From light microscopy to multi-photon imaging:

Old and novel approaches for systems biology

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Overview

- Systems Biology and Imaging
- Confocal and Multi-Photon Imaging and modeling applications
- Bright field microscopy applications
- Method validation

Yet another definition of “Systems Biology”

- Integrating information: from single molecules to networks
- across the scales: from molecules to cell populations
- with high-throughput methods: Multi-modality data acquisition of spatio-temporal information about multiple molecules in single experiments
- using advanced data analysis: Large amounts of multi-dimensional data require standardized and efficient quantitative data analysis methods
- and modeling&simulation: Design and simulation of realistic models to explain and predict biological behaviour

Imaging and Systems Biology

"Classical" view of Systems Biology:

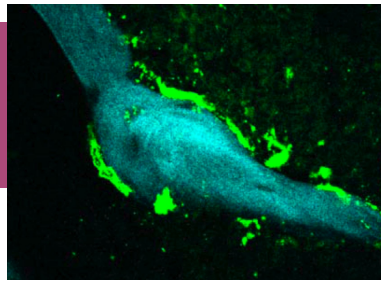
- "Omics"-approaches: Genomics, Proteomics, i. e. high-throughput technologies for quantitative measurements of molecular components, used to generate comprehensive (network) representations of biological function.
- Imaging not considered of particular importance for systems biology!
- Maybe because conventional, i. e. manual image analysis mostly yields *qualitative* results!
- However ... imaging methods in combination with quantitative computer-aided image analysis methods highly compatible with needs of systems biology!!!

Imaging and Systems Biology

- Integrating information across the scales: analysis of subcellular structures, single cells and cell populations
- with high-throughput methods: data acquisition of spatio-temporal information of multiple cells in single experiments
- using advanced data analysis: From “scores” to “measurements”: Computer-aided objective, standardized, quantitative image analysis methods
- and modeling&simulation: Design and simulation of realistic *image-data-based* models to explain and predict biological behaviour

Microscopy-based experiments

spatio-temporal information on biological processes



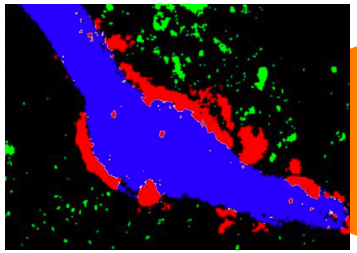
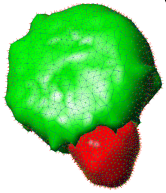


Image Analysis

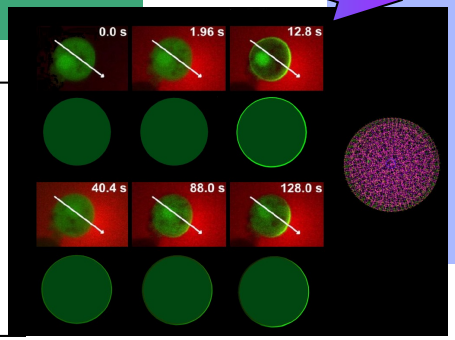


Parameter estimation (dynamics of localization, concentration of proteins, cell geometry model generation)

Quantitative results/ conclusions

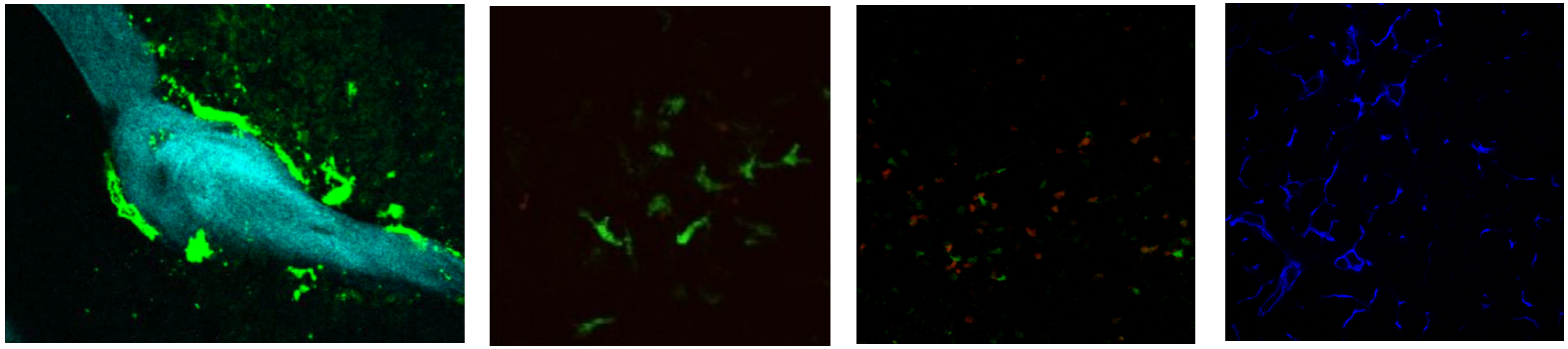
Modeling and Simulation

Simulation results/ conclusions



quantitative model validation: direct comparison of simulation and experimental results

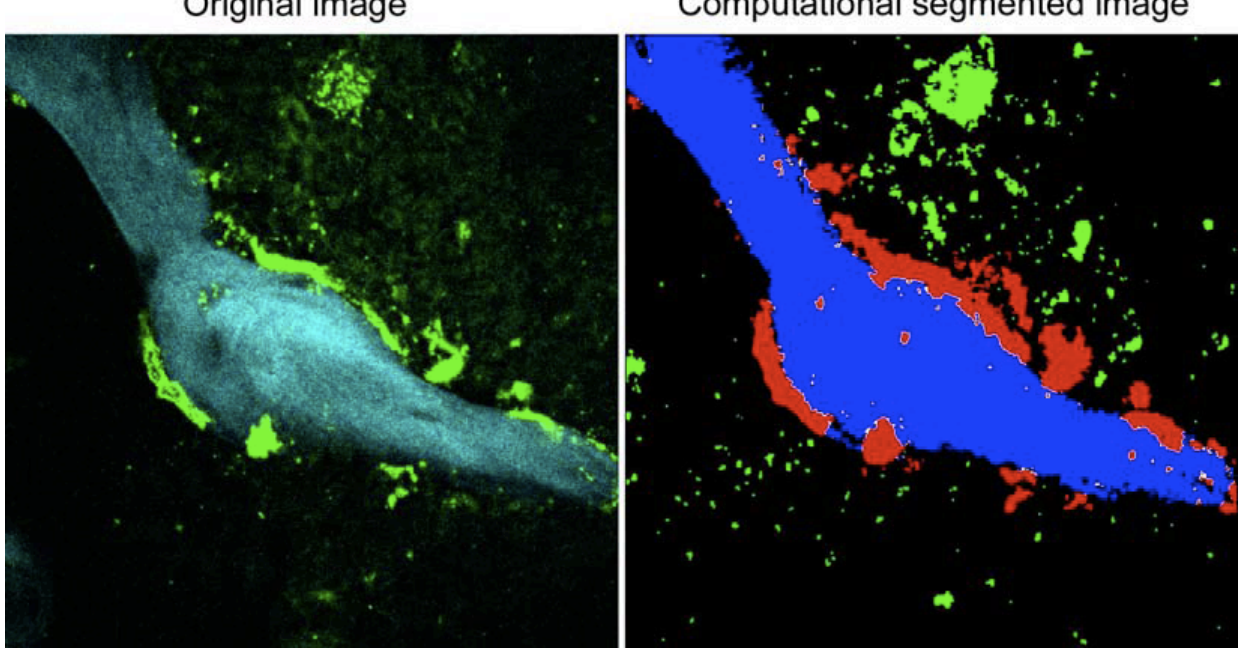
Cell-Cell/Cell-Tissue Interaction Image Analysis (confocal/two-photon microscopy data)



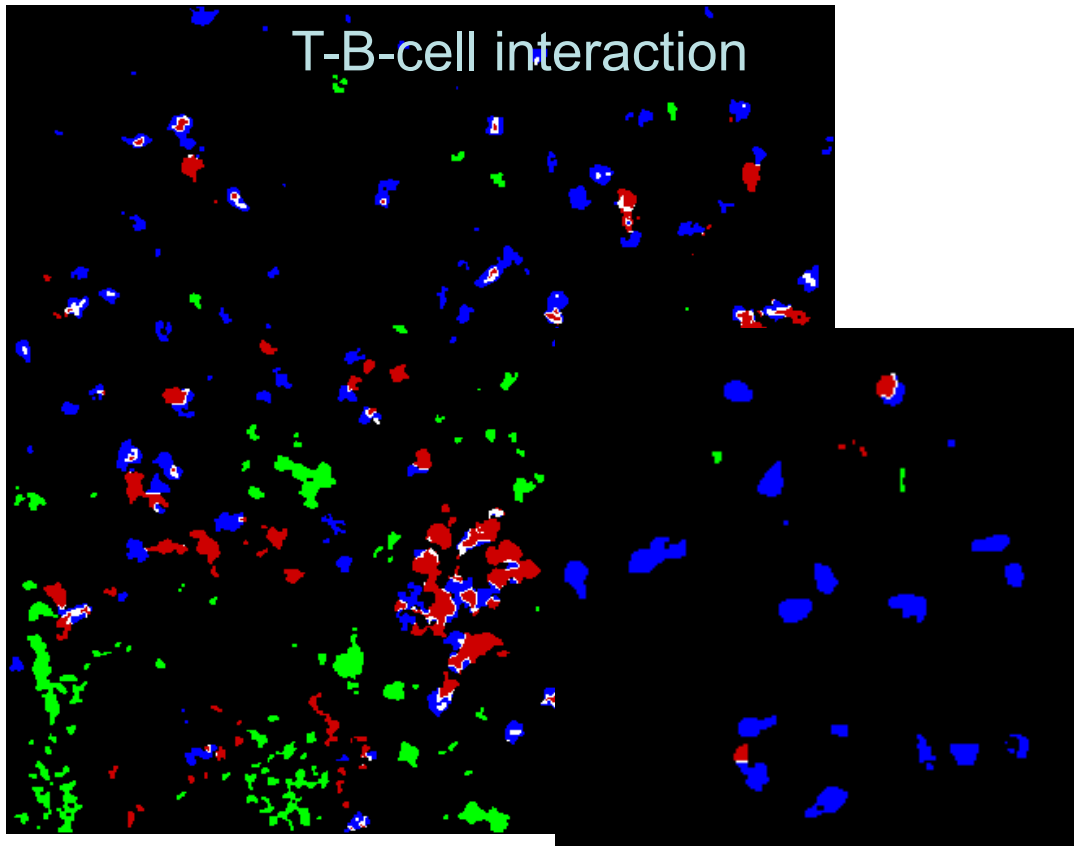
- Consists of four modules:
  - Signal-background separation by adaptive threshold segmentation
  - Single cell/object detection and removal of noise/artefacts
  - Adaptive channel merging
  - Image feature quantification

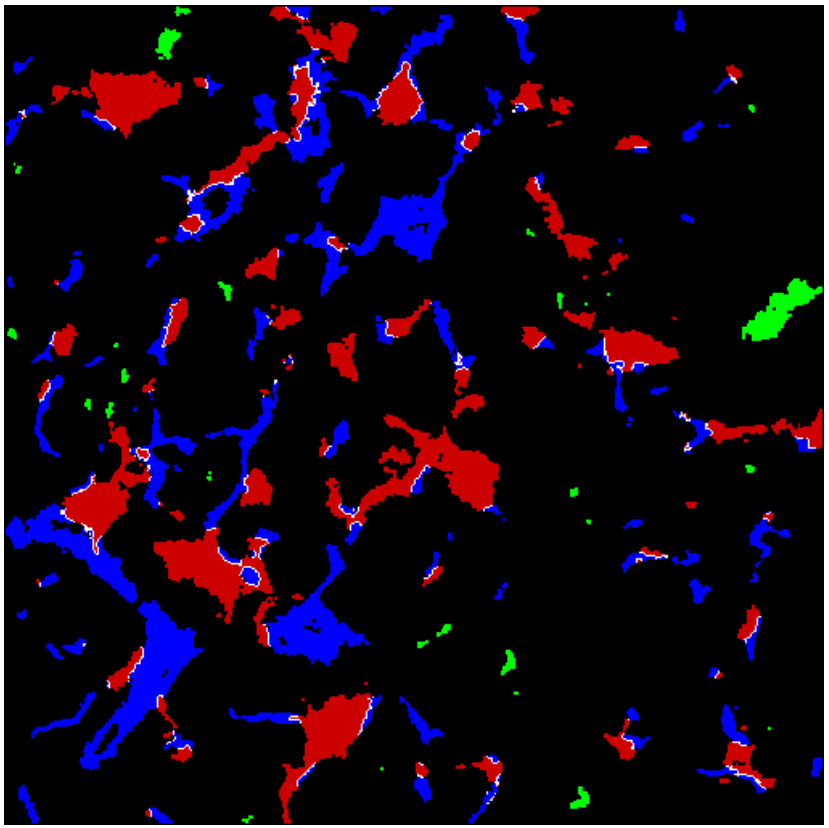
- This allows for:
  - Standardized, robust analyses: works for different fluorescent probes, image acquisition parameters, image qualities (z-stack intensity inhomogeneities)
  - Automated, no user intervention necessary (optimization by user possible)
  - Capable of processing large 3-/4-D data sets and detect and quantify even subtle phenomena (differences between experimental groups)
  - Basis for standardized morphology feature reconstruction for realistic modeling & simulation of cellular processes

Applications



bone-osteoclast interaction



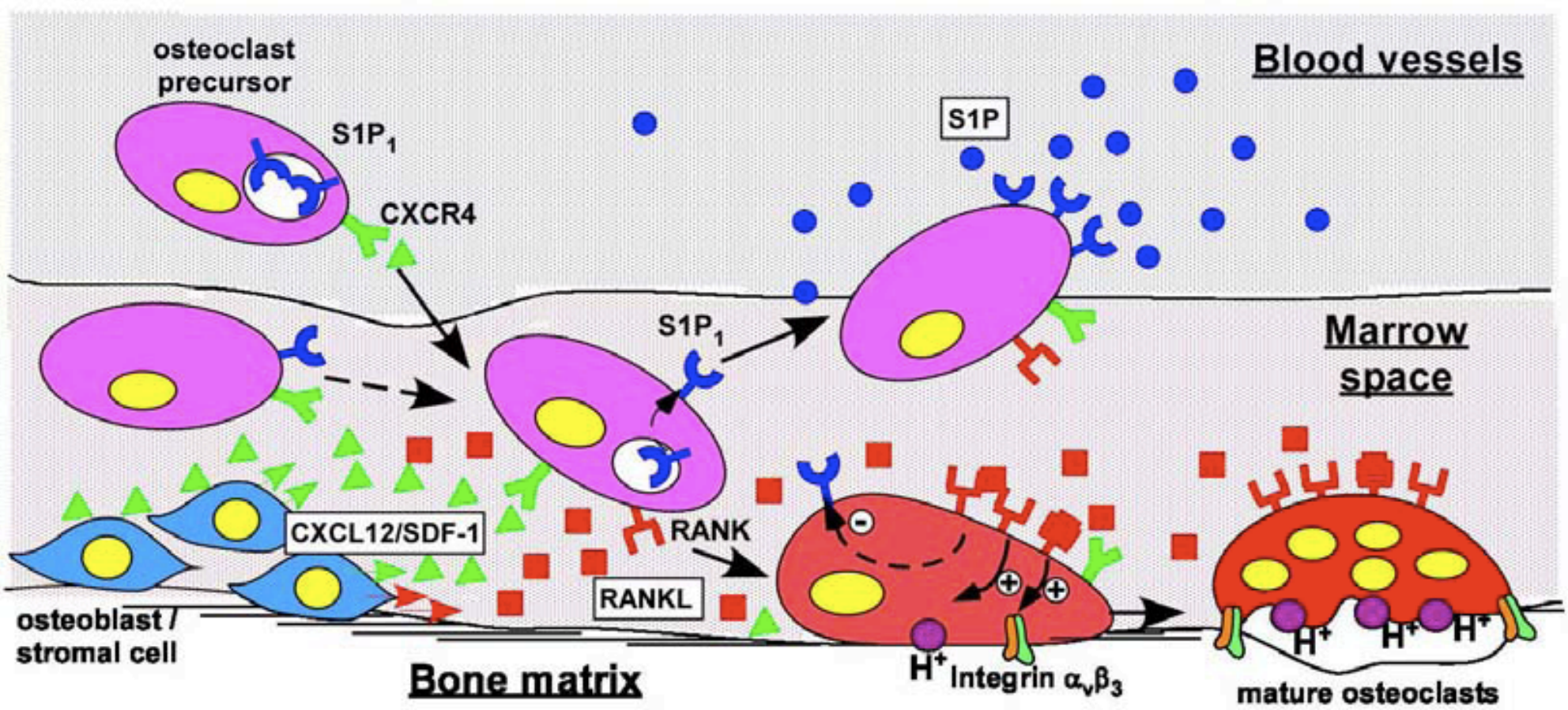


DC - fiber interaction in LN

Quantitative analysis of bone homoeostasis

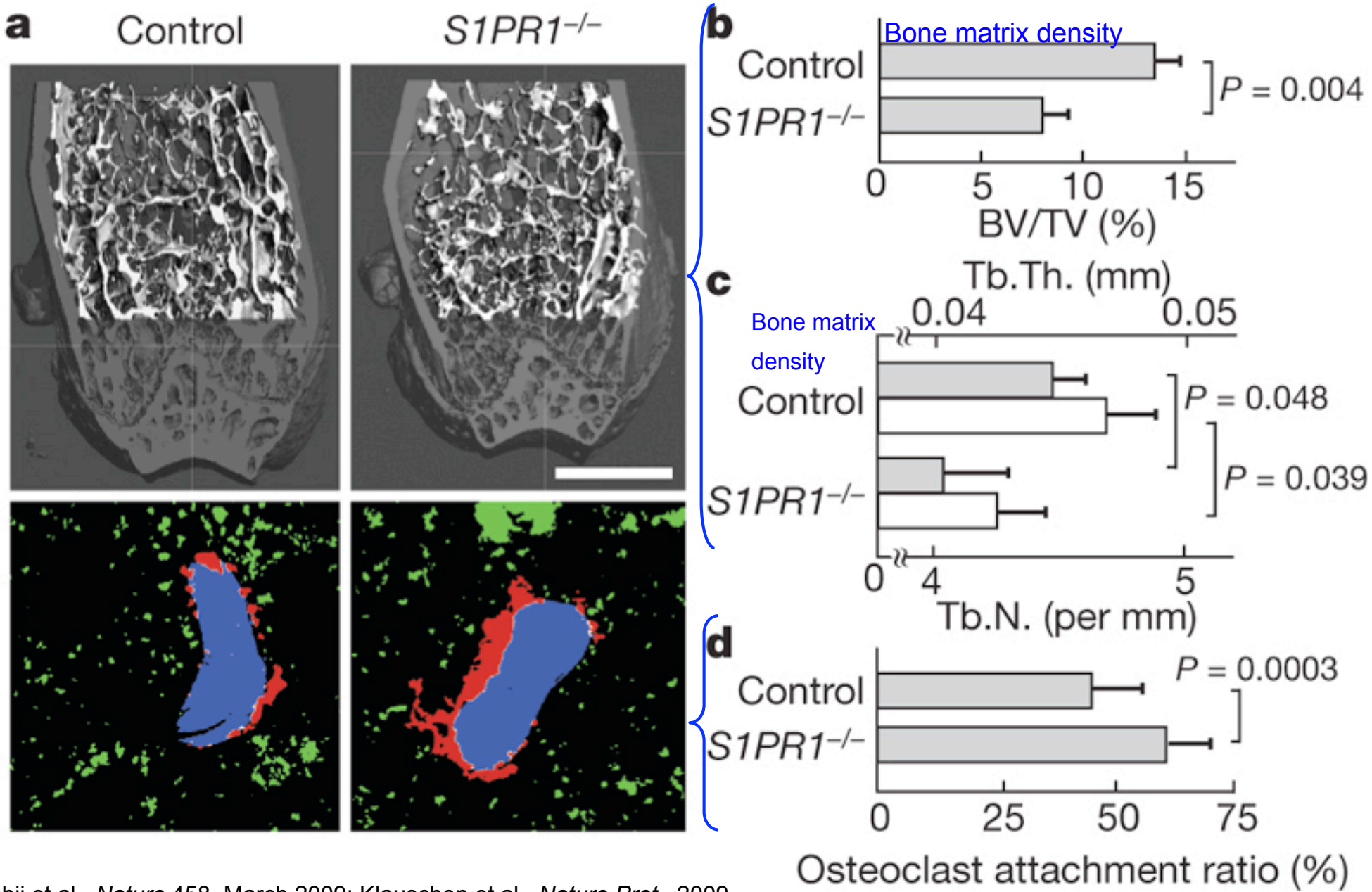
RANK activation promotes osteoclast attachment and bone resorption

Sphingosine-1-Phosphate-Rec. ($S1P_1$) activation inhibits osteoclastogenesis



Ishii, Egen, Klauschen et al., *Nature* 458, March 2009

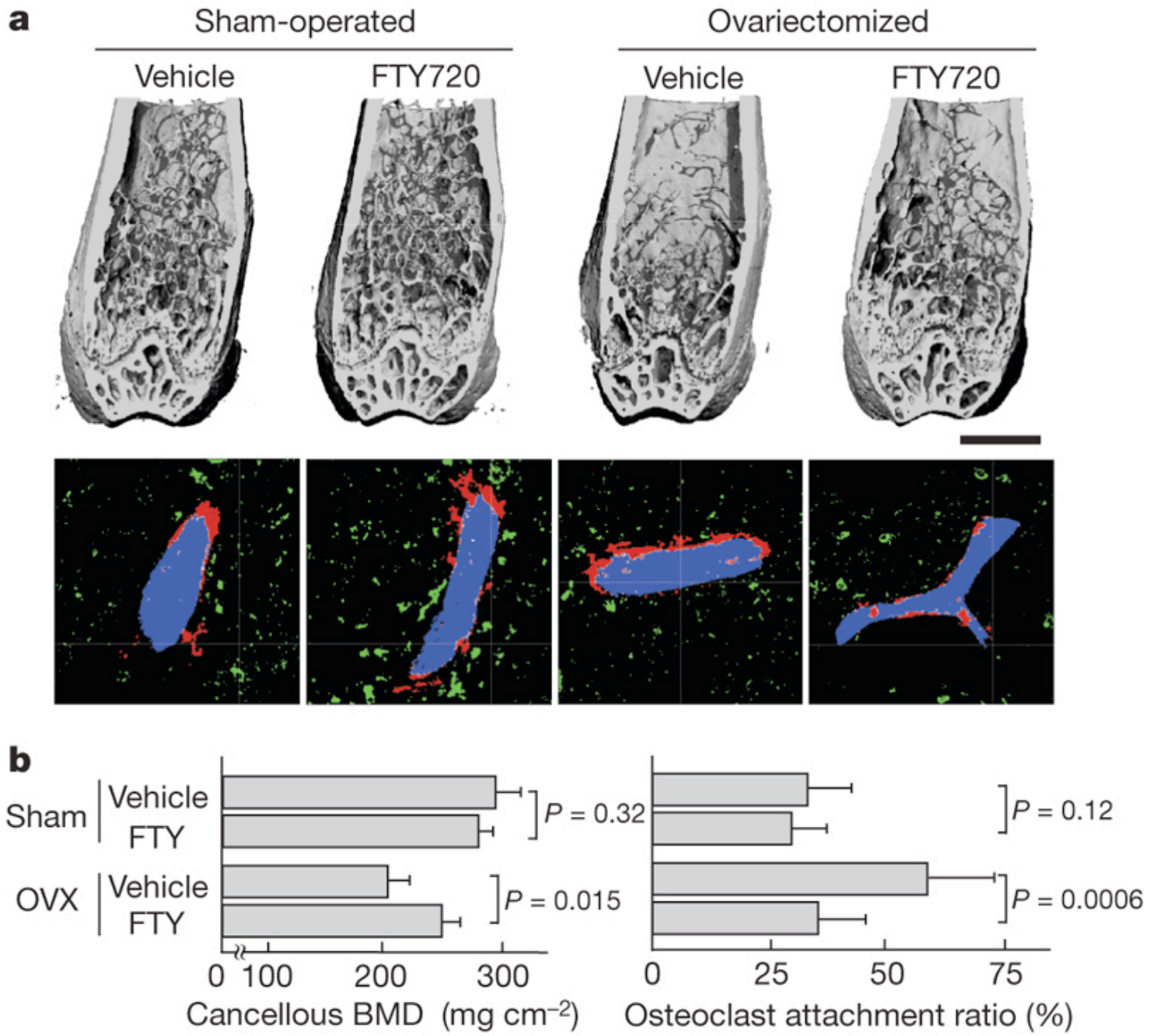
Relevance of $S1P_1$ for bone homoeostasis



Ishii et al., *Nature* 458, March 2009; Klauschen et al., *Nature Prot.,* 2009.

Use of $S1P_1$-activation in pathological conditions?

$S1P_1$-agonist FTY720 reduces effects of estrogen-deprivation induced osteoporosis in mice!

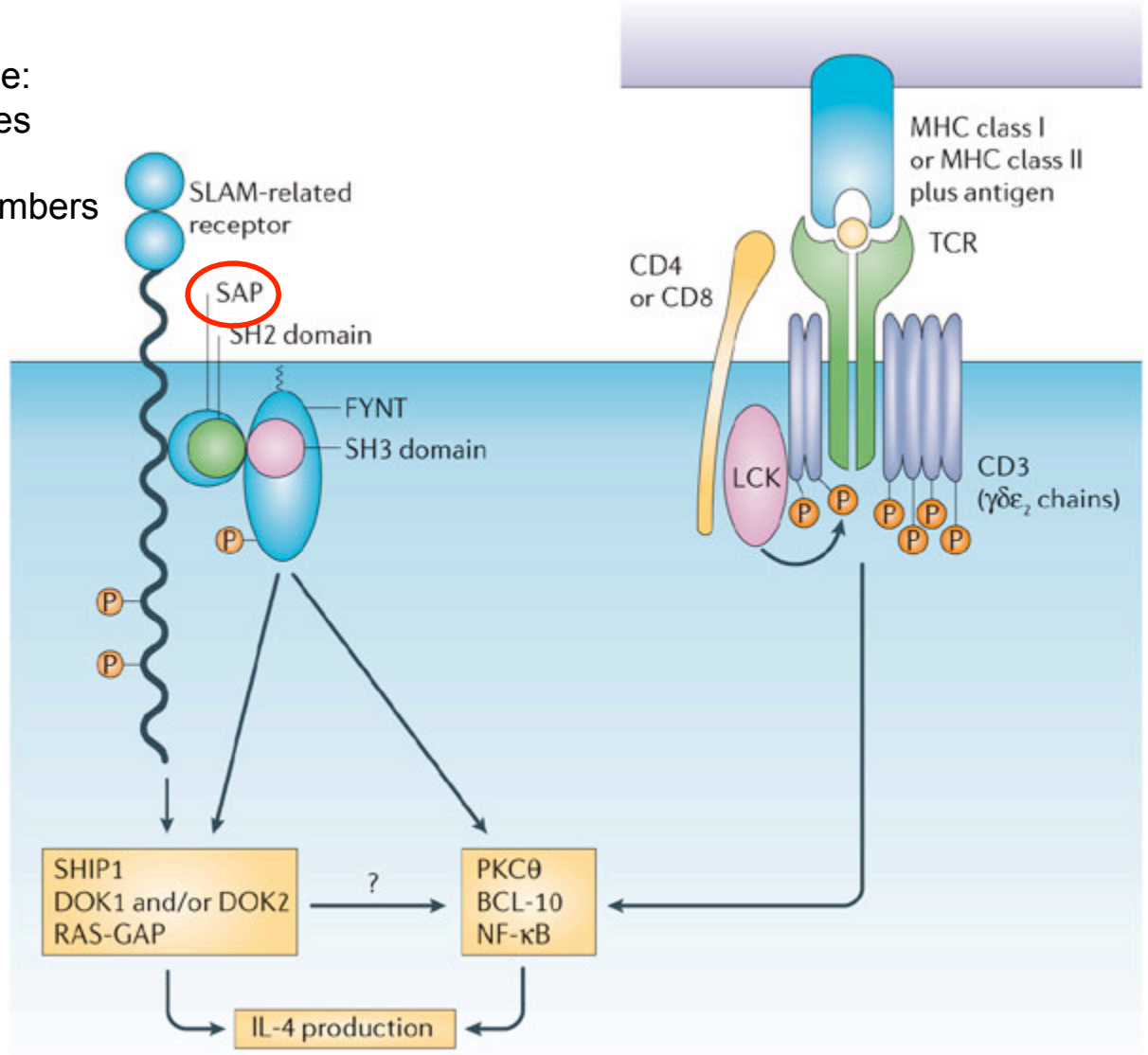


Ishii et al., *Nature* 458, March 2009;
Klauschen et al., *Nature Prot.,* 2009.

Influence of SAP on T-B-cell interaction

SAP-mutation:
X-linked lymphoproliferative syndrome:
- defective humoral immune responses
- defective germinal centre formation
- reduction in IgG+ memory B-cell numbers

SAP: SLAM-associated-protein
SLAM: Signaling lymphocyte assoc. molecule



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Nature Reviews | Immunology

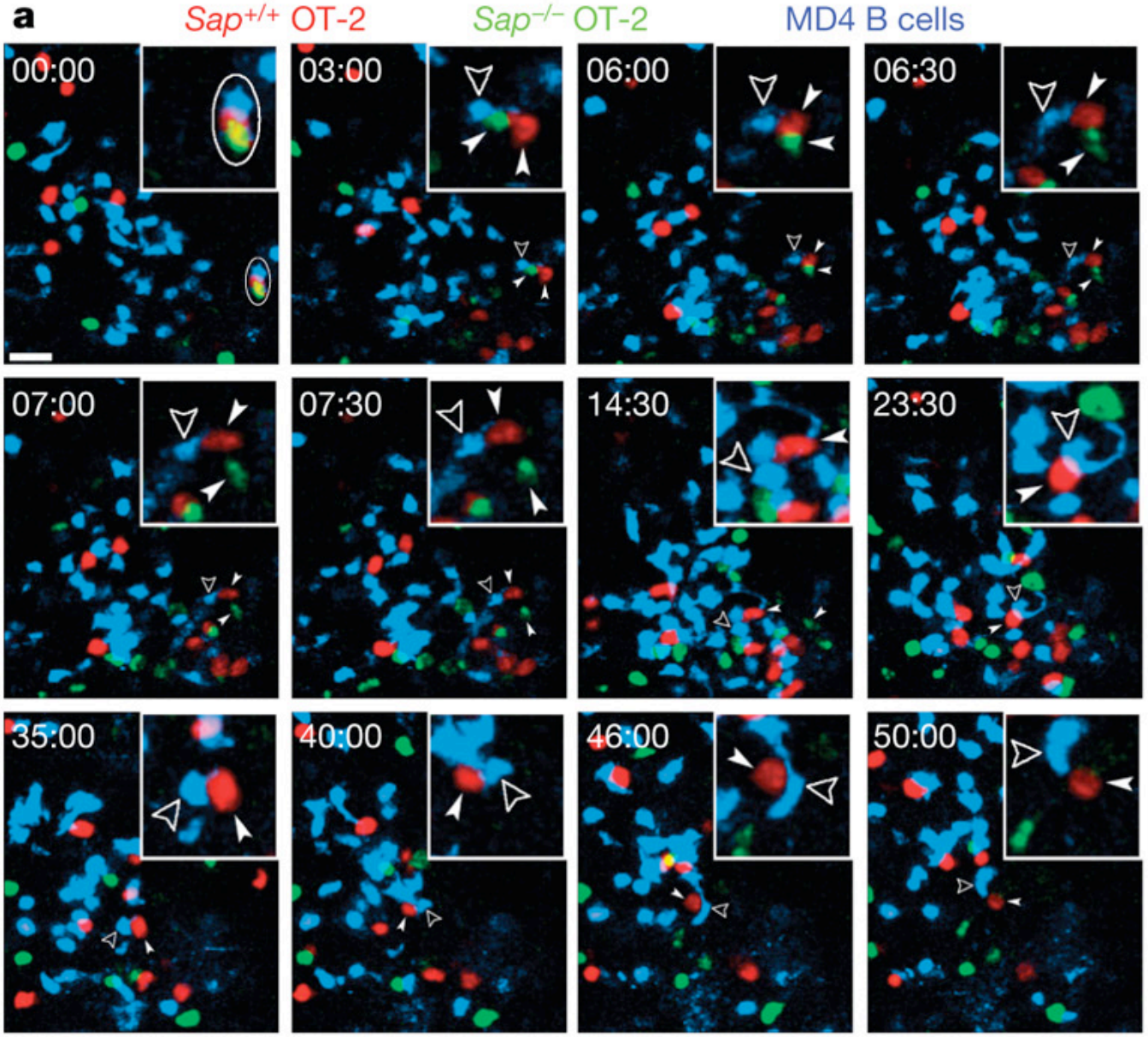
André Veillette
Nature Reviews Immunology 6, 56-66 (January 2006)

Influence of SAP on T-B-cell interaction

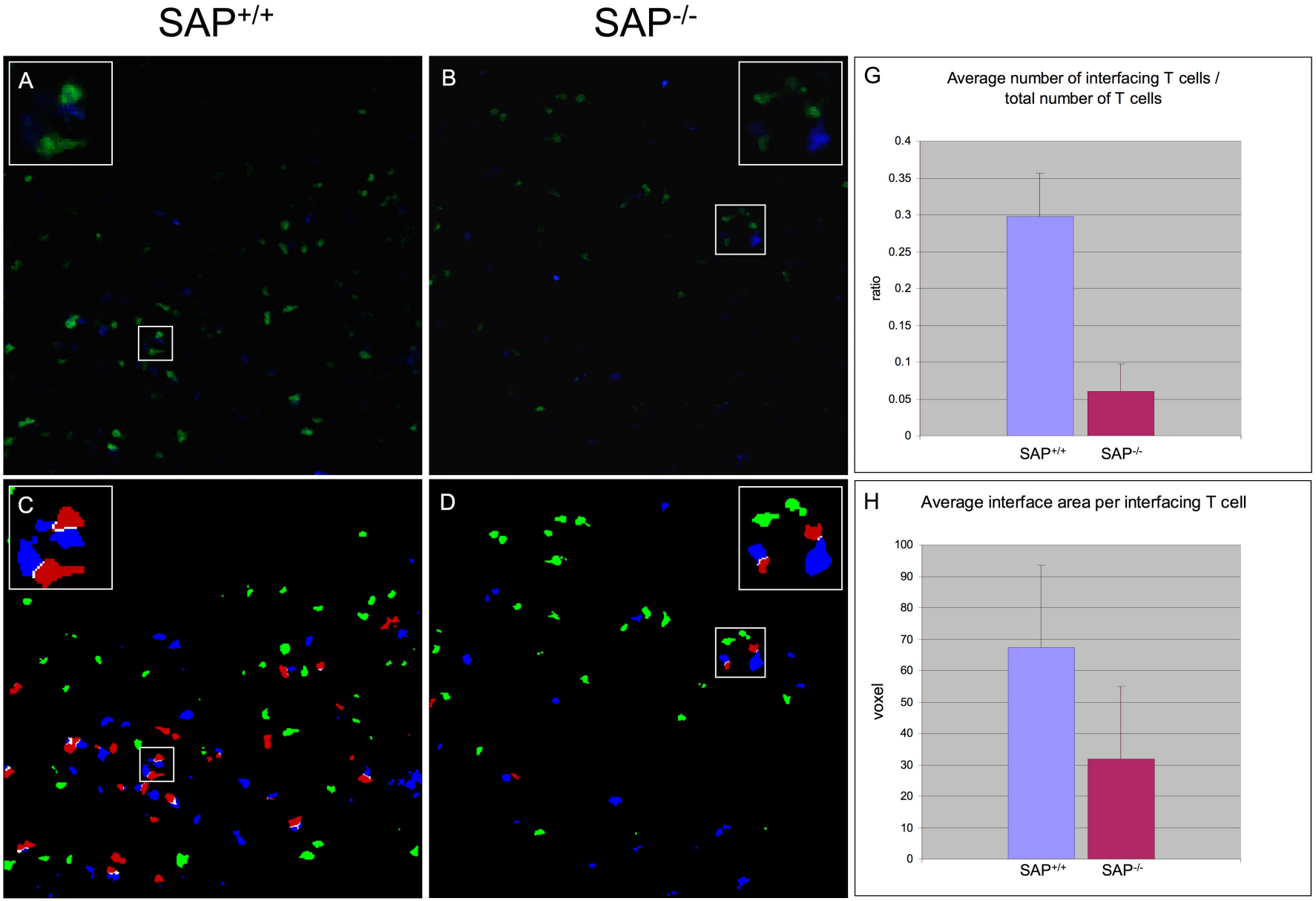
SAP is needed for germinal center formation

SAP deficiency reduces stability of T-B-interaction

Qi, Cannons, Klauschen et al., *Nature* 2008 Oct 9

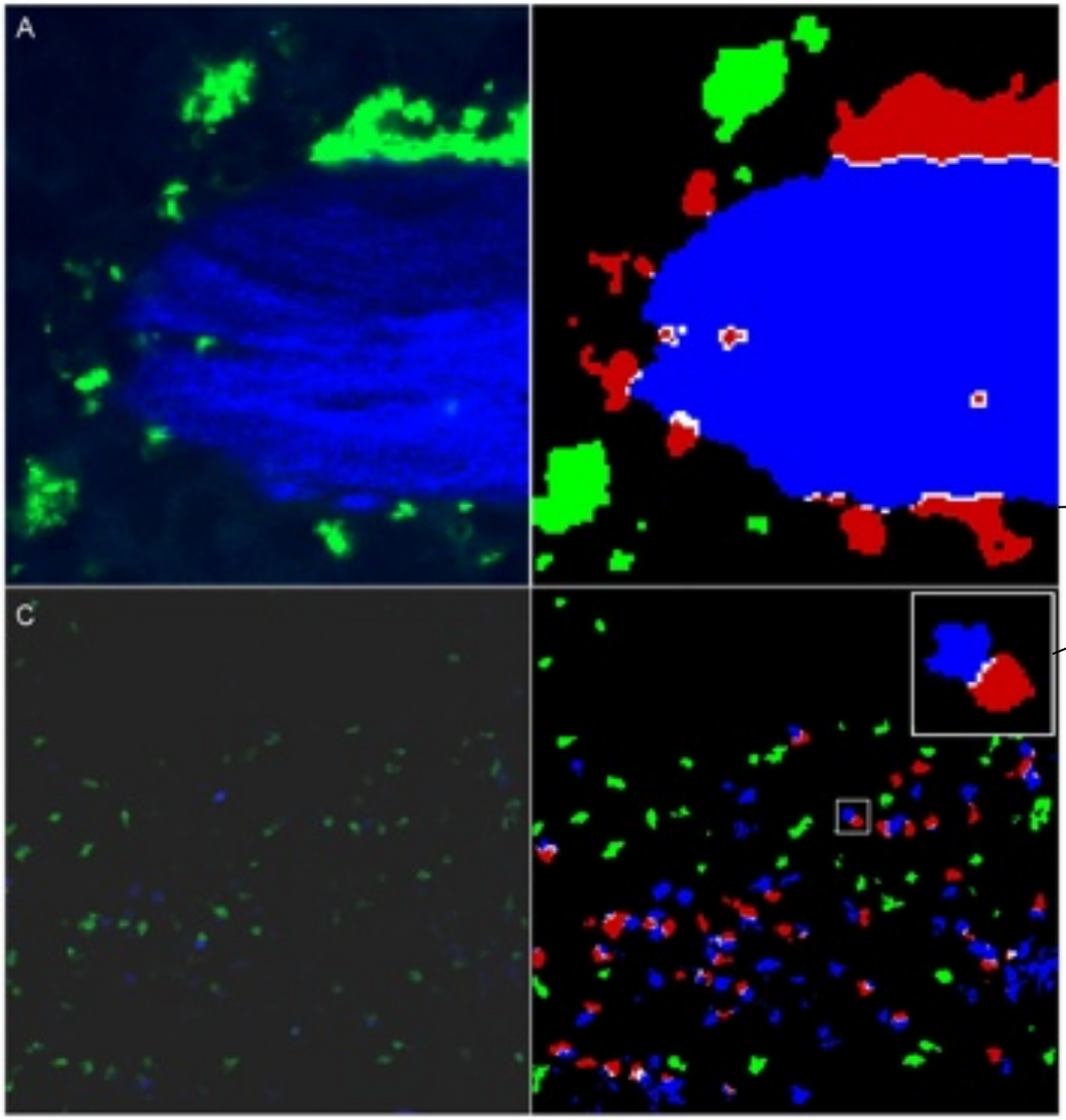


SAP influences synapse size of interacting B and T cells



(unpublished data)

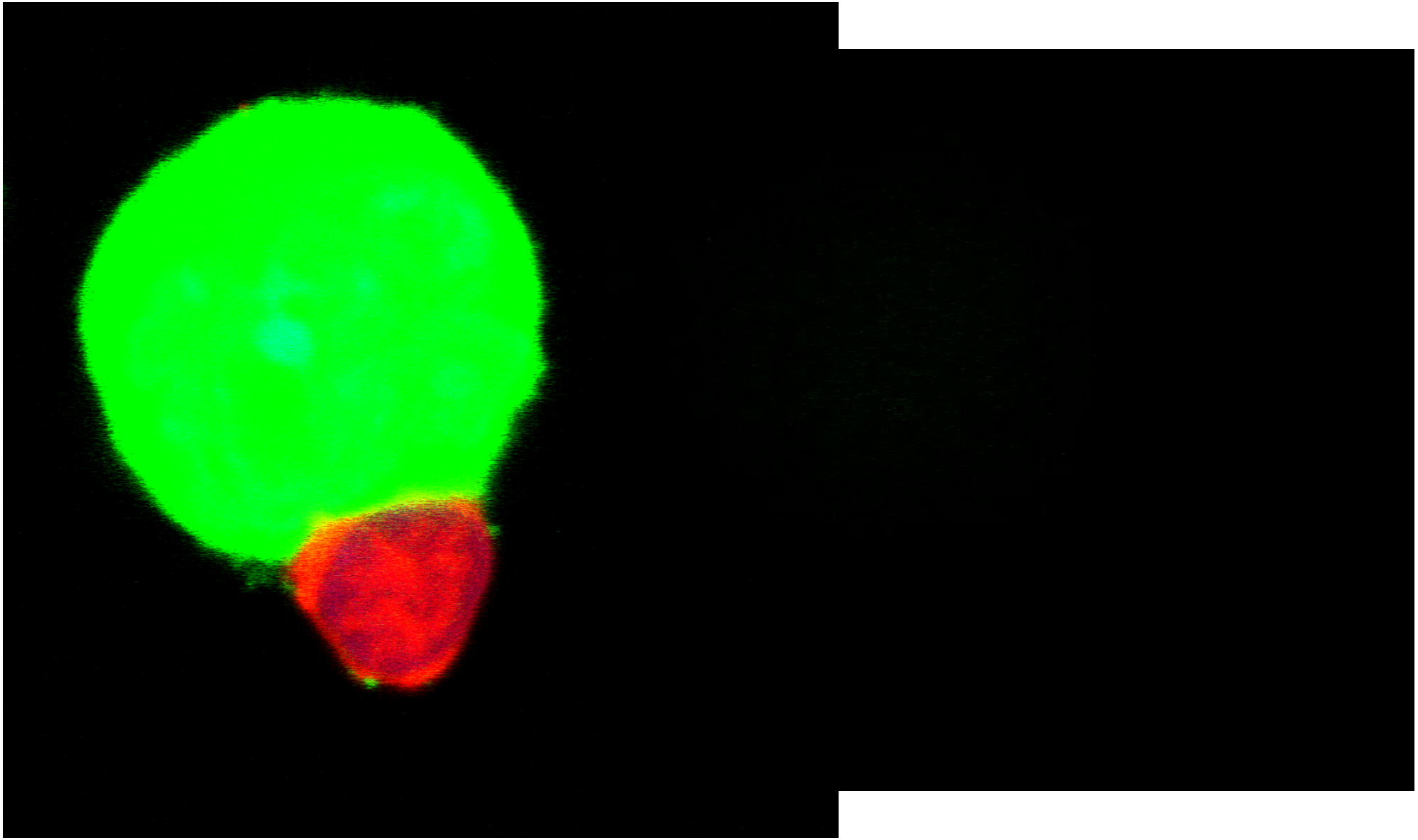
From descriptive and correlative models to the understanding of underlying mechanisms using realistic computational models



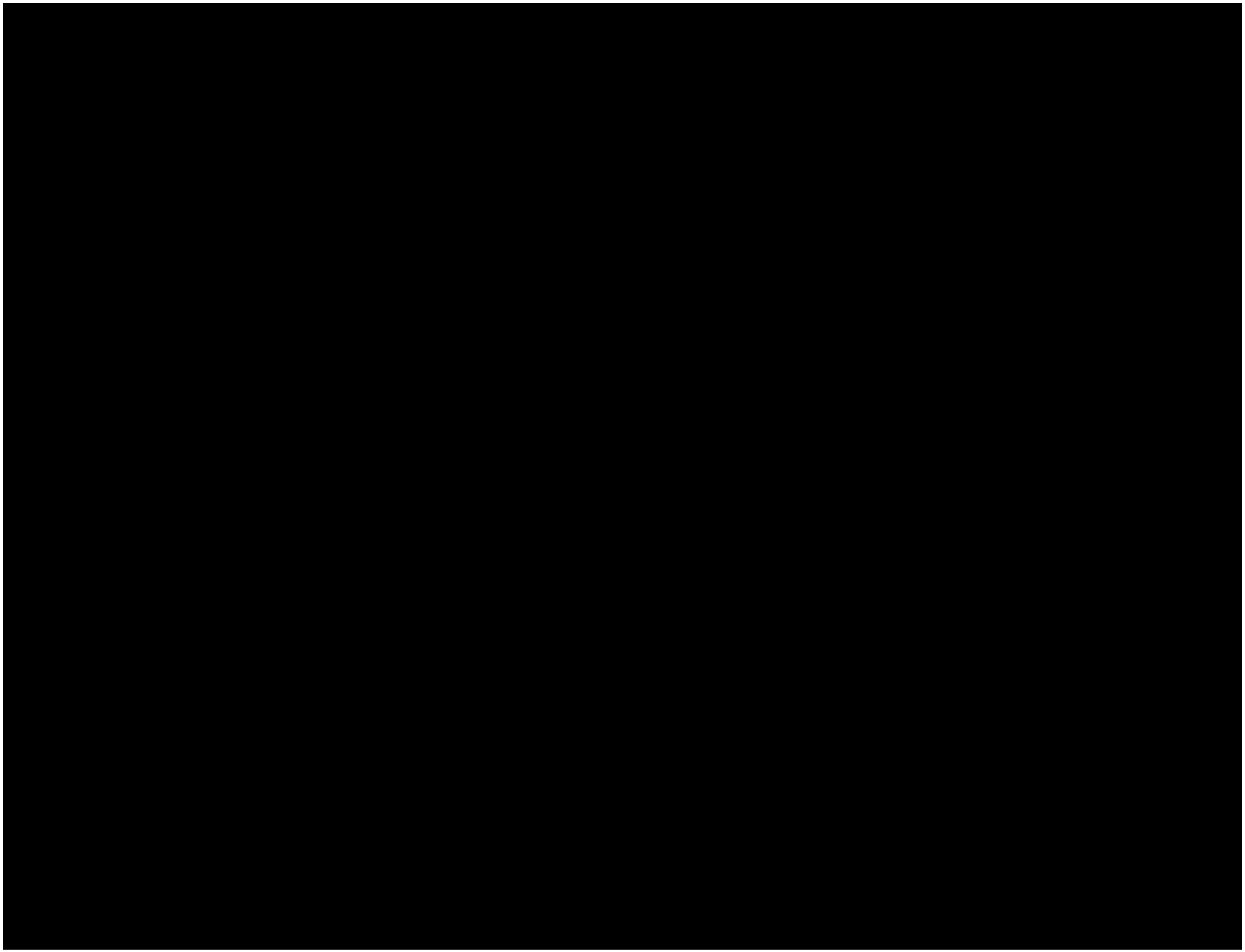
quantitative analysis of interface area allows for correlation of experimental condition with biological response

use spatial information as basis for mechanistic systems analyses of cellular processes

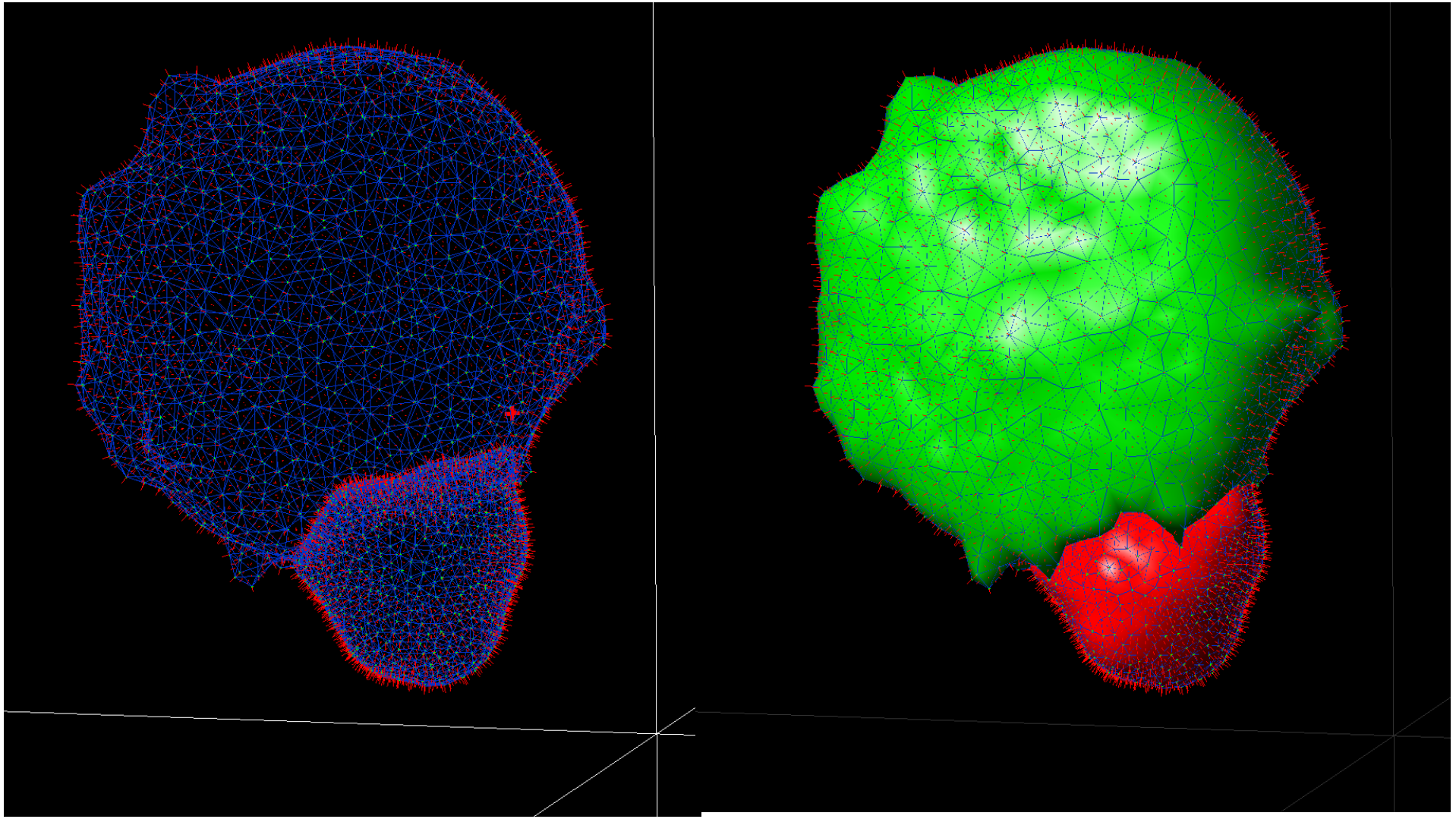
Construction of a spatial cell model from fluorescence microscopy data



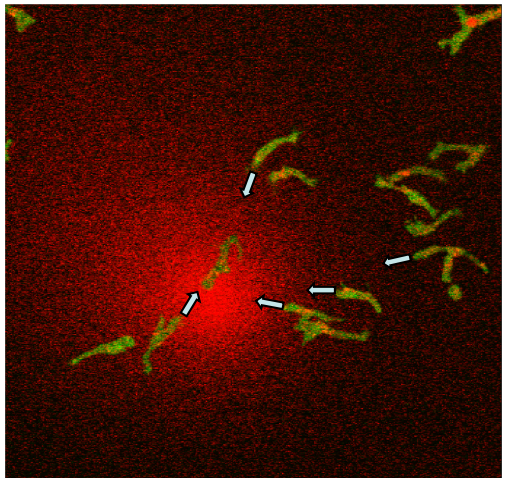
Construction of a spatial cell volume model



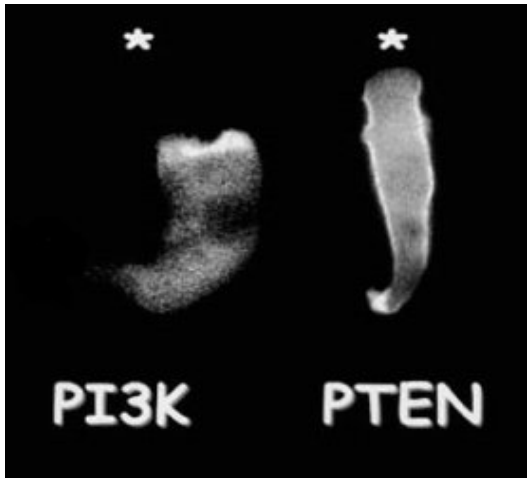
A realistic surface - volume model of T cell - APC interaction



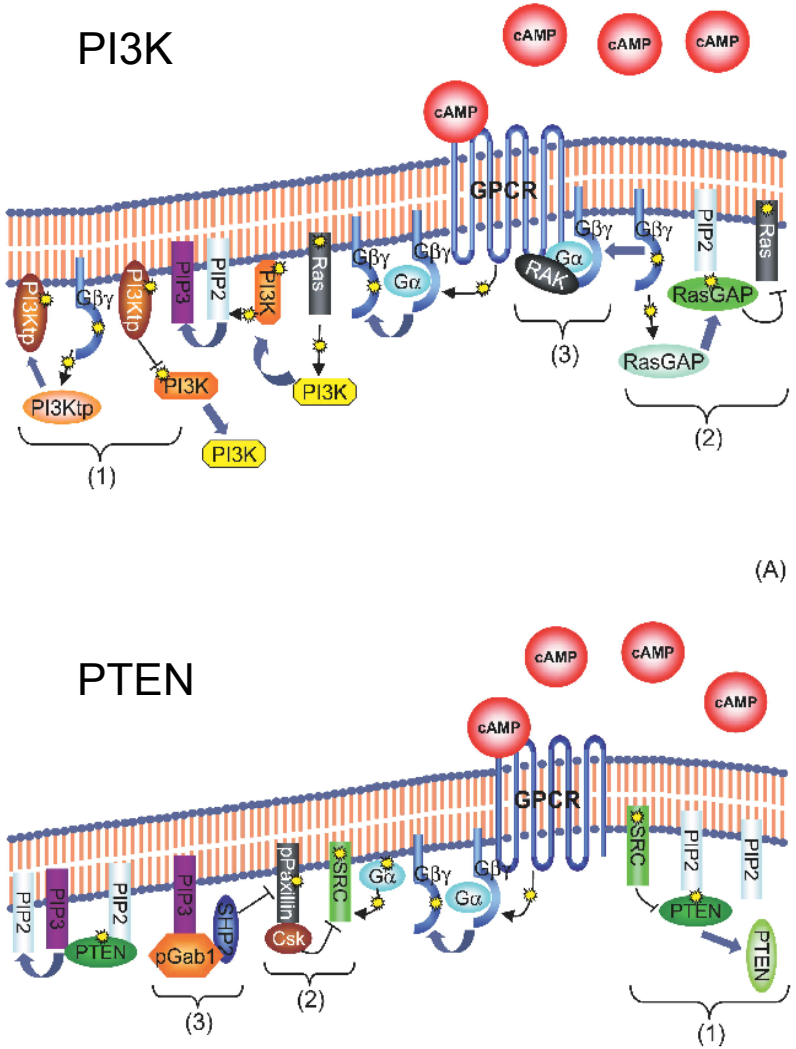
Spatial simulation of chemotaxis signalling



Chemotaxis, Xu and Jin, 2006

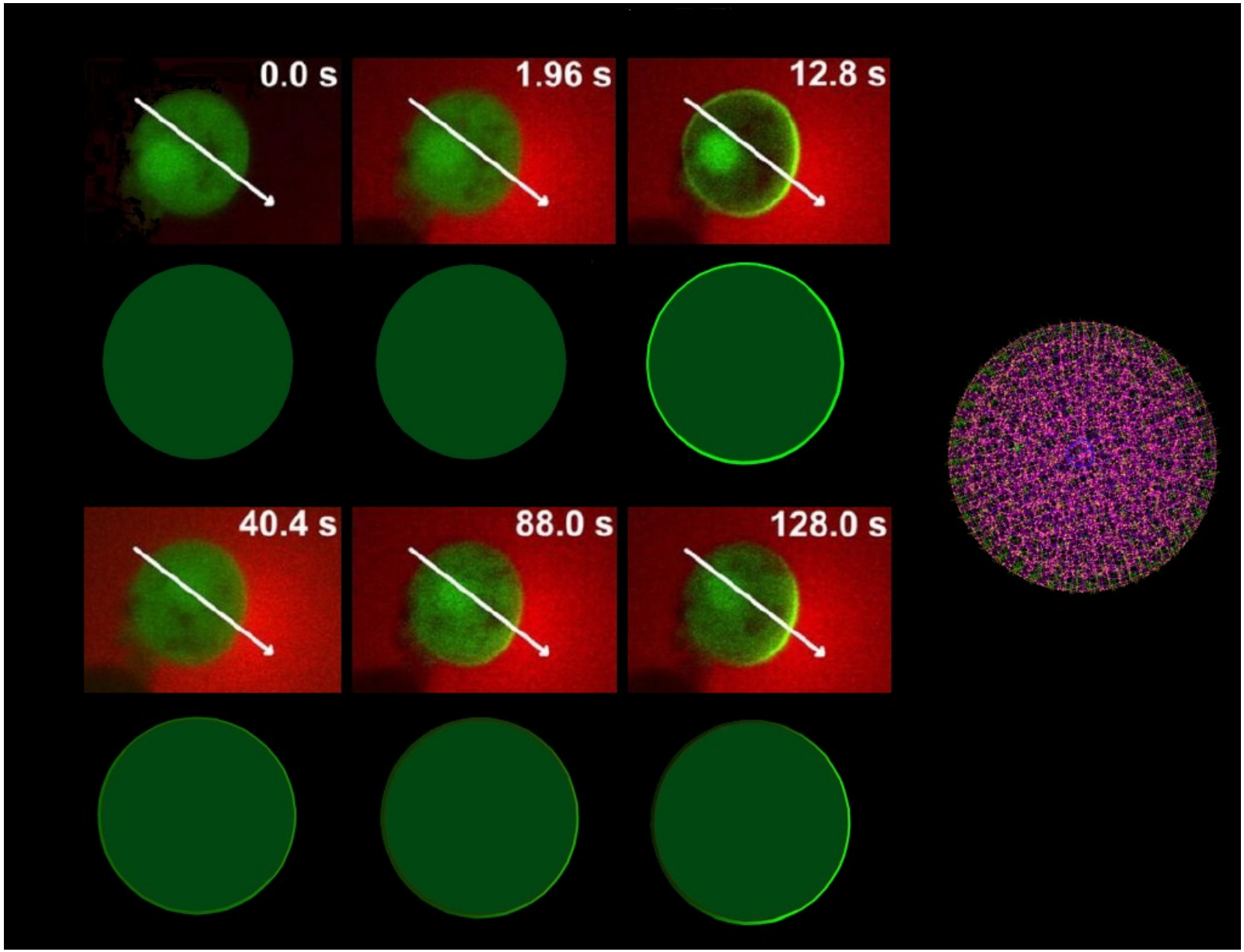


Comer and Parent, Cell, 2002



Meier-Schellersheim et al., PLOS CompBiol 2006

Spatial simulation of chemotaxis signaling



Klauschen, F. and Meier-Schellersheim, M..,
manuscript in preparation

Bright-field vs. fluorescence microscopy

- Bright-field offers high-throughput capability in a single slide!
- Requires color channel separation and segmentation based on morphology features
- Max. 1 to 2 simultaneous colors
- 2-D data

Next generation imaging:
Multi-color wide-field 3-D confocal microscopy