High-throughput screening for the investigation of the biological effects of millimetre-wave radiation

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Summary

This thesis reports on the design and evaluation of a high-throughput screening system for investigating the biological effects of microwave and millimetre-wave radiation. The approach presented here differs significantly from others with the deployment of a continuous-culture device and sample handling technology that allows for the rapid presentation of test samples in a uniform physiological state. The millimetre-wave exposure cell was operated as a flow-through device. To remove convolution-by-flow effects this was combined with a segmentation scheme. A sensitive bioluminescence-based reporter monitored the temporal evolution in each assay segment using optical detector arrays mounted at three observation points: - pre-millimetre-wave exposure, syn- exposure and post-exposure. With this approach, the continuous monitoring of bioluminescence may be used on different time scales to measure energy metabolism, gene expression and growth. Exposed and control samples were monitored and any combination of stimulus parameters, namely, radiation frequency, intensity and polarization (or any modulation of these), which induced a response that exceeded a noise threshold of the system could be further investigated automatically in real-time. The device could screen systematically or use feedback to automatically investigate a region of interest at increasing resolution in parameter space. Environmental parameters such as temperature and magnetic and electric fields were carefully controlled. The instruments operation was characterized over the 26 and 40 GHz frequency range and this gave confidence that the technique could be applied to the entire mm-wave range. This sensitive “active” search system may have application in industry, biomedical research and environmental health.

Declaration

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

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STATEMENT 1
This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and the summary to be made available to outside organisations.

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“I believe that this concept (i.e. quantum coherence) may have a much wider range of application, in particular, in systems which are relatively stable but not near thermal equilibrium, and which show an organised collective behaviour which cannot be described in terms of an obvious (static) spatial order”

H. Fröhlich, Proceedings of the first International Conference on Theoretical Physics and Biology, Versailles, 1967

Publications

Several papers based on the work contained within this thesis have been published or are in the process of being published.

D. Pooley, B. Ellison, C. Gibson and W. Stewart. “A cell-culture exposure system for millimetric wavelength radiation” Proceedings of the European Conference on Microwave Technology, London, 2001

D. Pooley, C. Gibson, W. Stewart, J. Magee and B Ellison. “Biological effects of millimeter-wave radiation: a high-throughput screening system”. Review of Scientific Instruments, March 2003. (Also featured in March 2003 edition of the Virtual Journal of Biological Physics Research).

D. Pooley, C. Gibson, W. Stewart, D. Lloyd, M Rayner-Brandes and G Jones. “The continuous culture of photobacterium”. Submitted. Biosensors and Bioelectronics January 2003

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Glossary

ATP

A nucleotide derived from adenosine. It is the major source of energy for cellular reactions.

Average SAR

Volume-averaged specific absorption rate

Bioluminescence

Luminescence (light emission) produced by physiological processes (as in the firefly).

Bose-Einstein condensation (BEC)

A phenomenon wherein the bosons making up a substance merge into the lowest energy level, into a shared quantum state. More generally, it refers to the tendency of bosons to occupy the same energy level.

Boson

Bosons are sub-atomic particles that have integral spin. They include mesons (e.g., pions and kaons) and nuclei of even mass number (e.g., helium-4).

Cell membrane

A thin membrane around the cytoplasm of a cell; controls passage of substances in and out of the cell

Chemostat

Device used for the continuous-culture of cells.

Chromosome

A threadlike body in the cell nucleus that carries the genes in a linear order

Circadian biology

Of or relating to biological processes occurring at 24-hour intervals

Classical coherence

Order introduced through a phase transition for example boiling.

Conformation (macromolecular conformation)

Non-oscillatory change in shape

Continuous-culture device

Device use to culture cells in constant physiological state over extended periods

Cytoplasm

The contents of a cell external to the nuclear membrane

DNA

A nucleic acid found in the nucleus of a cell and consisting of a polymer formed from nucleotides and shaped like a double helix; associated with the transmission of genetic information

Dielectric

A material with extremely low electrical conductivity

Electron transport chain

A complex chain of events and reactions and the basis of the cell's ability to derive ATP from metabolic oxidation.

Enzymes

Any of several complex proteins that are produced by cells and act as catalysts in specific biochemical reactions

Eukaryote

An organism with cells characteristic of all life forms except primitive microorganisms such as bacteria; i.e. an organism with 'good' or membrane-bound nuclei in its cells

Exponential phase culture

Culture that is not nutrient-limited

Extremely high frequency (EHF)

Electromagnetic radiation with frequency between 30 and 300 GHz also referred to as millimetre-wave radiation.

Fröhlich (Bose-Einstein-type) condensation

Theoretically formed in biological systems at physiological temperatures and akin to Bose-Einstein condensation; a macroscopic quantum effect.

Hydrogen bond

A chemical bond consisting of a hydrogen atom between two electronegative atoms (e.g., oxygen or nitrogen) with one side be a covalent bond and the other being an ionic bond.

Local SAR

Spatial distribution of energy

Luciferase

The enzyme system required for light emission

***Lux* operon**

Genes related to bioluminescence

Lysogeny

Type of life cycle that takes place in a phage (bacterial virus) following its infection of certain types of bacteria

Millimetre-wave or mm-wave

Electromagnetic radiation with frequency between 30 and 300 GHz sometimes referred to as “EHF”.

Organelle

A specialized and usually spatially localised part of a cell; analogous to an organ

Phage

Bacterial virus

Phonon

A quantum of lattice vibrational energy that in analogy to a photon, can be viewed as a wave packet with particle-like properties.

Prokaryote

A unicellular organism having cells lacking membrane-bound nuclei; bacteria are the prime example but also included are blue-green algae and actinomycetes and mycoplasma.

Radiometry and passive radiometry

A device used to detect and measure radiant energy (In this electromagnetic radiation in the microwave and millimetre-wave spectral range).

Soliton

A non-linear wave that propagates as a coherent entity

Specific Absorption Rate or SAR

Absorbed power W kg^{-1}

Stationary phase culture

Nutrient limited high density cell culture

Ultradian biology

Oscillatory biological processes with a periodicity less than 24 hr

Vibrational modes

A regular periodic variation in value about a mean

Background

Over the last thirty years a number of insights into the biological effects of interaction with mm-wave radiation have been presented, although their interpretation remains controversial (Gos et al. 1997b). In many cases, these publications relate to incident power exposures at levels below that recommended for human exposure (< 10 mW cm^{-2}) (Polk 1995b). Induced effects are often reported to have nonlinear power and frequency dependencies inconsistent with induction through heating (Polk 1995b). However, these may be related to what has been termed *complex behaviour* in biological systems.

Theoretical models such as the Fröhlich condensate (Fonseca et al. 2000; Frohlich 1982a; Mesquita, Vasconcellos, & Luzzi 1998b) or the Davydov soliton (Scott, Williams, & Lloyd 1983) are discussed in past papers in connection with experimental observations, although these have largely proved inconclusive with poor reproducibility and several confounding factors were identified (Furia, Hill, & Gandhi 1986b; Gos, Eicher, Kohli, & Heyer 1997b). Spectroscopic approaches to determine the existence of Fröhlich-condensate-type vibrational modes have been unsatisfactory, since their frequency coincides with the Brillouin zone boundary (Mesquita, Vasconcellos, & Luzzi 1998b).

The potential for human exposure to mm-wave radiation at frequencies and power levels that have been associated with biological effects will increase significantly in the coming decades. In part, this is due to the recognition of the significant commercial potential that this region offers for telecommunication systems, automobile collision avoidance radar, aviation radar and weather monitoring and other applications. These are all free-space transmission technologies in which human exposure is inherent and will greatly exceed background levels. In addition, millimetre-wave radiation is also used therapeutically in some countries. This is in contrast to the situation in 1986 (Furia 1986), where the scope

for human exposure was limited to satellite communications systems and military equipment.

The existence of non-thermal interactions would be highly significant as the current public-health exposure guidelines are thermally based, and non-thermal interactions could represent a potential avenue for the selective interaction with biological systems. This would have the potential for considerable social and economic impact.

Experiments involving millimetre-wave radiation and biological systems are, by necessity, complex and time-consuming. It was also recognized at the beginning of the project that historically, attempts to replicate millimetre-wave induced effects have met with failure. Rather than attempting to replicate prior experimental studies *per se*, the approach here is to develop a methodology that is flexible, providing the basis for experimentation on both eukaryotic and prokaryotic cultures and over a wide spectral range.

The thesis has been written so that it is accessible to a general scientific audience and specialists in radio frequency engineering and biology. A glossary is provided and words that appear in the glossary are indicated with bold type in the text.

Aims: -

Design and construct an instrument that is flexible and which can: -

(a) be deployed in the investigation of a wide variety of culturable cell types.

(b) employ a sensitive and higher integrative reporter to detect biological effects.

(c) systematically screen parameter space in reasonable time scales.

(d) attain high level of physiological reproducibility.

(e) incorporate feedback into the analysis and control system.

(f) be used to determine the existence or otherwise of non-thermal interactions in biological systems with confidence.