Laser Systems & Applications

MSc in Photonics & Europhotonics

Lasers for biomedical applications

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ICFO Institut de Ciències Fotòniques

Goals

- To provide a broad overview of the many applications of lasers in the life sciences (biology and medicine).
  - Therapeutic
  - Diagnostic
  - Safety

- To describe a few recent practical examples.

- To provide further reading.

Main applications of lasers in life sciences

- Photomedicine: therapeutic and diagnostic use of light
  - Therapeutic
    - Neurophotonics (neurosurgery, optogenetics=drugs activated by light)
    - Surgery (hart, vision, cancer, etc.)
      - Vision correction (laser-based procedures to reshape the cornea, to re-attach retinas, to treat cataracts, etc.)
    - Light therapies (photodynamic therapy, photobiomodulation, photothermal treatments, etc.)
      - Dermatology (hair and tattoo removals, treatment of port wine stains)
  - Diagnostic
    - Imaging (microscopy, fluorescent, photoacoustic, etc.)
    - Sensors (flow cytometry, nanosensors)

- Safety (drugs, food) & environmental monitoring

Therapeutic applications of laser light

- Drugs activated by light
  - Optogenetics: controlling neurons with light
  - Photodynamic therapy (PDT): uses nontoxic light-sensitive compounds –photosensitizing agents- that, when exposed to light, they become toxic to target malignant cells.

- Light therapies: exposure to light leads to beneficial clinical effects (e.g. analgesia: diminish inflammation, relieve pain).

- Surgery, dermatology, ophthalmology & dental medicine

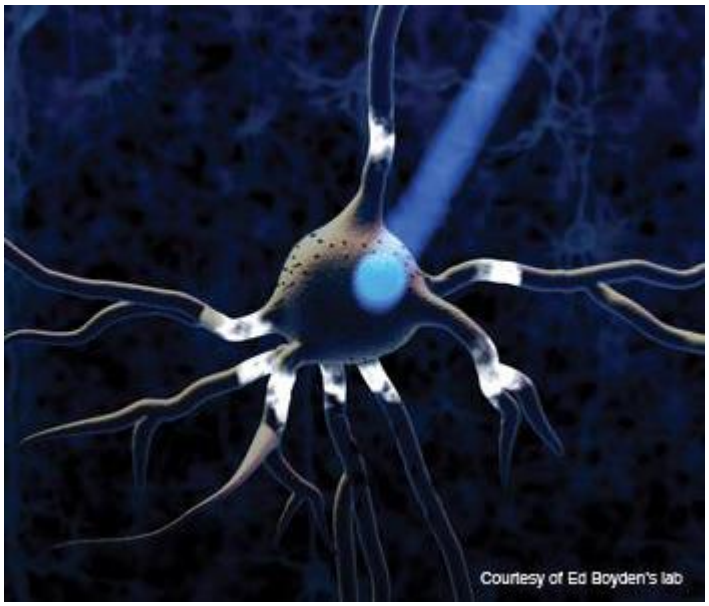
Outline

- Drugs activated by light
  - Optogenetics
  - Photodynamic therapy
- Light therapies
- Light sources for bioimaging
- Laser-based sensors and safety

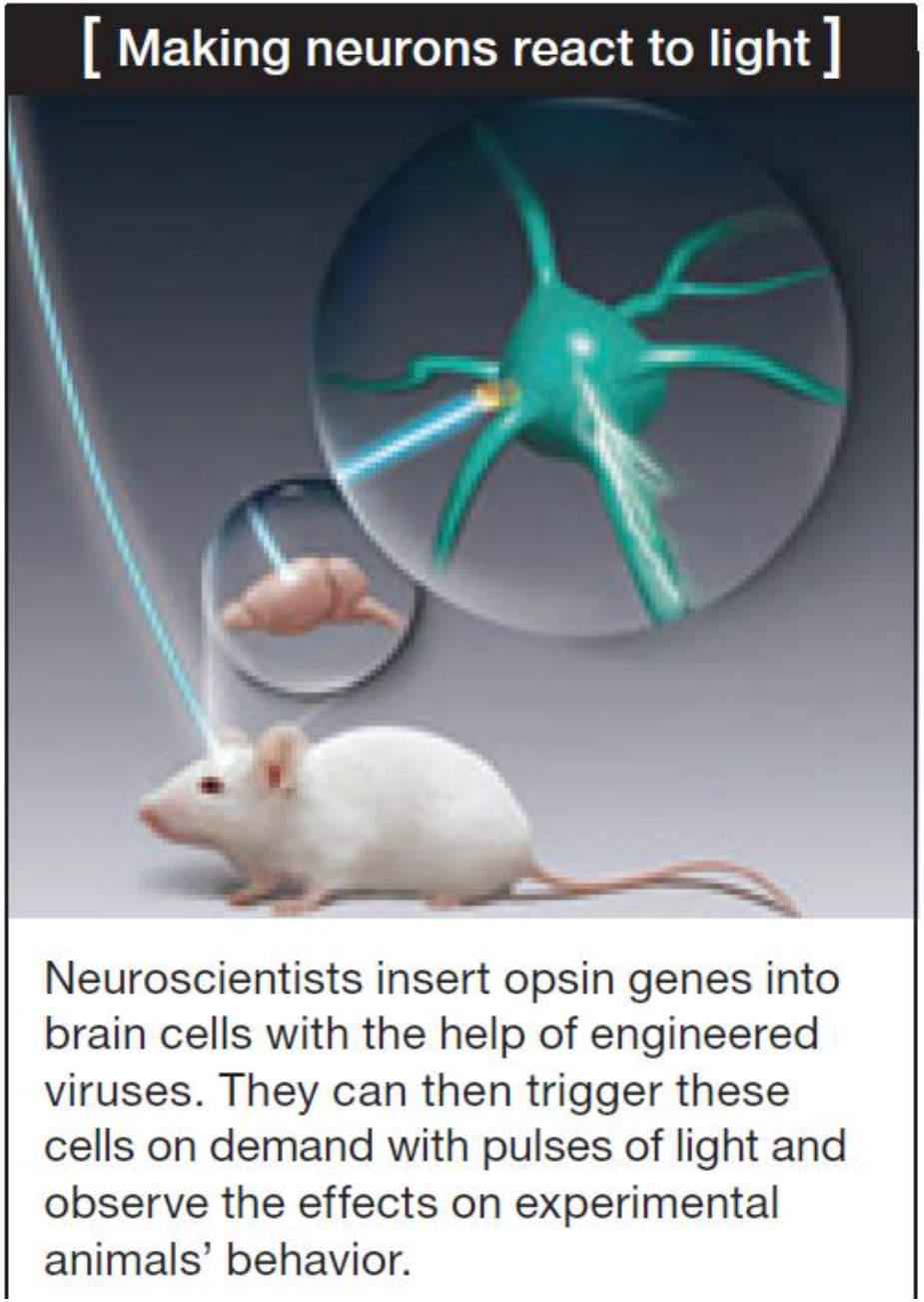
DRUGS ACTIVATED BY LIGHT - OPTOGENETICS

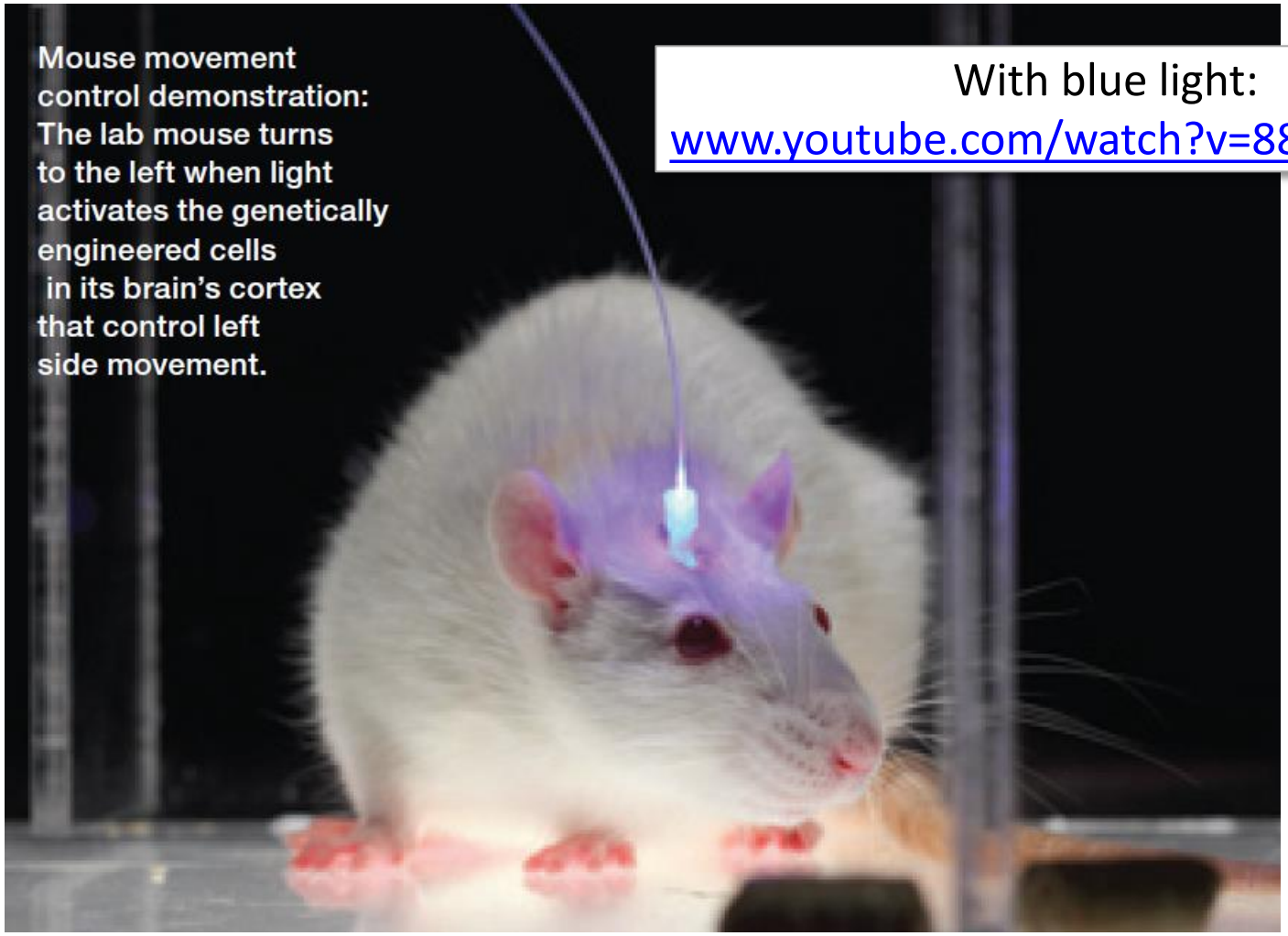
Optogenetics

Controlling neurons with light



Opsin: light activated protein





With blue light:
www.youtube.com/watch?v=88TVQZUfYGw

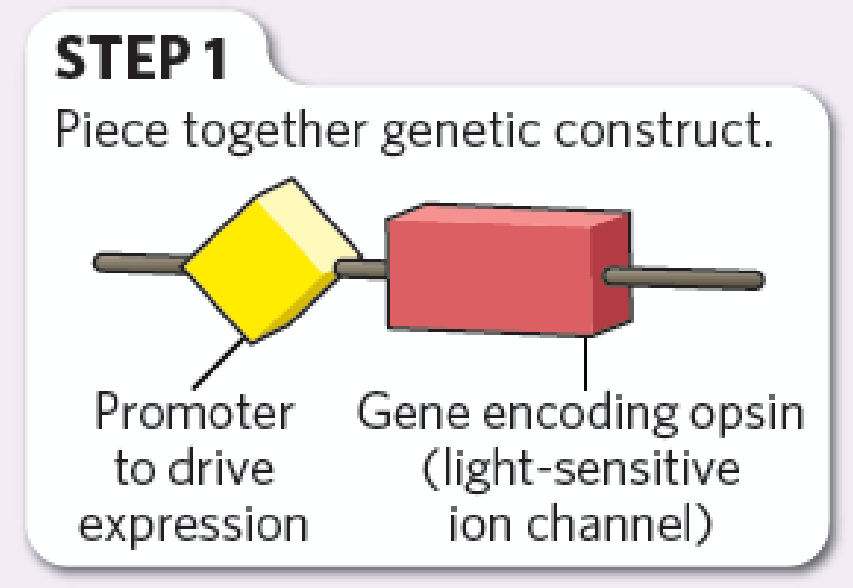
Nature May 2010; OPN August 2011

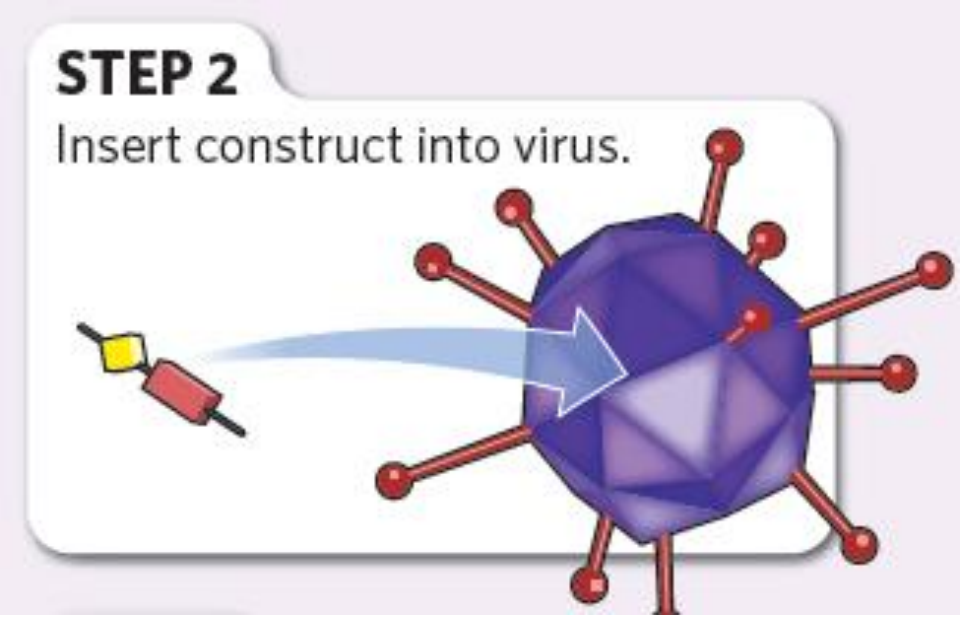
More info: TED TALK "A light switch for neurons"
https://www.ted.com/talks/ed_boyden?language=en

How does it work?

- Genes that code for light responsive proteins (known as opsins) are inserted into cells.

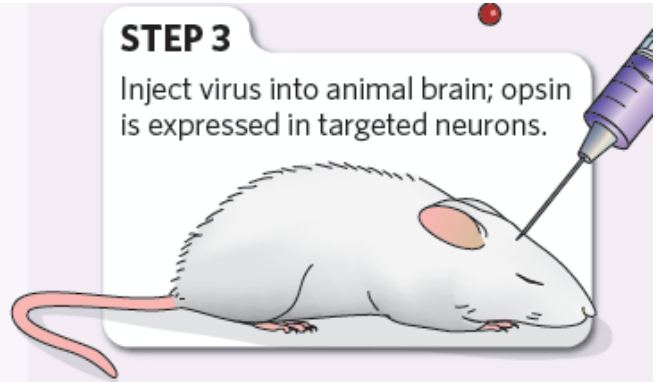
- Photo-excitation and/or photo-inhibition of these proteins causes them to alter cell function.

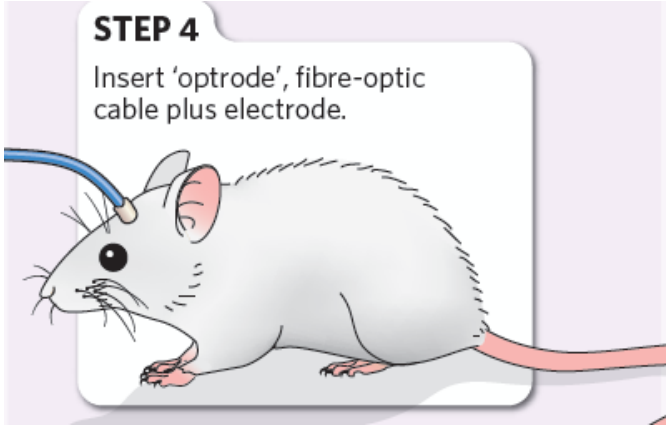


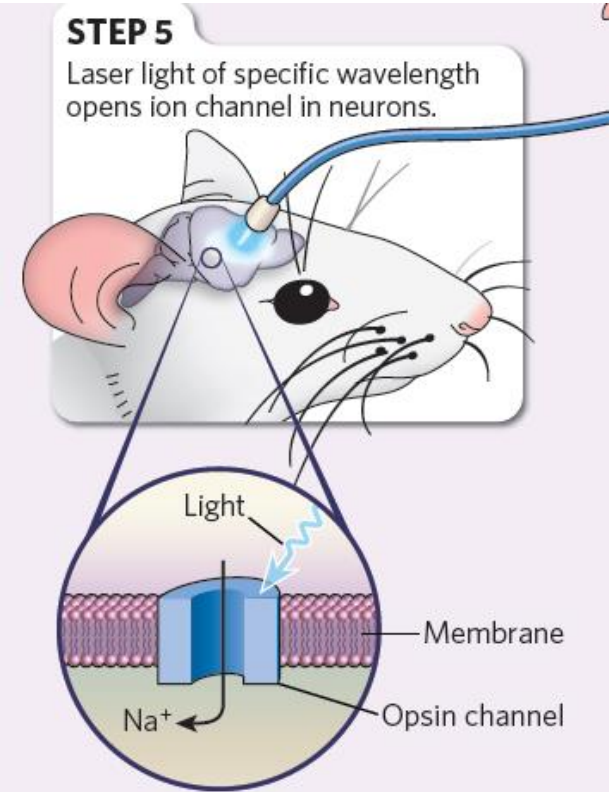


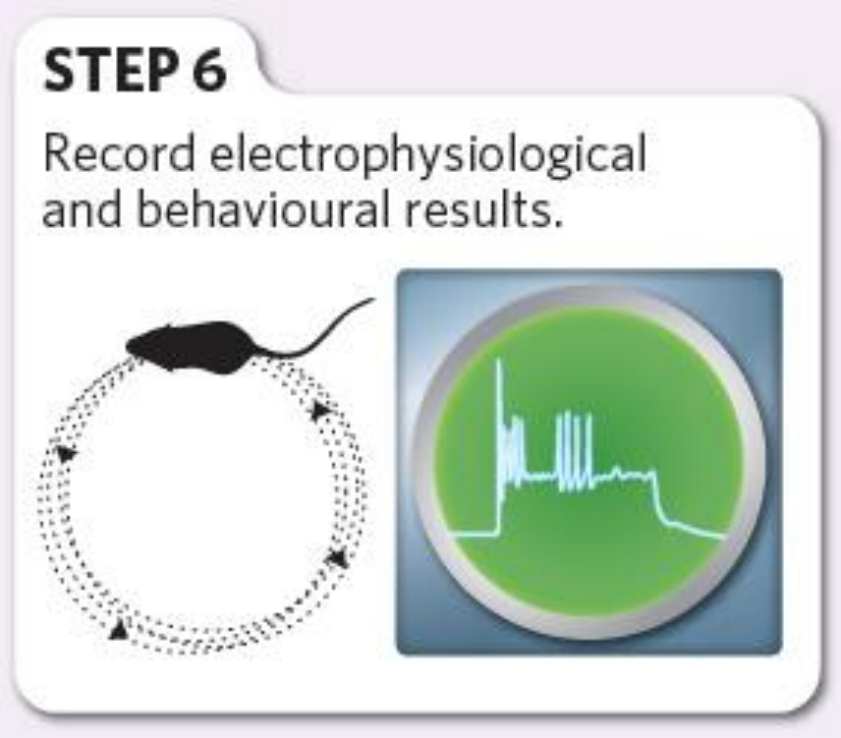
Nature Vol. 465, 6 May 2010

Six steps to optogenetics









Which light source?

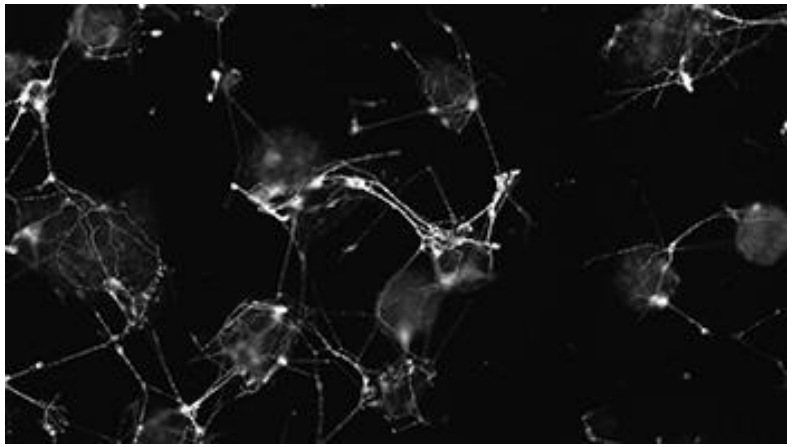
- Choice of light source depends on the light sensitive protein (opsin) used.
- Good spectral, temporal, and spatial control is important.
- Diode lasers at 405 nm and 488 nm and diode-pumped solid state lasers at 473 nm, 532 nm, 561 nm and 593.5 nm are commonly used.
- For illuminating a small number of neurons in the brain, the laser beam needs to be focused to a very small spot.
- Usually two laser wavelengths are required: one to excite cells and one to inhibit them.
- Optogenetics requires good temporal control: the light source must be turned off and on in a very precise manner.
- In experiments involving multiple opsins, narrow emission spectrum is important to selectivity activate each opsin.
- Drawback of diode lasers: mode-switching noise, sensitivity to back reflections and speckle noise (random spatial intensity variation at the illuminated site)

LED advantages for optogenetics

- The emission spectrum of most LEDs is from 10 to 30 nm, a spectral width that allows the selective activation of multiple opsins.
- They are cheaper than laser diodes.
- Because LEDs don’t have resonant cavities, don’t exhibit noise related to mode switching or instability from back reflection. They also don’t have the problem of speckle noise.
- Drawback: spectrally less efficient.

Recent development: optogenetic control of stem cell differentiation

- Engineered mouse embryonic stem cells (ESCs) turn into nerve cells when exposed to blue light from a LED matrix.
- Blue light turns on Brn2 gene
- Varying the duration and strength of the light controls the amount of Brn2 expression; and with the right level of expression, ESCs differentiate into nerve cells.
- Short pulses ignored but constant 24-hour light exposure induced rapid cell differentiation.



A brain-like network of neural progenitors 96 hours after being stimulated to turn into neurons. *Source: Thomson lab/UCSF*

Read more: http://www.osa-opn.org/home/newsroom/2015/august/optogenetic_control_of_stem_cell_differentiation/

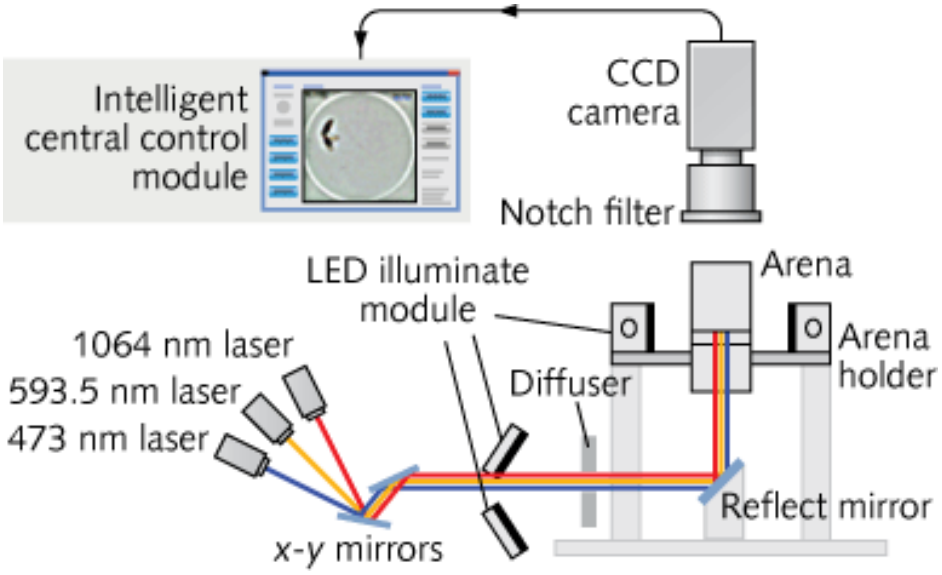
Optogenetic setup for studying memory in the common fruit fly

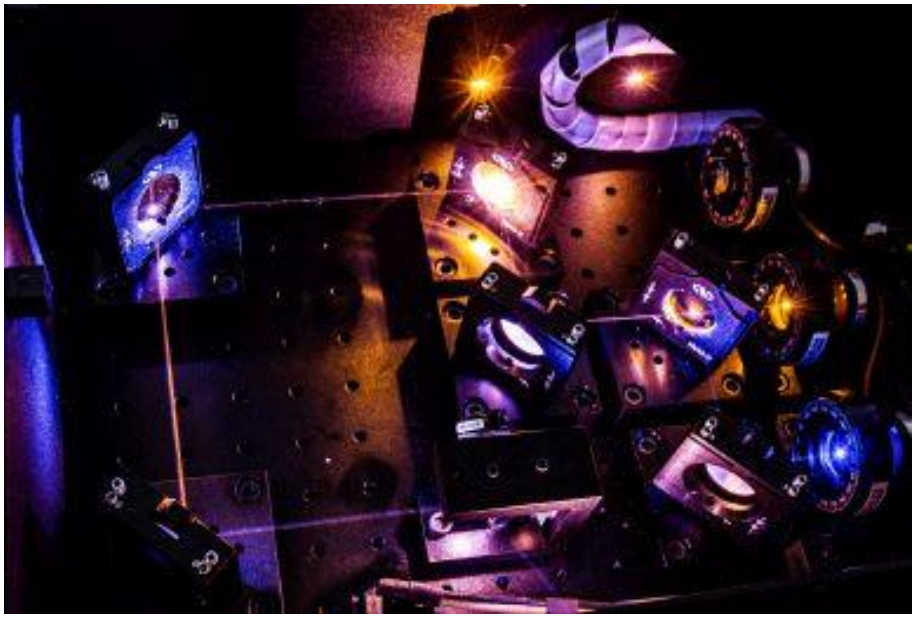
Automated laser-tracking and optogenetic-manipulation system (ALTOMS)

- Noninvasive system that can simultaneously
  - deliver heat “punishment” (serving as an incentive for the fly to alter its behavior)
  - activate ChR2 and a red-shifted variant of ReaChR, which serve to activate or inhibit specific neurons.

- Uses three wavelengths: 1064 nm for inflicting heat punishment and 473 and 593 nm to activate ChR2 and ReaChR.

- ALTOMS also includes a real-time image-analyzing system to track the flies.





A three-color laser-based optogenetic system for testing fruit fly behavior includes lasers, beam-steering mirrors, an image-capture module, an intelligent central-control module that analyzes the video images and aims the lasers accordingly, and an experimental arena.

Read more: LFW april 2015

http://www.laserfocusworld.com/articles/print/volume-51/issue-03/world-news/optogenetics-lasers-control-fruit-flies-for-behavioral-studies.html

Laser-control of single neurons

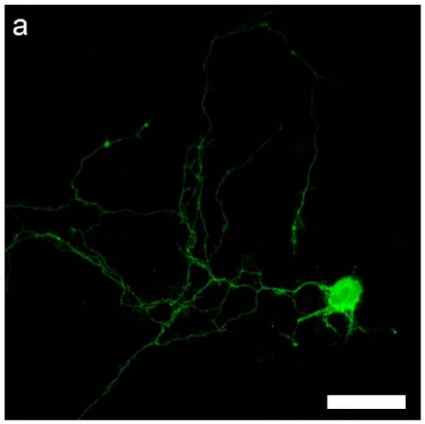
Fast targeted gene transfection and optogenetic modification of single neurons using femtosecond laser irradiation

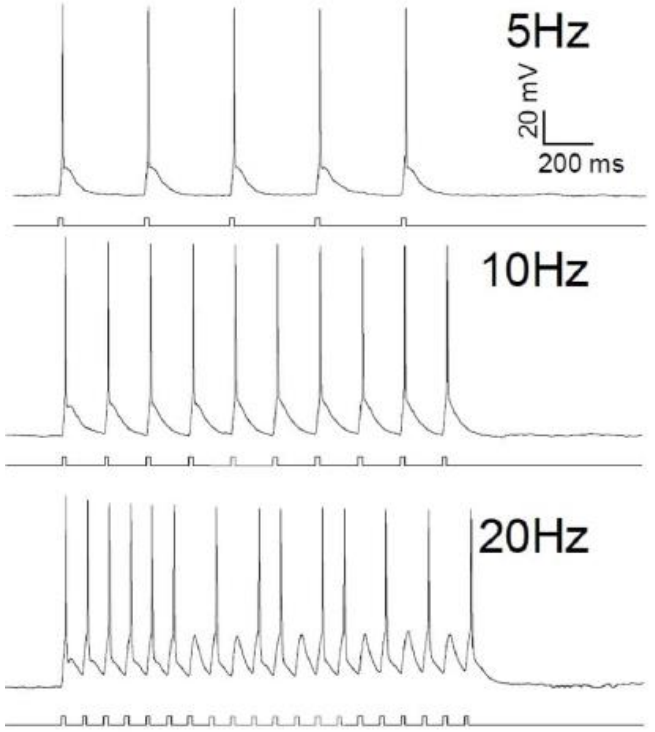
Maciej Antkowiak[1,2,4], Maria Leilani Torres-Mapa[2], Emily C. Witts[3], Gareth B. Miles[3], Kishan Dholakia[2] & Frank J. Gunn-Moore[1]

[1]University of St Andrews, School of Biology, St Andrews, UK, [2]SUPA, University of St Andrews, School of Physics & Astronomy, St Andrews, UK, [3]University of St Andrews, School of Psychology and Neuroscience, St Andrews, UK, [4]SULSA, Scottish Universities Life Sciences Alliance.

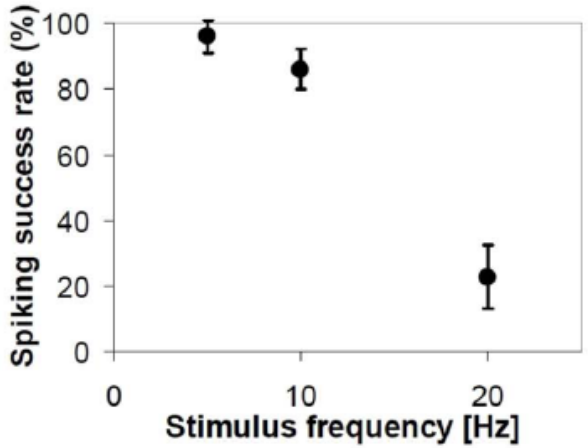
A prevailing problem in neuroscience is the fast and targeted delivery of DNA into selected neurons. The development of an appropriate methodology would enable the transfection of multiple genes into the same cell or different genes into different neighboring cells as well as rapid cell selective functionalization of neurons. Here, we show that optimized femtosecond optical transfection fulfills these requirements. We also demonstrate successful optical transfection of channelrhodopsin-2 in single selected neurons. We extend the functionality of this technique for wider uptake by neuroscientists by using fast three-dimensional laser beam steering enabling an image-guided "point-and-transfect" user-friendly transfection of selected cells. A sub-second transfection timescale per cell makes this method more rapid by at least two orders of magnitude when compared to alternative single-cell transfection techniques. This novel technology provides the ability to carry out large-scale cell selective genetic studies on neuronal ensembles and perform rapid genetic programming of neural circuits.

SCIENTIFIC REPORTS | 3 : 3281 | DOI: 10.1038/srep03281





- Fluorescence (YFP) image of a cultured cortical neuron expressing YFP-tagged CHR2(H134R) 48 hrs post-transfection. Scale bar represents 30 mm.
- Representative trains of spikes evoked by pulsed (T = 10 ms) blue light excitation (λ=470 nm, P=0.45 mW/mm^2).
- Spiking success rate at various stimulation frequencies

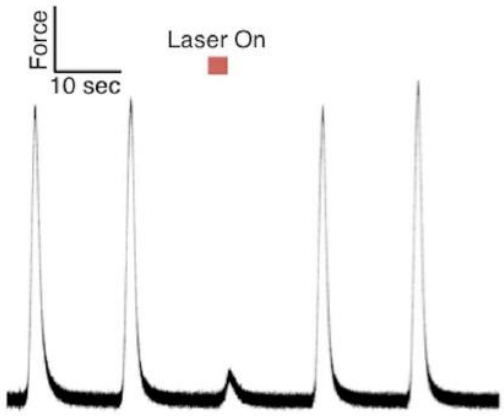


Transient and selective suppression of neural activity with infrared light

Austin R. Duke[1], Michael W. Jenkins[2], Hui Lu[3], Jeffrey M. McManus[3], Hillel J. Chiel[3,2,4] & E. Duco Jansen[1,5]

[1]Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, USA, [2]Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH, USA, [3]Department of Biology, Case Western Reserve University, Cleveland, OH, USA, [4]Department of Neurosciences, Case Western Reserve University, Cleveland, OH, USA, [5]Department of Neurological Surgery, Vanderbilt University, Nashville, TN, USA.

Analysis and control of neural circuitry requires the ability to selectively activate or inhibit neurons. Previous work showed that infrared laser light selectively excited neural activity in endogenous unmyelinated and myelinated axons. However, inhibition of neuronal firing with infrared light was only observed in limited cases, is not well understood and was not precisely controlled. Using an experimentally tractable unmyelinated preparation for detailed investigation and a myelinated preparation for validation, we report that it is possible to selectively and transiently inhibit electrically-initiated axonal activation, as well as to both block or enhance the propagation of action potentials of specific motor neurons. Thus, in addition to previously shown excitation, we demonstrate an optical method of suppressing components of the nervous system with functional spatiotemporal precision. We believe this technique is well-suited for non-invasive investigations of diverse excitable tissues and may ultimately be applied for treating neurological disorders.



Infrared inhibition of electrically-evoked muscle contraction (Scientific Reports 2013)

Big money

Is being invested in the US and in the EU in brain research:

- in the US: 100 M proposed by Obama for the BRAIN Initiative (Brain Research through Advancing Innovative Neurotechnologies)
- In the EU: 10 year Human Brain Project (54 M€ for the rump up phase, 2013-2016)

http://thebraininitiative.org

http://www.humanbrainproject.eu

DRUGS ACTIVATED BY LIGHT
- PHOTODYNAMIC THERAPY (PDT)

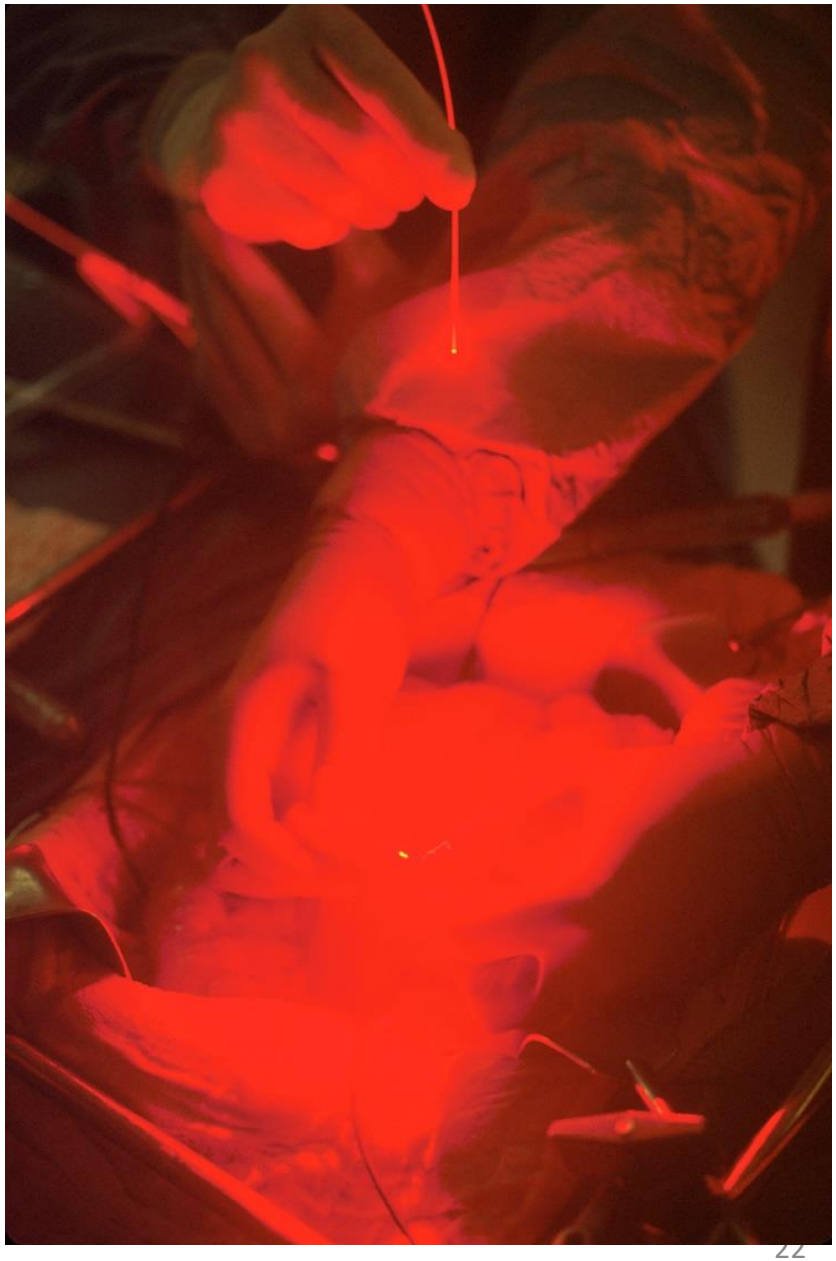
Photosensitizing agents are activated by light to kill cancer cells

How does it work?

- Patients are injected with a drug containing a photosensitizer that is selectively retained by cancer cells.
- Exposing the cancer to laser light activates the photosensitizer.
- The presence of tissue oxygen produces a toxic reaction that destroys the tumor without irreparably damaging the surrounding normal cells.
- Most modern PDT applications involve the combination of these three components (a photosensitizer, a light source and tissue oxygen) to trigger the chemical destruction of any tissues which have both selectively taken up the photosensitizer and have been locally exposed to light.
- The wavelength of the light source needs to be appropriate for exciting the photosensitizer to produce reactive oxygen species.

Close up of surgeons' hands in an operating room with a beam of light traveling along fiber optics for photodynamic therapy. A patient is given a photosensitive drug that is absorbed by cancer cells. During the surgery, the light beam is positioned at the tumor site, which then activates the drug that kills the cancer cells.

Source: wikipedia



Light sources for PDT

- Careful control of and exposure to the light source is crucial for the success of the treatment.
- Because of the high precision required, lasers are often used due to their focused output.
- Visible diode lasers are predominantly used in PDT treatments (630-760 nm).
- However, other types of light sources (LED arrays, lamps) can also be used, delivered to the treatment site via an optical fiber.

Source: BioOptics World

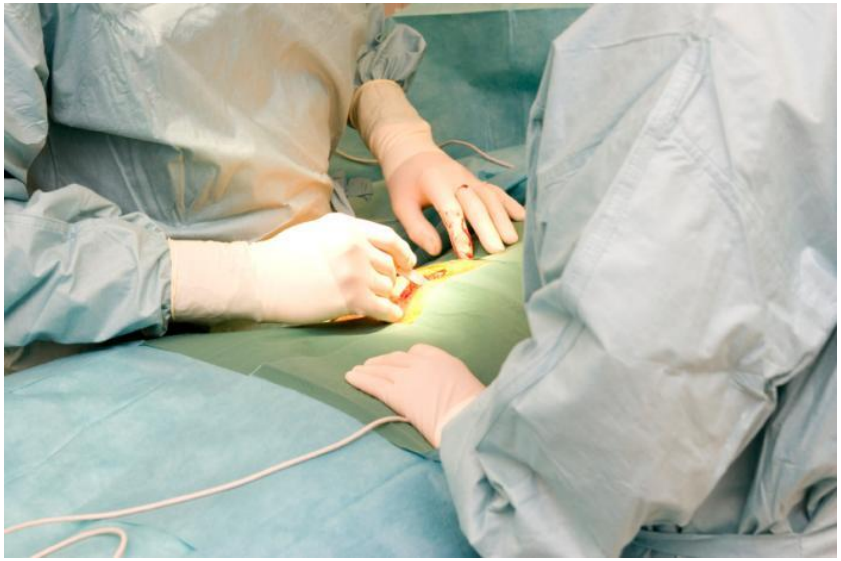
How to detect and kill cancer cells simultaneously?

- Some organic compounds (porphyrins) accumulate in cancers and fluoresce if illuminated with a particular wavelength of light to show where the cancer is.

- If these same compounds are then illuminated with a different wavelength they become toxic and kill the cancer cells.

A fluorescent agent could help surgeons remove all of tumor first time

- A fluorescent marker is injected into a tumor.
- The marker attaches only to cancer cells, which glow blue when illuminated by appropriated light.
- This method makes tumor more visible to surgeons wearing special glasses, improving their ability to fully remove the tumor.



Current imaging methods (MRI, CT scans) do not always detect all the cancerous tissue at the margins of a tumor, so in the first operation some harmful cells can be left behind.

Read more: http://www.medicalnewstoday.com/articles/304776.php
January 2016

-LIGHT THERAPIES

Light therapy (also known as phototherapy)

- Therapeutic radiation used to treat skin conditions (psoriasis)
- Also used to treat circadian rhythm (sleep) disorders, depression.

Treatment for neonatal jaundice (yellowish pigmentation of the skin): photo-induced degradation of bilirubin molecules.
Source: H. Failache, UDELAR, Uruguay

How does it work?

- Laser irradiation over *chromophores* (the part of a molecule that is responsible for its color, that absorbs certain wavelengths and transmits or reflects the rest).

- Results in: photon-induced chemical reactions and/or photon-induced alterations (stimulating or inhibiting cellular functions).

- Goal: to chose a wavelength to reach chromphores in target cells without the photons being absorbed by other substances.

Light sources

- A light box which emits up to 10,000 lux of light at a specified distance, much brighter than a normal lamp.

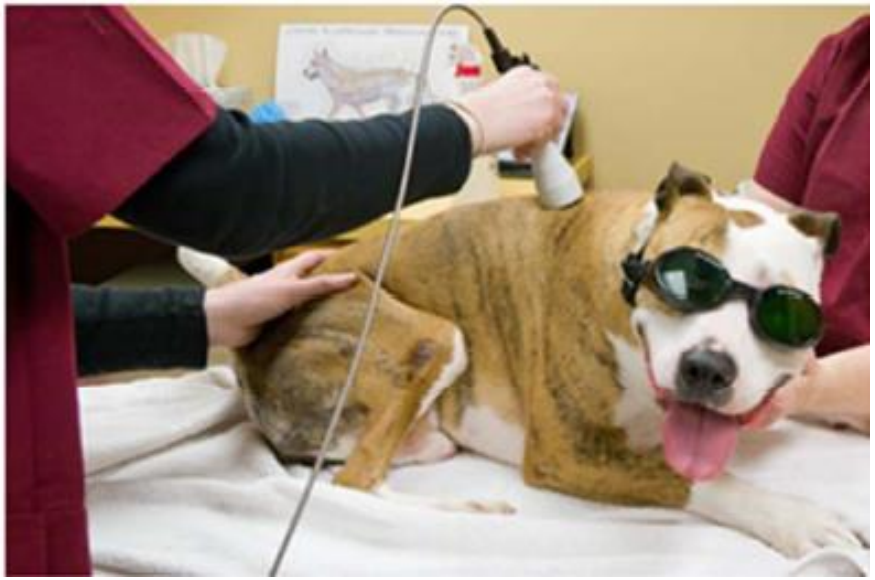
- Specific visible wavelengths, from the blue (460 nm) to the green (525 nm).

High intensity blue light (425 nm) used for the treatment of acne.

A light box in use for depression, seasonal affective disorder, or a circadian rhythm sleep disorder. Source: wikipedia

Photo-bio-modulation, also known as Low Level Laser/Light Therapy (LLLT)

- Used on sports injuries, arthritic joints, back and neck pain, nerve injuries, spinal cord injuries, etc.
- “laser acupuncture”: lack of consensus over its scientific validity
- Uses lasers or LEDs to improve tissue repair, reduce pain & inflammation.
- The intensity of LLLT lasers and LED's is not high like a surgical laser (there are no heating effects)
- Usually applied by a doctor, therapist or technician, treatments take about 10 minutes and should be applied two or more times a week.
- More info: http://www.aslms.org/public/LowLevelLight.shtml



Source: Bioptics World

Transcranial Light/Laser Therapy (TLT)

- Noninvasive delivery of light into the brain for the treatment of
  - Brain injuries
  - Chronic neurological diseases
  - Mental illness
- Red and NIR light is used because it penetrates the scalp, skull and brain.
- cw or pulsed,
- To the entire head or to target specific areas of the brain.

Source: Bioptics World

PRECLINICAL TLT PROCEDURE

Tested in different models of induced Acute Ischemic stroke - multiple labs

New Zealand white rabbits – over 400 animals

Sprague Dawley® rats – over 1000 animals

Preclinical TLT Dose – Therapy Parameters

PARAMETER at cortical surface	Rat[2]	Rabbit[2]
Radiant power	14 mW	14 mW
Power density	10 mW/cm^2	10 mW/cm^2
Treatment duration	120 seconds	120 seconds
Wavelength	808 nm	808 nm

Demonstrated that TLT was safe and effective in the treatment of induced Acute Ischemic Stroke in rats and rabbits.

Next steps: *in vitro* studies and *in vivo* clinical trials

Source: J. Anders, Bioptics World

TLT boosts cognitive function following brain injury

- Two patients with **chronic traumatic brain injury** were treated with transcranial LEDs.
- The patients showed significant improvement in concentration and memory.
- Light source: a LED console device, containing 52 near-infrared (870 nm) and nine red (633 nm) diodes for a total output power of 500 mW continuous wave.
- But the patients' improvements vanished if they stopped the treatment.

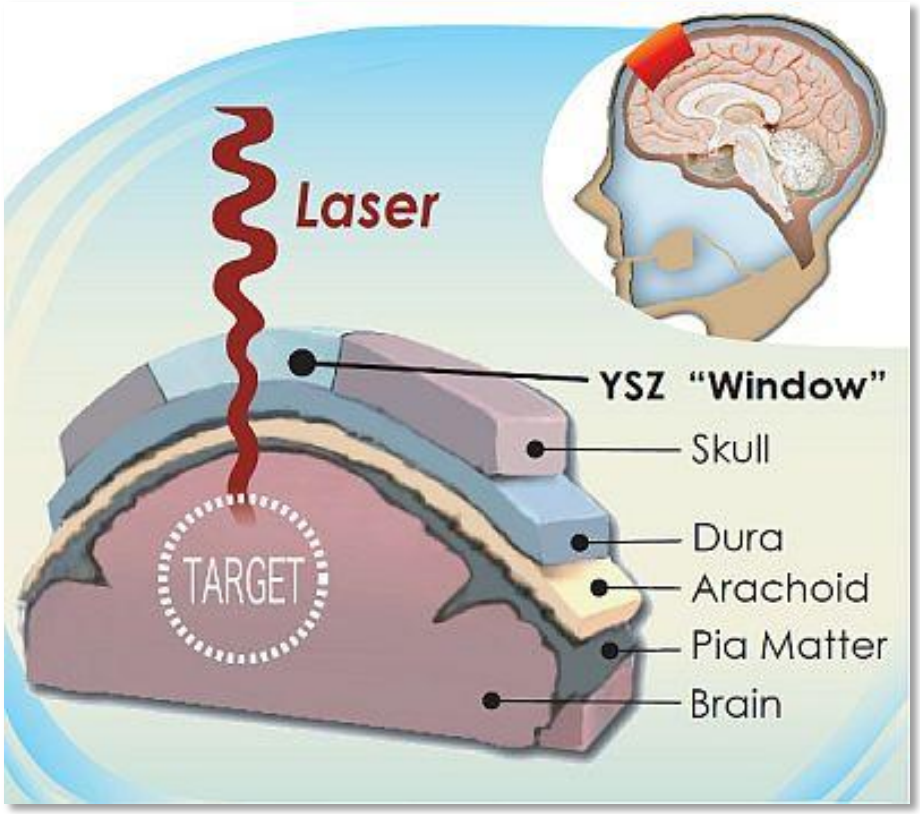
Photomedicine & Laser Surgery (doi:10.1089/pho.2010.2814)

How does it work?

- Exact mechanism unknown
- In rabbits, NIR laser light (808 nm) applied transcranially resulted in significantly higher levels of the energy-containing molecule ATP.
- Light energy is converted into chemical energy (stored in ATP).
- This energy is then available to power processes such as growth and repair.
- Thus, simultaneous repair and growth by neural cells fueled by ATP in response to light treatment.
- Also, increased blood circulation in response to light therapy.

A transparent permanent window to the brain

- Yttria-stabilized-zirconia (YSZ) is a ceramic material, which is well tolerated and used in hip implants and dental crowns.
- It was modified to make it transparent.
- The modified YSZ prosthesis can provide a permanent window through which doctors can aim light-based treatments for the brain without having to perform repeated craniectomies.



Y. Damestani et al, Nanomedicine: Nanotechnology, Biology and Medicine Volume 9, Issue 8 , Pages 1135-1138, November 2013

Photo-thermal treatments: laser light removes port-wine stains

The argon laser light is selectively absorbed by the red pigment in the abnormally enlarged blood vessels that cause the stain.

Dilated vessels are burned and sealed off while normal skin tissue is undamaged.

In dermatology, the type of laser and wavelength depend on the type of lesion being treated and what the main absorber is within it. The wavelength also depends on the patient's skin type.

Other uses: in **angioplasty** (patients with blocked or narrowed coronary arteries), to remove blood-vessel plaque.

Many other light-activated therapies

- Lasers can be used to burn a tiny hole on a cell surface, allowing exogenous substances to enter the cell.
  - Example: perforate egg walls to assist in vitro fertilization.
  - A laser is focused to a diffraction limited spot (~1 μm diameter). The membrane of a cell is then exposed to this highly focused light for a small amount of time (typically tens of milliseconds to seconds), generating a transient pore on the cell membrane
  - This transient pore allows exogenous objects (DNA, RNA, QDs) to enter the cell.
  - In this technique, one cell is treated at a time.
  - Demonstrated using a variety of lasers, including 800 nm femtosecond pulsed Ti:Sapphire and 1064 nanosecond pulsed Nd:YAG.

- NIF laser light can deliver extremely localized heat to cancer cells through biocompatible nanoparticles, which can also act as sensors to monitor the amount of heat delivered.

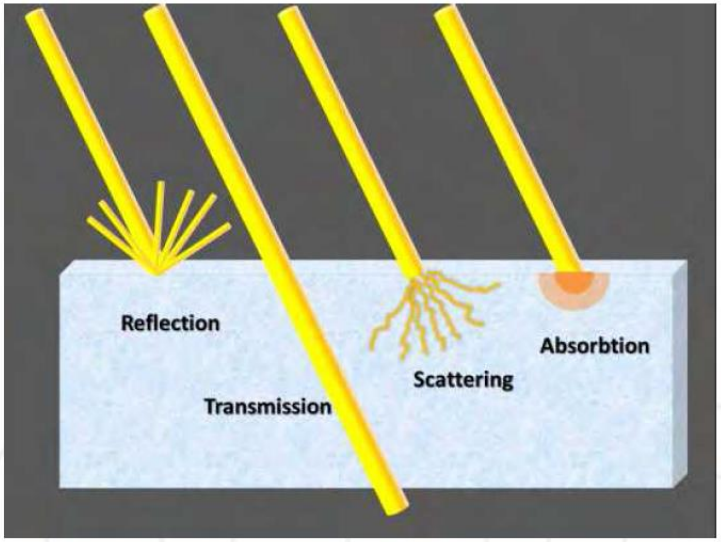
TF

- In optogenetics, light activates natural chromphores, and their excitation induces alterations in cell function.
- Photodynamic therapy (PDT) requires the use of a photosensitizer.
- PDT requires oxygen to produce a toxic reaction that destroys the tumor.
- Transcranial Light Therapy uses white and blue light sources.
- Light therapy uses light of appropriate wavelength, which is absorbed by chromophores, inducing chemical reactions or cellular alterations.
- Photo-bio-modulation requires high-intensity light sources to induce heating in tissue.

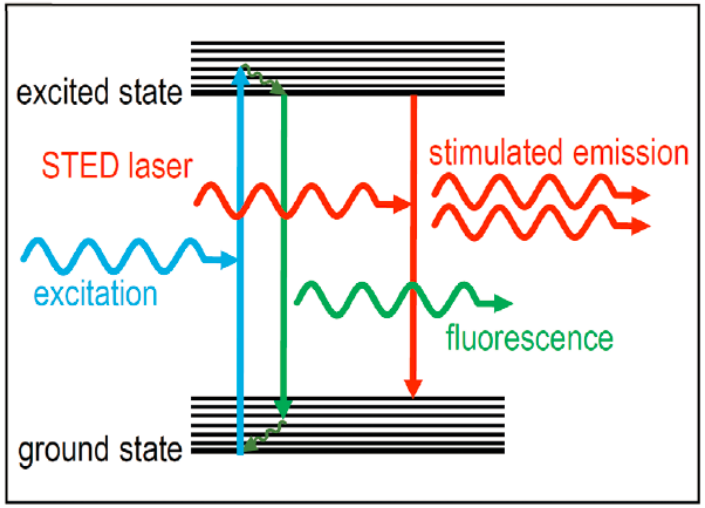
-LIGHT SOURCES FOR BIOIMAGING

Main operation principles

- Techniques based on light transport



- Fluorescent techniques based on bioluminiscence



Sources: C. B. Sener, www.intechopen.com
J. Bewersdorf, Europhysics New, Dic. 2015

Non-fluorescence techniques

- **Raman spectroscopy**: is based on inelastic (Raman) scattering of laser light (visible, near infrared, or near ultraviolet). The laser light interacts with molecular vibrations, phonons or other excitations in the system, resulting in a shift of the photons' energy that gives information about the molecular vibrational modes.
- **Photo-acoustic**: when laser light strikes the target tissue, some of the energy is converted to heat by the cells, resulting in a mechanical wave that can be processed into an image.

  Because different types of tissue absorb laser energy at different rates, lasers can be tuned to target specific tissues.
- **Optical coherence tomography (OCT):** a laser delivers pulses of NIR light into the tissue; the resultant optical scattering is processes to create high-resolution images at the micrometer scale.

  OCT is analogous to ultrasound, but, while ultrasound provides a resolution of tenths of millimeter, OCT can achieve orders-of-magnitude finer resolution (on the micrometer scale).

Fluorescence microscopy

- light-activated fluorescent molecules

FIGURE 1. Nonlinear microscopy has inherent 3D capabilities. With a green beam (532 nm) coming from the right, single-photon absorption excites fluorescence along the entire light path. But with a near-infrared (NIR) beam (1057 nm) coming from the left, two-photon absorption excites fluorescence only at the small intense focal spot indicated by the arrow. (*Image courtesy of Brad Amos, Science Photo Library, London*)

BioOptics World june 2014

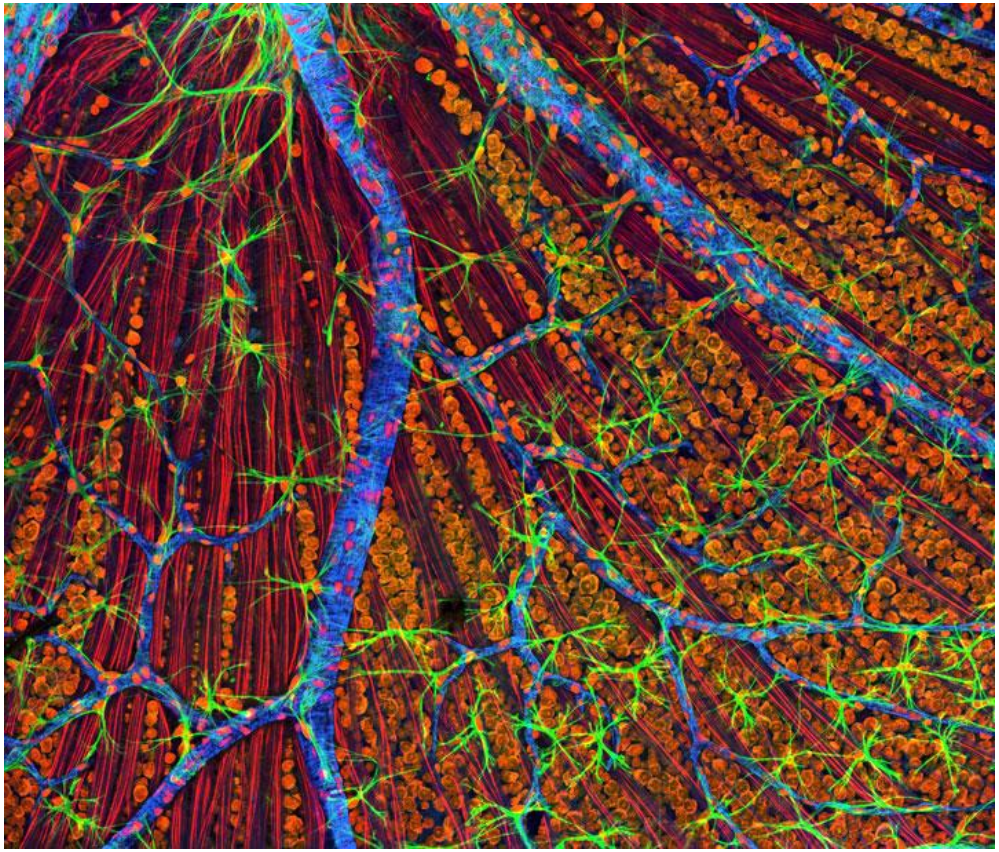
Other optical imaging techniques

- **Spectral imaging**: generates many images of the same object, each measured at a different wavelength.

- **Confocal microscopy**: a technique for increasing the contrast of microscope images (better resolution that OCT but less tissue penetration).

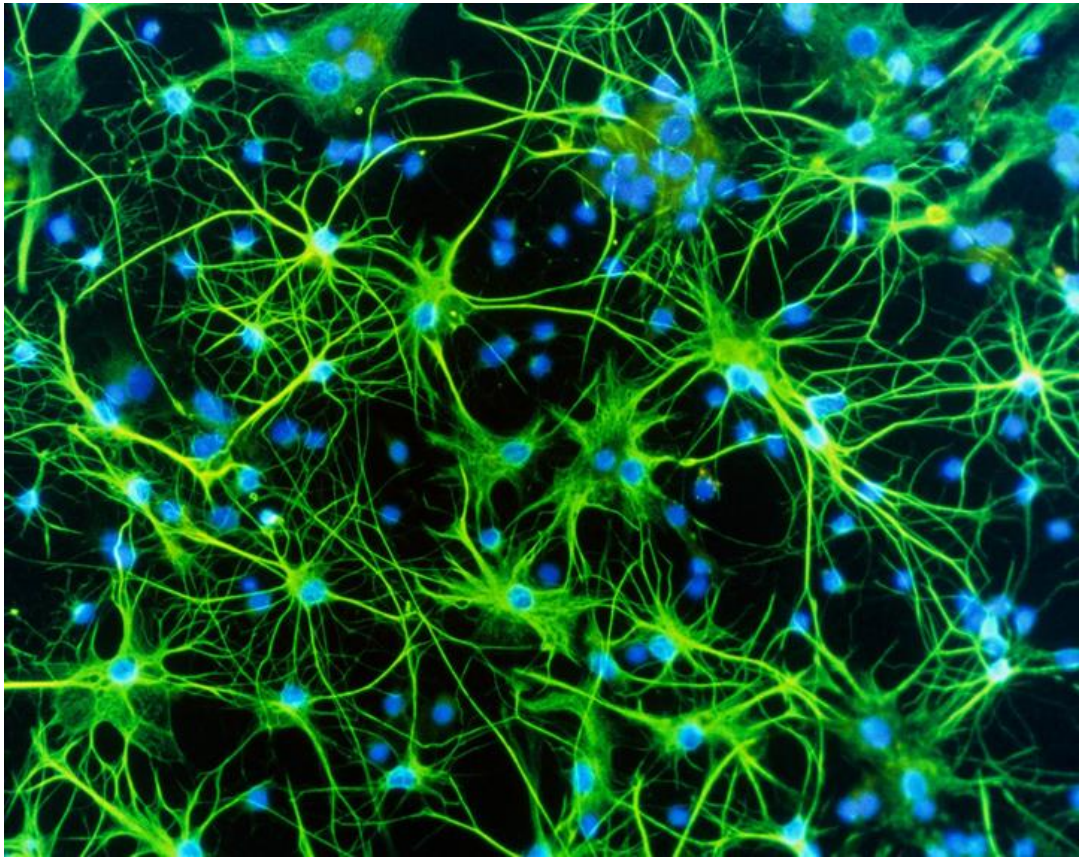
- **Plasmon** imaging using nano-particles (gold): plasmons are collective oscillation of electrons on a conductive surface that can be excited by light.

- **Multimodal** systems: no single imaging modality can provide all the information in one image.



A laser scanning, or "confocal" microscope scans a sample point-by-point or line-by-line at once, assembling the pixel information to generate one image. This allows for a very high-resolution and high-contrast image in three dimensions. The image is from a **laser scanning microscope** of a **mouse retina**, where the cells have been stained with **fluorescent dye**.

http://lightexhibit.org



Astrocytes are the star-shaped cells found in spinal cord and the brain. In this image of astrocytes, the **nucleus** of each cell has been stained blue while the **cytoplasm** (the fluid that fills the cell) has been colored green. To achieve this, the process of immuno-fluorescence was used (antibodies are used to attach fluorescent dyes to specific molecules in the cells).

http://lightexhibit.org

Protozoa are single-celled animals found throughout the world in many different habitats. They play a key role in maintaining and balance of bacteria, algae, and other microbial life. This photograph illuminates one particular type of protozoa called vorticella. In this image, a technique called "**dark field microscopy**" was used. This technique blocks out the direct light from the source, so that **only light scattered** by the specimen is observed, enabling brilliant bright images to be seen again a dark background.

Optical imaging: advantages & drawbacks

- Advantages:
  - Relatively low cost.
  - Wide range of spatial resolution: μm – mm (molecular to physiological information).
  - Time-resolved measurements.

- Drawback:
  - Limited depth penetration: about 10-fold loss in photon intensity for every centimeter of tissue depth.

EXAMPLES

The Nobel Prize in Chemistry 2014

Eric Betzig Stefan W. Hell William E. Moerner

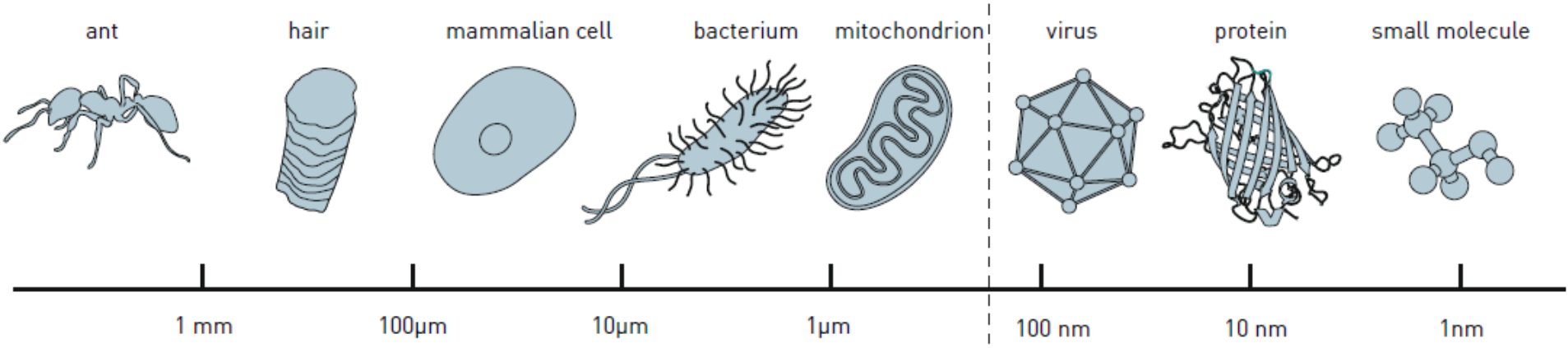
"*for the development of super-resolved fluorescence microscopy*".

United Nations Educational, Scientific and Cultural Organization

International Year of Light 2015

The classical limit for visualizing the biological world

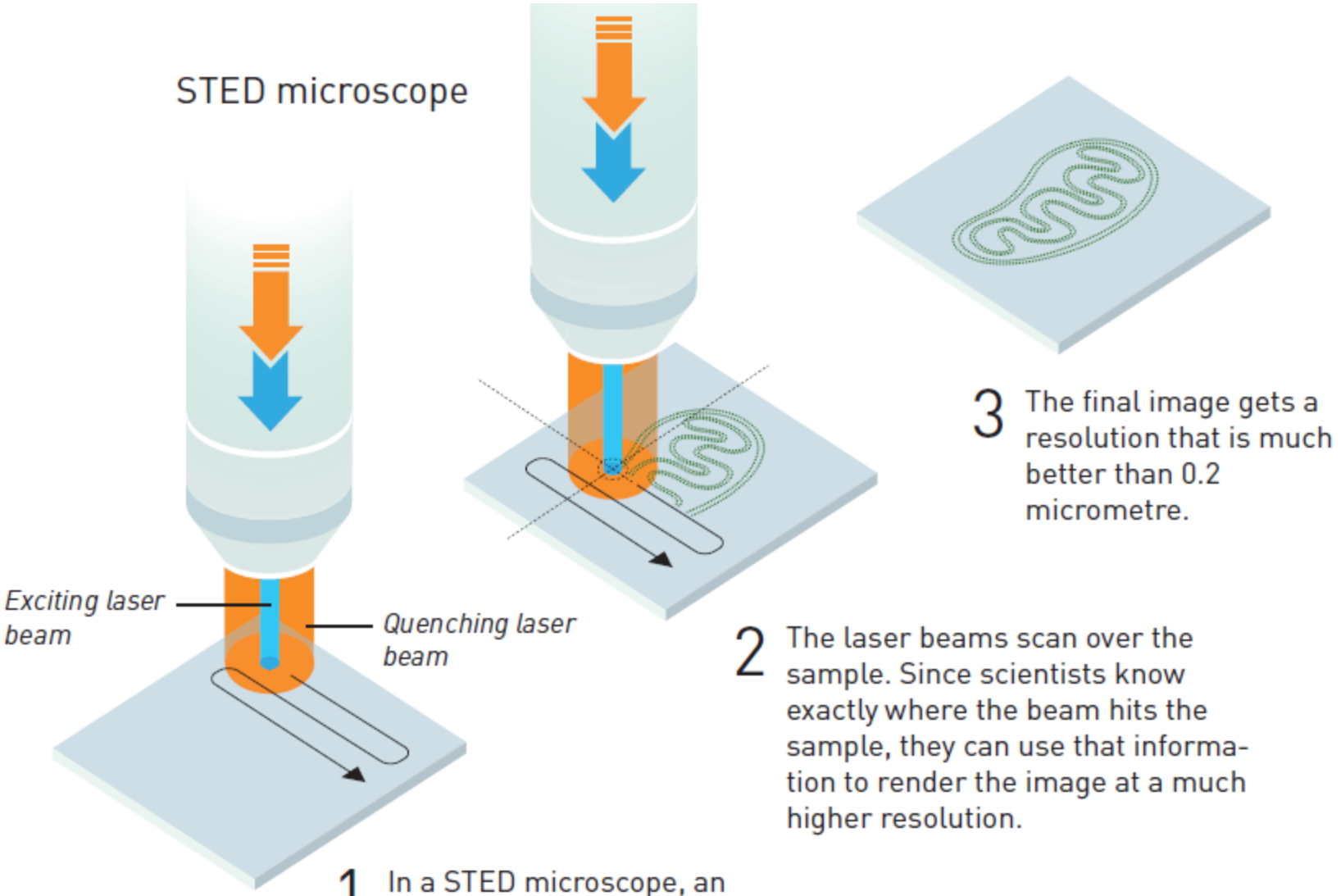
- About half the wavelength of light, i.e., about 0.2 μm (d=λ/2NA; λ=400 nm, NA = 1.4)



Beating the diffraction limit via **stimulated emission** of **fluorescent molecules**

- Stimulate Emission Depletion (**STED**) microscopy (developed by **Stephan Hell**):

  molecules are first excited by a focused laser beam and then de-excited by a second laser with a doughnut-shaped focus, so that only few molecules in the center of the doughnut remain in their excited state and their fluorescence serves as measure signal.


STED microscope
Exciting laser beam
Quenching laser beam
1 In a STED microscope, an annular laser beam quenches all fluorescence except that in a nanometre-sized volume.
2 The laser beams scan over the sample. Since scientists know exactly where the beam hits the sample, they can use that information to render the image at a much higher resolution.
3 The final image gets a resolution that is much better than 0.2 micrometre.

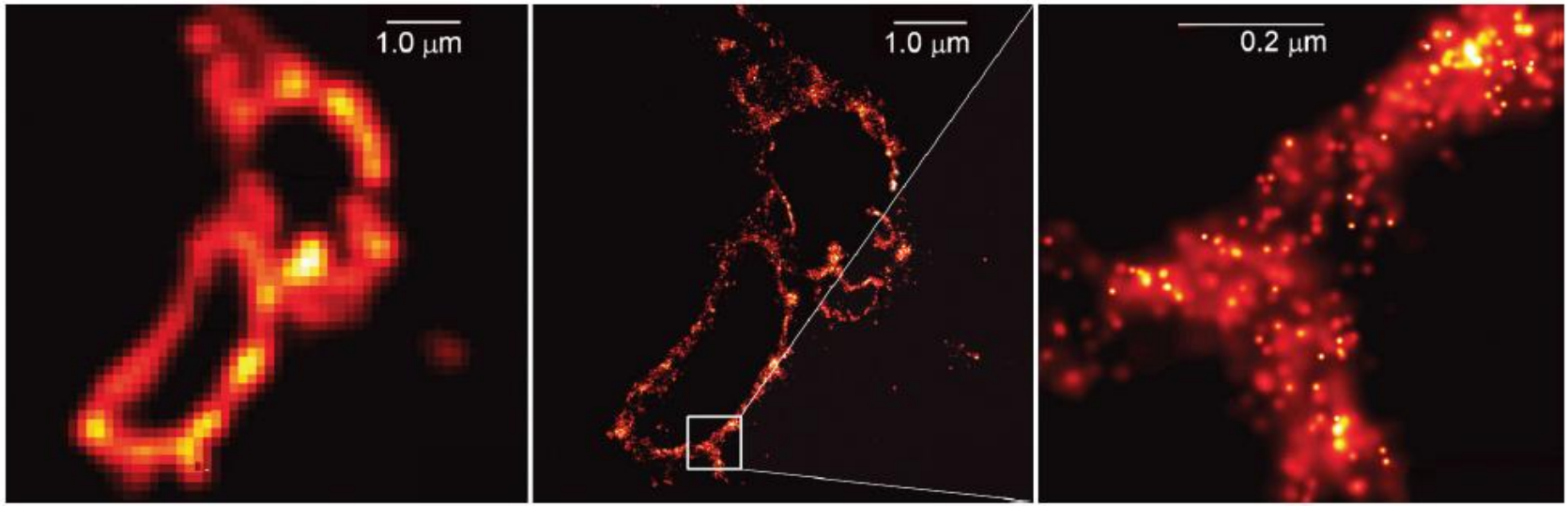
The path to single-molecule microscopy

- **William E. Moerner** discovered that it is possible to optically control fluorescence of single molecules.
- The fluorescence of one variant of a *green fluorescent protein* (GFP) could be turned on and off at will.
- The strong green color of a GFP protein appears under blue and ultraviolet light.
- When Moerner excited the protein with light of wavelength 488 nanometres the protein began to fluoresce, but after a while it faded.
- Regardless of the amount of light he then directed at the protein, the fluorescence was dead.
- But light of wavelength 405 nm could bring the protein back to life again.
- When the protein was reactivated, it once again fluoresced at 488 nm.

$\Rightarrow$ individual molecules act like tiny lamps with switches

And by using light pulses

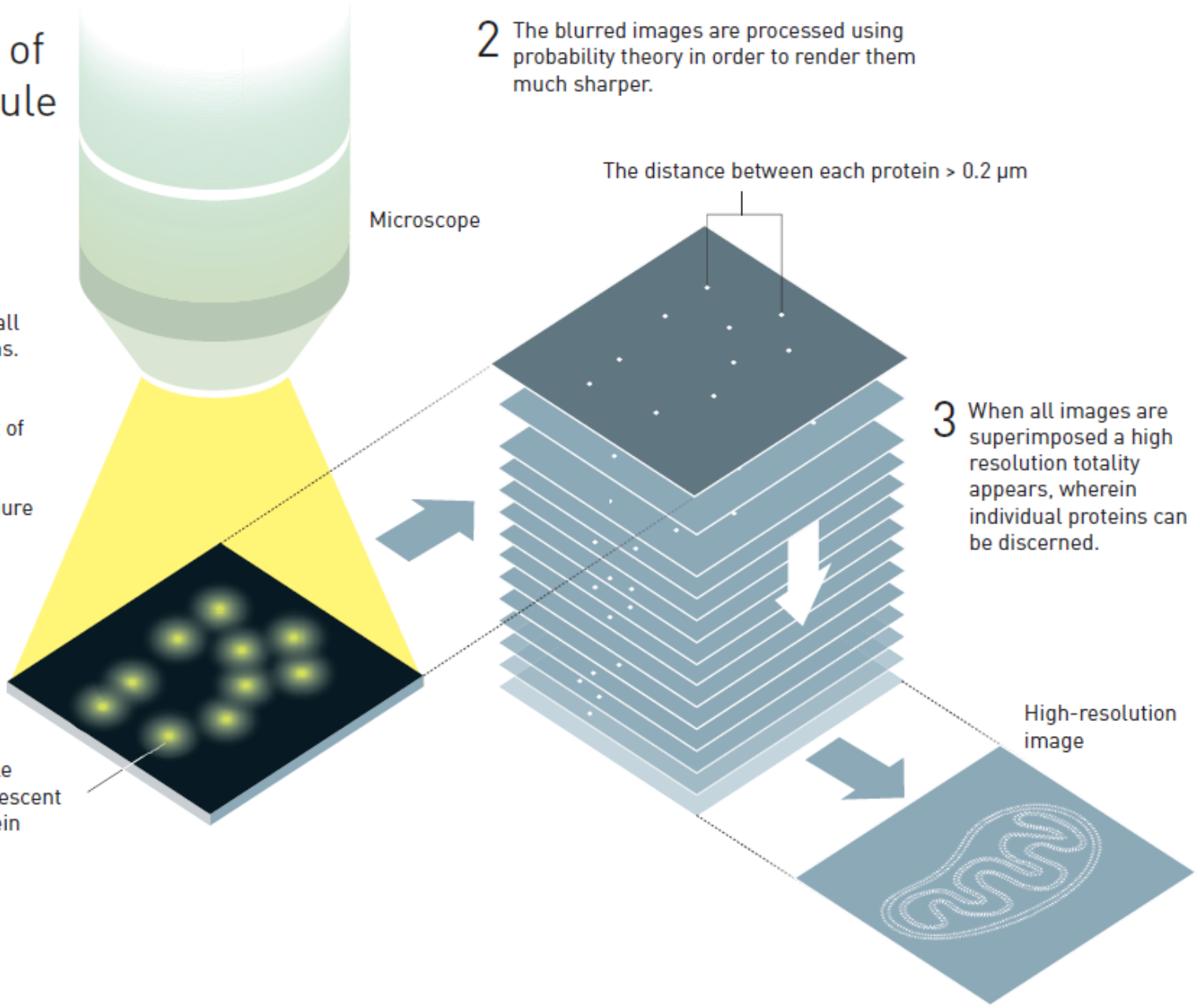
- **Eric Betzig**: a super-resolution image can be obtained by using fluorescent molecules that fluoresce at different times & superimposing the images.



conventional microscopy single-molecule microscopy

Science 313:1642–1645 (2006)

The principle of single-molecule microscopy



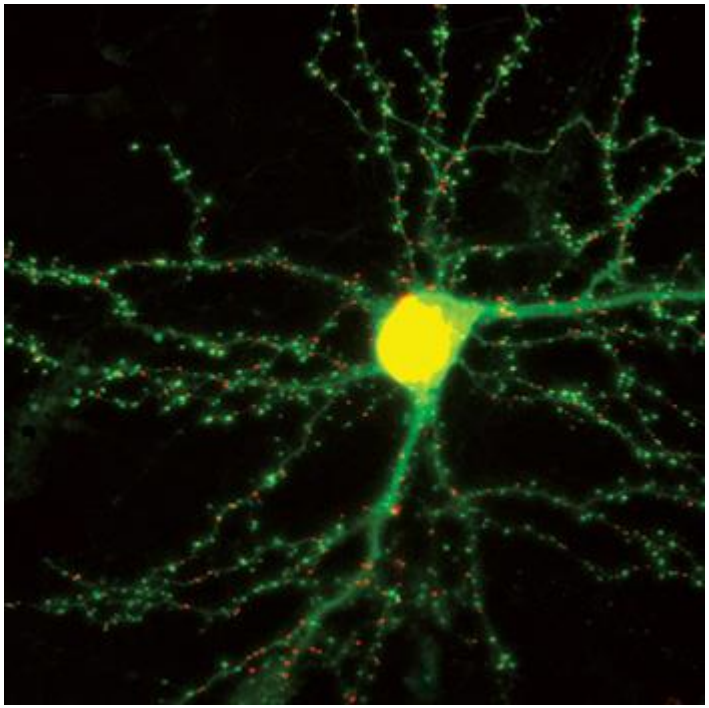
Hell, Moerner & Betzig are still active mapping the secrets of the biological world

- Stefan Hell has studied the inside of living nerve cells in order to better understand brain synapses.
- William E. Moerner has studied proteins in relation to Huntington's disease.
- Eric Betzig has tracked cell division inside embryos.

More info: Prof. Betzig video, ceremony of the International Year of Light
https://www.youtube.com/watch?v=o6U7v3knbb0

Fluorescing live synapses shed light on learning, memory formation

By using a green fluorescent protein (GFP), which glows brightly when exposed to blue light, researchers studied structural changes in the brain when we make a memory or learn something (and found that what gets changed is the distribution of synaptic connections).



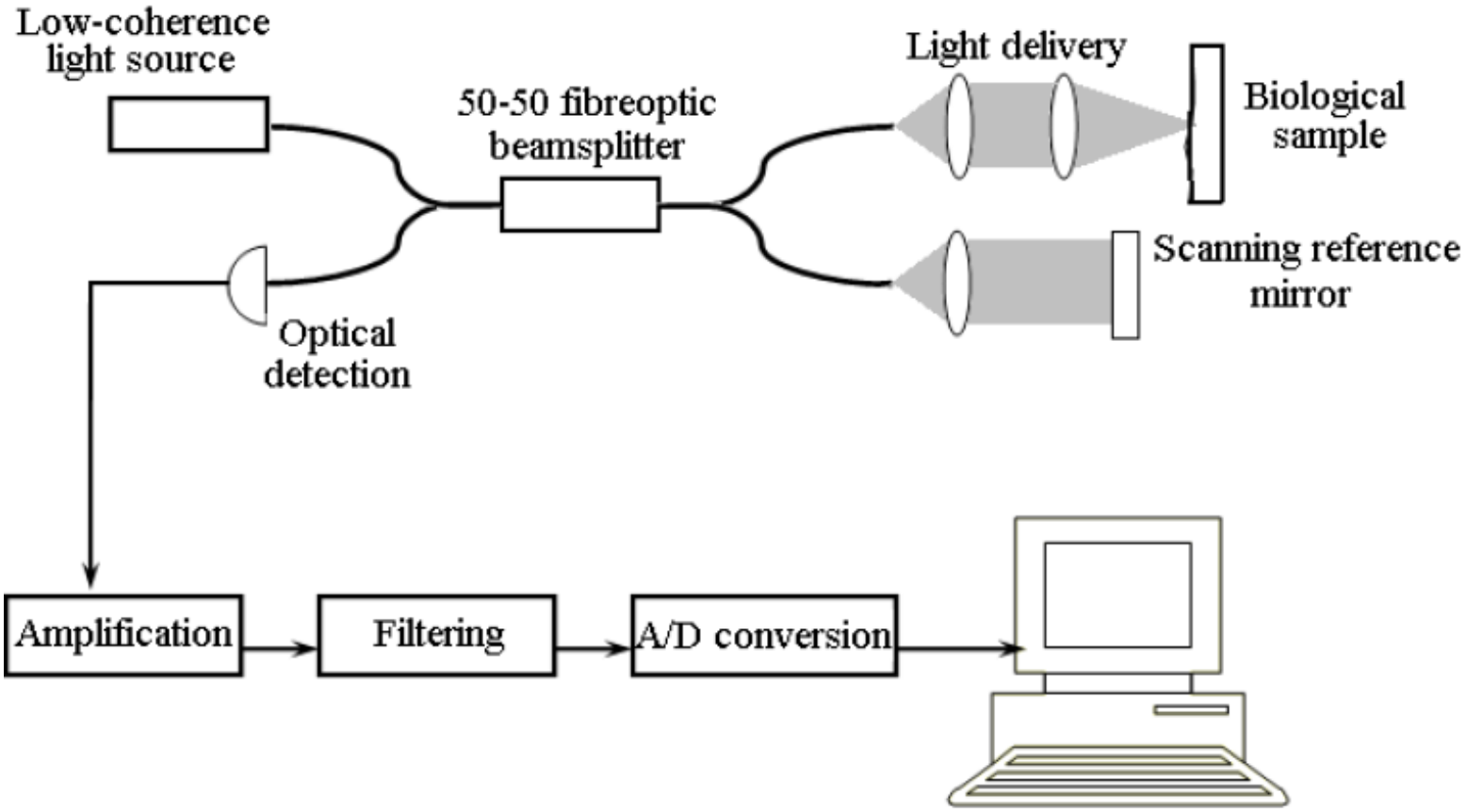
G.G. Gross et al., *Neuron*, 78, 6, 971–985 (2013).

Tissue-level imaging: tomography

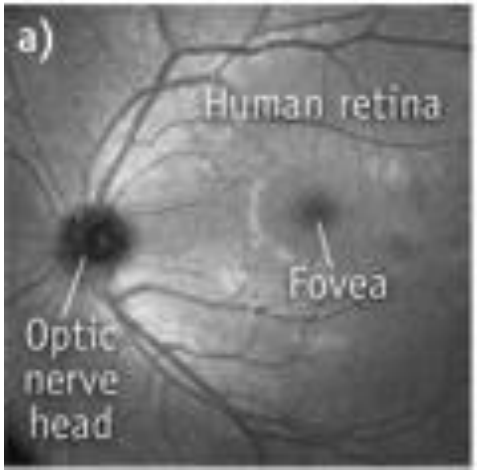
- Slices of tissue can be imaged by focusing a source onto the focal plane, while structures in other planes appear blurred.
- The source can be optical, x-ray, ultrasound, gamma rays, electrons, or magnetic resonance, or a combination of them.
- A tomogram (2-D slice) is produced from image reconstruction algorithms. 3-D reconstructions are made by scanning a detector across the sample.
- Many types of tomography for many types of imaging.
- Type used is highly dependent on information desired.

Optical coherence tomography (OCT)

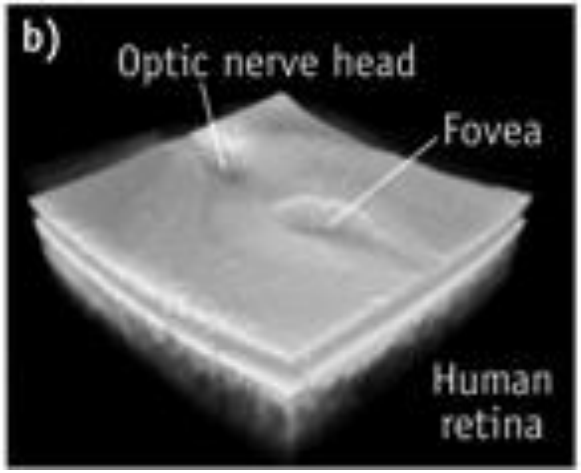
Based on optical scattering of tissue, typically uses infrared light and offers μm-resolution.



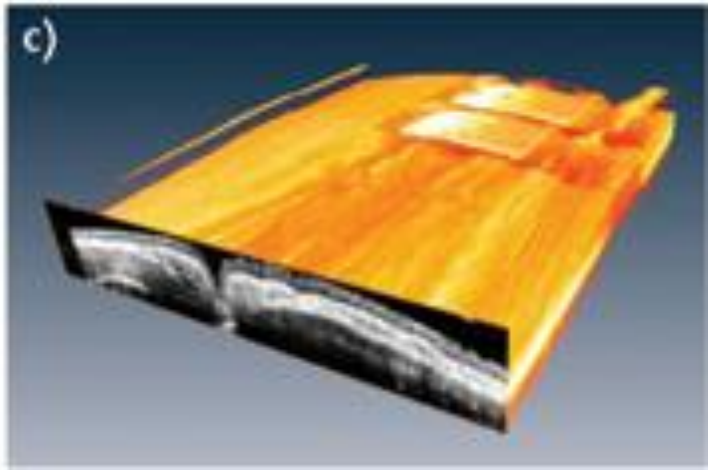
Light sources in the 900 - 1500 nm can achieve sub-micron resolution.



OCT fundus image produced at a 1.2 MHz axial scan rate. *in vivo* human retina obtained with a 1050 nm VCSEL.



400 kHz axial-scan-rate 3D volume of *in vivo* human retina obtained with a 1050 nm VCSEL was motion-corrected and averaged from four volumes.

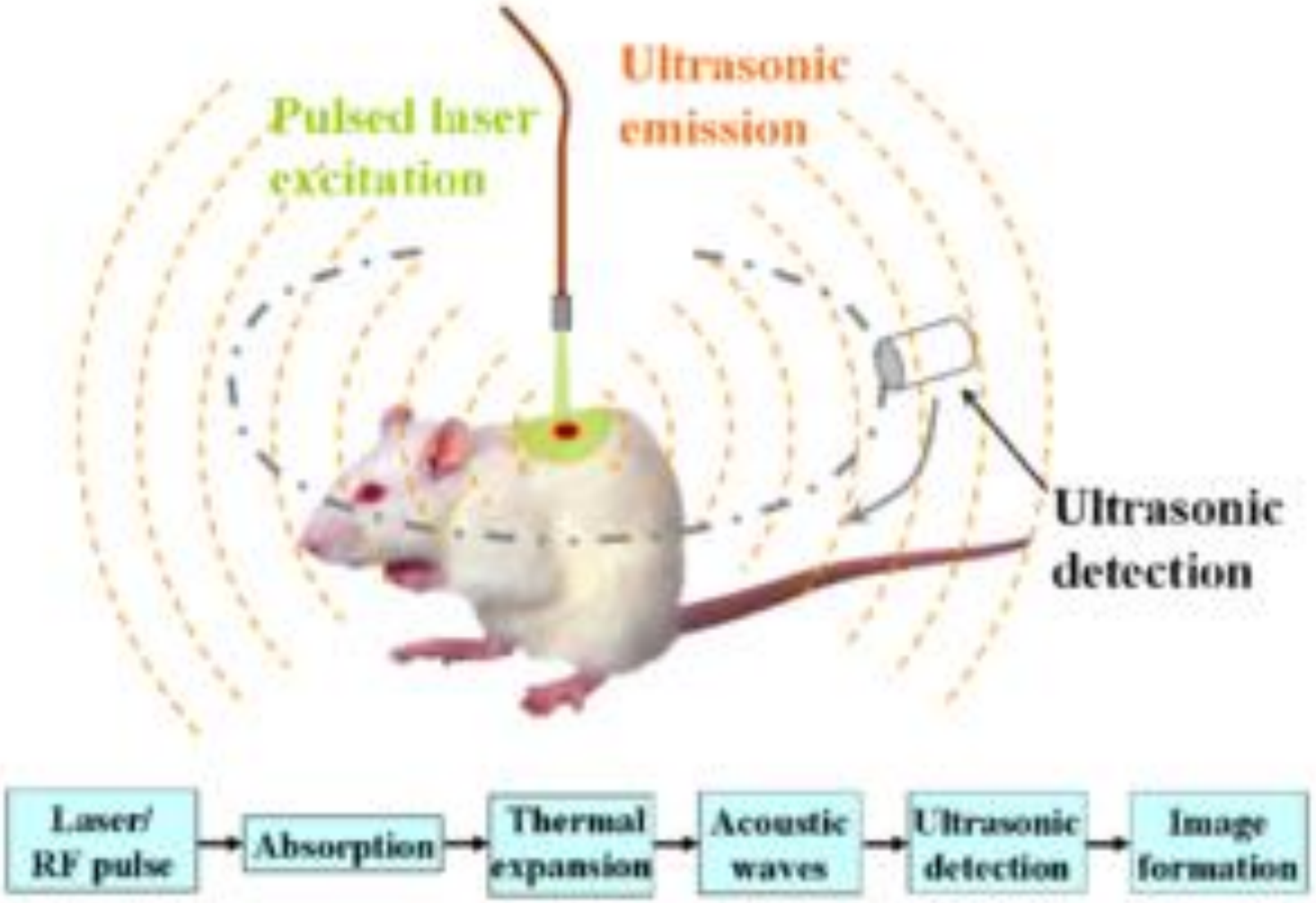


1 MHz axial-scan-rate 3D volume of *in vivo* rabbit stomach was obtained with a miniature endoscopic probe with a 1310 nm VCSEL.

BioOptics World July 2013

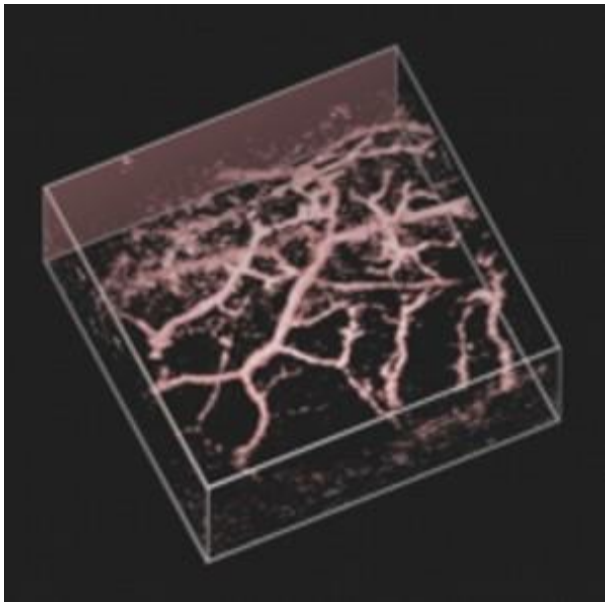
Photoacoustic tomography/microscopy

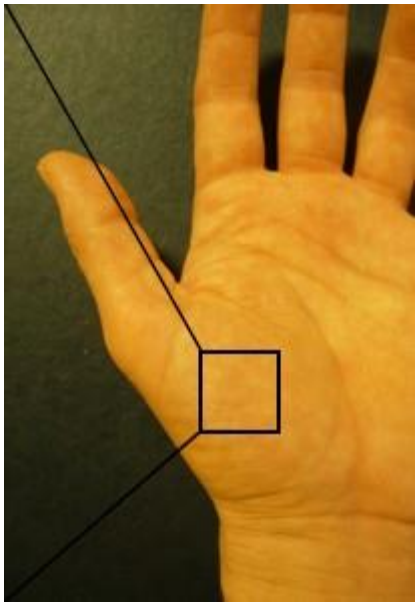
- Non-invasive imaging based on the photo-acoustic (PA) effect.
- Radio-frequency or **optical pulses** are transmitted into the tissue. Upon absorption of the pulse, a sound wave is produced due to energy deposition.
- The generated acoustic waves allow visualising the internal structure and function of soft tissues.
- Potential applications include the clinical assessment of breast cancer, vascular disease, skin abnormalities, etc.
- The PA signal depends on optical properties, thermal diffusivity/expansion and elastic properties.
- Returning ultrasound is detected at tissue surface by a transducer (single transducer, a line array, or a circular array can be used for detection).
  - backward mode: the sensor head is transparent to the excitation laser pulses, enabling the PA signals to be detected on the same side of the tissue that is irradiated.
- PA systems image cms into tissue: can offer 100 μm resolution at 4 cm.

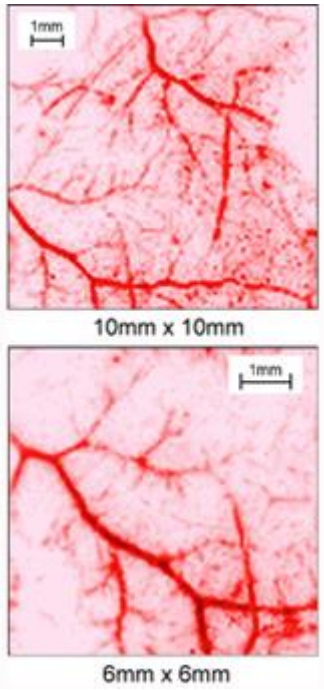


Source: Wikipedia

Photoacoustic imaging is particularly well suited for visualising blood vessels







Volume rendered *in vivo* photoacoustic image of the vascular anatomy in the palm of the hand. Image volume: 20mm x 20mm x 6mm. Excitation wavelength λ=670nm. Incident fluence: 8mJ/cm^2. No signal averaging was used. Image acquisition time: 15 minutes.

Source: Photoacoustic Imaging Group, Univ. College London

Researchers at Washington University (St. Louis, US) have developed a **hand-held** photoacoustic microscopy device that can be used directly on a patient and accurately measure how deep a melanoma tumor extends into the skin.

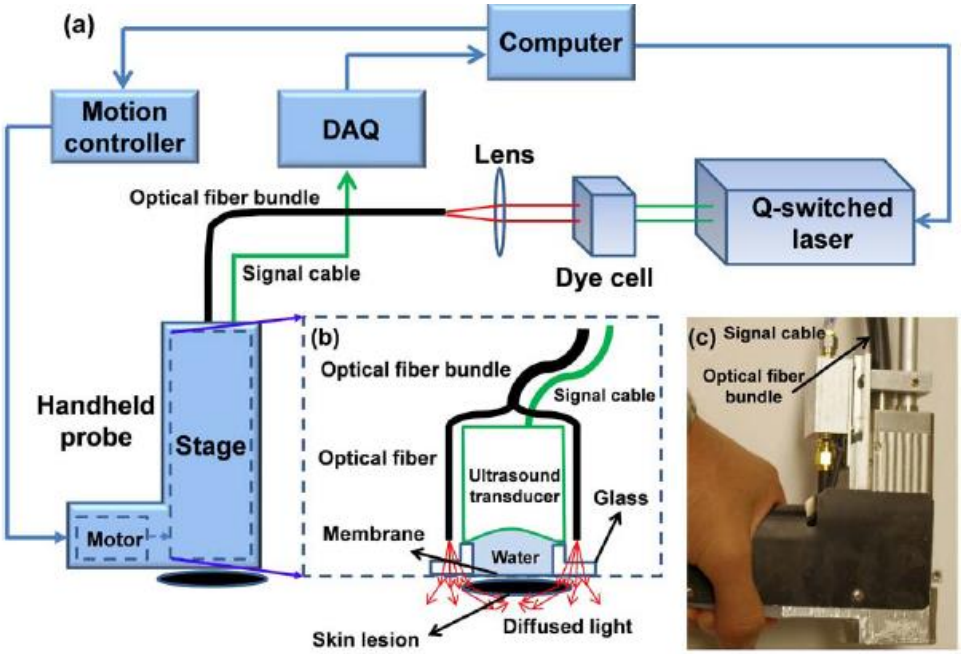
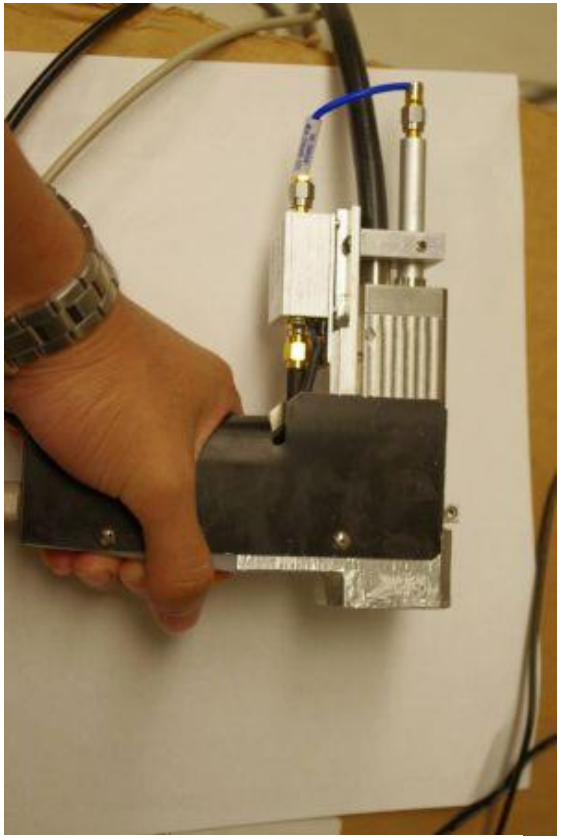


Fig. 1. Experimental handheld photoacoustic microscopy (PAM) system. (a) Schematic of the handheld PAM system. (b) Components held by the translation stage in the handheld probe. (c) Photograph of the handheld probe.



BioOptics World 12/2014

OPTICS LETTERS / Vol. 39, No. 16 / August 15, 2014

-LASER SYSTEMS FOR SENSING & SAFETY

Drug verification & food safety

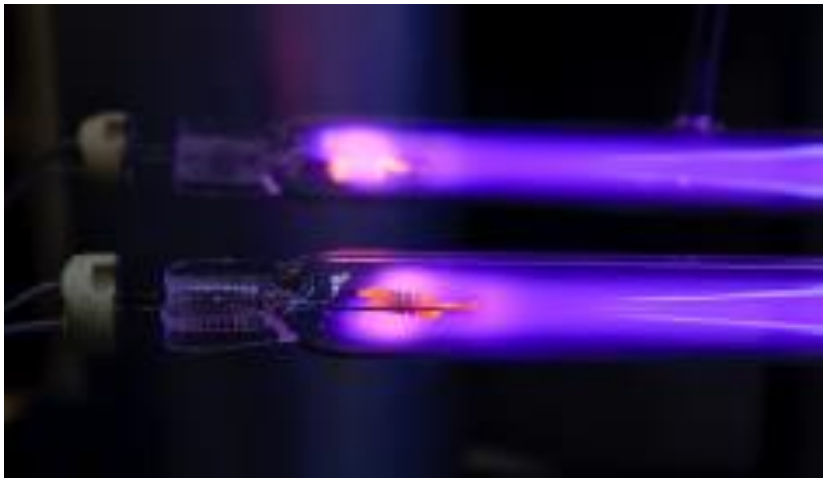
- Instruments employing lasers emitting a several frequencies use spectroscopy methods (eg, Fourier Transform Infrared Spectroscopy, FTIS) to identify different materials and verify the composition of different drugs.

- Hand-held spectrometers allow detecting chemical compounds during drug development and testing.

- Optical sensors also routinely used to ensure food quality and safety
  - to measure the oxygen levels inside sealed packaged food to ensure that the food inside the package will not spoil
  - Spectrometers allow to identify chemical signatures in food, to detect contaminants

UV light used for sterilizing water

- UV light's ability to alter virus DNA to halt its replication has made UV germicidal irradiant (UVGI) devices possible for water purification and medical equipment sterilization.
- UVGI devices could also disinfect hospital and healthcare rooms from deadly diseases such as Ebola, Tuberculosis and Lassa



Source: ISTOCK

Current light sources: lamps that produce UV radiation by ionizing low pressure mercury vapor. These lamps are similar to typical fluorescent household lighting fixtures but do not have the phosphorescent coating which imparts the soft white light. Ionized mercury emits at 254nm -- s an ideal wavelength for disrupting the DNA of microorganisms. Blue LEDs can eventually replace UV lamps

Flow cytometry: Fluorescence Activated Cell Sorter (FACS)

- Is routinely used to diagnose blood cancers and other diseases
- Employed in cell counting, cell sorting, biomarker detection and protein engineering, by suspending cells in a stream of fluid and passing them by an electronic detection apparatus.
- It allows simultaneous multiparametric analysis of the physical and chemical characteristics of up to thousands of particles per second.

FACSCalibur. Source: Wikipedia

A flow cytometer has:

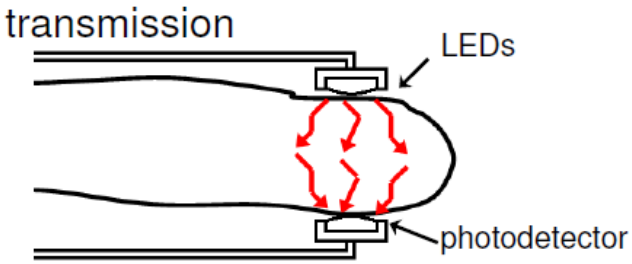
- A flow cell: liquid stream which carries and aligns the cells so that they pass single file through the light beam for sensing
- A measuring system : commonly used are measurement of impedance (or conductivity)
- Optical systems: lamps (mercury, xenon); high-power water-cooled lasers (argon, krypton, dye laser); low-power air-cooled lasers (argon (488 nm), red-HeNe (633 nm), green-HeNe, HeCd (UV)); diode lasers (blue, green, red, violet)
- A detector and Analogue-to-Digital Conversion (ADC) system - which generates forward-scattered light (FSC) and side-scattered light (SSC) as well as fluorescence signals from light into electrical signals that can be processed by a computer
- An amplification system and a computer for analysis of the signals.

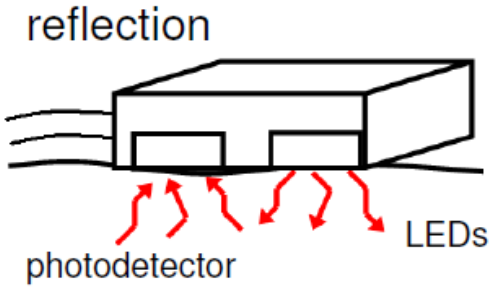
Pulse Oximetry

Non-invasive method that gives information of blood oxygenation.

- A clip on the fingertip (or earlobe) is used, with a red (660nm) and IR (910 nm) LEDs facing a photodetector.
- Can be operated in reflection or transmission mode.

Source: M. Leahy (University of Limerick)





- Absorption at these wavelengths varies significantly for oxy and de-oxy haemoglobin.
- From the ratio of the absorption of the red and infrared light the oxy/deoxyhaemoglobin ratio can be calculated.
- Many conditions can be studied (depth of anesthesia, blood loss).

Other applications

Dental DNA analysis

- Take sample by swiping teeth with a toothpick like piece of paper.
- Placing the sample in a device that amplifies the DNA with the polymerase chain reaction (PCR) fluorescent labeling plus a laser diode and a photo-detector can identify 11 types of bacteria.
- It can be used to select the best antibiotic treatment.

Laser-based optical tweezers

- Have been used to trap everything from viruses, bacteria, small metal particles, and strands of DNA.
- Promising for cancer diagnostic

Pillcam: vitamin-sized capsule

Capsule endoscopy with the PillCam SB video capsule enables your doctor to examine your entire small intestine. Your doctor will have you ingest a video capsule that has its own camera and light source. You can move freely during the exam, which lasts about eight hours. While the video capsule travels through your body, it sends images to a data recorder you will wear close to your waist. Afterwards, your doctor will view the images on a video monitor.

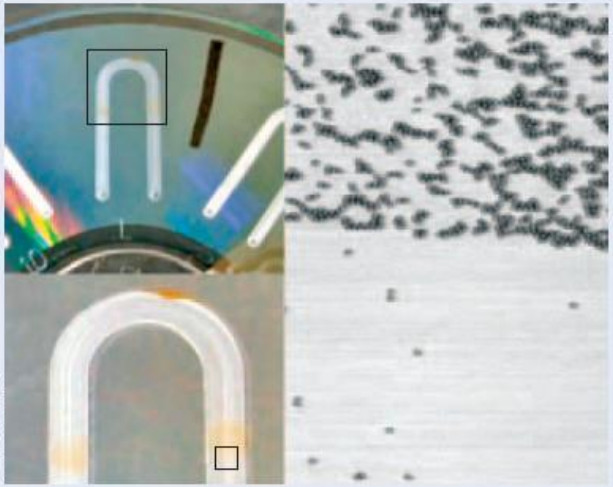
Source: wikipedia

BIOIMAGING

Lab on a DVD

Scientists based in Europe have succeeded in converting a commercial DVD drive into a laser scanning microscope that can analyse blood and perform cellular imaging with one-micrometre resolution (*Lab Chip*, doi: 10.1039/C3LC41360H; 2013). Harisha Ramachandraiah and the team from KTH Royal Institute of Technology in Sweden and the companies, Plarion in the UK and Lingvitae in Norway, say that their 'lab-on-a-DVD' system offers affordable and convenient cellular diagnostic testing for diseases such as HIV.

The approach makes two important modifications to the DVD drive and standard DVD media. First, an extra photodiode is added to the drive to detect transmitted and forward-scattered light through the disk. Second, the DVD media is replaced with a disposable, multilayer, semi-transparent polymer disk that contains fluidic microchannels



in addition to the usual 0.74-μm-wide spiral track.

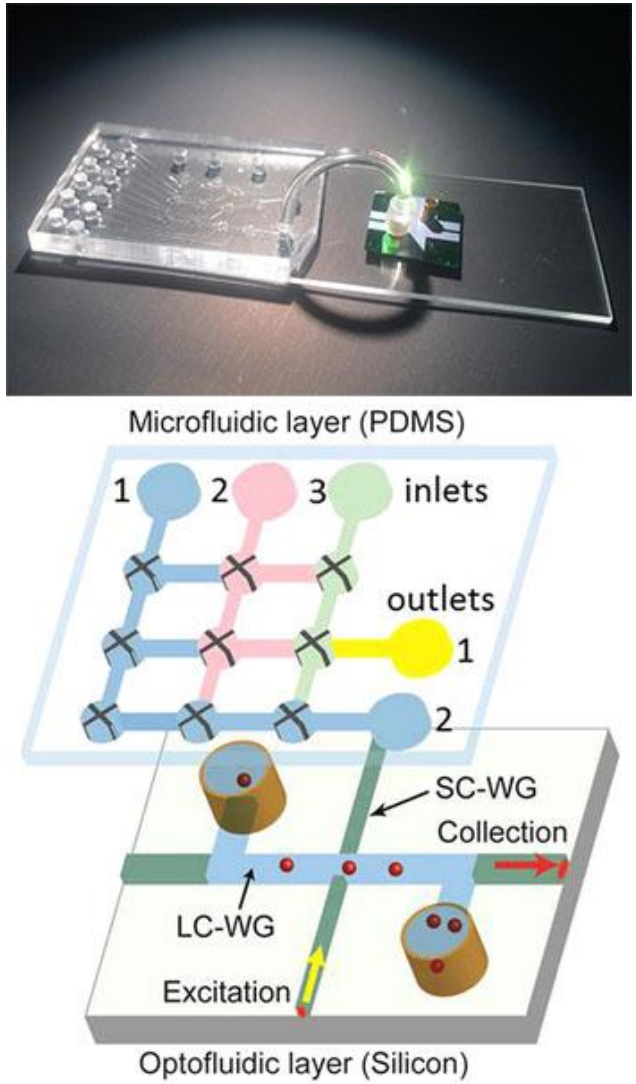
Before performing experiments, the inner surfaces of the fluidic channels are functionalized to allow surface attachment of the desired cells or particles. Samples of blood or another liquid of interest are then pumped into the channels and the DVD drive is switched on. The added photodiode records the amount of light from the drive's 658-nm semiconductor laser that is transmitted through the disk as it spins. The result is a two-dimensional image, which is saved to a computer hard drive for analysis. Cells or particles that have been successfully bound to the treated channels show up in the resulting images. To date, the team has tested their system by using it to image polymer beads of various sizes (1, 2.8 and 5 μm) suspended in a solution as well as $CD4^+$ cells in blood, which are an important marker for the HIV virus.

The researchers are now working on extending the system to handle larger sample volumes so that low-concentration species such as circulating tumour cells can be analysed in a fully integrated approach that automates the tasks of channel surface modification, washing and sample preparation.

OLIVER GRAYDON

A Chip-Based System for Detecting Ebola

- The device, which combines microfluidics and optics in a modular setup, reportedly can achieve sensitivities and dynamic range comparable to those of commonly used, far more complex and time-consuming lab techniques.



More info: http://www.osa-opn.org/home/newsroom/2015/september/a_chip-based_system_for_detecting_ebola/

Take home message

- Laser-based imaging methods provide early diagnoses of diseases before the point when costly medical treatments are necessary.
- The emerging fields of neurophotonics and optogenetics will drastically advance our understanding of the brain.
- Nowadays drugs can be delivered and/or activated by light.
- Optical sensors are fast, reliable and sensitive for detection of harmful substances, as well as valuable diagnostic tools: can monitor through breath analysis; can test drinking water for contamination, etc.
- Lab-on-a-chip devices and low-cost microscopes incorporated to smart phones could revolutionize diagnosis and track infectious diseases.

THANK YOU FOR YOUR ATTENTION !

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Universitat Politecnica de Catalunya

http://www.fisica.edu.uy/~cris/