MEASUREMENT OF SINGLE-CELL DYNAMICS

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- Background
- Dynamic processes and measurement technology
- Model-led data integration and analysis
- Conclusions

BACKGROUND

- Populations of cells are almost always heterogeneous in function and fate. To understand the plasticity of cells, it is vital to measure quantitatively and dynamically the molecular processes that underlie cell-fate decisions in single cells. Early events in cell signalling often occur within seconds of the stimulus, whereas intracellular signalling processes and transcriptional changes can take minutes or hours. By contrast, cell-fate decisions, such as whether a cell divides, differentiates or dies, can take many hours or days. Multiparameter experimental and computational methods that integrate quantitative measurement and mathematical simulation of these noisy and complex processes are required to understand the highly dynamic mechanisms that control cell plasticity and fate.

DYNAMIC PROCESSES AND MEASUREMENT TECHNOLOGY

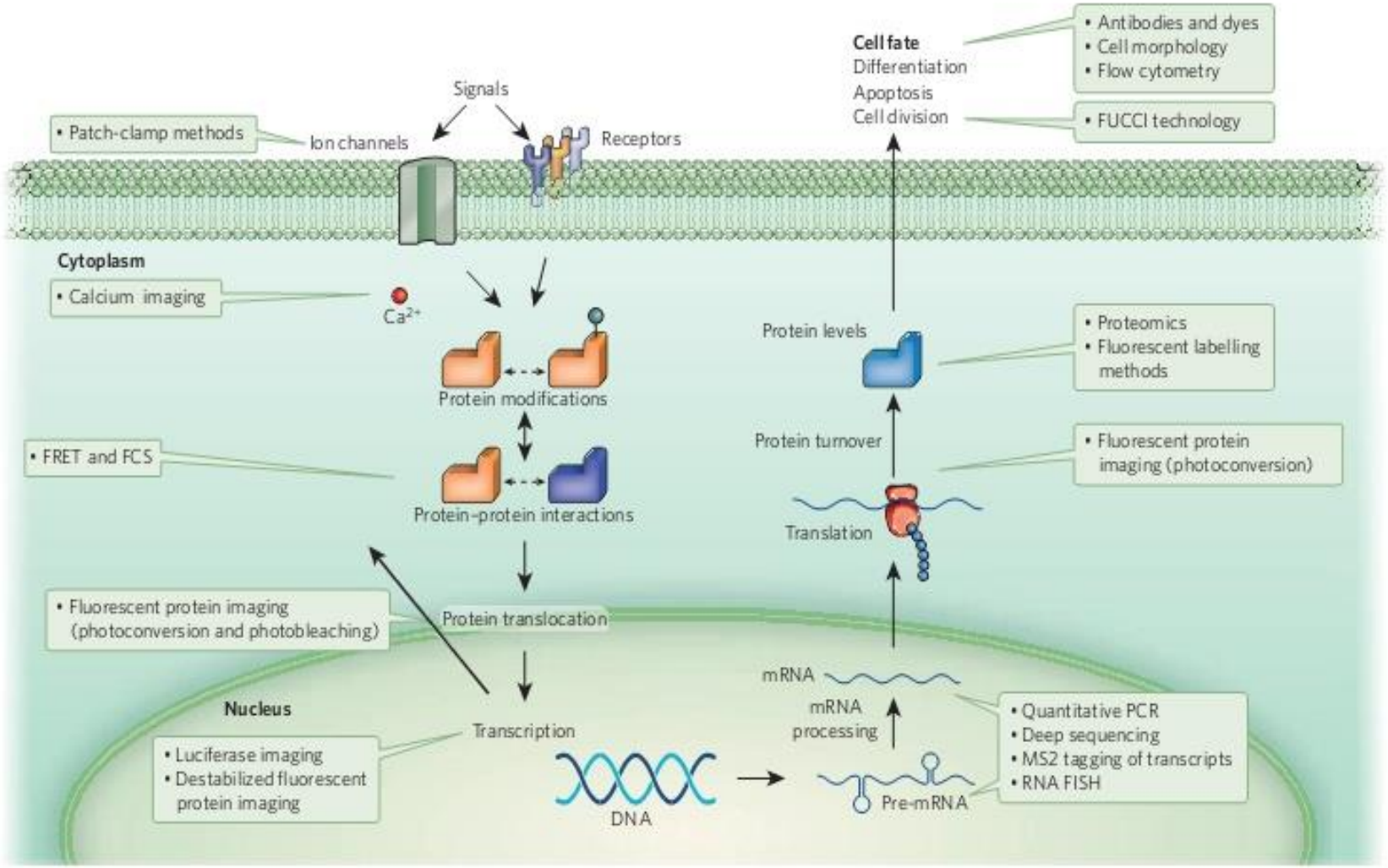


图1 活细胞动力学过程以及量化这些步骤的测量技术

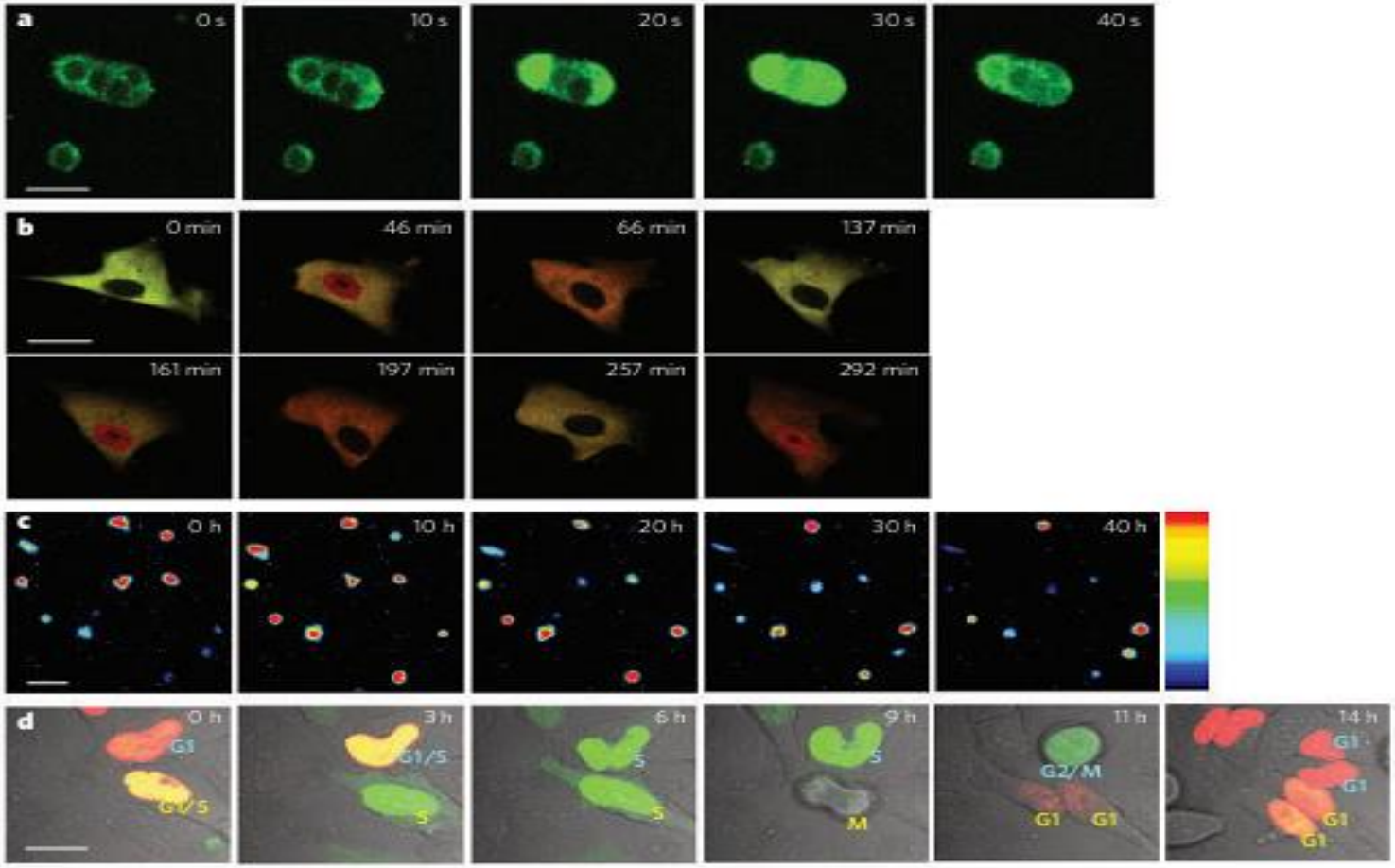


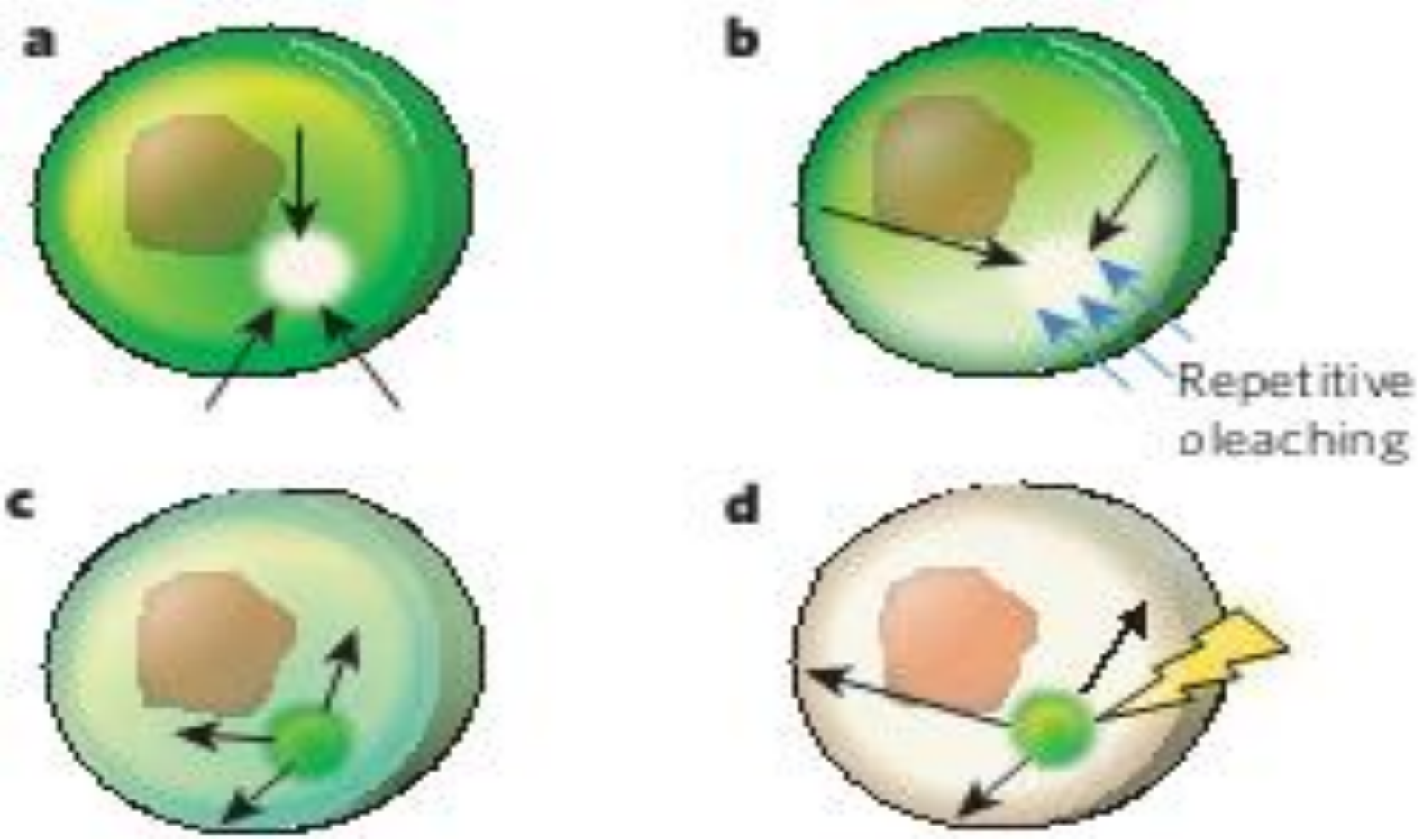
图2 单细胞时差成像的例子

a图是用促甲状腺释放因子处理过的腺垂体细胞的钙成像图（早期信号传导）

b图是用肿瘤坏死因子处理过的神经细胞瘤的荧光蛋白图（转录因子转位）

c图是腺垂体细胞在人类催乳激素基因的启动子的控制下表达的荧光素酶的低光像图（转录解析）

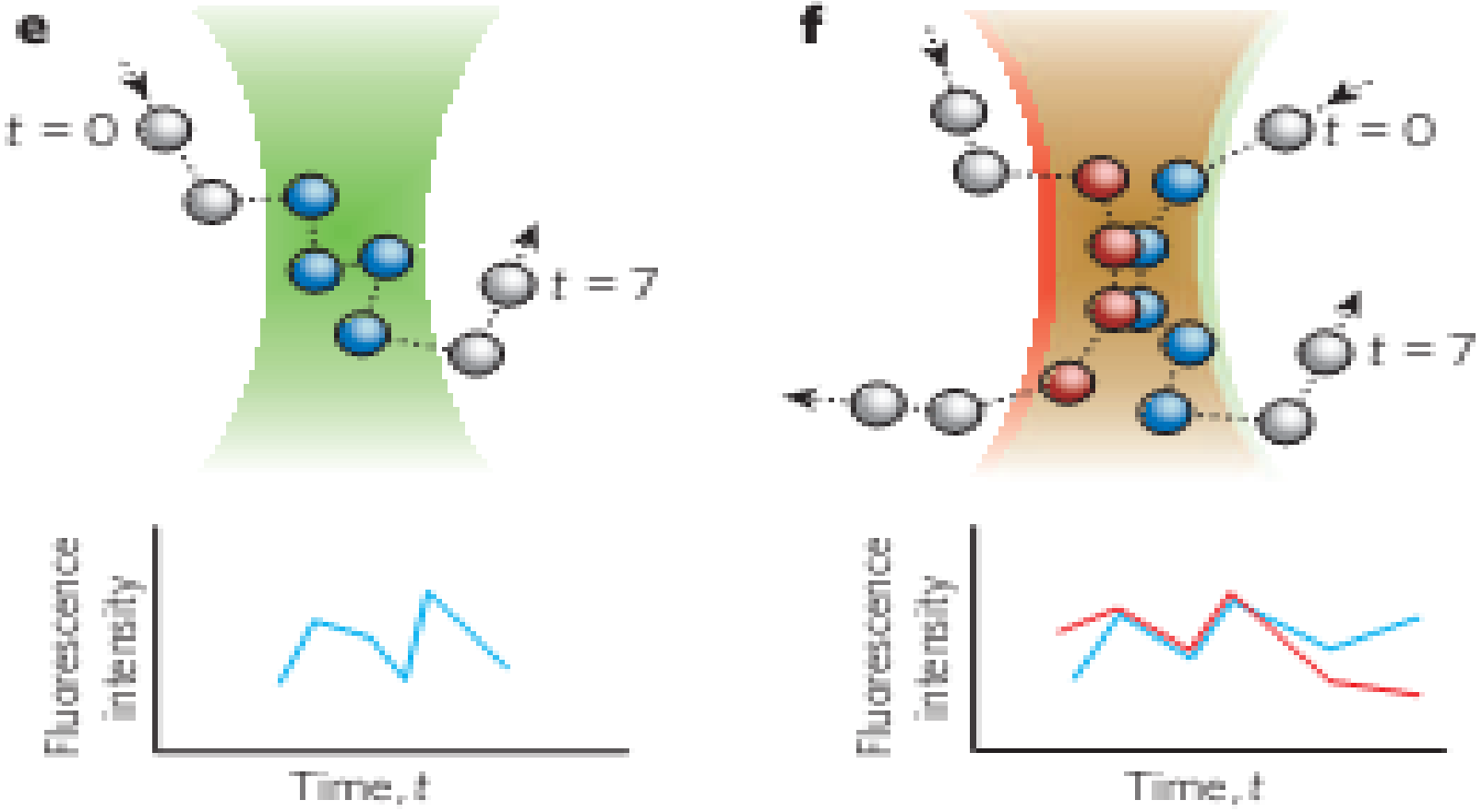
d图是用FUCCI技术（活细胞周期成像技术）处理上皮细胞的图像（细胞分裂)



图a和图b是用荧光漂白恢复（FRAP)和光漂白（FLIP)技术

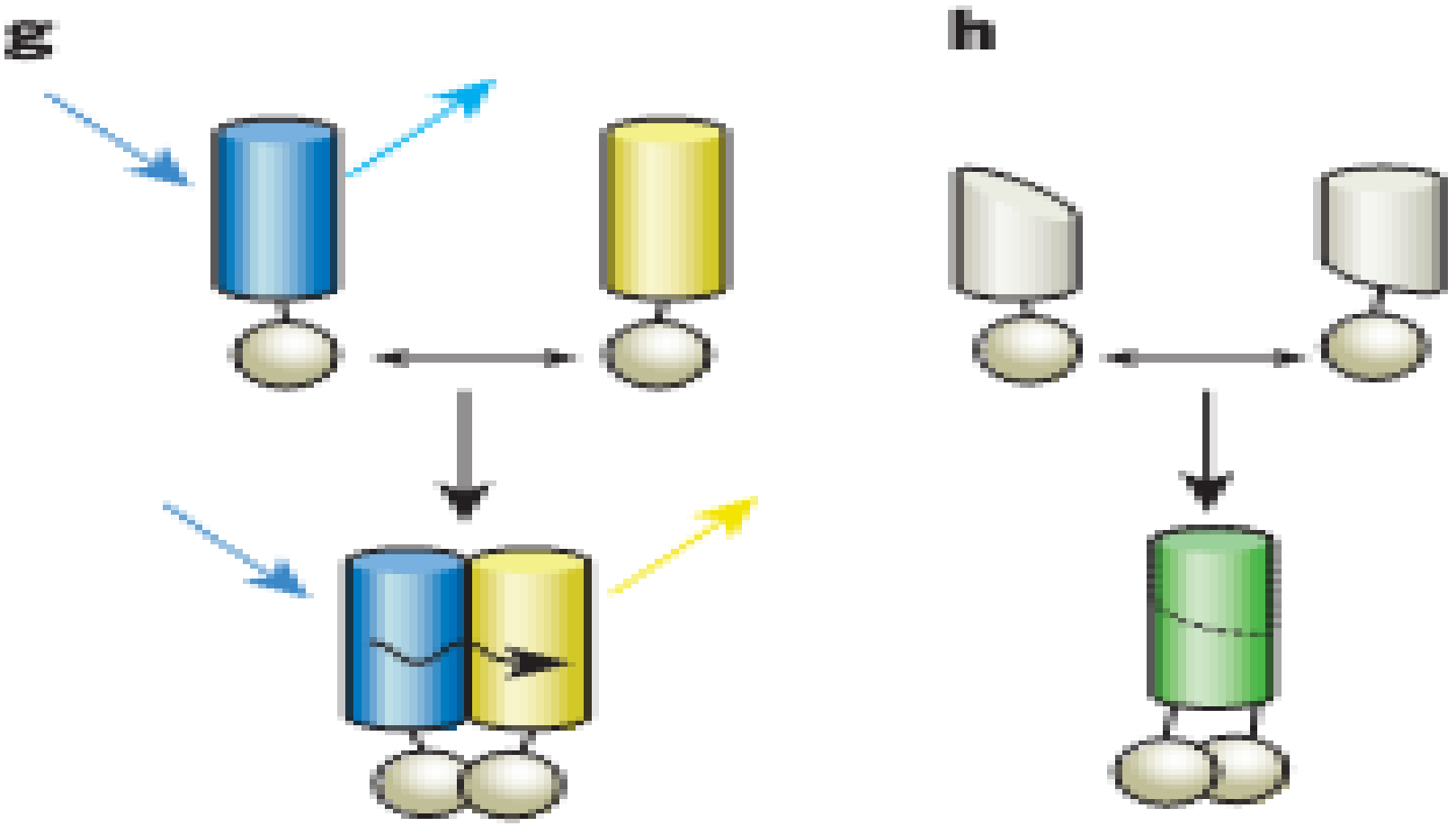
图c是FRAP技术的反向利用

图d是用光活化（Photoactivation)和光转换(Photoconversion)技术



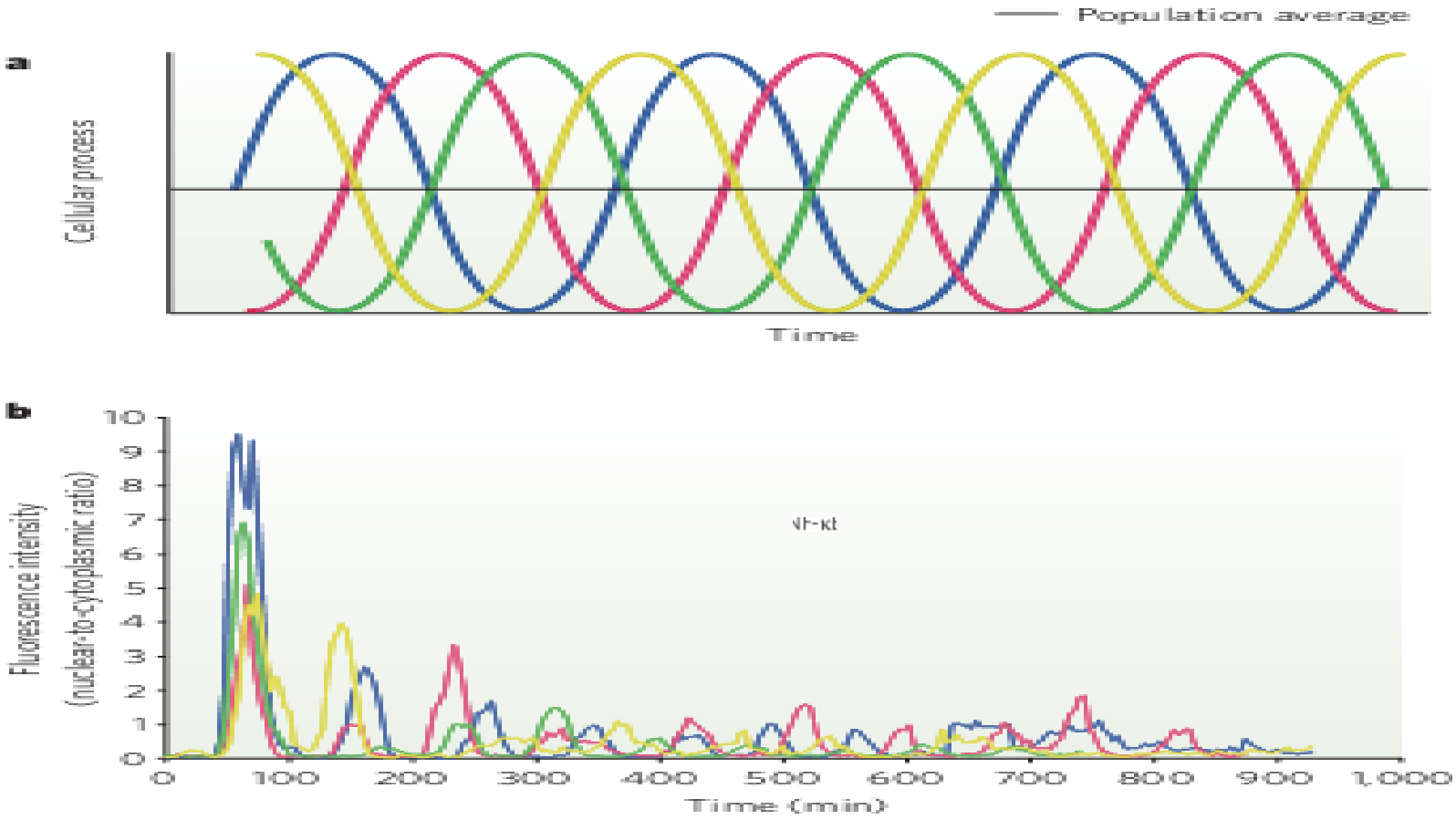
图e是用荧光相关光谱学技术（FCS)

图f是用荧光交叉相关光谱技术（FCCS)



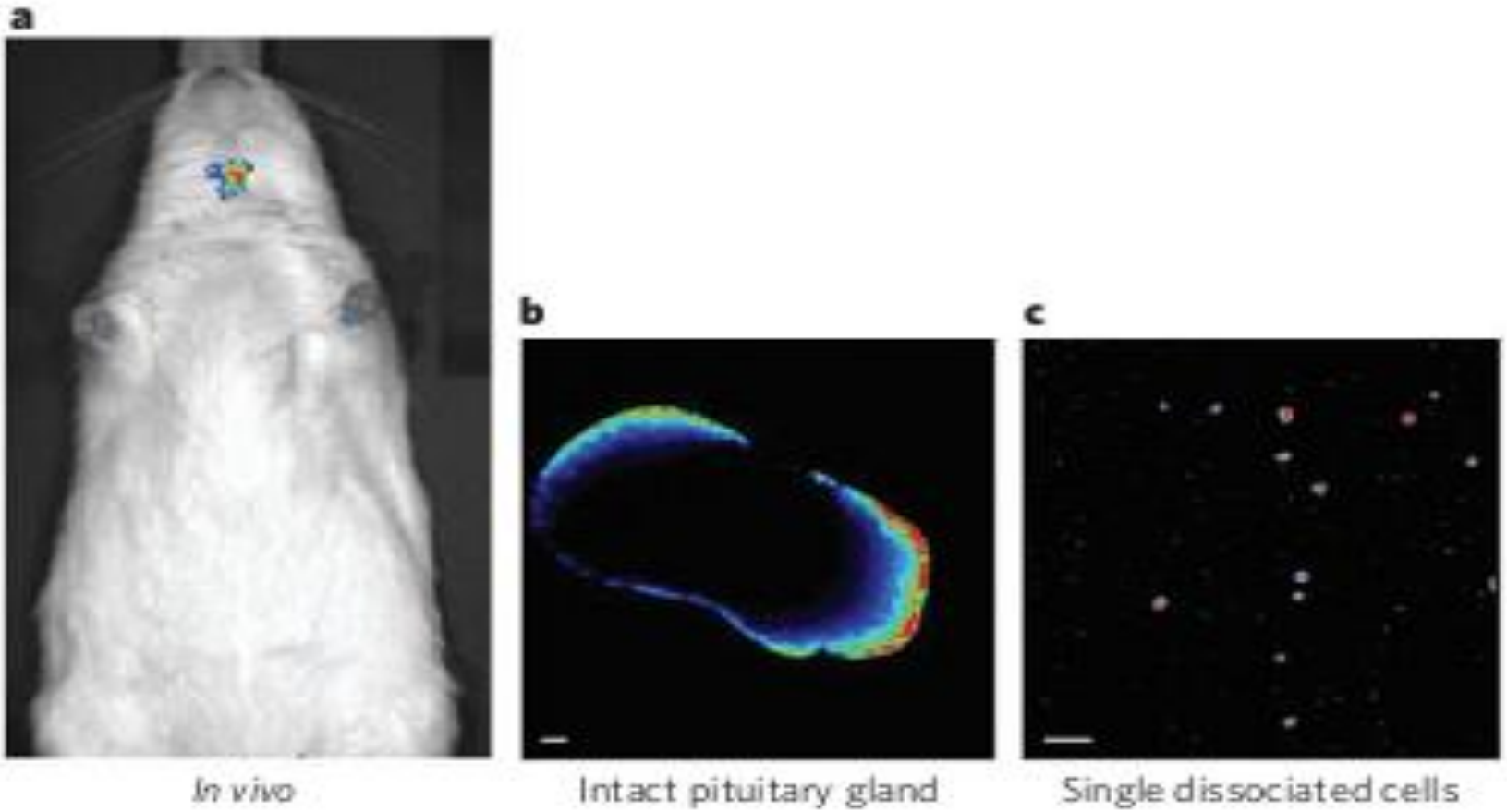
图g用的是荧光共振能量转移技术(FRET)

图h用的是蛋白质片段互补技术（PCA)



图a显示的是单细胞异相脉动

图b显示的是真实例子的脉动，是神经细胞瘤经过肿瘤坏死因子的刺激后，细胞中转录因子kB从细胞质到细胞核再到细胞质的转位

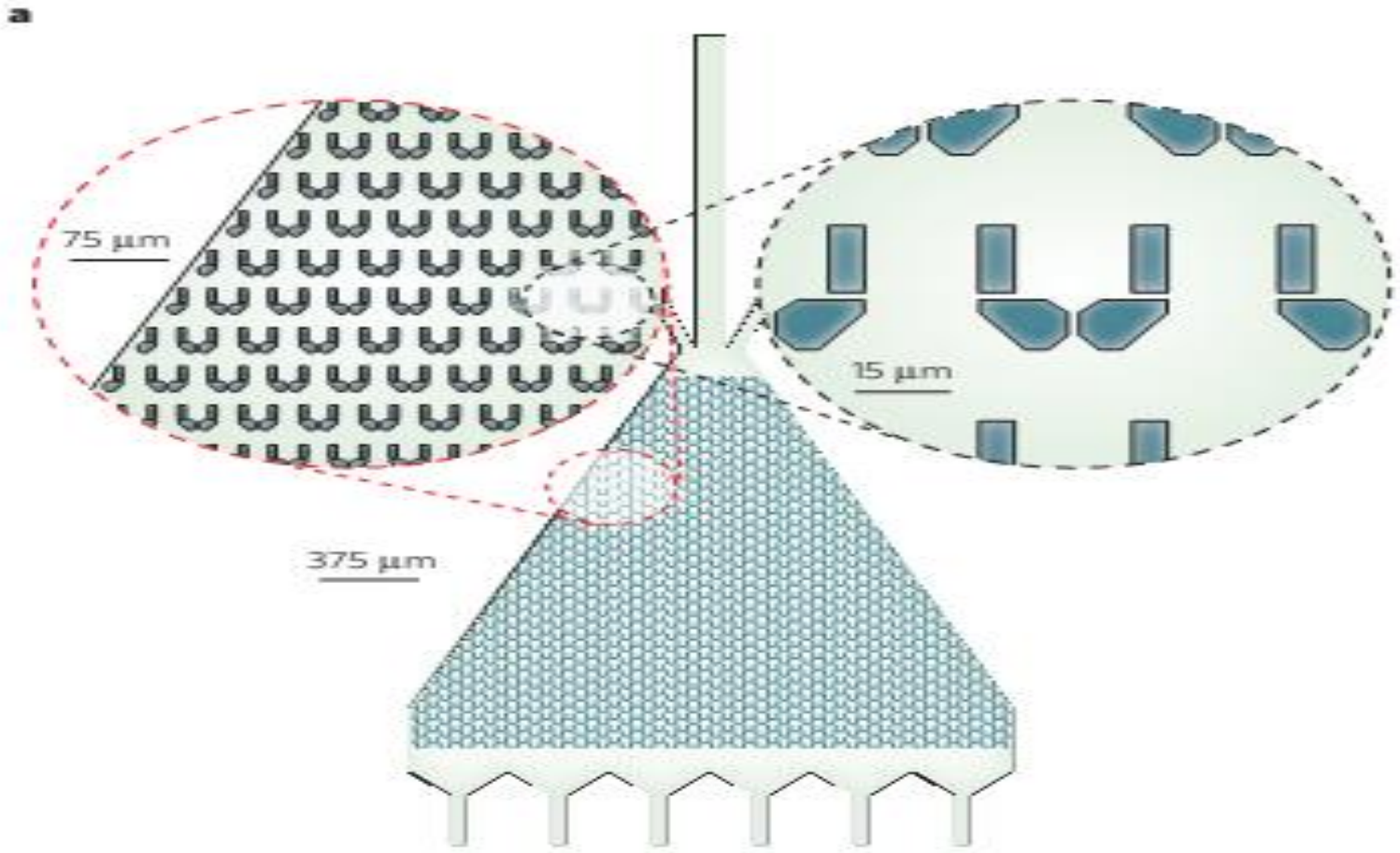


该图是体内和体外的冷光成像图

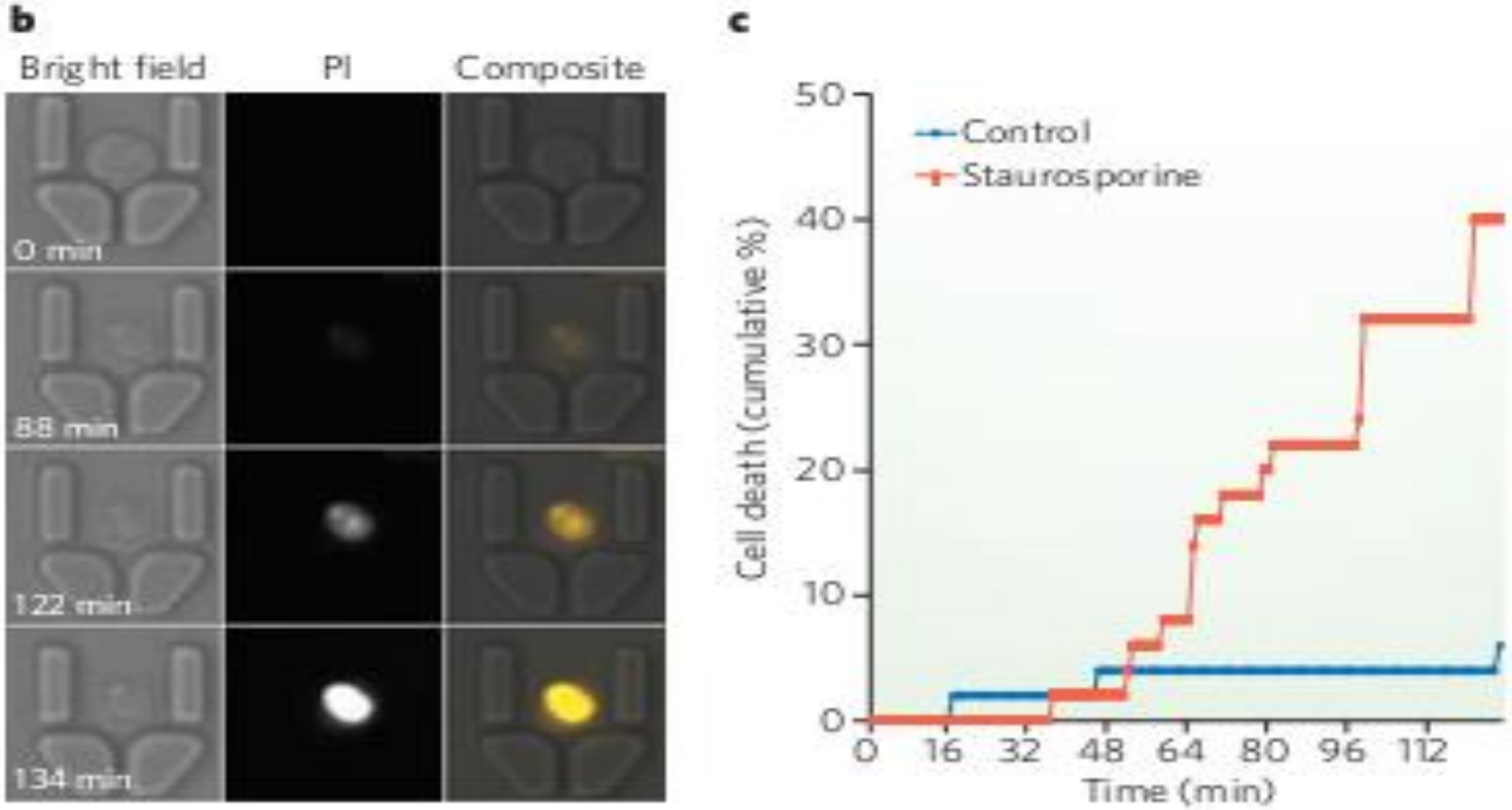
图a是转基因大鼠的腺垂体细胞在人类催乳激素基因的启动子的控制下，细胞中表达的荧光素酶的图像

图b是图a中的转基因大鼠的完整的腺垂体

图c是大鼠单个腺垂体细胞



图a用于调控和长期观察单细胞的微流装置



图b是早幼粒细胞性白血病细胞被捕获到“小监狱”中，并用诱导细胞死亡的星形胞菌素进行处理，用碘化丙啶（不能对活细胞染色）进行染色。

图c是在“小监狱”中的死细胞的积累率的绘图

MODEL-LED DATA INTEGRATION ANANALYSIS

- The quantities of experimental data and the number of reactions that regulate cell fate pose a major challenge to understanding cellular plasticity. Mathematical modelling and model-based data analysis are required to understand the behaviour and design principles of complex systems that show nonlinear behaviour in space and time (for example patterns, oscillations, switching and stochasticity).
- Modelling is also crucial for processing, integrating and interpreting complex high-dimensional data sets.Time-lapse measurements of single cells are ideal data sets from which to develop dynamic mathematical models. Deterministic mathematical models (for example using differential equations) can accurately simulate dynamic cellular subsystems.

- Cellular heterogeneity, however, is a common problem, and deterministic models cannot accurately simulate population-level or single-cell data unless the cells are relatively synchronous. Deterministic models basically assume that all cells are the same, which has the same outcome as experimental measurements of cell populations, which essentially average the data.

- Therefore, stochastic models often need to be used in combination with deterministic models, in order to take into account cell-to-cell variation and noise, as well as to explain cellular heterogeneity.

- All of these models need to be in a common format so that they can be more easily understood and integrated together into larger models.

CONCLUSIONS

- The diverse technologies used for single-cell measurements reflect the complexity and variety of the processes that need to be probed if we are to understand the basis of cellular plasticity. There remain many areas where the technologies that are available have limited the ability to make accurate measurements of important processes, including the quantification of protein–protein interactions, post-translational modifications and the absolute levels of key proteins.Isolated cells in vitro lack the correct environment that organizes and directs their behaviour in vivo .

- In this way, there is an increasing need for single-cell analysis of molecular and cellular dynamics, coupled to an integrative and interdisciplinary systems-biology approach. This seems to be the most promising way to understand these important cellular decision-making processes.

Thank you!