

## MAGNETO-OPTICAL RELAXATION OF FERROFLUIDS

C. Groß<sup>1</sup>, E. Romanus<sup>1,2</sup>, G. Glöckl<sup>1</sup>, P. Weber<sup>2</sup>, and W. Weitschies<sup>1</sup>

<sup>1</sup>*Institute of Pharmacy, Ernst-Moritz-Arndt-Universität Greifswald, Jahnstr.17, D-17487 Greifswald, Germany,* <sup>2</sup>*Institute of Solid State Physics, Friedrich-Schiller-Universität Jena, Helmholtzweg 5, D-07743 Jena, Germany*

**Introduction:** A variety of different methods for the determination of biological binding reactions are available due to the great interest in biology, biochemistry and medicine. One approach to the determination of biological binding reactions is based on the use of single domain magnetic nanoparticles (MNP) as signal generators conjugated with one of the components of the binding reaction [1-3]. The measured signal is the relaxation of the magnetization of the magnetic nanoparticles after switching off a magnetizing field. As the concentrations of the binding partners are usually very low, the measured magnetic relaxation signals are extremely weak. Therefore, the detection of such magnetic relaxation signals requires a sophisticated measurement setup based on superconducting quantum interference devices (SQUIDs) as the currently most sensitive, magnetic field sensors.

Ferrofluids become birefringent when a magnetic field is applied perpendicular to the optical axis of light impinging the fluid, as the magnetic nanoparticles contained in the ferrofluid tend to align in the direction of the external field. This causes an optical anisotropy (Cotton-Mouton-effect) [4]. After switching off the magnetizing field a relaxation of the optical birefringence can be observed due to Brownian motion. Recently, it has been demonstrated that magneto-optical relaxation measurements can be used for the determination of binding reactions of biological molecules attached to magnetic nanoparticles [5]. The aim of the present study was to evaluate this novel approach for the determination of biological binding reactions of antibody-conjugated MNP.

### **METHODS: Magnetic nanoparticles.**

Magnetic nanoparticles with a core of iron oxide (DDM 128N, Meito Sangyo, Japan) and a shell of carboxydextran were magnetically fractionated at 50 mT, as described elsewhere [6]. The coupling of streptavidin onto the nanoparticles was achieved by oxidation of the carboxydextran molecules on the surface of the nanoparticles and reaction of streptavidin with

the aldehyde groups formed during oxidation of the carboxydextran shell. Briefly, 15 mg sodium periodate were dissolved in a citrate/phosphate buffer (pH 5.0) and added to 2 ml of the magnetic nanoparticles. The mixture was incubated at 4°C for 40 min. Thereafter, the buffer was exchanged to 10 mM phosphate buffer pH 7.4 by size exclusion chromatography. Then, 2 mg of streptavidin were added. After 2 h incubation at 4°C dimethylborane (150 mM) and ethanolamine (0.5 M) were added. Finally, the magnetic nanoparticles were purified via magnetic separation and stored in 0.1 % BSA/PBS at 4°C. For the determination of the binding reaction between an antibody and its antigen the biotinylated antibody was attached to the nanoparticles via the binding between biotin and streptavidin.

**Measurement setup.** The measurement setup for the determination of the magneto-optical relaxation of ferrofluids (MORFF) consists of a laser (L), a polarizer (P), a  $\lambda/4$  plate (R), a cuvette (C) containing the sample, an analyzer (A) and a detector (D) mounted on an optical bench. Figure 1 shows a scheme of the measurement setup. The polarizer and analyzer are aligned orthogonally and at 45° to the magnetic field axis (or, more precisely, the axis of birefringence). The polarizer is parallel to the slow axis of the  $\lambda/4$  plate. The cuvette is placed into a magnetization coil generating a pulsed magnetic field with a magnetic flux density of 10 mT at a frequency of 20 Hz and with a duty cycle of 25 % corresponding to a magnetization time of 12.5 ms. The relaxation of the birefringence is recorded by a photodiode. The measurement runs continuously without any delay between magnetization and detection.

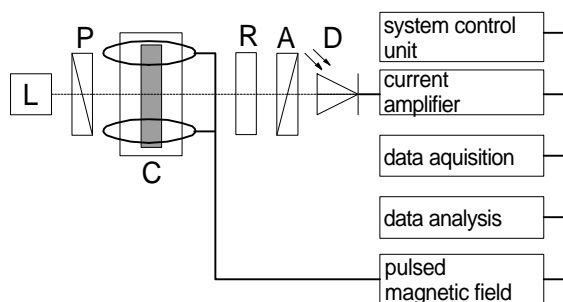


Fig. 1: Measurement setup for the detection of the magneto-optical relaxation in ferrofluids.

**Results:** The mean hydrodynamic particle diameters obtained by MORFF measurements for the binding experiments are shown in Figure 2. The data show that during the observed incubation time of 6 h the mean particle sizes of the samples incubated with 10 ng, 100 ng, 1  $\mu$ g and 10  $\mu$ g of hIgG are increasing. The maximum increase in particle size was found for an added amount of 1  $\mu$ g. For higher amounts of hIgG an increasing number of binding sites becomes saturated and, thus, cannot further contribute to the crosslinking.

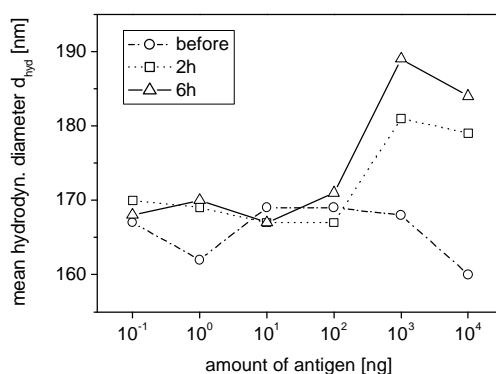


Fig. 2: Hydrodynamic particle diameters calculated from magneto-optical measurements before, 2 h and 6 h after addition of different amounts of antigen (hIgG) to magnetic nanoparticles conjugated with an antibody against hIgG.

**Discussion and Conclusions:** The presented experiments confirm that the determination of the relaxation of the transient field-induced birefringence of magnetic nanoparticles can be used as a novel tool for the investigation of

biological binding reactions, as long as these reactions result in an increase of the particle size of magnetic nanoparticles due to aggregation. Compared to magnetic nanoparticle relaxation measurements the separation of stimulation (magnetic) and signal detection (optical) is of great advantage, as the optical measurement system is comparatively simple, robust and compact. The application of optical measurements is restricted by optical properties of the sample due to scattering or absorption of the laser beam. Nonetheless, magneto-optical nanoparticle relaxation measurements seem very feasible for *in vitro* investigations of binding reactions.

**Acknowledgments:** This research project is supported by the Deutsche Forschungsgemeinschaft (DFG), No. WE 2555/2.

**References:** <sup>1</sup>Weitschies, R. Kötitz, T. Bunte, and L. Trahms (1997) *Determination of relaxing or remanent nanoparticle magnetization provides a novel binding-specific technique for the evaluation of immunoassays* Pharm. Pharmacol. Lett. **7**: 5-8. <sup>2</sup>R. Kötitz, H. Matz, L. Trahms, H. Koch, W. Weitschies, T. Rheinländer, W. Semmler, and T. Bunte (1997) *SQUID Based Remanence Measurements for Immunoassays* IEEE Trans. Appl. Supercond **7**: 3679-3681. <sup>3</sup>Y.R. Chemla, H.L. Grossman, Y. Poon, R. McDermott, R. Stevens, M.D. Alper, and J. Clarke (2000) *Ultrasensitive magnetic biosensor for homogeneous immunoassay* PNAS **97** 14268-14272. <sup>4</sup>A. Cotton, H. Mouton (1907) *Nouvelle propriété optique (biréfringence magnétique) de certains liquides organiques non colloïdaux* Comptes Rendus hebdomadaires des Séances de l'Académie des Sciences Paris **45**: 229-231 <sup>5</sup>E. Romanus, C. Groß, R. Kötitz, S. Prass, J. Lange, P. Weber, and W. Weitschies (2001) *Monitoring of biological binding reactions by magneto-optical relaxation measurement* Magnetohydrodynamics **3**: 328-333 <sup>6</sup>T. Rheinländer, J. Justiz, A. Haller, R. Kötitz, W. Weitschies, W. Semmler (1999) *Dynamic properties of fractions yielded by magnetic fractionation of magnetic fluids* IEEE Trans. Magn. **35**: 4055-4057