Introduction
Systemic Lupus Erythematous (SLE) is an autoimmune disease, where the detection of circulating auto-antibodies against ds-DNA is used as an important diagnosis criterion. However, current methods for detecting anti-ds-DNA antibodies in blood samples are primarily based on ELISA procedures utilizing surface chemistries (i.e. immobilization of antigen), multiple steps and cumbersome optimization. Here we present a simple and immobilization-free procedure, termed Flow-Induced Dispersion Analysis (FIDA), for detecting auto-antibodies against ds-DNA directly in 85% plasma samples.

FIDA is a new capillary-based technology for measuring in-solution binding under native conditions in complex solutions (e.g. plasma). FIDA is based on Taylor dispersion in a pressure-driven flow of a ligand (termed indicator, e.g. ds-DNA) interacting with the analyte of interest (e.g. anti-ds-DNA antibody). The indicator alone appears small (i.e. it has a small hydrodynamic radius) when it is not bound to the antibody, but upon binding it will appear larger (i.e. the complex has a larger hydrodynamic radius). The change in apparent size forms the basis for an accurate measure of analyte concentration and interaction.

Materials & Methods
488 nm Laser-Induced Fluorescence detection. FIDA-coated capillary (i.d.: 75 µm, L: 75 cm, Lcap: 65 cm).
100 mM phosphate buffer pH 7.9 was used as working buffer, three different ds-DNA sequences (32 bp) labelled with atto488 was used as indicators (50-100 nM), monoclonal antibody against ds-DNA (0-1000 nM) was spiked into 0-85% human plasma (healthy control) and used as analyte, 85% plasma from six SLE patients was used as analyte [1].

Sample analysis was performed by filling the capillary with an analyte zone, followed by injection of 26 nL indicator, which was mobilized towards the detector with analyte at 50 mbar.

Results
The apparent size (hydrodynamic radius) of the ds-DNA indicator was plotted as a function of antibody concentration (Figure 1) in 0, 20 and 85% healthy donor plasma respectively. The obtained dissociation constants (Kd) were 236 nM, 278 nM and 362 nM in 0, 20 and 85% plasma respectively, and thus correlated well.

Figure 1: Binding curves for the interaction between ds-DNA-atto488 and monoclonal anti-ds-DNA antibody in 0, 20 and 85% healthy donor plasma (adapted from [1]).

Plasma samples from six SLE patients were analysed by FIDA using three different DNA sequences (indicators), see Figure 2. A-samples (positive) and B-samples (negative) were tested with ELISA and CLIFT. FIDA positive samples were patient 2 and 3. Furthermore, antibody heterogeneity was observed and may be used for patient stratification.
Figure 2: FIDA analysis of six SLE patient samples [1].

References