Exploring human pluritotent stem cell heterogeneity using a multi-scale imaging and informatics pipeline

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Disclosures: None

Acknowledgments

The promise of human pluripotent cells

hPS cells

Bone Marrow

Nerve Cells

Insulin-producing Cells

Heart Muscle Cells

Directed differentiation is inefficient and variable.

Cellular replacement therapy

Human disease modeling

Drug testing
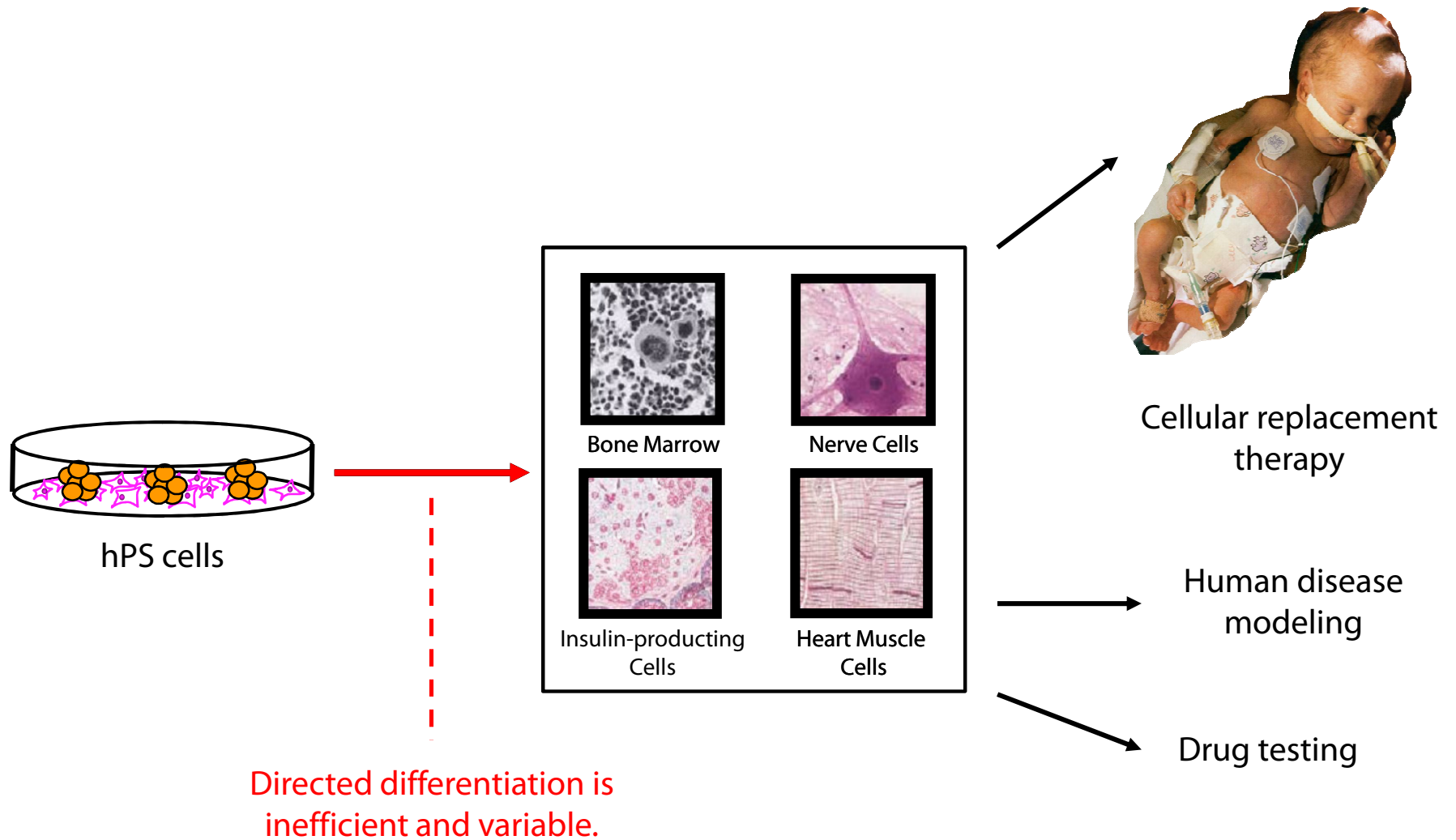
Directed differentiation is unreliable due to noisy input

hPS cells are highly heterogeneous

Bone Marrow

Nerve Cells

Heart Muscle Cells
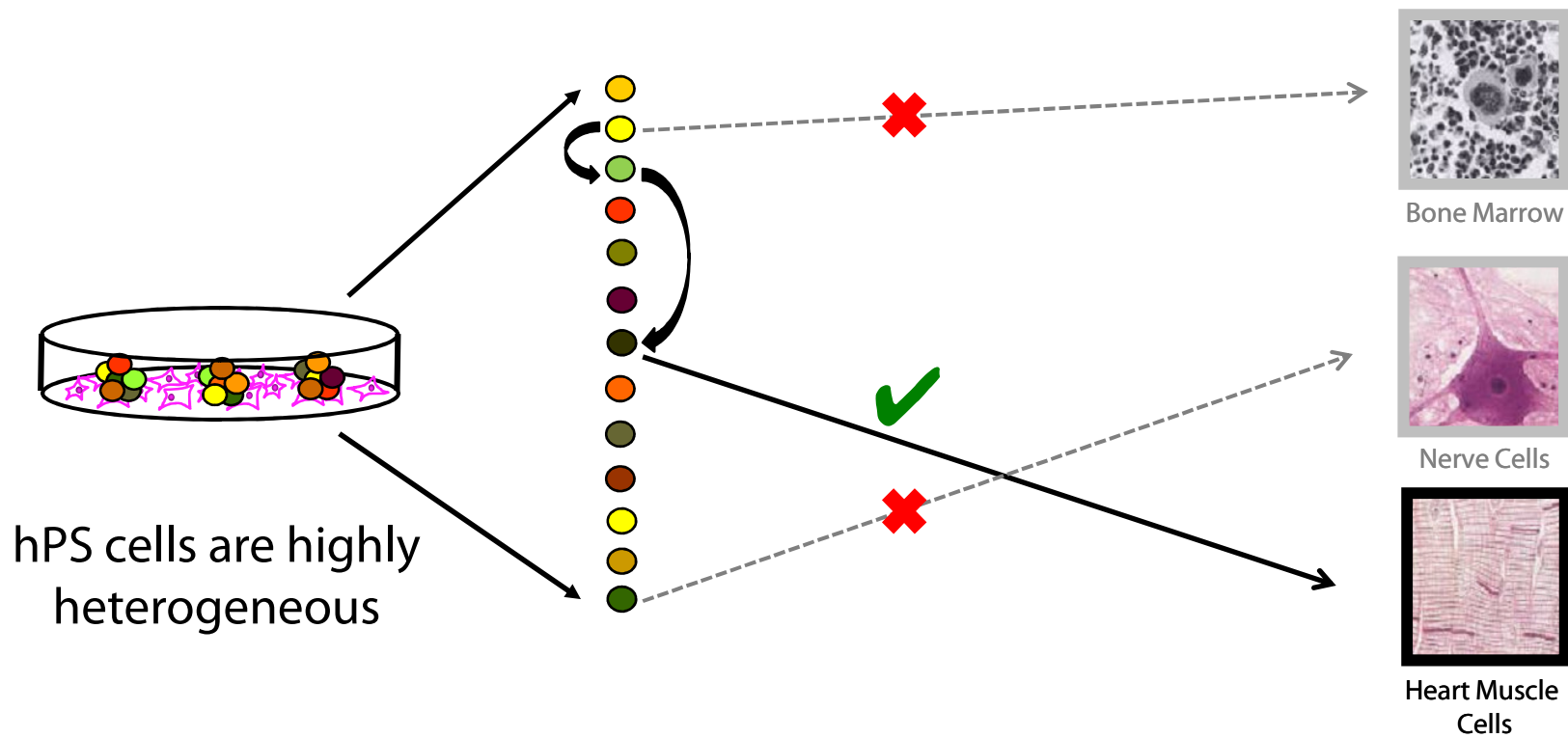
Differentiation to Definitive Endoderm (DE) and subsequent derivatives is progressively inefficient

in vitro

(Nodal, WNT) Activin A, CHIR99021		(Nodal) Activin A			
POU5F1 (Oct4)	T (Brachyury) (Mesendoderm)		SOX17 FOXA2 **(Definitive Endoderm)**		INS
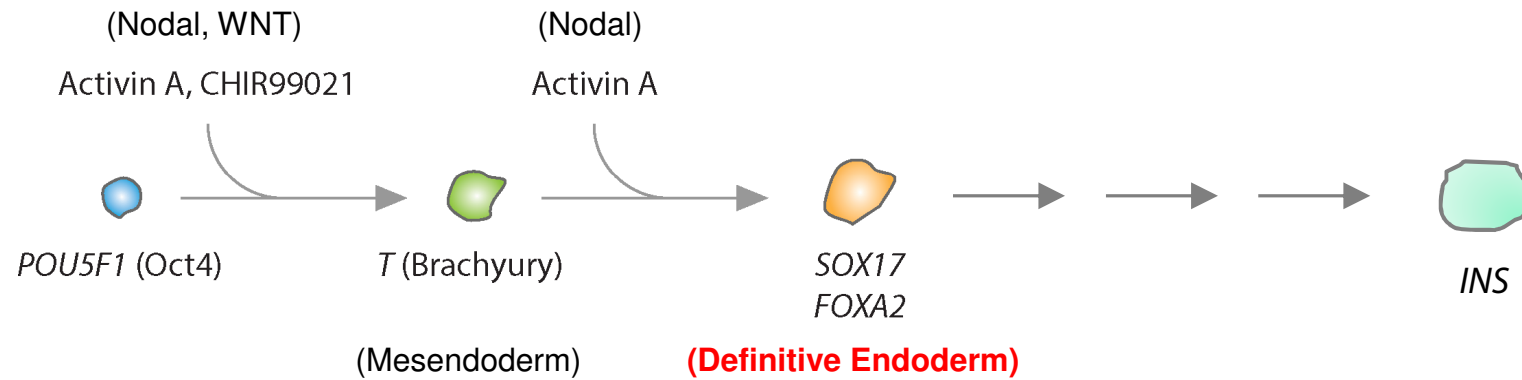
Efficiency:	90%	80%	50%	25%	10%

Populations of human ESCs show heterogeneity in their differentiation status

Oct4 Sox17 Foxa2
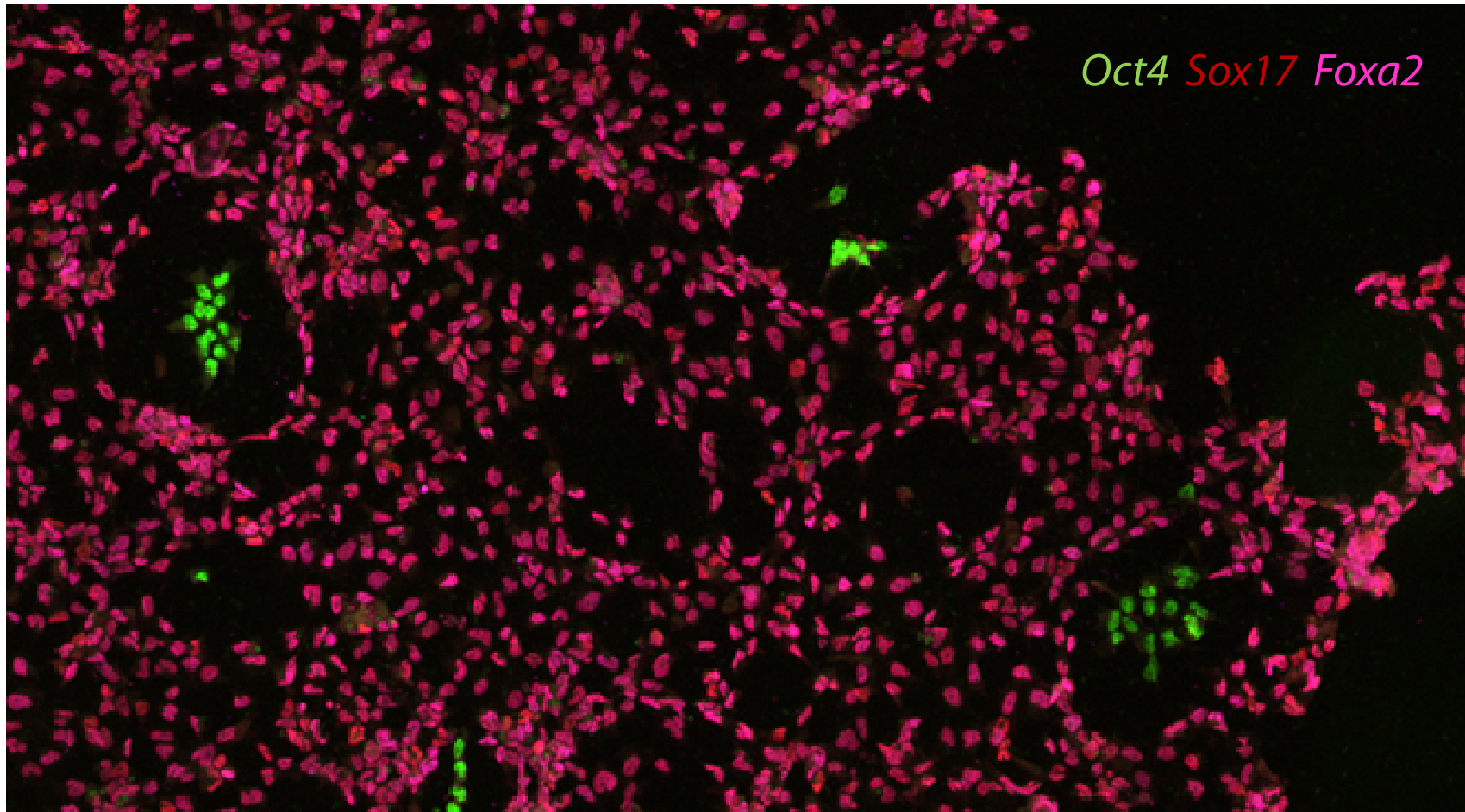
Human H9 ESCs, Day 4

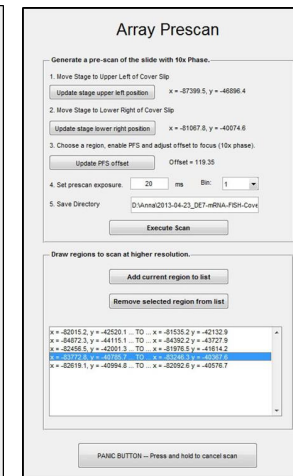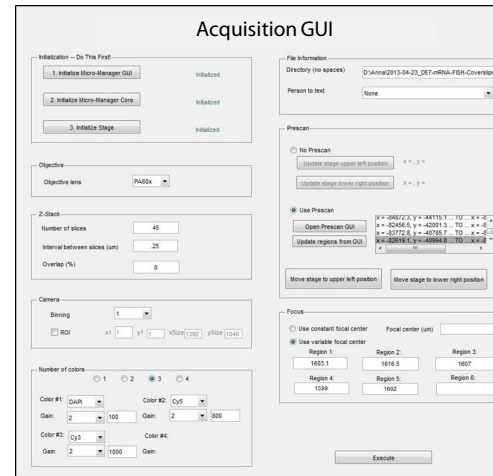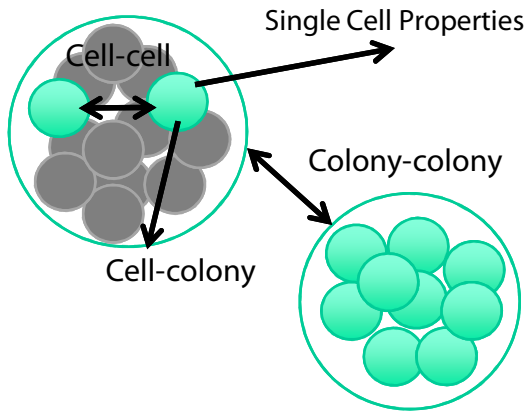# Immediate vs. delayed induction of differentiation influences the degree of heterogeneity
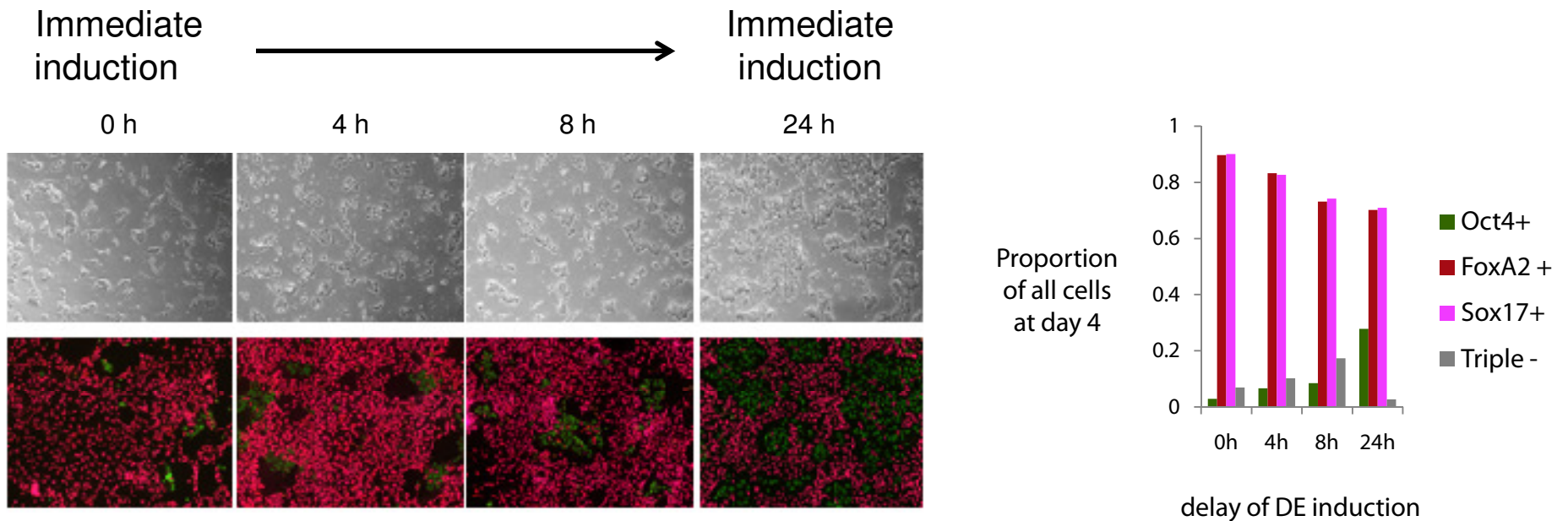
What is the origin of heterogeneity during DE differentiation?

How are spatial organization and differentiation status related?

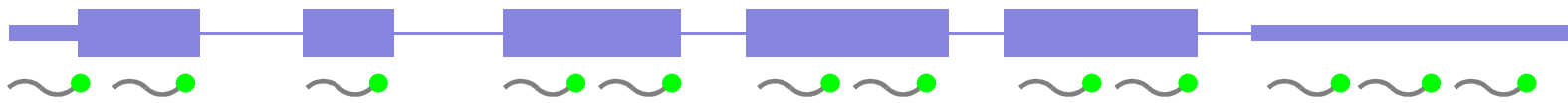# Multi-scale imaging and informatics pipeline for in situ pluripotent stem cell analysis
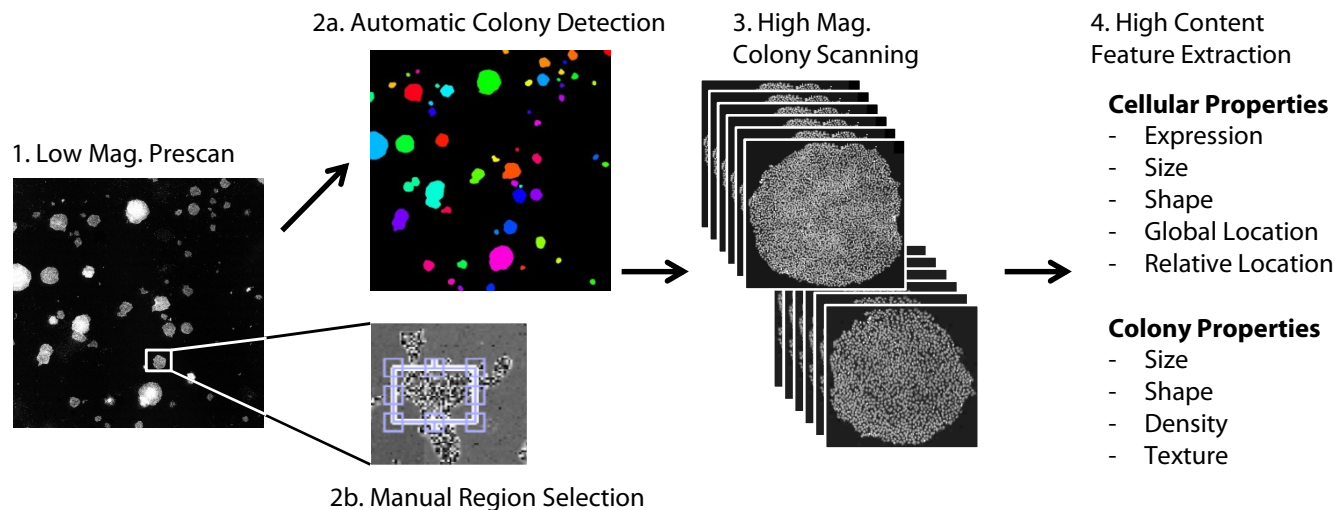
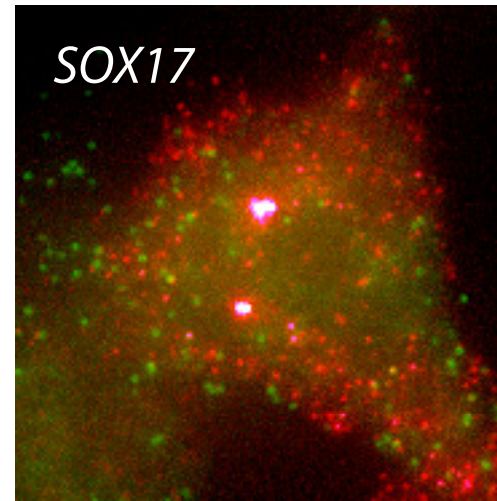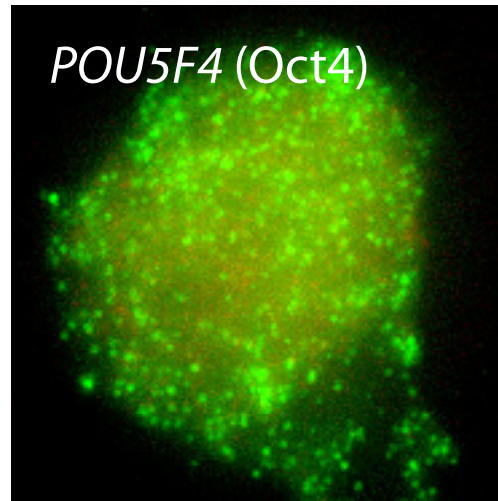# Single-molecule mRNA FISH quantifies expression of differentiation markers

POU5F4 (Oct4)

SOX17

Single-molecule FISH allows robust quantification of expression of differentiation markers
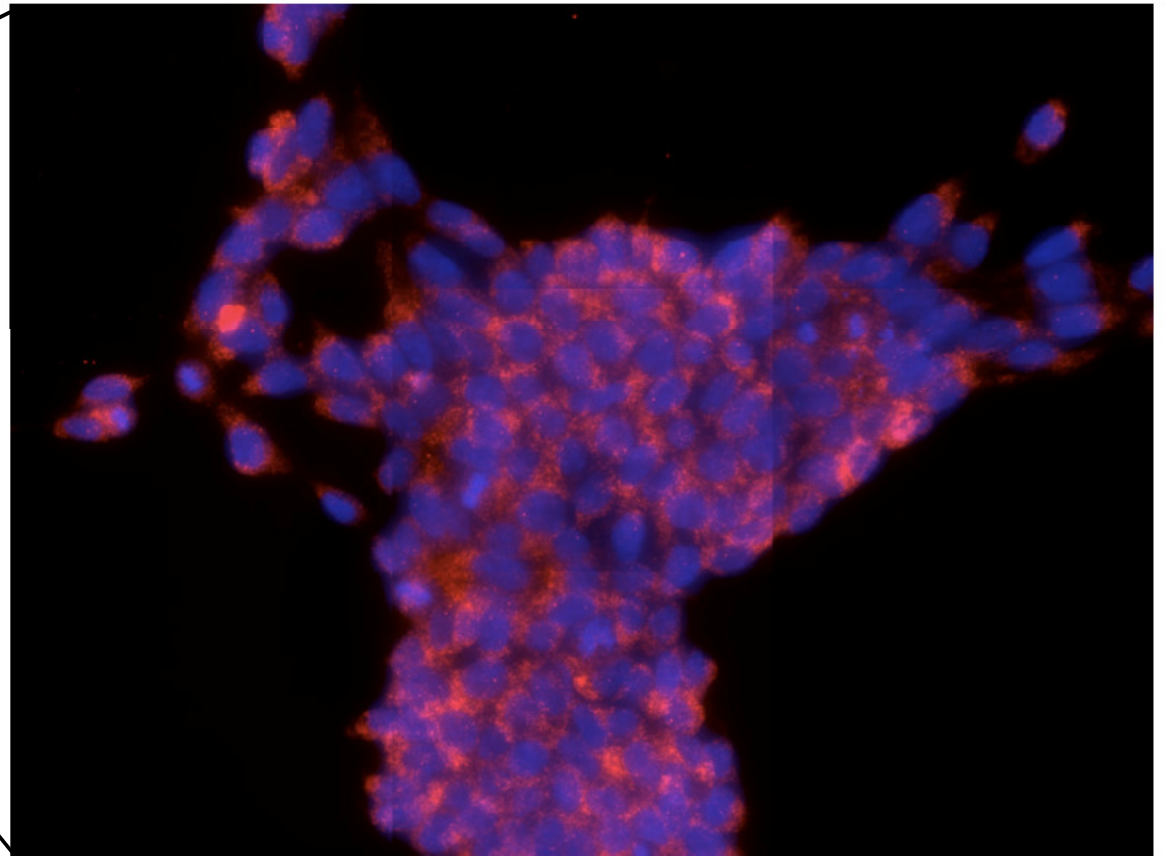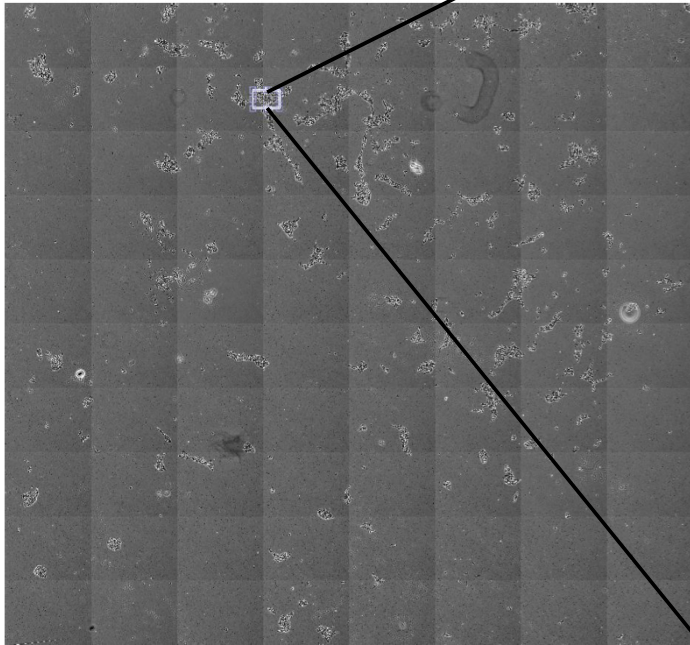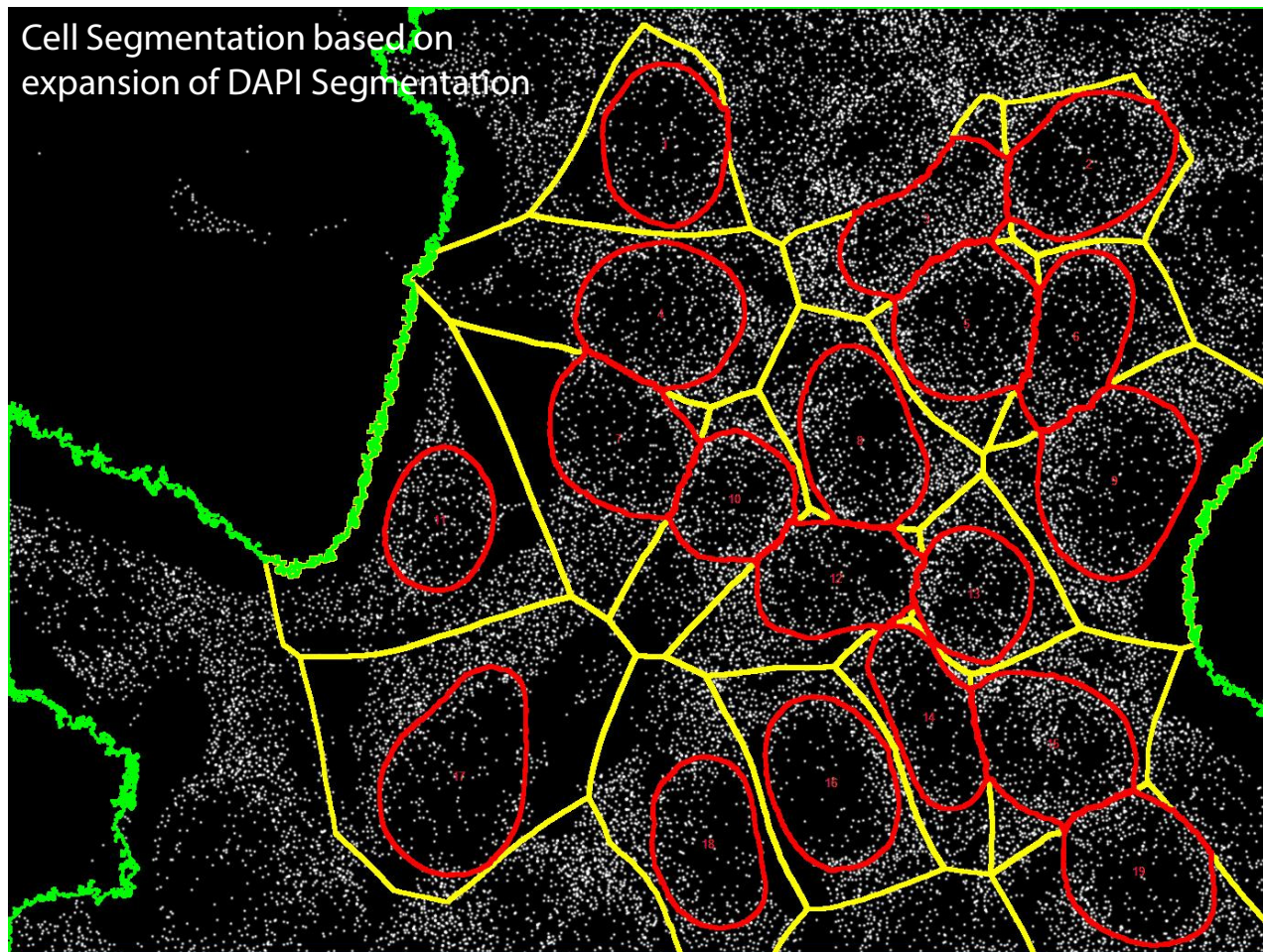

467 Oct4 transcripts   270 *SOX17* transcripts

Single-molecule FISH allows robust quantification of expression of differentiation markers

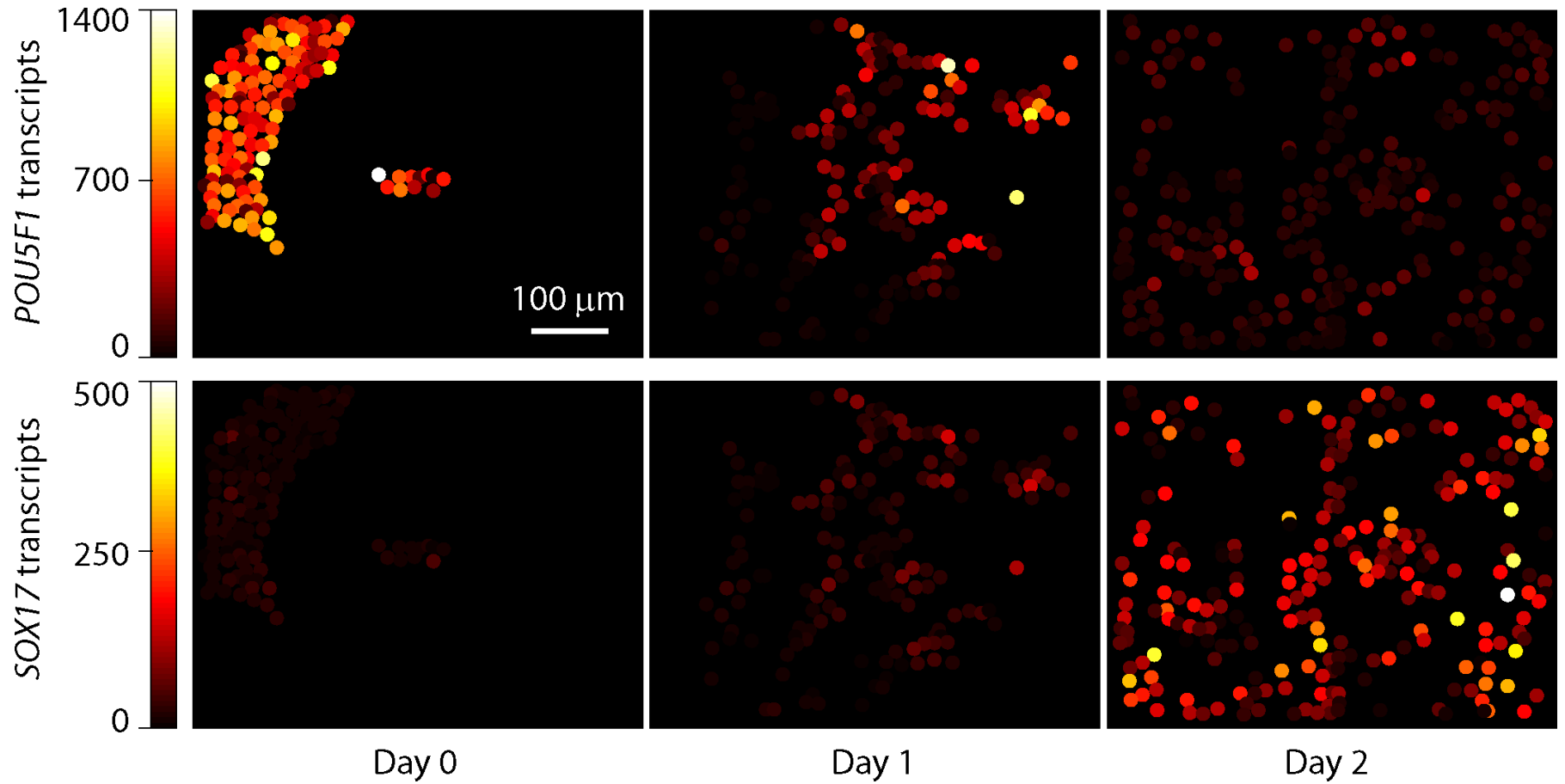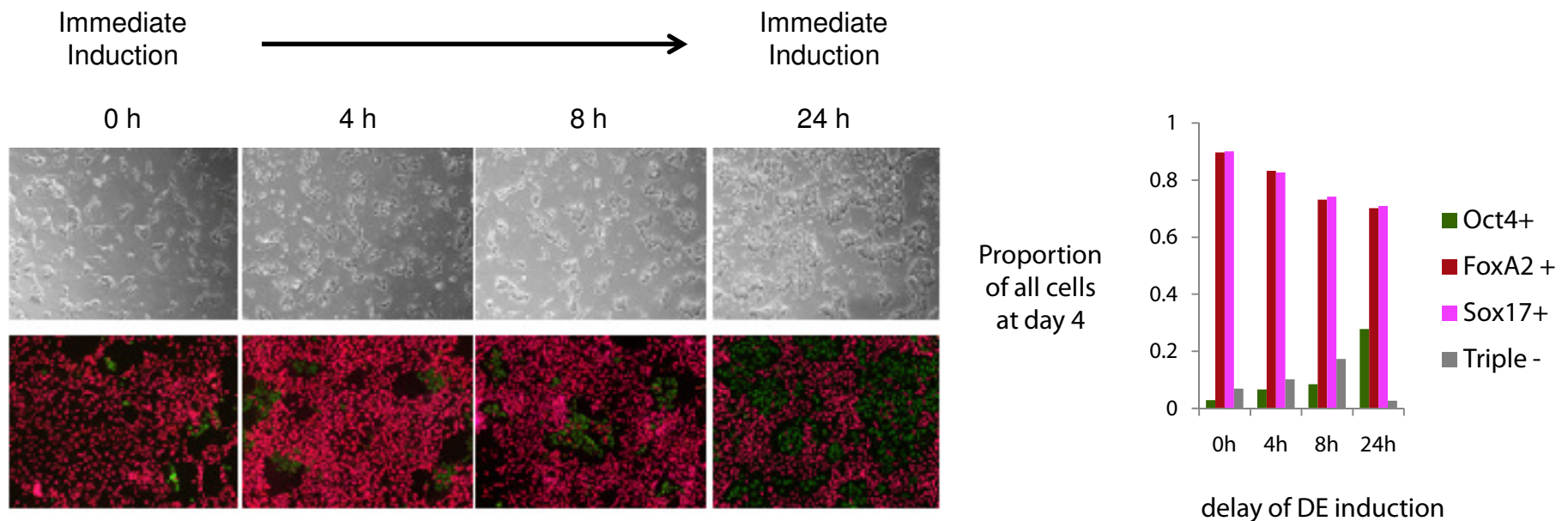# Single-molecule FISH allows robust quantification of expression of differentiation markers

Cell Segmentation based on expansion of DAPI Segmentation

Processed Data Visualized

Immediate vs. delayed induction of differentiation influences the degree of heterogeneity

Immediate Induction → Immediate Induction

0 h   4 h   8 h   24 h

Proportion of all cells at day 4

- Oct4+
- FoxA2 +
- Sox17+
- Triple -

delay of DE induction

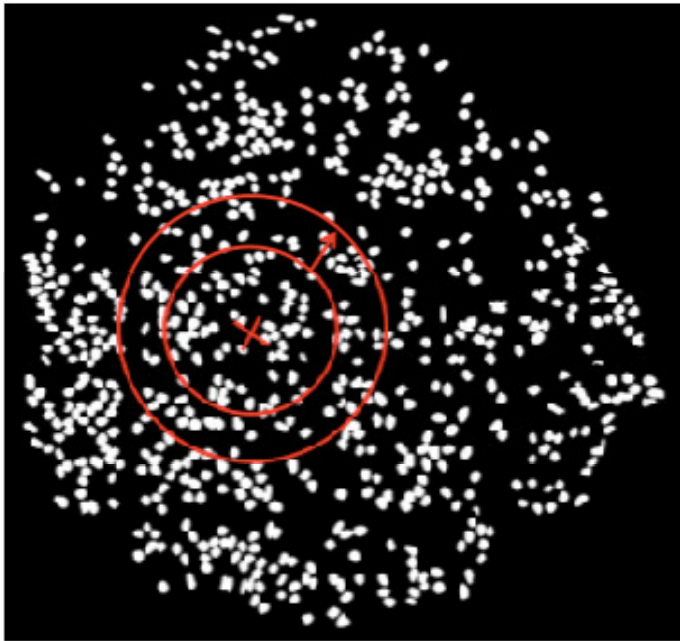What is the origin of heterogeneity during DE differentiation?

How are spatial organization and differentiation status related?

Delayed induction of DE differentiation increases heterogeneity
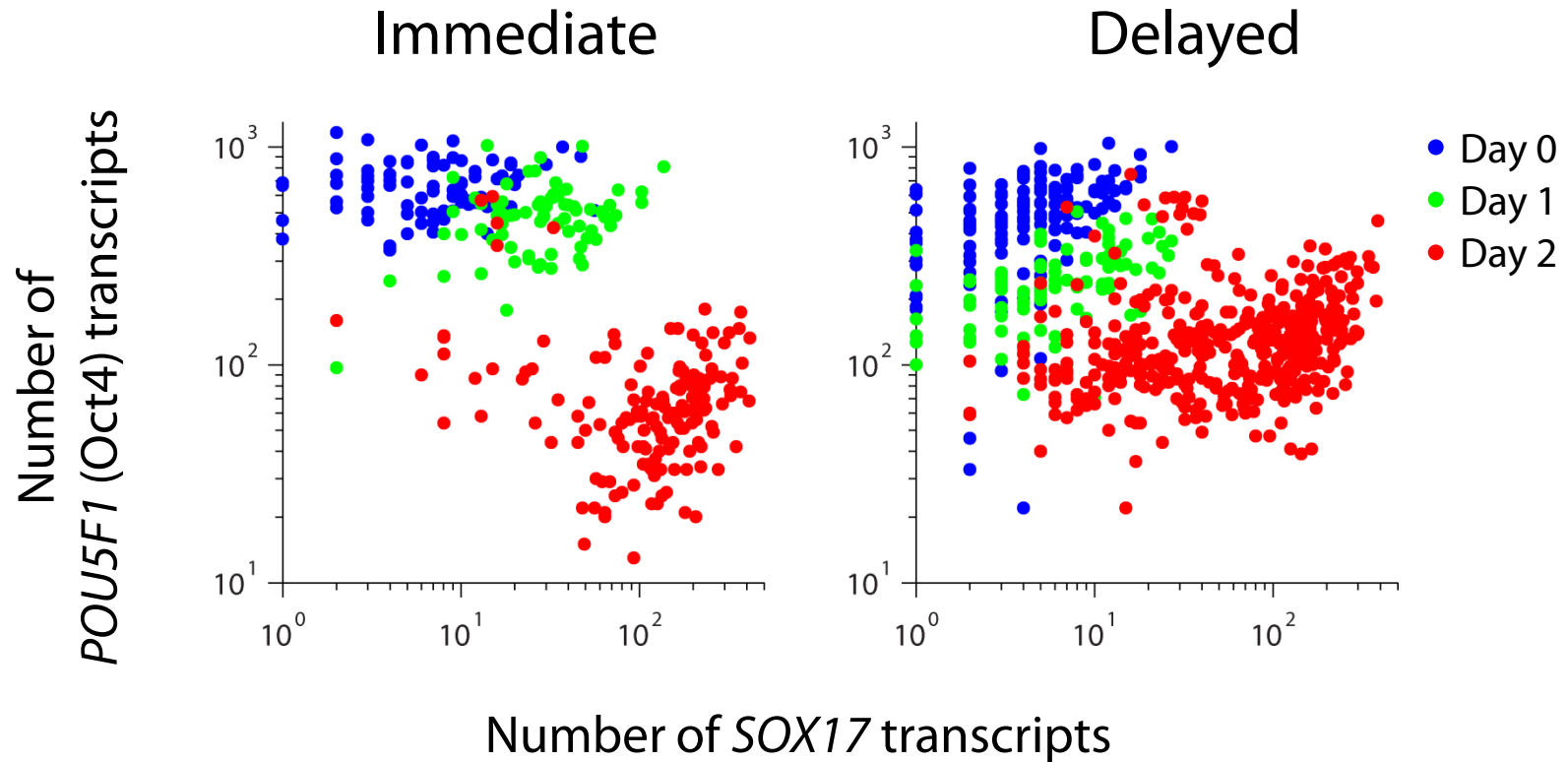
Spatial statistics of cell features

- If a cellular feature distribution is uniform and continuous, then it may be modeled as a spatial poisson process.
- Thus, if a random point is chosen in a colony and a circle of radius r is extended outward, the probability of k cells belonging to a given cell state contained in the circle, X(S):

$$P\{X(S) = k\} = \frac{[\lambda \pi r^2]^k \, e^{-\lambda \pi r^2}}{k!}.$$

- The (observed) distribution of the arrival times of cells with a specific feature into the expanding circle is statistically compared (K-S test) against an exponential distribution (random).

mRNA sequencing defines intermediate stages and expression diversity during DE differentiation
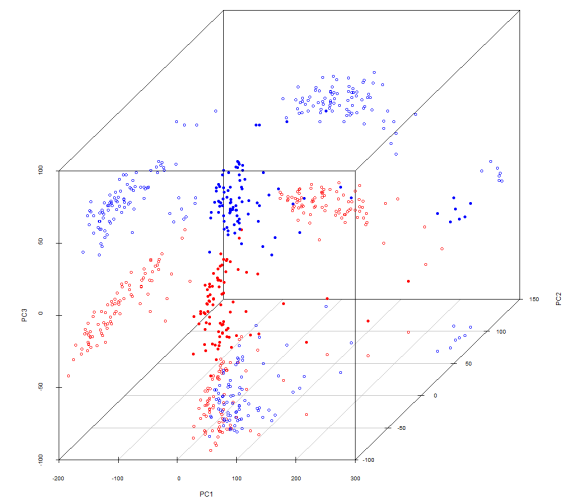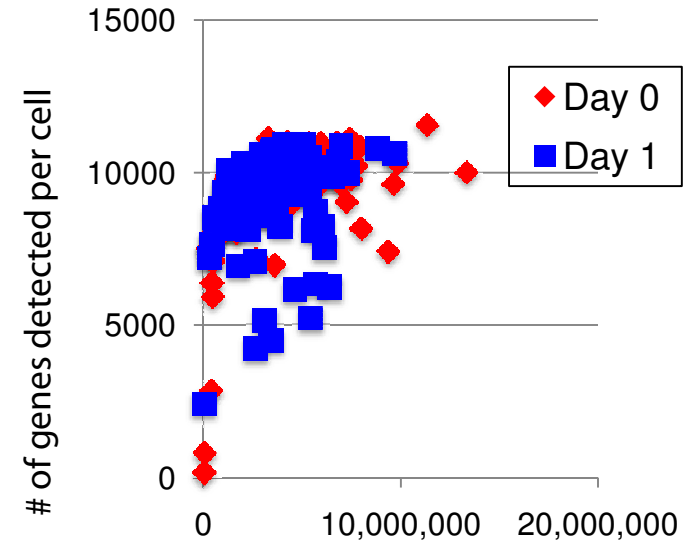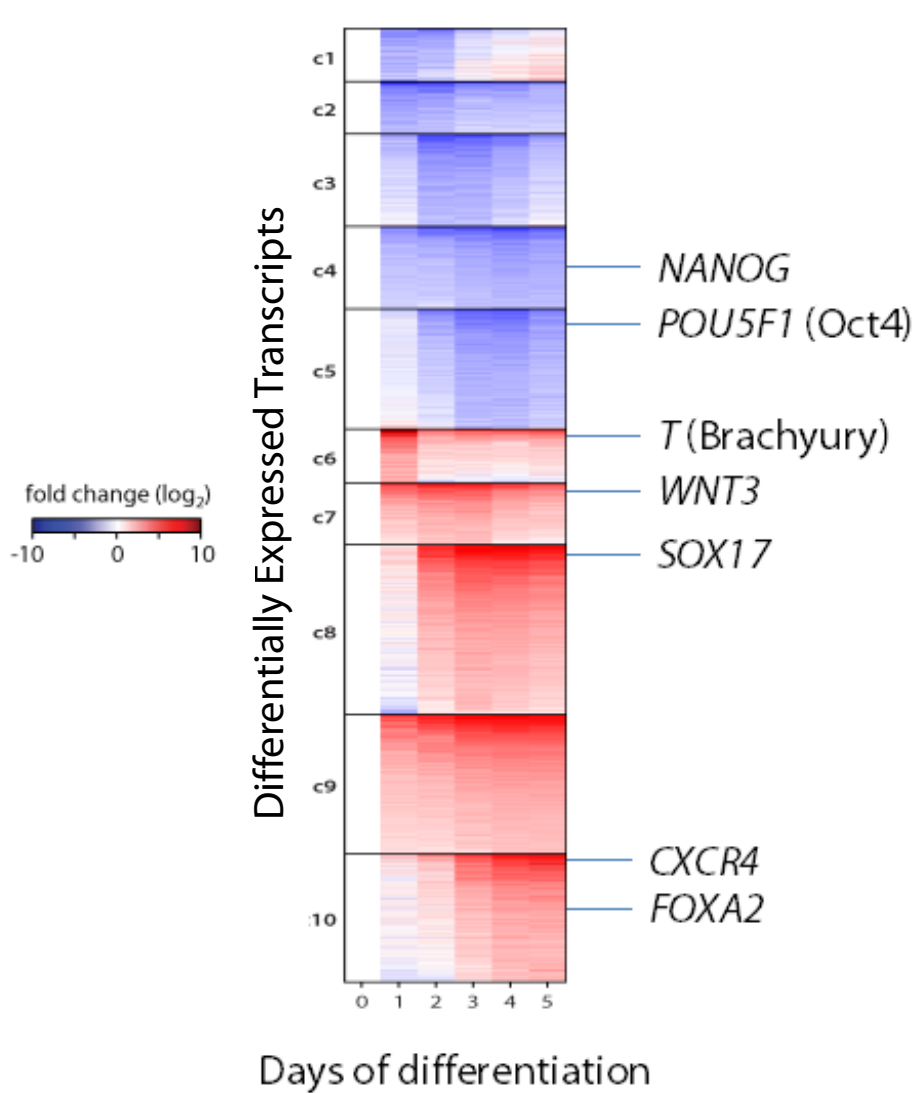
Conclusion

- hPS differentiation is inefficient in part due to instrinsic heterogeneity

- We hypothesize that improved understanding of the origin and dynamics of heterogeneity will lead to improved differentiation protocols

- In situ single cell analysis is essential for understanding the role of the cellular micro-environment during differentiation

- We have developed a customized high content imaging system adapted to hPSCs that allows us to perform multi-scale analysis (e.g. features of the colonies in which a cell resides to number of transcripts within each cell)

- We are using bulk and single-cell mRNA seq to define the expression heterogeneity during DE differentiation and hope to identify novel genes that make give insight into the cellular dynamics we have begin to observe.

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