12^{th} International Bioelectrics Symposium

September 6-10, 2015

Portoroz, Slovenia

ONKOLOŠKI INŠTITUT LJUBLJANA INSTITUTE OF ONCOLOGY LJUBLJANA

Members of International Bioelectrics Consortium

Founding members of the consortium:

1. **Old Dominion University (ODU)**

Norfolk, Virginia, USA
Principal investigator: Richard Heller

2. **Karlsruhe Institute of Technology (KIT)**

Karlsruhe, Germany
Principal investigator: Wolfgang Frey & Georg Mueller

3. **Kumamoto University**

Kumamoto, Japan
Principal investigator: Hidenori Akiyama

Joined members of the consortium:

4. **University of Missouri (MU)**

Columbia, Missouri, USA
Principal investigator: Robert Druce

5. **Leibniz Institute for Plasma Science and Technology (INP)**

Greifswald, Germany
Principal investigator: Juergen F. Kolb, Klaus-Dieter Weltmann

6. **Institute of Pharmacology and Structural Biology (IPBS)**

Toulouse, France
Principal investigator: Marie-Pierre Rols

7. **University of South Florida (USF)**

Tampa, Florida, USA
Principal investigator: Richard Gilbert

8. **Institute Gustave-Roussy**

Paris, France
Principal investigator: Lluis M Mir

9. **Institute of Oncology Ljubljana (OI)**

Ljubljana, Slovenia
Principal investigator: Gregor Serša

10. **Institute of Plasma Physics (IPP)**

Prague, Czech Republic
Principal investigator: Peter Lukes

11. ENEA-Frascati & Institute of Translational Pharmacology (IFT)
Frascati & Rome, Italy
Principal investigator: Gian Piero Gallerano & Alfonsina Ramundo-Orlando

12. Institute for Electromagnetic Sensing of the Environment (IREA)
Naples, Italy
Principal investigator: Maria Rosaria Scarfi

13. Lisbon Engineering Superior Institute (GIAAPP)
Lisbon, Portugal
Principal investigator: Luis Redondo

14. University of Copenhagen, Herlev Hospital (C*EDGE)
Herlev, Denmark
Principal investigator: Julie Gehl

15. University of Technology Eindhoven (TUE)
Eindhoven, Netherlands
Principal investigator: A. J. M Pemen

A Brief History of the International Bioelectrics Consortium

Bioelectrics has emerged as a new, exciting research field. It deals with the use and effect of intense, pulsed electric fields on biological cells and tissue. Although some effects of pulsed electric fields on cells have been known for more than 50 years, the emphasis was generally on electroporation of the plasma membrane using pulses with duration in excess of microseconds. Advances in pulsed power technology, involving the generation of intense, ultrashort electrical pulses, provided an opportunity to explore and utilize the effects of nanosecond electric pulses. The effects of such short pulses have been shown to reach into the cell interior, opening up new applications and allowing the use of novel pulse delivery devices such as wideband antennas.

First efforts to create a consortium to foster this new research field originated with three research institutions which are known for vibrant pulse power research: the research group at Old Dominion University's Frank Reidy Research Center for Bioelectrics, the Pulsed Power Science team at Kumamoto University, Japan, and the Institute for Pulsed Power and Microwave Technology at the Forschungszentrum Karlsruhe, Germany.

Cooperation among the three groups, which were led by Karl H. Schoenbach, Hidenori Akiyama, and Hansjoachim Bluhm, respectively, on the topics of bioelectrics began in 2002, with mutual visits and joint research projects by scientists of the three institutions. In 2005, this cooperative work had reached a level which made it desirable to have a formal research agreement establishing an International Bioelectrics Consortium. A Memorandum of Understanding was created which serves as the basis for international cooperation among the original members of the consortium and any new members.

The Memorandum of Understanding

The first three paragraphs of this MOU read:

"This agreement provides the basis for the formation of a research consortium ("Bioelectrics") in bioelectric research, in order to develop broad international research collaboration among the participating institutions in the emerging field of bioelectrics. The goals of the consortium are to: (1) Strengthen the field of bioelectrics by forming a consortium with worldwide visibility and impact; (2) Encourage the establishment of Centers of Excellence focused on bioelectrics; and (3) Provide the basis for successful grant proposals to national and international funding agencies. The consortium members will interact through tele-videoconferences and annual/bi-annual workshops. Themes of joint

activities and the conditions for their execution (e.g., provisions for utilizing the results achieved) and arrangements for specific visits, exchanges of personnel or information and other forms of cooperation will be agreed to by the parties concerned in writing on a case-by-case basis. Old Dominion University (hereafter "ODU"), Norfolk, Virginia, USA, will perform the function of "Coordinator" for the consortium, to facilitate the activities of the consortium.

The following institutions will be the initial members of the consortium:
Forschungszentrum Karlsruhe GmbH (Institute for Pulsed Power and Microwave Technology, IHM) - Karlsruhe, Germany;
Kumamoto University (Graduate School of Science and Technology, 21st Century COE Programs on Pulsed Power Science and on Cell Fate Regulation Research and Education Unit) - Kumamoto, Japan;
Old Dominion University (Center for Bioelectrics) - Norfolk, Virginia, USA.

Other institutions may join the consortium by submitting an appropriate application to the Coordinator followed by unanimous agreement of the initial consortium members."
This Memorandum of Understanding was signed on November 11, 2005, by the President of Old Dominion University, Roseann Runte; the Deputy Chairman of the Executive Board, Sigurd Lettow, and a member of the Executive Board, Reinhard Maschuw, at the Forschungszentrum Karlsruhe; and finally, in a ceremony at Kumamoto University by the President of this University, Tatsuro Sakimoto.

In September 2006, another powerhouse in pulsed power technology, the **University of Missouri, Columbia**, joined the consortium as a full member. The Memorandum of Understanding was signed by Jim Thompson, Dean of the College of Engineering at this university.

In October of 2007, the **Institute for Low-Temperature Plasmaphysics (Institut fuer Niedertemperatur-Plasmaphysik e.V) in Greifswald, Germany**, joined the consortium, bringing a wealth of expertise in the use of low-temperature plasmas for medical and environmental applications to the International Consortium. Klaus-Dieter Weltmann, the Director of the Institute, signed the Memorandum of Understanding.

And in November 2008 at a teleconference of the five members, the applications of three new full members were approved. They are: the **Center for Molecular Delivery at the University of South Florida**, with Richard Gilbert as director; the **Laboratory of Vectorology and Gene Transfer, CNRS, Institut Gustave-Roussy and University of**

Paris XI, Villejuif, with Louis Mir as Director; and IPBS **Universite P Sabatier/CNRS in Toulouse, France**, with Justin Teissie as leader of the Cellular Biophysics group. The new members brought a wealth of knowledge in basic bioelectric science as well as in applications, particularly in electrochemotherapy, to the consortium.

In October 2010 two additional members joined the consortium: **Institute of Plasma Physics, Academy of Sciences of the Czech Republic, Prague, Czech Republic**, with Petr Lukes as director; and the **Department of Experimental Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia**, with Gregor Sersa as director.The Institute in Prague has a long history of research in the use of plasma and shockwaves for medical and environmental applications. The Institute in Ljubljana is well known for its research on medical applications of bioelectrics.

The Bioelectrics Consortium, approved at the 2012 symposium in Kumamoto the addition of the **Radiation Sources Laboratory, ENEA (Italian National agency for new technologies, energy and sustainable economic development), Frascati, Italy**, with Gian Pierro Gallerano as director. The expertise on electromagnetic radiation in this center promises to extend the research on bioelectrics into the high frequency, ultrashort pulse duration range.

The newest members of the Consortium are: **Lisbon Engineering Superior Institute, Pulsed Power Advanced Applications Research Group Lisbon, Portugal**, with Luis Redondo as Director; **Eindhoven University of Technology, Department of Electrical Engineering, Eindhoven, The Netherlands**, with A. J. Pemen as Director; **CNR - Institute for Electromagnetic Sensing of the Environment, (IREA), Naples, Italy**, with Maria Rosaria Scarfi as Director; and **Center for Experimental Drug and Gene Electrotransfer, Copenhagen University Hospital Herlev, Denmark**, with Julie Gehl as Director. The new members brought excellent new knowledge in basic bioelectric science as well as applications in the medical and environmental fields. This covers pulse power, plasma technology, electromagnetic sensing and molecule delivery.

To foster research cooperation, videoconferences between the consortium members, and workshops (now symposia) on bioelectrics were held. The first Workshop on Bioelectrics, with Karl H. Schoenbach as chair, was held in Norfolk (Frank Reidy Research Center for Bioelectrics) in March of 2005, with members of the research teams at the Center for Bioelectrics at Old Dominion University and the University of Kumamoto University participating. The second and third International Workshops on Bioelectrics, with Hidenori

Akiyama and Sunao Katsuki as chairs, were held in Kumamoto, Japan, in November 2005, and February 2007, respectively, with representatives of all the members of the consortium and invited guests. The fourth Workshop on Bioelectrics was held in Karlsruhe, as part of the IEEE International Conference on Plasma Science in June of 2008, and the fifth workshop/symposium on Bioelectrics was conducted at the University of Missouri, Columbia, in June 2009. In June 2010 the Bioelectrics Conference was again held in Norfolk, VA, at the Frank Reidy Research Center for Bioelectrics, where the conference was established. Richard Heller, the director of the center, chaired this conference. In 2011, the symposium was held for the first time in Europe, in Toulouse, France, with Justin Teissie chairing it. The following year, in September 2012, the Symposium was held again in Kumamoto, with Hidenori Akiyama as chair. The 2013 International Bioelectrics Symposium will be hosted by the Karlsruhe Institute for Technology with Wolfgang Frey as chair. The 2014 conference was hosted by the University of Missouri with Robert Druce as chair.

Not only the name has changed -- from Bioelectrics Workshop to International Bioelectrics Symposium -- the number of participants also has changed. Whereas only a small group, less than 20 scientists and engineers, attended the first workshop in 2005, the number of participants has dramatically increased. More than 150 participants attended the 2012 Bioelectrics Symposium in Kumamoto. Another indication for the growing interest in this new field of research was the successful publication of a special issue on Bioelectrics in the IEEE Transactions of Dielectrics and Electrical Insulation in 2009, with Wolfgang Frey, Richard Heller, and Karl H. Schoenbach as guest editors. The special issue with 20 publications on bioelectrics, was, as expected from publications in an IEEE Journal, focused on the physics and engineering side of this research. A special issue of the Journal of Bioelectrochemistry, published in 2015 was a strong forum for the biology aspect of this interdisciplinary research. Guest editor for this special issue were Richard Gilbert, Wolfgang Frey, and Hidenori Akiyama.

The 12th International Bioelectrics Symposium in Portoroz at the World Congress

Since the Consortium is growing and recently many new members have joined, we felt that an open discussion on the work that the groups perform would be appropriate. For this reason each of the centers was asked to fill in the form which includes information on their available instrumentation, methods that they use and most importantly their field of interest. Copies of the forms are included in this brochure. Based on the available data we have prepared a table with the fields of interest of all centers, which presumably reflects also their instrumentation. The table is included and will be updated during the discussion and then sent to all the participating centers.

Besides this, we have drawn a chart of existing collaborations between the centers. Based on the presentations of the centers we anticipate that the collaborations will strengthen, based on the mutual interests, which is also the aim of the consortium. The International Bioelectrics Consortium is very much active, and through its collaboration between the centers it strengthens also its importance in broader community. In relation to this the afternoon session will be devoted to discussion about the possible collaborations, and applications for funding.

We hope that you will find the meeting interesting, and most enjoyable.

Yours,
Gregor and Richard

Fields of interest and corresponding facilities

	Theoretical app. of pulsed power science	Technology research and development	Biological and other effects of pulsed power science				Technology transfer to industry and medicine	Biomedical applications and treatment
			Magnetic f.	EP	Plasma	Shock waves		
Heller (ODU)	X	X		X	X		X	X
Frey (KIT)				X			X	
Akiyama (Kumamoto)	X	X					X	
Rols (IPBS)				X			X	X
Lukes (IPP)					X	X		
Scarfi (IREA)		X	X	X				X
Gilbert (USF)		X		X	X			X
Sersa (OI)				X	X			X
Gallerano (ENEA&CNR-IFT)		X						X
Kolb (INP)		X		X	X		X	X
Redondo (GIAAPP)	X	X		X			X	X
Gehl (C*EDGE)		X		X				X
Mir (IGR)	X	X		X			X	X
Pemen (TUE)		X			X			X
Druce (MU)		X				X	X	X
	4	11	1	10	6	2	8	12

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Institution name: Old Dominion University
Web page: http://www.odu.edu/bioelectrics

Center Director: Richard Heller

Investigators: Stephen Beebe, John Catravas, Hai-Lan Chen, Siqi Guo, Barbara Hargrave, Loree Heller, Chunqi Jiang, Sunil Joshi, Michael Kong, Muhammad Malik, Andrei Pakhomov, Olga Pakhomova, Karl Schoenbach, Michael Stacey, Thomas Vernier, Shu Xiao, Christian Zemlin

Brief description of area of expertise (up to 500 words):
Therapeutic in vivo DNA electrotransfer to several tissues
Protein and RNA expression analysis, plasmid design and construction
Clinical and molecular microbiology
Bioelectromagnetics, electrophysiology, live cell imaging, cell and membrane effects of nsPEF, cell death mechanisms
Gene Immunotherapy
Cancer models
In vitro 3D tissue models
Treatment of ischemic tissue – Peripheral and Coronary
Cellular immunity at host-pathogen / cancer interface. Developing novel and efficient methods of antigen presentation to T cells.
Biomarkers of cancer for the prediction of prognosis, stratification of the level of risk and the choice of therapeutic regimen. Biomarkers of metastatic cancer.
Activated B cells as a promising candidate for a cancer vaccine eliciting functional systemic CTLs. IL-17 production in T. Cruzi infection.
Identify and understand the molecular-level interactions between external electromagnetic fields and biological systems. We concentrate on the effects of very short (nanosecond), intense (megavolt-per-meter) pulsed electric fields on cells and tissues, combining experimental observations with molecular dynamics simulations. delineation and characterization of the biophysical mechanisms that govern electric field-driven, nondestructive perturbations of biological membranes.
Atomic Force Microscopy measurements on cell and protein structures and the influence of pulsed
Hemichannels in the role of cell communication pre and post pulse power applications electric fields.
Environmental applications of nonthermal palsmas: specifically, air purification, water purification, fuel reforming and bacterial load reduction from wounds and surfaces by employing nonthermal plasmas.
Fundamental and applied research of non-thermal plasma sources and nanosecond pulsed power technology support

Facilities:
In vivo imaging (IVIS, laser doppler and IV 100).
Multiview-400 multiple probe AFM
AutoMACS, X-ray Irradiator, Blood Chemistry analyzer
Animal facilities (large and small species) including Surgical suites and Catherization suite
Patch clamp, confocal and ratiometric microscopy, a variety of nsPEF generators and electrodes for in vitro treatments, multi-mode microplate reader Synergy-2, cell counter Cellometer Vision
Flow cytometry including cell sorting

Gene electro transfer instrumentation and electrodes
Pulse power device development
Plasma device development

Area where interaction is needed:
Immunology expertise
nsPEF delivery and dosimetry
tissue modeling for the profile of ablation or gene expression in vitro and in vivo after nanosecond or micro/mili-second electric fields plus heat or other physical energy forms.
oncologists and surgeons interested in the development of therapies for pancreatic cancer, metastatic cancer.
dermatologists and pathologists with expertise in psoriasis or other skin immune disease.
Artificial planar lipid bilayers and vesicles
AFM expertise for discussion on data interpretation

Existing collaborations:
University of Buenos Aires and Buenos Aires Institute of Technology (molecular simulations of lipid electropore dynamics);
Massachusetts Institute of Technology (macroscale modeling);
University of Copenhagen (calcium electroporation);
Max Planck Institute for Colloids and Interfaces (electromanipulation of lipid bilayers);
Mount Saint Vincent University (quantum mechanical modeling);
Karlsruhe Institute of Technology (electropermeabilization mechanisms);
University of Limoges (ultra-fast pulse generation and metrology);
University of Ljubljana (intracellular electroperturbation);
University of California, Merced (modeling of interfacial water);
IREA-CNR, Naples (terahertz biostimulation and spectroscopy);
Institut Gustave Roussy, Paris (membrane peroxidation and permeabilization);
University of Nevada, Reno (nanoelectrostimulation of electrically active cells);
ENEA-CNR, Rome (abscopal effects of nanoelectropulse exposure);
University of Rome “La Sapienza” (molecular simulations of electropore conductance);
Institute of Pharmacology and Structural Biology, Toulouse (membrane permeabilization biophysics);
Ruđer Bošković Institute, Zagreb (molecular simulations of lipids at charged interfaces).
N2 Biomedical LLC.
Electroblate, Inc.
Institute of Oncology, Ljulbjana, Slovenia
Eastern Virginia Medical School
University of California San Francisco, Cancer Center
Christopher Newport University
University of South Florida, College of Engineering
Radio Frequency Bioeffects Branch, Bioeffects Division, Human Effectiveness Directorate, Air Force Research Laboratory, Fort Sam Houston, TX.

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Institution name: Karlsruhe Institute of Technology (KIT)
Institute for Pulsed Power and Microwave Technology (IHM)
Web page: www.ihm.kit.edu

Principal investigators: Georg Mueller and Wolfgang Frey

Staff members: Martin Sack, Christian Gusbeth, Aude Silve, Alfons Weisenburger, Annette Heinzel, Christian Eing, Ralf Straessner, Klaus Leber, Ruediger Wuestner, Johannes Fleig, Martin Hochberg and Sarah Rocke

Brief description of area of expertise (up to 500 words):
The Institute for Pulsed Power and Microwave Technology (IHM) is a part of KIT and employs 45 scientific staff members, subdivided into a Pulsed Power and a Microwave Department. The Pulsed Power Department contributes to the national Helmholtz-Program "Renewable Energies". The main subject is to advance science and technology for the generation of high-power, repetitive high-voltage-pulses for large mass-flow applications in energy-, environmental-, and bio-technology. Current topics are surface modification of materials by pulsed electron beams, pulsed electric field (PEF) processing of energy plants and microalgae for dewatering, cell disruption and component extraction. Specifically, the following areas of expertise is covered:
- *Pulsed electric field treatment of microalgae biomass for extraction of valuable intracellular substances.* This activity focuses on downstream processing of microalgae biomass for energetic use. Key objective is to improve extraction of intracellular lipids and other valuable substances like proteins and carbohydrates from microalgae by PEF treatment. Appropriate analytical methodes to quantify the intracellular components have been established, like the colorimetric Sulfo-Phospho-Vanillin assay for lipids, the Anthrone-Test for carbohydrates and the Bradford or Lowry methodes for proteins. The work also includes photobioreactor cultivation of microalgae as feedstock for experiments on lab and pilot scale and implementation of fluorescence-optical and biochemical diagnostics for lipid and cell content monitoring.
- *Stimulation of cellular stress responses by sublethal pulsed electric fields.* In paricular cells and organisms respond with stress reactions, like Calcium emission or cytoskeleton reorganization and ROS generation. Besides intracellular single cell reactions basic mechanisms for growth stimulation on plant seedlings and applications to proliferate growth of single cell suspensions are under investigation. Principal electrical parameters have been identified.
- *Nanosecond time resolved measurement of the membrane charging.* Diagnostics like pulsed laser fluorescence microscopy (PLFM) with a temporal resolution of 5 ns is used. For measurement the cell membrane is stained by a voltage sensitive dye. Current basic research deals with charging properties during the first hundred of nanoseconds of a pulse and with molecular dynamics of voltage sensitive dyes. Evalution of the stationary value of the transmembrabe voltage value is beeing investigated and the data are used to validate theoretical models.
- *Concepts and devices in the field of Pulsed-Power technology.* For different innovative fields of application as for example processing of plant tissue there is a need for pulsed-power generators and loads tailored to fit the specific application. Suitable pulsed power generators, components and scaleable electroporation reactors are under investigation. Prototype industrial scale facilities with a mass flow of t´s/h have been build.
- Inactivation of bactria by pulsed electric field and underwater corona treatment. Studies on the correlation of treatment parameters and inactivation efficiency.

- Material investigation and processing to mitigate corrosion and electrode errosion (e. g. spark gaps). In paricular a surface modification process by pulsed electron beams combined with a fully equiped metalurgical lab is availible.

Facilities:

- Mobile electroporation facility KEA-WEIN, (1 kJ/pulse, 20 Hz) throughput: 800 l/h.
- Mobile electroporation device KEA-Mobile (1 kJ/ pulse, 15 HZ) batch 2 kg
- Electroporation facility for micro algae treatment (1.2 kJ/pulse, 10 HZ) throughput: 100 l/h
- Different lab scale devices for ns to ms-pulses, 1 – 100 kV/cm, several J/pulse
- Patch clamp facility
- Pulsed laser fluorescence microscope (PLFM)
- optical and fluorescence microscopy and diagnostic
- out-door photobioreactors 5 x 200 l for microalgae cultivation
- in-door photobioreactors 2 x 30 l for microalgae cultivation
-diverse pulsed electron beam facilities (GESA1-5)
- metallurgical lab (SEM, XRD, LM..)
- Full equipped S1 certified biological lab
- Mechanical and electrical workshop

Area where interaction is needed:

-Indusrial scale continues exctaction process (lipid, proteins..)
- Diognostics to determine cell wall porosity/permeability

Existing collaborations within consortium:

Lluis Mir, IGR

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Institution name: **Institute of Pulsed Power Science**
Kumamoto University
Kumamoto 860-8555, Japan
Web page: **http://www.ipps.kumamoto-u.ac.jp/English/index.html**

Principal investigator: Prof. Hidenori Akiyama, PhD (Pulsed Power)

Staff members:
Director of the Institute: Prof. Hidenori Akiyama
Vice Director of the Institute: Prof. Tsutomu Mashimo (Chemical)
Professors, PhD: Sunao Katsuki (Bioelectrics), Ken-ichi Yana (Biology), Hamid T. Hosseini (shock wave), Takashi Sakugawa (Pulsed Power), Kazuyuki Hokamoto (explosives), Ichiro Akai (Physics), Hiroshi Kubota (Semiconductor),
Associate Professor, PhD: Douyan Wang (Plant), Takao Namihira (Pulsed Power), Mitsuru Sasaki (Supercritical fluid), Kosumi Daisuke (Photosynthesis), Nobuaki Kawai (High pressure)
Assistant Professor, PhD: Hiromoto Kitahara (Material), Morotomi Keiko (Biology), Alexis Guionet (Biology), Petre Hoffer (Plasma)

Brief description of area of expertise (up to 500 words):
Mission of Institute of Pulsed Power Science:
Our mission is to promote basic research into and new theories of pulsed power science through international-oriented problem solving on an interdisciplinary basis as well as to train globally active young researchers and engineers.
Goals:
The Institute of Pulsed Power Science has had over the 10 years since its establishment the following six goals:
1. Exploration of scientific principles of materials, including biological materials at the boundary of the pulsed power reaction field.
2. Development of new medical technologies and discovery of new materials using the boundary of the pulsed power reaction field.
3. Creation of new science through the fusion of pulsed power science and leading-edge scientific fields.
4. Strategically apply researchers and research results of pulsed power science for extensive problem-solving in the international community.
5. Formation of an international research center on comprehensive pulse power science through activities of an international consortium.
6. Development of young researchers and engineers able to demonstrate international leadership.

Facilities:
1. Pulsed Power Research Infrastructure Equipment
2. Bioelectrics Research Facility
3. Explosive Experiment Facility
4. Supergravity Generation Equipment
5. Supercritical Fluid Equipment

Area where interaction is needed:

We need interections to develop new medical technologies using the boundary of the pulsed power reaction field and to create new science through the fusion of pulsed power science and leading-edge scientific fields.

Existing collaborations:

1. About 25 collaborations with Japanese universities
2. About 20 collaborations with Japanese Company
3. About 10 collaboration with Universities outside of Japan

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Institution name:
Leibniz Institute for Plasma Science and Technology e.V. (INP Greifswald)
Felix-Hausdorff-Strasse 2
17489 Greifswald
Germany
Web page: www.inp-greifswald.de

Principal investigator: Prof. Dr. Klaus-Dieter Weltmann (Director)

Staff members:
Research Program Manager Plasma Medicine: Prof. Dr. Thomas von Woedtke (Pharm)
Research Program Manager Decontamination: Prof. Dr. Juergen F. Kolb (Phy)
Deputy Research Program Managers Decontamination: Dr. Ronny Brandenburg (Phy), Dr. Jörg Ehlbeck (Phy)
Researchers (in Bioelectrics): Jie Zhuang, PhD (Eng), Camelia Miron, PhD (Eng), Petra Macikova, PhD (Biol), Jörn Winter PhD (Phy)
Young researchers (in Bioelectrics): Anna Steuer (Biophy), Robert Banaschik (Pharm), Katja Zocher (Pharm), Fukun Shi (Phy)
Engineering/Laboratory Assistance (in Bioelectrics): Jana Kredl (PTA), Tilo Schulz (Eng), Liane Kantz (MTA)

Brief description of area of expertise (up to 500 words): The Leibniz Institute for Plasma Science and Technology (INP Greifswald) is the largest non-university research institute in Europe that is dedicated to research and development in the field of non-thermal plasmas and related technologies. The mission of the institute is application-driven basic research from idea to prototype. Accordingly, research is conducted with a close look at industry-needs. Two Research Divisons are focused on Materials & Energy and on Environment & Health, respectively. The three priorities that are pursued by the second devision with dedicated research programs are the development and understanding of bioactive surfaces, the basics and applications of plasma medicine and novel approaches for the decontamination of gases, water, food and sensible surfaces. In this respect research on Biolectrics is primarily conducted for medical and environmental applications. The focus of fundamental studies in Bioelectrics are hereby **biophysical phenomena** that are instigated by the interaction of cells with short pulsed electric fields and/or non-thermal plasmas, and the study of subsequently induced biochemical processes. As such we investigate **dielectric properties** of cells, **membrane charging** mechanisms, and **transport phenomena as they occur for example in the gap junction mediated intercellular communication**. A more recent topic is the study of induced mechanical effects for the exposure of mammalian cells. In a complementary effort we develop **nanosecond high voltage pulse generators** and **delivery systems** for the exposure of cells suspensions, cell monolayers and tissues. These systems are design with respect to individual experimental or treatment requirements. Main applications that are currently pursued are the decontamination and disinfection of water by pulsed discharges and the combination of nanosecond pulsed electric field treatments with non-thermal plasma treatments. Pulsed discharges that are generated directly in water have shown great potential for the degradation of pharamceutical residues and water borne bacteria in bacteria. For the use of pulsed electric fields against bacteria it was found that the combination with a treatment by non-thermal plasma results in synergistic effects that can be exploited for a more efficient treatment of wounds.

Facilities: Core facilities at the INP include a fully equipped microbiological laboratory (S2) and 3 cell culture laboratories with the ability to conduct also some molecular biology studies. The laboratories are equipped with biosafety cabinets, CO2 and incubators, several centrifuges, microplate readers, spectrophotometers, cell-migration assays, imaging systems etc. The core expertise of the INP Greifswald is the diagnostic of physical and biophysical phenomena. Accordingly state-of-the-art equipment such as for example TALIF, MS, XPS is available for research. Techniques that are in particular dedicated to research in Bioelectrics and respective equipment are: dielectric spectroscopy (e.g. TDR spectrometer Agilent DCA 86100D, precision impedance analyzer Agilent 4294A, Zeta-potential analyzer Horiba SZ-100Z); terrahertz spectrometry (Batop Optoelectronics TDS1008); inverted fluorescence microscopy (e.g. Zeiss Axio Observer D1) together with miroinjection system or microscope incubators; confocal microscopy (e.g. Leica TCS SP5 II); pulsed laser imaging (Radiant Dyes NarrowScan K, Continuum Sherline I): microscope (fluorescent) imaging with 5 ns resolution; automated patch-clamp (Nanion Technologies Port-a-Patch); scanning electron microscopy (Phenom ProX); bio atomic force microscopy (JPK Instruments Nano Wizard AFM, Zeiss Axio Observer D1); high voltage pulse generators (e.g. FID Technologies FPG 150-1NK, DEI PVX-4110, BTX Harvard Apparatus ECM 830): covering a pulse range from 10-ns/150-kV to 10-s/5-V); fast high voltage pulse diagnostics (e.g. Tektronix DPO7204D); fast imaging (e.g. 4 Channel Multiple Framing Camera SIMD8 – UV, Specialised Imaging); GC-MS (Agilent 7890A), HPLC-MS (Agilent 1260 Infinity).

Area where interaction is needed: The INP is looking in particular for partners to conduct animal experiments.

Existing collaborations: In the area of Bioelectrics, the INP is currently collaborating in particular with

- Petr Lukes; Department of Pulse Plasma Systems, Institute of Plasma Physics AS CR, Za Slovankou 3, 182 00 Prague 8, Czech Republic;
- Ludek Blaha, Pavel Babica; Masaryk University, RECETOX - Research Centre for Toxic Compounds in the Environment, Kamenice 753/5, pavilon A29, 625 00 Brno, Czech Republic;
- Michael Stacey; Center for Bioelectrics, Old Dominion University, 4211 Monarch Way, 23529 Norfolk, VA.

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Institution name:
Institute of Pharmacology and Structural Biology (UMR 5089)
Department of Structural Biology and Biophysics
Cellular Biophysics Group
CNRS/ University Paul Sabatier
205, route de Narbonne
31077 Toulouse cedex
France

Web page:
http://www.ipbs.fr/?-Biophysique-cellulaire-Marie-

Principal investigator: Dr Marie-Pierre Rols

Staff members:
Head of the Group and of the Department : Dr. Marie-Pierre Rols
Permanent staff: E. Bellard (IE2, 40%), V. Ecochard (MCU-UPS), M. Golzio (CR1-CNRS), L. Hellaudais (AJT, 50%), C. Ladurantie (T), L. Paquereau (Pr2-UPS), M-P. Rols (DR1-CNRS), J. Teissié (DRCE-CNRS Emeritus).
Post-docs: L. Gibot (2012-), N. Joncker (2013-), F. Pillet (2013-), D. Lacorre (2015-).
PhD students: A. Tamra (2013-), M. Madi (2013-), I. Dupont (2013-), J-F Guillet (2014-), A. Ric (2014-), M. Bocé (2015-), C. Lamarche (2015-).
Abbreviations: CR, junior scientist; DR, senior scientist; IE, engineer; MCU, assistant-professor; Pr, professor; T, technician.

Brief description of area of expertise (up to 500 words):
Our group develops a multidisciplinary approach combining cell biology and biophysics to determine the mechanisms of membrane perturbations induced by transmembrane potential modifications, i.e. by the so-called “electroporation” or “electropermeabilization” technique (EP). In addition, complementary approaches, including DNA molecules called aptamers and glycoprotein such as lectines, are under development in our group. These studies bring new approaches and guidelines for safe and efficient delivery of therapeutic molecules into cells and tissues.
Our strategy is to adapt and develop different complementary systems with increasing complexities (model membranes, cells, spheroids and tissues in living mice) and to integrate various imaging tools to visualise and define the mechanisms of membrane perturbations along molecule delivery processes.
GUVs (Giant Unilamellar Vesicles) *represent a convenient way to study membrane properties* such as lipid bilayer composition and membrane tension. We showed that different membrane perturbations (pores, vesicles and tubules) are associated to electropermeabilization. As a direct consequence of membrane permeabilization is membrane fusion, another emerging aspect of our project concerned GUVs as models to study the mechanisms of electrofusion, with a direct interest to their use as vehicles to deliver molecules.
Cells in culture revealed more complex ways involved in molecule electro-mediated uptake than GUVs can do. By using single cell imaging, we showed that the uptake of nucleic acids and antitumor drugs takes place in well-defined membrane regions and depends on their chemical and physical properties. Our work raises differences between 1) the delivery of small molecules and of macromolecules and 2) between the mechanisms of transfer involved on cells in culture and on

tissues. DNA entrance into cells is a multi-step process with a crucial initial stage of interaction of DNA with the membrane and cytoskeleton plays a key role in that process.
We implemented and developed *multicellular spheroids as a model to mimic the behavior of cells in a complex 3D organized system*. We showed that spheroids can be used as an original and convenient approach to access *in vivo* electropermeabilization. 3D cell culture models are indeed more relevant concerning *in vivo* cell organization since cell-cell contacts are present as well as extracellular matrix. We showed relevance of the model to address and improve the electrotransfer processes.
In addition, we investigated and demonstrated the ability of an *anti-MUC1 fluorescent aptamer to efficiently label multicellular spheroids of breast cancer* as tumor models. The anti-MUC1 aptamer is able to penetrate inside these 3D tumor models and thereafter internalize into the cancer cells. This approach has interests for targeting of tumours and diagnostics.
Because *in vivo organization of tissues is much more complex than spheroids, we also need to evaluate the effects of EP in vivo.* We indeed adapted imaging systems (dorsal window chamber, fluorescence micro and macroscopies) and we have shown that EP induces the leakage of intravenously injected large molecules by transient permeabilization of normal vessels with concomitant changes in the blood flow. Our work is of a interest for further use of EP in clinical applications and molecular targeted delivery.

Facilities:
IPBS supports a broad array of technological facilities and state-of-the-art equipments designed to advance the research efforts of the Institute and of external investigators. In order to provide the capability to analyze biological materials using the most sophisticated technologies, our facilities are operated by experienced personnel and some of these facilities conduct their own research and development programs.
The Institute hosts four technological platforms, all of them having the IBiSA label (national coordination of the life science platforms, http://www.ibisa.net). The optical imaging and spectroscopy facility at the IPBS campus is part of the Toulouse regional imaging network (TRI), an IBiSA labed technological platform (Head: P Cochard) belonging to Genotoul.

The major goal of the optical imaging platform is to bring cutting-edge support to scientists in the field of life science imaging. This facility provides a wide range of equipments that allow observations from the molecular level (microspectrofluorimetry) to the intact organism (in vivo and intravital observations). The time scale range can be from the nanosecond scale (fluorescence lifetime) up to weeks by time lapse observations on living animals. The network develops new technologies in the field of molecular and cellular spectroscopy and living animal imagery and keeps the equipments into a world competitive level.

Main Electroporation related research techniques and equipment present in our group:
12 Pulse generators: Jouan, Cliniporator, Betatech
3 inverted fluorescence microscopes equipped with different sensitive and fast camera (CDD, scMOS camera)
2 light microscopes
2 stereomicroscopes
fast kinetic imaging system
cell culture room
4 laboratories for experiments on liposomes, bacteria, mammalian cells and small animals

Research topics, techniques and equipment not related to Electroporation present in our group:
Part of research is dedicated to:
- develop, select, produce and test DNA aptamers that can be used to recognize relevant targets both in vitro and in vivo using SELEX or non-SELEX methods.

- develop nanocontainers (obtained from a mushroom lectine) targeting epithelial cancer cells.

Available facilites present in our group:
laboratory for human and animal cultures
laboratory for bacterial, yeast and algae cultures
laboratory for giant unilamellar vesicles formation
animal facilities
all the equipement for biochemistry and molecular biology

Area where interaction is needed:
Since our aim is both to fully understand the basics of electropermeabilization processes and to translate the knowledge gained in our studies into clinical trials and industries, we need interactions and collaborations with mathematicians, physicists, clinicians and people from companies.

Existing collaborations:
Toulouse
• Picometrics Technology (Dr J.C. Garcia)
• Pierre Fabre Dermo-cosmetics (Dr. G. Josse)
• French aerospace lab, ONERA (Dr X Orlik)
• Veterinary school, ENVT (Pr. Tamzali)
• IMRCP, CNRS UPS (Dr. M Blanzat)
• Coordination Chemistry lab, LCC, (Pr. Majoral)
• IMRCP (Dr.Patricia Vicendo)
• Laboratory for analysis and architecture of systems, LAAS (Dr. Etienne Dague)
• LAAS (Dr. David Dubuc)
National
• CEA Gramat (Dr. R. Vezinet)
• ITHPP Thegra (Dr. C Goepfert)
• Université de Bordeaux (Dr. F. Couillaud)
• Université de Bordeaux (Pr. D. Dean)
• IGR CNRS, Villejuif (L Mir)
• XLim CNRS (P Leveque)
• IUT Laval, Université du Maine (V Blanckaert)
• Beta tech, l'Union
• Université François-Rabelais, Tours, (Dr. Ayache Bouakaz)

International
• COST TD1104 European network for development of electroporation-based technologies and treatments (EP4Bio2Med)
• International Bioelectrics Consortium (coordinator ODU Norfolk, R Heller)
• European Associated Laboratory (LEA) "Pulsed Electric Fields Applications in Biology and Medicine

• Institut of Oncology, Ljubljana (Pr. G Sersa)
• Faculty of electrical engineering, Ljubljana (Pr. D Miklavcic)
• Herlev Hospital, Copenhagen University (MD PhD, J Gehl)
• University Campus Bio-Medico, Rome (Dr. Emmanuella Signori)
• University C Davila, Bucarest (Pr. M Moisescu)
• University of Konstanz (Pr. Zumbusch)
• Frank Reidy Research Center for Bioelectrics, Old Dominion University (Norfolk, USA) (Dr. Thomas Vernier)

Existing collaborations:

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Institution name:
Center for Molecular Delivery
College of Engineering,University of South Florida
Tampa, Florida USA

Center Director: Richard Gilbert Professor Chemical and Biomedical Engineering

Web page:

Center members:
Professor Mark Jaroseski; Department of Chemical and Biomedical Engineering
Professor Andrew Hoff ; Department of Electrical Engineering
Dr. Timothy Fawcett; Senior Research Engineer, University Computational Research Department
Young researchers: Reginald Atkins
Graduate Student Researchers: The Center uses the talents of various graduate students when the Center's efforts can be mapped to the students' specific degree efforts and interests.

Brief description of area of expertise (up to 500 words):
The Center for Molecular Delivery uses combinations of interdisciplinary faculty to address transport related challenges. The Center's general goal to transform applied research efforts into patents that are then licensed through the University of South Florida to interested companies. Previous success in these efforts include patented RO technology, non-contract semiconductor wafer metrology tools, and a family of patents addressing applicators and delivery protocols for electric field mediated drug and gene delivery for clinical applications. Current work is focused on non-contact applicators for electric field mediated agent delivery and the development of a fast impedance spectrometer for clinical and clinical related research applications.

Facilities:
The Center for Molecular Delivery operates out of the College of Engineering at the University of South Florida with easy cooperative access to facilities in the various colleges that pertain to the Center's research effort. This access includes various research based hospitals on campus as well as animal facilities associated with these facilities. The Center also has easy access to various metrology laboratories as dictated by project needs. Relative to Center efforts with electric field mediated agent delivery, the first drug delivery clinical trial in the United States as well as the first gene delivery clinical trial in the world were conducted by Center members at clinics on the USF campus.

Area where interaction is needed: The Center of Molecular Deliver welcomes interactions with any group that has similar interests. Typically our interest has a transport or metrology challenge where the results adds now information about the system under investigation. The Center can partner in clinical trials if the target technology makes sense to clinicians at USF.

Existing collaborations:
Relative to the drug and gene delivery research area the Center for Molecular Delivery is continuously working with the Frank Reidy Research Center.
Membership in International Societies:

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Institution name:
Institute of Oncology Ljubljana,
Department of Experimental Oncology
Zaloska 2
Si-1000 Ljubljana
Slovenia

Principal investigator: Prof. Gregor Sersa, PhD (Biol.)

Web page: http://www.onko-i.si/eng/sectors_of_the_institute_of_oncology/research_and_education/department_of_experimental_oncology/

Staff members:
Head of the Depatment: Prof. Gregor Sersa, PhD (Biol.)
Deputy head: Prof. Maja Cemazar, PhD (Biol.)
Researchers: Simona Kranjc, PhD (Biol.), Urska Kamensek, PhD (Biol.), Vesna Todorovic, PhD (Biochem.), Tanja Dolinsek, PhD (Biochem.), Lara Prosen, PhD (Pharm.), Masa Bosnjak, PhD (Pharm.)
Young researchers: Monika Stimac, MMolBiol, Andreja Brozic, BSc (Biol.), Spela Kos, MPharm, Ursa Lampreht, DVM, Ajda Prevc, MFuncBiol
Engineer in laboratory medicine: Mira Lavric
Secretary of Editorial Board of Radiology and Oncology: Mira Klemencic
Technical assistant: Elizabeta Dzekovic

Brief description of area of expertise (up to 500 words):
Our group has been extensively involved in development of **biomedical applications of electroporation**. The technology utilizes application of electric pulses as a drug delivery system, and it can be applied for delivery of drugs or plasmids to tumors and other tissues. **Electrochemotherapy** is aimed at delivery of chemotherapeutic drugs, such as bleomycin or cisplatin to tumors, and has reached wide clinical acceptance. **Gene electrotransfer**, on the other hand, is still in development, but holds a great potential for clinical application, especially for gene therapy of cancer and DNA vaccination. It has already been tested in first clinical trials, utilizing plasmid encoding immunomodulatory interleukin12 (IL12), and in the second one, plasmid encoding antiangiogenic AMEP, where safety and feasibility of the gene electrotransfer was proven.
Vascular targeted therapies already proved to be effective for cancer treatment, also in combination with radiotherapy, however, due to undesirable toxicity of currently used vascular targeted therapeutics, new targets are being sought, that target cellular pathways unconnected to VEGF pathway. One of such potential new target is endoglin, a coreceptor of TGFβ. We have shown that silencing of endoglin leads to prevention of new growth and the disruption of existing tumor blood vessels. Besides new targets, gene therapy could be made more specific by using tissue specific promoters that would provide also better safety profile of the therapy. Among the promoters that in line with our targets are promoters of endothelin, smooth muscle gamma actin and collagen, which are specifically targeting vasculature, muscle and skin.
For delivery of therapeutic plasmids, we use our standard method **electroporation**, however, also **new non-viral gene delivery systems** are being sought, such as the use of cold plasma, where the research in gene therapy is only in its beginnings and is especially applicable to skin and the other one, the use of mesenchymal stem cells for specific tumor targeting. Stem cells in and around the

tumor will be evaluated. Many of the biological therapies are already in the clinics as **adjuvant therapy to radiotherapy**. Among these, bevacizumab is an example, as antiangiogenic approach using antibodies. The other way of combining antiangiogenic approaches or immunomodulatory ones, is with gene electrotransfer. This was already demonstrated by our group using plasmid encoding IL12. The whole area of combining vascular targeting gene therapy with radiation therapy has not been explored yet, and holds great promise.
Based on our experience with **preparation and execution of clinical trials in veterinary and human oncology**, we anticipate to translate the knowledge gained in preclinical studies into clinical trials. Clinical trials on use of electrochemotherapy in treatment of deep seated tumors are needed, both in veterinary and human oncology. Gene electrotransfer on the other hand is still in early stages of clinical testing. Therefore, clinical studies that will prove the safety and effectiveness of gene electrotransfer will first be performed in veterinary oncology, since such studies are the first step for the effective translation of new therapies into human clinic.

Facilities:
Institute of Oncology Ljubljana is a public health institution providing health services on the secondary and tertiary level as well as performing educational and research activities in oncology in Slovenia. Department of Experimental Oncology is the only full-time research department at the Institute of Oncology Ljubljana. The mission of the department is to develop new treatment approaches preclinically, test them clinically, and introduce them into clinical practice. One of the most important approaches is non-viral delivery system for introducing molecules in to the cells and tissues, called electroporation.The department includes both laboratories and offices, where researchers with expertise in biology, molecular biology, microbiology, biochemistry, veterinary medicine and pharmacy are employed.
Laboratory: The principal investigator has access to three (~5000 sq ft) laboratories. The laboratories are equipped with a biosafety cabinets, CO_2 and microbiological incubators, several centrifuges. The laboratories are divided by type of experimental work on cell culture laboratory, bacterial laboratory, laboratory for molecular biology and laboratory for animal work.
Core equipment in the **cell culture laboratory** include spectrofluorimeter (microplate reader) Tecan, incubators, freezing chamber, freezing chamber Polar, biological safety cabinet, laminar air flow, incubator Sanyo multi gas, incubator CO_2 Heraus Heracell 240, inverted microscope, fluorescent inverted microscope, centrifuge Multifuge 1s-R Kendro, centrifuge citospin, spectrophotometer (microplate reader) Epoch, oscilloscope, photometer, inverted microscope with camera Olympus, UV spectrophotometer, luminometers, electric pulse generator GT-01.
Core equipment in **bacterial laboratory** includes a high speed centrifuge with rotors, biological containment cabinets (1) for bacterial work, ultracentrifuge, mikrocentrifuge, PCR, thermomixer Eppendorf, centrifuge, incubator for bacteria, laminar air flow, water bath (2), ultrafreezer, freezers and refrigerators.
Core equipment in **Laboratory for molecular biology** includes ultracentrifuge, thermomixer, fume hood, gene documentation system G: Box, RT PCR system 7300, balance, microbiological protection chamber, pH meters, microwave, electrophoresis equipment, sonificator, balance analytical Denver, precision balance, electrophoresis, chemical fume hood, iBlot® Dry Blotting System for Western Blot and XCell SureLock® Mini-Cell system for SDS-PAGE, tissue processor, cryostat, FACSCanto II flow cytometer.
Animal: An animal facility is located at the Department of Experimental Oncology at the basement of building B of the Institute of Oncology Ljubljana. The facility includes procedure room and surgical facilities as well as an imaging suite. The department is registered as a user organization for experimentation on laboratory animals and also an organization with license to work with the GMO at safety level 2.
Core equipment appropriate to rodent experiments includes a fluorescent stereomicroscope Zeiss, anesthesiology apparatus Drager Sulla 808V, animal chamber, fluorescence microscope, biological

safety cabinet, Cliniporator, electroporator βtech ELECTRO CELL B10, individually ventilated animal caging system.
Irradiator: Additional available equipment at the Department includes X-ray for irradiation DARPAC, autoclave and sterilizator GE 446, cryomycrotome and cold chamber.

Area where interaction is needed:
Since our aim is to translate the knowledge gained in preclinical studies into clinical trials in veterinary and human oncology, we need interactions and collaboration with veterinary and human clinicians. In order to develop suitable electrodes for the electroporation and to gain the optimum electric field distribution in treated tissue, we need interactions and collaboration with electrical engineers and companies, which could supply us with safe and quality electromedical devices.

Existing collaborations:
University of Ljubljana: Veterinary Faculty, Faculty of Electrical Engineering, Faculty of Pharmacy, Biotechnical Faculty
University of Primorska: Faculty of Health Sciences
National Institute of Biology, Ljubljana
Jožef Stefan Institute, Ljubljana
National Institute of Chemistry, Ljubljana
University Medical Centre Ljubljana, Ljubljana
University Medical Centre Maribor, Maribor
Frank Reidy Research Center for Bioelectrics, Old Dominion University (Norfolk, USA)
Herlev Hospital (Herlev, Denmark)
Institut Gustave Roussy (Paris, France)
Institute of Pharmacology and Structural Biology (Toulouse, France)
Membership in International Societies: LEA EBAM, COST EP4 Bio^2 Med, BIOELECTRICS, INSPECT

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Institution name:
Institute of Plasma Physics
The Czech Academy of Sciences
Department of Pulse Plasma Systems
Za Slovankou 3
18200 Prague 8
Czech Republic

Web page: www.ipp.cas.cz/lps/

Principal investigator: Petr Lukes, PhD (Chem.) (lukes@ipp.cas.cz)

Staff members:
Head of the Department: Petr Lukes, PhD (Chem.)
Researchers: Vaclav Babicky, MSc (Phys.), Martin Clupek, PhD (Phys.), Eva Dolezalova, PhD (Biochem.), Petr Hoffer, PhD (Phys.), Vitaliy Stelmashuk, PhD (Phys.), Milan Simek, PhD (Phys.), Irena Sisrova, PhD (Chem.), assoc. prof. Pavel Sunka, PhD (Phys.), Olexander Frolov, PhD (Phys.), Karel Kolacek, PhD (Phys.), Vaclav Prukner, PhD (Phys.), Jiri Schmidt, PhD (Phys.)
Young researchers: Vladyslava Fantova, MSc (Phys.), Anna Kuzminova, MSc (Phys.)
Technicians: Antonin Baumruk, Ales Kolar, Zdenek Kopecky, Jaroslav Straus

Brief description of area of expertise (up to 500 words):
Our research team at the Department of Pulse Plasma Systems (IPS) is focused on the research a non-equilibrium (low-temperature) plasma generated by various types of pulse high-voltage electrical discharges in gases, liquids and gas/liquid environments. The physical and chemical processes intentionally initiated by such discharges are studied in dependence on the type of discharge, pulsed power, and the nature and the chemical composition of the surrounding environment in order to achieve specific effects and utilization in different applications (environmental, biological, medical). The main research topics that are related to Bioelectrics include 1) Chemistry and physics of plasma/liquid interactions; and 2) Physics and biological effects of focused shock waves in water.
In the frame of the research of plasma/liquid interactions the IPS team is focused on the characterization of physical, chemical and biological processes induced in electrical discharge plasmas in contact with aqueous solutions and the research on mechanisms of plasma interactions with living cells and tissue. The IPS research group has long term experiences in the research of elementary physical and chemical processes induced by plasma of electrical discharges either directly in the liquid phase or in the gas phase in close proximity to the liquid surface and their effects on model organic compounds and microorganisms. The research group is highly qualified in design, operation and diagnostics of different high pulse power devices, including investigation of fast transient phenomena, emission spectroscopy diagnostics as well as chemical analysis of aqueous liquids, organic compounds and transient chemical products of electrical discharge plasma in water. The chemistry of ROS and RNS produced by air discharge plasmas in aqueous solutions is studied in detail. Mechanisms of bacterial inactivation induced by plasma in water are investigated focusing particularly on the effects of the reactive species generated by the air plasma on the bacterial cell membrane integrity.
The second main topic is the study of physical and biomedical effects of focused shock waves in water. In 1980's IPS team has pioneered method of the extracorporeal shock wave lithotripsy in non-

invasive treating patients with stone deceases (mostly kidney stones), which is up to now used in the commerical lithotripters Medilit (MEDIPO, Czech Republic) at approximately 20 hospitals in the Czech and Slovak Republics with more than 150 000 patients treated. Based on these expereinces we continue on the research of interaction of shock waves with soft tissues and possible applications in noninvasive treatment of cancer tissues and a targeted drug delivery by shock waves. Special type of multichannel pulsed-electrohydraulic discharge generator with cylindrical ceramic-coated electrodes developed at IPS is used as a source of focused shock waves and their biological effects are studied in vitro and in vivo on different cancer cells and tumors using various animal models. We proved that focused tandem shocks waves are capable to cause localized lesions at a predictable location deep within soft tissue in the focus and can significantly delay growth of tumors. Focused shock waves were also determined promising for potentiation of cytotoxic effects of cytostatic drugs on tumors. Currently, the first clinical tests of focused shock waves are under preparation in collaboration with the First Medical Faculty of Charles University Prague.

Facilities:

The Institute of Plasma Physics is the sole entity in the Czech Republic specializing in the research and applications of plasma. The research includes both experimental and theoretical studies of artificially generated plasma in a broad range of temperatures, densities, and lifetimes. The IPS group is equipped with a variety of pulse power supply and analytical instrumentation for use on generation and diagnostics of electrical discharge plasma in gases and liquids as fast time resolved system of data acquisition and processing, spectrometric systems with gated intensifiers and instrumentation for chemical and biological analysis.

Lab-scale pulse power generators for generation of electrical discharges in liquid:
- monopolar and bipolar (~µs) pulse high-voltage generators with spark gap switch (± 40 kV, 1-100 Hz, 0.2-5 J/pulse);
- monopolar (~µs) pulse high-voltage MPC generator (30 kV, 1-500 Hz, 0.3 J/pulse)
- rectangular pulse forming generator with thyratron switch (2 µs, load 2 kΩ, 50 Hz, 30 kV);

Physical diagnostics of discharge plasma:
- high-voltage measurement and fast time resolved systems for data acquisition and processing;
- fast multichannel emission spectroscopy and LIF diagnostics;
- characteristics of shockwaves by PVDF, FOPH hydrophones and time-resolved Schlieren and shadowgraphy methods;

Instrumentation for chemical analysis in liquid and gaseous mixtures:
– HPLC chromatography with photo diode array UV/Vis and fluorescence detection;
– ion chromatography with simultaneous UV and suppressed conductivity detection;
– GC chromatography with mass spectrometry and He plasma ionization detection;
– scanning UV/Vis spectrophotometry of aqueous analytes;

Instrumentation for biological analysis in aqueous solutions:
– microbiological facilities for cultivation and analysis of bacteria;
– multi-mode microplate reader with UV/Vis, fluorescence and luminescence detection;
– real-time PCR (qPCR);

Area where interaction is needed:

We have already well-established cooperation with several consortium members specialized on the research of non-equilibrium plasma in gases and liquids and on shock waves. Nevertheless, we are interesting in closer collaboration with the consortium members specialized on biology in order to develop our better understanding of interactions of plasma and shock waves with biological cells and tissues. Although we cannot probably fully utilize all the methods specifically designed for the research of biological effects of pulsed electric fields, we think that some of them could be applicable, e.g. for the study of the biocidal effects of reactive species (RONS) generated by the air plasma in liquids – e.g., their effects on the bacterial cell membrane integrity, or interactions of shock waves with soft tissues, where membrane perforation mechanisms or intracellular sonodynamic effects are expected to play an important role. On the other hand, we believe that some of our expertise in the area of physics and chemistry of electrical discharges in liquids and focused shock waves might be of interests and applicable for the consortium members specialized in other research fields.

xisting collaborations:

Consortium BIOELECTRICS: INP Greifswald (Germany); Kumamoto University (Japan)

Other collaborations (national): University of Chemistry and Technology Prague; Czech Technical University; Charles University Prague - First Medical Faculty;

Other collaborations (international): Florida State University (USA); Comenius University (Bratislava, Slovakia); University of Bologna (Italy); Ruhr University Bochum (Germany); University of Bari (Italy);

Membership in International Societies: COST TD1208; COST MP1101; EUROATOM; EUROfusion; LASERLAB;

Institution name:
National Research Council
Institute for Electromagnetic Sensing of the Environment (IREA)
Via Diocleziano, 328 – 80124 Naples, Italy

Web page: www.irea.cnr.it

Principal investigator: Dr. Maria Rosaria Scarfì (Biologist)

Staff members:
Head of the Bioelectromagnetic laboratory: Maria Rosaria Scarfì
Researchers: Olga Zeni, PhD (Biologist)
Young Researcher: Stefania Romeo, PhD (Engineer)
Associate Researcher: Luigi Zeni, PhD (Engineer)
Technician: Anna Sannino, PhD (Biologist)

Brief description of area of expertise (up to 500 words):
The activity at IREA started in 2007, moving from the idea to set-up a modified version of the classical Blumlein pulse-forming network, based on a double-switch system, which allows to generate high voltage ns pulses with variable duration and polarity. Since then, our group has been involved in the following activities: **a) design and realization of ns pulse generation systems** to expose cell suspensions with electroporation cuvettes and with microscope slides based electrodes; **b) investigation on the intracellular effects**, including effects on cell membranes, of ns pulsed electric fields (ns PEFs); **c) use of electrophysical model** for the numerical analysis of electroporation onset and dynamics, and of pore distribution and density under variable pulsing conditions. Further, in the framework of **electrochemotherapy (ECT**), we are also involved in the identification of equivalent electric protocols, ECT protocols for new drugs and for drug-resistant cancer types.

Facilities: The bioelectromagnetic group at IREA has a long experience in biological and dosimetric aspects of the electromagnetic fields (EMF) for health risk evaluation and clinical applications.
Laboratories
Cell biology - Cell culture handling and maintenance (laminar flow cabinets, CO_2 incubators, cryopreservation systems,...); instruments for the analysis of samples (fluorimeter, spectrophotometers, flow cytometer,....); microscopy (bright field, fluorescence, confocal)
Electromagnetics – Static magnetic fields, Low and high frequency EMF exposure systems (Radiofrequency and Extremely Low Frequencies generators and amplifiers, power meters, waveguides, wire patch cells, Helmholtz coils, ...); **measurement instruments** (Spectrum analyzer, Vectorial network analyzer, Oscilloscopes, gaussmeters, termocouples and fiber optic thermometers, ...); **high voltage ns and µs pulse generation systems** (cuvette-based and microscope slides-based; CLINIPORATOR); **THz time domain spectroscopy system.**

Area where interaction is needed:
Concerning the investigation on intracellular effects of nsPEFs, the interaction with research groups with expertise in molecular biology is needed in order to go deep into interaction mechanisms. In the framework of electrochemoterapy, collaborations and interactions with laboratories involved in animal studies should be very useful to test *in vivo* the equivalent electric protocols identified in *in vitro* investigations.

Existing collaborations:
Second University of Naples, Dept of Industrial and Information Engineering; Aversa, Italy
University of Salerno, Dept of Electrical and Information Engineering, Salerno, Italy
ProdAl S.c.a.r.l., Salerno, Italy
Herlev Hospital, Herlev, Denmark
IGEA – Clinical Biophysics, Carpi, Italy
Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk, USA

Membership in International Societies: COST EP4 Bio^2 Med; COST EMF-MED; Bioelectrics, EBEA, BEMS

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Institution name:
Pulsed Power Advanced Applications Research Group, GIAAPP, from ISEL (Lisbon Engineering Superior Institute, Portugal)
Web page: http://www.giaapp.isel.pt

Principal investigator: Luis Redondo

Staff members: Hiren Canacsinh, Marcos Pereira, José Lopes Ana Paula Barros

Brief description of area of expertise (up to 500 words):
Solid-state pulsed power design and assembling
Electrotroporation (mass tranfer and inactivation) in food, microalgae and medical applications
Non-thermal plasma for decontamination of water
Plasma based ion implantation for surface engineering
Magnetic forming and cutting of thin metal sheets

Facilities:
Solid-state modulator, up to 10 kV monopolar and up to 25 kV bipolar, for microseconds pulse width, up to 250 Hz.
25 ns pulse width solid-state generator, 10 kV monopolar, up to 10 kHz
50 kA resonant generator, 150 microseconds
-20 kV, microseconds pulse solid-state generator

Area where interaction is needed:
Non-thermal plasma for decontamination of water
Platelet activation for regenerative medicine
Microalgae

Existing collaborations: H2020

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Institutions name:
ENEA-Frascati
Sources, Antennas & Diagnostics Laboratory
Via Enrico Fermi 45, 00044 Frascati-Italy
Web page: http://www.enea.it/en/home?set_language=en&cl=en
CNR-Institute of Translational Pharmacology (IFT)
& Via del Fosso del Cavaliere 100, 00133 Rome-Italy
Web page: http://www.cnr.it/istituti/DatiGenerali.html?cds=116

Principal investigators: Dr. Gian Piero Gallerano & Dr. Alfonsina Ramundo-Orlando

Staff members:
ENEA-Frascati
Director of Sources, Antennas & Diagnostics (SAD) Laboratory: Gian Piero Gallerano
Staff members: Gian Piero Gallerano (Director), Andrea Doria (Senior Researcher), Emilio Giovenale (Senior Researcher), Ivan Spassovky (Senior Researcher), Alberto Petralia (Researcher), Marco Zerbini (Senior Researcher)
IFT-CNR
In charge of Liposome Research Laboratory: Alfonsina Ramundo-Orlando
Young researchers: Martina Albini, BSc (Biol.), Simone Dinarelli (PhD Biophysics), Andrea Lucibello (PhD Engineering)
Technical assistant: Mauro Fabiani

Brief description of area of expertise (up to 500 words):
The ENEA group of SAD Laboratory has a long term expertience in the realisation of electron based sources of coherent electromagnetic radiation and, among the others, they have realised two Free Electron Lasers (FEL) operating in the millimetre and sub-millimetre wave range. The group has realised an extensive variety of applications in different scientific and technological fields, like living plants biology, human biology, solid state physics and cultural heritage protection.
The most notable activity was the pioneering EU THz BRIDGE project (2001 – 2004), that opened the way to the study of the interaction of THz radiation with biological systems following a streamline of increasing complexity, from bio-molecules to living cells and tissues. Current applications are carried out in the frame of the GREAM project, financed by the Ministry of Defence, and the THz-ARTE project financed by the Ministry Foreign Affairs. The GREAM activity is realised in collaboration with the Army Medical and Veteri nary Research Centre in Rome, and the Roma 3 University and is focused
on the study of potential genotoxic effects induced on different kind of living cells after irradiation with the Compact FEL source. The THz-ARTE activity is conducted in the frame of the bilateral collaboration agreement between Italy and Japan. For this project the ENEA THz Laboratory has realized an innovative imaging system for the analysis of artwork samples. An important existing partnership has been established with the Clarendon Laboratory, Oxford University (UK), with which ENEA signed a memorandum of understanding for an extensive collaboration on THz Spectroscopy, based on the time -domain techniques, with a focus on fusion plasma diagnostics.
The IFT-CNR group has been extensively involved on development of membrane model systems and protocell from both research and educational point of view. Our lab made major contributions to the enhance of understanding of biophysical mechanisms of interaction between extremely-low frequency (ELF) EMF and membrane models. Using a simpler model than cell membrane it is possible

to reveal in real time relevant changes in the function and structure of lipid bilayer under exposure to EMF at different frequencies. Experimental studies on giant liposomes, with cell dimension, are already underway that build on the results obtained via participation in the EU THz-BRIDGE ptoject. In this case Compact FEL has been successfully employed in the study of the effects of 130 GHz radiation on the increase of permeability of enzyme-loaded liposomes. It has been shown that short pulses of THz radiati on delivered by this source can yield a peak electric field greater than 2 kV/cm at the sample surface when the THz beam is focused to a spot size of about 0.5x1cm2. This relatively high value of the field amplitude is capable of inducing a voltage drop across a lipid bilayer, which cannot be considered negligible when compared to the natural membrane potential. We have developed a new model system to reconstitute gap junction (e.g. connexin-32) between two apposite liposomes as experienced in natural cells. An International Training School on Advanced Topics on Cell Model Systems for the development of early pharmaceutical research, biomaterials, tissue engineering and molecular simulation is annually organized by our lab. Focus of the school is the creation of models representing cellular structure and function, and predicting cell responses to physiological stimuli and to pharmaceutical agents that could help speed discovery of new drugs.

Facilities:

The ENEA group has several facilities hosting numerous devices. A Compact FEL is based on a microtron type accelerator source capable of generating a relativistic electron beam of 300 mA, with 4 ms pulse duration, at energy of 2.3 MeV. A magnetic undulator provides the energy transformation from the electron kinetic energy into electromagnetic radiation. This FEL generates radiation in the range 100-150 GHz with a peak power of 1.5 kW over the pulse duration of 4 ms, but due to the fact that the microtron is a Radio-Frequency (RF) accelerator, a sub-structure inside the pulse generates a train of micro-pulses of about 60 ps (separated by 330 ps) with a peak radiation power of about 10 kW. The average CW power can be varied according to the repetition rate of the RF system driving the accelerator giving about 6 mW/Hz. The second FEL source is named FEL-CATS (Compact Advanced THz Source); it is a compact source based on a Linac type accelerator (2.3 MeV of energy and 250 mA of average current over 5 ms), but it includes a further RF device (called PMD), downstream the Linac, capable of manipulating the electrons in the longitudinal phase-space in such a way to create an adequate level of order in the bunch to enhance the electromagnetic generation mechanism. This unique source extends its tuning capability over almost one octave. In fact, it generates radiation from 0.7 THz to 0.4 THz with a power level of several kW over the microsecond pulse duration. Beside FEL sources the THz laboratory has an extensive assortment of other coherent sources; the first to be mention is a Far InfraRed (FIR) gas laser based on a CO2 laser pumping a gas cell. This is a versatile device because the pumping laser can excite with different lines and, moreover, the active media (the gas) can be changed making this laser the most widely tunable (on a series of individual lines). It can, in principle, operate from 40 mm up to about 1.1 mm wavelength. The most powerful line is at a wavelength of about 119 mm with an average CW power of about 130 mW. This laser has been recently re furbished with a new power supply that can be externally controlled, by a waveform generator, for a proper tailoring of the radiation time structure. A second important laser system is a Ti:Sa laser oscillator that generates a train ultra-short pulses (about 130 fs) in the Near InfraRed (NIR) region at about 800 nm (@80 Mhz in modelocking) with an average CW power in excess of 1W. This source is now used to generate THz radiation by means of coherent optical rectification process in non-linear antennas and, due to the high repetition rate it has commonly become a spectroscopic tool by means of the so-called Time Domain Spectroscopy (TDS) technique. Other compact, and portable, solid state sources are available in the THz laboratory to cover the low frequency spectrum: they are an YIG oscillator that emits in the range 20-40 GHz with an average CW power of 5-10 mW. An IMPATT diode at 97 GHz with about 80 mW of output power and a second IMPATT diode emitting at 140 GHz with about 20 mW of power.
IFT- The IFT is one of the eleven Institute of CNR Research Establishment based at Rome-Tor Vergata, located very close to the Second University of Rome; the Italian National Agency for new

technologies, Energy and sustainable Economic development (ENEA)-Frascati; the National Institute of Nuclear Physics and ESA-ESRIN centre for Earth observation. The IFT comprises 91 units of research staff (52 researchers / 19 technologists and administrative technicians), 20 units including Temporary Research Fellow, PhD candidate and personnel in training for master and bachelor thesis and 19 associate researchers. Research activities of the IFT focus on mechanisms of insurgence of human disorders, particularly of neoplastic, immuno-degenerative, infective and neurogenetic diseases, and on the development of innovative preventive/therapeutic strategies, also considering the translation to clinic. At IFT there are fully equipped facilities for molecular and biological investigations and the safe handling of biological materials.
Liposome Research Laboratory: The Laboratory comprises two separate rooms divided by type of experimental work:
Biochemical/Molecular biology:
1. Biochemical studies, isolation of sub-cellular organelles, purification of proteins (e.g. chromatography, ultra-centrifugation, gradients, electrophoresis units);
2. Development of model membrane systems different in size and lamellarity (e.g. LIPOSOMAT, Extruder, Sonicator, GUV electroformation cell, Evaporator, AC-field Generator).
Electromagnetic Exposure:
1. Electromagnetic field exposure devices for studies in the range from 0 to 100 Hz at 0-70 microTesla intensity comprising of triaxial coils and equipped with amplifiers, magnetic probe and computer control system; -MW Irradiation studies at 2.45GHz (strip-line adaptable to the cell sample of spectrophotometer); - MW Irradiation studies at 53.37 and 42.22 GHz (MM band low intensity device IGM by Micro Med Tech); -Network Analyzer HP 8510 C;
2. Spectrophotometers (e.g. CARY50 fitted with an optical probe of 1 meter long to be used for measurements in real time during irradiation studies).
3. A special thermoregulatory system is also available.
4. A specially constructed magnetically shielded room (3x8x2.4 m). Its mumetal shielding is capable of reducing external electromagnetic fields, both 'man made' and geomagnetic, to picoTesla values within the room.

Area where interaction is needed:
Since our aim is to provide a unified view of ultrafast electromagnetic interactions with cellular systems, we need interactions and collaborations with different teams involved on studies with high voltage, short duration electric pulses at different biological level. In order to compare our studies on membrane models with pulsed carrier waves between 30 GHz and 3 THz with same electric field amplitude and pulse duration and thus demonstrate that cellular systems can rectify rapidly oscillating electric fields at frequencies which have equivalent time durations ranging from pico-tomicroseconds.

Existing collaborations:
ENEA-Frascati
-Celio Army Hospital in Rome (It) and the Roma Tre University (It) for the GREAM project.
- NICT of Tokio (Jp) and CNR-IFAC of Florence (It) for the THz Arte project
- Oxford University (UK) for the Eurofusion Enabling Research project

IFT-CNR
-International (University of Karachi, Pakistan; University of Teheran, Iran; University of Fukui, Japan)
-European (CNRS University of Rennes, France)
-National (Polito University of Turin; University of Bari; University of Federico II, Naples; University of Cosenza; ENEA-Frascati; CNR-IREA Naples; CNR-ISM Roma; University of Roma3)
Memberships: COST Action EMF-MED B1309, BIOELECTRICS, ICEmB

Bioelectrics 2015 - The 12th International Bioelectrics Symposium

Institution name:
Laboratory: Vectorology and Anticancer Therapies, joint research unit (UMR) 8203 of CNRS, University Paris-Sud and Gustave Roussy
TEAM 1: Vectorology of anticancer drugs and nucleic acids
GROUP: Physical vectorology
Web page:

Principal investigator: Lluis M. MIR

Staff members of the Group:
CNRS: Isabelle Leray, Thierry Ragot, Franck André, Isabelle Croquison
Post-docs and researchers with temporary positions: Marie Breton, Caterina Merla, Tomas Garcia-Sanchez
Ph.D. students: Antoine Azan, Hanna Hanna, Florian Gailliègue, Khaouter Tounekti

Brief description of area of expertise (up to 500 words):
Physics, chemistry and biology of the membrane impermeability rupture caused by electric pulses.
Basic mechanisms of cell electroporation and cell electropermeabilization: modeling, development of optical analytical approaches based on Raman spectroscopy and microscopy, detailed analysis of the chemistry involved in the membrane impermeability rupture,…
Electrotransfer of anticancer drugs and nucleic acids: applications and analysis of the mechanisms of the molecules electromediated transport, applications of gene electrotransfer.
Analysis of the interactions of electromagnetic fields with biological objects

Facilities of the Group:
Microsecond and nanosecond pulse generators; Fluorescence microscopes equipped with time-lapse video equipment, various illuminations systems and high sensitivity cameras; FACS; unique, laboratory-built Coherent Antistockes Raman Scattering microscope and spectrometer, etc.
Facilities of the team and the laboratory:
complete cell culture facilities (3 rooms, 8 laminar hoods, 8 incubators including hypoxic incubators, etc…);
complete molecular biology facilities with qPCR, thermocyclers, nanodrop;
material to work with nanoparticles including ultracentrifuges and nanozetasizer;
facilities to work with viruses including a P3 laboratory;
mass spectrometer, classical spectrophotometers as well as absorbance, fluorescence and chemiluminescence plate readers;
basic resources (6 freezers -80°, cryopreservation under liquid nitrogen in special rooms, 2 cold rooms at 4°, radioactivity room, storage rooms, …);
access to a cell and animal imaging platform, equipped with cell sorters, IVIS, all sort of classical microscopes (confocal, biphotonics);
access to animal housing facilities for small laboratory animals, with in-house production of nude and NOD-SCID mice, transgenic mice maintenance, isolators to treat mice under P3 conditions, etc;

Area where interaction is needed:
Interaction with groups possessing an expertise in the generation and the application of cold plasmas to cells and tissues

Existing collaborations:
Collaborations with the other groups of the European Associated Laboratory (LEA) on the applications of the Electric pulses in Biology And Medicine (LEA EBAM) of the CNRS and 8 other institutions in France and Slovenia.
Collaborations in France with mathematicians, groups specialized in linear and non linear optics, ...
Colaborations with other groups in the EU (Germany, Spain).