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Major Goals: 1. Development of a novel THz spectroscopy unit for real time in-situ rapid and sensitive bio-sensing. The proposed THz spectroscopy system without moving time-delay parts can speed up the measuring and analyzing times for obtaining THz spectra of biological and chemical agents. The proposed THz spectroscopy system with a multiple reflection (White Cell) sample chamber can be used for detection of very low concentration biological and chemical agents because the White Cell can significantly increase the optical path of a THz beam through target agents and enhance the detection ability.
2. THz spectroscopy unit without moving time-delay parts and with a White Cell sample chamber will be used for real-time in-situ sensing of low concentrations of bacteria, spores, and other harmful chemical and biological materials for applications in battlefields, home land security and environmental protection.
3. To investigate and understand the THz spectroscopy of bio-molecules in bacteria under optical pumping by femtosecond pulses such as proteins (probing tryptophan)and water. The impact of the THz spectral kinetics allows for understanding the changes of molecular structure and dynamics associated with the vibrational, rotational, and torsional modes of bio-molecules in the bacteria during induced photodynamical and structural changes caused by UV and NIR radiation.

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RAPID AND SENSITIVE THz BIO-SENSING

PI – Robert Alfano, researchers Dr Pavel Shumyatsky and Wells Crandall

THz-Pulse Grating Time-Delay Method

Abstract

This is the final report on the progress achieved under ARO grant to develop a novel, compact Terahertz (THz) spectroscopy system with and without moving parts for real time, in-situ, rapid, and sensitive bio and condensed matter sensing. The proposed THz unit would increase the speed of measurements and improve analysis times to obtain THz spectra of biological and chemical agents as well as analysis of the absorption and refraction indices of those agents.

We focused on investigating the actual THz-spectrometer with and without a grating time delay unit. The grating, by its nature, creates a natural time delay. We included a grating in a real THz device and investigated the parameters of THz pulse time delayed by the grating.

After that, with the grating included in the real THz device we investigated THz spectra of real biological agents i.e. Alzheimer-Disease (AD) brain tissue and the Breast Cancer Cells (BCC). A paper was published on AD and Normal Brain and breast cells .

Method

The schematic drawing of setup for investigating the THz pulse/Grating time delay is shown in **Fig.1**. The IRMA femtosecond laser, previously used, was changed due to the lack of power to generate enough of a THz signal with the grating. A near infrared (NIR) Mira Seed-Rega Coherent Laser (λ=800nm) is used as the source of pump and probe radiation. The radiation is directed into the THz-Spectrometer [1] as shown at **Fig.1**. The output power of the Coherent NIR pump-probe laser was in the range of 600-700 mW average power, with 2 μj at 250 KHz repetition rate.

The output beam of this laser is divided by beam-splitter (BS) in two optical arms: 1. pump-beam and 2. probe-beam in 50/ 50 ratio. During the experiments the output power of laser radiation was reduced to $\leq$ 400mW, so in each of optical arms (pump and probe) was directed no more than 200mW to prevent damage of both the emitter and detector ZnTe-crystals. The pump beam is reflected by the BS at the emitter of the THz radiation, installed in the focus point of the first Parabolic Mirror. This Parabolic Mirror collects the THz-radiation and creates a parallel THz-beam between both of the Parabolic Mirrors. The second Parabolic Mirror focuses the THz-beam into the detector crystal. Between both of the Parabolic Mirrors are installed two THz lenses for concentrating the THz field inside small objects of interest. These lenses are fabricated from material transparent in THz-range. We used the lenses fabricated from Tsuruppical. This plastic material has high transparency in THz and visible spectral ranges and has the same index of refraction value in either range.

The probe beam goes through the BS, diffracted by the Grating and directed through the hole in the center of the second Parabolic Mirror into the ZnTe detector crystal. The Grating (N=600 1/mm) is installed at a normal position to the incident probe beam. The m=1 order of diffraction is used. The incident beam spot on the grating was limited by an aperture in the range 1-4mm. The diffracted beam by the Grating has high angle divergence and an elliptical geometric form of the beam-spot. This spot looks like a strip around 2cm long at a distance (~70cm) up to the input in the parabolic mirror hole for the case of the Aperture Dia=2mm. Two lenses were used for focusing this diverging beam: the cylindrical long focus (30cm) lens and the spherical long focus (60cm) lens. The focused beam spots on the surface of the detector-crystal were around 2mm and 3mm for each Aperture Diameter of 2mm and 3mm, correspondingly. The mirrors and optical lenses are used to make the adjustment for the pump and probe beams overlapping inside the detector-crystal. The CCD-camera was used for the best control of overlapping the pump and probe beam spots.

The ZnTe-detector works as an optical Kerr gate [2]. This gate is opened when THz and optical probe beams and pulses coincide in time and space. The gated beam goes through a λ/4-plate, a Wollaston prism and enters in to a balanced detector (BD). The BD is connected via a Lock-in Amplifier with a PC.

Photographs of our experimental device are shown in Fig. 2 and Fig. 3. Fig. 2 shows the main part of pump arm of the setup. Fig. 3 shows the probe arm and THz-space of this setup.

The experimental results are shown on Figs. 4 to 6.

However, the value of THz signal in the new THz-Grating spectrometer (set-up without moving parts) is weaker at 20 to 50 times in comparison with the THz-spectrometer with regular set-up with moving time–delay part.

Our experiments using new THz-Grating spectrometer showed that THz-pulse, which passed through the biological object is so small, that output S/N ratio is around ~1. Because of this, our next measurements of the absorption spectra of Tryptophan in biological tissue in (0.2-2.5) THz-range were done by using regular set-up with moving time–delay part.

During the experiments for investigation of THz spectra of real biological agents we measured the THz-spectra of Alzheimer-Disease (AD) brain tissue and the Brest Cancer Cells (BCC) using time delay method..

The setup of the experiments is shown schematically in Fig.7. The Ti-Sph femtosecond pulse laser (1) is used as the pump generator for THz-source. The output linear polarized light of the pump laser is divided by the beam splitter(4) into two parts, so that the higher portion (pump optical arm- 70%) of the pump beam power goes into THz-wave emitter (6) on the bases Zn-Te(110) nonlinear crystal, that have the thickness 2mm. The lower portion (30%) of the beam goes into receiver optical arm. The pump beam is focused into emitter-crystal by means short focus lens (5). The emitted by the crystal THz-waves are collimated by the parabolic mirror (7) and are focused into a sample (9) by means the THz-lens(8) manufactured from TPX-material. The THz-wave, refracted and partially absorbed by a sample, collimated by the second THz-lens (8) and focused by second parabolic mirror (10) on the Zn-Te detector. This Zn-Te crystal is the same (2mm thickness, 110-orientation) as emitter. Both of the Zn-Te crystals (emitter and detector) are placed at the focal points of offset parabolic mirrors (7, 10). The polarization of infrared (800nm) pump beam is parallel to the ZnTe-emitter crystal (110) direction. The polarization of emitted THz-wave is the same as polarization of pump beam and Zn-Te-detector crystal orientation.

The pump optical arm and receiver optical arm must have the same optical length. For precise equalizing of both of arm lengths are used moving back-reflectors (prism-mirrors) installed on the translated stages (2, 12). The receiver optical arm beam has commonly used name probe beam, and described method has commonly used name pump-probe. The probe beam focused by long-focus lens (11) goes through the hole in the center of parabolic mirror (10) collinearly to the THz-beam and shots on the detector crystal at the same point as the focused THz-beam.

The THz-source-detection is based on the method of pump-probe electro-optical detection of a THz-signal [3]. The THz-pulse transmitted through a sample goes into nonlinear detector crystal, which has a high birefringence. The electric field component of the THz-pulse modulates the amplitude of the birefringence. The infrared probe beam pulse going through the detector crystal changes own polarization (from linear to elliptical) synchronously to variation of amplitude of birefringence modulation, other words synchronously to variation of intensity of electric field component of THz-pulse. This change in polarization of infrared probe beam is analyzed by the $\lambda/4$-waveplate (14) and the Wollaston prism (15). Owing to change of polarization of probe beam takes place a changing of intensity of two infrared beams going from Wollaston prism into Balanced detector (16). The THz-signal appears as a derivative curve at the output of Balance detector, goes into Lock-in-amplifier and analyzed by PC.

Results and Discussion

Fig.4 shows the regular THz pulse with moving mirror time delay [1]. In this case, the grating (Fig.1) was replaced by a regular mirror. Fig.4 is shown for comparing the parameters of the regular THz pulse, obtained by using mirror moving time delay device, with the Grating delayed pulse, obtained by using the grating, which were recorded in the same experimental conditions.

Fig.5 shows the THz pulse obtained by the grating time delayed device, in other words without a moving mirror. The aperture diameter was 2 mm. The measured value of grating time delay ($\delta\tau$ measured) is equal to 3.6 ps. The calculated value ($\delta\tau$ calc) is defined from equation (1), for m=1, λ=800nm, D=2mm (aperture), N=1/d = 600 1/mm: $\delta\tau_{calc}$ = 3.2 ps.

We can see from the curve traces that both of these values match very well.

Fig.6 shows the THz pulse obtained by the grating time delayed device without moving mirror parts. The aperture diameter is 3.2 mm. The measured value of grating time delay ($\delta\tau$ measured) is equal to 5.2 ps. This value match very well with the calculated value $\delta\tau_{calculated}$ = 5.16 ps.

We can compare the values of THz signals showed in Figs. 5 to 6 with the curve of the THz signal showed in Fig.4. In Fig. 5 THz signal is weaker 20 times and in Fig.6 signal is weaker 50 times in comparison with the Fig. 4 signal. For larger apertures (close to 4 mm), THz signal reduction is even more significant.

The result of the measurements, shown in Figs. 5 and 6, demonstrates for the first time experimental evidence of the THz-pulse / Grating Time delay effect. The out come of the reseach was the measurement of AD brain and Breast cells using

moving THZ parts. The laser system crashed and we could put it back into operation until Dec 2019 with purchase of new Monaco Coherent fs laser,

The Overview of the project :

- We completed the THz-spectroscopic device for our experiments which is shown schematically in Fig.1 and in the photographs in Figs.2 and 3.
- We measured the real THz pulse/ Grating time delay without the moving mirror part, shown in Figs.5-6. The measured values of the Grating time delay ($\delta\tau_{measured}$) coincide very well with the calculated values ($\delta\tau_{calculated}$).
- We demonstrate results of our experiments in Figs.5 and 6. It is the First Experimental Evidence of the THz-pulse / Grating Time Delay Effect. Such evidence of the effect supports possibility to create the THz-Grating unit with White Cell – a spectrometer without moving parts.

  Based on these experimental results we were planning to make measuremets to produce a more advanced THz / Grating-spectroscopic device with a longer time delay and include the 2D-CCD and White Cell. The setup for this advanced THz-device is shown schematically on Fig.7 , The laser crashed so we could not completed this work.

We did measured the absorption and refraction of mouse brain tissue for AD-tissue and Normal (N) tissue samples. The slices of tissue have thickness 150 µ and were installed on the TPX-film substrate. This film substrate has the thickness 250 µ. The THz-wave absorption in AD and N tissue samples were compared with the absorption in the pure film-substrate (without tissue) samples at the same experimental conditions.

The THz-box was filled by the Nitrogen (Fig.7) for eliminating of water vapor absorption in the THz-spectra of the samples.

The Results of these bio -Experiment

The results of our measurements of THz-radiation power transmitted through the AD-tissue and N-tissue samples in comparing with substrate (TPX) samples, as function of frequency in the range 0.5-2.5 THz, are shown on Figs. 8 and 9. The Fig. 8 shows the scans of THz-pulse signals vs time-delay in the range 0-6 ps. The Fig. 9 shows the power transmitted through the AD-tissue and N- tissue. Both of curves show the decrease of power transmitted through the tissue samples, in compare with substrate samples, in the frequency range 1.4-2.2 THz. The calculated index of absorption (cm-1) is shown in Fig.10. The figure curve shows three maximums of absorption in AD-samples (1.4, 1.8 and 2.1 THz) that well coincide with the Tryptophan torsional-vibrational modes (1.435, 1.842 and 2.114 THz). In the Fig. 11 are shown the calculated (on the bases of our experimental dates) values for refraction index (n) for AD and N-tissues. We can see the difference in the refractive Indexes for AD-tissues and N-tissue.

As the result we concluded, that absorption (cm-1) spectrum and refraction index (n) of AD-tissue at the THz-modes 1.44, 1.8 and 2.11 THz can be used as the fingerprint for diagnosis Alzheimer disease tissue.

1. Terahertz Absorption Spectrum and Refractive Indexes of Brest Cancer Cells

In this part of the report we will describe our new experiments for measurements of Tryptophan level in the Brest Cancer Cells (BCC) using THz-waves. The same TTDS method was used in our measurements of Tryptophan level at THz-frequency modes. We measured the absorption spectra of Tryptophan in (0.2-2.5) THz-range. The new results for the level of absorption frequency modes and refraction indexes for BCC are presented below. These results show three dominating torsional-vibrational modes at around 1.4, 1.8 and 2.1 THz. These three THz-absorption modes could be used as BCC-fingerprint.

Experimental Method

The setup of experiments is shown schematically in Fig.7. The explanation of the method is the same as above. The only difference was that we used the BCC deposited on the quartz plate substrates.

The results of our measurements of THz-radiation power transmitted through the BCC and Healthy (Normal) Cells samples in comparing with pure substrate (quartz plates without cells) samples, as function of frequency in the range 0.5-2.5 THz, are shown in Figs. 12, 13 and 14. The Fig.12 shows the power transmitted through Cancer and Healthy Cells and through the quartz substrate for the reference. These curves show the decrease of power transmitted through the BCC samples, in compare with substrate without cells samples, in the frequency range 1.4-2.2 THz. The calculated values (on the bases of our experimental dates) for absorption index (cm-1) and refraction index (n) are shown in Fig.13 and Fig.14. The curves of these figures show the rise of absorption index in the tissue samples in the same range 1.4-2.2 THz. We can see three maximums of absorption in BCC-samples (1.4, 1.8 and 2.1 THz) that well coincide with the Tryptophan torsional-vibrational modes (1.435, 1.842 and 2.114 THz). The curves of the Fig.14 shows the big difference between the n-values for both of tissue-samples .

As the result we can conclude, that absorption (cm-1) spectrum and refraction index (n) of the BCC in the THz-range(1.44-2.11 THz) can be used as the fingerprint for diagnosis of the Brest Cancer Cells.

This research work was performed by:

- Dr. Pavel Shumyatsky (all parts of the work), project leader;
- Richard Crandall, graduate physics student (cooling system of pump laser (800nm), adjustment of this laser, PC software).

The authors are grateful to Mr.Yury Budansky for help building the apparatus and to Dr. V.Kartazaev for help maintaining Mira Seed-Rega Coherent Laser system.

References:

1. Pavel Shumyatsky and Robert R. Alfano, “Terahertz sources”, Journal of Biomedical Optics, v.16(3), 033001-1-9 (2011).
2. P.P.Ho and R.R.Alfano, “Optical Kerr effect in liquids”, Phys.Rev.A”, v.20, 2170-2187, 1979.

3. B.Yu, F.Zeng, Y.Yang, Q.Xing, A.Chechin, X.Xin, I.Zeylikovich, and R.R.Alfano, “Torsional vibrational modes of Tryptophan studied by Terahertz Time-Domain Spectroscopy”, Biophis.J.,86,1649-1654, (2004).
4. Lingyan Shi, P.Shumyatsky, A.Rodriguez-Contreras and R.Alfano, “Terahertz spectroscopy of brain tissue from a mouse model of Alzheimer`s disease”, JBO, 21(1), (2016).

Publications :

1. Pavel Shumyatsky and Robert R. Alfano, “Terahertz sources”, Journal of Biomedical Optics, v.16(3), 033001-1-9 (2011).
2. Lingyan Shi, P.Shumyatsky, A.Rodriguez-Contreras and R.Alfano, “Terahertz spectroscopy of brain tissue from a mouse model of Alzheimer`s disease”, JBO, 21(1), (2016).

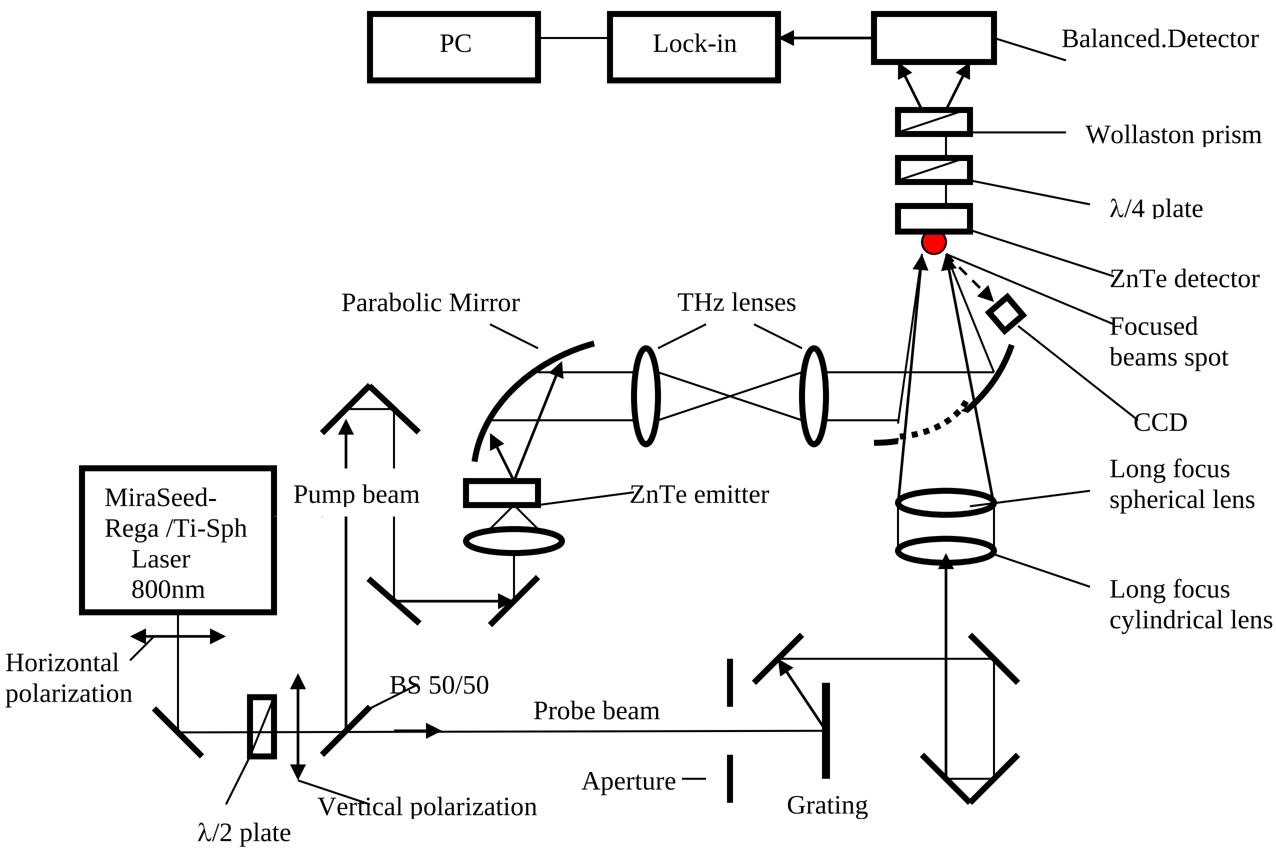


Fig.1

The setup of the experiment for THz-pulse / Grating time-delay

Fig.2

Picture of the pump-arm / 800nm laser of Setup

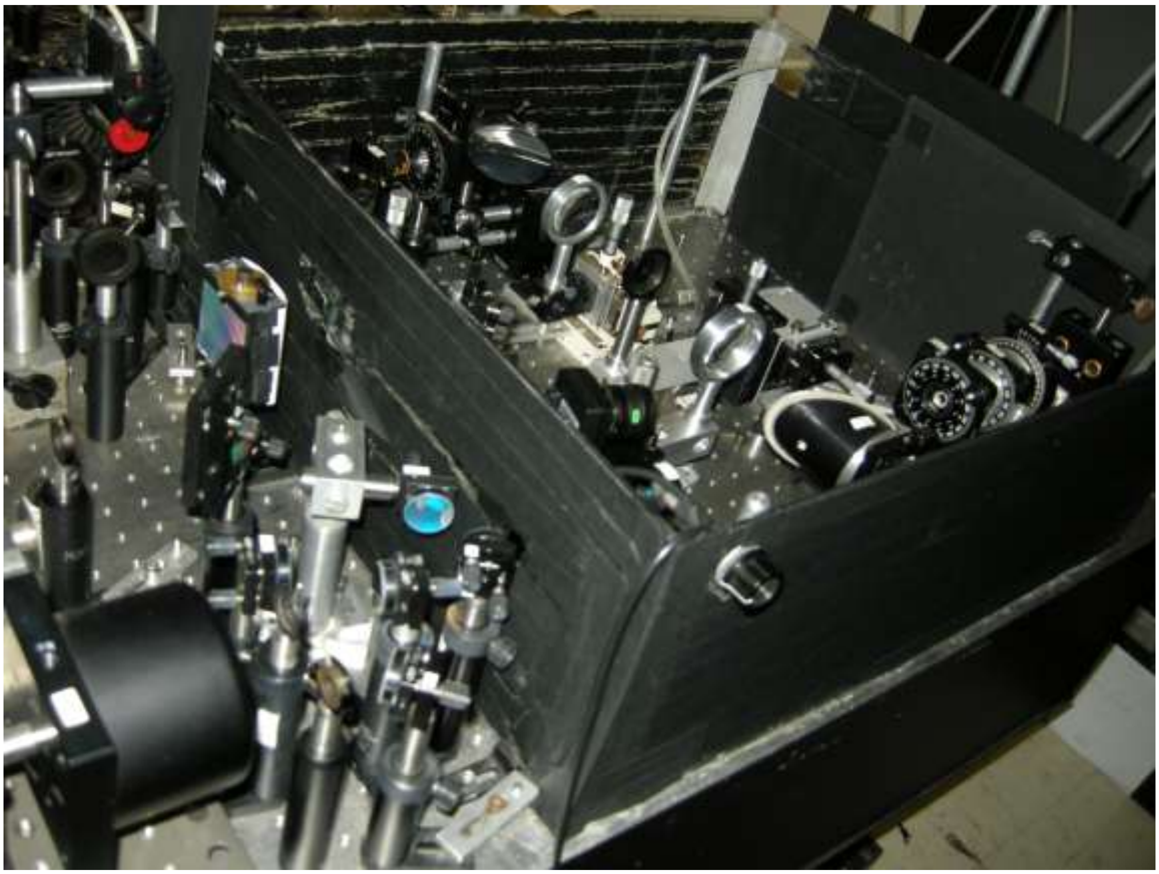


Fig.3

Picture of the probe-arm / THz-space of Setup

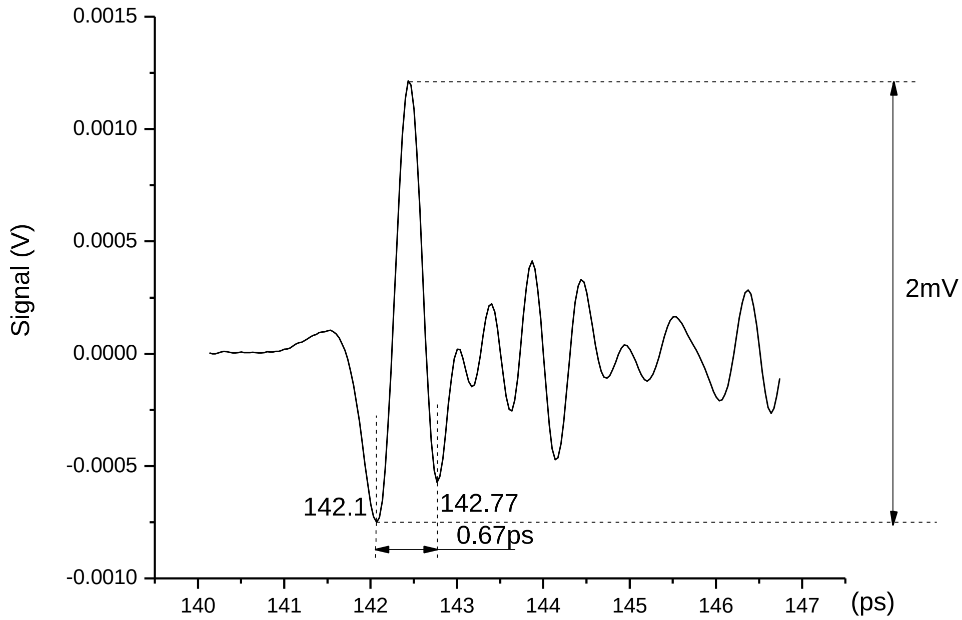


Fig.4

THz pulse e obtained by the regular THz -device with moving mirror time delay.

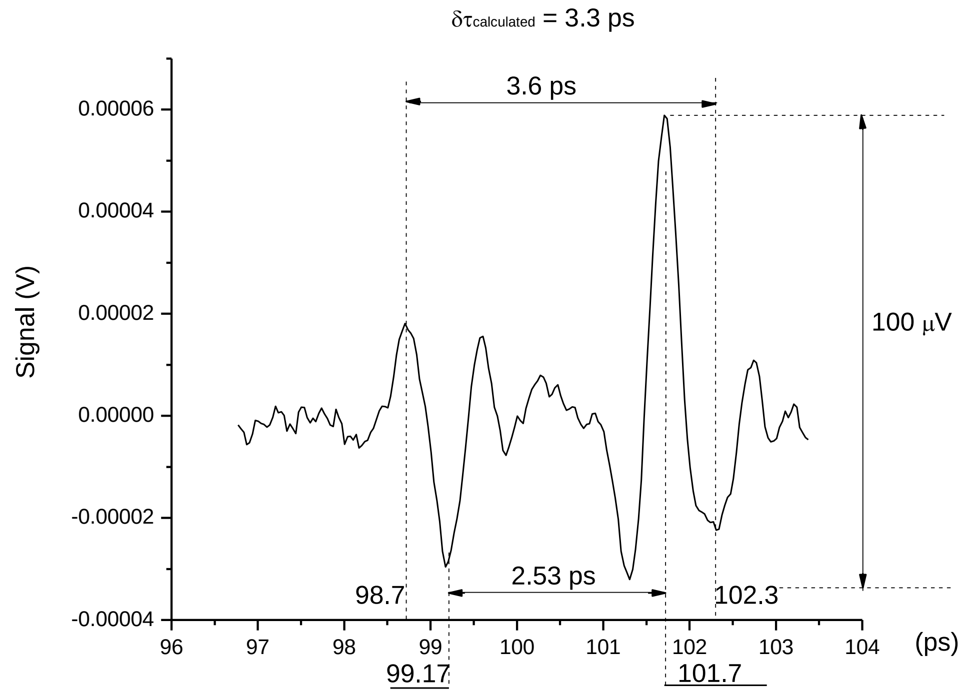


Fig.5

THz-pulse obtained by the grating time delay THz device.

Aperture Diameter = 2 mm
δτcalculated = 3.3 ps
δτmeasured = 3.6 ps

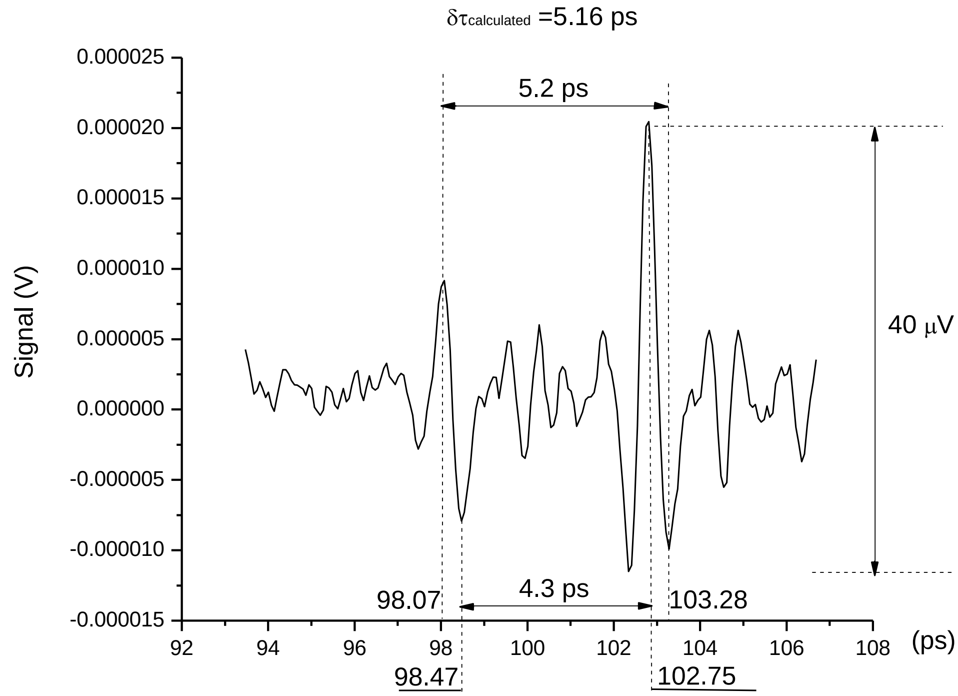


Fig.6

THz pulse obtained by the Grating time delay THz device.

Aperture Diameter = 3.2 mm
δτcalculated = 5.16 ps
δτmeasured = 5.2 ps

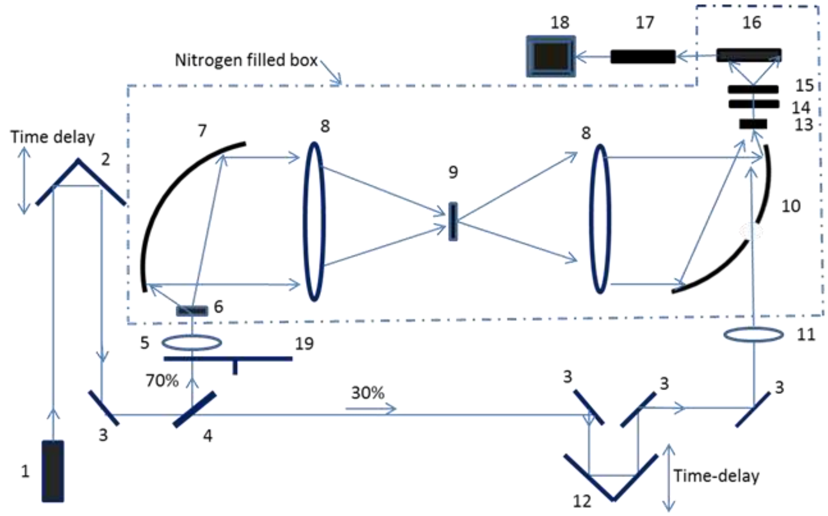


Fig.7

Experimental setup of the experiments performed on Alzheimer-Disease (AD) brain tissue and the Brest Cancer Cells (BCC). No grating scanning delay time type
1. Ti-Sph Laser (800nm) 2. Translated-stage prism-mirror 3. Mirror 4. Beam-splitter(T/R = 30%/70%) 5. Short-focus lens 6. THz(Zn-Te) emitter 7. Parabolic mirror 8. THz-lens 9. Sample 10. Parabolic mirror with the hole 11. Long focus lens 12. Soft-driven translated-stage prism-mirror 13. Zn-Te detector. 14.λ/4-waveplate 15. Wollaston-prism 16. Balanced detector 17. Lock-in-Amplifier 18. PC 19. Chopper

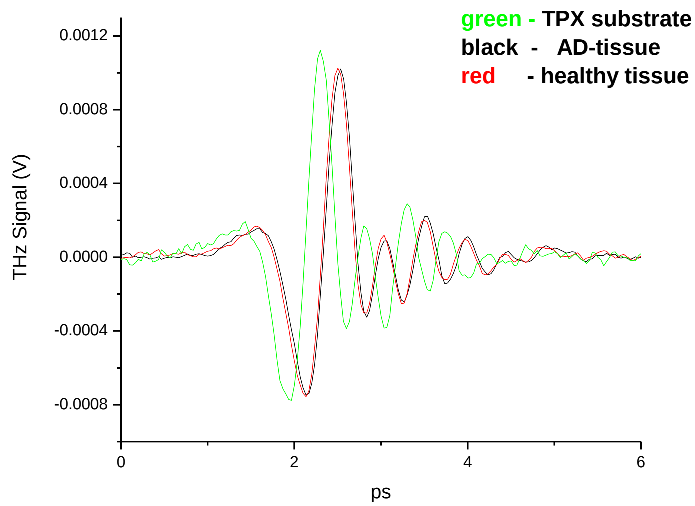


Fig. 8

Scan of the THz-pulse signal passing through the sample:
Green-pulse passed through the substrate without tissue;
Black-pulse passed through the substrate with AD-tissue;
Red- pulse passed through the substrate with healthy-tissue.

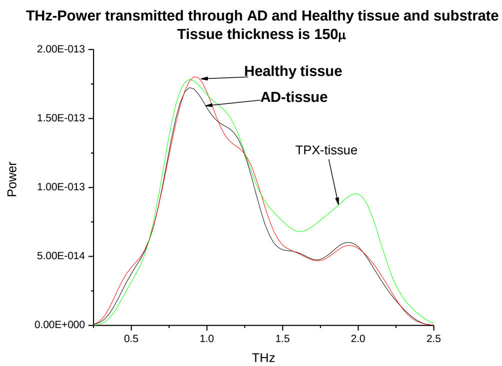

THz-Power transmitted through AD and Healthy tissue and substrate
Tissue thickness is 150µ
Healthy tissue
AD-tissue
TPX-tissue
Power
2.00E-013
1.50E-013
1.00E-013
5.00E-014
0.00E+000
0.5
1.0
1.5
2.0
2.5
THz

Fig. 9

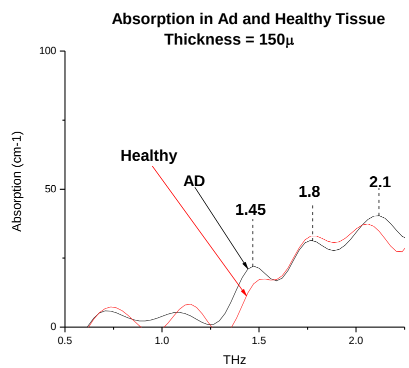

Absorption in Ad and Healthy Tissue
Thickness = 150µ
Absorption (cm-1)
100
50
0
Healthy
AD
1.45
1.8
2.1
0.5
1.0
1.5
2.0
THz

Fig. 10

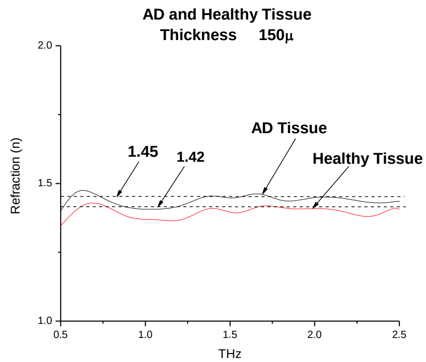

AD and Healthy Tissue
Thickness 150µ
Refraction (n)
THz
AD Tissue
Healthy Tissue
1.45
1.42
2.0
1.5
1.0
0.5
1.0
1.5
2.0
2.5

Fig. 11

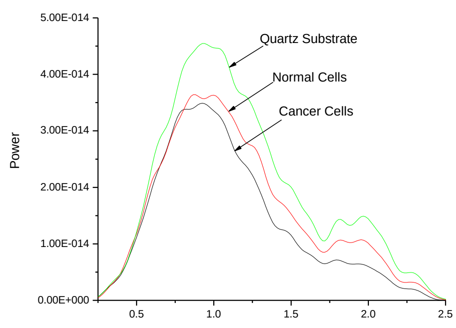
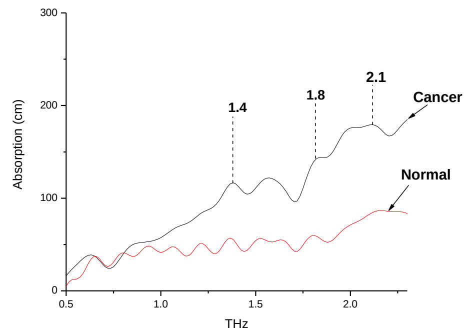
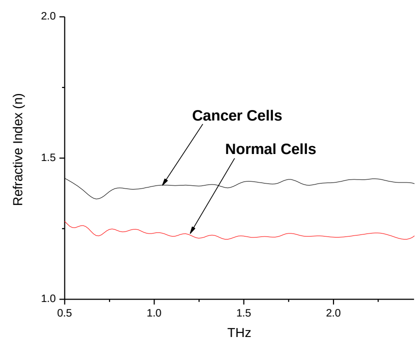

Power
THz
Quartz Substrate
Normal Cells
Cancer Cells
5.00E-014
4.00E-014
3.00E-014
2.00E-014
1.00E-014
0.00E+000
0.5
1.0
1.5
2.0
2.5

Fig. 12



300
200
100
0
0.5
1.0
1.5
2.0
Absorption (cm)
THz
1.4
1.8
2.1
Cancer
Normal

Fig. 13



2.0
1.5
1.0
0.5
1.0
1.5
2.0
Refractive Index (n)
THz
Cancer Cells
Normal Cells

Fig. 14