Two colour single particle tracking: methods and applications

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Annual Report III year

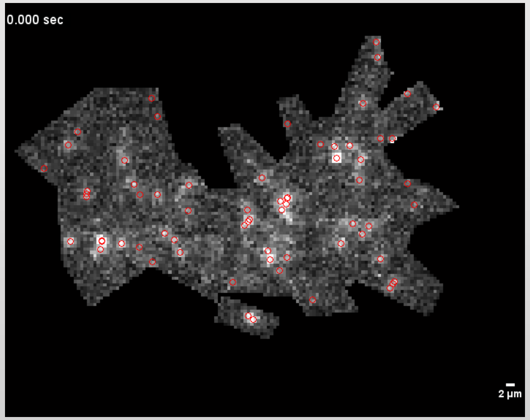
Supervisor Dr.Stefano Luin

Message passing parallel approach

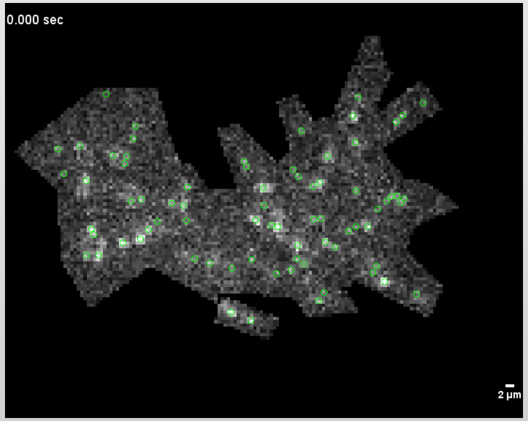
MatLab SPMD: single program multiple data

Client

Worker 1

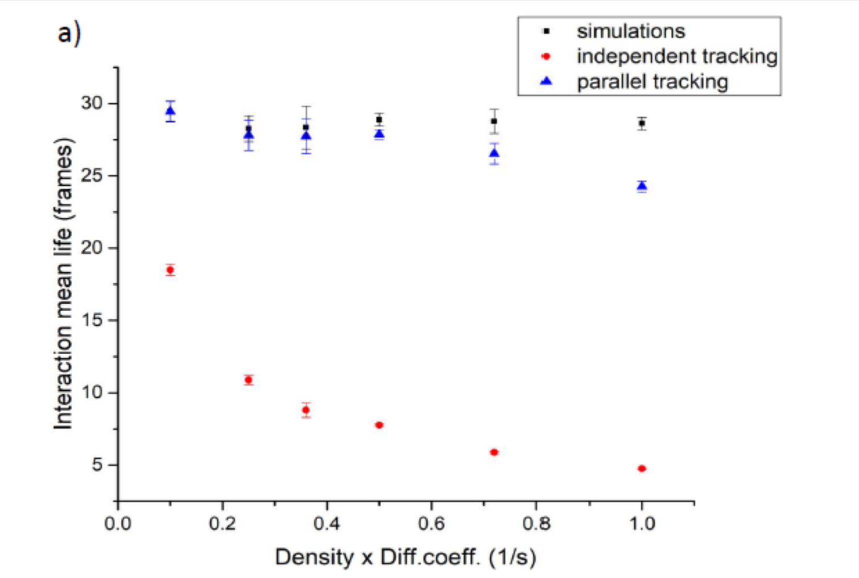


Worker 2



LabSendReceive:
explicit managment of communication

Severe underestimation of interaction mean lifetime with an independent tracking in the two channels

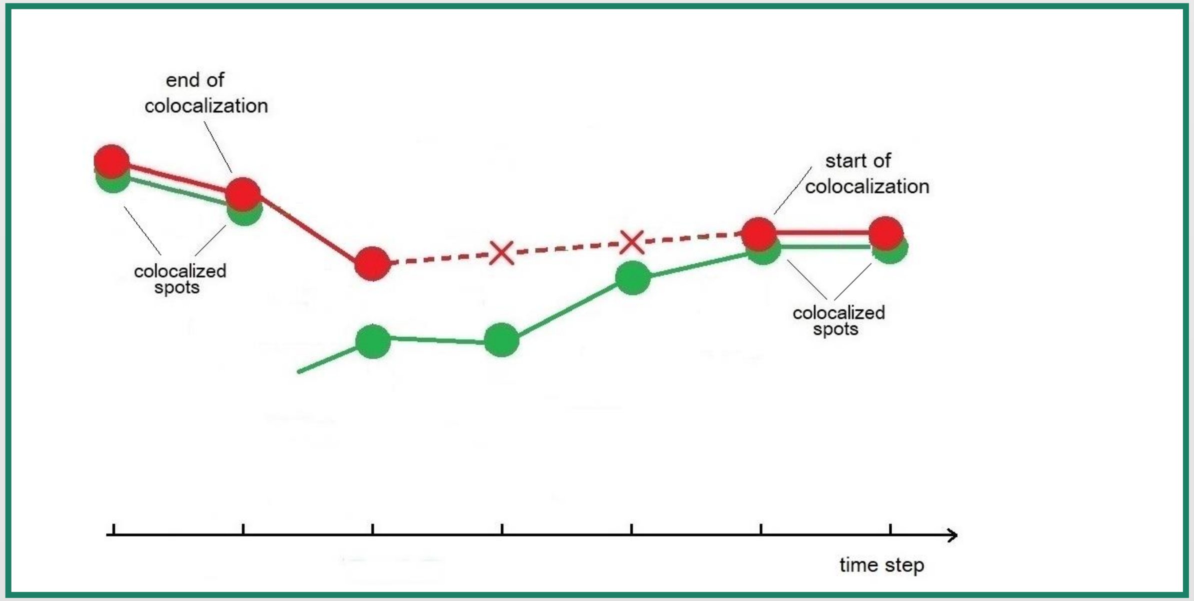


Mean life of the interaction (mean± std.dev.) versus the product of density and diffusion coefficient.

Method presented in an oral presentation at SPAOM19 Spanish and Portuguese Advanced Optical Microscopy (November 2019)

Inside a detail of the algorithm

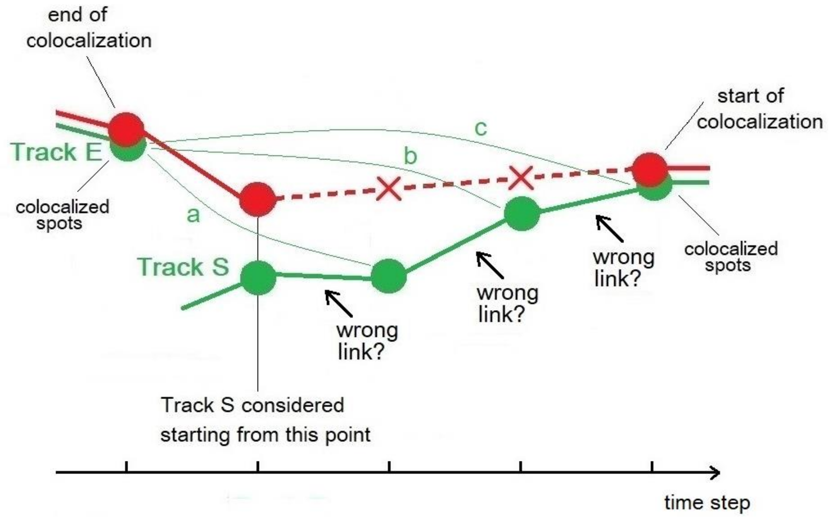
In the Sending channel



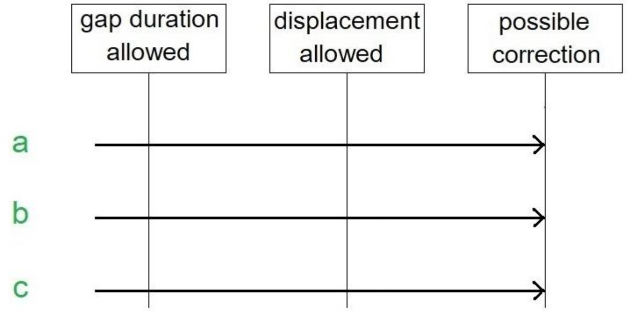
Inside a detail of the algorithm

In the Receiving channel

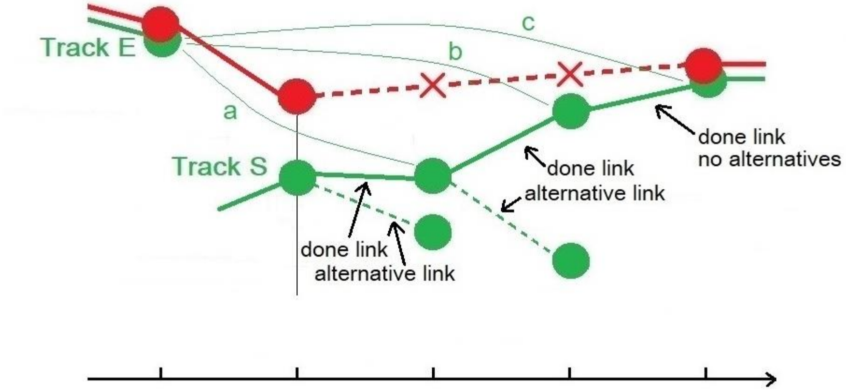
1. Find all possible connections



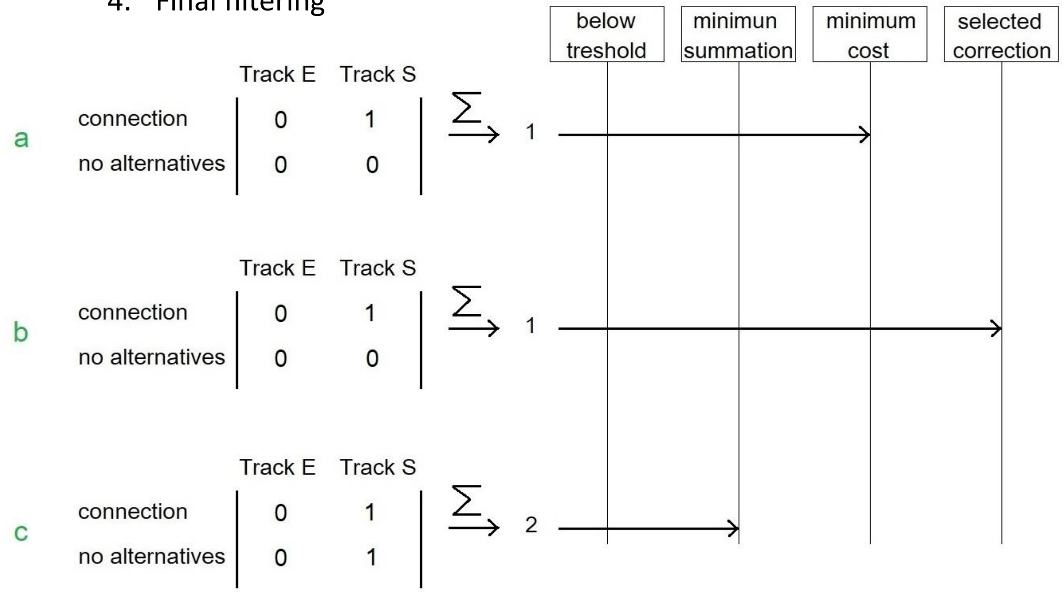
2. Filtering



3. Check alternative connections



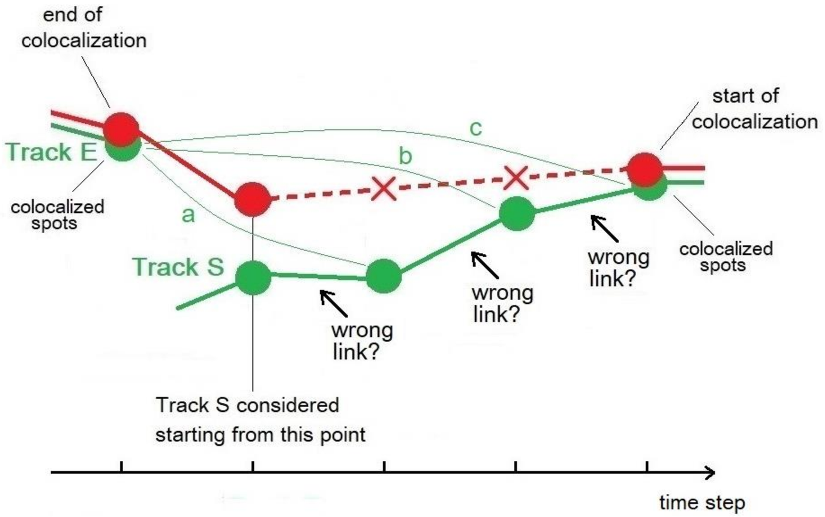
4. Final filtering



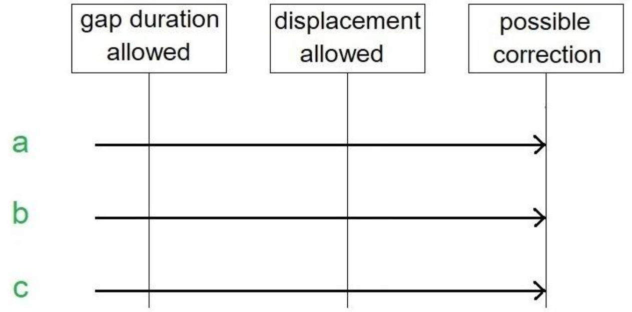
Inside a detail of the algorithm

In the Receiving channel

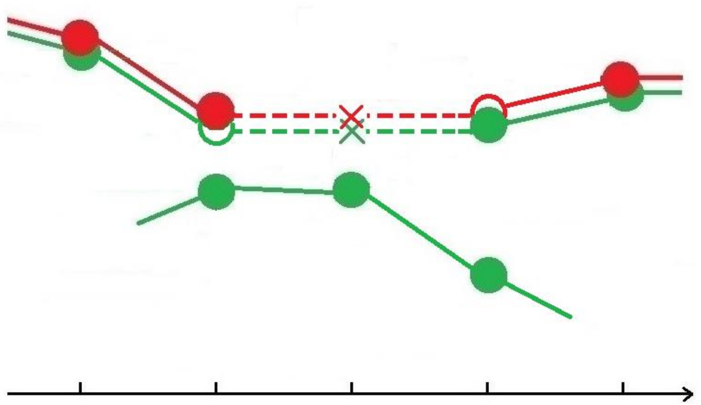
1. Find all possible connections



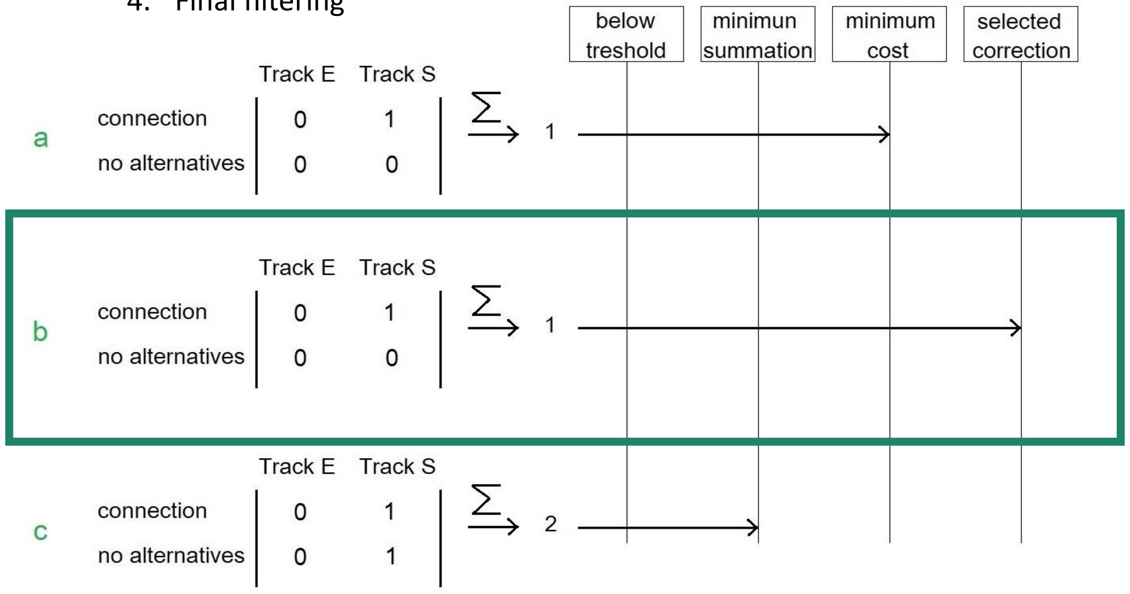
2. Filtering



Reconstructed interaction



4. Final filtering



Post tracking analysis

Random colocalization

Convolution of random and true colocalization

$$f(x) = p_1\lambda_1 e^{-\lambda_1 x} + p_2 \frac{\lambda_2\lambda_3}{\lambda_2 - \lambda_3}\left(e^{-\lambda_3 x} - e^{-\lambda_2 x}\right)$$

$$\lambda_3 \cong \lambda_1$$

$$y(t) = \int_t^{+\infty} f(x) = \left(p_1 + \frac{p_2\lambda_2}{\lambda_2 - \lambda_1}\right)e^{-\lambda_1 t} - \frac{p_2\lambda_1}{\lambda_2 - \lambda_1}e^{-\lambda_2 t}$$

Biexponential fitting $Ae^{-\lambda_1 t} + Be^{-\lambda_2 t}$

Estimation of interaction off-rate $k_{off} = \lambda_2 - k_{bd1} - k_{bd2}$

Tracks breaks in the two channel

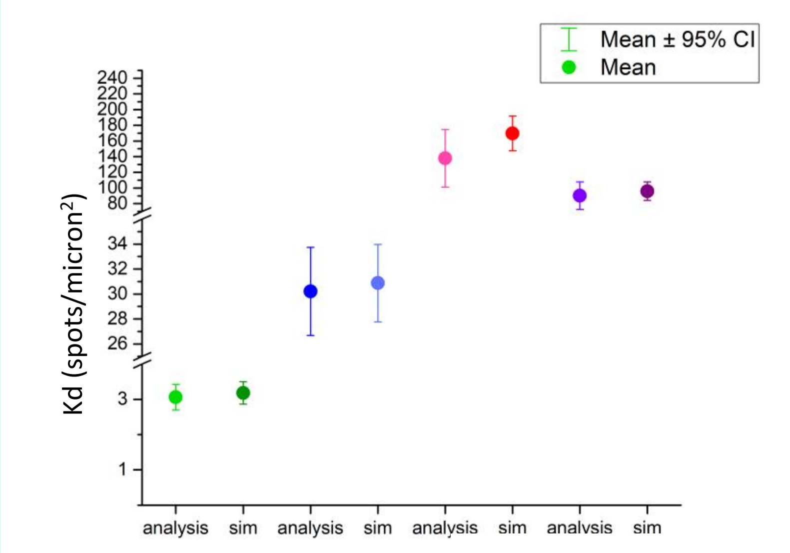
Estimation of true colocalizations and dissociation constant

$$F = \frac{B\left(1 - \frac{\lambda_2}{\lambda_1}\right) e^{-\lambda_2}\left(1 + \frac{1}{\lambda_2}\right)}{\left(A + B\frac{\lambda_2}{\lambda_1}\right)e^{-\lambda_1}\left(1 + \frac{1}{\lambda_1}\right) + B\left(1 - \frac{\lambda_2}{\lambda_1}\right) e^{-\lambda_2}\left(1 + \frac{1}{\lambda_2}\right)}$$

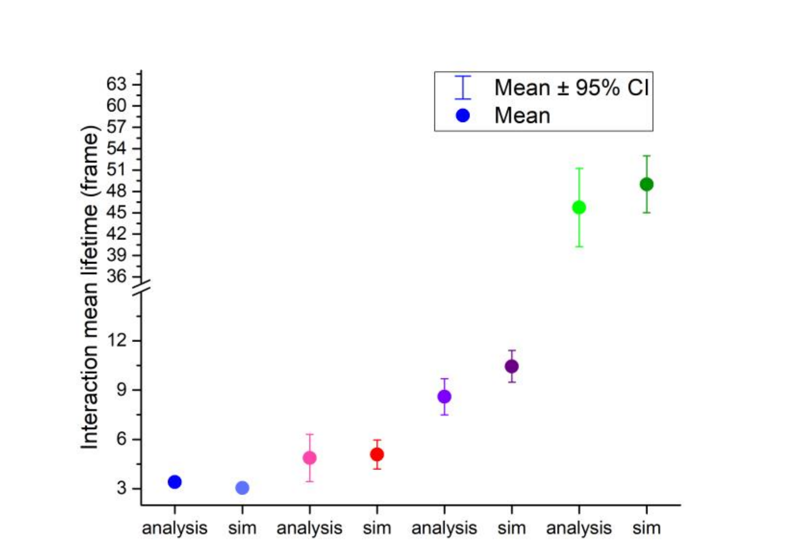
Temporal resolution and convolution effects included

$$C_T(t) = \frac{F \times c(t)}{e^{-k_{bl}t}} \qquad K_D = \frac{(N_1 - \bar{C}_T)(N_2 - \bar{C}_T)}{\bar{C}_T}$$

Bleaching correction



Kd estimated in range: 1-10^2 molecules/micron^2

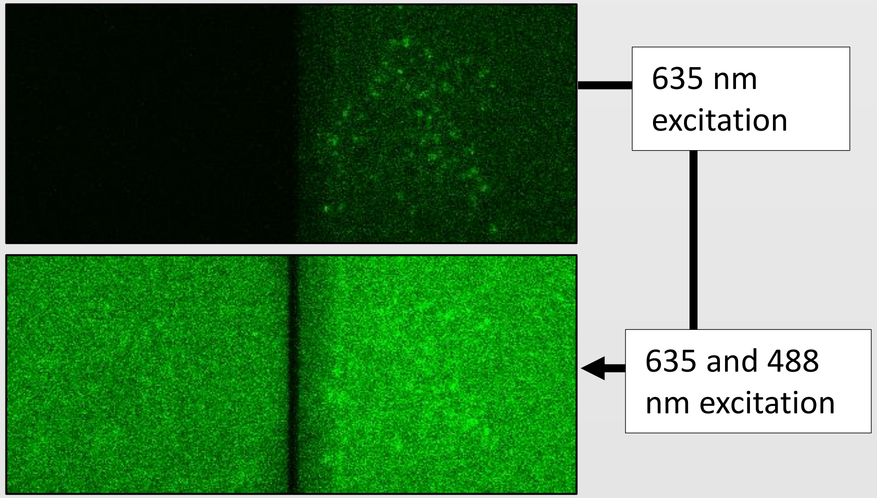


Interaction mean lifetime estimated in range 90-1500 ms

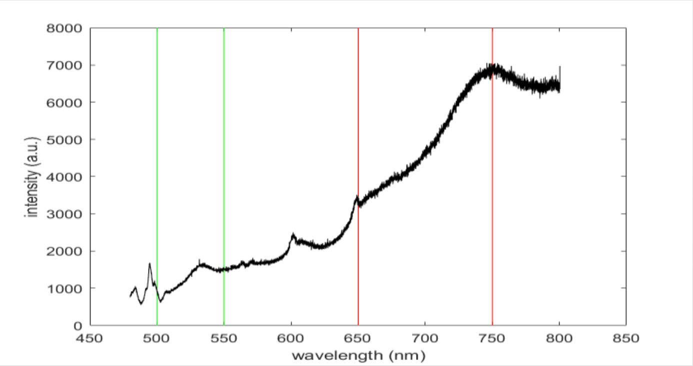
(time resolution 30 ms)

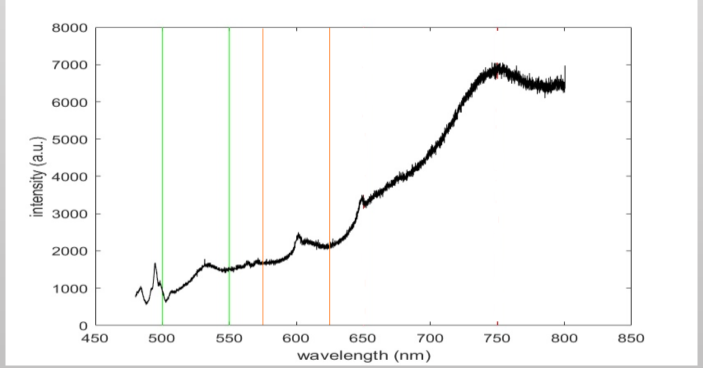
Sungkaworn et al. "Single-molecule imaging reveals receptor–G protein interactions at cell surface hot spots." *Nature* 550.7677 (2017). Limits: fractions of interacting particles of at least 0.2 and lifetime of at least 284 ms

Optimization of the experimental setup

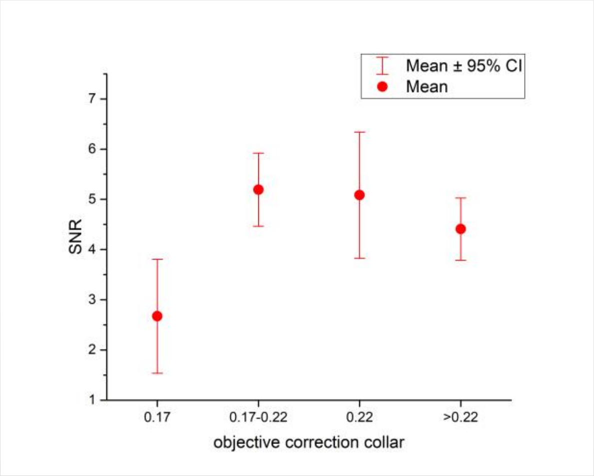


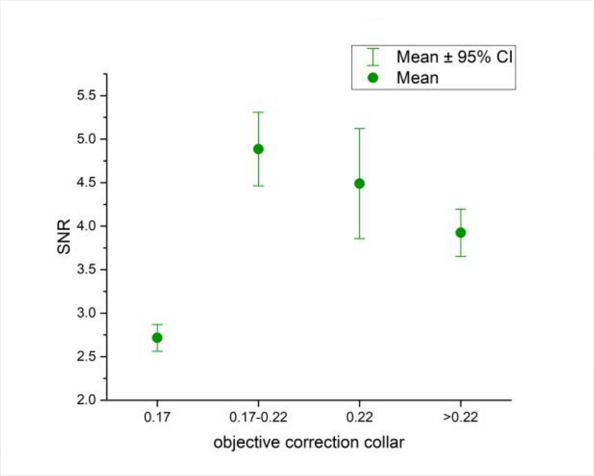
N-PK51 coverglass. Halved autofluorescence in the green channel.
Refractive index 1.530 (546 nm) instead of 1.515.
Optimizations of objective collar and immersion oil for aberration correction

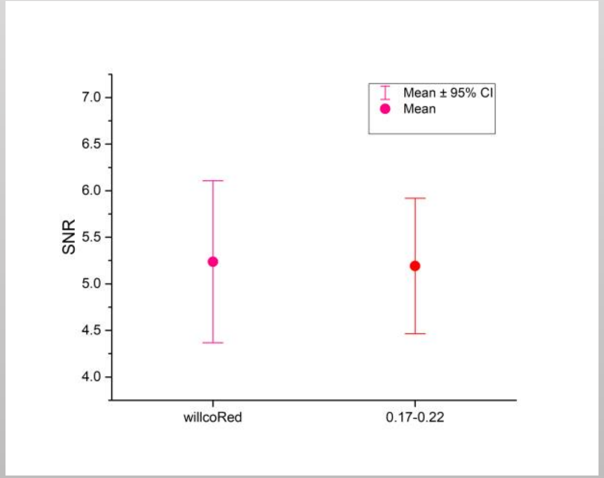


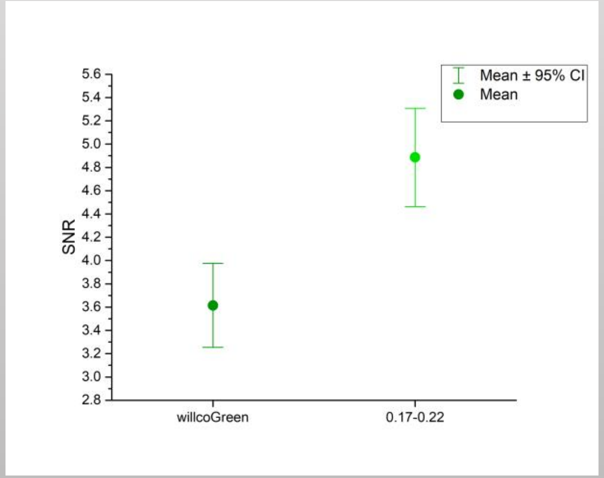


Doubled SNR in the second channel

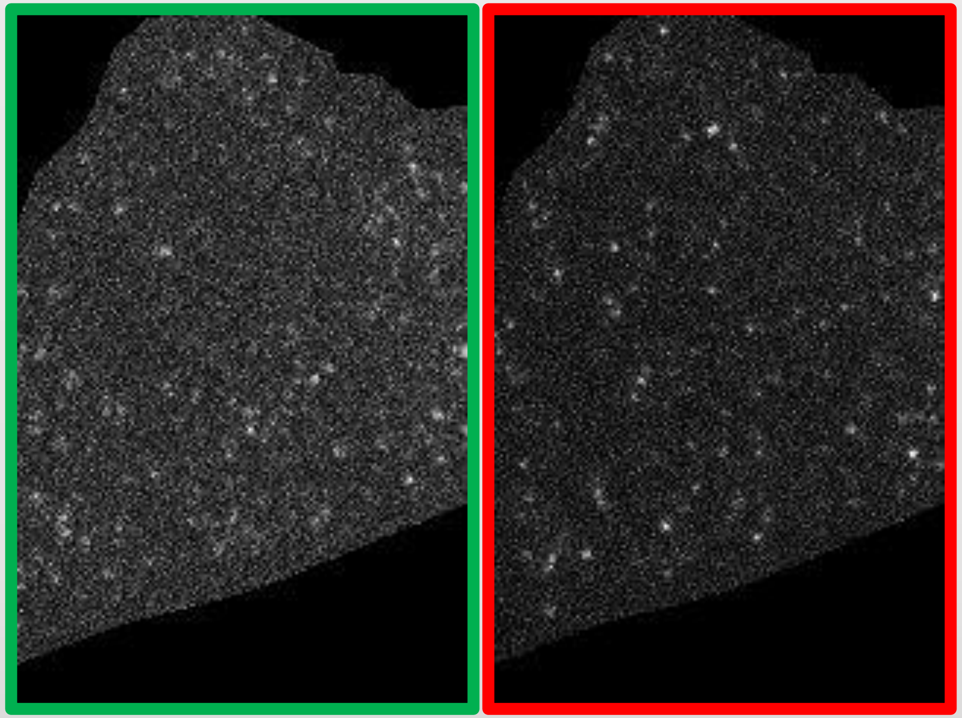


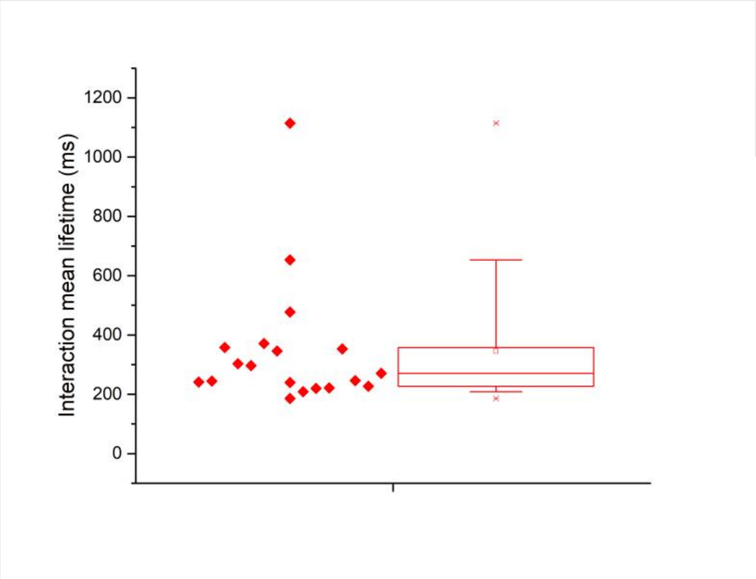


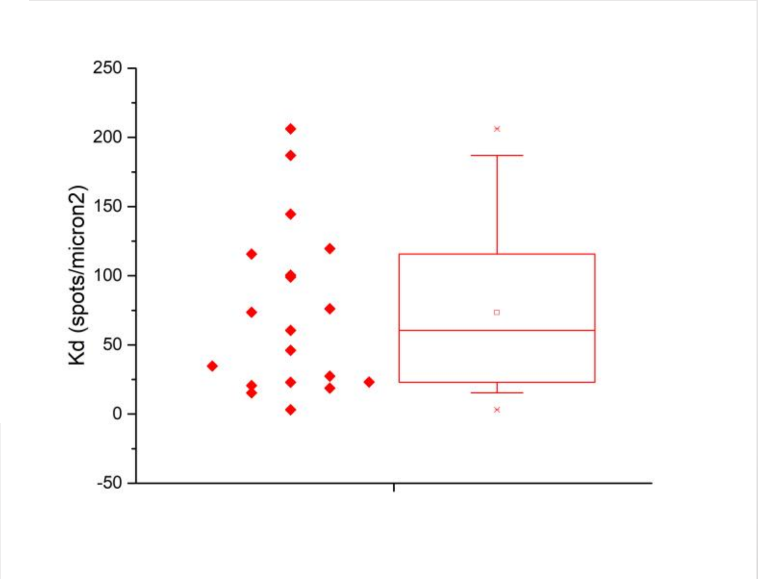




Measure of the homodimerization of TrkA receptor







$$K_D = 73 \pm 29\ molecules/\mu m^2$$
$$\tau = 346 \pm 55\ ms$$

$$M + M \rightarrow D$$

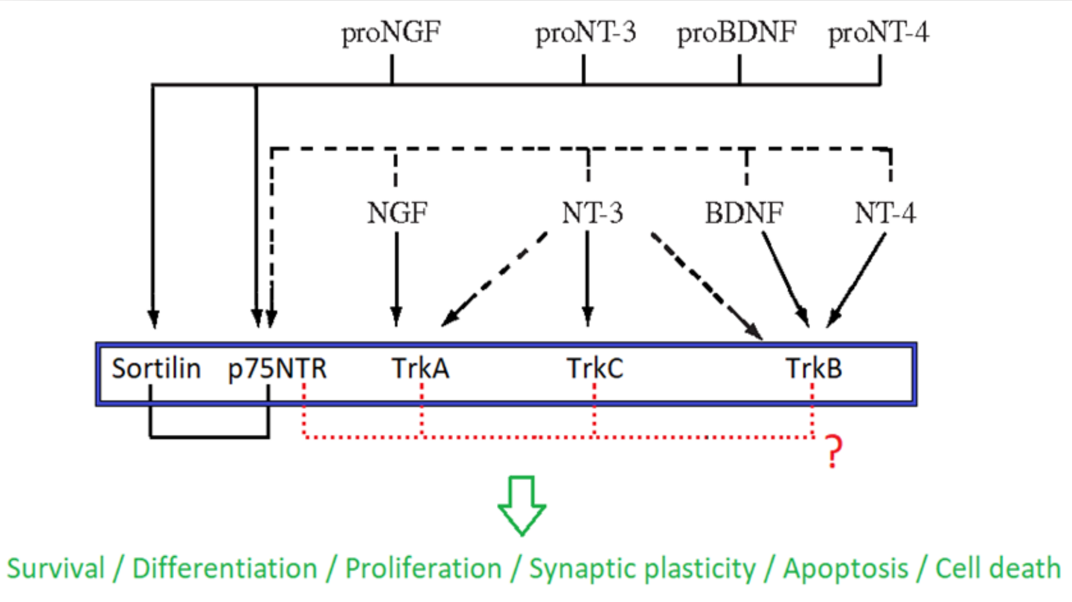
$$k_d = \frac{[M]^2}{[D]} = \frac{([R]+[G])^2}{[RR]+[GG]+[RG]} \qquad r = [R]+[RR] \qquad g = [G]+[GG]$$

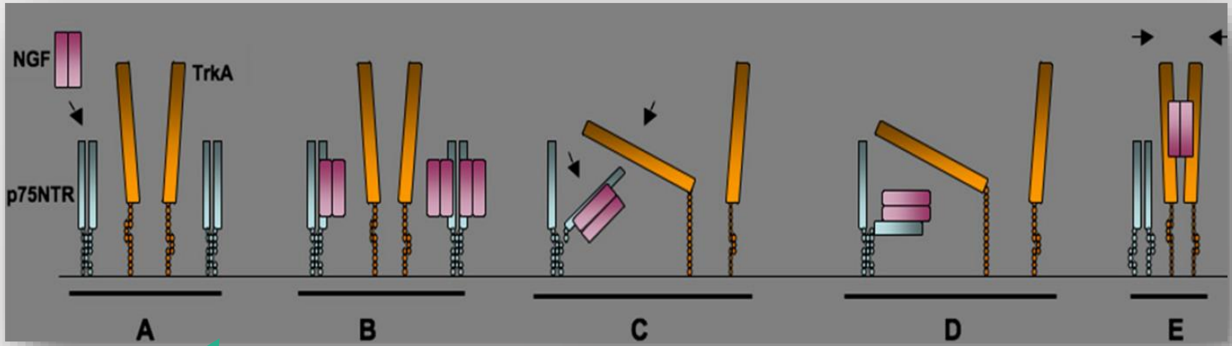
$$\Rightarrow k_d = \frac{(4[r][g]-[RG]^2)^2}{2[RG](2[g]+[RG])(2[r]+[RG])}$$

Ahmed, Fozia, and Kalina Hristova. "Dimerization of the Trk receptors in the plasma membrane: effects of their cognate ligands." *Biochemical Journal* 475.22 (2018)

$$K_D = 132 \pm 37\ molecules/\mu m^2$$
$$Range\ 12 - 227\ molecules/\mu m^2$$

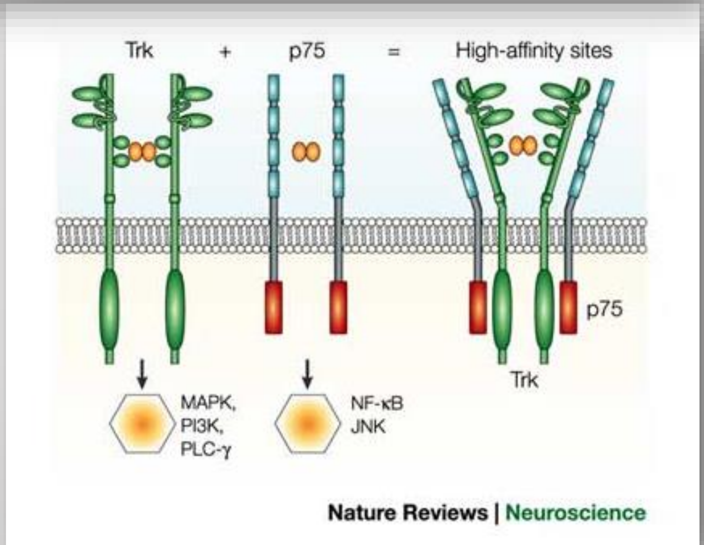
Next year
The neurotrophic signalling





"ligand passing" model

conformational model



Next experiments: ready to start!

1. **TrkA and p75 receptors observed in absence and in presence of ligands NGF and proNGF**
2. **TrkA and ligand (NGF / proNGF) observed in absence and in presence of p75**
3. **P75 and ligand (NGF / proNGF) observed in absence and in presence of TrkA**

Acknowledgments:
Stefano Luin, Rosy Amodeo, Aldo Moscardini