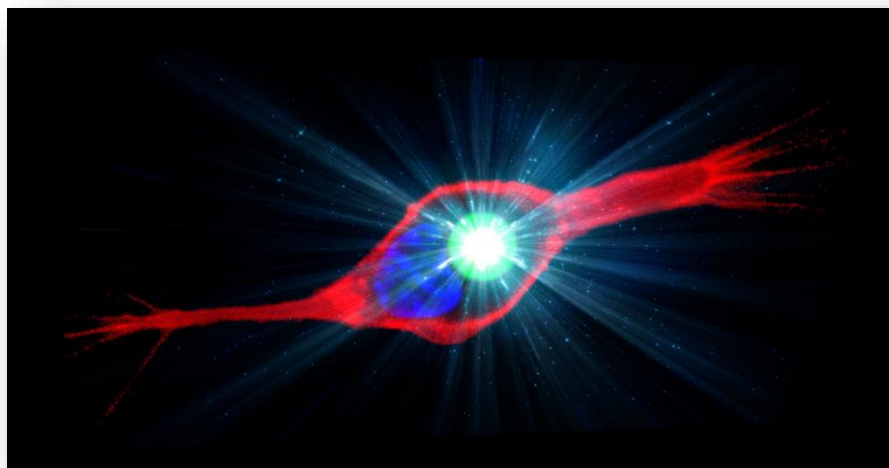


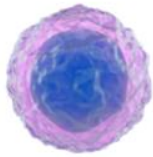
Bio-integrated micro-laser particles for sensing, imaging and cell barcoding



Matjaž Humar

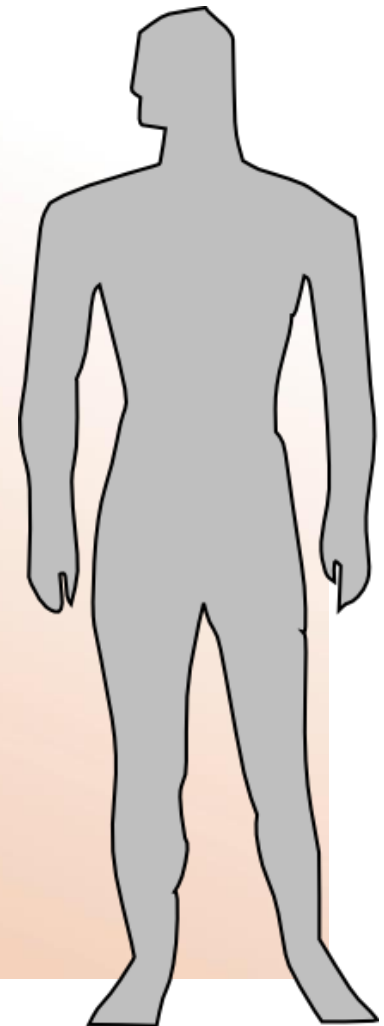
Jožef Stefan Institute, Slovenia
Faculty of Mathematics and Physics, University of Ljubljana, Slovenia

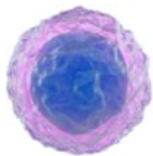




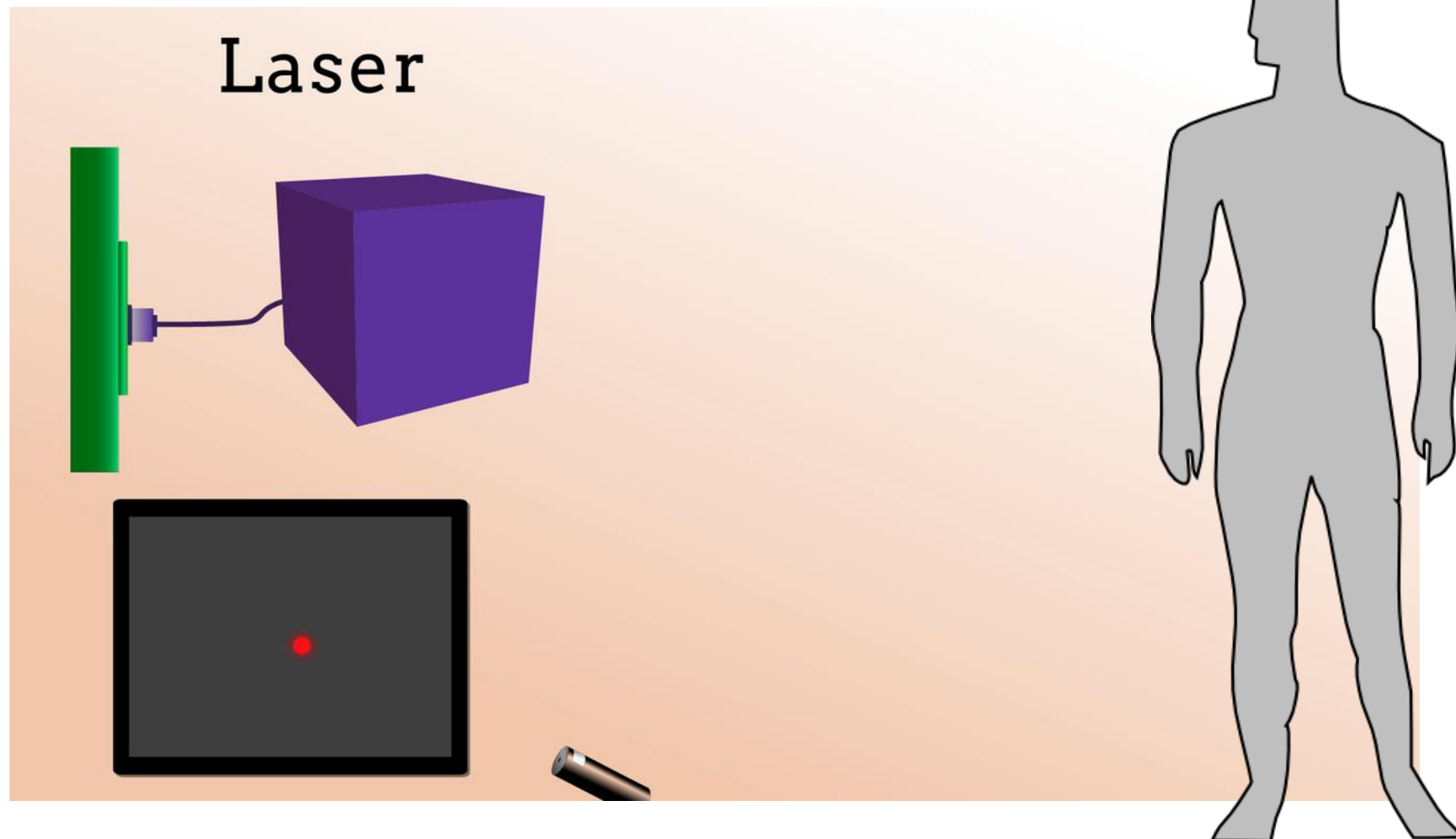
Laser inside biological tissues – paradigm shift

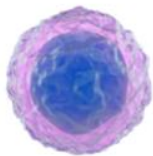
Laser





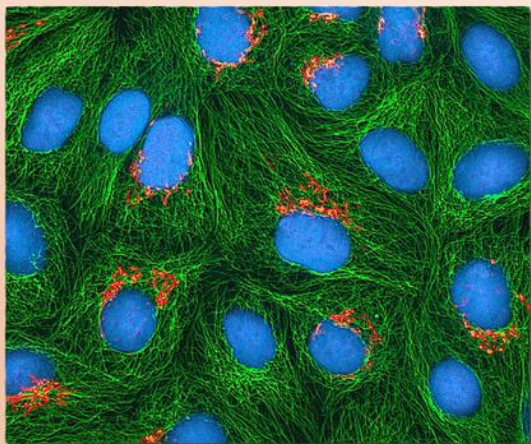
Laser inside biological tissues – paradigm shift





Fluorescent probes vs lasers

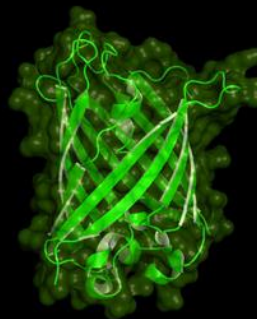
Luminescent probes



Quantum dots

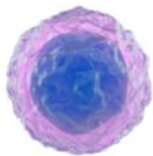


Bioluminescent molecules



Plasmonic nanoparticles

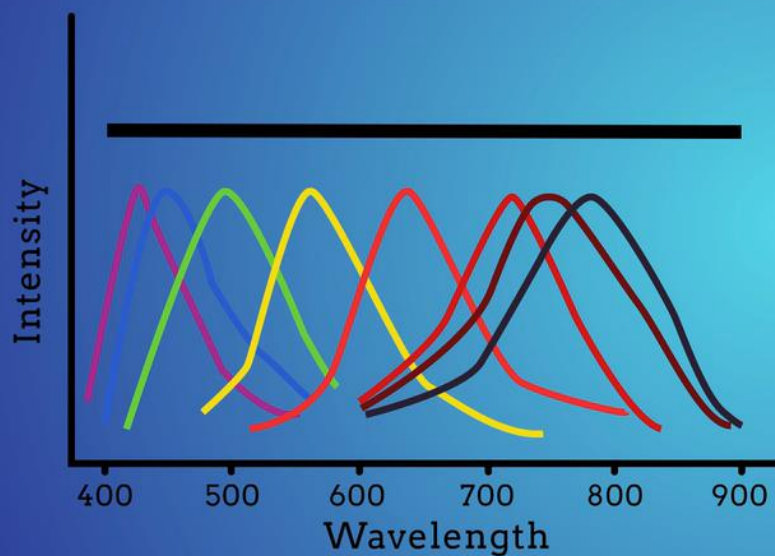




Fluorescent probes vs lasers

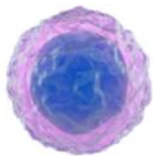


Broad range of wavelengths



Current probes



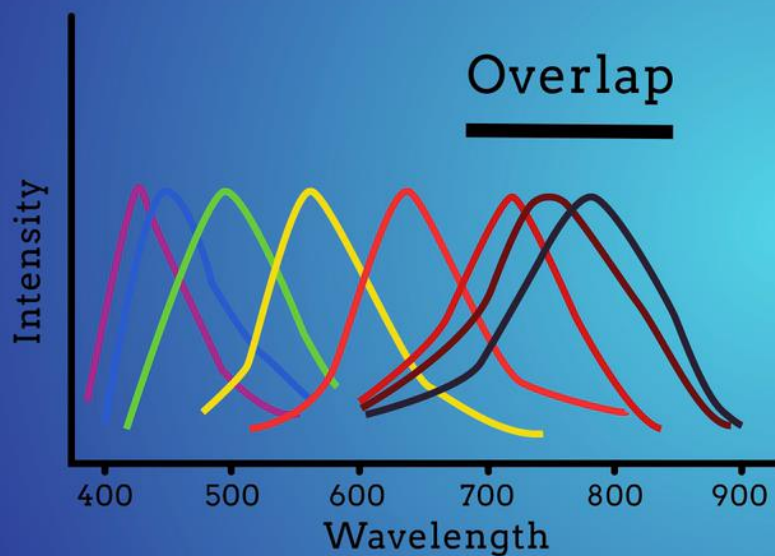


Fluorescent probes vs lasers



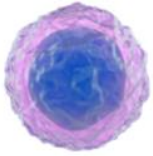
Broad range of
wavelengths

Overlap

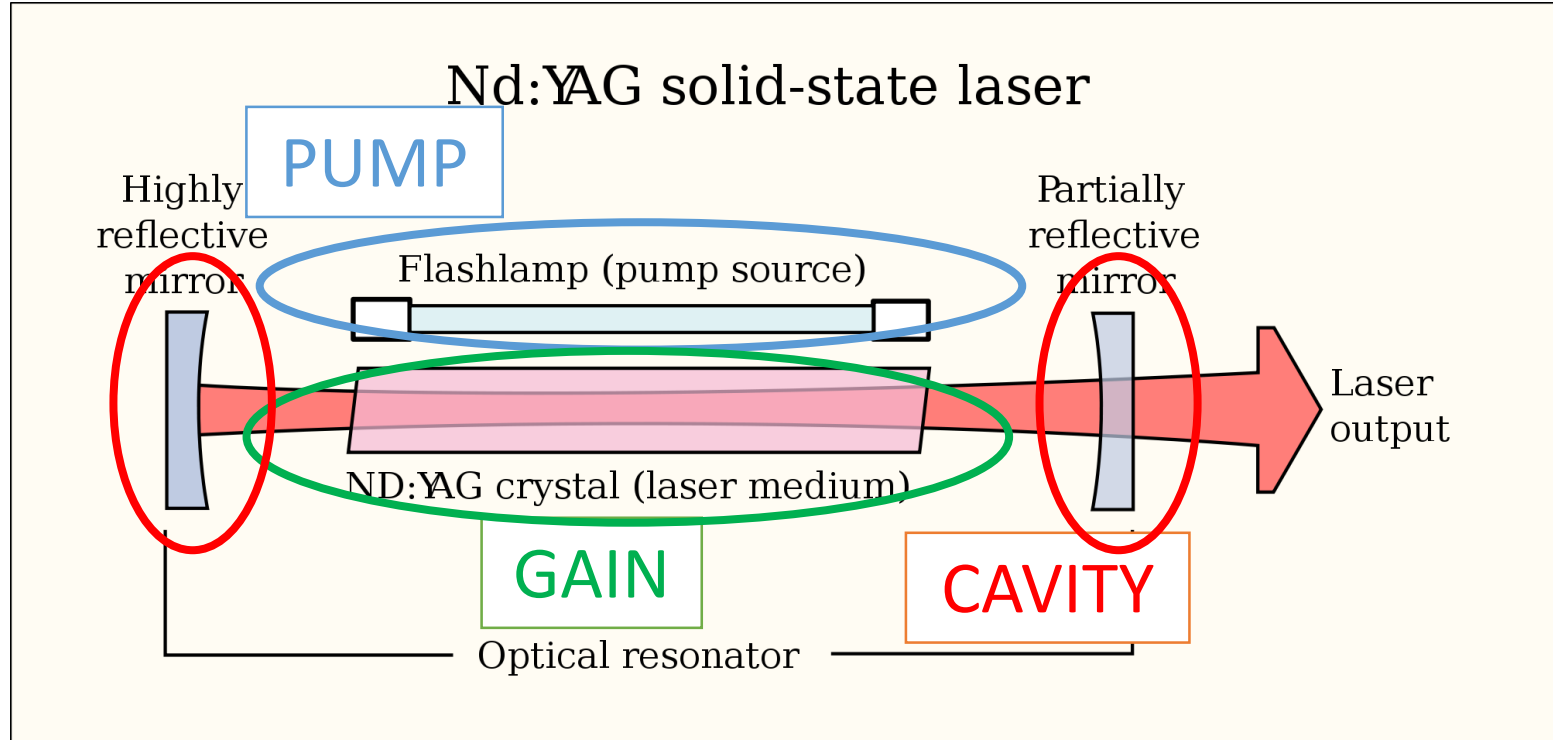


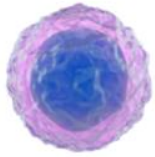
Current probes





Lasers made completely out of biological materials





Bio-integrated photonics

Biophotonic device

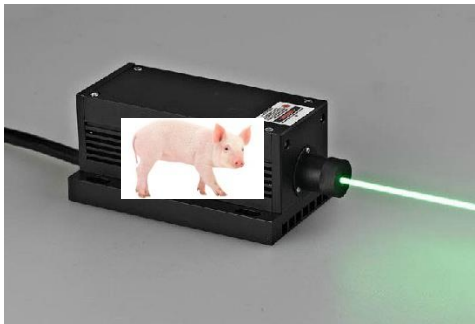


Treatments, diagnostics



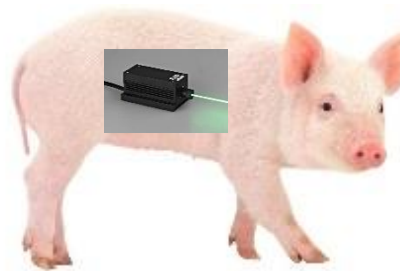
Biological system

Biosystem as part of the device



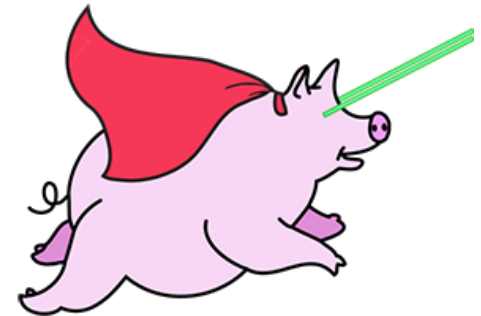
Example: cell inside a laser

Device inside a biosystem



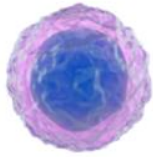
Example: optical waveguide in tissue

Device = Biosystem



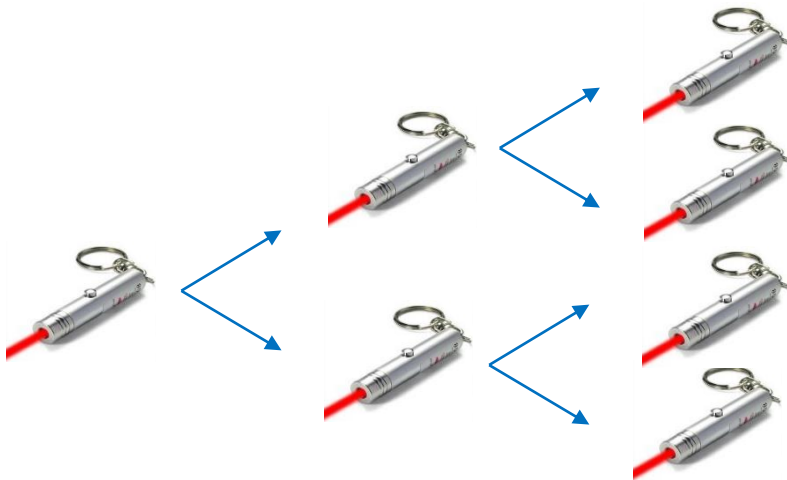
Example: cell as laser



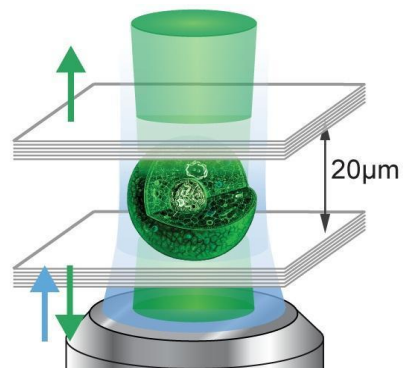
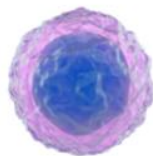


Why bio-integrated photonics

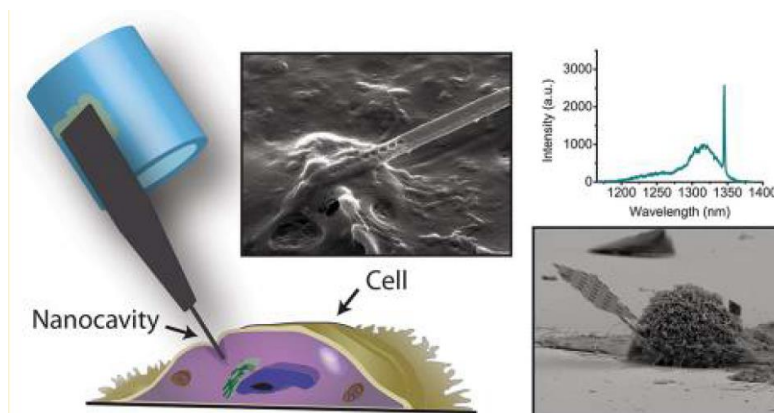
- Better coupling between the device and the biological system
 - Better sensors
 - Targeted medical treatments
- Live lasers: Self-reproduction, self-assembly, adaptation, self-healing
- New human-robot interfaces (Cyborg)



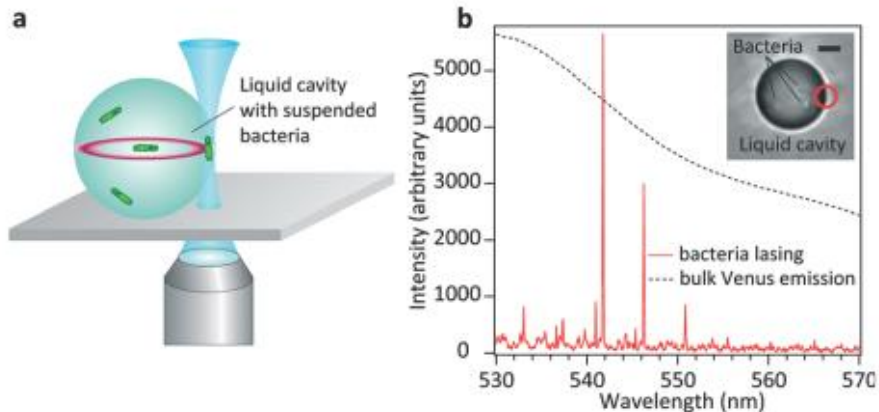
Previous research on cell lasers and bio-lasers



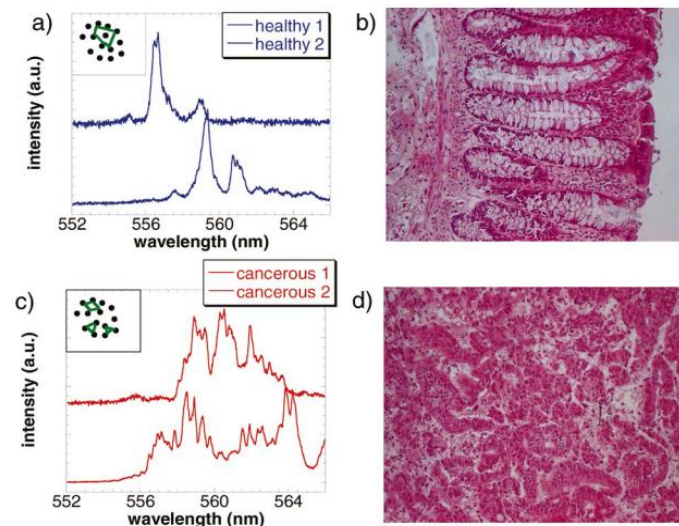
M. Gather, S.H. Yun, Single cell biological lasers. *Nature Photonics* 5, 406-410 (2011).



Shambat G et al. Single-cell photonic nanocavity probes. *Nano letters*, 13, 4999 (2013).



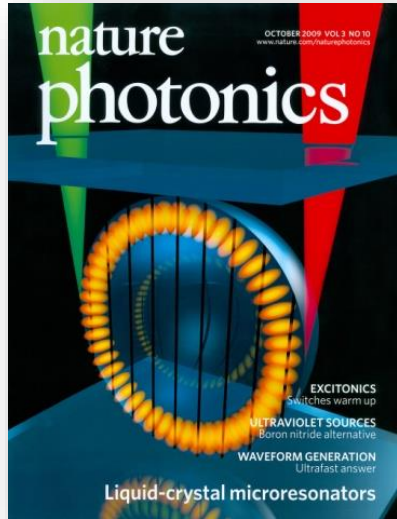
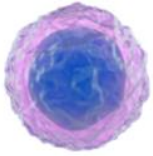
A. Jonáš et al., *Lab on a Chip* 14, 3093 (2014).



Polson, R. C. & Vardeny, Z. V. *Appl. Phys. Lett.* 85, 1289–1291 (2004).

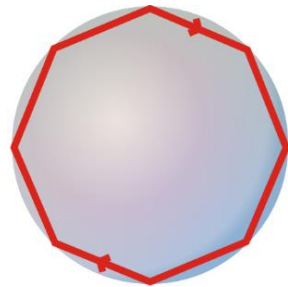


WGM cavities in cells

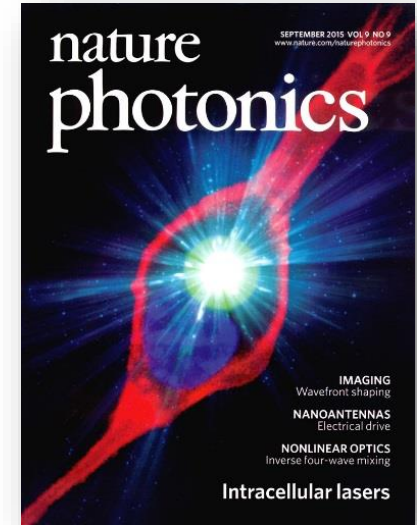
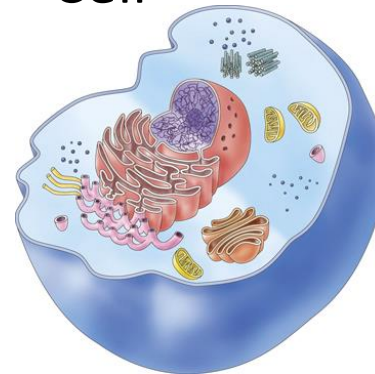


Whispering-gallery mode laser

- Light circulates due to total internal reflection
- Fluorescent dye as gain
- External laser pumping



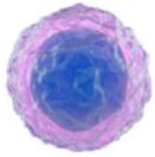
Cell



M. Humar, M. Ravnik, S. Pajk, and I. Muševič, Electrically tunable liquid crystal optical microresonators, *Nat. Photonics* 3, 595–600 (2009).

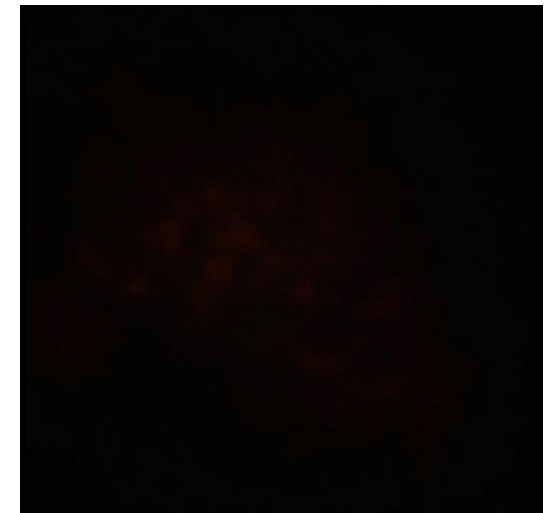
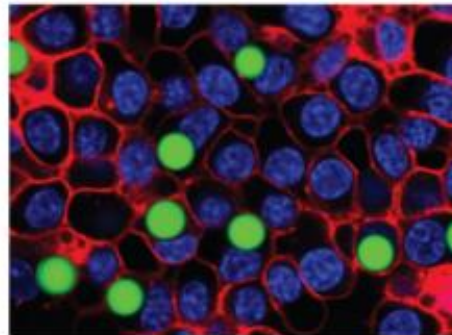
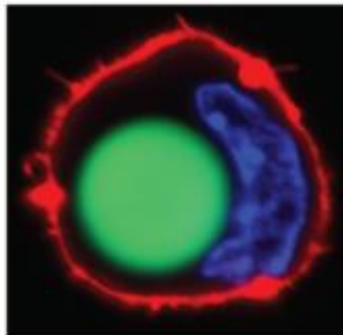
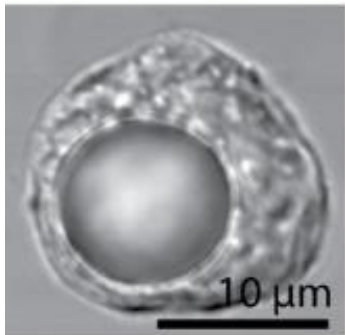
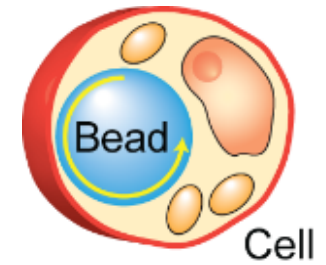
M. Humar, S.-H. Yun, Intracellular Microlasers, *Nature Photonics* 9, 572–576 (2015).

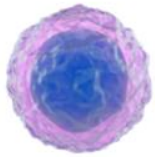




Solid beads – uptake by cells

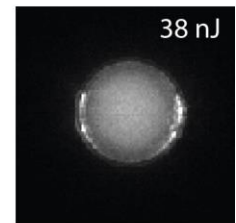
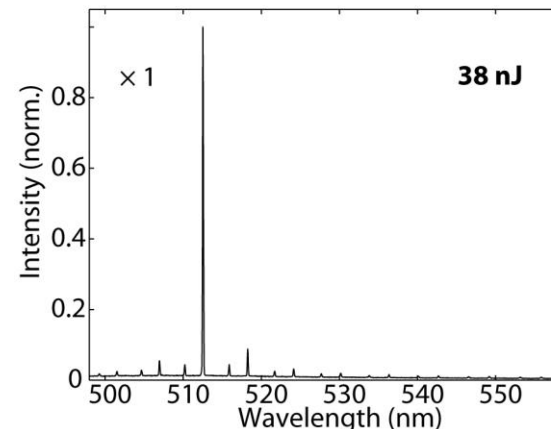
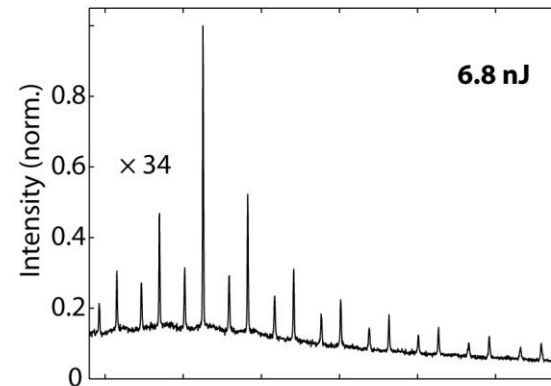
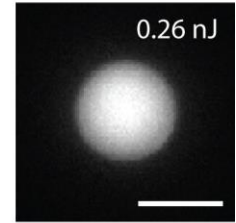
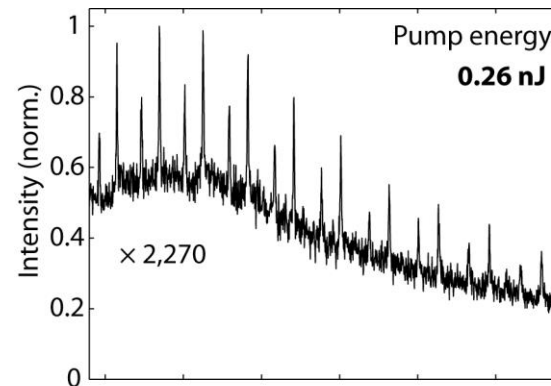
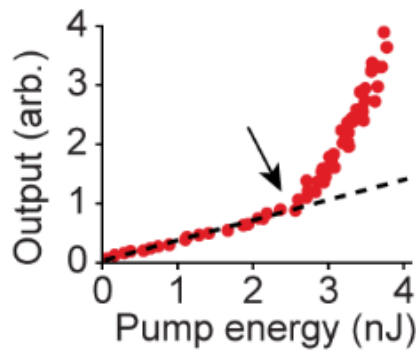
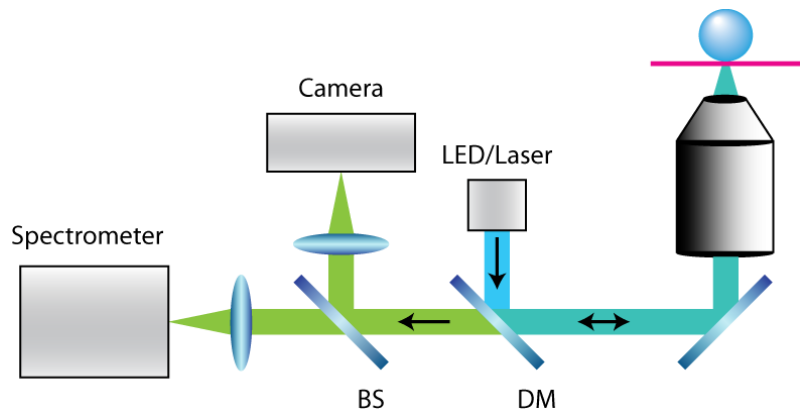
- Commercially available microbeads (1 – 15 μm)
- Materials: polystyrene, glass, BaTiO_3
- Engulfed by macrophage/non-macrophage cells by phagocytosis





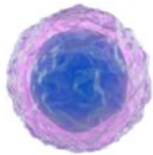
Solid beads - lasing

- Pumping with an external laser
- Sharp spectral peaks
- Whispering gallery modes
- High intensity above lasing threshold



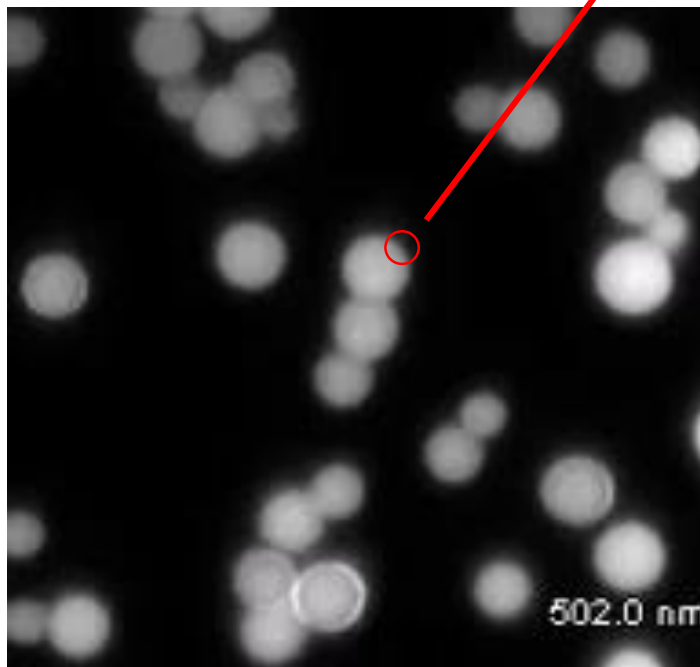
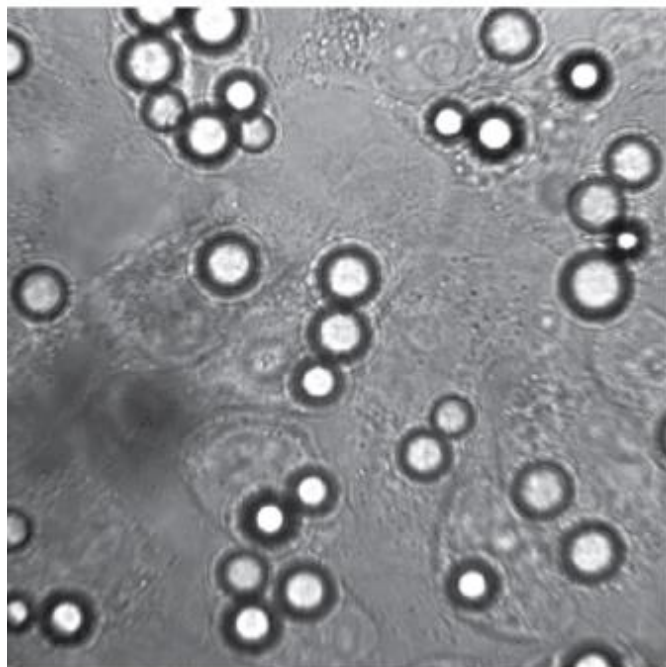
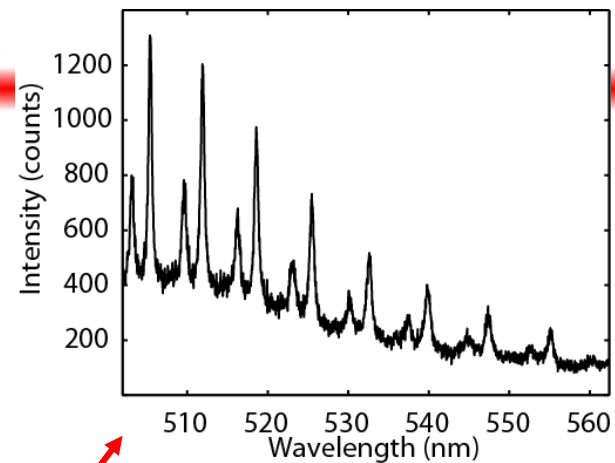
Increasing pump energy





Cell tagging

- Hyperspectral confocal fluorescence image
- Blinking corresponds to WGMs

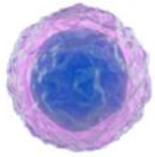


Fitting



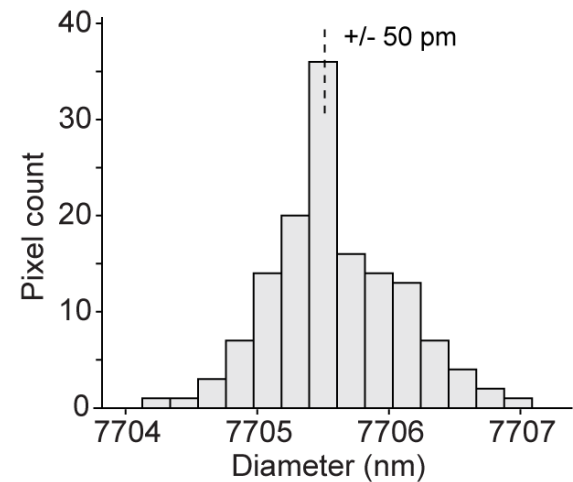
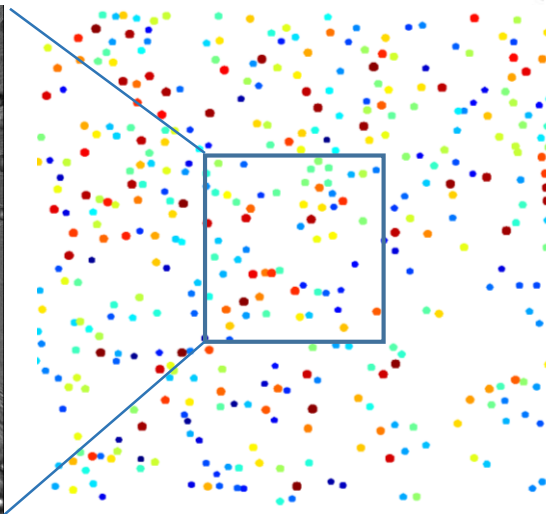
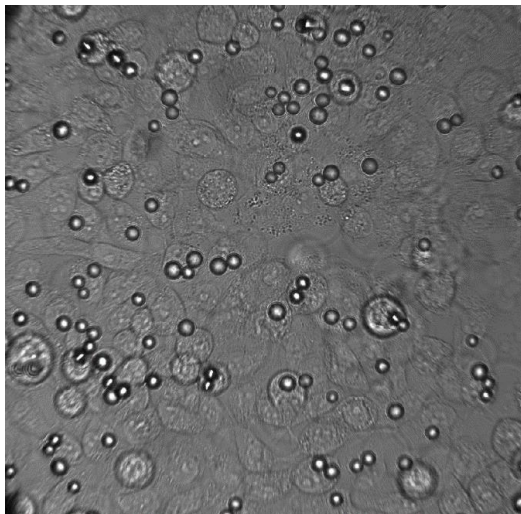
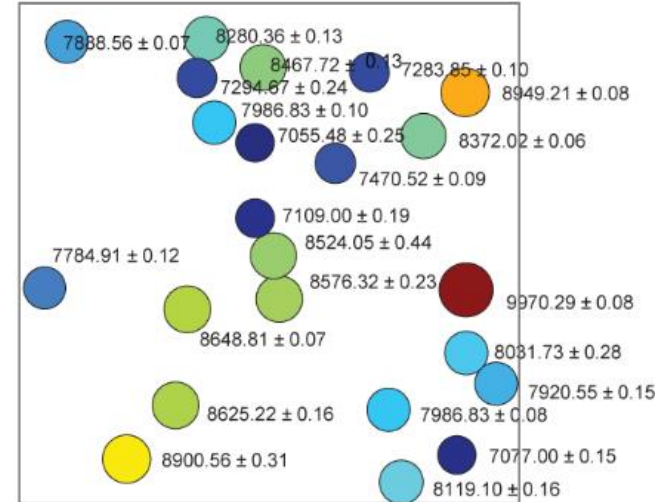
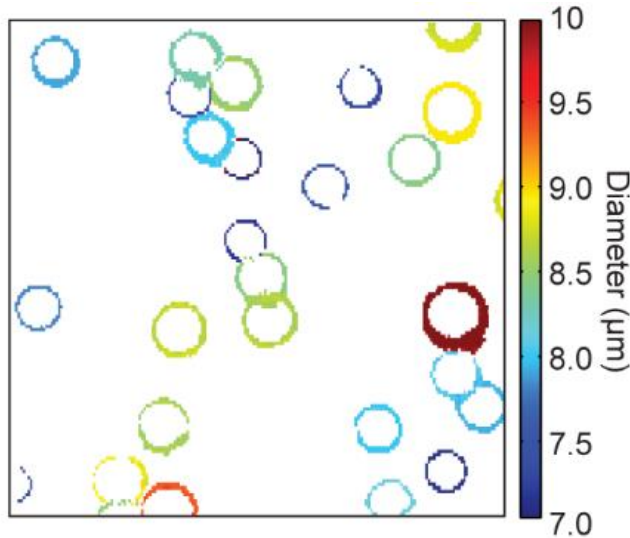
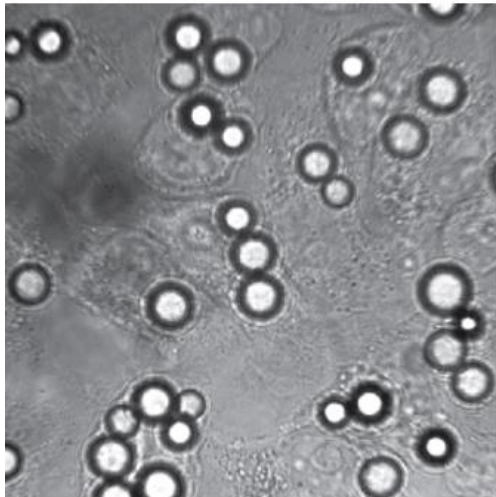
$d = 8576.32 \pm 0.23 \text{ nm}$



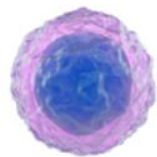


Hyperspectral imaging

- Calculate bead diameter
- Sensitivity: $50 \text{ pm} / 7.7 \text{ } \mu\text{m} = 6.5 \times 10^{-6}$

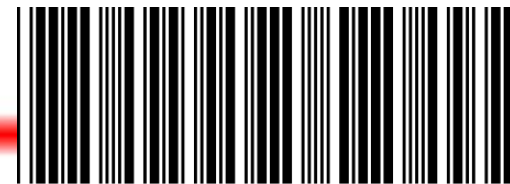
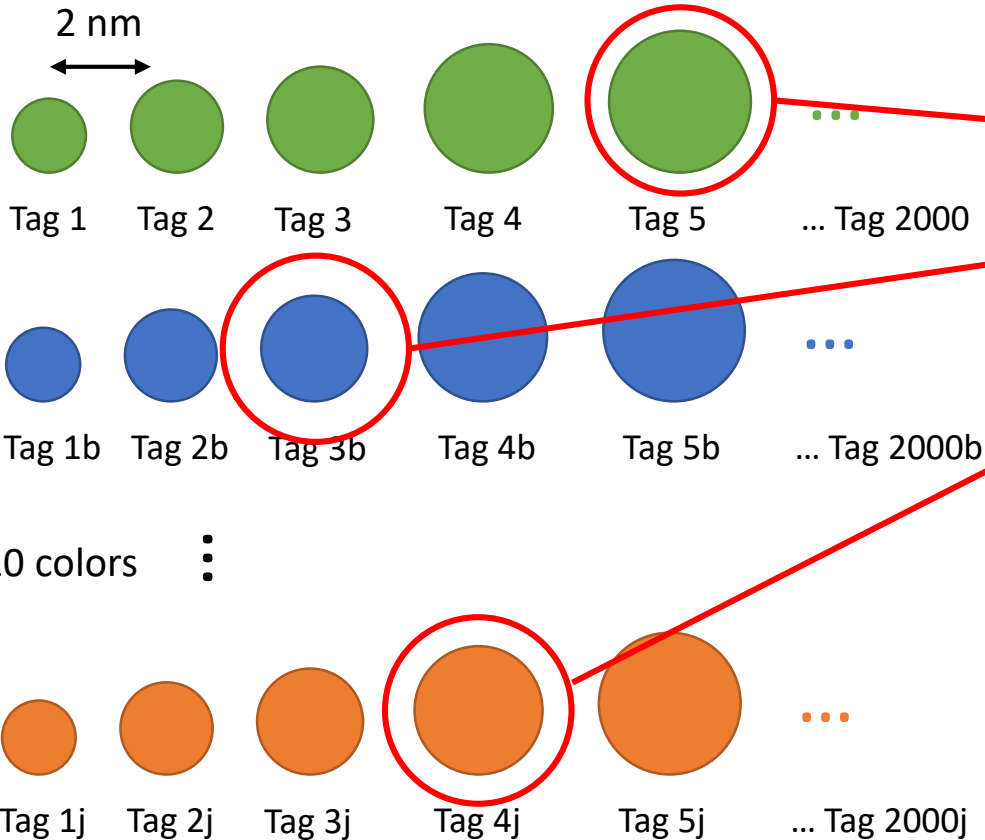


Cell tagging

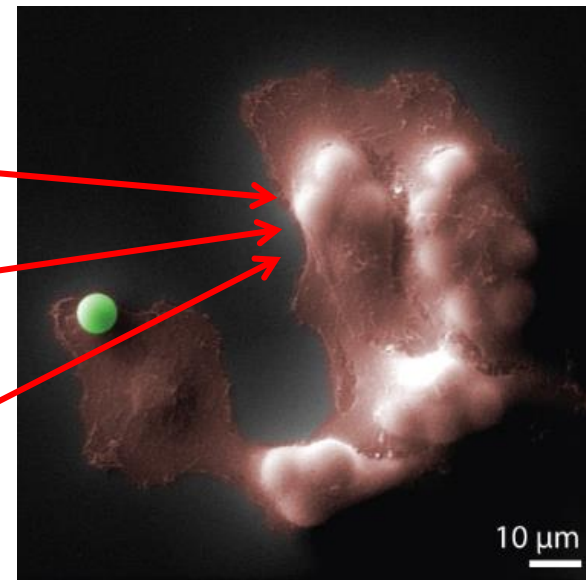


8 μm - 12 μm

2 nm

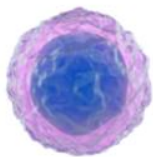


39123439



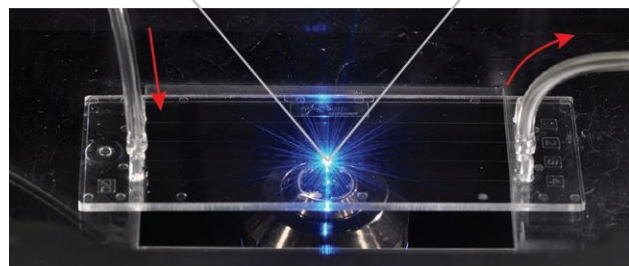
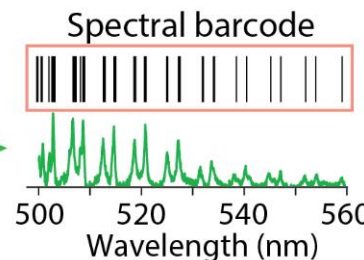
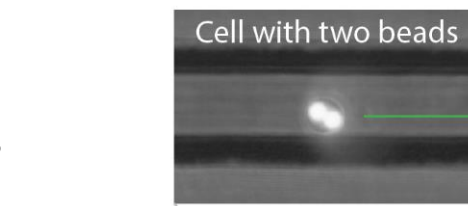
- By multiplexing: $\binom{2,000 \times 10}{3} \approx 10^{12}$
 - 10 different dyes/colors
 - 3 beads per cell
- On the same order as the number of cells in the human body





Cell tagging in microfluidics

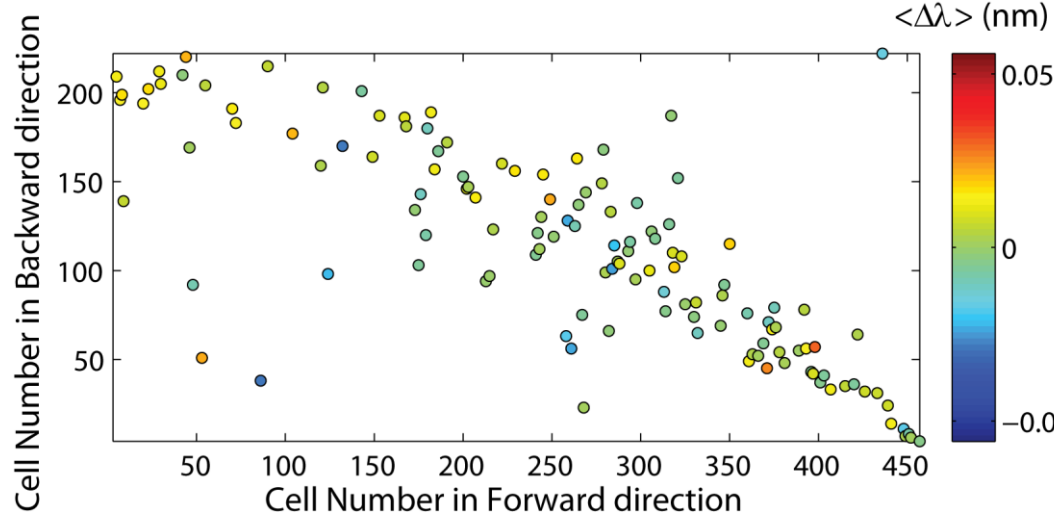
- Cells containing beads
- Flow through a microfluidic channel
- Flow back to identify individual cells
- Identification based on spectral line positions
- Identification accuracy: >99%

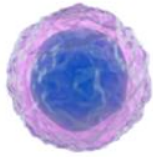


Forward run (reference)



Backward run (sample)





Intracellular sensing

- Whispering gallery modes are sensitive to surrounding refractive index
- Proof of concept measurement
 - Increase of external osmolarity
 - Decrease in cell volume
 - Increase in refractive index
- We can measure change in refractive index down to 2.9×10^{-4} RIU



Hypotonic

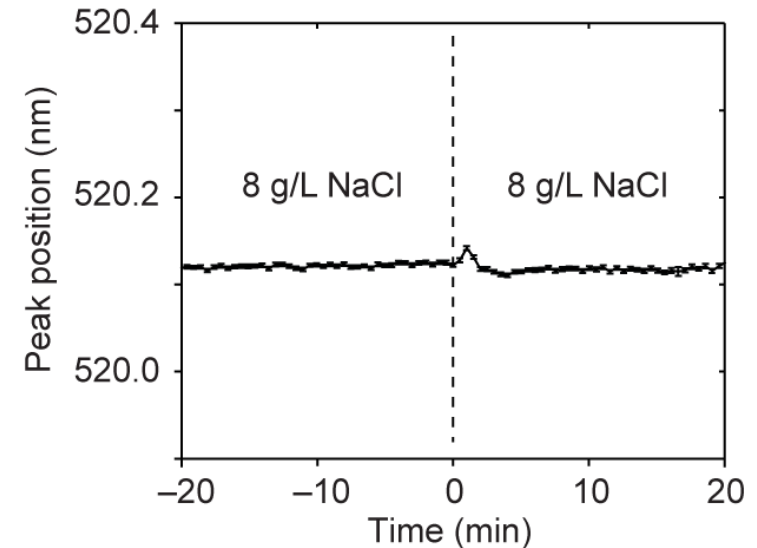
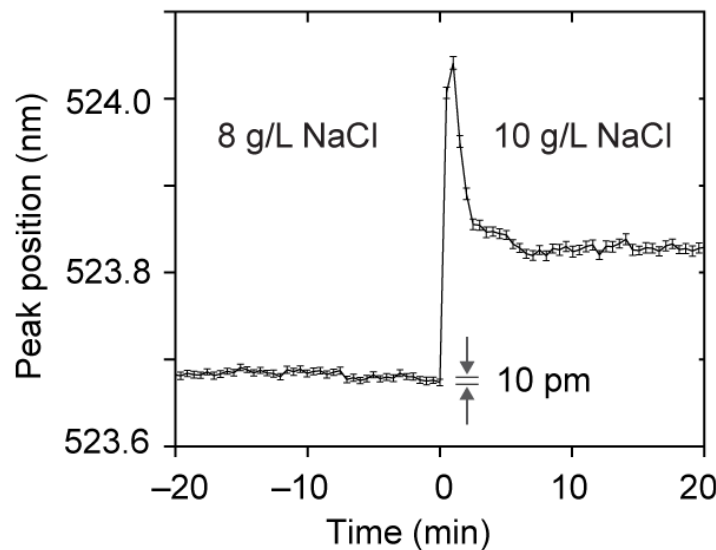
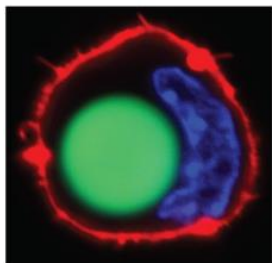


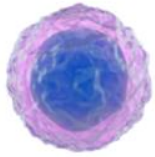
Isotonic



Hypertonic

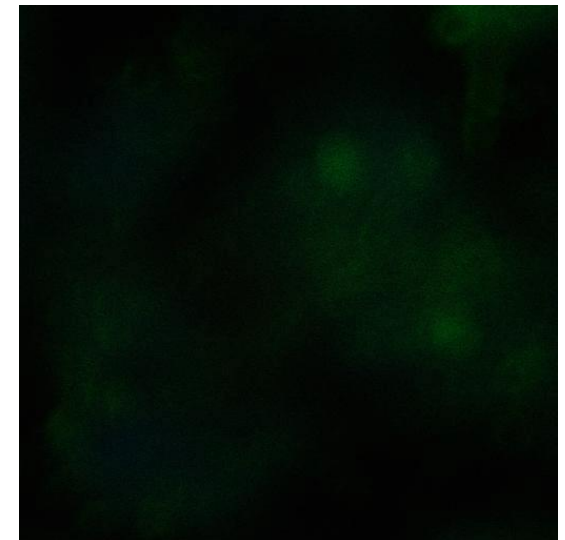
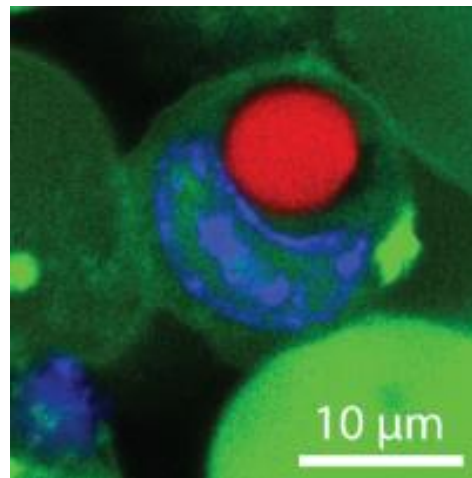
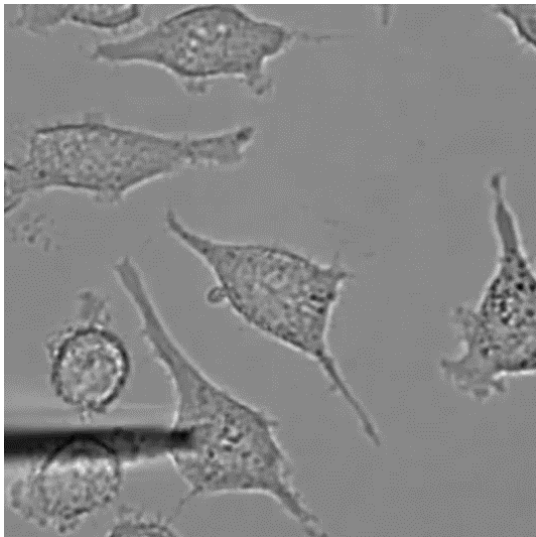
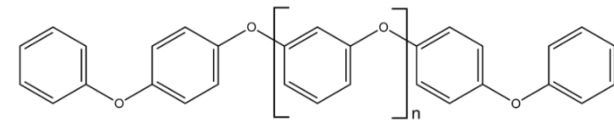
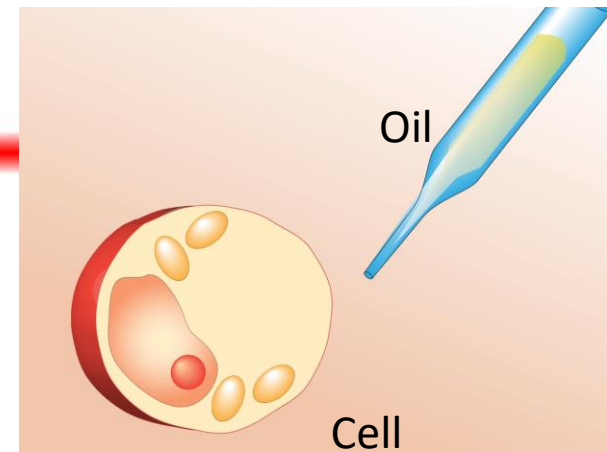
<http://medical-dictionary.thefreedictionary.com>

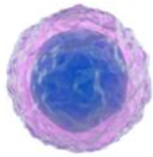




Injected high index oil

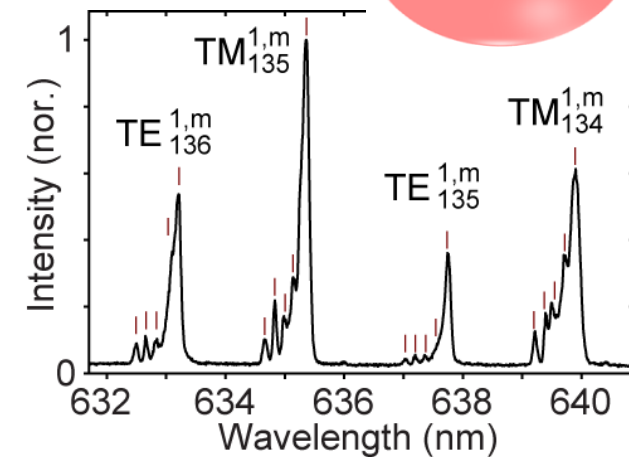
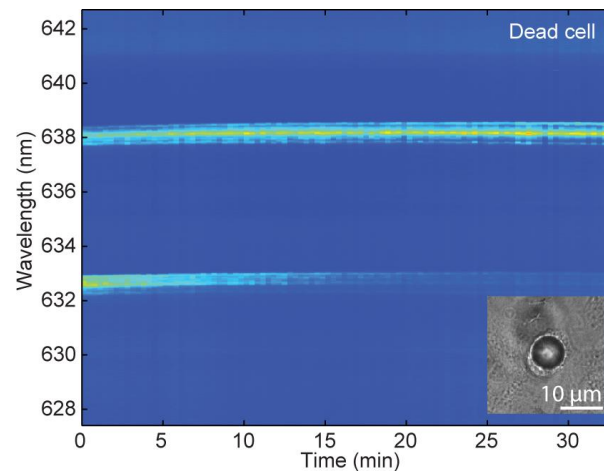
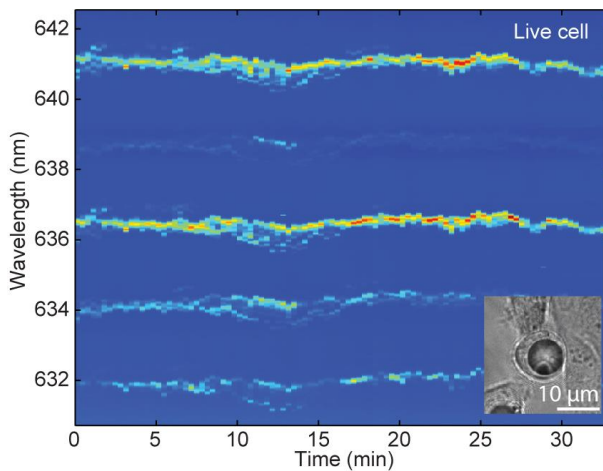
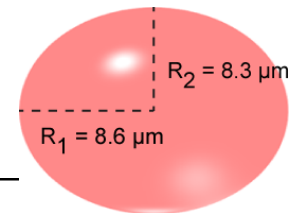
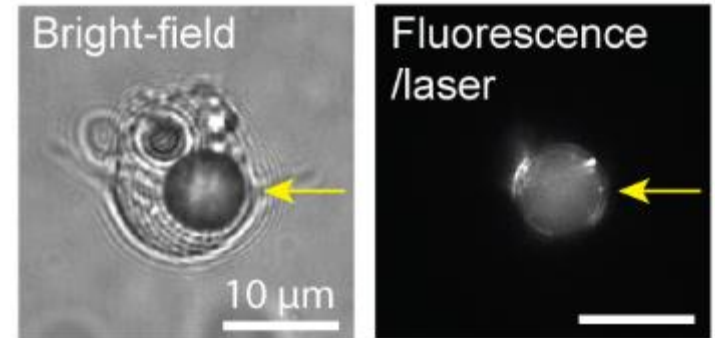
- Standard microinjection apparatus
 - Typically used to inject DNA
 - Glass micropipette with tip diameter $\sim 1 \mu\text{m}$
- Polyphenyl ether oil
 - High refractive index ($n = 1.69 @ 500 \text{ nm}$)
 - Low viscosity, Insoluble in water, Nontoxic
 - Nile red fluorescent dye, 5 mM

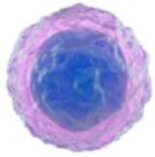




Injected high index oil - lasing

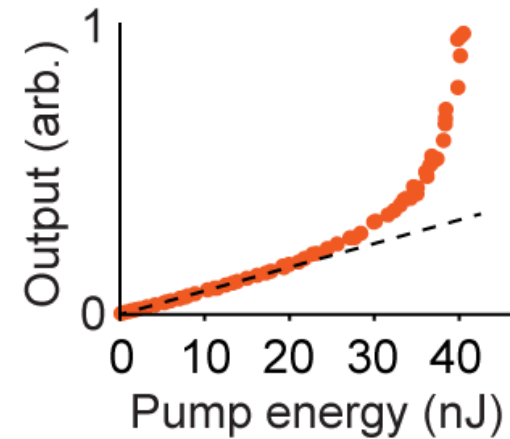
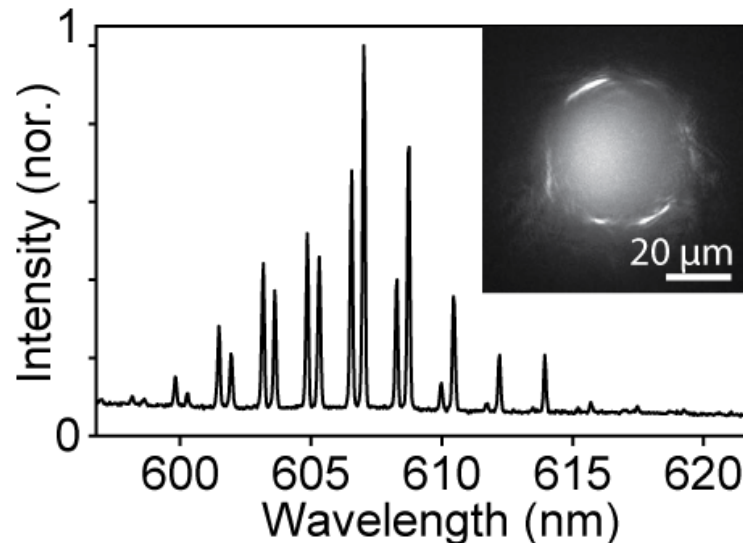
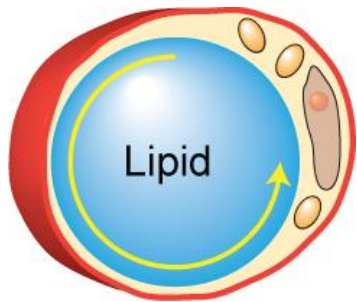
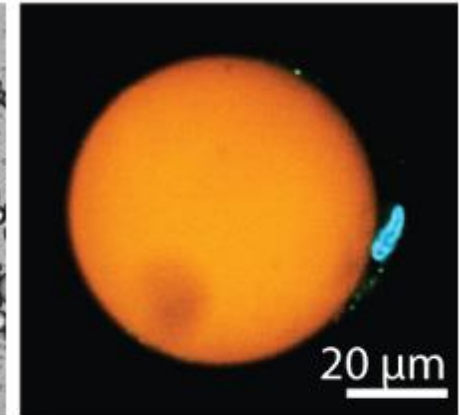
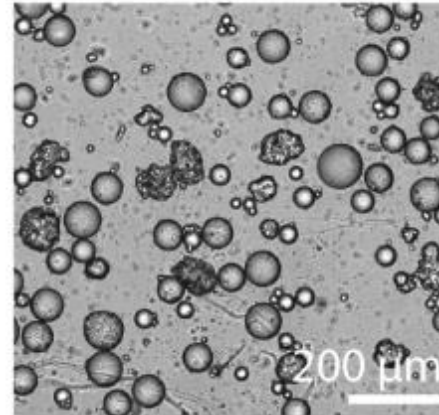
- Droplet deformation causes splitting of spectral lines
 - Enables approximate force calculation
 - Real time intracellular force tracking
- Force fluctuation sensitivity: $40 \text{ pN}/\mu\text{m}^2$ (40 Pa)

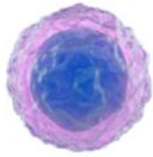




Adipocytes – fat tissue cells lasing

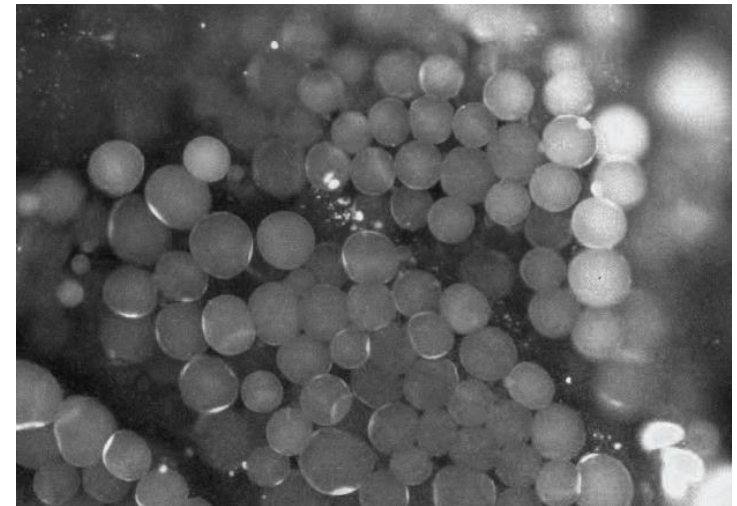
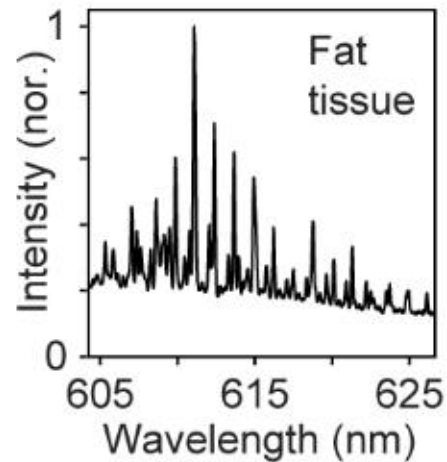
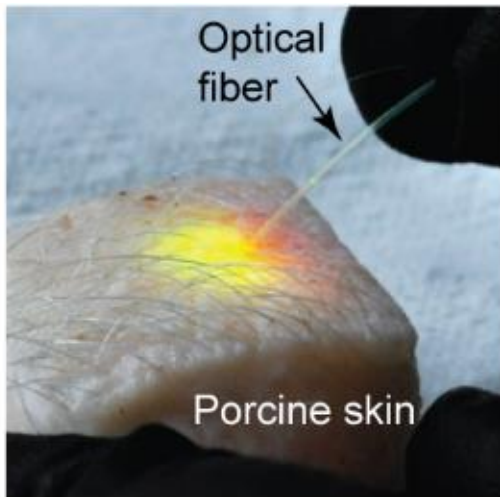
- Adipocyte cells naturally contain large lipid droplets
- Adipocytes were harvested by collagenase digestion from porcine subcutaneous fat tissue
- Stained with Nile red
- Pumped with external laser

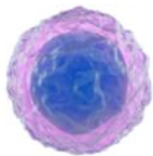




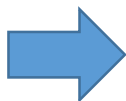
Lasing from subcutaneous fat tissue

- Inject Nile red dye
- Insert optical fiber
- Pump and collect laser light through the fiber

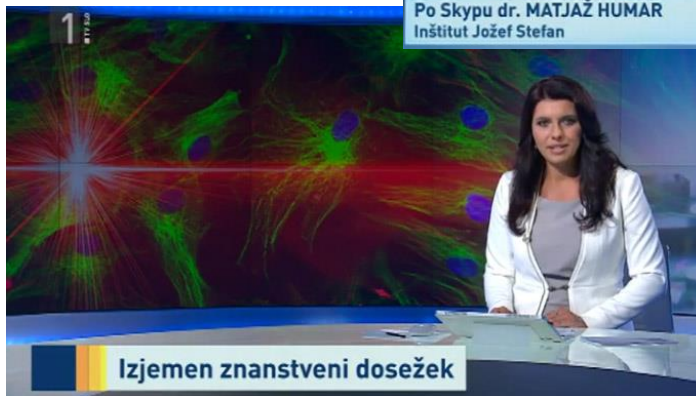
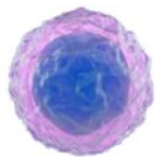




Rewriting science fiction

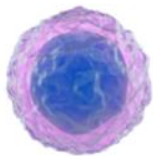


Media impact



Osebnost Primorske meseca julija

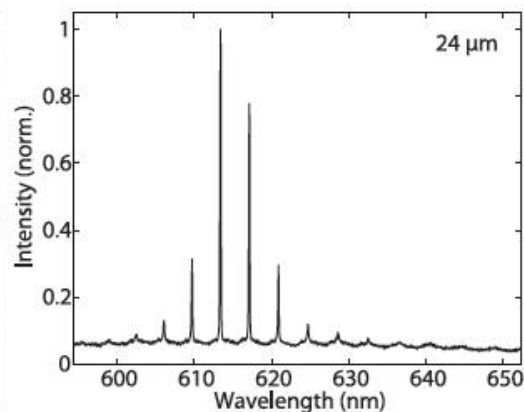
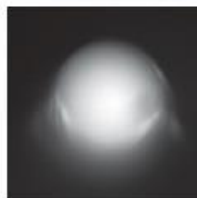
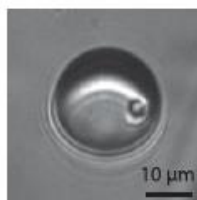
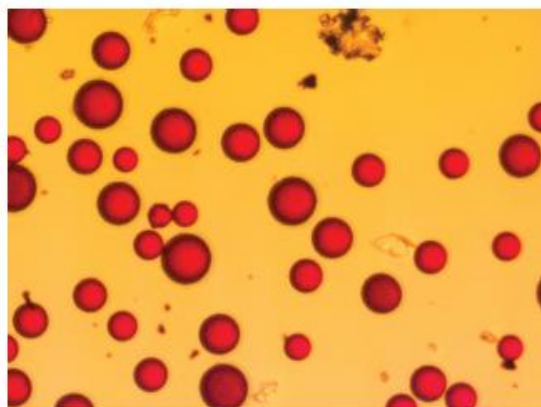




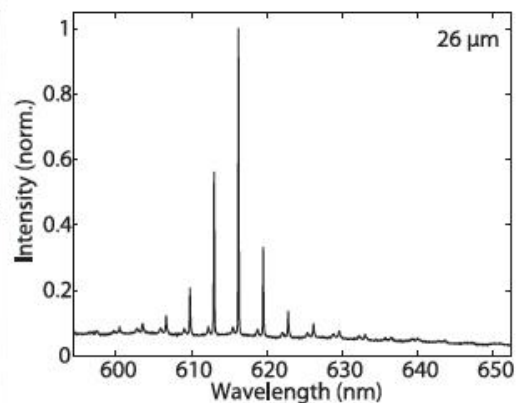
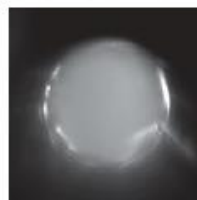
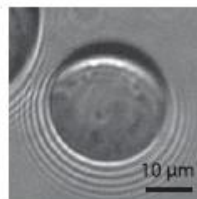
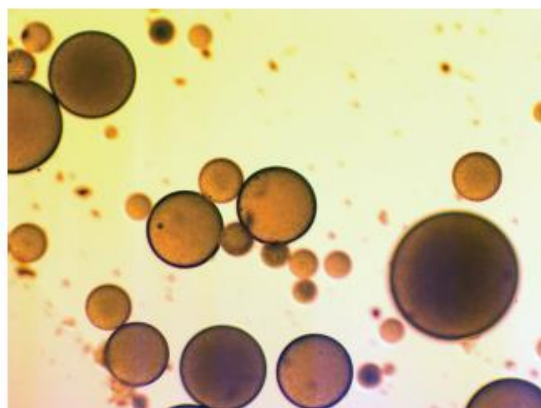
Biodegradable lasers

- Poly(lactic-co-glycolic acid) (PLGA) or Poly-L-lactide (PLLA)
- Dissolved in dichloromethane (DCM) + fluorescent dye
- Oil in water dispersion
- The minimum size for lasing: 20 μm

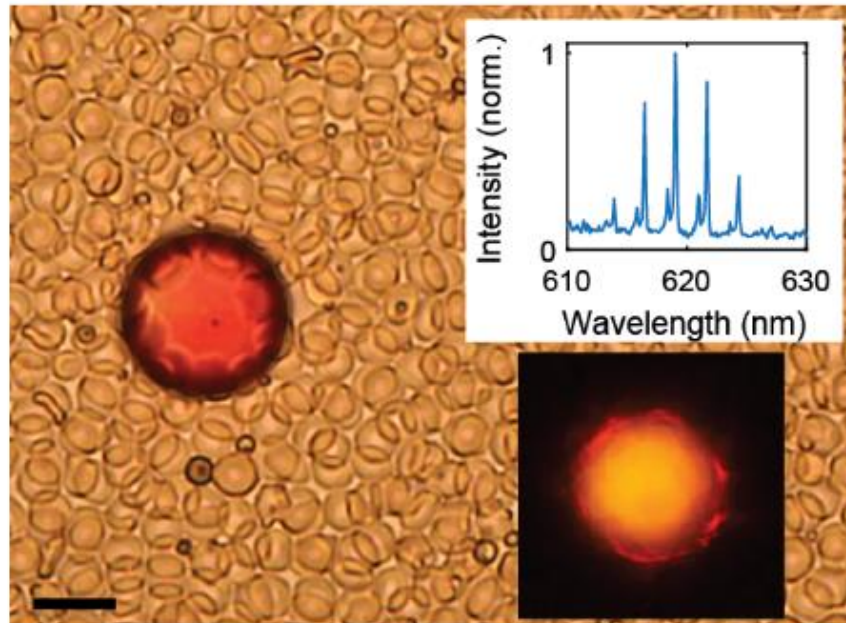
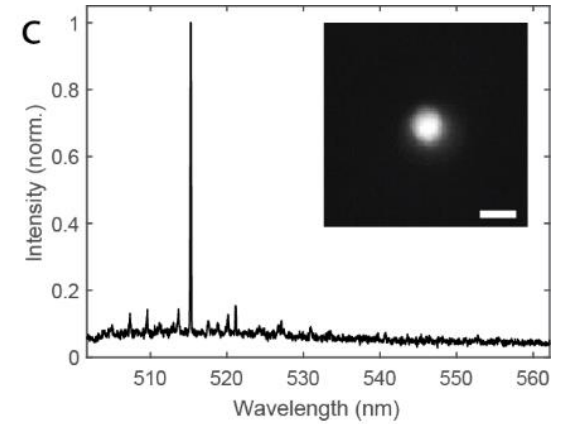
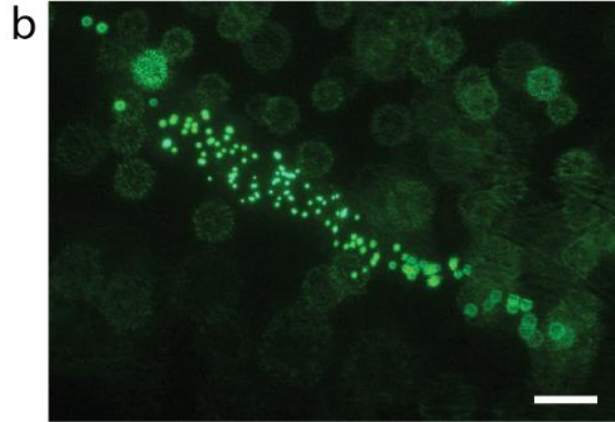
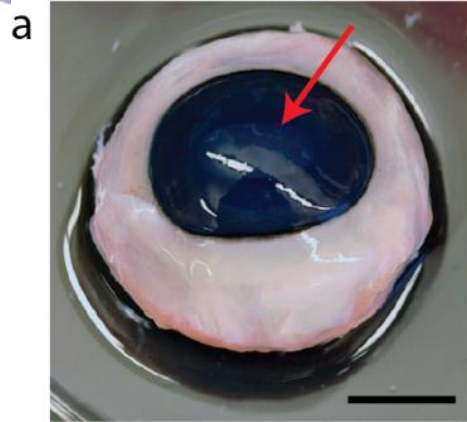
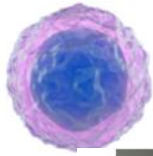
PLGA

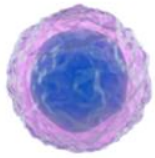


PLLA



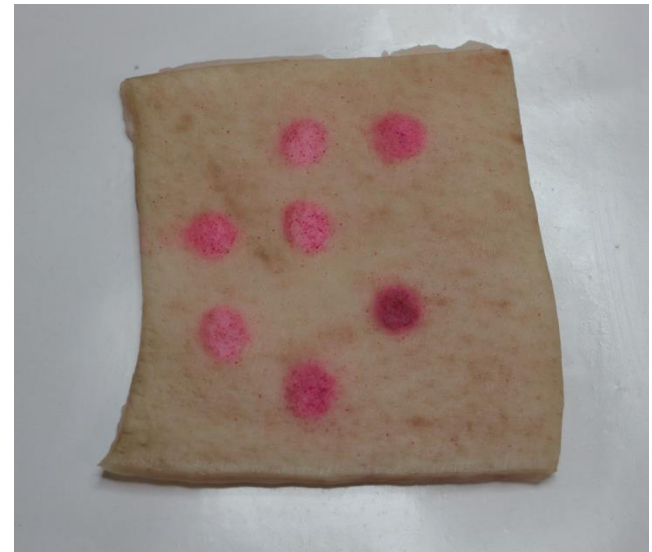
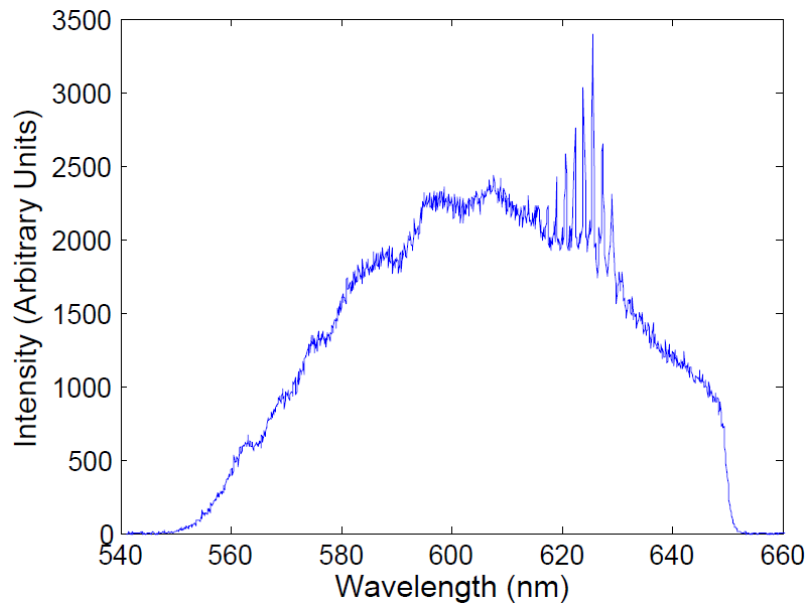
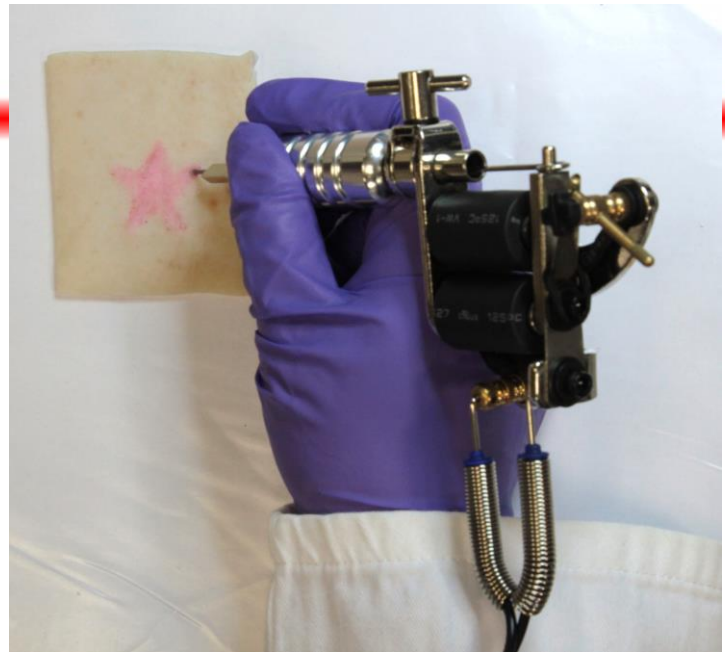
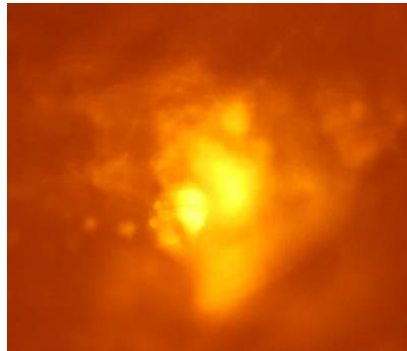
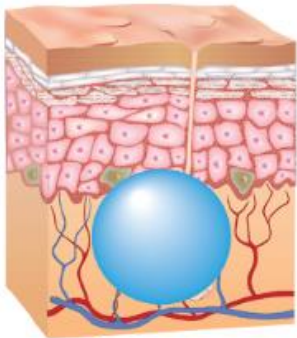
Operation of lasers in eye and blood



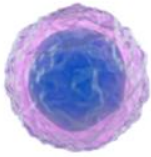


Laser tattoos

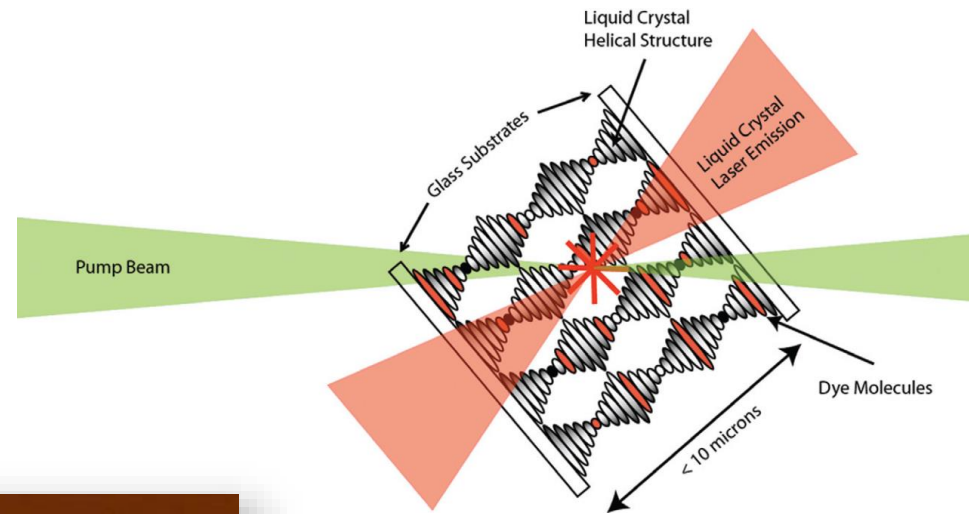
- Implantation of biocompatible lasers into skin using standard tattoo gun



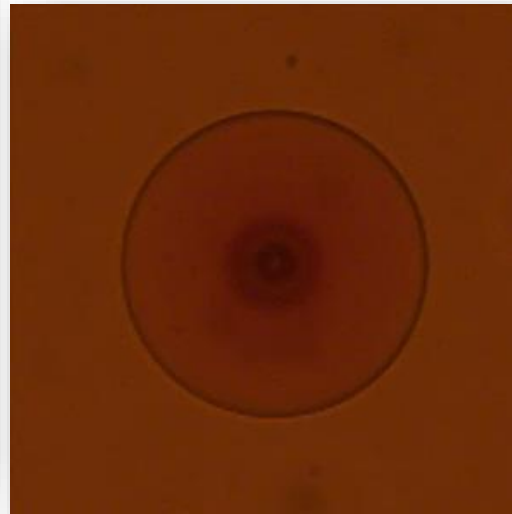
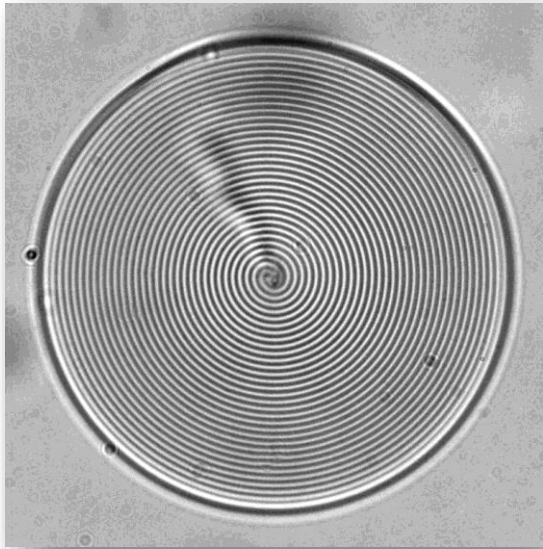
Cholesteric liquid crystal droplet laser



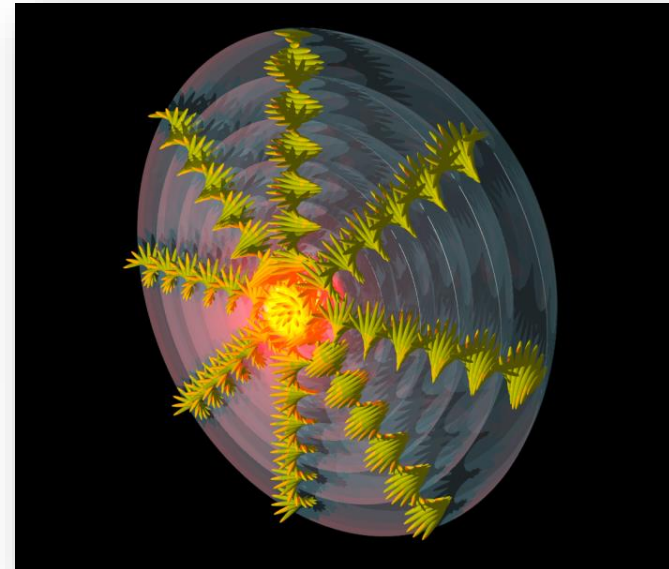
- Helical twist -> photonic crystal
- Dye doped LC droplet is a Bragg onion laser
- Single mode lasing
- Omnidirectional emission
- Smallest laser size: 14 μm



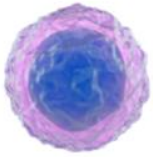
A. D. Ford, et al, *Materials Today* 9, 2006.



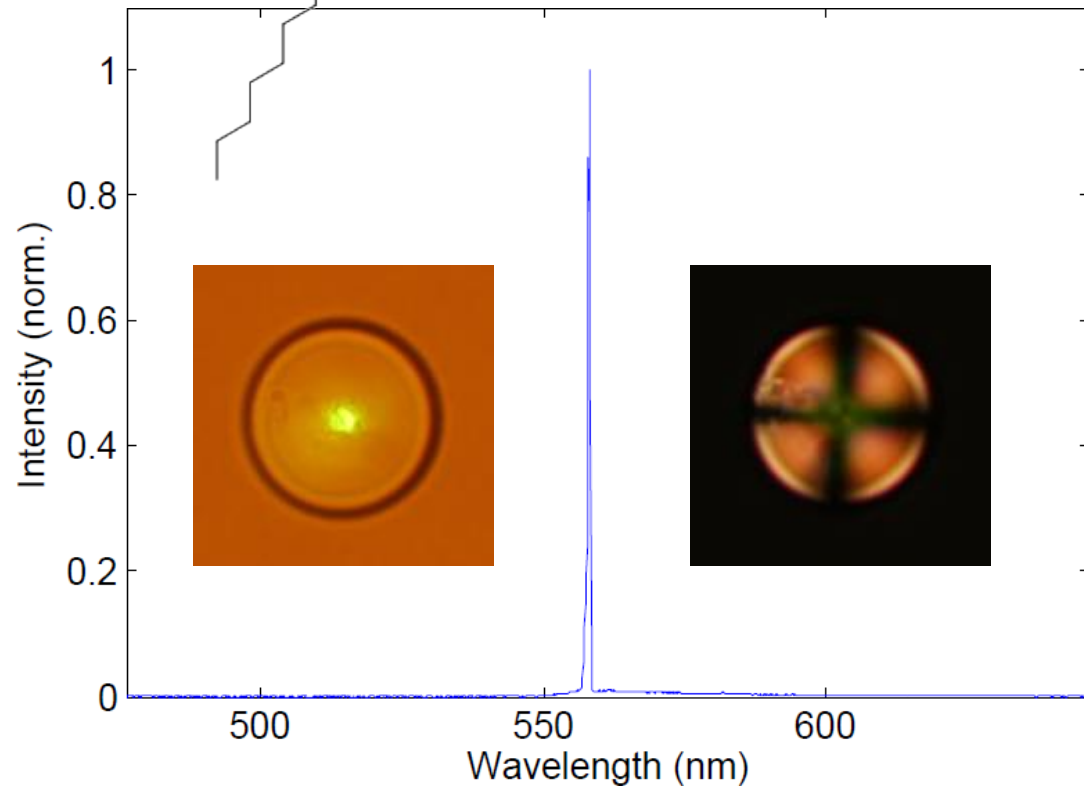
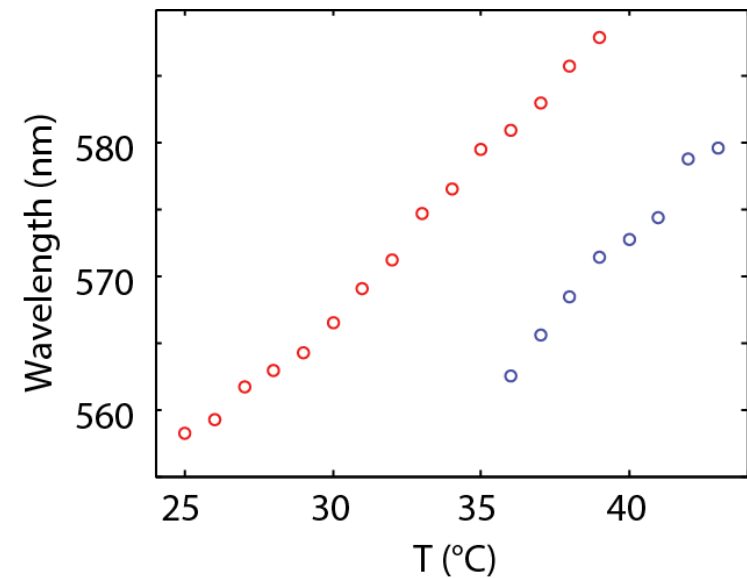
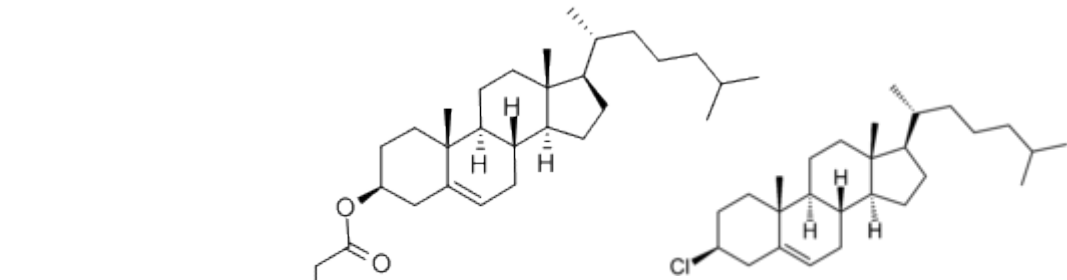
M. Humar and I. Muševič, 3D microlasers from self-assembled cholesteric liquid crystal microdroplets, *Opt. Express* **18**, 26995-27003 (2010).

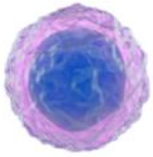


Cholesterol laser

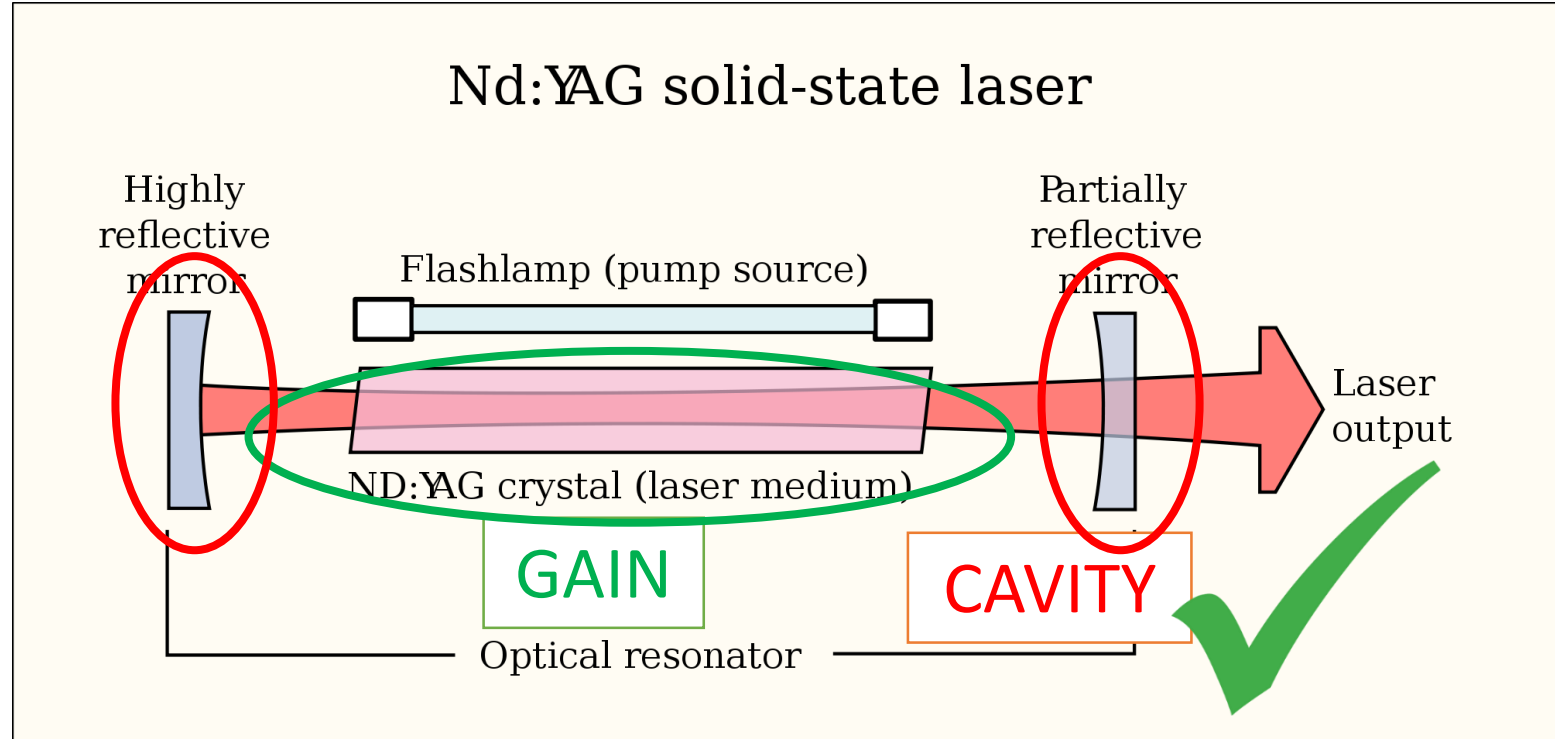


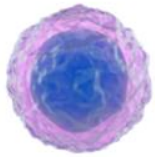
- Cholesteryl chloride, cholesteryl nonanoate and cholesteryl oleyl carbonate
- Biocompatible, since already present in our body





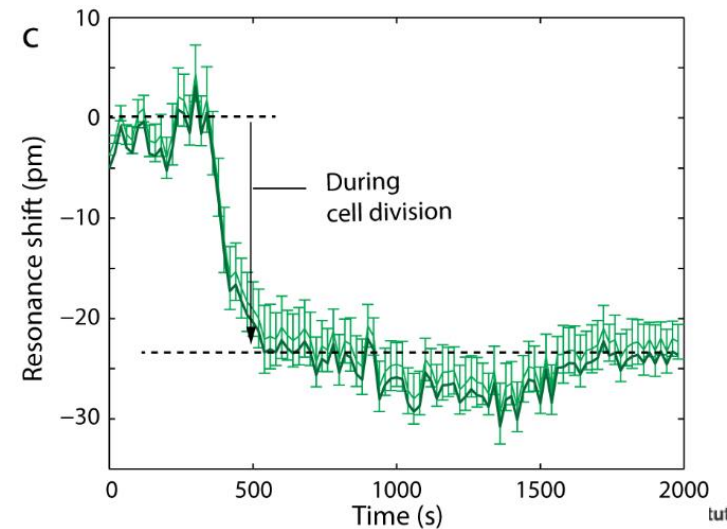
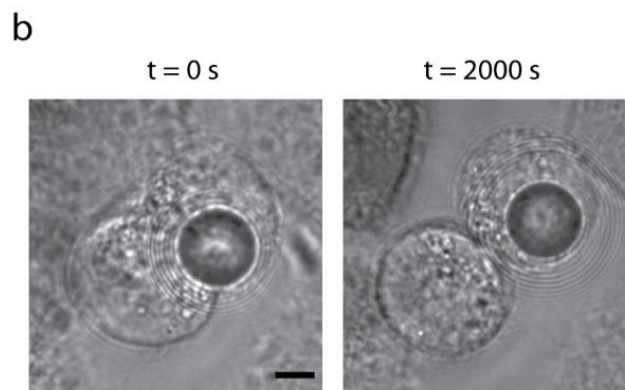
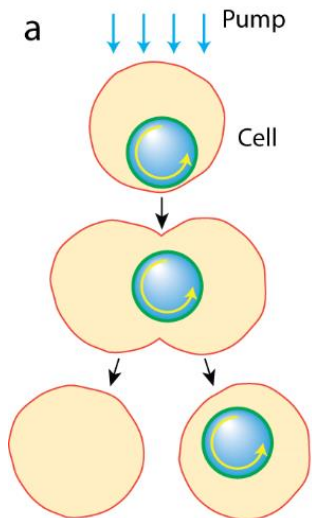
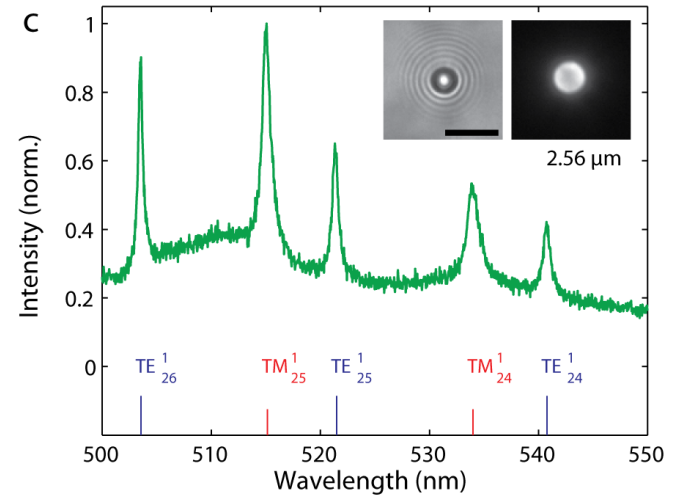
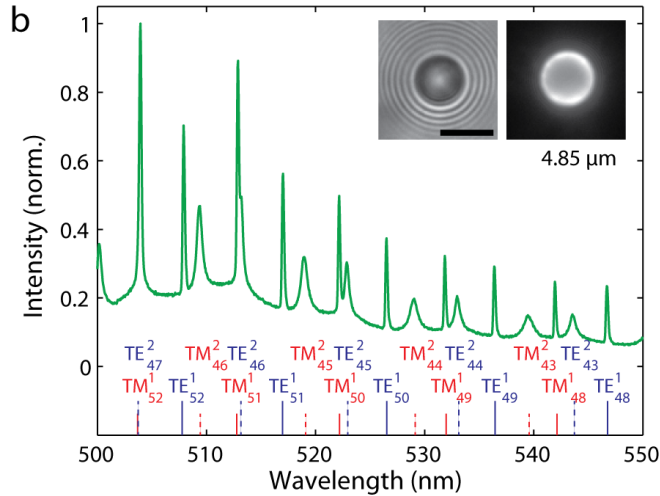
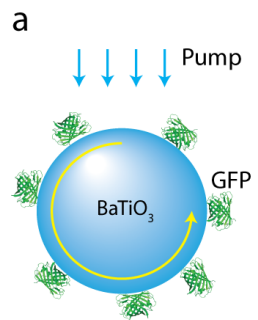
Lasers made completely out of biological materials



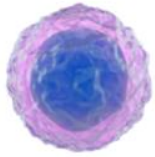


GFP WGM "lasers"

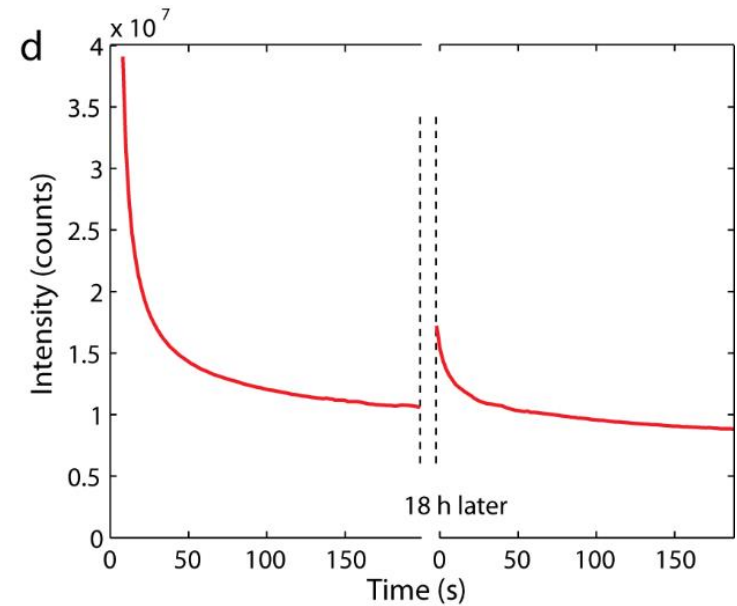
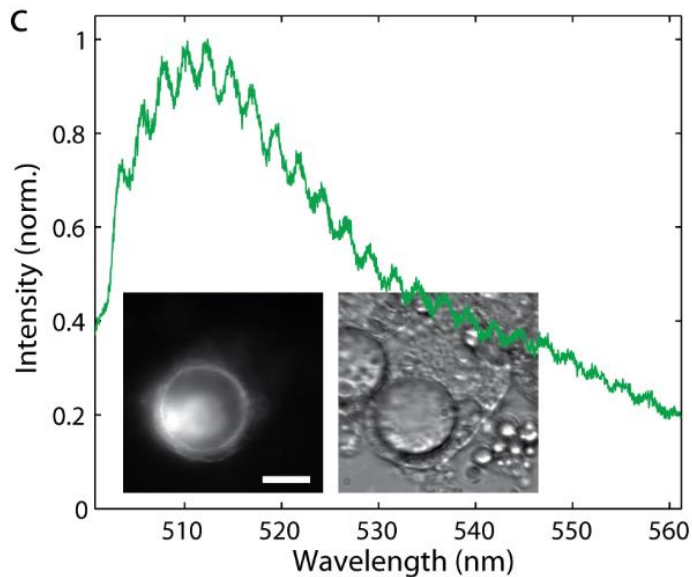
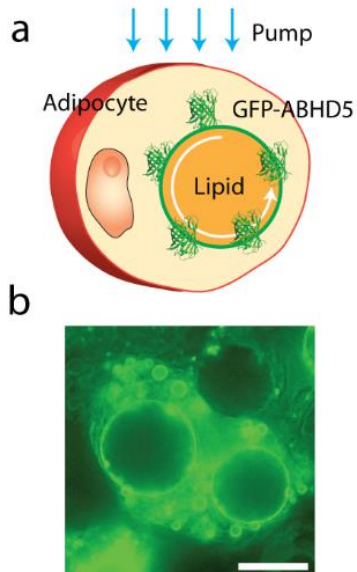
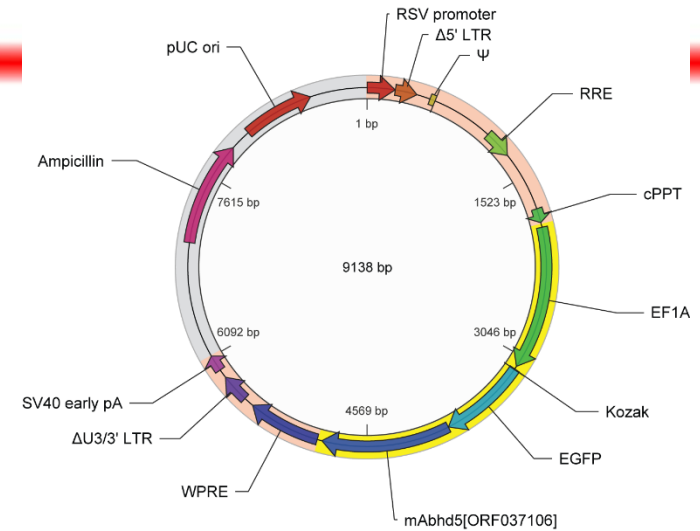
- GFP chemically linked to the surface of beads ($\text{BaTiO}_3\text{-APTES-GA-GFP}$)
- Whispering gallery modes generated



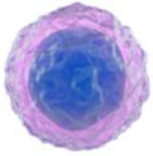
GFP in adipocytes



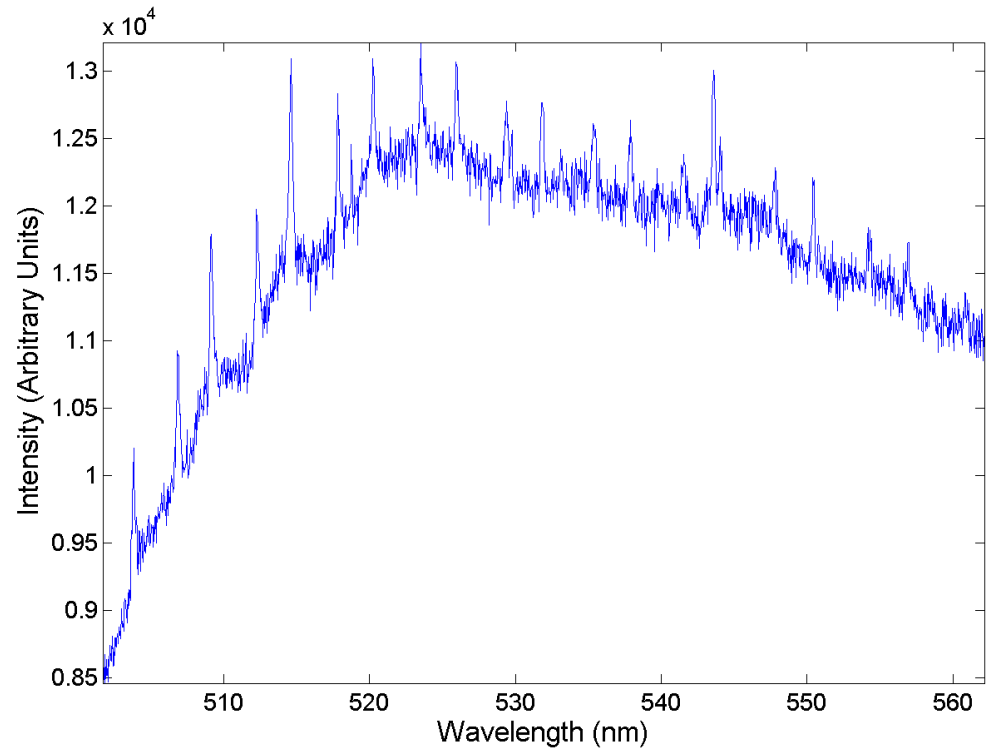
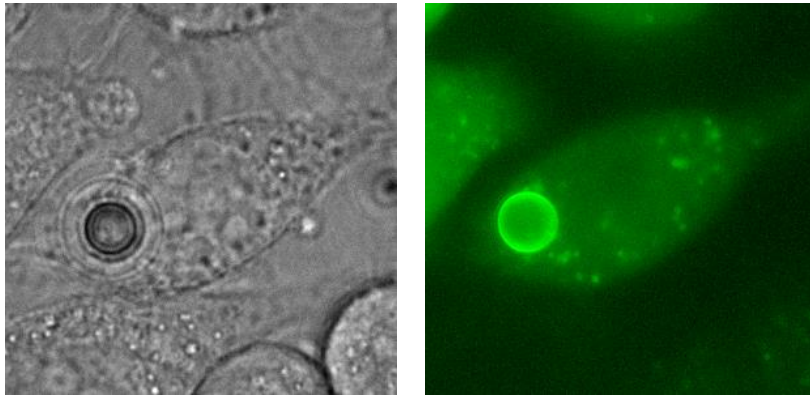
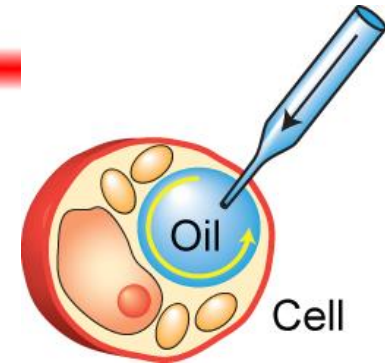
- Can we combine
 - Bio-cavity – lipid droplets in adipocytes
 - Bio-gain – GFP transfection
- We designed a custom fusion protein: Abhd5-GFP
- Abhd5 is known to bind to the surface of lipid droplets
- Completely bio-cavity, self-healing

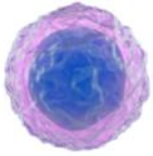


GFP in cell + artificial droplet

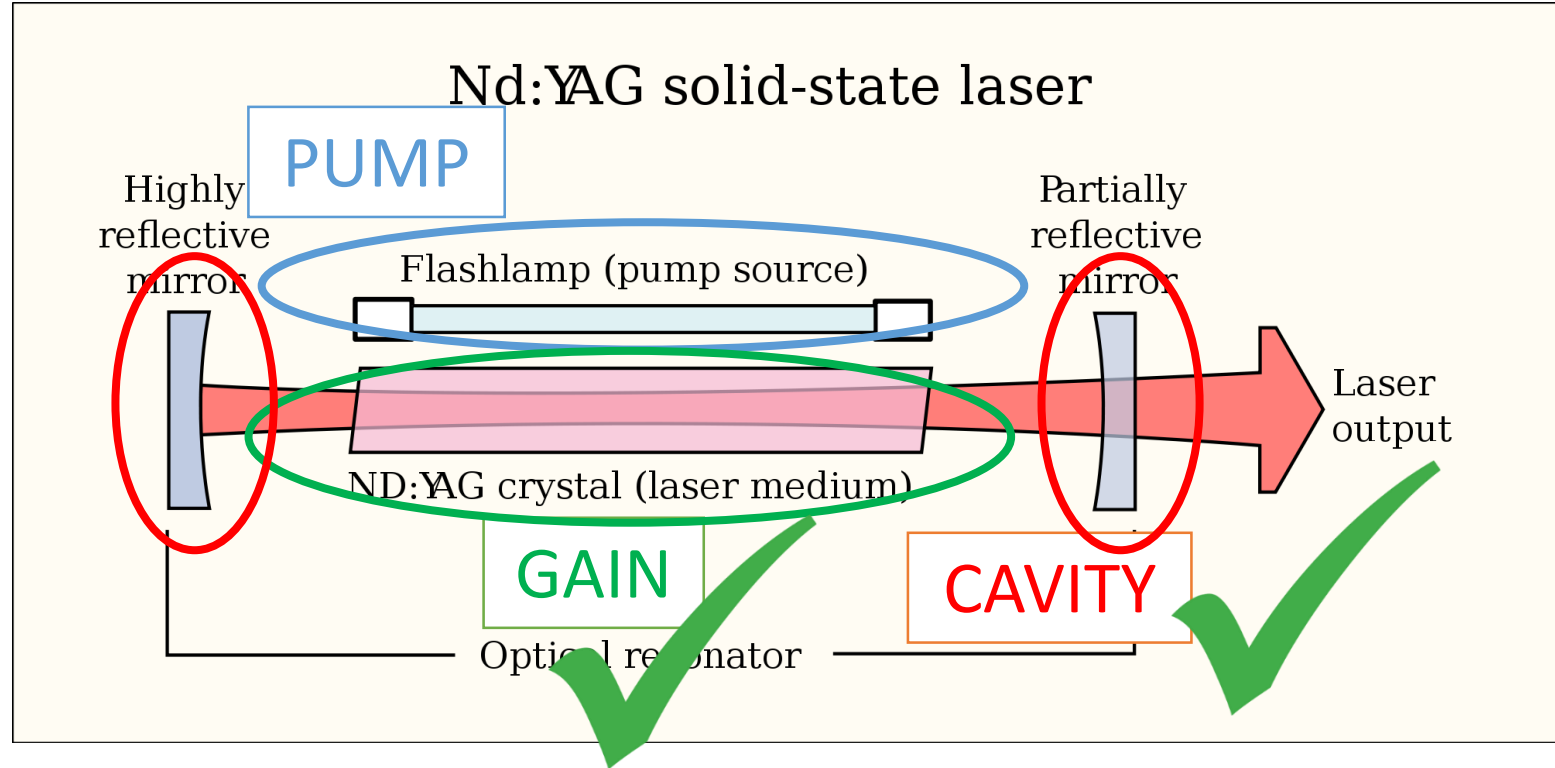


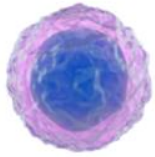
- Abhd5-GFP fusion protein
- Polyphenyl ether oil
- WGMs visible
- Self-healing



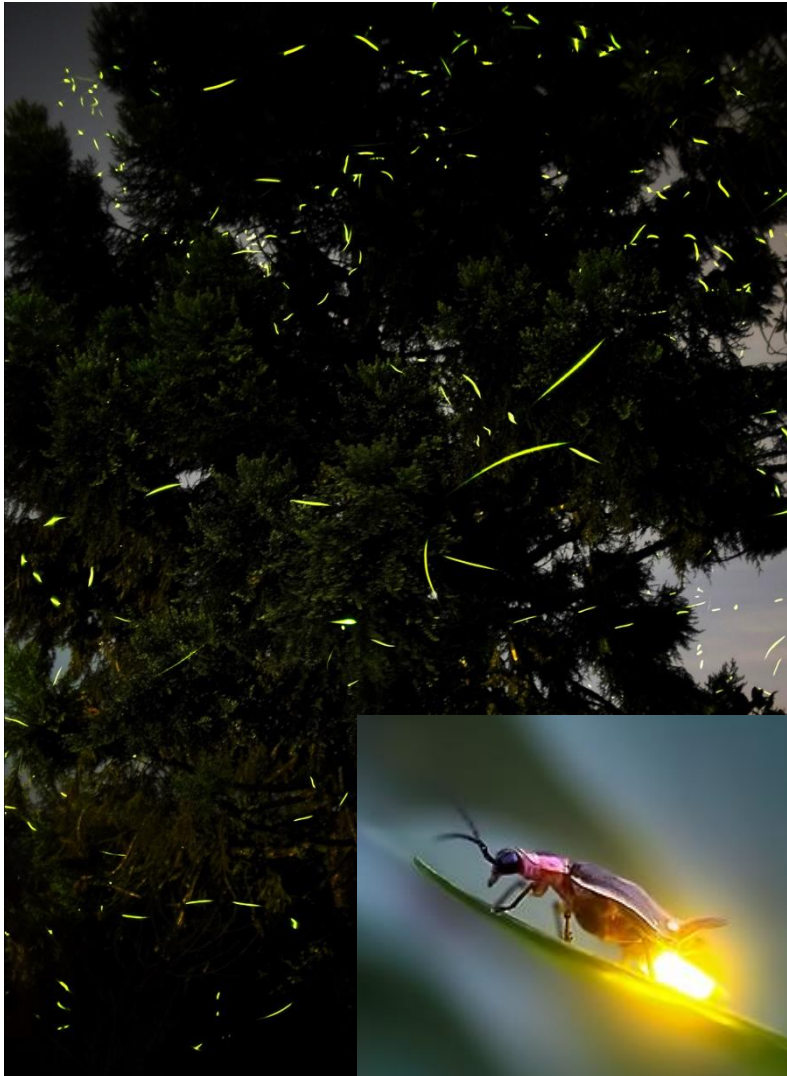


Lasers made completely out of biological materials

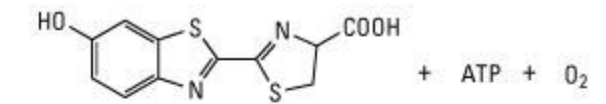




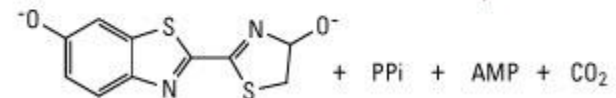
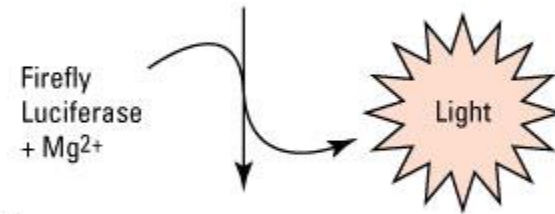
Bioluminescence



luciferase + luciferin \rightarrow light

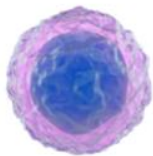


D-Luciferin



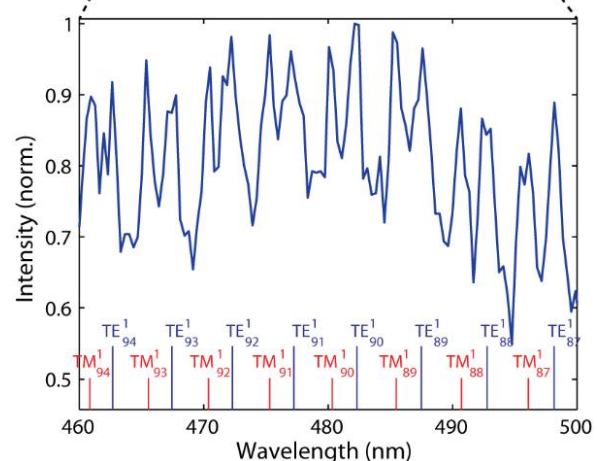
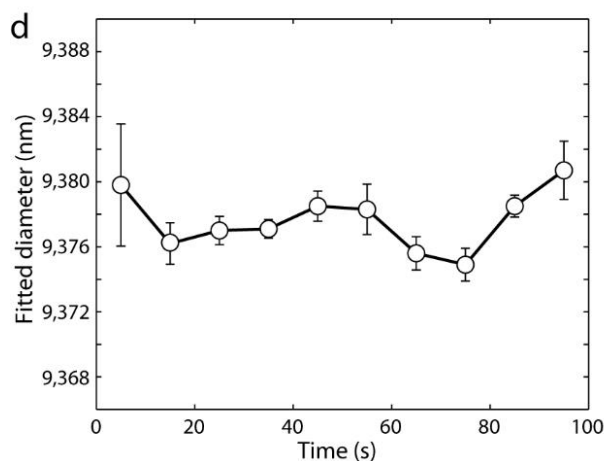
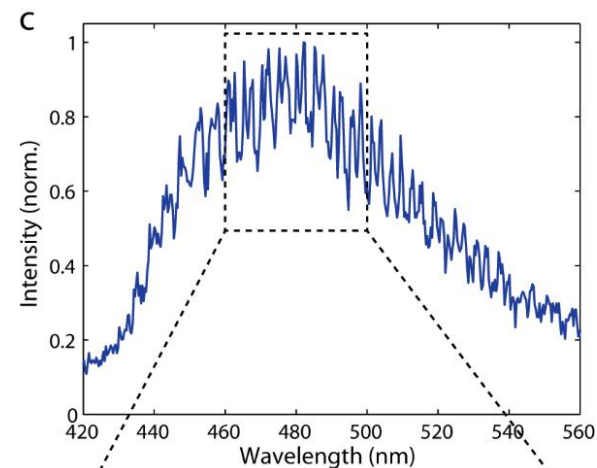
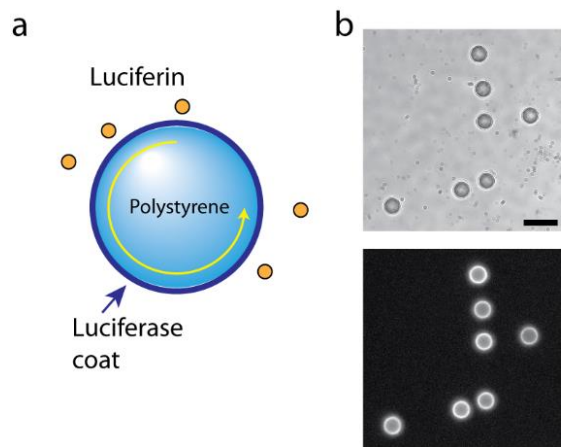
Oxyluciferin

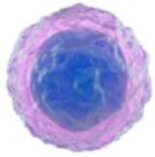




Bioluminescence pumped WGMs below threshold

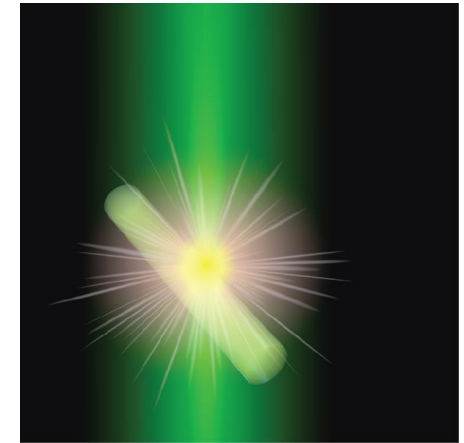
- Beads coated with luciferase
- Introducing luciferin into solution
- Light generated is coupled to WGMs
- Laser like emission



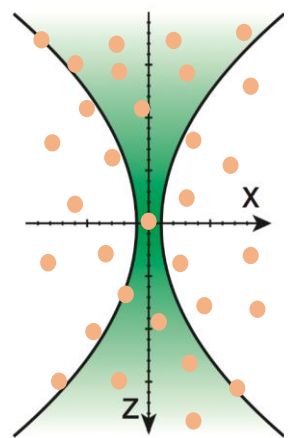


Laser particle stimulated emission (LASE) microscopy

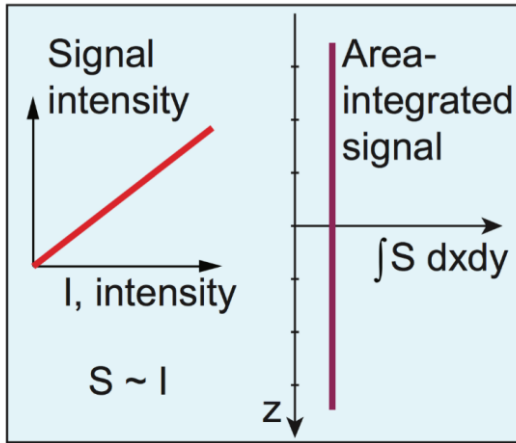
- Using nanolasers instead of fluorescent probes
- Scanning with a laser beam
- High nonlinearity
- Sub-diffraction resolution, optical sectioning, high background rejection



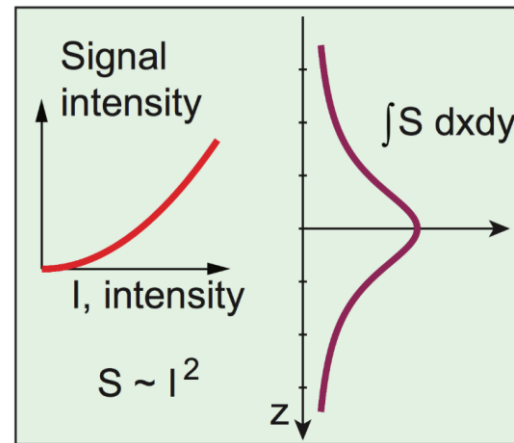
a Excitation beam



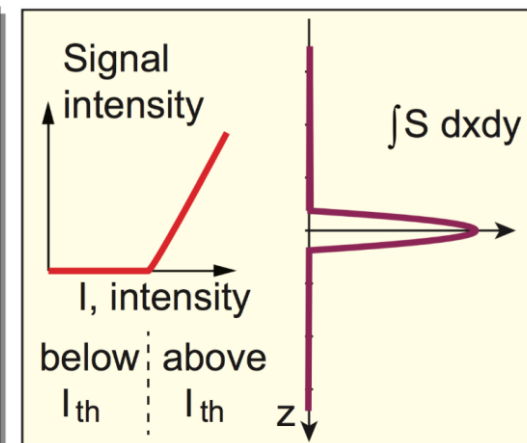
b Bright-field imaging

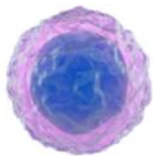


c Two-photon imaging



d LASE imaging





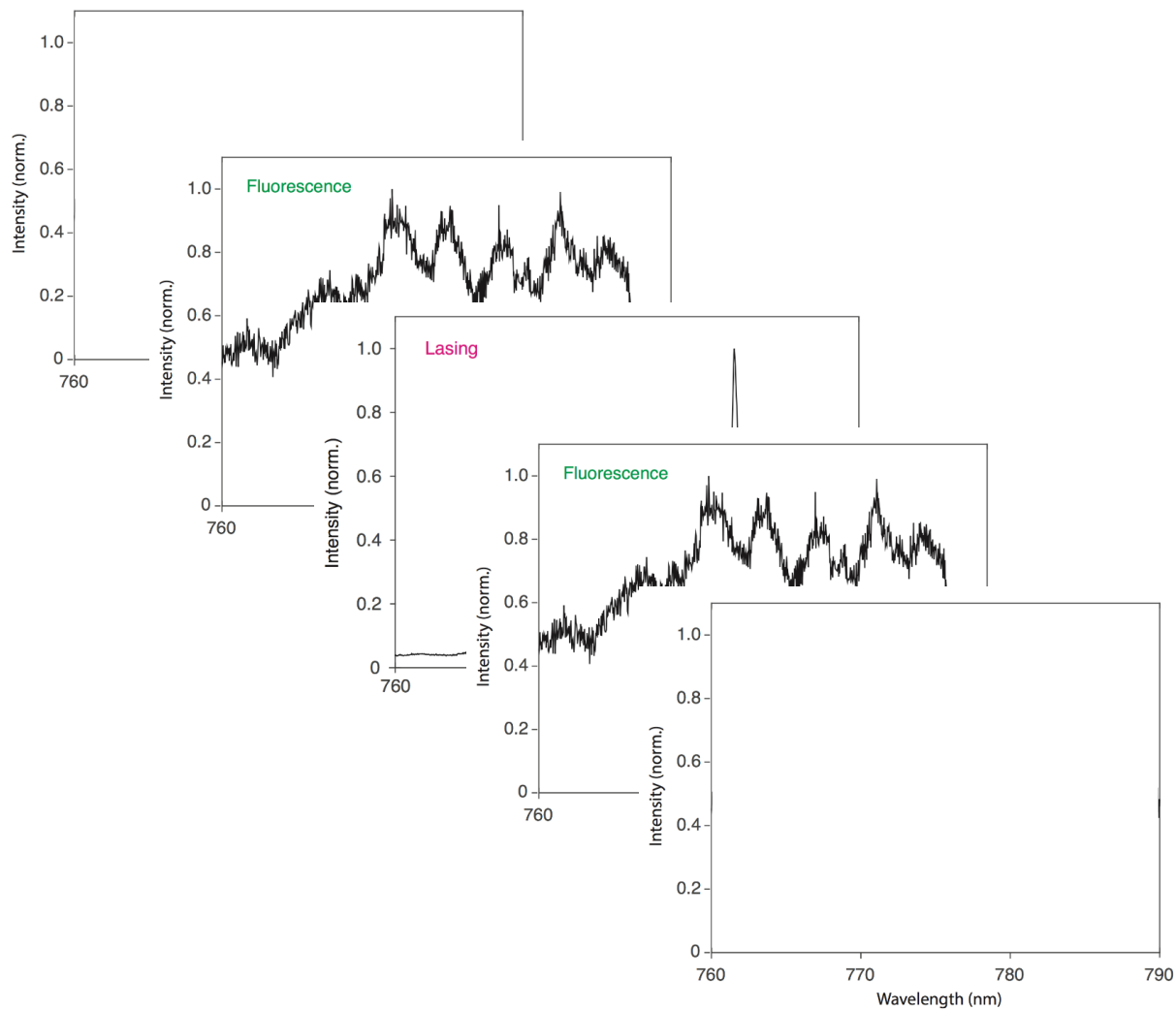
Experimental validation

1D measurement with elliptical beam

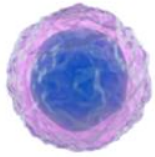


NANOWIRE

Illumination x axis: $2.4 \mu\text{m}$
Illumination y axis: $9.5 \mu\text{m}$



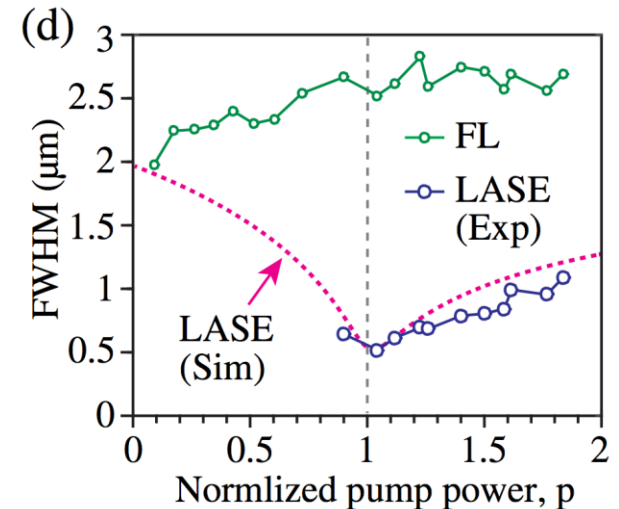
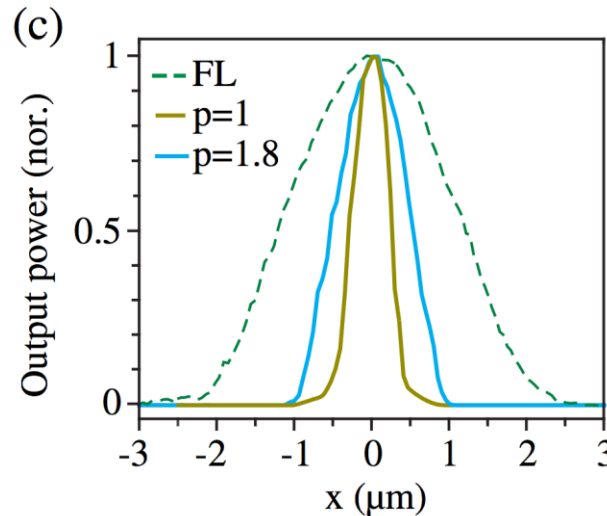
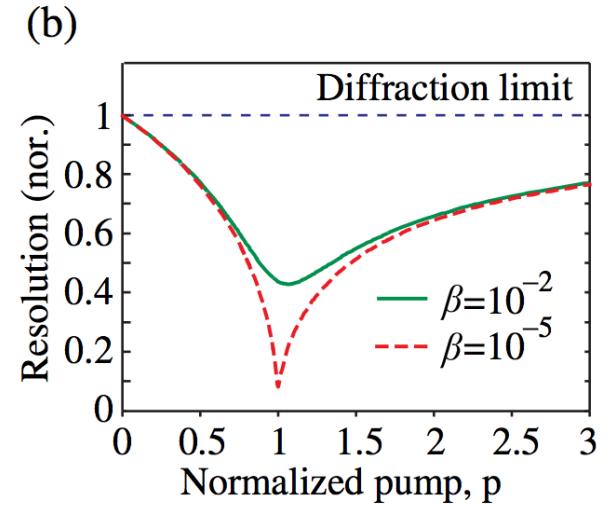
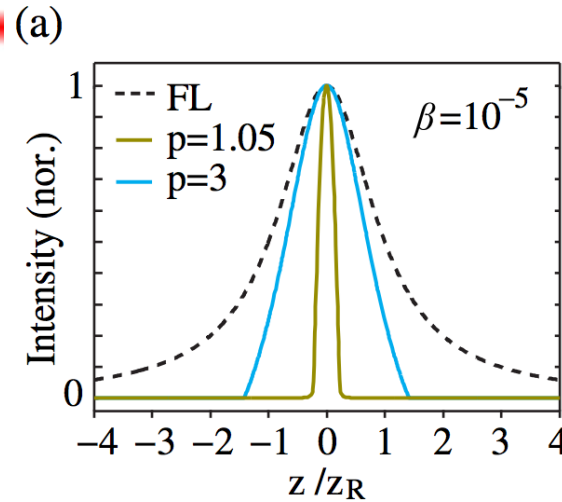
Laser particle stimulated emission (LASE) microscopy



$$\frac{\Delta_{LASE}}{\Delta_0} \approx \beta^{0.25}$$

β is the spontaneous emission factor

$$p = \frac{P}{P_{th}}$$

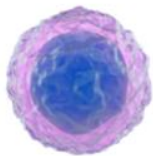


Diffraction-limited resolution: $\Delta_0 = 2.5 \mu\text{m}$

LASE measurement resolution: $\Delta_{LASE} = 0.52 \mu\text{m}$ @ $p = 1.1$ (≈ 5.9 improvement)

$\Delta_{LASE} = 1.1 \mu\text{m}$ @ $p = 1.8$ (≈ 2.3 improvement)





Acknowledgements

- Seok-Hyun Andy Yun
- Sangyeon Cho
- Nicola Martino
- Avinash Upadhya
- Xiangwei Zhao
- Sedat Nizamoglu
- Malte Gather
- Anja Dobravec
- Igor Muševič

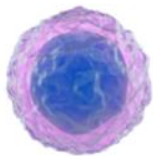
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Faculty of Mathematics and Physics
Wellman Center for Photomedicine,
Harvard Medical School,
Massachusetts General Hospital

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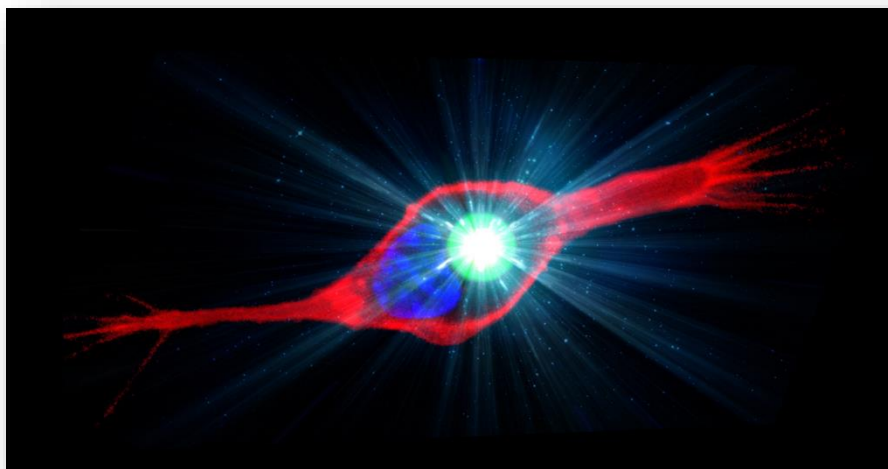


Marie Curie International Outgoing Fellowship
Marie Curie Reintegration Fellowship
Director's Fund from J. Stefan Institute





Thank you!



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