Cell migration in the immune system

Rob J de Boer, Utrecht University

Theoreticians:

Joost Beltman (UU, LU), Ioana Niculescu (UU),

Johannes Textor (UU) & Stan Marée (Norwich).

Experimental collaborators:

Jennifer Lynch & Mark Miller (St Louis)

Sarah Henrickson & Ulrich Von Andrian (Harvard)

Silvia Ariotti & Ton Schumacher (NKI, Amsterdam)

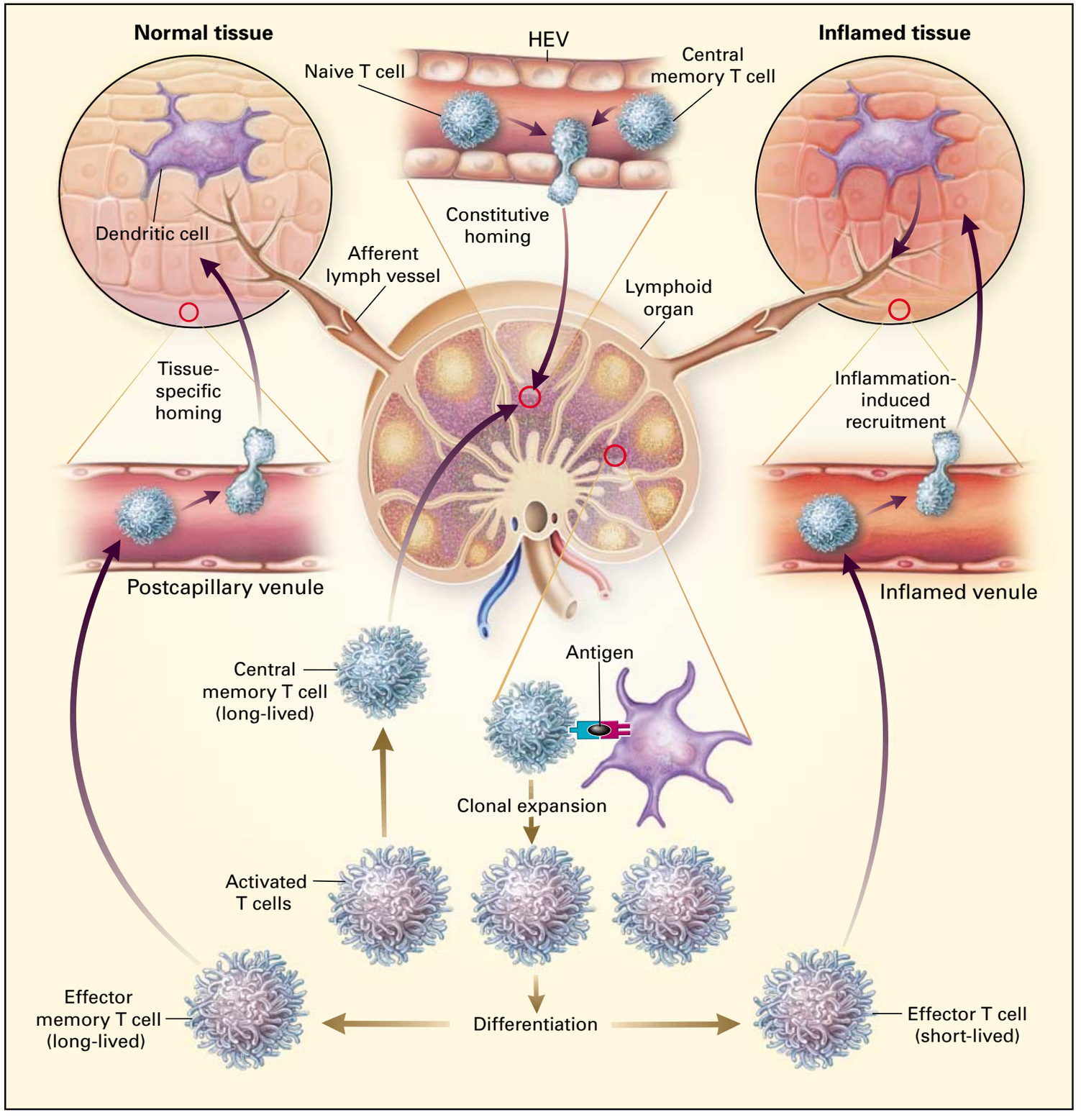
Immune responses develop in draining lymph nodes

Dendritic cells (DC) scan peripheral tissues, and migrate to draining lymph nodes to present their antigens.

Millions of different naive T cells migrate through lymph nodes, and bind these DC.

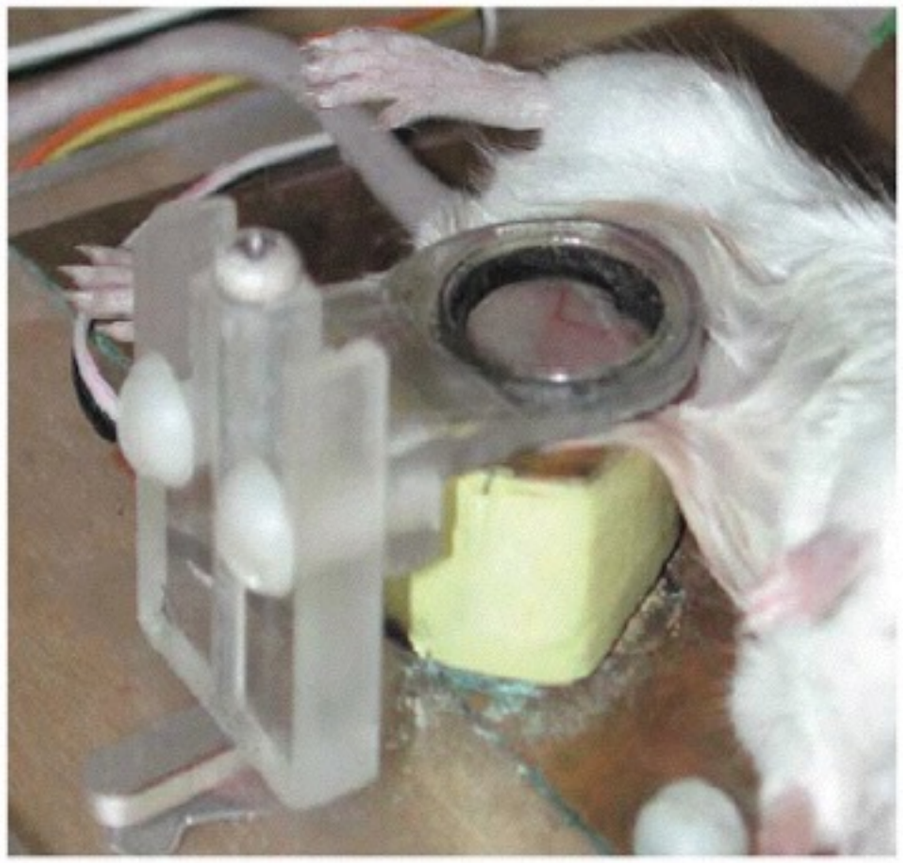
Only 1:100000 T cells will become activated, expand, and emigrate as effector cells that move back to the inflamed tissues.

Needle in a haystack problem: how long would it take to initiate an immune response?

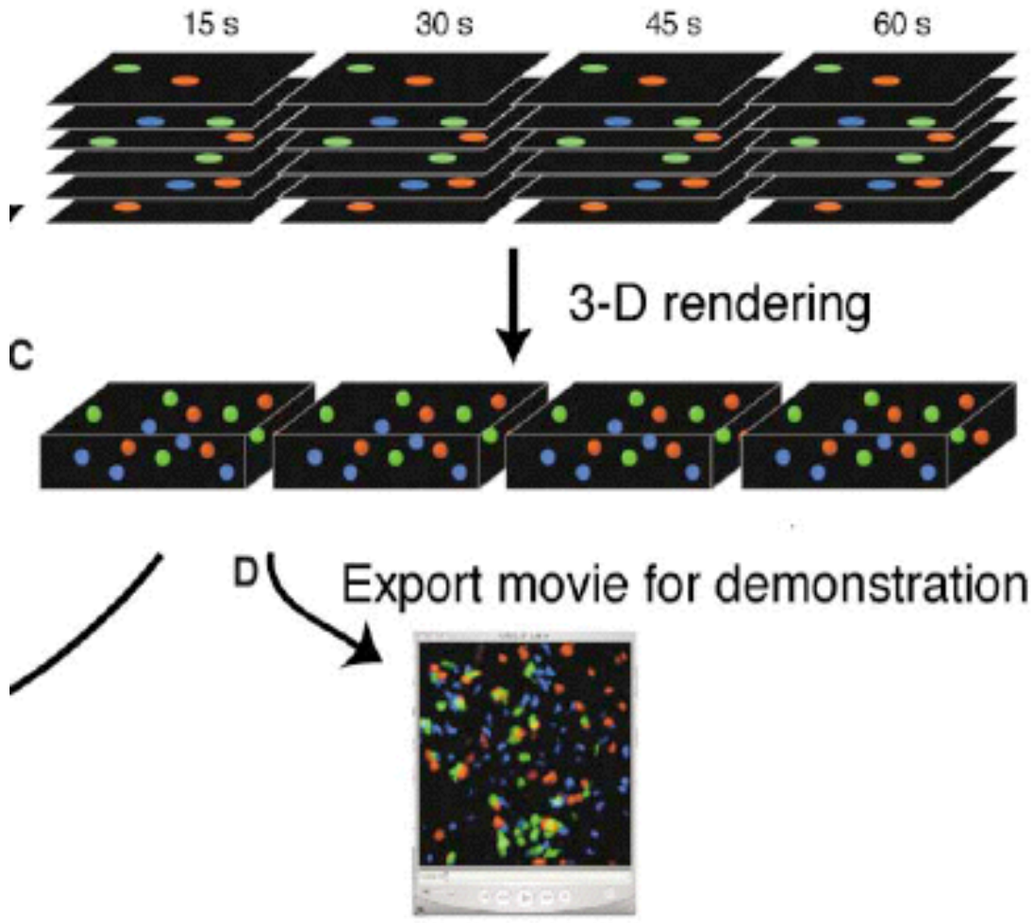


Von Andrian & Mackay, NEJM, 2000

Two photon microscopy: 2PM



Cahalan et al. Curr Op Immunol (2003)



Sumen et al. Immunity (2003)

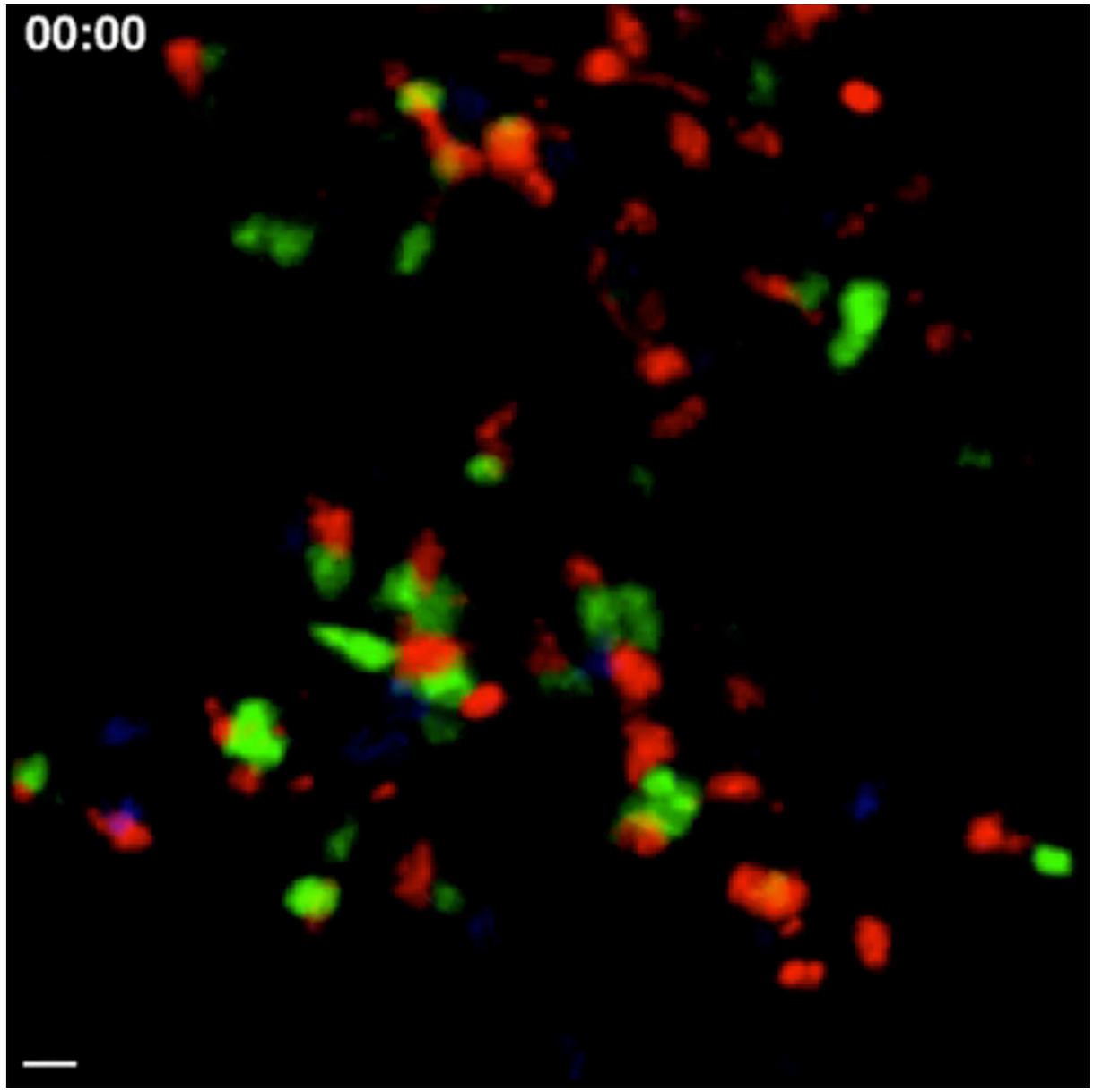
Label cells with fluorescent marker, inject, wait until they arrive in lymph node

Use different colors for T cells and dendritic cells

Use tracking software to translate videos into cellular trajectories

Delivers rich data sets that are difficult to quantify

Vivid movies of migrating cells in LT

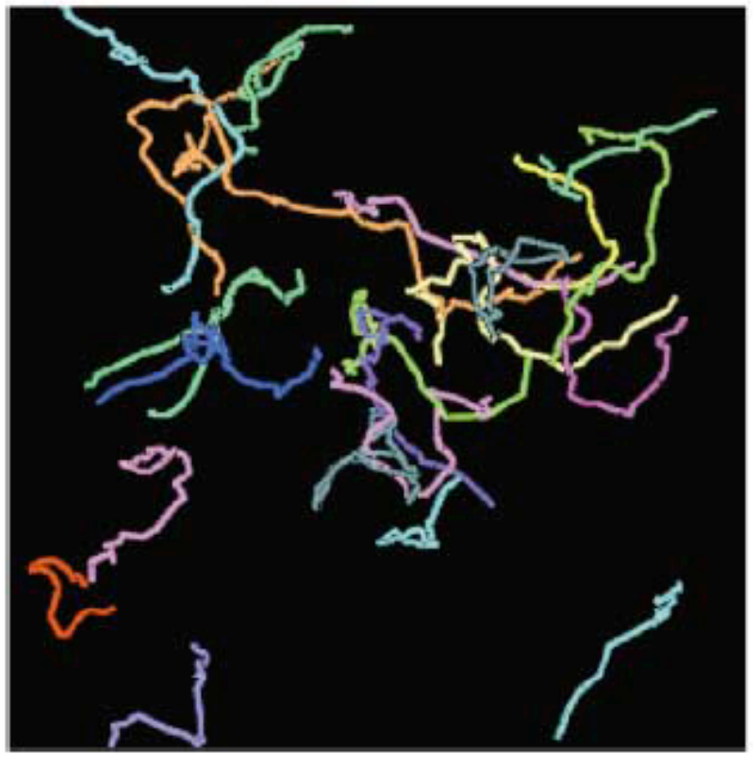


Henrickson et al.
Nat. Imm (2008)

Green: Ag specific CD8 T cells, Blue control cells, and Red DC

Small volume and short time period: tracks are biased samples

Software tracks the cells "automatically"

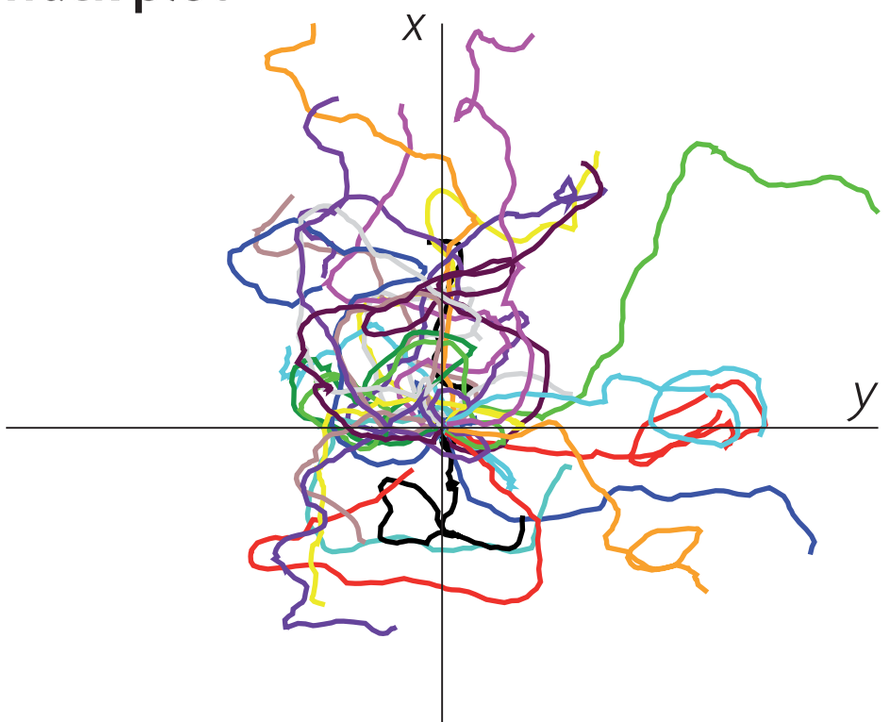


Export tracks

Track	Time-point	Coordinate X	Y	Z
1	1	127.1	62.8	4.4
1	2	124.2	59.3	3.8
1	3	122.2	60.7	3.1
1	4	119.2	63.3	3.6
1	5	116.8	63.2	4.4
1	6	114.1	63.1	3.1
1	7	113.7	60.5	3.4
1	8	114.9	60.4	2.1
1	9	115.1	62.5	1.5
2	5	112.3	95.4	2.4
2	6	112.5	93.5	1.4
⋮				

Compute motility parameters

Track plot



Quantify cell migration by:

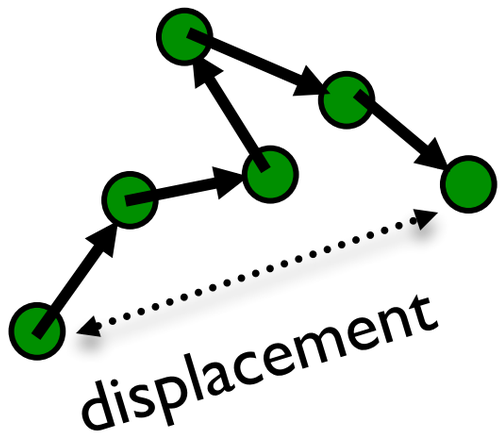
plot tracks

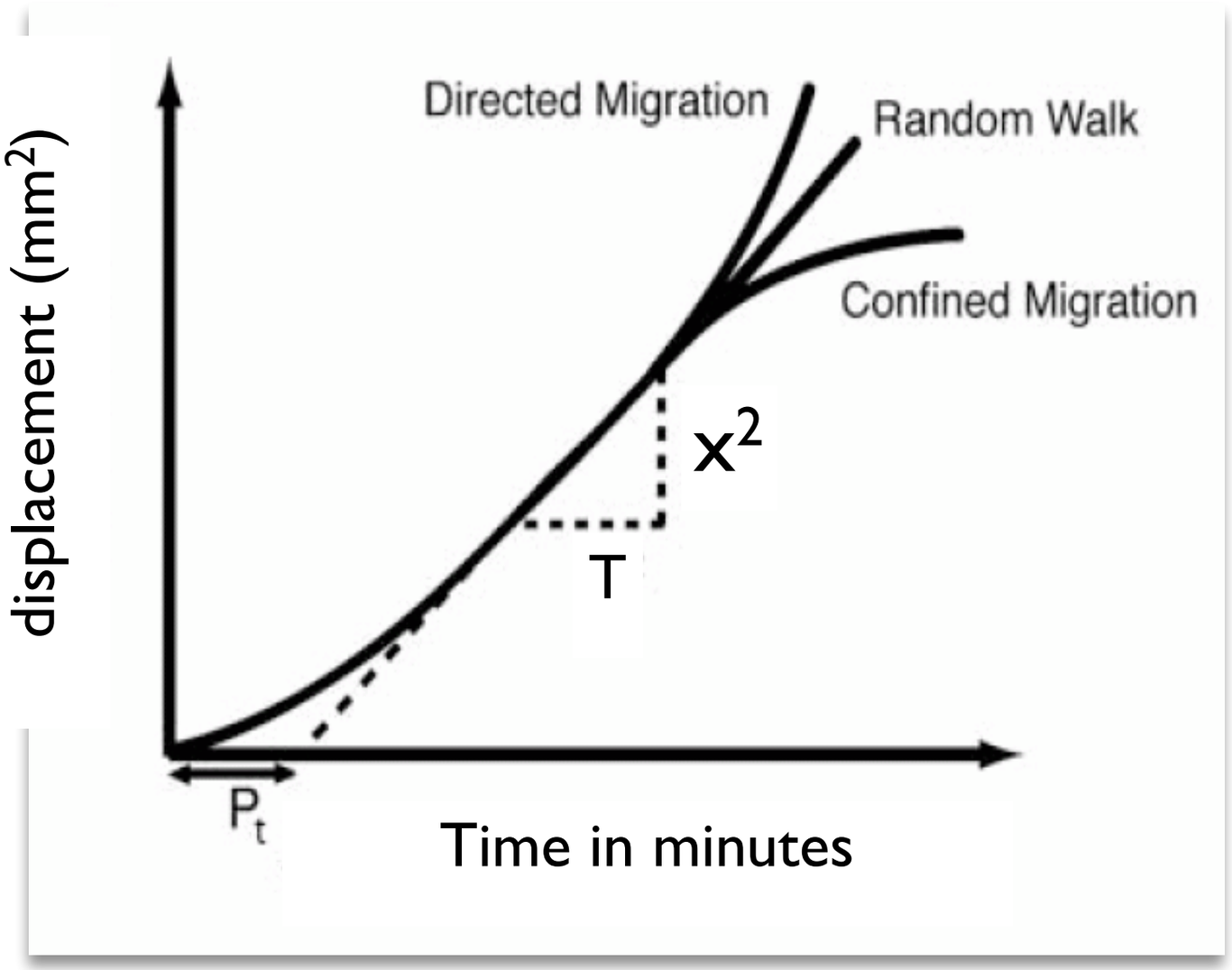
record speeds

angles of migration

mean square displacement plot

Mean square displacement suggests a random walk





Motility (diffusion) coefficient:

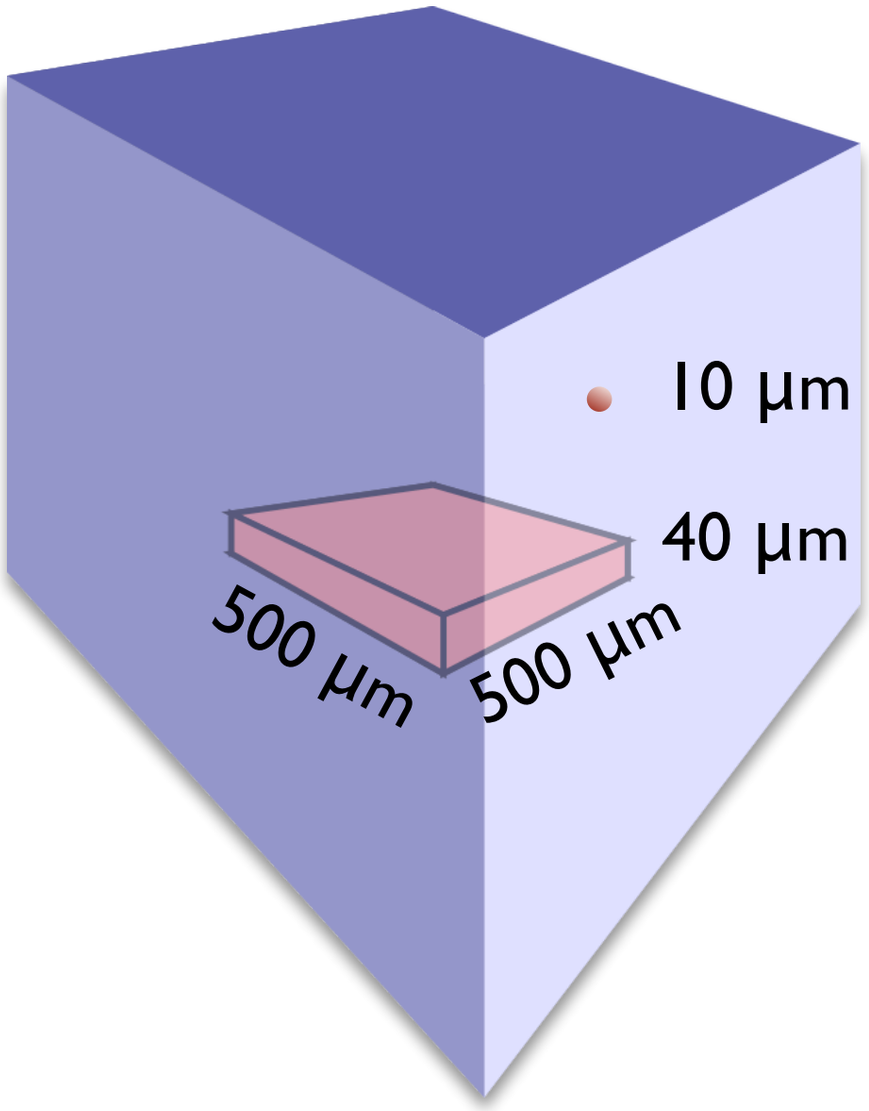
$$M = \frac{\overline{x^2}}{2dt}$$

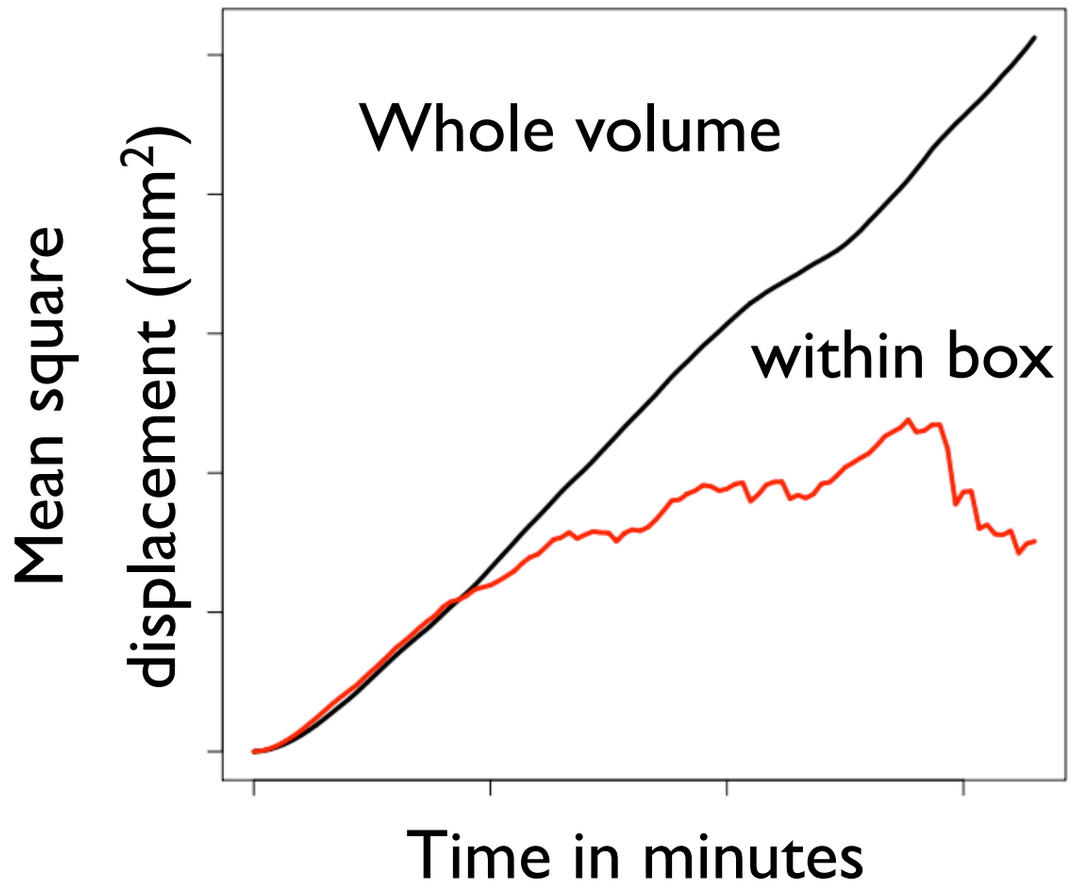
d: dimension,

x: displacement,

t: time

Mere imaging in a small volume gives a bias

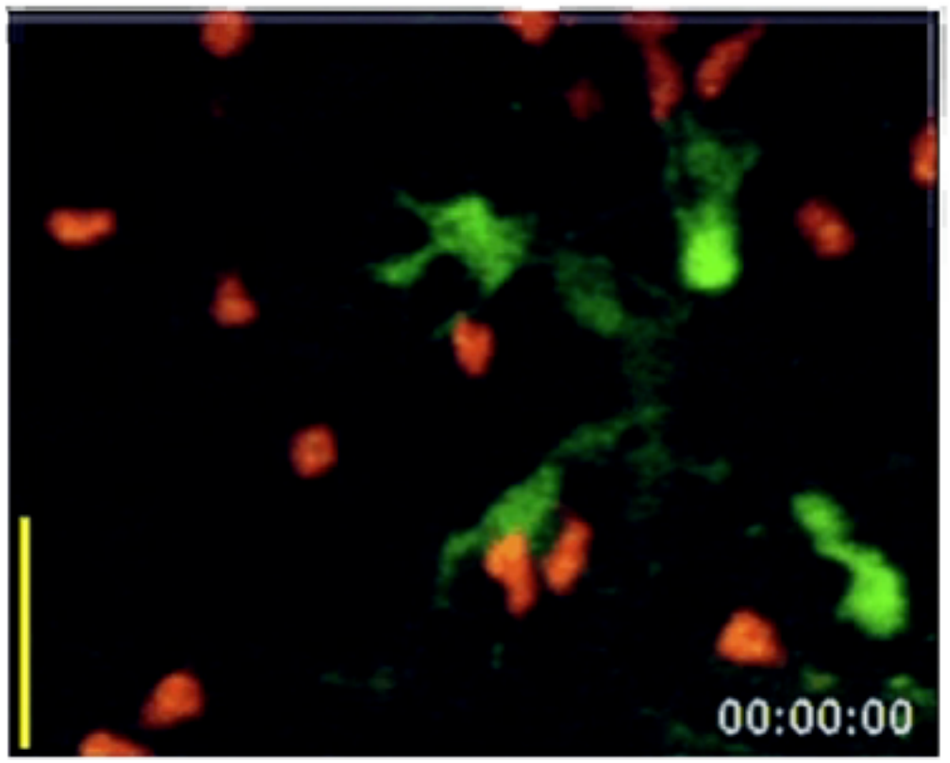




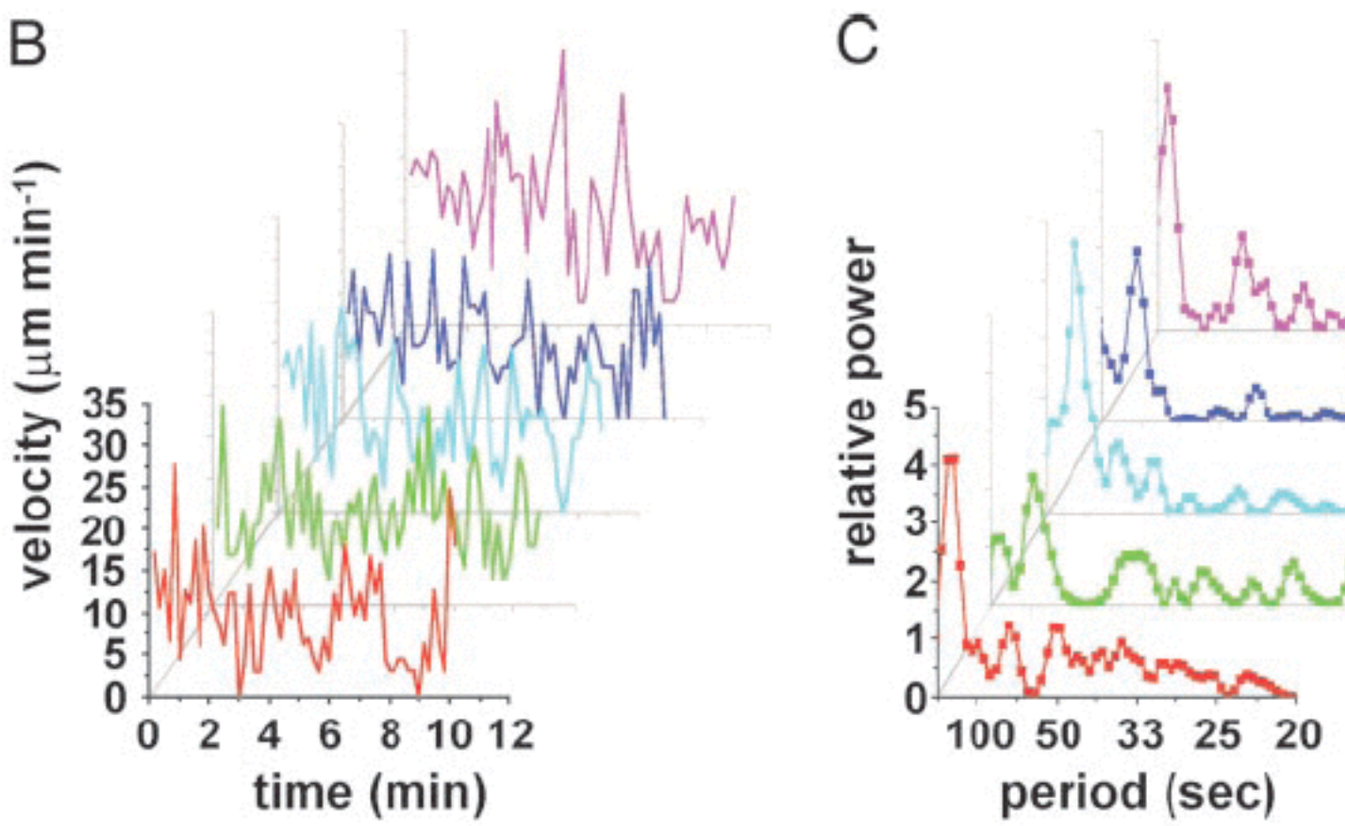
At large time intervals the displacement plot will be biased towards slow cells that tend to stay in the box.

This looks like confined migration

T cells migrate randomly in the absence of antigen



Red: T cells, **Green**: Dendritic cells (DC)



Maximum intensity projection giving a top-view

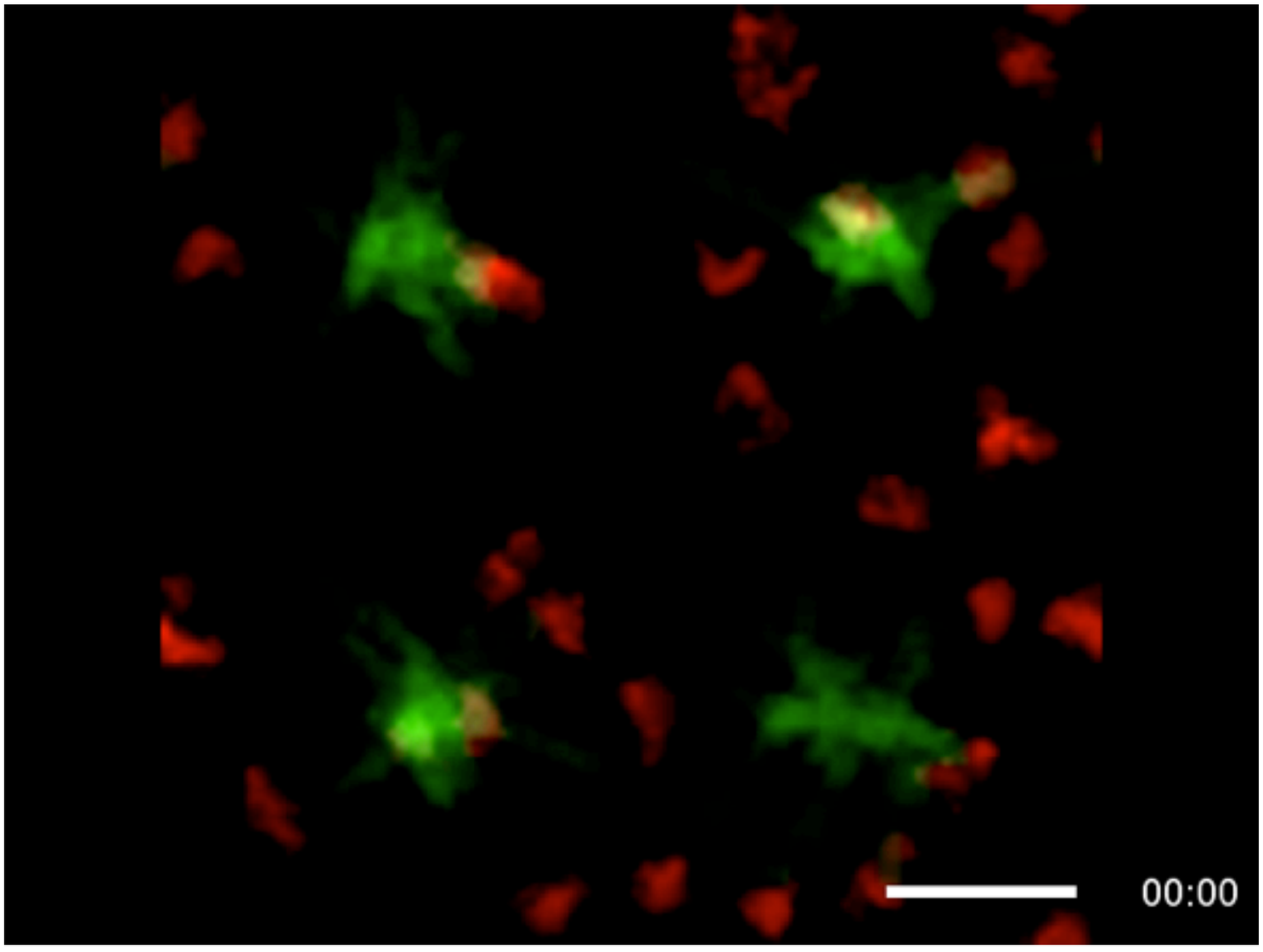
Random walk, irregular velocities, speed one cell diameter min^{-1}

no overall directionality, no collective motion

stop-and-go movement: peak in Fourier spectrum at ~ 1 min

Miller et al. PNAS (2003)

Cellular Potts Model: in silico movie where we "labeled" a small subset of the cells

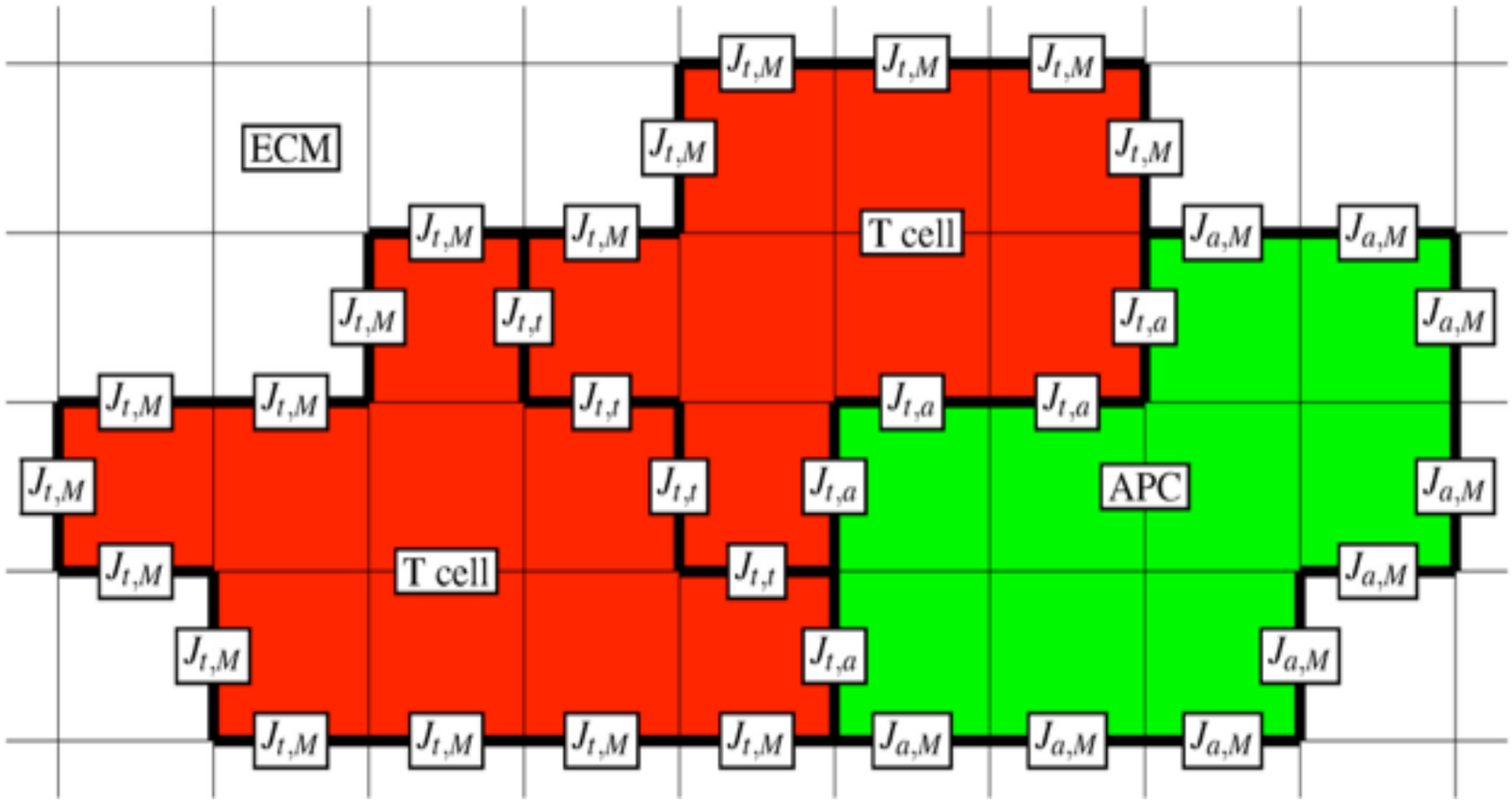


Red: T cells **Green**: Dendritic cells (DC)

Similar maximum intensity projection

Beltman et al. J Exp Med (2007)

Cellular Potts Model: grid based



$$H = \Sigma J + \lambda(v\text{-}V_T)^2$$

Surface energies: Hamiltonian

System minimizes its energy

ΔH determines probability of copying (Boltzmann distribution)

Cellular Potts Model

0	0	3	3	3	3	3
0	0	0	3	3	3	3
0	0	0	5	3	3	3
0	5	5	5	5	5	3
5	5	5	5	5	5	5

Cellular Potts Model

0	0	3	3	3	3	3
0	0	0	3	3	3	3
0	0	0	5	3	3	3
0	5	5	5	5	5	3
5	5	5	5	5	5	5

Cellular Potts Model

0	0	3	3	3	3	3
0	0	0	3	3	3	3
0	0	0	5	3	3	3
0	5	5	5	5	5	3
5	5	5	5	5	5	5

Cellular Potts Model

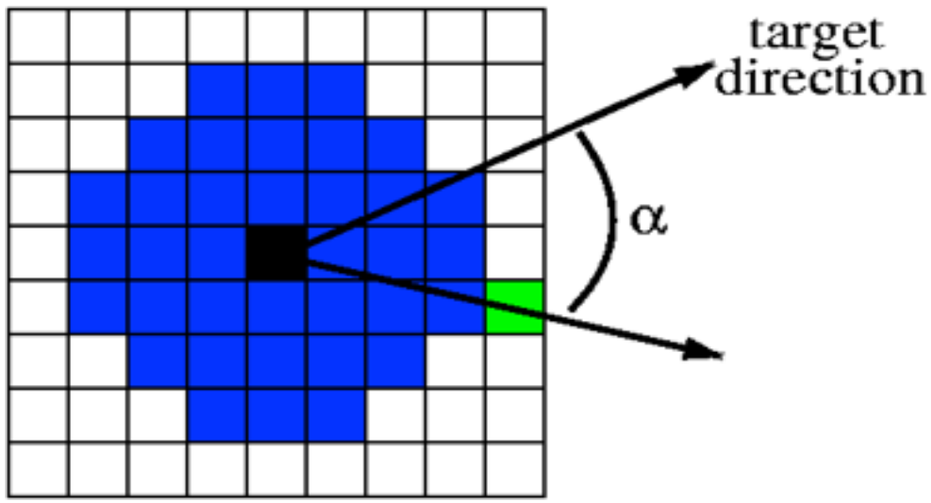
0	0	3	3	3	3	3
0	0	0	3	3	3	3
0	0	0	3	3	3	3
0	5	5	5	5	5	3
5	5	5	5	5	5	5

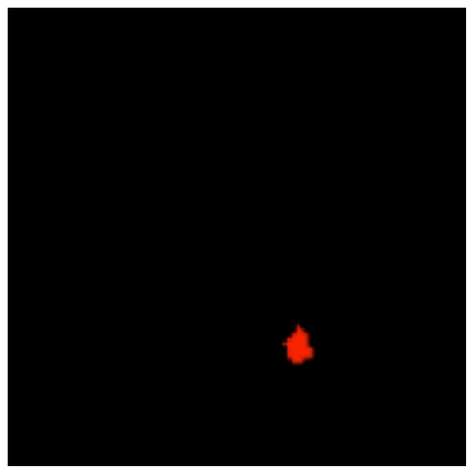
Cellular Potts Model

0	0	3	3	3	3	3
0	0	0	3	3	3	3
0	0	0	3	3	3	3
0	5	5	5	5	5	3
5	5	5	5	5	5	5

To move T cells have a target direction

$$\Delta H = - \mu \cos(\alpha)$$



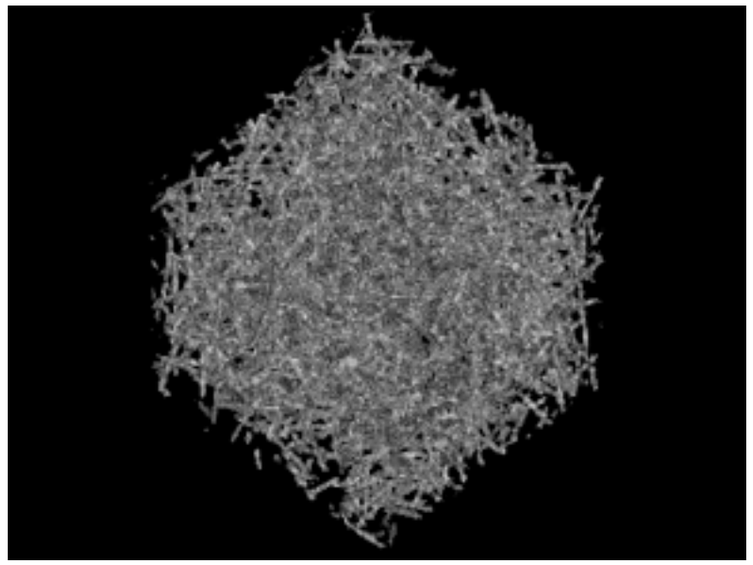


Target direction is adjusted according to recent displacement (directional persistence)

New “actin inspired” model:

http://tbb.bio.uu.nl/ioana/cpm/

T cell area in lymph nodes has a static reticular network



1 pixel = 1 μm^3

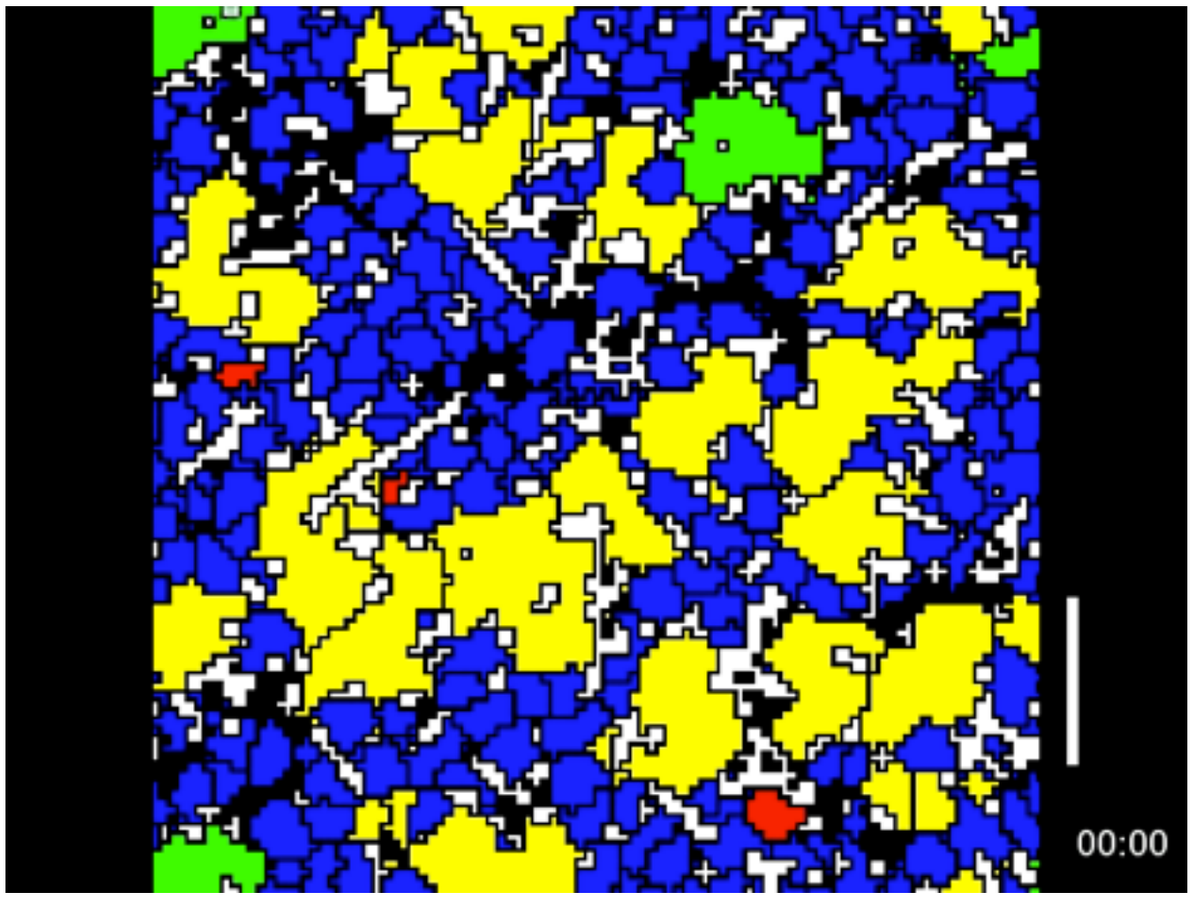
T cell: 150 μm^3, DC: 2200 μm^3

torus: 100 µm x 100 µm x 100 µm

reticular network: randomly oriented rods

Cell populations in the CPM

cross-section:



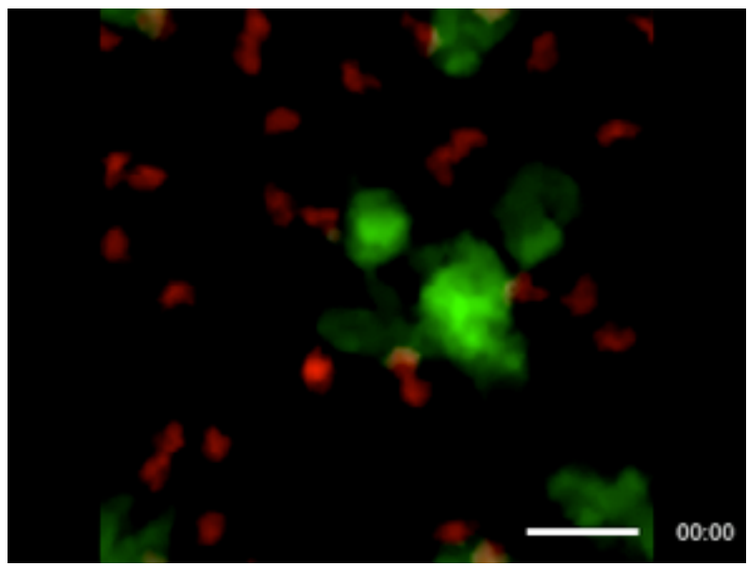
- rods (reticular network)
- extracellular matrix
- non-labeled DCs
- **labeled** DCs
- non-labeled T cells
- **labeled** T cells

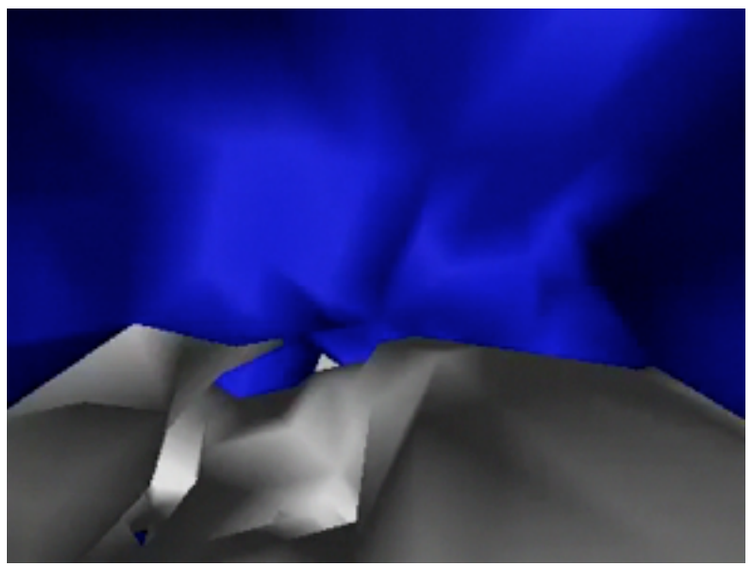
These were all the rules of the game (all assumptions)

We have tuned the adhesion parameters

model is phenomenological!

normal X-ray view and true 3D view





bar: 20 µm

labeled DCs

labeled T cells

reticular network

non-labeled T cells

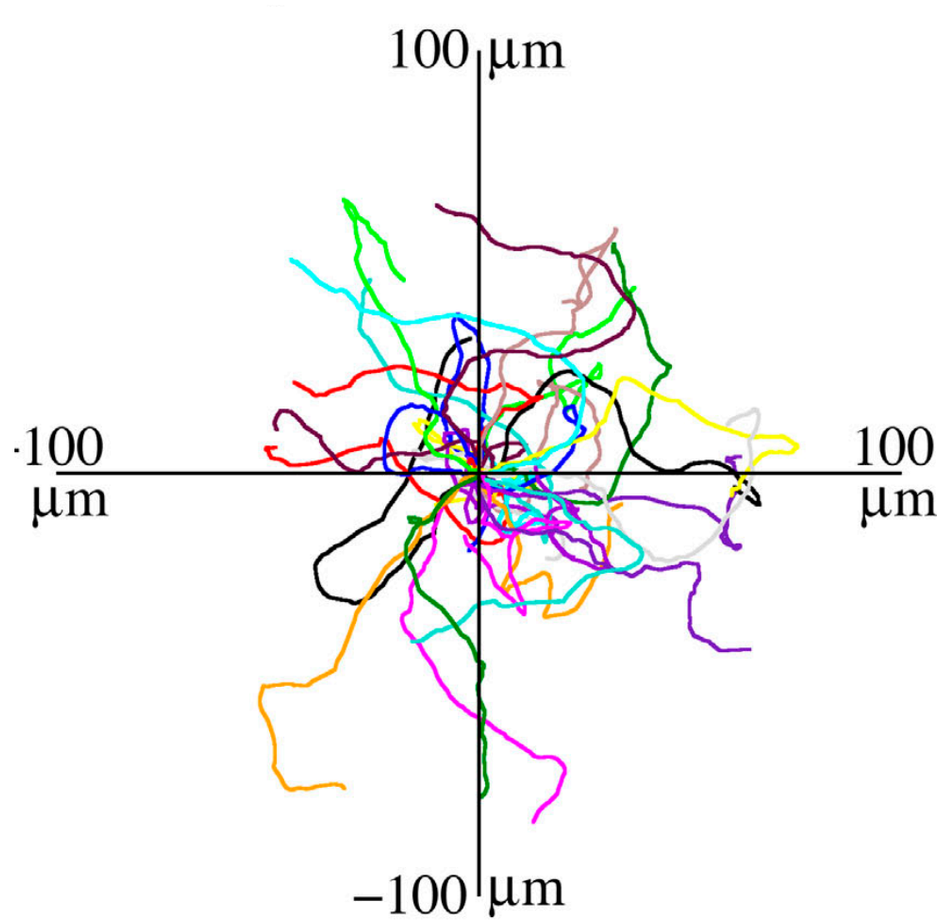
non-labeled DCs

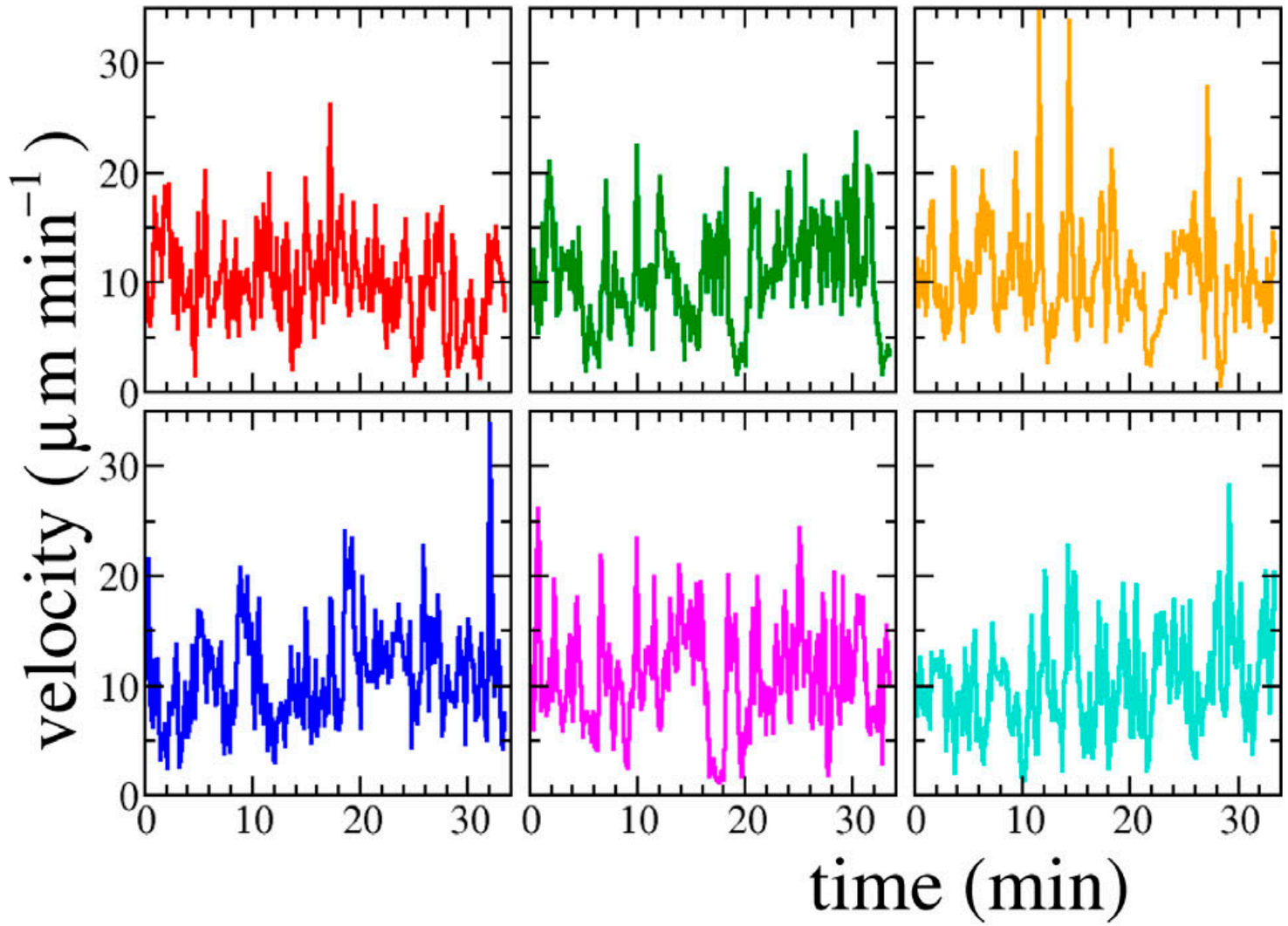
Grey: reticular net, Blue: T cells, Green/Yellow: DCs

Because we now see all the cells we appreciate much better that this is a densely packed environment!

Beltman et al. J Exp Med (2007)

T cell tracks in the model: automatic



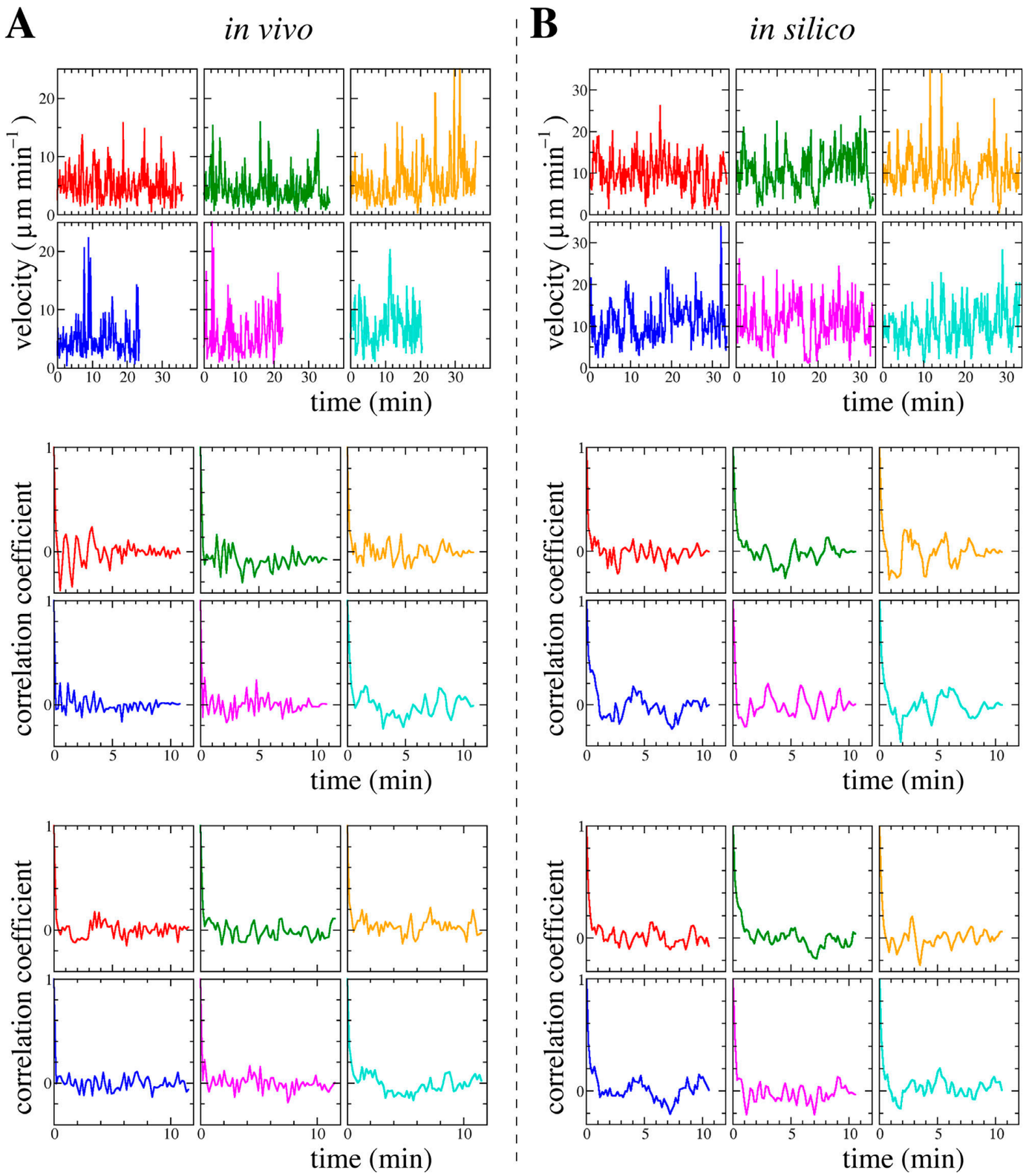


Very similar persistent motion in short term.

Very similar irregular velocities.

But no stop-and-go encoded in the model?

Stop-and-go just due to collisions



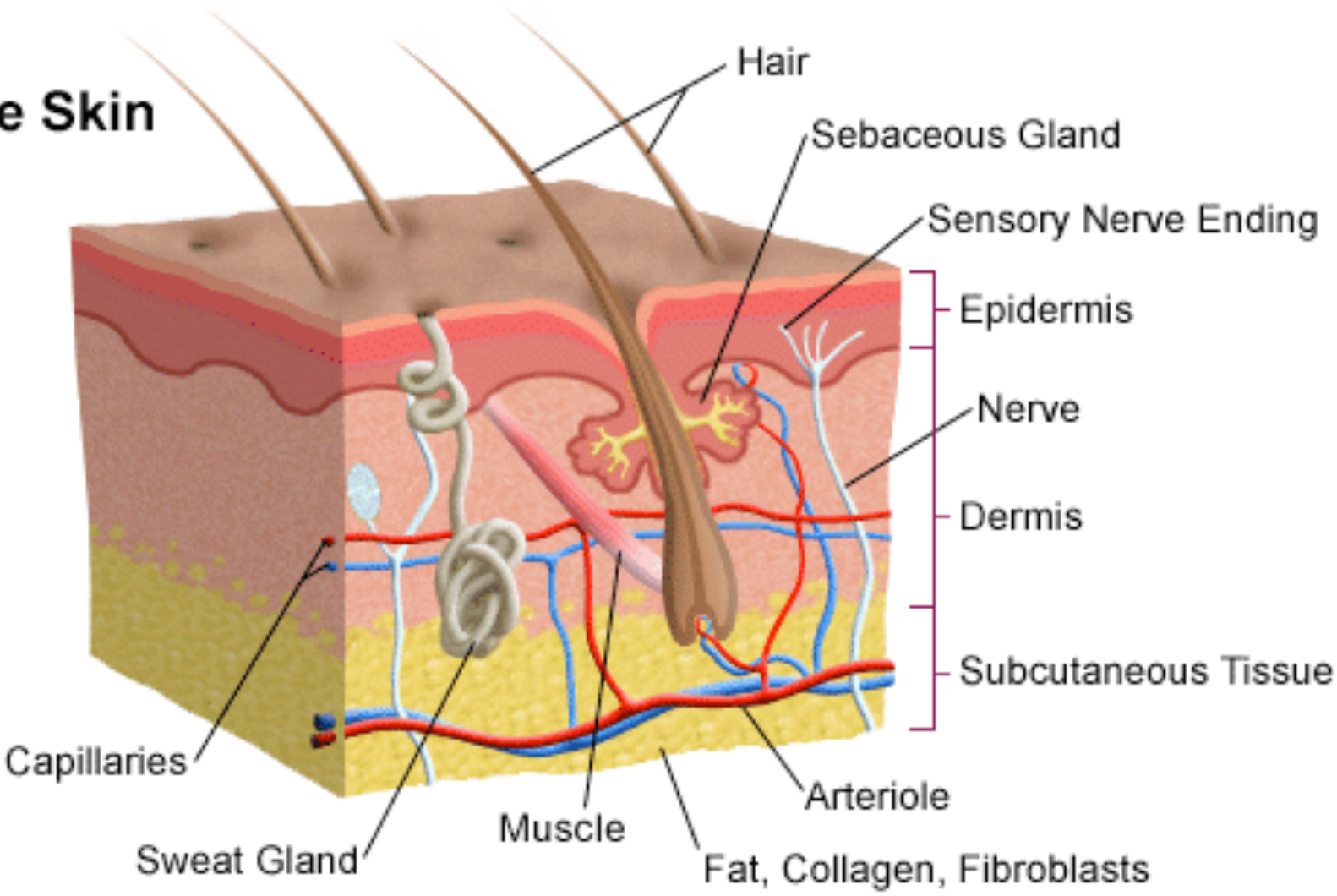
Longer time series

Autocorrelation on first 64 data points

Autocorrelation on all data points

Beltman JEM 2007

HSV infection in skin epidermis



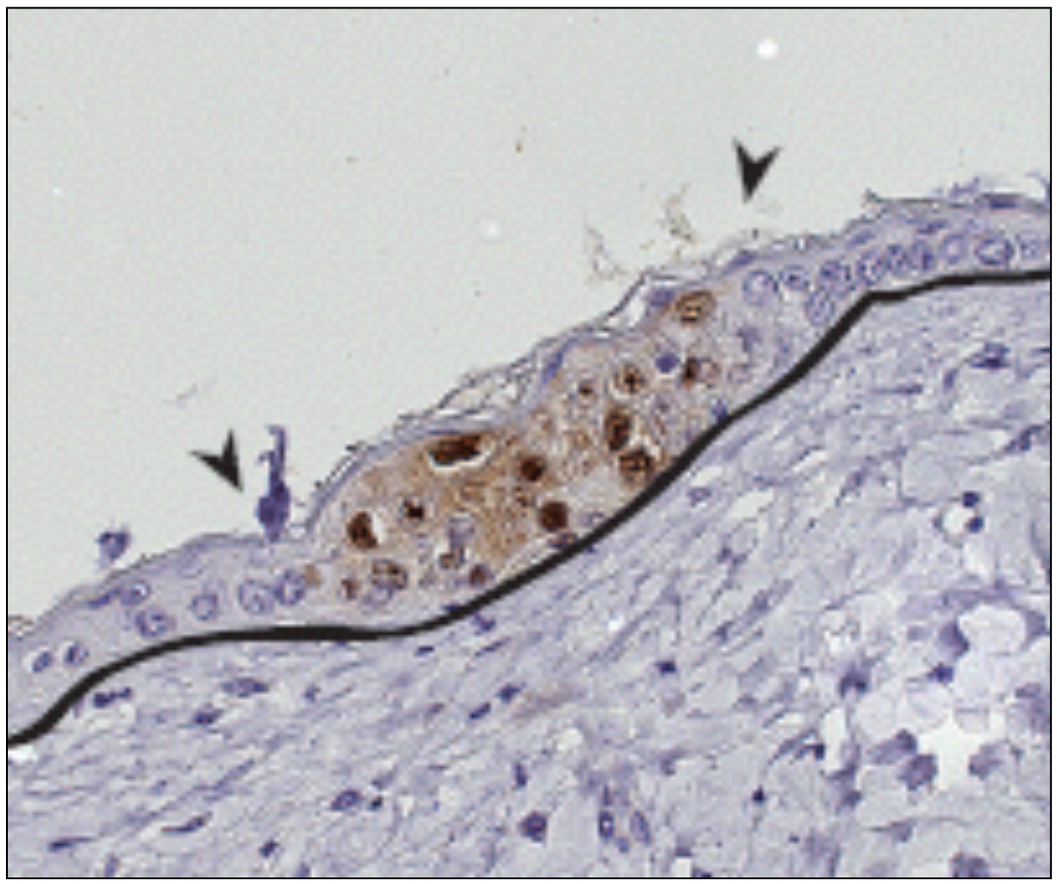
Infect epidermis with Herpes Simplex Virus (HSV-1)

Visualize infected skin + effector T cells

Silvia Ariotti & Ton Schumacher (NKI, Amsterdam)

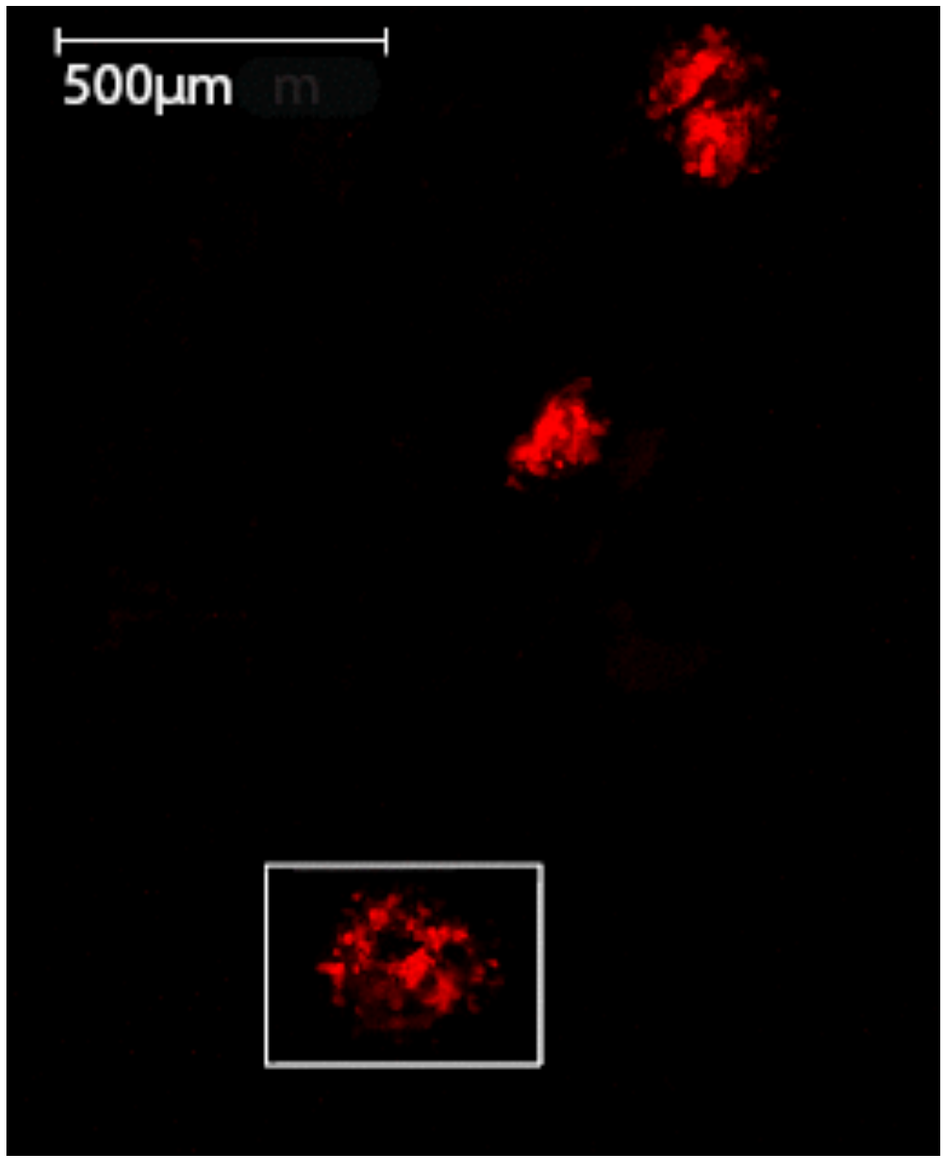
Patches of HSV infection

Immunohistochemistry staining with anti-HSV antibody

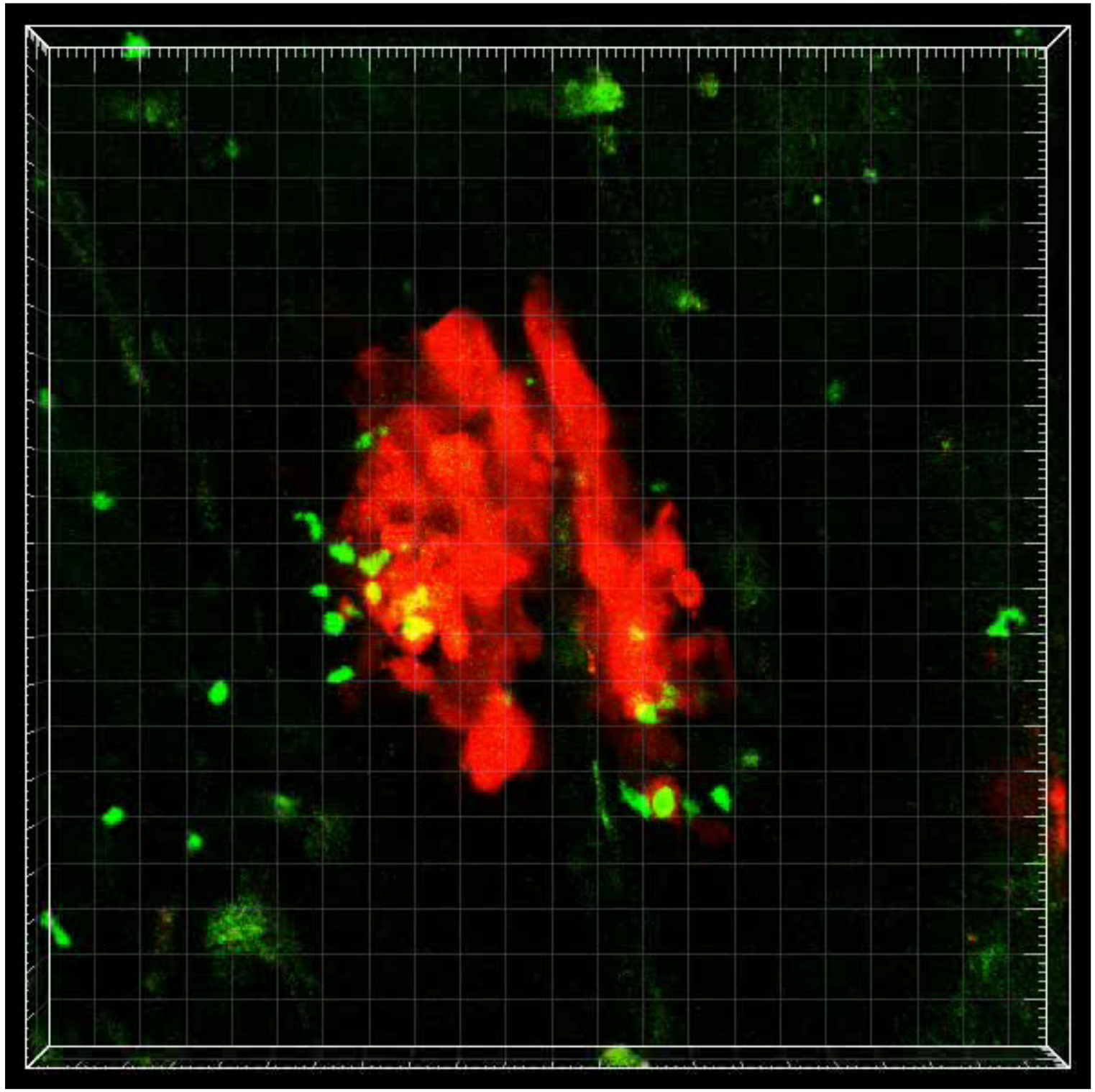


Black line: basal membrane

Confocal microscopy



Silvia Ariotti & Ton Schumacher (NKI, Amsterdam)



specific T cell

HSV (virus)

≈100 min

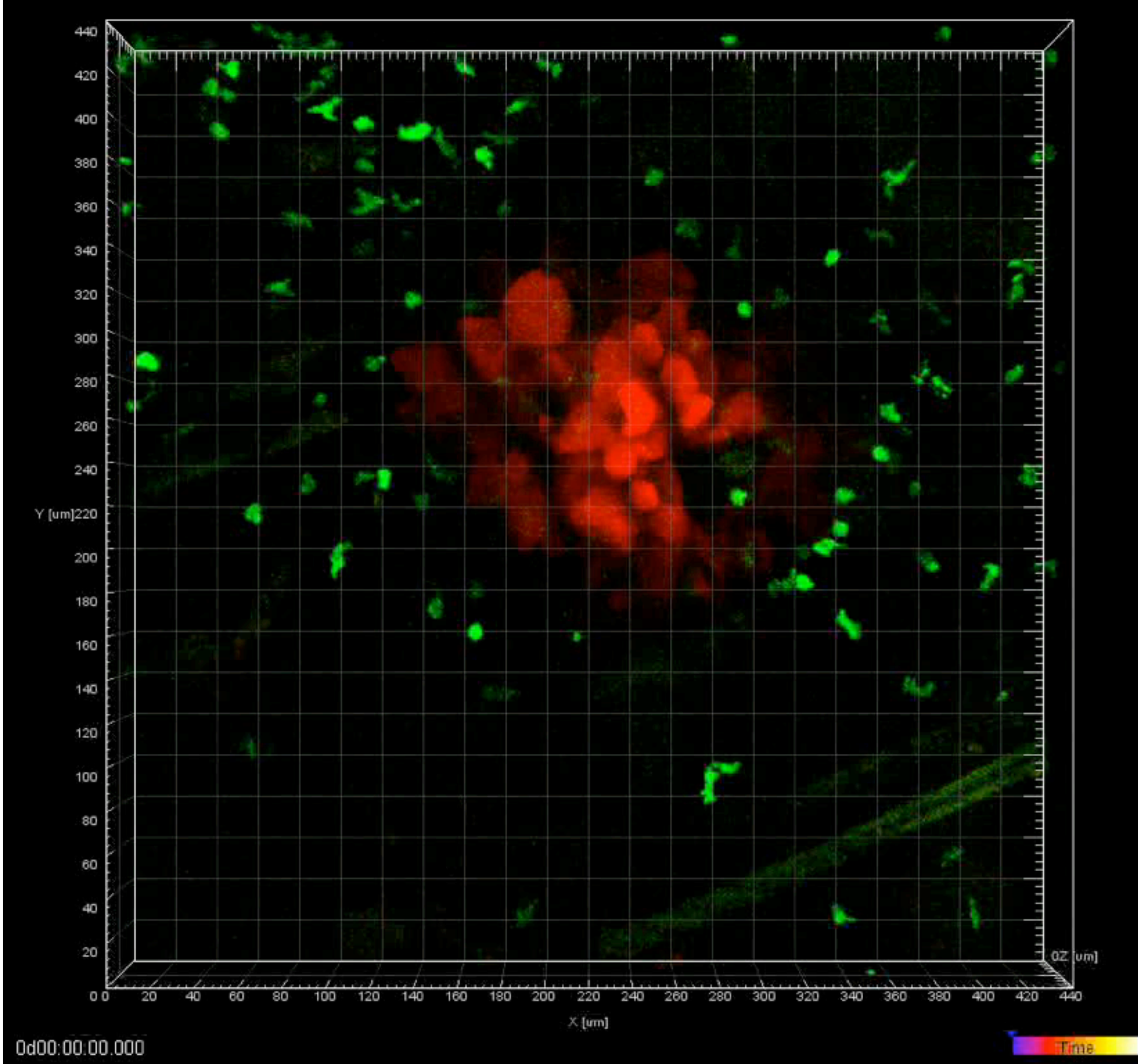
440x440x30µm

other T cell

HSV

≈60 min

440x440x35µm



How do T cells reach microlesions?

By random or directed migration?

Differences close to/far away from infection?

Differences by presence of matching antigen?

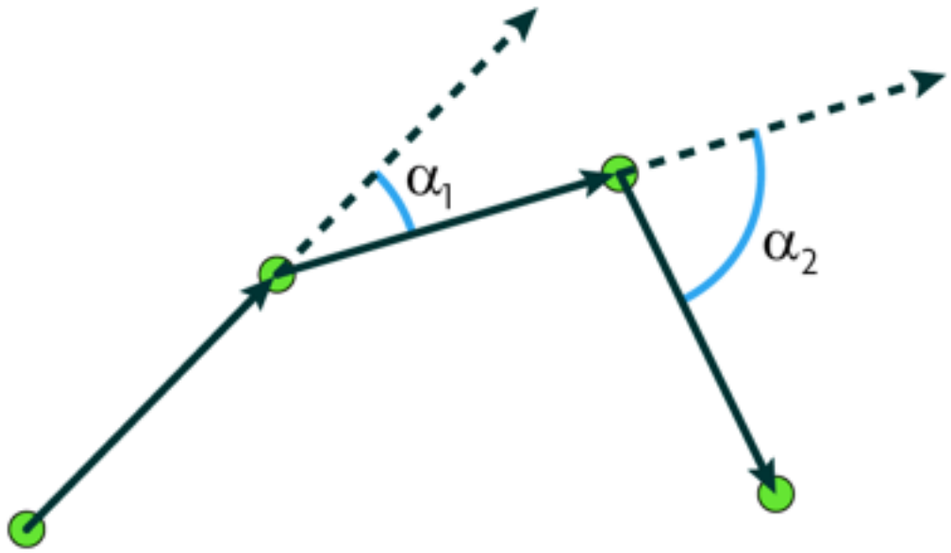
Not apparent from visual inspection.

Quantitative analysis on tracked cells is required

Calculate for each movement step:

1. speed

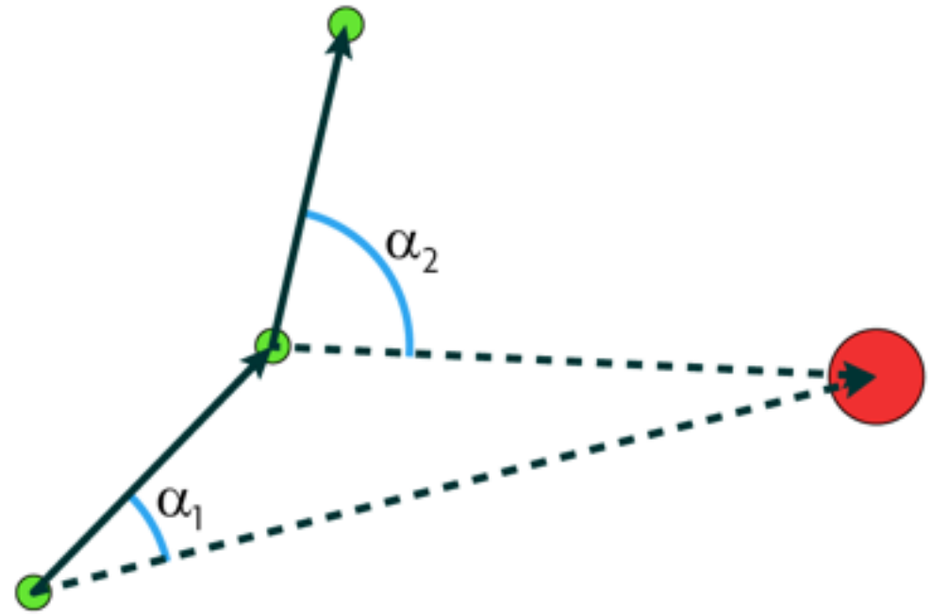
2. turning angle



Persistent motion:

<90 degrees

3. angle to infection



Random migration:

≈90 degrees

4. displacement towards infection

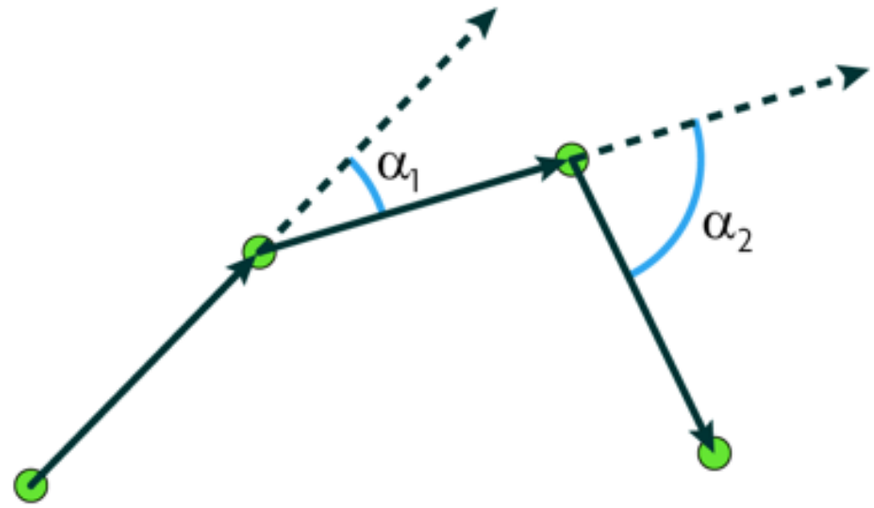
Project movement step onto vector toward infection

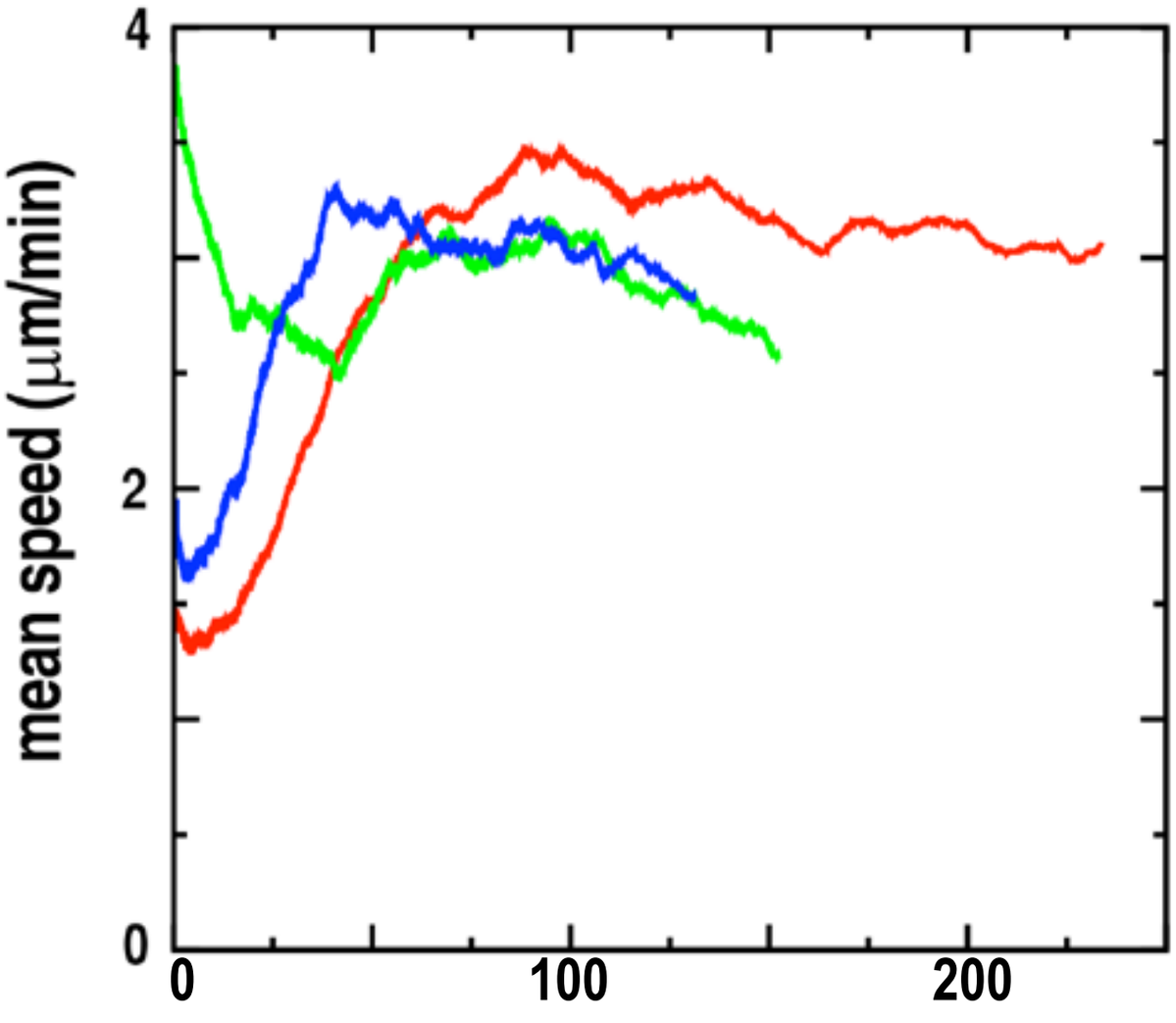
Antigen specific arrest

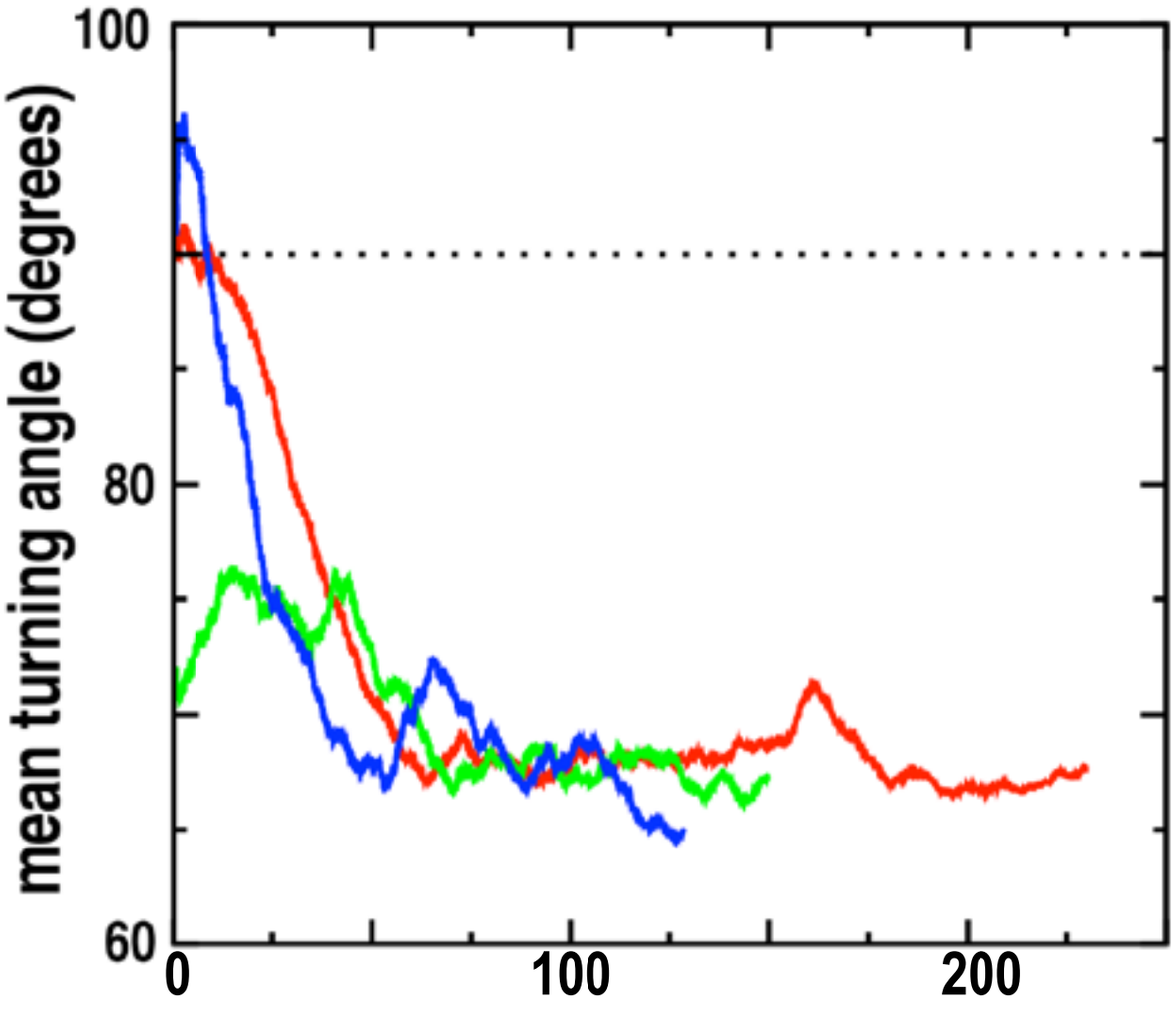
specific cell

non specific cell (OTI)

specific cell (OTI)

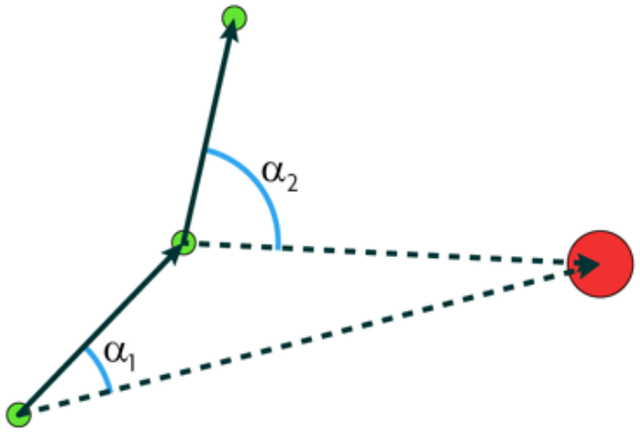






distance from infection (μm)

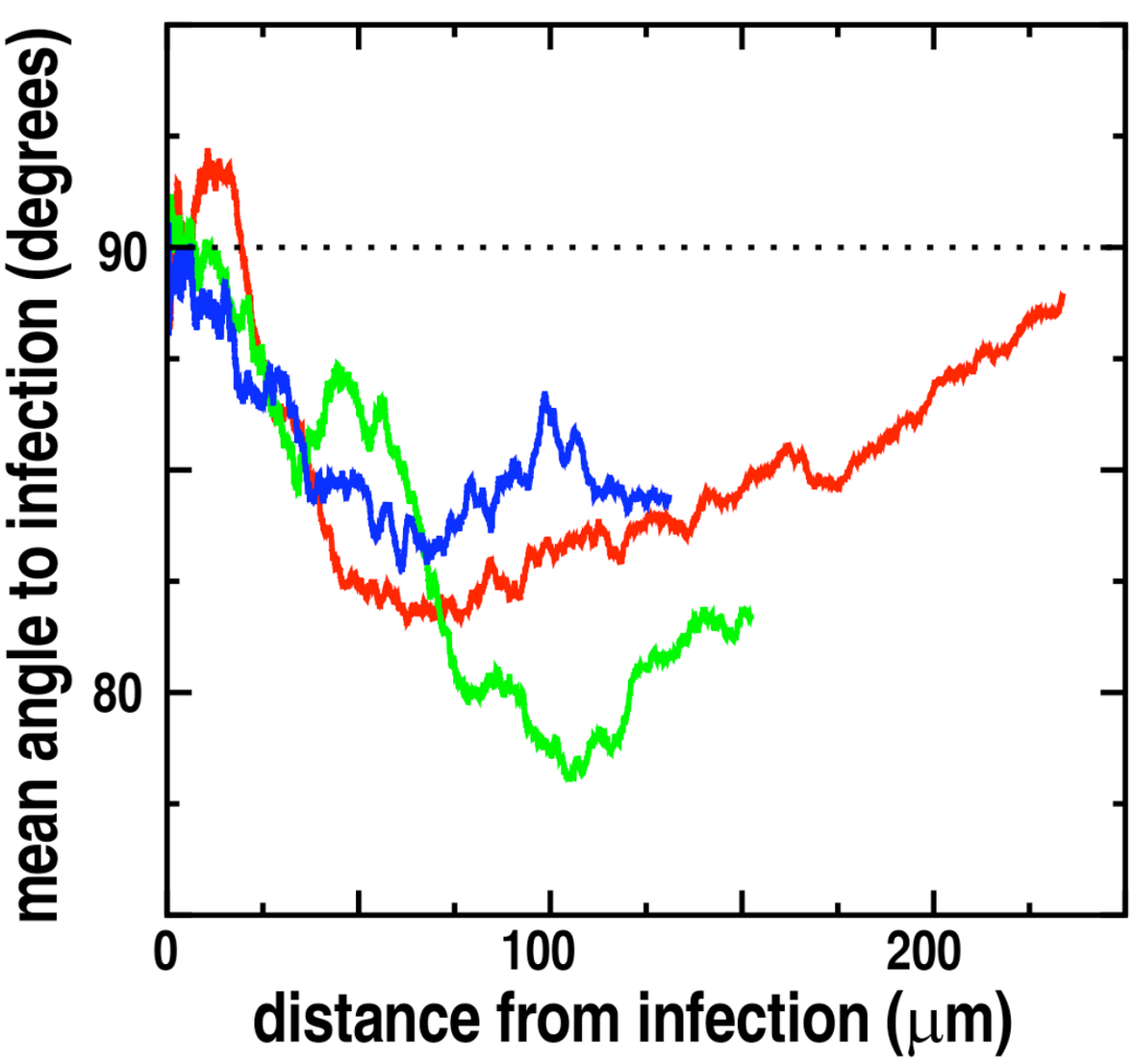
There is a small preference for all cells to migrate towards the microlesions

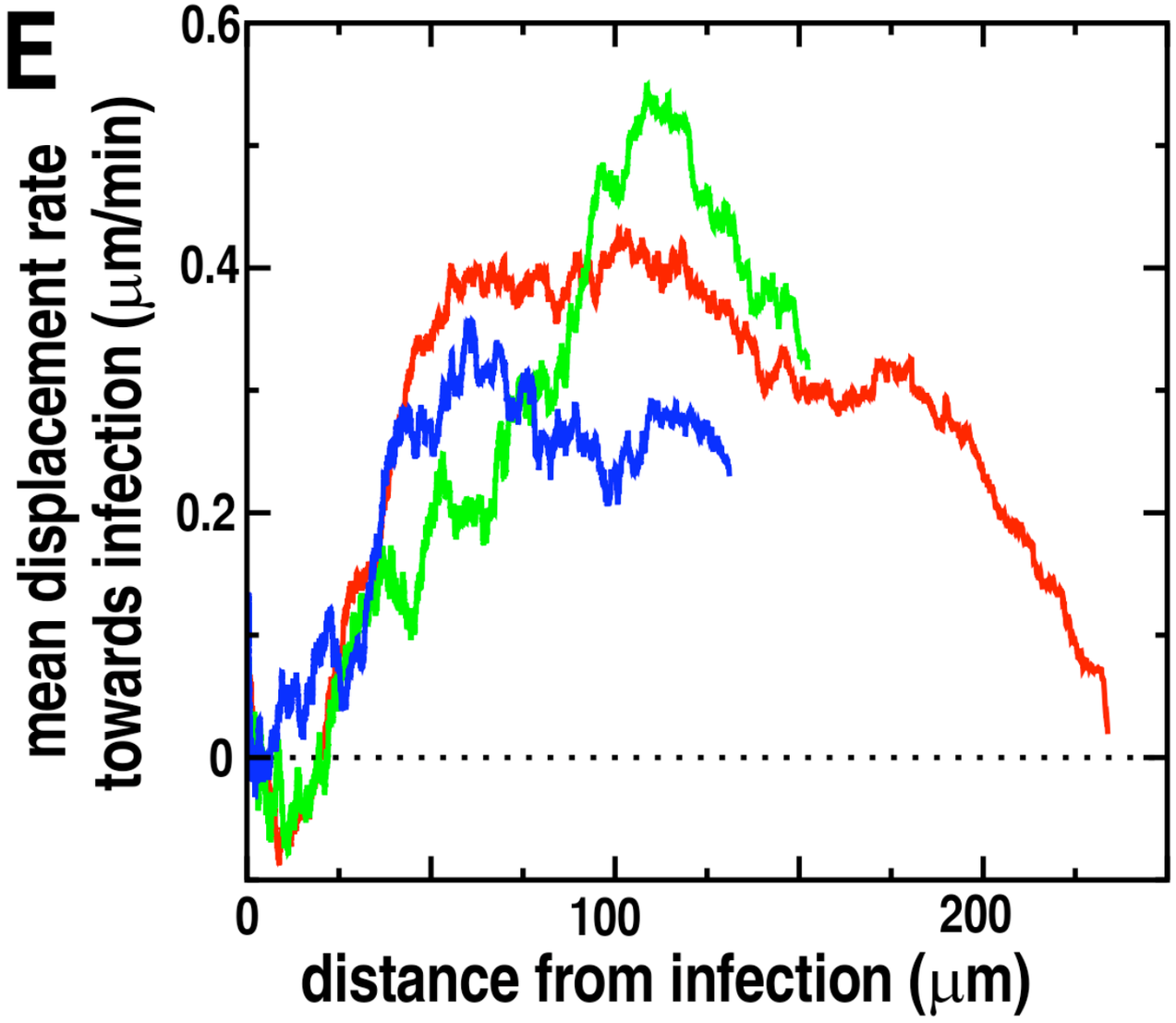


specific cell

non specific cell (OTI)

specific cell (OTI)





There is a small preference to travel towards the microlesions

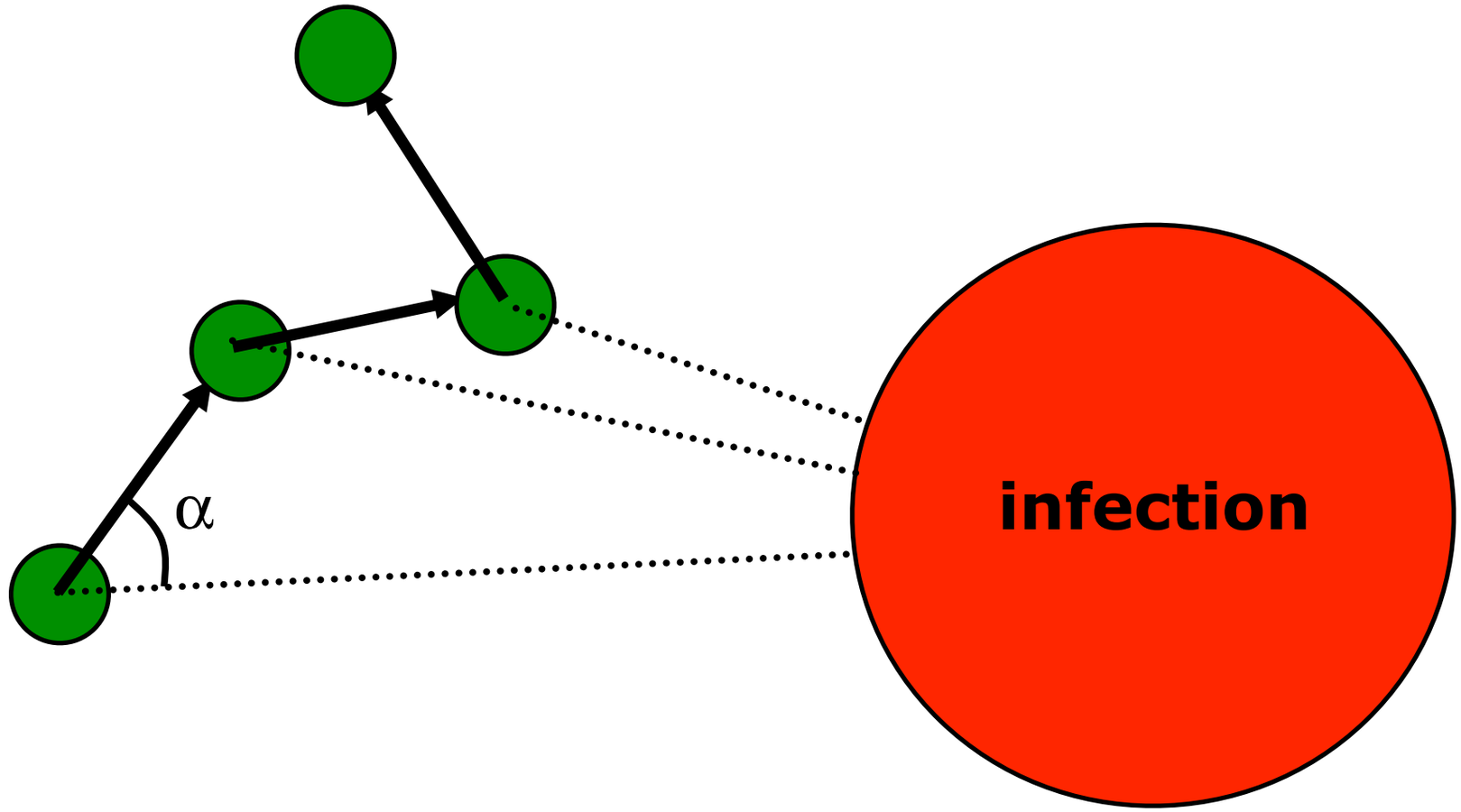
This is not antigen specific.

Difficult to appreciate in videos.

Is such a small preference relevant?

Model of cell migration to construct long tracks and estimate impact on arrival

Bootstrap the experimental data

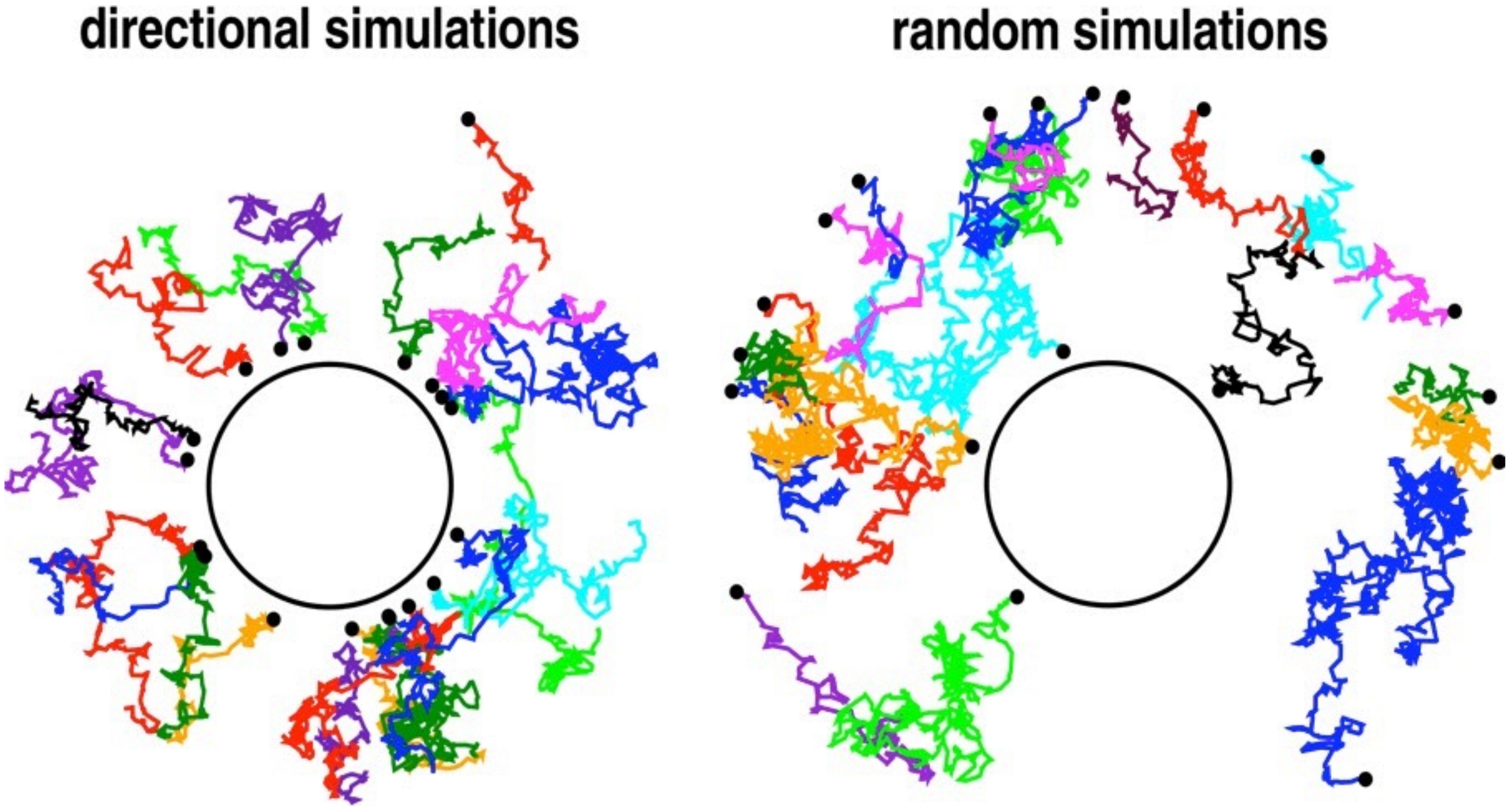


Choose speed + '**angle to infection**' combinations

Accept according to **turning angle** distribution

Combination depends on distance to infection

In silico 2D tracks (also 3D)



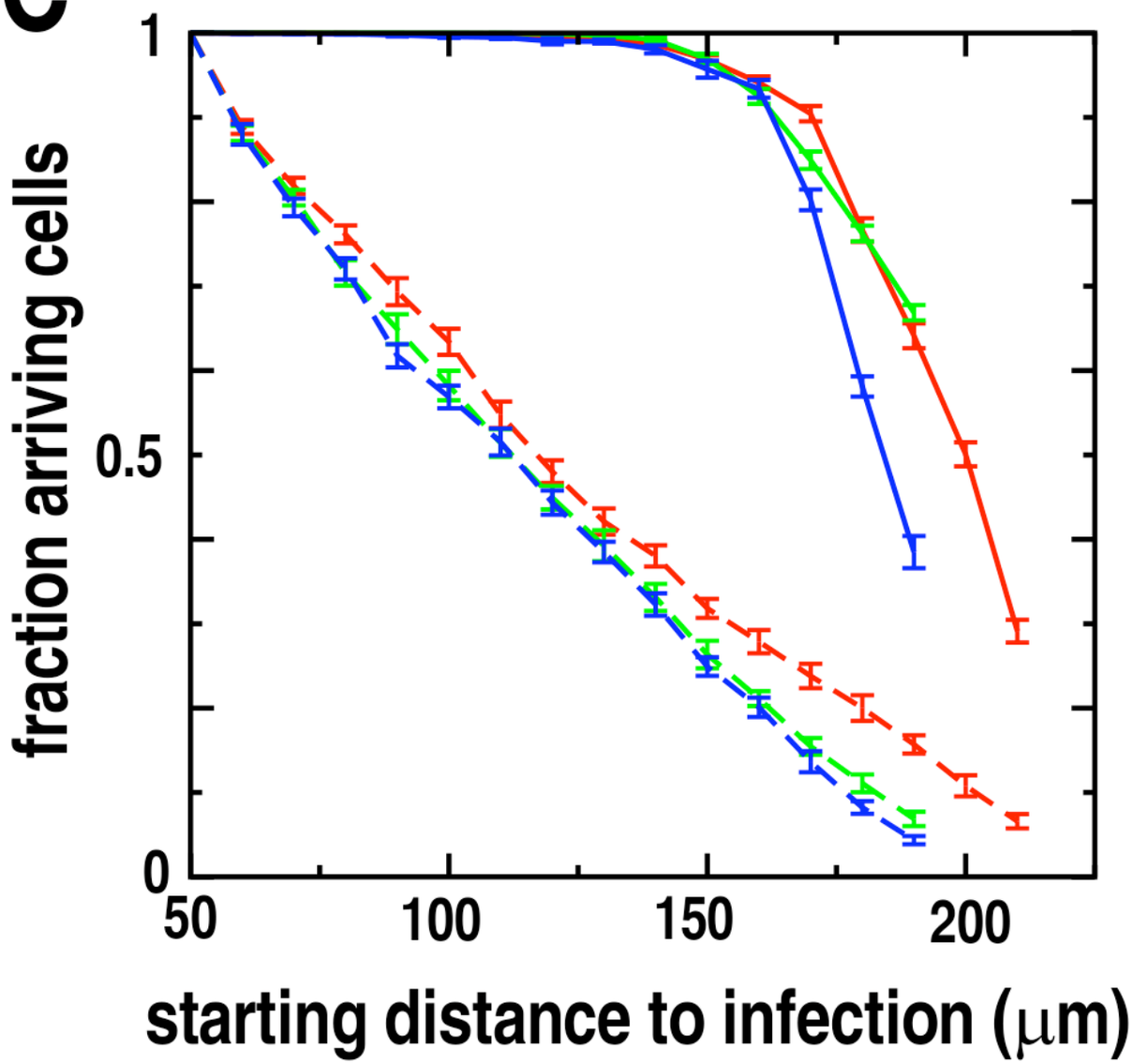
Random tracks: use random ‘angles to infection’ but maintain speed+persistence

Directionality strongly contributes to arriving at microlesions

specific cell
non specific cell (OTI)
specific cell (OTI)

directional simulation
random simulation

C



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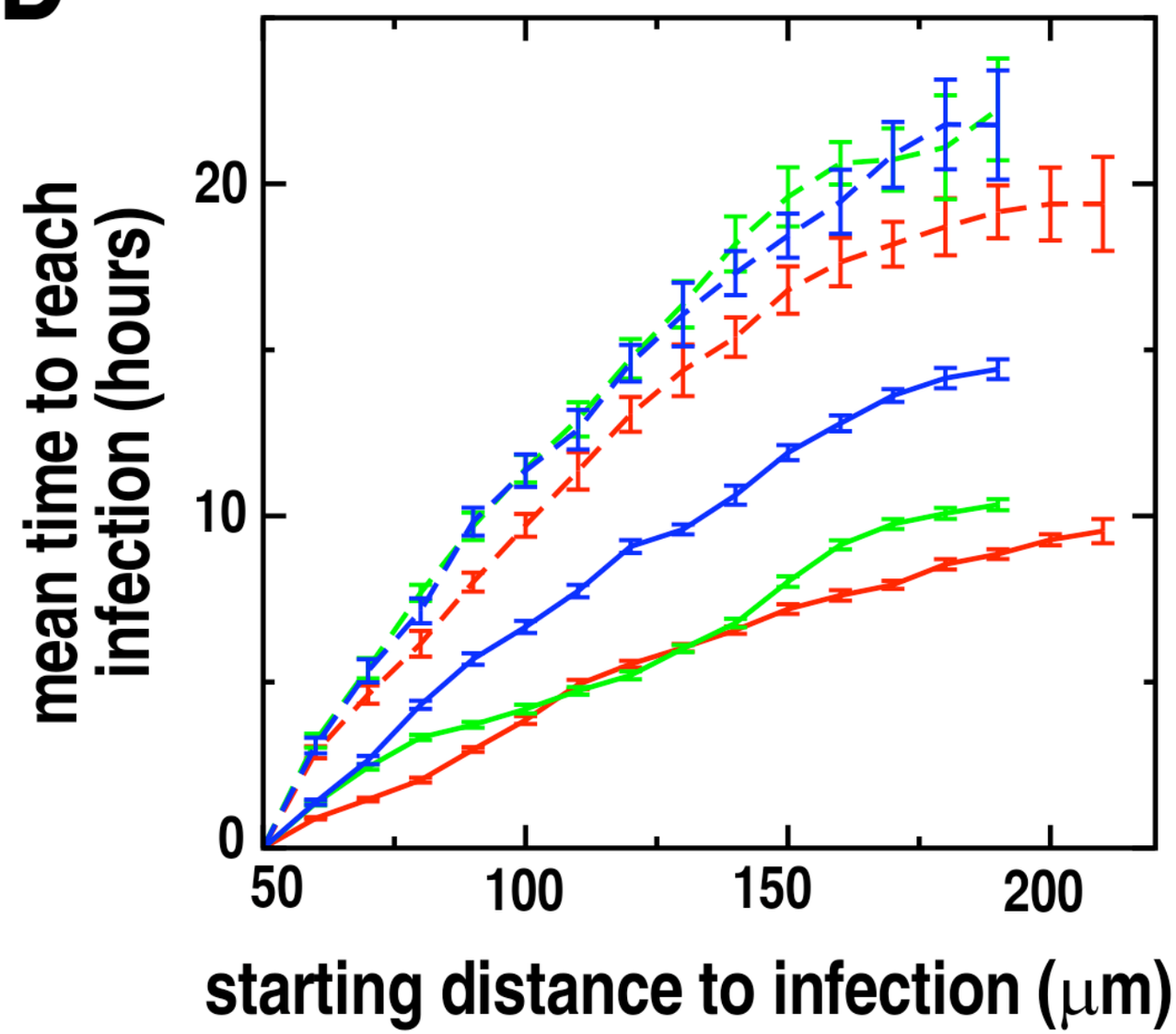

Conclusions

Stop-and-Go just due to collisions (not encoded)

Effector T cells are attracted towards microlesions independent of antigen specificity.

Small directionality allows a much larger fraction of cells to arrive faster at the site of infection.

Utrecht Center for Quantitative Immunology

Lymphocyte dynamics (modeling deuterium labeling)
life spans of naive and memory T cells

Lymphocyte migration (quantifying 2PM videos)
http://2ptrack.net/: open analysis tool

Epitope identification (NetMHCpan)
predict pMHC complexes of HIV and cancers

T cell repertoire sequencing (diversity)
RTCR: flexible pipeline with better recall than MiTCR

Universiteit Utrecht

http://tbb.bio.uu.nl/ucqi

Universitair Medisch Centrum
Utrecht