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Biophotonics in veterinary medicine: the first steps toward clinical translation

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ABSTRACT

In this paper, we analyzed the current situation and the potential of biophotonics and biomedical optics in veterinary medicine. Promising optical techniques such as optical coherence tomography, pulse oximeter, and hyperspectral imaging have been clinically translated into human medicine. But even though human and small animal medicine share personalized and state-of-the-art approach, biophotonics remains rarely exploited in the canine and feline medicine. However, there are some biophotonics studies in veterinary oncology which addressed tumor diagnosis (skin and subcutaneous tumors), prognosis (lymphoma), and therapy (clear surgical margins). Visible and near-infrared spectroscopy served for measuring various physiological parameters related to circulation, and photobiomodulation therapy was often used for the management of wounds, skin conditions, and orthopedic problems. In the research, the most popular clinically translated technique is thermography which was applied for the diagnosis of orthopedic problems and diseases as infections and hyperthyroidism. The future optical devices for small animals such as dogs and cats should be robust and resilient to damage (e.g., due to biting, chewing), offering user-friendly and short measurements. In veterinary oncology, biophotonics could replace invasive fine-needle aspiration procedure. The potential of a pulse oximeter for pet monitoring has yet to be explored. What is more, photobiomodulation efficiency should be tested in an extensive clinical (*in vivo*) study. The technique would be very beneficial in dentistry which currently requires expensive and risky anesthesia.

Keywords: biophotonics, biomedical optics, veterinary medicine, clinical translational, small animals, pulse oximeter, optical coherence tomography, dogs

1. INTRODUCTION

Recently, we have seen a great advance in biophotonics and biomedical optics¹. The promising technologies as optical coherence tomography (OCT), diffuse optical spectroscopy and imaging (also hyperspectral imaging, HSI), fluorescence imaging, and photoacoustic microscopy have been translated to the clinics in order to improve human health and well-being². Especially, oncology, dermatology, surgery, urology, and dentistry benefited substantially.

Even though many technologies (including MRI and CT) were introduced to small animal veterinary medicine, biophotonics remains rarely exploited. It is obvious that most techniques are primarily developed for humans. Therefore, only if a specific technique can be directly or with minor adjustments applied to animals, it gets translated into veterinary medicine (Figure 1). Since animal health and well-being needs differ from those of human's, a clinical translation should be realized directly.

In this paper, we briefly describe the field of veterinary medicine compared to human medicine. We continue with the review of the recent literature describing applications of biophotonics in veterinary medicine. Here, we focus only on the studies with a clear goal to solve a veterinary challenge. Therefore, if animals were used as models or if the animalistic purpose was not clear, the paper was not taken into account. Further, we explored the opportunities of biophotonics in small animal medicine. Finally, our recent work with a pulse oximeter for canine monitoring is briefly presented.

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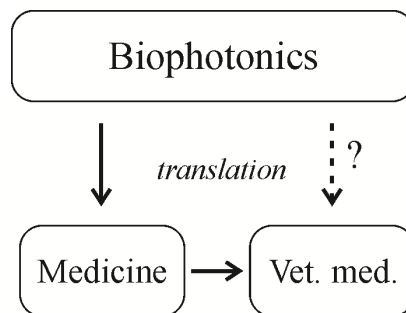


Figure 1. Biophotonics techniques are first translated to (human) medicine. There are rare cases when the optical device is designed especially for animals and translated directly to veterinary medicine.

2. CHARACTERISTICS OF VETERINARY MEDICINE

Veterinary medicine handles non-human aspects of medicine, including preventive, diagnostic and, therapeutic procedures. It also cooperates closely with medical institutions to control diseases, which can be dangerous to humans (i.e., zoonotic diseases)³. Zoonosis can be transmitted by air (e.g., influenza), contact (e.g., rabies), or with food (e.g., *Salmonella* or *Campylobacter* infection). Therefore, an important aspect of veterinary medicine is also monitoring animal-origin food quality and safety⁴. Besides meat, veterinarians inspect eggs, honey, seafood and, dairy products.

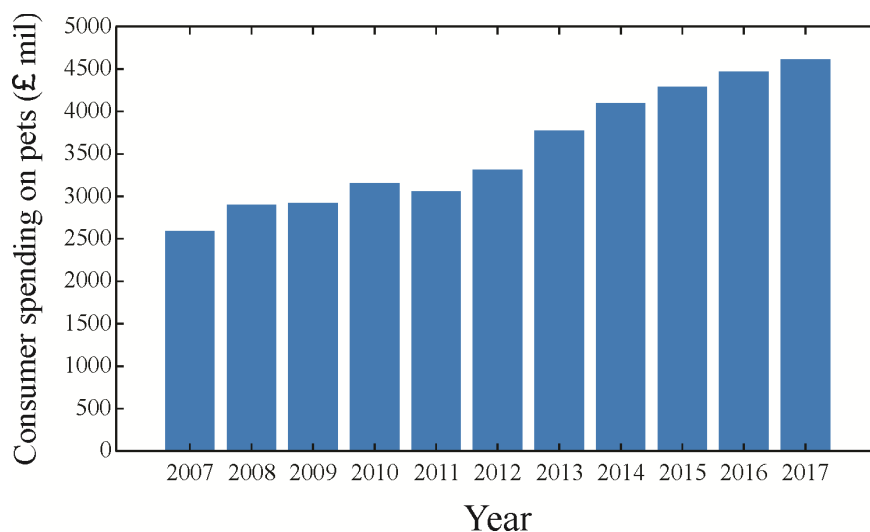


Figure 2. Consumer spending on pets in the UK between 2007 and 2017⁵.

More than 117,000 veterinarians worked in private and public veterinary institutions in the USA in 2017⁶. In Europe, there are around 243,000 veterinarians, responsible for 157 million companion animals and 342 million farm animals⁷. Yearly revenues per European veterinarian in 2015 were a bit more than €110,000 (ca. \$125,000). 72% of revenue is generated by companion animal-related services⁷. In the last ten years, consumer spending on pets and related products in the United Kingdom almost doubled (Figure 2)⁵. However, the average veterinary practice is small as more than 80% of clinics employ less than ten people. In the US, the average veterinary practice has only 2.38 veterinarians⁸. Additionally, the revenues from pet food sales in Europe in 2017 were €20.5 billion with the average growth rate of 2%⁹. Various food diets which correspond to different animal activity levels or medical conditions (e.g., diseases, allergies), are especially popular.

2.1 Companion animals

In the European Union, the most common companion animals are dogs and cats⁷. However, other pets as horses, rabbits, rodents, birds and, reptiles often need medical help as well. The veterinary approach is similar to human since companion animals do not possess just monetary but also a psychological and social value to owners. That calls for personalized and state-of-the-art medicine what is reflected in many published professional guidelines from the veterinary organizations like American Animal Hospital Association (AAHA), American Veterinary Medical Association (AVMA), British Small Animal Association (BSAVA), which are expected to be followed by the practitioners. At the average veterinary clinic, despite small in size, a pet can receive a complete set of preventive (e.g., vaccination), diagnostic (e.g., physical examination, blood analysis, x-ray) and therapeutic services (e.g., sterilization, drug administration, surgery).

Modern veterinary clinics are equipped with state-of-the-art medical technologies such as digital x-ray, blood analyzer and, ultrasound. Almost every clinic possesses a microscope, which serves for the inspection of blood smears, plucked hair (i.e., trichogram), swabs and other tissues taken by the fine-needle procedure (also, biopsy). Additionally, Wood's lamp¹⁰ can help to diagnose dermatophytosis due to the autofluorescence of fungus *Microsporum canis*¹¹. Some clinics have various therapeutic lasers, mostly applied for wound management. Electronic health record systems (EHR) are becoming the dominant way of tracking health documentation and providing business functionalities¹². Moreover, telemedicine¹³, webpage and social media are important communication channels between caregivers and pet owners.

2.2 Farmed animals

Although medicine for companion animals is highly personalized, the same cannot be said for farmed animals (also farm animals, livestock). Farmed animals exhibit mainly monetary (also economic) value; therefore, the choice of veterinary services depends on the ratio between animal value and service cost. Since livestock such as chicken, rabbits and small ruminants have low monetary value, the veterinary approach is often preventive, and it focuses on the whole herd (e.g., antiparasitic treatments, vaccinations). Animals with higher monetary value (e.g., cattle) get basic medical services as infection therapy and simple surgery. Only very precious animals (e.g., animals with specific breeding potential) receive more sophisticated and expensive veterinary treatment. In general, reproduction management and feed conversion efficiency are the main interest since they impact the economics the most.

3. CURRENT STATE-OF-THE-ART: A BRIEF REVIEW

In this brief review, we focused on novel papers up to ten years old, describing biophotonics applications exclusively for veterinary medicine. We identified 43 papers which can be placed into four categories concerning oncology (tumors), measuring various physiological parameters, photobiomodulation, and thermography (Table 1).

Table 1. Recent studies on biophotonics in veterinary medicine.

Category	Main focus
Oncology	diagnosis (skin and subcutaneous tumors), prognosis (lymphoma), therapy (clear surgical margins)
Physiology	various circulation indices (StO ₂ , rSO ₂ , HbI, CRT)*
Photobiomodulation	therapy (wounds, skin conditions, orthopedics), results are contradictory
Thermography	diagnosis (orthopedics, diseases as infections and hyperthyroidism)

*StO₂, (tissue oxygen saturation) rSO₂ (regional saturation of oxygen), HbI (total hemoglobin index), CRT (capillary refill time)

3.1 Oncology

Cancer is the main cause of companion animal morbidity and mortality¹⁴. As in humans, biophotonics can contribute to the cheaper, faster and, less painful oncological diagnosis, prognosis and, therapy.

Diagnosis

Diffuse reflectance spectroscopy (DRS) has been applied for *in vivo* and *in vitro* detection of canine malignant skin and subcutaneous tumors with the sensitivity up to 90%. However, specificity was quite low, only between 54.6% and 73.5%¹⁵. A higher specificity of 94.6% (sensitivity 85.7%) was obtained with hyperspectral imaging which served for differentiation between malignant and benign mammary tumors in 22 dogs¹⁶. Additionally, canine and feline sarcomas were studied by optical coherence tomography (OCT). Texture-based OCT image processing metrics were different for sarcomas, muscles and adipose tissue^{17,18}. Undirectly, tumors can also be detected via certain blood markers. UV absorbance and photoluminescence spectra of serum albumin, globulins, and hemoglobin were acquired to distinguish between cancer and normal patients. 70% of all cancer patients were correctly identified (20% false positives)¹⁹.

The histological examination of biopsy normally gives a final tumor diagnosis. Here, RGB imaging with fluorescence can help to visualize mast cell tumor (MCT) structures. Mast cell granules emitted a specific yellow color²⁰.

Prognosis

Biodynamic imaging (BDI) was used *ex vivo* on 19 dogs with non-Hodgkin lymphoma (NHL) to predict their response to chemotherapy (CHOP therapy)²¹. The prediction accuracy was 84%. The authors believe that results could personalize the chemotherapy and by that, avoid unnecessary interventions.

Therapy

Tumors are often removed by conventional surgery where it is important to achieve clear surgical margins. In practice, this is hard to deliver. RGB or near-infrared (NIR) fluorescence imaging can be beneficial to detect residual cancer cells in surgical margins. In one study, fluorescence LUM015 agent assisted the differentiation between sarcomas and mast cell tumors in 19 dogs. In 92% of cases, the system correctly detected cancerous or normal tissue²². In the second study, NIR fluorescence images matched histopathological interpretation of the diverse group of tumors (perivascular tumors, sarcomas) in 15 dogs²³. There are also many near-infrared fluorescent contrast agents which have potential in veterinary oncology²⁴.

3.2 Physiological parameters

Monitoring decreased tissue oxygen saturation (StO₂) is crucial in critically ill patients for maintaining the adequate oxygen delivery²⁵. Noninvasive StO₂ monitoring methods include near-infrared spectroscopy (NIRS), dark field videomicroscopy, or Doppler flowmetry. In dogs, we found only studies with NIRS applied. Apart from a pulse oximeter, NIRS based devices rely more on wavelengths between 700 and 1000 nm^{25,26}. Sartorius muscle was a recommended measurement site due to ease of positioning, limited hair, and minimal skin pigment²⁷. Expectedly, lower StO₂ was measured in patients with various traumatic conditions as acute hemorrhagic shock and hypoxemia²⁸⁻³⁰.

Additionally, two different NIRS monitors were compared³¹. The comparison resulted in various and generally lower StO₂ values of one monitor which point to unsteady measurements. The potential future work in veterinary medicine can be an evaluation of patients with trauma, sepsis, and surgical diseases, and to detect occult shock²⁵. Additionally, cerebral and skeletal muscle oxygenation (regional saturation of oxygen (rSO₂) and total hemoglobin index (HbI)) was measured by finger-held NIRS device in small dogs (Chihuahua)³². Measuring oxy- and deoxy-hemoglobin with functional NIRS (fNIRS) can observe canine behavior changes³³.

3.3 Photobiomodulation therapy (PBMT)

Despite the fact that photobiomodulation therapy (PBMT) is more than 50 years old, it is one of the most controversial biophotonics techniques³⁴. The positive effects of low-level laser therapy (LLLT), the old name for PBMT, on cells and tissues have been shown *in vitro* or on the models (mostly rats), but the *in vivo* results are often conflicting and the PBMT therapeutic benefit questionable. Some studies claim that many negative trials appeared due to the variations in study designs³⁵.

PBMT has become an important part of veterinary medicine³⁶⁻³⁸. PBMT, as a therapeutic method, was studied on wounds, bone³⁹ or cartilage, arthritis, ligament or tendon, analgesia, nociceptors, nerves, and spinal cord. So far, the most promising results appear in the management of cartilage and nerve injuries, and lick granulomas (also lick dermatitis)³⁶.

Recent studies have expressed doubts about PBMT. First, laser therapy, initially described in 1968, showed potential for more effective wound management by normalizing cell function, stimulating collagen alignment and enhancing the local blood flow³⁷. However, PBMT did not appear to influence the healing time of acute or surgically created wounds in dogs^{40,41}. Similarly confusing are studies on postoperative recovery of dogs undergoing hemilaminectomy (i.e., surgical procedure after intervertebral disk herniation) and osteotomy. On the one hand, studies showed that PBMT reduces time to ambulation due to reducing pain, edema, and inflammation⁴² and increase gait functionality⁴³. On the other hand, there was no difference in recoveries and pain of dogs, which received PBMT and sham therapy^{44,45}.

In veterinary dermatology, PBMT was used for the treatment of various dermatitis (acral lick, pyotraumatic, folliculitis), anal sacculitis, hematoma, pemphigus, alopecia, and cellulitis^{37,46,47}.

3.4 Thermography

Thermography is based on the infrared range between 9 and 14 μm . On the other hand, biophotonics includes techniques with the upper wavelength range of 10.6 μm ¹. Therefore, thermography is more borderline than typical biophotonics technique. However, it seems that thermography is one of the most studied optical (imaging) method in veterinary medicine⁴⁸; therefore, we decided to dedicate one section to this field as well.

In animals, thermography represents a potentially safer and cheaper alternative to x-ray, CT and, MRI^{49,50}. The technique was applied to study various viral or bacterial diseases, orthopedic conditions like laminitis, tissue perfusion, reproduction issues, production, and welfare⁴⁸. In dogs, thermography was used for diagnosing bone cancer (sensitivity 100 %, specificity 80 %)⁵⁰. The rest of the problems addressed by thermography belonged to orthopedics where an anterior cruciate ligament (ACL)⁴⁹, hips, stifles, elbows, and disc disease were studied^{51,52}. In cats, thermal imaging detected hyperthyroidism with 87.5% accuracy⁵³. Thermography is popular also in horses, where the technique was used to detect various diseases (e.g., Horner's syndrome, neurectomies) and to address various challenges in equine orthopedics^{54,55}.

3.5 Other applications

Fluorescence spectroscopy was used for analyzing urine from healthy dogs and dogs with various disorders (cancer, portosystemic shunt, fever, renal failure, and Cushing's disease). The authors discovered that the studied disorders impact urine fluorescent attributes⁵⁶. Besides urine, blood samples were analyzed by Fourier transform infrared spectroscopy (FTIR) in order to distinguish between healthy dogs, dogs with nonfood-induced canine atopic dermatitis, and dogs with non-allergic inflammation⁵⁷. Additionally, visible and NIR spectroscopy was used for detection of chondrodystrophoid canine intervertebral discs⁵⁸.

4. FUTURE CHALLENGES

As showed in Section 3, biophotonics has just started its journey across the field of veterinary medicine and by that, many opportunities arise in every (veterinary) direction. One of the biggest issues in the field is patient behavior. With humans, relative physical and emotional serenity can be achieved for measurements. That certainly cannot be said for pets since they like moving, avoiding, jumping, barking, and scratching, not just due to fear, sometimes also due to positive excitement. Therefore, simple and short procedures as temperature measurement (rectally) or blood collection (from the cephalic vein on the front leg) can become very challenging. Also, devices and prototypes are in danger and are often in for repair.

Non-invasive techniques would be especially welcomed. For example, diagnosing superficial tumors (e.g., skin and subcutaneous tumors) requires fine-needle aspiration (FNA) procedure. Since benign tumors represent at least 38% of all tumors (a pet can often have multiple benign lesions)⁵⁹, FNA is not very practical. Conventional spectroscopy (DRS) has already been tested for the tumor diagnostics¹⁵, but the more portable and user-friendly system would be convenient. If economically sound, handheld OCT, currently being introduced to human clinics⁶⁰, could be a solution. What is more, fluorescence could accelerate and facilitate classical tumors diagnostics (i.e., histopathology), *in vivo*⁶¹ or when biopsy has already been performed. Finally, optical techniques can detect tumor biomarkers^{62,63} or tumorous cells directly⁶⁴.

Table 2. Identified main challenges of biophotonics in veterinary medicine.

Category	Challenge
Device design	Robust optical devices which will offer simple and short measuring procedures
Oncology	Can biophotonics replace FNA?*
Pulse oximeter	further sensor exploitation for pet monitoring
Photobiomodulation	extensive clinical (<i>in vivo</i>) study needed, less painful approach in dental medicine

*FNA (fine-needle aspiration procedure)

As discussed in the next section, pulse oximeter has big potential in pet monitoring⁶⁵. We think that new probes adjusted to animal anatomy and physiology should be developed and clinically tested. That will allow performing more specific PPG studies on pulse transit times (PTT), PPG pulse shape, etc. For example, the acquired data could serve for optical blood pressure estimation⁶⁶.

PBMT is highly promoted in veterinary medicine, but good clinical (*in vivo*) studies are missing⁶⁷. In major books, only separate clinical cases are presented. However, it seems that lasers can be applied less painfully, e.g., when performing surgery of maxillary and mandibular bone³⁹. Another beneficial PBMT contribution would be in veterinary dentistry since most of the dental procedures require (expensive) anesthesia. Treating periodontal disease could be especially welcomed.

5. RECENT RESULTS

Recently, we have been exploiting the monitoring potential of a pulse oximeter in small animal medicine. First, we studied photoplethysmography (PPG) signal quality on several canine and feline measurement sites that would be better tolerated than the oral sensor placement by conscious animals⁶⁵. We discovered that a pet monitor with pulse oximeter could be applied on the canine metacarpus or the tail. In cats, the digit could be used. Secondly, an optical device was tested on various measurement sites for the estimation of canine capillary refill time (CRT)^{68,69}. CRT is a part of the cardiorespiratory examination and can reflect pathological conditions like shock or anemia. In both studies, we identified the metacarpal pad, digit, or tail as a potential site for CRT estimation by the proposed device. However, many measurements were affected by heavy pigmentation, motion artifacts, and other contact-pressure related effects⁷⁰.

Currently, we are testing if the pulse oximeter can improve CADESI-4 scale⁷¹ for canine atopic dermatitis severity by introducing objective erythema index (EI) estimation. Since our pulse oximeter included green and red LEDs, two EI equations were employed⁷²:

$$EI_1 = R / G, \quad (1)$$

$$EI_2 = (G - R) / (G + R), \quad (2)$$

where G and R are green and red optical signals, respectively. We applied linear regression between clinician estimated-erythema level (EL, 0-3 or 0-100%) as an independent variable and measured EI_1 or EI_2 . The results with eight dogs (inguinal measurement site) are presented in Table 3 and Figure 2. R-squared was between 0.54 and 0.71 what points to the potential of a pulse oximeter to benefit CADESI-4 scale.

Table 3. R-squared (r^2) of linear regression analysis.

	EI_{rg}		EI_{bc}	
	r^2 (%)	r^2 (0-3)	r^2 (%)	r^2 (0-3)
Inguinal	0.56	0.69	0.54	0.71 ¹

¹Figure 3

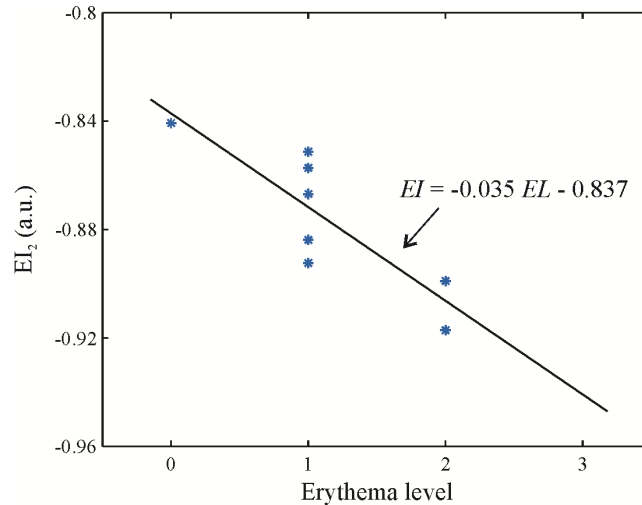


Figure 3. Linear regression fitted line between erythema level (EL) and erythema index (EI).

6. CONCLUSION

Biophotonics and biomedical optics are predominantly applied in human medicine. However, veterinary science is developing rapidly as well, because pets are becoming more and more emotionally valuable. We believe that biophotonics should be translated directly into veterinary practice accordingly to peculiar animal needs (different anatomy and behavior). We think that various optical methods can contribute to veterinary oncology (e.g., spectral imaging), monitoring (e.g., pulse oximeter), and dentistry (e.g., photobiomodulation).

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