

# Rejet du fond fluorescent dans les tissus par microscopie de fluorescence à excitation biphotonique par aberration différentielle (DA-TPEF)

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Lille*

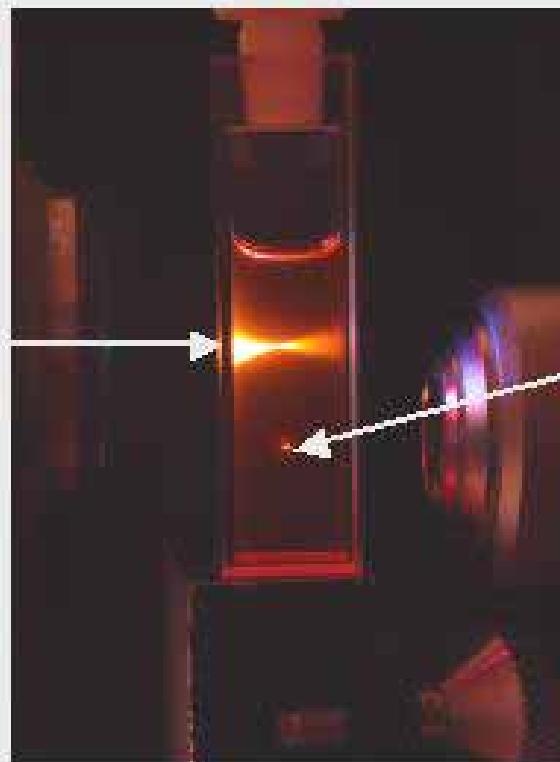
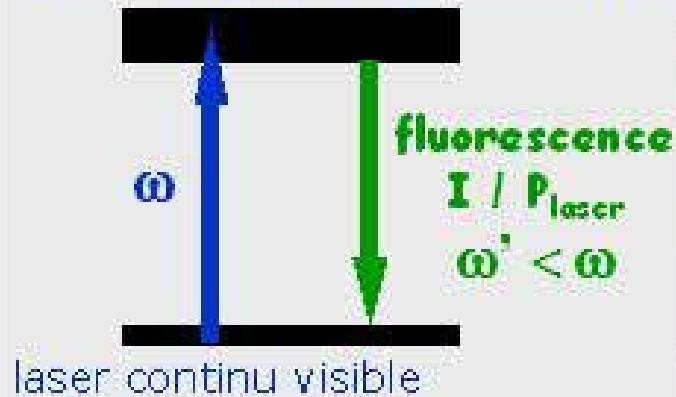


<sup>(1)</sup> *Department of Biomedical Engineering  
Biomicroscopy Lab  
Boston university*



# Intérêt de la microscopie de fluorescence par excitation biphotonique (TPEF)

excitation monophotonique



excitation biphotonique



excitation spatialement non sélective

$$I_{\text{plan}} = (P_{\text{laser}}/S) \xi S = \text{cste}$$

microscopie confocale

collection sélective (trou confocal)

AVANTAGES  
DU MULTIPHOTON

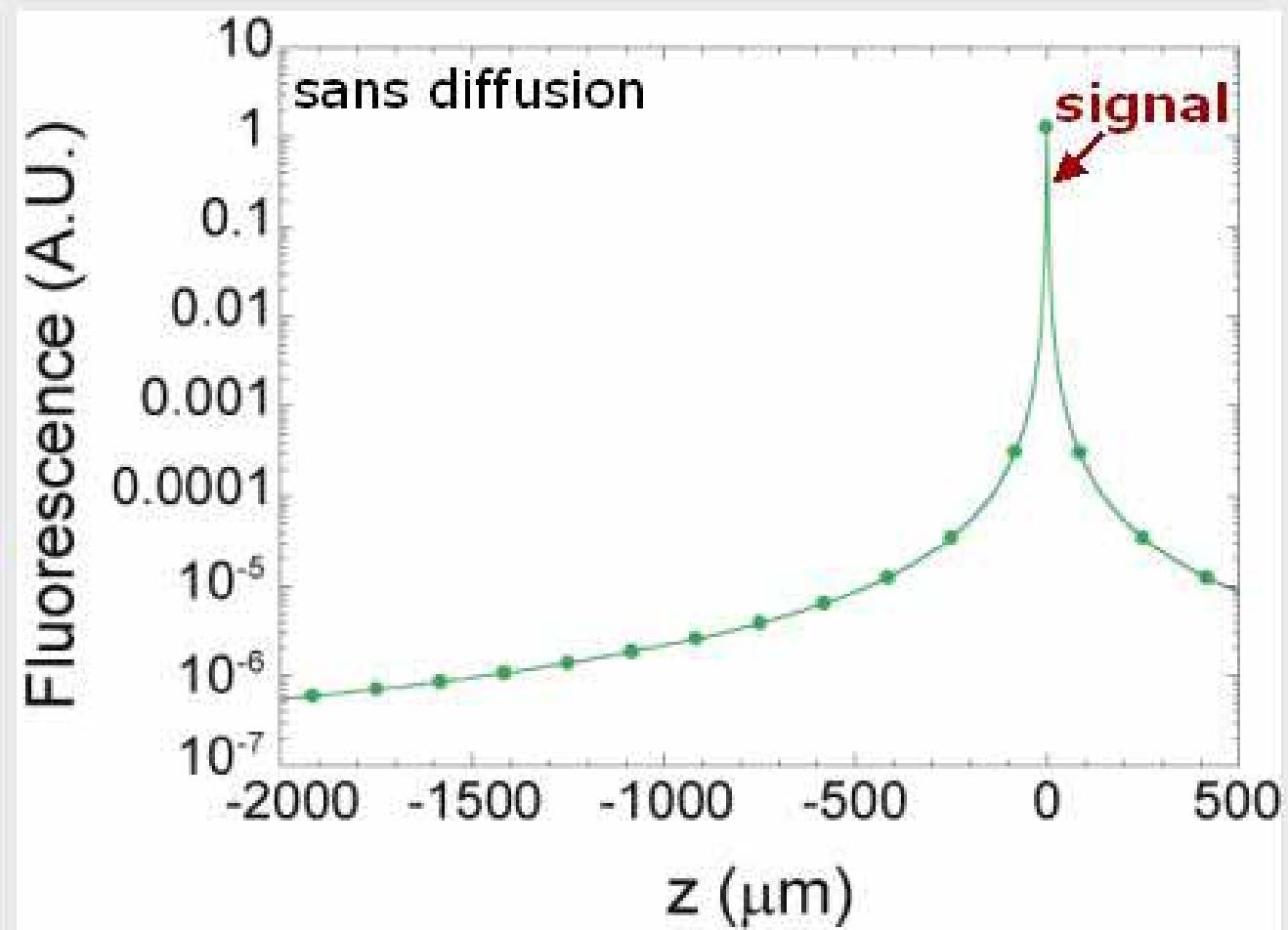
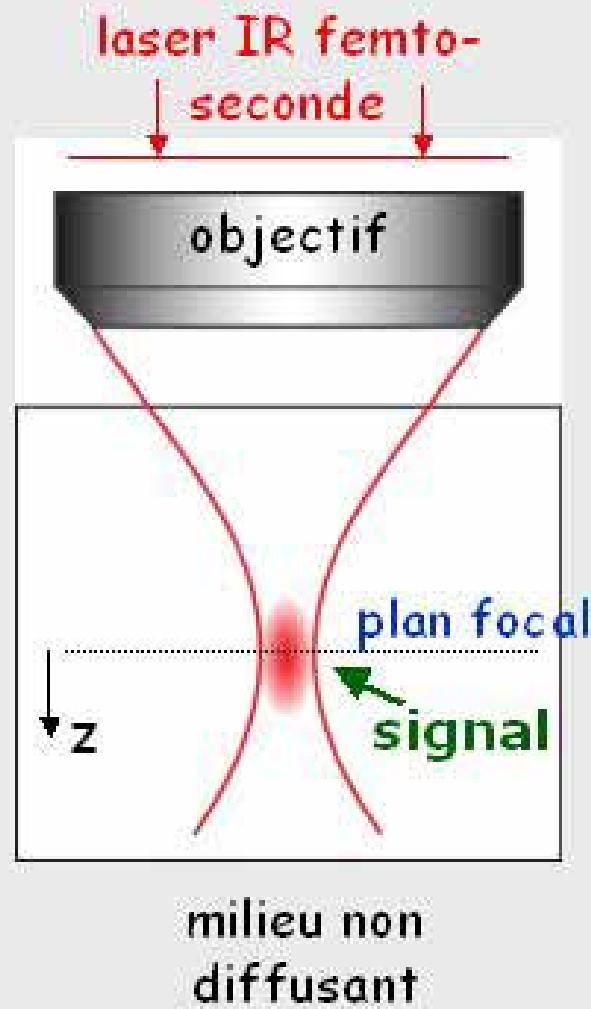
excitation spatialement sélective

$$I_{\text{plan}} / (P_{\text{laser}}/S)^2 \xi S = P_{\text{laser}}^2/S$$

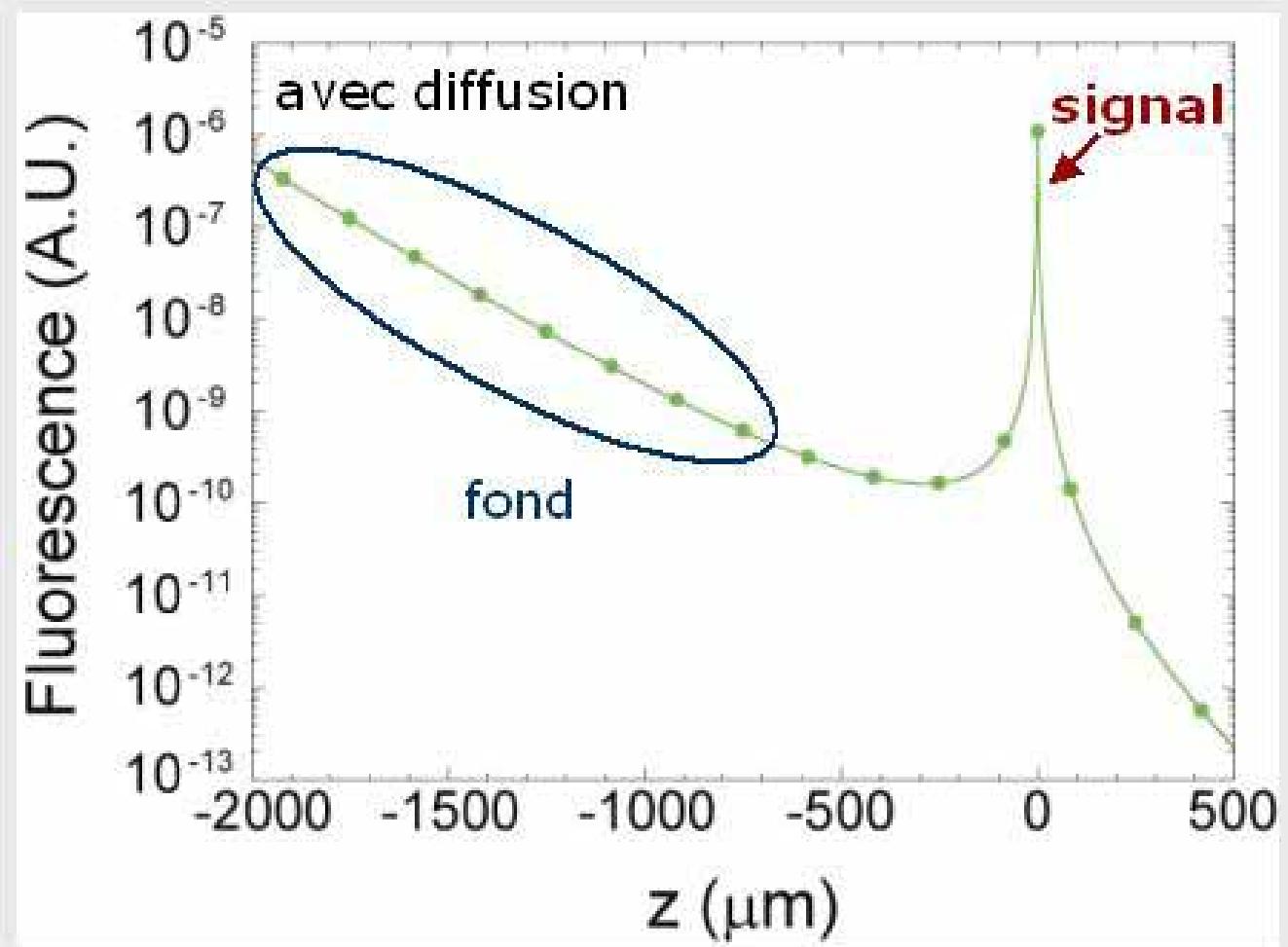
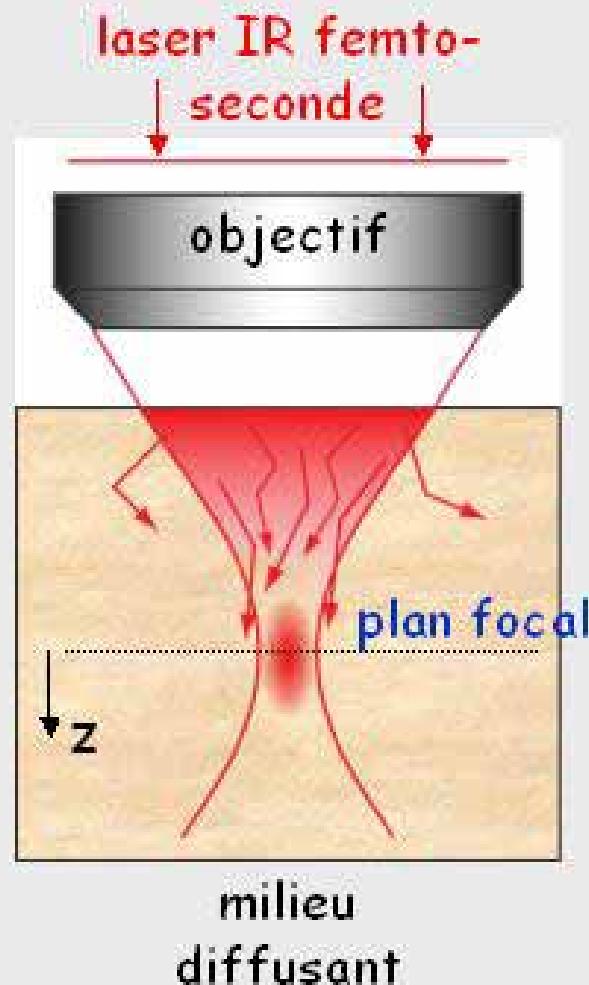
microscopie biphoton

- détection en champ large
- $\lambda_{\text{exc}}$  IR  $\Rightarrow$  moins phototoxique
- plus grande profondeur d'imagerie (500 $\mu\text{m}$ )

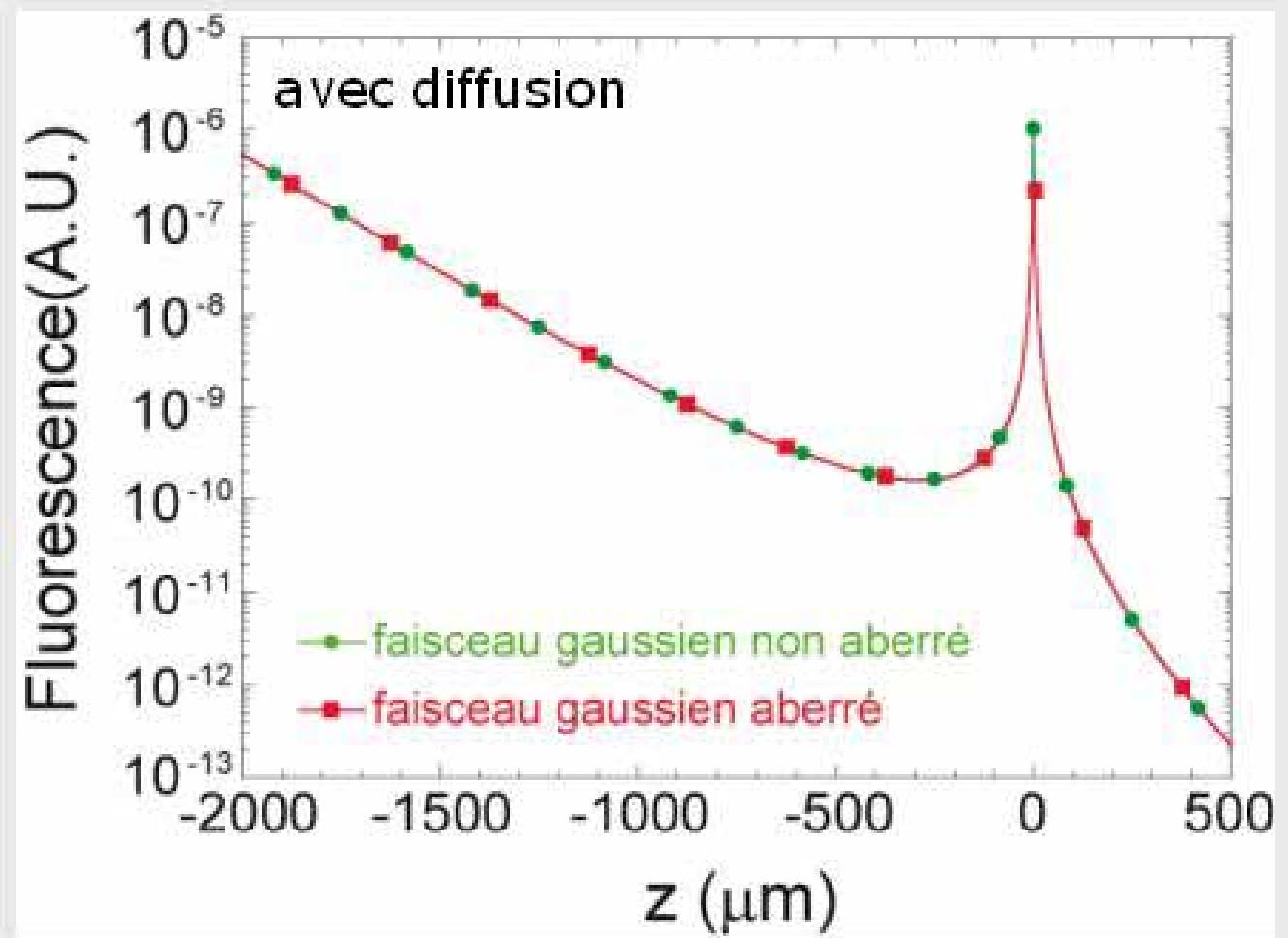
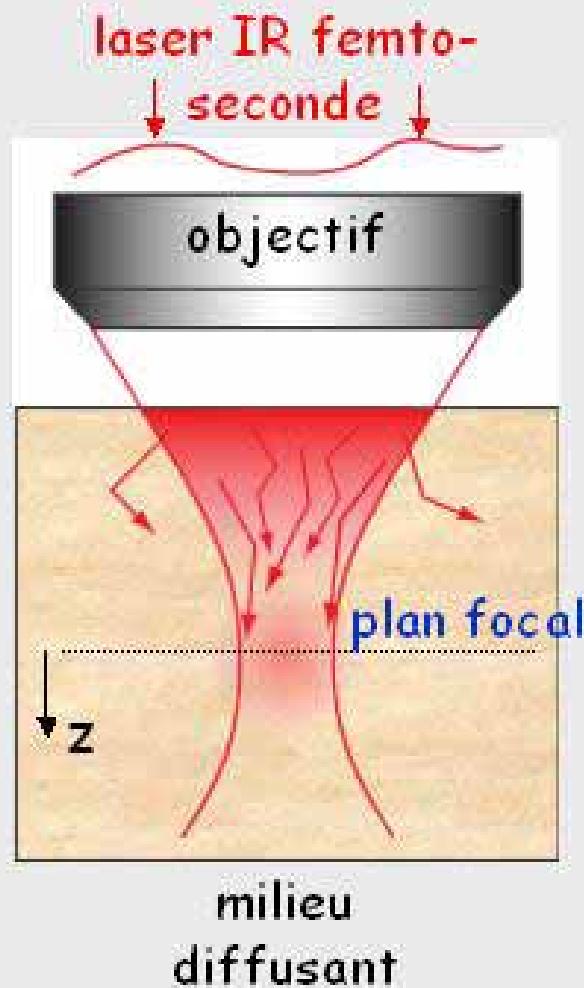
# Profil de la TPEF dans un milieu transparent



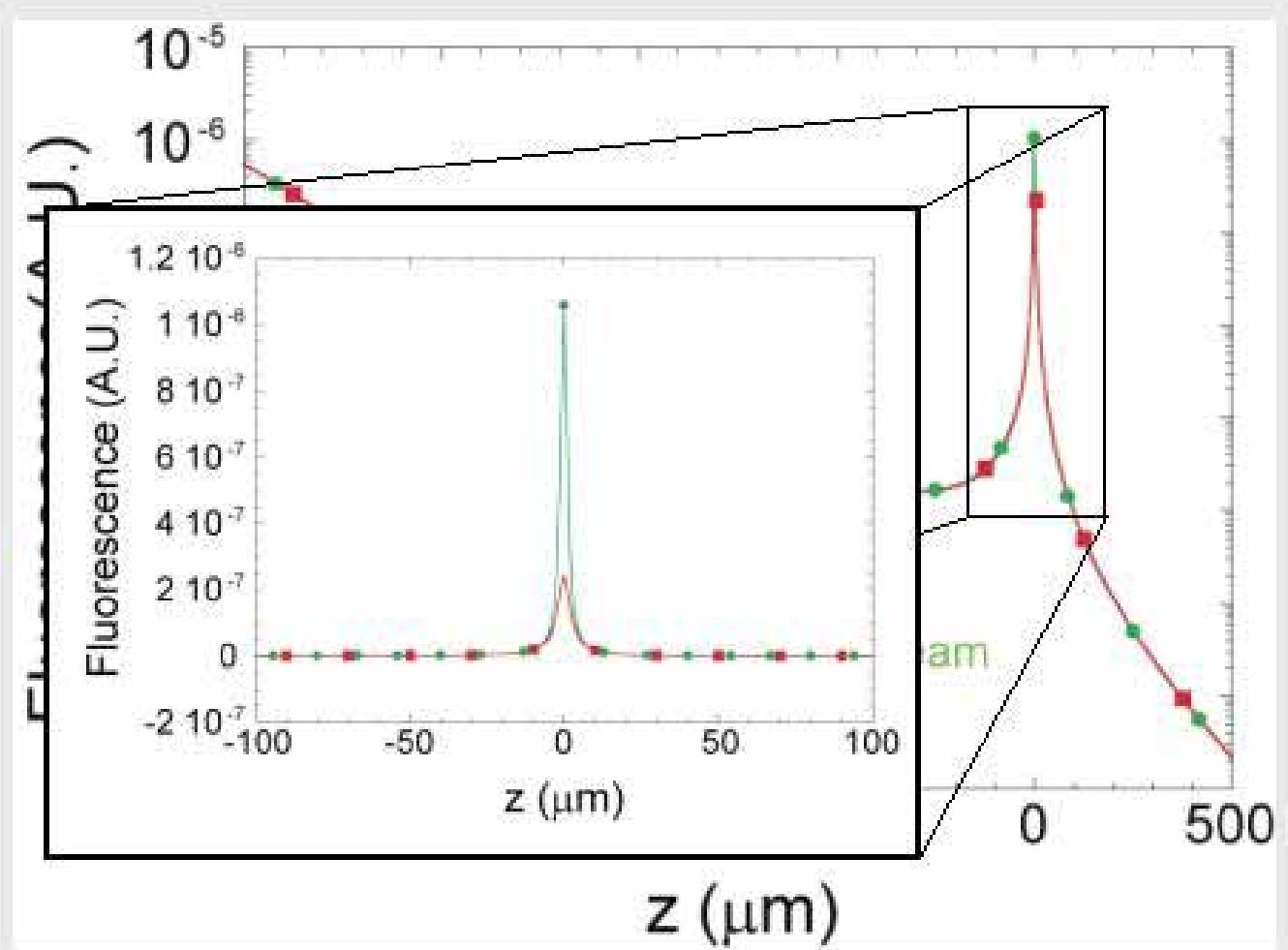
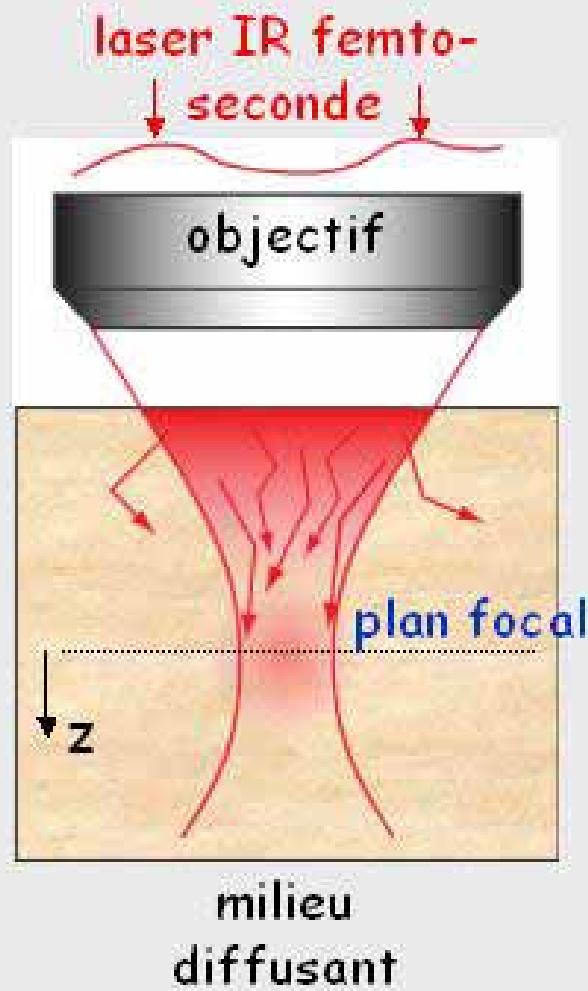
# Profil de la TPEF dans un milieu diffusant



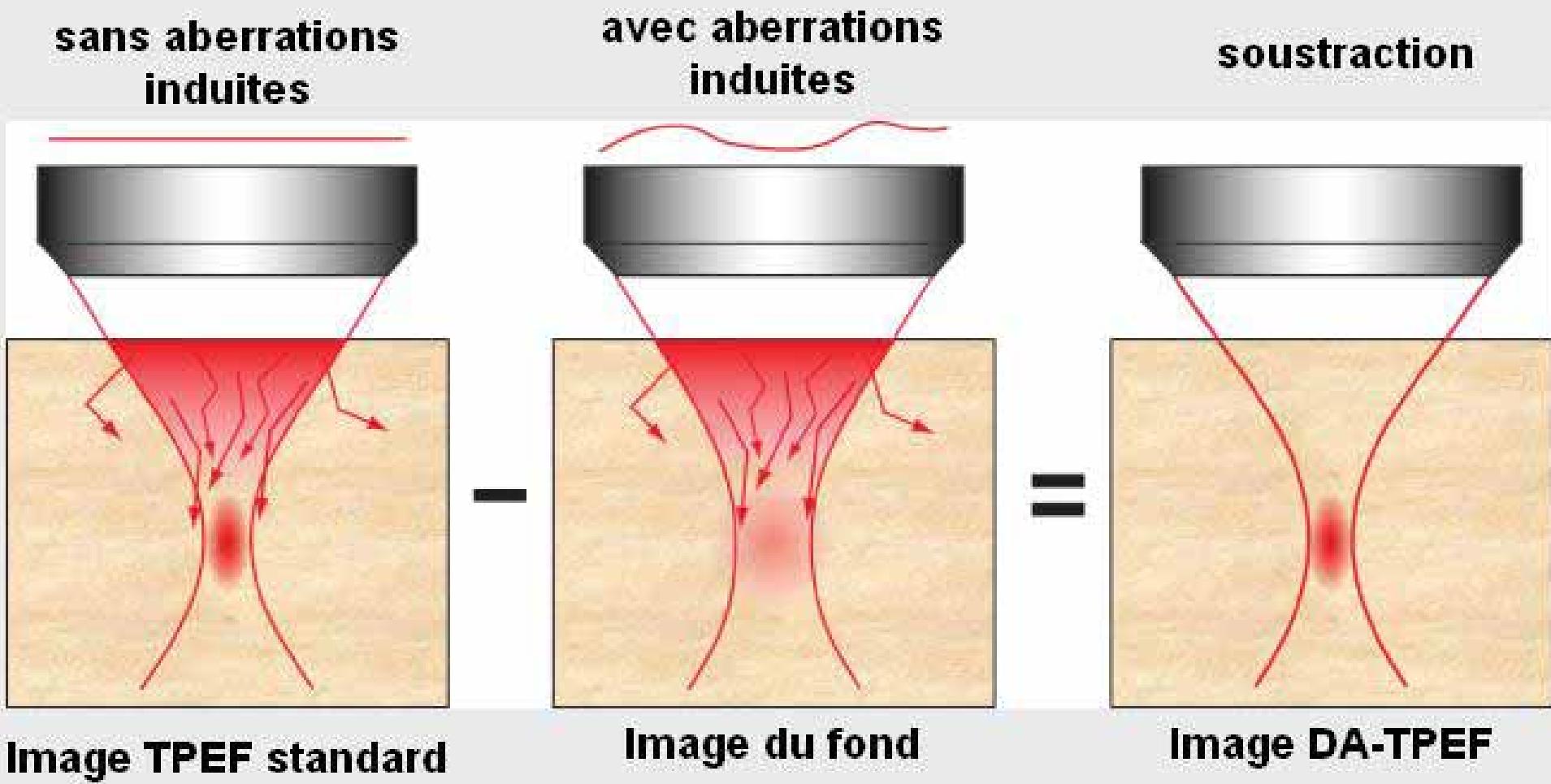
# Profil de la TPEF dans un milieu diffusant



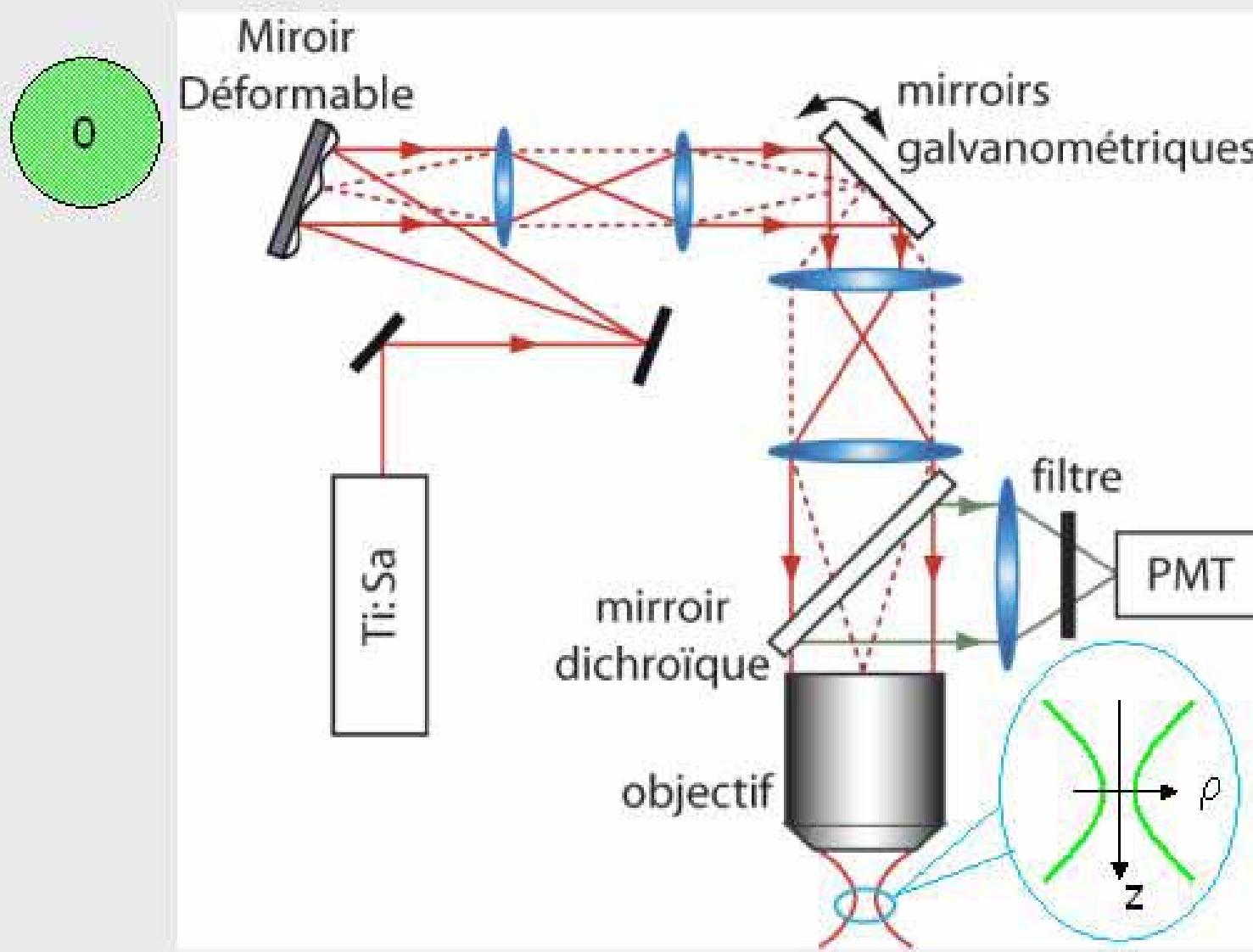
# Profil de la TPEF dans un milieu diffusant



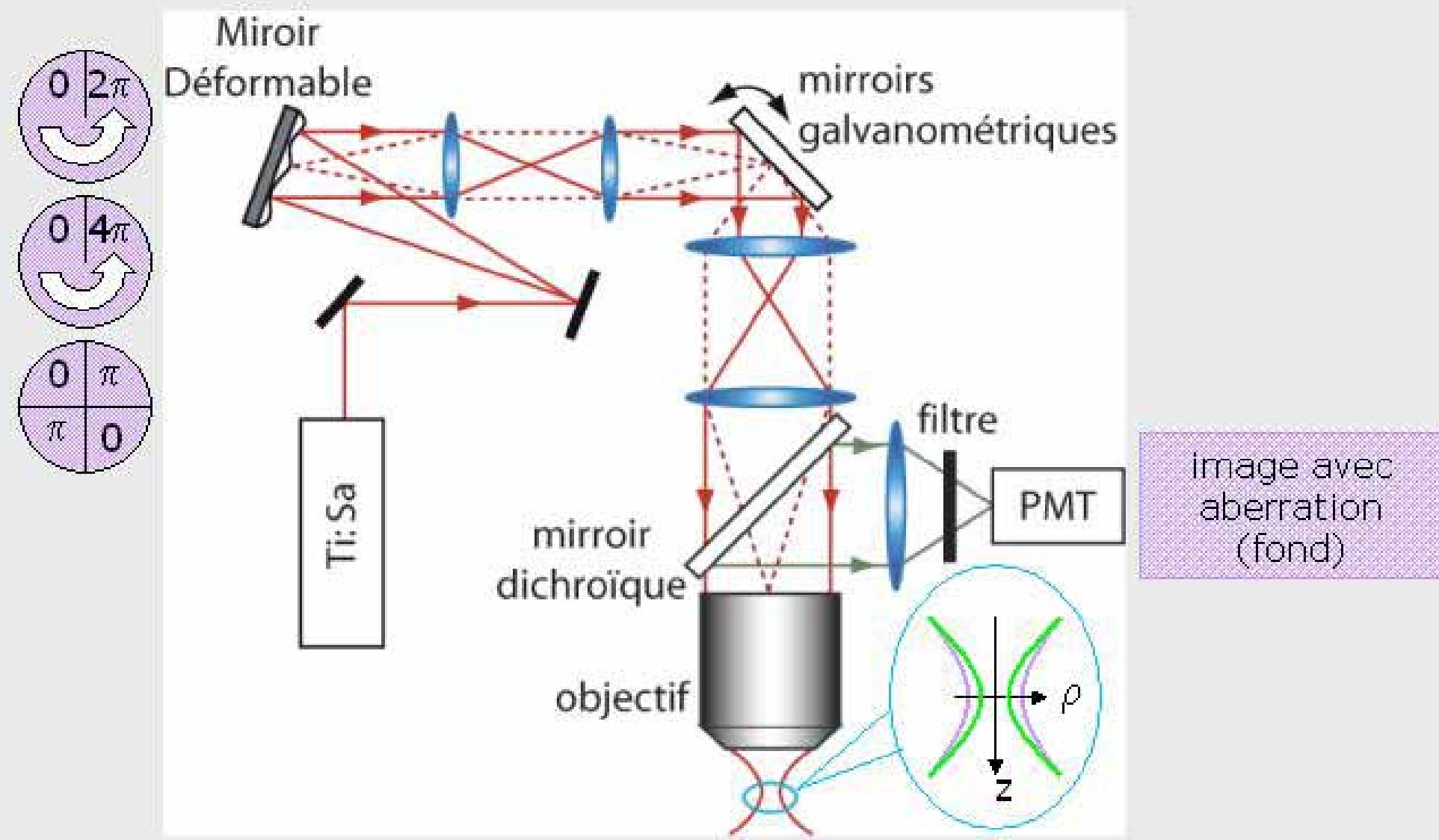
# Imagerie TPEF par aberration différentielle (DA-TPEF): principe



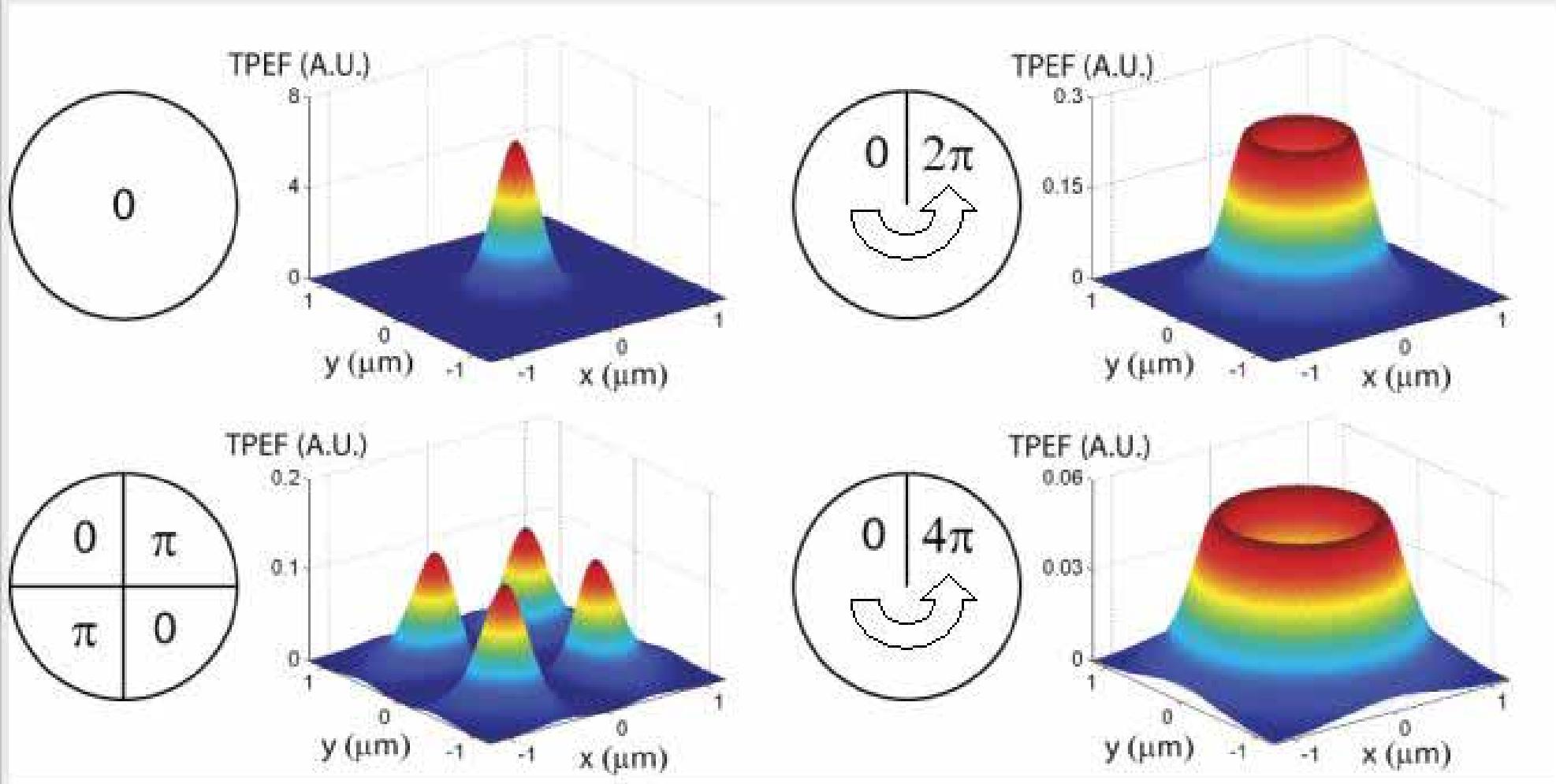
# Imagerie DA-TPEF : montage expérimental



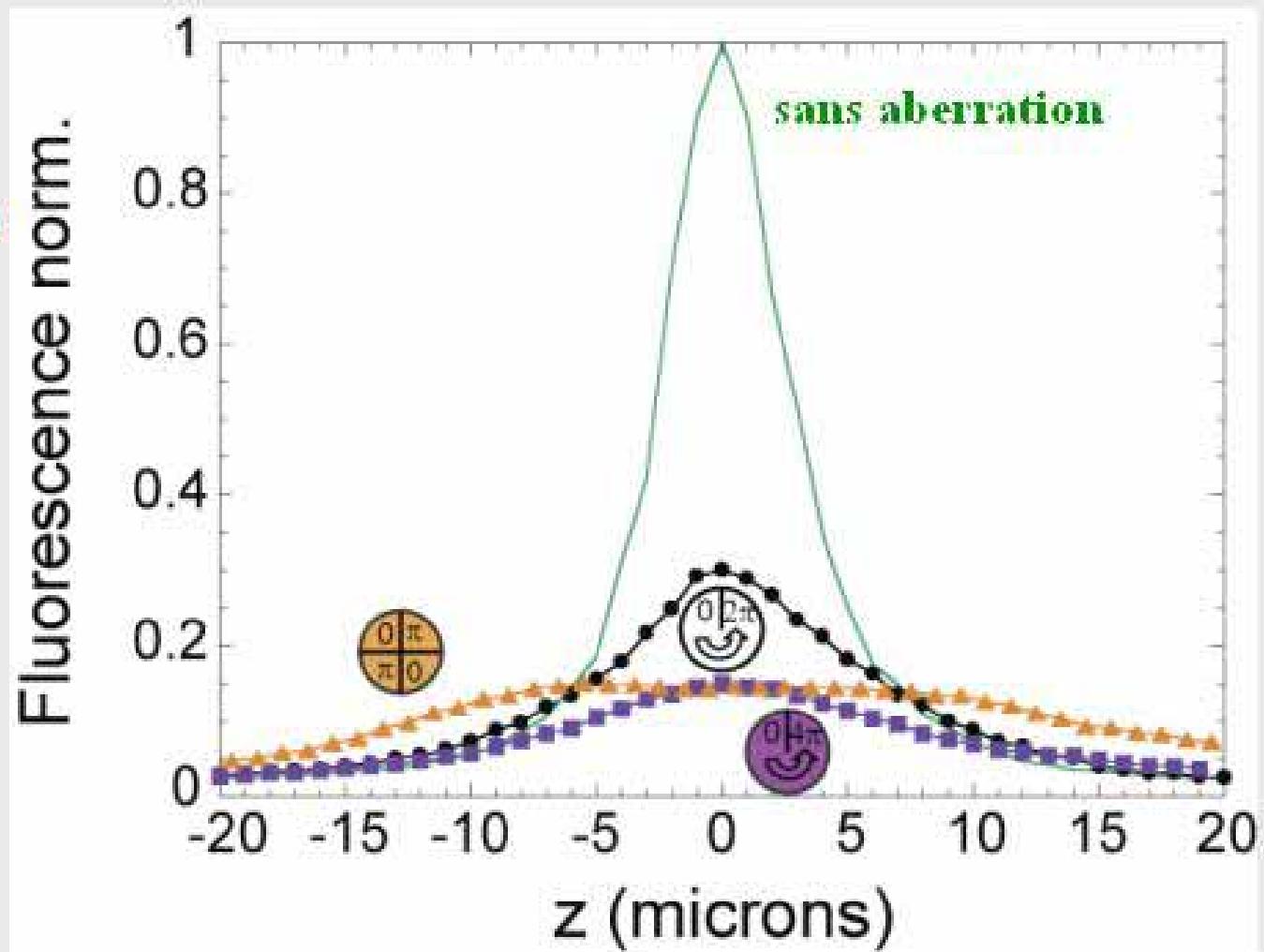
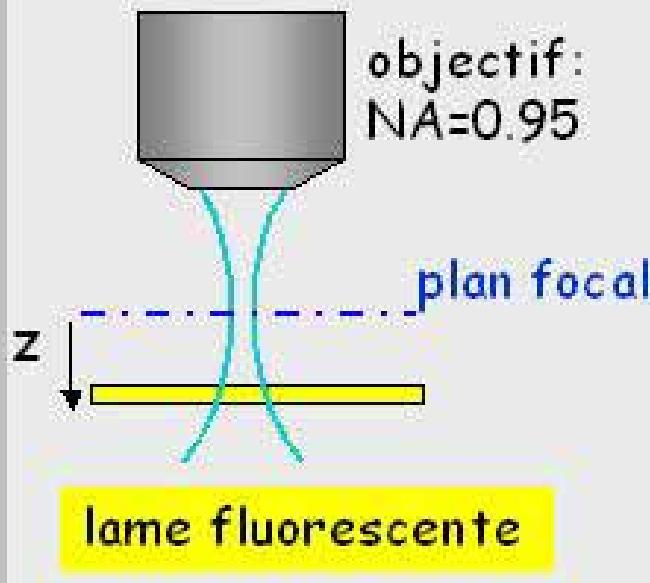
# Imagerie DA-TPEF : montage expérimental



# Distributions de la TPEF dans le plan focal

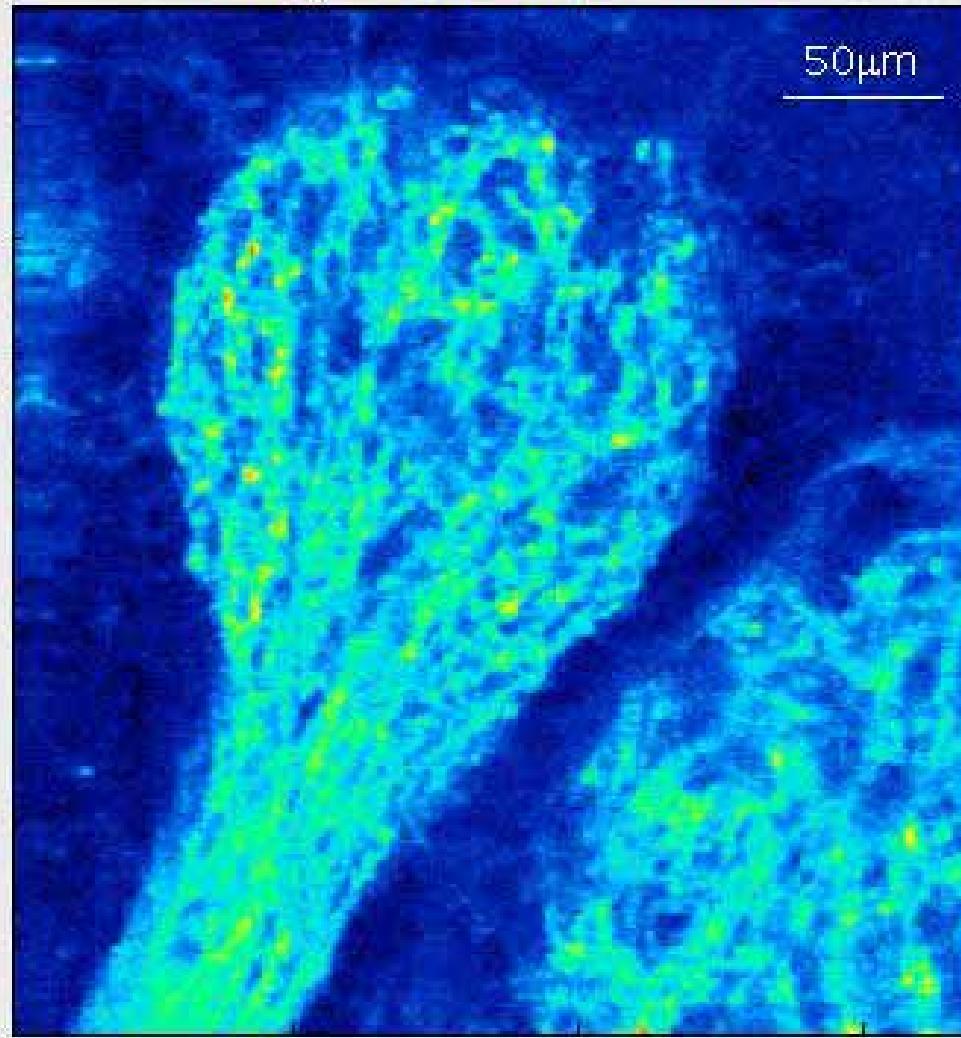


# TPEF émise par une lame: résultats expérimentaux



# Imagerie d'un glomérule marqué à la GFP dans un bulbe olfactif de souris

image TPEF standard

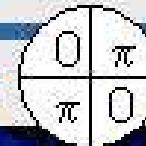


échantillon préparé  
par M. Wachowiak lab

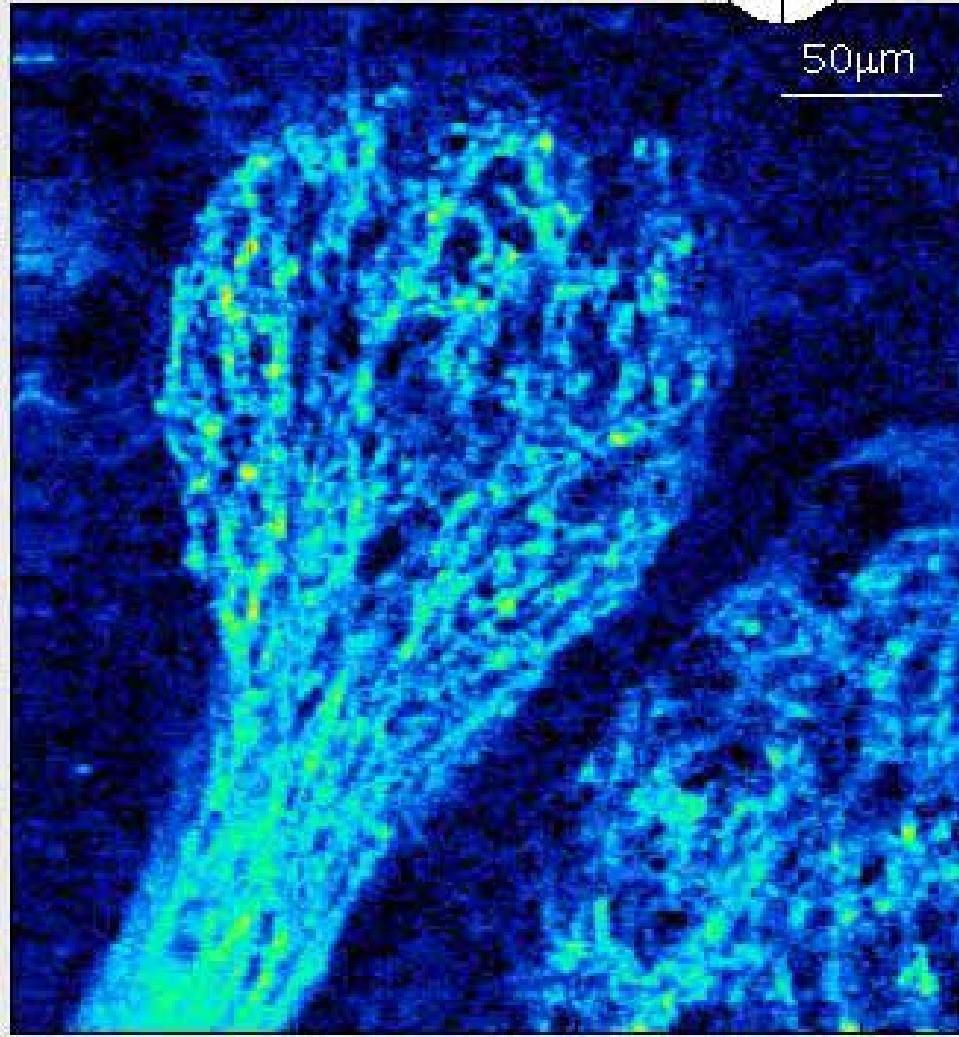
20x, objectif à  
immersion à eau  
(NA=0.95)  $\lambda=810\text{nm}$

# Imagerie d'un glomérule marqué à la GFP dans un bulbe olfactif de souris

image DA-TPEF avec



50μm



échantillon préparé  
par M. Wachowiak lab

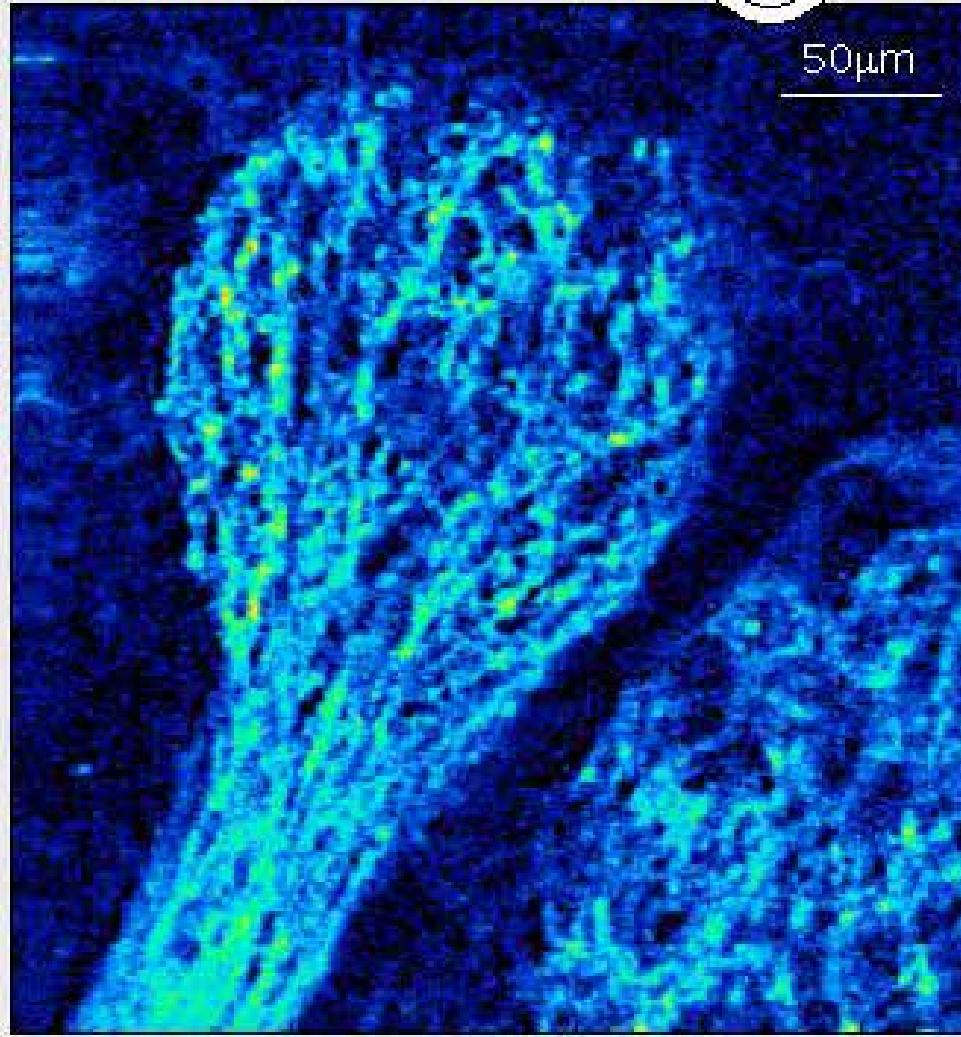
20x, objectif à  
immersion à eau  
(NA=0.95)  $\lambda=810\text{nm}$

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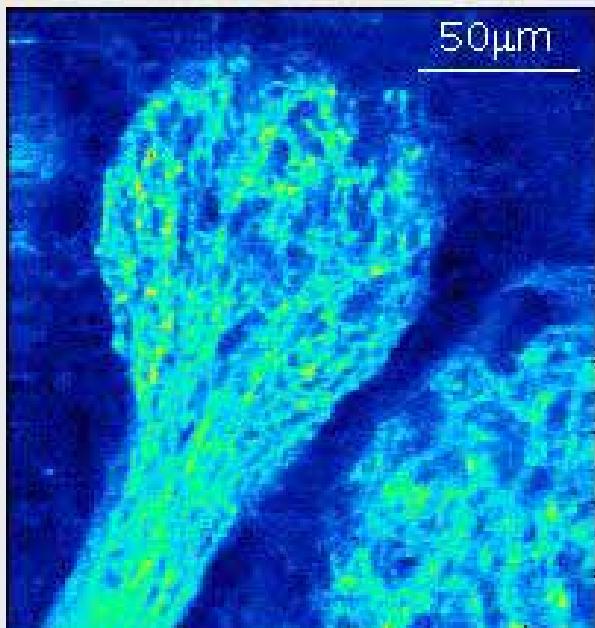


échantillon préparé  
par M. Wachowiak lab

20x, objectif à  
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image DA-TPEF

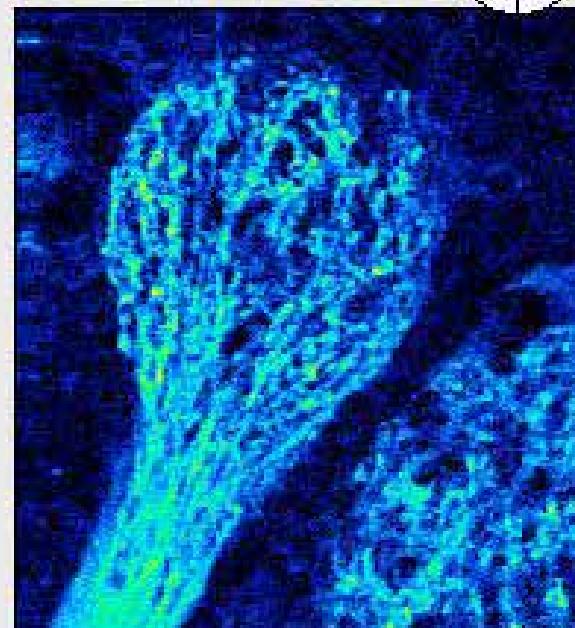


image DA-TPEF

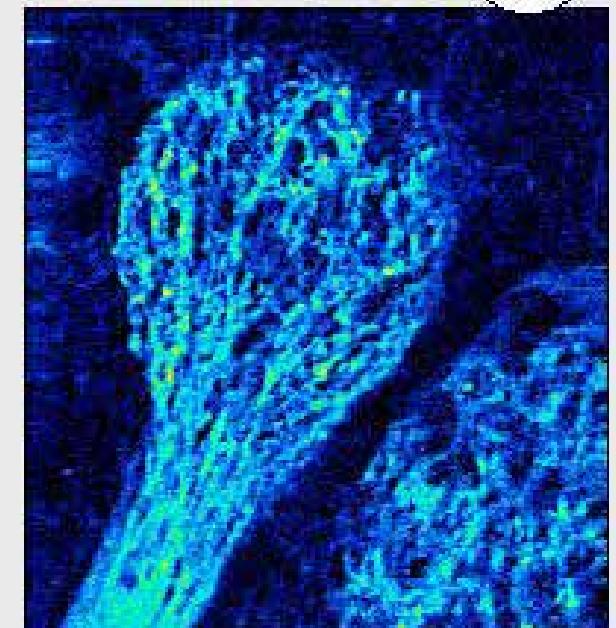
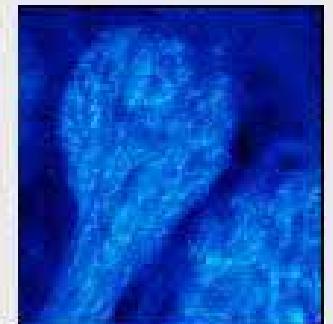


image avec  
aberration  
(fond)



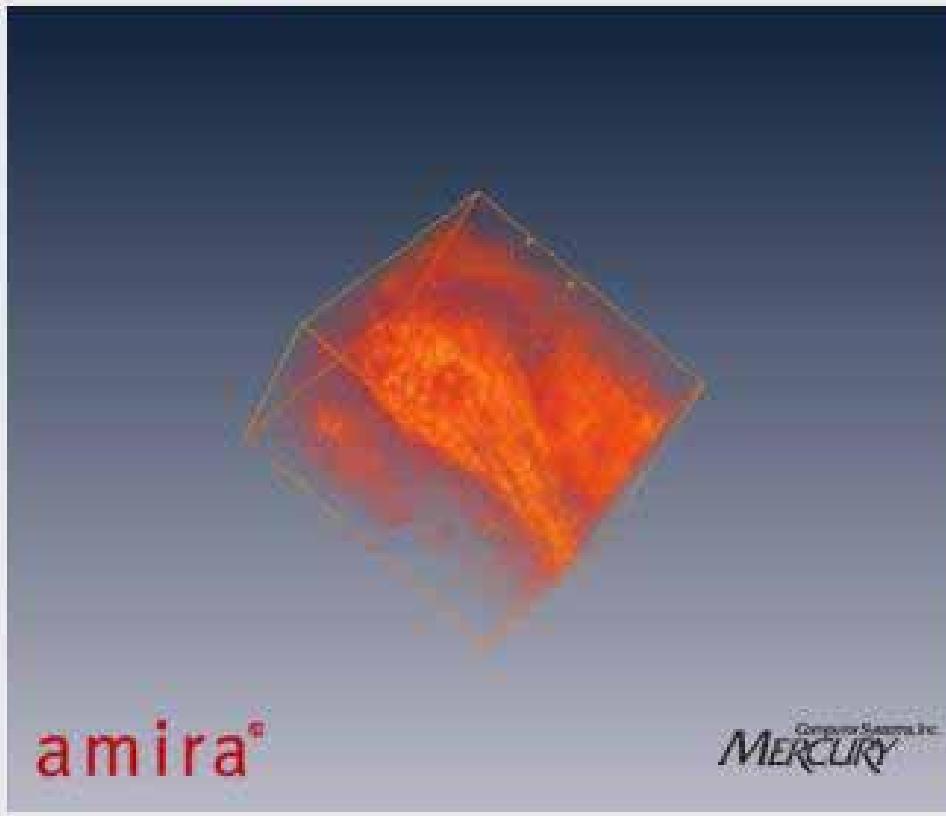
image avec  
aberration  
(fond)



A. Leray, K. Lillis and J. Mertz, *Biophys. J.*, accepted (2007)

# Reconstructions 3D d'un glomérule marqué à la GFP dans un bulbe olfactif de souris

image TPEF standard



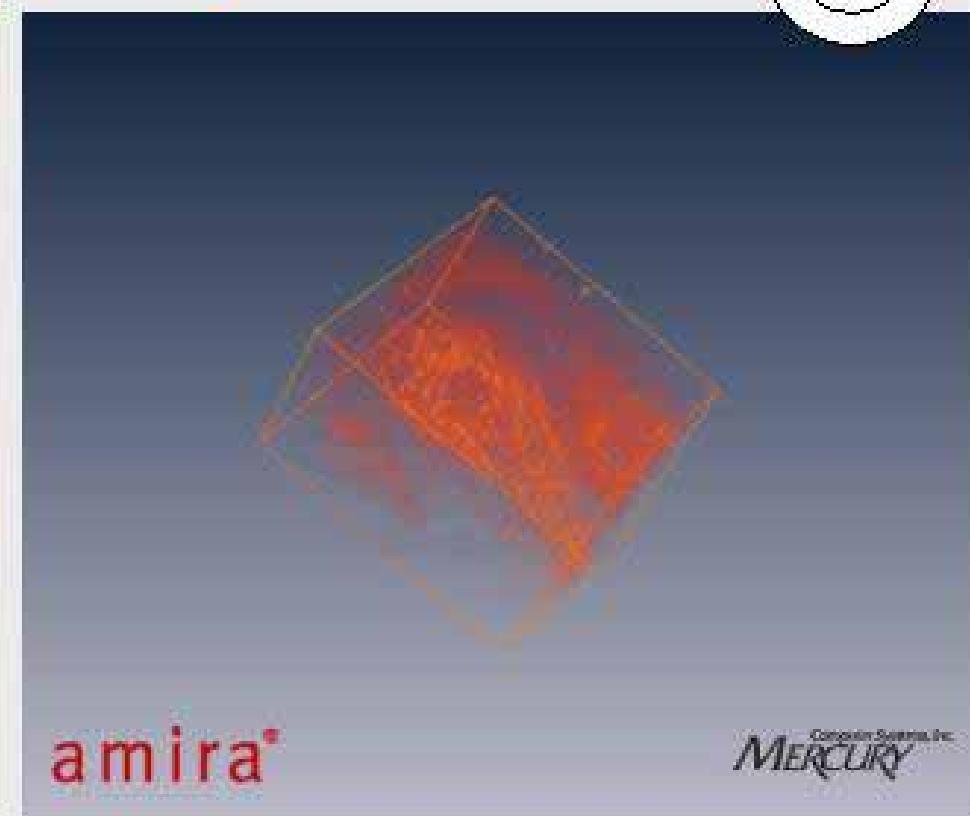
amira®

MERCURY

50 images ( $2\mu\text{m}$  entre deux images)

échantillon préparé par M. Wachowiak lab

image DA-TPEF avec



amira®

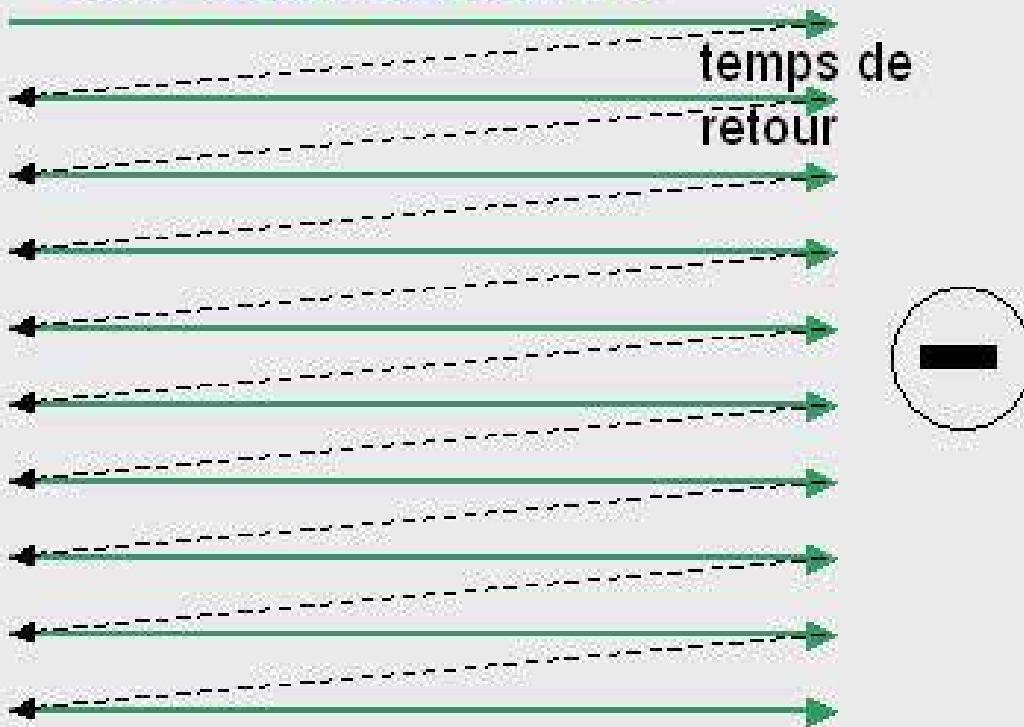
MERCURY

20x, objectif à  
immersion à eau  
(NA=0.95)  
 $\lambda=810\text{nm}$

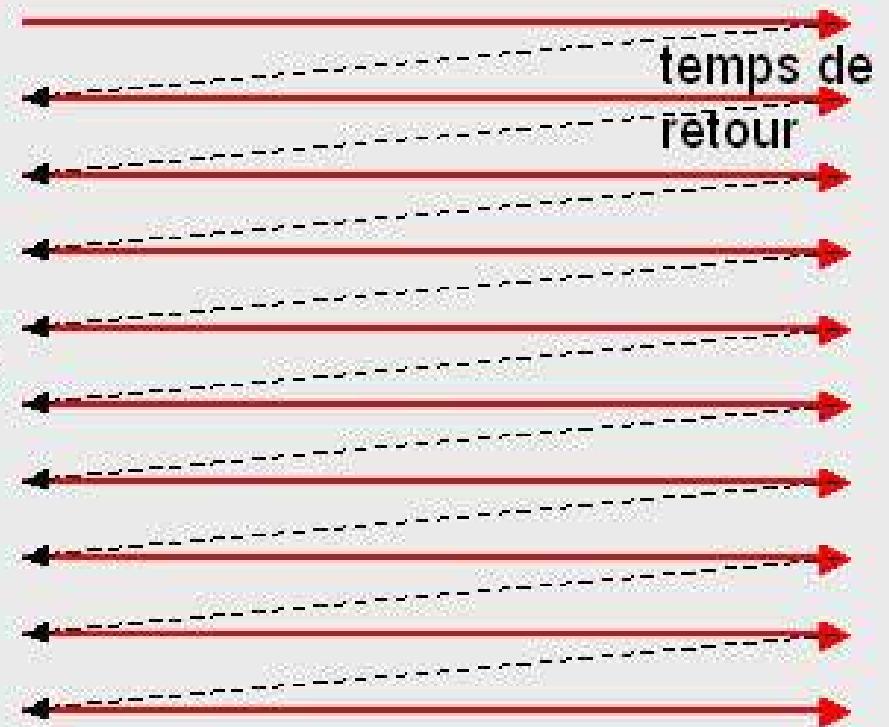
# Imagerie DA-TPEF image par image

Problème: temps d'acquisition doublé

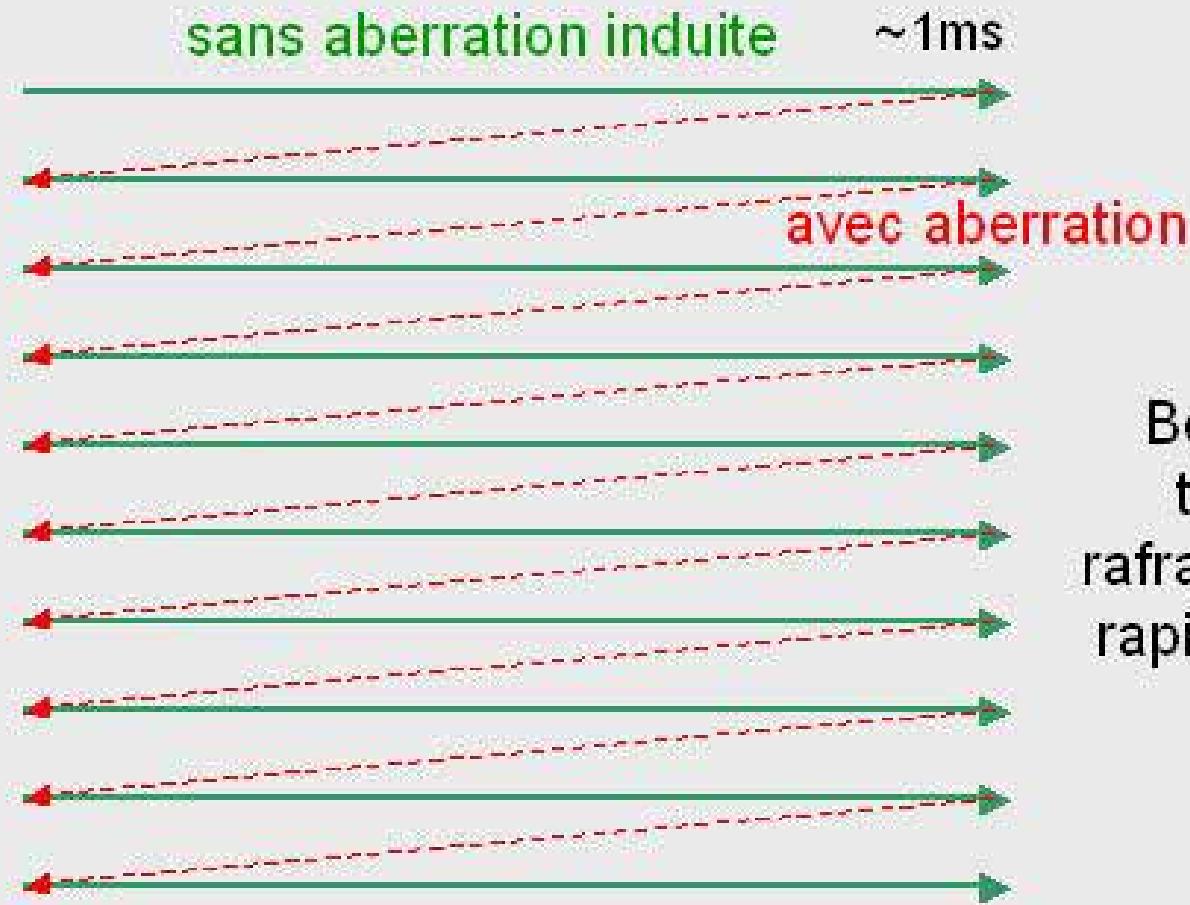
sans aberration induite



avec aberration



# Imagerie DA-TPEF ligne par ligne



Le miroir déformable est suffisamment rapide !  
(pour le quadrant uniquement)

# Patch Clamp d'une cellule pyramidale marquée au calcium green dans une tranche de cerveau de rat

image TPEF standard

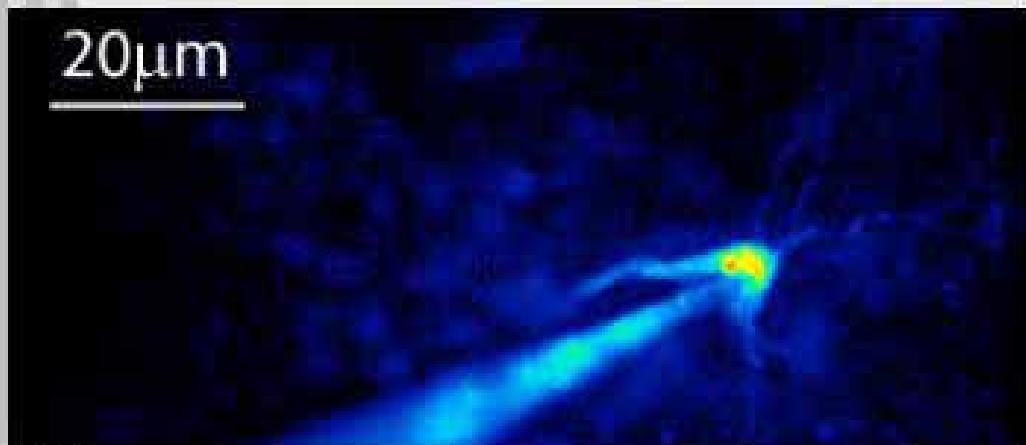


image DA-TPEF

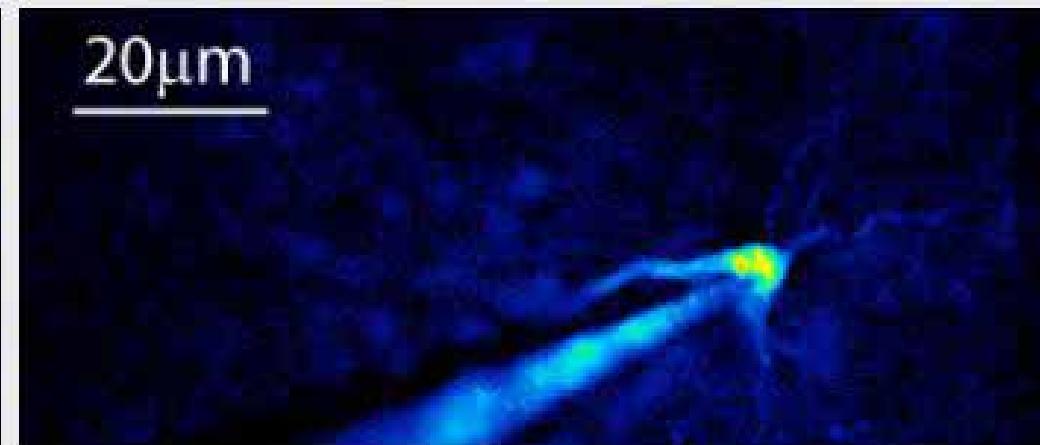


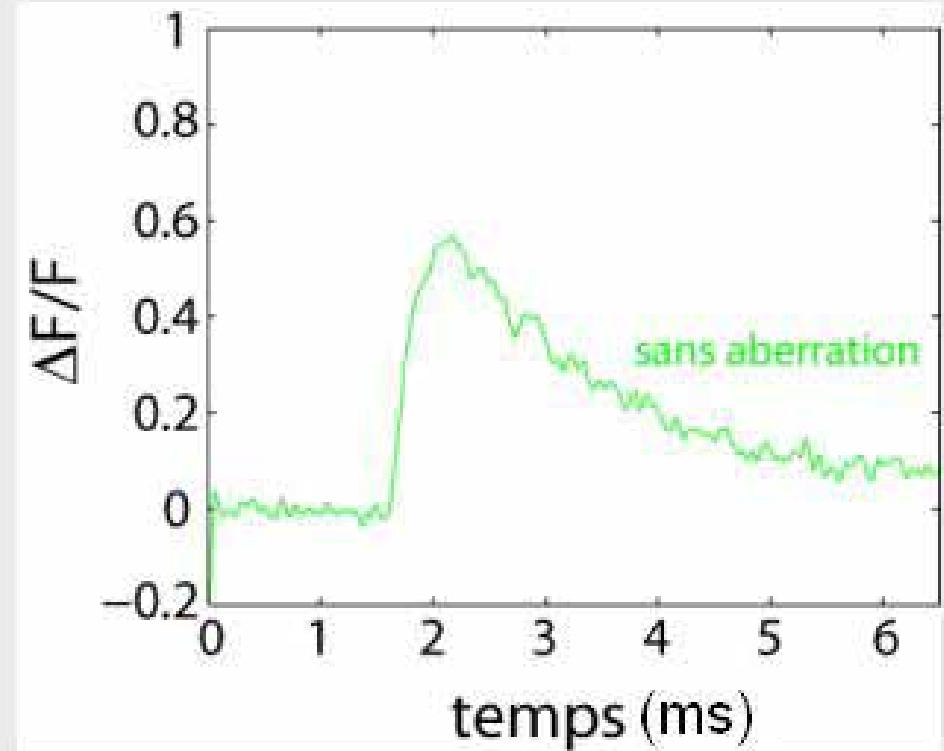
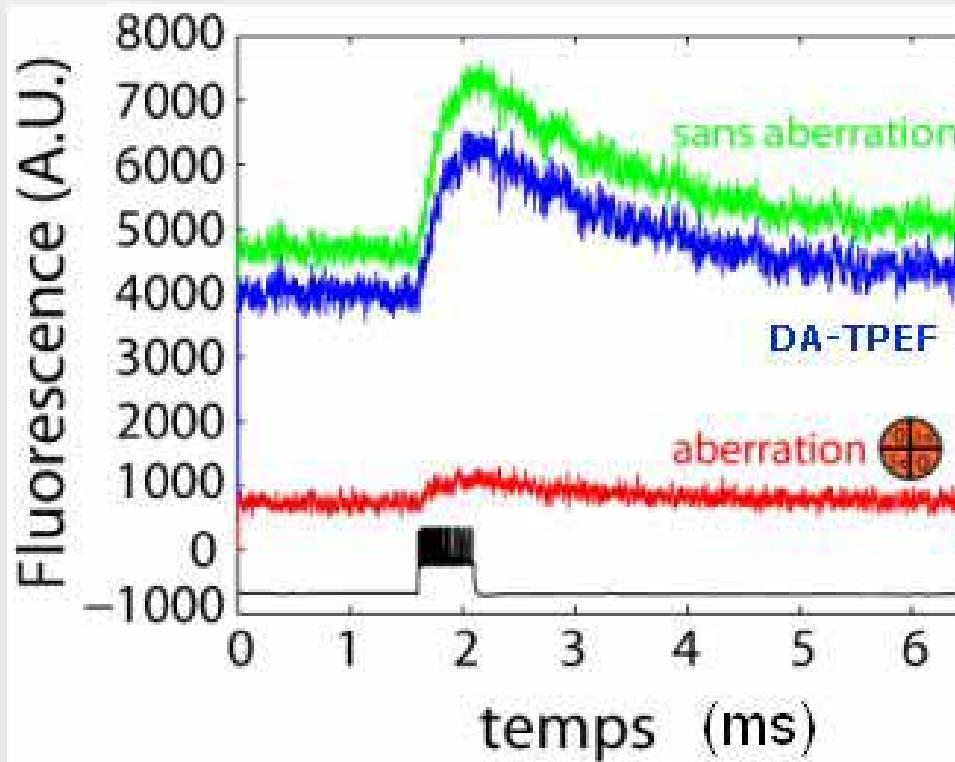
image avec  
aberration (fond)



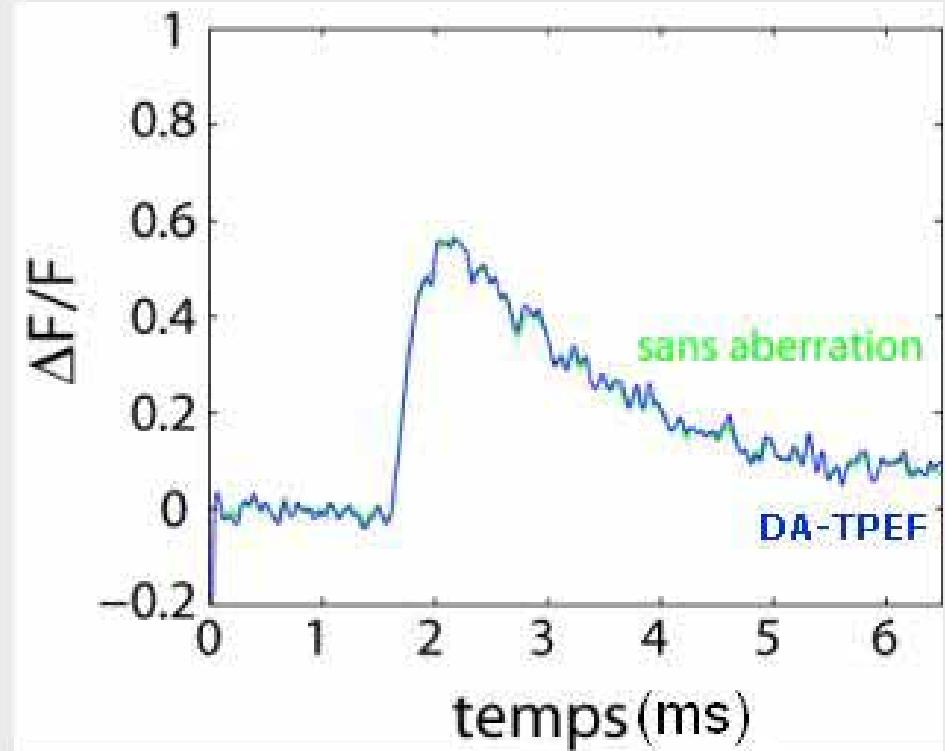
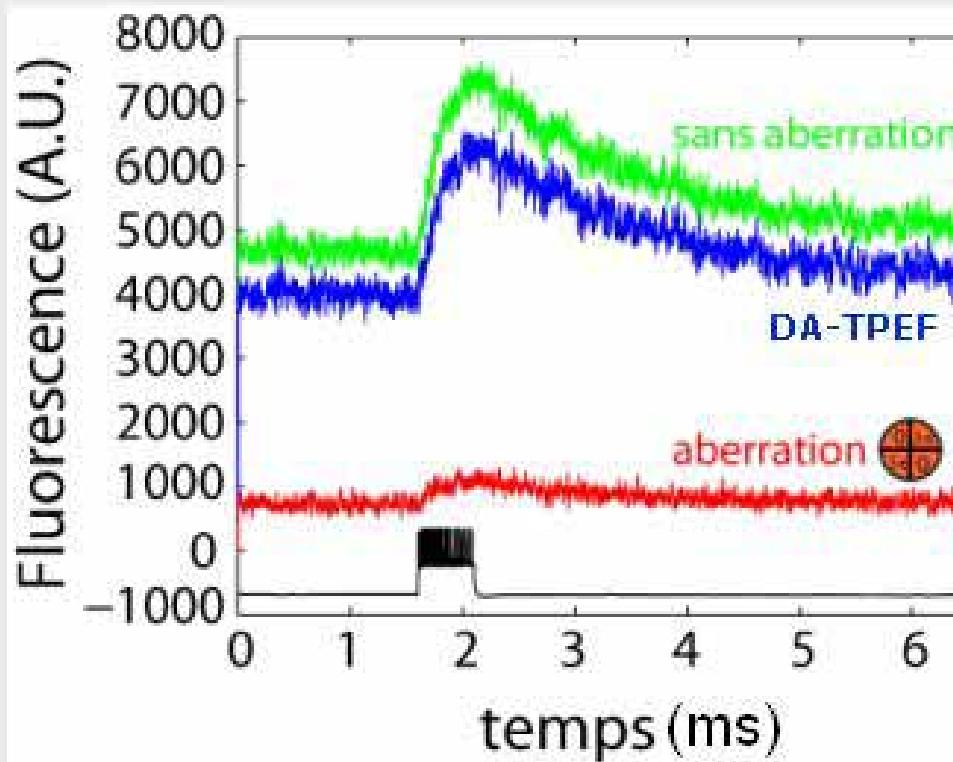
20x, objectif à  
immersion à  
eau (NA=0.95)

$\lambda=810\text{nm}$

# Dynamique calcique : résultats expérimentaux



# Dynamique calcique: résultats expérimentaux

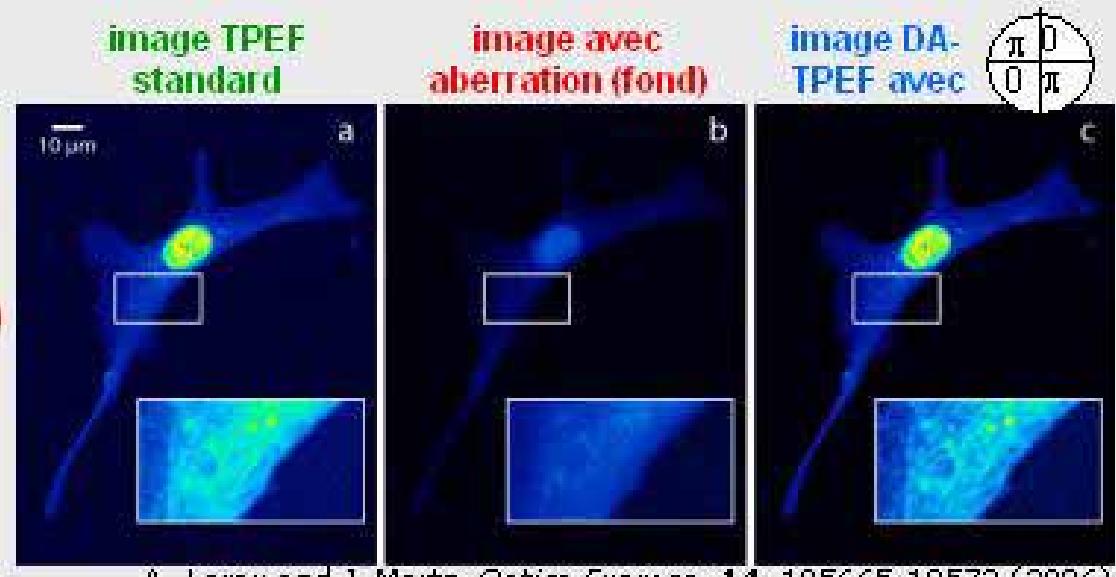


injection d'un pulse de courant  
de 500pA pendant 500ms

# Conclusion

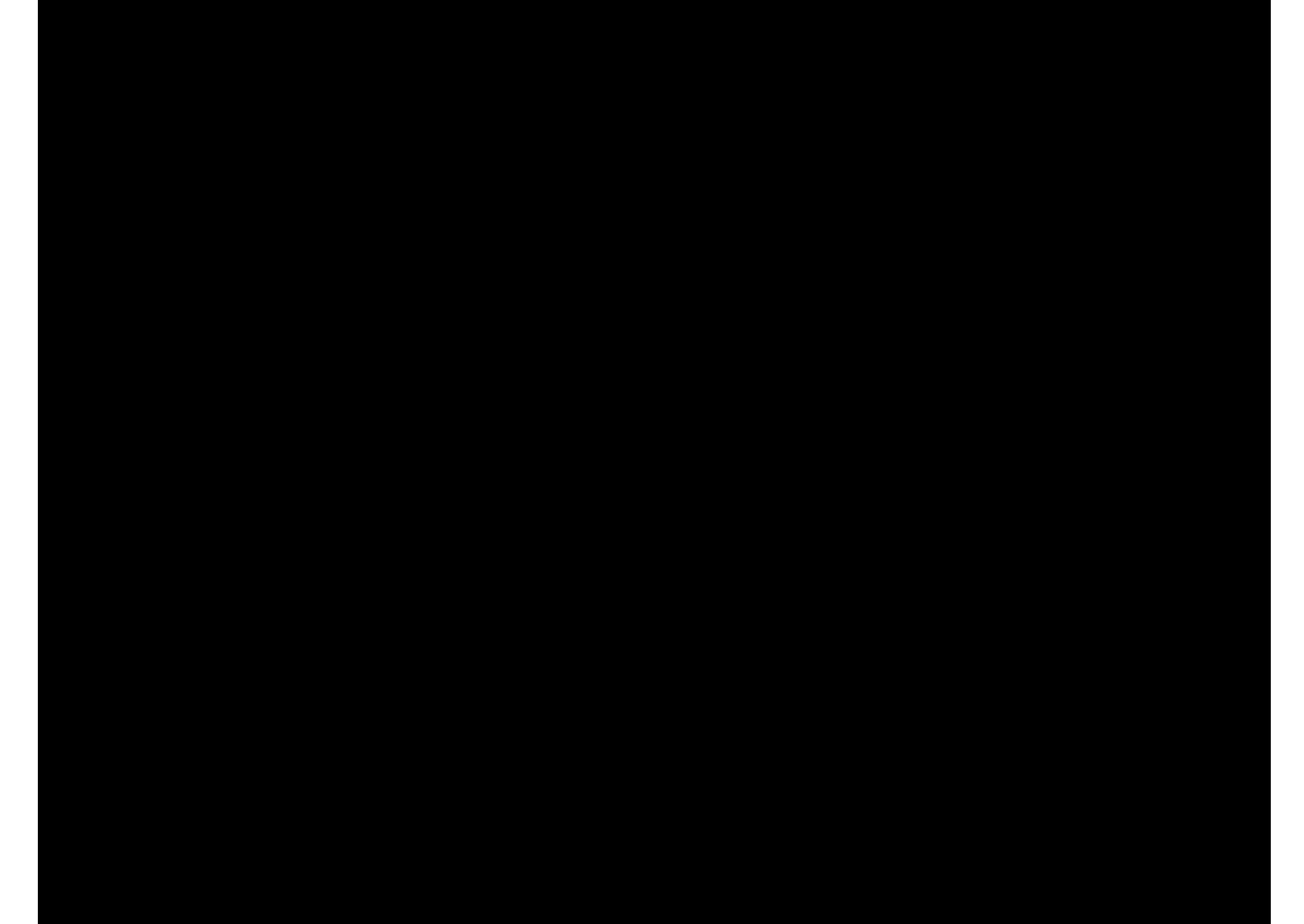
Avantages de la technique DA-TPEF :

- Rejet du fond fluorescent
- Facile à implémenter (pas de boucle fermée ni de correction de front d'onde)
- Ne ralentit pas le temps d'acquisition
- Meilleure résolution apparente



A. Leray and J. Mertz, *Optics Express*, **14**, 105665-10573 (2006)

Merci à Tom Bifano et Boston Micromachines Corporation de nous avoir prêté le miroir déformable!!!



# Imagerie DA-TPEF : montage expérimental

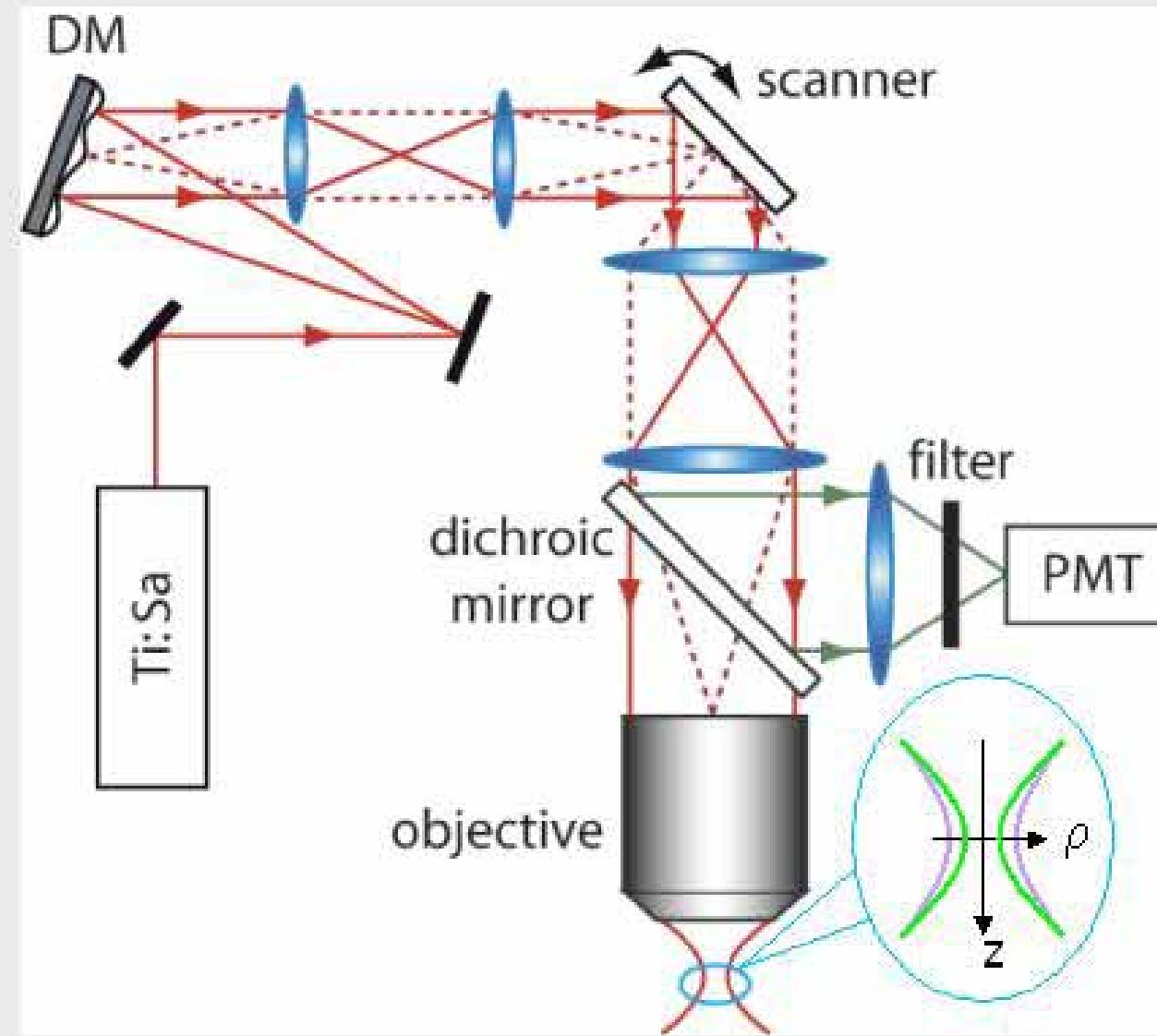


image TPEF  
standard (sans  
aberration)

image avec  
aberration  
(fond)

image DA-TPEF  
(soustraction)

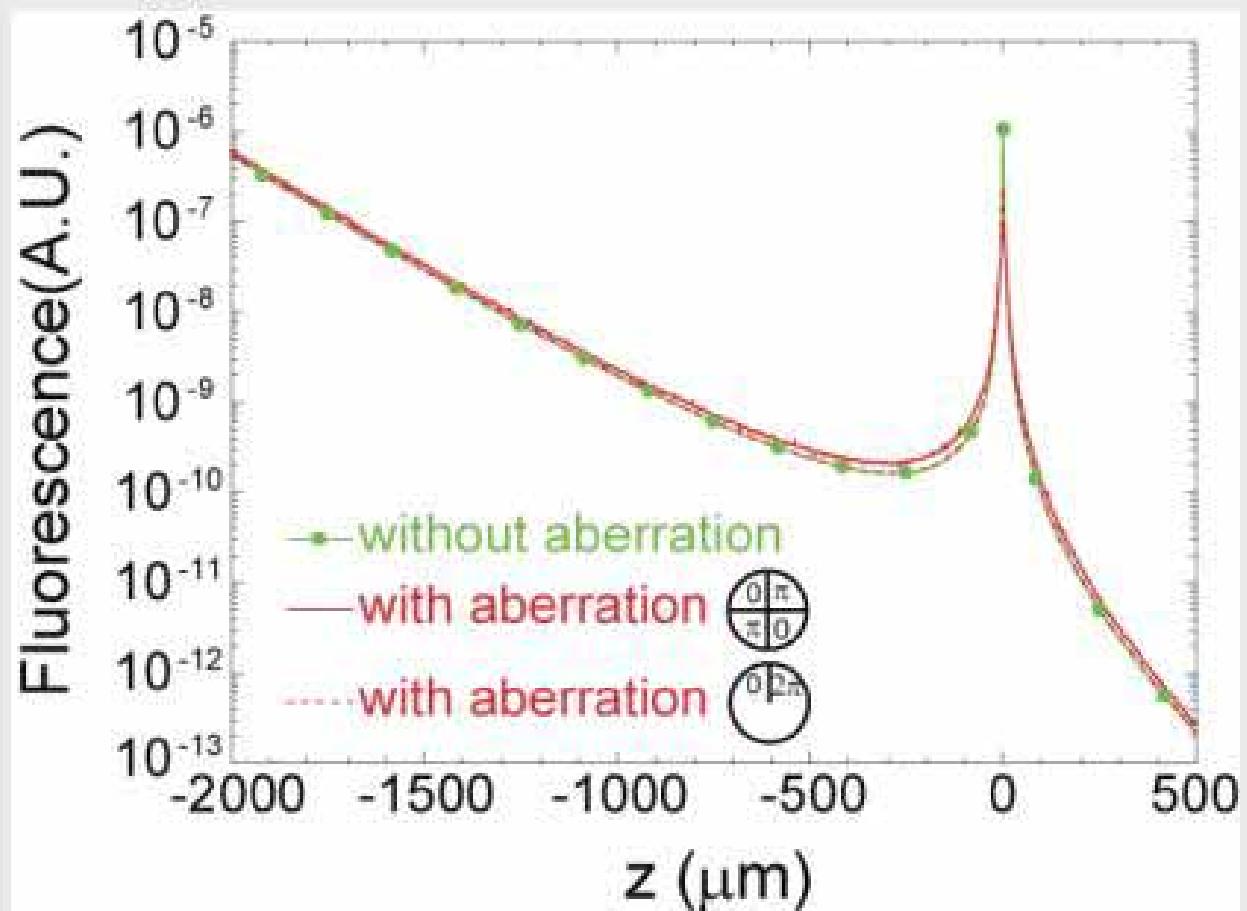
# Main advantage: two-photon fluorescence background rejection

In the paraxial approximation, for ballistic (non scattered) photons in a uniformly fluorescent and scattering medium:

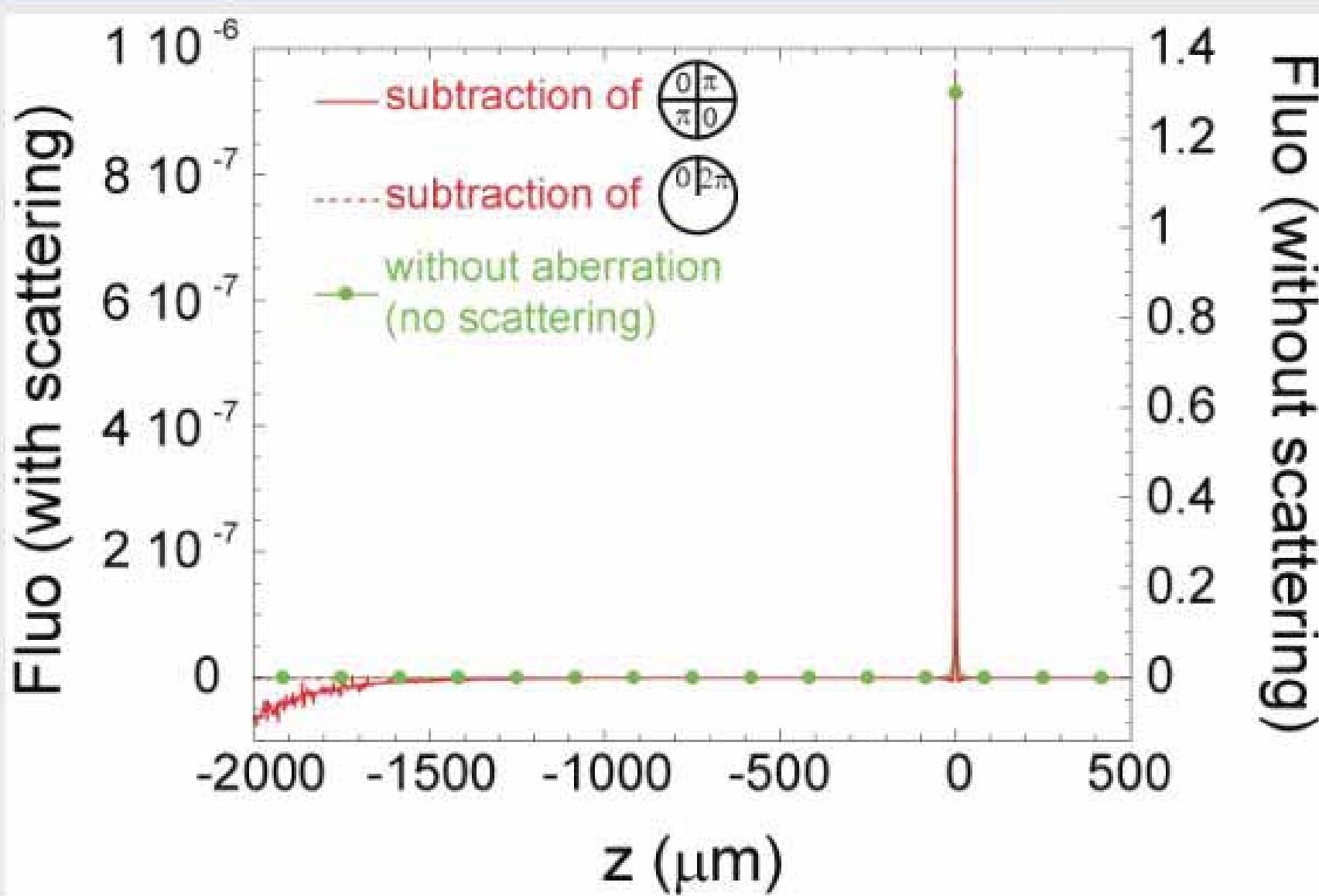
$$I_b(\vec{\rho}, z) = PSF(\vec{\rho}, z) e^{-\mu_s(z+z_s)}$$

Two photon fluorescence profiles:

$$F(z) = \int_0^{\infty} I_b^2(\vec{\rho}, z) 2\pi \rho d\rho$$



# Main advantage: two-photon fluorescence background rejection

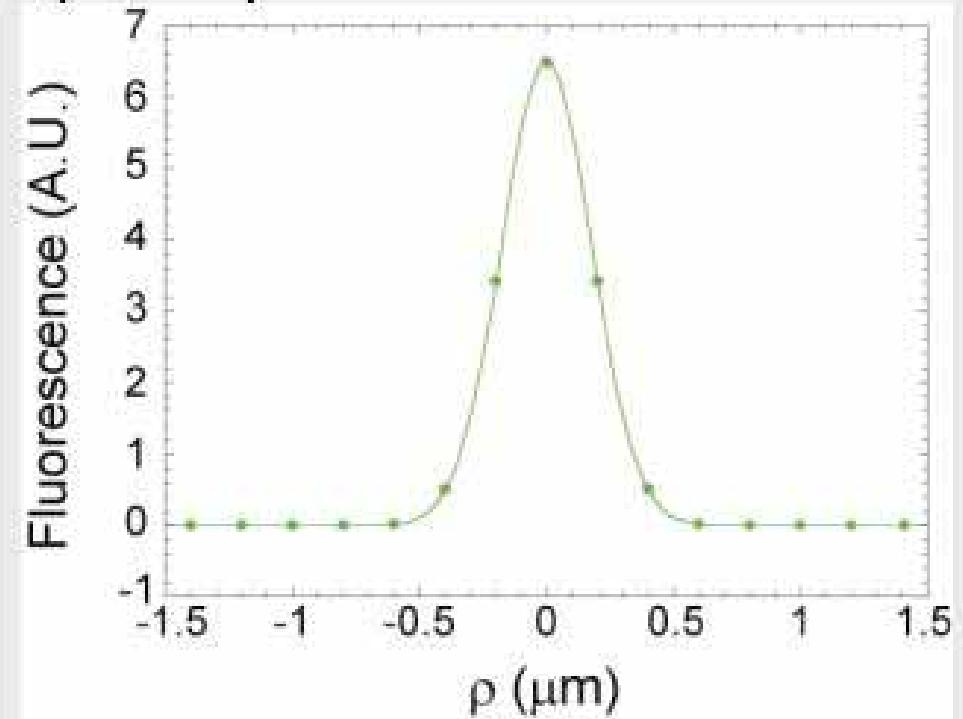
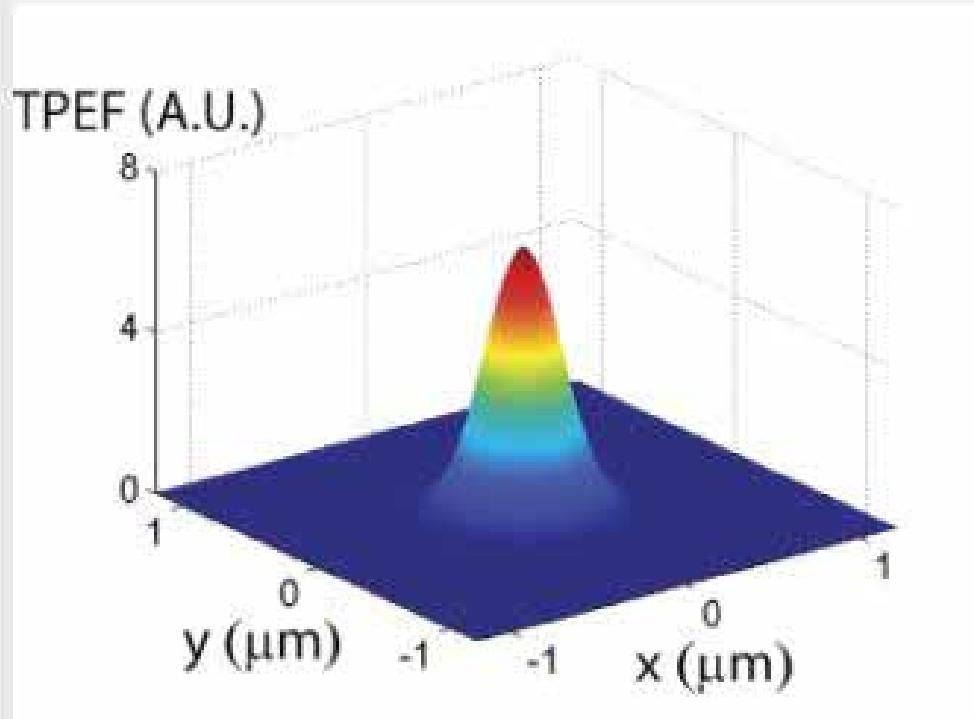


# First advantage: better resolution

without aberration

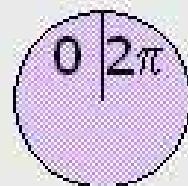
$$PSF_0^2(\bar{\rho}, z) = \frac{4}{(\pi\omega^2(z))^2} e^{-\frac{\rho^2}{\omega^2(z)}} \quad \text{with} \quad \omega(z) = \omega_0 \left( 1 + \left( \frac{z}{z_R} \right)^2 \right)^{1/2}$$

$\omega_0$ : beam waist  
 $z_R$ : Rayleigh range



# First advantage: better resolution

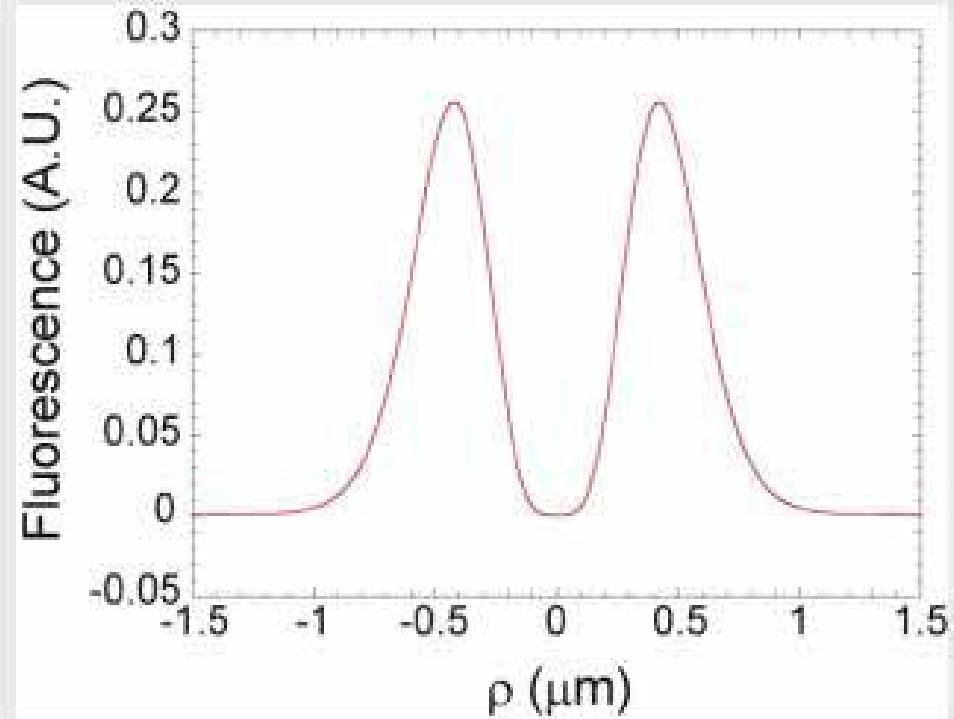
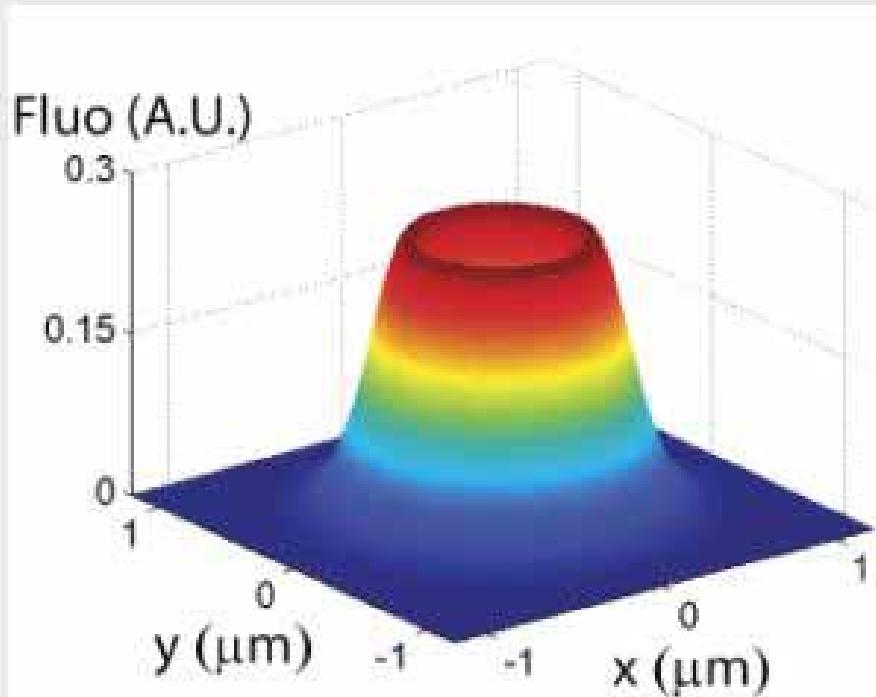
with aberration



$$PSF_0^2(\rho, z) = \frac{\rho^4}{4\omega^6(z)\omega_0^2} e^{-\frac{\rho^2}{2\omega^2(z)}} \left| I_0\left(\frac{\rho^2}{8A}\right) - I_0\left(\frac{\rho^2}{8A}\right) \right|^2$$

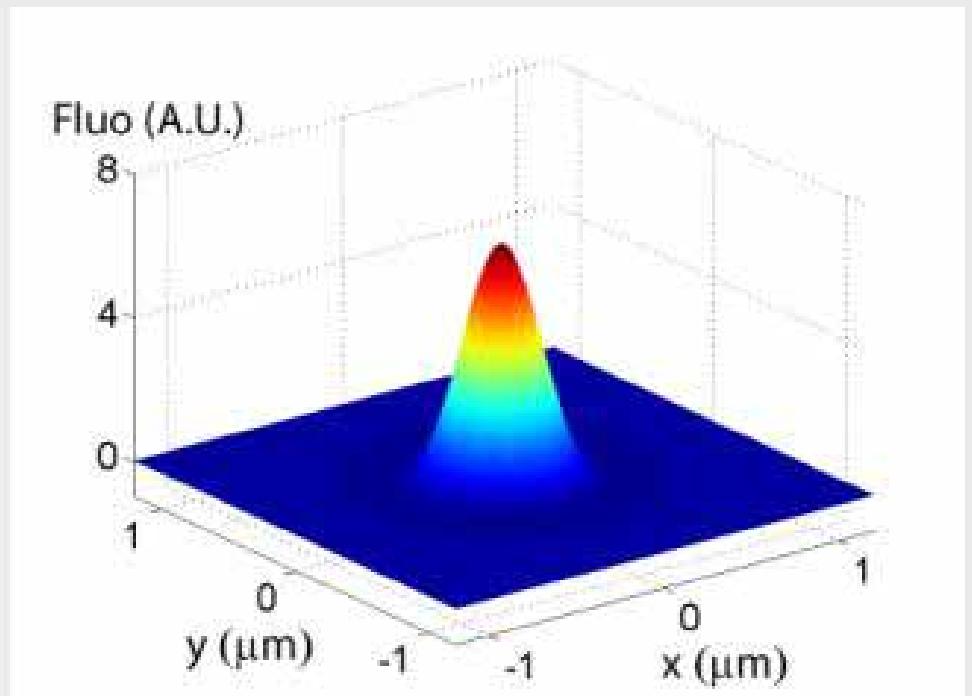
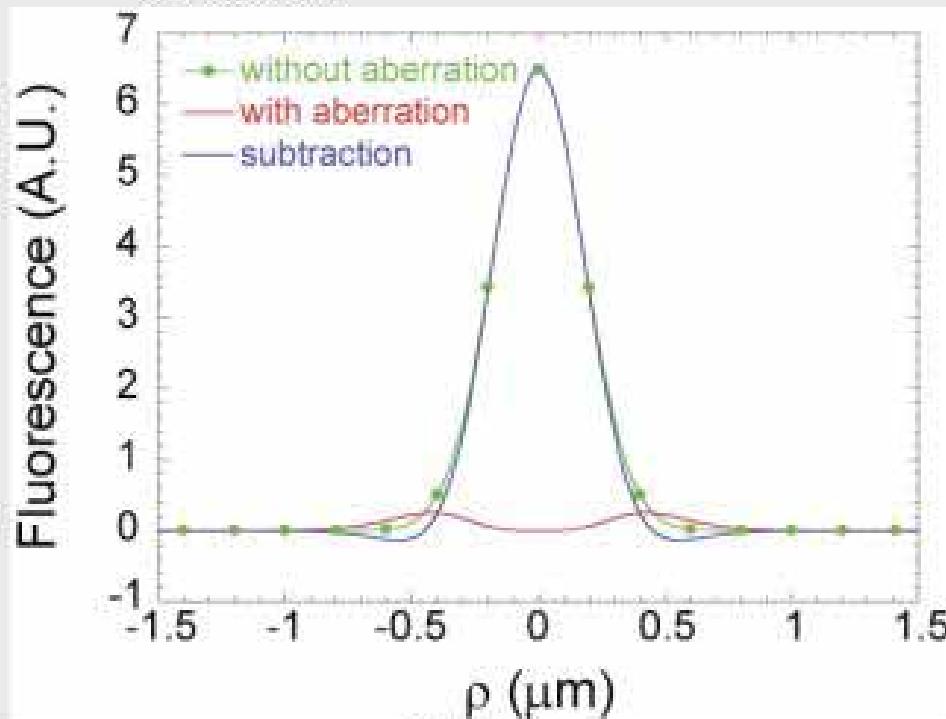
$$\text{with } A = \frac{\omega_0^2}{4} \left( 1 + \frac{iz}{z_R} \right)$$

$I_n$ : Bessel function of the first kind



# First advantage: better resolution

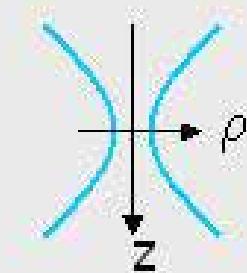
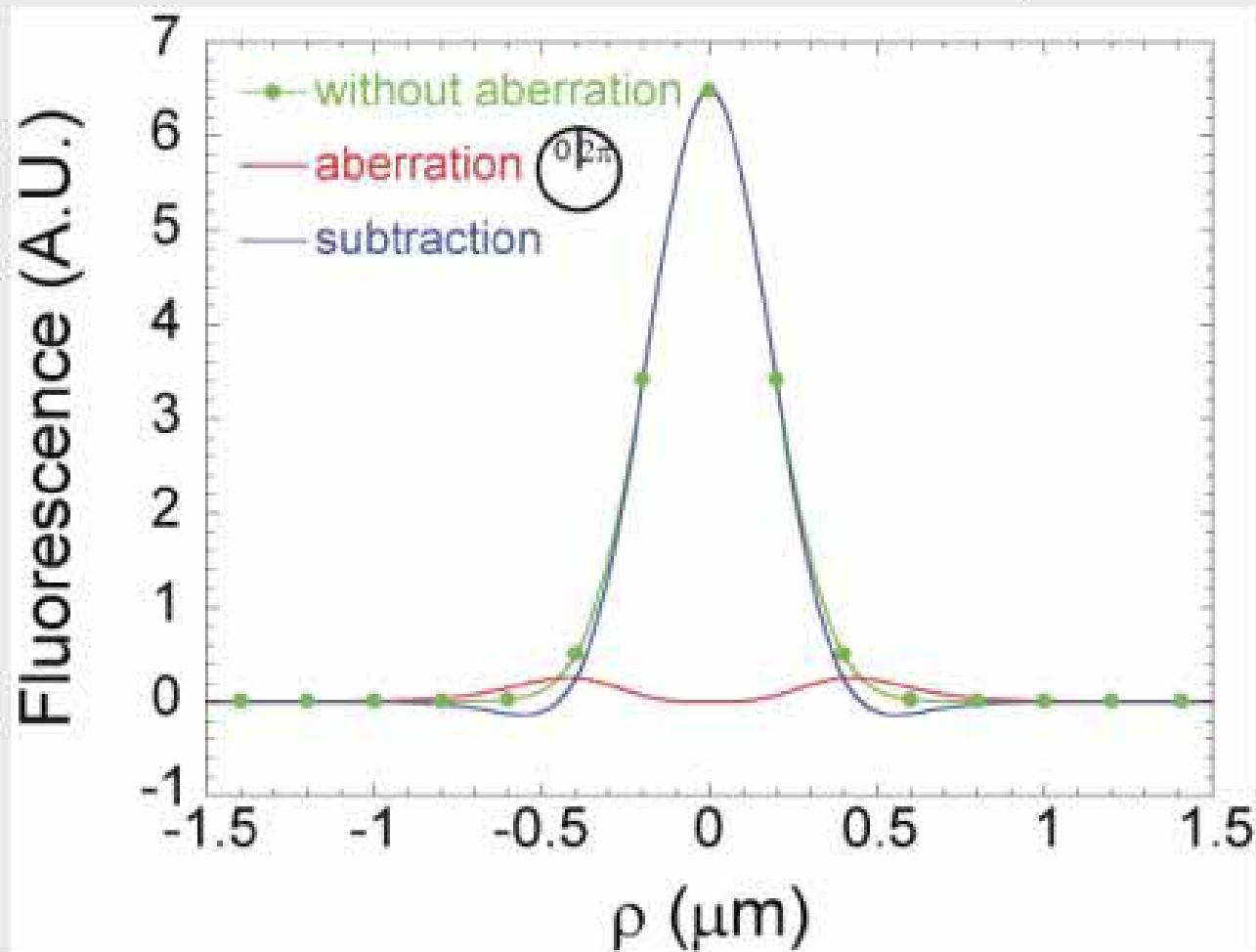
From Fresnel diffraction formula



Slight **improvement** of the **resolution**

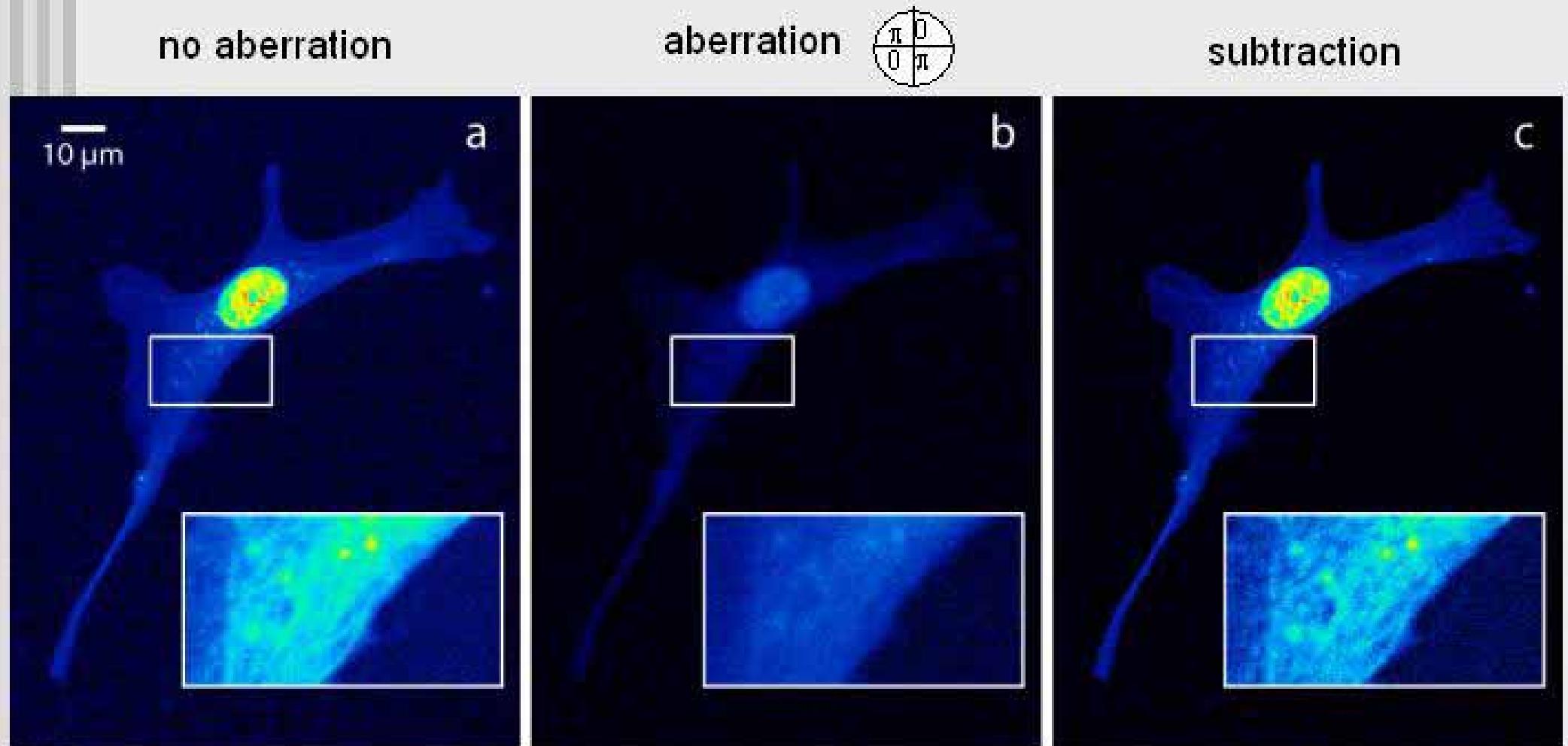
# First advantage: better resolution

From Fresnel diffraction formula:  $PSF^2(\vec{\rho}, z) = \left| \frac{1}{(2\pi)^2} e^{ikz} \int P(\vec{k}_\perp) e^{i\vec{k}_\perp \cdot \vec{\rho}} \frac{k^2}{2k} d^2 \vec{k}_\perp \right|^2$



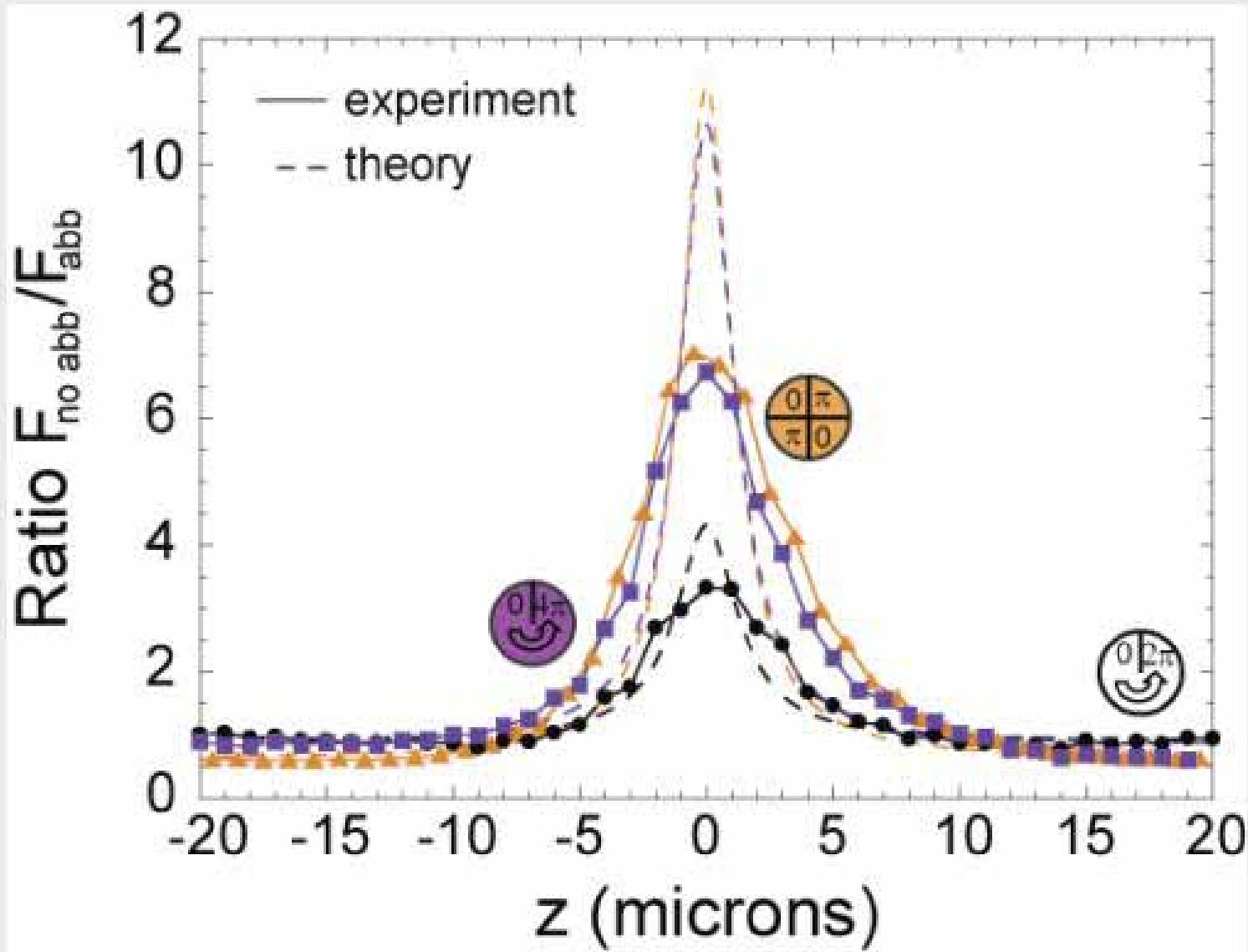
for a Gaussian beam ( $\omega_0=500\text{nm}$ ) in water ( $n=1.33$ ), in the focal plane

# imaging of bovine pulmonary artery endothelial cells labeled with 3 different fluorescent dyes (for a transparent medium)

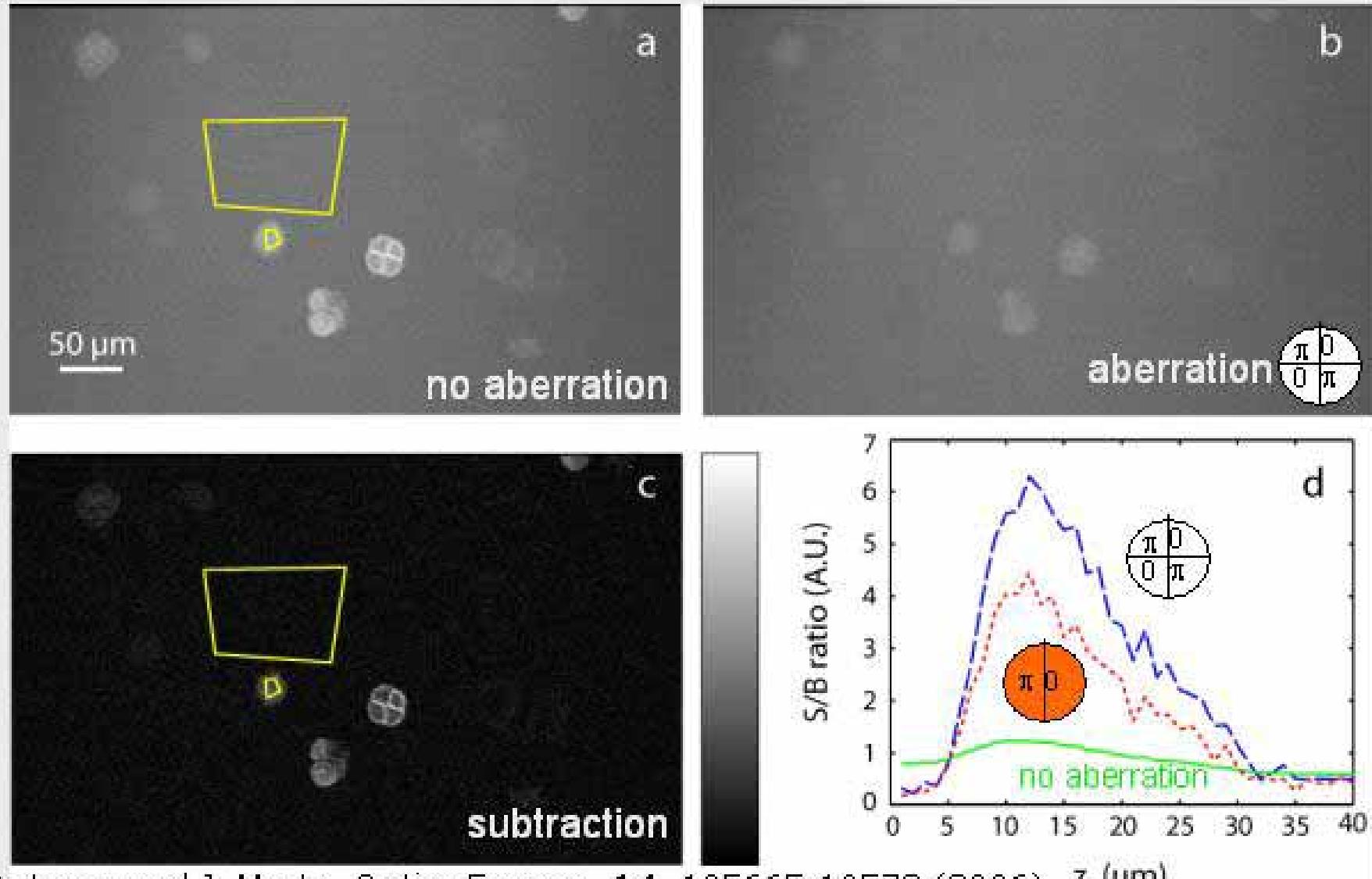


A. Leray and J. Mertz, *Optics Express*, **14**, 105665-10573 (2006)

# Fluorescence from a plane: comparison with theory



imaging of pollen grains labeled with different fluorescent dyes  
(for a scattering and fluorescent medium)

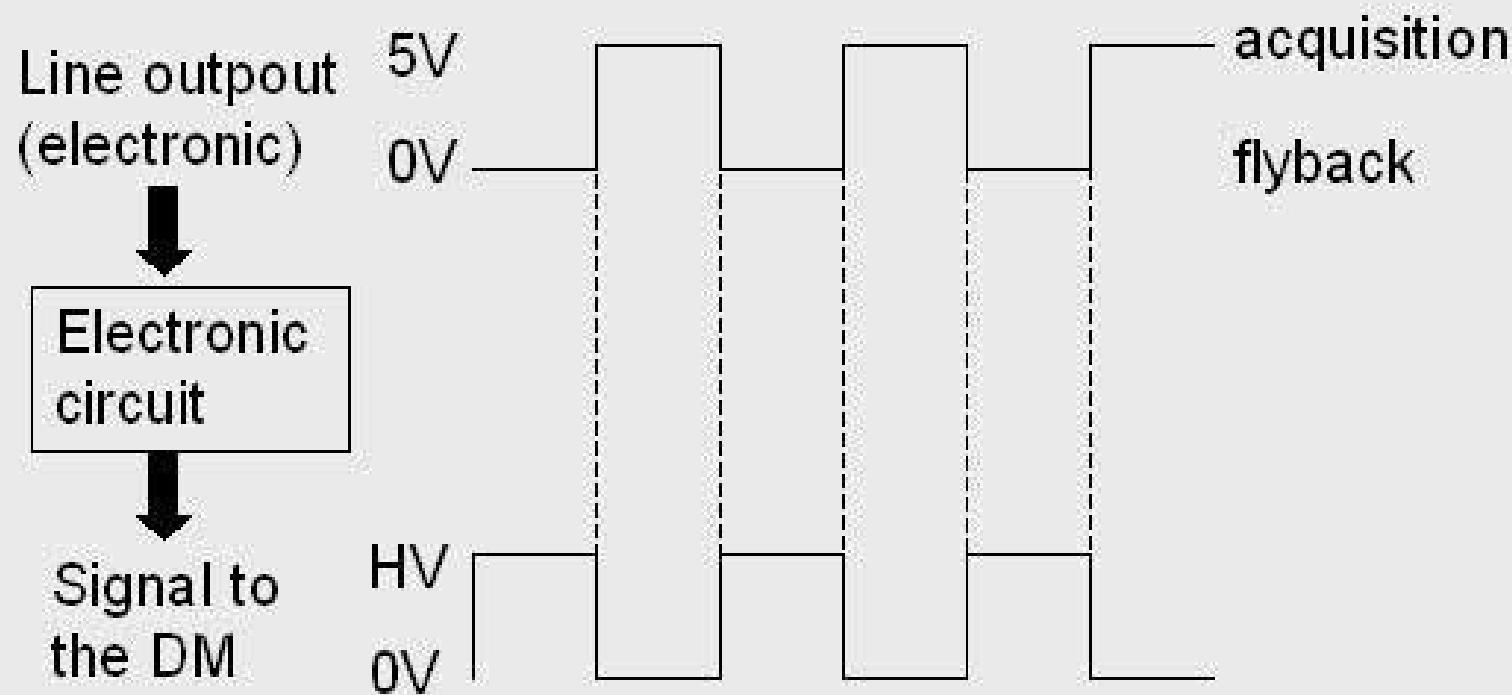


A. Leray and J. Mertz, Optics Express, 14, 105665-10573 (2006)

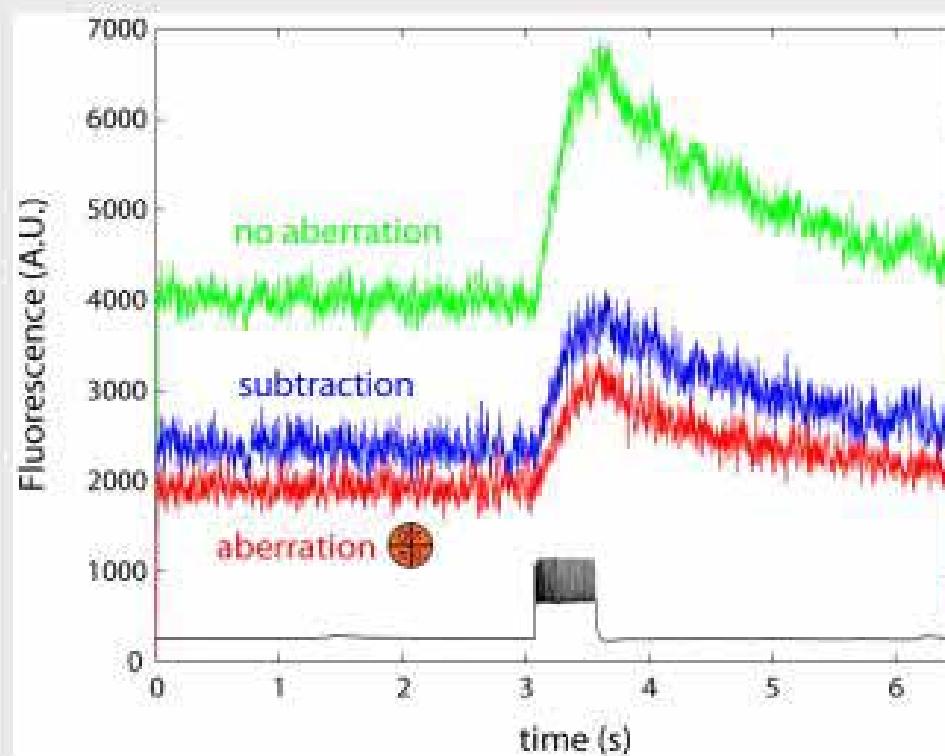
# Experimental setup

New DM mount with a switch for each actuator :

- switch off  $\rightarrow$  0V (always)
- switch on  $\rightarrow$  HV (choose)



# Experimental results



500pA, 500ms pulse of  
current injected

