Short Note

f.sight™ | Fluorescence intensity-based isolation of single cells with assured clonality for CLD workflows

f.sight™

Fluorescence-based clonal cell line development (CLD) workflow

Clonal cell line development is a step crucial to many applications, including biopharmaceutical generation (e.g., monoclonal antibodies). The host cell line is transfected with the gene of interest (GoI) and selection pressure is performed to separate cells that significantly produce GoI. Thousands of clones must be screened to select for further analysis and production. Regulatory authorities also require that cell lines for biopharmaceutical production must originate from a single clone. Thus, single-cell cloning is performed to obtain monoclonal cell linesfor screening. In recent years, workflows have increasingly incorporated fluorescence-based cell line development to provide additional assurance of clonality. Here, cells are stained fluorescently to improve single-cell detection, particularly after deposition into multiwell plates. Fluorescent staining can be leveraged to distinguish between living and dead cells, and often, GFP expression is correlated to GoI expression, enabling better selection of producers. Therefore, incorporating fluorescence in cell line development is a robust method of improving efficiency and reducing time in the CLD workflow.

f.sight™

The f.sight™ is a single-cell printer™ that addresses the growing need for a fluorescence-based CLD workflow. The f.sight™ enables fully automated isolation and fluorescent sorting of single cells into 96- and 384-well plates. The patented technology uses high-resolution optics and image recognition to dispense single-cell-containing, free-flying small droplets in an inkjet-like process. With an integrated computer, the benchtop-sized instrument readily fits in a biosafety cabinet. A sterilized, disposable dispensing cartridge containing a microfluidic chip is continuously imaged at the nozzle to identify droplets containing single cells, enabling image-based assurance of clonality.

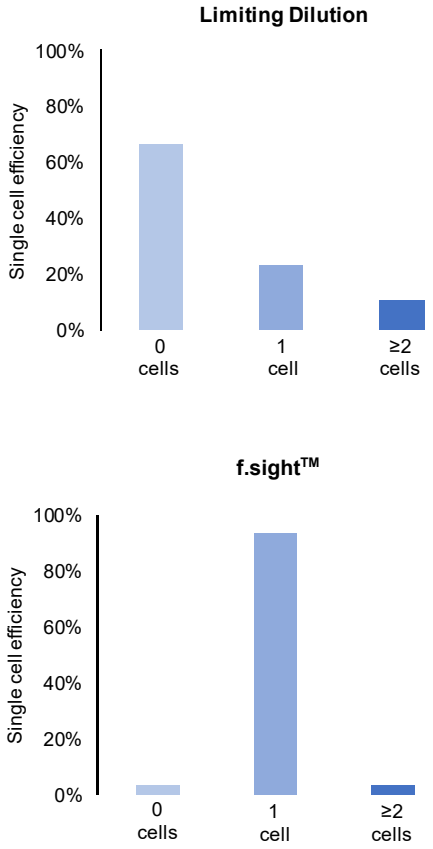

Figure 1. f.sight™ and limiting dilution single-cell efficiencies

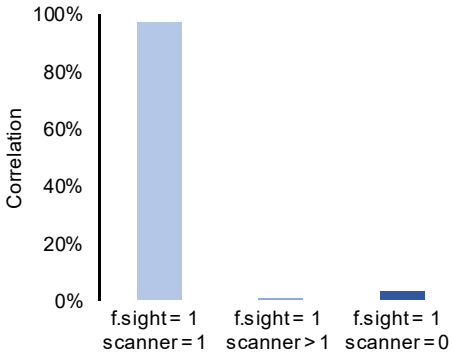

Figure 2. Correlation between f.sight™ and scanner images.

Single-cell efficiency and detection accuracy

The f.sight™ enables high single-cell efficiency compared to standard methods, including limiting dilution. Here, the f.sight™ isolated more than three times as many single cells compared to limiting dilution (Figure 1). Single-cell efficiency

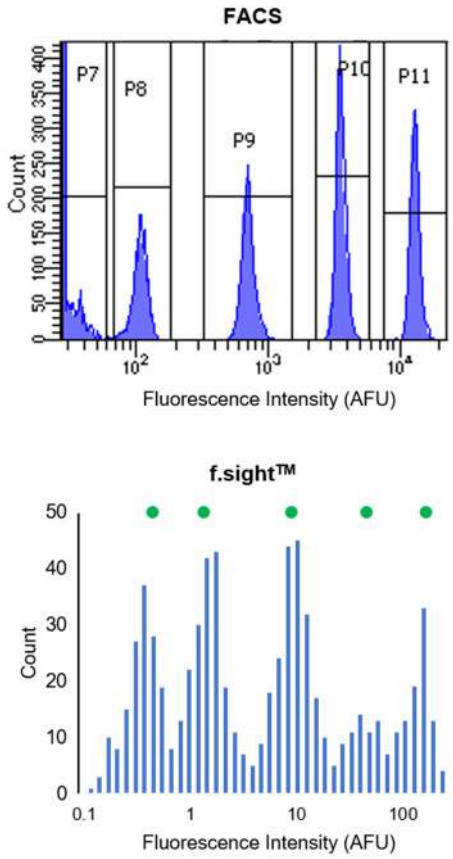

Figure 3. Benchmarking f.sight™ against FACS with FACS calibration beads. The green points denote the manufacturers stated average intensity for each bead population.

is important in CLD, as the lower single-cell efficiencies associated with limiting dilution results in slower workflows, as well as additional labor and resources. The f.sight's™ automatic dispensing of single cells can be processed in approximately 5 min for a 96-well plate and 15 min for a 384-well plate with optimized cell concentrations. Additionally, the f.sight™'s image-based assurance of clonality is very accurate. In conjunction with a collaborator, plates imaged with a scanner after dispensing showed that over 95% of the time, scanner images correlated with the f.sight™ nozzle images (Figure 2). The correlation is in line with similar data produced with the first-generation instrument, the single-cell printer™ (scp™)[1], highlighting the accuracy of image-based assurance of clonality with nozzle images.

f.sight™ high sensitivity and precise sorting

The f.sight™ can isolate both unlabeled and fluorescently labeled cells with high efficiency and sensitivity. This is achieved with a novel dual-camera system that captures and saves brightfield and fluorescence information simultaneously at full resolution. Both cameras can be individually controlled, and the built-in laser is freely adjustable. The system has a high dynamic range and is compatible with green fluorophores. The nontransparent flap protects fluorescent dyes and cells from ambient light during dispensing, and laser excitation is restricted to cells in the microfluidic chip nozzle. This minimizes the effects of bleaching and phototoxicity on the cells and dyes. The f.sight™ and its fluorescent capabilities were benchmarked with standard fluorescence-activated cell sorting (FACS) calibration beads, which contain five intensity-specific populations of beads. The f.sight™ identified all five populations, demonstrating its large high-sensitivity dynamic range that is capable of detecting low intensity beads (Figure 3).

The sensitivity of the f.sight™ enables the use of lower dye concentrations, which is important for cell types that are more sensitive to staining procedures. Here, the sorting capabilities were tested with CHO-K1 cells stained with 1 µM of CellTracker™ Green, a cell viability stain. In addition to cell size and roundness, selection parameters supported by the scp™, the software enables selection of the desired fluorescence intensity. Prior to dispensing, a scatter plot can be generated to determine the fluorescence intensity distribution for a given cell population. Figure 4 shows the fluorescence intensity of dispensed cells that were not stained (No stain) and cells stained but not sorted (Stained, No Sort), as well as cells dispensed from the same population sorted for high (Stained, High) and low (Stained, Low) fluorescence intensity. From the original population (Stained, No Sort), the f.sight™ can easily sort cells with both high and low fluorescence.

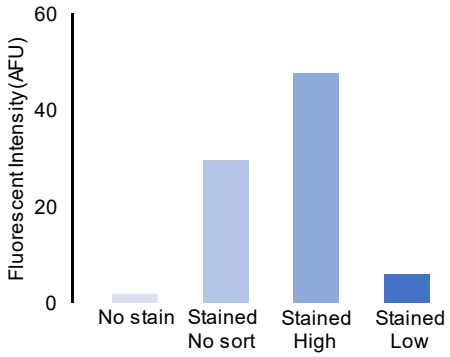

Figure 4. Fluorescence intensity of f.sight™ sorted cells.

Summary

The ability of the f.sight™ to readily sort cells by fluorescence intensity while achieving high single-cell efficiencies makes it compatible with fluorescence-based CLD workflows. The system's flexibility and automation enable its integration with downstream applications like single-cell cloning after CRISPR gene-editing or other fluorescence-based workflows outside of CLD.

Reference

1. Yim M., Shaw D., Achieving greater efficiency and higher confidence in single-cell cloning by combining cell printing and plate imaging technologies, Biotechnol Prog. 2018.

CONTACT

cytena GmbH
Neuer Messplatz 3 | 79108 Freiburg | Germany
Phone: +49 761 70 88 90 -0
Email: info@cytena.com