

Modernize your cell line development with high-throughput image cytometry

Cell line development (CLD) encompasses an optimized process that balances cell line productivity and cell product quality over a consistent and reproducible timeline. This literature review summarizes areas of cell line development where image cytometry has provided workflow advantages and made the entire process more efficient.

There are several solutions available for research groups within cell line development. For most manufacturers, CROs, and large-scale operations, there can be a daily need to process stacks of plates utilizing a high-throughput solution.

As bioprocessing moves towards high-throughput and automation solutions – it seems prudent to replicate already established methodology from leaders in cell line generation.

CLD workflow automation

Though single-cell cloning is a hallmark of the cell line development community, the entire process of engineering a cell line is composed of multiple stages (Figure 1). Researchers at Genentech, Inc. are often attributed to originating and continuing to pioneer methodology that shapes cell line development. Recent publications disclose Genentech's automated single-cell cloning workflow with implementation of imaging plates at the time of seeding to immediately identify wells likely to containing single clones.^{1,2,3}

This process was optimized by fluorescent live-cell staining with CMFDA, which increased the accuracy of identifying single cells within wells. Plates were imaged in brightfield and fluorescence, and growth was tracked until confluent wells could be picked. Monoclonality was visually

confirmed prior to banking of any cell lines. Genentech utilized Revvity's Celigo™ image cytometer within their automation workflow.^{1,2,3}

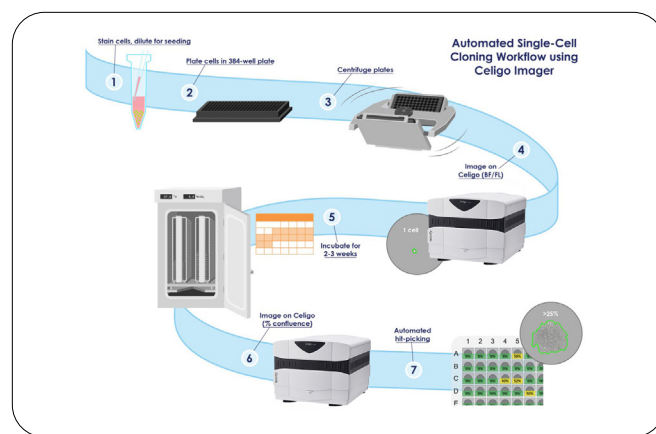


Figure 1: Flow chart of the cell line development workflow for single-cell cloning. Single-cell (4) to single-colony (7) verification can be obtained in automation. The entire process of single-cell cloning and automated workflow using the Celigo image cytometer has been published by researchers at Genentech, Inc.¹

Fluorescent labeling

Though debated within the cell line development community, the authenticity of single-cell clones can be enhanced with the use of a fluorescent marker. The WHO Expert Committee on Biological Standardization (ECBS) have stated that any changes to the cell culture process should not affect product quality.⁴ Therefore, fluorescent labeling should be limited to reagents that will not interfere with the integrity of the cell line. The only tracer dyes that should be used are transient ones unless a cell line has been engineered to fluoresce for non-clinical research applications.

Researchers at Genentech have thoroughly vetted the safety and integrity of utilizing CMFDA for verifying clonality.¹ CMFDA is a green cell tracer dye that is transiently retained inside live cells. CMFDA is a cell-permeable compound that is rapidly hydrolyzed inside live cells converting it to a fluorescent marker. The dye becomes difficult to detect after approximately 3-5 generations. Studies strongly suggest that the use of CMFDA during single-cell cloning does not interfere with safety or overall product quality, but rather reinforces the assurance of monoclonality. Ready-to-use CMFDA solution (avoid errors during resuspension and tedious calculations) for direct use on an image cytometer or for another fluorescent application can be obtained from Revvity (ViaStain™ CMFDA part number CS1-P0001-1).

Single-cell to single-colony verification

Without image cytometry, limiting dilutions are performed until the point at which, statistically, one cell should be present per well – though still not guaranteed with possible product contamination. In single-cell cloning of hybridomas, for example, it is vital to ensure that a single product of interest is generated; inclusion of more than one hybridoma cell per well could lead to the production of non-monoclonal antibodies. Working with cell banks that have a high assurance of monoclonality can help avoid serious disruptions in manufacturing. Image cytometry allows for the early evaluation of clones with quantitative information that the manufacturer can use to select the most promising clones for scaling. Tracking proliferation rates and phenotypic stability of single-cell colonies through image cytometry can provide early indications for the quality of the final products.

In automation, it is good to have an image cytometer that can perform rapid whole-well imaging – especially when tracking the growth of a single-cell to a colony in larger well formats. In 96-well or larger area wells, poor image contrast near the edge can prevent cell detection especially since cells often settle near the edge of a well. With the galvanometer mirrors and F-theta lens, the Celigo image cytometer was designed to perform rapid whole-well imaging with excellent edge contrast.

With whole-well imaging, single-cells sitting near the edge of a well can be easily detected in both brightfield and fluorescent channels (Figure 2). The Celigo image cytometer is frequently used as a powerful tool that can provide documentation to meet FDA requirements in a single round of cloning and expansion.

Rapid and accurate analysis

A common goal within the cell line development community is to transition processes to high-throughput instruments that can efficiently scale up productivity without sacrificing validation. The Celigo image cytometer has served within this capacity as a reliable screening tool within the cell line development community.

Current configurations of the Celigo allow for fast scan and analysis times that are used in automation to capture images for manual inspection over long periods. Researchers at Genentech have published articles that demonstrate this ability by setting up projects that screen over 45,000 samples while utilizing low volume 384-well Corning 3542 plates, which are ideal for miniaturizing single-cell cloning assays.³

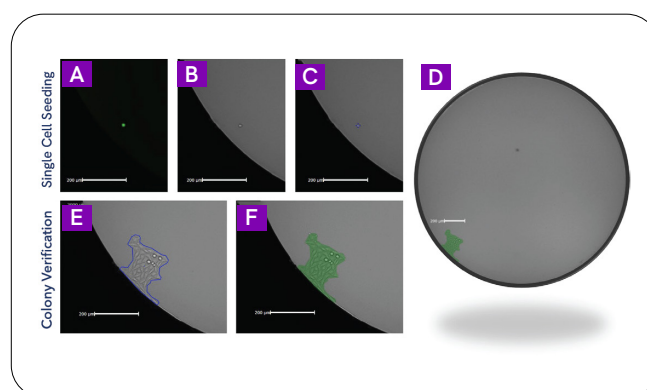


Figure 2: Verification of a single CHO-K1 cell clone at the edge of one well within a 384-Well Corning 3542 plate by the Celigo image cytometer. (A) A single fluorescent (transient or transfected) cell plated in a well can be easily visualized after single-cell deposition. (B) Enlarged image of a single cell near the edge of a well. (C) Label-free identification of the cell; single-cell seeding was verified by the software using only brightfield analysis. (D) A whole-well image acquired on a Day 4 scan shows a colony in the bottom left of the well (artificial color overlay added for contrast). (E) Single-colony identified at the edge of a well with excellent contrast (outlined). (F) Artificial color overlay on the brightfield image to aid in visual verification of an identified colony.

This method that researchers at Genentech published allows for multi-channel scan times of less than three minutes per 384-well plate; it is possible to comfortably process over 5,000 samples per hour in automation. Other cell line development groups also utilize multiple the Celigo system, some in automation, to function in cloning and other supportive projects.

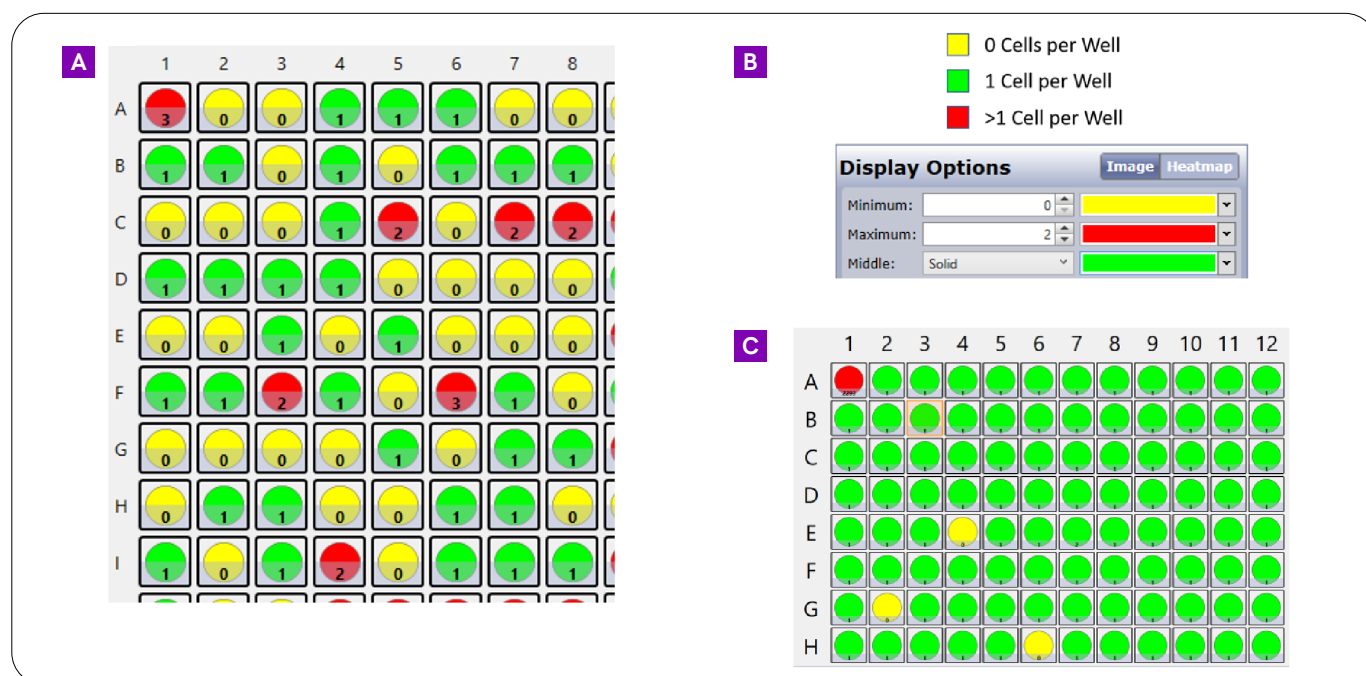


Figure 3: Example of heatmaps generated by the Celigo image cytometer after analysis of single-cell plating. (A) Reported cell counts following a limiting dilution protocol in 384-well Corning 3542 plates. (B) Heatmap legends and user-defined settings for identifying 1 cell per well. Wells containing more than 1 cell per well were flagged “red”. (C) A 96-well plate heatmap where the Celigo was used to optimize cell printing settings – bringing the efficiency of single-cell seeding up to 97%. Visual confirmation of the number of cells in each well showed 100% accurate reporting.⁵

Cell printing and imaging

Limiting dilution can produce reliable single clones, however the entire process generates a low percentage of successful single clones per attempt. Cell printing or sorting a single cell into a well is the ideal workflow that reduces the number of plates used as well as the number of raw high-resolution images. In addition to raw images, the Celigo image cytometer can generate heatmaps to immediately provide the user with confirmation of seeding density (Figure 3).⁵

Researchers at Genentech have demonstrated that combining manual image verification following image analysis from two instruments, one from single-cell printing and the other analysis tracked on the Celigo image cytometer, can yield an assurance of 99.9% that the clonal cell lines are derived from single cells.^{1,2}

Early screening tool

A US patent application by Selexis S.A. (US 2020/0109421 A1) reveals the significant role image cytometry has played in the early screening of cells for the production of recombinant therapeutic proteins.⁶ After transfection and selection, researchers at Selexis labeled their cells with CMFDA followed by imaging on the Celigo platform – the same transient tracer dye and protocol used by researchers at Genentech to verify monoclonality.

The patent also presents a unique use of the Celigo image cytometer, to detect the vitality of the cell lines being generated through a secretion application. The cell secretion application within the Celigo platform allows for evaluation of protein production by cells at a significantly earlier stage than conventionally practiced.⁷ Screening methodologies employing image cytometry can contrast antibody binding signals directly from living cells and avoid any nonspecific signals. As a high-throughput image cytometer, the Celigo platform has been implemented as a highly sensitive and versatile tool for hybridoma screening, capable of detecting antibody production at concentrations as low as 5 ng/mL.⁸

The Celigo platform continues to be the instrument of choice for hybridoma screening directly because of its ability to process large volumes of samples rapidly in an automated fashion; the Celigo image cytometer has even been used by researchers at the FDA to study hybridomas.⁹ Early implementation of high-throughput screening through image cytometry can greatly facilitate antibody and therapeutic discovery.

Support for CRISPR/cas9 editing

Image cytometry is frequently used in automation to track growth rates prior to batch clone screening and mass production of therapeutic products. Image cytometry has also been integral to several other aspects of cell line development especially in the generation of CRISPR/Cas9 edited cell lines. Researchers at Novo Nordisk Foundation Center for Biosustainability have published several articles about the use of the Celigo image cytometer in their genome technology.^{10,11}

Some of these applications include measuring transfection efficiency rates for mCherry-positive cells within their fluorescent enrichment protocols, cell cycle analysis with G2/M cell cycle arrest to test effects on CRISPR/ Cas9-mediated genome editing in CHO cells, and to validate maintenance of cell viability. Examining cell health, including apoptosis rates and reactive oxidative stress, is also a common application for image cytometry in cell line development.^{12,13,14,15}

Overall, the Celigo Image cytometer can help validate and accelerate several processes, while allowing for higher throughput evaluation of targeted genes aimed at enhancing therapeutic and antibody production.

Regulatory considerations

Regulatory processes for novel therapeutics continue to emphasize the importance of single-cell cloning and proof of monoclonality before generating a master cell bank (MCB). Researchers at the FDA have identified clonality as one of the most crucial steps in guaranteeing quality and safety.

The FDA researchers recommend that two rounds of limiting dilution or FACS be used along with imaging to supplement the clonality data.^{17,18} The use of these additional verification steps should greatly increase the probability of monoclonality. The WHO ECBS has also stated that cloning procedures should be fully documented with appropriate statistics and detailed images.⁴ Assurance of clonality is a crucial part of the overall control strategy to ensure product safety, stability, quality, and efficacy. Reviewers do an assessment of the clonality of the MCB during the Investigational New Drug application stage.

This review includes information on the cloning process, clone expansion, selection, and clone testing in accordance with ICH Q5D and EMA/CHMP. The FDA also requests that assurance of clonality be submitted along with the Biologics License Application.^{17,18} Image cytometry can be useful in supporting each of the steps within this process.

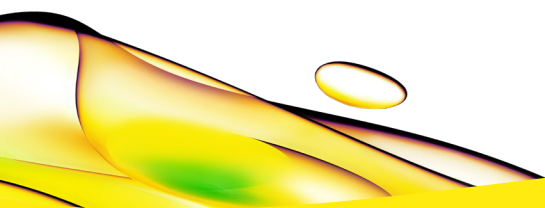
High-throughput solution

The Celigo image cytometer provides fast, time-saving, high-throughput analysis and imaging with the ability to document monoclonality and expansion with great ease.

The Celigo system is the “Swiss Army knife for cell line generation,” states Jean Qiu, founder of Nexcelom Bioscience (now part of Revvity) in and out of automation, the Celigo image cytometer has an extensive track record of successfully aiding in single-cell cloning, growth tracking, phenotypic stability, scale-up projects, and providing supporting documentation to meet FDA, ICH, and WHO standards. Revvity is committed to supporting the cell line development community by continuing to provide complete image cytometry solutions to advance research and therapeutics.

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