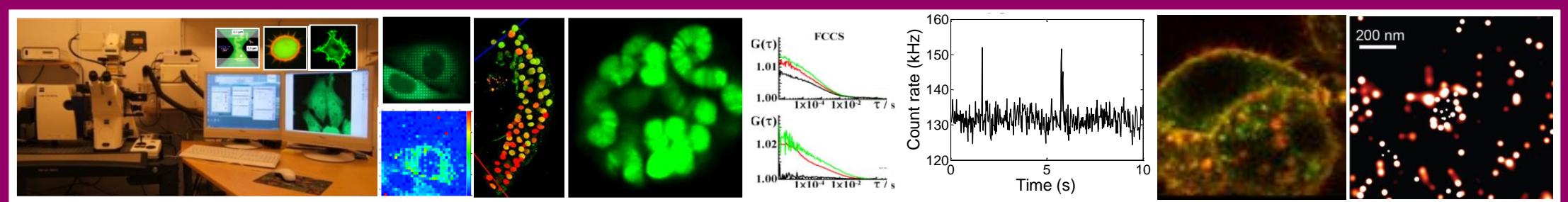


# Quantitative Scanning-Free Confocal Microscopy with Single-Molecule Sensitivity and Fluorescence Lifetime Imaging for the Study of Fast Dynamic Processes in Live Cells

Stanko N. Nikolić, Aleksandar J. Krmpot, Sho Oasa, Andrew H. A. Clayton,  
Lars Terenius, Milivoj R. Belić, Rudolf Rigler, Vladana Vukojević

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BPU 11 Congress, Aug 28<sup>th</sup> – Sep 1<sup>st</sup> 2022, Serbian Academy of Sciences and Arts – SASA



# Karolinska Institutet, a medical university with a mission to contribute to the improvement of human health through research, education and information



## Research goals

The goal for our research is to achieve scientific breakthroughs that change the view of human health and disease, as well as normal vital processes. Research results should lead to innovations and practical applications that can be implemented within the health service sector.

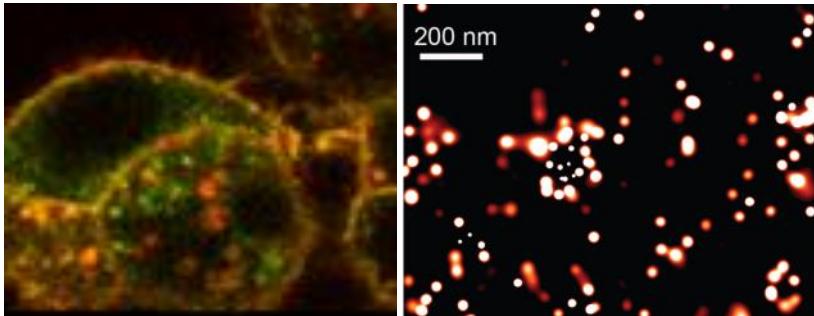
## Educational goals

The goal for our educational programs is to strengthen their link to research, and to prepare the students for engagement in research. They should provide the best possible conditions to work in, lead and continue to develop activities in collaboration with other professions.

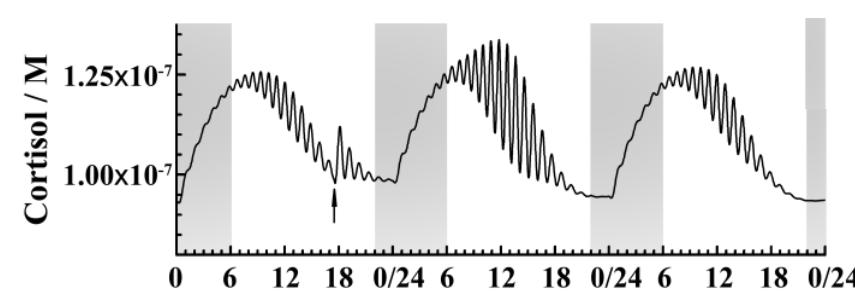
# Experimental alcohol and drug dependence research

My research focuses on understanding cellular and molecular mechanisms that underly the development of alcohol use disorders (AUD), with a particular interest on understanding the opioid system role in the development and management of AUD. To this aim, we use methods with single-molecule sensitivity and approaches from dynamical systems theory.

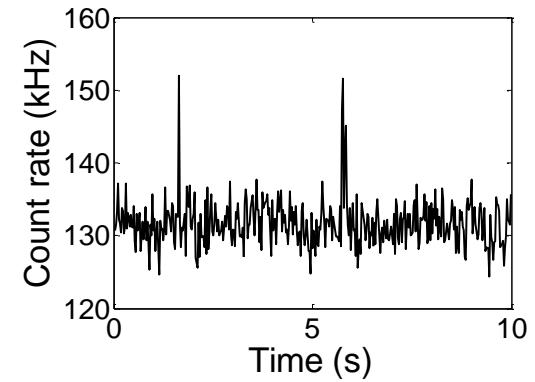
## Functional Fluorescence Microscopy Imaging (fFMI)



## Dynamic self-regulation of the neuroendocrine system



## Early biomarker of amyloid diseases

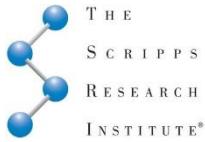




Karolinska  
Institutet



National Institute  
on Alcohol Abuse  
and Alcoholism



## Ethanol effect on the opioid receptor function at the nanoscale level

1. Tobin SJ, Cacao EE, Hong DW, Terenius L, **Vukojević V**, Jovanovic-Talisman T.  
Nanoscale Effects of Ethanol and Naltrexone on Protein Organization in the Plasma Membrane Studied by Photoactivated Localization Microscopy (PALM)  
PLoS One. 2014 9:e87225.
2. Rogacki MK, Golfetto O, Tobin SJ, Li T, Biswas S, Jorand R, Zhang H, Radoi V, Ming Y, Svenssonsson P, Ganjali D, Wakefield DL, Sideris A, Small AR, Terenius L, Jovanović-Talisman T, **Vukojević V**.  
Dynamic lateral organization of opioid receptors (kappa, muwt and muN40D ) in the plasma membrane at the nanoscale level  
Traffic 2018 **19(9)**: 690-709.
3. Tobin SJ, Wakefield DL, Terenius L, **Vukojević V**, Jovanović-Talisman T.  
Ethanol and Naltrexone Have Distinct Effects on the Lateral Nano-organization of Mu and Kappa Opioid Receptors in the Plasma Membrane  
ACS Chem Neurosci. 2019 **10(1)**: 667-676.
4. Laurent A, Bindslev N, Vukojević V, Terenius L.  
Iso- $\alpha$ -acids in Nonalcoholic and Alcoholic Beer Stimulate Growth of Neuron-like SH-SY5Y Cells and Neuroepithelial Stem Cells.  
ACS Bio Med Chem Au 2021 **1(1)** 11–20.
5. Radoi V, Jakobsson G, Palada V, Nikosjkov A, Druid H, Terenius L, Kosek E, **Vukojević V**.  
Non-Peptide Opioids Differ in Effects on Mu-Opioid (MOP) and Serotonin 1A (5-HT1A) Receptors Heterodimerization and Cellular Effectors (Ca<sup>2+</sup>, ERK1/2 and p38) Activation.  
Molecules. 2022 **27(7)**:2350.



# Mathematical Modeling of HPA Axis Dynamics Using Approaches from Dynamical Systems Theory

- 1.** Čupić Ž, Marković VM, Maćešić S, Stanojević A, Damjanović S, Vukojević V, Kolar-Anić L.  
Dynamic transitions in a model of the hypothalamic-pituitary-adrenal (HPA) axis  
*Chaos* 2016 26(3):033111.
- 2.** Čupić Ž, Stanojević A, Marković VM, Kolar-Anić Lj, Terenius L, Vukojević V.  
The HPA axis and ethanol: a synthesis of mathematical modelling and experimental observations.  
*Addict. Biol.* 2017 22(6):1486-1500.
- 3.** Abulseoud OA, Ho MC, Choi D-S, Stanojević A, Čupić Ž, Kolar-Anić Lj, Vukojević V.  
Corticosterone oscillations during mania induction in the lateral hypothalamic kindled rat – experimental observations and mathematical modeling.  
*PLoS One* 2017 12(5): e0177551.
- 4.** Stanojević A, Marković VM, Maćešić S, Kolar-Anić Lj, Vukojević V.  
Kinetic modelling of testosterone-related differences in the hypothalamic–pituitary–adrenal axis response to stress  
*Reac. Kinet. Mech. Cat.* 2018 123: 17.
- 5.** Stanojević A, Marković VM, Čupić Ž, Kolar-Anić Lj, Vukojević V.  
Advances in mathematical modelling of the hypothalamic–pituitary–adrenal (HPA) axis dynamics and the neuroendocrine response to stress.  
*Current Opinion in Chemical Engineering* 2018, 21:84–95.



# Single-molecule detection of structured amyloidogenic aggregates in body fluids for early diagnosis of amyloid diseases

1. Tiiman A, Jelić V, Jarvet J, Järemo P, Bogdanović N, Rigler R, Terenius L, Gräslund A, **Vukojević V.**  
Amyloidogenic nanplaques in blood serum of patients with Alzheimer's disease revealed by time-resolved Thioflavin T fluorescence intensity fluctuation analysis  
*J. Alzheimer's Dis.* 2019 **86(2)** 571-582
2. Bonito-Oliva A, Schedin-Weiss S, Younesi SS, Tiiman A, Adura C, Paknejad N, Brendel M, Romin Y, Parchem RJ, Graff C, **Vukojević V**, Tjernberg LO, Terenius L, Winblad B, Sakmar TP, Graham WV.  
Conformation-specific antibodies against multiple amyloid protofibril species from a single amyloid immunogen.  
*J Cell Mol Med.* 2019 Jan 20. doi: 10.1111/jcmm.14119.
3. Aksnes, M; Müller, EG; Tiiman, A; Edwin, TH; Terenius, L; Revheim, ME; **Vukojević, V**; Bogdanović, N; Knapskog, AB.  
Amyloidogenic Nanplaques in Cerebrospinal Fluid: Relationship to Amyloid Brain Uptake and Clinical Alzheimer's Disease in a Memory Clinic Cohort  
*J Alzheimer's Dis.* 2020 **77(2)**: 831-842
4. Aksnes M, Tiiman A, Edwin TH, Terenius L, Bogdanovic N, **Vukojević V**, Knapskog AB.  
Comparison of Cerebrospinal Fluid Amyloidogenic Nanplaques with Core Biomarkers of Alzheimer's Disease.  
*Front. Aging Neurosci.* 2021 **12** 608628 1-11
5. Aksnes M, Aass HCD, Tiiman A, Edwin TH, Terenius L, Bogdanović N, **Vukojević V**, Knapskog AB.  
Cerebrospinal fluid amyloidogenic nanplaques and cytokines are associated in Alzheimer's disease. 2021  
*Transl. Neurodegener.* 2021 **10(1):18**
6. Aksnes M, Aass HCD, Tiiman A, Terenius L, Bogdanović N, **Vukojević V**, Knapskog AB.  
Serum Amyloidogenic Nanplaques and Cytokines in Alzheimer's Disease: Pilot Study in a Small Naturalistic Memory Clinic Cohort  
*J Alzheimers Dis.* 2022 **86(3)**: 1459 – 1470



## Functional Fluorescence Microscopy Imaging (fFMI)

1. Vitali M, Bronzi D, Krmpot AJ, Nikolić S, Schmitt F-J, Junghans C, Tisa S, Friedrich T, **Vukojević V**, Terenius L, Zappa F, Rigler R. A single-photon avalanche camera for fluorescence lifetime imaging microscopy and correlation spectroscopy. *IEEE J. Sel. Top. Quantum Electron.* 2014 20(6): 344-353.
2. Papadopoulos DK, Krmpot AJ, Nikolić SN, Krautz R, Terenius L, Tomancak P, Rigler R, Gehring WJ, **Vukojević V**. Probing the kinetic landscape of Hox transcription factor-DNA binding in live cells by massively parallel Fluorescence Correlation Spectroscopy. *Mech Dev.* 2015 138 Pt 2: 218-225. pii: S0925-4773(15)30029-0.
3. Krmpot AJ, Nikolić SN, Oasa S, Papadopoulos DK, Vitali M, Oura M, Mikuni S, Thyberg P, Tisa S, Kinjo M, Nilsson L, Terenius L, Rigler R, **Vukojević V**. Functional Fluorescence Microscopy Imaging (fFMI). Quantitative Scanning-Free Confocal Fluorescence Microscopy for the Characterization of Fast Dynamic Processes in Live Cells. *Analytical Chemistry* 2019 91(17):11129-11137
4. Oasa S, **Vukojević V**, Rigler R, Tsigelny IF, Changeux JP, Terenius L. A strategy for designing allosteric modulators of transcription factor dimerization. *Proc Natl Acad Sci U S A.* 2020 117(5): 2683-2686.
5. Oasa S, Krmpot AJ, Nikolić SN, Clayton AHA, Tsigelny IF, Changeux JP, Terenius L, Rigler R, **Vukojević V**. Dynamic Cellular Cartography: Mapping the Local Determinants of Oligodendrocyte Transcription Factor 2 (OLIG2) Function in Live Cells Using Massively Parallel Fluorescence Correlation Spectroscopy Integrated with Fluorescence Lifetime Imaging Microscopy (mpFCS/FLIM). *Analytical Chemistry* 2021 93(35):12011-12021.
6. Nikolić SN, Oasa S, Krmpot AJ, Terenius L, Belić MR, **Vukojević V**, Rigler R. Mapping the direction of nucleocytoplasmic transport of glucocorti-coid receptor (GR) in live cells using two-foci spatial cross-correlation and massively parallel Fluorescence Correlation Spectroscopy (mpFCS) *Manuscript*

# Outline

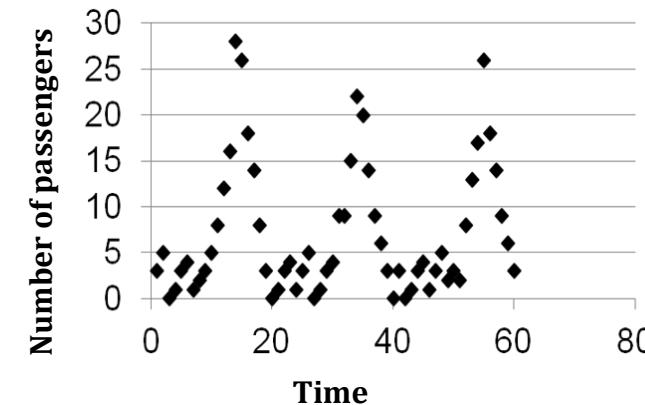
- **Part 1:** Introduction to Fluorescence Correlation Spectroscopy (FCS) and its use for the study of fast dynamical processes and molecular interactions in solution and live cells
- **Part 2:** Limitations of single-point FCS and recent advances in the development of massively parallel FCS (mpFCS) with examples of applications
- **Part 3:** Future perspective and the development of dc-mpFCCS

# Fluorescence Correlation Spectroscopy (FCS) is a fluctuation analysis technique

FCS is a quantitative analytical method with the ultimate, single-molecule sensitivity that relies on time-resolved detection of fluorescence intensity fluctuations around a stationary state and their analysis to characterize the dynamics of processes that give rise to the fluorescence intensity fluctuations.



3 5 0 1 3 4 1 2 3 5 8 12 16 28 26 18 14 8 3 0 1 3 4 1 3 5 0 1 3 4 9 9  
15 22 20 14 9 6 3 0 3 0 1 3 4 1 3 5 2 3 2 8 13 17 26 18 14 9 6



<http://www.arlotto.com/newsletter/filmshot.png>

Elliot L. Elson, all papers!

<http://www.fcsxpert.com/>

<http://www.biophysics.org/portals/1/pdfs/education/schwille.pdf>

T. Wohland, S. Maiti, R. Macháň, An Introduction to Fluorescence Correlation Spectroscopy, 2020

# Milestones in FCS development

<b>1871</b>	<b>Lord Rayleigh (John Strutt) describes scattering of light by small particles</b>
<b>1905/1906</b>	<b>Einstein and von Smoluchowski work on the fluctuation theory of light scattering</b>
<b>1911</b>	<b>Svedberg observed fluctuations in the number of colloidal gold particles under a microscope</b>
<b>1913</b>	<b>Perrin anticipated fluorescence fluctuation studies</b>
<b>1957</b>	<b>Laser development</b>
<b>1961</b>	<b>Confocal microscope</b>
<b>1961/1964</b>	<b>Solid state single photon detectors</b>
<b>1967</b>	<b>Autocorrelator</b>

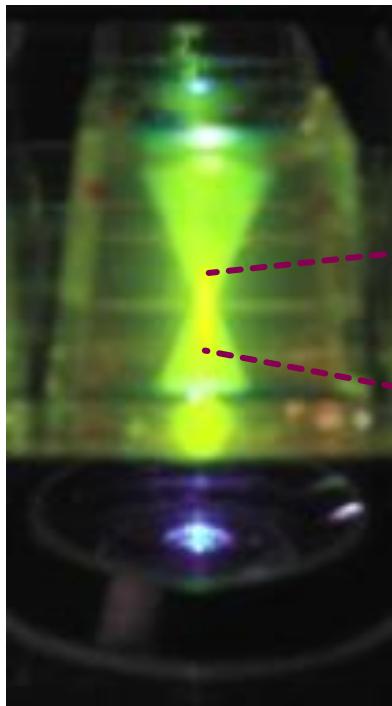
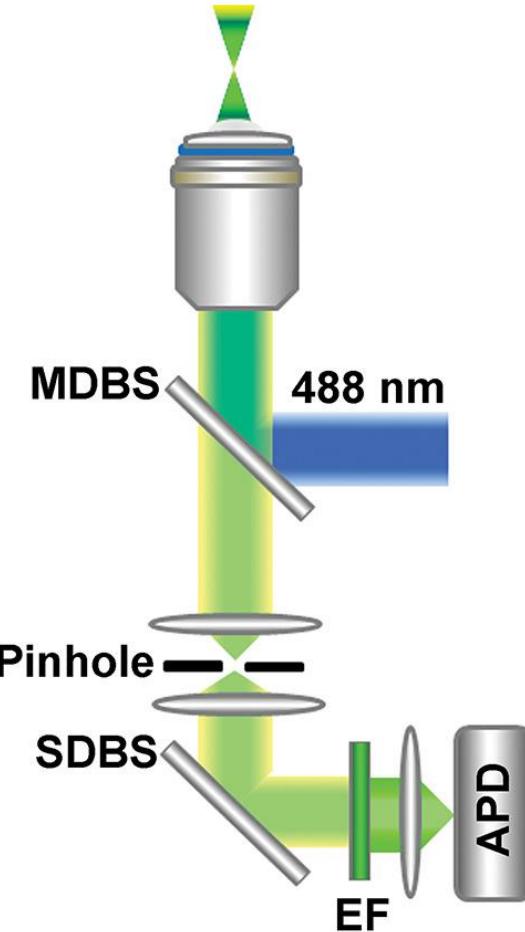
## Modern era of FCS

Magde D, Webb W W, Elson E. (1972) *Phys. Rev. Lett.* **29**, 705-708.

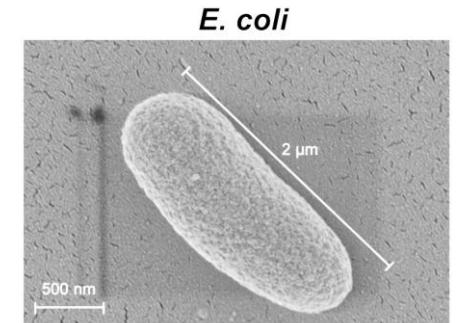
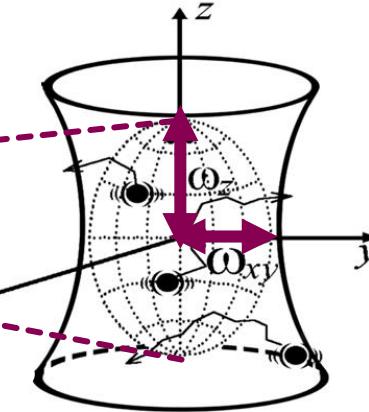
Elson EL, Magde D. (1974) *Biopolymers* **13**, 1-27.

Ehrenberg M, Rigler R. (1974) *Chem. Phys.* **4**, 390-401.

# FCS coupling with confocal microscopy – short measurement time and single-molecule sensitivity

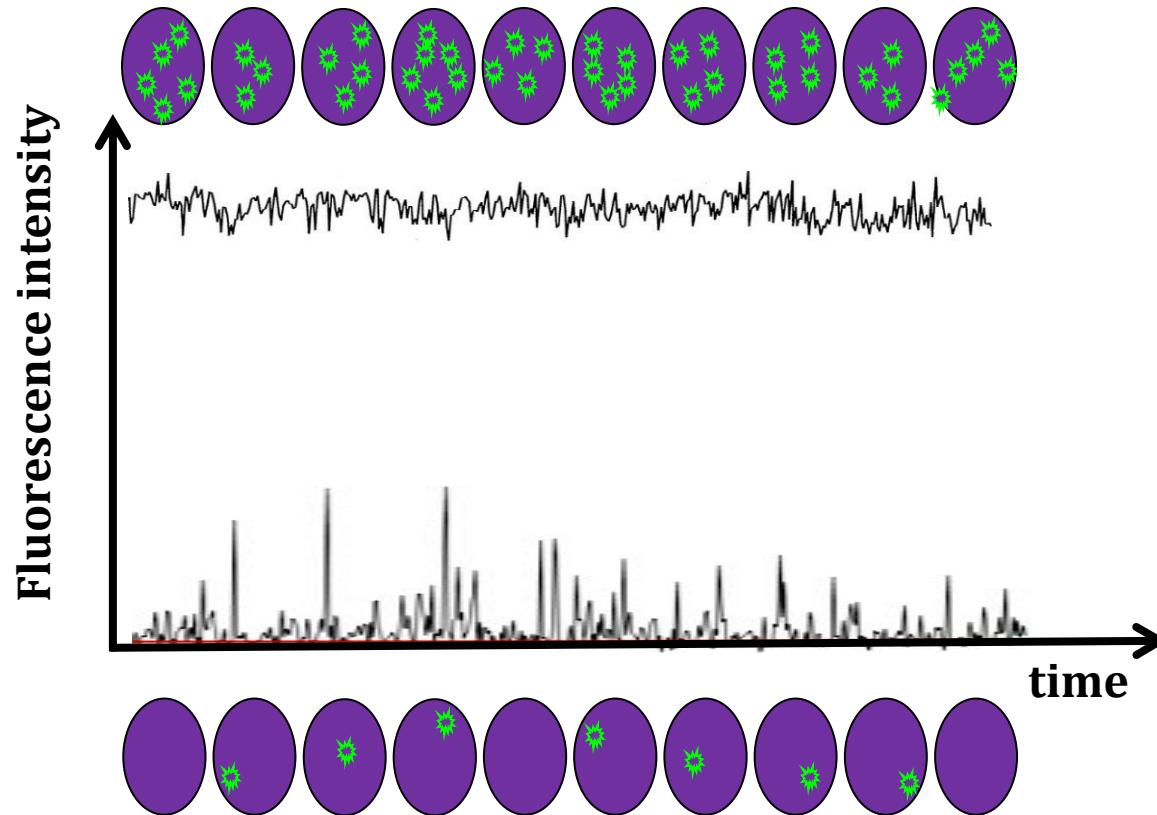


$0.5 \mu\text{m} \times 0.5 \mu\text{m} \times 1.5 \mu\text{m}$   
 $V_{\text{OVE}} \approx 2 \times 10^{-19} \text{ m}^3 \approx 0.2 \text{ fl}$   
 $c = 10 \text{ nM}; N_{\text{ave}} = 1$



**Single-molecule sensitivity**  
**Real time analysis**  
**Live cells**  
**Limited overview**

# Fluctuation analysis can give quantitative information about the investigated system, but where is this information hidden and how can we retrieve it?



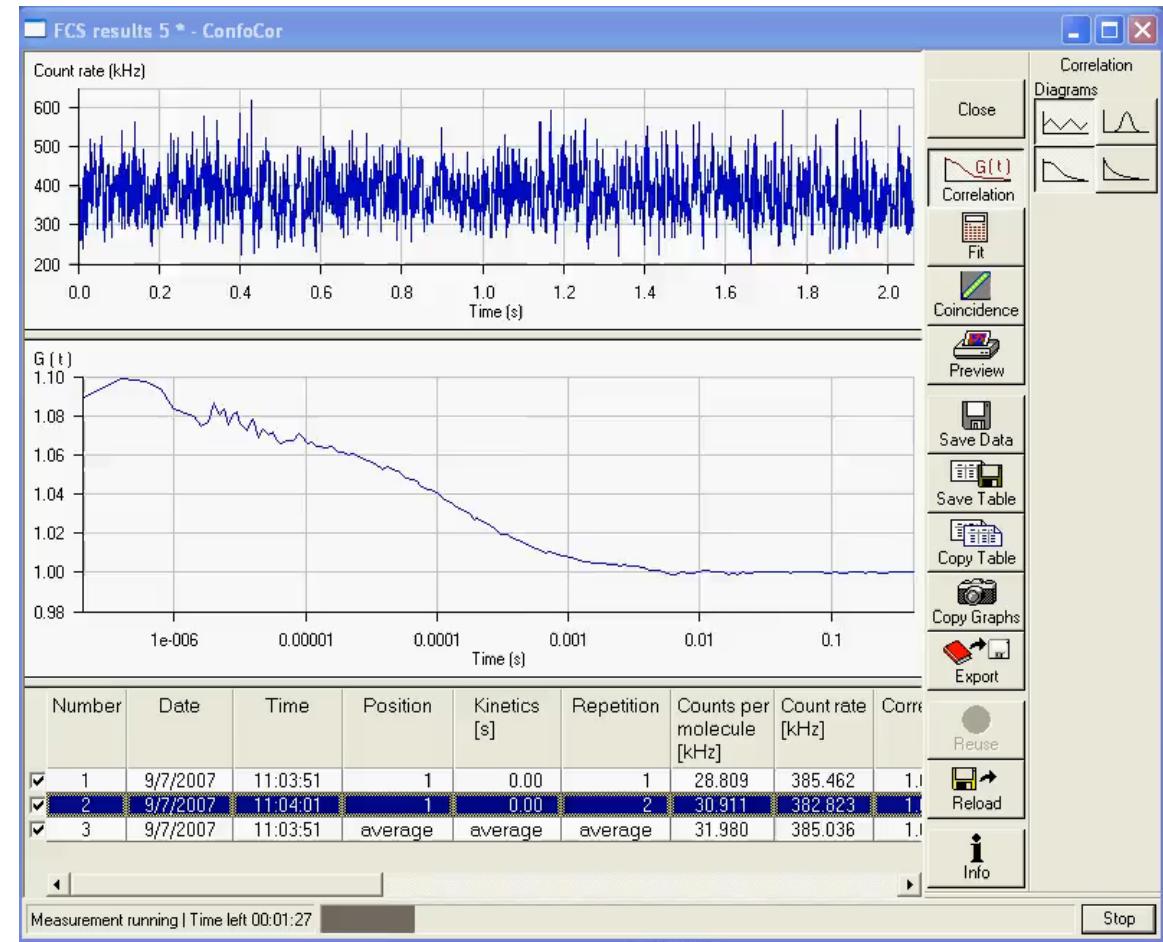
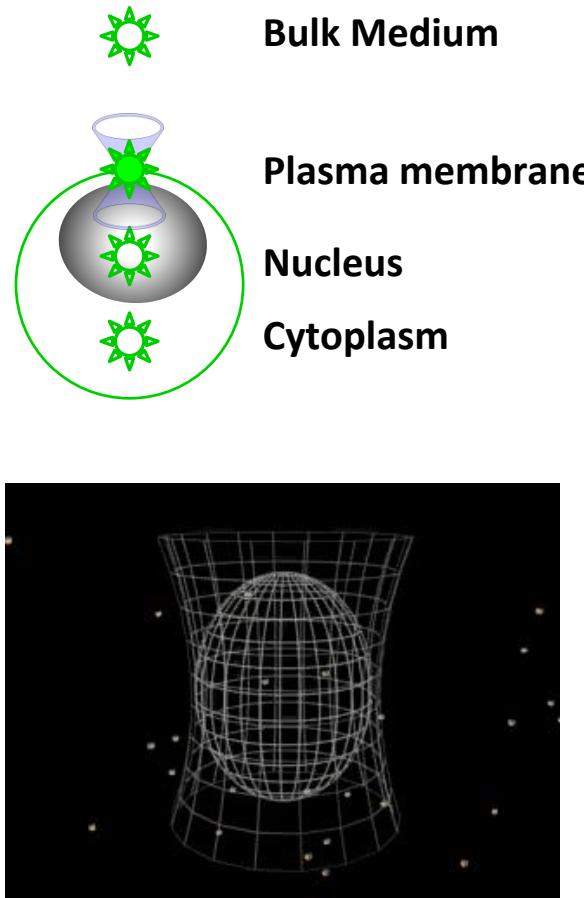
$$G(\tau) = \frac{C(\tau)}{\langle F \rangle^2} = \frac{\langle F(t) \cdot F(t + \tau) \rangle}{\langle F \rangle^2}$$

$$F(t) = \langle F \rangle + \delta F(t)$$

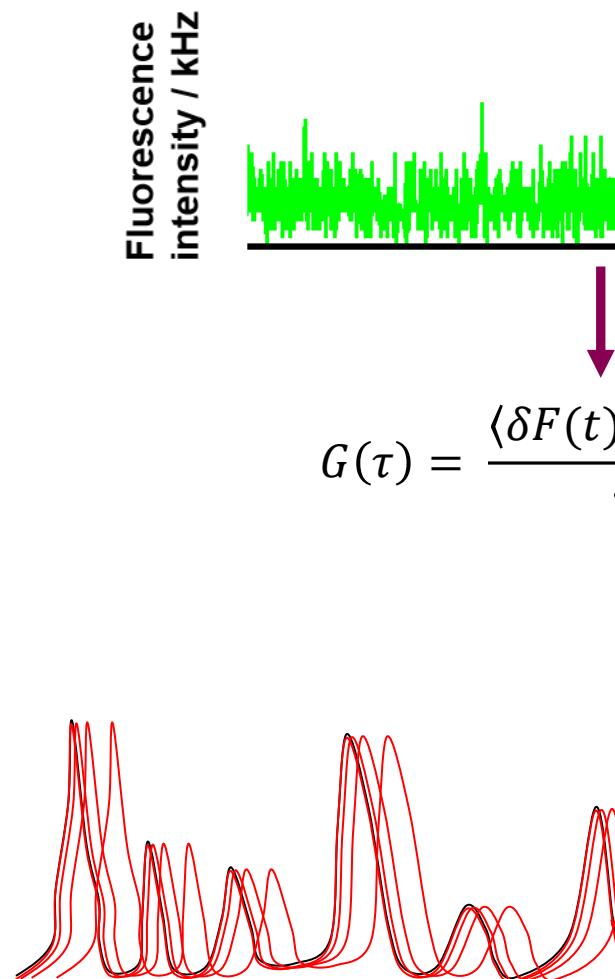
$$F(t + \tau) = \langle F \rangle + \delta F(t + \tau)$$

$$G(\tau) = 1 + \frac{\langle \delta F(t) \cdot \delta F(t + \tau) \rangle}{\langle F \rangle^2}$$

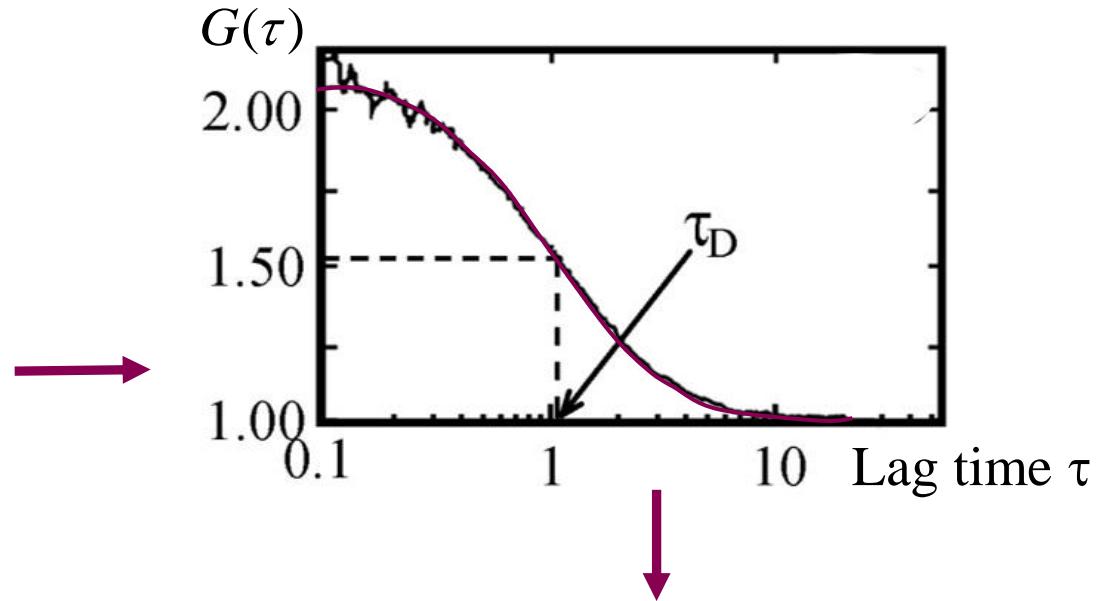
# FCS measurement in a solution of one fluorescent species (one component) undergoing free 3D diffusion



# Temporal auto-correlation analysis of fluorescence intensity fluctuations generated by free 3D or 2D diffusion of molecules



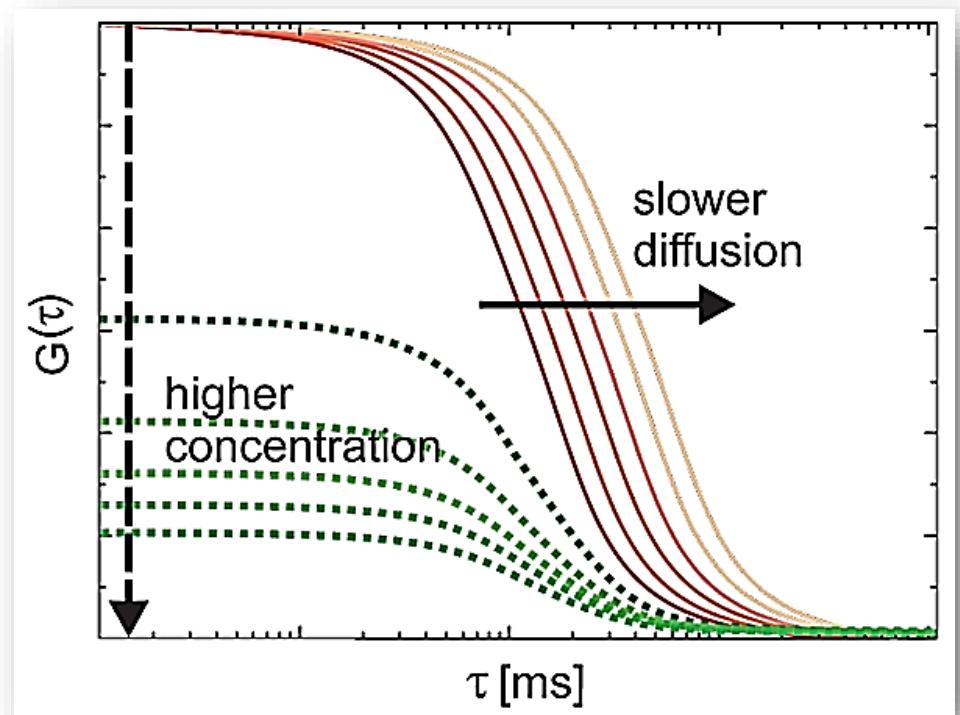
$$G(\tau) = \frac{\langle \delta F(t) \cdot \delta F(t + \tau) \rangle}{\langle F(t) \rangle^2}$$



$$G(\tau) = 1 + \frac{1}{N} \cdot \frac{1}{\left(1 + \frac{\tau}{\tau_D}\right) \sqrt{1 + \frac{\omega_{xy}^2}{\omega_z^2} \cdot \frac{\tau}{\tau_D}}}$$

$$G(\tau) = 1 + \frac{1}{N} \cdot \frac{1}{\left(1 + \frac{\tau}{\tau_D}\right)}$$

# Analysis of the temporal autocorrelation curve (tACC) acquired in a system where one fluorescent species (one component) undergoes free 3D diffusion

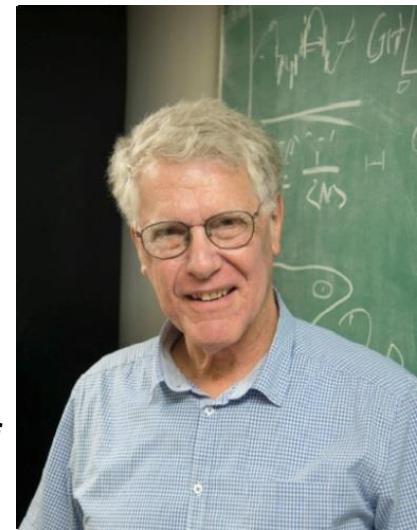


$$G(\tau) = 1 + \frac{1}{V_{eff} \cdot c} \cdot \frac{1}{\left(1 + \frac{\tau}{\tau_D}\right) \sqrt{1 + \frac{\omega_{xy}^2}{\omega_z^2} \cdot \frac{\tau}{\tau_D}}}$$

When  $V_{eff}$  is about 0.17 fl ( $0.17 \times 10^{-15}$  dm<sup>3</sup>), the average number of molecules in the observation volume is 1 when the concentration is 10 nM.

c / nM	N <sub>molecules</sub> / 0.17 fl (temporal average)	ACC <sub>amplitude</sub>
0.1	0.01	100
1.0	0.1	10
10	1	1
100	10	0.1
1000	100	0.01

# FCS for the study of fast chemical reactions



**Elson EL. 40 Years of FCS: How It All Began, Methods Enzymol. 2013 518:1-10.**

*"A primary motivation (for FCS development) was to develop a method that could measure the kinetics of chemical reactions in systems in equilibrium and thereby evade the need to perturb the state of the system, for example, by temperature jump, as is required for conventional measurements of chemical kinetics."*

**Elson EL, Magde D. (1974) Biopolymers 13,1-27.**

**Ehrenberg M, Rigler R. (1974) Chem. Phys. 4, 390-401.**

## Recommended reading:

**Elson EL.** Introduction to fluorescence correlation Spectroscopy-Brief and simple. Methods. 2018 140-141:3-9.

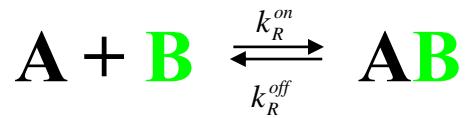
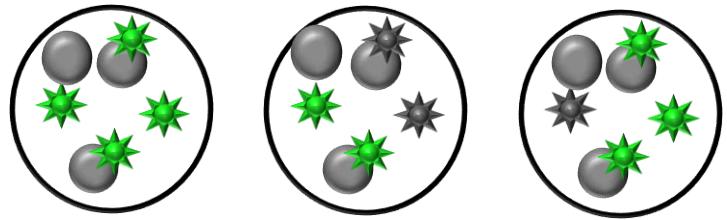
**Elson EL.** Brief introduction to fluorescence correlation spectroscopy. Methods Enzymol. 2013;518:11-41.

**Elson EL.** Fluorescence correlation spectroscopy: past, present, future. Biophys J. 2011 101(12):2855-70.

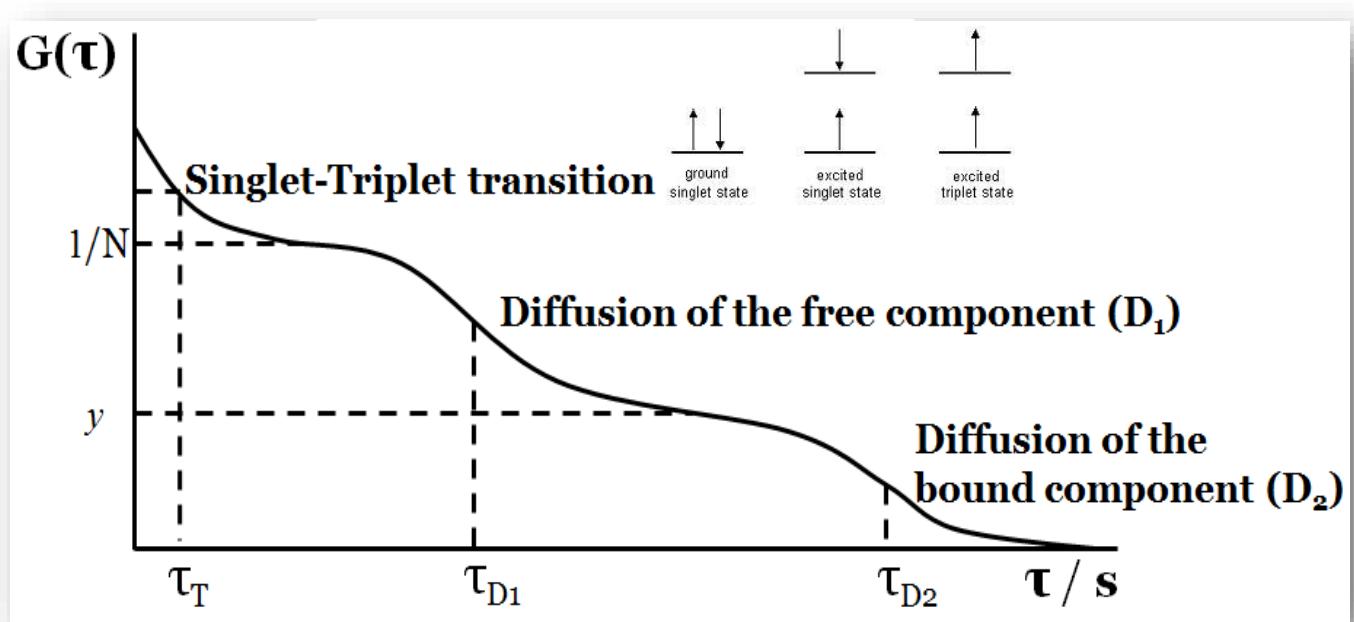
**Elson EL.** Quick tour of fluorescence correlation spectroscopy from its inception. J Biomed Opt. 2004 9(5):857-64.

**Elson EL.** Fluorescence Correlation Spectroscopy Measures Molecular Transport in Cells. Traffic 2001 2: 789-796

# FCS for nondestructive characterization of processes in complex systems

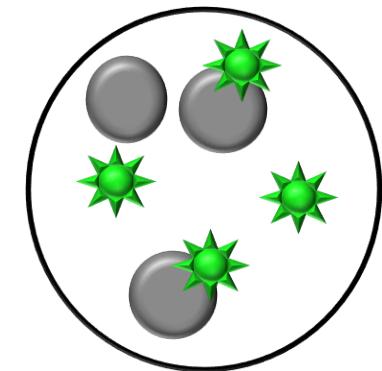


$A \gg B$

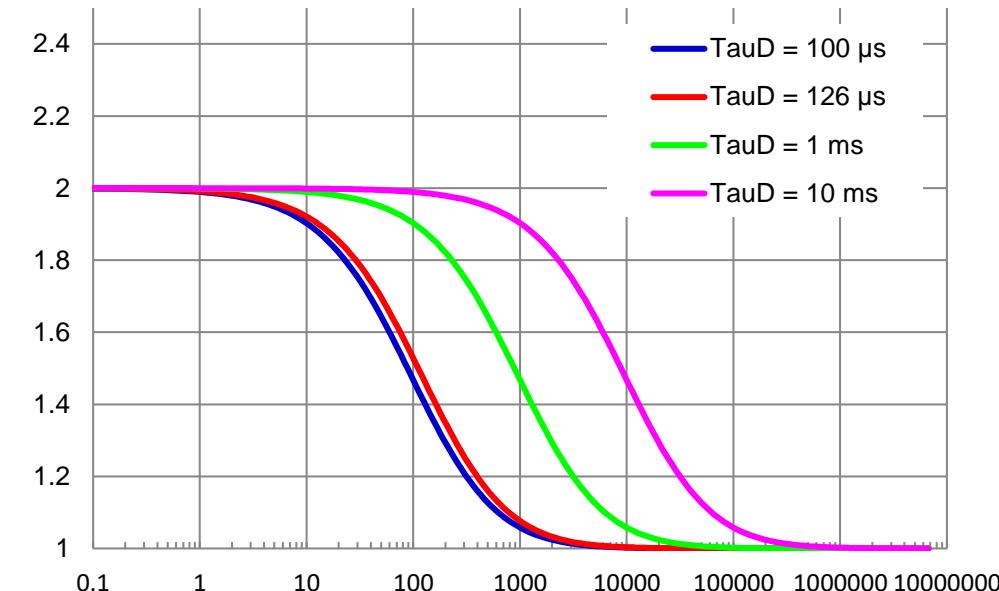
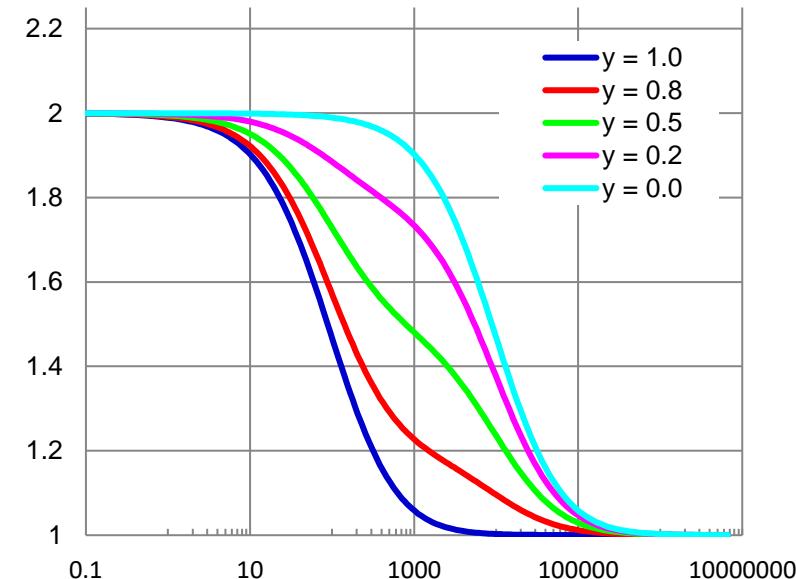


$$G(\tau) = 1 + \frac{1}{N} \cdot \left[ 1 + \frac{F}{1-F} \exp\left(-\frac{\tau}{\tau_{Chem}}\right) \right] \cdot \left( \frac{y}{\left(1 + \frac{\tau}{\tau_{D1}}\right) \sqrt{1 + \frac{w_{xy}^2}{w_z^2} \frac{\tau}{\tau_{D1}}}} + \frac{1-y}{\left(1 + \frac{\tau}{\tau_{D2}}\right) \sqrt{1 + \frac{w_{xy}^2}{w_z^2} \frac{\tau}{\tau_{D2}}}} \right)$$

# Limitations of single-color FCS for the study of molecular interactions in live cells



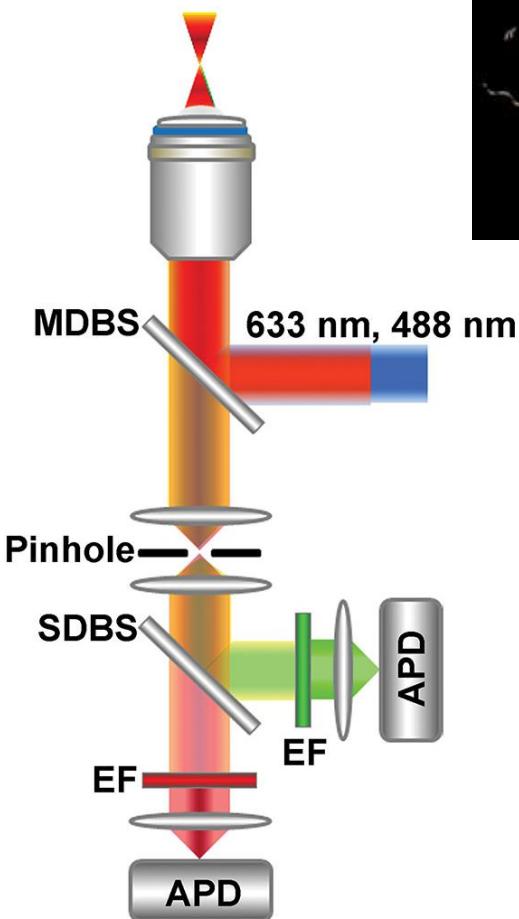
$\tau_D^{free} \ll \tau_D^{bound}$



$$\tau_D = \frac{\omega_{xy}^2}{4 \cdot D} \quad D = \frac{k_B T}{6\pi\eta r} \quad r \propto \sqrt[3]{M}$$

$$\tau_D \propto \frac{3\pi\omega_{xy}^2\eta}{2k_B T} \cdot \sqrt[3]{M} \quad \frac{\tau_{D2}}{\tau_{D1}} = \sqrt[3]{\frac{M_2}{M_1}}$$

# Fluorescence Cross-Correlation Spectroscopy (FCCS)



$$A_{\text{red} + \text{gr}} = 0.08$$

$$A_{\text{green} + \text{gr}} = 0.05$$

$$A_{\text{cc}} = 0.02$$

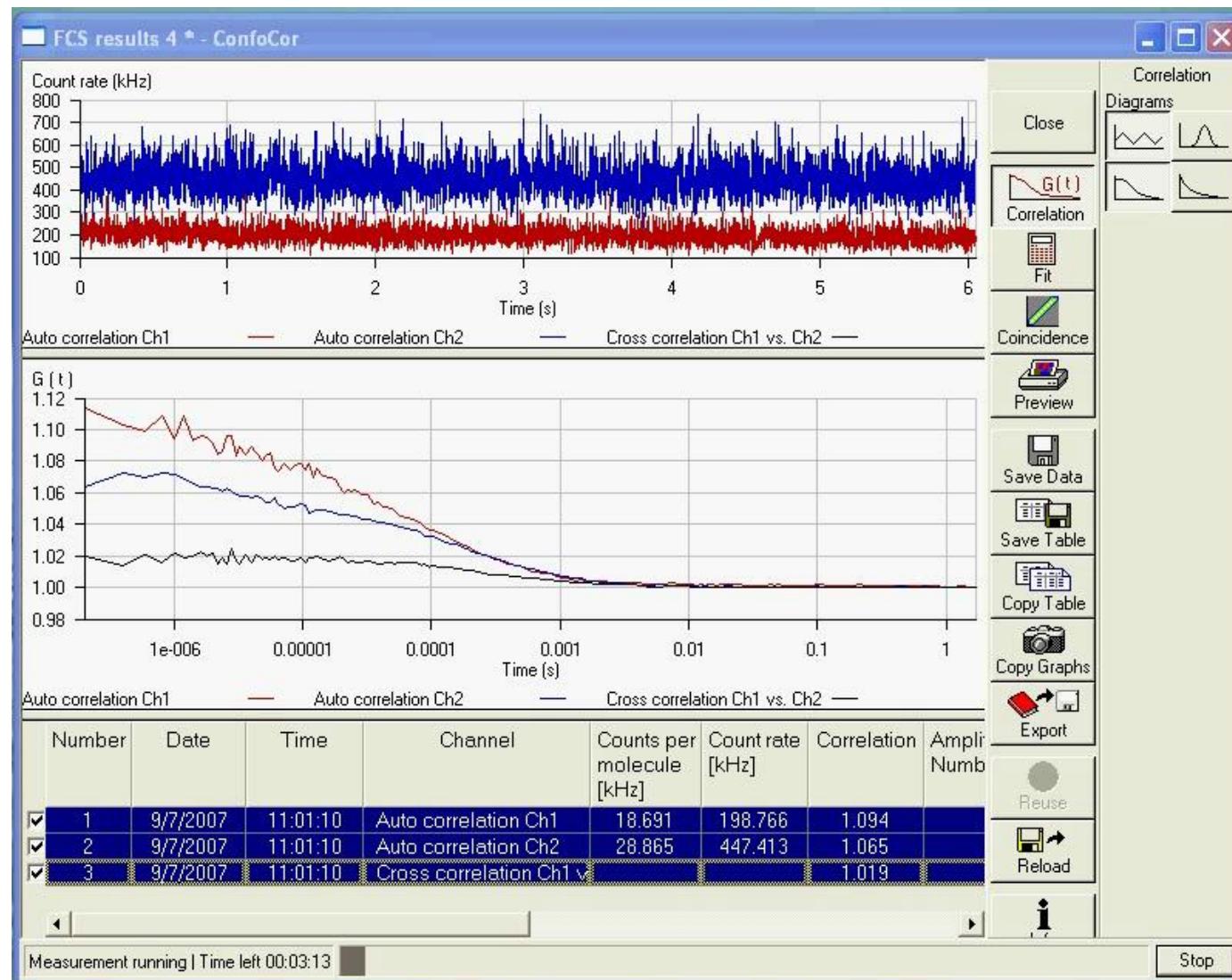
$$\frac{1}{N_{\text{cc}}} = \frac{N_{\text{gr}}}{(N_{\text{gr}} + N_g)(N_{\text{gr}} + N_r)}$$

$$N_{\text{gr}} = A_{\text{cc}} / A_{\text{r+gr}} \cdot A_{\text{g+gr}}$$

$$N_{\text{green, red}} = 5$$

$$N_{\text{red}} = 12.5 - 5 = 7.5$$

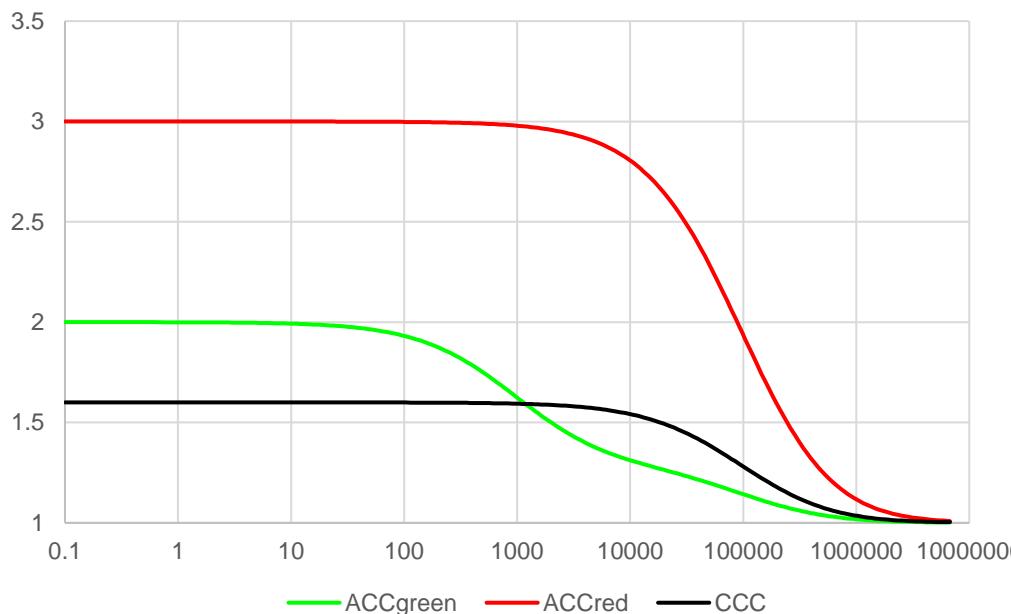
$$N_{\text{green}} = 20 - 5 = 15$$



# FCCS for nondestructive characterization of molecular binding



FCCS - no triplet and no cross-talk between the channels



$$G_g(\tau) = 1 + \frac{\langle \delta F_g(t) \cdot \delta F_g(t + \tau) \rangle}{\langle F_g \rangle^2}$$

$$G_r(\tau) = 1 + \frac{\langle \delta F_r(t) \cdot \delta F_r(t + \tau) \rangle}{\langle F_r \rangle^2}$$

$$G_{gr}(\tau) = 1 + \frac{\langle \delta F_g(t) \cdot \delta F_r(t + \tau) \rangle}{\langle F_g \rangle \cdot \langle F_r \rangle}$$

$$N_{gr} = \frac{G_{gr}(0) - 1}{(G_g(0) - 1) \cdot (G_r(0) - 1)}$$

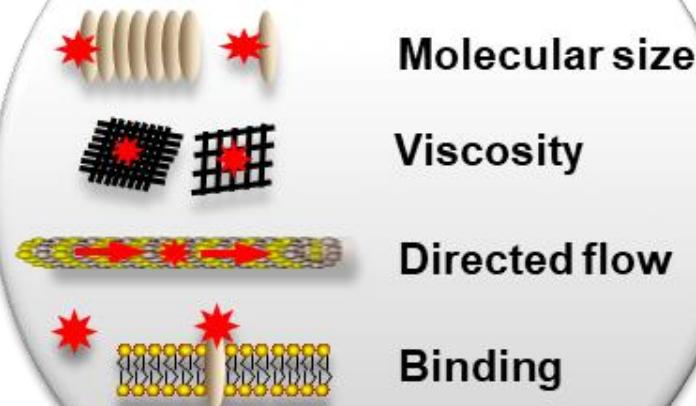
$$K_D = \frac{[A]_g^{free} \cdot [B]_r^{free}}{[AB]_{gr}}$$

# Which processes can be studied by FCS

Concentration measurements



Mobility – diffusion time



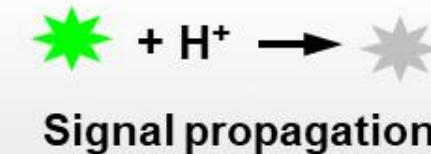
Molecular size

Viscosity

Directed flow

Binding

Kinetics of biochemical reactions

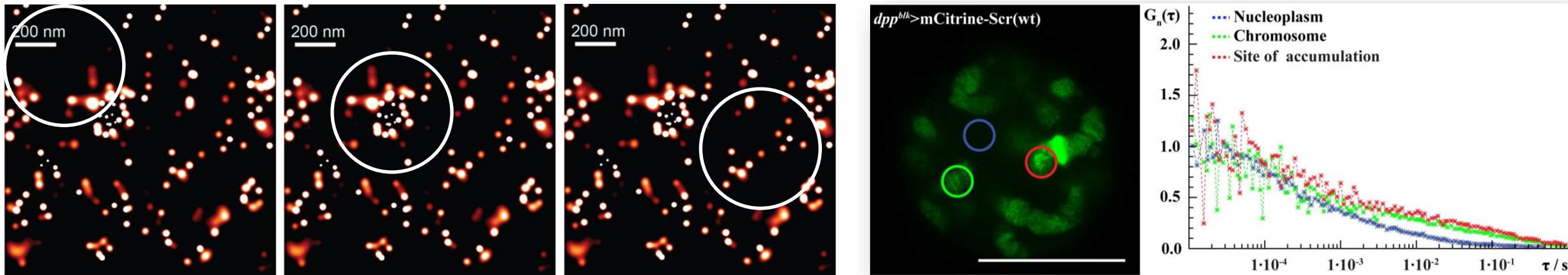


Signal propagation



$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}$$

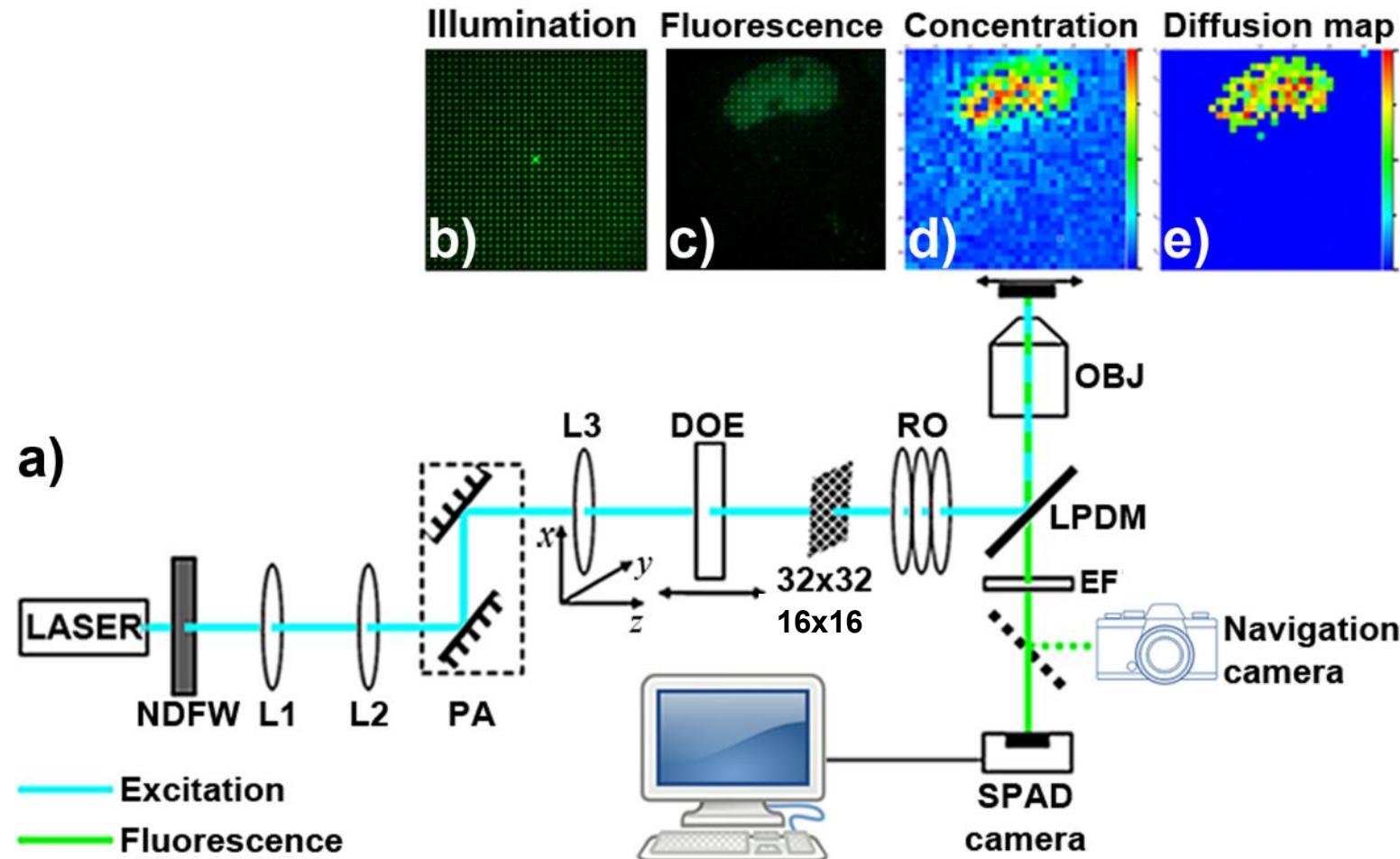
# Limitations of conventional single-point FCS



University of Belgrade

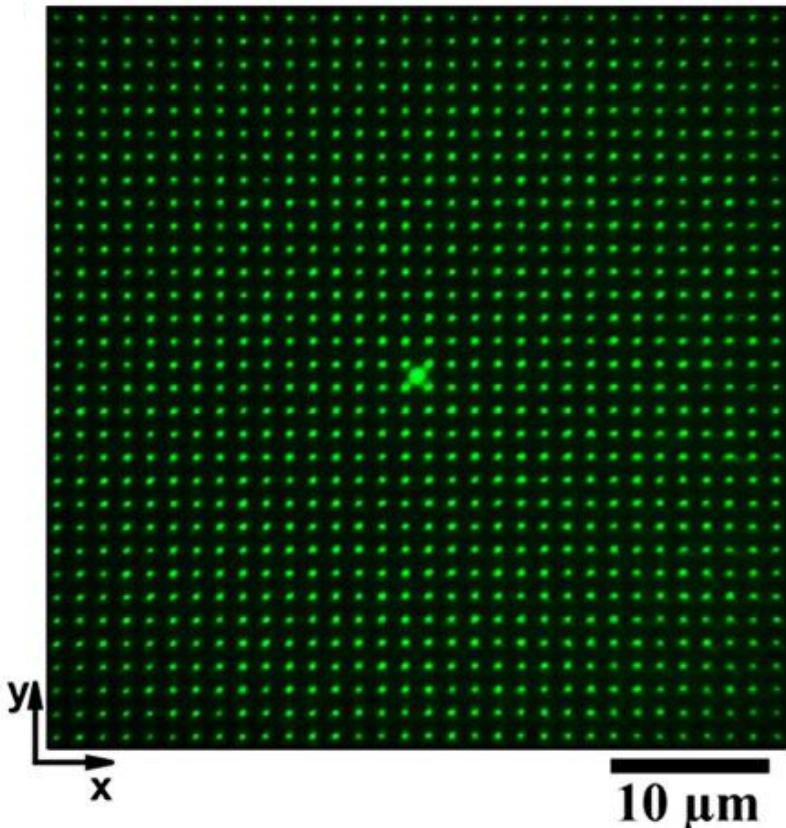


# Massively parallel FCS (mpFCS) for quantitative scanning-free confocal fluorescence microscopy imaging of dynamic processes

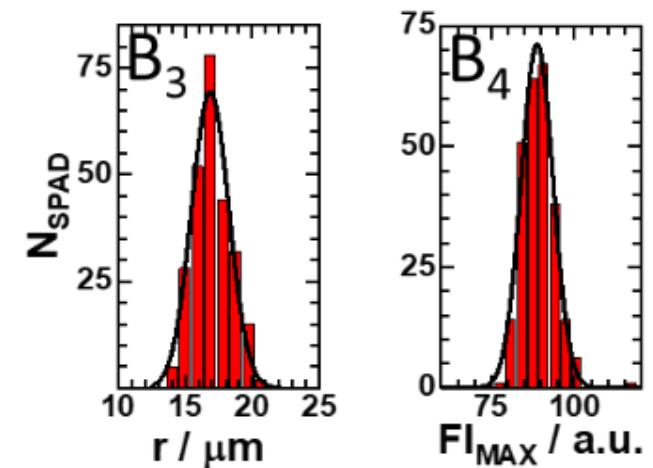
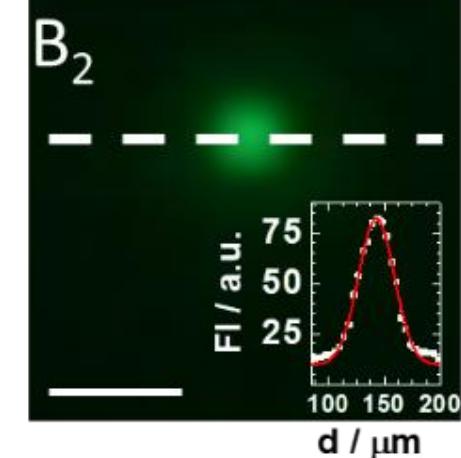
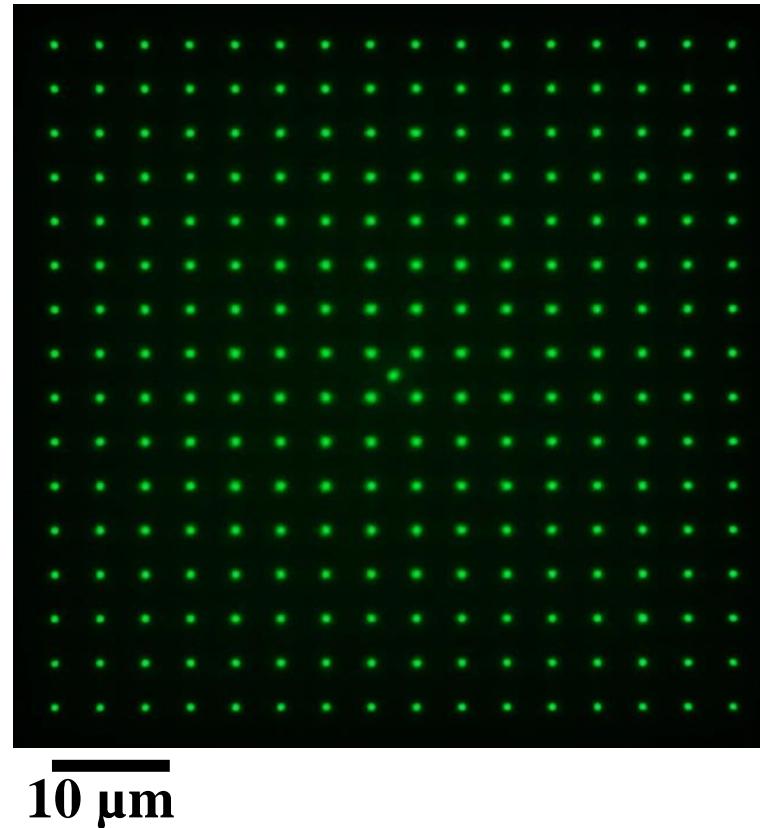


# Characterizing the mpFCS illumination matrix

**32 × 32**



**16 × 16**

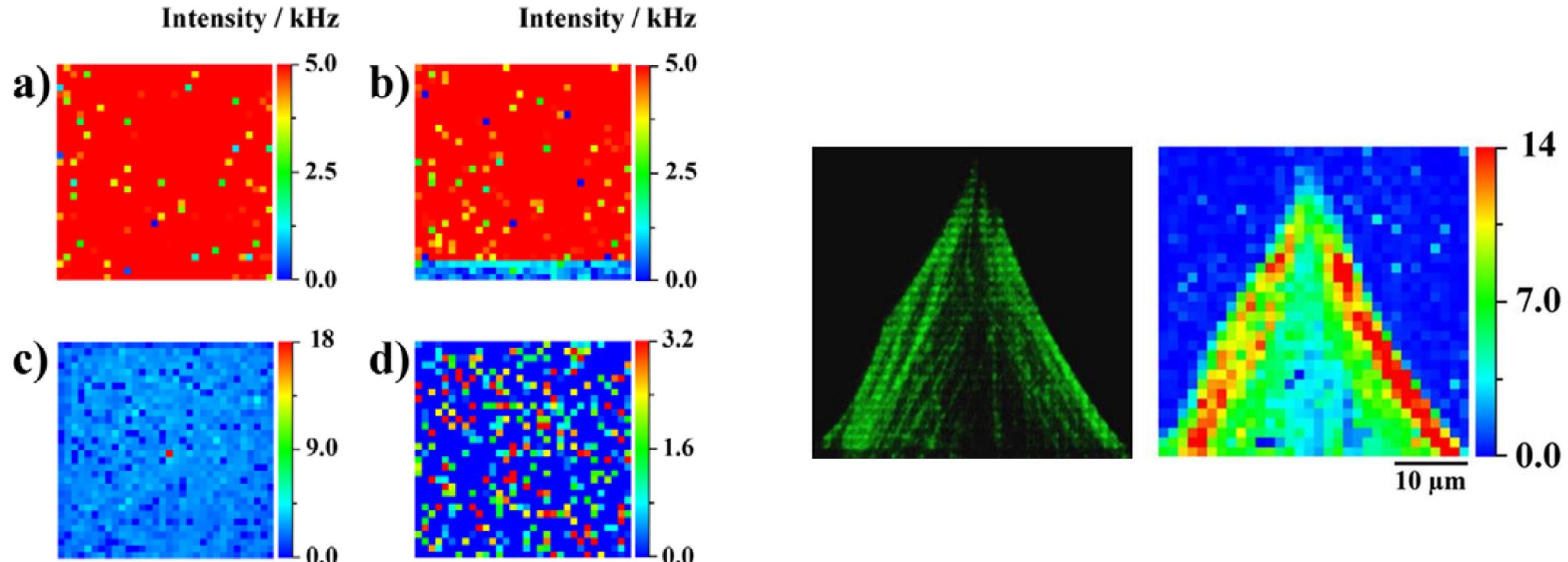


# Single-Photon Avalanche Diode (SPAD) cameras

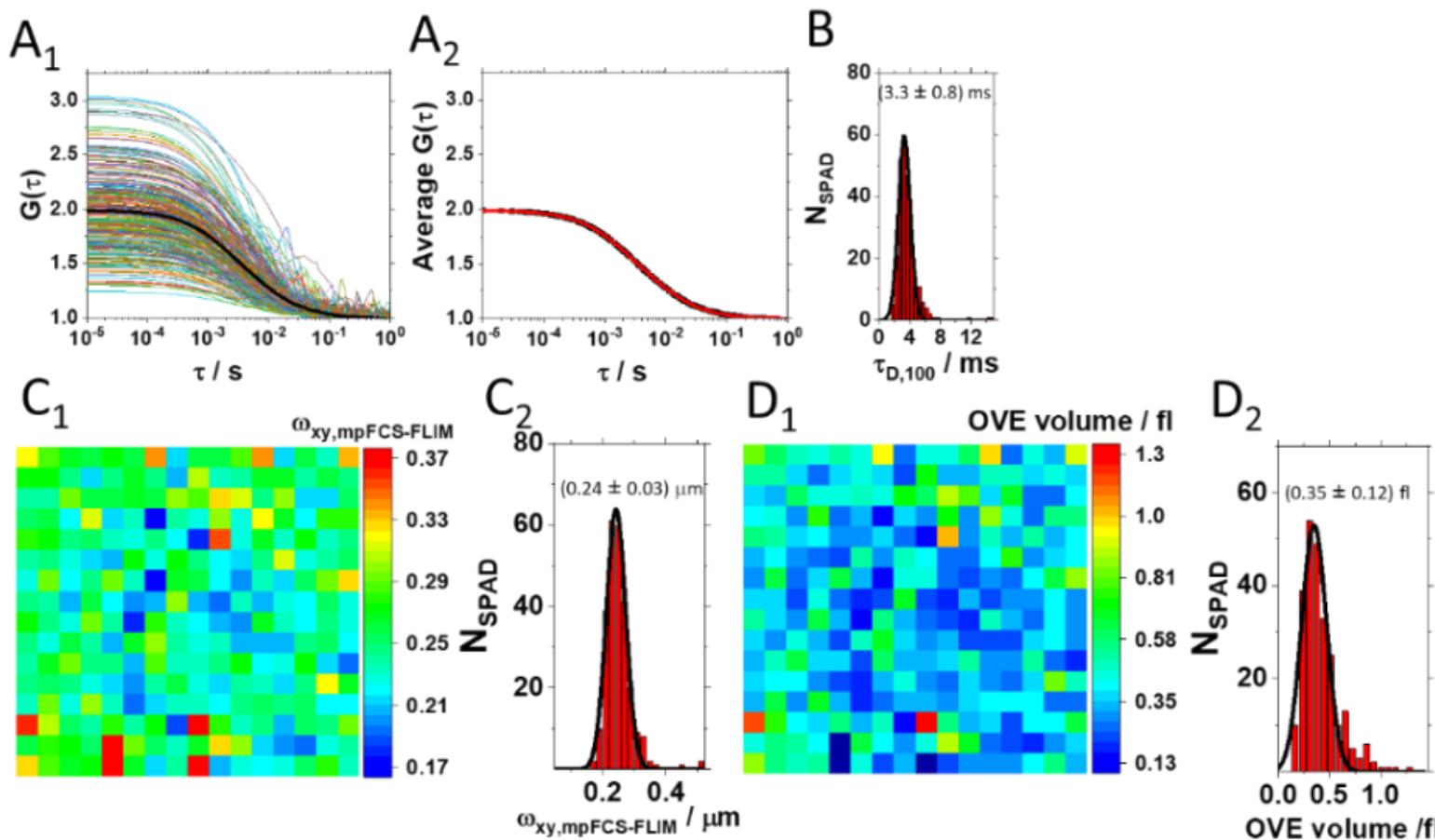
	SPC <sup>2</sup> (32x32)	SPC <sup>3</sup> (64x32)
Photon Detection Efficiency	38 % at 400 nm	50 % at 400 nm
Detector background	50 % of pixels < 4 kHz 75 % of pixels < 25 kHz	50 % of pixels < 150 Hz 95 % of pixels < 300 Hz
Temporal resolution / Frame rate	20.41 µs / 49 kframes / s	10.42 µs / 96 kframes / s
Inter-frame dead-time	20 ns	10 ns
Diameter of SPAD active area	20 µm	30 µm
SPAD pitch	150 µm	150 µm
SPAD dead-time	200 – 600 ns	50 - 125 ns
Afterpulsing probability	5 % at 200 ns SPAD dead-time	1 % at 50 ns SPAD dead-time
Photon Counting Dynamics	8 bits @ 49 kframes / s 12 bits @ 3 kframes / s 16 bits @ 191 frames / s	8 @ 96 kframes / s 12 @ 6 kframes / s 16 @ 375 frames / s
Readout noise	No	No
Internal memory	128 MiB ( $2^{20} \approx 134$ MB)	128 MiB ( $2^{20} \approx 134$ MB)
Time-Gated FLIM mode		
Gate Width		1.5 – 20 ns
Gate Steps		800
Step Size		20 ps
Measurement Time range		16 ns
Laser Sync Out		50 MHz

# mpFCS alignment and information loss due to interspaced sampling

## Deliberate instrument misalignment



# mpFCS calibration and observation volume element (OVE) size determined using 100 nm fluospheres



$$\tau_D = \frac{\omega_{xy}^2}{4D}$$

$$\omega_{xy} = (255 \pm 40) \text{ nm}$$

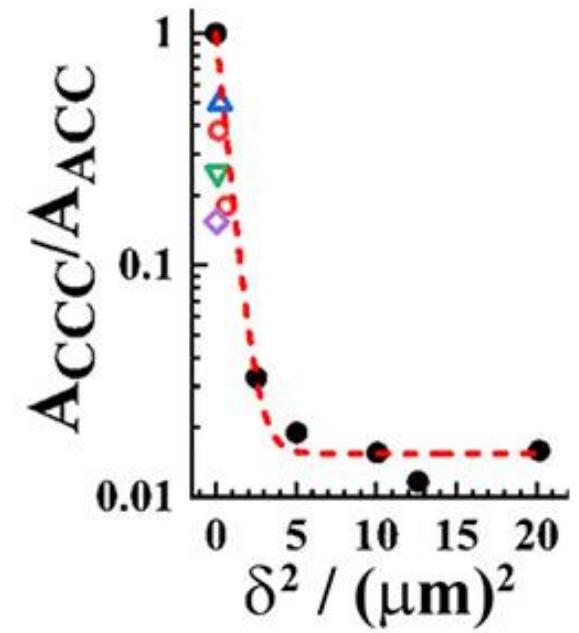
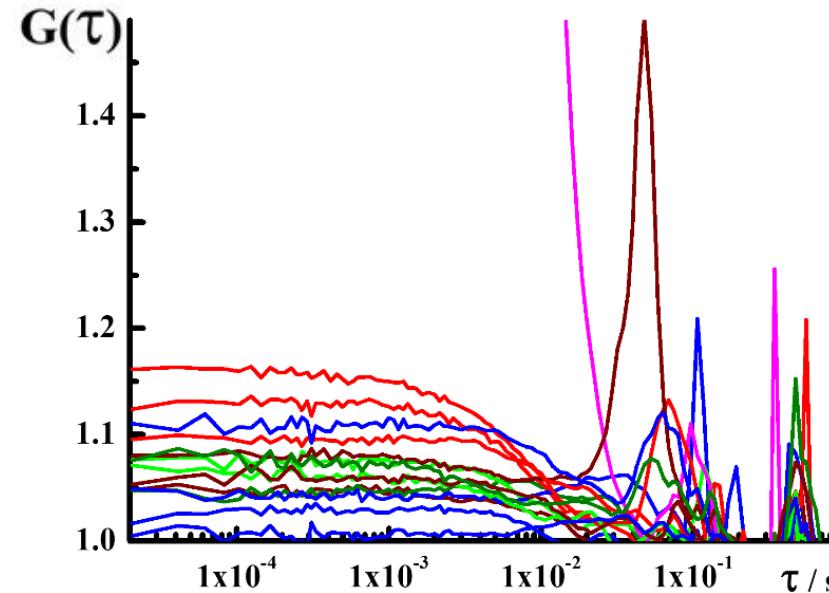
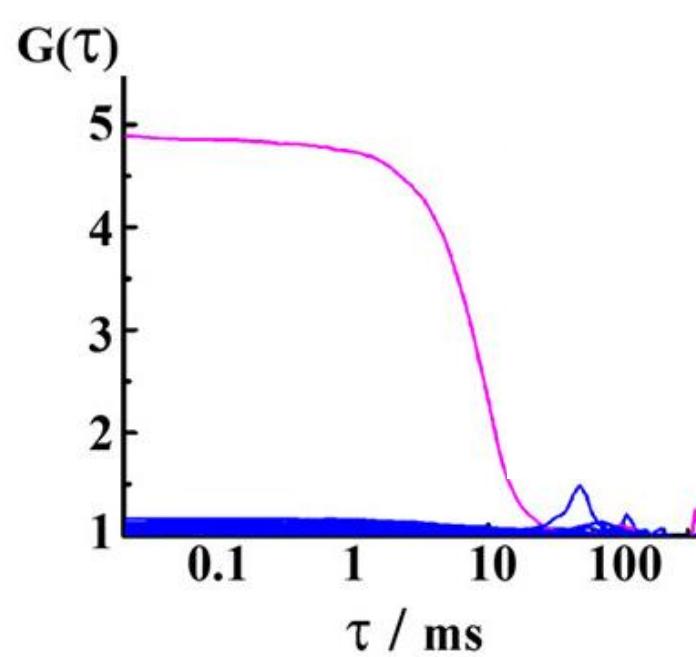
$$\frac{\omega_z}{\omega_{xy}} = 4.28$$

$$\omega_z = (1.1 \pm 0.2) \mu\text{m}$$

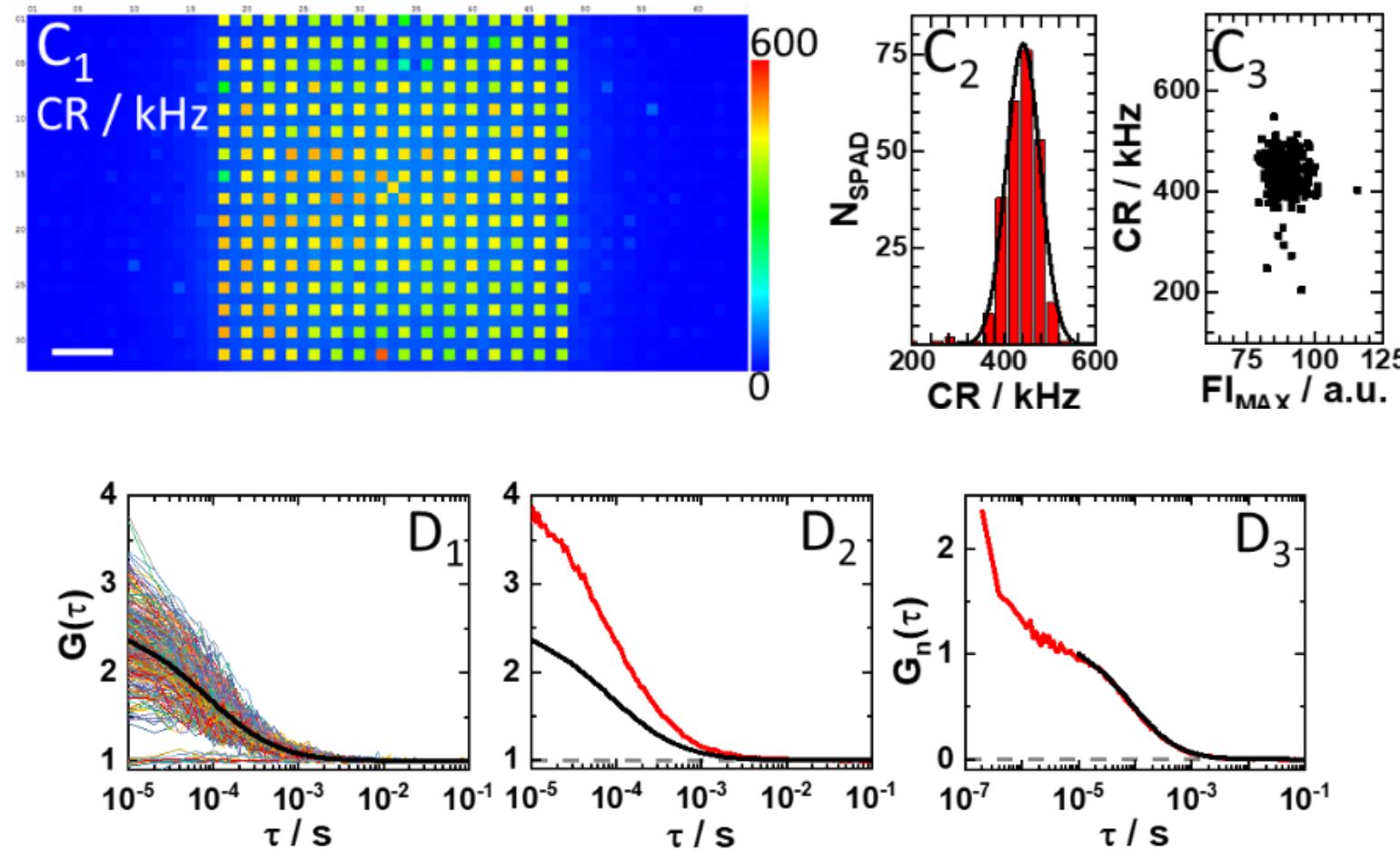
$$V_{eff} = \pi^{3/2} \cdot \omega_{xy}^2 \cdot \omega_z$$

$$V_{eff} = (0.4 \pm 0.2) \times 10^{-15} l$$

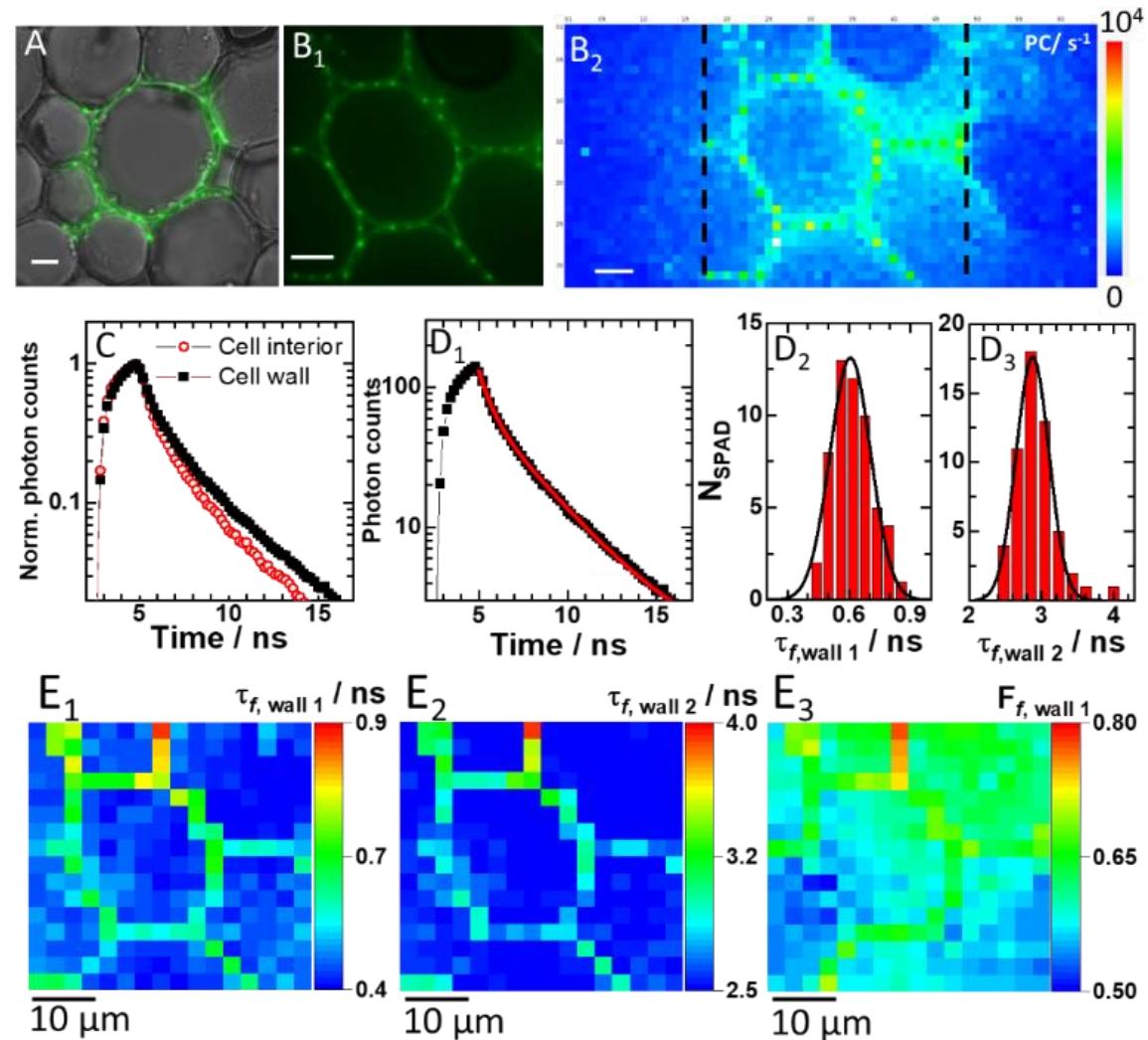
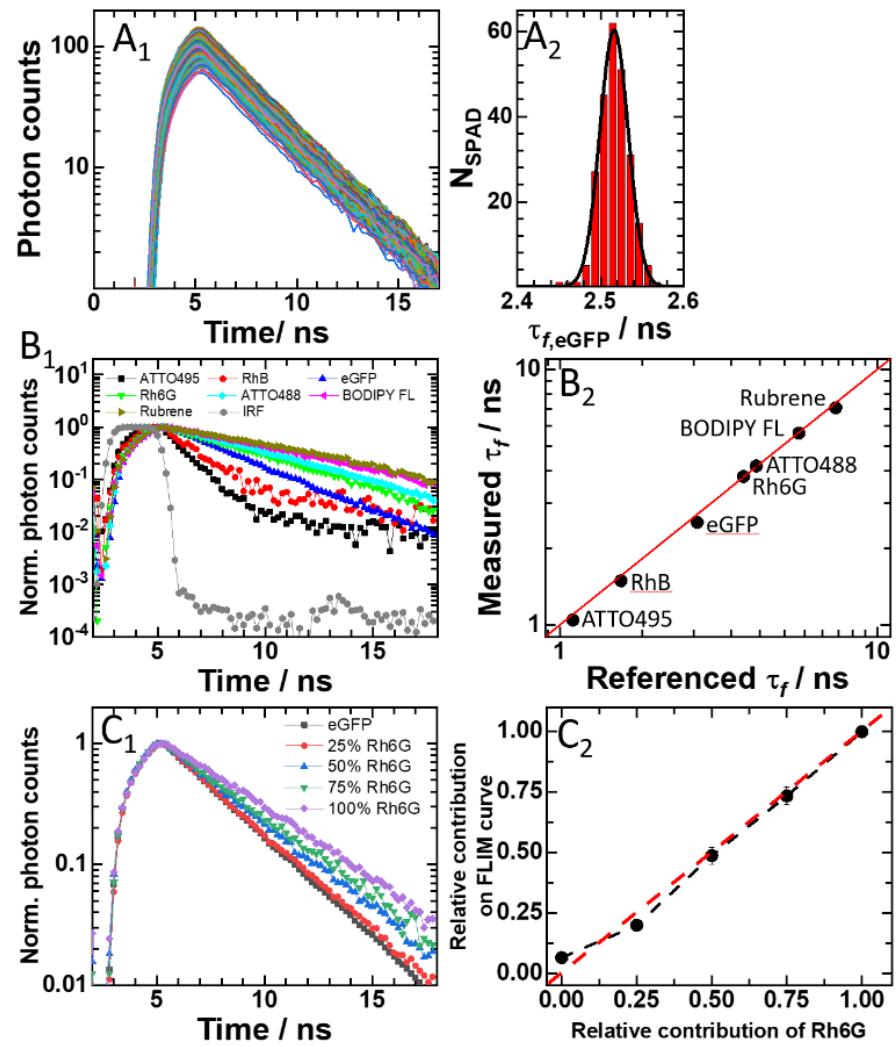
# mpFCS – crosstalk between adjacent pixels



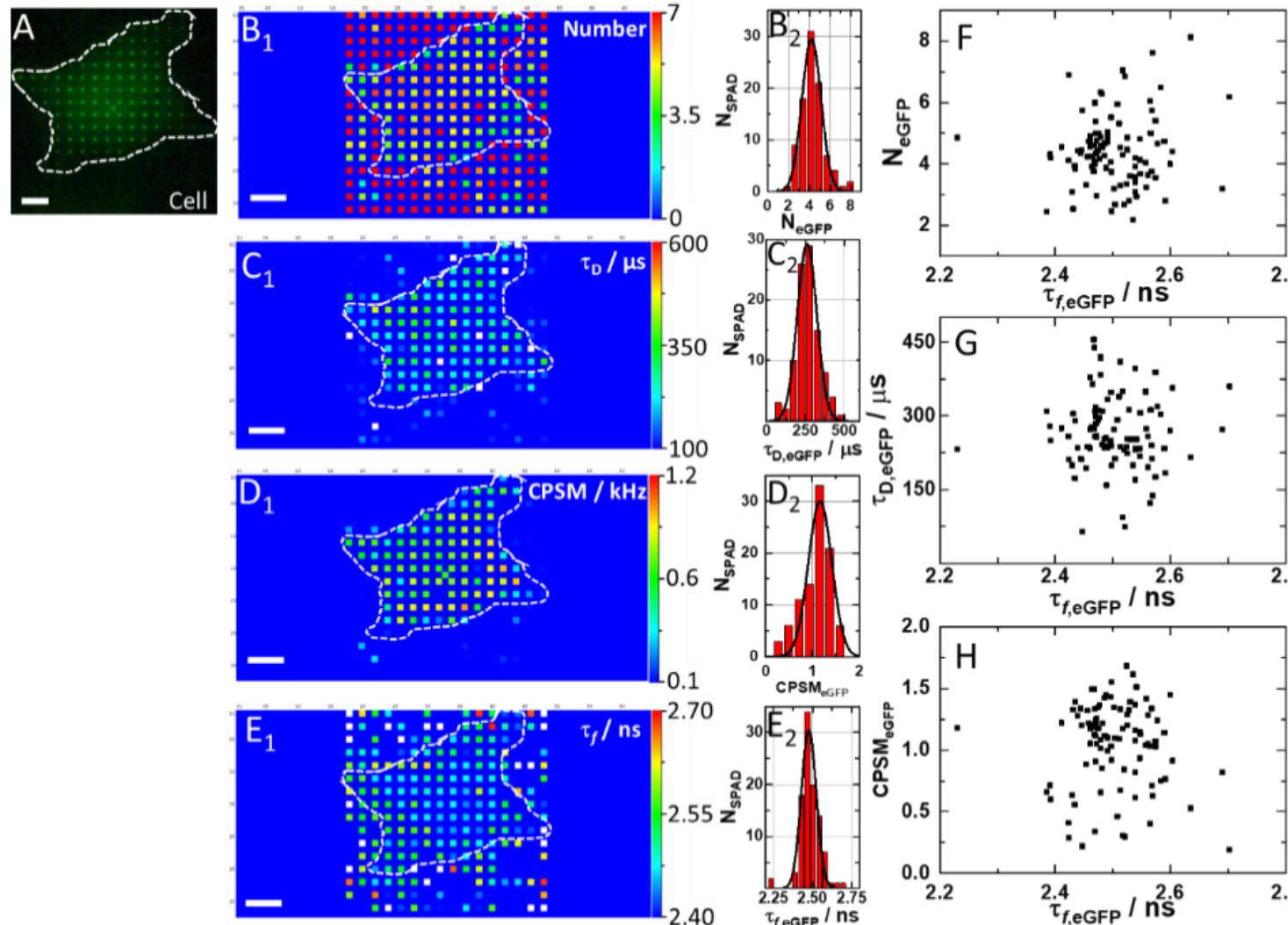
# Imaging eGFP concentration and diffusion *via* mpFCS



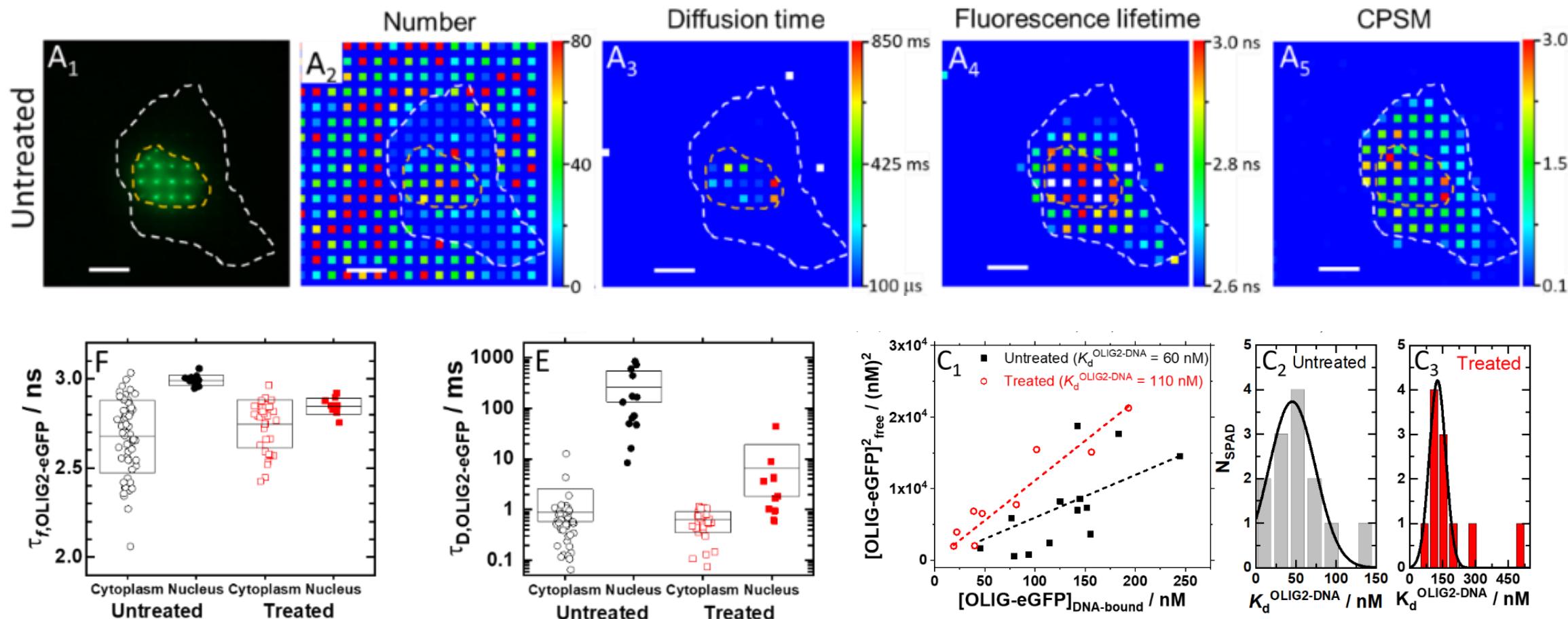
# mpFCS integrated with FLIM



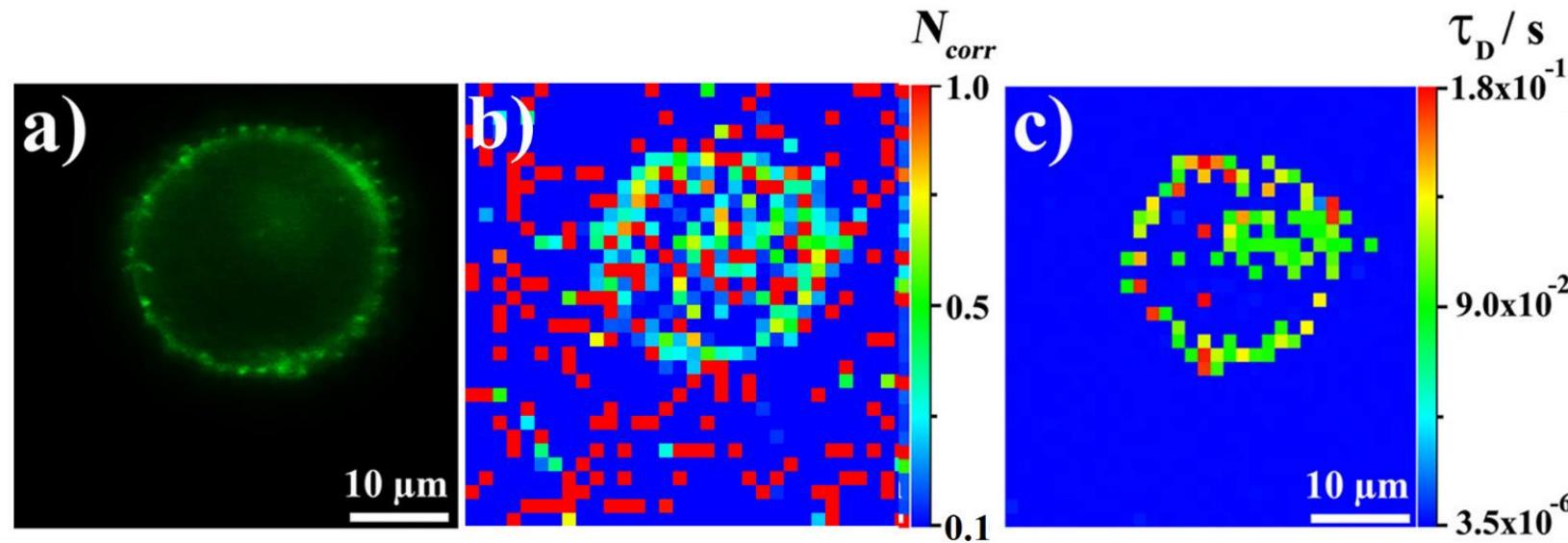
# Spatial map of eGFP concentration, diffusion, brightness and fluorescence lifetime in a live HEK cell



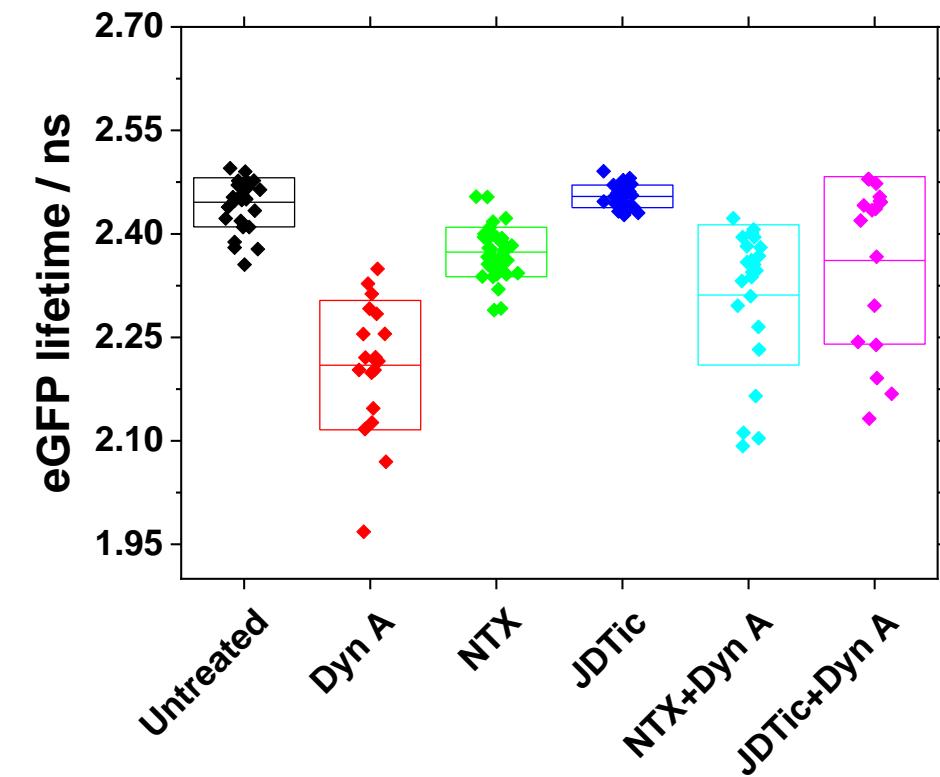
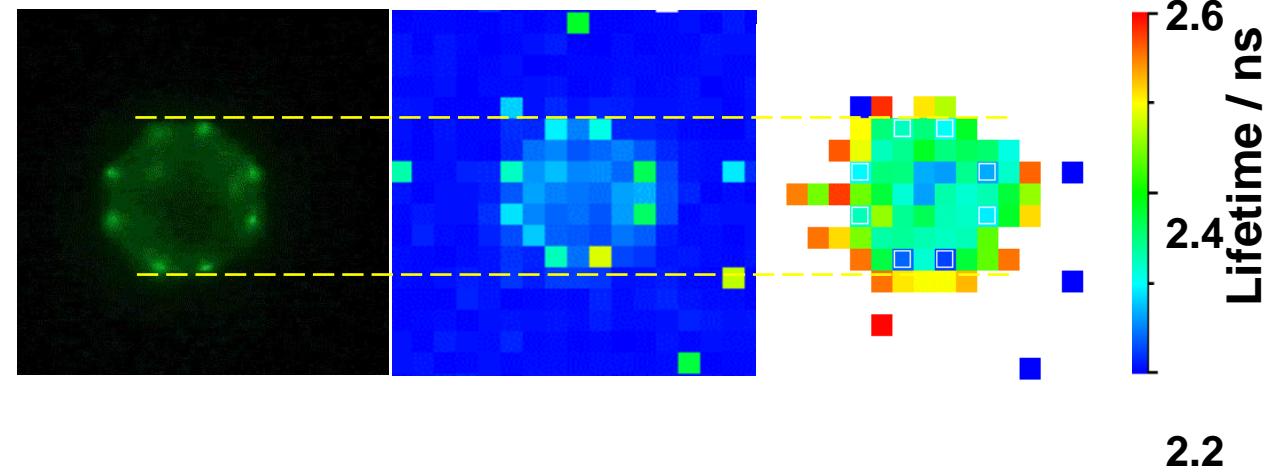
# OLIG2-eGFP concentration, diffusion, brightness and fluorescence lifetime in a live HEK cell. Effect of NSC 50467



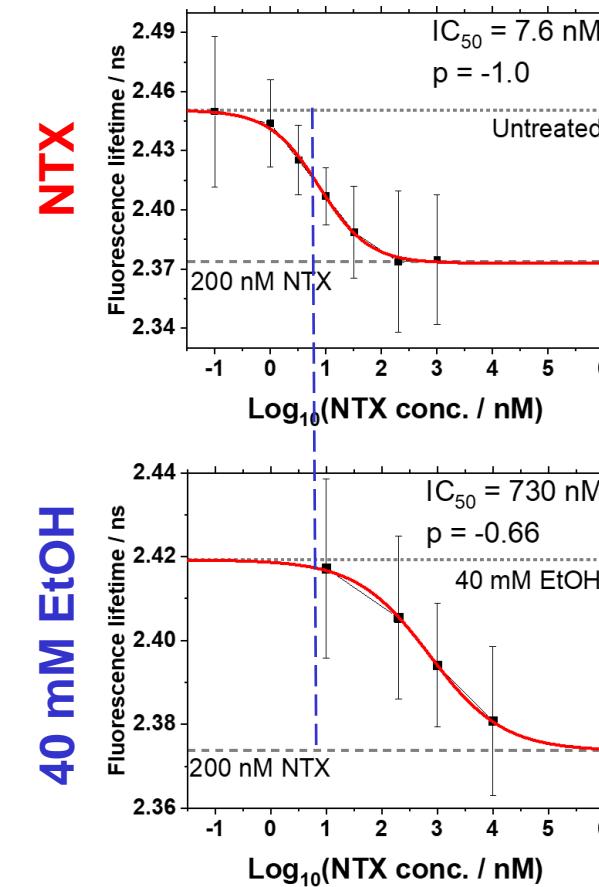
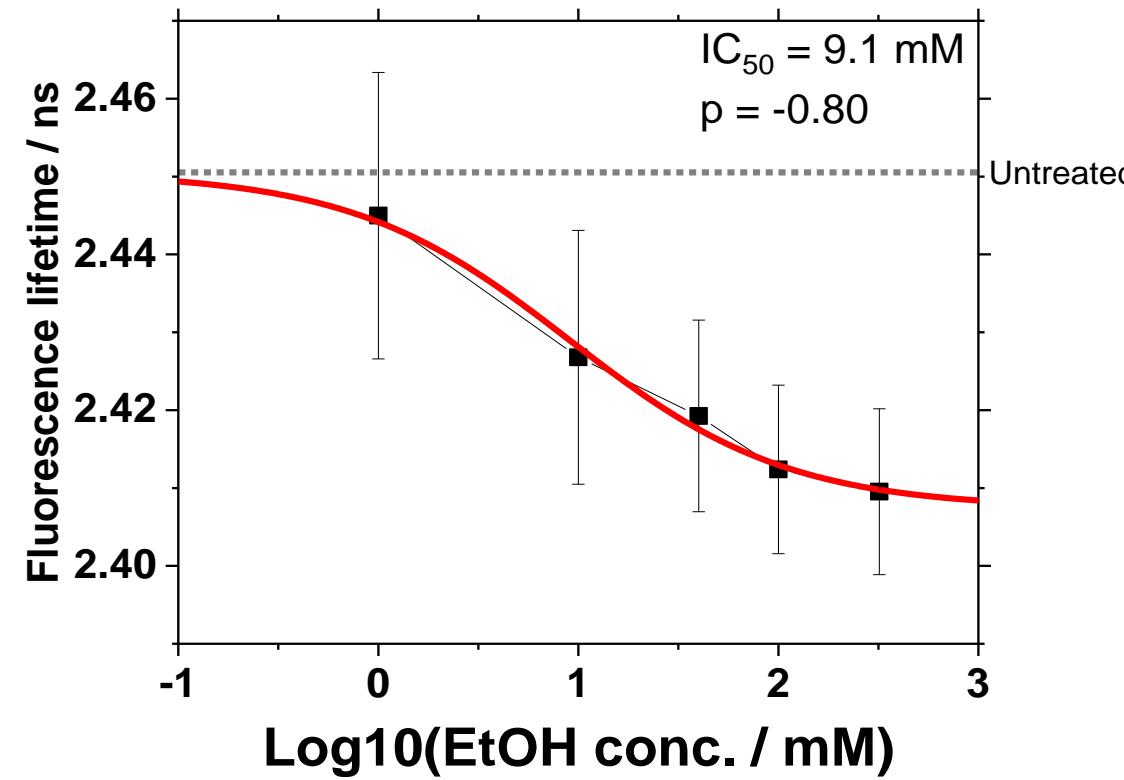
# Mapping the concentration and diffusion of MOP-eGFP in live cells



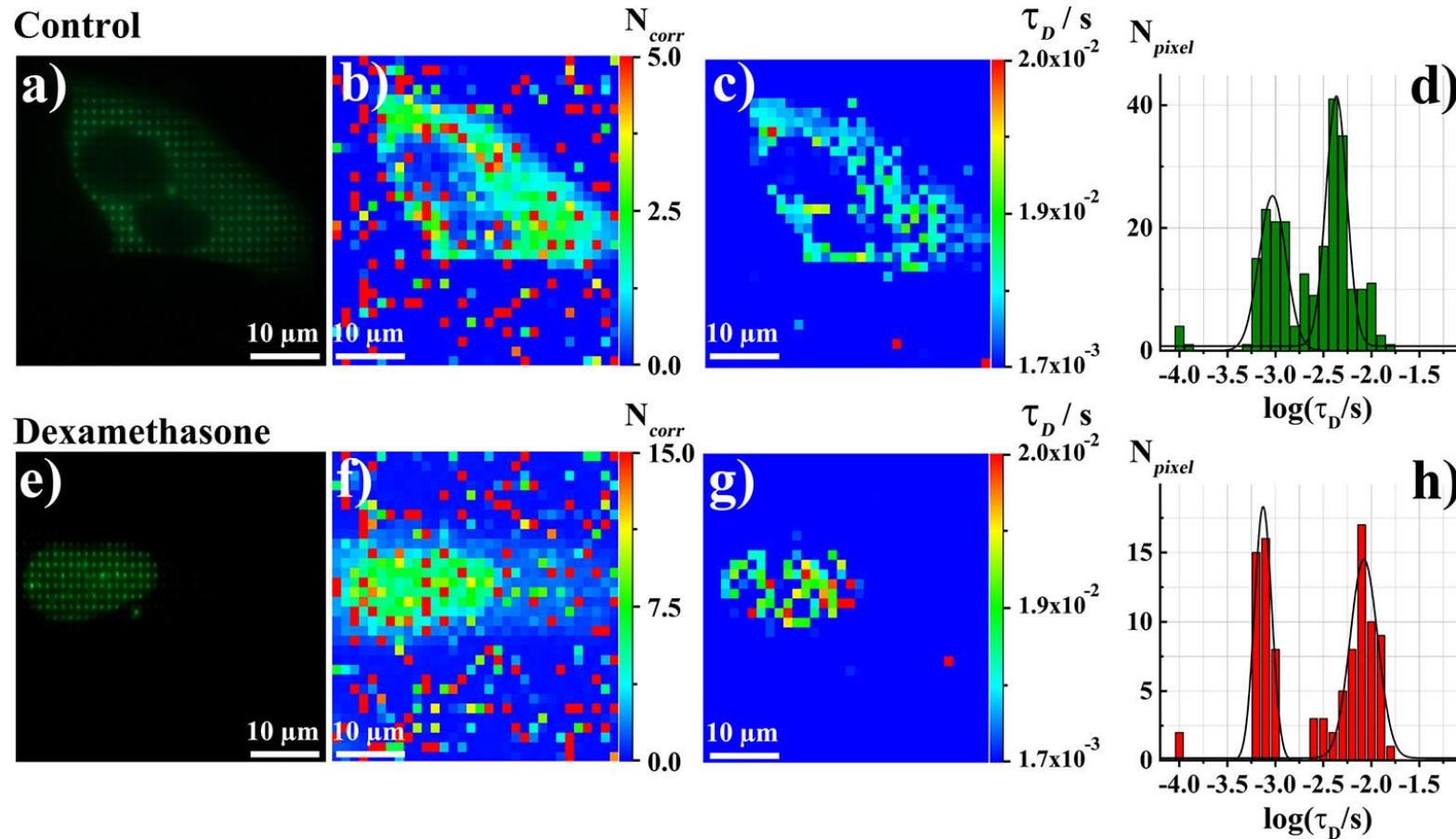
# Ligand effect on eGFP fluorescence lifetime in live PC12 cells stably expressing KOP-eGFP using mpFCS/FLIM



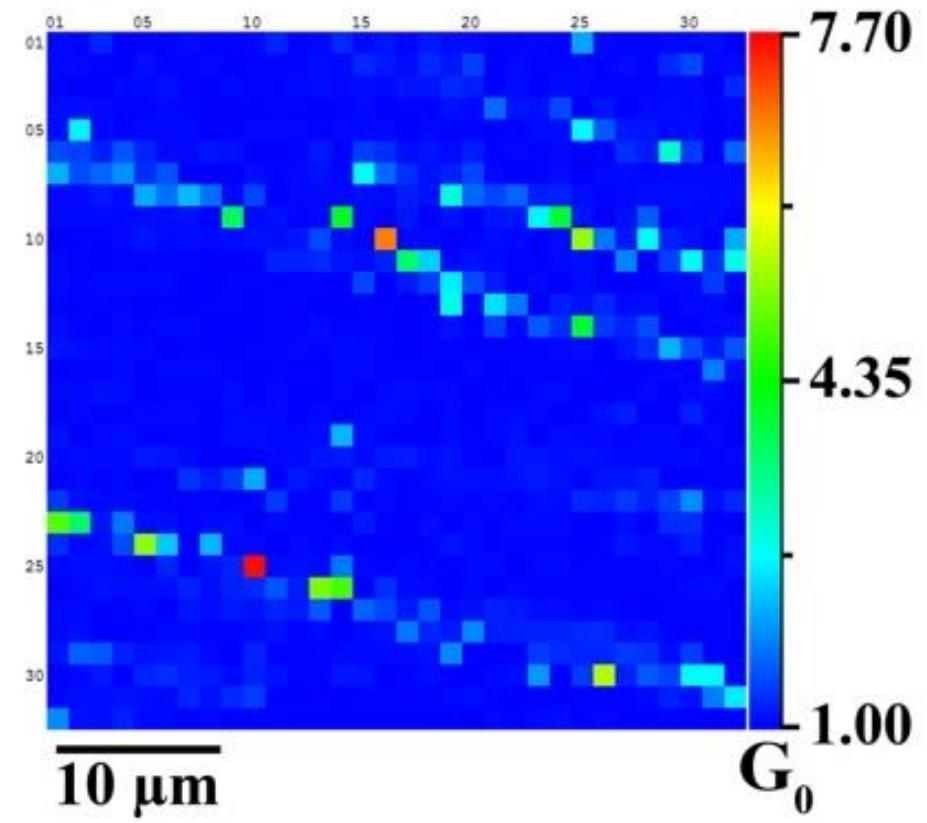
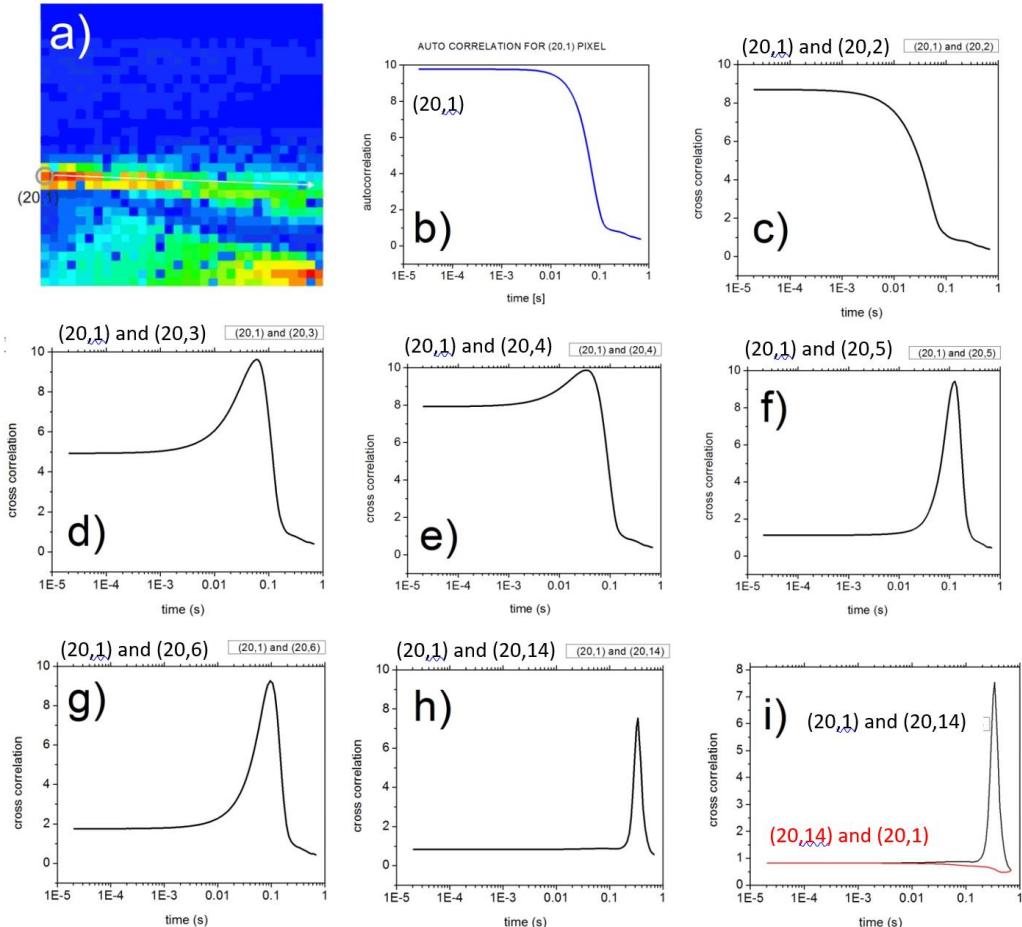
# Dose-dependent effect of kappa-opioid receptor antagonist on EtOH-induced change in eGFP fluorescence lifetime



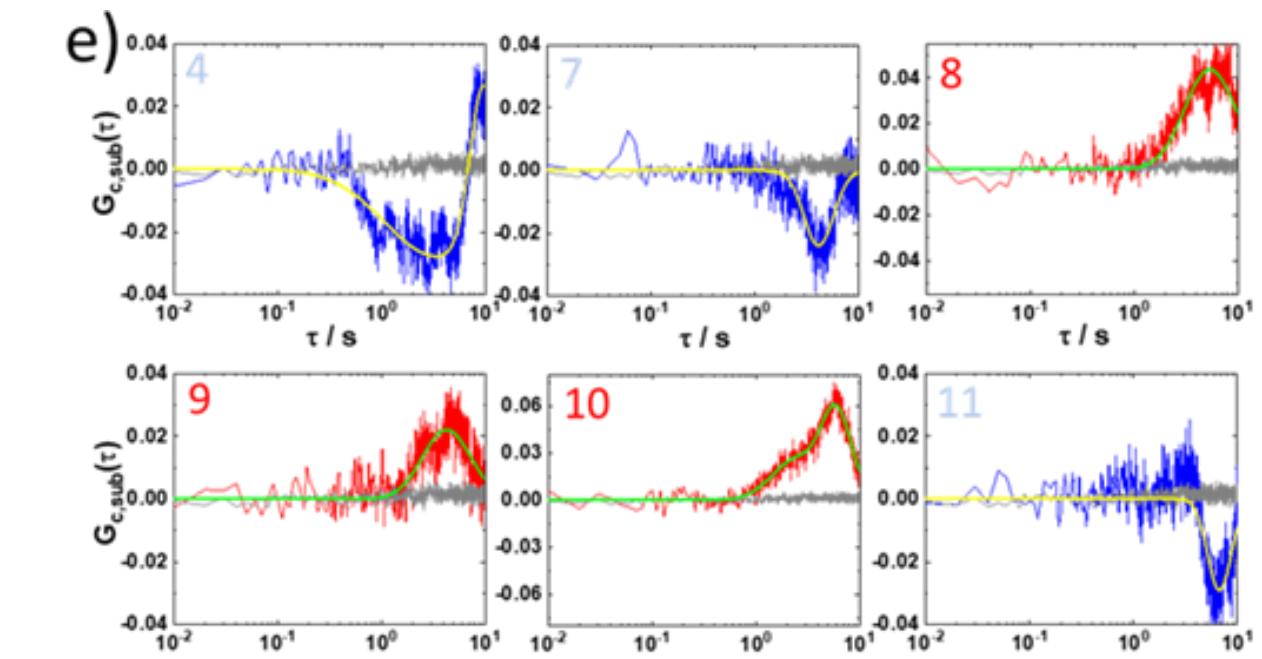
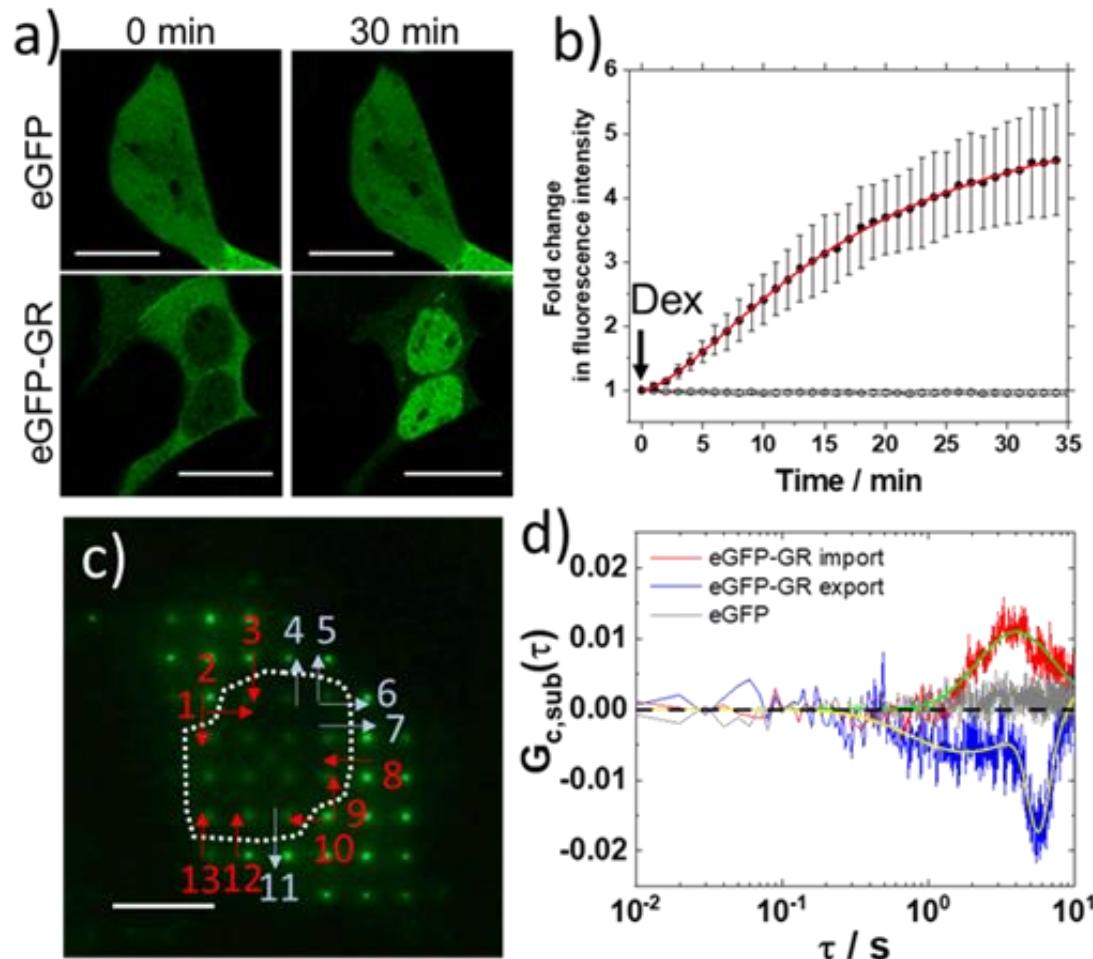
# Mapping the concentration and diffusion of glucocorticoid receptor (GR-eGFP) in live cells



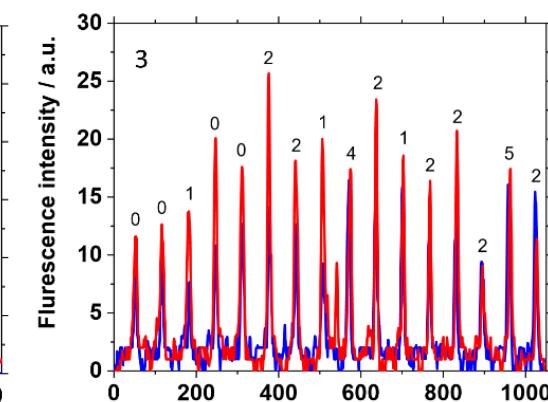
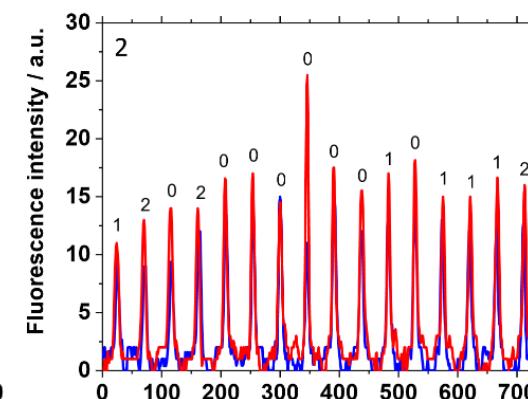
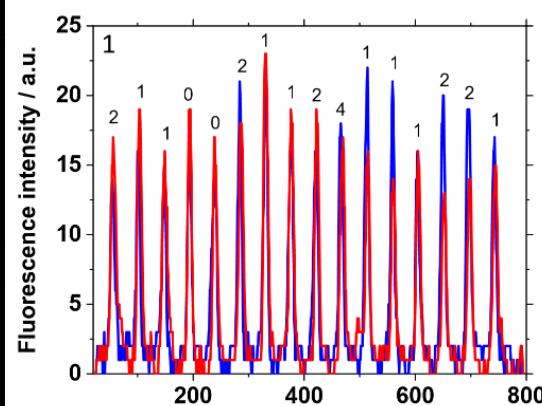
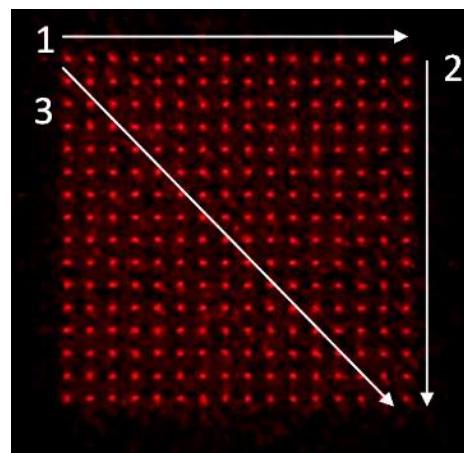
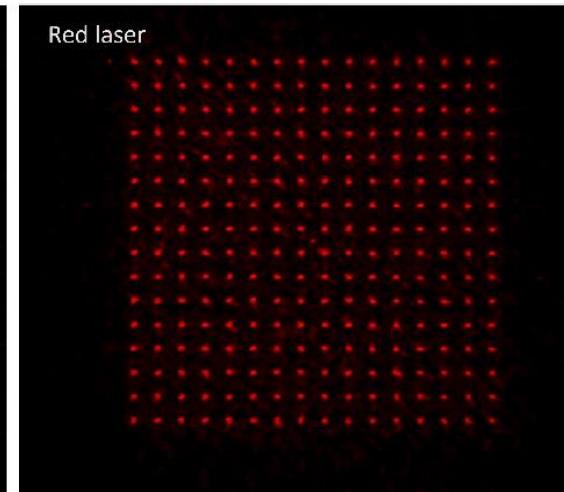
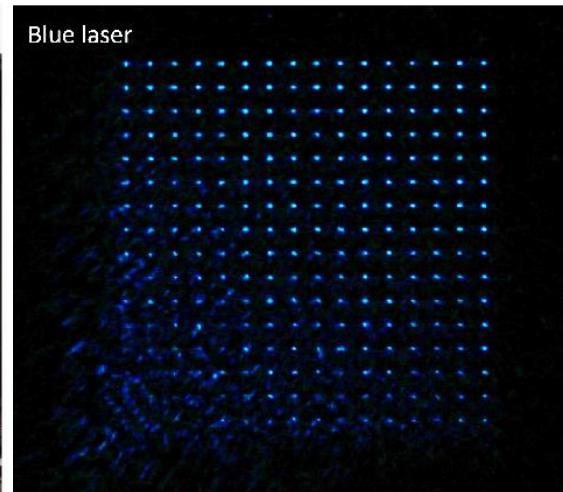
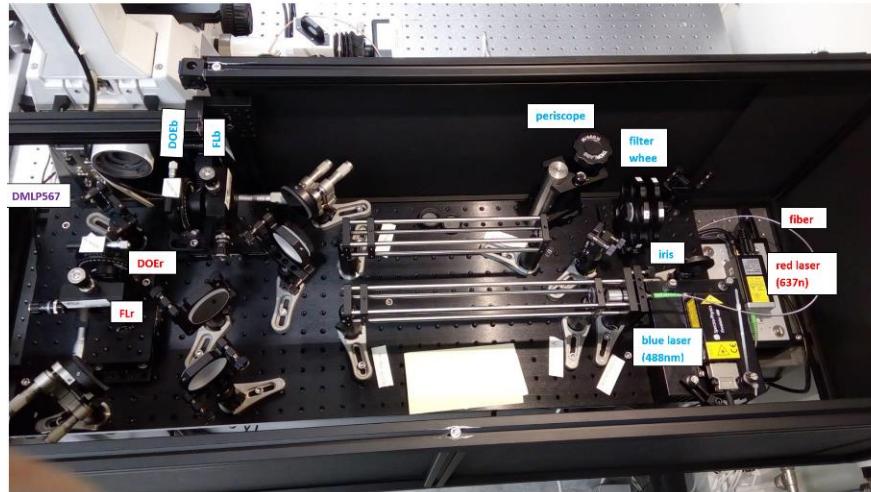
# Mapping the directional motion of fluospheres by two-foci spatial cross-correlation using mpFCS



# Mapping the direction of ligand-dependent nuclear translocation of glucocorticoid receptor in live cells by two-foci spatial cross-correlation using mpFCS



# Dual color massively parallel Fluorescence Cross-Correlation Spectroscopy (dc-mpFCCS)



Distance: 0 – perfect overlap; 1 = 68 nm; 2 = 136 nm

# Acknowledgements



## Karolinska Institutet

Per Svenningsson (CNS)  
Eva Kosek (CNS)  
Nenad Bogdanović (NVS)  
Vesna Jelić (NVS)  
Tomas Ekström (CNS)  
Per Nilsson (NVS)

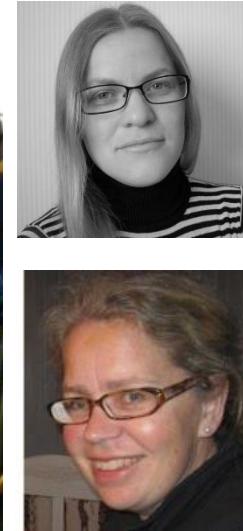
## Sweden

Astrid Gräslund (SU)  
Ludmilla Morozova-Roche (UmU)  
Ülo Langel (SU)



## International

Tijana Jovanović-Talisman (CoH, USA)  
Thomas Sakmar (Rockefeller University, USA)  
Osama Abulseoud (NIH/NIDA, USA)  
Claudio D'Addario (Teramo, Italy)  
Thomas Friedrich (TU Berlin, Germany)  
Marco Vitali (Sicoya, Germany)  
Dimitrios Papadopoulos (HGU Edinburgh, UK)  
Masataka Kinjo (Sapporo, Japan)  
Ljiljana Kolar-Anić (FFH, Serbia)  
Željko Čupić (IHTM, Serbia)



**THANK YOU!**