Photothermal and Photochemical Uses of the FEL for Biomedical Problems

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- Biomedical research and treatments require selective interactions at the cellular and molecular levels. FEL is a unique tool to achieve these. For example --
- Selective photothermal targeting
  - Lipid-rich tissues
  - nanoparticles
- Multiphoton excitation for
  - microscopy
  - photodynamic therapy

“Selective Photothermolysis”

RR Anderson, JA Parrish Science 220:524-527 (1983)

Selective Absorption → Wavelength

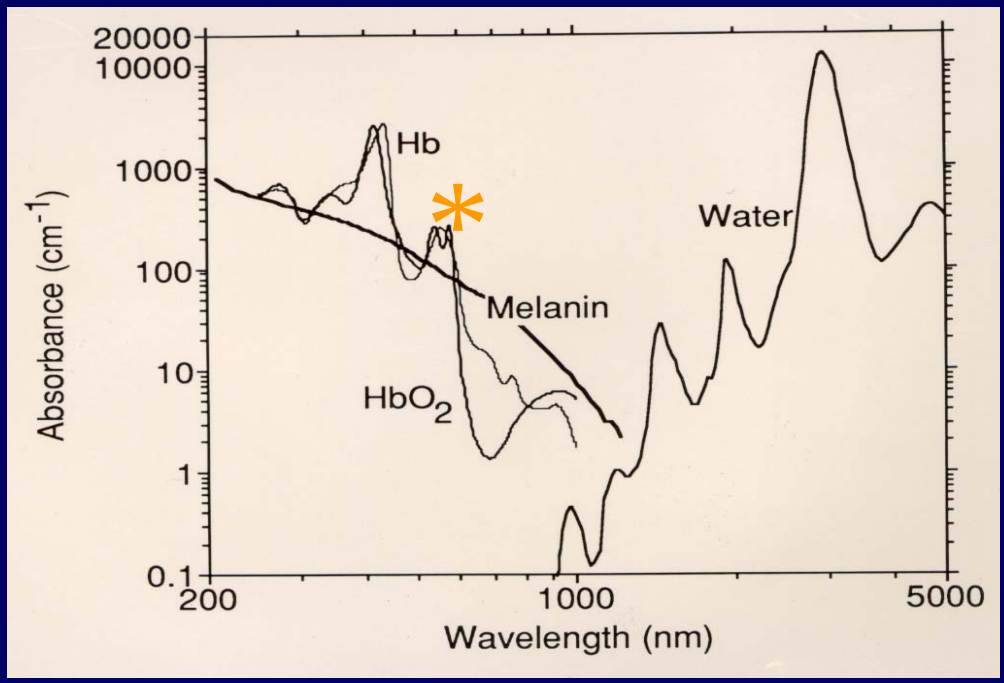
Thermal Confinement → Pulse duration

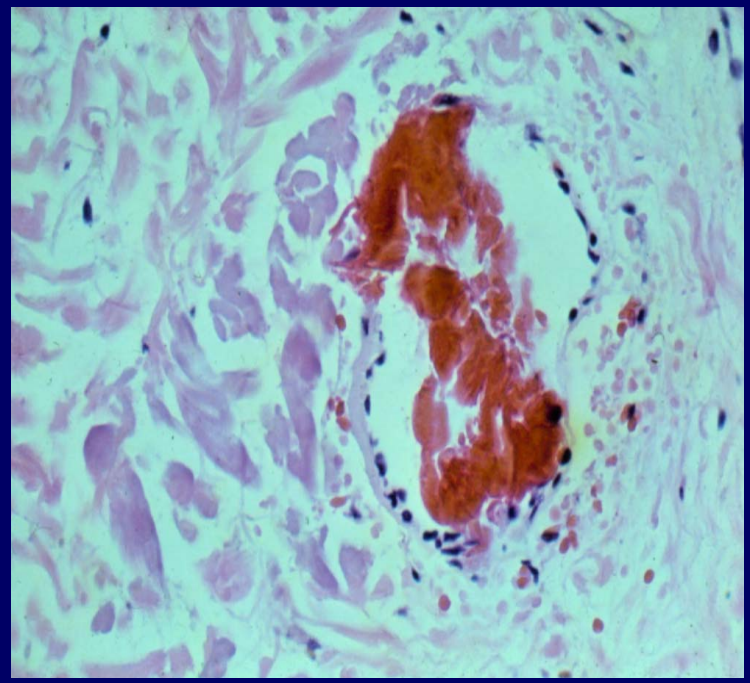
~ 1 million treatments / month for skin, eye, larynx, GI diseases

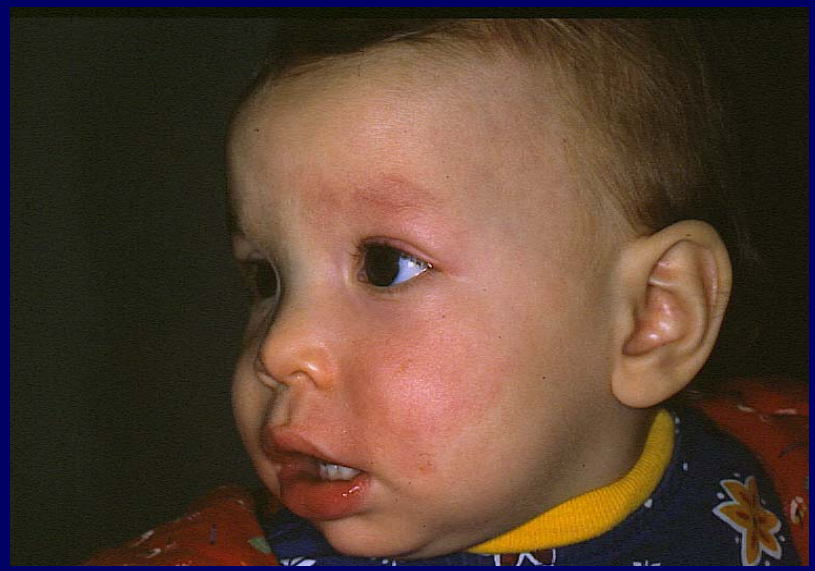
Blood vessels Pigmented Cells

Tattoos Hair removal

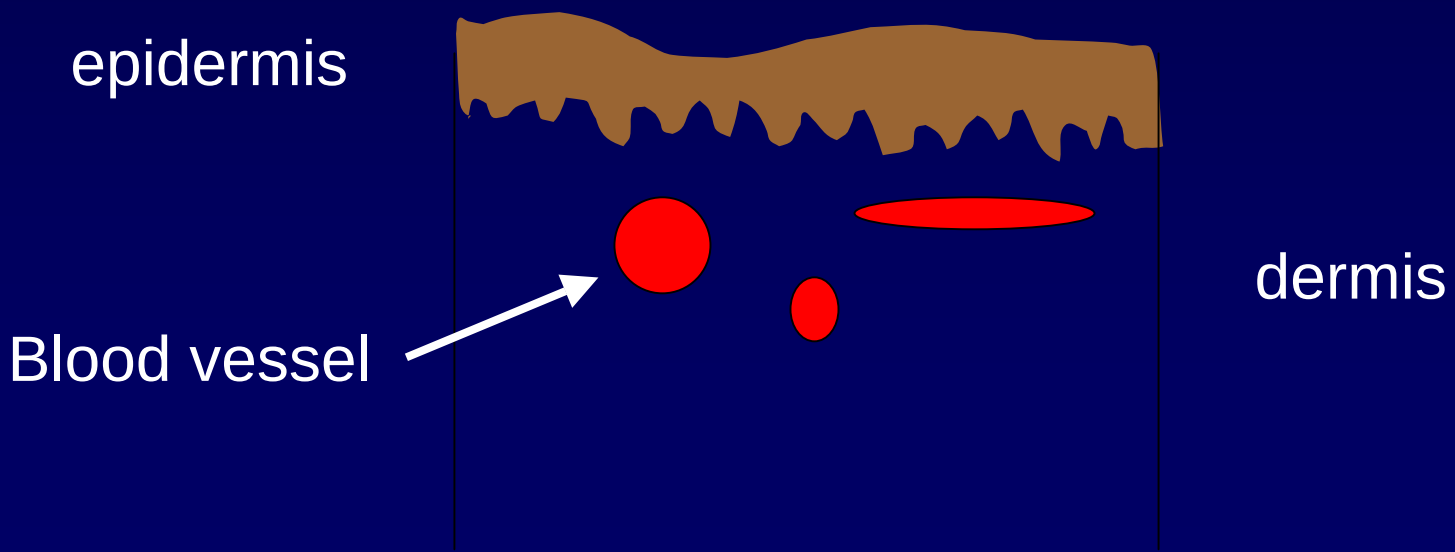
Fat and Acne (two problems of fatty tissue)?


20000
10000
1000
100
10
1
0.1
Absorbance (cm^{-1})
Hb
Melanin
HbO_2
Water
200
1000
5000
Wavelength (nm)





Selective light absorption
→ very local heating



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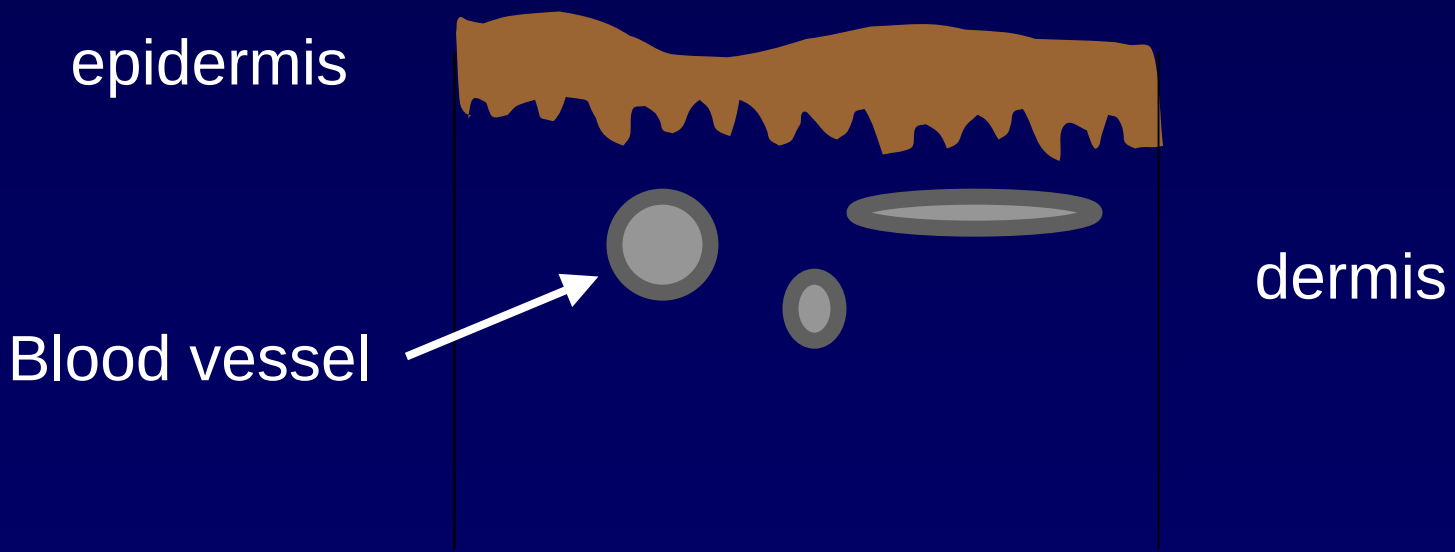
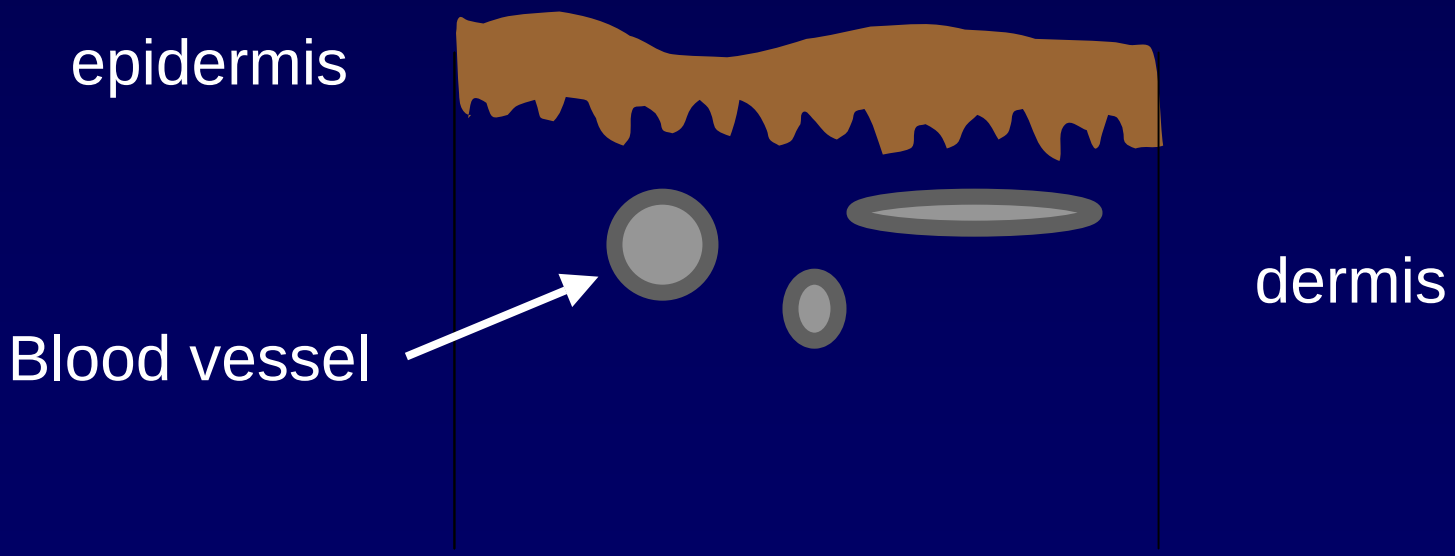


Selective light absorption
→ very local heating
→selective repair



Three fatty tissue targets:

- Sebaceous Glands – cause of acne
- Subcutaneous fat – liposuction is #1 plastic surgery in the US
- Atherosclerotic plaque – lipid-rich plaques kill 40% of the US population by stroke or heart attacks.

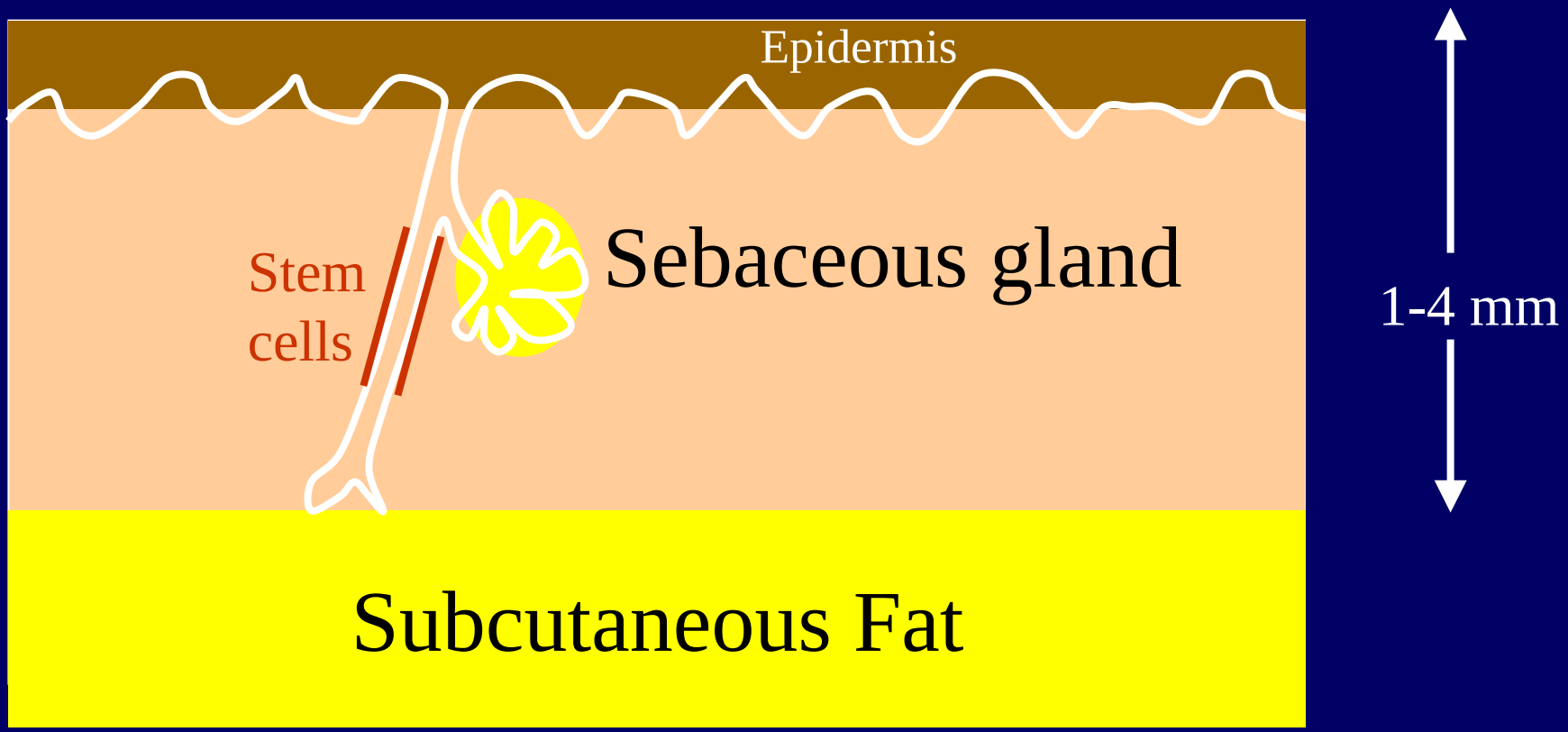


Photo-thermal Excitation

$$\Delta T = (E\mu_a/\rho c)$$

ΔT: Temperature rise

E: Energy density

μ_a: Absorption

ρ: Density

c: Heat capacity

$\rho_{fat} = 0.85\ g\ cm^{-3}$

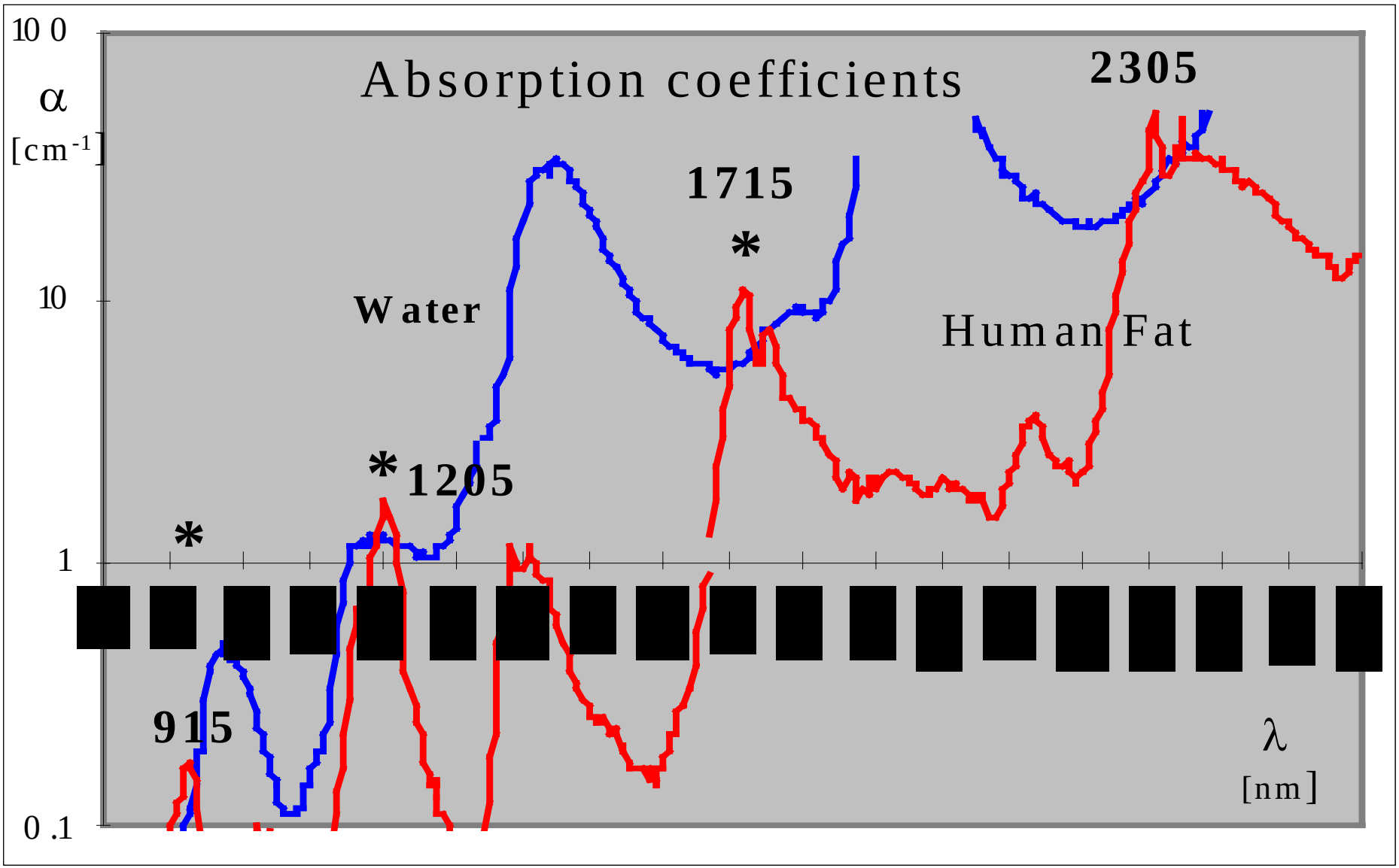
$\rho_{dermis} = 1.08\ g\ cm^{-3}$

$c_{fat} = 2.3\ J\ g^{-1}\ K^{-1}$

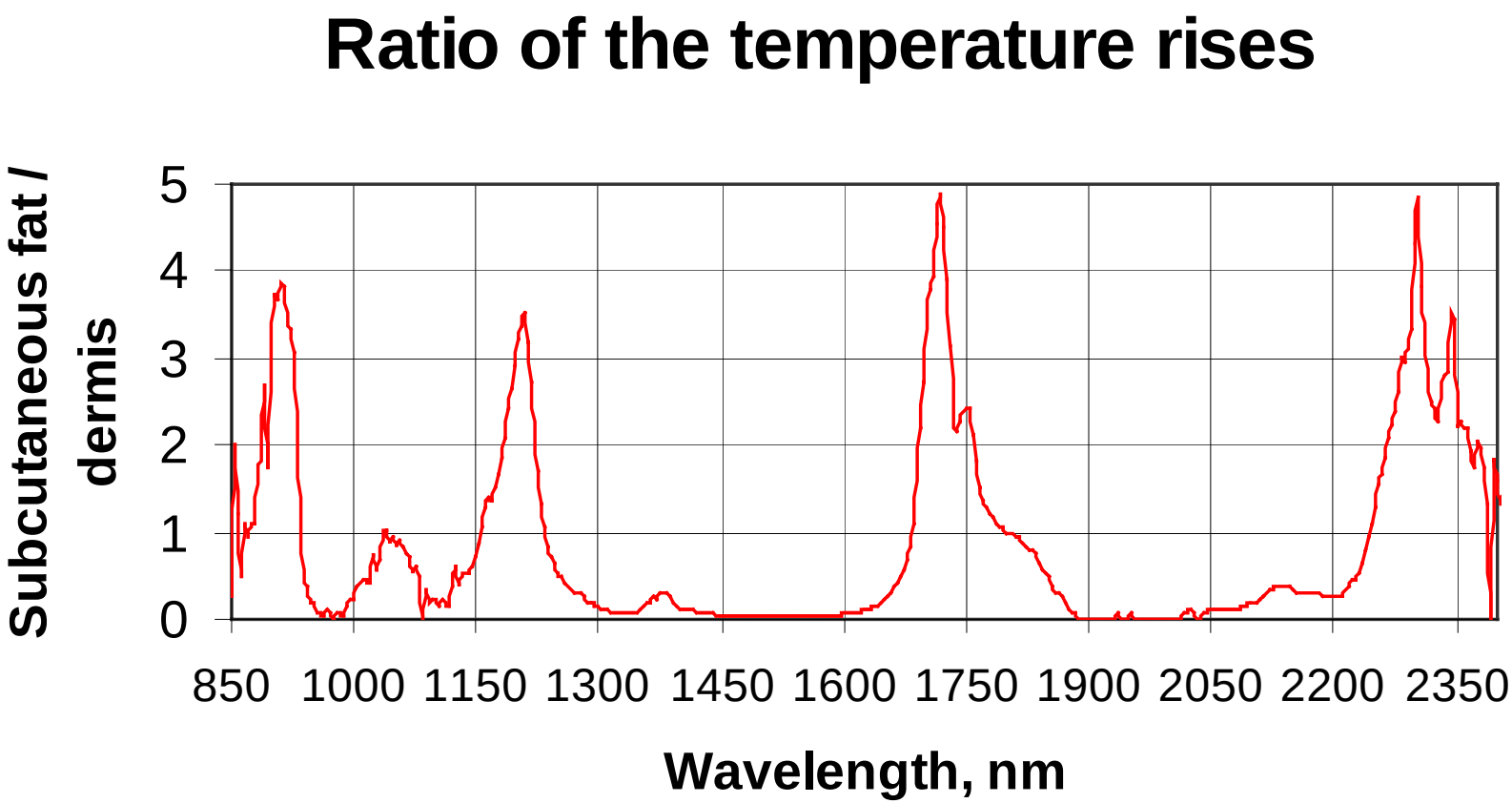
$c_{dermis} = 3.5\ J\ g^{-1}\ K^{-1}$

Because of lower ρc, fat should be prone to photothermal heating.

Fat and Water have nice “colors” in the NIR

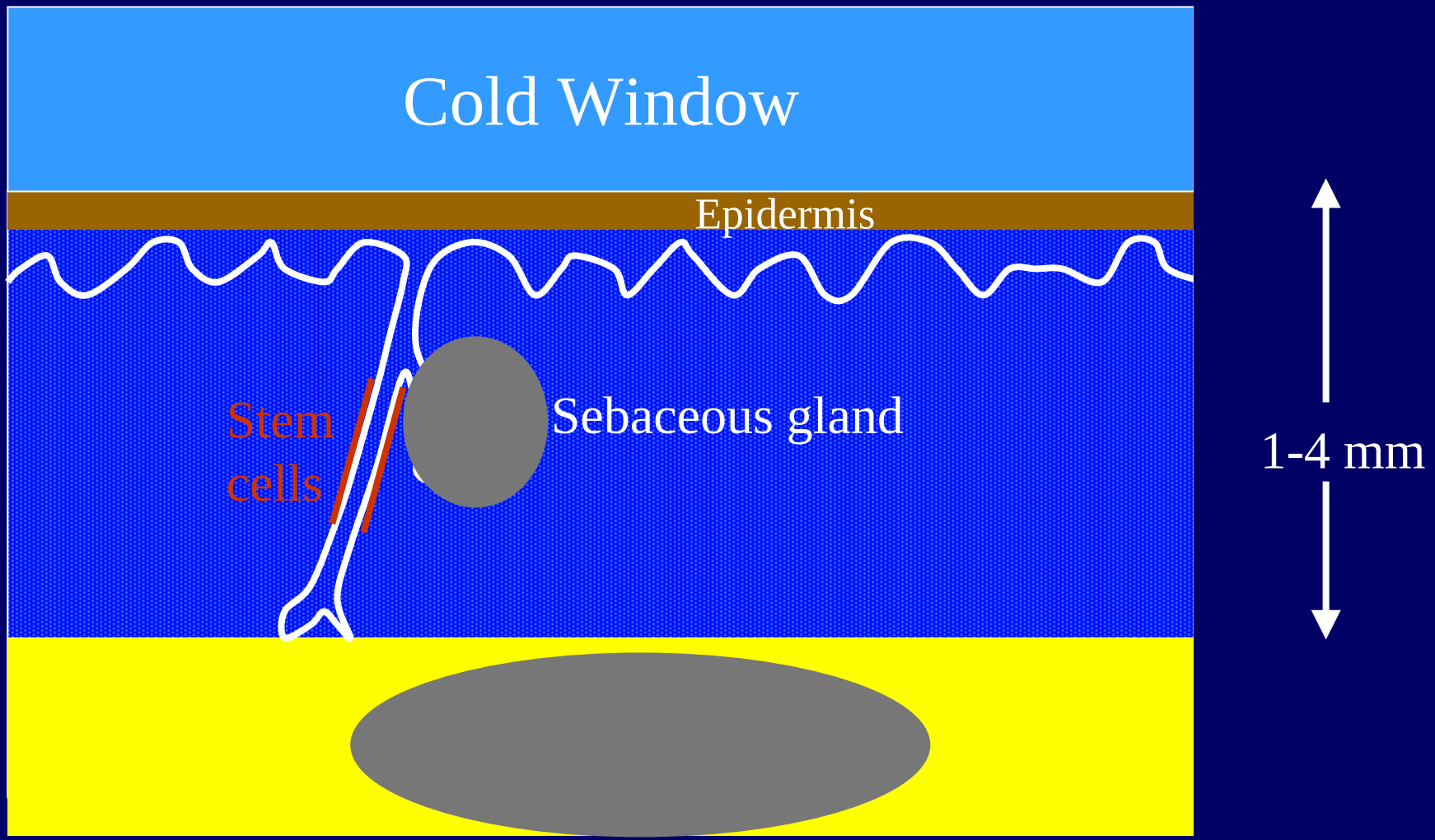


Ideally, ratio of photothermal heating for fat vs. water



Can we ‘target’ sebaceous glands and fat?

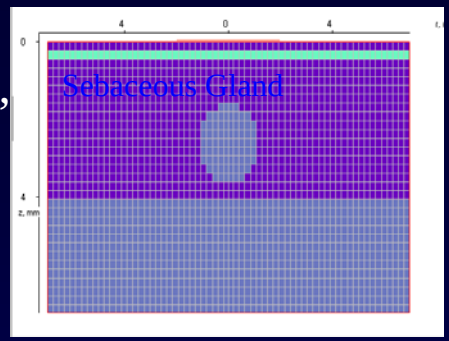
CH-selective Laser



Selective Fatty Tissue Targeting

Monte Carlo Simulations :

Sebaceous gland (depth = 2.5 mm, radius = 1.0 mm n=1.45, μ_a=0.17 /mm, μ_s'= 0.58 /mm) below epidermis (n=1.4, μ_a=0.039 /mm, μ_s'= 0.79 /mm) and capillary layers (n=1.37, μ_a=0.04 /mm, μ_s'= 0.3 /mm) within 3.8 mm thick dermis (n=1.4, μ_a=0.035 /mm, μ_s'= 0.2 /mm) irradiated by focused beam (λ = 1200 nm r= 2 mm, focusing depth = 3.5 mm)

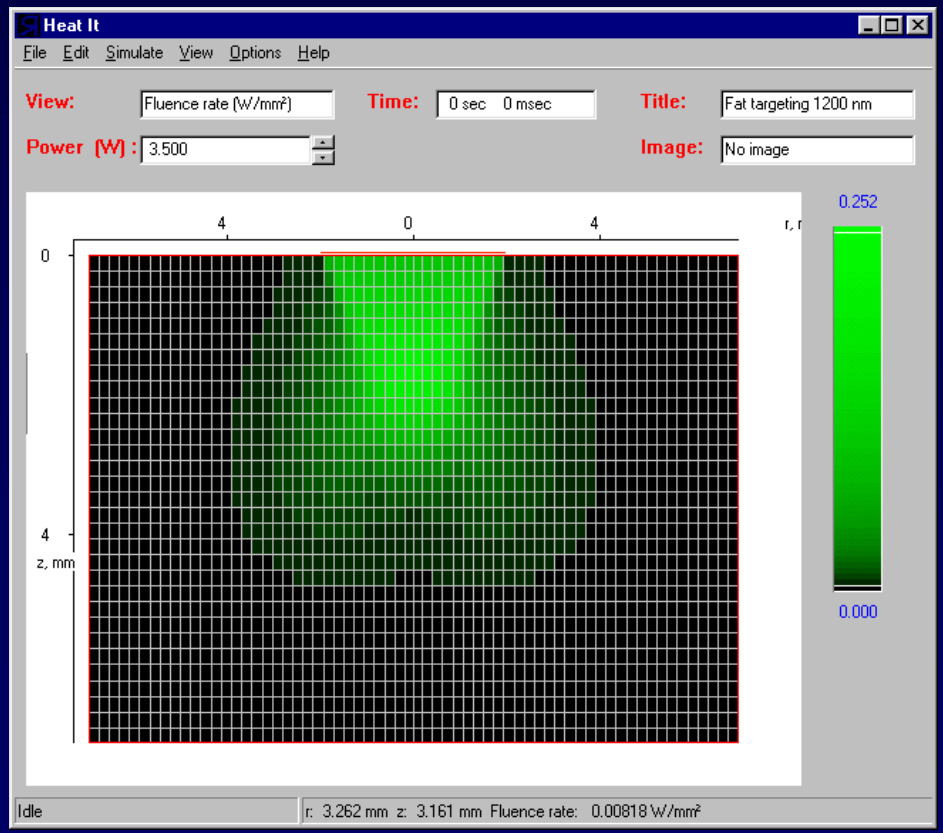


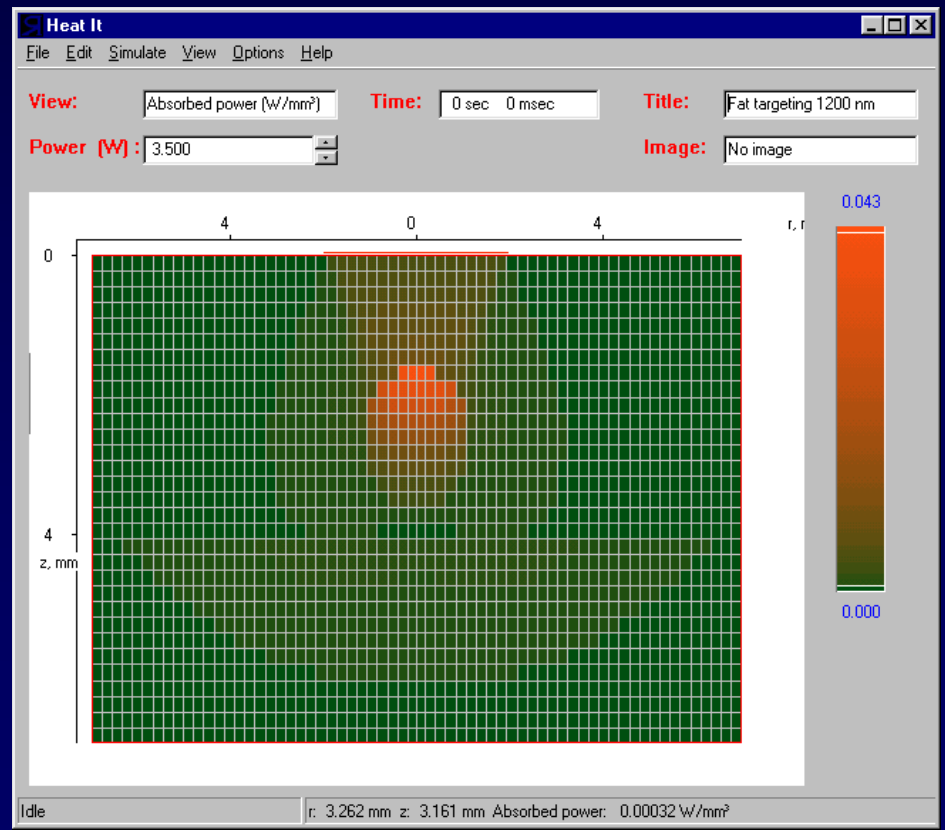
Epidermis

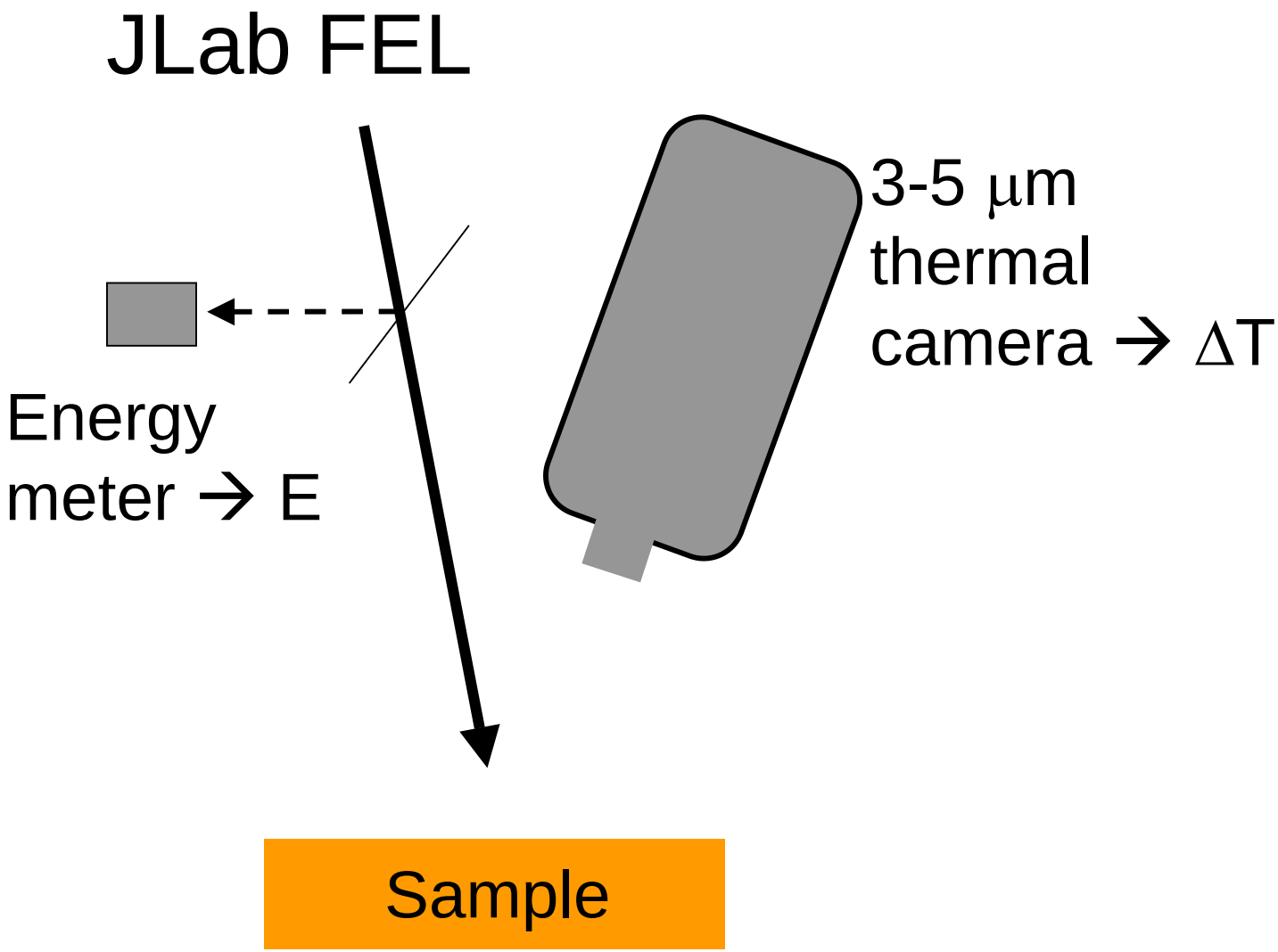
Blood

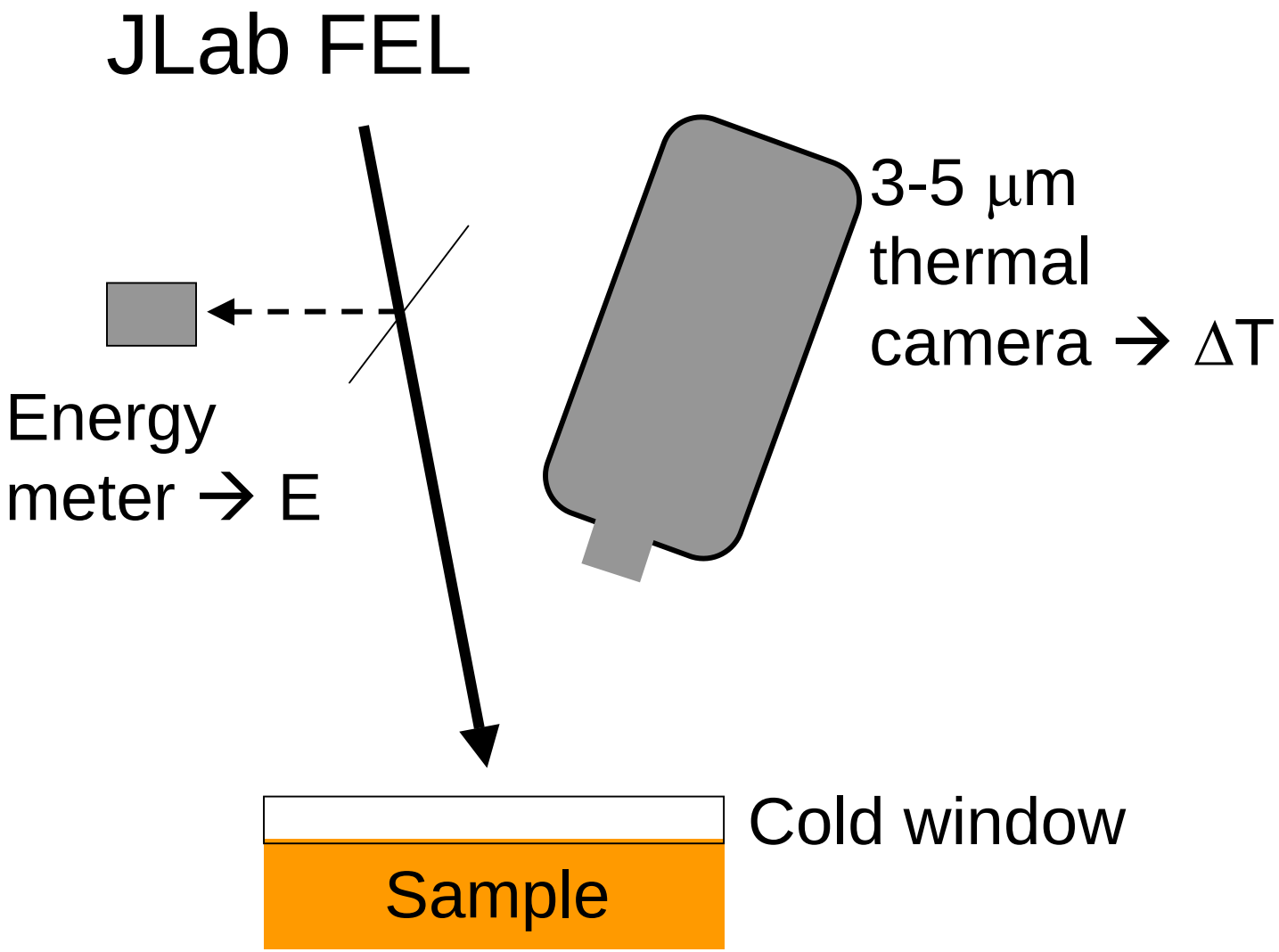
Dermis

Subcutaneous Fat

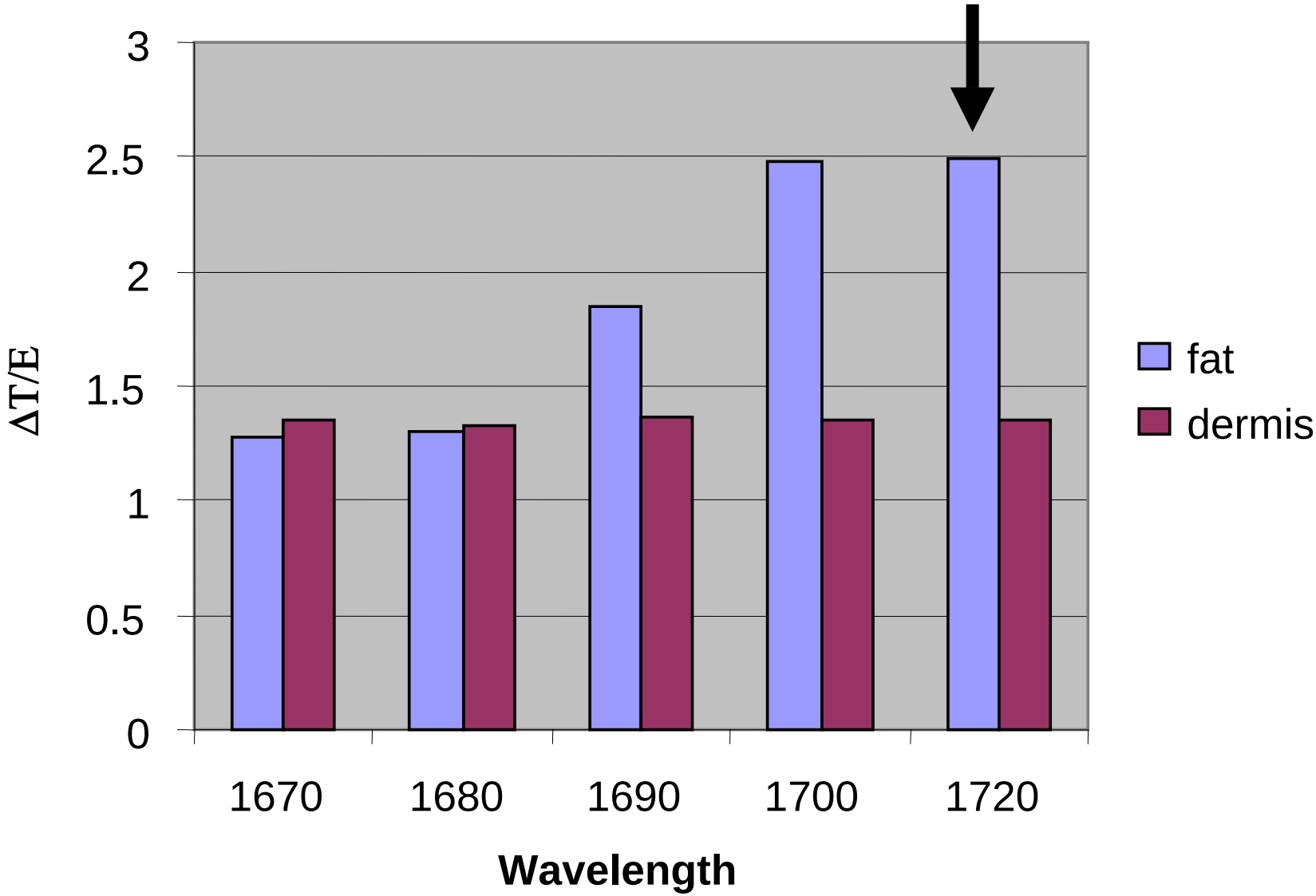





JLab FEL
3-5 μm
thermal
camera → ΔT
Energy
meter → E
Sample


JLab FEL
3-5 μm
thermal
camera → ΔT
Energy
meter → E
Cold window
Sample

Photothermal excitation spectrum taken with J-Lab FEL shows “where” to build a fat-seeking laser



Nanoscale Particle Targeting

Thermal Confinement

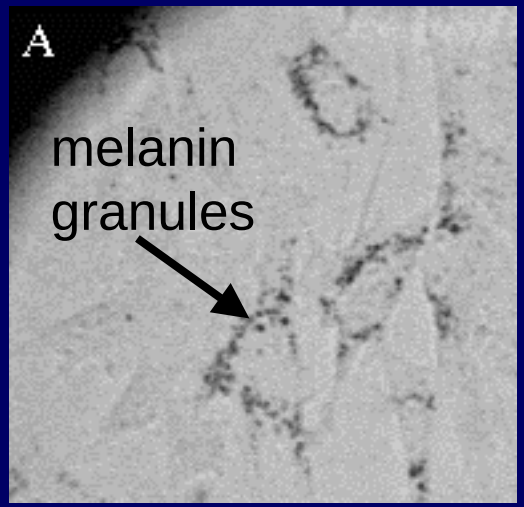
- Optical pulse < Thermal relaxation time (τ_t)
- $\tau_t \cong d^2/4\kappa$ (κ is thermal diffusivity)
- 1 μm object cools in ~ 1 μs

Inertial Confinement

- Optical pulse < Acoustic relaxation time (τ_a)
- $\tau_a \cong d/v$ (v is sound velocity)
- 1 μm object relaxes in ~ 1 nanosecond
- FEL is an ideal source for nanoparticle targeting

Laser pulse targeting at the single cell level:

Cytoplasmic cavitation



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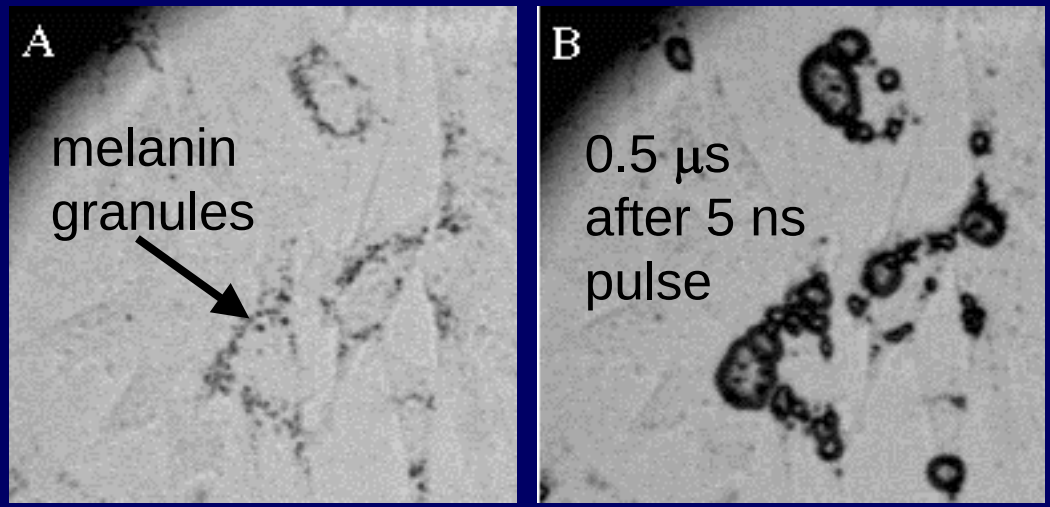
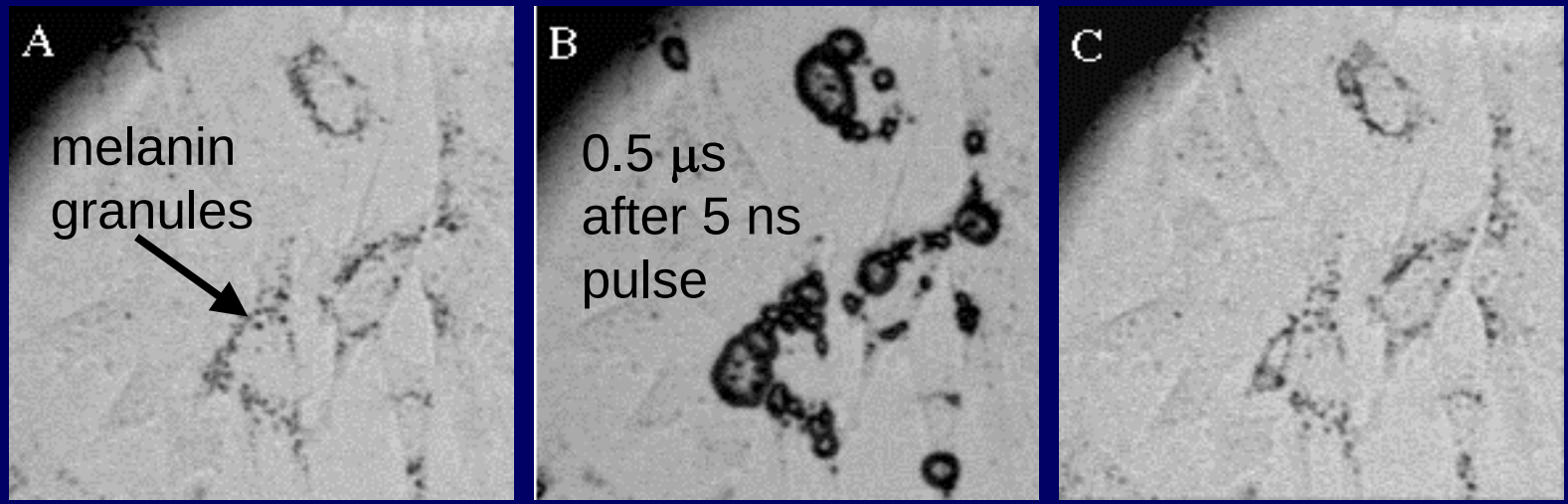


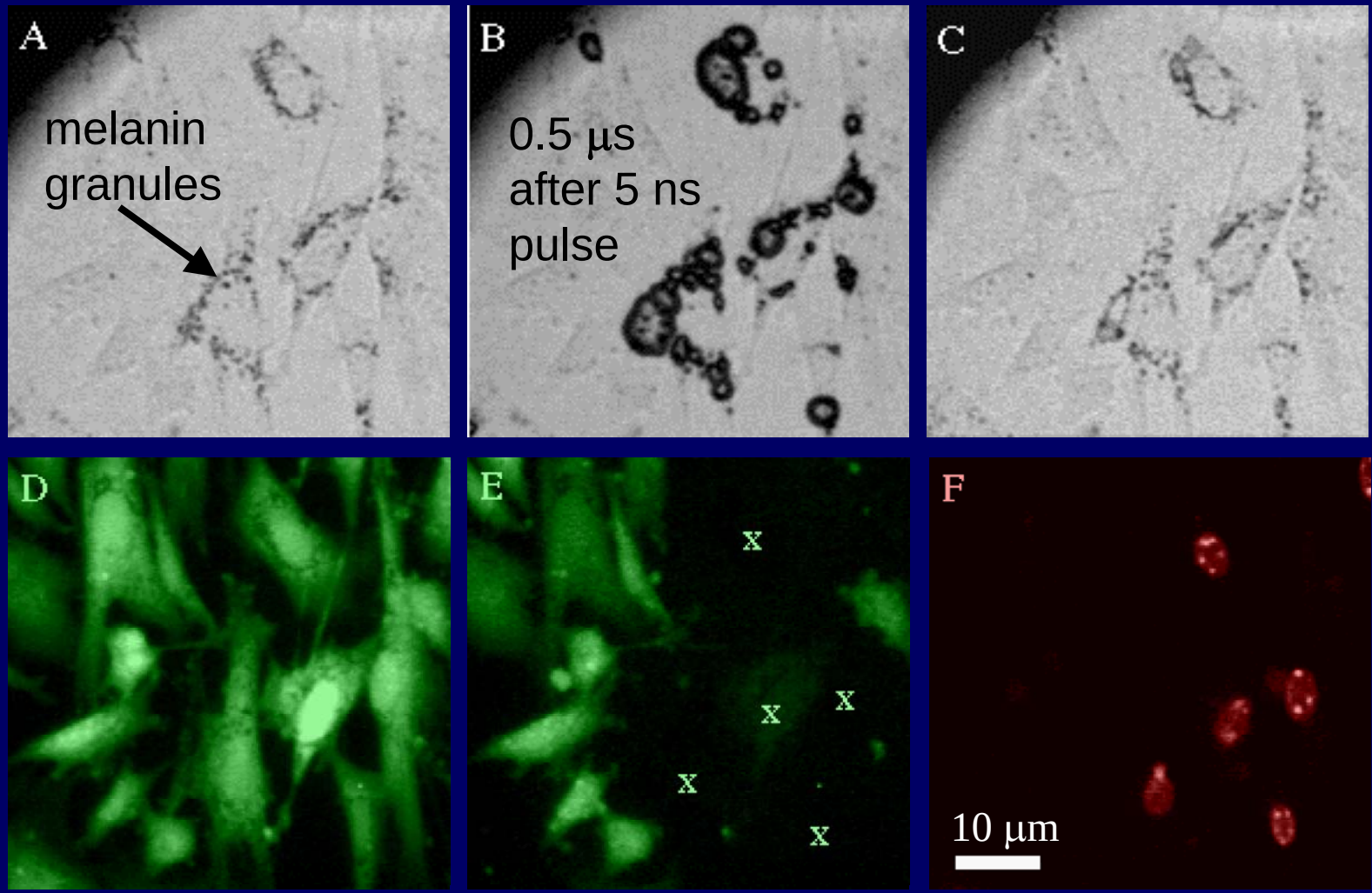
Laser pulse targeting at the single cell level:

Cytoplasmic cavitation



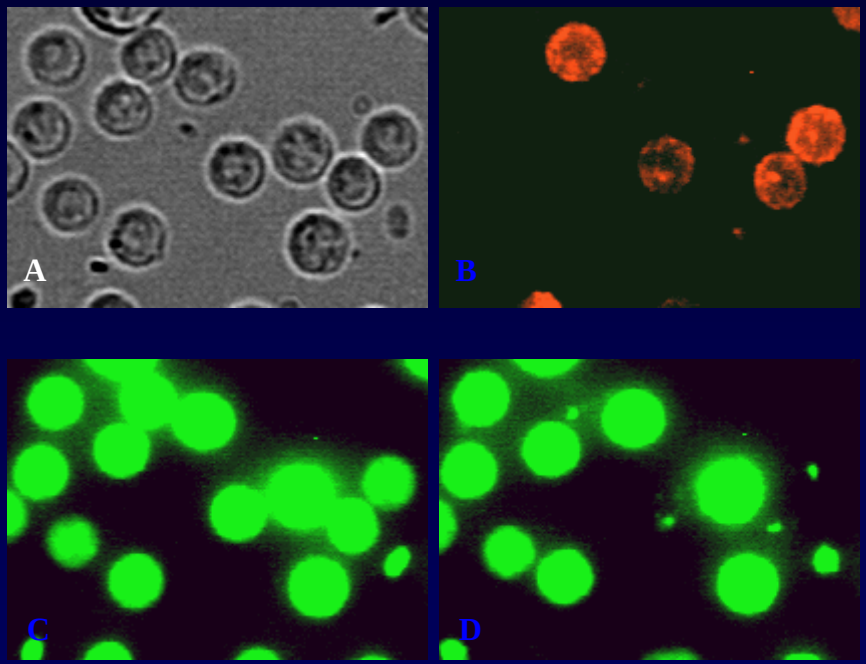
Laser pulse targeting at the single cell level:

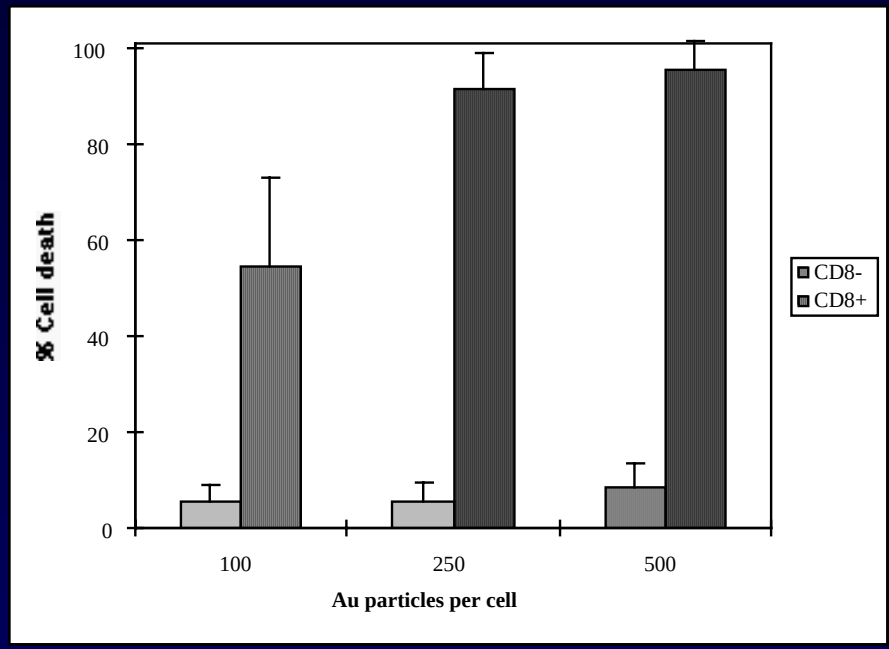
→ New treatment of glaucoma



Nanoparticle “laser knock out” of immune effector cells. Selective Targetinging of CD8+ T Cells

(30 nm gold Nanoparticles)



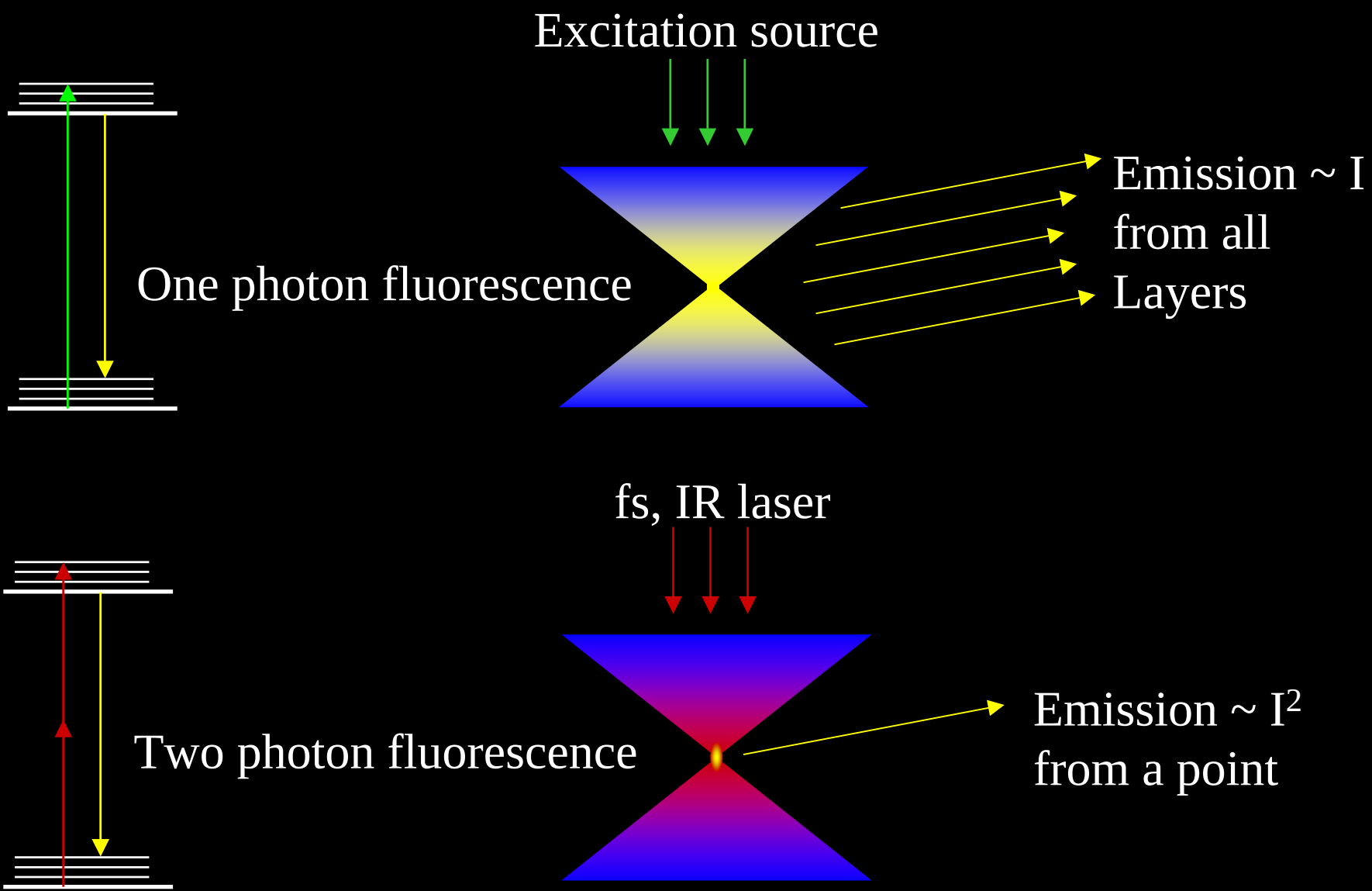


- (A) T lymphocytes labeled with 30 nm gold particles
- (B) Cells double-labeled with anti-CD8 phycoerythrin (PE) fluorescent probe
- (C) and (D) Cells are irradiated with 20 nsec, 565 nm laser pulses at a fluence of 0.5 J/cm^2. Calcein-AM fluorescence before and after irradiation indicates loss of viability in CD8+ cells.
- (E) Results of selective killing of human lymphocytes using 30 nm gold particles directed against the CD8 membrane receptor.

Multiphoton excitation for microscopy and drug activation ("photodynamic therapy"

- Requires high power, femtosecond, tunable pulses.
- Current work is limited by Ti:sapphire and similar laser technology

Multiphoton Fluorescence Microscopy



A recent example of 2-photon in-vivo microscopy at Wellman

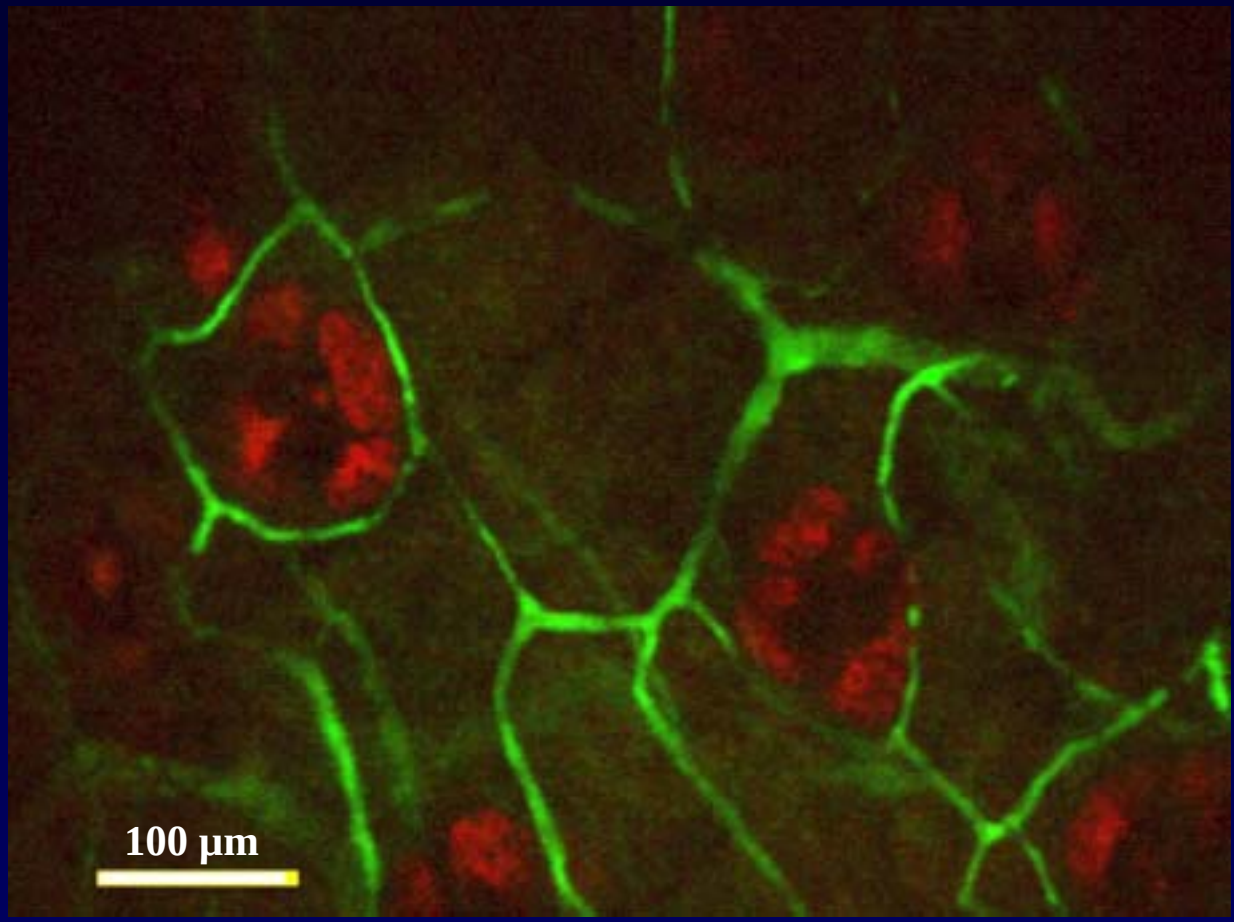
This image (published in Nature) is the live bone marrow imaged through a mouse's skull, intact. The green GFP-labelled cells are metastatic cancer. We found that cancer metastasizes by using the same microdomains employed by normal bone marrow stem cells. This has major implications for developing new cancer therapy.

Sipkins DA, Wei X, Wu JW, Runnels JM, Cote D, Means TK, Luster AD, Scadden DT, Lin CP. In vivo imaging of specialized bone marrow endothelial microdomains for tumor engraftment. Nature 2005;435:969-73.

Why use the FEL for femtosecond laser microscopy?

- Deeper imaging will be possible
- Wider range of probe dyes
- Can take spectra, which reveal chemical changes and micro-environments *in vivo*
- Spectroscopy and microscopies can be combined by tuning and wavelength combinations, e.g. Coherent Anti-Stokes Raman Spectroscopy (CARS) microscope

Combined CARS and two-photon fluorescence



Green is fluorescence from FITC-labeled dextran in bloodstream, excited @ 817 nm (7 ps, 50 mW @ 76 MHz), detected @ 500 – 535 nm.

Red is CARS from CH_2 stretch vibration at 2845 cm^{-1}, pump 817 nm, Stokes 1064 nm (both 50 mW @ 76 MHz), detected @ 663 nm.

Multiphoton excitation for Photodynamic Therapy

- Activate drugs, e.g. for cancer treatment, and natural pathways by multiphoton absorption
- Minimizes collateral damage because the rate of photochemistry $\propto I^2$, which is confined
- Demonstrated in cultured cells, but not in vivo due to *lack of high average power, tunable fs lasers*
- Requires on-site biomedical facilities not yet available at JLab
- *A multiphoton excitation source with the power and spectrum of JLab FEL is unprecedented for photomedicine and photobiology research.*

Thank you,
Fred Dylla and
the JLab FEL team