ESP Photobiology School

June 20 – 25, 2016

Brixen/Bressanone

Programme

Abstracts

European Society for Photobiology

Organizing Committeé

Kristian Berg (NO, Chair)
Lesley Rhodes (UK, Chair - Education and Training Committee)
Rex Tyrrell (UK, ESP President)
Giorgia Miolo (IT, Local Organizer)
Francesco Ghetti (IT, Treasurer)
Santi Nonell (ES, ESP President-Elect)

Faculty

Stefan Anderson-Engels (SE), Kristian Berg (NO), Janet Bornman (AU), Giovanni Checcucci (IT), Luca Dall'Osto (IT), Thierry Douki (FR), Amparo Faustino (PT), Francesca Giuntini (UK), Giorgia Miolo (IT), Miguel Miranda (ES), Tomas Morosinotto (IT), Carlo Musio (IT), Santi Nonell (ES), Jacques Piette (BE), Lesley Rhodes (UK), David Russell (UK), Evelyne Sage (FR), Herbert Stepp (DE), Franz Trautinger (AU), Massimo Trotta (IT), Rex M.Tyrrell (UK), Christiano Viappiani (IT), Georges Wagnieres (CH), Peter Wolf (AU)

Important locations in Brixen/Bressanone

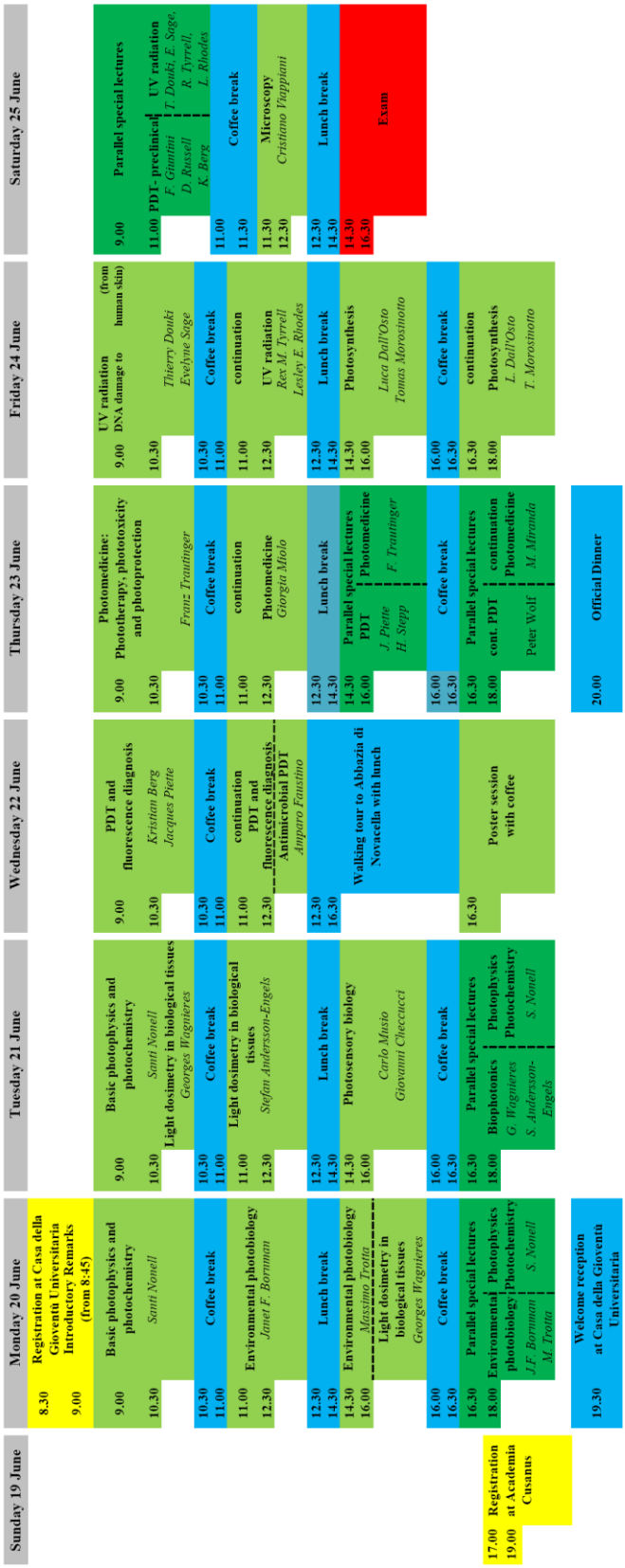
School venue:
Casa della Gioventù, Università di Padova,
Address:
via Rio Bianco 6.
39042 Bressanone BZ, Italy

Accomodation:
Academia Cusanus
Address:

Sponsor

European Society for Photobiology

ESP Photobiology School - Time Schedule



Light green: plenary lectures
Dark green: special lectures

ESP PHOTOBIOLOGY SCHOOL
SCIENTIFIC PROGRAM

Sunday June 19

Time: **17:0-19:00:**

Registration - at Academia Cusanus

Monday June 20

Time: **8:30-9:00: Registration**
Time: **8:45-9:00: Welcome and Introductory remarks**

Time: **9:00-10:30**

Topic, plenary: **Basic photophysics and photochemistry**
Santi Nonell, Institut Químic de Sarrià, Universitat Ramon Llull, Barcelona, Spain.

Time: **10:30-11:00: Coffee break**

Time: **11:00-12:30**

Topic, plenary: **Environmental photobiology**
Janet Bornman International Institute of Agri-Food Security (IIAFS), Curtin University, Perth, Australia.

12:30 – 14:30 Lunch

Time: **14:30-16:00**

Topic, plenary: **Environmental photobiology**
Massimo Trotta, Institute for Chemical-Physical Processes, Italian National Research Council (CNR), Bari, Italy

Topic, plenary: **Light dosimetry in biological tissues**
Georges Wagnieres, Institute of Chemical Sciences and Engineering, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland.

Time: **16:00-16:30: Coffee break**

Time: **16:30-18:00**

Parallel sessions

I. Topic, special: **Environmental photobiology**
Janet Bornman International Institute of Agri-Food Security (IIAFS), Curtin University, Perth, Australia.
Massimo Trotta, Institute for Chemical-Physical Processes, Italian National Research Council (CNR), Bari, Italy

II. Topic, special: **Photophysics and photochemistry**
Santi Nonell, Institut Químic de Sarrià, Universitat Ramon Llull, Barcelona, Spain.

Time: **19:30:**

Welcome reception - at the School venue

Tuesday June 21

Time: **9:00-10:30**

Topic, plenary: Basic photophysics and photochemistry

Santi Nonell, Institut Químic de Sarrià, Universitat Ramon Llull, Barcelona, Spain.

Topic, plenary: Light dosimetry in biological tissues

Georges Wagnieres, Institute of Chemical Sciences and Engineering, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland.

Time: **10:30-11:00: Coffee break**

Time: **11:00-12:30**

Topic, plenary: Light dosimetry in biological tissues

Stefan Andersson-Engels, Department of Physics, Lund University, Lund, Sweden.

12:30 – 14:30 Lunch

Time: **14:30-16:00**

Topic, plenary: Photosensory biology

Giovanni Checcucci, Istituto di Biofisica, Consiglio Nazionale delle Ricerche, Pisa Italy
Carlo Musio, Consiglio Nazionale delle Ricerche - Istituto di Biofisica Fondazione Bruno Kessler, Trento, Italy

Time: **16:00-16:30: Coffee break**

Time: **16:30-18:00**

Parallel sessions

I. Topic, special: **Biophotonics**

Stefan Andersson-Engels, Department of Physics, Lund University, Lund, Sweden.
Georges Wagnieres, Institute of Chemical Sciences and Engineering, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland.

II. Topic,special: **Photophysics and photochemistry: Measurement, simulation, and analysis of spectroscopic data**

Santi Nonell, Institut Químic de Sarrià, Universitat Ramon Llull, Barcelona, Spain.

Wednesday June 22

Time: **9:00-10:30:**

Topic, plenary: **Photodynamic therapy and fluorescence-based diagnosis**
Kristian Berg, Oslo University Hospital - Radium Hospital, Department of Radiation Biology, Oslo, Norway

Time: **10:30-11:00: Coffee break**

Time: **11:00-12:30:**

Topic, plenary: **Photodynamic therapy and fluorescence-based diagnosis**
Jacques Piette, Laboratory of Virology & Immunology, University of Liege, Liege, Belgium

Topic, plenary: **Antimicrobial photodynamic therapy**
Amparo Faustino, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal.

Time: 12:30 – 16:30: Walking tour to Abbazia di Novacella with lunch

Time:16:30 – 18:00: Poster session with coffee

Thursday June 23

Time: **9:00-12:30**

Topic, plenary: **Photomedicine (Phototherapy, phototoxicity and photoprotection)**

Time: **9:00-10:30** **Basic photodermatology**

Franz Trautinger, Karl Landsteiner Institute for Dermatological Research, St. Poelten, Austria

Time: **10:30-11:00** **Coffee break**

Time: **11:00-12:30** **Photoreactivity and phototoxicity of drugs**

Giogia Miolo, Department of Pharmaceutical Sciences, University of Padova, Padova, Italy.

12:30 – 14:30 Lunch

Time: **14:30-18:00**

Parallel sessions

I. ***Topic, special:*** **PDT/Photodiagnosis – preclinical/clinical**

PDT – cell signaling

Jacques Piette, Laboratory of Virology & Immunology, University of Liege, Liege, Belgium

PDT/Photodiagnosis – clinical experience

Herbert Stepp, Laser-Forschungslabor, Klinikum der Universität München, München, Germany.

II. ***Topic, special:*** **Photomedicine (Phototherapy, phototoxicity and photoprotection)**

Phototherapy: Specific treatment modalities

Franz Trautinger, Karl Landsteiner Institute for Dermatological Research, St. Poelten, Austria

Time: **16:00-16:30** **Coffee break**

I. ***Topic, special: PDT – clinical***

Photodynamic Therapy in Dermatology

Peter Wolf, Research Unit for Photodermatology, Department of Dermatology, Medical University of Graz, Graz, Austria.

Impact of immunology on the outcome of photodynamic therapy

Peter Wolf, Research Unit for Photodermatology, Department of Dermatology, Medical University of Graz, Graz, Austria.

II. ***Topic, special:*** **Photooxidative reactions of drugs with biomolecules**

Miguel Miranda, Universidad Politecnica de Valencia, Departamento de Quimica, Valencia, Spain

Time: 20:00: Official dinner

Friday June 24

Time: **9:00-12:30**

Topic, plenary: **UV (from cells to skin tissue)**

Time: **9:00-10:30**

Thierry Douki, Laboratoire Lesions des Acides Nucleiques, CEA-Grenoble, France.
Evelyne Sage, Institut Curie – Recherche, Centre Universitaire, Orsay – France

Time: **10:30-11:00** **Coffee break**

Time: **11:00-12:30**

Rex Tyrrell, Department of Pharmacy & Pharmacology,University of Bath, United Kingdom
Lesley Rhodes, Photobiology Unit, Dermatological Sciences, Salford Royal Foundation Hospital, University of Manchester, Manchester - United Kingdom.

12:30 – 14:30 Lunch

Time: **14:30-16:30**

Topic, plenary: **Photosynthesis**

Time: **14:30-16:00:**

Luca Dall'Osto Department of Biotechnology, University of Verona, Italy

Time: **16:00-16:30:** **Coffee break**

Time: **16:30-18:00:**

Tomas Morosinotto, Department of Biology, University of Padova, Italy

Saturday June 25

Time: **09:00-11:00**

I. Topic, special: PDT

Photosensitizers in Photodynamic Therapy

Francesca Giuntini, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK.

Nano-systems for photodynamic therapy

David Russell, School of Chemistry, University of East Anglia, Norwich, Great Britain

Photochemical internalization (PCI) – from photodynamic targeting of lysosomes to clinical utilization of PCI

Kristian Berg, The Norwegian Radium Hospital, Department of Radiation Biology, Oslo, Norway

II. Topic, special: UV (from cells to skin tissue)

Title

Thierry Douki, Laboratoire Lesions des Acides Nucleiques, CEA-Grenoble, France.

A role for UVA on skin cancer

Evelyne Sage, Institut Curie – Recherche, Centre Universitaire, Orsay – France

Endogenous and exogenous protection against UV generated oxidative stress

Rex Tyrrell, Department of Pharmacy & Pharmacology,University of Bath, United Kingdom

Balancing the benefits and risks of solar UVR exposure

Lesley Rhodes, Photobiology Unit, Dermatological Sciences, Salford Royal Foundation Hospital, University of Manchester, Manchester - United Kingdom.

Time: **11:00-11:30** **Coffee break**

Time: **11:30-12:30**

Topic, plenary: Microscopy

Christiano Viappiani, Dipartimento di Fisica e Scienze della Terra, Università di Parma, Parma, Italy

12:30 – 14:30 Lunch

Time: **14:30-17:30**

EXAM

Abstracts

Preferential reading has been highlighted in bold

Monday morning, *topic, plenary:*

Photophysics and Photochemistry (Nonell)

Light
Nature and properties of light. Light sources in photobiology. Light conditioning and measuring

Interaction of light with biomolecules
Outcomes of the interaction of light with matter. Light absorption: creating excited states. The properties of excited states. Absorption spectra and action spectra

Photophysics
The fate of excited states: radiative and non-radiative unimolecular decay processes. Rate constants, quantum yields, and lifetimes. A closer look to fluorescence. Emission techniques: steady-state and time-resolved. Fluorescent proteins. Photothermal techniques

Photochemistry
General aspects. Classification of photochemical reactions. Selected examples in Photobiology

Environmental photobiology (Bornman)

Current and future environmental change and global consequences: how are UV radiation, climate and the ozone layer involved?

Janet F. Bornman, Chair Agri-Food Security, Curtin University, Western Australia
(janet.bornman@curtin.edu.au)

Without the stratospheric ozone layer (10-50 km above the Earth's surface), life on Earth as we know it today would not be possible, since ozone effectively screens out most of the damaging ultraviolet (UV) radiation. Therefore any decrease in this ozone layer can have serious consequences for the biosphere. For the past 40 years the threat of a depleted ozone layer has been a major environmental concern after the discovery that man-made chemicals, the chlorofluorocarbons, which were widely used as refrigerants, solvents, aerosol-spray propellants, and foam-blowing agents, were breaking down the stratospheric ozone layer. Other chemicals, such as methyl bromide, halons, and carbon tetrachloride, were also found to be destroying the ozone.

Some of the consequences of a depleted ozone layer include increased skin cancers and cataracts, reduced agricultural production, changes in productivity and biodiversity of terrestrial and aquatic ecosystems that sustain life, and increased degradation or breakdown of many materials used outdoors.

In the years that followed this discovery by the scientists, Mario Molina, Sherwood Rowland and Paul Crutzen, the Montreal Protocol gathered countries together to collectively work towards

protecting the ozone layer. Along the way, research has uncovered many complex interactions between ozone dynamics and UV radiation, and climate, that are continuing to affect the environment. Can the damage be repaired? Is climate geoengineering part of the solution?

The Montreal Protocol has prevented catastrophic damage in what has been termed 'the world avoided.' However, because of the complex interactions, the issues around some existing ozone depleting substances and their substitutes, and the contribution of an increasingly warming climate, many challenges remain.

This lecture will explore some of the 'world avoided' outcomes that could have occurred, and illustrate how the emerging connections between ozone dynamics and UV radiation, together with climate change are altering the environment in many inter-linked and intricate ways. The second lecture will look at some of the negative and positive effects these changes are having on human health, agricultural and natural ecosystems as well as projections into the future.

Suggested reading

Ajavon, A-L., Bornman, J.F., Maranion, B., Paul, N.D., Pizano, M., Newman, P.A., Pyle, J.A., Ravishankara, A.R. & Woodcock, A. 2015. Synthesis of the 2014 Reports of the Scientific, Environmental Effects, and Technology and Economic Assessment Panels of the Montreal Protocol. http://ozone.unep.org/en/Assessment_Panels/SynthesisReport2014.pdf

Velders, G.J., Ravishankara, A.R., Miller, M.K., Molina, M.J., Alcamo, J., Daniel, J.S., Fahey, D.W., Montzka, S.A. and Reimann, S., 2012. Preserving Montreal Protocol climate benefits by limiting HFCs. *Science*, *335* (6071), pp.922-923.

Williamson, C., Zepp, R., Lucas, R., Madronich, S., Austin, A.R., Ballaré, C.L., Norval, M., Sulzberger, B., Bais, A., McKenzie, R., Robinson, S., Häder, D-P., Paul, N.D., Bornman, J.F. 2014. Solar ultraviolet radiation in a changing climate. Nature Climate Change 4, 434–441.

Monday after lunch, *topic, plenary*

Environmental photobiology (Trotta)

1. DEFINITION OF PHOTOBIOLOGY (LIFE UNDER THE SUN)
2. BRIEF INTRODUCTION IN A PLAUSIBLE BREAKDOWN OF TOPICS INCLUDED UNDER THE TERM PHOTOBIOLOGY
   - 2.1. The photobiology daisy
   - 2.2. Emerging topics
3. 3. FOCUS ON ENVIRONMENTAL PHOTOBIOLOGY
   - 3.1. Light and planet energy
   - 3.2. Terrestrial photosynthetic systems
   - 3.3. Aquatic photosynthetic systems
4. LIGHT DOMINATED ECOSYSTEMS
5. CLIMATE ISSUES INCLUDING OZONE CHEMISTRY
6. SUMMARY

Light dosimetry in biological tissues (Wagnieres)

Course description:
The main objective of this course is to convey a broad introduction to the principles governing the propagation and dosimetry of light, as well as its interactions with biological tissues.
This course will provide an excellent background to enable the communication and interaction between the students and industrial/laboratory specialists, as well as with medical or clinical partners, thanks to a good understanding of the vocabulary, principles and instruments used in these fields.
Key concepts will be addressed during the plenary symposium, whereas a special symposium will be dedicated to advanced concepts in the field of light dosimetry in biological tissues.

Plenary symposium:
The fundamental optical parameters characterizing biological tissues will be defined and described following a brief introduction in the field of radiometry and photometry. A presentation of the methods used to measure these parameters will be followed by an introduction to the photophysical processes involved in photomedicine and a review of the different types of laser-tissue interactions.
Following a brief presentation of the approaches used to predict, model and measure the light distribution in biological tissue, we will describe how to determine the light dose for different types of light-based treatments.
The instrumentation and techniques used to illuminate or to measure the light in biological tissues will be described. Finally, selected illustrative applications will be presented.

In this session:
1. Introduction (GW; general)
2. Radiometry/photometry (GW; general)

References:
I. Bigio and S. Fantini, "Quantitative Biomedical Optics", (Cambridge Uni. Press, 2016).

Chapter 1 in the PHD thesis from Tomas Svensson and Chapter 1&2 from the PhD thesis of Johan Axelsson. The theses can be found at the following addresses:
http://www.atomic.physics.lu.se/biophotonics/publications/phd_theses/
http://www.optics.arizona.edu/Palmer/rpfaq/rpfaq.htm

A.J. Welch & M.J.C. van Gemert, "Optical-Thermal Response of Laser Irradiated Tissue ", (2nd Ed., Springer, 2011).

V. Tuchin, "Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis", volume PM166, SPIE Press, 2nd Edition, 2007.

J. Sandell and T. Zhu, "A review of in-vivo optical properties of human tissues and its impact on PDT", J Biophotonics", 4(11-12), pp 773–787, 2011.

Monday after lunch, *topic, special:*

Environmental photobiology (Bornman, Trotta)

The interplay of UV radiation, climate events and ozone dynamics: implications for human health, agricultural and natural ecosystems

Janet F. Bornman, Chair Agri-Food Security, Curtin University, Western Australia
(janet.bornman@curtin.edu.au)

Changes in ozone depletion and recovery, consequent altered levels of UV-B radiation, and interactive effects of climate are posing many unknowns for life on Earth. Although there has always been climate change, the current frequency and intensity of extreme climate events are increasing, resulting in more and extended drought periods, floods, and large temperature fluctuations in many parts of the world.

These changes are leading to a wide range of consequences for plants and animals, among them shifts in seasons, decreased water availability, spreading of vector-borne diseases into new areas, altered insect and pathogen incidence, and changes in species and their distribution. Apart from the direct UV-screening by the ozone layer, exposure to UV radiation is changing because of factors other than ozone, such as changes in climate (clouds, increased aridity, erosion, ice melting), land-use and agricultural practices, deforestation, pollution, and in the case of humans, factors such as lifestyle choices are leading to either increased or decreased UV exposure.

UV radiation can have unfavourable and beneficial effects on plants and human health. For humans, high levels of solar irradiation may cause cataract of the eye and other eye diseases; different types of skin cancers, including malignant melanoma and non-melanoma cancers. UV exposure also suppresses the immune system with both adverse and beneficial outcomes. On the other hand, insufficient exposure to solar radiation leads to a deficiency in vitamin D, since UV irradiation of the skin is the main source of the vitamin. A lack of vitamin D has been implicated in bone disease, rickets and some internal cancers.

The interplay of UV radiation, climate and ozone dynamics on plants is reflected in their separate and combined effects in a number of ways. For example, the UV-B component (280-315 nm) has regulatory roles during plant development that are modified by co-occurring climate factors such as water availability and temperature. This can result in differences in plant hardiness, plant resistance to herbivores and pathogens, and even improved quality of agricultural crops. Solar radiation also increases the decomposition or breakdown of plant litter especially in arid and semi-arid ecosystems, releasing nutrients and carbon, and thus may contribute to global warming. In the Southern Hemisphere, ozone depletion is modifying climate through effects on rainfall and wind, which in turn have consequences for plant growth and ecosystems.

In addition to discussing some of the effects from the interplay of UV radiation, climate and ozone dynamics on human health, ecosystems and food production, the lecture will also briefly address positive spin-offs from adapting to climatic risk and UV radiation.

Suggested reading

Thomas, P., Swaminathan, A. and Lucas, R.M., 2012. Climate change and health with an emphasis on interactions with ultraviolet radiation: a review. *Global Change Biology*, *18*(8), pp. 2392-2405.

Wargent, J.J. and Jordan, B.R., 2013. From ozone depletion to agriculture: understanding the role of UV radiation in sustainable crop production. *New Phytologist*, *197*(4), pp.1058-1076.

Environmental photobiology (Trotta)

1. ARTIFICIAL PHOTOSYNTHESIS
   1.1. Energy Conversion
   1.2. Photocatalysis
   1.3. Biosensing
2. Hybrid Systems
3. Biotechnological Application of photosynthetic organisms

Photophysics and photochemistry (Nonell)

Measurement, simulation, and analysis of spectroscopic data

Solar irradiance data and the UV Index. Absorption spectra of the aminoacids and nucleobases. Absorption spectra of UV photoprotectors. Absorption spectra of endogenous photosensitisers. Assessing donor-acceptor pairs for Förster resonance energy transfer. Analysis of fluorescence decay data. The singlet oxygen simulator.

Please make sure you have the PhotochemCAD software installed on your computer at the beginning of the course (available at www.photochemCAD.com)

Bibliography

J. M. Dixon, M. Taniguchi, J. S. Lindsey, PhotochemCAD 2. A Refined Program with Accompanying Spectral Databases for Photochemical Calculations," Photochem. Photobiol. 2005, 81, 212-213.

<u>Tuesday morning</u>, *Topic, plenary:*

Basic photophysics and photochemistry (Nonell)

Energy transfer
Bimolecular decay processes. Quenching and the Stern-Volmer relationship. Energy transfer: concepts and mechanisms. FRET and its applications. Singlet oxygen: production, characterisation and detection.

Electron transfer
Thermodynamic and kinetic aspects. Charge-transfer derived Reactive Oxygen Species (ROS). Time-resolved absorption techniques: Detection of radicals and radical ions.

Bibliography

G. Cox. *Optical Imaging techniques in Cell Biology*, CRC, Boca Raton, 2007

Peter Klán and Jakob Wirz, *Photochemistry of Organic Compounds*, John Wiley & Sons 2009

Brian Wardle, Principles and Applications of Photochemistry, John Wiley & Sons 2009
N. J. Turro, V. Ramamurthy, J. C. Scaiano, *Modern Molecular Photochemistry of Organic Molecules*, University Science Books; 2009.

Lars Olof Björn (Ed.), Photobiology, *The Science of Life and Light*, Springer, 2008

Handbook of Photochemistry, Third Edition (Hardcover)

Marco Montalti, Alberto Credi, Luca Prodi, M. Teresa Gandolfi, Handbook of photochemistry 3rd Edition, Marcel Dekker, 2006

Photobiology Sciences Online, http://www.photobiology.info/

Light dosimetry in biological tissues (Wagnieres, Anderson-Engels)

Continuation from Tuesday after lunch
In this session:

9:45 – 10:30 Optical parameters characterizing biological tissues
11:00 – 11:45 Methods to measure the tissue optical parameters
Introduction to the photophysical processes involved in Photomedicine
11:45 – 12:30 Light-tissue interactions (SAE; Plenary)
Prediction and measurement of the light dose

Tuesday after lunch, *topic, plenary:*

Photosensory biology (Musio and Checcucci)

PHOTOSENSORY BIOLOGY IN ANEURAL ORGANISMS (Giovanni Checcucci)

(giovanni.checcucci@ nano.cnr.it)

Gathering information on the surrounding environment is of vital importance for any living organism. Therefore also aneural organisms (prokaryotic and eukaryotic unicellular microorganisms, fungi, plants) have developed ways of perceiving and reacting to the luminous stimulus. The reaction in microorganisms is usually a change in movement pattern, that mainly evolved because of the necessity of optimizing photochemical energy production or avoiding photoinduced damage, but that may also be correlated to the presence of preys (e.g. photosynthetic algae), predators or favour the possibility of reproducing. In sessile organisms responses to light are even more important and allow the individual to react to changes in light quality (tip elongation), quantity (leaves orientation, chloroplasts movement), duration (flowering) and direction (phototropism).

This lecture will mainly deal with the basic principles of photoperception and photoreaction, analysing the structure and function of known photoreceptors, the molecular pathways that transduce the light stimulus to a chemical signal, and how this affects and modulates the effector.

REFERENCES

www.photobiology.info - Web site entirely devoted to photobiology. Of particular interest for photosensory biology of aneural organisms are the modules, "Photomorphogenesis" and "Photomovement".

CRC Handbook of Organic Photochemistry and Photobiology, Third Edition (Griesbeck A., Oelgemöller M., Ghetti F., eds), CRC Press. - Several chapters of the photobiology section deal with photosensory biology.

Möglich A., Yang X., Ayers R.A., Moffat K. (2010) Structure and function of plant photoreceptors. Annual Review of Plant Biology, 61: 1-27

Sgarbossa A., Checcucci G., Lenci F. (2002) Photoreception and photomovements of microorganisms. Photochemical and Photobiological Sciences, 1: 459-467.

Phototransduction Motifs in Metazoa: From Non-Visual Photoreception to Image-Forming Vision

Carlo Musio

CNR Istituto di Biofisica, c/o Fondazione Bruno Kessler, 38123 Trento, Italy [carlo.musio@cnr.it]

Photoreception is the first key step in seeing (*i.e.* the perception of color, shape and motion). It takes place in photoreceptor cells capable to sense directly ambient light. Visual pigments are integral membrane proteins of photoreceptor cells, which absorb photon energy and finally convert it into an electrical signal toward the central nervous system. Photoreception is phylogenetically one of the oldest sensory systems due to the remarkable ubiquity, in all animal *phyla*, of morphological, functional and molecular systems (from simple invertebrate light-sensitive cells to more complex vertebrate eyes) that respond to environmental luminous stimuli. Basically, although in a frame of different structure-function relationship, in vertebrate and invertebrate visual cells photoreception starts with the photoisomerization of the retinal cromophore of the photopigment, usually an opsin-

based pigment. This process triggers the binding of the opsin with a G-protein which leads an enzymatic visual cascade culminating in the production of a second messenger. The latter gates light-sensitive ion channels in order to modulate and shape the electric signal toward the nervous system. In addition to conventional eyed-structures, vertebrates and invertebrates have supplementary non-visual photoreceptor (NVP) systems for non-image forming function. Photic information mediated by NVP integrates visual activity and is involved in temporal (time-of-day) and behavioral physiology of the animal (*e.g.*, photoperiodism, photoentrainment of circadian rhythms). NVP cells, formerly named extraretinal or extraocular photoreceptors, are currently termed as non-visual (non-image-forming) photosensitive cells in invertebrates and non-rod non-cone photoreceptors in vertebrates (after the discovery of photosensitive retinal ganglion cells ipRGCs). NVP cells are mainly located within nervous system districts and share with retinal photoreceptors common evolutionary origin and light-sensing modalities: above all the same superfamily of opsin-based photopigment although with variations in structural motifs and related phototransductive elements. The searching for novel opsins (e.g., melanopsin) supplying non-image-forming photoreception is a new challenging field in photosensory biology and in vision research. Surprisingly, these pigments have been identified in cells beyond the retinal photoreceptors in several vertebrates and in extraretinal tissues of invertebrates. Recently, genetically-engineered visual (rhodopsin), non-visual (melanopsin), and microbial opsins (e.g., channelrhodopsins), have been successfully proved as suitable optogenetic tools to control the physiology and the behavior of cells and organisms. This lecture will survey similarities and differences among visual and non-visual systems in both vertebrates and invertebrates, focusing on the role of opsins and how almost similar structural and functional mechanisms could serve different physiological tasks. Recent insights on optogenetic control by opsin-based photopigments will be also presented.

SELECTED READINGS

Fain GL, R Hardie, SB Laughlin (2010) Phototransduction and the evolution of photoreceptors. *Current Biology* 20 :R114-24 [Open Access: http://www.sciencedirect.com/science/article/pii/S0960982209021253]

Koyanagi M, Terakita A. (2014) Diversity of animal opsin-based pigments and their optogenetic potential. *Biochim Biophys Acta* 1837(5):710-6 [Open Access: http://www.sciencedirect.com/science/article/pii/S000527281300159X]

Musio C & S Santillo (2012) Nonvisual photosensitivity and circadian vision. In: *CRC Handb. Organic Photochem. Photobiol.*, 3rd ed (Griesbeck A, Oelgemöller M, Ghetti F, eds), CRC Press, pp. 1195–1210. [Open Access: https://www.researchgate.net/profile/Carlo_Musio/contributions]

Santillo S, P Orlando, L De Petrocellis, L Cristino, V Guglielmotti & C Musio (2006) Evolving visual pigments: hints from the opsin-based proteins in a phylogenetically old eyeless invertebrate. *BioSystems* 86:3-17. [Open Access: https://www.researchgate.net/profile/Carlo_Musio/contributions]

Díaz NM, LP Morera, ME Guido ME (2016) Melanopsin and the non-visual photochemistry in the inner retina of vertebrates. *Photochem Photobiol.* 92(1):29-44 [Full-Text Online: http://onlinelibrary.wiley.com/doi/10.1111/php.12545/abstract]

Terakita A (2005) The opsins. *Genome Biol.* 6:213 http://genomebiology.com/2005/6/3/213

Tuesday after lunch, *topic, special*:

Light dosimetry in biological tissues (Andersson-Engels)

Course description:
The main objective of this course is to convey a broad introduction to the principles governing the propagation and dosimetry of light, as well as its interactions with biological tissues.
This course will provide an excellent background to enable the communication and interaction between the students and industrial/laboratory specialists, as well as with medical or clinical partners, thanks to a good understanding of the vocabulary, principles and instruments used in these fields.
Key concepts will be addressed during the basic symposium, whereas a special symposium will be dedicated to advanced concepts in the field of light dosimetry in biological tissues.

Special symposium:
This symposium will be dedicated to the detailed presentation of the most important models used to: 1) describe the propagation of light in biological tissues, and 2) to derive the geometric distribution of the light dose. Finally, direct and inverse problems in light dosimetry will be presented.
References:
I. Bigio and S. Fantini, "Quantitative Biomedical Optics", (Cambridge Uni. Press, 2016).

Chapter 1 in the PHD thesis from Tomas Svensson and Chapter 1&2 from the PhD thesis of Johan Axelsson. The theses can be found at the following addresses:

http://www.atomic.physics.lu.se/biophotonics/publications/phd_theses/

http://www.optics.arizona.edu/Palmer/rpfaq/rpfaq.htm

A.J. Welch & M.J.C. van Gemert, "Optical-Thermal Response of Laser Irradiated Tissue ", (2nd Ed., Springer, 2011).

V. Tuchin, "Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis", volume PM166, SPIE Press, 2nd Edition, 2007.

J. Sandell and T. Zhu, "A review of in-vivo optical properties of human tissues and its impact on PDT", J Biophotonics", 4(11-12), pp 773–787, 2011.

Photophysics and photochemistry: Measurement, simulation, and analysis of spectroscopic data (Nonell).

Solar irradiance data and the UV Index. Absorption spectra of the aminoacids and nucleobases. Absorption spectra of UV photoprotectors. Absorption spectra of endogenous photosensitisers. Assessing donor-acceptor pairs for Förster resonance energy transfer. Analysis of fluorescence decay data. The singlet oxygen simulator.
Please make sure you have the PhotochemCAD software installed on your computer at the beginning of the course (available at www.photochemCAD.com)

Bibliography

J. M. Dixon, M. Taniguchi, J. S. Lindsey, PhotochemCAD 2. A Refined Program with Accompanying Spectral Databases for Photochemical Calculations," Photochem. Photobiol. 2005, 81, 212-213.

<u>Wednesday morning</u>, *topic, plenary:*

Photodynamic medicine: From basics to practice (Berg, Piette and Faustino)

Basic PDT and use in cancer detection and treatment (Kristian Berg)

Photodynamic therapy (PDT) is a clinically approved, minimally invasive therapeutic procedure that can exert a selective cytotoxic activity toward malignant cells. The procedure involves administration of a photosensitizing agent followed by irradiation at a wavelength corresponding to an absorbance band of the sensitizer. In the presence of oxygen, a series
of events lead to direct tumor cell death, damage to the microvasculature, and induction of a local inflammatory reaction. This lecture will present the basic mechanisms involved in PDT and fluorescence diagnosis (PD) such as formation of reactive oxygen species, cellular uptake mechanisms and intracellular localization of the photosensitizers, cellular formation of photosensitizers, pharmacokinetics of photosensitizers, the impact of light penetration and light sources on therapeutic effects and some information on the clinical use of PDT and PD.

Berg et al. (2005) Porphyrin-related photosensitizers for cancer imaging and therapeutic applications. *J Microscopy* 218(Pt 2):133-47.
Agostinis et al. (2011) Photodynamic Therapy Of Cancer: An Update CA: A Cancer Journal for Clinicians. 61(4):250-81.
http://www.photobiology.info/Kessel.html

Overview of the molecular and cellular mechanisms of the anti-cancer effects of PDT.

Jacques Piette
Laboratory of Virology & Immunology, GIGA-Research, University of Liège, B-4000 Liège, Belgium.
e-mail: jpiette@ulg.ac.be

Photodynamic therapy (PDT) is an important modality in the treatment of solid tumors. It is based on the use of a visible light absorbing compound, the photosensitizer, a red-light emission device (lamp or laser) and molecular oxygen. This leads to the generation of singlet oxygen inside the tumor cells. The singlet oxygen production can be localized at various cellular places depending on the photosensitizer which is used. Several authors have unambiguously shown that singlet oxygen is the main cytotoxic reactive oxygen species in PDT. The mechanisms of cytotoxicity are now rather well-described and mostly depend on two main pathways: apoptosis and necrosis. Several photosensitizers are also known to induce autophagy which is in most cases a pro-survival mechanism. However, in several conditions, autophagy can also be part of the observed cytotoxicity mediated by PDT.
PDT can also stimulate an immune response and tumor cells can be effectively destroyed by the immune system. The link between cell death caused by PDT and the exposure of danger associated

molecular patterns (DAMPs) in now rather well defined, and it is established that an efficient tumor eradication rely on a strong immune response initiated by DAMPs exposure on tumor cells. PDT based on photosensitizers located in the endoplasmic reticulum turns out to generate an important immune response and applications are now in progress to use PDT as a modality to vaccinate cancer patients.

This lecture will provide to students an overview of (i) the cell death mechanisms and (ii) the immune response elicited by PDT.

References:

1. D. Kessel (2015) PPS 14, 1397
2. A.D. Garg et al. (2015) PPS 14, 1410.
3. 3. A.D. Garg & P.A. Agostinis (2014) PPS 13, 474

Antimicrobial photodynamic therapy (PDT) (Amparo Faustino)

Photodynamic therapy (PDT) is emerging as a promising and efficient alternative treatment for microbial infections, a problem which is presently exasperated by the increasingly widespread diffusion of antibiotic-resistant microbial strains. Studies on the relationship between the chemical structure of photosensitizing agents and their phototoxicity against microbial pathogens led to identification of a selected number of compounds with optimal cytocidal effects. In particular, the use of red light-absorbing photosensitizers as photodynamic antimicrobial agents is characterized by various favourable features, including: (a) the broad spectrum of antimicrobial action of selected phenothiazines, porphyrins, and phthalocyanines, which promote the photosensitized inactivation of Gram(+) and Gram(-) bacteria, fungi, mycoplasmas, and parasitic protozoa in both vegetative and cystic stages, by using one phototherapeutic protocol and mild irradiation conditions; (b) porphyrins/phthalocyanines display no appreciable toxicity in the dark of photochemically active doses; (c) microbial cell death is primarily a consequence of membrane photodamage through a typically multi-target process, which minimizes the risk of both the onset of mutagenic processes and the selection of photoresistant cells; (d) such photosensitizers act with essentially identical efficiency against both wild and antibiotic resistant strains, whereas no selection of photoresistant microbial pathogens has been observed; (e) a combination between antibiotic-based and photodynamic therapy is possible. The primary interaction of the photosensitizer takes place with the outer wall that surrounds most microbial cells. This wall is characterized by broadly different levels of complexity and three-dimensional architecture, as well as of permeability to external molecules. A typical example of PS-sensitized photoinactivation of methicillin-resistant *Staphylococcus aureus* (MRSA) is provided. MRSA has become a predominant infective agent even in nosocomial environments due to its ability to develop high levels of resistance to several classes of antibiotics through different pathways, including mutation, conjugation, transduction or transformation. PDT appears to represent an efficacious alternative modality for the treatment of localized microbial infections through the *in situ* application of the photosensitizer followed by irradiation of the photosensitizer-loaded infected area. Proposed clinical fields of interest of antimicrobial PDT include the treatment of chronic ulcers, infected burns, acne vulgaris, and a variety of oral infections, such as oral candidiasis and periodontitis.

Selected readings

Jori G., Camerin M., Soncin M., Guidolin L., Coppellotti O. (2011). Antimiocrobial photodynamic therapy: basic principles. In “Photodynamic inactivation of microbial pathogens: medical and environmental applications” (Michael R. Hamblin & Giulio Jori Editors). Comprehensive Series in Photochemistry and Photobiology, Volume 11, 1- 18. RSC Publishing, Cambridge, UK.

Alves E., Faustino M.A.F., Neves M.G.P.M.S., Cunha A., Tomé J.P.C, Almeida A. (2014). An insight on bacterial cellular targets of photodynamic inactivation. *Future Medicinal Chemistry*, **6** (2) 141-164.

Alves E., Faustino M.A.F., Neves M.G.P.M.S., Cunha A., Nadais H., Almeida A. (2015). Potential applications of porphyrins in photodynamic inactivation beyond the medical scope. *J. Photochem Photobiol. C: Photochem. Rev.*, **22**, 34-57.

Almeida A., Faustino M.A.F., Tomé J.P.C. (2015). Photodynamic inactivation of bacteria: finding the effective targets. *Future Medicinal Chemistry*, **7** (10),1221-1224.

Almeida J., Tomé J.P.C., Neves M.G.P.M.S., Tomé A.C., Cavaleiro J.A.S., Cunha A., Costa L., Faustino M.A.F., Almeida A. (2014) Photodynamic inactivation of multidrug-resistant bacteria in hospital wastewaters: influence of residual antibiotics. *Photochem. Photobiol. Sci.*, *13*, 626-633

Pereira M.A., Faustino M.A.F., Tomé J.P.C., Neves M.G.P.M.S., Tomé A.C., Cavaleiro J.A.S., Cunha A., Almeida A. (2014) Influence of external bacterial structures in the efficiency of photodynamic inactivation by a cationic porphyrin. *Photochem. Photobiol. Sci.*, **13**, 680-690.

Wednesday lunch: Walking tour to Novacella

Wednesday after lunch: Poster session

<u>Thursday morning</u>, *topic, plenary:*

Photomedicine (Trautinger and Miolo)

Basic photodermatology (Trautinger)

Abstract: Photodermatology deals with the clinical consequences of the interaction of ultraviolet radiation with the various molecular and cellular components of human skin. These responses can be either physiologic (e.g. Vitamin D production, tanning) or lead to adverse reactions and disease (e.g. sunburn, polymorphic light reaction, and other photosensitivity diseases). Furthermore, sunlight and ultraviolet radiation from specific artificial sources (UVB, narrow-band UVB, UVA1) alone or in combination with photosensitizing drugs (psoralen-photochemotherapy) can be therapeutically employed for the treatment of a wide range of skin and other diseases. Finally, photodermatology also includes photoprotection through avoidance, clothing, and sunscreens with the main aim to prevent photoaging and photocarcinogenesis.
The lecture will provide basic knowledge about these major areas in photodermatology.

Readings:
Effects of ultraviolet radiation, visible light, and infrared radiation on erythema and pigmentation: a review
Lindsay R. Sklar, Fahad Almutawa, Henry W. Lim and Iltefat Hamzavi, Photochem. Photobiol. Sci., 2013, 12, 54-64
DOI: 10.1039/C2PP25152C

Photoreactivity and phototoxicity of drugs (Miolo)

The lecture will give insights on the mechanisms of some drugs able to interact with light giving positive (therapeutic) or negative (adverse effect) biological effects in humans.
The photosensitizing properties, *i.e.* phototoxic-photoallergic effects of some class of topical or systemic drugs, as a consequence of patient sunlight exposure, will be presented. The photoactive chemical can be the parent drug or an excipient in a drug formulation, or it can be a metabolite, an impurity, or a degradant able to absorb UVB, UVA or visible radiation (290-700 nm). Some drugs can also induce subthreshold photoeffects (e.g., DNA damage) that are not visible to patients and pose a long-term risk for adverse skin effects with photocarcinogenic potential.
Photosafety evaluation of topically and systemically administered drug products and photostability measurements are indeed required in the pharmaceutical industry to predict adverse effects of drugs in the presence of light and to characterize the effects of light on the degradation and efficacy of their products.
Some *in vitro* tests useful both to predict the photosensitizing activity of the compounds (Photosafety Evaluation of Pharmaceuticals) and to elucidate the mechanism underlying drug phototoxicity/photoallergy will be described. These include photocytotoxicity assays on specific cell lines, photoreactivity experiments (photomodification, *i.e.* phototoxidation, photoaddition) towards target substrates (DNA; proteins and amino acids; lipids; membranes) and the evaluation of the reactive oxygen species involved.

Examples of photodegradation of some drugs and the role of their photodegradation products on the photosensitizing side effects, as well as the loss of therapeutic activity, will be also reported. Attention will also be dedicated to some Photostability Testing of new drug substances and products.
In the case of phototherapy, the main mechanisms of psoralens in combination with UVA light (PUVA therapy) and the experimental methods to reveal how psoralens are able to interact with their biological targets under UVA irradiation will be also discussed.

References:

Aaron M. Drucker and Cheryl F. Rosen, Drug-Induced Photosensitivity, Culprit Drugs, Management and Prevention, Drug Saf 2011; 34 (10): 821-837

Guidance for Industry, Photosafety Testing, U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER) May 2003, Pharmacology and Toxicology

Thursday after lunch, *topic, special*:

PDT (Piette, Stepp and Wolf)

Cell death signaling induced by Photodynamic Therapy in cancer cells.

Jacques Piette.
Laboratory of Virology & Immunology, GIGA-Research, University of Liège, B-4000 Liège, Belgium.
E-mail: jpiette@ulg.ac.be

Singlet oxygen which is generate inside tumor cells by photodynamic therapy (PDT) has been shown by many authors as the main reactive oxygen species responsible for the direct cytotoxic effect. How singlet oxygen generated by PDT is causing cell death is the main topic of the lecture. Two main cell death modalities have been well-characterized: apoptosis and necrosis.
Apoptosis induced by PDT has been shown to cause cell death via two main intrinsic and extrinsic pathways. The signaling intermediates in these two pathways have been well-characterized in cancer cell lines but evidences that apoptosis can generate cell death in vivo are much less abundant. By opposition, most the cell death seen in tumors in vivo is occurring via necrosis. Recently, necrosis has been demonstrated as being well controlled and the signaling cascades leading to the regulated necrosis (called necroptosis) are being uncovered.
Finally, this lecture will provide information on the induction of autophagy by PDT. Autophagy is fundamentally a cytoprotective process supporting cell survival because autophagy blocks the induction of apoptosis. However, in several situations, autophagy or autophagy-related proteins may help in inducing apoptosis or necrosis, leading to autophagic cell death.

References

1. J. Piette (2015) PPS 14, 1510.
2. I. Coupienne et al. (2011) PPS 10, 1868.

PDT - clinical implementation (Herbert Stepp)

The transfer of the photodynamic principle into clinical practice is hampered by a couple of issues to be carefully considered. In vitro, drug concentration and light dose can be precisely measured and controlled. This is not the case in vivo. Light distribution over the surface and into the depth of the target tissue is not easy to keep under precise control. Light absorption and scattering determine the light intensity impinging on sensitized cells. The photosensitizer on the other hand is delivered topically or systemically, but the final concentration in the target cells is difficult to measure and may be rather heterogeneously distributed. With all these uncertainties, it should be guaranteed that while the target tissue is effectively treated, side effects on adjacent normal tissue remain tolerable. We will discuss the most important of these aspects and illustrate suggested solutions on practical examples.
There are also biological effects, which determine the clinical outcome: tissue optical properties, tissue oxygenation, tissue vascularization, stimulation of immune response, role of cancer stem cells or drug accumulation enhancement to name a few. Different clinical indications require different

property profiles of the photosensitizer. We will strive through experimental and established clinical applications of PDT and speculate on whether and how improvements may be achieved, if these parameters could be properly assessed and optimized.

More key words: light dosimetry, photobleaching, interstitial irradiation, fractionated irradiation, oxygen diffusion, light sources, light applicators, immune checkpoint inhibitors, membrane transporters.

Literature:

1. Wilson BC, Patterson MS. The physics, biophysics and technology of photodynamic therapy. Physics in medicine and biology. 2008 May 7;53(9):R61-109. PubMed PMID: 18401068.
2. Jacques SL. How tissue optics affect dosimetry of photodynamic therapy. J Biomed Opt. 2010 Sep-Oct;15(5):051608. PubMed PMID: 21054082. Pubmed Central PMCID: 2973987. Epub 2010/11/09. eng.
3. Huang Z. A review of progress in clinical photodynamic therapy. Technol Cancer Res Treat. 2005 Jun;4(3):283-93. PubMed PMID: 15896084. Pubmed Central PMCID: 1317568.
4. Bown SG. Photodynamic therapy for photochemists. Philosophical transactions Series A, Mathematical, physical, and engineering sciences. 2013 Jul 28;371(1995):20120371. PubMed PMID: 23776302.
5. Nokes B, Apel M, Jones C, Brown G, Lang JE. Aminolevulinic acid (ALA): photodynamic detection and potential therapeutic applications. The Journal of surgical research. 2013 May;181(2):262-71. PubMed PMID: 23510551.

Photodynamic Therapy in Dermatology (Wolf)

Photodynamic therapy (PDT) has become an important treatment modality in dermatology. In particular, topical PDT with the porphyrin precursor 5-aminolevunic acid (ALA) or methyl aminolaevulinate (MAL) is a standard in the treatment of actinic keratoses, Morbus Bowen (in situ squamous cell carcinoma) and superficial basal cell carcinoma. PDT favorably competes with surgical methods and other non-invasive anti-cancer treatment modalities such as topical 5-fluorouracil [5-FU], diclofenac, imiquimod (TLR-7 agonist), and ingenol mebutate. Topical PDT is known for its favorable cosmetic results in particular compared to surgical methods, including cryosurgery and excisional surgery. The recent introduction of day light PDT has further boostered the use of PDT in dermatology. In comparison to standard PDT day light PDT is associated with much less pain, a drawback of the former modality in particular in the treatment of multiple skin lesions in field-cancerized areas. Nowadays topical PTD is also used more and more in cosmetic dermatology for the treatment of acne, sebaceous gland hyperplasia, and skin rejuvenation.

- The lecture will comprehensively cover the principles of topical PDT and its use in dermatology in the treatment of skin cancer and beyond

- Advantages and disadvantages of PDT compared to other dermatological treatments will be discussed

Recommended Literature:

Morton C A, Szeimies R M, Sidoroff A, Braathen L R. European guidelines for topical photodynamic therapy part 1: treatment delivery and current indications - actinic keratoses, Bowen's disease, basal cell carcinoma. J Eur Acad Dermatol Venereol 2013: 27: 536-544.

Morton C A, Szeimies R M, Sidoroff A, Braathen L R. European guidelines for topical photodynamic therapy part 2: emerging indications--field cancerization, photorejuvenation and inflammatory/infective dermatoses. J Eur Acad Dermatol Venereol 2013: 27: 672-679.

Wiegell S R, Wulf H C, Szeimies R M, et al. Daylight photodynamic therapy for actinic keratosis: an international consensus: International Society for Photodynamic Therapy in Dermatology. J Eur Acad Dermatol Venereol 2012: 26: 673-679.

Karrer S, Kohl E, Feise K, et al. Photodynamic therapy for skin rejuvenation: review and summary of the literature--results of a consensus conference of an expert group for aesthetic photodynamic therapy. J Dtsch Dermatol Ges 2013: 11: 137-148.

Photodynamic Therapy and Immunology (Wolf)

Pre-clinical and clinical studies have demonstrated that PDT is capable of affecting both the innate and adaptive arms of the immune system. Depending on the circumstances and light delivery PDT may be immune suppressive or stimulatory. The immune suppressive effects are a theoretical concern with the regard to skin carcinogenesis though there is no convincing clinical evidence for it. On the other hand, the immune stimulatory properties of PDT may increase its beneficial therapeutic effects. Besides stimulating tumor-specific cytotoxic T-cells capable to destroy distant untreated tumor cells, PDT leads to development of anti-tumor memory immunity that can potentially prevent the recurrence of cancer. The immunological effects of PDT may make the therapy more effective also when used for treatment of bacterial and fungal infections (at presence experimental indications), due to an augmented infiltration of neutrophils into the infected regions that may potentiate the outcome of the treatment.

- The lecture will cover basic (photo) immunology and the effect of PDT on the immune system

Recommended literature:

Reginato E, Lindenmann J, Langner C, et al. Photodynamic therapy downregulates the function of regulatory T cells in patients with esophageal squamous cell carcinoma. Photochem Photobiol Sci 2014: 13: 1281-1289.

Reginato E, Wolf P, Hamblin M R. Immune response after photodynamic therapy increases anti-cancer and anti-bacterial effects. World J Immunol 2014: 4: 1-11.

Photomedicine (Trautinger and Miranda)

Phototherapy: Specific treatment modalities (Trautinger)

The lecture will provide an overview of the various currently used methods of phototherapy and psoralen photochemotherapy. Treatment of psoriasis, atopic dermatitis, cutaneous T-cell lymphomas, vitiligo and other skin diseases will be covered. Special emphasis will be given to

extracorporeal photochemotherapy (photopheresis). Methods of radiation delivery, mechanisms of action, clinical results, and adverse reactions will be discussed.
Photopheresis (extracorporeal photochemotherapy)
Franz Trautinger, Ulrike Just and Robert Knobler, Photochem. Photobiol. Sci., 2013, 12, 22-28
DOI: 10.1039/C2PP25144B

Photooxidative reactions of drugs with biomolecules (Miranda)

- Oxidative damage photosentitized by drugs. Type I and type II mechanisms.
- Drug-mediated photooxidation of lipids. Polyunsaturated fatty acids and cholesterol.
- Photoreactivity of drugs with proteins. Oxidation of amino acid residues.
- Covalent photobinding of drug to proteins. Photoantigen formation and its implications in photoallergy.
- Photooxidative DNA damage mediated by drugs. Reactions at the purine bases and at the deoxyribose units.
- Drugs as triplet sensitizers for the photodimerization of pyrimidine bases in DNA.
- Repair of damaged DNA by photoinduced electron transfer. Drugs with potential photolyase activity.

<u>Friday morning</u>, *topic, plenary:*

UV(from cells to skin tissue) (Douki, Sage, Tyrrell and Rhodes)

Formation and repair of DNA damage induced by solar UV radiation in human cells (Douki)

Induction of damage to cellular DNA is a major deleterious event in cells and skin exposed to solar radiation. Indeed, modification of the chemical structure of DNA may lead to the blocking of vital biological processes such as transcription and replication. These arrests may result in cell death. In case of survival of a damaged cell, errors are likely to occur upon replication of the damaged DNA templates leading to irreversible mutations in the progeny of the cell. These mutagenic events represent the earliest step of carcinogenesis. DNA damage is also implicated in other adverse effects of exposure to solar radiation such as immunosuppression. Fortunately, cells are equipped with a series of DNA repair systems that are able to restore the integrity of the genome in most cases. Evidence has been obtained for a major role played by the ultraviolet part of the solar spectrum and the type of DNA lesions was found to greatly depend on the wavelength of the incident photons. The effects of UVB (280-320 nm) mostly result from a direct absorption of the radiation by DNA, leading to dimerisation of pyrimidine bases. In contrast, DNA lesions induced by UVA (320-400 nm) are mostly mediated by photosensitized oxidation reactions. The purpose of this session is to present the main chemical modifications induced within DNA upon exposure to sunlight and to provide information on their repair.

Solar UV-induced mutagenesis and carcinogenesis (Sage)

Solar UV radiation, UVB (295-320 nm) and UVA (320-400 m), is able to induce a plethora of damage types to biomolecules, including DNA, proteins and lipids, with collateral harmful consequences, such as skin aging and carcinogenesis. Acute cellular responses allow to mitigate the long term adverse effects of UV exposure. In this regard, the maintenance of genome integrity which is essential to minimize heritable mutations and for viability of cells and the health of organisms, is insured by surveillance mechanisms such as DNA repair, cell cycle checkpoints and stress signalling cascades. However, when left unrepaired, DNA damage may lead to mutations. DNA damage and mutations represent early genetic events in photocarcinogenesis process. After excessive exposure, massive cell death (apoptosis) may occur and result in peeling of the skin after a few days. This process will prevent heavily damaged cells from contracting mutations. The transcription factor p53 tumor suppressor protein is a key factor in all this signaling network. It has been found mutated in a majority of skin cancer and the p53 mutation spectrum in such tumors carries a “UV signature”. A UV signature has been observed in a large majority of non-melanoma skin tumours and, recently, through a genome wide analysis, in malignant melanoma as well. The loss of p53 function appears to be an early event in UV carcinogenesis. Other oncogenic pathways are also activated.

Special reading :

J. Cadet, E. Sage et T. Douki (2005).Ultraviolet radiation-mediated damage to cellular DNA. Mutat. Res. 571, 3-17.
de Gruijl FR, van Kranen HJ, Mullenders LH. (2001) UV-induced DNA damage, repair, mutations and oncogenic pathways in skin cancer. J Photochem Photobiol B. Oct;63(1-3):19-27.
Ridley A J, Whiteside J R, McMillan T J and Allison S L (2009) Cellular and sub-cellular responses to UVA in relation to carcinogenesis. International Journal of Radiation Biology 85, 177-195.
Bennett DC (2008) Ultraviolet wavebands and melanoma initiation. Pigment Cell Melanoma Res. 21; 520–524.
Pfeifer G & Besaratinia A (2012) UV wavelength-dependent DNA damage and human non-mélanome and melanoma skin cancer. Photochemical & Photobiological Sciences, 11 (1), 90-97.
Sage E, Girard PM, Francesconi S. (2012) Unravelling UVA-induced mutagenesis. Photochemical & Photobiological Sciences, 11 (1), 74-80.
UV signature mutations.
Brash DE. (2015) Photochem Photobiol. Jan-Feb;91(1):15-26. doi: 10.1111/php.12377. Epub 2014 Nov 28.

Solar UV generation and biological significance of reactive oxygen species (Tyrrell)

Solar UVA as well as UVB radiations cause damage to skin cells and skin tissue. UVA generates distinct types of damage often associated with oxidative stress and mediated by reactive oxygen species (ROS) which are central to the interaction of UVA with biological material . There are many cellular molecules which absorb UVA and generate reactive oxygen species .Singlet oxygen is undoubtedly the major species generated directly by UVA but other species such as superoxide and hydrogen peroxide are also generated and there are many ways in which these species can interconvert or form the highly diffusible oxidant hydrogen peroxide .UVA also leads to the release of free iron and free heme which are pro-oxidant catalysts and further exacerbate this oxidative stress. A major effect of UVA radiation is to oxidise proteins throughout the epidermis and the dermis and cause damage to the extracellular matrix .Lipids are also oxidised by low levels of UVA and this can set off lipid chain oxidation reactions Such processes can generate many lipid messenger molecules which activate other proteins and enzymes and this includes not only enzymes such as oxidases which generate additional ROS but also sustained activation of proteases which can lead to chronic damage including photaging of human skin

Tyrrell, R.M. (1991) UVA (320-380 nm) radiation as an oxidative stress *in* "Oxidative Stress : Oxidants and antioxidants" (ed. H. Sies), pp 57-83, Academic Press, London.

Solar UVR-induced vitamin D synthesis (Rhodes)

Vitamin D synthesis is the best established beneficial effect of solar UVR exposure. The synthesis of vitamin D is initiated in skin following absorption of UVB by the chromophore 7-dehydrocholesterol (7-DHC, pro-vitamin D), resulting in its photochemical conversion to pre-vitamin D. A slower thermal conversion of pre-vitamin D to vitamin D then follows. Further photochemical reactions occur, of pre-vitamin D and vitamin D to inactive metabolites, in which UVA plays a role. Acquisition of vitamin D through cutaneous synthesis is governed by external and personal factors, the latter being both physiological and behavioural.

This talk will focus on the photobiological aspects of vitamin D, comprising both the accepted scientific theory and recent experimental findings. The pathways for cutaneous synthesis and metabolism will be presented and the action spectrum of pre-vitamin D synthesis will be discussed, including the influence of UVA on this UVB-induced beneficial effect. The influence of the predictable factors of latitude, season and time of day on solar zenith angle, and of the atmospheric variables of ozone, cloud and pollution, which determine the amount of ambient UVB available, will be considered, in addition to personal attributes affecting vitamin D production, including skin pigmentation and age. Recent data on the impact of sun exposure behaviour and photo-protective measures on vitamin D synthesis will be discussed, and will include the length of time of exposure and the skin surface area exposed.

Reading:

Rhodes LE and Webb AR. Ultraviolet radiation and vitamin D. In: Handbook of Organic Photochemistry and Photobiology, 3rd Edition. Eds. Griesbeck A, Oelgemoeller M, Ghetti F. CRC Press, 2012, pp1435-1444

Webb AR. Who, what, where and when - influences on cutaneous vitamin D synthesis. Prog Biophys Mol Biol. 2006; 92: 17-25

Further Reading:

Holick MF, MacLaughlin JA, Clark MB, Holick SA, Potts Jr JT, Anderson RR, Blank IH, Parrish JA, Elias P. Photosynthesis of previtamin D3 in human skin and the physiologic consequences. Science 1980; 210: 203-205

Norval M, Bjorn LO, de Gruijl FR. Is the action spectrum for the UV-induced production of previtamin D3 in human skin correct? Photochem Photobiol Sci 2010; 9: 11–17

<u>Friday after lunch</u>, *topic, plenary*:

Photosynthesis (Dall'Osto and Morosinotto)

Photosynthesis (Dall'Osto)

This part of the lecture will instead focus on the regulation of photosynthesis light reactions. In a natural environment, light for photosynthetic organisms represents not only an energy supply but also a source of reactive oxygen species, when light absorbed is in excess. Plants are particularly exposed to oxidative stress because they live in a land environment where illumination is generally stronger and oxygen diffusion is faster with respect to water. To avoid cellular damages different photoprotection mechanisms evolved, allowing plants survival in these conditions. Differences with other photosynthetic organisms living in water will also be reviewed. In particular, we will describe recent findings on the faster among photoprotection mechanisms called NPQ (Non Photochemical Quenching). This is particularly interesting because it allows modulation of light harvesting efficiency within a few seconds without requiring protein synthesis or post-translational modifications. This is a valuable example on how protein conformational changes are able to drastically modulate quantum yield of pigments they are binding.
The relevance of the clarification of regulation of photosynthesis in the exploitation of photosynthetic organisms for biofuels production will be also discussed.

Bibliography for Photosynthesis

Chapter 12 on Photosynthesis from "Biochemistry and Molecular Biology of Plants" by Bob B. Buchanan, Wilhelm Gruissem, and Russell L. Jones. ASPB publications

Nelson N. Photosystems and global effects of oxygenic photosynthesis. Biochim Biophys Acta. 2011 Aug;1807(8):856-63. doi: 10.1016/j.bbabio.2010.10.011. Epub 2010 Oct 16.

Eberhard S, Finazzi G, Wollman FA. The dynamics of photosynthesis. Annu Rev Genet. 2008;42:463-515.

Silvia de Bianchi, Matteo Ballottari, Luca Dall'Osto and Roberto Bassi. Regulation of plant light harvesting by thermal dissipation of excess energy. Biochem Soc. Trans. 2010

Li Z, Wakao S, Fischer BB, Niyogi KK. Sensing and responding to excess light. Annu Rev Plant Biol. 2009;60:239-60. Review.

Niyogi KK, Truong TB. Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. Curr Opin Plant Biol. 2013 Jun;16(3):307-14. doi: 10.1016/j.pbi.2013.03.011

Photosynthesis (Morosinotto)

This part of the lecture will review of the light dependent reactions of oxygenic photosynthesis and how light is absorbed by photosynthetic complexes and converted into photochemical energy. It will also describe protein composition of photosystems, the molecular machineries catalysing the

primary steps of light conversion into the chemical bond energy of organic compounds. In higher plants, algae and cyanobacteria, these steps are operated by two pigment-protein supercomplexes localised in the thylakoids membranes, called Photosystem I (PSI) and II (PSII). Their activity leads to the transport of electrons from the water to a final acceptor with higher potential (NADP+) as well as to a asymmetric protons and charge distribution, which is the motor force for ATP synthesis. The peculiarity of Photosystems with respect to the other chloroplast complexes is their binding of massive amounts of pigmented compounds, chlorophylls and carotenoids.

Photosystems are composed by two moieties: attention will be first dedicated to the core complexes, which are responsible of conversion of light into chemical energy. Later we will describe antenna systems, which are composed by pigment binding proteins responsible of increasing light harvesting capacity. While core complexes are conserved, antenna systems diverged during evolution and differences between different organisms capable of oxygenic photosynthesis (cyanobacteria, algae and plants) will be described. Particular attention will also be dedicated to how proteins of the photosynthetic apparatus are able to modulate pigments biophysical properties.

<u>Saturday morning</u>, *topic, special*:

PDT – preclinical (Giutini, Russel, Berg)

Photosensitizers in Photodynamic Therapy

Francesca Giuntini (Liverpool John Moores University)

Since the early days of photodynamic therapy, much effort has been devoted to the elucidation of the relationships between the molecular structure of a photosensitiser and the outcome of photodynamic treatment. This led to the discovery of numerous photosensitising moieties, whose ability to inactivate cells in response to irradiation has been extensively probed by tuning the physicochemical properties *via* chemical modifications.
In this session, we will survey the different classes of photosensitisers used for the photodynamic inactivation of cells: we will focus on their mechanism of action as PDT agents (e.g., photosensitisation pathway, intracellular localisation, etc.) and examine examples of chemical approaches adopted to improve their efficacy.

Suggested reading:
Castano AP, Demidova TN, Hamblin MR. Mechanisms in photodynamic therapy: part one—photosensitizers, photochemistry and cellular localization. Photodiagnosis Photodyn Ther. 2004;1(4):279-93.
Detty MR, Gibson SL, Wagner SJ. Current Clinical and Preclinical Photosensitizers for Use in Photodynamic Therapy. J Med Chem. 2004;47(16):3897-915.
Yoon I, Li JZ, Shim YK. Advance in Photosensitizers and Light Delivery for Photodynamic Therapy. Clin Endosc. 2013;46(1):7-23.
Bonnett R. Photosensitizers of the porphyrin and phthalocyanine series for photodynamic therapy. Chem Soc Rev. 1995;24(1):19-33.
Josefsen LB, Boyle RW. Photodynamic Therapy and the Development of Metal-Based Photosensitisers. Met-Based Drugs. 2008;2008.
Lovell JF, Liu TWB, Chen J, Zheng G. Activatable Photosensitizers for Imaging and Therapy. Chem Rev. 2010;110(5):2839-57.

Nano-systems for photodynamic therapy

David A. Russell
School of Chemistry, University of East Anglia, Norwich, NR4 7TJ, U.K.
Email: d.russell@uea.ac.uk

One of the areas where nanotechnology is likely to have a profound influence in the development of novel therapies is within medicine. Nanomedicine has now become a discipline where numerous groups Worldwide have focused research activity. This presentation will provide a review of some of the activities within nanomedicine that have been developed for photodynamic therapy (PDT). Nano-systems such quantum dots, silica and metallic nanoparticles and upconverting nanoparticles will all be considered. The development of multifunctional nano-systems where a targeting molecule, such as an antibody, has been added with the photoactive photosensitiser will be

discussed. An assessment will be made of the importance of nanomedicines for the efficient delivery of photosensitiser agents and their therapeutic efficacy.

Suggested reading:

1. Nanoparticles as vehicles for delivery of photodynamic therapy agents, D. Bechet, P. Couleaud, C. Frochot, M-L Viriot, F. Guillemin and M. Barberi-Heyob, *Trends in Biotechnology*, **2008**, *26*, 612-621.
2. Nanoparticles in photodynamic therapy: An emerging paradigm, D. K. Chatterjee, L. S. Fong and Y. Zhang, *Advanced Drug Delivery Reviews*, **2008**, *60*, 1627-1637.
3. Nanoparticles in photodynamic therapy, T. Nann, *Nano Biomed. Eng*., **2011**, *3*, 137-143.
4. Nanoparticles for photodynamic cancer therapy. G. Obaid and D. A. Russell in *Handbook of Photomedicine*, Eds. M. R. Hamblin and Ying-Ying Huang, **2013**, Chapter 32, pp 367-378, CRC Press (Taylor Francis Group), Boca Raton, (ISBN: 978-1-4398-8469-0).
5. Metallic nanoparticles for targeted delivery of photosensitisers for photodynamic therapy. P. García Calavia and D. A. Russell in *Photodynamic Medicine: From bench to clinic*, Eds. H. Kostron and T. Hasan, 2016, Chapter 7, pp 113-135, Royal Society of Chemistry, Cambridge.

Photochemical internalization (PCI) – from photodynamic targeting of lysosomes to clinical utilization of PCI (Berg)

The fluorescing properties of photosensitizers can be used to evaluate their intracellular localization and treatment effects. Some photosensitizers localize intracellularly in endocytic vesicles such as endosomes and lysosomes. The treatment effect of light exposure of photosensitizers localized in these vesicles depends on the structure of the photosensitizer. Some photosensitizers may rupture these vesicles without causing substantial damage to the matrix contents of these vesicles. In these cases the contents of these vesicles, e.g. containing endocytoses therapeutics, may be released into the cytosol in a functionally active form that may exert therapeutic effects. This is the basis for the PCI technology. The basic mechanisms and the clinical utilization of this technology will be presented.

Høgset et al. (2004) Photochemical internalisation in drug and gene delivery. *Adv. Drug Deliv. Rev*. 56(1):95-115.

Selbo et al. (2010) Photochemical internalization provides time and space controlled endo-lysosomal escape of therapeutic molecules. J.Control Release. 148: 2-12.

UV(from cells to skin tissue) (Sage, Tyrrell and Rhodes)

Formation and repair of DNA damage induced by solar UV radiation in human cells (Douki)

Induction of damage to cellular DNA is a major deleterious event in cells and skin exposed to solar radiation. Indeed, modification of the chemical structure of DNA may lead to the blocking of vital biological processes such as transcription and replication. These arrests may result in cell death. In case of survival of a damaged cell, errors are likely to occur upon replication of the damaged DNA templates leading to irreversible mutations in the progeny of the cell. These mutagenic events represent the earliest step of carcinogenesis. DNA damage is also implicated in other adverse effects of exposure to solar radiation such as immunosuppression. Fortunately, cells are equipped with a series of DNA repair systems that are able to restore the integrity of the genome in most cases. Evidence has been obtained for a major role played by the ultraviolet part of the solar spectrum and the type of DNA lesions was found to greatly depend on the wavelength of the incident photons. The effects of UVB (280-320 nm) mostly result from a direct absorption of the radiation by DNA, leading to dimerisation of pyrimidine bases. In contrast, DNA lesions induced by UVA (320-400 nm) are mostly mediated by photosensitized oxidation reactions. The purpose of this session is to present the main chemical modifications induced within DNA upon exposure to sunlight and to provide information on their repair. **In-depth presentation of the topics included in the general part of the program.**

Solar UV-induced DNA damage, repair, mutagenesis and carcinogenesis (Sage)

Solar UV radiation, UVB (295-320 nm) and UVA (320-400 m), is able to induce a plethora of damage types to biomolecules, including DNA, proteins and lipids, with collateral harmful consequences, such as skin aging and carcinogenesis. Acute cellular responses allow to mitigate the long term adverse effects of UV exposure. In this regard, the maintenance of genome integrity which is essential to minimize heritable mutations, for viability of cells and the health of organisms, is insured by surveillance mechanisms such as DNA repair, cell cycle checkpoints and stress signalling cascades. However, when left unrepaired, DNA damage may lead to mutations. DNA damage and mutations represent early genetic events in photocarcinogenesis process. After excessive exposure, massive cell death (apoptosis) may occur and result in peeling of the skin after a few days. This process will prevent heavily damaged cells from contracting mutations. The transcription factor p53 tumor suppressor protein is a key factor in all this signaling network. Its gene has been found mutated in about half of skin tumors and the p53 mutation spectrum in such tumors carries a “UV signature”. A UV signature has been observed in a large majority of non-melanoma skin tumours and, recently, through a genome wide analysis, in malignant melanoma as well. The loss of p53 function appears to be an early event in UV carcinogenesis. Other oncogenic pathways are also activated.

The course will 1- define the mechanisms of DNA damage formation, including chemical aspects, 2- present the repair mechanisms available on UV-induced lesions, 3- explain how a DNA lesions which is a transient modification of DNA, can be transformed into a mutation, an heritable change in DNA, 4- provide information on the molecular and genetic aspect of melanoma and non-

melanoma skin carcinogenesis. **The special symposium will be devoted to some specific aspects of UVA.**

Special reading :
Special symposium
Ridley A J, Whiteside J R, McMillan T J and Allison S L (2009) Cellular and sub-cellular responses to UVA in relation to carcinogenesis. International Journal of Radiation Biology 85, 177-195.
Sage E, Girard PM, Francesconi S. (2012) Unravelling UVA-induced mutagenesis. Photochemical & Photobiological Sciences, 11 (1), 74-80.
Schuch et al (2013) DNA damage as a biological sensor for environmental sunlight. Photochemical & Photobiological Sciences, 12 (8), 1259-1272.

Endogenous and exogenous protection against UV generated oxidative stress (Tyrrell)

Homeostatic maintenance of cellular redox state as well as iron and heme levels requires exquisite control to avoid potential cell and tissue damage. Both oxidative (e.g. UVA) damage to cells and tissue as well as inflammatory responses appear to disturb such homeostatic mechanisms and lead to activation of distinct stress responses such as the activation of the antioxidant and anti-inflammatory enzyme, heme oxygenase 1. While DNA repair is crucial to reversal of UVB damage, constitutive endogenous antioxidants (eg glutathione) and constitutive and inducible antioxidant enzymes are major factors in protecting cells and tissue against UVA damage. Various naturally occurring antioxidants have the potential to protect against solar damage. In addition to the antioxidant vitamins, these include carotenoids which scavenge singlet oxygen and phenolic compounds (eg flavonoids) which have strong free radical scavenging properties (by virtue of favourable reduction potentials) and hydroxyl groups which can chelate iron. Substantial information on protection of human cells and skin by a variety of natural antioxidants has accumulated

Reeve,V. and R.Tyrrell (2007). UVA and inducible protection in "Biophysical and Physiological Effects of Solar Radiation on Human Skin" pp 293-310 (ed.P.Giacomoni) RSC press,UK.
Stahl W and Sies H β-Carotene and other carotenoids in protection from sunlight. Am J Clin Nutr.2012 96 1179S-1184S.publications, Cambridge, UK.

Balancing the benefits and risks of solar UVR exposure (Rhodes)

Solar UVR exposure has the well-established beneficial health effect of cutaneous vitamin D synthesis, but also a range of harmful effects including melanoma and non-melanoma skin cancer, photosensitivity and photo-ageing. However, vitamin D is essential for bone health, with low levels causing rickets and osteomalacia and also showing associations with a range of malignant, immune-mediated and systemic disorders. Low amounts of vitamin D are usually obtained through diet, and different recommended levels of oral intake are set in European countries and the USA. On the other hand, vitamin D is efficiently synthesised following relatively short sunlight exposures containing the requisite UVB.

This talk will examine what represents a "healthy" vitamin D status, and how this may be attained and maintained whilst minimising health risks. The findings of recent interventional and observational research and of modelling studies examining UVR dose-vitamin D relationships in

humans will be evaluated. This includes examination of winter and summer target vitamin D status and strategies to achieve these. Data will also be compared of the relative efficacy of UVR and vitamin D supplements in raising vitamin D status. Current recommendations on sunlight exposure and vitamin D will be reviewed, with examination of their appropriateness in different population groups.

Reading:

Cancer Research UK SunSmart website http://info.cancerresearchuk.org/healthyliving/sunsmart/

Petersen B, Wulf HC, Triguero-Mas M, Philipsen PA, Thieden E, Olsen P, Heydenreich J, Dadvand P, Basagaña X, Liljendahl TS, Harrison GI, Segerbäck D, Schmalwieser AW, Young AR, Nieuwenhuijsen MJ. Sun and ski holidays improve vitamin D status, but are associated with high levels of DNA damage. J Invest Dermatol. 2014; 134: 2806-13.

Further reading:

Farrar MD, Webb AR, Kift R, Durkin MT, Allan D, Herbert A, Berry JL, Rhodes LE. Efficacy of a dose-range of simulated sunlight exposures in raising vitamin D status in South Asian adults: implications for targeted guidance on sun exposure. Am J Clin Nutr. 2013; 97: 1210-1216

Webb AR, Kift R, Berry JL, Rhodes LE. The Vitamin D Debate: Translating Controlled Experiments Into Reality For Human Sun Exposure Times. Photochem Photobiol 2011; 87: 741-745.

Saturday morning, *topic, plenary*:

Microscopy (Viappiani)

Cristiano Viappiani (cristiano.viappiani@unipr.it)

The invention of the microscope in the 17th century marked the beginning of modern natural sciences, disclosing to the eyes of scientists the nature of the inner structure of living beings and their constitutive elementary components. Since then, the optical microscope has been one of the most important tools in life-science. Unlike other imaging methods, such as electron microscopy, optical microscopy techniques generally do not require sample preparation that eventually results in cell death. However, in comparison to electron microscopy, spatial resolution in optical microscopy was held back by a physical restriction as to what size of structures are possible to resolve, the so called Abbe's law. The resolution of a light microscope is in fact limited to about half the wavelength of the light in use, which typically amounts to about 200–350 nanometers.
Recent advances in optical microscopy have demonstrated that this limitation can now be overcome using fluorescent molecules and specialized illumination schemes and geometry. In this lecture are described the physical basis of the modern fluorescence microscopy methods for life science. Methods based on structured illumination and stochastic single molecules activation are discussed, emphasizing the role of molecular photophysical and photochemical processes that are exploited to reach subdiffraction resolution.

Further readings

Patterson, G.; Davidson, M.; Manley, S.; Lippincott-Schwartz, J. Superresolution imaging using single-molecule localization *Annual Review of Physical Chemistry* **2010**, *61*, 345-367.

Eggeling, C.; Willig, K.I.; Sahl, S.J. and Hell, S.W. Lens-based fluorescence nanoscopy *Quarterly Reviews of Biophysics* **2015***, 48*, 178–243.