TERAHERTZ SENSING IN BIOLOGY AND MEDICINE

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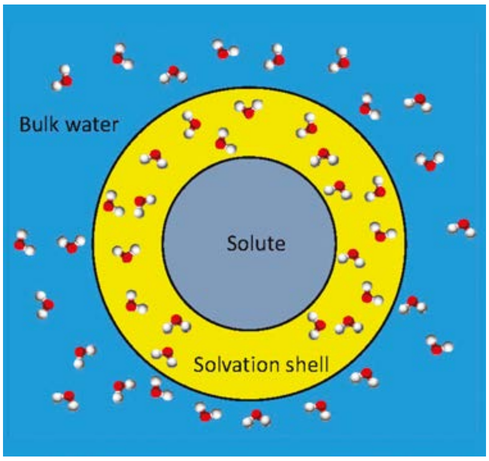

Terahertz radiation offers new contrasts with biological systems, without markers or staining, at the molecular, cellular or tissue level. Thanks to technological advances, it is increasingly emerging as a solution of choice for directly probing the interaction with molecules and biological solutions. Applications range from dynamics of biological molecules to imaging of cancerous tissues, including ion, protein and membrane sensors.

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Many imaging techniques are currently available to observe biological systems in 2D or 3D (microscopy, OCT, X-ray, NMR, PET, ...), each having advantages and drawbacks. The terahertz wave domain (0.1 to 10 THz) is one of the least studied regions of the electromagnetic spectrum, and this is even more notable in the field of sensing and imaging. Until now it has been challenging to generate and detect terahertz radiation. However, these difficulties are gradually being overcome by new commercial terahertz sources and detectors, increasing the development of applications in life sciences. The terahertz range offers new imaging contrasts thanks to a strong coupling with molecular vibrational modes. Water plays a paradoxical central role. On the one hand, its strong absorption significantly limits the penetration depth through biological samples. But on the other hand, the modification of its dielectric properties is a marker for sensing and imaging. After discussing the origin of this contrast, we will present the major techniques used to investigate biological systems. Then, we will detail applications from the sensing of fundamental biological molecules to medical imaging.

THz INTERACTION WITH BIOLOGICAL MOLECULES

Pure water composes about 62% of a human body, followed by proteins/peptides (17%), lipids (14%) and ions (6%). Pure water is a polar liquid and thus strongly absorbs and disperses terahertz radiation. The dielectric constant of bulk water is well fitted by an overdamped Debye-type relaxation model which reflects the coupling with the hydrogen bounding (HB) network in liquid water and several spectrally broad vibration modes (HB binding, stretching, hindered rotation, ...) [1]. The absorption spectrum of water is very broad, with higher absorption at higher frequencies. However, water is never found pure in biology, but associated with solutes such as proteins, peptides and ions. A simple model can explain the modification of the dielectric constant of water by the solutes. Solute molecules, in particular large biomolecules such as proteins, have a dielectric constant different from bulk water. But they also alter the properties of a layer of water molecules close to the solute: the solvation shell. Therefore, the alteration of the terahertz dielectric properties of the solution originates from both the substitution of •••

bulk water by solute molecules, and from the relative importance of the solvation shell [2]. Small molecules such as ions mostly contribute to the solvation shell, whereas proteins bring their specific vibration modes. Therefore, the nature and concentration of solutes in liquid water is the main contrast factor for biological systems For instance, lipids absorb less than water, which can be used for cancer imaging purpose [3, 4]. In solution, biomolecules (proteins, DNA, ...) exhibit large vibrational modes due to inhomogeneous broadening. Therefore, many investigations are performed in dry or cryogenic states for more accurate spectral analysis, at the expense of preserving the same properties as in-vivo.

SENSING BIOLOGICAL SYSTEMS

TERAHERTZ SPECTROSCOPY

The expansion of terahertz spectroscopy originally started in the 1990's with the development of the terahertz Time Domain Spectroscopy (THz-TDS), either with photoconductive antennas or optical rectification, and based on femtosecond pulsed lasers. THz-TDS probes matter with short pulses of terahertz radiation. Accessible frequencies range from 0.03 to 30 THz. At room temperature, it provides a high precision and consistent measurement of the absorption and refractive index of the material, in transmission or reflection geometries. Fiber based compact systems are now commercially available. A terahertz pulse is recorded in the time-domain, and the complex Fourier transform of the terahertz signal provides the complex dielectric constant of the sample. In biology, the reflection geometry is often preferred due to strong water absorption. Classical Fourier transform infrared spectrometer (FTIR), with their Michelson-like scheme, are still widely used nowadays, in particular above 3 THz. However, they are of less interest for biology because water absorption is very high in their area of interest. High resolution spectroscopy can be achieved by photomixing. Two continuous lasers are mixed together and focused on a photomixer chip which generates tunable frequencies with spectral resolution down to 1 MHz. A wide variety of other terahertz sources can also be used [5]: electronic systems such as klystron, gyrotrons, free electro lasers, BWO, synchrotrons. Terahertz laser sources also include quantum cascade lasers (QCL), semiconductor such as CMOS or gas lasers.

TERAHERTZ MICROSCOPY

To image small samples, the terahertz systems must compete with the diffraction limit in the far field. The far-field resolution Δl is given by Rayleigh criterion $\Delta l = 1.22\,\frac{\lambda f}{D}$ where λ is the wavelength, f the focal length and D the aperture diameter of the lens, making very difficult to obtain a resolution better than the wavelength (*i.e.* 300 μm at 1 THz). This is enough to image large samples such as skin or biopsy samples, but not to resolve individual cells with a typical size of the tens of micrometers. Near-field techniques can break the diffraction limit by coupling the light to a subwavelength object and can reach resolutions as small as $\lambda/100$ [6]. Near-field techniques imply an important loss of energy, thus they require very high signal-to-noise-ratio measurements and a trade-off between signal and resolution. Examples are: near-field imaging with micro-probes or apertures [7], subwavelength electro-optic crystals and photoconductive antennas, terahertz nanoscopy coupled with Scanning Near-Field Microscopy (SNOM) [8], or evanescent waves in Attenuated Total Reflection [9]. Another aspect of microscopy is to reach remote samples, for instance the interior of an organ or a cavity of the body. The terahertz radiation has to be delivered directly into the organ by the help of endoscopes using hollow core fibers, metallic waveguides, or by generating and detecting the terahertz probe directly into the organ using optical fibers.

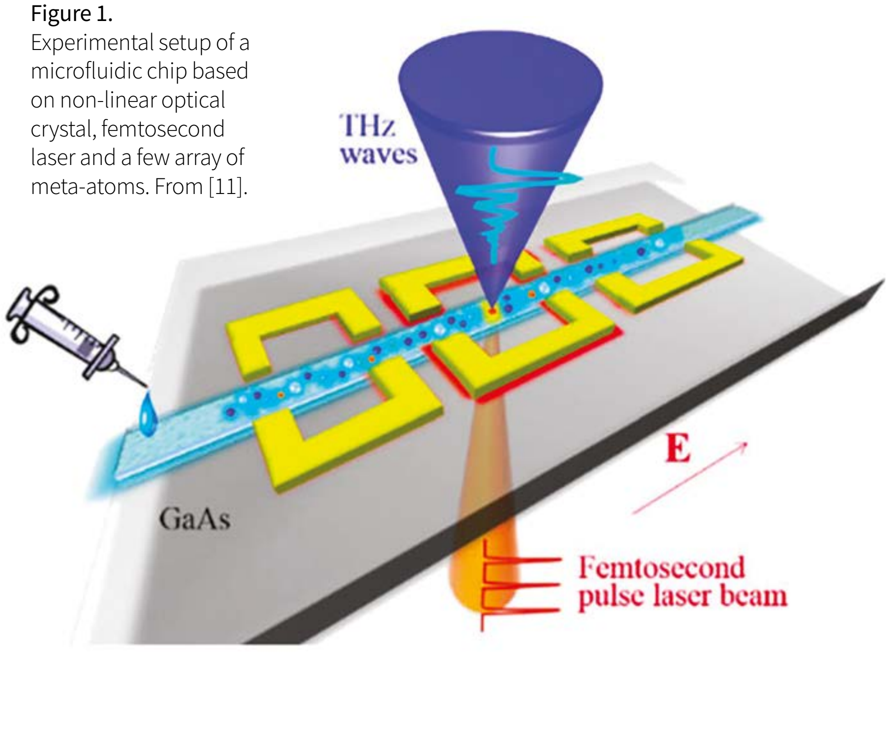

Figure 1.
Experimental setup of a microfluidic chip based on non-linear optical crystal, femtosecond laser and a few array of meta-atoms. From [11].

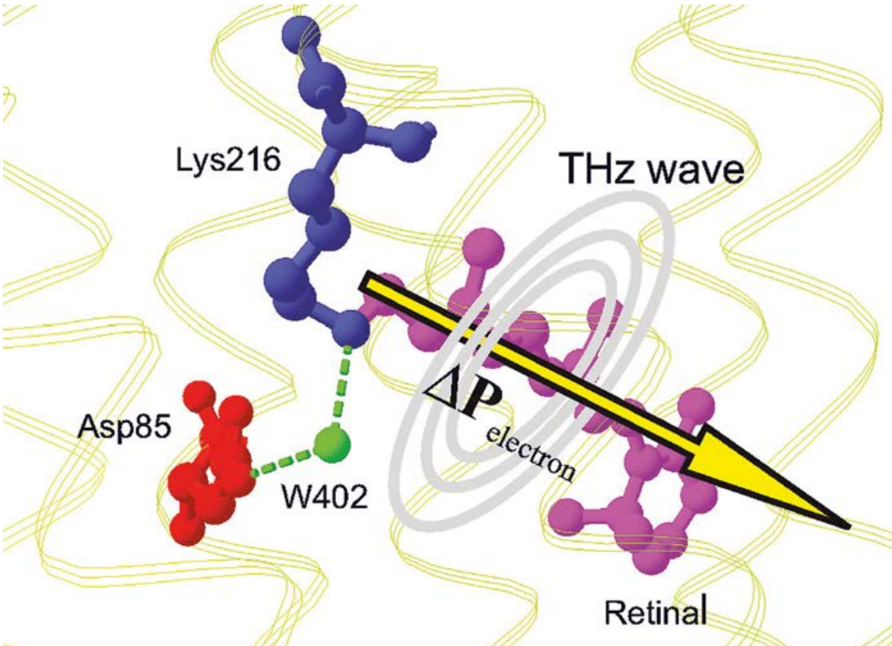

Figure 2.
Deconvoluted 3D image of the axon obtained with near-field technique with aperture, using finite element method (FEM) simulations. From [13]. Copyright (2008) National Academy of Sciences, U.S.A.

BIOSENSORS

Terahertz biosensors are sensors probing molecules of biological interests (proteins, enzymes, antigen, ions, ...) using terahertz radiation. They measure the change of the terahertz permittivity due to biomolecules. Terahertz biosensors focus on two main objectives: improving sensitivity (*i.e.* reaching lower concentration level of biomolecules), and reducing the volume of samples since biomolecules are often found in small quantities. Spectral shift can be used to differentiate molecules, such as hybridized and denatured DNA [10]. Metamaterials can increase the sensitivity of detection by several orders of magnitude, such as split-ring resonators, meta-atoms [11], plasmonic effect in nanoparticles, photonic bandgap sensors, plasmonic antennas. A second aspect is the reduction of the probed volumes. Microfluidic devices (in silicon, PDMS, ...) are of particular interest to transport small quantities of aqueous samples, down to the femto-mole (10^{-15}) level of ions. Combined with metamaterials, microfluidics with enhanced sensitivity capability was used for testing liver cancer biomarkers. Furthermore, microfluidic chips have the potential for parallel sorting and sensing (fig. 1).

APPLICATIONS IN BIOLOGY AND MEDICINE

FUNDAMENTAL PHENOMENA IN BIOLOGICAL SYSTEMS

Fundamental applications in biology involve the probing at the vibrational level of biomolecules, such as proteins, DNA or peptides. This information is related to their dynamics and thus functions *in-vivo*. Retinal molecules are the chromophores in the photoactive proteins rhodopsin and bacteriorhodopsin. Photoinduced isomerization of the chromophore molecule is the primary step in their photocycles. The spectroscopy of 3 isomers of retinal, the key molecule of the vision, was performed by THz-TDS, and related to vibrational modes characteristics [12]. The primary charge translocation phenomena that take place in the proton pump were investigated in bacteriorhodopsin [13]. The authors observed light-induced coherent terahertz radiation from bacteriorhodopsin with femtosecond time resolution, related to an excited-state intramolecular electron transfer within the retinal chromophore. In spectroscopic studies of biomolecules, water strongly modifies the vibrational response of the molecules. Resonances are intensely broadened and mostly disappear, due to the coupling with water molecules. Experiments can be performed on dehydrated or cryogenic samples to study the vibrational modes, but in non-biological conditions. On the other hand, studies in solution are more involved on the solvation of biomolecules. In solid state samples, spectroscopy of amino-acids was reported at room and cryogenic temperature and compared to DFT vibration modes calculation [14]. This allows a better understanding of empirical force fields in biological molecules, which are essential for the modelling of complex molecules such as proteins or DNA. Direct spectroscopy measurements were also performed on proteins such as lysozyme in solid state. In solution, the hydration number is the number of water molecules interacting with a solute molecule. A solvation shell is found around the solute, with hydration numbers different from bulk water. The spectral and dynamical properties of the solute and hydration shell (hydration number, solvation shell thickness, ...) can be inferred from terahertz measurements. Combined by molecular dynamics simulation, the hydration number and hydration shell thickness are important parameters related to the dynamics and thus biochemical properties of biomolecules in solution, such as sugars and peptides, carbohydrates, proteins, ions, RNA [2, 15] (Fig. 2).

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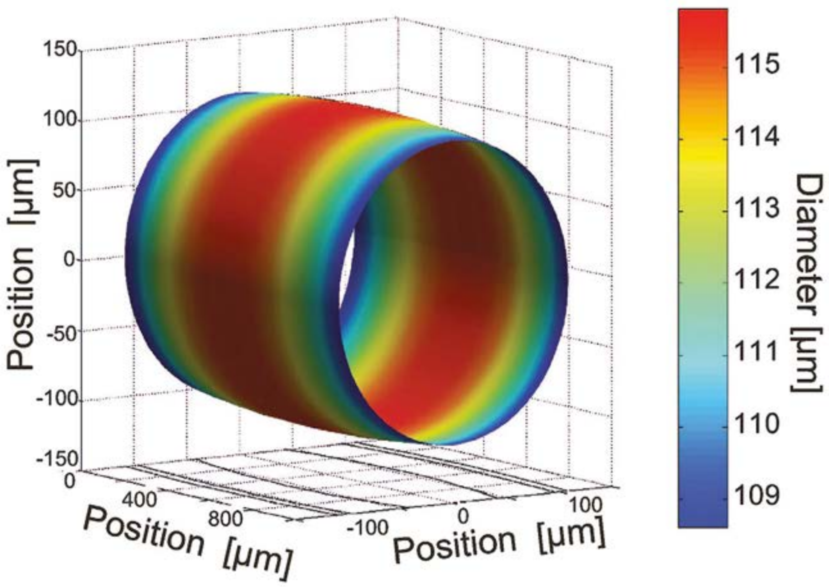
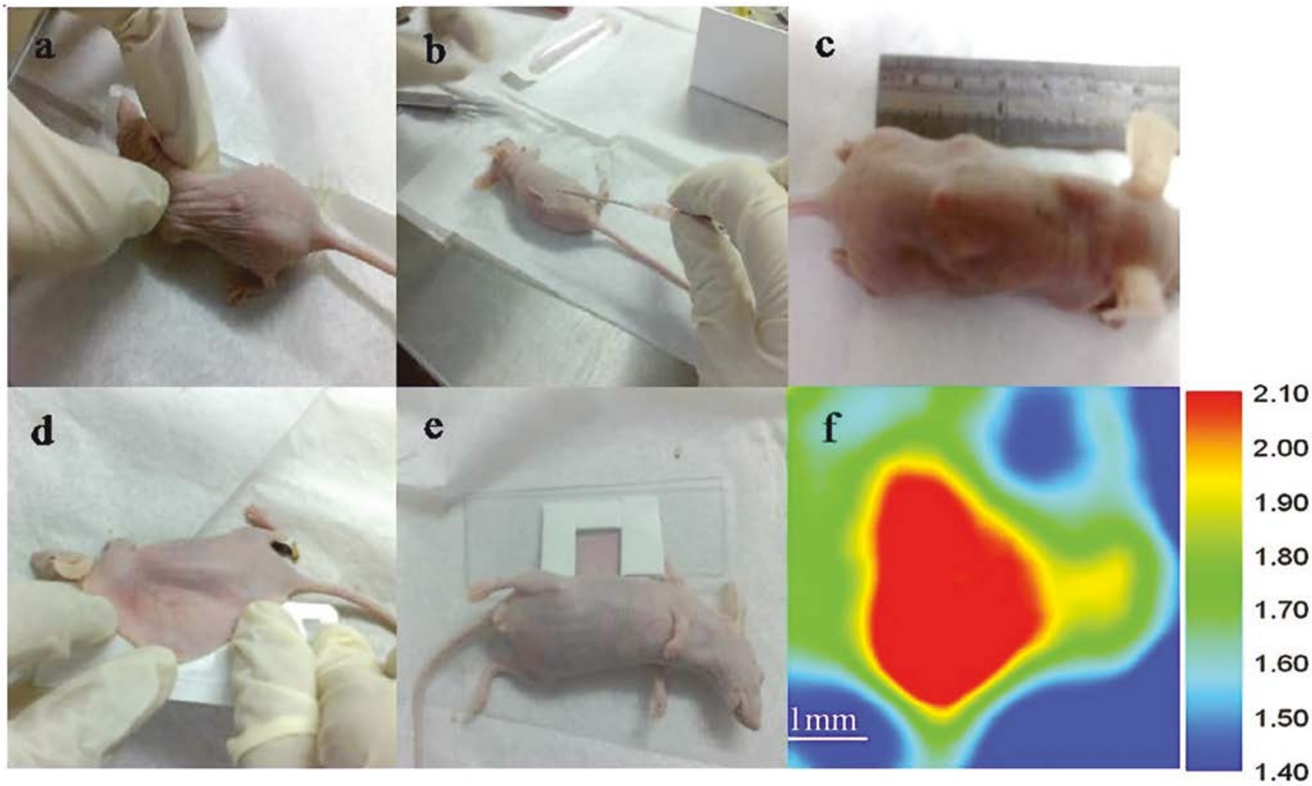


TERAHERTZ IMAGING

Profiting from the contrast given by the water content of tissues or by solutes, terahertz imaging investigated a wide range of biological systems [1]. Cancer has been studied by many groups. The goal is to detect cancerous tissues containing more water and absorbing more terahertz radiations, due to abnormal angiogenesis compared to healthy ones. Many studies used reflection techniques to reduce absorption in in-vivo experiments, while others employed thin or partially dehydrated biopsies [16]. Skin carcinoma received many attention [4,17], as well as breast cancer [3,18]. Other types of cancers studied are glioma, or ovarian. More specific systems were also studied. Neuron was imaged with near-field ionic contrast [7]. Corneal tissue hydration was also investigated. Most terahertz imaging measurements imply to deal with a lot of information, in particular when time-domain or spectroscopic signals are involved.

Figure 3.
Deconvoluted 3D image of the axon obtained with near-field technique with aperture, using finite element method (FEM) simulations. From [7]. Copyright (2006) National Academy of Sciences, U.S.A.



The choice of the relevant parameters is often very important for an accurate analysis. Mathematical statistical methods such as principal component analysis (PCA) can minimize the correlated variables into useful uncorrelated variables (Figs. 3,4).

BIOLOGICAL SENSORS

A last field of applications is related to biosensors seeking to detect low amount of biomolecules with high precision, and to correlate the measurements with biological and medical relevant indicators. Microfluidic circuits compatible with ●●●

Figure 4.
Imaging of a human breast tumor implanted in a mouse. (a) a visible tumor. (e) Stretched tumor between two cover glasses. (f) In-vivo terahertz imaging. From [18], © OSA.

terahertz radiation have also been developed to merge small volumes and sorting capability. Sensors can be based on spectral analyses, by a change of permittivity or a shift of resonance in human cancer cells or blood cells. Other demonstrations involved near-field time-dependent measurement of ionic flow during auricular muscle electrical activity [19] or plasmonic antennas on bacterial layers allowing the selective recognition of the Gram type of the bacteria. Sensitivity enhancement by plasmonic techniques is also very promising. Integrated planar terahertz probing demonstrated an enhancement of the detection threshold allowing the differentiation of denatured and hybridized DNA in solution [10]. THz scattering-type scanning nearfield optical microscopy (THz s-SNOM) demonstrated sub-attomole sensitivity on crystalline lactose and offers the detection of fingerprints of biomolecules at very low volumes. Very low volume detection was also achieved with split-ring resonators (SRR) [11], as low as 30 fmol of ions in 300 pmol of water. SRR were also integrated with microfluidics circuit for live cancer biomarkers. High-Q factor meta-sensors based on toroidal surface plasmon resonance enhanced by gold nanoparticles also targeted biomarker proteins.

CONCLUSION

Water plays a central role in life, as well as in the probing of biological systems by terahertz radiation. Thanks to the development of better terahertz sources and detectors, the analysis of the dielectric constant of solutions brings new solutions for imaging and sensing biomolecules and tissues, in addition to existing and more conventional techniques.

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