New trends in biophotonics

András Kincses
Institute of Biophysics
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Biophotonics by Wikipedia: „The term denotes a combination of biology and photonics with photonics being the science and technology of generation, manipulation, and detection of photons, quantum units of light.”

**BIOPHOTONICS**

(classical usage)

„PHOTOBIONICS”

(alternative usage)

Both terms denote a combination of biology and photonics.

development and application of optical techniques, particularly imaging, to the study of biological molecules, cells and tissue

application of biomaterials in the development of optical devices
OUTLINE OF THE TALK

Optics in biology
- What is light?
- Role of light in biology
- Biological/ medical applications of light

Biology in optics
- Integrated optics
- All-optical logic gates
- Optical biosensors
- Optogenetics
What is light?

Light usually refers to visible light, but this is just a part (from ~380 nm to ~700 nm) of the electromagnetic radiation.

- wave-particle duality
- electromagnetic wave (wavelength, frequency)
- photon

![Image of the electromagnetic spectrum from gamma rays to radio waves, highlighting visible light between ~380 nm and ~700 nm]
Light has an important role in almost every vital process.

- provides energy
- controls biological clock
- perception
- phototaxis
Perception of light

Light is an important information carrier: e.g. for humans it is the primary source of the detection of the environment.

- Visible spectrum for human: ~400 nm – ~700 nm
- Many species can sense light with frequencies outside the human visible spectrum, for example birds, bees and many other insects detect UV light, and snakes sense infrared.
**Phototaxis**

Movement of an organism (e.g. bacteria, insects) toward or away from a source of light.

**Phototropism**

Phototropism is advantageous for plants as they can orient themselves towards light to receive light most efficiently for photosynthesis.
On Earth the main source of light is the Sun.

the average intensity of solar energy hitting the ground is around 1.1 kW/m².

Incandescent light bulbs radiate 40 to 100 watts.

Total amount of Sun’s energy per year over all earth is $1.6 \times 10^{18}$ kWh.

Global energy consumption is about $9 \times 10^{13}$ J in a year.
Light has an important role in the energy cycle for life:

1. Chloroplasts of plants absorb energy from the Sun light.
2. Energy is used in chloroplasts to create energy-rich sugar molecules.
3. Sugars available to plants to use their own purposes.
4. Sugars also available to animals for food.
5. Animals use the sugar to produce their own "energy currency" through the mitochondria.
6. ATP production.
The relationship of life and light based on complex biological and chemical processes:

Transforming light energy to chemical energy: plants, bacteria (e.g. *Halobacterium salinarum*), algae (e.g. *Chlamydomonas reinhardtii*)
Optical microscopy

Optical microscopes use visible light and a system of lenses to magnify the sample.

Several techniques

For example:

• bright field: absorbance of light in the sample

• dark field: light scattered by the sample

• phase contrast: interference of light of different path lengths through the sample

• fluorescence
Optical tweezer

Light propagates as a stream of photons, and every photon carries mechanical momentum:

\[ p_{\text{photon}} = \frac{h}{\lambda} \]

Light exerts a small force on objects:

![Diagram of optical tweezer](image)
Optical tweezer

Transparent objects – e.g. microbeads, cells, cell organelles, bacteria, etc… - with higher refractive index than the medium (usually water) can be trapped in the focus of a laser beam.

Trapped object is kept around the trap center by a linear restoration force:
3D manipulation of biological objects:
- study RBC infection by malaria parasites
- positioning of pathogens to macrophage cells for the observation of phagocytosis
- indirect optical trapping of live cells with microfabricated polymer microtools

Force measurements in biological systems:
- RBC stiffness measurement
- DNA torsional stiffness measurement
- Cell membrane stiffness measurement
- Force measurement on single kinesin molecules
Biophotonics

Light emitting diodes, fluorescent lamps, etc.
- Phototherapy:  
  - skin disorders  
  - sleep disorders  
  - neonatal jaundice

Laser
- medical diagnosis:
- eye surgery
- soft tissue surgery
- laser scalpel
- dentistry: caries removal, tooth withening
- cosmetic surgery: removing tattoos, scars, wrinkles  
  birthmarks, hairs
Biology in optics – Integrated optics – Photobionics

- the future of microelectronics
- new trends, optical communication, dawn of integrated optics
- passive and active components
- the slab waveguide
- active components, modulators
- nonlinear optical materials, crystals, dyes and materials of biological origin
- photobionics
- bacteriorhodopsin
- all-optical photobionic switching, idea and realization
- biosensor application
- bioelectric phenomena
- optogenetics
Moore's law is the observation that, over the history of computing hardware, the number of transistors in a dense integrated circuit doubles approximately every two years.

Resolution of photolithographic procedures is limited by diffraction.

Size of focal spot: \[ d = 1.22 \frac{f \lambda}{D} = 1.22 \lambda \cdot \left( \frac{f}{D} \right) \]

Deep-UV excimer lasers: KrF (248 nm), ArF (193 nm)

**Problems:** air absorption below 185 nm, SiO\(_2\) bandgap at 127 nm (free electrons)

Recent technology: high-index immersion lithography; 30 nm feature size (2006, IBM)

Moore’s law hits the wall...
Miniatrurization of electronic components can not be continued forever
Optical communication, integrated optics (IO)

Analogy between electronics and optics

Information carrier: **light**

**Passive components:**
Electric conductors $\rightarrow$ optical waveguides

**Active components:**
Transistors, diodes $\rightarrow$ optical modulators, switches

**Integrated circuits**

*IBM Research*

*BRC*

*Fujitsu Laboratories Ltd., Japan, 2012*
The slab optical waveguide

Waveguiding layer of high refractive index, thickness ~200 nm

Provides the optical analogon of electrical wire, can be used “as is” or can be integrated in several distinct types, according to application requirements. Light is propagating in the thin guiding layer, by law of total internal reflection (TIR).

The reflected elementary rays interfere with each other, building up the so-called guided modes.
Although the radiation is confined in the guiding layer, a small part of the propagating wave (*the evanescent wave*) penetrates the surrounding media (substrate and adlayer). Propagation properties of the guided modes depend not only the parameters of the waveguide, but on those of the adlayer (sample).

Cross-sectional intensity distribution

The propagating mode „feels” the refractive index of the adlayer through the evanescent wave.
The slab optical waveguide

Guided modes are characterized by their **effective refractive index** \(N\) which depends on the waveguide parameters (thickness, refractive indices of substrate and guiding layer) **AND** on the refractive index of the **adlayer**.

Only discrete modes are allowed to propagate.

Waveguide parameters: \(n_S = 1.52, \ n_F = 1.77, \ n_A = 1, \ \lambda = 632.8\ \text{nm}\)
Optical Waveguide Lightmode Spectroscopy (OWLS)

The incident monochromatic light is coupled into the waveguide by a grating coupler. Intensity peaks of guided modes are detected at the end of the waveguide. The refractive index of the adlayer can be calculated from the incoupling angles.

**OWLS:** measurement of refractive index (change) via detection of guided modes
OWLS „in vivo”

- bR adlayer
- guiding layer
- substrate
- grating coupler

Grating
Dried sample
Guided mode
Waveguide
Rotating sample holder
Active integrated optical components

Fabrication techniques of the passive components are well established, the bottle-neck of integrated optics is the research of nonlinear optical materials that can be used in the active, light-controlling parts of IO devices.

Control of light through electro-, magneto-, thermo- and acousto-optic phenomena

**Kerr effect**: change of material **refractive index** upon application of an electric field (requires **birefringent** materials without central symmetry) used in wave plates, optical modulators, shutters

**Cotton–Mouton effect**: magneto-optical analogon of the Kerr effect

**Acousto-optic control of light**: Interaction of light wave with periodically modulated material properties (e.g. density) induced by (ultra)sound waves.

(optical gratings, reflectors, refractors, deflectors)
Active integrated optical components

The most important requirements against active optical materials:

- high nonlinear optical coefficients (e.g. Kerr-constant, nonlinear refractive index, ...)
- optical and mechanical stability (against radiation and mechanical stress)
- sensitivity (excitable by relatively low intensities)
- „infinite” excitability (re-excitable without degradation)
- speed (in change of properties)

Commonly used active optical materials:

- organic and inorganic crystals
- natural and artificial dye molecules
- biological materials (?)

Can biology help information technology?

B. Coe, Univ. of Manchester, UK
Photonics by Wikipedia: "The word 'photonics' is derived from the Greek word "photos" meaning light; it appeared in the late 1960s to describe a research field whose goal was to use light to perform functions, that traditionally fell within the typical domain of electronics, such as telecommunications, information processing, etc."

Bionics by Wikipedia: "The application of biological methods and systems found in nature to the study and design of engineering systems and modern technology"

Photonic "devices" are already created by Nature

What can we learn from these natural devices? Can we reproduce them artificially? Or even, can we exploit some of their intrinsic features?

Photobionics
Nowadays, the switching speed of electronic devices is around several GHz (~ ns).

Time-scale of initial processes in light-sensitive biological molecules is in the fs – ps range (e.g. charge separation, isomerization, etc.).

To improve the switching speed of hybrid (electronic – optical) devices, we must eliminate the „weak link”, electronics. We need a new technique...

**All-optical data processing**

Usually, biological molecules, proteins become very unstable (even „dead”) outside of their natural environment.

**Or... Are there some exceptions we know about...?**
Bacteriorhodopsin (bR)
Discovered in *Halobacterium salinarum*, 7 α-helices, retinal chromophore. The simplest light-sensitive ion (H⁺) pumping protein known so far.

*In vivo* role: generation of electrochemical gradient across the cell membrane.
During the proton pumping process, the protein undergoes several conformational substates with distinct absorption spectra. According to the Kramers – Krönig relations, the shift of absorption peaks leads to refractive index changes between these substates.
Bacteriorhodopsin (bR)

Calculated refractive index changes between some intermediate states (I, K, L, M) and the ground state (BR)
Photobionic switching and modulation

1) We have an integrated optical device that can detect refractive index changes
2) We also have a protein that changes its refractive index when excited by light

Let’s combine them...

What do we expect?

1) Angle of incidence is set and fixed in the vicinity of the guided mode
2) The sample is excited, its refractive index changes
3) The incoupling peak is shifted to a new position, corresponding to the altered propagation conditions
4) Intensity of the outcoupled light changes
Photobionic switching and modulation

Intensity changes between the BR→M transition of bacteriorhodopsin, measured at the two sides of the incoupling peak.

The switching speed is limited by the formation of the M state, ~ 50 μs

Too slow for a switch...
How can we exploit the speed (ps) of the early transitions of *bacteriorhodopsin*?

Ultrafast pump – probe technique
Ultrafast photobionic switch

- Rotational table
- Pump beam 532 nm
- Fabry-Pérot interferometer
- Probe beam 790 nm
- Delay line between pump and probe
Photobionic wavelength modulation

Since the ultrafast (ps) probe beam is of a broad-band, the intensity doesn’t change due to the altered propagation conditions.

With a broad-band probe beam we can not detect intensity change, only a shift in the wavelength of the incoupled light.
Photobionic intensity modulation

The Fabry-Pérot and its output spectrum (only illustration)

After narrowing the spectrum of the probe beam with a Fabry-Pérot interferometer, the intensity modulation can clearly be detected.

Grey: probe intensity without excitation
Red: with excitation of bR
All-optical switch with bacteriorhodopsin
Logic gate performs logical operation on one or more logical inputs, and produces a single logical output:

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<td>$X_2$</td>
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<td>$\text{NOT } X_1$</td>
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Truth table of logic operations
Logic 0: low voltage level
Logic 1: high voltage level

Logic 0: low light intensity
Logic 1: high light intensity

**Switch:** Voltage level or intensity level changes from low to high/ high to low.

In integrated electronic circuits logic gates are primarily implemented using diodes or transistors acting as **electronic switches**.

Optical logic gates – optical switches
Excitation of the bR layer by a continuous laser beam shifts the dynamic equilibrium between the concentrations of its ground (BR) and excited (M) states, consequently, the effective refractive index of the propagating mode is altered.

Refractive index alteration:

- Perturbs the frequency of the interacting resonant modes.
- Develops phase difference between the two arms.

Mach-Zehnder interferometer with the controlling bR layer.
The input values of the logic device were represented by the light of two 532 nm continuous wave diode lasers.

The key step of the switch between the bi- and tri-state logic is a proper preadjustment of the phase shift of the light propagating in one arm of the MZ interferometer. This can be realized by changing the refractive index of the bacteriorhodopsin adlayer via an external control signal.
All-optical interferometric logic gate – Binary mode

INVERTER

XOR GATE

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**COMPARATOR**

**Comparator** is a device that compares two input values and output indicates which one is larger.

In ternary mode output could be -1, 0, 1.
Optical interference is a phenomenon in which two electromagnetic waves superpose to form a resultant wave of greater or lower amplitude. The resultant amplitude depends on the phase difference between the waves.

The light is split into the two arms and the light beams interfere at the output of the interferometer. The output intensity depends on the phase difference between the arms.
The basis of operation of a MZ-based biosensor is that whenever an adsorption process takes place on only one branch of the interferometer (the measuring arm), a thin adlayer is formed on the waveguide surface. The accompanying effective refractive index change gives rise to a phase difference between the two branches causing an intensity change at the output of the interferometer that can be sensitively detected.
Integrated optical interferometric biosensor
Bioelectric phenomena

Accompanying biological energy- and signal transduction phenomena can be traced back to the level of cell membranes: ion channels and pumps.
Firing of neurons can be induced by electric stimulation

Disadvantage: insufficient spatial resolution, only depolarization

Suggestion for solution: optogenetics
Optogenetics by Wikipedia: „Optogenetics (from Greek optos, meaning "visible") uses light to control neurons which have been genetically sensitised to light. It is a neuromodulation technique employed in neuroscience that uses a combination of techniques from optics and genetics to control and monitor the activities of individual neurons in living tissue — even within freely-moving animals — and to precisely measure the effects of those manipulations in real-time.”
Optogenetic proteins: cation channeling and anion pumping rhodopsins isolated from microorganisms
Inserting retinal proteins into neural cells of the brain

Deisselroth et al., 2005, at Wikipedia
Optogenetics

Light-induced depolarization

Baratta et al. from Optogenetics, Wikipedia
Light-induced hyperpolarization

Baratta et al. from Optogenetics, Wikipedia
Fields of applications

Brain research (in-vivo investigation of neural networks)

Neurological and psychiatric diseases (Parkinson’s disease, epilepsy, depression, schizophrenia)

Blindness (retinitis pigmentosa, macular degeneration)

Selective excitation of muscle cells and tissues

Controlled cell motility
Method of the Year 2010: Optogenetics - by Nature Video
Thank you for your attention!

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