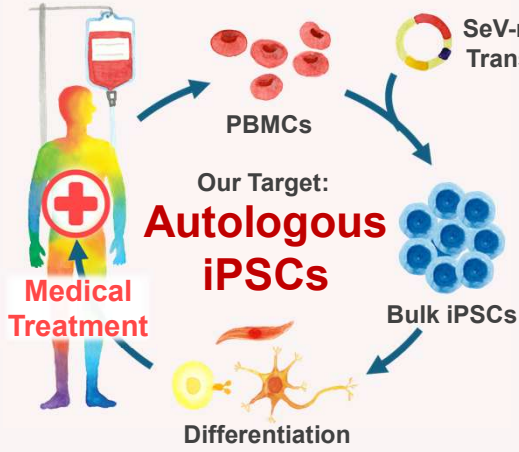
# Field-Level Image Morphomics Enable Scalable Frameworks for Non-Invasive Quality Control of Human iPSC Cultures

Moe OKAMOTO, Eiko SHIMIZU, Risa HIBINO, Yuri NAKATANI, Masayoshi TSUKAHARA
CiRA Foundation Research & Development Center. CONTACT: moe.okamoto@cira-foundation.or.jp

CiRA Foundation

myIPS®

## Background : Autologous iPSC Manufacturing - my iPS® Project -






**Current Achievement**

We have generated research-grade iPSCs from healthy donor whole blood using a **fully closed, automated** cell-processing system.

Shimizu et al., *Cytotherapy* (2026).

**my iPS® Project: What Benefits Could it Bring?**

- ◆Lower Immune Rejection Risk
- ◆Lower Manufacturing Costs
- ◆Patient-specific Therapies
- ◆More Consistent Production

**Broader Access to Regenerative Therapies**

## Question

**How Can we *Tailor* Differentiation Conditions to Individual iPSC Cultures?**



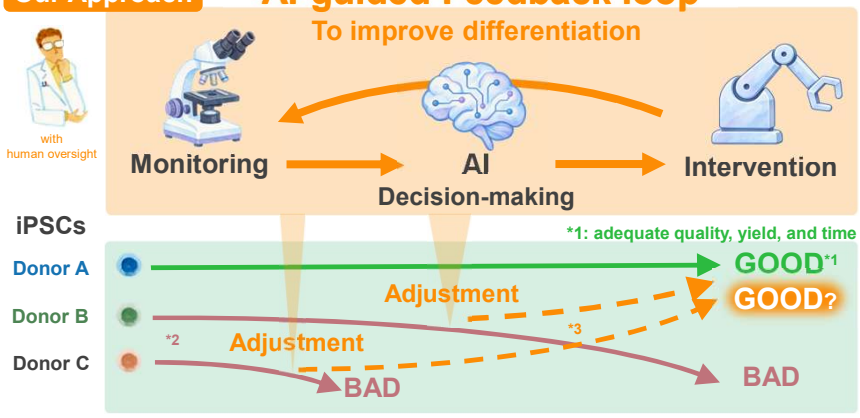
**Our starting material**
Patient-specific Bulk iPSC cultures without clonal selection

## Proposed Approach

### - AI-Guided Adaptive Differentiation Using Non-invasive Monitoring -

**Challenge** Differentiation responses vary across iPSC cultures.

**Our Approach** **AI-guided Feedback loop**




*2 Red solid: current path for B & C *3: Orange dashed: a possible course correction

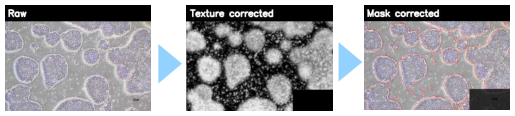
## Preliminary Studies

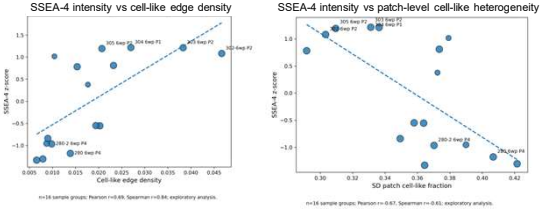
**For AI model training, we investigated relationships between cell images and flow cytometry measurements.**

### iPSCs maintenance cultures

Representative iPSC culture images showing differences in cell-like edge density

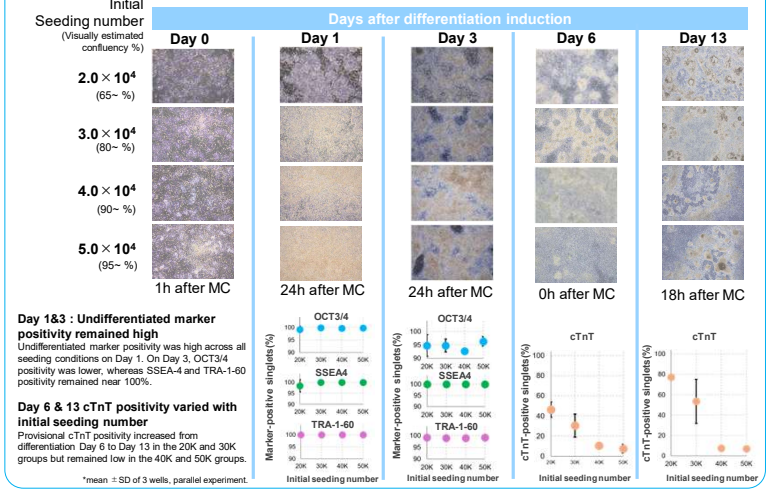
Cell-like edge density was calculated as: $\text{Cell–like edge density} = \frac{\sum P_{i,\text{external}}}{\sum A_i}$ [pixel$^{-1}$]




Across 16 iPSC sample groups, image-derived features were associated with SSEA-4 fluorescence intensity. Cell-like edge density showed a positive correlation (Spearman ρ = 0.84), while variability in patch-level cell-like coverage showed a negative correlation (ρ = −0.61).

### Cardiomyocytes Differentiation




**Day 1&3 : Undifferentiated marker positivity remained high**
Undifferentiated marker positivity was high across all seeding conditions on Day 1. On Day 3, OCT3/4 positivity was lower, whereas SSEA-4 and TRA-1-60 positivity remained near 100%.

**Day 6 & 13 cTnT positivity varied with initial seeding number**
Provisional cTnT positivity increased from differentiation Day 6 to Day 13 in the 20K and 30K groups but remained low in the 40K and 50K groups.

*mean ± SD of 3 wells, parallel experiment.

## Discussion

**Image-based iPSC assessment:**
Preliminary associations between image-derived features and pluripotency marker expression suggest potential for noninvasive assessment. Reliable identification of cell regions and exclusion of dead cells and debris require further validation.

**Differentiation dynamics:**
Differences in cTnT-positive cell fractions among initial seeding densities were observed at the earlier sampled time point. Whether these differences can be reduced through interventions during differentiation remains to be tested.

**Next steps:**
We will compare interventions at selected time points with a no-intervention control to evaluate their effects on differentiation outcomes. These experiments will support future AI-guided protocol optimization with our collaborators.

## Acknowledgements

This research was supported by AMED under Grant Number JP26bm1323001.We would also like to express our sincere gratitude for the generous donations made to the CiRA Foundation, which greatly contributed to this research.

**COI:**
**Conflict of Interest: The authors declare no conflicts of interest.**

