

Annual report 2015 SSF FLU-ID

Project goals: The purpose of this interdisciplinary project is the development of a low-cost and portable nano-diagnostics unit, which will provide an efficient analytical platform for rapid detection of pandemic influenza directly at the point of care. Summary of work packages:

1. RCA and Assay development
2. Detector development
3. Lab on a chip development (start 6/2015)
4. Integration and benchmarking (start 2/2016)

The FLU-ID partner contract has been approved by all partners and formally registered. The consortium collaborated significantly in the report period, and a joint publication, to which all partner groups contributed, was submitted.

Activities and Results in 2015:

Uppsala University, Work package 1: Magnetic Nucleic Acid- (NA-) assay development (JA, MN, MS, Year Y1-Y5, Milestones M1,3,4)

1) We have investigated possibilities for protocol simplifications for a DNA biodetection principle relying on hybridization of magnetic nanobeads to rolling circle amplification (RCA) products. It was found that beads could be present during amplification without noticeably interfering with the enzyme used for RCA (ϕ 29 polymerase). As a result, the bead-coil hybridization could be performed immediately after amplification in a one-step manner at elevated temperature within a few minutes prior to read-out in an AC susceptometer setup. Moreover, by recording the phase angle $\xi = \arctan(\chi''/\chi')$, where χ' and χ'' are the in-phase and out-of-phase components of the AC susceptibility, respectively, normalization of AC susceptibility data is not needed.

2) An optomagnetic method for rapid and cost-efficient qualitative biplex detection of bacterial DNA sequences has been demonstrated. Within less than two hours, the assay gives an answer to whether none, both, or only one of the bacterial DNA sequences is present in the sample. The assay relies on hybridization of oligonucleotide-functionalized magnetic nanobeads of two different sizes to RCA products originating from two different bacterial targets. The different bead sizes are equipped with different oligonucleotide probes, complementary to only one of the RCA products, and the read-out is carried out in the same sample volume. In an optomagnetic setup, the frequency modulation of transmitted laser light in response to an applied AC magnetic field is measured.

3) Detection of a *Vibrio cholerae* DNA-sequence using an optomagnetic read-out exploiting the dynamic behavior of magnetic nanobeads along with two turn-on data analysis approaches has been demonstrated. The optomagnetic method uses a weak uniaxial AC magnetic field of varying frequency applied perpendicular to the optical

path and measures the modulation of laser light passing through a cuvette containing the sample with oligonucleotide-tagged magnetic beads and macromolecular coils of single-stranded DNA. The presence of target gives rise to a change of the 2nd harmonic component, $V_2 = V_2(') + iV_2('')$, of the transmitted light. We demonstrated that by using the phase angle ξ defined as $\xi = \arctan V_2(') / V_2(')$ in the low-frequency region we obtain a LOD of 10pM for an RCA time of only 20min corresponding to a total assay time of 60min. Moreover, we showed that the approach based on ξ is significantly more robust than the analysis based on a turn-off of the signal due to free magnetic nanobeads used in previous work where a limit of detection of 10pM was obtained for an RCA time of 60min.

4) We have presented a measurement and data analysis procedure reducing the read-out time for the volume-amplified magnetic nanobead detection assay from ~30 min to only 2 min, providing fast, sensitive detection of DNA molecules. The molecular detection and amplification protocol was verified using samples containing rolling circle-amplified DNA products formed from synthetic *Vibrio cholerae* target DNA, with a limit of detection of 5 pM. The developed read-out method could be used to rapidly identify pathogens in a variety of applications including target screening in hospitals with limited resources, in out-patient settings and in the field. Work Submitted!

5) We have optimized an optomagnetic bioassay strategy utilizing 100 nm functionalized magnetic nanoparticles in order to obtain higher detection sensitivity for DNA molecules and bacteria. Measurements at low MNP concentrations require adjustments of the photodetector sensitivity to avoid saturation as well as a careful blocking of the sample container. We showed that an equivalent effect is obtained by including larger target-nonspecific MNPs with a diameter of 250 nm and that the concentration of 100 nm MNPs could be significantly reduced when adding 250 nm beads. Under optimized conditions, a LOD of 780 fM of DNA coils and 105 CFU/mL of *Salmonella* cells was achieved. Work submitted!

6) Earlier reported initial studies were continued and completed:

- Demonstration of singleplex detection of bacterial DNA sequences in a portable AC susceptometer with low picomolar concentrations.
- Stability studies in 4°C and RT of oligofunctionalized nanoparticles.
- Quantification of DNA coils formed by rolling circle amplification on-chip by Brownian relaxation measurements on magnetic nanobeads.

Publications in 2015/Q1 2016

Journals

Magnetic nanobeads present during enzymatic amplification and labeling for a simplified DNA detection protocol based on AC susceptometry.* R.S. Bejhed, M. Strømme, P. Svedlindh, A. Ahlford, M. Strömberg, *AIP Advances* **5 (2015) 127139.

Optomagnetic bioassay for qualitative bplex detection of bacterial DNA sequences.* R. Bejhed, T. Zardán Gómez de la Torre, P. Svedlindh, M. Strömberg *Biotechnology Journal* **10 (2015) 469-472.

Turn-on optomagnetic bacterial DNA sequence detection using volume-amplified magnetic nanobeads.* R. Bejhed, T. Zardán Gómez de la Torre, M. Donolato, M. Hansen, P. Svedlindh, M. Strömberg. *Biosensors & bioelectronics* **66 (2015) 405-411.

Quantification of rolling circle amplified DNA using magnetic nanobeads and a Blu-ray optical pick-up unit, M. Donolato, P. Antunes, T. Zardán Gómez de la Torre, E.-T. Hwu, C.-H. Chen, R. Burger, G. Rizzi, F. Giacomo Bosco, M. Strømme, A. Boisen., M. Fougth Hansen, *Biosensors&Bioelectronics* **67** (2015) 649-655.

Novel readout method for molecular diagnostic assays based on optical measurements of magnetic nanobead dynamics, M. Donolato, P. Antunes, R. Bejhed, T. Zardán Gómez de la Torre, F. Østerberg, M. Strömberg, M. Nilsson, M. Strømme, P. Svedlindh, M. Hansen, P. Vavassori, *Analytical Chemistry* **87** (2015) 1622-1629.

On-chip detection of rolling circle amplified DNA molecules from Bacillus globigii spores and Vibrio cholera, F.W. Østerberg, G. Rizzi, M. Donolato, R. S. Bejhed, A. Mezger, M. Strömberg, M. Nilsson, M. Strømme, P.Svedlindh and M. F. Hansen, *Small* **10** (2014) 2877-2882.

A magnetic nanobead-based bioassay provides sensitive detection of single- and biplex bacterial DNA using a portable AC susceptometer, M. Strömberg, T. Zardán Gómez de la Torre, M. Nilsson, P. Svedlindh, M. Strømme, *Biotech Journal* **9** (2014) 137-145.

*Newly published since last status report

Conferences

Functional nanomaterials in Life Science and Energy Storage applications, M. Strømme, Joint Symposium Tokyo Tech – Uppsala University 16-17/9-2014, Uppsala. Section Joint Renewable Energy

FLU-ID: Development of a low-cost and portable nano-diagnostics unit for detection of pandemic influenza, A. Kalabukhov, A. Jesorka, A.-S. Velasco, D. Winkler, J. Schneiderman, C Johansson, J. Blomgren, A. Ahlford, M. Nilsson, J. Albert, T. Zardán Gómez de la Torre, M. Strømme, 16th Nordic-Baltic Conference on Biomedical Engineering and Medical Physics & 10th Medicinteknikdagarna, October 14-16, 2014, Göteborg, Sweden

Rapid detection of Vibrio Cholerae DNA molecules, T. Zardán Gómez de la Torre and M. Strømme, 5th International NanoMedicine Conference, July, Sydney

Molecular diagnostics based on magnetic nanobead clustering dynamics monitored using a Blu-ray optomagnetic readout system, M. Donolato, P. Antunes, R. S. Bejhed, T. Zardán Gómez de la Torre, A. Ahlford, M. Strömberg, M. Nilsson, M. Strømme, E. T. Hwu, C. Chen P. Svedlindh, A. Boisen, P. Vavassori, M.F. Hansen, International Conference on the Scientific and Clinical Applications of Magnetic Carriers 2014, 10th international meeting, Dresden

A magnetic biodetection using magnetic multi-core nanoparticles and RCA coils. C. Johansson, J. Blomgren, A. Sarwe, C. Jonasson, F. Ahrentorp, S. Sepehri, E. Eriksson, A.

Kalabukhov, A. Jesorka, D. Winkler, J. Schneiderman, M. Nilsson, J. Albert, T. Zardán Gómez de la Torre, M. Strømme. 2016 Applied Superconductivity Conference (ASC'16) 4-9 September 2016, Denver, USA (abstract accepted).

Stockholm University, Work package 1: Magnetic Nucleic Acid- (NA-) assay development (JA, MN, MS, Year Y1-Y5, Milestones M1,3,4)

SU is mainly engaged in developing probes and amplification systems for specific and sensitive detection of Influenza virus (WP1), and contribute to chip integration (WP3). The challenges are sample preparation, sensitivity, and assay complexity for integration.

Progress during 2015 towards these challenges:

1) We have developed a new design approach targeting multiple sites of the viral genomes all at once and accumulate all successful detection events for increased signal from individual viral particles.

2) We have investigated different pre-amplification procedures, but will most likely target RNA directly to reduce assay complexity.

3) We have developed a new protocol that allows for efficient and specific probing directly on RNA, circumventing the cDNA synthesis step that currently adds assay complexity and inefficiency. We are investigating whether this approach can be patented.

4) In other projects (IMI-project RAPP-ID and FP7 project DIATOOLS), we have developed automated systems for sample preparation and probing, which we can learn from. One of the main lessons learnt is that we will gain a lot by reducing assay complexity!

5) We have developed a new approach for increased sensitivity of magnetic nanoparticle readout in collaboration with FLU-ID associated partner Mikkel F. Hansen at DTU, published in ACS Nano.

6) SU and KI have formed a work group on nucleic acid assay design, which has had close and regular interactions over email and face-to-face meetings. The work commenced earlier in the project has been continued and completed:

- Development of novel strategies for sample preparation and capture.
- Simplifications of molecular protocol, including enrichment of RNA by different capture approaches, detection of up to 10 influenza target sequences, increase of useable fraction of the extracted patient sample, pre-amplification of the RNA, using different approaches, direct probing of RNA
- Magnetic nano particle optimizations by means of real-time labelling
- Development of microfluidic devices for RCA protocol automation

Publications in 2015/Q1 2016

Journals

Scalable DNA-Based Magnetic Nanoparticle Agglutination Assay for Bacterial Detection in Patient Samples.* Mezger, A., Fock, J., Antunes, P., Osterberg, F.W., Boisen, A., Nilsson, M., Hansen, M.F., Ahlford, A. & Donolato, M. ACS Nano **9, 7374-7382 (2015).

Karolinska Work package 1: Magnetic Nucleic Acid- (NA-) assay development (JA, MN, MS, Year Y1-Y5, Milestones M1,3,4)

The PI continued the earlier initiated collaboration with the partner at Stockholm U. and the Dept of Clinical Microbiology at the Karolinska University Hospital. The latter provides reference materials, clinical samples and current routine influenza assays for development and benchmarking of the FLU-ID test. Through a contract between KI and Clinical Microbiology the FLU-ID has been carried out through part time work of one molecular biologist (Malin Grabbe) and one technician (Binnaz Acar) who are employed at Clinical Microbiology. However, Malin Grabbe has been on maternity leave during part of 2015 and Binnaz Acar left her position in the fall of 2015. To fill these gaps Eva Eriksson, also employed at the hospital, has worked part time in FLU-ID during the fall.

We perform nucleic acid assay design in WP1 and testing and have further initiated support of benchmarking in WP4. During this reporting period we have:

- 1) Generated large batched stocks of four relevant influenza strains. The four strains were kindly provided from the Public Health Agency of Sweden (FoHM) and represent the major circulating variants in the season 2014/2015, i.e. influenza A H1N1, A H3N2, B Yamagata and B Victoria.
- 2) Quantified the virus levels in the four virus stocks. This has been done by limiting dilution experiments using the commercial Simplexa influenza assay and Poisson distribution calculations.
- 3) Determined the analytical sensitivity of the Simplexa influenza assay which is our target sensitivity for the FLU-ID assay.
- 4) Worked closely with Mats Nilsson's group on assay design, i.e. sample handling and preparation, primer/probe design, amplification set-up, etc.
- 5) Earlier reported work on development of sampling protocols and materials, as well as improving test sensitivity have been continued and completed.

Publications

Conferences

A magnetic biodetection using magnetic multi-core nanoparticles and RCA coils. C. Johansson, J. Blomgren, A. Sarwe, C. Jonasson, F. Ahrentorp, S. Sepehri, E. Eriksson, A. Kalabukhov, A. Jesorka, D. Winkler, J. Schneiderman, M. Nilsson, J. Albert, T. Zardán Gómez de la Torre, M. Strømme. 2016 Applied Superconductivity Conference (ASC'16) 4-9 September 2016, Denver, USA (abstract accepted).

Chalmers University of Technology

Detection - Work Package 2: Detector development (DW, CJ, Y1-Y5, M2,3)

1) Successful operation of high- T_c SQUID gradiometers in an open-cycle, two-stage MEMS-based Joule-Thomson micro-cooler with a base temperature of 75 K was demonstrated. The micro-cooler system is based on a commercial desktop CryoLab from Kryoz Technologies BV. We have performed measurements of the SQUID noise with the SQUID gradiometer placed on the cold stage of the micro-cooler inside a magnetically shielded room (MSR) and did not observe a significant contribution to the magnetic flux noise as compared with one recorded in a stand-alone liquid-nitrogen cryostat. Our results prove that a MEMS micro-cooler system can be successfully utilized for cooling the high- T_c SQUIDs in a prototype of point-of-care molecular bio-diagnostics system.

2) Theoretical simulations of optimal microchannel geometry have been performed as a function of channel shape, distance and relative position to the SQUID. The simulations are based on spatial dependency of the SQUIDs sensitivity to isolated magnetic dipoles placed in relatively small distance to it. The model is an idealized system which is composed of a rectangular SQUID loop of width a and calculates the magnetic flux threading the loop from every individual magnetic nanoparticles randomly distributed in the sample solution. [DW1] The results suggest that for different heights from the SQUID there is an optimum loop area to collect the maximum flux. It also calculates the optimum geometry of the ferrofluid to maximize the magnetic flux threading the SQUID loop.

3) Dilution experiments of non-functionalized magnetic nanoparticles (Micromod BHF Starch 80 nm) were performed in our AC susceptibility measurement setup using a high- T_c dc SQUID gradiometer. Microfluidic channels of about 3 μ l volume were fabricated in collaboration with the Physical Chemistry group at Chalmers (Aldo Jesorka). The channels were used to handle magnetic nanoparticles in the AC susceptibility measurement setup. From the dependence of the signal amplitude on concentration of these magnetic nanoparticles, we were able to estimate equivalent sensitivity to iron content of our system to about 5×10^5 magnetic nanoparticles at 10 Hz.

4) For the first time, we have performed measurements of oligonucleotide-functionalized magnetic nanoparticles and binding reactions with RCA coils in our SQUID measurement setup. The measurements were performed using the same microfluidic channel. We observed a significant suppression of the signal at the resonance peak of the functionalized magnetic nanoparticles after they were hybridized with RCA coils of 500 and 50 pM in concentration, in excellent agreement with predicted result and benchmark experiments in the DynoMag system. We are currently performing measurements of binding reactions with various amounts of RCA coils to estimate sensitivity to the target molecules.

Dilution experiments and binding reactions with RCA coils were benchmarked in the DynoMag AC susceptibility system at Acreo Swedish ICT AB (Christer Johansson). RCA coil samples were prepared by Uppsala University (Maria Strömme, Teresa Zardán). To learn protocol of binding reactions, PhD student Sobhan Sepehri and MSc student Emil Eriksson visited Uppsala University for 2 days in February 2016.

Microfluidics - Work Package 3: Lab-on-a-chip and sample processing (DW, Y1-Y5, M2,4)

1) We developed a microfluidic test platform with sample size between 1-5 μl , which covers the volume region of highest sensitivity. A replica molding process was developed and microfluidic chip devices for delivery of magnetic particles to the SQUID test platform were fabricated. The devices were designed to optically cover the available detection area, and previously unreached sensitivities were achieved. A master thesis project joint between the microfluidics and detector development groups was coupled to this task.

2) The microfluidic concept of the sample handling unit was further developed, and hardware and software for a multichannel flow control unit were developed in collaboration with external partners. The flow controller is near completion, electronics circuits and software functions need to be integrated. Publication is anticipated after a patent application, following the previously reported patent application WO2014132139-A2.

3) We engaged in development and application testing of the open-volume platform (multifunctional pipette - Biopen). Testing was performed under various field operation conditions, in particular the combination with different probes and detection methods, was investigated. The results of the studies were published.

4) We continued the development of acoustofluidic devices based on Aluminum Nitride (ALN) MEMS resonators ($\sim 3\text{GHz}$) to fully replace the pressure based fluidics as well as the current generation of low frequency acoustofluidic devices. The new generation of chips combines several fluidic functions such as acoustic focusing and pump-less flow generation, particle focusing and others, which are expected to replace bulky peripheral instrumentation. This is crucial for the development of small, desk-top or portable devices. A specially outfitted sputter fabrication tool (Tool 428) has been remodeled for our purposes in the Nanofabrication Laboratory (NFL) at

MC2. In parallel, we evaluated the in-liquid performance of ALN-based chips, using devices fabricated by a collaborator. The results are submitted for journal publication, and were also accepted as conference proceedings.

5) Project parts earlier initiated were continued and completed.

- Developed of a new open-volume platform for sample collection and processing. The development was performed and concluded in collaboration with the Institute for Micromechanics at Stuttgart, Germany (Hahn-Schickard Society) and the Göteborg-based SME Fluicell AB.
- FEM simulations regarding valve performance and flow stability were performed

Undergraduate Education

Emil Eriksson is currently completing his 30p MSc project, in a joint project spanning the detector and microfluidics groups, on integration of microfluidic chip and SQUID system November 2015 - April 2016.

Publications

Journals

A Microfluidic Pipette for Studying Platelet Activation at the Single Platelet Level. Donati, Alessia; Kustanovich, Kiryl; Jeffries, Gavin; Jesorka, Aldo; Reviakine, Ilya*. Submitted to Analytical Chemistry, Manuscript ID: ac-2016-008363.

Ahemaiti, A.; Wigstrom, H.; Ainla, A.; Jeffries, G. D. M.; Orwar, O.; Jesorka, A.; Jardemark, K., Spatial characterization of a multifunctional pipette for drug delivery in hippocampal brain slices. *Journal of Neuroscience Methods* 2015, 241, 132-136;

Xu, S.; Ainla, A.; Jardemark, K.; Jesorka, A.; Jeffries, G. D. M., A Heating-Superfusion Platform Technology for the Investigation of Protein Function in Single Cells. *Analytical Chemistry* 2015, 87 (1), 381-387;

Xu, S.; Kim, A.; Jeffries, G. D. M.; Jesorka, A., A rapid microfluidic technique for integrated viability determination of adherent single cells. *Analytical and Bioanalytical Chemistry* 2015, 407 (5), 1295-1301.

Comparison of the In-liquid Performance of ALN-based Thin Film Electroacoustic Resonators. T. Mirea, V. Yantchev, J. Olivares, B. Díaz-Durán, M. Clement, A. Jesorka and Enrique Iborra. Submitted to *Sensors and Actuators B*, 2016.

Conferences

High coupling phononic SH-SAW resonators for in-liquid operation. V. Yantchev, T. Mirea, E. Iborra and A. Jesorka. in Proc. 2016 European Freq. and Time Forum (EFTF), York, UK (conference proceeding in Print).

A magnetic biodetection using magnetic multi-core nanoparticles and RCA coils. C. Johansson, J. Blomgren, A. Sarwe, C. Jonasson, F. Ahrentorp, S. Sepehri, E. Eriksson, A. Kalabukhov, A. Jesorka, D. Winkler, J. Schneiderman, M. Nilsson, J. Albert, T. Zardán Gómez de la Torre, M. Strømme. 2016 Applied Superconductivity Conference (ASC'16) 4-9 September 2016, Denver, USA (abstract accepted).

Acree Work Package 2: Detector development (DW, CJ, Y1-Y5, M2,3)

- 1) Made the initial system design for the induction coil detection system. We have identified suppliers of key components, such as capillary tubes, for later integration with the microchip.
- 2) Performed FEM simulations to study and optimize the induction coil design (shape and number of turns) for different sample geometries
- 3) Investigated alternative measurement routines in the DynoMag in order to increase the measurement speed without sacrificing too much of sensitivity
- 4) Designed and done initial tests of sample heating methods compatible with the DynoMag system. A sample heating system enables real-time monitoring and optimization of binding reactions at elevated temperatures, for instance binding of MNP to coils during rolling circle amplification. Two different concepts have been investigated; warm air flow through the coil system and using a detection coil frame made from Shapal / Aluminium Nitride, which is a good heat conducting ceramic.
- 5) Rebuilt DynoMag to handle a plug flow sample in a tube. Developed a setup and written software for plug flow sample handling and developed a routine to locate the optimal sample position inside the detection coils.
- 6) Developed a flow chart model for calculating the expected detection limit of the total system. The spreadsheet covers the MNP properties, the target molecule amplification process and MNP binding yield to calculate an expected susceptibility change of the sample. The susceptibility change is compared with the susceptibility resolution of the induction coil and SQUID measurement methods, to give an expected target molecule detection limit of the total system.
- 7) Developed a double relaxation model for the dynamic response in the case of a mix of free particles and particles bound to DNA coils.
- 8) Initial tests with different mixing ratios of functionalized magnetic nanoparticles and RCA targets and comparing the results of the dynamic magnetic measurements using the DynoMag system (Acree) and SQUID based system (Chalmers).

Publications

Conferences

A magnetic biodetection using magnetic multi-core nanoparticles and RCA coils. C. Johansson, J. Blomgren, A. Sarwe, C. Jonasson, F. Ahrentorp, S. Sepeshri, E. Eriksson, A. Kalabukhov, A. Jesorka, D. Winkler, J. Schneiderman, M. Nilsson, J. Albert, T. Zardán Gómez de la Torre, M. Strømme. 2016 Applied Superconductivity Conference (ASC'16) 4-9 September 2016, Denver, USA (abstract accepted).

Summary of project efforts:

The project is successfully pursuing the project goals on time, progress has been made on all tasks. No major delays have been encountered, and the activities are within the predicted financial framework. The milestone of having an optimized assay ready has been reached. 13 journal publication and 6 conference contributions have been achieved in the reporting period. Options for patent applications have been identified and are being pursued.