Chapter 3
Detection and measurement of biogenic ultra-weak photon emission

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Abstract: The chapter provides the reader with a link to better comprehend the interacting concepts and the implications of electromagnetic radiation for biocommunicative processes. The issues presented herein can be also considered as tools to elaborate new perspectives and to understand the issues presented in successive chapters. In practical terms, the reader will be introduced into the technical aspects and challenges involved when dealing with the detection of ultra-weak photon emissions. Hence, this section looks at various types of detectors available and discusses advantages as well as disadvantages of their usage for one-dimensional measurements (1D). It also includes a short outlook for areal (2D) and spatial (3D) imaging. The chapter concludes with an elaboration on how to register and record photonic emissions from biotic samples, and highlights the basic building-blocks of a state-of-the-art detection system, its modes of usage, and issues of calibration.

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1. The concept of a photon

The particle-like view of nature is the predominant way to envision the flow of matter such as atoms, ionic species or (macro-)molecules through biological structures. Yet, the interaction of electromagnetic radiation (EMR) with matter involves both particle-like as well as wave-like properties. Their manifestations in matter and the resulting consequences constitute the min-
imum “set of tools” for entering into the fascinating area of exploring the interplay between electromagnetic fields and living cells.

According to Quantum Mechanics, there is no distinction between a wave and a particle. However, the field aspect of EMR is more evident at lower frequencies, whereas the particle aspects become more evident at higher frequencies (see Figure 4 of Ch.2). Accordingly, it is legitimate to introduce here – apart from electron, neutron and proton – another kind of particle, which is denoted as the photon. This particle however, has no resting mass and as such is never at rest – it always propagates with velocity “c” (Feynman et al., 2010). Hence, as a wave it carries the energy \(E = h \nu\). Due to the electromagnetic properties of matter, and only upon striking it, will the photon reveal its particle-like nature. When the photon’s energy is converted to another form, the photon no longer exists. An example where the particle-like nature prevails is the photoelectric effect – see Figure 13-e of Ch.2. In the wake of this particle-wave duality, the photon should therefore be envisioned as an interactive process of an EM-field – thus the term “wavicle” would perhaps better denote these underlying features.

Now, how can this particle-wave duality be embedded into a biophysical context? From a classical perspective the assembly of molecules giving rise to cell membranes and other complex structures relies on covalent bonding, van der Waals forces etc. In such a setting, biochemical reactions are regarded as signal-driven processes in a stochastic temperature bath. Some authors claim that the often-quoted “randomness” in a cell’s metabolism is never able to fully encompass the complexity of life in its entire form. Dürr et al. (2002) and Ho (2003) state that movements of the molecules within cells have to be coordinated. Indeed, biology is also a philosophy within which the facts are organized into a unified conceptual framework that attempts to relate them all into a consistent concept of reality. This issue still dominates the quest of how all the neurons in a brain integrate and work together to produce coherent functioning (Becker & Marino, 1982). Already Fröhlich (1968) noted that, when energy is supplied to a system – either from metabolism or from external sources – above a critical rate and under certain conditions it is channelled into the lowest frequency mode, thereby resulting in coherent excitation of the vibratory components. This well-known established concept in Quantum Electro Dynamics (QED) is known as a Bose-Einstein condensate and manifests itself in macroscopic effects like lasing, superconductivity as well as other macroscopic quantum coherence phenomena. Particularly, weak condensates (those involving enzyme kinetics) could in fact be induced by biochemical energy (Reimers et al., 2009) or EMR, especially among microtubuli (Pokorný et al., 2001, Pokorný, 2004).

One way to shed light on Fröhlich’s observation is to show how cells utilize EMR for intra- and extra-cellular modes in “biocommunication”. The
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act of monitoring such phenomena is accomplished by using photon-counting devices. Yet, these devices somehow elude us, as these make us believe that the product of the transmitted energy equates to the “clicking” noise of a particle. Surely, this process correlates with the detection of a photon - but again, the driving principles behind it are less than obvious. When envisioning these processes, namely the emission of photons, as being part of all life-forms, it is necessary to find out why a particular emission occurs at a specific point in time. This is an issue that will be better understood when integrating biophysical aspects into biochemistry (Dürr et al. 2002).

Although beyond commonly accepted understanding, Ho (1997) and other authors proposed that QED offers solutions that help to comprehend why an autopoietic entity, such as a cell or a cellular cluster, can act in organismic fashion without falling apart. Popp (2005) for example, hypothesized that the presence of an underlying photonic field helps to explain the rather low rate of mutations during cell divisions. Every second, roughly $10^4$ reactions take place within each cell. Orchestration of these reactions requires some kind of coordination. So he suggested that coherent fields within the cell sustain coordination of these reactions. In addition it is assumed that any of these fields are coupled to resonating structures with high Q-values (see Section 3 of Chapter 2). Physics teaches us that a high Q-value of the resonating structure is required for the specificity of an EM-field to couple with a given biological structure.

On a higher hierarchical level, these fields were proposed to account for the efficient and cooperative act among the roughly $37.2 \times 10^{13}$ cells that constitute the human body (Bianconi et al., 2013). Doing so would involve a broad range of frequencies coupled together in such a way as to effectively converge into a single degree of freedom – so much so as to prevent life from disintegrating (Ho, 1997). By following this thread, it becomes more plausible that the common denominator is rooted in the concept of wholeness, which encompasses the genotype (genome) as well as the phenotype (proteome, metabolome and epigenome). It somehow seems to be embedded in an underlying field potential that plays a crucial role in the shape and the structure of every organism (Ho, 2003). Birnbaum & Sanchez-Alvarado (2008) for example, refer to the toti- and pluripotency of stem-cells in that if less vital parts are removed from an organisms, it is in principle able to regenerate the missing parts. Already Becker & Seldon (1985) wrote about the regenerative capabilities of amputated fingertips of young children, which has only recently being picked up again by Rinkevich et al. (2011) who documented similar effects in amputated digits of adult mammalia. According-
regenerate the whole from a part. Although beyond the level of accepted knowledge, it is possible to relate the underlying field-potential with the photon as the relational entity.

As outlined in section 4 of Chapter 2, matter at the subatomic level not only interacts with EMR, it basically is made out of it. Here, matter does not exist with certainty at definite places, but rather shows’ tendencies to exist’. These 3D-probability-waves collapse into a local entity to constitute its particle nature (thus, the particle is merely a local condensation of the underlying field; a concentration of energy that can be quite stable even in geological timescales or very labile as in radioactive decay patterns). As such, it cannot be regarded as isolated; rather it has to be understood as an integrated part of the whole that is, connected via electromagnetic coupling (Capra, 1975, Zukav, 2007). Accordingly, matter can be envisioned as a “coagulated potentiality” or in simpler terms as “condensed standing waves” usually in the form of a crystalline matrix where coherent phonon coupling among molecules accounts for the “hardness” of matter. While abiotic structures can be considered mere standing waves frozen in space-time, biotic structures – in particular the liquid-crystalline matrix of living tissues (Ho, 1997) – are dynamic entities, which are able to “harvest” EMR for specific needs. Well-known examples regard antenna complexes in thylakoid membranes for photosynthesis, or rhodopsin molecules in the rods and cones of the retina. Already Schrödinger (1943) wrote that organisms feed on negative entropy, by extracting energy from the environment to build order (an issue that will be discussed in more details in Chapter 4). Such order, regardless of their nature, i.e. biological primary (plants) or secondary (animal) matter, in their source terms are EMR-driven. So it should not come as a surprise that most biotic structures upon exposure to external stimuli emit light at a steady rate from a few photons per cell per day to several hundred photons per second (Musumeci et al., 1992). So, rather than speaking of ultra-weak photon emissions, especially when originating from living tissues, the word "biophotons" has been coined already 70 years ago to express the fact that light emissions from living samples (greek: bios) correlate with metabolic activity (Gurwitsch & Gurwitsch, 1943). Depending on the site of photonic origin, spectral emissions have been observed for various biological species and cellular fractions (van Wijk & Schamhart, 1988). Already Gurwitsch & Gurwitsch (1943) as well as Colli & Facchini (1954), documented this phenomenon but only later did Presman (1970), Becker & Seldon (1985), and Popp & Li (1993) develop concepts on how EMR is involved in cell interaction and cell-to-cell communication.

Recent experiments actually provide evidence for the bio-communicative role of light within and among cell populations (Farhadi et al., 2007; Fels, 2009, 2012; Salari et al., 2011; Albrecht-Buehler, 1991, 1992, Rossi et al., 2011; Madl & Witzany, 2014). Standard biological thermodynamics attests that all living systems are out of thermal equilibrium (Kondepudi & Prigo-
gie, 1981), and as such are likely to be more sensitive to external stimuli, including EMR (Kondepudi, 1982), than systems at linear steady states – an issue also elaborated in Chapter 4.

Although not too many studies have been performed in this field, it is possible to show that biophotonic emissions are somewhat correlated with the cell cycle and other functional states of cells. In reverse, biophotonic emissions of cells correlate with the extent and duration of many external stimuli of stresses (Roschger & Klima, 1985). Both properties can be observed in-vitro using highly sensitive photon-detecting devices.

2. Measurement of EM-fields and photons in live biotic samples

Hereafter, detection and measurement techniques of EMR are presented that emphasize photon capturing, either in the visible (VIS), infrared (IR) or ultraviolet (UV) ranges. Detection of longer wavelengths such as radio- and decimetre waves (referring to Fig. 1 of Chapter 2), require discrete macroscopic structures that act as antennas. According to Faraday’s law, detection of magnetic fields employ loops that transform the time-derivative of the magnetic field flux density into currents. Likewise, detection of the complementary electric fields, demands linear antennas, or electric dipoles that convert the electric field to voltages. In order to extract imprinted information such as a modulated signal from a radio wave – apart from inductances \( L \) and capacitances \( C \) required to tune into a specific resonance band – engineers use special demodulators to decode a useful signal. To envision the sensitivity of such devices, one just needs to recall that the Pioneer-spacecraft series relied just on 40 W-transmitters to relay data down to Earth from distances as far as from the edge of our solar system.

As outlined above, for shorter wavelengths (e.g. below the IR-range), EMR increasingly manifest its particle-like properties. Here, detection does not rely anymore on a matching relationship between wavelength and its corresponding resonator geometry but on the resonance modes of harmonically coupled atomic/molecular oscillators. In that regime, the energy of the radiation has to match at least one energy level of electronic orbitals (see Fig.13 of Chapter 2), whereby a resonating “LC-equivalent” can only be assigned if the molecular/atomic properties are considered. Such minute “antenna-like properties” are found among photosensitive chemicals, in gas-filled ionisation chambers, among fluorescence/phosphorescence properties of certain materials, volatile fluids of bubble chambers, gases of Geiger-tubes, in semi-conducting solid-state counters and so forth.

With regards to photons in the VIS range, real progress in the field of biophotonic research was made possible by Colli & Facchini (1954), Colli et al (1955) and later by Ruth’s device when it was possible to quantify these emissions (Ruth, 1977). The employed emission-type photomultiplier was
able to detect light intensities as low as $10^{-17}$ W (comparable with photonic emissions of a single firefly at a distance of 10 km).

Their elusive properties – very low intensities (Popp et al., 1988), ranging from a few to up to some hundreds photons s$^{-1}$ cm$^{-2}$ along with their wide spectral occurrence, covering the UV- VIS- and near IR windows (van Wijk & Schamhart, 1988) – call for detection devices that meet these requirements. Nowadays, various detector types are available and range from photomultiplier tubes (PMTs) and channeltrons that are fitted with photon-converting cathode interfaces to photodiode arrays (PDA) suitable for 1D-resolution (Fig. 1). The latter can be classical, avalanche or even made as a hybrid type. All are widely used standard optical detectors. For areal (2D) or spatial (3D) resolution, PDAs, charged-coupled devices (CCDs as used in digital cameras) and micro-channel plates (MCPs) can be utilized. MCPs are closely related to electron multipliers in that these amplify single photons by the multiplication of electrons via secondary emissions (Kobayashi, 2013). Signal intensities of PDAs or CCDs can be boosted substantially not only by cooling the device to reduce noise but additionally by simply combining MCPs with CCD-technology. Thus, MCPs are much more sensitive than comparable PDAs or CCDs and could one day become a real alternative to currently used PMTs. Yet, still, 2D-yield is much lower per detector element than in 1D-detectors as in the latter the full detector surface area (representing one large pixel) is used for photon capturing. In order to obtain usable signals in 2D, these detectors require prolonged exposure times, as a reduction in detection area results in a drastic loss of integration time – the functional dependence is proportional to diameter$^{-2}$. More recent advances include visible light photon counters (VLPCs) and superconducting tunnel junctions (STJs) – both have good yield but require operation at almost absolute zero temperature (Hadfield, 2009).

Since PMTs can be operated at reasonable conditions at very low noise and relatively high photon detection efficiency of up to 40%, they still remain the workhorses for ultra-weak photon detection (Swain, 2010).

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3. Concept of a 1D-ultra-weak photon emission detector (UWPE) system

The core of a UWPE-System with 1D-resolution consists of a highly sensitive PMT, which allows photon counting of biological as well as non-biological samples. Figure 2 displays the principal components of such a system, with the PMT constituting the heart of the system (Fig. 3). Mechanically, a PMT is simply an assembly of electrodes in a sealed, evacuated glass tube, which houses a photo-cathode conversion layer, several dynodes, and an anode. In order to yield the photo-multiplying effect, this setup must be operated using a high voltage power supply. Incident photons strike the photo-cathode material, which is present as a thin deposit on the entry window of the device. For wide range applications, the photo-cathodic window is made of a multi-alkali alloy (e.g. Sb-Na-K-Cs) that is sensitive over a wide spectral range from 200 to 800 nm (Popp et al, 1984).
Due to the photoelectric effect, this layer emits primary electrons when hit by incoming UV-VIS-IR radiation. Yet, detection efficiency over this range is not the same all over. Here, quantum efficiency (QE) – which is the ratio of the number of produced primary electrons over the number of photons striking the photo-cathode – characterizes the sensitivity of the photo-converting layer. Referring to Planck’s equation ($E = h \nu$), photons with shorter wavelengths have higher energy than those with longer wavelengths. This means that the QE is higher at shorter wavelengths than for longer ones. Thus, not all photons are energetically strong enough to trigger the release of primary electrons. These shortcomings contribute to the overall reduced QE of most common PMTs, which – depending on the wavelength sensitivity – is typically less than 30%. Thus, the energy of primary electrons corresponds to the incident photonic energy minus the conversion function of the photo-cathode. The emitted primary electrons in turn are directed to the focusing electrode and toward the electron multiplier unit via a process known as secondary emission. This unit consists of a number of serially arranged
electrodes (various dynode stages). Each dynode is held at a more positive voltage than the previous one, with the most distal dynode stage—depending on the PMT-type—reaching a maximum potential of approx. 2 kV\text{DC}. Acceleration via the applied electrical field gradient assures that the electrons hit the first dynode stage with much greater energy than originally released by the photo-converting cathode. Upon striking the first dynode and due to the existing potential gradient more electrons are emitted, which in turn are accelerated toward the successive dynode. The geometry of the dynode chain is such that with every dynode and thus potential increase more and more electrons are being produced. Upon reaching the anode, the avalanche of electrons is typically amplified by a factor of $10^6$, where the accumulation of charge results in a sharp current pulse that correlates to the incident photon.

![Figure 3](http://en.wikipedia.org/wiki/Photomultiplier_tube)

**Figure 3.** Functional design of a classical PMT. It consists of a photon-electron converting cathode to which a series of dynodes are attached. The dynodes are connected to a voltage cascade that increases in stepwise manner as it approaches the anode. The latter can reach a potential of up to 2 kV.²

QE, though, is not the most important delimit when detecting ultra-weak photon emissions. Even more important is a low signal to noise ratio (SNR) of the PMT. For simplicity, it can be condensed to a simple ratio of “signal over noise”. SNR follows a Poisson distribution and is highly dependent on the type of PMT used, its gating and the employed signal amplifier. To improve SNR, one needs to restrict the signal to a few photons per gate and count photons for many successive gating intervals. Obviously, this introduces the inconvenience of longer measurement times. Yet, doing so

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usually far offsets the small gain in SNR, which would result from a single photon count.

Thermoionic background noise from the photocathode – in the lower kHz-range when operated at ambient temperature – can be further reduced by cooling the PMT to well below freezing: typically -25 °C. This is achieved via Peltier-elements, which causes the spontaneous emission of temperature-related primary electrons to fall below 10 dark-count pulses per second. Only then is it possible to observe ultra-weak emissions from live samples.

As PMTs operate on the basis of a potential gradient, these detectors are sensitive to magnetic fields (>100 mT). Thus shielding of the detector should be considered to maintain proper gains in signal strength. Furthermore, to extend the lifespan of a PMT, it should never be operated at maximum potential, rather 300–400 V below this value (Swain, 2010).

Figure 4. Channel Photomultiplier. Cross-sectional view (left) and external view with and without encapsulation (right).

A more modern design concerns the channel photomultiplier (CPM). It still preserves the advantages of the classical PMT, yet instead of the complicated dynode structure, a bent, thin semi-conductive channel acceler-

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ates the electrons through the channel. Secondary emissions are emitted each time electrons are obstructed by the undulating geometry of the tube, resulting in the same avalanche effect as in the classical dynode design (Fraden, 2011). As depicted in Figure 4, the CPM is polled with encapsulation material and is quite rugged compared to the fragility of classical PMTs. Other advantages of CPM technology include: i) very low background noise due to different dynode design; ii) being made of a monolithic semi-conductive channel structure, there are no charge-up effects. As with PMTs, however, cooling is again unavoidable if one wants to reduce thermal emissions of the photocathode.

With the absence of dynode noise, thermoelectrically cooled CPMs enable clean separation between real events created from the conversion of a photon to a photoelectron, which leads to high stability of the signal over time. However, these ruggedized detectors still do not yield the same detector efficiency as comparable PMTs.

Since active (window) diameters are quite smaller than in larger PMTs, CPMs are suitable for 2D imaging. An array of several CPMs in parallel provides a 2D detector surface with a very coarse resolution. The drawback however, is evident: the reduced surface area per detector translates into a $1/d^2$ lower yield compared to a large 1D-PMT.

Regardless of the detector employed and as shown in Figure 2, additional amplification using an electronic amplifier is necessary. Only then can the discriminator unit convert the current spikes into a computer-compatible transistor-transistor-logic (TTL) signal. Since recovery times of these detectors are very fast, the number of TTL signals per given sampling interval (ranging from ms to days) corresponds to the intensity of photon emission (Yu, 2002).

### 4. Experimental procedures

Prior to measurements and to avoid the additive effect of ambient bias, any sample (e.g. quartz-glass cuvette housing the cell suspension) should be kept in a dark chamber for at least 15 minutes. With respect to the spectral window, quartz-glass cuvettes are preferred for liquid samples over standard glass, as it allows UV radiation originating from the sample to actually reach the detector.

For biological samples, it is often required to operate the measurement cycle under controlled temperature conditions. Thus, the dark chamber (as shown in Fig. 2) is fitted with temperature sensors, a PID controller and peltier elements to enable accurate adjustment to comply with physiological constraints – usually in a range from 0 to 50 °C.

Upon placing a biological specimen into the detector chamber, as conceptualized in Figure 2, two modes of operations are possible. The first
concerns conditioning the sample with a light source prior to measurement (delayed luminescence, DL-mode), whereas the second operates without activation 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 with a luminous flux rating in the order of 1–2 klm). A suitable optic fiber cable routes 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 to select the desired narrow spectral window (monochromatic DL-mode). Illumination with monochromatic light stimulates resonant structure only that best interact with the incident radiation and thus provide additional information with respect to the most active re-radiated spectral 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 (Scholz et al., 1988).

Calibration of the detector is crucial and can be achieved by using reference emission sources. Usually, it is sufficient to turn towards readily available $^{14}$C isotopes ($\beta$-emitters) in combination with fluorescent organic solutions that are frequently utilized in calibration procedures for scintillation counters. The isotope comes in a range from 1–2 kBq (27–54 nCi), which needs to be coupled to the fluorescing scintillation solutions consisting of 2,5-Diphenyloxazole and 1,4-bis-(2-methylstyryl)-benzene. The $\beta$-radiation from the isotope induces weak fluorescence, which is recorded by the detector. Calibration of the detector assures reproducibility and reduces measurement errors to levels of a few counts per second (Popp et al., 1984; Yu, 2002).

5. Conclusion

In this chapter the focus was laid on how EMR emitted by living entities – both within cells as well as outside the organism – could play a vital role in inter- and intra-cellular communication as well as in the organization of living systems. Such ultra-weak photon emission (also known as biophotons) can be measured with highly sensitive devices called photomultipliers. This type of detector has shown to be a reliable tool for diagnostic purposes within the field of biophotonics. Yet, further research efforts and improved detector efficiencies are urgently required to achieve better signal to noise ratios and enhanced photon-conversion yield. The emerging 2nd-generation detectors will eventually make it possible to explore biophysical properties in living organisms even beyond existing limitations. This will both include
measurements of the spectral intensities of these emissions as well as 2D-dynamics within cell cultures during growth and development or during normal metabolic activity.

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