Real-time Eu/Di-Stress Monitoring

Bicommunication and UwPE detection

Contributed by
Pierre MADL
University of Salzburg
Dep. of Physics & Biophysics

Pierre MADL (PhD, MSc, EE)
University of Salzburg
Dep. Of Physics & Biophysics
Hellbrunnerstr. 34
A-5020 Salzburg
pierre.madl@sbg.ac.at
URL: biophysics.scb.ac.at/talk/Why_QFT_matters.pdf

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www.autreef.com

Ultraweak Photon Emission (Photon Flux)

…. and how to measure in real-time living matter under stress ….

What is UwPE and **where** does it originate from and **why** does it matter?

Why:
i) involved in Bio-Communication (exchange of information, that does not primarily rely on energy),
i) is an intrinsic feature of all **Life-forms** across kingdoms (most Eubacteria Protists, Fungi, Plantae, Animalia)

Where:
i) originates from within cells (DNA, Microtubuli, Proteins, etc.),
i) during cellular metabolism
i) form spontaneously, but also during oxidative reactions (ROS)

What:
i) according to the Theory of
- Imperfection …. by-product of photo-biochemistry (QM)
- Coherence …. by-product of coherent delocalized EMF (QED)

i) few quanta per second and cm^2 (typ. 100 photons·sec^{-1}·cm^{-2})
i) within the UV-VIS-IR-range (200-800nm @ 1.67 – 3.41 eV) thus with very low intensities (see previous slide)

Bischof & DelGiudice, 2013
Cifra & Bosposil, 2014

Why: The problem of bio-communication has been addressed in recent times within the frame of the molecular paradigm, which states that a living organism is an ensemble of appropriate molecules kept together solely by chemical forces, whose essential features are that they can be always reduced to pairwise interactions [1].

1) the existence of chemical codes remains unexplained since no reason is given why a molecule is able to encounter its molecular partner in the sequence underlying the given biological cycle just in the right place at the right time;

2) spreading of information about each molecular event to the other component molecules of the organism would require the emission of signals, such as chemical messengers or electromagnetic signals, whose formation would require energy. The huge ensemble of all the signals necessary to keep other parts of the organism informed about what is going on in one part …. would demand an immense consumption of energy.

There are two opposite "theories" about biophoton emission, i.e., the "imperfection theory" and the "coherence theory“[1] …. See later slides

Ultra-weak photon emission originates from the oxidative metabolic reaction in microbial, plant and animal cells. It is generally considered that electronically excited species formed during the oxidative metabolic processes are solely responsible for the ultra-weak photon emission. Spontaneous photon emission without any special dedicated high-intensity-luminescent enzymatic systems (e.g. luciferin/luciferase) is what distinguishes ultra-weak photon emission from ordinary bioluminescence. Photon emission without external stimulation by light is a feature that distinguishes ultra-weak photon emission from fluorescence and phosphorescence. Experimental evidence for other types of luminescence than chemiluminescence (for instance mechano-luminescence and electroluminescence in biological systems is very limited[2].

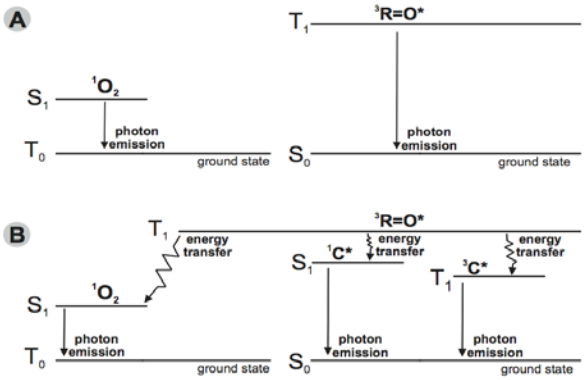
Source: [1] Bischof M, DelGiudice E (2013). Communication and the Emergence of Collective Behavior in Living Organisms: A Quantum Approach. Molecular Biology International, Vol. 2913, ID 987549: 1-19. doi:10.1155/2013/987549.

[2] Cifra M, Pospsil P (2014). Ultra-weak photon emission from biological samples: Definition, mechanisms, properties, detection and applications. J Photochem Photobiol B. Vol. XX pii: S1011-1344(14)00046-3. doi: 10.1016/j.jphotobiol.2014.02.009.

Classical QM-Transition:

- particle-like (ensemble of quanta in a coarse-grained structure): imperfection theory …. classical photo-biochemistry

S_0 .... singlet ground state,
S_1 .... singlet excited state,
T_1 .... triplet excited state
of electronically excited species formed during oxidative metabolic and oxidative stress processes
a) excited carbonyles ($^3R=O^*$)
b) singlet oxygen ($^1O_2$)



Cifra & Pospisil, 2014

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This review attempts to summarize molecular mechanisms, spectral and intensity properties, detection techniques and applications of ultra-weak photon emission. Ultra-weak photon emission is the chemiluminescence from biological systems where electronically excited species are formed during oxidative metabolic or oxidative stress processes. It is generally accepted that photons are emitted (1) at near UVA, visible, and near IR spectral ranges from 350 to 1300nm and (2) at the intensity of photon emission in the range of several units to several hundreds (oxidative metabolic process) and several hundreds to several thousands (oxidative stress process) photons s-1cm-2. Current development in detection using low-noise photomultiplier tubes and imaging using highly sensitive charge coupled device cameras allows temporal and spatial visualization of oxidative metabolic or oxidative stress processes, respectively. As the phenomenon of ultra-weak photon emission reflects oxidative metabolic or oxidative stress processes, it can be widely used as a non-invasive tool for monitoring of the physiological state of biological systems.

Image: Singlet and triplet excited level of electronically excited species formed during oxidative metabolic and oxidative stress processes in cells. (A) The energy level of triplet excited carbonyles ($^3R=O^*$) and energy level of singlet oxygen ($^1O_2$). (B) Alternatively, the energy of excited triplet carbonyl can be transferred to pigment forming singlet excited or triplet excited pigment or to molecular oxygen forming singlet oxygen. Singlet or triplet excitation energy of electronically excited species is emitted in the form of near UVA, visible or infrared photons. S_0 .... singlet ground state, S_1 .... singlet excited state, T_1 .... triplet excited state.

BPs are the result of energy-matter interaction; i.e.: absorbed electromagnetic radiation (EMR) results in an excited atomic state (quantum jump), while de-excitation emits same or slightly less EMR. Light is generally emitted form an excited atom or molecule, when an electron in the outermost shell, having been promoted to an excited energy level by, say, a collision with another molecule or absorption of energy by other means, falls back into a lower energy level ($E_e = E_1-E_o = h\cdot\nu$). Light emission does not always occur, however. The excited electron can often start to move, thus becoming an electric current, or it can be involved in a chemical reaction.

Source: Cifra M, Posposil P (2014). Ultra-weak photon emission from biological samples: Definition, mechanisms, properties, detection and applications. J Photochem Photobiol B. Vol. XX pii: S1011-1344(14)00046-3. doi: 10.1016/j.jphotobiol.2014.02.009.

Classical QM-Transition:

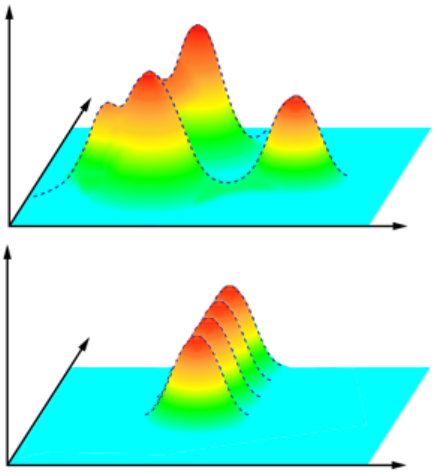
- particle-like (ensemble of quanta in a coarse-grained structure): imperfection theory …. classical photo-biochemistry

Coherence & QFT - *Heisenberg* relation:

- wave-like (phase-correlation grouping particle-wave-function together): delocalized coherent EMF …. Modern Quantum Electro-Dynamic approach

$$\delta N \cdot \delta \varphi \geq \frac{1}{2}$$

Bischof & DelGiudice, 2013



There are two opposite "theories" about biophoton emission, i.e., the "imperfection theory" and the "coherence theory“[2]:

•The imperfection theory explains biophoton emission on the basis of photobiochemistry, i.e, as weak luminescence in terms of rare and random metabolic aberrations which lead to excited compounds in the visible range of the electromagnetic spectrum, e.g. electronic excitations of radicals. The emission of photons can then be assigned to the permanent tendency of excited living matter to return to thermal equilibrium.

•The coherence theory, on the other hand, based on the physics of interactions of weak radiation in and with optically dense matter, claims that biophoton emission originates from a delocalized coherent electromagnetic field within living tissue, in particular from its optical modes. In contrast to imperfection theory, this field is claimed to stabilize around a threshold between a "chaotic" and an "ordered" regime far away from thermal equilibrium in the sense of "dissipative structures". Essentially, coherence theory takes account of the laws of Cavity Quantum Electrodynamics.

The relation between these two representations is expressed by the uncertainty relation, similar to the Heisenberg relation between position and momentum, connecting the uncertainty δN of the number of quanta (particle structure of the system) and the uncertainty δφ of the phase (which describes the rhythm of fluctuation of the system). Consequently, the two representations we have introduced above correspond to the two extreme cases[1]:

(1) If , the number of quanta is well defined, so that we obtain an atomistic description of the system but lose the information on its capability to fluctuate, since becomes infinite. This choice corresponds to the usual description of objects in terms of the component atoms/molecules.

(2) If , the phase is well defined, so that we obtain a description of the movement of the system but lose the information on its particle-like features which become undefined since becomes infinite. Such a system having a well-defined phase is termed coherent in the physical jargon.

Source: [1]Bischof M, DelGiudice E (2013). Communication and Emergence of collective Behavior in Living Organisms: A Quantum Approach. Mol.Biol.Int. Vol. 2013, Article ID 987549, 19 pages, 2013. doi:10.1155/2013/987549

[2]Popp FA (1992). Some essential Questions of Biophoton Research and possible answers. In: Popp FA, Li KH, Gu Q (eds) Recent Advances in Biophoton Research and its Applications. World Scientific Publ., Singapore.

Originally termed “mitogenic radiation”,
today better known as **U**ltra-**w**eak **P**hoton **E**mission (UwPE, UPE) or Biophotons:

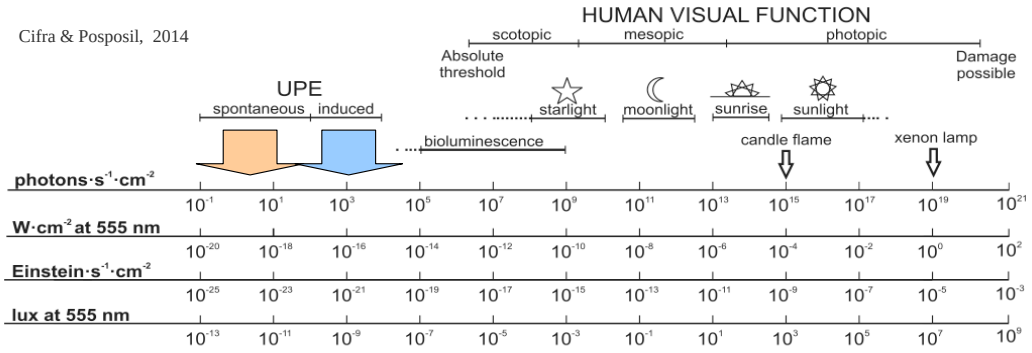


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Image: Radiometric and photometric units comparing ultra-weak photon emission intensity with that of common light phenomena. First three axis (photon flux [photons $s^{-1}\cdot cm^{-2}$], radiant flux [$W\cdot cm^{-2}$], photon flux or irradiance in Einsteins ([mol of photons $s^{-1}\cdot cm^{-2}$) are radiometric units which are easily interconvertible from one to another. Conversion between radiometric units is as follows: number of photons $s^{-1}\cdot cm^{-2}$ = [$W\cdot cm^{-2}$] $h\cdot c\cdot \lambda$ = einstein. 6.022 1023 $s^{-1}\cdot cm^{-2}$. [$W\cdot cm^{-2}$] at 555 nm on the second axis does not mean that the radiation from given sources only at 555 nm is considered, but that we calculate number of photons from given radiant .ux as if every photon from the source in the visible region of the spectrum had the wavelength of 555 nm. Lux is a photometric unit, unit of illuminance, related to sensitivity of human eyes and varies strongly with wavelength. Only the visible spectrum contributes to illuminance. Under approximation that all incoming photons would have wavelength of 555 nm, one can use conversion number of $s^{-1}\cdot cm^{-2}$ = 2.45 1012 lux.

Note: threshold for human vision ~ $1\cdot E^6$ $s^{-1}\cdot cm^{-2}$ → bioluminescence is not normally visible → need special setups to be detected: dark rooms and photon counting devices.

Source: Cifra M, Pospsil P. (2014) Ultra-weak photon emission from biological samples: Definition, mechanisms, properties, detection and applications. J Photochem Photobiol B. Vol. XX pii: S1011-1344(14)00046-3. doi: 10.1016/j.jphotobiol.2014.02.009.

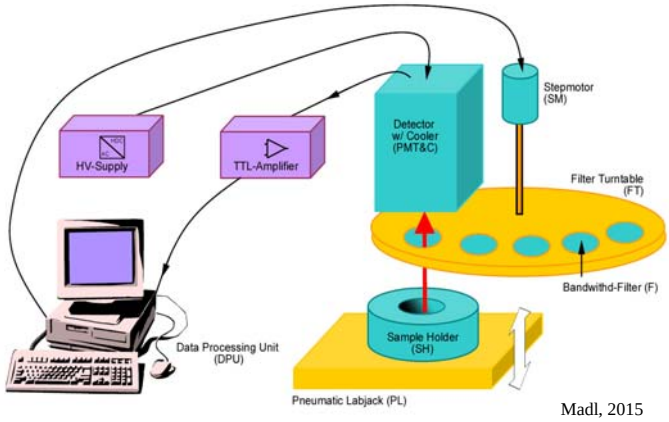
Methodological approach to induce and measure

in-vitro
Stress Reactions

i) Detector @ PLUS

Setup:
Block Diagram

Results:
i) in the final assy stage



Madl, 2015

i) Detector @ PLUS

Setup:
SW-HW-Interface Subunit &
Dark-Chamber (open)

Results:
i) in the final assy stage
i) new LED-driver board
i) rH-sensor in the making
i) strobe-light in the assy stage

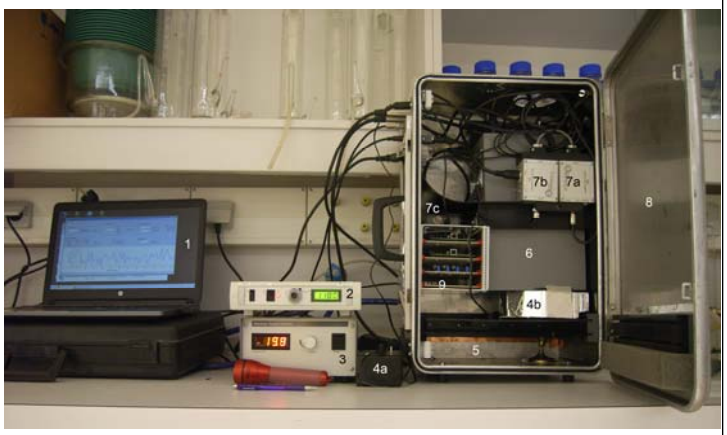


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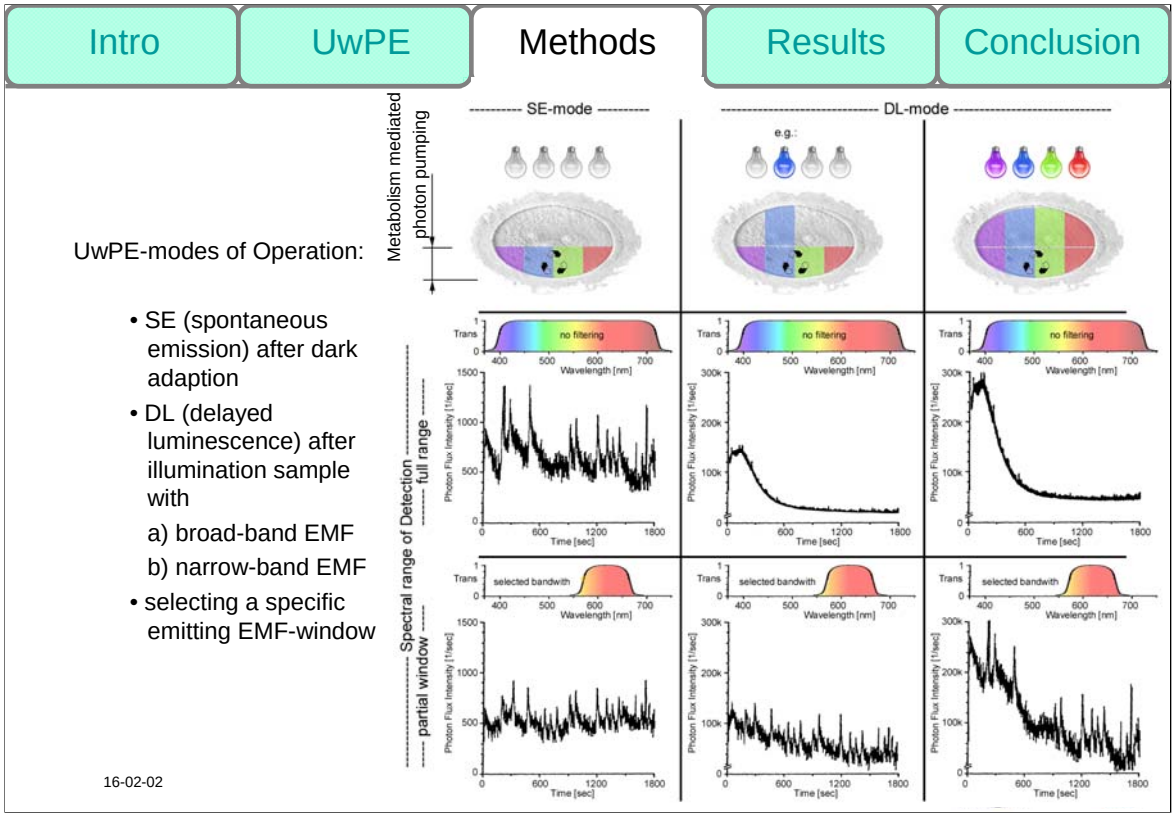
Figure 6.2: Actual pre-prototype of the CM in operation:

Working units of the pre-prototype CM – besides the Navetta a crucial component of RACES: (1) data processing unit;(2) High Voltage Power Supply; (3) PE-Power Supply; (4a) shutter control unit; (4b) high speed electromechanical shutter; (5) dark chamber compartment where Navetta should slide into; (6) PMT-housing with detector and quartz window; (7a) signal amplifier; (7b) signal discriminator; (7c) TTL signal converter; (8) photon-proof aluminium lid cover; (9) electronics for LED-batteries, PE unit in dark chamber & data interfaces to computer.

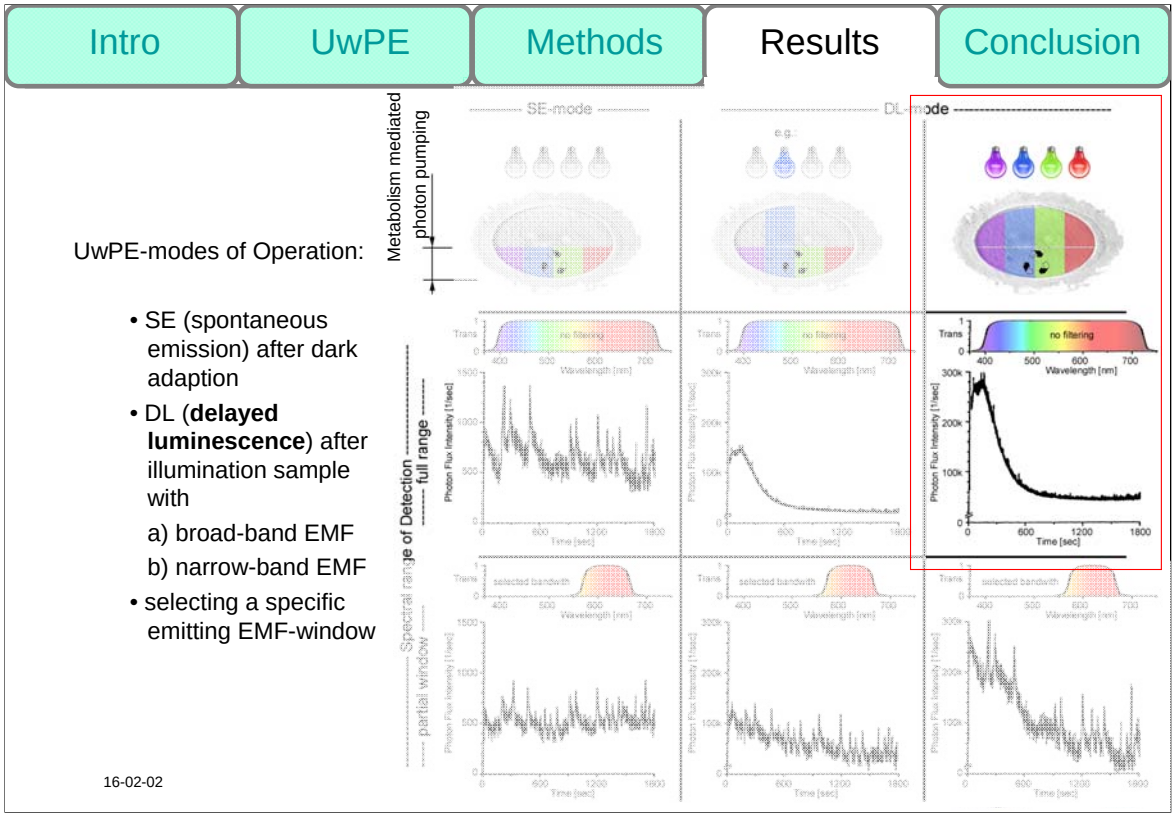


Upon placing a biological specimen into the detector chamber, as conceptualized in Figure 2, two modes of operations are possible. The first concerns activation of the sample with a light source (delayed luminescence, DL-mode) prior to measurement, whereas the second operates without an external light source and aims to detect spontaneous emissions (SE-mode).

Illumination in the DL-mode requires a focused light source with a spectral range covering UV, VIS and IR (e.g. xenon-lamp or high-yield polychromatic LEDs with a power rating in the order of 50-150 W). A suitable optic fiber cable routs the beam of light to the sample. The optical link, as shown in Figure 2, is recommended as this cuts off specific wavelengths; e.g. above 720 and below 310 nm. In addition, the light source can be used in full spectral mode (polychromatic DL-mode) or via a monochromator split into the desired narrow spectral window (monochromatic DL-mode). Illumination with monochromatic light provides additional information with regard to the most active spectral luminescence window. Each measuring cycle should start with an irradiating phase that lasts from 1 to several minutes. After excitation, the subsequent DL-emission are then recorded and evaluated in a time slot ranging from 0.7 to 60 seconds. For statistical purposes, every sample should be measured at least three times.

Source: Madl P. (2015) Detection and measurement of biogenic ultra-weak photon emission. In: Fels D, Cifra M, Scholkmann F (eds). Fields of the Cells. Research Signpost Press.

Results of abiotically induced stress on selected Organisms



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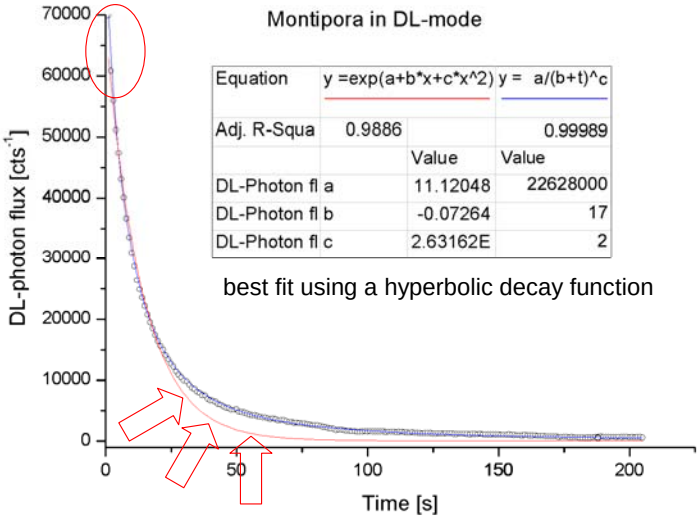
Montipora sp. ….

…. **DL-mode** (delayed luminescence)

…. HQI 400W



best fit using a hyperbolic decay function

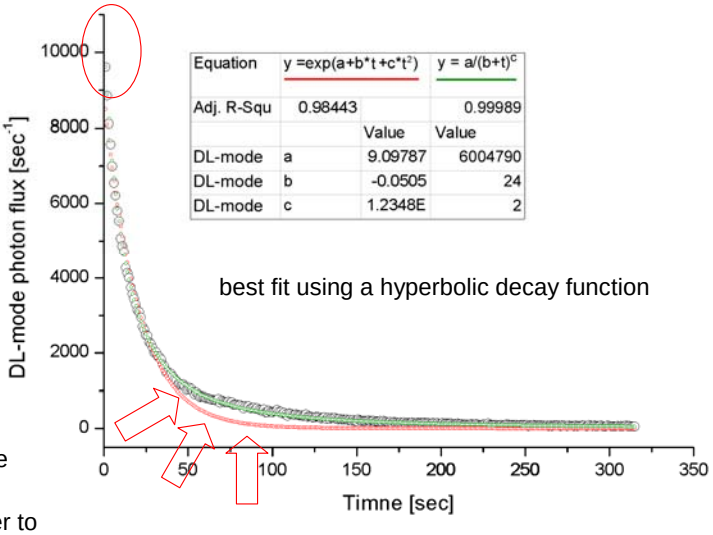
Lobaria sp. ….

…. **DL-mode** (Strobe 40 flashes) after DEP-exposure (Week-5)

things to do:

i) DL-excitation source to be integrated into the detector

i) Integrating bandwidth filter to determine wavelength dependency



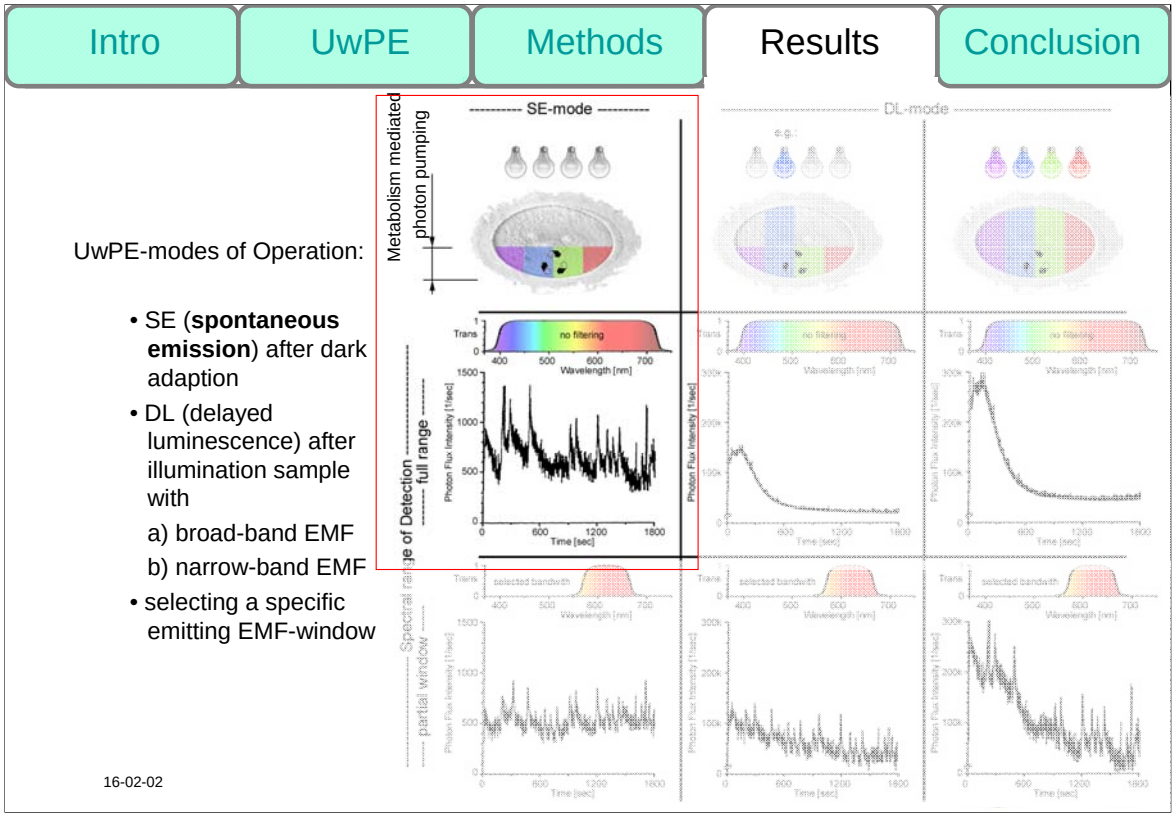
Equation	y =exp(a+b*t+c*t²)		y = a/(b+t)^c
Adj. R-Squ	0.98443		0.99989
		Value	Value
DL-mode	a	9.09787	6004790
DL-mode	b	-0.0505	24
DL-mode	c	1.2348E	2

best fit using a hyperbolic decay function

Attempting a DL-measurement using the 400W HQI lamp:

Red fitting: exponential decay function

Green fitting: hyperbolic decay function



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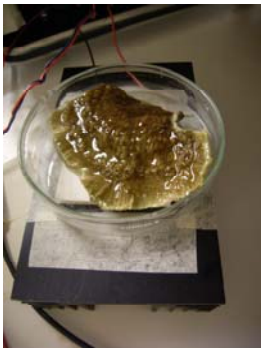
Montipora sp. exposed to temperature fluctuations

…. **SE-mode** after 18 /24 / 48 / 72 h ….



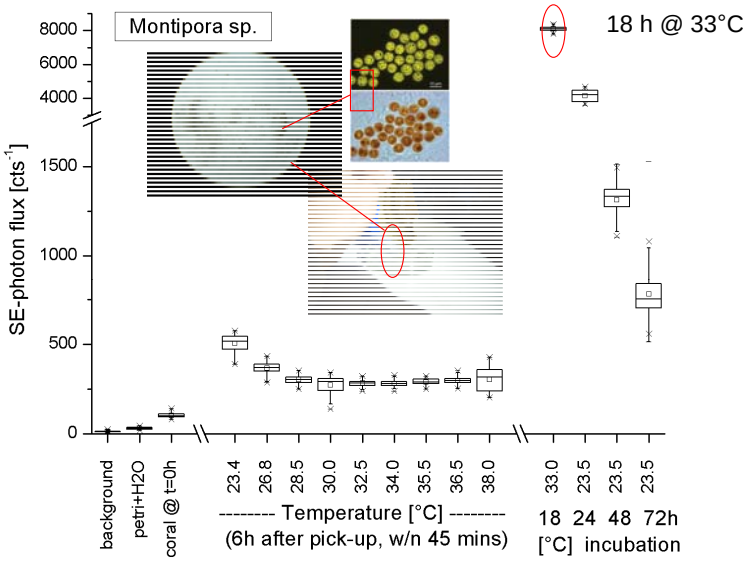
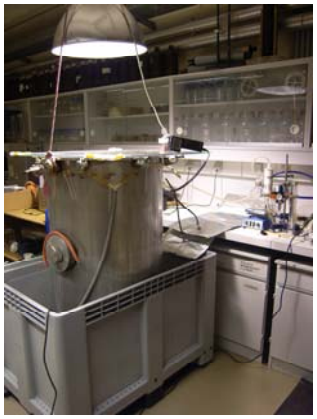


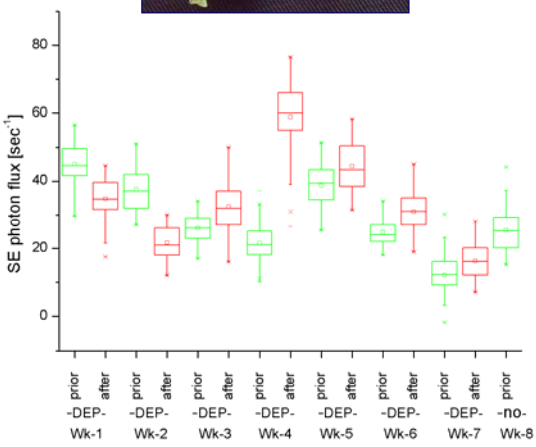
Image: Light and confocal images of Symbiodinium cells in hospite (living in a host cell) within scyphistomae of the jellyfish Cassiopea xamachana. This animal requires infection by these algae to complete its life cycle. The chloroplast imaged in 3-D is highly reticulated and distributed around the cell's periphery

Source: https://en.wikipedia.org/wiki/Symbiodinium

Lobaria sp. Exposed to Diesel Exhaust Particles

…. **UwPE**





The sample graph displayed here was obtained using always the same (and only one species) of Lobaria. Rather than point samples from the thallus, the applied method (UwPE) integrates the entire surface area of the thallus (matched with the aperture dimension of the 1D-PMT).

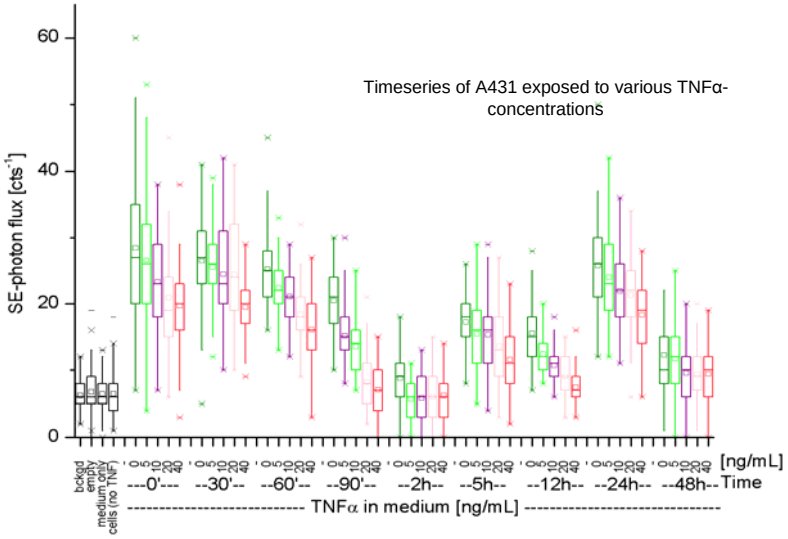
When considering the initial lower concentration of 15L DEP-tedlar bags the overall photon flux from the organism before and after DEP-exposure seemed to indicate a stabilizing trend. Once the concentration has been increased to 30L DEP-tedlars, the trend reverses and the organism shows severe signs of stress (highlighted by the increase in photon fluxes towards the end of each subsequent exposure window.

Note: one measurement got lost as the operator to get the measurement done on that day (week-6) was unavailable.

i) A431
epidermoid carcinoma cells

Setup:
UwPE Detector w/
monolayer of cells in petri

Results:
i) SE-mode measurements working



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This follow-up series shows the same cell lines, yet with different TNFα exposure concentrations along with increase time-resolution. i.e. the five plates housing this cell type have been exposed to 0 / 5 / 10 / 20 / 40 ng/mL TNFα. The cells stressed using these five different concentrations have been measured immediately after medium exchange at 0, 30, 60, 90, 120 min break and again after 2, 5, 12, 24 as well as 48h. In-between these measurements the plates have been incubated again at 36.7 °C with a pCO_2 of 4.9 % at 94.9% rH.

Each colored dataset shown here consists of 60 individual measurements, whereas those in black & white (representing background values of chamber @ opened shutter, empty dish-holder, 4mL medium-filled petri dish and petri with cells and TNF α -free medium) consist of 180 single measurements.

Interestingly, the cells react both to the change of medium as well also the TNFα. While the former was not as evident during the first trials (here it can be distinguished from the TNFα stress-exposure), the latter reveals that the cells react more strongly to the change of medium than to the stressor itself. Indeed, it seems that the higher the stress-concentration, the less vigorously the cells emit photons. In addition, the effect fades off after 2 hours, yet somehow reverberates further on till a full day has passed, indicating that the cells may indeed “recall” their past experience of being exposed to a given TNFα -concentration.

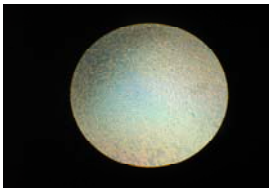
i) A549 adenocarcinomic human alveolar basal epithelial cells

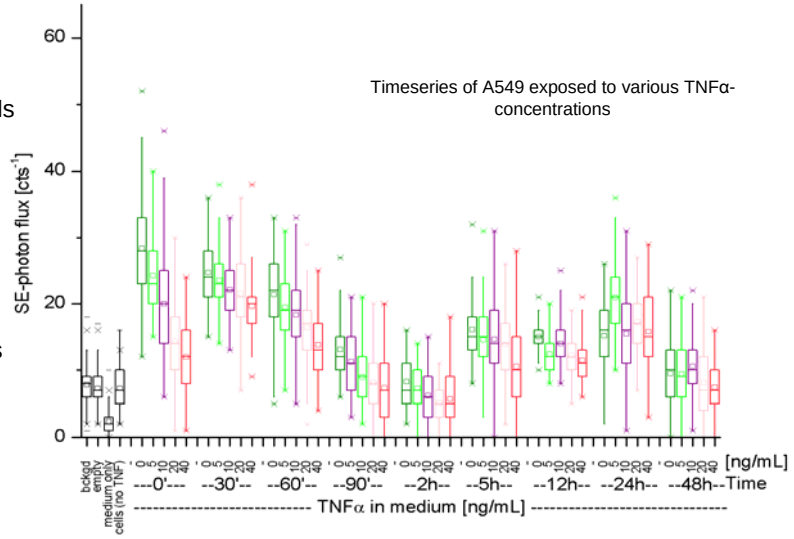
Setup:

UwPE Detector w/ monolayer of cells in petri

Results:

i) SE-mode measurements working

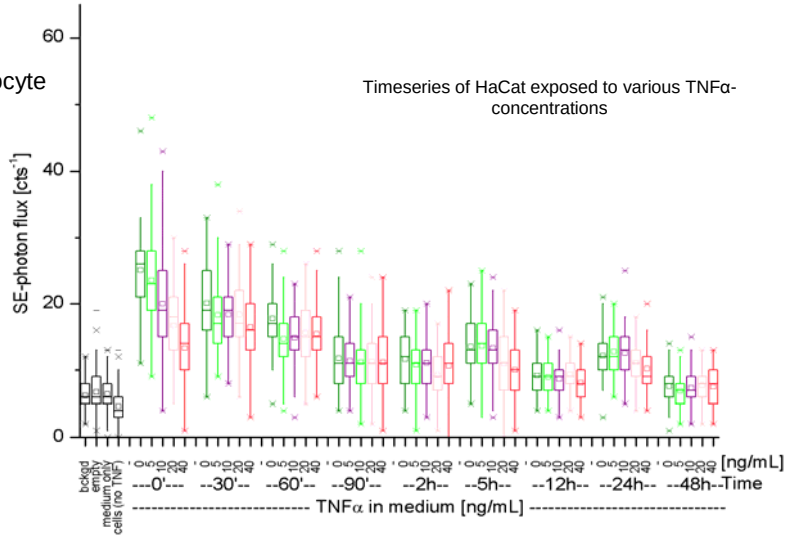




i) HaCat
aneuploid immortal keratinocyte cells from human skin

Setup:
UwPE Detector w/ monolayer of cells in petri

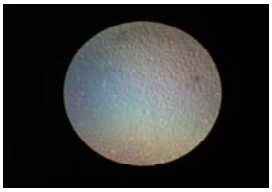
Results:
i) SE-mode measurements working

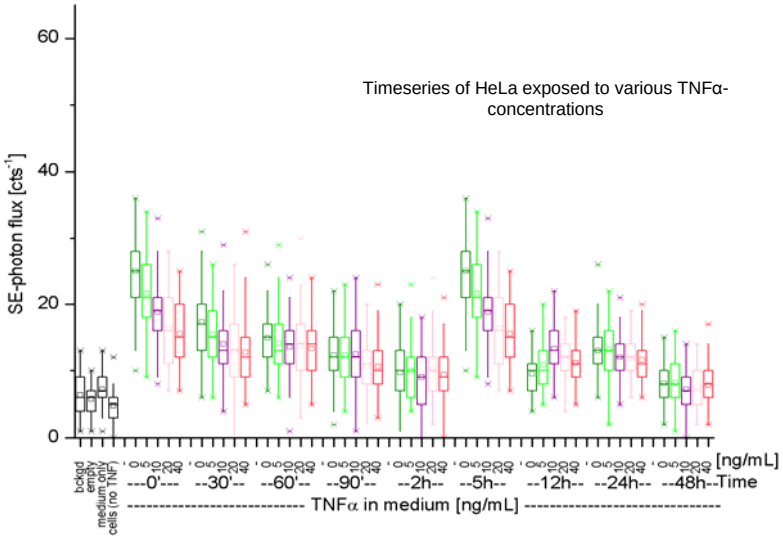


i) HeLa
cervical cancer cells

Setup:
UwPE Detector w/
monolayer of cells in petri

Results:
i) SE-mode measurements working





Concluding remarks

…. in a nutshell ….

Conclusion

In a Nutshell
UwPE ….

- is a **rapid** detection device (quasi real-time) ;
- relying on electromagnetic radiation, it is n**on-invasive** ;
- both SE- & DL-modes yield information on the **state of stress** when subjected to **live** a/biotic stimuli (predation, diseases, sedimentation, temperature fluctuation, etc.;
- Is an essential tool to investigate **coherence** in biotic systems,
  i) **viability** as a measure of the **Froehlich state**, i.e. long-range interaction, **BEC**-like state, resonance coupling, etc.);
  i) metabolic **activity** , mitotic events, nutrient fluctuations (“memory” effects);
- in tandem mode, can be used to monitor the
  i) propagation of **biocommuciative** signaling *in-vitro in both* **intra- & inter-specific**;
  i) scaled down & in **water-proof housing** enables investigating these effects *in-situ*;
- fitted with filters may even enable identification of “behavioral”-specific **wavelengths**;

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HAUS DER NATUR SALZBURG

Thank You for your attention