Adaptive optics for fluorescence correlation spectroscopy (FCS)

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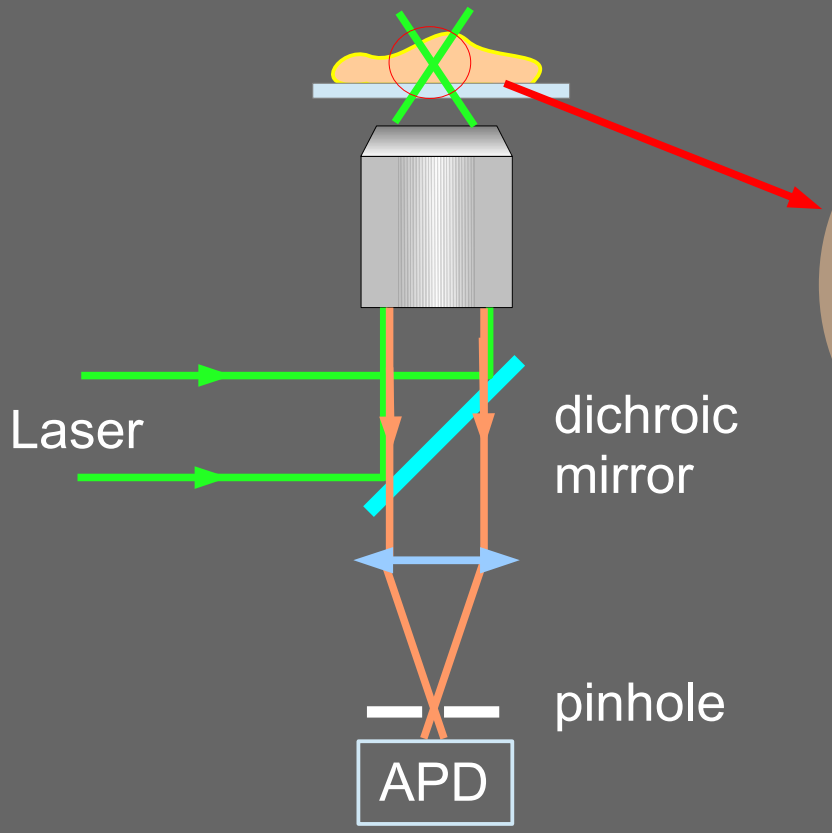
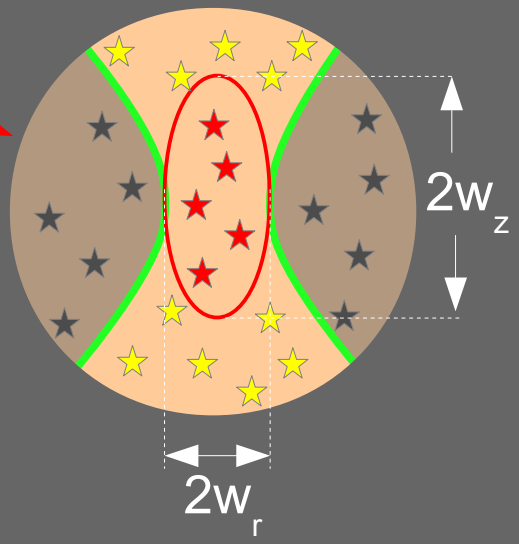
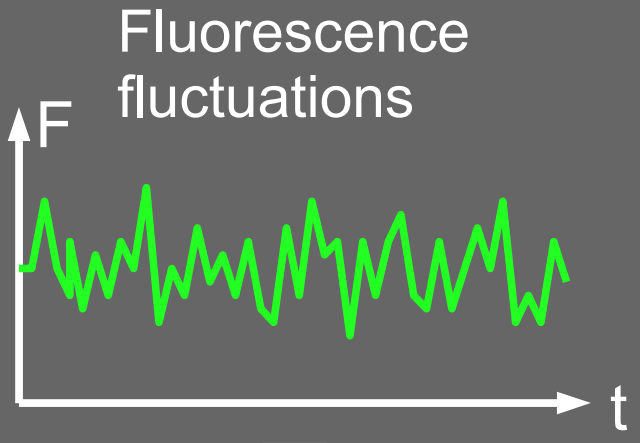
LIPhy (Grenoble)

Fluorescence correlation spectroscopy (FCS)

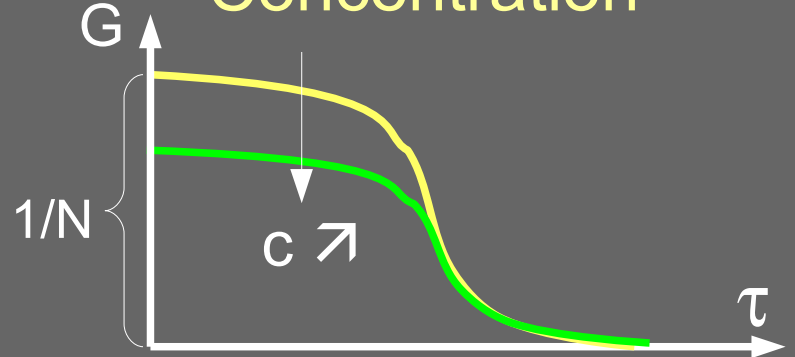


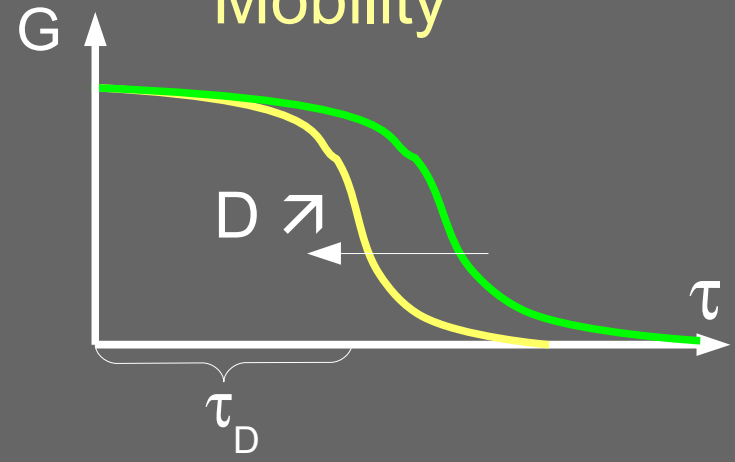




Autorrelation function

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





Why do we need adaptive optics ?

aberrations

Measured parameters

N, τ_D

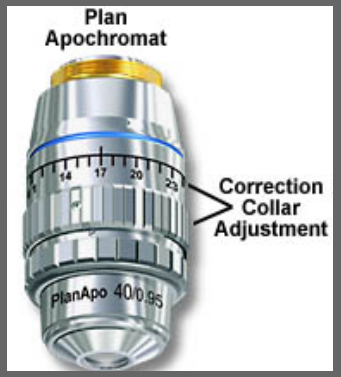
Calibration : w_r, w_z

$$c = \frac{N}{\pi^{3/2} w_r^2 w_z} \qquad D = \frac{w_r^2}{4\tau_D}$$

Physical quantities

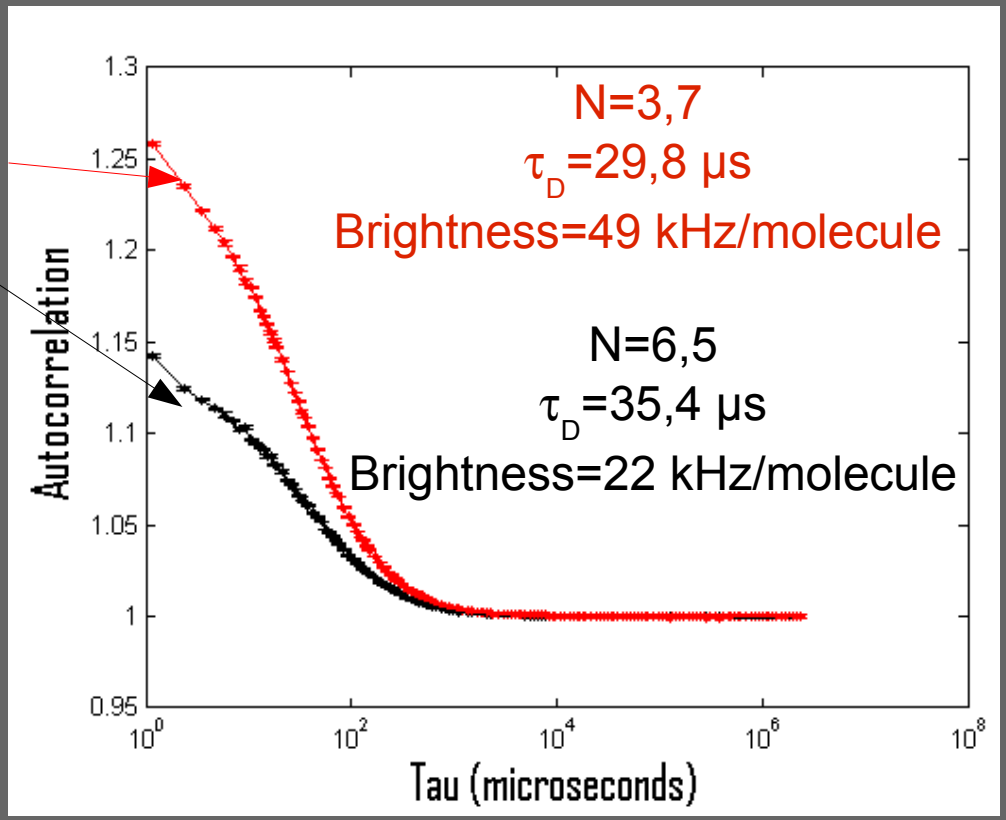
c, D

Example: incorrect adjustement of the objective coverslip correction collar



Coverslip thickness : 150 µm

- Correct adjustment of the collar
- Collar adjusted to 170 µm (spherical aberrattion ~60 nm rms)

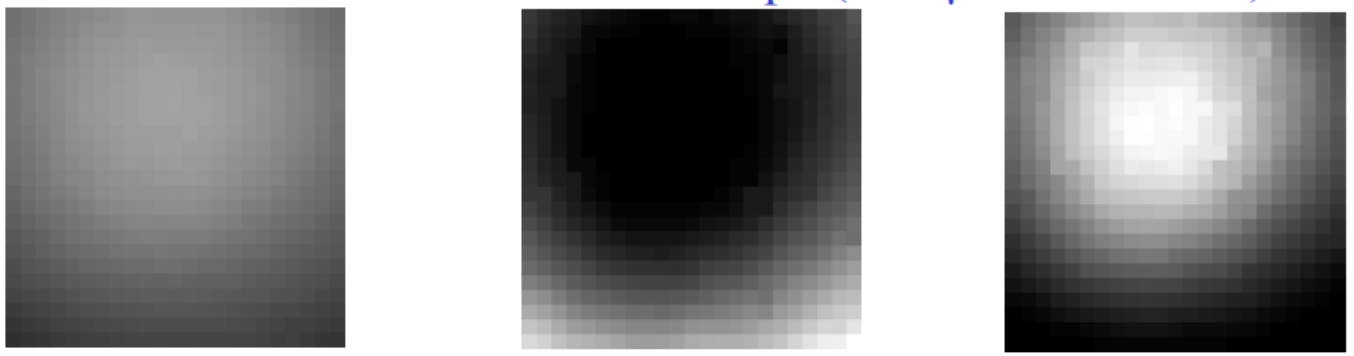


FCS is very sensitive to optical aberrations

Why do we need adaptive optics ?

Another example : Raster Image Correlation Spectroscopy

RICS measurements performed at CRI U823 lab. (in solution)
on a commercial FFM microscope (350 µm scan field).



Intensity, scaling from
52 (bottom right corner) to 156

Number of molecules, scaling from
2.8 (bottom right corner) to 0.59

Brightness, scaling from 18
(bottom right corner) to 263

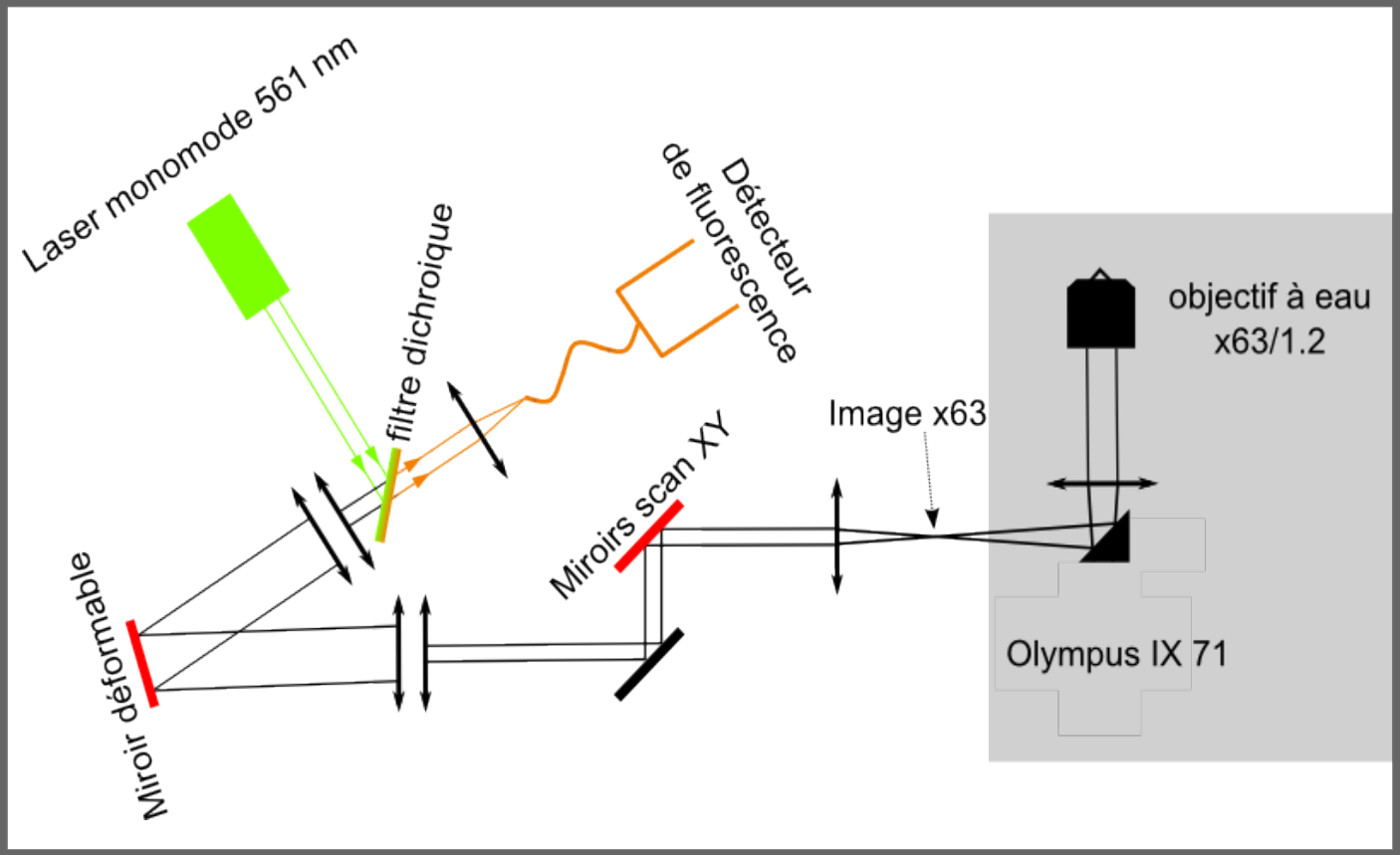
(Alexei Grichine)

In the field of view :

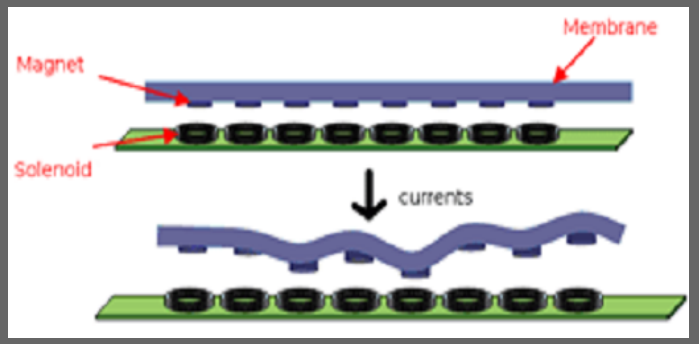
- Number of molecules varies by a factor of 5
- Brightness varies by a factor of 15

Home-built confocal microscope

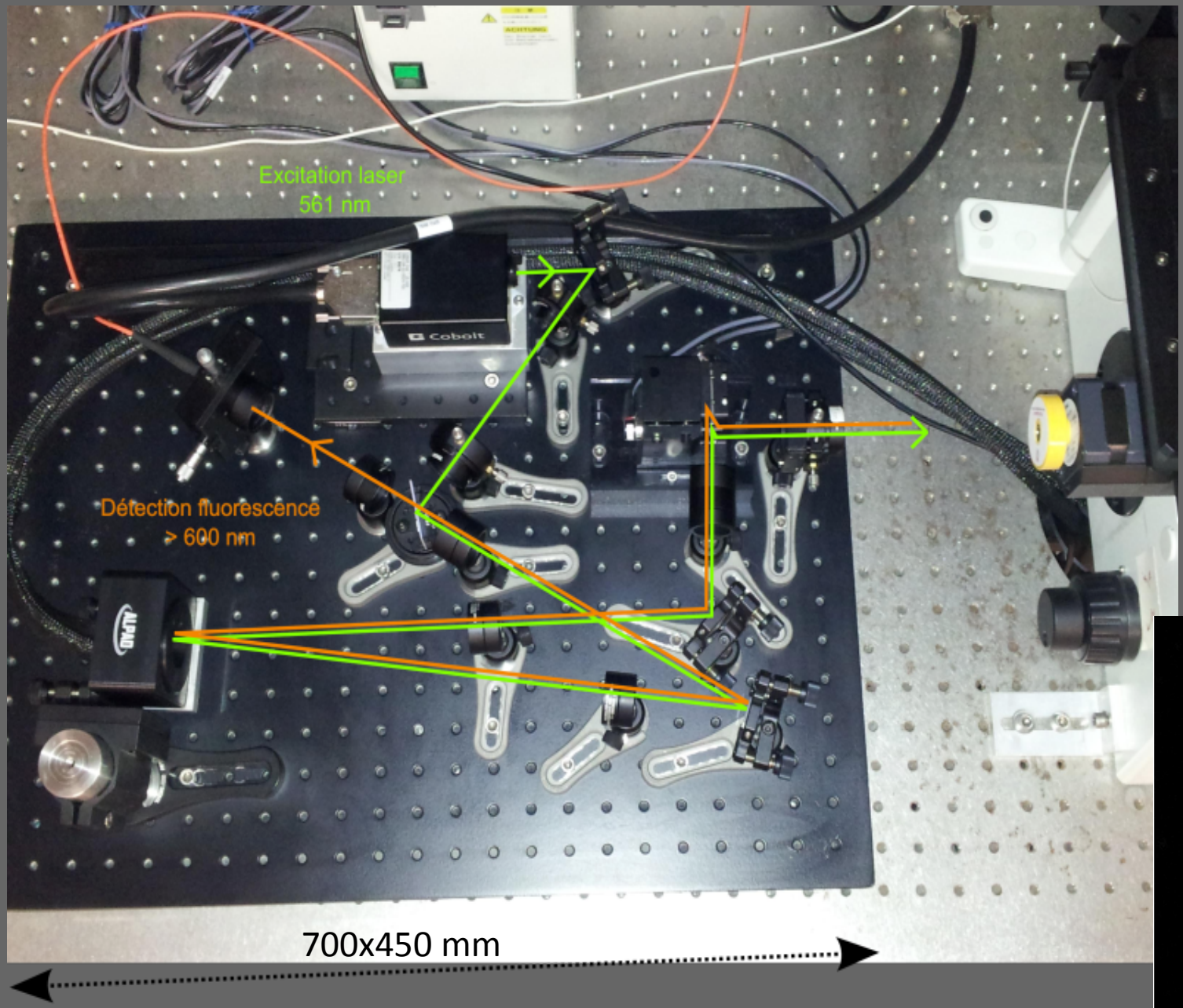
A deformable mirror is used on both excitation and fluorescence paths



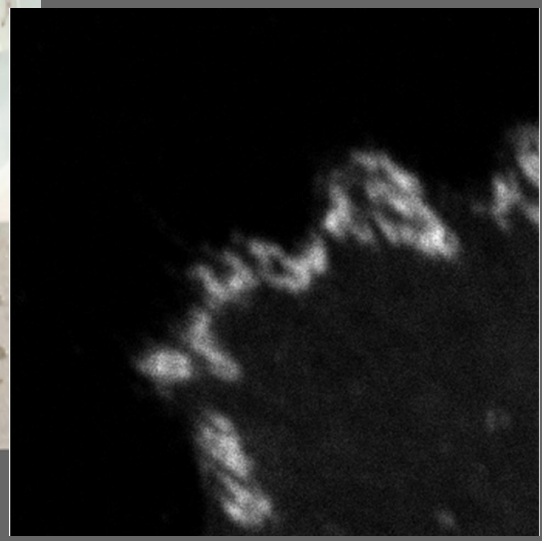
Deformable mirror : 97 actuators (ALPAO)



Experimental setup

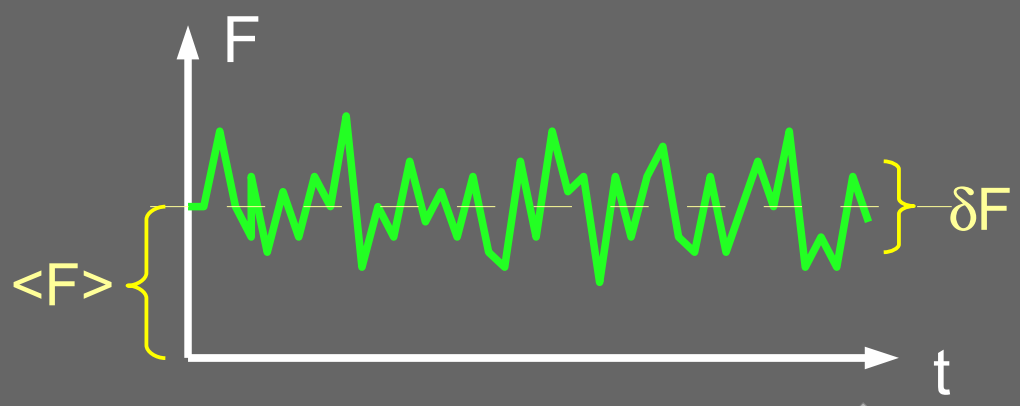


MEF Paxillin-mCherry



Sensorless adaptive optics scheme

What metric ?



Count rate

$\langle F \rangle$

Average number of molecules

$\langle N \rangle$

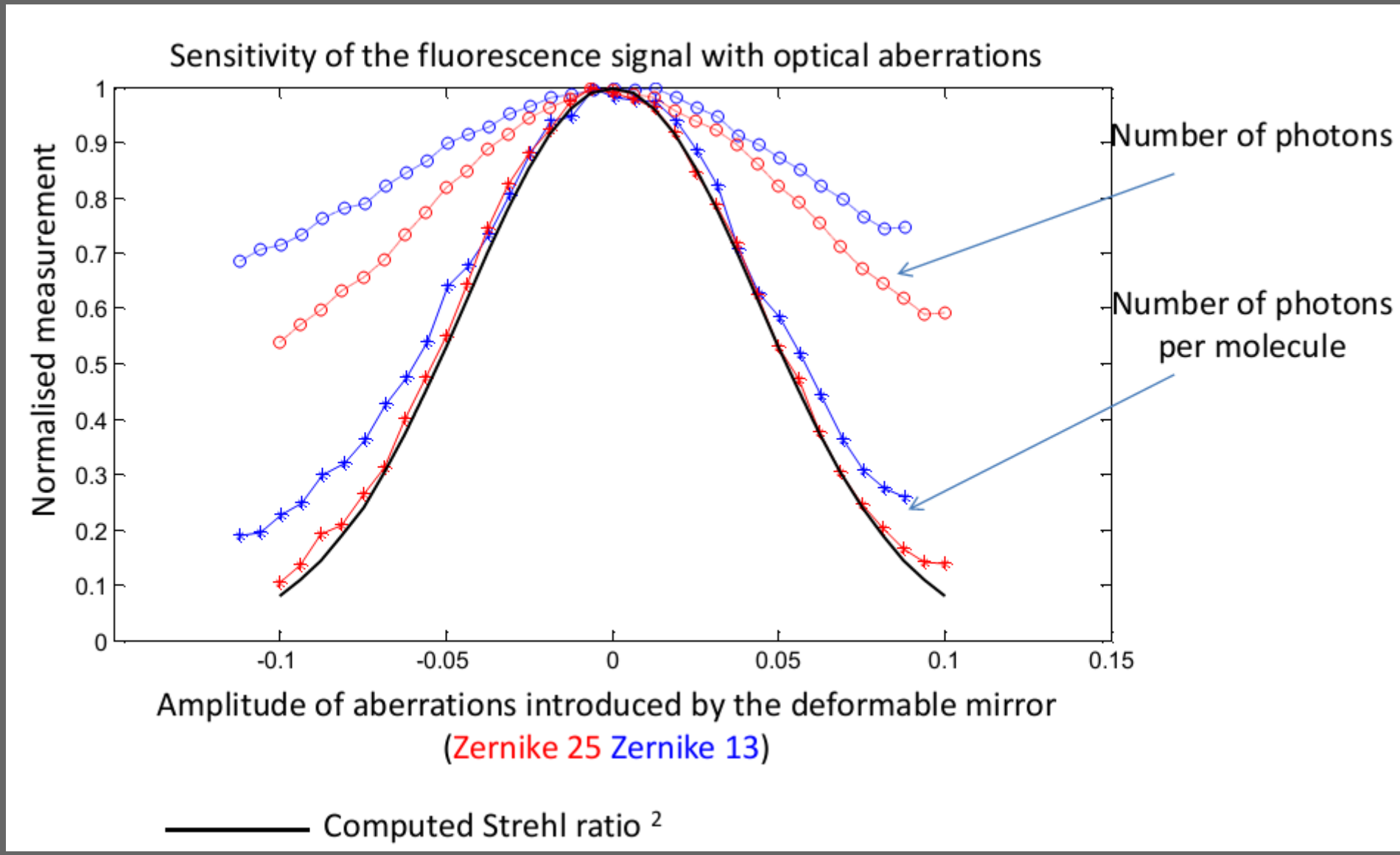
Molecular brightness
(count rate per molecule)

$$CRM = \frac{\langle F \rangle}{\langle N \rangle}$$

FCS provides a quality metric without images

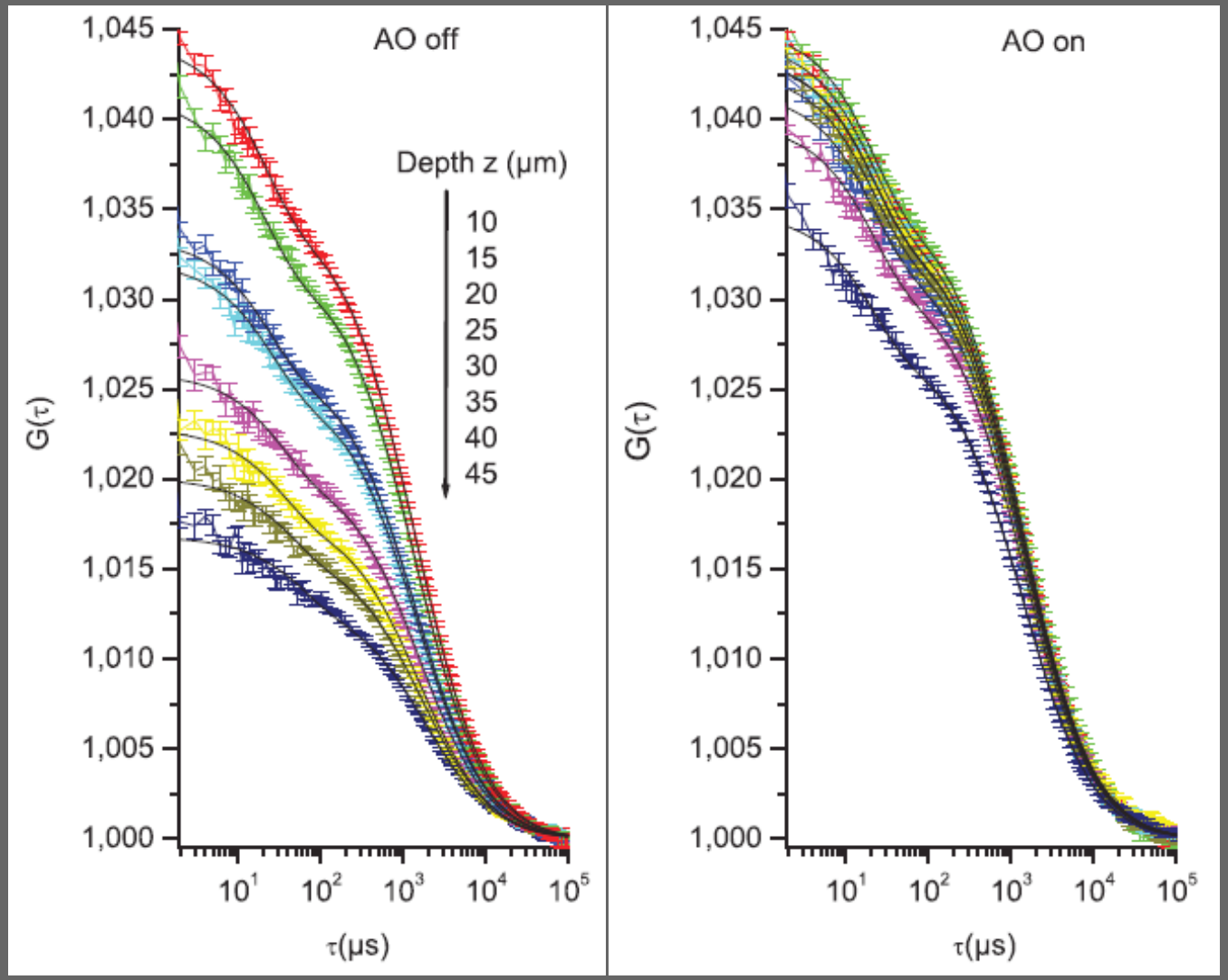
$$CRM \propto Strehl^2 \approx \exp\left[-2\left(\frac{2\pi\sigma}{\lambda}\right)^2\right]$$

Wavefront quadratic error



Adaptive optics correct focusing depth induced errors

FCS measurements in a water-glycerol mix (n=1.435) with a water immersion objective (n=1.33)



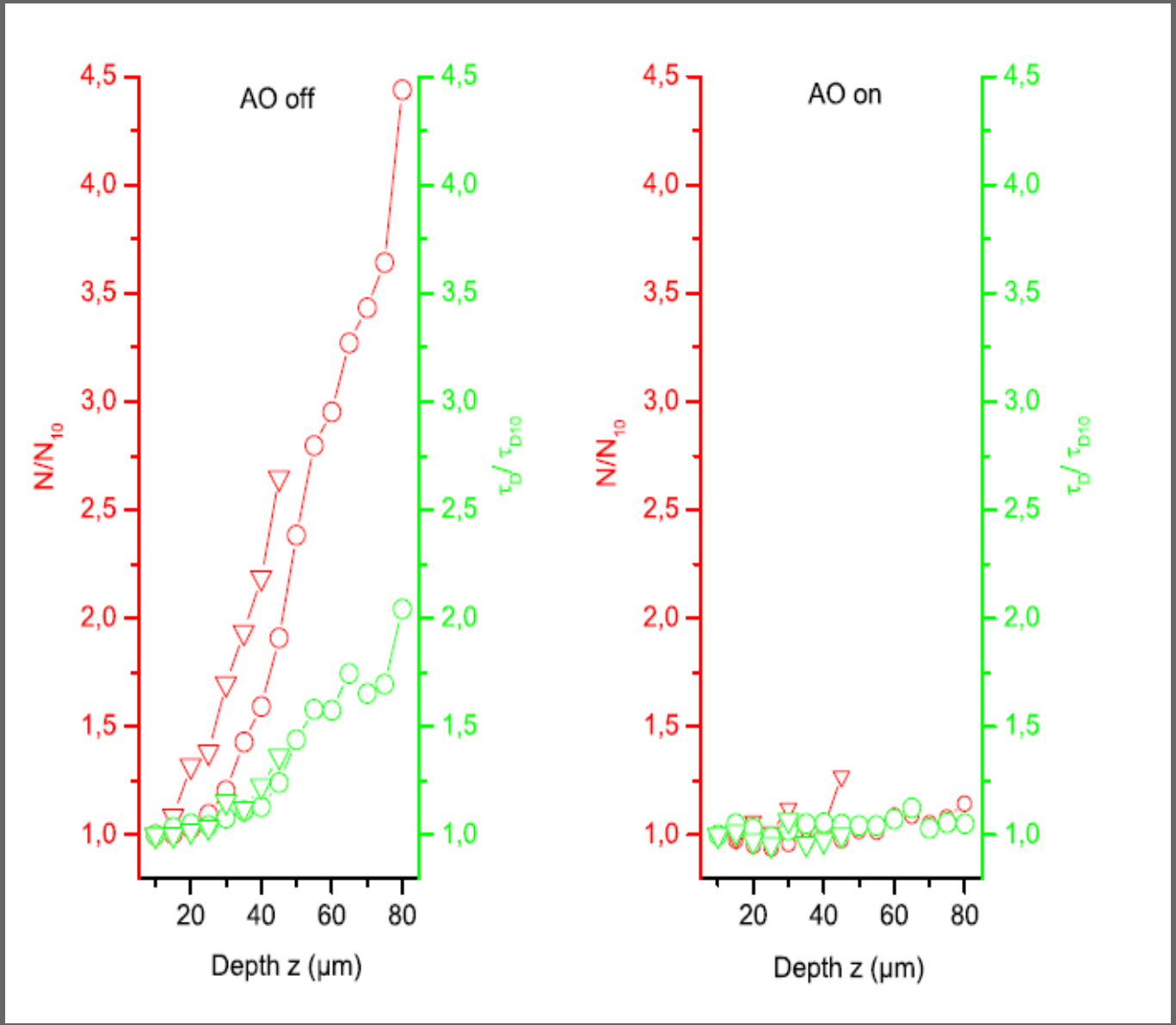
Optimization cost :

3 data points
x 1 sec
x 7 Zernike modes
x 2 iterations
= 42 secs

To be compared to the time needed for a FCS acquisition :
10 x 10 secs

C.-E. Leroux *et al.*, Opt. Exp. 19 (2011) 26839

Stabilization of estimated parameters thanks to AO



Conclusion

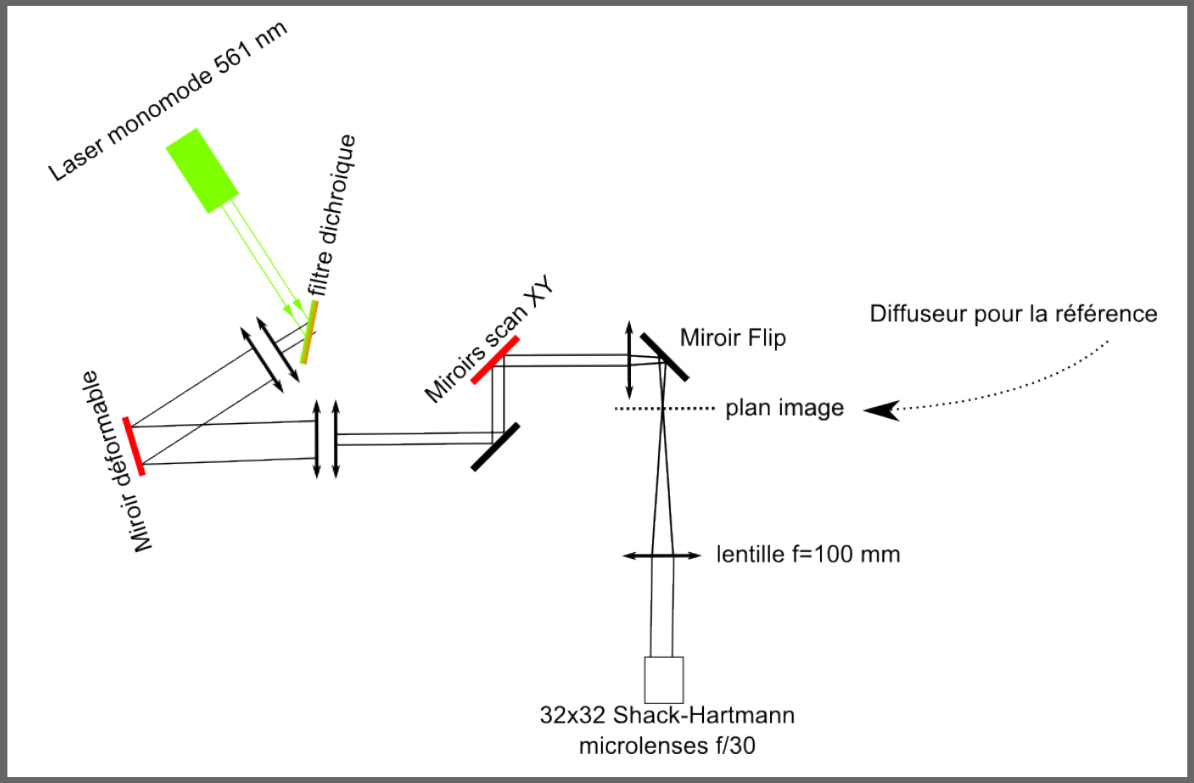
- FCS is very sensitive to optical aberrations
- Sensorless AO is efficient to stabilize FCS parameters
- Molecular brightness is an image quality metric

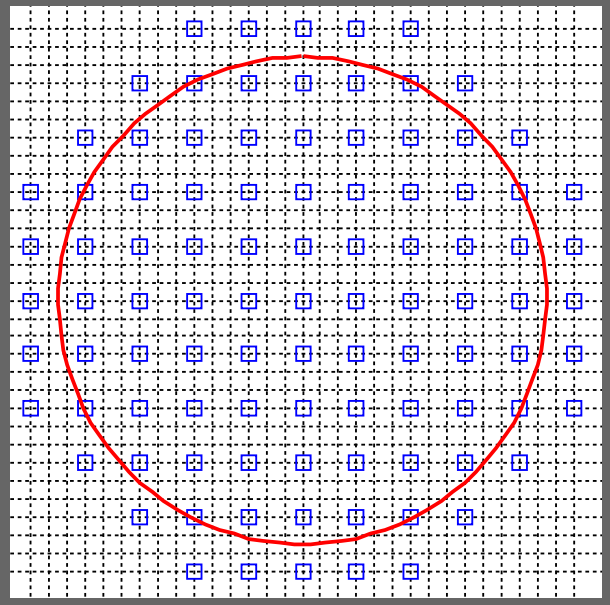
$$CRM \propto Strehl^2$$

In progress...

- Application to living cells
- Extension to other fluctuation techniques

Calibration du miroir déformable ALPAO DM 97-15





Diamètre de pupille: 13.5 mm
9 actuateurs, 26 lentilles
3 lentilles/actuateur

Aqueous glycerol solutions of A647

Spherical aberrations (a_{10}) and the residuals (r_{10}) *vs* depth

A bit of theory

$$CR = \eta \times C \times S_{tr}^2 \int PSF_{con} d\vec{r}$$

PSF(0) = 1 S_{tr} = single pass Strehl ratio

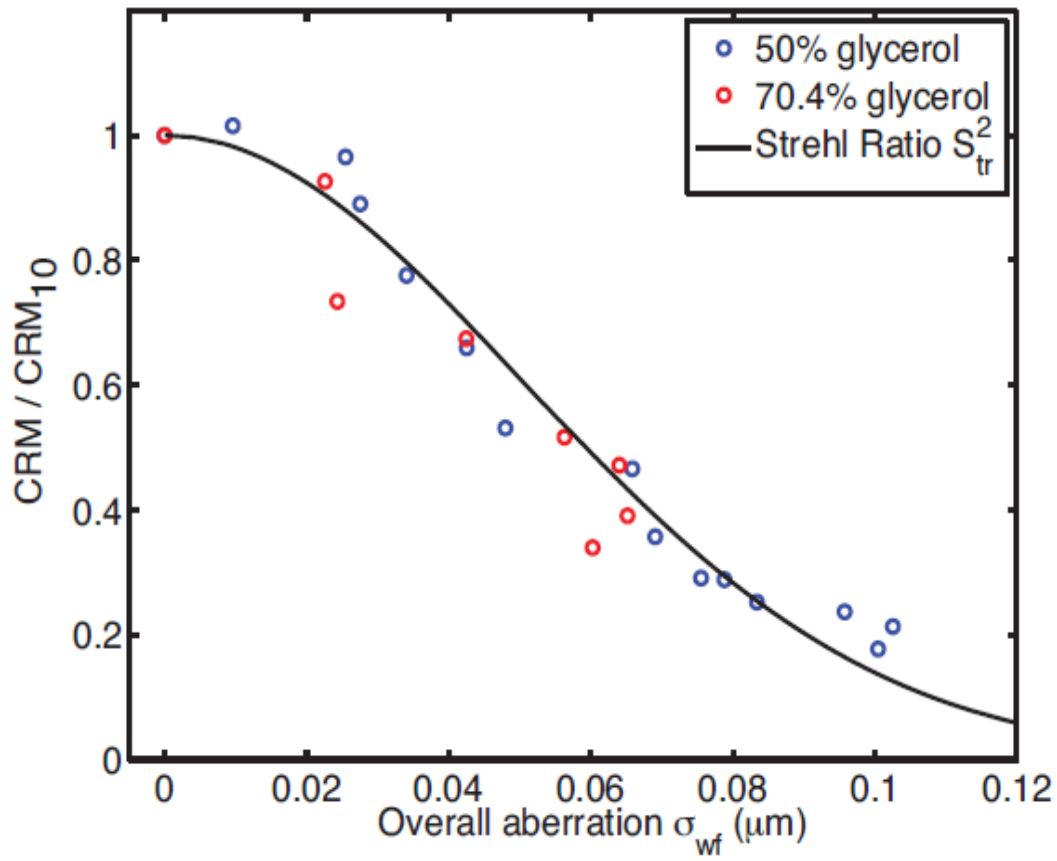
$$CRM = CR/(C \times V_{fcs})$$

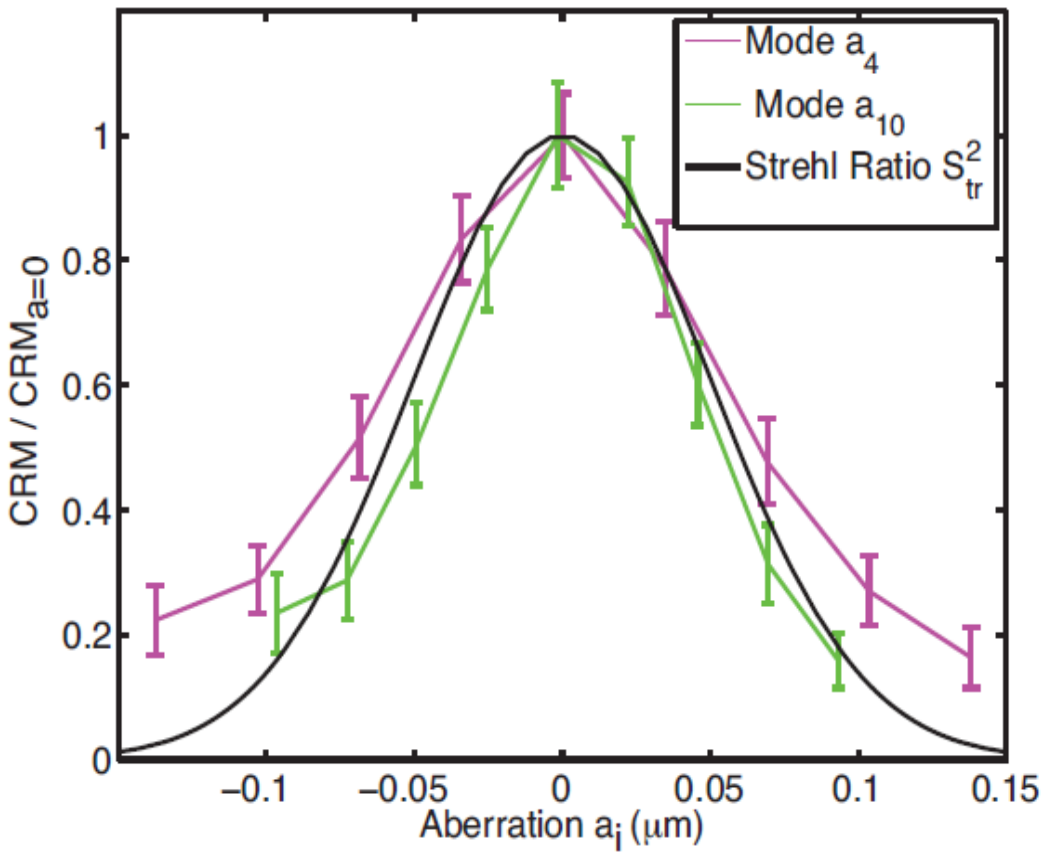
Gaussian PSF

where $$S_{tr} \simeq \exp^{-\left(\frac{2\pi \times \sigma_{wf}}{\lambda}\right)^2}$$

with σ_{wf} = aberration amplitude (RMS)

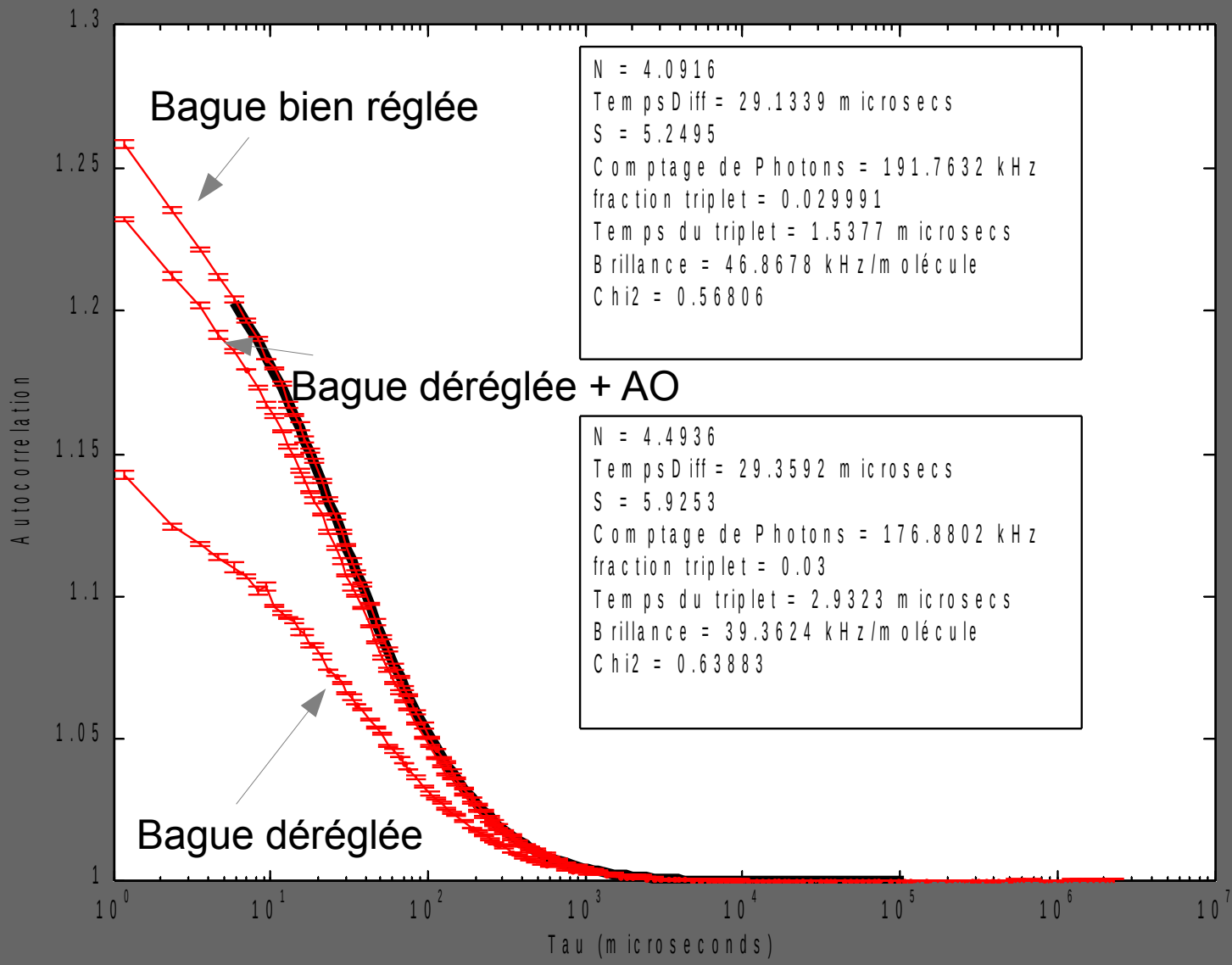
Experiment *vs* theory





Aberrations due to refraction index mismatch

Aberrations generated on purpose by the deformable mirror


Bague bien réglée
N = 4.0916
TempsDiff = 29.1339 microsecs
S = 5.2495
Comptage de Photons = 191.7632 kHz
fraction triplet = 0.029991
Temps du triplet = 1.5377 microsecs
Brillance = 46.8678 kHz/molécule
Chi2 = 0.56806
Bague déréglée + AO
N = 4.4936
TempsDiff = 29.3592 microsecs
S = 5.9253
Comptage de Photons = 176.8802 kHz
fraction triplet = 0.03
Temps du triplet = 2.9323 microsecs
Brillance = 39.3624 kHz/molécule
Chi2 = 0.63883
Bague déréglée
Autocorrelation
1.3
1.25
1.2
1.15
1.1
1.05
1
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
Tau (microseconds)