

A look at some systemic properties of self-bioluminescent emission

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ABSTRACT

Self-bioluminescent emission (SBE) is a type of biological chemiluminescence where photons are emitted as part of chemical reactions occurring during metabolic processes. This emission is also known as biophoton emission, ultraweak photon emission and ultraweak bioluminescence. This paper outlines research over the past century on some systemic properties of SBE as measured with biological detectors, photomultiplier detectors and ultra-sensitive imaging arrays. There is an apparent consensus in the literature that emission in the deep blue and ultraviolet (150-450nm) is related to DNA / RNA processes while emission in the red and near infrared (600-1000nm) is related to mitochondria and oxidative metabolisms involving reactive oxygen species, singlet oxygen and free radicals in plant, animal and human cells along with chlorophyll fluorescent decay in plants. Additionally, there are trends showing that healthy, unstressed and uninjured samples have less emission than samples that are unhealthy, stressed or injured. Mechanisms producing this emission can be narrowed down by isolating the wavelength region of interest and waiting for short-term fluorescence to decay leaving the ultraweak long-term metabolic emission. Examples of imaging this emission in healthy versus unhealthy, stressed versus unstressed, and injured versus uninjured plant parts are shown. Further discussion poses questions still to be answered related to properties such as coherence, photon statistics, and methodological means of isolating mechanisms.

Keywords: bioluminescence, biophotons, biophoton emission, biofields, chemi-bioluminescence, self-bioluminescence, self-bioluminescent emission, auto-bioluminescence, biological chemiluminescence, ultraweak bioluminescence, ultraweak photon emission

1. INTRODUCTION

Self-bioluminescent emission (SBE) is a type of biological chemiluminescence where photons are emitted as part of chemical reactions occurring during metabolic processes. It is a weak emission and is referred to by various names such as ultraweak photon emission, biophoton emission or simply biophotons. This radiation is not stimulated by chemical or optical markers and is distinctly different from luciferin/luciferase reactions used in screening of gene mutants [1] and tumor detection [2] through the expression of the luciferase “firefly” gene [3].

SBE exists in all living organisms and persists at a weak steady-state level as part of living metabolic processes. Its amplitude can be orders of magnitude below that of luciferin/luciferase reactions and has been measured in all types of plant, animal and human cells. This radiation is strongly correlated with cellular function (as first noted by Gurwitsch [4]) and state of health [5-16]. Hundreds of studies have looked at properties of this radiation. However, there is much confusion on this subject because of the different terminology that gets used. Those who want to think of it as something different from biological chemiluminescence to distinguish it from the popular topic of luciferin-luciferase reactions add to the confusing terminology. There is also the added confusion that the terms biophoton and biophotonic have become popularized to encompass any type of light emission and measurement of light from biological objects. The term this paper uses is self-bioluminescent emission or self-bioluminescence because the generation of this emission does not require any extra or outside agents or stimulants. (Auto-bioluminescence would also work.) The distinction of what type of emission we are talking about is important, whereas the actual term is not.

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The kinds of questions often posed about this emission are usually related to “what is the mechanism behind what you are measuring?” There have been a lot of studies since the development of the photomultiplier tube (PMT) detector that have looked into specific mechanisms, and an historical outline of some of them will be provided in Section 2. When Fritz-Albert Popp and colleagues coined the term biophotons [17] and tied it back to Gurwitsch many thought that something had been rediscovered. Yet when you go back through the literature there is a continuous trail from Gurwitsch to Popp to the present. This is very encouraging. The main difference that emerges is that the biophoton community concentrates on measurements in situ of live organisms and most of the post World War II research focuses on measurements from cell cultures or prepared samples to isolate a specific type of metabolic response so that specific mechanisms can be studied. The leap then, and I propose that it is not a big leap, is to go from measurements on sliced and diced cells to those of fully functioning organisms.

The reason that we have been able in the last couple of decades to look at fully functioning organisms is that we now have much more sensitive measurement equipment than was previously available. Beginning in the 1970’s and 1980’s significant improvements were made to photomultiplier tube detectors with improved materials and electronics. In the 1990’s there were breakthroughs in low light-level imaging with extremely sensitive cameras designed for astronomy. The current decade has also brought improved imaging arrays with intensified cascading gain essentially acting like a photomultiplier array (see for example [18, 19]). Even though these imaging arrays are less sensitive than a high-end photomultiplier, their noise levels are close to counting photons at each pixel.

Currently, the imaging of self-bioluminescence requires total darkness and an approximately 1-minute exposure of a plant surface or a 15-minute exposure from human skin requiring a subject to remain still for a long time. With the trends in cameras it is likely in another decade that exposure times will be reduced by another factor of 10 or 20.

Looking back through the literature there are trends in looking at different types of systemic properties of this emission. At the lowest level, studies measure amplitudes of the emission. The next step up is wavelength measurement, and then amplitudes of different wavelengths relating to different mechanisms. Since the 1970’s there has been an added interest in measuring the statistics of this emission so as to determine more of its systemic properties like the probability distribution function of the photons arriving at a photomultiplier, the correlation in photon counts, the coherence, and the states of the photons (ie. are they classical modes or squeezed states?). But what other properties would it be useful for us to look at?

This paper begins with a historical outline of research on self-bioluminescence. It continues on to show examples of measurements, and then discusses some of the systemic properties of this emission.

2. HISTORICAL BACKGROUND AND LITERATURE REVIEW

Let me provide a disclaimer and note that it is impossible in a paper of this type to cover all the previous research since there have been multiple edited books on the subject. I have compiled a bibliography of more than 200 papers stretching over the last 97 years, but only a subset is mentioned in this paper, and there are thousands of others. I will undoubtedly not mention something another researcher thinks is most important. Given the plethora of research in this field it would be difficult for someone to have read every paper. And in the last generation, the field has branched in many directions. The study of mechanisms at the cellular level using special cell preparations is still very fruitful and enables us to begin to understand how to characterize different metabolic functions and different mechanisms in a wide variety of cell types.

Among the many reviews on this subject I want to point out those by Van Wijk [20], Musumeci et al. [21, 22], Slawinska and Slawinski [23], and the books edited by Popp et al. [24], Wolken [25] and Jezowska-Trzebiatowska et al. [26, 27].

2.1 Early Research

The earliest work on self-bioluminescence has been attributed to Alexander Gurwitsch. From his observations, Gurwitsch put forth the idea that “radiation generates cell division” as early as 1911 [20, 28]. His studies utilized onion roots as both radiation emitters and biological detectors [4, 29]. He coined the term “mitogenetic” radiation and hypothesized that this emission was related to mitosis. He noted that the cellular division increased exponentially on the sides of the roots facing one another while in shadow areas the cellular division rate was less [30]. To show that it was

due to effects of radiation rather than a chemical effect, quartz plates were placed between the roots. In later studies it was shown that the radiation passed through quartz plates and not through regular glass indicating that the stimulating radiation was in the UV portion of the spectrum [20, 29].

The implications of Gurwitsch's 1925 theory [4] that plants emitted weak radiation undetectable by current optical detectors and that this weak radiation could possibly also be regulating biological processes created a stir. Over the next 12 years, more than 600 studies were published on this topic including one by Egon Lorenz [31, 32]. However, because the radiation was not visible to the naked eye, and biological detectors only work under certain conditions, there were very mixed results from these studies. Both the controversy over the subject and World War II intervened to essentially shut down this research.

Meanwhile, in the late 1940's after WWII the photomultiplier and photon-counting devices were introduced by Engstrom and further improved by Morton and Mitchell and Westoo and Wiedling [33-35]. These new detectors opened up a whole new realm of research because now experiments could be devised and implemented that were able to count the number of photons. These detectors do not make an image. Measurements are made at a "single point" collecting all the incident radiation over the area of the anode which is usually around a square centimeter in size.

The field of biochemistry was coming into its own during this era, and the focus shifted towards looking at particular biochemical reactions and their by-products. Light absorption and emission were considered part of the chemical processes and were generally not looked at as mediators of reactions, but rather as by-products [36, 37].

By the 1950's the new photon-counting photomultiplier detectors were beginning to be put to use for studying biological functions. In 1951 Strehler and Arnold [38] reported their measurement of radiation from plants relating to photosynthesis. Their spectral measurements show a peak around 700 nm (now well known to be due to chlorophyll) and another peak around 400 nm (further corroborated by other authors [39] and now thought to be attributed to DNA / RNA processes).

In 1954 Colli et al. published their first results using a photomultiplier to measure weak spontaneous emission from different kinds of plants [40, 41]. In their studies, beans that had been cut into pieces were found to emit 2-3x more light than whole beans. The seedlings had been grown in the dark to eliminate effects due to chlorophyll. The peak output was found around 550 nm in the green. Their detector was not sensitive to red or near infrared. It has since been corroborated by many authors that damaged and injured plants emit more than healthy plants as will be discussed in Section 2.3. For the rest of this section this paper will focus on different aspects and systemic properties of self-bioluminescent emission.

2.2 Emission at Different Wavelengths

One of the first systemic properties that were closely evaluated was the wavelength of the emissions. The general consensus among researchers is that emissions in the ultraviolet (UV) portion of the spectrum are related to different metabolic functions that those in the near infrared portion of the spectrum (NIR).

2.2.1 DNA / RNA and Ultraviolet Emission

The emission from plants (animals) in the deep blue and UV has since been associated with DNA [42, 43]. It is well-known that when DNA unwinds (denatures) it gives off UV light and that emission from DNA can be enhanced using higher-frequency UV light [44]. When DNA renatures, less light is emitted.

Rattemeyer and Popp [42] have shown that DNA unwinding produces more photonic (ultraweak) emission. Utilizing the hypothesis that damaged or unhealthy tissue emits more radiation, we would expect that denatured (unwinding) DNA to put off more photons because the denaturing process stresses the DNA.

Research into the functional properties of DNA has focused on measurements in the UV [42, 45, 46]. These measurements require low-noise, high-gain detectors that are sensitive down to as short as 200nm. Typically, specially designed photomultiplier detector anodes going down to 200nm are used for these measurements and are more sensitive than imaging sensors. The most sensitive types of imaging cameras utilized for astronomical purpose are comprised of back-illuminated silicon as the sensing medium having a range from 200-1100nm. It is possible to get sensors with UV enhanced coatings that boost the response in the deep blue and UV by 10-20%. The big problem with imaging is finding a well-corrected, low F/#, quartz imaging lens over the region of interest. Most imaging systems are limited by the lens.

2.2.2 *Near Infrared Emission*

The other band of the spectrum with lots of research interest is in the near infrared with a peak around 700 nm. This puts it right at the far-red end of human vision and beyond.

It is well-known that in plants this emission is related to chlorophyll [47]. Experimental evidence seems to point to singlet oxygen (free radicals) as the source of this emission. However, there are other processes involving chlorophyll that can also produce near infrared emission.

Of interest is what processes can produce emission after many hours in the dark once the chlorophyll emission has subsided. Etiolated plants (those grown in darkness) have an emission spectrum shifted into the visible with less output in the infrared. Fluorescence from photosynthesis only lasts a few minutes in total darkness so there must be something else involved. According to Hideg and colleagues, mitochondria are another possible explanation for emission peaking in the red and near IR [48, 49]. As pointed out in the rest of this section, oxidative processes involving singlet oxygen, reactive oxygen species and free radicals are also important contributors in this wavelength range.

2.3 Response to Stress and Injury

It has been well-documented in the literature that cells under stress or that have been injured emit more photons than healthy cells [5, 6]. In general, a healthy system emits fewer photons than an unhealthy or injured system and is in more of an equilibrium state. Furthermore, the spectral characteristics, and decay of the emission depend upon the state of health of the tissue.

One example is studies of mechanical injury to plants where it is clearly seen that emission near an injury is greater than in uninjured tissue [7, 13]. The research groups of Boveris et al. and Slawinska et al. have independently shown that stressed plants emit more radiation in the red and that the kinetic response is non-stationary (emission varies with time) [7, 8].

Salin and Bridges [13] describe the similarities between emission from plant and root tissue and emission from leukocytes from animal cells. They show common links of oxygen free radicals, an increase of emission with the presence of oxygen, a decrease of emission in a nitrogen environment and the implication of peroxidase in plant tissue (myeloperoxidase in leukocytes). This is consistent with enhanced oxidative metabolism from wounded plant tissue. Wounded plant tissue responds by increasing oxidative metabolism as the tissue responds to the injury. It is hypothesized that this is part of a defense response by plants to seal off the wounded area and generate new tissue.

Emission also changes with temperature. Another finding is that chilling plants causes them to emit more light [9]. They point out that the increased emission reflects increased oxidative stress. This is evidenced by the presence of a larger concentration of free radicals. Furthermore, Iyozumi et al. have found there is also a shift of spectral characteristics as part of the defense response in plants [10]. Additional studies have shown that when seeds are stressed by heat as they are warmed, they emit more radiation [50].

Many groups have studied different kinds of stressors such as toxic substances and disease. Studies with algae show that when in contact with a poison the algae emit more than untouched algae [11]. Cancerous cells have been shown to emit more than healthy cells and the emission decays differently [12].

Function has further been studied by looking at seeds. Germinating seeds emit quite a bit more than denatured seeds [8, 39] reflecting their higher metabolic rate. Changes in the amount of emission have also been found to correlate to physiological changes in tissue such as variations in surface potential [51].

2.4 Imaging of self-bioluminescent emission

Since the development of multi-channel plates and position sensitive multi-anode photon-counting detectors, imaging has enabled studying the localization of SBE emission [52-56]. With ultra-sensitive low noise imaging CCD's developed for astronomical purposes and further developed for luciferin/luciferase measurements, a new breed of imaging arrays enables high-resolution images of SBE and noise sensitivity close to counting of photons [57-59]. These new imaging arrays require a longer integration time but offer the ability to image an entire leaf, multiple plant parts, or human body parts such as hands, so that variation with time and space can be measured. Although the exposure time means events are watched over minutes rather than seconds, it is possible to see details in samples and pinpoint areas to compare over time [58, 60].

2.5 Mitochondria

Mitochondrial functions are of interest because they emit in the red and near infrared and therefore can be monitored using either photomultiplier detectors or highly sensitive imaging detectors. Mitochondria are the respiratory center of cells converting 'food' (glucose) into ATP and energy. They are fundamental to cellular energy metabolism in both animals and plants. When metabolism increases, mitochondria activity increases [61].

Mitochondria serve a vital role in the healthy functioning of cells and energy metabolism. Research points out effects of by-products of mitochondrial mechanisms such as free radicals, reactive oxygen species and singlet oxygen [61-63]. These by-products have been linked with weakened immune function and various diseases.

2.5.1 Relationship between emissions from mammalian and plant cells

A common link between biological mechanisms in mammalian and plant cells is mitochondria. Both types of cells have been shown to emit this ultraweak self-bioluminescent emission [48, 49]. Mitochondria from rat and human cells also show this emission [64, 65].

Another link is a correlation between singlet oxygen production and increased photon emission [66-68]. Singlet oxygen has been shown to emit in the red part of the spectrum [67] and emission measured in human blood is linked to reactive oxygen species [69]. There is a lot of other experimental evidence pointing towards other oxidative mechanisms similar in plants and animals.

Thus, there are similar emissions in all types of biological tissue that are related to similar mechanisms. Mitochondrial functions and their by-products of singlet oxygen and reactive oxygen species have been shown to be the predominant mechanisms producing this emission in the red and near infrared while DNA / RNA processes are the predominant mechanisms in the UV.

3. IMAGES OF SELF-BIOLUMINESCENT EMISSION

As examples of this type of emission, this section contains images showing plants in varying states of health, stress, and injury. These images clearly show variations in emission as a function of time and state of health. The imaging system utilized for these examples is comprised of a Princeton Instruments VersArray 1300B camera system consisting of a back-illuminated silicon CCD with 1340x1300 pixels and a standard Nikon 50mm F/1.2 lens enclosed in a light-tight dark box [57, 70]. The camera has a quantum efficiency (QE) of greater than 0.3 over a wavelength range of 200-900nm cutting off at 1100nm, a maximum QE of 0.9, no UV enhancement coating, and an overall useful transmission range with the glass lens of approximately 420-1000nm. This low-noise camera system is cooled to -90°C to reduce thermal and readout noise enabling the camera to essentially count photons from 430-760nm ($\text{QE} > 0.8$) [70].

For the first example, similarly sized healthy and unhealthy leaves from a single geranium plant are compared in Figure 1. Figure 1(a) shows a color image of the leaves taken with a Canon digital camera. The unhealthy leaf on the left is yellowish green with brown spots, while the healthy leaf on the right is deep green. * Figure 1(b) is a 1-minute exposure taken with the VersArray camera in total darkness showing the chlorophyll fluorescence. Figure 1(c) shows a 10-minute exposure after 27 minutes in complete darkness after the chlorophyll fluorescence has decayed. For comparison Figure 1(b)-(c) have been scaled the same. Note that while the healthy leaf fluoresces more, the unhealthy leaf has a greater self-bioluminescent emission (SBE).

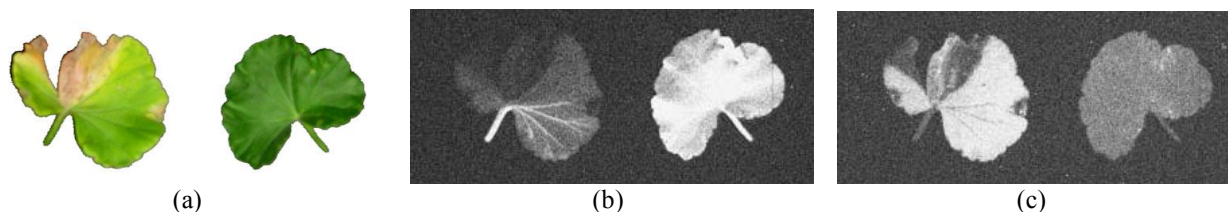


Figure 1. Unhealthy (left) and healthy (right) geranium leaves. (a) Digital camera photograph. (b) Chlorophyll fluorescence (1-minute exposure) in darkness. (c) SBE image (10-minute exposure) after 27 minutes in darkness.

* To see the images in color please consult the online pdf version available at spiedl.org.

The next example shows some string beans. The beans were obtained fresh from the grocery store and chosen to be in the same visual state of health. The top 4 beans in each picture were kept at room temperature, the middle 4 beans were kept in the refrigerator overnight, and the bottom 4 beans were frozen in the freezer overnight. All beans were taken out and placed into the light-tight chamber, and then a series of images were taken over a 48-hour period without opening the chamber. Some of the images are shown below. The images have each been scaled and mapped with a pseudo-color scale. The minimum amplitude color of black is at the 5% level of the total amplitude scale and the maximum amplitude color of white is at the 95% level. This method stretches the scale so that we can see relative values and clips the highest and lowest points. Figure 2(a) is a 1-minute exposure, (b) is a 10-minute exposure, and (c-e) are 1-hour exposures. Figure 2(f) is a color photograph taken with a Canon digital camera at the end of the experiment after 48 hours of data taking in the dark. Note that the left side of these images has a non-fluorescing white paper background while the right side has a black background. The white background can be seen to scatter more of the emission towards the camera than the black background. Also note that the previously frozen beans are quite moldy and rotting after 48 hours while the fresh beans still look fine and the refrigerated beans are a little limp. During the course of the experiment the photonic emission activity shifts from the refrigerated beans to the frozen ones at about 20 minutes. The fresh ones have a fairly continuous emission. By the 16-hour mark the individual beans seeds are obvious indicating that the emission is coming from deep within the bean at that point.

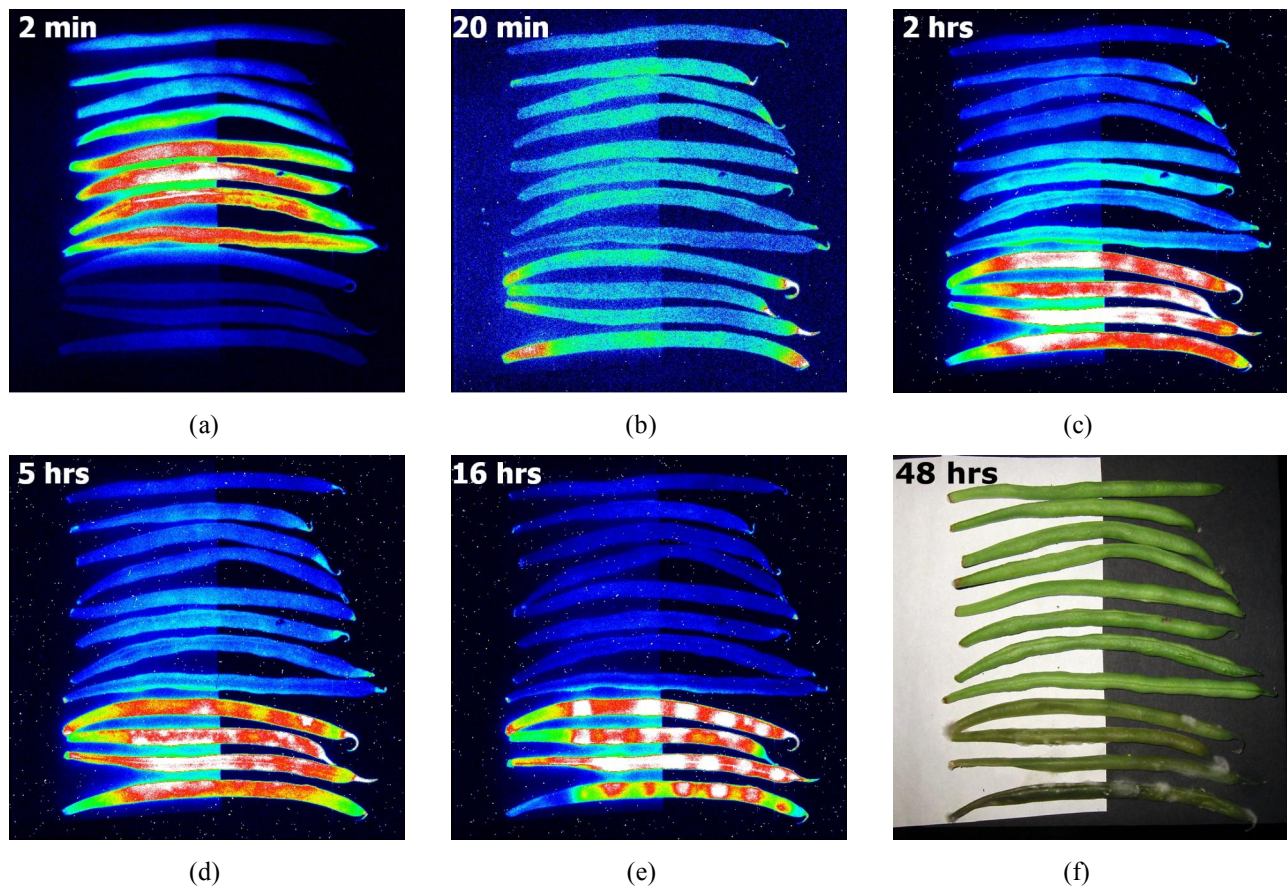


Figure 2. Time series of string beans. Top 4 beans are fresh, middle 4 are refrigerated, and bottom 4 have been frozen. Exposures (a)-(e) have been taken in total darkness. Their scales have been stretched from 5-95% and colored with a pseudo-color scale where black is low and white is high. (a) 1-minute exposure after 2 minutes. (b) 10-minute exposure after 20 minutes. (c)-(e) 1-hour exposures after time delays of 2, 5, and 16 hours. (f) Digital white light color photograph of beans after 48 hours showing white non-fluorescing paper on left, black paper on right, and the previously frozen beans are now moldy.

Figure 3 shows a geranium leaf cut into 4 sections using a blunt edge and severely injured with a hammer blow in the middle of the right side showing noticeable damage. The chlorophyll fluorescence image [Figure 3 (b)] indicates noticeably more activity along the edges of the leaf. With time the SBE activity moves to different parts of the leaf sections as can be seen by comparing Figure 3 (c) and (d). There is more SBE in the less traumatized left half of the leaf. In the right half of the leaf the activity migrates more to the edges over time. These images illustrate that activity increases as a result of a cut injury. With time the leaf focuses its energy where it needs it most at the edges of the severe trauma wounds leaving less activity in adjacent areas.

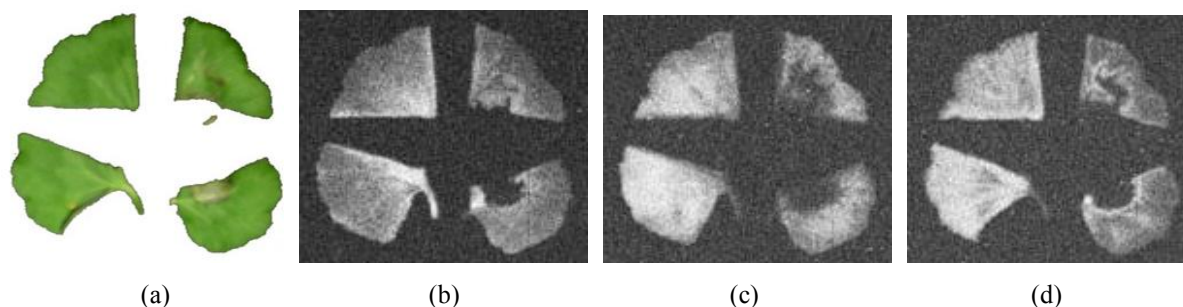


Figure 3. Geranium leaf injured with a hammer blow and cut with a blunt edge into 4 sections. (a) Digital camera photograph. (b) Chlorophyll fluorescence (1-minute exposure). SBE images (10-minute exposures) after (c) 22 minutes and (d) 116 minutes in total darkness.

All of these examples point out the variation of SBE with time for leaves in various states of health, stress, and injury. It is possible to extract quantitative data from these time series to analyze the activity as a function of position and/or time [58].

Since the imaging system used for these examples has a standard glass lens in front of the back-illuminated, uncoated silicon sensor, its wavelength sensitivity is from 420-1000 nm. The systemic properties of interest therefore are those mostly within the red and NIR (The measured response in the deep blue is minimal [58]). The variations in SBE for these images are most likely due to fluorescence of chlorophyll during the first 20 minutes in the dark. After this time, the most likely mechanisms are those related to mitochondria, singlet oxygen, and reactive oxygen species as outlined in Section 2. These examples also clearly illustrate that after 20 minutes in the dark during the ultraweak emission stage, unhealthy, stressed and injured tissue tends to emit more photons than healthy, unstressed, and uninjured tissue.

These examples corroborate the results of many other researchers' work outlined in Section 2 utilizing photomultiplier tube detectors that measure at single points over a limited area.

4. DISCUSSION

From Section 2 it is obvious that there is a long history of research on self-bioluminescent emission (SBE). The early studies by Gurwitsch were on live whole plant subjects. After the invention of photomultiplier detectors much of the research focused on prepared plant and animal samples as they were collecting and measuring total output over a given area of the sample. It should be noted that since many studies have found more emission from stressed and damaged plant parts than from unstressed, whole plant parts, the preparation of the sample has a lot to do with what mechanisms will be measured as does whether the cells and tissues are still live. Systemic properties such as wavelength, amplitude, and possible coherence and photon statistics will depend upon how the sample is processed. From the body of literature we would expect similar results from sliced and diced cells and cut, wounded tissue that would be different from healthy, untouched tissue.

The predominant cellular mechanisms that emit SBE studied have been DNA / RNA processes in the deep blue and UV (150-450nm) and chlorophyll fluorescence, mitochondria, and oxidative metabolisms indicating singlet oxygen, reactive oxygen species and free radicals in the red and NIR (600-1000nm). It should be pointed out that in the red and NIR this

SBE is greater than would be expected from a blackbody radiator. Since plants emit about 200 photons/cm²/sec (steady state after chlorophyll fluorescence decays) and human skin emits about 30 photons/cm²/sec. [20] blackbody radiation would not be a consistent hypothesis. Humans have a higher body temperature than plants and would be expected to emit more blackbody radiation than plants at room temperature.

If we wish to study mechanisms in vivo and track the emission with time, we need to take care of how the samples are prepared and what it is we are looking at. Looking at whole organisms and tracking healing mechanisms is still a young and fertile area of study. Methodologies need to be developed that can enable tracking of a particular mechanism and property without interfering with the health and state of being of the biological sample. Both imaging and multiple-point simultaneous measurement with photomultiplier detectors are good candidates for this type of work [57, 58, 71, 72].

In the literature review I mentioned coherence and photon statistics. Given the scope of this paper, there is not room to go into a long discussion of this. Photons are essentially mass-less blobs having a single quanta of energy that carry information from previous interactions with matter [73]. There has been much speculation about the role of SBE for communication with and in between cells of an organism. When in a steady healthy state, the emission is lower which suggests that photons are absorbed and re-emitted as part of a cyclic process. At these times they are said to be more "coherent" using the coherence theory of Dicke [74], which have certain photon statistics, photon states, photon correlations and hyperbolic decay time [75-78]. As the state of health deteriorates, and stress and damage to the cells increase, the coherence declines, and the photon statistics and decay characteristics change [20, 79]. In order to correlate these findings with specific cellular metabolic mechanisms, there needs to be more basic research to tie this all together. This basic research would enable routine measurements of SBE and its properties as an array to help with various health diagnoses.

5. CONCLUSION

In this paper I have outlined the historical milestones in the measurement and understanding of self-bioluminescent emission. Results from the literature delineating many of the systemic properties and mechanisms behind SBE have been outlined. Examples of some of these properties as measured with a highly sensitive imaging system were presented. And further discussion of my interpretation of the current consensus of this field was outlined.

However, there are many questions that come to mind that haven't been addressed enough to have a consensus. Some of them are listed here. In terms of measurement of photon statistics, when are they Bose-Einstein (classical) or Poisson? Are the photons spaced or bunched (a measure of the variance relative to the mean)? What aspects of basic metabolic process correspond to these different states? In terms of coherence, what are the spatial and temporal characteristics of this emission? Do we see speckle (coherent) effects or interference phenomena? Is this emission polarized? How does the phase of the photons relate to the emission during different states of stress and wellness and how does this relate to different mechanisms? Does the wavelength of the emission strictly depend on the metabolic mechanism? How does it vary? Can this emission be used as a deterministic assay? Or do we have to continue to rely upon looking at emission stimulated by optical and chemical markers?

Although this area of research still has a lot of unanswered questions, the potential is large. Technology for these measurements is improving orders of magnitude each decade. Methodological studies continue to help us understand how to control parameters of importance. The application of our knowledge of photons and light with careful methodologies in basic science experiments with live subjects will enable us to understand a lot better the role of light in biological processes and how we may use that information to determine state of health. Ultimately, the measurement of self-bioluminescent emission could have a large impact on how we test for various types of disease.

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