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Perspective

Perspective on Terahertz Applications in Bioscience and Biotechnology

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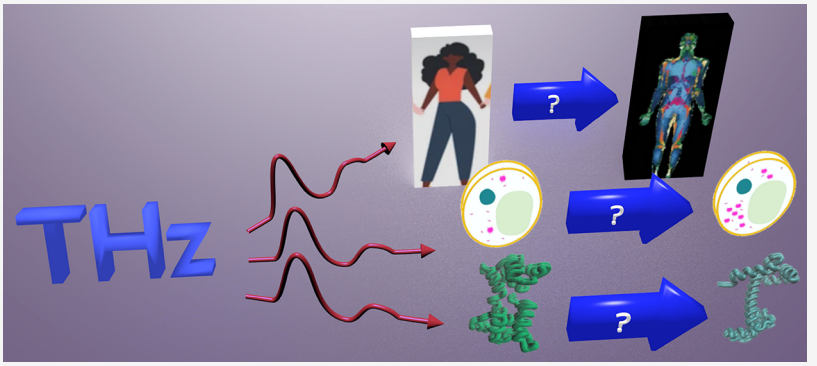

ABSTRACT: Within the field of terahertz science and technology, one of the most active areas of current research focuses on the intersection of terahertz measurements and methods with the world of biology and medicine. Current activities revolve around numerous diverse questions, ranging from studies of the vibrational spectra of biomolecules and macromolecular complexes to biosensing to medical diagnostics based on noninvasive imaging techniques. Unlike many other areas in which terahertz science is now making inroads, this research domain has been plagued with a number of misleading ideas, which originated at least two decades ago and continue to crop up in current literature. In the worst case, these unfortunate notions can distract from, and even obscure, fascinating and meaningful results. The purpose of this Perspective is to highlight a few of these mistaken concepts and, more importantly, to distinguish them from the many interesting works that continue to emerge from the fruitful marriage of terahertz with biology and medicine.

KEYWORDS: *terahertz, biophotonics, biomedical, imaging, spectroscopy*

I. INTRODUCTION

Over the last 30 years, there has been a significant maturation of the field of terahertz science and technology. The state of the art in the technology has progressed dramatically, affording far easier access for researchers in all fields to the terahertz spectral range (roughly speaking, 1−200 cm^{-1}).[1,2] When the authors of this Perspective first began their research careers in terahertz science, the spectrometers, based on the technique of time-domain spectroscopy, required femtosecond mode-locked dye lasers, pumped by large-frame argon ion lasers.[3] Today, these cumbersome and challenging lasers are long gone, replaced by more compact and robust mode-locked solid-state or fiber lasers. This transition was key to the commercialization of terahertz spectrometers, which began in about 2000.[4] Today, there are at least eight companies selling time-domain spectrometers, for a wide variety of uses in both commercial and academic settings. Meanwhile, the excitement surrounding time-domain spectroscopy quickly inspired similar progress in other areas of terahertz technology. Since about the year 2000, major steps have been taken in the maturation of solid-state terahertz sources,[5] terahertz cameras,[6] and theoretical tools for elucidating terahertz light−matter interactions.[7] All of these efforts have substantially lowered the bar for entry into the field. As a consequence of this progress, the ideas of terahertz science have found their way into many different disciplines. Inevitably, this has produced some exciting results, as well as some with more dubious value.

In this Perspective, we have chosen to focus on one of the more challenging (and perhaps controversial) of these areas, which is the application of terahertz techniques in biology and medicine. Terahertz measurements of biomaterials are motivated in part by the overlap of fundamental excitations

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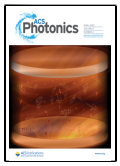

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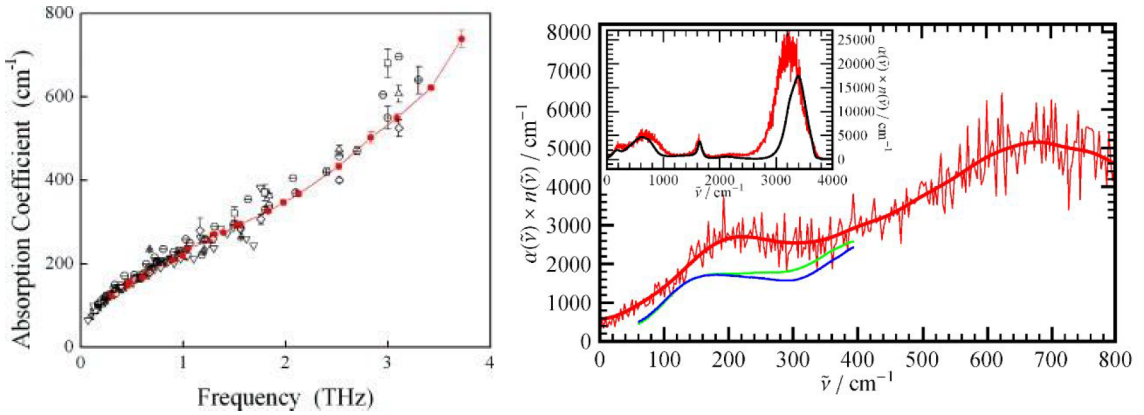

Figure 1. Room temperature absorption spectrum of liquid water in the terahertz range, in the lower portion of the frequency range (left) and the higher portion (right). Left panel: Measurements obtained using several different techniques. Reproduced with permission from ref 8. Copyright 2006 AIP Publishing. Right panel: Blue curve: experimental absorption spectrum of liquid H_2O. Green curve: experimental absorption spectrum of liquid D_2O. Red curve: ab initio molecular dynamics (AIMD) simulation. The inset shows the comparison to AIMD over a larger frequency range. Adapted with permission from ref 9. Copyright 2010 NAS. Note that the spectrum is essentially featureless and monotonic at all frequencies below ~5 THz.

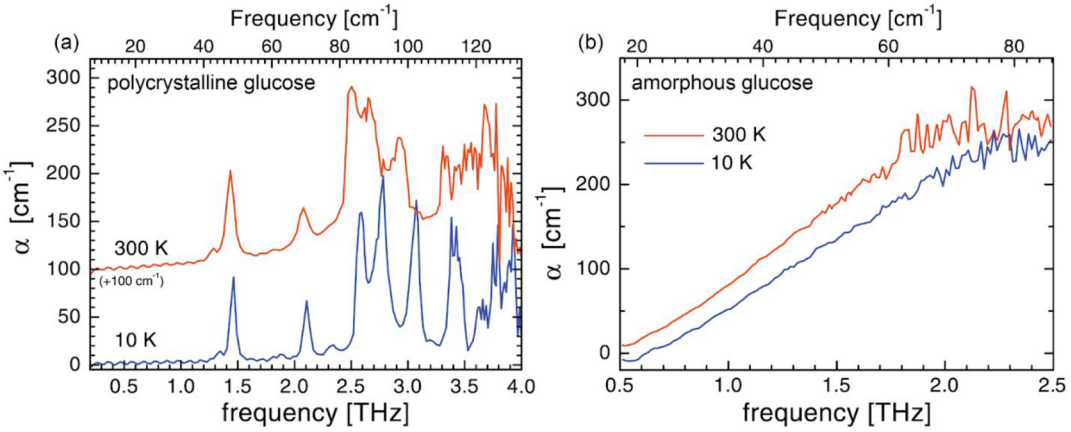

Figure 2. A comparison of the absorption spectra for crystalline and amorphous glucose. The peaks in (a) are not associated with motions of a single molecule, but rather with lattice vibrations. In a disordered system of the same molecule, these vibrational signatures are absent. Adapted with permission from ref 27. Copyright 2003 Elsevier.

with this energy range. Intermolecular and intramolecular vibrational and librational excitations often lie in this range. Further, if chemically or biologically relevant contrast can be established, THz could then be broadly applied without concern of specimen damage, as the photon energy is well below that of chemical bonds. The key challenge here is relatively easy to understand: biology happens in water, but liquid water absorbs quite strongly across the entire terahertz range. This absorption coefficient is frequency dependent, increasing smoothly and monotonically up to about 5 THz (167 cm^{-1}),[8,9] as shown in Figure 1. Yet, even at a relatively low THz frequency, say 0.5 THz, the penetration depth of radiation into liquid water is quite small, only 63 μm (at 22 °C).[8] While this is not the only issue, the extremely large attenuation due to the absorption coefficient of liquid water is an ever-present consideration in any discussion of spectroscopy or imaging of biological media. Obviously, since living tissue is not the same as pure water, the penetration depth is correspondingly larger, reaching as high as ~200 μm at 0.5 THz for healthy skin tissue.[10] While indeed larger, this is still the dominant limiting factor both in interpreting spectroscopic results and in biomedical applications. In the case of tissues, the issue of scattering from wavelength-scale inhomogeneities becomes increasingly important, as this scattering can not only attenuate the signal reaching the detector, but can also obscure spectroscopic signatures, or even induce false ones.[11]

In recent years, there have been a number of review articles on the general topic of terahertz applications in biology and medicine.[12−15] Here, we take a somewhat different approach from most of these earlier reviews. As this is a Perspective, rather than a review, we do not aim to provide a comprehensive overview. At this point, the field is simply too large, and the reported results too numerous, to do so. Instead, we begin our discussion by advancing the notion that at least some (though by no means all) of the publications in the field contain misleading assertions or irreproducible results. As a key illustration of this unfortunately not-uncommon issue, we consider the question of the existence in the THz range of spectrally narrow, well-resolved vibrational resonances in biological media. Despite numerous claims to the contrary, in both older[16] and more recent[17,18] publications, condensed-phase material systems that lack long-range crystalline order (including living cells and tissues, as well as biological molecules in solution or randomly aggregated on a surface)

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simply cannot exhibit well-resolved peaks in linear absorption spectra at frequencies up to a few terahertz. Such claims abound in the literature (e.g., refs 15, 19, and 20), generally without the minimal corroborating evidence of being reproduced by independent researchers in a different lab, suggesting that reports of these resonant features are easy to publish, if not easy to substantiate. As another example, a theoretical discussion from about 10 years ago of the effects of terahertz waves on DNA[21] has become quite influential, although the analysis treated the molecule classically and ignored such basic physics as intramolecular vibrational relaxation and the coupling of the solvated DNA molecule to the surrounding water bath. As a result of these oversimplifications, the conclusions have no implications for the real world. Yet, the paper (and subsequent press coverage[22]) has led many to the erroneous implication that terahertz radiation can "unzip" DNA molecules in living cells. Even today, many calculations of vibrational modes of molecular solids are based on an analysis of the molecule in the gas phase, neglecting interactions with its environment,[23,24] an approach that is well-known[25,26] to produce inaccurate and often meaningless results. Unfortunately, these false notions persist in the zeitgeist.

The idea that noncrystalline condensed-phase systems can exhibit well-resolved THz resonances in measured absorption spectra represents a profound misunderstanding of the nature of light-matter interactions in this frequency range. Of course, resonant absorption features can (and do) exist for individual molecules in the gas phase, where rotations (or motions associated with coupled rotational−vibrational excitations) are not hindered by steric interactions. However, for small molecules (<1 kDa) in condensed phases, these unhindered motions become highly overdamped, and the resonant peaks disappear, replaced by broad featureless continua, or (in the case of crystalline materials) by collective vibrational modes associated with long-range order (i.e., lattice phonons; see Figure 2). In large biomolecules in solution or in a noncrystalline state, underdamped intramolecular vibrations can exist, but they are always embedded within a highly congested spectrum reflective of a continuum vibrational density of states, such that individual modes can never be spectrally resolved in a conventional linear absorption measurement. This key distinction between ordered crystalline materials and other condensed-matter materials has been clarified over the last two decades in several works using both terahertz techniques[27] and other methods.[28]

In fact, this idea is not the only misleading notion to be found in the recent literature. Another disturbing discussion centers on the nonthermal alteration of biological processes by exposure to THz radiation. It is of course well-known that illumination of any absorbing medium by an electromagnetic wave can produce heating, and that changes in temperature can influence many biological processes. For long-wavelength radiation, where the photon energy is far below that needed to induce chemical reactions (e.g., bond energies), and even below that of thermal photons at ambient temperature, one might wonder if any nonthermal mechanisms exist that can induce any biologically relevant changes. This question has been the topic of extensive studies for many years at lower frequencies[29] and also, more recently, in the THz regime.[30−32] In most of the cases when the data have been interpreted to show a nonthermal effect arising from exposure to THz light, the experiments did not employ the needed controls and statistics to rule out a more prosaic interpretation, such as heating.[33−40] These are exceedingly challenging experiments, as (for example) it is almost impossible to rule out the possibility of spatially nonuniform transient heating with significant temperature variations on a subcellular length scale. Most notably, none of the purported nonthermal effects have ever been verified by a second independent research laboratory, and no plausible underlying mechanism has been proposed for how such effects could be induced by photons with only a few millivolts of energy. Field-induced effects such as electroporation are of course a different matter and are addressed in more detail in Section V.

To be clear, none of the above critiques are intended to cast aspersions on the motivations of any researcher in the field. Even the most well-intentioned scientist (including, for example, one of us) can be mistaken or misled, and find himself as a coauthor on a paper containing claims that are just flat-out wrong.[41] The point here is not to scold, but to caution. Many current articles, even those containing solid and reasonable science, continue to cite papers containing these incorrect results,[42−44] perpetuating their misleading influence on the field.

Having said all of that, we do not wish to convey the impression that the entire field of THz biology is meaningless. Although there are a number of published articles containing claims that are based on either ruthlessly oversimplified computational models or obvious errors in experimental technique or data analysis, it would be most unfair if these unfortunate examples tainted the entire field. Indeed, the goal of this Perspective is to highlight some of the excellent research that has been, and continues to be, reported in the field. We will describe a few truly innovative approaches to mitigating the challenges noted above, which have enabled THz measurements that have revealed new science about biologically relevant material systems. The marriage of terahertz with biology is indeed exciting, and with proper caution about extraordinary claims, one can easily cite many fascinating results. In this Perspective, we discuss a few examples and speculate about the most promising future directions.

II. BIOMEDICAL IMAGING

The first and perhaps most obvious idea in the application of terahertz techniques to problems in medical science involves the generation of images. This idea has some intrinsic appeal; for one, terahertz radiation is inherently nonionizing. Unlike X-rays, there are no cumulative effects of exposure, and short-term exposure limits are determined only by tissue damage that would result from heating. Indeed, the low penetration depth implies that exposure to terahertz radiation is even safer than exposure to RF or microwave radiation, since the thermal damage would be limited to only the uppermost skin layers.

The earliest work in this area focused on the identification of subcutaneous dermal carcinoma. The goal was to provide a noncontact method for defining the extent of a tumor, avoiding the need for histological analysis.[45] Numerous similar studies have been performed more recently, for example on excised breast cancer tissue samples.[46] While initially promising, this idea has not been widely adopted. The contrast mechanism in such images is likely associated with a change in tissue water content, due to the increased vascularization of cancerous tissue compared to healthy tissue. An image based on water content does not contain a lot of information, as this is a pretty nonspecific contrast mechanism. This fact, combined with the

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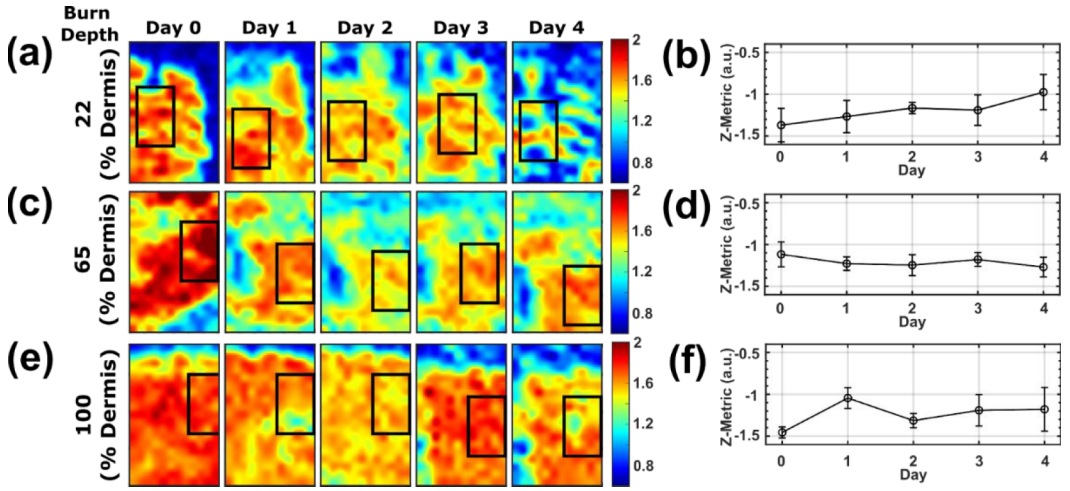

Figure 3. Burns imaged in reflection using terahertz pulses. Panels (a), (c), and (e) show burns of increasing severity, imaged over the course of several days. Panels (b), (d), and (f) show the evolution of a hyperspectral metric defined in ref 55, which offers the possibility of noncontact discrimination of burn severity. Adapted with permission from ref 55. Copyright 2022 Wiley; https://creativecommons.org/licenses/by/4.0/.

issues of absorption and scattering noted above, have so far limited the value of this diagnostic approach. Nevertheless, several interesting in vivo imaging studies based on this contrast mechanism have been reported,[47] and it could be that this idea will eventually prove valuable.

We note in passing that the suggestion that one could obtain more specific information through imaging of biomarker concentration variations (by relying on resonant absorption features of specific molecules) is often cited.[18,48,49] But, that proposal relies on the unfounded idea discussed above that resonant absorption peaks of a molecule in the gas phase (or in a crystallized solid state) persist even when that same molecule is randomly dispersed in a liquid or other amorphous medium.

Beyond cancer detection, researchers have recently discussed several other ideas based on terahertz imaging for quantification of moisture in exposed tissue, such as skin. One interesting recent example involves the study of diabetic foot syndrome, through reflection imaging of local water content on the sole of the patient's foot.[50] Many diabetics experience a loss of sensation in the lower limbs due to neurological deterioration, and this condition is accompanied by skin dehydration. This condition is the most common cause of limb amputation after traumatic accidents. A recent study of diabetic foot involving terahertz imaging of 178 patients represents the largest human population imaged with terahertz radiation for medical purposes. This work demonstrates that terahertz imaging can be a valuable tool for early diagnosis of this debilitating condition.[51] Terahertz reflection imaging can also be used for characterizing the water content of the cornea, which could be valuable for early detection of a number of different corneal diseases. This proposal was first advanced about 10 years ago with ex vivo[52] and then in vivo[53] studies and continues to be a topic of current research interest.[54]

Another example in which noninvasive terahertz reflection imaging measurements could be valuable is the noncontact imaging of wounds to evaluate healing (potentially, without the need to remove bandages).[56] In the case of burns, there can be an additional contrast mechanism, beyond just changes in water content. Numerous skin structures, both at the surface and buried in the tissue volume, are of the scale of the THz wavelength in size. Burns can change the concentration of such structures, producing a wavelength-dependent contrast mechanism due to light scattering, that can be exploited in addition to the (less wavelength-dependent) contrast due to hydration level (see Figure 3).[57] Following the first discussion of this idea,[58] a variety of in vivo studies on animal models including rats[59] and pigs[56] have established the possibility of measuring burn depth using terahertz techniques, which can be difficult to assess by other methods in a clinical setting. Terahertz reflection studies also can distinguish between third-degree "full thickness" burns and less severe burns in a noncontact fashion, through measurements of water depletion and accumulation in the minutes and hours immediately after the injury.[60]

III. BIOMOLECULAR DYNAMICS

The very strong absorption of liquid water mentioned above can, in certain cases, be exploited in interesting ways. For instance, THz spectroscopy is now used somewhat routinely to characterize water structure and dynamics on different length scales. A well tested multicomponent model for the dielectric response of water along with the development of precision measurements techniques, have led to methods for determining the number of water molecules strongly bound to a biological membrane or biomolecule,[61,62] the effective extent of the solvation shells for different structural motifs, and secondary structure content, all without the need for tagging with local probes.[63] While local probes such as time-resolved fluorescence[64] can provide detailed information about the heterogeneous water structure, broadband THz measurements can distinguish multiple contributions to the response including local, intermediate range and long-range effects.[65] Even though the technique is nonlocal, some surface resolution can be attained by local perturbation comparisons such as ligand binding, and reorganization time scales can be determined using pump−probe techniques.[66] In such measurements, THz absorption sensitivity to solute concentration due to changes in bound water fraction are subtle; results obtained over only a single decade of frequency range, or single path length cells, are unlikely to produce reliable results.

Of course, there is keen interest in whether THz can provide any unique insight into the dynamics of biomacromolecules themselves. While investigators had shown that collective vibrations of structured biomacromolecules such as RNA

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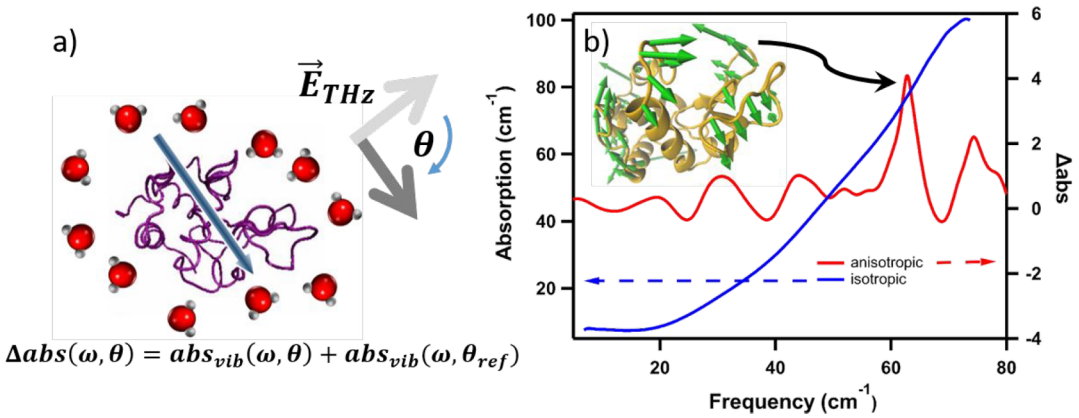

Figure 4. Distilling a congested spectrum using anisotropic absorption. Panel (a) illustrates the selective absorption for polarization angle along the vibration's dipole transition vector. (b) The isotropic (blue, left axis) and anisotropic (red, right axis) absorption spectra for lysozyme. Adapted with permission from ref 67. Copyright 2021 American Chemical Society.

polynucelotides and proteins can indeed be underdamped even in aqueous solution,[28,68−72] experimental characterization has been extremely challenging because of their dense vibrational density of states (VDOS) and their small THz absorption strength (relative to water). Standard linear scattering and absorption measurements of solvated proteins reveal a broad smooth and mostly featureless response, as in the left axis data in Figure 4b.[73,74] Nevertheless, THz measurements of hydrated powders, hydrated films and solutions have provided insight both into hydration and structural dynamics.[75−80] An outstanding question for molecular biology is whether and how biomolecular vibrations impact biological outcomes. To answer this question one must achieve some spectral definition, as has been done with some success using, for example, Mossbauer techniques.[70] One interesting method for attaining vibrational selectivity in the THz range is polarization spectroscopy, which can isolate the vibrations based on the direction of their transition dipole moment. By measuring the change in the absorption with polarization, the water background can be removed and spectral structure reflecting the directionality of the motions is attained. Figure 4a illustrates the concept and Figure 4b shows how it increases spectral structure, using a particular realization called anisotropic terahertz microspectroscopy.[67] Alignment of solute molecules is most easily achieved using protein crystals, regular arrays of molecules in a hydrated environment similar to a crowded cell.[81] However, robust modeling accounting for the configurational heterogeneity of the molecules finds that the anisotropic motions persist even in solution.[82] Other promising approaches include nonlinear correlation methods such as 2D THz spectroscopy,[83] an emerging technique which is now beginning to reveal important dynamical processes in liquids.[84]

IV. BIOSENSORS

Over the years, there have been a very large number of studies that have discussed the use of terahertz techniques for biosensing, ranging from sensing of biomolecules and biopolymers to sensing specific bacteria or viruses. As with much of the work discussed above, there are some truly interesting and innovative ideas here, but they are sometimes obscured by other works containing claims that are unsubstantiated, or just plain wrong. Recent publications, for example, trumpet the claim that disease-causing viruses exhibit spectroscopic absorption features in the terahertz range that can be used to detect the virus and even to distinguish one virus from another, based on their terahertz spectroscopic fingerprints.[85,86] Such claims are mistaken, and should be treated with an appropriate level of skepticism.

Because of the lack of terahertz spectroscopic features in most biological systems, the primary method for biosensing is based on changes in dielectric contrast. A typical strategy is to employ an artificial structure such as a metasurface or a nanogap array, which exhibits a spectrally narrow resonance in the terahertz range. The introduction of a biological or biochemical medium in the vicinity of the resonator can cause a resonance shift, due to the changed dielectric environment.[87] This general scheme has been studied by many authors, and can offer an impressive level of sensitivity.[88] However, this scheme offers little specificity, since the only thing that is measured is the average dielectric of the medium, usually at just one particular frequency (the resonant frequency of the artificial structure). Nevertheless, claims of specificity, even the ability to distinguish different subtypes of a particular strain of flu virus, can easily be found in the recent literature.[89,90] These claims are obviously incorrect; a featureless dielectric spectrum cannot be distinguished from another featureless dielectric spectrum by measuring their respective values at one particular frequency. This is especially true in situations where the strength of the interaction of the analyte with the resonant structure is uncalibrated, due to factors such as the rapid spatial variation of the local electric field, and the density of the biological material (which correlates strongly with dielectric constant for many liquids and amorphous materials in the terahertz range[91] and which is generally neither controlled nor measured in sensors of this type).

Of course, enhanced specific detection of, for example, a particular protein, can be achieved by functionalizing the surface of the resonant sensor, or that of nearby metal particles, with complementary binding sites[92] via an immobilized bioreceptor. This well-known approach is not specific to terahertz sensing, and so it is unclear if it offers greater improvement when compared to sensing modalities at other wavelengths (e.g., conventional SPR sensors). Even so, the strategy of employing a resonant light-matter interaction (to enhance sensitivity) combined with analyte-specific binding (to provide selectivity) has produced some interesting and potentially promising results in the THz range. For example,

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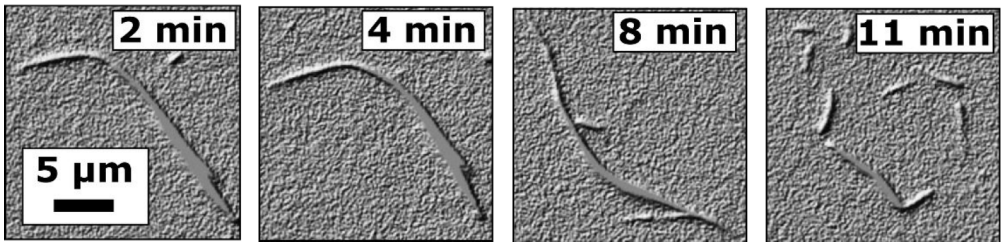

Figure 5. High magnification time-series images of a single motion-tracked microtubule (MT) highlights the structural disassembly of polymerized and chemically stabilized MTs. Here, exposure to a 1 kHz train of picosecond THz pulses with peak electric field of ∼230 kV/cm causes disassembly of the MT within 11 min. Adapted with permission from ref 42. Copyright 2021 The Optical Society.

one recent study reports the sensitive and selective detection of virus proteins, including that of SARS-CoV-2, using a THz biosensor incorporating metallic nanoparticles functionalized with the appropriate antibody to provide selectivity.[93] One intriguing advantage of using terahertz radiation for this approach is that there is no need for an optical tag; a dielectric change will always occur with binding.

Another strategy for biosensing relies on adapting microfluidic platforms for terahertz spectroscopy. This approach has the attractive feature that the path length of the terahertz beam through the sample can be carefully controlled, and optimized for aqueous solutions. Some of the earliest work on terahertz microfluidics was focused on the study of nonpolar liquids such as hydrocarbons,[94] for applications in the petrochemicals industry. However, with a thin (∼100 μm) sample path, the attenuation due to the absorption by liquid water is manageable, and changes in the attenuation due to the introduction of solvated biomolecules, cells, or other biological structures into the microfluidic chamber can therefore be observed.[95] As noted above, these signatures are never in the form of narrow resonant absorption peaks, despite some recent (highly implausible) claims to the contrary.[96] Even in a microfluidic channel, solvated molecules cannot manifest resonant absorption peaks at frequencies below 5 THz (and neither can liquid water). Rather, the observed changes are broadband and largely uniform across the frequency spectrum, representing a shift in the overall average dielectric constant of the liquid.[97,98] Such changes may be due to, for example, an increase in the density of hydrogen bonds.[99] As a consequence, careful calibration steps are required in order to extract quantitative information from such measurements. The combination of microfluidics with resonant structures such as metasurfaces can provide enhanced sensitivity to these dielectric shifts,[100] offering interesting possibilities for THz biosensing in aqueous environments.

V. BIOMEDICAL THERAPIES AND APPLICATIONS

Another area of growing activity involves the general idea of exploiting terahertz radiation for its therapeutic possibilities. At first thought, this may seem highly implausible, given the small penetration depth into living tissue, and the photon energy which is far too small to induce any chemical reaction. Yet, at either high power or high peak electric field, the possibility becomes more plausible. In this context, we note that many THz biomedical investigations have used the terminology "nonthermal effects". This phrase has been used with several different meanings, so clarity of language is important. Earlier in this Perspective, we used the term to indicate hypothetical effects induced in cells by exposure to THz radiation that are caused by some mechanism other than a change in temperature. However, others have used the same term to acknowledge that high-power steady-state illumination with THz radiation can lead to substantial heating relative to the environment due to the strong absorption of the radiation by liquid water.[101] The effects are "nonthermal" in the sense that cells or tissues are locally heated by the radiation field, and are therefore out of equilibrium with the ambient environment. Such effects, which might more properly be termed "non-equilibrium heating", are of course quite plausible. They must be distinguished from "photonic effects" which would involve the excitation of an underdamped resonance at a particular frequency (with the implication that illumination at other nearby frequencies would have dramatically different effects).[35,37] As noted above, these photonic effects are highly implausible due to, among other things, the broad and featureless absorption spectra of all forms of biological media. Nevertheless, it is clear that THz radiation of sufficiently high intensity can perturb cellular biochemistry by rapidly (and perhaps nonuniformly) changing the local temperature distribution experienced by cells.

A second mechanism involves the use of high-peak-field pulsed illumination, where the average power in the THz beam may remain low, but the peak electric field is large, on the order of tens of kV/cm or larger (essentially, the regime of nonlinear optics). In this case, several groups have demonstrated nonthermal effects associated with the extremely large electric field amplitude.[102−104] These results are exciting, although independent verification and statistical reliability remain lacking in most cases. We emphasize that such effects are generally restricted to the case of illumination with ultrashort (picosecond-scale) terahertz pulses. The reason for this is simple: a continuous (not pulsed) electromagnetic wave with a sufficiently large peak field of 10 kV/cm corresponds to an intensity exceeding 100 kW/cm^2, well beyond the threshold for thermal destruction. With the recent proliferation of techniques for producing high-field picosecond THz pulses, this field-induced mechanism, along with the nonequilibrium heating mechanism described above, could provide unique therapies if properly explored and developed.

A number of studies have shown transcription manipulation with high field/high power THz illumination.[105−107] Recently, several plausible (i.e., not relying on excitation of a particular resonance) mechanisms have been proposed for the observed effects. One involves the effects of rapid energy absorption in the solvent, which can lead to local inhomogeneous heating as well as impulsively initiated pressure variations.[101] Another interesting possibility is that these short ultrahigh-field pulses could induce spatially selective electroporation. Cell membrane electroporation is a laboratory tool for transformation and molecular insertion.[108] Studies have indicated an inverse

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relationship between the field strength needed for pore formation and the pulse duration.[109,110] Moreover, there is some evidence of electroporation using low-frequency radiation,[111,112] but the mechanism of the transcription manipulation in the aforementioned studies has not yet been firmly established. This approach could provide an exciting tool for in vitro studies. However, with the rapid advance of mRNA and Crispr Cas9 targeted expression technologies, it is difficult to foresee therapeutic applications of uncontrolled gene manipulation with THz radiation.

Finally, several studies have put forward the possibility of locally inducing cell death, for example, in cancerous tissue, using terahertz radiation. This would be based on manipulation of actin or tubulin filaments,[42,113,114] as illustrated in Figure 5. The rapid localized heating/cooling of the solvent may provide a mechanism for in vitro filament manipulation. In the case of in vivo studies, the cells were illuminated with high field pulses (~60 kV/cm), which is sufficient for some net polarization of small molecules and also for electroporation, as noted above. In this case, rapid changes in pH and ionic strength as well as redistribution of cellular contents could contribute to the observed changes. Regardless of the mechanism, the application of these treatments to skin pathologies may warrant further investigation.

VI. CONCLUSION

We hope that the above discussion has provided a useful overview of some of the exciting and important ways in which terahertz techniques are having an impact in areas relevant to the biological sciences. Admittedly, our discussion has also made note of some less inspiring examples. One may be led to ask, why do such examples persist in the literature? Rather than speculate as to the cause, we suggest that it would be more productive to focus on the solution. We would, for example, urge journal editors to reject any manuscript that claims to have obtained biologically relevant information through studies of dried films or lyophilized powders. Manuscripts that rely on gas-phase calculations of terahertz molecular vibrational modes to inform measurements of molecules that are not in the gas phase should never be sent out for review. Data purporting to show well-resolved and isolated absorption peaks due to resonant vibrational motions in noncrystalline condensed-matter systems should be viewed with the appropriate degree of skepticism. Studies which claim to have observed non-thermally induced changes in biological function from THz exposure should be held to the same standards as are common in the medical literature, with well-established guidelines for statistics, double-blind studies, repeatability, and so on. These and similar basic sanity checks, uniformly applied, would go a long way toward resolving the issues noted here.

Ultimately, we are highly optimiztic and enthusiastic about the current and future activities in this discipline. We feel that THz techniques can provide answers to some vexing and interesting questions, properly posed. For example, why is it that the water librational and rotational relaxational excitations exquisitely overlap the biomolecular intramolecular vibrations? Why do biomolecules not have unique isotropic absorption signatures? What contrast mechanisms other than water content could be exploited for diagnosis of disease or injury? Terahertz research may provide foundational understanding of biomaterials, and could underpin valuable new technologies to improve human health. As with many other areas in which terahertz science is making inroads, we look forward to an exciting future.

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