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Some Medical and Biological Applications of Ultrafast Lasers

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Abstract

Ultrashort lasers are finding many applications in medicine and biology. Pulses from such systems can be used directly or after non-linear modification. Direct utilisation includes time-resolved fluorescence spectroscopy and imaging. Such studies can be performed for tissue diagnosis in the field of malignant diseases as well as in the cardiovascular sector. Two-photon induced fluorescence provides a basis for high-quality optical microscopy, and optical coherence tomography with ultra-short and thus broadband pulses allow a radar-like depth-ranging into tissue. Photon propagation in scattering media also has important applications in optical mammography, dosimetry for photodynamic therapy and species concentration assessment. Intense continua of electromagnetic radiation of very brief duration are formed in the interaction of focused ultra-short and intense laser pulses with matter. Focusing into water, or better, a photonic band-gap fibre, leads to the generation of a light continuum through self-phase modulation. The propagation of white light through tissue was studied addressing questions related to tissue chromophore concentration measurements employing their absorptive imprint in the light. Even free gas enclosures in solids and liquids give rise to an absorptive signature, which, however, is typically 10^4 times narrower than spectral structures in the matrix material. Such sharp features are best detected by wavelength modulation of a single-mode laser, and many new applications of gas-in-scattering-media absorption spectroscopy (GASMAS) are developing. When terawatt laser pulses are focused onto a solid target with high nuclear charge Z, intense X-ray radiation of few ps duration and with energies exceeding hundreds of keV is emitted. Biomedical applications of this radiation are described, including differential absorption and gated-viewing imaging.

1 Introduction

We will start our discussion of biological and medical applications of short-pulse optical radiation with some background regarding the interaction between electromagnetic radiation and biological tissue. Such interactions are discussed, e.g., in [1]. The interaction is governed by absorption and scattering processes. Absorption occurs mainly through the interaction with the major tissue constituents. Water absorbs all radiation below about 190 nm and is transparent up till about 1400 nm. Haem absorbs through the visible region with a maximum at about 400 nm (the Soret band) till about 600 nm, where the absorption falls off drastically, rendering blood its characteristic red colour. Aminoacids and melanin absorb through the UV and visible regime with an absorptivity falling of towards the red and near IR-regions. Practically, this means that the region from about 600 - 1400 nm is particularly transparent to radiation. Here scattering is instead the dominant interaction leading to the well-known phenomena of diffuse transmission of red light through tissue. While red light readily penetrates through a finger no shadow of the bones can be seen. When high-energy levels of CW radiation (more than 10W) in focused beams are used, strong tissue heating occurs, leading to coagulation or carbonisation of the tissue. Thermal treatment is the most wide-spread laser modality used, e.g. in surgery and eye treatments. Intense pulses in the UV region, where the penetration depth into tissue is very low, lead to ablation where cell layer by cell layer can be lifted off with high precision, e.g. in refractive eye surgery. Ultra-short laser pulses also lead to ablative interaction due to strong non-linear effects, and femto-second lasers are finding many applications for precision surgery.

A further laser-matter interaction mechanism of non-thermal nature is the photodynamic action. Photodynamic therapy (PDT) provides a method to selectively eradicate malignant cells while leaving the normal cells intact [2-4]. Here a particular chemical agent, a sensitiser, is administered to the body and localises selectively to malignant cells. Using light matching an electronic transition of the agent, it is excited. The excitation energy is transferred to oxygen molecules, which normally are in their ground triplet state. The resulting singlet oxygen is very toxic to tissue and induces necrosis. There is a certain parallel to photosynthesis in the environmental field. There the combination of a particular chemical agent, chlorophyll, and light brings about the build up of organic material out of carbon dioxide and water in a cold photochemical procedure. PDT is from a certain aspect the opposite process; the agent in combination with light instead breaks down organic material into its components, also in a cold process.

PDT was first introduced at the turn of the last century. However, with the development of pure and efficient sensitisers combined with laser sources at the relevant wavelengths, the field has expanded a lot, first through the work of T. Dougherty, demonstrating the treatment efficacy of haematoporphyrin derivative (Photofrin), absorbing at 630 nm. Actually, the sensitiser absorption must fall within the range 600 nm to 1400 nm to allow penetration into tissue. In practice it must also be below 1 μm in order to ensure enough energy to allow the energy transfer to oxygen. While most sensitisers such as mesotetrahydroxy phenyl chlorine (Foscan; 652 nm), benzoporphyrin (Verteporfin; 690 nm) and lutetium texaphyrin (Lutrin; 732 nm) must be administered intravenously, the

protoporphyrin IX (PpIX) precursor δ-amino levulinic acid (ALA) has the advantage of additional possibilities of topical or oral administering. ALA is a natural constituent in the body, utilised as the starting material in the haem cycle. Strongly fluorescing and photodynamically active PpIX is produced at higher concentrations in skin tumours after applying an ALA-containing cream over the tumour a few hours before irradiation with light at 635 nm. The very efficient ALA-PDT was introduced by Z. Malik and J.C. Kennedy [5,6] and has been used widely also in our group [7-9].

Sensitising agents frequently exhibit a strong and characteristic fluorescence signal that can be utilised for localising and demarcating tumours. Porphyrins are best excited at the peak of their Soret band around 400 nm. If a shorter excitation wavelength is used (optimally around 340 nm), autofluorescence from endogenous chromophores can be used for tumour diagnostics, since the signatures do frequently differ from normal tissue. Fibre-optic fluorosensors with a compact pulsed nitrogen laser emitting at 337 nm have been much utilised. The laser light is transmitted to the tissue through the fibre, which also picks up and conducts the fluorescence signal to the entrance slit of a gated and intensified detector, displaying the full LIF spectrum for each laser shot. Instrument constructed in this way are described, e.g. in [10,11]. A simplified variety employs a violet diode laser at 397 nm to induce fluorescence, which is recorded by a compact integrated spectrometer with array detector read out [12].

Most medical diagnostic devices provide image information. It is also possible to build multi-spectral fluorescence imaging systems. Then a larger tissue area is illuminated and the fluorescence is simultaneously recorded in several fluorescence bands using special beam-splitting optics [13-15]. Tumour tissue is characterized by a strong intensity increase at 635 nm due to the sensitizer, at the same time as the tissue autofluorescence level around 470 nm is reduced. Three detection bands are used. By subtracting signals recorded at 635 nm by the signal at about 600 nm a background-free peak intensity is obtained, and this intensity is then divided by the blue intensity, resulting in a dimensionless quantity featuring enhanced contrast and immunity to detection geometry etc.

Fluorescence imaging can also be performed using a push-broom sensor, where multi-spectral imaging is performed line-wise in a similar way as in a satellite imaging spectrometer. Instead of using the platform movement for the line advancement, it is achieved by tilting a scanning mirror. This technique employing the strong mercury line at 365 nm for excitation has been applied to gynaecology [16]. By ALA instillation into the bladder, it is possible to detect dysplasia and carcinoma *in situ* using plain visual inspection, as performed in a commercial system by Storz.

2 Time-Resolved Fluorescence Spectroscopy and Imaging

An excited state is characterised not only by its energy but also by its lifetime. In the measurements just discussed only the spectral distribution is utilised. However, the decay time of the fluorescence, excited by a short laser pulse, can also be employed. Tissue

consists of numerous constituents which contribute to the spectral shape of tissue and also to the decay characteristics. The excited state lifetimes in a number of the major constituents have been determined using picosecond laser excitation followed by time-resolved single-photon detection [17-19]. Frequently, several overlapping excited states with different lifetimes are excited, and several decay components with different time constants in the range ofhundreds of picoseconds to 10 ns are observed.

The different temporal characteristics of excited states have also been used in fluorescence imaging experiments by Cubeddu et al. A short-pulse nitrogen laser is used to illuminate a larger tissue area, and the fluorescence is observed through a filter with a time-gated intensified CCD detector. By recording the images with different delays it is possible to generate a lifetime image. In tissue prepared with ALA, a tumour region is then characterised by longer lifetimes than the surrounding normal areas due to the longer lifetime of porphyrins [20].

The same type of lifetime measurements are performed on a microscopic level, frequently utilising two-photon-induced fluorescence. Such instruments have a very high optical resolution and are little vulnerable to scattering from out-of-focus regions. Commercial equipment employing also the temporal characteristics are available.

3 Ultra-Short Laser Pulse Propagation in Scattering Media

Multiple light scattering in weakly absorbing media is a common phenomenon observed in clouds, fog and in water. While some light is transmitted (there is still daylight on a cloudy day) image contrast disappears (the sun disc is not observed through the cloud). Milk is also a strongly scattering medium, and the presence of, e.g. a cherry, held by the stem inside a glass of milk, cannot be assessed in normal transillumination. If the image blurring due to scattering somehow could be defeated, red light tissue transillumination would be attractive for optical mammography without the use of ionising radiation. Also it would be very interesting to localise haematoma after trauma to the skull. Preceding such imaging applications, primary studies concerned the study of brain oxygenation [21]. First imaging recordings with scattering rejection through living tissue were reported in 1990 [22]. The basic idea behind imaging through turbid media using time-resolved spectroscopy is to restrict the detection to the photons which travel through the medium without being scattered. This corresponds to the first emerging photons following short-pulse injection. The non-scattered "ballistic" photons are recorded as the transmitter - detector line-of-sight is scanned across the sample. If an object of different absorbing and scattering properties is hidden inside the scattering medium the ballistic signal is changed.

Optical mammography using time-resolved detection of transmitted light has been demonstrated. In one approach a pulsed diode laser system was used in transillumination of breast specimen placed between two glass plates. The diode laser operated at 815 nm and produced 30 ps pulses at a rate of 10 MHz. The single-photon time-correlated detection technique was used. A ductal carcinoma could be revealed in time-gated

transillumination, while the image corresponding to the time integrated signal did not contain any diagnostic value [23]. Many techniques for time-gated imaging have been developed, including the use of optical Kerr gating, stimulated Raman amplification and second-harmonic cross correlation. Other techniques, which focus on the distinct phase properties of the non-scattered light, are heterodyne detection and light-in-flight holography. Different aspects of light in scattering media such as tissue are covered in [24]. Time-resolved photon propagation studies have also been performed in green leaves from plants [25].

Related to time-resolved propagation studies in scattering media is the field of optical coherence tomography (OCT). This technique was first developed for probing the human fundus. An experiment by Fujimoto *et al.* in 1986 can be seen as a precursor to this technique [26]. Here short pulses were propagated into the optical system of an eye and reflexes at the passages through the optical elements were recorded similarly as in laser radar sounding. The arrival time of the echoes were detected by frequency doubling of the radiation if the backscattered light and a delayed reference pulse arrived simultaneously in a non-linear crystal, as is frequently utilised in autocorrelators. It was subsequently realised that an improved result could be obtained by using broad-band CW light in a Michelson interferometer and observe the intensity increase when the interferometer arms were equally long and all frequency components interfere constructively [27]. The broader the wavelength distribution, the better the spatial resolution. This has reintroduced the use of pulsed lasers in OCT, since a short femto-second laser pulse covers a spectral bandwidth of 100 nm or more. By carefully compensating for group-velocity dispersion a spatial resolution of 1 μm can be achieved [28].

4 Species Concentration Measurements in Turbid Media

Frequently it is of great interest to be able to measure the concentration of a substance in the presence of heavy scattering. The scattering results in the optical path length being undefined and the normal Beer-Lambertian law does not pertain to the measurements. It is then important to be able to "orthogonalise" the effects of absorption and scattering to be able to access both. This can be made by solving the transport equation in the turbid medium, or by relying on Monte-Carlo simulations. It is clearly necessary to measure at many different wavelengths, since absorption and scattering obviously respond to wavelength shifts differently. A number of discrete wavelengths can be employed, but even better "all" wavelengths, e.g. white light. When high-power laser pulses are focused into a condensed medium such as water, a light continuum is generated through self-phase modulation. The origin of the phenomenon is the dependence of the index of refraction on the light intensity, leading to frequency chirps when the pulse intensity is rising and falling. Following the injection of such pulses into strongly scattering media, such as tissue, the time dispersion of the photons can be studied. The spectral contents and the time duration of the radiation can be measured with a streak camera coupled to the exit slit of a spectrometer. A two-dimensional representation of time versus colour is obtained as demonstrated in [29].

In the white-light propagation studies, a chirped-pulse amplification (CPA) laser system based on Ti:Sa is conveniently used. The CPA technique allows to achieve extremely high optical intensities in compact laser systems. Recently, the introduction of photonic bandgap fibres allows a very efficient white-light generation, and a mode-locked femtosecond lasers can be used directly without the need for pulse amplification.

This type of measurements can be used for studying absorption spectra of sensitisers *in situ* in living tissue [30]. A rat tumour model was used, where a human adenocarcinoma, inoculated on the hind leg muscle produced tumours of about 15 mm diameter. Optical fibres were used to inject the light in the tissue and to collect scattered light emerging again at a distance of about 8 mm. The rats were injected with disulphonated aluminium phtalocyanine at a concentration of 2.5 or 5 mg/kg body weight and measurements were performed both on the tumour and normal tissue. From the experimental recordings the absorption and scattering coefficients can be extracted. This allows the plotting of the absorption profile of the sensitizer *in vivo* and an assessment of the tumour/normal tissue uptake ratio. Similar techniques can be used to extract tissue absorption and scattering properties related to optical mammography [31]. A further application is the assessment of the concentration of certain near-IR-absorbing molecules in scattering powders. Pharmaceutical preparations are of particular interest [32].

5 Gas in Scattering Media Absorption Spectroscopy (GASMAS)

The spectral absorptive features of solids and liquids are broad and seldom sharper than 10 nm due to the interactions between the participating atoms. Thus the spectroscopic equipment used for the study of such samples seldom has a very high resolution. However, an many samples there are structures which are 10,000 times sharper - enclosures of free gas. Enclosures mean that the materials are then also scattering due to the changes in refraction index. Thus there is again no straight-forward Beer-Lambertian law to analyse the results. Instead, the techniques wellknown in tissue optics as just discussed can be used. We have recently introduced a new spectroscopic method of considerable potential, the Gas in Scattering Media Absorption Spectroscopy (GASMAS) [33,34]. Here a light from a single-mode diode laser is conducted by a fibre to the sample, which is placed in proximity to the photocathode of a photomultiplier tube, in order to allow a maximum detectivity of the diffusely transmitted light, which might be very faint. By wavelength- or frequency-modulation techniques it is possible to sensitively detect the sharp absorptive imprint due to the gas. Our first measurement have concerned normal atmospheric oxygen molecules, which were studied in matrices as diverse as polystyrene foam, wood, and fruits. Concentration assessment can be achieved by also performing time-resolved measurements with a pulsed laser at the same wavelength using a set-up very similar to an optical mammograph. Then the time-dispersion curve will give information on the "history" of different photons travelling short or long distances through the sample. The mean travel time through the sample can be evaluated to be used for concentration evaluation.

A particularly interesting application is to study gas diffusion through porous material. Then the sample is first stored in a nitrogen atmosphere for few hours, e.g. by placing it in a plastic bag, which is briefly flushed from a nitrogen tank and sealed with a tape. After several hours the sample is taken out and placed in the GASMAS apparatus, and the successive reinvasion of the normal air into the sample can be followed by the oxygen signal [35]. E.g., it was found in studies of 10 mm thick samples of Norwegian Spruce and Balsa wood, that the latter exhibits a much longer time constant, probably related to well sealed individual cell compartments. Clearly, diffusion can be characterised by several time constants. The influence of surface coatings such as paints or plastic films can be readily studied. Internal pressure in a sample can also be monitored non-intrusively through the pressure-broadening of the absorption lines [33].

The monitoring of concentration ratios of two gases absorbing at close-lying lines eliminates the need for a detailed understanding of the photon transport through the sample and could give new insights in field as diverse as plant physiology and catalyst research.

Additional information on applications of laser spectroscopy to biology and medicine can be found, e.g. in [36-39].

6 Laser-Produced X-Rays

Medical application of X-rays has developed during 100 years and now constitute one of the most important diagnostic modalities. X-ray generation is accomplished through acceleration of electrons into a material with a high nuclear charge Z. With the development of short-pulse lasers with output powers in the terawatt range it has become possible to generate hard X-rays by focussing the radiation to a small spot on the surface of a high-Z material [40]. While this generation scheme is obviously more complicated and costly it can present some advantages which are mostly related to the small source size and the potential to reduce the X-ray dose by viewing techniques in an analogue way as discussed for optical mammography in Sect. 3. Early high-resolution X-ray imaging was reported in [41,42].While it is easy to understand why scattered photons need to be eliminated in the optical regime due to the obvious and massive light scattering, it is not so evident for X-rays which are known to produce sharp images of ribs etc. However, in whole-body X-ray radiography without any special precautions actually 90% of the photons reaching the plate are Compton scattered and contribute only to a diffuse background. This background is frequently reduced using anti-scattering (straight-vision) grid plates between the patient and the recording plate, to reduce the photons having the wrong angle. The price is obviously that the patient has to be exposed to a higher dose of ionising radiation. The potential of medical X-ray gated viewing was first pointed out in [43]. We have performed detailed studies of gated X-ray detection using a 10 Hz high-energy laser system in combination with an X-ray streak camera [44], and also demonstrated gated tomographic X-ray imaging [45].

Contrast agents are frequently used in radiography. By utilising the sharp K emission lines from the laser-produced plasma and employing two different target materials so that the K-lines bridge the K absorption line of a contrast agent (at 33 keV for iodine, at 50 keV for gadolinium; both approved contrast agents). By dividing two images taken with different target materials only the contrast agent survives the division, and demonstrations of this kind are presented in [46]. Presently, medical image plates, which are much more sensitive than photographic plates and also re-usable, have been employed. The future for laser-produced X-rays in medicine will depend on the possibility to develop compact and efficient laser systems and gateable image plates. Recent experiments with a 1 kHz relatively compact system gave encouraging results [47].

A very important aspect in the potential use of laser-produced X-rays is the demonstrate that these widely spaced and extremely powerful X-ray spikes do not present an additional detrimental ionising radiation effect for the same integrated Gray dose. A first experiment using V79 Chinese hamster cells showed that this does not seem to be the case [48].

7 Discussion

Ultrafast laser provide many new possibilities for diagnostics and analysis in biology and medicine. Sometimes a moderate temporal resolution is sufficient, such as in time-resolved fluorescence imaging, while other applications, such as white-light or X-ray generation, benefit from the fastest possible pulses. Certain applications, such as two-photon-induced fluorescence microscopy and OCT, as well as certain aspects of precision medical surgery, have already reached a commercial stage. Very compact, rugged and reliable diode-laser pumped femto-second lasers are becoming readily available and will strongly influence the wide-spread use of the technology. Also large CPA-based terawatt systems have the potential for important applications in medicine. Induction of nuclear reactions leading to the production of short-lived radioactive isotopes for single-photon emission computerised tomography (SPECT) and positron emission tomography (PET) is an attractive possibility as is laser-driven acceleration of protons for tumour therapy [49].

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