BIODIAGNOSTICS

SIXTH FRAMEWORK PROGRAMME

Contract: NMP4-CT-2005-017002

BIODIAGNOSTICS

Biological diagnostic tools using microsystems and supersensitive magnetic detection

Specific Targeted Research Project
PRIORITY 3 – NMP
Nanotechnologies and nano-sciences, knowledge-based multifunctional materials and new production processes and devices
Bio-sensors for Diagnosis and Healthcare

Twelve Months Publishable Executive Summary

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Dag Winkler
Chalmers University of Technology

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1. Executive summary

1.1. Summary description of project objectives

The objective of the proposed project is to develop new medical diagnostic tools based on the most sensitive detector technologies available today. A common denominator for the technologies is reading out the biological state in the magnetic domain. This will be accomplished by using both magnetic nanoparticles as substrates or tags for biological reactions and reading out the atomic nuclear spin. The latter technology is a development from the inductive readout in Nuclear Magnetic Resonance (NMR) and Magnetic Resonance Imaging (MRI). The technology used in this project is a pure magnetic field read-out system, which quite contrary to the inductive method has a sensitivity which is more or less independent of frequency and magnetic field down to very low fields. The several competing technologies will be benchmarked against each other with a model system consisting of biotin and streptavidine in order to gain a unique understanding of the differences and advantages with the different systems. To validate the concept(s), real diagnostic test will be done on in vitro detection of F1 antigen (y. pestis) Mycobacterium tuberculosis in urine samples and detection of Yersinia pestis F1 antigen in environmental samples (bio terrorism). Further to the validation, neuroimaging and instrumentation for diagnosis of neurological diseases will be investigated in vivo.

1.2. Contractors involved

List of participants:

1.	Chalmers University of Technology	Sweden
2.	Imego AB	Sweden
3.	Karolinska Institute	Sweden
4.	The Royal Institute of Technology	Sweden
5.	Forschungzentrum Juelich	Germany
6.	Juelicher SQUID GmbH	Germany
7.	Senova GmbH, Jena	Germany
8.	Physikalische Technische Bundesanstalt	Germany
9.	Chemicell GmbH	Germany
10.	Friedrich-Schiller-Universität Jena	Germany
11.	Micro - Sphere S.A.	Switzerland
12.	Royal Holloway University London	Great Britain
13.	MIC/DTU	Denmark

1.3. Co-ordinator contact details

Co-ordinator name: Dag Winkler
Co-ordinator organisation name: Chalmers University of Technology
e-mail: dag.winkler@mc2.chalmers.se
fax: +46 31 772 3471

1.4. Work performed and results achieved so far

Chalmers University of Technology

The microstructure of Chemicell and KTH particles has been determined using both scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

A system has been built around a high-T_c SQUID magnetometer with a white noise of 0.75 pT/Hz$^{1/2}$. In this system, successful magnetophysiology measurements on brain slices have previously been performed. The system is being retrofitted to suit the magnetic nanoparticle measurements. Another cryostat is being tested and will be wired up shortly.

Numerical simulations of the motion of magnetic nanoparticles in a magnetic field gradient including the Brownian motion have been performed. Particle density as a function of time has been detected in optical measurements to verify the numerical simulations.

IMEGO

Imego has developed an integrated dynamic susceptometer for magnetic nanoparticle characterization. The instrument uses induction techniques in order to detect the dynamic magnetic susceptibility. System software governs the whole experimental set up and the collection of the data and presentation of the result.

Karolinska Institute

Initial studies have been performed to examine the contrast enhancing properties of superparamagnetic iron oxide nanoparticles in solution and in dispersed in agar gel and optimize the conditions for MRI recordings performed by a 4.7 Tesla magnet.

Recently, they have started to test the properties of different batches of fluidMAG nanoparticles infused intravenously into the rat. The attenuation of T2 relaxation signals in the whole brain during the initial 40 min after the nanoparticle infusion was clearly visible.

The Royal Institute of Technology

KTH have prepared samples of iron oxide nanoparticles by co-precipitation method.

A new synthesis technique was optimized in order to obtain thermally blocked iron oxide nanoparticles with an average particle diameter of 17 nm. A narrow particle size distribution, with an average particle diameter of 17 nm, was obtained.

KTH has been involved in the characterization of the synthesized nanoparticles with several techniques in respect of their crystal structure, chemical composition, size, morphology and size distribution. XRD, photon correlation spectroscopy, TEM, SEM and XPS are some of the analysis that were performed in order to characterize the prepared materials.

Forschungzentrum Juelich

Development of a HTS rf SQUID instrument with a microscopic HTS rf SQUID sensor configuration in gradiometric mode for readout of immunoassays based on coated magnetic nanoparticles is ongoing.

In order to selectively improve the ac-field sensitivity of a HTS SQUID system, a *LC* resonant circuit has been developed as a single-loop flux transformer. With the *LC* circuit, an equivalent magnetic field sensitivity of the HTS SQUID of 2.5 fT/$\sqrt{\text{Hz}}$ at 88 kHz was achieved.

Planar gradiometers with a single step-edge Josephson junction fabricated by epitaxial laser-deposited YBCO on single-crystal $LaAlO_3$ substrates have been chosen as suitable sensors for the magnetic particle detection. Several gradiometers with different baseline lengths have been fabricated.

A novel concept for sample handling is currently under development. It involves a bent glass capillary, which is guided into the vacuum space in close proximity to the SQUID.

Juelicher SQUID

The project work conducted thus far includes the development of an rf SQUID readout electronics with special emphasis on a fast triggered reset and a fast feedback loop. The assembly of a first version of the rf SQUID electronics is currently in progress.

Software development for user control of the setting of the voltage controlled oscillator (VCO), the attenuator (VCA), and the integrator offset has been started.

Senova

SENOVA GmbH has successfully bound streptavidin to filter material using various covalent binding strategies. The most successful method for binding is achieved when protein is coupled to filter material by the EDC-method.

It has been found that the magnetic detection of the Chemicell magnetic beads is superior to those supplied by Chemagen. Chemicell beads have a diameter of ~100nm whereas Chemagen beads range between 0.5-1.0µm in size.

Physikalische Technische Bundesanstalt

Magnetic nanoparticle preparations made by Chemicell have been characterized using a commercial susceptometry measurement system and the homemade PTB magnetorelaxometry (MRX) system. MRX has been employed to assess the binding kinetics of functionalized magnetic nanoparticles.

PTB has made extensive investigations on the improvement of low-T_c SQUID sensor designs for magnetic detection techniques. A white flux noise density of 0.6 $\mu\Phi_0/\sqrt{Hz}$ @ 4.2 K and a corresponding current noise of 1.2 $pA/\sqrt{Hz}$ have been demonstrated.

For NMR applications PTB is developing a series of SQUID current detectors which is robust and easy to handle. A very high sensitivity and bandwidth can be achieved by using SQUID arrays and two stage SQUID configurations.

Chemicell

Chemicell has developed for and supplied magnetic nanoparticles to the following partners: a) Imego AB / Sweden, b) Karolinska Institute / Sweden, c) Physikalische Technische Bundesanstalt (PTB) / Germany, d) MIC/DTU / Denmark.

Friedrich-Schiller-Universität Jena

The work of FSU Jena is focused on temperature-dependent Magnetorelaxometry (MRX). Magnetic nanoparticles were characterized by measurements of the Néel relaxation signal. Their volume and size distribution have been found to be in agreement with the values determined by TEM or AFM.

An anticryostat was recently built with a design to minimize the distance between the SQUID gradiometer operating at 4.2 K and the sample holder which is at temperatures between 4.2 K and 350 K.

Phantom measurements have been performed using eight different columns with immobilized magnetic particles from SENOVA. The columns were measured with the 195 channel vector magnetometer system at the Biomagnetic Centre Jena. The dipoles of the magnetic beads could be properly located.

Micro-Sphere

Particles with sizes around 100 nm and very narrow distribution have been realised after several synthesis trials. A first trial to perform lipid encapsulation/modification of magnetic particles has been performed.

Royal Holloway University London

Measurements have been carried out on a water sample of volume ~ 0.1 ml. Signals have been observed down to 93 nT, corresponding to a Larmor frequency of 4 Hz. A systematic study of the frequency dependence of the linewidth has been carried out. They have measured the longitudinal relaxation time T_1 in water at room temperature using two different methods.

A magnetically screened enclosure has been purchased to house a new helium Dewar with a small warm-cold distance for SQUID NMR/MRI measurements on room temperature samples.

MIC

The work has focused on systematic theoretical studies of the magnetic field from bead assemblies (D3.6) and of the sensor performance and optimization (D3.7). The first batch of wafers with sensors for a systematic experimental study has been fabricated at INESC-MN in Portugal. A deposition system for in-house sensor fabrication has been purchased for an instrument grant from the Danish Research Councils and will be installed at MIC, DTU during the Spring/Summer 2007.

1.4.1. Deliverables during the first twelve months

D1.1: Applications, material and magnetic detection requirements; D2.1: Report on stable suspension of uncoated magnetic nanoparticles with narrow size distribution; D3.6: Simulations and modelling of nanobeads fringe fields and sensor geometry; D3.7: Planar Hall sensor design; D5.1: Microfludic system definition; D5.2: Technology selection for SQUID encapsulation; D5.3: Development processes of MEMS technology for microfluidic system; D5.6: LTS SQUID gradiometer for unshielded spatial resolved MRX; D6.7: System definition and evaluation of the MRI system; D6.9: Demonstration of feasibility of magnetic nanoparticles for MRI imaging; D8.1: Project presentation; D8.2: Dissemination and use plan

1.5. Expected end results, intentions for use and impact

The technologies that will be developed will be exploited across a range of carefully chosen applications. Of particular importance are NMR and MRI as the programme will aim to enhance this field in several directions. The work on tailored magnetic nanoparticles will allow the development of new target specific contrast agents for NMR and MRI.

SQUID systems will also be used to provide low noise readout schemes for low-frequency broadband NMR, and MRI, which can be applied in lower magnetic fields and with improved spatial resolution.

Instrumentation based on SQUID sensors, which senses the dynamics of functionalized magnetic nanoparticles will be built to read out immunoassay arrays, and to detect the transport of magnetic nanoparticles *in vivo*. The SQUID-packaging and the liquid sample handling will be built from MEMS circuits, where the interfacing between the SQUID sensor and the specimen will yield ultimate readout properties. The project will develop and use the strong SQUID sensor development in Europe based both on low and high transition temperature superconductors (LTS and HTS) depending on the application. In new configurations the SQUID magnetometer or gradiometer will serve as readout for the dynamics of magnetic nanoparticles dressed with e.g. antibodies, which will either be immobilized through a reaction to a pre-patterned surface or have a change in their hydrodynamic volume from the reaction. Since the immobilized or the reacted particles have different magnetic dynamics compared to the unreacted particles they can be detected in the same volume, which is impossible with most other techniques. Studies have shown a detection improvement of an order of magnitude compared to the commonly used ELISA, and predictions point to two or possibly three orders of magnitude in improvement. The studies could be performed *in vitro* or *in vivo*. The program will make advances in four major scientific directions, which combined will initiate studies in cross-disciplinary fields between biophysics, medicine, electronics, and motivate fundamental studies in nanoscience. The combination of these research fields together with the end user perspectives will ensure the relevance of the application as well as utilize top ranked expertise in each different field in order to achieve the individual and cross-disciplinary goals.