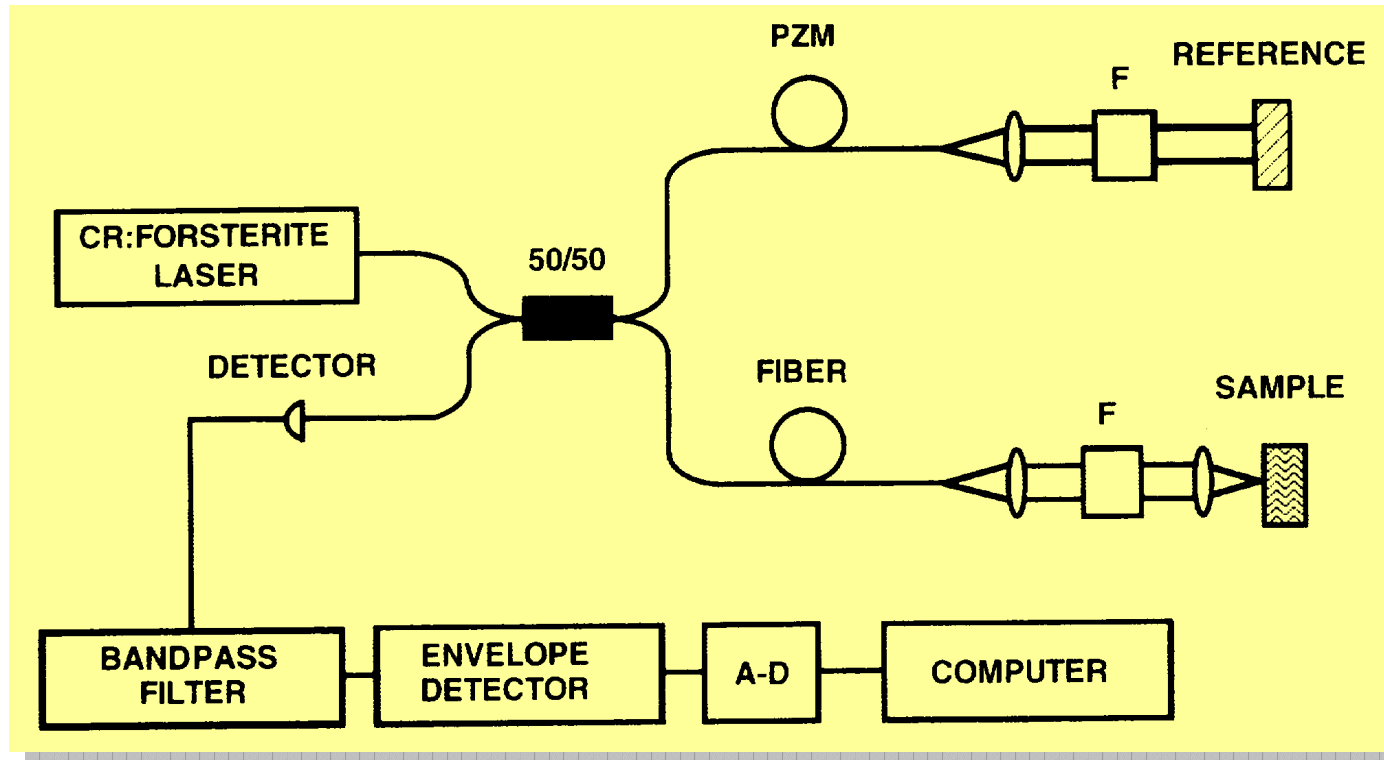


# Techniques d'imagerie à base d'impulsions femtosecondes

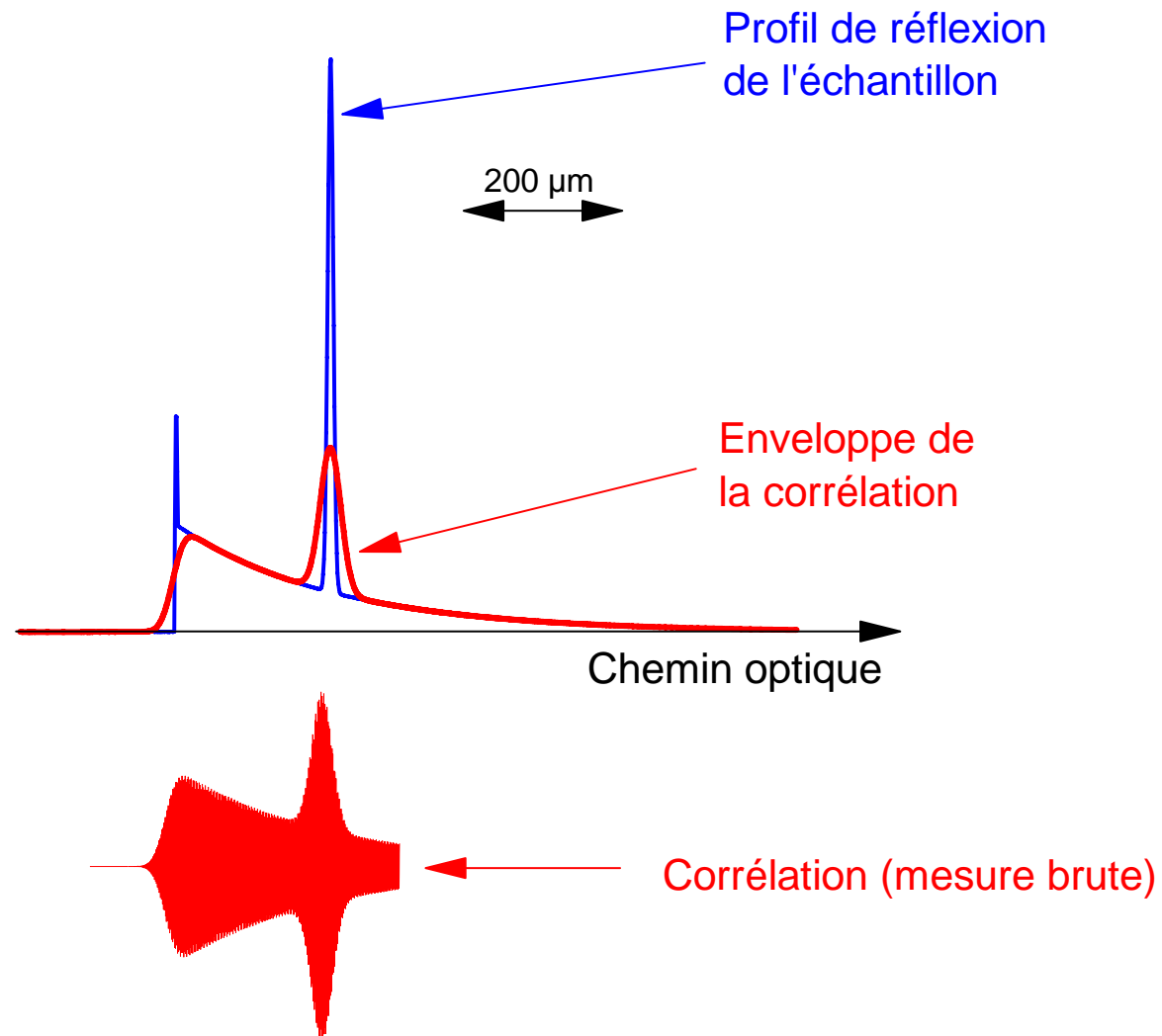
1. Tomographie cohérente optique
2. Microscopie à deux photons
3. Microscopie par génération d'harmoniques
4. Microscopie CARS
5. Microscopie non-linéaire cohérente

# Tomographie cohérente optique

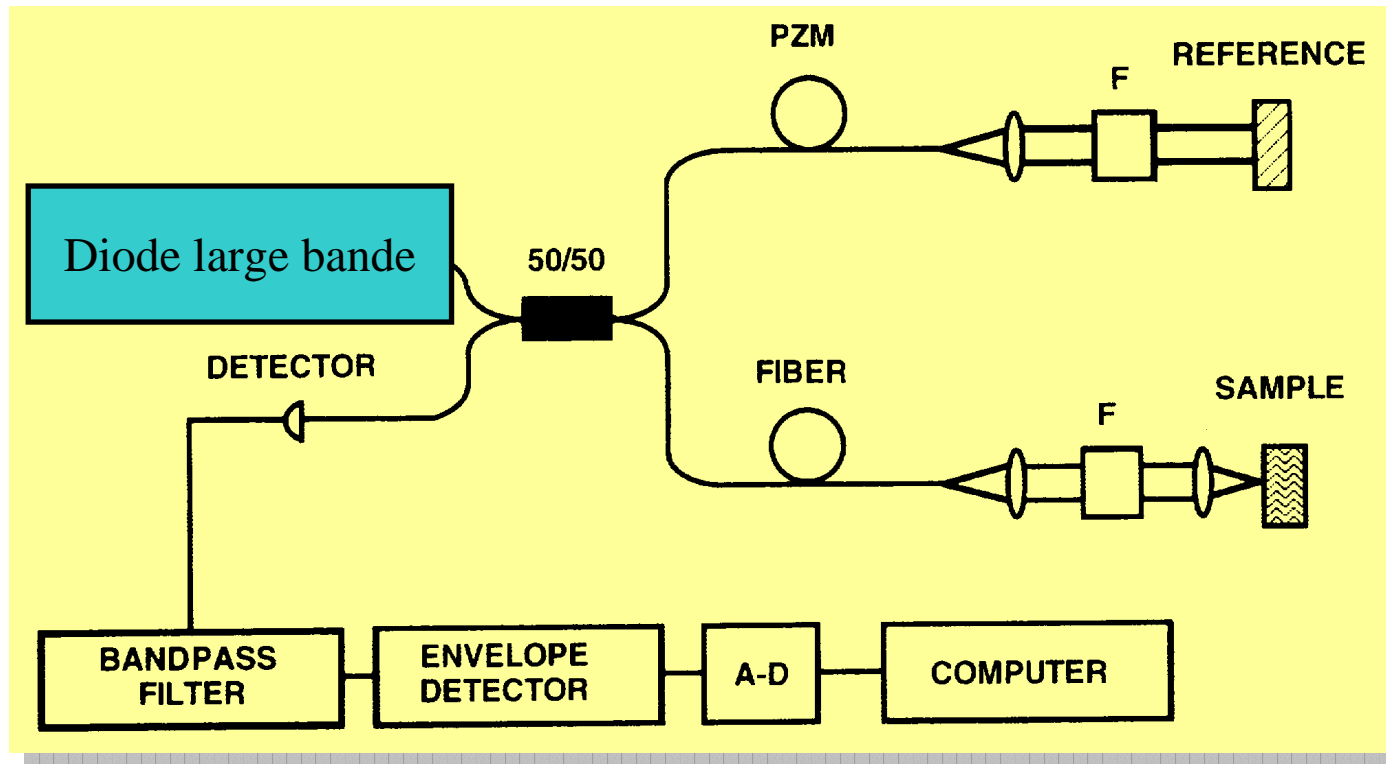


G.J. Tearney et al., Opt. Lett. **21**, 1408 (1996)

# Tomographie cohérente optique : principe

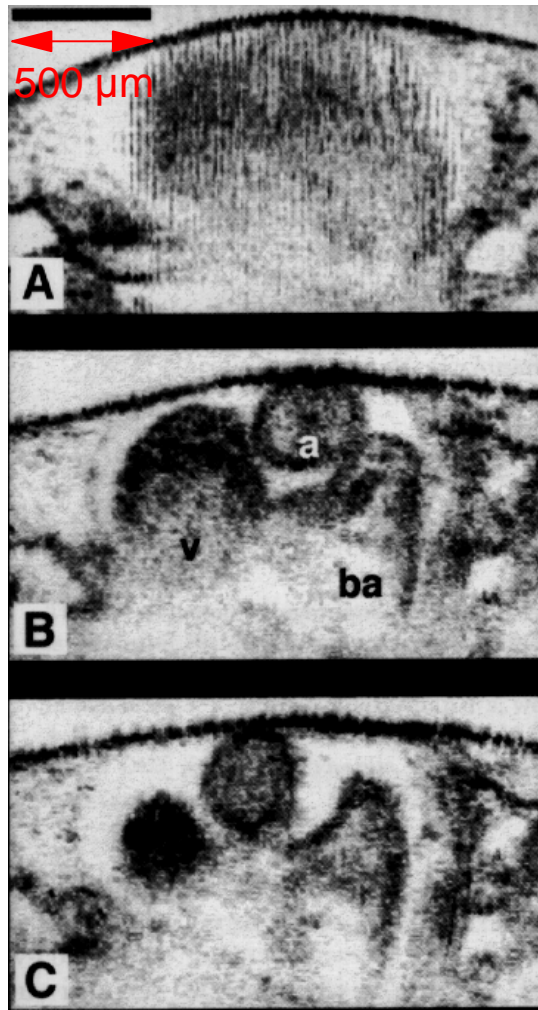


# Intérêt du laser femtoseconde ?



G.J. Tearney et al., Opt. Lett. **21**, 1408 (1996)

# Tomographie cohérente optique : imagerie du cœur d'un têtard

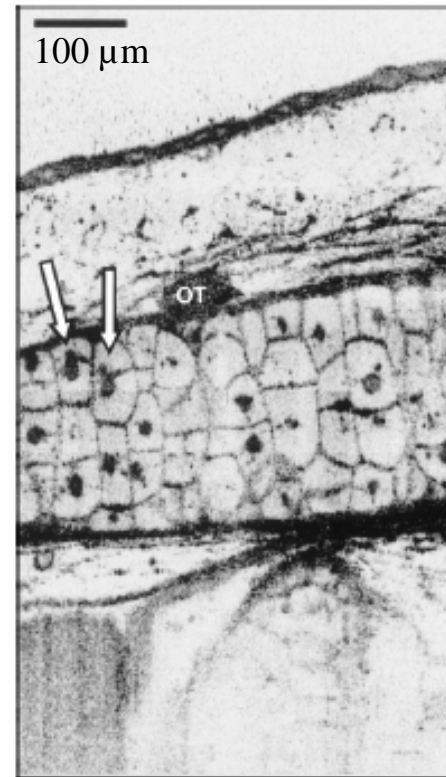
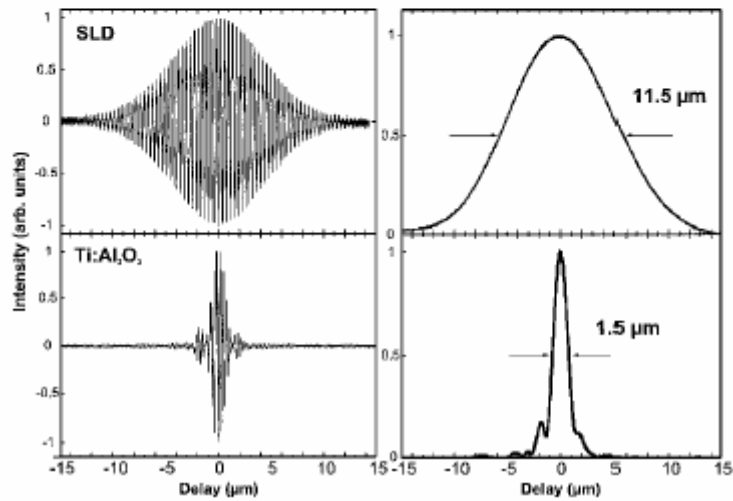
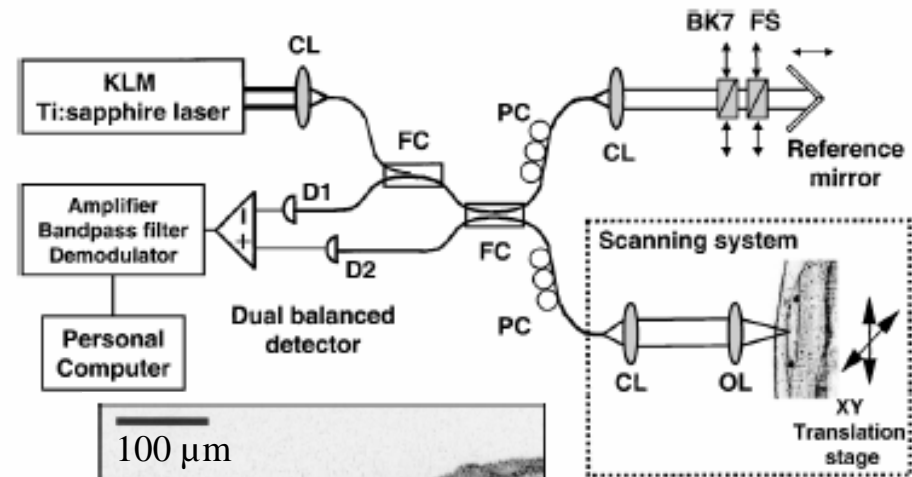
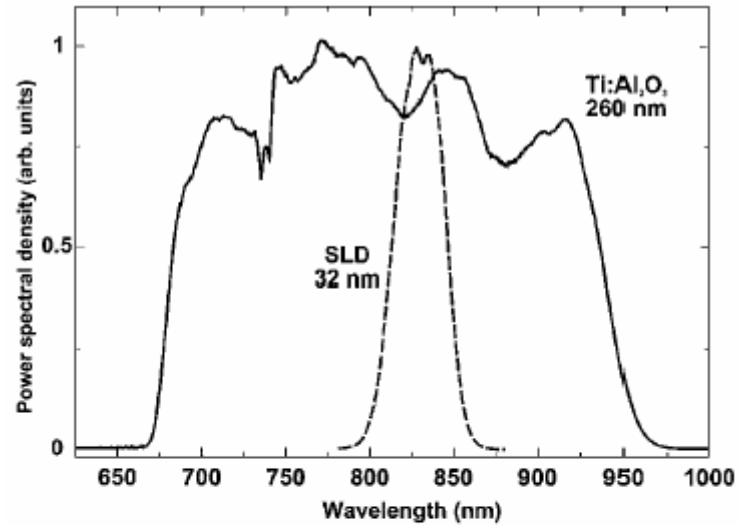


*Images d'un embryon de grenouille obtenues par tomographie cohérente optique. La zone représentée correspond au coeur de la grenouille.*

*L'image A, correspondant à un temps d'acquisition assez long, est brouillée en raison des mouvements du coeur.*

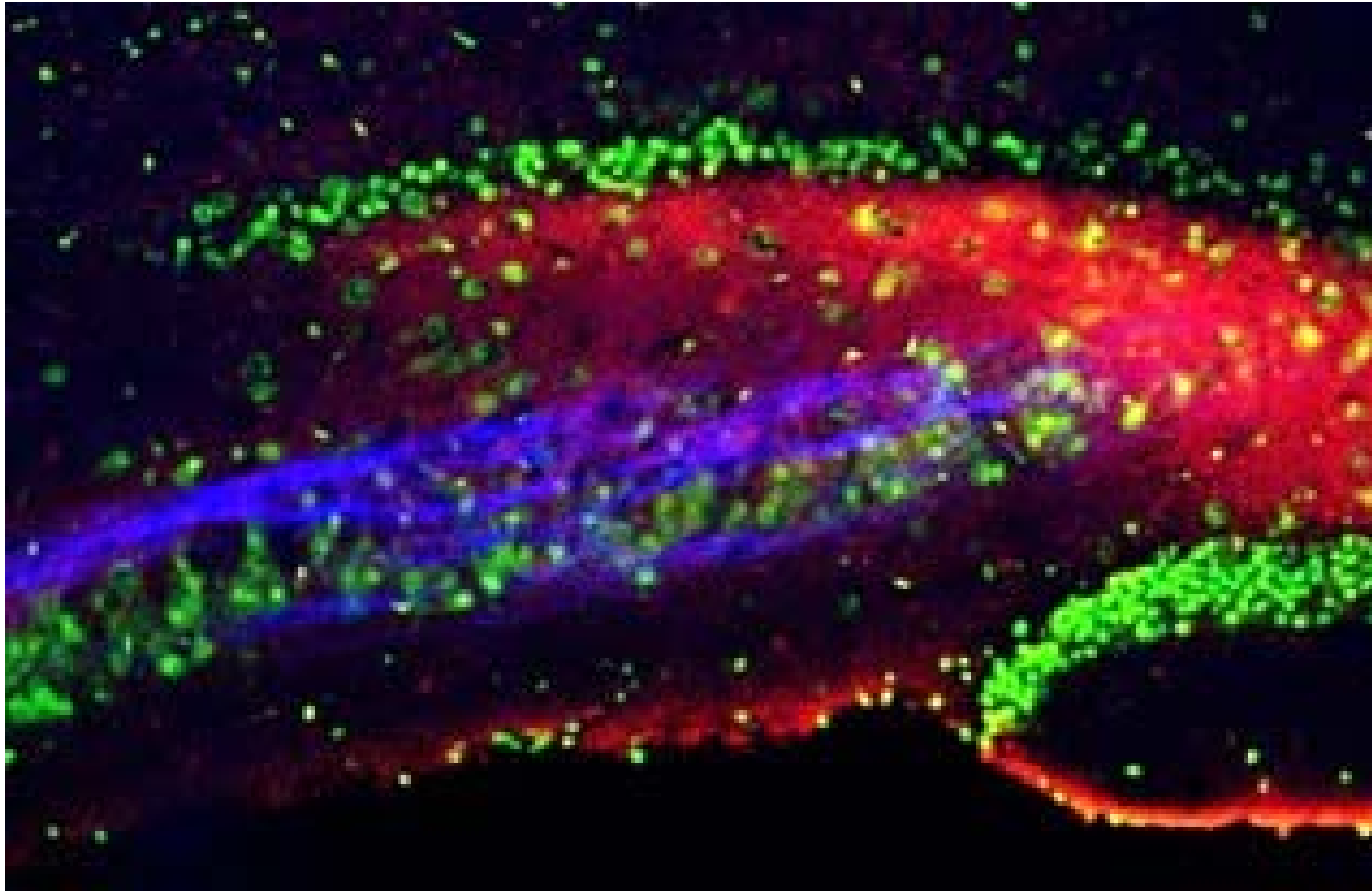
*Au contraire, les images B et C ont été obtenues en une fraction de seconde, montrant l'état du coeur de la grenouille à deux instants successifs. En montant de telles images les unes à la suite des autres, il est possible de reconstituer un film des battements du coeur. Ces images sont extraites d'un article de G.J. Tearney et al., du Massachusetts Institute of Technology *Optics Letters* 21, 1408 (1996)).*

# Tomographie cohérente optique à haute résolution



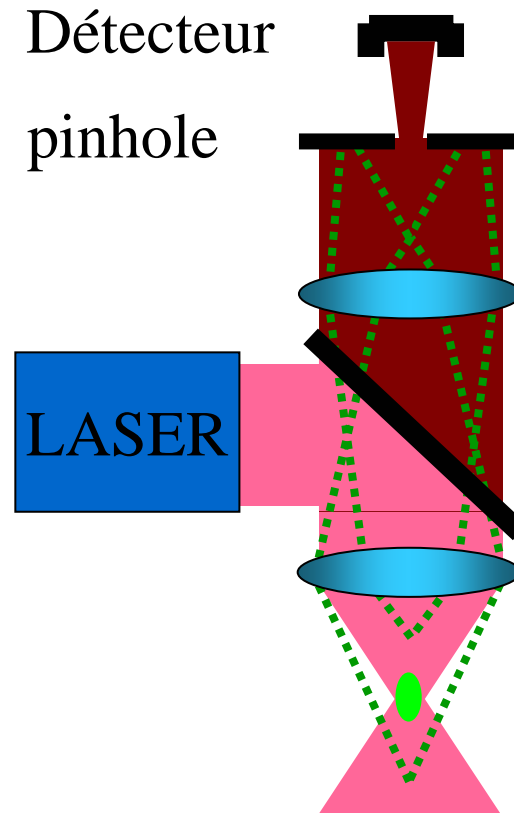
W. Drexler et al., Opt. Lett. **24**, 1221 (1999)

## *2. Microscopie à deux photons*



<http://www.drbio.cornell.edu/pastcovers.html>

# Rappel : microscopie confocale





# Microscopie confocale à "pinhole" virtuel

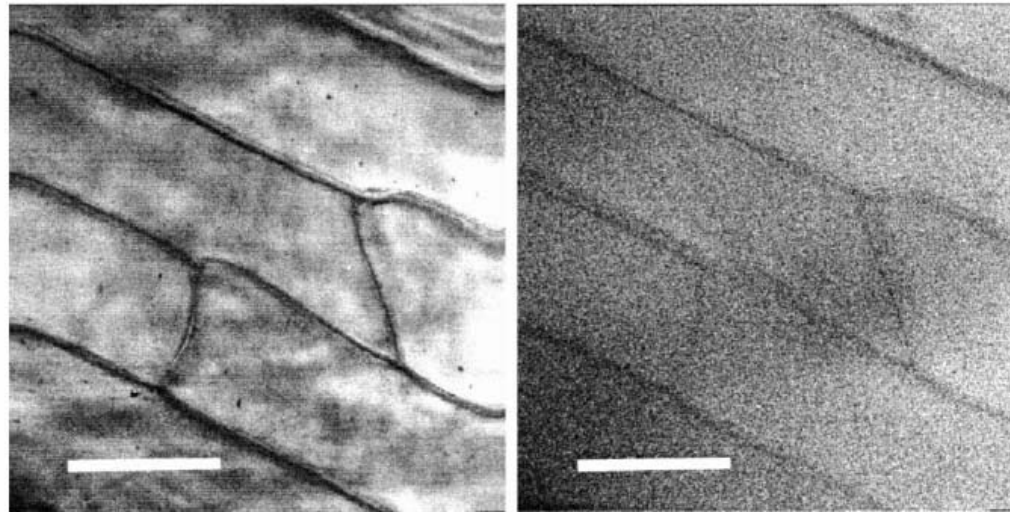
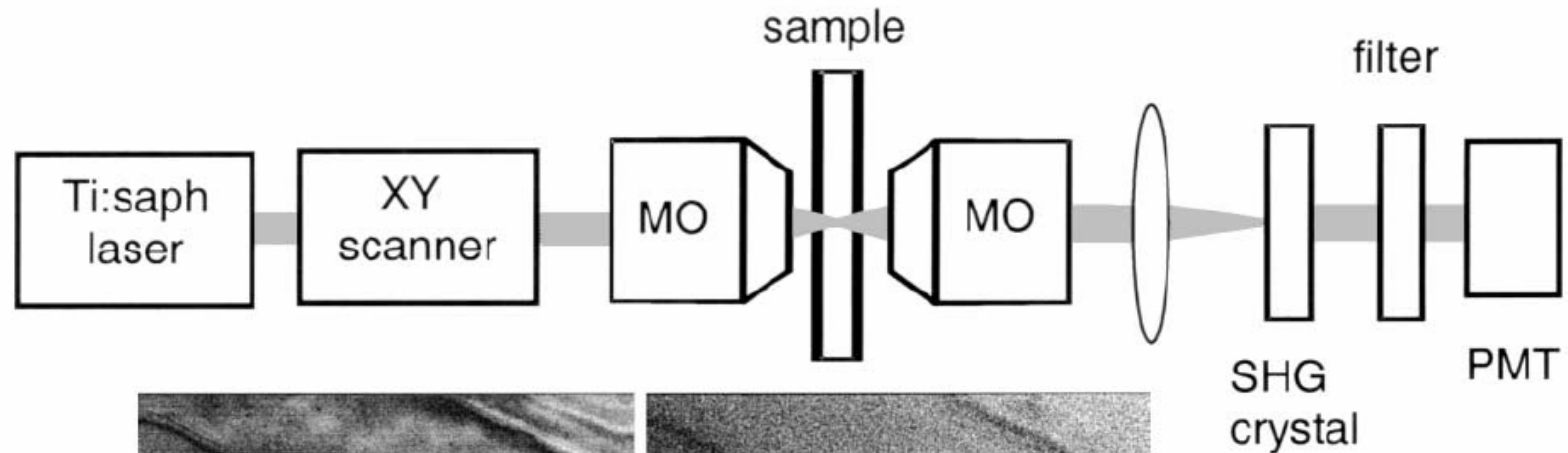
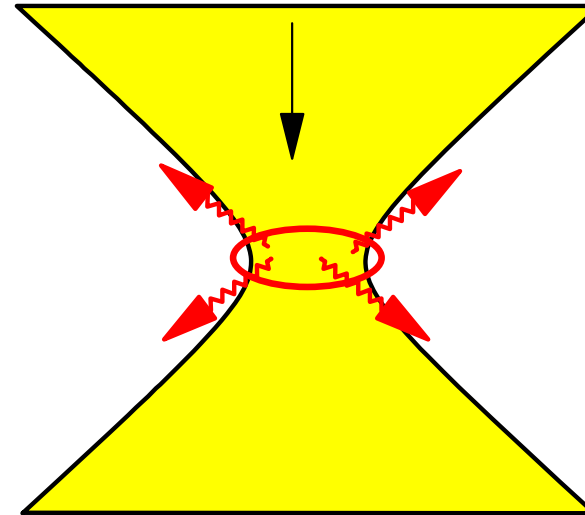
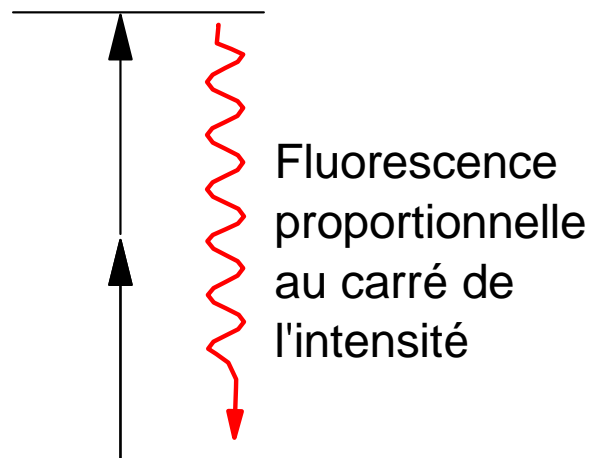


Fig. 4.  $x$ - $y$  images of an onion slice beneath a 200- $\mu\text{m}$  agarose slab of 1- $\mu\text{m}$  latex beads obtained left, by SHG detection and right, by direct  $P^2$  detection. Scale bars, 100  $\mu\text{m}$ .

# Fluorescence par excitation à deux photons



- ✓ Résolution intrinsèquement tri-dimensionnelle.
- ✓ Seule la zone effectivement observée est excitée.

# Fluorescence par excitation à deux photons

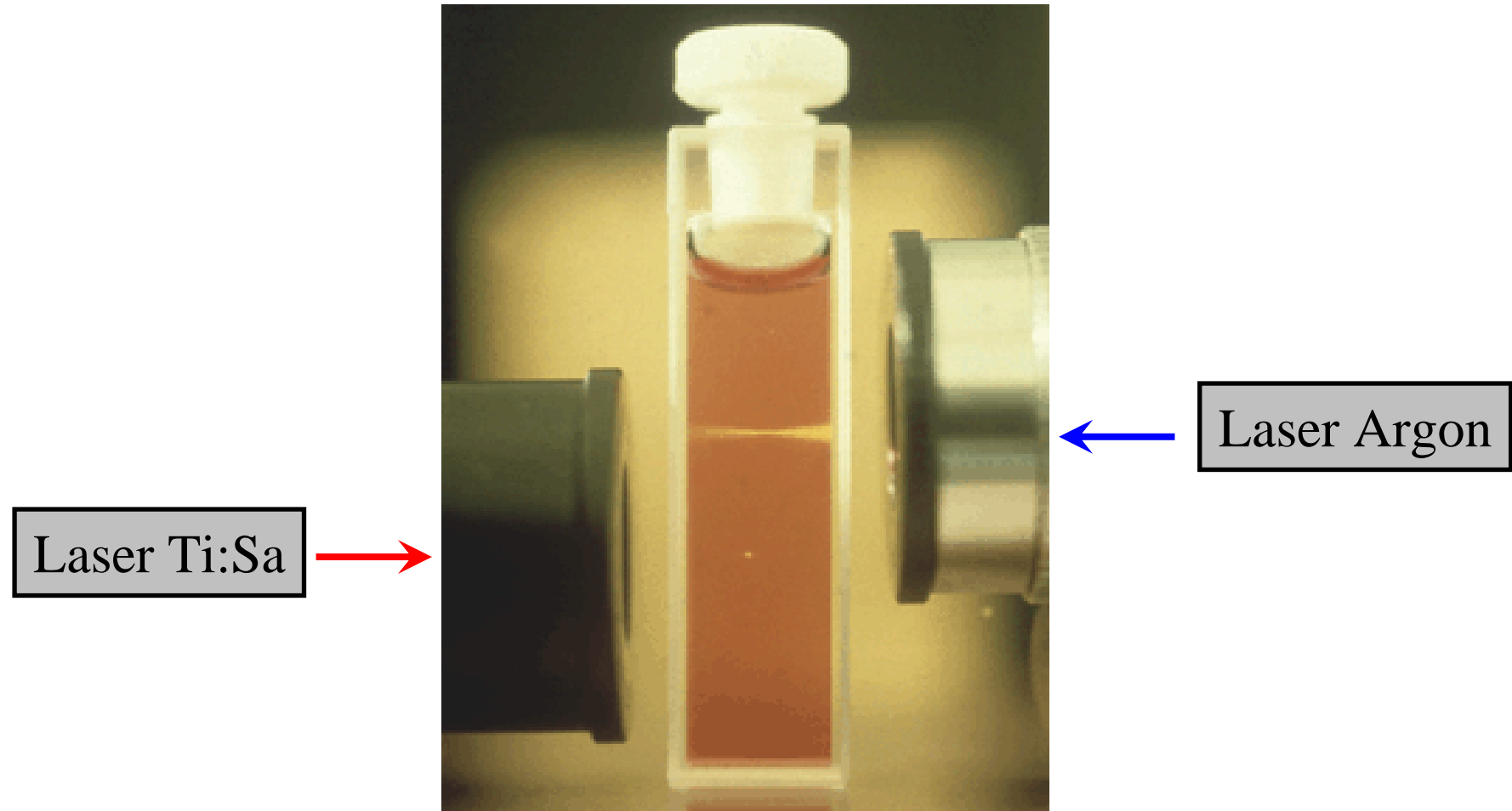
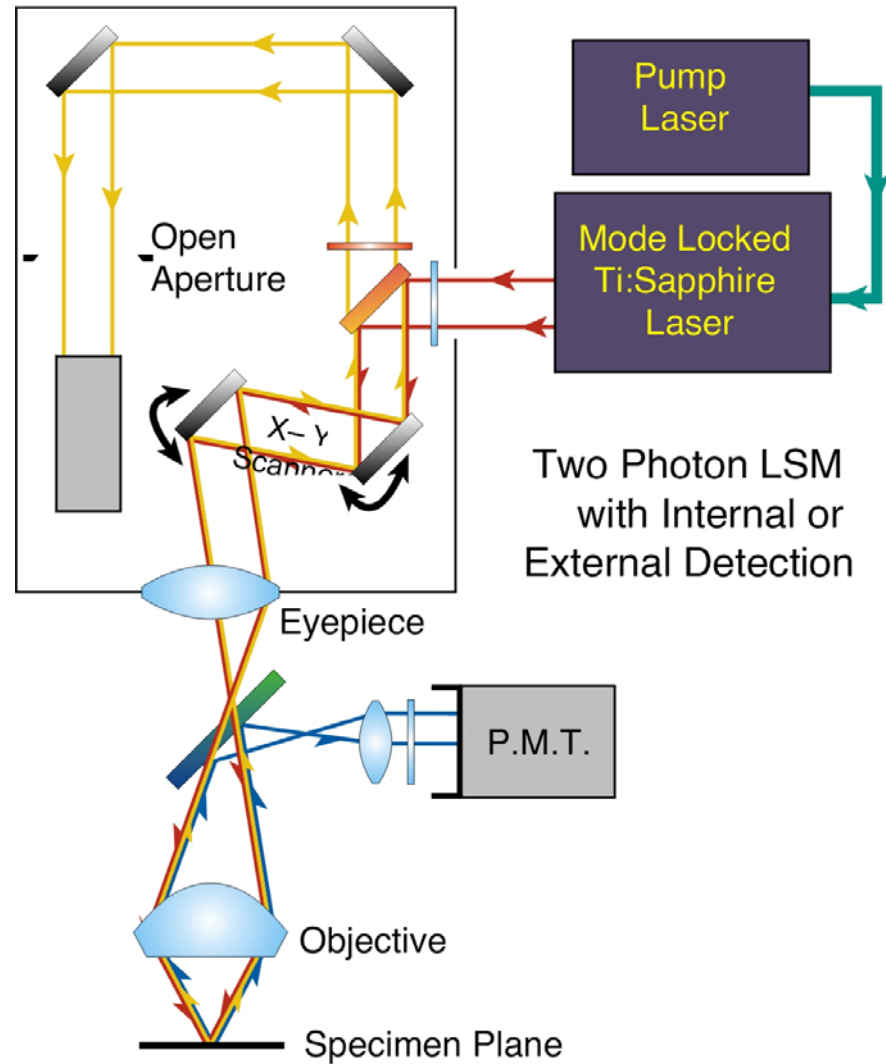


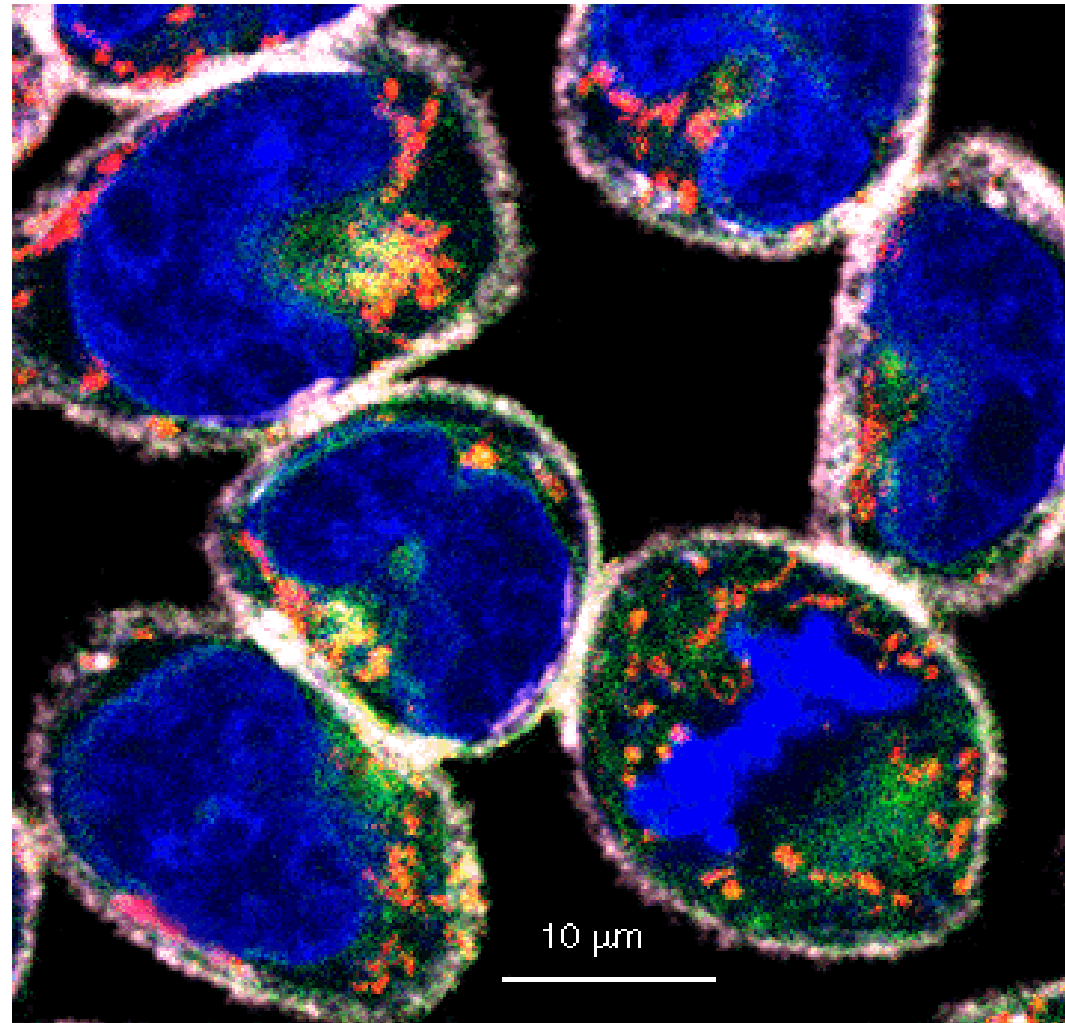
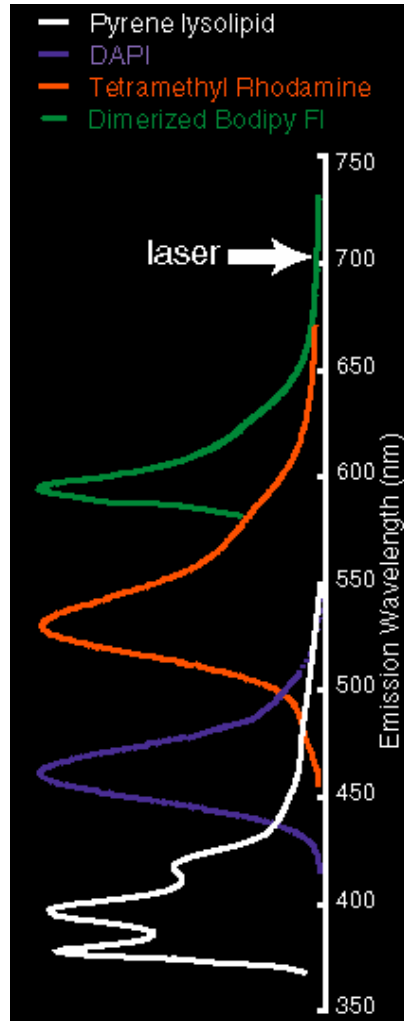
Photo: Brad Amos, MRC, Cambridge

# Microscopie à deux photons : schéma de principe



W. Denk, J. H. Strickler, and W. W. Webb, *Science* **248**, 73 (1990)

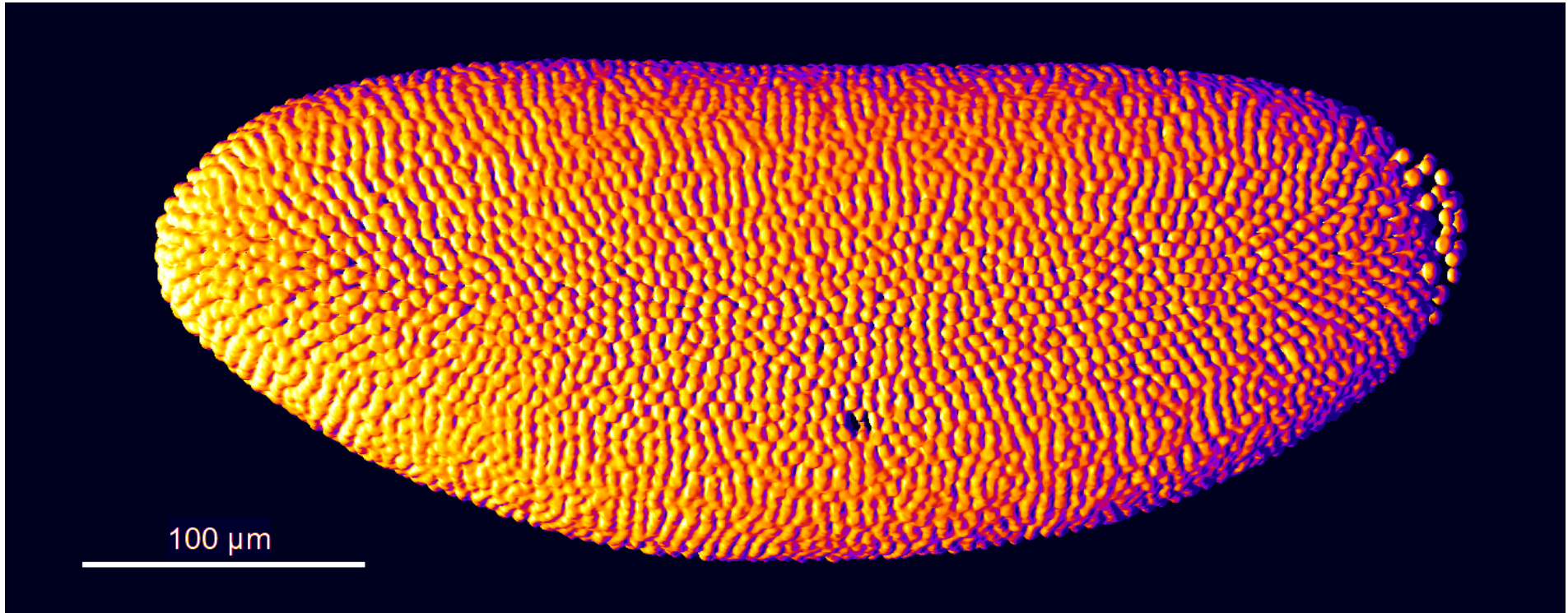
# Microscopie par excitation à deux photons



C. Xu, W.W. Webb (1995)

# Développement d'un embryon de Drosophile

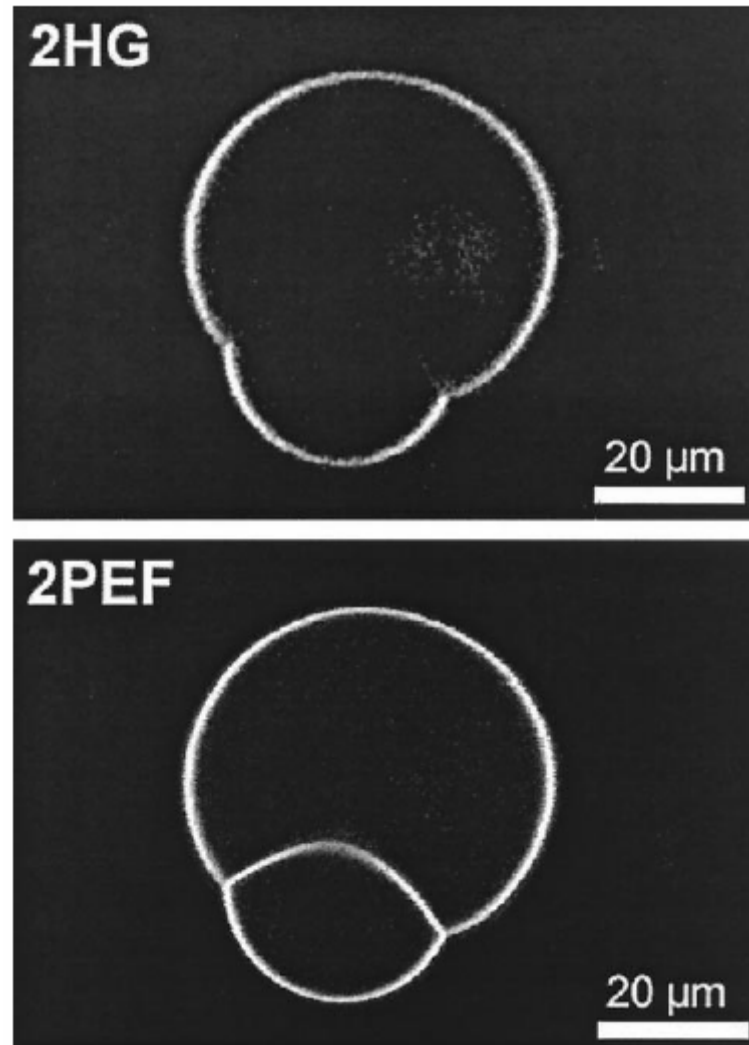
Reconstruction tridimensionnelle



*W. Supatto, D. Débarre, B. Moulia, E. Brouzés, J.-L. Martin, E. Farge, E. Beaurepaire*  
Proc. Nat. Acad. Sci. (USA) **102**, 1047 (2005)

### *3. Microscopie par génération d'harmoniques*

# Microscopie par génération de second harmonique



L. Moreaux et al., *Biophys. J.* **80**, 1568 (2001)



# Microscopie par génération de second harmonique

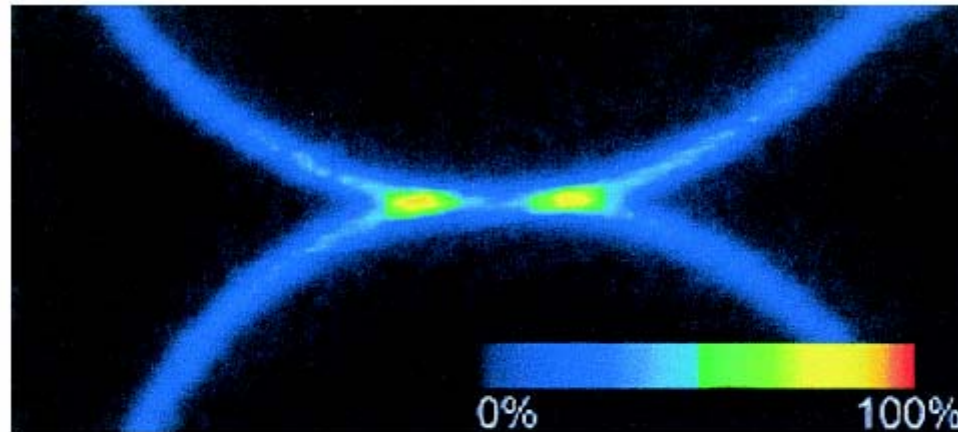
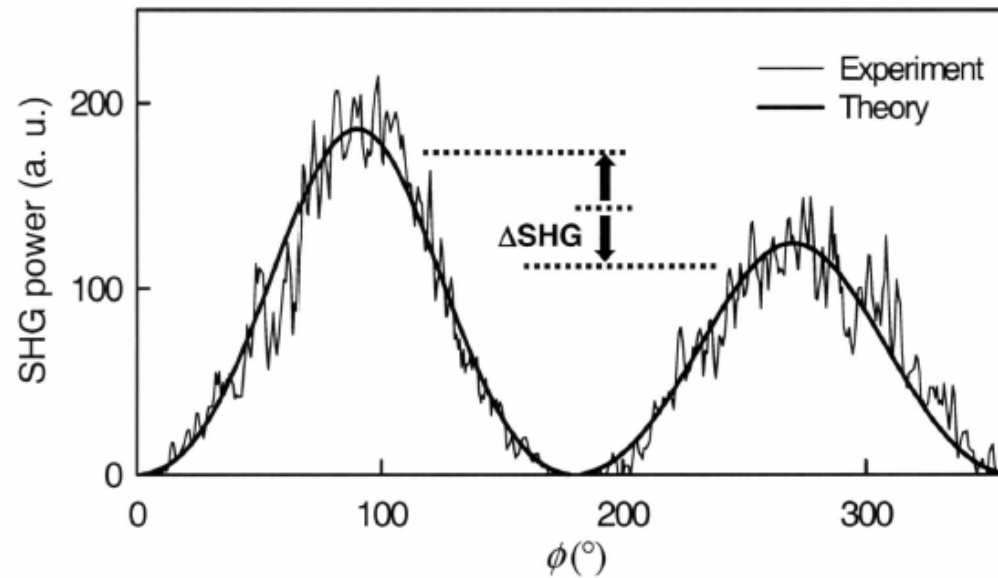
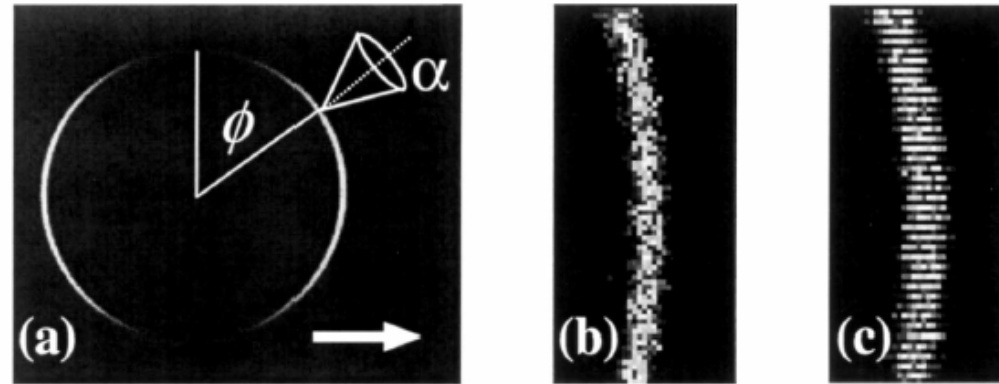


FIGURE 4 Two GUVs are brought into close proximity, occasioning partial destructive or constructive interference of the 2HG signal depending on the separation of the GUV membranes. 2HG provides an accurate measure of the local separation for distances smaller than the focal spot size of the excitation beam. The illumination power is  $<1$  mW.

L. Moreaux et al., *Biophys. J.* **80**, 1568 (2001)

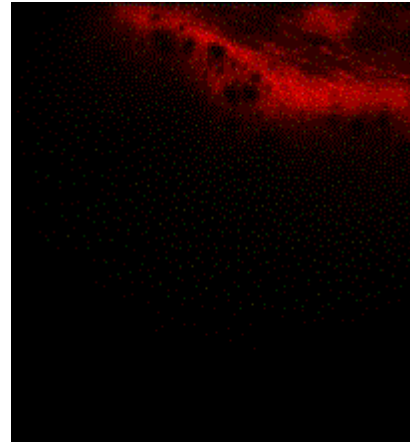
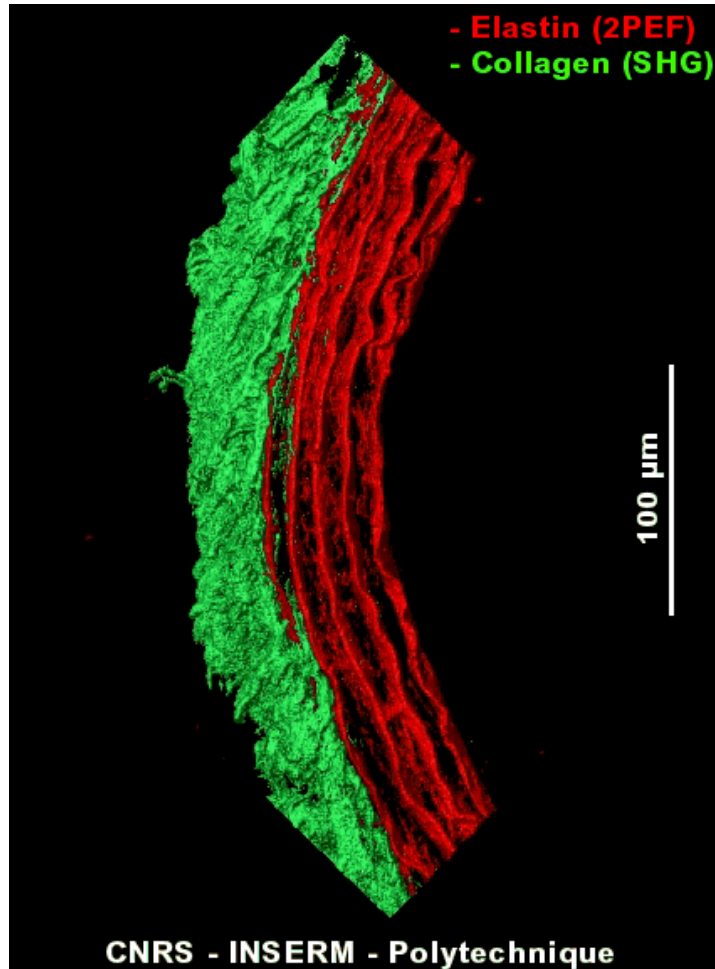
# Microscopie par génération de second harmonique

*Sensibilité au potentiel transmembranaire*



L. Moreaux et al., Opt. Lett. **28**, 625 (2003)

# Microscopie par excitation à deux photons et génération d'harmoniques



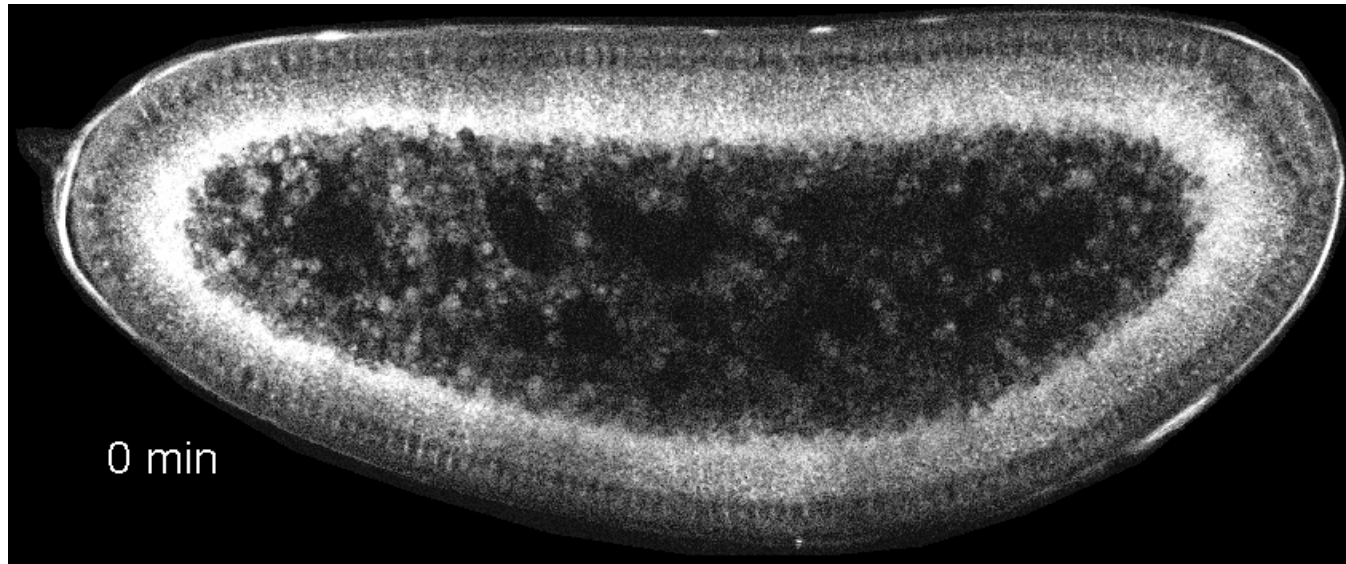
Une série de tranches enregistrées à des profondeurs successives permet ensuite d'obtenir une image tridimensionnelle.

Paroi d'artère de Rat non marquée

*E. Beaurepaire, T. Boulesteix, A.-M. Pena, M.-P. Sauviat, M.-C. Schanne-Klein*

# Morphogénèse dans un embryon de drosophile

Microscopie par génération de troisième harmonique

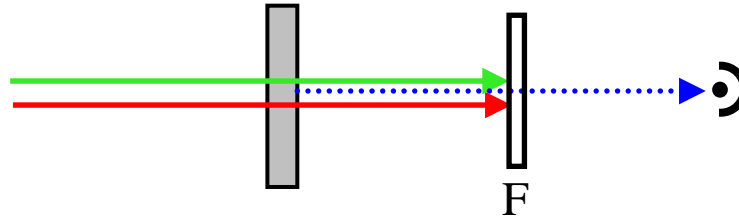


W. Supatto, D. Débarre, B. Moulia, E. Brouzés, J.-L. Martin, E. Farge, E. Beaurepaire  
*In vivo modulation of morphogenetic movements in Drosophila embryos with femtosecond laser pulses*  
Proc. Natl. Acad. Sci. USA **102**, 1047 (2005)

<http://www.lob.polytechnique.fr>

## *4. Microscopie CARS*

# Coherent Antistokes Raman Scattering

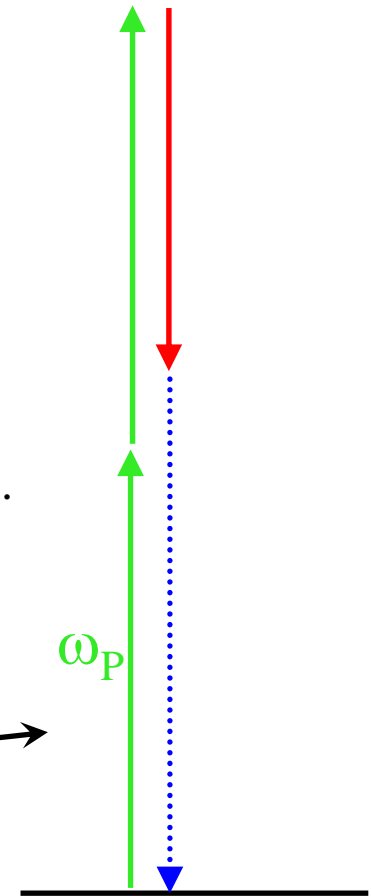
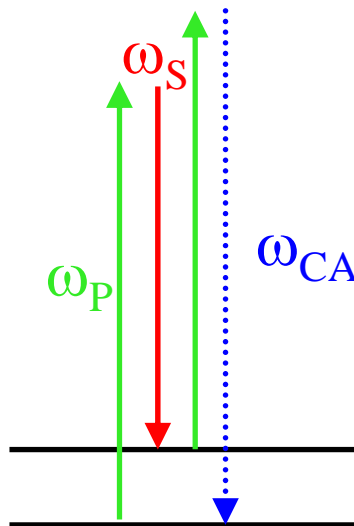


Avantages pour la microscopie :

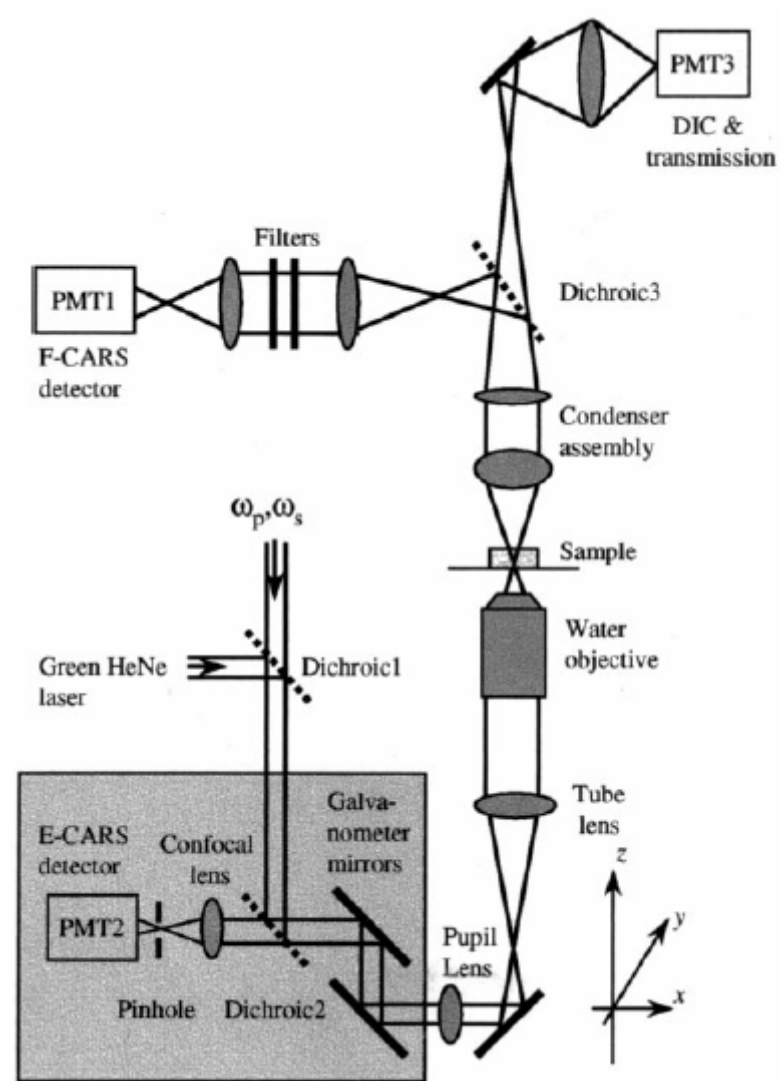
- ✓ Processus non-linéaire
- ✓ Ne nécessite pas de marqueur fluorescent
- ✓ Emission cohérente

Inconvénients :

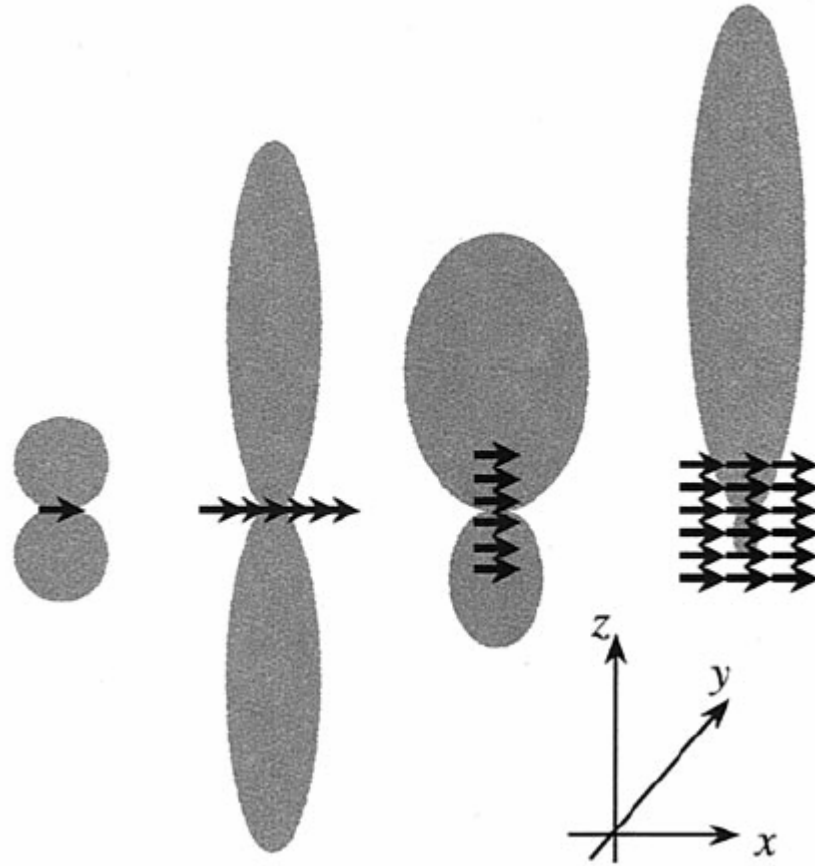
- ✓ Nécessite deux lasers picosecondes synchronisés.
- ✓ Contribution non-résonnante importante



# Microscopic CARS



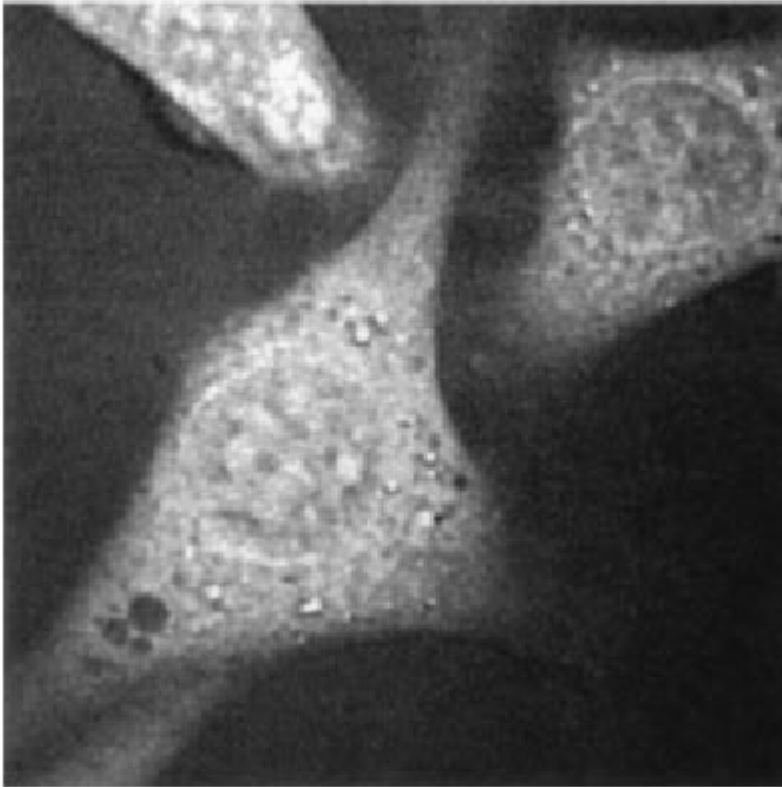
# Diagrammes de rayonnement



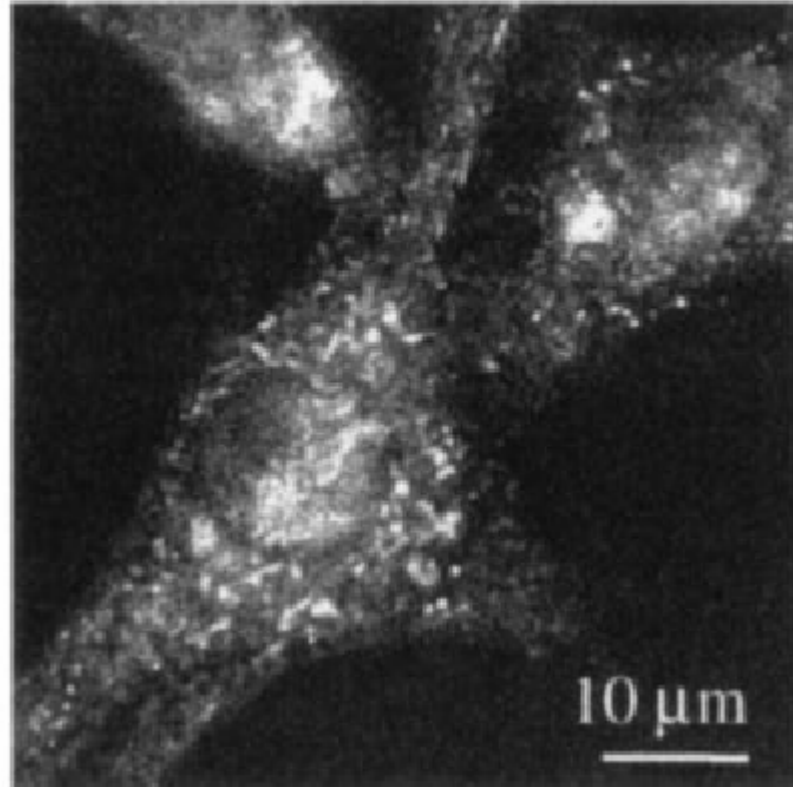


## Comparaison émissions avant et arrière

**F-CARS**



**E-CARS**



# Visualisation des chromosomes

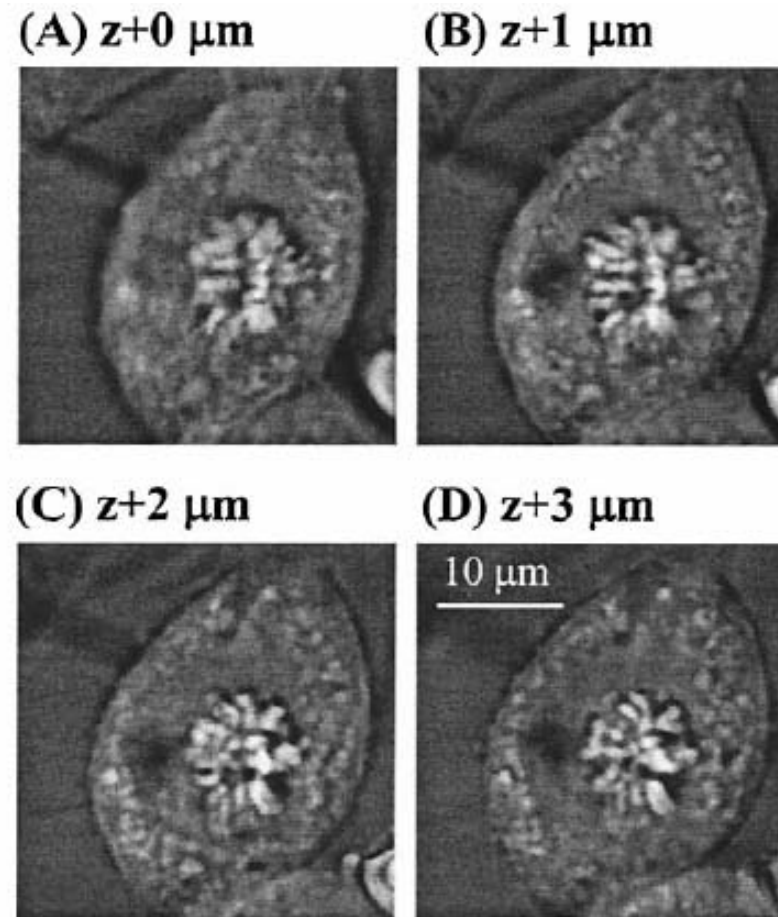
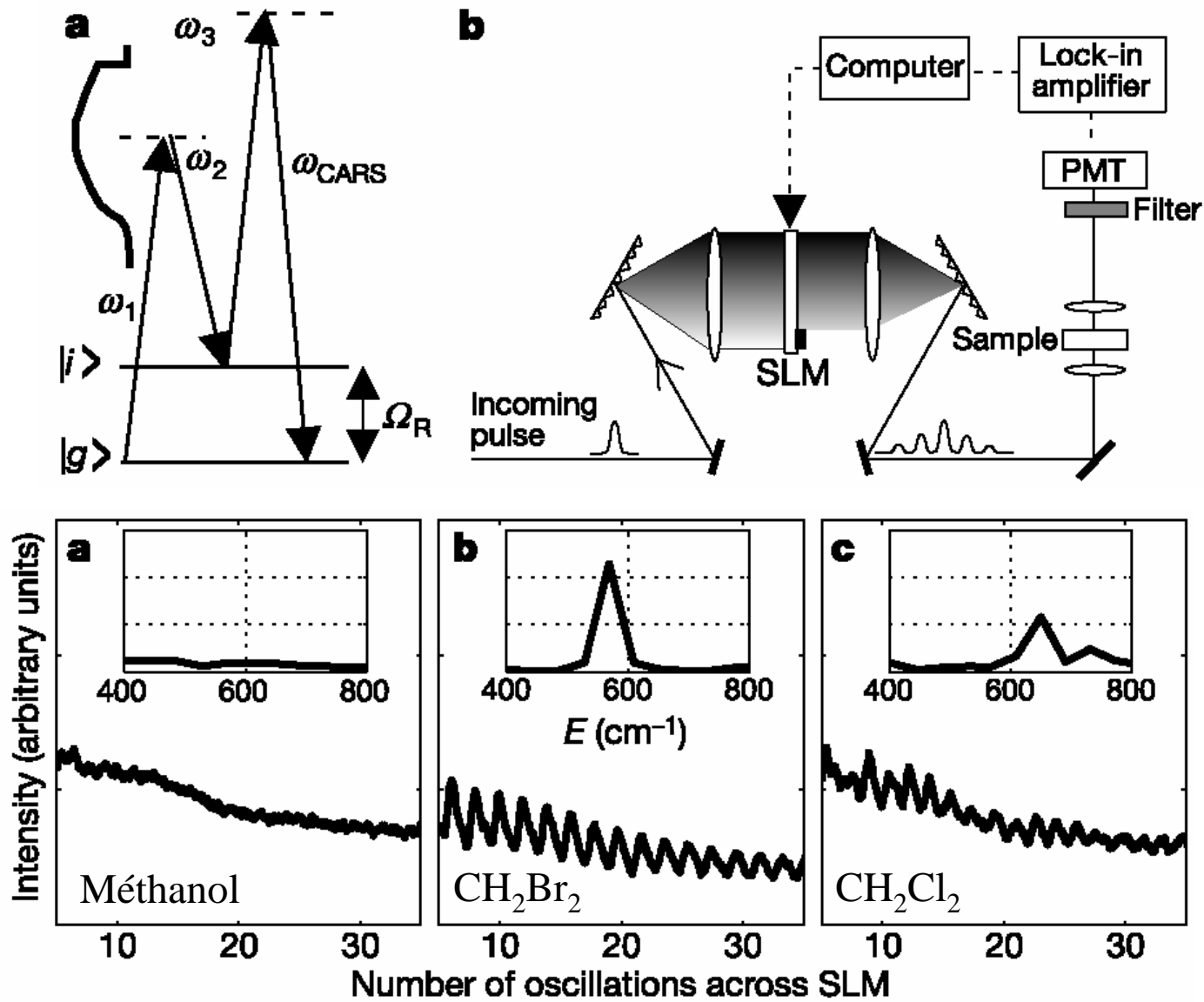


FIGURE 6 F-CARS images of a NIH 3T3 cell in metaphase at different depths.  $\omega_p - \omega_s$  was tuned to the  $\text{PO}_2^-$  symmetric stretching vibrational frequency at  $1090 \text{ cm}^{-1}$ . The pump frequency was  $13,593 \text{ cm}^{-1}$  and the Stokes frequency was  $12,503 \text{ cm}^{-1}$ . The acquisition time was 16.9 s for each image of  $29.6 \times 29.6 \mu\text{m}^2$ . The pump and Stokes power were 40 and 20 mW, respectively.

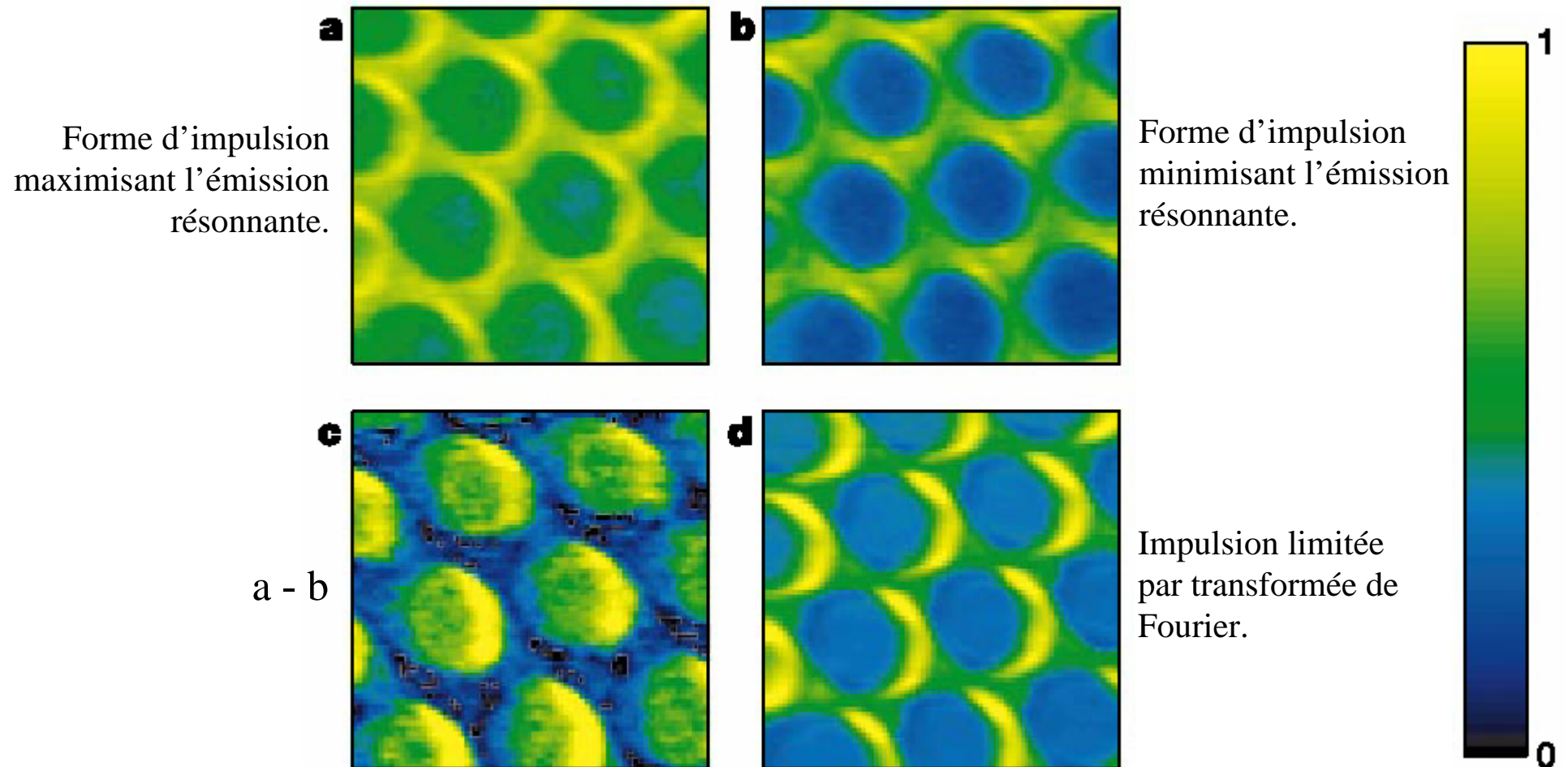
## *5. Microscopie non-linéaire cohérente*

# Microscopie CARS à impulsions façonnées

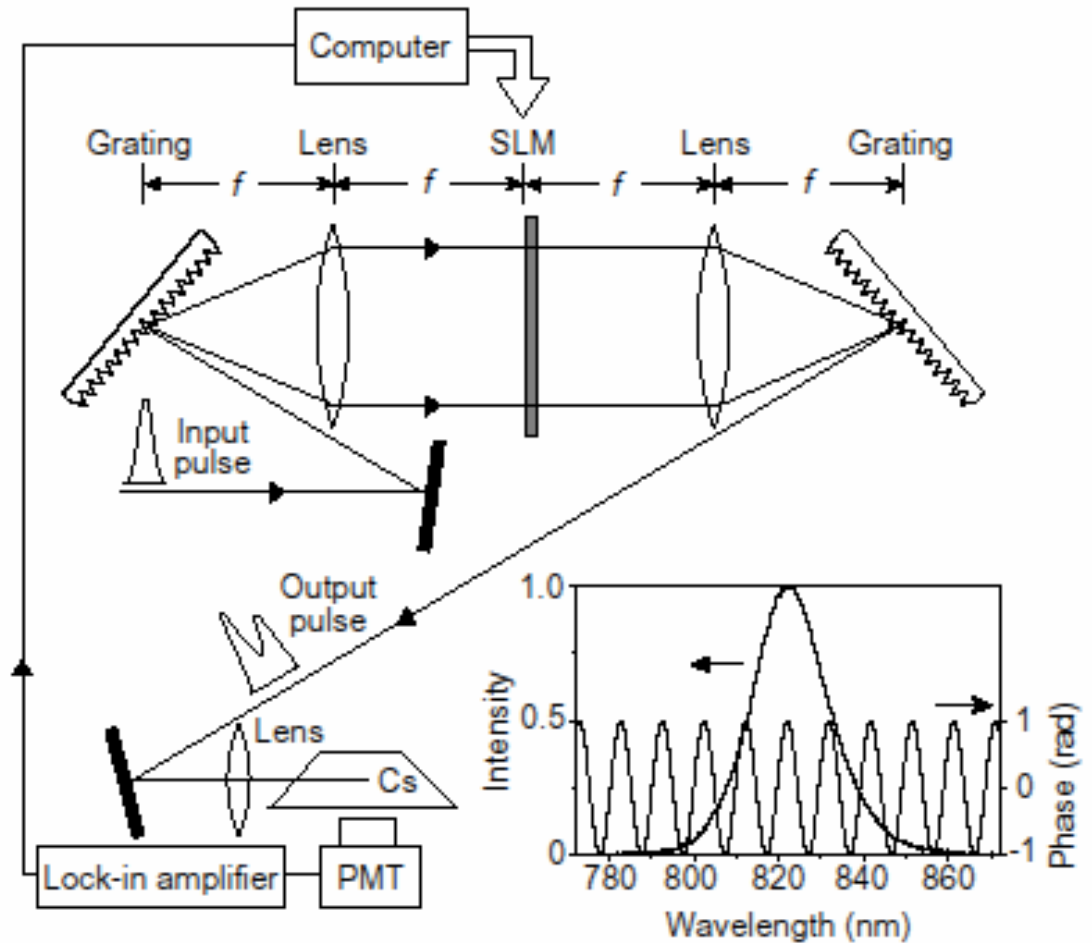
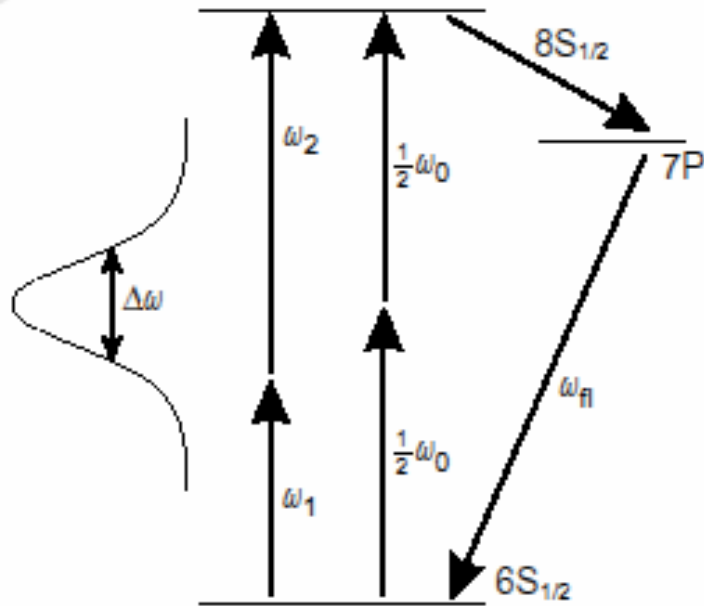


N. Dudovich, D. Oron, Y. Silberberg, Nature **418**, 512 (2002).

# Microscopie CARS à impulsions façonnées



# Contrôle cohérent de l'absorption à deux photons



D. Meshulach et Y. Silberberg, Nature **396**, 239 (1998)

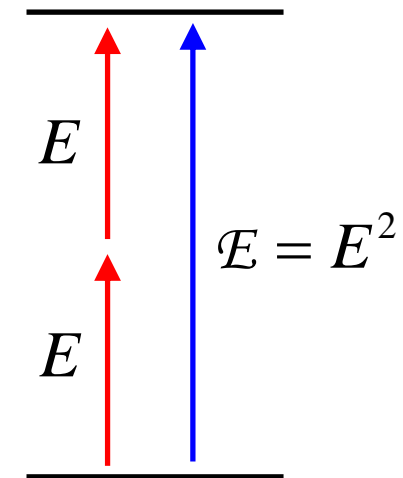
# Quantum interference effects in two-photon spectroscopy\*

M. M. Salour

*Department of Physics and Gordon McKay Laboratory, Harvard University, Cambridge, Mass. 02138*

## When there is no intermediate resonant level

It is shown, however, (Salour, 1977a) that the two-photon transition between the ground state  $|g\rangle$  and the excited state  $|e\rangle$  induced by two counterpropagating waves of amplitude  $E$  and frequency  $\omega$ , for a separation of  $\omega_0 = 2\omega$ , is equivalent to the problem of a two-level system  $|1\rangle$  and  $|2\rangle$ , separated by  $\omega_0$  (where  $\omega_0$  is the Bohr frequency of the atomic transition), with an “effective” dipole moment  $\mathfrak{D}$ , excited by a field  $\mathcal{E}e^{-i(2\omega t - \theta)}$ , where  $\mathcal{E}$  is proportional to  $E^2$ ,  $\theta$  is a phase depending on the mirror reflecting the incident wave, and  $\mathfrak{D}$  is proportional to  $\sum_r \langle e | \mathbf{D} | r \rangle \langle r | \mathbf{D} | g \rangle / (E_g + \omega - E_r)$ , where  $\mathbf{D}$  is the atomic dipole moment. (To simplify calculations, we take  $\hbar = 1$  throughout this paper.)



# Spectre d'absorption à deux photons

$$\text{Champ doublé : } E_{SHG}^{(2)}(t) \equiv E(t)^2 = \int E_{SHG}^{(2)}(\omega) \exp(-i\omega t) \frac{d\omega}{2\pi}$$

$$E_{SHG}^{(2)}(\omega) = \int E\left(\frac{\omega}{2} + \Omega\right) E\left(\frac{\omega}{2} - \Omega\right) \frac{d\Omega}{2\pi}$$

$|E_{SHG}^{(2)}(\omega)|^2$  Spectre doublé, ou spectre à deux photons

$$\text{Signal} \propto \int g^{(2)}(\omega) |E_{SHG}^{(2)}(\omega)|^2 \frac{d\omega}{2\pi}$$

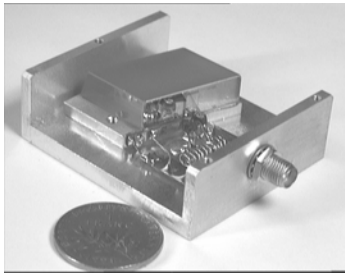
$g^{(2)}(\omega)$  Spectre d'excitation de la fluorescence à deux photons

I. Pastirk, J.M. Dela Cruz, K.A. Walowicz, V.V. Lozovoy, M. Dantus, *Opt. Expr.* **11**, 1695 (2003)

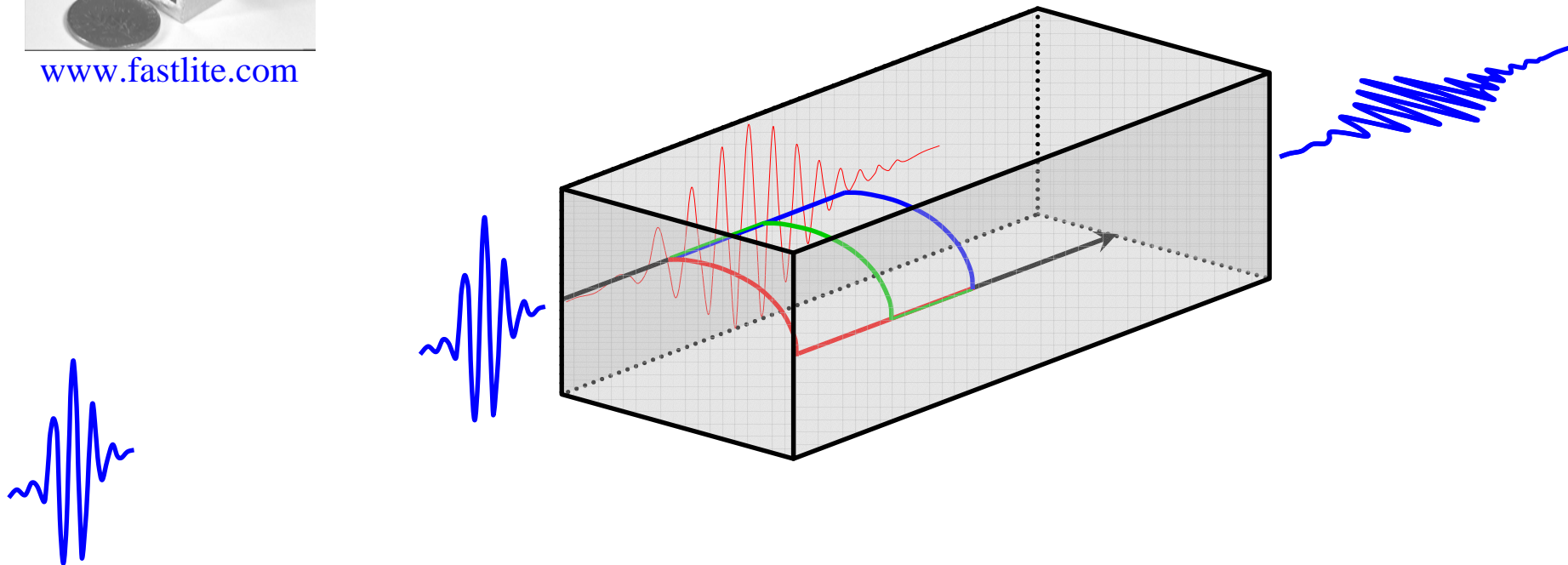
T. Brixner, N. H. Damrauer, B. Kiefer, G. Gerber, *J. Chem. Phys.* **118**, 3692 (2003)



# Utilisation du dazzler avec un oscillateur

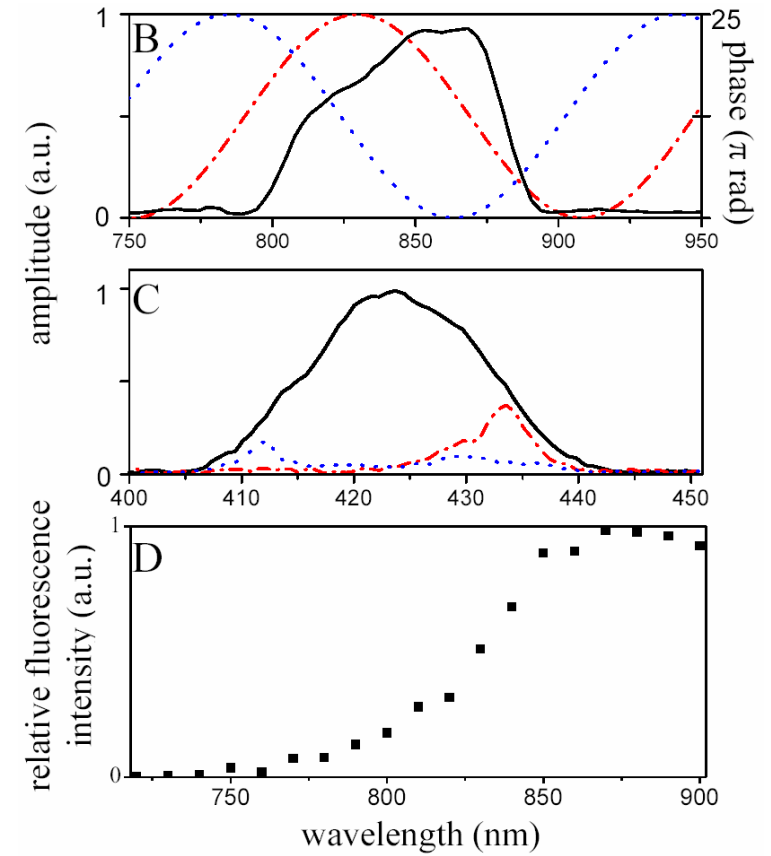
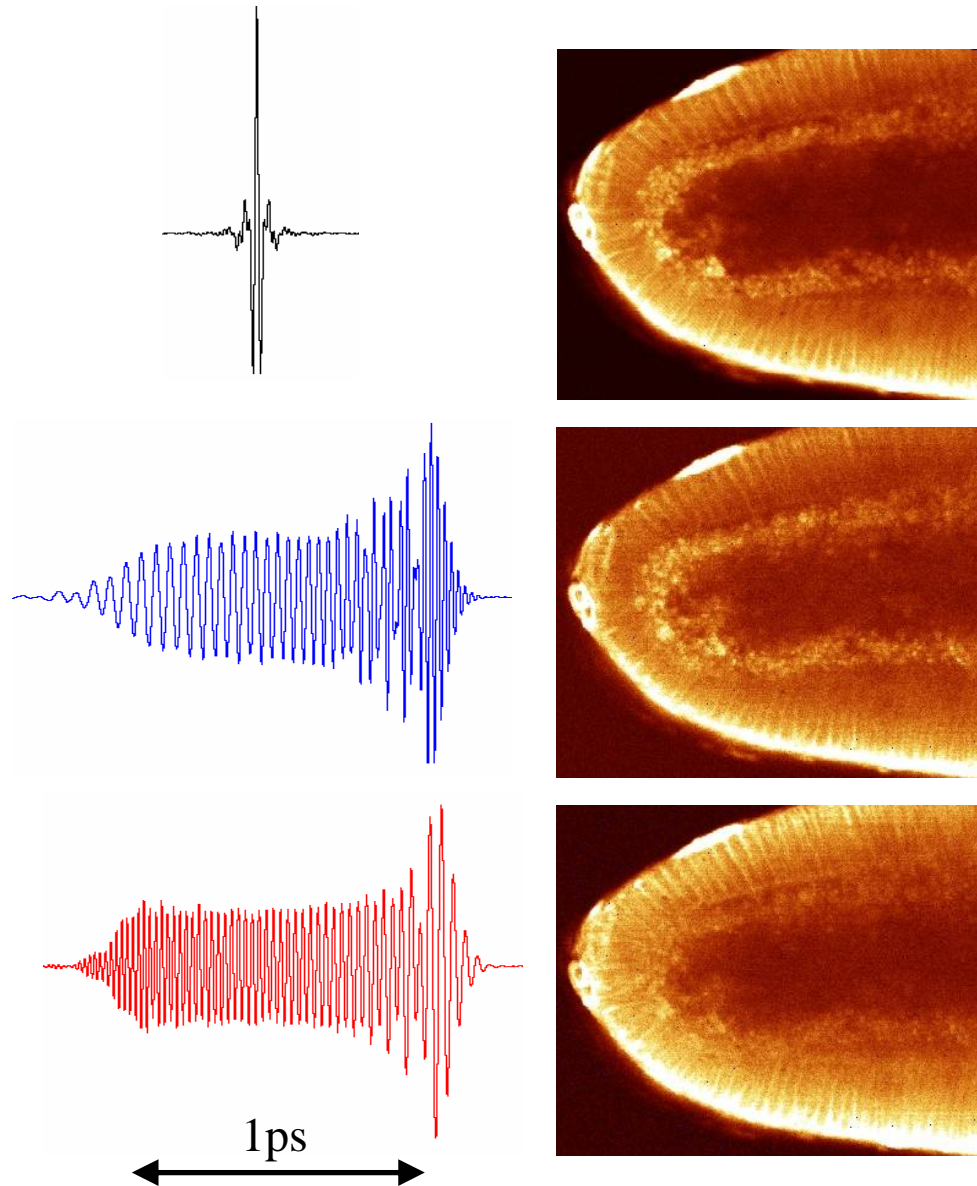


[www.fastlite.com](http://www.fastlite.com)

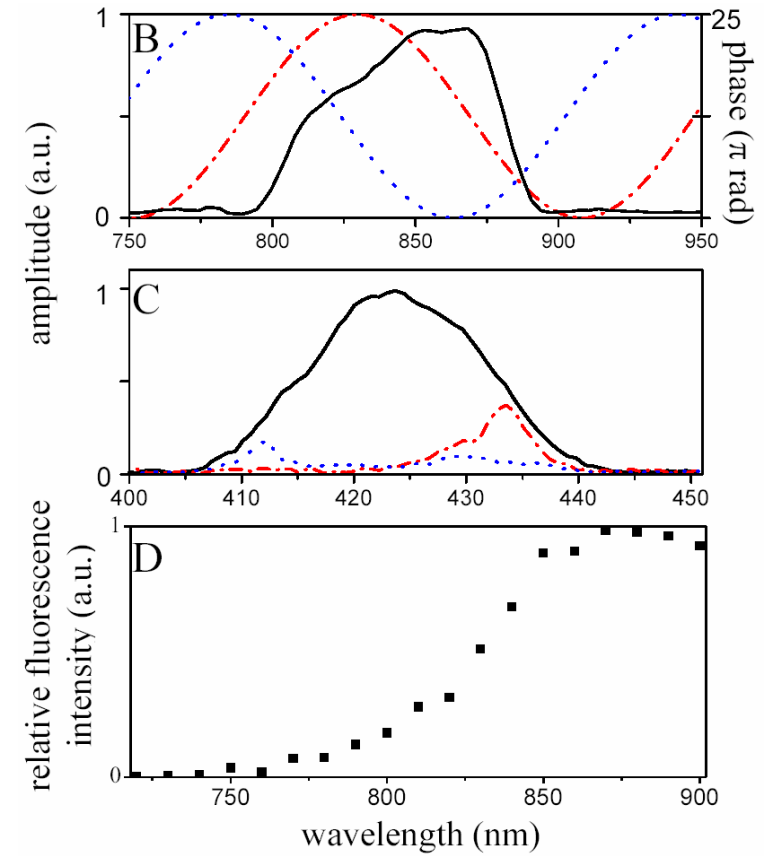
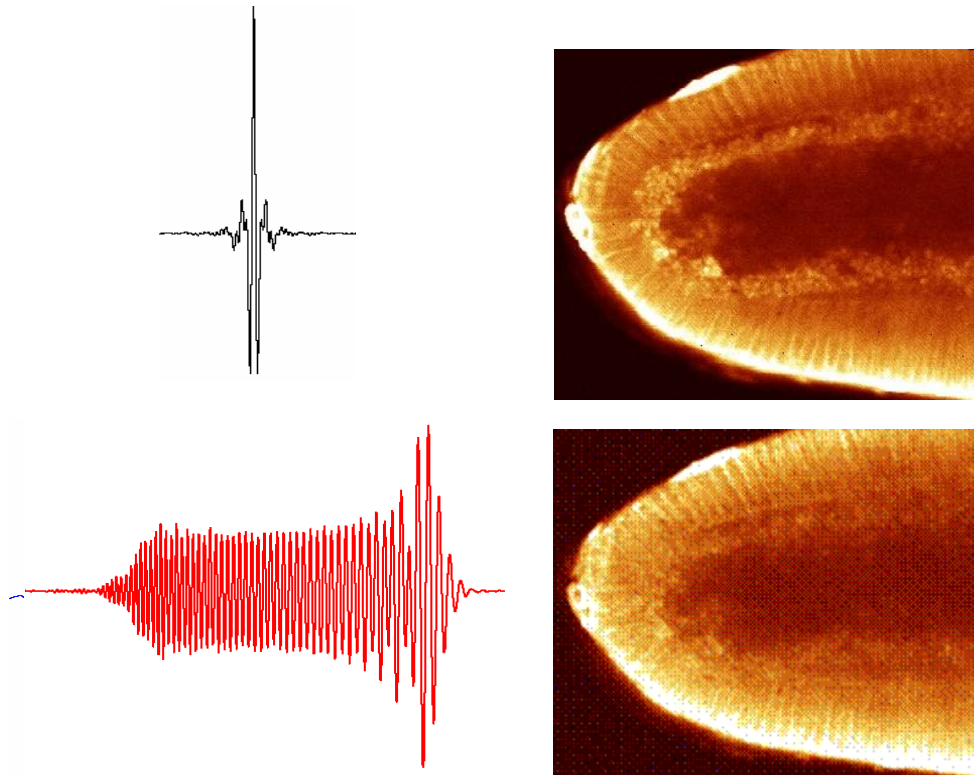


- Il faut sélectionner les impulsions correctement mises en forme.

# Images obtenues pour différentes phases spectrales



# Images obtenues pour différentes phases spectrales



Le dazzler permet de commuter entre deux impulsions à 10 kHz.

# Microscopie non-linéaire cohérente à 2 photons

