Homework

H26 journal seminar

Current topic of Nonlinear optical microscopy

6/10 Hase

①Demirhan Kobat, Nicholas G. Horton, and Chris Xu, “In vivo two-photon microscopy to 1.6-mm depth in mouse cortex”, Journal of Biomedical Optics **16**, 106014 (2011).

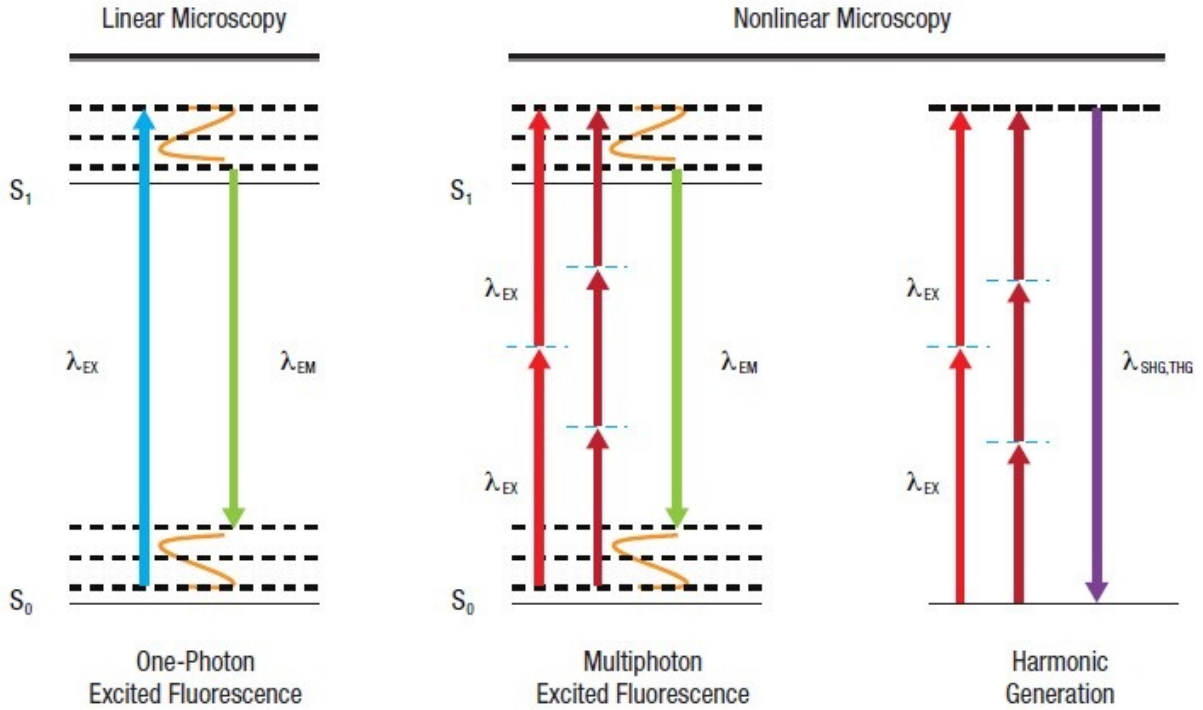
②Nicholas G. Horton, Ke Wang, Demirhan Kobat, Catharine G. Clark, Frank W. Wise, Chris B. Schaffer and Chris Xu, “In vivo three-photon microscopy of subcortical structures within an intact mouse brain”. Nature Photonics **20**, 1 (2013).

③Adrian F. Pegoraro, Aaron D. Slepkov, Andrew Ridsdale, Douglas J. Moffatt and Albert Stolow, “Hyperspectral multimodal CARS microscopy in the fingerprint region”. Journal of Biophotonics **7**, 49 (2014).

Homework

- Spatial resolution of
  - Two Photon Fluorescence
  - Three Photon Fluorescence
- Spectral focussing CARS

Multi Photon Fluorescence



http://www.thorlabs.com

- Two Photon absorption

$$\frac{dI(z)}{dz} = -\frac{3\omega}{2n_0^2 c^2 \varepsilon_0}\chi_I^{(3)}(-\omega;\omega,-\omega,\omega)I(z)^2 \qquad \alpha_2 = \frac{3\omega}{2n_0^2 c^2 \varepsilon_0}\chi_I^{(3)}(-\omega;\omega,-\omega,\omega)$$

$$\boxed{\frac{dI(z)}{dz} = -\alpha_2 I(z)^2}$$

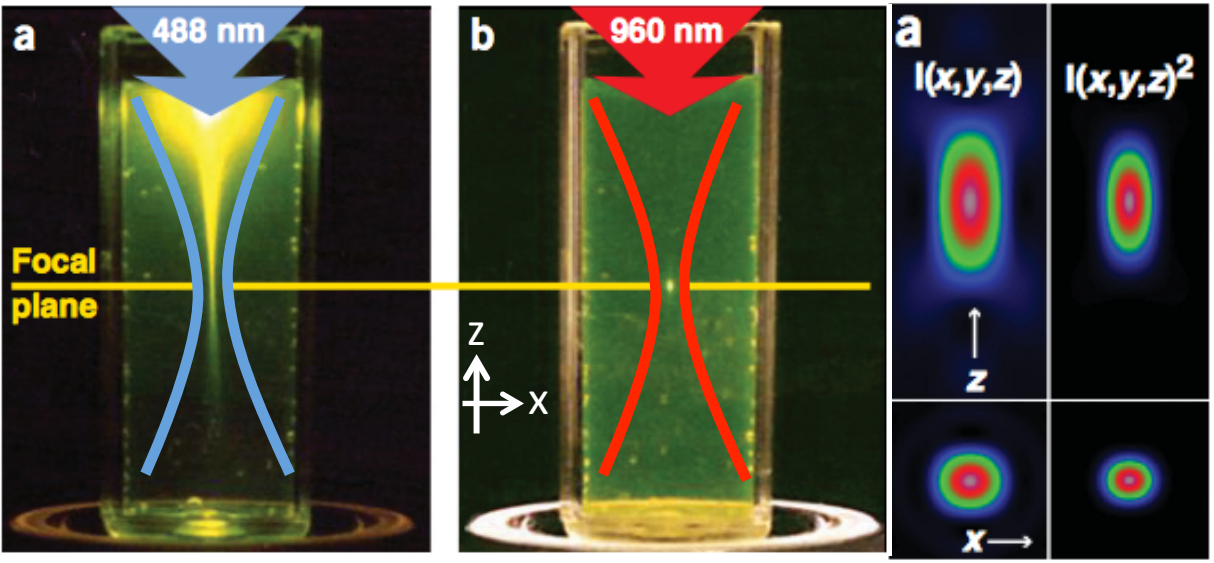
- Three Photon absorption

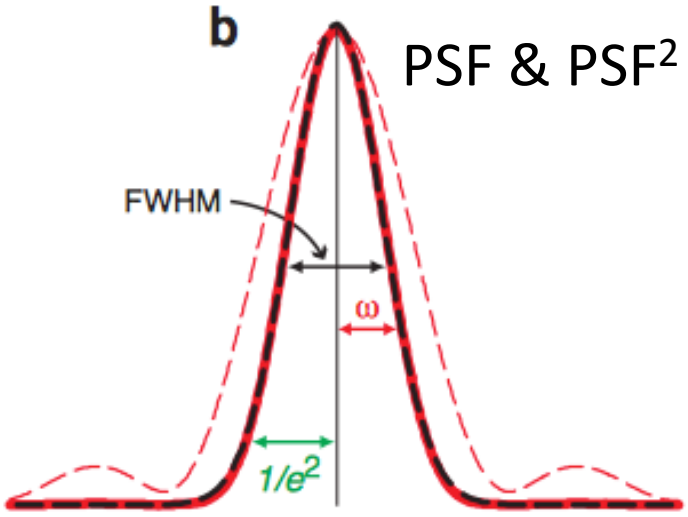
$$\boxed{\frac{dI(z)}{dz}} = -\frac{5\omega}{2n_0^3 c^3 \varepsilon_0^2}\chi_I^{(5)}(-\omega;\omega,-\omega,\omega,-\omega,\omega)I(z)^3 = \boxed{-\alpha_3 I(z)^3} \qquad \alpha_3 = \frac{5\omega}{2n_0^3 c^3 \varepsilon_0^2}\chi_I^{(5)}(-\omega;\omega,-\omega,\omega,-\omega,\omega)$$

Spatial resolution

One Photon

Two Photon





$PSF_{one\text{-}photon} = PSF(v, u)$

$$PSF_{confocal} = PSF_{illumination} \times PSF_{detection} \approx PSF^2(v,u)$$

$$PSF_{two\text{-}photon} = (PSF_{illumination})^2 \approx PSF^2(v/2, u/2)$$

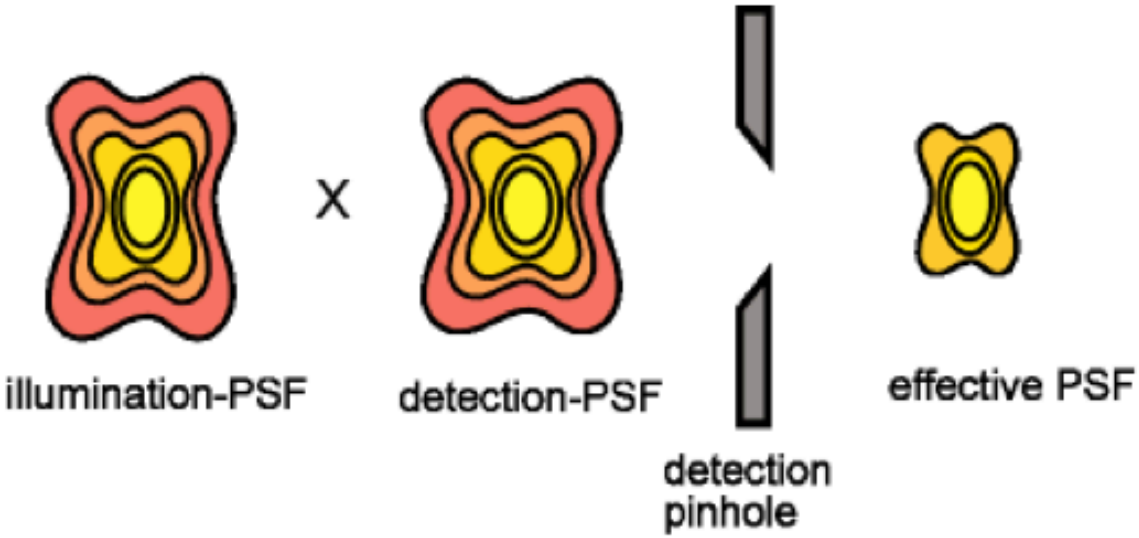
$PSF_{Three\text{-}photon} = (PSF_{illumination})^3 = PSF^3(v/3, u/3)$

$v = k\,(NA)\,r$

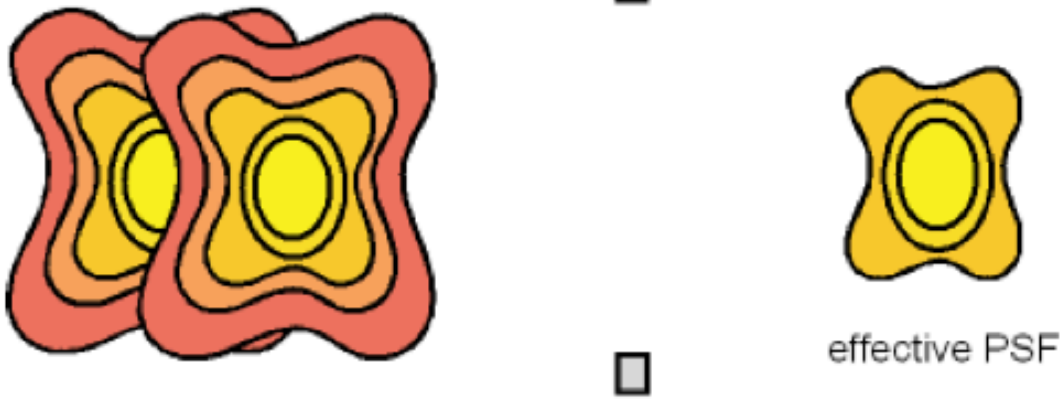
$u = k\,(NA)^2\,z$

$k = 2\pi/\lambda$

radial and axial normalized coordinates



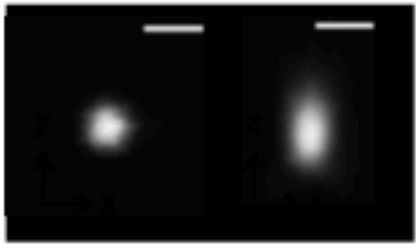
confocal microscopy



2P microscopy

Resolution in multiphoton microscopy

1PE 543 nm

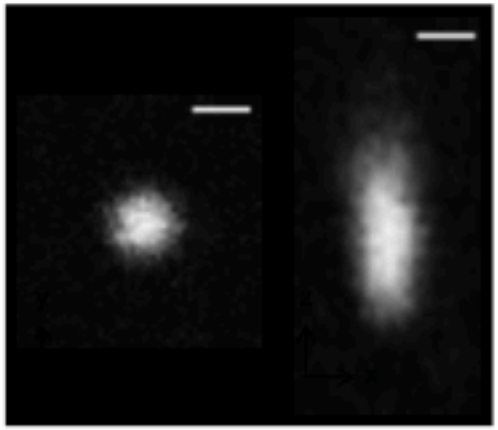


$FWHM_{xy}$= 0.25 µm

$FWHM_z$ = 0.50 µm

scale bar = 0.50 µm

2PE 900 nm



$FWHM_{xy}$= 0.40 µm

$FWHM_z$ = 1.1 µm

scale bar = 0.50 µm

Orange bead (Ø 0.18 µm) imaged with 60x/1.4 oil objective

11.1. Comparison of resolution between two-photon and three-photon scence microscopy under the condition of the equal fluorescence wavelength, v are normalized by the fluorescence wavelength. $v_{1/2}$ and $u_{1/2}$ are the transverse half widths at half maximum of the 3-D IPSF. $\Delta u_{1/2}$ is the half width at half mum for an axial response to a thin layer, γ the gradient of the image intensity at face of a thick layer, γ the gradient of the image intensity at the edge of a thin and γ'' the gradient of the image intensity at the edge of a thick edge

		Conventional (1-photon)	Confocal (1-photon)	Conventional (2-photon)	Confocal (2-photon)	Conventional (3-photon)	Confocal (3-photon)
idth of SF	$v_{1/2}$	1.62	1.17	2.34	1.34	2.86	1.41
	$u_{1/2}$	5.56	4.01	8.02	4.62	9.87	4.89
	$\Delta u_{1/2}$	∞	4.3	8.6	5.1	9.98	5.67
tion	γ	0	0.09	0.045	0.093	0.047	0.082
erse	γ'	0.27	0.417	0.208	0.361	0.171	0.349
tion	γ''	0	0.333	0.167	0.340	0.166	0.305

ble 11.2. Comparison of resolution between two-photon and three-photon orescence microscopy under the condition of the equal illumination wavelength, v d u are normalized by the incident wavelength. The others conditions are the same hose in Table 11.1

		Conventional (1-photon)	Confocal (1-photon)	Conventional (2-photon)	Confocal (2-photon)	Conventional (3-photon)	Confocal (3-photon)
l-width	$v_{1/2}$	0.81	0.58	1.17	0.67	0.953	0.47
3-D IPSF	$u_{1/2}$	2.78	2.0	4.01	2.31	3.29	1.63
al olution	$\Delta u_{1/2}$	∞	2.15	4.3	2.50	3.33	1.89
	γ	0	0.18	0.09	0.186	0.141	0.246
nsverse olution	γ'	0.54	0.84	0.416	0.722	0.513	1.047
	γ''	0	0.67	0.334	0.68	0.498	0.915

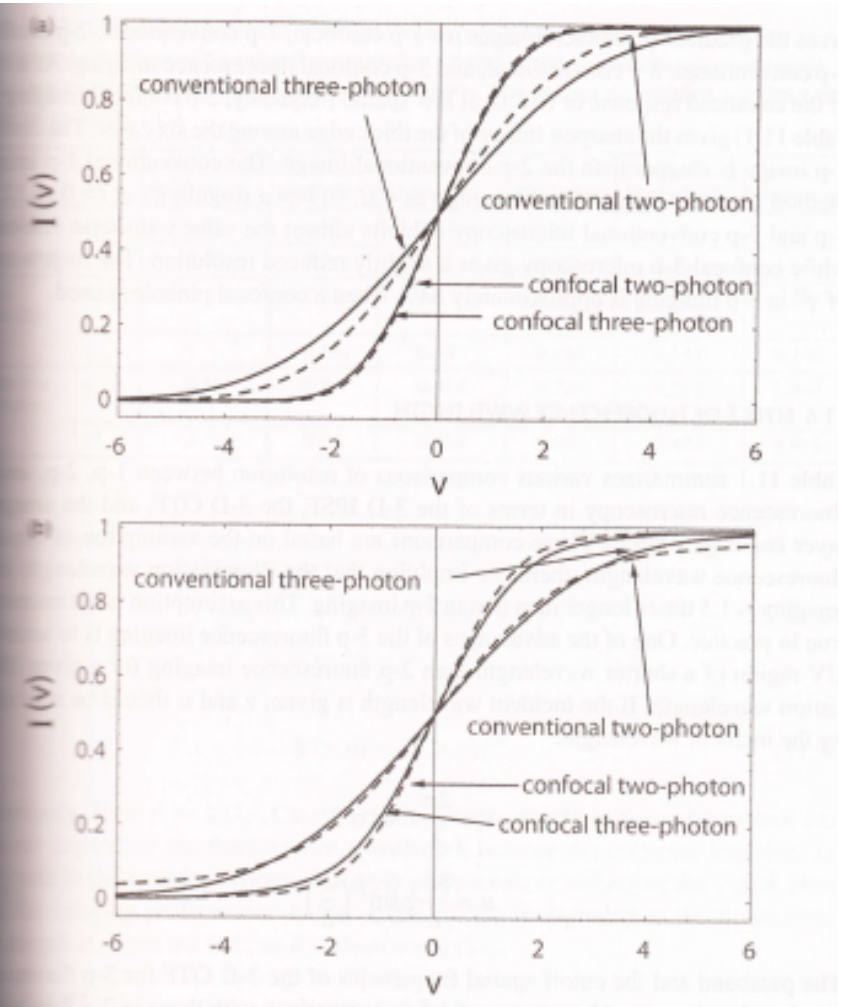
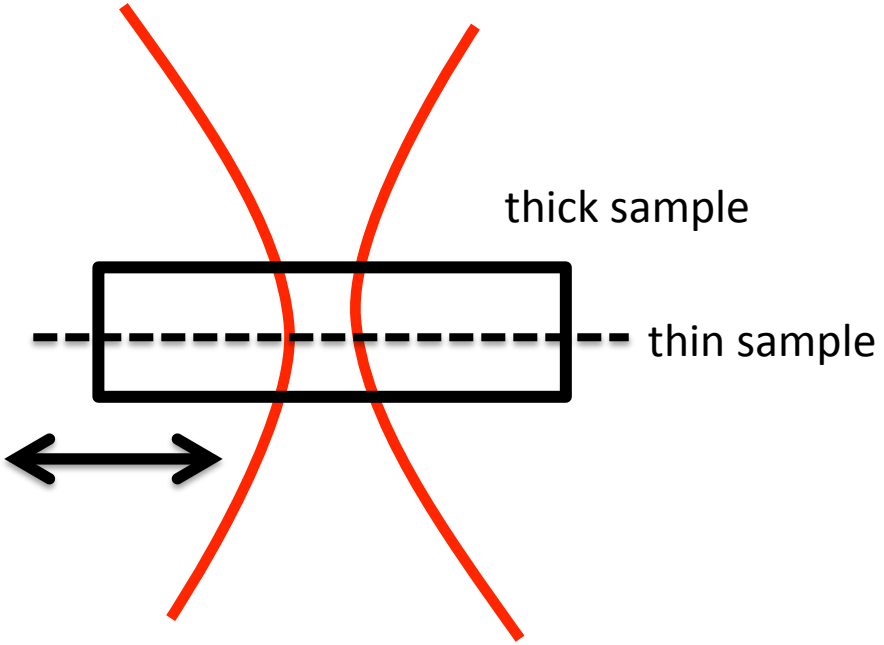


Image intensity of thin (a) and thick (b) fluorescent edges. The other ... are the same as those in Figure 11.1.



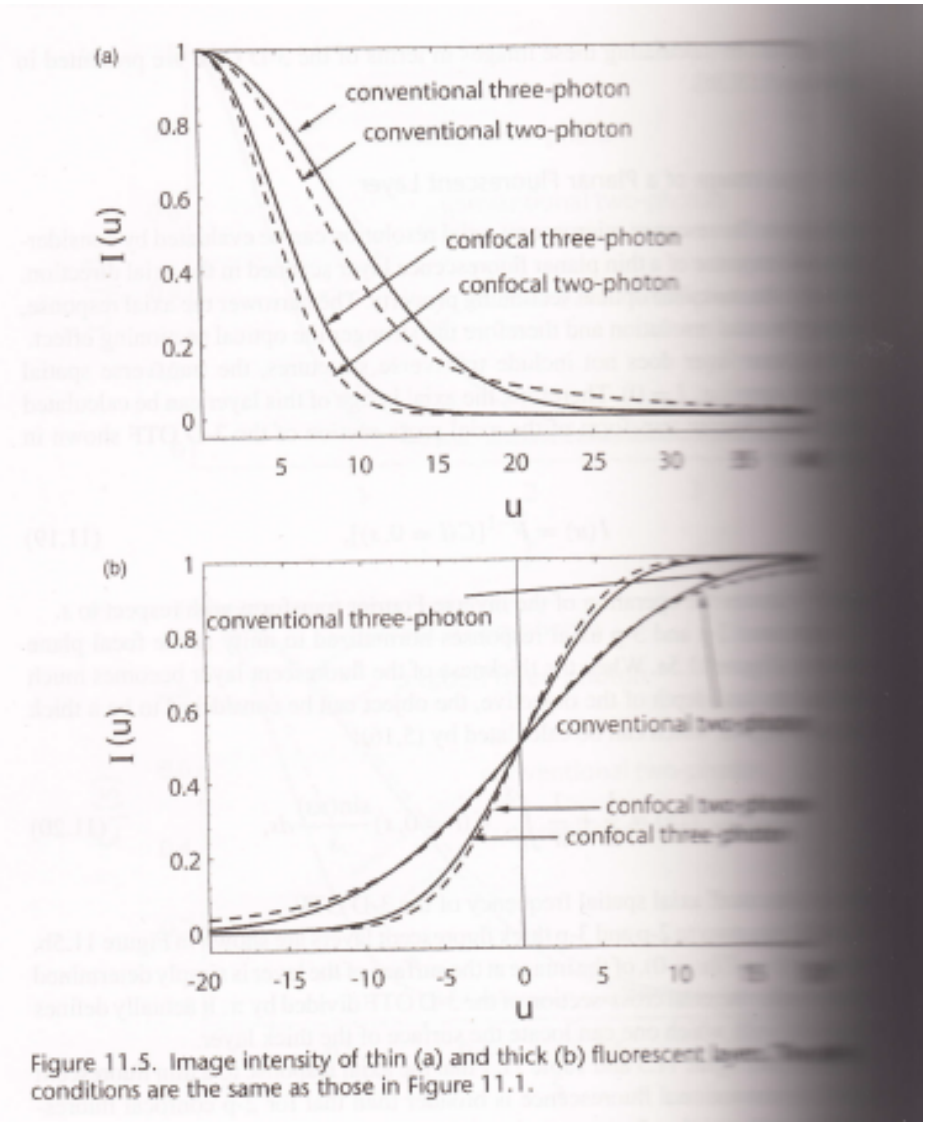
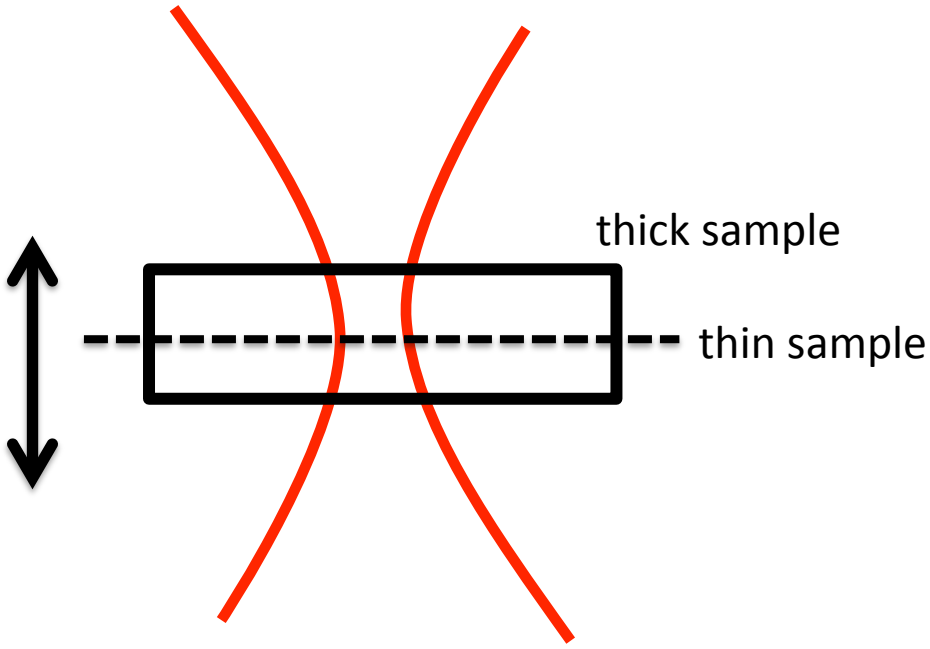
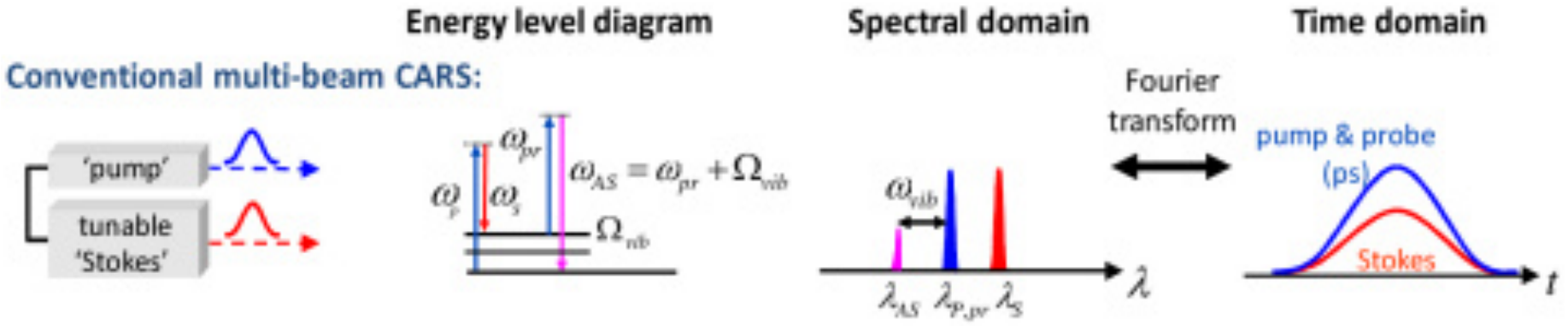


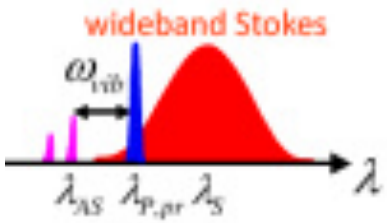
Figure 11.5. Image intensity of thin (a) and thick (b) fluorescent layer. The
conditions are the same as those in Figure 11.1.

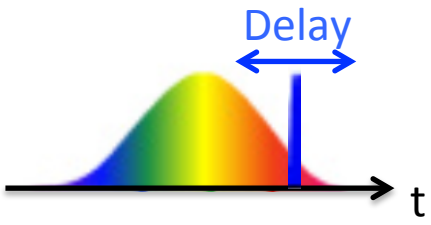


Spectral focussing CARS



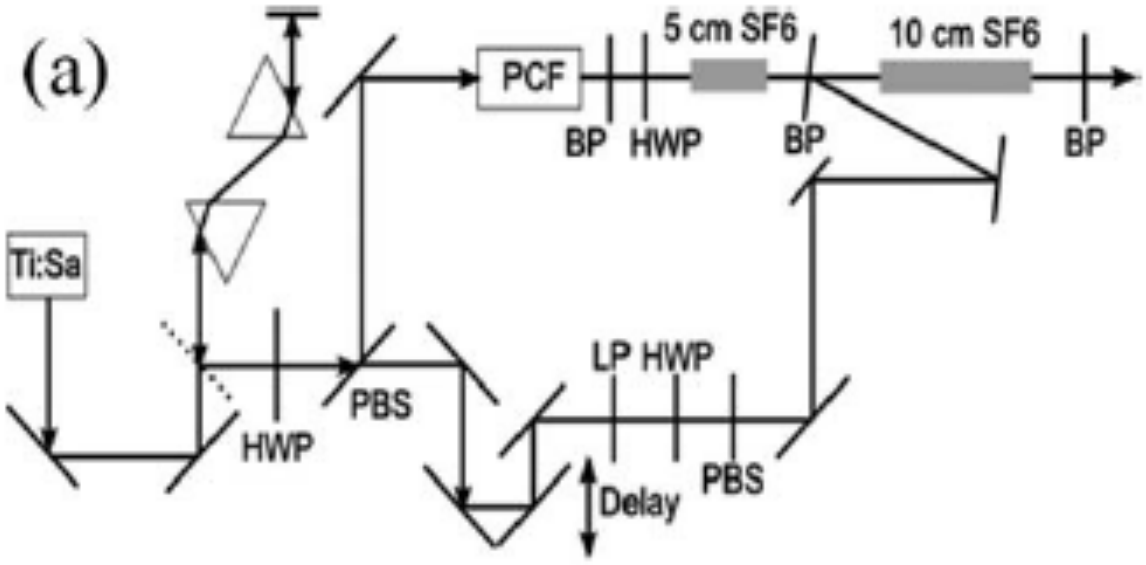
This paper





chairp pulse

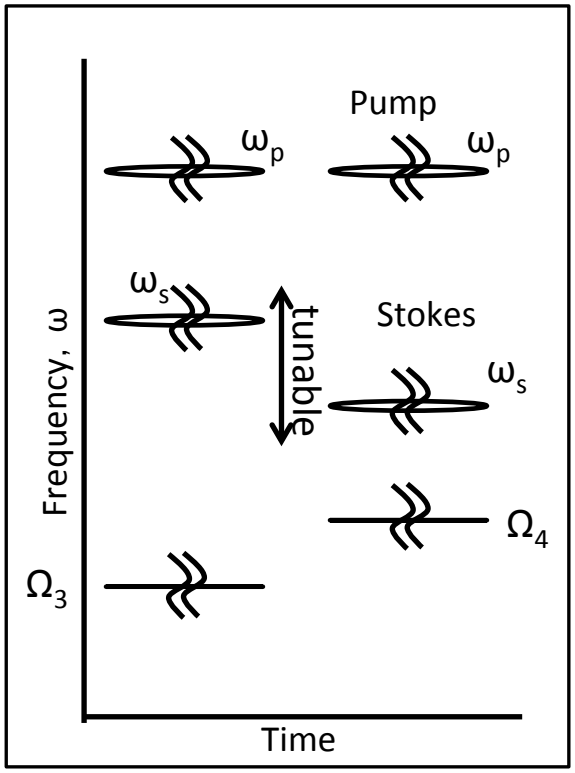
60 fs
80 MHz



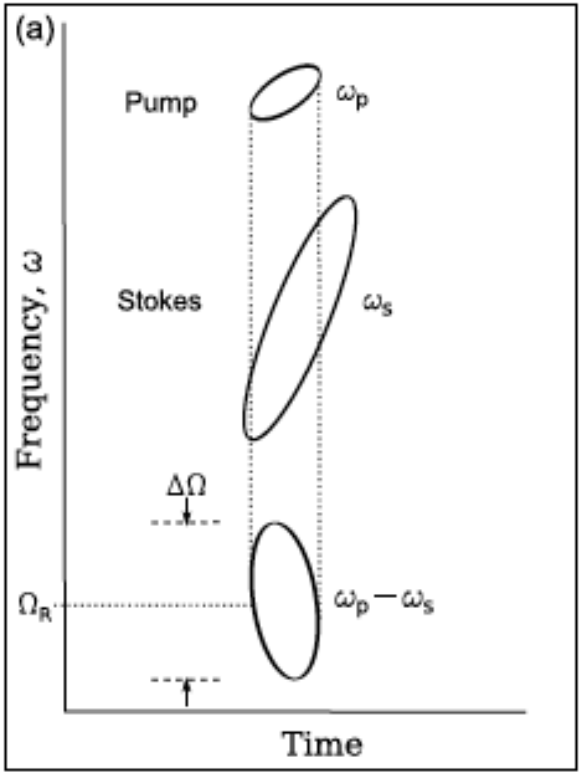
Spectral focussing CARS

Raman active vibrational mode: $\omega_p - \omega_s = \Omega$

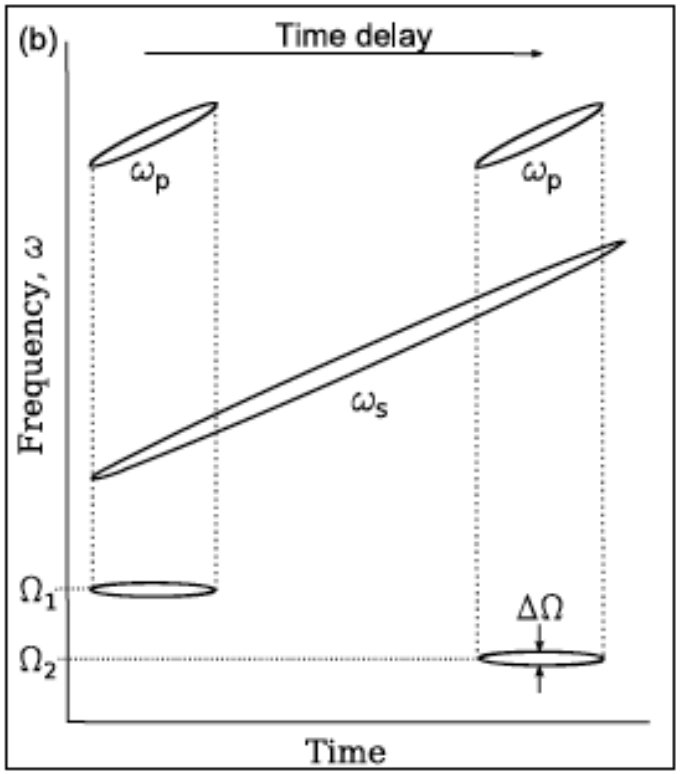
Conventional ps CARS



unmatched chirps fs CARS



Chirp matched fs CARS



The spectral resolution $\Delta\Omega$ is determined by the total height of the ellipse $\omega_p - \omega_s$.