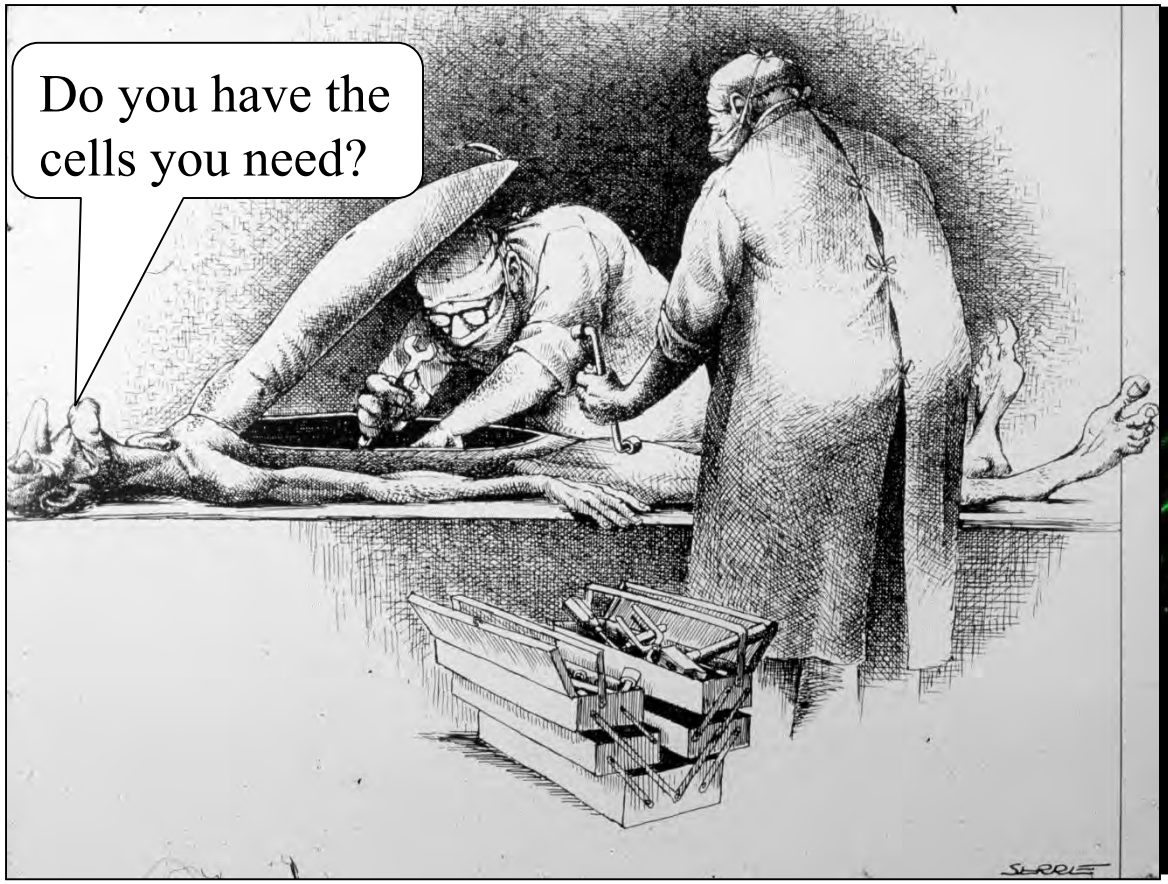
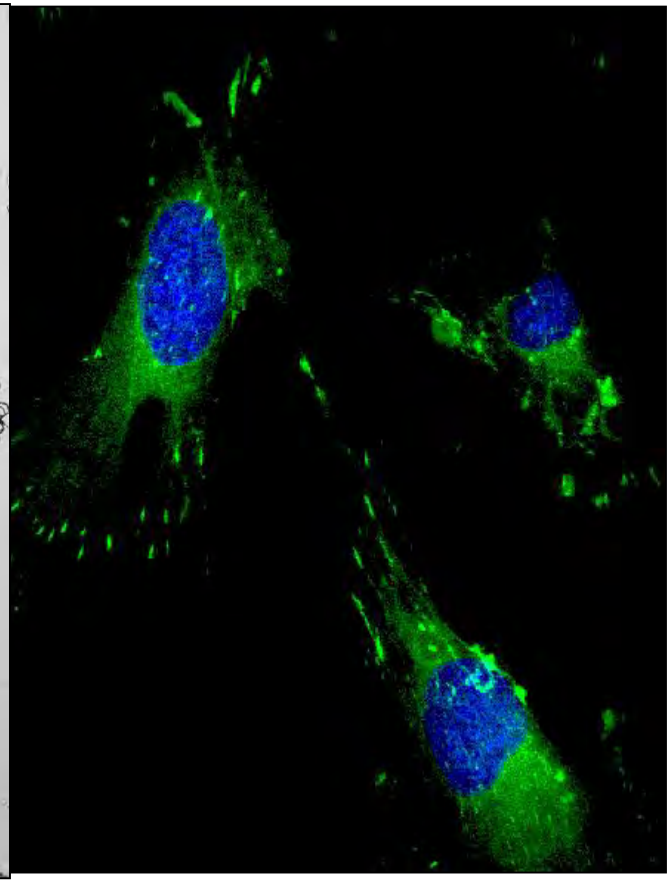
Enabling Cell Therapy

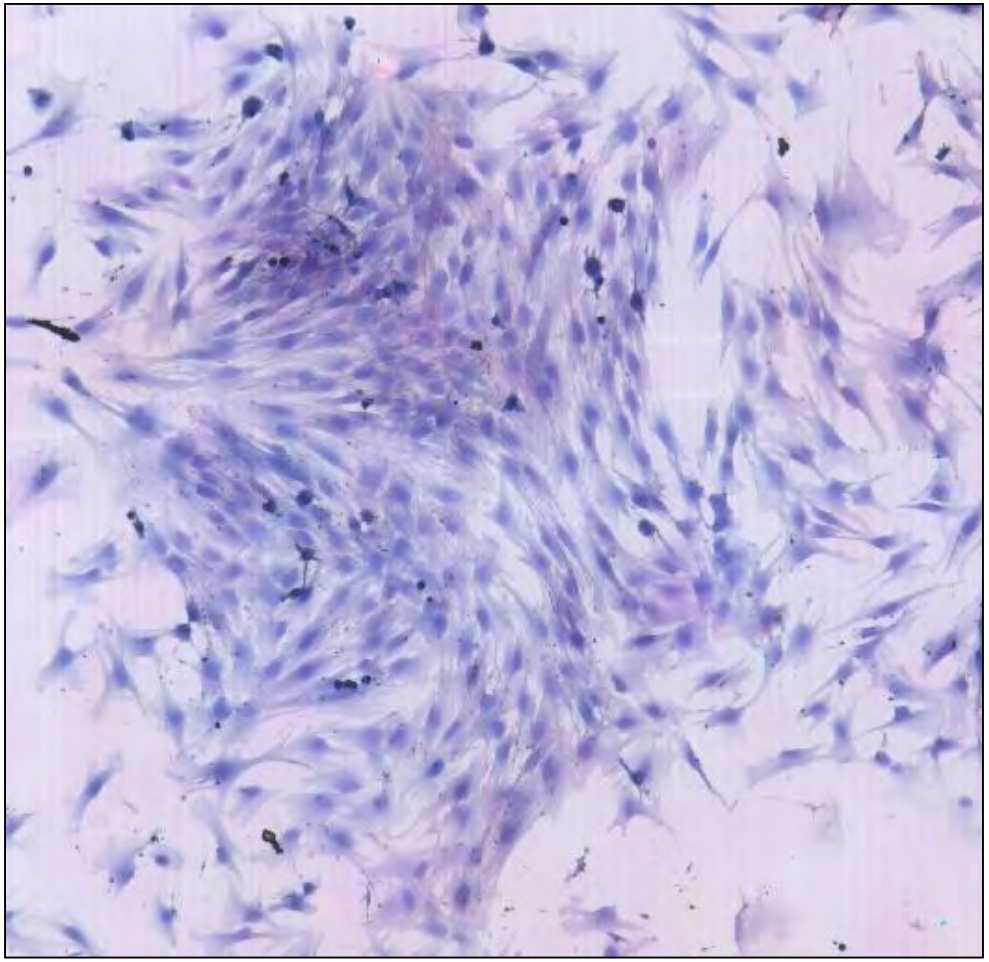
Finding and Processing the Cells We Need





George F. Muschler, M.D. - Cleveland Clinic

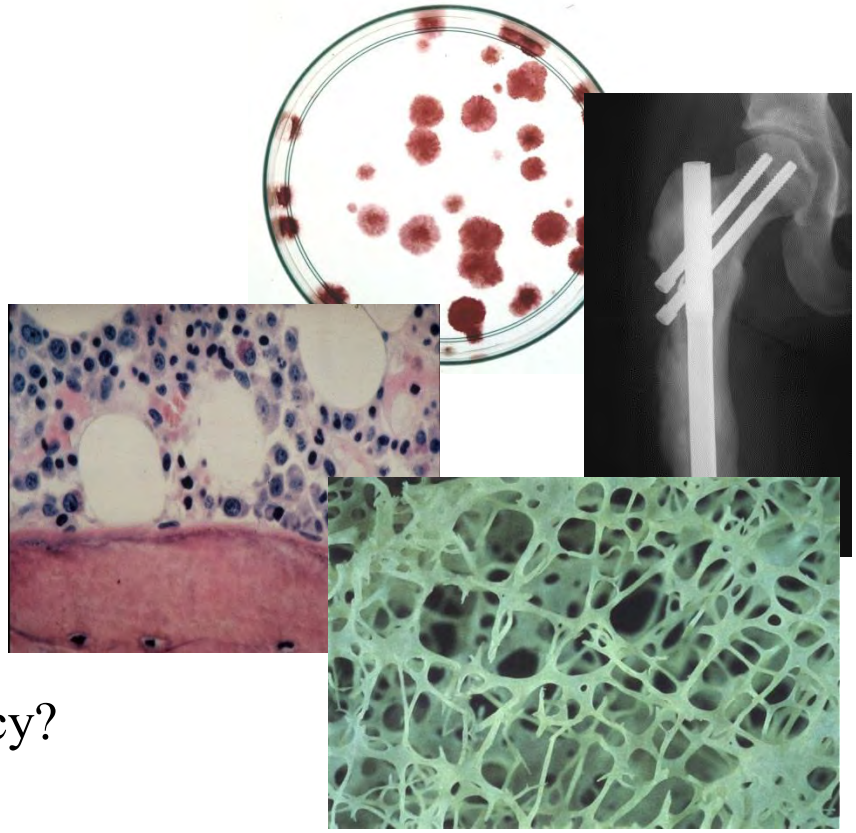
Integrated Cellular Imaging, Analysis and Processing Tools for Regenerative Medicine



- Harvest
- Assay
- Isolation
- Attachment
- Proliferation
- Migration
- Differentiation
- Transplantation
- Homing
- Kinetics

Questions – Problems - Opportunity

- What cells do we want?
- How can they assayed?
- Where are they?
- How many are there? Patient Variation
- Harvest Technique? Yield?
- Transplantation Environment?
- Processing/Selection Methods?
- Transplantation Method?
- Clinical Setting of Need?
- Preclinical Testing – Safety and Efficacy?
- Regulatory Barriers – Uncertainty?
- Market Size, IP Protection, Price?
- Clinical Trials – Time/Money/ROI?



Questions – Problems - Opportunity

- Ask Important Questions
- Solve Important Problems
- Create Opportunity

Questions – Problems - Opportunity

- Ask Important Questions ⇨ Sources of Variation?
- Solve Important Problems ⇨ Methods for Management
- Create Opportunity ⇨ Safe and Efficient Products

Cell Therapy Paradigm

"Life is a River"

Tissue

Cell Therapy Paradigm

"Life is a River"

Tissue → Tissue Loss

Senescence
Degeneration
Destruction
Trauma

Cell Therapy Paradigm

"Life is a River"

Stem Cell → Progenitor Cell → Tissue → Tissue Loss

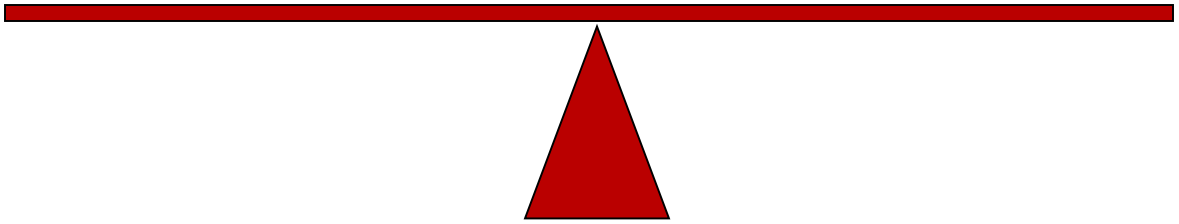
Activation
Proliferation
Migration
Differentiation
Survival

Senescence
Degeneration
Destruction
Trauma

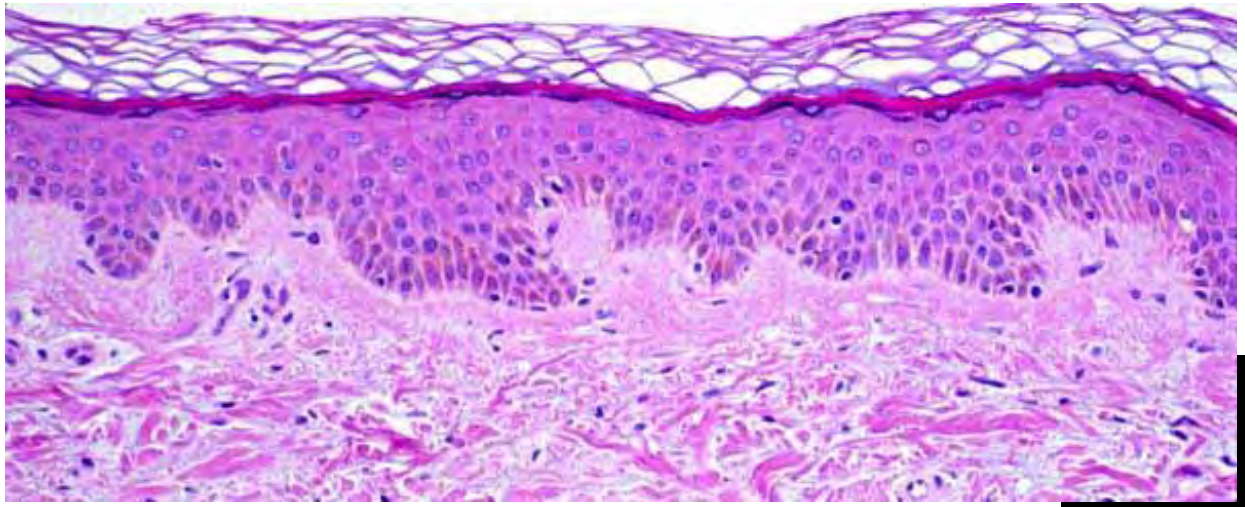
Cell Therapy Paradigm

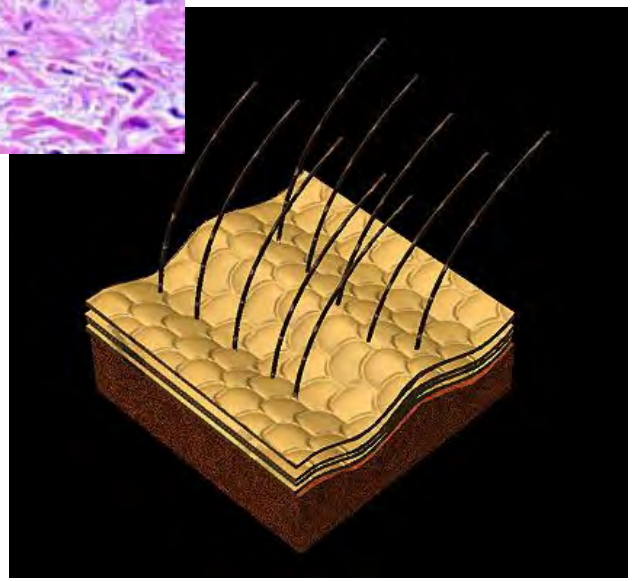
"Life is a River"

Stem Cell → Progenitor Cell → Tissue → Tissue Loss

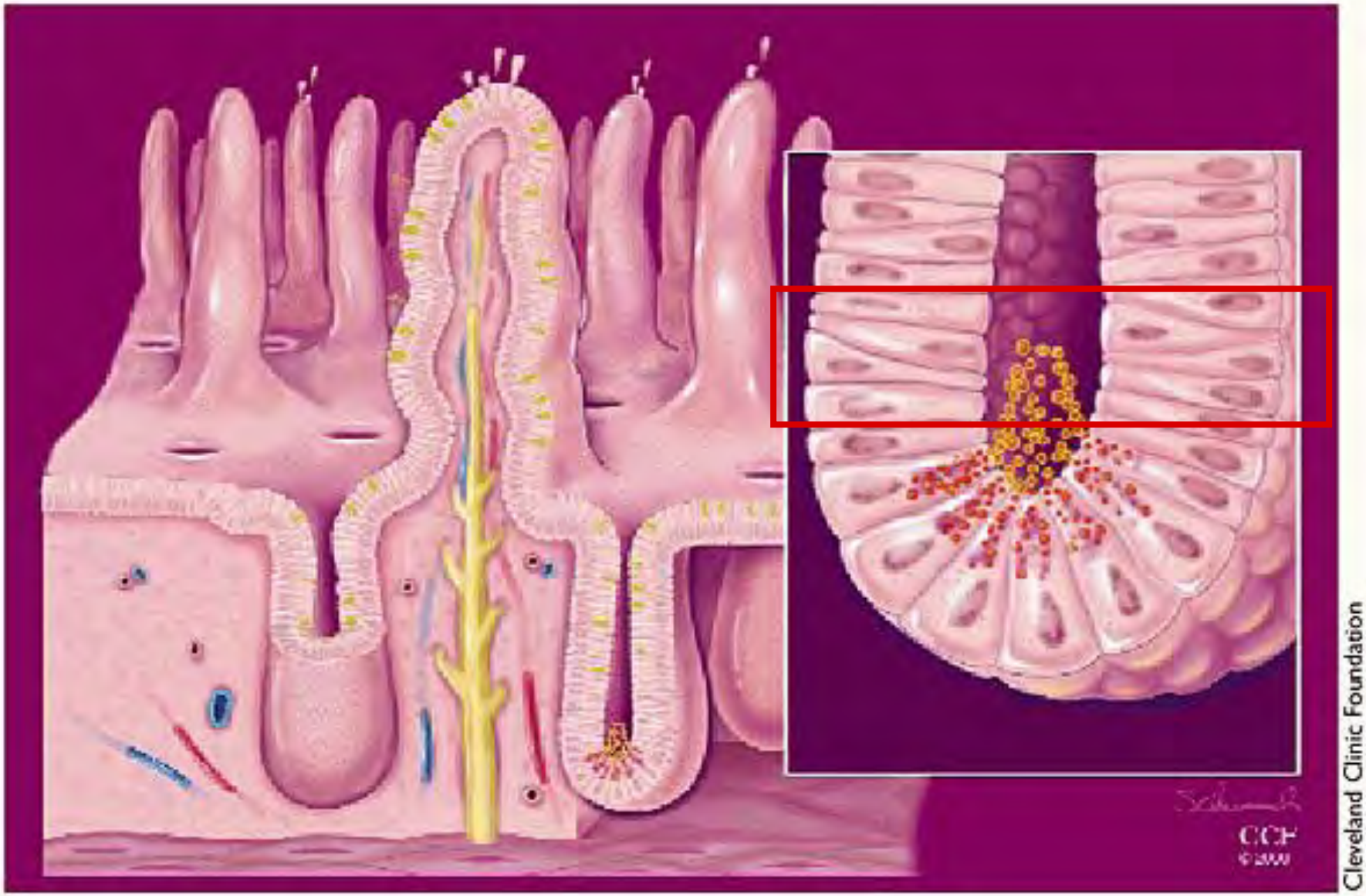


Skin



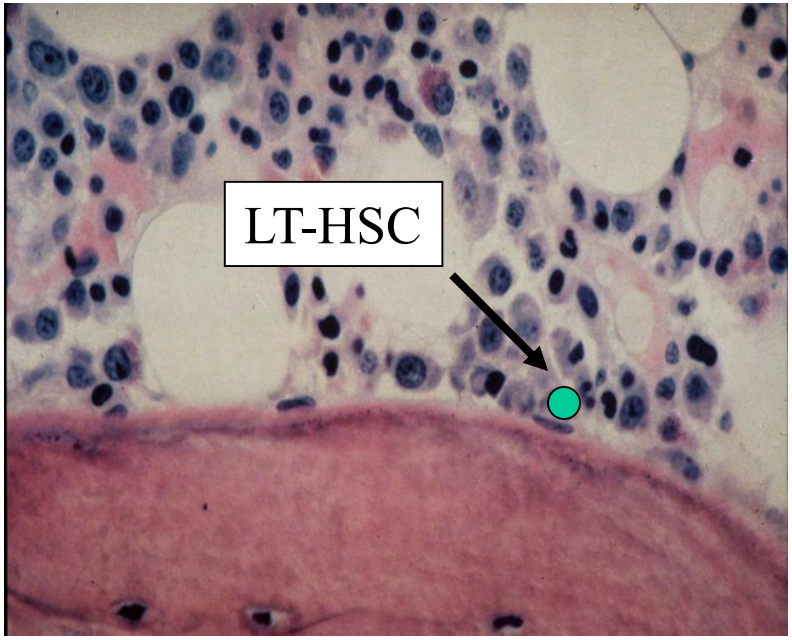


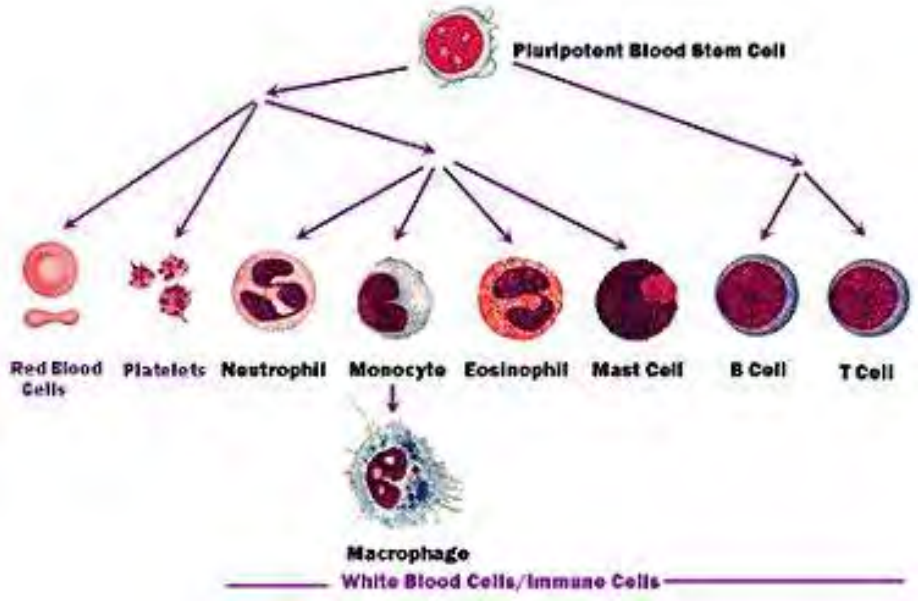
Intestinal Lining Cells



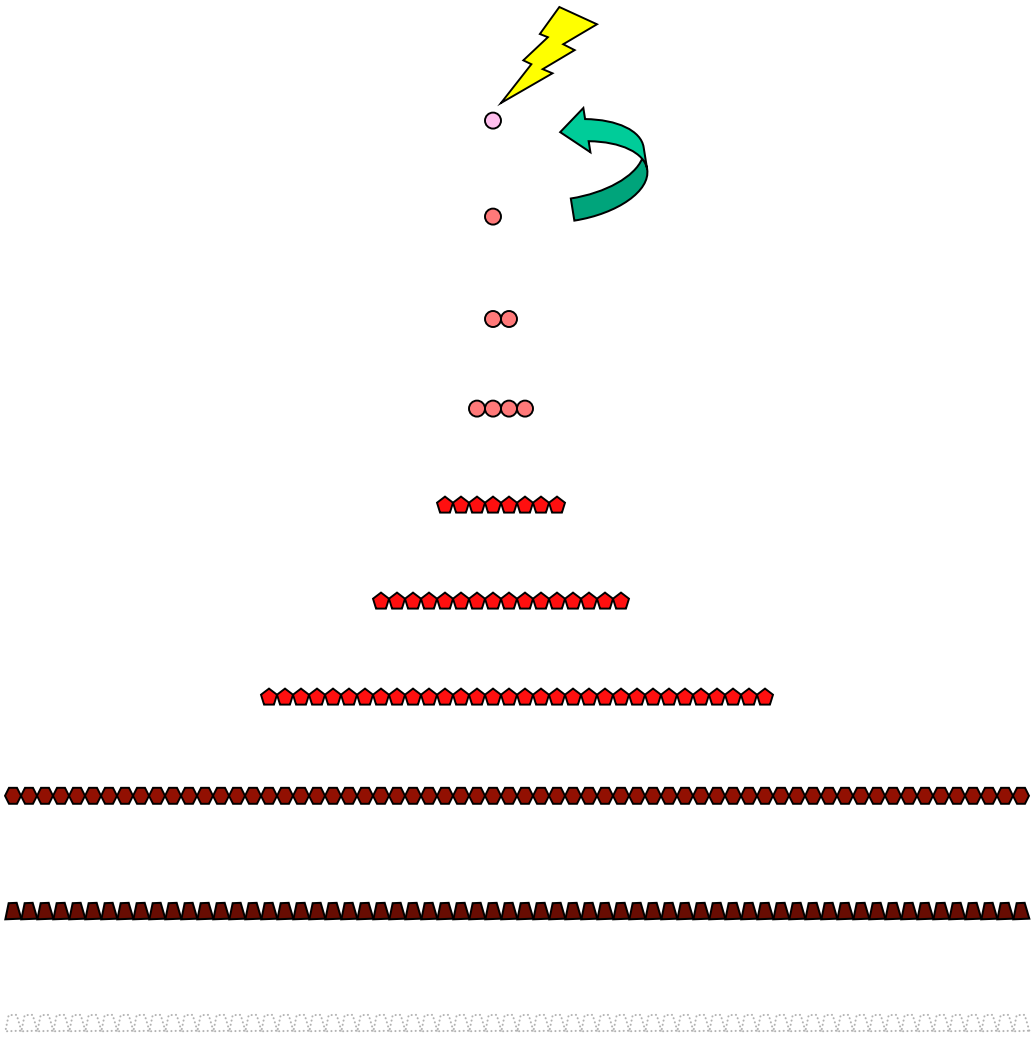
Marrow and Blood

Hematopoietic Stem Cell (HSC)





The Stem Cell Life Cycle



Activation

Self Renewal

Proliferation

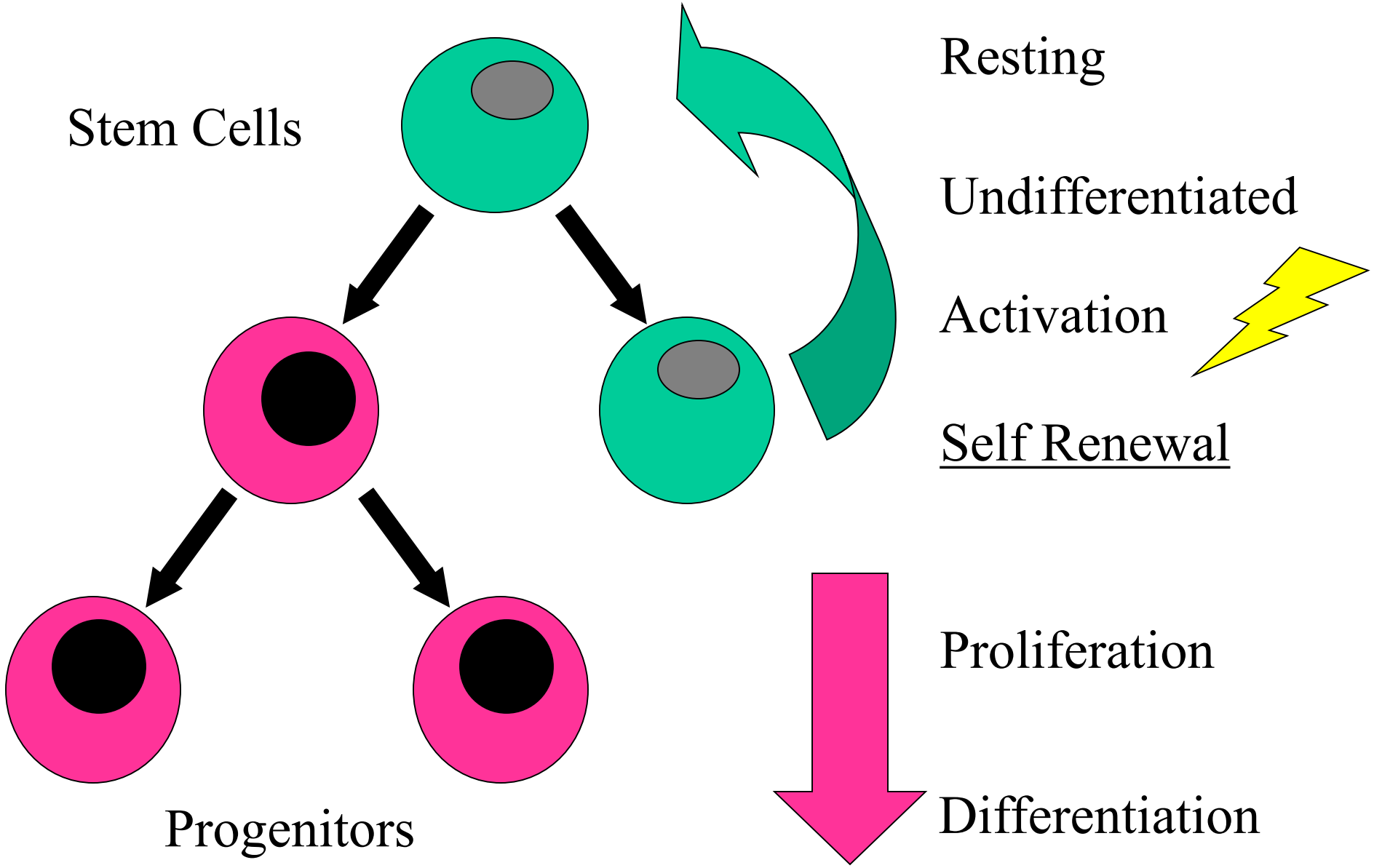
Migration

Commitment

Terminal Differentiation

Death

What is a Stem Cell?



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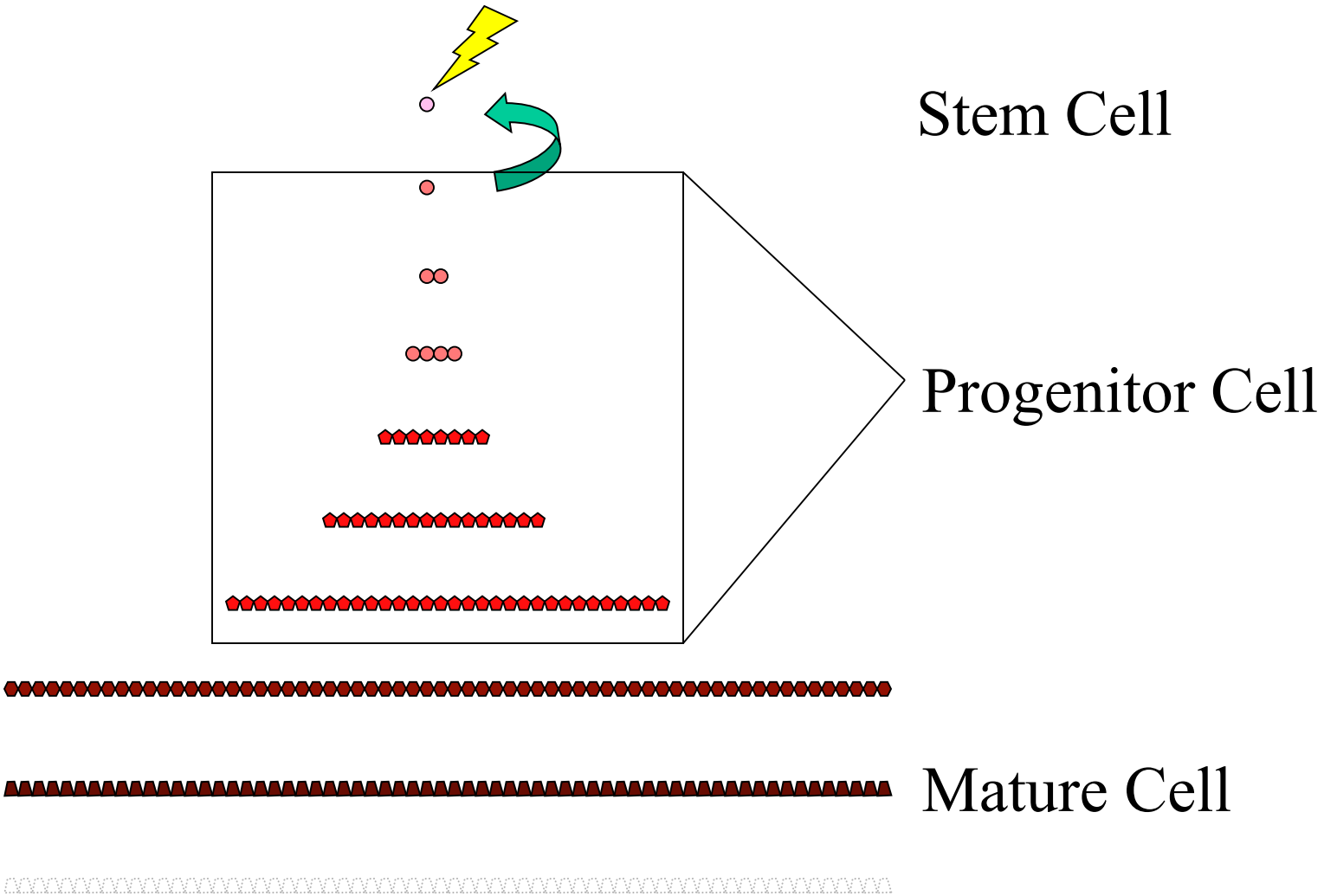
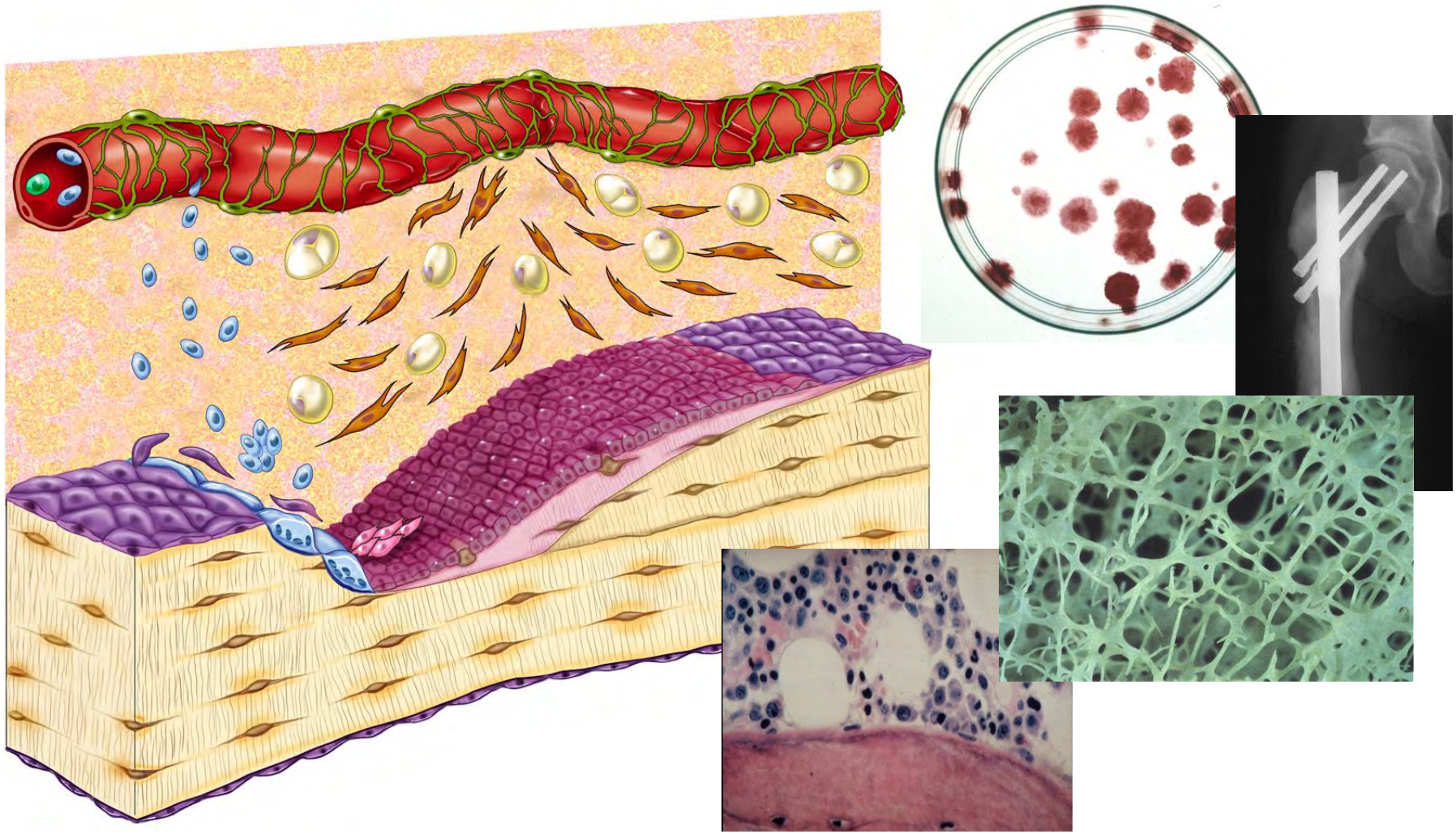
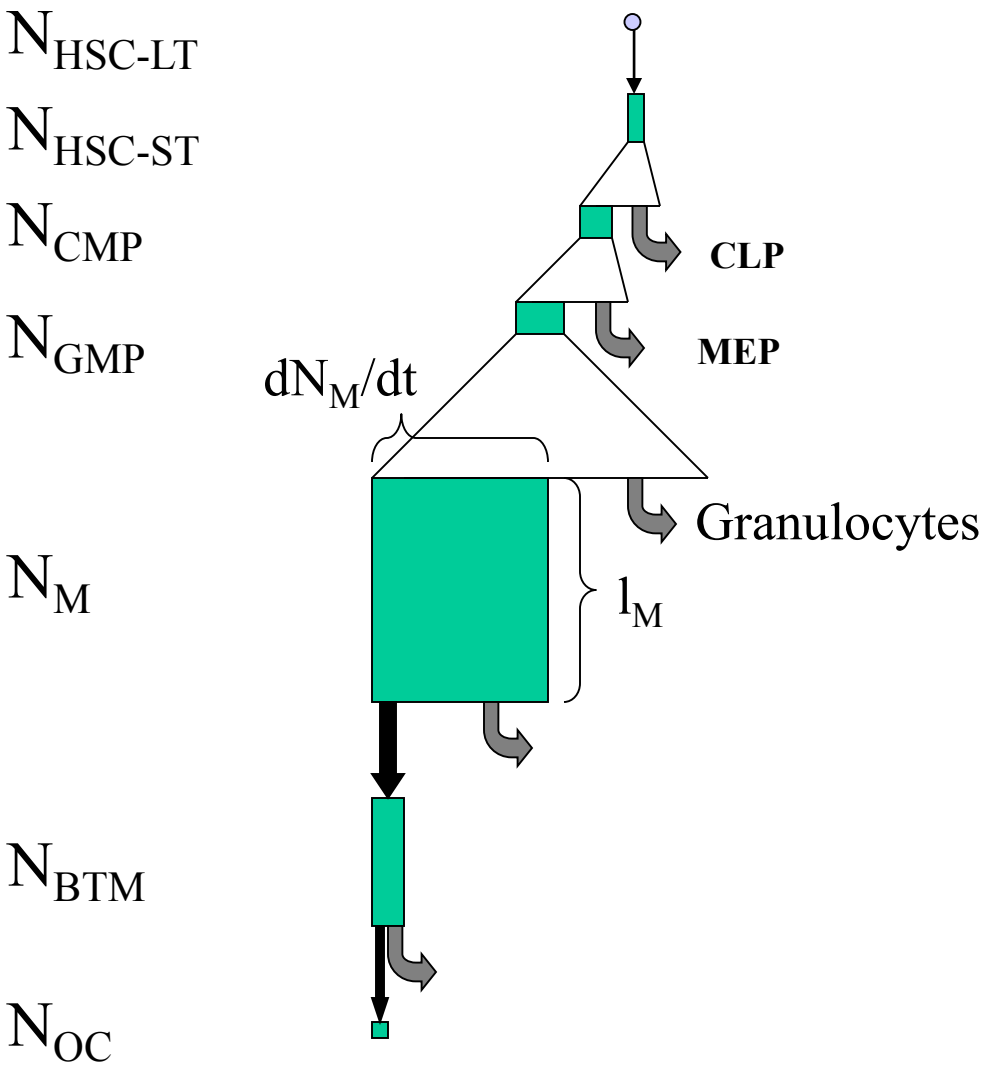


Bone



Stem and Progenitor Cell Compartment Kinetics



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REVIEW ARTICLE

Practical Modeling Concepts for Connective Tissue Stem Cell and Progenitor Compartment Kinetics

George F. Muschler,[1,2]* Ronald J. Midura,[2] and Chizu Nakamoto[2]

[1]*Department of Orthopaedic Surgery (A-41), The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195, USA*
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Received 13 September 2002; revised 15 January 2003; accepted 23 January 2003

Stem cell activation and development is central to skeletal development, maintenance, and repair, as it is for all tissues. However, an integrated model of stem cell proliferation, differentiation, and transit between functional compartments has yet to evolve. In this paper, the authors review current concepts in stem cell biology and progenitor cell growth and differentiation kinetics in the context of bone formation. A cell-based modeling strategy is developed and offered as a tool for conceptual and quantitative exploration of the key kinetic variables and possible organizational hierarchies in bone tissue development and remodeling, as well as in tissue engineering strategies for bone repair.

THE PARADIGM OF STEM CELLS AND PROGENITOR CELLS

Stem cells and progenitors are essentially present in all normal tissues [1, 2, 3, 4, 5, 6, 7]. "Stem cells" are defined, in general, as resting cells (not actively proliferating) that are present in small numbers in normal tissues. They share one important feature: the capacity for "asymmetric" cell division and "self-renewal" [8, 9]. In this process, a stem cell is activated by some signal or event to leave its normal resting state and to divide. However, the result of this cell division provides two daughter cells that are not identical. One daughter cell proliferates symmetrically, often for many cell divisions, to produce an abundance of progeny referred to as progenitors. These progenitors subsequently differentiate to form a mature tissue. In contrast, the second daughter cell returns to the original resting state of the mother cell until a new activating signal or event occurs. It retains a stem cell phenotype and all of the capabilities of the original mother cell in a process referred to as "self-renewal." This process is critically important to the preservation of the stem cell compartment. If both daughter cells were to become progenitors, then the pool of stem cells would be progressively depleted with each activation event. Such an outcome would rapidly deplete the stem cell population that is necessary to support ongoing tissue remodeling and repair required for long-term health.

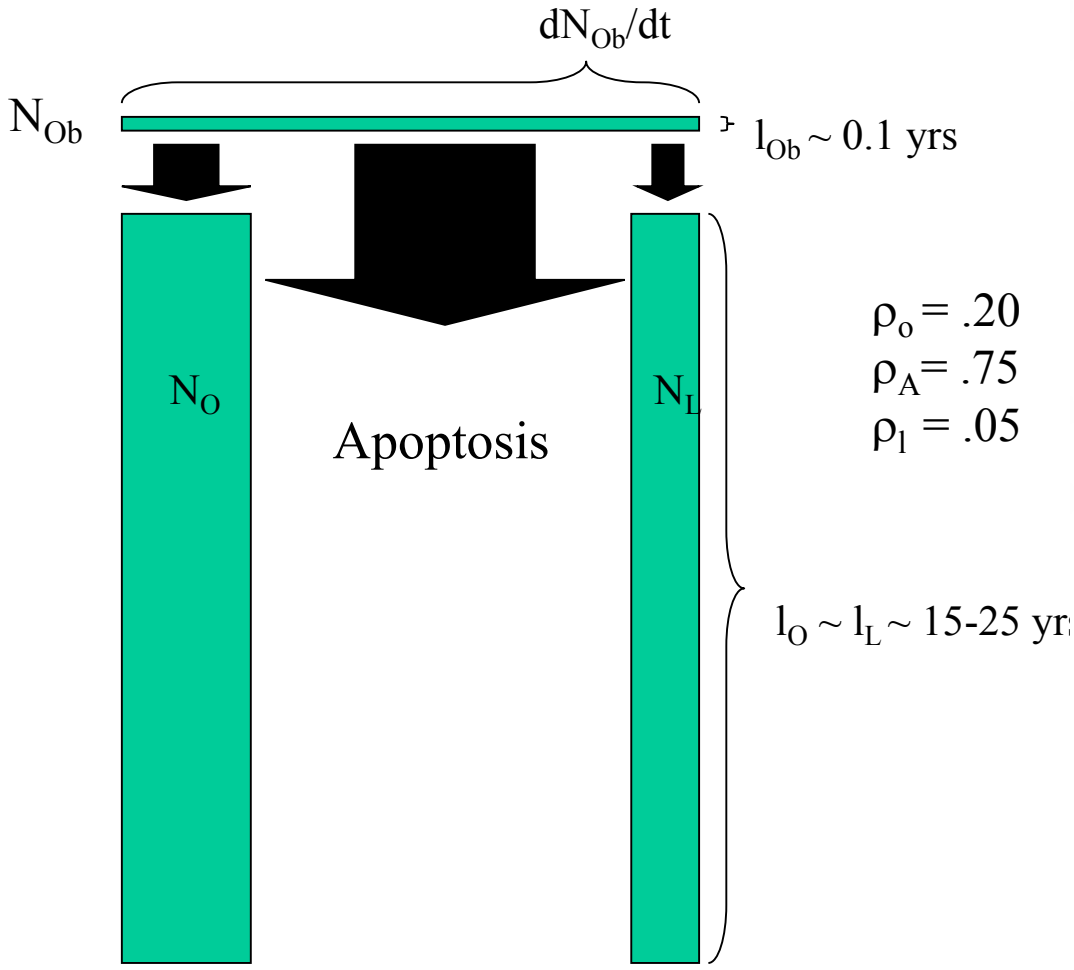
During embryonic development, cells of the inner mass of the blastocyst retain the capability to regenerate an entire individual, and are therefore "totipotent" in their differentiation potential. However, convention has held that as the progeny of these totipotent stem cells become dispersed throughout the organism and localized within specific tissues or organs, the stem cells in each of these tissues become progressively determined and confined transiently or permanently within defined stem cell compartments or niches. Stem cell populations initially become committed as "pleuripotent" stem cells confined to selected groups of tissues within a developing embryo (endoderm, ectoderm, or mesoderm). As development proceeds, some stem cell populations may remain "multipotent," capable of differentiation along one of several cell lineages (eg, cell populations in the neural tube, neural crest cells, hemangioblasts, and the mesenchymal mass of fetal limb buds). Other stem cell populations become intrinsically limited to the generation of only one mature cell type (eg, intestinal endothelium or skin keratinocytes). Such monopotent or unipotent stem cells were considered to be "committed," "restricted," or "determined" as a result of irreversible changes in the cell nucleus.

The transient pleuripotent and multipotent stem cell populations of embryonic and fetal life have appeared to disappear in postnatal life, leaving behind populations of more restricted adult stem cells that support virtually every organ system (eg, skin, intestinal mucosa, liver, vascular endothelium, the central nervous system, hematopoietic stem cells in bone marrow, and connective tissue or mesenchymal stem cells) [1, 2, 3, 4, 5, 6, 7]. These adult stem cell populations are of central importance in adult health and in all settings requiring tissue repair, remodeling, or regeneration. In fact, the health of a given tissue might even be defined by the state and kinetics of the supporting infrastructure of stem cells and progenitors.

2002

Stem and Progenitor Cell Compartment Kinetics

Compartments Downstream From Osteoblasts



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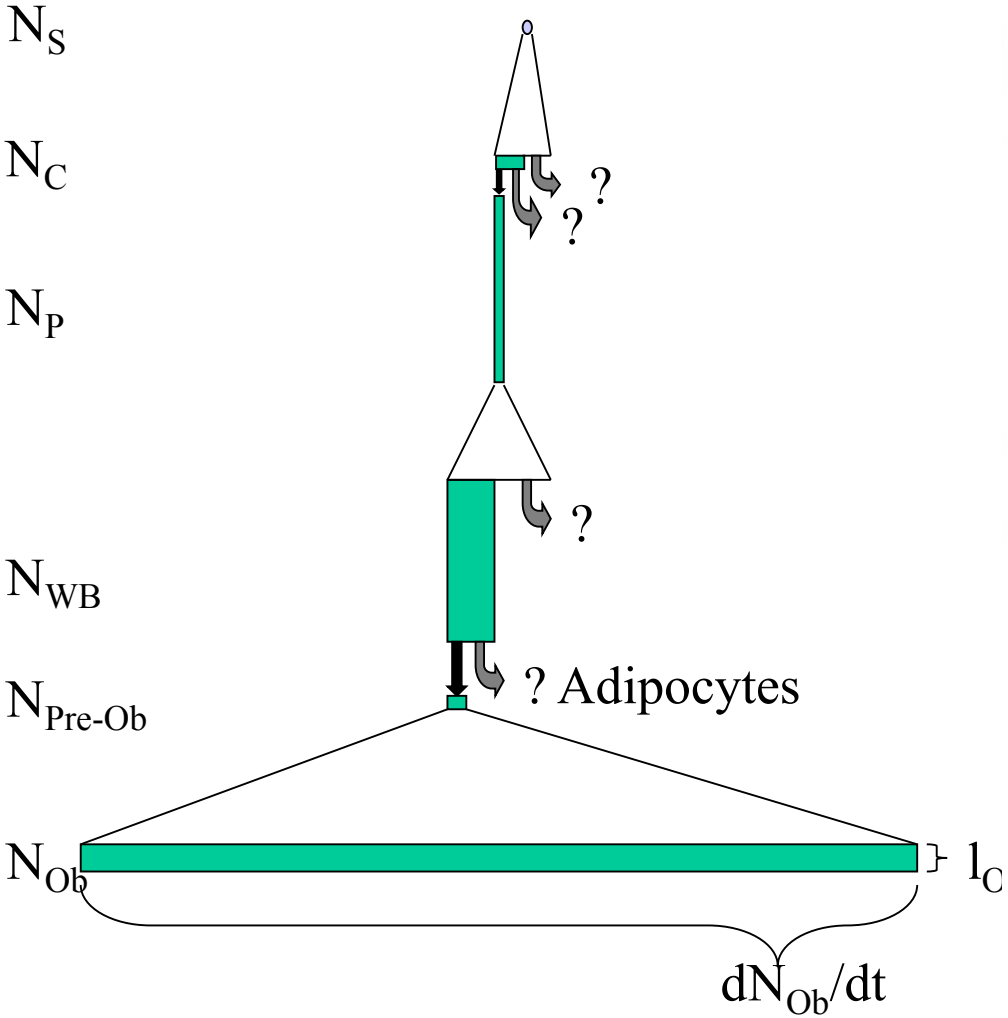

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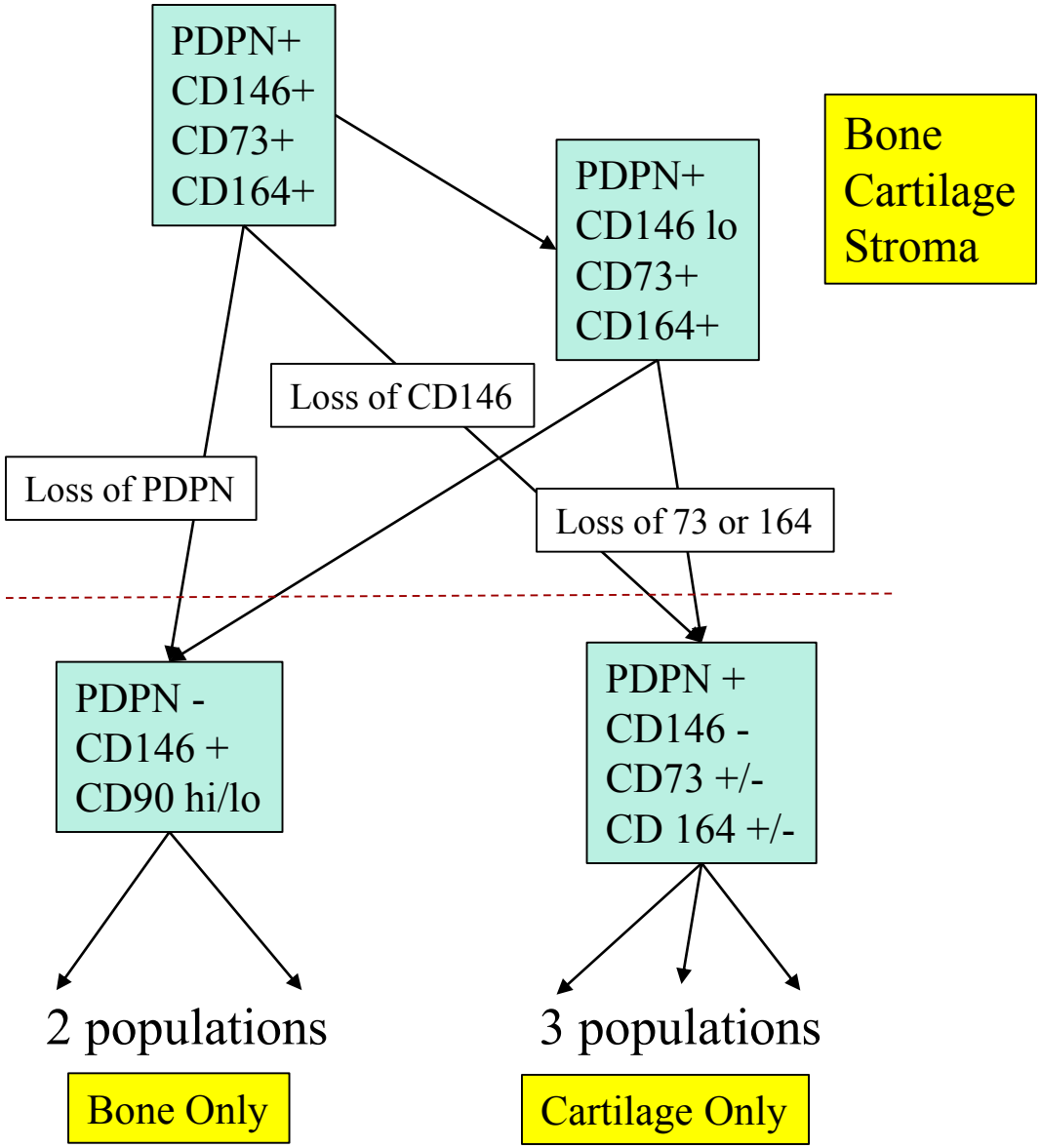
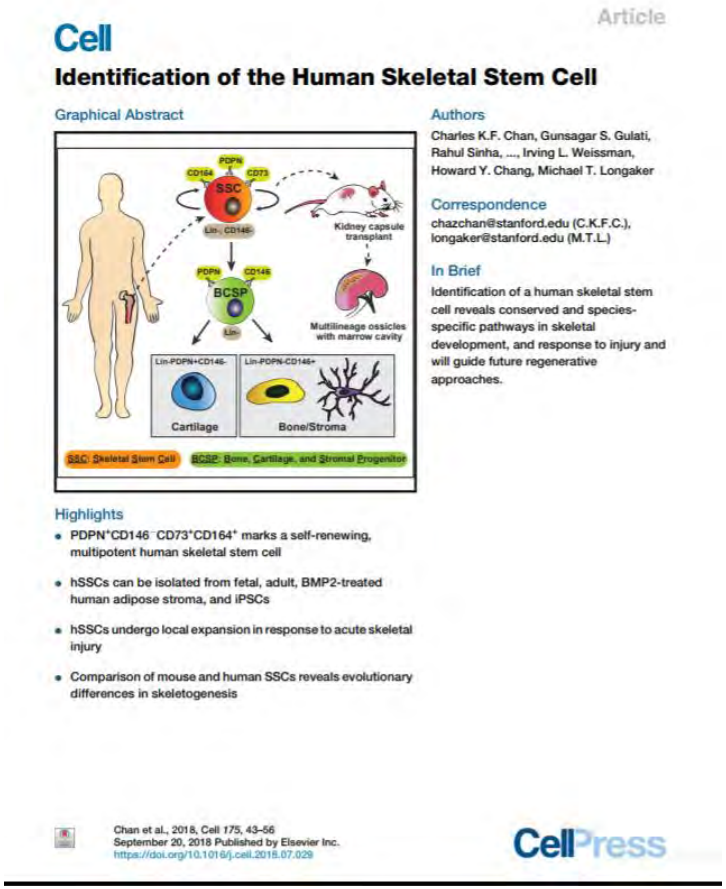
Stem and Progenitor Cell Compartment Kinetics





Article

Cell

Identification of the Human Skeletal Stem Cell

Graphical Abstract

Authors

Charles K.F. Chan, Gunsagar S. Gulati, Rahul Sinha, ..., Irving L. Weissman, Howard Y. Chang, Michael T. Longaker

Correspondence

chazchan@stanford.edu (C.K.F.C.), longaker@stanford.edu (M.T.L.)

In Brief

Identification of a human skeletal stem cell reveals conserved and species-specific pathways in skeletal development, and response to injury and will guide future regenerative approaches.

Highlights

- PDPN+CD146-CD73+CD164+ marks a self-renewing, multipotent human skeletal stem cell
- hSSCs can be isolated from fetal, adult, BMP2-treated human adipose stroma, and iPSCs
- hSSCs undergo local expansion in response to acute skeletal injury
- Comparison of mouse and human SSCs reveals evolutionary differences in skeletogenesis

Chan et al., 2018, Cell 175, 43–56
September 20, 2018 Published by Elsevier Inc.
https://doi.org/10.1016/j.cell.2018.07.029

CellPress

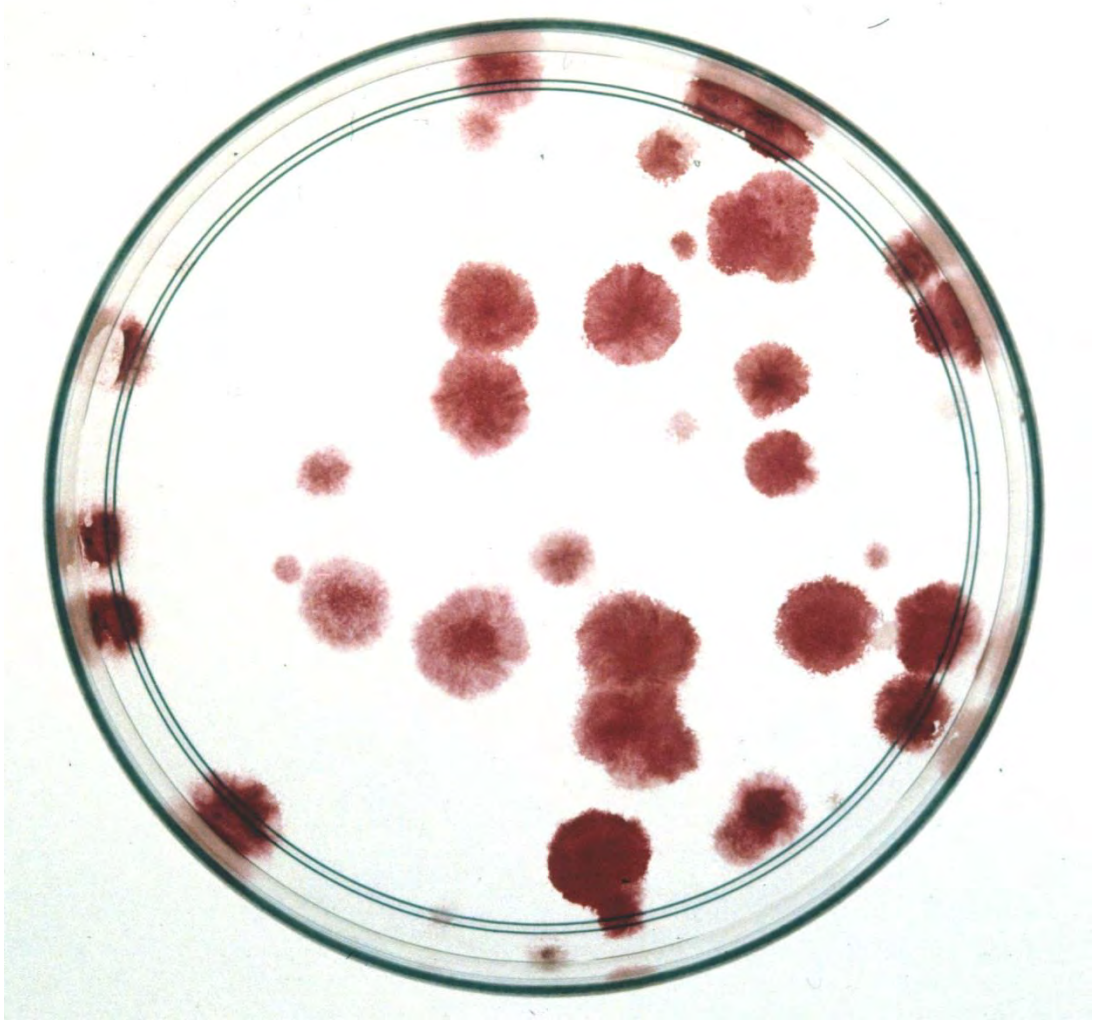
Sources of Variation in Cell Sourcing and Selection

Patient/Donor	Age Gender Diagnosis Stage Site History Medications Diet Comorbidities Systemic Health Phenotype Genotype
Harvest Tissue	Site Tissue Health Tissue Turnover State Clone Diversity
Harvest Technique	Precision Sampling Efficiency Yield
Transport Conditions	Temperature Humidity Container Medium Mechanical
Processing Method	Mechanical Thermal Chemical Enzymatic
Cell Selection Criteria	Density Size Granularity Adherence Markers Performance
Assay Technique	Visual/Manual Imaging/Manual Imaging/Automated Biochemical
Expansion Conditions	Media Surfaces Activators Inhibitors Mechanical
Storage Conditions	Freeze Rate Thaw Rate Temp Duration Medium
Preclinical Models	Species Age Gender Site Size Methods Outcome Parameters
Clinical Model	Inclusion/Exclusion Methods Outcome Parameters
Clinical Use	Patient/Donor Patient Co-Morbidities Methods Skill Outcome Parameters

Sources of Variation in Cell Sourcing and Selection

Patient/Donor	Age	Gender	Diagnosis	Stage	Site	History	Medications	Diet
	Comorbidities	Systemic Health	Phenotype	Genotype				
Harvest Tissue	Site	Tissue Health	Tissue Turnover State	Clone Diversity				
Harvest Technique	Precision	Sampling Efficiency	Yield					
Transport Conditions	Temperature	Humidity	Container	Medium	Mechanical			
Processing Method	Mechanical	Thermal	Chemical	Enzymatic				
Cell Selection Criteria	Density	Size	Granularity	Adherence	Markers	Performance		
Assay Technique	Visual/Manual	Imaging/Manual	Imaging/Automated	Biochemical				
Expansion Conditions	Media	Surfaces	Activators	Inhibitors	Mechanical			
Storage Conditions	Freeze Rate	Thaw Rate	Temp	Duration	Medium			
Preclinical Models	Species	Age	Gender	Site	Size	Methods	Outcome Parameters	
Clinical Model	Inclusion/Exclusion	Methods	Outcome Parameters					
Clinical Use	Patient/Donor	Patient Co-Morbidities	Methods	Skill	Outcome Parameters			

Colony Forming Unit Assay

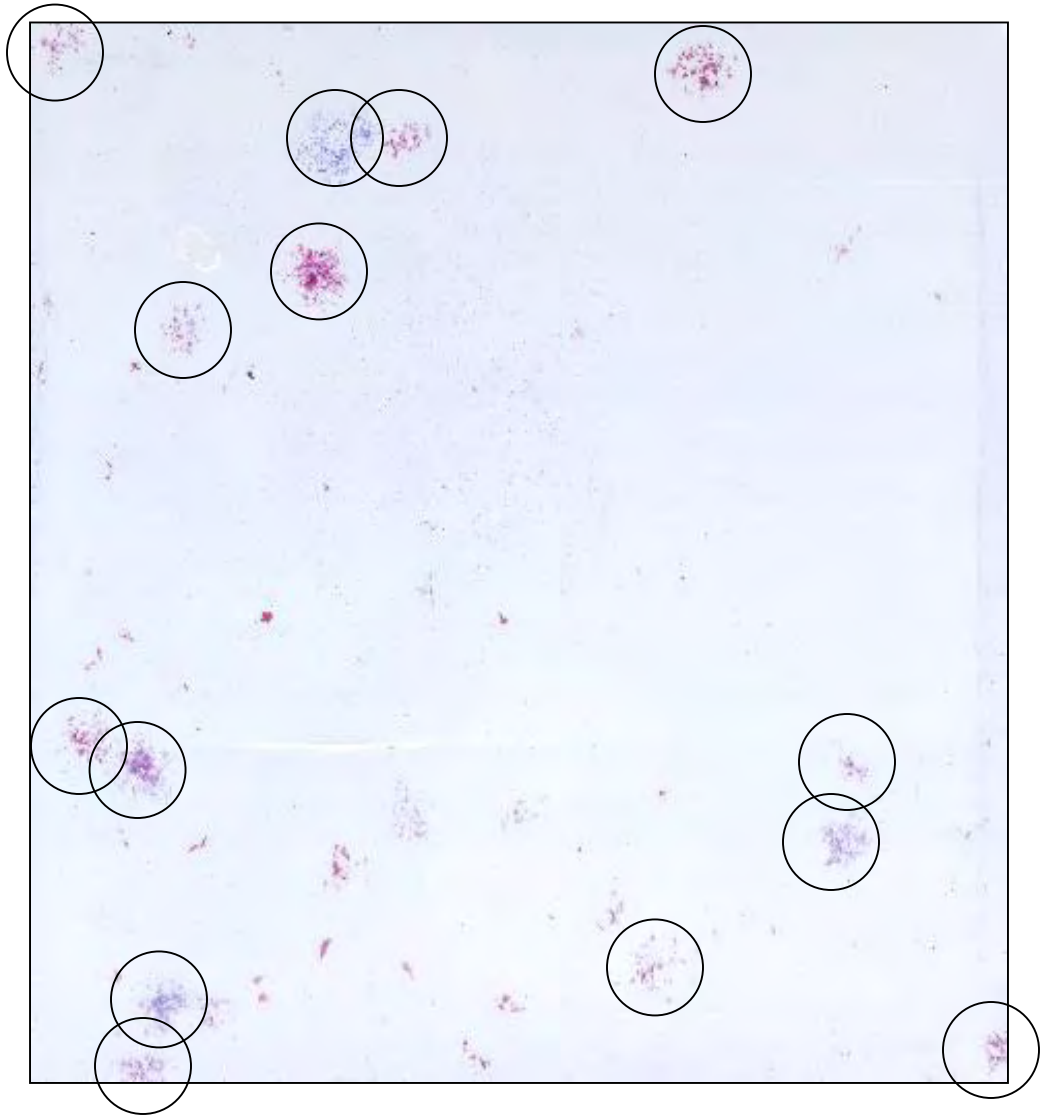


Alk Phos

Hand Count CFU Assay

- ✓ Utility
- Accuracy?
- Reproducibility?
- Efficiency?

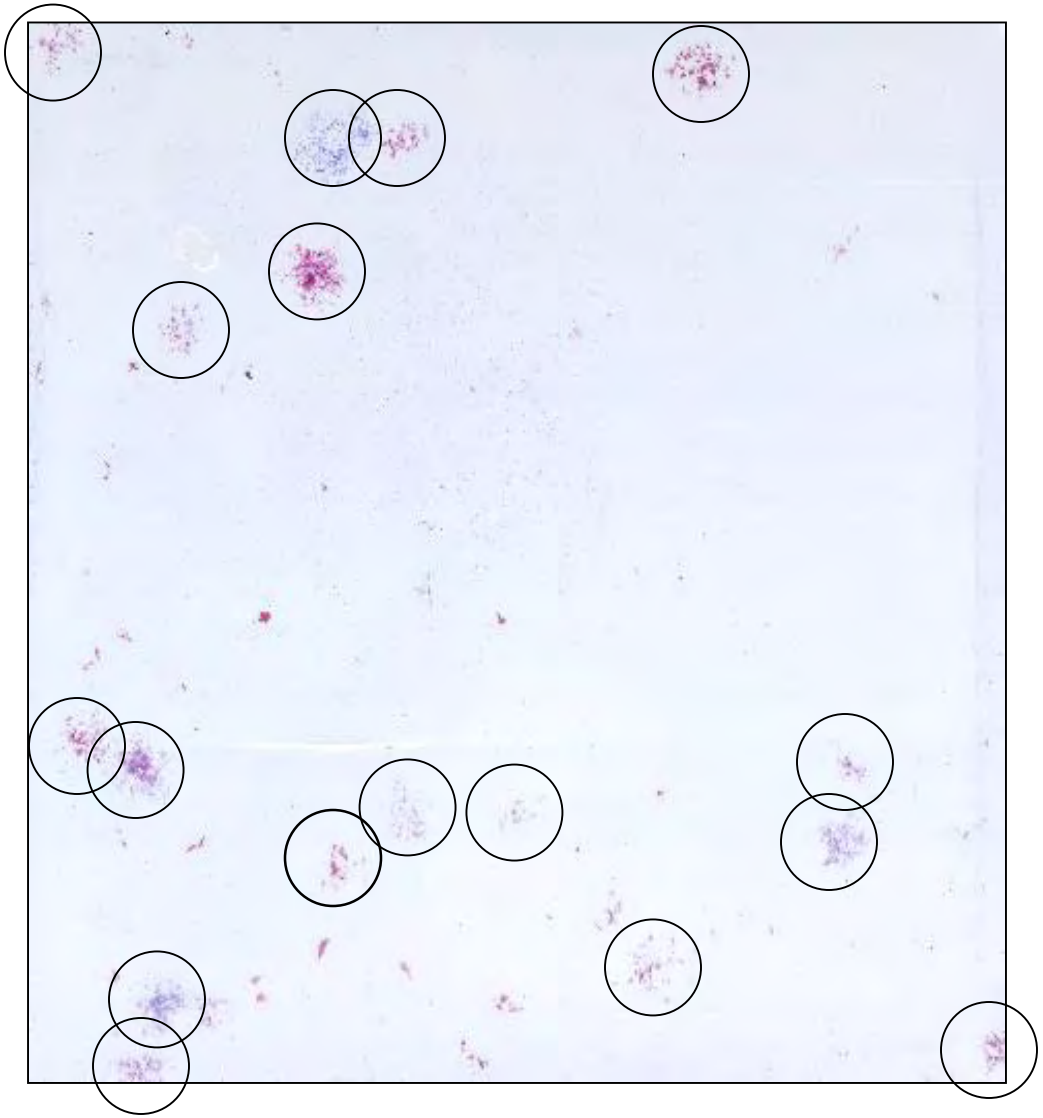
Hand Count CFU Assay



CFU = 14

- ✓ Utility
- Accuracy?
- Reproducibility?
- Efficiency?

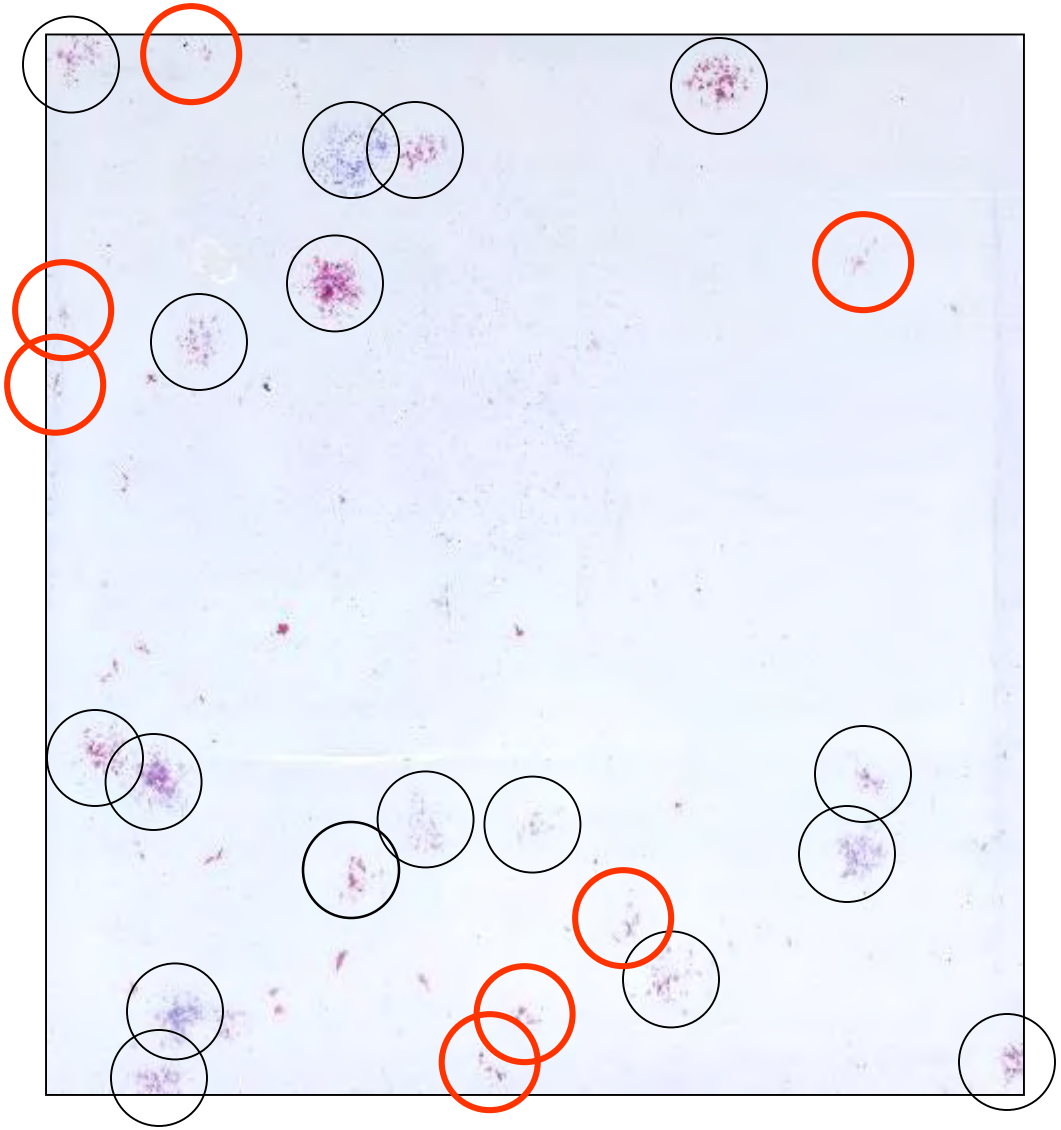
Hand Count CFU Assay



CFU = 17

- ✓ Utility
- Accuracy?
- Reproducibility?
- Efficiency?

Hand Count CFU Assay



CFU = 24

✓ Utility

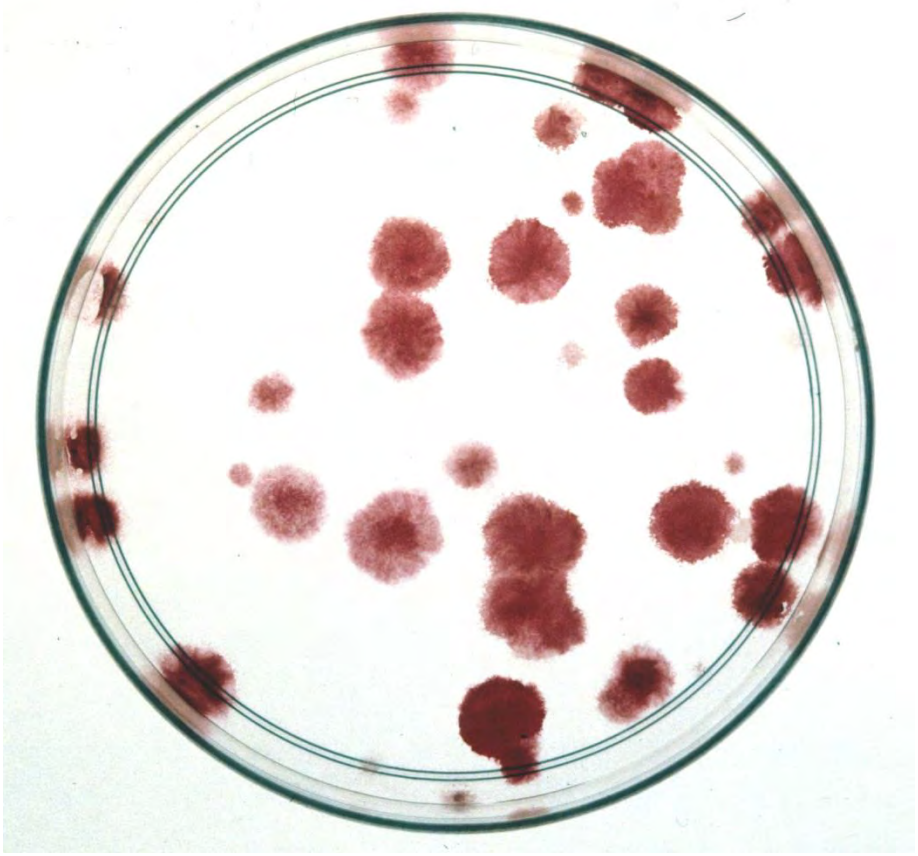
Accuracy?

Reproducibility?

Efficiency?

CFU Assay Upgrade?

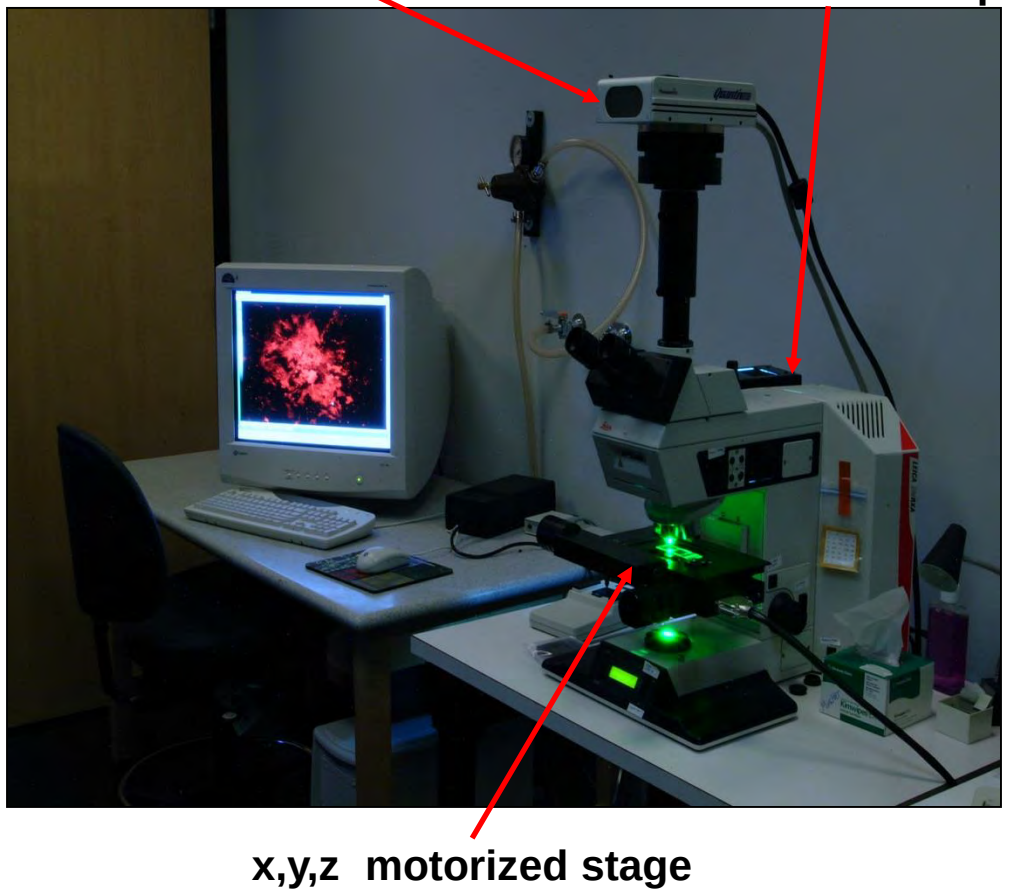
Colony Counting → Population Characterization



- Concentration
- Prevalence
- Activation Efficiency
- Proliferation
- Migration
- Differentiation
- Response to Stimuli
- Heterogeneity

Automated Image Acquisition

- Automated slide scanning
- 10x magnification
- 884 individual image tiles per chamber



CFU Analysis: Image Processing

480 Individual Images were montaged to produce one entire image of the cell culture

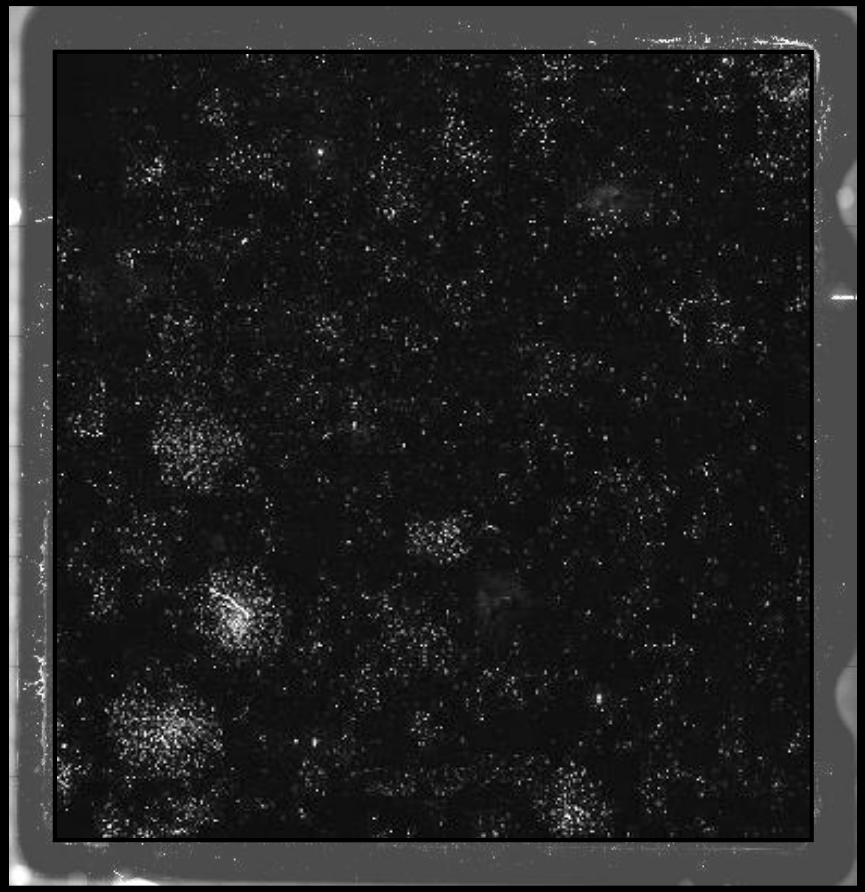
Background Correction

Image obtained by subtracting a smoothed, unlabeled background image from each individual image

An ROI within the boundaries of the cell culture was defined

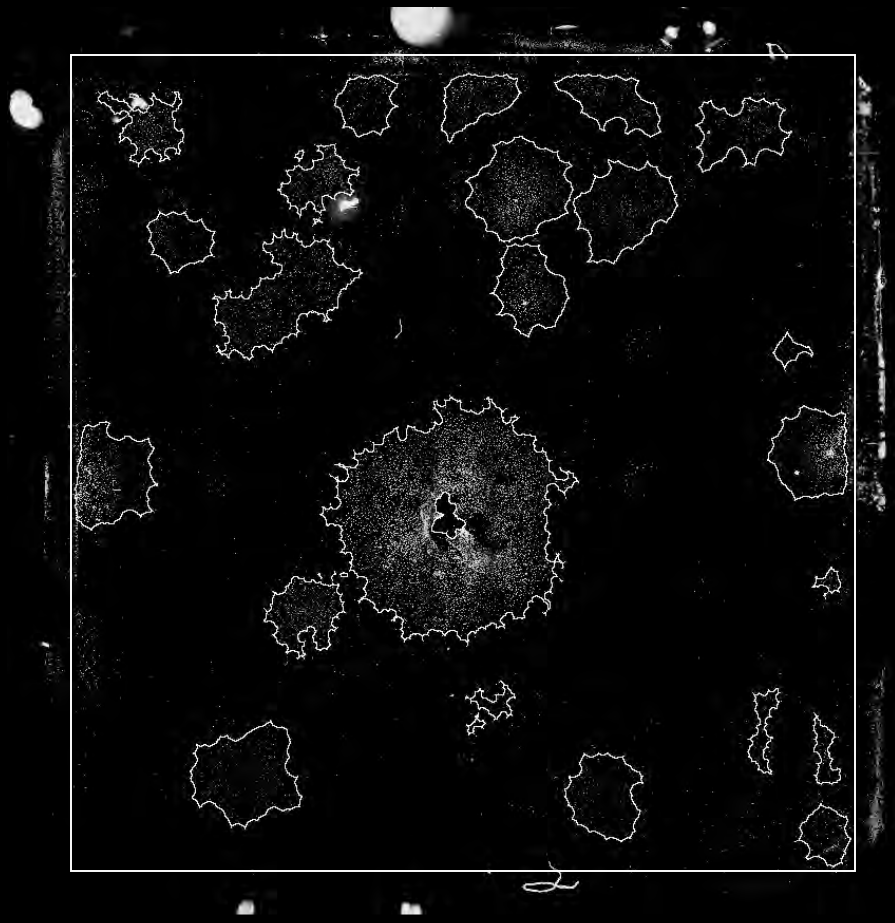
Nuclear segmentation using gray level threshold

Removal of lint debris, apoptotic debris, and glass aberrations (size and shape segmentation)



Culture Chamber

Montaged Culture Chamber

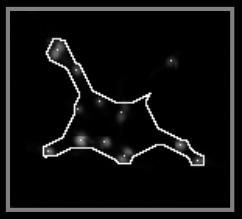


Processed Montage

20x24 Matrix of Images (160MB)-8 bit gray level-10x objective -pixel size =3.56μm

Colony Metrics

Large Colony
378 cells
Colony area = 2.07mm^2
Density = 182 cells/mm^2
Nuclei Area = 621μm^2

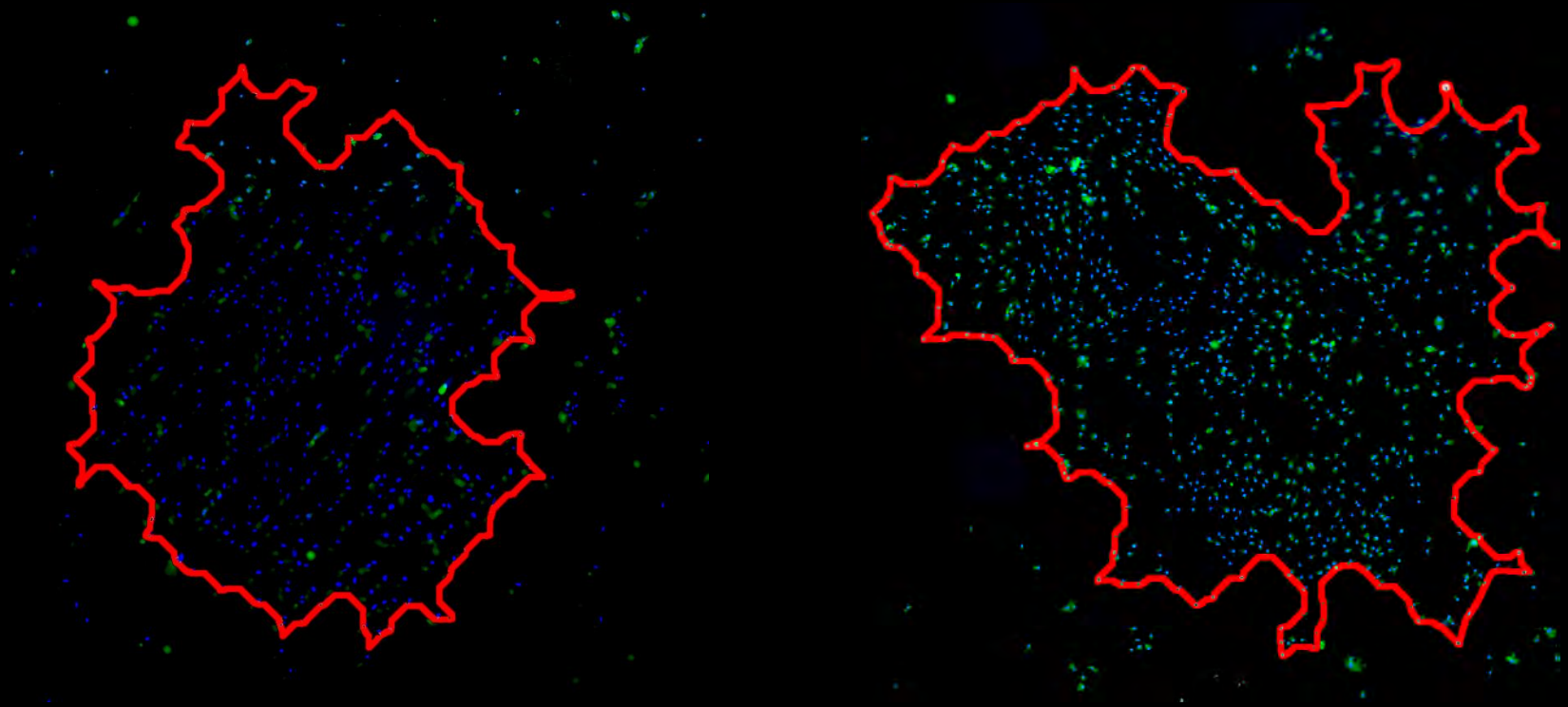


Small Colony
13 cells
Colony area = 0.037mm^2
Density = 351 cells/mm^2
Nuclei Area = 570μm^2

Identified colonies containing **eight** or more cells where each nuclei was under **six nuclear distances** (6*142.2μm= 852μm) to its nearest neighbor

Cartilage Progenitor Assays

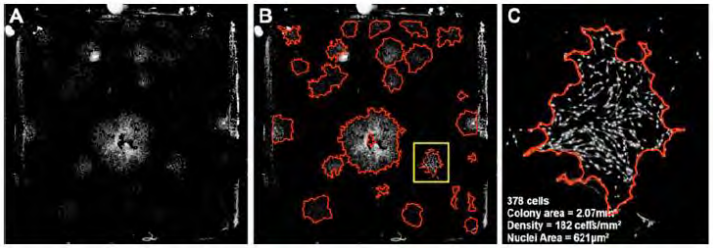
Variation Between Cell Colonies



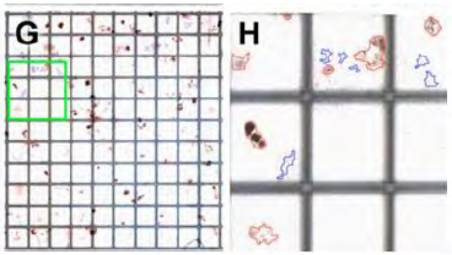
Proteoglycan Synthesis – Acridine Orange

Designation: F2944 – 12

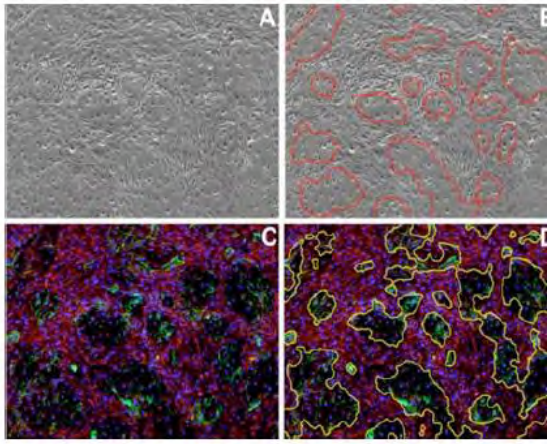
Standard Test Method for Automated Colony Forming Unit (CFU) Assays—Image Acquisition and Analysis Method for Enumerating and Characterizing Cells and Colonies in Culture[1]



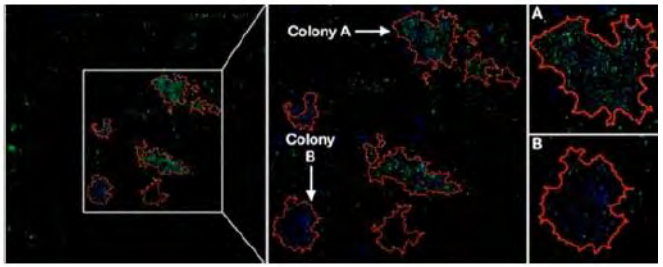
Osteogenic CFUs



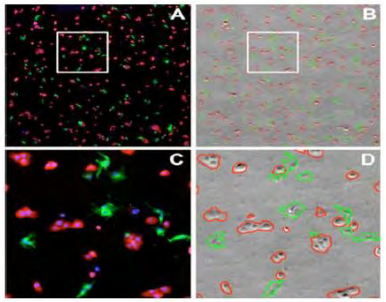
Hematopoietic CFUs



Fibroblasts/Endothelial Cells

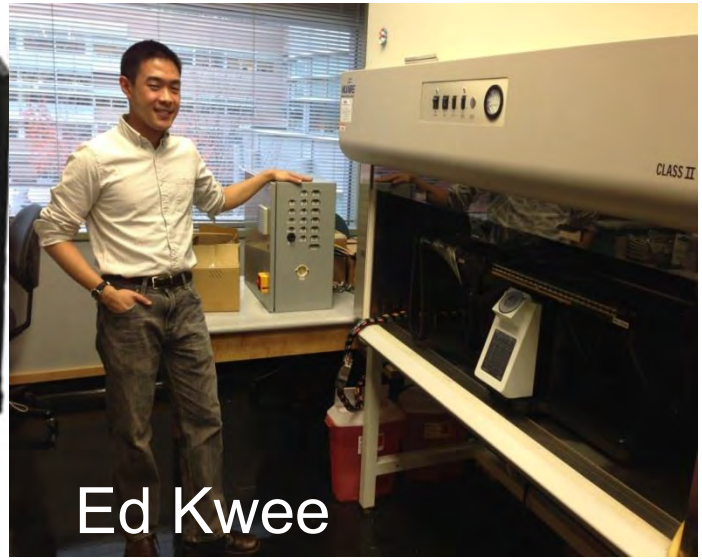


Chondrogenic CFUs



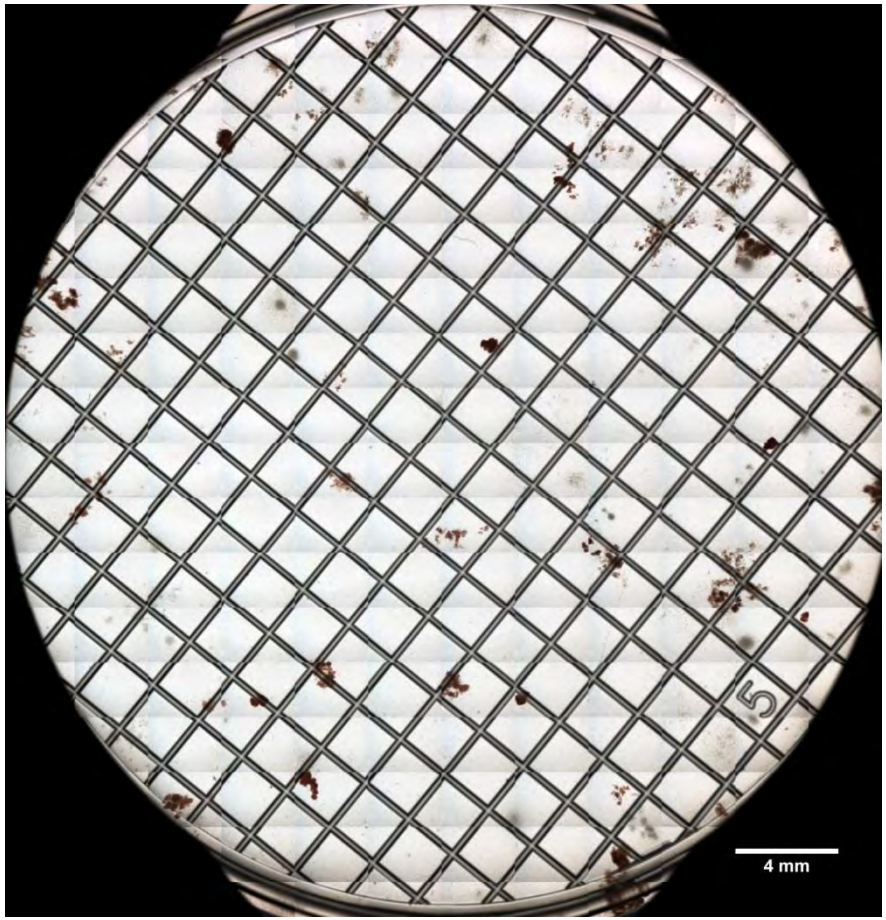
Keratinocytes/Melanocytes

“Cell X” Robot



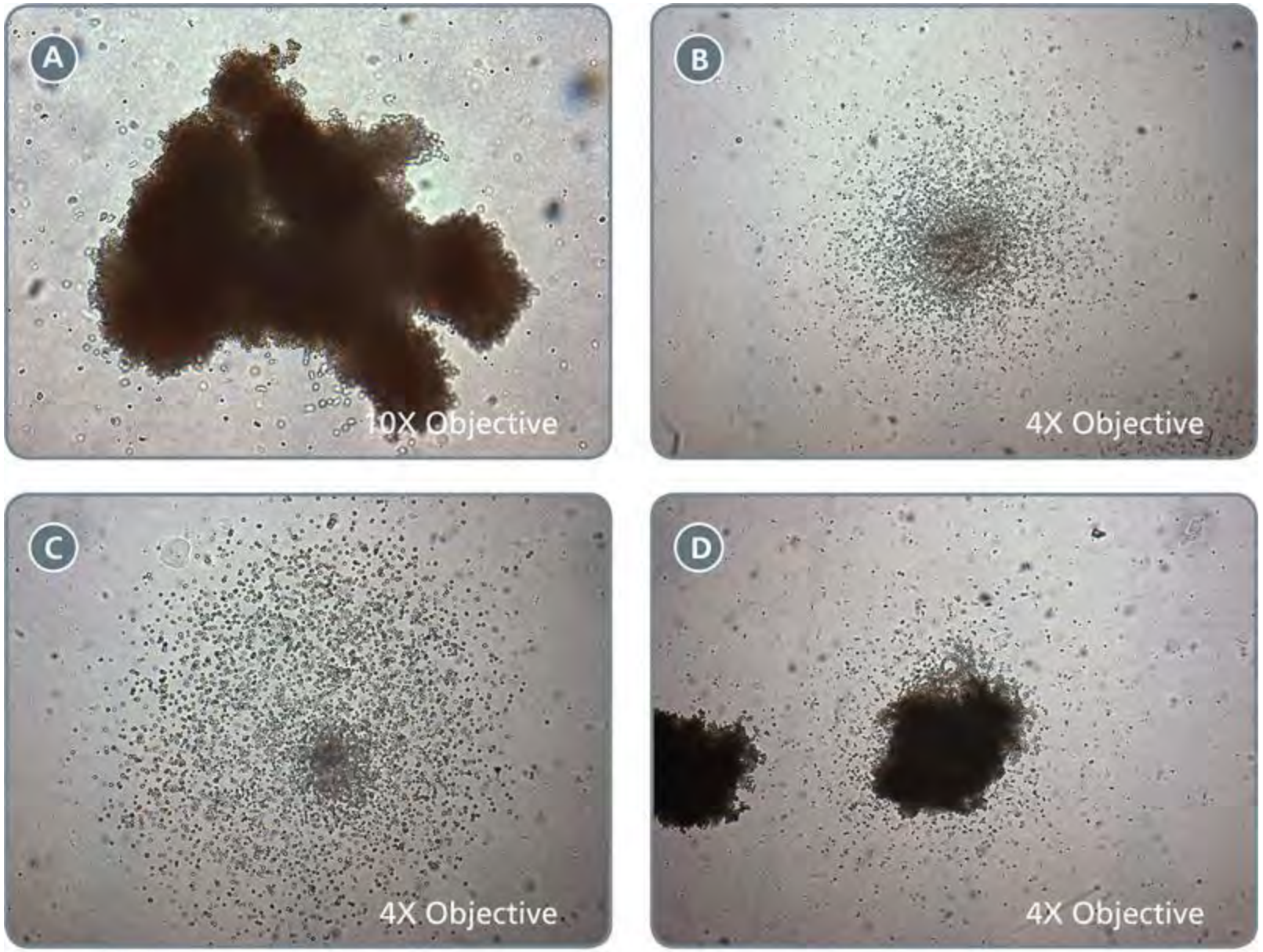
Automated Cell and Colony Management

UBC HSC Assay

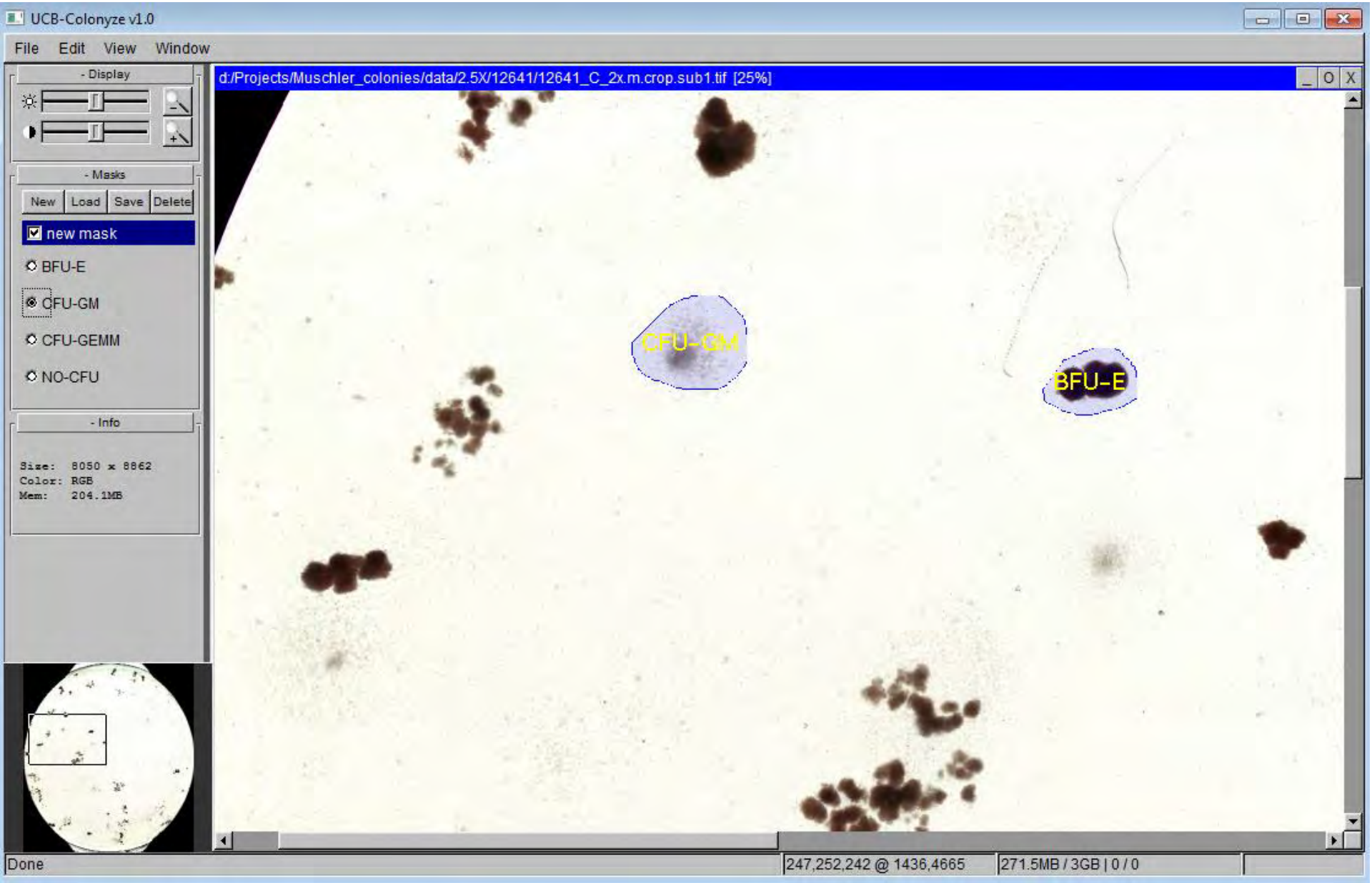


What is the inter- and intra-observer variation?

UBC HSC Assay



UBC HSC Assay



Reviewer Variation

Plate A

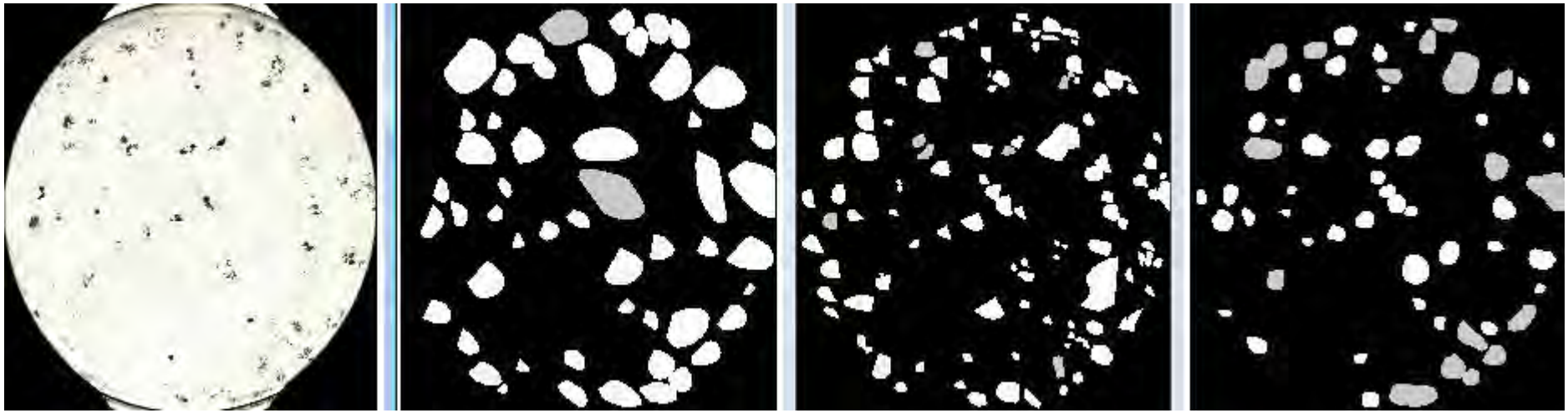
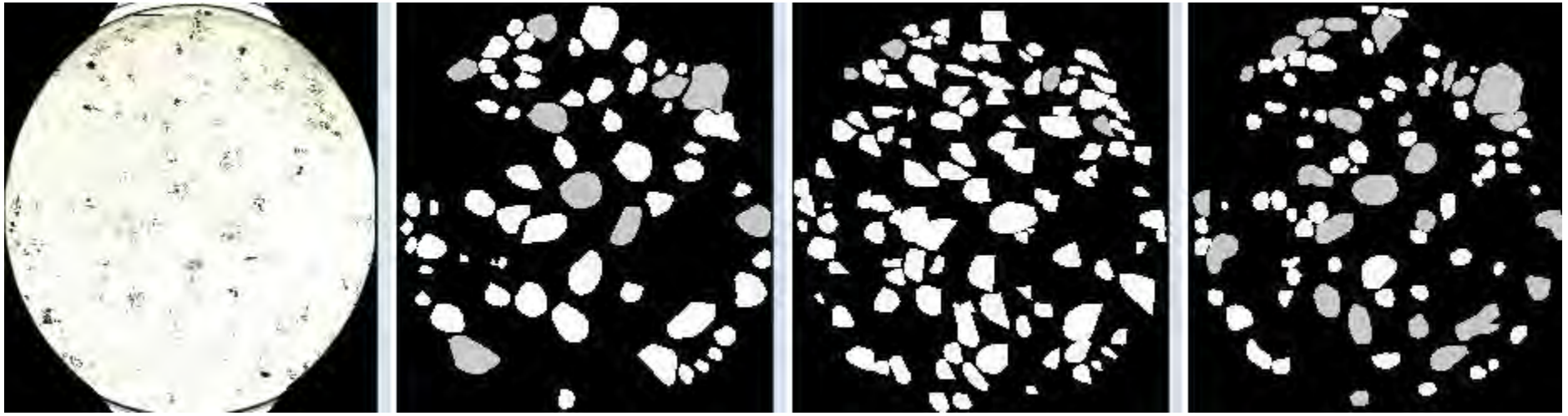


Plate B



1 2 3

Reviewer Variation

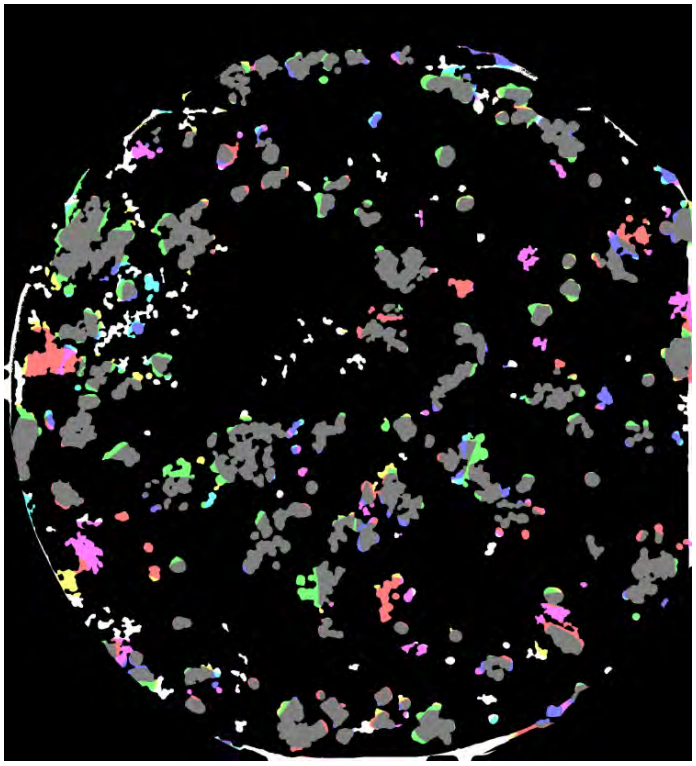


Plate 3

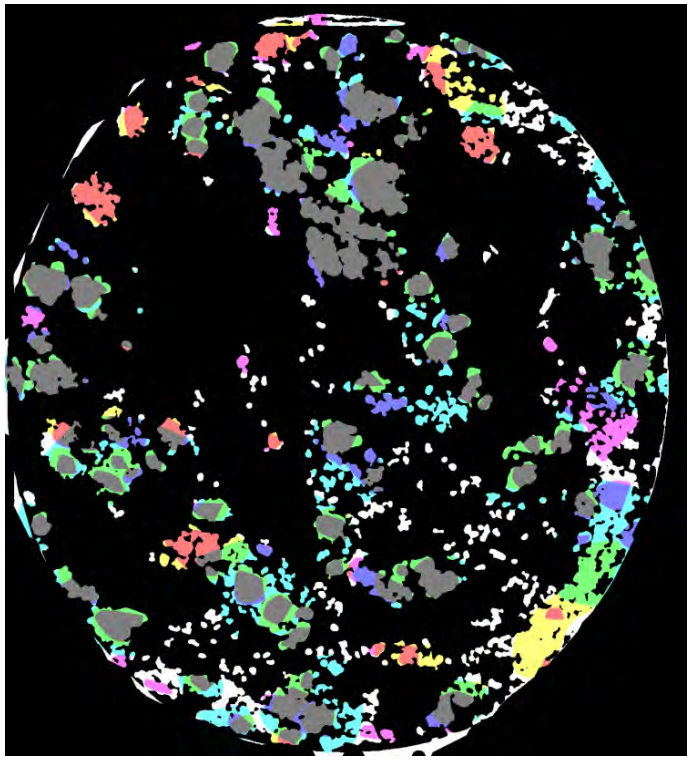
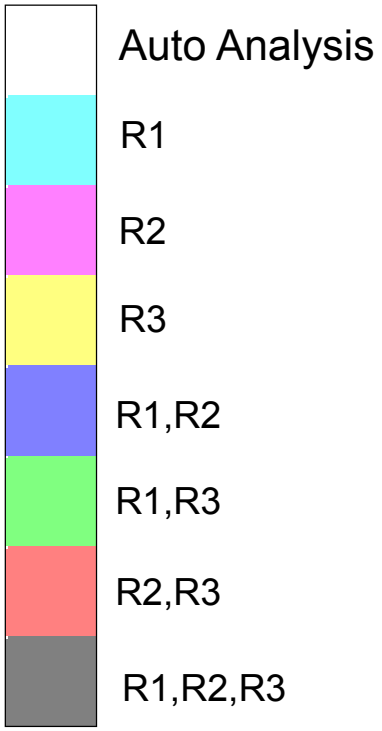
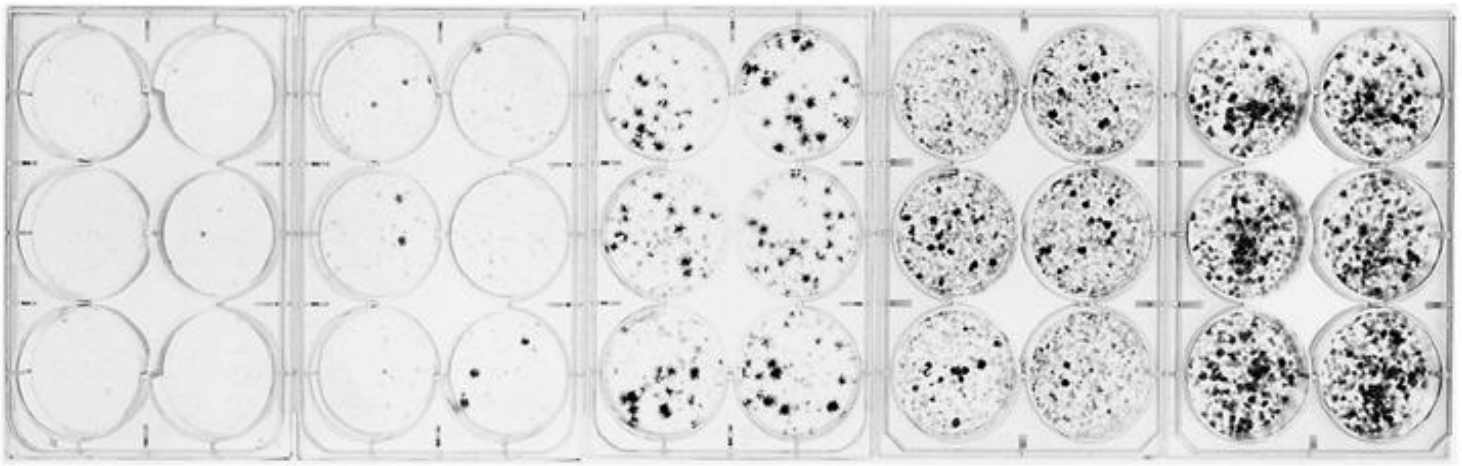


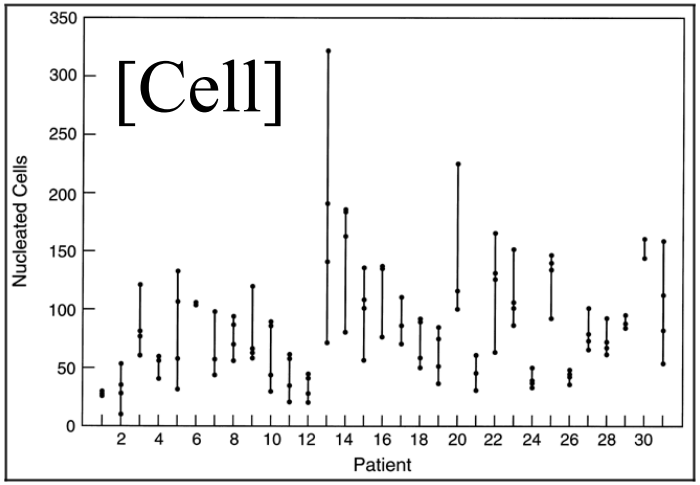
Plate 4

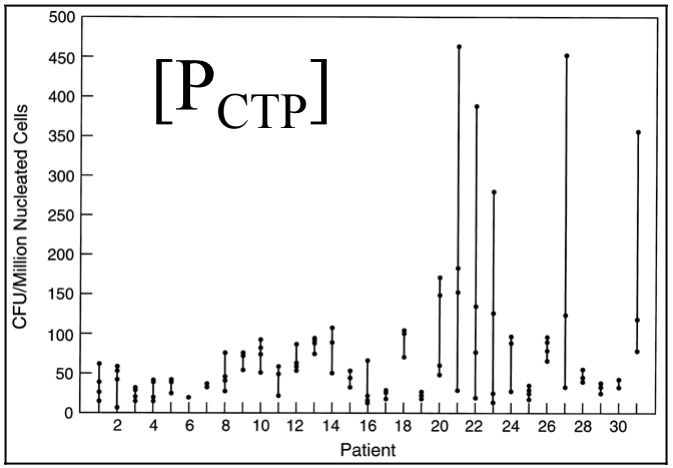


Grey = 70% agreement on presence of a colony (excluding colony type)

Variation between Patients and Aspirates in [Cells] and P_{CTP}







70% due to variation between patients
20% due to variation between aspirates

J Bone Joint Surg [Am] 1997; 79-A; 1699-1709

Variation between Patients and Aspirates in [Cells] and P_{CTP}

ELSEVIER

Journal of Orthopaedic Research 19 (2001) 117–125

Journal of Orthopaedic Research

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Age- and gender-related changes in the cellularity of human bone marrow and the prevalence of osteoblastic progenitors

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Received 28 June 1999; accepted 13 March 2000

Abstract

Bone marrow harvested by aspiration contains connective tissue progenitor cells which can be induced to express a bone phenotype in vitro. The number of osteoblastic progenitors can be estimated by counting the colony-forming units which express alkaline phosphatase (CFU-APs). This study was undertaken to test the hypothesis that human aging is associated with a significant change in the number or prevalence of osteoblastic progenitors in the bone marrow. Four 2-ml bone marrow aspirates were harvested bilaterally from the anterior iliac crest of 57 patients, 31 men (age 15–83) and 26 women (age 13–79). A mean of 64 million nucleated cells was harvested per aspirate. The mean prevalence of CFU-APs was found to be 55 per million nucleated cells. These data revealed a significant age-related decline in the number of nucleated cells harvested per aspirate for both men and women ($P = 0.002$). The number of CFU-APs harvested per aspirate also decreased significantly with age for women ($P = 0.02$), but not for men ($P = 0.3$). These findings are relevant to the harvest of bone marrow derived connective tissue progenitors for bone grafting and other tissue engineering applications, and may also be relevant to the pathophysiology of age-related bone loss and post-menopausal osteoporosis. © 2001 Orthopaedic Research Society. Published by Elsevier Science Ltd. All rights reserved.

Introduction

Bone marrow harvested by aspiration contains connective tissue progenitor cells which can be selectively isolated and induced to express a bone phenotype in vitro. Many investigators have demonstrated that bone marrow contains connective tissue progenitors, which can differentiate into osteoblast-like cells in vitro [1,2,10,20,21]. These cells are likely to be responsible for the finding in many bone grafting models that bone marrow delivered by injection or combined with other matrix materials significantly improves the bone healing response [6,7,11,22]. Several clinical reports support the potential value of bone marrow as a graft material. Connolly et al. [6] reported the successful treatment of 18 of 20 tibial non-unions using injection of aspirated bone marrow combined with either use of cast or fixation with an intramedullary nail. Healey et al. [11] also demonstrated five of eight non-unions using injections of bone marrow obtained from iliac crest. Recognizing the potential clinical value of cells available from bone marrow by aspiration, many surgeons have begun using bone marrow as part of many bone grafting procedures, despite the fact that no prospective randomized clinical studies have yet been performed to definitively document the efficacy of this practice. Bone marrow has also been proposed as a potential source of connective tissue progenitors for a broad range of tissue engineering applications related to connective tissues [26].

The number of potential osteoblastic progenitors can be estimated by counting colony-forming units which express alkaline phosphatase (CFU-APs) [18]. Alkaline phosphatase is an early marker for the osteoblastic differentiation [4,16,25]. In a previous study, we have shown that the technique of aspiration has a significant effect on the concentration of CFU-APs harvested from bone marrow, primarily due to dilution with peripheral blood [19]. We further found evidence that individuals differed significantly from one another with respect to both the cellularity of bone marrow aspirates (the number of nucleated cells harvested per aspirate) and in the prevalence of CFU-APs among the nucleated cells harvested. Though large variation existed between aspirates from a single individual, approximately 70% of

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E-mail address: muschler@bme.ri.ccf.org (G.F. Muschler).

PII: S0736-0266(00)00010-3

2001

The Efficiency of Bone Marrow Aspiration for the Harvest of Connective Tissue Progenitors from the Human Iliac Crest

Thomas E. Patterson, PhD, Cynthia Boehm, BS, Chizu Nakamoto, MD, PhD, Richard Rozic, BS, Esteban Walker, PhD, Nicolas S. Piuzzi, MD, and George F. Muschler, MD

Investigation performed at the Cleveland Clinic, Cleveland, Ohio

Background: The rational design and optimization of tissue engineering strategies for cell-based therapy requires a baseline understanding of the concentration and prevalence of osteogenic progenitor cell populations in the source tissues. The aim of this study was to (1) define the efficiency of, and variation among individuals in, bone marrow aspiration as a means of osteogenic connective tissue progenitor (CTP-O) harvest compared with harvest from iliac cancellous bone, and (2) determine the location of CTP-Os within native cancellous bone and their distribution between the marrow-space and trabecular-surface tissue compartments.

Methods: Eight 2-mL bone marrow aspiration (BMA) samples and one 7-mm transcortical biopsy sample were obtained from the anterior iliac crest of 33 human subjects. Two cell populations were obtained from the iliac cancellous bone (ICB) sample. The ICB sample was placed into αMEM (alpha-minimal essential medium) with antibiotic-antimycotic and minced into small pieces (1 to 2 mm in diameter) with a sharp osteotome. Cells that could be mechanically disassociated from the ICB sample were defined as marrow-space (IC-MS) cells, and cells that were disassociated only after enzymatic digestion were defined as trabecular-surface (IC-TS) cells. The 3 sources of bone and marrow-derived cells were compared on the basis of cellularity and the concentration and prevalence of CTP-Os through colony-forming unit (CFU) analysis.

Results: Large variation was seen among patients with respect to cell and CTP-O yield from the IC-MS, IC-TS, and BMA samples and in the relative distribution of CTP-Os between the IC-MS and IC-TS fractions. The CTP-O prevalence was highest in the IC-TS fraction, which was 11.4-fold greater than in the IC-MS fraction ($p < 0.0001$) and 1.7-fold greater than in the BMA fraction. However, the median concentration of CTP-Os in the ICB (combining MS and TS fractions) was only 3.04 ± 1.1-fold greater than that in BMA (4,265 compared with 1,402 CTP/mL; $p = 0.00004$).

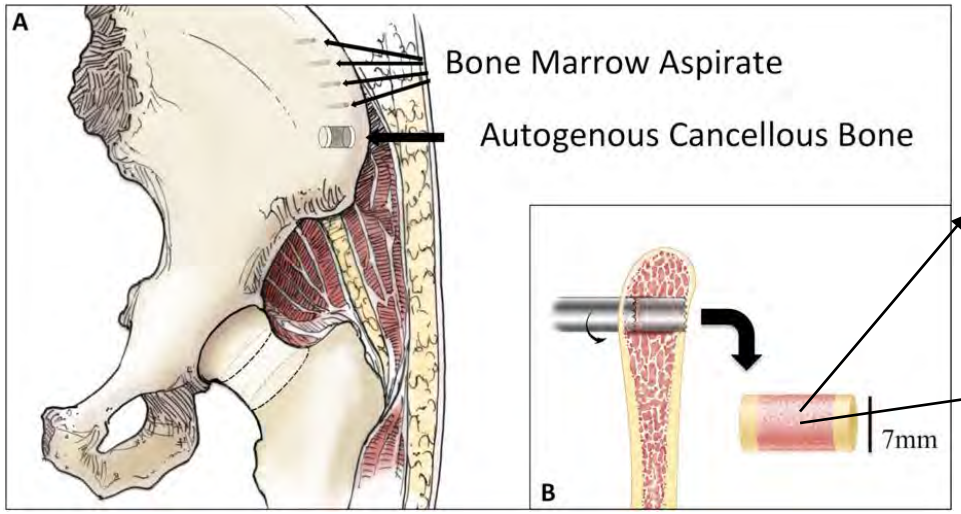
Conclusions: Bone marrow aspiration of a 2-mL volume at a given needle site is an effective means of harvesting CTP-Os, albeit diluted with peripheral blood. However, the median concentration of CTP-Os is 3-fold less than from native iliac cancellous bone. The distribution of CTP-Os between the IC-MS and IC-TS fractions varies widely among patients.

Clinical Relevance: Bone marrow aspiration is an effective means of harvesting CTP-Os but is associated with dilution with peripheral blood. Overall, we found that 63.5% of all CTP-Os within iliac cancellous bone resided on the trabecular surface; however, 48% of the patients had more CTP-Os contributed by the IC-MS than the IC-TS fraction.

Tissue regeneration and effective remodeling in all settings requires the effective recruitment and activation of stem cells and progenitor cells, whose progeny are capable of generating the new tissue or tissues that are required[1,2]. As a result, the rational development of cell-based therapy strategies that target or use progenitor cell populations requires an understanding of the concentration, prevalence, biological potential, heterogeneity, and distribution of stem cells and progenitor cells that may be of use[3,4]. The term *connective tissue progenitor (CTP)* has been defined and used in this effort. CTPs are defined

Disclosure: This work was performed through the support of NIH AR049687, Epidemiology of Connective Tissue Progenitor Cells (09/26/2002 to 08/31/2007), and NIH 1R01AR063733-01, Epidemiology of Human Chondrogenic Progenitor Cells (01/1/14 to 12/30/17). On the **Disclosure of Potential Conflicts of Interest** forms, *which are provided with the online version of the article*, one or more of the authors checked "yes" to indicate that the author had other relationships or activities that could be perceived to influence, or have the potential to influence, what was written in this work (http://links.lww.com/JBJS/E344).

2017



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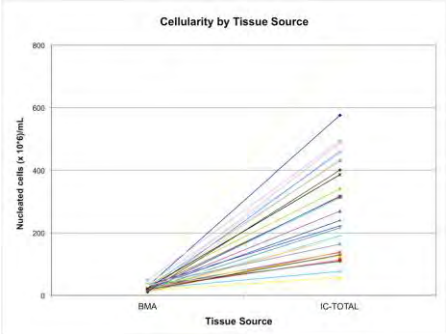
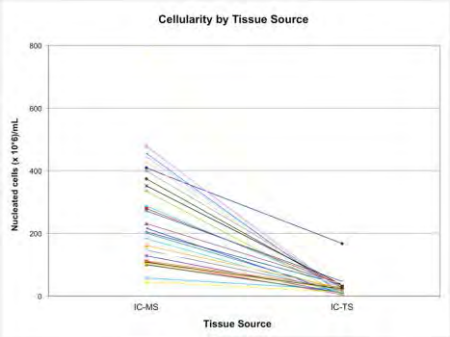
Tissue regeneration and effective remodeling in all settings | that target or use progenitor cell populations requires an un-

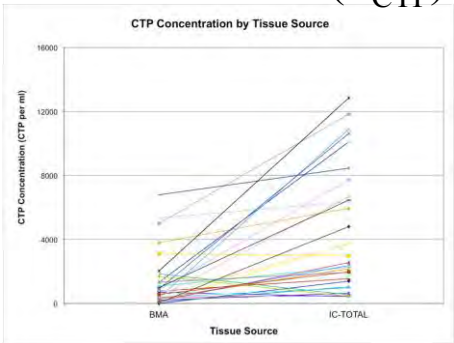
JBJS 2017

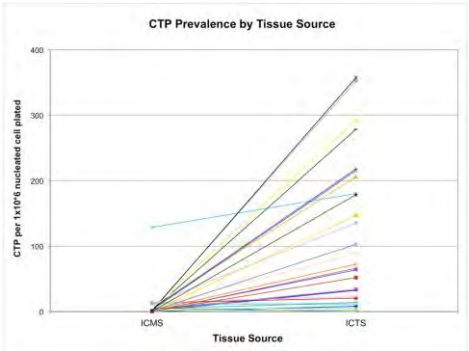
Cellularity ([Cell])



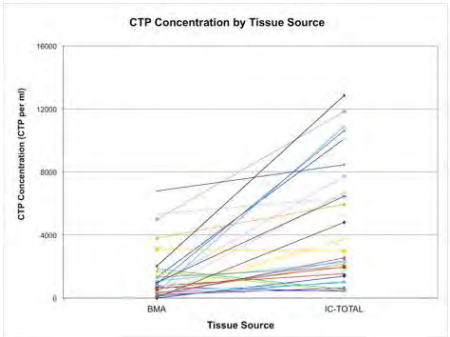


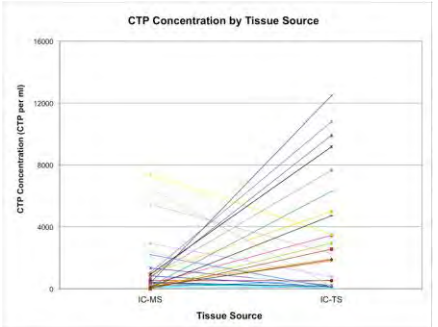
CTP Prevalence (P_{CTP})

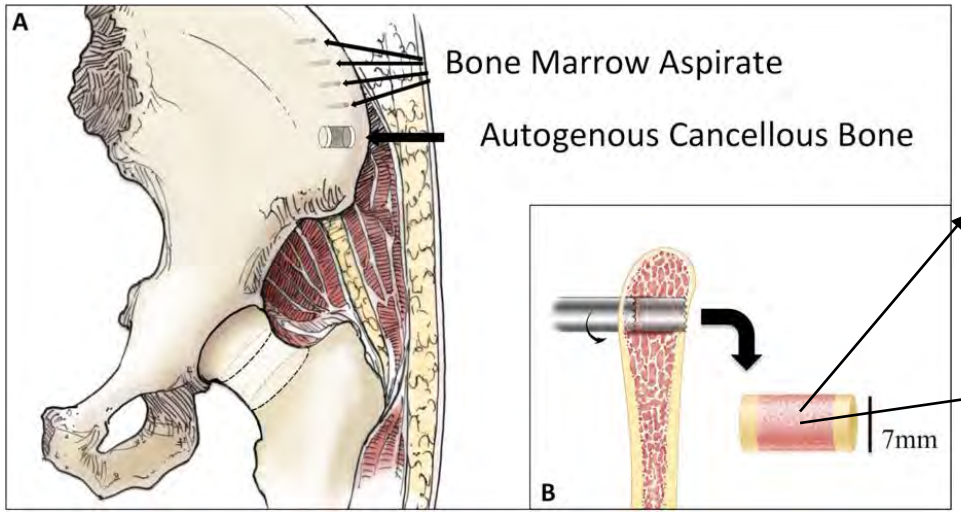


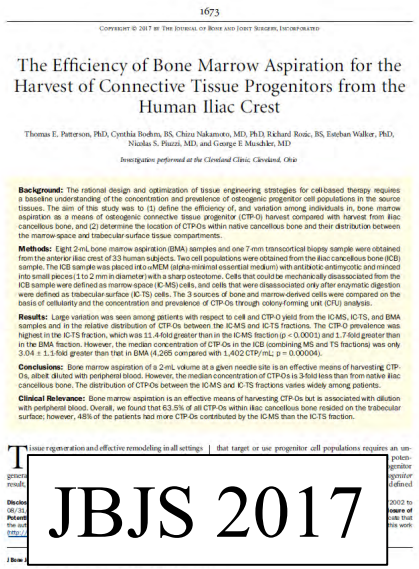


CTP Concentration ([CTP])

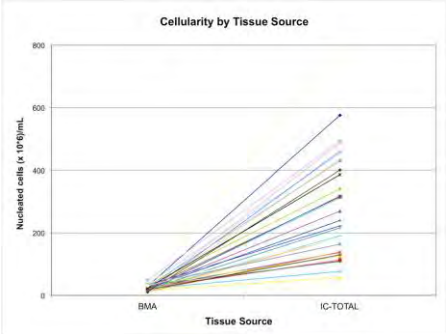


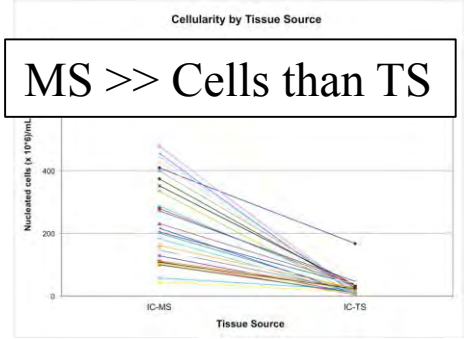






Cellularity ([Cell])

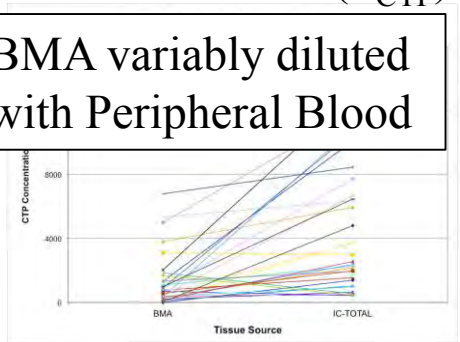


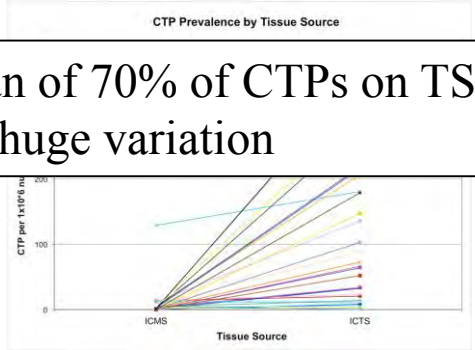


MS >> Cells than TS

CTP Prevalence (P_{CTP})

BMA variably diluted with Peripheral Blood

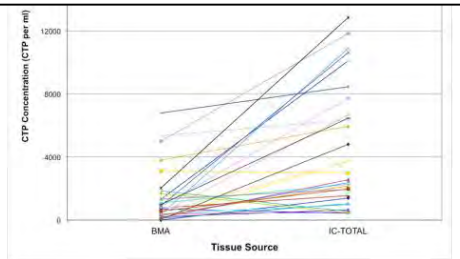


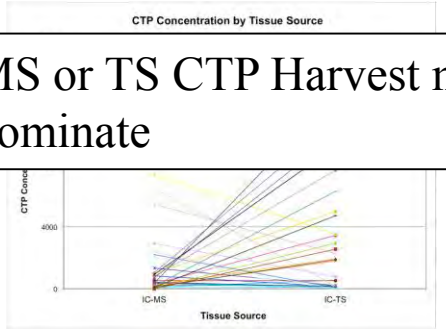


Mean of 70% of CTPs on TS, But huge variation

CTP Concentration ([CTP])

Mean BMA 3 fold lower that ICB



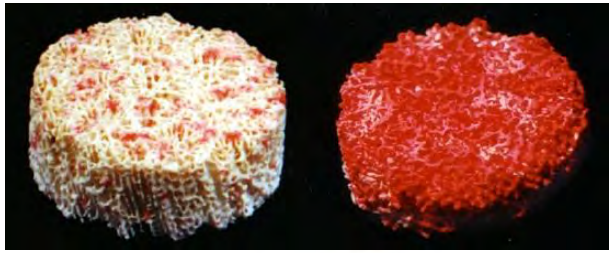


MS or TS CTP Harvest may dominate

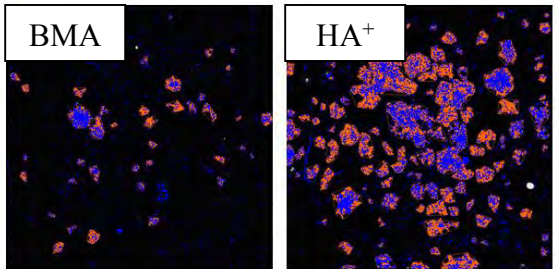
Rapid Clinical Cell Processing Methods

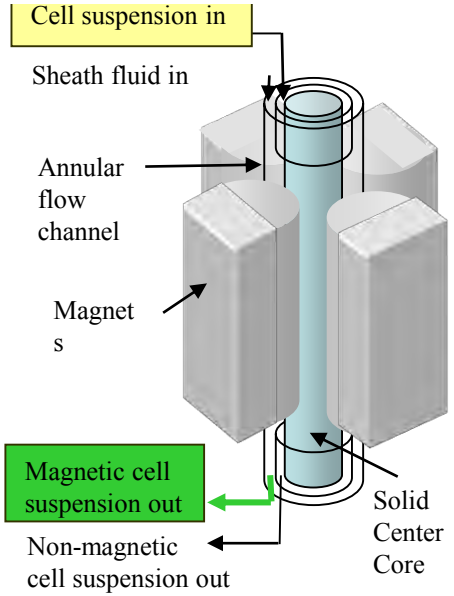
Density Separation

Selective Retention



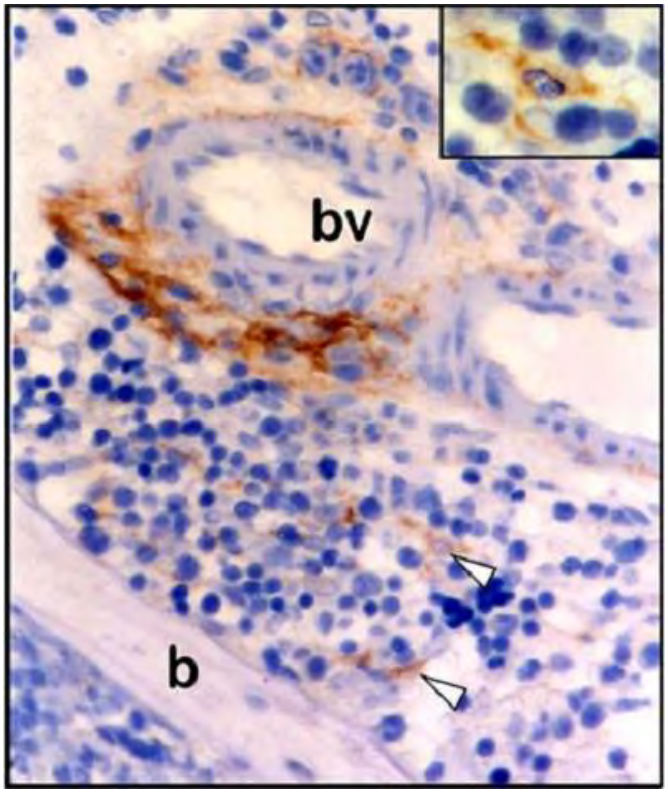
Magnetic Separation

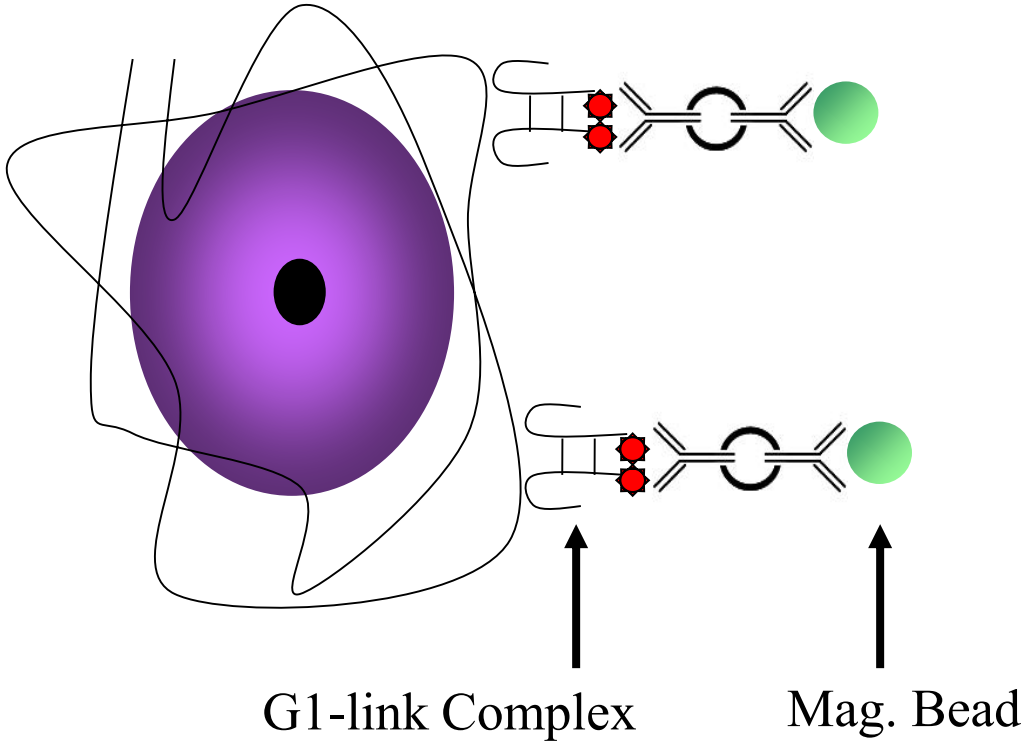




CTP Selection Using Magnetic Separation

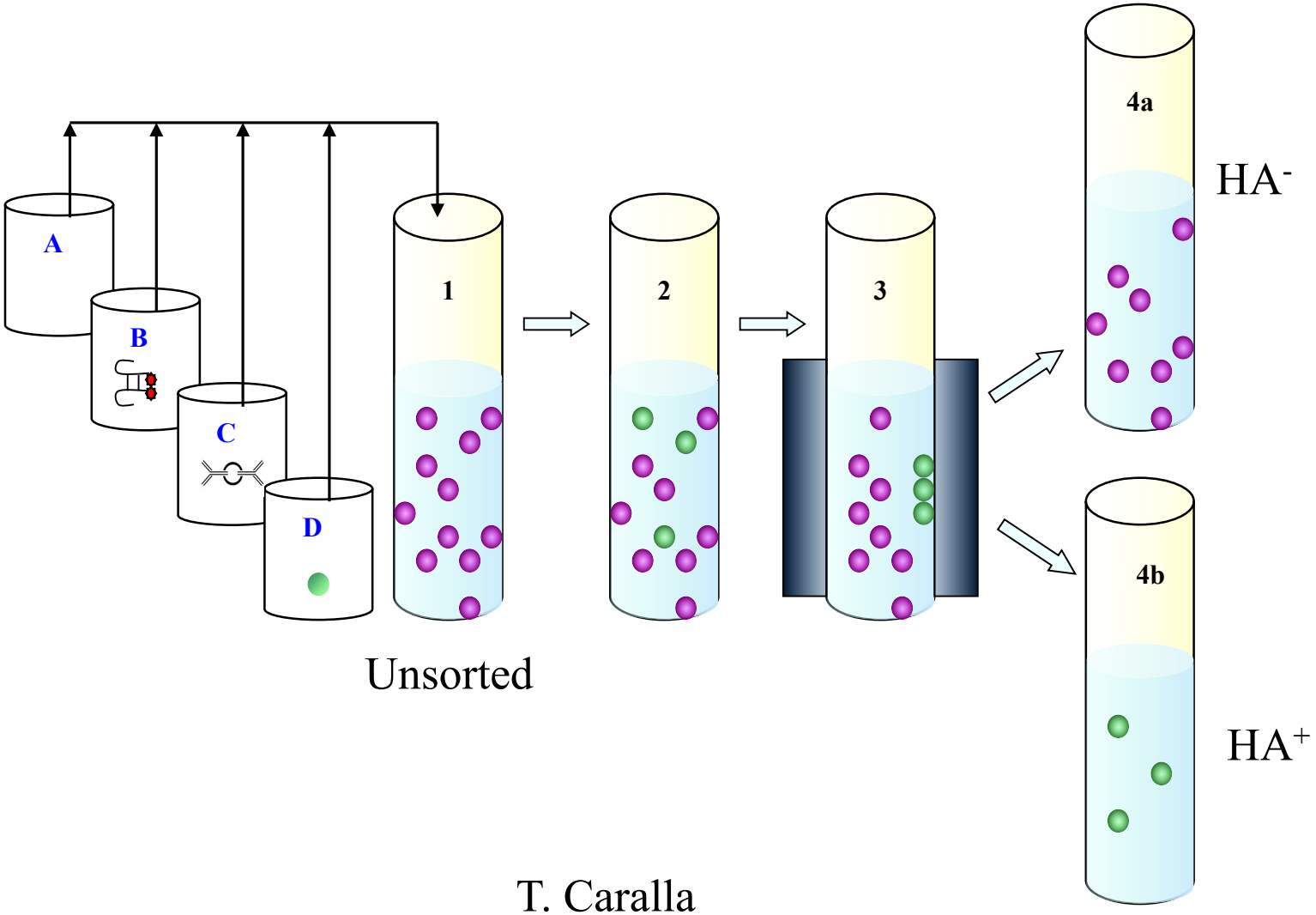
Hyaluronic Acid





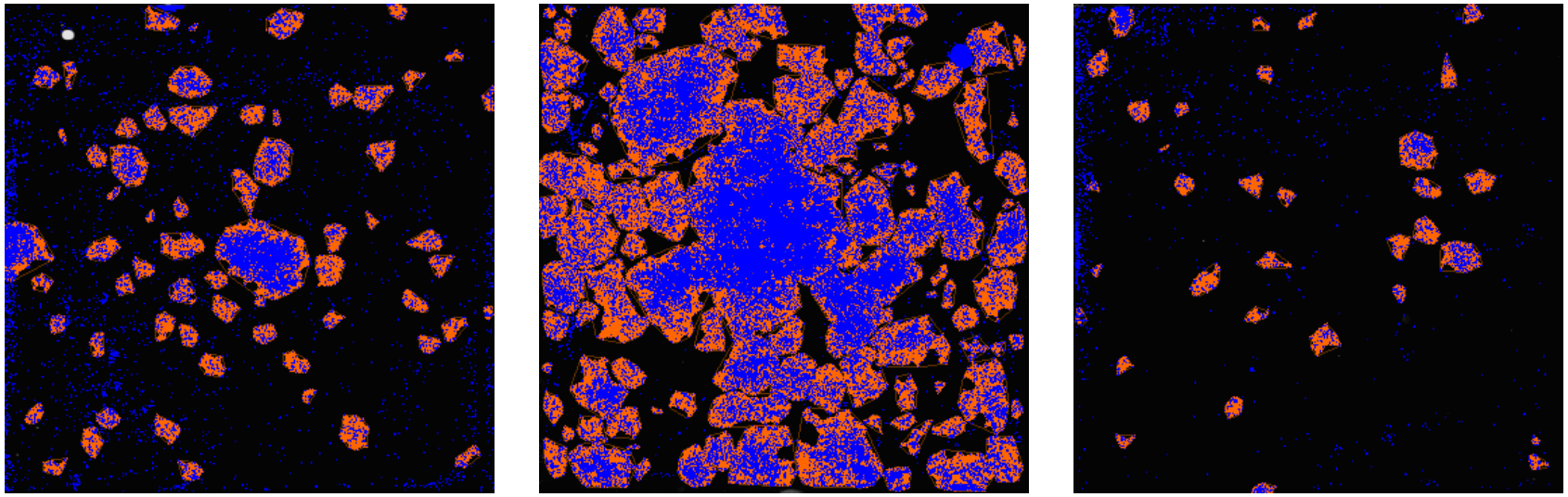
T. Caralla

CTP Selection
Using Magnetic Separation



T. Caralla

CTP Selection Using Magnetic Separation

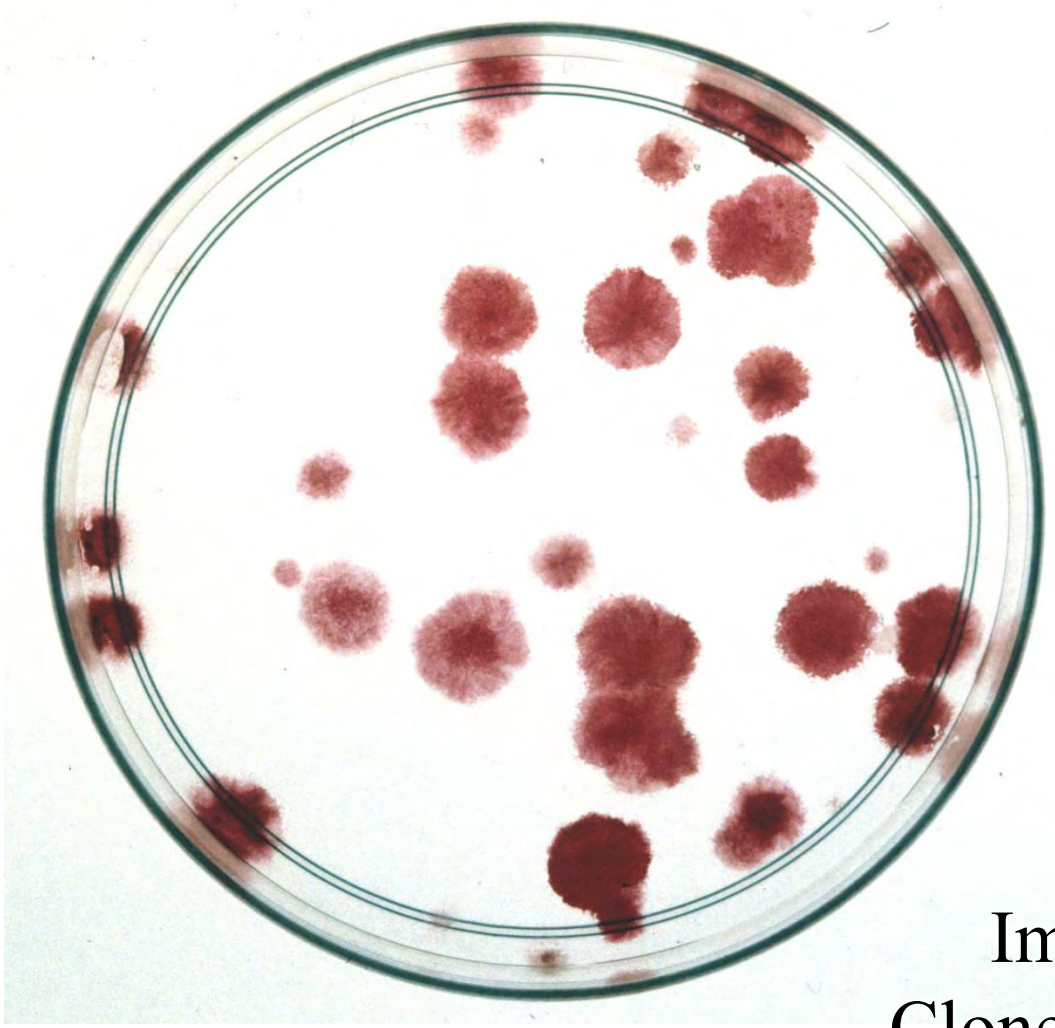


Unselected HA^{+} HA^{-}

P_{CTP} - 4-20 fold
Cells per colony (Proliferation)
AP per cell (Differentiation)

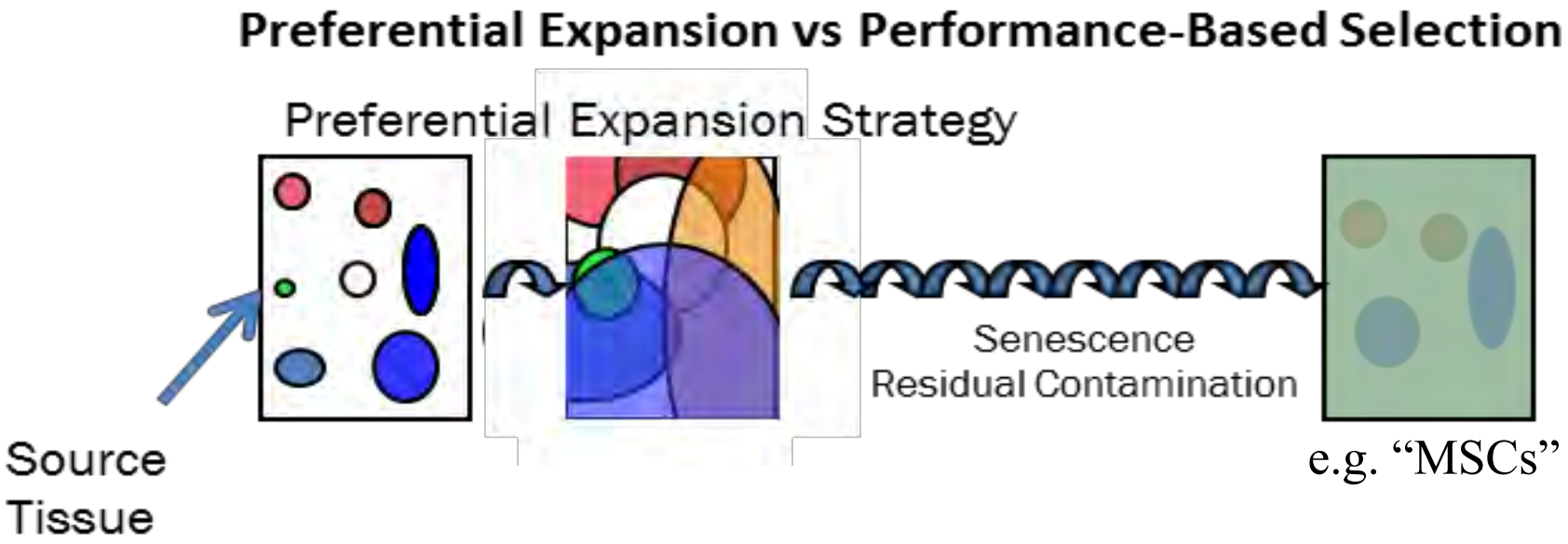
Caralla et al, Annals of Biomedical Engineering, 40(12): 2559–2567, 2012
Caralla T, et al: Tissue Eng Part A. 2013 Jan;19(1-2):125-34.

In Vitro Expansion Methods?



Impact of
Clone Diversity?

Moving to Performance-Based Selection



How repeatable and reproducible is this Preferential Expansion?

Adherent Culture Expanded "MSCs" Far More Variation than Reported

Patient #-Cell Source	CD105	CD73	CD90
1-MS	99.0	100.0	100.0
1-TS	100.0	100.0	100.0
1-AT	100.0	100.0	100.0
2-MS	99.5	100.0	100.0
2-TS	99.8	100.0	100.0
2-AT	99.9	100.0	100.0
3-MS	99.9	100.0	99.9
3-TS	97.7	98.1	97.8
3-AT	100.0	100.0	100.0
4-MS	99.9	100.0	100.0
4-TS	99.9	100.0	100.0
4-AT	100.0	100.0	100.0
5-MS	94.5	100.0	100.0
5-TS	99.7	100.0	100.0
5-AT	97.7	100.0	100.0
6-MS	99.1	99.9	100.0
6-TS	99.7	100.0	100.0
6-AT	99.9	100.0	100.0
7-MS	97.2	100.0	100.0
7-TS	99.7	100.0	100.0
7-AT	99.7	99.9	100.0
8-MS	99.3	100.0	100.0
8-TS	99.6	99.9	100.0
8-AT	90.0	99.6	97.3

P2 "MSC" cultures from 8 subjects

Marrow Space (MS)
Trabecular Surface (TS)
Adipose Tissue (AT)

Adherent Culture Expanded (ACE) Cells
Far More Variation than Reported

Patient #-Cell Source	CD105	CD73	CD90
1-MS	99.0	100.0	100.0
1-TS	100.0	100.0	100.0
1-AT	100.0	100.0	100.0
2-MS	99.5	100.0	100.0
2-TS	99.8	100.0	100.0
2-AT	99.9	100.0	100.0
3-MS	99.9	100.0	99.9
3-TS	97.7	98.1	97.8
3-AT	100.0	100.0	100.0
4-MS	99.9	100.0	100.0
4-TS	99.9	100.0	100.0
4-AT	100.0	100.0	100.0
5-MS	94.5	100.0	100.0
5-TS	99.7	100.0	100.0
5-AT	97.7	100.0	100.0
6-MS	99.1	99.9	100.0
6-TS	99.7	100.0	100.0
6-AT	99.9	100.0	100.0
7-MS	97.2	100.0	100.0
7-TS	99.7	100.0	100.0
7-AT	99.7	99.9	100.0
8-MS	99.3	100.0	100.0
8-TS	99.6	99.9	100.0
8-AT	90.0	99.6	97.3

Traditional MSC Markers are ALL near 100%!

Adherent Culture Expanded (ACE) Cells
Far More Variation than Reported

Patient #-Cell Source	CD105	CD73	CD90
1-MS	99.0	100.0	100.0
1-TS	100.0	100.0	100.0
1-AT	100.0	100.0	100.0
2-MS	99.5	100.0	100.0
2-TS	99.8	100.0	100.0
2-AT	99.9	100.0	100.0
3-MS	99.9	100.0	99.9
3-TS	97.7	98.1	97.8
3-AT	100.0	100.0	100.0
4-MS	99.9	100.0	100.0
4-TS	99.9	100.0	100.0
4-AT	100.0	100.0	100.0
5-MS	94.5	100.0	100.0
5-TS	99.7	100.0	100.0
5-AT	97.7	100.0	100.0
6-MS	99.1	99.9	100.0
6-TS	99.7	100.0	100.0
6-AT	99.9	100.0	100.0
7-MS	97.2	100.0	100.0
7-TS	99.7	100.0	100.0
7-AT	99.7	99.9	100.0
8-MS	99.3	100.0	100.0
8-TS	99.6	99.9	100.0
8-AT	90.0	99.6	97.3

Traditional MSC Markers are **NOT** a Measure of Quality

Adherent Culture Expanded (ACE) Cells
Far More Variation than Reported

Patient #-Cell Source	CD105	CD73	CD90	Oct3/4	Sox2	Nanog	SSEA-4	SSEA-1	SSEA-3	Cripto-1	E-Cadherin	Ep-CAM	CD146	HA
1-MS	99.0	100.0	100.0											
1-TS	100.0	100.0	100.0											
1-AT	100.0	100.0	100.0											
2-MS	99.5	100.0	100.0											
2-TS	99.8	100.0	100.0											
2-AT	99.9	100.0	100.0											
3-MS	99.9	100.0	99.9											
3-TS	97.7	98.1	97.8											
3-AT	100.0	100.0	100.0											
4-MS	99.9	100.0	100.0											
4-TS	99.9	100.0	100.0											
4-AT	100.0	100.0	100.0											
5-MS	94.5	100.0	100.0											
5-TS	99.7	100.0	100.0											
5-AT	97.7	100.0	100.0											
6-MS	99.1	99.9	100.0											
6-TS	99.7	100.0	100.0											
6-AT	99.9	100.0	100.0											
7-MS	97.2	100.0	100.0											
7-TS	99.7	100.0	100.0											
7-AT	99.7	99.9	100.0											
8-MS	99.3	100.0	100.0											
8-TS	99.6	99.9	100.0											
8-AT	90.0	99.6	97.3											

?

Traditional MSC Markers are **NOT** a Measure of Quality

Adherent Culture Expanded (ACE) Cells
Far More Variation than Reported

Patient #-Cell Source	CD105	CD73	CD90	Oct3/4	Sox2	Nanog	SSEA-4	SSEA-1	SSEA-3	Cripto-1	E-Cadherin	Ep-CAM	CD146	HA
1-MS	99.0	100.0	100.0	2.7	99.4	100.0	7.5	3.1	0.9	2.9	18.3	3.9	22.8	0.1
1-TS	100.0	100.0	100.0	22.9	96.3	100.0	43.6	25.4	0.7	19.8	63.6	11.7	38.3	5.5
1-AT	100.0	100.0	100.0	27.1	99.3	100.0	6.8	17.5	2.1	32.1	84.2	16.0	8.0	8.5
2-MS	99.5	100.0	100.0	83.5	98.3	99.4	78.8	60.4	8.9	47.8	74.9	25.2	63.2	37.5
2-TS	99.8	100.0	100.0	17.3	87.2	99.8	62.2	44.2	1.6	16.2	65.3	5.1	13.0	8.4
2-AT	99.9	100.0	100.0	22.3	47.5	99.7	32.5	49.3	0.4	31.9	80.8	9.8	14.2	2.3
3-MS	99.9	100.0	99.9	92.4	99.7	99.5	69.4	89.1	4.5	92.6	99.0	81.8	96.5	67.2
3-TS	97.7	98.1	97.8	99.2	99.9	99.8	90.9	89.9	4.4	92.1	96.8	55.8	96.0	76.5
3-AT	100.0	100.0	100.0	97.2	99.9	99.1	96.9	97.9	24.0	85.9	99.2	54.6	95.2	95.7
4-MS	99.9	100.0	100.0	18.4	16.1	64.5	87.0	42.8	1.8	48.4	87.0	9.0	69.0	4.2
4-TS	99.9	100.0	100.0	21.4	16.8	83.2	71.5	43.0	4.6	42.6	66.0	8.6	80.1	7.5
4-AT	100.0	100.0	100.0	9.9	24.6	92.2	69.7	56.7	4.6	60.0	93.5	17.1	56.2	12.2
5-MS	94.5	100.0	100.0	4.3	37.1	58.2	43.2	25.2	0.3	14.8	79.5	3.0	20.1	2.3
5-TS	99.7	100.0	100.0	8.0	63.1	76.7	31.8	20.3	0.4	10.6	43.9	3.5	31.0	0.7
5-AT	97.7	100.0	100.0	8.2	56.5	68.0	13.4	5.3	0.1	5.7	34.0	0.9	9.1	0.8
6-MS	99.1	99.9	100.0	4.1	78.6	91.1	24.6	5.0	0.1	16.7	80.1	4.0	37.8	18.8
6-TS	99.7	100.0	100.0	20.0	96.2	74.6	78.0	35.2	1.8	40.2	87.2	17.1	55.1	16.8
6-AT	99.9	100.0	100.0	20.8	78.8	82.6	50.7	55.3	1.7	24.3	65.9	4.2	32.8	2.1
7-MS	97.2	100.0	100.0	49.6	99.3	94.7	93.6	53.7	1.7	50.3	73.4	5.8	64.0	54.5
7-TS	99.7	100.0	100.0	79.3	99.7	99.5	92.6	35.4	2.2	63.9	75.3	9.4	58.5	32.5
7-AT	99.7	99.9	100.0	91.7	98.9	96.9	72.6	15.7	1.3	68.4	70.8	5.2	56.8	99.3
8-MS	99.3	100.0	100.0	8.6	40.3	96.1	18.8	3.9	0.3	29.9	33.2	2.3	27.0	28.1
8-TS	99.6	99.9	100.0	4.1	3.7	72.9	61.0	3.5	0.4	16.1	39.4	1.8	20.1	15.1
8-AT	90.0	99.6	97.3	4.6	3.7	12.6	16.1	0.1	0.1	17.0	2.1	0.1	7.1	7.3

Adherent Culture Expanded (ACE) Cells
Far More Variation than Reported

Patient #-Cell Source	CD105	CD73	CD90	Oct3/4	Sox2	Nanog	SSEA-4	SSEA-1	SSEA-3	Cripto-1	E-Cadherin	Ep-CAM	CD146	HA
1-MS	99.0	100.0	100.0	2.7	99.4	100.0	7.5	3.1	0.9	2.9	18.3	3.9	22.8	0.1
1-TS	100.0	100.0	100.0	22.9	96.3	100.0	43.6	25.4	0.7	19.8	63.6	11.7	38.3	5.5
1-AT	100.0	100.0	100.0	27.1	99.3	100.0	6.8	17.5	2.1	32.1	84.2	16.0	8.0	8.5
2-MS	99.5	100.0	100.0	83.5	98.3	99.4	78.8	60.4	8.9	47.8	74.9	25.2	63.2	37.5
2-TS	99.8	100.0	100.0	17.3	87.2	99.8	62.2	44.2	1.6	16.2	65.3	5.1	13.0	8.4
2-AT	99.9	100.0	100.0	22.3	47.5	99.7	32.5	49.3	0.4	31.9	80.8	9.8	14.2	2.3
3-MS	99.9	100.0	99.9	92.4	99.7	99.5	69.4	89.1	4.5	92.6	99.0	81.8	96.5	67.2
3-TS	97.7	98.1	97.8	99.2	99.9	99.8	90.9	89.9	4.4	92.1	96.8	55.8	96.0	76.5
3-AT	100.0	100.0	100.0	97.2	99.9	99.1	96.9	97.9	24.0	85.9	99.2	54.6	95.2	95.7
4-MS	99.9	100.0	100.0	18.4	16.1	64.5	87.0	42.8	1.8	48.4	87.0	9.0	69.0	4.2
4-TS	99.9	100.0	100.0	21.4	16.8	83.2								7.5
4-AT	100.0	100.0	100.0	9.9	24.6	92.2								12.2
5-MS	94.5	100.0	100.0	4.3	37.1	58.2								2.3
5-TS	99.7	100.0	100.0	8.0	63.1	76.7	31.8	20.3	0.4	10.6	43.9	3.5	31.0	0.7
5-AT	97.7	100.0	100.0	8.2	56.5	68.0	13.4	5.3	0.1	5.7	34.0	0.9	9.1	0.8
6-MS	99.1	99.9	100.0	4.1	78.6	91.1	24.6	5.0	0.1	16.7	80.1	4.0	37.8	18.8
6-TS	99.7	100.0	100.0	20.0	96.2	74.6	78.0	35.2	1.8	40.2	87.2	17.1	55.1	16.8
6-AT	99.9	100.0	100.0	20.8	78.8	82.6	50.7	55.3	1.7	24.3	65.9	4.2	32.8	2.1
7-MS	97.2	100.0	100.0	49.6	99.3	94.7	93.6	53.7	1.7	50.3	73.4	5.8	64.0	54.5
7-TS	99.7	100.0	100.0	79.3	99.7	99.5	92.6	35.4	2.2	63.9	75.3	9.4	58.5	32.5
7-AT	99.7	99.9	100.0	91.7	98.9	96.9	72.6	15.7	1.3	68.4	70.8	5.2	56.8	99.3
8-MS	99.3	100.0	100.0	8.6	40.3	96.1	18.8	3.9	0.3	29.9	33.2	2.3	27.0	28.1
8-TS	99.6	99.9	100.0	4.1	3.7	72.9	61.0	3.5	0.4	16.1	39.4	1.8	20.1	15.1
8-AT	90.0	99.6	97.3	4.6	3.7	12.6	16.1	0.1	0.1	17.0	2.1	0.1	7.1	7.3

10-1000 fold Variation !

Variation in Other Markers is most likely to be predictive

Adherent Culture Expanded (ACE) Cells
Far More Variation than Reported

Patient #-Cell Source	CD105	CD73	CD90	Oct3/4	Sox2	Nanog	SSEA-4	SSEA-1	SSEA-3	Cripto-1	E-Cadherin	Ep-CAM	CD146	HA
1-MS	99.0	100.0	100.0	2.7	99.4	100.0	7.5	3.1	0.9	2.9	18.3	3.9	22.8	0.1
1-TS	100.0	100.0	100.0	22.9	96.3	100.0	43.6	25.4	0.7	19.8	63.6	11.7	38.3	5.5
1-AT	100.0	100.0	100.0	27.1	99.3	100.0	6.8	17.5	2.1	32.1	84.2	16.0	8.0	8.5
2-MS	99.5	100.0	100.0	83.5	98.3	99.4	78.8	60.4	8.9	47.8	74.9	25.2	63.2	37.5
2-TS	99.8	100.0	100.0	17.3	87.2	99.8	62.2	44.2	1.6	16.2	65.3	5.1	13.0	8.4
2-AT	99.9	100.0	100.0	22.3	47.5	99.7	32.5	49.3	0.4	31.9	80.8	9.8	14.2	2.3
3-MS	99.9	100.0	99.9	92.4	99.7	99.5	69.4	89.1	4.5	92.6	99.0	81.8	96.5	67.2
3-TS	97.7	98.1	97.8	99.2	99.9	99.8	90.9	89.9	4.4	92.1	96.8	55.8	96.0	76.5
3-AT	100.0	100.0	100.0	97.2	99.9	99.1	96.9	97.9	24.0	85.9	99.2	54.6	95.2	95.7
4-MS	99.9	100.0	100.0	18.4	16.1	64.5	87.0	42.8	1.8	48.4	87.0	9.0	69.0	4.2
4-TS	99.9	100.0	100.0	21.4	16.8	83.2	71.5	43.0	4.6	42.6	66.0	8.6	80.1	7.5
4-AT	100.0	100.0	100.0	9.9	24.6	92.2	69.7	56.7	4.6	60.0	93.5	17.1	56.2	12.2
5-MS	94.5	100.0	100.0	4.3	37.1	58.2	43.2	25.2	0.3	14.8	79.5	3.0	20.1	2.3
5-TS	99.7	100.0	100.0	8.0	63.1	76.7	31.8	20.3	0.4	10.6	43.9	3.5	31.0	0.7
5-AT	97.7	100.0	100.0	8.2	56.5	68.0	13.4	5.3	0.1	5.7	34.0	0.9	9.1	0.8
6-MS	99.1	99.9	100.0	4.1	78.6	91.1	24.6	5.0	0.1	16.7	80.1	4.0	37.8	18.8
6-TS	99.7	100.0	100.0	20.0	96.2	74.6	78.0	35.2	1.8	40.2	87.2	17.1	55.1	16.8
6-AT	99.9	100.0	100.0	20.8	78.8	82.6	50.7	55.3	1.7	24.3	65.9	4.2	32.8	2.1
7-MS	97.2	100.0	100.0	49.6	99.3	94.7	93.6	53.7	1.7	50.3	73.4	5.8	64.0	54.5
7-TS	99.7	100.0	100.0	79.3	99.7	99.5	92.6	35.4	2.2	63.9	75.3	9.4	58.5	32.5
7-AT	99.7	99.9	100.0	91.7	98.9	96.9	72.6	15.7	1.3	68.4	70.8	5.2	56.8	99.3
8-MS	99.3	100.0	100.0	8.6	40.3	96.1	18.8	3.9	0.3	29.9	33.2	2.3	27.0	28.1
8-TS	99.6	99.9	100.0	4.1	3.7	72.9	61.0	3.5	0.4	16.1	39.4	1.8	20.1	15.1
8-AT	90.0	99.6	97.3	4.6	3.7	12.6	16.1	0.1	0.1	17.0	2.1	0.1	7.1	7.3

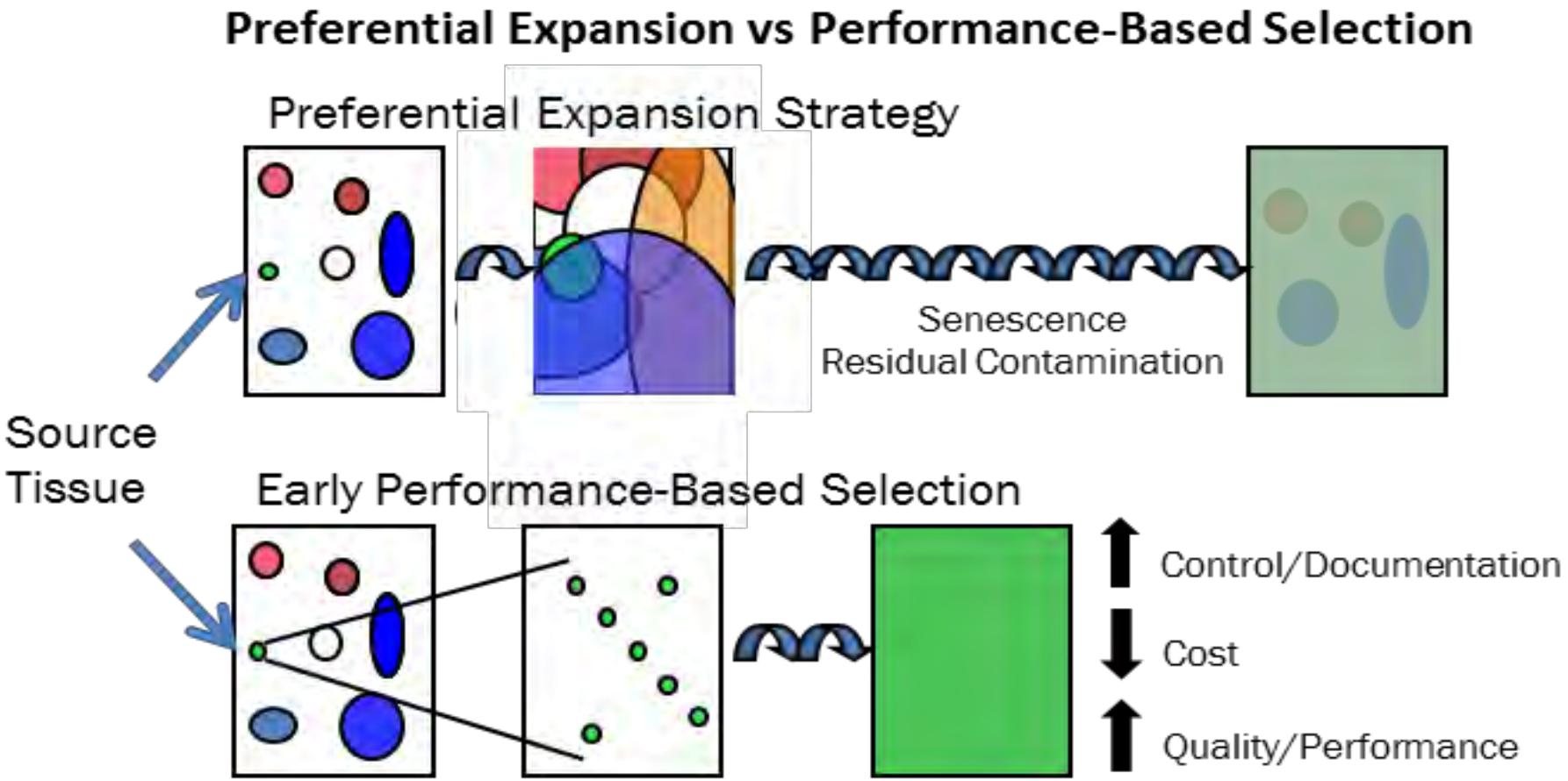
Some good donors and some poor donors

Adherent Culture Expanded (ACE) Cells
Far More Variation than Reported

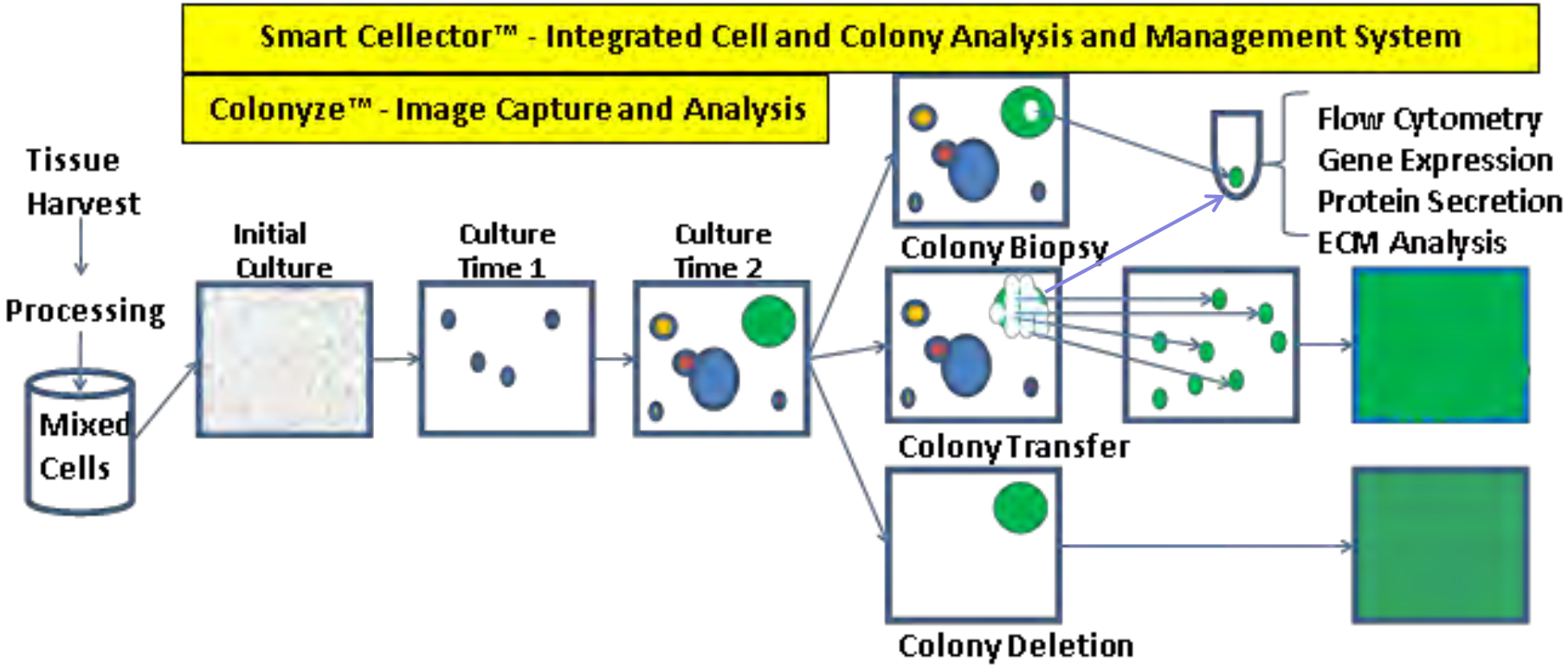
Patient #-Cell Source	CD105	CD73	CD90	Oct3/4	Sox2	Nanog	SSEA-4	SSEA-1	SSEA-3	Cripto-1	E-Cadherin	Ep-CAM	CD146	HA
1-MS	99.0	100.0	100.0	2.7	99.4	100.0	7.5	3.1	0.9	2.9	18.3	3.9	22.8	0.1
1-TS	100.0	100.0	100.0	22.9	96.3	100.0	43.6	25.4	0.7	19.8	63.6	11.7	38.3	5.5
1-AT	100.0	100.0	100.0	27.1	99.3	100.0	6.8	17.5	2.1	32.1	84.2	16.0	8.0	8.5
2-MS	99.5	100.0	100.0	83.5	98.3	99.4	78.8	60.4	8.9	47.8	74.9	25.2	63.2	37.5
2-TS	99.8	100.0	100.0	17.3	87.2	99.8	62.2	44.2	1.6	16.2	65.3	5.1	13.0	8.4
2-AT	99.9	100.0	100.0	22.3	47.5	99.7	32.5	49.3	0.4	31.9	80.8	9.8	14.2	2.3
3-MS	99.9	100.0	99.9	92.4	99.7	99.5	69.4	89.1	4.5	92.6	99.0	81.8	96.5	67.2
3-TS	97.7	98.1	97.8	99.2	99.9	99.8	90.9	89.9	4.4	92.1	96.8	55.8	96.0	76.5
3-AT	100.0	100.0	100.0	97.2	99.9	99.1	96.9	97.9	24.0	85.9	99.2	54.6	95.2	95.7
4-MS	99.9	100.0	100.0	18.4	16.1	64.5	87.0	42.8	1.8	48.4	87.0	9.0	69.0	4.2
4-TS	99.9	100.0	100.0	21.4	16.8	83.2	71.5	43.0	4.6	42.6	66.0	8.6	80.1	7.5
4-AT	100.0	100.0	100.0	9.9	24.6	92.2	69.7	56.7	4.6	60.0	93.5	17.1	56.2	12.2
5-MS	94.5	100.0	100.0	4.3	37.1	58.2	43.2	25.2	0.3	14.8	79.5	3.0	20.1	2.3
5-TS	99.7	100.0	100.0	8.0	63.1	76.7	31.8	20.3	0.4	10.6	43.9	3.5	31.0	0.7
5-AT	97.7	100.0	100.0	8.2	56.5	68.0	13.4	5.3	0.1	5.7	34.0	0.9	9.1	0.8
6-MS	99.1	99.9	100.0	4.1	78.6	91.1	24.6	5.0	0.1	16.7	80.1	4.0	37.8	18.8
6-TS	99.7	100.0	100.0	20.0	96.2	74.6	78.0	35.2	1.8	40.2	87.2	17.1	55.1	16.8
6-AT	99.9	100.0	100.0	20.8	78.8	82.6	50.7	55.3	1.7	24.3	65.9	4.2	32.8	2.1
7-MS	97.2	100.0	100.0	49.6	99.3	94.7	93.6	53.7	1.7	50.3	73.4	5.8	64.0	54.5
7-TS	99.7	100.0	100.0	79.3	99.7	99.5	92.6	35.4	2.2	63.9	75.3	9.4	58.5	32.5
7-AT	99.7	99.9	100.0	91.7	98.9	96.9	72.6	15.7	1.3	68.4	70.8	5.2	56.8	99.3
8-MS	99.3	100.0	100.0	8.6	40.3	96.1	18.8	3.9	0.3	29.9	33.2	2.3	27.0	28.1
8-TS	99.6	99.9	100.0	4.1	3.7	72.9	61.0	3.5	0.4	16.1	39.4	1.8	20.1	15.1
8-AT	90.0	99.6	97.3	4.6	3.7	12.6	16.1	0.1	0.1	17.0	2.1	0.1	7.1	7.3

No clear difference between MS, TS, AT sources

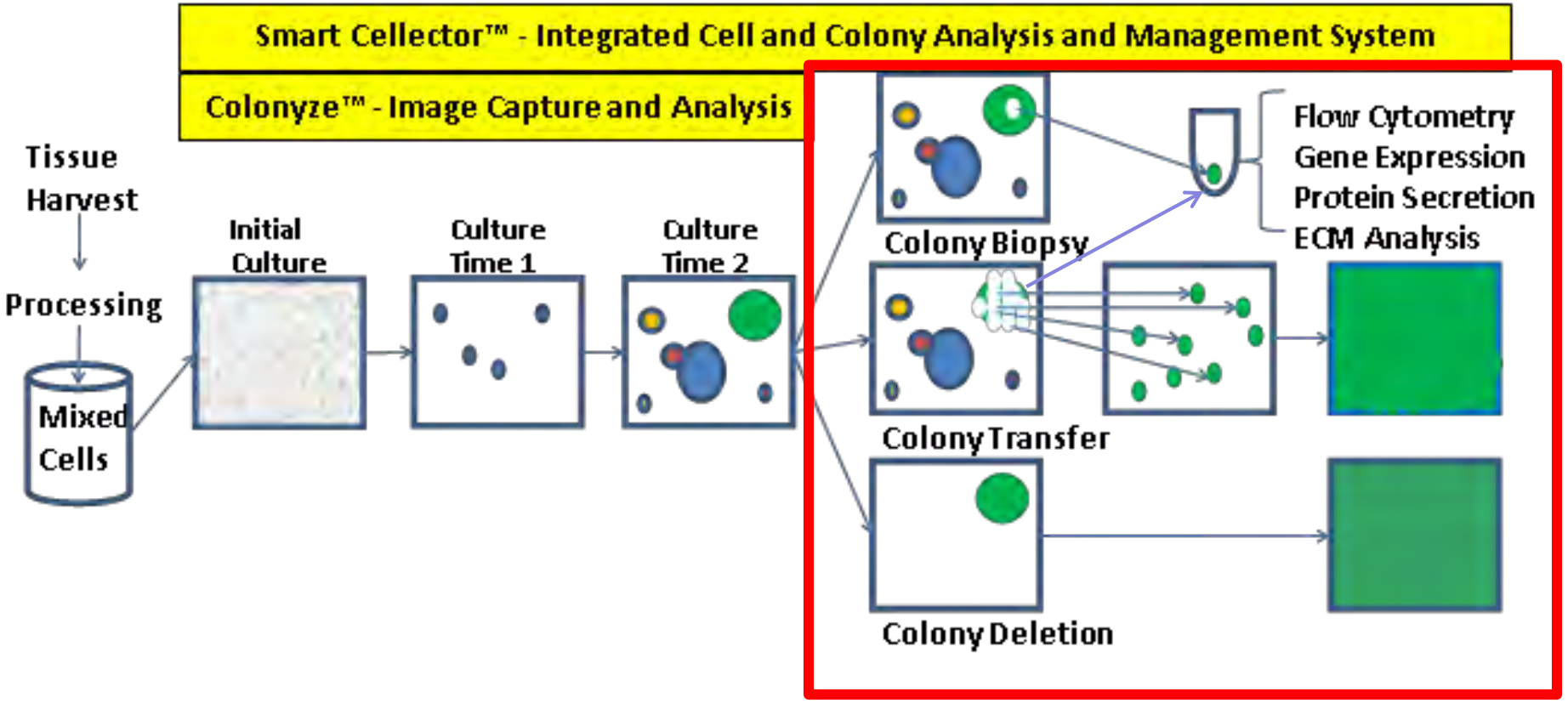
Moving to Performance-Based Selection



Moving to Performance-Based Selection

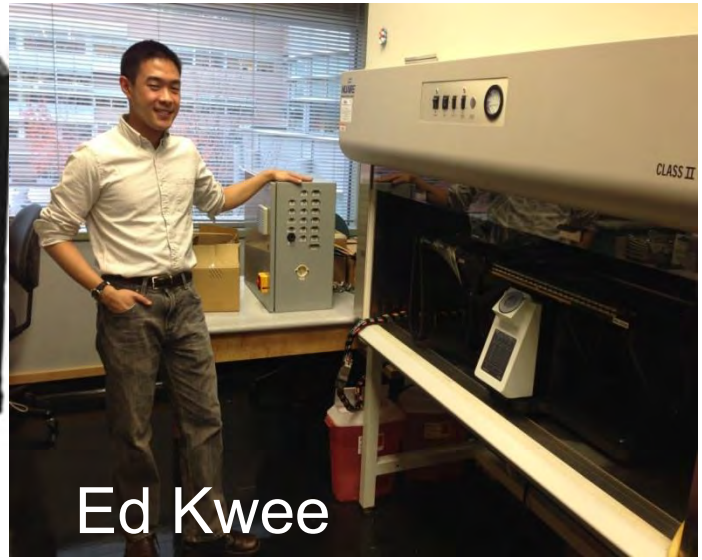


Moving to Performance-Based Selection

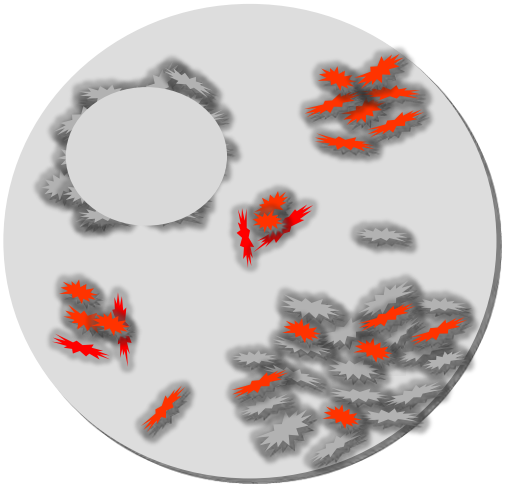


Cell X™ Robot

Colonyze ™ Software



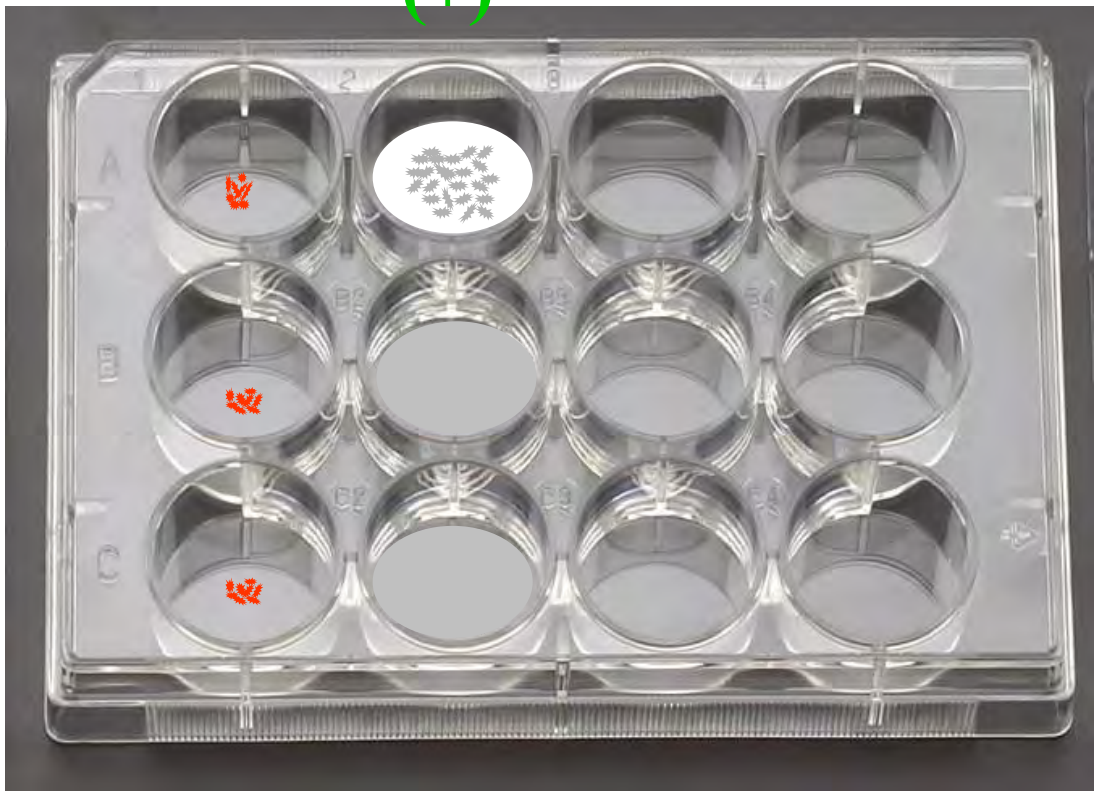
Automated Cell and Colony Management



Marker

(-)

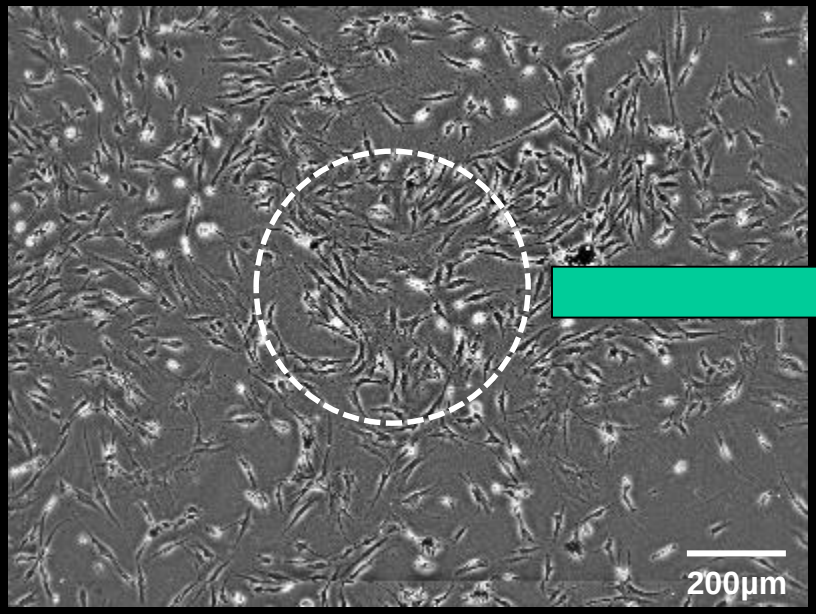
(+)



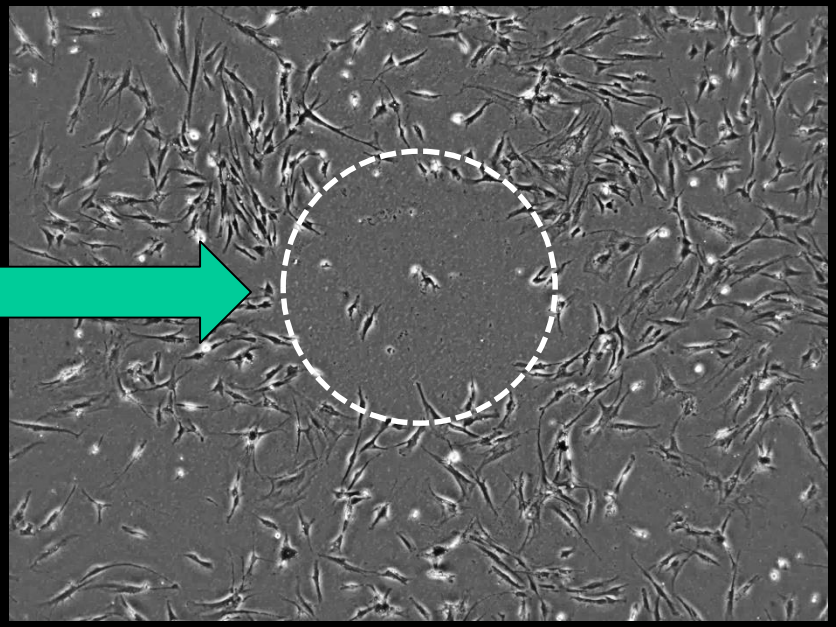
Cell X Picking

Sub-Confluent Cultures

Pick Site



After pick

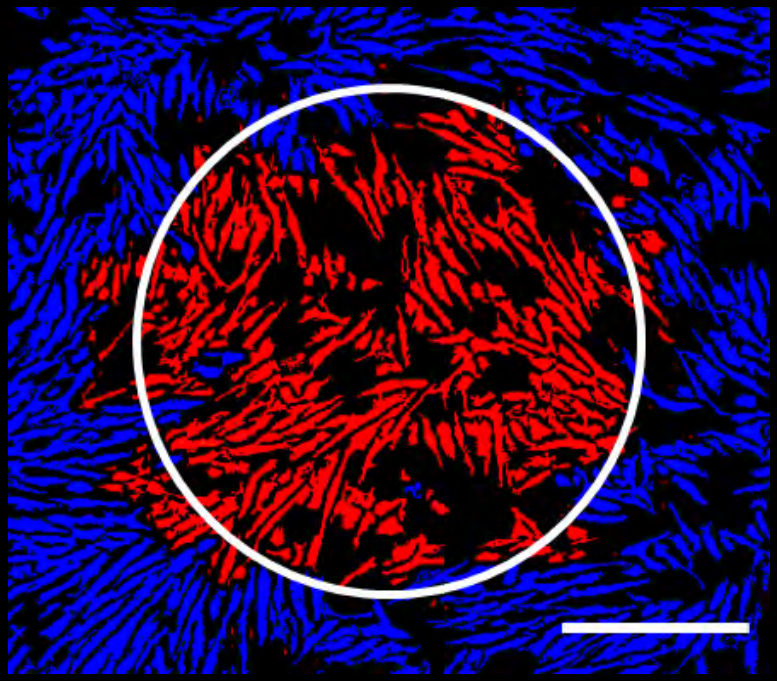


500 µ ID Tool

- **90% efficiency**

Cell X Picking

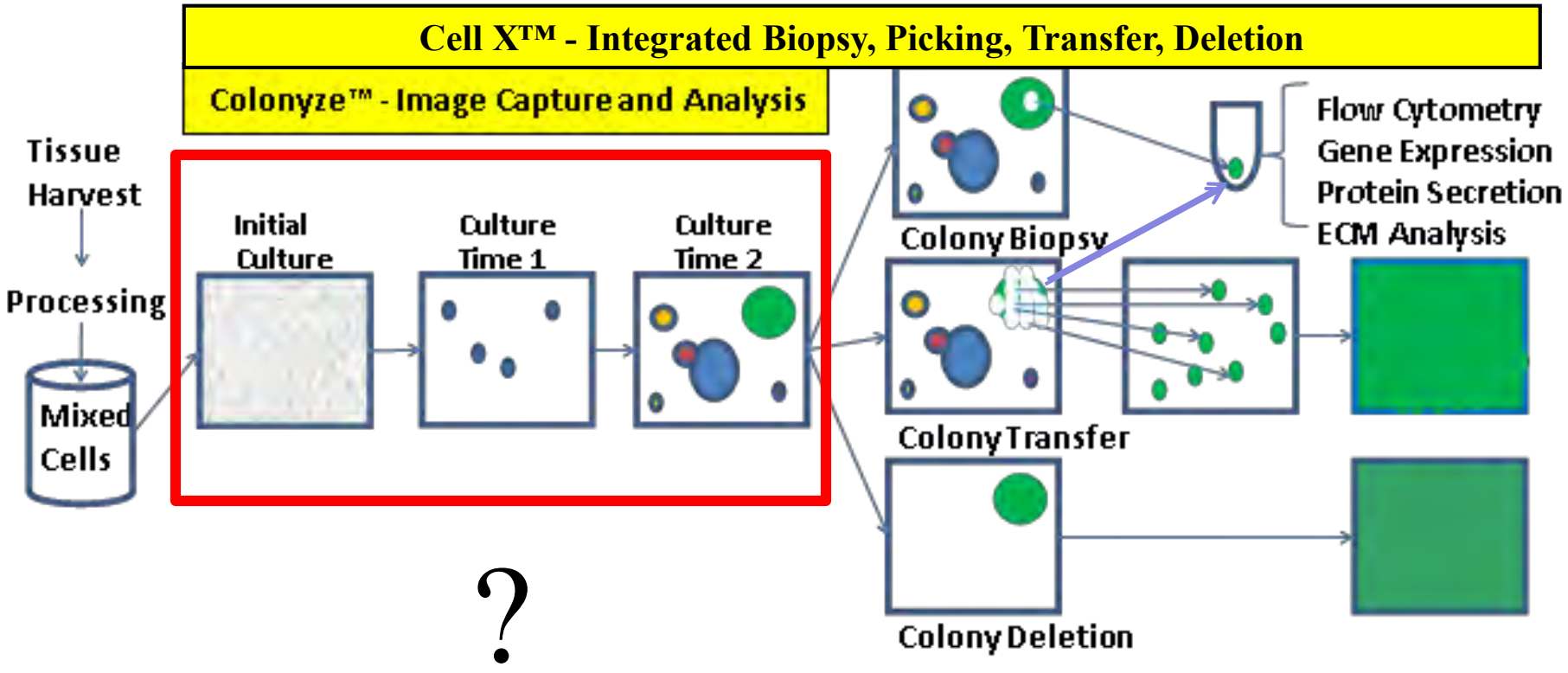
Picking Efficiency



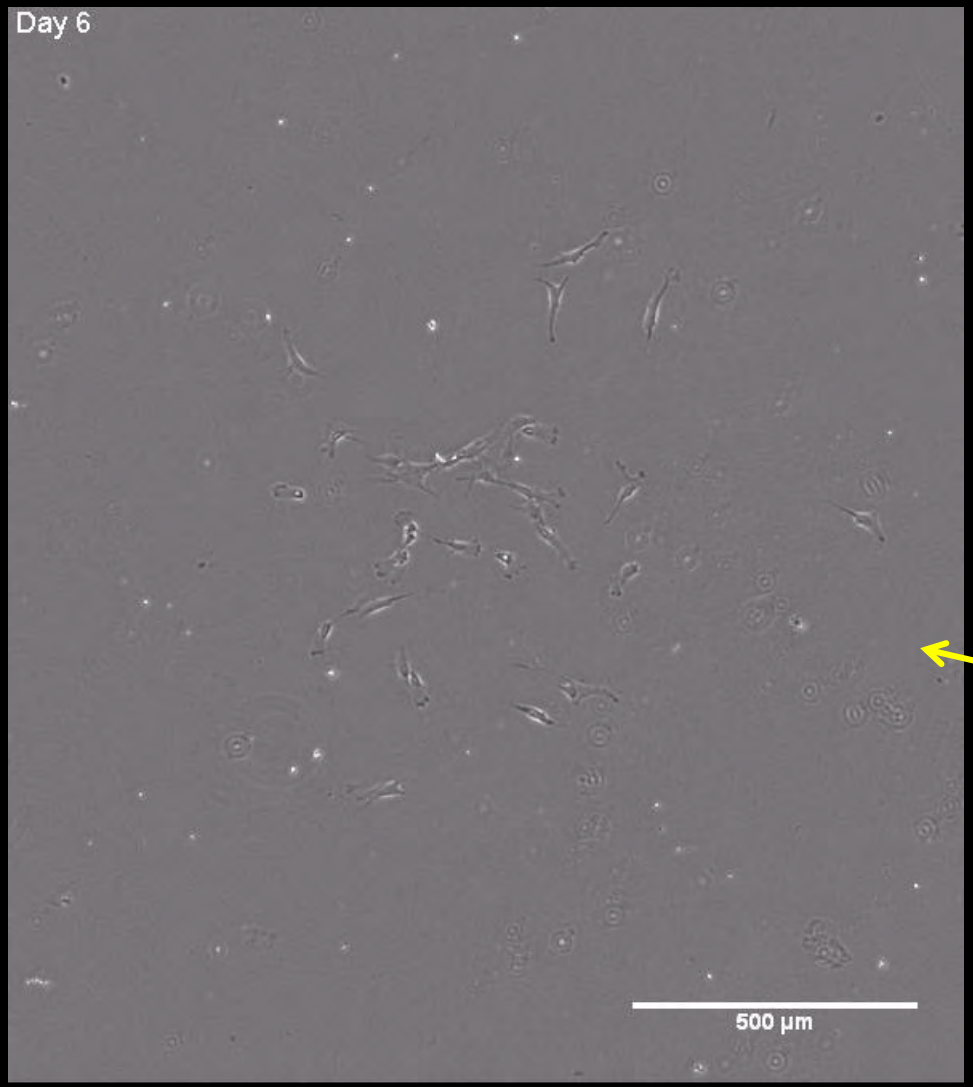
Picking Efficiency

- Cell Area Before: 293000 μm^2
- Cell Area Removed: 227000 μm^2
- Picking Efficiency: 92.1%

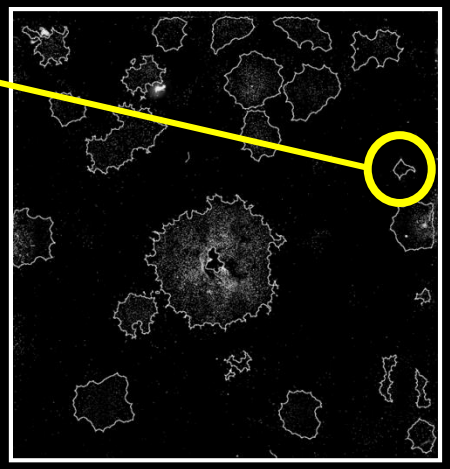
Moving to Performance-Based Selection



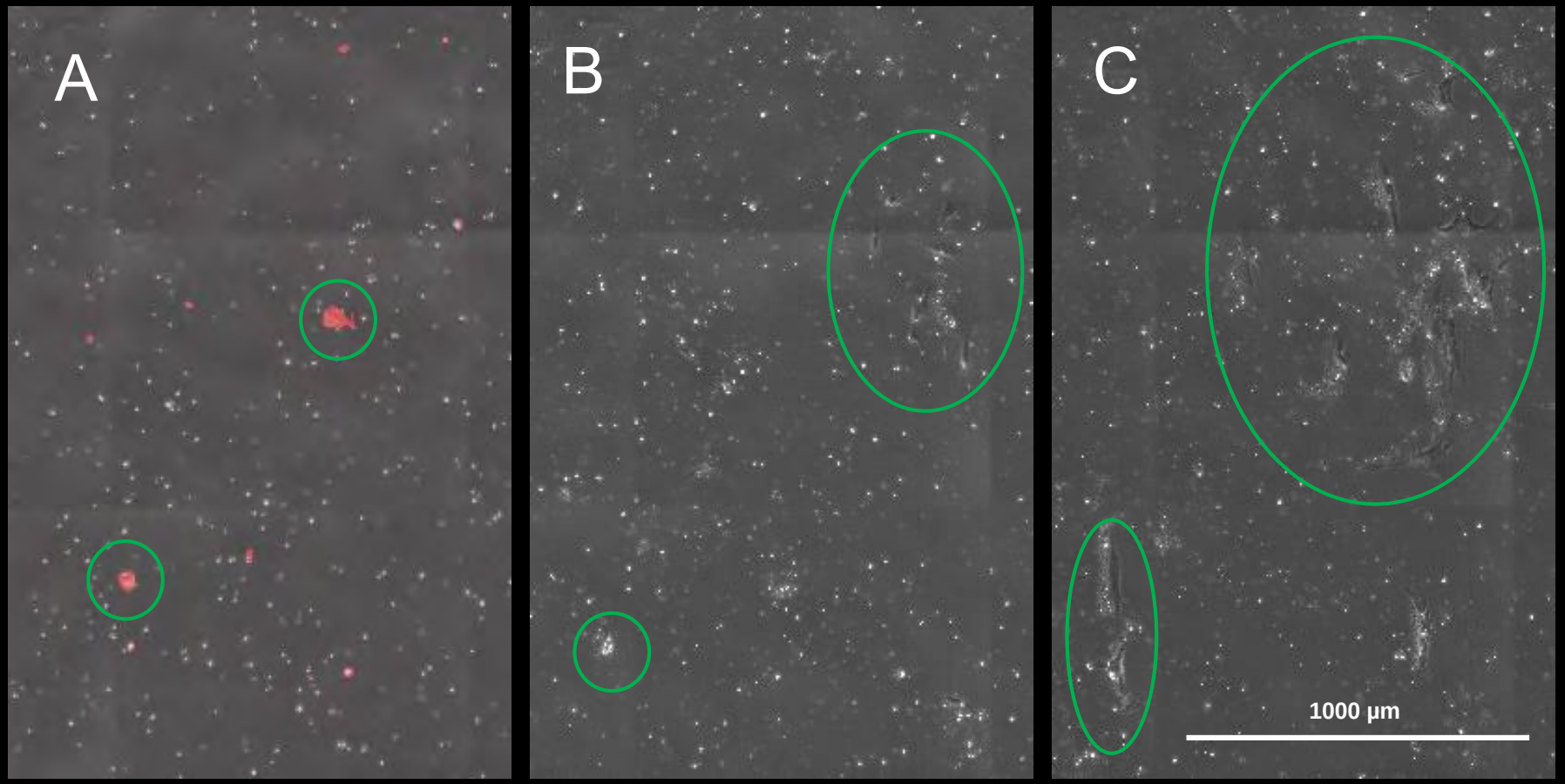
Reverse Time Lapse



- **Track and identify colony founding cell.**
- **Cell division does not start until day 3.**
- **Effective proliferation rate: 1.6 days^{-1}**



In vitro Videomicroscopy



Day 1

Day 3

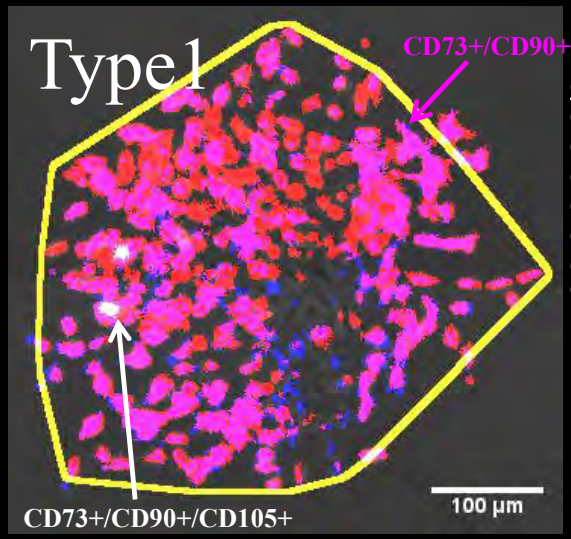
Day 6

CD90

Cartilage Derived CTP-C Typing

CTP Morphology Metrics (type1)

Area	426.5 μm^2
Circularity	0.34
Feret Diameter	46.7 µm
CD73	Positive
CD90	Negative
CD105	Negative

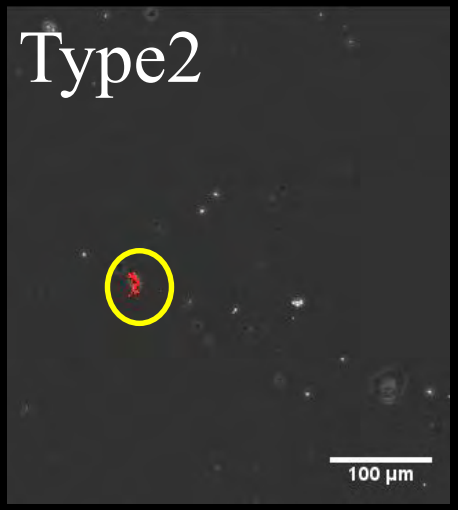


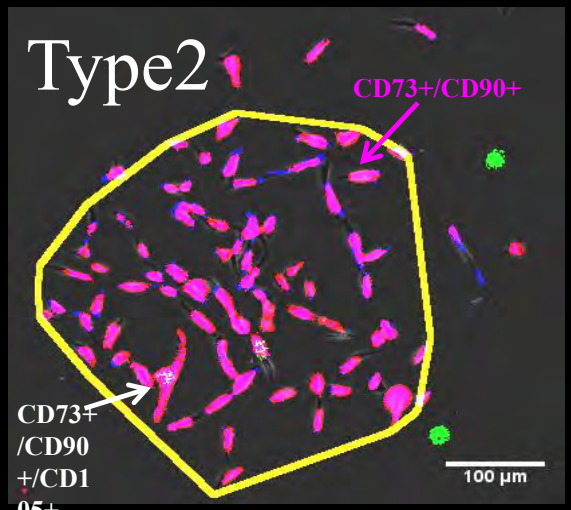
Colony Metrics (Type 1)

Colony Area	73000 μm^2
Cell Area	35000 μm^2
Cell Density	47.7 %
CD73 (Area/Percent)	43000 μm^2/71.4%
CD90 (Area/Percent)	55000 μm^2/92.2%
CD105 (Area/Percent)	172 μm^2/0.28%
Triple Positive (Area/Percent)	172 μm^2/0.28%

CTP Morphology Metrics (type2)

Area	87.5 μm^2
Circularity	0.312
Feret Diameter	30.5 µm
CD73	Positive
CD90	Negative
CD105	Negative

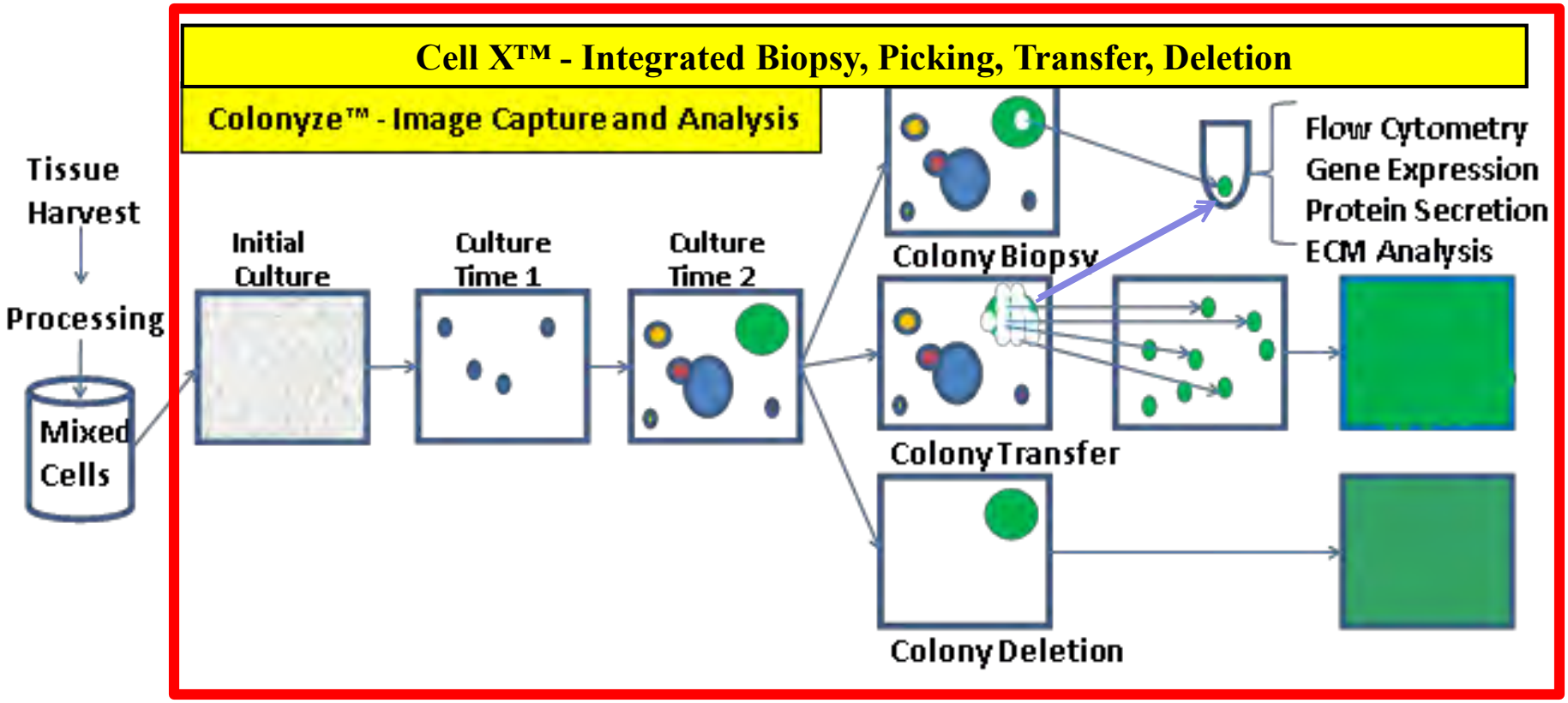




Colony Metrics (Type2)

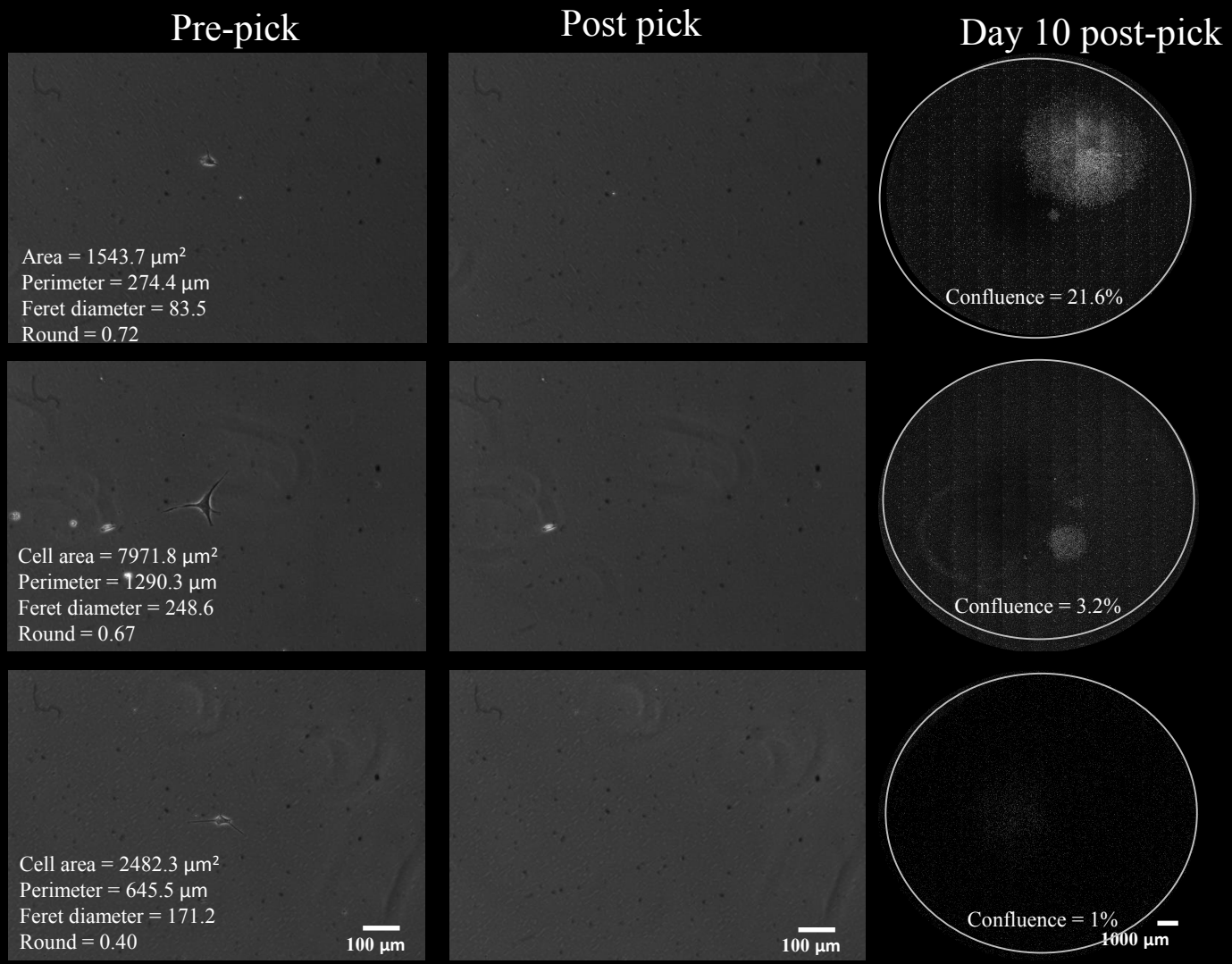
Colony Area	67000 μm^2
Cell Area	6262 μm^2
Cell Density	9.3 %
CD73 (Area/Percent)	10400 μm^2/66.1%
CD90 (Area/Percent)	7500 μm^2/48%
CD105 (Area/Percent)	104 μm^2/0.66%
Triple Positive (Area/Percent)	104 μm^2/

Moving to Performance-Based Selection

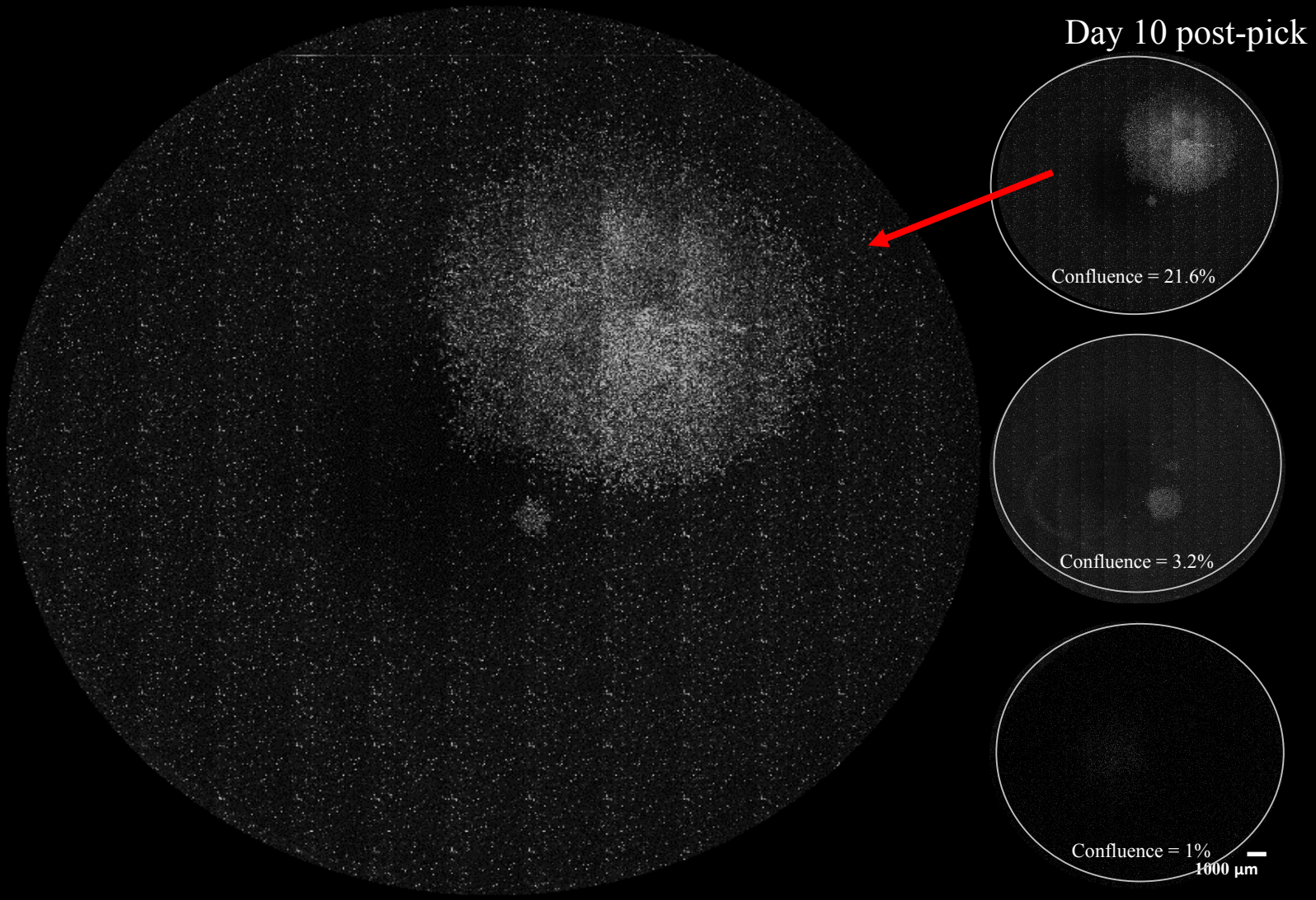


?

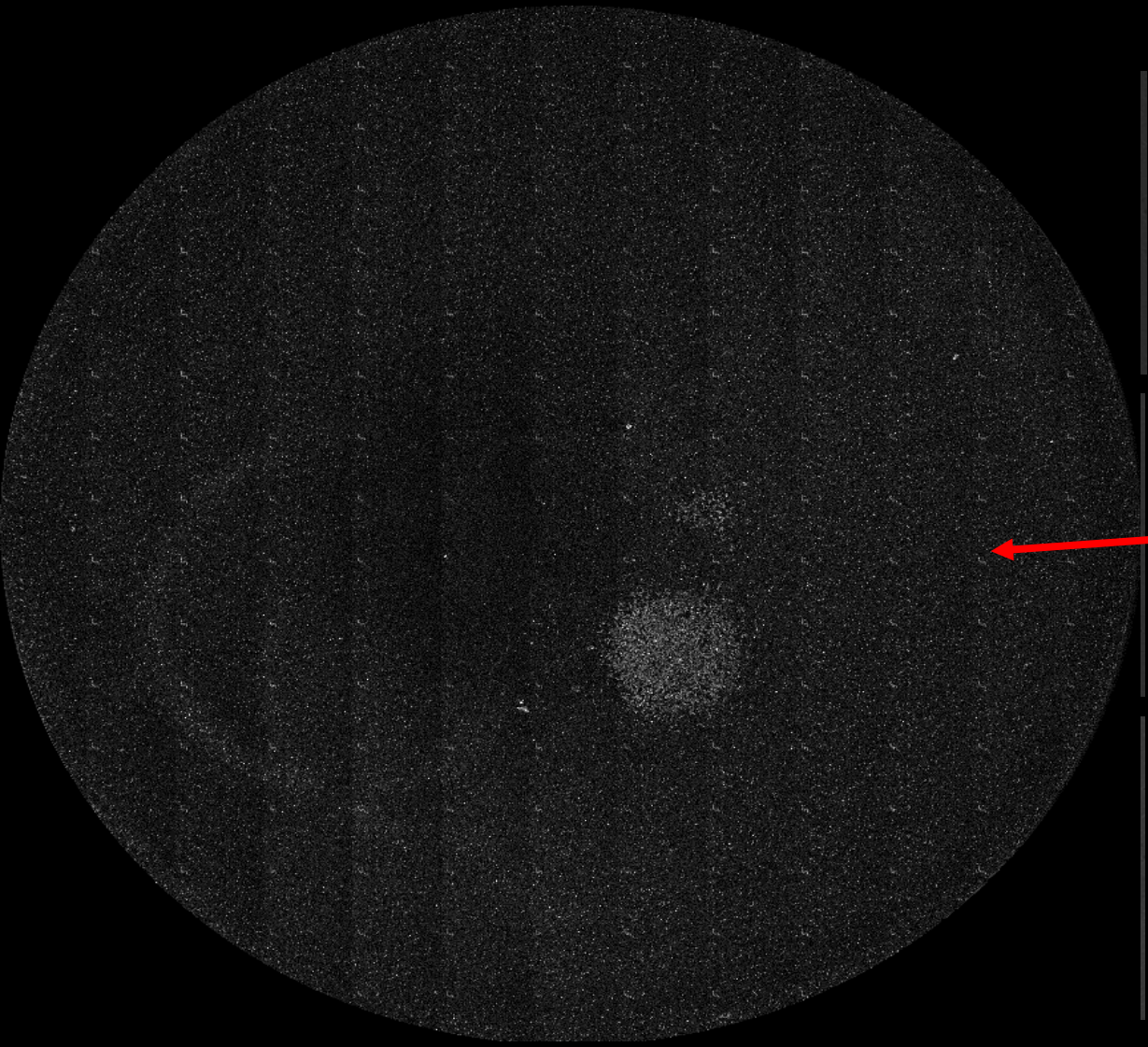
Single Cell Picking



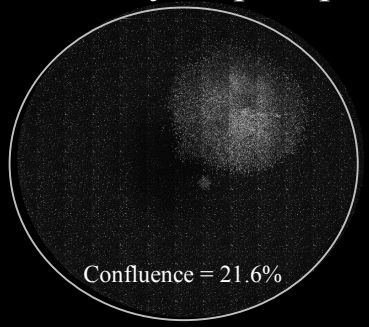
Single Cell Picking

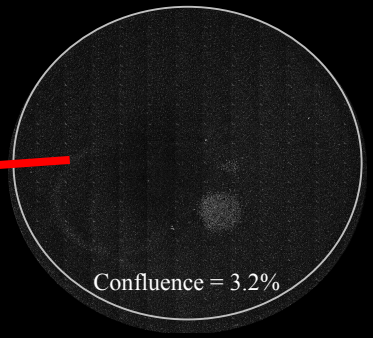


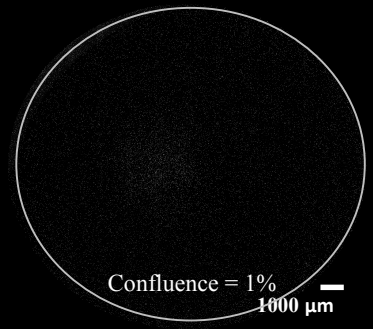
Single Cell Picking



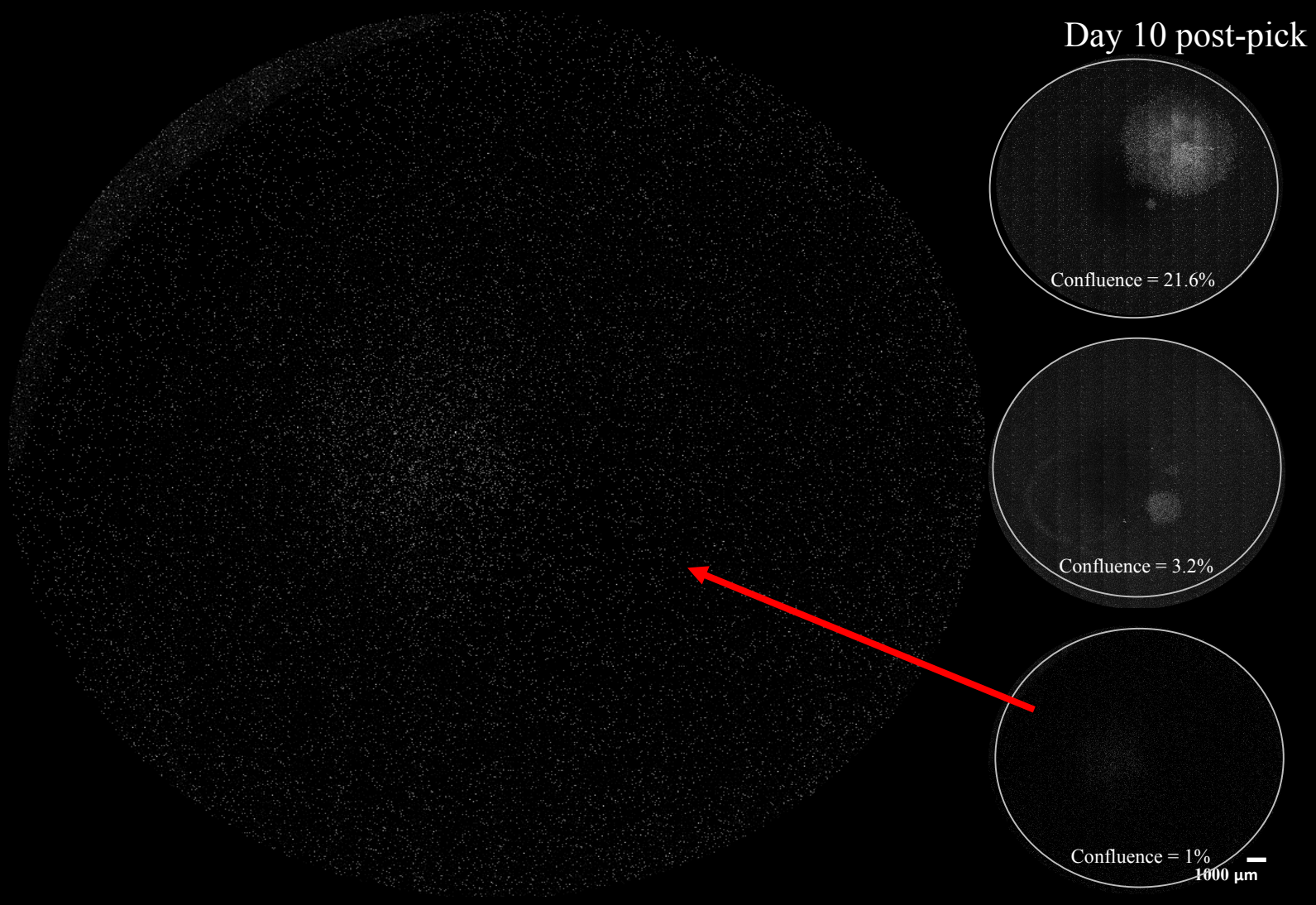
Day 10 post-pick



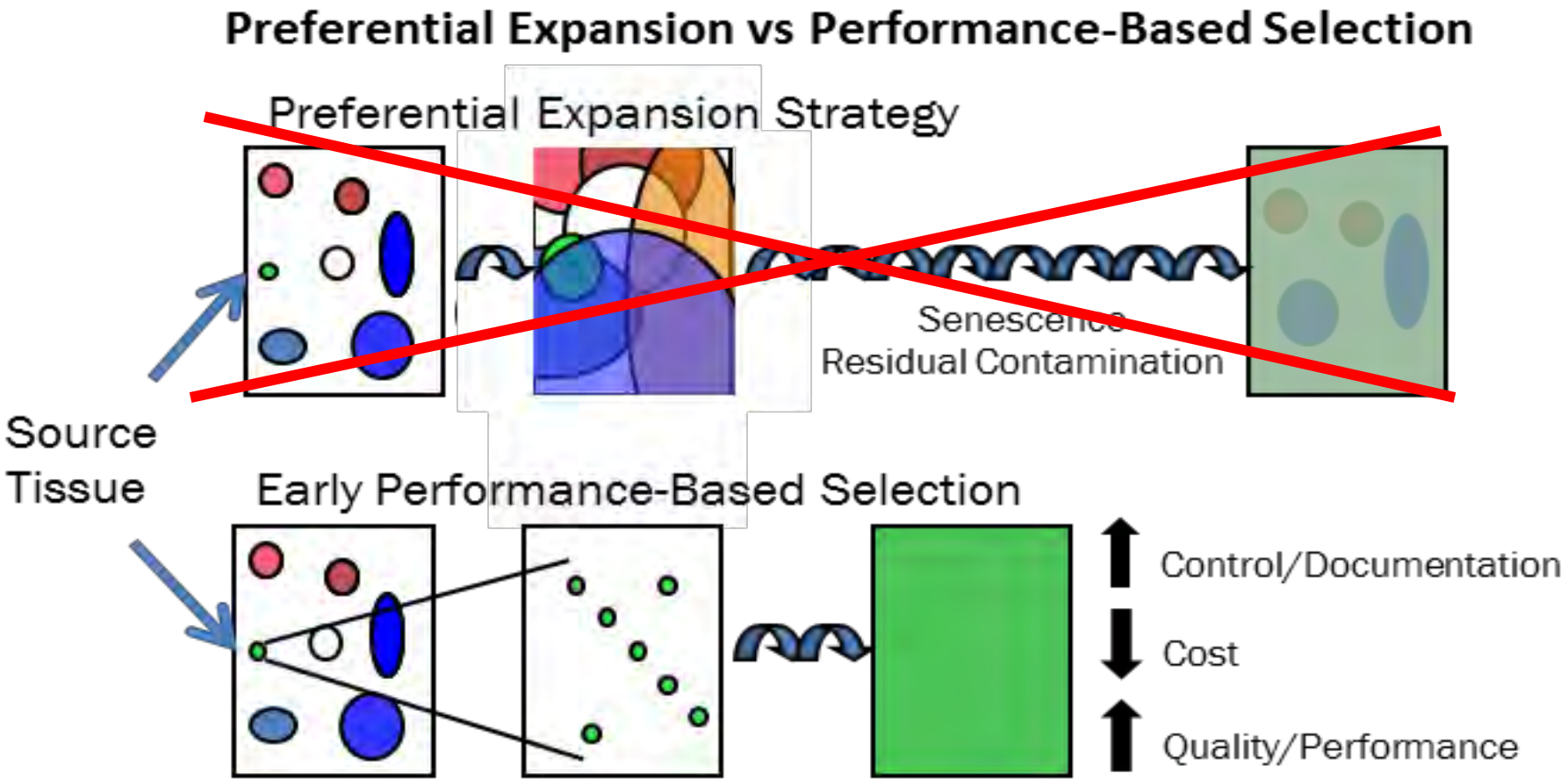




Single Cell Picking

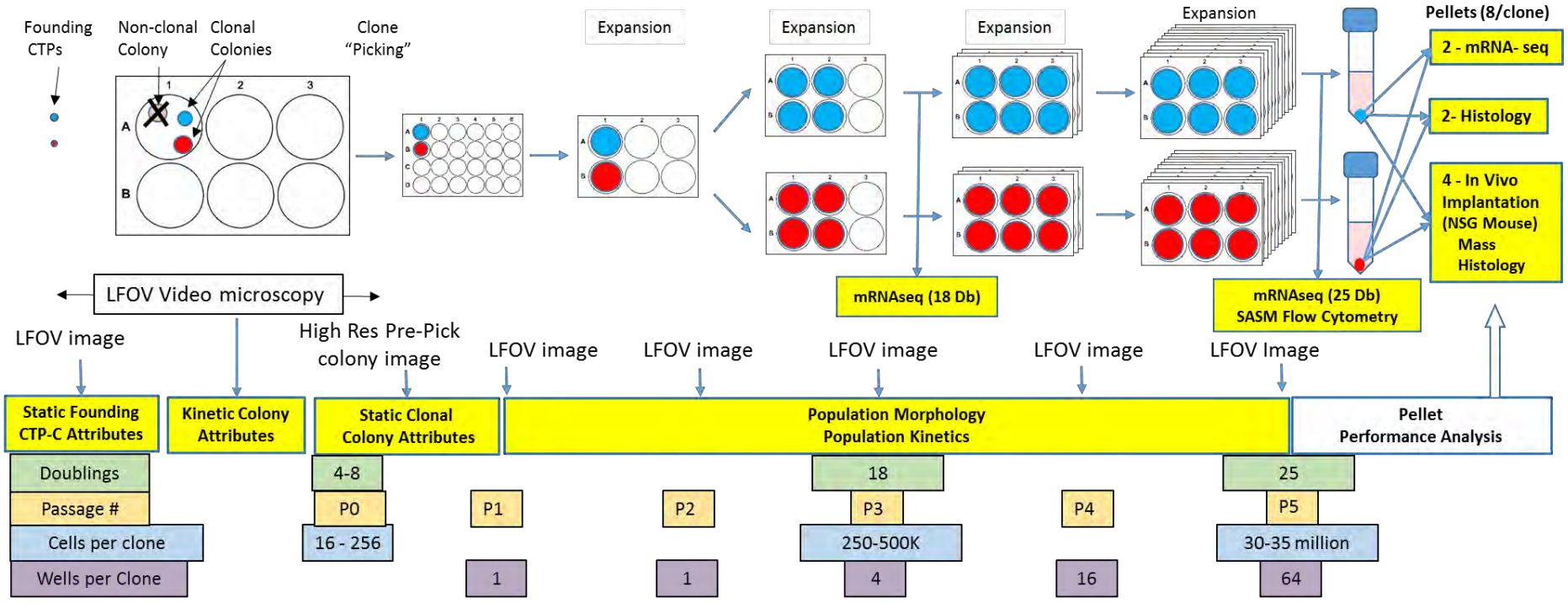


Moving to Performance-Based Selection



Moving to Performance-Based Selection

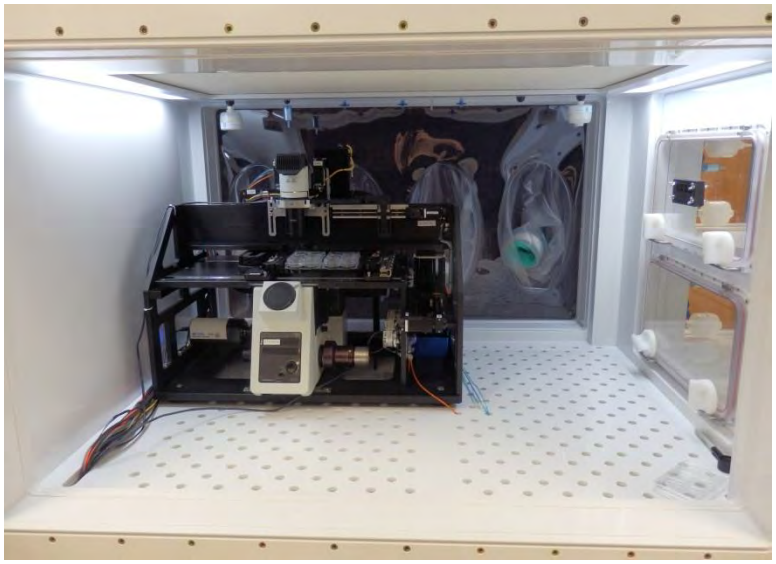
Connecting Early Attributes with Downstream Performance

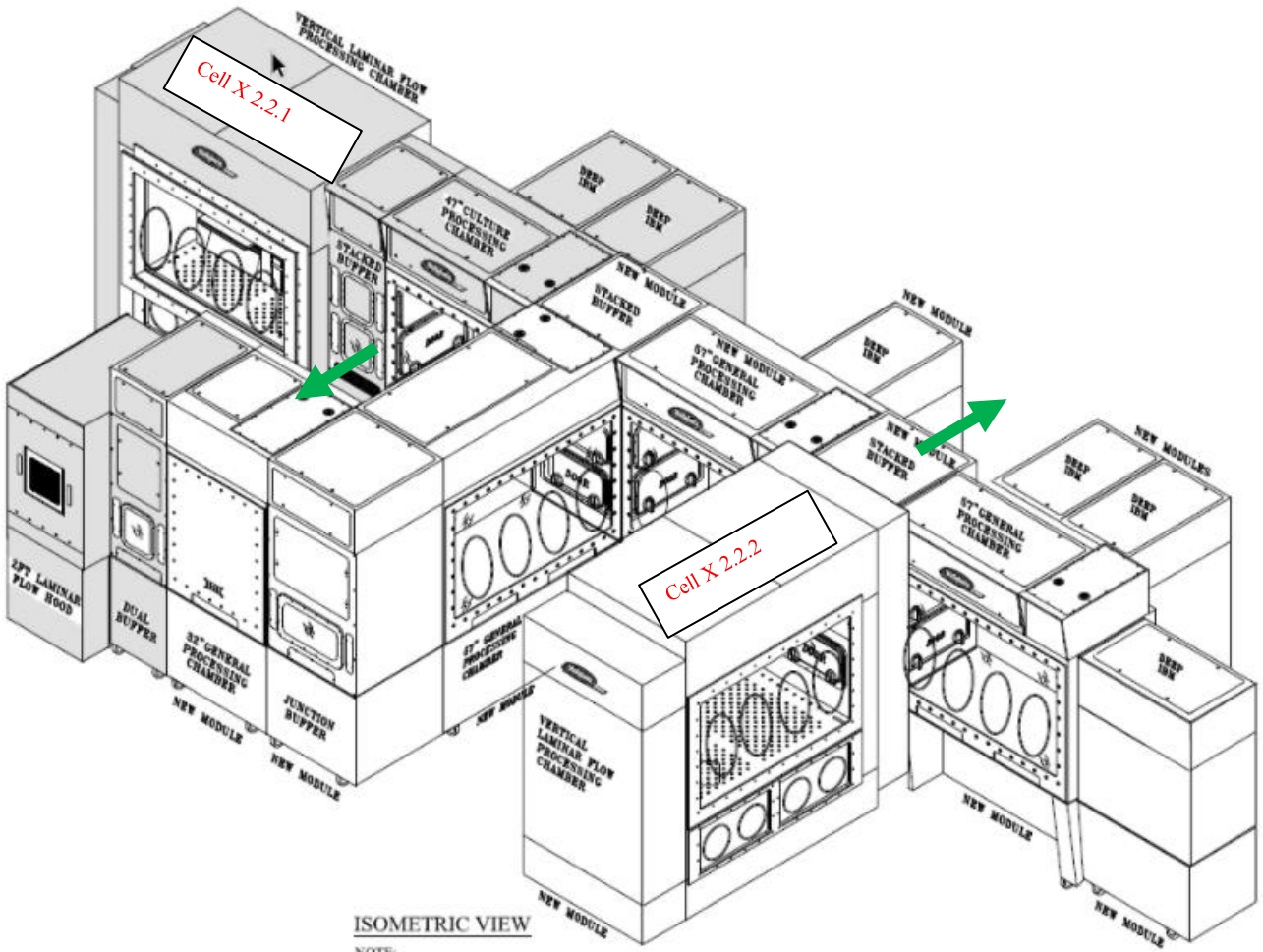


Overview of Experimental Design and Data Collection. The "Life Cycle" of an individual cartilage-derived CTP-C through clonal expansion and analysis at 18 Db or 25 Db, through pellet generation and analysis is outlined. The data sets contributing to analysis at each stage are identified (Yellow Boxes). Sources of cells and data elements are tracked (Blue Arrows). Logistics of doublings, passage #, cells per clone and the number of wells required at each step are itemized.

Cell X Xvivo System Module

Biospherix





ISOMETRIC VIEW

NOTE:
1. MATERIAL OF CONSTRUCTION: POLYPROPYLENE

REV NO	DESCRIPTION	DR BY	DATE
2	Added Adapter Plate to Mod 15	NLE	09/08/17
1	SYSTEM UPGRADE	NLE	08/23/17

BIOSPHERIX MEDICAL, LTD.

X2 CLOSED INCUBATION SYSTEM

ISOMETRIC VIEW

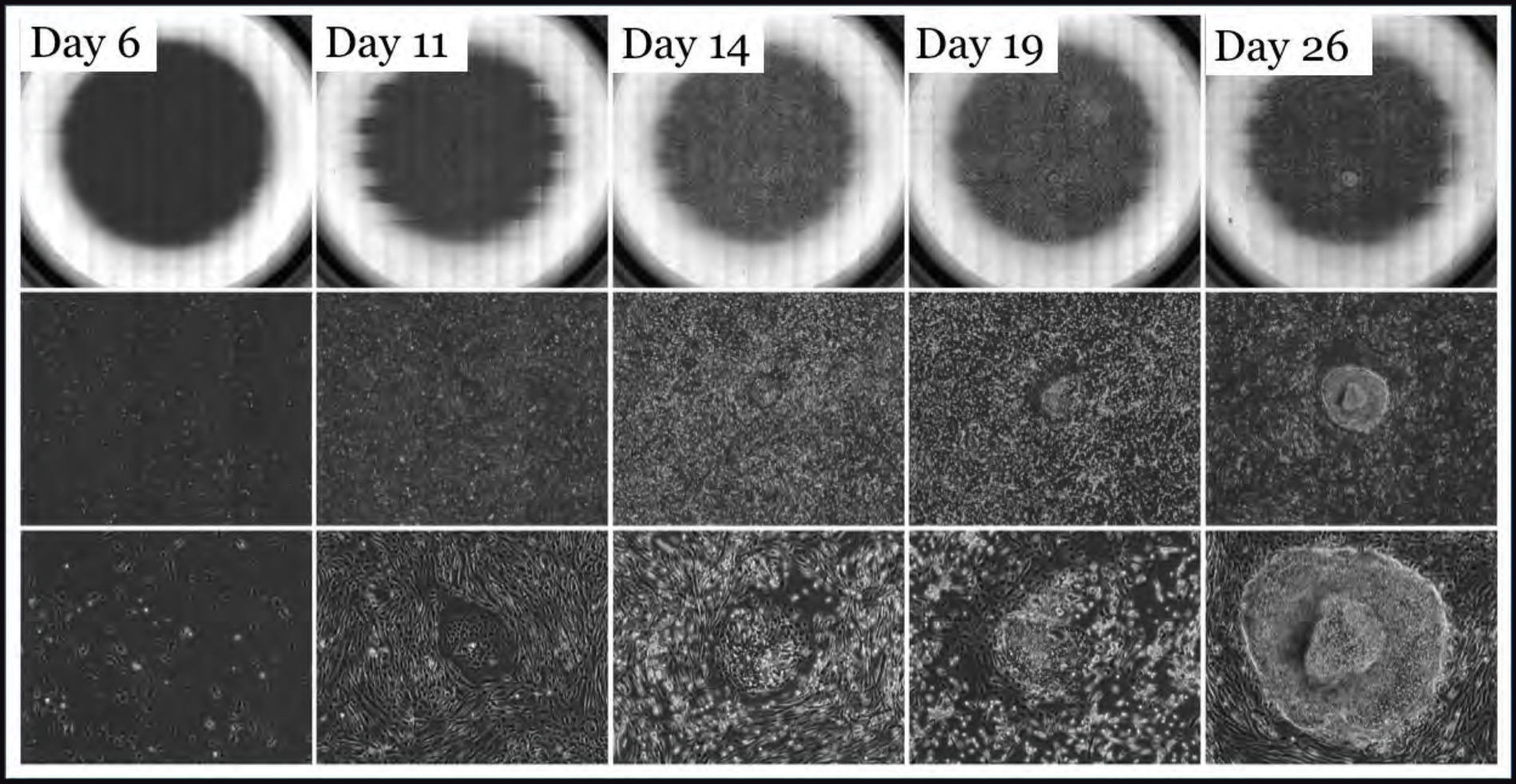
UNIVERSITY OF IOWA

BioSpherix Medical, Ltd.

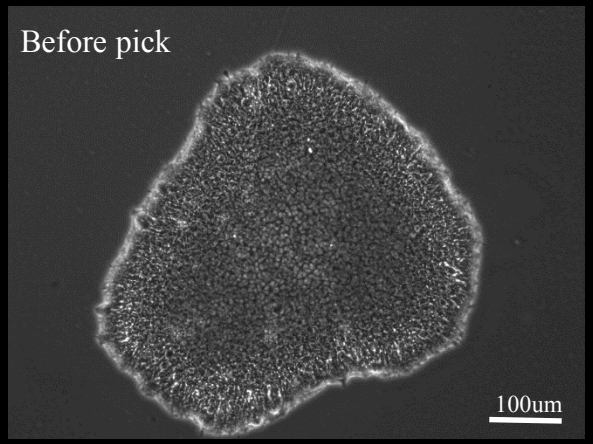
PO Box 249, Parish, NY 13131
25 Union Street, Parish, NY 13131
Phone: (315)625-8025 Fax: (315)625-8098
Toll Free US/CANADA 800-441-3414
www.BioSpherixMedical.com support@biospherix.com(email)

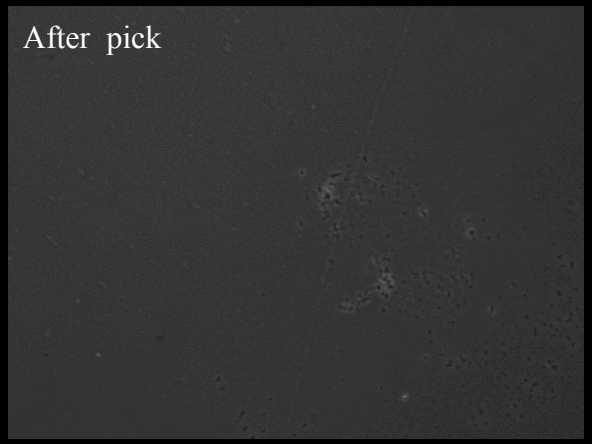
ENG. APPR.	MFG. APPR.	QC APPR.	CHKD. BY	DR. BY	SCALE	SHEET	DWG. NO.
				NLE	NONE		X2-CIS-IS01
DATE	DATE	DATE	DATE	DATE 11/07/16	UIN X2-110716-1-01	REV NO. 2	

Performance-Based iPS Clone Selection

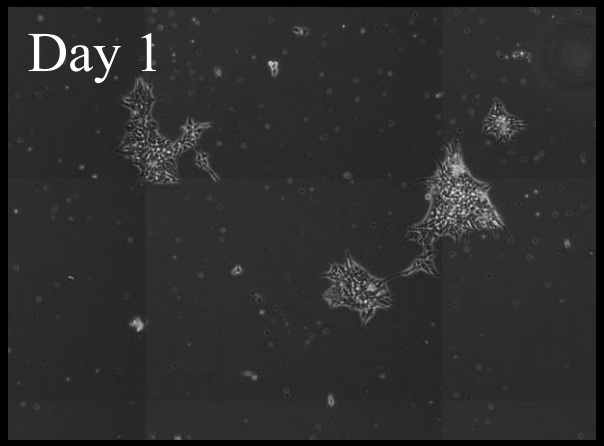


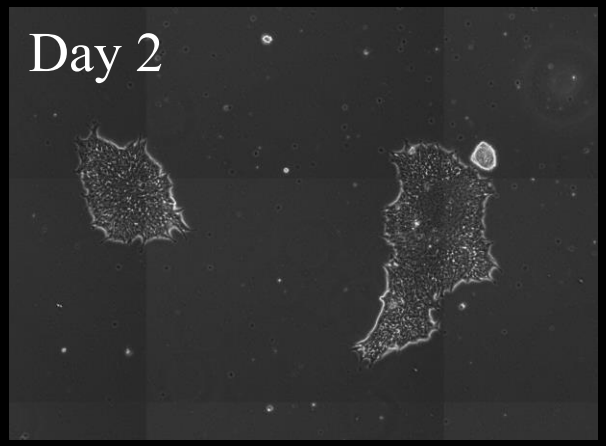
iPS Performance-Based Passaging

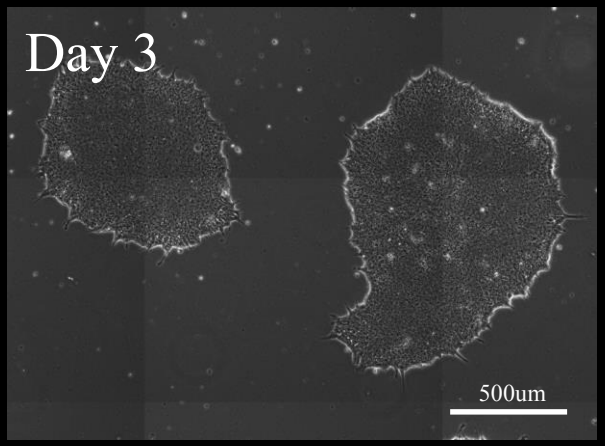




Pick parameters	Values
Tip ID	533 um
Aspirate height off bottom	16.99um
Aspirate volume	200 ul
Aspiration flow rate	150 ul/sec
Dispense height	20 um
Dispense volume	200 ul
Dispense flow rate	100 ul/sec

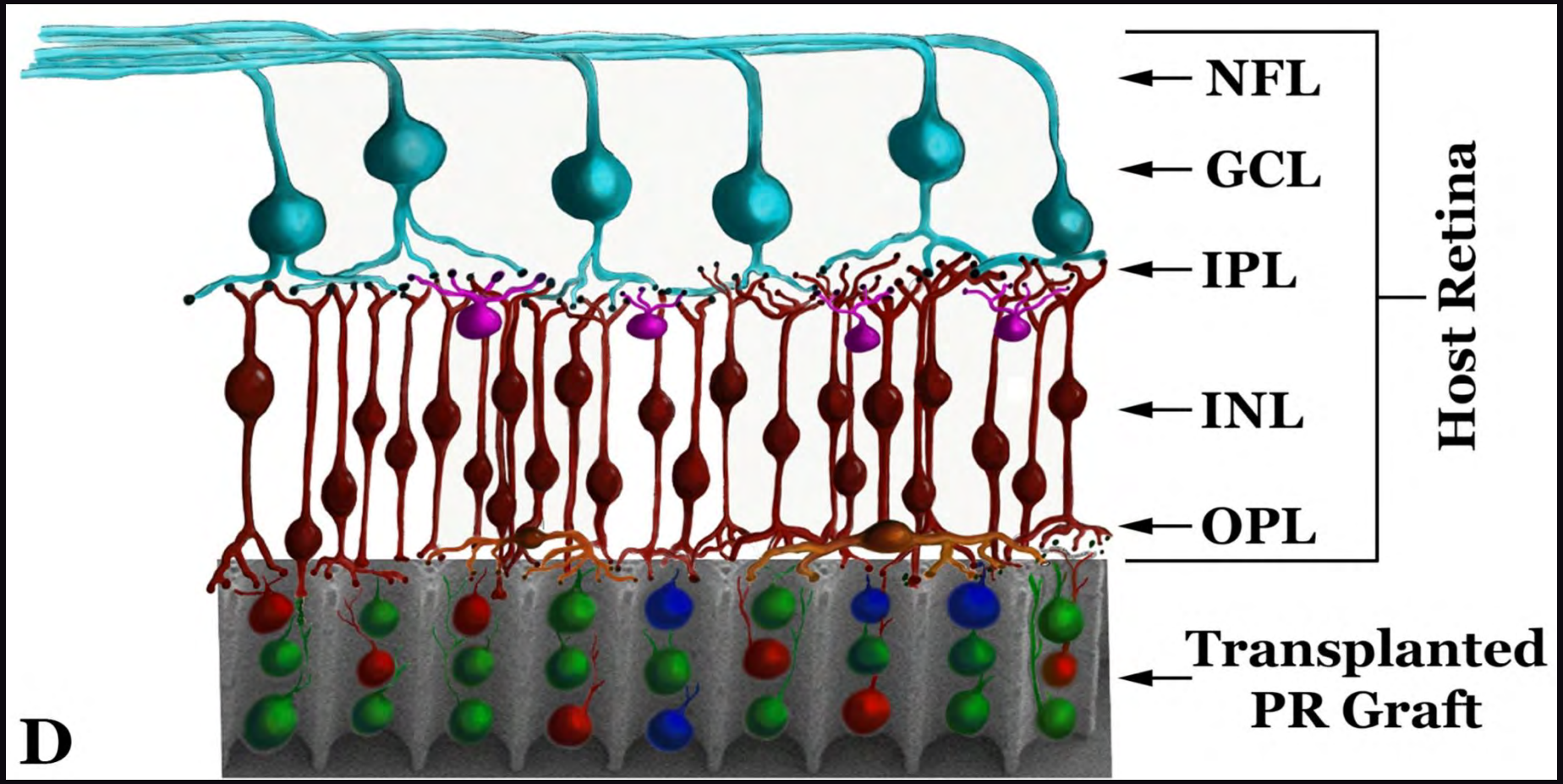


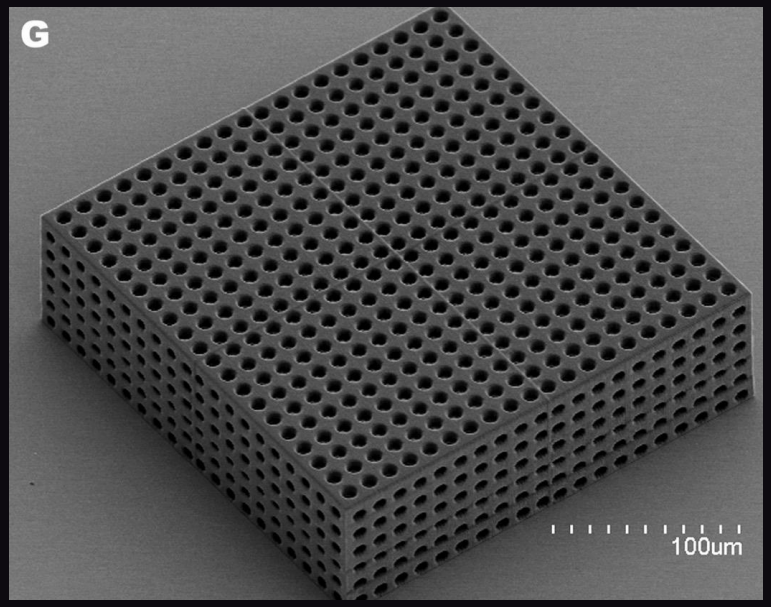


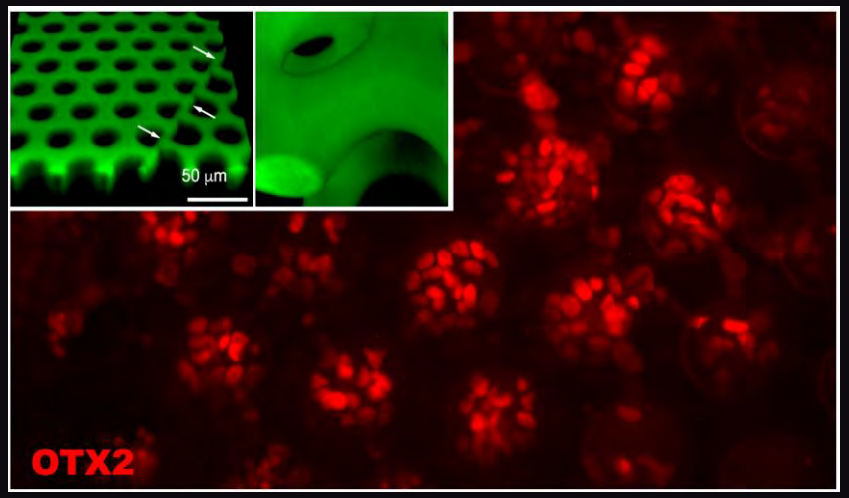


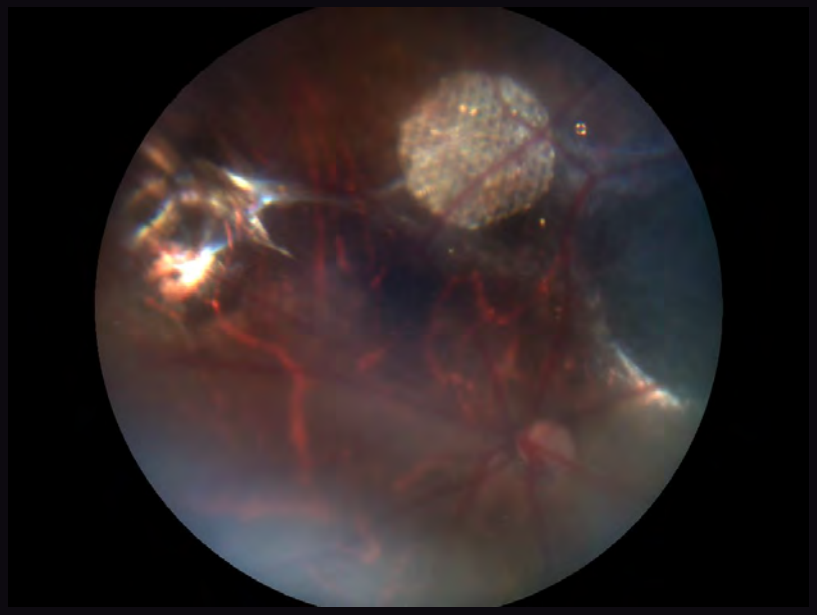
Neural Organoid Quality Attributes

Wiley, et al., *Scientific Reports*, 2016


NFL
GCL
IPL
INL
OPL
Host Retina
Transplanted
PR Graft
D

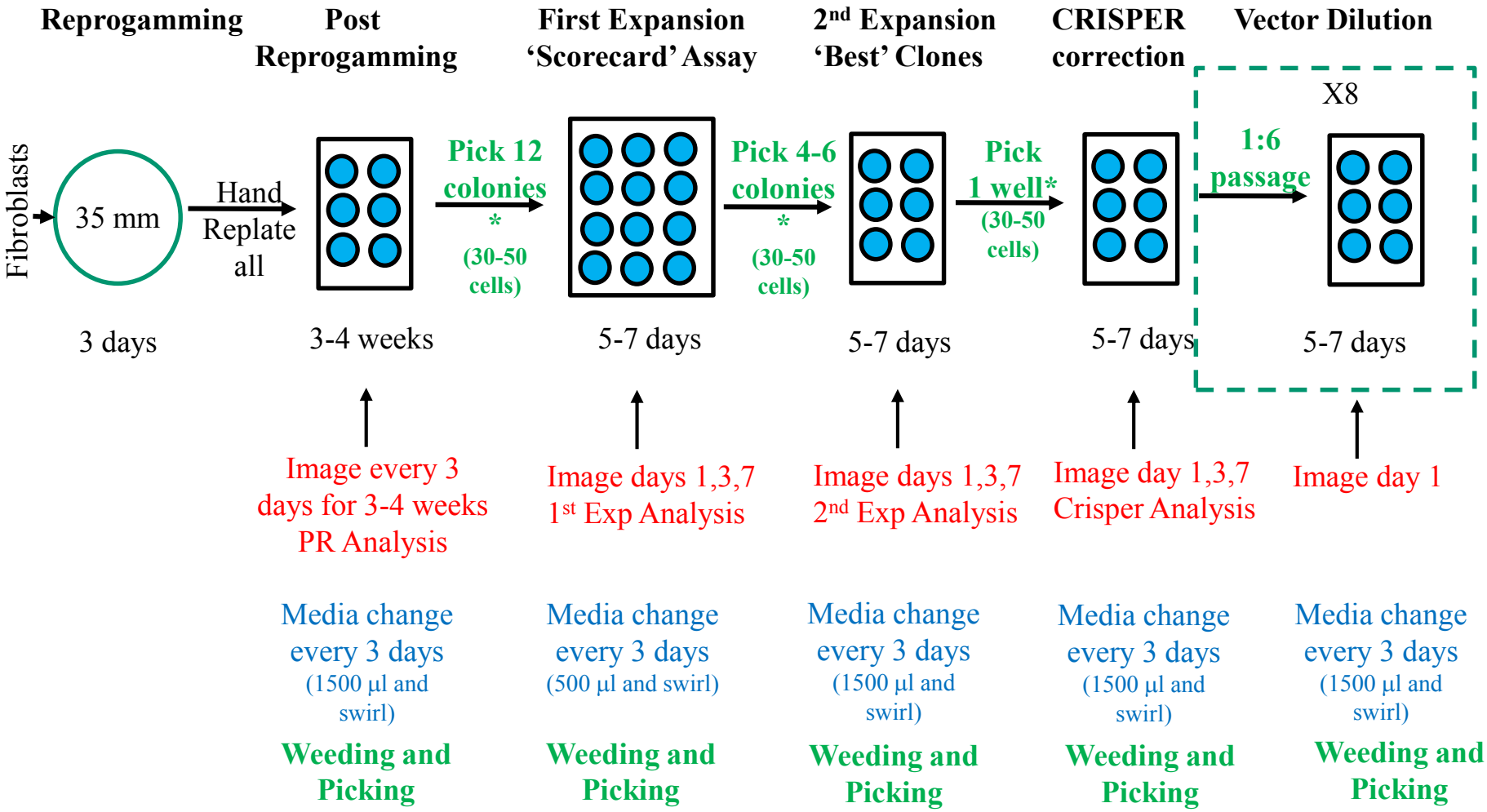

G
100um


50 µm
OTX2



iPS Cells

Selection - Expansion - Correction



Muschler Lab

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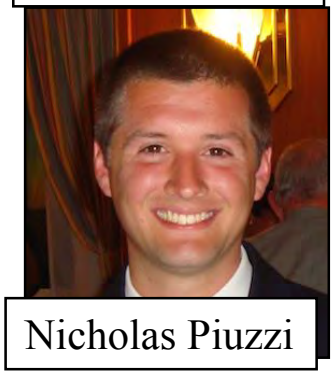
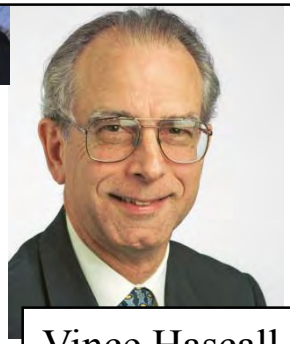


Nicholas Piuzzi

Ed Kwee

Venkata Mantripragada



Vince Hascall

Eben Alsberg

Maha Qadan

Alan Sumski

Selvam Selvaanish

Terri Zachos

Bob Sah

Cell X™ Robot

Colonyze ™ Software

Parker

Cleveland Clinic

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Lisa Mosley Foundation

Clinical Precision Bioprocessing (CPB) Team

Cleveland Clinic – Parker Hannifin - Biospherix

Parker Hannifin

- Precision Movement
- Precision Fluidics
- Automation Platforms
- Design Engineering
- System Integration

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- Large Field of View Imaging
- Quantitative Image Analysis
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- Human Cell Sourcing
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- GMP Manufacturing Enclosures
- Custom Modularity

Integrated Tools and Solutions

Human Cell Sourcing	Cell Processing	Quantitative Assay	Process Controls	Environmental Controls	Process Documentation

Potency | Efficacy | Repeatability | Reproducibility

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