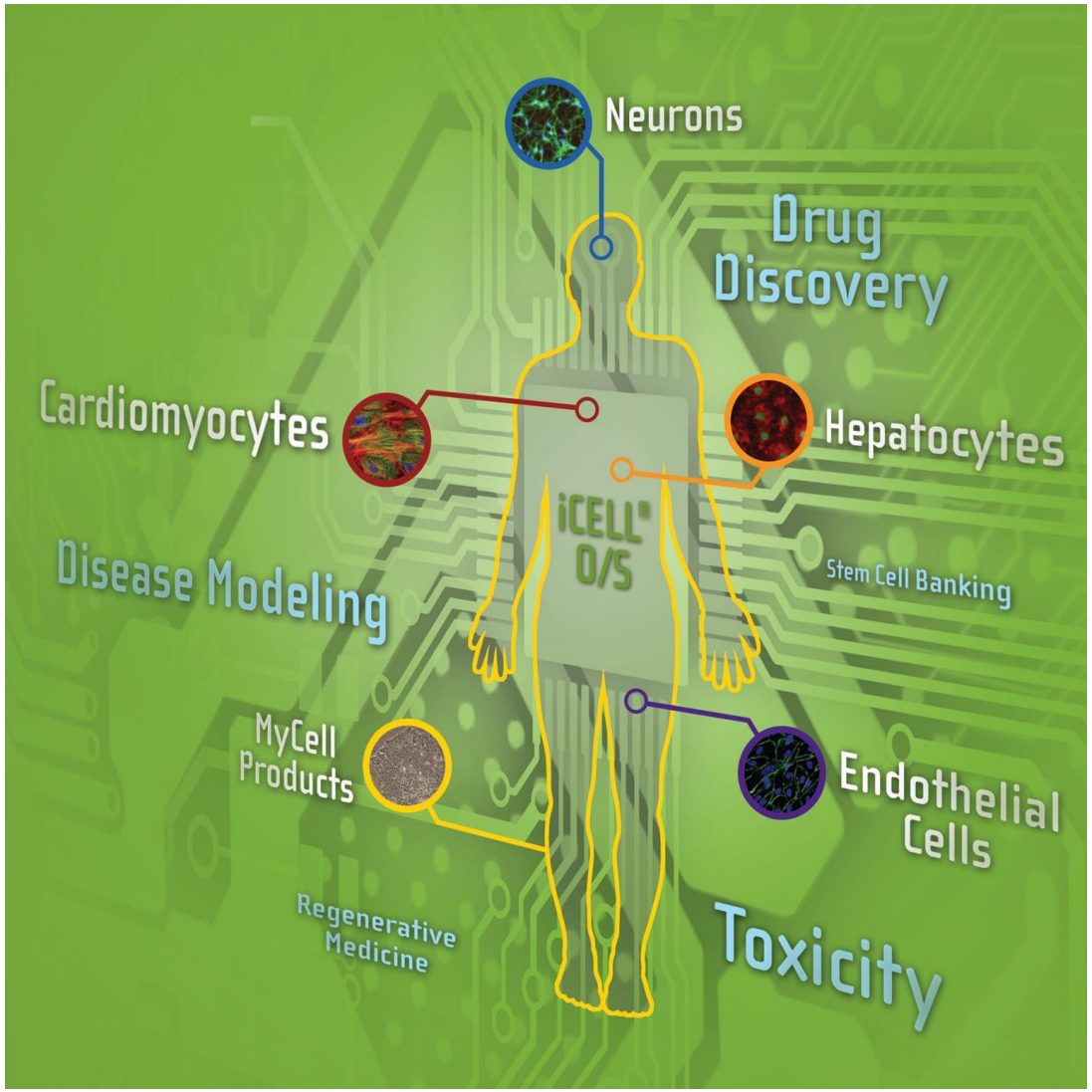
Cell Counting

Cellular Dynamics International
A Fujifilm Company

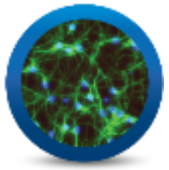
Company Overview

- Cellular Dynamics International (CDI) is the world's largest producer of human iPS cells and iPS cell-derived cell types
- Acquired by FUJIFILM in April of 2015
- Headquartered in Madison, WI , with a site in Novato, CA
- Currently employs ~175 total staff
- ~750 yrs human stem cell experience
- >900 patents (owned or licensed) to enable FTO
- Life Sciences and Therapeutics divisions
- Core competencies
  - **Creation and culture of human iPS cells**
    - Normal and disease phenotypes
  - **Genetic engineering of iPS cells**
    - SNP repair, Indels, knock-out, knock-in and more
  - **Development of new differentiation protocols**
    - Differentiated cells from all three germ layers
  - **Manufacture of human iPS cell-derived cell types**
    - Scalable production of highly purified cells

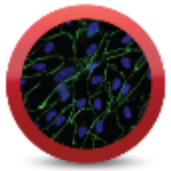


Life Science Research: Current Product Portfolio

iCell Cardiomyocytes

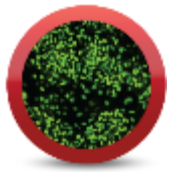


iCell Neurons

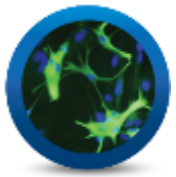


iCell Endothelial Cells

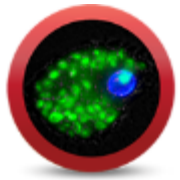
iCell DopaNeurons



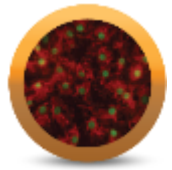
iCell Cardiac Progenitor Cells



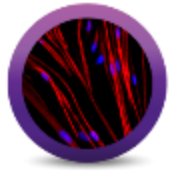
iCell Astrocytes



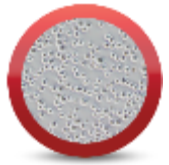
iCell Macrophages



iCell Hepatocytes



iCell Skeletal Myoblasts



iCell Hematopoietic Progenitor Cells

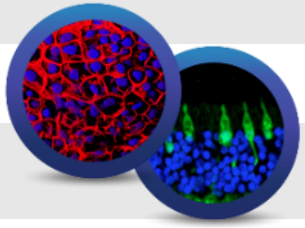
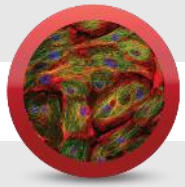
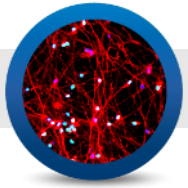
Additional undisclosed cell types

Essential 8 Medium
Episomal Reprogramming Kit
Vitronectin

MyCell Products

- Quality Management System; Selected guidance from both ISO and GMP
  - ISO – ideal for research phase
  - GMP – required for clinical phase
- 100% complete batch record and traceability; all material, incubator, operator, refrigerator, freezer, instrument, etc.
- Material management risk profile for every reagent on BOM, qualified reagents, 2nd vendors, etc.

Cellular Therapeutics: Active Programs

Indication	Therapeutic	Discovery	Pre-clinical	IND	Phase I
OCULAR					
Age-related Macular Degeneration					
Retinitis Pigmentosa and Cone Dystrophies					
CARDIAC					
Heart Failure (ischemic cardiomyopathy)					
NEUROGENERATIVE					
Parkinson's Disease					

Disease Modeling: 250 Donor Panel Flow Diagram





Blood sample collection

Cell Count

Cell Selection and expansion

Cell Count

Reprogramming

Cell Count

Colony picking and expansion

Cell Count

iPSC line Cryo and QC tests

Nuclease-mediated genome engineering

Cell Count

Engineered line Cryo

Cell Count

Selection and Expansion

Cell Count

Cardiomyocyte differentiation

Cell Count

Cardiomyocyte Purification

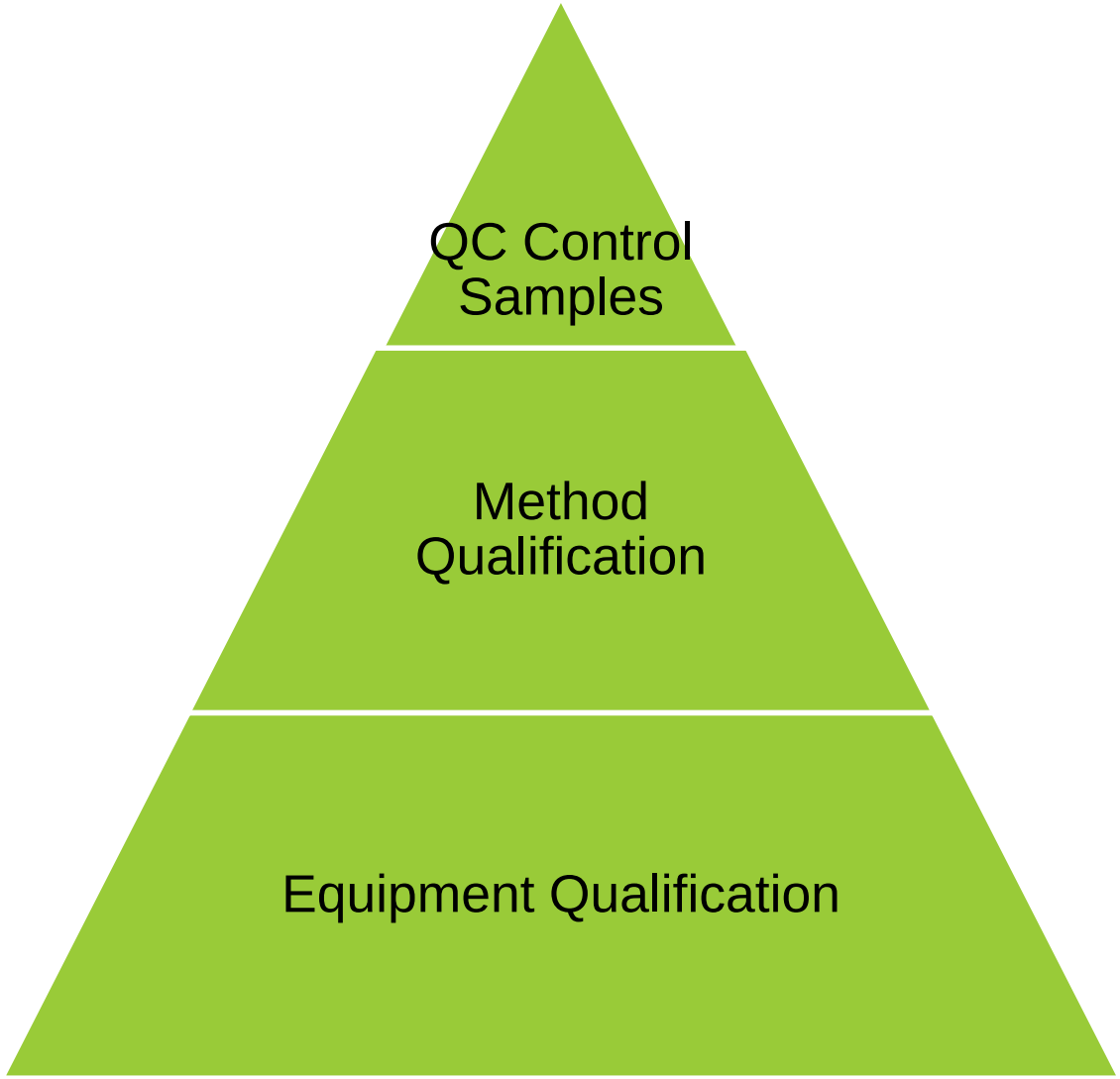
Cell Count

Cardiomyocytes Cryo and QC tests





Basis for Data Quality for Cell Counting



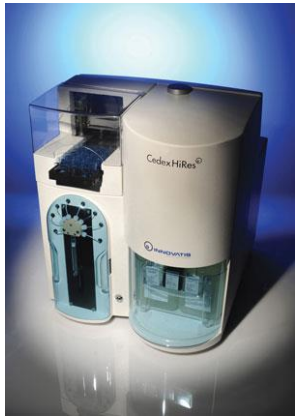
Daily calibration
QC control sample run before each release QC cell counting test

Robustness
Accuracy
Precision
Operator
Device
Product lot
Product vial
Assay reagent

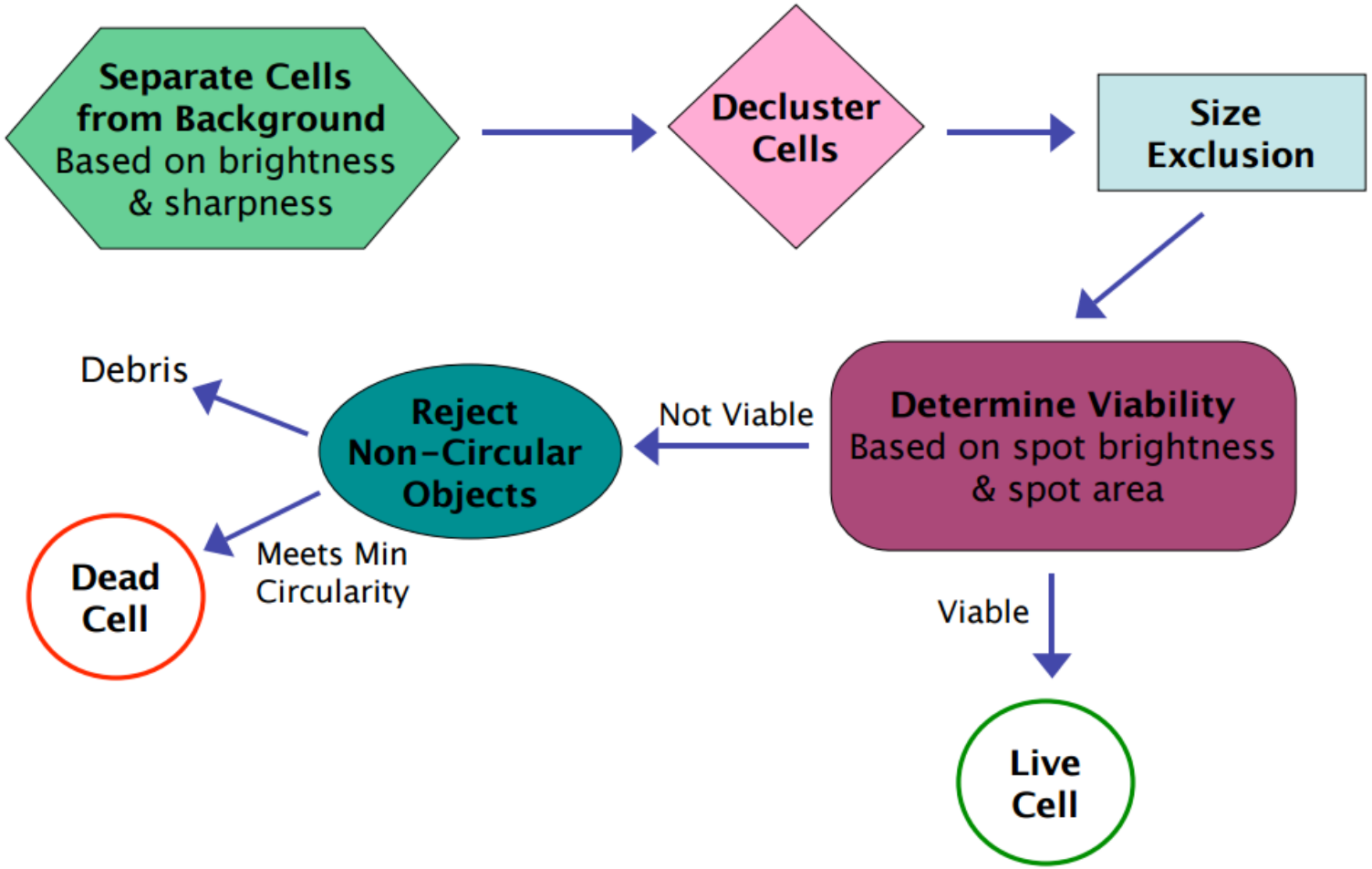
IQ
OQ
PQ

Transition from Cell Counting Device Cedex to ViCell

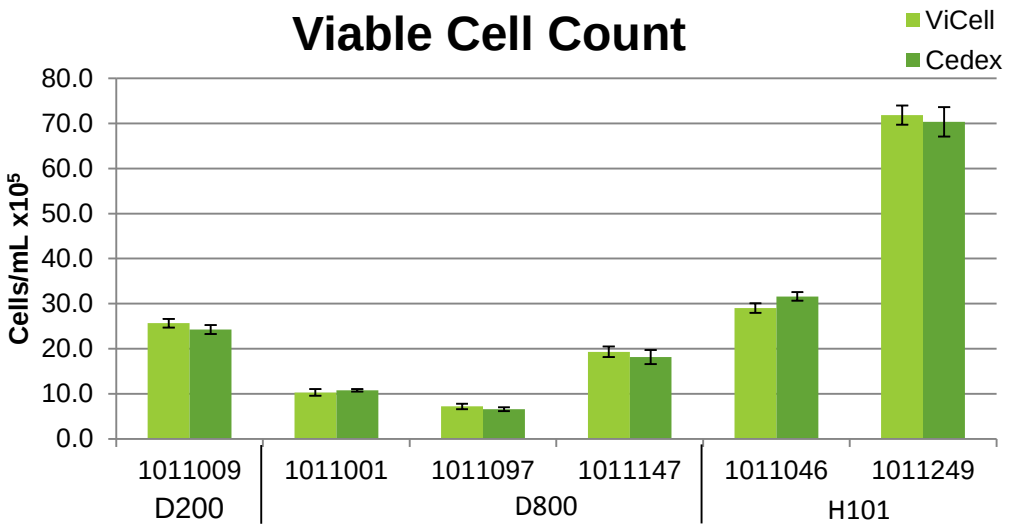
- Keep using Cedex for existing manufacturing processes and develop new processes with ViCell
- Adjust ViCell settings to match cell count numbers from Cedex from the same sample
- Adjust ViCell settings to reflect the true cell count, understand the cell count differences between Cedex and Vicell from the same samples for a certain process step and adjust process cell number ranges accordingly

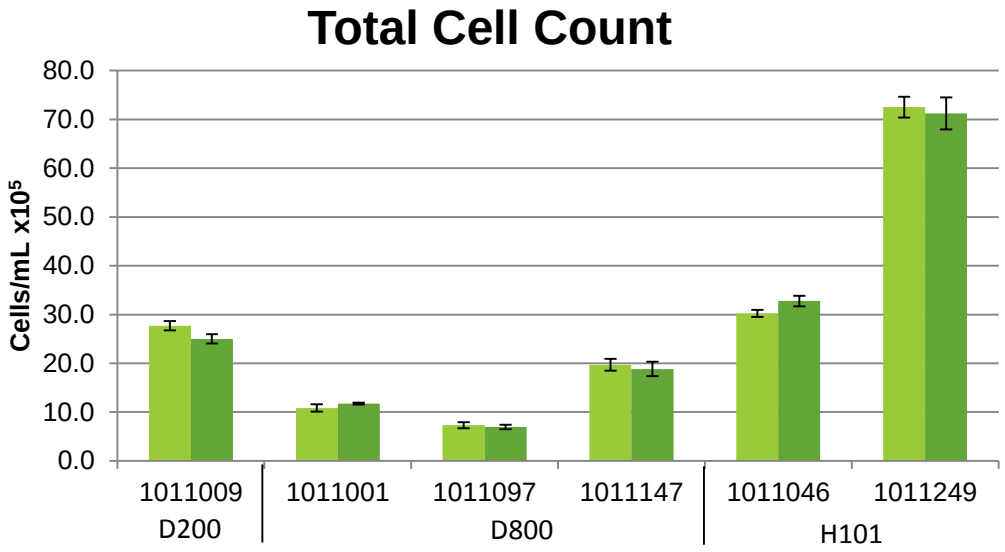


Vi-CellXR Cell Image Analysis Scheme



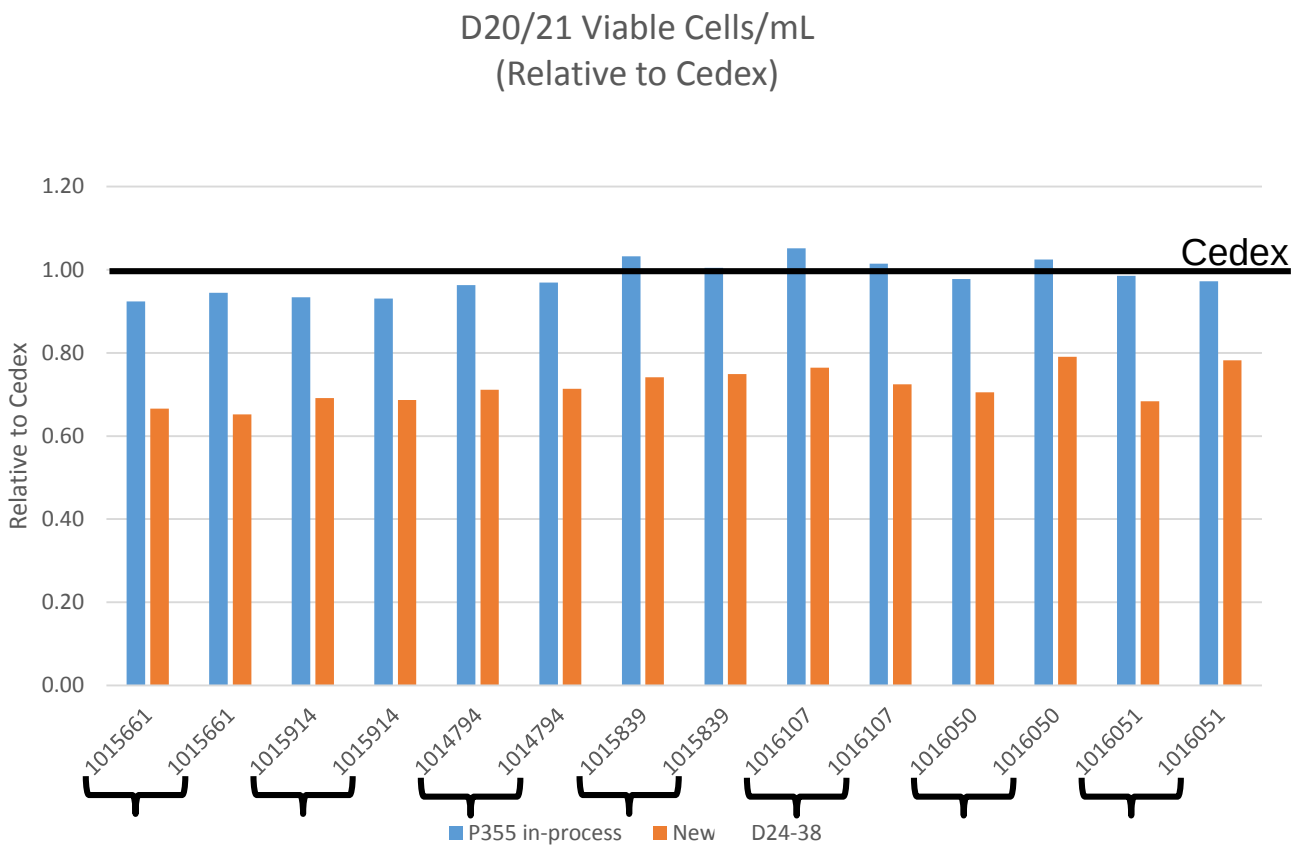
Adjust ViCell Settings To Match Cell Count Numbers From Cedex

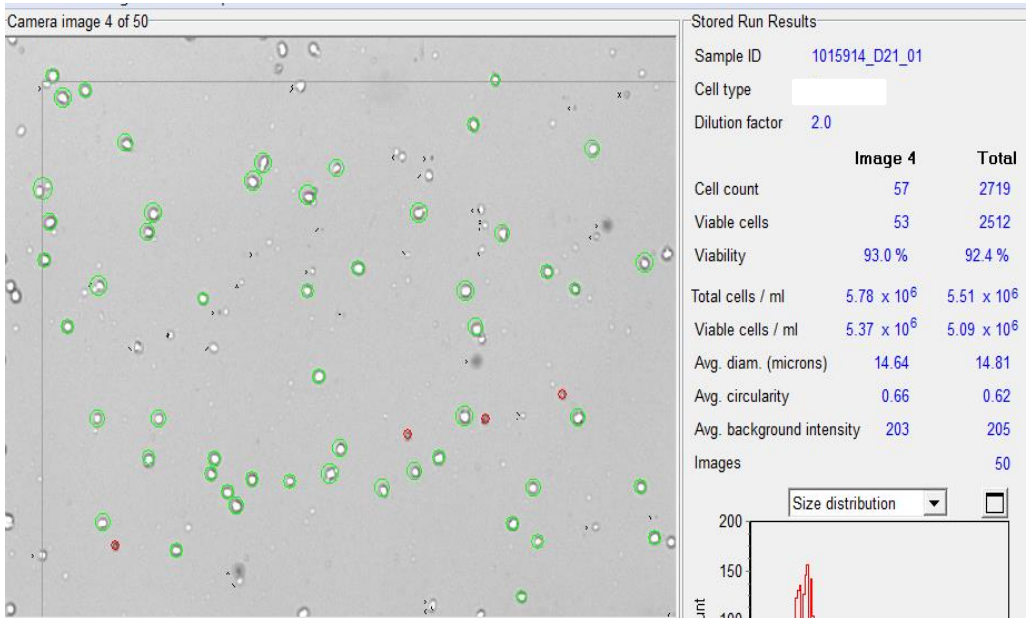




ViCell Settings	Cell Type I
Min Dia	7
Max Dia	50
Cell Brightness	75
Cell Sharpness	40
Viable Cell Spot Brightness	80
Viable Cell Spot Area	20
Min Circularity	0.3

Differentiation Process Example I- Day21 Vicell Setting

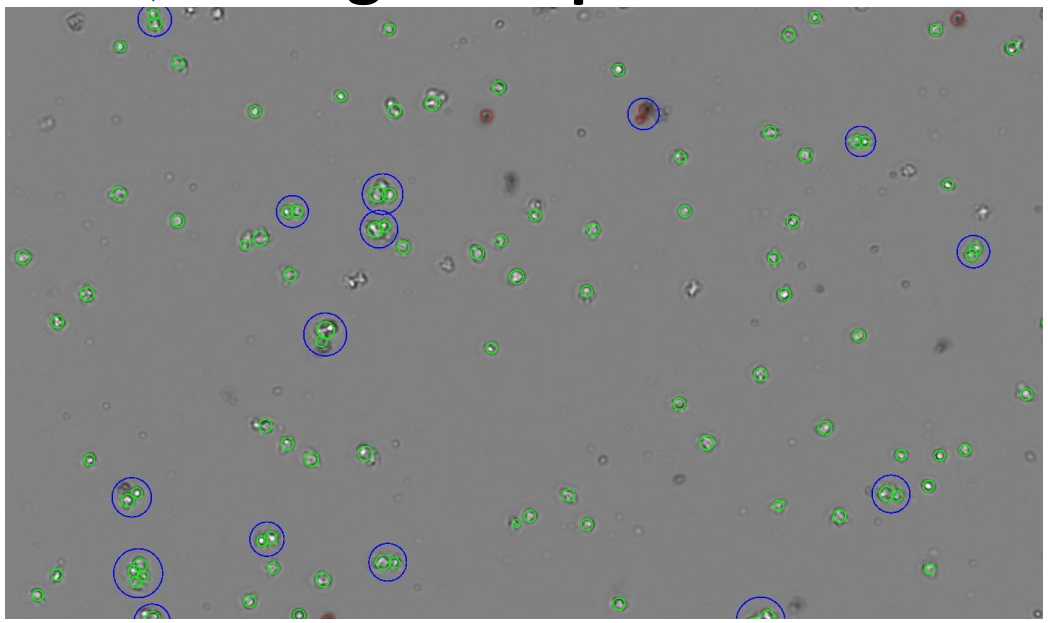


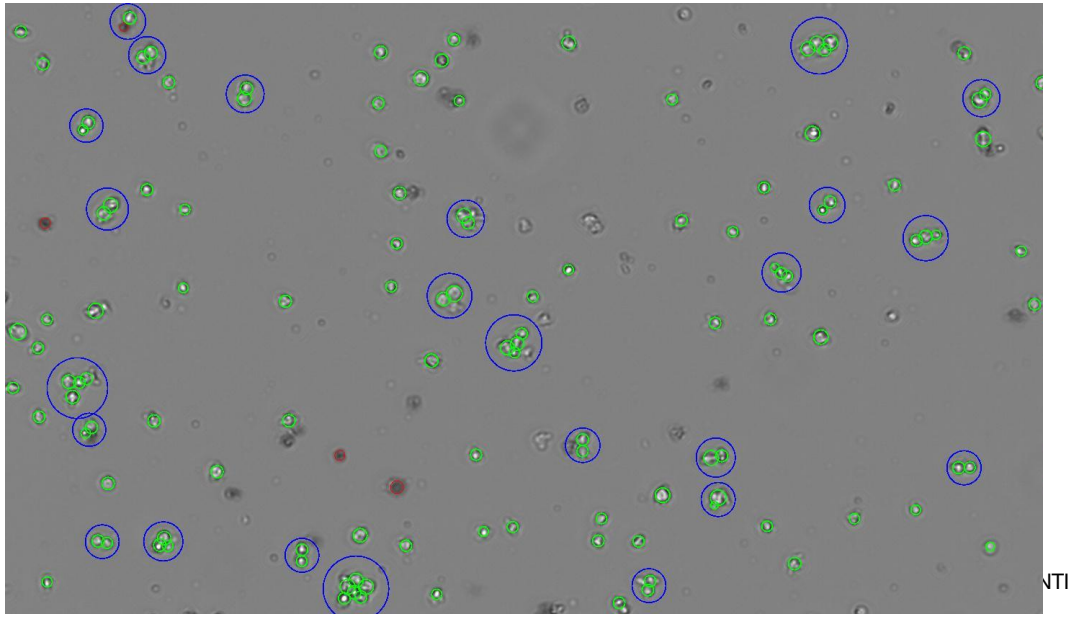


- Vicell Cell Setting A in-process setting matches the Cedex counts very well (within 8%)
- Vicell Cell Setting A in-process setting matches the reality well enough.
- Recommend this setting for D21 counts

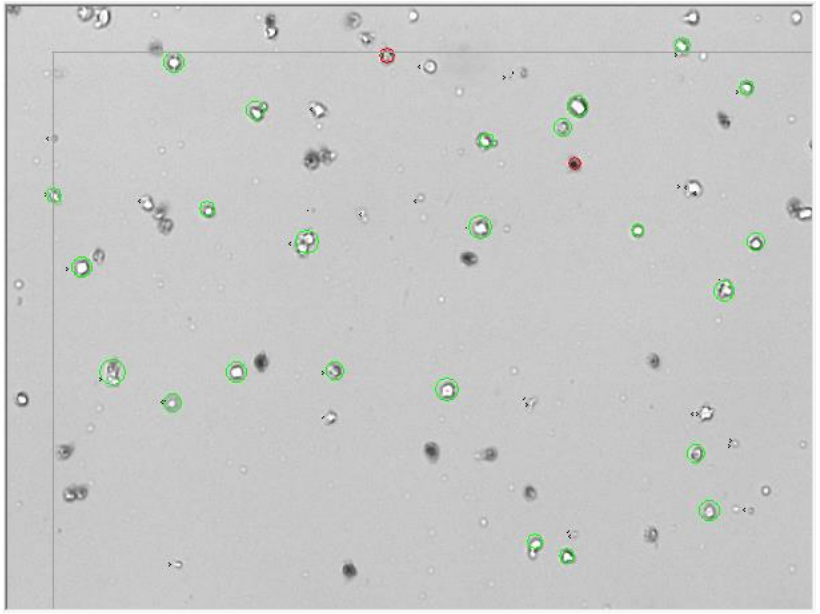
Differentiation Process Example II-
Image comparison: 1018075 s1 d11

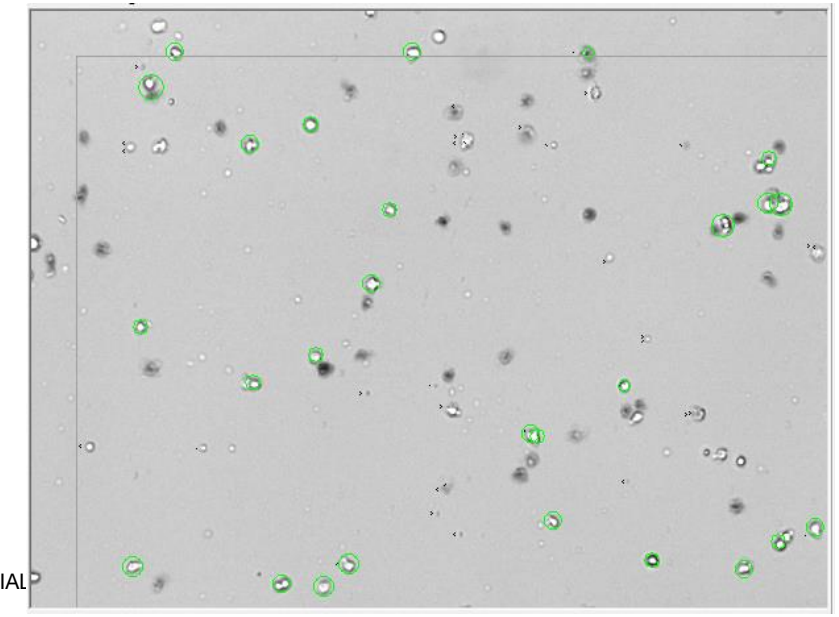
CEDEX





ViCell

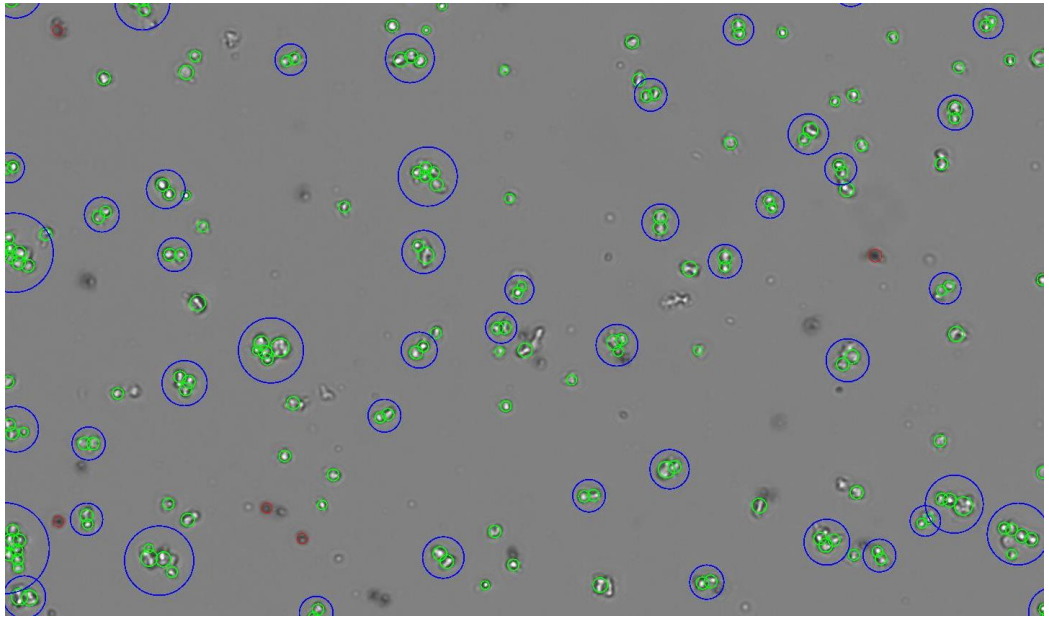


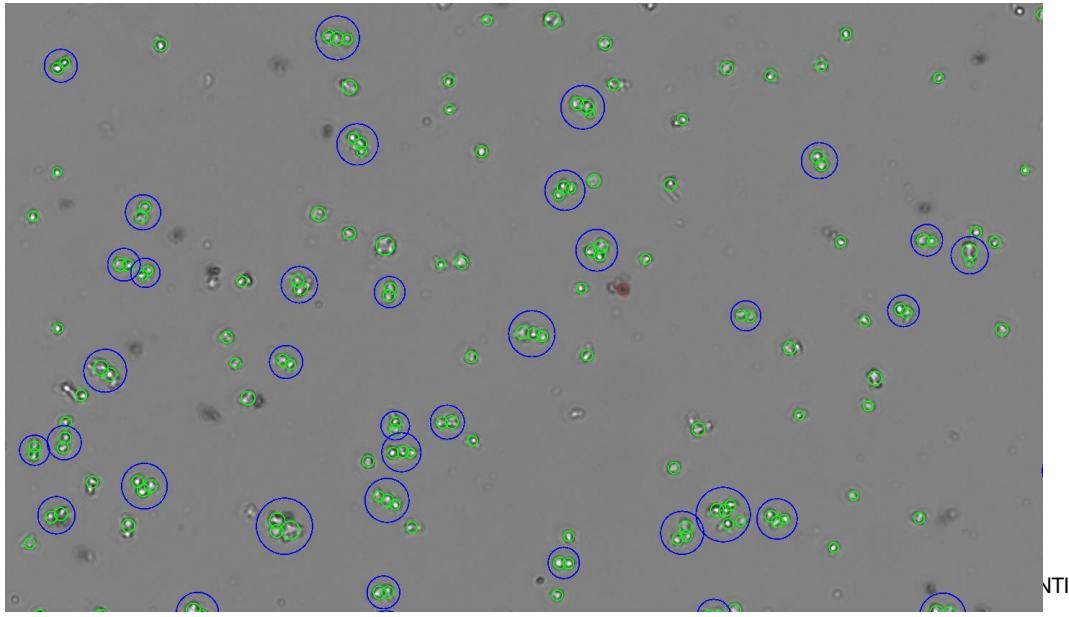


Differentiation Process Example II-

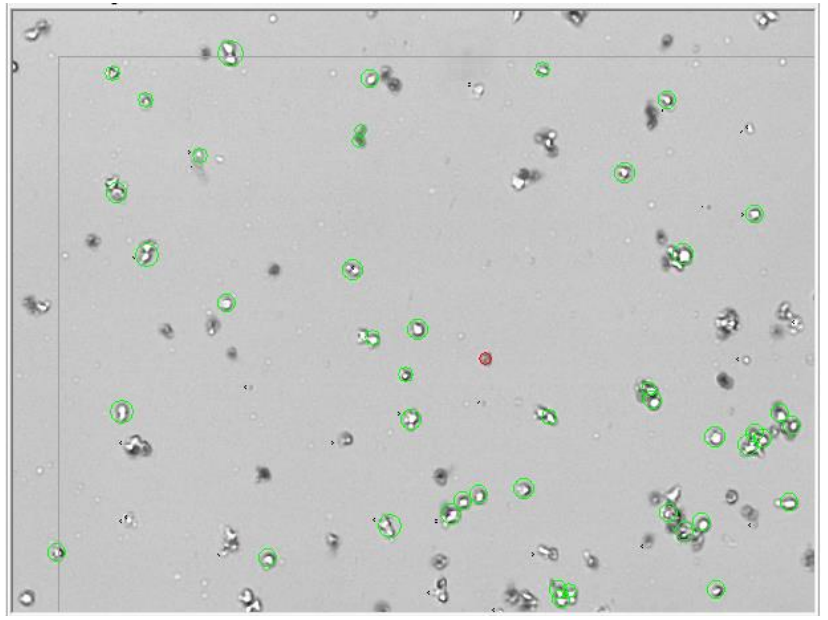
Image comparison: 1018136 s1 d11

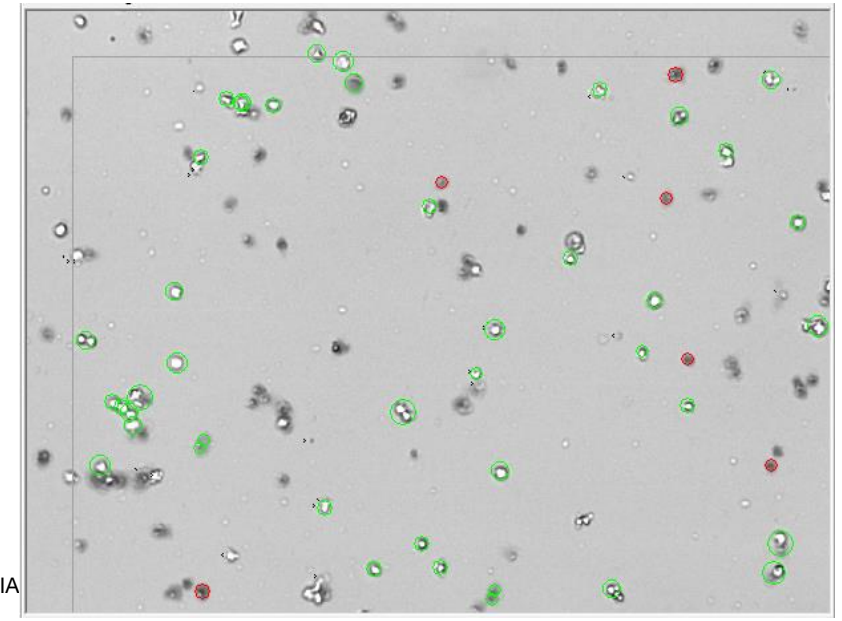
CEDEX





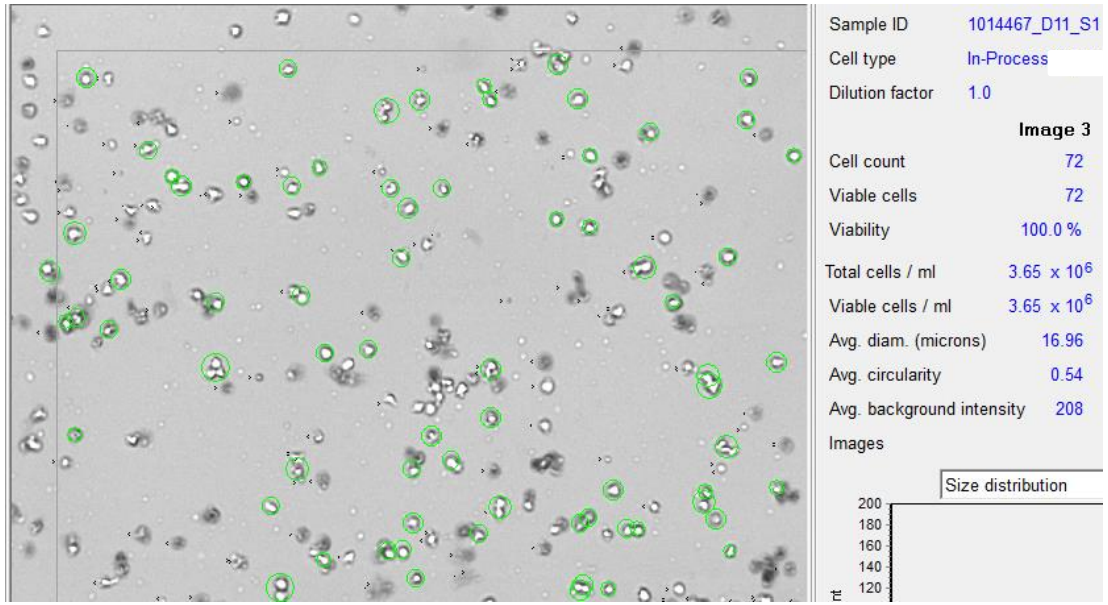
ViCell



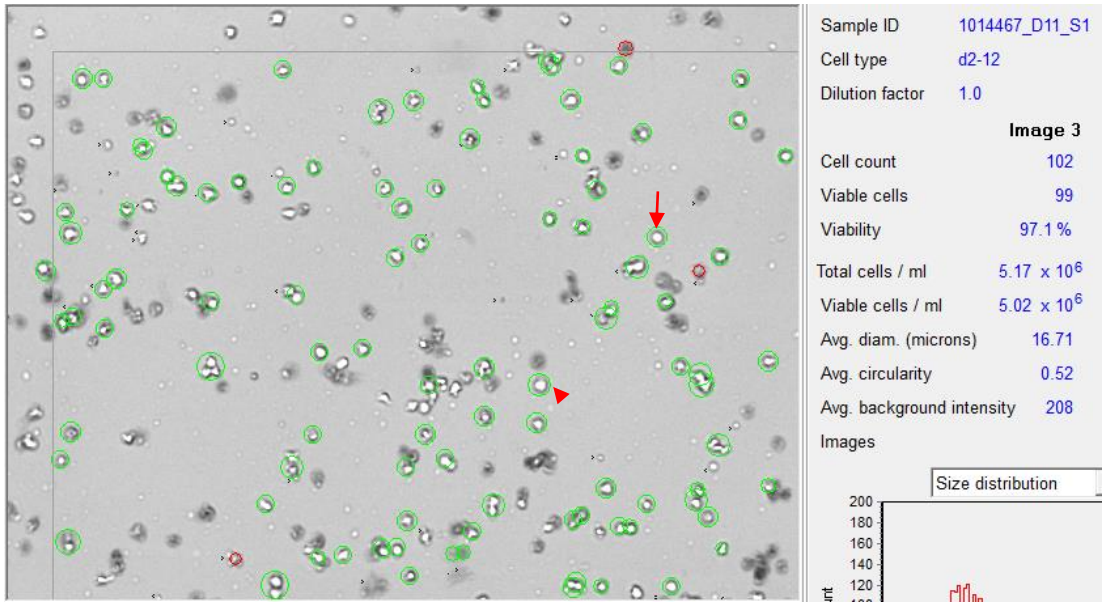


Adjusting Cell Settings for ViCell

Cell Setting A



Cell Setting B



Lesson's Learned

- Choose cell counting platform wisely to avoid changes:

Automated
Meet sample throughput
Reliable and objective results over manual hemacytometer methods Cell
Record keeping
21 CFR 11 compliance
Audit trail
Electronic signature capability
Secure user sign-on
User level permissions
Administrative configuration tools
Easy software update
Good technical and service support
PM

Thank You!