Statistical analysis of protein patternation on cell membranes during immunological synapse

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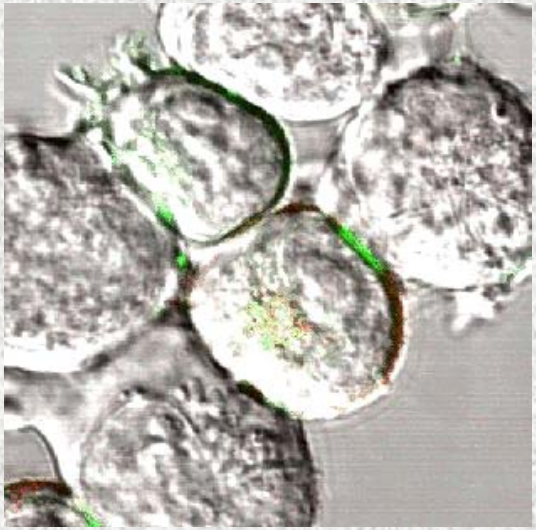
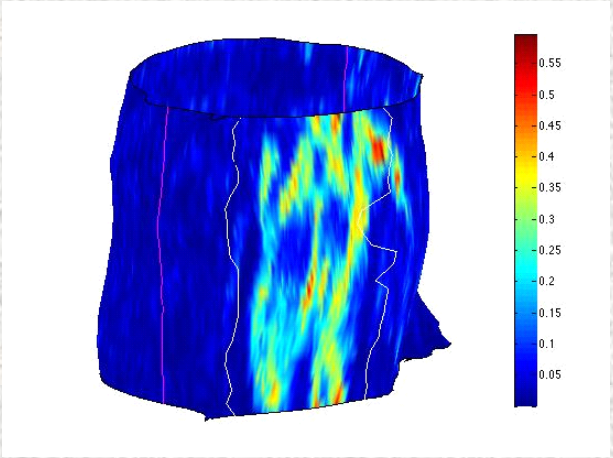
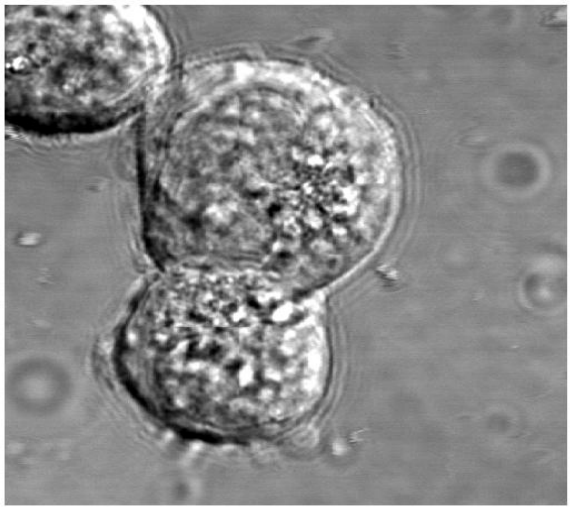


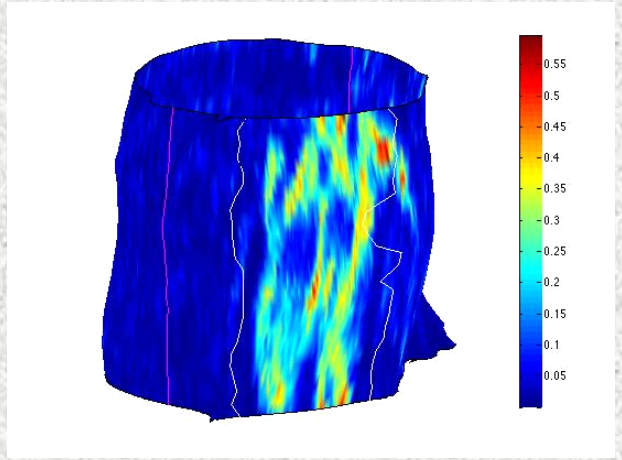


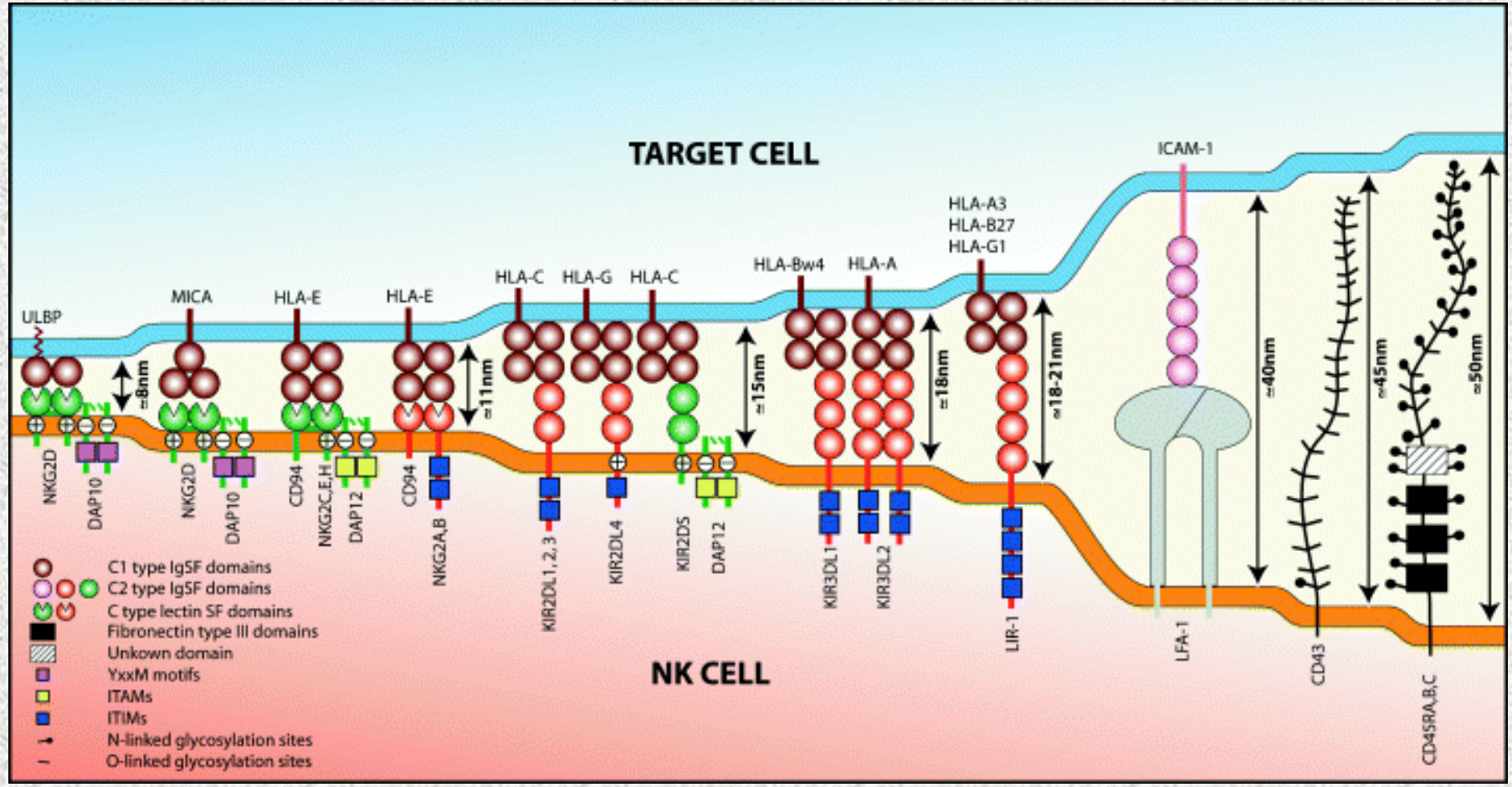
The immunological synapse



As a part of immune response a lymphocyte (immunological cell: T-cell, NK-cell) attaches to an antigen-presenting cell (APC).

The *immunological synapse* is the interface between a lymphocyte and an APC.

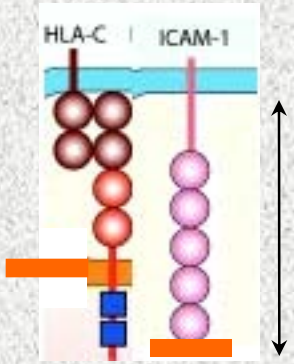




McCann et al Immunological reviews 189, 2002, 179.

KIR-HLA complex

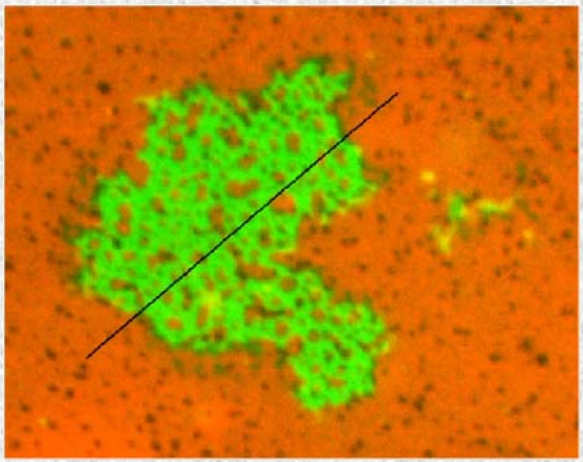
~12nm

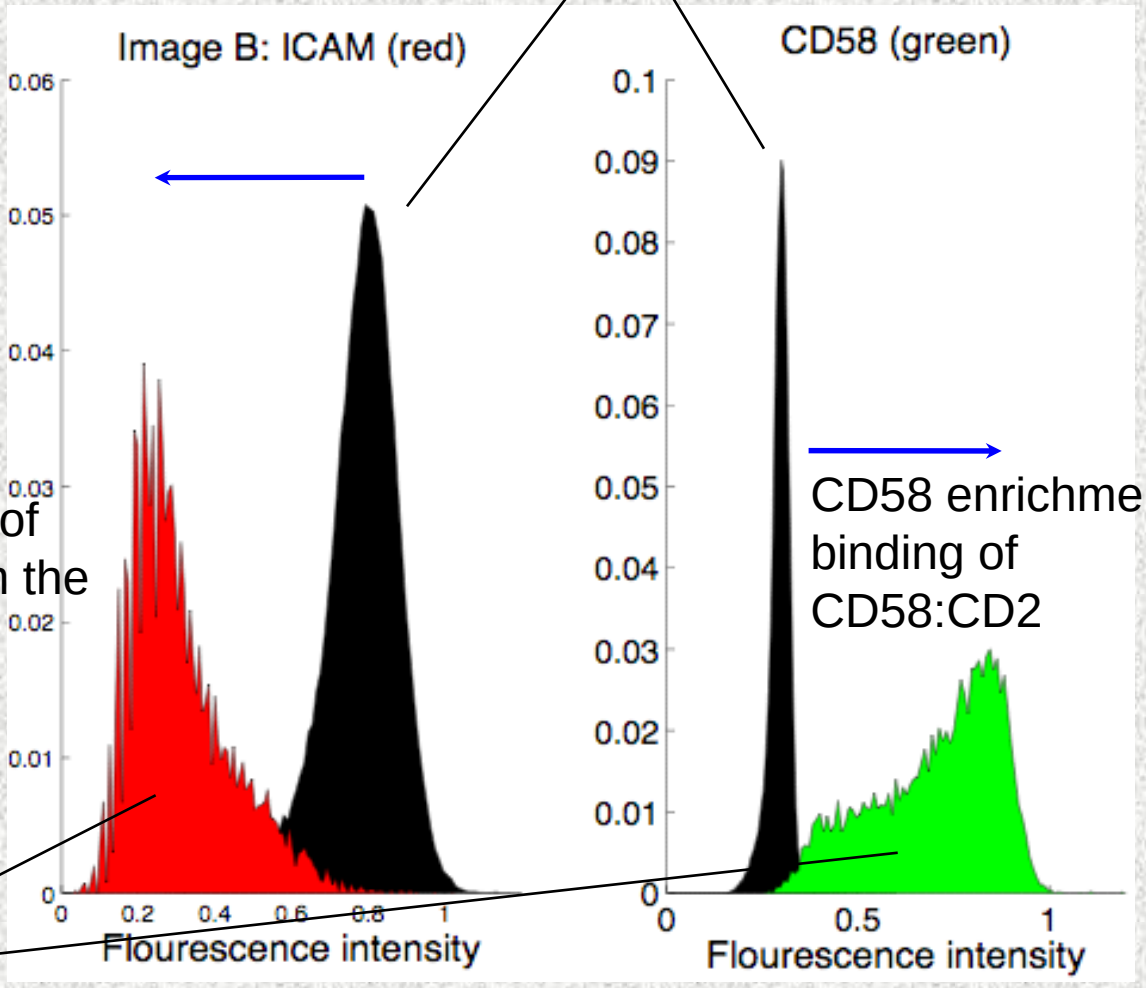


ICAM unbound

~18nm

The immunological synapse. T-cell data.



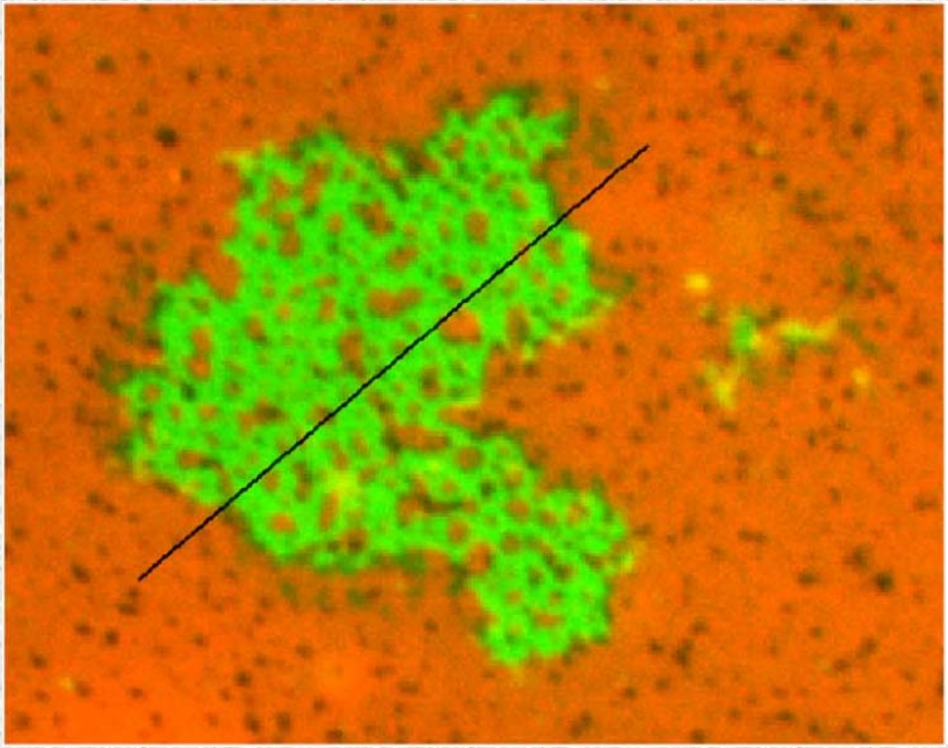


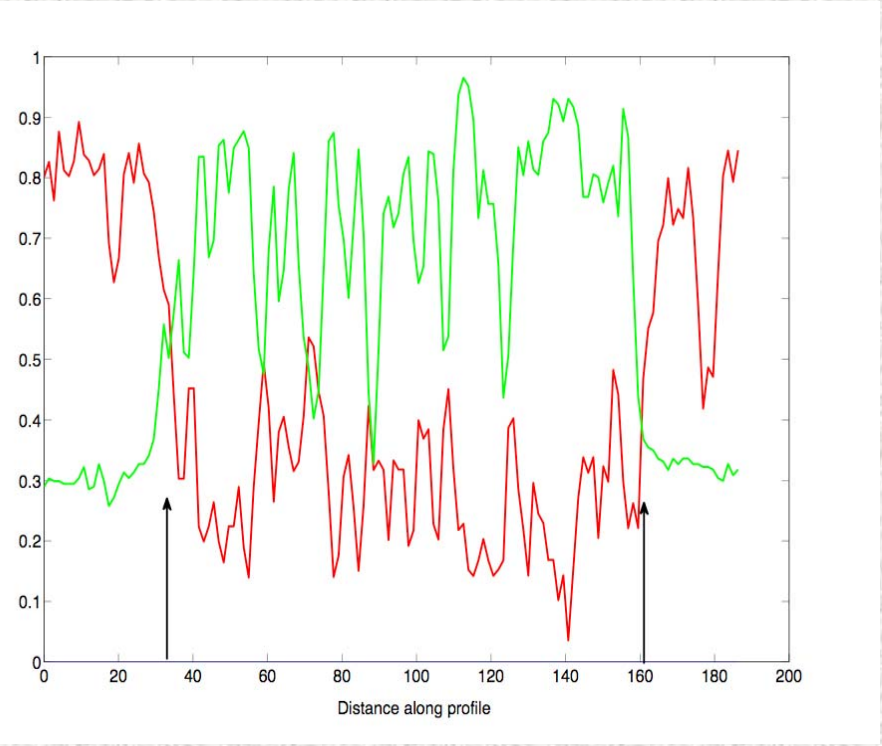
Exclusion of ICAM from the interface.

CD58 enrichment: binding of CD58:CD2

Contact interface

Synapse environment modeling and quantification





Two colour fluorescence image of a synapse pattern (Shaw and Dustin 1997) with labeled ICAM1 (Red) and CD58 (green). Segregation by size: CD58-CD2 ~13nm and ICAM-LFA1 ~40nm.

Synapse environment modeling and quantification

- Total green fluorescence is produced by free CD58 ligands and CD58:CD2 complexes:

$$\mathbf{E}\left(F_{1,FS}(x)\right) = \alpha_1 L_{1,\,free},$$
$$\mathbf{E}\left(F_{1,CI}(x)\right) = \alpha_1 \left(L_{1,\,free} + C(x)\right),$$

- Red fluorescence corresponds to ICAM1 and is only from unbound ligands:
-

$$\mathbf{E}\left(F_{2,FS}(x)\right) = \alpha_2 L_{2,\,free}.$$
$$\mathbf{E}\left(F_{2,CI}(x)\right) = \alpha_2 L_2(x).$$

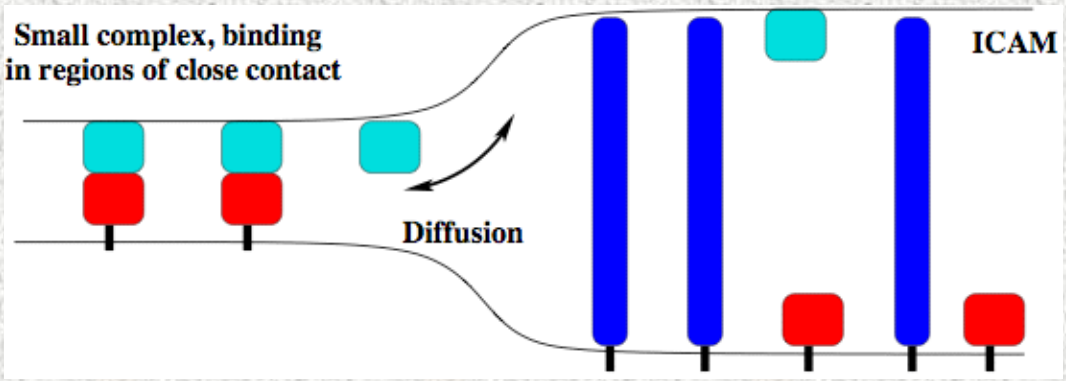
Here $L_{1,\,free}, C(x), L_{2,\,free}, L_2(x)$ are expected local molecular densities.

We use the Boltzmann distribution to determine the energy of ICAM and the CD58:CD2 complex in the area corresponding to pixel x

$$C(x) = C_{max}\exp\left(-G_{58:2}(x)\right)$$
$$L_2(x) = L_{2,\,free}\exp\left(-G_{ICAM}(x)\right)$$

C_{max} is maximum complex concentration, and $L_{2,free}$ is ICAM concentration on free surface

Exclusion by size.



The *hypothesis of exclusion by size* states that cell-surface molecules and molecule complexes are segregated (excluded) according to their size.

Here we model CD2:CD58 bond stretching and ICAM molecule compression as elastic springs:

$$G_{582}(x) = \frac{\kappa_{582}}{2k_BT}\left(z(x) - l_{582}\right)^2, \quad G_{ICAM}(x) = \frac{\kappa_{ICAM}}{2k_BT}\left(l_{ICAM} - z(x)\right)^2$$

l is the length of a molecule/complex
z is the local distance between the cell membranes,
κ is an elasticity parameter.

Problem statement

The hypothesis of exclusion by size results in the linear dependence between $g_1 = \sqrt{G_{58:2}}$ and $g_2 = \sqrt{G_{ICAM}}$:

$$\sqrt{\kappa_{ICAM}}\, g_1 + \sqrt{\kappa_{58:2}}\, g_2 = \left(\frac{\kappa_{58:2}\kappa_{ICAM}}{2\mathrm{k}_B T} \right)^{1/2} \left| l_{ICAM} - l_{58:2} \right|$$

Relating the variables g_1, g_2 ***with experimental fluorescence data we want to estimate*** g_1, g_2 ***and the parameters of the linear dependence***

Statistical inference

The dependence between the exclusion potentials and fluorescence intensities for a particular pixel x is assumed to be stochastic:

$$F_{1x} \sim \alpha_1 Poisson\left(L_1\left(1 + C_1' \exp\left(-g_{1x}^2\right)\right)\right),$$
$$F_{2x} \sim \alpha_2 Poisson\left(L_2 \exp\left(-g_{2x}^2\right)\right)$$

It is more convenient to work with continuous distributions so we switched to normal approximations of Poisson distribution

$$F_{1x} \sim Normal\left(\alpha_1 L_1\left(1 + C_1' \exp\left(-g_{1x}^2\right)\right), \alpha_1^2 L\left(1 + C_1' \exp\left(-g_{1x}^2\right)\right)\right),$$
$$F_{2x} \sim Normal\left(\alpha_2 L_2 \exp\left(-g_{2x}^2\right), \alpha_2^2 L_2 \exp\left(-g_{2x}^2\right)\right).$$

Bayesian approach. Separate analysis.

We have the conditional distributions

$$p\left(F_{1\mathrm{x}}\middle|\alpha_1, L_1, C_1', g_{1\mathrm{x}}\right), \quad p\left(F_{2\mathrm{x}}\middle|\alpha_2, L_2, g_{2\mathrm{x}}\right),$$

and data set $\left\langle F_{1x}, F_{2x}\right\rangle_{x\in X}$.

One approach is to estimate g_1, g_2 based on the posterior distributions

$$p\left(g_{1\mathrm{x}}\middle|F_{1\mathrm{x}}\right), \quad p\left(g_{2\mathrm{x}}\middle|F_{2\mathrm{x}}\right)$$

and then estimate the parameters of linear dependence.

As the global parameters are not known we will have to deal with the full joint distributions

$$p\left(\left\{g_{1\mathrm{x}}\right\}, \alpha_1, L_1, C_1'\middle|\left\{F_{1\mathrm{x}}\right\}\right), \quad p\left(\left\{g_{2\mathrm{x}}\right\}, \alpha_2, L_2\middle|\left\{F_{2\mathrm{x}}\right\}\right).$$

Introducing the dependence into the model. Joint analysis.

As an alternative to the separate reconstruction we can also introduce an extra equation describing the linear dependence into the model

$$\begin{cases} F_{1x} \sim Normal\left(\alpha_1 L_1\left(1+\tilde{C}_1 \exp\left(-g_{1x}^2\right)\right), \alpha_1^2 L\left(1+\tilde{C}_1 \exp\left(-g_{1x}^2\right)\right)\right), \\ F_{2x} \sim Normal\left(\alpha_2 L_2 \exp\left(-g_{2x}^2\right), \alpha_2^2 L_2 \exp\left(-g_{2x}^2\right)\right), \\ g_{2x} = a + b g_{1x}. \end{cases}$$

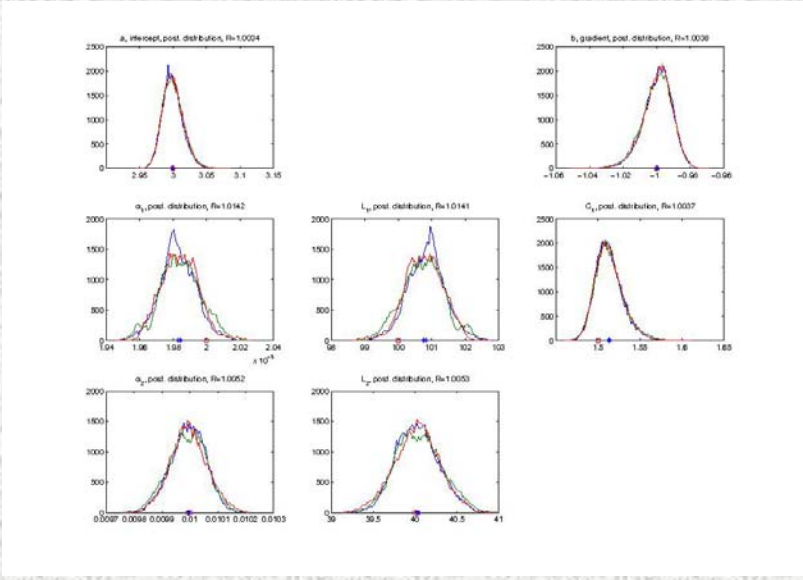
MCMC inference algorithm

In case of separate analysis to estimate the variables g_1, g_2 we sample their values from the posterior distribution using *single-component Metropolis - Hastings MCMC* algorithms separately for different fluorescence channels.

For the joint analysis the coefficients of the linear dependence are the parameters of the model and are estimated with the other unknown parameters. A joint MCMC algorithm is developed to analyse the fluorescence channels together.

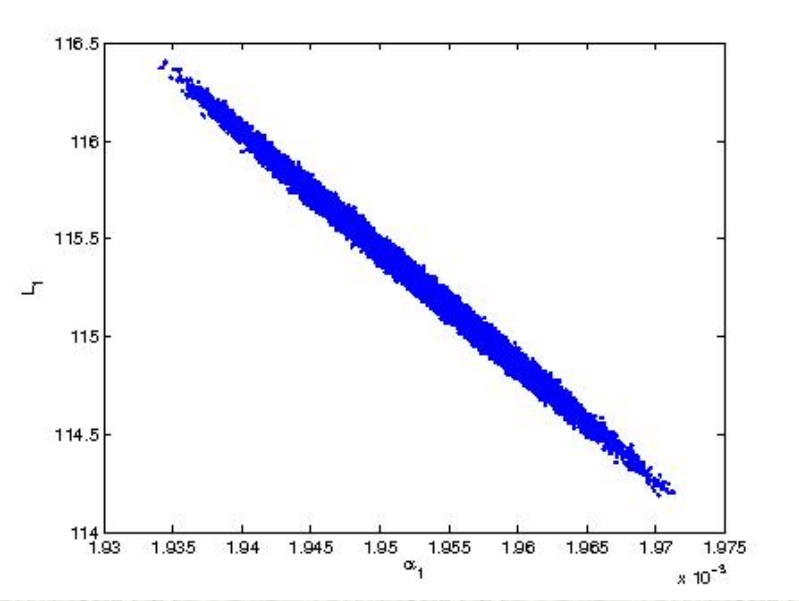
The full conditional distributions for the components are not analytically tractable therefore a *random walk algorithm* is used to update the variables with normally distributed steps. The variance for the RW steps is “tuned” during burn in period.

MCMC algorithm. Tests and improvements.



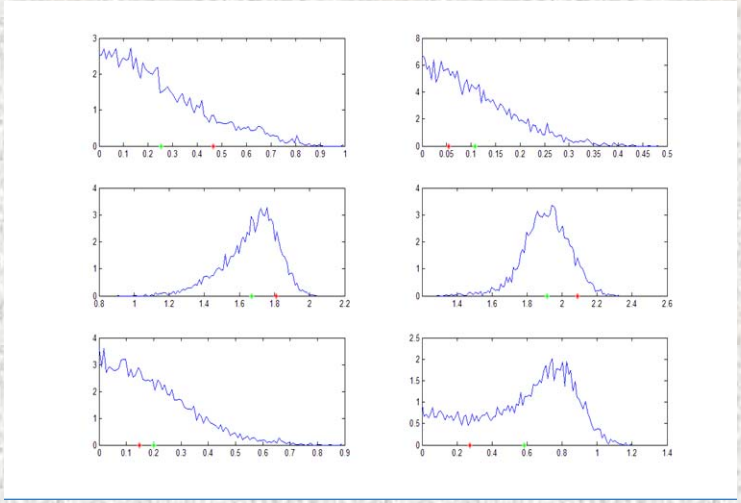
Preliminary test were run on simulated data to assess the quality of parameter reconstruction and possible convergence problems.

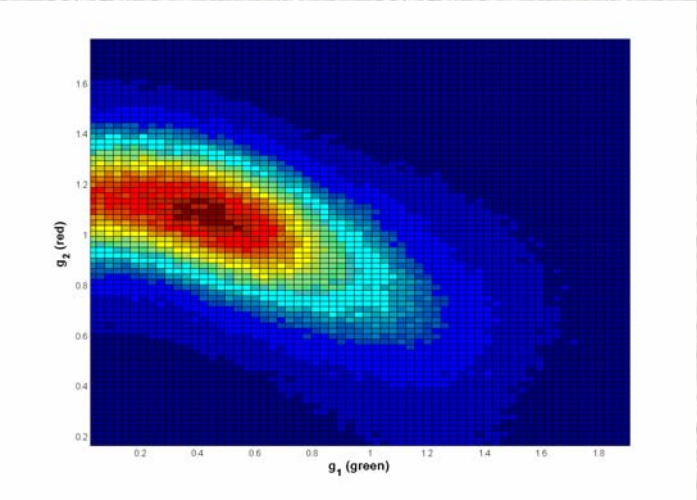
Some parameters showed very strong correlation that results in slow convergence in case of single-component update. To improve convergence the variables are updated along the dependence directions that are estimated during the burn in period.



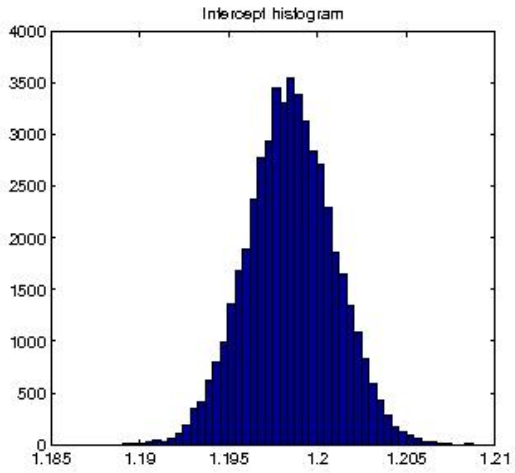
Output of the MCMC algorithm

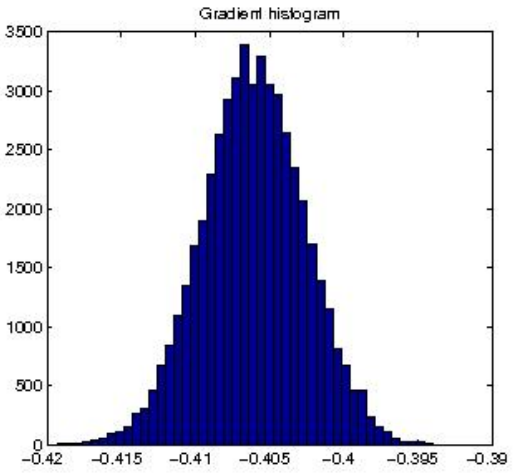
As a result we obtain samples from the posterior distributions for the variables g_1, g_2 that can be used afterwards to estimate the parameters of the linear dependence:

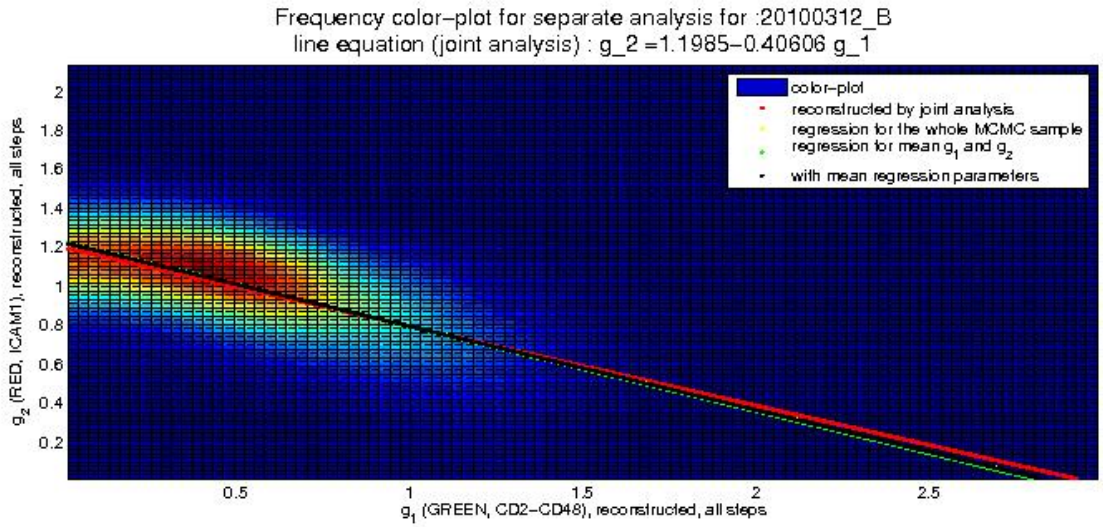




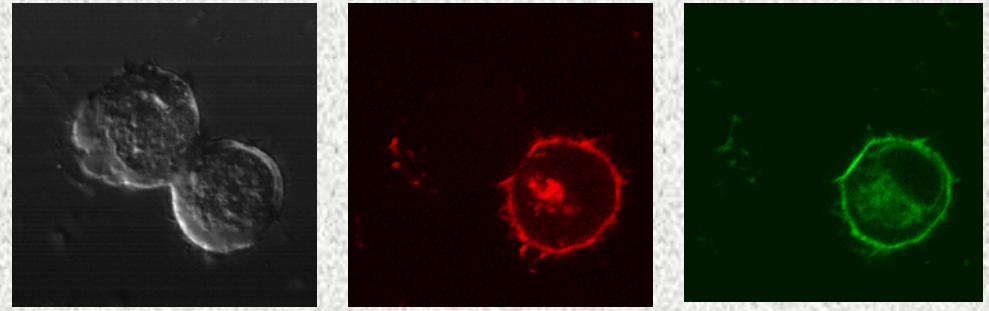
Results of separate and joint analysis







Extending to 3D stacks. NK-cell data



We track the surface and measure fluorescence in the contact region and on a free surface.

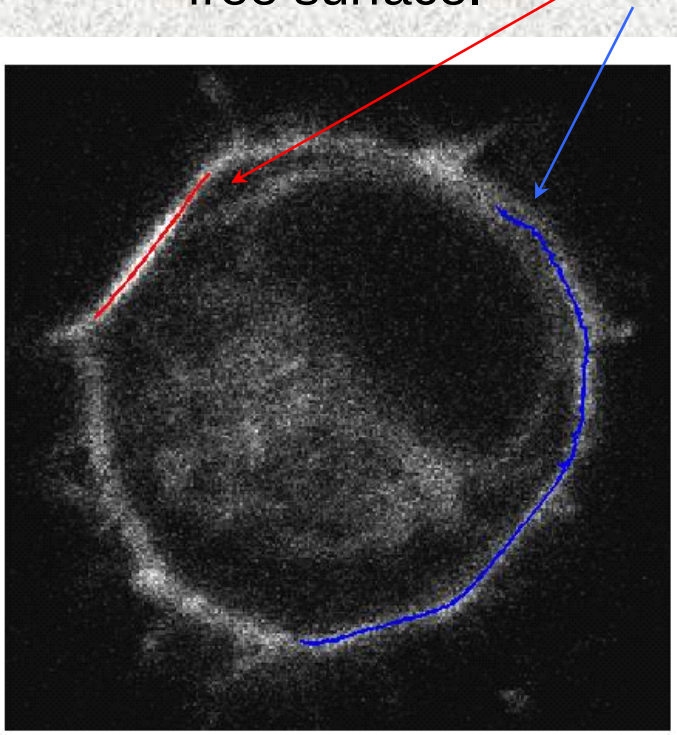
NK cells with 221 cells.

We have 3D scanning confocal images in 2 fluorophores:

ICAM-Cherry (red)

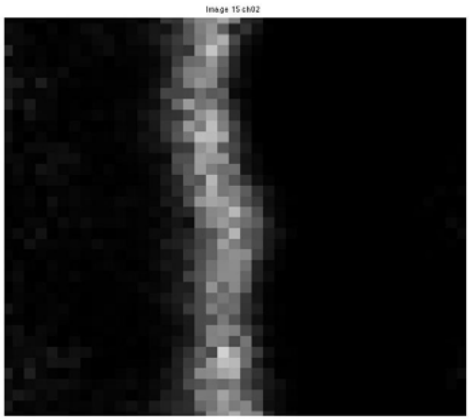
HLA-cw6-GFP (green)

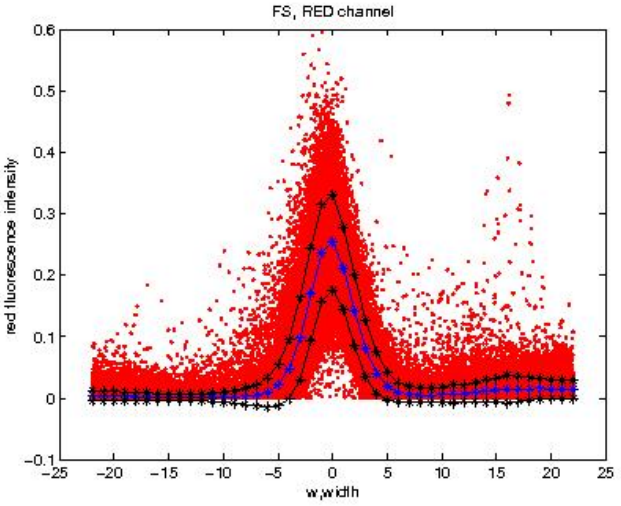
from which we extract exclusion energies.



(s,w) data representation

Switching to stack data we have to switch from 2d surface densities to 1d slice surface densities. The main problem is how to preprocess fluorescence data as the fluorescence is distributed across the cell membrane.





We introduced the concept of membrane width:

$$L(s,w) = L(s)\exp\left(-\frac{\tau(s)w^2}{2}\right)$$

where the parameter $\tau(s)$ characterizes the width of the membrane at the location s.

Membrane width modeling

- Green fluorescence is produced by free ligands on the free surface of the cell and by free ligands together with the complexes in the contact interface:

$$\mathbf{E}\left(F_{1,FS}(s,w)\right) = L_{1,\,free}\exp\left(-\frac{\tau_{FS}(s)w^2}{2}\right),$$

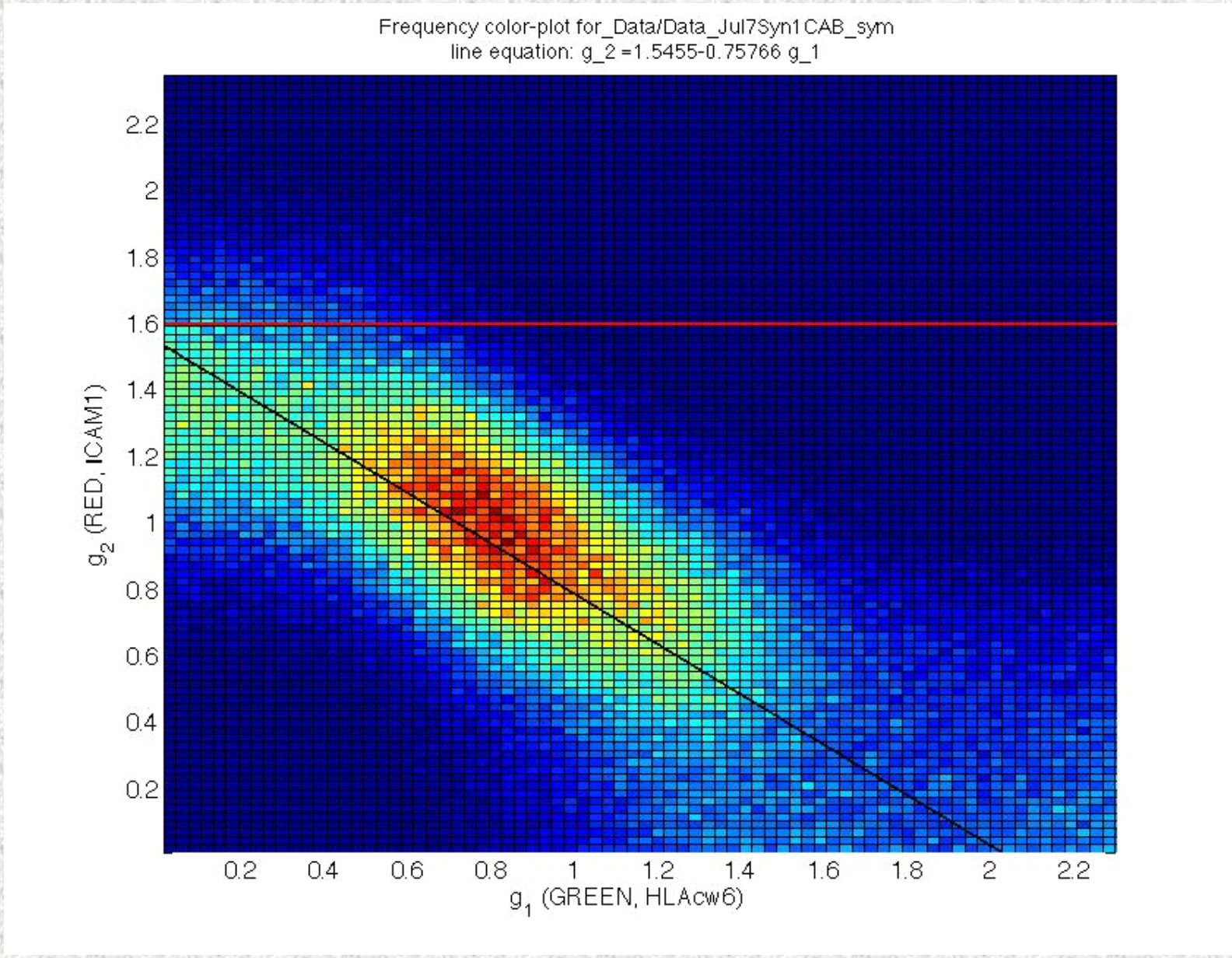
$$\mathbf{E}\left(F_{1,CI}(s,w)\right) = \left(L_{1,\,free} + C_{\max}\exp\left(-G_1(s)\right)\right)\exp\left(-\frac{\tau_{CI}(s)w^2}{2}\right)$$

- Red fluorescence is only from the unbound ICAM1 ligand, which is freely diffusing on the free surface and partly excluded in the contact interface:

$$\mathbf{E}\left(F_{2,FS}(s,w)\right) = L_{2,\,free}\exp\left(-\frac{\tau_{FS}(s)w^2}{2}\right),$$

$$\mathbf{E}\left(F_{2,CI}(s,w)\right) = L_{2,\,free}\exp\left(-G_2(s)\right)\exp\left(-\frac{\tau_{CI}(s)w^2}{2}\right)$$

g_1 vs g_2 plot for NK-cell data



Collaborators:

Functional characterisation and transformants: Anton van der Merwe, Shiqiu Xiong (Sir William Dunn, Oxford)

Imaging and transformants: Dan Davis, Karsten Kohler (Division of Cell and Molecular Biology, Imperial)

Keith Gould (Faculty of Medicine, Imperial).

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