Pushing and pulling: the molecular basis for force generation in cells

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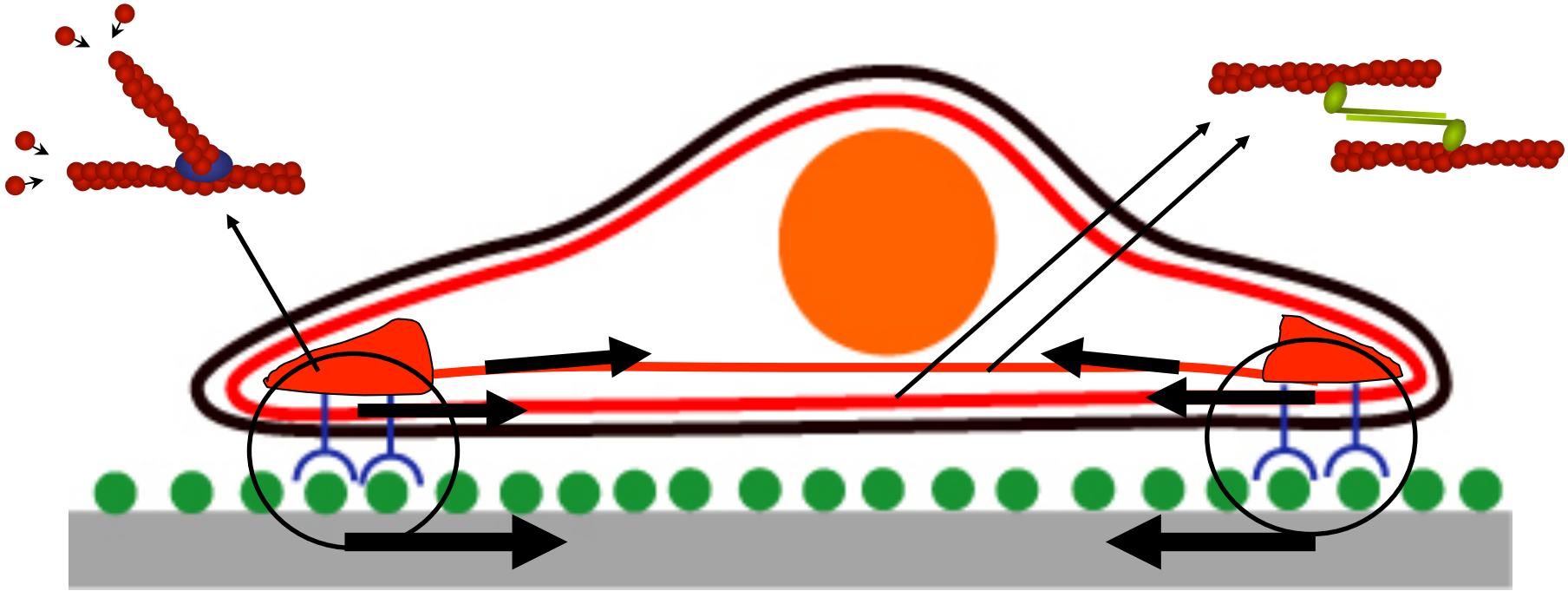
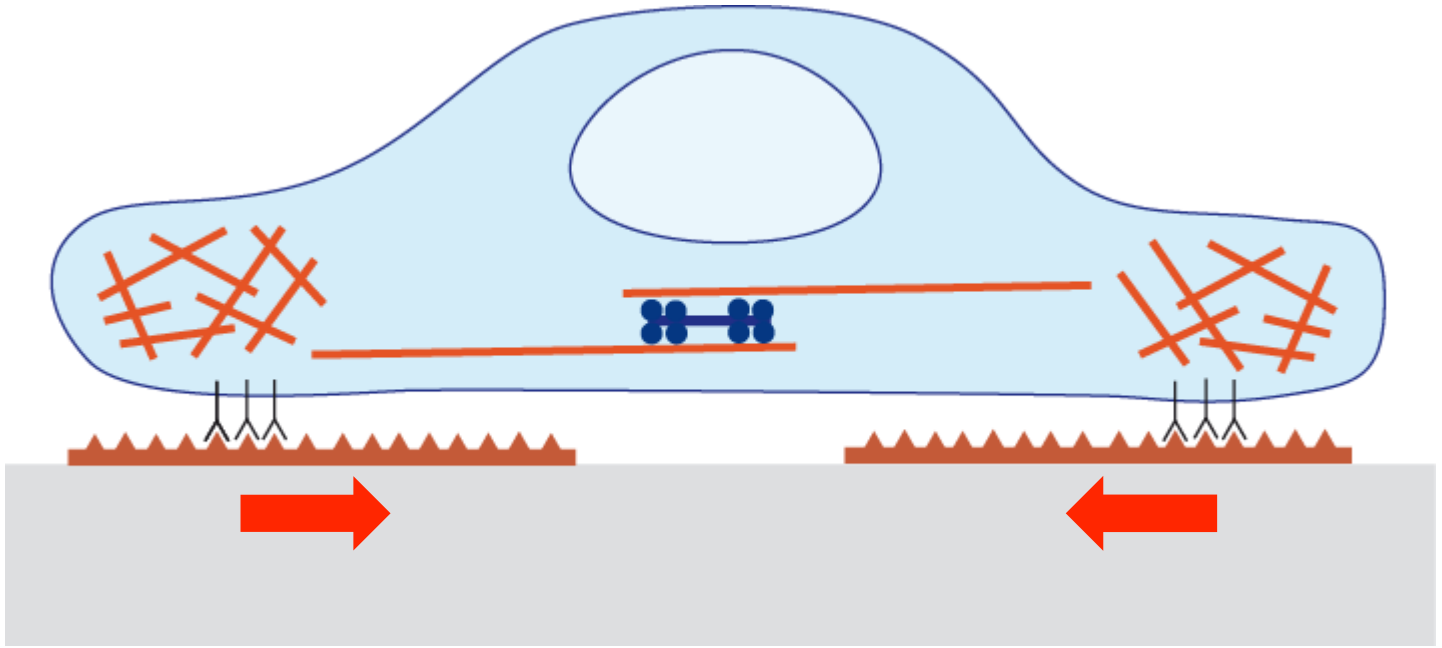
Reminder conference talk

Active processes during cell adhesion



- actin polymerization pushes out **lamellipodia**
- contractile forces generated in **stress fibers** and **actin networks**
- force is transmitted to substrate through **focal adhesions**

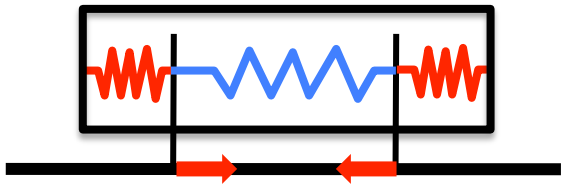
Overall force balance in the cell



Network polymerization: compressed spring

Motor contraction: tensed spring

Whole system: contraction force dipole

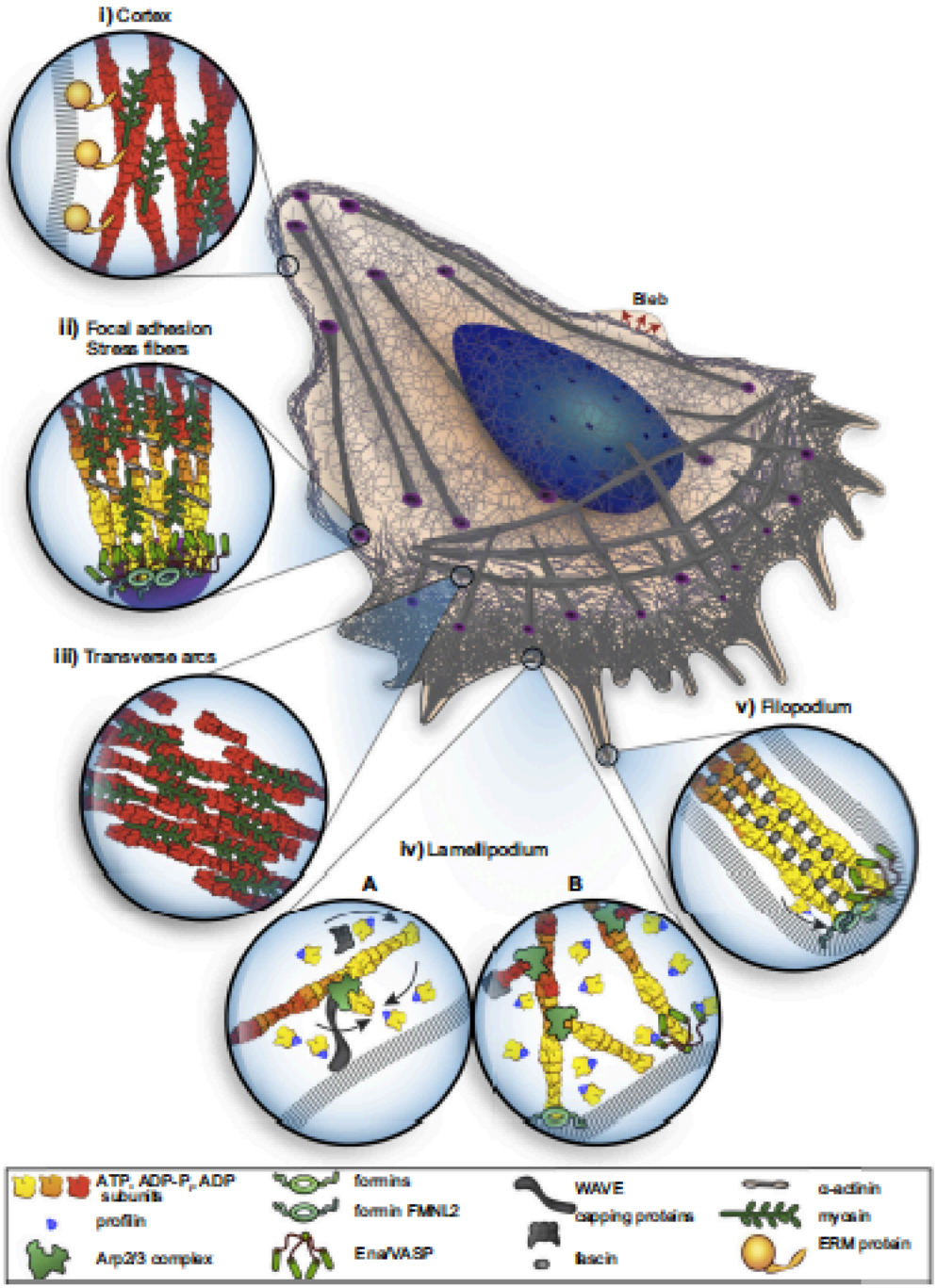


[Schwarz and Safran RMP 2013]

Different modelling approaches to contractile matter

- Mechanical (polymer) networks
- Active cable networks
- Contour models
- Active gels
- Active elasticity
- Cellular Potts Model
- Phase field or level set models

All of these coarse-grained models need to be informed by our understanding of the molecular basis of force generation.



A molecular view on force generation in the cell

It‘s the actin cytoskeleton, stupid !

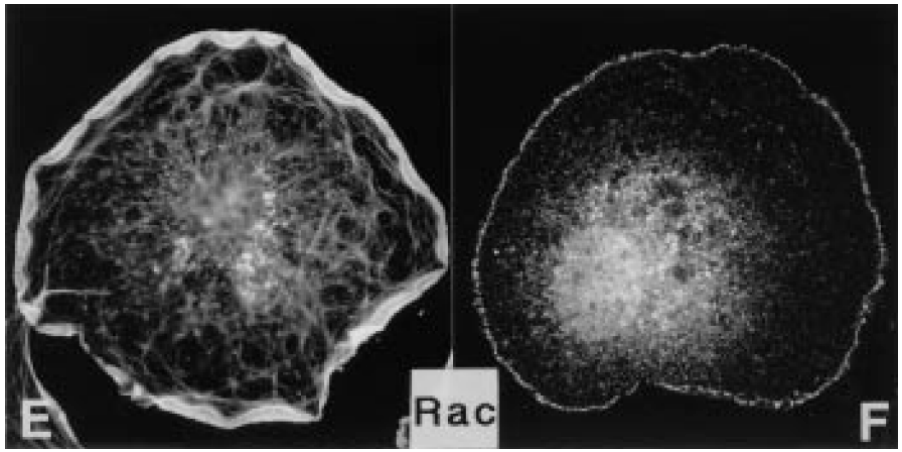
[Blanchoin et al. Physiol. Review 2014]

Regulation of the actin cytoskeleton through small Rho-GTPases

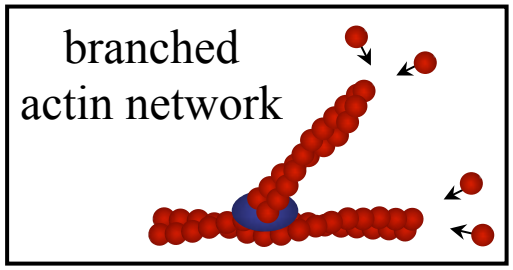
Ridely and Hall and coworkers Cell 1992

Rac: spreading and migration

pushing module



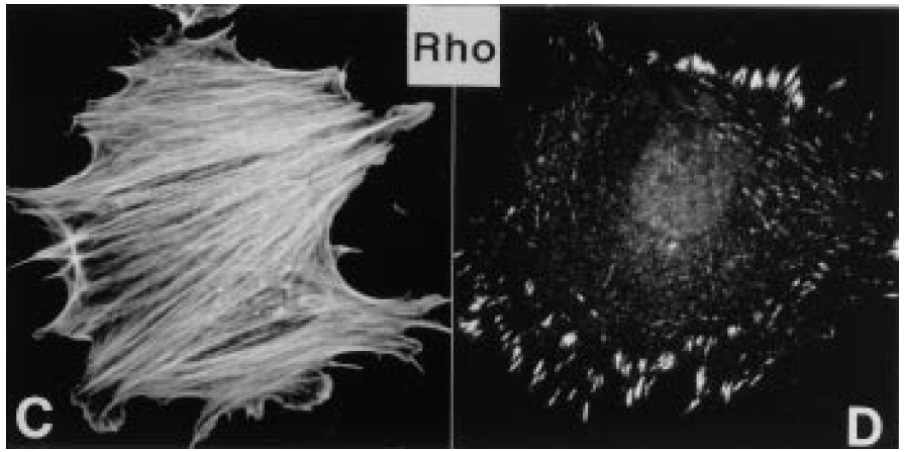
lamellipodia and focal complexes



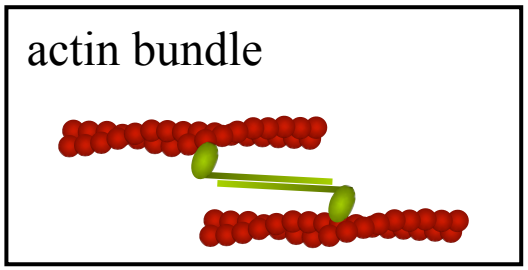
Arp2/3

Rho: mature adhesion

pulling module



stress fibers and focal adhesions

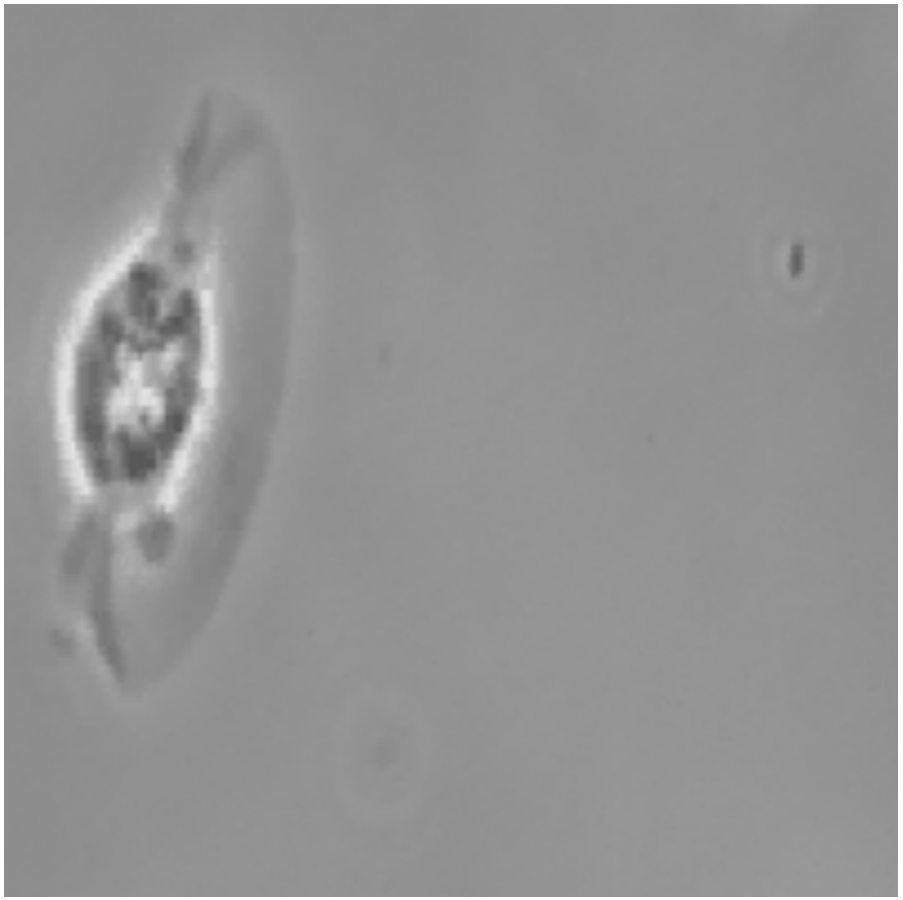


myosin II

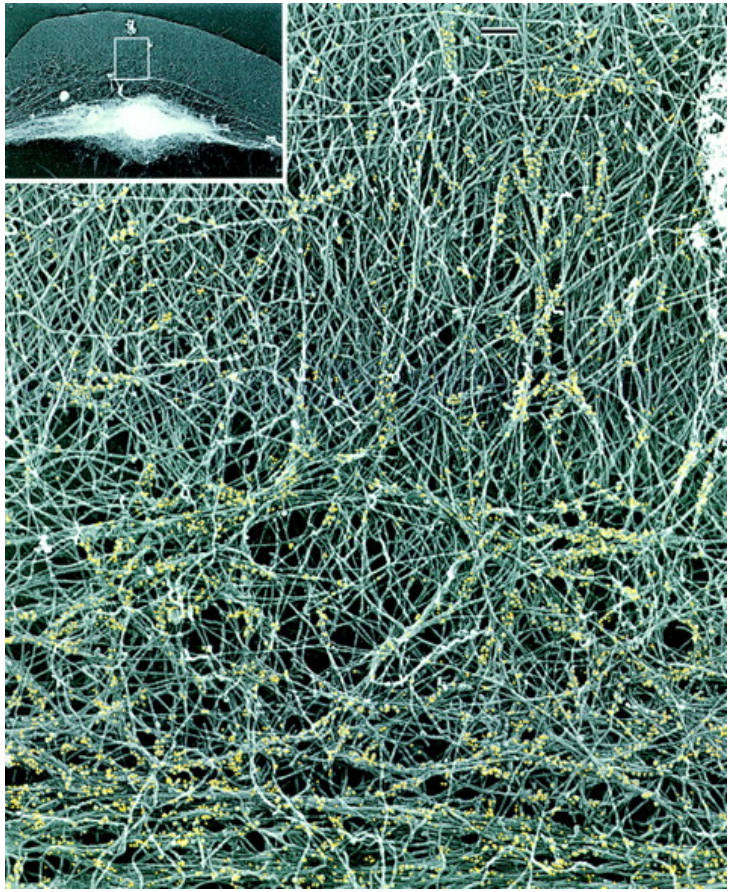
Pushing – actin polymerization

[Weichsel and Schwarz, PNAS 2010;
Weichsel et al., Cytometry A 2012;
Weichsel et al. PRE 2013;
Weichsel and Schwarz NJP 2013]

Cell use growing actin networks to move

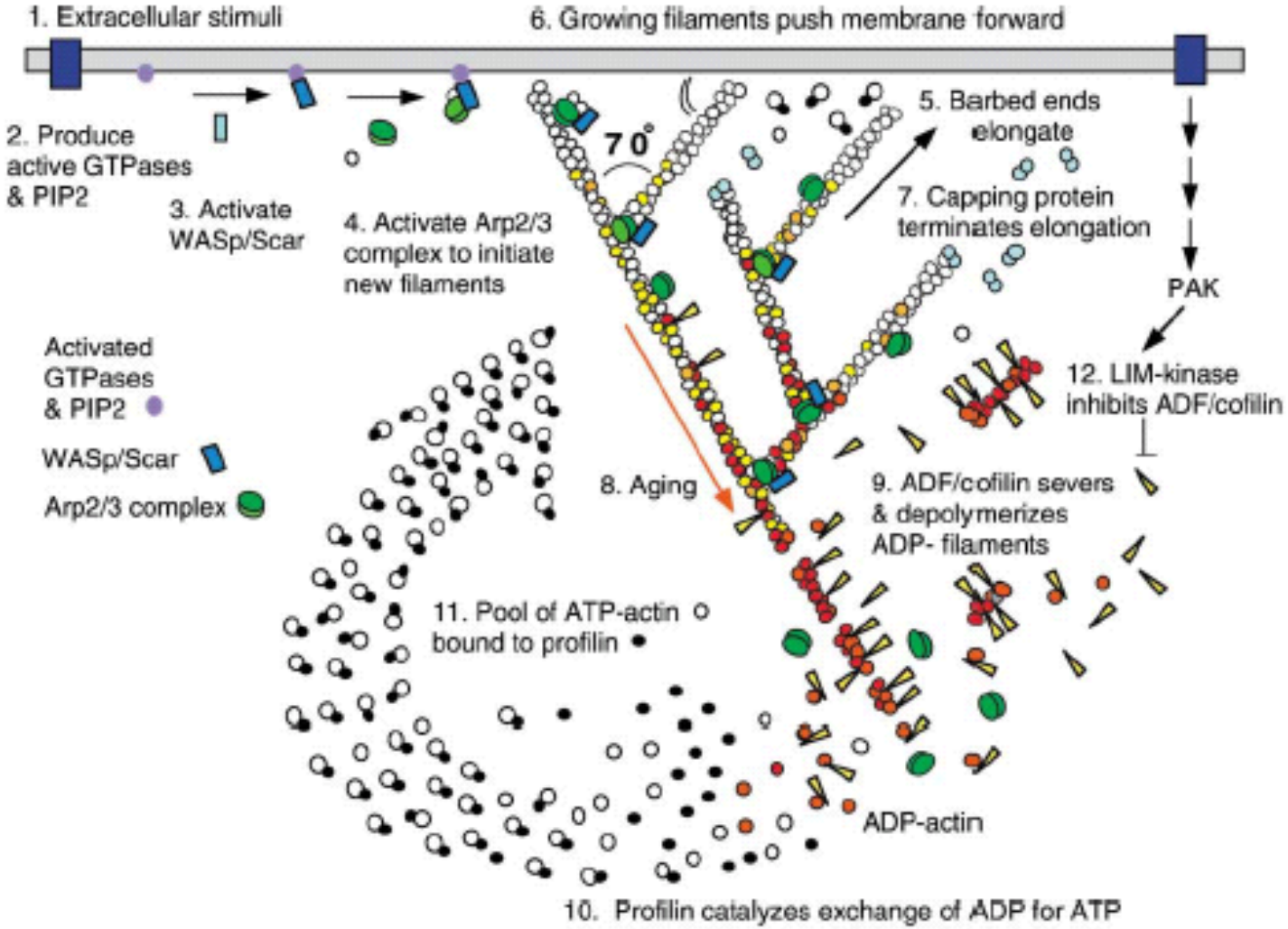


Migrating keratocyte
(Julie Theriot)



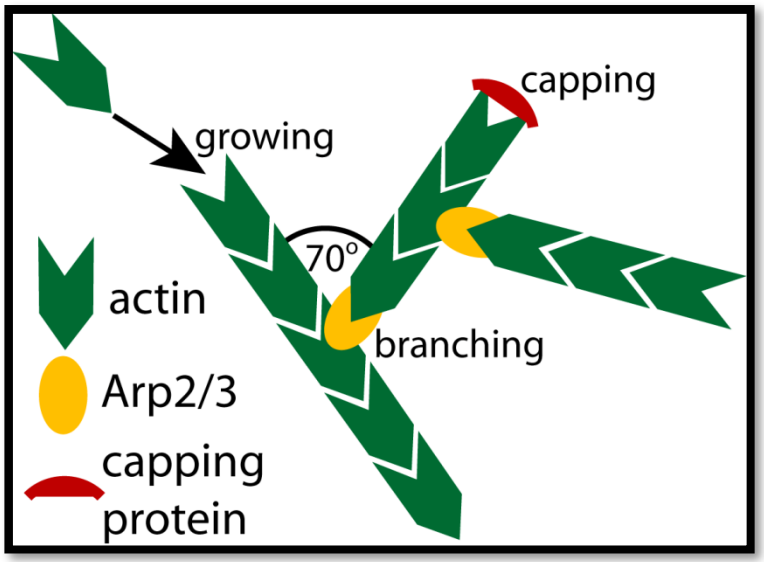
Electron micrograph
of actin network
[Svitkina and Borisy JCB 1999]

Dendritic nucleation model



[Borisy and Pollard Cell 2003]

Dendritic nucleation model revisited



Elementary processes:

1. Growth (gain)
2. Branching (gain)
3. Capping (loss)
4. Outgrowth (loss)

Because there is both gain and loss, the system develops into a steady state.

Our main model assumptions:

1. Capping is a first order reaction in the number of actin filaments (capping protein exists in excess)

2. The order of the branching reaction is variable (extreme cases: autocatalytic and zero order)

3. The N_{front} topmost filaments are stalled; this results in a well-defined network protrusion velocity v_{nw}

[Weichsel and Schwarz, PNAS 2010; Weichsel et al. PRE 2013]

Kinetic model for branching

Number of bound but not branched Arp2/3s:

$$\frac{dA}{dt} = k_+ N_{\mathrm{fil}} - \left(k_- + \frac{v_{\mathrm{nw}}}{d_{\mathrm{br}}}\right) A - \tilde{k}_{\mathrm{b}} A\, P\ ,$$

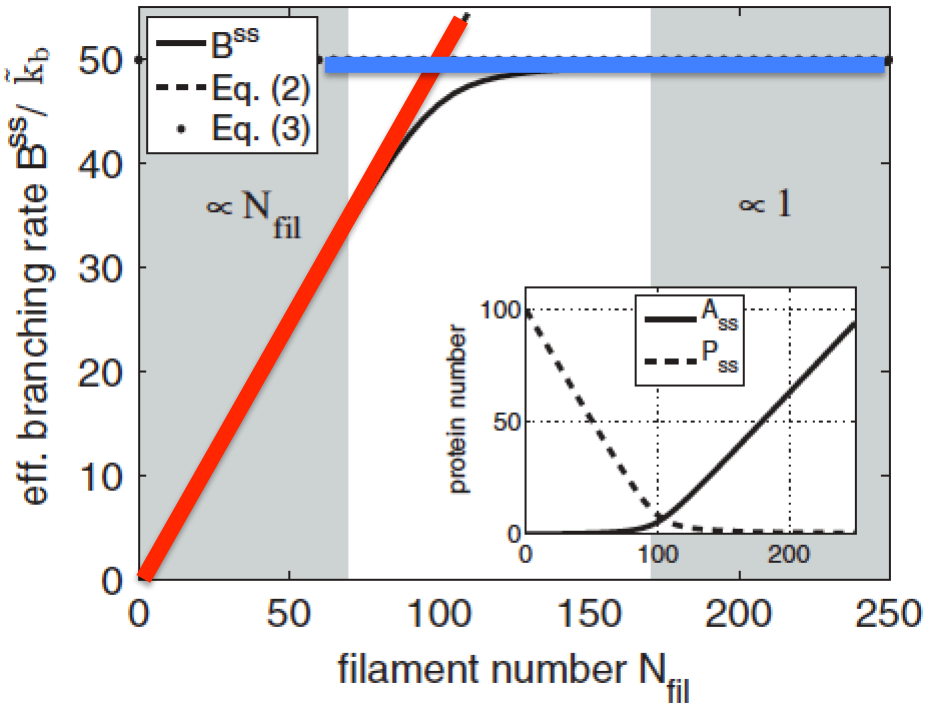
Number of nucleation promoting factors:

$$\frac{dP}{dt} = -\tilde{k}_{\mathrm{b}} A\, P + k_{\mathrm{act}}(P_0 - P).$$

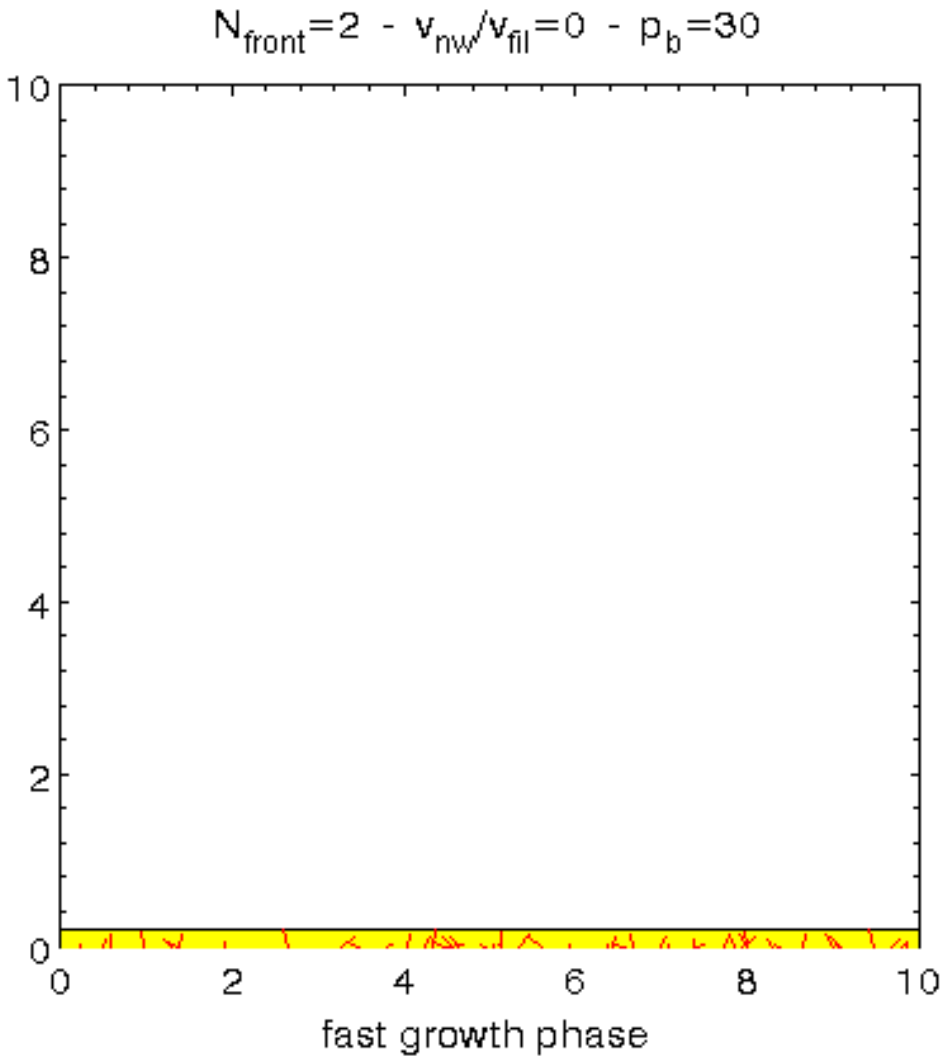
Two different regimes of the branching reaction:

Autocatalytic regime: 1st order branching

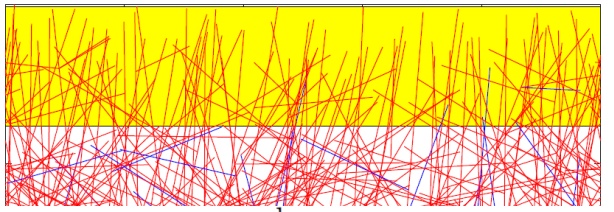
Zero order regime: 0th order branching



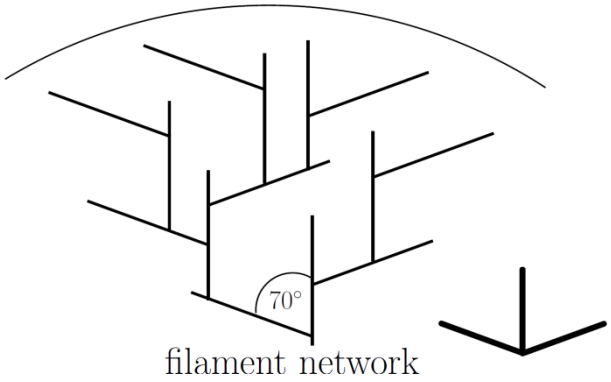
Stochastic network simulations (zero order regime)



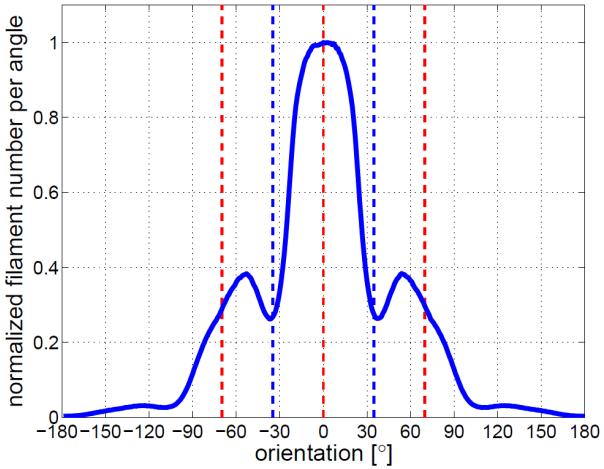
Stochastic network simulations

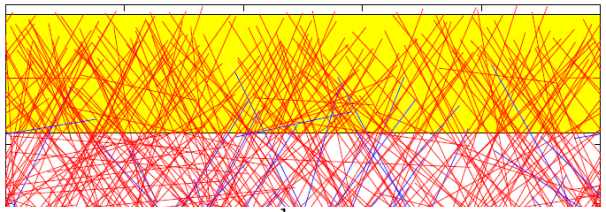


membrane

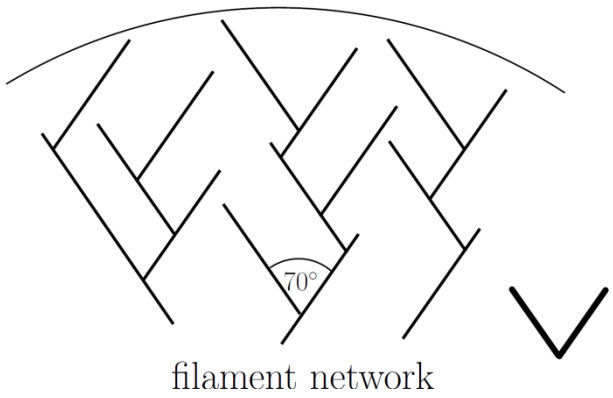


$v_{nw}/v_{fil} \cong 0.95$

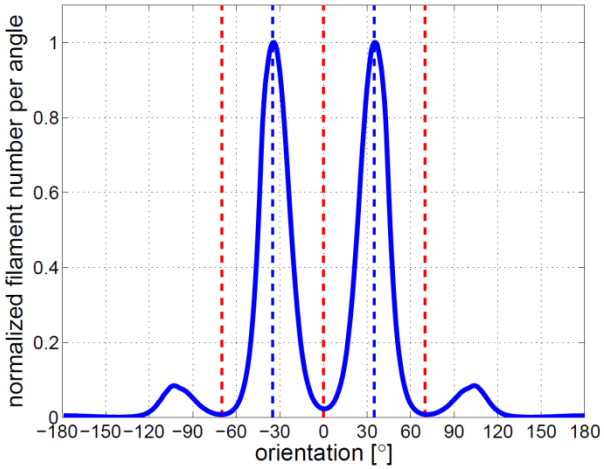


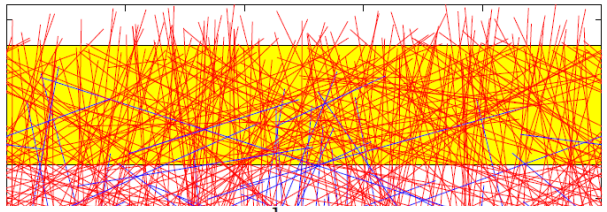


membrane

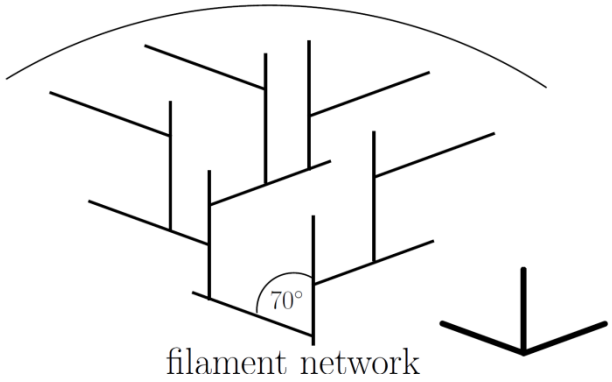


$v_{nw}/v_{fil} \cong 0.76$

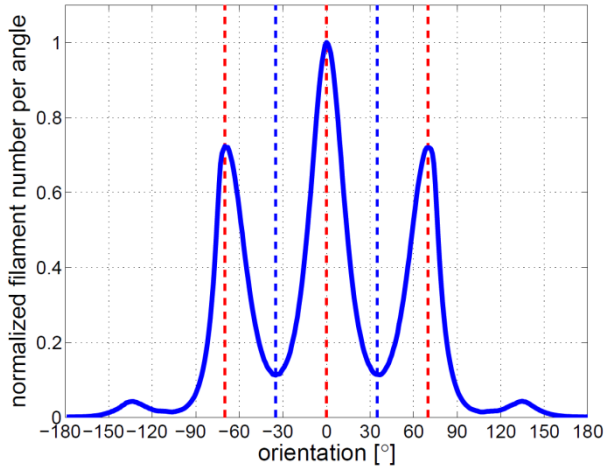




membrane



$v_{nw}/v_{fil} \cong 0.30$



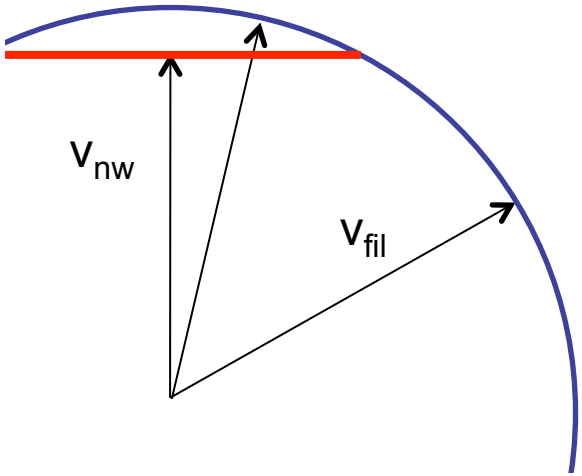
Competition between growth in different directions – outgrowth rate

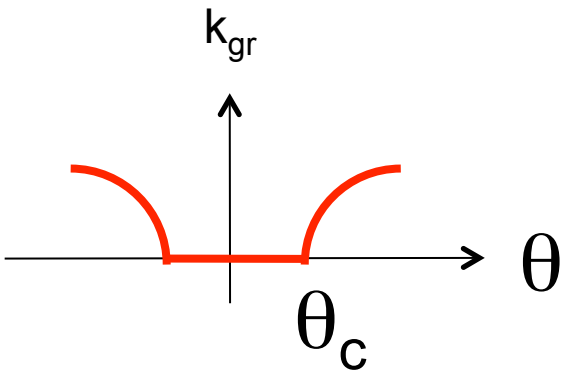
Critical angle at which the single filament cannot follow the network anymore:

$$\theta_c = \arccos(v_{nw} / v_{fil})$$

Rate of outgrowth:

$$k_{gr}(\theta) = \begin{cases} 0 & if \quad |\theta| \le \theta_c \\ \dfrac{v_{nw} - v_{fil}\cos\theta}{\delta_{fil}} & if \quad |\theta| > \theta_c \end{cases}$$





Rate equation approach

Full model

$$\frac{\partial N(\theta,t)}{\partial t} =$$

$$\frac{k_b}{\hat{N}_{tot}} \int_{-\pi}^{+\pi} w(\theta,\hat{\theta}) N(\hat{\theta},t) d\hat{\theta} \quad \text{branching}$$

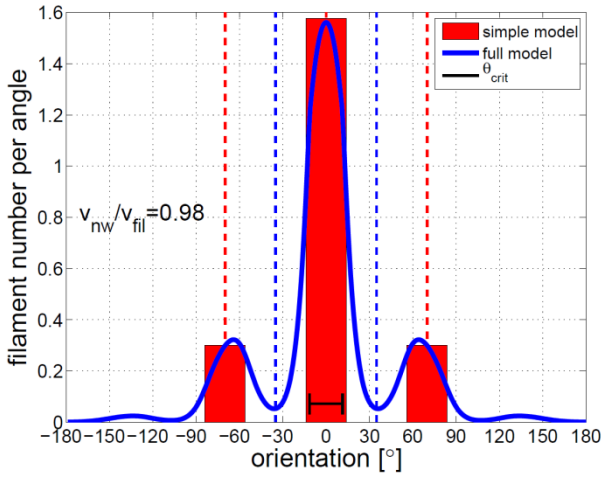
$$-k_c N(\theta,t) \quad \text{capping}$$

$$-k_{gr}(\theta, v_{nw}) N(\theta,t) \quad \text{outgrowth}$$

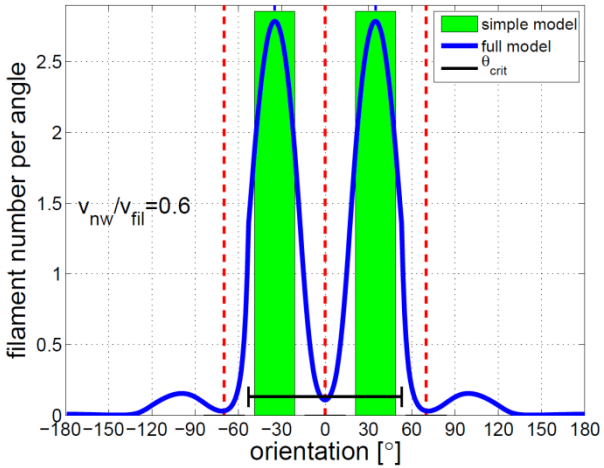
Simple model

$$\begin{aligned}
\frac{\partial N_{-70^\circ}}{\partial t} &= \hat{k}_b N_{0^\circ} - \left(k_c + k_{gr}(70^\circ, v_{nw})\right) N_{-70^\circ} \\
\frac{\partial N_{-35^\circ}}{\partial t} &= \hat{k}_b N_{+35^\circ} - \left(k_c + k_{gr}(35^\circ, v_{nw})\right) N_{-35^\circ} \\
\frac{\partial N_{0^\circ}}{\partial t} &= \hat{k}_b \left(N_{-70^\circ} + N_{+70^\circ}\right) - k_c N_{0^\circ} \\
\frac{\partial N_{+35^\circ}}{\partial t} &= \hat{k}_b N_{-35^\circ} - \left(k_c + k_{gr}(35^\circ, v_{nw})\right) N_{+35^\circ} \\
\frac{\partial N_{+70^\circ}}{\partial t} &= \hat{k}_b N_{0^\circ} - \left(k_c + k_{gr}(70^\circ, v_{nw})\right) N_{+70^\circ} ,
\end{aligned}$$

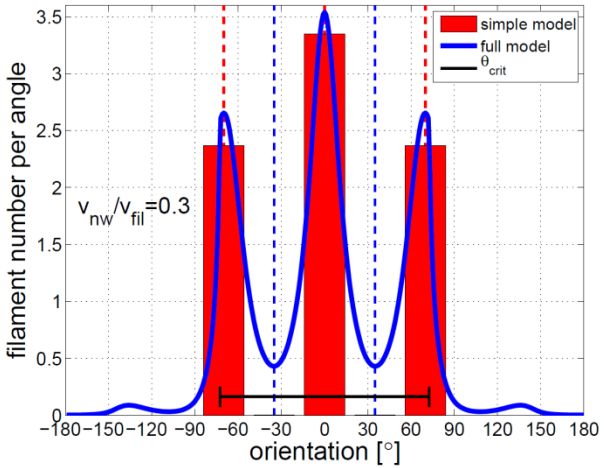
$+70/0/-70$



± 35

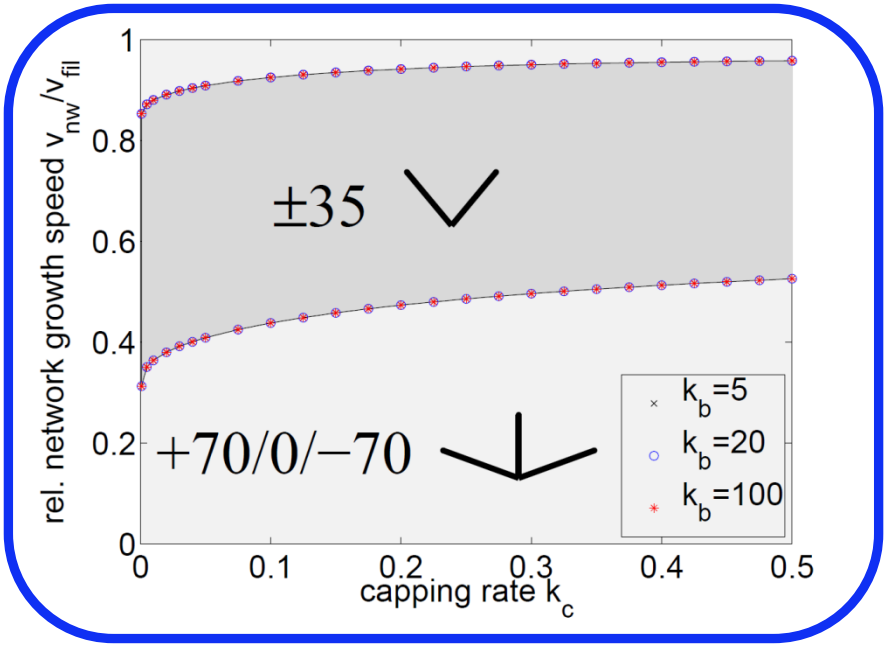


$+70/0/-70$

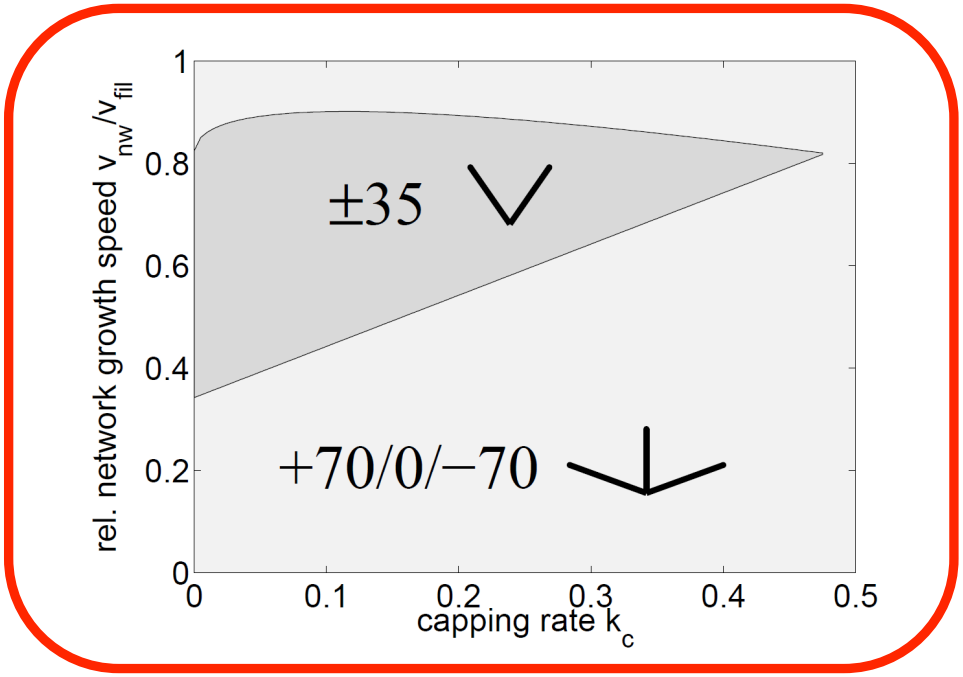


Phase diagrams

Full model

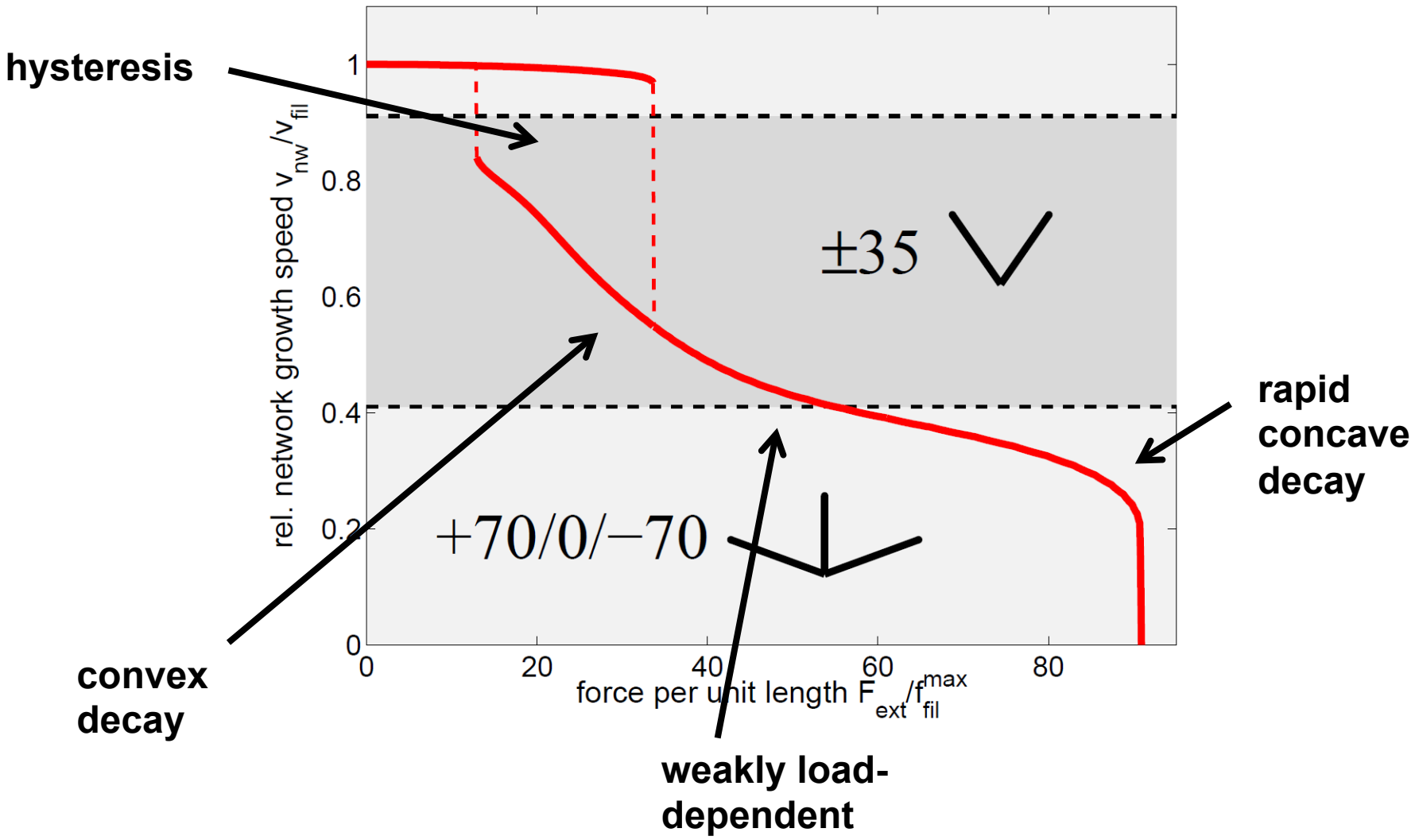


Simple model



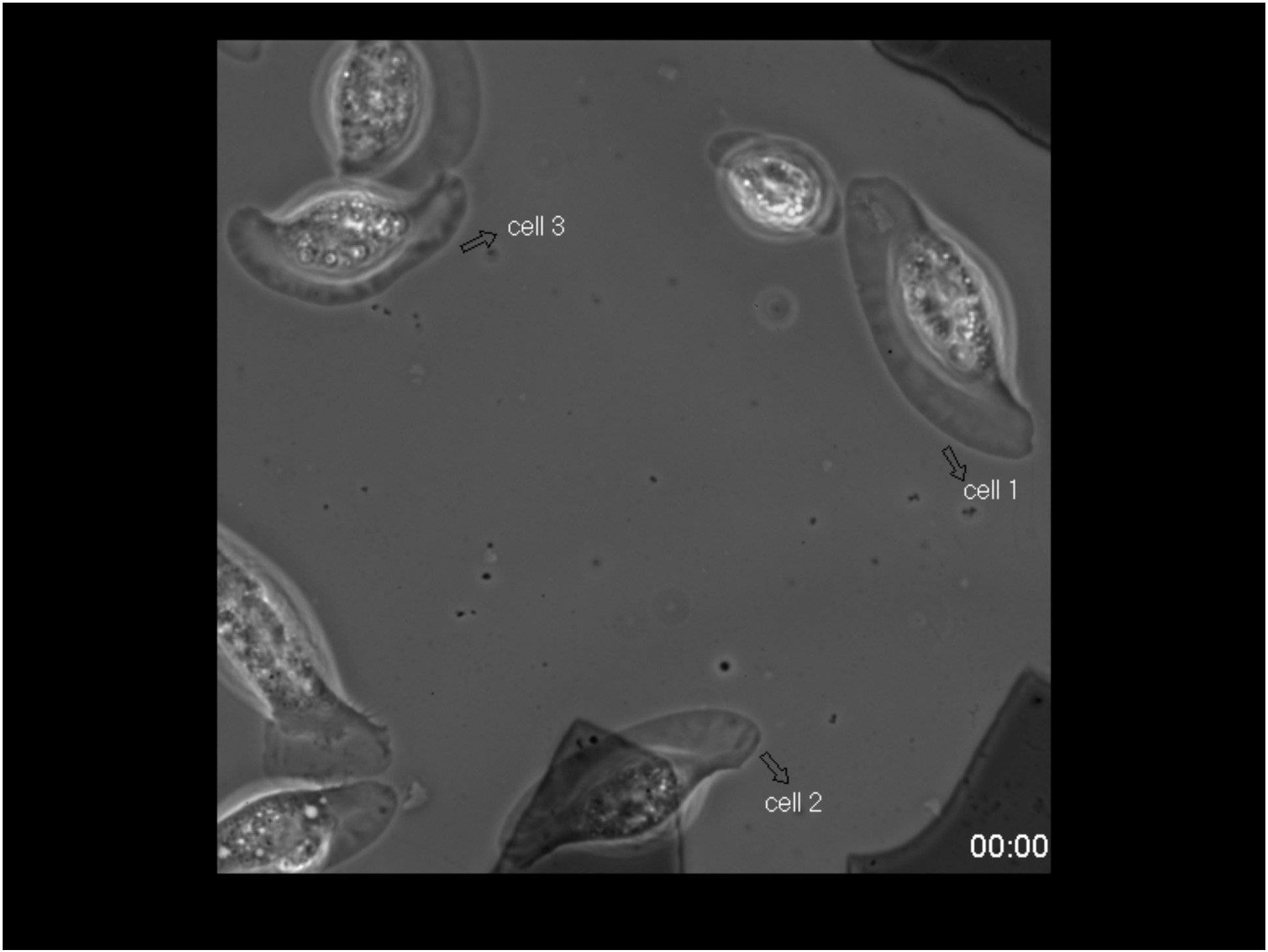
Network growth velocity is the main parameter determining network architecture (PNAS 2010); phase diagram does not depend on the order $0 \leq \mu \leq 1$ of the branching reaction (PRE 2013)

Force-velocity relation from full model



Comparision with exp. data

- Electron microscopy is still the only method to resolve actin filaments (although super resolution light microscopy is getting better)
- Correlative microscopy: freeze or fix cells at an interesting stage
- Individual actin filaments can be reconstructed with image processing


cell 3
cell 1
cell 2
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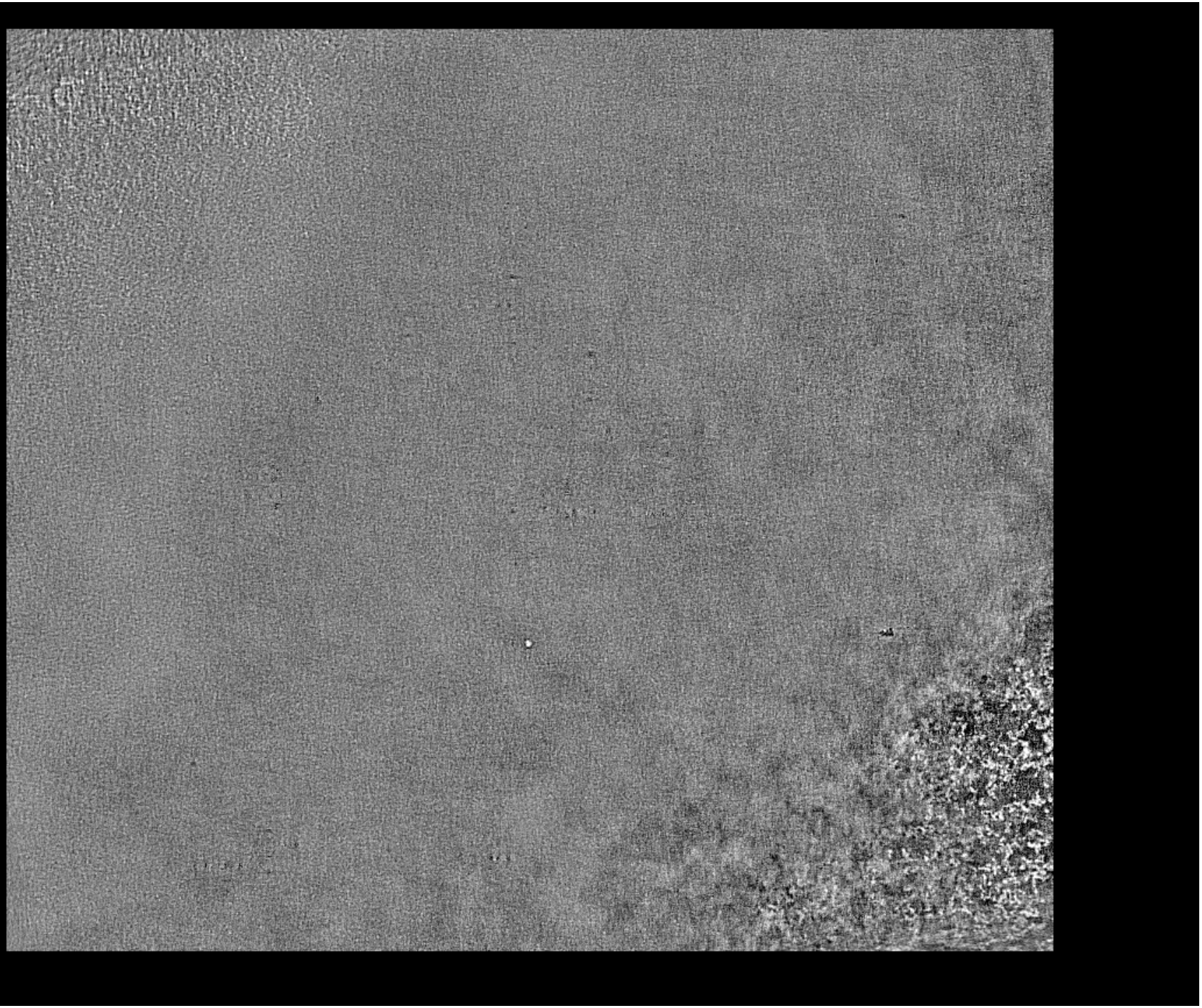
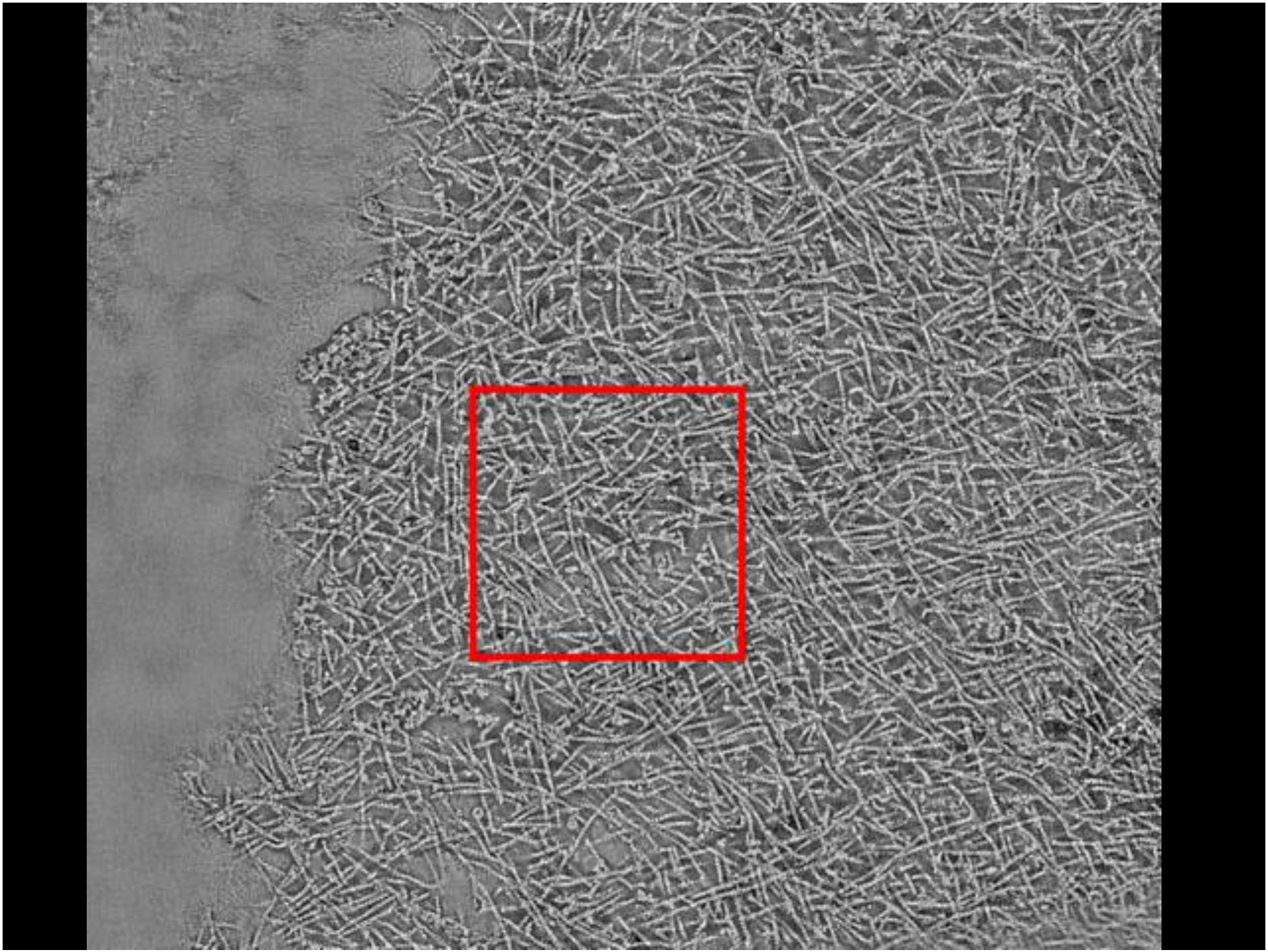
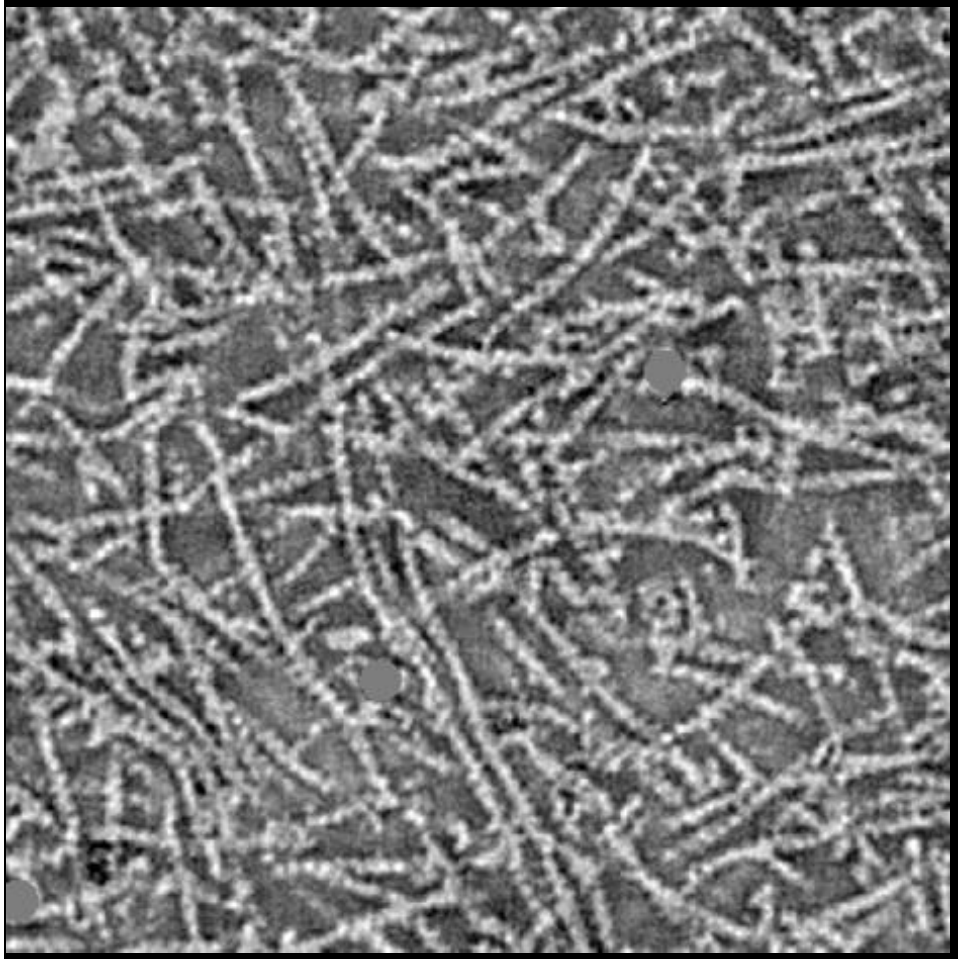
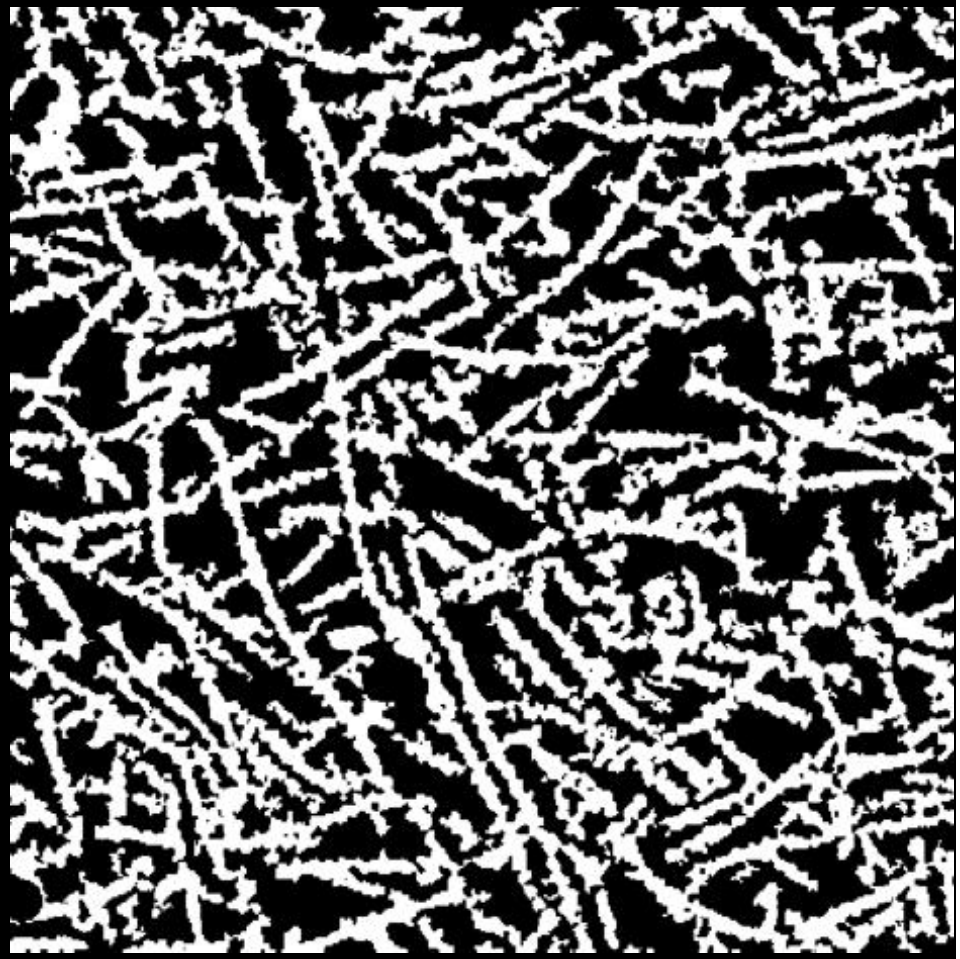
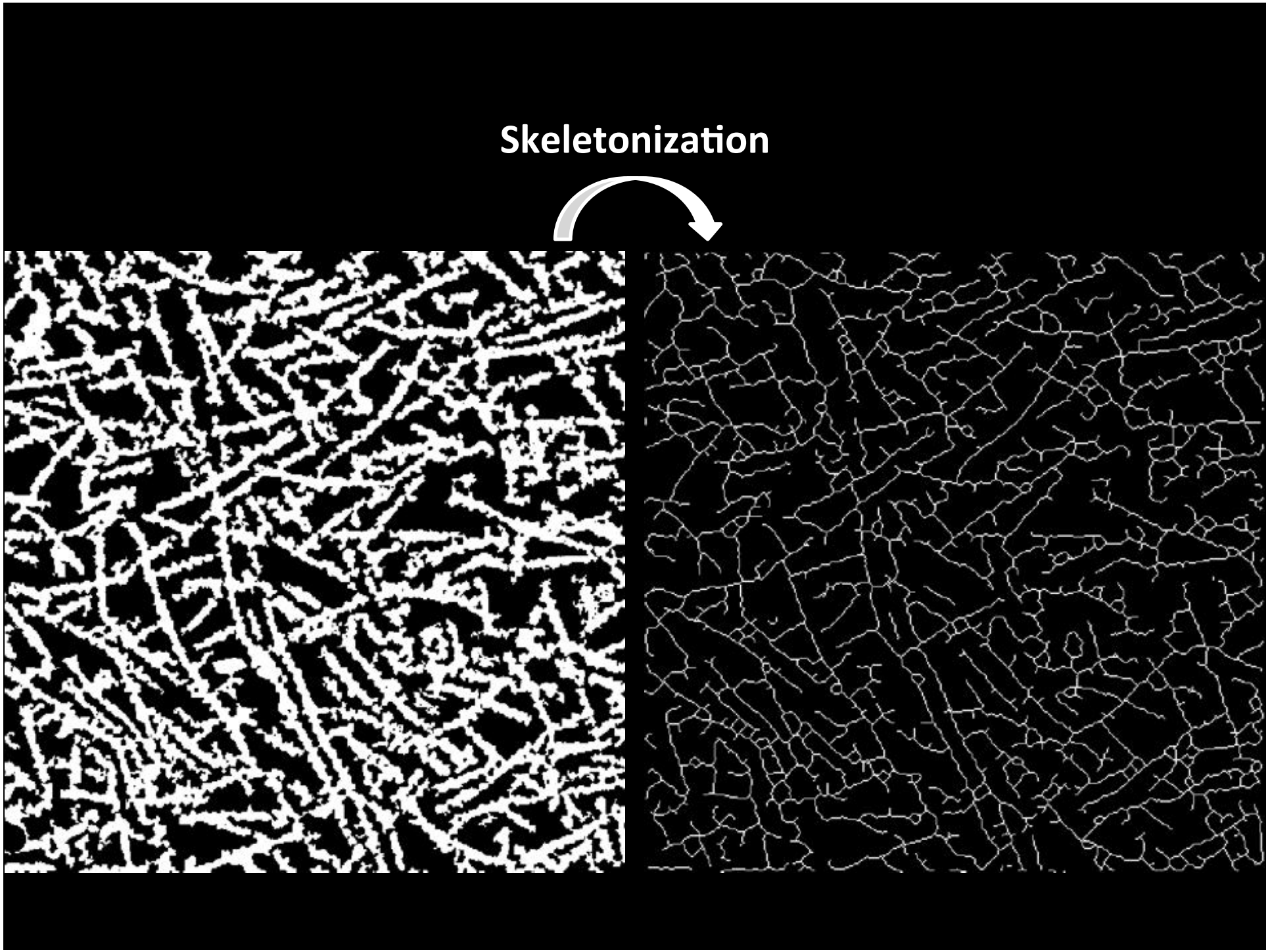


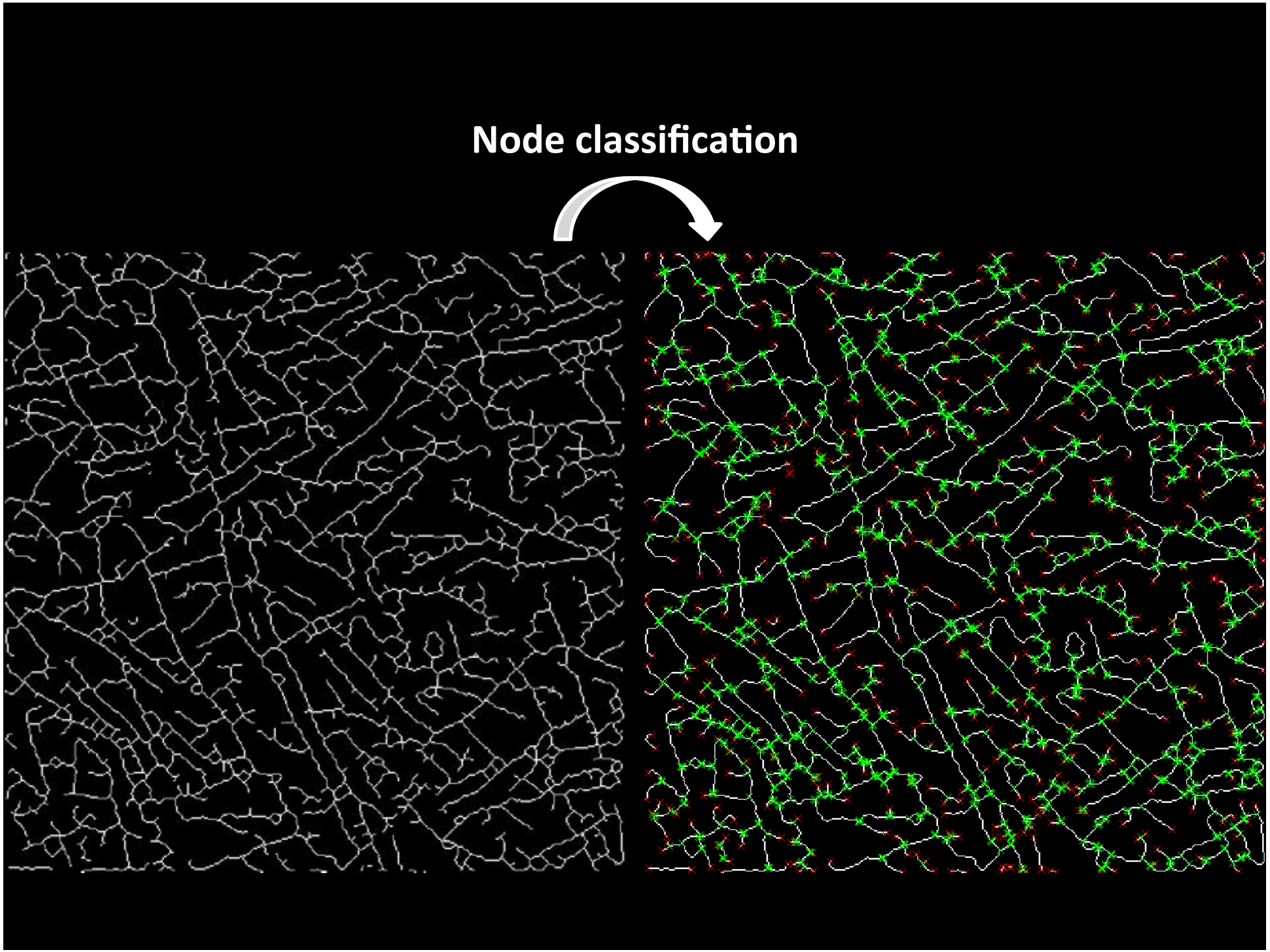


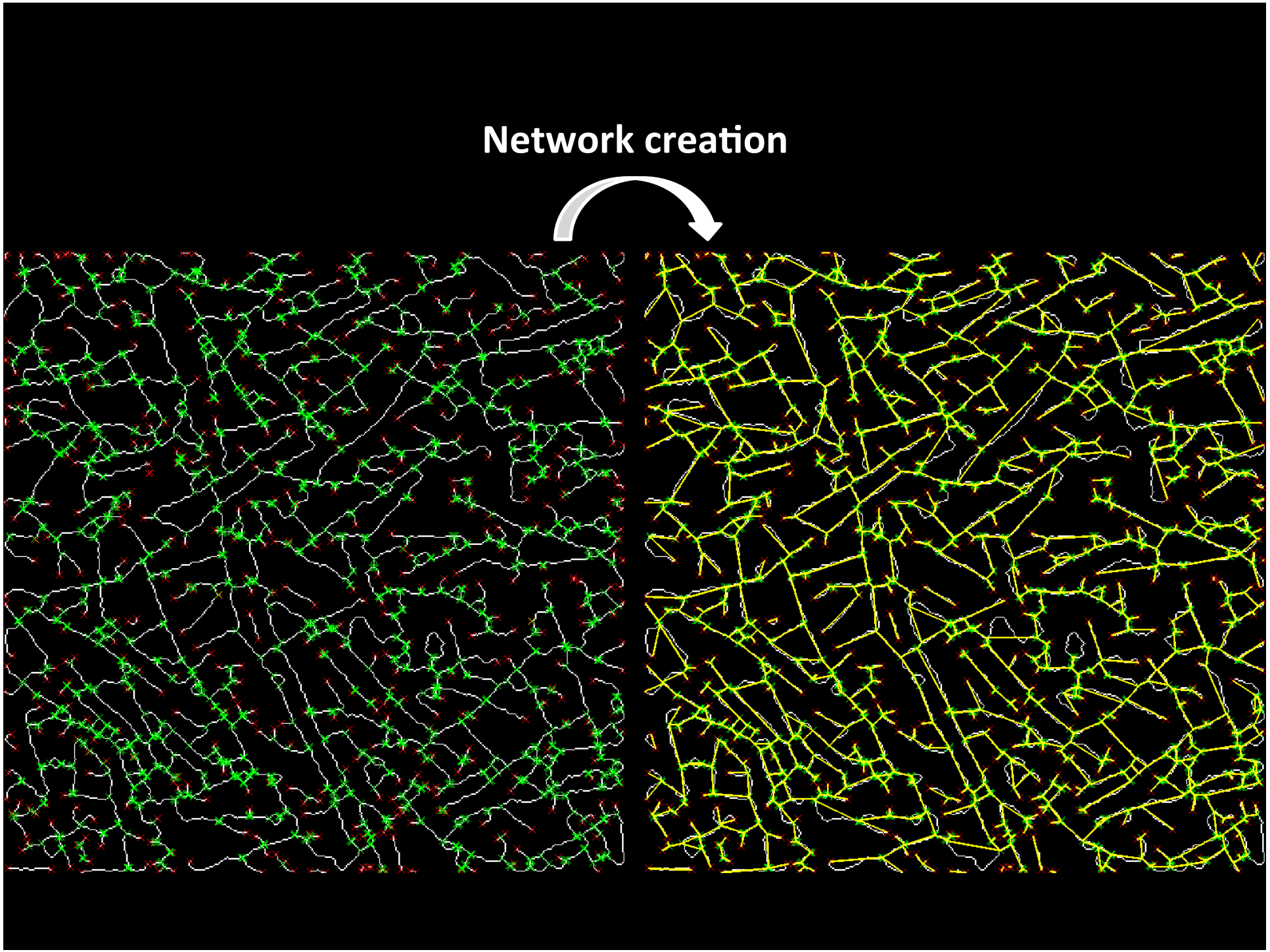
Binarization



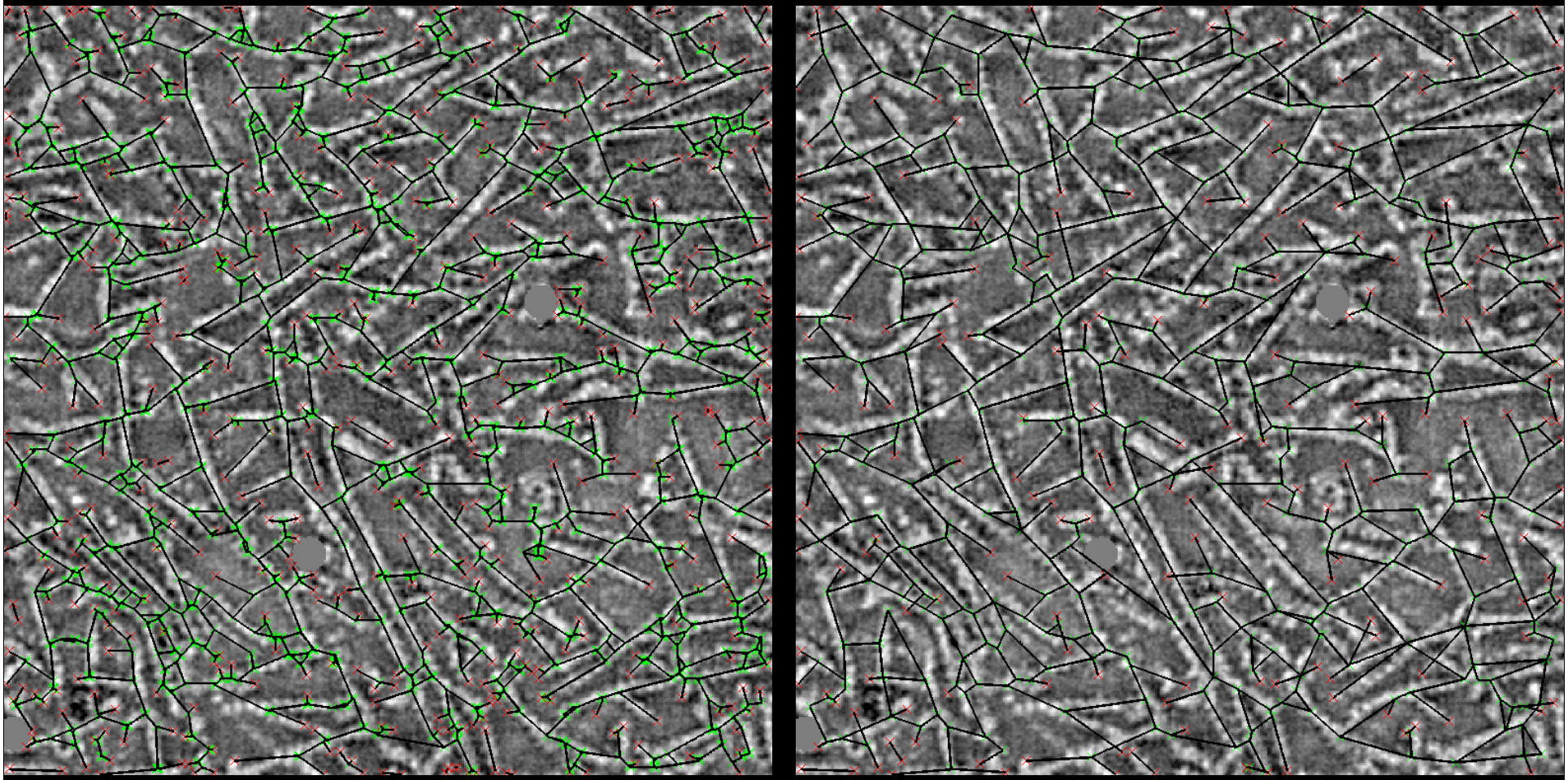


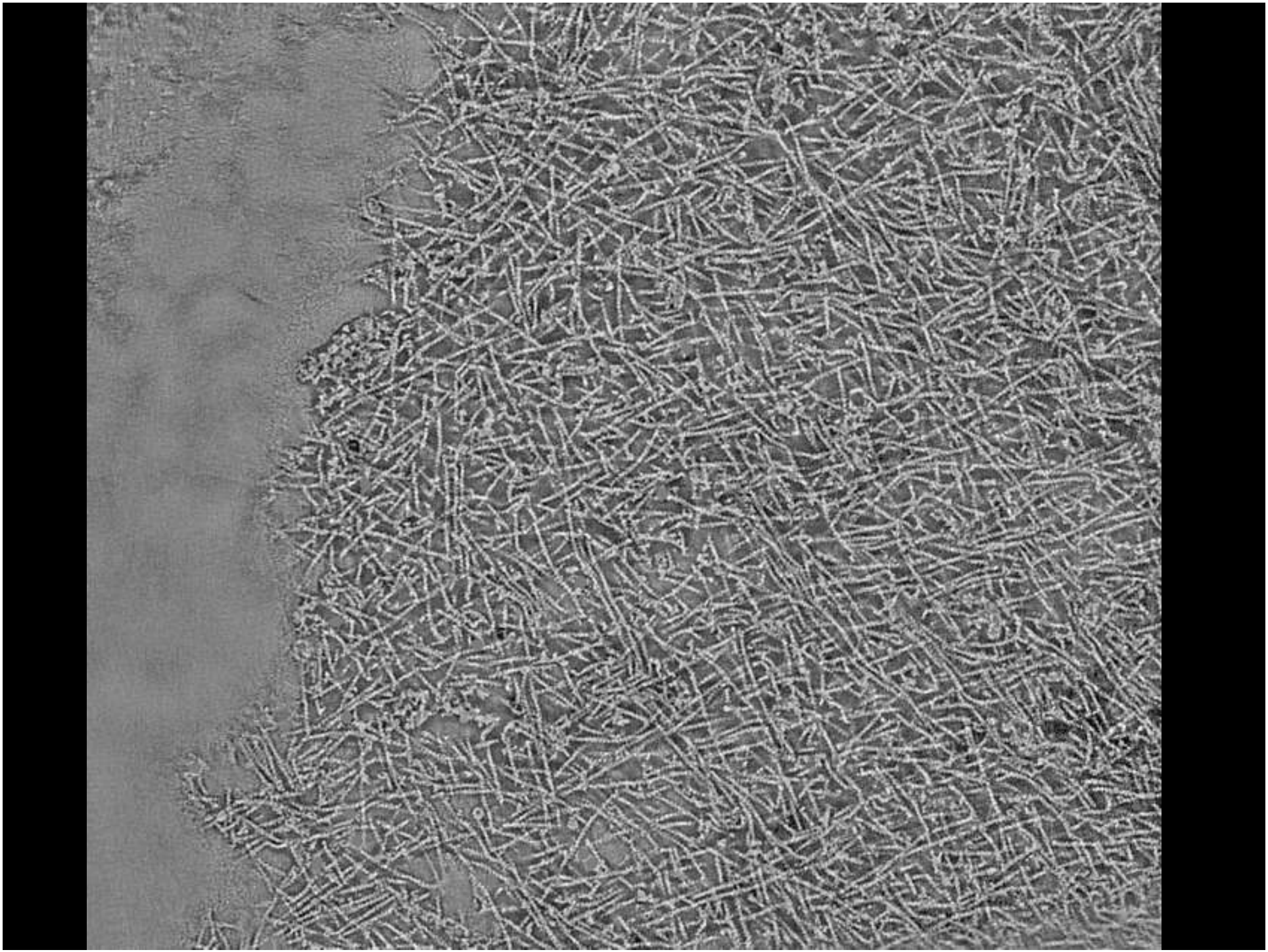

Skeletonization

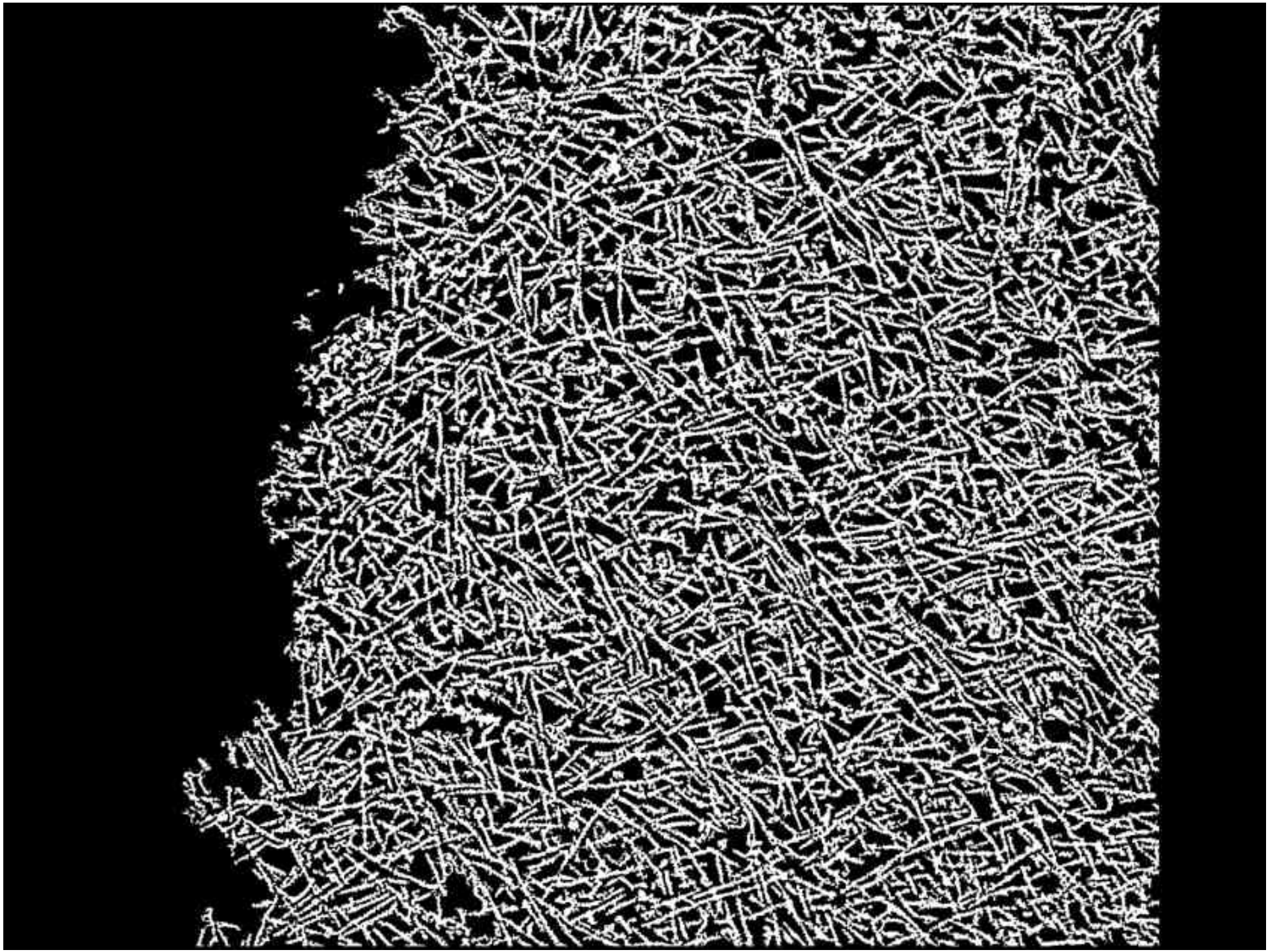

Node classification

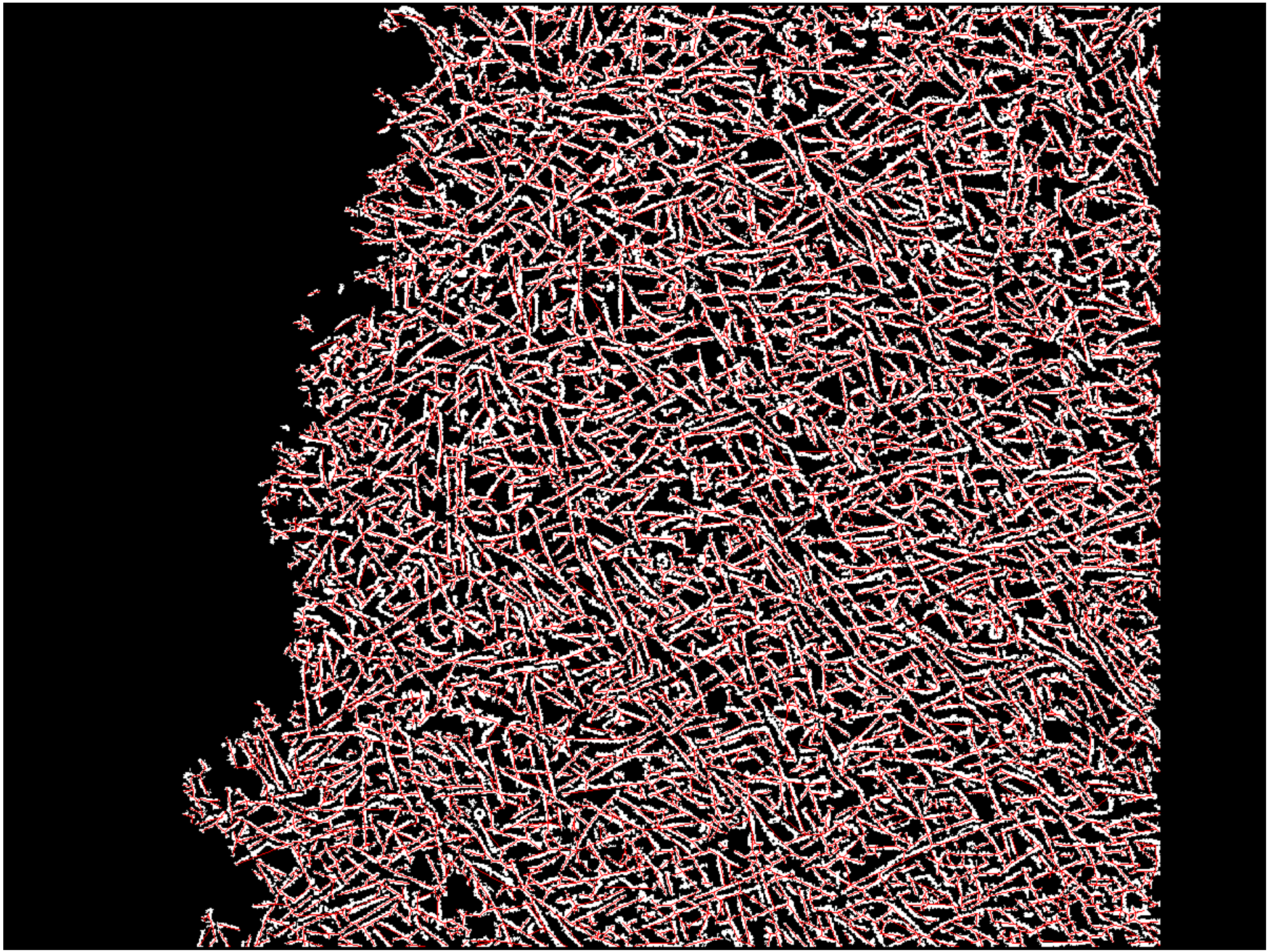

Network creation

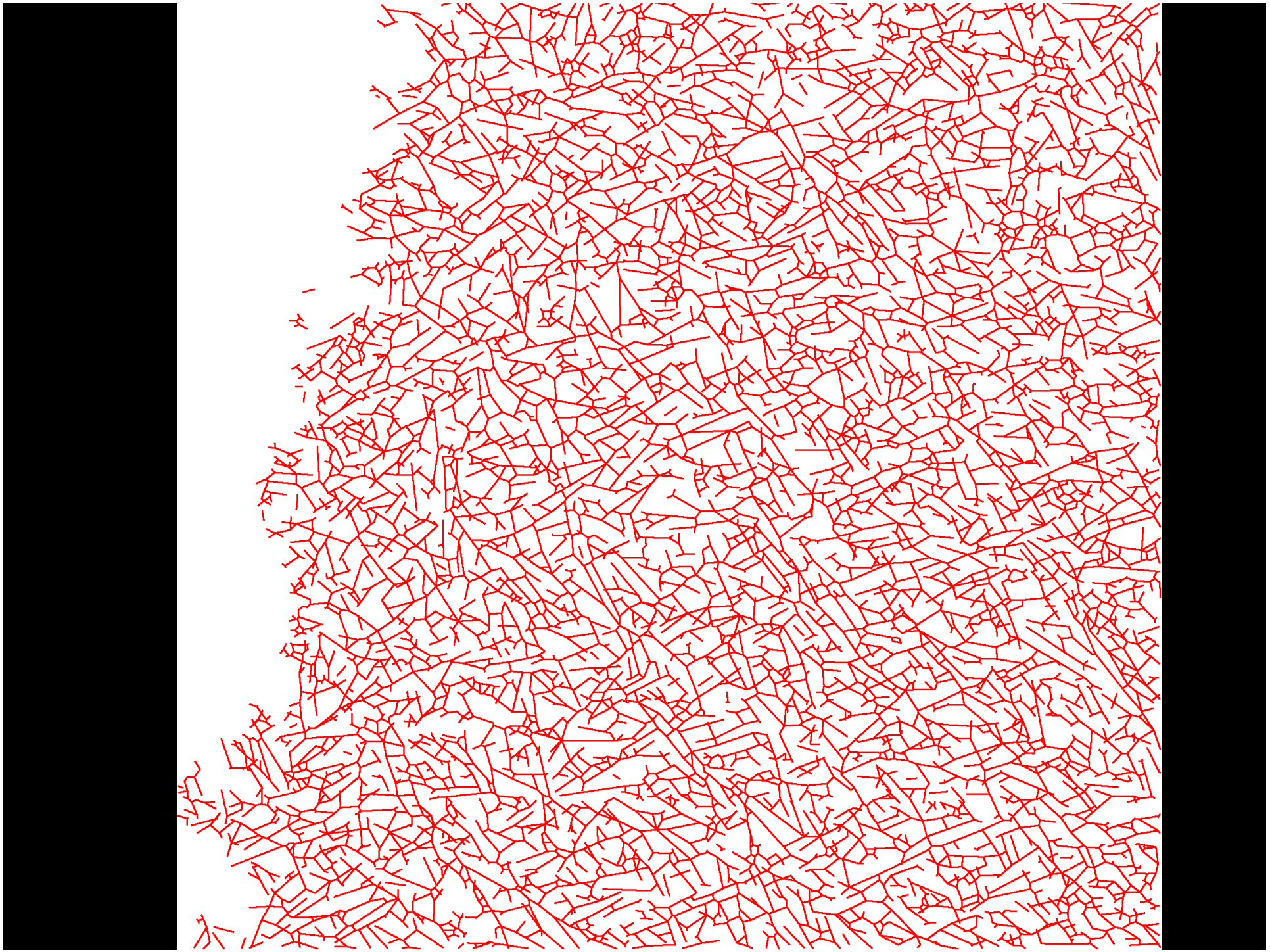
Network simplification

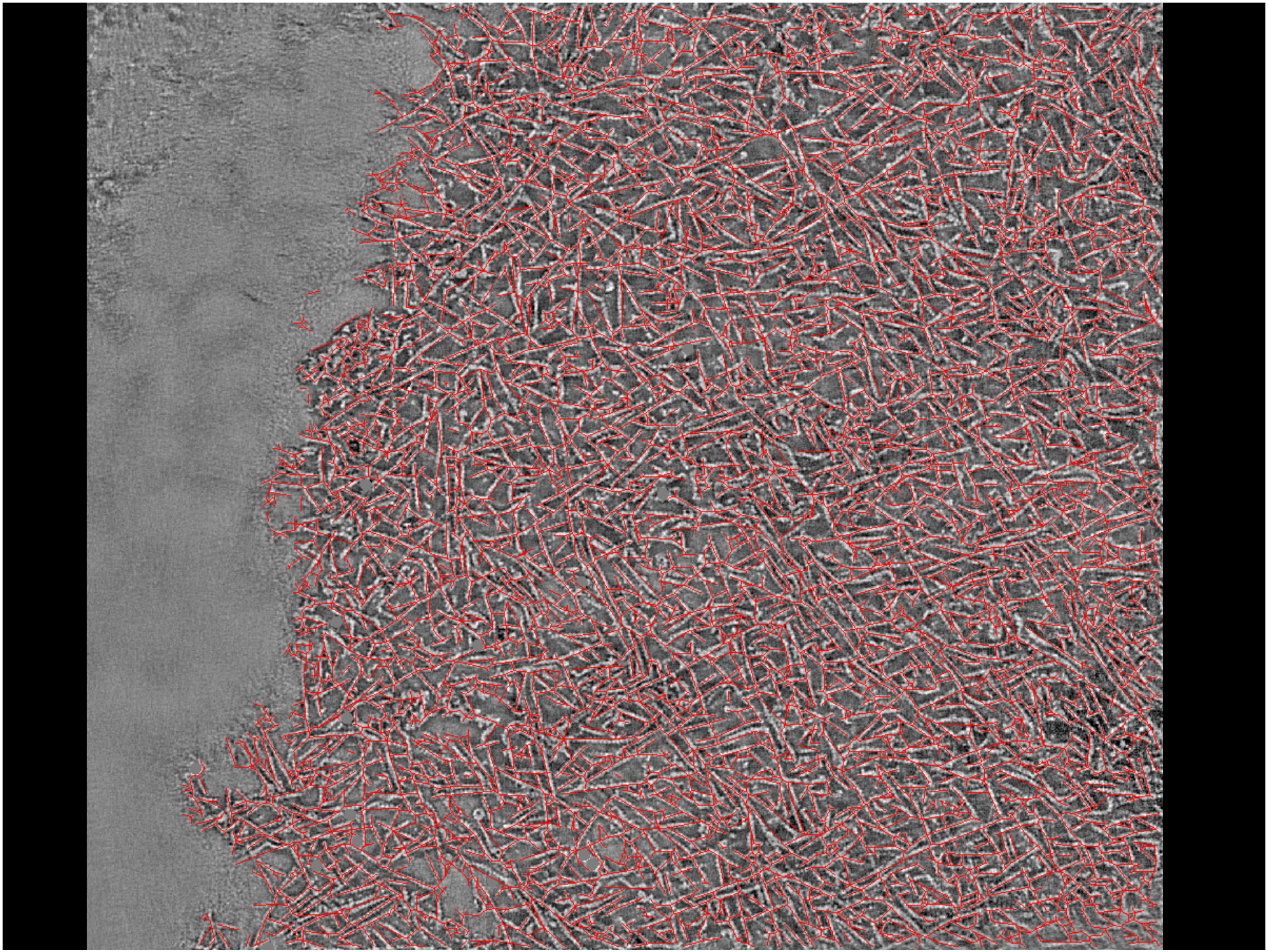


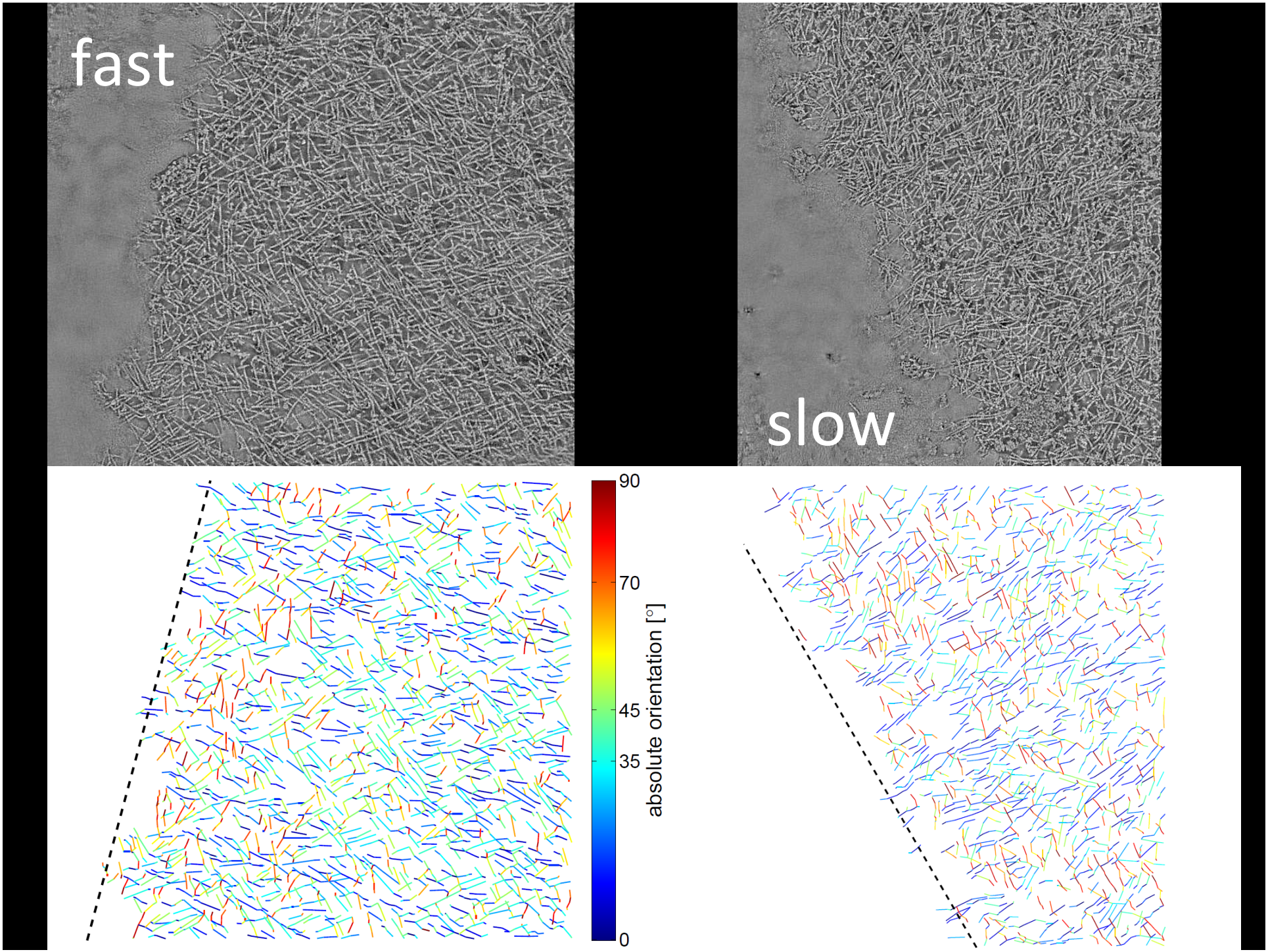


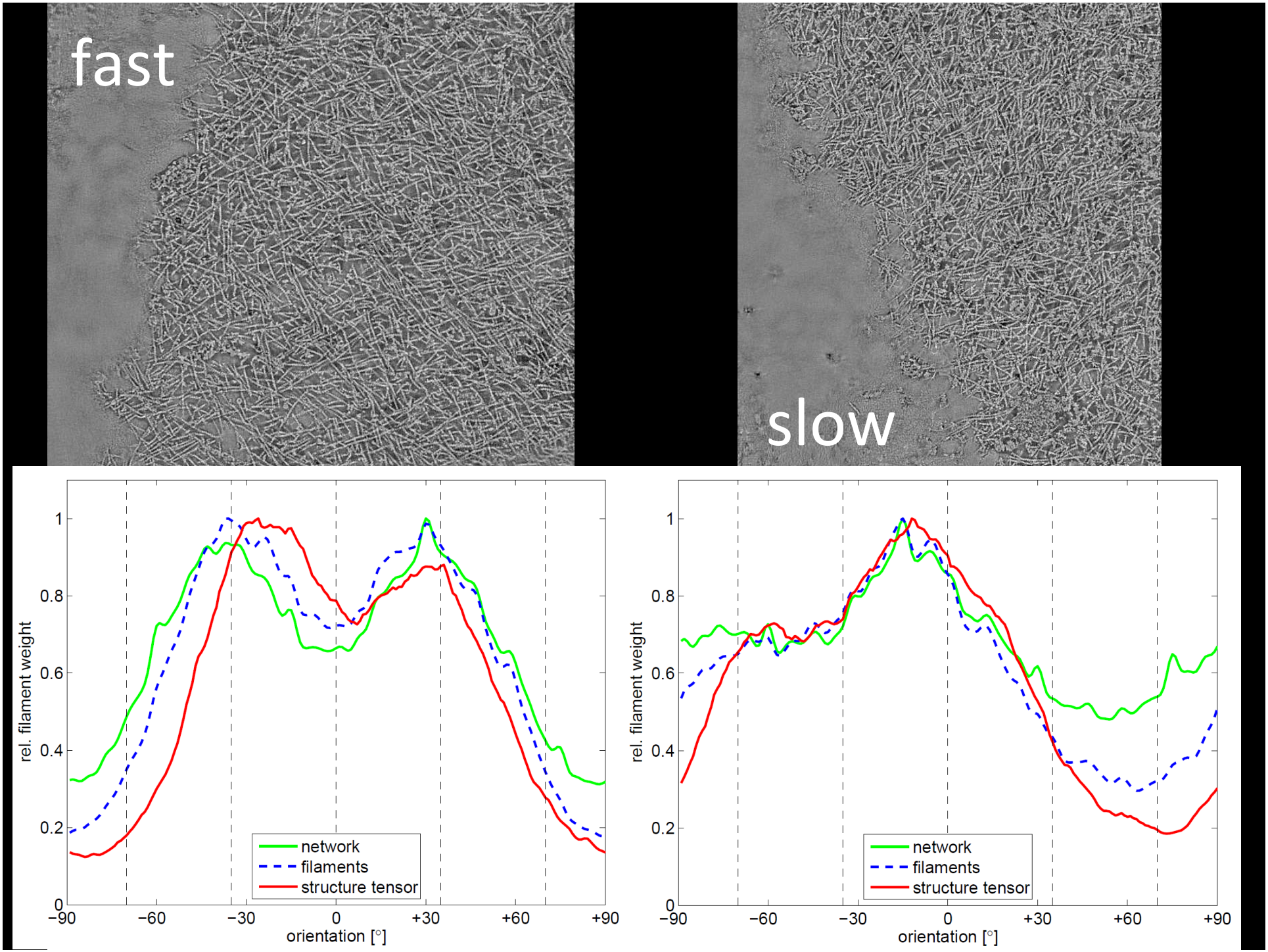




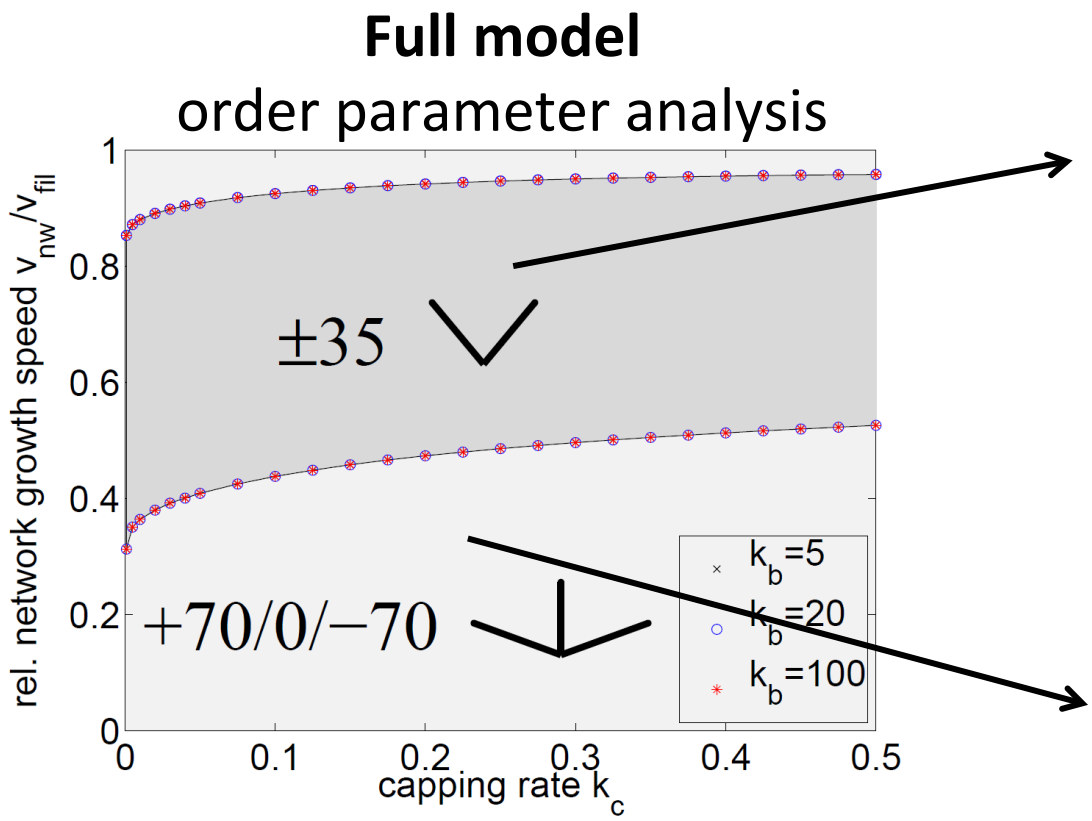


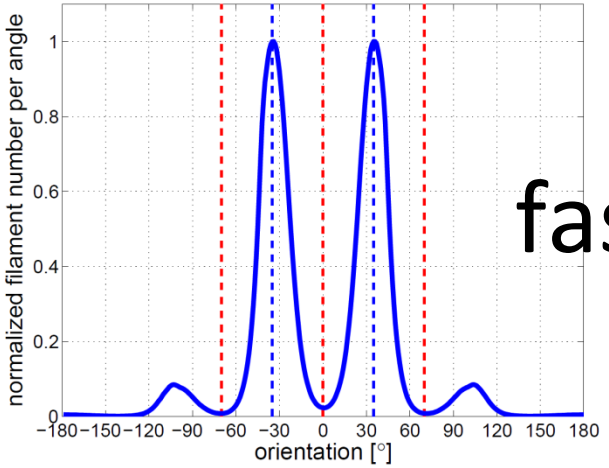



fast
slow
90
70
45
35
0
absolute orientation [°]

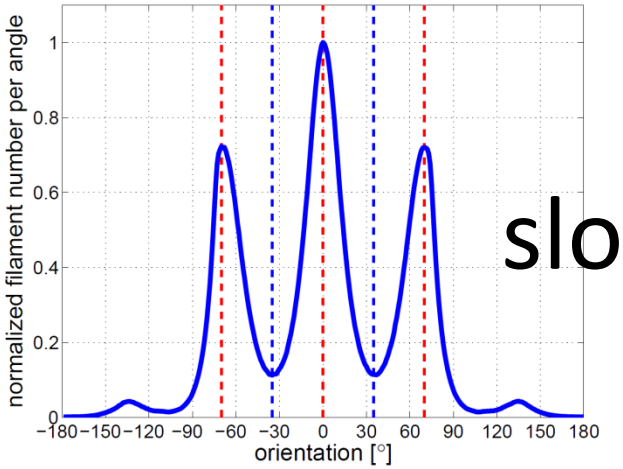

fast
slow
rel. filament weight
orientation [°]
network
filaments
structure tensor
−90
−60
−30
0
+30
+60
+90
0
0.2
0.4
0.6
0.8
1

Interpretation within the model





fast

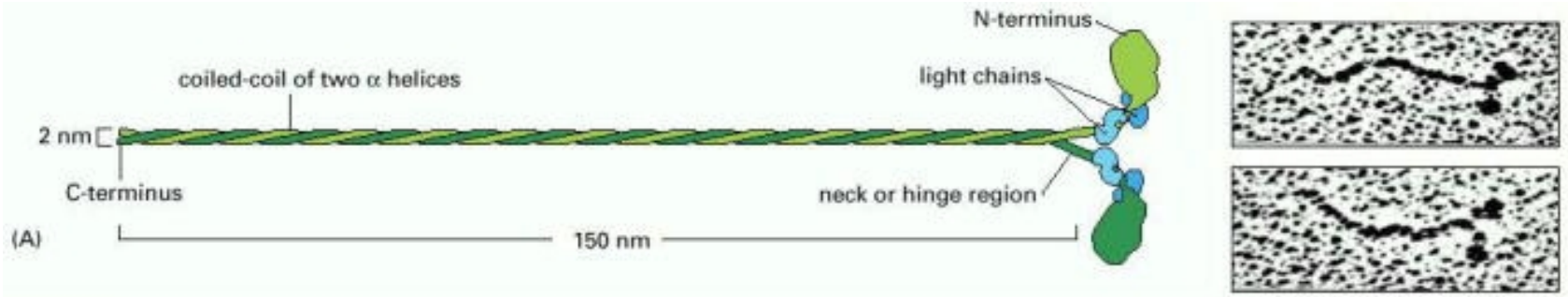


slow

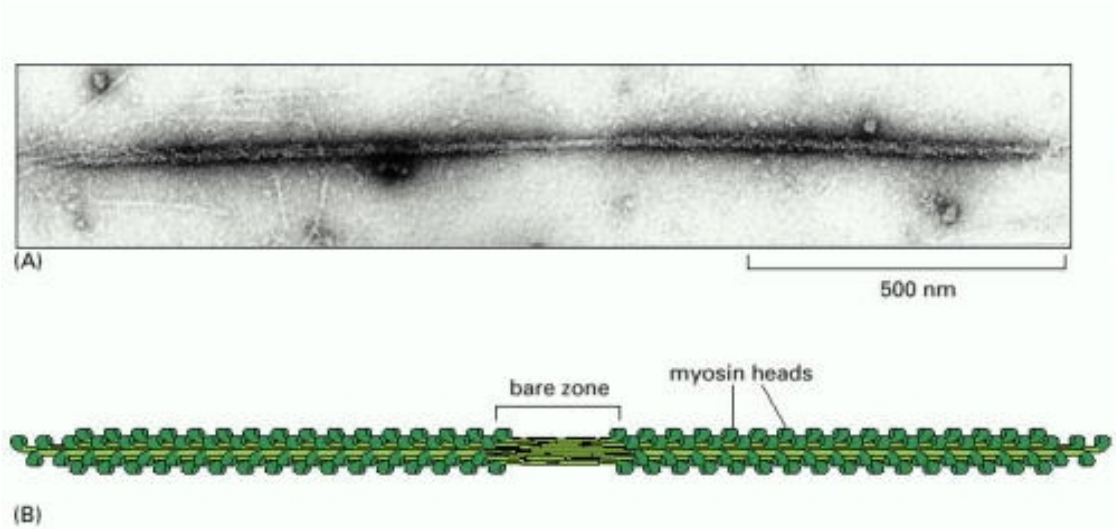
Pulling – motor contractility

[Erdmann and Schwarz PRL 2012;
Erdmann et al. JCP 2013]

Myosin II



Myosin II is a dimer with a long tail and two heads.

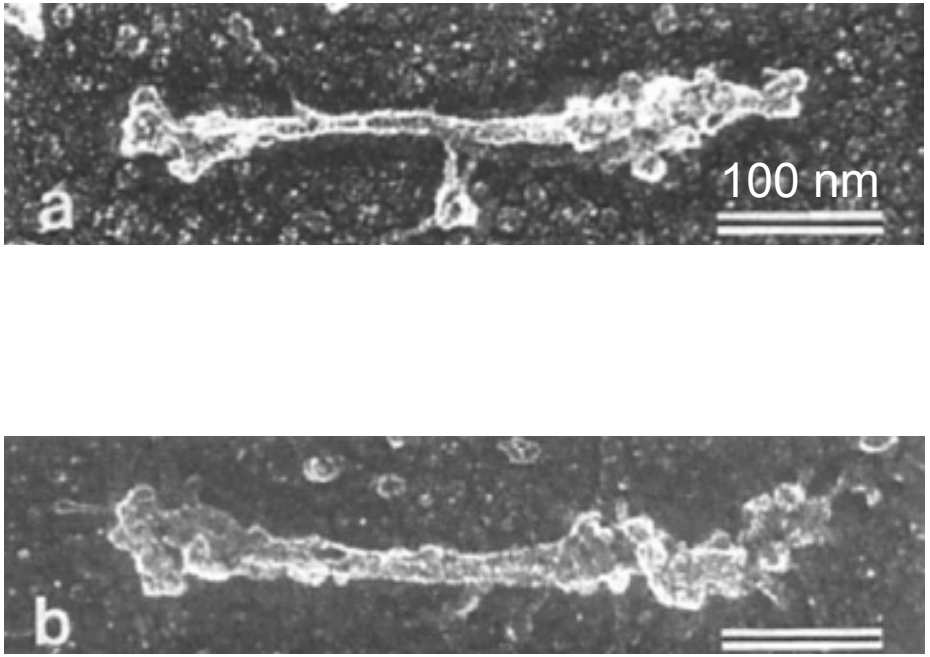


In skeletal muscle, it assembles into thick filaments.

[Alberts]

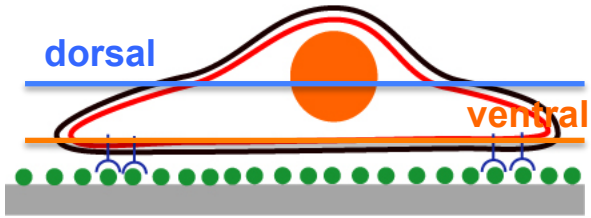
Myosin II minifilaments in the actin cytoskeleton of non-muscle cells

Myosin mini-filaments works in groups of 10-30 motor heads in non-muscle cells

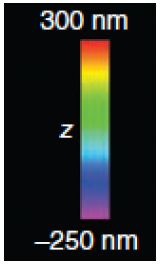


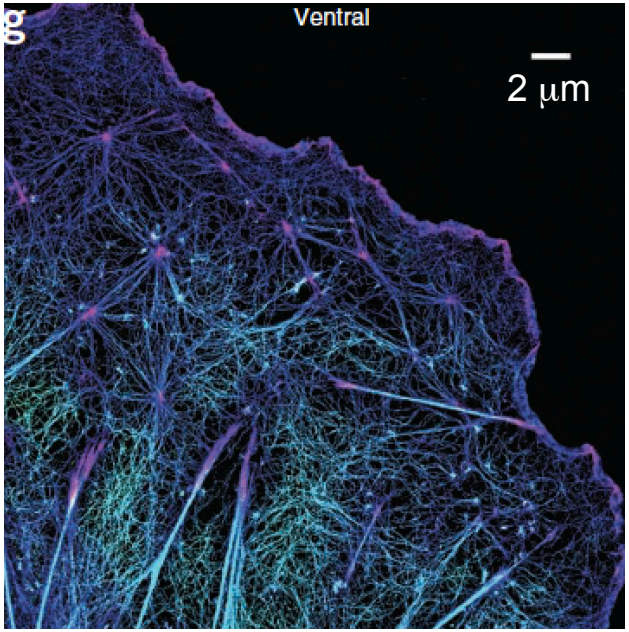
[Verkhovsky & Borisy JCB 1993]

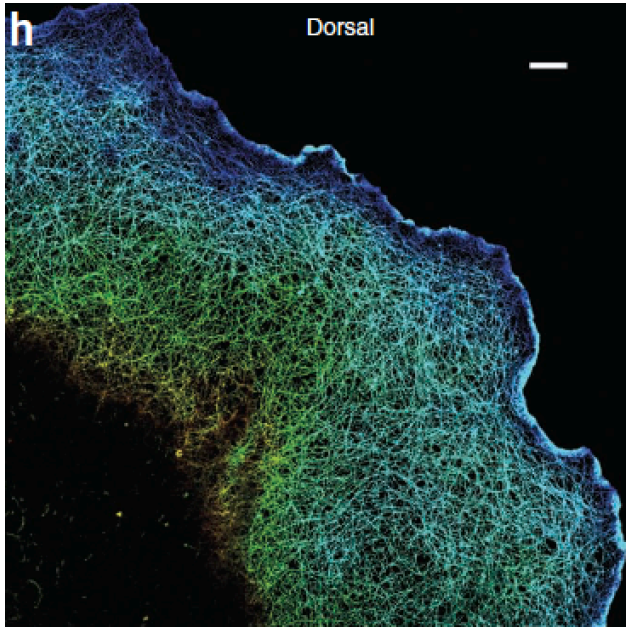
Network contraction by myosin II



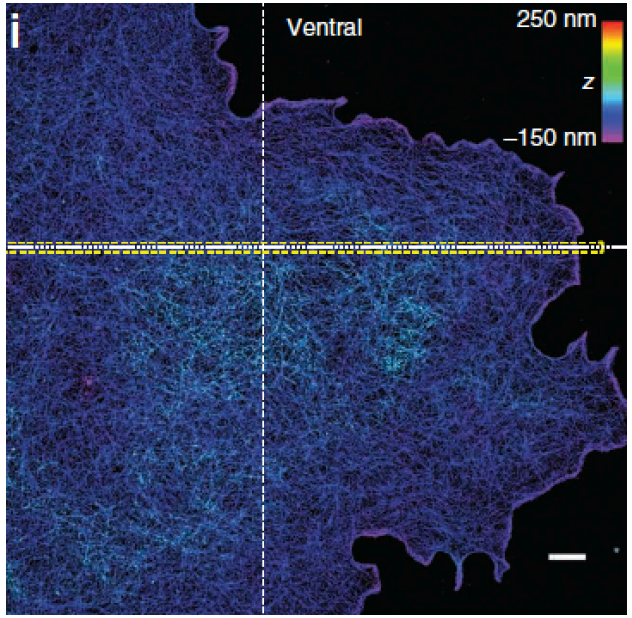
wildtype

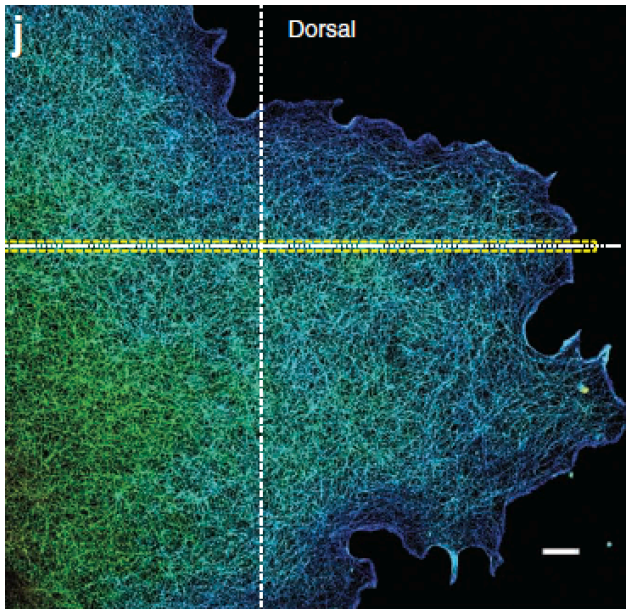






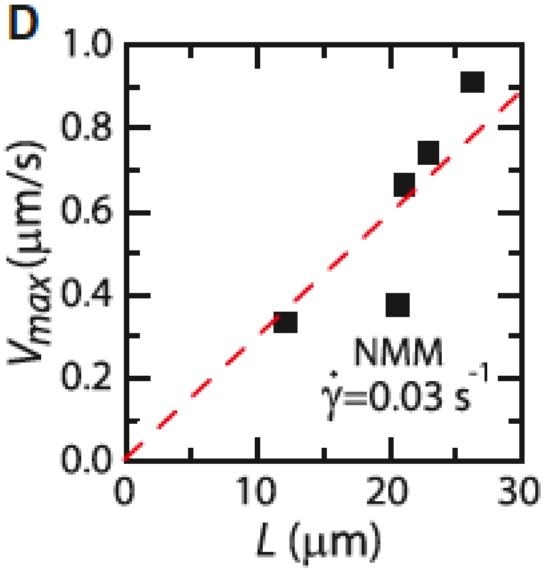
myosin II inhibition

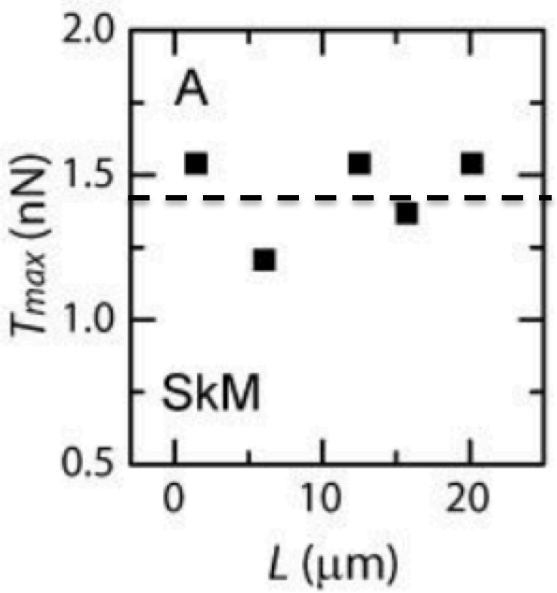


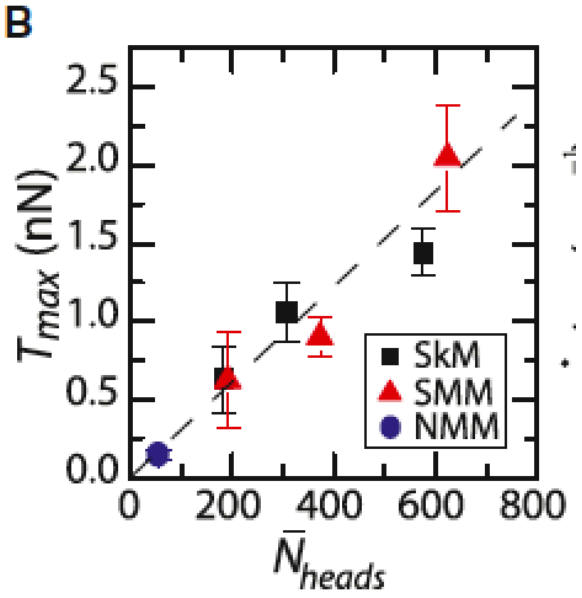


[Xu et al. Nature Methods 2012]

Reconstituted actin bundles are dominated by the single minifilaments



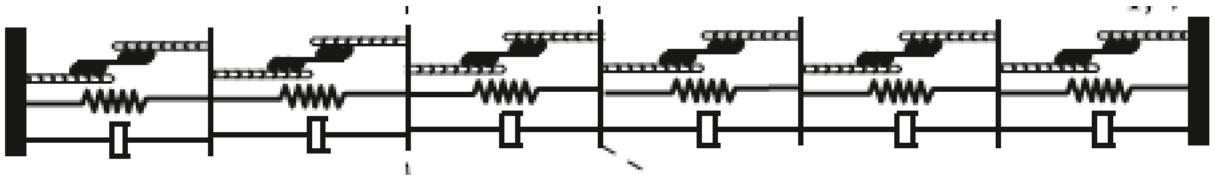




Unloaded velocity versus length

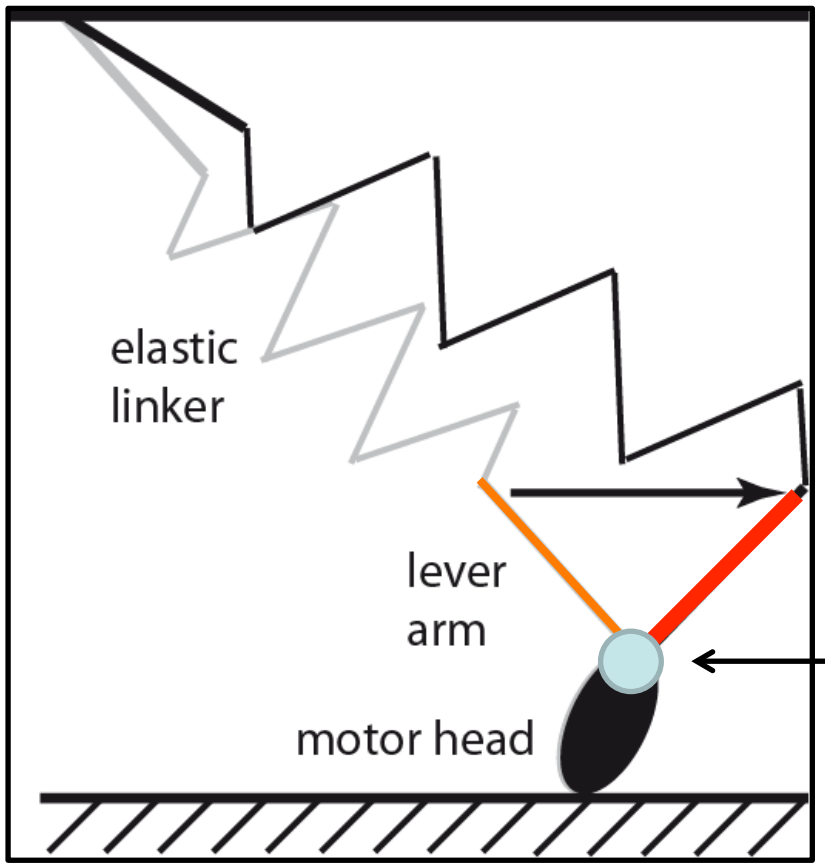
Maximal tension versus length

Maximal tension versus # motor heads



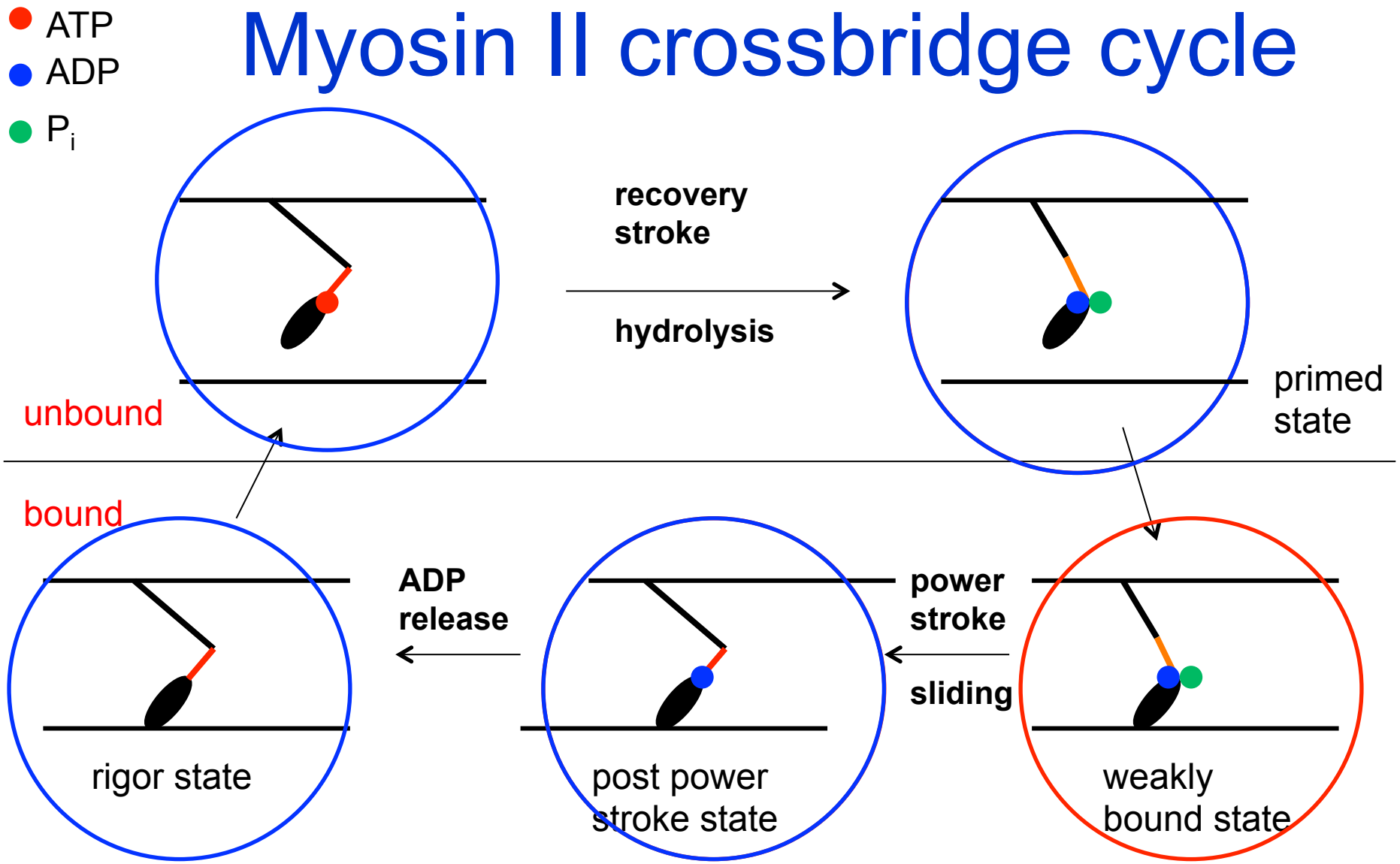
[Thoresen et al., BPJ 2013]

Mechanics of a single motor



The powerstroke of the lever arm amplifies small configurational changes in the motor head and strains the elastic neck linker.

Myosin II crossbridge cycle

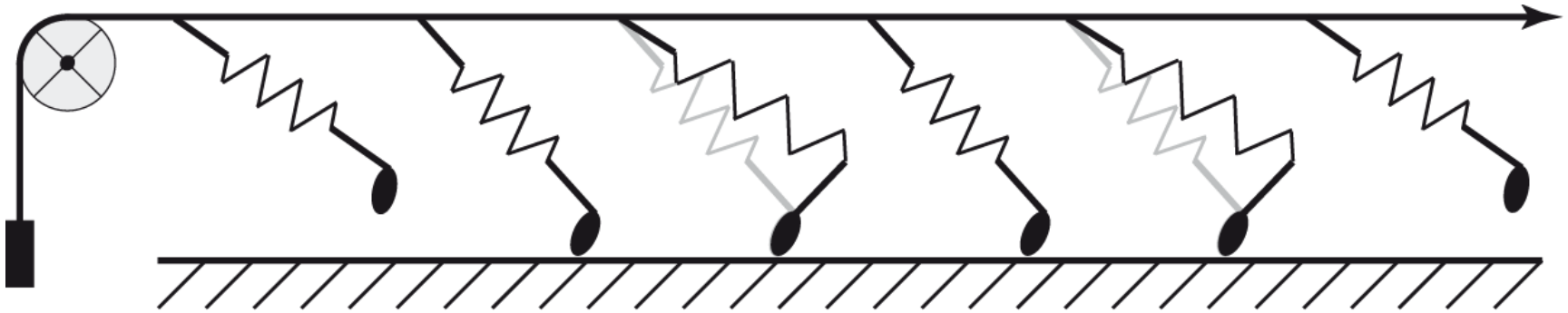


three state model
Duke PNAS 1999

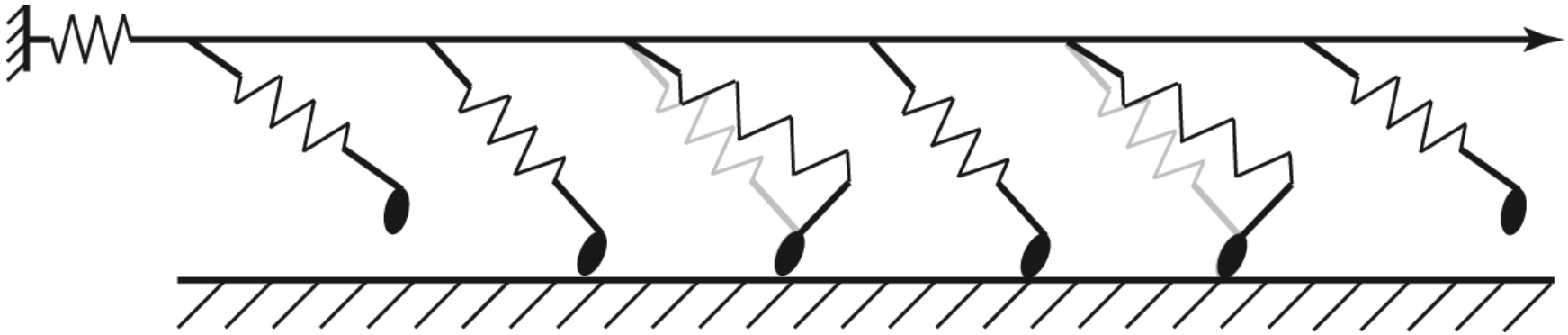
four state model
Walcott et al. BPJ 2012

Two paradigmatic loading situations

constant force permits constant velocity

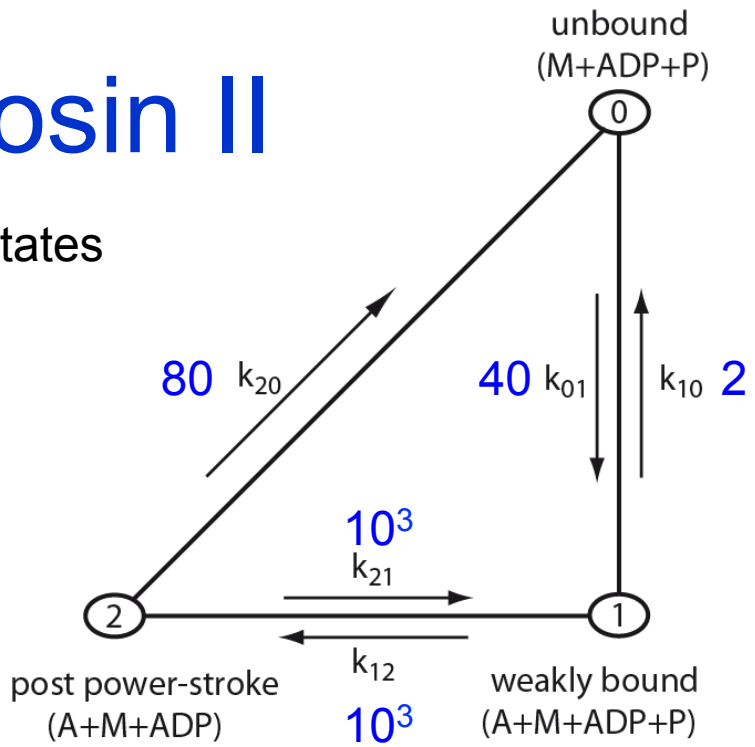


linear force leads to stalling



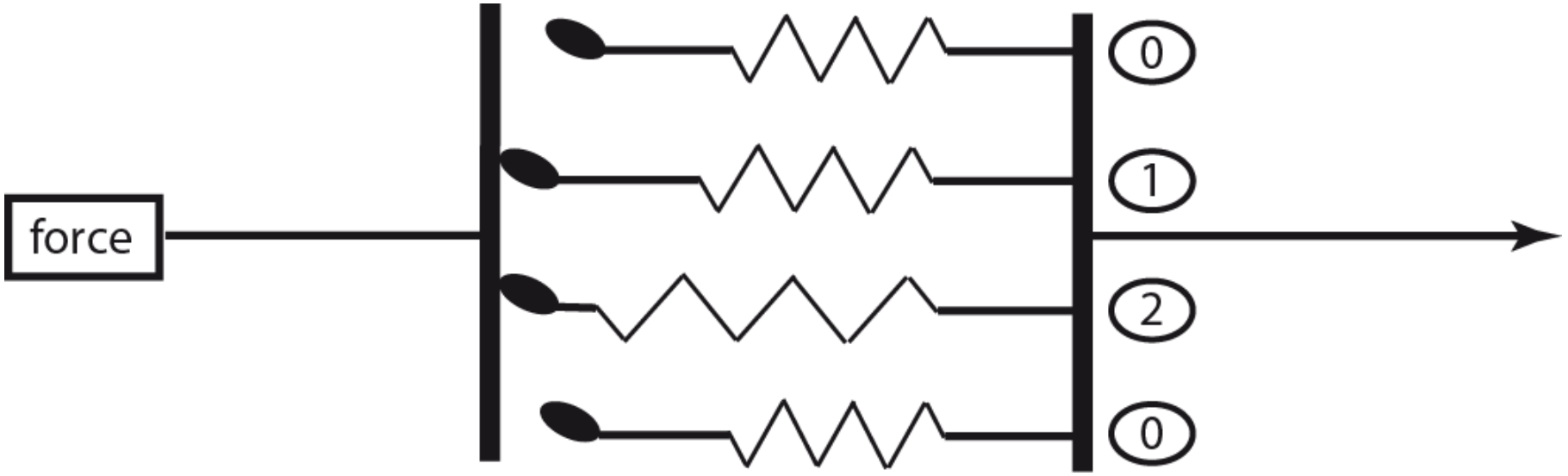
Motor cycle of myosin II

Three main mechano-chemical states



parallel cluster model

powerstroke corresponds to change in rest length, strain is homogenized



Network of stochastic transitions

N_t motors

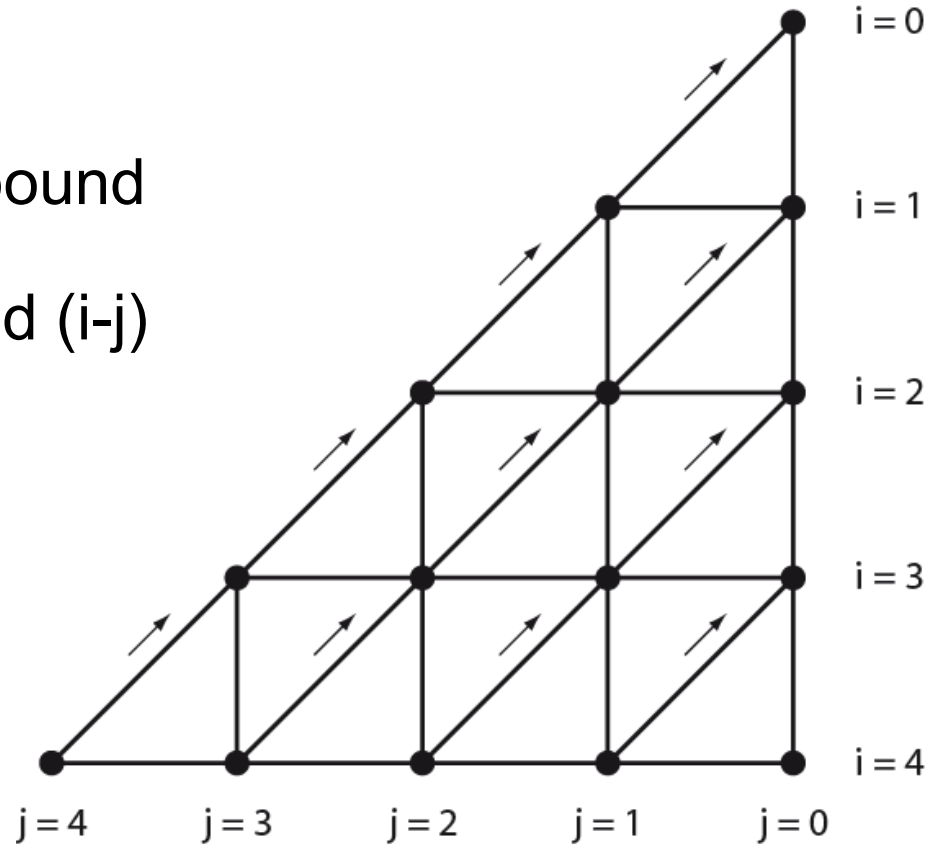
i are bound and (N_t-i) unbound

j are post-powerstroke and (i-j) weakly bound

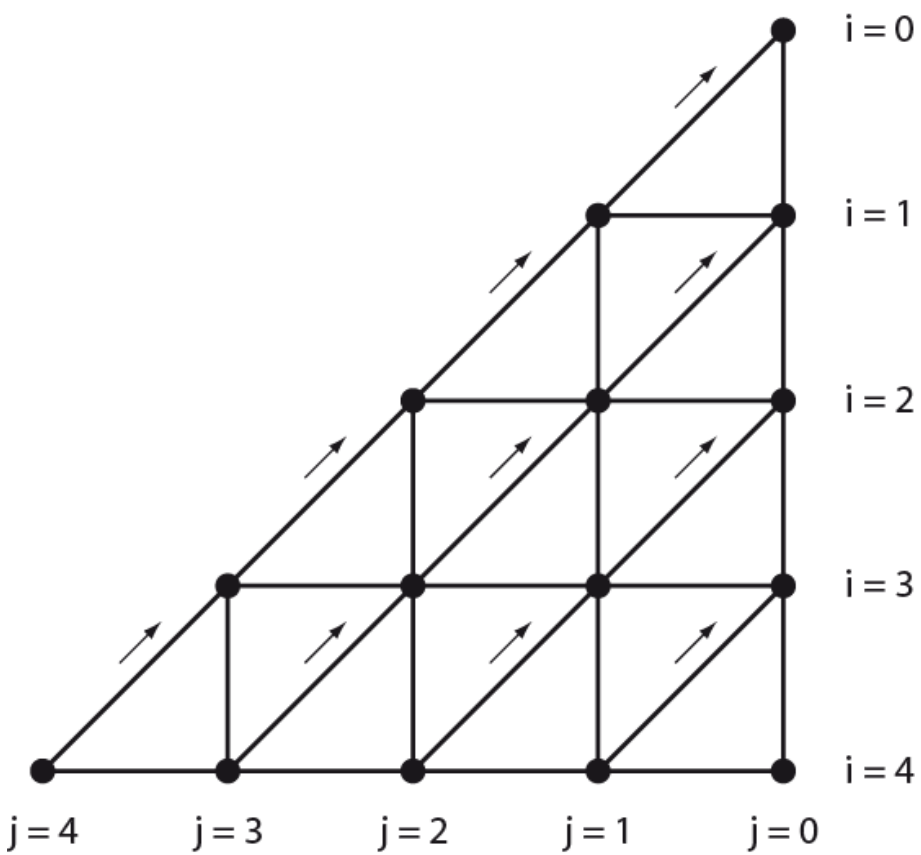
#states = $(N_t+1)(N_t+2)/2$

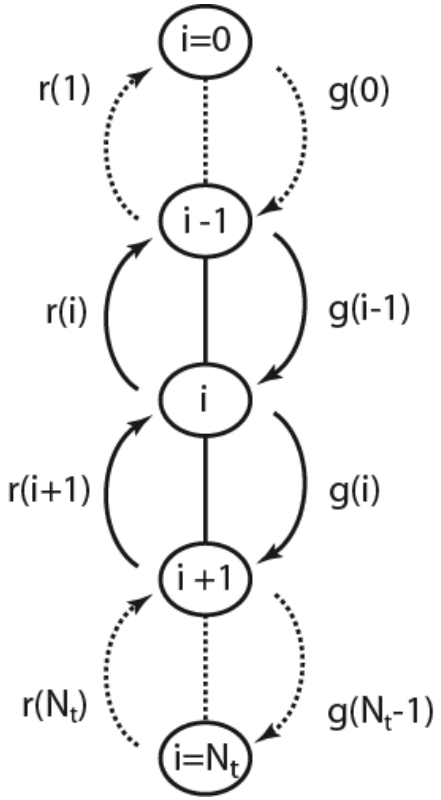
#transitions = $3N_t(N_t+1)/2$

(2)->(0) is irreversible



Model reduction due to assumption of local thermal equilibrium between bound state



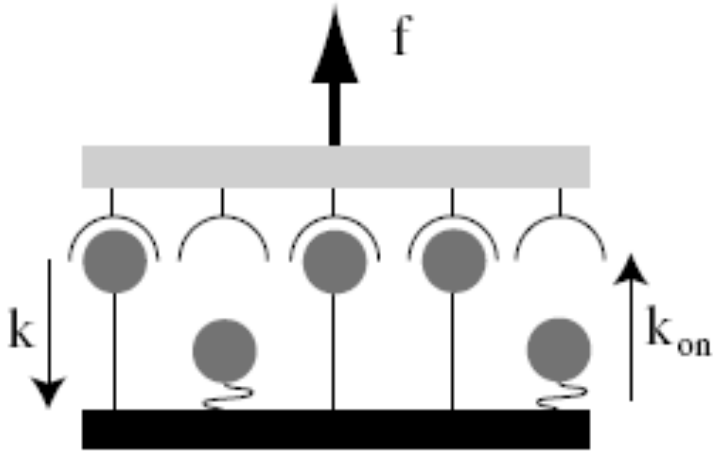


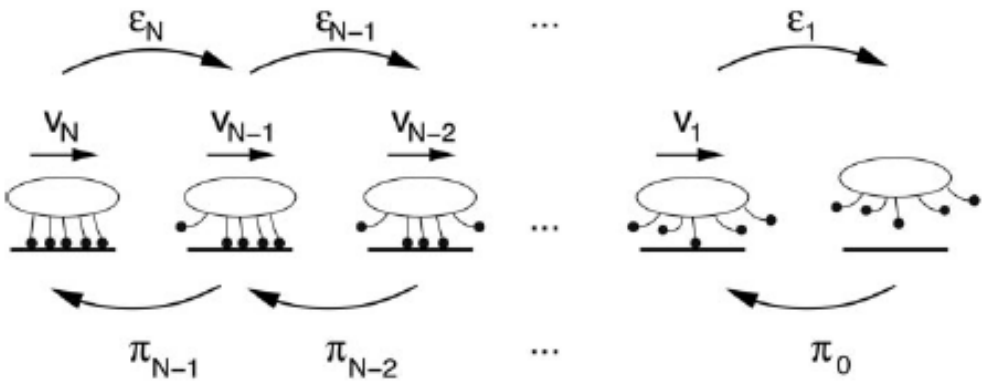
one-step master equation

The power of the one-step master equation

Stability of an adhesion cluster under force

[Erdmann and Schwarz PRL 2004]





Cooperative transport by teams of processive motors

[Klumpp and Lipowsky PNAS 2005]

Local thermal equilibrium between bound states

Conditional probability for j motors after the powerstroke if i motors are bound:

$$p(j|i) = \frac{1}{Z_i} \exp\left(-G(i,j)/k_B T\right)$$

Free energy is sum of elastic energy and energy gained by ATP-hydrolysis:

$$G(i,j) = E(i,j) + j\Delta G$$

Partition sum for fixed i: $$Z_i = \sum_{j=0}^{i} \exp\left(-G(i,j)/k_B T\right)$$

State probabilities: $$p(i,j)(t) = p(j|i)(t)p_i(t)$$

One-step master equation

$$\frac{d}{d\tau}p_i = r(i+1)p_{i+1} + g(i-1)p_{i-1} - (r(i)+g(i))p_i$$

Dissociation rate: $r(i) = \langle r(i,j)\rangle_j = \sum_{j=0}^{i} r(i,j)p(j|i)$

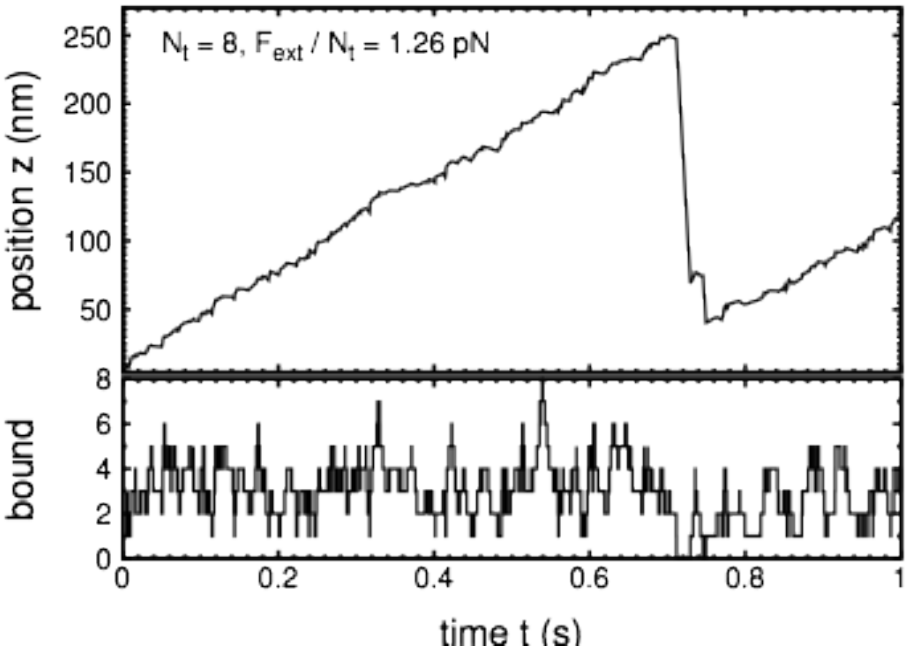
$$r(i,j) = (i-j)k_{10}(i,j) + jk_{20}(i,j)$$

$$k_{10}(i,j) = k_{10}$$

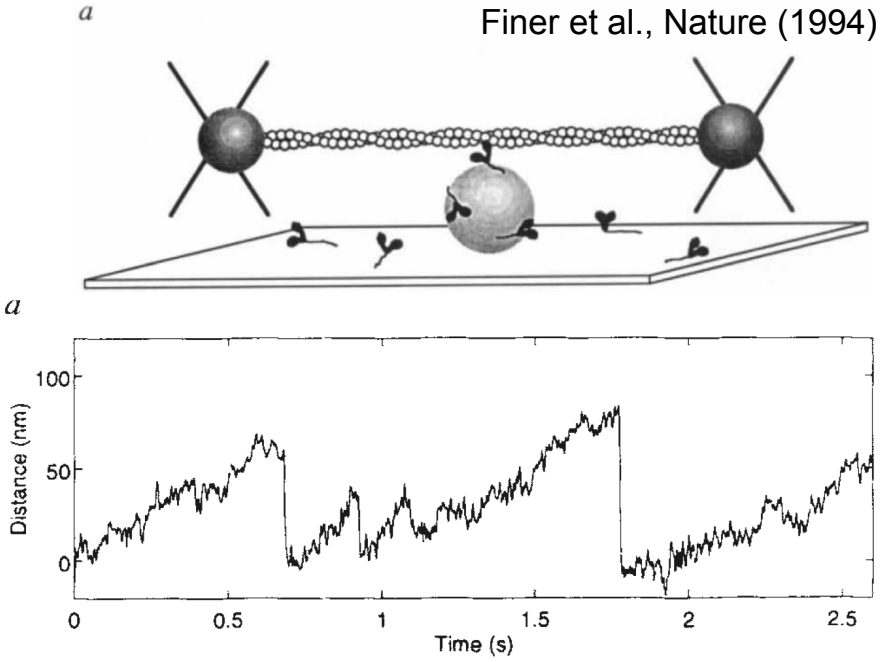
$$k_{20}(i,j) = k_{20}\exp\left(-F_{pp}(i,j)/F_\delta\right)$$ catch bond

Association rate: $g(i) = (N_t - i)k_{01}$

Stochastic trajectory for constant force



Finer et al., Nature (1994)

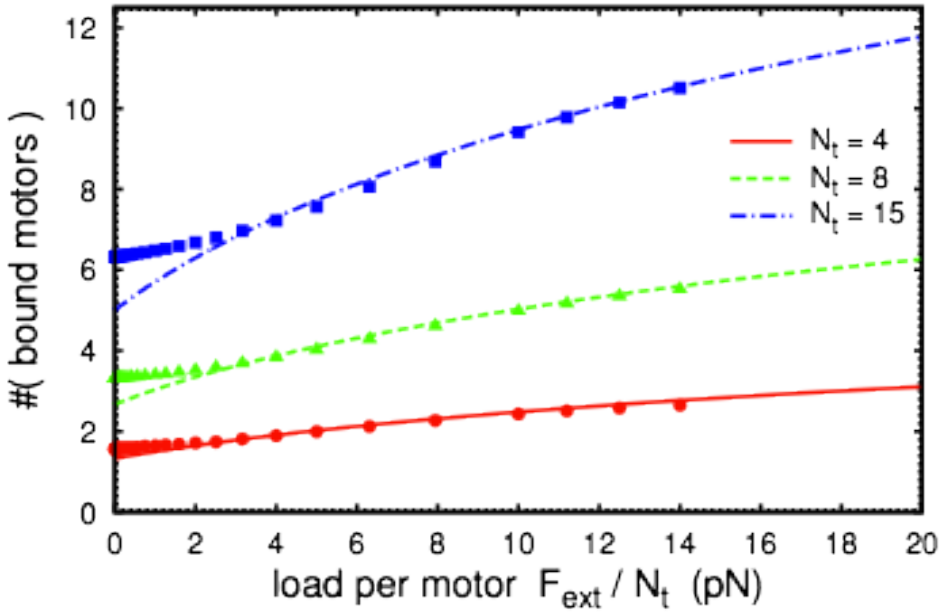


Bound ensemble move with fluctuating velocity

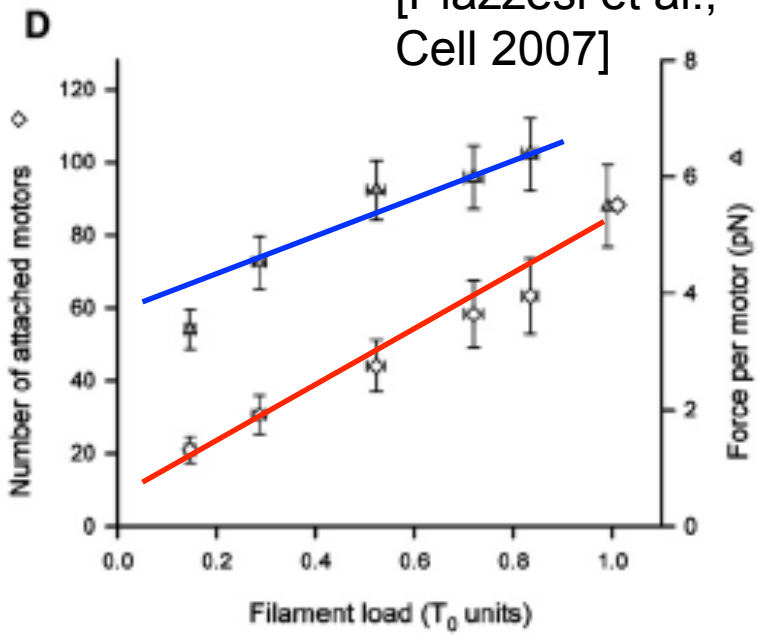
Large backward step at unbinding

Sufficiently large ensembles move processively

Catch bonding



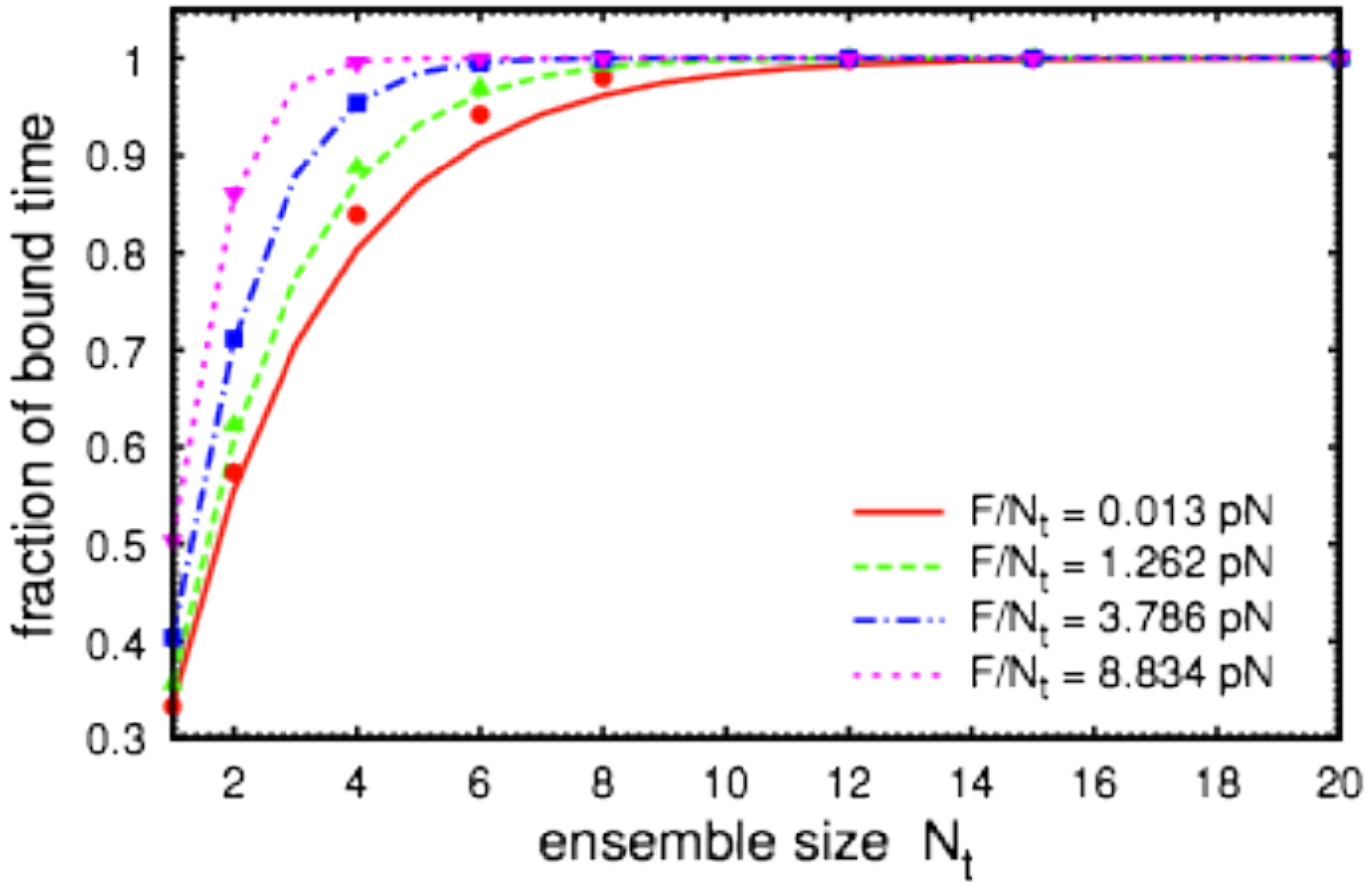
[Piazzesi et al., Cell 2007]



Number of bound motors increases with force because of the catch-bond character of myosin II

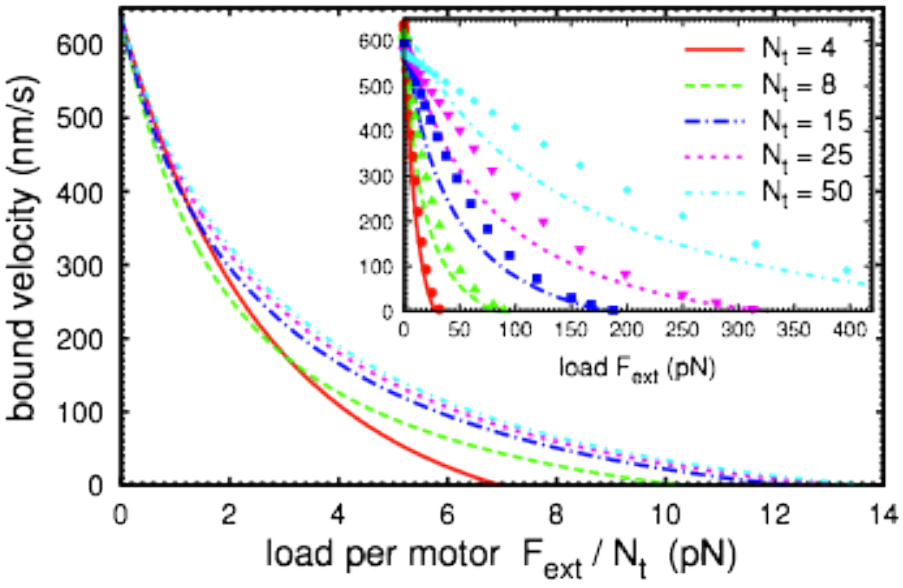
Symbols are computer simulations with individual motor strains (good agreement with analytical results)

Relevance of ensemble size

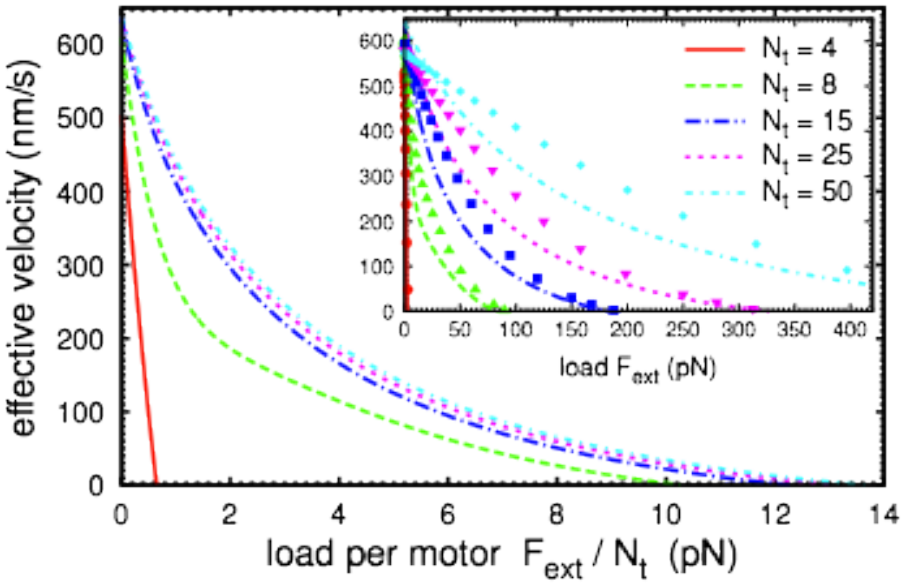


Duty ratio rises quickly with N_t → stochastic effects important for $N_t \leq 15$

Force-velocity relation



without backward sliding

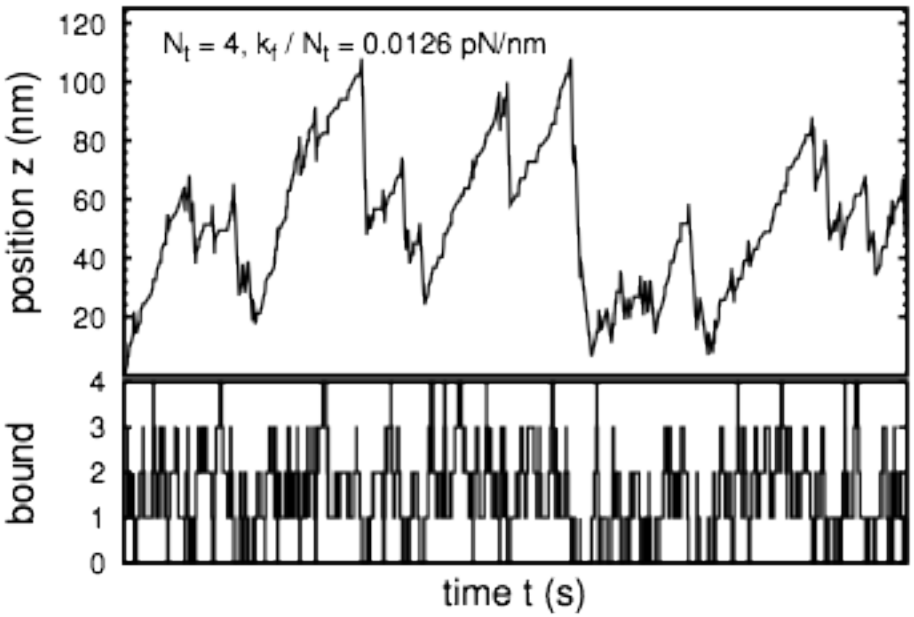


with backward sliding

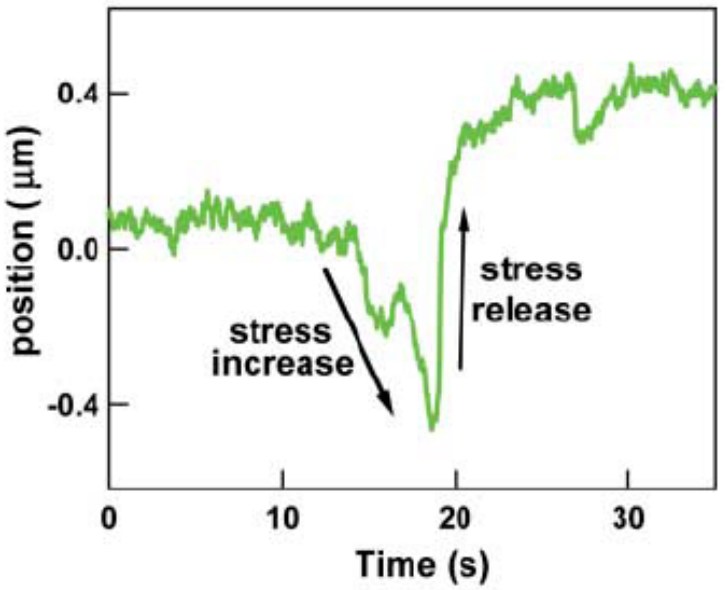
Upward convex shape due to increase of the number of bound motors under force

Velocity and stall force increase with ensemble size but stay constant above $N_t \approx 15$

Stochastic trajectory for linear force



[Mizuno et al. Science 2007]



Sequence of forward motion and unbinding

At large forces the catch-bonds stabilize the ensemble

Conclusion

- **Cells push by growing actin networks**
- Our model predicts bistability between two different network architectures
- Changes in migration velocity or force can induce structural transitions (cells shift gears)
- **Cell pull through small myosin II minifilaments**
- Under increasing force, the ensemble becomes more stable
- This might be one of the key elements in mechanosensing

Funding

www.thphys.uni-heidelberg.de/~biophys/