



Venkata Mantripragada, Viviane Luangphakdy, George Muschler, Travis Schneider, and Brian Handerhan

Introduction

Cleveland Clinic and Parker Hannifin have collaborated to create the Cell X™ and Colonyze™ platform as resource tools for cell source management and quality control.

Cells are the core resource for biological products & biomanufacturing. No new tissue is formed without cells.

Cell populations from different donors/tissues vary highly in concentration, prevalence, & biological potential. The challenge: managing this variation in design, fabrication, & quality control of all cell therapies.

Innovators of new therapies must have control over cell sources, quality, & biological attributes of cells entering/leaving the processing chain.

The cell sourcing and product quality mantra:

*Know what you have.
Pick what you want.
Show what you've done.
Know how to do it again.*

Quantitative metrics must be used to define critical quality attributes (CQAs) of cell sources and cell products.

Capabilities

Fully automated fluorescence microscope integrated with robotic platform & image analysis system that enables:

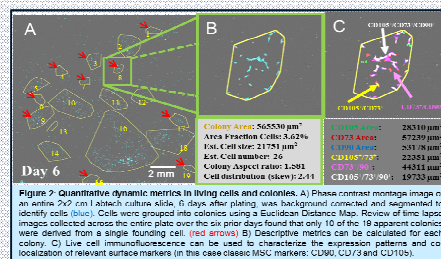
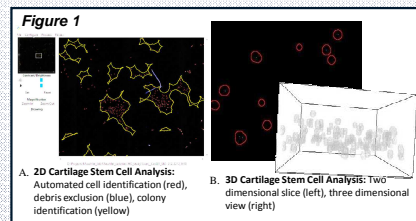
- Automated quantitative high resolution large field of view imaging of cell populations in culture (Figure 1).
- Quantitative automated image analysis of cells and colonies per ASTM standards (Figures 1 & 2).
- GMP Quality environmental controls (Figure 3).
- Automated processing to biopsy or pick selected cells or remove ("weed") undesired cells (Figure 5 & 6).
- Automated fluid management for media changes and rigorously documented timed addition of reagents.
- Rigorous time oriented documentation of process steps and process imaging

Automated Cell and Colony Analysis Software System (Colonyze™)

PROBLEM: Existing cell and colony counting methods were inconsistent, time-consuming and imprecise.

SOLUTION: Automated imaging and analysis is quantitative, standardizable, and reproducible Colonyze™ Imaging and Analysis Software provides:

- Color and grayscale imaging and processing
- 2D and 3D interactive graphics (Figure 1A, 1B)
- Time-lapse image processing and analysis
- Batch process images
- Automated or Interactive review
- Integrated multi-fluorophore analysis (Figure 2)
- Spreadsheet-ready output



"Cell X™ Robot" (Cell X™) Automated Cell and Colony Manipulation

PROBLEM: Manual manipulation is time consuming, imprecise, highly variable, and undocumented.

SOLUTION: Automated manipulation of cells is rapid, precise, repeatable and rigorously documented. Integration of automated quantitative imaging and automated picking enables rapid early performance based selection of cell sourcing (Figure 3-5).

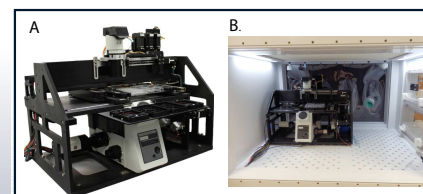


Figure 3 Cell X™ developed in collaboration with Parker Hannifin (Irwin, PA). Integrated cell imaging, analysis, and cell selection system (A). Weight and dimensions were designed to fit in a laminar flow hood or Xvivo oxygen controlled processing environment (Biospherix, Parish, NY) (B).

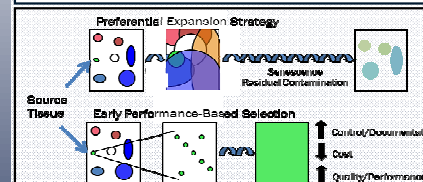


Figure 4 Process workflow: Typical cell therapies workflows involving preferential expansion are time consuming. Early performance based selection enabled by this technology rapidly isolates a cell population of interest.

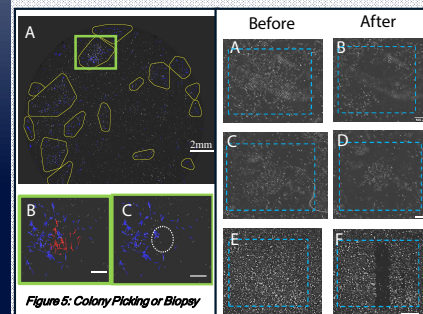


Figure 5: Colony Picking or Biopsy

A single colony may be identified from a large mixed culture (5A), based on quantitatively defined features. The colony can be picked or just sampled for analysis and the cells included in the sample documented by comparison of the before and after images. (5B & C) Sampled cells colored red. This colony can then be used or excluded from use, based on the biopsy data.

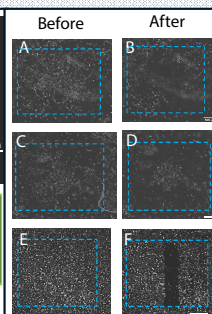


Figure 6: Automated Cell Selection

Three options are illustrated:
1. Pick (select) desired cells and move to a new location for processing. (6A & 6B)
2. Remove (weed) unwanted cells, preserving desired cells in situ (6C & 6D)
3. Biopsy Remove cells from a defined area (6E & 6F)

Future Project Direction

OBJECTIVE: Deploy the Cross-Cutting Cell X™ platform to academic centers and commercial partners. Cell X™ is directly related to four thrust areas: Cell Selection and Sourcing, Bioprocess Automation, Tissue/Product Finishing, and Testing, and can be readily integrated into a Biofabrication Platform processes and controls.

Cell X™ features would enable our partner to:

1. Improve the reproducibility and the quality of the cells they include in their research or products.
2. Reduce the variation and cost of manufacturing associated with variation in starting materials (cell sourcing decisions).
3. Improve process documentation and controls, eliminating operator bias.
4. Define quantitative imaging-derived in process quality attributes as well as CQA –based metrics for release criteria their unique cells or products.

Relevance & Need

User cases for Cell X™ and Colonyze™ will include:

1. Early performance-based colony selection to reduce large batch-to-batch & donor-to-donor variation in MSC population.
2. Optimize & standardize the process of iPSC selection using standardized criteria derived from live-cell imaging.
3. Systematic, reproducible & fast removal ("weeding") of differentiating cells during iPSC expansion.
4. Development of standardized image-based profiles for a range of cell types based on ASTM standards for use as in process controls & CQA-driven release criteria.
5. Design of rapid scalable automated processes with defined validation histories for applications to Cell Banks, CLIA certification of laboratories, & automated GMP fabrication processes.