CellPress

Review

Biomedical Applications of Terahertz Spectroscopy and Imaging

Xiang Yang,[1,2] Xiang Zhao,[1] Ke Yang,[1,2] Yueping Liu,[1] Yu Liu,[1] Weiling Fu,[1,*] and Yang Luo[2,*]

Terahertz (THz = 10^{12} Hz) radiation has attracted wide attention for its unprecedented sensing ability and its noninvasive and nonionizing properties. Tremendous strides in THz instrumentation have prompted impressive breakthroughs in THz biomedical research. Here, we review the current state of THz spectroscopy and imaging in various biomedical applications ranging from biomolecules, including DNA/RNA, amino acids/peptides, proteins, and carbohydrates, to cells and tissues. We also address the potential biological effects of THz radiation during its biological applications and propose future prospects for this cutting-edge technology.

Trends

THz spectroscopy has proven to be an innovative tool for providing new insights into the hydration shell in the solvation dynamics of protein solutions.

THz in-line digital holography, THz near-field imaging modality, and THz endoscope prototypes have been utilized to identify abnormal tissues faster and more accurately.

Increasing applications of artificial modeling and numerical computation are becoming essential supplements for THz biological effect studies.

Terahertz Radiation: An Emerging Technology for Biomedical Research

THz radiation generally refers to the frequency band spanning 0.1–10 THz, which lies between the microwave and infrared regions of the electromagnetic spectrum (Figure 1). Due to the lack of effective sources and detectors, this 'THz gap' between electronics and photonics remained unexplored until advances in physics during the 1980s [1]. Specifically, the rapid development of ultrafast lasers contributed to the establishment of modern terahertz time-domain spectroscopy (THz-TDS), which has been widely utilized in applications such as astronomy, microelectronics, and biomedical science.

Excited by low-frequency molecular vibrations from intra/intermolecular domains connected by weak and conformation-related interactions, including hydrogen bonds, van der Waals, and nonbonded (hydrophobic) interactions, THz radiation has unique properties that are well suited to biomedical research (Box 1) [2]. According to the underlying detection and signal processing method, THz technologies can be classified into two categories: THz spectroscopy and THz imaging. Although a recent review summarized several key macroscopic and molecular applications [3], a more comprehensive and critical review is presented here, illustrating potential biomedical applications.

THz Spectroscopy: A Cutting-Edge Method for Biomolecule Recognition

There has been great interest in applying THz spectroscopy to probe and characterize various biomaterials in recent decades because most low-frequency biomolecular motions, including vibration and rotation of the molecular skeleton, lie in the same frequency range as THz radiation. Therefore, various biomolecules can be effectively recognized and characterized according to their distinctive spectral fingerprints. Additionally, by sensitively probing the fast hydration dynamics around biomolecules whose key large-amplitude motions coincidently occur on the picosecond timescale of THz frequencies, THz spectroscopy has demonstrated unique advantages for detecting the coupling between biomolecules and their hydration shells when

[1]Department of Laboratory Medicine, Southwest Hospital, Third Military Medical University, Chongqing 400038, China
[2]Medical Research Center, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

*Correspondence: fwl@tmmu.edu.cn (W. Fu) and luoyang@tmmu.edu.cn (Y. Luo).

 http://dx.doi.org/10.1016/j.tibtech.2016.04.008

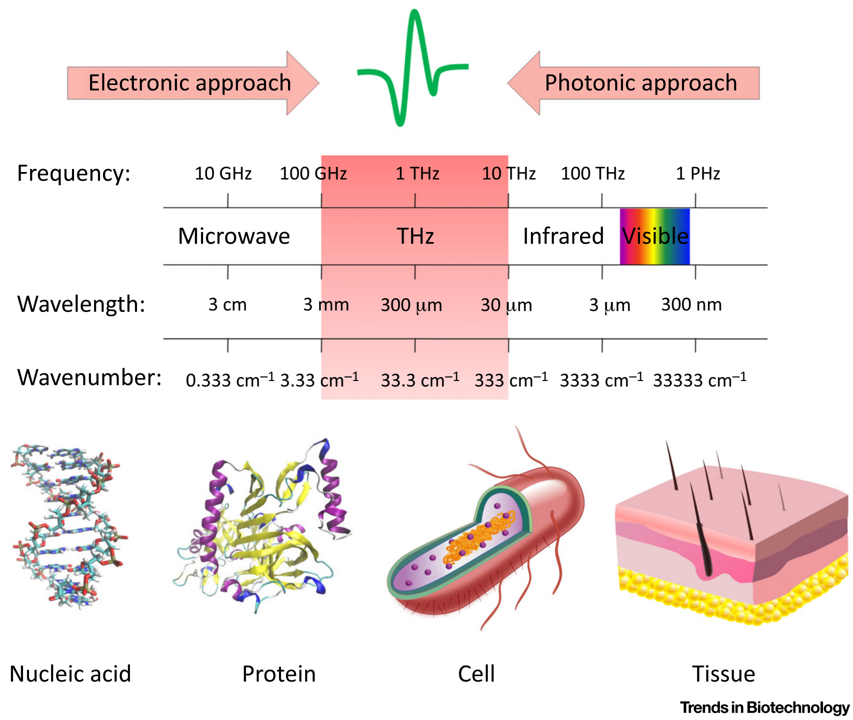


Figure 1. The Terahertz (THz) Region and Applications of THz Radiation. The THz region typically includes frequencies from 100 GHz to 10 THz on the electromagnetic spectrum. Converted to other units, the THz region can be defined as 30 μm–3000 μm (wavelength) or 3.33 cm^{-1}–333 cm^{-1} (wavenumber). THz generation approaches in both electronics and photonics have been successfully developed to explore the so-called 'THz gap', including the upconversion of electronic radiofrequency sources and the downconversion of optical sources. THz technology has been exploited to detect various biomaterials, illustrated by nucleic acids, proteins, cells, and tissues.

compared with conventional dielectric spectroscopy, X-ray crystallography, or nuclear magnetic resonance spectroscopy [4].

Nucleic Acids

The low-frequency molecular motions that originate from the hydrogen bonds of nucleic acid base pairs and nonbonded interactions are sensitive to the base composition and conformational state of nucleic acids; this phenomenon has been utilized as the basis of THz spectroscopy for nucleic acid detection. The four nucleobases [adenine (A), cytosine (C), guanine (G), and thymine (T)] have distinct absorption coefficients in the THz range and, thus, can be clearly profiled (Figure 2A) [5,6]. Additionally, a slight blueshift and several additional adjacent narrow bands are observed when decreasing the sample temperature from 300 K to 10 K, suggesting that temperature influences the analytical results. Furthermore, aqueous DNA molecules have been effectively measured with respect to two strong and repeatable THz signatures (400 and 720 GHz) using waveguide confinement techniques that have partially overcome the effect of water absorption, which currently limits THz technology [7]. In addition to experimental observations, various theoretical studies have also demonstrated the different absorption features of single-stranded (ss) and double-stranded (ds) DNA [8].

With the elucidation of the mechanisms that determine the THz fingerprints of biomolecules, THz spectroscopy has been exploited for qualitative and quantitative analyses of various

Box 1. Attractive Characteristics of THz Radiation for Biomedical Research

Spectral Fingerprint

The photon energy of the THz wave largely coincides with energy levels corresponding to low-frequency motions, such as the vibration, rotation, and translation of the molecular skeleton. These molecule-specific motions identify biomolecules by measuring their characteristic spectral signatures in the THz range.

Transparency of Materials Comprising Nonpolar Molecules

Materials such as paper and plastic, which are opaque to visible and near-infrared waves, may be transparent in the THz range. This property enables the detection of hazardous substances without opening packages because THz radiation can easily penetrate common packing materials.

Strong Absorption by Water

Polar molecules, such as water, exhibit strong absorption in the THz range. Specifically, the THz absorption coefficient of water at 1 THz is approximately 220 cm^{-1} at room temperature, exceeding that of common biomolecules. Normal tissues and cancer tissues can be accurately differentiated because their water contents are different. Moreover, THz radiation has the potential to rapidly assess the living state of bacteria (live or dead) according to their different hydration levels.

Excellent Time and Spatial Resolution

THz radiation interrogates characteristic frequency features with spatial resolution of several micrometers by near-field spectroscopic modalities and reveals time-resolved dynamics on the subpicosecond to picosecond timescales. Thus, THz spectroscopy permits time-resolved investigations of the collective vibration modes of biomolecules in solution with unprecedented sensing capability.

Noninvasive and Nonionizing Properties

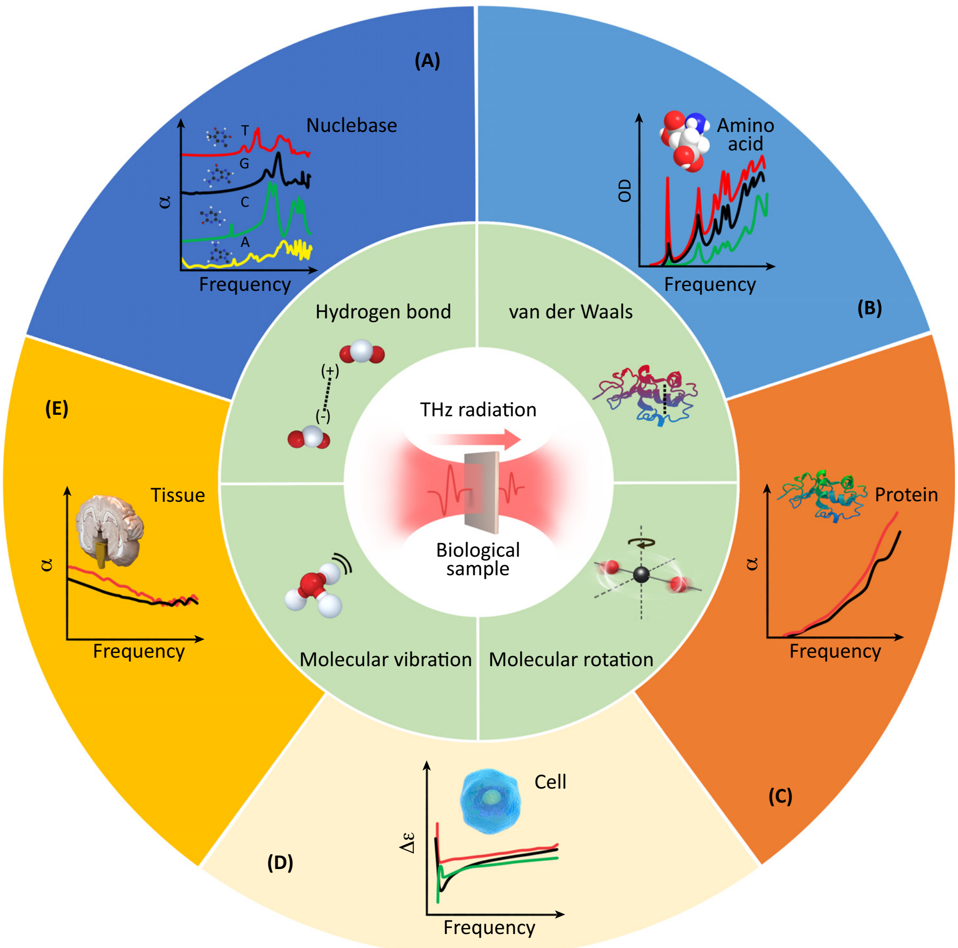
The low-energy photons of THz radiation (i.e., $\approx$1–10 meV) make it suitable for medical imaging because the noninvasive imaging modality can be applied for *in vivo* real-time diagnosis without causing ionization damage, unlike X-rays. Moreover, THz wavelengths are longer than infrared and visible light, and scattering losses in biological tissues are negligible.

nucleic acids. Hybridized DNA films have been observed to exhibit a higher refractive index than denatured DNA films [9], demonstrating the ability of this method to recognize nucleic acid-binding states and suggesting the possibility of developing a THz DNA biosensor. Moreover, the detectable spectral discrimination between single-base changes in DNA molecules indicates the potential to detect DNA mutations [10]. Finally, the absorption coefficients of DNA samples in aqueous solution decreases as their concentration increases because of the replacement of highly absorbing water molecules by less-absorbing DNA molecules. Hence, THz spectroscopy has been successfully used for the quantitative detection of DNA fragments in aqueous solutions at a sensitivity of 0.1 ng/μl with a minimum volume of 10 μl [11].

Much recent effort has focused on enhancing the detection sensitivity. For instance, utilizing resonant THz structures as sample carriers, nucleic acid hybridization can be probed with femtomolar sensitivity [12], which is comparable to fluorescence tagging methods. Metallic mesh structures have been applied to observe the different optical properties of ssDNA and dsDNA with dramatically enhanced sensitivity (microgram level) [13]. Moreover, narrow absorption features with a 10-GHz line width have been successfully observed for RNA samples suspended in buffer solution using a nanofluidic channel as the sample cell [14]. All of these breakthroughs pave the way for developing innovative nucleic acid assay methods in the near future.

Amino Acids, Carbohydrates, and Peptides

Various studies have been conducted to measure the THz absorption features of amino acids, revealing that the 20 naturally occurring amino acids share a dense set of absorption features in the 1–15 THz range [15]. Although obtaining the low-frequency vibrational spectra of amino acids in aqueous solutions, thus mimicking their natural state, would be preferable, the intrinsic absorption by amino acids can be substantially masked by the stronger absorption of water.



Trends in Biotechnology

Figure 2. Biomedical Applications of Terahertz (THz) Spectroscopy. Due to the weak interactions including hydrogen bonds and van der Waals lying in the THz range, the low-frequency vibration and rotation of biomolecules could be probed by THz spectroscopy. (A) Absorption coefficients of the nucleobases adenosine (A), cytosine (C), guanine (G), and threonine (T) recorded at 10 K. For clarity, the spectrum for A has been multiplied by a factor of 3, and the spectra for C, G, and T have been vertically offset in the figure [5,6]. (B) Optical density (OD) of L-glutamic acid (L-Glu) at 0.314 mol/L (green), 0.585 mol/L (black), and 0.837 mol/L (red). Sharp peaks at 1.22, 2.03, 2.46, 2.68, 2.80, and 3.24 THz were observed. The ODs of L-Glu were found to be linearly proportional to the concentration [17]. (C) Absorption coefficients (∝) for hen egg white lysozyme (HEWL) solution (red) and HEWL+ triacetylglucosamine (3NAG) solution (black) at 270 K. A slight decrease was observed when HEWL was bound to 3NAG [29]. (D) Differences in the complex dielectric constants between distilled water and cultured human cancer cells, including DLD-1 (red), HEK293 (black), and HeLa (green) (ε_water–ε_cell) [31]. (E) Absorption coefficients of paraffin-embedded brain gliomas (red) and normal tissues (black). Absorption coefficients of glioma tissue were higher than those of normal tissue at frequencies below 1 THz, although the differences were not distinct at higher frequencies [43]. All figure panels were adapted, with permission, from the references indicated.

Accordingly, a polymer membrane able to filter water has been developed for the THz transmittance spectroscopy of amino acids, which appears to be a pilot study for detecting amino acids in aqueous solution [16]. In addition, quantitative analyses of L-glutamic acid were reported based on their concentration-dependent molar absorption coefficients (Figure 2B) [17]. Recently, the study of amino acids using THz technology has gradually shifted to the identification and classification of amino acids, including detecting isomers of various amino acids, investigating cocrystals of different amino acids, and nondestructive quantitative testing of packaged amino acids.

The sensitive and selective discrimination of carbohydrate molecules with micromolar sensitivity was demonstrated by a THz nano-antenna sensing chip, possibly serving as an alternative tool for highly sensitive blood-sugar monitoring [18]. Furthermore, the interactions between the water hydrogen bond network and saccharide solutes were quantitatively evaluated by THz time-domain attenuated total reflection (ATR) spectroscopy, which potentially provides a window into global hydration states and a better understanding of the mechanism by which saccharides confer a sense of sweetness [19,20].

Studies of alanine-rich peptides in aqueous forms have reported obvious vibrational bands, but the sharp spectral features of peptide mixtures containing more than ten amino acids were difficult to detect due to masking by increasingly hydrogen-bonded water [21,22]. Lyophilized forms of the peptide analogs of oxytocin and vasopressin were also measured using THz-TDS. Small changes in the specific amino acid sequence of the peptide chain were found to potentially affect the THz absorbance spectra, making THz-TDS a potential tool to investigate the structure of new peptides [23].

Proteins

THz studies of proteins date back to the 1990s, most of which addressed protein conformational changes and intermolecular interactions [24]. Conformational changes, which are essential for protein function, directly affect the dielectric response in the THz range. The advent of THz pulse spectroscopy enabled measurements of the low-frequency dielectric response of strongly attenuating protein solutions, yielding a convenient method to explore protein conformational changes. For instance, the transformation of an α-helix into a β-sheet during the fibrillation of amyloid proteins, which is vital for the clinical diagnosis of, and drug discovery for, Alzheimer's disease, produces as an obvious increase in the absorption coefficient and refractive index spectra [25].

THz technology has also been applied to monitor intermolecular interactions, including protein hydration and protein–ligand binding. The collective vibrational modes of water and biomolecules coincidently lie in the THz frequency range and occur on picosecond timescales; thus, THz spectroscopy enables time-resolved measurements of these interactions (1 ps^{-1} = 1 THz) [26]. It has been demonstrated that hydration water in the immediate vicinity of, and interacting strongly with, biomolecules has a slower response time than that of the remaining bulk water [27]. Due to its distinctive spectral responses to THz radiation, the dynamic hydration shell can be precisely determined by probing protein-induced fast solvation dynamics by THz spectroscopy [4]. A broader hydration shell than previously determined by molecular dynamics simulations has been detected by THz spectroscopy, highlighting its potential for estimating protein hydrophobicity [28]. In addition, the protein–ligand binding between hen egg white lysozyme (HEWL) and triacetylglucosamine (3NAG) was successfully probed at 270 K [29], showing that HEWL + 3NAG has smaller absorption coefficients than free HEWL (Figure 2C) and proving the feasibility of monitoring protein–ligand binding in solution using THz spectroscopy.

Despite the successful reports of the detection of nucleic acids, amino acids, proteins, and other biomolecules, particularly widely implemented applications in detecting the hydration shell, several issues remain to be solved. First, strong water absorption is the primary obstacle to applications in biomolecular research, especially protein detection. Second, standardized sample preparation procedures are urgently needed to facilitate obtaining comparable results. Third, a unified and authorized THz spectral database of biomolecules must be established as a reference for evaluating the results of different studies.

Cells

The spectroscopic features of various blood cells in the THz range have been obtained, and excellent linearity can be observed between THz signals and erythrocyte concentrations [30], demonstrating the potential of this method for quantitative cell concentration analysis. Moreover, various cultured human cancer cells can be distinguished by their different complex dielectric constants (Figure 2D) [31]. Notably, the THz system has demonstrated greater sensitivity in measuring tiny structural changes in cultured cell monolayers than conventional optical phase-contrast microscopy and electric cell-substrate impedance sensing [32]. Therefore, noninvasive, *in situ*, real-time investigation of the dynamics of cytoplasm leakage without staining or labeling can be realized by THz-ATR measurements [33]. Furthermore, the intracellular water dynamics of human cancer cells and the ratio of hydrating water molecules in the intracellular water can also be revealed by ATR spectroscopy [34].

THz radiation can also differentiate various types of bacteria according to their distinct spectral signatures. For instance, spores of *Bacillus* species [35], *Escherichia coli*, and *Bacillus subtilis* [36] have different absorption spectra. Additionally, the absorption differences between thermally treated and untreated cells of the same species can be used to assess the living state of bacteria [36]. A quantitative bacterial detection scheme has been reported in which a THz fiber was used to sense *E. coli* with a detection limit of 10^4 cfu/ml [37], and bacterial monolayers were qualitatively detected with THz plasmonic antennas based on their different dielectric responses [38]. Furthermore, the sensitive detection of bacteria in both ambient and aqueous environments was realized using THz metamaterials, by investigating the resonant frequency shifts resulting from the alterations of the effective dielectric constants of the metamaterial patterns [39].

The cellular hydration state and permeabilization of living cells, which are closely related to cellular activity and pathological states, have been effectively measured by THz-ATR technology. However, most cellular absorption spectra in the THz range were merely collected as monotonically increasing curves without any characteristics peaks. The limit in specificity should be addressed before this technique can serve as an alternative tool for distinguishing different types of cell.

Tissues

An *in vivo* study of human skin showed that various skin tissues had significantly different absorption coefficients and refractive indexes due to discrepant hydration levels [40]. Additionally, healthy skin, dysplastic nevi, and nondysplastic nevi have been demonstrated to exhibit different dielectric parameters, hinting at the possibility for early, noninvasive diagnostics of pigmentary skin nevi [41].

Paraffin-embedded tissues are frequently measured to reduce the influence of water, and numerous data revealed that cancer tissues display higher absorption coefficients (Figure 2E) and refractive indexes than normal tissues [42,43]. However, cancerous gastric tissues exhibit higher absorption than normal tissues only at relatively low THz frequencies [44]. Furthermore, snap-frozen brain tissues from patients with Alzheimer's disease appeared to be less absorptive than healthy tissues [45]. Hence, differences in spectral signals are determined by not only varying tissue water content, but also different tissue compositions and structures.

Tissue detection by THz spectroscopy is inherently limited by small detection areas and the heterogeneity of biological samples. By contrast, THz imaging is more visual and convenient than THz spectroscopy and, thus, can more accurately analyze tissues. Integrating THz spectroscopy and THz imaging into a single system would enable the collection of more comprehensive information for clinical applications.

THz Imaging: A Useful Tool for Biomedical Characterization

THz technology is an image-spectrum merging modality, by which intrinsic properties and morphological characteristics can be extracted from amplitude and phase information synchronously. Compared with X-ray scanning, which struggles with limited sensitivity for tumors without calcium deposits and constitutes a potential radiation hazard, THz imaging can distinguish tumors with much-improved sensitivity in a nonionizing manner [46]. Moreover, THz imaging is superior to magnetic resonance imaging (MRI) by having an appropriate penetration depth (a few hundred microns) for superficial neoplasms, with the ability to develop miniaturized instruments for intraoperative imaging and the potential for distinctive molecular fingerprints identification.

Imaging Mechanisms

Differences in water content and structural variations in tissues are essential mechanisms of THz biomedical imaging. (i) Water content: cancerous or diseased tissues may contain more interstitial water as a result of abundant vascularity or tissue edema [47], as demonstrated by positron emission tomography, MRI, and frequency-domain photon migration. Therefore, these tissues display obviously different THz absorption from that of normal tissues. (ii) Structural variations: variational tissue microenvironments [48], deteriorative cellular morphologies [49], and mutative biomolecules [50] can alter the quality and quantity of the image contrast. According to previous research on liver cirrhosis tissues, 50–66% of the contrast could be assigned to the inner structural and compositional variations [48]. (iii) Artificial contrast enhancement: glycerol and targeted gold nanorods have been successfully utilized as imaging contrast-enhancing agents [51,52]. The absorption of water molecules in THz range increases significantly when the temperature rises [53]. Therefore, based on the thermal effects of the targeted gold nanorods and the subsequent water absorption enhancement, preclinical epidermoid carcinoma could be sensitively and accurately identified by THz molecular imaging [52]. Moreover, tryptophan, the elevated consumption of which is involved in mechanisms of tumor immune escape, showed strong resonant absorptions at 1.42 and 1.84 THz [50], and the imaging contrast was enhanced by designing an appropriate continuous wave (CW) THz imaging system.

Current Status of THz Imaging Studies

Over the past 5 years, various tissues, especially cancer tissues, have been increasingly detected by THz imaging modalities (Table 1). Taking pathological biopsy as the gold standard for reference, THz *in vitro* imaging has been extensively applied in digestive system [54–64], integumentary system [65,66], reproductive system [67–70], respiratory system [57], and nervous system [49] neoplasms. Frozen sections [54,56], formalin fixations [57], and paraffin sections [67] have typically been applied as specimen pretreatment processes to overcome the strong water absorption loss.

Fresh tissues directly excised from specimens enabled the comprehensive assessment of both water content and structural variations [71]. Fresh human early gastric cancer tissues were successfully differentiated based on their higher THz reflection intensities compared with normal mucosa and correlated well with pathologically mapped images, except for signet ring cell carcinoma [56]. Although the influence of water content can be excluded for dehydrated samples, cancer tissues can be differentiated from fibro and fatty tissues and exhibit stronger contrast (darker color) because of their structural variations (Figure 3A) [67]. Notably, fibrosis was noticeable in the THz hologram of frozen hepatocellular carcinoma tissues, an indication of cirrhosis that may develop into hepatocellular carcinoma without appropriate intervention (Figure 3B) [62]. By rejecting Fresnel reflections, which mostly come from the glass–air interface and contain no sample information, the cross-polarized THz image of nonmelanoma skin cancer can display the approximate location of the tumor as well as the level of contrast (Figure 3C) [65].

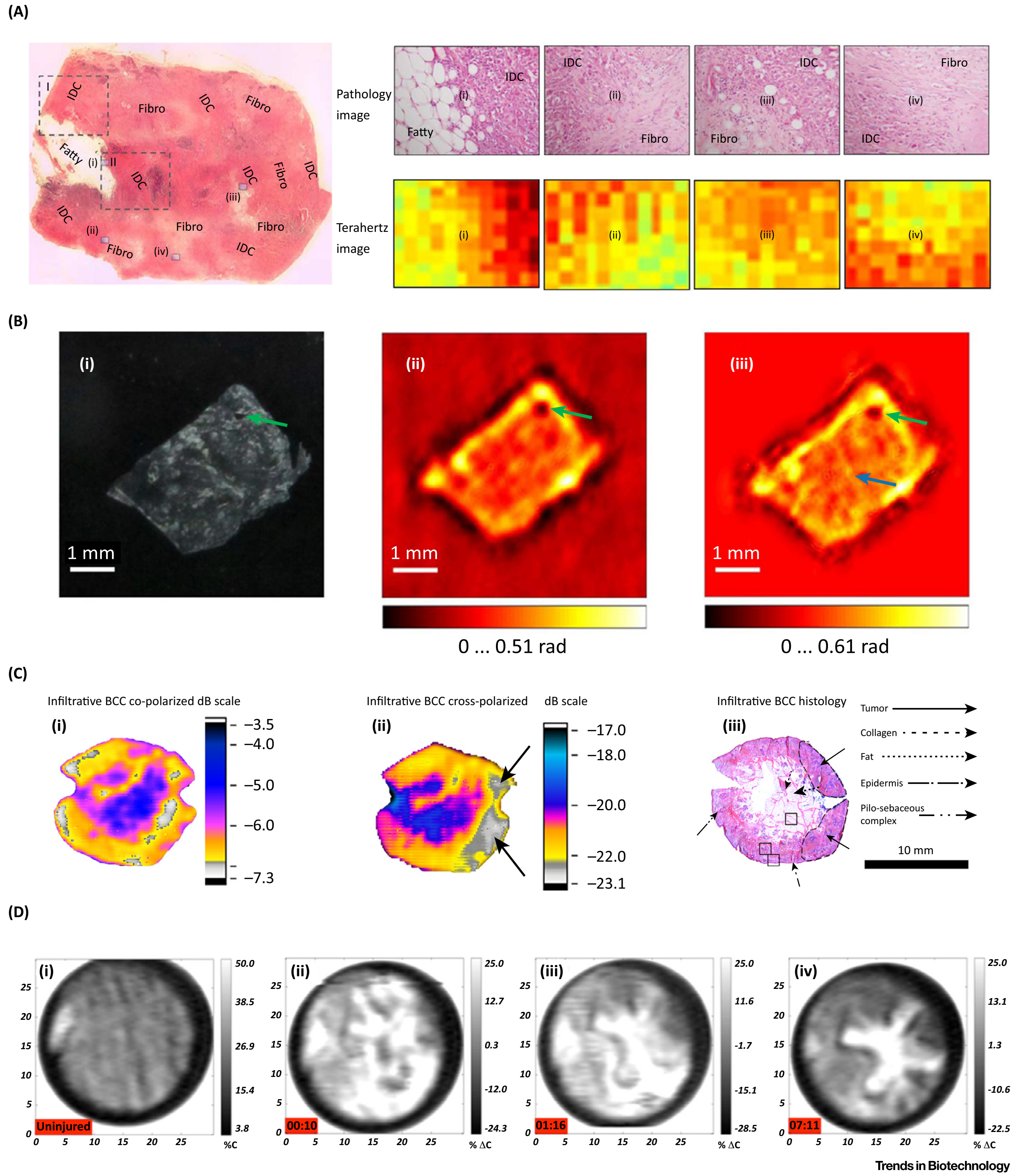
Table 1. Biomedical Applications of THz Imaging in Tissues over the past 5 Years[a]

Anatomical System/ Animal Model	Tissue	Specimen Sources	Instrumentation	Spatial Resolution	Year	Refs
Digestive system	Mouth neoplasms: mucoepidermoid and SCC	Frozen section	TPI reflection system	250 μm[b]	2013	[54]
	Colonic neoplasm	Paraffin section	CW THz imaging system	500 μm	2016	[64]
		Formalin fixation	CW THz endoscopic system	100 μm[b]	2014	[63]
		Frozen section	THz near-field imaging system	220 μm	2015	[61]
		Freshly excised	CW THz imaging system	600 μm	2013	[60]
		Freshly excised	Portable TPI reflection system	–[e]	2011	[55]
	Stomach neoplasms: EGC	Freshly excised	TPI reflection system	500 μm[b]	2015	[56]
	Liver neoplasm	Frozen section	THz near-field imaging system	240 μm	2013	[59]
	Liver neoplasm: hepatocellular carcinoma	Frozen section	THz in-line digital holography system	158 μm	2015	[62]
	Pancreatic neoplasm: IDC	Paraffin section	TPI reflection system	500 μm[c]	2010	[57]
Integumentary system	Skin neoplasms: BCC and SCC	Freshly excised	CW THz imaging system, Polarized light imaging system	100 μm[b]	2014	[65]
	Skin neoplasm: nonmelanoma	Frozen section	CW THz imaging system	390 μm	2011	[66]
Reproductive system	Breast neoplasm: IDC	Paraffin section	TPI reflection system	50 μm[b]	2015	[67]
	Breast neoplasm: IDC and papillary tumor	Frozen section	THz near-field imaging system	240 μm	2011	[69]
	Breast neoplasm	Freshly excised/ formalin fixation	CW THz imaging system	1000 μm[c]	2013	[70]
	Uterine cervical neoplasm: SCC	Freshly excised	TPI reflection system	500 μm[b]	2011	[68]
Respiratory system	Lung neoplasm: SCC	Paraffin section	TPI reflection system	500 μm[c]	2010	[57]
Motor system	Normal skeleton	Air dried	THz computed tomography system	2700 μm	2012	[82]

Table 1. (continued)

Anatomical System/ Animal Model	Tissue	Specimen Sources	Instrumentation	Spatial Resolution	Year	Refs
Swine	Normal skin, subdermal fat, and muscle	Freshly excised	THz QCL interferometry imaging system	50 μm	2014	[58]
	Normal cornea	Freshly excised (immersed in polyethylene glycol solutions)	TPI reflection system	1200 μm[c]	2011	[79]
Rat	Normal gastrointestinal tract	Freshly excised	TPI reflection system	250 μm[b]	2014	[71]
	Breast neoplasm (xenograft)	Freshly excised	TPI transmission system	1100 μm[d]	2011	[47]
	Glioblastoma	Freshly excised	TPI reflection system	–	2014	[49]
	Liver cirrhosis	Freshly excised/formalin fixation	TPI reflection system	–	2010	[48]
	Normal skin	Burn	TPI reflection system	–	2012	[76]
	Normal vessels in ear	*In vivo*	THz near-field imaging system	500 μm	2015	[77]
Rabbit	Cartilage	Formalin fixation	TPI reflection system	–	2010	[81]
	Normal cornea	*In vivo*	TPI reflection system	1000 μm[c]	2015	[78]

[a]Abbreviations: BCC, basal cell carcinoma; EGC, early gastric carcinoma; IDC, infiltrating ductal carcinoma; SCC, squamous cell carcinoma; TPI, terahertz pulsed imaging.
[b]Scanning resolution.
[c]Spot size.
[d]Lateral resolution.
[e]–, not given.



Trends in Biotechnology

Figure 3. Biomedical Applications of Terahertz (THz) Imaging. (A) THz images of breast cancer tissue diagnosed with poorly differentiated infiltrating ductal carcinoma (IDC). Low-power pathology image shows the macroscopic distributions of IDC, fatty, and fibro. (i–iv) show selected locations of high-power pathology (upper) and THz frequency-domain image at 1.5 THz (lower) [67]. (B) THz holography of human hepatocellular carcinoma tissue. (i) Digital photograph of the carcinoma tissue; (ii) phase-shift distributions reconstructed from the normalized hologram; and (iii) phase-shift distributions reconstructed from the extrapolated hologram. The green arrow indicates a hole in the top left corner, which could be easily identified in all three figures. The blue arrow indicates a vertical line in the middle of the tissue that is obvious only in (iii) and could be attributed to fibrosis in the carcinoma tissue [62]. (C) THz polarized reflectance images of infiltrative basal cell carcinoma tissue. (i) THz co-polarized

(Figure legend continued on the bottom of the next page.)

In brief, THz *in vitro* imaging has been utilized as a label-free method complementing pathological examination [72].

Although THz *in vivo* imaging has mainly been applied to epidermal tissue because of its limited penetration depth, several meaningful breakthroughs have been achieved. Again, water absorption has a dominant role in shaping the variable angle ellipsometry or polarized reflectometry THz spectrum in a human skin layer model [73,74]. A recent simulation study found that InN-enhanced THz imaging enables ultrahighly sensitive diagnosis of early-stage skin cancer at a scale comparable to tiny water content changes [75]. Experimentally, dynamic changes in rat skin burns were clearly observed by THz *in vivo* imaging, which satisfies an unmet need in skin burn assessment [76]. Initial edema imaging showed a highly reflected signal due to postinjury responses, which are characterized by an inrush of edema (mostly water); subsequently, the edematous response began to organize and the shape of the burn became visible (Figure 3D). Finally, the zone of stasis containing the dehydrated tissue became discernable in THz images due to structural variations. Blood vessels [77] and corneal tissue water content [78,79] were also clearly resolved using a THz imaging system, which has promising *in vivo* applications for the long-term monitoring of changes in blood and quantitative measurements of corneal tissue water content. In addition to THz *in vivo* imaging, this modality has been expanded to many other medical fields. For instance, drug tracing within the skin [80], cartilage damage imaging [81], and skeletal computed tomography [82] have been performed. These trials have attracted intensive attention and support prospective application of this technique in clinical settings.

Perspective on Clinical Adoption

Current THz imaging investigations are neither standardized nor comparable due to the differences in sample preparations and measurements. Hence, standard operating procedures and reliable databases should be established to ensure that results are repeatable and unbiased; these procedures and databases should fully account for slice thickness, water content, storage conditions, and instrumentation. Meanwhile, a credible imaging modality able to precisely differentiate the border between normal and tumor tissues *in situ* is urgently needed. Although a THz endoscope prototype could satisfy this requirement [63], further improvements are required for this technique to be embedded in gastrointestinal endoscopy or other endoscopic surgery instruments, including a compact transceiver. In addition, it is believed that, after coupling the THz molecular imaging modality to enhance the imaging contrast, the intense attenuation by water should be overcome to realize real-time intraoperative monitoring. Specifically, prospective THz imaging investigations should focus on estimating tumor stage and type [64] and not simply be confined to differentiating between benign and malignant tissues.

Given its poor, diffraction-limited spatial resolution, THz biomedical imaging remains in its infancy. Biomolecule fingerprinting is critical for precision medicine and could be acquired at nanometer spatial resolution by THz pulse near-field microscopy [83], which can simultaneously probe both propagating and evanescent waves. However, owing to strong signal attenuation in aqueous environments, this technology cannot yet be exploited for practical applications. Indeed, some obstacles remain in obtaining biomolecular and single cell THz images although epithelial cell monolayer images have been collected using a THz-ATR system [33].

reflectance image; (ii) THz cross-polarized reflectance image, and (iii) H&E stained histology image. The low reflectance areas indicated by a solid arrow in THz cross-polarized image are consistent with the size and shape of the tumor region [65]. (D) THz images of a rat skin burn. Panels (i–iv) show THz images of unburned skin, skin burned after 10 min, >1 h, and >7 h, respectively. The lighter areas correspond to edematous response areas post-burn due to the higher reflected signal of local water [76]. All figure panels were adapted, with permission, from the references indicated.

Biological Effects: Increasing Concerns

With the growing applications of THz detective modalities, concerns about the biological effects of THz radiation have been raised by researchers in the field [84]. Subsequently, several studies at the organism, tissue, cell, and biomolecule levels were conducted to identify the potential health hazards of THz radiation, formulate safety guidelines, and guarantee the safe use of THz systems [85].

The biological effects of THz on superficial tissues and cells, mainly determined by its power densities, draw concerns because they are the parts of the human body most readily exposed to THz radiation. An *in vitro* study found that low-power density THz exposure (1.89 THz, 189.92 mW/cm^2) induced no tissue damage in porcine skin and few coagulation areas in egg whites [86]. By contrast, high-power density THz exposure (0.1–1.0 THz, 2000–14000 mW/cm^2) produced tissue damage in wet chamois cloths at a damage threshold of 7160 mW/cm^2 [86], along with an obvious temperature rise. Similar studies on cells also revealed that low-power densities had no adverse effects on the morphology, attachment, proliferation, or differentiation of human ocular cell lines and embryonic stem cells (0.02–0.37 mW/cm^2) [87], whereas continuous exposure to 84.8 mW/cm^2 power density THz radiation for 80 min was sufficient to raise the temperature by approximately 3 °C and led to almost 10% cell death in human primary dermal fibroblasts [88]. Therefore, tissue damage and cell death can result from the thermal effects of high-power densities [84]. It should be mentioned that the energy of the THz pulses used in typical THz biomedical applications and representative laser-based THz-TDS systems are several orders of magnitude lower than those reported above; thus, temperature increases in the human body during real applications would be negligible [89].

However, THz irradiation may induce some changes at the biomolecule level, such as DNA damages [89,90], gene expression alterations [91], and protein changes [92]. Given that no obvious temperature rise was observed in these studies, most of the results are associated with proposed nonthermal effects of low-level THz irradiation, wherein THz radiation induced low-frequency collective biomolecular vibrations [90]. However, the most recent evidence indicated no obvious DNA damages or negligible gene changes for some ranges of THz radiation, such as 2.3 THz [93]; additionally, the observed DNA damages may be effectively repaired [89]. These controversial results can be attributed to our limited understanding of the nonthermal effects of THz at the biomolecule level because of a lack of applicable modern tools [85].

The influences of THz irradiation at the organism level have also been studied. Behavioral alterations, such as increased levels of depression (0.15 THz, 3 mW/cm^2, 60 min) [94], were observed in a mouse model. Moreover, inflammatory responses and perturbation of the wound-healing process in mouse skin under THz irradiation have also been verified [95,96]. These findings indicate that THz radiation poses a slight potential health hazard. By contrast, a new endometrial ablation technique using THz irradiation instead of microwaves was proposed by converting the electromagnetic energy of the THz band into thermal energy [97]. Theoretical analyses showed the potential for clinical application, but technical challenges remain. Furthermore, gender may directly influence the effects of THz irradiation; stressed females showed significantly increased survival rates [98] and decreased platelet activity [99] under THz irradiation in *Drosophila melanogaster* [98] and rats [99]. No obvious temperature changes were observed during these studies, and these influences should be attributed to the nonthermal effects of THz radiation [99].

Although the biological effects of THz radiation have been intensively studied, the actual elucidation of their impact on human body is far from complete [84]. To better determine the thermal and nonthermal effects of THz radiation, several key elements, such as reliable THz equipments and dosimetric tools, as well as standardized designs and analyses, must be carefully considered in future experimental studies [85]. As a complementary method, the recently developed theoretical modeling used in the thermal analysis of tissue damage thresholds and temperature developments should have a more important role in future studies [100,101]. Moreover, few studies concerning measures to protect researchers from THz radiation have been reported due to a lack of risk assessment data. Thus, more evidence from further systematic investigations and simulations are required to fill the gap in risk assessment in the future.

Concluding Remarks and Future Perspectives

Impressive progress has been achieved in THz radiation-mediated macromolecule detection and tissue imaging. Moreover, great potential has also been shown for clinical applications, such as the label-free identification of pathogenic bacteria and real-time imaging during surgical operations. However, several challenges must be overcome before this technology can be widely applied in the biological sciences.

Cost-effective THz systems with good operating performance are preferable for various applications. Currently, the relatively high cost of such systems impedes their routine application in diverse healthcare settings and hospitals. Continuous physics and materials science developments will further lower the hardware costs. Meanwhile, improving the accuracy and repeatability of the analytical results using equipment with accelerated acquisition speed, enhanced signal:noise ratios, and higher power will facilitate clinical applications. Portable and cost-effective CW systems, especially some solid-state CW devices, would be particularly suitable for situations where space is limited.

The strong absorption of water throughout the THz frequency range is a double-edged sword. On the one hand, some cancer tissues can be accurately differentiated from normal tissues by recognizing their different THz absorption contrasts originating from their water contents. On the other hand, the THz signal of water is stronger than that of biological tissues, thus compromising precise detection. In situations where water absorption affects the measurement, sample pretreatments, such as freezing or paraffin embedding, and the use of THz penetration-enhancing agents, such as glycerol, may help. Furthermore, microfluidic and nanofluidic devices can be utilized to minimize the water absorption loss, and ATR-mode THz spectroscopy can also provide some relief.

Another obstacle is data analysis and interpretation. Computational modeling by molecular dynamics simulation has helped improve our understanding of the mechanism underlying the interactions between samples and THz radiation. Currently, numerical simulations are available for modeling THz spectra using commercial software [102]. Moreover, chemometrics, such as principal component analysis, used for other frequencies, could be extended to THz spectroscopy, and more theoretical models for appropriate interpretation could be developed. This interdisciplinary, cutting-edge science will undoubtedly achieve a historic breakthrough in the near further by overcoming these existing limitations (see Outstanding Questions).

Outstanding Questions

How can we build a spectral database without influences from experimental conditions, such as temperature and humidity?

How can we develop portable THz systems with good performance for clinical applications, such as THz endoscopy for intraoperative imaging?

How can we establish a standardized evaluation system for the biological effects of THz radiation and establish damage thresholds for the human body?

Acknowledgments

This work was partially supported by the National Basic Research Program of China (2015CB755400), the National Natural Science Foundation of China (81430054, 81572079, 81371899), and the Science Foundation for Distinguished Young Scholars of Chongqing (CSTC2014JCYJJQ10007).

References

1. Auston, D.H. and Nuss, M.C. (1988) Electrooptical generation and detection of femtosecond electrical transients. *IEEE J. Quantum Electron.* 24, 184–197
2. Qin, J. *et al.* (2013) The detection of agricultural products and food using terahertz spectroscopy: a review. *Appl. Spectrosc. Rev.* 48, 439–457
3. Shuting, F. *et al.* (2014) The growth of biomedical terahertz research. *J. Phys. D Appl. Phys.* 47, 374009
4. Born, B. *et al.* (2009) The terahertz dance of water with the proteins: the effect of protein flexibility on the dynamical hydration shell of ubiquitin. *Faraday Discuss.* 141, 161–173

5. Fischer, B.M. *et al.* (2002) Far-infrared vibrational modes of DNA components studied by terahertz time-domain spectroscopy. *Phys. Med. Biol.* 47, 3807–3814
6. Pickwell-MacPherson, E. and Wallace, V.P. (2009) Terahertz pulsed imaging—a potential medical imaging modality? *Photodiagn. Photodyn.* 6, 128–134
7. Zhang, W. *et al.* (2013) Observation of terahertz absorption signatures in microliter DNA solutions. *Appl. Phys. Lett.* 102, 023701
8. Globus, T. *et al.* (2006) Terahertz Fourier transform characterization of biological materials in a liquid phase. *J. Phys. D Appl. Phys.* 39, 3405–3413
9. Brucherseifer, M. *et al.* (2000) Label-free probing of the binding state of DNA by time-domain terahertz sensing. *Appl. Phys. Lett.* 77, 4049–4051
10. Tang, A.M. *et al.* (2015) Terahertz spectroscopy of oligonucleotides in aqueous solutions. *J. Biomed. Opt.* 20, 095009
11. Arun, A. *et al.* (2012) Terahertz-time domain spectroscopy for the detection of PCR amplified DNA in aqueous solution. *Analyst* 137, 575–579
12. Michael, N. *et al.* (2002) Integrated planar terahertz resonators for femtomolar sensitivity label-free detection of DNA hybridization. *Appl. Optics* 41, 2074–2078
13. Hasebe, T. *et al.* (2012) Metallic mesh-based terahertz biosensing of single- and double-stranded DNA. *J. Appl. Phys.* 112, 094702
14. Brown, E.R. *et al.* (2010) Narrow THz Spectral signatures through an RNA solution in nanofluidic channels. *IEEE Sensors J.* 10, 755–759
15. Kutteruf, M.R. *et al.* (2003) Terahertz spectroscopy of short-chain polypeptides. *Chem. Phys. Lett.* 375, 337–343
16. Kikuchi, N. *et al.* (2009) A membrane method for terahertz spectroscopy of amino acids. *Anal. Sci.* 25, 457–459
17. Ueno, Y. *et al.* (2006) Quantitative measurements of amino acids by terahertz time-domain transmission spectroscopy. *Anal. Chem.* 78, 5424–5428
18. Lee, D-K. *et al.* (2015) Highly sensitive and selective sugar detection by terahertz nano-antennas. *Sci. Rep.* 5, 15459
19. Shiraga, K. *et al.* (2015) Quantitative characterization of hydration state and destructuring effect of monosaccharides and disaccharides on water hydrogen bond network. *Carbohyd. Res.* 406, 46–54
20. Shiraga, K. *et al.* (2013) Evaluation of the hydration state of saccharides using terahertz time-domain attenuated total reflection spectroscopy. *Food Chem.* 140, 315–320
21. Plusquellic, D.F. *et al.* (2007) Applications of terahertz spectroscopy in biosystems. *Chemphyschem* 8, 2412–2431
22. Tao, D. *et al.* (2010) Terahertz and far infrared spectroscopy of alanine-rich peptides having variable ellipticity. *Opt. Express* 18, 27431–27444
23. Fuglewicz, B. *et al.* (2014) Selected nonapeptides in terahertz light. *Opt. Appl.* 44, 159–171
24. Xie, L. *et al.* (2014) The application of terahertz spectroscopy to protein detection: a review. *Appl. Spectrosc. Rev.* 49, 448–461
25. Liu, R. *et al.* (2010) Insulin amyloid fibrillation studied by terahertz spectroscopy and other biophysical methods. *Biochem. Biophys. Res. Commun.* 391, 862–867
26. Wilmink, G.J. *et al.* (2011) Development of a compact terahertz time-domain spectrometer for the measurement of the optical properties of biological tissues. *J. Biomed. Opt.* 16, 047006
27. Pal, S.K. *et al.* (2002) Biological water at the protein surface: dynamical solvation probed directly with femtosecond resolution. *Proc. Natl. Acad. Sci. U.S.A.* 99, 1763–1768
28. Sushko, O. *et al.* (2015) Sub-terahertz spectroscopy reveals that proteins influence the properties of water at greater distances than previously detected. *J. Chem. Phys.* 142, 055101
29. Chen, J.Y. *et al.* (2007) Terahertz dielectric assay of solution phase protein binding. *Appl. Phys. Lett.* 90, 243901
30. Reid, C.B. *et al.* (2013) Terahertz time-domain spectroscopy of human blood. *IEEE J. Biomed. Health Informat.* 17, 774–778
31. Shiraga, K. *et al.* (2014) Characterization of dielectric responses of human cancer cells in the terahertz region. *J. Infrared Millim. Tech.* 35, 493–502
32. Liu, H-B. *et al.* (2007) Sensing minute changes in biological cell monolayers with THz differential time-domain spectroscopy. *Biosens. Bioelectron.* 22, 1075–1080
33. Grognot, M. and Gallot, G. (2015) Quantitative measurement of permeabilization of living cells by terahertz attenuated total reflection. *Appl. Phys. Lett.* 107, 103702
34. Shiraga, K. *et al.* (2015) Hydration state inside HeLa cell monolayer investigated with terahertz spectroscopy. *Appl. Phys. Lett.* 106, 253701
35. Zhang, W. *et al.* (2014) Narrow terahertz attenuation signatures in *Bacillus thuringiensis*. *J. Biophotonics* 7, 818–824
36. Globus, T. *et al.* (2012) Sub-THz vibrational spectroscopy of bacterial cells and molecular components. *Am. J. Biomed. Eng.* 2, 143–154
37. Mazhorova, A. *et al.* (2012) Label-free bacteria detection using evanescent mode of a suspended core terahertz fiber. *Opt. Express* 20, 5344–5355
38. Berrier, A. *et al.* (2012) Selective detection of bacterial layers with terahertz plasmonic antennas. *Biomed. Opt. Express* 3, 2937–2949
39. Park, S.J. *et al.* (2014) Detection of microorganisms using terahertz metamaterials. *Sci. Rep.* 4, 4988
40. Ibtissam, E. *et al.* (2013) Using a portable terahertz spectrometer to measure the optical properties of in vivo human skin. *J. Biomed. Opt.* 18, 120503
41. Zaitsev, K.I. *et al.* (2015) Terahertz spectroscopy of pigmentary skin nevi in vivo. *Opt. Spectrosc.* 119, 404–410
42. Wahaia, F. *et al.* (2015) Study of paraffin-embedded colon cancer tissue using terahertz spectroscopy. *J. Mol. Struct.* 1079, 448–453
43. Meng, K. *et al.* (2014) Terahertz pulsed spectroscopy of paraffin-embedded brain glioma. *J. Biomed. Opt.* 19, 077001
44. Hou, D. *et al.* (2014) Terahertz spectroscopic investigation of human gastric normal and tumor tissues. *Phys. Med. Biol.* 59, 5423–5440
45. Png, G.M. *et al.* (2009) Terahertz spectroscopy of snap-frozen human brain tissue: an initial study. *Electron. Lett.* 45, 343–344
46. Jacoby, M. (2015) Biomedical terahertz imaging advances in far-infrared spectroscopy could aid cancer diagnosis. *Chem. Eng. News* 93, 10–14
47. Chen, H. *et al.* (2011) High-sensitivity in vivo THz transmission imaging of early human breast cancer in a subcutaneous xenograft mouse model. *Opt. Express* 19, 21552–21562
48. Sy, S. *et al.* (2010) Terahertz spectroscopy of liver cirrhosis: investigating the origin of contrast. *Phys. Med. Biol.* 55, 7587–7596
49. Oh, S.J. *et al.* (2014) Study of freshly excised brain tissues using terahertz imaging. *Biomed. Opt. Express* 5, 2837–2842
50. Joseph, C.S. *et al.* (2009) Terahertz spectroscopy of intrinsic biomarkers for non–melanoma skin cancer. In *Proceedings of SPIE 7215, Terahertz Technology Applications II 7215*, pp. 72150I
51. Oh, S.J. *et al.* (2013) Measurement depth enhancement in terahertz imaging of biological tissues. *Opt. Express* 21, 21299–21305
52. Oh, S.J. *et al.* (2012) Cancer diagnosis by terahertz molecular imaging technique. *J. Infrared Millim. Tech.* 33, 74–81
53. Ronne, C. *et al.* (1997) Investigation of the temperature dependence of dielectric relaxation in liquid water by THz reflection spectroscopy and molecular dynamics simulation. *J. Chem. Phys.* 107, 5319–5331
54. Sim, Y.C. *et al.* (2013) Terahertz imaging of excised oral cancer at frozen temperature. *Biomed. Opt. Express* 4, 1413–1421
55. Reid, C.B. *et al.* (2011) Terahertz pulsed imaging of freshly excised human colonic tissues. *Phys. Med. Biol.* 56, 4333–4353
56. Bin Ji, Y. *et al.* (2015) Feasibility of terahertz reflectometry for discrimination of human early gastric cancers. *Biomed. Opt. Express* 6, 1398–1406
57. Brun, M.A. *et al.* (2010) Terahertz imaging applied to cancer diagnosis. *Phys. Med. Biol.* 55, 4615–4623
58. Lim, Y.L. *et al.* (2014) High-contrast coherent terahertz imaging of porcine tissue via swept-frequency feedback interferometry. *Biomed. Opt. Express* 5, 3981–3989

59. Chen, H. *et al.* (2013) The diagnosis of human liver cancer by using THz fiber-scanning near-field imaging. *Chinese Phys. Lett.* 30, 030702
60. Doradla, P. *et al.* (2013) Detection of colon cancer by continuous-wave terahertz polarization imaging technique. *J. Biomed. Opt.* 18, 090504
61. Chen, H. *et al.* (2015) Diagnose human colonic tissues by terahertz near-field imaging. *J. Biomed. Opt.* 20, 036017
62. Rong, L. *et al.* (2015) Terahertz in-line digital holography of human hepatocellular carcinoma tissue. *Sci. Rep.* 5, 8445
63. Doradla, P. *et al.* (2014) Single-channel prototype terahertz endoscopic system. *J. Biomed. Opt.* 19, 080501
64. Wahaia, F. *et al.* (2016) Terahertz absorption and reflection imaging of carcinoma-affected colon tissues embedded in paraffin. *J. Mol. Struct.* 1107, 214–219
65. Joseph, C.S. *et al.* (2014) Imaging of ex vivo nonmelanoma skin cancers in the optical and terahertz spectral regions. *J. Biophotonics* 7, 295–303
66. Joseph, C.S. *et al.* (2011) Continuous wave terahertz transmission imaging of nonmelanoma skin cancers. *Lasers Surg. Med.* 43, 457–462
67. Bowman, T.C. *et al.* (2015) Terahertz imaging of excised breast tumor tissue on paraffin sections. *IEEE Trans. Antenn. Propag.* 63, 2088–2097
68. Jung, E. *et al.* (2011) Terahertz pulse imaging of micro-metastatic lymph nodes in early-stage cervical cancer patients. *J. Opt. Soc. Korea* 15, 155–160
69. Chen, H. *et al.* (2011) Performance of THz fiber-scanning near-field microscopy to diagnose breast tumors. *Opt. Express* 19, 19523–19531
70. Peter, B.S. *et al.* (2013) Development and testing of a single frequency terahertz imaging system for breast cancer detection. *IEEE Trans. THz Sci. Technol.* 3, 374–386
71. Ji, Y.B. *et al.* (2014) Terahertz spectroscopic imaging and properties of gastrointestinal tract in a rat model. *Biomed. Opt. Express* 5, 4162–4170
72. Grootendorst, M. *et al.* (2015) The use of a handheld Terahertz pulsed imaging device to differentiate benign and malignant breast tissue with a view to reducing re-operation rates in breast-conserving surgery. *Eur. J. Surg. Oncol.* 41, S262
73. Ney, M. and Abdulhalim, I. (2010) Does human skin truly behave as an array of helical antennae in the millimeter and terahertz wave ranges? *Opt. Lett.* 35, 3180–3182
74. Ney, M. and Abdulhalim, I. (2011) Modeling of reflectometric and ellipsometric spectra from the skin in the terahertz and submillimeter waves region. *J. Biomed. Opt.* 16, 067006
75. Ney, M. and Abdulhalim, I. (2015) Ultrahigh polarimetric image contrast enhancement for skin cancer diagnosis using InN plasmonic nanoparticles in the terahertz range. *J. Biomed. Opt.* 20, 125007
76. Tewari, P. *et al.* (2012) In vivo terahertz imaging of rat skin burns. *J. Biomed. Opt.* 17, 040503
77. Tseng, T-F. *et al.* (2015) Near-field sub-THz transmission-type image system for vessel imaging in-vivo. *Opt. Express* 23, 25058–25071
78. Taylor, Z.D. *et al.* (2015) THz and mm-wave sensing of corneal tissue water content: in vivo sensing and imaging results. *IEEE Trans. THz Sci. Technol.* 5, 184–196
79. Bennett, D.B. *et al.* (2011) Terahertz sensing in corneal tissues. *J. Biomed. Opt.* 16, 057003
80. Kim, K.W. *et al.* (2012) Terahertz dynamic imaging of skin drug absorption. *Opt. Express* 20, 9476–9484
81. Kan, W-C. *et al.* (2010) Terahertz pulsed imaging of knee cartilage. *Biomed. Opt. Express* 1, 967–974
82. Bessou, M. *et al.* (2012) Three-dimensional terahertz computed tomography of human bones. *Appl. Optics* 51, 6738–6744
83. Moon, K. *et al.* (2015) Subsurface nanoimaging by broadband terahertz pulse near-field microscopy. *Nano Lett.* 15, 549–552
84. Hintzsche, H. and Stopper, H. (2012) Effects of terahertz radiation on biological systems. *Crit. Rev. Environ. Sci. Technol.* 42, 2408–2434
85. Wilmink, G.J. and Grundt, J.E. (2011) Invited review article: current state of research on biological effects of terahertz radiation. *J. Infrared Millim. Tech.* 32, 1074–1122
86. Dalzell, D.R. *et al.* (2010) Damage thresholds for terahertz radiation. In *Proceedings of SPIE 7562, Optical Interactions with Tissues and Cells XXI 7562, 75620M*.
87. Williams, R. *et al.* (2013) The influence of high intensity terahertz radiation on mammalian cell adhesion, proliferation and differentiation. *Phys. Med. Biol.* 58, 373–391
88. Wilmink, G.J. *et al.* (2011) In Vitro investigation of the biological effects associated with human dermal fibroblasts exposed to 2.52 THz radiation. *Lasers Surg. Med.* 43, 152–163
89. Titova, L.V. *et al.* (2013) Intense THz pulses cause H2AX phosphorylation and activate DNA damage response in human skin tissue. *Biomed. Opt. Express* 4, 559–568
90. Alexandrov, B.S. *et al.* (2010) DNA breathing dynamics in the presence of a terahertz field. *Phys. Lett. A* 374, 1214–1217
91. Alexandrov, B.S. *et al.* (2013) Specificity and heterogeneity of terahertz radiation effect on gene expression in mouse mesenchymal stem cells. *Sci. Rep.* 3, 1184
92. Cherkasova, O.P. *et al.* (2009) Influence of terahertz laser radiation on the spectral characteristics and functional properties of albumin. *Opt. Spectrosc.* 107, 534–537
93. Bogomazova, A.N. *et al.* (2015) No DNA damage response and negligible genome-wide transcriptional changes in human embryonic stem cells exposed to terahertz radiation. *Sci. Rep.* 5, 7749
94. Kirichuk, V.F. *et al.* (2009) Effect of high power terahertz irradiation on platelet aggregation and behavioral reactions of albino rats. *Bull. Exp. Biol. Med.* 148, 746–749
95. Hwang, Y. *et al.* (2014) In vivo analysis of THz wave irradiation induced acute inflammatory response in skin by laser-scanning confocal microscopy. *Opt. Express* 22, 11465–11475
96. Kim, K-T. *et al.* (2013) High-power femtosecond-terahertz pulse induces a wound response in mouse skin. *Sci. Rep.* 3, 2296
97. Neelakanta, P.S. and Sharma, B. (2013) Conceiving THz endometrial ablation: feasibility, requirements and technical challenges. *IEEE J. Biomed. Health Informat.* 17, 813–819
98. Weisman, N.Y. *et al.* (2015) Terahertz radiation improves adaptation characteristics in *Drosophila melanogaster*. *Contemp. Probl. Ecol.* 8, 237–242
99. Kirichuck, V.F. *et al.* (2008) Sex-specific differences in changes of disturbed functional activity of platelets in albino rats under the effect of terahertz electromagnetic radiation at nitric oxide frequencies. *Bull. Exp. Biol. Med.* 145, 75–77
100. Saviz, M. *et al.* (2013) Theoretical estimations of safety thresholds for terahertz exposure of surface tissues. *IEEE Trans. THz Sci. Technol.* 3, 635–640
101. Spathmann, O. *et al.* (2015) Numerical computation of temperature elevation in human skin due to electromagnetic exposure in the THz frequency range. *IEEE Trans. THz Sci. Technol.* 5, 978–989
102. El Haddad, J. *et al.* (2013) Review in terahertz spectral analysis. *Trends Anal. Chem.* 44, 98–105