

IMPROVEMENT OF UPCONVERSION DEEP-TISSUE IMAGING WITH OPTICAL CLEARING

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Facts about Finland



Independent: 1917

European Union: 1995

Euro: 1999

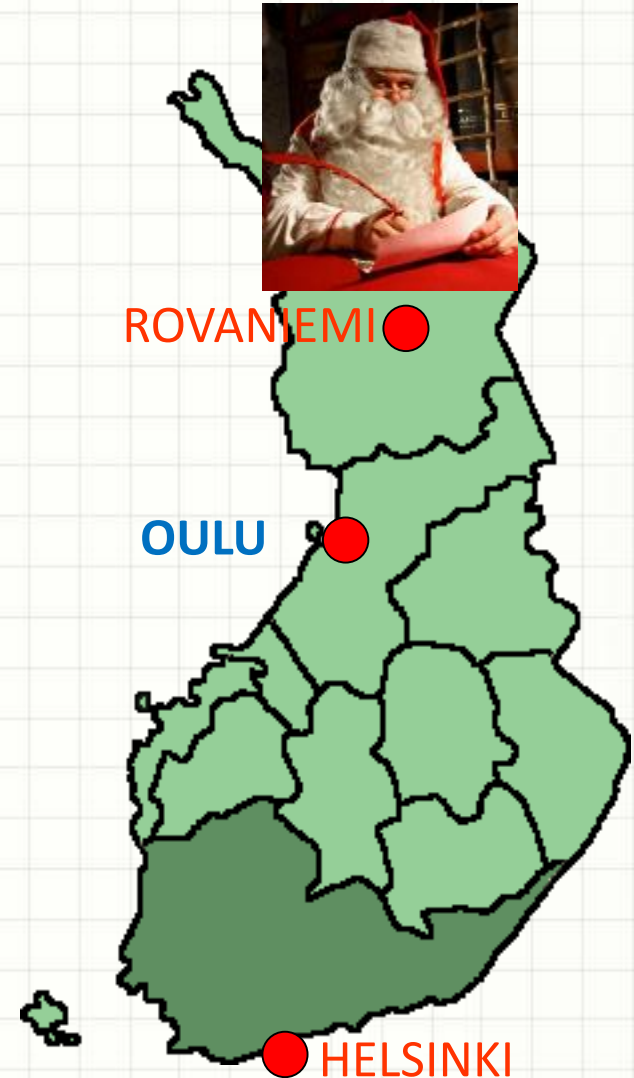
Population: 5.3 million

Marked area: 63% of population

Helsinki & beyond: 1 million

Official languages: Finnish, Swedish

Neighbours: Russia, Sweden, Norway



Facts about Oulu

Founded: 1605 by King Karl IX of Sweden

Population: 190.000 (No. 4 in Finland)

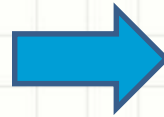
Location: by Gulf of Bothnia

Helsinki - 650 km, Arctic Circle – 200 km

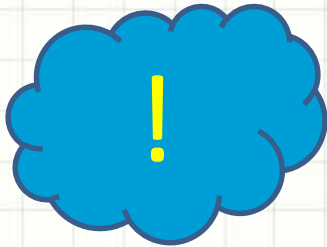


Motivation

Diagnostic imaging



Scattering & absorption



Solution



Near-infrared spectral range
(650-1100 nm)

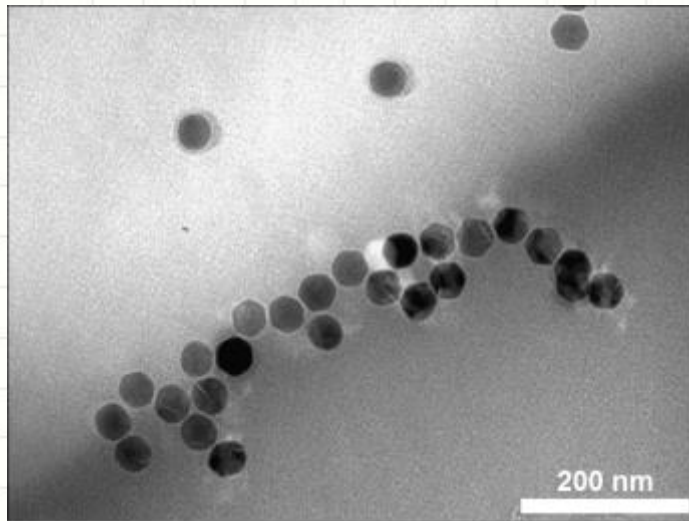


Upconversion luminophores
Excitation: 975 nm
Emission: 800 nm

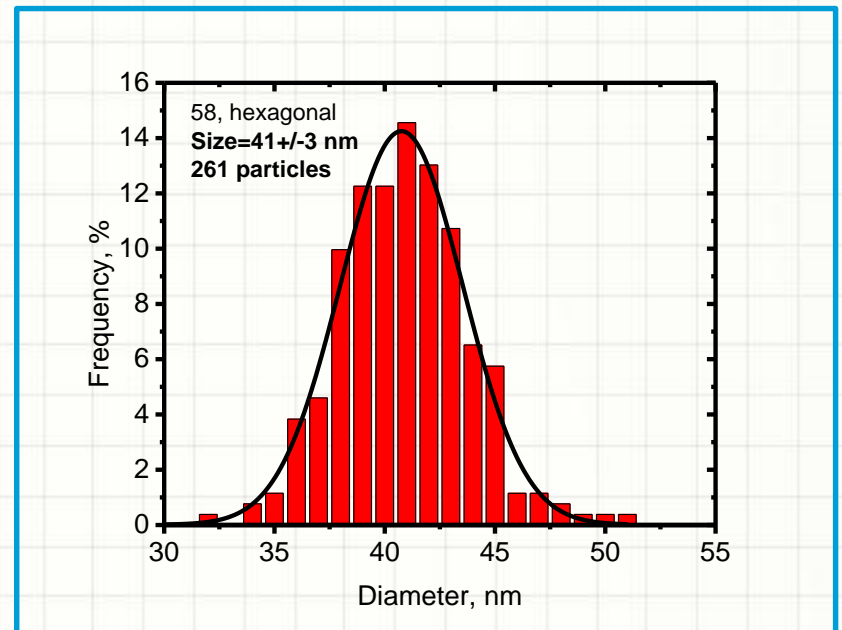


Optical clearing

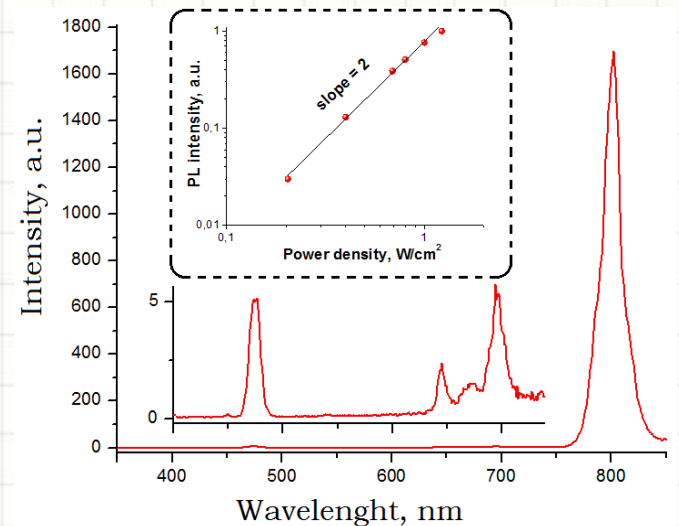
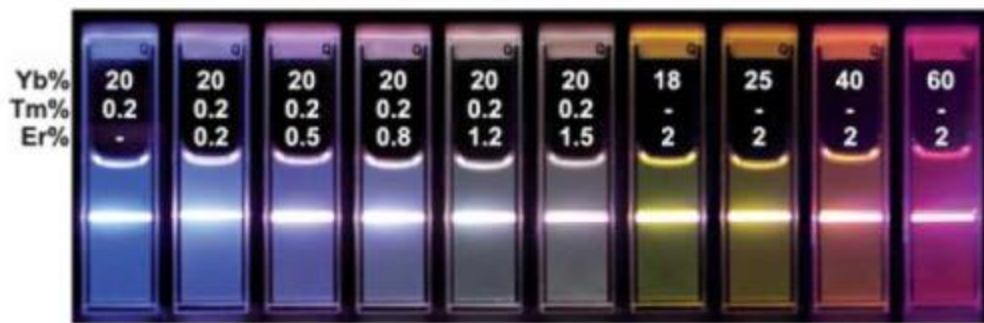
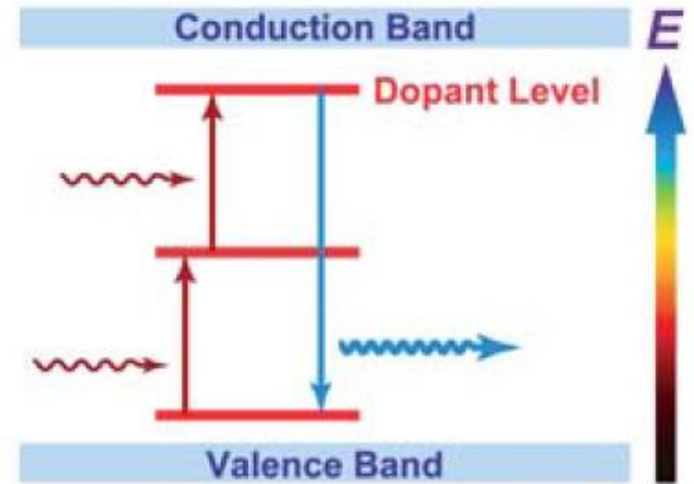
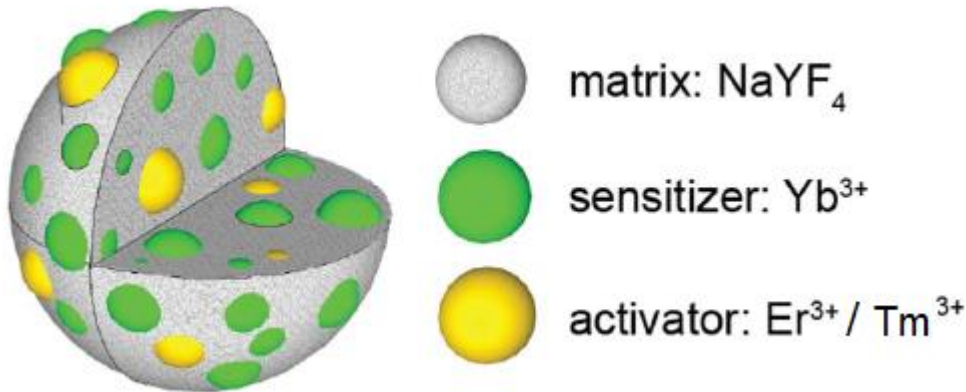
Upconversion nanoparticles



TEM micrograph and corresponding size distribution of in-house fabricated $\text{NaYF}_4:\text{Yb}^{3+},\text{Tm}^{3+}$ nanoluminophores



Upconversion nanoparticles



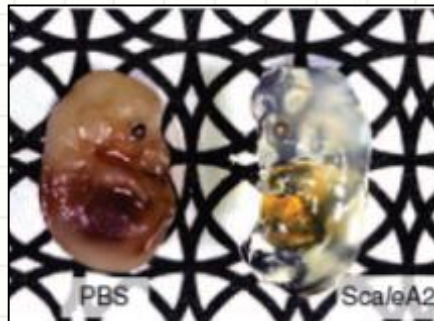
Optical clearing

⇒ **Refractive index matching of tissue components and interstitial fluid (ISF)**
due to:

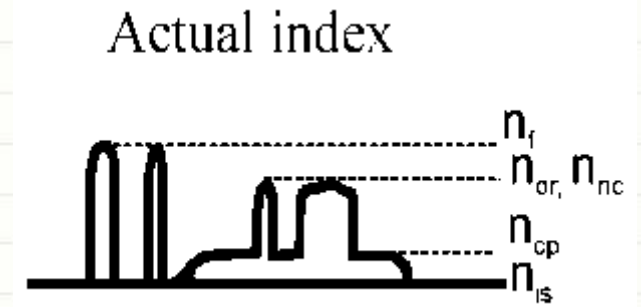
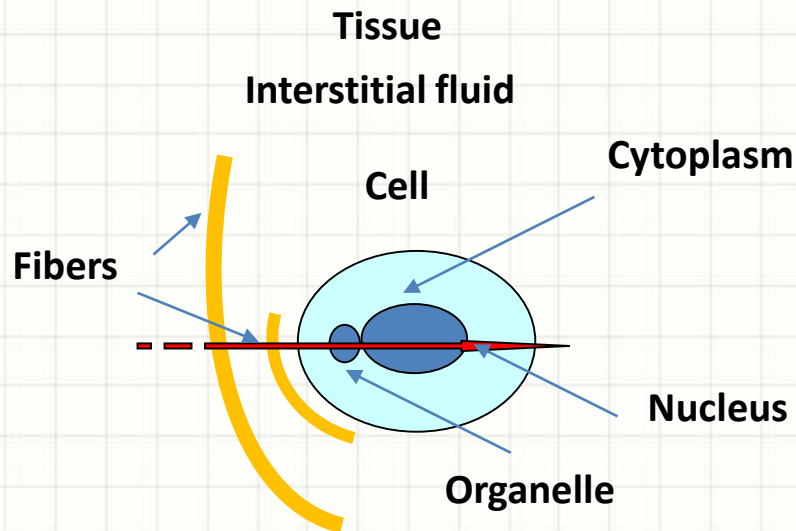
1) agent diffusion into the tissue (mostly to ISF)

2) tissue dehydration (osmotic action of solutes or water displacement
caused by compression)

⇒ **Tissue shrinkage** (less **thickness** and better **ordering** of tissue compounds)
also may have a significant effect at both – immersion and compression
optical clearing



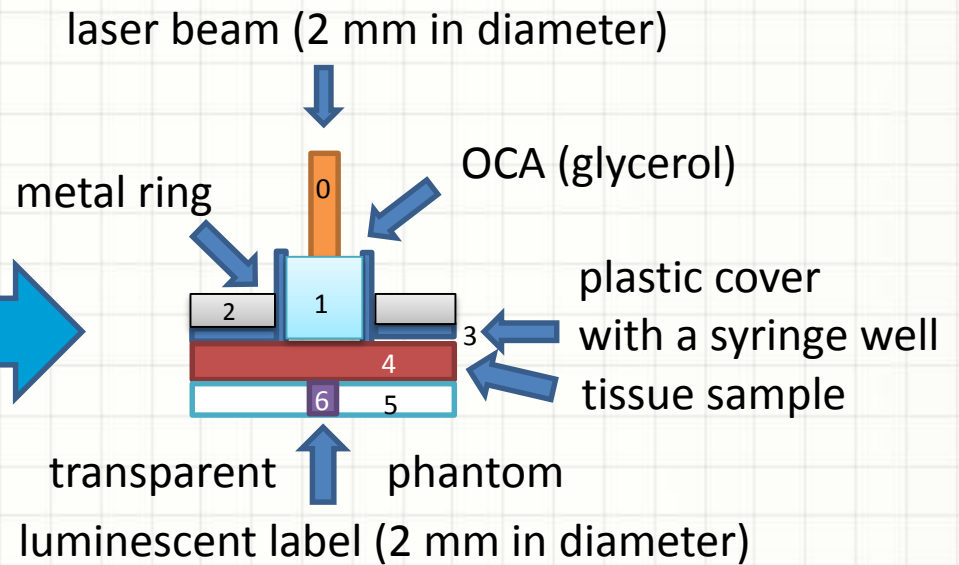
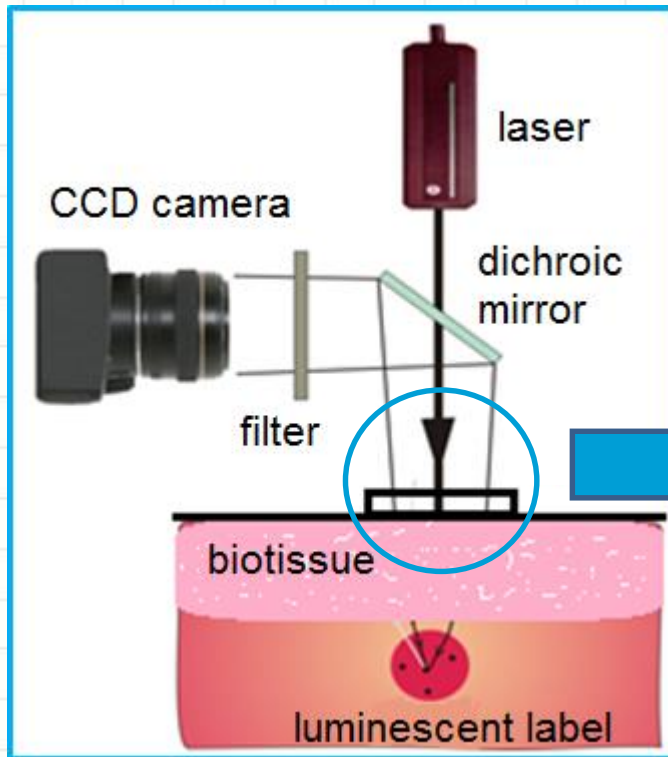
Optical clearing mechanism



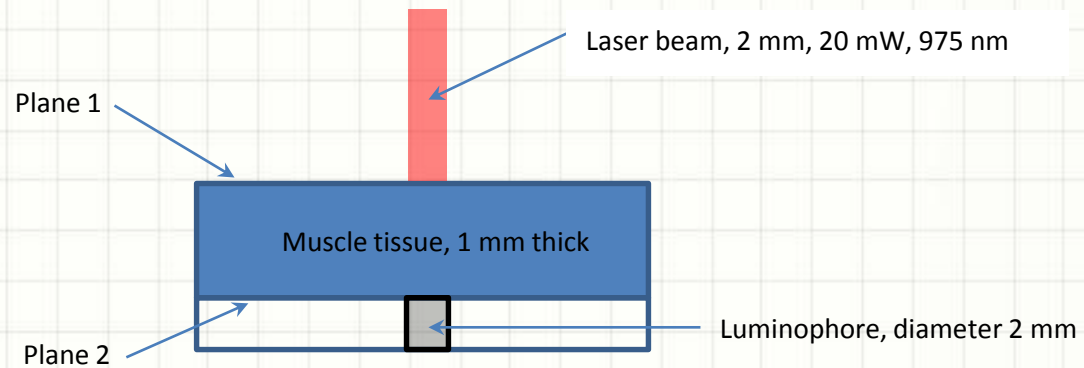
$$\mu_s' \sim d^2 \rho (d/\lambda)^{0.37} (m - 1)^2$$

$m \equiv n_s/n_0$ defines the scattering coefficient

Setup for OCA-enhanced luminescence

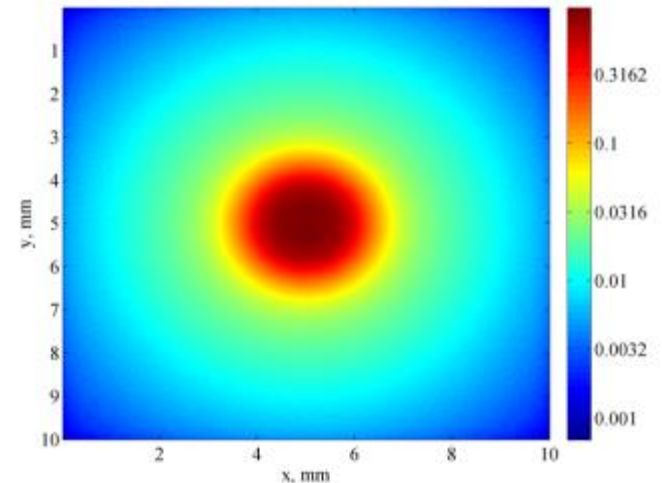
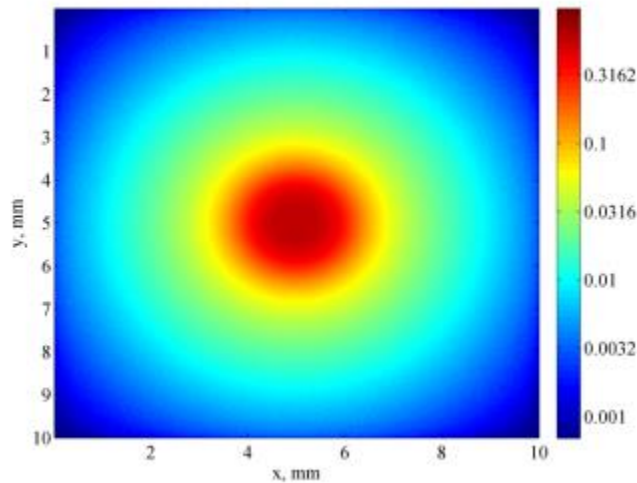


Monte Carlo simulations



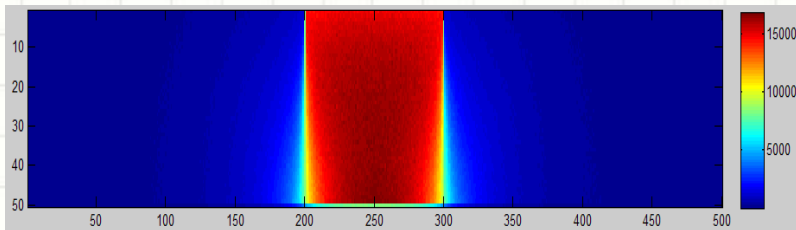
Before clearing

After clearing

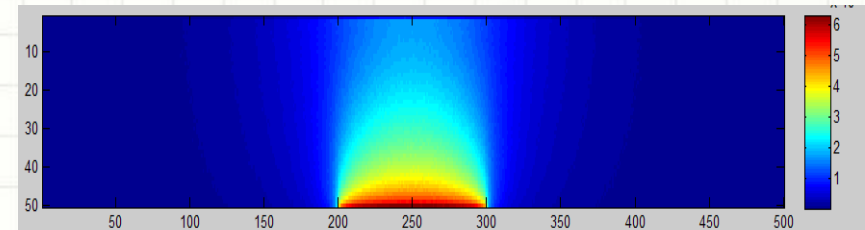


Monte Carlo simulations

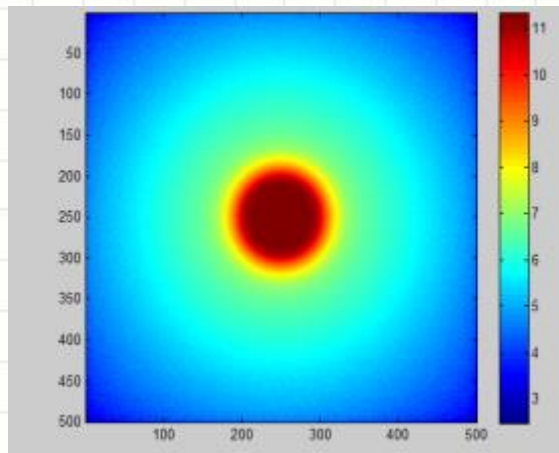
Excitation: in-depth (input beam: 2 mm)



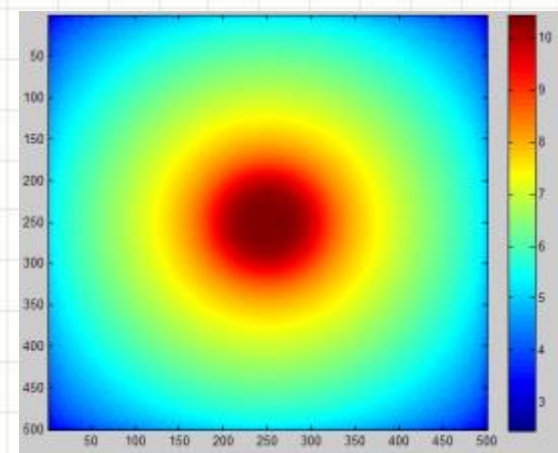
Luminescence: in-depth



Excitation: bottom (spot: 2 mm)

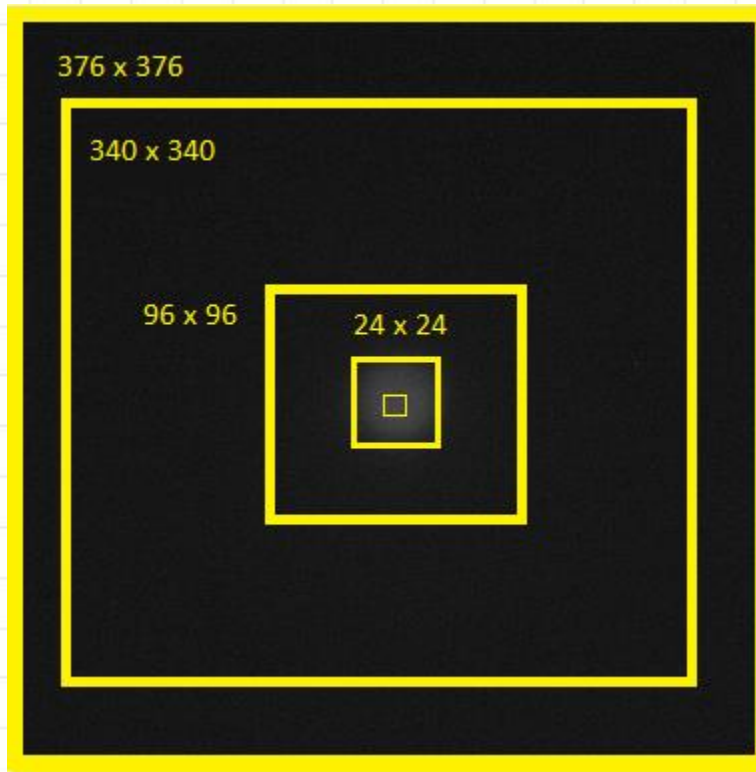


Luminescence: surface



Incident beam: 10^9 photons

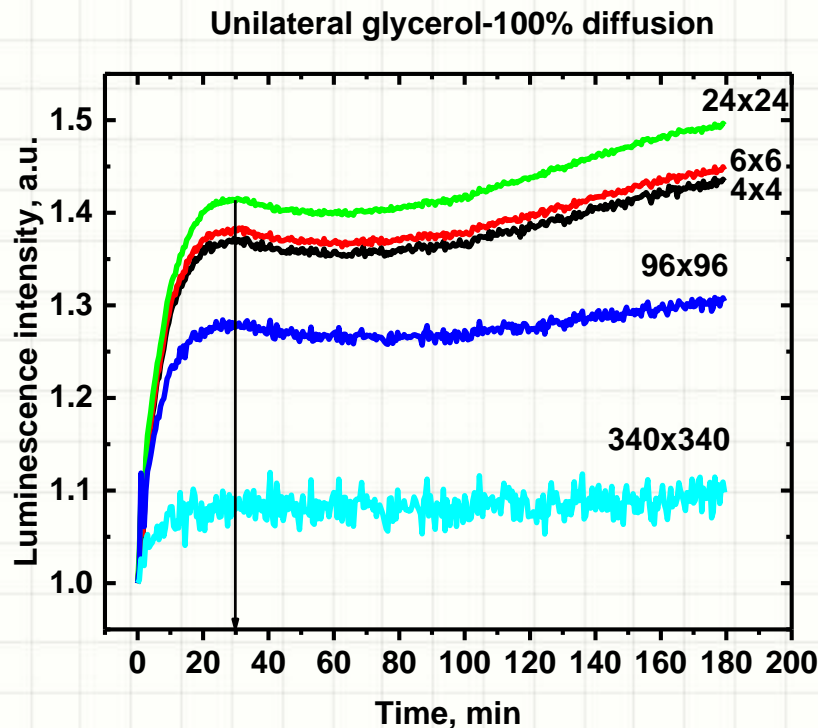
Optical clearing *in vitro*: effect of detection aperture



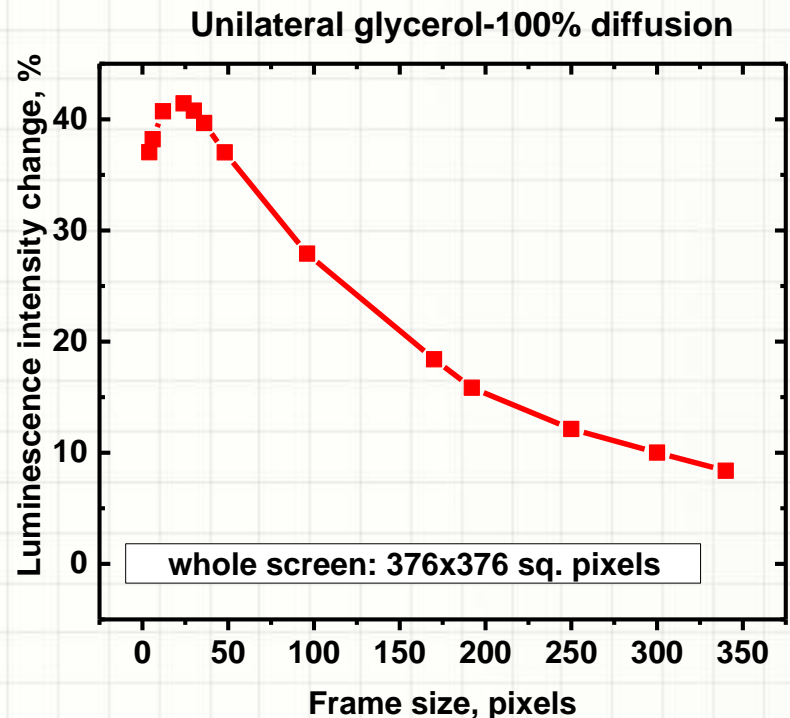
CCD camera field of view: 340 x 340 sq. pixels

Smaller areas were chosen to evaluate optical clearing efficacy

Optical clearing *in vitro*: effect of detection aperture



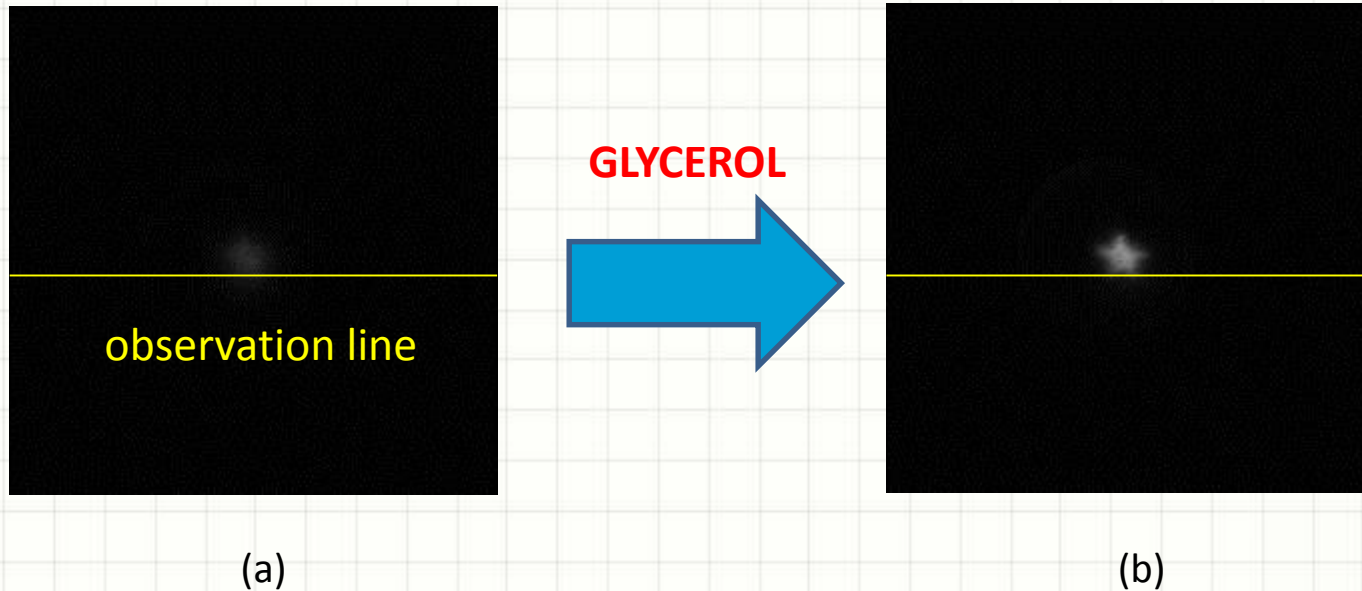
(a)



(b)

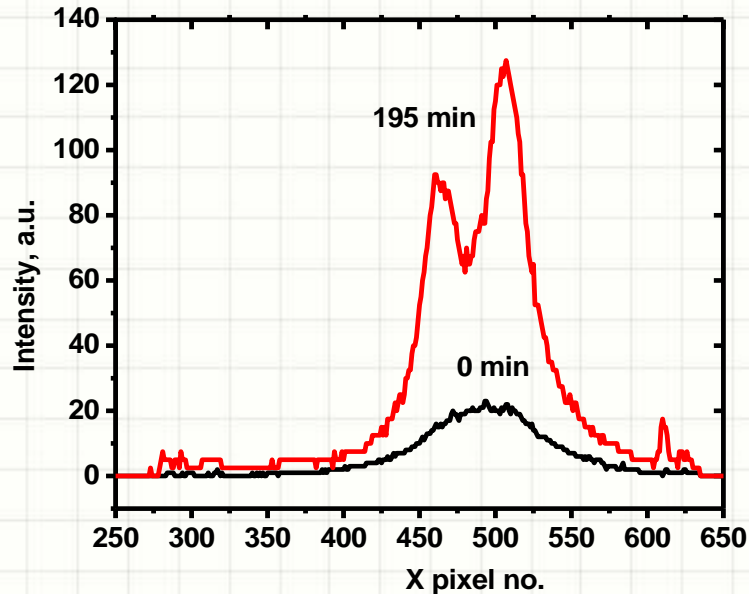
Kinetics (a) and amplitude (b) of enhancement of NALs luminescent intensity during unilateral optical clearing of muscle tissue (1.1 mm) with 100% glycerol depends on the CCD camera aperture.

Optical clearing *in vitro*: thick samples

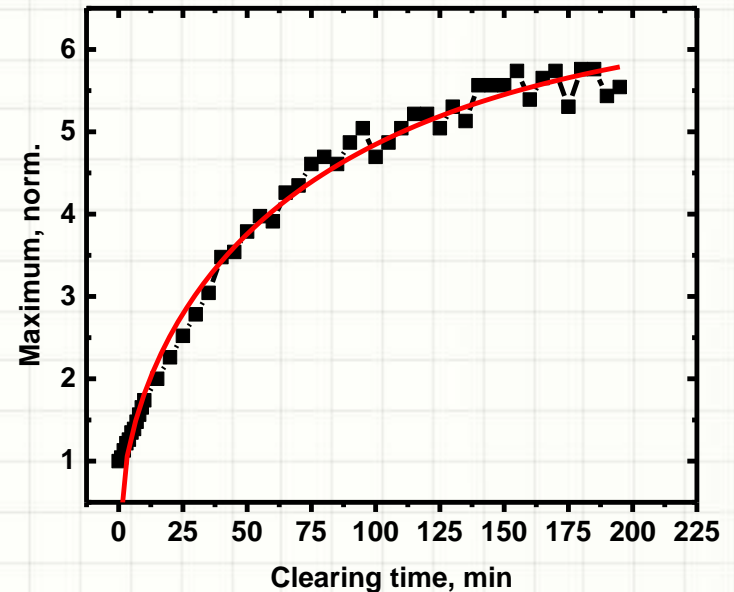


Upconversion luminescence of a **star-shaped** label @ 800 nm before (a) and after 195 min (b) of **glycerol** clearing **6-mm-thick** porcine muscle tissue *in vitro*

Optical clearing *in vitro*: thick samples



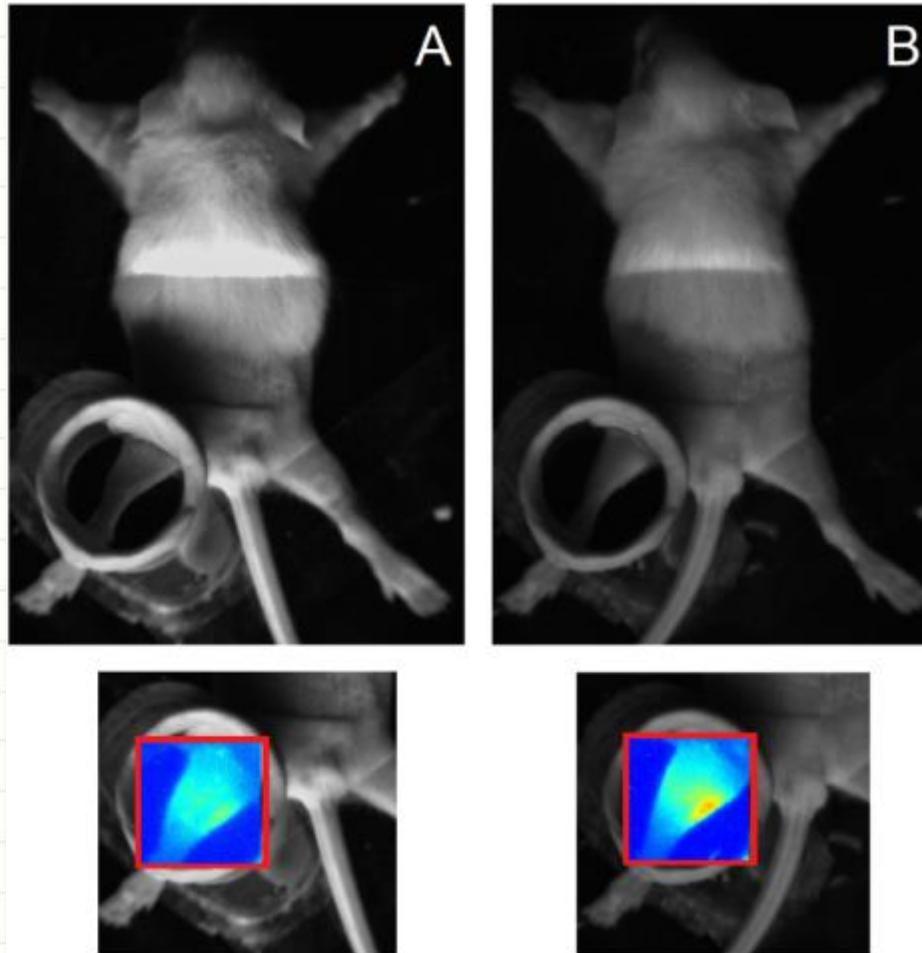
(a)



(b)

Intensity of the lowermost beams of the **star-like** upconversion luminescent label (a) and the corresponding optical clearing kinetic curve (b).

Optical clearing *in vivo*



White-light (upper row) imaging and upconversion luminescence @ 800 nm before (left) and after 255 min (right) of glycerol clearing mouse leg *in vivo*.

Summary

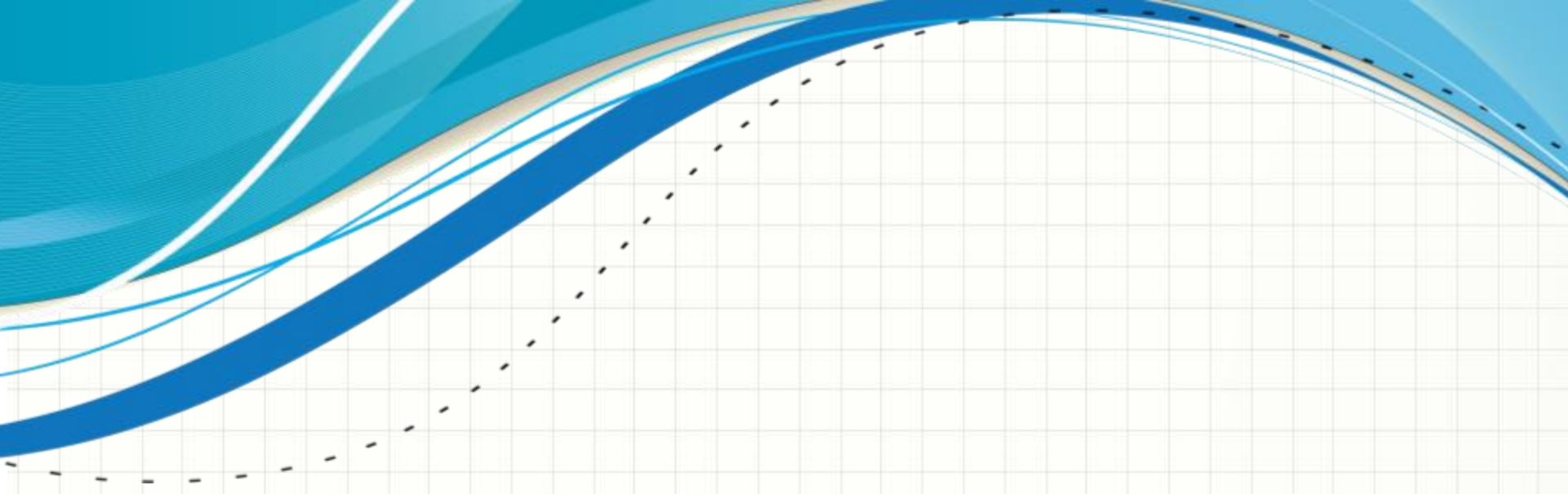
- Novel Tm-doped upconversion phosphors
- Glycerol as optical clearing agent



- increase in visibility
- increase in maximal signal intensity



Promising for precise detection of tissue-embedded labelled inhomogeneities



THANK YOU!