FLUORESCENT NANOPARTICLES FOR IMAGING, TREATMENT AND MANIPULATION AT CELLULAR LEVEL

J. García Solé

Fluorescence Imaging Group: Dpto de Física de Materiales , C-IV, Facultad de Ciencias.Universidad Autónoma de Madrid, 28049, Madrid.

INTERNATIONAL SUMMER SCHOOL ON FLUORESCENT NANO-PARTICLES IN BIO-MEDICINE

15-20 July 2012

Residencia La Cristalera, Miraflores de la Sierra, Madrid

Multidisciplinar Research

Department of Chemistry and Biochemistry, Concordia University, Canada




Nicoleta Bogdan Rafik Naccache John A. Capobianco Emma Martín Rodríguez

INRS, Université de Québec. Canada

Fiorenzo Vetrone

Departamento de Biología, UAM


Francisco Sanz Rodríguez Mª Ángeles Juarranz

Departamento de Fisiología, Facultad de Medicina , UAM

Mª.C. Iglesias de la Cruz

Univ. Sonora, Mexico


M. Barboza K. Santacruz

Departamento de Física de Materiales, UAM




J. García Solé

L.Martínez Maestro D.Jaque J.Y. Chooi[1]


Ning Dong A. Benayas B. De Rosal

Universita Degli Studi di Verona , Italy

Marco Pedroni
Fabio Piccinelli

Patricia Haro


Marco Bettinelli Adolfo Speghini

U. Federal de Alagoas, Brasil

U. Rocha

-Nanoparticles in medicine: Nanomedicine

-IR excitation: Two-photon fluorescence imaging

-Fluorescent nanothermometers.

- **-Nanoheaters: Controlled Hyperthermia.**
- **-Controlled cell heating by optical trapping**

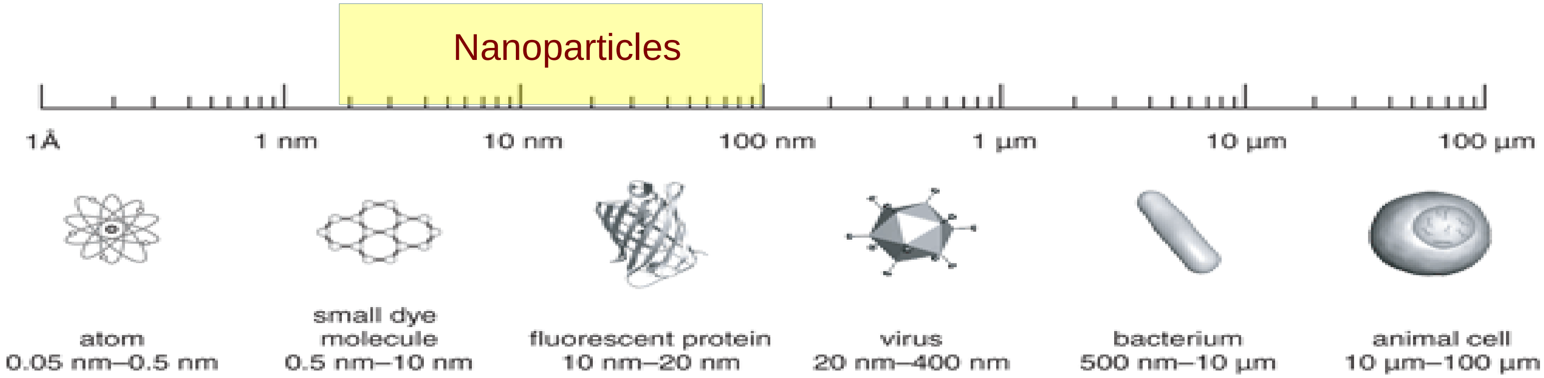
-Deep tissue imaging

-Fast Imaging: Lifetime imaging nanoparticles

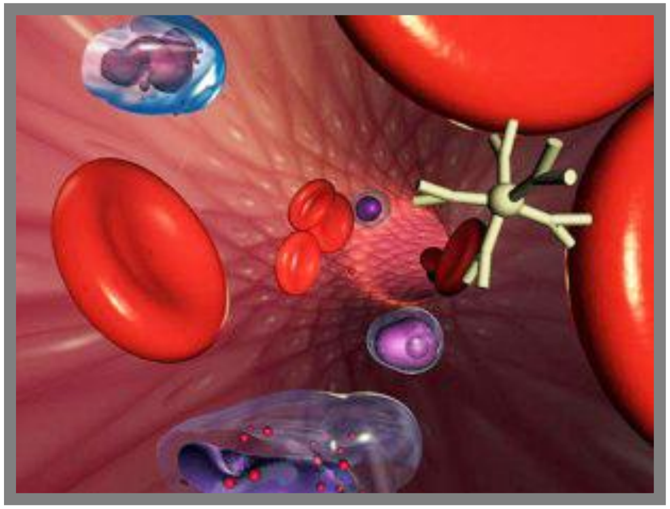
- Summary

Nanoparticles in medicine: Nanomedicine

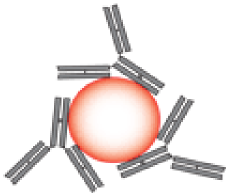
Why nanoparticles?



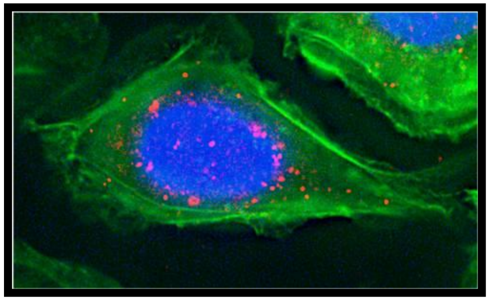
-Move in the blood and are preferentially accumulated in tumors, due to its permeability
⇒ It is avoided the devastating action of the usual drugs.



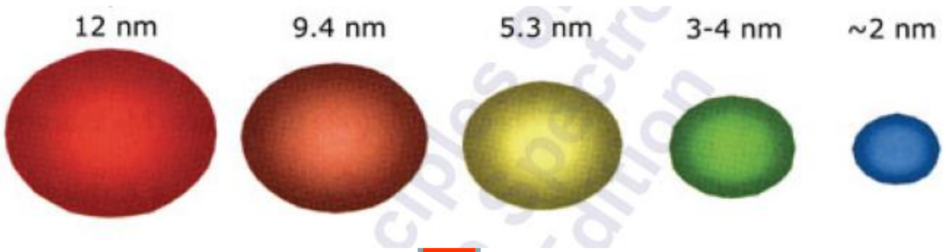
-Large surface to accommodate functional groups (diagnosis, therapeutic…)



- Interact in a singular way with biomolecules, proteins and are easily up-taken by cells.



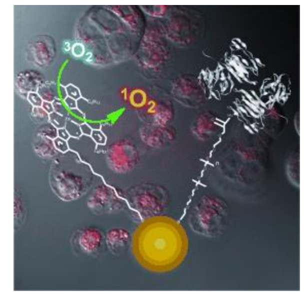
- Spectral properties (semiconductors and metals) depend on the particle size.



NANOMEDICINE

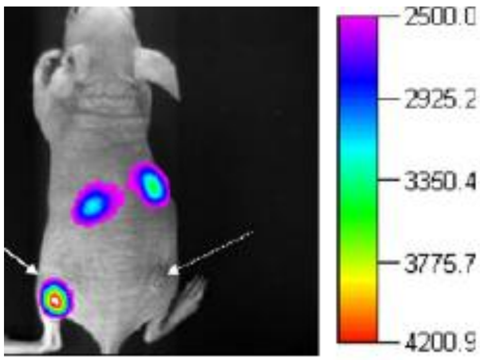
SOME APPLICATIONS OF NANOMEDICINE IN CANCER THERAPEUTICS

Non- invasive therapies

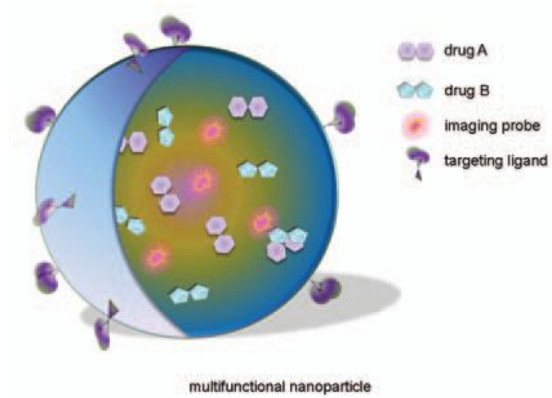


Photodynamic
Photothermal,
RadioFrequency induced.
thermal therapy.

Cancer detection (cellular level)

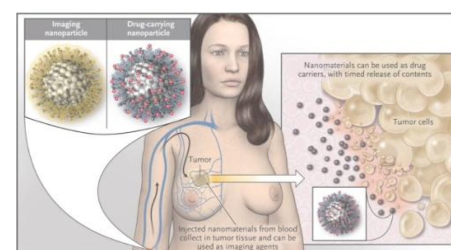


Drug delivery



Surgical efficacy

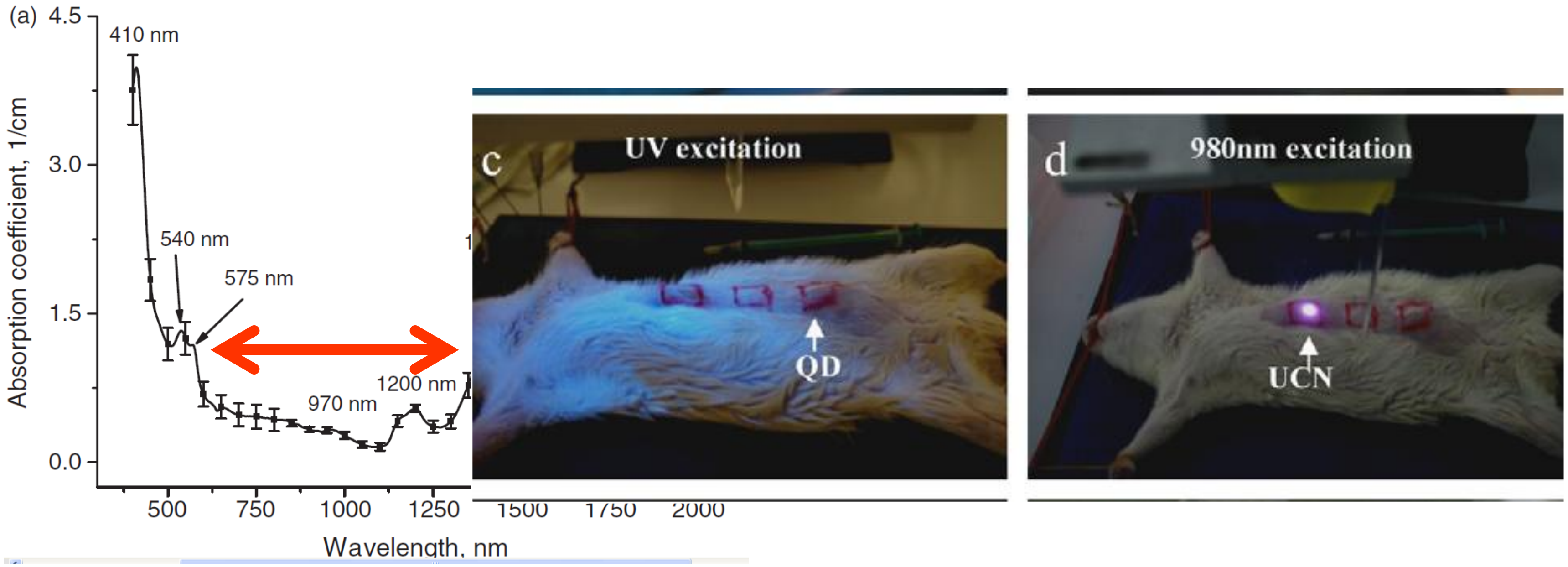
Real time evaluation of therapeutic



IR excitation: Two-photon fluorescence imaging

- IR excitation is less harmful for cells and organelles.

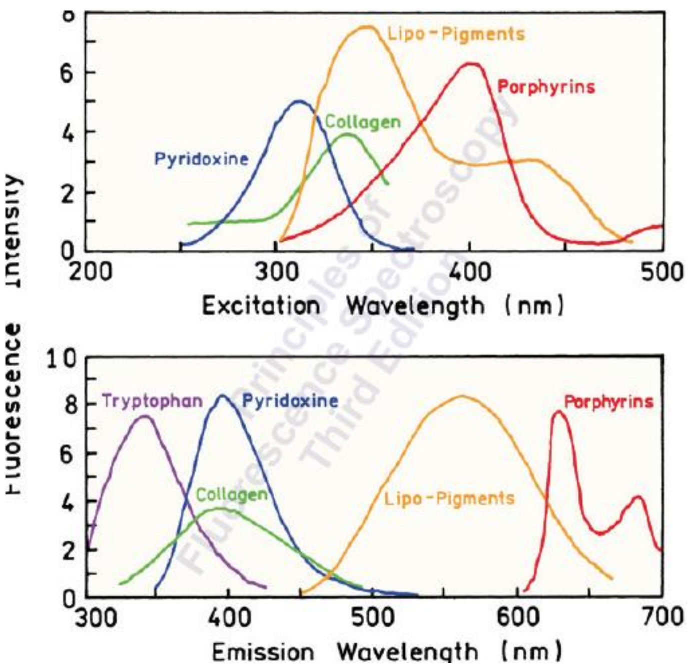
- Depper penetration (700- 1250 nm biological spectral window).



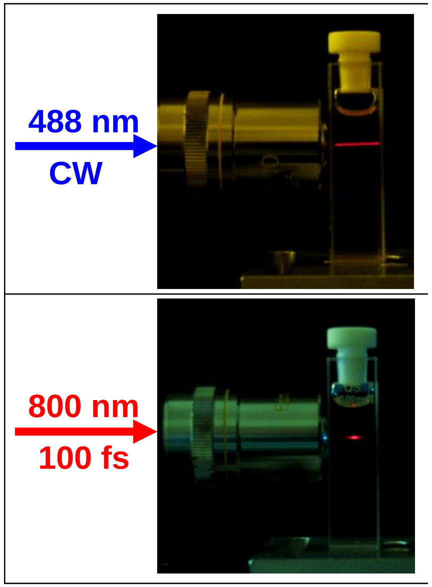
- Almost no autofluorescence.

Excitation spectra

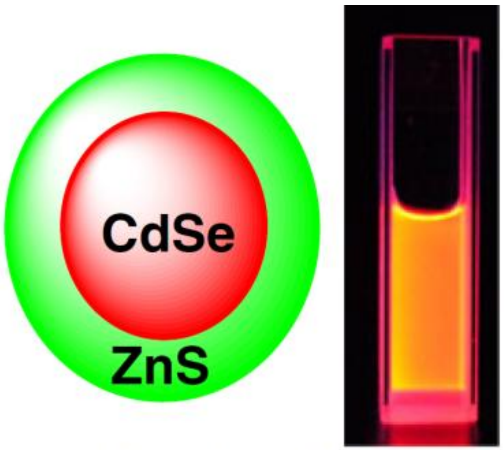
Emission spectra



- Better resolution.

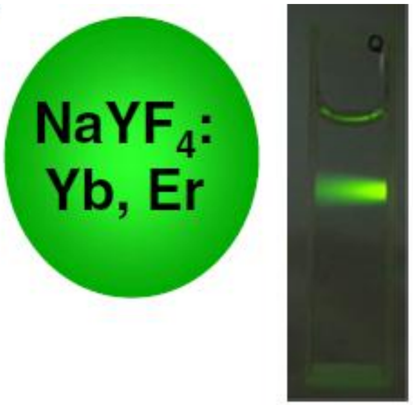


Nanoparticles for multi-photon excitation



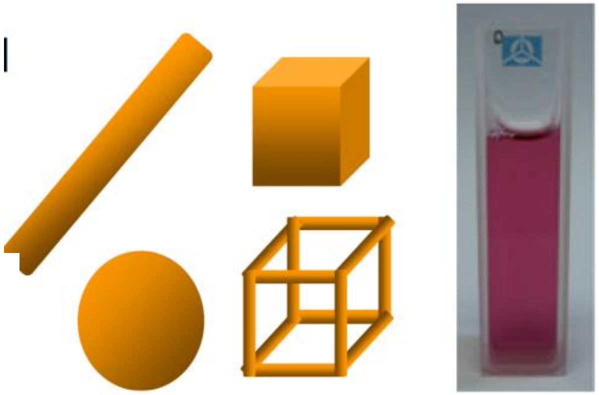
Quantum Dots

CdS, CdSe, ZnS, ZnSe, PbSe, PbS



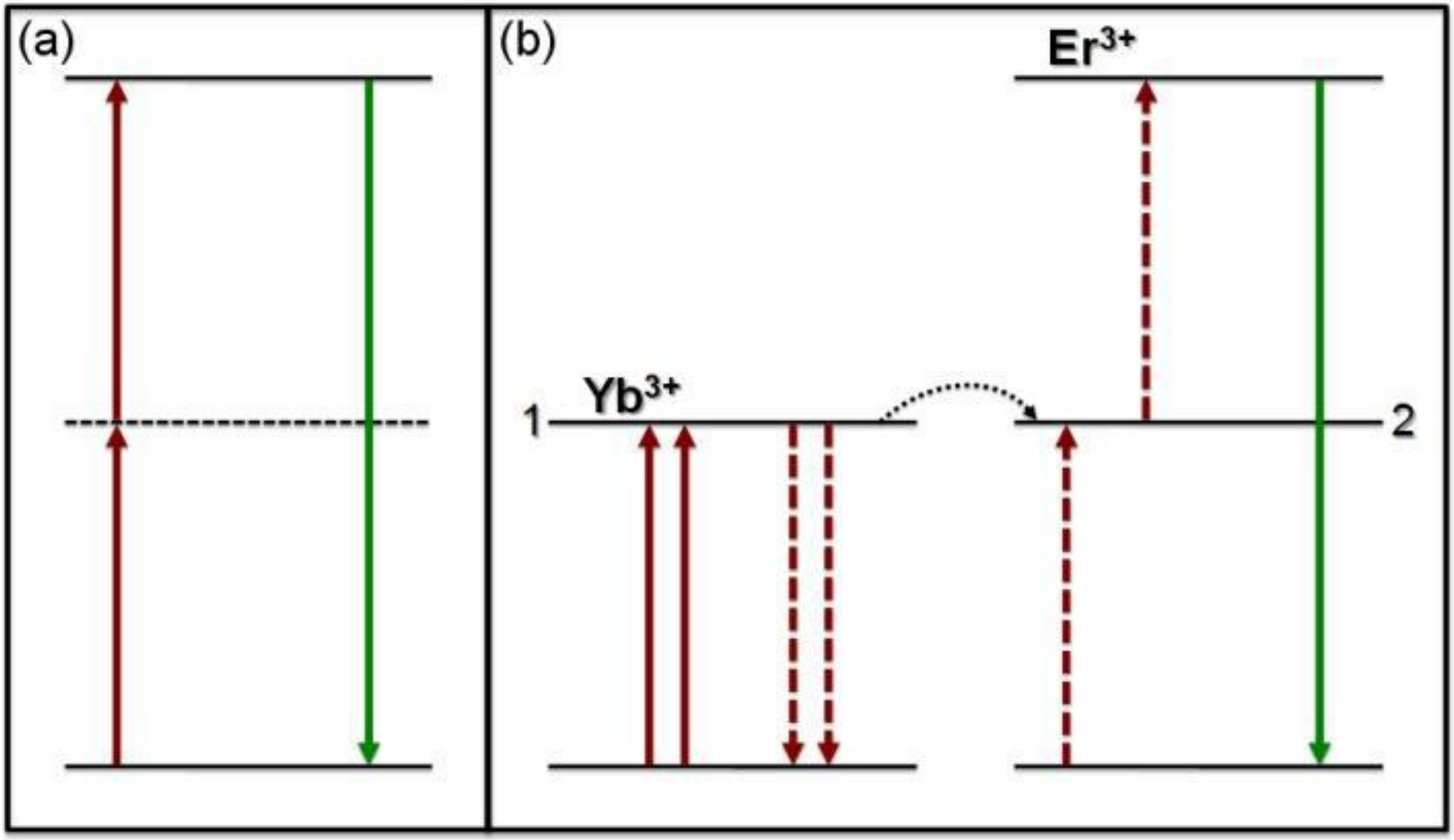
Up-conversion Nanoparticles

$NaGdF_4$, $NaYF_4$, CaF_2
Dopants : Yb, Er, Nd, Tm



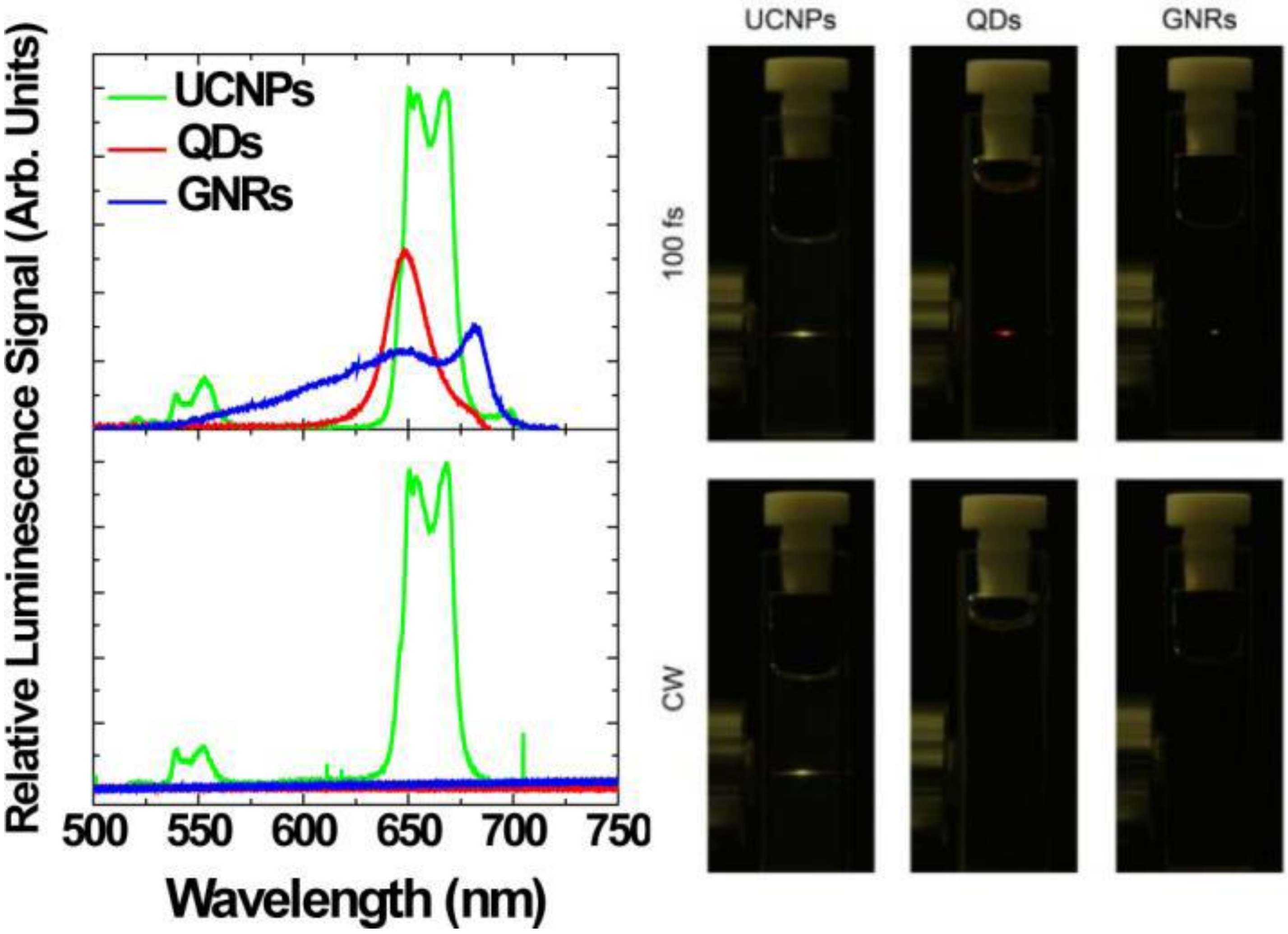
Gold Nanostructures

Excitation Mechanisms



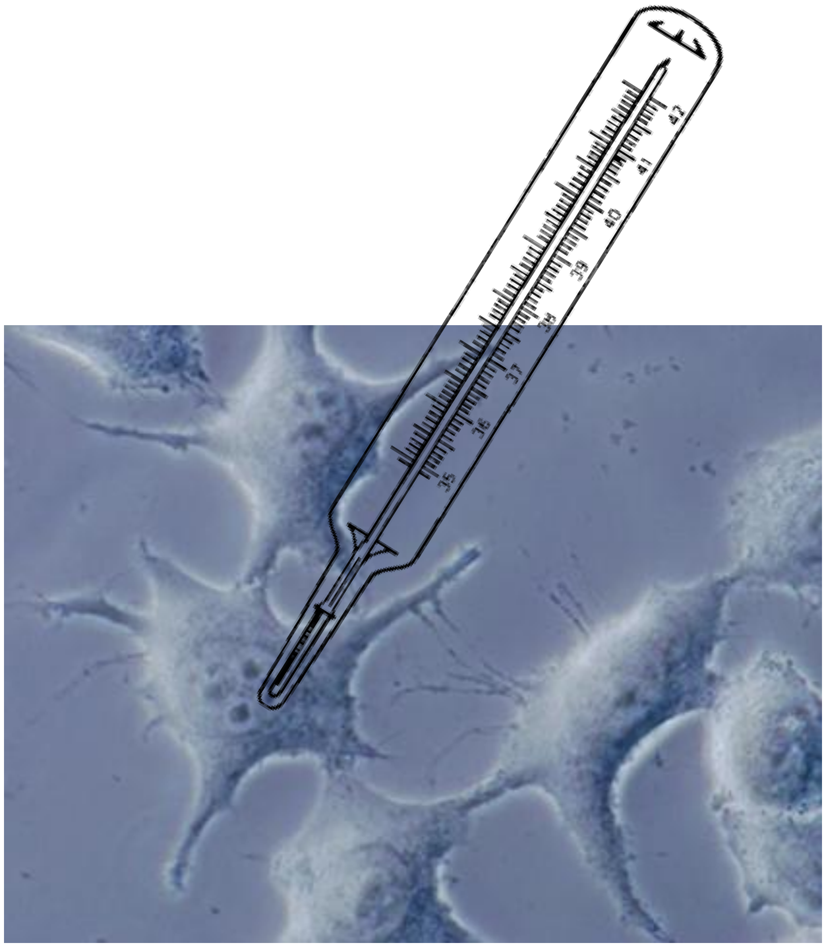
QDs and Metals

Up converting (Yb, Er) nanoparticles



L.M. Maestro et al. Optics Express, (2010)

Fluorescent Nanothermometers



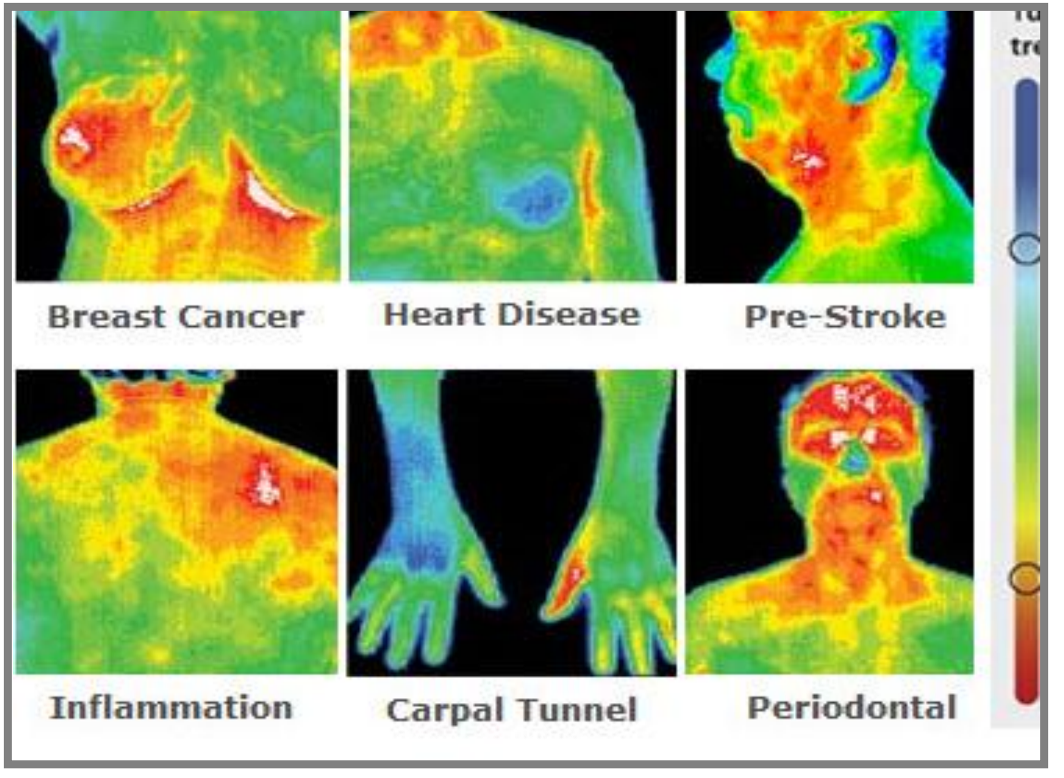
Several features in the emission of nanoparticles are sensitive to temperature

Non contact fluorescent thermometers at (potentially) Nanoscale

Nanothermometers

Importance of determining temperature at micro/ nano scale

Thermal imaging

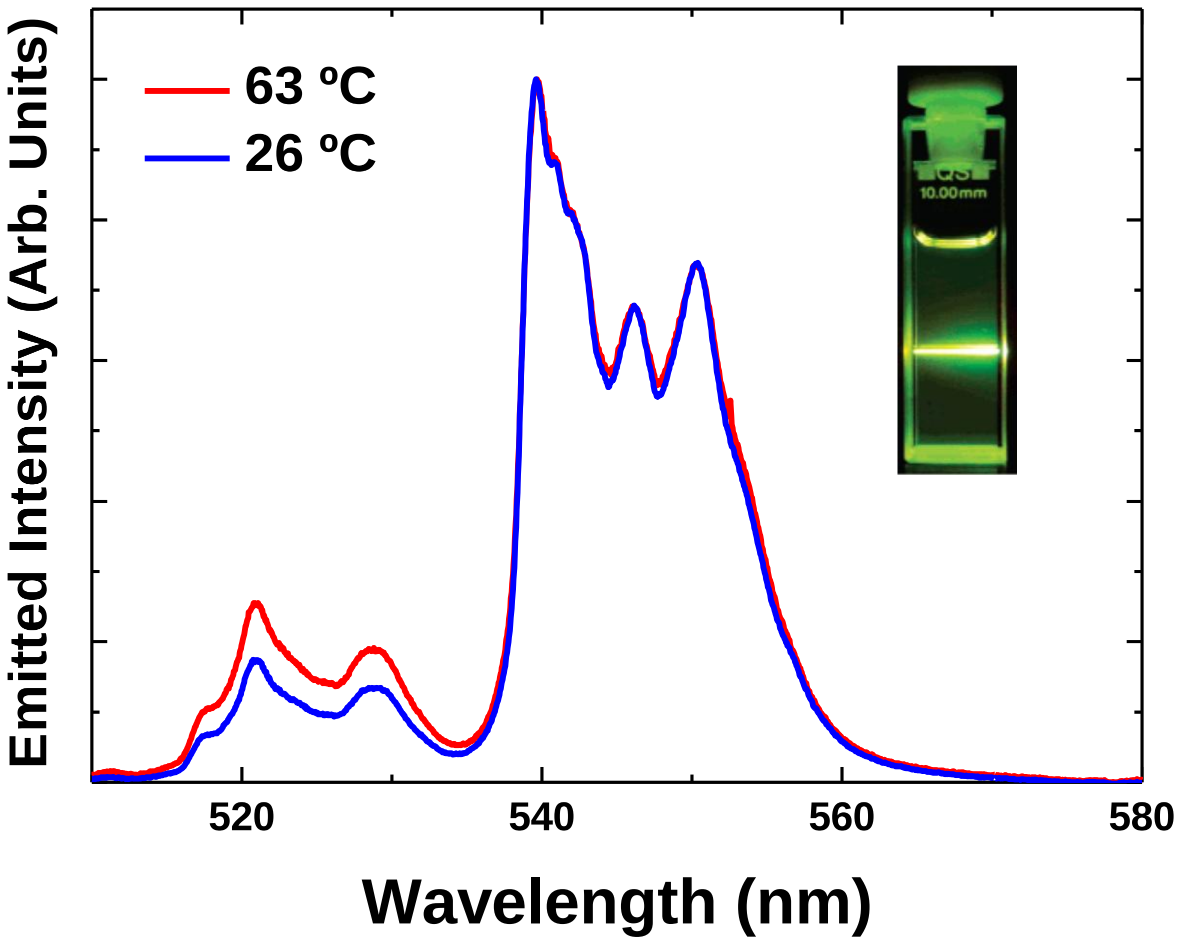


Tumor temperatures are slightly higher due to higher blood flow and larger metabolic activity

¡ Nano-thermo-imaging would be useful do detect cancer at cellular scale!

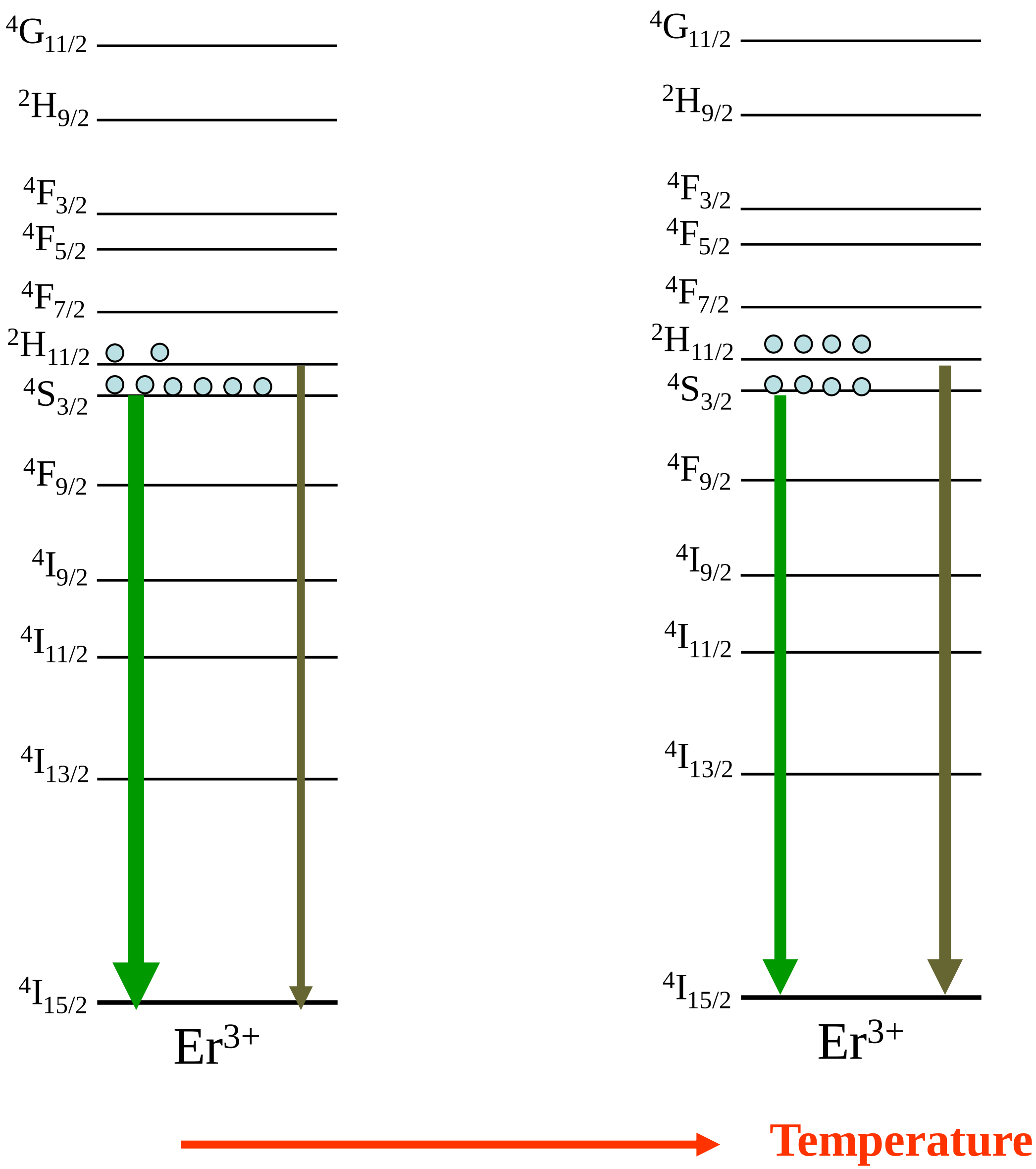
The first ($NaYF_4$:Yb, Er) Nanothermometers

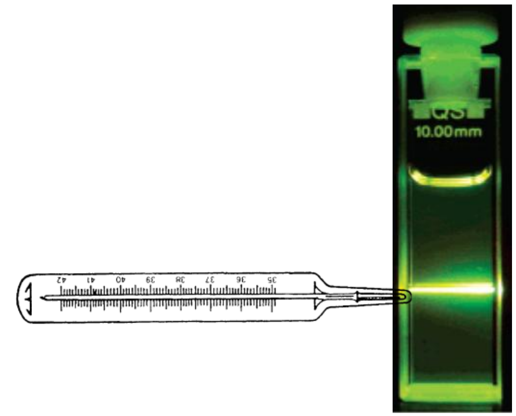
The green-Er^{3+} ion-emission is sensitive to temperature changes in the physiological range

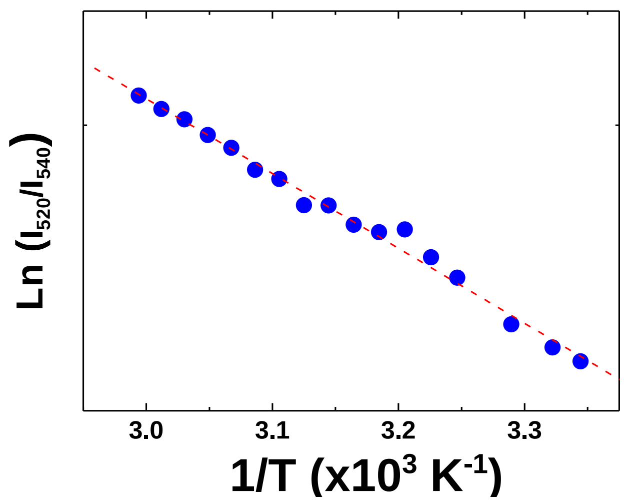


Vetrone et al. ACS Nano *vol.4, No. 6 (2010)*

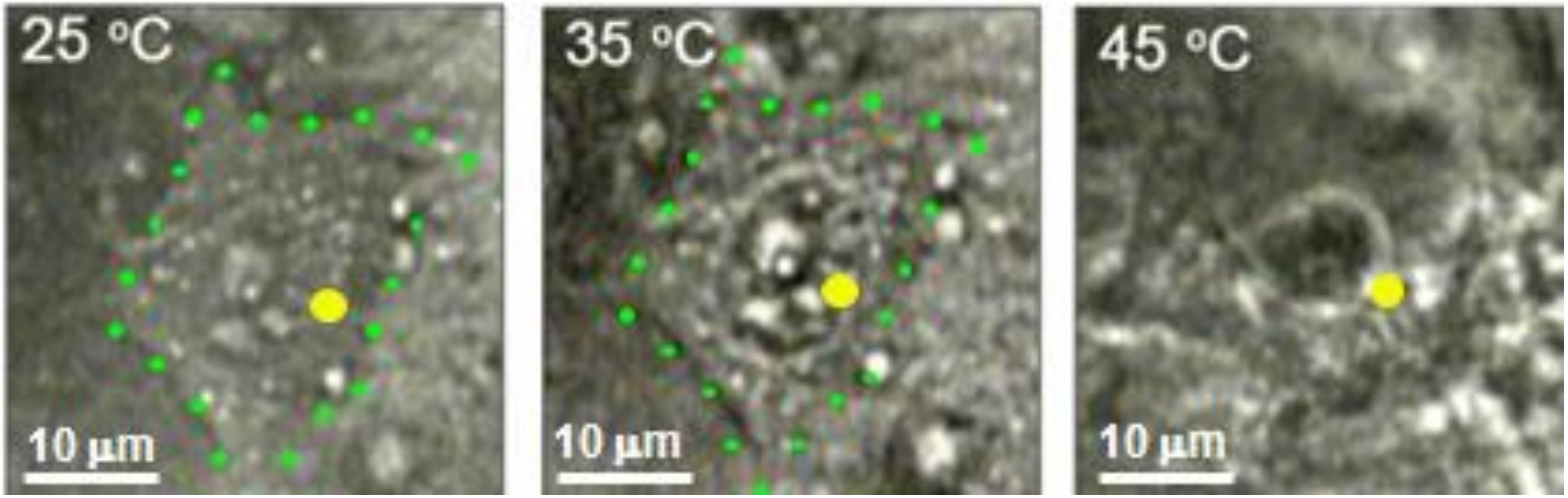
$$\frac{I_{525}}{I_{545}} = C\exp\left(-\Delta E/kT\right)$$





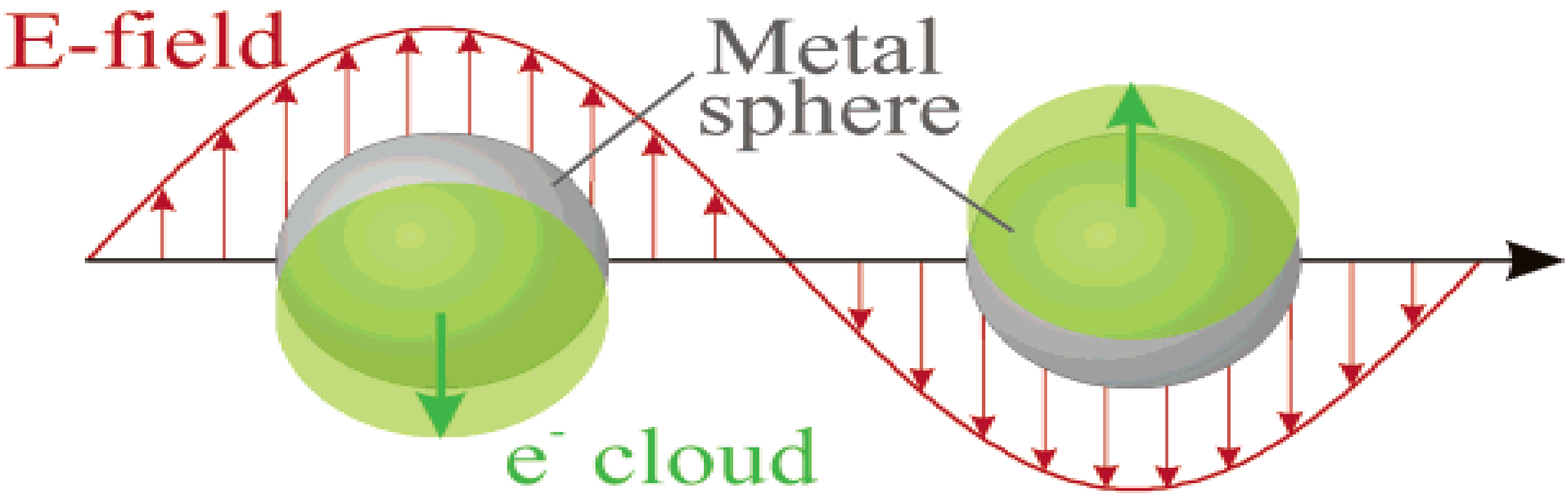


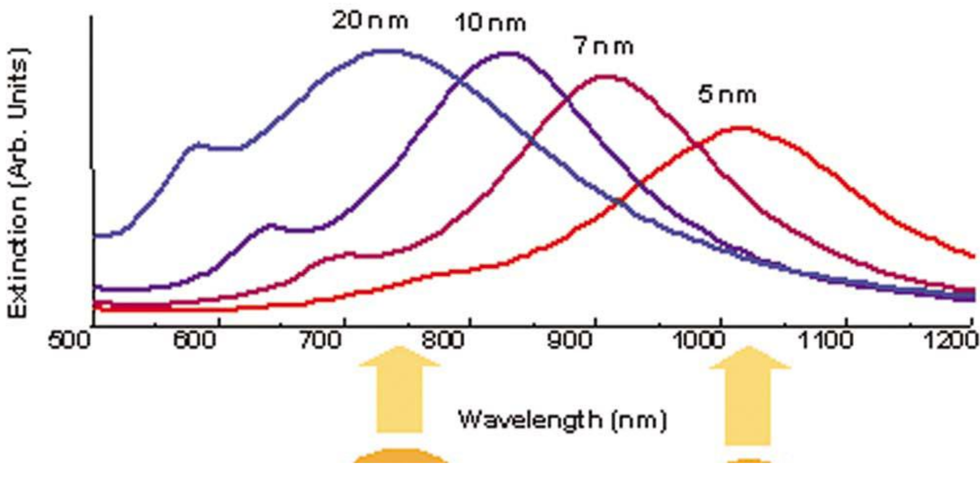
Intracellular temperature measurements
(External heating by a resistor)

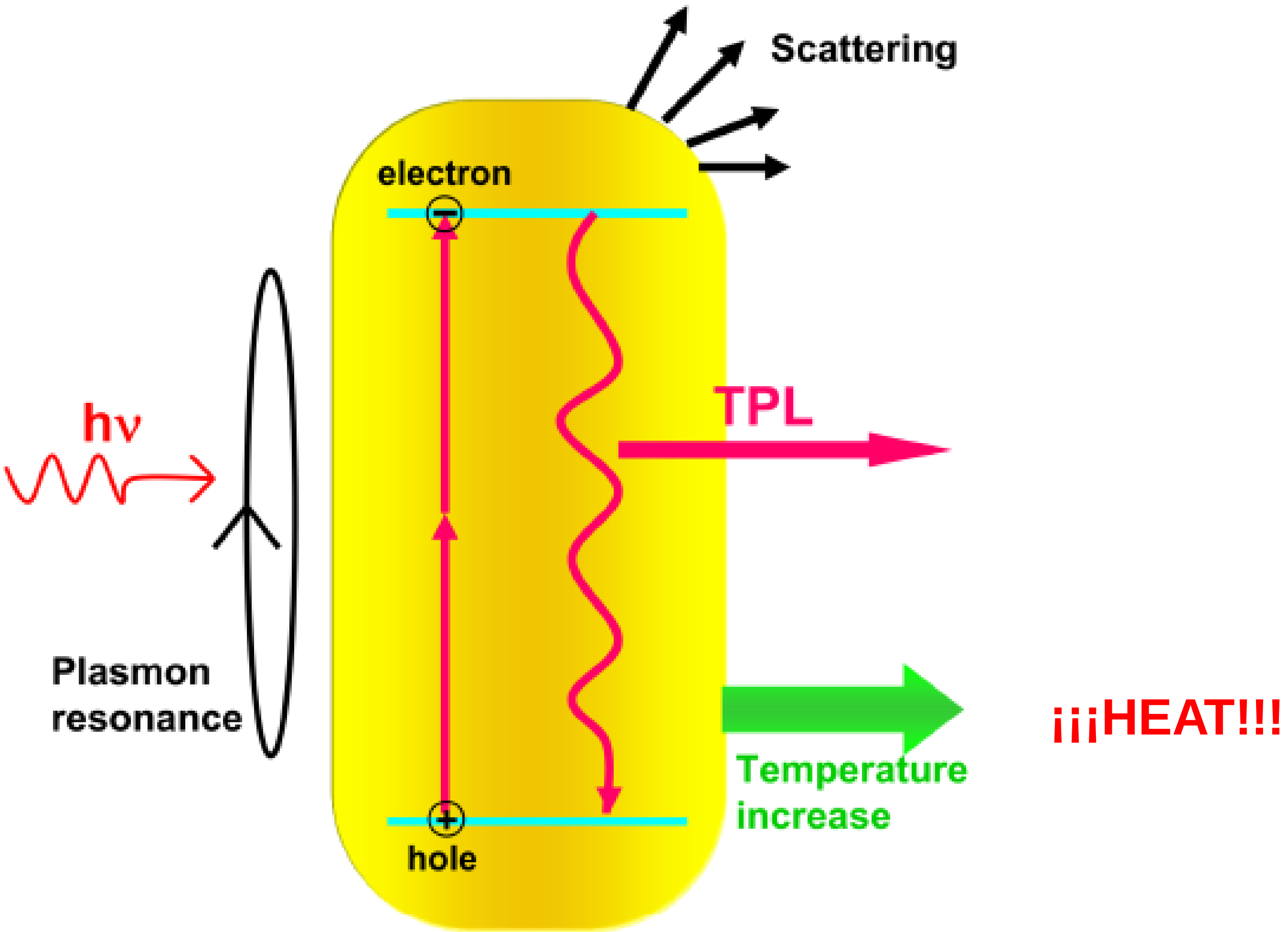


Sensitivity : ±1.5 º

Metals: Nanoheaters

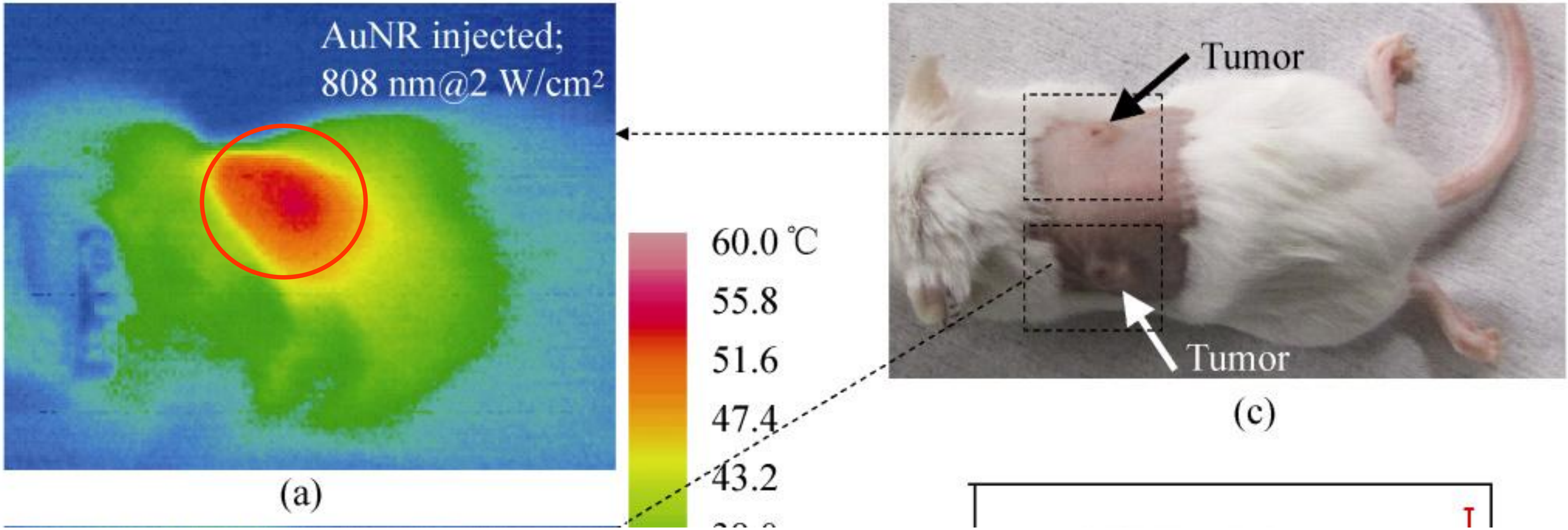






Gold Nanoparticles (mostly nanorods) are very efficient “Nanoheaters”

Light induced Hyperthermia



Thermal imaging of tumor during photo-thermal treatment (Gold Nanorods)

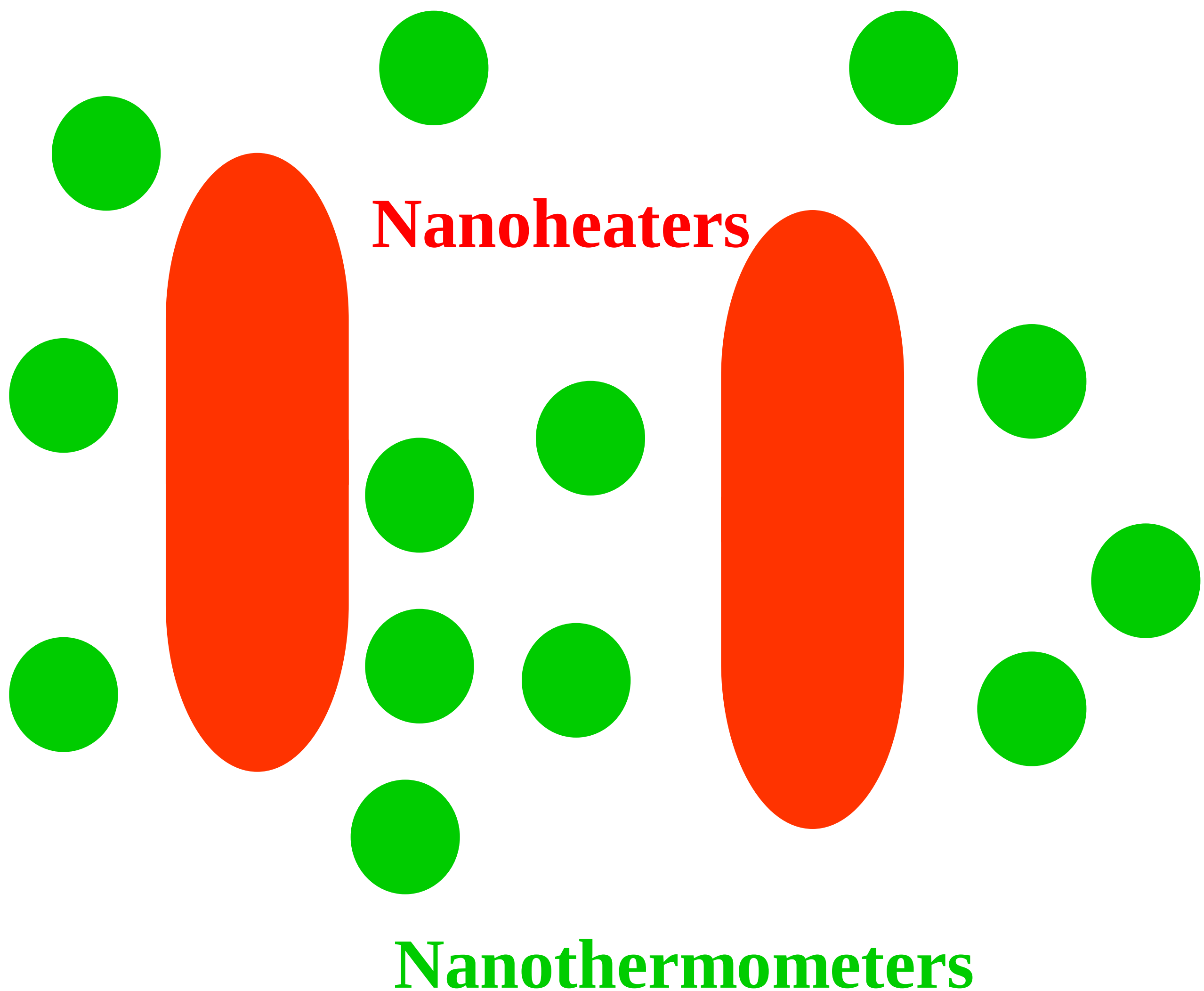
J.T. Robinson et al. Nano. Res., 3 (11), 779 (2010)

Temperature measured with a thermocouple

Uncontrolled heating area

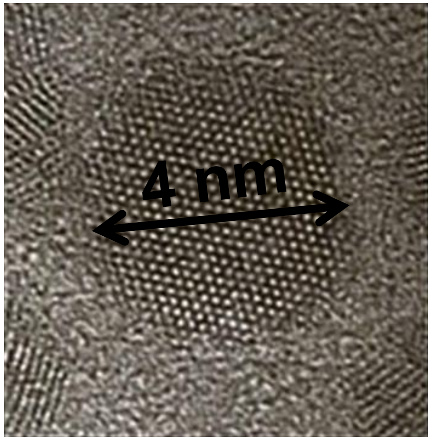
¡Need of temperature control at tumour scale!

Controlled Hyperthermia



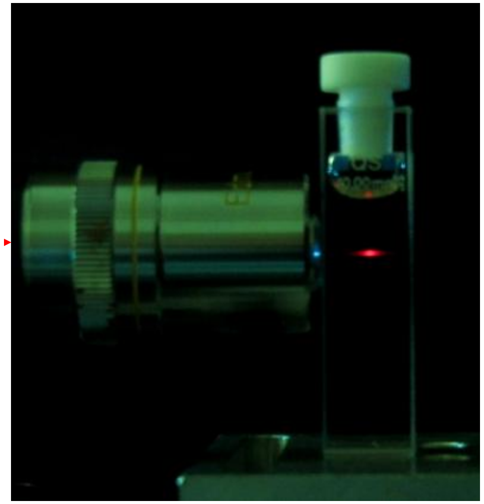
Looking for the best Nanothermometer...

CdSe-4 nm-QDs Nanothermometers



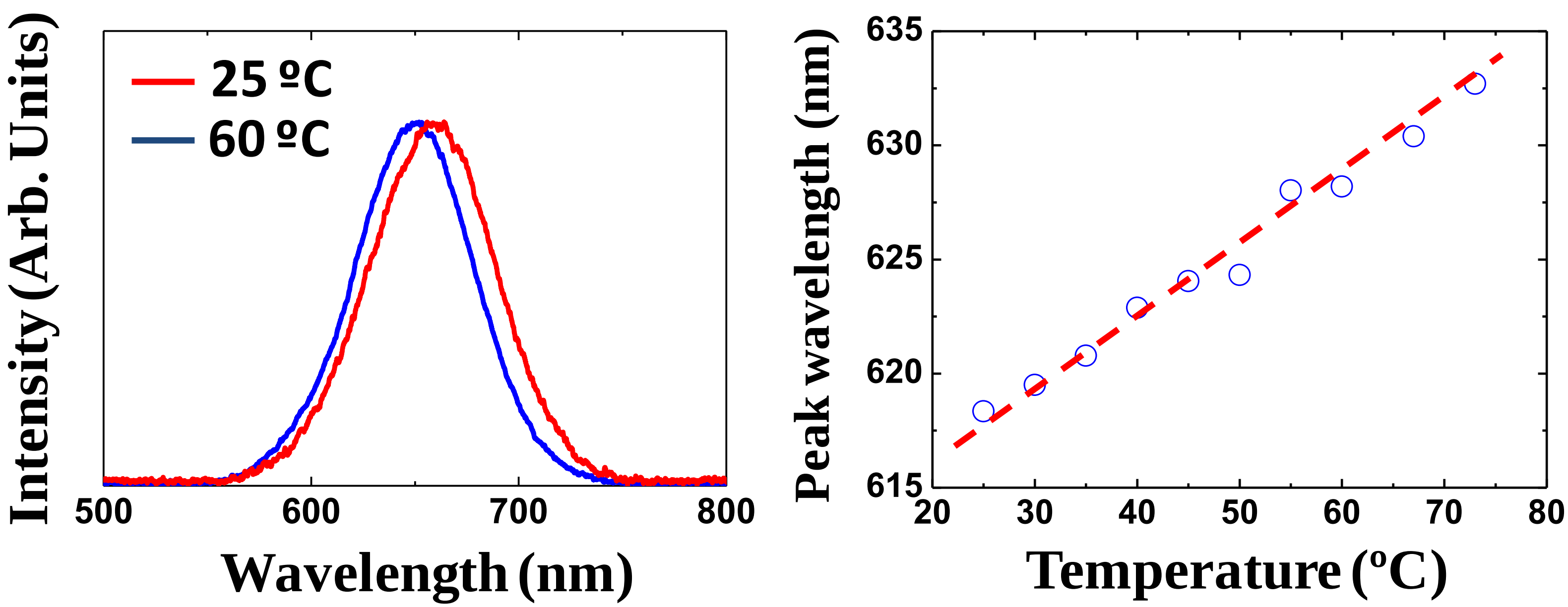
Quantum Dots improve the thermal sensitivity of UCNPs

920 nm
100 fs



CdSe-QDs show a very strong two-photon excited emission

CdSe (4 nm)-QDs



D. Jaque et al. J. Luminescence. (2012)

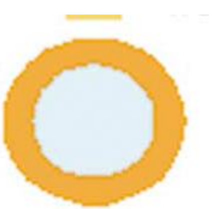
Looking for the best Nanoheaters ...

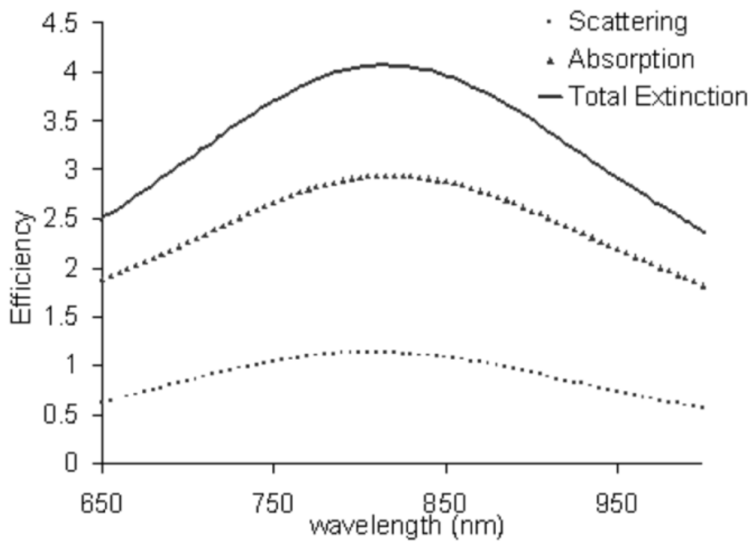
Efficiency of Gold Nanoheaters

$$\phi = \frac{\alpha_{abs}}{\alpha_{ext}}$$

Best Nanoshells

Core radius = 50 nm; shell thickness = 10 nm).



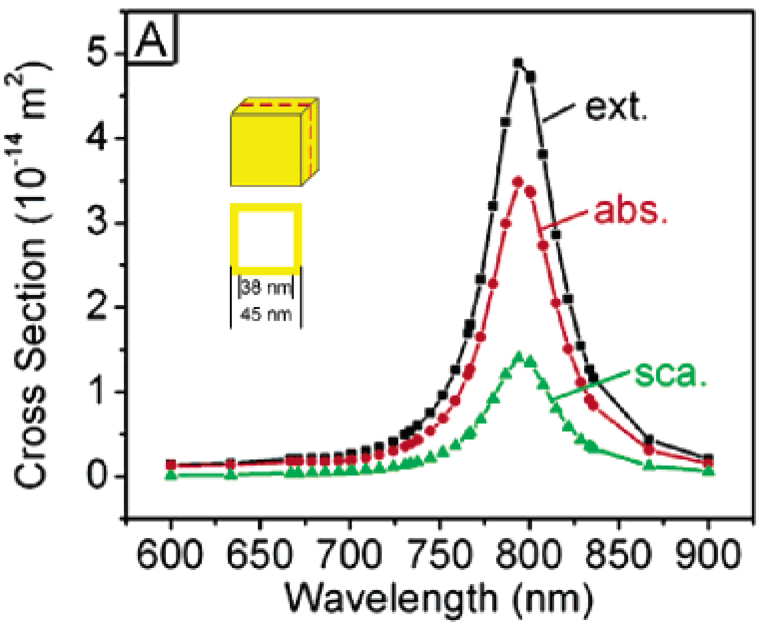


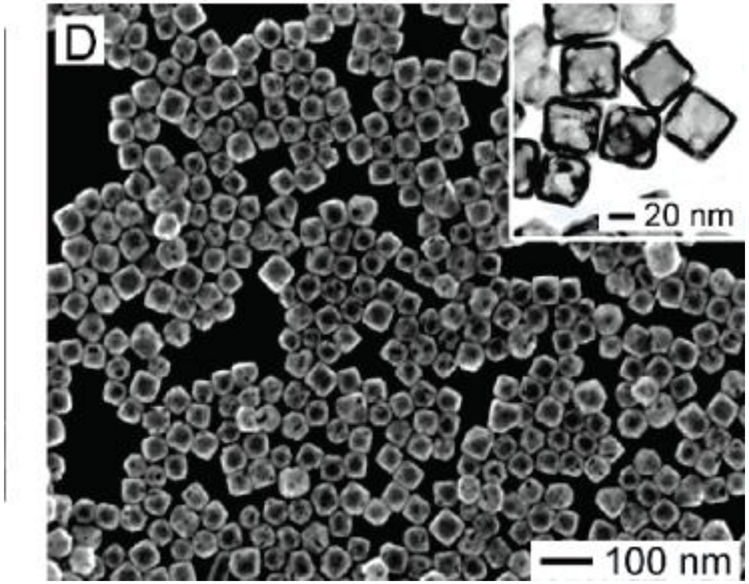
$$\alpha_{ext} = \alpha_{abs} + \alpha_{scat}$$

$$\phi = 67\%$$

C. Loo et al., Technology in Cancer Research & Treatment Vol.3, Number 1, February (2004)

Best Nanocages

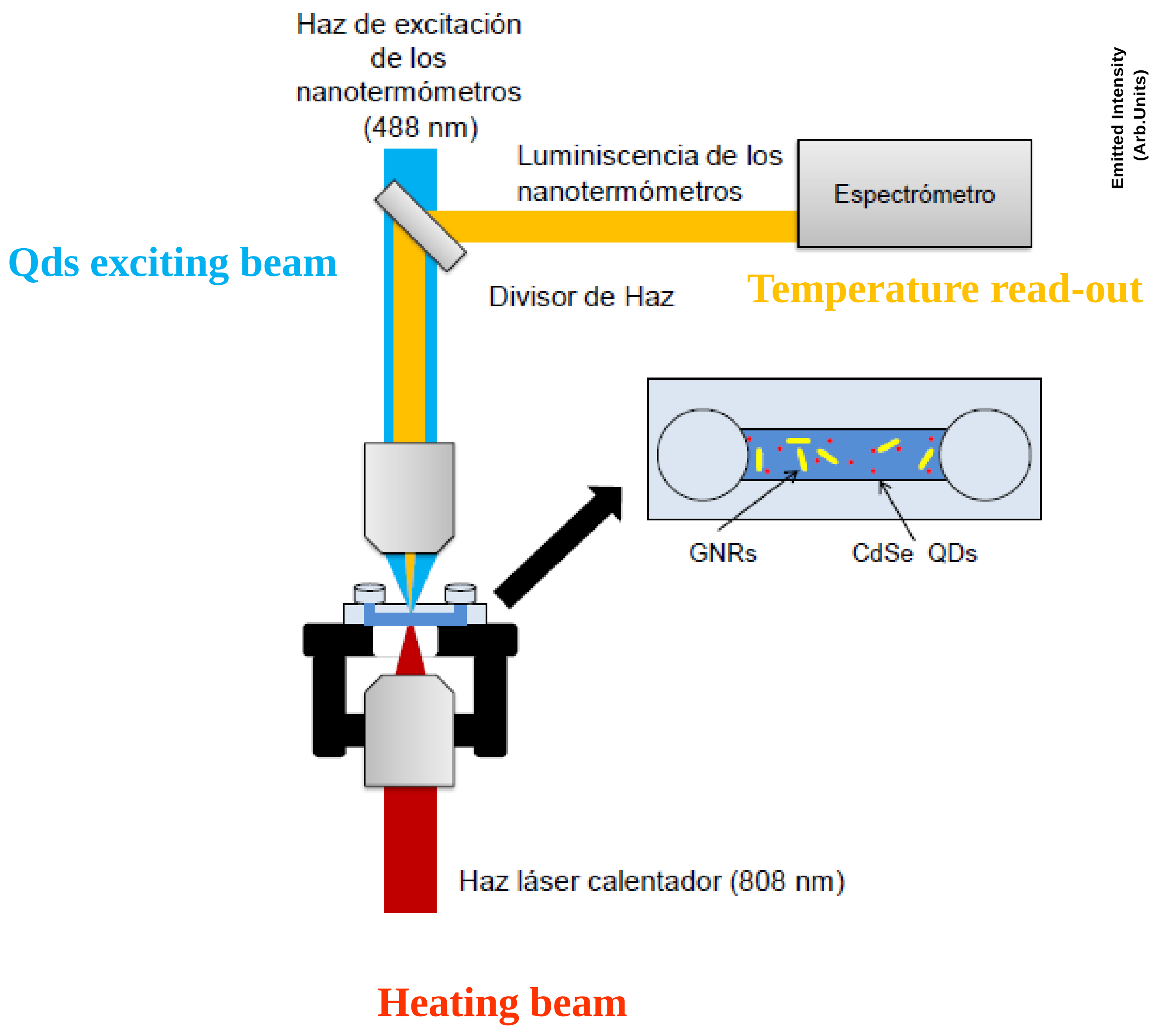


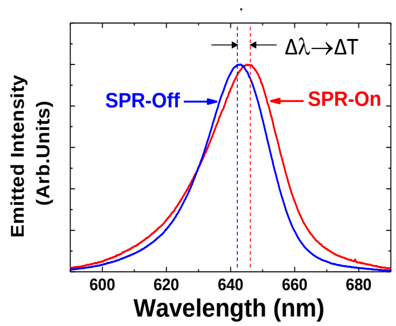


J. Chen et al Nanoletters, Vol 7, No. 5, 1318 (2007)

$$\emptyset = 74\%$$

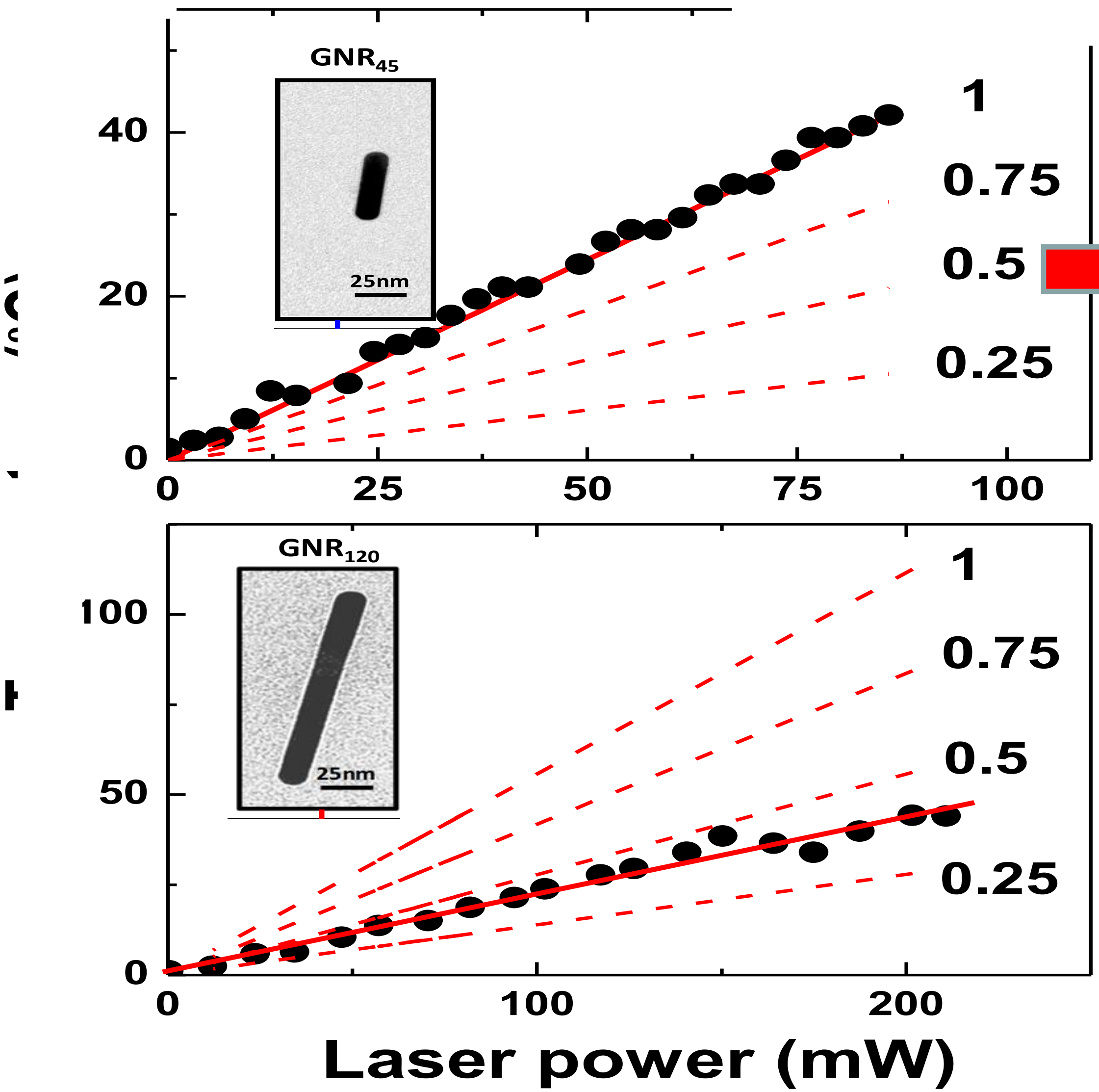
QDs sensed laser heating experiments to look for the Best GNRs



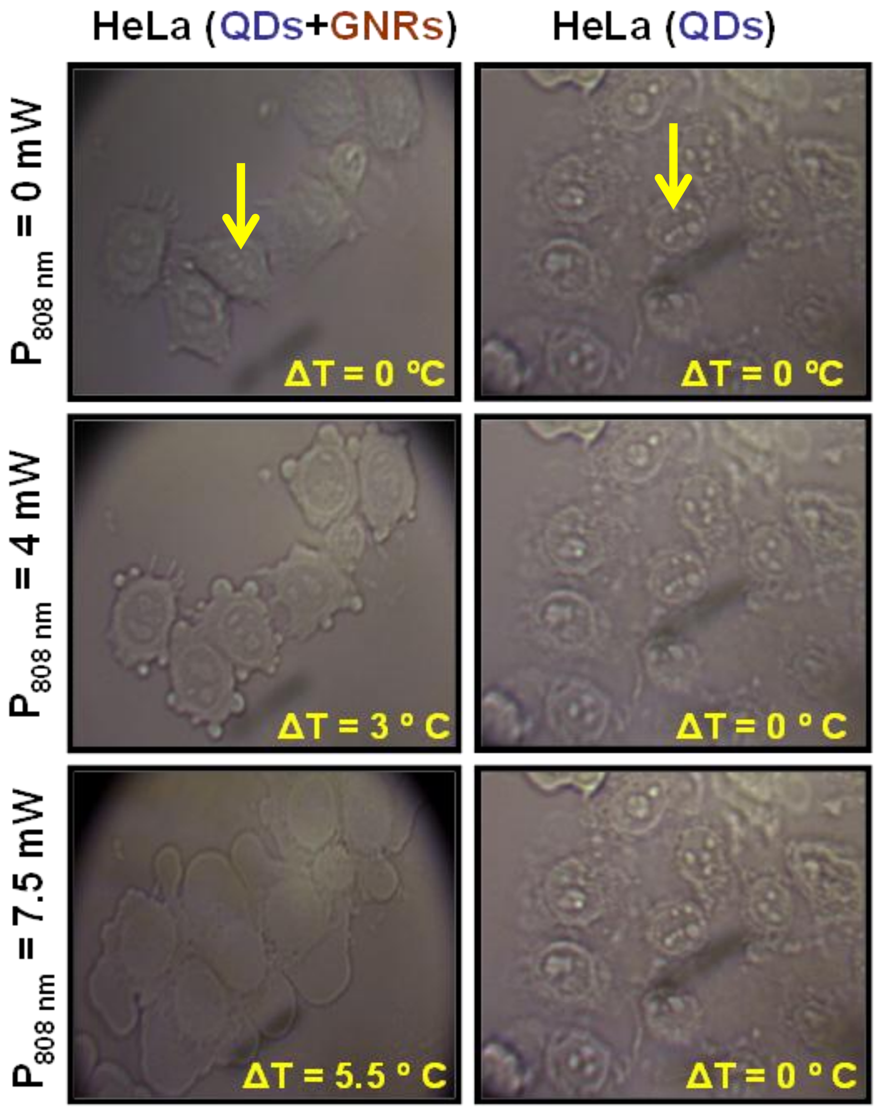


ΔT_{focus}

$$\Delta T_{focus} = \frac{P_{in} \cdot \alpha_{abs}}{2 \cdot \pi \cdot K} \cdot Ln\left[\frac{D}{w_l}\right] = \frac{P_{in} \cdot \Phi_{abs} \cdot \alpha_{ext}}{2 \cdot \pi \cdot K} \cdot Ln\left[\frac{D}{w_l}\right]$$



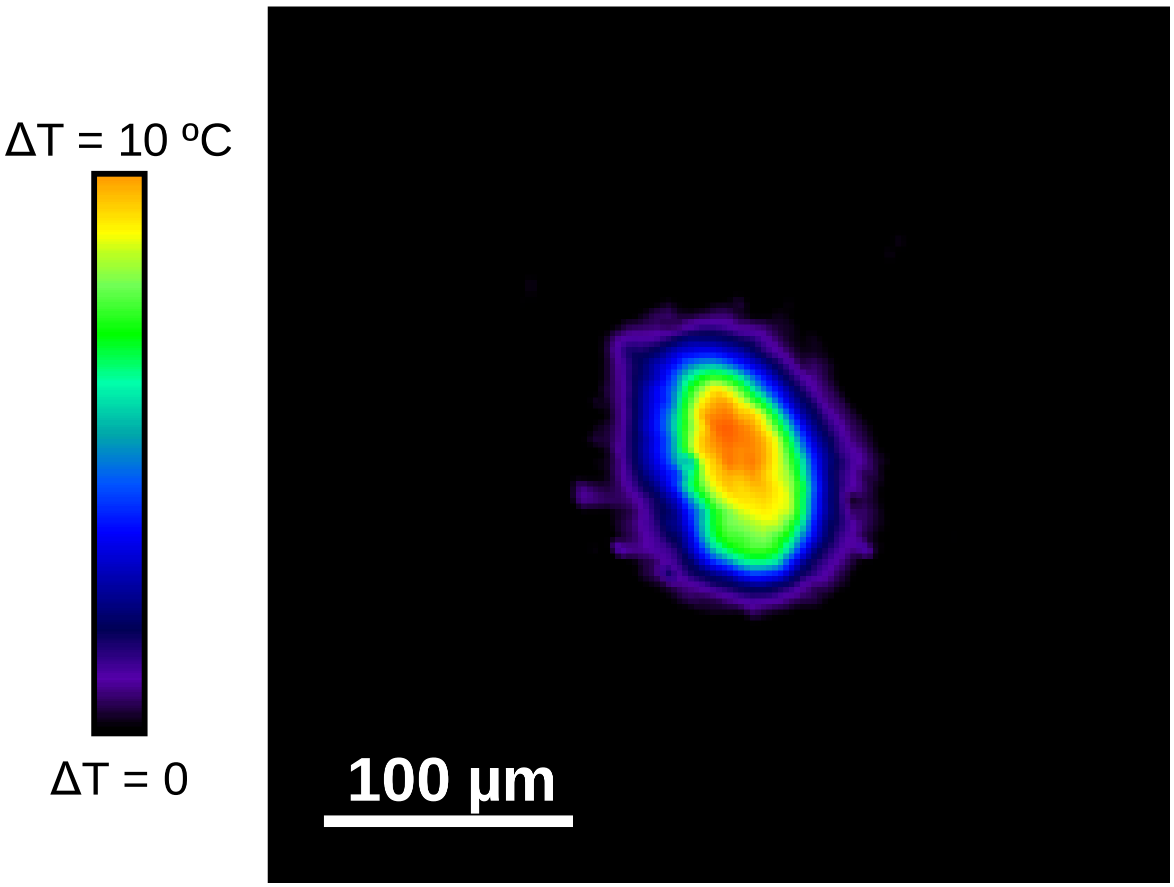
¡¡GNRs (45nm x10 nm) have a light to heat efficiency close to 1!!



Cell death

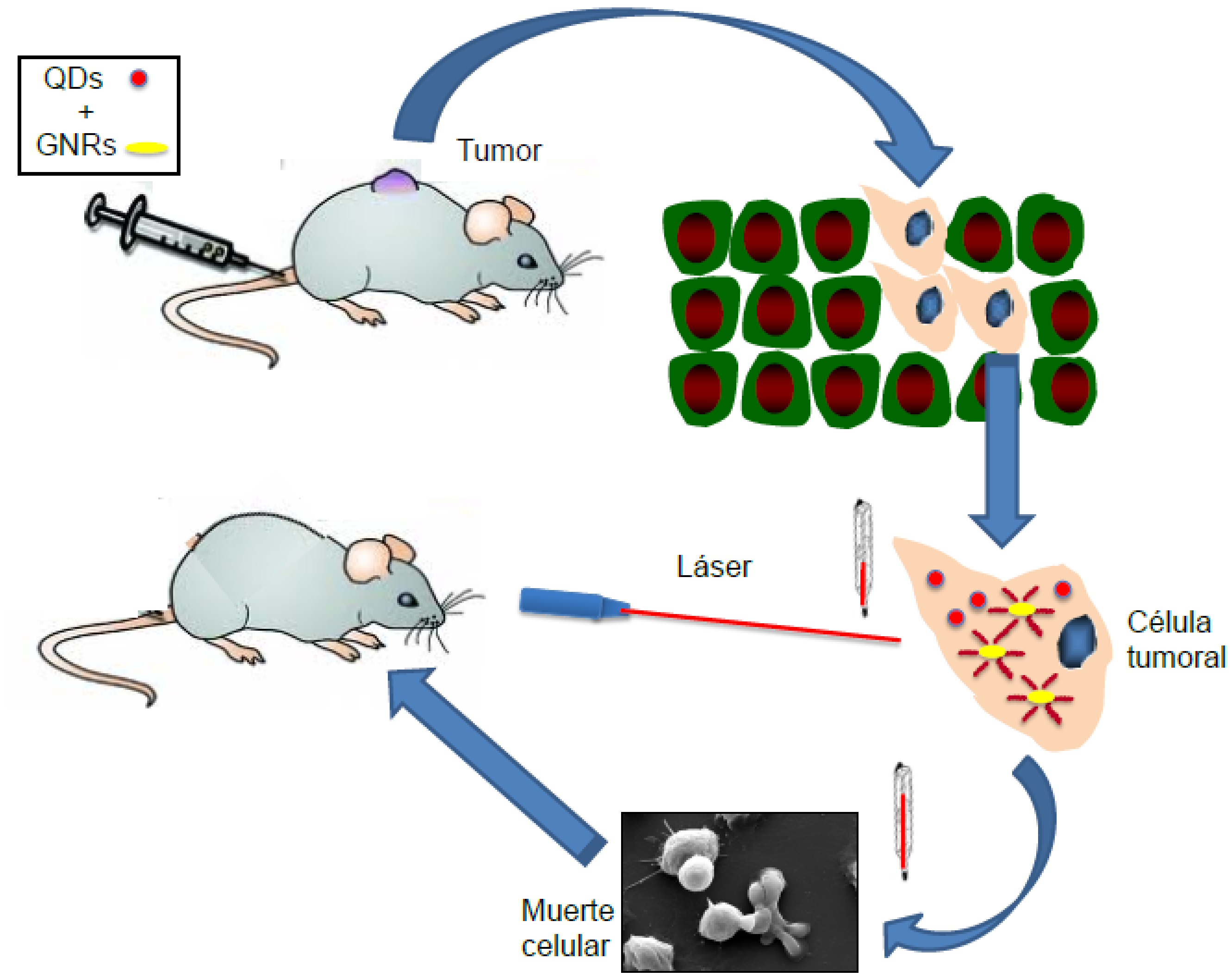
Cells unaffected

Spatial distribution of temperature



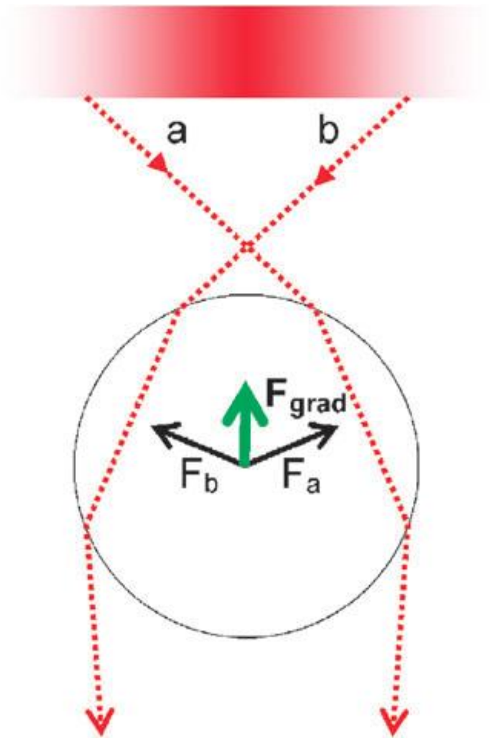
Spatial distribution of temperature increments caused by a 808 nm laser beam tightly focused within a GNR+QDs:PBS solution. Experiments were carried out by using the same concentrations as those used for the incubation of HeLa cells.

Project of “in vivo” Controlled Hyperthermia



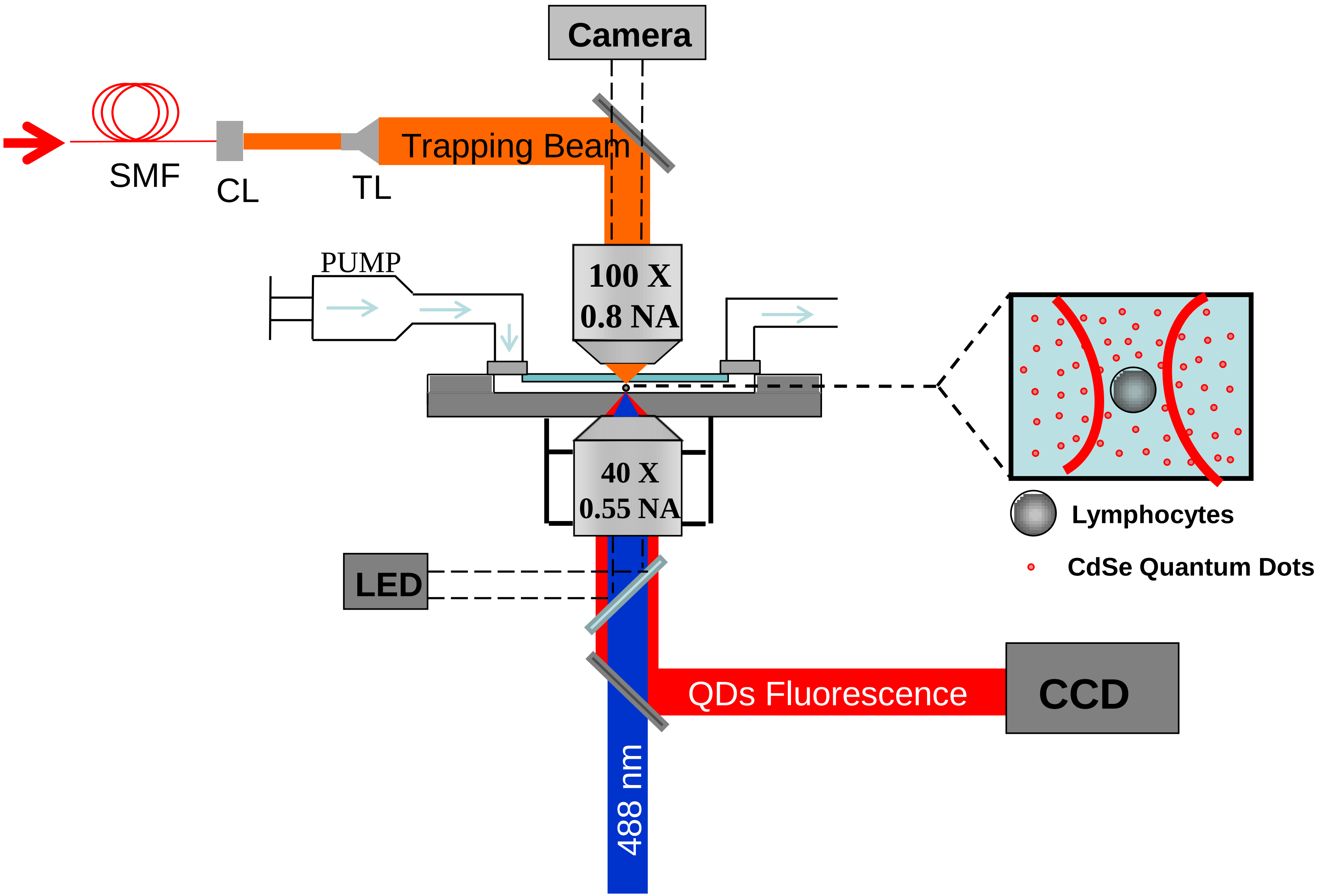
Controlled cell heating by optical trapping

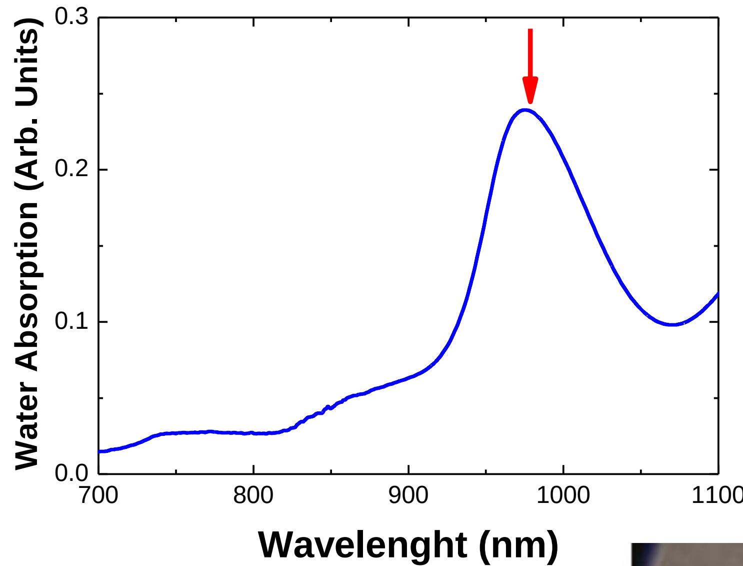
An optical trap results when a high-numerical aperture lens is used to focus a laser beam to a diffraction-limited spot

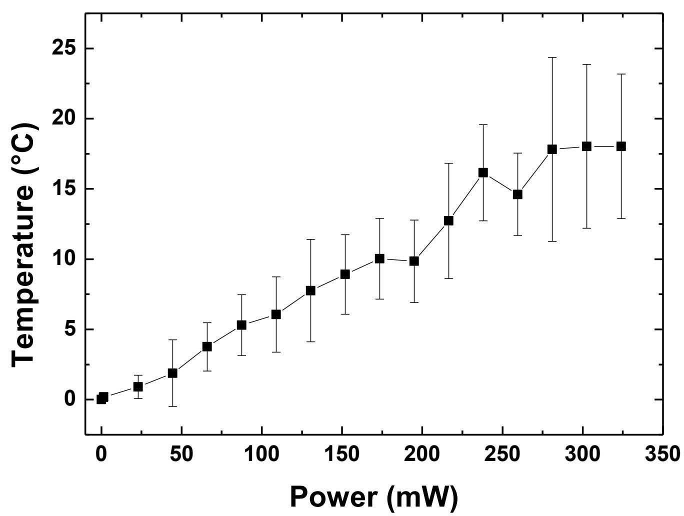


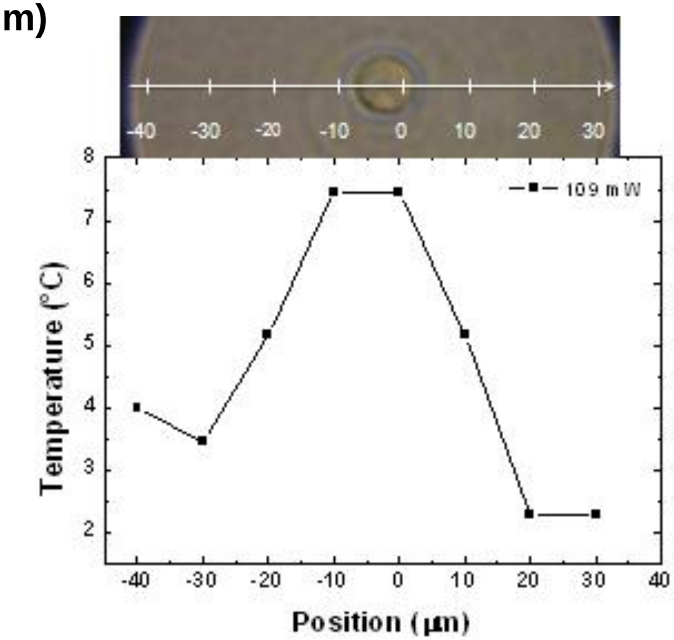
Gradient Force on a spherical particle : Forces the particle towards the higest intensity region

Lymphocites are quasi-spherical cells



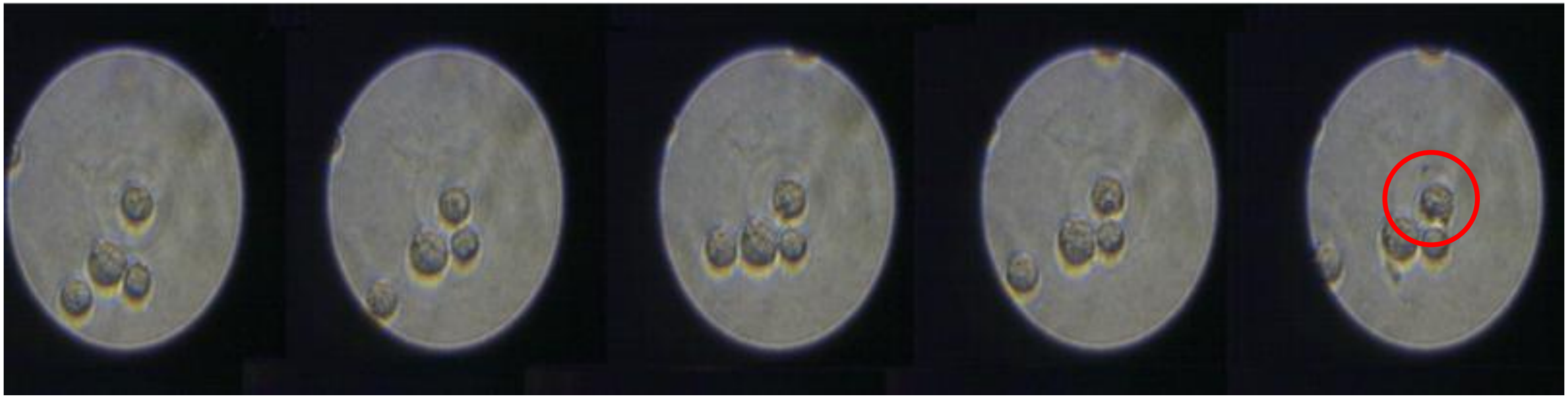

Water Absorption (Arb. Units)
0.0
0.1
0.2
0.3
700
800
900
1000
1100
Wavelenght (nm)


Temperature (°C)
0
5
10
15
20
25
0
50
100
150
200
250
300
350
Power (mW)

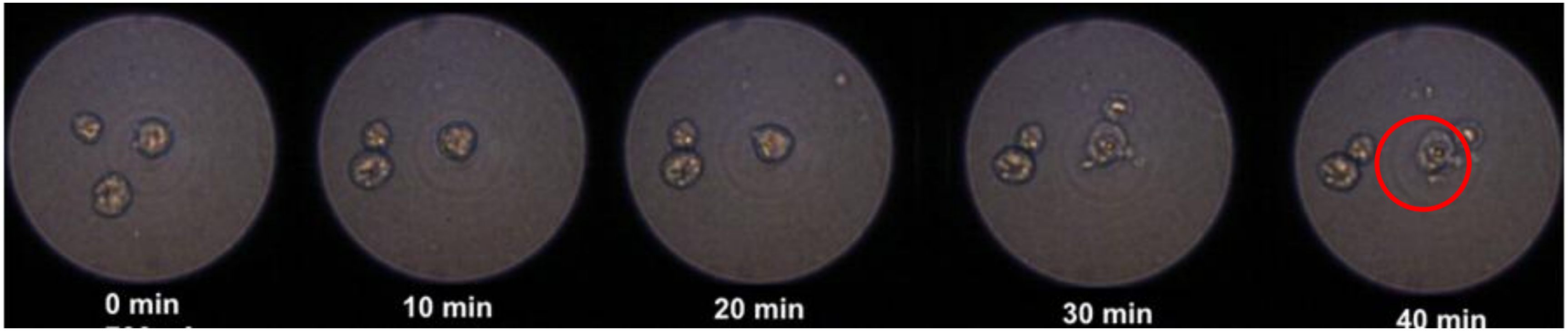

-40
-30
-20
-10
0
10
20
30
109 mW
Temperature (°C)
2
3
4
5
6
7
8
-40
-30
-20
-10
0
10
20
30
40
Position (µm)

Temperature induced cell damage during trapping

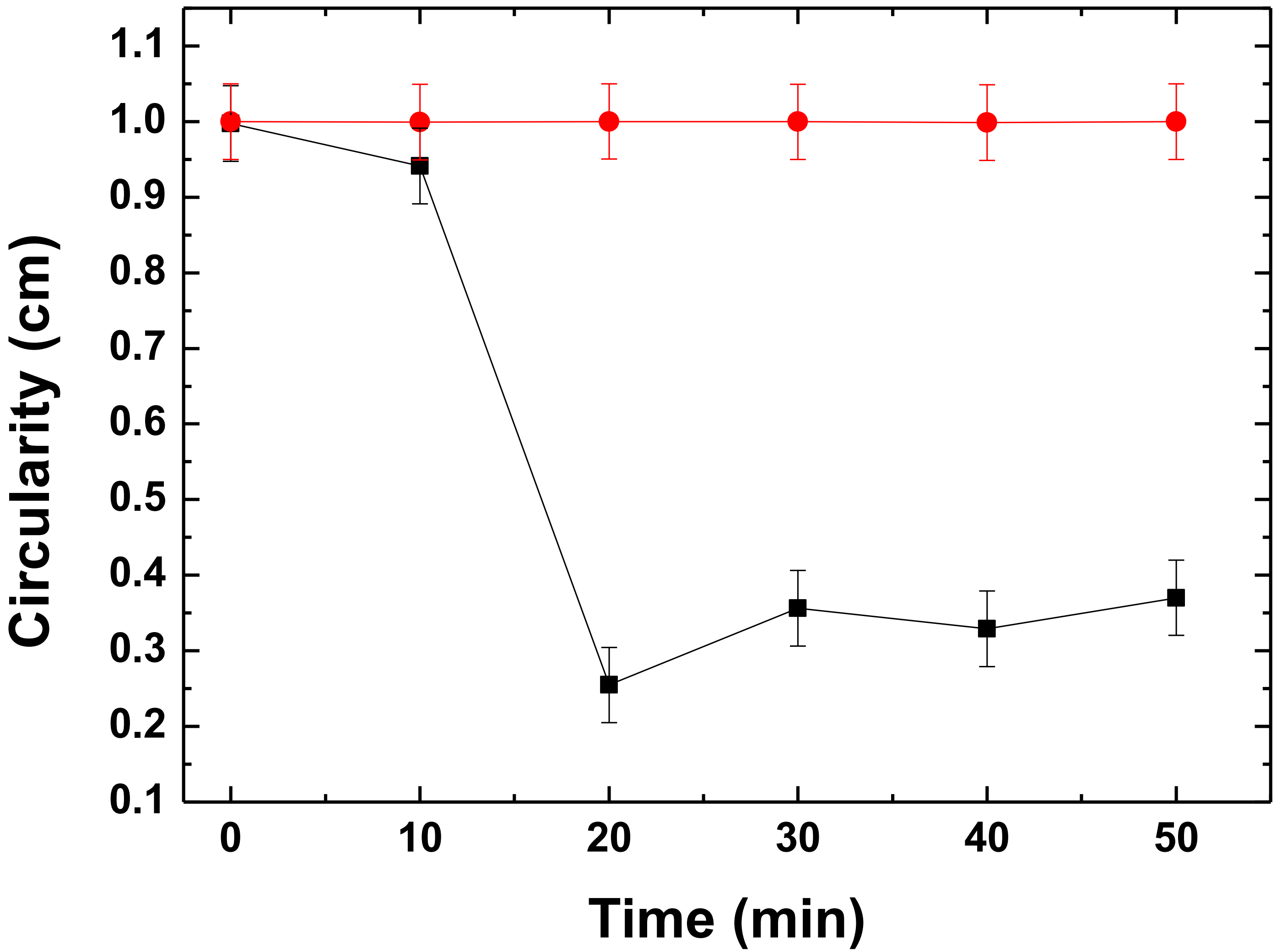
Low power (< 100 mW)



Power (110 mW)



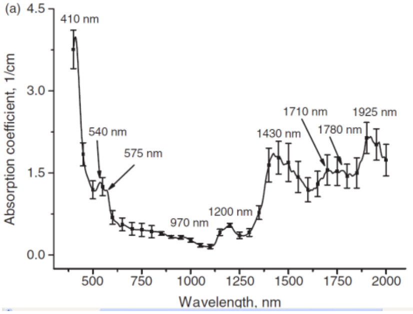
Optical trapping of lymphocytes with 110 mW reveals a significant reduction in circularity due to the irregular shape (apoptosis) induced by trap/heating.

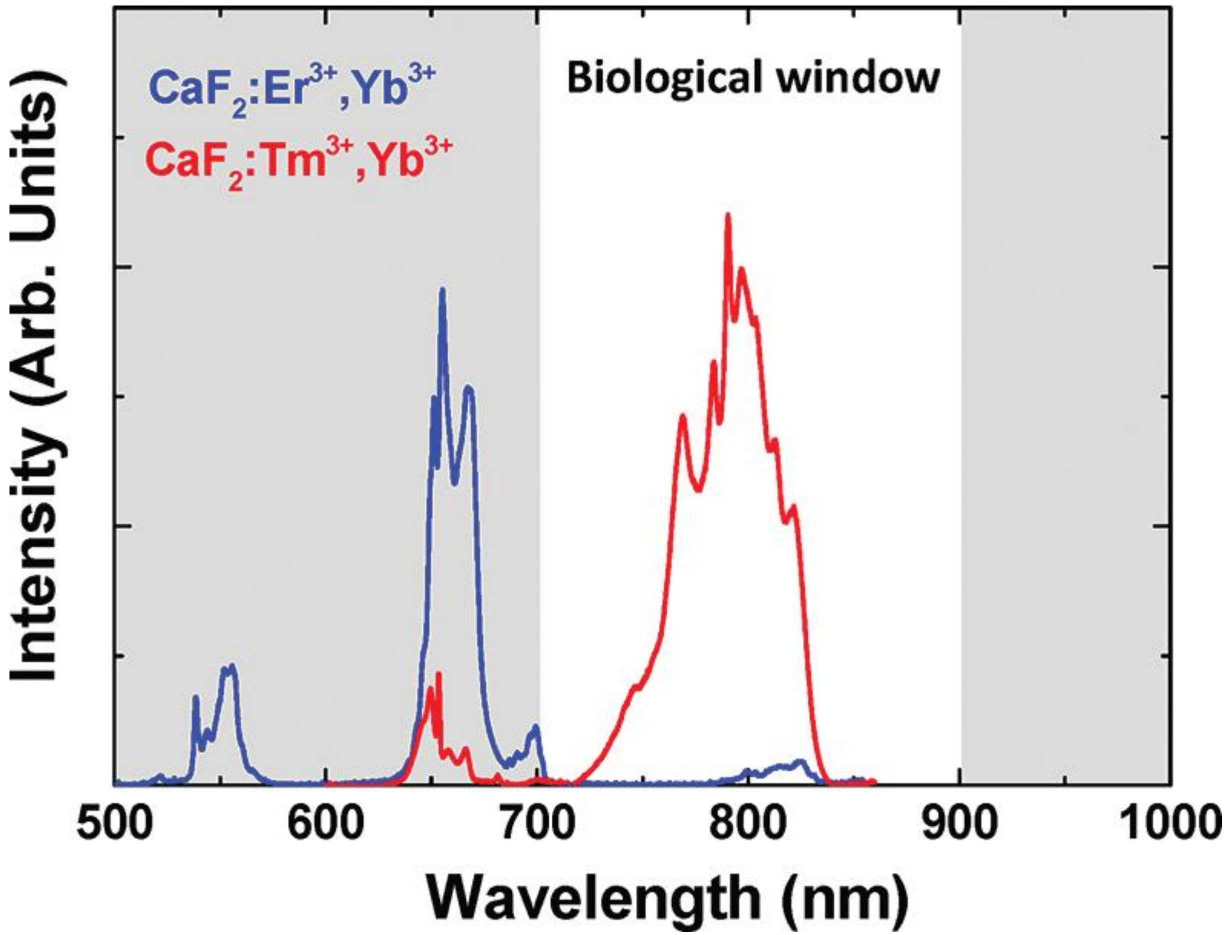


Deep tissue imaging

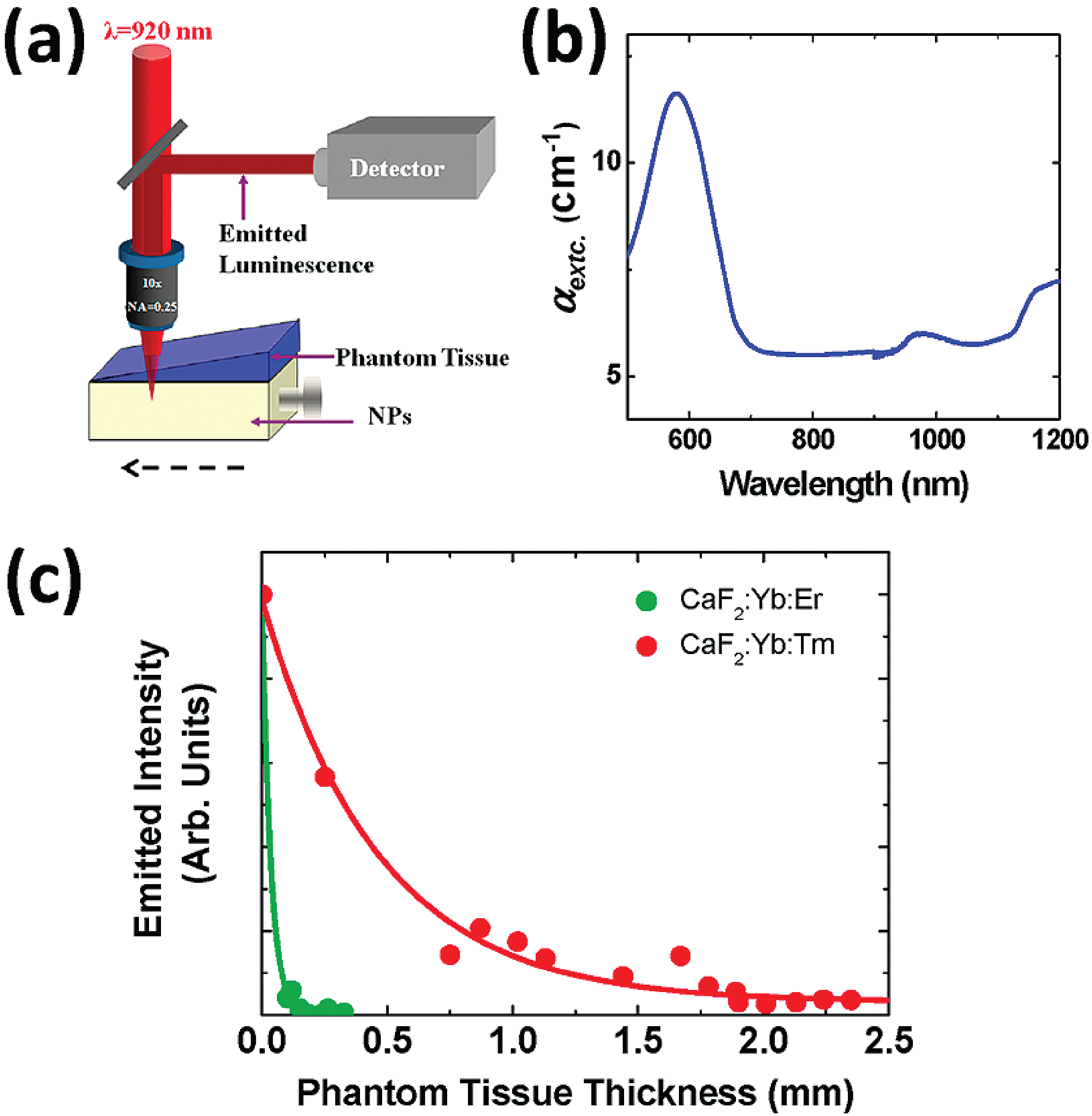
Novel IR –IR probes

Tm^{3+} vs Er^{3+}: Deeper tissue penetration

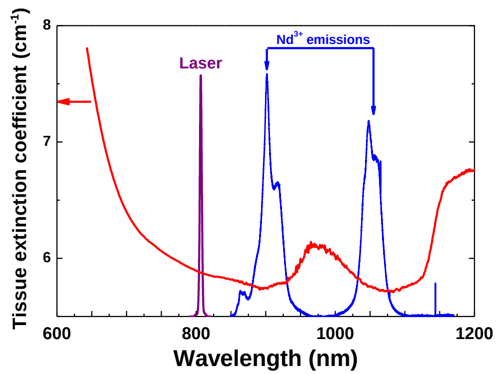


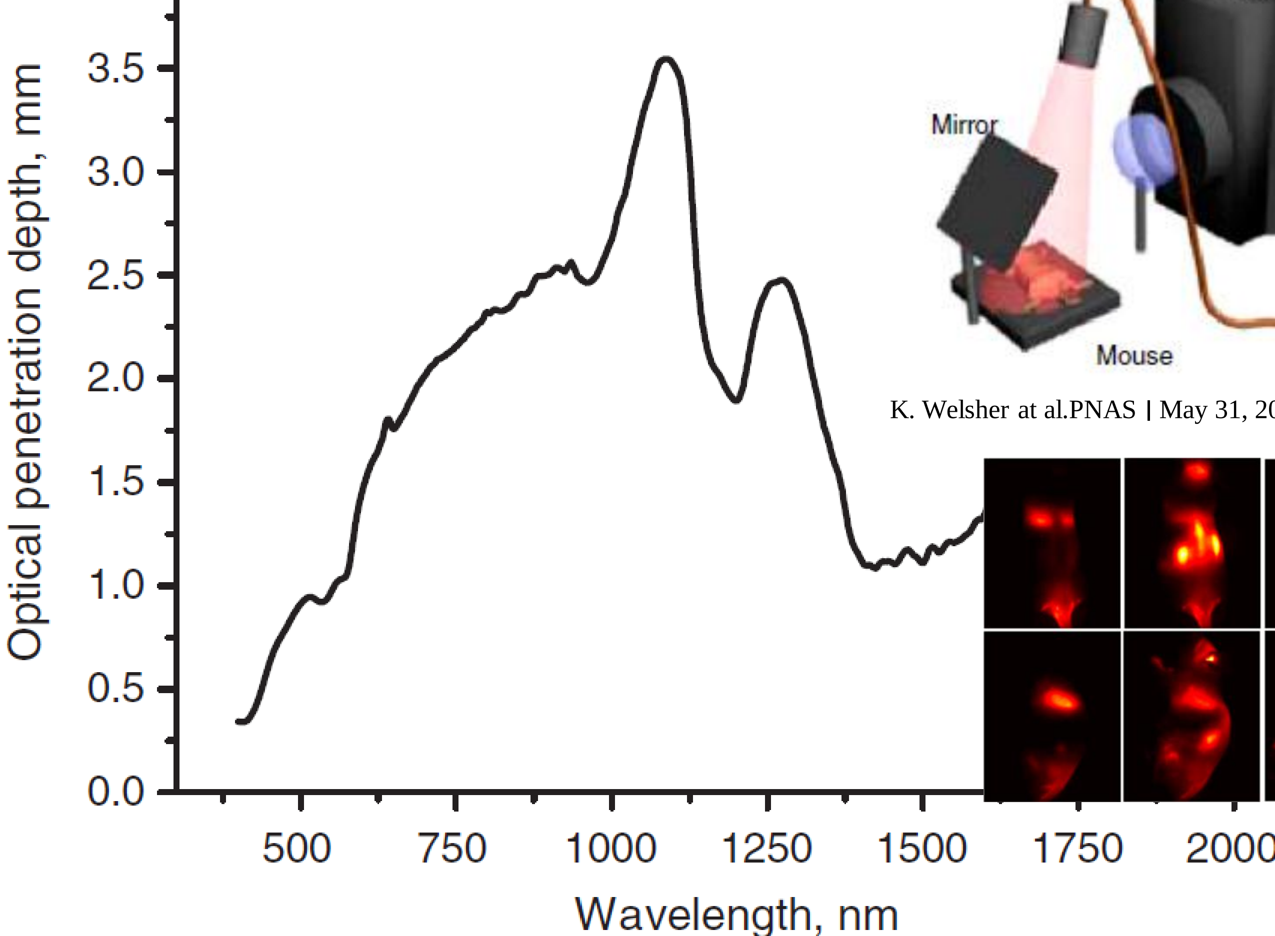


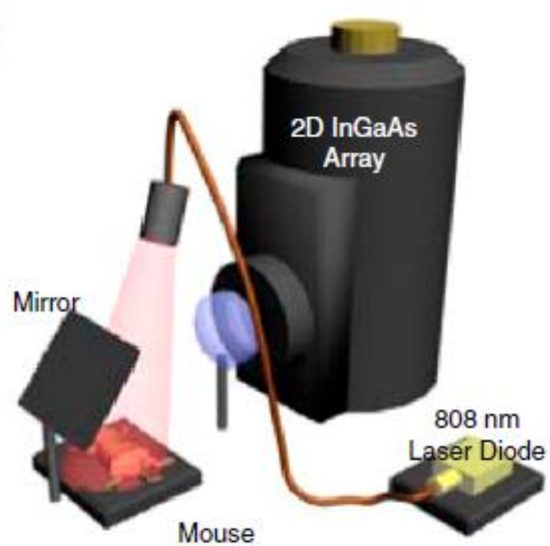
Dong, Pedroni et al, ACS Nano, 15, 8665 (2011)



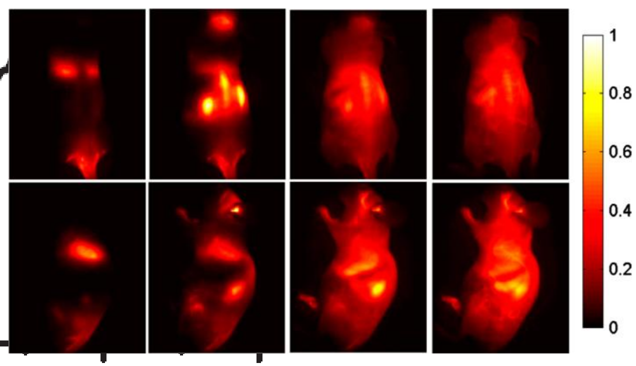
(a)
λ=920 nm
Detector
Emitted
Luminescence
10x
NA=0.25
Phantom Tissue
NPs
(b)
$\alpha_{extc.}$ (cm^{-1})
10
5
600
800
1000
1200
Wavelength (nm)
(c)
CaF$_2$:Yb:Er
CaF$_2$:Yb:Tm
Emitted Intensity
(Arb. Units)
0.0
0.5
1.0
1.5
2.0
2.5
Phantom Tissue Thickness (mm)

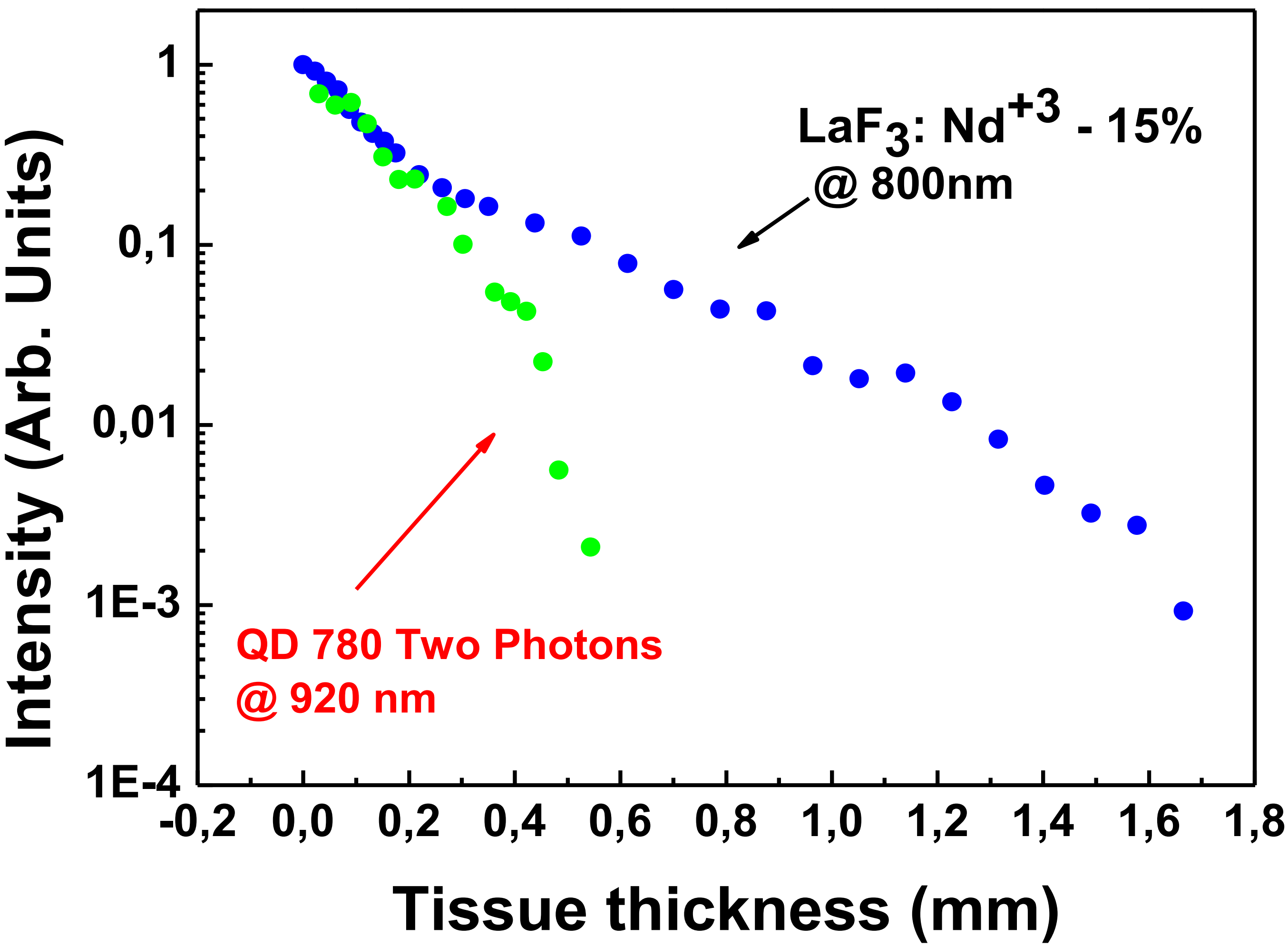






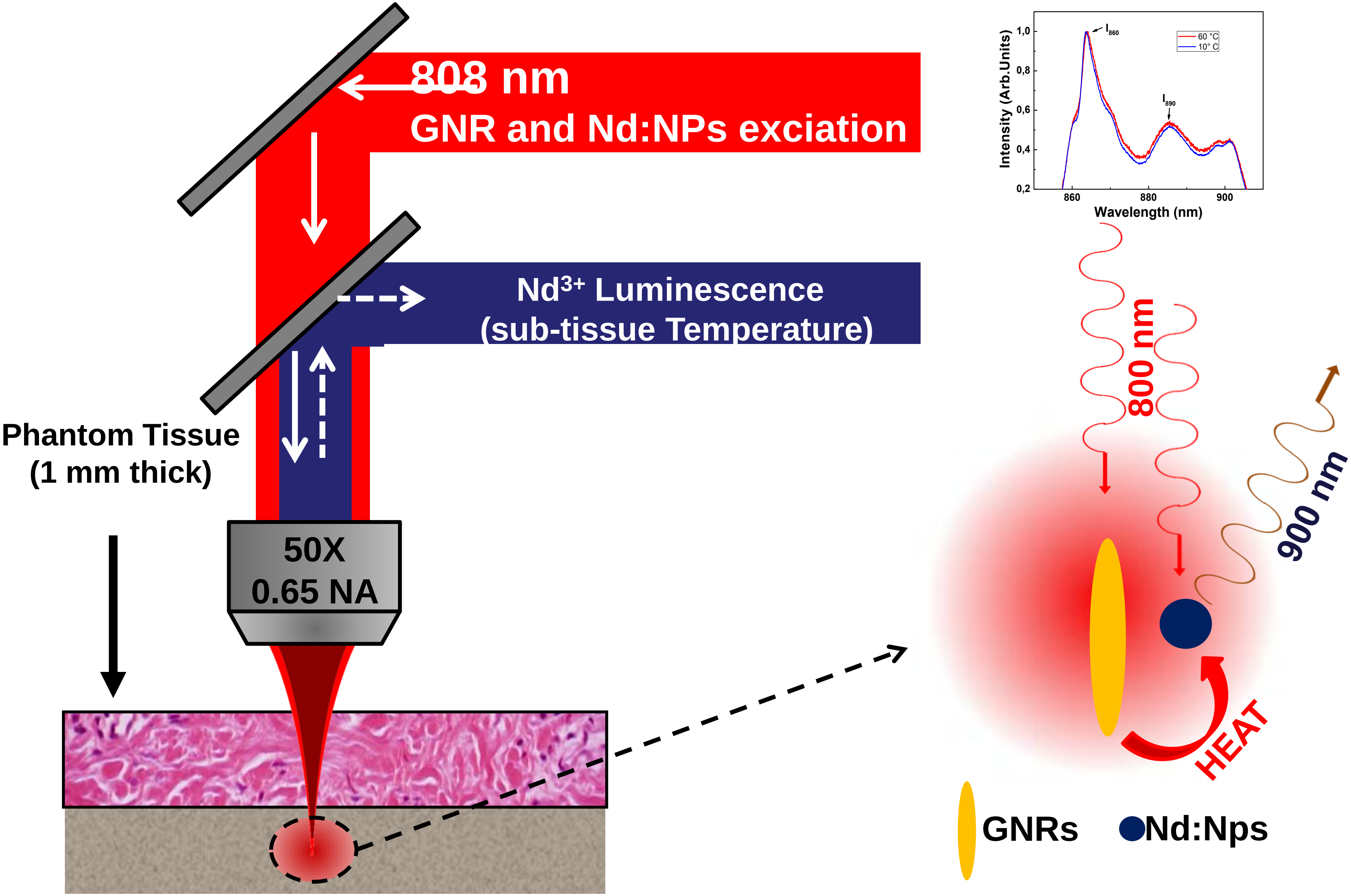
K. Welsher at al.PNAS | May 31, 2011 | vol. 108 | no. 22 | 8943

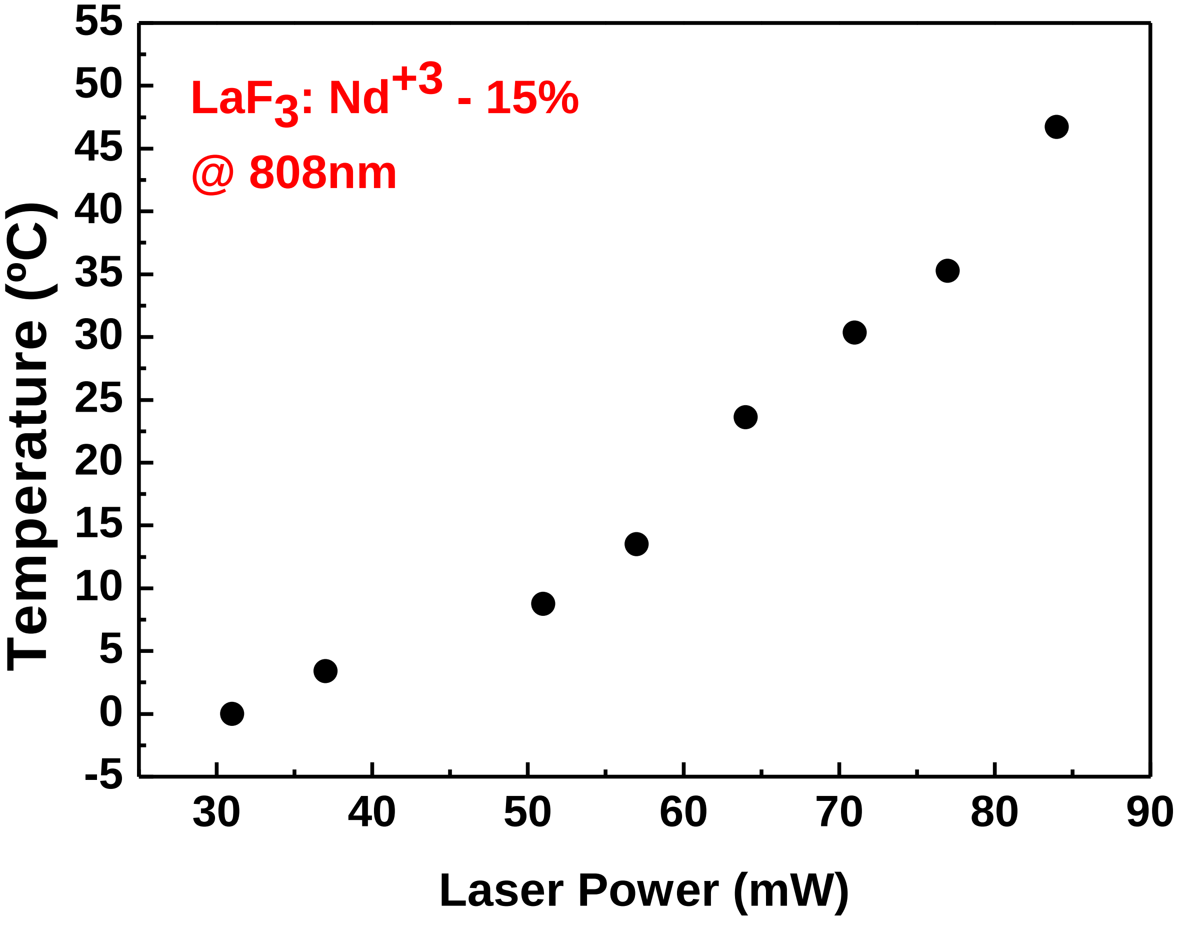




Intensity (Arb. Units)
1
0,1
0,01
1E-3
1E-4
-0,2
0,0
0,2
0,4
0,6
0,8
1,0
1,2
1,4
1,6
1,8
Tissue thickness (mm)
LaF3: Nd+3 - 15%
@ 800nm
QD 780 Two Photons
@ 920 nm

Across tissue temperature measurements

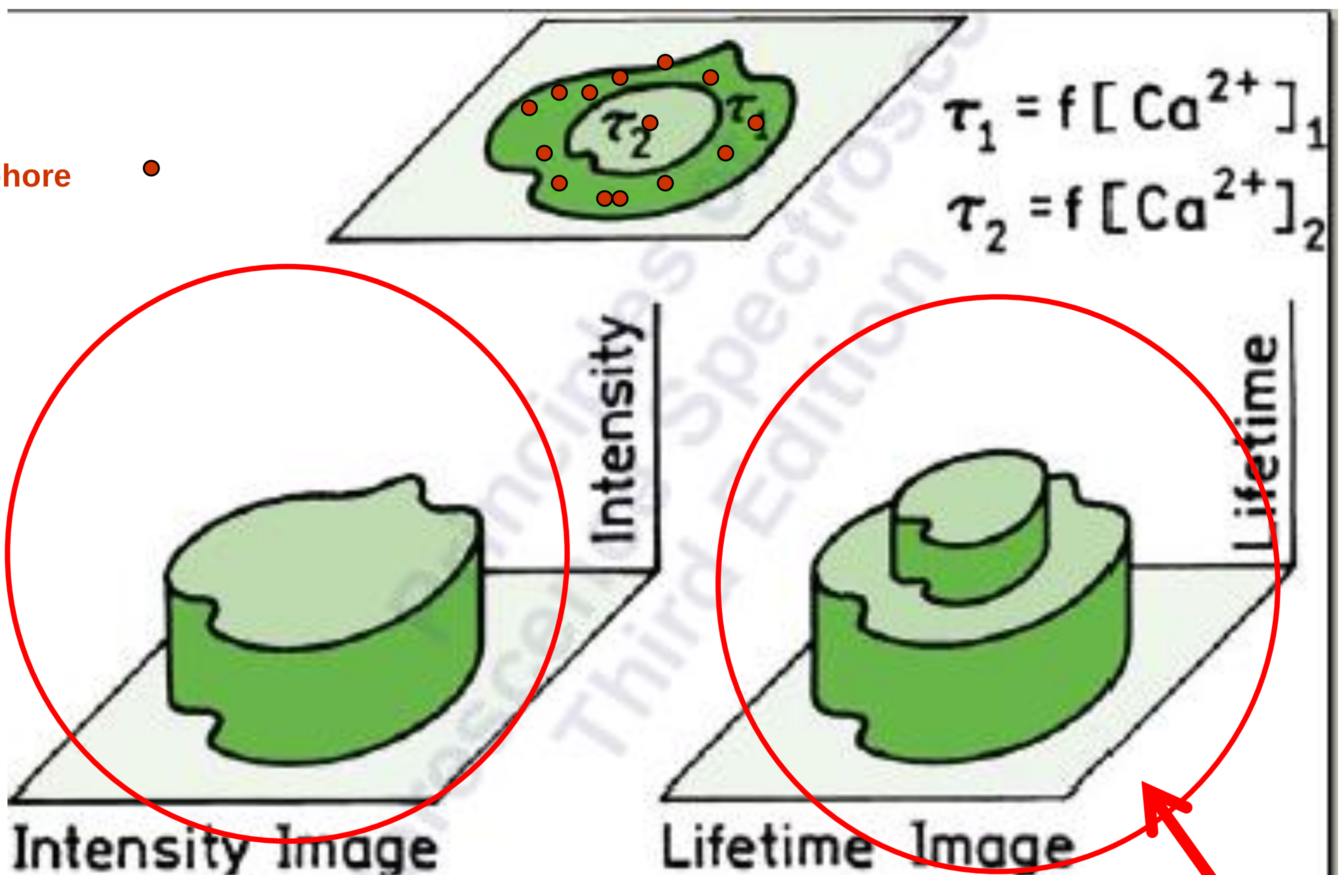




LaF3: Nd+3 - 15%
@ 808nm
Temperature (ºC)
Laser Power (mW)
55
50
45
40
35
30
25
20
15
10
5
0
-5
30
40
50
60
70
80
90

Fluorescence Lifetime Imaging

fluorophore

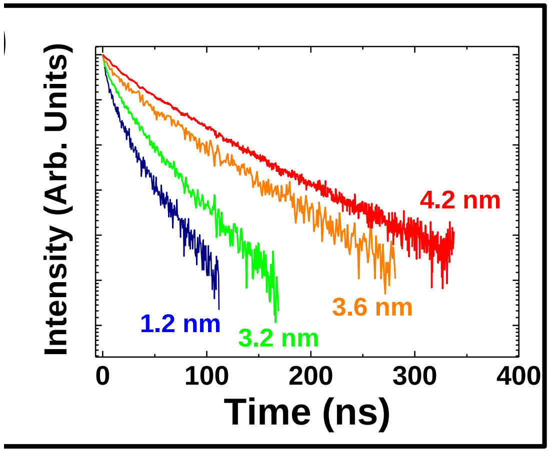


Imaging faster than the regular Fluorescence Imaging

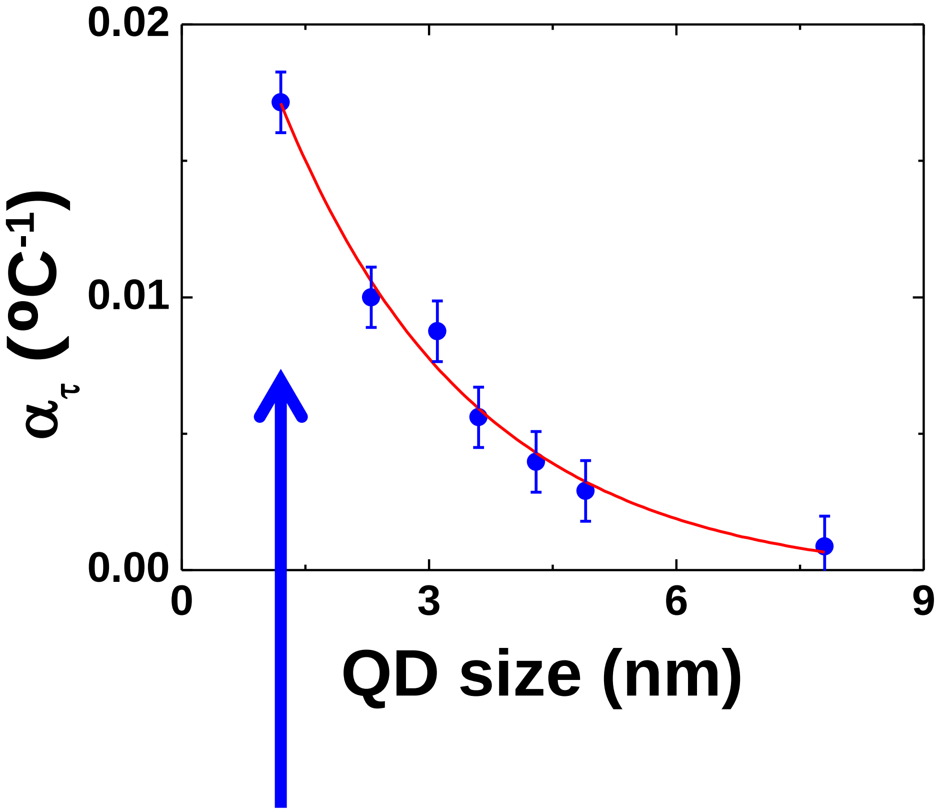
¡Dependent on fluorophore concentration!

¡Independent on fluorophore concentration!

CdTe-QDs for Lifetime imaging



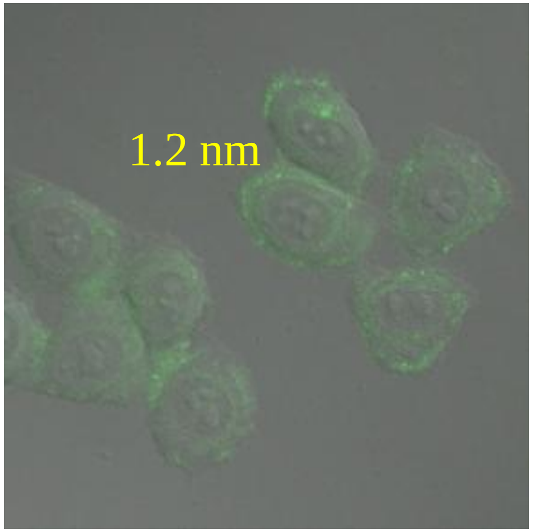
P. Haro et al, Small (2012)

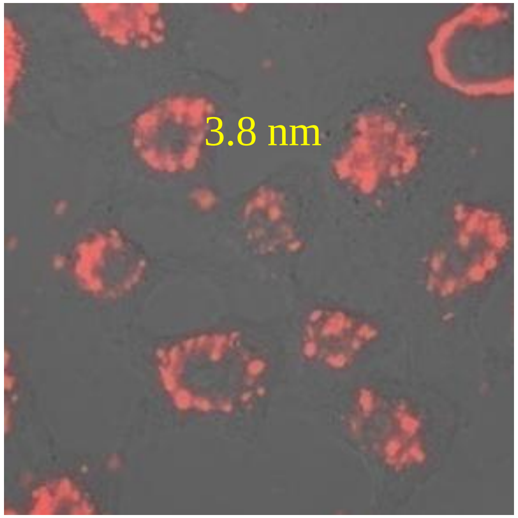


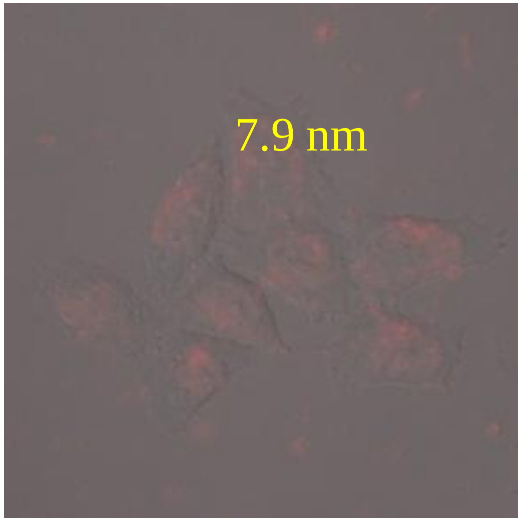
Most suitable QDs for Lifetime Imaging

¡The size also plays a role for brightness!

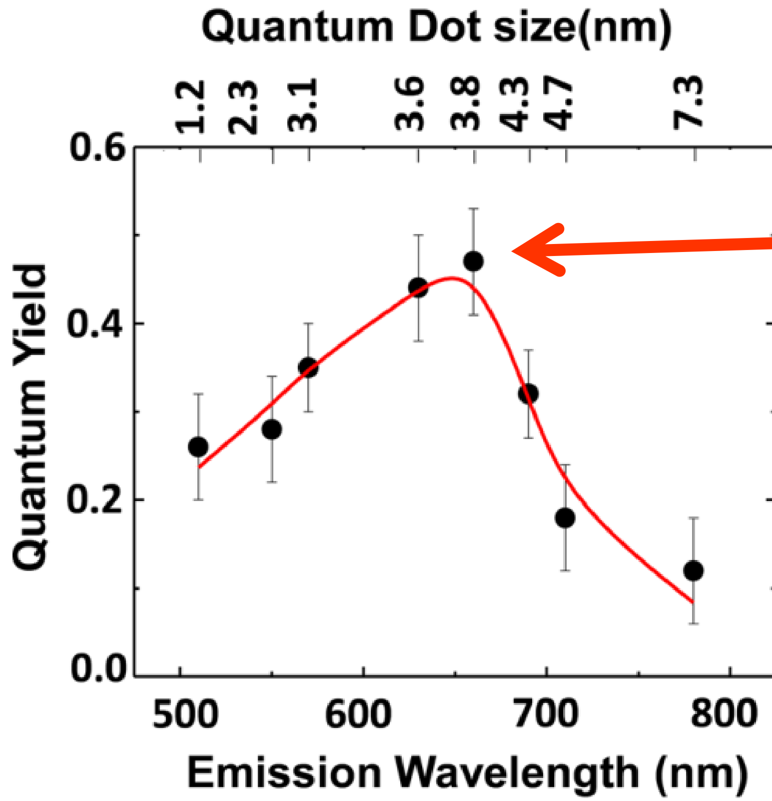
CdTe-QDs



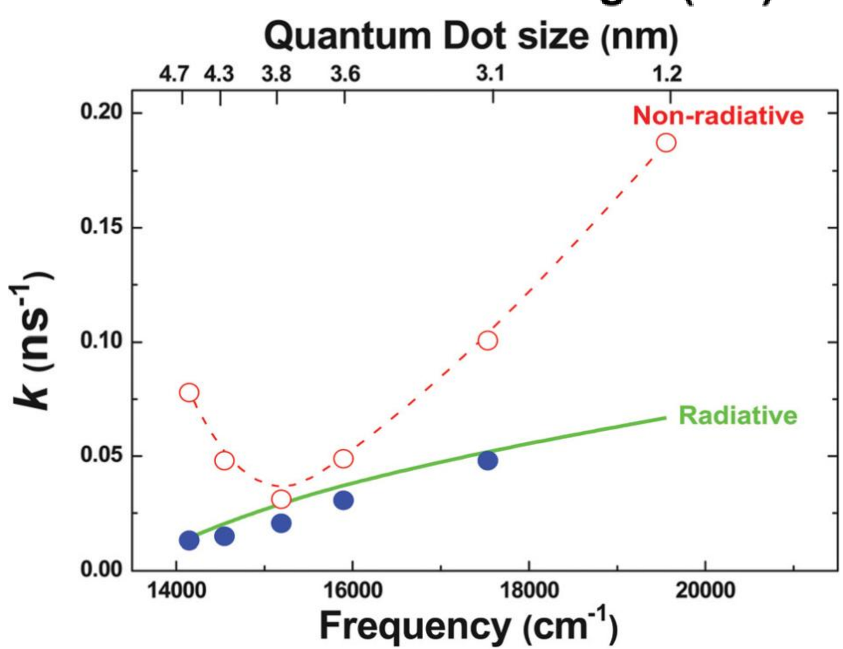




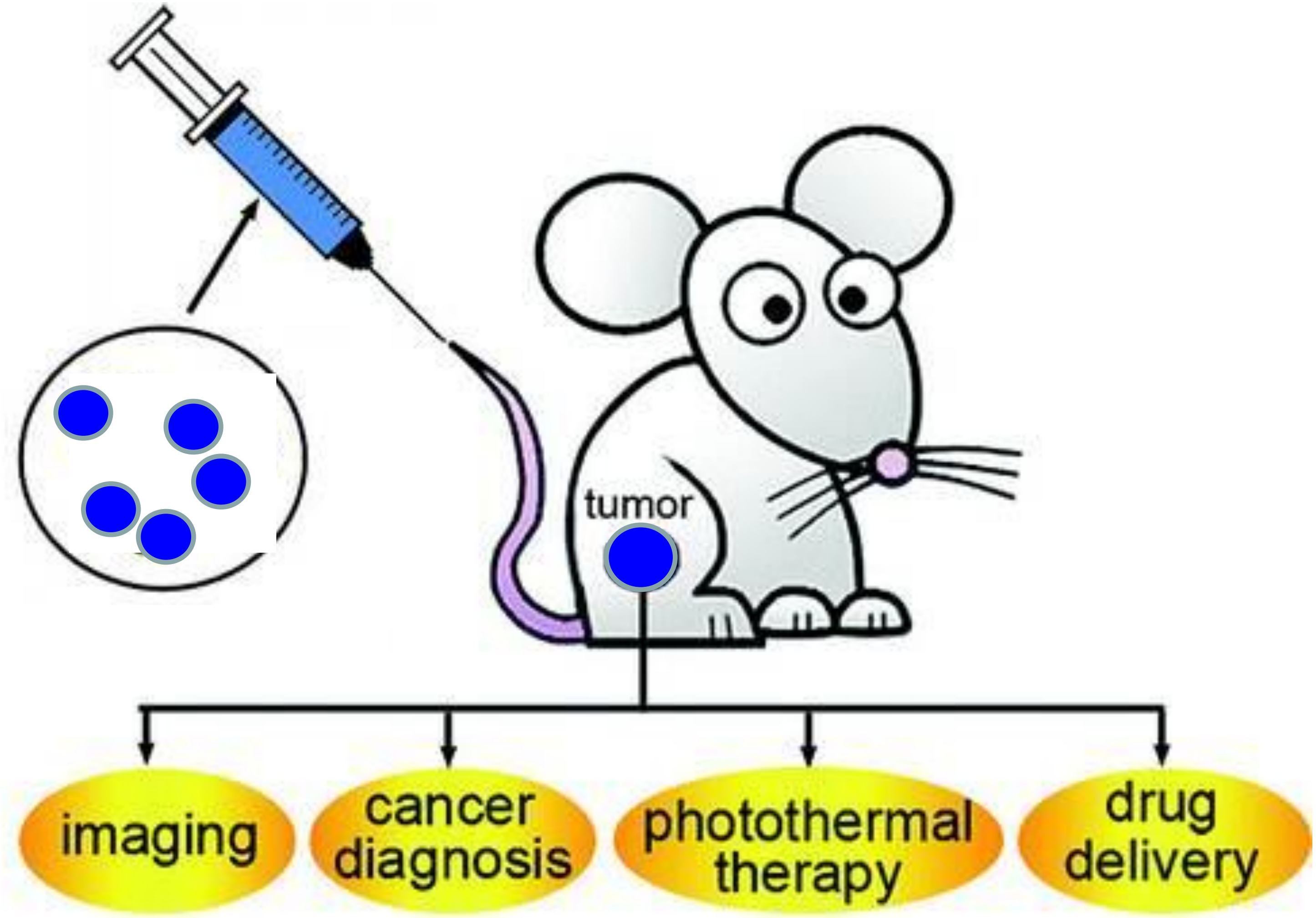
L. M. Martinez Maestro et al. Journal of Applied Physics., 111, 023513(2012).



Optimum size (3.8 nm)



Final Goal: Multifunctional Nanoparticles



SUMMARY

-Inorganic Nanoparticles (QDs and UCNPs) are excellent probes for multi-photon excited fluorescence thermal imaging : “Nanothermometers”

- GNRs (41 x 10 nm) are the best optical nanoheaters.

- Mixed solutions of Gold Nanorods + CdSe Quantum Dots allow for controlled hyperthermia of cancer at cellular level.

-The temperature increase of cell optically trapped can be controlled by nanothermometry: Selecting the proper wavelength apoptosis can be induced

- Nd-Activated nanoparticles have strong potential for deep tissue imaging

- CdTe are promising nanoparticles for lifetime imaging. Much faster and accurate detection