Terahertz sensing based on metasurfaces

*Miguel Beruete* and *Irati Jáuregui-López*

Dr. M. Beruete, Irati Jáuregui-López 1, 2,
1 Antennas Group-TERALAB, Universidad Pública de Navarra, Campus Arrosadía, 31006 Pamplona, Spain
2 Multispectral Biosensing Group, Navarrabiomed, Complejo Hospitalario de Navarra (CHN), Universidad Pública de Navarra (UPNA), IdiSNA. Irunlarrea 3, 31008 Pamplona, Navarra, Spain
E-mail: miguel.beruete@unavarra.es

Dr. Miguel Beruete
Institute of Smart Cities (ISC), Public University of Navarra, 31006 Pamplona, Spain

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The terahertz band has very attractive characteristics for sensing and biosensing applications, as it is not a non-ionizing radiation and sensitive to weak interactions, complementing typical spectroscopy systems at infrared. However, a fundamental drawback is its relatively long wavelength (10 – 1000 μm) which makes it blind to small features, hindering seriously both thin-film and biological sensing. Recently, new ways to overcome this limitation have become possible thanks to the advent of metasurfaces. These artificial structures are planar screens usually made of periodic metallic resonators and whose electromagnetic response can be controlled at will by design. This design freedom allows metasurfaces to overcome the limitations of classical THz spectroscopy, by creating fine details comparable to the size of the thin-films of microorganisms under test. The strong field concentration near these small metasurface details at resonance make them highly sensitive to tiny variations in the nearby environment, allowing for an enhanced detection more accurate than classical THz spectroscopy. In this review, the main advances in THz metasurface sensors from a historical as well as application-oriented perspective are summarized. The focus is put mainly in thin-film and biological sensors, with the aim to cover the most recent advances on the topic.
1. Introduction

The terahertz (THz) band is the portion of the electromagnetic spectrum placed between microwaves/millimeter-waves and infrared radiation. Although there is not complete consensus about its exact location [depending on the reference consulted, one can find that the lower and upper frequency limits are put either at 100 GHz - 10 THz (3 mm - 30 µm) or 300 GHz - 30 THz (1 mm - 10 µm)], it encompasses the radiation with wavelengths ranging from a few tens to thousands of microns. Historically, this band has been elusive, to the point that it was known as the “terahertz gap”, due to the difficulty to generate and detect this radiation efficiently at ambient temperature. To make things worse, THz was largely eclipsed by the vast development of microwave and infrared technology. All these factors together restricted THz applications in the past to narrow specific niches mainly related with basic science like astronomy and spectroscopy.[1,2]

Luckily, a series of recent breakthroughs have reverted the situation putting THz in the focus of research, as demonstrates the dramatically increasing activity related to the topic in worldwide research. Efficient THz sources and detectors are now available as well as commercial instrumentation specifically designed to operate at THz frequencies, such as Time Domain Spectroscopy (THz-TDS) systems,[3] complementing Fourier Transform Infrared (FTIR) Spectroscopy and providing ultimately a new impulse to THz research. Exciting applications are thus constantly foreseen and some of them coming gradually to light in different fields including medicine, biology, communications, security, defense, food industry, etc.[1,3,12–15,4–11] This might be just the tip of the iceberg and killer THz applications might be just around the corner.

THz radiation has some characteristics that make it a suitable technology for sensing applications, complementing or enhancing conventional solutions.[7,10,15] Unlike x-rays used in medical diagnosis, THz waves are non-ionizing and do not damage biological tissues, a property of outmost importance for health applications and critical for biodetection in
Moreover, THz waves are sensitive to weak resonances such as hydrogen bonds, van der Waals forces and non-bonding (hydrophobic) interactions not detectable by classical mid-infrared (MIR) spectroscopy, offering new means for substance identification and opening new paths for identification of macromolecules like amino acids, proteins, RNA or DNA in biological and/or medical investigation. Furthermore, THz instrumentation gives both magnitude and phase information, allowing for a direct extraction of the refractive index and attenuation constant of the materials or substances under test. In addition, the strong sensitivity of THz waves to water is perhaps the most exploited feature in biomedical applications, and has been intensely investigated due to the promising perspectives it offers in cancer detection but also in other diseases like otitis media. Finally, THz waves can penetrate materials composed of non-polar molecules such as paper, plastic and textile materials (opaque to visible and near-infrared (NIR) waves), enabling easier security checks and safer inspection without direct contact between the operator and the measured substance. These properties have given rise to a powerful research line and, indeed, the list of biological applications of THz is extensive and in continuous development so the interested reader is referred to some recent books and reviews devoted to the topic.

Nevertheless, a challenge still open is how to achieve reliable sensing when the measured sample amount is very small. In this situation, the substance under test simply does not interact sufficiently with the electromagnetic waves inducing negligible changes in the response and making detection extremely difficult or even impossible with conventional techniques. This problem, which arises in every sensing application, is especially serious in the THz band, because of its relatively long wavelength. This type of measurement is called thin-film sensing and becomes indispensable in the characterization of dangerous samples (i.e. toxic substances, explosives, etc.) that use very small amounts of the substance or when the samples are functionalized because monolayers are easier to process as thin-films. Moreover,
thin-films may improve the sensitivity compared to bulk samples, due to purity or crystallinity properties and can be complementary to other conventional techniques, like ellipsometry.\cite{26} In this context, metamaterials (and more specifically, metasurfaces) are emerging as true game changers for reliable and ultrasensitive label-free thin-film detection.\cite{26} Metamaterials can be defined as artificial materials whose electromagnetic properties can be controlled by the shape, geometry or orientation of its constituents, called metaatoms. This fascinating research topic revolutionized the field of electromagnetics after the seminal papers of Pendry\cite{27-29} and Smith\cite{30} that made possible the artificial materials predicted in 1968 by Veselago\cite{31} leading to the first realization of a negative index medium (see\cite{32} for a historical review). After nearly two decades of intensive research, metamaterials have given rise to provoking concepts such as enhanced lenses\cite{33} or invisibility cloaks,\cite{34} and have been recognized as one of the century photon milestones.\cite{35}

Nowadays, metamaterials research is progressively leaning towards metasurfaces\cite{36-40} (the two-dimensional counterpart of volumetric metamaterials) that have given rise to a new wave of revolutionary ideas like generalized Snell’s law structures, planar lenses, enhanced spin-Hall effect, wavefront mathematical computation or even ultrathin invisibility cloaks.\cite{40-43}

In addition to those exciting realizations, metasurfaces are emerging as a true game changer for reliable and ultrasensitive sensing platforms, all along the electromagnetic spectrum, but with special relevance at THz frequencies. The key idea behind this type of sensors is to engineer (usually metallic) patterns with small details (slots or patches) to produce a strong field concentration at localized spots under an external source illumination. This intense field confinement enhances light-matter interaction with the analyte (i.e. the substance under test), giving rise to a strong change in the spectral response.

In this review, we will summarize the advances achieved in THz sensing using metasurfaces from both a historical and application-oriented perspective. The initial part, Section 2, is dedicated to the important topic of thin-film sensing using metasurfaces, due to its historical
importance, as it was crucial in launching the topic and merging metamaterials and metasurfaces with sensing applications. Several strategies are highlighted, starting from the classical split-ring resonators (SRRs) arrays and the evolution of the topic towards more sophisticated phenomena and structures such as Fano and extraordinary transmission resonances as well as the recently proposed metageometries. Then, Section 3 is focused on the important topic of biological sensing based on metasurfaces at THz frequencies. As it will thoroughly explained there, metasurface biosensors have been crucial to alleviate one of the biggest problems for THz biological sensing, which is the stark difference between the typical size of microorganisms (~ 1 μm) and the wavelength at THz (~ 10 - 1000 μm) that makes this radiation largely myopic to these small details. Thus, classical THz spectroscopy of microorganisms needs many individuals and the response is taken as an average, making it very difficult to distinguish subtle differences in complex tissue samples, not to mention the identification of a single microorganism. Metasurface biosensors hold the promise to overcome these limitations taking advantage of the unprecedented freedom to engineer the metasurface parts properly. Due to the broad reach of the topic, the information is presented in several subsections, dealing with biomolecule sensing, microorganisms sensing and cancer and tumor detection. Section 4 covers what is possibly one of the most disruptive manufacturing techniques for metasensors with extremely high sensitivity, the so-called atomic layer lithography. This revolutionary technique is capable of achieving devices with a size in the centimeter scale and at the same time with a level of detail in the nanometer scale. This stark contrast allows for an exceptionally high field concentration and hence extraordinary sensitivity. A final section is presented at the end summarizing the main conclusions of this review.

2. Thin Film sensing with metasurfaces

2.1. Motivation of thin film sensing
The characterization of very thin homogenous samples by THz radiation is attracting a high interest from the scientific community in recent years; especially in areas where measuring substances in a thin-film form becomes essential, as in biomedicine or biological sensing. In addition, thin film sensing becomes crucial in situations where traditional THz sensing methods, such as THz-TDS, do not reach enough sensitivity to obtain sufficiently good results. Some of these circumstances, as mentioned in\cite{26} are: when the amount of sample is very small (for instance, when one want to characterize biological samples, such as DNA, microorganisms or has to manipulate dangerous samples); when the sample is easier to be processed in thin-film form; when a thin-film sample has different properties from its bulk counterpart; when one can obtain better sensitivity using a thin film; or when the sample characterization is complementary to other thin-film techniques, such as atomic layer deposition.

Although different solutions have been proposed (resonators, plasmonic structures, etc.), metasurfaces have emerged as a revolutionary alternative due to their exciting properties, as explained in the introduction of this work. More detailed examples are provided in this and the next sections of this review with the aim to present an updated portrait of the advances achieved during the last decade, that have been enormous. In metasurface devices, when radiating with THz waves on very thin samples, small changes occur in the spectral response with respect to the empty structure. Therefore, one of the biggest challenges in thin-film sensing is to obtain designs with high sensitivity and Figure of Merit (FOM). Although it can be defined in multiple ways and there is not a single universally accepted definition, generally speaking the sensitivity represents the variation in frequency (or, in some cases amplitude) in the spectral response of a sensor device when an analyte (i.e. the substance under test) is deposited on it. The FOM is a more accurate parameter defined as the ratio between the sensitivity and the full width at half maximum (FWHM). This parameter gives usually a better measure of the performance of a sensor, because not only is important to have a noticeable
frequency shift in the spectral response but also to have resonance with narrow FWHM (narrow peaks or dips) to minimize the overlapping between the detection thresholds.

2.2. From metaatoms to metageometries

The first metasurface design reported in the literature specifically used for thin-film sensing applications was proposed by Driscoll et al. in 2007.[44] Their design consisted in a square periodic matrix of gold SRRs patterned on a silicon (Si) substrate of thickness equal to 1 mm and coated with a thin layer of 6 μm of benzocyclobutane, used as a low-loss adhesion layer for the metallization, see Figure 1(a). The empty metasurface was experimentally measured in a FTIR spectrometer using s-polarized radiation impinging at 45° and showed a resonance dip at 1.20 THz corresponding to the magnetic mode of the SRR (i.e. its fundamental quasi-static resonance).[32] To evaluate the sensing performance, the metasurface was then covered with Si nanospheres of 50 nm diameter diluted in an ethanol solution. After the deposition, the sample was heated at 60 °C in order to evaporate the ethanol and obtain an homogeneous layer of Si nanospheres, many times thinner than the skin depth of THz radiation.[44] A redshift of 0.05 THz was observed in the spectral response of the device when a single layer of nanospheres was added with respect to the empty structure, as shown in the bottom panel of Figure 1(a). As the authors noted, the deposition of the nanospheres was made in multiples of 30 μl drop of solution, that contains an insignificant amount of Si (they estimated it below 1 ng) so small that it would be undetectable using a standard transmission-amplitude experiment. The SRR metasurface allowed the detection by transforming the typical transmission-amplitude change scheme to a new paradigm where the parameter that changes is the spectral location of the metasurface resonance frequency, which is often much more accurate.
This work was soon followed the same year, by another one by Debus et al.\textsuperscript{[45]} who proposed a metasurface made of asymmetric double split ring elements of 50 µm radius and two gaps with a 20 deg gap angle (see Figure 1(b)) working at 0.875 THz. The rationale behind this new particle was to break the symmetry by adding a second gap in the ring, opening the possibility to excite additional resonant modes. The study was based uniquely on numerical simulations and it was observed that when the metasurface was coated with a dielectric material of permittivity 3.2 (typical for organic systems) and thickness of 10 nm (corresponding to a typical monomolecular film) covering completely the area of the structure, a redshift of 0.005 THz appeared in the spectral response of the reflection coefficient magnitude, see top panel of Figure 1(b). Interestingly, it was observed that placing the analyte only in the ring gaps, which are the zones of highest electric field concentration, led to a shift of 0.004 THz, comparable to the previous case but using a much smaller amount of material, see bottom panel of Figure 1(b). Obviously, this strategy is optimal in terms of sensitivity, as it leads to a similar performance with a significantly smaller volume of analyte.

Both works were pioneering in the emerging topic of thin-film sensing using metasurfaces and, indeed, the strategy since then has evolved but using practically the same principles. In\textsuperscript{[46]} a systematic study of the sensing performance of SRR metasurfaces as a function of the dielectric thickness was performed. The SRR elements were made of aluminum with thickness 200 nm laying on 0.64 mm thick Si substrates and were characterized in transmission by a broadband THz-TDS at normal incidence with the electric field orthogonal to the ring gaps in order to excite the fundamental quasistatic resonance. To assess its sensing capability, the device was coated with a photoresist overlayer. Three resonances were observed in the spectral response, corresponding to the quasistatic, electric dipole and a weaker resonance related with the excitation of the inner SRR, and all of them experienced a shift towards lower frequencies when the photoresist layer was deposited on top. Although its thickness was gradually varied up to 90 µm, it was observed that the frequency shift saturated
at approximately 16 μm and no further significant shifts were observed afterwards. To corroborate those results, additional measurements depositing only a thin layer of B$_2$O$_3$ (with relative permittivity $\varepsilon_r = 3.6 \pm 0.2$ at 1 THz) with a thickness of the order of hundreds of nanometers were performed. The metasurface was similar to the previous case except that it had a 270 nm layer of silicon dioxide (SiO$_2$) on the Si surface to prevent diffusion of B$_2$O$_3$ into the substrate. This device showed a noticeable shift even with the thinnest 100 nm layer. However, the frequency shift was only 2 GHz, which represents a 0.40% shift with respect to the empty structure. This small effect is probably in the limit of reasonable detection, as the authors explain in the discussion, and suggests that a critical feature in the sensing performance of a metasurface sensor is the effect of the substrate permittivity and its thickness.

This was addressed in detail subsequently in$^{[47]}$ by analyzing two types of planar THz metamaterials manufactured on ultrathin silicon nitride (SiN$_x$) substrates and bulk Si substrates. The main idea behind this study is that high-permittivity substrates such as gallium arsenide and high resistance Si (typically used in metasurface sensors to that date), contribute a large capacitance to the resonator, hiding the spectral variation (typically a shift of a dip or peak) induced by the changes in the capacitance due to the analyte. In the thorough investigation done in$^{[47]}$ it was demonstrated that ultrathin substrates led to an order of magnitude improvement in the sensitivity of planar THz metasurface sensors. The designs proposed were made of 20 nm thick gold SRRs patterned on a 400 nm thick SiN$_x$ substrate, and on a 500 μm thick Si film, see Figure 2(a). Both designs were tuned to present their main resonance around 0.6-0.8 THz. When the SiN$_x$-based structure was coated with a silk fibroin film 1.5 μm thick, a frequency redshift of 0.115 THz was observed, corresponding to a 19.3% variation, a much more substantial change than the tiny 3.9% obtained with the thicker and higher permittivity Si substrate. Interestingly, the experimental measurements carried out with a TDS-THz system confirmed the simulation results with an excellent agreement.
Nevertheless, as the authors pointed out, even though the sensitivity of SiN$_x$-based metasurface was significantly better than that based on a Si substrate, protein monolayer sensing was largely out of reach. By extrapolating their results, the authors estimated that an analyte with a refractive index typical for organic systems ($n = 1.91$) and a film thickness of 10 nm, the resonance shifts of the SiN$_x$-based metasurface would reach only $7.7 \times 10^{-4}$ THz, rendering detection below the capabilities of standard measurement systems. However, in a scheme based on volumetric detection, one could deposit the analyte preferentially on the high electric field spots, following the same strategy commented in,$^{[45]}$ making it possible to detect small volumes such as for example 0.87 ng of silk with a density of 1.35 g/cm$^3$, which in the calculations performed would induce a measurable 5 GHz resonance shift for a single SRR on a SiN$_x$-SRR substrate.

In the quest towards finding biocompatible substrates, Tao et al. investigated metasurfaces based on silk$^{[48]}$ and paper.$^{[49]}$ To manufacture the metasurfaces, a shadow mask patterning manufacture process was employed, based on selective deposition of gold working as target material through SiN$_x$ microstencil-based shadow masks, overcoming this way the problem of conventional photolithography, where chemical solutions can degrade biocompatible substrates like silk or paper. Although the silk metasurface was proposed as a proof-of-concept of the new fabrication technique with promising potential for biosensing, the sensing capability of paper-based metasurface was actually tested both numerically and experimentally. The SRR metasurface was directly printed on a 280 $\mu$m thick paper substrate with predefined microstencils that were fabricated by the use of surface micromachining technology. Then, the microstencils were attached to the paper substrate and a thin layer of gold 150 nm thick was sprayed on the paper substrate. The experimental measurements were done at normal incidence, with the electric field perpendicular to the SRR gap to excite its fundamental resonance, using a bare paper as the reference. The sensing capabilities of the device were studied by coating the device with glucose solutions with different concentrations,
from low blood glucose level considered as hypoglycemia (3 mmol/L), to high blood glucose level considered as hyperglycemia (30 mmol/L). The devices were designed to have the fundamental resonance at 0.9 THz without any coating and it was observed that this value suffered a frequency shift of 0.3 THz for a sample coated with a 30 mmol/L glucose solution. To check the origin of this frequency shift, the authors measured the behavior when the metasurface was covered with pure water and noticed no change in the frequency resonance, suggesting that the 0.3 THz shift in the previous case could be attributed uniquely to glucose. This result opened the door to this kind of devices as biological or biochemical sensors in practical applications.

A challenge in reliable sensing is to achieve a high quality factor resonance and hence improve the sensitivity. A problem with the typically used far-field setups is that they require a minimal area of analyte deposited on the metasurface. This is even worse due to the fundamental limitation imposed by diffraction which restricts the smallest possible beam waist size to approximately half-wavelength in free space, although in practice the beam waist is usually larger. A direct consequence of this setup is that one needs a large volume of analyte to cover fully the area of excited resonators. This collective excitation causes resonance broadening due to enhanced scattering, decreasing ultimately the quality factor.

This limitation was overcome in\textsuperscript{150} by proposing a near-field experimental setup using a SRR metasurface sensor operating at 0.4 THz. The main concept is that with a near-field setup only a small number of resonators participate in the resonance process and therefore the analyte area can be reduced. The improvement of the quality factor comes as a consequence of the weak near-field interactions among a reduced number of resonators, leading to an effective sub-diffraction sensing capability. Putting these ideas into test, a successful detection of a thin-film with a thickness of only $\frac{\lambda}{375}$ was achieved.

In a further step towards improving the performance of THz metasurface sensors, it was found that absorbers working in reflection have a better performance than metasurfaces working in
transmission. This was analyzed in detail in [51] by comparing the behavior of metasurfaces working in absorption and transmission. The basic principle of operation of the proposed devices was based on the concept of perfect metamaterial absorbers that evolve from metamaterial absorbers and whose main mission is to minimize the reflection, enhancing this way the absorption by enforcing impedance matching to free space. At the same time, transmission can be avoided through the use of a metallic ground plane. Thanks to this configuration, the electromagnetic field can be locally stored inside the structure, thus improving the interaction with the analyte under test. Another advantage of the use of a ground plane is that it prevents the electric field decay inside high-index substrates leading to an enhanced interaction between the incident wave and the analyte. In [51] the performance of two different perfect metamaterial absorber sensors was compared with planar metasurface sensors working in transmission (i.e. without ground plane) with identical metallic pattern. The resonator elements were a cross-shaped absorber and its complementary cross-shaped absorber design with 4-fold rotational symmetry, both made of a 200 nm thick aluminum layer on a polyimide spacer and a Si substrate 500 µm thick. The polyimide layer was crucial in order to achieve impedance matching of the device, leading to the perfect absorption condition. In turn, the cross-shaped patterns chosen were very convenient for sensing purposes as they enhance the confinement of the electric field between the metallic strips of the cross in the surface, making them very sensitive to small changes in the vicinity when coating the surface with an analyte, as shown in Figure 2(b). The empty structures were designed to have the fundamental resonance between 0.7 and 0.8 THz. In the experimental characterization of the sensing performance, a photoresist of refractive index 1.6 was used. When the absorbers were covered with a photoresist layer 11 µm thick, a frequency shift of 0.077 THz and 0.068 THz was obtained for the cross-shaped and complementary cross-shaped absorber, respectively. In percentage terms, this means a frequency shift equivalent to 11% and 8.5% with respect to the empty structure resonance frequency. The study was
continued by evaluating numerically the response of the structures for various analyte thicknesses and refractive indices. The maximum frequency shift for both absorbers was fixed near 13%, much larger than the frequency shift obtained for the similar designs working in transmission (without ground plane) for which the maximum shift was around 5%. The enhanced sensitivity of the absorbers was mainly attributed to the intense resonant electric and magnetic field enhancement in the absorber cavity, which is an order of magnitude larger than that of metasurfaces.

Soon after, a similar study was reported in,[52] where a multispectral and flexible metamaterial absorber with enhanced sensing capabilities in the THz spectral range was numerically and experimentally studied. In this case, a flexible design using a 50 µm thick polyimide layer (with complex permittivity of εᵣ = 3+i0.15) as substrate material was implemented. The resonators were composed of an inner cut wire and an outer two-gap SRR made of aluminum with a thickness of 200 nm. The bottom of the substrate was fully covered with a 200 nm thick aluminum layer, acting as the ground plane responsible for preventing any type of transmission through the structure. The behavior of the structure was numerically and experimentally evaluated by exciting the absorber with a normally incident plane wave linearly polarized with the electric field parallel to the inner cut wire of the resonator. For the experimental results a THz–TDS in reflection configuration was used. The first evaluation was done for the empty structure and it was established that it presented three resonant modes in the frequency range from 0.15 to 0.85 THz. The sensing capability of the structure was then tested by coating the device with a thin film layer with refractive index 1.73 and varying the analyte thickness between 1 µm and 50 µm. In all cases, the frequency shift observed was larger for the highest frequency resonance. Concretely, a frequency shift of 0.14 THz was achieved for an analyte thickness of 50 µm. The performance of the absorber as a refractometer was also studied numerically. This work is a good example of the use of THz
metasensors implemented on flexible substrates, opening the possibility of THz sensors integrated on chip, as well as low cost mass production.

Aside from the previously mentioned near-field operation\textsuperscript{[50]} another strategy to increase the quality factor of metasurface sensors is based on the use of high-order resonances supported by asymmetric resonators following the strategy already pointed out in one of the pioneering articles.\textsuperscript{[45]} This idea was further followed in\textsuperscript{[53]} by Singh \textit{et al}. who achieved high quality factor sensing by exploiting quadrupole and Fano resonances excited by breaking the symmetry of the metamaterial unit cells, as shown in the left panel of Figure 2(c). The symmetry breaking was realized by an array of asymmetric split ring resonators made of aluminum 200 nm thick laying on a high-resistivity Si substrate 0.5 mm thick by using photolithography. The asymmetry in the resonators was introduced by displacing the lower gap 5 \( \mu \)m from the central vertical axis. The characterization of the structure was done using a broadband THz-TDS working in transmission mode at normal incidence. Depending on the polarization of the incident wave, either the quadrupole or the Fano resonance could be excited. The first one happened at 1.14 THz whereas the second was located at 0.52 THz for the empty structure. It is worth mentioning that the quadrupole resonance was tuned to coincide with the onset of the first diffraction order to enhance the quality factor. Next, the structure was spin coated with photoresist layers (refractive index of 1.6) of different thicknesses, ranging from 1 to 16 \( \mu \)m. A clear redshift of both quadrupole and Fano resonances was observed as the photoresist thickness was increased, getting a saturation when the analyte thickness was around 16 \( \mu \)m. Interestingly, the performance of the Fano resonance was found to be better suited for sensing purposes, as it led to higher sensitivity values. This important aspect could be attributed to the different electric field mode profiles in the capacitive gaps of the resonators. In a subsequent step, a simulation analysis reducing the Si thickness was carried out to evaluate the effect of the substrate. With this study, it was noticed
that the sensitivity of the Fano resonance could be enhanced by a factor of two and of the quadrupole resonance by a factor of three when the substrate thickness was below 20 μm for a 1 μm thick analyte. This can be explained because of the stronger interaction of analyte layer with the electric field in the gaps when the metasurface is implemented on ultrathin substrates. Nevertheless, high quality factor resonances are not restricted to asymmetric structures. Rather, a narrowband response (and hence high quality factor) can be found in simple symmetric structures but introducing the asymmetry in the excitation, by impinging for instance at oblique incidence. This was analyzed both numerically and experimentally in[54] using a symmetric aluminum cross-dipole array on a low-loss flexible polypropylene substrate with a thickness of 41 μm, permittivity of 2.25 and loss tangent of only 0.0005 in the considered band, see the schematic as well as a microphotograph of the manufactured prototype in the left and center panels of Figure 2(d). The metasurface was characterized in transmission mode using a THz-TDS system. An initial study at normal incidence was done by spin coating the structure with a photoresist whose permittivity and loss tangent were fitted by comparing the experimental measurements with numerical calculations, giving as a result 2.855 and 0.05, respectively. In this initial characterization, two different analyte thicknesses were evaluated, 9 and 20 μm, giving a noticeable redshift of the transmission dip in both cases with respect to the empty metasurface. A simulation study extrapolating the experimental results predicted a saturation of the response for an analyte thickness of 20 μm with a maximum frequency shift of approximately 0.175 THz. After this study, the metasurface was characterized at oblique incidence for both principal polarizations (TE and TM) of the impinging wave. It is remarkable that under TM incidence an extremely sharp dip not present at normal incidence was observed in the spectral transmission response. As elucidated in the paper, this narrow dip corresponds to the bent mode which is related to the excitation of an asymmetrically induced current due to the coupling between the horizontal and vertical
dipoles of the cross resonator, as shown in the right panel of Figure 2(d). The narrow spectral line associated with this mode is linked to a high quality factor which leads ultimately to relatively large values of sensitivity and figure of merit.

The path of Fano-based sensing was further extended using more refined and disruptive concepts such as toroidal resonances. Concretely, Gupta et al. presented in 2017 a metamaterial device based on a planar toroidal dipole.\textsuperscript{[55]} Originally, toroidal resonances are related with currents flowing on a torus surface. To implement the toroidal characteristic in two dimensions, Gupta et al. proposed a two dimensional metasurface made of mirrored asymmetric split ring resonators coupled through a toroidal moment due to an anti-aligned set of magnetic dipoles, see a schematic in the central panel of Figure 2(c). In the context of sensing platforms, an interesting feature of toroidal resonances is that they have a remarkably high quality factor. Hence, the toroidal metasurface introduces an improvement in both sensitivity and FOM with respect to typical sensing metasurfaces based on traditional uniform (as opposite to mirrored) Fano resonator arrays. For the implementation, the authors used asymmetric split ring resonator with a geometry similar to the one used in\textsuperscript{[53]} made of aluminum and with a thickness of 200 nm laying on a 0.5 mm thick Si substrate. As mentioned above, a crucial step was to mirror the resonators to excite the toroidal resonance.

The structure was excited by a normally incident THz beam with electric field polarized parallel to the non-gap arm of the asymmetric resonators and the measurements were done using a THz-TDS system. To evaluate the sensing performance, the toroidal metasurface was spin coated with a photoresist layer of refractive index 1.66 and thickness varying from 1.5 to 13.5 \( \mu \text{m} \). Without the photoresist film, the toroidal dipole resonance appeared at 0.402 THz and when the photoresist was deposited, this resonance experienced a redshift in the spectral response. With a thickness of 3 \( \mu \text{m} \), the frequency shift was of 0.0162 THz, which is equivalent to a relative displacement of 4\% with respect to the response of the empty structure.

It was noticed that the frequency shift increased exponentially when increasing the analyte
thickness until the device saturated at a thickness of approximately 9 µm. To determine the sensitivity for thin analyte layers, the toroidal metasurface was coated with a 0.25 µm thick layer of SiO₂ producing a redshift of 13.3 GHz (3.3%); and a 0.25 µm thick layer of germanium, achieving a displacement of 20.2 GHz (5%). Both materials were chosen due to the high contrast in their refractive index.

Up to this point, all the described sensing devices have been limited to the use of only the patterned surface of the metasurface, where the metallic resonator array is localized. Different solutions have been presented in the last years in which it is possible to use both the patterned and unpatterned surfaces of the structures, leading to a dual surface-sensing.\cite{56,57} In\cite{56} Srivastava et al. presented a dual surface flexible metasurface based on an ultrathin low refractive index polyimide substrate. To study the behavior of the designed structure in thin-film sensing applications, the metasurface was coated with a germanium layer 10 nm thick, with a refractive index of 4. Both sides of the structure were coated separately and the frequency shift of the fundamental resonance, situated at approximately 0.866 THz for the empty metasurface, was studied. In the first analysis, a frequency shift of 0.084 THz, corresponding with a 9.7% variation was achieved when depositing the germanium layer on the patterned side of the metasurface, while when coating the substrate side, a less significant redshift of only 0.006 THz (0.7%) was recorded. In the same work, a study on the influence of substrates with low or high refractive index was also made. The comparison was made between polyamide substrate with a refractive index of 1.72 and a Si substrate, with refractive index of 3.418. Comparing both transmission spectra, it was concluded that the results were more favorable in the case of lower refractive indexes, corroborating previous works.\cite{46–48,52,54,58}

Furthermore, focusing on the use of the unpatterned side of the structure to perform an effective sensing, the substrate thickness was reduced so that the electric field could penetrate it and reach the analyte.
Although all the previous examples reported here work well as sensing platforms, they present some limitations in their sensitivity and FOM. A stronger interaction with the dielectric analyte and hence better performance can be possible by enhancing the electric field confinement in the surrounding of the metallic parts of the structure. To tackle this challenge, more complicated geometries have been suggested, by proposing a paradigm shift from classical metaatoms to more elaborated metageometries resulting ultimately in an ultrasensitive performance. This disruptive idea was put to test in,\(^{[59]}\) by designing a labyrinth metasurface sensor working in absorption mode. The metasurface had an intricate labyrinth pattern to produce a high electric field confinement on the whole surface of the structure, and not only at discrete points. Concretely, the labyrinth metasurface was made of a convoluted aluminum strip with thickness of 400 nm laying on a polypropylene layer of 50 \(\mu\)m backed by an aluminum ground plane. The sensing behavior was evaluated by coating it with thin SnO\(_2\) films (\(\varepsilon = 4\)) with thicknesses ranging from 24 nm to 345 nm. The empty structure was designed to have a resonance dip in the reflection coefficient near 0.14 THz and when the analyte was deposited a minimum frequency shift of 0.7\% was recorded for the case of 24 nm and a maximum of 6\% for the case of a 345 nm thickness film (see Figure 2(e)). This means that the minimum detectable analyte has a thickness \(10^{-5}\) times the operation wavelength. This translates directly into superb sensing behavior, with an excellent sensitivity and FOM, as indicated at the comparison table at the end of this section (Table 1). This performance can be explained by noting that the convoluted geometry of the metallic strips and the inter-strip gaps noticeably increases their total length within the metasurface unit cell compared to non-convoluted geometries. From a circuit point of view, this means that the labyrinth shape increases the effective lumped inductance and capacitance, inducing a much higher frequency shift. Thus, from the previous discussion, it can be concluded that the use of metageometries is a promising concept to improve the sensing performance allowing the detection of
exceedingly thin dielectric samples making it possible to detect samples much thinner than the operation wavelength.

2.3. Extraordinary transmission-based devices

An alternative way to devise metasurface-based THz sensors with interesting and competitive characteristics is to exploit the so-called extraordinary transmission resonance that takes place in periodic structures made of small apertures on a metallic plate. The first report of extraordinary transmission was published by Ebbesen et al. in their seminal paper of 1998 where they described high transmittance peaks arising in the cutoff region of thin metallic plates perforated with a periodic matrix of subwavelength holes. This first experimental realization was done in the infrared range\(^6\) and was explained as a result of the coupling of the incident light to surface plasmons supported by the periodic metallic surface. However, it was soon noticed that the phenomenon was more general and could exist in other frequency ranges such as microwaves and millimeter-waves where metals do not strictly follow a Drude model and, rather, are usually described assuming high conductivity. Indeed, the theoretical derivation proposed in\(^6\) already predicted similar peaks even with a perfect electric conductor metal model. This opened the way towards the replica of the extraordinary transmission resonance all along the electromagnetic spectrum including frequency ranges very far from infrared such as millimeter waves\(^6\) and THz.\(^6\)

Generally speaking, the fundamental difference between the metasurfaces studied up to this point and extraordinary transmission hole arrays lies in the type of resonance supported by the structure. Conventional metasurface-based sensors rely on the excitation of local self-resonances supported by the unit cell elements. This means that the effect of the periodic structure is second-order as, loosely speaking, the exact frequency location is marked by each resonator and the coupling between them mainly affects the resonance bandwidth. In contrast, the extraordinary transmission resonance is a collective effect that arises due to the interaction of the overall structure because, by definition, each element is non-resonating (recall that the
extraordinary fact of this resonance is that the apertures are working in cutoff which inherently implies that the holes must be non-resonating, as explained in).[64] More specifically, the extraordinary transmission peak can be explained as the interaction between the incident wave and leaky-modes excited by the periodic hole matrix that run along the surface as they leak away energy from it.[65] In this sense, it is remarkable that the operation of metageometries such as the labyrinth metasurface mentioned above are midway between self-resonances and collective resonances. In fact, metageometries consist in the periodic replication of a unit cell but it is clear from the field analysis that the energy distribution is spread along the unit cell and not confined exclusively at some particular spots. In other words, there are internal interactions in the unit cell that contribute to the resonance process and, furthermore, although these interactions might change depending on the polarization of the incident wave, the frequency response remains unaltered, giving an indication of a collective response of the intricate structure that composes the unit cell.

Returning now to extraordinary transmission hole arrays and focusing now on sensing applications, the first realization of a thin-film sensor based on this resonance operating at the THz band was published by Miyamaru et al. in 2006.[66] The structure used in that work was a metal sheet 500 µm thick perforated with an array of circular holes of 680 µm in diameter distributed along a triangular array with a periodicity of 1130 µm. All the experiments were done by means of a THz-TDS instrument. With the mentioned dimensions, the bare structure was shown to present the extraordinary transmission resonance at 0.3 THz. To demonstrate the sensitivity of the metallic grid to variations near the surface, it was coated with two different paper sheets 240 µm thick, one clear and another printed. As the printed paper possessed an estimated ink thickness of less than 5 µm, the transmission response experienced a slight frequency shift to lower frequencies with respect to the bare paper. Remarkably, in the comparison between the bare hole array response and the hole array with a clear paper on top, it was noticed that the frequency response of both cases was almost identical. Thus, it was
concluded that the structure was capable of detecting extremely small amounts of substance (ink) in thin films. The study was then followed by coating the structure with a 120 µm thick layer of nylon and a small quantity of liquid glycerin (800 pl/mm²) with a refractive index of $n_{glyc} = 2.18$. By looking at the transmittance of the structure it was noted that the transmission peak experienced an amplitude attenuation when the volume of the liquid glycerin was increased, allowing for a quantitative evaluation of the amount of glycerin with high sensitivity. This was interpreted by the authors as a consequence of the leaky-wave (surface wave in their own words) excited by the structure that stays focused on the metal surface for more time than the transmitted THz wave, enhancing the sample absorption of the incident THz radiation.

Miyamaru et al. also studied the effect of dielectric thin-films on the reflection properties of hole arrays in 2010.[67] In particular, they investigated the dependence of the sensing performance of the structure on the HA thickness and the side on which the analyte is deposited. In this study, two plates with different thicknesses, 100 µm and 300 µm, perforated with a triangular lattice array of circular holes with a diameter of 500 µm and a periodicity of 1000 µm were analyzed. The results were experimentally obtained by the use of a THz-TDS system. First, the prototype with metal thickness 300 µm was employed. In order to study the thin-film sensing capability of the device, the analyte thickness was varied from 50 µm to 250 µm, and the frequency response of the reflection dip was recorded. For a polypropylene film of 250 µm, the resonant frequency shifted from 0.32 THz (bare structure response) to 0.23 THz, resulting in a high relative displacement of 28%. The transmission of the structure was also studied and, although the transmission peak also suffered a similar redshift in the frequency response, this was accompanied by a significant attenuation as the thickness of the polypropylene was increased. This attenuation can be explained due to the cutoff effect of the metal holes, and the mismatch between both sides of the structure. This effect does not become so important in the reflection spectrum, which shows that for sensing purposes,
working in reflection configuration may be a better option in terms of signal to noise ratio. However, when placing the polypropylene film on the non-illuminated side of the metal, the reflection dip became very weak due to the fact that the contribution of that side of the structure is much smaller. This presents a huge disadvantage for high sensitivity applications, even though this problem could be resolved by the use of thinner dielectric films, where the mismatch between both sides of the structure is reduced. The same study was repeated for the 100 µm thick metal structure. When depositing the polypropylene film on the illuminated side of the structure, the results obtained were similar to those obtained with a thicker metal. However, in the case of depositing on the non-illuminated side of the metal, the contribution of the waves reflected on that side of the structure turned out to be much higher than in the previous case. With this study, the potential of this type of structure for high sensitivity sensing in reflection configuration was demonstrated.

More recently, a study of a structure composed of two stacked hole arrays (i.e. the so-called fishnet structure) was evaluated for thin-film sensing applications, by depositing a thin film overlayer and studying numerically and experimentally both the amplitude modulation and the frequency shift of the spectral response. This study showed that both sensing strategies, amplitude and frequency modulation, could be successfully used for thin-film sensing purposes. The hole array proposed consisted of a pair of 35 µm thick copper layers periodically perforated with a hole array of 300 µm in diameter and a periodicity of 500 µm separated by a 100 µm thick dielectric layer. For the analysis, a THz-TDS system was employed. The structure was illuminated by a normally incident plane wave and to test its response, it was coated with a polyimide film 50 µm thick and with a refractive index of 1.41. A redshift of the extraordinary transmission peak was observed in both simulation and measured results. Particularly, the resonance peak was shifted from 557 GHz to 505 GHz (9.3%) in simulation and from 520 GHz to 490 GHz (5.8%) in the experiment. These results show that, along with the frequency displacements, there is an attenuation in the amplitude of
the peak that could be attributed to the two different mechanisms mentioned before: cutoff effect of the metal holes and mismatch between both sides of the structure. To examine in detail the behavior of the structure to changes in the film thickness, this was gradually increased from 5 µm to 50 µm with a step size of 5 µm. It was noted that the frequency shift grew almost linearly by increasing the analyte thickness. Similarly, the amplitude modulation also increased in a linear way as the analyte became thicker, reaching its maximum value for the case of a 50 µm thick film. The behavior of this device could be further improved by the use of thinner dielectric substrates, where the interaction of the analyte in the regions of high electric field concentration is stronger. In any case, an interesting conclusion of this work is that these changes both in frequency and amplitude can be exploited to perform thin-film sensing, obtaining two different types of sensitivity: frequency sensitivity (FS), and amplitude sensitivity (AS), as can be seen in the comparison at the end of this section.

Up to this point, we have only considered hole arrays with identical periodicity in both axes. Nevertheless, hole arrays with a rectangular unit cell such as the one shown in Figure 3(a) have a richer physical behavior and open novel possibilities for applications, amongst them sensing. One of the first reports on rectangular hole arrays was published in [69] in the context of miniaturized and low-loss fishnet metamaterials. However, the interest in rectangular hole arrays spurred some years later while studying the frequency response of doubly periodic single layer hole arrays supported by a polypropylene slab. Interestingly, two different resonances were identified, each one of them corresponding to orthogonal polarizations along the two principal unit cell lattice vectors. [63] The resonance associated with the polarization parallel to the large hole array periodicity was identified as the normal extraordinary transmission resonance and thus it was termed as regular extraordinary transmission. On the other hand, when the polarization was parallel to the short hole periodicity an unexpected resonance peak was noticed in the spectrum. This came as a surprise, because for this polarization the periodicity of the hole array would not be compatible with extraordinary
transmission and, in fact, it did not appear in previous reports.\textsuperscript{[69]} This fact led to the denomination of this resonance as anomalous extraordinary transmission. After several analytical and experimental studies, now it is clear that this phenomenon is tightly linked to the excitation of a grounded dielectric slab surface wave that becomes leaky due to the periodic structure.\textsuperscript{[70,71]}

The anomalous extraordinary transmission contributes to the rich variety of possibilities offered by hole arrays for the design and test of sensing devices. Before, we have seen examples where one can use one face or another for deposition, or excite different resonances or even use frequency or amplitude modulation for sensing. The question one can then ask is: what is the best strategy? The truth is that there is not a single optimal approach suitable for all cases and, rather, the best solution will depend on the application scenario. Therefore, and with the aim of trying to give a partial answer to the previous question, a comprehensive analysis of the sensing capability of subwavelength hole arrays operating at both the regular and anomalous extraordinary transmission resonances was performed in.\textsuperscript{[57]} To do it, thin layers of dielectric analyte slabs of different thicknesses were deposited on rectangular subwavelength hole arrays. Different scenarios were tested to demonstrate that the best thin-film sensing behavior is achieved when the structure operates in the anomalous extraordinary transmission resonance and the analyte is deposited on the non-patterned side of the metasurface. The structure analyzed in\textsuperscript{[57]} consisted of a periodic rectangular array of circular holes of diameter 105 \( \mu \text{m} \) and periodicity 115.5\( \times \)350 \( \mu \text{m} \) etched on an aluminum layer of thickness 0.4 \( \mu \text{m} \) laying on a polypropylene substrate of two different thicknesses, 50 \( \mu \text{m} \) and 75 \( \mu \text{m} \). These two substrate thicknesses were chosen this way because the appearance of the anomalous extraordinary transmission depends both on the period of the unit cell and the properties of the chosen materials, as well as on the thickness of the substrate. This parameter is critical because below a given substrate thickness the anomalous extraordinary transmission is in cutoff. For the hole array considered in\textsuperscript{[57]} with a substrate thickness of 50 \( \mu \text{m} \), the
anomalous extraordinary transmission resonance is deeply in cutoff whereas with 75 µm it is also below but near the cutoff condition.

Before characterizing its sensing behavior, a simulation study of the lossless structure was done, to compare the performance under regular extraordinary transmission (polarization parallel to the larger period of the unit cell) and anomalous extraordinary transmission (polarization parallel to the short period of the unit cell). The results of this preliminary study demonstrated that the anomalous extraordinary transmission was preferable for sensing. For this reason, the rest of the work was developed putting the main focus of attention on this resonance.

The other objective of the work was to assess the differences in terms of sensitivity between depositing the analyte on the metallization (patterned) side or on the other (unpatterned) side, as well as to study the effect of the substrate thickness. For this purpose both structures with different substrate thicknesses ($h_{PP} = 50$ µm and $h_{PP} = 75$ µm) were coated with a SnO$_2$ thin film deposited in both faces of the metasurface, varying its thickness from 3 µm to 13 µm. As can be observed in the left panels of Figure 3(b), the best performance was achieved when the analyte was deposited on the polypropylene side of the 75 µm thick metasurface, where a maximum redshift of 0.04 THz (4.7%) was achieved. This result can be easily explained by considering the mentioned effect of the substrate on the appearance of the anomalous extraordinary transmission resonance. As said above, to excite it one needs a minimum substrate thickness and in the case considered, 75 µm is near but not sufficiently thick enough. Certainly, depositing the analyte on top of the substrate layer can be considered equivalent to increasing the overall dielectric thickness, breaking the cutoff condition and exciting effectively the anomalous extraordinary transmission peak. On the other hand, when the analyte is deposited on the metallization side, the frequency shift of the anomalous extraordinary transmission resonance is negligible, as shown in the second column panels of Figure 3(b), making this configuration ineffective for thin film sensing purposes because the
analyte slab is too thin and unable to excite the anomalous extraordinary transmission resonance corresponding to that face.

For the 50 µm thick polypropylene structure with the analyte deposited on the non-patterned side – third column panels of Figure 3(b) – it is observed that increasing the analyte thickness leads to an increment of the peak amplitude but not a proper anomalous extraordinary transmission resonance, due to the fact that even with the largest analyte thickness it is still in cutoff. However, when placing the analyte on the patterned side of the metasurface there is not any frequency shift but, instead of that, a clear peak amplitude increase appears, as shown in the rightmost panels of Figure 3(b), making it possible to define an amplitude sensing by the use of an amplitude sensitivity. Although the data obtained by depositing on the substrate layer of the structure are significantly lower, this opens the possibility of designing structures capable of sensing analytes taking advantage of both sides of the structure. In addition, depositing on the substrate face can lead to some advantages such as the possibility of cleaning the structure without damaging the metallic pattern.

As a final step, a comparison of all the works mentioned using hole arrays and extraordinary transmission is presented in Table 1. In order to make a fair comparison in terms of sensitivity and FOM we define these parameters as frequency sensitivity, \( FS = \Delta f/(h_a n_a) \), where \( \Delta f = f_a - f_0 \) with \( f_a \) the resonance frequency at each analyte thickness, \( h_a; n_a \) the refractive index unit (RIU) of the analyte; and \( f_0 \) the resonance frequency of the empty structure; amplitude sensitivity, \( AS = \Delta A/h_a \), where \( \Delta A \) is the amplitude variation; FOM = FS/FWHM. Note that here we have different results than the ones presented in the referred articles due to our different method to calculate the sensitivity and FOM. As we can observe in the Table, the three most recent works\(^{[55,56,59]} \) are the ones with higher values of FOM. If we compare the two works in which the amplitude sensitivity is used, we can observe that the AS achieved by Jáuregui-López et al. in\(^{[57]} \) is much higher, improving the results achieved by Yahiaoui et al. in\(^{[68]} \) by a factor of more than 4.
3. Biological sensing with metasurfaces

3. 1. Introduction to THz biosensing

The THz band has some interesting features that make it an interesting and emerging technological alternative in the fields of life sciences, mainly biology, biomedicine and pharmaceutics, especially due to its high potential in biosensing applications. This is mainly because THz radiation has proven to be a non-invasive, contactless, and non-ionizing technique innocuous to living organisms exposed to it. Moreover, THz radiation is very sensitive to water, making it a good option for cancer and tumor treatments, since the tumor cells contain different aqueous percentage than healthy cells. Hence, nowadays THz technology stands out as a competitive option for replacing or complementing other techniques that have proven to be harmful, such as x-rays. In addition, absorption in THz is dominated by the excitation of unique intermolecular and intramolecular vibrations, such as hydrogen bonds or weak interactions such as Van der Waals forces, giving access to significant information about the chemical composition of substances and/or biomolecules not available using traditional detection methods such as infrared spectroscopy. Due to these characteristics, the biological-related THz research activity has spread throughout the world covering a wide variety of fields such as diagnosis of diseases,\textsuperscript{[72]} detection of protein concentrations,\textsuperscript{[73,74]} DNA sequencing,\textsuperscript{[75,76]} detection of cancer cells and tumors,\textsuperscript{[18,72]} or detection of microorganisms,\textsuperscript{[77,78]} among others. Metasurfaces have also emerged as excellent label-free sensing devices in biological applications, as they respond to changes in the refractive index of the device, a measurement that is relatively easy and cheap to perform, allowing for a quantitative and kinetic measurement of molecular interactions.\textsuperscript{[79]} This section is structured in three sub-sections, attending to the main application domain for THz metasurfaces-based biosensing: detection of biomolecules, detection of cancer cells and tumors, and detection of microorganisms.

3. 2. Biomolecules sensing with THz metamaterials
Biological sensing has always attracted a great interest from the scientific community, for obvious reasons. The fundamental process for chemical analysis and medical diagnosis is based on the specific interaction between molecules. As mentioned above, THz radiation has expanded the possibilities of biomolecular detection, thanks to its sensitivity to weak intermolecular interactions. This fact added to its strategic location in the middle of the electromagnetic spectrum that allows for an extrapolation of concepts borrowed from other bands, has led to a fast technological development of biosensing devices in this range. In this regard, metasurfaces are probably one of the most successful realizations thanks to their simplicity.

A pioneering antecedent of metasurface sensors can be found in the infrared range in the work of Brolo et al. who in 2004 proposed the use of arrays of nanoholes in a gold film to monitor the binding of organic and biological molecules to the metallic surface. This line was continued at THz by Debus et al., in the report that we have already discussed in a previous section. There, a thin layer of 10 nm of a dielectric slab with a permittivity typical of organic systems was used as analyte material, with good results in terms of sensitivity. From that work to the present, many articles have been published in this field. For example, a new method of analyte deposition by 3D printing was used to develop a label-free biosensor based on the resonant transmission of a metallic mesh with the objective of detecting a small amount of protein horseradish peroxide. The designed structure was composed by an array of metallic mesh of 6 µm thick electroformed nickel, with a grating period of 76.3 µm and a metallic strip width of 18.3 µm, in both axes. To characterize the sensing capability of the device, the protein horseradish peroxide was dissolved in sterilized pure water at different concentrations and then was deposited on the biosensor surface by means of a commercial printer. The transmission spectra of the bare structure showed a transmission peak at 3.37 THz when it was measured using a FTIR spectrometer. Different concentrations of horseradish peroxide were then printed on the surface, from 0.125 mg/mL to 1 mg/mL, and
the frequency response was measured. With the addition of the sample, the transmission dip frequency showed a tendency to decrease. A clear frequency shift was observed for a 500 pg/mm² horseradish peroxide concentration (11 fmol) with respect to the bare structure. The obtained results demonstrated that the designed device could achieve a sensitivity equal to that of a conventional method based on the use of antibody labeled horseradish protein. These results gave an evidence that metasurface sensors at THz are a cost-effective alternative for protein detection in biomedicine applications.

As in thin-film applications, the geometrical design and effect of the substrate on the performance of metasurface biosensors is also relevant in biosensing and thus it has been exhaustively analyzed. This is the case of the study carried out by Wu et al., where two different substrates of Si ($\varepsilon = 11.56$) and quartz ($\varepsilon = 4$) were compared in a label-free and specific sensor for streptavidin-agarose. This sensor was fabricated by the functionalization of octadecanethiols and biotins in the THz range, and its behavior was numerically and experimentally tested by the study of the first two resonant modes present in the designed metasurface. The unit cell of the device was composed by an array of gold U-shaped SRRs 80 nm thick with dimensions of 31 µm of length and width, and a gap of 5 µm. Two substrates, 500 µm thick Si and 800 µm thick quartz, were used. The metasurface was functionalized via a specific thiol chemical interaction to form a monolayer of thiol-terminated molecules so that the intermolecular interaction between the alkyl chain of the thiol and biotin could immobilize the latter onto the surface. This way, the biotin presented a specific interaction with the streptavidin-agarose by an affinity effect. All the experiments were carried out by a THz-TDS system with the polarization aligned along the SRRs gap. As mentioned before, two sharp transmission dips were found for the bare structure. The first one was located at 0.4 THz for the Si substrate and at 0.75 THz for quartz substrate; the second one appeared at 1.2 THz and 2 THz for Si and quartz substrates, respectively.
Interestingly, it was shown that the frequency shift towards lower frequencies became larger in the case of the second dip, and this was more evident for the quartz substrate. Concretely, a redshift of 2.77 GHz and 6.76 GHz was observed in the second dip for Si and quartz substrates respectively. This result implies a significant improvement in the obtained displacement from 0.23% to 0.55%. Focusing now on the first dip, the shifts observed were 0.75 GHz on Si and 1.58 GHz on quartz. Numerical simulations were also carried out in order to verify the experimental results. Due to the computational complexity of treating numerically very thin film samples in the nanometer scale, the streptavidin-agarose molecule was modelled as a thin layer with a thickness of 2 µm, and its dielectric permittivity was set to 1.2. The numerical simulations performed gave a redshift of both resonances, with a higher frequency shift of the second dip, in agreement with the experimental results. In particular, a shift of 5.4 GHz and 9.5 GHz were obtained for the Si substrate and quartz substrates, respectively. These results highlight the importance of the choice of the substrate materials when designing a biosensor, corroborating the findings of the thin-film metasurface sensors.\[47\]

The previous papers among others led to a surge of interest in the possibilities of metasurfaces for biosensing. Since then, numerous studies have been carried out in order to detect multiple types of substances, such as proteins, amino acids, sugars, etc. Among all these substances, there are some that present special interest for health. For example, carbohydrates perform essential roles in most living organisms in energy metabolism processes, or cell to cell communications. Nevertheless, they lead to an increment of the blood sugar and it is very important to have control on the intake amount to avoid hyperglycemia and illnesses related to it. This control becomes even more important in patients suffering from carbohydrate specific diseases such as diabetes. Therefore, finding new ways to detect and differentiate sugars is of great scientific and practical interest. This is the case of the work presented in\[75\] where a nano-slot antenna array working in the THz range was presented for selective
carbohydrates detection and discrimination method, able to discern molecules with concentrations ranging from hundreds to tens of moles.

Firstly, an exhaustive study of several carbohydrates was carried out using a commercial THz-TDS system, in which the transmission spectrum of each of the samples was obtained, and it was observed that these showed peaks of absorption at different frequencies, depending on the type of carbohydrate. With this study, it was determined that the hydroxyl functional group, present in molecules as D-glucose, fructose, and sucrose, has strong absorption peaks between 0.5 THz and 2 THz, as can be seen in the contour plot of Figure 4(a), left panel. In contrast, the polysaccharide group (present in molecules such as glycogen, cellulose, or amylose) showed no recognizable absorption peaks in the THz spectrum, due to the complexity of the chains between various structural groups and the lack of coordinated hydrogen-bond vibrations in the crystalline state.\(^{[75]}\) Among all the groups shown in the contour plot of the left panel of Figure 4(a), special attention was paid to the group mapped in red, which corresponds to the monosaccharide group. Within this, two frequencies of interest have been highlighted with dotted circles, which correspond to the absorption peaks of glucose and fructose. Because these two sugars present absorption features at different frequencies, two different antennas were designed, in order to match the fundamental antenna resonance with the absorption peak of each substance. The glucose antenna had a length of 40 \(\mu\)m to match with the D-glucose absorption peak at the frequency of 1.4 THz. Likewise, the fructose antenna was designed with a length of 35 \(\mu\)m in order to match its resonance with the absorption peak of 1.7 THz. Once the respective antennas were designed, their behavior as biosensors were tested by means of variations in the molecular concentrations of the different sugars, from 0 mg/dL to 500 mg/dL (27.5 mmol/L). These ranges of concentrations were defined in this way taking as reference the levels specified for blood sugar. The concentration of the normal fasting glucose in blood is 70–100 mg/dL (3.9 to 5.5 mmol/L),
whereas the concentration for patients with diabetic symptoms is 100–125 mg/dL (5.6 to 6.9 mmol/L).

The glucose nanoantenna with 1.4 THz resonance was applied to detect D-glucose molecules with concentrations varying from 0 to 4168 mg/dL. As shown in second column, top panel of Figure 4(a), the transmittance peak suffered an attenuation when increasing the concentration of the D-glucose molecule deposited on the surface. The glucose antenna was also applied to sucrose and sucrose molecules, but the results did not show such dramatic changes in the spectrum, as it is shown in the bottom panel of the second column of Figure 4(a). In a similar study, the fructose antenna with a fundamental resonance at 1.7 THz was used to measure different molecules. When a sample was coated in one region with D-glucose and fructose, the THz image obtained showed a higher attenuation in the fructose area, as shown in the third column of Figure 4(a). These results put into manifest the fact that these antennas, specifically designed for a certain sugar molecule, work properly for the targeted molecule with strong absorption features at the design frequency, but are insensitive to other molecules. Therefore, this type of sensor presents an efficient way to isolate molecules of interest by simply engineering the antenna at the optimal frequency. As a final study, the fructose antenna was used for the detection of sugars contained in various commercial sweetened drinks, as well as some versions with low sweeteners concentrations (diet version). The decrease in the obtained transmittance clearly showed that there were noticeable differences in the concentration of sugars in the different beverages, as can be seen in the rightmost panels of Figure 4(a).

As we have seen up to now, the detection of some biomolecules is of great importance as they might be related to specific diseases and an early identification can be crucial to treat them in time. Antibiotics have revolutionized healthcare and are one of the main factors for the rise of life expectancy during the last century. In fact, they are essential in the treatment of infections, fighting bacteria or other pathogenic microorganisms. However, an excessive use of
antibiotics can generate bacterial resistance decreasing the treatment effectiveness leading ultimately to a serious health risk. In order to keep a safe health environment, a proper control of the residues of antibiotics present in food becomes of vital importance. In recent years, it has been shown that metasurfaces are an interesting option in this field, with high sensitivity in the detection of some kinds of antibiotics. This was explored in\textsuperscript{[73]} where by the use of the extraordinary transmission resonance in a square-shaped hole array structure over a Si substrate, Xie \textit{et al.} were able to detect trace amounts of molecules of kanamycin sulfate, with a concentration as small as 100 pg/L. The structure used consisted of a periodic array of ring square apertures with width of 1 µm, length of 100 µm, and a period of 150 µm over a Si substrate. The kanamycin sulfate solutions used in the experiments were prepared by mixing the kanamycin sulfate with distilled water. A small volume of 30 µL was deposited on the metasurface and dried in air for each measurement, performed with a THz-TDS system. First of all, the THz transmission spectra of a Si wafer, without any metasurface on it and the same Si wafer coated with kanamycin sulfate deposited at a concentration of 0.1 g/L was measured. No change was noticed in the resulting frequency responses. However, with the incorporation of the metasurface on the Si wafer and with concentrations as fine as 10 ng/L, the same experiment resulted in a visible variation of the spectral response.

The sensing performance of the device was tested afterwards by coating the structure with different concentrations of kanamycin sulfate, ranging from 0 to 10 ng/L and observing the fractional transmission change of the structure by calculating the peak transmittance decrease. The results obtained showed that the peak transmittance changes with kanamycin sulfate concentration from $T = 47\%$ at the concentration of 1 pg/L to $T = 51\%$ for concentrations of 10 ng/L. With these results and taking into account the system stabilities, the minimum detectable kanamycin sulfate concentration was estimated to be around 100 pg/L. In order to make a comparison, similar measurements were carried out on the bare Si wafer without metamaterial, and the minimum detectable concentration in this case was estimated to be
around 1 g/L, which is $10^{10}$ times higher than that obtained for the metasurface case. This highlights the importance of metasurfaces for biological sensing, capable of improving the minimum detectable quantities of sample by a factor of more than nine orders of magnitude.

Aside from the detection of bacteria, drugs, or proteins, the study of transgenic foods has become very important in recent years, and there is a great debate about whether its use is beneficial or not for human health. Transgenic foods are those that have been produced from an organism modified by genetic engineering and to which genes from another organism have been incorporated to produce the desired characteristics. According to the World Health Organization, the transgenic foods available in the market have passed risk assessments and are unlikely to pose health risks. However, it seems important to perform more exhaustive studies and monitoring. In the work presented in,[76] a metasensor was designed to detect transgenic tomato DNA genome by THz time-domain spectroscopy. As mentioned there, one of the prominent challenges in plant biotechnology is the development of a rapid and flexible system for genetic analysis. Although the ability of THz to detect DNA sequences had already been demonstrated by the detection of vibrational modes in one of the pioneering THz spectroscopy works,[83] the distinction between a native DNA or a modified DNA is extremely challenging as they usually differ only in a very small fragment containing a few nucleotides. This implies that a sensor able to discern between native and transgenic DNA must have a very high sensitivity, due to the small amount of sample available for measurement. In[76] two circular metamaterial SRRs with single or double splits were designed and tested. The SRRs were made of a 200 nm thick gold deposited on a polyethylene terephthalate (PET) substrate 25 µm thick and had the following dimensions: inner radius of 24 µm, outer radius of 30 µm, width of 6 µm, and gap size of 2 µm. The genome obtained for its identification was a wild-type, near-isogenic lines of tomatoes. For the engineering of the transgenic genome, two types of DNA were mixed at a certain concentration in order to have a non-pure DNA sample. These genomes were prepared as a thin-film layer by depositing an
aqueous solution of DNA covering the metasurface and then the solution was evaporated at low temperature to avoid damaging the structure or the genome itself. A THz-TDS system was employed to obtain the THz transmission spectra with different concentrations of both genomes. The bare structure presented two resonances in the frequency response, located at 0.571 THz and 1.838 THz in the case of the single-gap SRR; and at 0.974 THz and 1.736 THz in the case of the double-gap SRR. In order to test the sensing behavior, a 5 µL layer of DNA was deposited on the structure, and a significant redshift was observed, in both single-gap and double-gap SRRs. Concretely, the redshift normalized to the resonant frequency of low and high resonances reached 6.5% and 3.8% for the single-gap SRR; and 7.9% and 5.5% for the double-gap SRR. These data show that double-gap structures are more sensitive, a result that can be explained by noticing that this structure doubles the areas of high electric field confinement. In addition, as the authors mentioned, the resonance produced at lower frequencies seems a better option for sensing, because the frequency displacement produced is greater, and its line width is much narrower than that of the second resonance. However, as commented in the thin-film section, what is important is to have high sensitivity values, which depend not only on the frequency displacement, but also on other parameters such as the thickness of the analyte, or its dielectric constant.

To further investigate the potential of the double-gap SRR in distinguishing between native and transgenic DNA, two overlayers were prepared with DNA solution in different volumes ranging from 0 µL to 9 µL. In this experiment, it was assumed that the thickness of the deposited film was proportional to the DNA volume. By measuring the frequency shift of the different transmission spectra of the different volumes for both DNA genomes, it was observed that the transgenic DNA could be distinguished from the native DNA due to its larger frequency shift. These differences could be attributed to the different refractive index of both genomes. In order to corroborate the experimental results, similar simulations were carried on, with the dielectric constants of native and transgenic DNAs set to 2.8 and 3.1
respectively, and showed a successful reproduction of the experimental results, demonstrating the capability of metasurface sensors to identify transgenic genome of plants with high sensitivity.

In the last years, new designs more complex than those presented so far have been proposed, based on geometries more elaborated than simple rings, with the aim to achieve a greater confinement of the electric field in more than a specific point of the structure, trying in all cases to enhance the sensitivity of the designed structures. In a metasensor based on four identical resonators was numerically and experimentally demonstrated by THz-TDS for measuring concentrations of protein bovine serum albumin (BSA). The unit cell of the metasurface was an aluminum top layer 0.2 µm thick consisting of an outer square split-ring resonator connected to an inner circular split-ring resonator by two short arms over a Si substrate. After an exhaustive study of the optimal dimensions of the gap, side length, and line width, they were set to \( g = 5 \) µm, \( L = 48 \) µm and \( W = 5 \) µm, respectively. To characterize the sensing performance of the biosensor, the transmission spectrum variation was numerically evaluated with CST Microwave Studio when an analyte layer with refractive index equal to 2 and several thicknesses ranging from 2 µm to 40 µm was put on top, assuming normal incidence of a wave polarized perpendicularly to the gaps. When increasing the overlayer thickness between 2 µm and 10 µm, a maximum frequency shift of 34 GHz took place. However, when the analyte thickness was larger than 10 µm, the device showed a saturation effect, corroborating that this type of metasensors are useful to measure small amounts of samples, which is the main interest in biosensing applications. In the experiment, the transmission spectrum of the bare structure was measured with a THz TDS system and a resonance dip was observed at 0.85 THz. The analyte employed in the experiment was a high molecular weight protein BSA and a liquid deposition method was employed to indirectly measure the concentration of the analyte. Different solutions with concentrations ranging between 1.5 µmol/L to 765 µmol/L were prepared in deionized water and then a 5 µL BSA
solution was dropped onto the metasensor surface and dried in the air for each concentration, in order to obtain an homogeneous BSA thin film. By observing the transmission spectrum for each concentration, it was demonstrated that the frequency shifted to lower frequencies when increasing the BSA concentration, until reaching a maximum displacement of 50 GHz for a BSA concentration of 764.7 µmol/L. However, saturation of the response was noticed for concentrations higher than 14.7 µmol/L. In any case, the minimum concentration detectable was 1.5 µmol/L, which led to a frequency shift of 2 GHz. This device could be used for rapid concentration measurements with the general advantages of metasurface-based sensors, such as the small shape, easy design and fabrication process, and simple experimental conditions.

Usually, medical examinations are performed by measuring the interactions between bio-samples and frequency-tuneable plasmons where distinct transmission spectra can be obtained for different tissues. In[85] a spiral-shaped plasmonic structure working in the THz region was presented in which, by rotating the spiral structure, highly tunable transmission bands were observed. The structure consist of a spiral bull’s-eye antenna with a subwavelength aperture surrounded by concentric grooves, as shown in the inset of the left panel of Figure 4(b). The main interest in this work was to characterize different mouse tissues.

The frequency tunable characteristic of the device was based on the spiral shape of the bull’s-eye structure that was designed to have a changing period depending on the in-plane angle. Owing to the spiral shape, the frequency of the transmission peak could be tuned by simply changing the rotational direction of the structure and hence changing the angle of polarization. The structure was designed to set the detection range between 1 and 1.4 THz. The outer diameter was set to 150 µm and the structure had 8 tips with an angle of 30º between them. First, a spectroscopic characterization was done in which the transmission spectra for a pharmaceutical tablet (bufferin) was measured. The results showed the same peaks and
valleys that those shown in far-field measurements results, demonstrating the capability of the
device to perform spectroscopic measurements with high concentration.

Next, and with the purpose of testing this device for biosensing applications, different mouse
tissues were excised and put into the spiral structure. The transmission spectra for different
samples such as skin, hearth, lung, or brain samples is shown in the left panel of Figure 4(b).
As observed, most of the samples showed transmission peaks in the frequency range between
1 and 1.3 THz, highlighting the interest of this frequency range for this type of applications.
As reported in\cite{85} transmission of brain, heart, and bone samples were overall lower than that
of other tissues, whereas skin samples showed the highest transmission between 1.2 and
1.3 THz. Taking advantage of the large difference between the transmission spectra between
skin and bone, a comparison of both samples was made by mapping them through the
metasensor. For this purpose, two mouse tail transverse sections with a diameter of 2.5 mm
where cut, see the samples in the top central panel of Figure 4(b). Then, the THz images were
obtained with a conventional mirror-based focusing setup, top right panel of Figure 4(b), and
with the designed spiral bull’s-eye, bottom right panel of Figure 4(b). The measurements
acquired with a conventional mirror-based focusing setup showed no difference between the
different tissues due to the low resolution of the system. Nevertheless, when placing the
samples on the spiral structure, it was possible to clearly distinguish hair, skin, and bone of
the mouse tail represented by the colors yellow and red for hair, light blue for skin, and dark
blue for bone. As a final experiment, a mouse-lung transmission mapping was carried out and
biological three-dimensional microstructures were visualized in the middle of the lung lobe,
which may represent lung pipes. All these experiments demonstrate the great value of the
antenna designed to carry out medical examination by means of spectroscopy, sensing, and
imaging in the THz range.

Although metasurfaces have proved to serve as a promising signal-enhancing tool with
important improvements in target detection, much effort is still needed to get designs with
competitive values of sensitivity. The addition of a graphene layer could be a good solution by taking benefit of the fact that graphene interacts with external molecules in its vicinity through delocalized $\pi$-electrons. With this premise, a biosensing platform by the integration of a graphene layer on a THz metamaterial absorbing cavity was presented in\cite{86} where, due to the strong interaction with the graphene layer, the introduction of a target layer introduces dramatic changes in the frequency response of the structure. It is noteworthy that the sensitivity of the device depends on how the external molecules interact with the monolayer graphene. Molecules with $\pi$-electrons like chlorpyrifos methyl molecule interact strongly with the $\pi$-electrons of graphene through $\pi$-stacking (in chemistry, this stacking refers to a stack ordering adopted due to interatomic interactions).

The structure designed consisted of square gold patches resonator arrays 200 nm thick over a thin polyimide spacer ended on a 200 nm thick aluminum film used as a ground plane, see scheme in the top left panel of Figure 4(c). First, a comparison of the empty structure and its natural behavior was carried out by depositing a layer of graphene on it. As can be seen in the bottom left panel of Figure 4(c), depositing a layer of graphene increases the reflectance dip of the structure considerably, which may present an advantage for sensing purposes. Then, a chlorpyrifos methil sample from a 10 $\mu$L with a concentration of 3 mg/L solution was prepared and deposited over the graphene layer. The reflection curves of the bare structure and the coated one were recorded by a THz-TDS system, and a clear blueshift of the reflection dip was observed, as shown in the top panel of the second column of Figure 4(c). It can be noticed that this result differs from the normal behavior of a metamaterial without a layer of graphene above, where the frequency shift produced goes usually towards lower frequencies (redshift), as shown in the bottom panel of the second column of Figure 4(c). This response from the graphene-metamaterial is caused by the interaction of the external molecules with the graphene structure. As explained in\cite{86} the interaction moves the Fermi
level toward the Dirac point and decreases carrier density in graphene, leading to a reduction of the graphene conductivity and in a resonance enhancement, resulting ultimately in a reduced and blueshifted reflection dip (increased absorption), as well as a higher Q-factor value.

It is also noteworthy that the sensitivity depends on the type of interaction between the analyte and the graphene monolayer. In general, molecules with $\pi$-electrons, such as the mentioned chlorpyrifos methyl, offer higher sensitivities than other molecules that do not possess this type of electrons, such as fructose. To demonstrate this point, samples of both substances were used and experimentally measured. As can be seen the third column of Figure 4(c), a concentration of 3 mg/L of chlorpyrifos methyl resulted in a variation of 35%, while the maximum change achieved by fructose solution was only 15%.

Due to the good biocompatibility of graphene, Xu et. al proceeded to check the behavior of the structure designed as an aptasensor. An aptamer is based on the technique of using single-stranded oligonucleotides, such as DNA, that can be linked to other substances with which they have good biocompatibility. In the aptamers were used to detect thrombin. As graphene is hydrophobic and DNA is hydrophilic, it was necessary to introduce pyrene in the sequence, so that it was used for the absorption of graphene via $\pi-\pi$ stacking. An atomic force microscope (AFM) was employed to verify if the binding was done correctly, and the aptamers were immobilized on graphene. As it can be seen in the AFM images represented in the top right panel of Figure 4(c), the aptamer region reveals the existence of some type of structures, due to the height change obtained which is approximately 40-50 nm. This fact suggests that the aptamers were succesfully added to the graphene layer, and can be measured in order to use the device as a biosensing platform. In fact, when measuring the THz reflection spectra with and without the presence of 100 nM thrombin concentration, a reflectance decrease of 4% was found when coating the structure with the thrombin layer, see the bottom right panel of Figure 4(c). These results demonstrate the great utility of using not
only metamaterials, but adding layers of graphene to detect some types of biological molecules, promising great potential in biological sensing applications.

In addition to the types of metasurfaces reviewed so far, another very popular alternative when designing sensing devices are plasmon-based sensors, based on surface waves. In particular, at THz regime, similar waves exist that are usually called spoof plasmons. The first time that these waves were used for sensing applications in the THz range was in 2004, when Saxler et al. presented the first time-domain study on metal surfaces covered or uncovered with dielectric films.\(^\cite{87}\) In that work, they used a TDS-THz system to generate and detect THz waves and demonstrate that small changes on the overlayer thickness lead to drastic modifications on the spoof plasmons field distribution. Although other techniques for the excitation of spoof plasmons have been exposed, such as the pump or prism coupling,\(^\cite{88,89}\) the first designs in THz were not made with the aim put in sensing applications. Furthermore, devices based in metal dielectric interfaces have proven to be a reliable technique for thin film sensing.

### 3.3. Microorganisms sensing

Microorganisms such as fungi, bacteria, or viruses are the cause of many human diseases, so the demand for tools to detect them in a quickly and effectively manner has grown and is continuously increasing over the years. Usually, the methods used for this purpose are very slow due to the growth time of the bacteria (from days to weeks). Although some techniques, such as molecular methods or mass spectrometry, have managed to reduce the time necessary for a correct detection, they are still relatively complex and difficult to apply in the point-of-care.

Metasurfaces operating in the THz regime are an emerging alternative in this regard, as they have already shown a great potential in high-speed and on-site detection of microorganisms. One of the first examples is found in\(^\cite{78}\) where a metasurface-based sensor for the high speed detection of live microorganisms was numerically and experimentally studied. The structure
proposed consisted of metallic arrays of a square ring with a gap in the center deposited over a Si substrate, as can be seen in Figure 5(a). The advantage of this type of structure with respect to direct detection without a metasurface is that the size of the microorganisms is very similar to the micro-gaps of the resonators. In this way, the microorganisms that are placed in the metallic gap, will undergo a high interaction with the incident wave due to the high electric field intensity in those areas, when the metasurface is at resonance, causing important changes in the frequency response. In the experimental demonstration, all the transmission spectra were obtained by THz-TDS techniques. First, a layer of penicillin with a density of 0.032 /µm² was deposited on a Si substrate, but no change in the frequency response was observed comparing it with that of the empty structure. This is because many microorganisms are transparent to the THz waves, and do not have any spectral fingerprint in this range, unlike what happened with chemical substances such as those studied in the previous section.

On the other hand, when depositing 0.09 /µm² of penicillin on the metamaterial structure, a frequency shift of 9 GHz, corresponding to 6% of the FWHM was obtained.

One of the advantages of sensing microorganisms is that they usually can be cleaned. Once the measurement of the resulting spectrum was obtained, the structure was treated with a fungicide, and the resulting spectrum returned to coincide with the original, showing the possibility to reuse metasurface sensors. As explained in[78] this technique could be extended to the detection of bacteria in aqueous environments, through the functionalization of the metasurface with receptors that allow the detection of certain bacteria. To validate this point, *E. coli* bacteria from a solution of 100 µg/mL were deposited on the designed structure functionalized with *E. coli* anti-body. As depicted in Figure 5(a), the structure was covered by a glass film and the water layer thickness was set to 55 µm by the use of a spacer. The THz transmission response showed a blue-shift of 23 GHz (15% of the FWHM) for an *E. coli* solution of 0.019 /µm², see the central panel of Figure 5(a). The reason why in this case the displacement occurs towards higher frequencies is explained due to the low dielectric constant
of *E. coli* compared to that of water in the range of THz (1.6 versus 4.2). It must be taken into account that in the case of not performing the functionalization of the structure with the *E. coli* anti-body, there are no observable changes in the obtained spectra, see the right panel of Figure 5(a). This means that it is possible to create structures sensitive to the desired microorganism by using the correct anti-body in the functionalization of metasensors. Obviously, the frequency shift obtained depends on the number of fungi and bacteria located in the area of the metal hole. For this reason, in the same work a study was conducted varying the number of microorganisms (*N*) of penicillin in the gap area, from 0 to 5. Several transmission spectra were obtained, for the average values of *N* of 0.59, 0.85, and 3.4, which correspond to densities of 0.02 /µm², 0.028 /µm², and 0.113 /µm² respectively, obtaining in all the cases a measurable frequency shift. It is remarkable that the correct detection of average values below the unity were carried out, which demonstrates the high sensitivity of these structures for biosensing applications. With the purpose of verifying the experimental results obtained, different finite-difference time-domain simulations were carried out, with the fungi modelled as spheres of 2 µm in diameter placed in the area of the metallic gap of the structure. Consistent with the experimental results, a clear redshift of the frequency resonance was observed, verifying the effectiveness of the technique in biosensing applications.

The same year, Park *et al.* extended the reach of metasurface biosensors to the detection of individual yeast cells and yeast films by the use of structures fabricated on various substrates with different dielectric constants, in order to study the effect of the substrate in the sensing performance of the device.[77] The antenna arrays consisted of slot antenna patterns with length of 100 µm and periodicity of 200 µm over two different substrates: a Si wafer 550 µm thick and a quartz slab 2000 µm thick. Transmission spectra of both structures with and without the deposition of yeast were experimentally measured by THz-TDS techniques. Comparing the bare and coated Si substrate structure, with a slot of *w* = 2 µm in width, and a fundamental resonance placed at 0.57 THz, a redshift of about 9 GHz was obtained when the
average number of yeast placed in the slot area was $N_{av} = 50$. Similar experimental results were carried out using a quartz substrate, with its fundamental resonance at 0.93 THz. Comparing both structures, it was observed that the frequency shift was much greater in the case of using quartz as the substrate material (about 3-fold higher than that of the Si substrate device). This fact can be explained because the relative change in the effective dielectric constant (weighted average between the dielectric constants of the substrate and air) with the presence of yeasts is higher in the case of using substrates with lower permittivity. As said in\textsuperscript{[77]} the contribution from the substrate index is about twice that of the air index, so the effective permittivity, $\varepsilon_{eff}$ was expected to be equal to 7.56 and 2.96 for the Si ($n_{si} = 3.38$) and quartz ($n_{q} = 1.93$) substrates, respectively. After obtaining the experimental results, different simulations were carried out to study the frequency shift as a function of the average number of yeasts located in the micro-gap of the designed structures. The results obtained using a structure with quartz as a substrate revealed to be 2.93 times more sensitive than in the case of using a Si substrate, a value that closely approximates the experimental results, in which the improvement of a quartz substrate with respect to Si was 2.83 times.

Even though it has been demonstrated that properly designed metasurfaces are capable of efficiently detecting microorganisms such as fungi or bacteria, the detection of viruses is more complicated, due to their extremely small size, typically less than 100 nm. This would imply the creation of new metasurfaces with a metal gap size $10^3$ orders of magnitude lower than the designs produced for the detection of bacteria, adding some difficulty to the manufacturing process. Three years after the work commented above, Park et al. carried out in\textsuperscript{[90]} THz-TDS measurements to demonstrate the detection of the bacteriophage viruses PRD1 and MS2, which have dimensions of 60 nm ($\lambda_{THz}/5000$) and 30 nm ($\lambda_{THz}/10000$), and are double-stranded DNA and single-stranded DNA viruses, respectively. The metamaterial patterns consisted of an array of SRRs with a gap of 200 nm, see scheme in the left panel of Figure 5(b). The viruses were deposited on the structure in a solution, and dried at 85° for 5 minutes.
To perform this type of detection, it is vital to know the dielectric properties of the samples under measurement. However, obtaining this data in the THz range is complicated, as there is not enough existing literature to consult. In\cite{90} the dielectric constant of the different viruses was obtained by studying the saturation-thickness behavior of the resonant frequency of THz metamaterials. Films of both virus types 40 µm thick, which is well above the saturation limit placed at approximately 10 µm, were deposited on a metasurface over a Si substrate having a resonance at 0.8 THz. As explained in\cite{90} using the relationship between the relative permittivity and the frequency normalized shift for metamaterial devices, the dielectric constants of the PRD1 and MS2 virus layers were found to be 3.48 and 3.83 at 0.8 THz, respectively. These values were confirmed by measuring the dielectric constants of thick films containing viruses in a high density. Comparing these values with those obtained in some typical bacteria (whose dielectric constants are between 1.2 and 1.53), it is found that the dielectric constant in the case of viruses is higher, which has advantages when performing high sensitivity sensing. With the dielectric properties of the samples known, a structure made of a quartz substrate with a resonance localized at 1.26 THz was used to measure low surface densities of PRD1 and MS2. To do this, 10 µl of a PRD1 virus solution of $10^9$ /ml was deposited on a 10 mm$^2$ coating area and a frequency redshift of 30 GHz was obtained for a surface density of 4 /µm$^2$. Similar experiments were done for MS2 viruses obtaining a larger frequency shift in this case. Concretely, a frequency shift of almost 60 GHz was achieved for a surface density of 4 /µm$^2$, which corresponds to double the displacement with respect to PRD1 viruses. According to the authors, the reason of the higher sensitivity is not apparent, because the dielectric constants of both substances are similar. One possible reason could be that the size of the MS2 is smaller (approximately half), so the samples would be closer to the substrate, and the concentration of the electric field at those points is stronger than as one moves away from the surface of the structure.
As an alternative study, the gap width of the designed structure was varied between 0.2 µm and 3 µm. As shown in the second and third panel of Figure 5(b) the response saturation depends on the gap size, although in all cases there is a redshift of the dip as the concentration increases. To present the results more clearly and simplify the comparison, the frequency shift for each gap width and surface density as well as the sensitivity as a function of the gap width are summarized in the fourth and fifth panels of Figure 5(b). As observed, the frequency shift is larger as the gap width decreases, due to the higher interaction with the viruses placed in that area. Therefore, the sensitivity of the device can be improved by optimizing the geometry of the metal pattern and the substrate. Despite the good results obtained in\textsuperscript{[90]} there is still a long way to go and numerical simulations would be necessary to understand the reasons behind the large difference between PRD1 and MS2 sensitivities. However, these simulations are expensive due to the small size of the samples compared to the THz waves. This confirms that the topic of microorganism sensing with metasurfaces is still a long-term research topic.

New investigations carried out showed that the sensitivity of this type of structures could be improved by the introduction of metallic wires in the geometry of the metamaterial structures. This is the case of the work presented in\textsuperscript{[91]} where silver nanowires were added to a hybrid slot antenna structure to increase the sensitivity obtained in the detection of microorganisms. The proposed design is based on arrays of slotted antennas, with the difference that silver nanowires are added protruding in the slot area, with the aim of increasing the concentration of the electric field and, with this, improving the sensitivity, see a scheme in the left panel of Figure 5(c). The slotted antenna was designed to have a length of 200 µm and a width of 15 µm, thus having a resonance at 0.46 THz. The slot manufacturing procedure is shown in Figure 5(c). First, the structure without the nanowires was coated with a poly-methyl methacrylate (PMMA) film 1 µm thick, and the transmission spectra was measured by a THz-TDS system, where a redshift of 2.8 GHz was observed. The same experiment was done but depositing the PMMA over the hybrid antenna, with nanowires of 5 µm in length, and the
frequency shift observed was 10 GHz, which is equivalent to an increase of 3.8 times with respect to the structure without nanowires on the slot of the antenna. This can be explained due to the greater concentration of electric field with the presence of nanowires. In fact, the empty structure with the presence of nanowires presents an enhancement in the quality factor from 2.2 to 3.5, thus improving the FOM by a factor of 5.4 times.

Next, a study of the behavior of the structure as a biosensor was made, by depositing some density of virus PRD1 on the surface of a structure whose slot average dimensions were 100 µm in length, and 3 µm in width. The deposition of PRD1 in a density of 6.3 µm\(^2\) led to a frequency shift of 32 GHz, see top central panel in Figure 5(c). By depositing the same amount of sample on the hybrid structure (with the presence of nanowires), this displacement increased to 54.2 GHz, as can be seen in the bottom central panel of Figure 5(c). To compare the performance, the frequency shift as a function of the virus concentration is plotted in the right panel of Figure 5(c). The frequency shift data can be translated into sensitivity values that have an increase from 12.8 GHz µm\(^2\)/particle for the simple antenna, to 32.7 GHz µm\(^2\)/particle for the hybrid antenna, which means an enhancement of 2.5 times.

According to the obtained data, the hybrid device would be able to detect PRD1 virus in a density lower than 0.1 unit/µm\(^2\) or 10\(^7\) unit/mL. Even though these values are enough to observe changes in the frequency spectrum, the sensitivity of these kind of devices should be improved to be used as biosensors, because, as mentioned in\(^{[91]}\) currently available assays are capable of detecting plasma viruses at a detection limit of 1000 unit/mL.

In order to corroborate these results, numerical simulations were carried out using a linearly polarized plane wave as source. The metallic parts of the antenna were considered as perfect electric conductor, and the polymer film was modelled by a 1 µm thick film with dielectric permittivity of 2.56. With these conditions, the normalized amplitude of the simple and the hybrid device were obtained, showing a 3.25 times higher sensitivity for the case of the hybrid device, in excellent accordance with the experimental results. Therefore, although there is still
a long way in the quest towards obtaining designs with better sensitivities for the correct
detection of viruses able to compete with standard techniques currently used, studies like this
represent a great step towards new structures capable of performing on-site detection of
microorganisms in various environments, in a high-speed and easy manner.

3.4. Cancer and tumor detection

Basal cell carcinoma (BCC) is the most common type of skin cancer as about eight out of ten
cases of skin cancer are BCCs. These cancers tend to grow slowly and, although it is very rare
for BCC to spread to other parts of the body, if a BCC is left untreated, it can spread to nearby
areas and affect the bones as well as other tissues under the skin. Detecting cancer when it is
in its early stages is often critical as it allows for the possibility of having more treatment
options with better life expectancy. Current diagnosis of BCC is usually based on visual
inspections, skin biopsies, or histologic techniques. Among the histological techniques, Moh's
micrographic surgery has proven to be the best reported method for this application.\textsuperscript{[92]} This
technique allows identifying the direction of tumor spread without the need for extensive
excisions and has reported cure rates of 99\% for primary tumors and 96\% for secondary
tumors after 5 years of treatment. This is a type of controlled microscopic surgery, in which
the patient remains in the operating room while the study of the extracted tissue is carried out.
This technique allows the removal of the exact cancerous tissue, saving the healthy tissue.
The main disadvantage is that Moh’s micrographic surgery is very expensive and time-
consuming.

Over the years, other imaging techniques have been proposed. The use of high-frequency
ultrasound allows an axial and lateral resolution of 80 and 200 \( \mu m \) respectively, and has a
penetration depth of 7 mm.\textsuperscript{[93]} However, it does not have chemical specificity, so it is not able
to differentiate between healthy and diseased cells. Magnetic resonance is only effective if the
tumor extends more than 15 mm below the surface. In that case, values of axial and lateral
resolution of 19 and 78 \( \mu m \) have been reported,\textsuperscript{[94]} with a penetration depth of 800 \( \mu m \).
Among the disadvantages of this technique are the need of the whole subject for the inspection, as well as the fact that it is an expensive and time-consuming technique. Several near infrared imaging techniques such as confocal microscopy have also been studied, able to provide a high resolution in real time with values of axial and lateral resolution of 4 and 2 \( \mu \text{m} \). The disadvantage is that this technique provides a very low penetration depth of just a few hundreds of micrometers.

The first report of THz spectroscopy applied for ex-vivo cancer detection was published several years ago\(^\text{[72]}\) using THz pulsed imaging (TPI) technique with the purpose of differentiating between BCC and normal tissue. For that, both a BCC and a healthy sample of the same patient were taken. Figure 6(a) shows the comparison of several samples images in the visible (top panels) and in the THz range (bottom panels). In these images, the areas marked by solid boundaries were those that indicate the area of damaged tissue, while the dashed boundaries were the indicators of healthy zones.

As can be seen, the THz image gives a lot more information. In fact, it was verified that within the areas marked as "affected" there were areas that were not affected at all. In the THz image, the damaged areas (in red color) were marked by rectangles. In contrast, the green areas corresponded to non-affected skin regions. These results are explained due to the fact that BCC absorbs more water and therefore shows an increased THz absorption in comparison with normal tissue. The results of this study show that the level of contrast obtained by means of TPI is more efficient than that obtained by histology techniques. After this work, other studies were published for ex-vivo skin cancer detection by the use of TPI. However, it was in\(^\text{[96]}\) where a systematic in vivo study of the response of THz radiation to normal skin was conducted, in order to evaluate the interaction of THz radiation with normal skin on the hand palm.

Expanding the reach of metasurface sensors, structures based on metasurfaces in detection and distinction between healthy and cancer cells have also been developed in recent years.
In a planar array of concentric subwavelength SRRs was employed to study the apoptosis in oral cancer cells by THz-TDS measurements. The device consisted of five concentric gold SRRs over a 10 µm thick polymide substrate. The period of the unit cell was 120 µm and the inner radius of the rings were 20, 28, 36, 44, and 52 µm respectively. In a first study, the designed metasurface was covered by a layer of oral cancer cells (HSC3) and normal epithelial cells (HaCaT) with the purpose of detecting both samples. The transmission spectra was measured in both cases, and the response with both cell types experienced a spectral redshift when the cell concentration increased. However, the frequency shift for the cancer cell was found to be higher. This could be explained by the difference in the water content for normal and cancerous cells. The potential of the biosensor designed as a cell apoptosis detector was also studied. For this, the transmission spectra when treating or not an oral cancer cell (SCC4) with cisplatin (a chemotherapeutic drug to induce cellular apoptosis used in clinical treatments) in a concentration of 5 uL, were compared. Obviously, a frequency shift and a change in the transmission peaks were observed when measuring the THz spectra from control and cisplatin treated samples after 24, 48, 72, and 96 hours. The results obtained showed that, despite the fact that the frequency shift did not increase with time, it suffered a slight decrease, indicating that the cell number did not increase due to the effect of the cisplatin. Another observation was that as time increased, the fourth resonance of the structure suffered a frequency shift decrease. As mentioned by the authors, this means that the dead cells start to break away from the THz metamaterial surface and float in the suspension, resulting in a decrease of the number of cells on the metasurface. As demonstrated in this type of metasensors are capable of determining the absolute value of cell apoptosis by the study of the frequency response.

Other geometries have been also studied for cancer cell detection. This is the case of where the authors designed a metasurface sensor based on symmetry-breaking double SRRs to perform oral cancer cells detection, see the scheme in the left panel of Figure 6(b). The
metallic SRRs were designed with a distance of 28 µm between both structure asymmetric gaps and were supported on a 25 µm thick polyimide film. Different concentrations of oral cancer cells (HSC3) were deposited on the metasensor surface in order to study the biosensing capability of the structure. The bare structure presented a resonant peak at the frequency of 1.65 THz. When the cell concentration increased, a frequency shift was observed. Particularly, with a cell concentration increase from $1 \times 10^5$ to $7 \times 10^5$, the frequency shift grew from 50 to 90 GHz, obtaining a maximum relative frequency shift of 5.4%. With these data, the maximum sensitivity obtained was 900 kHz/cell·mL$^{-1}$, which means that a cell per milliliter would lead to a 900 kHz frequency shift of the peak. As is well known, apoptosis is a very significant feature when it comes to evaluating the evolution of cancer cells. The existence of some cancer drugs have shown to promote cellular apoptosis whereby cells, before becoming cancerous, suffer a self-destruction process. In order to observe the behavior of the designed device in the detection of cellular apoptosis, a layer of HSC3 with a concentration of $2 \times 10^6$ cells/mL was deposited on the surface, and subjected to the action of different concentrations of cisplatin, used to promote cell apoptosis. Different cisplatin concentrations from 1 to 15 µM under an action time of 24 hours, as well as a cisplatin concentration of 1 µM under different action times ranging from 24 to 72 hours were measured, see the obtained transmission spectra in the central and right panels of Figure 6(b). It can be observed that when the cisplatin concentration increases, the resonant peak experiences a frequency blueshift of 70 GHz, suggesting that the killing effect of the cisplatin reduces the cell concentration. In the case of increasing the action time for a fixed cisplatin concentration, the resonant peak also exhibits a frequency blueshift, revealing a decrease of cancer cells concentration over time. These results demonstrate the effective cell killing over time with the action of cisplatin drug, and the significant potential of metasurface-based sensors in cancer treatment and detection.

4. New manufacturing techniques: atomic layer lithography
As discussed in the previous sections, there are currently many examples of THz metasurface sensors with very good performance in thin-film sensing applications, able to achieve high sensitivity as well as FOM values for thicknesses as fine as a few tens of nanometers. However, those designs suffer severe restrictions when the thickness of the thin-film analyte falls below 10 nm, due to the large dimensional difference between the thickness and THz wavelength. This fact renders metasurface designs based on conventional photolithography unable to detect extremely subwavelength analytes due to the insufficient interaction between the electromagnetic wave and the material.

To enhance this interaction, one should be able to squeeze the electric field within a very small volume and implement environments with a high electric field strength, increasing the light-matter interaction. In fact, this is at the core of the metasurface sensors discipline and the main reason for their success all along the electromagnetic spectrum. The problem is that it is intrinsically difficult to create sub-nanometer gaps uniformly distributed on a length scale of a few dozen microns using conventional manufacturing techniques.

This limitation has been circumvented recently by a new revolutionary manufacturing technique called atomic layer lithography\cite{99,100} based on atomic layer deposition and able to produce sub-nanometer details in large areas, of the order or millimeters or even centimeters. The key aspect of atomic layer lithography is that the sub-nanometer dimension is decoupled from the rest of the pattern. This way, it is possible to manufacture structures in the centimeter-scale with gaps in the atomic scale.

The first report on this type of manufacturing was presented in\cite{99} and consisted generically in the next steps: first, the initial metal pattern was defined using standard lithography; then, a nanometer-sized sacrificial alumina layer was deposited by using atomic layer deposition, covering in a conformal way the top and vertical sidewall metallic surfaces; after that, a second metallic layer was deposited and then an anisotropic ion milling is done to expose de alumina that separated metallic sidewalls; finally, the nanogaps were created by applying a
buffered oxide etchant to remove the alumina. Following this procedure, the nanogap could be accurately determined by the thickness of the conformal alumina layer with a nanometer-size precision, as corresponds to atomic layer deposition. Thanks to the ultrathin gap created, the authors in¹⁹⁹ were able to increase the local surface-enhanced Raman scattering by a factor of $10^9$ when the nanogap size was 5 nm.

A drawback of the previous structure is that the elements were hollow (i.e. slits or holes) and the nanogap was placed in the perimeter of the aperture (i.e. in the case of the holes is a narrow ring slot around the hole). Therefore, transmission measurements suffered from a significant background due to the direct transmission through the central aperture. In addition, the structural dimensions were not suited for THz frequencies. Those issues were addressed in¹⁰⁰ by implementing a new planarization scheme to obtain structures where all the apertures were nanogaps, avoiding any other hollow. As in the previous case, the first step was to define trenches using standard patterning techniques, e.g. conventional lithography. Then, the patterned substrate was coated with alumina using atomic layer deposition. After that, a metal layer was grown on the structure by using directional evaporation. A crucial aspect in this step was to keep the sidewalls vertical, to ensure that the first and second layer were not in contact. Once this condition was met, the top metal film layer was peeled off using a standard adhesive tape. This way, a structure consisting of metallic islands separated by alumina nanogaps was obtained. It is important to remark that this manufacturing technique can be applied to large areas, because the overall footprint is defined by standard lithography whereas the nanogap size depends uniquely on atomic layer deposition, which can have nanometer resolution.

Applying this method, slot arrays with width of 5 and 10 nm and various shapes and aspect ratios were successfully achieved in¹⁰⁰. In the THz characterization of the structures, an extraordinary field enhancement factor as high as 25000 was estimated from the experimental measurements for a 1 nm nanogap structure. Furthermore, it was anticipated that by inserting
molecules it was possible to enhance dramatically light–matter interaction, which can have a direct application on sensing platforms of extraordinary sensitivity.

Recently, Kim et al.\cite{101} proposed the implementation of a SRR array with sub-10 nm gap with a lattice periodicity of 100 µm a side length for each SRR of 80 µm and nanogap widths of only 5 and 10 nm, as shown in Figure 7(a), employing a more elaborated manufacturing technique, but sharing the main aspects of atomic layer lithography.

The response of the structures was experimentally analyzed by performing THz-TDS measurements with the electric field polarized perpendicular to the gap to excite the fundamental SRR resonance. Two resonance dips were identified in the transmission spectra recorded from 0.1 to 1.6 THz, the first one appearing at 0.25 THz and the second at 1.1 THz, for the 10 nm gap array, see the bottom panels of Figure 7(a). It is noteworthy that the array with 5 nm gap only presents the second resonance (near 0.96 THz) probably due to low frequency noise or loss that masks the dip associated with the first resonance. A numerical study was performed to complement the experimental findings and a field enhancement factor of approximately 11300 and 22100 was estimated at the 5 and 10 nm SRR nanogap, respectively. The experimental enhancement factor was also estimated for the fabricated 10 nm gap SRRs at the fundamental resonance. This factor was indirectly estimated from the measurements by two different methods: by guessing the incident electric field intensity when the in-gap electric field reaches the breakdown and by assuming that the transmission amplitude between an SRR array and a closed ring array is only due to the in-gap field and applying diffraction theory afterwards. With either method, the field enhancement factor was estimated as 7000.

These new manufacturing techniques open interesting avenues in thin-film sensing applications with exquisite sensitivity, as demonstrated in the work carried out by Park et al.\cite{102} There, they analyzed a structure made of ring slot arrays with diameter of 32 µm and an ultranarrow gap of only 2, 5 and 10 nm, periodically spaced with a 50 µm periodicity, as
shown in Figure 7(b). The fabrication of the samples was done using atomic layer lithography with a 150 nm thick gold film on a Pyrex glass substrate. The experimental characterization was done with a THz-TDS system from 0.1 to 1.0 THz. A field enhancement factor of 1250 inside the gap was estimated from the measurements of the 2 nm gap structure. The performance of the structure as a thin-film sensor was evaluated by depositing thin alumina overlayers on structures with a narrow gap varying from 2 to 10 nm. The thickness of the alumina was varied from 1 to 15 nm with steps of 1 nm and the transmission in each case was recorded, see the results in the top right panel of Figure 7(b). A redshift in the frequency response is clearly seen demonstrating that the structure is able to detect extremely thin alumina layers only 1 nm thick. In particular, a redshift of 5% was achieved when adding an ultrathin 1 nm thick layer. In addition, a structure with a larger gap of 1 µm was unable to detect thin layers, see the bottom right panel of Figure 7(b). As can be observed there, a hardly noticeable frequency shift appeared in the spectral response due to the weak electric field confinement. As an interesting complement to the experimental study, the behavior of the structure was numerically analyzed by applying an Hybridizable Discontinuous Galerkin method. This method solves the limitations found in traditional approaches, which usually fail in modelling properly structures with large differences in length scales, which in nanogap devices reaches up to 6 orders of magnitude. With this method, the authors found that the field in the 10 nm gap structure was enhanced by a factor of 3 with respect to the case of the 1 µm gap structure.

In summary, new manufacturing techniques amongst which the atomic layer lithography stands out, allow the design of increasingly complex structures based on nanogaps, which translates into the possibility of detecting ultrathin films with excellent sensitivity values and field confinement. This opens the door to designs capable of detecting materials with atomically thin thicknesses, such as graphene, or some biological samples.

6. Conclusions
To conclude, in this review a comprehensive summary of the origins as well as the latest advances of metasurface sensors working in the THz band has been presented, with the aim to provide the reader with an up-to-date compendium of this exciting and emergent topic. The main emphasis has been put first on thin-film sensors due to their historical and technological relevance. As a general rule, the initial works relied on classical metaatoms that evolved from the metamaterials research. Gradually, an evolution towards more complex structures was produced. In this respect, it seems remarkable the use of Fano and extraordinary transmission resonances as well as the more recent metageometries paradigm.

Also relevant is the research related with biosensors, as metasurfaces are crucial to detect small microorganisms and substances impossible to detect with traditional methods. Several topic have been covered in this review, starting with biomolecule sensing, followed by microorganisms sensing and cancer and tumor detection. Finally, the most recent manufacturing techniques, amongst which atomic layer lithography stands out, hold the promise to extend the reach of metasurface sensors, achieving sensitivity values much higher than traditional solutions. Thus, a bright future can be foreseen for the topic which started hardly a decade ago and that has grown nowadays to a stage of relative maturity.

**Acknowledgements**

(Acknowledgements, general annotations, funding. Other references to the title/authors can also appear here, such as “Author 1 and Author 2 contributed equally to this work.”)
References


Figure 1. (a) Different cross sections of the square SRRs fabricated in \[44\] showing the electric field confinement (top) and transmission response of the structure for different Si nanospheres concentrations (bottom). Reprinted from \[44\] with the permission of AIP Publishing. (b) Frequency shift obtained when coating an asymmetric double split ring element with a dielectric material with permittivity $\varepsilon = 3.2$. Differences between covering the whole surface (top) and covering a partial area at the position of maximum $E$-field concentration (bottom). Reprinted from \[45\] with the permission of AIP Publishing.
Figure 2. (a) Schematics of planar SRRs fabricated in\textsuperscript{47} on a thin silicon nitride substrate (top left) and on a bulk silicon substrate (top right). Measured transmission spectra of both structures for different silk fibroin analyte thicknesses (bottom). Reprinted from\textsuperscript{47} with the permission of AIP Publishing. (b) Schematic of the cross shaped absorber (top left) and its
complementary cross shaped absorber design (top left) fabricated in [51] and corresponding amplitude reflection spectra (bottom) of the bare sample (black) and the coated sample with an 11 µm thick photoresist (red). Reprinted from [51] with the permission of AIP Publishing.

(c) Different types of asymmetric square SRRs used for exciting Fano resonances: SRR used in [53] (left); toroidal resonator used in [55] (center); SRR used in [56] (right) The dashed line indicates the symmetry axis of the square. The red lines in the center panel indicates the surface currents in the metasurface. (d) Schematic of the unit cell reported in [54] with relevant dimensions: $d = 200 \, \mu m$; cross length, $L = 175 \, \mu m$; strip width, $w = 57 \, \mu m$; polypropylene substrate height, $h_{pp} = 41 \, \mu m$; analyte thickness $h_a$ (µm) (left). Photograph of the fabricated prototype (center). Simulated (solid line) transmission spectra obtained for oblique incidence (TM polarization) for the bare structure (black line) and different analyte thicknesses (right). Reprinted from [54] with the permission of AIP Publishing. (e) Simulated transmission coefficient spectra for the labyrinth metasurface absorber designed in [59] for different analyte thicknesses (left). Front view and cross section of the unit cell with relevant parameters (center). Wavelength shift as a function of the analyte thickness for extremely thin analytes, simulation (red) and measurements (blue), calculated as $\Delta \lambda = \lambda_a - \lambda_0$, with $\lambda_a$ the resonance wavelength at each $h_a$ and $\lambda_0$ the resonance wavelength without the analyte (right). Reprinted from [59] with the permission of 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
Figure 3. (a) Schematic of the hole array structure presented in\cite{70} and unit cell detail with the different polarizations that can be used in order to excite either the regular (left) or the anomalous extraordinary transmission (right). Reprinted from\cite{70} with the permission of AIP Publishing. (b) Transmission coefficient amplitude at the anomalous extraordinary transmission regime in the hole array metasurfaces designed in\cite{57} for two different substrate thicknesses: 75 μm (left), and 50 μm (right).under normal incidence for different analyte thicknesses. Simulated (top) and measured (bottom) results. The dashed lines highlight the regions where sensing based on frequency or amplitude shift id feasible. Reprinted from\cite{57} licensed under a Creative Commons Attribution 4.0 International License
Figure 4. (a) Absorption coefficients for different sugars grouped in different saccharide groups (left). Measured THz spectra with the D-glucose antenna presented in[75] for different glucose/sucrose concentrations (second-left column). Real photograph and normalized THz transmittance image of the nano antenna designed with a drop of fructose (upper-right corner) and D-glucose (lower-left corner) where the transmittance at the fructose area experiments a higher attenuation (second-right column). THz transmittance spectra (bottom-right) for different real market beverages containing different sugar concentrations (top-right).
(b) THz transmission spectra for different mouse tissues carried out with the spiral structure designed in[85] (left). Mouse sections photograph (top center) and x-ray image showing the tail and the location of the sections excised (bottom center). THz images obtained for the mouse tail sections shown in the center panel with a conventional mirror- based focusing setup (top-right panel) and with the designed spiral bull’s-eye (bottom right panel). In the image taken with the designed antenna, with much higher resolution, hair (yellow and red), skin (light blue), and bone (dark blue) tissues are clearly differentiated. Reprinted from[85] licensed under a Creative Commons Attribution 4.0 International License. (c) Schematic of the graphene metamaterial presented in[86] (top left) and reflection spectra of the structure with and without the graphene monolayer (bottom left). Measured spectra of the graphene metamaterial with and without methyl molecules (top center), and comparison with the same case of the metasurface without the graphene monolayer (bottom center). AFM images of a graphene-coated metamaterial with aptamer (top-right), where a change in the analyzed thickness can be observed and measured reflection spectra of the graphene-coated metamaterial with/without thrombin (bottom-right). Reprinted from[86] with permission from Elsevier.
Figure 5. (a) Schematic of the *E. coli* bacteria detector along with a microscope image of the metasurface proposed in [78](left). THz transmission amplitude before (blue) and after (red) the deposition of *E. coli* on the functionalized structure (center) and on the metasurface without functionalization (right). Reprinted from [78] licensed under a Creative Commons Attribution 4.0 International License. (b) Schematic of the nano-gap metasurface designed in [90] with a gap small enough to sense viruses (left). Normalized transmission amplitude of the metasurfaces with different gap size before and after coating the structure with PRD1 viruses at different concentrations (second-left and center). Frequency shift as a function of the gap size for different surface densities (second-right). Sensitivity as a function of the gap size (right). Reprinted from [90] with the permission of Biomedical Optics Express (open access document). (c) Fabrication process for the hybrid antenna presented in [91] (left). Comparison between the normalized transmission amplitudes through the bare structure (top center) and the hybrid antenna (bottom center) with (red) and without (black) the deposition.
of PRD1 viruses. Frequency shift as a function of the surface density of viruses for both
structures (right). As shown, the hybrid antenna offers much higher frequency shift. ).
Reprinted from[91] licensed under a Creative Commons Attribution 4.0 International License.
Figure 6. (a) Comparison between visible (top) and THz (bottom) images of different samples of skin tissue studied in[72]. The normal tissue is marked with a dashed boundary while the diseased tissue is marked by a solid boundary. As shown, the higher resolution of the THz image allows to discern more precisely the damaged areas, marked by squares named as $d_1$, $d_2$. Reprinted from[72] with permission from Elsevier. (b) Schematic of the unit cell as well as the geometric configuration of the metasurface sensor proposed in[98] with relevant dimensions: $p=50 \, \mu m$, $w=44 \, \mu m$, $t=25 \, \mu m$, $d=6 \, \mu m$, $g=4 \, \mu m$, $s=28 \, \mu m$ (left). THz transmission spectra of the designed biosensor under different cisplatin concentrations (center) and the same under different drug time action (right). Reprinted from[98] with permission from Elsevier.
Figure 7. (a) Schematic of the manufacturing procedure used to create the sub-10 nm gap SRR array used in\textsuperscript{[101]} along with a microscopic image of the fabricated structure (top). Measured (bottom left) and simulated (bottom right) transmission spectra for different gap...
sizes and field distribution at different cases. Reprinted (adapted) with permission from\textsuperscript{[101]} Copyright (2018) American Chemical Society. (b) Schematic of the annular gap array structure created with an Al\textsubscript{2}O\textsubscript{3} layer designed in\textsuperscript{[102]} as well as electric field distribution in the nanogap area of the unit cell (left). THz transmission amplitude for different gap sizes and different analyte thicknesses, from 1 to 15 nm (top-right). Measured THz amplitude for a gap size of 1 \textmu{}m with (red) and without (black) an Al\textsubscript{2}O\textsubscript{3} layer (bottom-right). Reprinted (adapted) with permission from\textsuperscript{[102]} Copyright (2015) American Chemical Society.
Table 1. Comparison of different values of sensitivity and FOM achieved in different works, calculated as $FS = \Delta f/(h_an_a)$, $AS = \Delta A/h_a$ and $FOM = FS/\text{FWHM}$. The FOM could not be obtained in some cases due to the impossibility of extracting the FWHM from the plots.

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Author Photograph(s) ((40 mm broad, 50 mm high, gray scale))

Miguel Beruete received his Ph.D. degree in telecommunication engineering from the Public University of Navarra (UPNA) in 2006. Currently, he is an Associate Professor in Antennas Group-TERALAB (UPNA), and head of the Multispectral Biosensing Group in Navarrabiomed. He has authored more than 130 JCR articles, 5 book chapters, over 250 conference communications (several invited), and holds 3 patents. His current research interests include terahertz sensing and communications using metamaterials, metasurfaces and plasmonics. He was a recipient of the Best Doctoral Thesis award (2006/07) from UPNA, three CST University Publication Awards (2005, 2012, and 2016), and several international conferences awards.

Irati Jáuregui López received her M. Sc. (Master of Science) in 2018 from the Public University of Navarra for which she received a third national prize for her final degree project. Since then, she has been working as researcher at the Antennas Group-TERALAB (UPNA) and the Multispectral Biosensing Group, Navarrabiomed. Currently she is a Ph. D candidate whose research focuses on the design of metasurfaces for sensing purposes.
This review covers the exciting topic of terahertz sensing using metasurfaces, using a historical as well as application-oriented perspective. Three main groups are covered: thin-film sensors, biosensors and the latest manufacturing techniques, able to achieve extreme sensitivity values. The compendium here presented should serve to frame this rapidly evolving topic with an updated as well as thorough historical view.

**Keyword** Metasurfaces

I. Jáuregui-López, M. Beruete*

**Title** Terahertz sensing based on metasurfaces

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