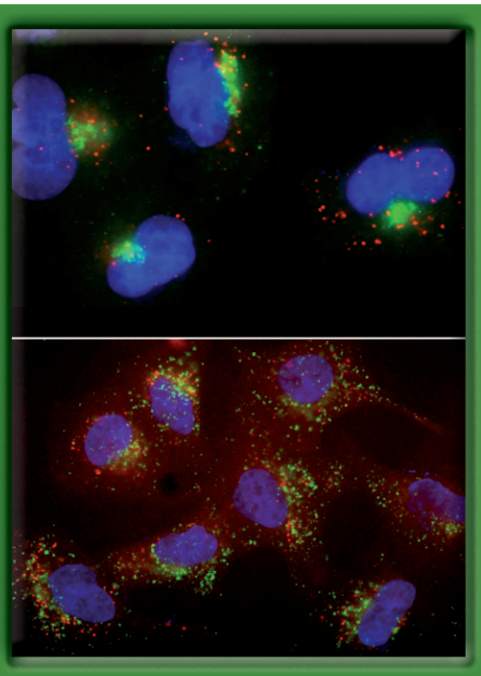
SCIENTIFIC PROGRAM AND BOOK OF ABSTRACTS

## BONSAI PROJECT SYMPOSIUM

# Breakthroughs in Nanoparticles for Bio-Imaging

**ENEA Research Centre of Frascati**
**Frascati (Rome), Italy**
**8th-9th April, 2010**



EC FP6 BONSAI
Project 037639

ENEA
Agenzia nazionale per le nuove tecnologie, l'energia e lo sviluppo economico sostenibile

*Cover image: Lung epithelial cells incubated with iron-oxide nano-particles loaded with TRITC, labelled for nucleus (blue) and Golgi apparatus or Lysosome (green). Cortesy of Dr. Ilaria Rivolta, University of Milan-Bicocca (Italy)*

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# *Breakthroughs in Nanoparticles for Bio-Imaging*

**ENEA Research Centre of Frascati**

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## Scientific Program and Book of Abstracts

## The BONSAI Symposium is supported by:

**ENEA, Italy**

**CEA, France**

**UCM, Spain**

**CSIC, Spain**

**University of Padova, Italy**

**MPIKG and MPIA, Germany**

**University of Milano-Bicocca, Italy**

**GUERBET, France**

**GPI-NSC, Russia**

**University of Freiburg-CAB, Germany**

**NANOVECTOR srl, Italy**

**TILL Photonics GmbH, Germany**

**INFLPR, Romania**

## BONSAI Symposium Organizers

E. Borsella-ENEA, Italy; N. Herlin-CEA, France; S. Veintemillas Verdaguer-CSIC, Spain; M. Garcia-UCM, Spain; G. Mattei-Univ. of Padova, Italy; D. Wang-MPIKG, Germany, F. Huisken-MPIA, Germany; G. Miserocchi & I. Rivolta-Univ. of Milan Bicocca, Italy; J. M. Idée-GUERBET, France; V. Pustovoy & V. Loschenov-GPI, Russia; K. Palme -Univ. of Freiburg, Germany; P. Gasco-NANOVECTOR srl, Italy; R. Uhl-TILL Photonics GmbH, Germany; R. Alexandrescu & I. Morjan-INFLPR, Romania.

## Symposium Secretariat

G. Bartolomei, M. Cimino- ENEA, Italy.

## Symposium Location

ENEA Research Centre of Frascati, Conference Room Bruno Brunelli, Via E. Fermi 45, Frascati (Rome), Italy

# Welcome to the Symposium

This volume collects the Abstracts presented as contributions to the Symposium entitled "Breakthroughs in Nanoparticles for Bio-imaging", held on Apr. 8-9, 2010 at the ENEA Research Centre of Frascati (Rome), Italy

Particles on the nanoscale exhibit extraordinary physical, chemical and biological properties significantly different from their conventional formulation on the micro-scale. These unique properties offer exciting opportunities and potential benefits in a wide range of applications, including visualization of cellular structures (in tissues and organs), receptors, inflammation, and characterisation of suspect lesions.

In order to take full advantage of nanoparticles for developing truly innovative bio-imaging techniques, it is necessary to address challenging questions, including surface functionalization, biocompatibility, cellular interaction and uptake of nanoparticles, effect of shape, size and coating in nanoparticle bio-distribution.

The FP6 Project BONSAI (Bio-imaging with smart multi-functional nanoparticles) was funded by EC in the fall 2006 with the objective of contributing to the progress in this key research area and it is now approaching its conclusion.

The aim of the Symposium is to gather the BONSAI Partners and scientists active in the field (chemists, material scientists, physicists, engineers, clinicians and biochemists) to discuss new ideas, experimental results and perspectives ranging from the preparation to the applications of functional nanoparticles for bio-imaging and cell labelling.

The abstracts here collected concern the main topics of the BONSAI Project, i.e. *Development of nanoparticles for bio-imaging*, *Cellular interaction and toxicity of nanoparticles* and *Bio-imaging using nanoparticles*.

We acknowledge all participants for their interest in the Symposium. A special tank to the invited speakers for their contributions. Our gratitude to the ENEA staff for the editorial support and for the organization of the event.

We would like to particularly thank the PO Dr. Philippe Jehenson for supportive and effective monitoring of the Project. This Symposium was made possible by the financial support of EC to the FP6 BONSAI Project N° 037639 in the Thematic Priority LifeSciHealth.

The BONSAI Partners

# BONSAI PROJECT SYMPOSIUM

EC FP6 BONSAI
Project 037639

# Scientific Program

BONSAI Project

# BONSAI SYMPOSIUM

## *Breakthroughs in Nanoparticles for Bio-Imaging*

## ENEA Research Centre of Frascati, Frascati (Rome), Italy

| Thursday, April 8, 2010 | |
|---|---|
| 8:15 am to 8:45 am | **Registration at Symposium Secretariat** |
| 8:45 am to 9:00 am | **Opening-Welcome Address**<br>Elisabetta Borsella,<br>ENEA, Research Centre Frascati, Frascati (Rome), Italy |
| 9:00 am to 11:00 am | **Session on Development of Nanoparticles for Bio-imaging: I Part.**<br><br>Chairs:<br>Dayang Wang MPI of Colloids and Interfaces, Potsdam, Germany<br>Alexander Douplik Friedrich-Alexander Universität Erlangen-Nürnberg, Germany. |
| I1<br>9:00 am to 9:30 am | **Invited Talk**<br>*From Inorganic nanoparticles towards their assembly in mesoscale structures designed for biological applications*<br>Teresa Pellegrino<br>National Nanotechnology Laboratory of CNR-INFM, Lecce, Italy and Italian Institute of Technology, Genova, Italy |
| I2<br>9:30 am to 10:00 am | **Invited Talk**<br>*Developing novel magnetic nanoparticles for MRI contrast agent*<br>Nguyen TK. Thanh<br>The Davy-Faraday Research Laboratory, The Royal Institution of Great Britain, Department of Physics and Astronomy, Univeristy College of London, London, UK. |
| | **Presentations** |
| S1-1<br>10:00 am to 10:20 am | *Magnetite Nanoparticles between 10 and 40 nm: synthesis, magnetic properties, dendron grafting and suspensions stability*<br>L. Truonc Phuoc, J. Santoyo Salazar, H. Mamlouk, D. Felder-Flesch, S. Begin-Colin, G. Pourroy<br>Institut de Physique et Chimie des Matériaux, UMR 7504 CNRS-ECPM-Université de Strasbourg, 23 rue du Loess, BP 43, 67034 Strasbourg Cedex 2, France<br>O. de Abril, L. Perez, M. Vazquez<br>Instituto de Ciencia de Materiales, CSIC, 28049 Madrid, Spain |
| S1-2<br>10:20 am to 10:40 am | *Synthesis of $MFe_2O_4$ (M = Fe, Mn) nanoparticles with tunable sizes*<br>Lourisa Cabrera[a], Álvaro Somoza[b], Carlos J. Serna[a] and M. Puerto Morales[a]<br>[a]Instituto de Ciencia de Materiales de Madrid/CSIC, Cantoblanco, Madrid, Spain<br>[b]IMDEA-Nanociencia, Facultad de Ciencias Módulo C-IX, Cantoblanco, Madrid, Spain |

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|---|---|
| S1-3<br>10.40 am to<br>11:00 am | *Continuous Production of magnetic nanoparticles for bio-applications by laser pyrolysis.*<br>Rodica Alexandrescu [1], Valentina Bello [2], Virginia Bouzas [3], Munish Chanana[4] , Rocío Costo-Cámara [5], Florian Dumitrache[1], Miguel Angel García [3], Maria del Puerto, Morales Herrero [5], Ion Morjan[1], Carlos J Serna [5] Sabino Veintemillas-Verdaguer [5], Dayang Wang[4]<br>[1]Natl. Inst. Lasers Plasma & Radiat. Physics Romania, [2]Dept. Physics, Univ. Padova, Italy<br>[3]Dept. Fis. Matt. Univ. Complutense Madrid, [4]Max Plank Inst. Colloids and Interfaces, Postdam, Germany, [5]Inst. Ciencia de Materiales de Madrid CSIC, Spain |
| 11:00 am<br>to 11:20 am | **Coffee Break** |
| 11.20 am<br>to 1:20 pm | **Session on Development of Nanoparticles for Bio-imaging: II Part.**<br><br>Chairs:<br>Friedrich Huisken, MPIA and University of Jena, Jena, Germany<br>Teresa Pellegrino, National Nanotechnology Lab. CNR-INFM, Lecce and IIT Genova, Italy |
| K1<br>11.20 am<br>to 11:50 am | **Keynote Address**<br>*Surface Processing of Nanoparticles for Bio-Imaging*<br>Dayang Wang and Haolan Xu<br>Max Planck Institute of Colloids and Interfaces, Potsdam, Germany |
| I3<br>11.50 am to<br>12.20 am | **Invited Talk**<br>*Size- and Composition-tunable Semiconductor Nanocrystals and Their Bio-applications*<br>Ming-Yong Han<br>Institute of Materials Research & Engineering, A-STAR, Singapore, Division of Bioengineering, National University of Singapore, Singapore |
| | **Presentations** |
| S1-4<br>12:20 am to<br>12:40 am | *Fluorescent labels based on nanosized silicon and diamonds*<br>Anna Fucikova[1,2,3,] Jan Valenta[1], Ivan Pelant[2], Vitezslav Brezina[3]<br>[1]Faculty of Mathematics and Physics, Charles University, Prague 2, CzechRepublic<br>[2]Institute of Physics AS CR, v. v .i., Prague 6, Czech Republic<br>[3]Institute of Systems Biology and Ecology AS CR, v. v .i., Nove Hrady, Czech Republic |
| S1-5<br>12:40 am<br>to 1:00 pm | *Luminescent Silicon based Nanoparticles by laser pyrolysis*<br>E. Borsella[1], R. D'Amato, M. Falconieri[2], N. Herlin[3], V. Maurice[3], O. Sublemontier[3], F. Huisken[4], T. Schmidt[4], G. Mattei[5], E. Trave[5]<br>[1] ENEA C. R. Casaccia, Roma, Italy<br>[2] ENEA C. R. Frascati, (RM), Italy<br>[3]Laboratoire Francis Perrin (CEA-CNRS URA 2453), SPAM, DSM, CEA Saclay, France<br>[4] Lab. Astrophysics and Cluster Physics Group, Inst. for Solid State Physics, F. Schiller-Universität Jena-Germany<br>[5] University of Padova, Dept. of Physics, Padova, Italy |
| S1-6<br>1:00 pm to 1:20<br>pm | *Fluorescence from silicon nanoparticles suspended in water: reactive co-deposition for the control of surface properties of clusters*<br>Klaus von Haeften, Atea Akraiam, Gauthier Torricelli, Anthony Brewer<br>Department of Physics and Astronomy, University of Leicester, Leicester LE1 7RH, UK |
| 1:20 pm<br>to 2:30 pm | **Lunch at ENEA Cafeteria** |

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|---|---|
| 2:00 am to 4:30 am | **Session on Cellular Interaction and Toxicity of Nanoparticles**<br><br>Chairs:<br>Florence Gazeau CNRS and Université Paris 7, Paris, France<br>Giuseppe Miserocchi Università di Milano Bicocca, Monza, Italy |
| I4<br>2:30 pm to 3:00 pm | **Invited Talk**<br>*Characterization of inorganic nanoparticles in regard to cytotoxicological screening*<br>Wolfgang Parak<br>Fachbereich Physik, Philipps Universität Marburg, Marburg, Germany. |
| K2<br>3:00 pm to 3:30 pm | **Keynote Address**<br>*Bio-testing of nanoparticles*<br>Giuseppe Miserocchi,<br>Università di Milano Bicocca, Monza, Italy |
| | **Presentations** |
| S2-1<br>3:30 pm to 3:50 pm | *Si-based Nanoparticles: a biocompatibility study*<br>I. Rivolta[1], B. Lettiero[1], A. Panariti[1], R. D'Amato[2], V. Maurice[3], M. Falconieri[2], N Herlein[3], E. Borsella[2] and G. Miserocchi[1]<br>1.Università di Milano Bicocca, Monza, Italy;<br>2. ENEA, Rome, Italy<br>3. CEA-CNR, Saclay, France |
| S2-2<br>3:50 pm to 4:10 pm | *Influence of iron oxide magnetite nanoparticles and fluorinated phosphates on red blood cells and CaCo-2 cells*<br>Daniel Moersdorf, Pierre Hougunenq, Ingolf Bernhardt<br>Laboratory of Biophysics, Saarland University, Saarbruecken, Germany |
| S2-3<br>4.10 pm to 4:30 pm | *The possible side-effects of iron oxide nanoparticles on cell functionality and MR signal*<br>S. J. Soenen[1], N. Nuytten[1], U. Himmelreich[2] and M. De Cuyper[1]<br>[1] Lab of BioNanoColloids, IRC, Katholieke Universiteit Leuven Campus Kortrijk<br>[2] Biomedical NMR Unit, Katholieke Universiteit Leuven. |
| S2-4<br>4:30 pm to 4:50 pm | *Towards ideal magnetofluorescent nanoparticles for bimodal detection of breast cancer cells*<br>Davide Prosperi,[1] Miriam Colombo,[1] Silvia Ronchi,[2] Fabio Corsi,[3] Clara De Palma,[4] Emilio Clementi.[4]<br>[1] Dipartimento di Biotecnologie e Bioscienze, Università di Milano-Bicocca, Milano (Italy).<br>[2] Istituto di Scienze e Tecnologie Molecolari, CNR, Milano.<br>[3] Dipartimento di Scienze Cliniche "Luigi Sacco", Università degli Studi di Milano, Ospedale L. Sacco, Milano.<br>[4] Dipartimento di Scienze Precliniche Lita Vialba, Università degli Studi di Milano, Ospedale L. Sacco, Milano. |
| 5:00 pm to 6:30 pm | **POSTER SESSION** |
| 6.45 | **Bus Departure to Frascati** |
| 8.00 | **Dinner at Restaurant Cacciani**<br>Via A. Diaz 13, Frascati |

| Friday, April 9, 2010 | |
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| 8:30 am to 10:50 am | **Session on Bio-imaging using Nanoparticles: I Part**<br><br>Chairs:<br>Matthias Taupitz, Universitätsmedizin Berlin, Berlin, Germany<br>Paolo Decuzzi Univ. of Texas, Medical School at Houston, USA-Univ. of Magna Graecia, Catanzaro, Italy. |
| I5<br>8:30 am to 9:00 am | **Invited Talk**<br>*Labelling, imaging and manipulating the cell with anionic magnetic nanoparticles*<br>Florence Gazeau<br>Laboratoire Matière et Systèmes Complexes, CNRS and Université Paris 7, Paris, France. |
| I6<br>9:00 am to 9:30 am | **Invited Talk**<br>*An integrated approach for the rational design of MRI contrast agents with large longitudinal relaxivity*<br>Paolo Decuzzi,<br>Dept. of Nanomedicine and Biomedical Engineering The University of Texas, Medical School at Houston, Houston, Texas, USA and Center of Bio-Nanotechnology and Engineering for Medicine, University of Magna Graecia, Catanzaro, Italy. |
| | **Presentations** |
| S3-1<br>9:30 am to 9:50 am | *Dendronised Iron Oxide Nanoparticles for biomedical Application*<br>B. Basly,[1] D. Felder-Flesch,[1] P. Perriat,[2] Claire Billotey,[3] J. Taleb, [3] G. Pourroy,[1] S. Bégin-Colin[1]<br>[1] Institut de Physique et Chimie des Matériaux de Strasbourg, UMR CNRS/UDS 7504, Strasbourg, France<br>[2] Groupe d'Etudes de Métallurgie Physique et de Physique des Matériaux, UMR 5510 CNRS-INSA de Lyon, Villeurbanne, France.<br>[3]UMR 5220, Inserm U 630, INSA-Lyon, Villeurbanne, France. |
| S3-2<br>9:50 am to 10:10 am | *Magnetic nanoparticle location and quantification in mice tissues after intravenous injection*<br>Lucía Gutiérrez, Lourisa Cabrera, Carlos J. Serna and M. Puerto Morales<br>Instituto de Ciencia de Materiales de Madrid/CSIC, Cantoblanco, Madrid, Spain<br>Raquel Mejías, Domingo F. Barber<br>Department of Immunology and Oncology, Centro Nacional de Biotecnología/CSIC, Cantoblanco, Madrid, Spain |
| S3-3<br>10.10 am to 10:30 am | *USPIO lipid coating by warm microemulsion: structure-activity relationship*<br>P.Gasco[1], N. Vivenza[1], G. Riccio[1], JM Idee[2], W. González[2], G. Miserocchi[3], I.Rivolta[3], G.Sancini[3], P.Mazzoldi[4] V. Bello[4], G. Mattei[4], MA Garcia[5], V. Bouzas[5], S. Veintemillas[6], I. Morian[7], E. Borsella[8]<br>[1]Nanovector srl – Torino (Italy) [2]Guerbet SA – Paris (France)<br>[3]Exp. Medicine Dep., Univ. of Milano Bicocca (Italy) [4]Dept. of Physics, Univ. of Padova (Italy)<br>[5]Univ. Complutense Madrid (Spain) [6]Instituto de Ciencia de Materiales de Madrid CSIC (Spain)<br>[7]Institute for Laser and Radiation Physics, Bucarest (Romania) [8] ENEA - Frascati (Italy) |
| S3-4<br>10:30 am to 10:50 am | *Targeting cells with MR imaging probes: cellular interaction and intracellular magnetic iron oxide nanoparticles uptake in brain capillary endothelial and choroidal plexus epithelial cells*<br>*I. Cambianica,**M. Bossi, ^P. Gasco, #W. Gonzalez, #JM. Idee, *G. Miserocchi, **R. Rigolio, @D. Wang , *G. Sancini |

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| | *Dept. of Exp. Medicine and **Dept. of Neuroscience, Univ. of Milano-Bicocca, Monza, Italy<br>^Nanovector S.r.l., Torino, Italy<br>#Guerbet Res, Roissy, France<br>@Max Planck Institute of Colloids and Interfaces, Dept. of Interfaces, Potsdam, Germany |
| 10.50 am to 11.10 am | **Coffee Break** |
| 11.10 am to 1:20 pm | **Session on Bio-imaging using Nanoparticles: II Part**<br><br>Chairs:<br>Wolfgang Parak, Philipps Universität Marburg, Marburg, Germany.<br>Ming-Yong Han A-STAR, Singapore, National University of Singapore, Singapore |
| I7<br>11.10 am<br>to 11:40 am | **Invited Talk**<br>*Electrostatically stabilized iron oxide nanoparticles for in vivo MRI*<br>Matthias Taupitz<br>Department of Radiology Charité , Universitätsmedizin Berlin, Berlin, Germany. |
| K3<br>11.40 am to<br>12.10 am | **Keynote Address**<br>*Ultrasmall Superparamagnetic Particles of Iron Oxide, Where We Are, Where We Want to Go: a Pharmaceutical Company Viewpoint*<br>Jean-Marc Idée<br>Guerbet, Research Division, Aulnay-sous-Bois, France |
| I8<br>12:10 am to<br>12:40 am | **Invited Talk**<br>*Luminescent Silicon Nanocrystals: Synthesis, Functionalization, and Applications in Bioimaging*<br>Mark T. Swihart<br>Department of Chemical and Biological Engineering and Institute for Lasers, Photonics and Biophotonicsb, University at Buffalo (SUNY), Buffalo, New York, USA. |
| | **Presentations** |
| S3-5<br>12:40 am<br>to 1:00 am | *Fate and biological effects of systemically administered quantum dots: impact of surface modification*<br>Markus Rehberg*, Marc Praetner*, Camila Ferreira Leite, Peter Bihari, Fritz Krombach<br>Walter-Brendel-Centre of Experimental Medicine, Ludwig-Maximilians-Universität München, Munich, Germany |
| S3-6<br>1:00 pm<br>to 1:20 pm | *Methods of silicon nanoparticles visualizations for in-vivo application*<br>Loshchenov V., Korovin S., Pustovoy V., Ryabova A., Vasil'chenko S., Grachev P<br>A.M. Prokhorov General Physics Institute RAS, Moscow, Russia |
| 1:20 pm<br>to 2:30 pm | **Lunch at ENEA Cafeteria** |

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| 2:30 am to 4:40 am | **Session on Bio-imaging using Nanoparticles: III Part**<br><br>Chairs:<br>Giovanni Mattei, University of Padova, Padova, Italy<br>Mark T. Swihart University at Buffalo (SUNY), Buffalo, New York, USA |
| I9<br>2:30 pm to 3:00 pm | **Invited Talk**<br>*Plasmonic nanostructures as contrast agents for bioimaging*<br>Alexander Douplik<br>Erlangen Graduate School in Advanced Optical Technologies (SAOT), Friedrich-Alexander Universität Erlangen-Nürnberg, Germany. |
| | **Presentations** |
| S3-7<br>3:00 pm to 3:20 pm | *Miniaturised devices for Au Nanorods detection*<br>V.Bouzas[1,2], M. A. Garcia[1,2], N. Carmona[1]<br>[1]Dpto. Física de Materiales, Universidad Complutense de Madrid<br>[2]Instituto de Cerámica y Vidrio, Consejo Superior de Investigaciones Científicas, Madrid, Spain |
| S3-8<br>3:20 pm to 3:40 pm | *Enhancing the sensitivity of DNA microarray by using dye-doped silica nanoparticles: application to Papilloma Virus detection*<br>F. Enrichi, R. Riccò, A. Meneghello, R. Pierobon, E. Cretaio<br>*CIVEN (Coordinamento Interuniversitario Veneto per le Nanotecnologie), Marghera (VE), Italia* |
| S3-9<br>3.40 pm to 4:00 pm | *Study of the photoluminescence bleaching in Si nanocrystals prepared by laser assisted pyrolysis*<br>Ayse Seyhan[1], Urcan Guler[1] ,Mauro Falconieri[2], Rosaria D'Amato[3], Elisabetta Borsella[3], Rasit Turan[1]<br>[1]Department of Physics Middle East Technical University, Ankara, Turkey<br>[2]ENEA C. R. Casaccia, Roma, Italy<br>[3]ENEA C. R. Frascati, Frascati, Roma, Italy |
| S3-10<br>4:00 pm to 4:20 pm | *Characterization of the transition dipole-moment of single semiconductor nanoparticles*<br>Anna M. Chizhik[1], Alexey I. Chizhik[1], Torsten Schmidt[2], Alfred J. Meixner[1], Friedrich Huisken[2]<br>[1] – Institute of Physical and Theoretical Chemistry, University of Tuebingen, Auf der Morgenstelle 8, D-72076, Germany<br>[2] – Laboratory Astrophysics Group of the Max Planck Institute for Astronomy at the Institute of Solid State Physics, University of Jena, Helmholtzweg 3, D-07743 Jena, Germany |
| S3-11<br>4:20 pm to 4:40 pm | *Innovative application of iron oxide nanoparticles for rapid biomarker isolation in life sciences*<br>Wang[1], B., Teale[1], W., Dürr[1], J., Verdaguer[3], S., Herlin-Boime[4], N., Wang[5], D., and Palme[1,2], K.<br>[1]Institute of Biology II, University of Freiburg, Freiburg (Germany), [2]Freiburg Institute of Advanced Studies FRIAS, University of Freiburg, Freiburg (Germany), [3]CSIC- Instituto de Ciencia de Materiales, Madrid (Spain), [4]CEA Saclay, SPAM/LFP Gif/Yvette (France), [5]Max Planck Institute of Colloids and Interfaces, Potsdam (Germany) |
| 4:40 pm to 4:50 pm | **Closing Address** |
| 5.00 pm | **Bus departure to Frascati** |

**POSTER SESSION**
**Apr. 8, 2010**
**5.00 pm to 6.30 pm**

P1-1 *Development of magnetic Fe@C nanocomposites obtained via the laser pyrolysis: structural and disaggregation properties*

Ion Morjan[1], R. Alexandrescu[1], F. Dumitrache[1], C. Fleaca[1], R. Birjega[1], I.Soare[1], V. Prodan[2], V. Kuncser[3], H. Xu[4], D. Wang[4]
[1]National Institute for Lasers, Plasma and Radiation Physics, 111 Atomistilor, MG-36, 077125 Bucharest, Romania
[2]Ovidius University of Constanta, Mamaia 124, Constanta, Romania
[3]National Institute of Materials Physics, Atomistilor MG-07, 077125 Bucharest, Romania
[4] Max Planck Institute of Colloids and Interfaces, Dept. of Interfaces, D – 14424 Potsdam, Germany

P1-2 *Iron oxide materials produced by laser pyrolysis*

Rodica Alexandrescu [1], Valentina Bello [2], Virginia Bouzas [3], Rocío Costo-Cámara [4], Florian Dumitrache[1], Miguel Angel García [3], Rosella Giorgi [5], Maria del Puerto Morales Herrero [4], Ion Morjan[1], Carlos J Serna [4] Sabino Veintemillas-Verdaguer [4]
Natl. Inst. Lasers Plasma & Radiat. Physics Romania
1) Dept. Physics, Univ. Padova, Italy
2) Dept. Fis. Matt. Univ. Complutense Madrid
3) Inst. Ciencia de Materiales de Madrid CSIC, Spain
5) ENEA, Rome

P1-3 *Magnetic properties of Fe Oxide Nanoparticles produced by laser pyrolysis for biomedical applications.*

M. A. García[1], R.Costo[2], S. Veintemillas[2], P. Morales[2], M. García-Hernández[2]
R. Alexandrescu[3] I. Morjan[3], P. Gasco[4]
1) Instituto de Cerámica y Vidrio CSIC & Dept. Fis. Mat. Univ. Complutense Madrid
2) Inst. Ciencia de Materiales de Madrid CSIC, Spain
3) Natl. Inst. Lasers Plasma & Radiat. Physics Romania
4) Nanovector S.r.l., Torino, Italy

P1-4 *Reproducibility of the synthesis of iron oxide nanoparticles by laser pyrolysis*

Rodica Alexandrescu [1], Virginia Bouzas [2], Rocío Costo-Cámara [3], Florian Dumitrache[1], Miguel Angel García [2], Maria del Puerto Morales Herrero [3], Ion Morjan[1], Carlos J Serna [3] Sabino Veintemillas-Verdaguer [3]
4) Natl. Inst. Lasers Plasma & Radiat. Physics Romania
5) Dept. Fis. Mat. Univ. Complutense Madrid
Inst. Ciencia de Materiales de Madrid CSIC, Spain

P1-5 *Recent Progress on the Preparation of Luminescent Silicon Nanoparticles for Bio-Imaging Applications*

V. Maurice[1], O. Sublemontier[1], N. Herlin[1], E. Doris[2], O. Raccurt[3], A. Sanson[2]
[1]Laboratoire Francis Perrin (CEA-CNRS URA 2453), SPAM, DSM, CEA Saclay, France
[2] SB2SM, Ibitec-S, DSV, CEA Saclay, France [3] LCSN, DTNM, LITEN, DRT, CEA Grenoble, France

P1-6 *Synthesis and characterization of light-emitting Si/Ge nanoparticles*

T. Schmidt, L. B. Ma, K. Potrick and F. Huisken
Laboratory Astrophysics Group of the Max Planck Institute for Astronomy,
Institute of Solid State Physics, Friedrich Schiller University, Jena, Germany

P1-7 *On the red photoluminescence emission from silicon-based nanoparticles*
M. Falconieri[1], E. Borsella[2], R. D'Amato[2], F. Fabbri[2], E. Trave[3] ,V. Bello[4], G. Mattei[4] Y. Nie[5], D. Wang[5]
1) ENEA C.R. Casaccia Roma, Italy. 2) ENEA C.R. Frascati , Frascati, (Roma), Italy. 3) Dip. Chimica Fisica, Università Ca' Foscari Venezia, Venezia, Italy 4) Dip. Fisica, Università degli Studi di Padova, Padova, Italy
5) Max Planck Institute of Colloids and Interfaces Potsdam, Germany

P1-8 *Synthesis and photoluminescence of Ytterbium-doped Silicon nanocristals*
R. D'Amato (1), M. Falconieri (2), E. Borsella (1)
(1) ENEA, CR Frascati, Frascati (Roma), Italy
(2) ENEA, CR Casaccia, Roma, Italy

P1-9 *Tunable luminescence from oxidized silicon nanoparticles.*
E. Kelm, S. Korovin, V.Pustovoy, A. Surkov, A. Vladimirov
General Physics Institute, Russian Academy of Sciences, Moscow, Russia

P1-10 *Optical properties of silicon nanoparticles covered with the dye layers*
Ryabova A., Vasil'chenko S., Korovin S., Loschenov V., Pustovoy V.
A.M. Prokhorov General Physics Institute RAS

P1-11 *Luminescence of the silicon based nanoparticles.*
E. Kelm, S. Korovin, V.Pustovoy, A. Surkov, A. Vladimirov
General Physics Institute, Russian Academy of Sciences, Moscow, Russia

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S. Korovin, R. Khasanshin*, A. Vladimirov
Natural Science Center of General Physics Institute, Moscow, Russia

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V.Bouzas[1,2], M. A. Garcia[1,2], N. Carmona[1]
[1]Dpto. Física de Materiales, Universidad Complutense de Madrid
[2]Instituto de Cerámica y Vidrio, Consejo Superior de Investigaciones Científicas, Madrid, Spain

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Katerina Polakova [1], Zdenka Markova [1,2], Radek Zboril [1,2], Ingrid Markova [3] and Miroslav Mashlan [1,4]
[1]Centre for Nanomaterial Research, Palacky University, Czech Republic, [2] Department of Physical Chemistry Palacky University, Olomouc, Czech Republic, [3]Department of Radiology, Faculty Hospital F.D.Roosevelt, Banska Bystrica, Slovak Republic, [4]Department of Experimental Physics, Palacky University, Czech Republic, *E-mail: kacka.polakova@email.cz

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B. Lettiero[1], A.Panariti[1], I. Morjan[2], R. Alexandreascu[2], S. Veintemillas[3], D. Wang[4], C Bucci[5], G. Miserocchi[1] and I Rivolta[1]
1. Università di Milano Bicocca, Milano, Italy; 2. NILPRPB, Bucarest, Romania;
3. CSIC, Madrid, Spain; 4. MPI, Postdam, Germany;
5. Università del Salento, Lecce, Italy

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A.Panariti[1], I. Rivolta[1], B. Lettiero[1], G. Chirico[1], P.Gasco[2] and G. Miserocchi[1]
1.Università di Milano Bicocca, Monza, Italy;
2. Nanovector, Turin, Italy

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Garzotto D.[1], Giacobini P. [1], Fubini B. [2], Tomatis M. [2], Quagliotto P. [2], De Marchis S. [1]
1- Dipartimento di Biologia Animale e dell' Uomo, Università di Torino, Torino
2- Dipartimento di Chimica Generale e Organica Applicata, Torino

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Mirka Safarikova and Ivo Safarik
Institute of Systems Biology and Ecology, Department of Nanobiotechnology, Ceske Budejovice, Czech Republic.

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Ryabova A.[1], Vasil'chenko S.[1], Volkova A.[1], Kaliya O.[2], Stratonnikov A.[1], Loschenov V.[1]
[1] A.M. Prokhorov General Physics Institute RAS
[2] Federal State Unitary Enterprise NIOPIK

P2-20 *Biocompatible carbon-coated 3-d metal nanocomposites for therapy of oncological deseases*
Ryabova A.[1], Vasil'chenko S.[1], Grachev P.[1], Ermakov A. [2], Stratonnikov A.[1], Loschenov V.[1]
[1] *A.M. Prokhorov General Physics Institute RAS*
[2] *Institute of Metal Physics, Ural Division RAS*

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Jan Valenta and Anna Fucikova
Department of Chemical Physics & Optics, Faculty of Mathematics & Physics,
Charles University, Prague 2, Czech R.

P3-22 *Nanoparticle characterization by using Tilted Microscopy Techniques*
D. Salerno, D. Brogioli, V. Cassina, F. Mantegazza
Dipartimento di Medicina Sperimentale,, Universita' di Milano - Bicocca, Monza (Milano), Italy

P3-23 *Confocal microscopy characterization of light-emitting nanostructures and X-ray imaging detectors based on color centers in lithium fluoride*
F. Bonfigli[1], S. Almaviva[1] and R. M. Montereali[1]
[1]ENEA, C.R. Frascati, Via E. Fermi 45, 00044 Frascati (Rome) Italy

P3-24 *Confocal spectroscopy and luminescence decay lifetime imaging of single semiconductor nanoparticles*
Alexey I. Chizhik[1], Anna M. Chizhik[1], Torsten Schmidt[2], Alfred J. Meixner[1], Friedrich Huisken[2]
[1] – Institute of Physical and Theoretical Chemistry, University of Tuebingen, Germany.
[2] – Laboratory Astrophysics Group of the Max Planck Institute for Astronomy at the Institute of Solid State Physics, University of Jena, Jena, Germany.

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Valentina Bello[1] Giovanni Mattei[1], Paolo Mazzoldi[1]
Nicoletta Vivenza[2], Paolo Gasco[2,] Jean Marc Idee[3], Caroline Robic[3], Elisabetta Borsella[4]
[1] Department of Physics, University of Padova, Padova, Italy - [2] Nanovector S.r.l., Torino, Italy
[3] Guerbet , Villepinte (Paris),France - [4] ENEA, Frascati (Rome), Italy

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Giovanni Pellegrini, Giovanni Mattei and Paolo Mazzoldi
CNISM, Department of Physics, University of Padova, Via Marzolo 8, I-35131 Padova, Italy

P3-27 *Aluminum Phthalocyanine Nanoparticles for Fluorescent Diagnostics in Dentistry and Skin Autotransplantology*
Vasilchenko S., Volkova A., Ryabova A., Loschenov V.
A.M. Prokhorov General Physics Institute RAS

P3-28 *Nanoscale magnetic, luminescent and plasmon detectable markers and drug delivery systems for cell recognition, labelling and treatment: oligoperoxide based synthesis*
A. Zaichenko, N. Mitina, O. Shevchuk, O. Shapoval
Lviv Polytechnic National University, Ukraine

# BONSAI PROJECT SYMPOSIUM

EC FP6 BONSAI
Project 037639

# Abstracts

**I1**

# From Inorganic nanoparticles towards their assembly in mesoscale structures designed for biological applications

Teresa Pellegrino

National Nanotechnology Laboratory of CNR-INFM, via per Arnesano, 73100 Lecce (Italy)

and

Italian Institute of Technology, via Morego 30, 16163, Genova (Italy)

Current trend in nanotechnology aims at developing nanostructured materials in which various components, having different properties (such as optical, chemical, and magnetic) are properly tailored and combined into single nano-objects. These nanoscale "devices" able to perform, in parallel several tasks will be exploited in fields ranging from biomedicine to material science. In particular, the development of nanostructures which comprise magnetic nanoparticles are of particular interest for biomedical field as indeed such nanostructures can be exploited as contrast agent in magnetic resonance imaging (MRI), as magnetic carrier for drug or gene delivery, and as heat mediators in hyperthermia treatment for killing tumour cells. In this talk we will focus mainly on two types of iron oxide-based nanostructures.

The first types of nanostructures display both fluorescent and magnetic features as they include together to the iron oxide nanoparticles, the magnetic domain, the fluorescent portions which are based on organic fluorophores, or quantum dots.

The second types of magnetic nanostructures are carrier nano-systems able to deliver their cargo under defined stimuli, like pH or temperature. Preliminary results on the development of magnetic nanostructures based on stimuli responsive polymer which are able to deliver in a controlled manner their payloads (DNA or doxorubicin and magnetic nanoparticles) will be also reported.
Example of applications of such nanostructures in biological assays will be also provided.

# I2
# Design, Synthesis, Characterization of Novel Magnetic Nanoparticles for Biomedical Applications

DR NGUYEN TK THANH
Royal Society University Research Fellow, Reader
The Davy-Faraday Research Laboratory, The Royal Institution of Great Britain
Department of Physics and Astronomy, Univeristy College London.

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Magnetic nanoparticles (MNP) have been attracting great interest due to their potential applications in healthcare, such as hyperthermia treatment of solid tumours, magnetic resonance imaging (MRI), and targeted drug delivery. One of my research interests is to produce new magnetic nanomaterials, with novel properties for high-sensitivity MRI and efficient magnetic fluid hyperthermia treatment. We have developed fabrication method for novel bio-compatible MNPs using "wet chemistry". Besides the possibility to obtain a large scale of product, chemical syntheses also permit the control of morphology and size of NPs which allow further manipulations such as bio-functionalisation. We are developing different synthetic methods using single or bimetallic precursors to fabricate MNPs with enhanced intrinsic magnetic responses of NPs.

Current chemical synthesis produces excellent transition metal MNPs, but these are only soluble and stable in organic solvents. In water these NPs rapidly (minutes) deteriorate and lose their magnetic properties. Single transition metals (*e.g.,* Co) or alloys (*e.g.,* CoFe) have exceptional magnetic properties which hitherto have not been accessible to the aqueous and biological worlds. In our group, we have synthesised water-soluble Co NPs using an alkyl thioether end-functionalised poly(methacrylic acid) which has shown enhanced MRI relaxivities.

The marco-molecule ligands (*e.g.,* peptides, polymer) lend themselves to the design of novel surfaces and, importantly, gaining access to new composition and shapes of water soluble MNPs. The bio-molecule toolbox would allow these materials to be used in those environments. My group have successfully synthesised water-soluble CoPt using peptides. They are extremely stable in physiological conditions (for at least a few months). Antibodies could attached to these NPs for further biological studies.

In a nanoscale object, shape is an additional feature, control of which allows the physical properties of the material to be tuned so as to maximise its uses in applications. For biomedical applications, shape-variant NPs have immediate utility in biomedical immuno-electron microscopy (EM). Shapes with tunable sizes in the range 5-15 nm dramatically increase labelling options that are currently limited to 5, 10 and 15 nm spherical NPs. This means that many more protein or lipid targets can be simultaneously labelled within a single sample, allowing direct comparison of members of signalling networks or multi-subunit molecular machines. Incorporating other features such as magnetism opens the way for correlative approaches between *in vivo* imaging and high-resolution EM analysis.

We have succeeded in generating monodispersed anisotropiccally shaped MNPs of different compositions in the range of 5-50 nm. The heating capacity depends on (i) the size of a chosen composition of MNPs, (ii) the composition of the MNP of the same size, and (iii) shape anisotropy. We would like to carry out systematic studies of these materials to look for the best candidates for hyperthermia cancer treatment.

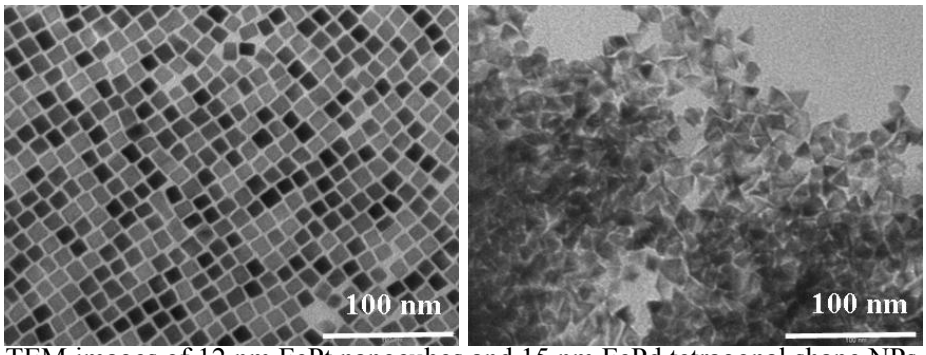


Figure 1. TEM images of 12 nm FePt nanocubes and 15 nm FePd tetragonal shape NPs .

*Email: ntk.thanh@ucl.ac.uk*

**S1-1**

# Magnetite Nanoparticles between 10 and 40 nm: synthesis, magnetic properties, dendron grafting and suspensions stability

**L. Truonc Phuoc, J. Santoyo Salazar, H. Mamlouk, D. Felder-Flesch, S. Begin-Colin, G. Pourroy**
Institut de Physique et Chimie des Matériaux, UMR 7504 CNRS-ECPM-Université de Strasbourg, 23 rue du Loess, BP 43, 67034 Strasbourg Cedex 2, France
**O. de Abril, L. Perez, M. Vazquez**
Instituto de Ciencia de Materiales, CSIC, 28049 Madrid, Spain

Batches of iron oxide nanoparticles with sizes between 10 and 40 nm were obtained by a co-precipitation route followed (or not) by a hydrothermal treatment at 250°C with $N(CH_3)_4OH$ (T-Methyl), $N(C_2H_5)_4OH$ (T-ethyl) or $N(C_3H_7)_4OH$ (T-Propyl) bases [1]. The synthesis parameters and conditions such as pH, Base/(Ferrous+Ferric), rotation and injection speed were controlled in order to improve monodispersity. The mean sizes decrease when going from T-methyl to T-Propyl-substituted base. Comparison with the lattice parameters of magnetite $Fe_3O_4$ and maghemite $\gamma$-$Fe_2O_3$, equal to 0.8396 nm (JCPDS file 19-629) and 0.8346 nm (JCPDS file 39-1346) respectively, show that the samples are mainly made of magnetite. A careful study of the structure and the composition has shown that the nanoparticles are made of a magnetite core surrounded by an oxidized layer close to maghemite. The distribution in size has been estimated by specific surface measurements, X-ray diffraction and electron microscopy techniques. Magnetization at H= 18 kOe decreases from 84 emu/g up to 52emu/g as the size decreases from 40 nm to 10 nm.
First generation pegylated dendrons have been grafted on the nanoparticles through a phosphonate coupling agent[2]. This strategy allows obtaining hybrid entities on which various functional groups can be attached later on. The grafting mechanism has been investigated in view to produce biocompatible magnetic nano-objects for biomedical applications[3]. Grafting has been demonstrated to occur by interaction of negatively charged phosphonate groups with positively charged groups and hydroxyl at the iron oxide surface. The isoelectric point of the suspension of dendronized iron oxide nanoparticles is shifted towards lower pH as the amount of dendron increases. Furthermore, it has been shown that grafting through a phosphonate improves the magnetization of the iron oxide due to super–super exchange interactions through the phosphonate group. Finally, a study of the suspensions stability will be presented.

* This work was supported by the European community through the NANOMAGDYE project, CP-FP 214032-2

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Daou, T. J.; Grenèche, J.; Pourroy, G.; Buathong, S.; Derory, A.; Ulhaq-Bouillet, C.; Donnio, B.; Guillon, D.; Begin-Colin, S. Chem. Mater. 2008; 20(18) 5869-5875
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# S1-2

## Synthesis of $MFe_2O_4$ (M = Fe, Mn) nanoparticles with tunable sizes

Lourisa Cabrera[a], Álvaro Somoza[b], Carlos J. Serna[a] and M. Puerto Morales[a]

[a]*Instituto de Ciencia de Materiales de Madrid/CSIC, Sor Juana Inés de la Cruz 3, Cantoblanco, Madrid 28049, Spain*

[b]*IMDEA-Nanociencia, Facultad de Ciencias Módulo C-IX, 3ª planta, Cantoblanco, Madrid 28049, Spain*

In the last years, $MFe_2O_4$ ferrite nanoparticles (with M = Fe, and Mn) have become of great interest for biomedical applications, such as hyperthermia cancer treatment and MRI (magnetic resonance imaging) because of their outstanding tunable magnetic properties, chemical stability and low level of toxicity in biological systems [1]. For this matter, nanoparticles should be monodisperse and have uniform chemical composition for controlled biodistribution, bioelimination, and contrast effects [1].

Synthesis of monodisperse ferrite nanoparticles of narrow size distribution can be achieved in a one-step synthesis by thermal decomposition of metal organic precursors [2] in the presence of a surfactant using a high boiling organic solvent [1-5]. Following this approach, we have prepared $Fe_3O_4$ and $MnFe_2O_4$ nanoparticles using Mn (II) oleate and Fe (III) oleate as precursors by thermal decomposition in the presence of oleic acid with the size tunable from 5 to 20 nm. These nanoparticles were functionalized for potential biomedical applications.

Metal-oleate complexes were prepared from the reaction between metal chlorides and sodium oleate. $Fe_3O_4$ and $MnFe_2O_4$ nanoparticles were nucleated by heating the metal complexes at 312ºC using octadecene as solvent. The particle sizes were controlled between 5 and 20 nm by varying the concentration of the metal precursor during the reaction. Nitrogen gas flow was placed during the 4 hour reaction process.

The ferrites nanoparticle surface functionalization was performed by surfactant exchange. Oleic acid was displaced by a number of bifunctional molecules with one carboxylic group and another functional group to stabilize the particles in aqueous media (thiol, maleimide). The molecules used were dimercaptosuccinic acid (DMSA), 11-mercaptoundecanoic acid (MUA), 16-mercaptohexadecanoic acid (MHA), and bis(carboxymethyl)(2-maleimidylethyl)ammonium 4-toluenesulfonate (MATS). In general, the capping process was done through simple mixing of the nanoparticles with a large excess of the surfactant of interest using a chloromethane as interchange solvent. The surfactants bind to the iron oxide surface through their carboxylic bonding. Elemental analysis indicates the amount of iron present in the material, as well as the ratio of Mn:Fe in manganese ferrite nanoparticles which is very close to 1:2. Particle size was measured from transmission electron microscopy (TEM) micrographs (Figure 1). It was observed that as the amount of metal-oleate complex was increased in the reaction solution, the particle size increased as well. All particles showed a cubic morphology with a very narrow size distribution. Dynamic laser scattering (DLS) showed in all cases that the hydrodynamic diameter was larger than the size determined from TEM. IR spectra for $Fe_3O_4$ and $MnFe_2O_4$ with oleic acid and treated with DMSA, MUA, MHA and MATS confirm the presence of the surfactants on the surface of the nanoparticles. On the other hand, magnetic measurements show the difference in coercivity and magnetic saturation between the materials generated due to their nature or due to the surface modification.

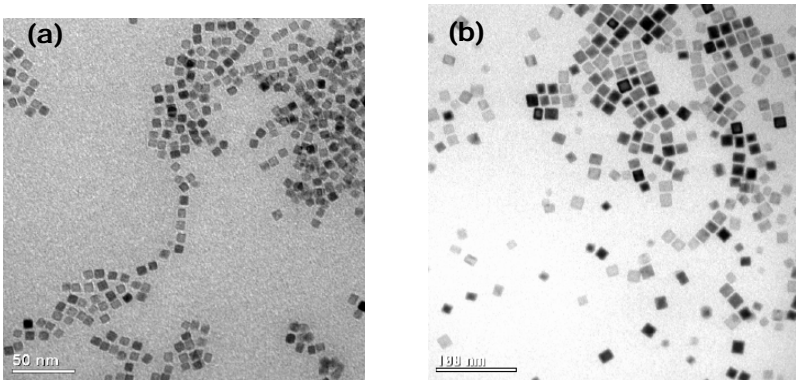


Figure 1. Morphology and size distribution of (a) $Fe_3O_4$ and (b) $MnFe_2O_4$ nanoparticles in function of the amount of precursor used.

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**S1-3**

# Continuous Production of Magnetic Nanoparticles for Bio-Applications by Laser Pyrolysis

Rodica Alexandrescu [1], Valentina Bello [2], Virginia Bouzas [3], Munish Chanana[4] , Rocío Costo-Cámara [5], Florian Dumitrache[1], Miguel Angel García [3], Maria del Puerto Morales Herrero [5], Ion Morjan[1], Carlos J Serna [5] Sabino Veintemillas-Verdaguer [5], Dayang Wang[4]

1) Natl. Inst. Lasers Plasma & Radiat. Physics Romania
2) Dept. Physics, Univ. Padova, Italy
3) Dept. Fis. Matt. Univ. Complutense Madrid
4) Max Plank Inst. Colloids and Interfaces, Postdam, Germany
5) Inst. Ciencia de Materiales de Madrid CSIC, Spain

ABSTRACT

The laser pyrolysis technique allows the production of nanoparticles in a continuous form provided that a suitable volatile precursor could be available. The intersection of the reactants with the laser beam generates step temperature gradients that enable the formation of very fine and uniform nanoparticles. In the development of the BONSAI project iron oxide samples and iron carbon nanocomposites were produced using this technique and delivered to other partners for their characterization, dispersion and bio testing. The iron oxide samples were in the range of 2-10 nm of particle size. All of them were superparamagnetic but with high differences in the saturation magnetization depending on the experimental conditions of synthesis (unfocused and focused laser beams). Two representative samples of both procedures were selected and fully characterized by TEM, HRTEM, IR, ATD-TG, DRX, and XPS, prepared in 10 g amounts in reproducible form and delivered to the rest of the BONSAI partners when necessary along the project. New carbon encapsulated iron/iron-oxide nanoparticles were also obtained by using laser pyrolysis method using vapour mixtures of ethylene-acetylene-iron pentacarbonyl or aerosols of ferrocene solutions in toluene carried by ethylene-oxygen to the reaction zone. These processes generated, in a single step, a nanocomposite formed by amorphous carbon nanoparticles in which isolated iron based nanoparticles are located. Often, core-shell structures may be observed. The magnetic cores have variable proportions of iron, iron carbides and iron oxides and particle sizes ranging from 7-9 nm depending upon the process conditions. $Fe_3C$-cementite phase is characterized by a higher mean crystallite dimensions. With increased residence time, the chemical content shifts towards the formation of the crystalline (about 9 nm diameter). The as prepared samples were characterized by standard techniques as XRD, TEM, IR, TG and elemental analysis.

Applications in biology require that all the magnetic nanoparticles (MNP's) produced in the BONSAI project are well dispersed and stable in physiological media, and great effort was devoted to the preparation of stable colloidal solutions of magnetic nanoparticles. In order to achieve this goal, the MNP's were dispersed in water + citrate or alternatively in hexane/toluene + oleic acid oleylamine with no alteration of their magnetic properties. The water-citrate samples reached the USPIO level (D < 40 nm) after the removal of aggregates, and presented good stability at high concentrations (>5mg Fe/ml). The organic-oleate approach produced colloids with D<200 nm. Both of them are able to be redispersed after drying with little change in the aggregate size distribution. Alternative further coatings have been developed in order to make the colloids stable and biocompatible in the body. In the BONSAI project the citrate of the water–citrate colloids was exchanged by L-Dihydroxyphenylalanine (L-dopa). This compound was chosen due to the affinity of its catechol group towards the iron oxide and its non toxic nature. The L-dopa coated nanoparticles are stable at physiological salt concentration of 150mM NaCl, and stable in culture medium.

**K1**

## Surface Processing of Nanoparticles for Bio-Imaging

Dayang Wang and Haolan Xu

Max Planck Institute of Colloids and Interfaces, D-14424, Potsdam, Germany

Email: dayang.wang@mpikg.mpg.de and Fax: +49 331 5679202

To apply nanoparticles for biological application it is essential to modulate their surface chemical nature to adapt complicated biological media that usually bear numerous proteins and electrolytes in a relatively high concentration. The present talk will summarize the current development of how to process the surfaces of nanoparticles to better dispersibility and colloidal stability in aqueous media and underline the experimental challenges for the surface processing of nanoparticles, specific to the production protocol, the chemical nature and the particular biological use of the particles and possible solutions to circumvent the challenges. Three nanoparticles – silicon quantum dots, magnetic iron oxide nanoparticles, and gold nanoparticles – will be specifically discussed. Polymer coating will be specifically highlighted as it imparts nanoparticles with robust steric stabilization, ease and flexibility to be functionalized to meet the demands of different applications, new particle collective properties, and new functionality arising from the polymer coating itself.

**I3**

## Size- and Composition-tunable Semiconductor Nanocrystals and Their Bio-applications

Dr. Ming-Yong Han
Institute of Materials Research & Engineering, A-STAR, Singapore 117602
Division of Bioengineering, National University of Singapore, Singapore 117574
E-mail: my-han@imre.a-star.edu.sg

Colloidal semiconductor nanocrystals (quantum dots) have attracted great attention for their distinguished roles in fundamental studies and technical applications such as biological labeling and optoelectronic devices. In the last decade, the main efforts have been focused on the preparation of size-tunable binary or core-shell nanocrystals with different emission colors. In our research, we also focus on the development of highly luminescent composition-tunable quantum dots across the whole visible spectrum. The resulting high-quality size- and composition-tunable quantum dots have been successfully used as multicolor biological nanoprobes for imaging, sensing, and drug delivery applications.

Biography:

Dr Han Ming-Yong is holding a joint appointment with Institute of Materials Research & Engineering and National University of Singapore. His research interests are to develop functional nanomaterials and multi-color biological nanoprobes for biomedical and optoelectronic applications. He has published over seventy peer-reviewed papers in the field of functional nanostructured materials/nano-biotechnology. His papers have been cited over 3,000 times, and received over sixty news/comments from Nature, Nature Biotechnology, Nature Asia Materials, Scientific American, Chemical & Engineering News, New York Times, etc. He also holds 20 patents or patent applications.

**S1-4**

# Fluorescent labels based on nanosized silicon and diamonds

Anna Fucikova[1,2,3,] Jan Valenta[1], Ivan Pelant[2], Vitezslav Brezina[3]
[1]Faculty of Mathematics and Physics, Charles University, Ke Karlovu 3, 121 16 Prague 2, CzechRepublic
[2]Institute of Physics AS CR, v. v .i., Cukrovarnicka 10, 162 53 Prague 6, Czech Republic
[3]Institute of Systems Biology and Ecology AS CR, v. v .i., Zamek 136, 373 33 Nove Hrady, Czech Republic
Corresponding author: anna.fucikova@email.cz

Fast and easy fluorescent coloring of organelles or tracking of single events on the cellular level is very important for progress in our understanding of functionality of biological systems and consequent applications in medicine. We are developing new non-toxic fluorescent labels based on (i) nanocrystalline silicon (Si-NCs), suitable for in vivo studies due to their biodegradability, and on (ii) nanodiamonds (ND), intended mainly for in vitro use due to their long-term stability and nondegradability.

State of the art in nanolabeling are various commercially used quantum dots (CQD) (e.g. cadmium containing quantum dots like CdS, CdSe, CdTe etc.) or fluorescent dyes (e.g. rhodamine) embedded in various shells. These nanolabels are more or less toxic according to the latest results and cannot be applied in vivo.

Our labels based on nanosized silicon (Si-NCs) overcome all disadvantages of CQD and FDs. Photoluminescence (PL) emission bands of Si-NCs range from ultraviolet to near infrared spectral regions. We are mostly interested in yellow-orange luminescence band with slow stretched-exponential decay, the life time of excited state of Si-NCs is in 10 to 100 μs range at room temperature. The luminescence stability is much better than CQD (represented in this work by eFlor$^{TM}$605$^{NC}$ (eBioscience) with maximum of PL band around 600 nm). Si-NCs can be exposed to daylight without significant luminescence loses. PL intensity is sufficient to observe single dots in florescence microscopy. Si-NCs have a crystalline core with size between 1 to 5 nm according AFM measurement and their surface is most often covered by $SiO_2$. Various methods of surface activation, normally used for CQD, are useable for $SiO_2$ surfaces. Si-NCs can be removed from living body by metabolic pathways, in contrast to CQD.

Nanodiamonds (ND) are produced by NanoCarbon UDD-TAH. They emit in blue region of the visible part of spectrum. ND with PL peak between 600-800 nm are also described in literature. The size of NDs is around 10 nm. Size of CQD with the envelope is mostly in 10-40 nm range, much more than Si-NCs and ND.

We use following cell culture lines for testing biocompatibility of nanoparticles: L929 mouse fibroblast and HeLa cells (human cervical cancer cells). Cytotoxicity tests show that Si-NCs and ND are biocompatible and no significant damage to the cell culture is observed. In case of Si-NCs, we observe a slight shift of the PL emission in the spectra when Si-NCs are interacting with internal environment of the cell. We also study the position of nanoparticles in the cell with use of various florescent stains (e. g. DAPI, Phalloidin).

Properties of Si-NCs and NDs show promising application potential as fluorescent labels.

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**S1-5**

## Luminescent Silicon based Nanoparticles by laser pyrolysis

E. Borsella[1], R. D'Amato, M. Falconieri[2], N. Herlin[3], V. Maurice[3], O. Sublemontier[3], F. Huisken[4], T. Schmidt[4], G. Mattei[5], E. Trave[5]

[1] ENEA C. R. Casaccia, Roma, Italy
[2] ENEA C. R. Frascati, (RM), Italy
[3]Laboratoire Francis Perrin (CEA-CNRS URA 2453), SPAM, DSM, CEA Saclay, France
[4] Lab. Astrophysics and Cluster Physics Group, Inst. for Solid State Physics, F. Schiller-Universität Jena-Germany
[5] University of Padova, Dept. of Physics, Padova, Italy

In the field of bio-imaging, the BONSAI Project aims at proposing alternatives to replace the toxic quantum dots (like CdSe) with light emitting Si and Si-based NPs having a broad excitation band, size dependent optical emission and a reduced tendency to photo bleaching.
For this purpose, Si nanoparticles produced from laser pyrolysis appear as good candidates. The laser pyrolysis, based on the use of a laser beam to initiate and sustain the chemical reactions that generate the NPs in the gas phase, has already proved to be an efficient method for the production of such nanoparticles. We present here how the optimisation of experimental parameters, in particular precise localization of the reaction zone in the reactor and the effect of time of reaction, allowed to improve the production rates up to 500 mg/hour for sizes less than 5 nm in diameter, while maintaining good quality (i.e. small size and low size dispersion).

The PL emission of Si nanoparticles falls in the range 600-1000 nm (well apt to *in vivo* application) and typical radiative lifetimes are in the range 0.05-0.3 ms. The quantum yield of these nanoparticles is usually low and efforts were done in order to passivate the surface, thus improving the quantum efficiency. Excitation of these nanoparticles is usually done in the UV-visible range, not compatible with *in vivo* applications. Recently, it was shown that it is possible to get two-photon excitation of the Si NPs in the IR (at about 900 nm) where the human skin transmittivity is high, which is an important point for *in-vivo* applications. Another point concerns the long emission decay time of silicon nanoparticles making detection of signal rather difficult on conventional microscopes. Some composite nanoparticles made of Ge and Si, exhibiting luminescent properties, were obtained using the laser pyrolysis method. In these new nanocrystals the decay time is reduced, compared to silicon, making them attractive for imaging.

The various results will be presented and compared to the state of the art in the literature.

**S1-6**

# Fluorescence from silicon nanoparticles suspended in water: reactive co-deposition for the control of surface properties of clusters

Klaus von Haeften, Atea Akraiam, Gauthier Torricelli, Anthony Brewer
Department of Physics and Astronomy, University of Leicester, Leicester LE1 7RH, UK
kvh6@le.ac.uk

The purpose of our research is to develop novel approaches to control the properties of nanoclusters. Here, our particular interest was to produce fluorescent silicon nanoparticles. Bulk silicon is an indirect bandgap material and therefore a poor light emitter. The discovery of intense fluorescence of porous Si[1] caused immense research activity because of the manifold possible applications. For example, there is recent interest from the semiconductor industry to replace electric signal transmission in integrated circuits by optical transduction and by using Si based light emitters[2]. A second, similarly important field for application of fluorescent silicon nanoparticles are thin film solar cells. These types of cells use only a limited fraction of the solar light spectrum and to improve their conversion efficiency it has been suggested to employ fluorescent nanoparticles to down-convert ultraviolet light into a wavelength to which the solar cell responds to[3].

Our approach to achieve luminescent silicon is to generate quantum confinement using cluster beams because the degree of confinement can be controlled by changing the average cluster size. Free silicon clusters are known to exhibit dangling bonds that quench fluorescence and therefore we passivate the cluster surface in-situ in a second step. In first experiments we produced Si clusters using a gas aggregation sputtering cluster source and co-deposited Si with a beam of water vapour onto a cold target in UHV. Melting of the ice yielded an aqueous suspension of a few ml that fluoresces at 420 nm when excited with UV light. The silicon nanoparticles produced in this way show a number of remarkable properties, for instance, their fluorescence remains stable in intensity for more than a year[4]. Characterisation by UV/VIS, PL and PLE spectroscopy revealed a structure of a Si core of 1.4 nm in diameter and a SiO shell. The band at 420 nm was assigned to the $T_1$ -> $S_0$ transition of Si lone pair defects in the SiO layer at the cluster surface being excited via energy transfer from a previously unreported state, presumably an interface exciton. Furthermore, we observed water Raman emission and we found that the intensity of the Raman lines were enhanced by the presence of nanoparticles. AFM images of the nanoparticle suspension exposed on HOPG confirmed our structural assignment and showed that the clusters (i) have a disk-like shape and (ii) are agglomerated to a fractal shaped network. Whether this is an effect of the surface interaction or an inherent property of the clusters in suspension remains to be investigated.

Our approach of using co-deposition in UHV provides great flexibility. We believe that our results are transferable to other passivation agents and, possibly, other nanoparticles. The fact that the Si/SiO nanoparticles fluoresce in an aqueous suspension is relevant for bio-medical applications. The fractal type shape seen in the AFM images suggests that linking to, for instance, antibodies is possible.

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**I4**

## Characterization of inorganic nanoparticles in regard to cytotoxicological screening

Wolfgang Parak
Fachbereich Physik, Philipps Universität Marburg, Germany
wolfgang.parak@physik.uni-marburg.de

For a detailed understanding on how cells and living matter interact with colloidal nanoparticles a profound characterization of the nanoparticles is crucial. Only from pure and well characterized samples defined information can be gathered. Characterization involves in particular colloidal properties of the particles, such as their dispersion and colloidal stability. We will describe one well defined particle system - polymer-coated inorganic nanoparticles - and describe some aspects of their cytotoxicity.

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**K2**

# Biotesting of nanoparticles

Giuseppe Miserocchi
Univ. of Milan Bicocca
Milan-Italy

BONSAI represented a wonderful occasion for integrative studies focused on the potential biomedical use of nanoparticles for imaging. In fact, various disciplines with different background, such as material sciences, cellular biology, physiology, applied pharmacology and nanoparticles (NPs) producers strived to collaborate in order to set a basis to identify a translational roadmap from producers to clinical practice. The task has been challenging, though stimulating, and I will summarize for you some of the concepts and facts from the biological standpoint emerging from this 3.5 years experience.

Iron based, silicon based and solid lipid nanoparticles were considered for potential use. Initial selection was made by producers based on emission/detection properties of NPs.

We decided to consider the perturbation induced in target cells at the level of the plasma membrane that represents the interface between medium and cytoplasm. We considered a gross level of perturbation by estimating the increase in the extracellular medium of a cytoplasmic enzyme (Lactic Dehydrogenase, LDH) of relative high molecular weight (400000 Da) whose escape represents a sensitive index of lesion of plasma membrane. We also considered a fine level of perturbation by estimating through electrophysiological techniques the alteration in plasma membrane electrical potential and ion channel fluxes. This technique is sensitive enough to detect the sub-threshold perturbation that might allow to monitor an early host-membrane chemical/physical interaction triggering a signal-transduction response.

We also evaluated through various imaging techniques the uptake and intracellular distribution of nanoparticles. Analytical treatment of the data allowed to gather indications on the mechanisms responsible for cellular uptake and to model the kinetics of intracellular accumulation.

An important development of these studies was the concept of “latent toxicity” in the evaluation of risks/benefits ratio in biomedical applications.

**S2-1**

# Si-based Nanoparticles: a biocompatibility study.

**I. Rivolta[1], B. Lettiero[1], A. Panariti[1], R. D'Amato[2], V. Maurice[3], M. Falconieri[2], N Herlein[3], E. Borsella[2] and G. Miserocchi[1]**

1.Università di Milano Bicocca, Monza, Italy;
2. ENEA, Rome, Italy
3. CEA-CNR, Saclay, France

Exposure to Si-NPs may occur in professional working conditions or for people undergoing a diagnostic screening test. Despite the fact that silicon is known as a non-toxic material, in the first case the risk is mostly related to the inhalation of nanoparticles, thus the most likely route of entry is across the lung alveolar epithelium whose surface area averages 70 $m^2$. In the case of diagnostic imaging, nanoparticles are usually injected intravenously and Si-NPs could impact on the endothelial wall.

In our study we investigated the interaction between selected Si-based NPs and an epithelial lung cell line. We evaluated the biocompatibility of nanoparticles by measuring the integrity of the plasma membrane. Our data showed that not all the silicon based NP are safe at the same level. Different preparation of particles may rise significantly difference in the cell membrane permeability (30%±6.5 vs 95%±1.6 of LDH release) for the same concentration (0.1 mg/ml) and time of exposure (24hrs). Since NPs impact on physiological barriers, another important question is whether they interfere with the integrity of the whole monolayer of the cells when they come in contact with. Trans Epithelial Resistance (TER, $\Omega/cm^2$) is a good parameter to estimate perturbations induced on the integrity of the monolayer since it is directly proportional to the integrity of the junctions between the cells. Indeed we found that the same particles that increased the membrane permeability, also decreased the TER by almost 20%, indicating that their "action" is not only at cellular level, but also concerned the junction between the cells.

We also want to investigate whether these particles alter the ionic fluxes across the plasma membrane and in order to reach this goal we will study single cells currents through the patch clamp technique.

Our data showed that despite the overall silicon biocompatibility, however accurate studies of the potential toxicity induced by the nanostructure and engineered surface characteristics need to be accurately investigated before Si nanoparticles can be safely used for in vivo applications as bio-imaging, cell staining and drug delivery.

**S2-2**

# Influence of iron oxide magnetite nanoparticles and fluorinated phosphates on red blood cells and CaCo-2 cells.

Daniel Moersdorf, Pierre Hougunenq, Ingolf Bernhardt

Laboratory of Biophysics, Saarland University, Campus, Building A2.4, 66123 Saarbruecken, Germany

**Purpose**

We studied the interaction of nanoparticles and components of complex co-polymers with two different cell types (red blood cells, CaCo-2 cells). We focused on two important physiological parameters, the intracellular pH and the intracellular $Ca^{2+}$ content. The investigations are part of the EC project NanoMagDye coordinated by the "Institut de Physique et Chimie de Matériaux de Strasbourg" (IPCMS).

**Material & Methods**

The nanoparticles and fluorinated phosphates used have been synthesized at the IPCMS. We investigated different nanoparticles with an iron oxide core and grafted with organic particles. In addition, we studied phosphate molecules (co-polymers) that will be grafted on nanoparticles. For analysing the physiological parameters we labelled the cells with a $Ca^{2+}$ sensitive (fluo-4 AM) or a pH sensitive (BCECF AM) fluorescent dye and measured the fluorescence intensity with a fluorescence microscope (Eclipse TE2000-E, Nikon, Japan) as well as a confocal microscope (LSM 510 META, Zeiss, Germany) for single cell measurements. Flow cytometer (FACScalibur, Becton Dickinson Bioscience, USA) measurements have been carried out for a higher amount of cells.

**Results**

The studied nanoparticles with an iron oxide core did not show an effect on the internal pH and the intracellular $Ca^{2+}$ content of both red blood cells and CaCo-2 cells. The phosphate molecules did also not affect the internal pH of both cell types and the $Ca^{2+}$ content of CaCo-2 cells. However, the co-polymers affected the red blood cells by increasing the $Ca^{2+}$ content significantly leading to a haemolysis of the cells.

**Conclusion**

Nanoparticles or components of them can influence physiological parameters of cells. Therefore, the nanoparticles have to be tested in respect of unspecific interactions with cells before applying them to target cells.

The research leading to these results has received funding from the European Union, 7$^{th}$ Framework Programme.

**S2-3**

## The possible side-effects of iron oxide nanoparticles on cell functionality and MR signal.

S. J. Soenen[1], N. Nuytten[1], U. Himmelreich[2] and M. De Cuyper[1]

[1] *Lab of BioNanoColloids, IRC, Katholieke Universiteit Leuven Campus Kortrijk*
[2] *Biomedical NMR Unit, Katholieke Universiteit Leuven.*

Iron oxide nanoparticles are routinely exploited as $T_2/T_2$* contrast agents[1]. One of the hottest topics in this biomedical research area is the non-invasive imaging of pre-labelled stem or therapeutic cells upon transplantation in an animal model of interest[2]. To this end, commercial particles such as Endorem® or Resovist® are frequently employed, although the particles display several characteristics which makes them less suitable for in vitro labelling.
In the present work, the effects on cell physiology of in-house produced cationic magnetoliposomes (MLs), i.e. 14-nm diameter iron oxide cores each individually enwrapped by a lipid bilayer containing a certain percentage of cationic lipid[3], are compared with the effects of Resovist, Endorem and citrate-coated iron oxide particles. In this study, the uptake efficiency of the different particles is investigated in a variety of cell types being: NIH 3T3 fibroblasts, murine C17.2 neural progenitor cells, primary human blood outgrowth endothelial cells (hBOECs) and rat pheochromocytoma (PC12) cells. In a first part, it is shown that MLs are fully biocompatible and allow an efficient and long-term labelling[3], where the flexibility of the lipid coating allows to easily modify and control uptake characteristics and potential cytotoxic effects[4]. Next, it is shown that high intracellular levels of iron oxide drastically affects cellular well-being[4]. The effects are concentration-dependent and seemingly independent of the type of particle used and can generally be ascribed to alterations in actin fiber and microtubule arrangements, distorting focal adhesion kinase-mediated signalling pathways. In a second part, it is shown that intracellular stability of the coating molecules is of primordial importance. The results in vitro show that citrate-coated particles are degraded very fast, whereas dextran-coated ones are more stable, but still less than the lipid-coated MLs. The degradation of the particles can be shown by the increase in free ferric ions, which also distort the r1/r2 ratio of the particles, hampering their use for long-term imaging. Next to signal alterations, the free ferric ions also affect the functionality of PC12 cells.
In conclusion, the results indicate that cells appear to have an intrinsically limited capacity for the amount of nanomaterial they can handle, impeding the use of high doses of contrast agent. Furthermore, the type of coating material used is highly important with regards to maintaining cell functionality and stability of the label. Further characterization of cell-nanoparticle interactions is both warranted and needed[5].

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**S2-4**

# Towards ideal magnetofluorescent nanoparticles for bimodal detection of breast cancer cells.[†]

Davide Prosperi,[1] Miriam Colombo,[1] Silvia Ronchi,[2] Fabio Corsi,[3] Clara De Palma,[4] Emilio Clementi.[4]

[1] *Dipartimento di Biotecnologie e Bioscienze, Università di Milano-Bicocca, Piazza della Scienza, 2 – 20126 Milano (Italy). Tel. (+39)02.6448.3302; Fax (+39)02.6448.3565.*
*Email davide.prosperi@unimib.it*
[2] *Istituto di Scienze e Tecnologie Molecolari, CNR, Via Fantoli 16/15, 20138 Milano.*
[3] *Dipartimento di Scienze Cliniche "Luigi Sacco", Università degli Studi di Milano, Ospedale L. Sacco, Via G.B. Grassi 74, 20157 Milano.*
[4] *Dipartimento di Scienze Precliniche Lita Vialba, Università degli Studi di Milano, Ospedale L. Sacco, Via G.B. Grassi 35, 20157 Milano.*

An increasing number of novel molecular markers based on nanomaterials for tumor diagnostics has been developed in recent years. Many efforts have been spent towards the achievement of site-targeted bioconjugated nanoparticles. In contrast, the mechanisms of toxicity, endocytosis and degradation pathways are still poorly understood, albeit their primary importance for clinical translation. We present the results of a recent study conducted in our laboratory, in which we have designed and fabricated three different model nanoscale magnetofluorescent particle systems (MFNs). These nanoparticles were evaluated in terms of size, morphology, zeta potential, fluorescence efficiency, capability of enhancing $T_2$ relaxivity of water protons and stability. Accordingly, two of them were developed and the mechanism of internalization, the intracellular fate and the toxicity in MCF-7 adenocarcinoma cells were investigated. Besides the well-documented size effect, we found that the anionic charge seems to be a crucial factor for particle internalization, as MFN penetration through the cell membrane could be modulated in dependence of their surface charge. Ultrastructural analysis of transmission electron micrographs combined with evidence from confocal microscopy revealed that MFNs were internalized by clathrin-mediated endocytosis and macropinocytosis. Moreover, MFNs were found in EEA1-positive endosomes and in lysosomes indicating that they followed a physiological pathway of endocytosis and were not rejected by the cell. Magnetorelaxometric analysis demonstrated that our MFNs enabled the detection of $5 \times 10^5$ cells $mL^{-1}$ after treatment with particle dosages as low as 30 μg $mL^{-1}$. Hence, MFN appears as a valuable and safe bimodal contrast agent to be developed for noninvasive diagnosis of breast cancer.

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**I5**

## Labelling, imaging and manipulating the cell with anionic magnetic nanoparticles

Florence Gazeau, Claire Wilhelm
*Laboratoire Matière et Systèmes Complexes,*
*UMR 7057 CNRS and Université Paris 7, Paris, France.*
florence.gazeau@univ-paris-diderot.fr

Magnetic labelling of living cells creates opportunities for numerous biomedical applications as individual cell manipulation, magnetic control of cell migration, intracellular heating or MRI cell tracking. The unique advantage of magnetic-based methods is to activate or monitor cell behaviour by a remote stimulus, namely the magnetic field. Cell labelling methods using superparamagnetic nanoparticles have been developed, showing no adverse effect on cell proliferation and functionalities, while conferring magnetic properties to various cell types. We will describe a non specific labelling technique based on anionic magnetic nanoparticles (AMNP) and the subsequent magnetic properties of cells. We will review the effects of different magnetic fields on the labelled cells: - non invasive MRI detection at the single cell level (in vitro and in vivo), - intracellular manipulation by rotating field, - cell targeting by magnetic attraction.

These approaches will be discussed in the context of their applications for cell therapy and tissue engineering (non invasive tracking of cell implants, cell delivery assisted by magnetic forces).

**I6**

# An Integrated Approach for the Rational Design of MRI Contrast Agents with Large Longitudinal Relaxivity

Paolo Decuzzi

Center for the "Rational Design of Particulate Systems for the Imaging and Hyperthermia of an Inflamed Endothelium" & Dept. of Nanomedicine and Biomedical Engineering
The University of Texas Medical School at Houston
Houston, Texas - USA

and
Center of Bio-/Nanotechnology and -/Engineering for Medicine
University of Magna Graecia, Catanzaro - ITALY

Magnetic resonance imaging (MRI) has evolved into one of the most powerful, noninvasive diagnostic imaging technique in medicine and biomedical research. The superior resolution and in-depth anatomical details provided by MRI are essential for early diagnosis of many diseases. Chemical contrast agents (CAs) have been widely used for improving the sensitivity and diagnostic confidence in MRI. Generally, these CAs contain paramagnetic metal ions that exhibit time-dependent magnetic dipolar interaction with the surrounding water protons and improve the MRI sensitivity by decreasing the relaxation time *T1* of water protons in and around their vicinity. The most widely-used clinical CAs use gadolinium ions ($Gd^{3+}$) as the paramagnetic ion. In spite of the enormous progress achieved in the design and synthesis of clinical MRI CAs, the current agents suffer from several limitations including low circulation time, insufficient contrast generation and potential toxicity.

In this talk, a new class of MRI CA will be demonstrated derived by combining together conventional and non-conventional Gd-based CAs with microfabricated silicon particles (SiMPs). Different sizes and shapes of SiMPs will be considered, namely hemispherical and discoidal with a characteristic size ranging between 0.6 and 1.6 μm, and their behavior in the vascular network will be assessed through an integrated approach comprising in-silico (mathematical) calculations, in-vitro dedicated assays and in-vivo small animal models. The new class of CAs proposed offer superior MRI performances with longitudinal proton relaxivities, *r1*, established in the range of ~ 150 – 200 $mM^{-1}s^{-1}$ per $Gd^{3+}$ ion (~ $2 - 4 \cdot 10^{+9}$ $mM^{-1}s^{-1}$ per construct) at 1.41 T. These relaxivity values (per $Gd^{3+}$ ion) are about 40 – 60 times larger than that of Gd-based clinically-available MRI agents (~ 4 $mM^{-1}s^{-1}$ per $Gd^{3+}$ ion/particle).

BONSAI Symposium "Breakthroughs in Nanoparticles for Bio-Imaging" Frascati (Rome) – Italy April 8-9, 2010

# DENDRONISED IRON OXIDE NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

B. Basly,[1] D. Felder-Flesch,[1] P. Perriat,[2] Claire Billotey,[3] J. Taleb,[3] G. Pourroy,[1] S. Bégin-Colin[1]

[1] Institut de Physique et Chimie des Matériaux de Strasbourg, UMR CNRS/UDS 7504, 23 rue du Loess BP 43, 67034 Strasbourg Cedex2, France
[2] Groupe d'Etudes de Métallurgie Physique et de Physique des Matériaux, UMR 5510 CNRS-INSA de Lyon, F-69621 Villeurbanne Cedex, France.
[3]UMR 5220, Inserm U 630, INSA-Lyon, 7 avenue J Capelle, bat. Blaise Pascal F-69621 Villeurbanne cedex, France.

Superparamagnetic iron oxide nanoparticles (SPION) with appropriate surface coating have been widely used for numerous *in vivo* applications such as magnetic resonance imaging contrast enhancement, tissue repair, immunoessay, hyperthermia, drug delivery. In this field most work has been achieved improving the materials biocompatibility but only a few investigations and developments have been carried out in improving the quality of the magnetic nanoparticles, their size distribution and their shape and in studying the effect of their functionalization on their structural and magnetic properties. Also the nature of the nanoparticle's surface coatings determines not only the overall size of the colloid but plays a significant role in the nanoparticle's biokinetics and biodistribution inside the body.

Iron oxide nanoparticles with sizes around 12 nm have been synthesized by two methods: the co-precipitation of iron chlorides by a base and by the thermal decomposition of iron stearate. After co-precipitation, the stripped iron oxide nanoparticles are stable in water suspension at pH below 5 or above 7 ($NP_{cop}$) (IEP = 6.8). The thermal decomposition method leads to nanoparticles covered with oleic acid molecules in an organic solvant ($NP_{td}$). These both nanoparticles have then been covalently coated with a hydrophilic polyethyleneglycol-based dendron having a phosphonic acid as a focal point.[1] The functionalization step has been determined to obtain stable suspension of iron oxide nanoparticles in water and osmolytic conditions.

For nanoparticles synthesized by co-precipitation, the grafting has been demonstrated to occur at pH 5 by interaction of negatively charged phosphonate groups with hydroxyl and positively charged groups at the iron oxide surface.[2] Their corresponding suspension's isoelectric point was shifted towards lower pH as the amount of dendron increased leading to stable suspension at pH = 7.[3] For nanoparticles obtained by thermal decomposition, a ligand exchange process including a phase transfer in water has been optimized/perfected.

The optimisation of grafting conditions has conducted to very stable water suspensions of iron oxide nanoparticles at pH=6.8. Both functionalized nanoparticles have been carefully characterized (XRD, TGA, IR, TEM, Elemental analysis, UV-visible, Zeta potential). The grafting step has been shown to preserve the magnetic properties of the iron oxide nanoparticles due to super-super exchange interactions through the phosphonate group. Finally, the relaxation properties of the colloidal suspensions have been studied in order to evaluate the possible use of these materials as MRI contrast agents. Indeed, NMR measurements revealed significantly reduced water proton relaxation times $T_1$, $T_2$ under a 1.5T magnetic field with a quite satisfactory ratio $r_2/r_1$ of 25 for nanoparticles obtained by co-precipitation. These values are lower for iron nanoparticles synthesized by thermal decomposition and these have been related to their structural and magnetic properties.

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**S3-2**

**Magnetic nanoparticle location and quantification in mice tissues after intravenous injection.**

Lucía Gutiérrez, Lourisa Cabrera, Carlos J. Serna and M. Puerto Morales
*Instituto de Ciencia de Materiales de Madrid/CSIC, Sor Juana Inés de la Cruz 3, Cantoblanco, Madrid 28049, Spain*
Raquel Mejías, Domingo F. Barber
*Department of Immunology and Oncology, Centro Nacional de Biotecnología/CSIC, Cantoblanco, Madrid 28049, Spain.*

Nowadays, a great effort is being performed in the development and optimization of drug delivery systems containing magnetic nanoparticles for controlled local drug release, in particular in cancer therapy [1]. Besides verifying *in vitro* that these systems fulfil their specific function, it is necessary to check whether they reach their target in a living organism. Moreover, it is also desirable to study their quantitative distribution in tissues in the presence or absence of a magnet in order to understand therapeutic effects. In this work, the localization and quantification of the magnetic carriers in tumorous tissues after their intravenous injection to mice has been performed.

Magnetic nanoparticles were synthesized by thermal decomposition of iron acetylacetonate in an organic medium, which assured formation of uniform nanoparticles with enhanced magnetic properties (Fig. 1). Further modification of the particles with dimercaptosuccinic acid (DMSA) provided with high stability in aqueous media and free ligand groups for subsequent biomolecule conjugation [2]. Then, cytokines were attached to the DMSA-coated magnetic nanoparticles for controlled local drug release, as they improve the immune response to solid tumours when administered in the vicinity of neoplastic cells [3]. The compound was intravenously injected to mice that were previously treated in order to contain tumorous tissues in both back legs. In order to concentrate the particles in a specific tissue, a magnet was externally attached to one of the legs, while the other, without the external magnet, was used as control. Then the tumororus tissue from both legs was characterised magnetically and by TEM.

Aggregates of magnetic nanoparticles have been observed in the targeted tissue (Fig. 1). Some particles were also detected in the control leg tissue, but undoubtedly in a less amount than the one observed in the leg with the external magnet. In spite of the endogenous iron from the tissues, the magnetic characterisation also allows the identification of the injected particles in the tissues, due to their different magnetic properties. It can be concluded that the presence of an external magnet helps to concentrate the magnetic particles in the tissue of interest. Besides, the study of the tumorous tissue size reduction obtained with this treatment is in progress and the results will be presented soon.

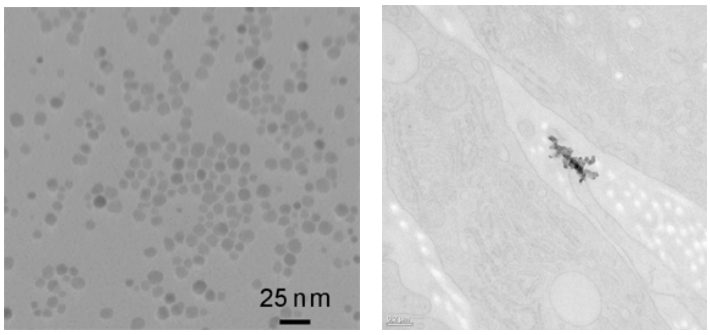


*Fig 1. TEM observation of the injected particles (left) and an aggregate of particles in the tumorous tissue (right).*

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**S3-3**

# USPIO lipid coating by warm microemulsion: structure-activity relationship

P.Gasco[1], N. Vivenza[1], G. Riccio[1], JM Idee[2], W. González[2], G. Miserocchi[3], I.Rivolta[3], G.Sancini[3], P.Mazzoldi[4] V. Bello[4], G. Mattei[4], MA Garcia[5], V. Bouzas[5], S. Veintemillas[6], I. Morian[7], E. Borsella[8]

[1]Nanovector srl – Torino (Italy)
[2]Guerbet SA – Paris (France)
[3]Experimental Medicine Dep., University of Milano Bicocca (Italy)
[4]Dept. of Physics, University of Padova (Italy)
[5]Universidad Complutense Madrid (Spain)
[6]Instituto de Ciencia de Materiales de Madrid CSIC (Spain)
[7]Institute for Laser and Radiation Physics, Bucarest (Romania)
[8]Advanced Physics Technologies and New Materials Dep. – ENEA - Frascati (Italy)

During the last years Ultrasmall SuperParamagnetic Iron Oxide (USPIO) have been studied and developed as promising contrast agent in MRI. Many different USPIO synthesis processes have been proposed as well as different coating solutions have been pointed out for stability and bioavailability improvement.

Warm microemulsion technique has been first developed to obtain Solid Lipid Nanoparticles as carriers for drug delivery.

In EC granted project BONSAI different kind of USPIO's have been prepared and warm microemulsion process has been applied to such different kinds of USPIO in order turn them into SLN or to coat them by lipid layer (USPIO-SLN)

Many different experiments have been performed on obtained USPIO-SLN in cooperation with BONSAI partners, for characterizing their physico-chemical structures and their activity in biological environment: summary of major evidences reached during BONSAI activities will be shown in present session.

**S3-4**

**Targeting cells with MR imaging probes: cellular interaction and intracellular magnetic iron oxide nanoparticles uptake in brain capillary endothelial and choroidal plexus epithelial cells**

*I. Cambianica,**M. Bossi, ^P. Gasco, #W. Gonzalez, #JM. Idee, *G. Miserocchi, **R. Rigolio, @D. Wang ,……..*G. Sancini

*Dept. of Experimental Medicine and **Dept. of Neuroscience, Univ. of Milano-Bicocca, Monza, Italy
^Nanovector S.r.l., Via Livorno, 60-10144 Torino, Italy
#Guerbet Res, F-95943 Roissy, France
@Max Planck Institute of Colloids and Interfaces, Dept. of Interfaces, Potsdam, Germany

Magnetic iron oxide nanoparticles are considered for various diagnostic and therapeutic applications in brain including their use as contrast agent for magnetic resonance imaging or as tool for magnetic drug delivery. The endocytic mechanism by which cells internalize specific NP such as iron oxide nanoparticles remains still unknown as well as the exact relationship between NP surface charges and pathway is unknown. In delivery application, the critical step is the transport across cell layers and the internalization of nanoparticles into specific cells, a process often limited by poor targeting specificity and low internalization efficiency.

The development of the models of brain endothelial cells and choroidal plexus epithelial cells in culture has allowed us to investigate into these mechanisms. Our strategy is aimed at exploring different routes to the entrapment of iron oxide nanoparticles in these brain related cells. Here we demonstrated that not only cells endowed with a good phagocytic activity like activated macrophages but also endothelial brain capillary and choroidal plexus epithelial cells do internalize iron oxide nanoparticles. Our study of the intracellular trafficking of nanoparticles by TEM, and confocal microscopy showed that nanoparticles are mainly internalized by the endocytic pathway.

Iron oxide nanoparticles were dispersed in water and coated with 3,4 – dihydroxyl – L – phenylalanine (L-DOPA) using standard procedures. Magnetic lipid nanoparticles were prepared by NANOVECTOR: water in oil in water (W/O/W) microemulsion process has been applied to directly coat different iron based nanoparticles by lipid layer or to encapsulate them into Solid Lipid Nanoparticles (SLNs). By these coating/loading the colloidal stability was improved without strong alteration of the particle size distribution. Magnetic lipid nanoparticles could be reconstituted after freeze drying without appreciable changes in stability. L-Dopa coated nanoparticles are stable in PBS and in MEM (Modified Eagle Medium) medium. The magnetic properties of the nanoparticles were not altered by the coating processes. We investigated the cellular uptake, cytotoxicity, and interaction of these nanoparticles with rat brain capillary endothelial (REB4) and choroidal plexus epithelial (Z310) cells. By means of widefield, confocal microscopy and flow cytometry we studied the cell uptake of magnetic solid lipid nanoparticles derivatized with a fluorescent reporter molecule and of L-Dopa-TRITC coated nanoparticles. Inhibition of the caveolae-mediated pathway by preincubation with filipin and nystatin did not modify the cellular uptake of these nanoparticles in both cell lines. Furthermore a mild decrease of the nanoparticles cell uptake was obtained after chlorpromazine and $NaN_3$ pretreatment, which interferes with clathrin and energy-dependent endocytosis, and cytochalasin and amiloride pretreatment which interfere with macropinocytosis. Particle size as such can strongly affect the efficiency of cellular uptake and the mode of endocytosis. Considering that our L-Dopa and magnetic solid lipid nanoparticles display a medium hydrodynamic sizes of 120 nm with a polydispersity index of 0.2, we can assume that the cell uptake process of these NP may develop, depending the particle size, both via clathrin mediated endocytosis and macropinocytosis and only to less extent via the pathway of caveolae-mediated endocytosis.
Taken together these results let us to conclude that SLN iron loaded and iron based L-Dopa coated nanoparticles are internalized into brain endothelial and choroidal plexus epithelial cells and this might provide the first step of an intracellular trafficking to transport these NPs between blood and brain.

**I7**

## Electrostatically stabilized iron oxide nanoparticles for in vivo MRI

Matthias Taupitz, Jörg Schnorr, Eyk Schellenberger, Susanne Wagner
Department of Radiology Charité - Universitätsmedizin Berlin, Berlin, Germany.

Electrostatic stabilization has the advantage of allowing generation of even smaller magnetic nanoparticles for use as markers in magnetic resonance (MR) imaging than steric stabilization, which is used to prepare the known ultrasmall superparamagnetic iron oxides (USPIO). However, electrostatically stabilized particles pose a challenge in terms of optimizing their tolerance for clinical use. Our group has prepared electrostatically stabilized very small superparamagnetic iron oxide particles (VSOP) with citrate coating. The particles have a core diameter of about 4 nm and a hydrodynamic diameter of about 7 nm. The ultimate aim is to use these particles for development of an intravenously administered contrast medium for MR imaging. In an initial experimental phase, the particles were optimized in terms of pharmacokinetic properties, high ratio of T1 to T2 relaxivity, and tolerance. The preclinical results were favorable to allow initiation of a first clinical trial. The clinical phase I trials showed the new nanoparticle preparation to be well tolerated and safe. With regard to the potential of VSOP for use as a blood pool contrast agent, a clinical phase IB showed the particles to produce excellent T1 contrast, allowing high-resolution imaging of the entire heart including the coronary arteries with a free-breathing technique. Experimental investigations have demonstrated the potential of VSOP for other uses: a study in a rabbit model of atherosclerosis has shown the particles to accumulate in atherosclerotic plaque with signs of destabilization as early as one hour after IV injection. Moreover, it has been shown that, in addition to the blood pool phase, VSOP can be used for first-pass MR angiography following bolus administration. The citrate-stabilized particles were also used for the development of specific probes consisting of VSOP coupled to annexin V, which can be used to label apoptotic tissue. In yet another approach to developing a specific probe, VSOP were modified in such a way that they precipitate in the presence of matrix metalloproteinase activity. This property might be exploited to identify tissues with enhanced degradation of extracellular matrix such as areas of tumor invasion or inflammation. In summary, the electrostatically stabilized particles as outlined above offer interesting properties for use as an MR contrast agent and as a component for the development of specific probes.

**K3**

## Ultrasmall superparamagnetic particles of ion oxide : where we are, were we want to go: a pharmaceutical company viewpoint

Jean-Marc Idée, Guerbet, Research Division, Aulnay-sous-Bois, France

The introduction of superparamagnetic iron oxide nanoparticles as contrast agents for magnetic resonance imaging (MRI) opens up very exciting clinical prospects because of their intrinsic capacity to target inflammatory lesions through macrophage labelling.
The concept of translational medicine ("from bench to bedside") has become a major issue in modern medical research and greatly benefits from the advances in medical imaging, especially molecular imaging.

Molecular imaging allows the longitudinal and non-invasive follow up of complex biological processes targeted by new molecular entities as well as the stratification of patients before the implementation of treatment, for a better clinical management of cancers or cardiovascular diseases. Indeed, this leads to the concept of clinical biomarker. The qualification of biomarkers is a major challenge for pharmaceutical companies involved in the field of the development of new therapeutic and diagnostic agents.

The capacity for superparamagnetic iron oxide nanoparticles to target inflammation, a very general physiopathological process, makes them a major tool for the follow up of a large number of diseases which are all characterized by the presence of inflammatory foci (cancer, neuroinflammatory diseases such as Alzheimer Disease, vulnerable atheroma plaques, etc.).
The preparation of iron oxide nanocrystalline structures requires a highly reproducible industrial process resulting in a homodisperse population of magnetic nanoparticles without any laborious purification steps. The stability of the coating in various media (water, saline, cell culture media and biological media) is crucial. Indeed, a high safety profile, first in vitro then in vivo, is mandatory before starting any further study.

An extensive multi-disciplinary and "multi-cultural" experimental work has been implemented as part of the BONSAI Project and will be discussed.

**I8**

## Luminescent Silicon Nanocrystals: Synthesis, Functionalization, and Applications in Bioimaging

Mark T. Swihart[a,b], Folarin Erogbogbo[a], Ken-Tye Yong[b], Indrajit Roy[b], Rui Hu[b], Wing-Cheung Law[b], Hong Ding[b], and Paras N. Prasad[b]

*Department of Chemical and Biological Engineering[a], and Institute for Lasers, Photonics and Biophotonics[b], University at Buffalo (SUNY), Buffalo, New York 14260-4200*

Photoluminescent silicon nanocrystals, or "quantum dots" (QDs) have great potential in applications ranging from low-cost flexible photovoltaics and light emitters to biological imaging and diagnostic applications, where they provide an attractive alternative to fluorescent organic dyes or potentially toxic heavy-metal-containing quantum dots. However, because of challenges related to the synthesis and surface modification of free-standing Si QDs, they have been much less studied than more easily prepared QDs of compound semiconductors such as CdSe. In all of their potential applications, stability and tunability of optical and electronic properties are important. In biological systems, the QDs must also remain stably dispersed in water and biological fluids, over a wide range of pH and salt concentration. High luminescence quantum yield at near-infrared wavelengths, where tissue is relatively transparent, is also a key requirement. Meeting these requirements requires engineering both the crystalline silicon core and its interface with the surroundings. This talk will outline our efforts in developing scalable, economical methods of preparing and modifying Si QDs, with an emphasis on biological and nanomedicine applications. This includes high-rate laser driven aerosol synthesis, solution phase etching and surface modification, and encapsulation of surface-modified nanocrystals within micelles for bioimaging. In vitro cytotoxicity assays and preliminary in vivo studies have confirmed the biocompatibility of phospholipid-micelle-encapsulated Si QDs (MSiQDs). This has enabled demonstrations of *in vivo* targeted tumor imaging and sentinel lymph node mapping in live mice. Biodistribution studies in tumor-bearing mice show that non-targetted MSiQDs accumulate primarily in the liver, whereas MSiQDs conjugated with a cyclic RGD peptide showed substantial accumulation in the tumor over a 40 hour period, with reduced accumulation in the liver. More recently, we have explored encapsulation of SiQDs with low-cost, biocompatible pluronic block copolymers, co-encapsulation of SiQDs with magnetic iron oxide, nanoparticles, plasmonic gold nanoparticles, fluorinated compounds, FRET dyes, or chemotherapeutic agents for multimodal imaging and treatment.

**S3-5**

## Fate and biological effects of systemically administered quantum dots: impact of surface modification

Markus Rehberg*, Marc Praetner*, Camila Ferreira Leite, Peter Bihari, Fritz Krombach

Walter-Brendel-Centre of Experimental Medicine, Ludwig-Maximilians-Universität München, Munich, Germany

**Purpose:** This study was designed to investigate the impact of surface modification (i) on blood/tissue kinetics and microvascular localisation of quantum dots (QDs) and (ii) on the effects of QDs on leukocyte recruitment in an *in vivo* setting.
**Materials & Methods:** For all experiments, QDs (emission 655 nm, size 20-30 nm) with carboxyl (carboxyl-QDs), amine (amine-QDs), or polyethylene glycol (PEG-QDs) surface coating were injected intra-arterially (3 pmol/g body weight) into anesthetized male C57BL/6 mice (n = 6 per group; control mice received vehicle). To assess blood and tissue kinetics, fluorescence was quantitatively measured in blood and tissue samples at subsequent time points. To get information on the fate and effects of QDs in the microcirculation, intravital transillumination and fluorescence microscopy on the cremaster muscle as well as 2-photon laser-scanning microscopy on abdominal wall and heart muscle were performed. Leukocyte rolling, adhesion, and transendothelial migration were analyzed in postcapillary venules of the cremaster muscle at different time points. In additional experiments, QD-treated animals were given an anti-ICAM monoclonal antibody or an inhibitor of mast cell degranulation (cromolyn). At the end of the experiments, diameters of analyzed microvessels, centreline blood flow velocity, and systemic leukocyte counts were measured and wall shear rates calculated.
**Results:** The blood half-life of PEG-QDs was 513±152 min, whereas that of amine- and carboxyl-QDs was only 29±3 and 6±1 min, respectively. As assessed by *in vivo* fluorescence microscopy, all three types of QDs tested were found to be associated with the endothelium of postcapillary venules, but only carboxyl-QDs were rapidly taken up by perivascular macrophages. Interestingly enough, only carboxyl-QDs were found to be strongly associated with capillary endothelium in skeletal muscle as well as in the abdominal wall and the heart muscle. Moreover, intraarterial injection of carboxyl-QDs but not of amine- or PEG-QDs aggravated leukocyte adhesion and transendothelial migration in cremasteric venules. This carboxyl-QD-amplified leukocyte recruitment was almost completely blocked in animals treated with either an anti-ICAM-1 monoclonal antibody or an inhibitor of mast cell degranulation.
**Summary and Conclusion:** Taken together, these *in vivo* findings show that carboxyl-modified QDs (i) are rapidly cleared from the circulation, (ii) are taken up by perivascular macrophages in very fast manner, (iii) are strongly associated with capillary endothelium, and (iv) amplify ICAM-1-dependent and mast cell-driven leukocyte adhesion and (subsequent) transmigration in postcapillary venules. In conclusion, these data strongly corroborate the view that the surface chemistry of nanomaterials strongly affects their fate as well as their biological effects *in vivo* and thus is a crucial parameter to be considered with regard to biomedical applications.

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*Both authors contributed equally to this study.

**S3-6**

# Methods of silicon nanoparticles visualizations for in-vivo application

Loshchenov V., Korovin S., Pustovoy V., Ryabova A., Vasil'chenko S., Grachev P.
*A.M. Prokhorov General Physics Institute RAS*
*E-mail: loschenov@mail.ru*

## Purpose

The production method of aqua-media-luminescent silicon nanoparticles was developed.

Video-fluorescence and spectral equipment and methods for silicon nanoparticles detection in biological objects including laboratory animals in-vivo were developed.

## Materials & Methods

A fiber optics spectrometer LESA-01 Biospec was applied for investigation of luminescence spectra of silicon nanoparticles in biological media and tissues in-vivo. This system was previously applied for investigations of molecular photosensitizers promising for photodynamic therapy and fluorescent diagnostics. In the current research the system was modified to apply a 532 nm laser source instead of 633 nm for more effective luminescence excitation of silicon nanoparticles. In spite of the lower penetration depth in biological tissues at 532 nm than at 633 nm the efficiency of light absorption by silicon nanoparticles increases as a wavelength decreases. This system will be used for measurements of both nanoparticles samples and tissues in-vivo.

A video-fluorescent system from Biospec was modified for visualization of silicon nanoparticles luminescence at high concentration in a whole mouse. The system manufactured for skin observation was modified by increasing the output power of a LED excitation source (400 nm and 630 nm) and using the special band pass filters compatible with emission of silicon nanoparticles at 700-900 nm.

We developed a high sensitive luminescence system with application of an integrating sphere with 2 parallel registration channels for imaging and for photo electron multiplier.

The results on biological application of other nanoparticles will also be presented. Within this work the original methods of nanoparticles covering with the special biopolymers were developed. The different methods of the nanoparticles transformation were used: active methods (by exogenous influence) and passive ones (by metabolic processes taking place in the nearest environment of the nanoparticles). Thus the method of substance transformation inside biotissue in-vivo from nano- to molecular form and inversely was developed.

## Results

All the measurement systems have sufficient sensitivity for detection of silicon nanoparticles at high concentration and bright luminescence.

## Conclusion

The three methods were developed and modified to optimize luminescence measurements from silicon nanoparticles (spectral measurements, LED imaging system, advanced imaging system with an integrating sphere).

**I9**

## Plasmonic nanostructures as contrast agents for bioimaging

Sasha Douplik
Erlangen Graduate School in Advanced Optical Technologies (SAOT)
Friedrich-Alexander Universität Erlangen-Nürnberg
Paul-Gordan-Straße 6, 91052 Erlangen

Abstract:

Gold metal nanopacticles (GNP) have been experimentally tested as absorption and scattering contrast agencies for the visible and infrared ranges in biological experiments both ex vivo and in vivo. The results have shown a reasonable contrast both at local and systemic administration spectroscopy and imaging acquisitions. These experimental studies prove the concept of applicability of noble metal nanoparticles as contrast agencies for imaging in vivo and opens the prospects of establishing nanoparticle based malignancy contrast imaging techniques for diagnostics and surgical navigation.

**S3-7**

# Miniaturised devices for Au Nanorods detection

V.Bouzas[1,2], N. Carmona[1], M. A. Garcia[1,2]

[1]Dpto. Física de Materiales, Universidad Complutense de Madrid
[2]Instituto de Cerámica y Vidrio, Consejo Superior de Investigaciones Científicas, Madrid, Spain

Gold nanoparticles exhibit an intense absorption band associated with the excitation of surface plasmon resonance that allows detecting small amounts of nanoparticles (even single nanoparticles in optima conditions). This absorption can be tuned through the particle geometry. Non spherical nanoparticles as nanorods shows tow absorption bands (associated with longitudinal and transversal surface plasmons). One of these bands falls in the IR part of the spectrum where blood and biological tissue scarcely absorbs open the possibility to detect nanorods in vivo. To this purpose, miniaturised devices are required to image or energize the nanorods locally.

We present here some miniaturised devices designed for the detection of nanorods in water and blood media. The devices use light emitting diodes as monochromatic light sources and Si photodiodes for detection.

Devices working on transmission mode are able to detect nanorods concentration below micrograms/litre. Sensitivity is reduced is one or of magnitude for nanorods in blood.

We show detection of low nanorods concentration with devices working in reflection mode, opening the possibility to perform direct imaging of nanorods in vivo. The optima condition for detection and resolution limits are also discussed.

**S3-8**

# Enhancing the sensitivity of DNA microarray by using dye-doped silica nanoparticles: application to Papilloma Virus detection

F. Enrichi, R. Riccò, A. Meneghello, R. Pierobon, E. Cretaio

*CIVEN (Coordinamento Interuniversitario Veneto per le Nanotecnologie), via delle Industrie 5, 30175, Marghera (VE), Italia*

**Abstract**

DNA microarray is a high-throughput technology used for detection and quantification of nucleic acid molecules and other ones of biological interest. The analysis is resulting by specific hybridization between probe sequences deposited in array and a target ss-DNA usually amplified by PCR and functionalized by a fluorescent dye.

These organic labels have well known disadvantages like photobleaching and low signal intensities, which put a strong limitation to the lower amount of DNA material that can be detected. Quantum dots may be used as alternatives, but they present troubles like blinking, toxicity and excitation wavelengths out of the usual range of commercial instruments. Therefore for trace analysis the development of novel and more efficient biomarkers is required.

In this presentation we report an easy and cheap Stöber-like synthesis route for the incorporation of standard luminescent dyes into inorganic silica nanoparticles and their efficient application to the DNA microarray technology. We show one order of magnitude increase of the optical signal and decrease of the limit of detection (LOD) with respect to the use of free dyes or quantum dots in conventional instruments. This is due to the high number of molecules that can be accommodated into each nanoparticle, to the reduced photobleaching and to the improved environmental protection of the dyes when encapsulated in the silica matrix. These novel biomarkers, in comparison to the use of standard dyes or quantum dots were efficiently applied to the detection of Papilloma Virus, which is associated to the formation of cervical cancer, a leading cause of death by cancer for women worldwide.

The cheap and easy synthesis of these luminescent particles, the stability in water, the surface functionalizability and bio-compatibility make them very promising for present and future bio-labeling and bio-imaging applications.

**S3-9**

# Study of The Photo-Luminescence Bleaching in Si Nanocrystals Prepared by Laser Assisted Silane Pyrolysis

Ayse Seyhan[1], Urcan Guler[1] ,Mauro Falconieri[2], Rosaria D'Amato[3], Elisabetta Borsella[3], Rasit Turan[1]

[1]Department of Physics Middle East Technical University, Inonu Blvrd, 06531 Ankara, Turkey

[2]ENEA C. R. Casaccia, Via Anguillarese 301, 00123 Roma, Italy

[3]ENEA C. R. Frascati, Via E. Fermi 45, 00044 Frascati, Roma, Italy

## ABSTRACT

Si nanoparticles are attracting great interest for biological imaging applications since they emit photoluminescence in the red-infrared region (where tissue absorption is low) and their surface can be easily functionalized for bio-conjugation. Moreover, in comparison with other semiconductor quantum dots (Q. D.), silicon is inert, non-toxic, abundant and economical.

Here we report about the photo-bleaching of Si nanoparticles, a critical factor for biological studies, requiring special attention under a fluorescent microscope when the intensity variation is an important factor or temporally resolved imaging. Temporal variation of photoluminescence (PL) from free standing Si nanoparticles is studied under a strong laser illumination. Upon exposure to the laser beam, the PL peak intensity decays to a lower level in a time duration of a few seconds. The decay rate for the luminescence bleaching increases with higher laser intensity, and decreases with decreasing temperature. It is shown by a series of experiements that the bleaching is reversible when the laser exposure is switched off. The bleaching behavior is investigated for different laser wavelengths and intensities at different ambient temperatures. Si nanoparticles were prepared by the laser assisted silane pyrolysis technique. The high resolution transmission electron microscopy images confirmed the presence of both crystalline and amorphous nanoparticles with an average diameter of 5 nm. The chemical structure of the nanoparticles were identified by Raman spectroscopy which exhibited two bands corresponding Si-Si bonds in crystalline and amorphous form. The bleaching in Si nanostructures is generally attributed to a blinking process which is observed in a single nanoparticle populated by more than one excitons under a strong laser illumination. It is commonly accepted that the blinking occurs only in connection with and Auger assisted charge trapping. We discuss the observed PL bleaching in terms of exciton trapping at the interface between nanocrystal and the surrounding oxide layer.

**S3-10**

**Characterization of the transition dipole-moment of single semiconductor nanoparticles**

Anna M. Chizhik[1], Alexey I. Chizhik[1], Torsten Schmidt[2], Alfred J. Meixner[1], Friedrich Huisken[2]
[1] – Institute of Physical and Theoretical Chemistry, University of Tuebingen, Auf der Morgenstelle 8, D-72076, Germany
[2] – Laboratory Astrophysics Group of the Max Planck Institute for Astronomy at the Institute of Solid State Physics, University of Jena, Helmholtzweg 3, D-07743 Jena, Germany

Semiconductor nanoparticles (NPs) became one of the central object of investigations in a variety of fields from microelectronics to medicine. The investigation of photoluminescence of single particles requires exceptionally sensitive and at the same time relatively simple methods of the research.

A recently developed technique for the determination of the single-molecule or single nanoparticle dipole-moment orientation is based on scanning confocal fluorescence microscopy in combination with cylindrical vector beams (also known as higher order laser modes) [1, 2]. Recording just one fluorescence image of the sample surface we acquire full information about dipole moment orientations of all the nanoparticles in the selected region. At the same time the images provide information about blinking and bleaching processes of the single nanoparticles.

We investigated single $SiO_2$ NPs, as well as $Si/SiO_2$ and CdSe/ZnS core-shell systems. The obtained results clearly demonstrate the presence of the one-dimensional dipole moment of silicon dioxide nanoparticles [3], while the spherical CdSe/ZnS particles exhibit a two-dimensional transition dipole moment. Analysis of a large number of images reveals that the transition dipole moments of the silica nanoparticles are isotropically distributed and that there is no preferred direction with respect to the surface. Moreover, image series of the same sample area revealed the possibility for some silica particles to change their dipole-moment orientation from one image to another. Also we discuss the results obtained from single $Si/SiO_2$ NPs, since the emission characteristics of its transition dipole-moment give a new insight into the photoemission properties of the system and the origin of the its photoluminescence.

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**S3-11**

# Innovative application of iron oxide nanoparticles for rapid biomarker isolation in life sciences

Wang[1], B., Teale[1], W., Dürr[1], J., Verdaguer[3], S., Herlin-Boime[4], N., Wang[5], D., and Palme[1,2], K.

[1]Institute of Biology II, Faculty of Biology, University of Freiburg, D-79104 Freiburg (Germany), [2]Freiburg Institute of Advanced Studies FRIAS, University of Freiburg, D-79104 Freiburg (Germany), [3]CSIC-Consejo Superior de Investigaciones Cientificas, Instituto de Ciencia de Materiales, E-28049 Madrid (Spain), [4]CEA Saclay, SPAM/LFP Bat 522 (URA CEA-CNRS 2453), F-91191 Gif/Yvette (France), [5]Max Planck Institute of Colloids and Interfaces, D-14424 Potsdam (Germany)

Biological materials consist of a broad variety of molecules such as DNA, RNA, proteins and thousands of metabolites of different chemical composition. Recent advances allow the production of mass images of high resolution as well as the counting or imaging of several isotopes simultaneously. The separation and post-processing of proteins, nucleic acids, and natural products from complex reaction mixtures still remain a labor-intensive and costly process. In order to identify biomarkers occurring at wide dynamic range in biological fluids or to extract and isolate protein complexes highly selective and efficient affinity based purification techniques are needed for rapid and efficient isolation of such complexes for subsequent mass spectrometric analysis. This will allow to identify proteins with different functional specificity and to distinguish between ions of very similar masses. Efficient isolation procedures will provide the basis to both image and quantify molecules labeled with stable or radioactive isotopes within subcellular compartments and establish a breakthrough for modern life science research and biotechnology.

Bonsai partners have produced novel magnetized iron oxide based nanoparticles by laser pyrolysis which were subjected to dispersion and surface functionalization for further processing. Particles are colloidally stable, well-dispersed, and biocompatible with hydrodynamic sizes of 20-80 nm. The surface of particles was coated in order to allow covalent coupling of target proteins such as protein G. Protein G binds tightly antibodies. These beads are tested for highly selective affinity purification of protein complexes from fully sequenced model organisms such as Arabidopsis thaliana or others and subsequent mass spectrometric analysis. The potential of this approach for biotechnology will be discussed and compared to other techniques.

**P1-1**

## Development of magnetic Fe@C nanocomposites obtained via the laser pyrolysis: structural and disaggregation properties

Ion Morjan[1], R. Alexandrescu[1], F. Dumitrache[1], C. Fleaca[1], R. Birjega[1], I.Soare[1], V. Prodan[2], V. Kuncser[3], H. Xu[4], D. Wang[4]

[1]National Institute for Lasers, Plasma and Radiation Physics, 111 Atomistilor, MG-36, 077125 Bucharest, Romania
[2]Ovidius University of Constanta, Mamaia 124, Constanta, Romania
[3]National Institute of Materials Physics, Atomistilor MG-07, 077125 Bucharest, Romania
[4] Max Planck Institute of Colloids and Interfaces, Dept. of Interfaces, D – 14424 Potsdam, Germany

In previous work we have employed the laser pyrolysis technique for the preparation of carbon coated iron nanoparticles using iron pentacarbonyl as iron donor. This one-step synthesis technique combines the fast heating of gas phase processes with the sudden quenching of reaction products. We report here the successful control of the structural and magnetic properties of Fe@C nanocomposites by essentially varying the nozzle diameter of the emergent reactive gas flow The nano-sized Fe@C nanocomposites (carbon matrix with dispersed iron-based nuclei or core-shell structures) have been directly synthesized by the laser induced pyrolysis of iron pentacarbonyl and a mixture of acetylene/ethylene, both confined toward the flow axis by an argon stream. Since both $Fe(CO)_5$ and $C_2H_2$ do not absorb in the 10.6 μm region, C2H4 was used as sensitizer. A triple nozzle system was used. Different representative samples were synthesized by varying the diameter of the central nozzle (between 0.55 mm to 1.5 mm internal diameter). In case of increased nozzle diameter, XRD analysis indicates that the chemical content shifts towards the formation of the crystalline $Fe_3C$-cementite phase with higher mean crystallite dimensions (about 9 nm diameter). The same tendency is confirmed by particle histograms (lognormal distribution). TEM and HRTEM analysis show mostly particles which seem encapsulated in an amorphous matrix.

The magnetic properties and the Fe phase composition of the Fe-C samples were analyzed by temperature dependent Mössbauer spectroscopy.

The successful investigations carried on the disaggregation into water of the as-synthesized Fe@C nanoparticles are reported. Highly stable solutions were obtained by a multistep procedure comprising the dispersion of nanoparticles in THF solution (about 0.1-0.3 mg/ml) with added borane tetrahydrofuran complex (Sigma-Aldrich), repeated stirring and centrifugation, re-dispersion in water, high-energy ultrasonication and finally, neutral and basic solution of the extract.

**P1-2**

## Iron Oxide Materials Produced By Laser Pyrolysis

Rodica Alexandrescu [1], Valentina Bello [2], Virginia Bouzas [3], Rocío Costo-Cámara [4], Florian Dumitrache[1], Miguel Angel García [3], Rosella Giorgi [5], Maria del Puerto Morales Herrero [4], Ion Morjan[1], Carlos J Serna [4] Sabino Veintemillas-Verdaguer [4]

1) Natl. Inst. Lasers Plasma & Radiat. Physics Romania
2) Dept. Physics, Univ. Padova, Italy
3) Dept. Fis. Matt. Univ. Complutense Madrid
4) Inst. Ciencia de Materiales de Madrid CSIC, Spain
5) ENEA, Rome, Italy

In the development of the BONSAI FP6 EU Project the development of new magnetic colloids for biomedical applications demanded nanometric powders of magnetic iron oxides with the maghemite/magnetite structure. The laser pyrolysis technique was employed due to its capability of producing the nanoparticles in continuous form with a high degree of homogeneity and without the use of surfactants of potential toxicity. This technique consists in the laser driven rapid heating of an iron precursor in vapour phase in presence of oxygen. Different samples were prepared by changing the experimental conditions of synthesis. In particular we explored the effect of the laser power density, the shape of the laser beam, the input rate of the iron precursor (iron pentacarbonyl) the oxidation procedure and the nature of the sensitizer (a component added to the gas mixture who absorbs the $CO_2$ laser radiation). In broad terms we found that high crystallinity and good magnetic properties is attained at high density of the laser power and strong oxidation, being the particle size controlled by the residence time (focused beam). On the other hand, the use of low laser densities (unfocused beams) and soft oxidation conditions give in general smaller nanoparticles, poorly ordered. The particle sizes obtained were in the range of 2 to 9nm (TEM). All of the particles were superparamagnetic with values of the saturation magnetization at room temperature in the interval of 4-38 emu/g-sample. Usually the degree of order comes parallel to the particle size being the smaller the most disordered, whereas the degree of homogeneity of the powders (absence of big aggregates) decrease with the average particle size. Given that both characteristics were necessary for the fabrication of stable magnetic colloids, a compromise solution was attained.

The characterization of the samples was done using the standard techniques XRD TEM, IR and chemical analysis for screening the materials obtained. Selected samples were characterized by HRTEM for careful phase identification and XPS for surface characterization. The main conclusions obtained by these techniques are that all the samples, with the exception of some of those obtained using sulphur hexafluoride as sensitizer, were pure $Fe_2O_3$ maghemite and that the carbon who is the main impurity of the samples is present basically in the surface in the form of C=O bonds. All the information obtained is important in order to understand the behaviour of the particles in water suspension.

**P1-3**

# Magnetic properties of Fe Oxide Nanoparticles produced by laser pyrolysis for biomedical applications.

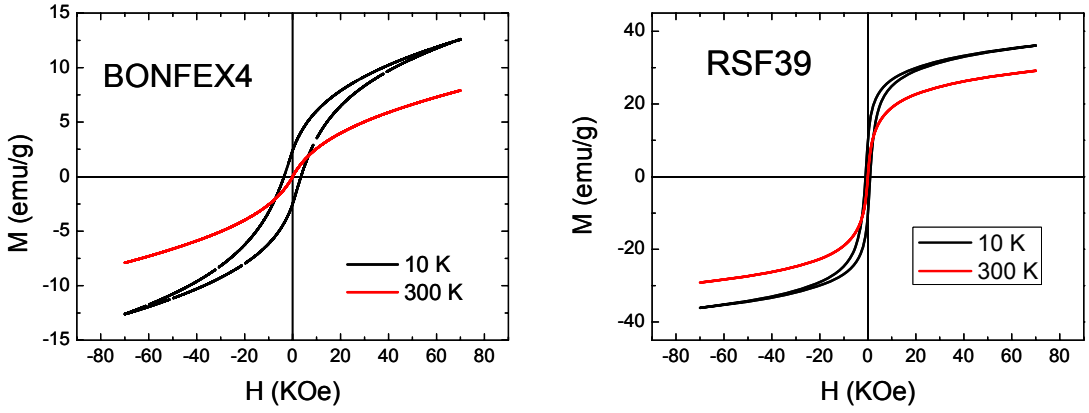
M. A. García[1], R.Costo[2], S. Veintemillas[2], P. Morales[2], M. García-Hernández[2]
R. Alexandrescu[3] I. Morjan[3], P. Gasco[4]

1) Instituto de Cerámica y Vidrio CSIC & Dept. Fis. Mat. Univ. Complutense Madrid
2) Inst. Ciencia de Materiales de Madrid CSIC, Spain
3) Natl. Inst. Lasers Plasma & Radiat. Physics Romania
4) Nanovector S.r.l., Torino, Italy

Iron oxide nanoparticles are commonly used in biomedical applications as contrast agent for Magnetic Resonance Imaging. The magnetic properties of the nanoparticles strongly depend on their features and small changes in shape and size can alter significantly the magnetic response of the nanoparticle. Therefore nanoparticle production methods with a narrow size distribution are required for these purposes. Among the different methods that can provide this kind of nanoparticles, laser pyrolysis has the advantage to produce homogeneous material in quantities fairly larger than other chemical methods that requires a long time to produce few milligrams and results expensive.

Here, we report on the preparation and magnetic characterization of Fe oxide nanoparticles by laser pyrolysis and the relationship between the preparation conditions and the magnetic response. It is shown that controlling the preparation conditions during the pyrolisis allows tuning the nanoparticles morphology and structure and consequently the magnetic properties of the nanoparticles.

A key step for the preparation of nanoparticles is the functionalization to ensure biocompatibility and avoid degradation. The effect of different procedures including the development of solid lipid nanoparticles on the magnetic properties is also discussed.




Magnetization curves of Fe oxide nanoparticle prepared by laser pyrolysis

**P1-4**

# Reproducibility of the Synthesis of Iron Oxiide Nanoparticles by Laser Pyrolysis

Rodica Alexandrescu [1], Virginia Bouzas [2], Rocío Costo-Cámara [3], Florian Dumitrache[1], Miguel Angel García [2], Maria del Puerto Morales Herrero [3], Ion Morjan[1], Carlos J Serna [3] Sabino Veintemillas-Verdaguer [3]

1) Natl. Inst. Lasers Plasma & Radiat. Physics Romania
2) Dept. Fis. Mat. Univ. Complutense Madrid
3) Inst. Ciencia de Materiales de Madrid CSIC, Spain

During the development of the BONSAI Project the need that other partners had of considerable amount (several gram quantities) of iron oxide nanoparticles pressed us to face the problem of the reproducibility in the production of nanoparticles. Given the fact that the productivity of the smaller, and more homogeneous nanoparticles (labelled BONFEX4) was low (in the range of 1g/day), it was necessary to repeat the synthesis many times. These repeated synthesis runs involved the use of three different $CO_2$ lasers (two with monomodal gaussian beams TEMoo mode with spot sizes of 4 and 3.5mm, respectively and one multimodal with 4mm spot size). By keeping constant all the other the parameters (including the laser density) we obtained similar powders with respect to the X-Ray diffraction pattern, and the particle size distribution but with different degree of order, as shown by the Infra-Red spectra and the magnetic properties, being the last the most sensitive to the type of laser employed (Figure 1). When the same laser was used the reproducibility of the hysteresis cycle increased significantly. These effects will be examined.

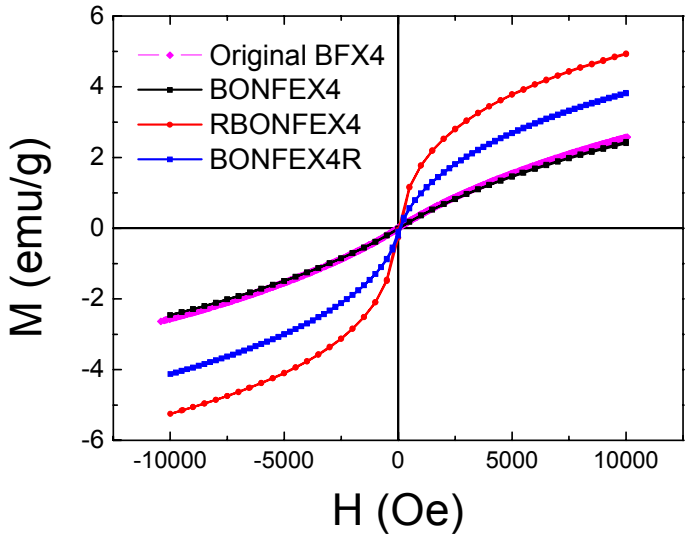



FIGURE 1

Hysteresis cycles at room temperature of various samples prepared with the same process parameters as the BONFEX4 sample, except the laser beam. The Original BFX4 and BONFEX4 were made using the 4mm TEMoo Laser, BONFEX4R with the 3.5mm TEMoo and RBONFEX4 with the multimodal 4mm.

**P1-5**

# Recent Progress on the Preparation of Luminescent Silicon Nanoparticles for Bio-Imaging Applications

V. Maurice[1], O. Sublemontier[1], N. Herlin[1], E. Doris[2], O. Raccurt[3], A. Sanson[2]
[1]Laboratoire Francis Perrin (CEA-CNRS URA 2453), SPAM, DSM, CEA Saclay, France
[2] SB2SM, Ibitec-S, DSV, CEA Saclay, France
[3] LCSN, DTNM, LITEN, DRT, CEA Grenoble, France

Until now, cellular components and processes are mostly visualized through organic dye based fluorophores. These dyes have several drawbacks such as rapid photo-oxidation, limited lifetime. Recently, semiconductor nanocrystals (quantum dots) have been introduced for bio-labelling. In fact, as a result of the quantum confinement, semiconductor NPs (*nanoparticles*) exhibit intense, size-tuneable emission in the visible and near-infrared optical range. The main advantage of using luminescent NPs for imaging rests on their photostability, increased sensitivity through longer life time and brightness. However, unfortunately as most widely used quantum dots (II-VI semiconductor QDs) are highly cytotoxic, limitations occur for *in vivo* application in living organisms.

It is possible to replace toxic quantum dots such as CdSe with light emitting Si and Si-based NPs having broader excitation band, size dependent optical emission and a reduced tendency to photo bleaching. In the study presented here, Si NPs have been synthesised by laser pyrolysis. This technique allows producing measurable amounts of particles of different materials. The particles produced are of high purity, and the experimental conditions allow to choose the characteristics of the particles: size between 3 and 50 nm, production rate ~200 mg/h for 5 nm particles. When the size of the particles is under 10 nm, the silicon has a photoluminescence in the range of 600-900 nm. It is precisely the range where the light is well transmitted by animal skin.

The as prepared Si NP exhibit only weak photoluminescence, due to surface defects hindering the radiative recombination of excitons. Therefore, the particles surface needs to be passivated to improve their emission properties. This can be done by surface oxidation in air or in liquid phase, or by surface reduction in HF; this results in different surface coverage: respectively $SiO_2$ and SiH. We chose the oxidation method, which is much easier to perform. After passivation of the surface, the Si NPs show a bright photoluminescence.

Another important issue is to ensure the colloidal stability of the particles in saline media and to protect the silicon core against water oxidation. This can be achieved by growing a silica layer around the particles, we present here two different processes, both involving the Stöber method. The first one leads to thin silica shells around the particles, and the other one leads to large monodisperse silica beads (~50 nm) containing several Si NP. The silica beads provide a better protection than the thin silica layer, and they are well dispersed in water. These beads are produced in microemulsion. Both silica beads and silica coated particles can be further functionalised by an amine layer, which allows grafting organic molecules such as polyethylene glycol to improve the colloidal stability.

We have shown that it is also possible to graft proteins on the surface of the particles, after amine functionalisation of the surface and using a polyethylene glycol based crosslinker. The reactions take place in a biological buffer. The protein is grafted using a thiol function. The evidence for the grafting has been given by fluorescence and electrophoresis.

**P1-6**

## Synthesis and characterization of light-emitting Si/Ge nanoparticles

T. Schmidt, L. B. Ma, K. Potrick and F. Huisken

*Laboratory Astrophysics Group of the Max Planck Institute for Astronomy,*
*Institute of Solid State Physics, Friedrich Schiller University,*
*Helmholtzweg 3, D-07743 Jena, Germany*

The intense photoluminescence (PL) of silicon nanocrystals (Si NCs) makes them a promising material for future devices in optoelectronics and photonics. Moreover, Si NCs with lateral dimensions between 2.5 and 8 nm are ideally suited to act as an efficient light source for bio-imaging techniques. Studies on naturally oxidized Si NCs selected in size revealed that the PL, located in the visible and near infrared spectral range (below 1.1 μm), is caused by quantum confinement.[1] This size-dependent effect allows flexible control over the energy and efficiency of the emitted light. Doping Si NCs with Ge, a larger luminescent range can be achieved due to a smaller band gap of bulk germanium (0.66 eV at 300 K) compared to bulk silicon (1.13 eV at 300 K). Thus, in principle, the band gap can be adjusted to any desired wavelength from the visible to 1.8 μm by varying the Ge concentration and the size of the $Si_{1-x}Ge_x$ NCs. Furthermore, theoretical calculations[2] predict a shorter PL lifetime and therefore a more efficient light emission in Ge-doped Si NCs.
The basics of laser-induced pyrolysis of silane ($SiH_4$) and germane ($GeH_4$) to synthesize luminescent $Si_{1-x}Ge_x$ NCs are briefly introduced. Using the capabilities of a cluster beam deposition apparatus, thin films of $Si_{1-x}Ge_x$ NCs are prepared. The size of the synthesized NCs is determined by in situ time-of-flight mass spectrometry (TOF-MS).[1,3,4] The optical properties of the as-prepared Si/Ge NCs are investigated by spectrally resolved PL emission and time-resolved decay measurements as a function of the Ge concentration. To identify the morphology high-resolution TEM as well as RBS to determine the amount of incorporated Ge are applied. These studies address the question whether the PL originates from radiative recombination of charge carriers in quantum-confined systems, realizing size-dependent light emission, or to what extent other mechanisms such as interface defects are operative. The observed aging characteristics can be explained in the frame of the quantum confinement model. The PL properties (peak position, FWHM, and lifetime) monitored over several weeks reveal stabilization after one month. Surface defects are passivated due to native oxidation in ambient air and, even if defect PL is operative, they play only a minor role. As predicted by *ab initio* calculations[2], the final radiative decay rate for Ge-doped Si NCs (~10 % Ge) is two times faster compared to pure Si NCs.[5] The bottom-up synthesis of mixed Si/Ge nanocrystals is a promising way to form improved Si-based quantum emitters fitting the particular spectral requirements for bio-imaging methods using nanoparticles.

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**P1-7**

# On the red photoluminescence emission from silicon-based nanoparticles

M. Falconieri[1], E. Borsella[2], R. D'Amato[2], F. Fabbri[2], E. Trave[3] ,V. Bello[4], G. Mattei[4] Y. Nie[5], D. Wang[5]

1) ENEA C.R. Casaccia via Anguillarese 301, 00123 Roma, Italy.

2) ENEA C.R. Frascati via E. Fermi 45, 00044 Frascati, (Roma), Italy.

3) Dip. Chimica Fisica, Università Ca' Foscari Venezia, Dorsoduro 2137, I-30123 Venezia, Italy

4) Dip. Fisica, Università degli Studi di Padova, via Marzolo 8, I-35131 Padova, Italy

5) Max Planck Institute of Colloids and Interfaces 14424 Potsdam, Germany

Abstract

Photo-luminescence (PL) emission from silicon nanocrystals is at the hearth of the widely investigated applications in fluorescence imaging. However the process responsible for the visible-near infrared PL emission in Si nanostructures has been generating significant controversy for years. The debate has focused on whether light emission is originated by band to band recombination of electrons and holes in Si nanostructures, or by recombination at defects located at the surface. It is experimentally difficult to distinguish the two contributions since both are size-dependent. Here we compare the spectroscopic properties of crystalline Si nanoparticles, as prepared by laser pyrolysis and after complete conversion to amorphous silica by oxidation-assisted alkali etching. The strong resemblance of the spectral and time-behavior of the red PL emission (in the range 600-1040 nm) in both systems, actually suggests that this emission is dominated by defects states localized at the $SiO_2$/Si interface. Here we show that the strongly non-exponential time behaviour of the photo-luminescence emission in both systems (nano-crystalline Si and oxidised amorphous sample), can be explained as the sum of exponential decays from three emitting non- bridging oxygen hole centres (NBOHC), thus ruling out the interpretation in terms of the so-called "stretched exponential" decay. The commonly observed red PL band is obtained from the superposition of the emissions from the three centres. Changes in bond angle and bond length in these centres due to size-dependent strain and chemical inhomogeneities, influence the emission energies and can give the false impression of a shift due to quantum confinement.

**P1-8**

**Synthesis and photoluminescence of Ytterbium-doped Silicon nanocristals**

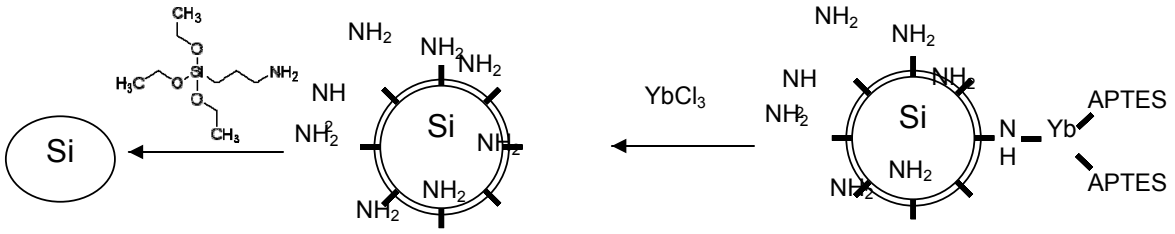
R. D'Amato (1), M. Falconieri (2), E. Borsella (1)

(1) ENEA, CR Frascati, via Enrico Fermi 45, 00044 Frascati (Roma), Italy
(2) ENEA, CR Casacia, via Anguillarese 301, 00123 Roma, Italy

The rare earth ion Ytterbium(III) is of particular interest because its ($^2F_{5/2}$) - ($^2F_{7/2}$) luminescent transition at 980 nm is commonly used in bioanalitycal applications. The f-f transitions are parity forbidden and, as a result, the absorption coefficients are very low and the emissive rates are slow, which results in long-lived and linelike emission bands. As a consequence, direct excitation of the lanthanide ions is unfavourable. A way to overcome the difficulties of low absorptivity and thermal relaxation is to incorporate lanthanide ions in a host matrix: the matrix is excited above the band-gap energy and, after energy transfer, the lanthanide ion emits.

Many methods have been developed for the preparation of discrete lanthanides-doped silicon nanocrystals (SiNC). We have developed an innovative approach for Yb and SiNC, based on the aptitude of Yb to form complexes with amino group.

SiNC produced by laser pyrolysis with diameter equal to 8 nm and 6 nm, were first functionalised with aminopropyltriethoxysilane (APTES) and then doped with Yb(III). It needs thermal treatment in order to obtain luminescence, as already reported in literature for sol-gel system containing Si and Yb, but at very high temperature. Our method is very convenient because the reaction is at room temperature and the thermal treatment is only at 300°C, so SiNC do not modified.




The Yb-doped nc-Si showed sharp emission peaks at wavelengths around 1 μm. We performed reaction by using different ratio SiNC/Yb, and we found that intensity of emission increased when quantity of Yb increased.

**P1-9**

**Tunable luminescence from oxidized silicon nanoparticles**.

E. Kelm, S. Korovin, V.Pustovoy, A. Surkov, A. Vladimirov
General Physics Institute, Russian Academy of Sciences, Moscow, Russia

The luminescent silicon nanoparticles with the luminescence covering red – near IR band was investigated. The particles with the size 2-6 nm were prepared by laser pyrolysis of silane with consequent chemical etching. With increasing photon energy of excitation from 660 nm to 365 nm, the PL peak shifted from 820 nm to 650 nm. That is allows using the silicon based particles for energy selected excitation in some practical application. In medicine it may be used as tunable optical markers.

**P1-10**

## Optical properties of silicon nanoparticles covered with the dye layers

Ryabova A., Vasil'chenko S., Korovin S., Loschenov V., Pustovoi V.
*A.M. Prokhorov General Physics Institute RAS*
*E-mail: nastya.ryabova@gmail.com*

**Purpose**
Study of silicon nanoparticles spectroscopic properties with the aluminum phthalocyanine molecules precipitated on their surface.

**Materials & Methods**
Silicon nanoparticles obtained by laser pyrolysis were used. The silicon nanoparticles were dried, added to the concentrated aluminum phthalocyanine solution in benzene. Thereby aluminum phthalocyanine molecules precipite on the nanoparticles surface. Further the optical spectra the obtained nanoparticles depending on the solvent polarity were investigated.

**Results**
Aluminum phthalocyanine molecules remarkable with the property to lose the luminescence capacity in the solid state as well as in the form of nanoparticles and in the form of dry film. However in the presence of polar solvent aluminum phthalocyanine molecules have some conformational freedom and are able to emit luminescence photons. We investigated the luminescence and absorption spectra of the silicon nanoparticles with aluminum phthalocyanine molecules precipitated on their surface. Reversible dependence of the nanoparticles optical activity on the solvent polarity was established.

**Conclusion**
Surface modification with fluorescence biocompatible molecules, not only with high fluorescence capacity, but also with photodynamic action, seems very perspective.

**P1-11**

## Luminescence of the silicon based nanoparticles.

E. Kelm, S. Korovin, V.Pustovoy, A. Surkov, A. Vladimirov

General Physics Institute, Russian Academy of Sciences, Moscow, Russia

The luminescent properties of silicon nanoparticles were studied. The particles were prepared by laser pyrolysis of silane in a gas flow reactor. Initially non-luminescent particles were treated by the chemical etching in mixture of fluoric and nitric acids. During the etching the silicon core size decreased to several nanometers. The high and stable photoluminescence was observed. With increasing etching time, the PL shifted its peak position to blue region due to the decrease of particles size. The results of spectral and TEM analysis are presented.

**P1-12**

## Optical properties dependence on interface states of silicon nanoparticles

**S. Korovin, R. Khasanshin*, A. Vladimirov**

Natural Science Center of General Physics Institute, Moscow, Russia,
Email: korovin@kapella.gpi.ru

In this paper we present the experimental results and theoretical analysis of the optical properties crystalline silicon nanoparticle's (c-n-Si). The optical properties of individual particles of c-n-Si covered by the natural oxide layer and one imbedded in water have been studied. In spite of the thickness of interfacial layers were about 1 nm the optical absorption spectra for each nanocomposites measured in the spectral range from 300 to 1300nm depend much on water consistence (we used of pure water and one with $H_2O_2$) and different metallic layers. The reason is because it depends on the surrounding media, mainly, but not on the particles microstructure. This character of optical absorption in the visible range is defined by the double charge layer near the surface which creates the strong static electric field inside the particles. The mechanism of the optical absorption is the composition of absorption by the surface electrons plasma waves and valence electron in strong static electric field created by double charge layer (Frantz – Keldysh effect).

**P1-13**

## Synthesis and characterization of Au nanorods for biomedical applications.

V.Bouzas[1,2], M. A. Garcia[1,2], N. Carmona[1]

[1]Dpto. Física de Materiales, Universidad Complutense de Madrid
[2]Instituto de Cerámica y Vidrio, Consejo Superior de Investigaciones Científicas, Madrid, Spain

Surface plasmon resonance (SPR) is the most outstanding feature of metallic nanoparticles (NPs). It consists on a collective oscillation of the conduction electrons inside the NPs. For the case of noble metal nanoparticles, the resonant frequency falls in the visible part of the spectrum. Therefore, when illuminating noble metal with visible light surface plasmons (SP) can be excited leading to a great light absorption. The SPR in gold nanoparticles is so strong that it enhances locally the light electric field several orders of magnitude; actually the cross-section of the absorption associated with the SP excitation can be up to 1000 times larger than its geometrical section. These effects, in addition with the high biocompatibility and easy functionalization rend gold nanoparticles as attractive elements for biomedical applications. The huge cross-section of SPR absorption allows detecting very small quantities of nanoparticles (even single NPs in the best experimental conditions) that can be used in diagnosis.

The main limitation for the use of Au nanoparticles in biomedicine is due to the fact that the SPR absorption band matches that of haemoglobin rending a huge task to detect Au nanoparticles in bloodstream. This limitation may be overcome by the synthesis of non spherical nanoparticles, as nanorods, for which the SPR bands splits into two: one matching that of spherical nanoparticles and a second one at the IR for which the position and intensity strongly depends on the nanorods geometry (mainly size and aspect ratio). On the contrary, fabrication of non spherical nanostructures is rather complicate, and only recent advances in colloidal synthesis methods allowed to fabricate this kind of nanostructures but the exact relationship between synthesis parameters and nanorods geometry is not well established.

In this work we analyze the effect of synthesis parameters as surfactants, stirring and time scale in the morphology and optical properties of the nanorods. It is found that, controlling these parameters, we may tune both position and intensity of the SPR bands of the nanorods.

**P1-14**

## Synthetic and biogenic magnetic nanoparticles for medical applications

Katerina Polakova [1], Zdenka Markova [1,2], Radek Zboril [1,2], Ingrid Markova [3] and Miroslav Mashlan [1,4]

[1]Centre for Nanomaterial Research, Palacky University, Slechtitelu 11, 771 46 Olomouc, Czech Republic, [2] Department of Physical Chemistry Palacky University, Svobody 26, 771 46 Olomouc, Czech Republic, [3]Department of Radiology, Faculty Hospital F.D.Roosevelt, Banska Bystrica, Slovak Republic, [4]Department of Experimental Physics, Palacky University, Svobody 26, 771 46 Olomouc, Czech Republic, *E-mail: kacka.polakova@email.cz, Phone: +420585634950, Fax: +420585634958.

Superparamagnetic iron oxide nanoparticles with an appropriate surface modification can be widely used in various applications including magnetic resonance imaging (MRI), drug targeting, hypertermia or cell labeling. Thermally induced solid state synthesis of magnetic nanocomposite consisting of superparamagnetic maghemite nanoparticles in the matrix of smectite mineral bentonite is presented as a simple and cheap method for preparation of SPIO (superparamagnetic iron oxides) negative oral contrast agent in MRI. The nontoxic biocompatible nanocomposite includes 20nm superparamagnetic noninteracting maghemite nanoparticles well dispersed and physically adsorbed on the surface of bentonite clay as confirmed by Mössbauer spectroscopy, TEM, SEM and magnetization measurements. The final experimental suspension, containing maghemite/bentonite nanocomposite, PEG, apple/carrot juice and water, represents an effective negative oral contrast agent fully comparable with a commercial preparation Lumirem. The results of the clinical tests unambiguously demonstrate its desirable applicability and high efficiency in imaging the small bowel mainly in MRCP (magnetic resonance cholangiopancreaticography) investigation. Currently, inflammatory bowel diseases by MREg (magnetic resonance enterography) are evaluated.

Microbial production and functionalization of biogennic magnetite from magnetotactic bacteria (*Magnetospirillum gryphiswaldense)* has been studied. Biogenic magnetite was coated with substances that make them biocompatible, biodegradable, stable, non-toxic and accessible for binding with various active biocomponents depending on particular bioapplication. Natural polymers and their derivates, such as chitosan, N-trimethylchitosan, carboxymethylchitosan or dextran, have been used in a coating procedure and the properties of the core-shell systems have been analyzed by TEM, SEM and SQUID magnetization measurements.

**P2-15**

## Uptake and intracellular distribution of functionalized iron oxide nanoparticles

B. Lettiero[1], A.Panariti[1], I. Morjan[2], R. Alexandreascu[2], S. Veintemillas[3], D. Wang[4], C Bucci[5], G. Miserocchi[1] and I Rivolta[1]

1. Università di Milano Bicocca, Milano, Italy;
2. NILPRPB, Bucarest, Romania;
3. CSIC, Madrid, Spain;
4. MPI, Postdam, Germany;
5. Università del Salento, Lecce, Italy

Due to their high magnetic response, iron nanoparticles are widely studied because of their potential application in bio-medicine as contrast agents for magnetic resonance imaging or for magnetothermia. To be suitable for these applications, the NPs must respect some requirements, such as a good dispersibility in physiological medium and biocompatibility.
In our study we investigated the interaction between lung epithelial cells and iron oxide NPs coated with L-Dihydroxyphenylalanina (L-Dopa)-TRITC.
We evaluate the particles biocompatibility by measuring the release of a cytoplasmic enzyme (Lactate Dehydrogenase, LDH) and found that, in a range of 10ng/ml up to 100µg/ml, the detection of the LDH in the medium was at maximum 20% higher compare to control condition (6 and 24hrs of incubation). The TRITC fluorophore allows to follow the particles in the cells when images are acquired with a confocal fluorescent microscope. Our data suggest that indeed the particles crossed the plasma membrane with an energy-dependent process, since the incubation at low temperature (4°C) prevented their entrance. The labelling of intracellular organelles revealed that the fluorescence due to the NPs may only be in proximity of lysosomes. We treated the cells with TRITC-NPs at different time (10 min to 4hrs) and found that the particles movement followed a centripetal direction, in fact in 4 hours they moved from 5.8±1.4µm to 2.7±1µm ($p<0.01$) considering the nucler membrane as reference point. Moreover, longer was the NPs exposure, larger was the dimension of the fluorescent intracellular aggregates (0.75±0.1 up to 1.91±0.5µm going from 10 minutes to 4 hours of incubation $p<0.01$).
Our goal now is to investigate in more details the mechanisms that govern the NP when crossing the cell membrane and their intracellular movement in order to have a complete picture of the uptake and intracellular distribution of these promising nanoparticles.

**P2-16**

## SLN as vehicle for an hydrophobic model drug: a biophysical study

**A.Panariti[1], I. Rivolta[1], B. Lettiero[1], G. Chirico[1], P.Gasco[2] and G. Miserocchi[1]**

1.Università di Milano Bicocca, Monza, Italy;
2. Nanovector, Turin, Italy

Solid Lipid Nanoparticles (SLN) are well defined nanocarriers that can be tailored in such a way to translocate a variety of lipophilic compounds to the close proximity of the cell membrane and to unload their content into the cell. We studied the distribution of the SLN load within the cell cytoplasm trying to shed some light on the internalization mechanism and on the average force field that acts on the internalized compound within the cell cytoplasm.
Epithelial lung cells were incubated with a non-toxic concentration (0.01mg/ml) of SLN loaded with 6-coumarine, a green fluorescent dye. The distribution of fluorescence within the cytoplasm, increased over 45 minutes observation as a function of the distance from the plasma membrane reaching the maximum in proximity of the nuclear membrane.
Lowering the temperature from 37°C to 4°C caused a 50% decrease of the uptake of the fluorophore suggesting that the uptake is not mediated exclusively by active processes.
Further insights on the origin of the observed distribution pattern was gained by altering the cell structure through a perturbation of the cytoskeleton. After reaching a steady state perinuclear accumulation condition for the fluorophore (45minutes), we treated the cells with cytochalasin D (2.0 μM for 1 h) to disintegrate actin boundless, avoiding cytotoxic damage. We found that while in the control condition the ratio between perinuclear and peripheral fluorescence was 5 fold, in the cells treated with cytoD, this value decreased to a plateau level of 1.6, suggesting that the integrity of the cytoskeleton is setting intracellular fluid-dynamics and mechanics condition causing the perinuclear accumulation.

**P2-17**

## The olfactory system as a route for nanoparticles to reach the brain

Garzotto D.[1], Giacobini P. [1], Fubini B. [2], Tomatis M. [2], Quagliotto P. [2], De Marchis S. [1]

1- Dipartimento di Biologia Animale e dell' Uomo, Università di Torino, Via Accademia Albertina 13, 10123 Torino 2- Dipartimento di Chimica Generale e Organica Applicata, Via Pietro Giuria 7, 10126 Torino

Nanoparticles are used in a wide range of human applications from industrial to bio-medical fields. However, the unique characteristics of nanoparticles, such as the small size, large surface area per mass and high reactivity raises great concern on the adverse effects of these particles on ecological systems and human health. There are several pioneer studies reporting translocation of inhaled particulates to the brain trough a potential neuronal uptake mediated by the olfactory nerve (Dorman et al., 2004; Elder et al., 2006; Oberdörster et al., 2004). However, no direct evidences have been presented up to now on the pathway followed by the nanoparticles from the nose to the brain. In addition to a neuronal pathway, nanoparticles could gain access to the central nervous system (CNS) trough extracellular pathways (perineuronal, perivascular and cerebrospinal fluid paths). In the present study we investigate the localization of intranasally delivered fluorescent nanoparticles in the olfactory epithelium and in the brain. To this purpose we used two different kind of nanoparticles: 1) titanium dioxide conjugated with the fluorescein isothiocyanate (FITC) and 2) carboxyl quantum dots as a model of innovative fluorescent semiconductor nanocrystals commonly used in cell and animal biology (Michalet et al., 2005). Intranasal treatments with nanoparticles were performed both subcronically for 5 days and acutely on adult CD1 mice. The olfactory epithelium and olfactory bulb were collected and analysed by confocal microscopy and ICP-AES at different survival time after treatment. Our results indicate that titanium dioxide nanoparticles delivered in the nose enter the brain. In vitro experiments on primary cultures of olfactory bulb neurons suggest that these cells are able to internalize nanoparticles, and ongoing studies are trying to characterize a possible toxic effects of these nanoparticles on olfactory neurons. We are also analysing their localization in the different cellular compartments of the olfactory epithelium. Data obtained following intranasal irrigation of quantum dots indicate that non neuronal compartments of the olfactory mucosa are preferentially involved in nanoparticles uptake, thus supporting the extracellular pathways as the most likely route to access the CNS. The possible selection of different penetration routes may be ascribed either at the different surface chemistry of the two kind of nanoparticles employed or to their difference in size and aggregation.

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**P2-18**

## Magnetic nanoparticles for surface modification of microbial cells

Mirka Safarikova and Ivo Safarik

Institute of Systems Biology and Ecology, Department of Nanobiotechnology, Na Sadkach 7, 370 05 Ceske Budejovice, Czech Republic. E-mail: mirkasaf@usbe.cas.cz

Biocompatible magnetic nanoparticles, which are mostly formed by magnetic iron oxides (magnetite, maghemite), can be used for postmagnetization (incorporation into the porous structure or attachment on the surface) of different kinds of biological, organic and inorganic materials. Such magnetically responsive materials exhibit magnetic properties and can be easily handled using a magnetic field.
Magnetic nanoparticles (maghemite, 10 – 15 nm) were attached on the surface of *Saccharomyces cerevisiae* and *Bacillus circulans* cells and such cells were used as magnetically responsive whole cell biocatalysts or as a producer of extracellular enzymes. Conditions for a distribution of magnetic nanoparticles within yeast cells were also studied.

Washed *Saccharomyces cerevisiae* cells under different physiological conditions were incubated with several types of water based ionic magnetic fluids. Internalization and cell-uptake of magnetic nanoparticles were investigated with the use of electron microscopy. Magnetically responsive cells were used for hydrogen peroxide decomposition and sucrose conversion (utilizing intracellular enzymes catalase and invertase). Dead magnetic yeast cells were also applied for biosorption of water-soluble organic dyes and heavy metal ions. Magnetic nanoparticles were adsorbed or covalently bound on the cell surface of *Bacillus circulans* cells and used for the production of extracellular enzyme cyclodextrin glucanotransferase (CGTase).

Distribution of magnetic nanoparticles within yeast cells was strongly dependent on their growth conditions. When cells were cultured before the treatment with magnetic fluid, only a small number of magnetic nanoparticles attached on the cell surface was revealed, whereas a significant amount of them was found inside the cells in the periplasmic space. When cells were not cultivated before the ferrofluid treatment, only very few magnetic nanoparticles were found within the cells.

The enzyme activities of catalase and invertase in magnetically modified yeast cells were higher or similar compared to cells entrapped in magnetic alginate beads (2-3 mm) or microbeads (50-100 μm). The yeast cells – magnetic nanoparticles complexes were stable for more then one-month storage, retaining their enzyme activities unchanged. The amount of magnetic nanoparticles adsorbed on the surface of *Bacillus circulans* cells decreased during incubation cycles and cells division. When magnetic nanoparticles were bound on cell walls covalently, they were not released into culture medium and a significantly enhanced enzyme yield and specific CGTase activity were achieved.

Cultivation step has been shown to be fundamental for magnetic nanoparticles (stabilized as ionic magnetic fluids) internalization by yeast cells. Magnetic nanoparticles can be used for the construction of magnetically responsive whole cell biocatalysts and for the magnetic modification of biosorbents. Such magnetic materials can be easily handled in external magnetic field.

**P2-19**

# Cobalt phthalocyanine nanoparticles capable of reversible aggregating in biotissues under physical action

Ryabova A.[1], Vasil'chenko S.[1], Volkova A.[1], Kaliya O.[2], Stratonnikov A.[1], Loschenov V.[1]
[1] *A.M. Prokhorov General Physics Institute RAS*
[2] *Federal State Unitary Enterprise NIOPIK*
*E-mail: nastya.ryabova@gmail.com*

## Purpose

Investigation of the cobalt phthalocyanine transformation dynamics under the influence of laser irradiation and weak electric fields *in vitro* and *in vivo*. Development of electrochemical and optical methods of influence on tumor tissue containing cobalt phthalocyanine under spectroscopic and thermal control.

## Materials & Methods

Cobalt phthalocyanine solutions and water colloids of water-insoluble cobalt phthalocyanine nanoparticles were used. The experimental setup for the diffuse backscattering spectroscopy and laser-induced fluorescence spectroscopy is made on the basis of a fiber-optic spectrometer LESA-01-Biospec. Methods of electrochemical and optical action on the tumor tissue containing cobalt phthalocyanine are tested on an experimental model of tumor carcinoma intramuscularly inoculated in laboratory mice. Controlling the tissues temperature was carried out using infrared cameras. The current and alternating voltage from a power supply was applied to the tumor through the needle-electrodes. A laser in the pulsed and continuous modes was used as a source of laser radiation.

## Results

Methods of current and alternating voltage action on tumor tissue containing cobalt phthalocyanine were developed. Application of the alternating voltage can locally increase the accumulation of cobalt phthalocyanine in the form of nano- and microparticles and enhance their catalytic activity.

Methods of pulse and continuous laser irradiation action on the tumor tissue containing cobalt phthalocyanine were developed. With the help of pulsed and continuous laser radiation one can also increase the cobalt phthalocyanine accumulation in tumors and enhance its catalytic activity.

## Conclusion

Physical action methods on the aggregates in the tumor cells *in vivo*: the electromagnetic action (current and alternating voltage) and photo action (continuous and pulsed irradiation in the absorption band of cobalt phthalocyanine) can be very promising as the additional method of selective influence.

**P2-20**

# Biocompatible carbon-coated 3-d metal nanocomposites for therapy of oncological deseases

Ryabova A.[1], Vasil'chenko S.[1], Grachev P.[1], Ermakov A.[2], Stratonnikov A.[1], Loschenov V.[1]
[1] *A.M. Prokhorov General Physics Institute RAS*
[2] *Institute of Metal Physics, Ural Division RAS*
*E-mail: nastya.ryabova@gmail.com*

## Purpose

Development of the pulsed laser phototherapy method with biocompatible carbon-covered 3-d metal nanocomposites.

## Materials & Methods

Carbon-coated nanoparticles of metals and metal oxides (Ag, Al, Fe, Ni, Co, Mg) covered with biopolymer are used.

Pharmacokinetic studies of the Me@C nanoparticles covered with biopolymer after systemic introduction to laboratory animals (mice BALB) was carried out by the optical spectroscopy.

Pulsed laser irradiation of the nanoparticles in the tumor over the skin was used (Ti-Sapphire laser, wavelength - 800 nm, pulse duration - 3 ps, repetition rate – 1 kHz, pulse energy – 2 mJ/cm$^2$, irradiation time -15 min, irradiation 4 days after nanoparticles input).

## Results

Pharmacokinetic studies of carbon-coated nanoparticles Ni, $Fe^2O^3$ and Al were carried out and the toxic effect of nanoparticles on the organism was evaluated. Owing to the presence of the carbon shells the nanoparticles have a high absorption coefficient in the visible and, most importantly, the red and near infrared spectral range where most biological tissues are transparent. This features allowed evaluating nanoparticles concentration in biotissues by measuring and analyzing of diffuse backscattering spectra.

The investigated nanoparticles showed low systemic toxicity at intravenous injection. The high selectivity of nanoparticles accumulation in the tumor was also revealed (Nanoparticles concentration in tumor is 6-7 times higher than that in the surrounding normal tissues one day after the intravenous injection of the nanoparticles). It was shown that the most promising model in terms of pharmacokinetic properties is carbon-coated iron nanoparticles. In studies on cell culture of mammary tumors (SkBr3 line) the highest cytotoxic activity was observed for iron nanoparticles. It was also shown that concentration dependence for aluminum nanoparticles of the growth suppression was the lowest and for the iron nanoparticles - the most pronounced.

The good therapeutic effect was obtained when pulsed laser irradiating the tumor tissue containing nanoparticles.

## Conclusion

The preliminary investigations on the application of biocompatible carbon-covered 3-d metal nanocomposites for diagnostics and therapy of oncological diseases were conducted. The investigated metal-carbon systems have shown the low systemic toxicity at intravenous introduction, the high selectivity of accumulation in tumor at fictionalization of the special biopolymers, the high therapeutic efficiency of tumors treatment in experimental animals with the use of lasers with short pulse duration for tumors photothermolysis.

**P3-21**

# Optical micro-imaging and spectroscopy of individual nanoparticles

**Jan Valenta and Anna Fucikova**

Department of Chemical Physics & Optics, Faculty of Mathematics & Physics,
Charles University, Ke Karlovu 3, CZ-121 16 Prague 2, Czech R.,
e-mail: jan.valenta@mff.cuni.cz

Optical spectroscopy can be applied to study individual nanoparticles in spite of poor resolution of the optical imaging. The key features of such experiments are (i) specially prepared samples with low concentration of optically addressed objects and very low background signal (at the spectral region of nanoparticle emission), (ii) high quality of optical imaging and optimized detection efficiency [1]. Sometimes, it is necessary to enclose sample in a chamber with optically thick windows, e.g. a cryostat, incubation chamber or pressure cell. Even such task can be solved by inserting optics inside a chamber or use a special optics outside [2]. Special micro-spectroscopy set-ups have been built by the authors and applied to study semiconductor nanocrystals [3], nanowires [4] photonic structures, cells etc. Several phenomena like luminescence fine-spectral structure, polarization, spectral diffusion and ON-OFF intermittency are unique features of single nano-object detection and cannot be seen in ensemble measurements. Moreover, surprising variability of single nano-object properties reflects "individuality" of each object caused by variations in size, shape, surface passivation, surrounding environment etc. The study of individual properties and their statistical distribution by means of optical micro-spectroscopy is extremely important for future applications of semiconductor nanostructures in e.g. bio-labeling or optoelectronic devices.

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**P3-22**

## Nanoparticle characterization by using Tilted Microscopy Techniques

D. Salerno, D. Brogioli, V. Cassina, F. Mantegazza

Dipartimento di Medicina Sperimentale,, Universita' di Milano - Bicocca, via Cadore 48, Monza (Milano), 20052 Italy

By using scattering in near field techniques, a microscope can be easily turned into a very useful device for the characterization of nanoparticle dispersions measuring static and dynamic light scattering. Usually, microscopy based techniques have been limited to forward scattering, up to a maximum of 30°. We present a novel optical scheme that overcomes this limitation, extending the detection range to angles larger than 90° (back-scattering). Our optical scheme is based on a microscope, a wide numerical aperture objective, and a laser illumination, with the collimated beam positioned at a large angle with respect to the optical axis of the objective (Tilted Laser Microscopy, TLM). We tested our instrument and our calculations with calibrated spherical and BONSAI nanoparticles, performing static and dynamic scattering measurements up to 110°. The measured static spectra and decay times are compatible with the Mie theory and the diffusion coefficients provided by the Stokes-Einstein equation.

**P3-23**

# Confocal microscopy characterization of light-emitting nanostructures and X-ray imaging detectors based on color centers in lithium fluoride

F. Bonfigli[1], S. Almaviva[1] and R. M. Montereali[1]

[1]*ENEA, C.R. Frascati, Via E. Fermi 45, 00044 Frascati (Rome) Italy*

Confocal Laser Scanning Microscope (CLSM) is a versatile and powerful optical instrument which is gaining a strong increase of interest in the scientific framework for biological investigations and also for the characterization of materials, microstructures and devices. The main reason that contributed to its popularity is the ability to operate an optical sectioning and to obtain tri-dimensional reconstruction of a great variety of biological and non-biological samples with sub-micrometric resolution. We present a characterization of light-emitting micro and nano-structures based on color centers in lithium fluoride (LiF) by using a CLSM system. These optical structures were obtained by irradiating LiF crystals and films with low penetrating ionizing radiation in different lithographic apparata. LiF material was also successfully proposed and used to realize innovative and versatile X-ray imaging detectors for biological applications, material characterizations and X-ray source diagnostics [1]. The peculiarities of these bi-dimensional LiF-based imaging detectors are the intrinsic high spatial resolution, in principle limited only by the point defect size, the large field of view, the wide dynamic range and the optical reading of color centers photoluminescence. CLSM was successfully used as an advanced optical system to detect X-ray micro-radiographies of biological specimens and nano-metric structures stored in LiF detectors [2]. The peculiarities of a CLSM system operating in fluorescence mode as optical reading instrument of LiF-based imaging detectors and for the characterization of light-emitting devices will be discussed.

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**P3-24**

## Confocal spectroscopy and luminescence decay lifetime imaging of single semiconductor nanoparticles

Alexey I. Chizhik[1], Anna M. Chizhik[1], Torsten Schmidt[2], Alfred J. Meixner[1], Friedrich Huisken[2]
[1] – Institute of Physical and Theoretical Chemistry, University of Tuebingen, Auf der Morgenstelle 8, D-72076, Germany.
[2] – Laboratory Astrophysics Group of the Max Planck Institute for Astronomy at the Institute of Solid State Physics, University of Jena, Helmholtzweg 3, D-07743 Jena, Germany.

The central scope of our work is spectral characterization of single semiconductor nanoparticles (NPs). We have investigated single $SiO_2$ NPs, as well as $Si/SiO_2$ and CdSe/ZnS core-shell systems. Furthermore, we present a detailed comparison of the photoluminescence (PL) properties of hollow $SiO_2$ shells and $Si/SiO_2$ NPs which leads to a new insight in the relation between two possible origins of the PL in the core-shell system. This is one of the key issues for the characterization of the optical properties of non-direct semiconductor nanostructures.

Commercially available CdSe/ZnS quantum dots demonstrate the dependence between the size of the core and the emission wavelength. Single NP PL spectra clearly show that the emission originates from quantum confinement. $SiO_2$ NPs were obtained from silicon nanocrystals which were synthesized by $CO_2$ laser pyrolysis of $SiH_4$ by full oxidation in water [1]. Samples of $SiO_2$ NPs embedded at low concentration in a thin polymer layer were prepared by spin-coating a dedicated solution on quartz cover slides. PL spectroscopy of single $SiO_2$ NPs revealed spectra with a double-peak structure consisting of an intense narrow zero-phonon line and a broader phonon band [2,3]. We attribute the phonon band to longitudinal optical phonons excited in the $SiO_2$ network. At the same time the PL of $Si/SiO_2$ core-shell systems can originate from the surface states or quantum confinement. Comparing these systems allows us to characterize the parameters (such as local environment or size of the NP), influencing on the emission of silicon nanostructure. Determination of the decay lifetime of single NP plays an important role, since it gives us another parameter to distinguish between the signals originating from defect or quantum confinement in the case of $Si/SiO_2$ NPs, which are known to lie in nanosecond and microsecond ranges respectively. Thus the characterization of a broad number of optical emission properties of single semiconductor NPs allows us to specify the origin of the observed PL.

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**P3-25**

## Transmission electron microscopy of lipid nanostructures for bio-imaging

Valentina Bello*, Giovanni Mattei, Paolo Mazzoldi
*Department of Physics, University of Padova, via Marzolo 8, I-35131 Padova, Italy*

Nicoletta Vivenza, Paolo Gasco
*Nanovector S.r.l., via Livorno, 60, I-10144 Torino, Italy*

Jean Marc Idee, Caroline Robic
*Guerbet , 15 rue des Vanesses, Zone Paris Nord I, 93420 Villepinte (Paris),France*

Elisabetta Borsella
*ENEA, Dept. FIM, via E. Fermi 45, I-00044 Frascati (Rome), Italy*

Lipid-containing nanostructures, in form of solid lipid nanoparticles (SLN) or iron oxide nanoparticles (NPs) are systems of paramount importance as drug delivery systems or as bio-compatible contrast agents. We have performed structural and compositional characterization of these systems by transmission electron microscopy, optimizing the staining procedure. In particular we have treated the systems with a negative (Phospshotungstic acid) or with a positive (Osmium Tetroxide) staining agent. For the iron-oxide NPs coated by the lipid shell negative staining has revealed to be more efficient with respect to the positive one. Nevertheless, in particular cases the combination of the two staining procedures allows to gain a more complete information (morphological and compositional) on the investigated systems.

* Corresponding author. Tel.:+39.049.8277039; fax: +39.049.8277003. E-mail: bello@padova.infm.it

**P3-26**

# Plasmonic Nanoshell Antennas for Enhanced Sensing Bio-Labeling

Giovanni Pellegrini*, Giovanni Mattei and Paolo Mazzoldi
*CNISM, Department of Physics, University of Padova, Via Marzolo 8, I-35131 Padova, Italy*

## Purpose

Ideal fluorescent bio-labels share the common properties of easy synthesis, strong light matter interaction both in absorption and emission, and high bio-compatibility. Real world labels, such as semiconductor Quantum Dots (QDs) or organic fluorophores, hardly posses all the relevant features. For these reasons it would be of high interest to devise a general strategy to boost the label relevant properties. In this framework plasmonic nanoantennas, (i.e. devices that convert localized energy in free propagating radiation and vice versa) are proposed as a promising solution to enhance all the fundamental characteristics of a wide spectrum of bio-labels.

## Materials & Methods

An extension of Generalized Multiparticle Mie theory is employed to model the optical properties of a general bio-label embedded in a gold plasmonic nanoshell. All the relevant quantities, i.e. absorption and emission enhancements, are calculated as a function of label position, dipole moment orientation, and emission wavelength, and then compared to the ones of an isolated bio-label.

## Results

Electrodynamics calculations show that flexible absorption and emission enhancement is obtained at the nanoshell resonance wavelength. Antenna performances are robust against different label positions and dipole orientations, therefore providing an ideal context for real world applications. Furthermore Gold shells yield high bio-compatibility and facile bio-conjugation through thiols chemistry, along with the possibility of hybrid thermal treatments.

## Conclusions

Theoretical simulations show that plasmonic nanoshell antennas are a promising candidate to enhance the optical and bio-compatibility properties of a broad range of bio-labels, going from semiconductor QDs to fluorophores and rare-earth doped silica nanospheres.

* Corresponding author. Tel.:+39.049.8277039; fax: +39.049.8277003. E-mail: pellegrini@padova.infm.it

**P3-27**

# Aluminum Phthalocyanine Nanoparticles for Fluorescent Diagnostics in Dentistry and Skin Autotransplantology

Vasilchenko S., Volkova A., Ryabova A., Loschenov V.
*A.M. Prokhorov General Physics Institute RAS*
*E-mail: vas_serg@inbox.ru*

## Purpose

The work discusses the possibility of aluminum phthalocyanine nanophotosensitizer application in clinical practice: in dentistry for diagnostics of tooth enamel microdamages and in transplantology for skin autotransplants engraftment quality. AlPc fluoresces in the molecular form but in the form of nanoparticles it does not. Separation of molecules from an AlPc nanoparticle and therefore fluorescence build-up occurs under effect of a number of biochemical and physical factors. Owing to this feature the application of AlPc nanoparticles followed by the measurement of fluorescence spectra is offered as a diagnostics method.

## Materials & Methods

Large-dispersed water-insoluble AlPc was used as a starting material for making nanoparticles. A certain amount of AlPc was introduced into physiological solution and subjected to ultrasonic dispergation that resulted to formation of colloidal solution of the AlPc nanoparticles.
To take fluorescent images a system UFF-630-01-Biospec was used. For the quantitative fluorescence measurements a fiber-optic spectrometer LESA-01-Biospec was used.
For approbation of the fluorescence diagnostics method of tooth enamel microdamages with the use of the AlPc nanophotosensitizer human teeth of different groups were used. The teeth had been previously extracted by the reason of clinical indications.
The possibility of the AlPc nanophotosensitizers application for diagnostics of the skin autotransplants engraftment quality was studied in experiment on mice.

## Results

AlPc fluorescence build-up occurs in the enamel microdamage area after the nanoparticles application on tooth surface. It was shown that 15 min. after the AlPc nanoparticles application the fluorescence intensity in the microdamage area is 2-3 times higher than that in the normal enamel area.
AlPc fluorescence building-up after the nanoparticles application on skin autografts testifies to the risk of rejection.

## Conclusion

The possibility of application of fluorescence diagnostics of tooth enamel microdamages with the use of the AlPc nanoparticles was discovered. It was shown that the observed effects are size-depended that will probably allow one to carry out differential diagnostics of different dental diseases associated with enamel structure change by using nanoparticles of different sizes. Also taking into consideration the lethal effect of PDT on bacteria, viruses, fungi and other microorganisms it seems appropriate to use the proposed technique not only for detecting microdamages but also for their antiseptic treatment in the framework of therapeutic interventions.
The possibility of use of the AlPc nanoparticles for assessing skin autografts rejection risk was shown. It was established that when colloidal solution of the AlPc nanoparticles is applied to autograft separation of fluorescent AlPc monomolecules from the nanoparticles takes place both in the case of inflammatory rejection and practically in all stages of ischemic rejection of autograft. It was shown that the observed effects depend on the sizes of the used AlPc nanoparticles.

**P3-28**

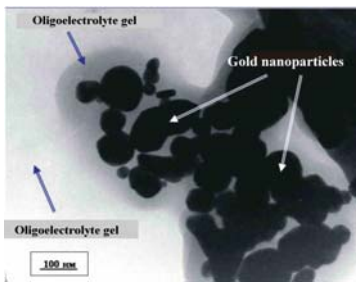
**Nanoscale magnetic, luminescent and plasmon detectable markers and drug delivery systems for cell recognition, labelling and treatment: oligoperoxide based synthesis**

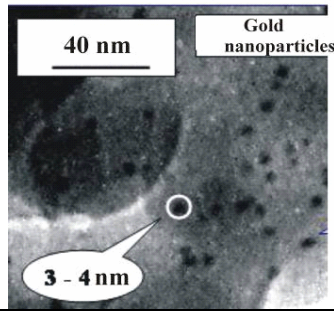
A. Zaichenko, N. Mitina, O. Shevchuk, O. Shapoval
Lviv Polytechnic National University, 12 S. Bandera Str., Lviv, 79013, Ukraine;

Molecular design of novel block, comb-like, and branched oligoperoxide based surfactants as well as derived coordinating complexes of rare earth metal cations for the synthesis of luminescent, colored, magnetic and other functional nanocomposites with controlled size distribution, functionality, and biocompatibility was studied.

Template synthesis of functional super paramagnetic (maghemite, magnetite), luminescent ($LaPO_4$, doped with Eu, Ce and other cations), plasmon detectable (Au, Ag) polymer – mineral nanoparticles in the presence of functional surfactants providing tailored particle size, functionality and reactivity was developed. Other approaches consisting in the encapsulation of organic luminophores (pyrazoline, fluorescein including anticancer drug doxorubicin, etc.) in functional polymeric nanoparticles or nanogels via water or organodispersion polymerization in the presence of oligoperoxide metal complexes as emulsifier, initiator and surface modifier simultaneously were developed and studied also.

Luminescent and plasmon resonance detectable functional polymeric and porous $SiO_2$ based nanogels of controlled size, hydrophily, and functionality were synthesized. Besides -COOH, $-SO_3H$, –SH, $-N(CH_3)_2$, -C (O) $NR_2$ ion forming groups, the developed nanocomposites contain desired amount of ditertiary peroxide fragments capable to form free radicals and initiate grafting functional reactive spacers of desired length for covalent attachment of cell recognizing vectors of natural origin (lectins, antibodies etc.). Composite nanoparticles comprising of $SiO_2$, $Fe_3O_4$, $Fe_2O_3$, Au core and functional polymeric nanogel shell were synthesized via polymerization of isopropyl acryl amide, dimethyl amino ethyl methacrylate and other functional monomers initiated from the nanoparticle surface. The polymeric nanogels (Fig. 1) as well as nanogel shell on the surface of polymer-mineral $SiO_2$ particles (Fig. 2) were filled with gold nanoparticles of controlled size range as well as with antimicrobial or anticancer drugs including water insoluble ones. These composite polymer-mineral nanogels are characterized by absorbance spectrum in near infrared field and capable to two photon resonance excitation. Functional polyelectrolyte and hybrid nanoscale gels that can bind specific proteins and interact with cell membrane, particularly with the apoptotic cells, bacteria and fungi were successfully examined as magnetic, luminescent and plasmon resonance capable markers and drug carriers *in vitro*.




Novel functional nanoparticles are studied by chemical, colloidal-chemical, and rheological methods, X-ray diffraction technique, luminescent spectroscopy, transmission and scanning electronic microscopy.

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e-mail: zaichenk@polynet.lviv.ua

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