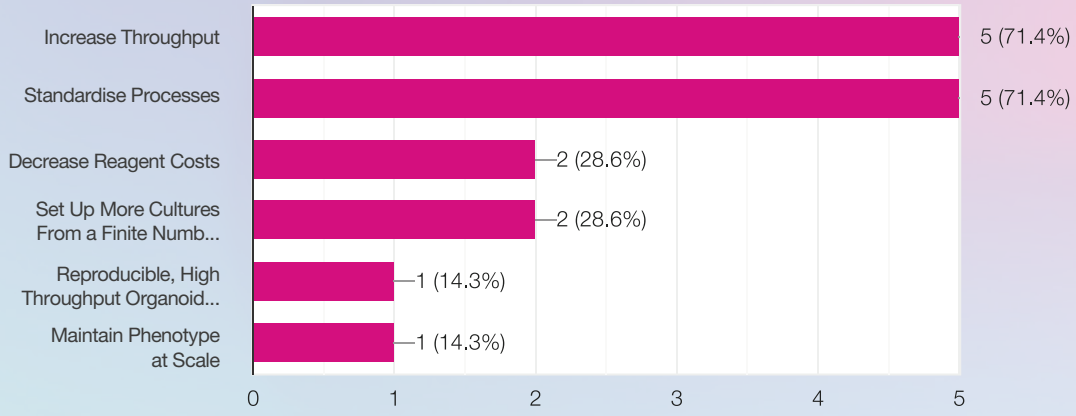
One issue that would benefit from advances in liquid handling technology is exchanging the cell culture media in screening wells. When working with slow metabolizing compounds in toxicology applications, it may take several days to see the impact on the organoid. During this period, the media in the wells needs refreshment to extend the screening window and allow time to assess the effect of compounds. It is technically extremely challenging to exchange the media on the plates without damaging the cell culture. Automation to support the media refreshment and extend the screening assay would be welcome.

### Participants noted available technologies designed to address this:

*Biotek has a plate washer that aspirates and adds fresh media. Organoids tend to be predictable in their position in the well, so setting automation to aspirate at a particular height and speed can work effectively to remove the cell culture media without disrupting the spheroid. Adding fresh media temporarily disturbs the 3D object, but eventually, gravity restores its position.*

*A NanoShuttle uses a magnetizing technology, making it easier to add and remove the solution by holding the 3D cells in a stationary position during aspiration.*

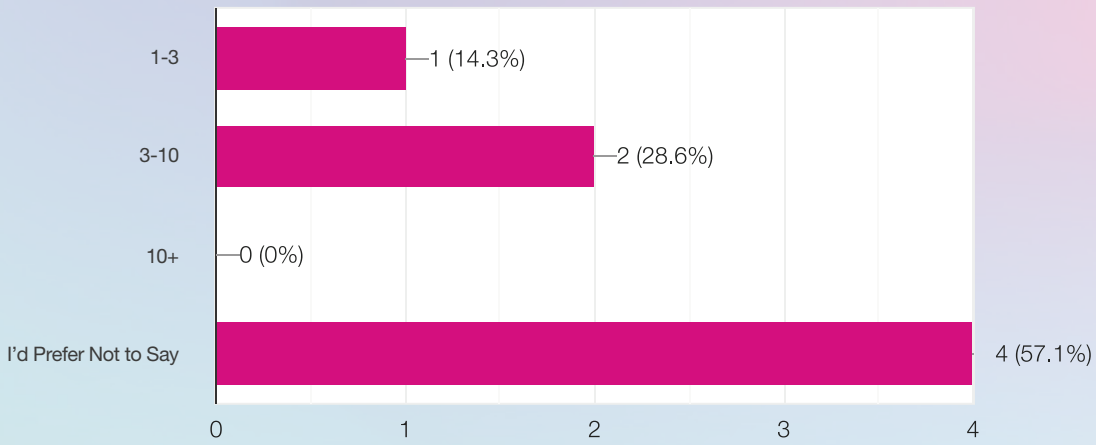
What best describes your laboratory's 3D cell culture goals?




## Do you perform any optimizations of your 3D cell culture conditions?

The discussion then turned to how Design of Experiments (DOE) could help optimize cell culture conditions. One variable requiring optimization for any 3D cell culture is how much media needs to be aspirated and at what point. Besides, the composition of the media, the number and ratio of cell types, the concentration of Matrigel and so on may also require optimization. While with scarce patient-derived cells, it may not be feasible to systematically test all variables one factor at a time, so a statistical approach may help narrow down the number of options.

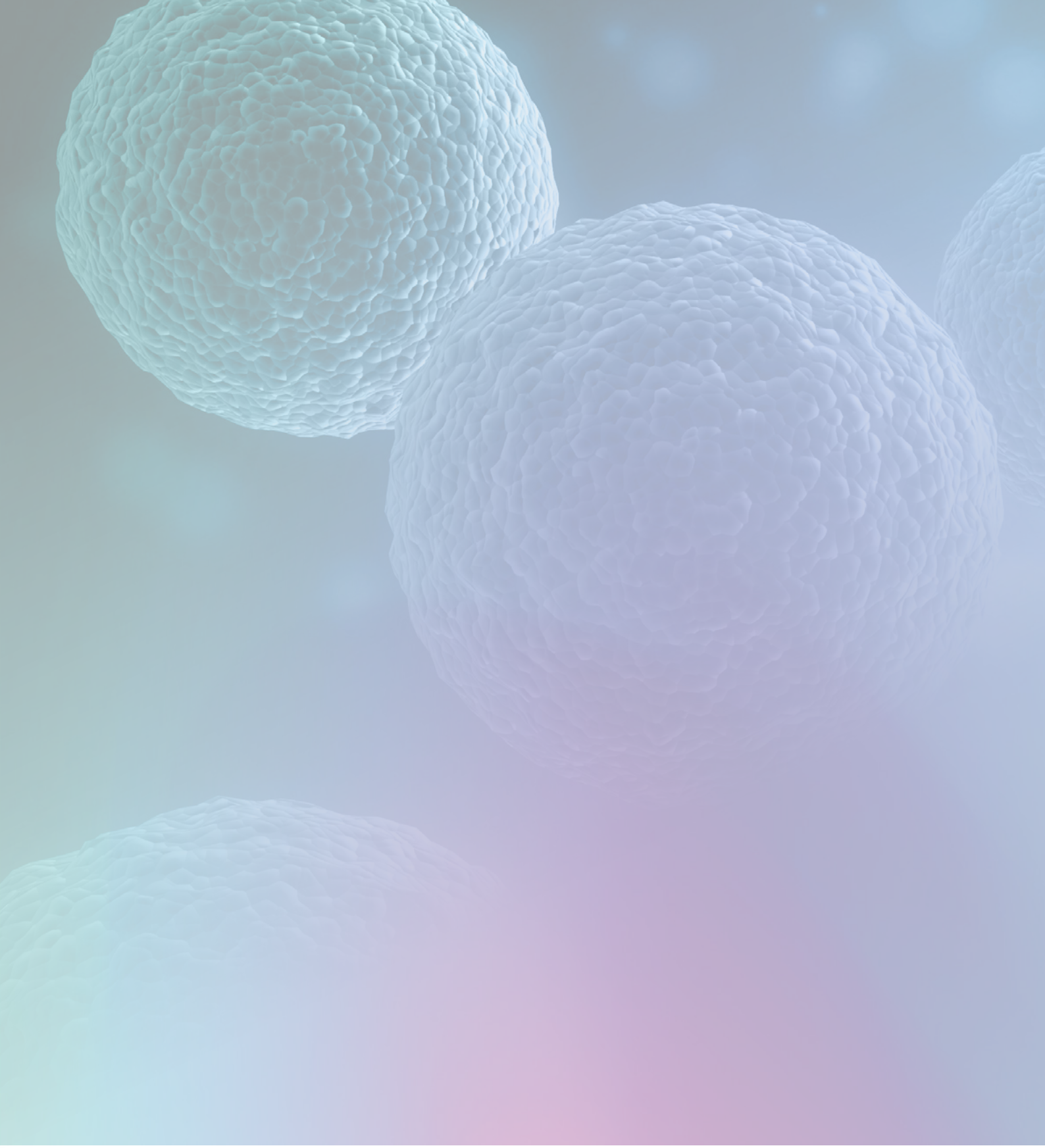
How many plates (96/384) of 3D cell cultures does your laboratory currently process per week?



# Summary

## Advances to support semi-automation are achievable

In summary, the discussion highlighted that application is key when considering automation and optimization for 3D cell cultures. Further, typical solutions are likely to involve semi-automation requiring a degree of user intervention rather than full end-to-end automation. Nevertheless, there are opportunities to realize process efficiencies through assay miniaturization and streamline 3D cell culture workflows through rapid and reliable reagent dispensing.



We thank participants for sharing their time and insights during this discussion and look forward to further advancing innovation in this area.

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