

Mixed effect modeling of neuronal cell differentiation kinetics from longitudinal phase-contrast imaging data

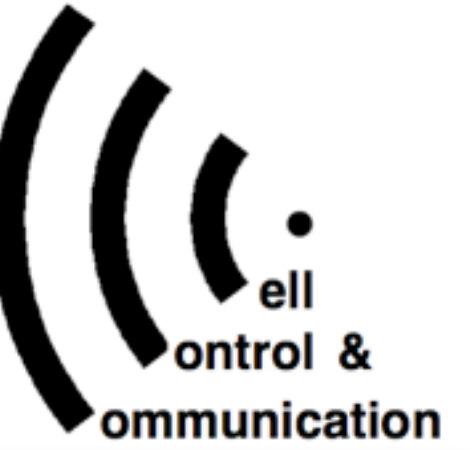
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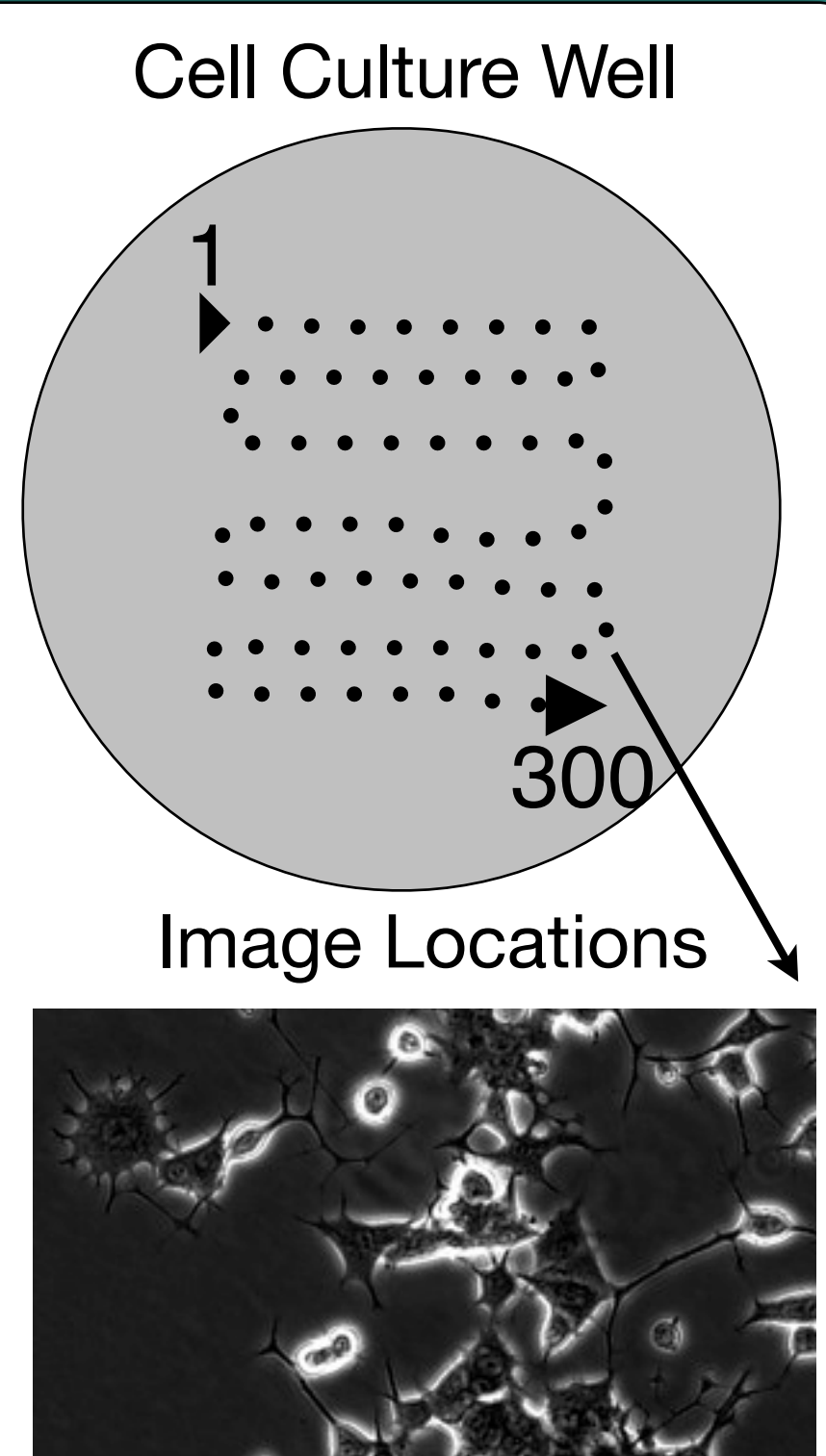


Neuronal Cell Differentiation

- For medical studies of neuronal development and disease the rat adrenal pheochromocytoma cells (PC12) are a well-established cell-line for *in vitro* studies [2] as model system
- The nerve growth factor (NGF) induces cell differentiation by *neurogenesis*, which is challenging to measure quantitatively
- Repeated measurements of images at fixed locations on a well allow for longitudinal data of cell sub-populations and an interpretation with a limited growth mixed effect model

Experimental Setup

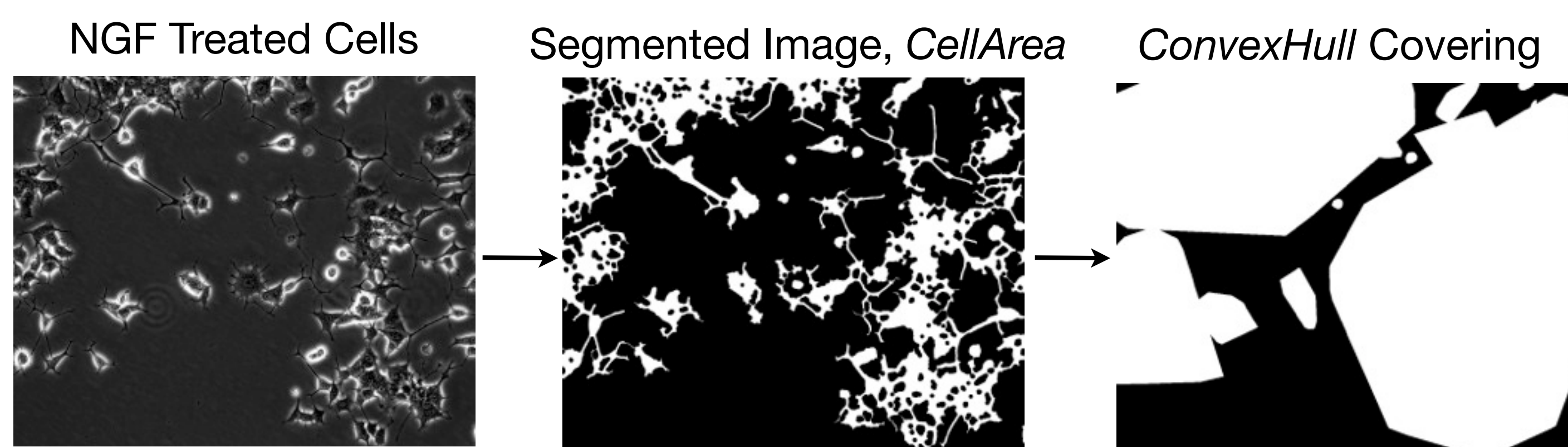
- PC12 cells are seeded on wells with NGF treatment and without as control, CTL
- From each well 300 images are recorded every 24h over a period of 6 days in a line-wise scanning pattern which stays constant
- The *unit of experiment* is a sub-population on the well of the overall cell population



Experimental Readout

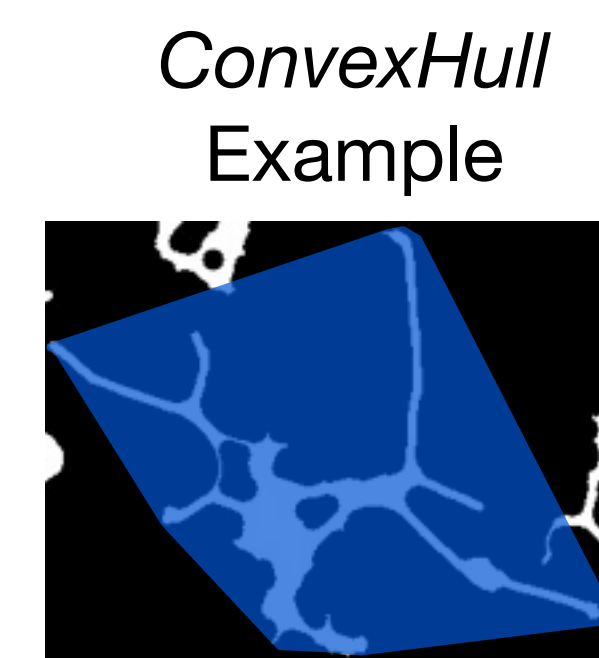
Cell Proliferation - CellArea

Images are segmented [1] and the number of pixels identified in segments measures the *cell area*



Cell Differentiation - ConvexHull

Each segment is covered by its *convex hull*, which is identical to itself only in case of round cells



Limited Growth Mixed Effect Model

Verhulst Equation

Resources for cell growth on well are limited, model fit by Monolix 4.1.2 [3] with one random effect per parameter

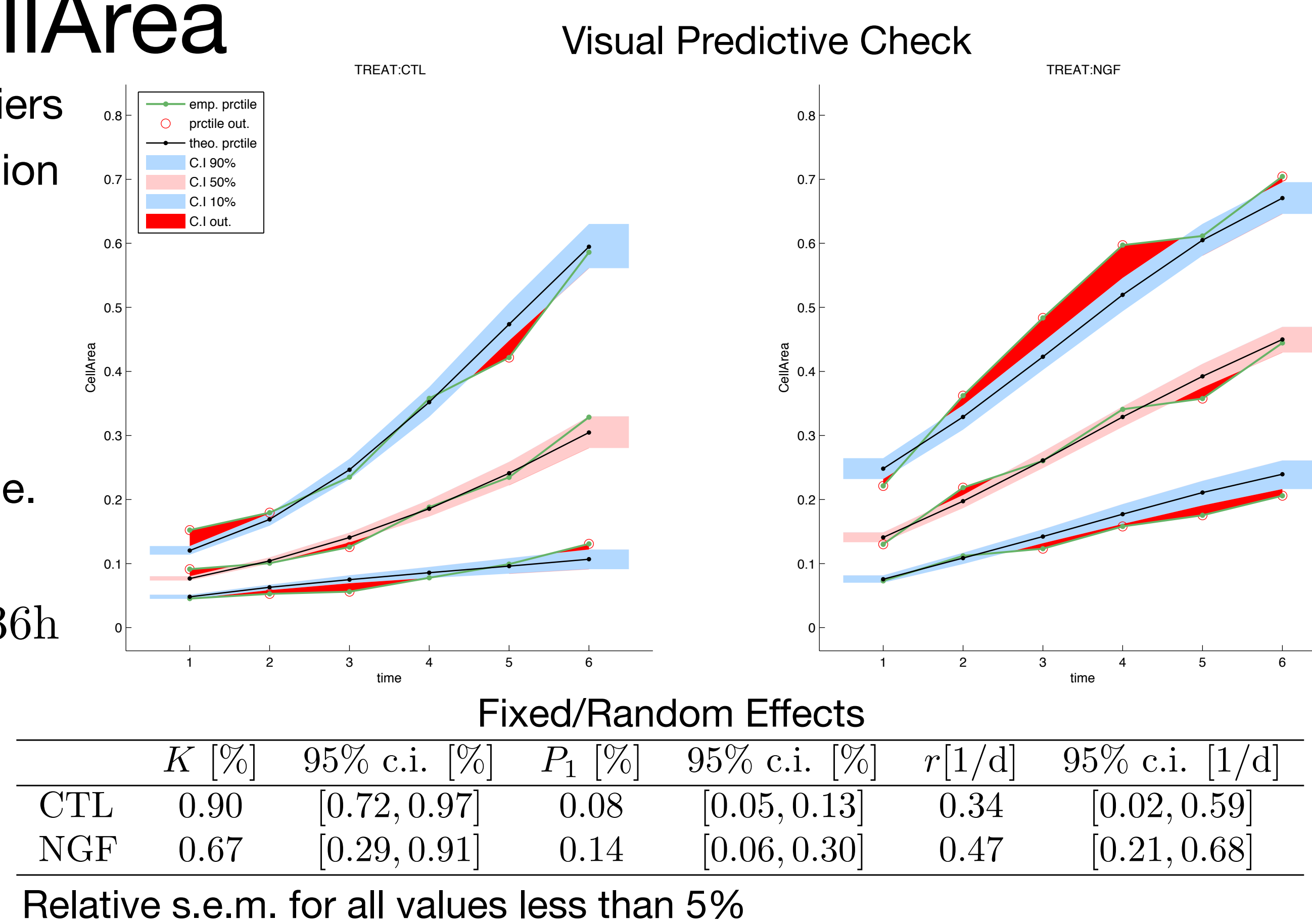
$$\dot{P} = r P (1 - P/K)$$

Parameter	Description	Distribution	Covariate
$P_1=P(t=1)$	Population t_1 [% pixels]	logit	Treatment
r	Growth rate [1/d]	lognormal	Treatment
K	Capacity [% pixels]	logit	Treatment

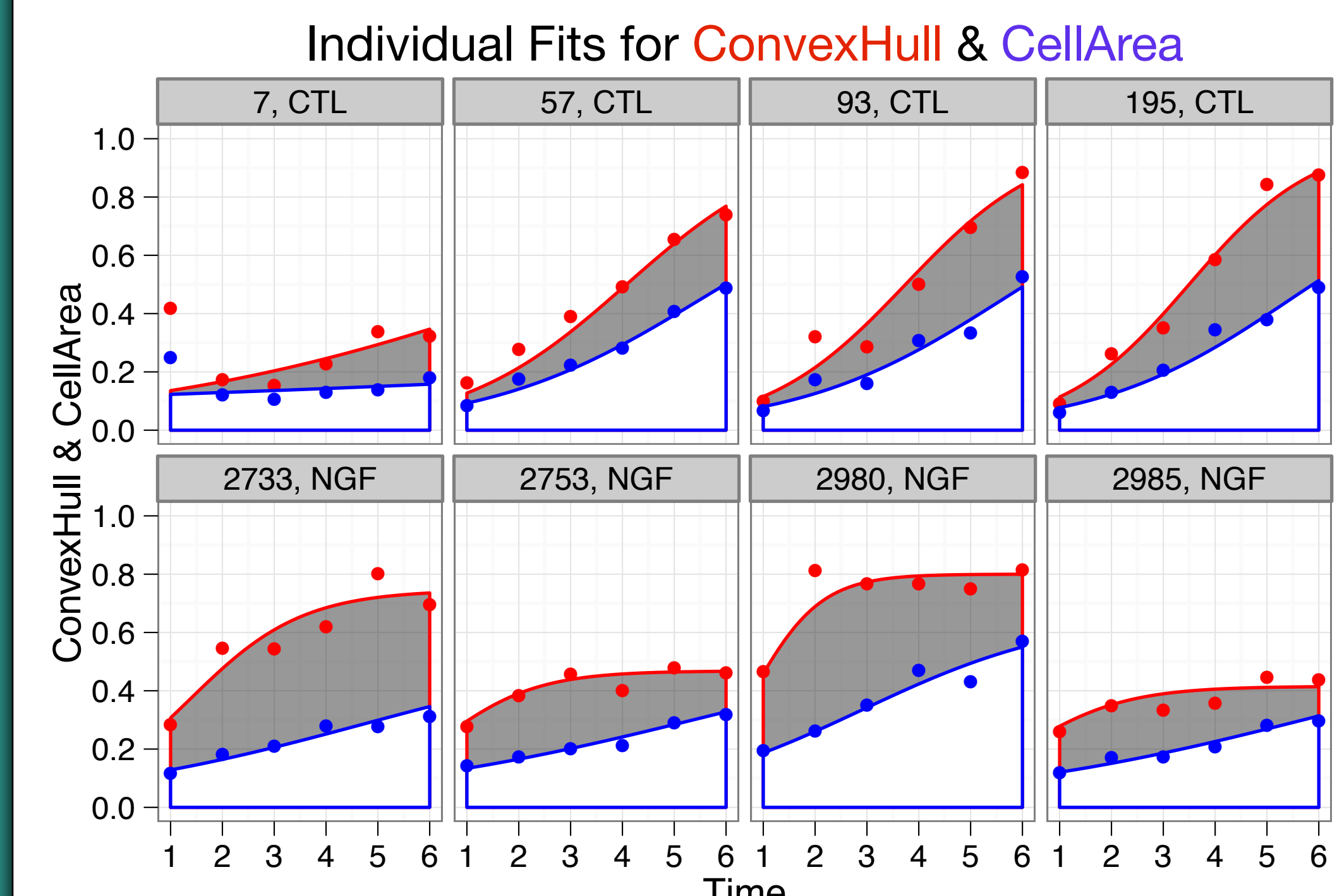
Cell Proliferation - CellArea

- Visual predictive check shows marginal outliers for the median simulated predictive distribution
- Treatment effect in all 3 model parameters:
 - P_1 increased 2x
 - K decreased 20%
 - r increased by 0.1/d
- Growth rate of CTL in physiological range, i.e. doubling time
$$T_2 \approx \ln(2)/r \rightarrow T_2^{\text{CTL}} = 48\text{h}, T_2^{\text{NGF}} = 36\text{h}$$
- Log-likelihood for nested vs full model

$$\Delta L = 295 \rightarrow p < 10^{-4}$$

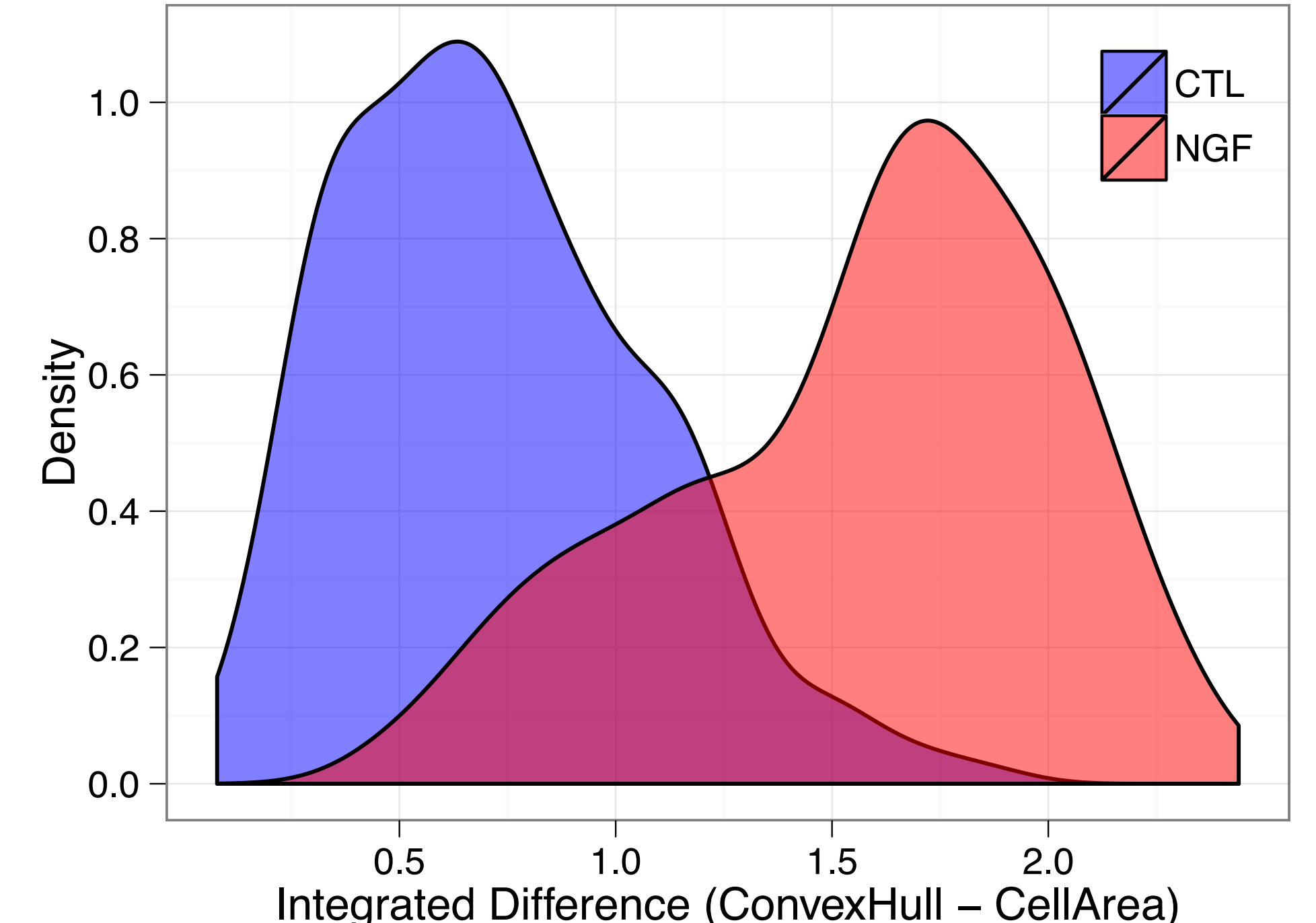


Treatment Effect



- The lengthy neurites of NGF treated cells lead to a faster increase of the ConvexHull area in comparison to CTL cells, i.e. the growth rate r doubles
- The ConvexHull is always larger than the CellArea and the integrated difference (grey area above) is a direct measure of their difference over time

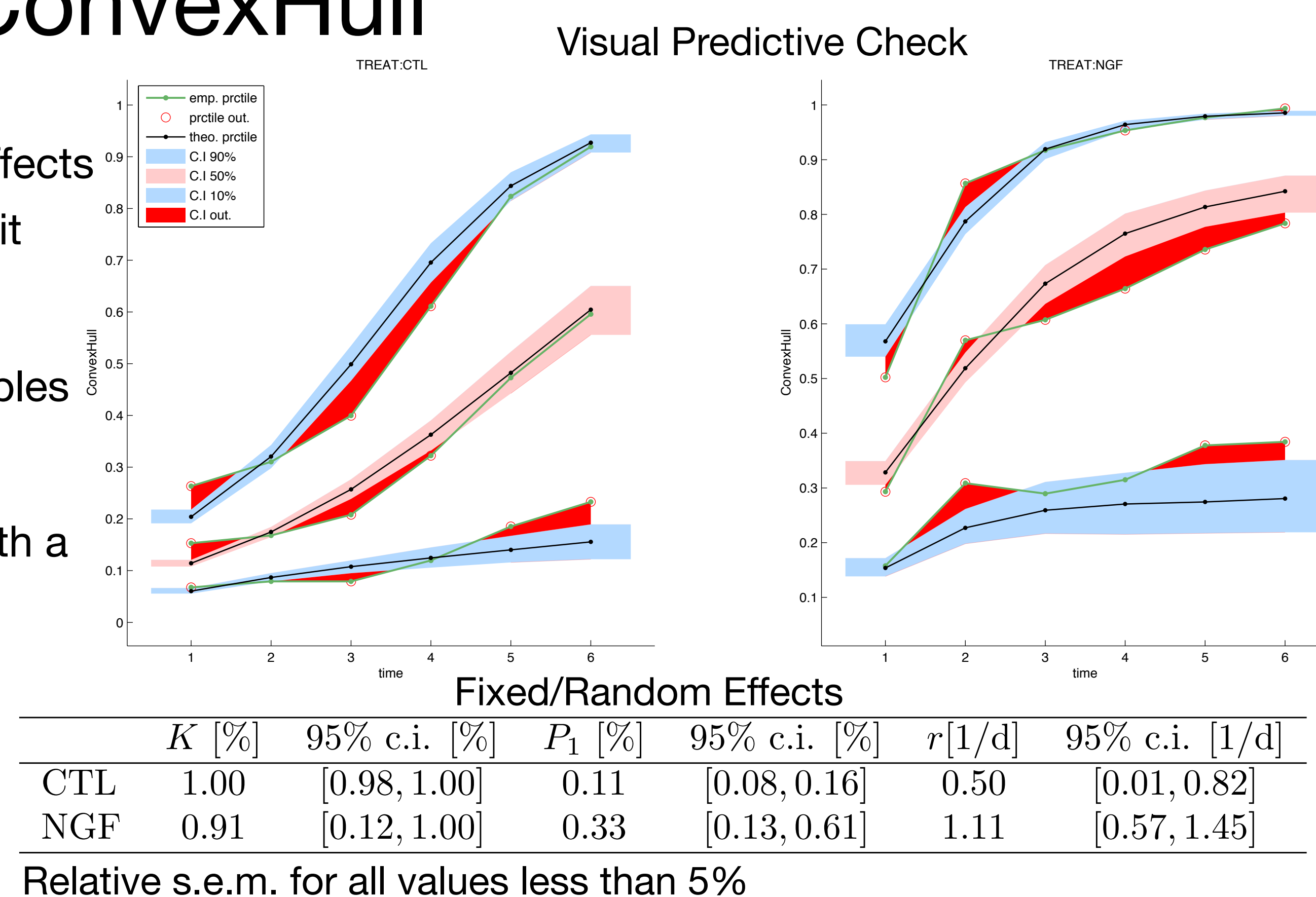
Summarized Treatment Effect



Cell Differentiation - ConvexHull

- ConvexHull much harder to fit due to discontinuous changes and discretisation effects close to 1, which is problematic with the logit error model used
- Treatment already on $t=1$ d in effect as P_1 triples
- NGF shows doubled growth rate r of the ConvexHull area, i.e. NGF cells grow fast with a large space need
- Log-likelihood for nested vs full model

$$\Delta L = 604 \rightarrow p < 10^{-4}$$



References

- [1] S. Weber, M.L. Fernandez-Cachon, B. Offermann, R.F. Murphy, M. Boerries, and H. Busch, (2012), Classification of neuronal cell differentiation from phase-contrast imaging, To be submitted
- [2] L.A. Greene and A.S. Tischler (1976), Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. PNAS, 73(7), 2424-2428.
- [3] Kuhn, E., and Lavielle, M. Maximum likelihood estimation in nonlinear mixed effects models. Computational Statistics and Data Analysis 49, 4 (2005), 1020-1038.

Acknowledgement

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Conclusions

- Using a limited growth mixed effect model we determined the treatment effect of NGF on cell *proliferation* and cell *differentiation*
- Non-destructive sampling enables longitudinal imaging data taking and allows for high-throughput, quantitative and highly sensitive analysis
- Further treatments are currently under study