Quantitative analysis of *C. elegans* embryogenesis

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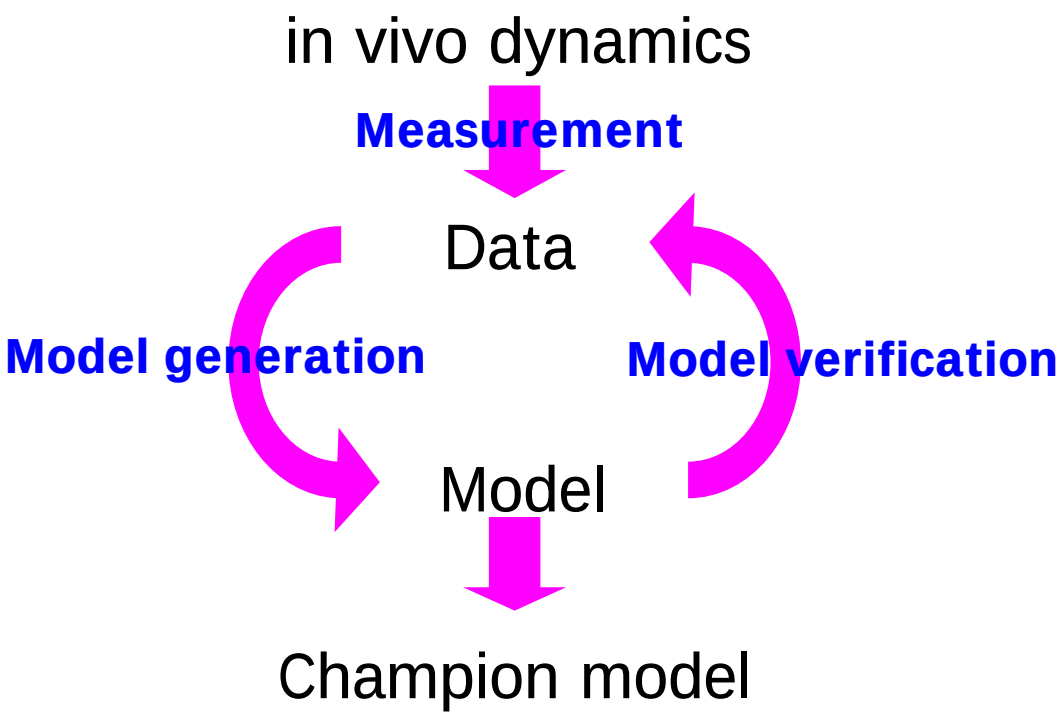
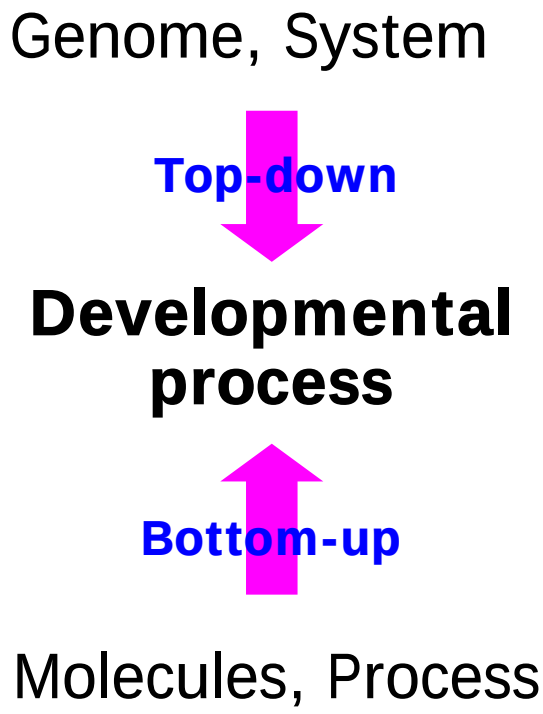
Caenorhabditis elegans

Strategy to understand developmental process





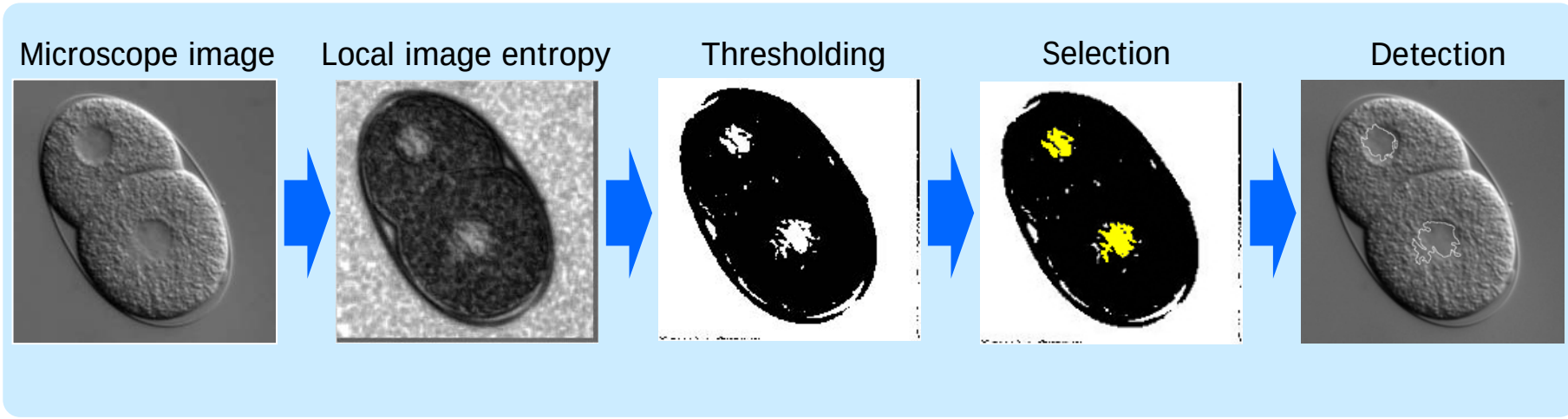
Computer image-processing and computer simulation provide great help!

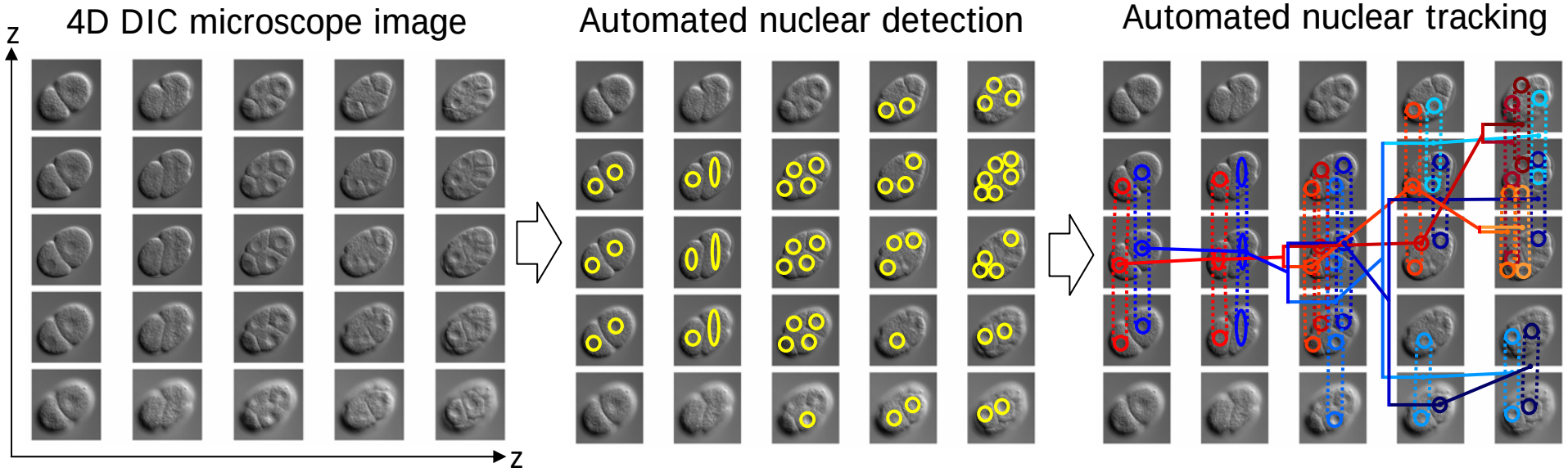
Problems in current phenotype analysis of gene knockdown embryos

- Objectivity of the data
- Versatility of the data

Cell division pattern measurement system

Detection of nuclei using image processing

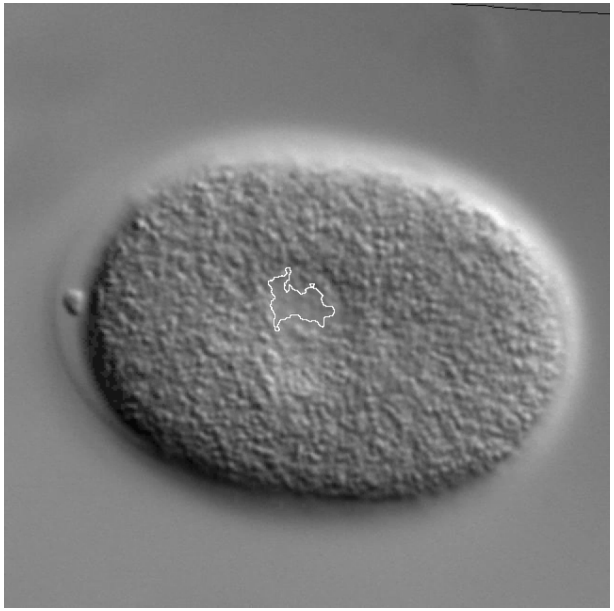




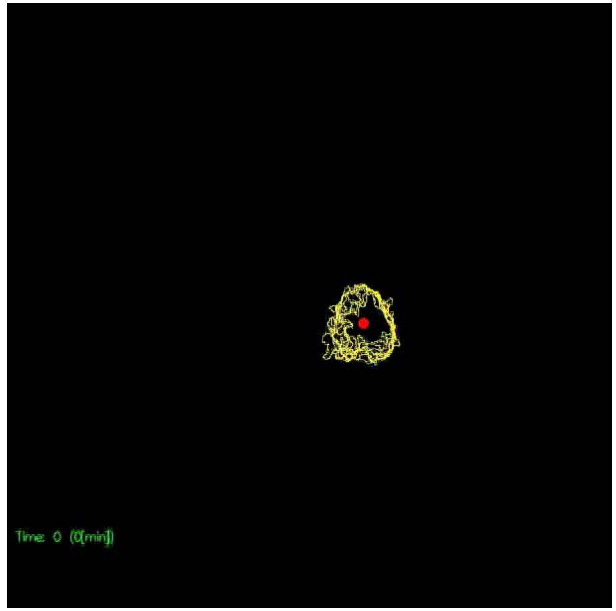
Onami et al.(2001) Foundations of Systems Biology, Hamahashi et al.(2005) BMC Bioinformatics

Cell division pattern measurement system

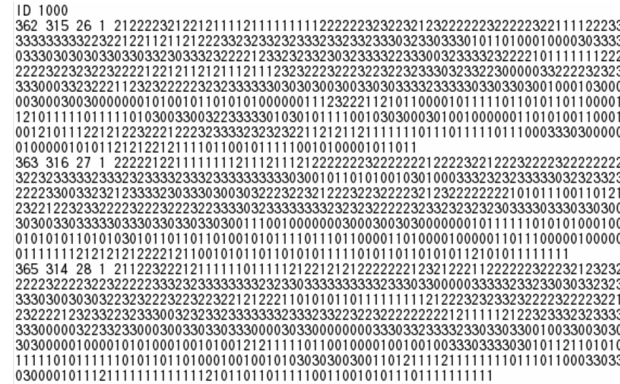
Detected nuclear regions



4D visualization of cell division pattern



Cell division pattern data (raw data)



- 3D outline of nucleus
- Tracking information
- Mother-daughter relationship

The system:

– provides **objective** information about structural change in early embryos.
  - Image processing based algorithm <u>objectively</u> detects nuclear regions.
– provides **versatile** information about structural change in early embryos.
  - Various kinds of information can be <u>calculated</u> from the cell division pattern data, e.g. the centroid, volume, and shape of nucleus.
– provides 4D cell division pattern up to **~24-cell stage**.

Onami et al.(2001) Foundation of Systems Biology, Hamahashi et al.(2005) BMC Bioinformatics

Collection of cell division patterns in RNAi embryos

Gene knockdown by RNAi

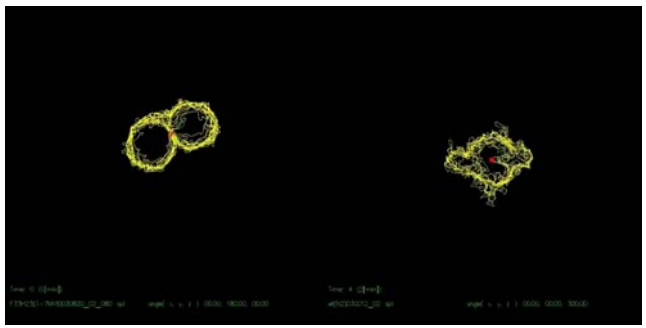
- 100% embryonic lethal genes on Ch.1 and Ch.3 (208 genes)

4D DIC microscope recording

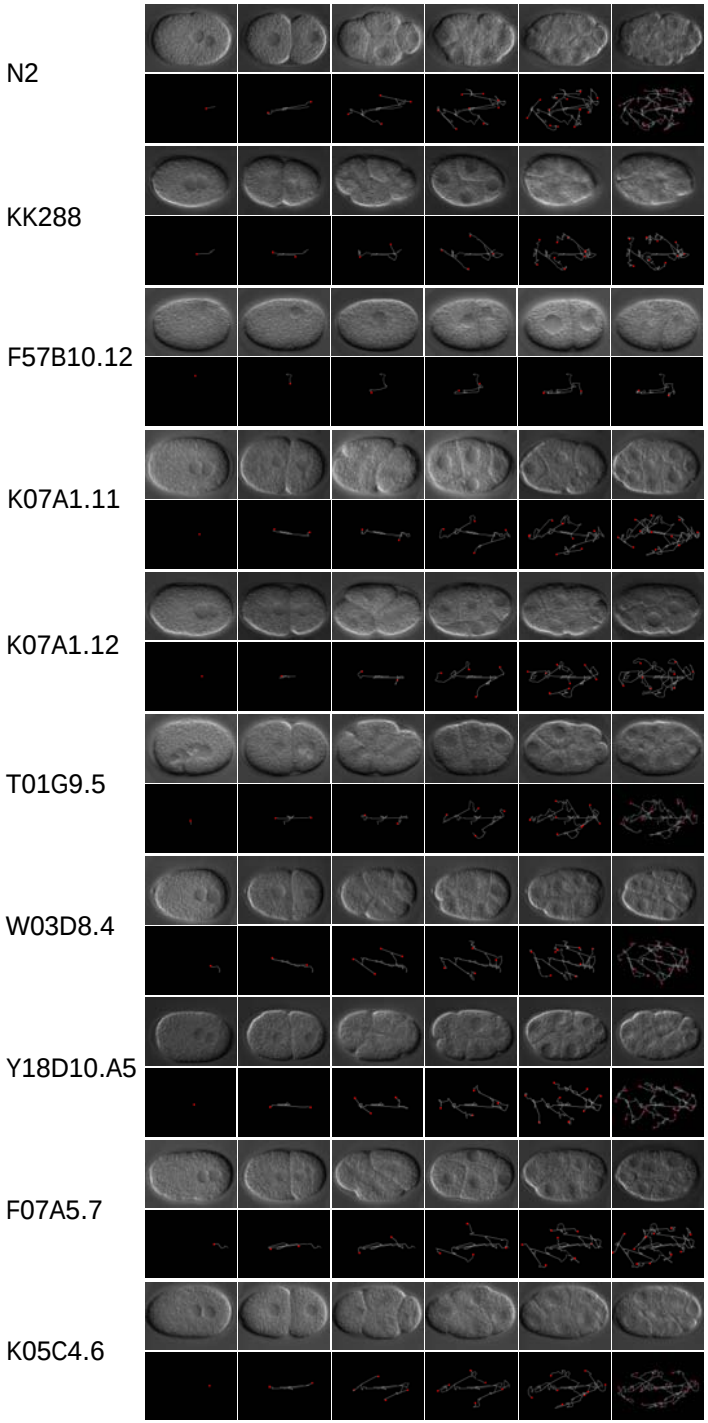
- 2-3 recordings/gene
- 22C

Cell division pattern measurement

- Ch.1: ~24cell stage
- Ch.3: ~8cell stage

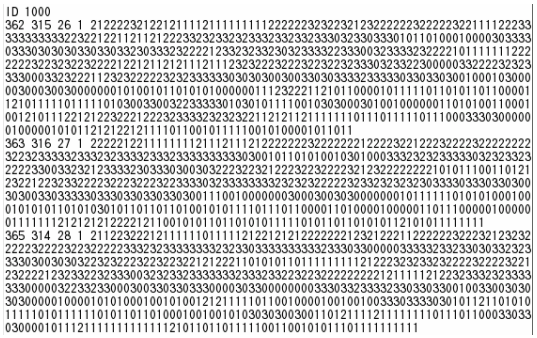


F33H2.5 WT



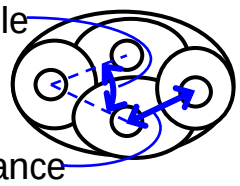
Objective phenotype analysis using cell division pattern data

Cell division pattern data



Calculation of signatures in cell division pattern

ex.

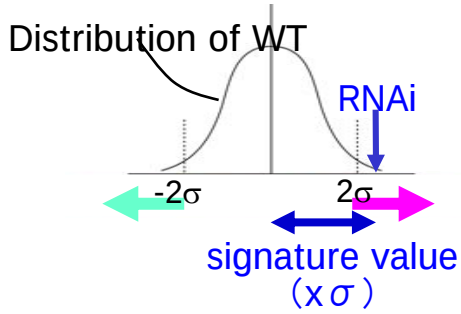


Distance: $D_{i,j} = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)^2}$

Angle:

$$\cos\theta_{i,j,k} = \frac{(x_i - x_j)(x_k - x_j) + (y_i - y_j)(y_k - y_j) + (z_i - z_j)(z_k - z_j)}{\sqrt{(x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)^2}\sqrt{(x_k - x_j)^2 + (y_k - y_j)^2 + (z_k - z_j)^2}}$$

Statistical comparison of signatures



|RNAi signature value|>2σ

⇨ Significant!

Computational cell division pattern analysis can examine all nuclear phenotypes in the past human-annotated analysis

44%(20/45) of all analyzed phenotypes can be examined.

Nuclear phenotypes

class	detect	Sonnichsen(2005)	this study	quantitative signature	explanation for the undetected phenotypes
P0 pronuclear centration	◎	1	1	Lack of centration (min. distance from center of embryo)	
P0 spindle irregular length	◎	1	1	P0 spindle irregular length (metaphase)	
P0 spindle positioning	◎	5	5	P0 spindle positioning during mitotic phase	
P1/AB nuclear separation	◎	2	1	Distance between P1 and AB	incomplete penetrance
AB nuclear migration	◎	1	1	Distance between AB and center of embryo	
P1 nuclear migration	◎	2	2	Distance between P1 and center of embryo	
P1/AB spindle assembly	◎	1	1	P1 or AB spindle thickness	
AB spindle orientation	◎	2	1	Division axis of AB cell	incomplete penetrance
P1/AB async. of divisions	◎	4	4	Delay between P1 and AB cell division	
4-cell stage configuration	◎	2	2	Division axis of AB/P1 cell	
Overall pace of events	◎	4	4	Periods of interphase and mitotic phase	
P0 spindle elongation	○	1	0	P0 spindle irregular length (telophase)	anaphase instead of telophase
4-cell stage cross-eyed	○	1	0	Distance between each nucleus and center of emrbyo	incomplete penetrance
P0 pronuclear rotation	○	0	0	Division axis of P0 cell	
P0 pronuclei number	△	4	-	Number of P0 pronuclei	
P0 pronuclei migration (f)	△	0	-	Movement of P0 pronuclear migration (f)	
P0 pronuclei migration (m)	△	1	-	Movement of P0 pronuclear migration (m)	
P0 pronuclear meeting	△	3	-	Meeting point	
P1/AB nuclear number	△	4	-	Number of P1/AB nuclei	
Unclear - multinucleate	△	1	-	Number of nuclei	

◎ detected, ○ detectable, △ in preparation

146 quantitative signatures

period of interphase AB
period of interphase P1
period of interphase ABa
period of interphase ABp
period of interphase EMS
period of interphase P2
period of mitotic phase P0
period of mitotic phase AB
period of mitotic phase P1
period of mitotic phase ABa
period of mitotic phase ABp
period of mitotic phase EMS
period of mitotic phase P2
number of nuclei
sphericity AB
sphericity P1
sphericity ABa
sphericity ABp
sphericity EMS
sphericity P2
division axis P0x
division axis P0y
division axis P0z
division axis ABx
division axis ABy
division axis ABz
division axis P1x
division axis P1y
division axis P1z
division axis ABax
division axis ABay
division axis ABaz
division axis ABpx
division axis ABpy
division axis ABpz

division axis EMSx
division axis EMSy
division axis EMSz
division axis P2x
division axis P2y
division axis P2z
division axis AB/P1
division axis ABa/ABp
division axis EMS/P2
division axis P0 θ
division axis AB θ
division axis P1 θ
division axis ABa θ
division axis ABp θ
division axis EMS θ
division axis P2 θ
division axis P0 spindle
division axis AB spindle
division axis P1 spindle
division axis ABa spindle
division axis ABp spindle
division axis EMS spindle
division axis P2 spindle
division timing AB/P1(m)
division timing AB/P1(t)
division timing ABa/ABp(m)
division timing ABa/ABp(t)
division timing ABa/ABp/EMS(m)
division timing ABa/ABp/EMS(t)
division timing EMS/P2(m)
division timing EMS/P2(t)

movement AB (inter)
movement P1 (inter)
movement ABa (inter)
movement ABp (inter)
movement EMS (inter)
movement P2 (inter)
movement AB (mitotic)
movement P1 (mitotic)
movement ABa (mitotic)
movement ABp (mitotic)
movement EMS (mitotic)
movement P2 (mitotic)
volume AB
volume P1
volume ABa
volume ABp
volume EMS
volume P2
volume AB/P1
volume ABa/AB
volume ABp/AB
volume ABp/ABa
volume EMS/P1
volume P2/P1
volume EMS/P2

distance AB/P1
distance ABa/ABp
distance ABp/P2
distance ABa/EMS
distance EMS/P2
distane ABa/P2
distance ABp/EMS
distance P0/AB
distance P0/P1
egg size
embryo size
embryo shape
spindle shape P0
spindle elongation P0
spindle rocking P0
spindle position P0
spindle shape AB
spindle elongation AB
spindle rocking AB
spindle position AB
spindle shape P1
spindle elongation P1
spindle rocking P1
spindle position P1
spindle shape ABa
spindle elongation ABa
spindle rocking ABa
spindle position ABa
spindle shape ABp
spindle elongation ABp
spindle rocking ABp
spindle position ABp

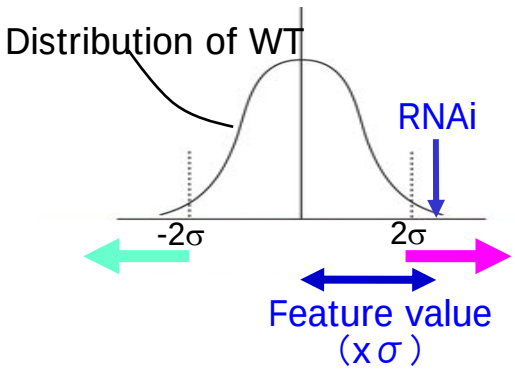
spindle shape EMS
spindle elongation EMS
spindle rocking EMS
spindle position EMS
spindle shape P2
spindle elongation EMS
spindle rocking EMS
spindle position P2
nuclear position P0
nuclear position AB
nuclear position P1
nuclear position ABa
nuclear position ABp
nuclear position EMS
nuclear position P2
centration P0
distance coe/AB
distance coe/P1
distance coe/ABa
distance coe/ABp
distance coe/EMS
distance coe/P2

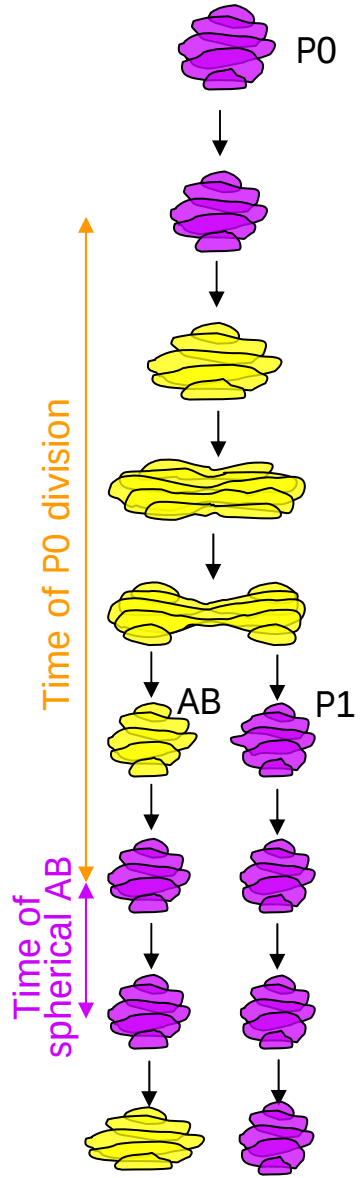
Computational cell division pattern analysis found detailed phenotypes in cell cycle progression

ORF	# cell *	period of interphase		period of mitotic phase		
		AB	P1	AB	P0	P1
F56D2.1	8	7.08	0.59	-0.78	0.27	0.70
T07C4.7	2	--	--	--	17.07	--
T20G5.2	8	7.08	2.26	-2.13	-0.71	-0.37
T20H4.5	4	9.15	7.23	7.52	7.09	6.04

AB lineage P lineage

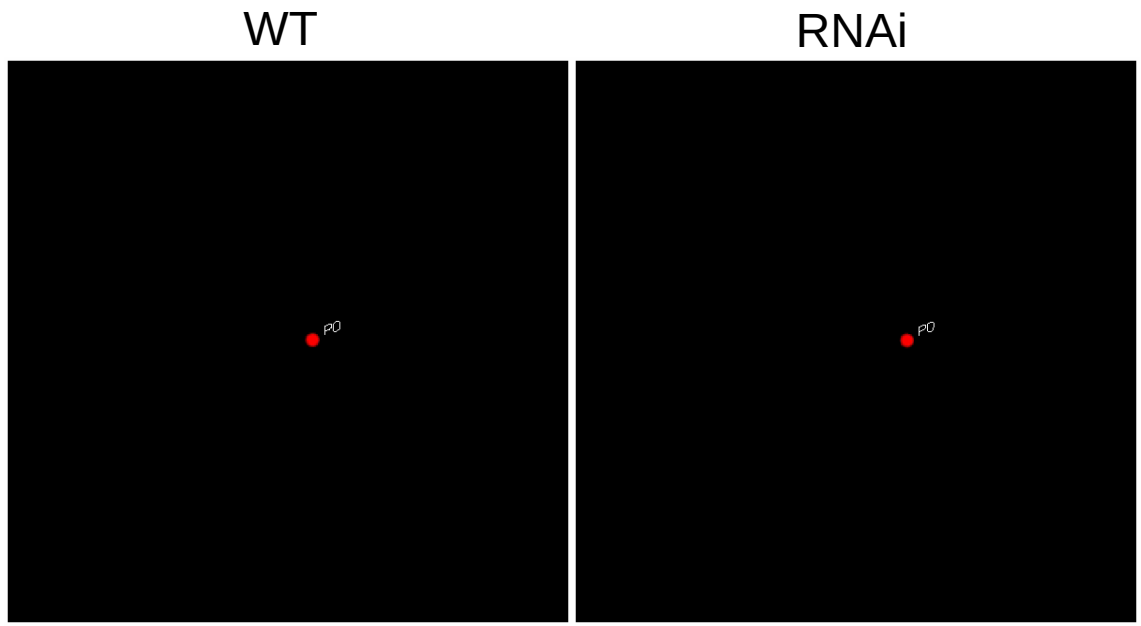
* number of cells when the number of cells is around 14 in wt embryo

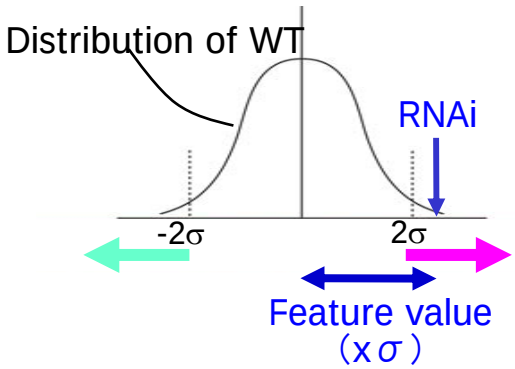




Computational cell division pattern analysis found phenotypes in cell division axis

ORF	LR component of division	
	ABa	ABp
B0361.10	2.11683	0.51708
C16C10.6	2.08450	-0.00934
C26E6.4	2.21194	0.39546
C34E10.2	2.11719	1.03871
F01F1.7	2.28395	0.85686
F11H8.4	2.24389	0.92590
Y37D8A.14	2.01229	1.03080
B0336.10	1.44615	-2.31990
R07E5.14	0.46546	-4.61461



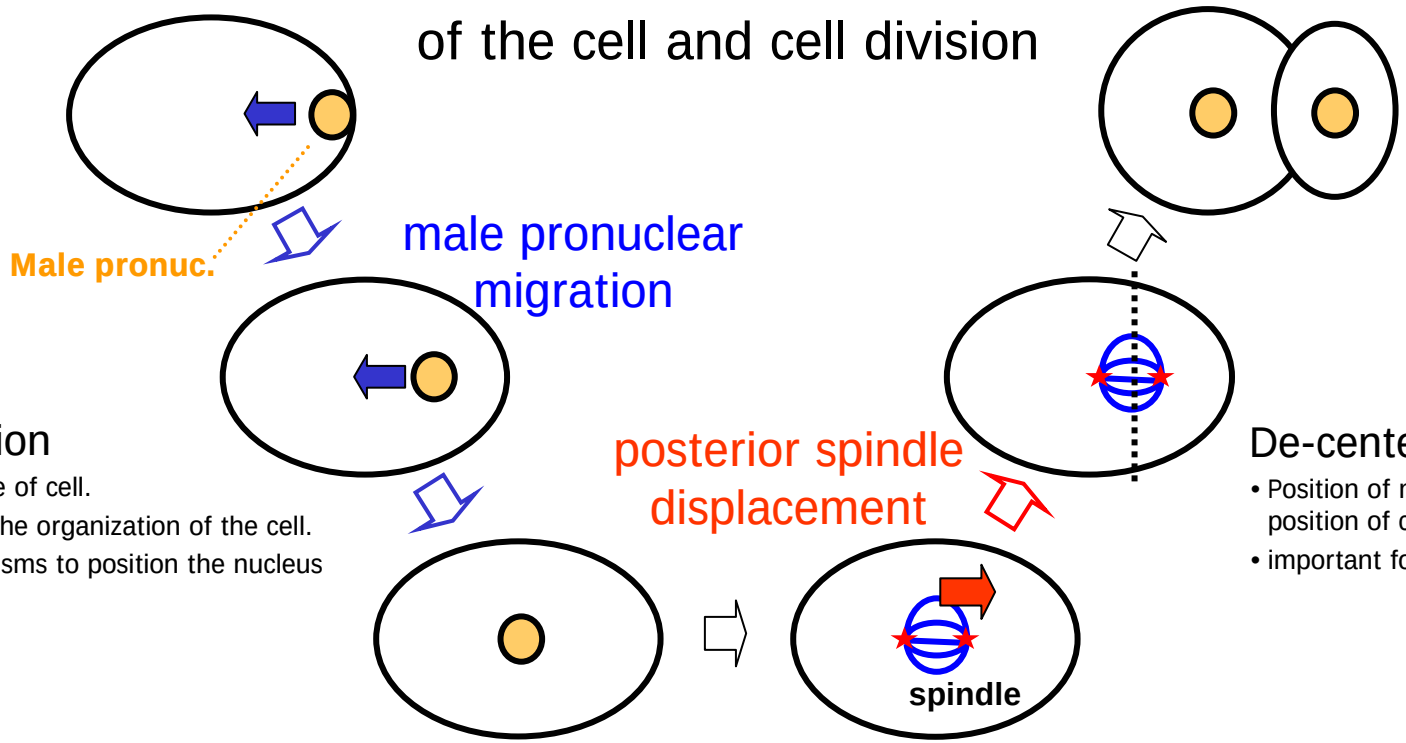


Summary

- Image-processing based approaches provide objective and versatile phenotype data.

Centerization and de-centerization of nucleus in one-cell embryo

Important for symmetry/asymmetry of the cell and cell division



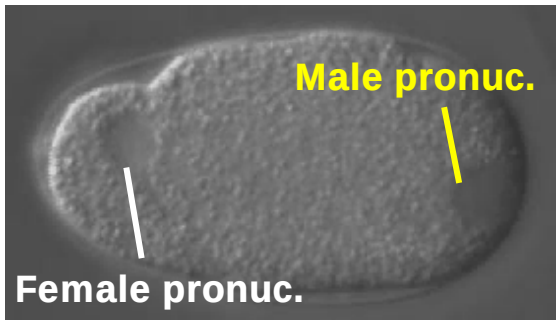
Centerization

- General feature of cell.
- Important for the organization of the cell.
- Active mechanisms to position the nucleus at the center

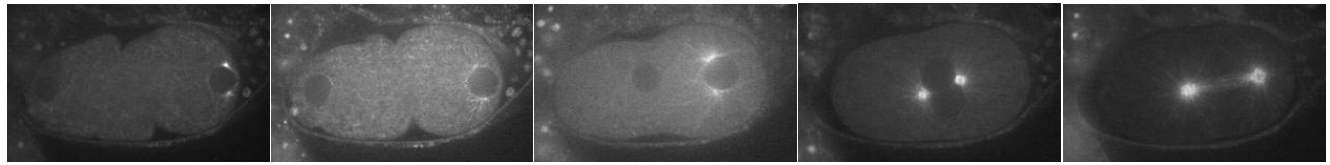
De-centerization

- Position of nucleus determines the position of cleavage plane.
- important for asymmetric division.

DIC



GFP::tubulin (white)



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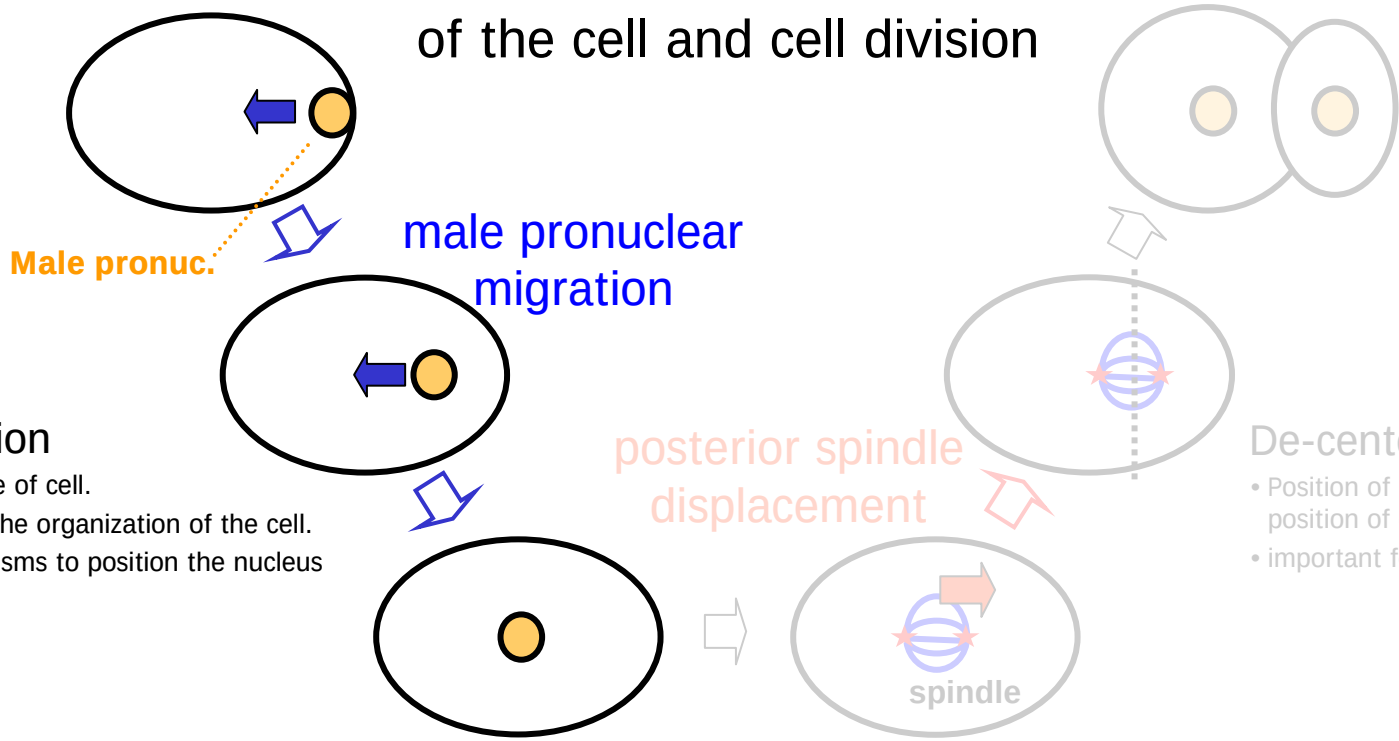

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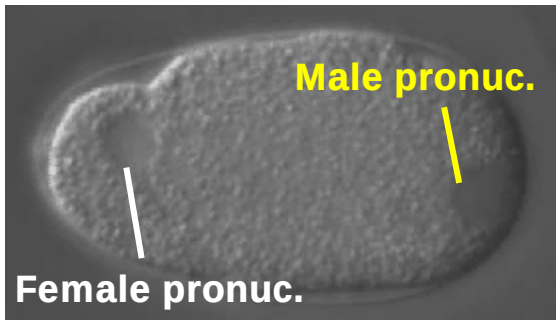

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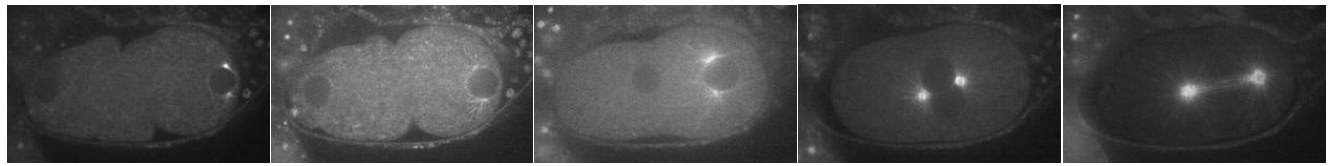
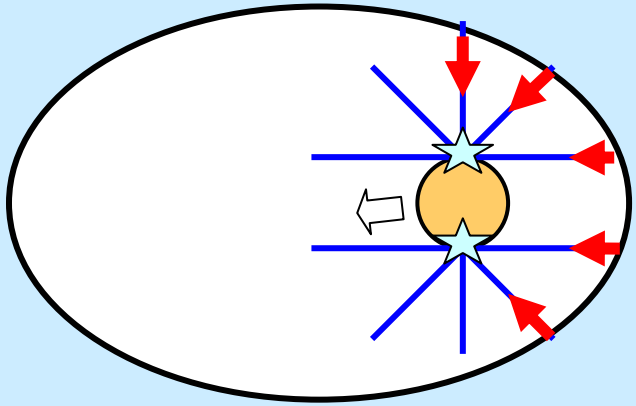


2 models for the centerization

Pushing model

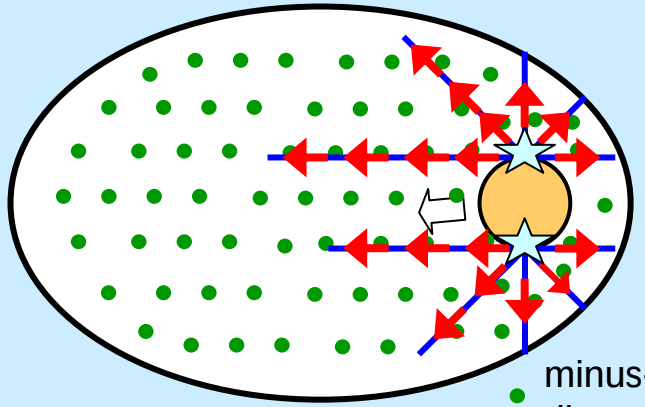


MT polymerization forces against the cell cortex

(Hamaguchi & Hiramoto (1980) Dev. Growth Differ.)

OR

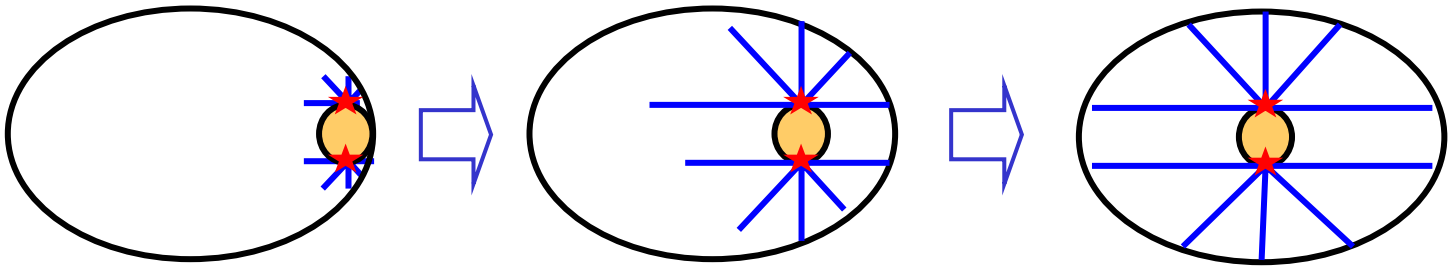
Pulling model



MT's length-dependent pulling forces by minus-end directed motor

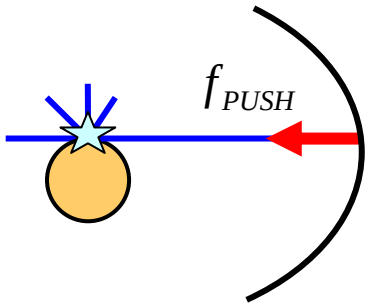
(Hamaguchi & Hiramoto (1986) Dev. Growth Differ)

Centerization = Male pronuclear migration



Simulation models were constructed based on physics

Theory of elasticity

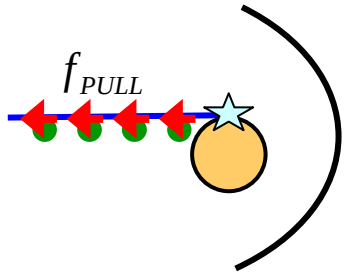


$$f_{PUSH} = A \cdot \kappa / L^2$$

A: prefactor for clamp quality
κ: flexural rigidity
L: length of the microtubule

(Dogterom & Yurke (1997) Science)

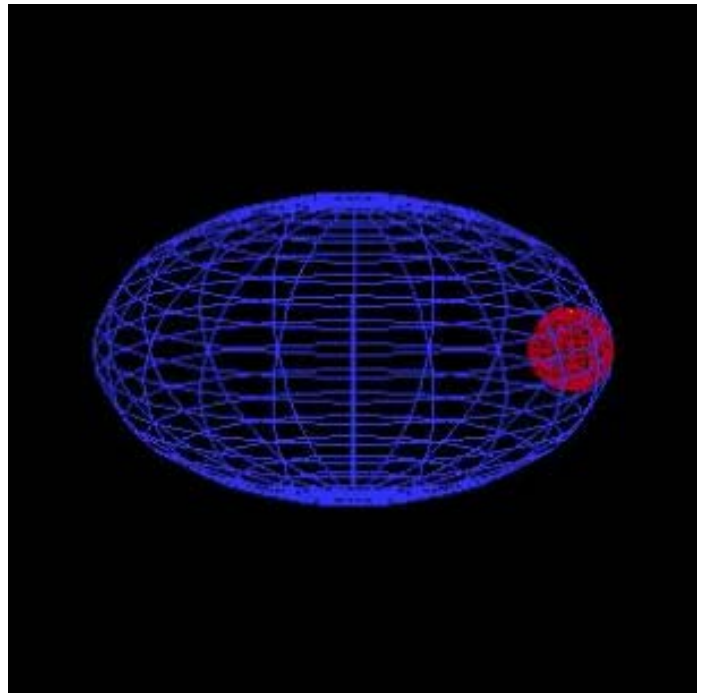
Length-dependent association of motors



$$f_{PULL} = F_{motor} \cdot D \cdot L$$

F_{motor}: force per motor
D: density of motors
L: length of the microtubule

(Reinsch & Gonczy (1998) J. Cell Sci.)



Movement at low Reynolds number

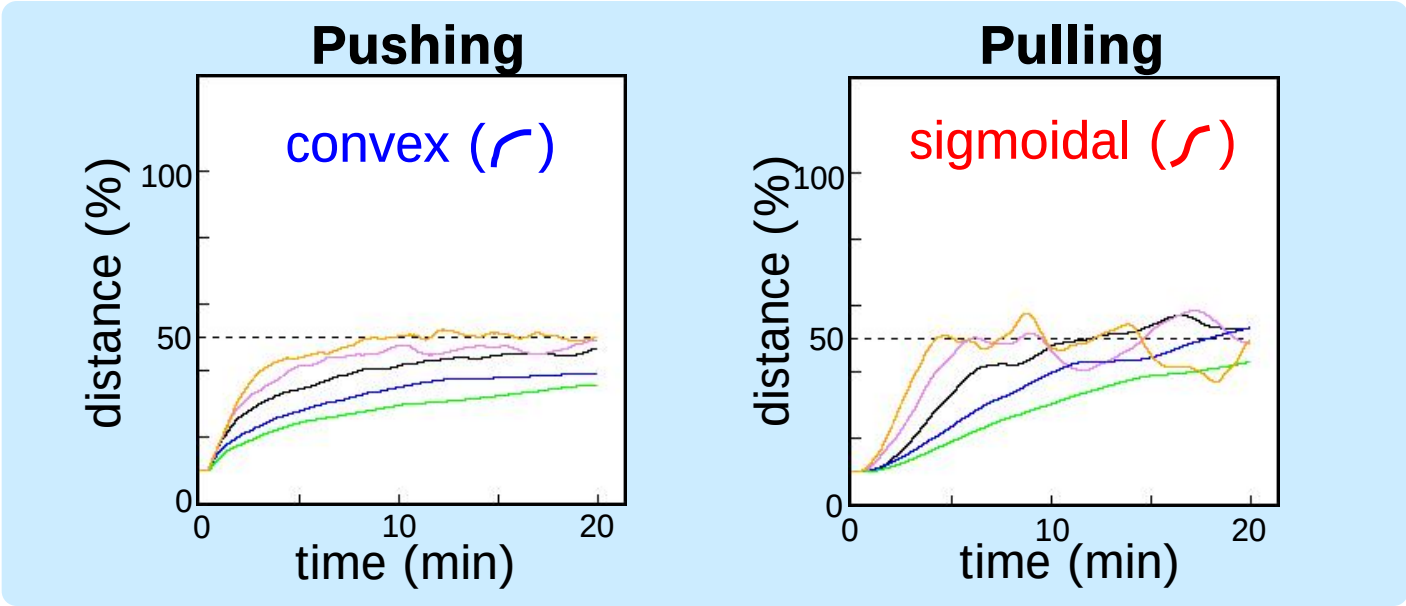
translational movement $\vec{F} = 6\pi r \mu \cdot \vec{V}$

rotational movement $\vec{M} = 8\pi r^3 \mu \cdot \vec{\omega}$

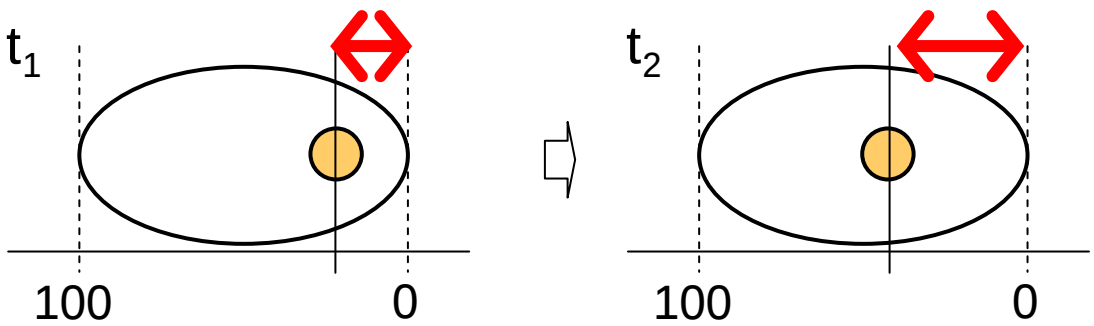
(Purcell (1977) Am. J. Phys.)

F: force (from microtubules)
M: moment (from microtubules)
r: (Stoke's) radius of pronucleus
μ: viscosity
V: (translational) velocity
ω: angular velocity

Intrinsic difference in the shape of distance-time graph between 2 models



Distance-time graph



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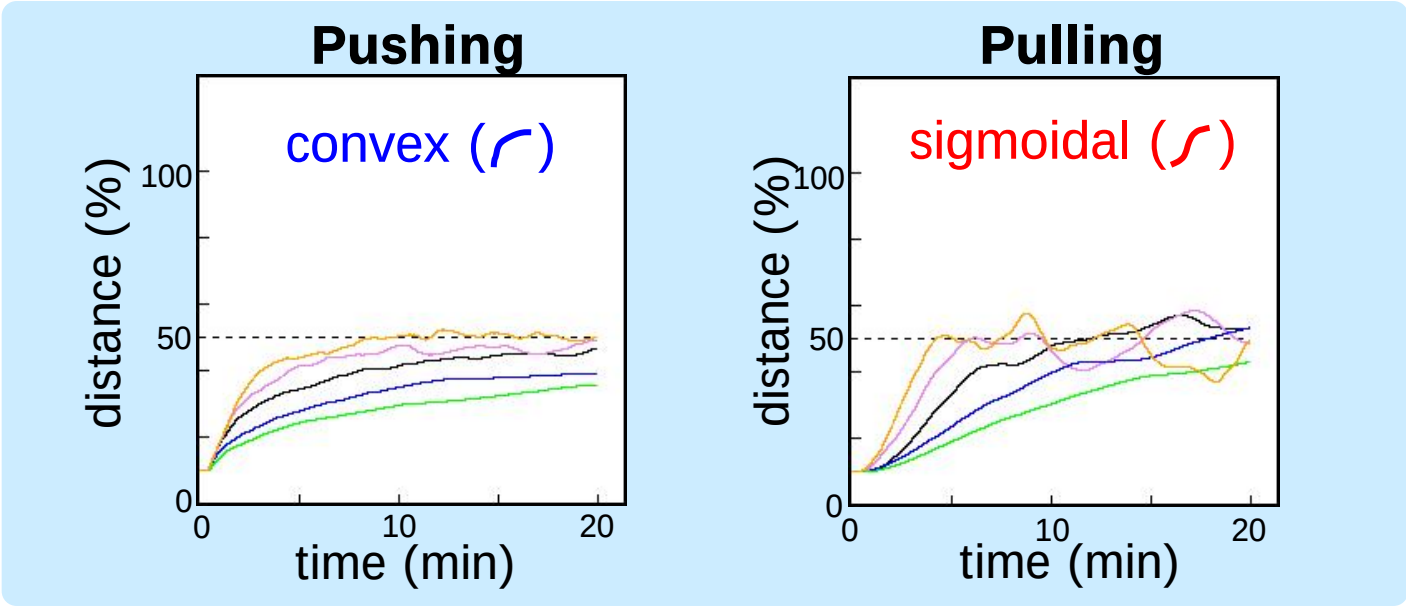
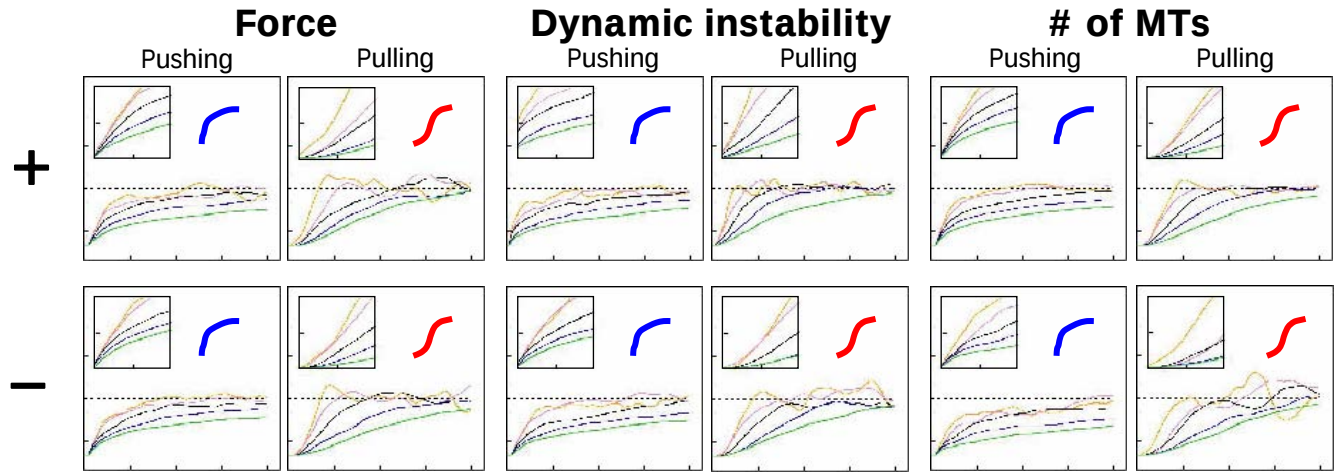
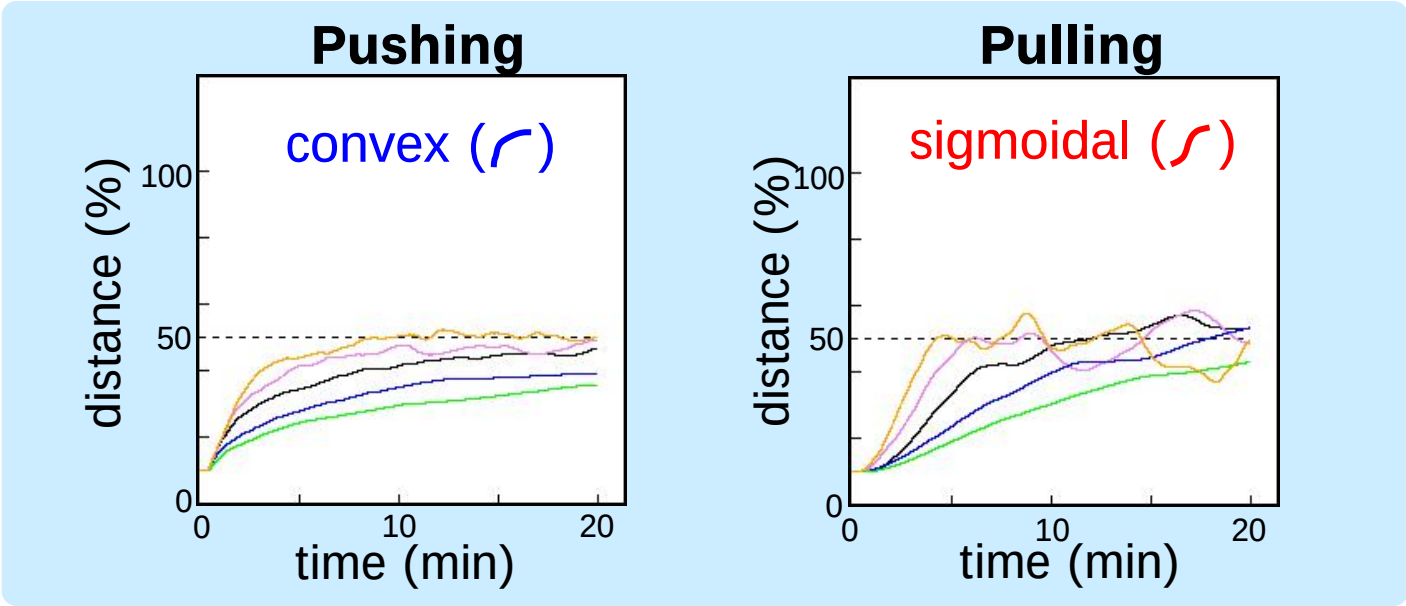


Difference does not depend on parameter values



Parameters
Dynamic instability
Number of MTs
Rigidity of MT
Elongation-rate parameter(A)
Elongation-rate parameter(B)
Stall force of motor
Maximum velocity of motor
Density of motor on MT
Viscosity of cytosol
Stoke's radius of pronucleus
Time step

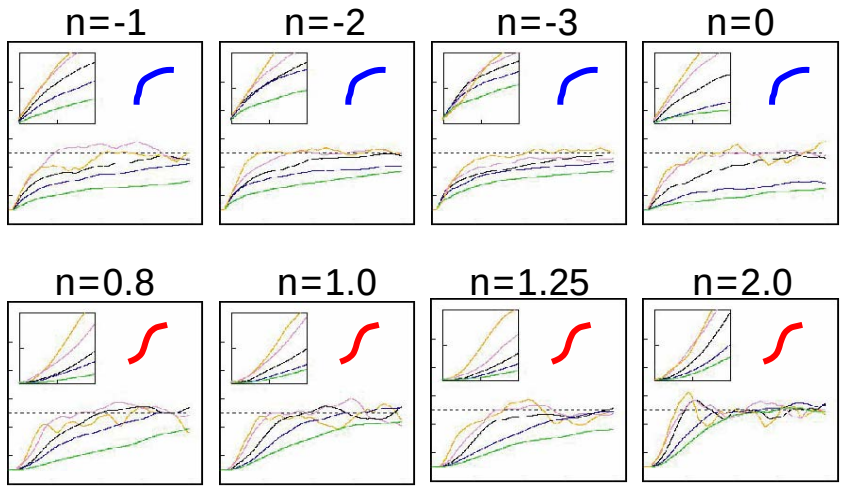
Intrinsic difference in the shape of distance-time graph between 2 models



Difference does not depend on specific definition of force

Pushing force: $f_{PUSH} = A \cdot \kappa \cdot L^n$

Pulling force: $f_{PULL} = F_{motor} \cdot D \cdot L^n$



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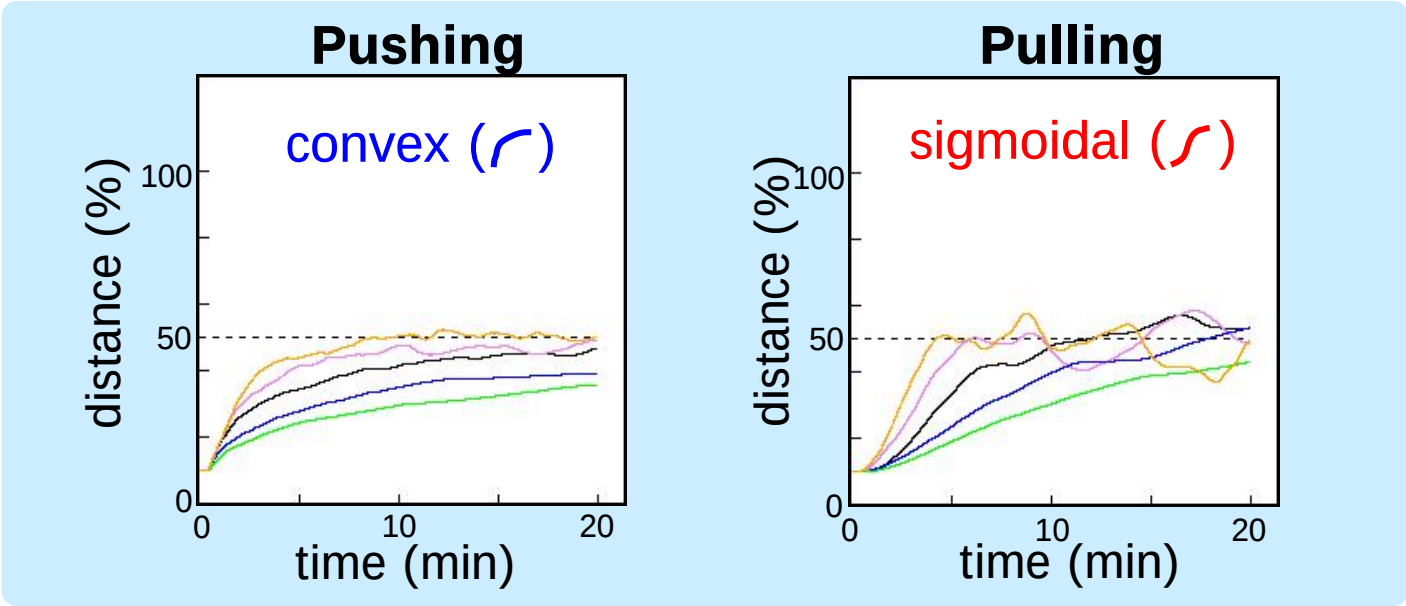
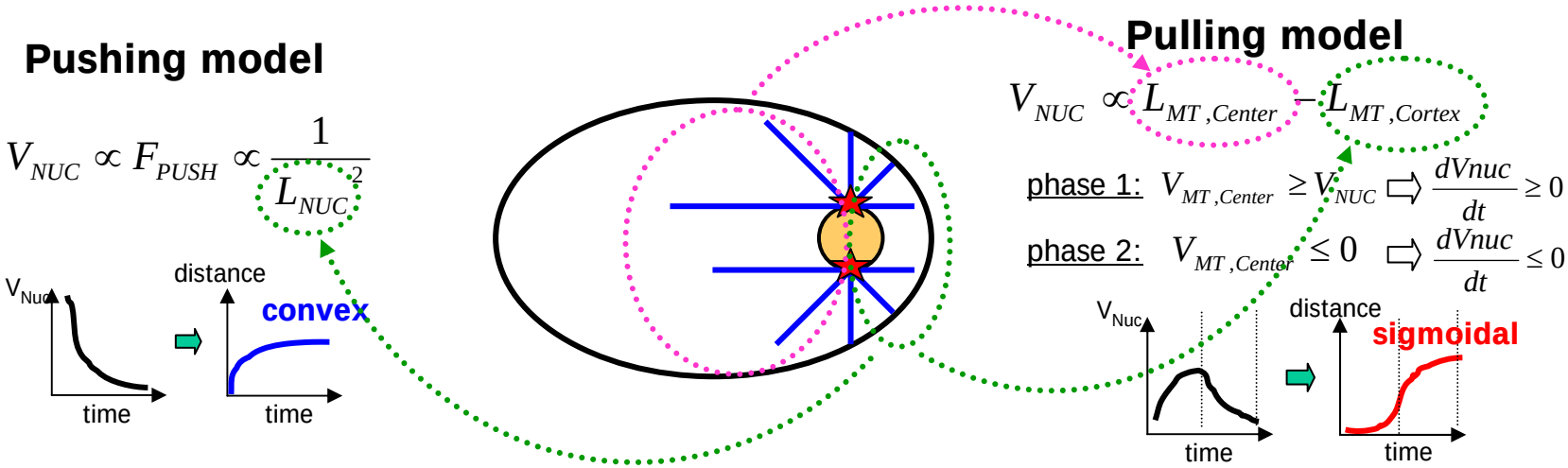
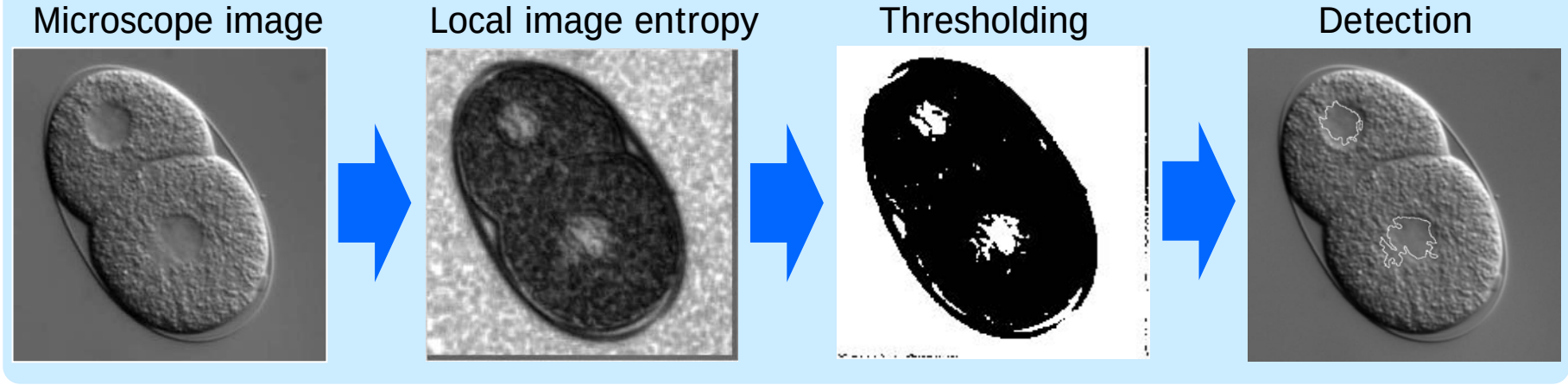


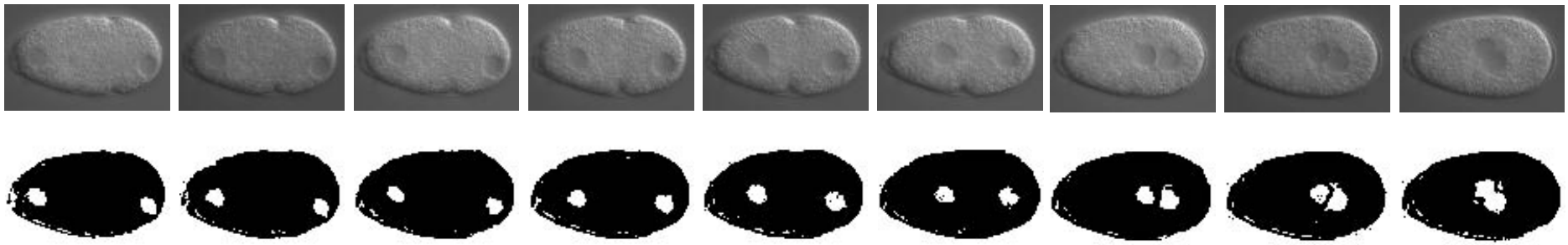
Difference can be explained theoretically



Measurement of in vivo migration using image processing

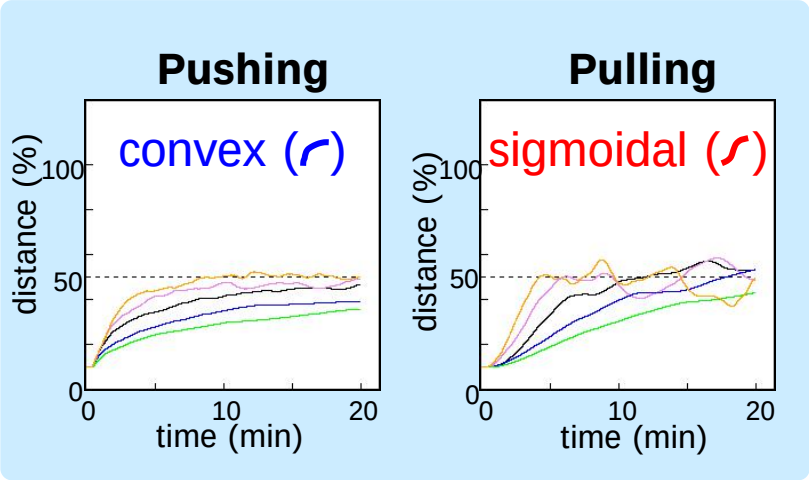
Detection of nuclei using image processing (Hamahashi et al. (2005) BMC Bioinformatics)



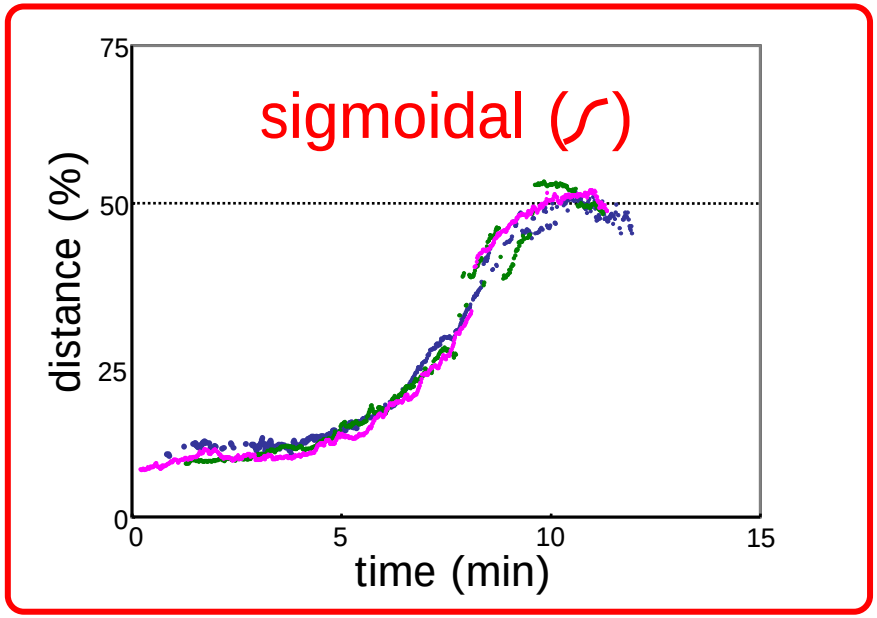


in vivo distance-time graph shows the shape of pulling model

in simulation

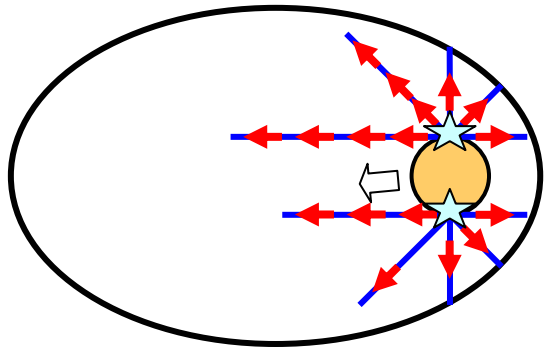


in vivo



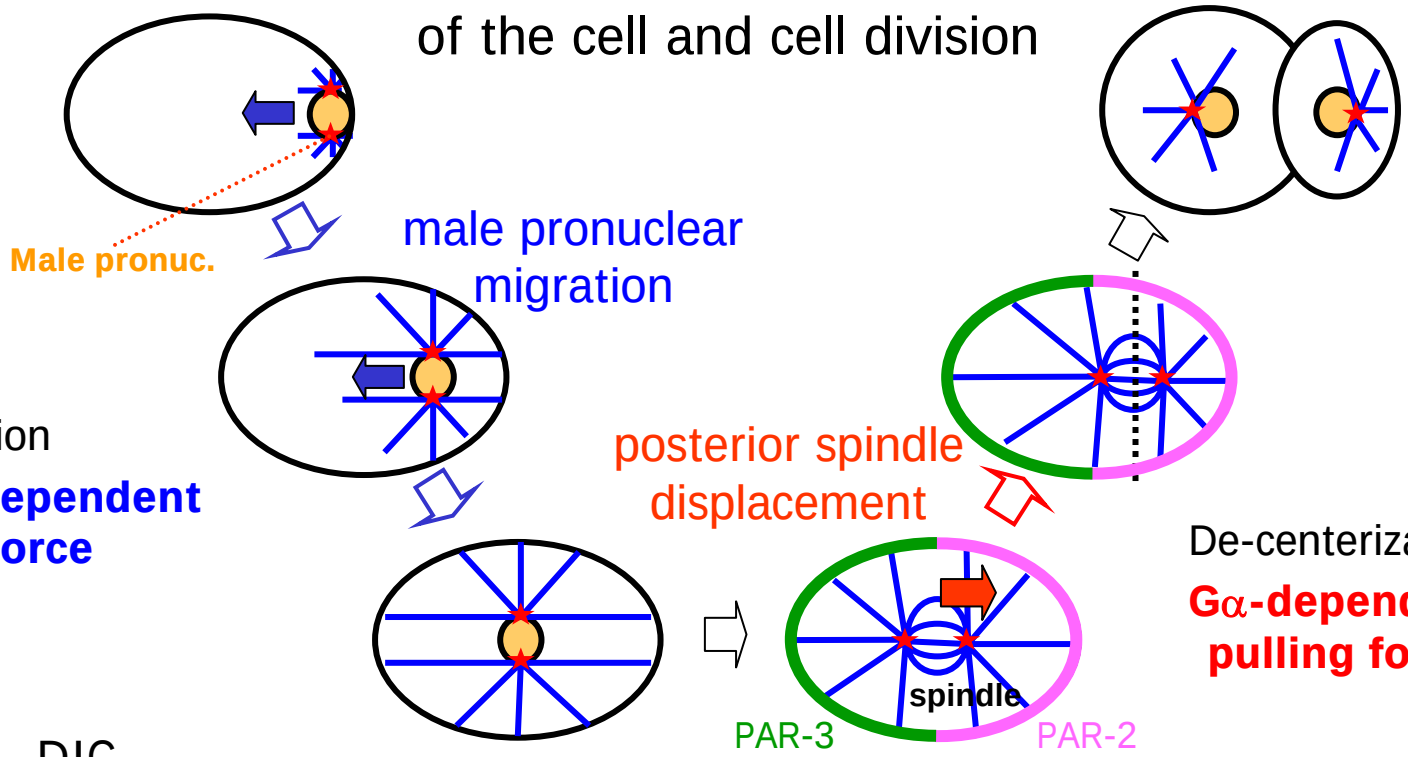
Pulling model is the primary contributor to male pronuclear migration

(Kimura & Onami (2005) Dev. Cell)

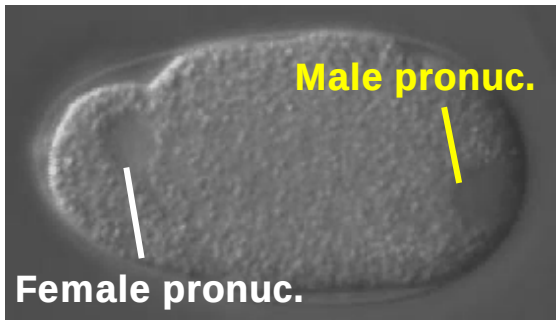


Centerization and de-centerization of nucleus in one-cell embryo

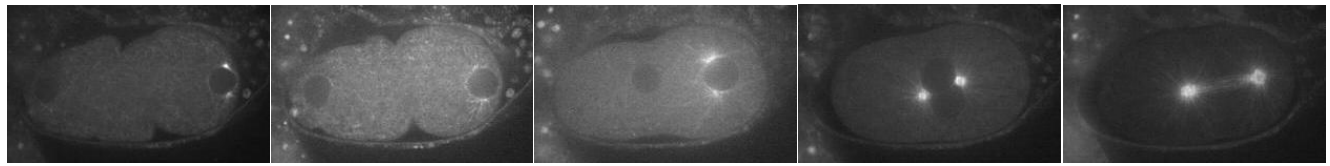
Important for symmetry/asymmetry of the cell and cell division



DIC



GFP::tubulin (white)



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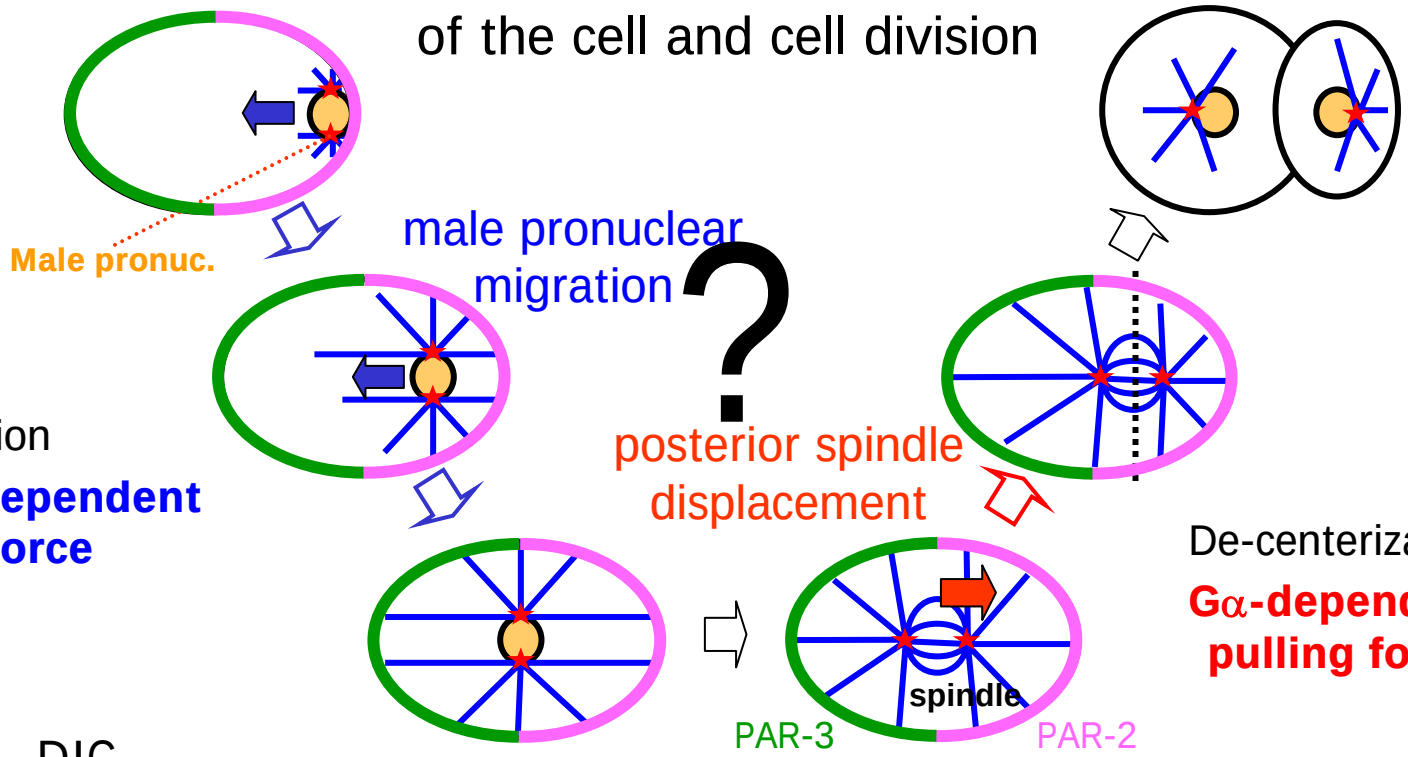


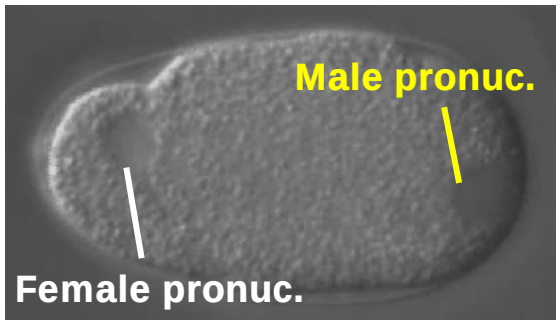
Centerization

Length-dependent pulling force

De-centerization

Gα-dependent cortical pulling force

DIC



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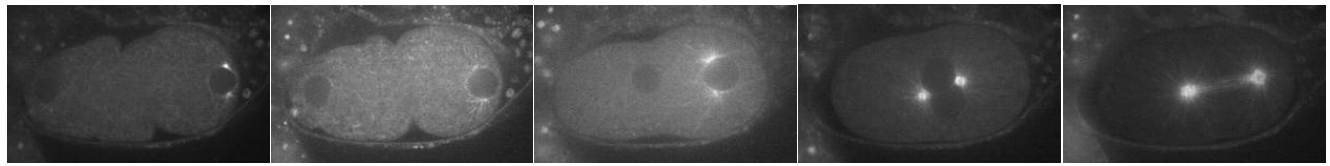
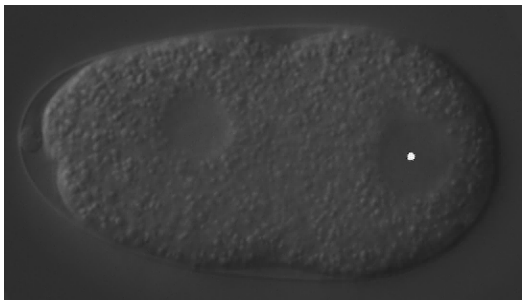
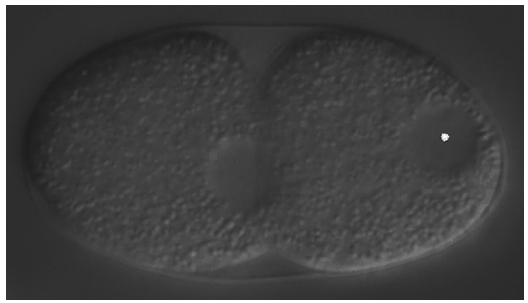
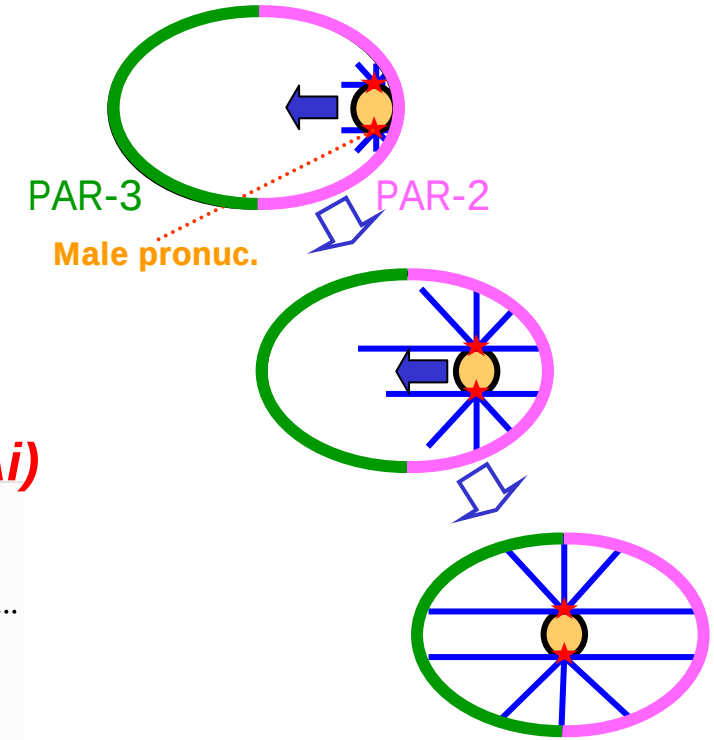
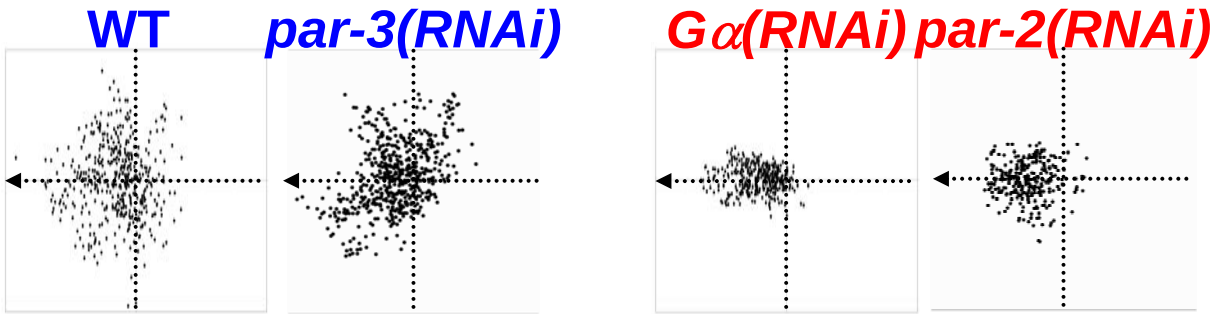


Micro-movements (MM) of male pronucleus reveal Gα-dependent cortical-pulling force during migration



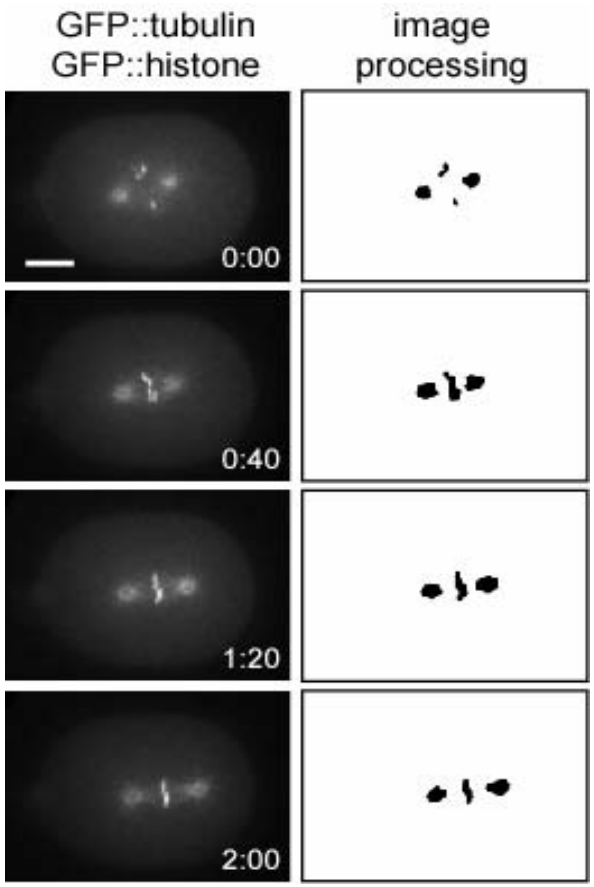






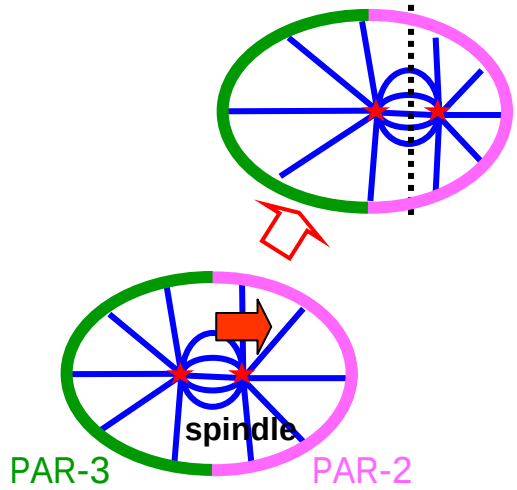
Cortical pulling force is functioning during male pronuclear migration.

Micro-movement (MM) of spindle before/during posterior displacement

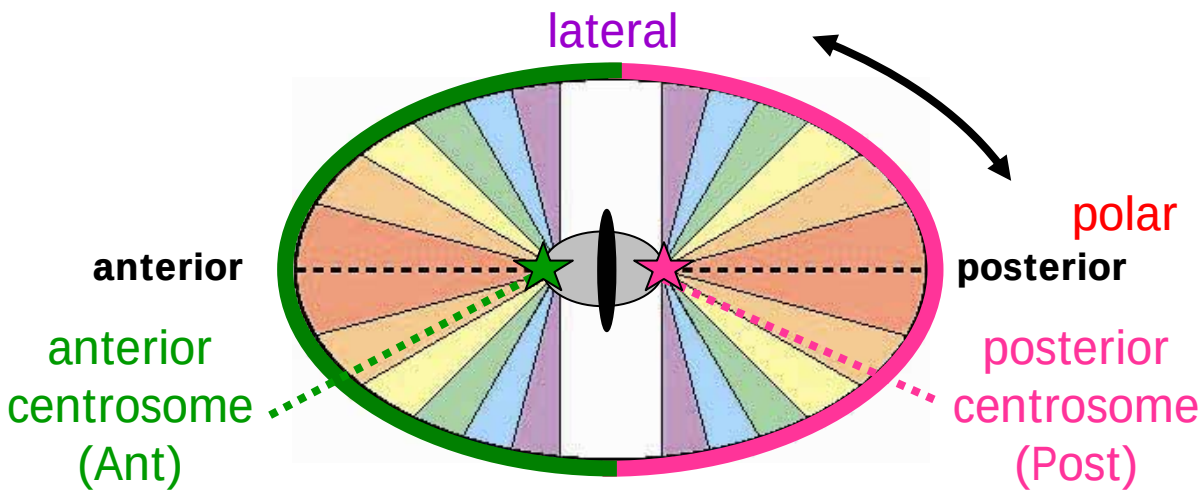


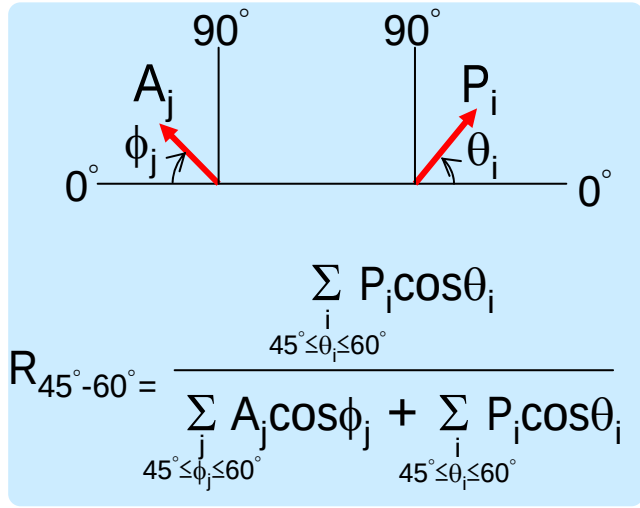
During

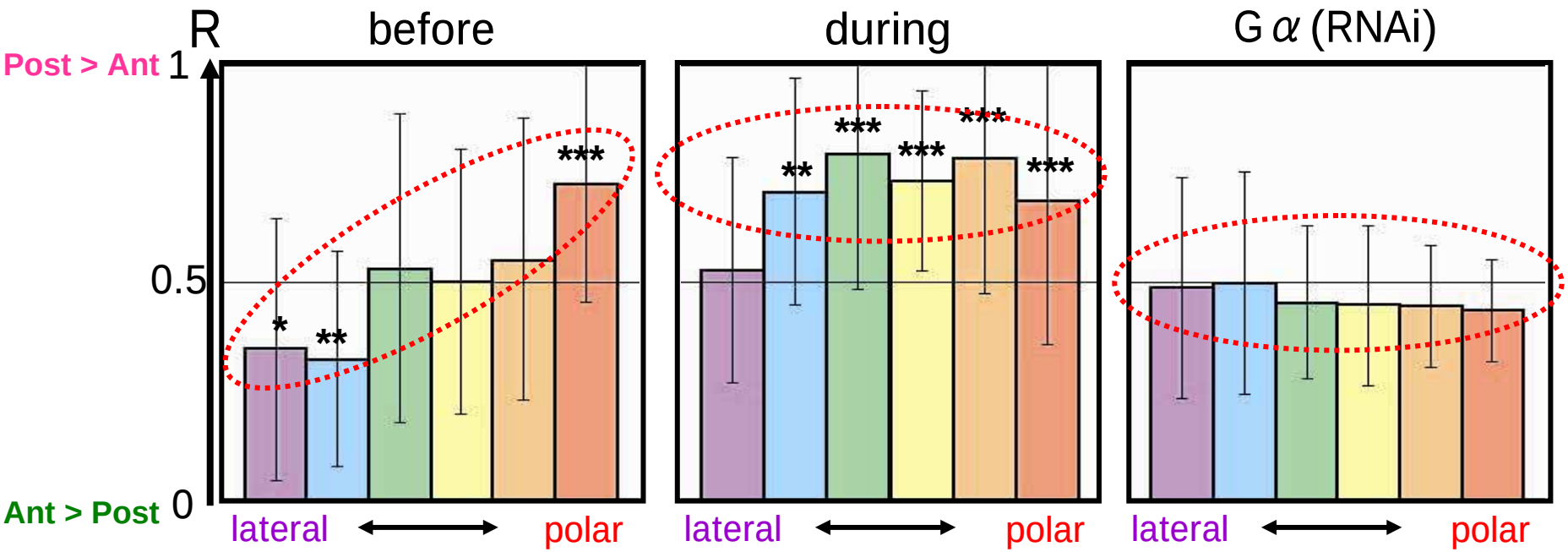
Before



Spatial distribution of MM of spindle before/during displacement

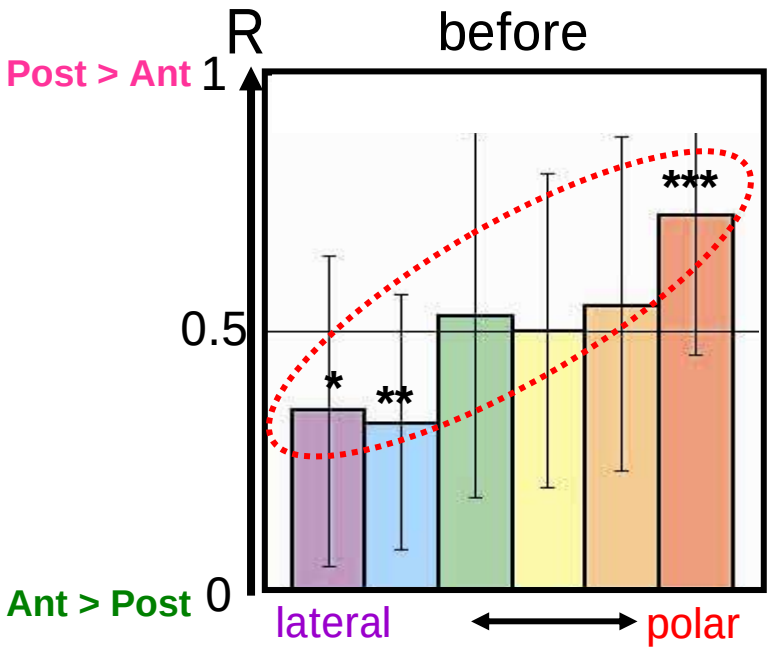


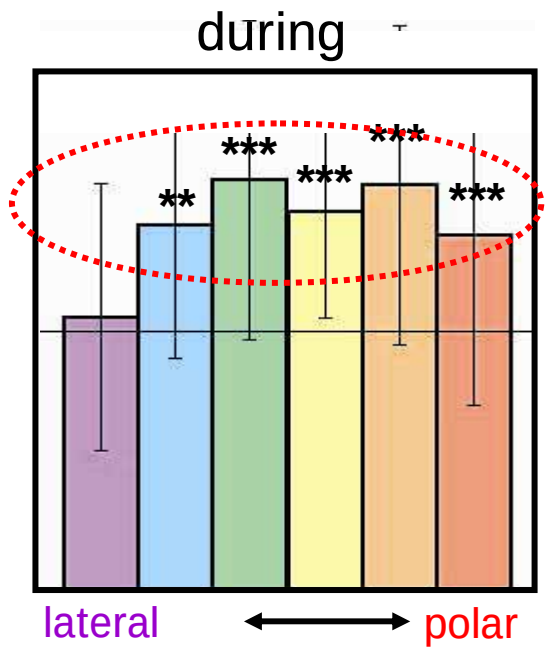


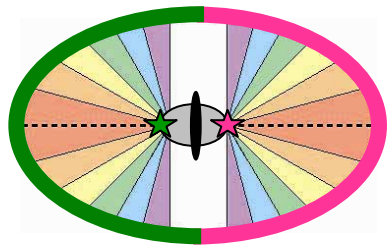


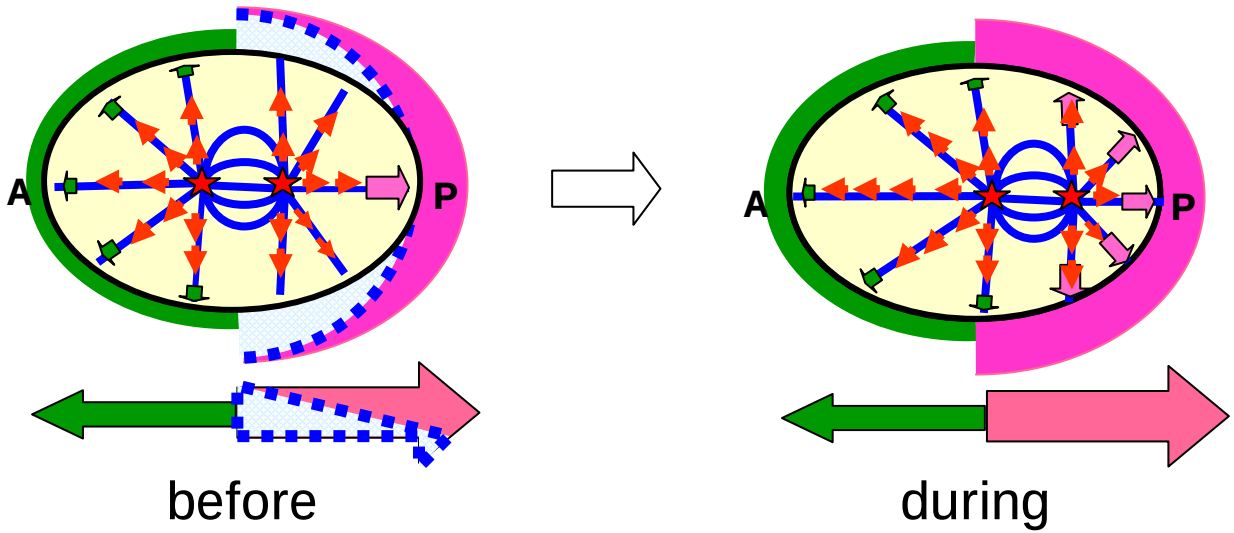
*** p<0.005, ** p<0.05, * p<0.1

Cortical force at lateral region controls the position of spindle.

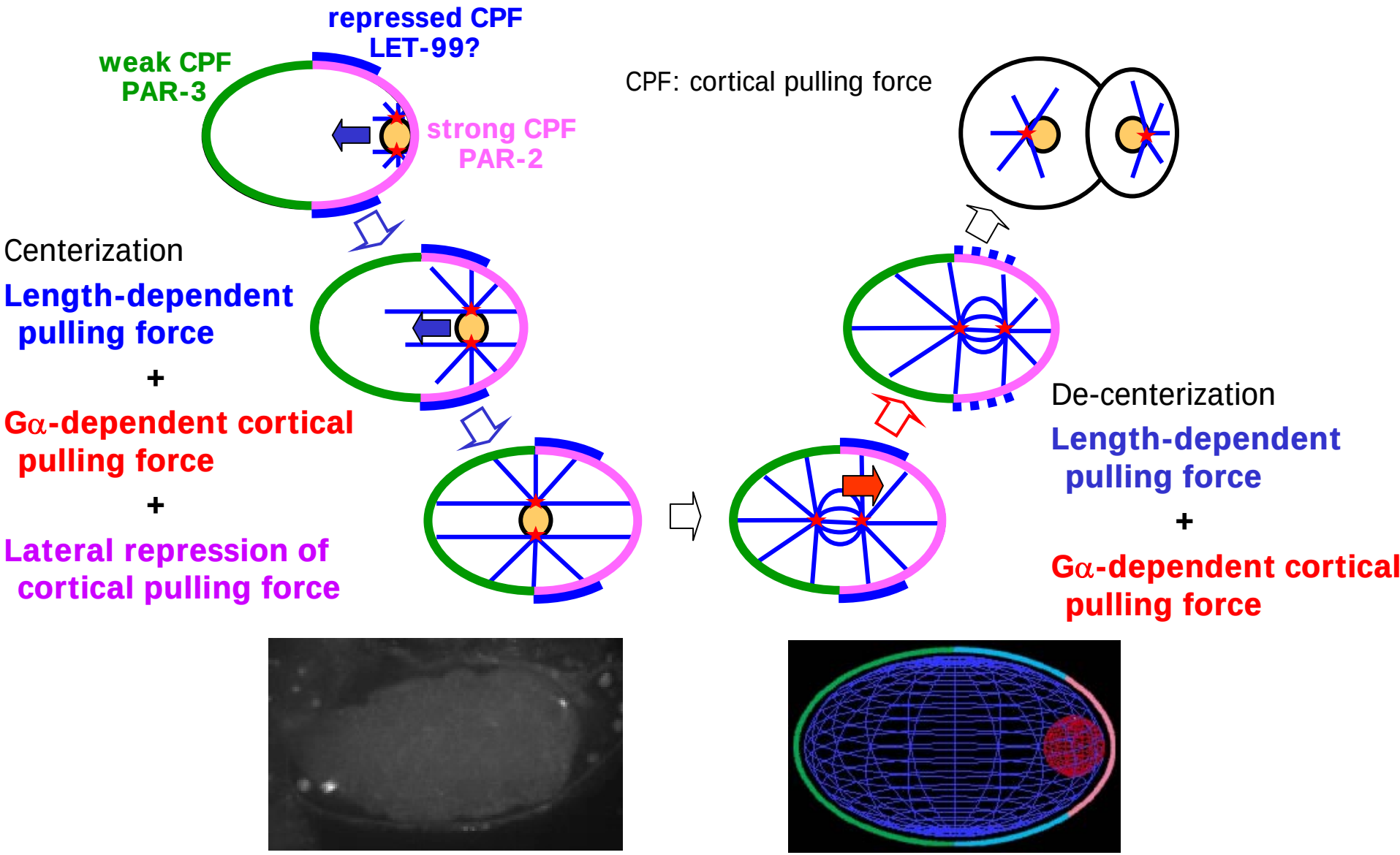








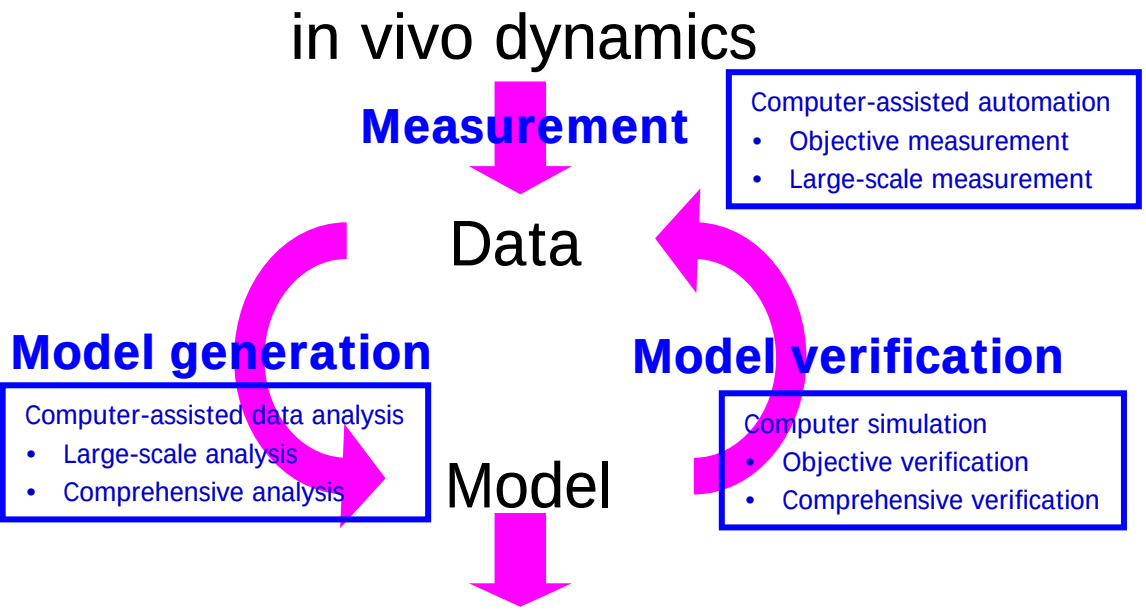
Model of centerization and de-centerization of nucleus in one-cell embryo

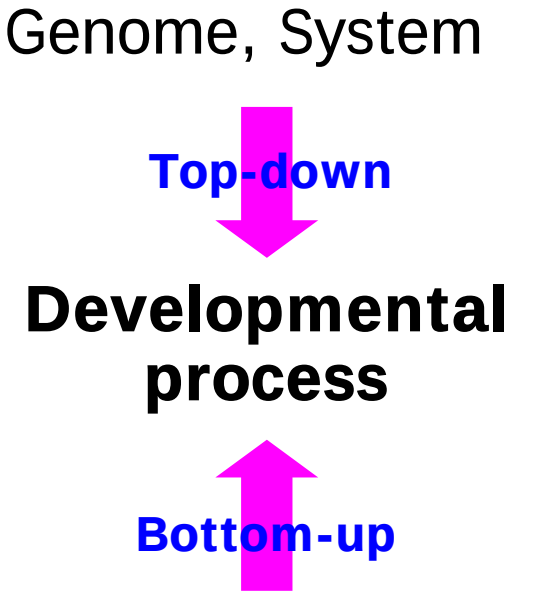


Summary

- A combination of computer simulation and image-processing based measurement is a powerful approach to understand developmental processes.

Strategy to understand developmental process





Contributors

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Koji Kyoda	Cell division pattern analysis
Akatsuki Kimura	Nuclear dynamics
Mitsuru Urai	Database

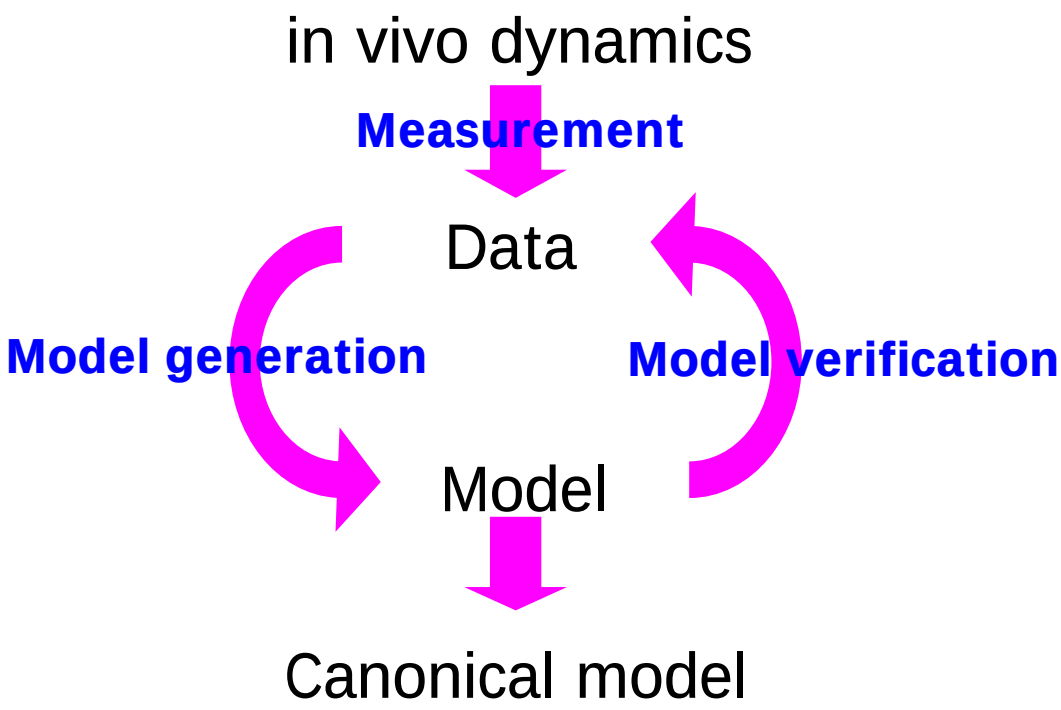
Acknowledgement

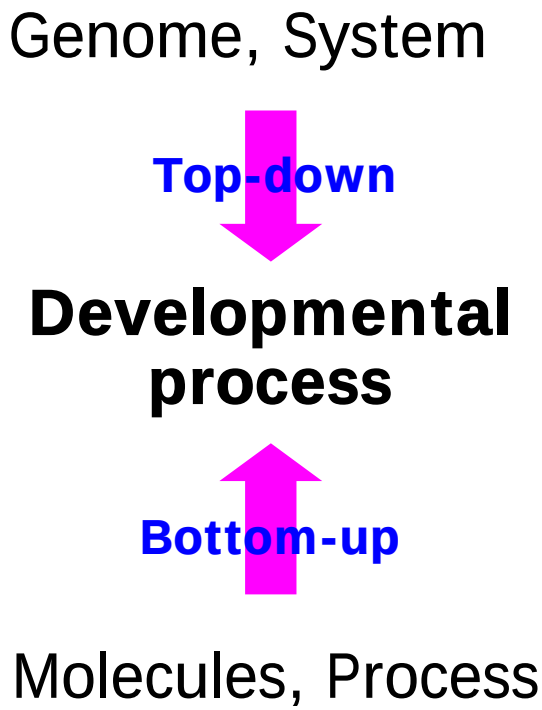
Hiroaki Kitano	Sony CSL
Asako Sugimoto	RIKEN CDB
Pierre Gönczy	ISREC
Yuji Kohara	NIG
Julie Ahringer	U Cambridge

CGC

BIRD JST

Strategy to understand developmental process





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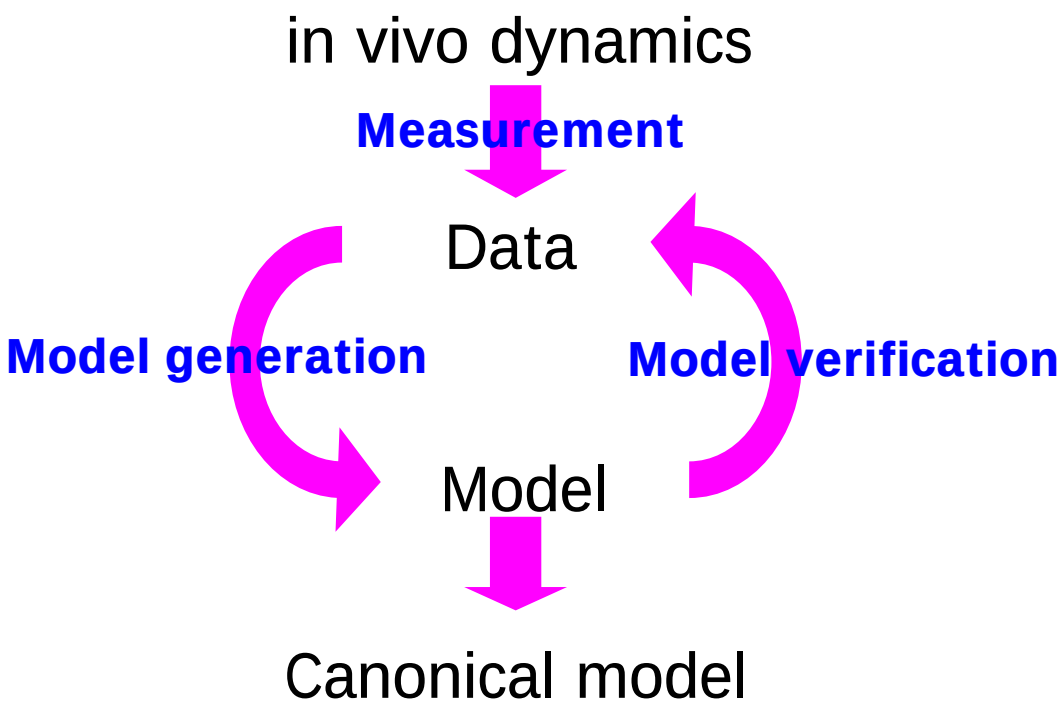
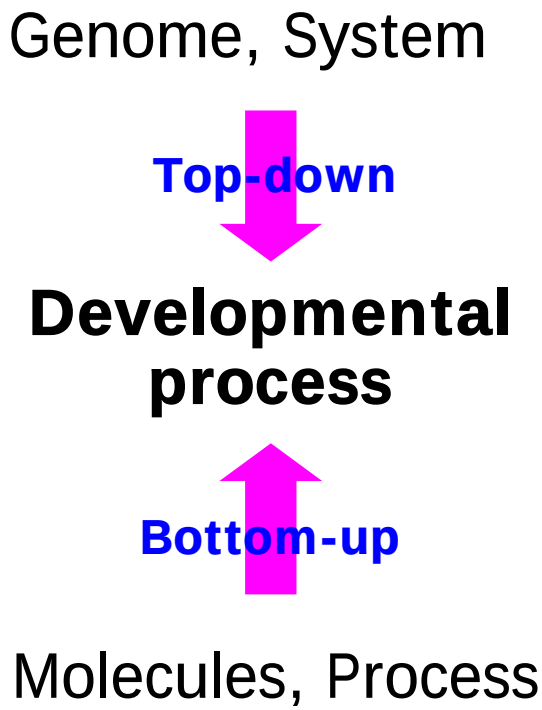
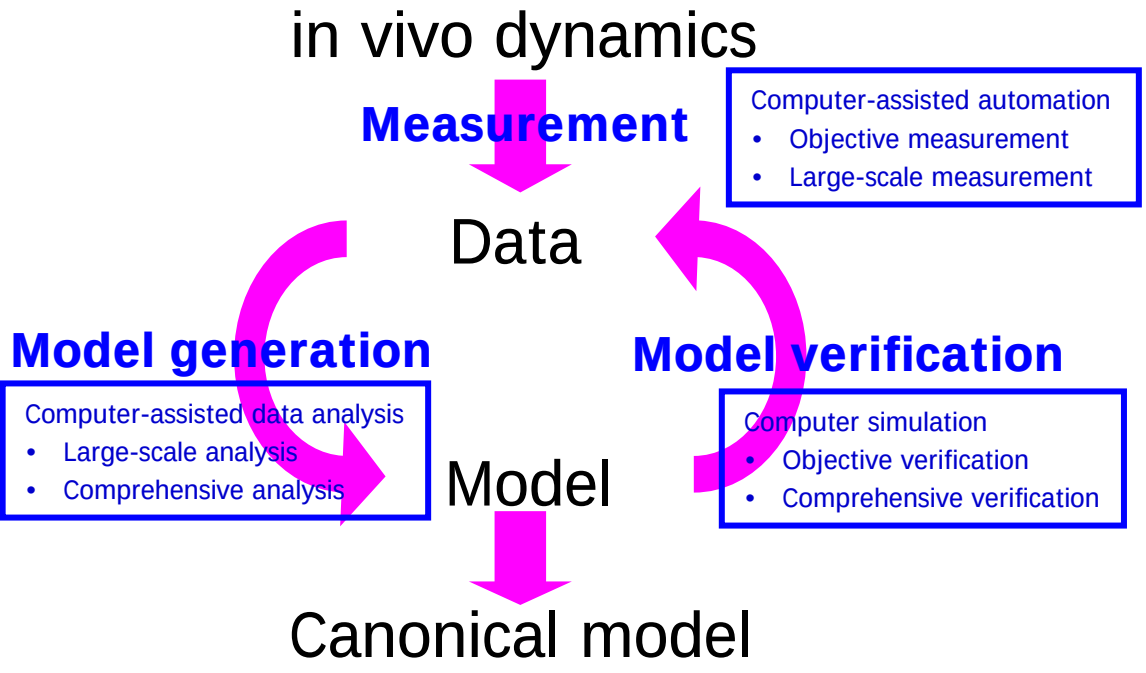
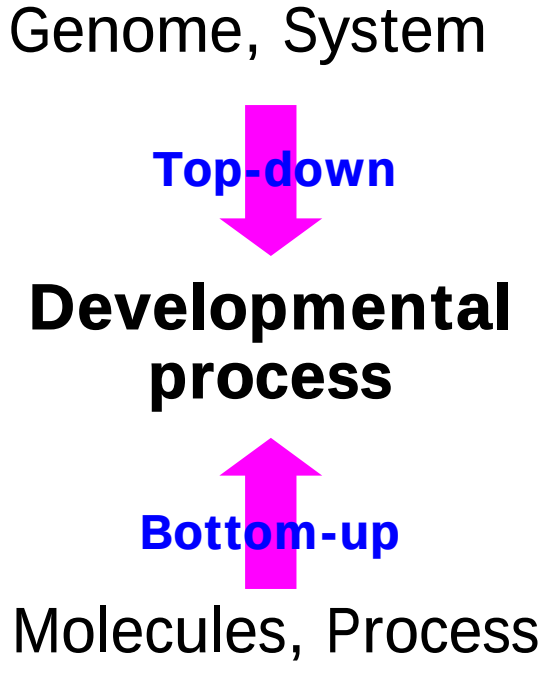




Summary

- Computers provide great help for quantitative studies of developmental process.
- Quantitative phenotype analysis provide more detailed information than human-annotated phenotype analysis.
- A combination of computer simulations and image-processing is a powerful approach for studying mechanism of development.





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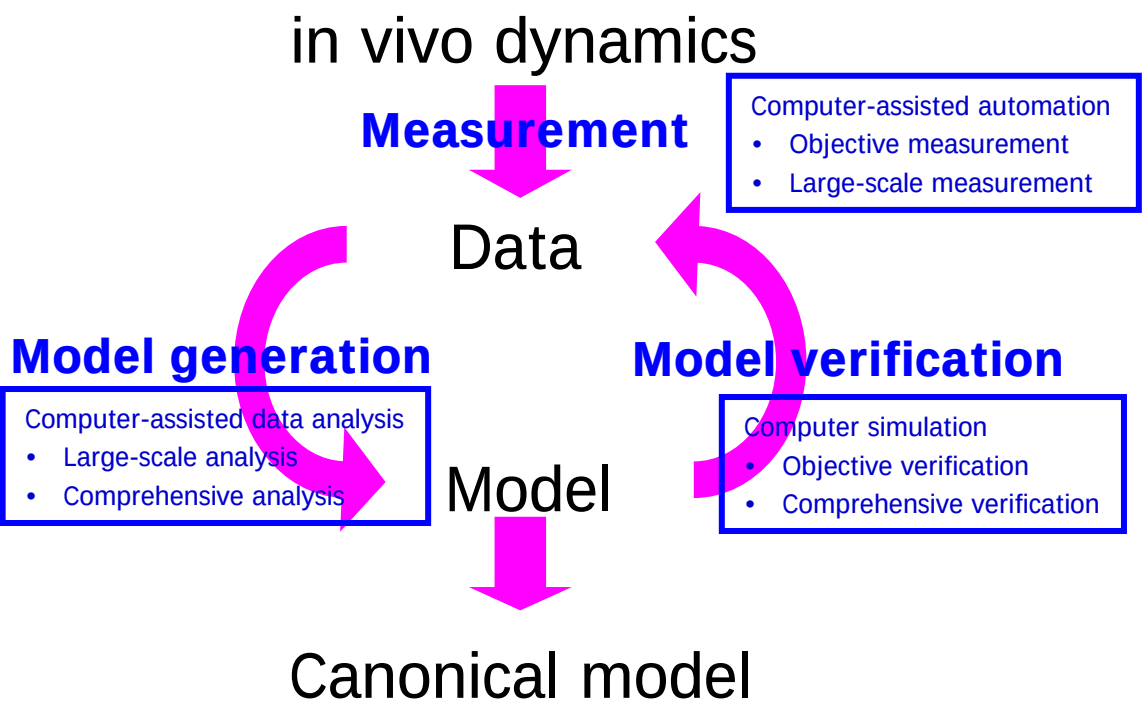
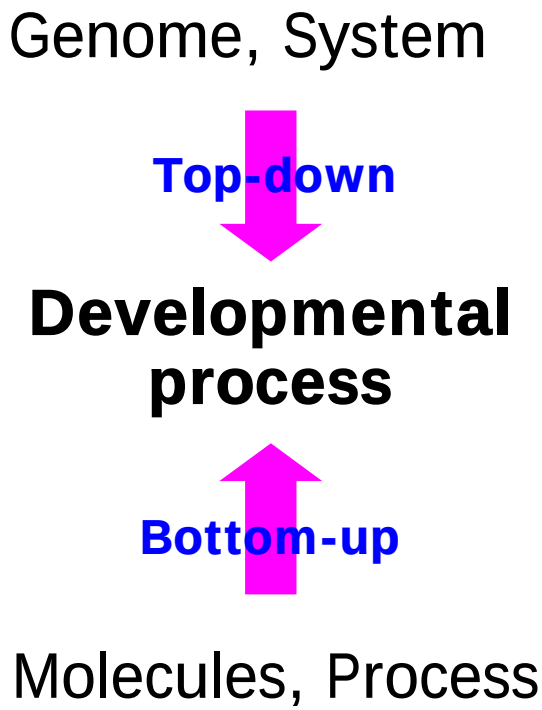
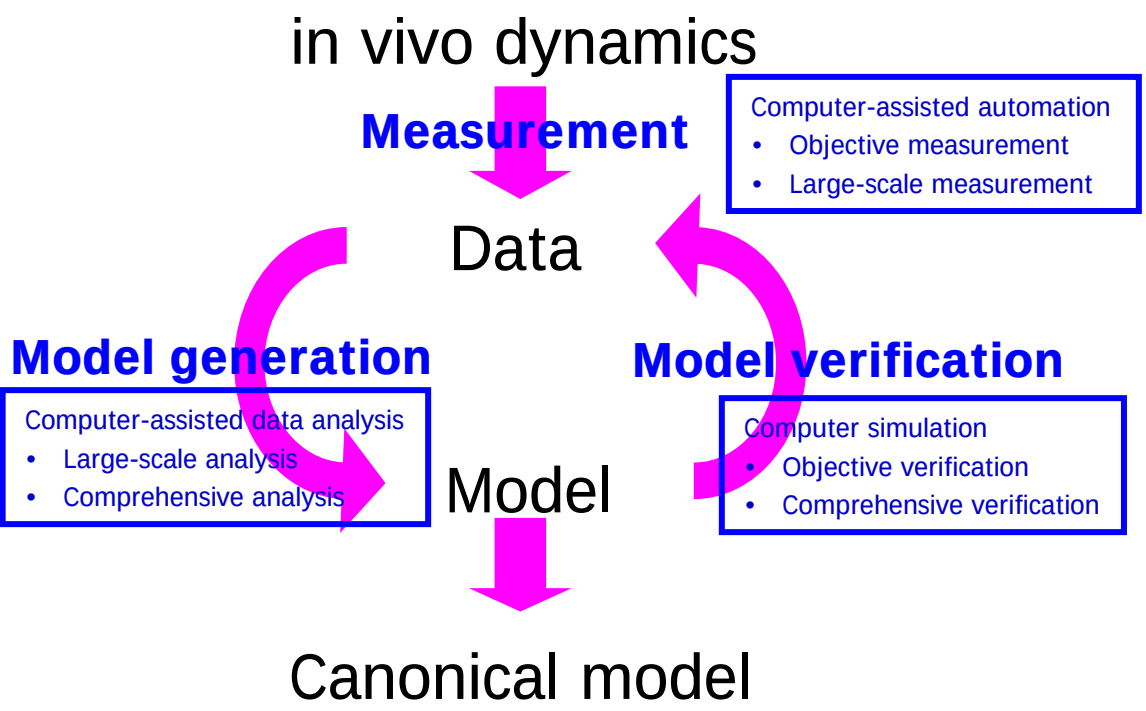
Computer-assisted top-down and bottom-up approaches

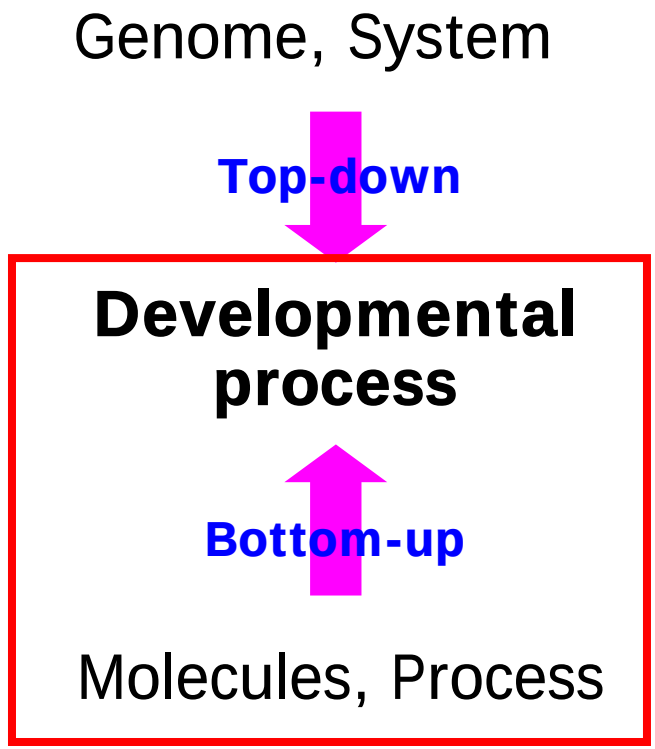




Bottom-up approach:

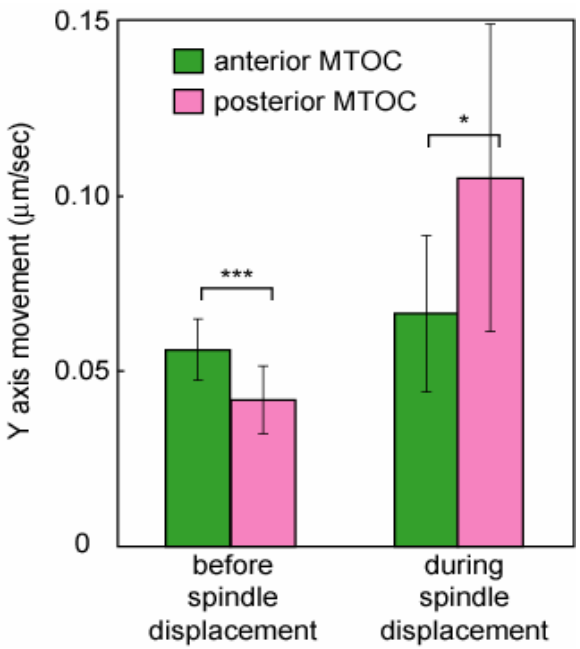
Nuclear positioning in one-cell embryo



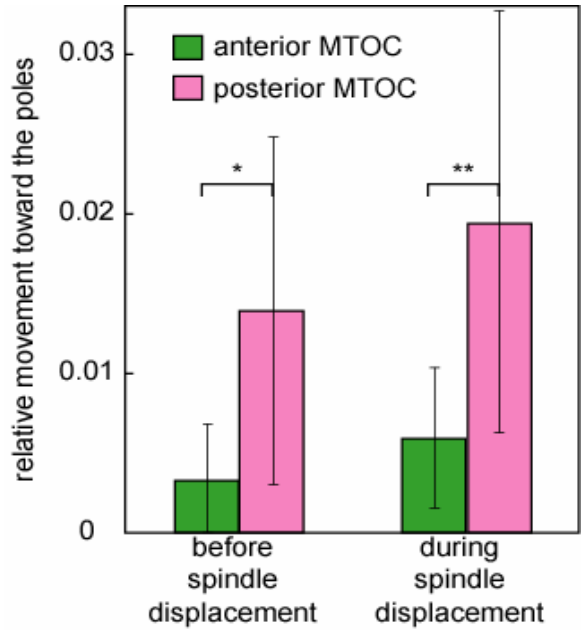


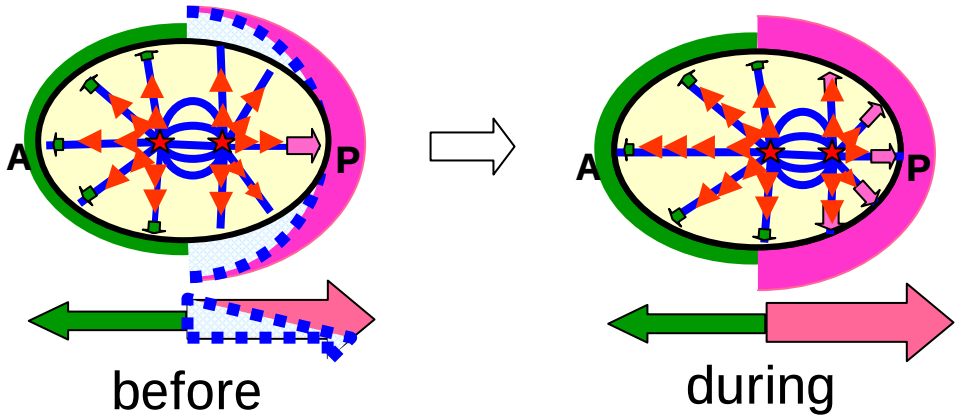
Cortical pulling force at lateral region is repressed before posterior displacement

lateral movement



polar movement





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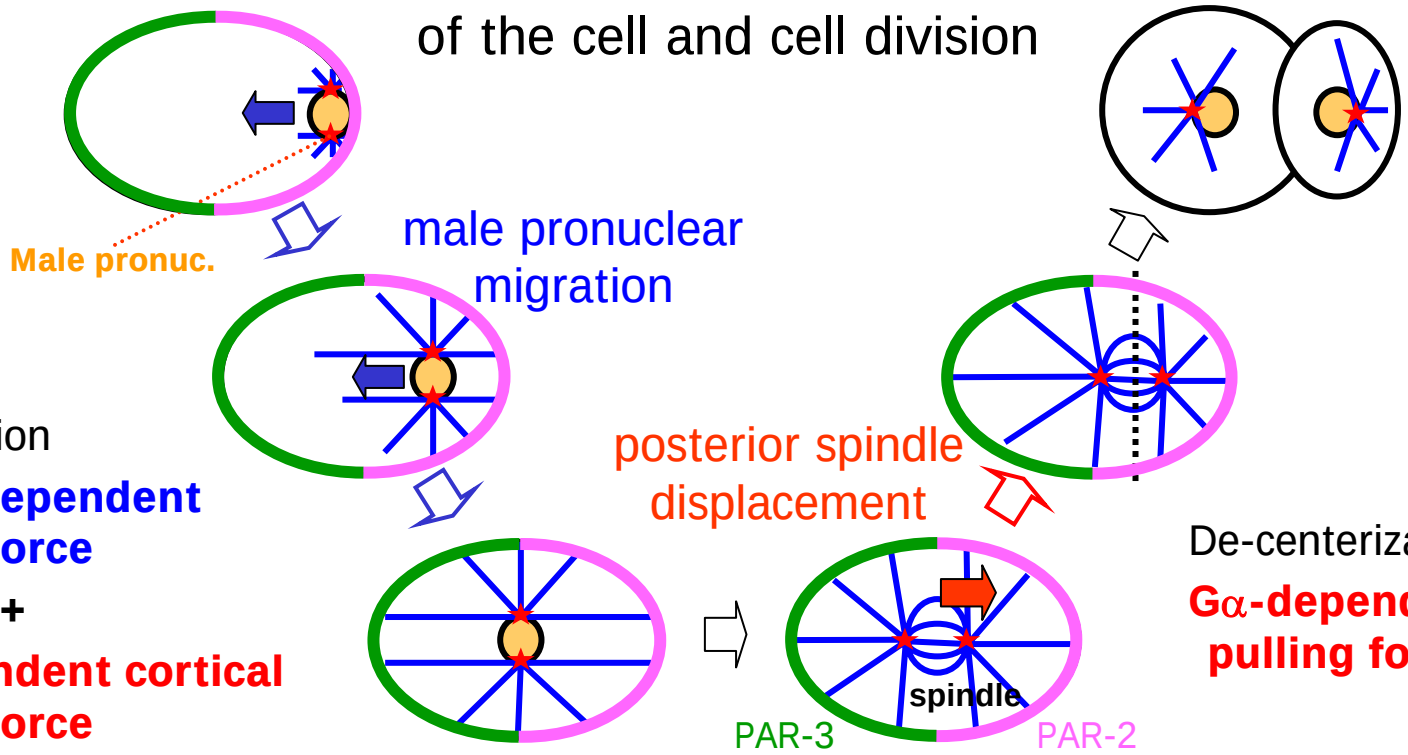


Centerization

Length-dependent pulling force

+

Gα-dependent cortical pulling force

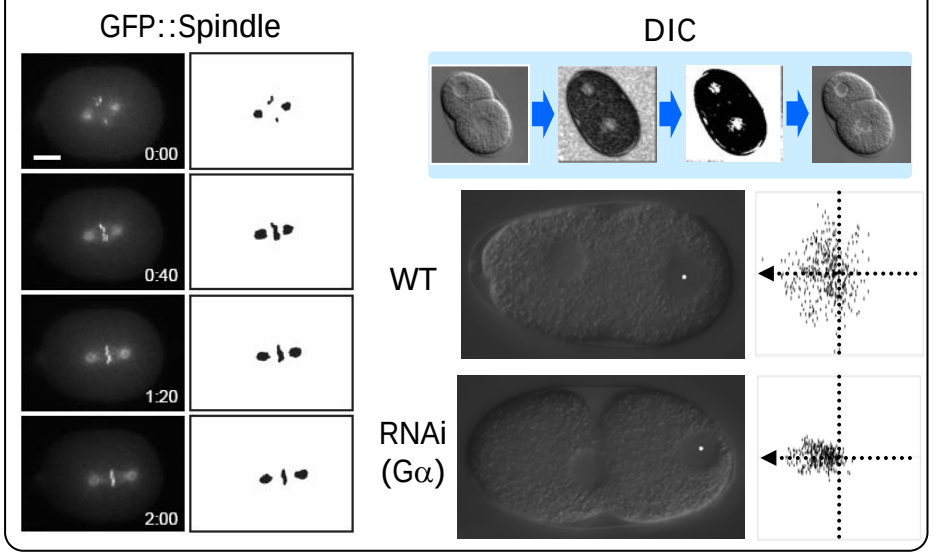
De-centerization

Gα-dependent cortical pulling force

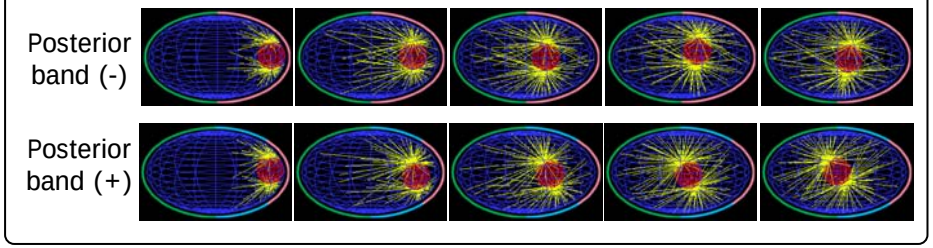
Why male pronucleus reach the center?

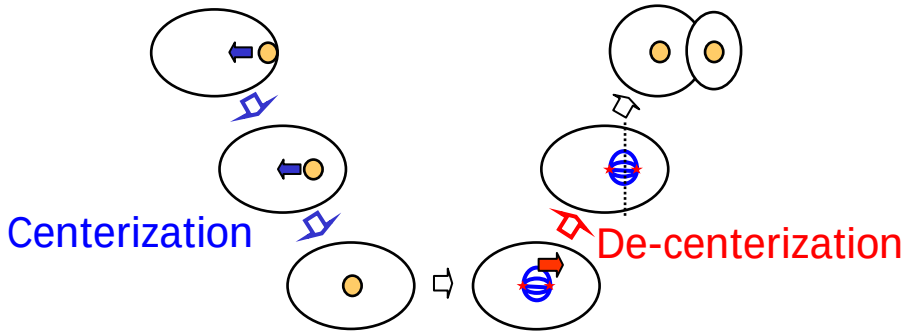
Computer simulation and image-processing create a new model for dynamics of nucleus in one-cell embryo

in vivo measurement using image processing

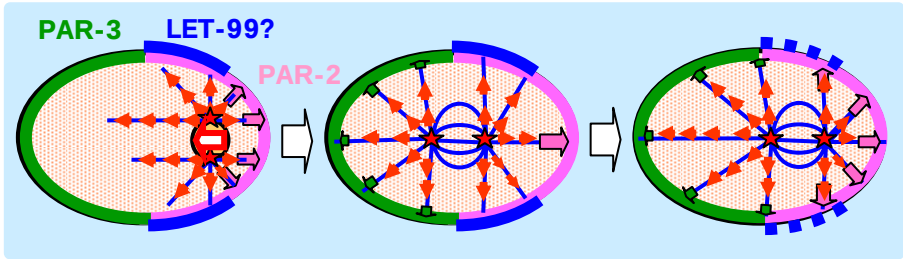


Computer simulation





A new model



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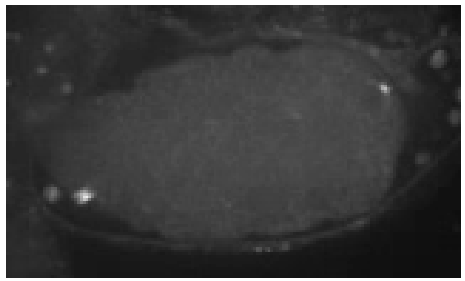
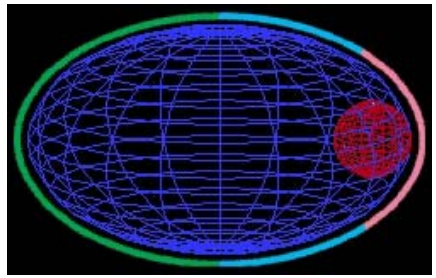


Simulation



Micro-movement analysis

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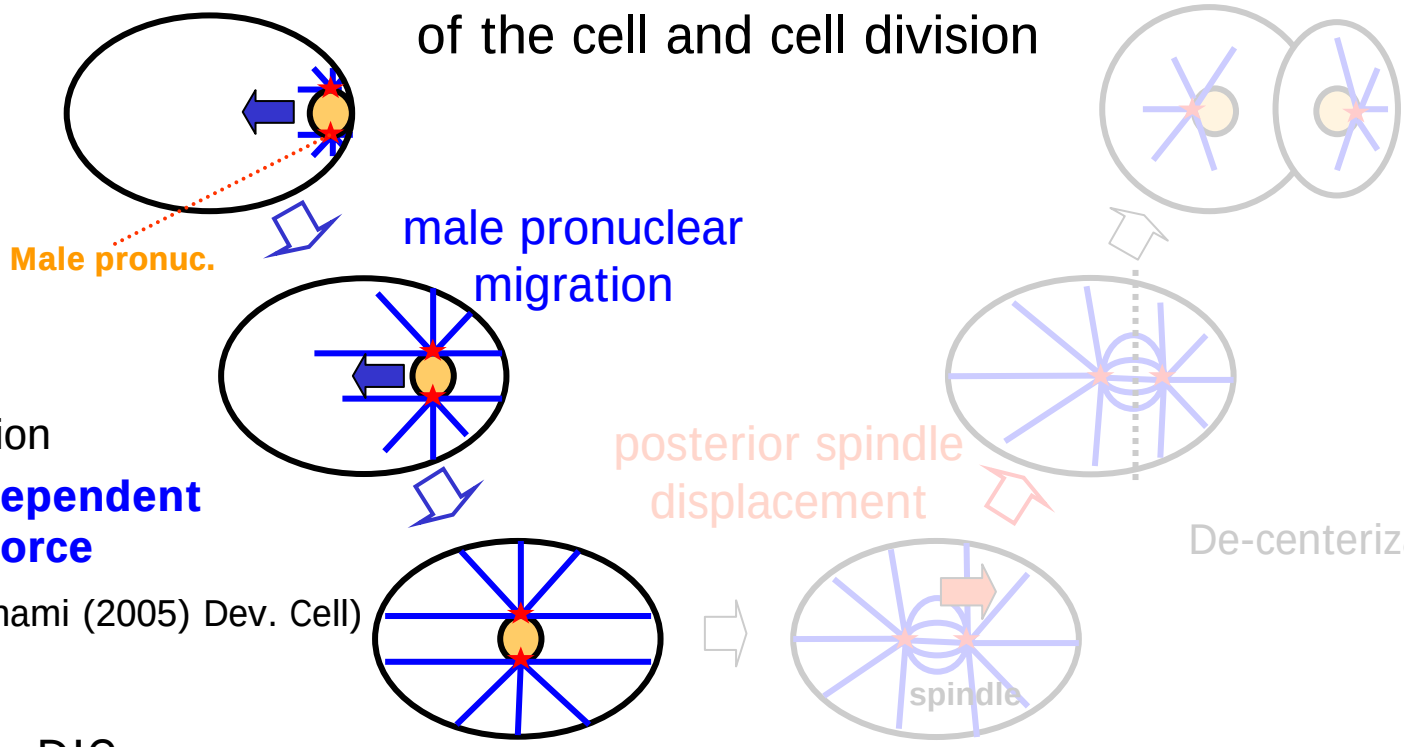
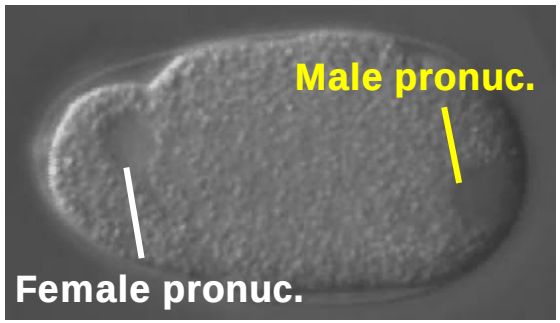
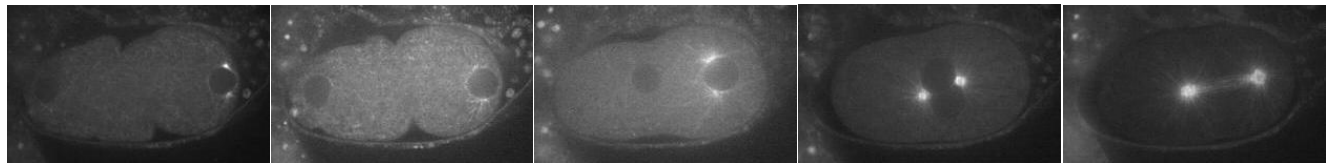


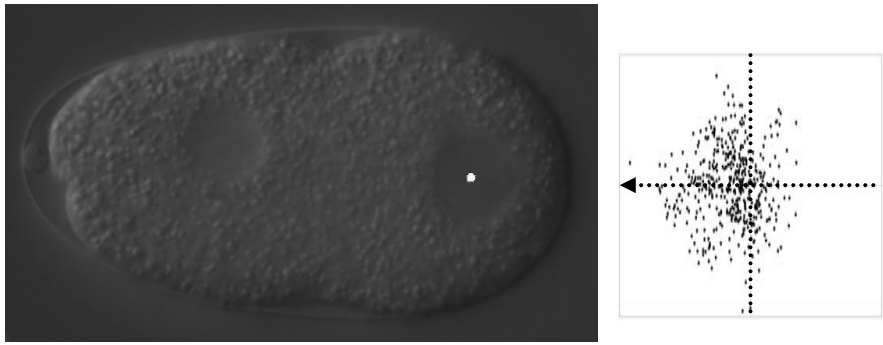
DIC



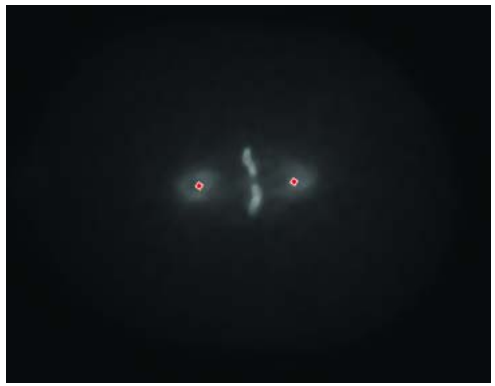
GFP::tubulin (white)



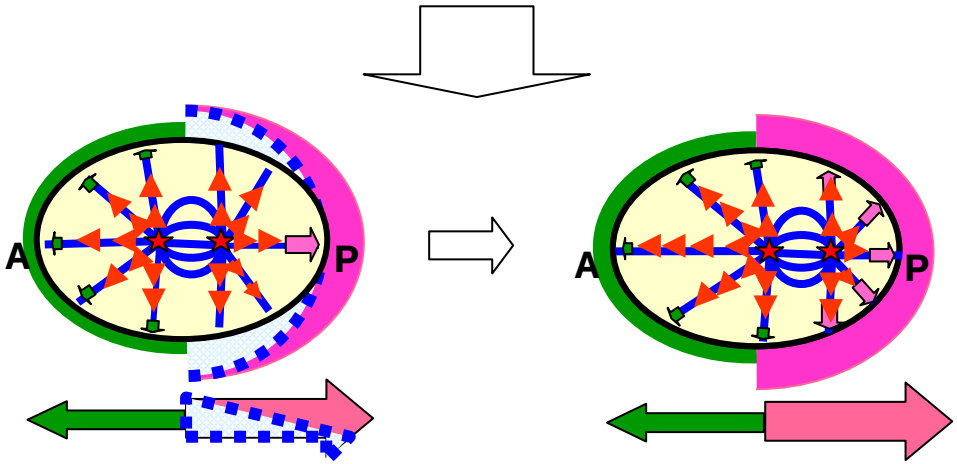
Micro-movement analysis



MM of male pronucleus

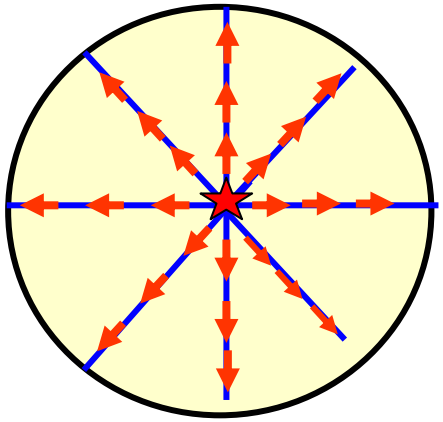


MM of spindle



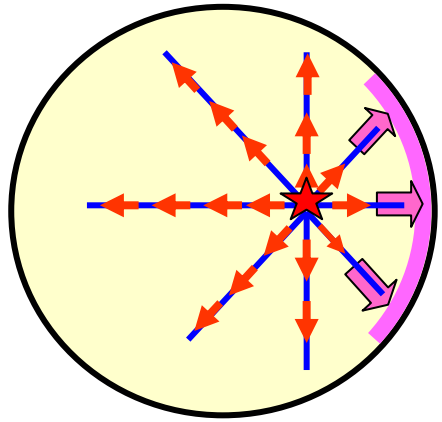
Temporal change in spatial distribution of cortical force that makes posterior displacement of spindle

General model of centrosome positioning



Length-Dependent Pulling Force (basal)

+



Cortical Pulling Force (modulation)

Top-down approach

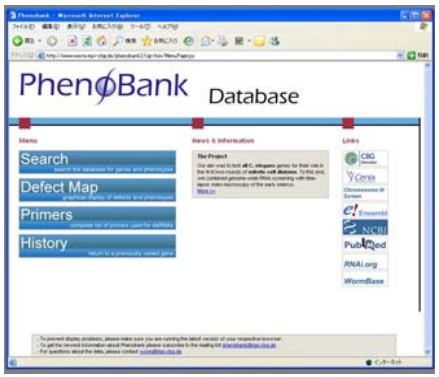
- We developed a system that objectively measure cell division pattern of C. elegans embryo upto 24-cell stage.
- We collected cell division pattern of 267 RNAi embryos.
- Computational analysis of cell division pattern data discovered many phenotypes that have not been detected in the past phenotypic analysis.

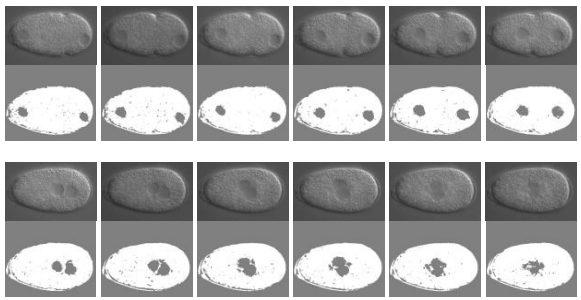
(2+1)D cell division pattern project

Collection of quantitative 2D cell division pattern up to 4-cell stage

Phenobank

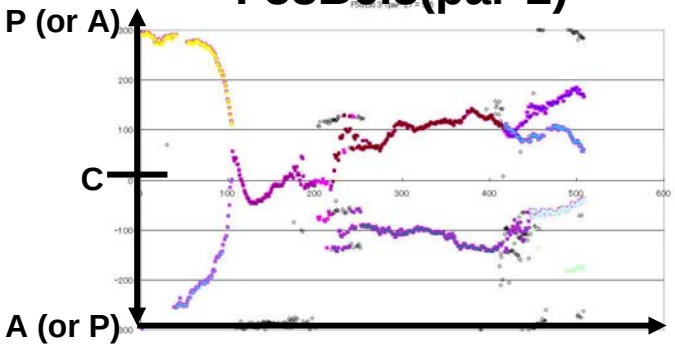
Movies of RNAi *C. elegans* embryos from one- to four-cell stage for almost all genes



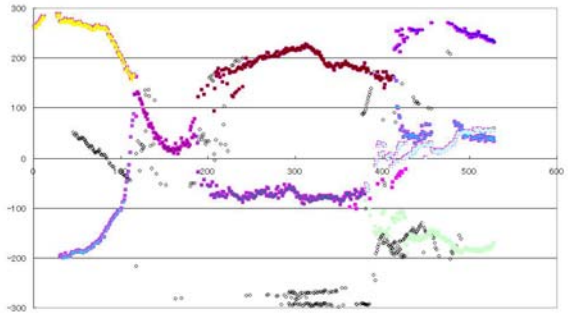


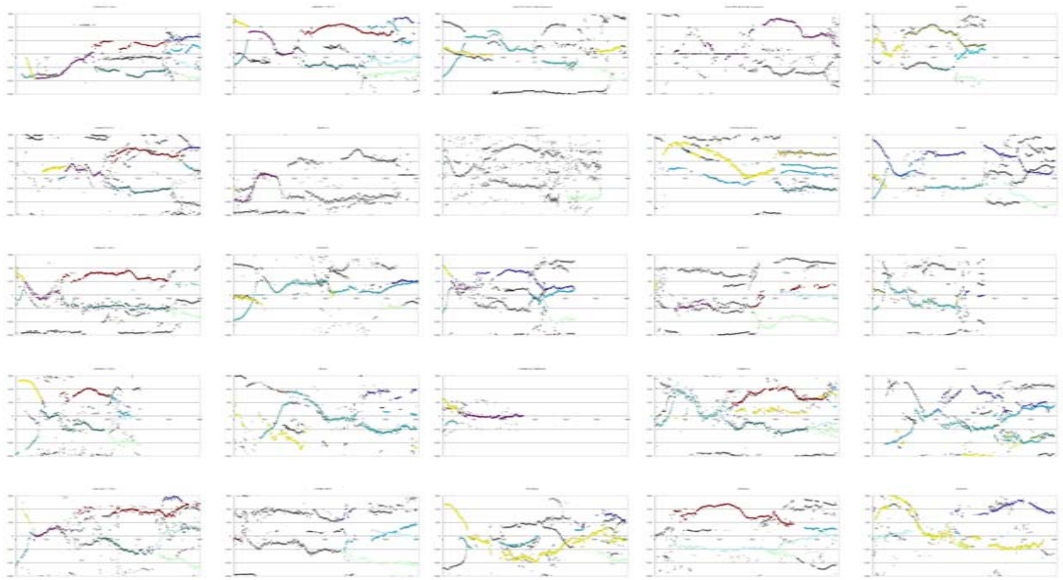
Quantitative difference in asymmetric cell division

F58B6.3(par-2)

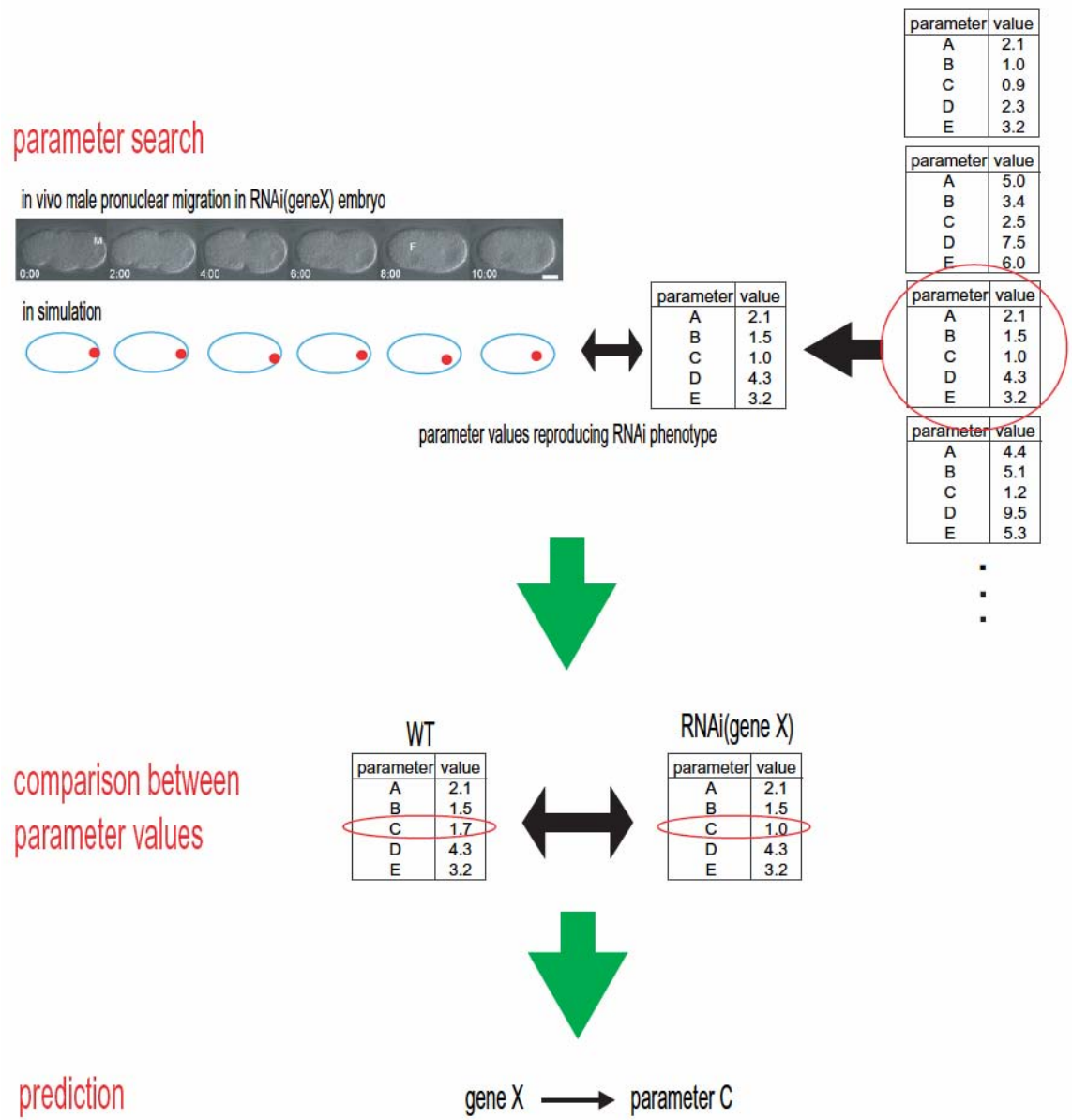


B0025.2





Prediction of gene function using parameters in simulation models



parameter	value
A	2.1
B	1.0
C	0.9
D	2.3
E	3.2

parameter	value
A	5.0
B	3.4
C	2.5
D	7.5
E	6.0

parameter	value
A	2.1
B	1.5
C	1.0
D	4.3
E	3.2

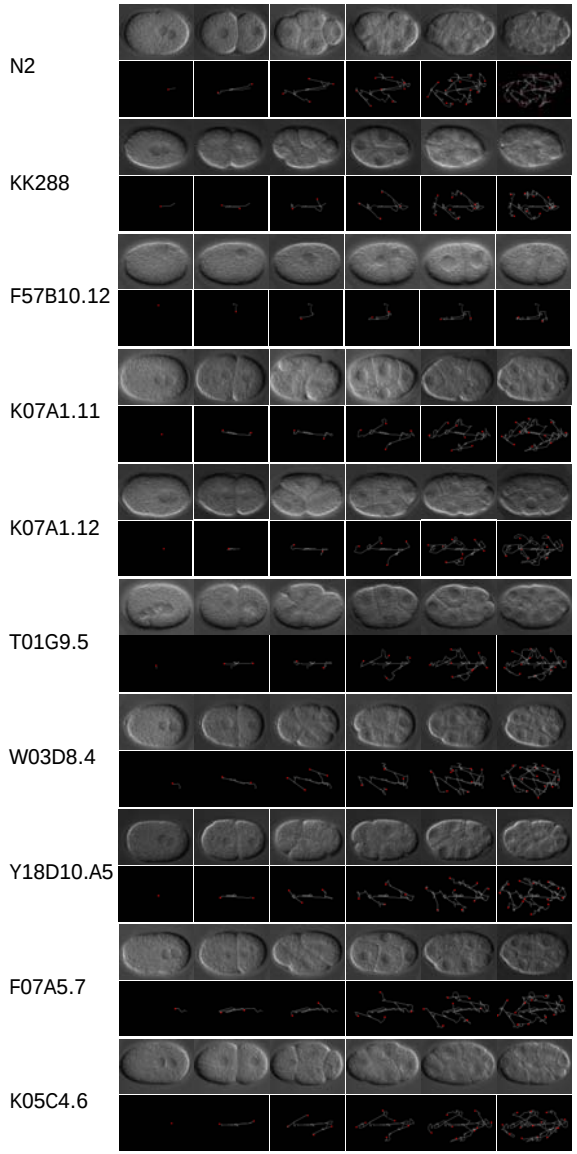
parameter	value
A	4.4
B	5.1
C	1.2
D	9.5
E	5.3

parameter	value
A	2.1
B	1.5
C	1.7
D	4.3
E	3.2

parameter	value
A	2.1
B	1.5
C	1.0
D	4.3
E	3.2

Knowledge extraction from cell division pattern data

Cell division pattern data



4D position etc.

Time	Connection		Position (μ m)		
	From	To	x	y	z
1	0	1000	12.1142	42.1350	8.34074
2	1000	2000	12.2759	41.7566	8.68689
3	2000	3000	13.9267	41.8832	8.18010
4	3000	4000	14.9502	41.4641	9.03192
5	4000	5000	16.4480	40.9389	10.4932
6	5000	6000	17.9468	40.8659	10.7559
7	6000	7000	20.1625	39.4095	10.7396
8	7000	8000	20.6087	38.9201	10.4093
9	8000	9000	22.1473	38.0135	10.4530

Comparison of quantified features

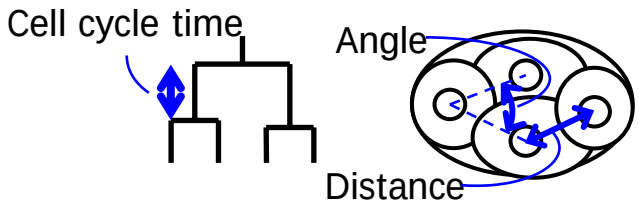
WT

feature	value
A	2.33
B	-0.41
C	0.98
D	**4.56**
E	3.19
F	-4.50
G	**2.11**
H	1.05

RNAi(geneX)

feature	value
A	2.33
B	-0.41
C	0.98
D	**1.42**
E	3.19
F	-4.50
G	**-0.01**
H	1.05

Quantification of features in cell division pattern



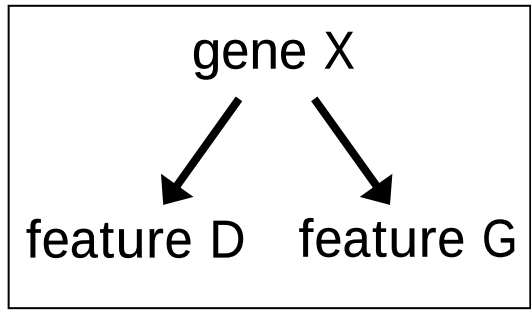
Cell cycle time: $T_{i,cct} = t_{i,last} - t_{i,first}$

Distance: $D_{i,j} = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)^2}$

Angle:

$$\cos\theta_{i,j,k} = \frac{(x_i - x_j)(x_k - x_j) + (y_i - y_j)(y_k - y_j) + (z_i - z_j)(z_k - z_j)}{\sqrt{(x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)^2}\sqrt{(x_k - x_j)^2 + (y_k - y_j)^2 + (z_k - z_j)^2}}$$

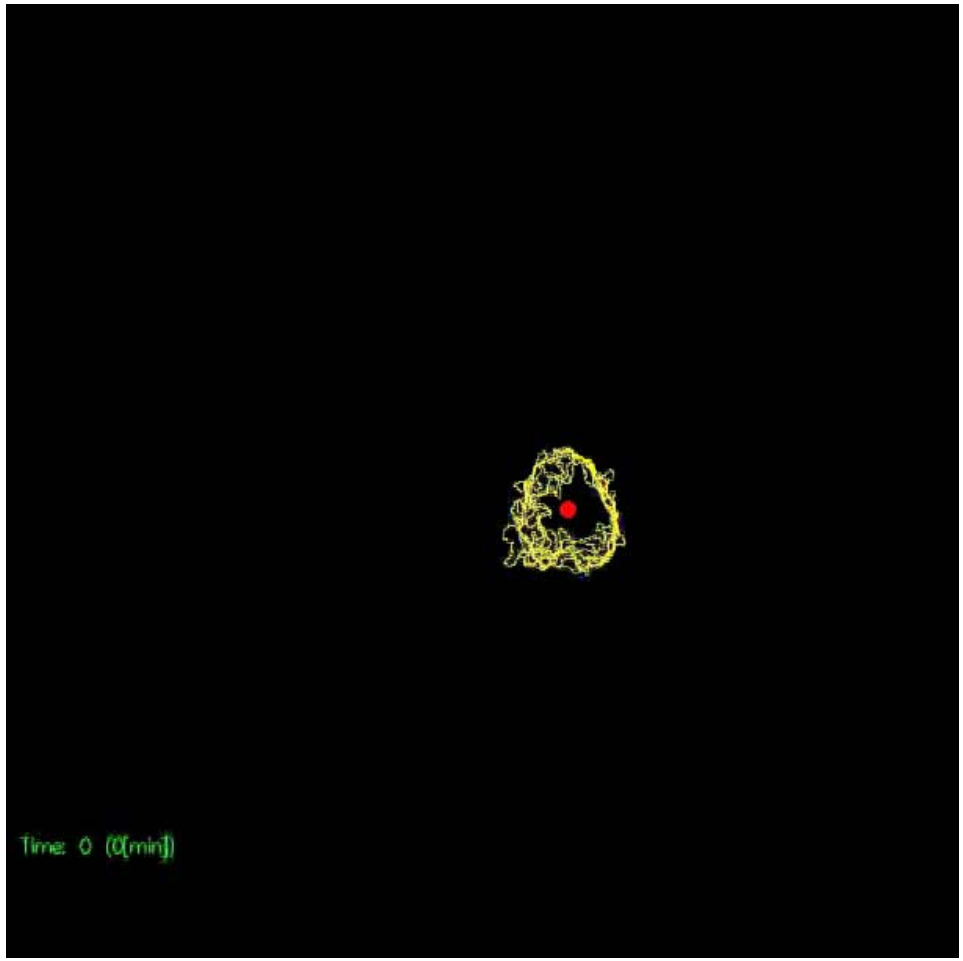
Prediction of gene function



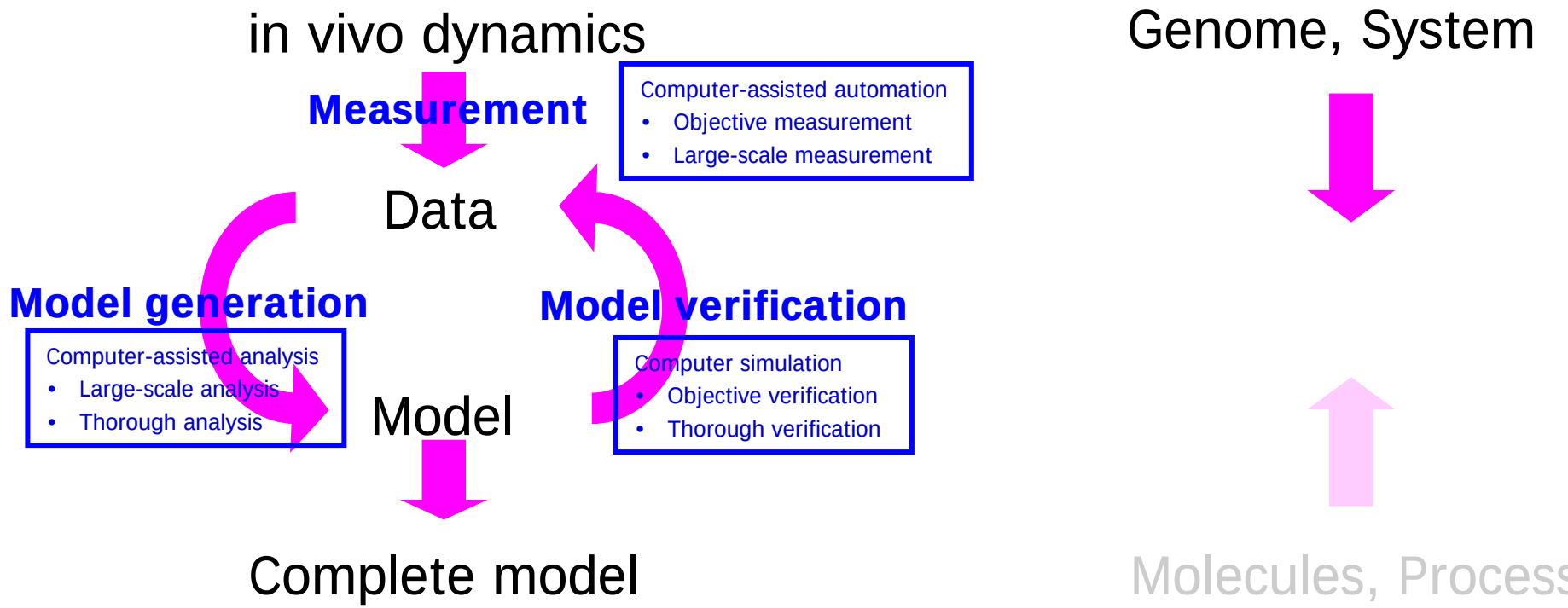
Objective and quantitative cell division pattern data

Time (x40s)	Connection		Position (μm)		
	From ID	To ID	x	y	z
1	0	1000	12.1142	42.1350	8.34074
2	1000	2000	12.2759	41.7566	8.68689
3	2000	3000	13.9267	41.8832	8.18010
4	3000	4000	14.9502	41.4641	9.03192
5	4000	5000	16.4480	40.9389	10.4932
6	5000	6000	17.9468	40.8659	10.7559
7	6000	7000	20.1625	39.4095	10.7396
8	7000	8000	20.6087	38.9201	10.4093
9	8000	9000	22.1473	38.0135	10.4530
10	9000	10000	25.6702	35.0143	10.3059
11	10000	11000	28.3613	32.6103	10.6428
12	11000	12000	30.5087	31.3147	11.3446
13	12000	13000	31.3288	30.8792	11.2539
14	13000	14000	31.1548	30.9600	11.3044
15	14000	15000	30.9064	31.5397	11.5984
16	15000	16000	30.0573	32.1259	11.7795
17	16000	17000	28.3158	33.5123	11.5346
18	17000	18000	27.1013	33.2146	11.5704
19	18000	19000	25.9500	34.7635	11.5519
20	19000	20000	27.5301	34.2762	11.4346
...	...	...	...	...	...

3D positions of nucleus every 40sec.

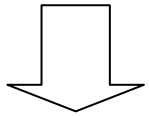


Top-down approaches



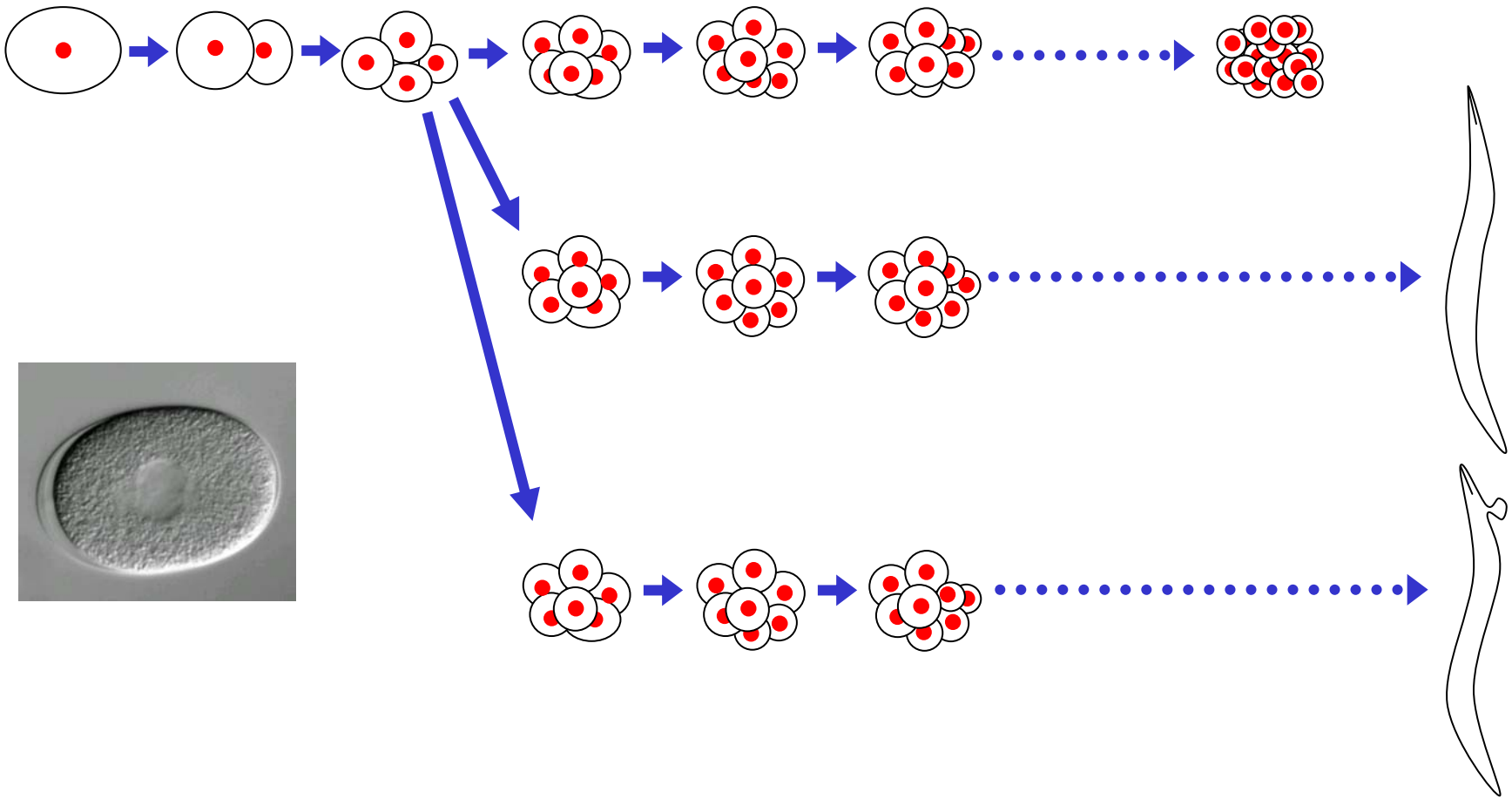
Quantitative analysis of embryonic structures

- Structural change is a major part of embryogenesis.
- Embryonic structure itself has not usually been a target of quantitative analysis.
  - Lack of technologies for quantification and analysis.



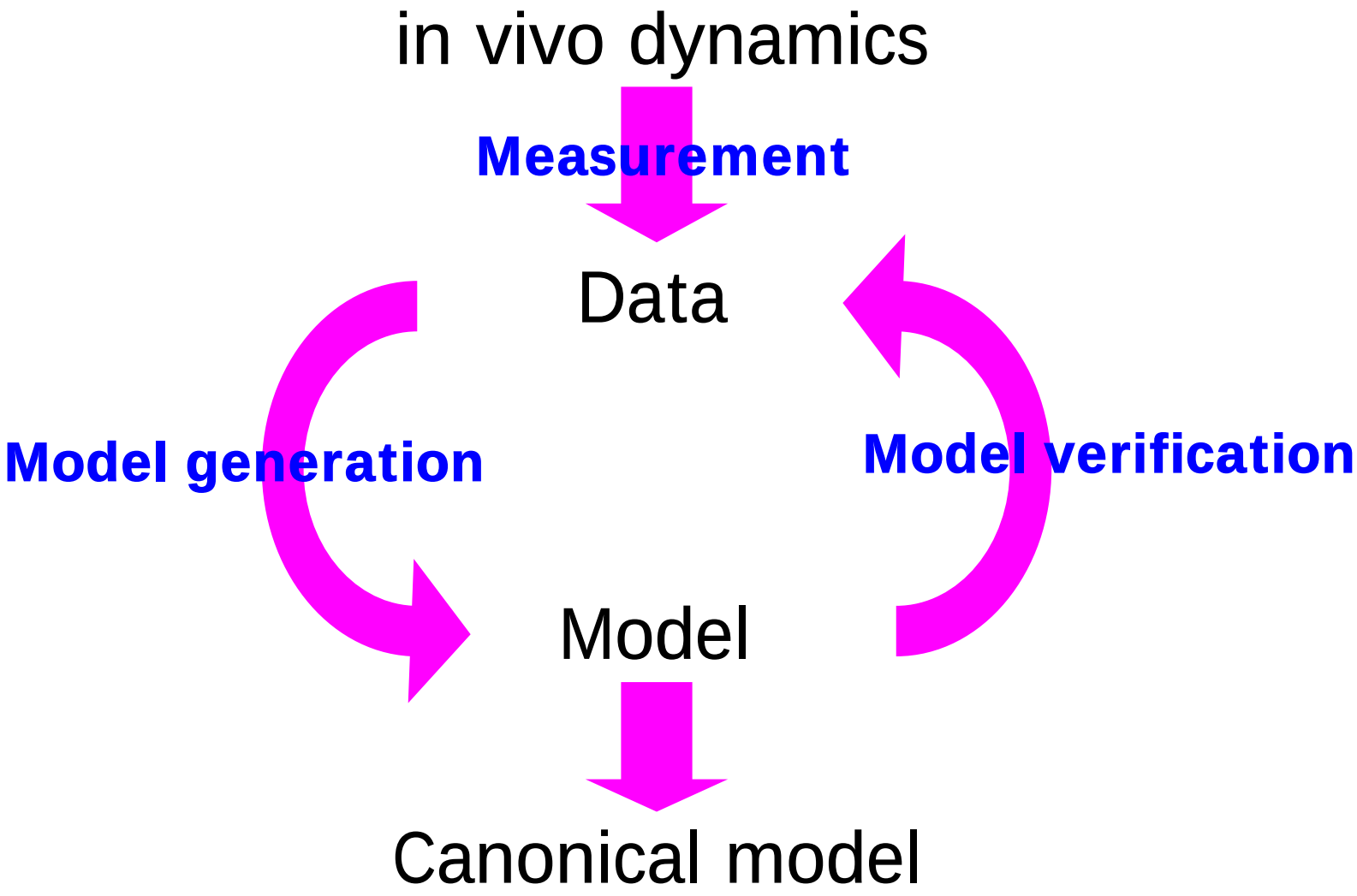
- Computer technologies enable quantitative analysis of embryonic structures.
  - Quantification using image processing
  - Computer simulation

Development is a highly controlled four-dimensional dynamic process



Question: How this for-dimensional process works?

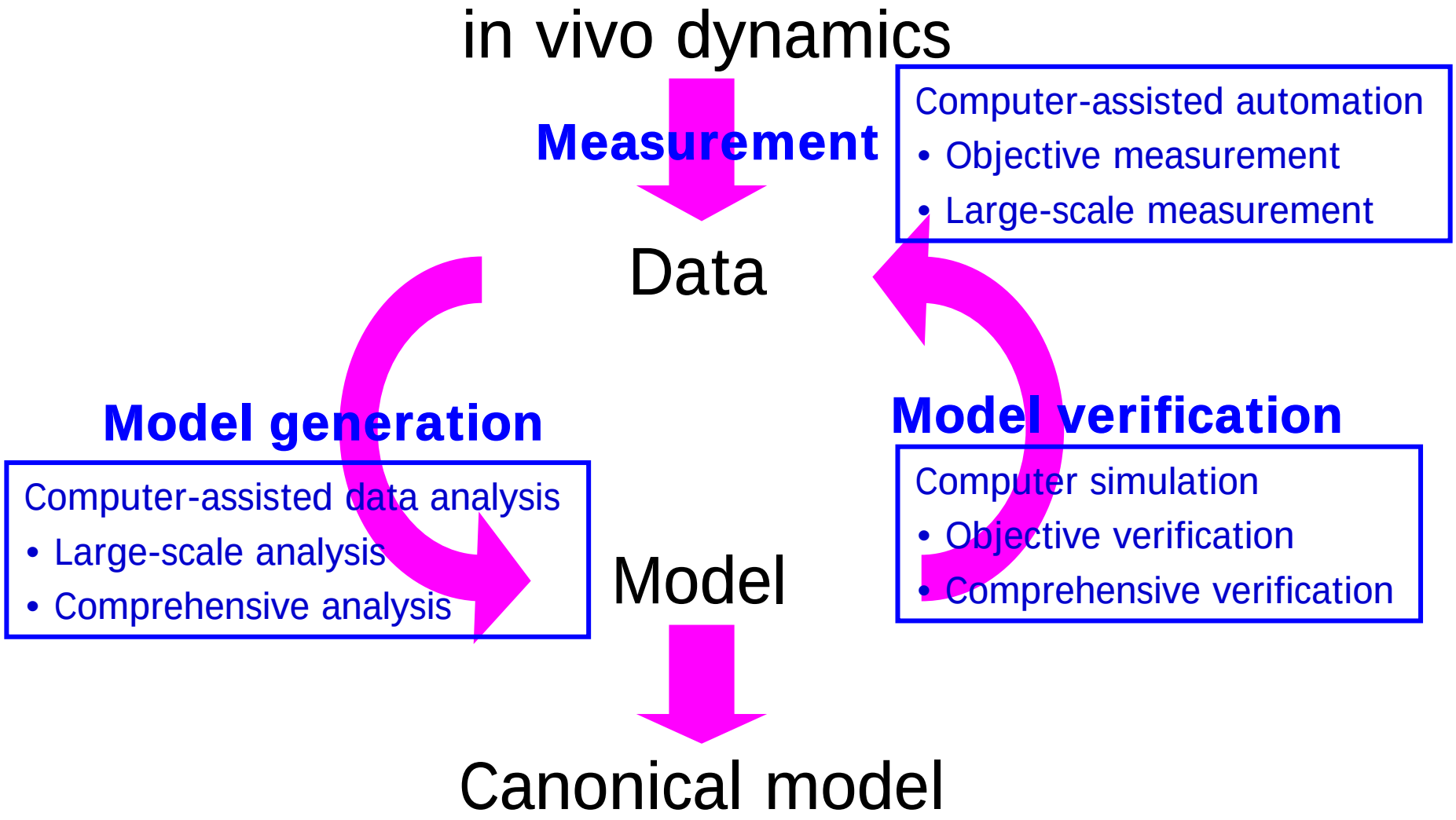
Strategy to understand developmental process



For-dimensionality makes every step difficult!

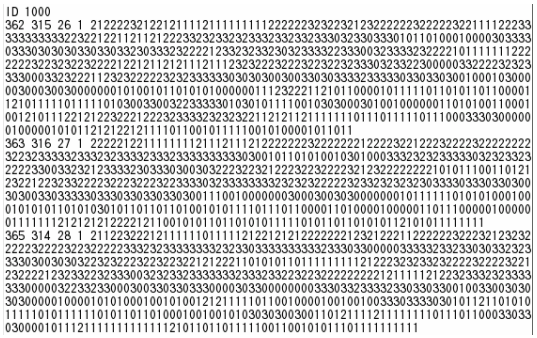
→ **Quantitative approaches are necessary**

Computers provide great help for quantitative studies of developmental processes

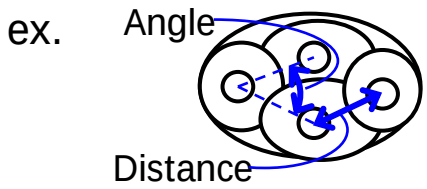


Objective phenotype analysis using cell division pattern data

Cell division pattern data



Calculation of signatures in cell division pattern



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Angle:

$$\cos\theta_{i,j,k} = \frac{(x_i - x_j)(x_k - x_j) + (y_i - y_j)(y_k - y_j) + (z_i - z_j)(z_k - z_j)}{\sqrt{(x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)^2}\sqrt{(x_k - x_j)^2 + (y_k - y_j)^2 + (z_k - z_j)^2}}$$

Comparison of calculated signatures

WT

feature	value
A	2.33
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RNAi(geneX)

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D	**1.42**
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G	**-0.01**
H	1.05

Cell division patterns of 29 WT embryos were measured for statistical analysis

Strategy to understand developmental process