



Non-Invasive Registration of Human Embryonic Stem Cell Differentiation Status



工業技術研究院
Industrial Technology
Research Institute

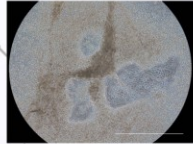
My IGERT Internship in Hsinchu, Taiwan: Summer 2007

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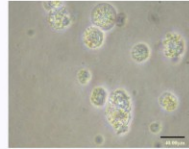
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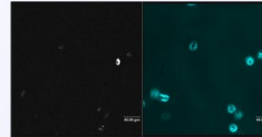
Rutgers Rehabilitation Lab



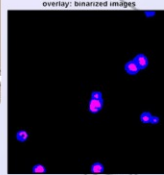
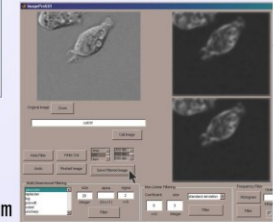
1. Learn to culture hES cell lines on mouse & human feeder cells



2. Simultaneous imaging via bright field & 2-Photon microscopies



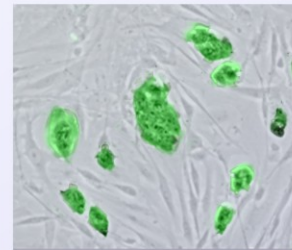
3. Create novel image processing softwares to filter, binarize, and perform feature extraction on images.



Which of these cells have differentiated?



What traits do stem cells exhibit when differentiating?



What approach to take in order to ID cell differentiation with minimum error?



Basic Parameters

Morphology

Area, Bounding Box, Major-Axis, Minor-Axis, Solidity, Eccentricity, Perimeter, Equivalent Diameter

Total I, Mean, Normalized Mean, Standard Deviation, Skewness, Kurtosis

Intensity Maximum, Minimum, Range
Total Overlap, Overlap Fraction

1° Statistics

2° Statistics Interaction

Image Moments

Treating each cell as its own physical species. Pixel value → Mass. Take the image moments (Zernike Polynomials)

$$Z_{nm} = \frac{n+1}{\pi} \sum_x \sum_y V_{nm}^*(x, y) I(x, y)$$

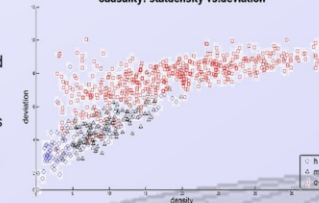
$$V_{nm} = \sum_{k=0}^{n-m} (-1)^k \frac{(n-k)!}{k! \left[\frac{n-2k+m}{2} \right]! \left[\frac{n-2k-m}{2} \right]!} (x^2 + y^2)^{\frac{(n-k)}{2}} e^{i\theta}$$

Statistical Pattern Recognition

causality: stastidney vs. deviation

Supervised Learning:
Cells were fixed and stained for differentiation markers

Attempts to segregate groups by the top-2 features (a total of 20-19 calculations)

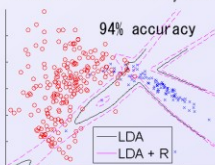


Moderate accuracy was achieved with some parameter combinations

Actual Label	Determined Label		Diff'd on HFCs
	Differentiated	Undifferentiated	
Differentiated	0.80	0.08	0.12
Undifferentiated	0.14	0.61	0.25
Diff'd on HFCs	0.06	0.31	0.63

Transformations Improve Accuracy

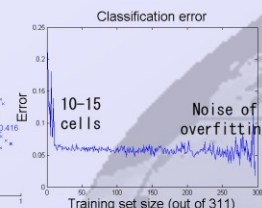
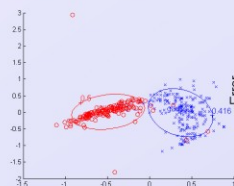
Classifier boundaries with rejection



Linear Discriminant Analysis (LDA)

Supervised linear classifier

Reduction of 20-3 most relevant factors



LDA is preferred to Principal Component Analysis, as PCA finds maximum variance, not necessarily model difference. Classification error was assessed at various training set sizes: 10-15 cells are sufficient for 95% classifier accuracy.

Preliminary Conclusions

Human embryonic stem cells ought to exhibit signature changes during differentiation because of mitochondrial relocation and/or changes in metabolic activity

Mitochondrial activity is detectable by 2-Photon Microscopy

Basic and advanced (image moments) feature extraction may permit accurate classification by differentiation status

May wish to seek a more robust labeling than SSEA-4 staining

Useful in an automated cell sorting/dissection paradigm

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