The Abdus Salam
International Centre
for Theoretical Physics

Thermal Lens Microscopy Combined with Intracavity Spectroscopy

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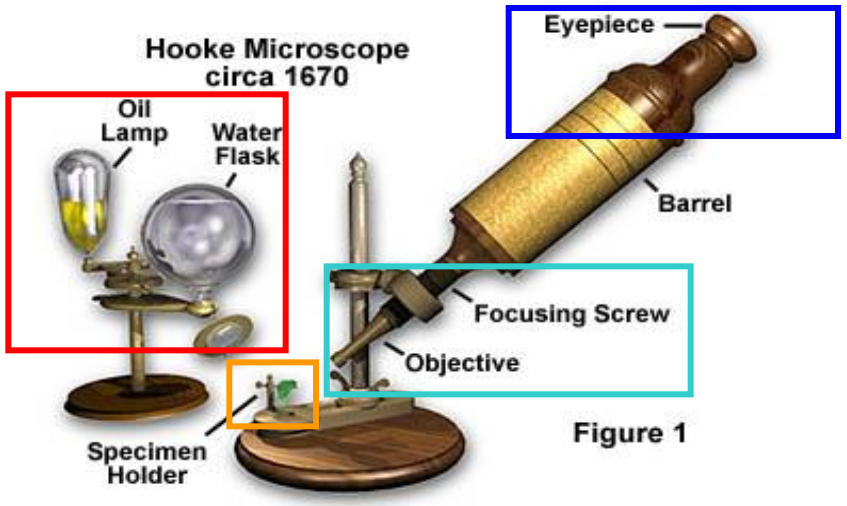
Preparatory School to the Winter Collegue on Optics: Optical Frequency Combs

- Introduction
- Basic components of the microscope and their functions
- Thermal lens microscopy
- Thermal lens microscopy combined with intracavity spectroscopy
- Typical examples and applications

Microscope Components

- Ocular
- Objective
- Condenser
- Numerical Aperture
- Optical Filters



Basic components and their functions

(1) **Eyepiece (ocular lens)**
(2) Revolving nose piece (to hold multiple objective lenses)
(3) **Objective lenses**
(4) And (5) **Focus knobs**
    (4) Coarse adjustment
    (5) Fine adjustment
(6) **Stage** (to hold the specimen)
(7) **Light source** (lamp)
(8) **Condenser lens** and diaphragm
(9) Mechanical stage (move the specimen on two horizontal axes for positioning the specimen)

The Concept of Magnification

Magnification of the Microscope

- M Microscope = M Objective X M Eyepiece X M Intermediate Factor

M = Magnification

- Example: Objective = 60 x

Eyepiece = 10 x

Intermediate Factor = 1 x

Overall M = 600 x

The characteristics of objectives

Figure 1

Objectives configurations

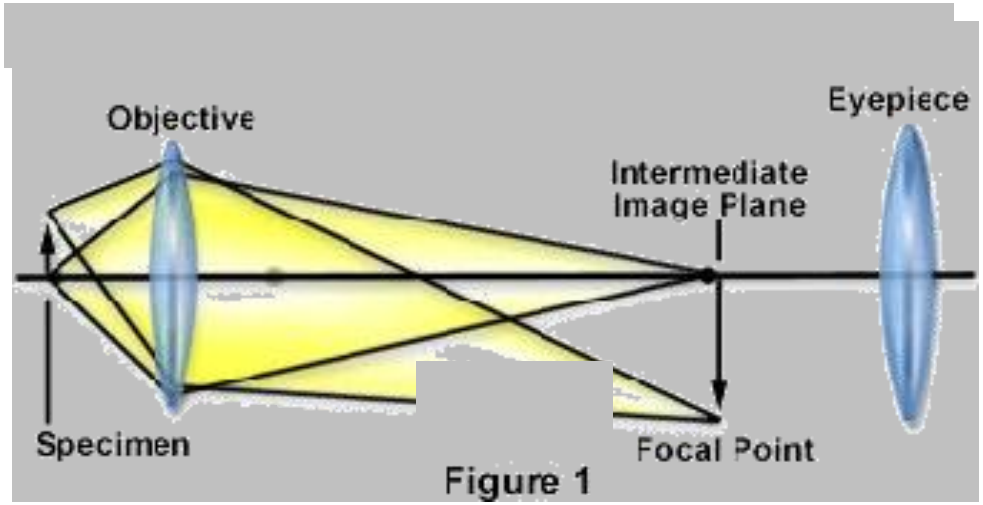


Figure 1

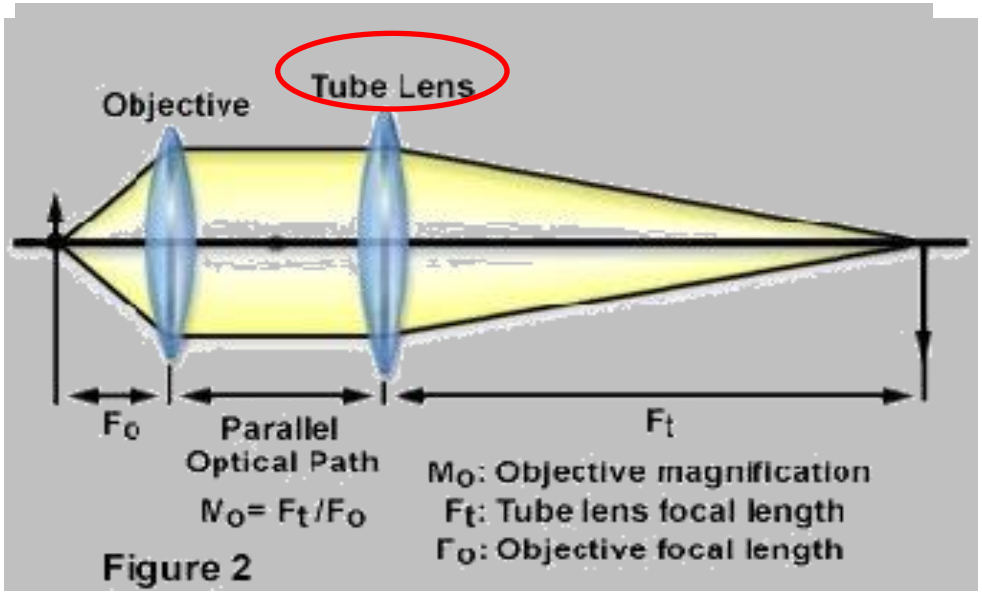


Figure 2

The characteristics of objectives

Figure 1

Numerical Aperture (N.A.)

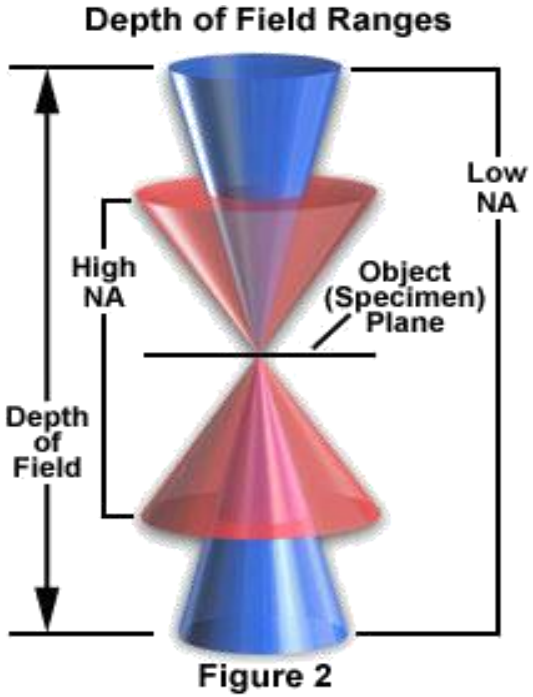


Figure 2

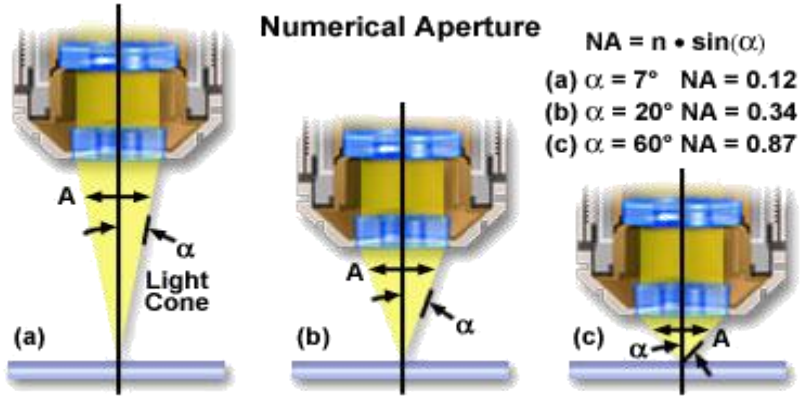


Figure 1

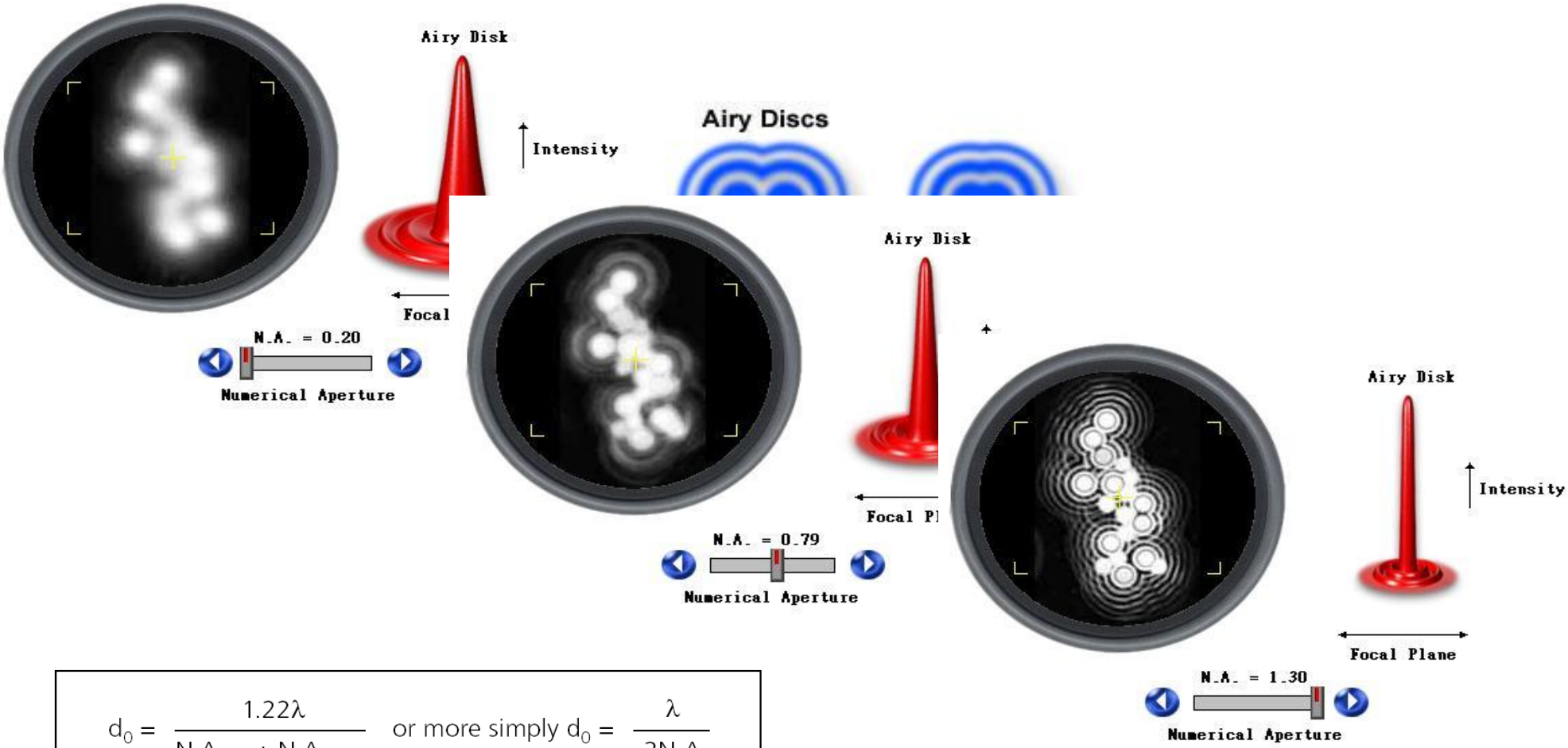
Numerical Aperture = N.A. = n · sin α

α is half the opening angle of the objective.

n is the refractive index of the immersion medium used between the objective and the object.

(n = 1 for air; n = 1.51 for oil or glass)

Resolution



$$d_0 = \frac{1.22\lambda}{N.A_{obj.} + N.A._{Cond}} \quad \text{or more simply } d_0 = \frac{\lambda}{2N.A}$$

λ = wavelength of light, e.g. 550 nm (green)

- **Resolving power, the limit up to which two small objects are still seen separately.**

Factors Affecting Resolution

- **Resolution (d_{min}) improves (smaller d_{min}) if $\lambda\downarrow$ or $n\uparrow$ or $\alpha\uparrow$**
- **Assuming that $\sin\alpha = 0.95$ ($\alpha = 71.8°$)**

Wavelength		Air (n= 1)	Oil (n = 1.515)
Red	650 nm	0.42 μm	0.28 μm
Yellow	600 nm	0.39 μm	0.25 μm
Green	550 nm	0.35 μm	0.23 μm
Blue	475 nm	0.31 μm	0.20 μm
Violet	400 nm	0.27 μm	0.17 μm
		Resolution $_{air}$	**Resolution $_{oil}$**

- **(The eye is more sensitive to blue than violet)**

Two sets of conjugate planes in the light microscope

Understanding the reciprocal relationship between the two sets of conjugate planes is crucial for properly understanding:

- Image formation
- Image resolution
- How phase-contrast and DIC work

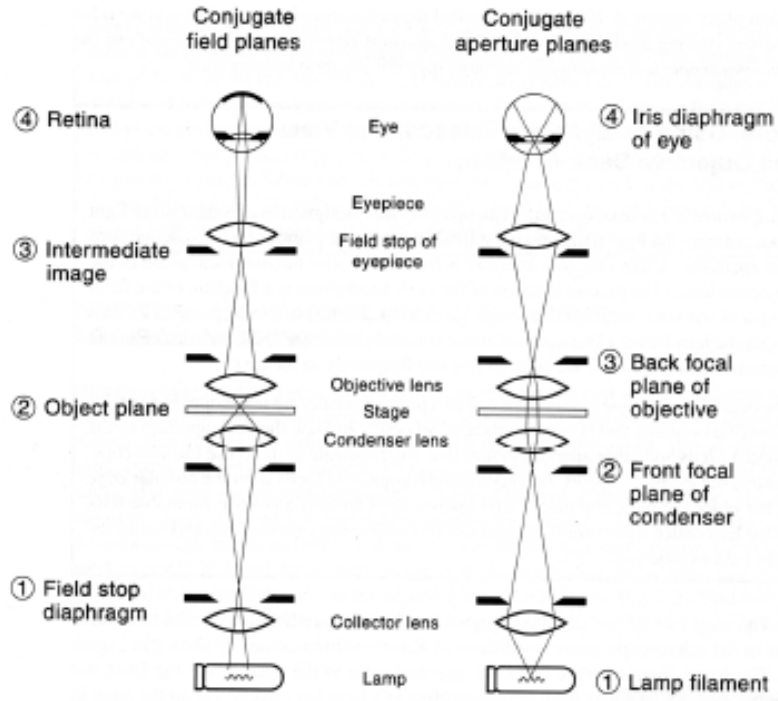


Figure 1-4

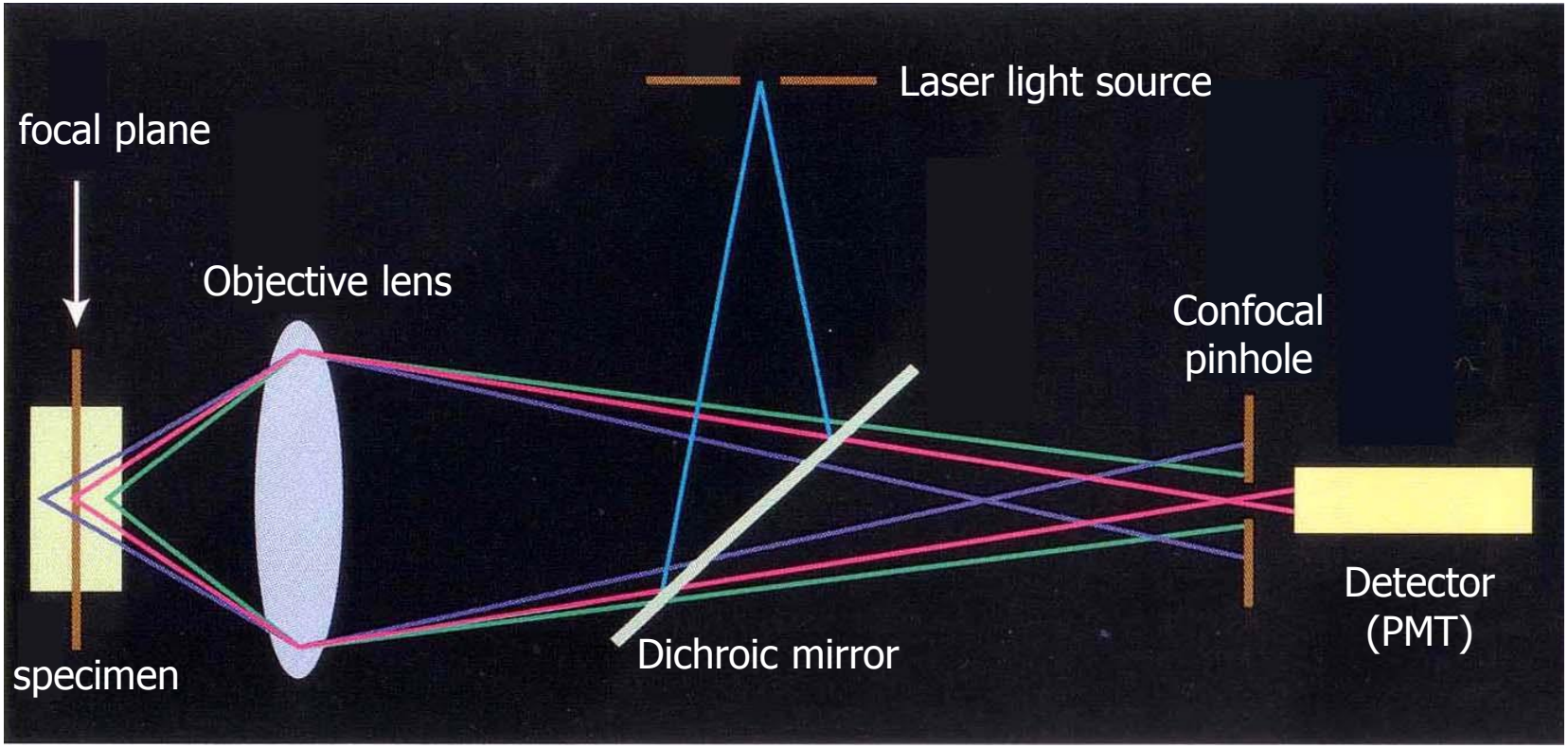
The locations of conjugate focal planes in a light microscope adjusted for Koehler illumination. Note the locations of four conjugate field planes (left) and four conjugate aperture planes (right) indicated by the crossover points of rays in the diagrams. The left-hand diagram shows that the specimen or object plane is conjugate with the real intermediate image plane in the eyepiece, the retina of the eye, and the field stop diaphragm between the lamp and the condenser. The right-hand drawing shows that the lamp filament is conjugate with aperture planes at the front focal plane of the condenser, the back focal plane of the objective, and the pupil of the eye.

Conjugate planes are "parfocal" with each other

When something is in focus in one set of conjugate planes, it is "maximally out-of-focus" in the other set of planes

These two sets are often called "reciprocal" or "transform" planes (with respect to each other)

Laser Scanning Microscope (Confocal System)



Light emitted from the focal plane

Light emitted from the out-of-focus region

Confocal Aperture

Decreasing the pinhole size rejects more out of focus light, therefore improving contrast and effective z resolution.

Decreasing the pinhole will increase x,y resolution (1.3x wide field)

Decreasing pinhole size decreases the amount of the Airy disk that reaches the detector. This results in less light from each point being collected

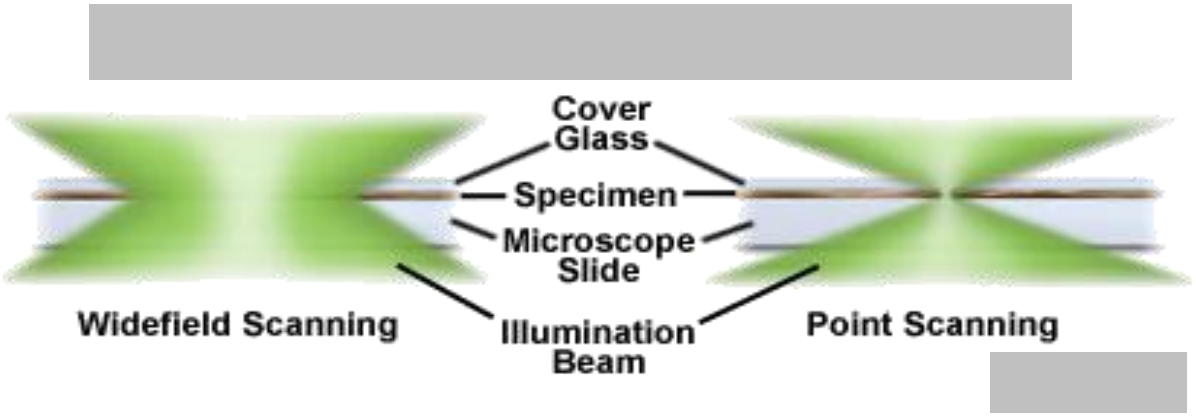
Generally, collecting the diameter of 1 Airy disk is considered optimal. This collects about 85% of light from a sub-resolution point.

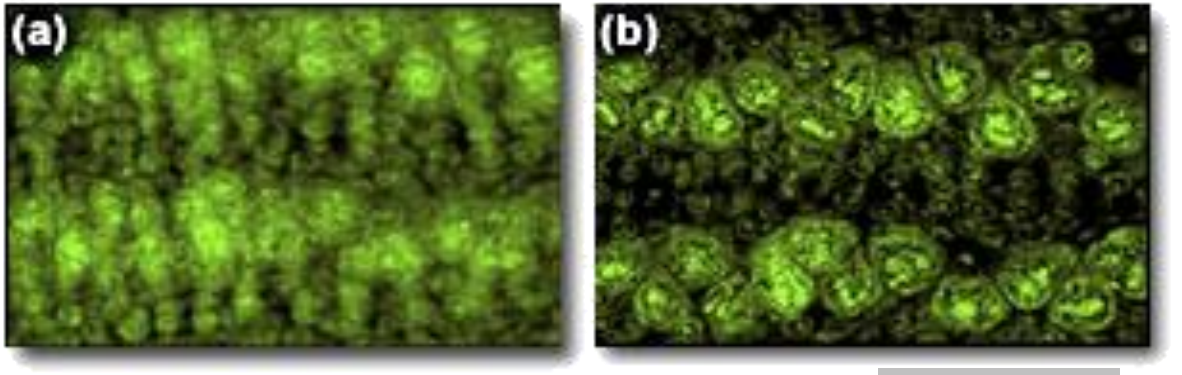
Limits:

Open pinhole: nearly wide field resolution (still some confocality)

Closed: no image

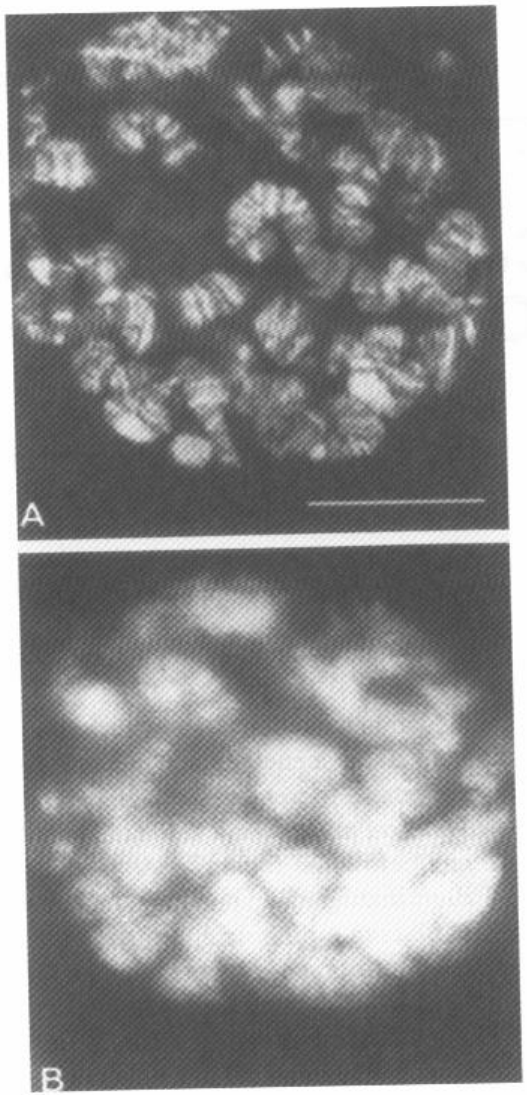
Wide field versus confocal scanning





Wide Field **Confocal**

WF vs C - Fluorescence Imaging



Confocal

Greatly reduces
Out of focus blur

Wide-field

Brighter but
No sectioning

More examples

widefield

confocal

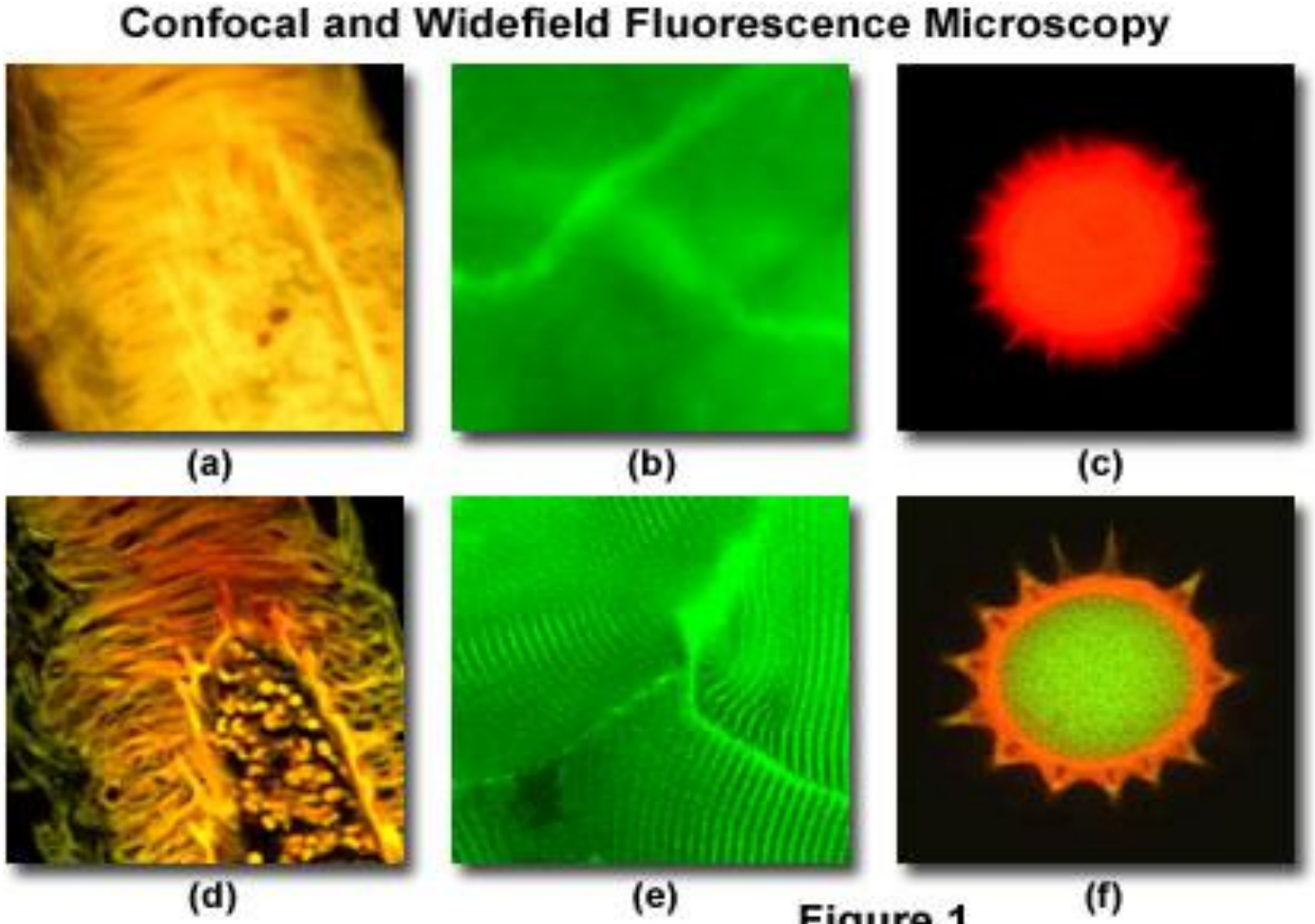
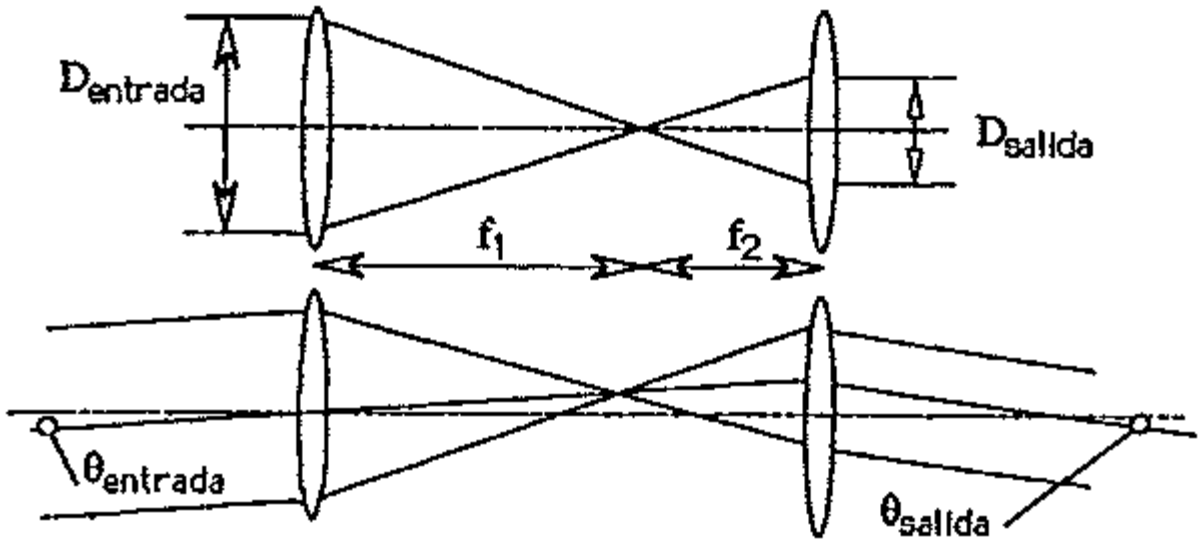


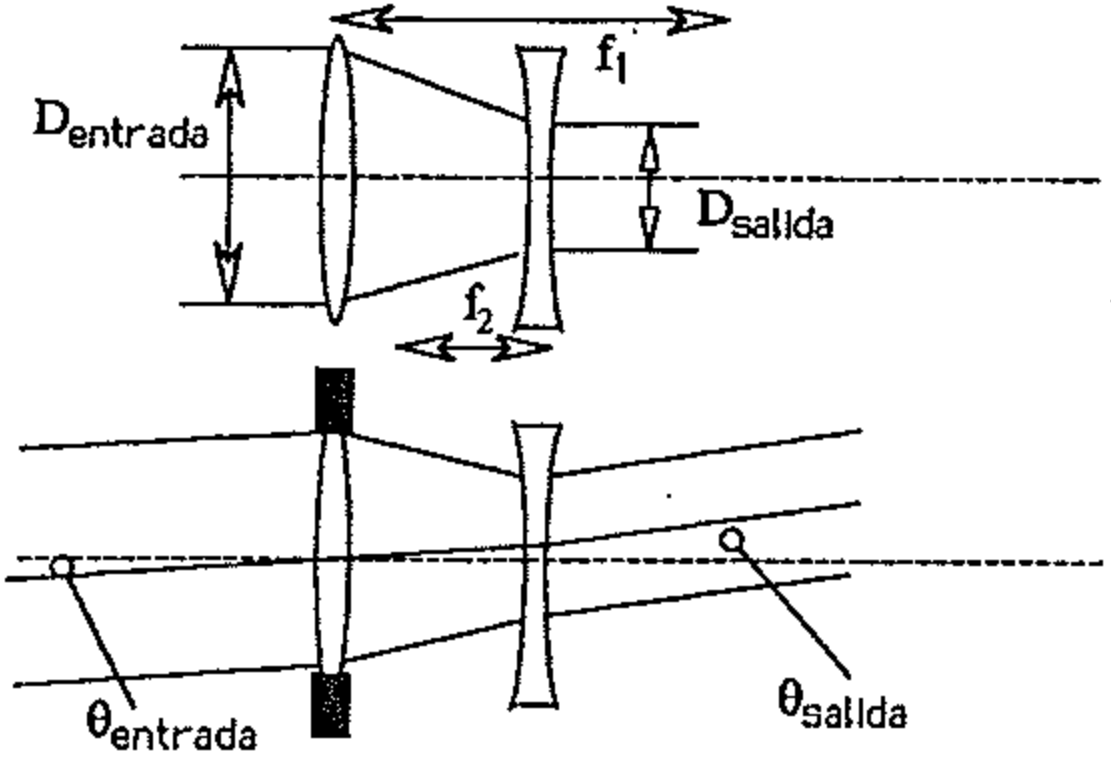
Figure 1

medulla muscle pollen

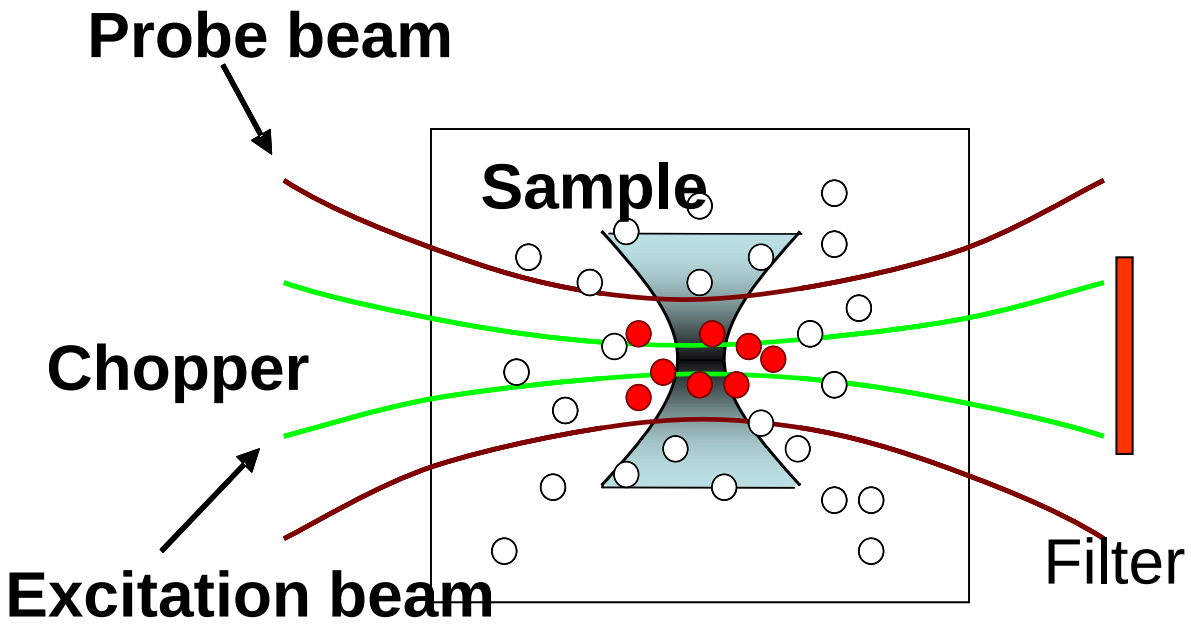
Kepler Telescope

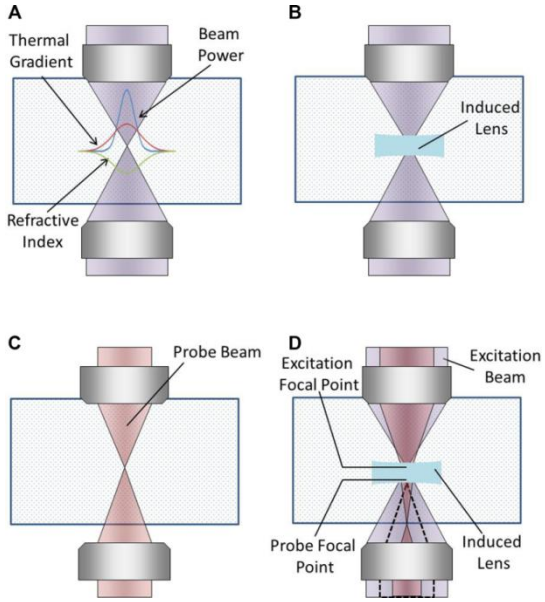


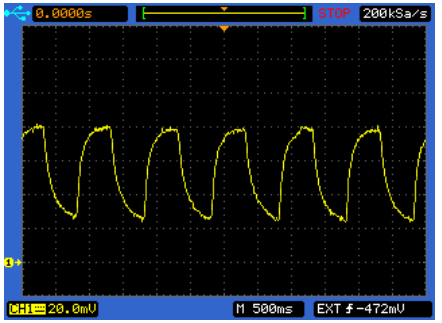
Galileo Telescope



Thermal lens effect and signal



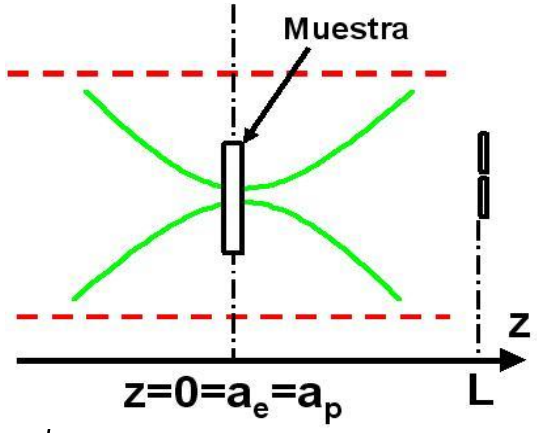




$$I(r) = \frac{2P_e}{\pi w_e^2} e^{-2r^2/w_e^2}$$

Physical mathematical model

$$z_p >> L >> z_e \quad ; \quad t \to \infty \quad ; \quad z_p \to \infty \quad ; \quad z = 0$$



$$S(z,t) = \Phi_0 \arctan\left\{ \frac{4m(z)\upsilon(z)t / t_c(z)}{\upsilon^2(z) + [1+2m(z)]^2 + \left[1+2m(z)+\upsilon^2(z)\right]2t / t_c(z)} \right\}$$

$$I(r) = \frac{2P_e}{\pi w_e^{\,2}} e^{-2r^2/w_e^2}$$

$$D = \kappa / \rho C_p$$

$$t_c(z) = \omega_e^{\,2}(z) / 4D$$

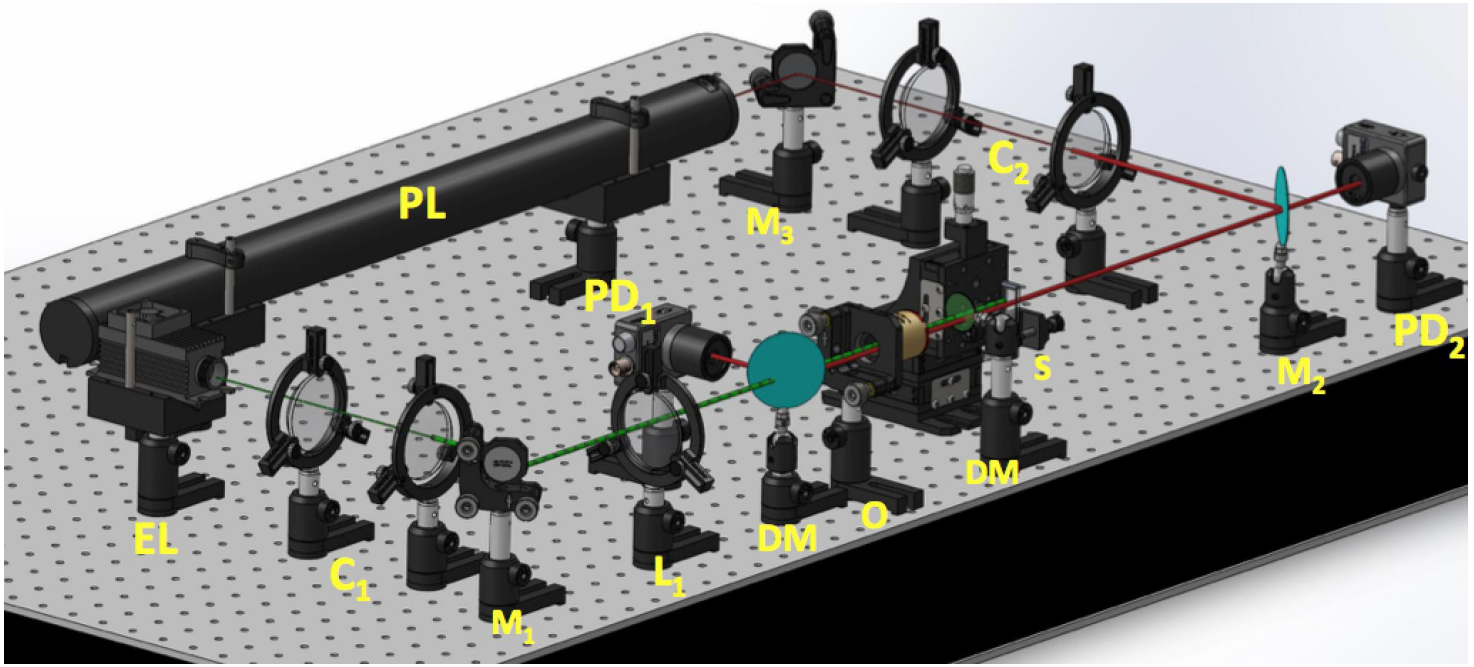
$$S_{max} = \pi \Phi_o / 2$$

$$\Phi_0 = \frac{P_e \alpha l}{\lambda_p k} \frac{dn}{dT}$$

H Cabrera, J. Opt. Soc. Am. B, 23, 1408 (2006).

H. Cabrera, Appl. Phys. Lett. **94** 051103, (2009).

Thermal lens microscopy set up



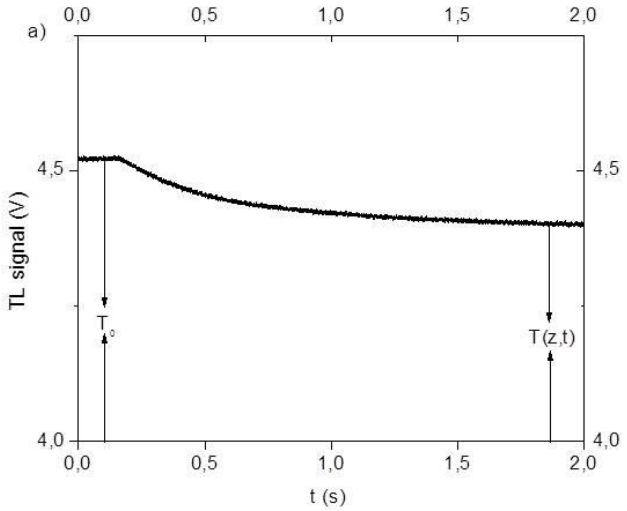
Schematic diagram of the TLM. S: sample; M_1 – M_3: mirrors; DM: dichroic mirror; L_1: lens; O: focusing objective lens; PD detector with pinhole and filter, C_1 and C_2 collimators; EL: excitation laser; PL: probe laser.

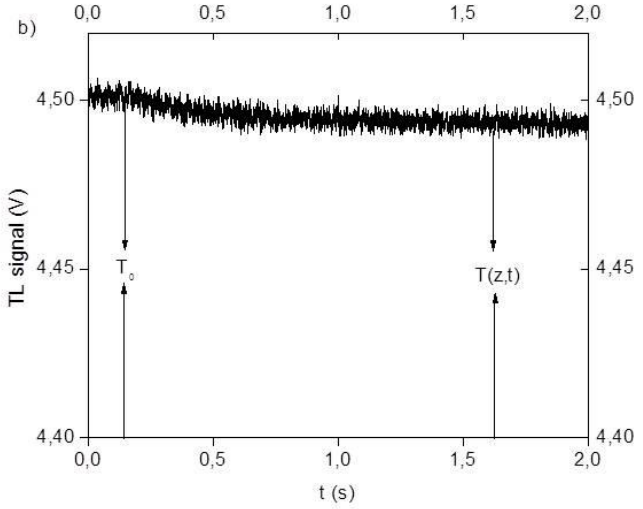
η

Determination of the intracavity amplification factor η

$$S(z,t) = \frac{\pi P_e \alpha \eta l}{2\lambda_p k} \frac{dn}{dT}$$

$$S(z,t) = \frac{\pi P_e \alpha l}{2\lambda_p k} \frac{dn}{dT}$$





TL signal for ethanol sample as a function of time for an excitation power of 22 mW. The values of the probe beam aperture's transmission in the absence, T_0, and in the presence, T(z,t), of the excitation beam are indicated, (a) TL signal for the sample inside the cavity and (b) TL signal for the sample without cavity.

$$S(z,t)=\frac{T(z,t)-T_0}{T_0}$$

Experimental

S=-0.0265

$dn/dT = -4\times10^{-4}\,^0C^{-1}$

$k = 1.67\times10^{-3} W cm^{-1}\,^0C^{-1}$

$\alpha = 6.8\times10^{-4} cm^{-1}$

l=0.2 mm

$\lambda = 632.8nm$

$\eta = 14.9$

$$S(z,t)=\frac{\pi P_e \alpha \eta l}{2\lambda_p k}\frac{dn}{dT}$$

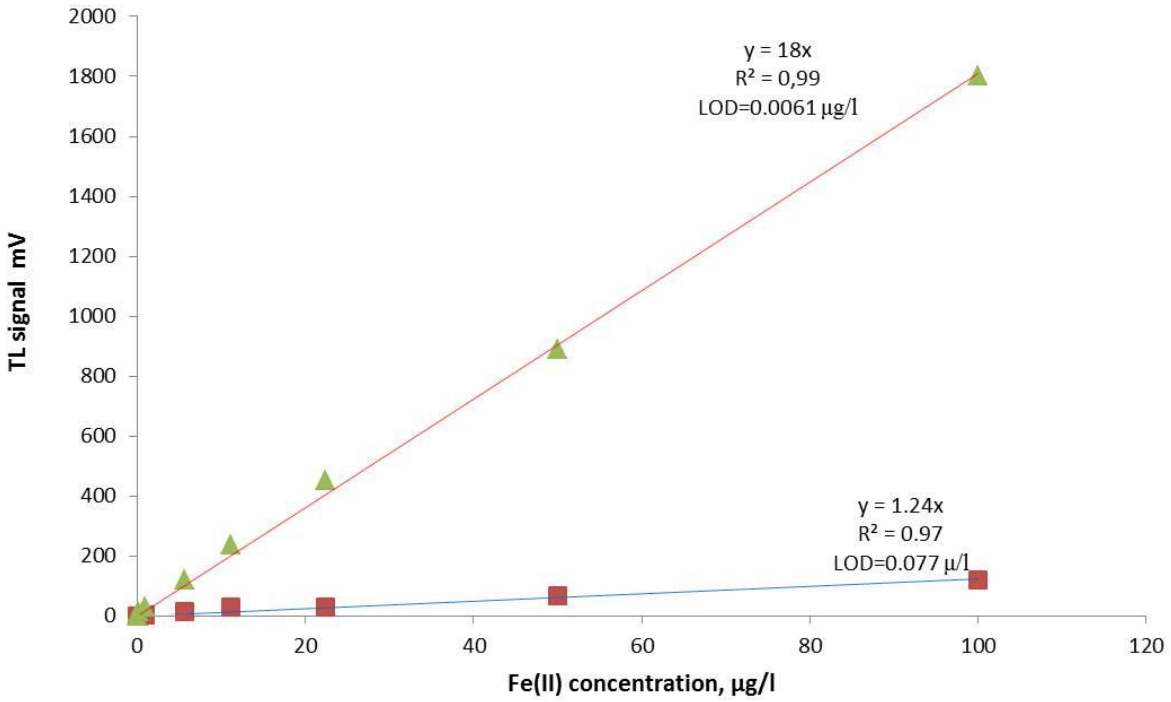
Theoretical

R_M= 0.87

T_M=0.13

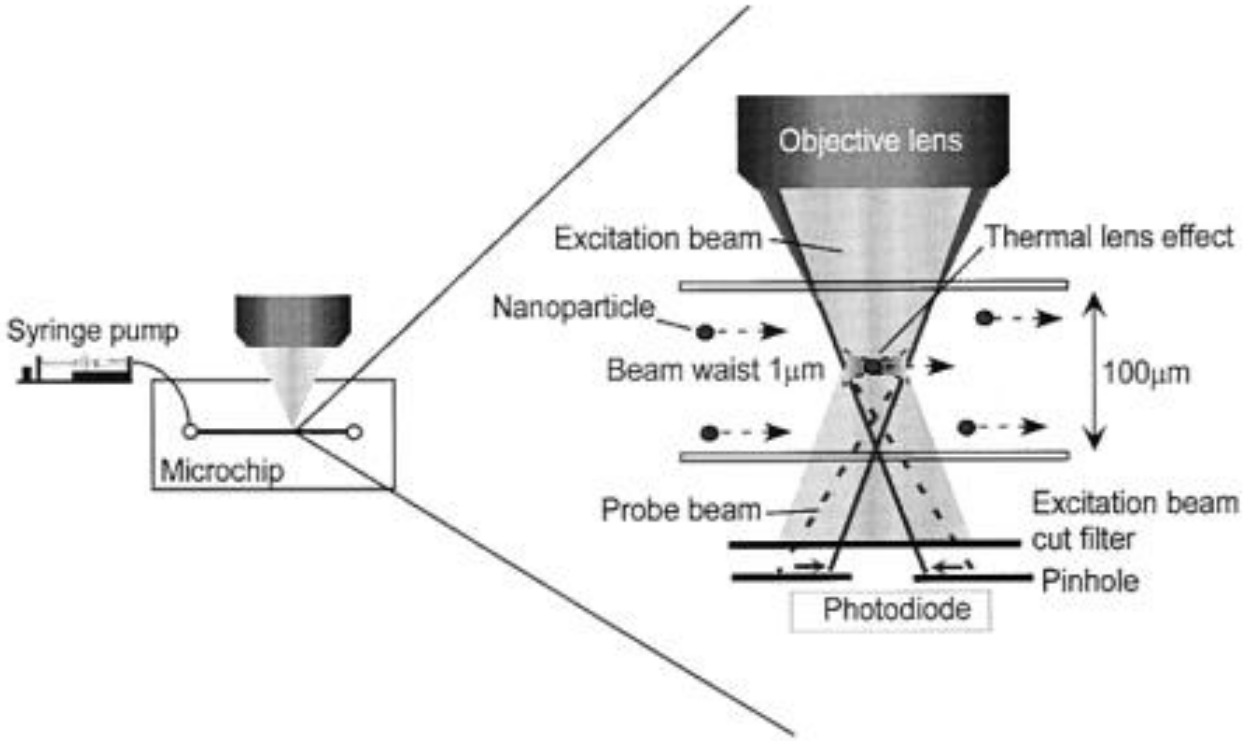
$\eta = 2/T_M = 15.4$

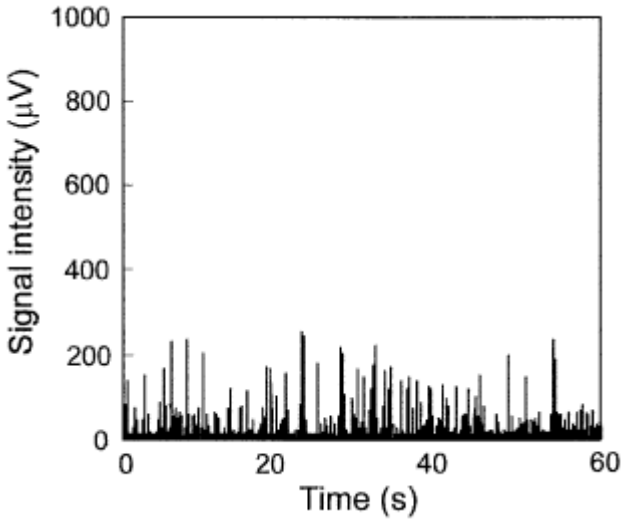
Applications



$$LOD = \frac{3SD_{blank}}{m}$$

Calibration curves for Fe(II) solutions in 80% water with the addition of 20% of acetonitrile in 0.2 mm cell at 532 nm for the sample without cavity and inside a cavity

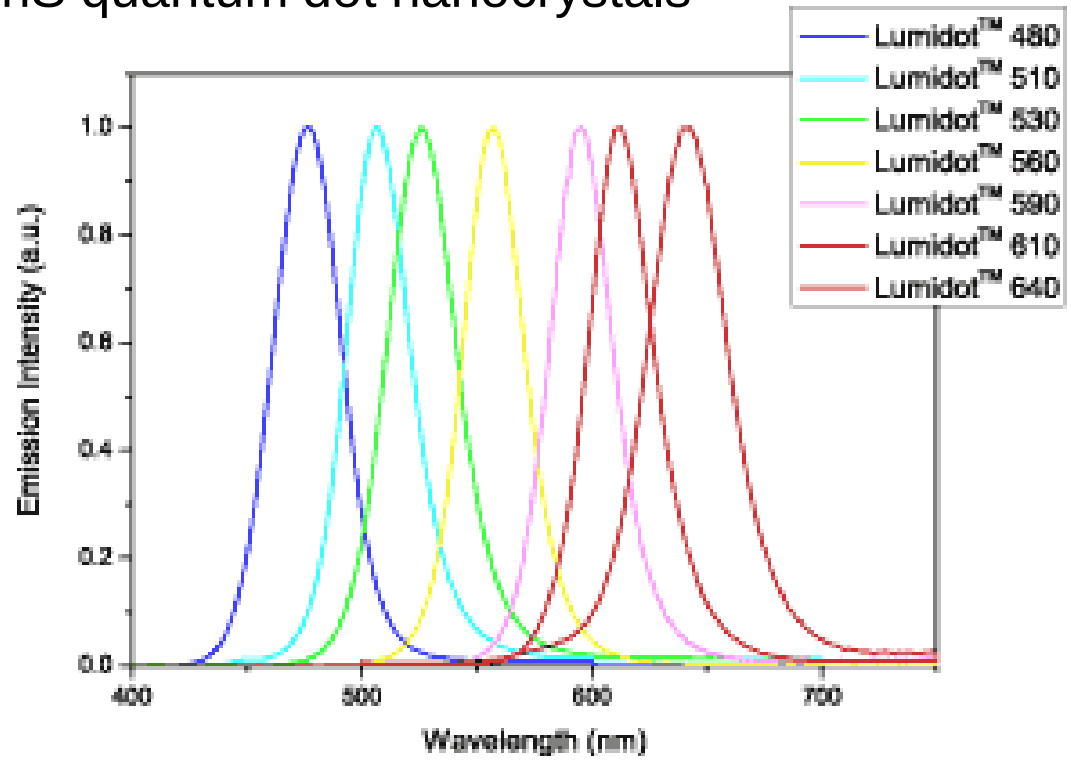

Objective lens
Excitation beam
Thermal lens effect
Nanoparticle
Beam waist 1μm
100μm
Syringe pump
Microchip
Probe beam
Excitation beam
cut filter
Pinhole
Photodiode


Signal intensity (μV)
1000
800
600
400
200
0
0
20
40
60
Time (s)

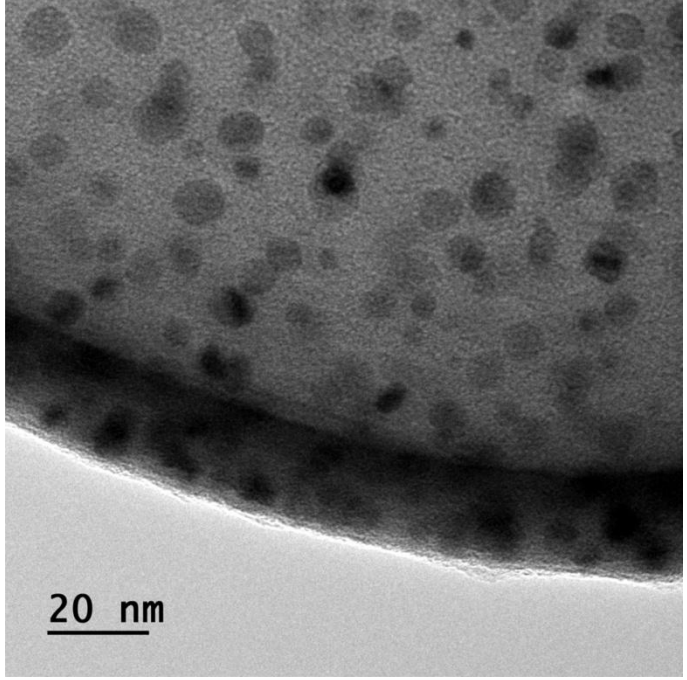
Encapsulation efficiency of CdSe/ZnS quantum dots by liposomes

Lumidots: Quantum Dot Nanocrystals

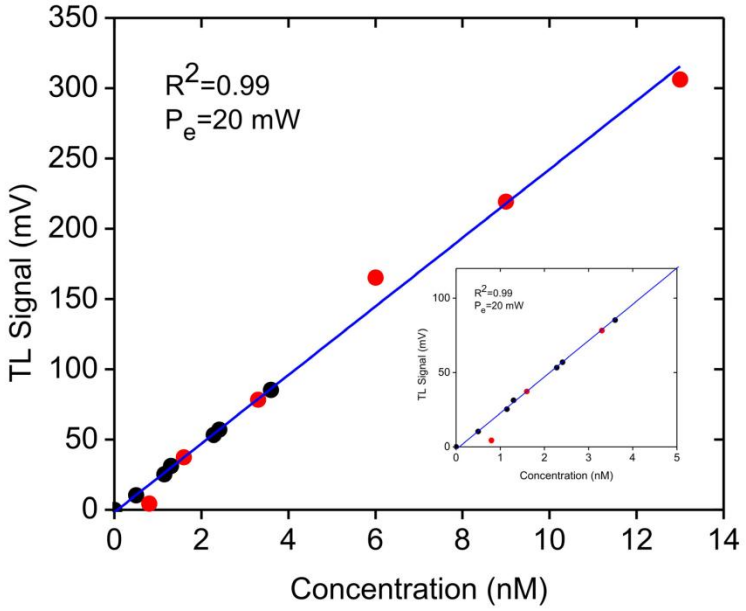
CdSe/ZnS quantum dot nanocrystals



Emission spectra of Lumidot™ CdSe/ZnS nanocrystals



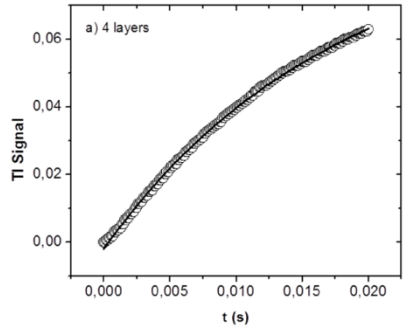
HR-TEM micrographs of the CdSe/ZnSe QDs encapsulated by liposome complexes (lipodots).

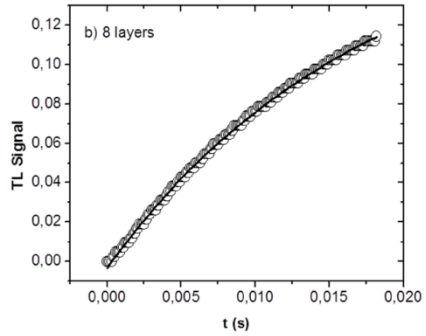


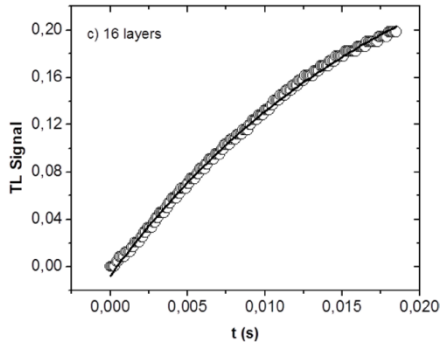
Calibration curve describing the TL intensity as a function of QDs concentration in aqueous solution 0 to 13 nM. The solid line represents a result of a best least squares linear fit with R2 = 0.99. Red circles correspond to measurements for QDs in water (without liposomes) and the black circles correspond to measurements for lipodots (QDs inside liposomes.

Jessica Batalla, Humberto Cabrera, Eduardo San Martín-Martínez, Dorota Korte, Antonio Calderón and Ernesto Marín, “Encapsulation efficiency of CdSe/ZnS quantum dots by liposomes determined by thermal lens microscopy”, Biomedical Optics Express **6(10),** 3898-3906 (2015).

Thermal diffusivity of few-layers graphene







Typical time evolution of the TL signal for four (a), eight (b) and sixteen (c) graphene layers. Solid curves are the results of the best least squares fits to equation

150 nm each layer

$$S(z,t)=\Phi_0 \arctan\left\{\frac{4m(z)\upsilon(z)t/t_c(z)}{\upsilon^2(z)+[1+2m(z)]^2+\left[1+2m(z)+\upsilon^2(z)\right]2t/t_c(z)}\right\}$$

$D=(6.5\pm 0.09)\times 10^{-4}\ m^2/s$ $D=(1.9\pm 0.07)\times 10^{-4}\ m^2/s$

$D=(1.9^3\pm 0.05)\times 10^{-4}\ m^2/s$

for four, eight and sixteen graphene layers respectively

Humberto Cabrera, Doroteo Mendoza, Jose Luis Benitez, Claudia Bautista, Salvador Alvarado Ramírez, Ernesto Marin, “Thermal diffusivity of few-layers graphene measured by an all-optical method”, Journal of Physics D: Applied Physics **48**, 465501 (2015).

Thanks for your attention!