Gene expression profiling in single cells

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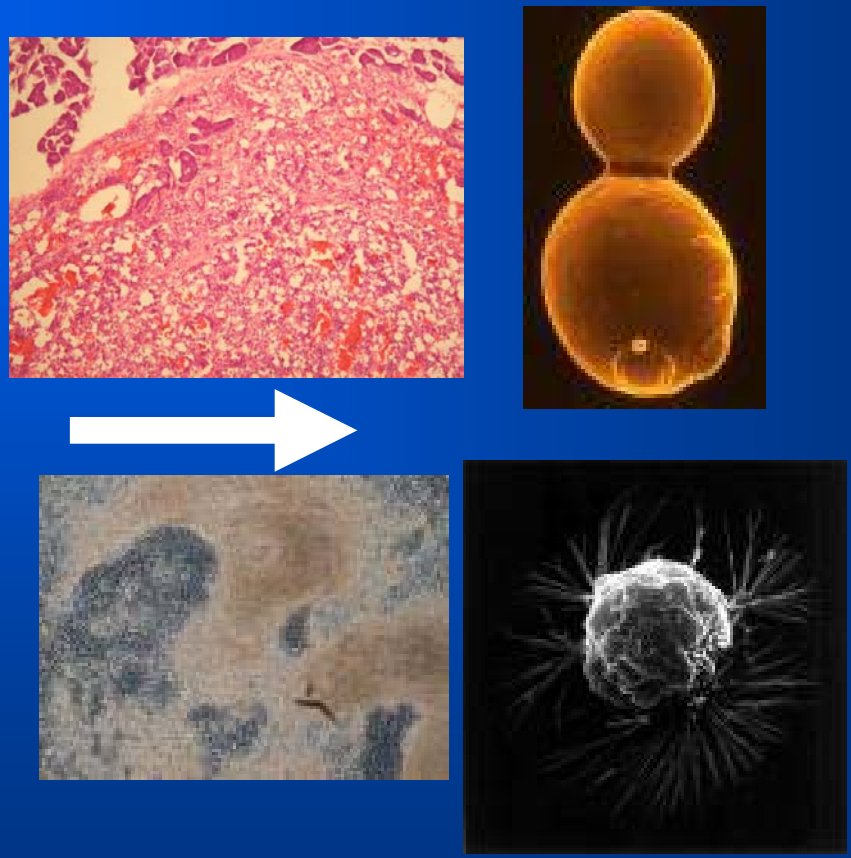
Outline

- Technical considerations
- Gene expression profiling
- Single cell biology

Why single cells?



Workflow – single cell analysis

Cell collection methods

- Patch clamp capillaries
- Flow cytometry
- Laser dissection

gents (Igepal, .)

- Physical disruption

erse transcription

producibility

fficient cDNA yield

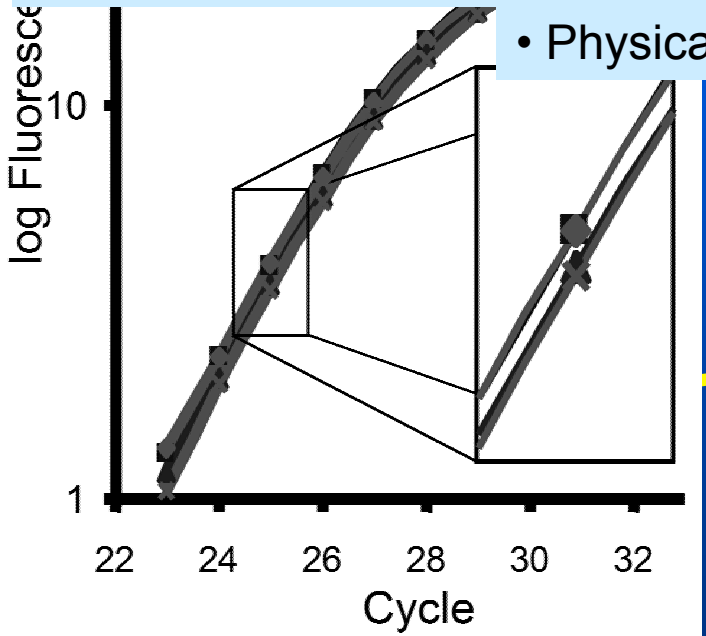
- Ba

Pre-amplification

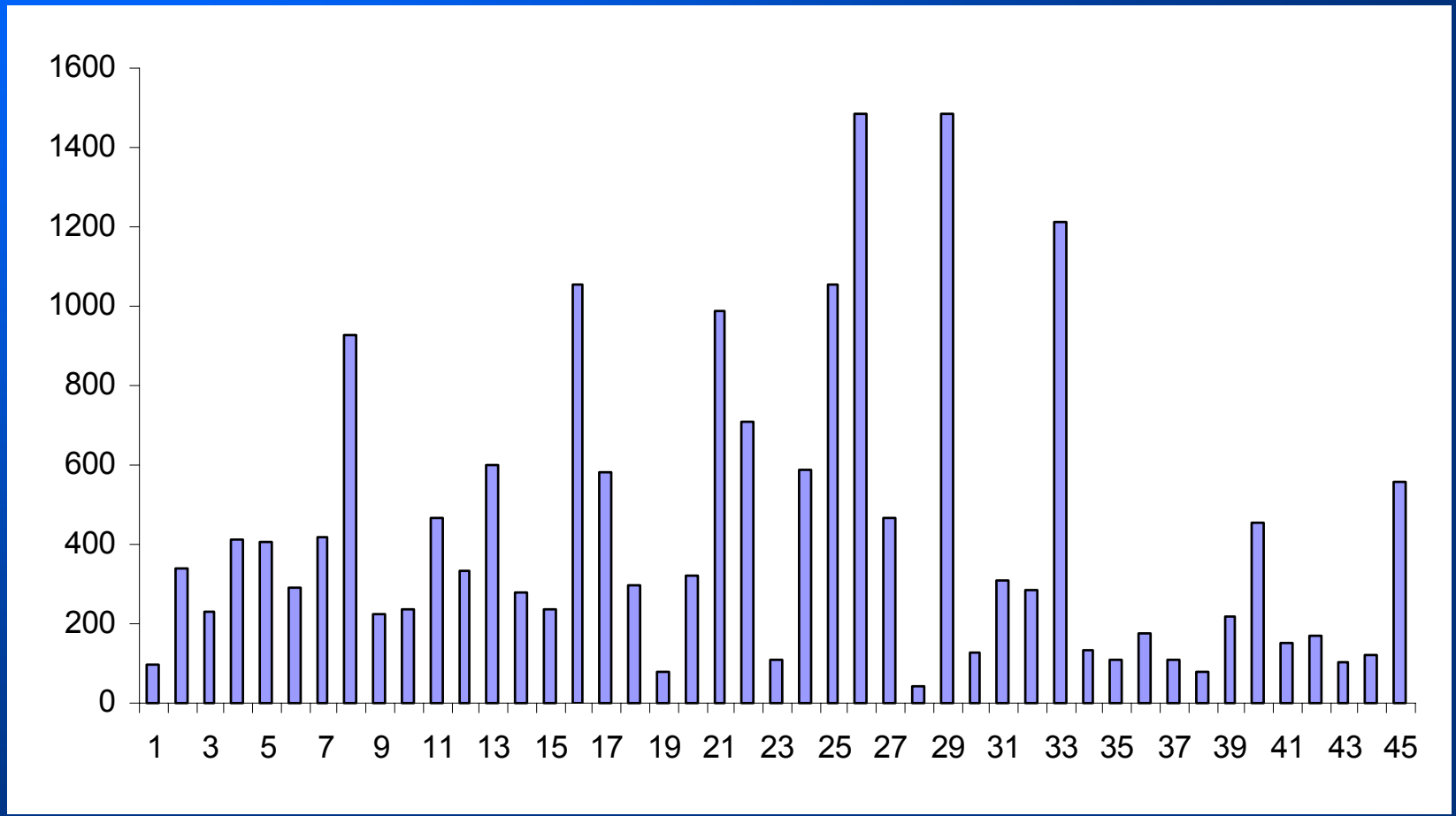
- Linear amplification at mRNA level
- Exponential amplification at cDNA level

Real-time

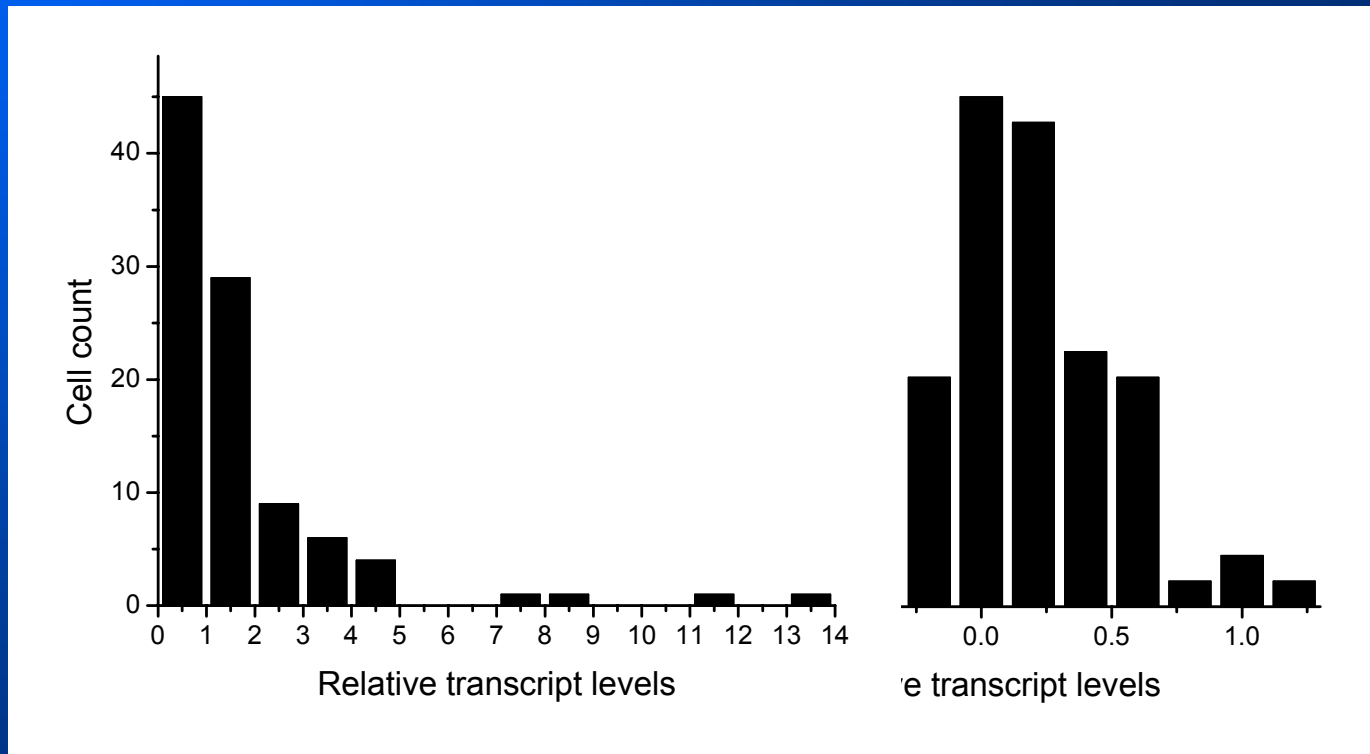
- Gene spe
- PCR inhib
- Limited amount of biological material
- Suitable controls



Gene expression in single cells

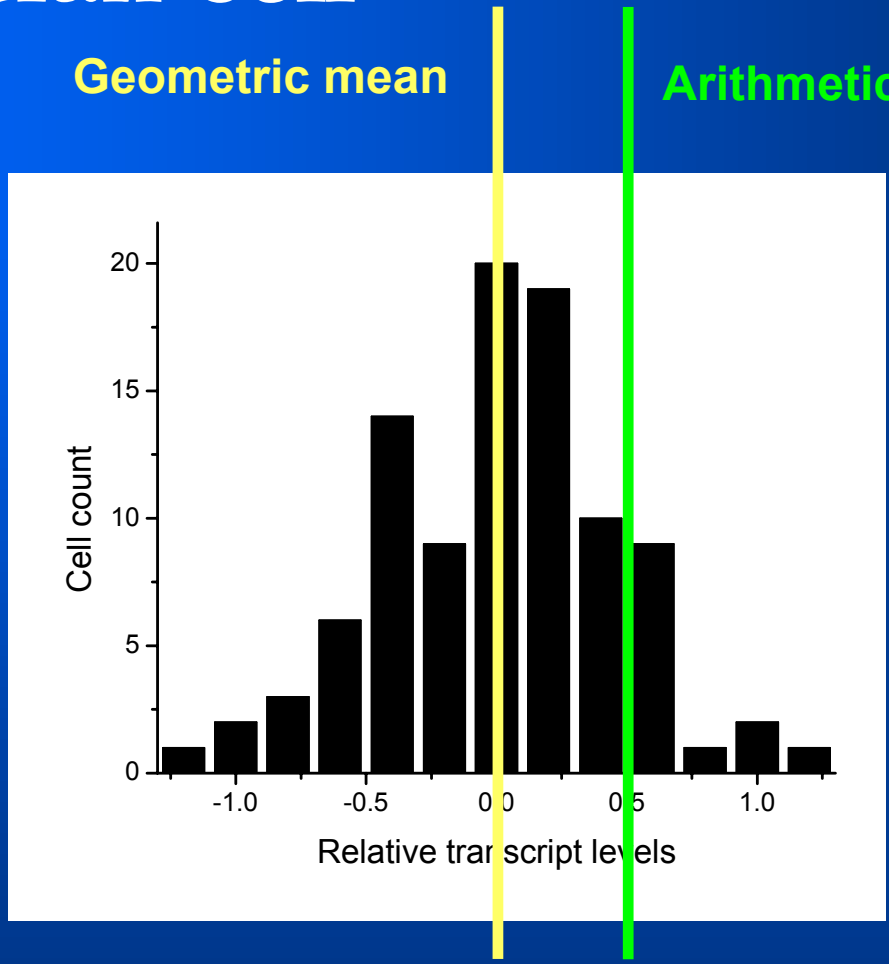


mRNA distribution

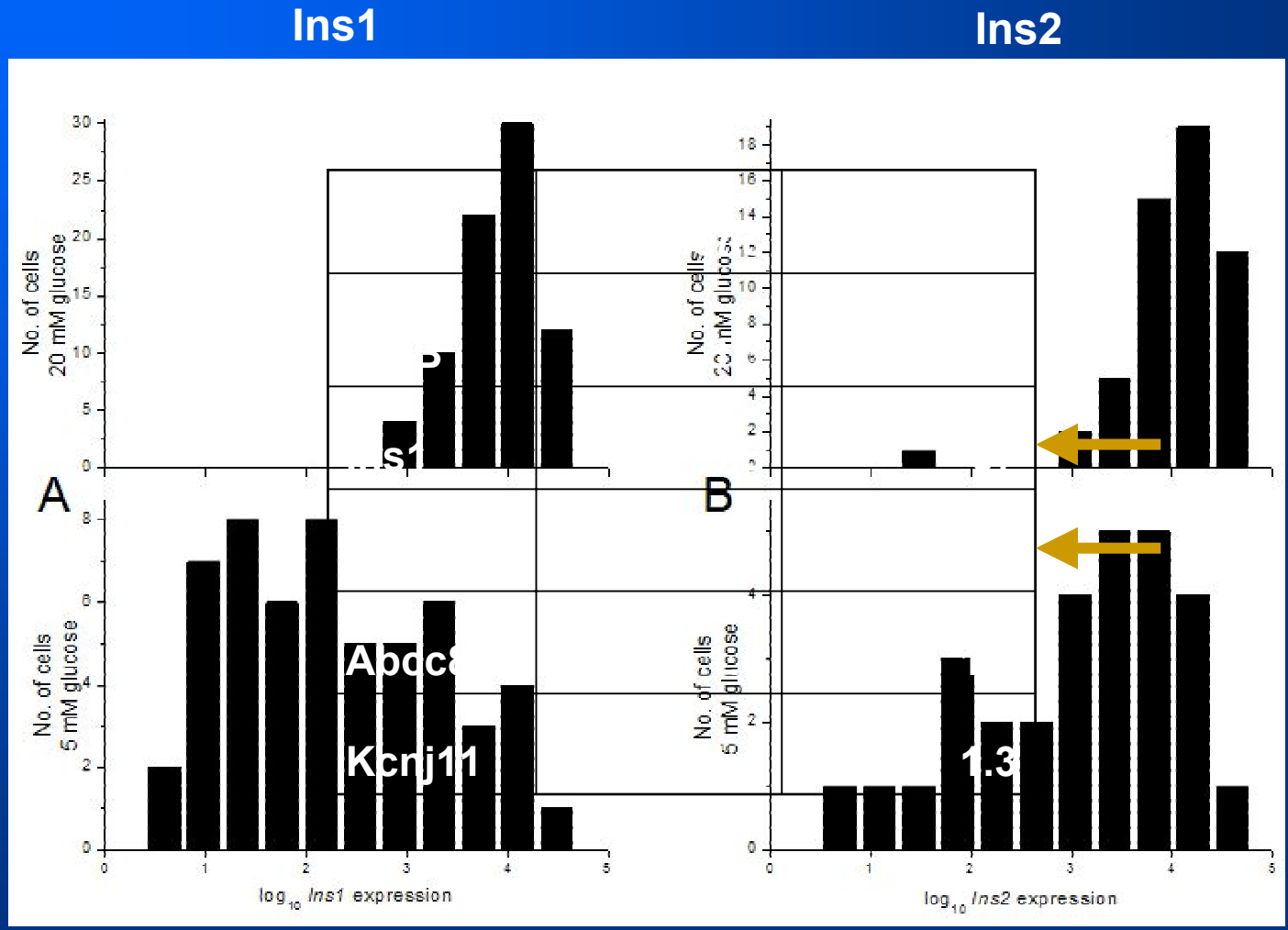


Linear scale **Lognormal scale**

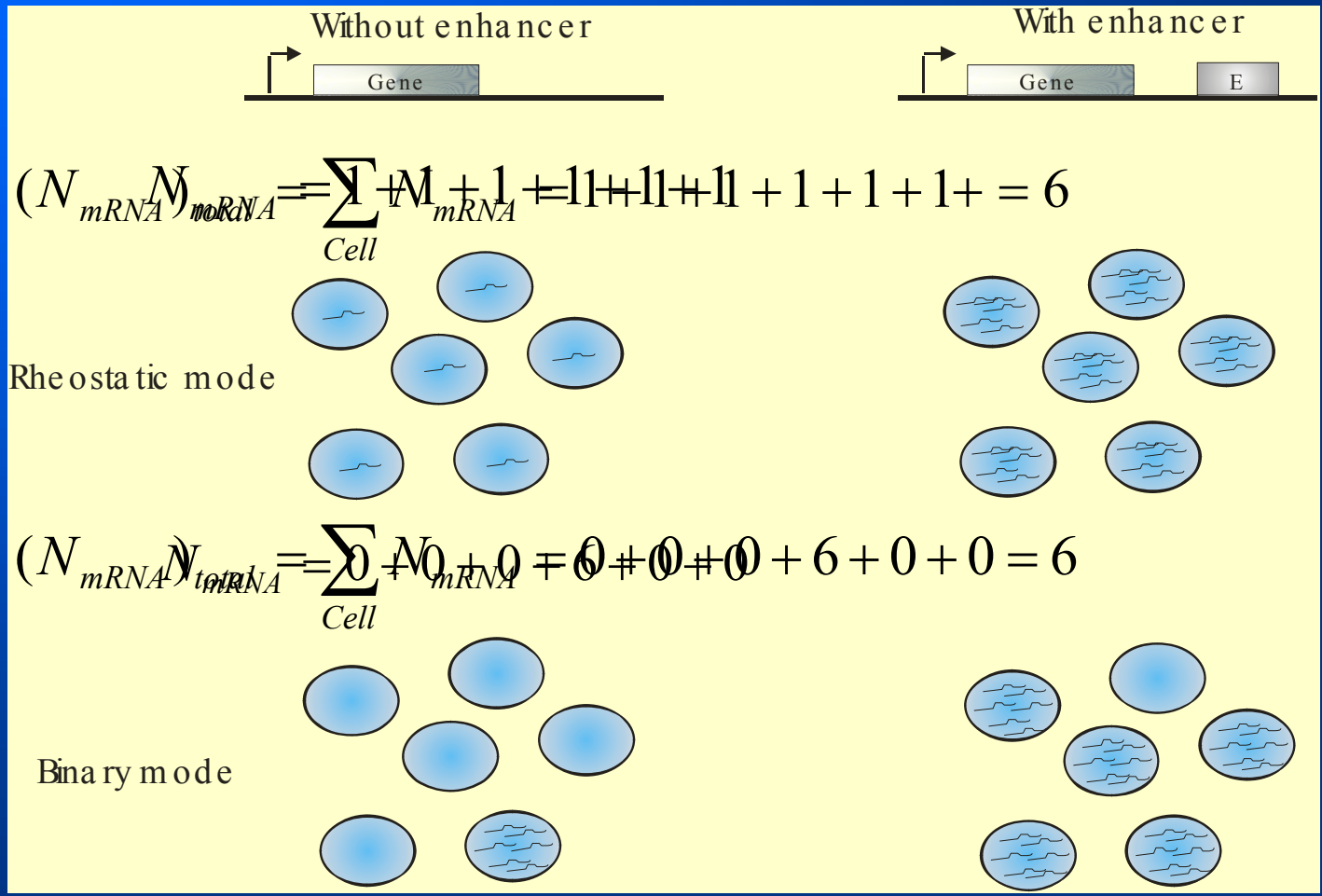
The median cell



Effect of glucose



Models of gene expression



Distribution skewness

Skewness = - 0.70

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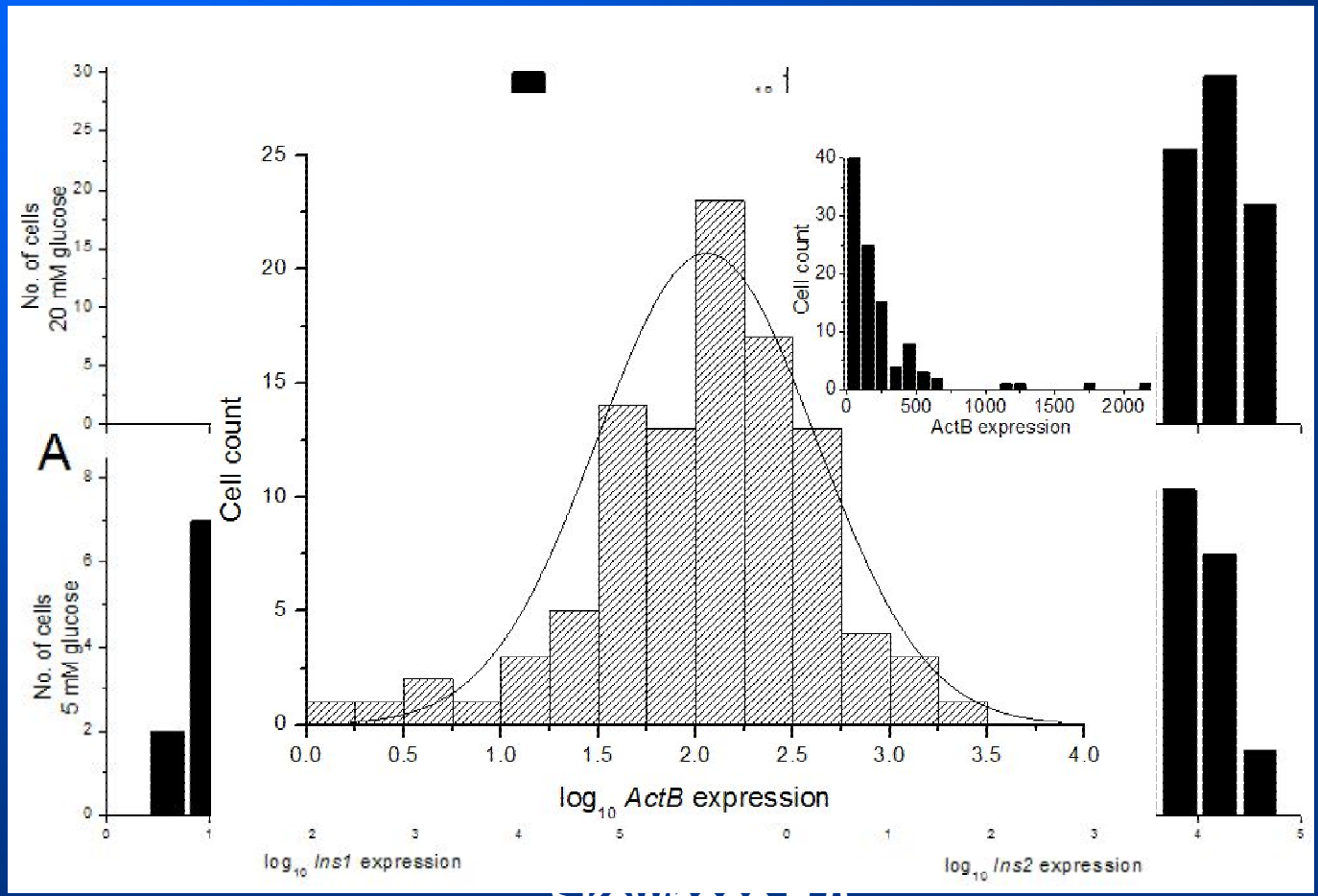


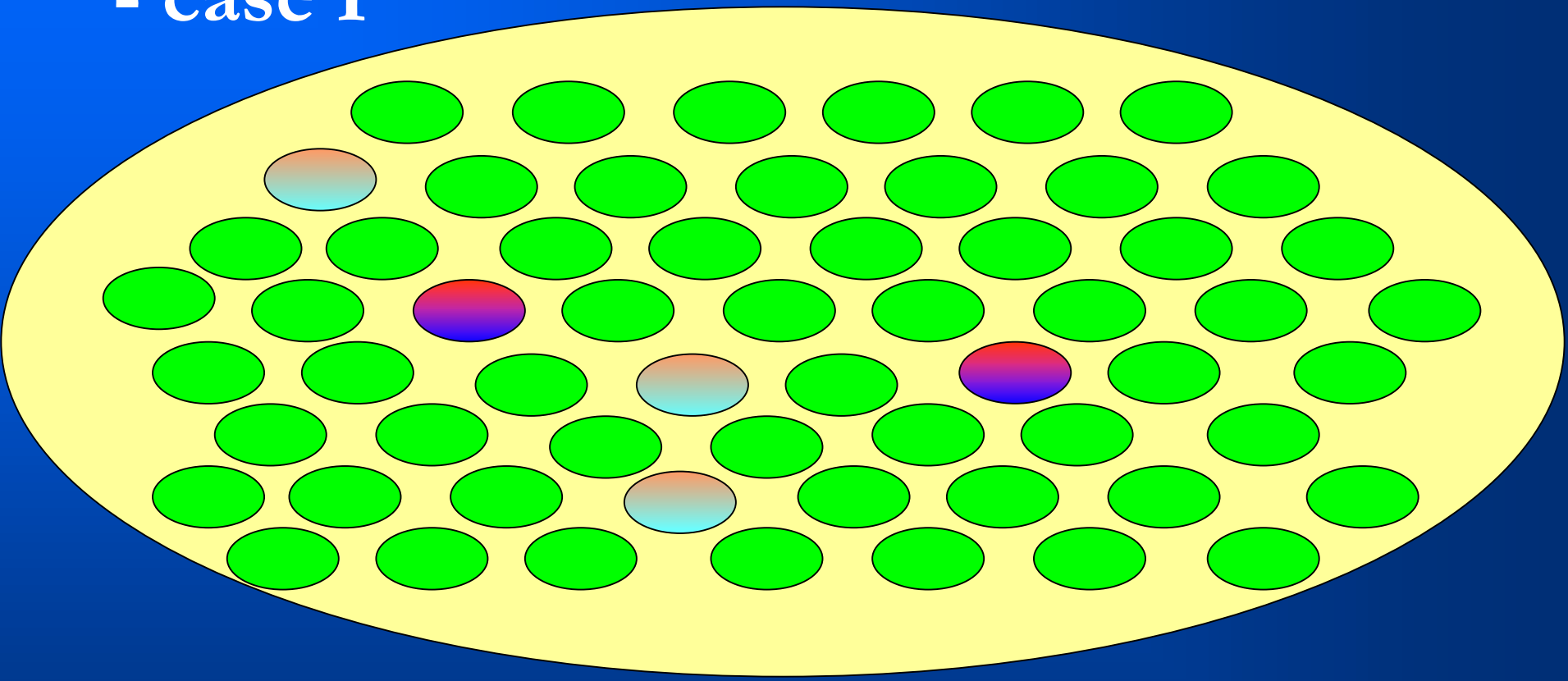
Skewness = 0.06

Skewness = - 0.12

Gene regulation at single cell level

	Arithmetic	*Geometric*
ActB	3.3	4.9
Ins1	5.5	37
Ins2	2.6	43
Abcc8	1.4	1.5
Kcnj11	1.1	1.3

Gene regulation
- case I



Low expression

Gene 1

Gene 2

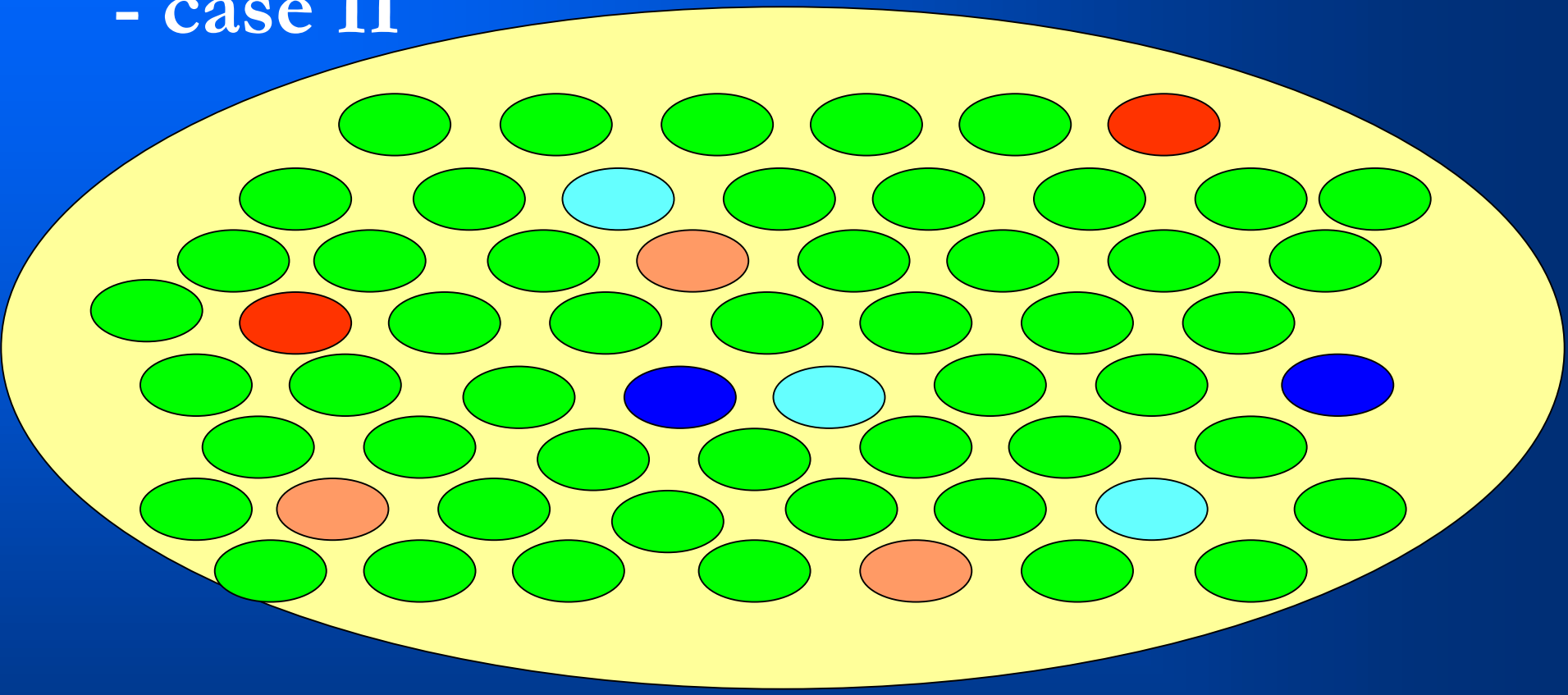
Intermediete expression

Gene 1

Gene 2

High expression

Gene regulation
- case II



Low expression

Gene 1

Gene 2

Intermediete expression

Gene 1

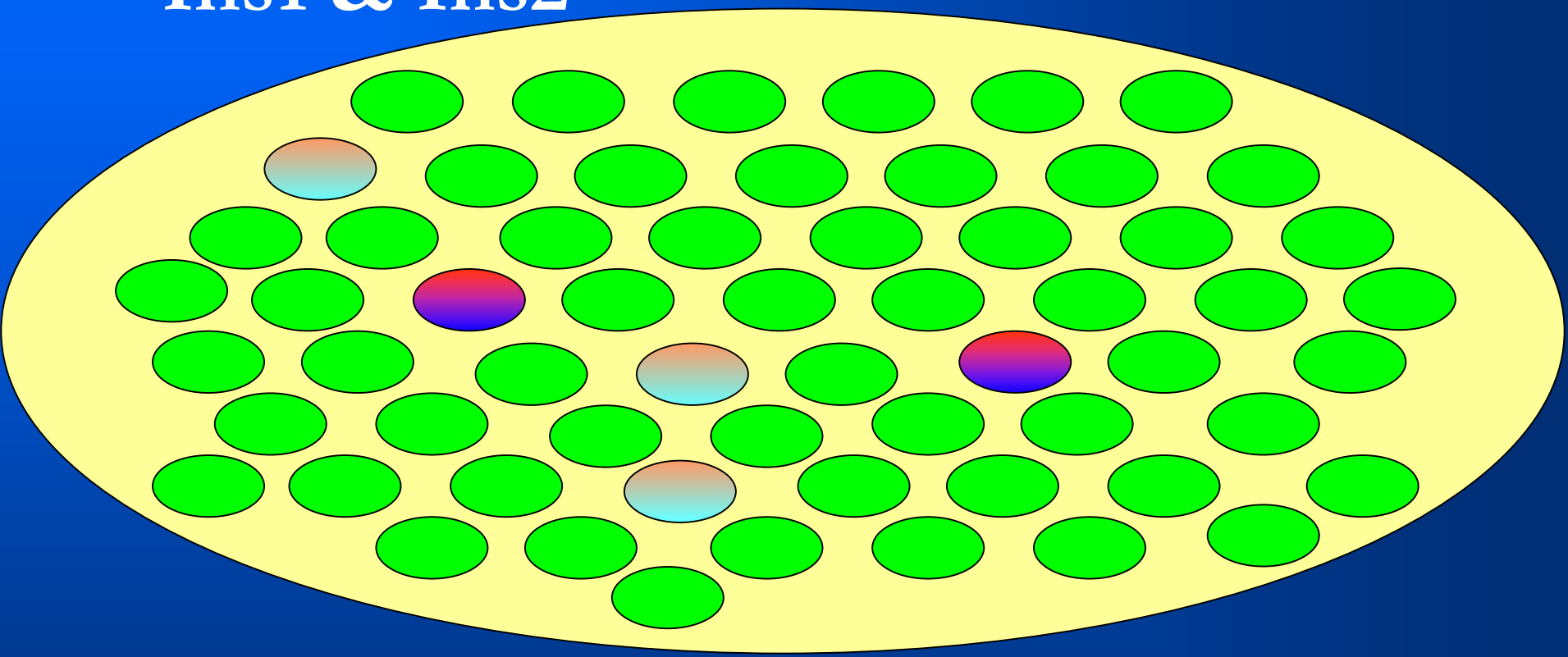
Gene 2

High expression

Gene regulation in individual cells

	ActB	Ins1	Ins2	Sur1	Kcnj11
ActB	1				
Ins1	0.15	1			
Ins2	0.12	0.90	1		
Sur1	-0.02	-0.01	0.06	1	
Kcnj11	0.11	-0.02	0.24	-0.15	1

Gene regulation
- Ins1 & Ins2

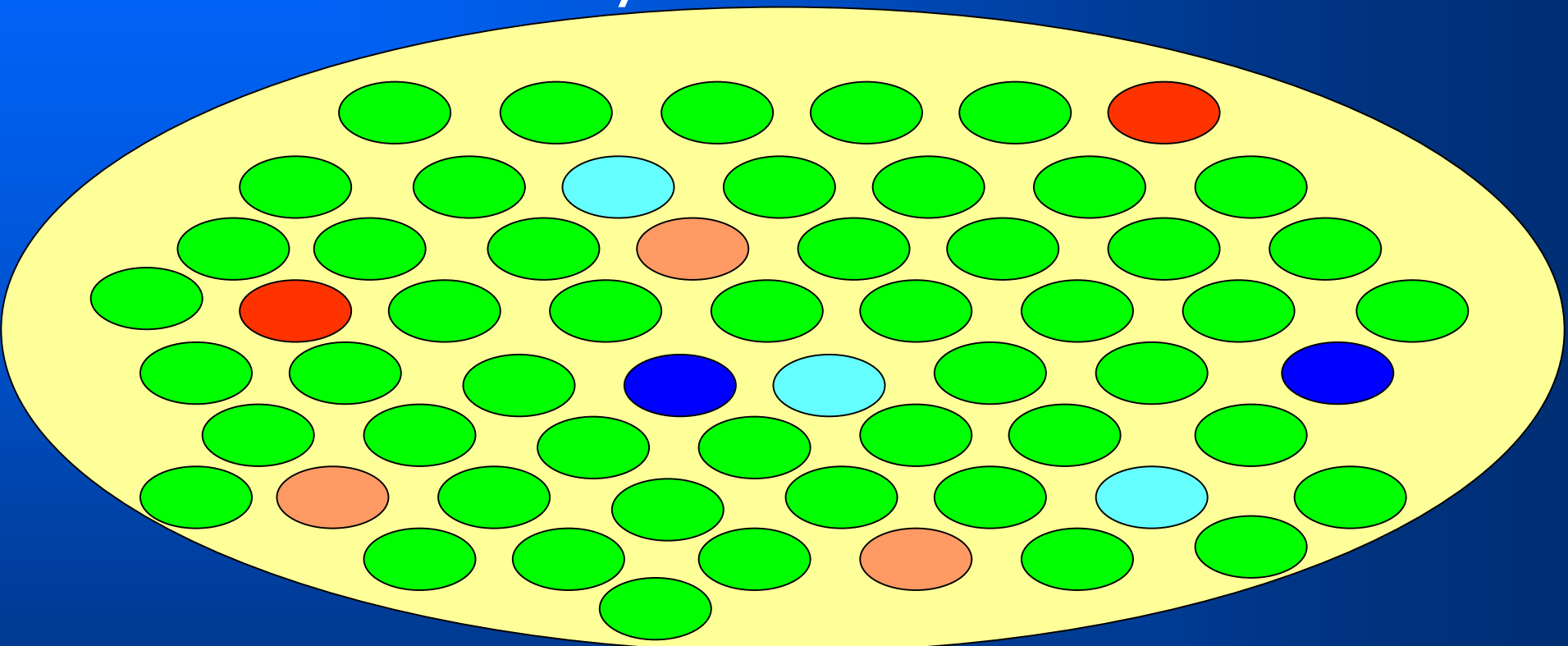


Low expression

Ins1
Ins2
Intermediete expression

Ins1
Ins2
High expression

Gene regulation
- Ins1 & ActB / Ins2 & ActB



Low expression

Ins1/2

ActB

Intermediete expression

Ins1/2

ActB

High expression

Conclusions

- The combination of patch-clamp capillaries and reverse transcription real-time PCR is suitable for gene expression studies in individual cells
- Gene expression is lognormally distributed at a single cell level
- Gene regulation at single cell level do not necessary correlate at cell population level
- Bengtsson M. *et al*. Genome Research, *in press*

Acknowledgments

- Martin Bengtsson
- Mikael Kubista
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