

Project Summary

Section 1: PROJECT IDENTIFICATION		NOT CONFIDENTIAL
Title of the project: <i>Tera-Hertz radiation in Biological Research, Investigations on Diagnostics and study on potential Genotoxic Effects</i>		
Acronym of the project: THz-BRIDGE		
Type of contract	RTD	Total project cost (in euro) 2.009.573 €
Contract number QLK4-CT-2000-00129	Duration (in months) 36 Months	EU contribution (in euro) 1.411.345 €
Commencement date 1 February 2001	Period covered by the progress report 1 February 2001 – 31 January 2004	
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Key words (5 maximum - Please include specific keywords that best describe the project.). Terahertz, spectroscopy, interaction, effects, safety		
World wide web address (the project's www address) www.frascati.enea.it/THz-BRIDGE		
List of participants (see next page)		

List of participants

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Objectives:

Recent technological break-through in the field of Terahertz radiation (T-rays) is triggering new applications in biology and biomedicine. Particularly, biological applications are based on the specific spectroscopic fingerprints of biological matter in this spectral region. The aim of this research project is to investigate the interaction of Terahertz (THz) radiation with biological systems following a streamline of increasing complexity, from bio-molecules to cells. The objectives of the project are:

- To provide a spectroscopic database for selected enzymes, proteins, biological membranes and cells in the frequency range from 100 GHz to 20 THz (the so-called "THz gap") under irradiation conditions that preserve the integrity and functionality of the biological samples.
- To identify, from the above data, critical frequencies, which might induce damages on biological systems, and to determine the spectral regions for optimal contrast in imaging applications.
- To assess potential risks due to the exposure of membranes, cells, and DNA to pulsed and CW THz radiation and to define safe exposure standards for THz biomedical imaging applications.

Results:

The spectroscopic activity carried out by the various groups in the project has yielded a systematic investigation of a variety of materials and biological systems. It has been found that polystyrene, a commercial plastic-ware material widely used in biology, exhibits excellent optical properties in the wavelength range between 200 μm and 3 mm (frequency range 1.5 THz to 100 GHz). Among the biological systems investigated, of particular relevance is a full characterization of amino-acids in the THz range. A concentrated effort has also been made to provide Time Domain (TD) spectra of thin samples of blood constituents compared to serum and saline solution. The spectral features of saline solution for different values of NaCl concentration have been investigated to clarify their role in the contrast mechanism in THz imaging applications. All spectral measurements performed during the project's life have been collected in a database and made available on-line to the scientific community. Further to this, the collaboration with a SME has permitted the development of infrared assays for the biomedical analysis of blood samples by mid-IR spectroscopy to determine blood glucose, cholesterol, urea, total protein, triglycerides and creatinine.

A full range of irradiation studies on DNA bases, human lymphocytes, membrane model systems and epithelial cell cultures has been carried out at the various partners' sites. The THz-induced biological end-points studied were alteration of membrane permeability of liposomes, induction of genotoxicity in lymphocytes as well as studying changes in cell activity, differentiation and barrier function in keratinocytes and neural cells. The results indicate that under various exposure conditions no biological effects could be detected. However, under some specific conditions of exposure, changes in membrane permeability of liposomes was detected and an induction of genotoxicity was observed to occur in lymphocytes. These studies suggest that medical imaging employing appropriate exposure parameters is most probably not harmful at least for single exposures. Since some effects were observed to be induced by the THz radiation at a relatively low intensity when compared to the limits set by the ICNIRP for exposure, these studies should be extended to establish more accurate dose-response relationships. This is expected to provide improved guidelines of exposure.

In particular, irradiation studies included the Micronucleus assay on human lymphocytes exposed in whole blood in the frequency band from 110 to 140 GHz. Incident power levels in the range 0.5 to 5 mW were extensively tested in different modulation conditions with an irradiation time of 20 min. The results obtained confirm the absence of both genotoxic effects and influence on cell proliferation under the adopted experimental conditions, as shown by comparing sham exposed cultures with exposed ones.. Attempts have been made to increase the exposure level by performing the Comet test on leukocytes separated from the aqueous part of the blood (serum) and directly exposed to THz radiation without the shielding effect of serum. However, the results of the Comet test on 12 healthy donors did not provide a clear answer, probably due to a lack of reproducibility in the exposure conditions.

As to human lymphocyte cultures, polystyrene culture flasks containing lymphocytes isolated from peripheral blood at 37 °C were exposed to 100 GHz radiation with different time duration at an average power density of 0.05 mW/cm² yielding an average SAR value of 3.2 mW/gr. The exposed cells were analyzed for changes in the level of aneuploidy (losses and gains of chromosomes), replication timing (early or late during s-phase) and asynchronous replication (coordination between the homologues) of centromeres of chromosomes 17 and 11. Both genotoxic and epigenetic effects were found in lymphocytes following exposure to CW 100 GHz radiation of 0.05 mW/cm² intensity when the exposure period exceeded one hour. Although the reported effects have been observed on cells directly exposed to THz radiation, without the shielding effect of the human body, they occurred at a relatively low intensity when compared to the exposure limits set by the ICNIRP guidelines (1mW/cm² for general public exposure and 5mW/cm² for occupational exposure)..

A database of the irradiation of epithelial cell cultures has been completed. For the exposure to THz radiation in the 0.1-10 THz range three different sources have been used. They were provided by Teraview Ltd, Cambridge, by at the Institute of Microwave and Photonics, University of Leeds, and by ENEA -Frascati. No changes compared with the unexposed controls, in terms of cell activity (Resazurin assay) or differentiation (Fluorescein Cadaverine assay), or barrier function in terms of the air liquid interface models (sodium fluorescein assay), was observed with any of the exposure rates employed, which exceed exposure times required to generate images in human patients by at least 5 times. It was also found that no damage in terms of cell activity, measured via oxidative stress, or differentiation was caused to ND7/23 cells or human keratinocytes.

A very simple membrane model, such as that provided by liposomes, has been used to study the permeability of a lipid bilayer in response to THz radiation in absence of interfering reactions. A systematic investigation on the effects of 130 GHz exposure of liposomes at different modulation conditions has been carried out. The results indicate that terahertz radiation can affect lipid bilayer permeability. An increase in substrate permeation rate across the liposome bilayer was observed over two minutes of 130 GHz irradiation with a pulse repetition rate of 7Hz and a delivered power of about 7.8 mW/cm². To verify the specific role of carrier frequency and pulse repetition rate in eliciting the THz-induced effect, additional experiments have been conducted changing the carrier frequency from 130 GHz to 3 GHz. Continuous wave (CW) irradiation was also performed at a carrier frequency of 150 GHz. No effects were observed in the two latter cases.

Results (cntd.)

The studies carried out within the THz-BRIDGE project have shown the importance of an interdisciplinary approach to tackle problems from different and complementary perspectives. The various scientific disciplines involved have also pointed out the necessity of carrying out a variety of biological and biomedical experiments using a variety of THz sources with different characteristics in terms of frequency, average and peak power, modulation conditions, which are usually not all easily available at the same site. The results of the project have been published or submitted for publication by the various research group in a number of papers

Finally, the response of a “Survey on the use of THz radiation” was analyzed by the project partners to collect information on the exposure conditions and main radiation parameters of THz sources (e.g. frequency range, power level, modulation), which are currently in use at various laboratories worldwide.

- 25 groups returned data on 34 different sources (37% of the responses came from the European Union; the remaining contributions came from the United States, Japan and Russia.).

Most of these groups are using terahertz radiation generated by short pulse solid-state lasers;

They are mostly interested in source development and biomedical applications;

Five groups expressed concerns of exposure to THz radiation.

- Mainly groups using Free Electron Lasers take precaution against THz radiation in the frame of more severe shielding requirements of the accelerators on which these sources are based.
- It appears currently that there are no records taken of occupational workers exposure to THz radiation.

Benefits and Beneficiaries:

The main objective of the THz-BRIDGE project is to provide a fundamental understanding of the interaction of THz radiation with biological systems. This is achieved by delivering a spectroscopic database and a timely risk assessment to the scientific community in view of the implementation of THz technology in the biological and biomedical field. In this respect the project has guided and accompanied the technological development of diagnostic devices in the above fields.

By providing spectroscopic databases on biological systems in the above frequency range the project has contributed to the EU policy of standardisation. By performing a basic risk assessment on the interaction of THz radiation with biological systems, the project has contributed to social objectives of the EU community such as safety requirements at specific occupational sites. A further contribution to the quality of life will derive from the development of biomedical imaging techniques based on THz radiation. Once the defined exposure standards are met, there is a great promise that such systems may provide sufficient information in diagnostics applications and may prove to be much less invasive than other commonly used techniques such as X-ray imaging. Biomedical applications will clearly also require adequate measurements to monitor the technical personnel involved and the future patient exposure.

Future Actions (if applicable):

The results of the THz-BRIDGE project will be disseminated through the Coordination Action EMF-NET funded under the Priority 8 of FP6, which gathers a number of projects on the interaction of EM fields with biological systems. EMF-NET should also help in providing indications to regulatory bodies.

The application of THz radiation and the realization of THz imaging systems is a rather new technology with a great potential for development in the coming years. Adequate training of young researchers in this field will be an incentive for the creation of new jobs in the Community. On the basis of the experience gained with the THz-BRIDGE project, plans are in progress to establish an interdisciplinary European New Research Infrastructure called THz-BEAM and based in Italy, specifically devoted to the development of THz sources and their application in biomedicine, material studies, security and telecommunication. Means will be provided to appoint young researchers at this site.