Biological applications of THz radiation: A review of events and a glance to the future

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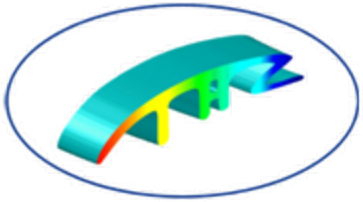
ENEA

Frascati, Italy

Journée Scientifique SFRP-RNI - Paris, 25 Janvier 2011

Outline

- Background: the THz-BRIDGE project
- Short electromagnetic vs. short electric pulses: "is a unifying view possible?"
- THz-Bio interaction: a review of events
- Some statistical data
- Recent work and trends in biomed applications
- Can the THz interaction with bio-systems play a role in other fields?
- Interdisciplinary applications, needs and priorities

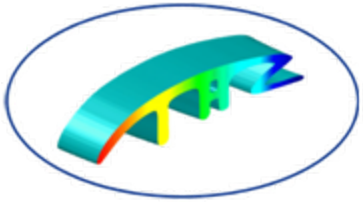


Background: the THz-BRIDGE project (2001 -2004)

FP5 – Quality of Life – Key Action 4 Environment & Health

" Tera-Hertz radiation in Biological Research, Investigation on Diagnostics and study on potential Genotoxic Effects "

- **To provide a spectroscopic database for selected biomolecules, biological membranes and cells** in the frequency range from 100 GHz to 20 THz under irradiation conditions that preserve the integrity and functionality of the biological samples.
- **To assess potential risks due to the exposure of membranes, cells, and DNA to pulsed and CW THz radiation** and to define exposure standards for THz biomedical imaging applications.
- **To monitor and recommend THz exposure conditions at specific occupational sites (survey on the use of THz radiation).**



www.frascati.enea.it/THz-BRIDGE

EMF-NET (2004 -2008)
THz Working Group

THz-BRIDGE results & further updates presented at

ICNIRP International Workshop on EMF Dosimetry and Biophysical Aspects Relevant to Setting Exposure Guidelines
20-22 March 2006, Berlin, Germany

"Terahertz radiation in biological and environmental studies"
LALS2007 - International Conference on Laser Applications in Life Sciences
June 11-14, 2007, Moscow, Russia

Several citations in international journals, two more papers on THz exposure of human lymphocytes and one on CA-loaded liposomes published after THz-BRIDGE end:

- ✓ "Terahertz radiation increases genomic instability in human lymphocytes", Radiat 844 Res, 170(2), 224-34 (2008)

  Korenstein-Ilan, A., Barbul, A., Hasin, P., Eliran, A., Gover, A. and Korenstein, R.

- ✓ "Cytogenetic Observations in human peripheral blood leukocytes following in vitro exposure to THz radiation: A pilot study" Health Phys. 92(4) 349-357 (2007)

  O. Zeni, G.P. Gallerano, A. Perrotta, M. Romanò, A. Sannino, M. Sarti, M. D'Arienzo, A. Doria, E.Giovenale, A. Lai, G. Messina and M.R. Scarfì

- ✓ "Permeability changes of cationic liposomes loading carbonic anhydrase induced by 130 GHz pulsed radiation" – Bioelectromagnetics 8, 501-508 (2007)

  A. Ramundo-Orlando, G.P. Gallerano, P. Stano, A. Doria, E. Giovenale, G. Messina, M. D'Arienzo, I. Spassovsky

THz-BRIDGE
Evaluation of Biological Effects In Vitro
summary of results

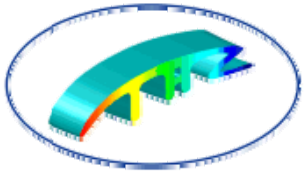
Whole blood exposure at 130 GHz– effects on human lymphocytes:

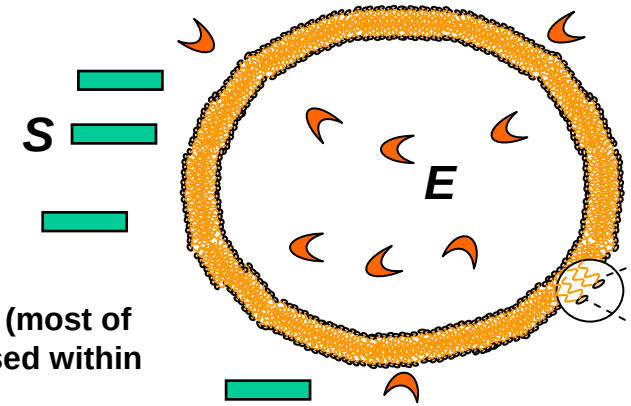
Absence of both genotoxic effects and influence on cell proliferation in the adopted experimental conditions, with 20 minutes exposure of whole blood samples at an average intensity in the range from 0.05 to 0.25 mW/cm^2 (SAR 0.4 to 2 mW/g)

Some genotoxic and epigenetic effects observed in lymphocytes cell cultures following 1 hour CW 100 GHz irradiation at an average intensity of 0.05 mW/cm^2

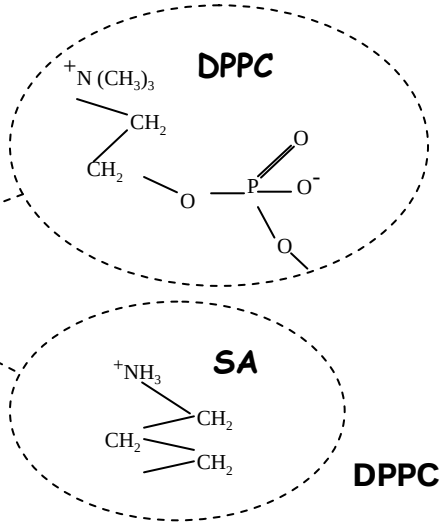
No evidence that the 100 GHz and 3 THz radiation, at more than 3 times the intensity to which human skin is exposed with commercial imaging systems, has any significant effects on human keratinocyte cells growth, differentiation, or cell adhesion capacity, in the presence or absence of neural cells, submerged or at the air liquid interface.

Liposomes as a simple model of the cell membrane





CA-loaded liposome (most of the enzyme is enclosed within the lipidic bilayer)



DPPC and SA polar head groups

- If the membrane permeability is altered by any external means, the substrate *S* penetrates the liposome and interacts with the enzyme *E* creating a product of reaction P.

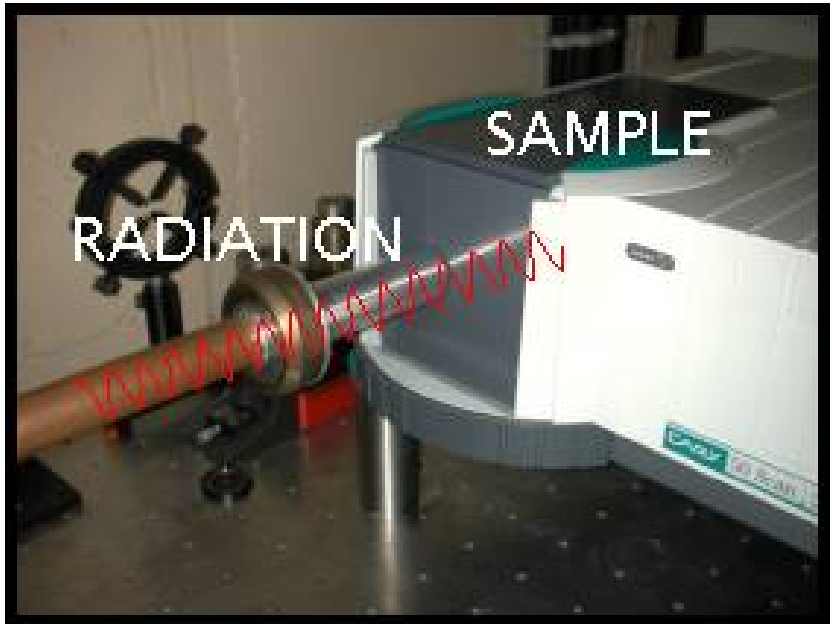
$$E + S \leftrightarrow P$$

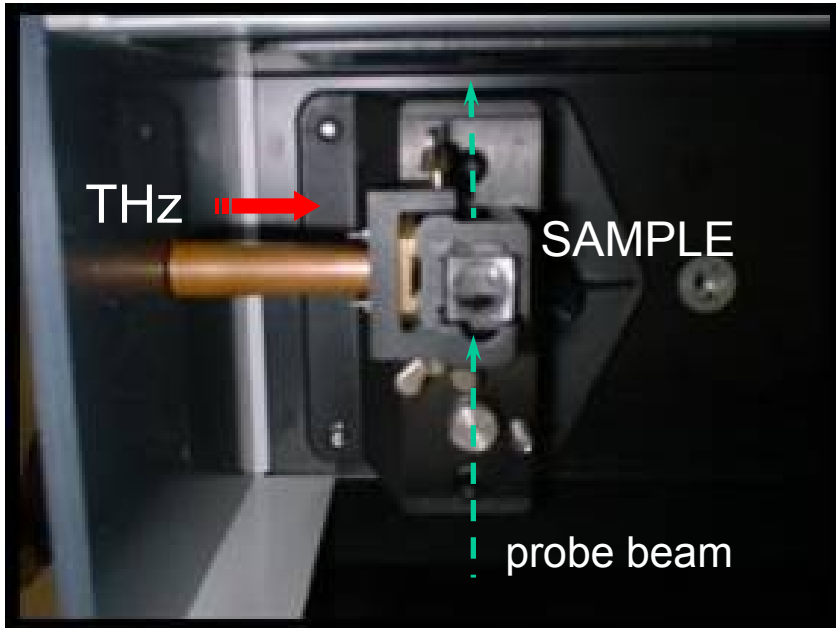
A UV spectrophotometer analyzes the absorbance of the product of reaction at λ= 400 nm as a function of time

THz exposure of CA-loaded liposomes

Bioelectromagnetics 8, 501-508 (2007)

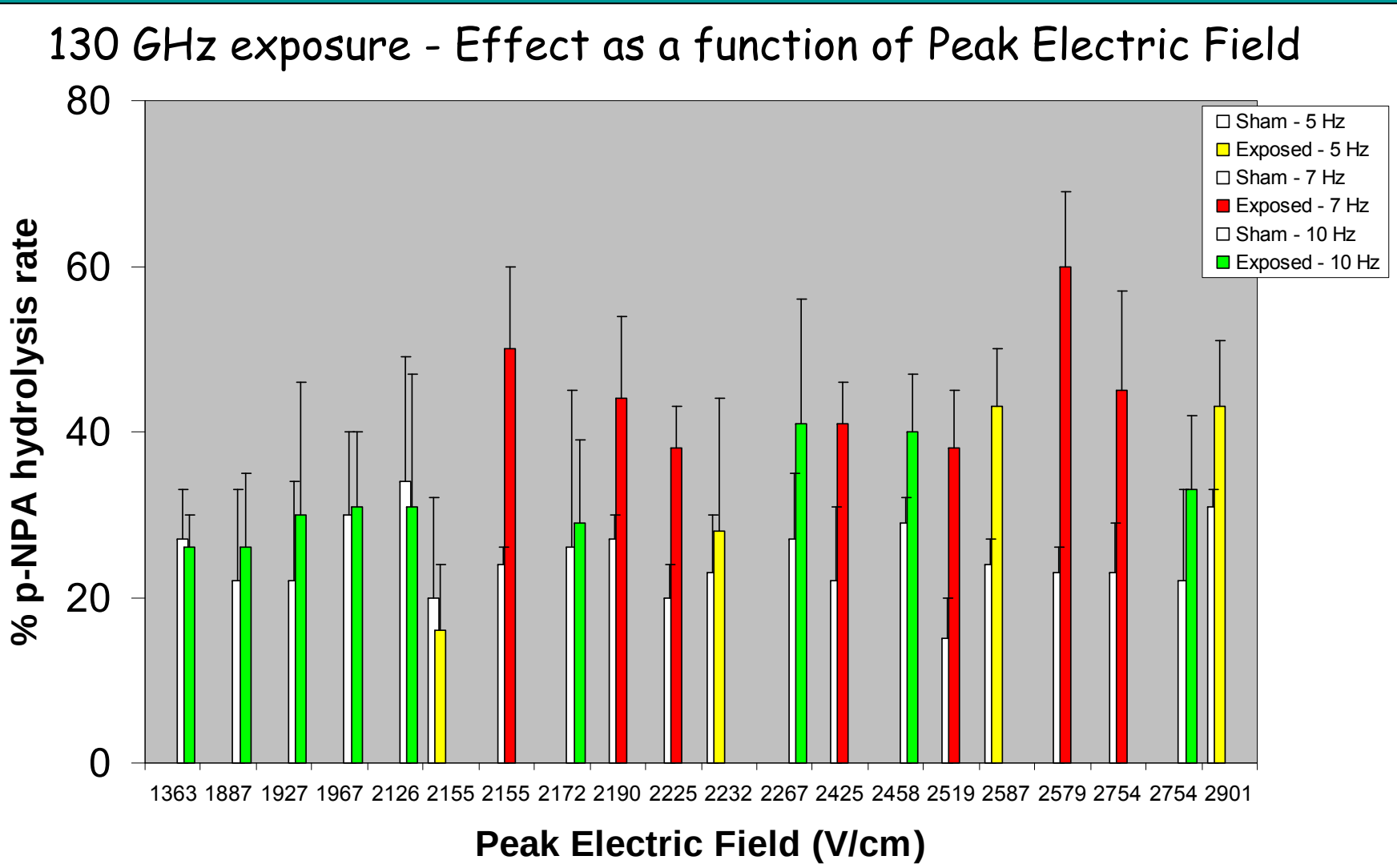
A systematic investigation on the effects of 130 GHz irradiation of Carbonic Anhydrase-loaded liposomes with different modulation conditions was carried out.





Irradiation of CA loaded liposomes at 130 GHz:
Increase in membrane permeability detected *over two minutes of 130 GHz irradiation with a pulse repetition rate of 5, 7 and 10 Hz* ***at an average incident intensity > 7.8 mW/cm^2 (60 ps pulses => Peak electric field up to 3 kV/cm)***

Can CA loaded liposomes rectify the THz pulse?



Comparison: short electric pulses

Electropermeabilization of cells by pulses of high field strength and ultra-short duration

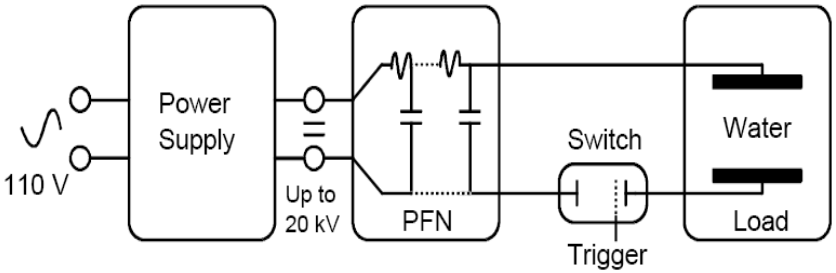
K.J. Müller, V.L. Sukhorukov, U. Zimmermann

Lehrstuhl für Biotechnologie, Biozentrum der Universität Würzburg, Germany

ns-Pulses

Nanopulser

- V_{max} = 15-20 kV
- square wave
- duration 10-100 ns
- $E_{max} \approx$ 200 kV/cm

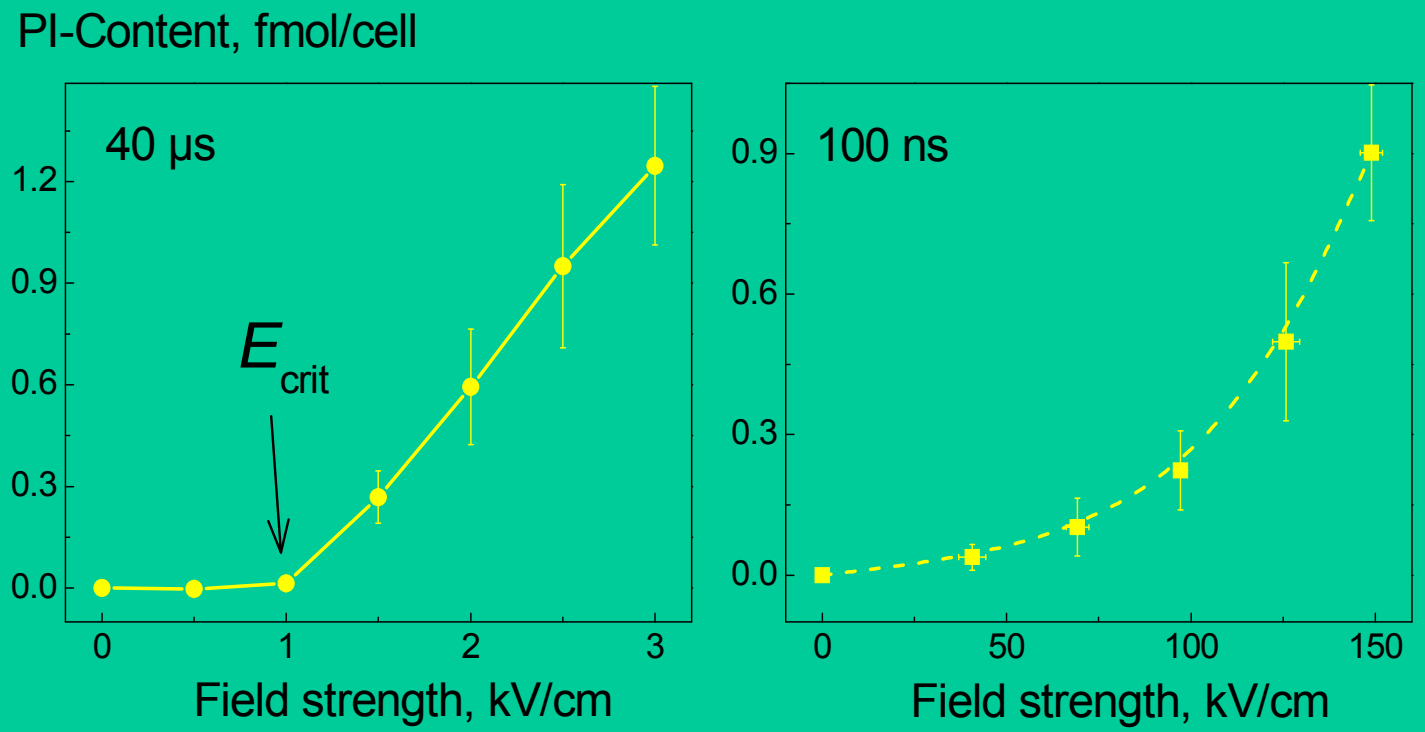


Schematic diagram of the nanopulser (Schoenbach et al.,1997)

Propidium iodide – a molecular indicator of electroporation

Electroinjection of propidium iodide into mouse myeloma cells

Field strength dependence of PI-uptake for ns- and μs-pulses



Is a unifying view possible?

- ✓ There is a need to compare the bio-effects of electric and e.m. pulses for a given:
  - duration
  - peak field amplitude

- ✓ Choose the carrier frequency to reach the target
  - choose duration to match target size
  - choose field amplitude to achieve the effect

- ✓ Classes of interesting phenomena and applications:
  - Drug delivery to cells
  - Electromanipulation of sub-microscopic vesicles and organelles

Past, recent & coming THz events on Bio-effects

- International THz-BRIDGE Workshop, Sept. 30 - Oct. 2, 2002, Capri, Italy
- Proceedings as Special Issue of J. Biol. Phys. 30 (2003)
- ICNIRP International Workshop on EMF Dosimetry and Biophysical Aspects Relevant to Setting Exposure Guidelines, 20-22 March 2006, Berlin, Germany
- BEMS 2007 - U.S. Air Force Workshop on Terahertz Bioeffects And Theory
- "Terahertz radiation in biological and environmental studies" LALS2007 - International Conference on Laser Applications in Life Sciences, June 11-14, 2007, Moscow, Russia
- Workshop "Terahertz Diagnostics and Treatment" TTD-2009, July 2009 Nyzhni Novgorod, Russia
- International Workshop of the Project "Genotoxic Effects of Terahertz Radiation in vitro?", Dec 6 -7, 2010, PTB - Braunschweig, Germany
- Journée Scientifique - SFRP-RNI – Paris, 25 Janvier 2011
  « Les ondes THz : effets biologiques, applications industrielles et médicales »
- Symposium "Terahertz sources, diagnostics and nonlinear interactions" July 9 - 16, 2011 Nyzhni Novgorod- St Petersburg Russia,

Julius-Maximilians-
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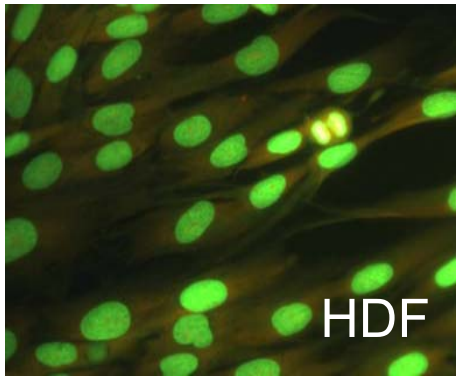
Project
Genotoxic effects of THz radiation in vitro

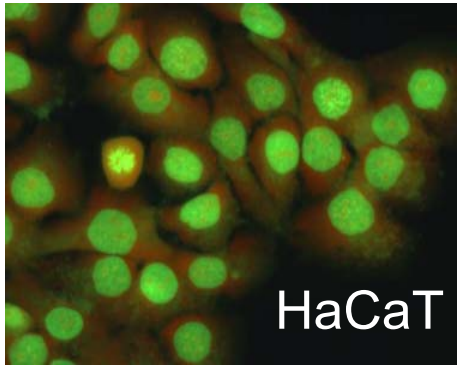
Helga Stopper & coworkers

PTB

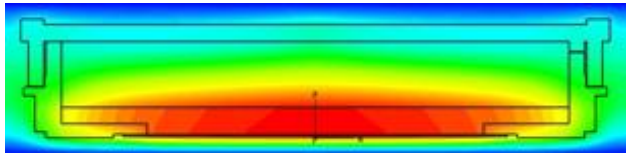
extension of THz-BRIDGE studies

• Cells	HDF, primary human skin fibroblasts HaCaT, human keratinocyte cell line
• Radiation	106, 380, 2520 GHz at 3 power densities ≤ 1mW/cm^2 for 2h and highest power density for 8h
• End points	Comet assay, micronucleus test





- 3 independent exposure series with sham controls
- untreated controls (12) and chemically-treated positive controls (9-12) during the time course of the project
- 2x1000 (Mn)/ 2x50 (Comet) cells analyzed from each exposure (coded slides/dishes)
- Mann-Whitney-U-Test for significance testing



Under the tested conditions, no genotoxicity was observed both in the comet-assay and in the micronucleus test

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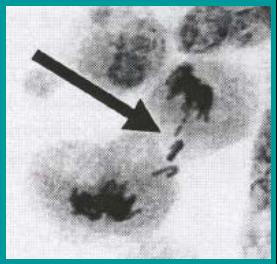
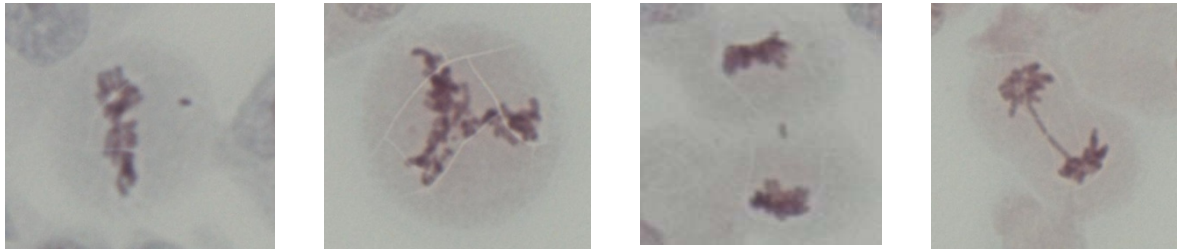
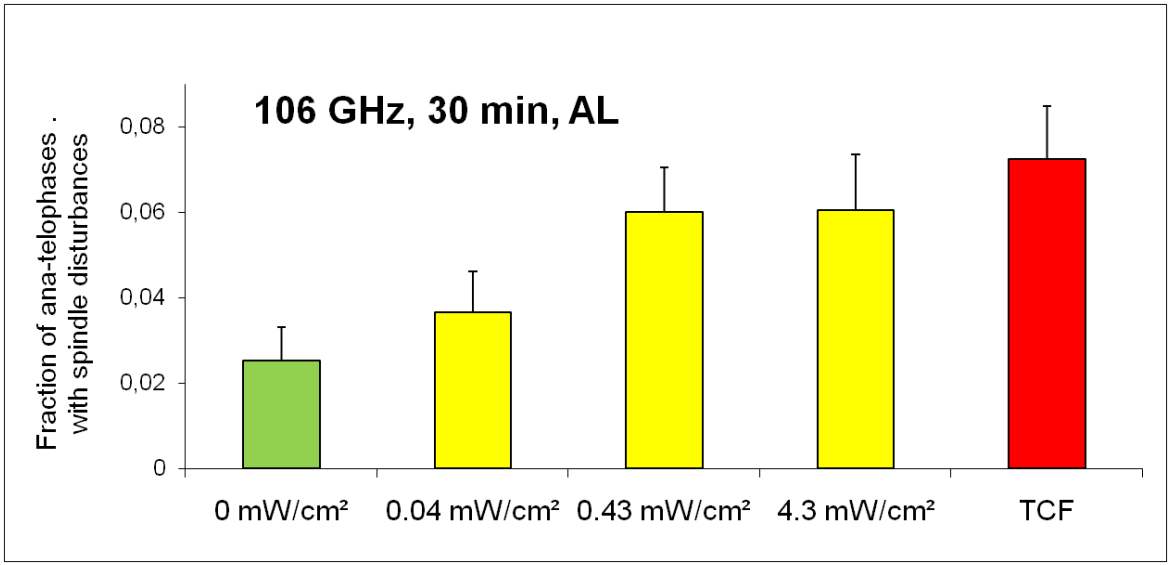
Mitotic disturbances



Method: Human-hamster hybrid Al cells are treated and fixed directly after that.
Mitotic figures are analyzed for the following categories of abnormalities:

Non-congression multipolar lagging non-disjunction





A correlation of the fraction of disturbances with the incident intensity was observed.

Why do these disturbances not lead to micronucleus formation?

Hintzsche et al., Radiation Research, accepted

Center for THz-Bio Application System

THz-Bio Center initiative in Korea

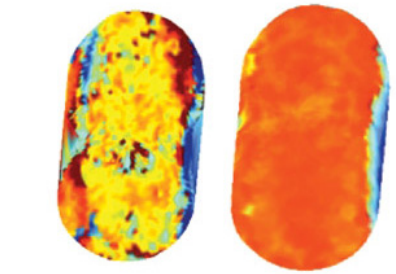
Prof. G.S. Park – Seoul National University

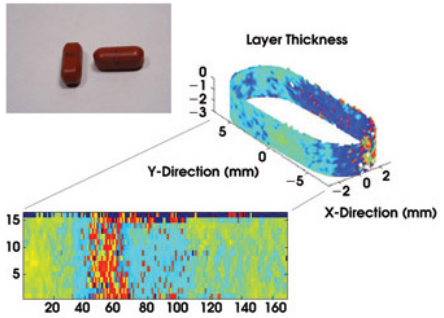
Network of 18 research groups in Korea

Opening Ceremony & Workshop
March 9 -10, 2010
Seoul National University

International
2nd THz-Bio Workshop
January 19~20, 2011
Hoam Faculty House
Seoul National University, Seoul, Korea

- Main projects:
  - ✓ THz Devices and Systems
  - ✓ THz-Bio Interaction
  - ✓ THz Clinical Imaging





THz-Bio Center initiative in Korea

Prof. G.S. Park – Seoul National University

- ## THz Clinical Imaging

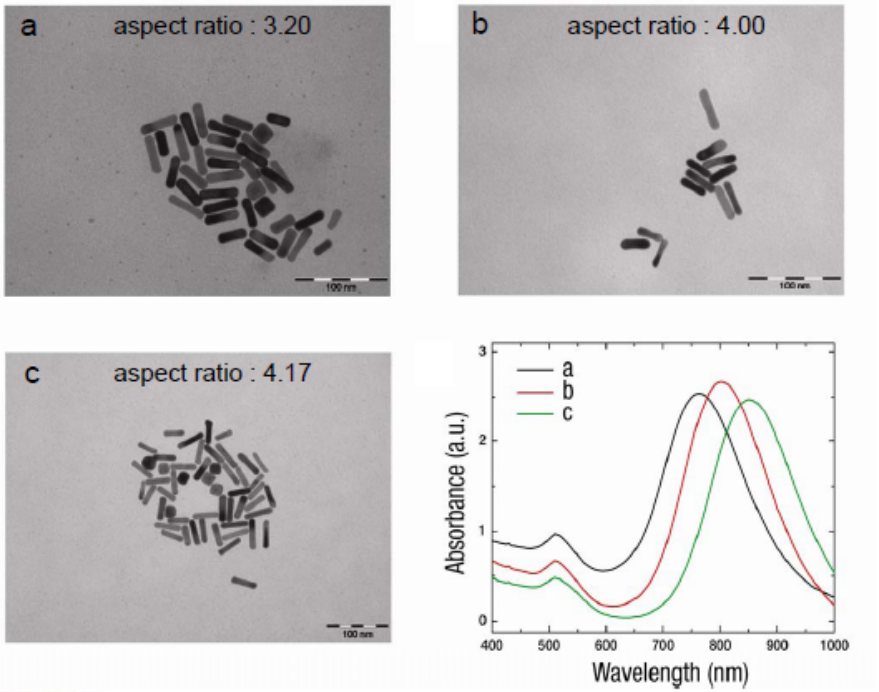
  ✓ 'Nanoparticle-enabled terahertz imaging for cancer diagnosis'
  Opt. Express, 17 (2009) 3469

Nanoparticle Contrast Agents

[Gold nano rods (GNRs) and their optical response]

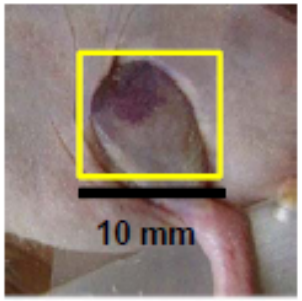
Injected gold nanorods preferentially concentrate in cancer tissue.

They locally increase the THz reflectivity of the sample providing a useful contrast mechanism



Courtesy of J.-H. Son, SNU Korea

In-vivo Cancer Image (medium size)



A431 (5X10^6 cells in 1 ml) in 50 µl PBS *

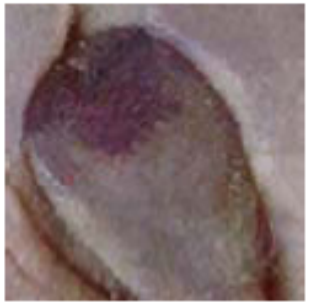
dosage of CET-PGNRs** : 54 µl (1mg of Au/ml)

imaged after 24 hours after injection

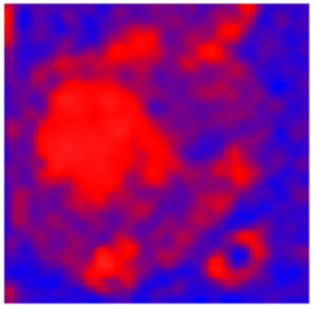
* phosphate buffered saline
** cetuximab PEGylated GoldNanoRods

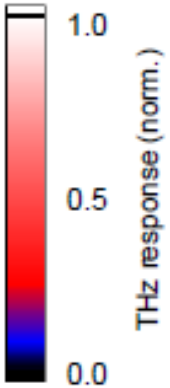
[THz Molecular Imaging]

visible imaging

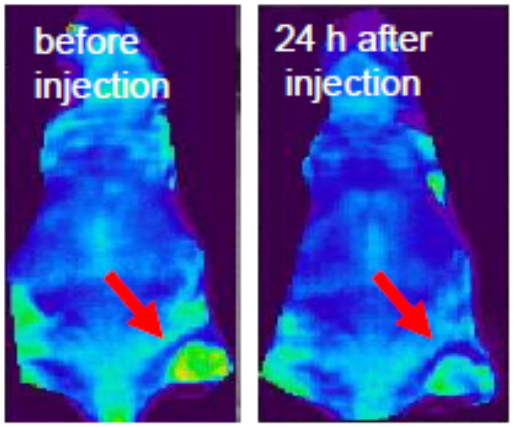


THz molecular imaging





[NIR Absorption Imaging]



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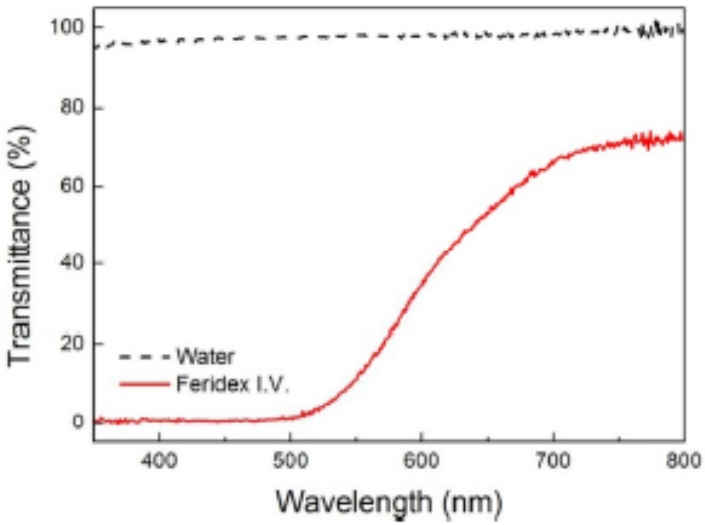
Courtesy of J.-H. Son, SNU Korea

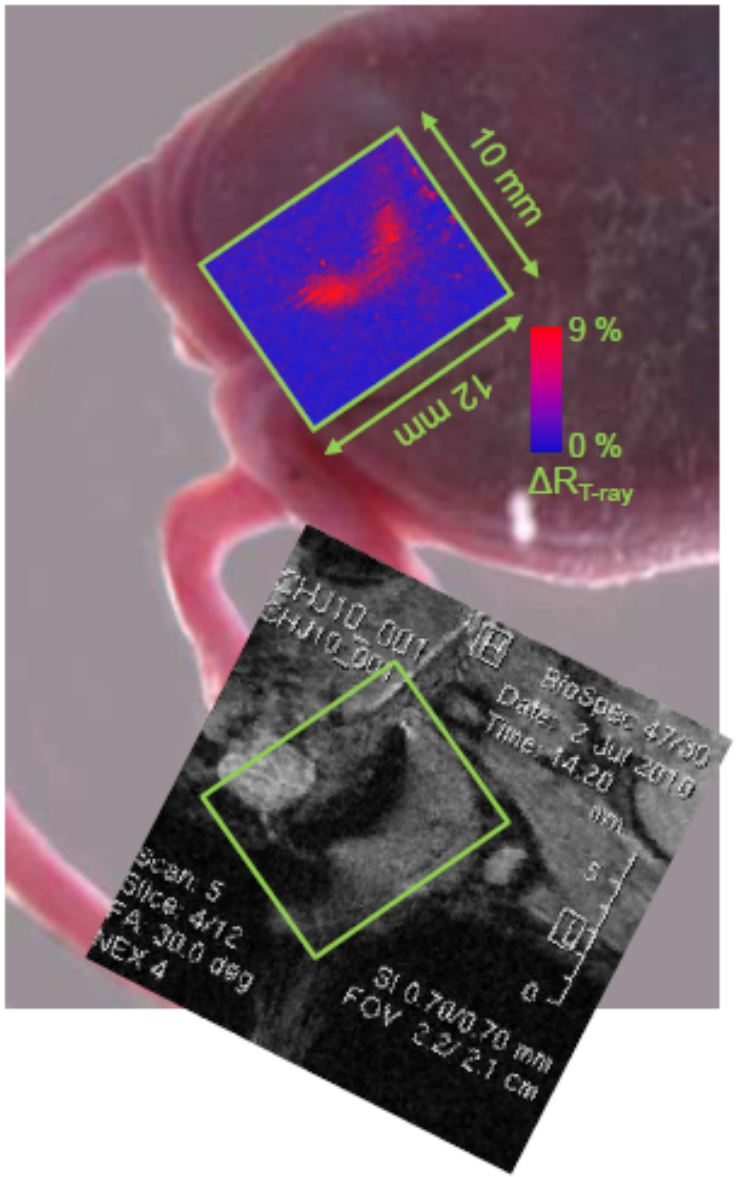
TMI is more sensitive than NIR absorption imaging

In Vivo THz & MR Images Using FERIDEX®

Human Approved Nanoparticle
Contrast Agent : FERIDEX®

[IR Transmittance]



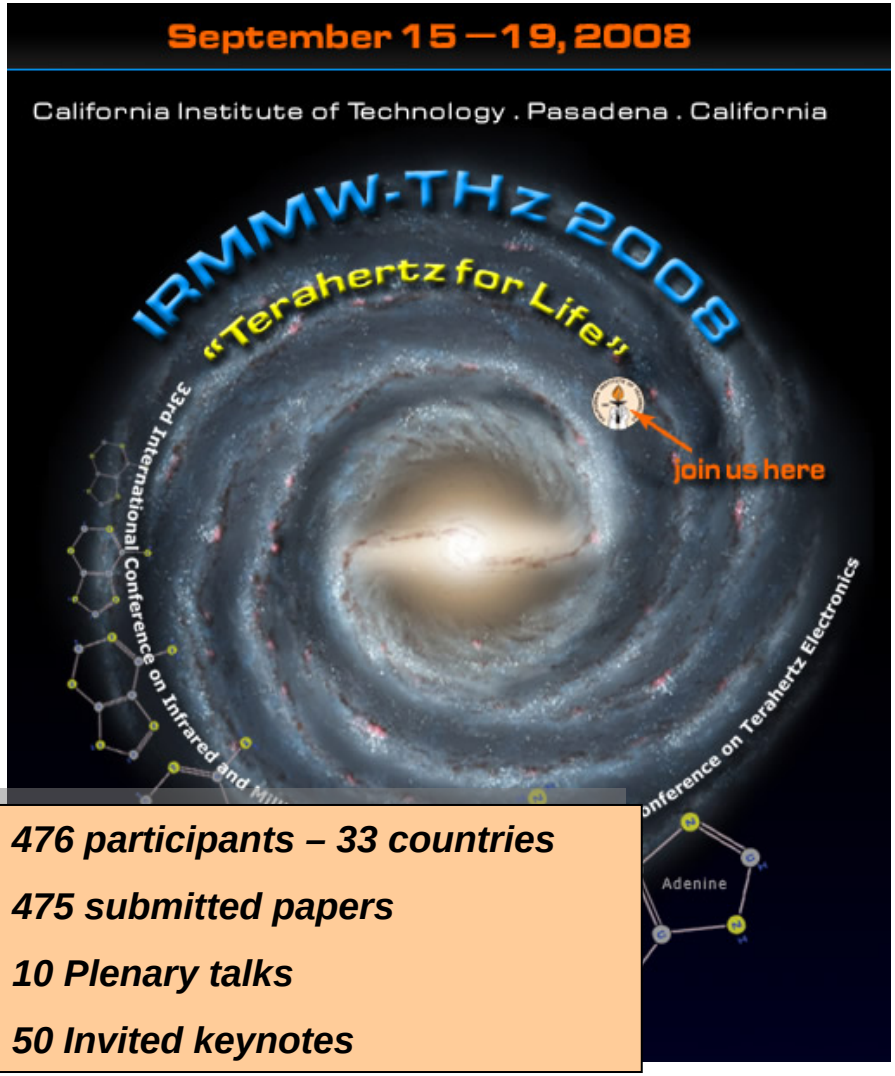


Collaboration with Dr. H. J. Choi at AMC

서울시립대학교 UNIVERSITY OF SEOUL | LABORATORY for TERAHERTZ BIOPHOTONICS

Courtesy of J.-H. Son, SNU Korea

IRMMW-THz conferences

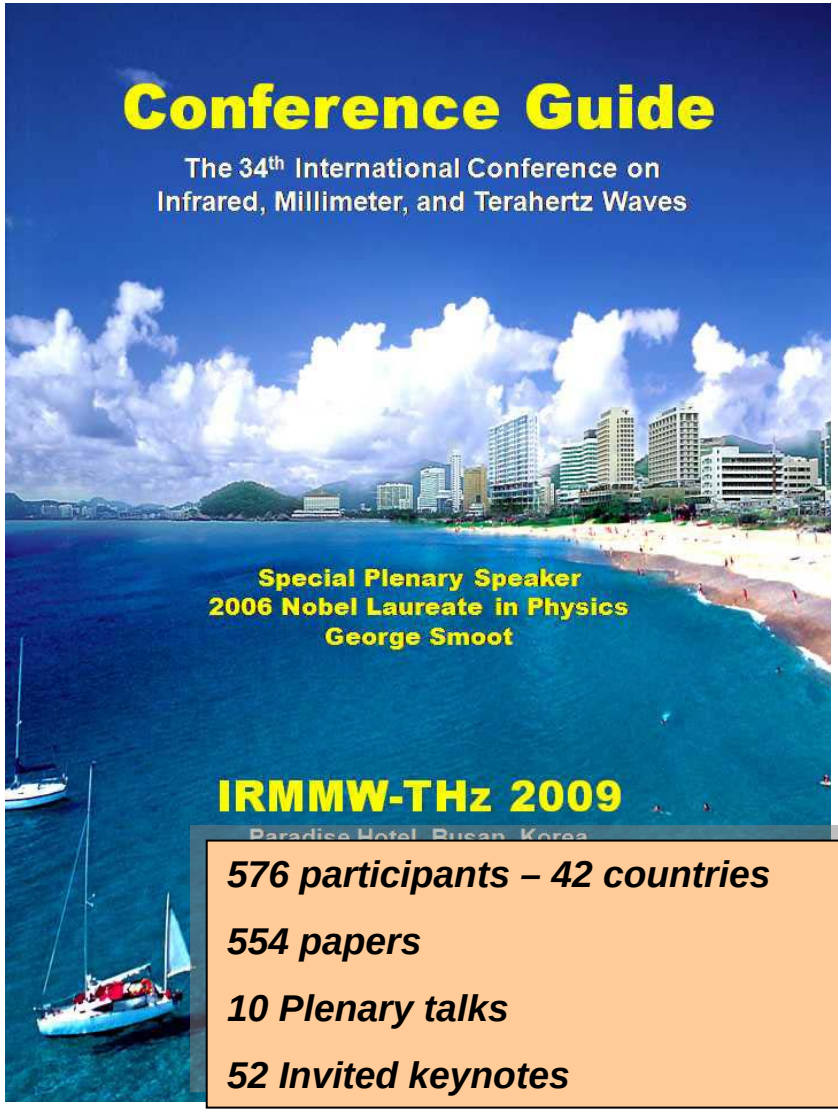


476 participants – 33 countries

475 submitted papers

10 Plenary talks

50 Invited keynotes



576 participants – 42 countries

554 papers

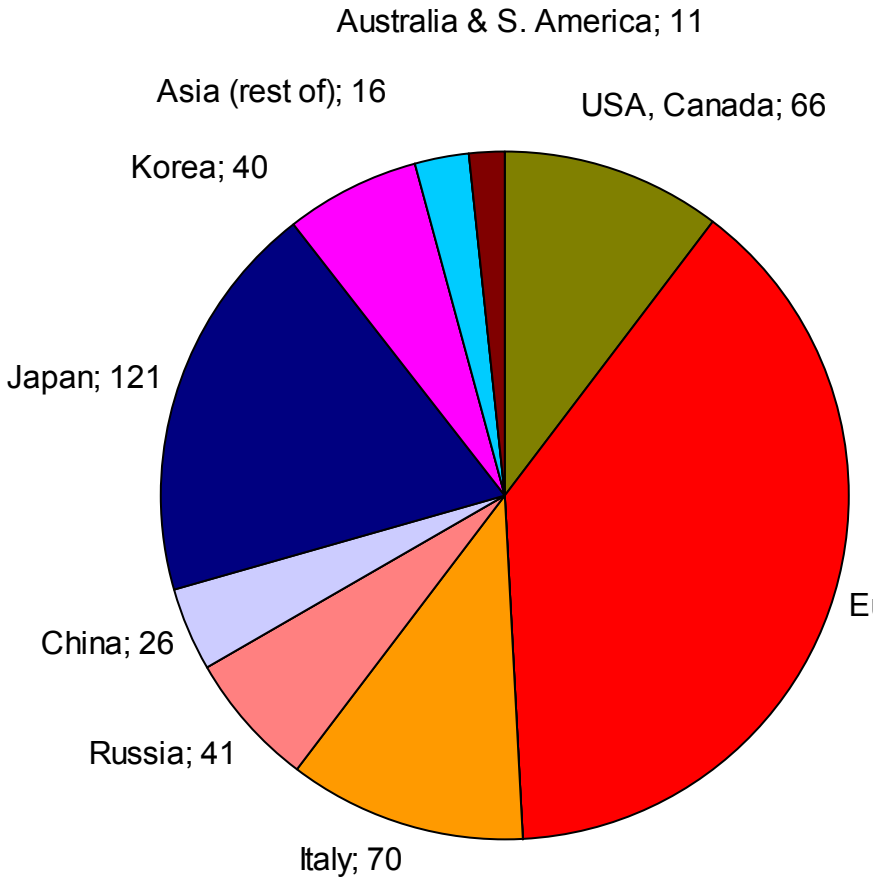
10 Plenary talks

52 Invited keynotes

IRMMW-THz Conferences attendance

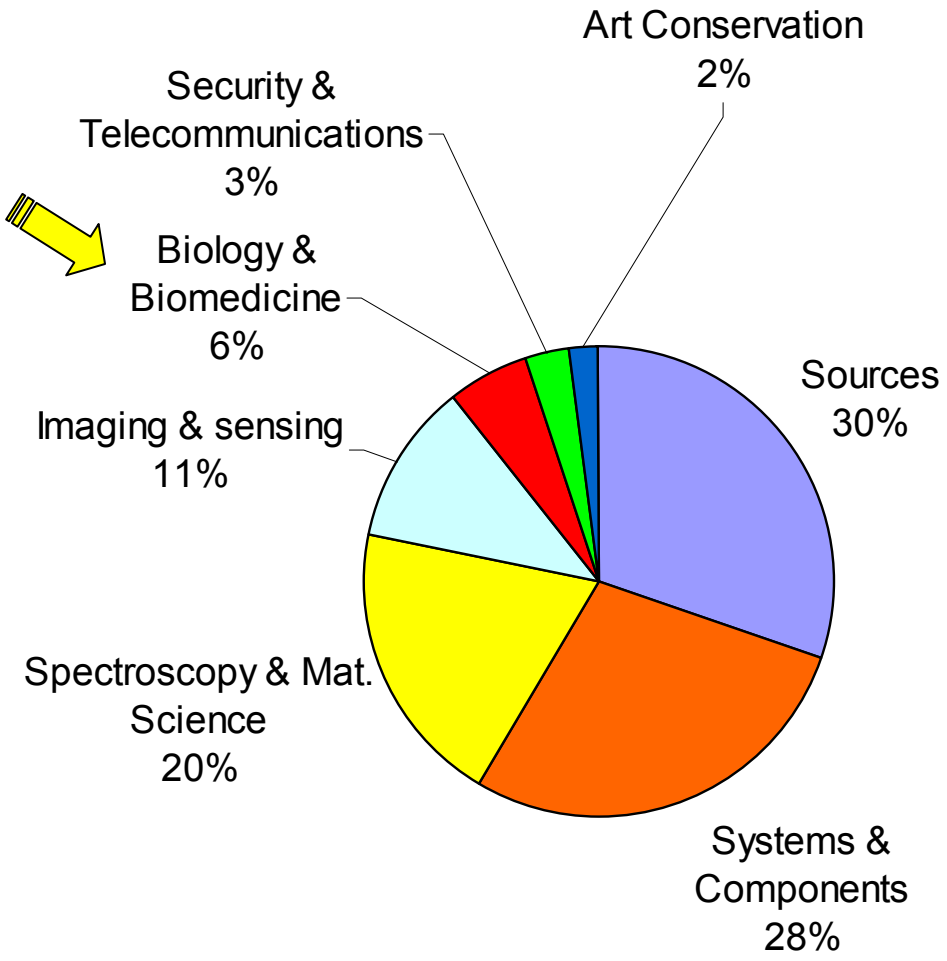
	USA - 2005	China - 2006	UK - 2007	USA - 2008	Korea - 2009	Italy - 2010
	Williamsburg (VA)	**Shanghai**	**Cardiff**	**Pasadena**	**Busan**	**Rome**
USA, Canada	101	60	55	124	73	**66**
Europe (rest of)	99	123	134 (Excl. UK)	157	139	**250** (Excl. IT)
Italy						**70**
Russia	58	48	52	46	51	**41**
UK			85			
China		224				**26**
Japan						**121**
Korea					100	**40**
Asia (rest of)	116	117 (Excl. China)	178	130	204 (Excl. Korea)	**16**
Australia & S. America	8	9	19	19	9	**11**
TOTAL	382	581	523	476	576	**641**

Country distribution of participants



of which 56 from France

IRMMW-THz 2010
Distribution of papers by topic



Plenary Talk

"The future of medical imaging:
Does Terahertz have a place"

V. Wallace, Univ. Western Australia

36 papers on bio-med applications:

21 – spectroscopic & dielectric properties

11 – imaging

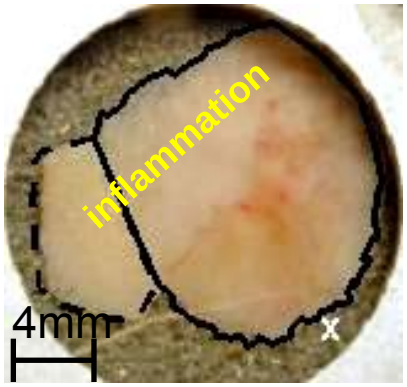
4- THz effects

THz imaging of cancer tissues

TERAVIEW developed a THz imaging system, working in reflection that provides quasi-3D images of suspect tissues.

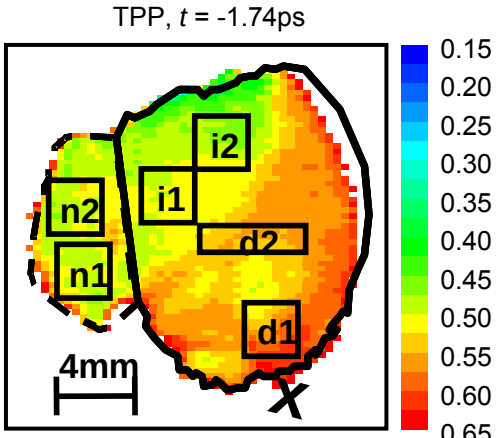
Diseased tissue appears to behave differently with respect to healthy tissues, showing a marked contrast, mainly due to water absorption in the sample. Histological analysis confirmed the results of THz scans.

(courtesy of Teraview Ltd)

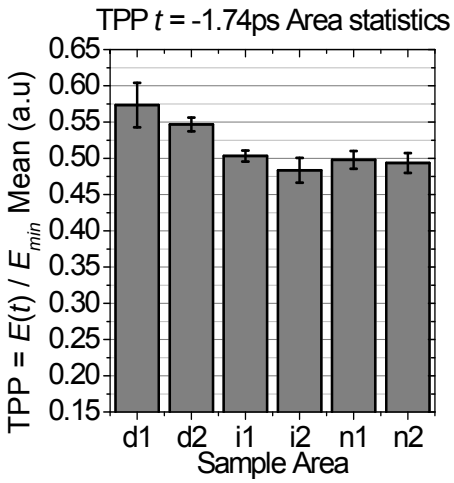


Visible image

Basal Cell Carcinoma



Time post pulse image

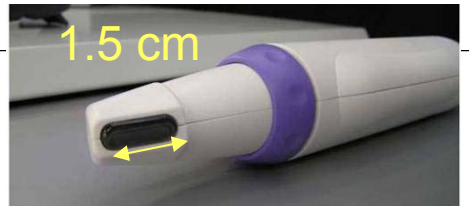


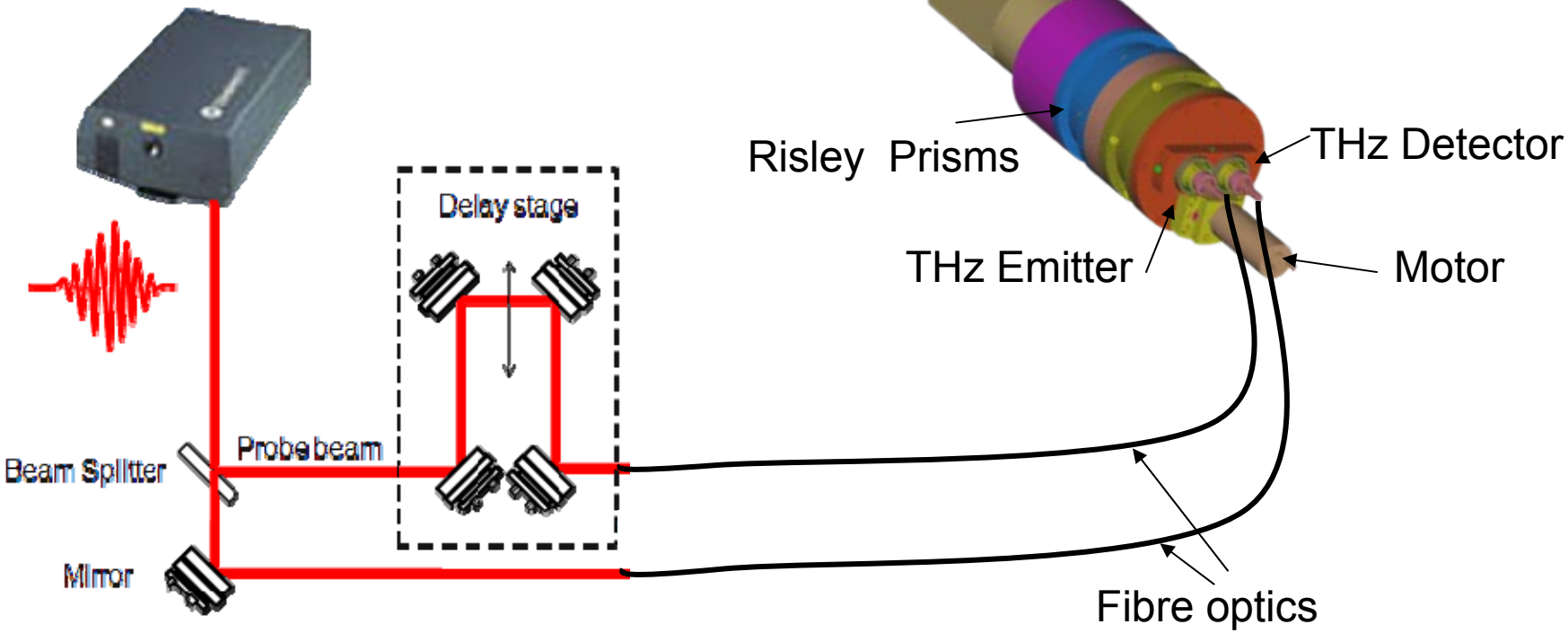
Terahertz Pulsed Imaging of Human Breast Tumors

A.J. Fitzgerald et al. Radiology 239, 533 (2006)

- The feasibility of using terahertz pulsed imaging to map margins of exposed breast tumors was investigated by imaging 22 excised human breast tissue specimens with carcinoma excised from 22 women.
- The size and shape of tumor regions on terahertz images were compared with those identified at histopathologic examination of the imaged section. Two image parameters were investigated: the minimum of the terahertz impulse function and the ratio of the minimum to the maximum of the terahertz impulse function.
- The correlation coefficient for the tumor area on images compared with that on a photomicrograph of all 22 samples was greater than 0.82 for both parameters. The shape of the tumor regions on terahertz images also correlated well with that on a photomicrograph (median Spearman rank correlation coefficient, 0.69).
- Findings of this study demonstrate the potential of terahertz pulsed imaging to depict both invasive breast carcinoma and ductal carcinoma in situ under controlled conditions
- Further studies are needed to determine the sensitivity and specificity of the technique.

Intraoperative probe design





Courtesy of V. Wallace

THz in biology & biomedicine
Review Papers

- A. Ramundo "Effects of Millimeter Waves Radiation on Cell Membrane - A Brief Review" - J Infrared Millimeter Terahertz Waves 31, 1400 (2010)
- A. Ramundo, G.P. Gallerano "Terahertz Radiation Effects and Biological Applications" – J Infrared Millimeter Terahertz Waves 30, 1308 (2009)
- D.F. Plusquellic et al. "Applications of THz spectroscopy in biosystems" Chem Phys Chem. 8: 2412 (2007)
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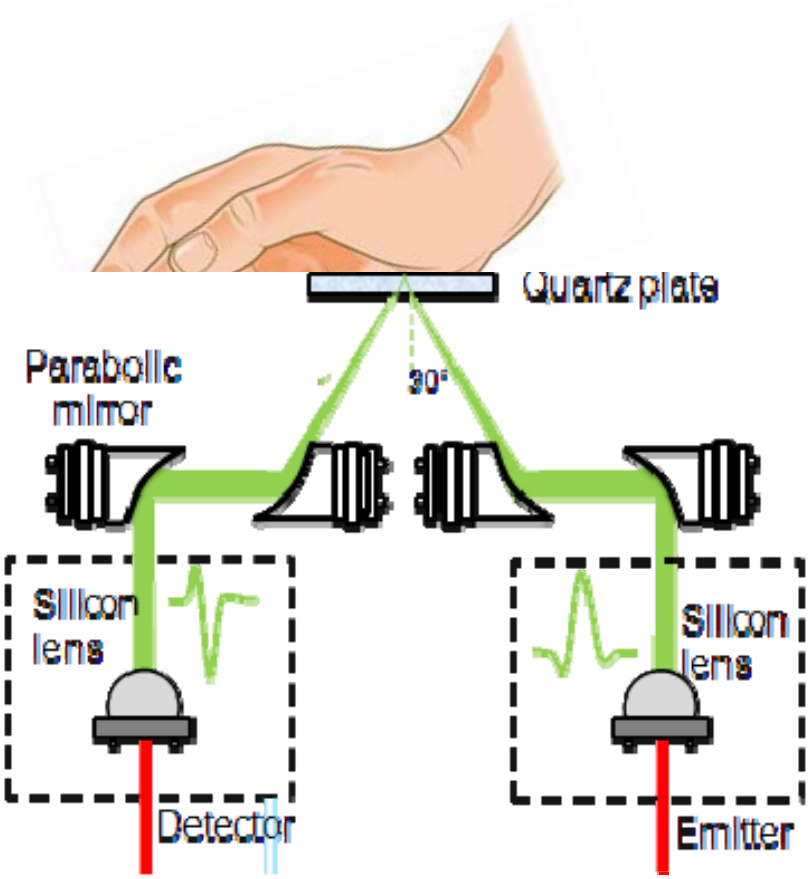
Issues in the definition of exposure limits at THz frequencies

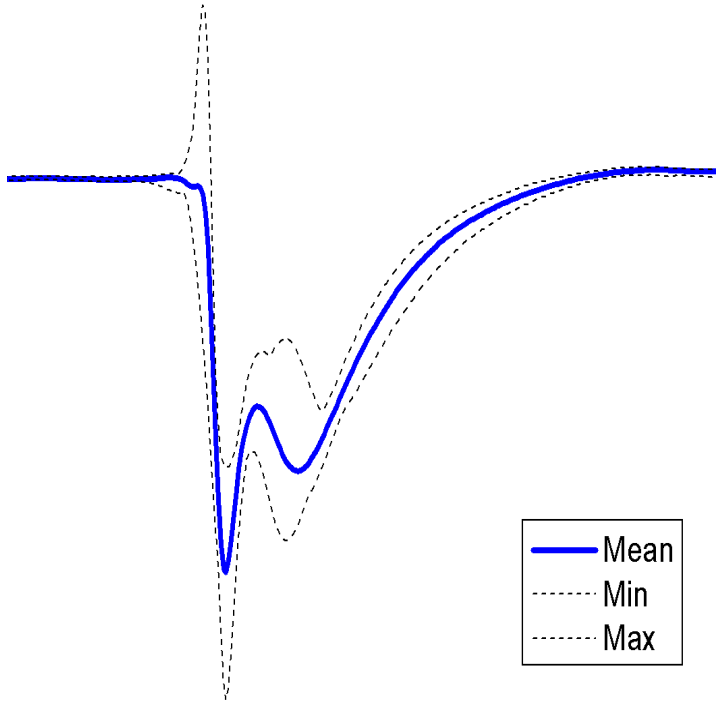
- Small radiation penetration through skin
- Water extinction coefficient greater than 50 cm^{-1} @ ν > 100 GHz
- Peak and average incident intensity (W/cm^2)
- Specificity of temporal structure of many THz sources (ps to fs pulses)
- Role of amplitude modulation
- Role of peak electric field

In vivo study of human skin using pulsed terahertz radiation

Pickwell *et al*
Phys. Med. Biol. 49, (2004) 1595-1607

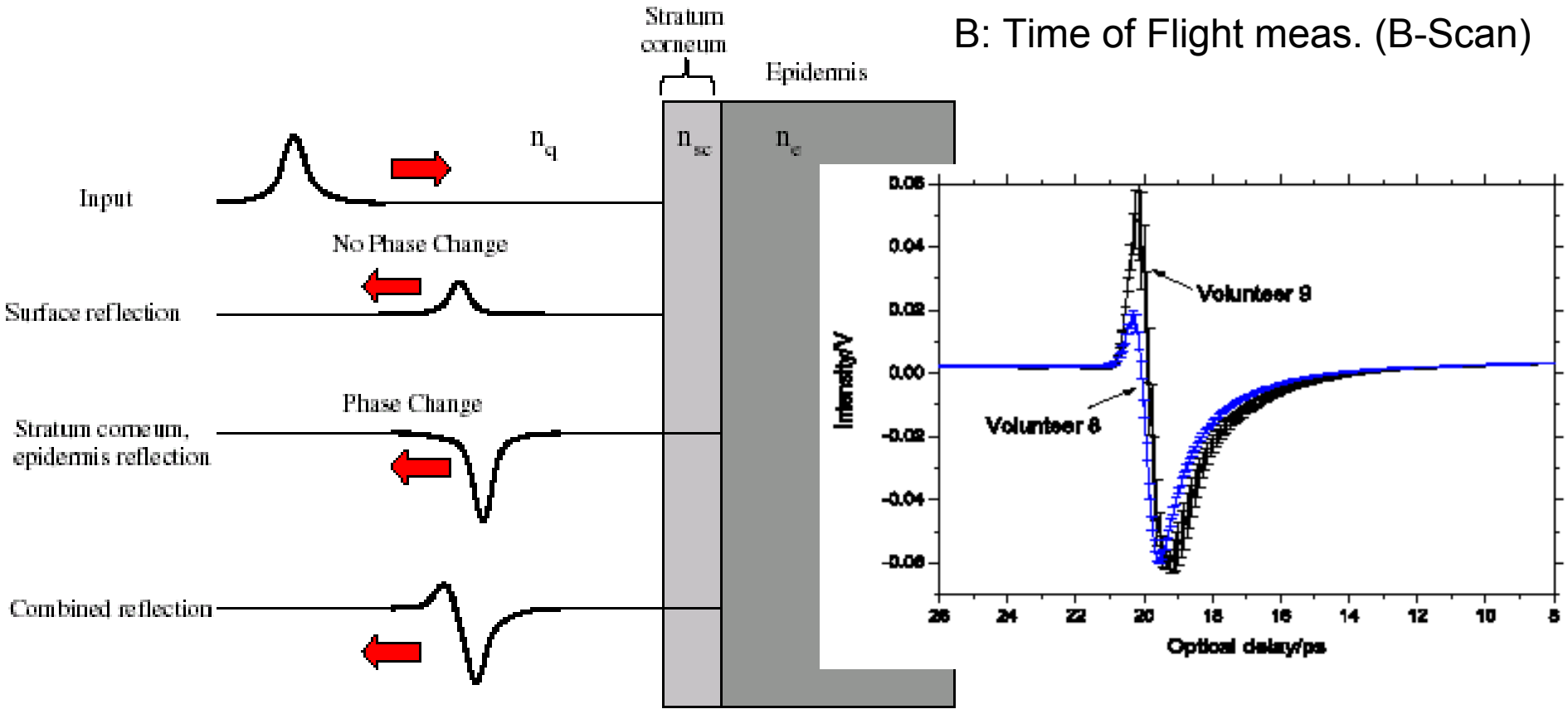


A THz point reflection (A-Scan)



In vivo study of human skin using pulsed terahertz radiation

B: Time of Flight meas. (B-Scan)



E. Pickwell et al., Phys. Med. Biol. 49 (2004) 1595–1607

Millimeter Wave Dosimetry of Human Skin

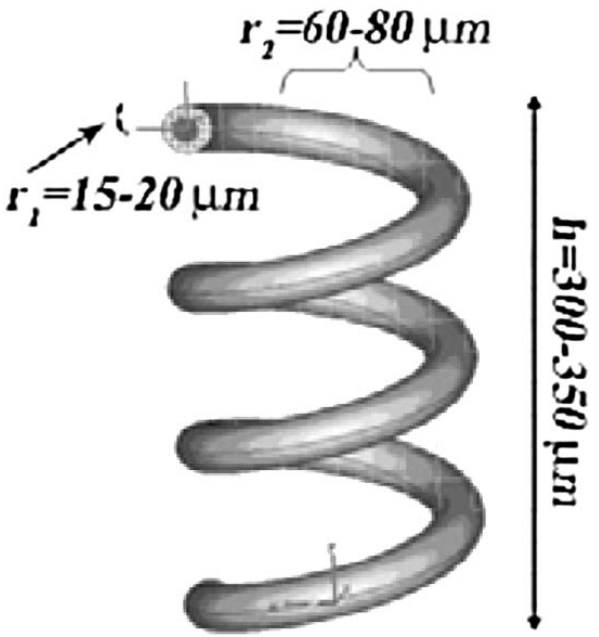
S.I. Alekseev, A.A. Radzievsky, M.K. Logani, and M.C. Ziskin

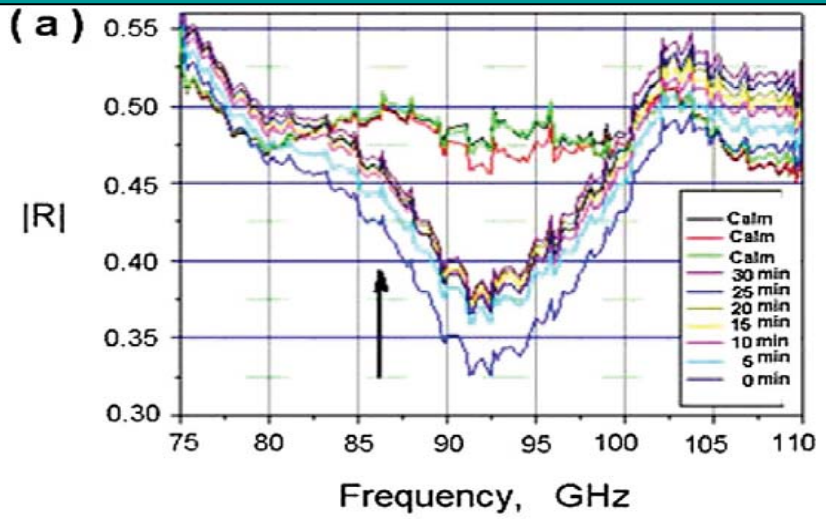
Bioelectromagnetics 29, 65-70 (2008)

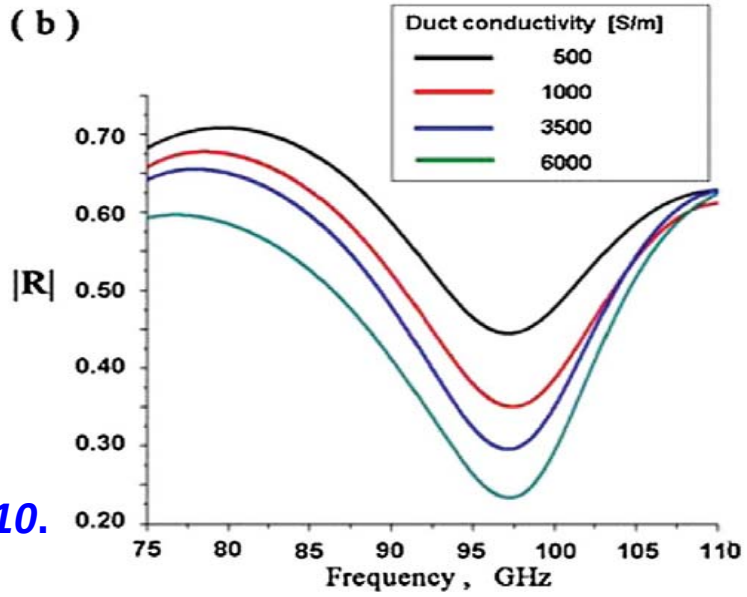
- The main characteristics of mm wave skin dosimetry - reflection, power density (PD), penetration depth (d), and specific absorption rate (SAR) - were calculated using a homogeneous unilayer model and two multilayer models of skin.
- The parameters of the models were found by fitting the experimental data obtained from measurements of mm wave reflection from human skin. The forearm and palm data were used to model the skin with thin and thick stratum corneum (SC), respectively.
- The thin SC produced little influence on the interaction of mm waves with skin. On the contrary, the thick SC in the palm played the role of a matching layer and significantly reduced reflection.
- The palmar skin manifested a broad peak in reflection within the 83–277 GHz range. The epidermis plus dermis, containing a large amount of free water, greatly attenuated the mm-waves. Therefore, the deeper fat layer had little effect on the PD and SAR profiles.
- A moderate SAR peak observed in the therapeutic frequency range (42–62 GHz) within the skin at a depth of 0.3–0.4 mm. Mm-waves penetrate into the human skin deep enough (d = 0.65 mm at 42 GHz) to affect most structures located in the epidermis and dermis.

Human skin as arrays of helical antennas in the 75-600 GHz

analogous work by K. Kawase presented in Braunschweig – Dec. 2010







Y. Feldman et al, PRL 100,128102 (2008)

E. Pickwell et al.,J. Applied Physics, 39 R301-R310.

Security applications… and related health concerns

Body Scanners

The ProVision™ Body Scanning System designed by L-3 SafeView screens people for concealed threats -- without exposure to harmful electromagnetic radiation. ProVision's **active millimeter wave imaging technology** penetrates clothing and packaging to reveal and pinpoint hidden weapons, explosives, drugs, and other contraband. With potential peak throughput levels of 600 people an hour, ProVision far outpaces alternative screening methods.

The system can easily be configured to meet specific throughput and facility requirements, from single scanner and single computer combinations to multilane configurations.

- Recognizes threats and contraband hidden anywhere on the body in single scan
- More reliable and less intrusive than pat-down searches
- Locates virtually any prohibited item or substance
- Generates scans in as little as 2 seconds
- Potential peak throughput of 600 people an hour
- User programmable algorithms ensure the preservation of privacy
- As safe to use as a cell phone

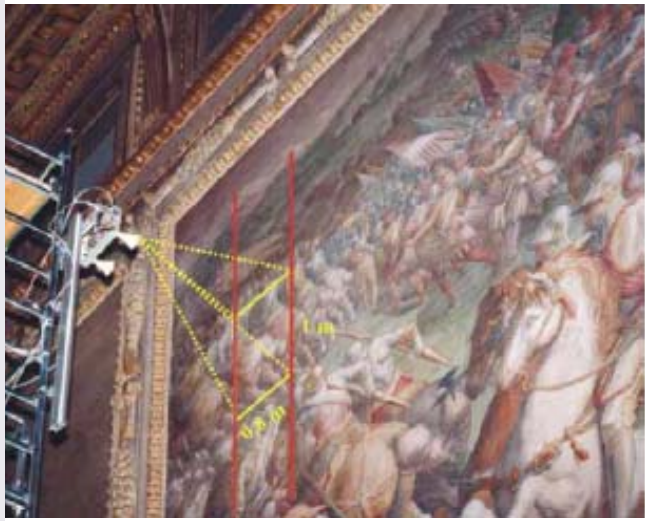
L3 communications
Security & Detection Systems

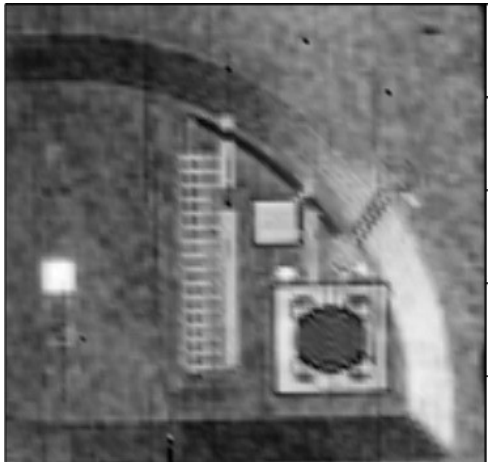
Open issues of body scanners

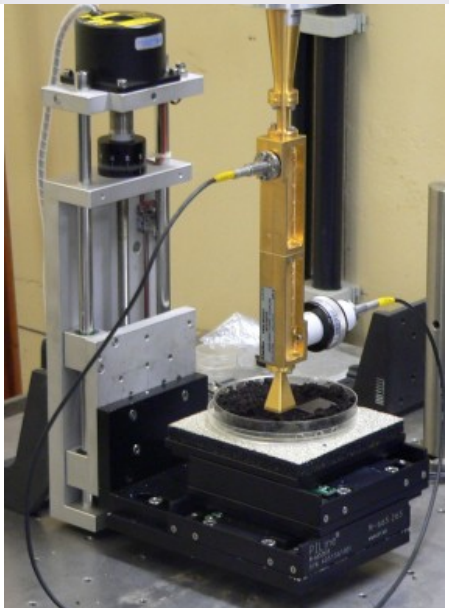
- Frequency of operation (24 – 30 GHz)
- Pulsed or CW
- MM-wave intensity at body surface

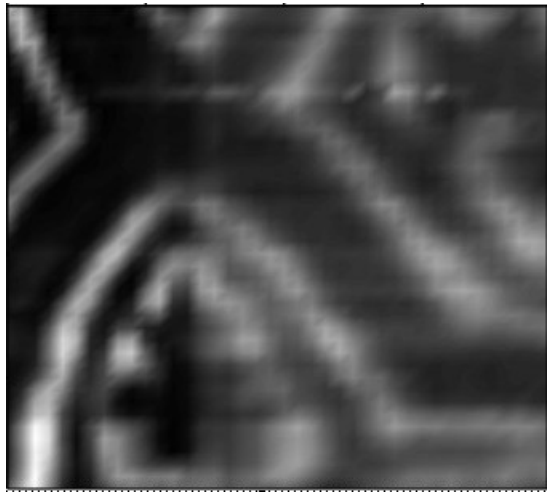
  347 μW/m^2 according to Apave, 640 μW/m^2 according to Emitech e 59,7 μW/m^2 according to TSA
- Interaction with epithelial cells (skin, cornea)
- Typical 10 s exposure time

Interdisciplinary THz applications









Biological Issues in art conservation studies

- Imaging of wooden art-crafts at millimeter wavelengths

  B. Bisceglia et al. – BEMS 2009, Davos (CH)
- Biodeterioration of a fresco by biofilm forming bacteria

  C. Milanesi et al., Int. Biodeterioration & Biodegradation 57 (2006) 168 – 173
- Progetto di recupero e conservazione della Villa Romana del Casale di Piazza Armerina

  Conservation Project of the Roman Villa del Casale – Piazza Armerina (Sicily)

  G. Meli – CRPR – Regione Sicilia (2007)

Can THz & mm-waves play a role?

Biological Issues in Art Conservation Studies

the case of mosaics

Villa del Casale – Piazza Armerina (Sicily)

Mosaics

Green patina in the Thermal bath (Apodyterium)

detachments, infiltrations underneath the mosaic

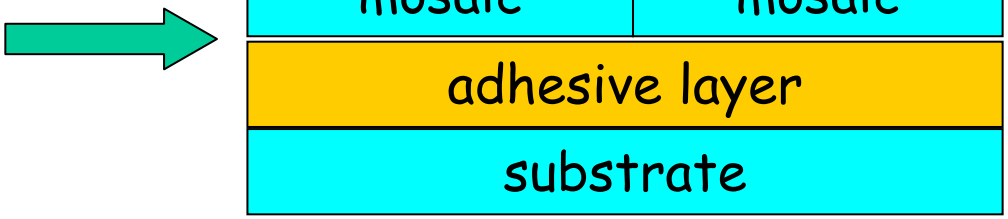
G.Miceli, L.M.Vinci, E.Guarneri - Laboratory *of* Microbiological Analysis , CRPR - Palermo

Biological Issues in Art Conservation Studies

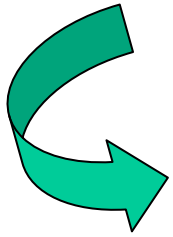
THz Diagnostics of mosaic biodegradation

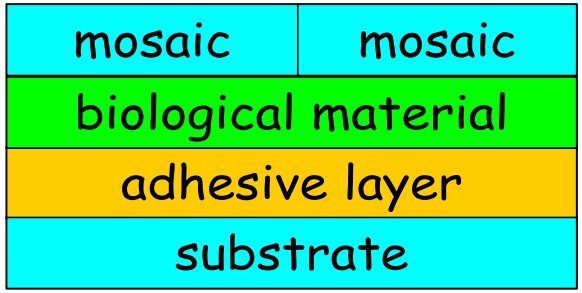
- The reflection by hydrated bio-materials can be exploited to map large areas of a mosaic and monitor its conservation state
- The frequency of the radiation can be chosen to optimize the penetration through the various materials

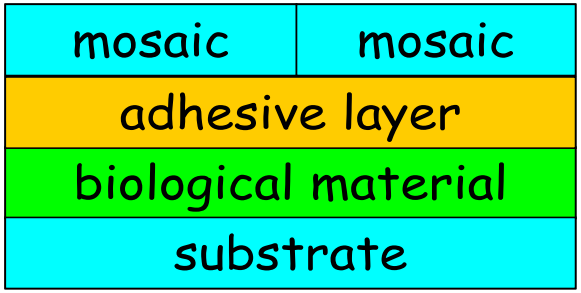
Imaging through covering layers is possible with a spatial resolution that can match the pattern of the *tesserae*.



Biological material (algae, lychens, etc) can be detected underneath the mosaic platelets







Summary needs and priorities

- ✓ Need of gathering scientists from different disciplines to develop applications of THz radiation in the fields of biomedicine, environment, material science, telecommunication, security
- ✓ Need of training young scientists from all over the world in an emerging field of technology
- ✓ Need of studies on the interaction mechanisms
  - ✓ Effects of high peak electric fields on cell membrane
  - ✓ Comparison of the effects of pulsed electric field and short electromagnetic pulses on biological membranes and cells
- ✓ Further extension of THz-BRIDGE and PTB studies
- ✓ Establish dose/response relationship & improve definition of relevant spectral ranges and ICNIRP exposure guidelines
- ✓ Integration & comparison of THz imaging techniques in different fields
- ✓ The development of emerging applications in the field of THz wireless communications, security and art conservation will require an assessment on exposure conditions of a wider public

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