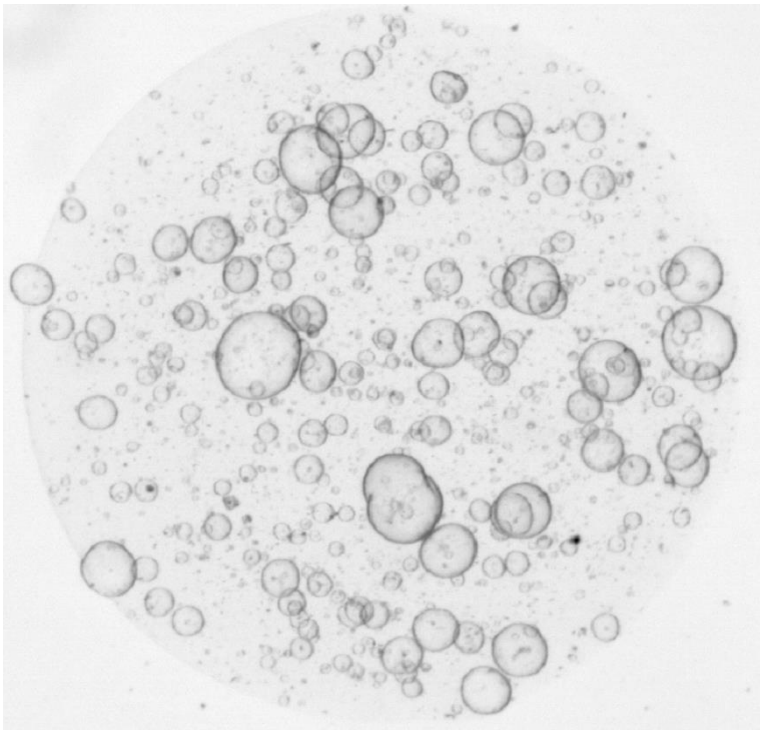
Quantifying Organoid Size and Counts: Solving a Common Issue with Automation

by Justin Croft

Organoid biology is a rapidly evolving and growing field. An organoid in the most basic definition means growing cells in 3-dimensions to produce cellular units that resemble a specific organ in function and structure. As organoids have become more prevalent and a more standard application base in research, a wide range of different organs and tissues have been recreated in vitro and been used for studying everything from basic developmental biology to translational research. Despite the assortment of organoids that have been generated and their multitude of potential uses, the field does face some common challenges.



A classic example of organoids growing within a 'dome' of soft agar, as viewed by GelCount

A multitude of the applications today using organoids look directly at kinetic cellular growth of organoids in different situations. These applications include the toxicity of specific drugs, microenvironment manipulation, and even comparing different organoid types to one another. These studies always come with a multitude of challenges with one of the most obvious being how to efficiently and precisely count and size 10's-100's of wells of organoids across an entire study.

Here we delve into this challenge fully to discuss how most researchers are currently counting and sizing their organoids with microscopy and review some challenges associated with counting organoids this way. We then look at an efficient, high throughput alternative using a machine counting platform, The GelCount, and discuss why this system may be a better option for most labs. Last, we briefly highlight and provide links to some of the most recent organoid publications who have used the GelCount to count and size their organoids.

While the article is written to ensure it is an easy read for anyone, we do quickly want to touch on whom the article was intended and written for. If you are a PI or lab manager looking to make your lab more efficient or are a researcher doing the menial task of counting thousands of organoids, then this article should be both topical and interesting to you. Also, if you are in a lab having issues with consistency of counts this again may be helpful to you. Last, if you are a lab who is considering working with organoids in the further then we think this will help prepare you for some of the challenges you will face.

The most frequently used techniques to count and size organoids

Manual counting of organoids can often be summed up like this: a well-trained researcher, under a microscope, finds an object and then determines if it is an organoid or not. If it is established to be an organoid, the researcher then ascertains if the organoid meets a minimum predetermined size. Again, if it does, then 1 is added to the running total and the researcher moves on to the next object. This next move needs to be extremely systematic as not to risk recounting previously counted organoids and the process is repeated for all objects across every well and plate. Further since organoids are grown in 3D space this requires the researcher to not only be methodical across an X and Y axis, but they must also be weary that the Z axis must also be considered as to not miss a substantial number of organoids at differing levels.

Counting using brightfield microscopy

This is the most common use case and as mentioned has a researcher scanning and counting organoids under a standard light microscope. Researchers generally look at one plane, count all the items of interest in that plane and then move down to the next plane until they reach the bottom of the plate. If the researcher is careful, they can avoid recounting objects, but it is not a particularly simple task and overlapping objects are nearly impossible to count. Regardless of a counters efficiency this is will inevitably be an extremely time-consuming responsibility.

It should also be noted, the repetitive and tedious task of counting organoids can cause both physical and mental fatigue which can seriously affect the accuracy and reproducibility of results, particularly in a large count. Studies have shown that the human error from investigative fatigue is such a serious problem that errors of 100% or more have been observed in lengthy counts. On top of this, manual counts can be further compromised, either wittingly or unwittingly, by the bias of a researcher whose expectations about the outcome of the experiment impact their counts.

Furthermore, the use of brightfield microscopy to visually count organoids offers a major problem for researchers which is it is often difficult to determine the acceptable organoid size across different samples. If an organoid is on the borderline of being counted is it counted or not? Is there potential in a set of particularly small organoids that a larger one that is under the minimum value will be counted? Adversely, in a large organoid sample is a smaller organoid over the minimum size limit counted? In practice, this means that an error of only ±10% in the visual estimate of organoid size across samples can result in +90% to –45% counting error. Since size can often be difficult to determine accurately, this issue can contribute to large errors when counting organoids.

Finally, without any actual quantitative sizing information being available in these counts there is a further issue that researchers cannot truly discuss the growth dynamics of organoids themselves. As many researchers undertaking counts using light microscopes will attest, while they may see differences in organoid sizes across treatment groups, without some way to quantify this, it is only an observational metric and generally not publishable.

Counting and sizing using confocal microscopy

Unlike the brightfield microscopy method for counting organoids, another way researchers often try and quantify their organoid size and number is with a confocal microscope. The confocal method has the user take several automated Z-stacks through the media and then piece the images together in 3D. Since the images are saved and the software packages associated with these systems are usually quite dynamic, these systems should never have an issue both quantifying and sizing organoids. While in theory this solves all the problems that were faced with the manual brightfield counting method above, this process suffers in that it is exceptionally slow in both imaging and analysis and makes even a small experiment of a few plates become a work project which will take several hours to complete. This one significant drawback makes using a confocal microscope for analysis of organoid counts and sizing feasible for very small-scale studies as throughput is minimal.

Beyond this another drawback of confocal microscopy for a simple count and sizing application is that these systems are often shared equipment and as such have some extra fees associated with them. Also, because they are shared systems and others do need to access them as well, it becomes even less feasible to tie up the system for days on end to complete an experiment whose overarching goal is just to count and size organoids.

Now before moving on, we do want to note that while confocal microscopy is far from an optimal tool from a throughput standpoint for a simple counts and sizing experiment, it certainly does have its place in organoid research to garner a better understanding of complex concepts at a sub-cellular level of resolution.

In summation, it is obvious the 2 most prominent methods to count and size organoids are extremely inefficient in doing this task. Beyond that in the case of the brightfield method, results may even be skewed completely unwitting by a fatigued, undertrained, biased researcher. So, what is the solution?

The GelCount: An automated alternative to microscope counting and sizing

The simplest place to begin is by answering the question “what is the GelCount exactly”?

The GelCount is an integrated hardware and additional PC software platform developed by Oxford Optronix for automated high throughput imaging and analysis of mammalian cell colonies (both adherent and non-adherent). Whether it be the counting and sizing of spheroid, tumor forming, or clonogenic assays, the GelCount has proven to be a highly capable system to do this. As such, the system is found in labs world-wide and has been cited in over 300 publications.

The GelCount itself, is quite different from other counting methods. After it takes its image, researchers can use the software to define specific thresholds (including minimum and maximum sizes) to be able to count exactly what they want to. These thresholds can then be automated to run across an entire experiment, counting each and every well with the exact same user defined specifications, meaning there will essentially be no variability in counts. Additionally, the systems learning curve is minimal and any one in the lab can be taught how to use the system in a short period of time. From a throughput standpoint, on average it takes the GelCount about 12 minutes to count and analyze four separate 6-well plates of non-adherent colonies (similar times for organoids).

The GelCount has only recently been found to be an extremely useful tool for researchers growing organoids as well. Since the system can count objects in 3D space (up to 5 mm deep) it has all the capability needed to image and resolve most organoid applications. Many organoid types tend to image similarly to what is seen in clonogenic assays and as such, makes them easily countable by the system. All the issues seen in the microscope counting techniques stated above (time consuming, counter reliability, low throughput, observer bias), the

GelCount entirely removes and will almost certainly provide a more consistent count while increasing throughput across an entire experiment. The system also provides other information about organoids including diameter and volume which as explained earlier can make a simple organoid counting experiment far more dynamic.

Last and likely of interest to many researchers, is while many labs do have their own GelCount unit, this is a system that is easily sharable between several labs. In a sharing scenario, the hardware of the system is often housed in a central location within a department or a core facility allowing many researchers to do their imaging in this location. Then once a researcher has completed the imaging, these images are sent to a home lab PC and analysis is done in that location, opening up the system for another next user.

Organoid publications using the GelCount with brief description

While the counting and sizing of organoids is a newer technique to the system, we are beginning to see more publications utilizing the GelCount for this application. Please note that most of the applications below have been completed by labs for cancer based organoid applications as the GelCount has been a staple in these types of labs for completing clonogenic/tumour forming assays for years. As such, it has been mostly these researchers who have been the primary early adaptors of using the GelCount for their organoid applications. While cancer organoid types are certainly one area we know has potential for numerous applications in the GelCount, it must be noted the system can extend itself well beyond just this area.

The list below contains some of the more notable organoid applications so far to use the GelCount.

Chakraborty G. et al. 2021
Attenuation of SRC kinase activity augments PARP inhibitor–mediated synthetic lethality in BRCA2-altered prostate tumors. Clinical Cancer Research
Used the GelCount to count patient-derived prostate organoids in 3D Matrigel (see Supplementary Figure S6)

Chakraborty G. et al. 2020
Significance of BRCA2 and RB1 co-loss in aggressive prostate cancer progression. Clinical Cancer Research
Used the GelCount to count patient-derived prostate organoids in 3D Matrigel (See figure 1. G)

Dustin D. et al. 2020
RON signalling promotes therapeutic resistance in ESR1 mutant breast cancer. British Journal of Cancer
Used the GelCount to count primary mouse tumour organoids in media (See figure 5. G-I and Figure 6. A-B)

Min J. et al. 2019
Heterogeneity and dynamics of active Kras-induced dysplastic lineages from mouse corpus stomach. Nature Communications
Used the GelCount to count and size mouse stomach corpus organoids in soft agar (See Figure 5. A-C)

Buczacki S. et al. 2018
Itraconazole targets cell cycle heterogeneity in colorectal cancer. Journal of Experimental Medicine
Used the GelCount to count and size mouse adenoma organoids in 3D Matrigel (See Figure 4. F)

Young M. et al. 2017
Subtle Deregulation of the Wnt-Signaling Pathway Through Loss of Apc2 Reduces the Fitness of Intestinal Stem Cells. Stem Cells Express
Used the GelCount to count intestinal organoids (See Figure 4. A and Figure 5. C)