VANDERBILT UNIVERSITY
MEDICAL CENTER

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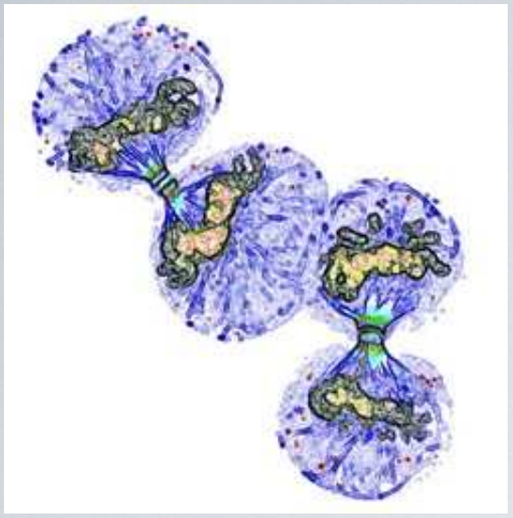
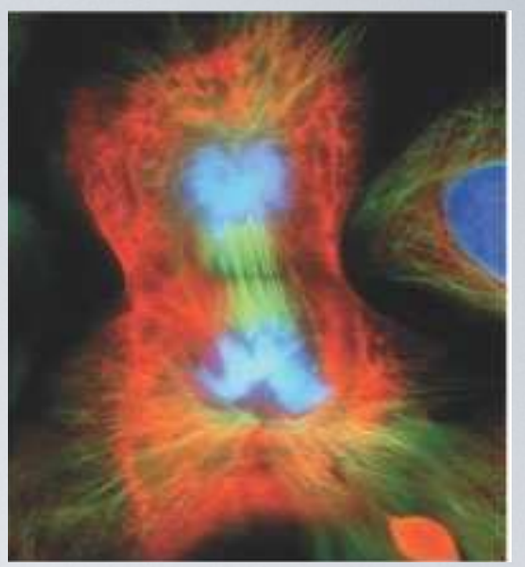
AN EMG MODEL OF CELL CYCLE TIME VARIABILITY IN CANCER DEVELOPED FROM LARGE DATASETS OF SINGLE-CELL MEASUREMENTS

Darren Tyson
Vanderbilt University

Mathematical Oncology Workshop
March 18, 2010
Fields Institute, Toronto, ON



CELL DIVISION



- The process of cell division has been studied since first described by Rudolf Virchow in 1855, "*omnis cellula e cellula*" ~ all cells come from cells

- Cancer is a disease of uncontrolled cell division

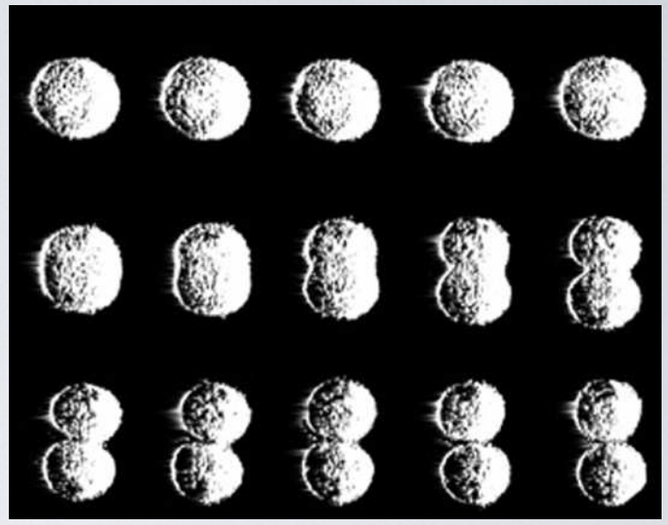
- Much is known about signaling pathways controlling cell division, especially due to the advent of "omics" technology coupled to mathematical models

OUR CHALLENGE AS CANCER CELL BIOLOGISTS

- How can we link signaling network dynamics or states to the decision of single cells to divide?

- First, it requires sufficiently large datasets of quantified cell cycle times

TIME LAPSE LIVE-CELL IMAGING

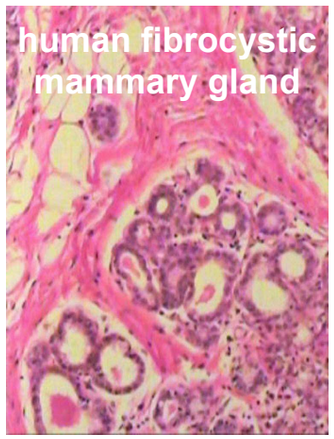


- Used for decades to study cell division
- Direct measurement of individual cells in a population (no need to model or estimate single-cell behavior!)
- Throughput limited by laborious manual tracking and challenges of automatic identification of cells in images from transmitted light microscopy
- Limitations alleviated by new instruments and computational tools

IMAGE ACQUISITION

- High-content imager (BD Pathway 855)
- H2BmRFP-expressing cells in complete culture medium
- Cells are washed with serum-free medium and medium is replaced with complete or serum-free medium
- Images are acquired every 6 min in confocal mode with 20X objective
- 3 cell lines, 2 conditions, 6 replicates = 36 wells

MODEL SYSTEM



spontaneous immortalization

MCF10A

V12 H-ras

MCF10AT

normal epithelium

Tumorigenicity

MCF10AT1

100 days

367 days

cloned & cultured
(MCF10AT1K.c12)

77 days

181 days

292 days

cloned in agarose

MCF10A-CA1a

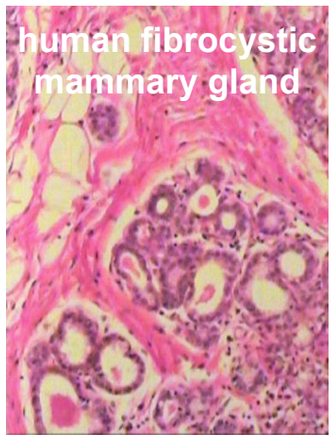
MCF10A-CA1d

invasive carcinoma

Dawson *et al.*, American Journal of Pathology 148: 313–319, 1996

Santner *et al.*, Breast Cancer Research and Treatment 65: 101–110, 2001

MODEL SYSTEM



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H2B-LABELED CELL IMAGING

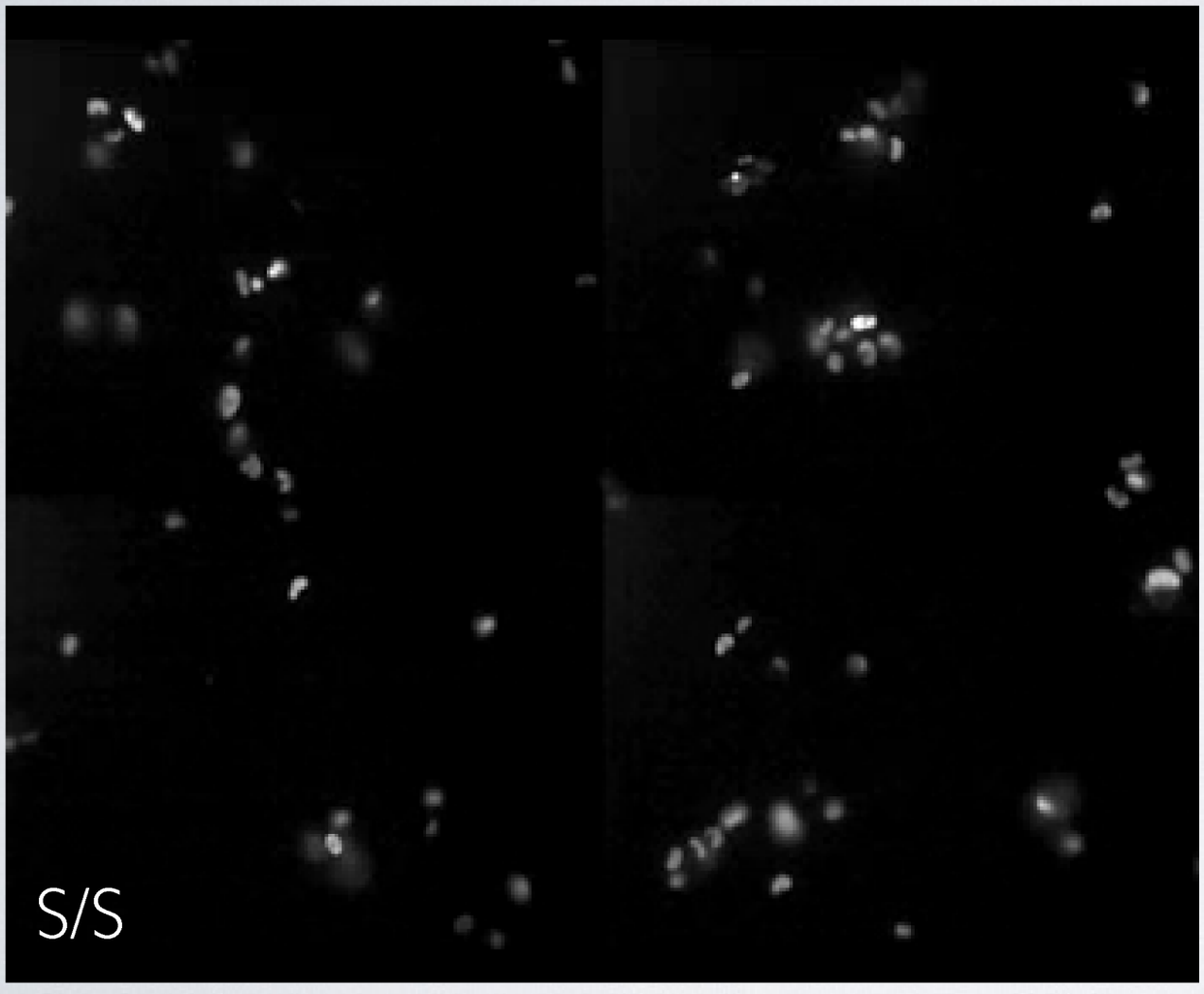
MCF10A

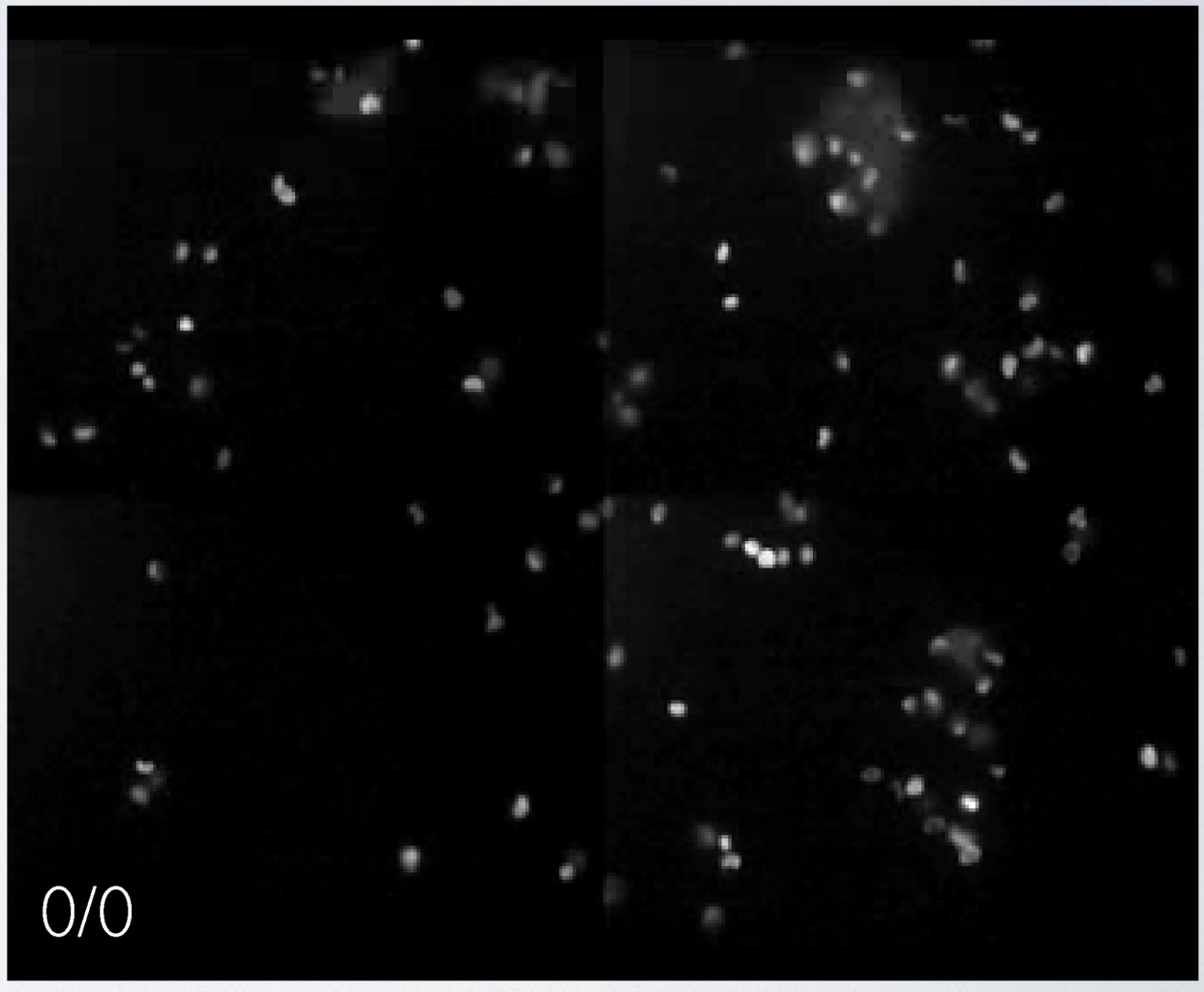
S/S

0/0

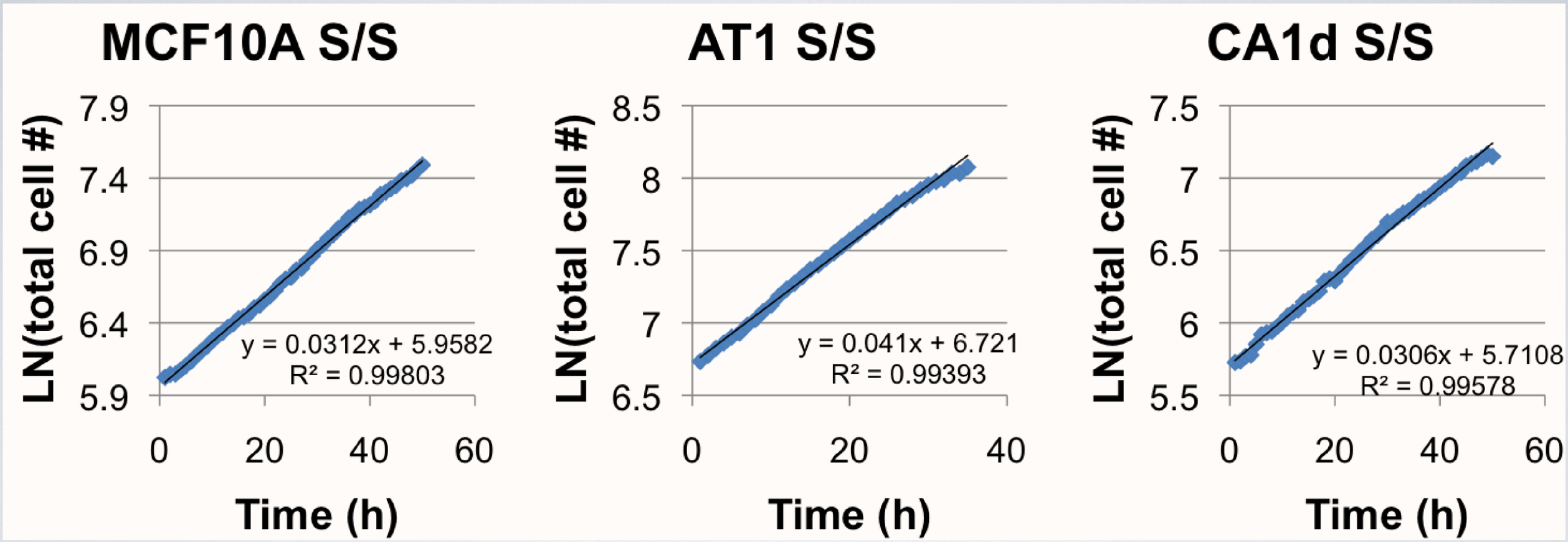
H2B-LABELED CELL IMAGING

MCF10A





OPTIMAL CONDITIONS



Data obtained by automatically counting nuclei in every 10th frame (1/h)
(discarding 90% of images from data set)

An exponential model is sufficient to describe proliferation of cell populations in optimal growth conditions

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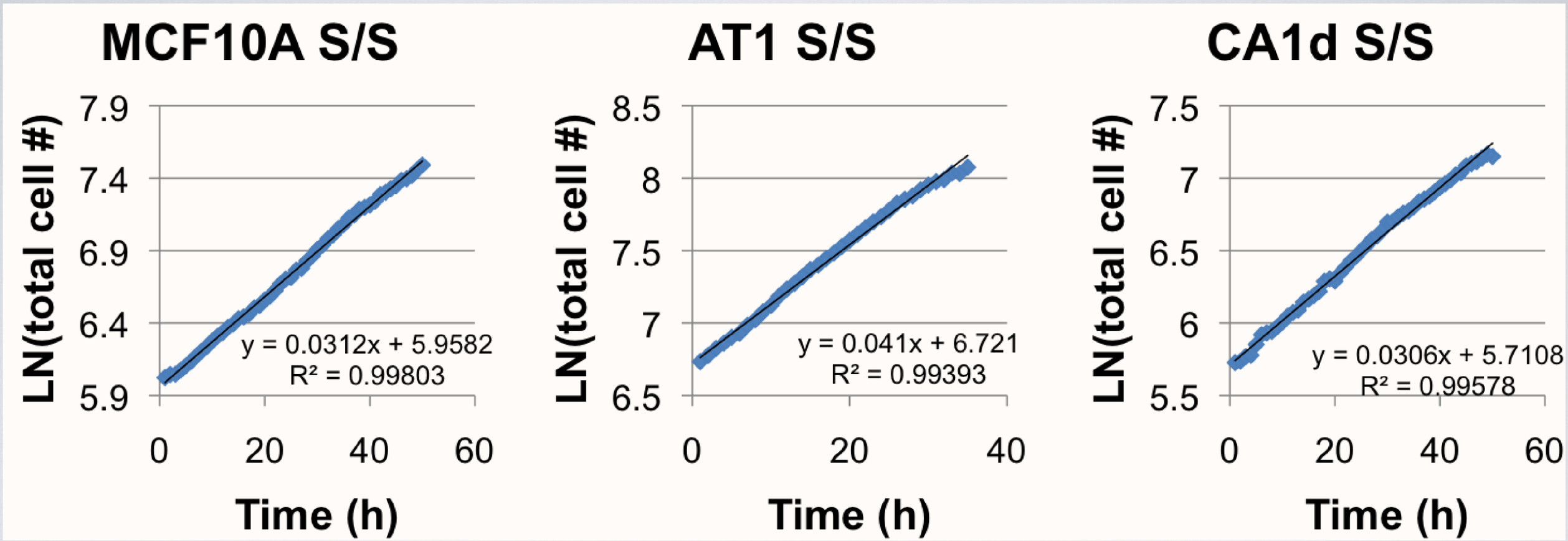

DT	22.22 h	16.91h	22.65 h

Data obtained by automatically counting nuclei in every 10th frame (1/h)
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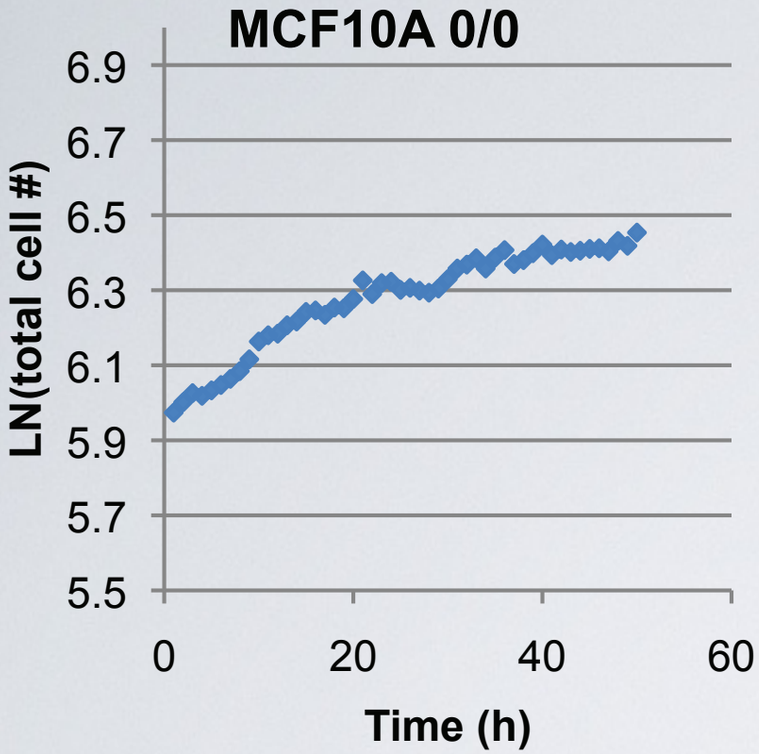
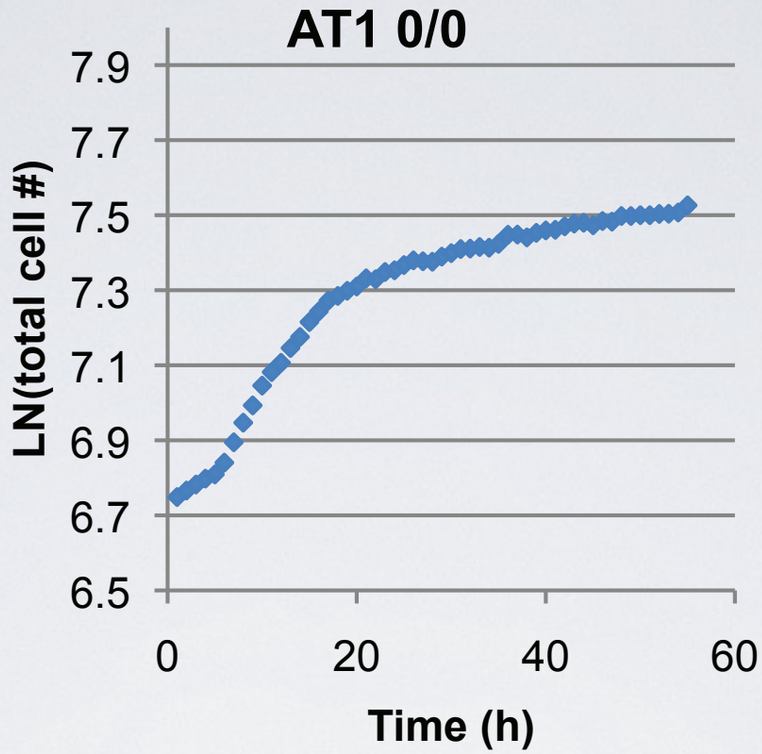
An exponential model is sufficient to describe proliferation of cell populations in optimal growth conditions

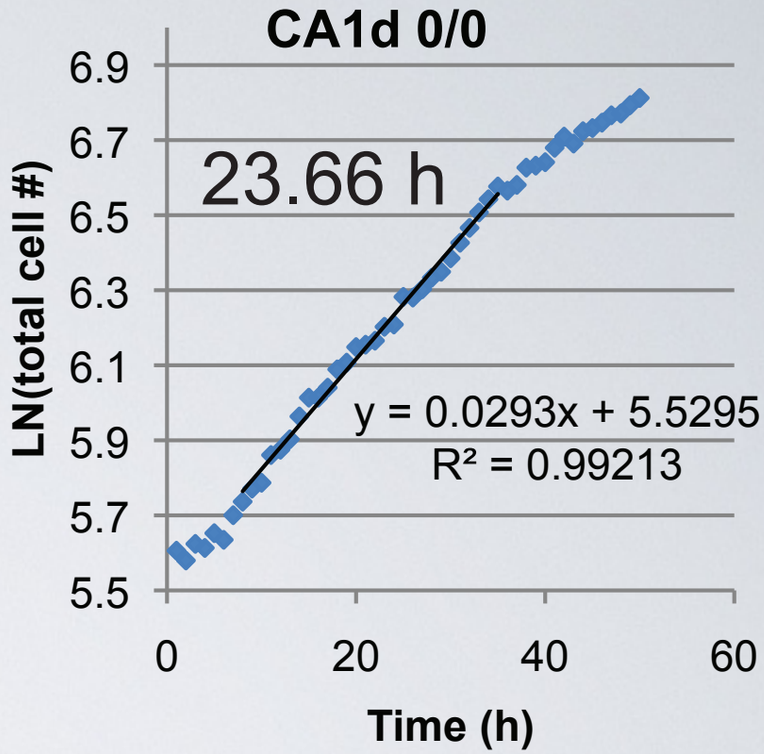
SERUM-DEPRIVED



period	DT
10–55 h	166.4h



period	DT
3–16 h	19.84 h
30–65 h (not space-restricted)	173.9 h



period	DT
3–40h	23.66 h
(Space-restricted >40h)	

Exponential model <u>not</u> sufficient to describe change in MCF10A and AT1 population size over time

QUESTIONS

- What is the range of cell cycle times of cells comprising the populations of exponentially dividing cells?
  - Are more slowly-dividing cells present?
  - Is the range of cell cycle times different for non-tumorigenic cells vs cancer cells?
- Exactly *how* is rate of proliferation changing in response to serum deprivation?
  - Are some cells no longer dividing (accumulation in G1)?
  - Are cell cycles longer in duration?

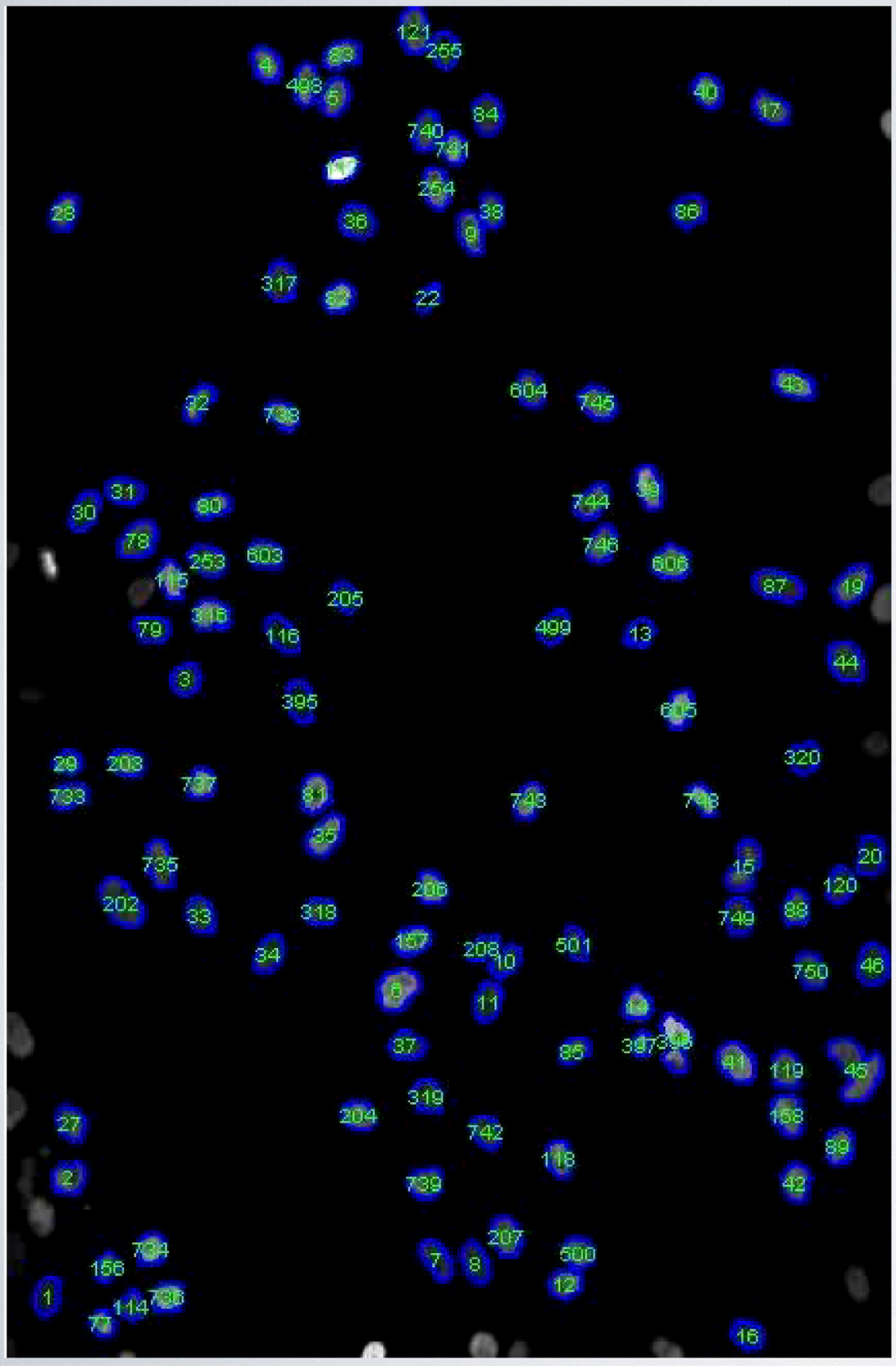
Need single-cell data to answer these questions

AUTOMATED ANALYSIS

1. ID & track individual cells
2. Detect mitotic events (using several criteria)
3. Assign daughter cells new IDs
4. Record ancestry
5. Generate image stack for verification (generation indicated by color)

W. Georgescu

AUTOMATED ANALYSIS



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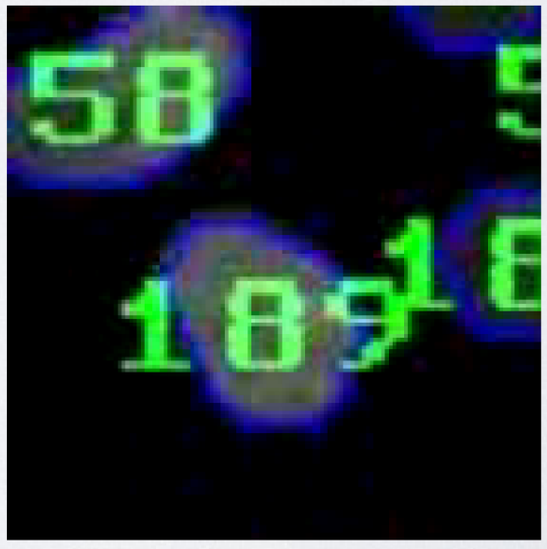

W. Georgescu

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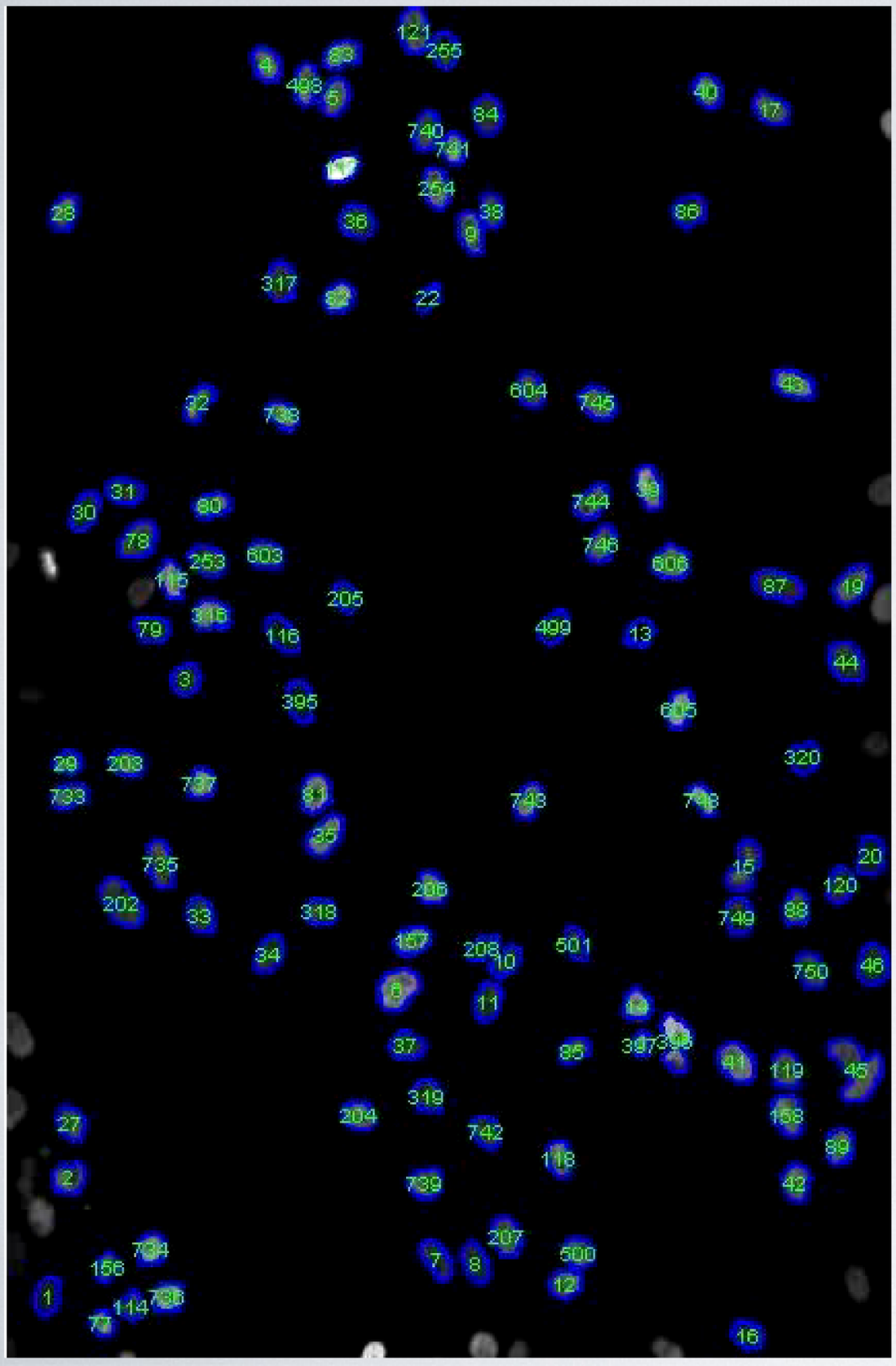

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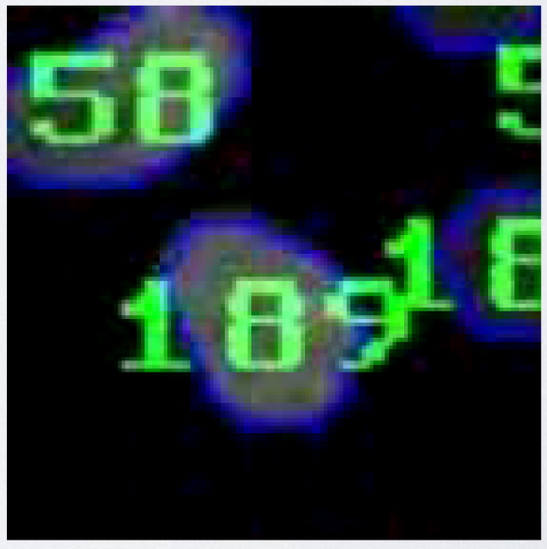

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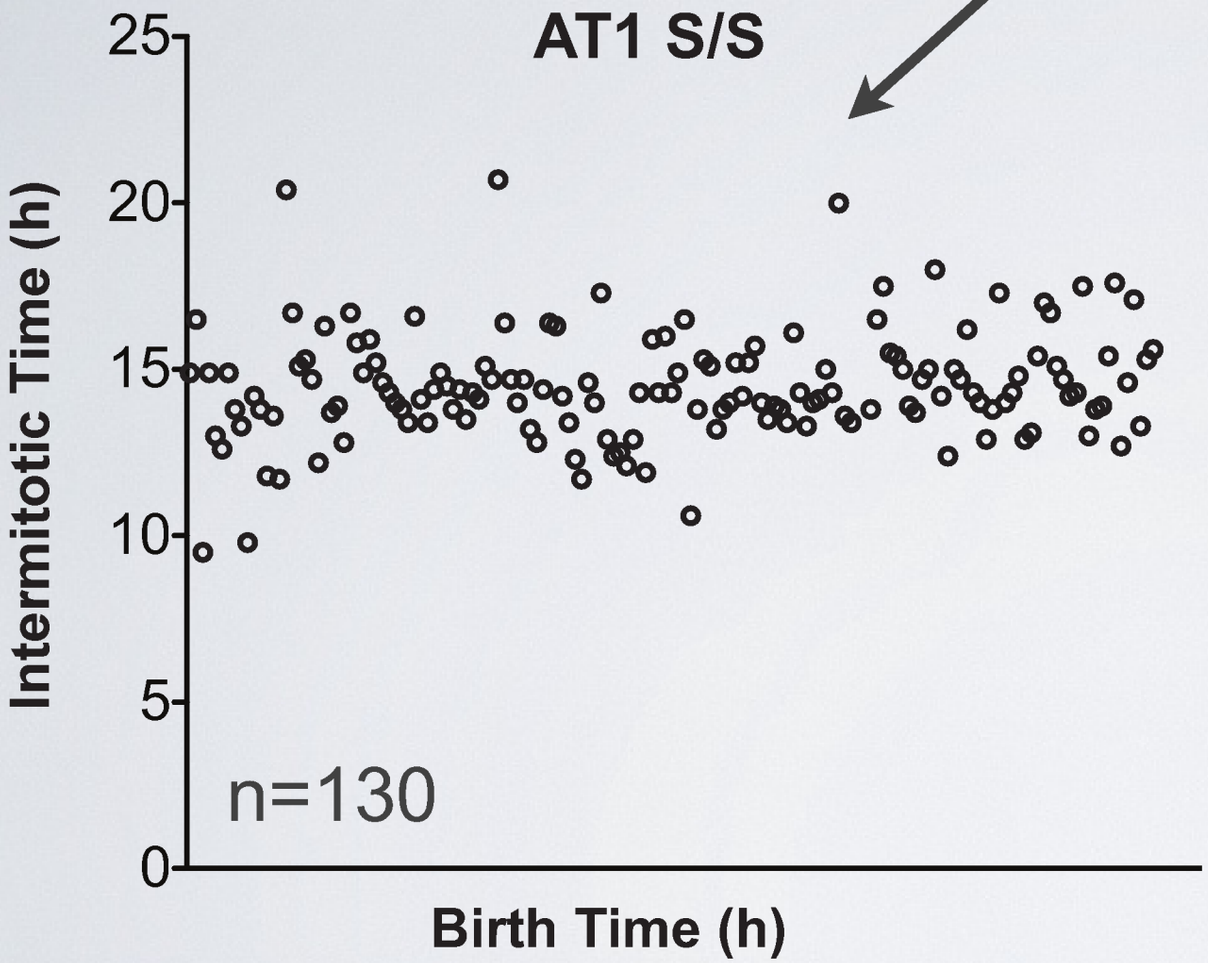
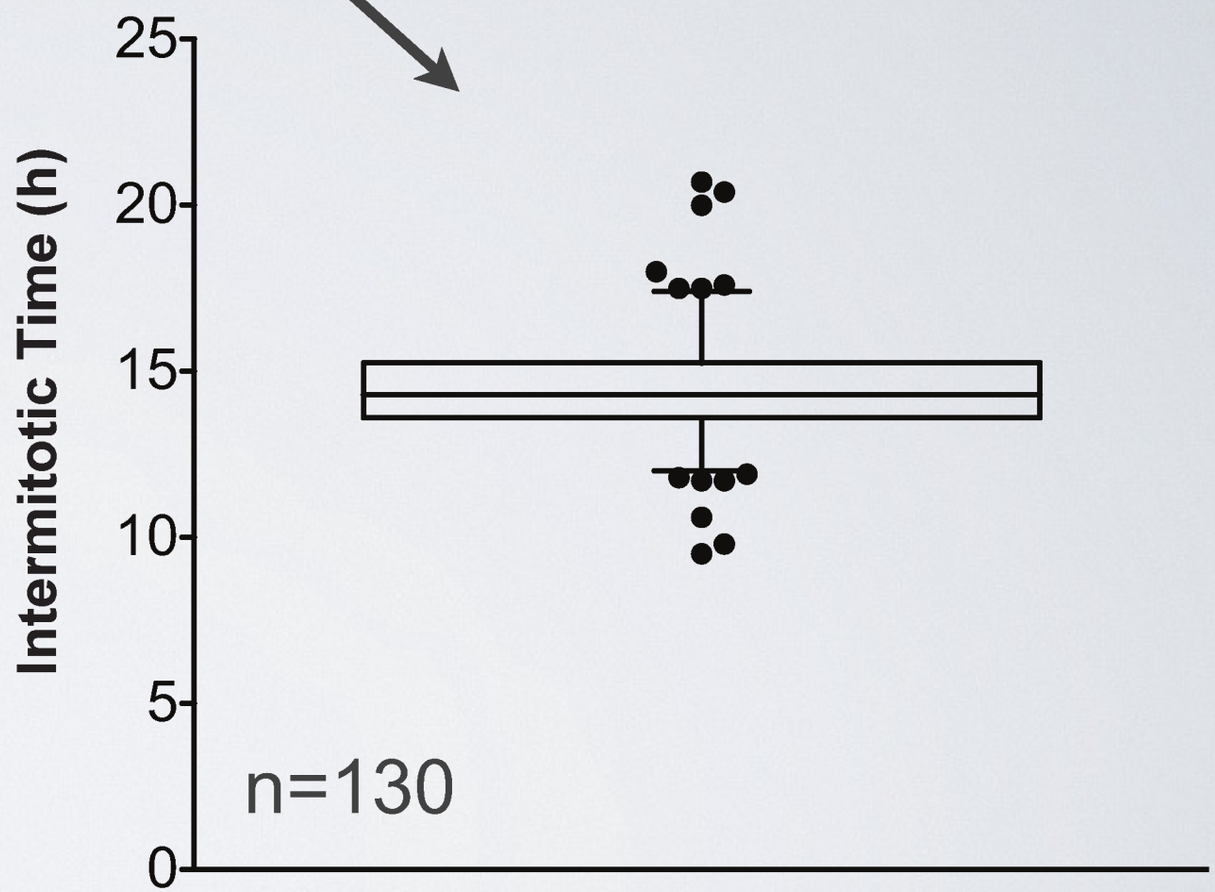
IMT DISTRIBUTIONS

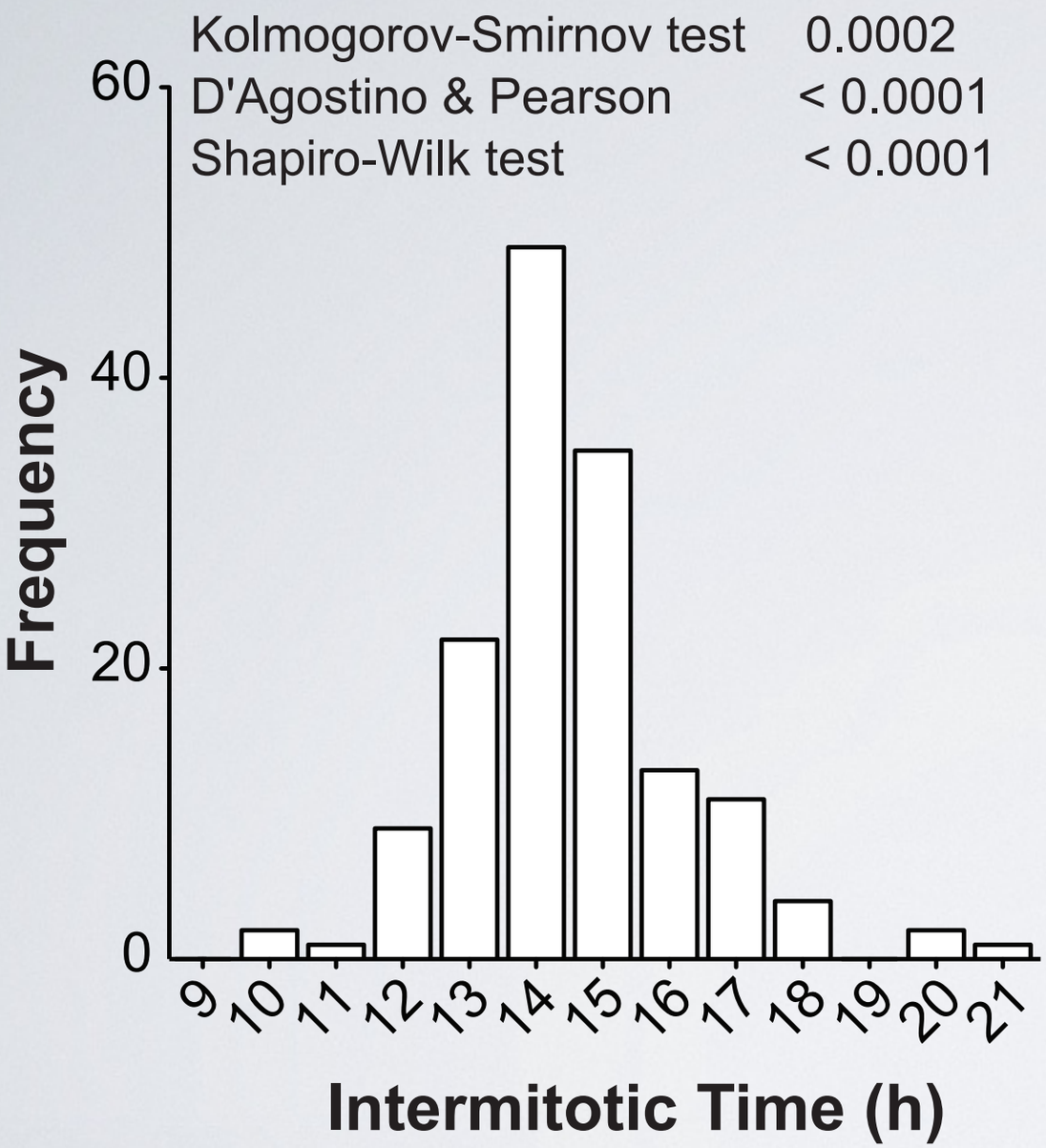
Same data

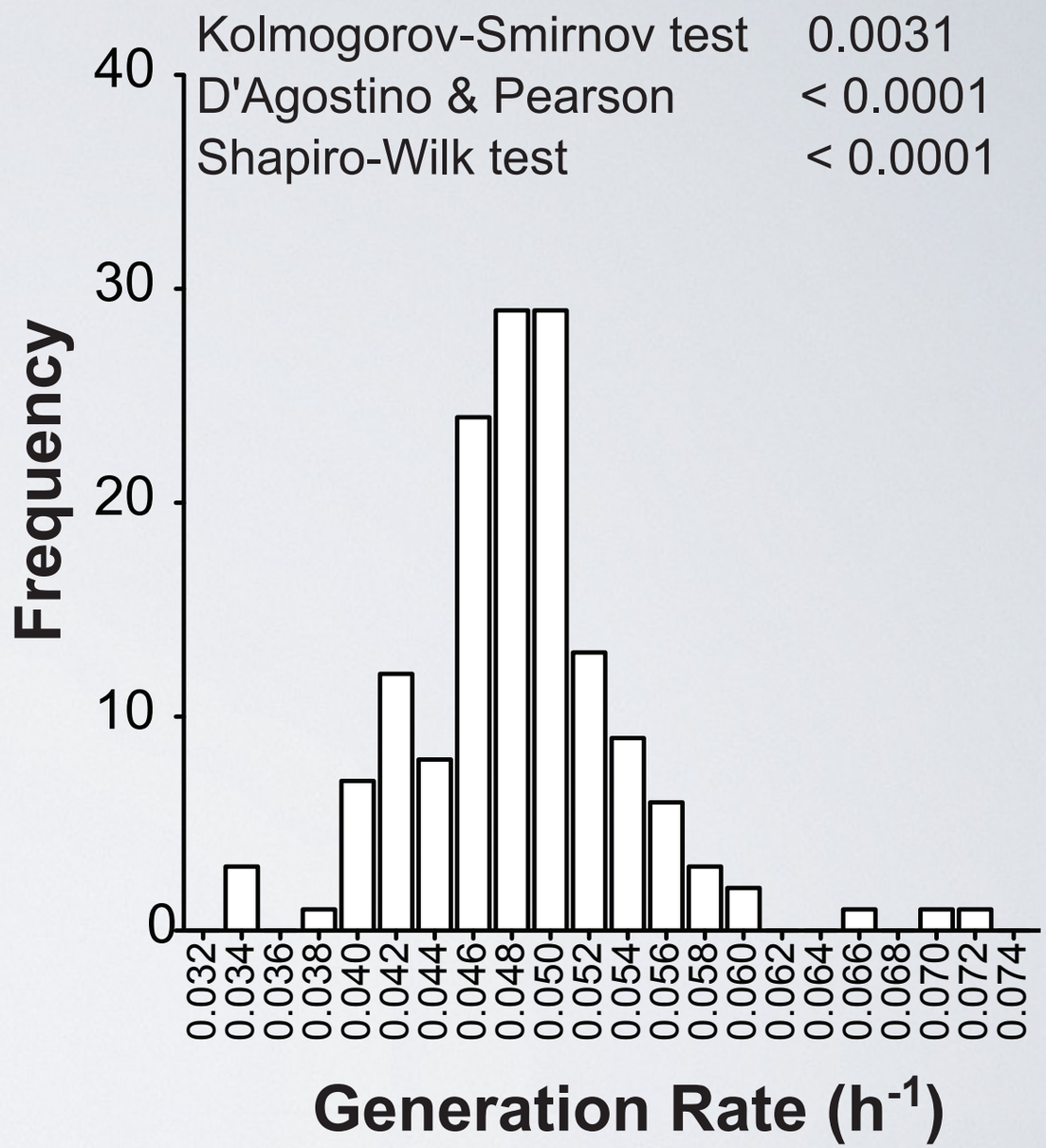




***Appears* normally distributed...**

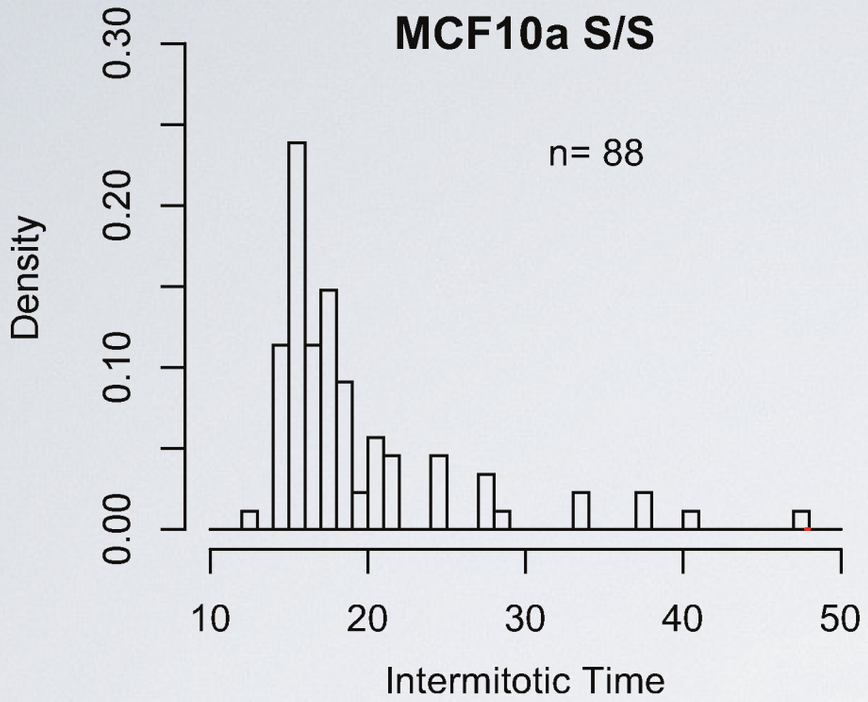
NON-GAUSSIAN

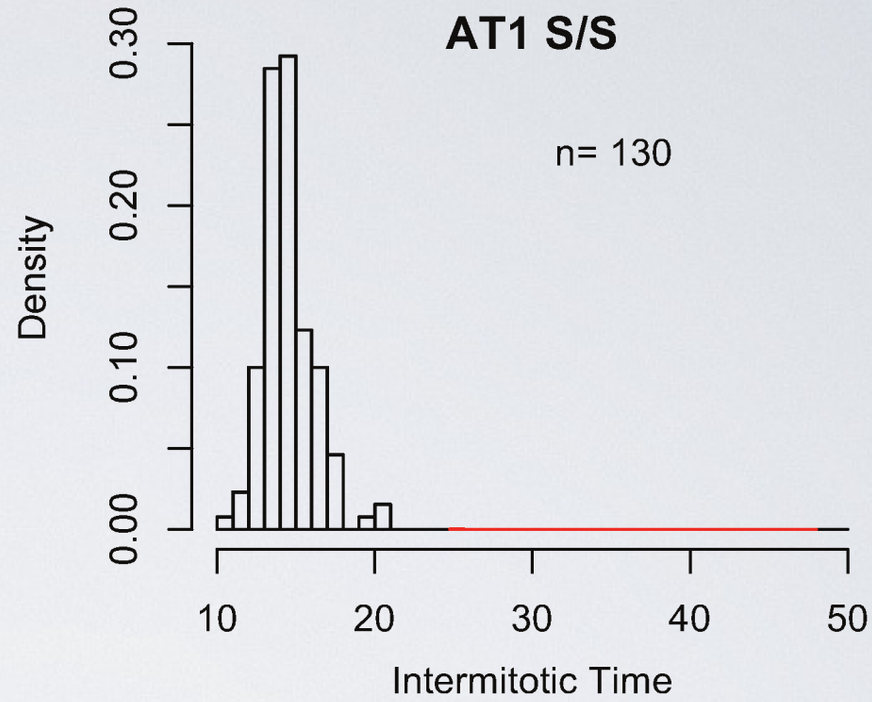


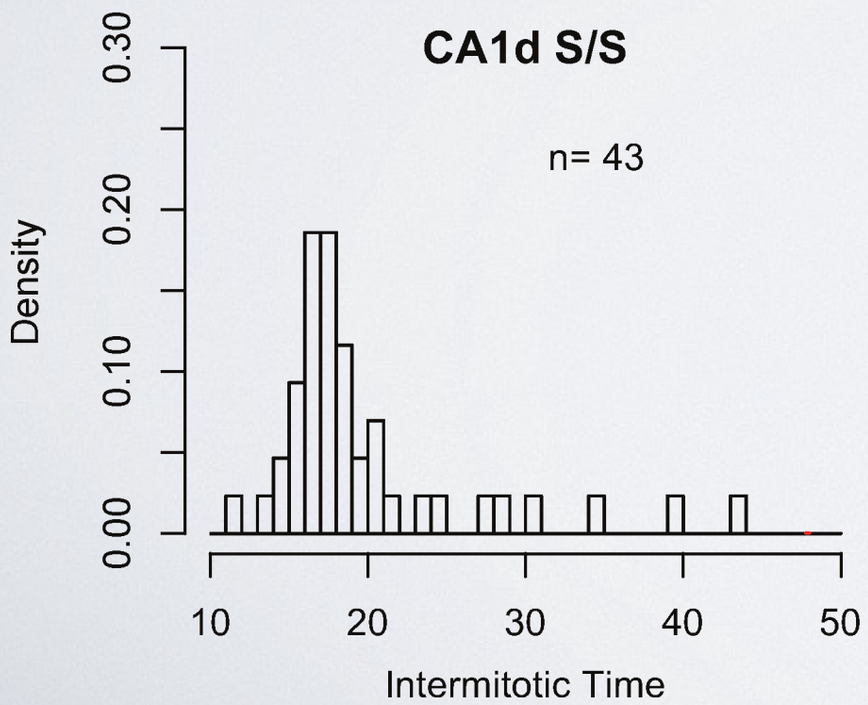


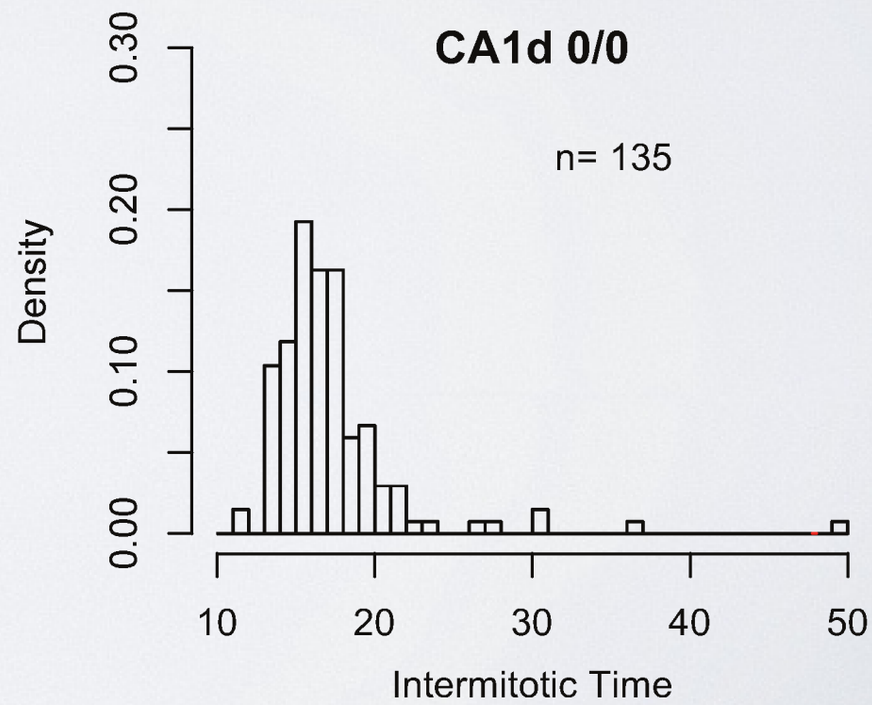
All normality tests failed

RIGHTWARD SKEW (TAIL)









IMT DISTRIBUTIONS

- The sum of many random processes would result in a Gaussian distribution (central limit theorum)

- A Gaussian distribution would be expected if the accumulation of one or more proteins are required at a certain level for cell division

- However, a Gaussian process (or inverse Gaussian) is insufficient to fit the distributions of intermitotic times, even those that appear normal

- Need additional component(s) to explain tails

OTHER DISTRIBUTION MODELS

Models	**KS test**	**parameters**
log normal	fail	2
inverse normal	fail	2
gamma	fail	2
exponentially-modified Gaussian	pass	3
gamma-modified Gaussian	pass	4

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EXPONENTIALLY-MODIFIED GAUSSIAN (EMG)

$$f(x|\lambda) = \lambda e^{-\lambda x} \quad (exponential\ distribution)$$

$$g(x|\mu,\sigma) = \frac{1}{\sigma\sqrt{2\pi})} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \quad (normal\ distribution)$$

$$emg(x|\lambda,\mu,\sigma) = \int_0^\infty f(t')g(t-t')dt' \quad (convolution)$$

$$emg(x|\lambda,\mu,\sigma) = \frac{\lambda}{2} e^{\frac{\lambda}{2}(-2x+2\mu+\lambda\sigma^2)} \left[Erfc(\frac{-x+\mu+\lambda\sigma^2}{\sigma\sqrt{2}}) \right]$$

λ = exponential component (rate parameter)

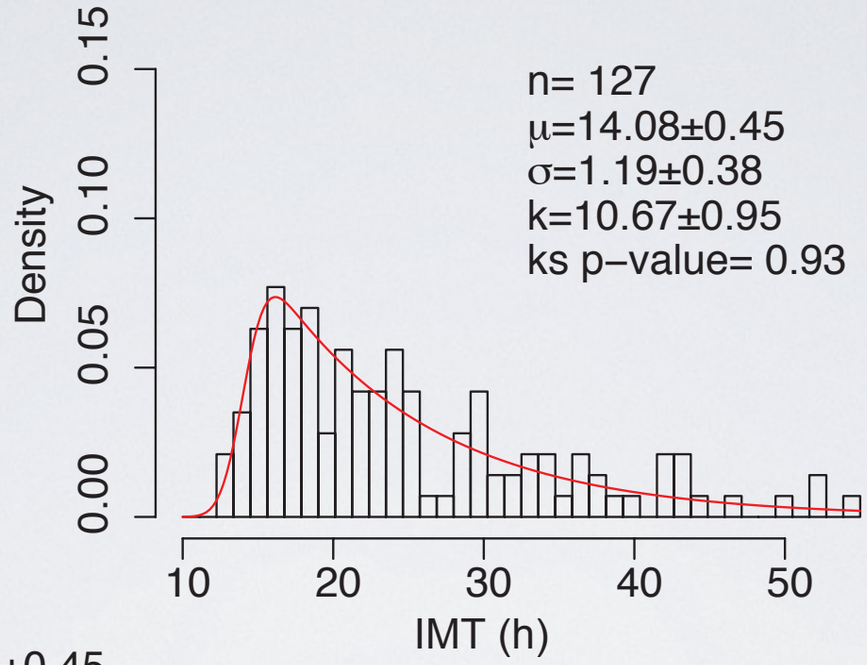
k = 1/**λ** (mean of exponential)

μ = mean of Gaussian

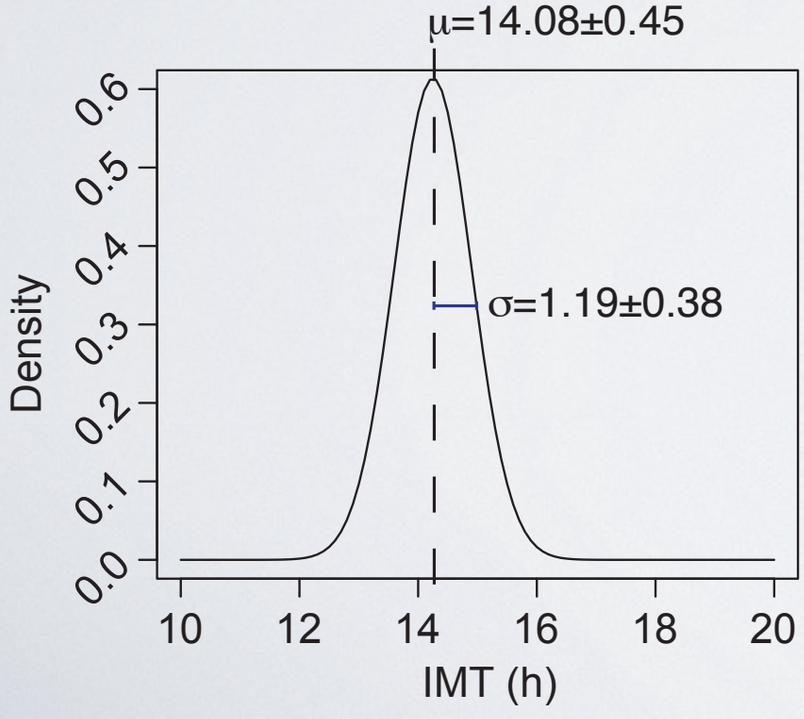
σ = Standard deviation of Gaussian

Shawn Garbett

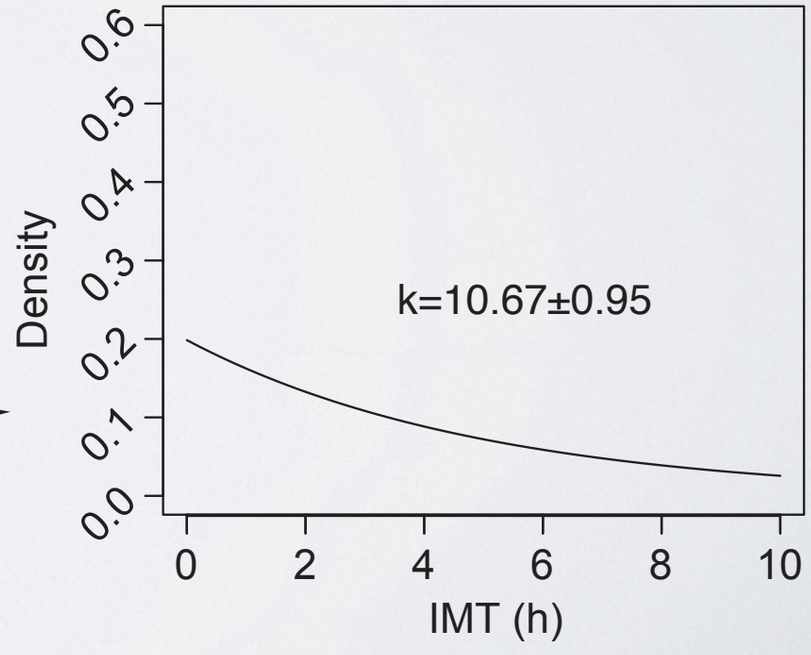
EMG COMPONENTS ARE SEPARABLE MATHEMATICALLY



Gaussian



Exponential



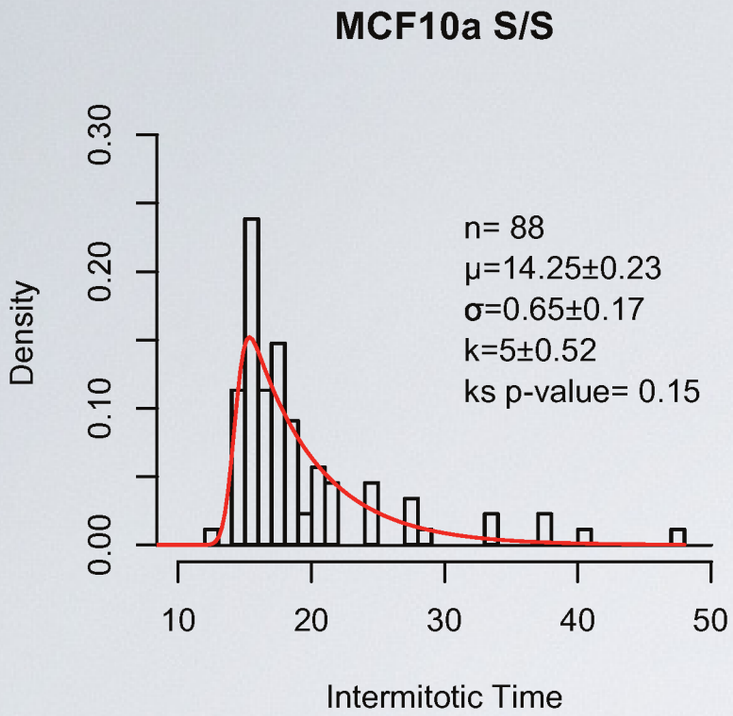
EMG COMPONENTS HAVE PLAUSIBLE BIOLOGICAL CORRELATES

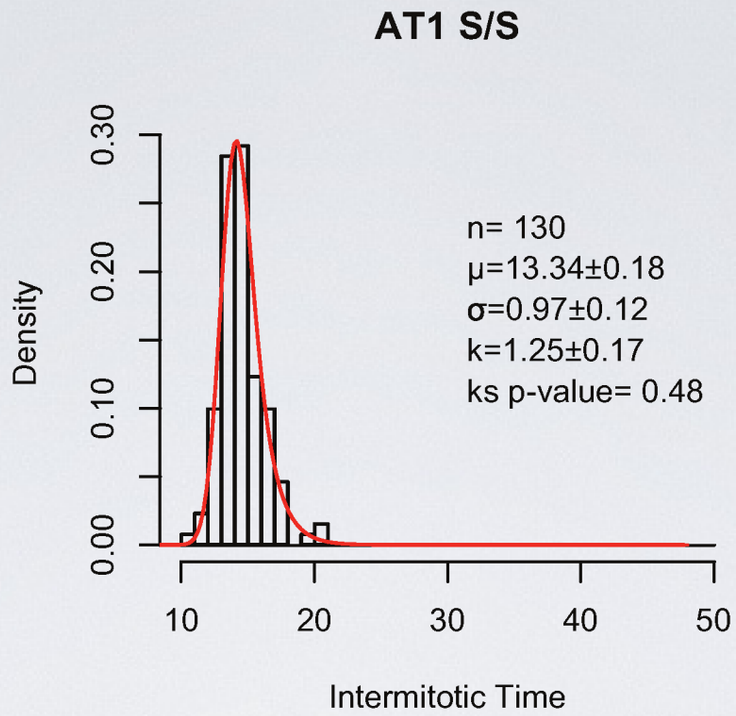
- *Gaussian* component could represent cell growth (biomass accumulation)

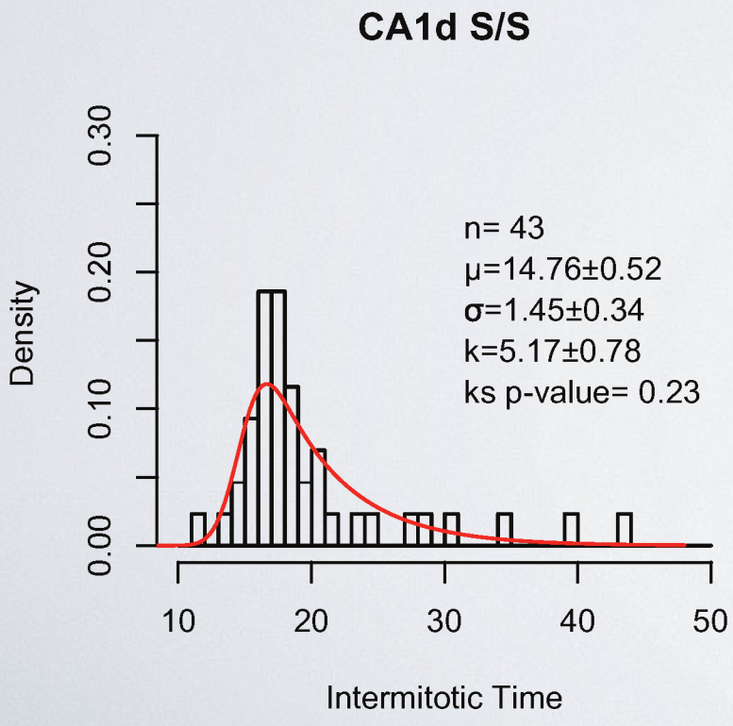
- *Exponential* component could represent a checkpoint function (requirements to be met, e.g. mitogens, space, nutrients, etc.)

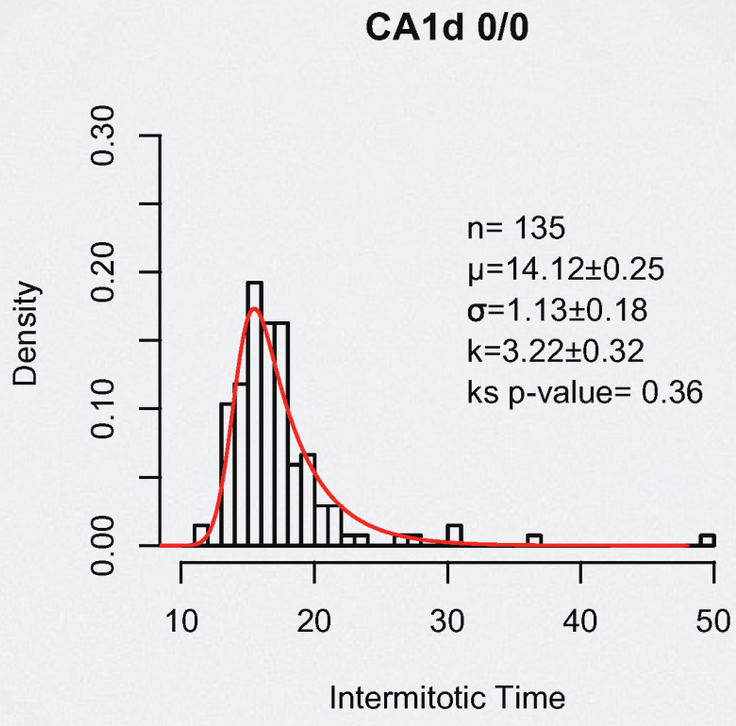
- Cell cycle times may be determined by a threshold of biomass accumulation and a rate of transition through a checkpoint(s)

EMG MODEL FITS DATA



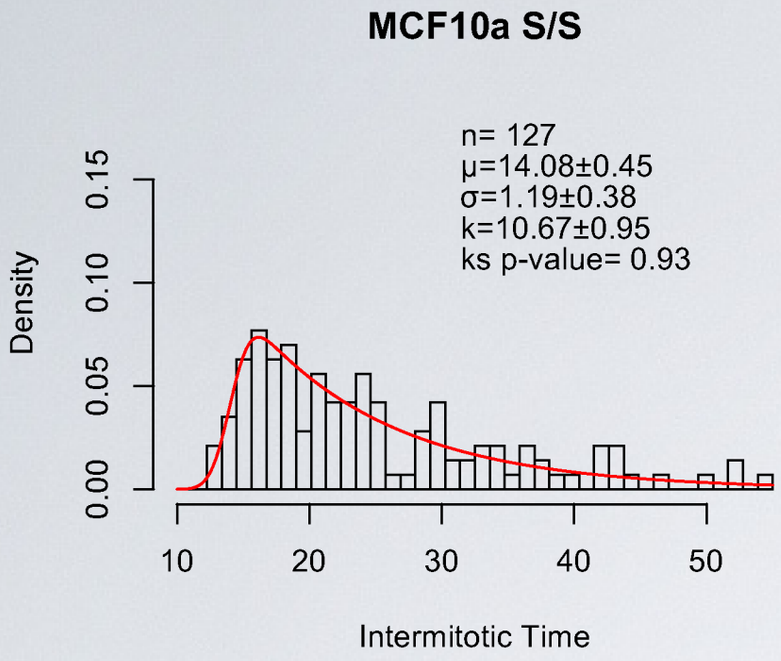


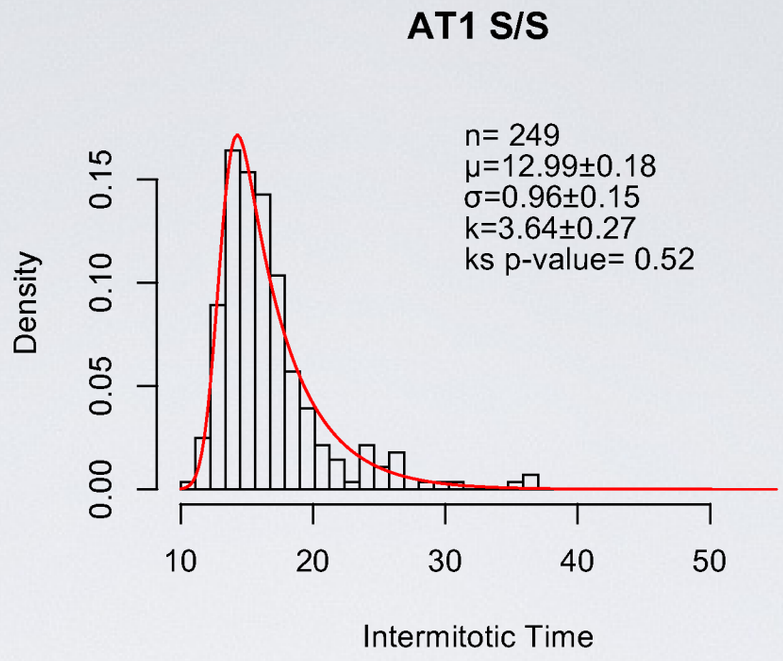


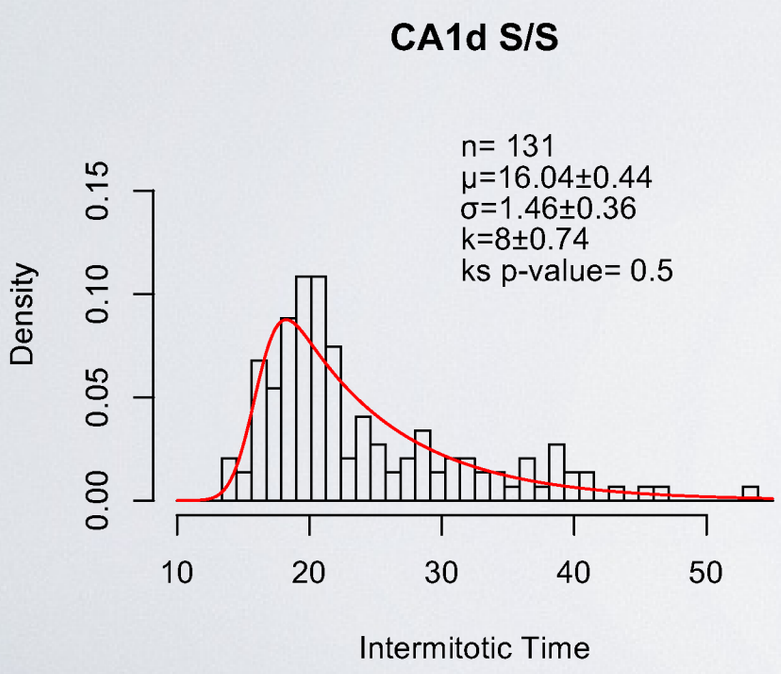


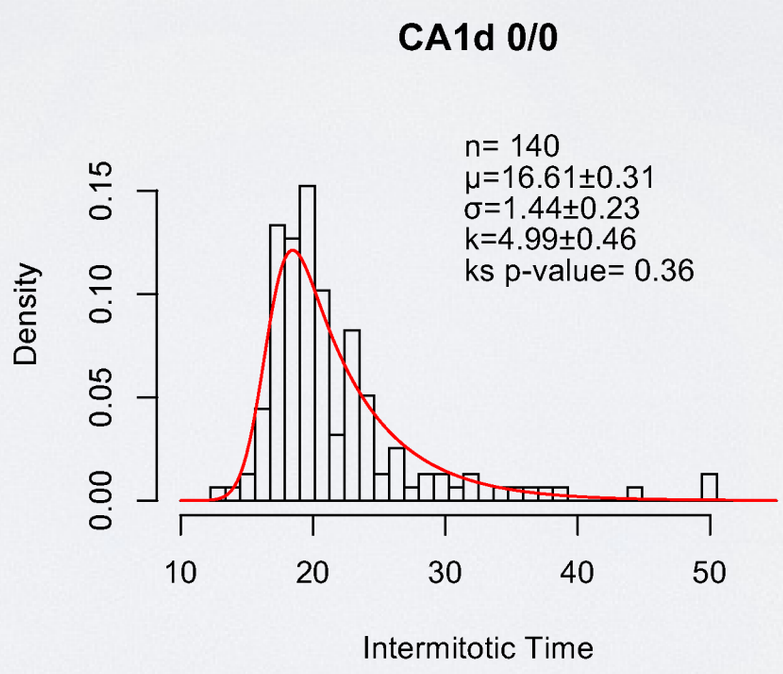
Data from populations in exponential growth phase collected after medium change

WASH AFFECTS E > G







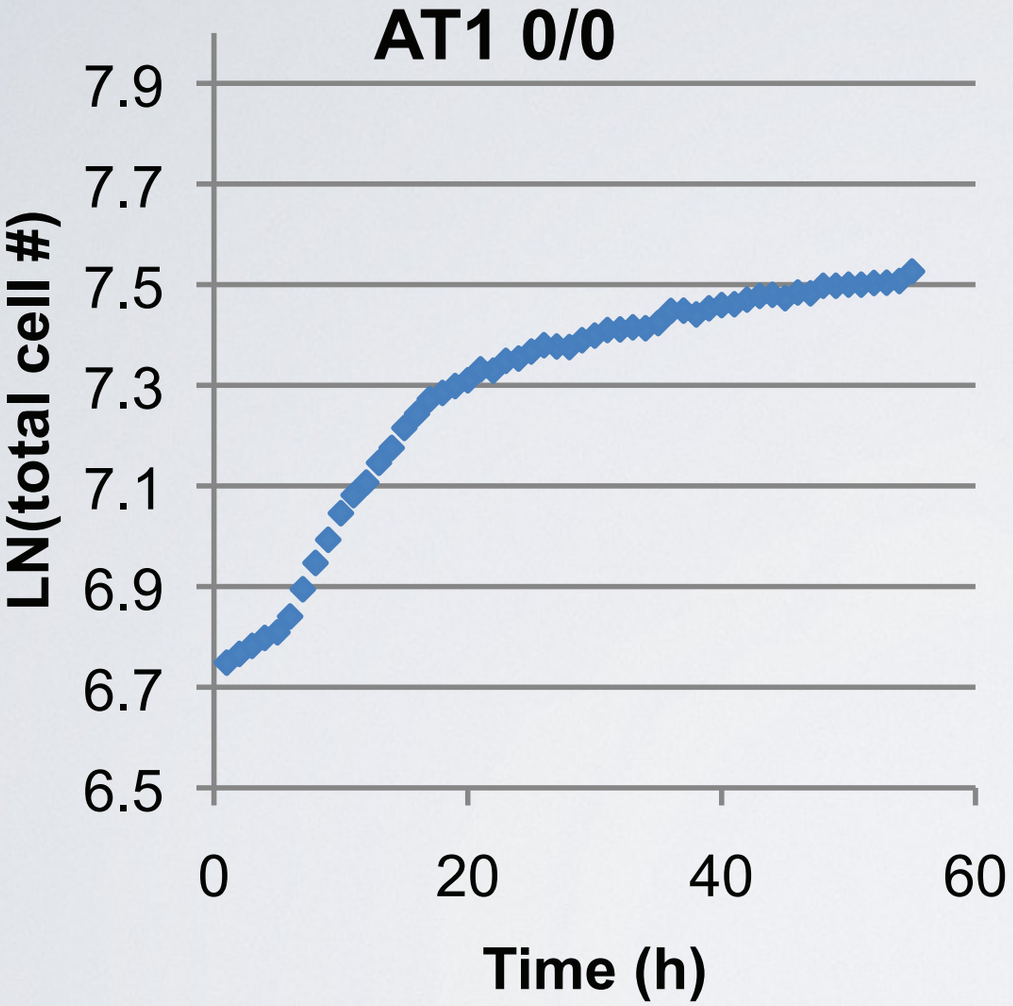


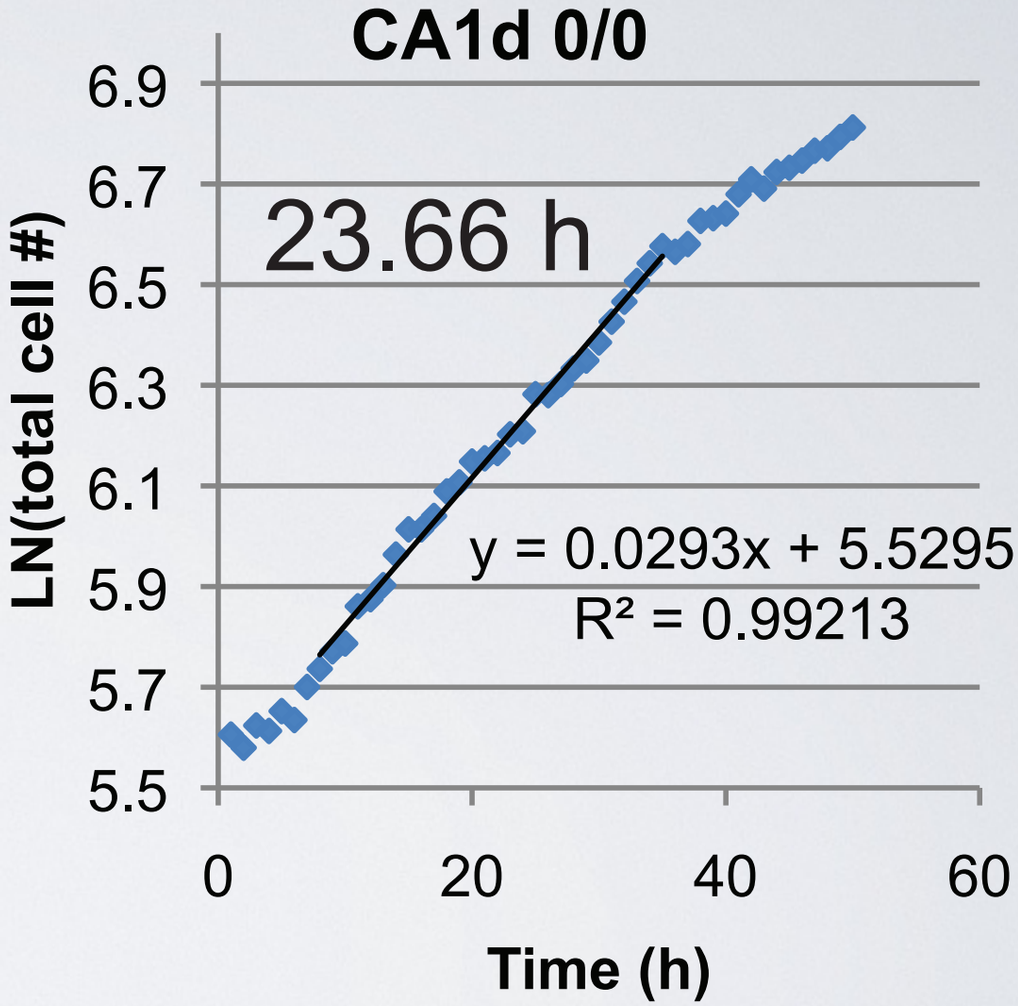
Data collected from 17 h before to 50 h after medium change

k values higher in each condition compared to previous

μ *values are only slightly increased in CA1d cells*

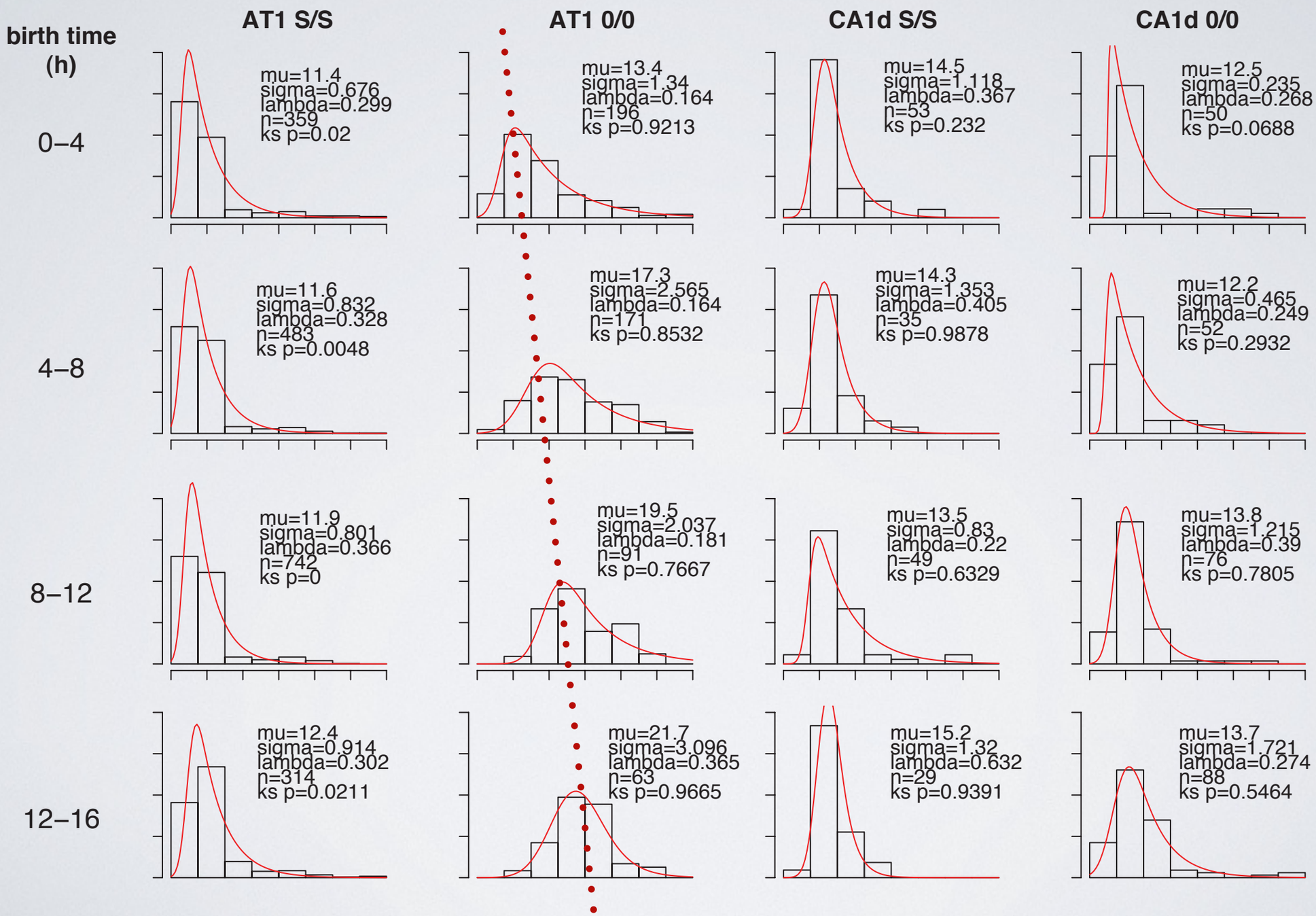
SERUM-DEPRIVED CONDITIONS?



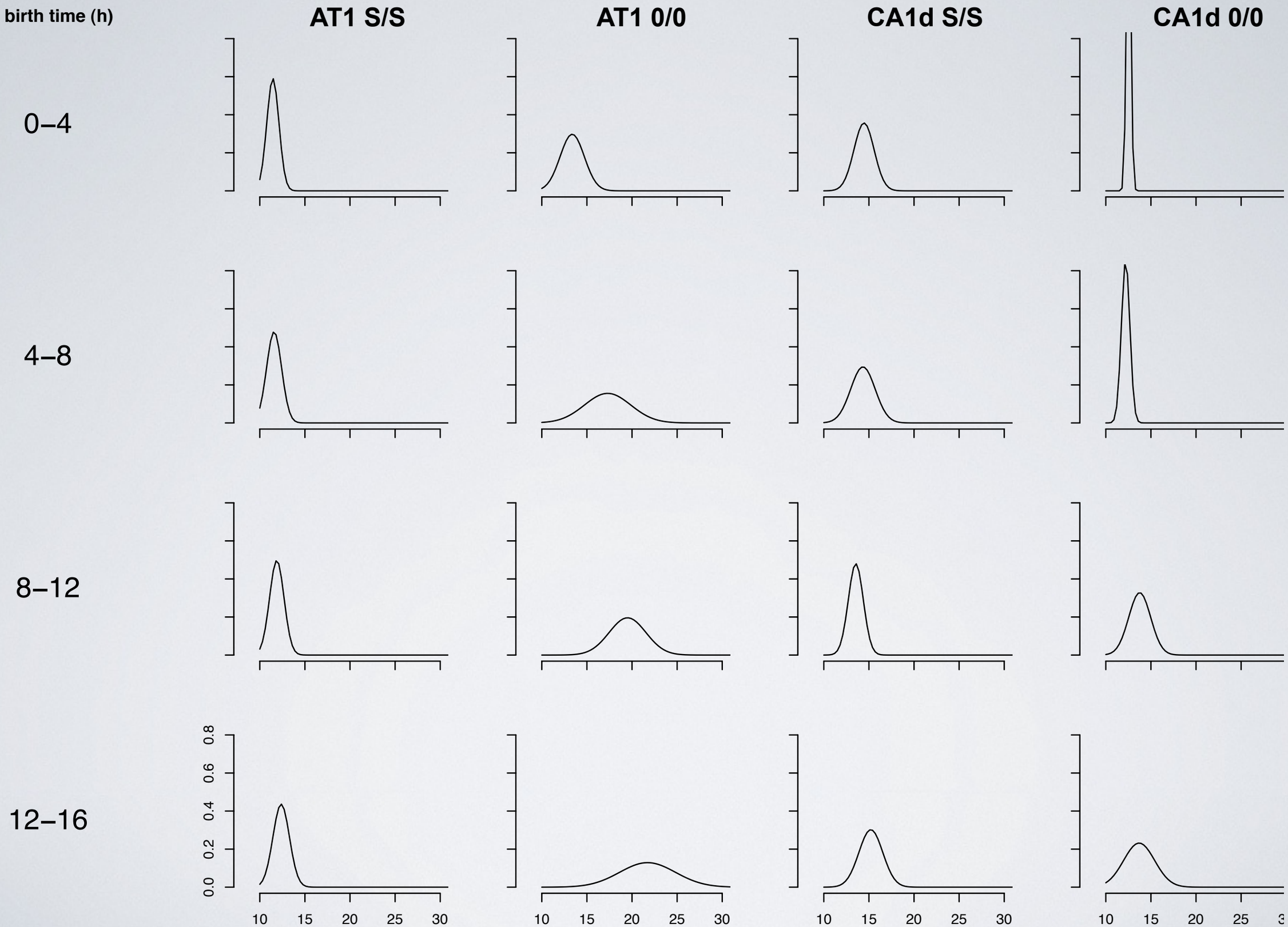


How does EMG fit data obtained during non-exponential growth (AT1 0/0)?

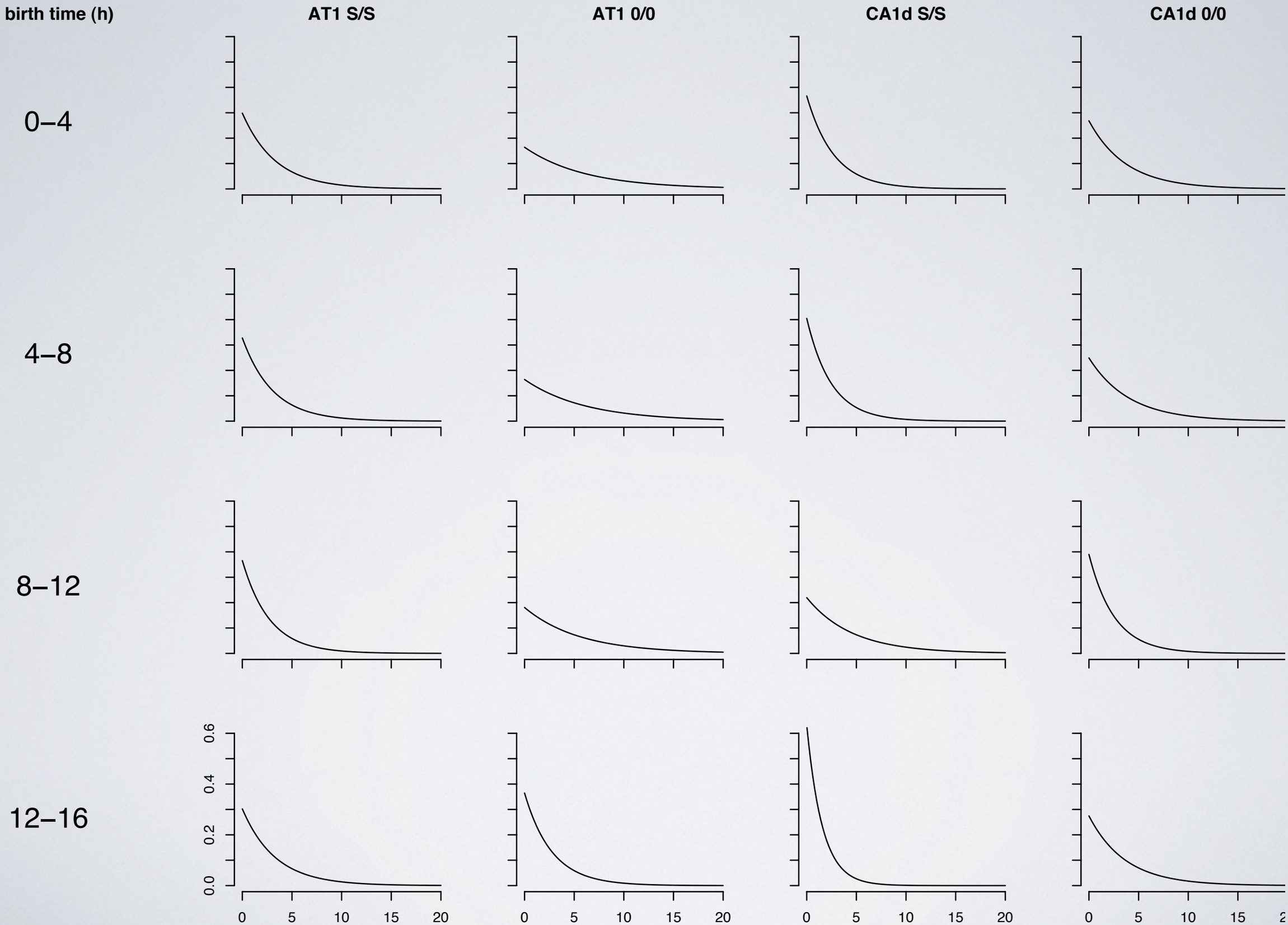
SERUM DEPRIVATION MAINLY AFFECTS G IN AT I CELLS



4/28+5/1 IMT Gaussian of EMG Fit



4/28+5/1 IMT Exponential of EMG Fit



CONCLUSIONS

- Large datasets of cell division obtained by time lapse fluorescence microscopic imaging provide sufficient power to distinguish among mathematical models
- EMG model can be separated into two components with plausible biological correlates
- EMG model provides a useful tool for dissecting the molecular underpinnings of cell cycle control

FUTURE WORK

- Explore the biological correlates of the exponential and Gaussian components using molecular-targeted drugs that affect signaling pathways altered in cancer
- Attempt to generate stochastic simulations of signaling network models that can explain the single-cell distributions
- Correlate signaling events with cell cycle progression in individual cells

ACKNOWLEDGMENTS

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- Jing Hao
- Jerome Jourquin

OME/OMERO

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- Dave Piston (Vanderbilt)
- Melissa Linkert (LOCI)
- Jason Swedlow (Dundee)

Immunofluorescence (MIT)

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