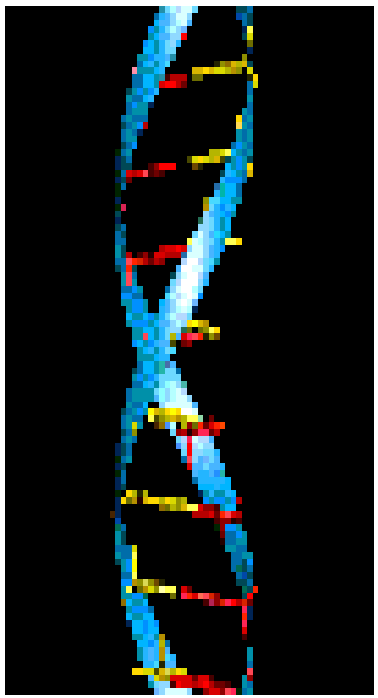
Non-thermal biological effects of microwaves: current knowledge, further perspective and urgent needs



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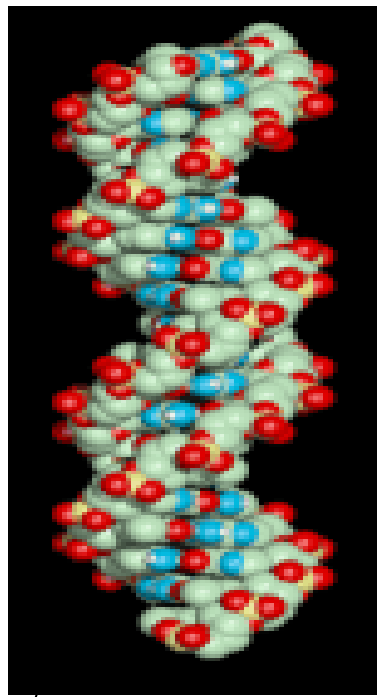


http://members.chello.se/igor.belyaev/

Workshop “Do sinusoidal versus non-sinusoidal waveforms make a difference?”
Zurich, Switzerland, February 17-18, 2005

Scope of this Lecture:

Overview

of diverse biological effects *of non-thermal microwaves*

and dependence of these effects *on various physical and biological parameters*

Information sources:

Books, handbooks, reviews, original publications

Chosen presentations from the latest meetings, own results

Electromagnetic exposures vary in many aspects:

-power (intensity, incident power density - thermal effects)
-wavelength / frequency
-near field / far field
- overall duration of exposure (continuous, interrupted), acute and chronic exposures
- polarization (linear, circular)
-continues wave (CW) and pulsed fields
(pulse repetition rate, pulse width or duty cycle, pulse shape, pulse to average power, etc.)
- modulation (amplitude, frequency, phase, complex)
-static magnetic field at the place of exposure?

All these parameters can affect biological effectiveness of exposure to non-thermal microwaves

Comments on microwave (MW) exposure safety standards

- "Dose" is not defined for microwave exposures and SAR (specific absorbed rate) or power density are used for guidelines
- Current safety standards are often based on thermal effects of microwaves in **acute** exposures.
- Safety standards significantly, up to 1000 times, vary between countries
- Why?

EVIDENCE FOR EFFECTS OF NON-THERMAL MICROWAVES (below ICNIRP (International Commission for Non-Ionizing Radiation Protection) restrictions based on thermal effects)

- Altered cell responses in laboratory *in vitro* studies
- Medical application (former Soviet Union countries)
- Hypersensitivity to electromagnetic fields
- Epidemiological cancer studies

Examples of non-thermal effects of microwaves on cells

Objects	Effects	Reference
Preloaded synaptosomes	Changes in calcium efflux	Lin-Liu and Adey, 1982
Reuber H35 hepatoma cells	Ornithine decarboxylase (ODC)	Byus et al. , 1988
rat brain cells	DNA damage as measured with comets	Lai H, Singh, 1997
AMA human epithelial cells	Cell proliferation	Velizarov et al., 1999
Human lymphocytes	53BP1 DNA-breaks co-localizing foci	Belyaev et al., 2005
Lymphocytes, *E. coli* cells	Chromatin conformation	Belyaev et al., 2000
rat atrocities and porcine brain capillary endothelial cells	permeability of an in vitro model of the blood-brain barrier (BBB)	Schirmacher et al., 2000
human mast cell line, HMC-1	expression of the proto-oncogene c-kit, the transcription factor Nucleoside diphosphate kinase B and the apoptosis-associated gene DAD-1.	Harvey and French, 2000
Human endothelial cell line	activation of the hsp27/p38MAPK stress pathway	Leszczynski et al., 2002
Human peripheral blood cultures	micronucleus frequency (only phase-modulated wave)	d'Ambrosio et al., 2002

Studies on Wireless Communication-related Signals Genetic Effects

Effect: d'Ambrosio (02); Goswami (99); Harvey & French (00); Gadhia (03); Ivaschuk (97); Maes (96a,b); Mashevich (03); Pacini (02); Phillips (98); Sykes (01); Tice (02)

No Effect: Antonopoulos (97); Bischt (02); Fritz (97); Gos (00); Li (01); Maes (01); Malyapa (97); McNamee (02a,b, 03); Morrisey (99); Takahaski (02); Vijayalaximi (01a,b, 03); Zeni (03)

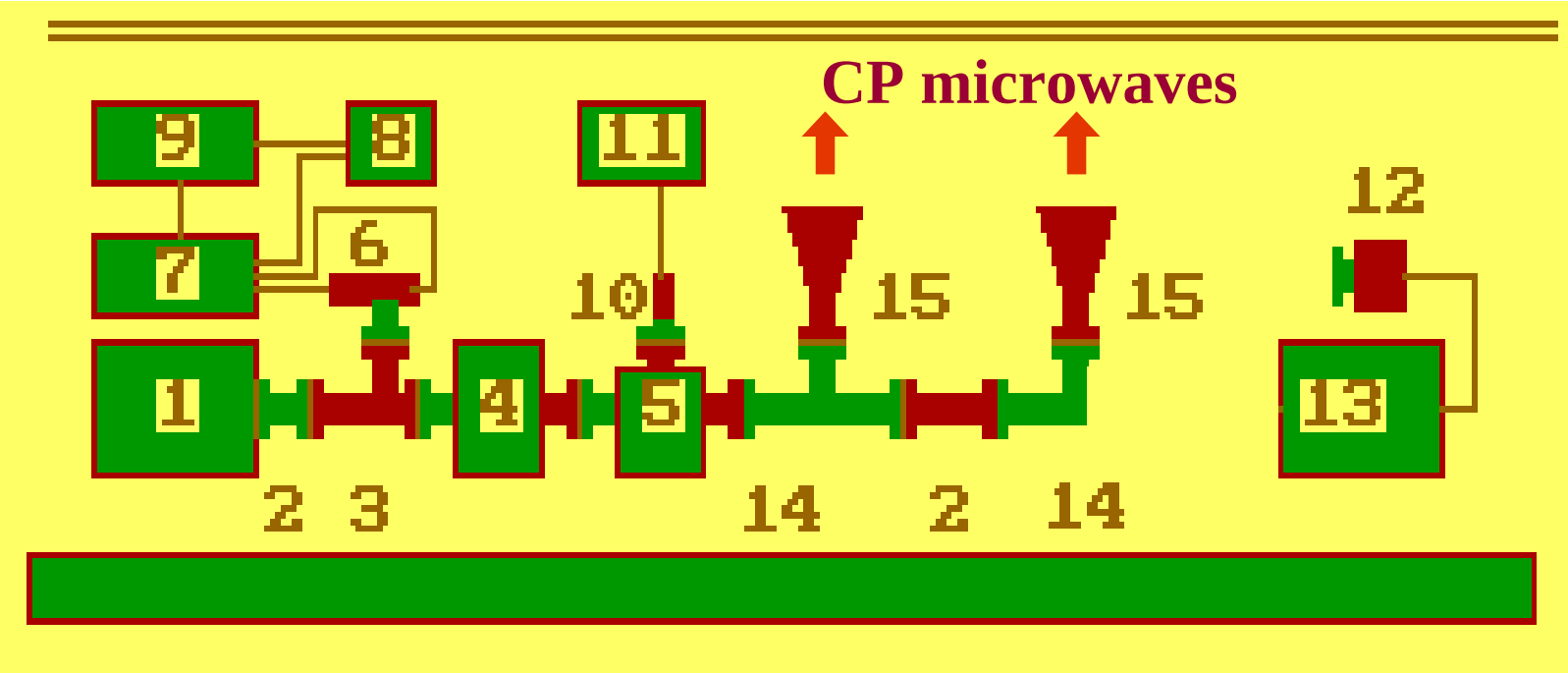
***Cell phone industry-sponsored studies in red**

From Henry Lai, BIOLOGICAL EFFECTS OF RADIOFREQUENCY RADIATION
University of Washington, Seattle, WA, USA, 2003

Non-thermal biological effects of microwaves

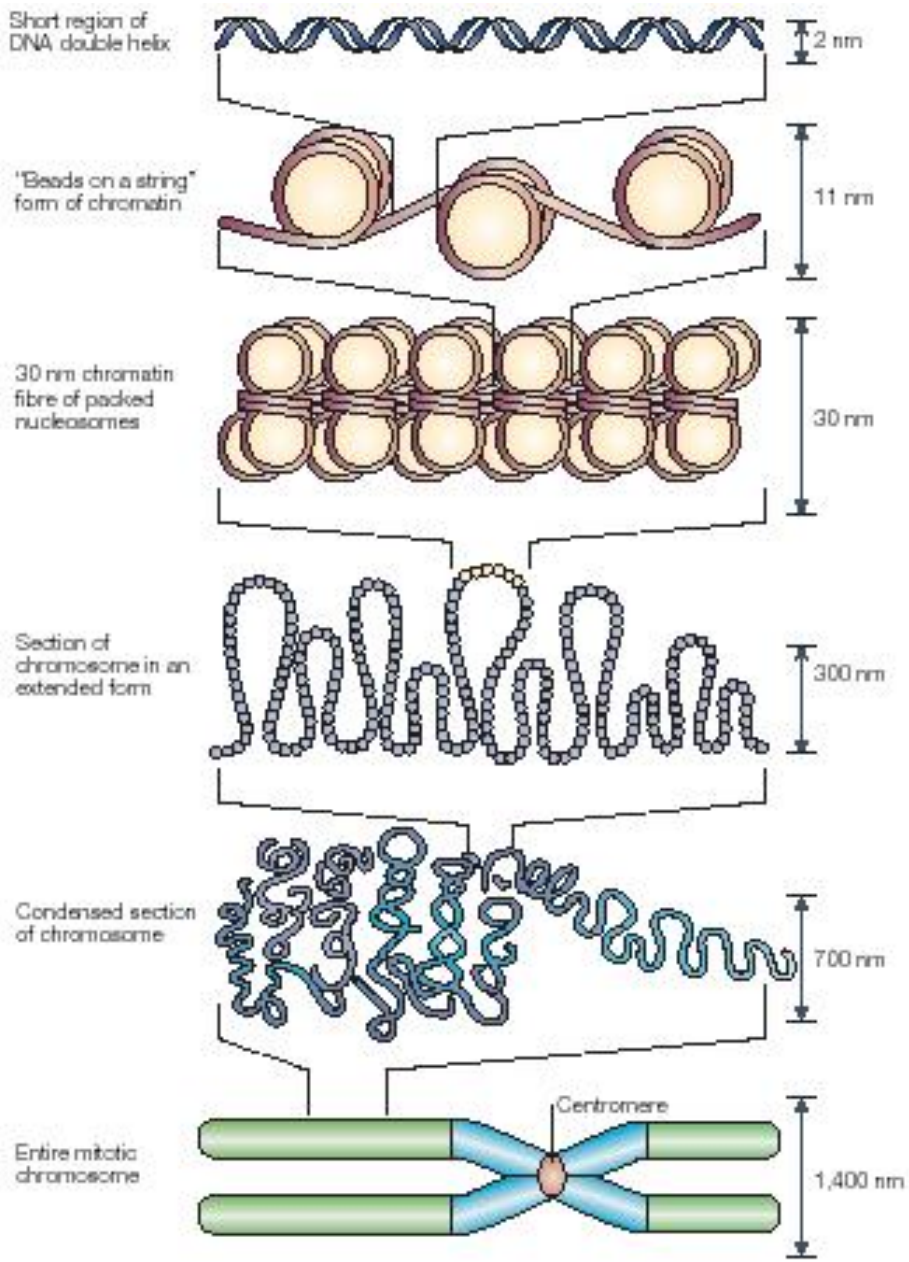
- Non-thermal biological effects of microwaves depend on several physical parameters and biological variables *[Belyaev et al, IEEE Transactions on Microwave Theory and Techniques, 48 (2000) 2172-2179]*
- Therefore, only results obtained under the same conditions of exposure should be compared in "replication" studies

Installation for exposure to linear and circular-polarised microwaves

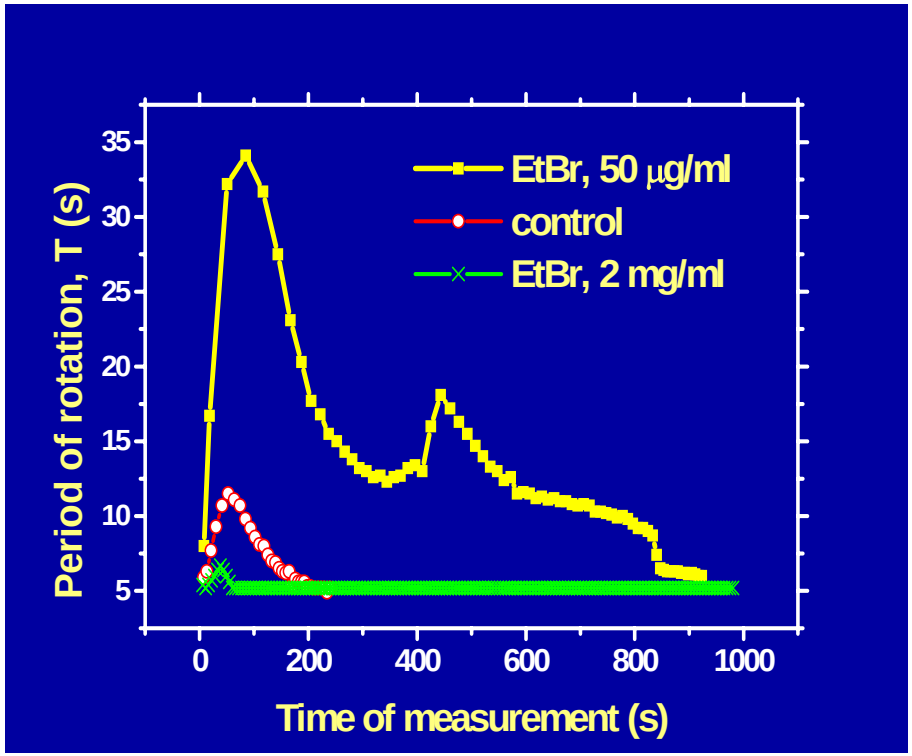


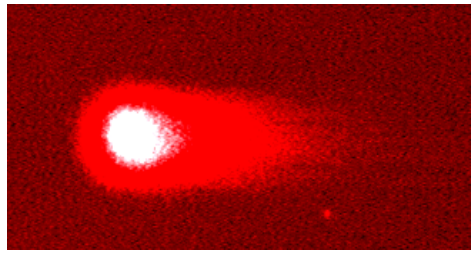
1 - oscillator; 2- waveguides; 3- directional coupler; 4- attenuators; 5- measuring line; 6- mixer; 7, 8 -frequency converters; 9- frequency meter; 10- detector; 11- voltage ratio meter; 12- thermistor detector; 13- power meter; 14- waveguides with quarter-wave mica plates; 15- conical horns.

Different levels of DNA-organization (DNA-loops)



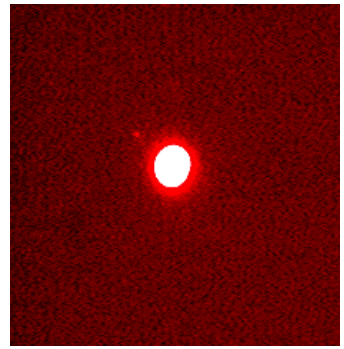
Anomalous viscosity time dependence (AVTD) similar to neutral comet assay provides possibility to measure relaxation and condensation of DNA loops

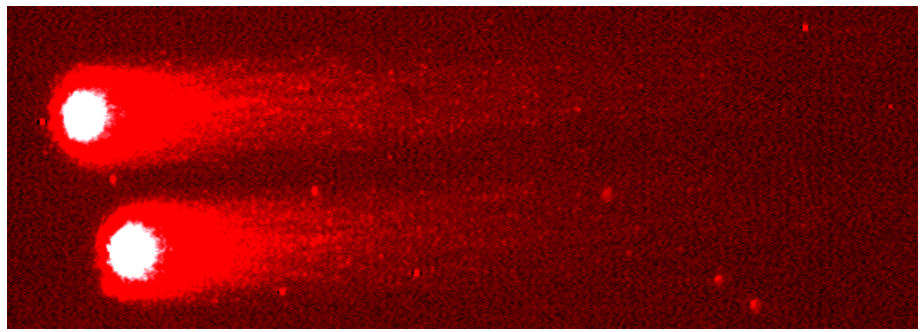




▲ Control

EtBr, 2 mg/ml, condensation ▶



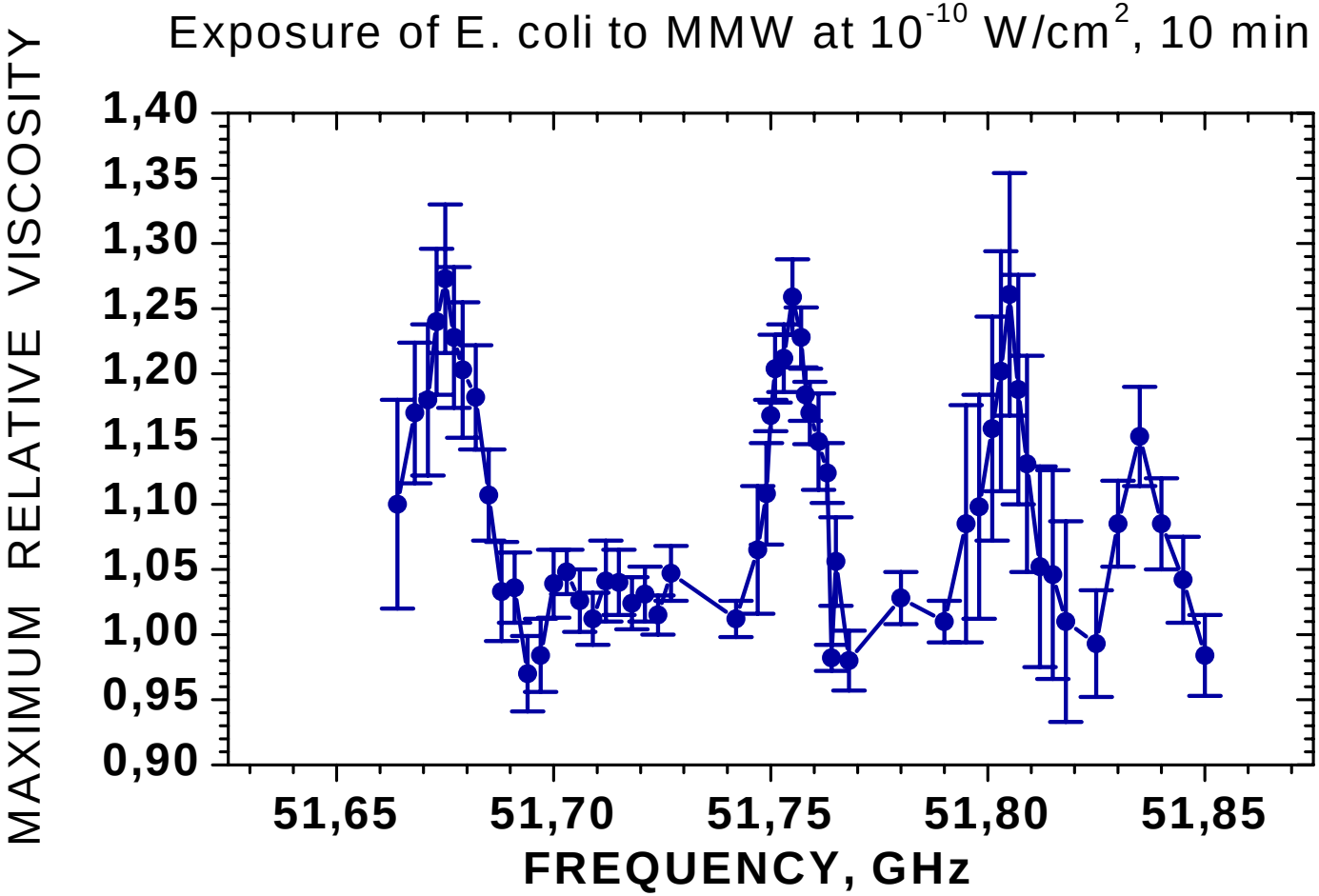


I. Y. Belyaev, S. Eriksson, J. Nygren, J. Torudd, and M. Harms-Ringdahl, *Biochim Biophys Acta*, vol. 1428, pp. 348-356, 1999

▲ EtBr, 50 μg/ml relaxation

Relaxation and condensation are characteristic responses to DNA damage and stress response

Frequency dependent effects of MWs at low intensities comparable with intensities produced by base stations



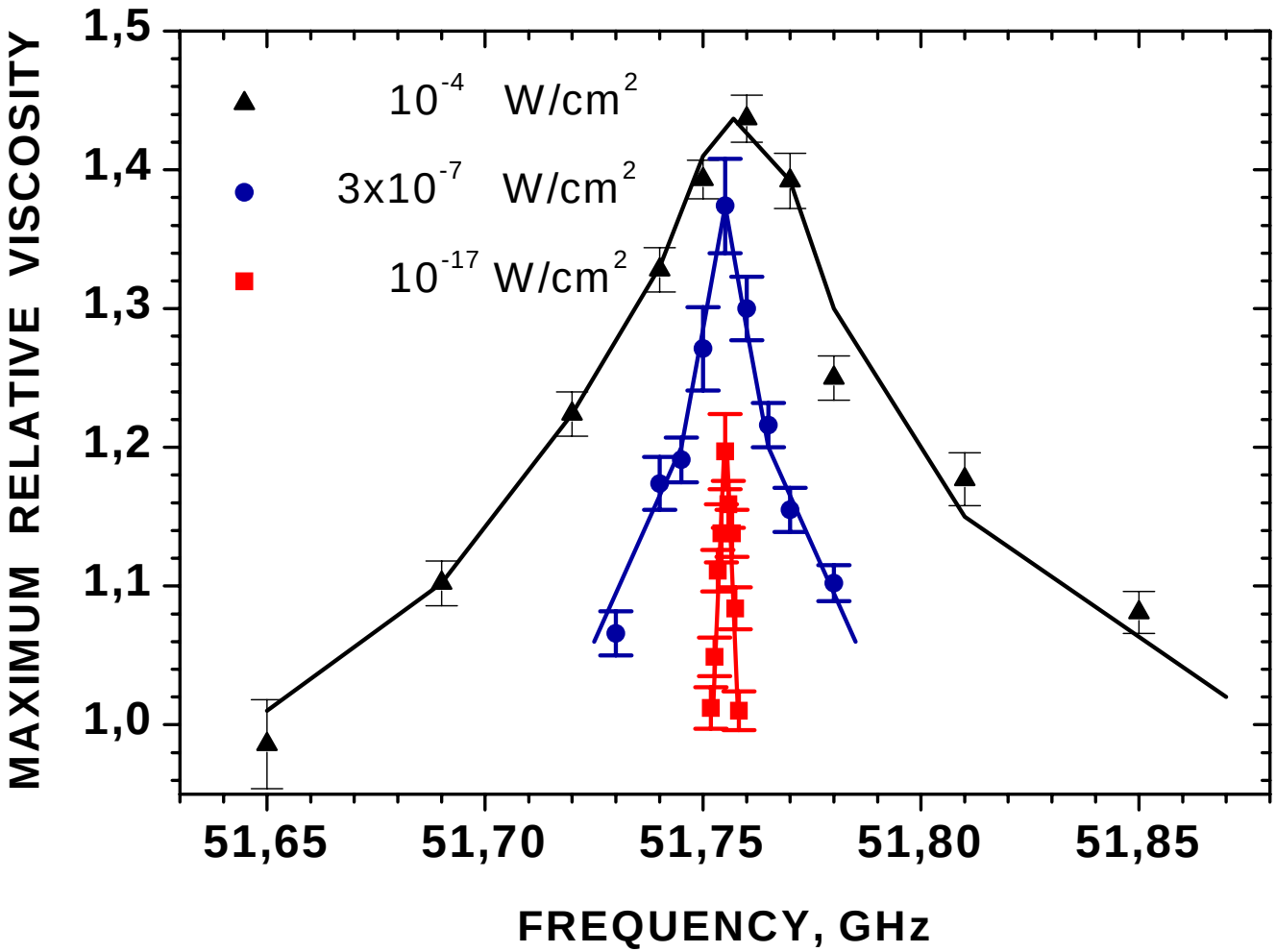
I. Y. Belyaev, V. S. Shcheglov, Y. D. Alipov, and V. A. Polunin, *Bioelectromagnetics*, vol. 17, pp. 312-321, 1996

Effective polarization in resonance responses of cells to non-thermal microwaves as measured with AVTD

Cells	Resonance frequency, GHz	Effective circular polarisation
E. coli K12 N99(λ,λ*imm*^{434}*bio*^{10})	41.277±0.002	Right-handed
Wistar rat thymocytes	41.303±0.001	Right-handed
E. coli K12 N99(λ)	41.305±0.001	Right-handed
E. coli K12 AB1157	41.32±0.01	Right-handed
E. coli K12 N99	41.324±0.001	Right-handed
Wistar rat thymocytes	41.61±0.01	Left-handed
E. coli K12 AB1157	51.675±0.001	Left-handed
E. coli K12 N99(λ,λ*imm*^{434}*bio*^{10})	51.723±0.001	Left-handed
E. coli K12 N99(λ)	51.740±0.001	Left-handed
E. coli K12 AB1157	51.755±0.001	Left-handed
E. coli K12 N99	51.765±0.002	Left-handed
E. coli K12 AB1157	51.805±0.002	Right-handed
E. coli K12 AB1157	51.835±0.005	Left-handed

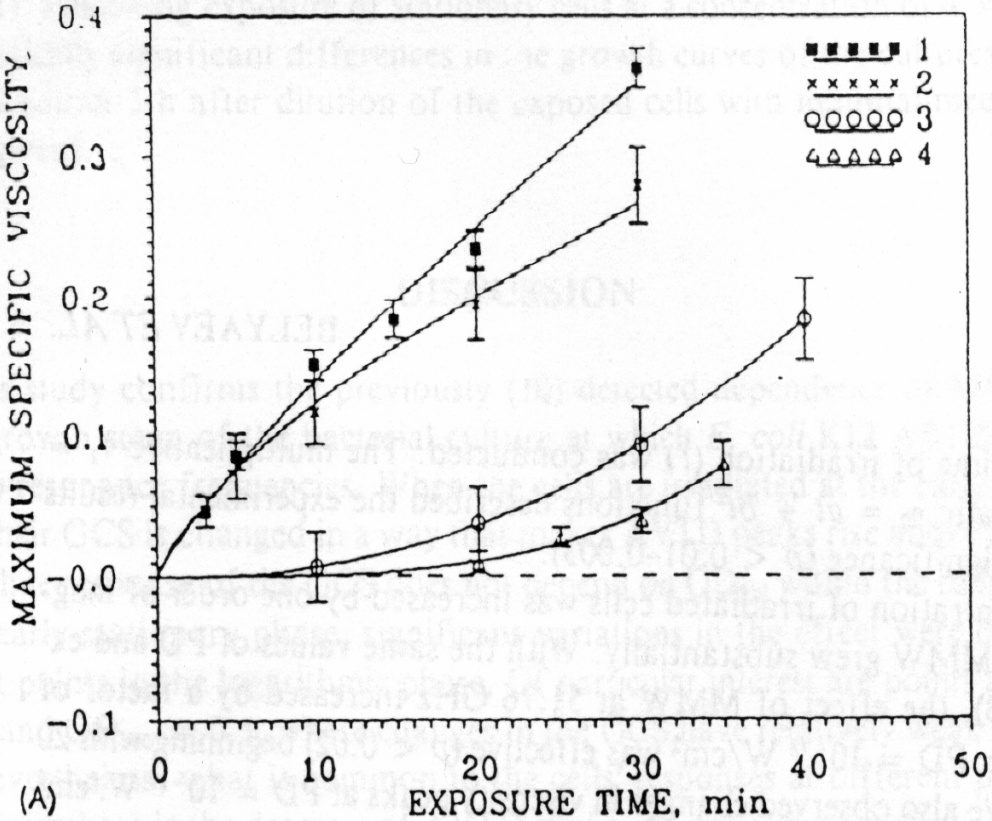
I. Y. Belyaev, V. S. Shcheglov, E. D. Alipov, and V. L. Ushakov, *IEEE Transactions on Microwave Theory and Techniques*, vol. 48, pp. 2172-2179, 2000

With decrease in intensity, the narrowing of microwave resonance windows was seen.



I. Y. Belyaev, V. S. Shcheglov, Y. D. Alipov, and V. A. Polunin, *Bioelectromagnetics*, vol. 17, pp. 312-321, 1996

Decreasing of intensity by orders of magnitude could be compensated by several-fold increasing of exposure time



(1) 10^{-14} W/cm^2;

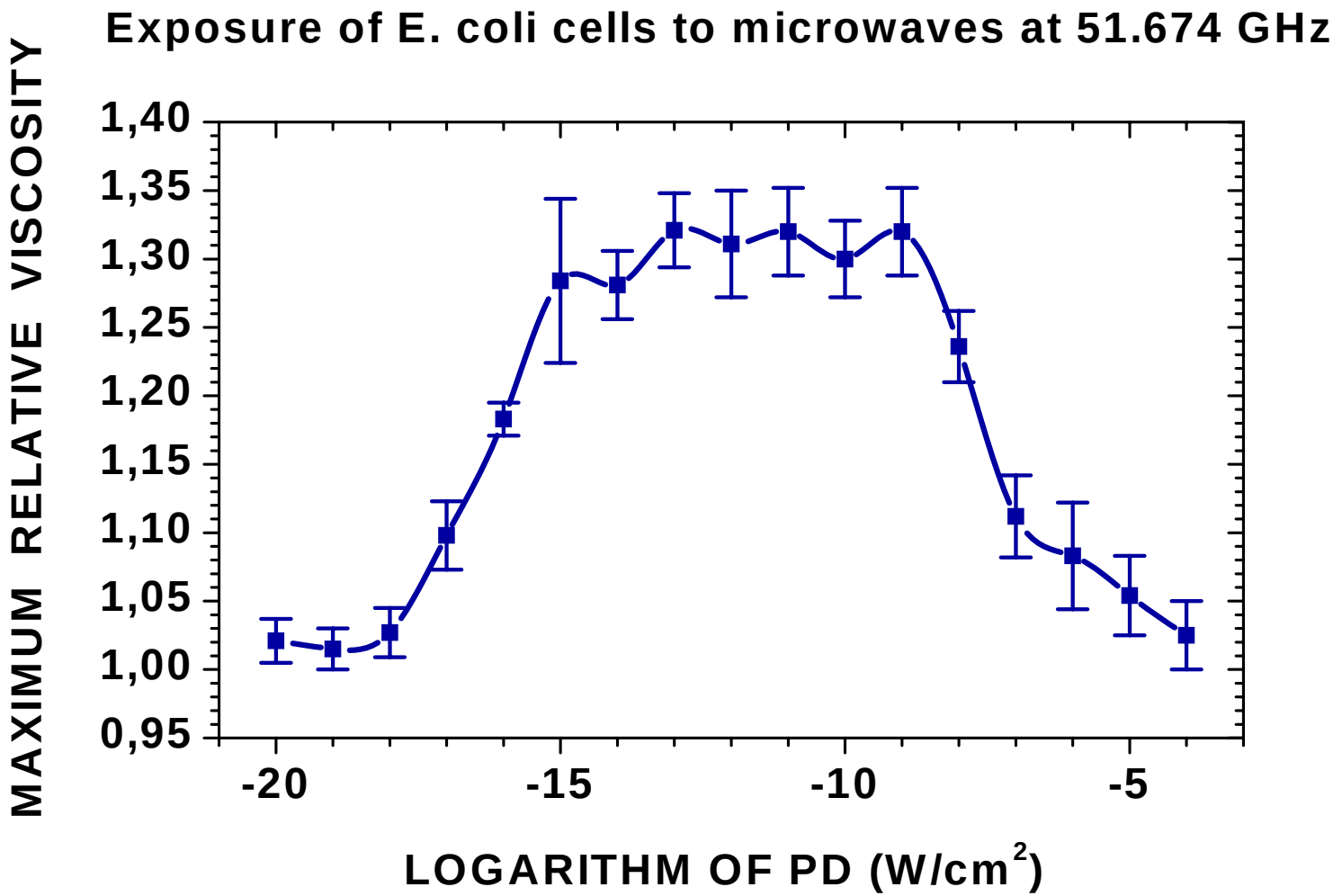
(2) 10^{-16} W/cm^2;

(3) 10^{-17} W/cm^2;

(4) 10^{-18} W/cm^2;

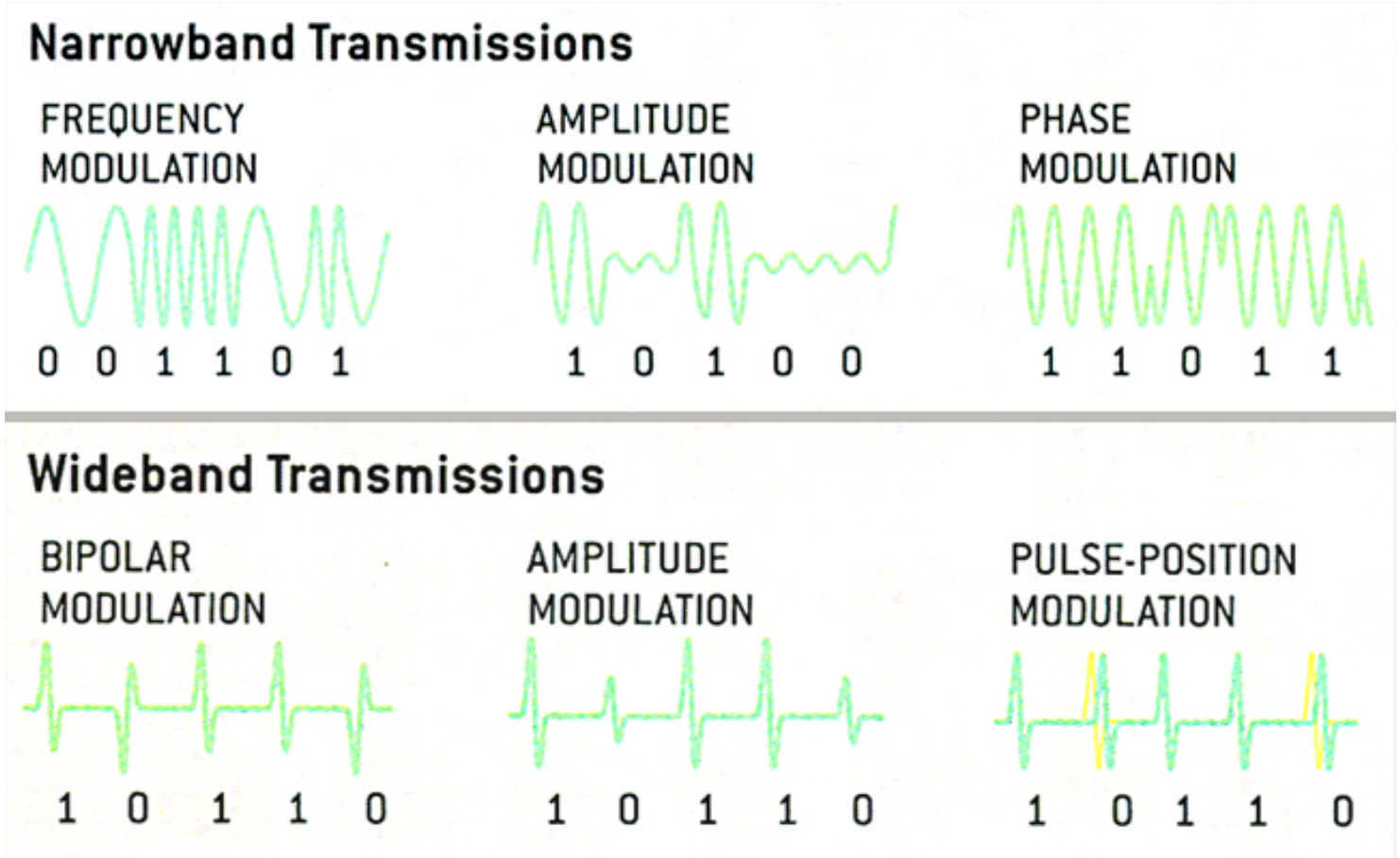
I. Y. Belyaev, Y. D. Alipov, V. S. Shcheglov, V. A. Polunin, and O. A. Aizenberg, *Electro- and Magnetobiology*, vol. 13, pp. 53-66, 1994.

Microwave effects are observed at specific ”intensity windows”



V. S. Shcheglov, I. Y. Belyaev, V. L. Ushakov, and Y. D. Alipov, *Electro- and Magnetobiology*, vol. 16, pp. 69-82, 1997

Narrowband & Wideband Modulation Schemes



D.G. Leeper, Sci Am 286 (5):68, 2002

Evidence for the role of modulation in effects of microwaves obtained in Soviet Union/Russia

•A.G. Pakhomov, M.B. Murphy, Comprehensive review of the research on biological effects of pulsed radiofrequency radiation in Russia and the former Soviet Union. In: Advances in Electromagnetic Fields in Living System, V.3 (J. C. Lin, ed.), Kluwer Academic/Plenum Publishers, New York, 2000, 265-290. …a number of good-quality studies have convincingly demonstrated significant bioeffects of pulsed microwaves. Modulation often was the factor that determined the biological response to irradiation, and reactions to pulsed and CW emissions at equal time-averaged intensities in many cases were substantially different.

•Grigoriev Yu. G., ROLE OF MODULATION IN BIOEFFECTS OF ELECTROMAGNETIC FIELDS (SUMMARY OF RUSSIAN STUDIES), ЕЖЕГОДНИК РНКЗНИ (Russian National Commission for Protection from Non-Ionizing Radiation), 2004, (review in Russian)

Examples for dependence of microwave effects on different types of modulation (Western scientists)

- **Amplitude modulation** 16 Hz but not 60 Hz or100 Hz modulated microwaves, 450 MHz, increased activity of ornithine decarboxylase (ODC) (Byus et al., Cancer Res, 48, 4222-6, 1988)

- **Speech-modulated** 835 MHz microwaves produced no effect on ODC as compared to typical signal from a TDMA digital cellular phone (Penafiel et al., Bioelectromagnetics, 18, 132-41, 1997).

- **Phase-modulated** GSM-1800 microwaves (Gaussian minimum shift keying, GMSK), 1.748 GHz, induced micronuclei in human lymphocytes while CW microwaves did not (d'Ambrosio et al., Bioelectromagnetics, 23, 7-13, 2002)

Important features of non-thermal MW effects

•Effects of resonance type within specific frequency windows.
•Dependence on type of signal, modulation, and polarization.
•Resonance effects are observed in specific intensity windows including super-low power densities (PDs) comparable with intensities from base stations/masts.
•With decrease in intensity, narrowing of the resonance windows occurs.
•The non-thermal MW effects are more sensitive to the duration of exposure than to the PD in the range of 10^{-17}-10^{-6} W/cm^2. Decreasing of PD by orders of magnitude can be compensated by several-fold increasing of exposure time. Therefore, duration of exposure may have significantly larger role as compared to PD.
•Some of these features indicate quantum-mechanical mechanism for non-thermal MW effects
•The effects depend on cell density suggesting cell-to-cell interaction during response to MWs. Theoretical modeling of experimental data suggested that electromagnetic field is involved in this intercellular communication and that cell density in tissues is determined by effective intercellular electromagnetic communication between cells in the millimeter-infrared frequency range.
•Radical scavengers/antioxidants have a potential to abolish MW effects.
•The effects depend on physiological conditions during exposure.
•Genomic differences influence response to MWs.

Were real signals as used in mobile communication tested for effects?

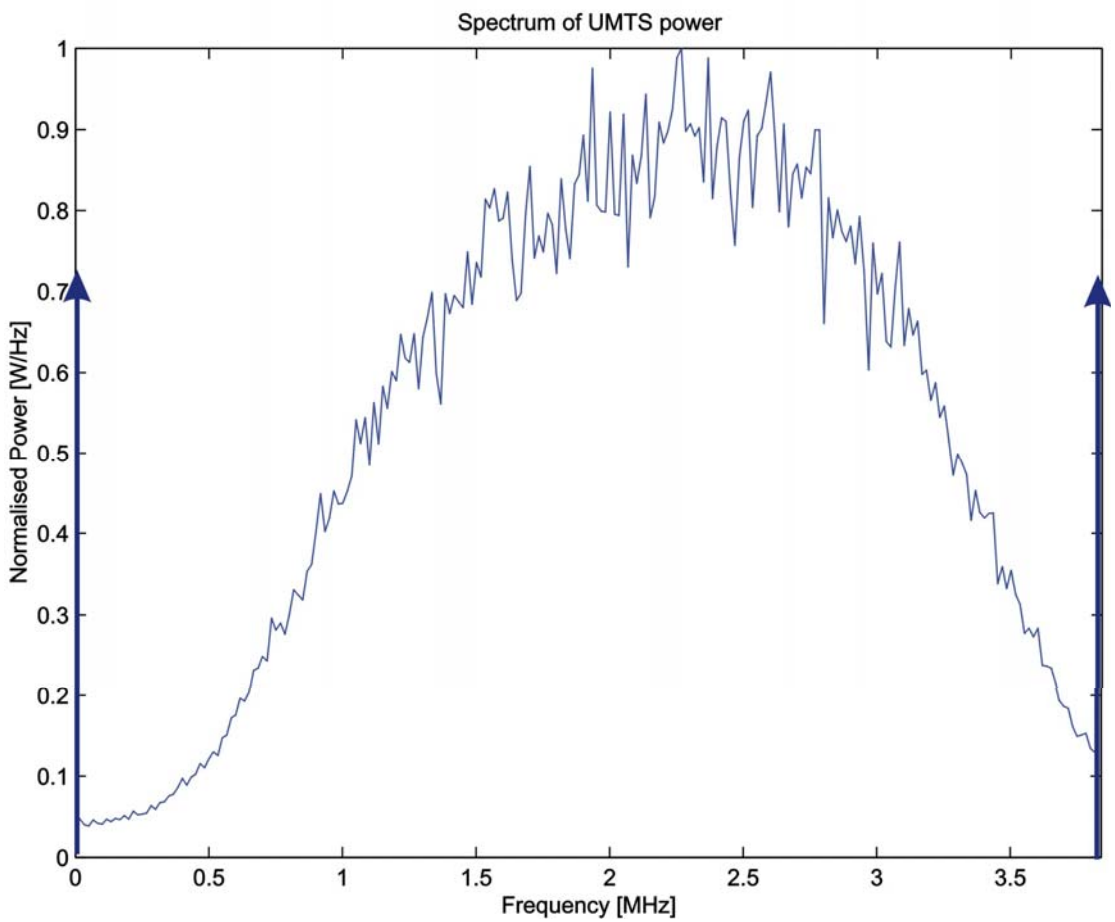
- Very little has been done with real signals. Most of them have not been tested so far

- We started to test real signals from GSM (Global System for Mobile Communication) mobile phones and UMTS (Universal Global Telecommunications System) mobile phones of the 3rd generation as used in Europe

During talks, GSM users are exposed to microwaves at different frequencies

•There are 124 different channels/frequencies, which are used in GSM900 (Global System for Mobile Communication). They differ by 0.2 MHz in the frequency range between 890 MHz and 915 MHz. Frequency is supplied by base station to a mobile phone user depending on amount of connected users. The frequency can be changed by base station during the same talk.

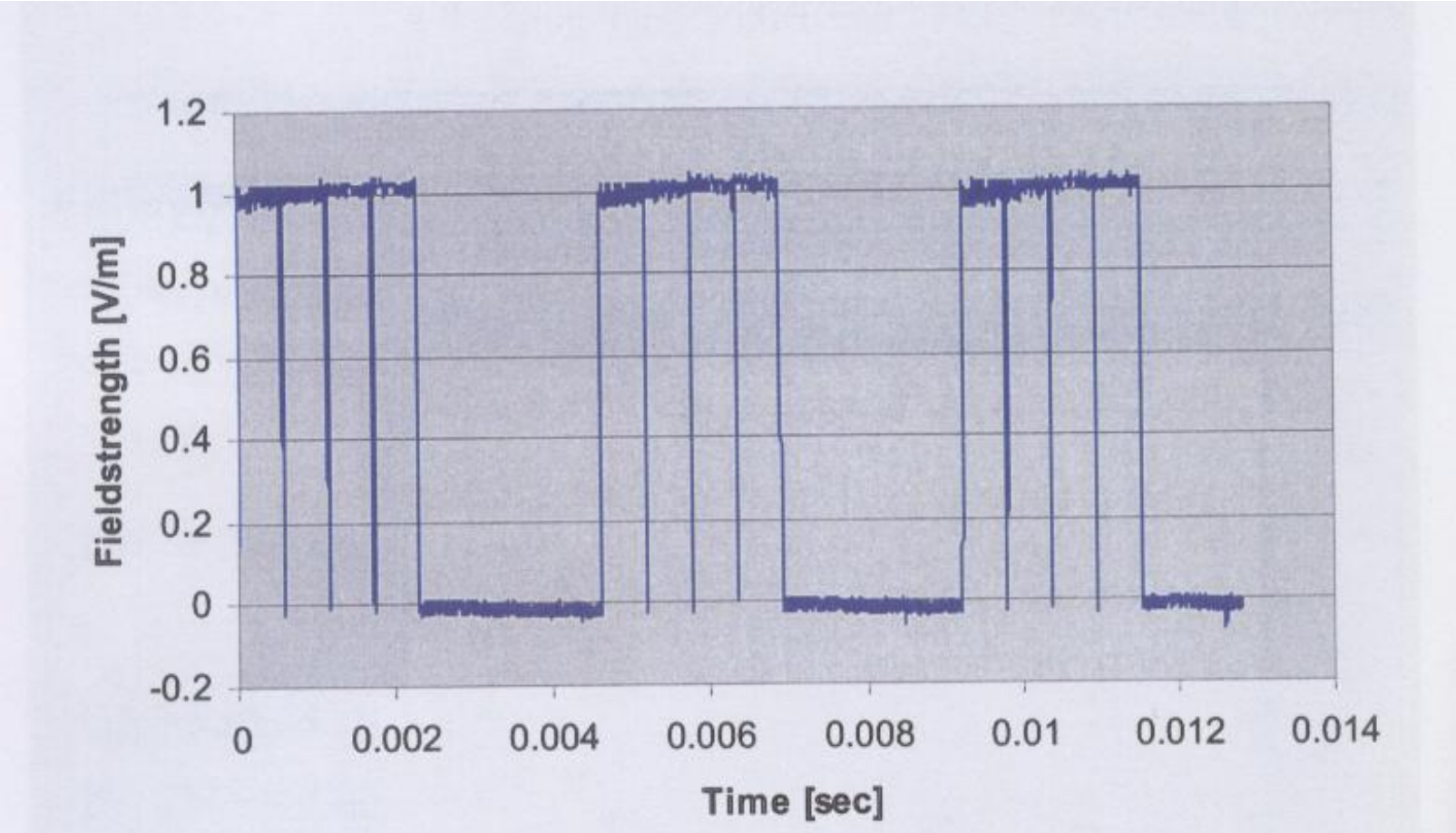
UMTS versus GSM



Contrary to GSM phones, mobile phones of the 3rd generation irradiate UMTS (Universal Global Telecommunications System) wide-band signal. UMTS MWs may result in higher biological effects because of eventual “effective” frequency windows.

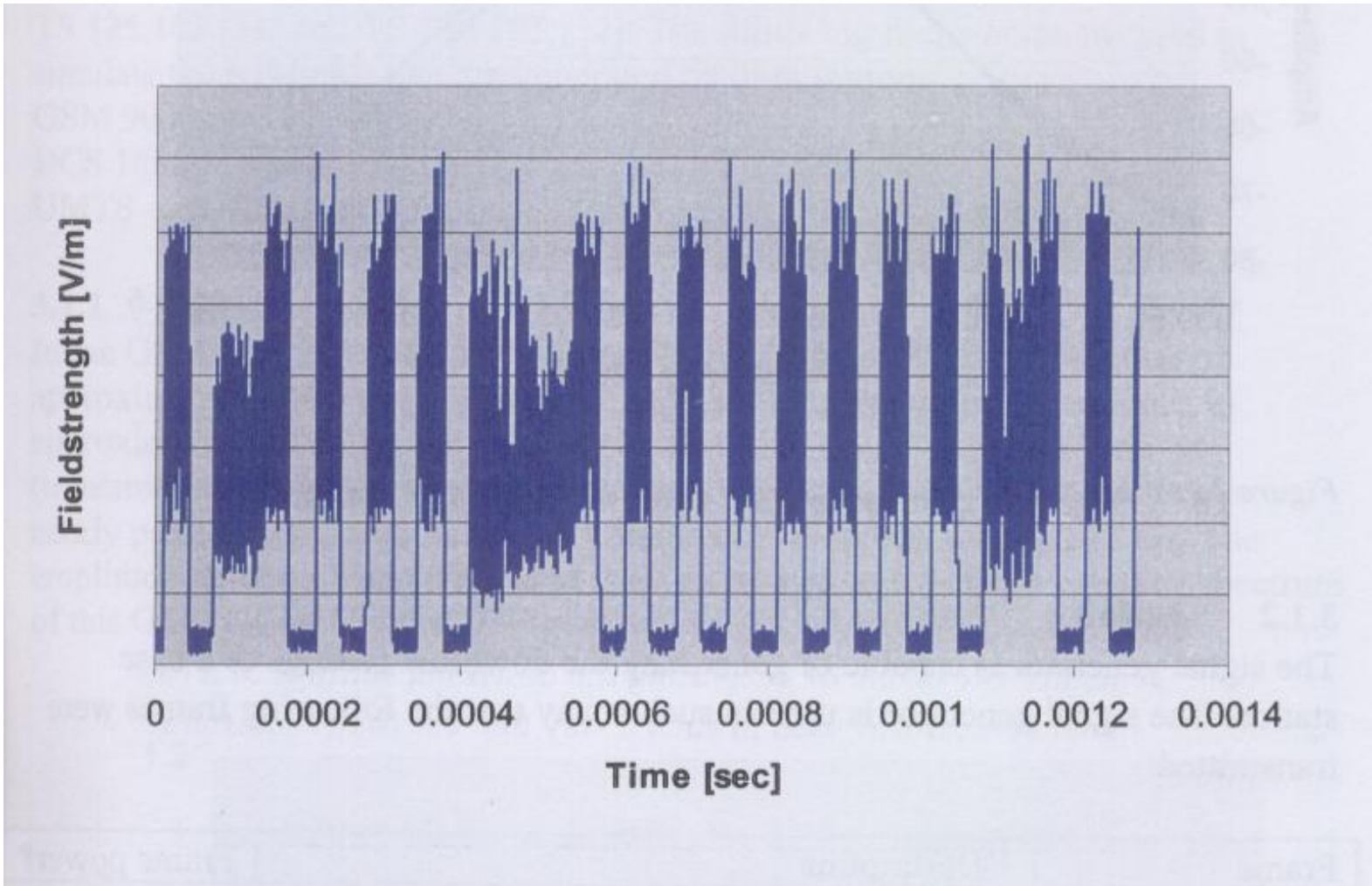
GSM modulations

GSM uses GMSK modulation (Gaussian Minimum Shift Keying)



UMTS modulations

W-CDMA (3G) uses essentially QPSK modulation



Effects of GSM and UMTS MWs and on human lymphocytes

- Lymphocytes from persons reporting hypersensitivity to MWs and matched healthy persons were exposed to GSM (905 MHz and 915 MHz) and UMTS (1947.4 MHz, middle channel), output power being the same, 0.25 W.
- Exposure, 1 h, was performed in a transverse electromagnetic transmission line cell (TEM-cell) using a GSM/UMTS test-mobile phone
- Changes in chromatin conformation, which are indicative of stress response and DNA damage, were measured by the method of anomalous viscosity time dependencies (AVTD).
- 53BP1 and γ-H2AX proteins that produce distinct foci co-localizing with DNA double strand breaks (DSBs) were analyzed by immunofluorescence laser confocal microscopy *in situ*.

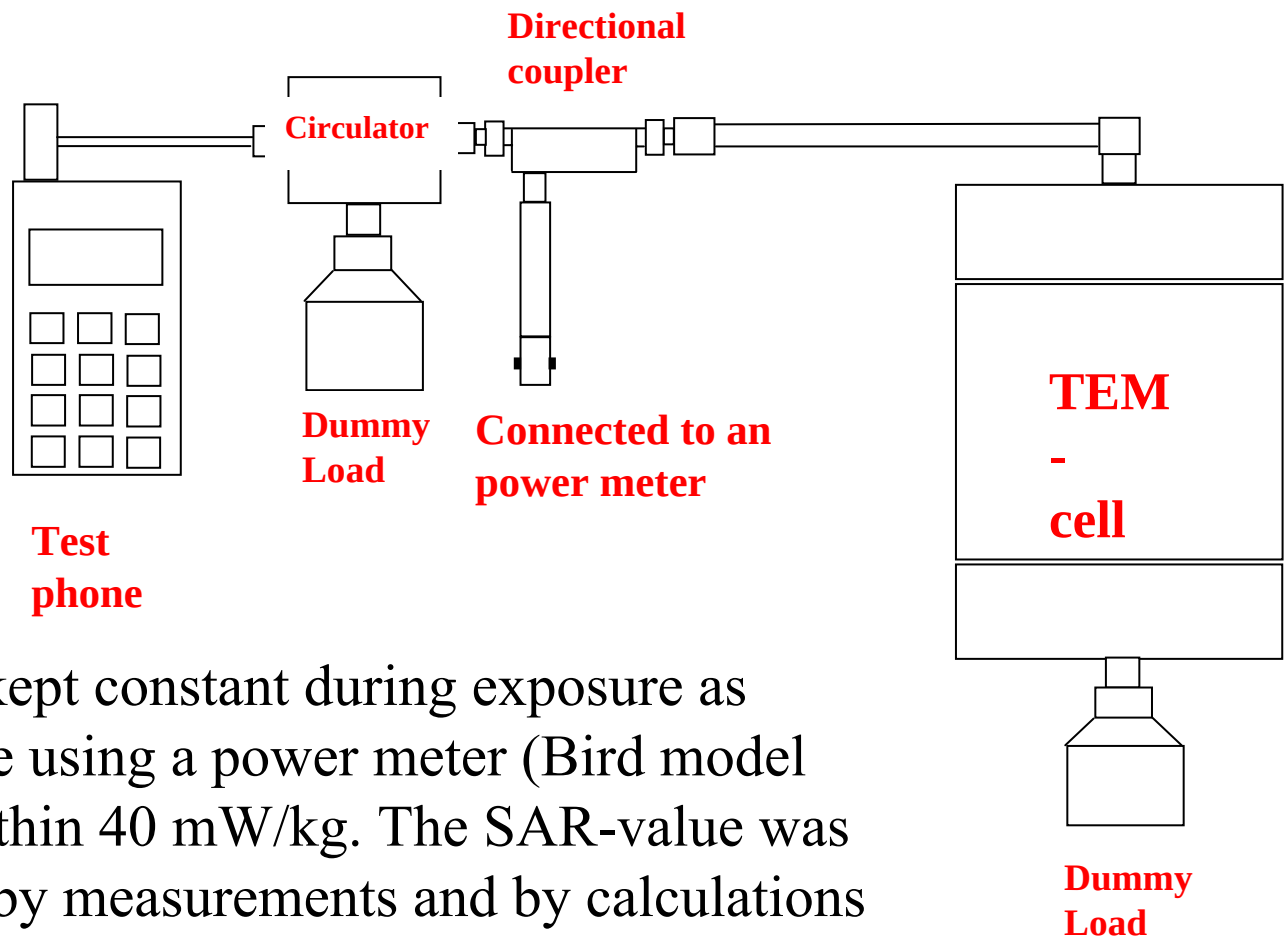
I. Y. Belyaev, L. Hillert, M. Protopopova, C. Tamm, L. Malmgren, B. Persson, G. Selivanova, and M. Harms-Ringdahl, ***Bioelectromagnetics,*** *in press*, 2005.

E. Markova, L. Hillert, L. O. G. Malmgren, B. R. R. Persson, and I. Belyaev, *Environmental and Health Perspective, submitted*, 2004.

R. Sarimov, L. O. G. Malmgren, E. Markova, B. R. R. Persson, and I. Y. Belyaev, *IEEE Transactions on Plasma Science*, vol. 32, pp. 1600-1608, 2004.

MICROWAVE EXPOSURE IN TEM-CELL BASED ON TEST-MOBILE GSM900/UMTS PHONE

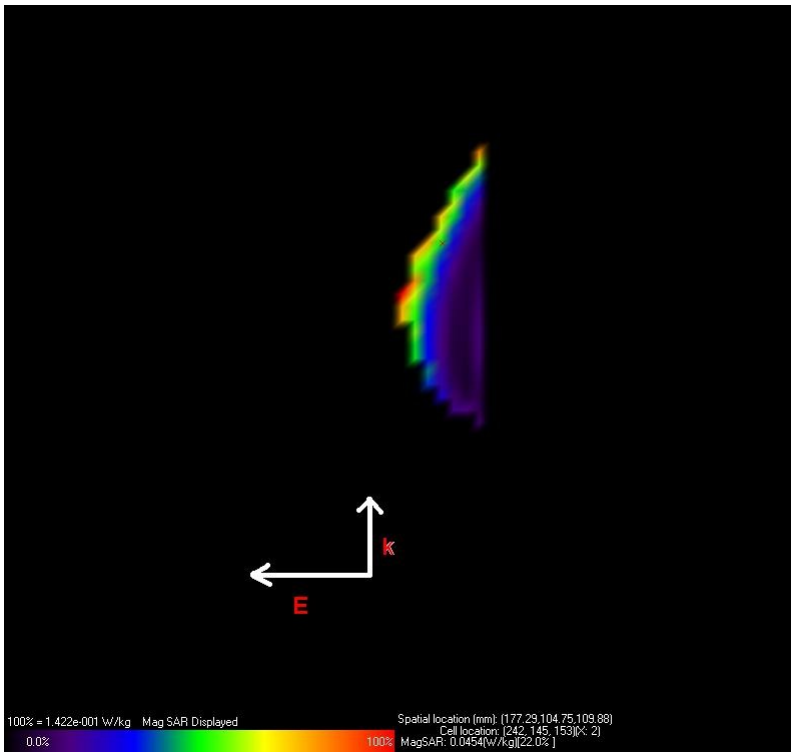
The test-mobile phone is programmed to choose the GSM frequency or UMTS band, and 0.25 W output power.



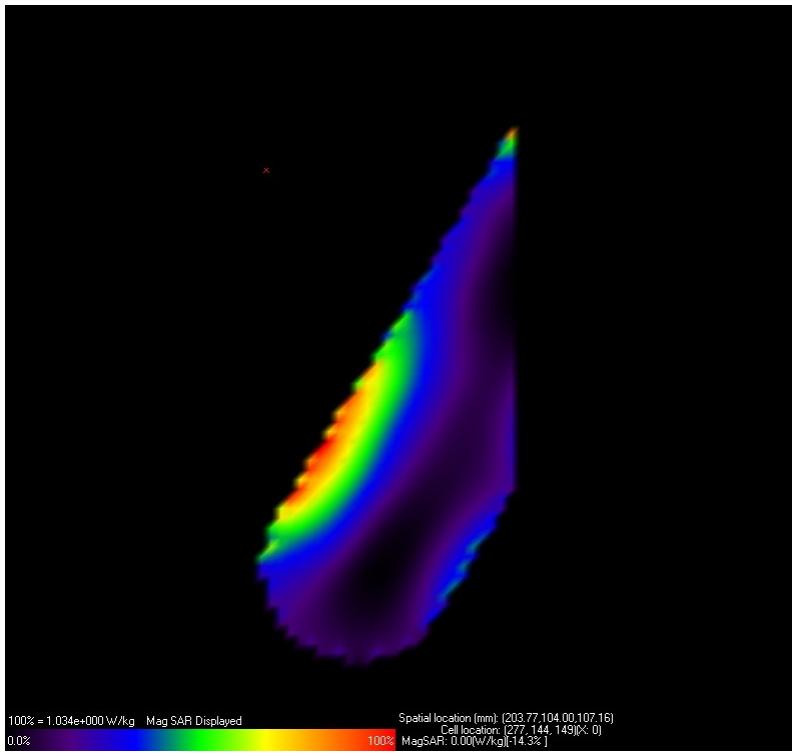
This power was kept constant during exposure as monitored on-line using a power meter (Bird model 43). SAR was within 40 mW/kg. The SAR-value was determined both by measurements and by calculations using the finite different time domain (FDTD)-method.

CALCULATIONS USING THE FDTD-METHOD

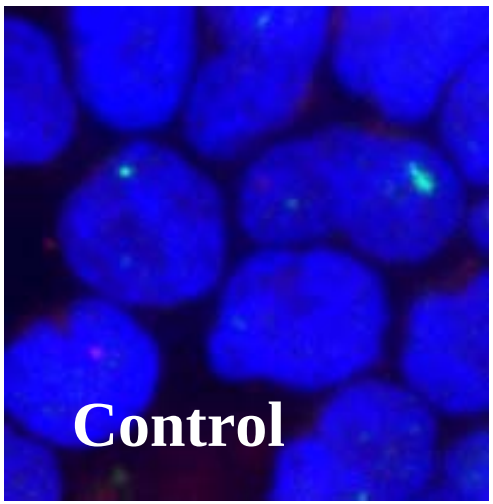
0.5 ml sample

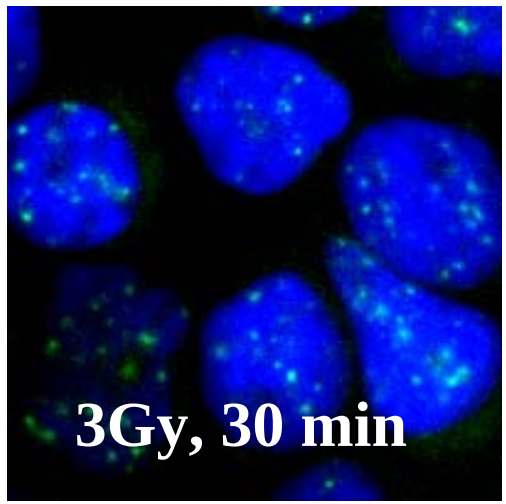


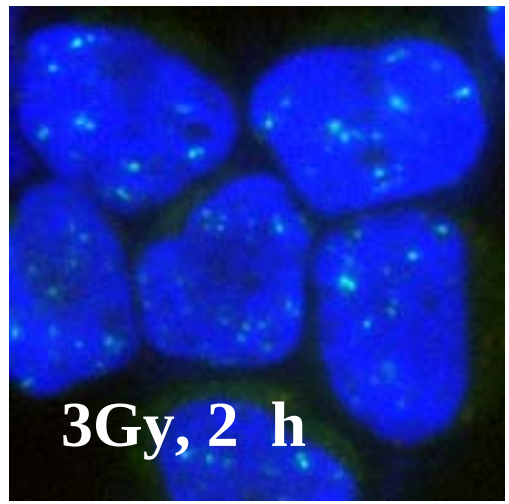
5 ml sample



ANALYSIS OF DNA DOUBLE STRAND BREAKS (DSB) WITH DSB-CO-LOCALIZING PROTEINS SUCH AS TUMOR SUPPRESSOR P53 BINDING PROTEIN 53BP1

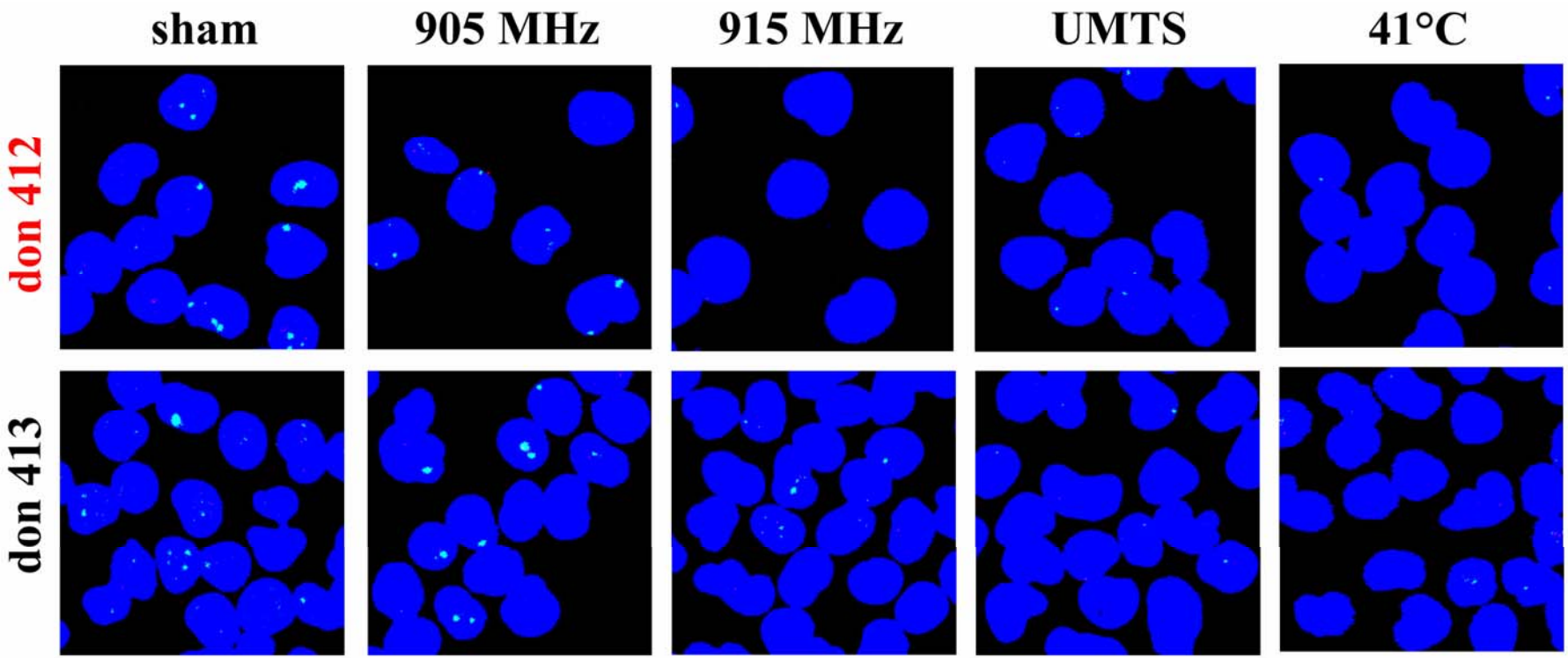






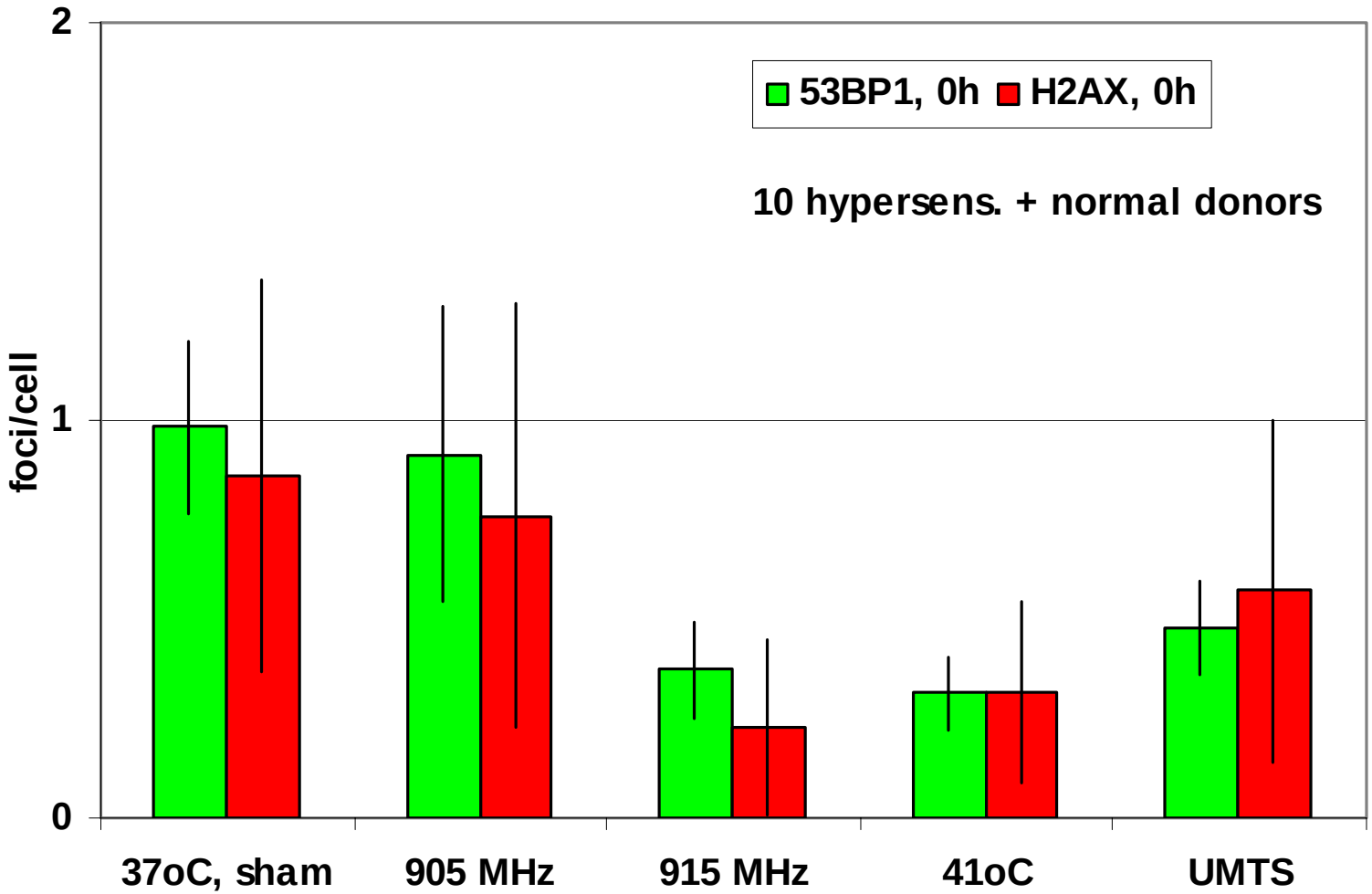
Formation of 53BP1 foci (stained in green) 30 min and 2 h after γ-irradiation of human lymphocytes (counterstained in blue) with 3Gy, as measured by immunostaining with antibody to 53BP1 protein that relocalizes to DNA double strand breaks

MICROWAVES AFFECT FOCI IN HUMAN LYMPHOCYTES DEPENDENT ON FREQUENCY AND SIMILAR TO HEAT

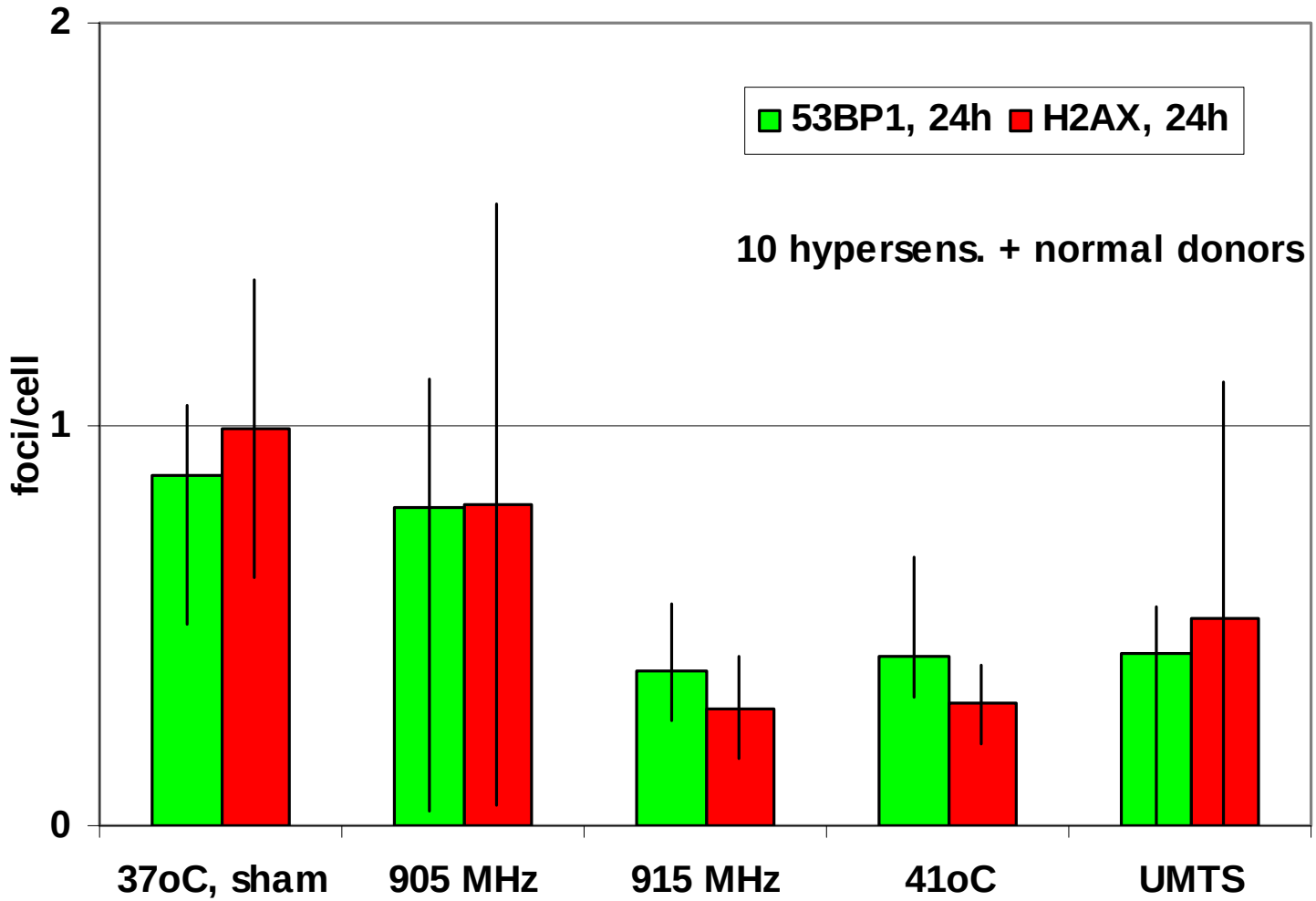


Formation of **53BP1 foci (stained in green)** and γ-H2AX foci (red) after 1-h exposure of human lymphocytes (counterstained in blue) with MWs, and heat shock

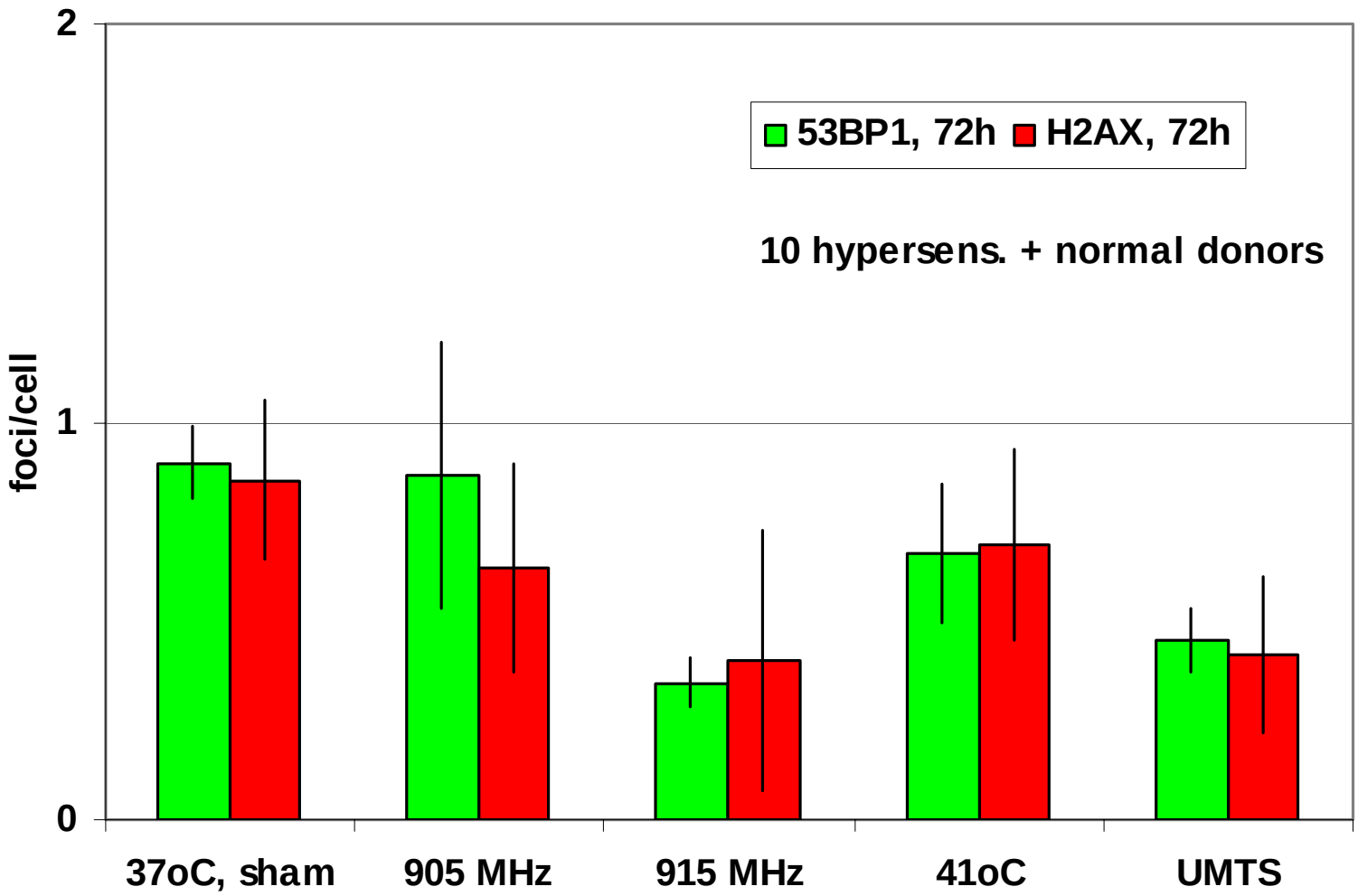
REDUCTION OF FOCI IMMEDIATELY AFTER 1 h EXPOSURE TO 915 MHz GSM, UMTS AND heat shock (no effect at 905 MHz)



EFFECTS OF 915 MHZ GSM AND UMTS ON FOCI IS OBSERVED 24 H AFTER EXPOSURE TO MWs



EFFECTS OF 915 MHZ GSM AND UMTS ON FOCI REMAIN EVEN 72 H AFTER EXPOSURE TO MWs



CONCLUSIONS

•Studies at cellular level have shown that non-thermal effects of MWs at lower levels than the ICNIRP (International Commission for Non-Ionizing Radiation Protection) safety standards depend on several physical and biological parameters. Frequency-dependent effects of non-thermal microwaves from GSM mobile phone on 53BP1/γ-H2AX foci and chromatin conformation in human lymphocytes are observed. UMTS MWs induce significant adverse effects in human lymphocytes similar to effects of heat shock and GSM MWs at effective frequencies. The obtained results are in line with hypothesis that UMTS MWs may affect cells more efficiently than GSM MWs because of the nature of signal.

•The effects of MWs from mobile phones on 53BP1/γ-H2AX foci persisted up to 72 h following exposure of lymphocytes. This long-lasting adverse effect on these important cells of our immune system can have strong relationship with health risk from mobile telephony.

•*In vitro* studies indicate that the duration of exposure can be more important for non-thermal effects than intensity and therefore, effects of MWs from base stations on primary human cells should be studied.

Comments: how mechanistic results can be used for interpretation of epidemiological cancer studies

•It is almost impossible to select control unexposed groups because whole population in industrial countries is exposed to wide range of MW signals from various sources such as base stations/masts, WILAN (Wireless Local Area Networks), WPAN (Wireless Personal Area Networks such as Bluetooth) wireless phones and given that duration of exposure (must be at least 10 years for cancer studies) may be more important for adverse health effects of non-thermal MWs than intensity. The adverse effects of "detrimental" MW signals are diluted because people are exposed to various signals/frequencies including non-effective or even hypothetically beneficial.

•Available mechanistic studies indicate that at this point, the epidemiological studies can be either inconclusive, if negative, or underestimate significantly the hazard of using specific MW signals, if positive.

Comments on urgent needs and perspective 1:

•ICNIRP continue disregarding non-thermal effects of MWs and their complex dependence on several physical and biological parameters. Usually, these important parameters are not controlled in so called "replication studies" of non-thermal effects of MWs and therefore, the data cannot be compared with the original results. The EU-program and the national programs for mechanistic studies of non-thermal effects of MWs (both from mobile phones and base stations) should be established. Those types and frequency channels/bands for mobile communication, which do not affect human should be identified.

•Because non-thermal MWs affect not only brain cells, but also blood cells and probably all human cells including cells of reproductive organs, the using of hands-free can not minimize all health effects. Possibilities to minimize the adverse effects of non-thermal MWs using different approaches should be studied.

Comments on urgent needs and perspective 2:

- To inform people regarding:

1. *evidence for biological effects of non-thermal MWs from mobile telephony*
2. *lack of comprehensive scientific knowledge to establish relevant safety standards for non-thermal effects from mobile telephony*

- To reduce usage of mobile communication (such as number and duration of calls) and especially UMTS signals of 3rd generation unless these specific signals have not been tested in experiments with primary human cells.
- Children may represent most sensitive group to non-thermal effects of mobile telephony. Before this issue will be investigated, precautions should be taken in erection of base stations in vicinity of schools. Creation of zones that are free of mobile communication for hypersensitive to EMF should be considered
- To include in education programs at different levels the results of studies of non-thermal effects of electromagnetic fields

COLLABORATIONS ON REPORTED DATA

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