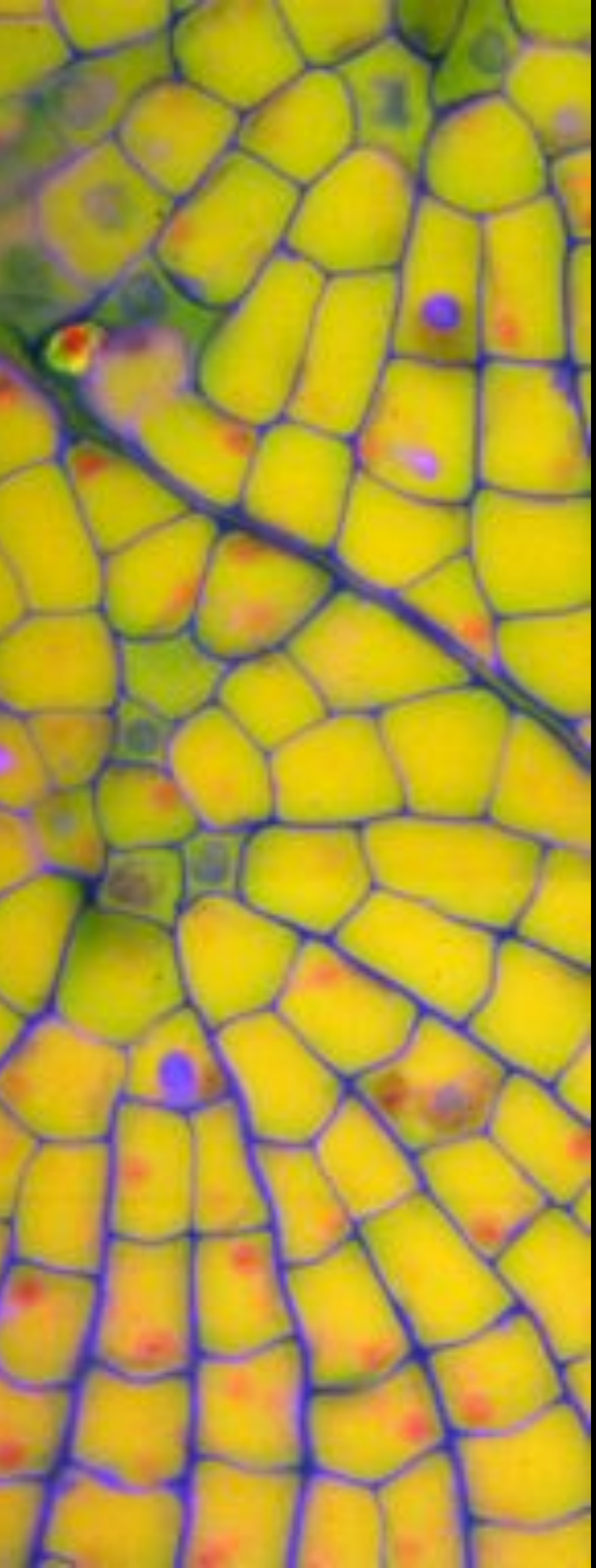


Synthetic Botany

Engineering plant form

Jim Haseloff
University of Cambridge

But...bespoke DNA assembly techniques are still common practice in the field after 40 years

Smithsonian museum exhibit: Stanley Cohen's laboratory bench

Construction of Biologically Functional Bacterial Plasmids *In Vitro*

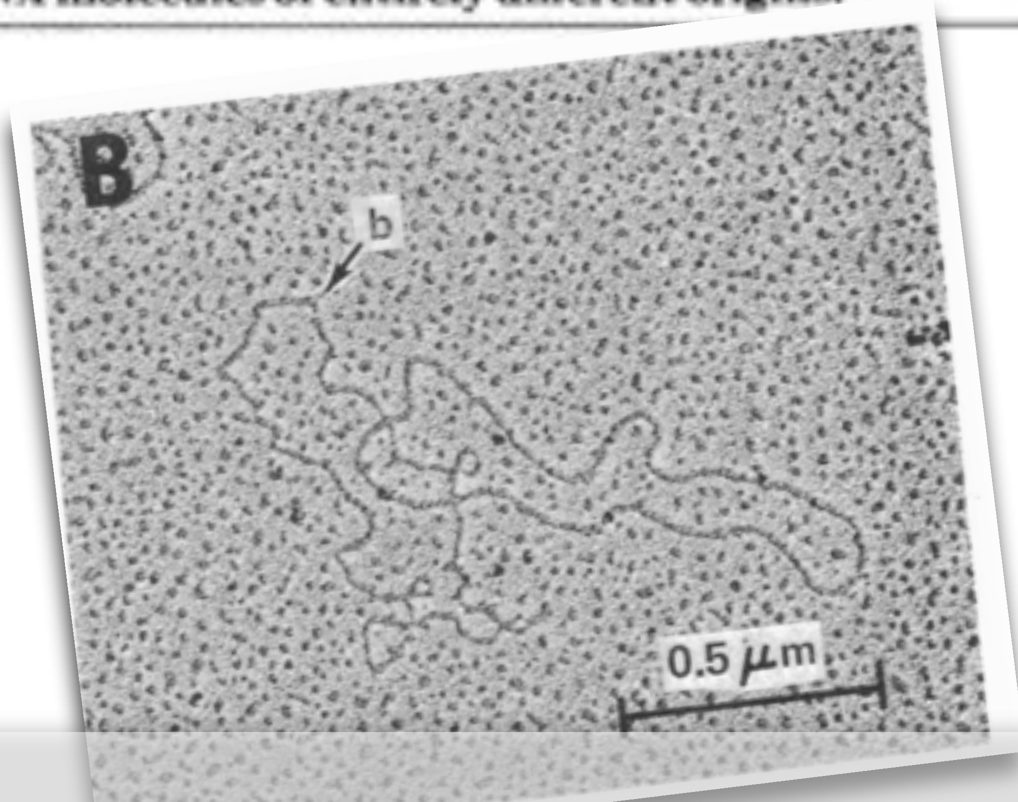
(R factor/restriction enzyme/transformation/endonuclease/antibiotic resistance)

STANLEY N. COHEN*, ANNIE C. Y. CHANG*, HERBERT W. BOYER†, AND ROBERT B. HELLING†

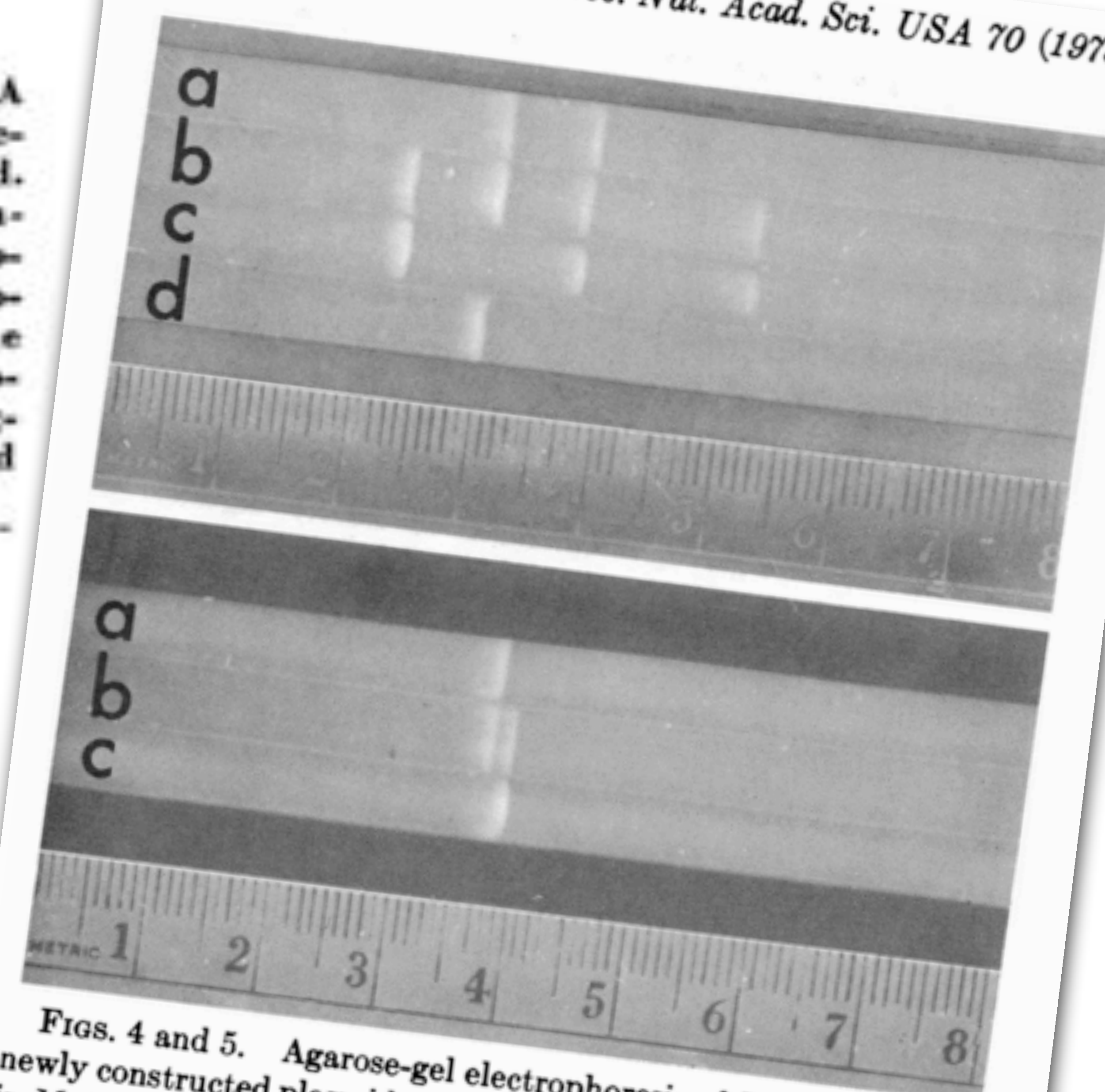
* Department of Medicine, Stanford University School of Medicine, Stanford, California 94305; and † Department of Microbiology, University of California at San Francisco, San Francisco, Calif. 94122

Communicated by Norman Davidson, July 18, 1973

ABSTRACT The construction of new plasmid DNA species by *in vitro* joining of restriction endonuclease-generated fragments of separate plasmids is described. Newly constructed plasmids that are inserted into *Escherichia coli* by transformation are shown to be biologically functional replicons that possess genetic properties and nucleotide base sequences from both of the parent DNA molecules. Functional plasmids can be obtained by reassociation of endonuclease-generated fragments of larger replicons, as well as by joining of plasmid DNA molecules of entirely different origins.



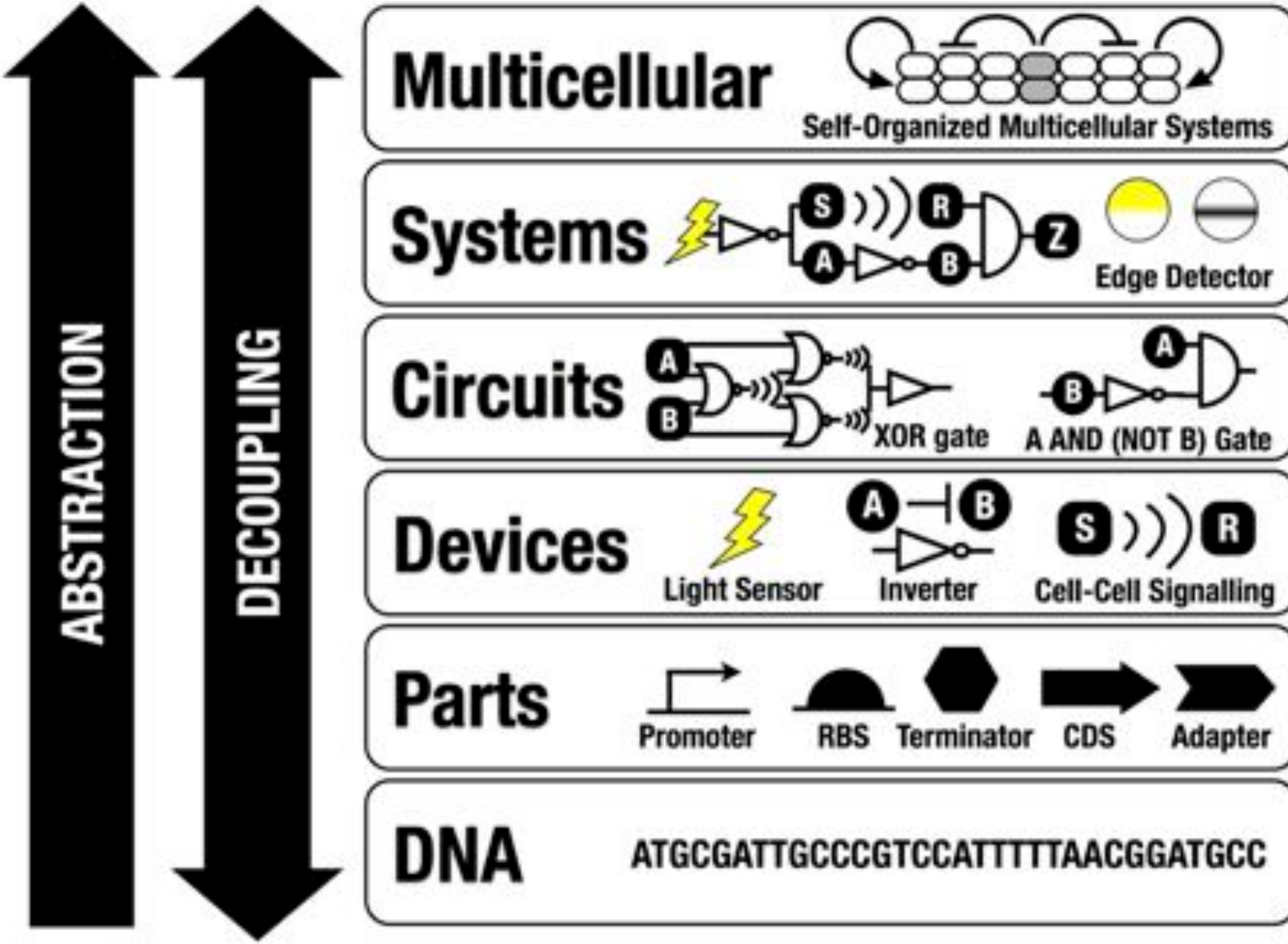
Proc. Nat. Acad. Sci. USA 70 (1973)



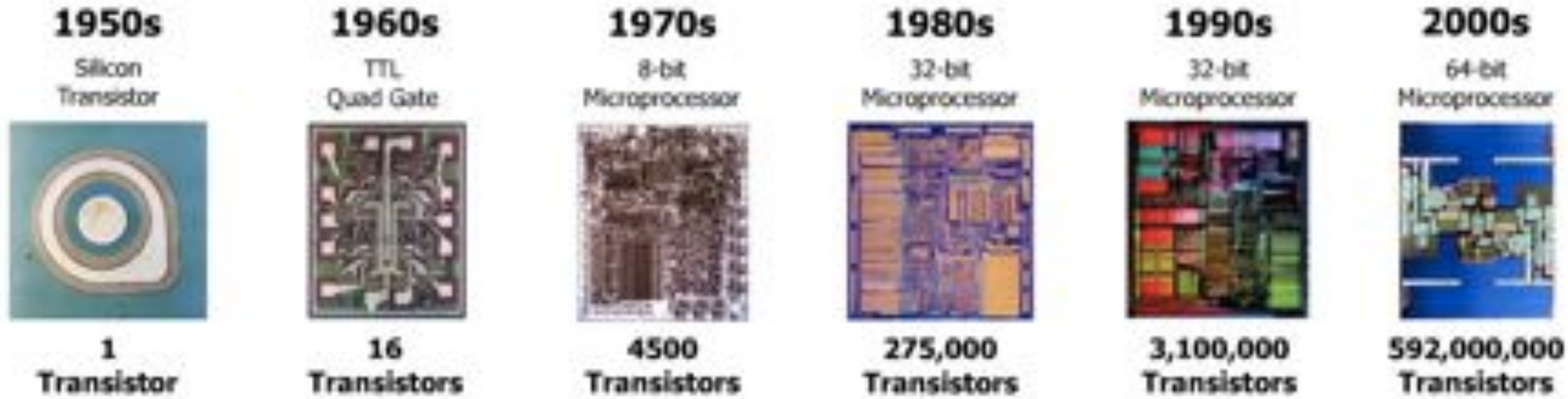
FIGS. 4 and 5. Agarose-gel electrophoresis of *Eco*RI digests of newly constructed plasmid species. Conditions were as described in *Methods*.

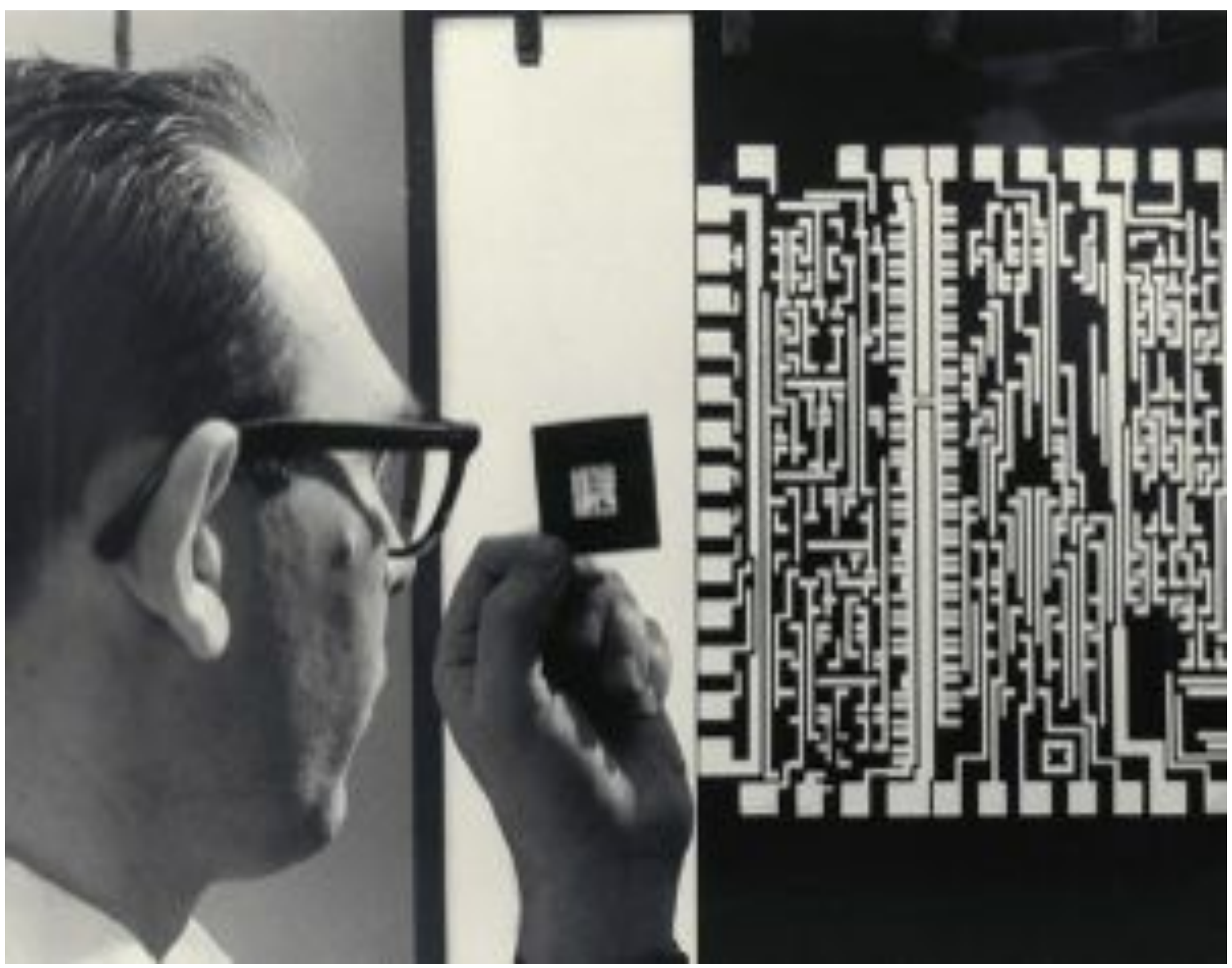
1973 - first molecular cloning experiments...

2003 Invention of standardised DNA parts...

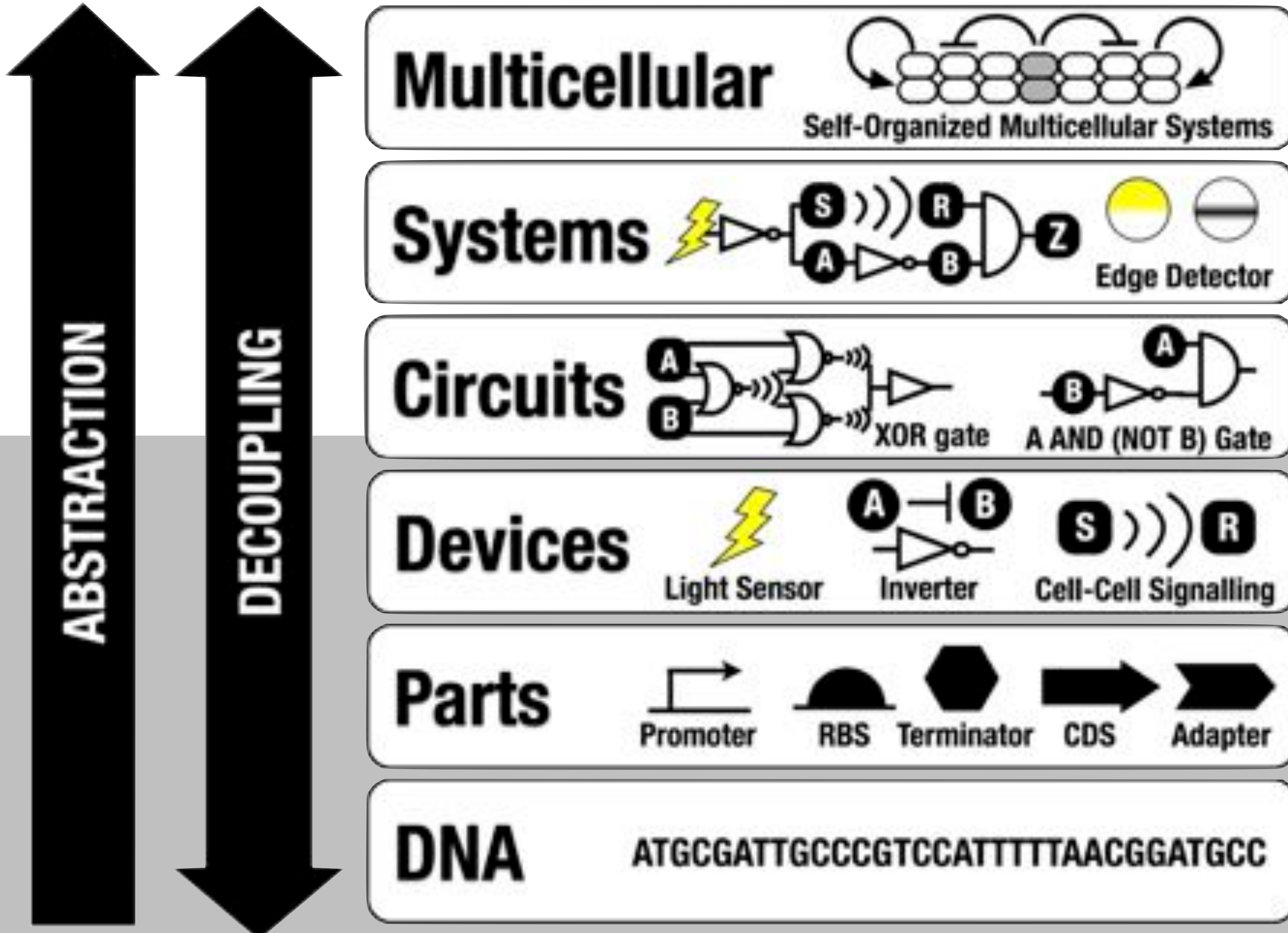


Federici, Rudge, Pollak, Haseloff, Gutierrez, 2013





2. Development of automated design tools and modular circuits to deal with increased complexity



Federici, Rudge, Pollak, Haseloff, Gutierrez, 2013

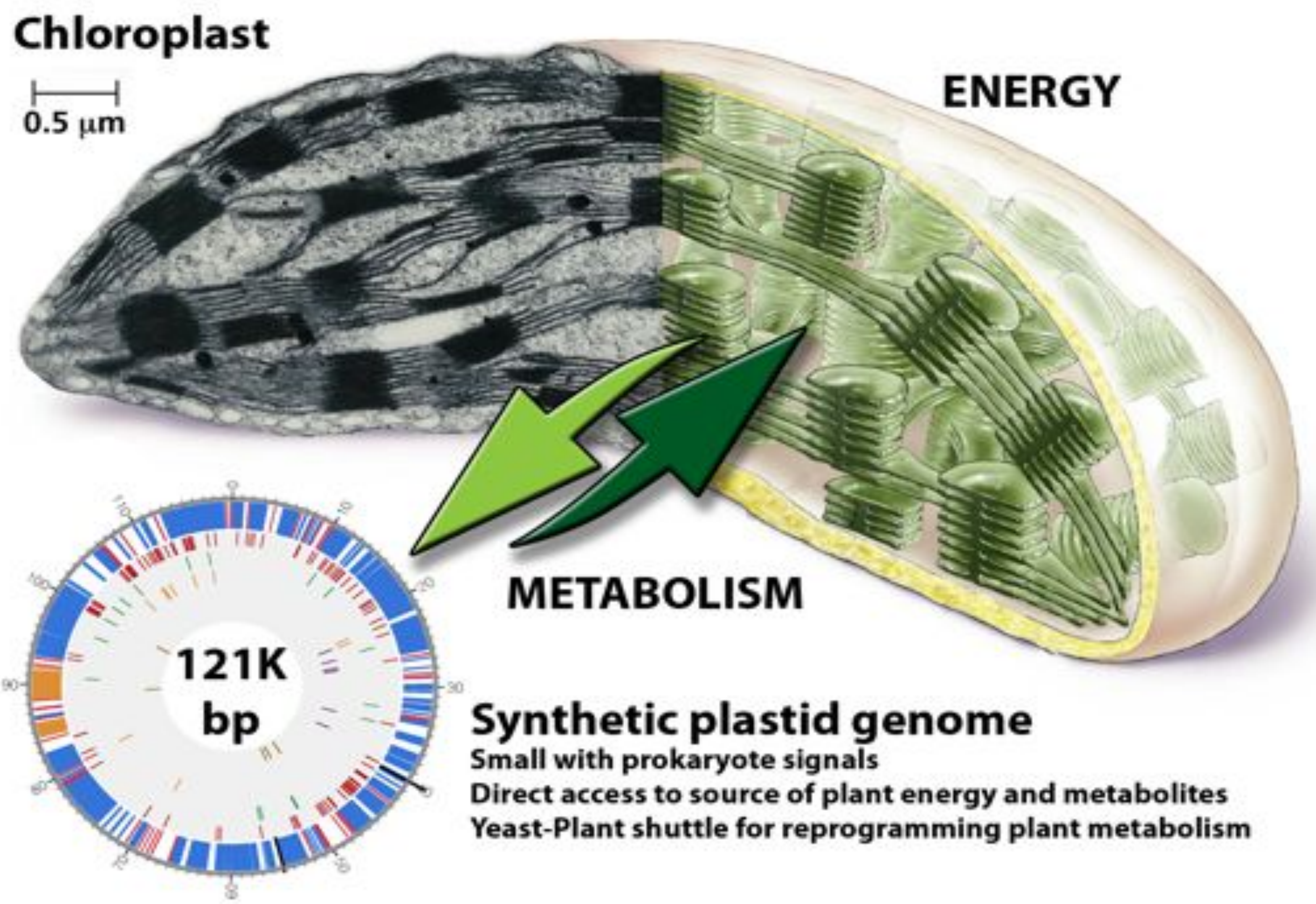
Applications for Synthetic Biology

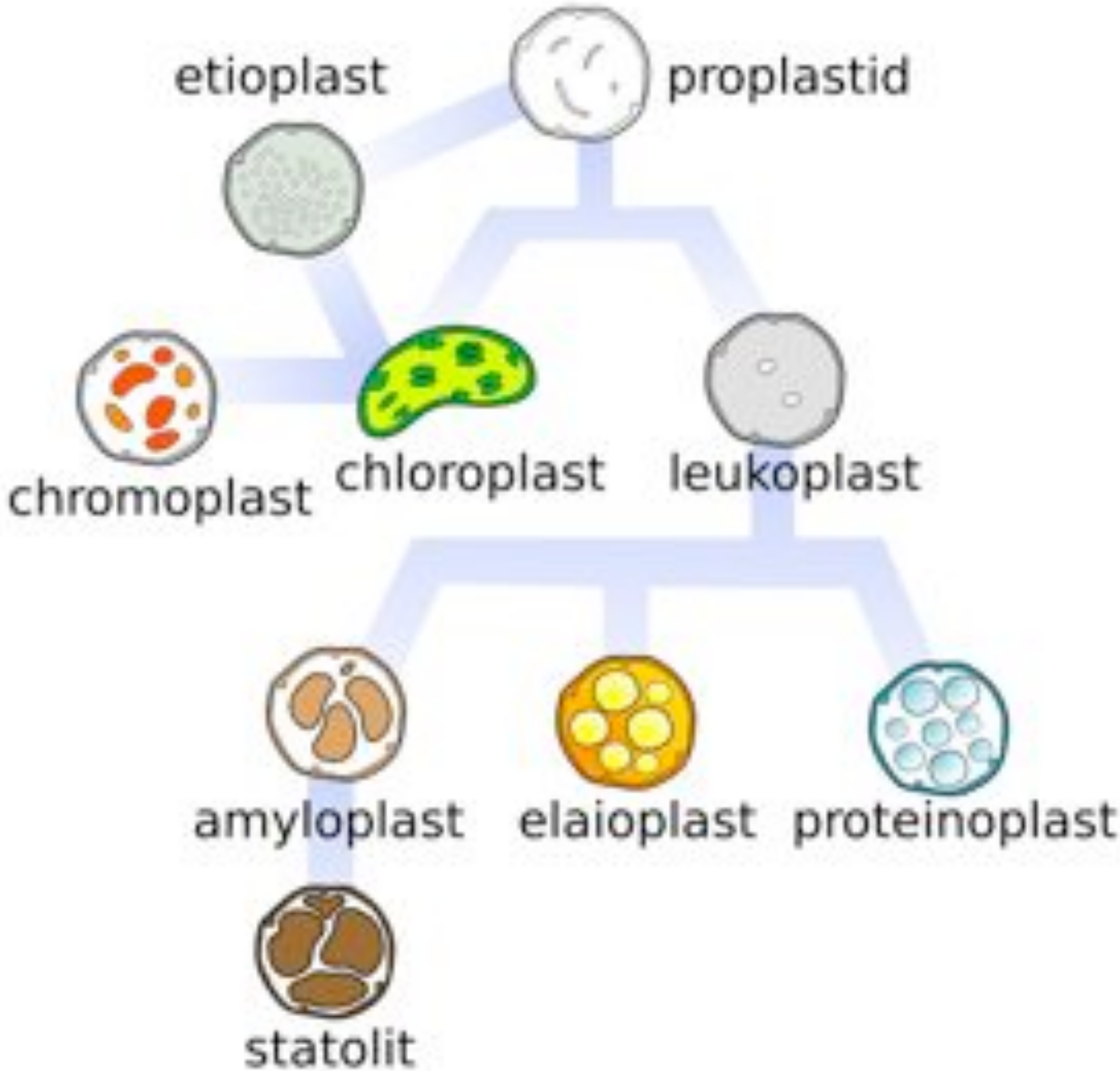
Cell autonomous genetic circuits with self-regulating properties

e.g. microbial engineering,
enviromental and biomedical sensors
engineering novel metabolic pathways

Morphogenetic circuits with self organising properties

e.g. microbial biofilms or self-organising communities for
bioremediation and bio catalysis
novel plant and algal feedstocks for bioproduction and bioenergy
tissue engineering


Chloroplast
0.5 μm
ENERGY
METABOLISM
121K bp
Synthetic plastid genome
Small with prokaryote signals
Direct access to source of plant energy and metabolites
Yeast-Plant shuttle for reprogramming plant metabolism


etioplast
proplastid
chromoplast
chloroplast
leukoplast
amyloplast
elaioplast
proteinoplast
statolit

Applications for Synthetic Biology

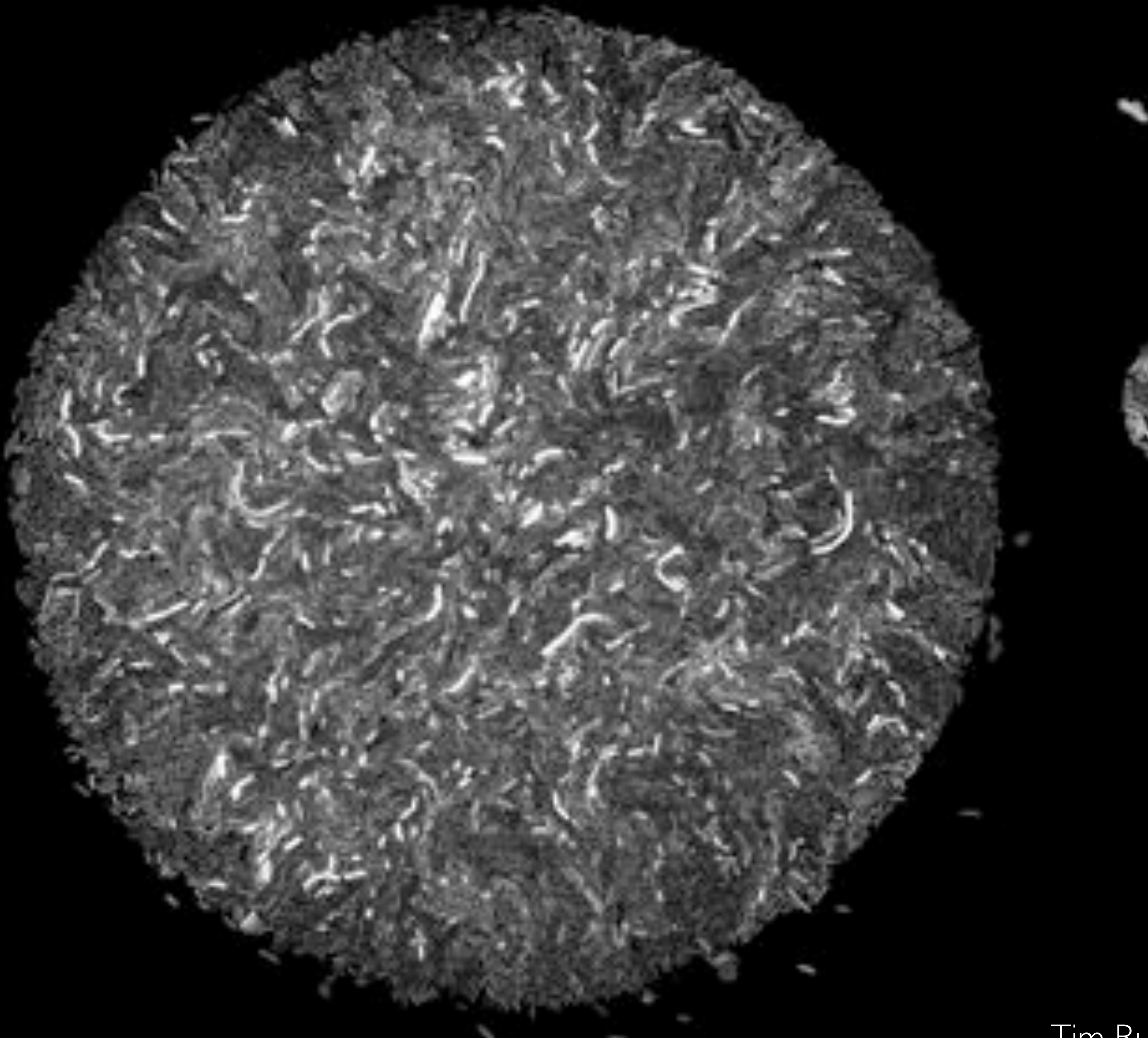
Cell autonomous genetic circuits with self-regulating properties

e.g. microbial engineering,
enviromental and biomedical sensors
engineering novel metabolic pathways

Morphogenetic circuits with self organising properties

e.g. microbial biofilms or self-organising communities for
bioremediation and bio catalysis
novel plant and algal feedstocks for bioproduction and bioenergy
tissue engineering

E. coli



Tim Rudge

Genetic program

Cellular automata model

Finite element model for cell wall mechanics

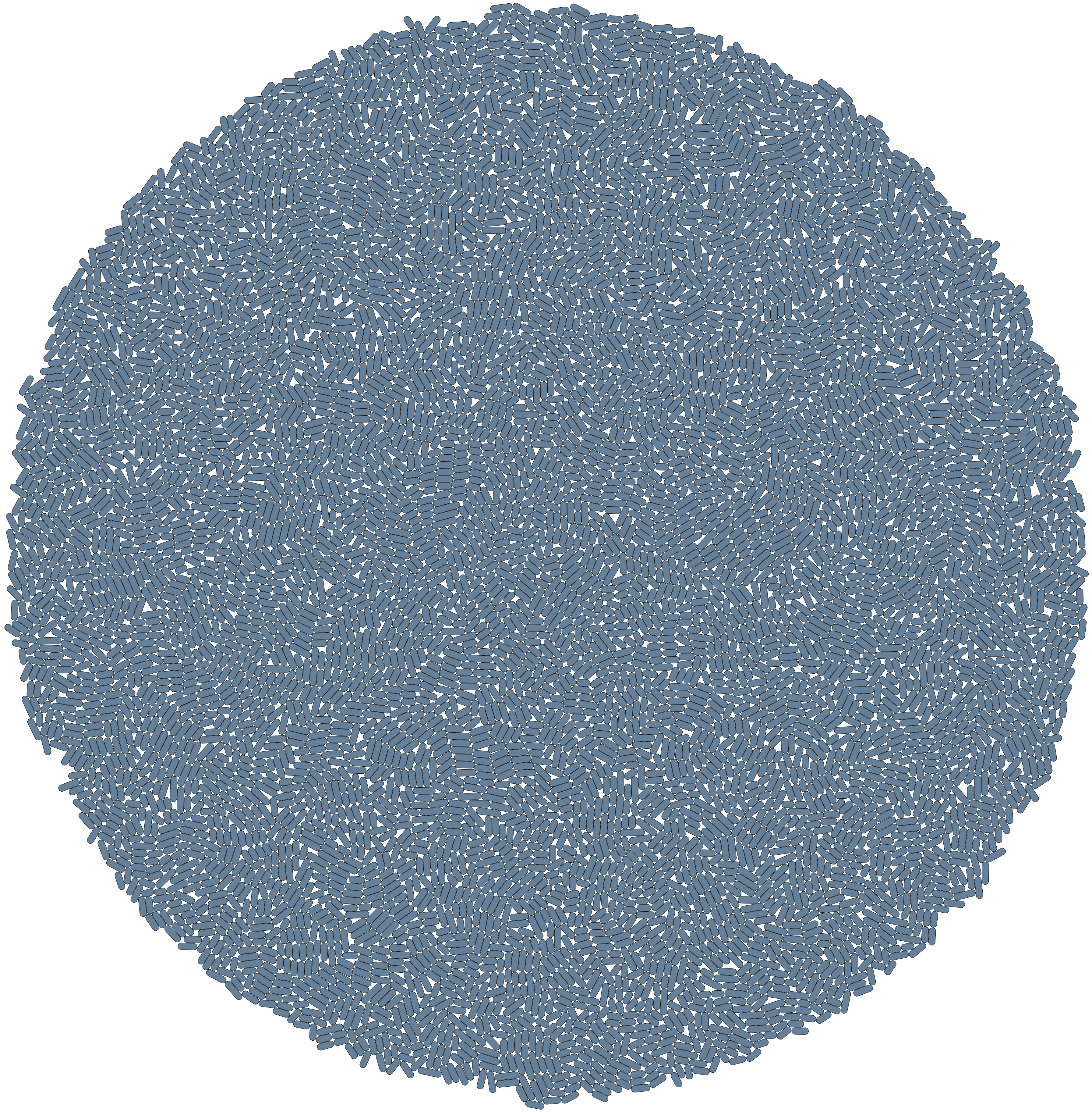
Algorithm for Cell Division

CELLMODELLER

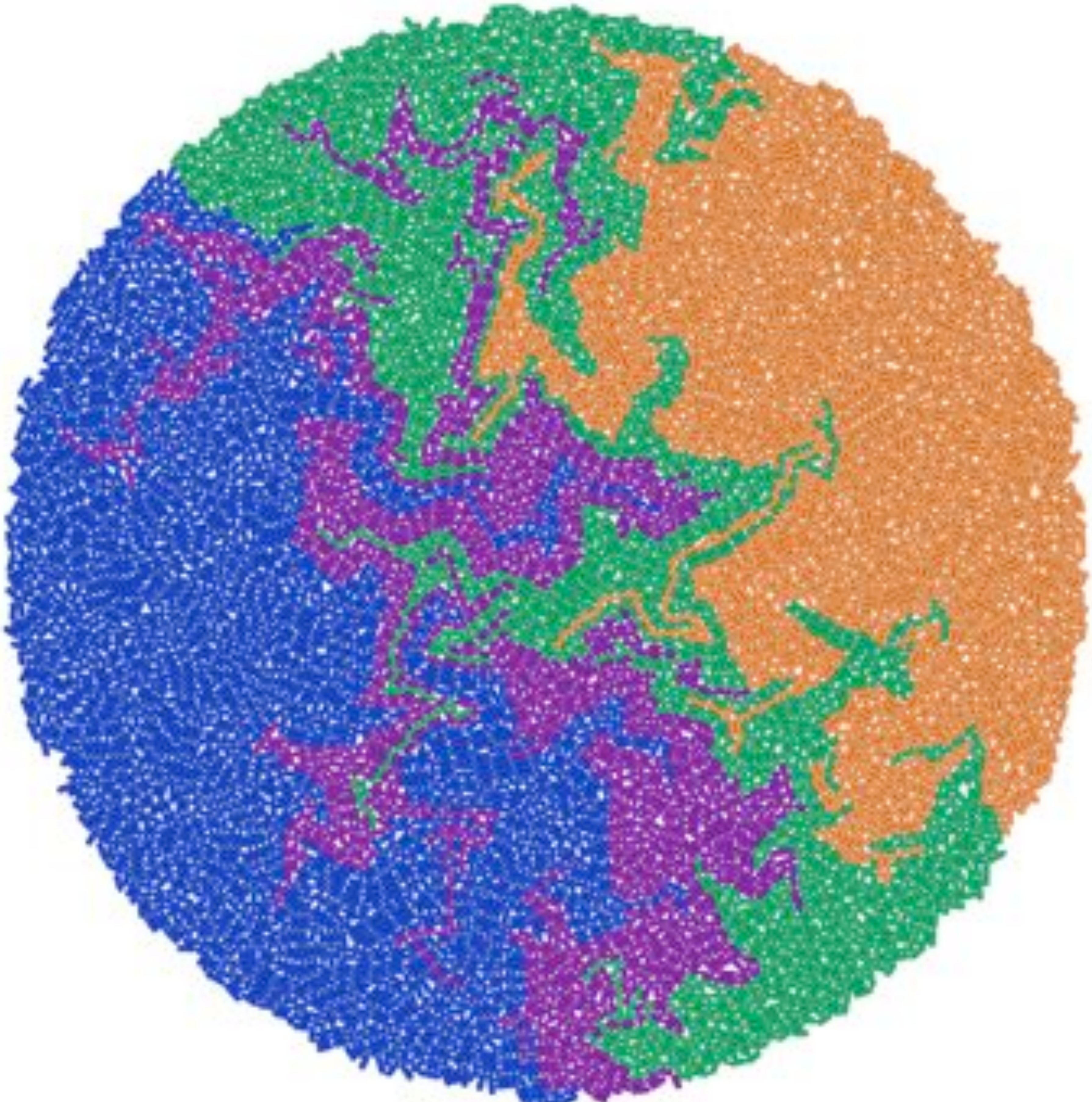
A software engine for programmed growth of multicellular tissues.

A tool for testing prototypes of DNA based morphogenetic programs.

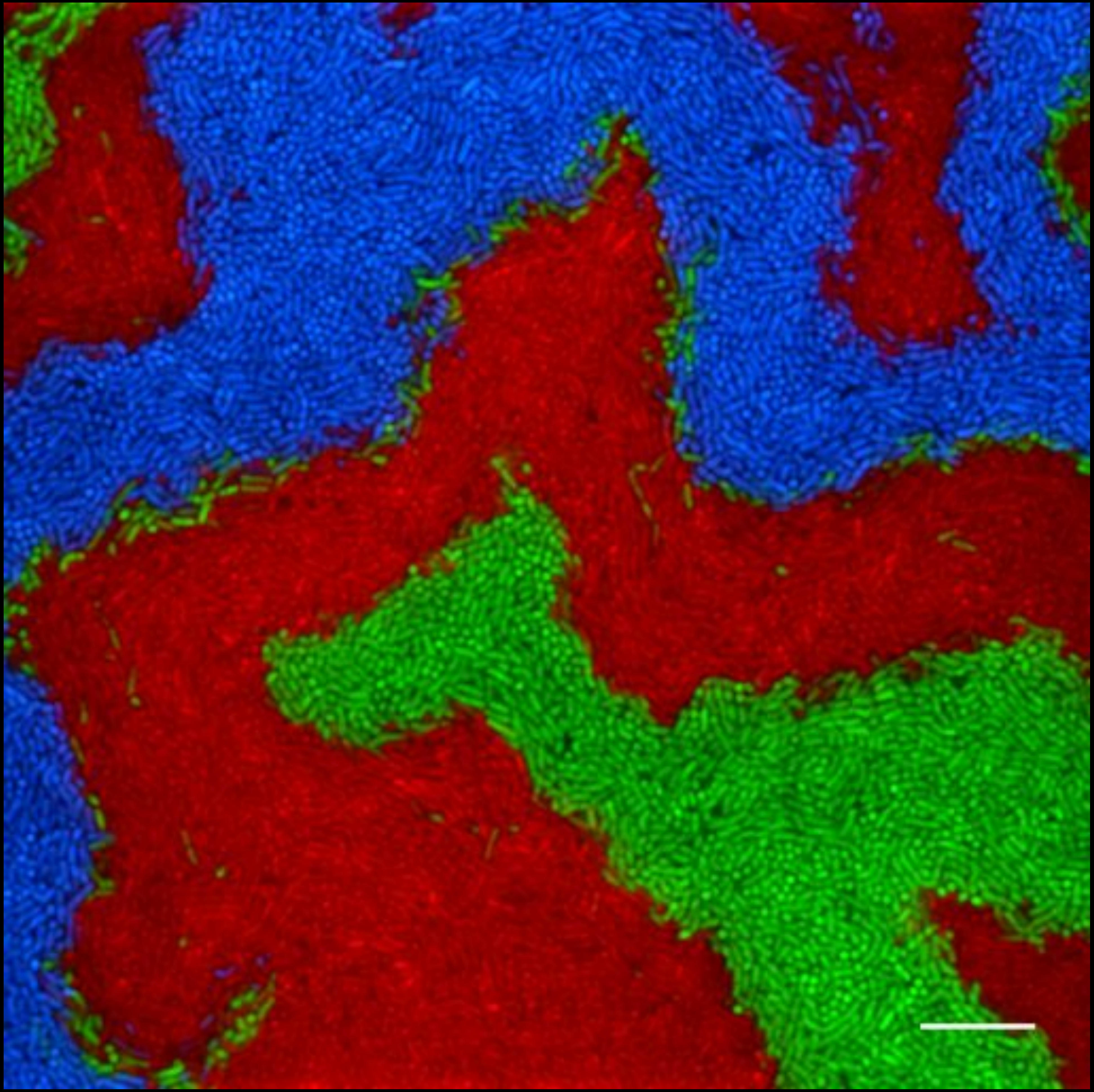
www.cellmodeller.org



Tim Rudge and PJ Steiner

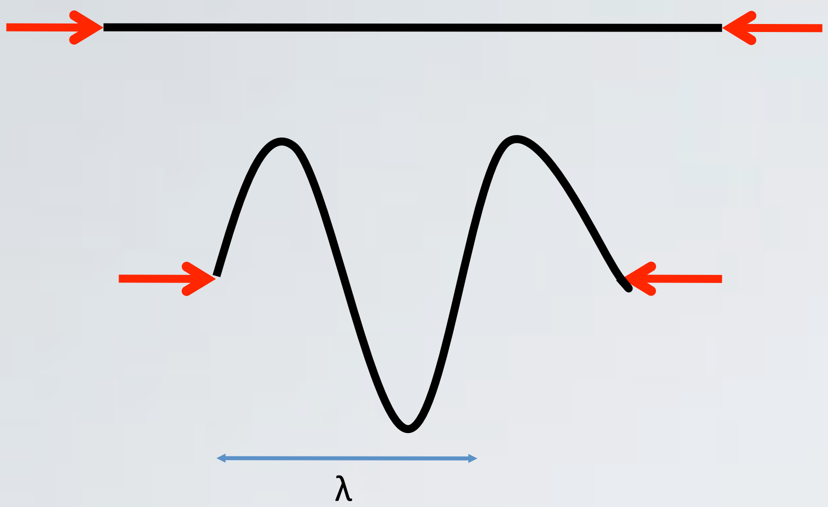


Tim Rudge and PJ Steiner

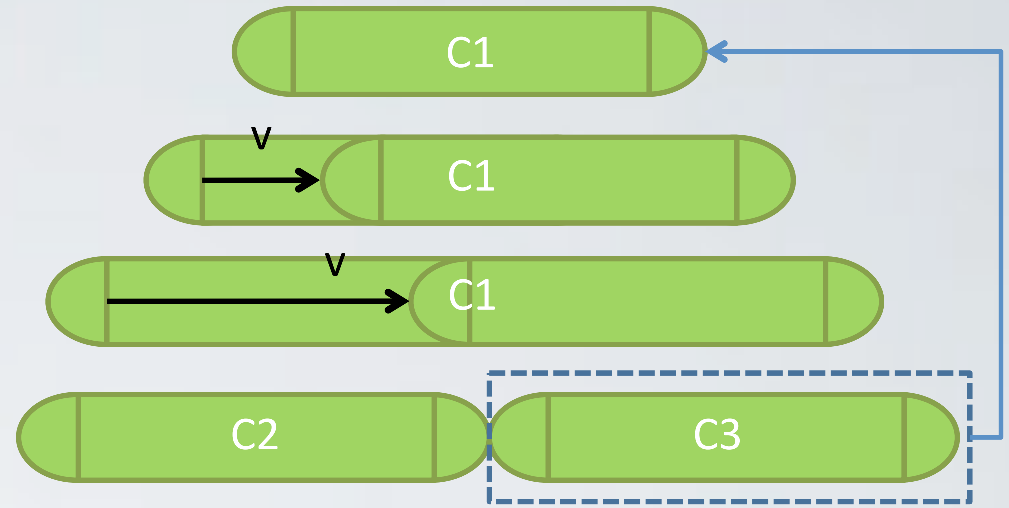


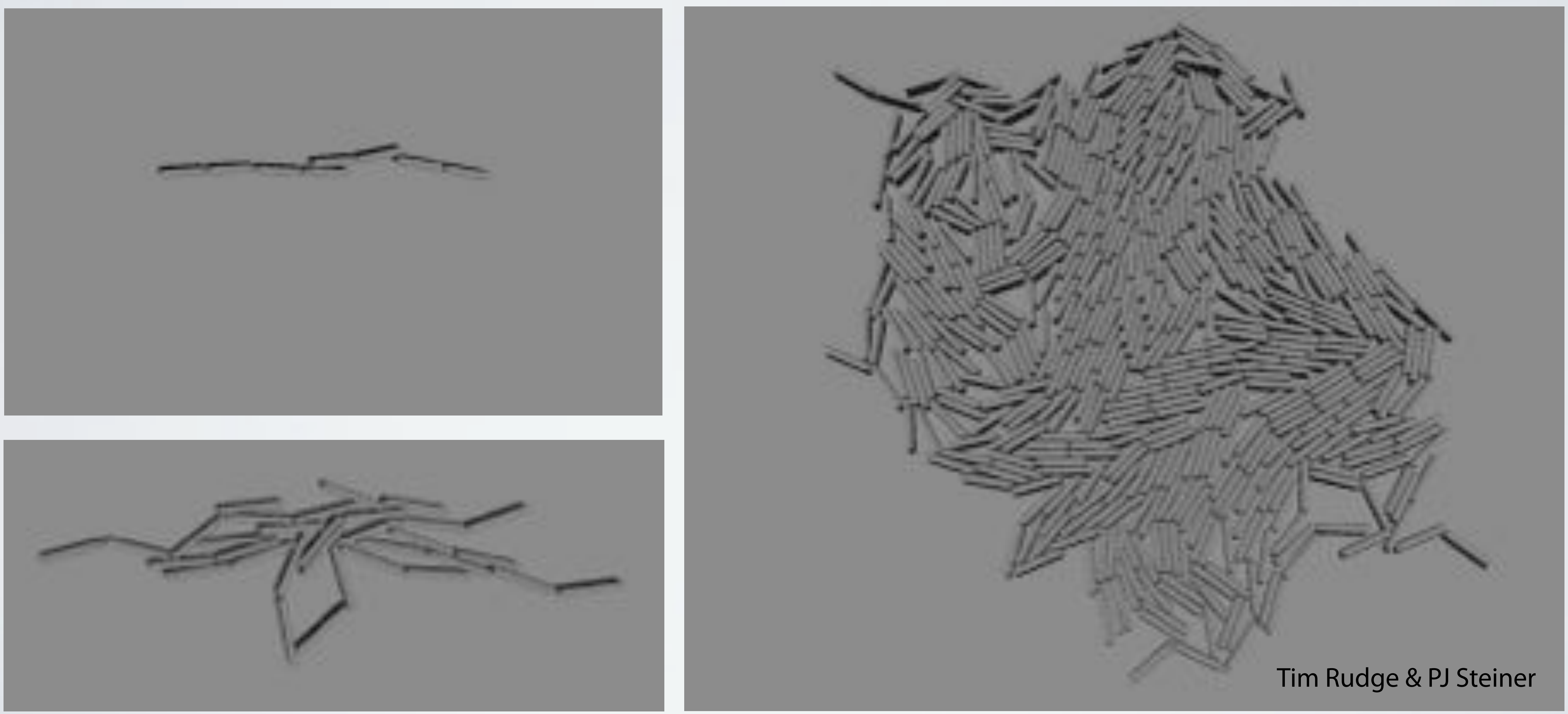
Fernan Federici

Folding growth pattern due to buckling



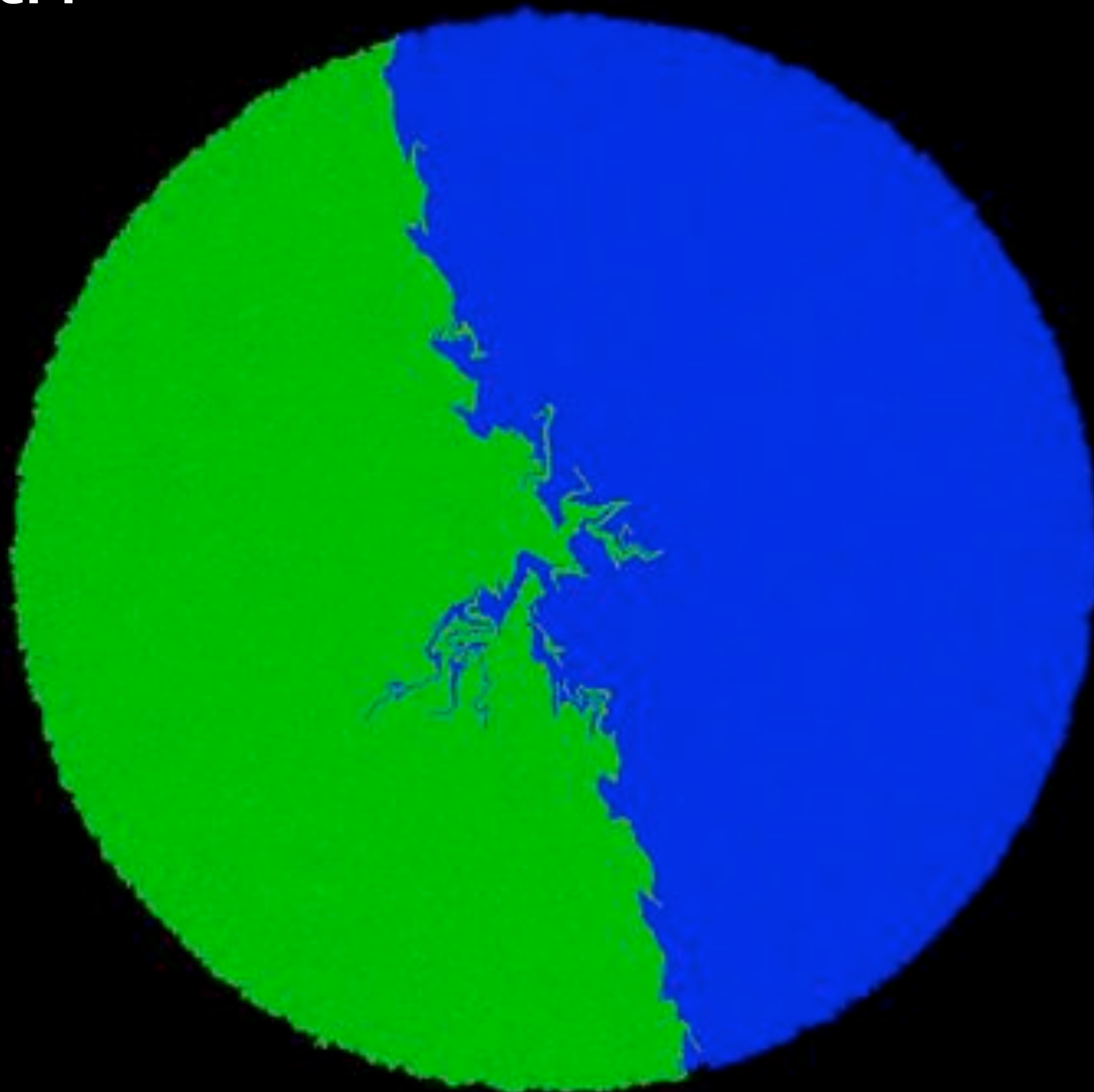
3D Rigid body dynamics





Software model for 3D growth of *Bacillus subtilis*

CellModeller4

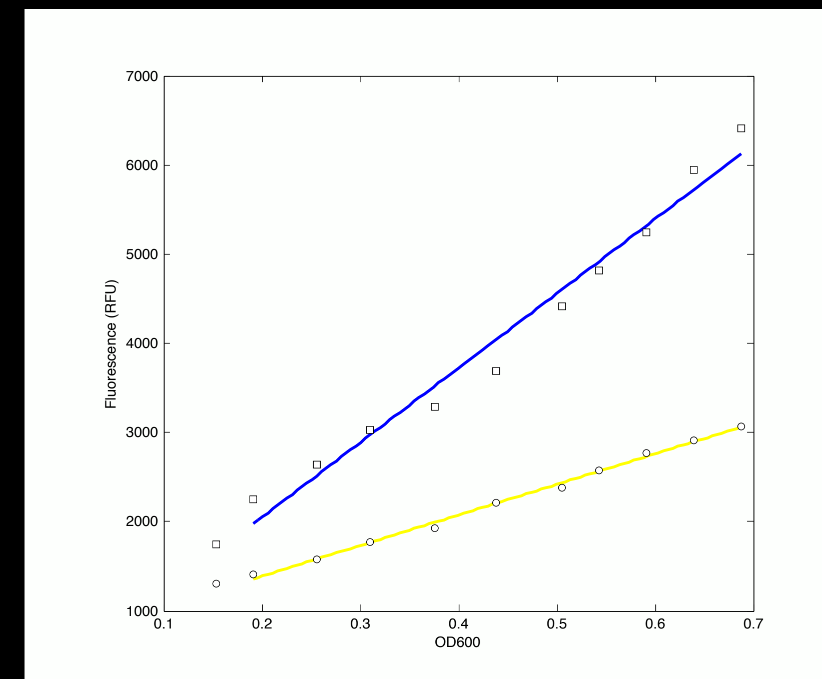


Growth of marked daughters into 100,000 cell colony

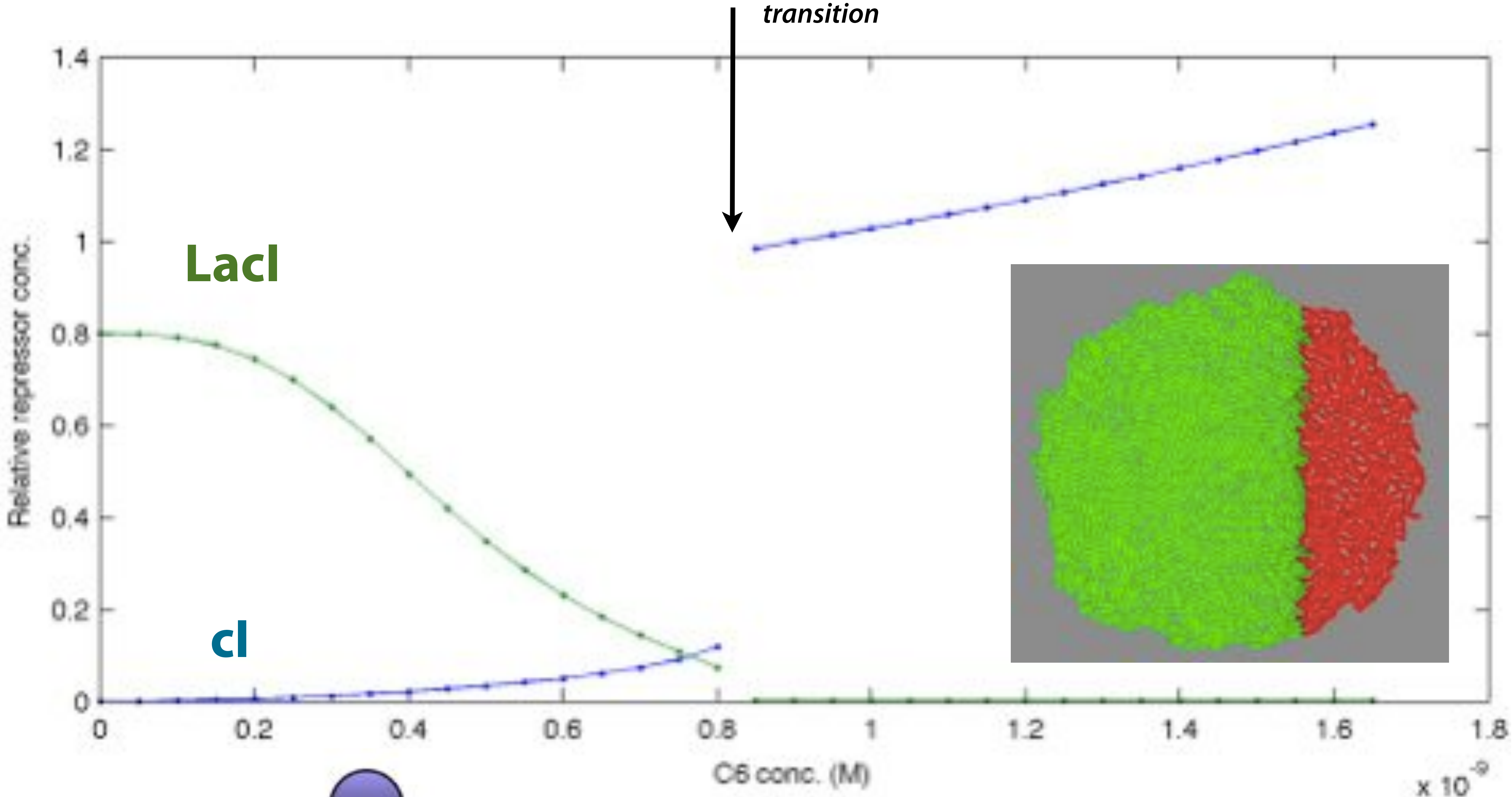
Tim Rudge & PJ Steiner

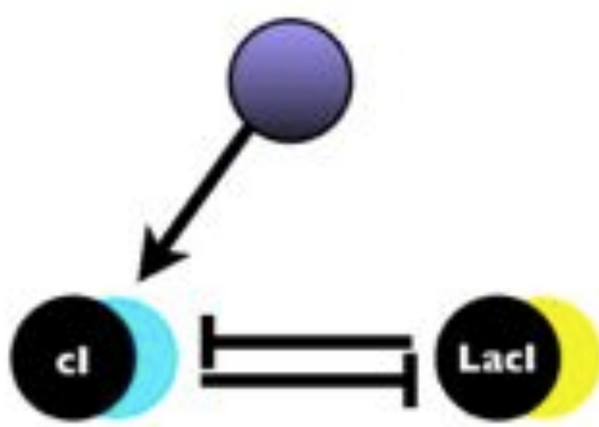
Measuring gene expression in living bacterial cells: ratiometric fluorescence measurement of promoter activity

James Brown & Fernan Federici



Modelling of metastable circuits





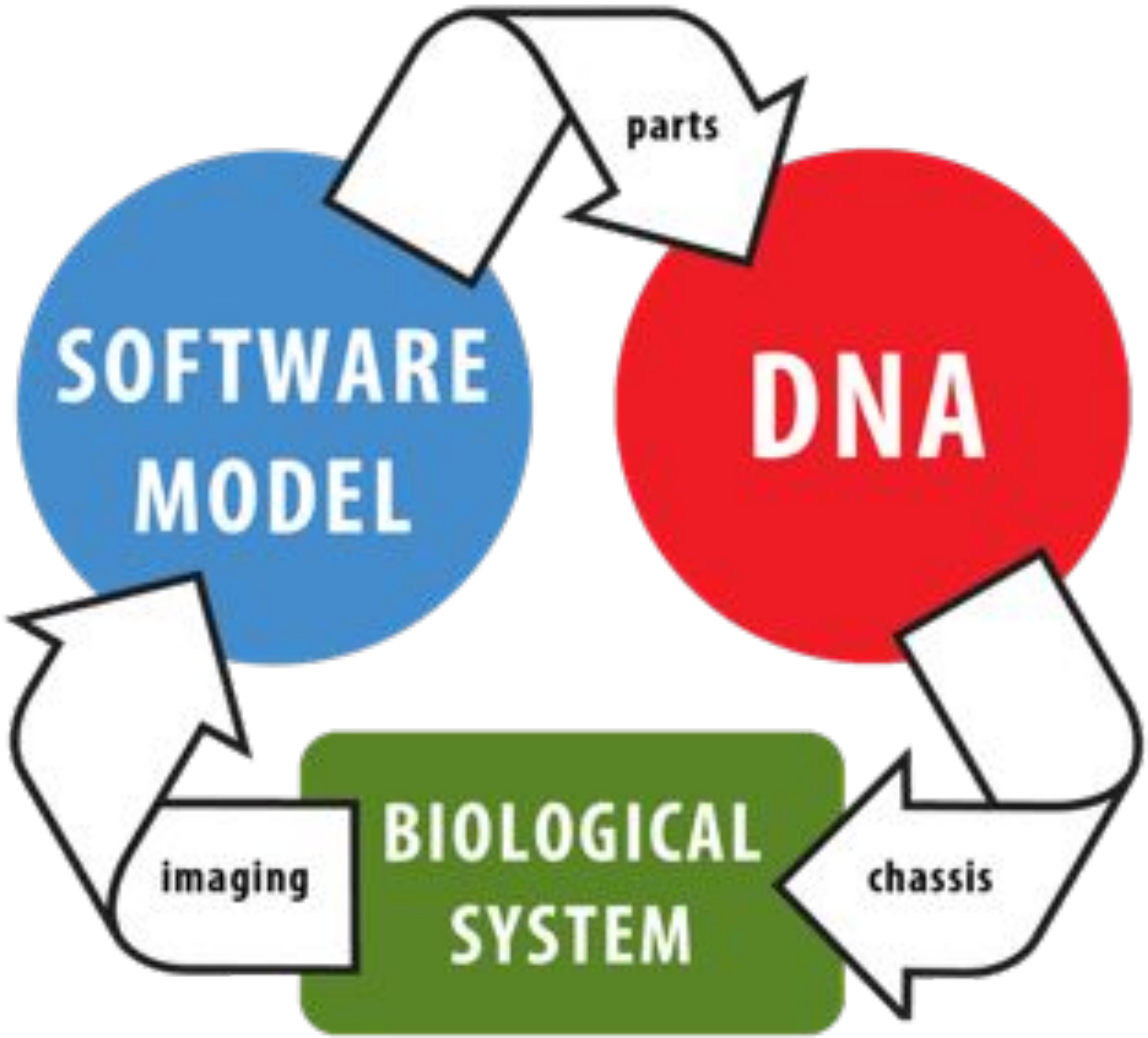
Fernan Federici, Tim Rudge and PJ Steiner

Design cycle for Synthetic Biology systems

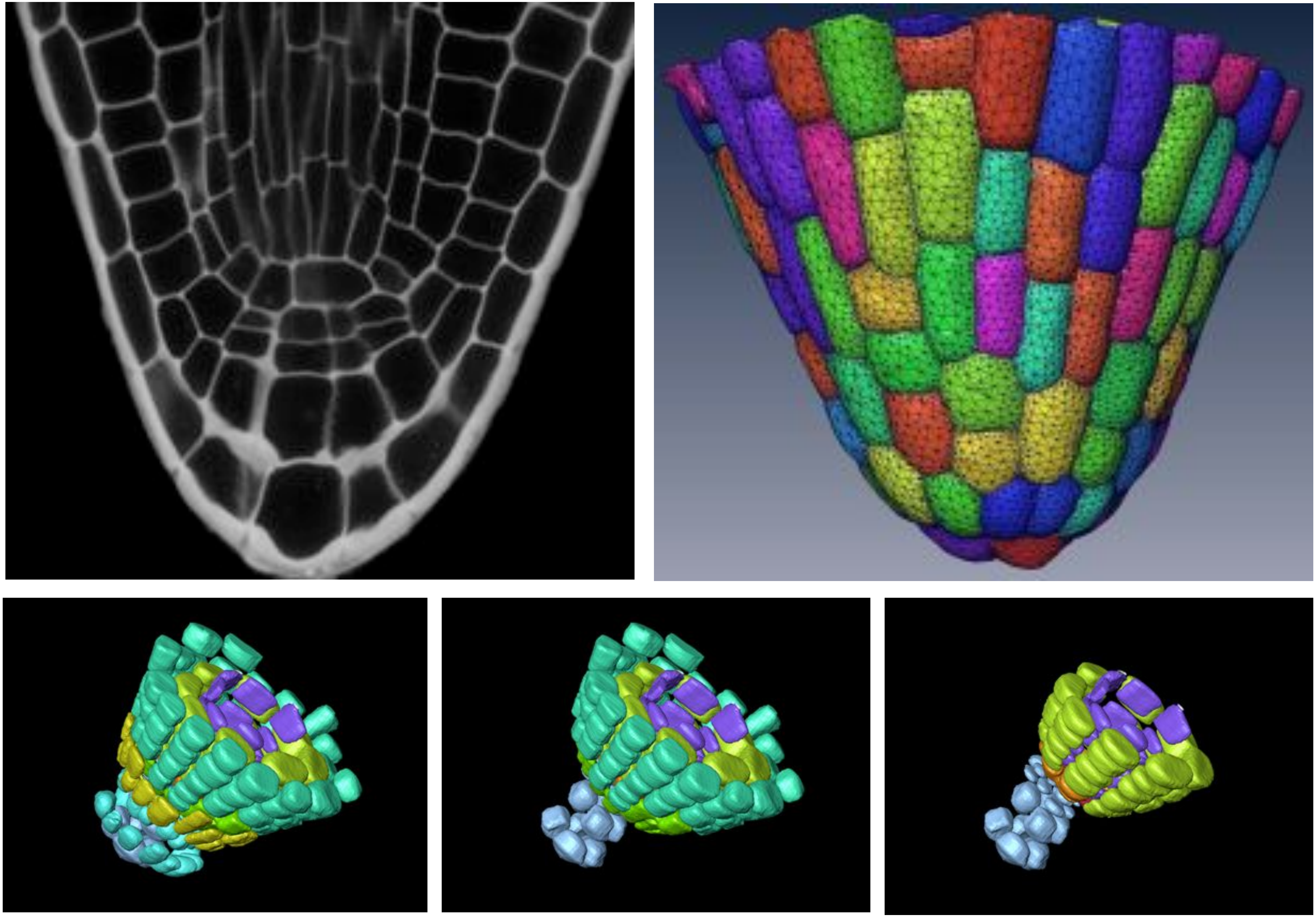
1. Specification and design of the system using computer models of the biological system

2. Construction of genetic circuits using standard DNA parts and high throughput assembly techniques

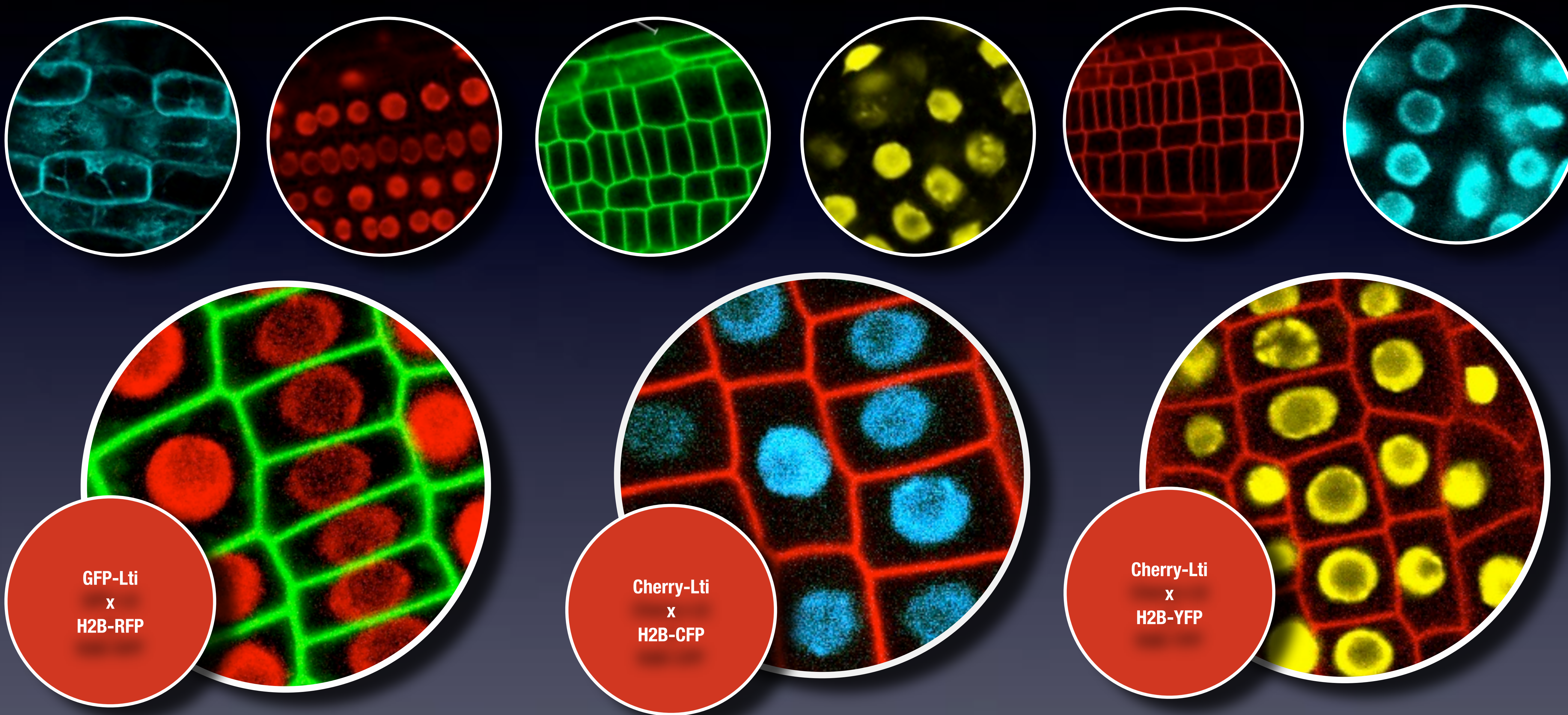
3. Transformation of chassis and visualisation of gene expression, cell states and phenotype



3D optical reconstruction of cellular features in plant tissues.



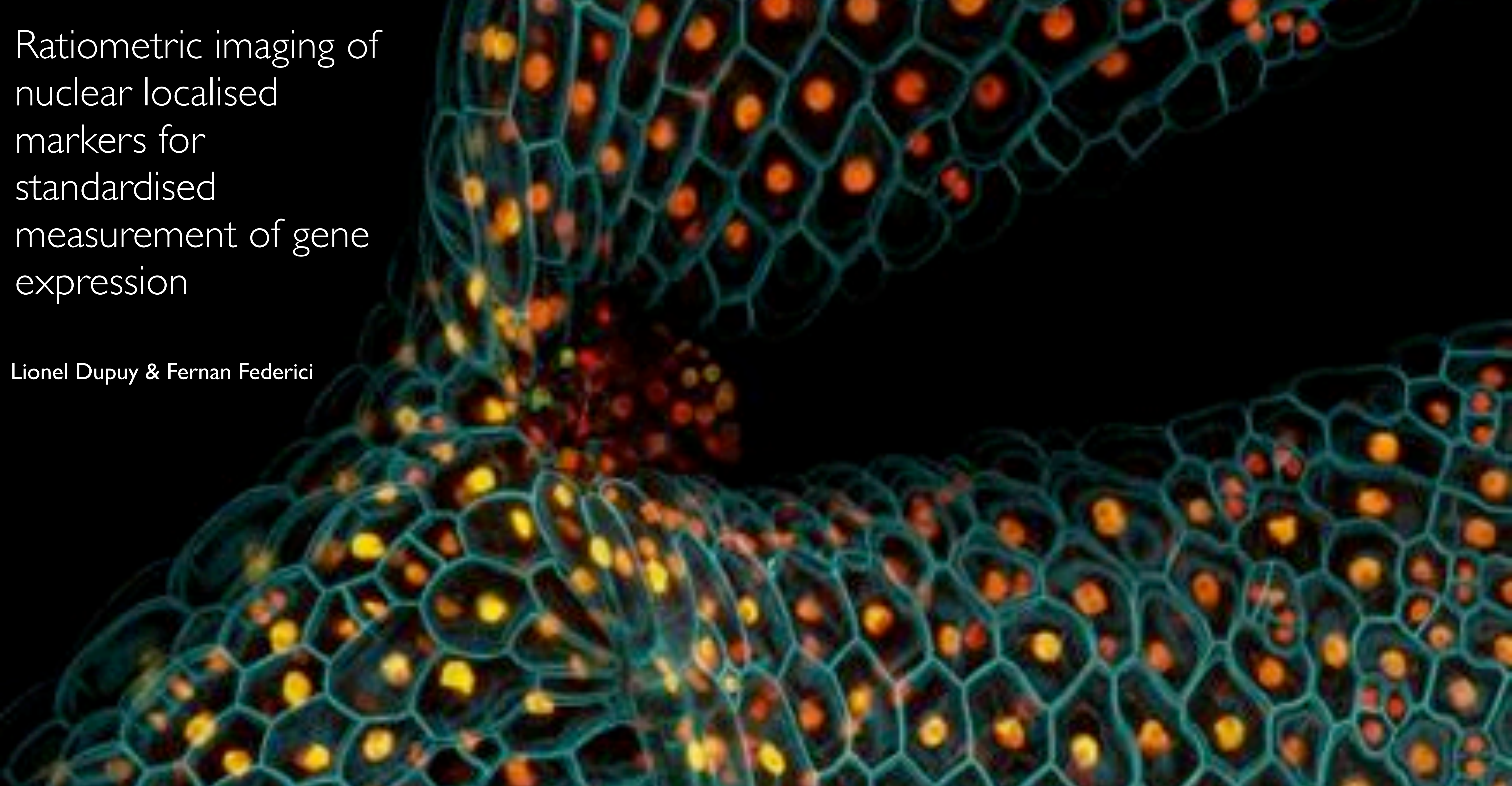
Live imaging of gene expression

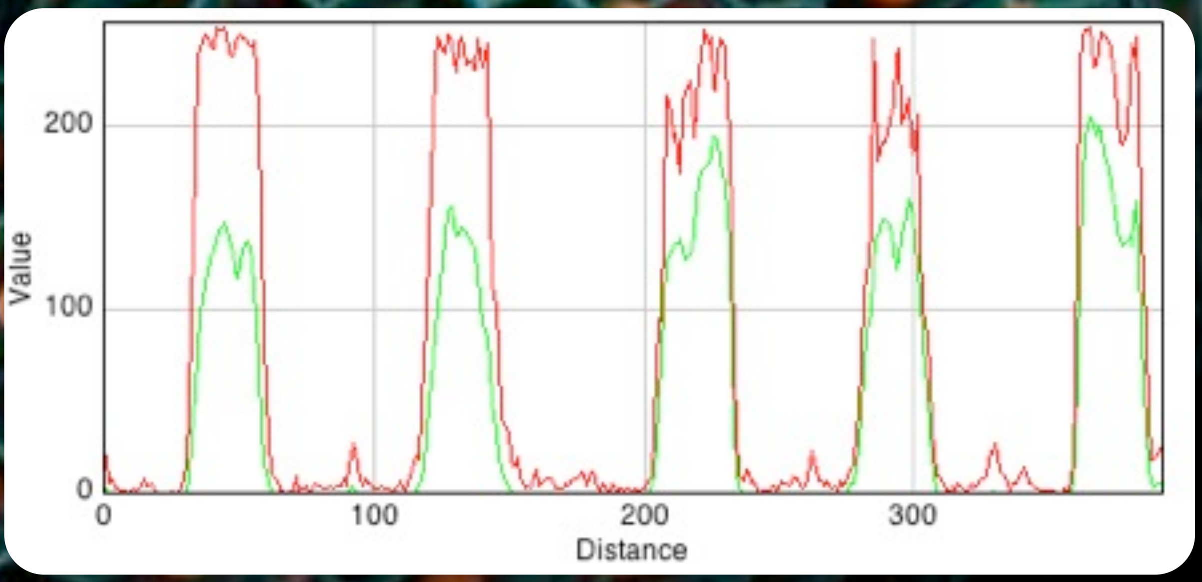


Fernan Federici

Ratiometric imaging of nuclear localised markers for standardised measurement of gene expression

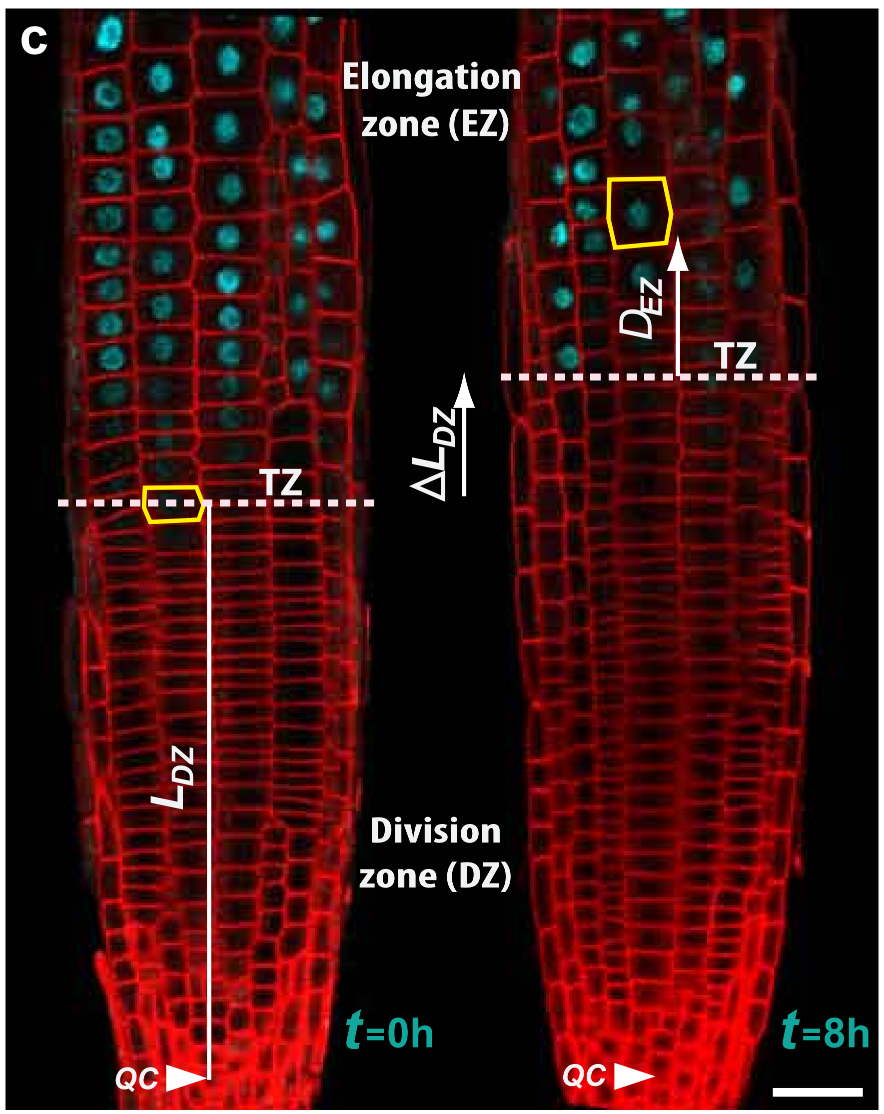
Lionel Dupuy & Fernan Federici

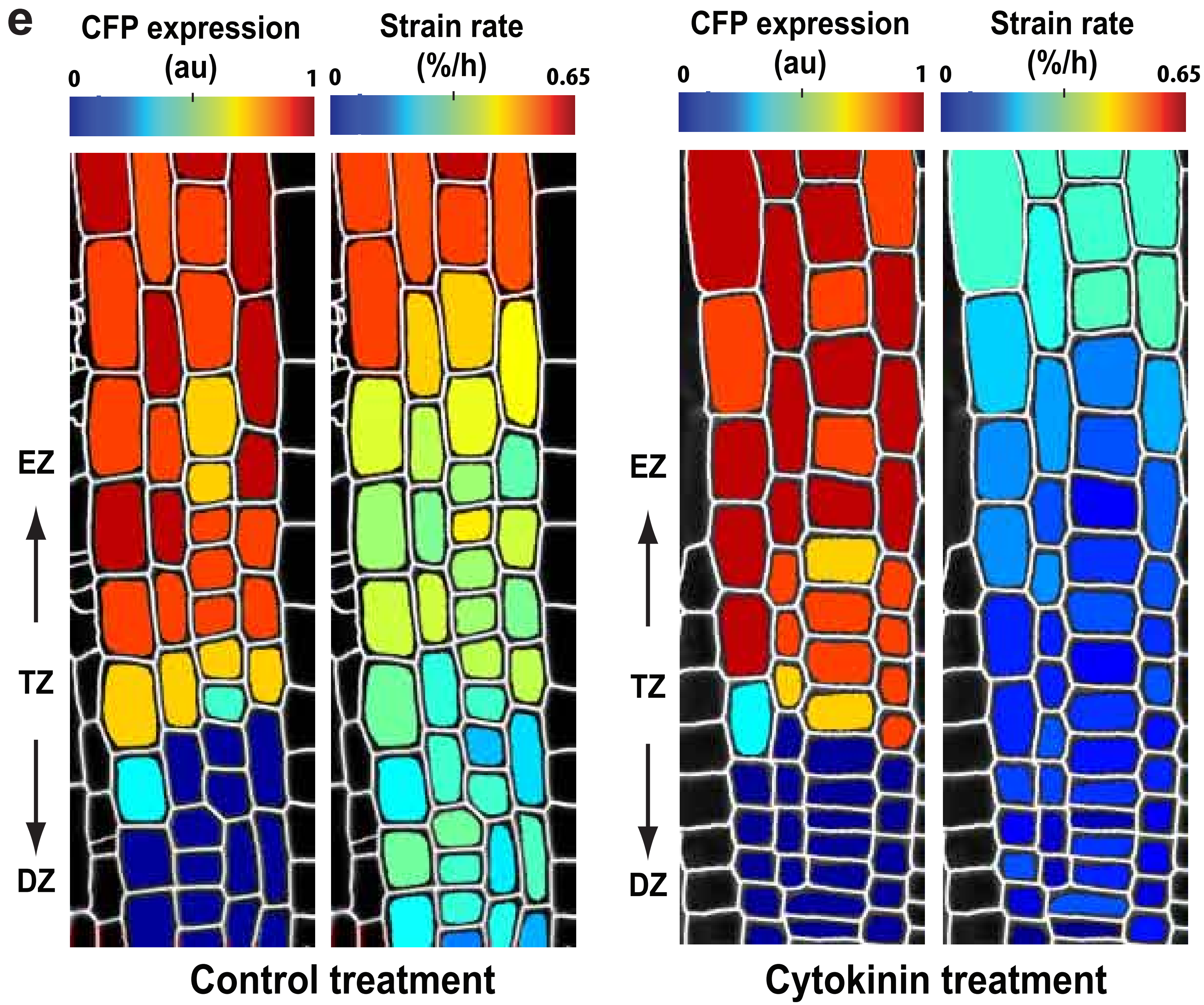




In planta cytometry

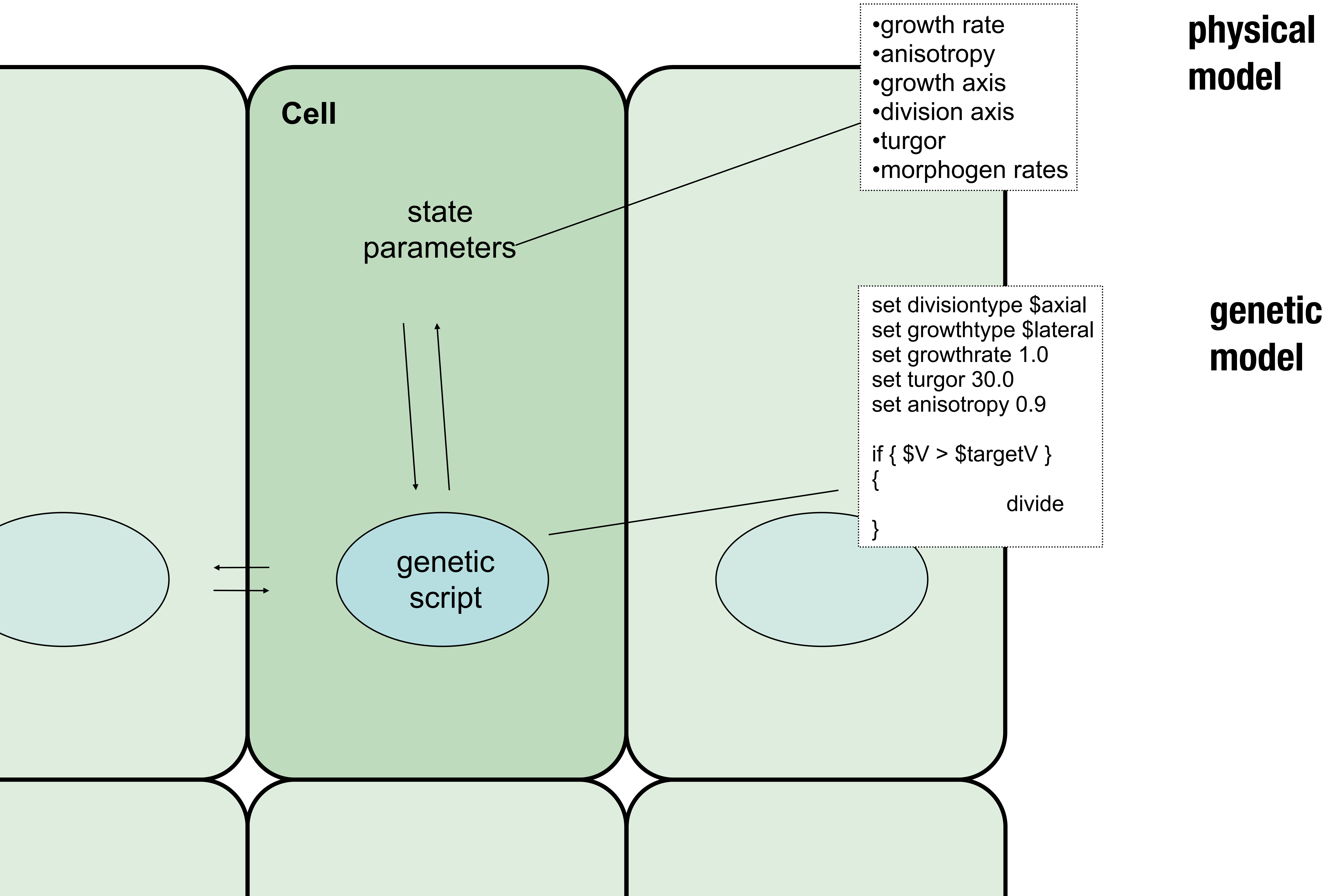
Provides a quantitative view of bacterial biofilms and plant microarchitecture and dynamics of cell shape and gene expression



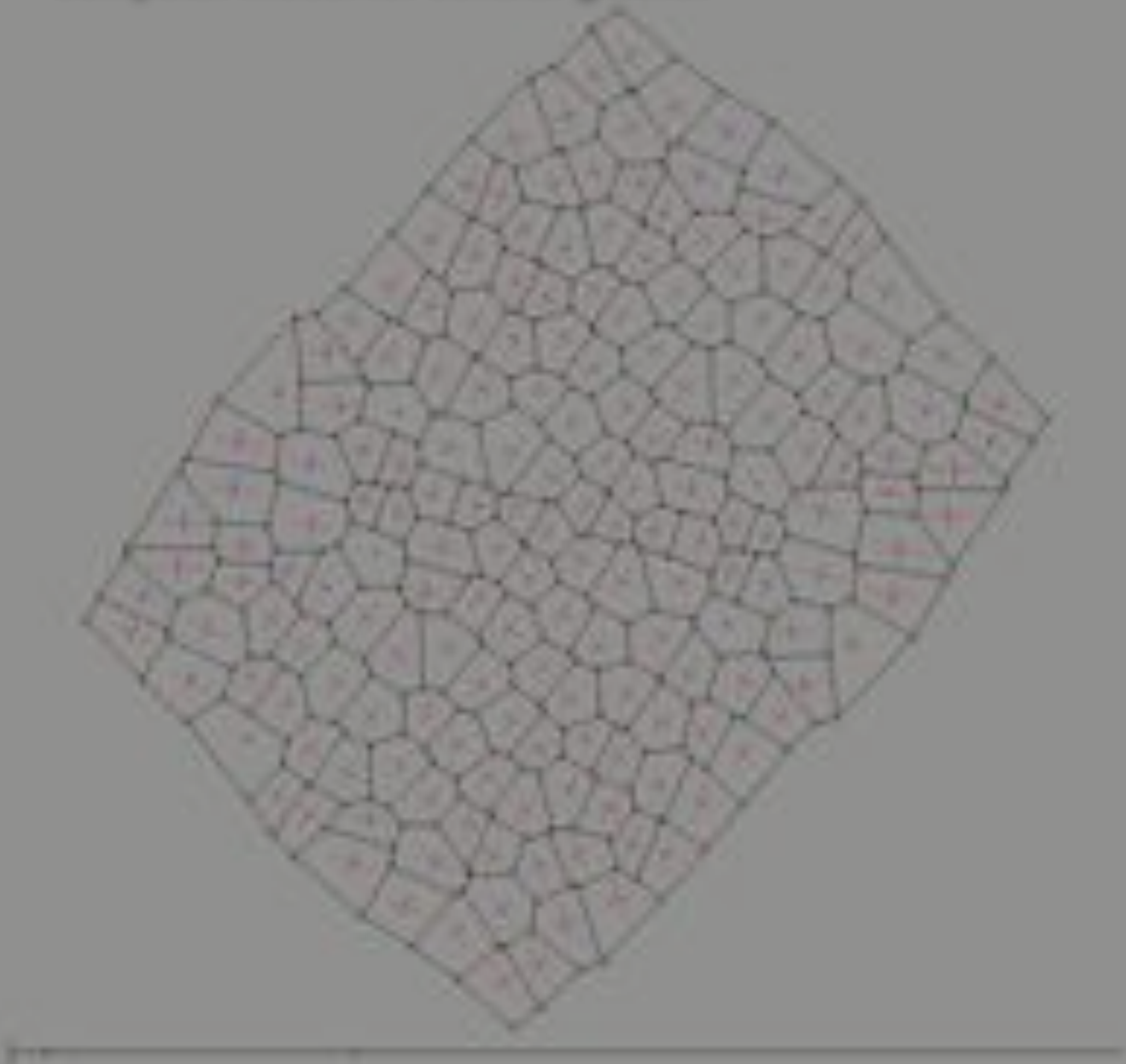


Integrated genetic and computation methods for in planta cytometry, **Nature Methods,** 2012
Fernán Federici, Lionel Dupuy, Laurent Laplaze, Marcus Heisler & Jim Haseloff

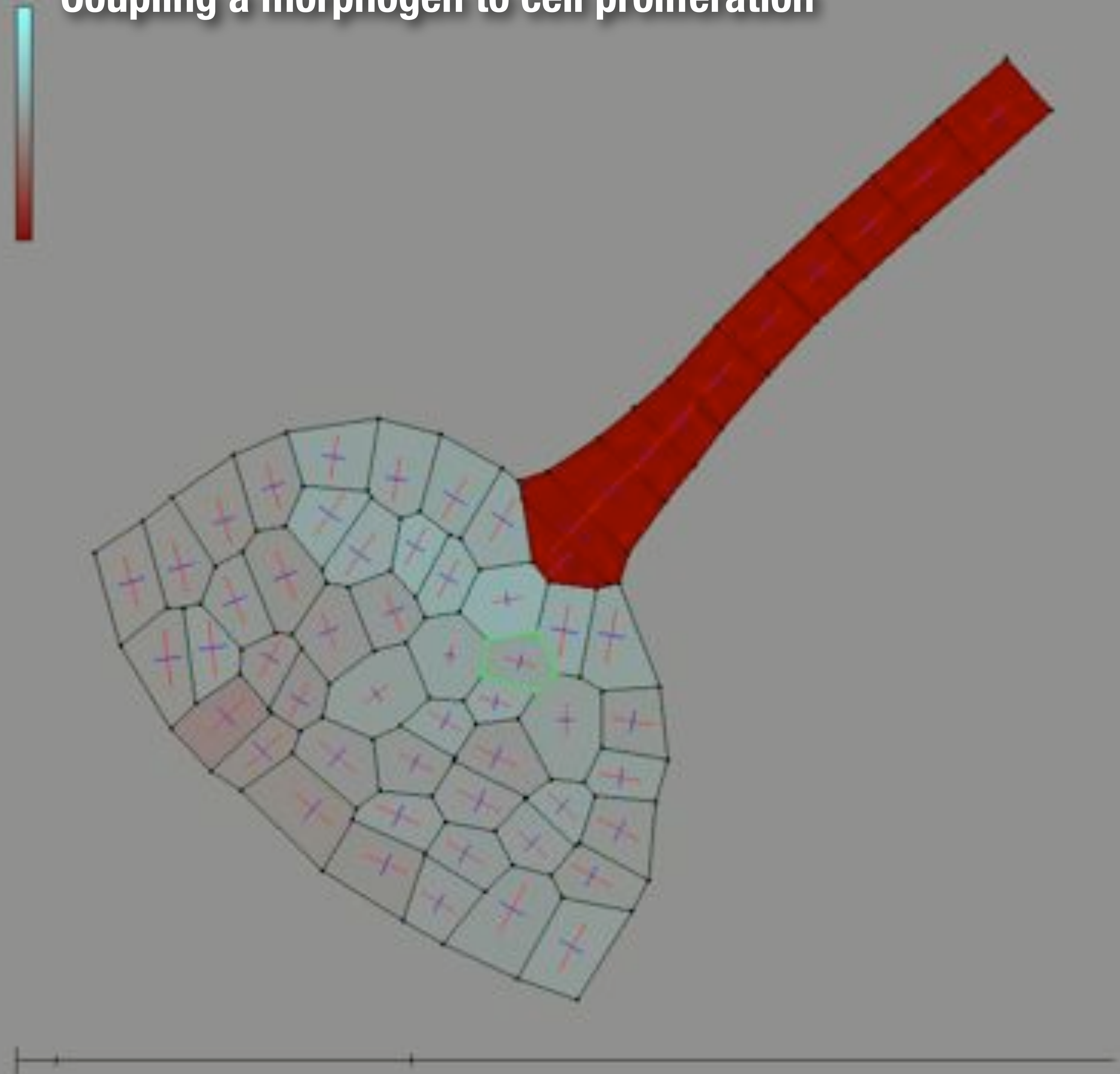
Cellular automata models for plant morphogenesis



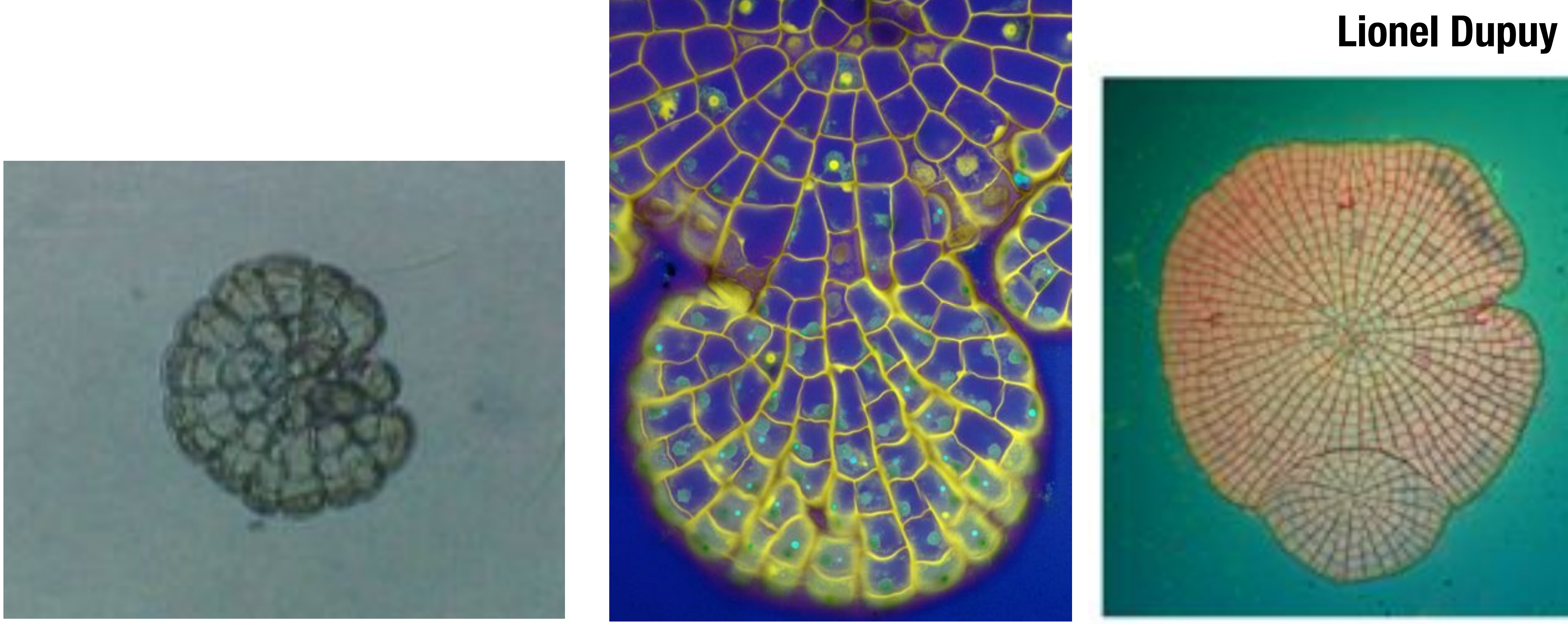
Computer model for cellular growth

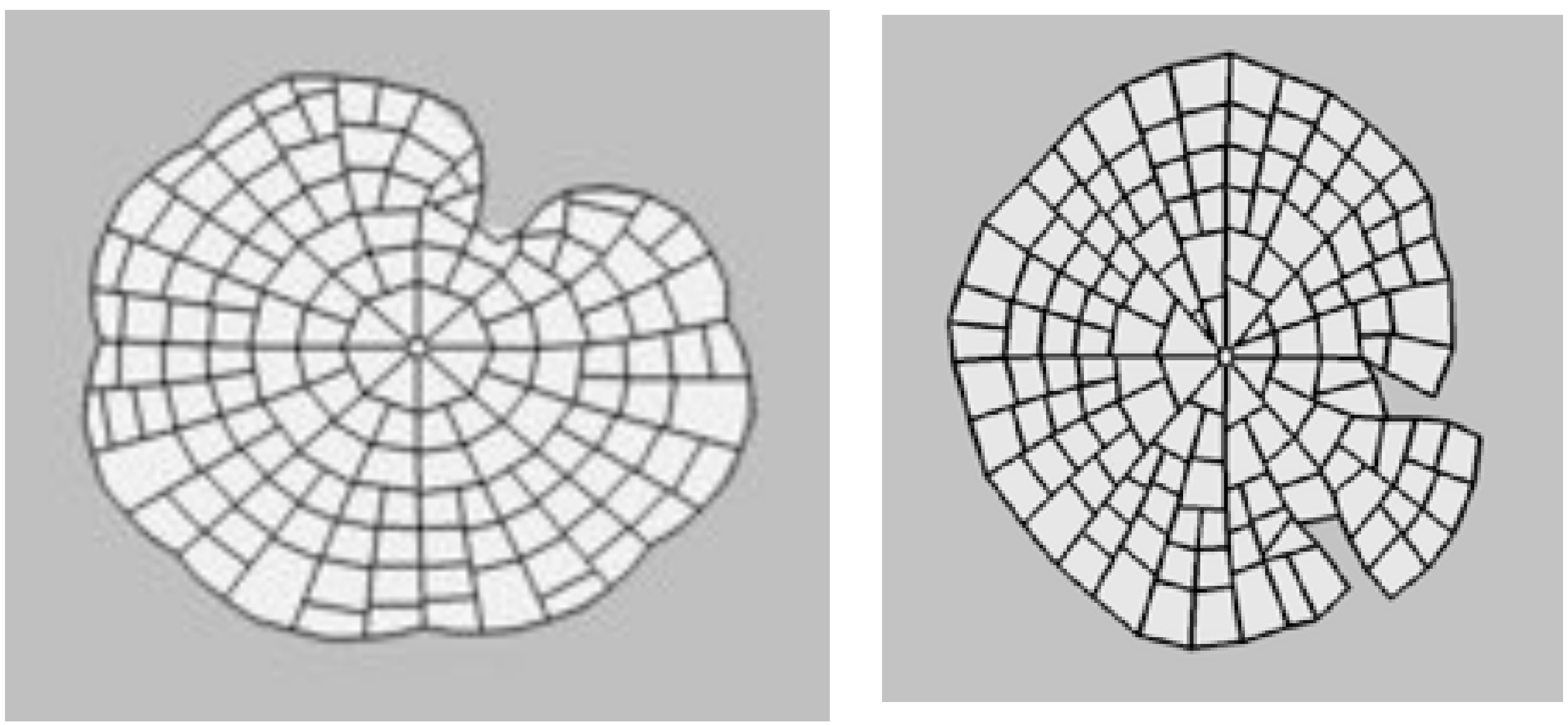


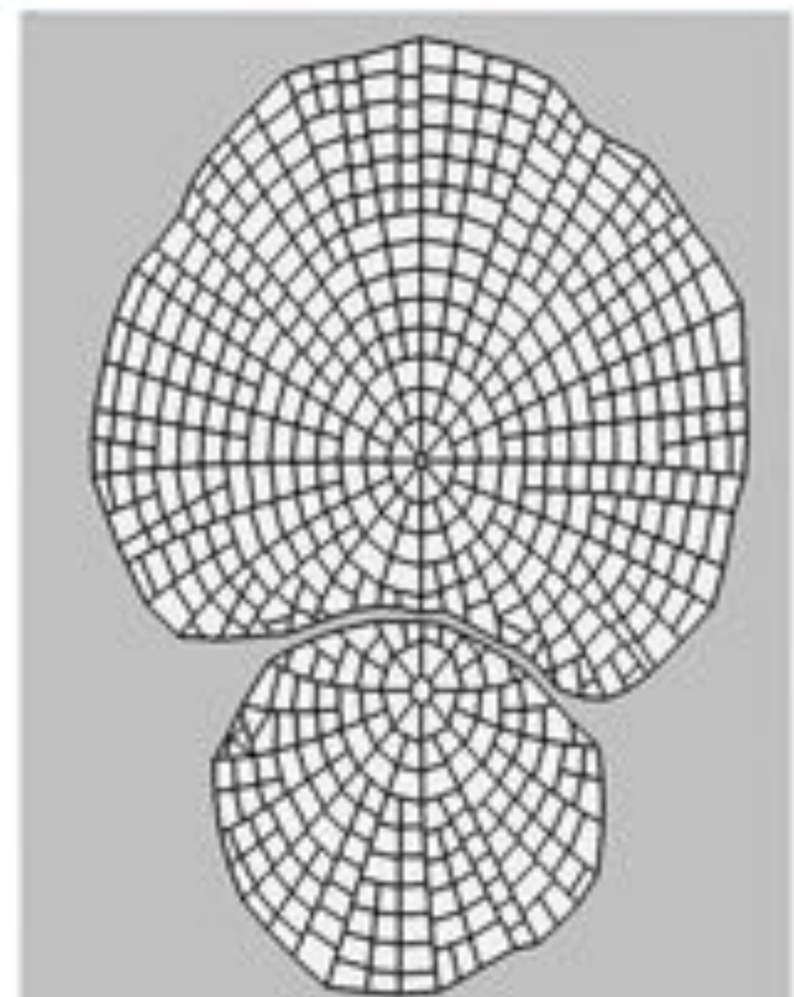
Coupling a morphogen to cell proliferation



Lionel Dupuy







Modelling growth of Coleochaete

Testbed for synthetic biology in plants

Testbed for synthetic biology in plants

Marchantia polymorpha

- Thalloid liverwort
- Descendant of earliest land plants
- Gametophyte dominant phase of lifecycle
- Haploid genetics
- Vegetative propagation by gemmae
- Easily propagated *in vitro*
- Gametes induced by far-red light
- Crossing easy
- Spores stable for >1 year
- EST collection available
- Easily regenerates in tissue culture
- High frequency transformation
- Y chromosome and plastid genomes seq'd
- 280 MB genome sequence due soon

www.marchantia.org

Marchantia has a highly simplified genome

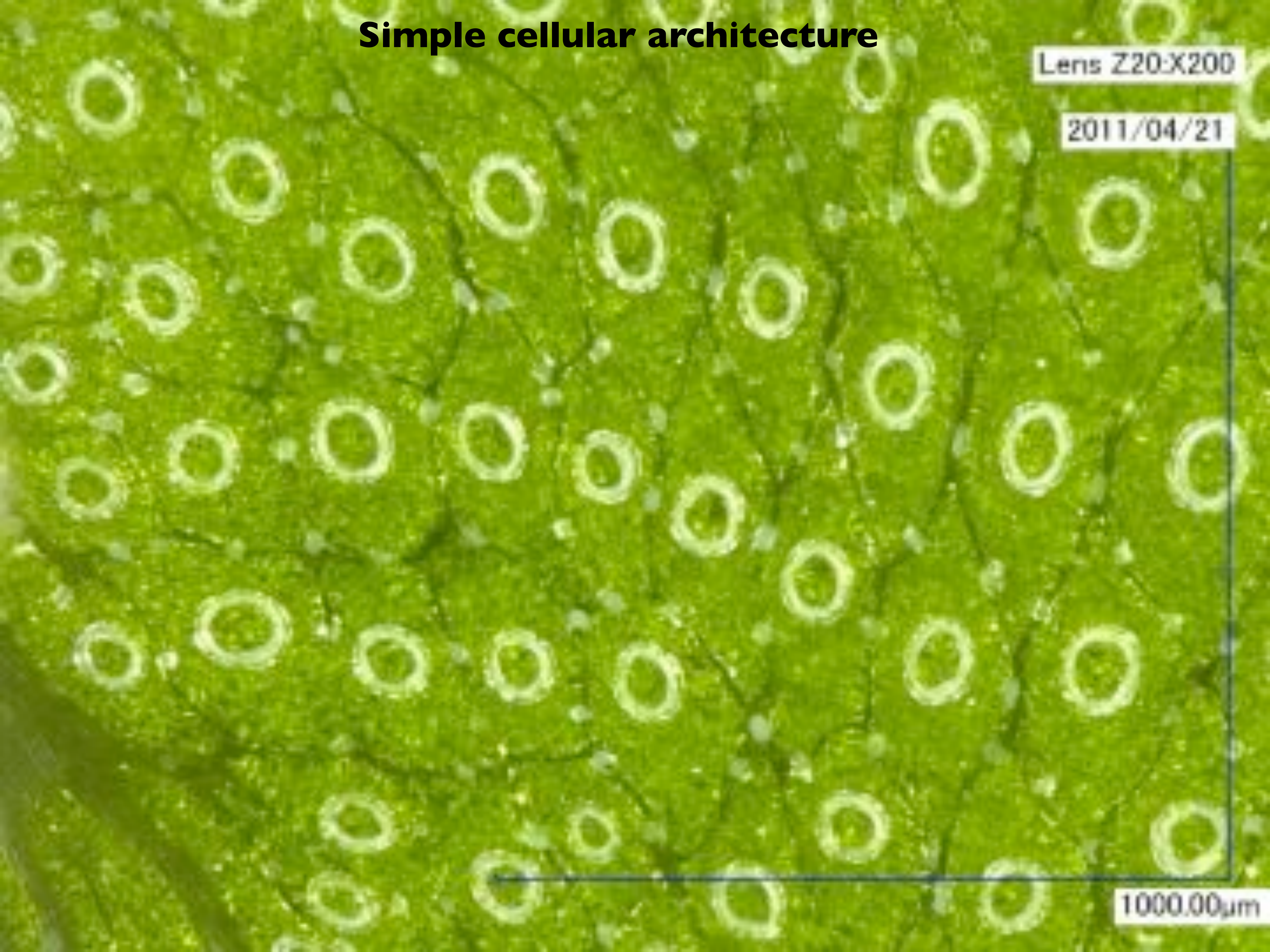
Gene Family	*Arabidopsis*	*Selaginella*	*Physcomitrella*	*Marchantia**
ARF	23	7	14	3
AUX/IAA	29	3	2	1
TIR1	6	2	4	1
TPL	5	3	2	1
TAA	3	1	4	1
GH3	19	17	2	1
Class I HD-Zip	17	4	17	1
Class II HD-Zip	10	2	7	1
Class III HD-Zip	5	3	5	1
Class IV HD-Zip	16	4	4	1
Class I KNOX	4	2	3	1
Class II KNOX	4	3	2	1
BELL homeobox	13	2	4	1
WOX	16	6	3	1
CLV3 (CLE)	28	6	4	1

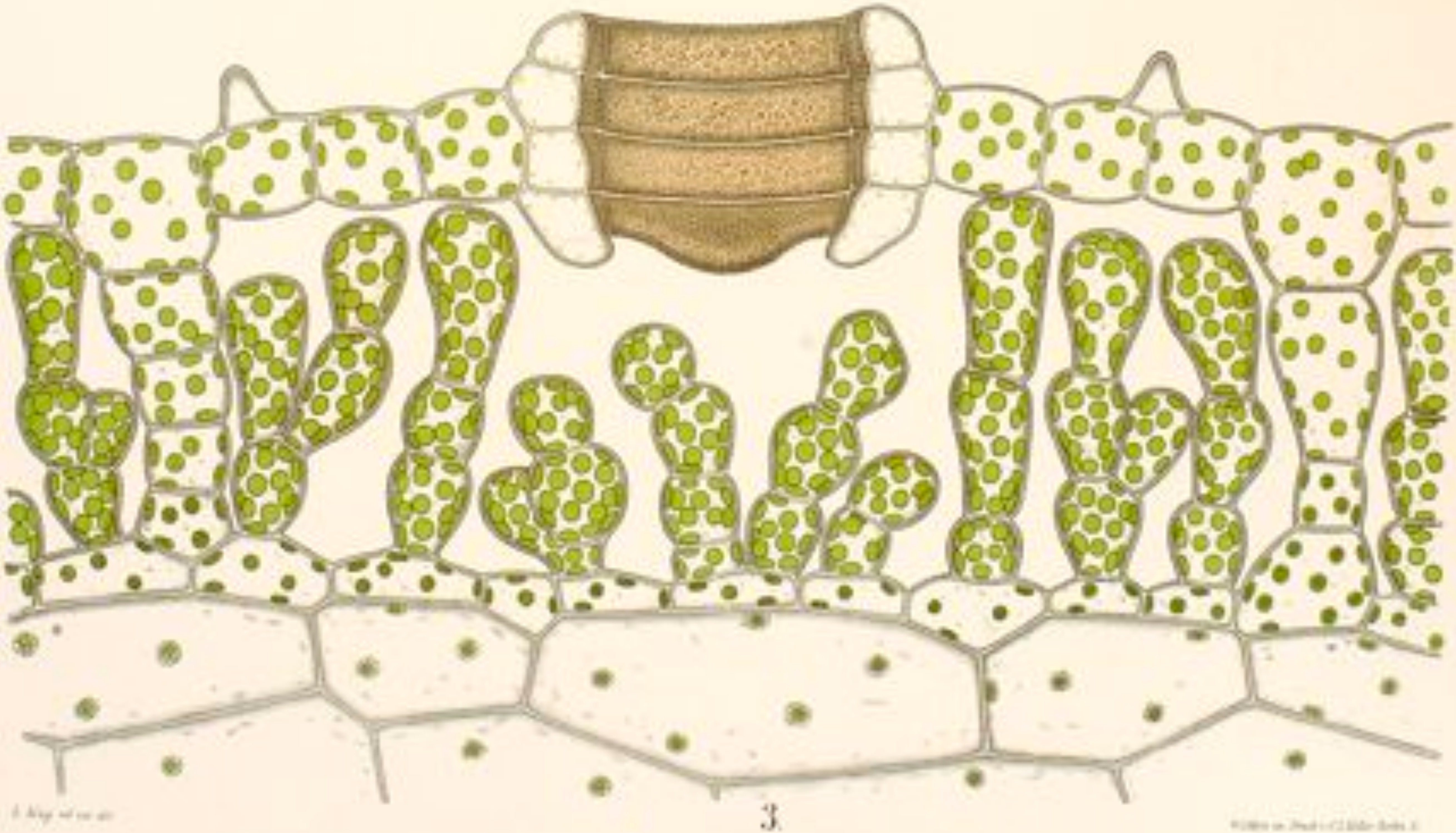
*Based on EST data (>2,300,000) represents a minimum number

Hirotaka Kato, Kimitsune Ishizaki, Takayuki Kohchi
Grad. Sch. of Biostudies, Kyoto Univ.

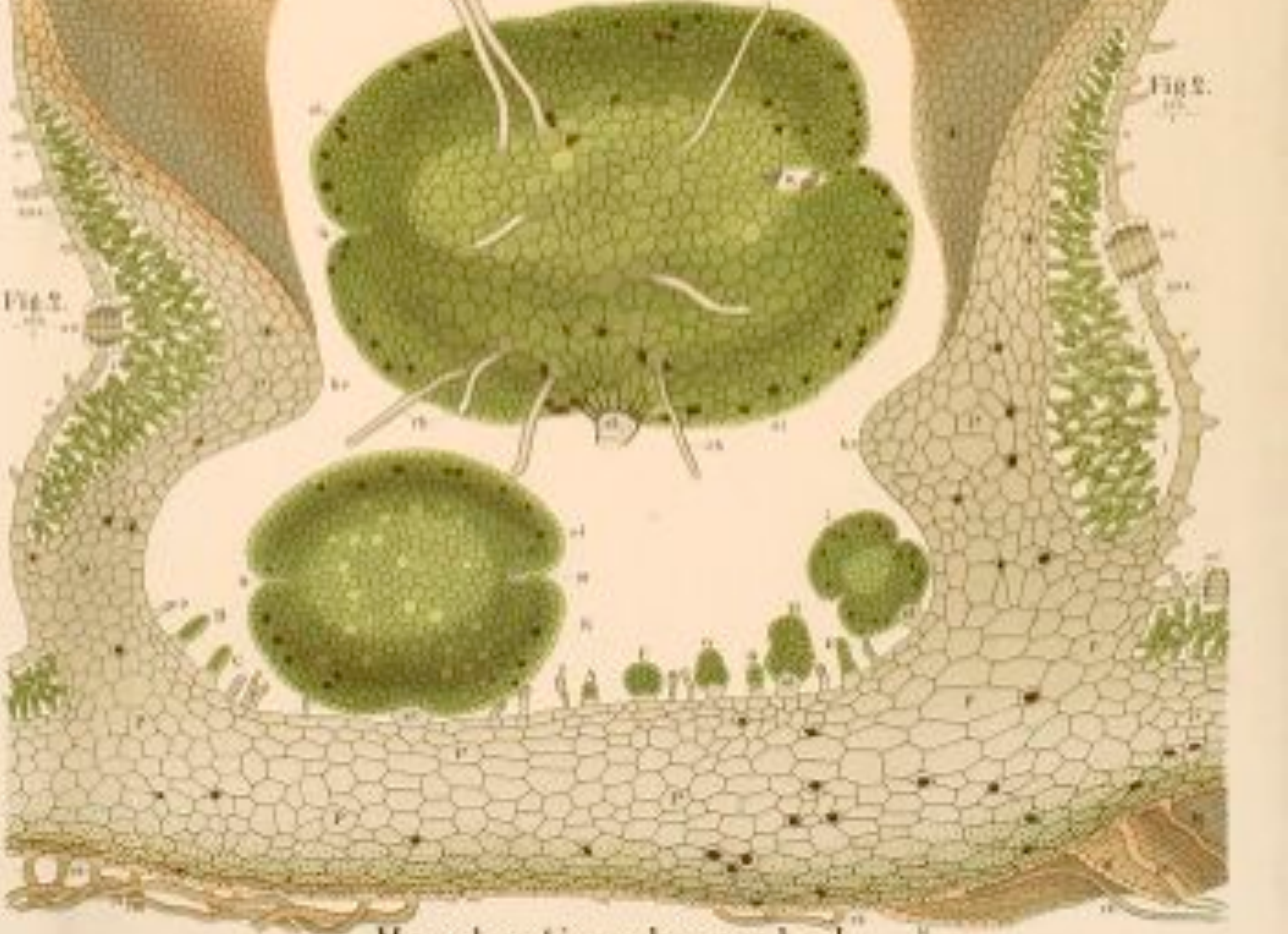
In collaboratioin with J. Bowman's lab, Monash

Simple cellular architecture



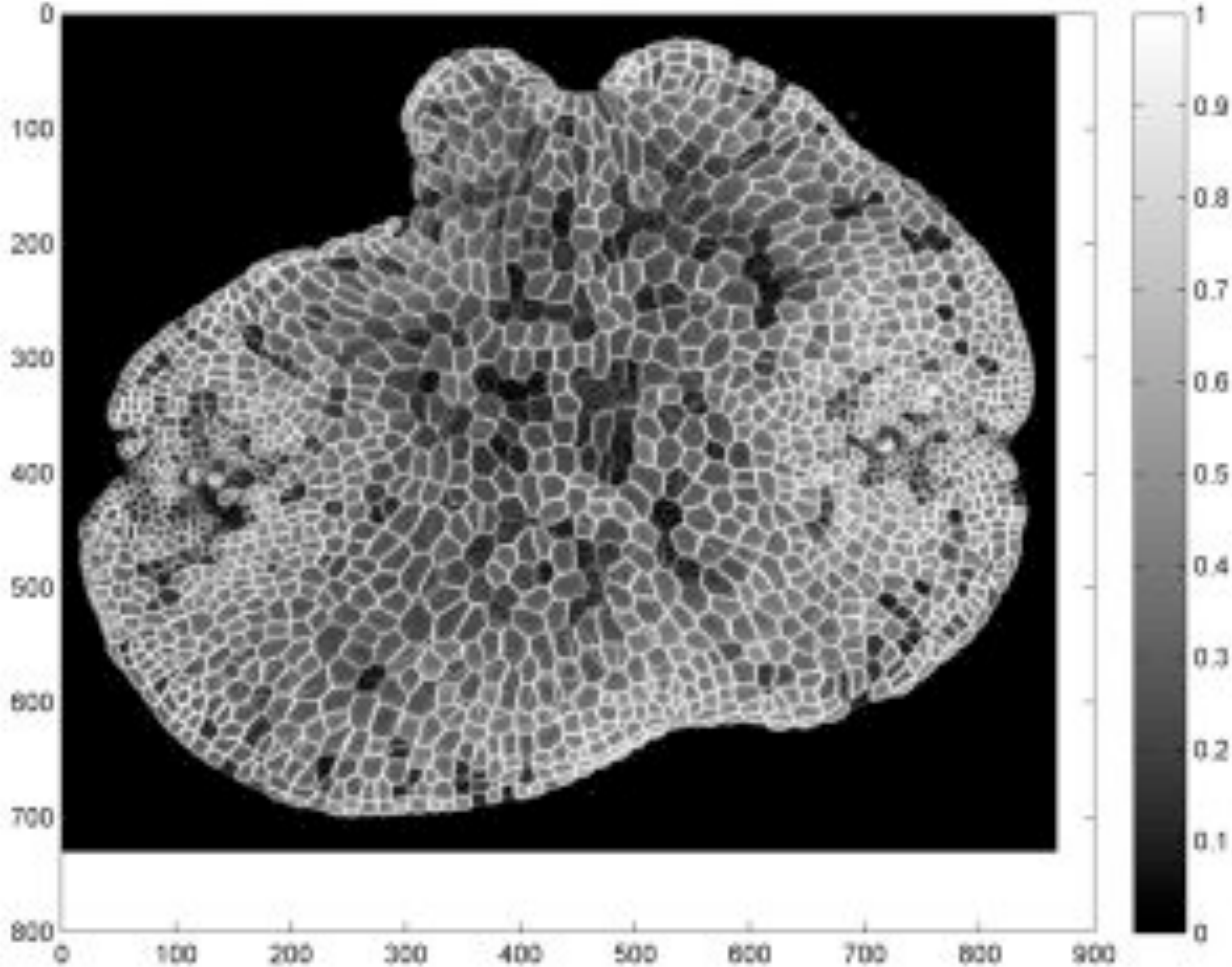


Transverse section of Marchantia polymorpha thallus (Leopold Kny)



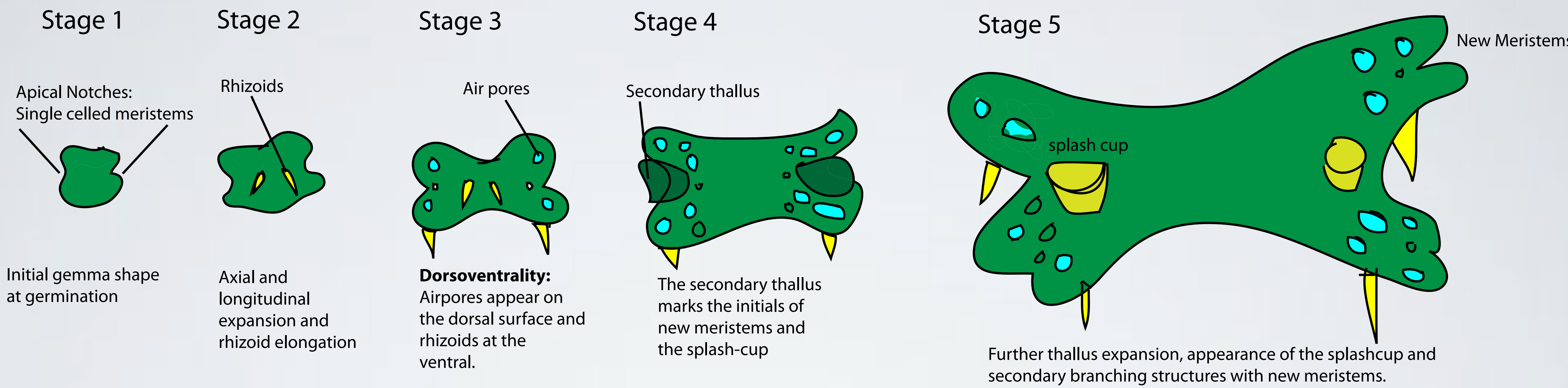
Marchantia polymorpha L.

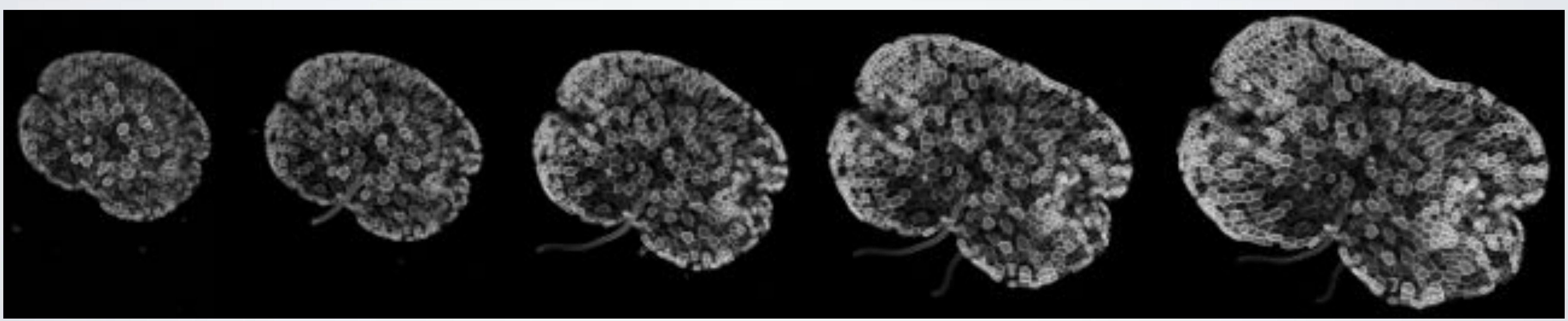
High resolution imaging of gemma



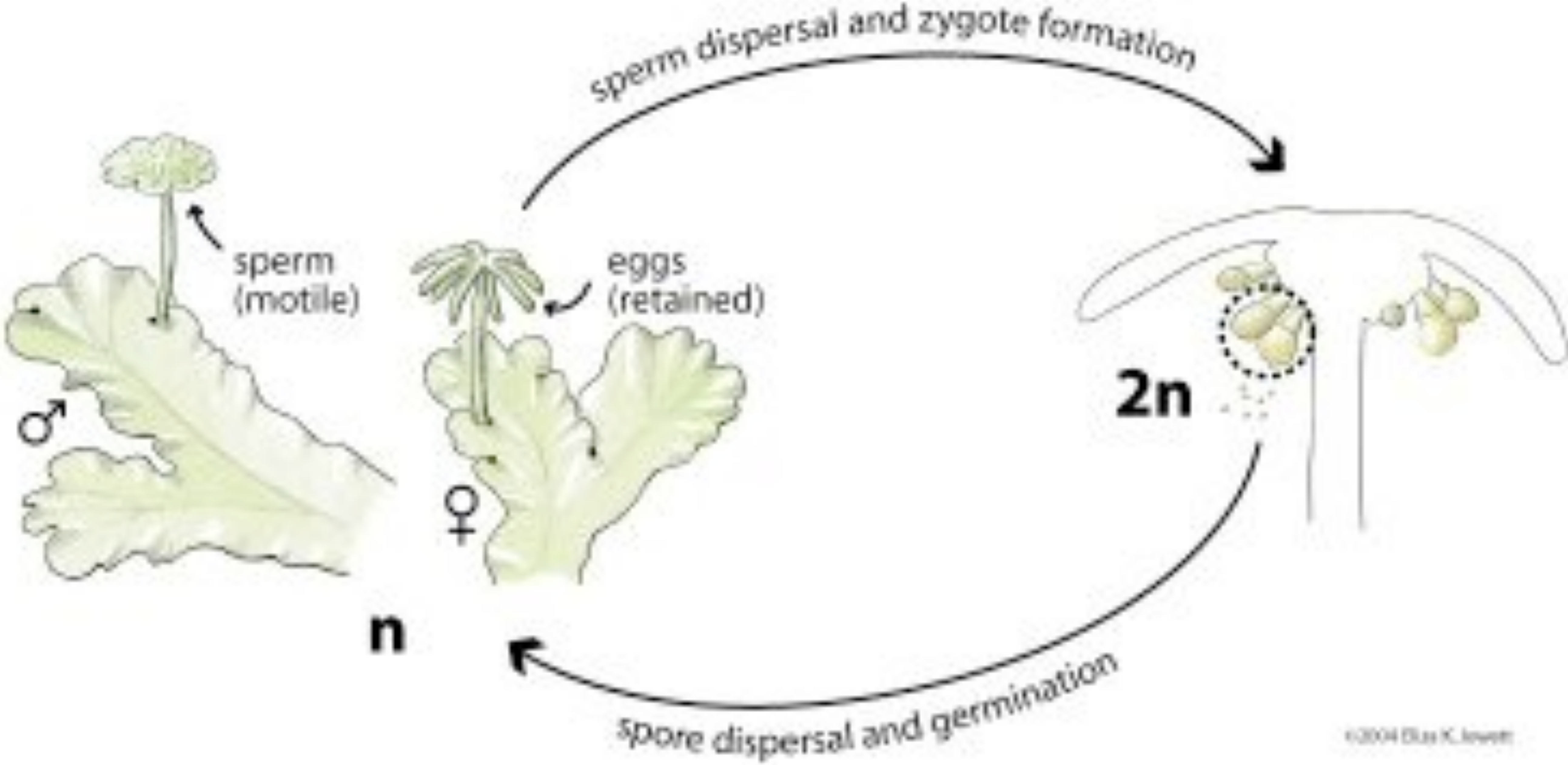
Nuri Purswani

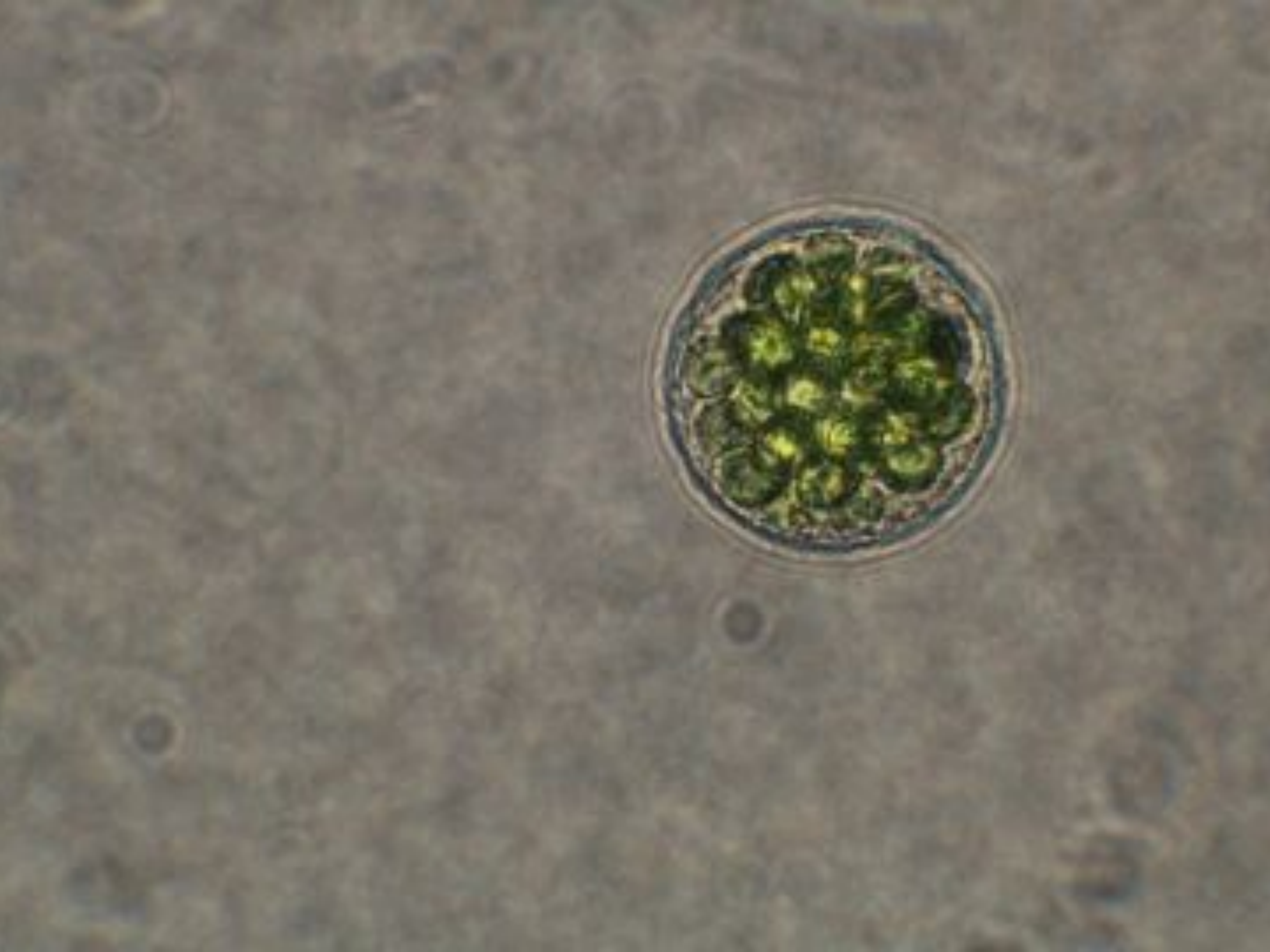
Staging of Marchantia gemma development





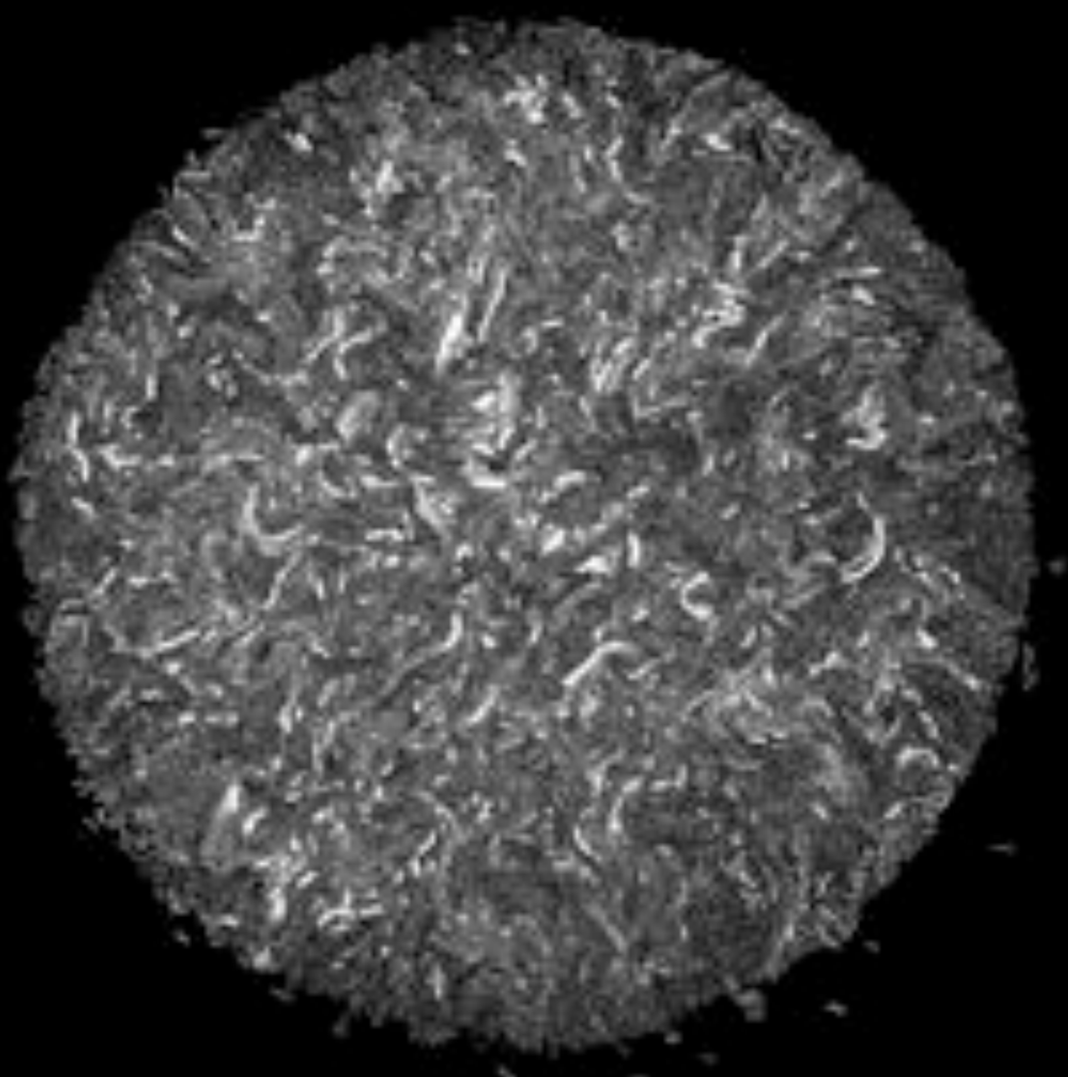
Nuri Purswani


sperm dispersal and zygote formation
sperm
(motile)
eggs
(retained)
♂
♀
n
2n
spore dispersal and germination

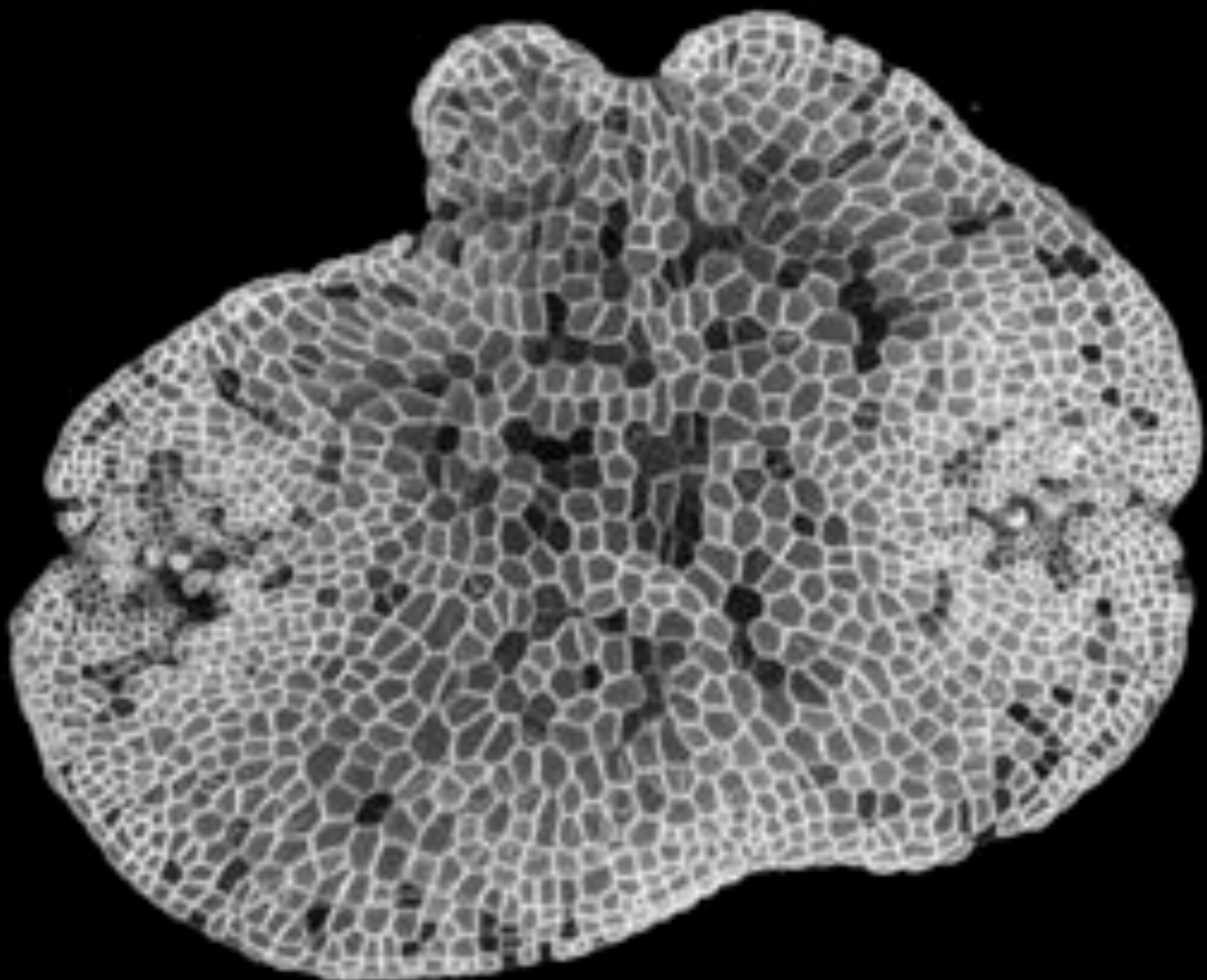


Model systems for engineering patterns in cell populations

microbes



plants



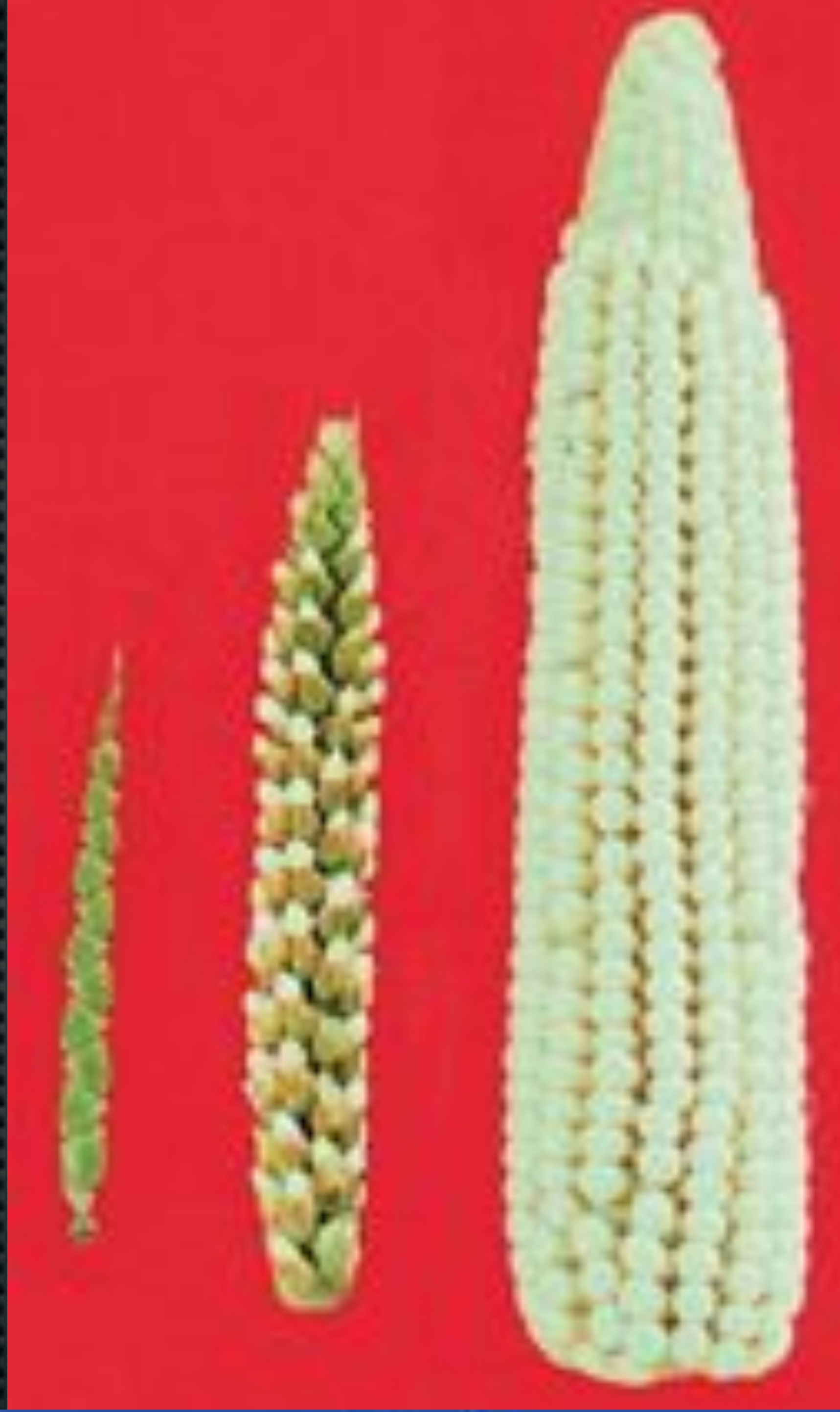
Rules and systems for engineering cell populations in plants

We need a new generation of feedback regulated genetic circuits with the ability to organise and coordinate cell cohorts.

GENERATION 1	promoter::gene
GENERATION 2	GAL4 > gene array
GENERATION 3	Turing-type population switch

Modern crop plants are derived from their natural ancestors by thousands of generations of selection and breeding.

What if we could reprogram the distribution of existing cell types in living systems?



James Brown

PJ Steiner

Paul Grant

Nuri Purswani

Fernan Federici

Li Hua Robertson

Tim Rudge

Michael Pedersen

Prof Chris Voigt
MIT

Dr Lionel Dupuy
SCRI, Dundee

Dr Jim Ajioka
Department of Pathology

Dr Andrew Phillips
Microsoft Research
Cambridge

Dr Neil Wipat
University of Newcastle

plus:

Judy Savitskaya
Anton Kan
Robyn Cooper