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WHITE PAPER

# Sensitive protein quantification in whole blood, dried blood spots, serum and plasma

White paper 3 | Version 2 | Kim Stevens, Kris Ver Donck, Filip Delport
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## Abstract

Fiber-optic surface plasmon resonance (FO-SPR) is a powerful tool that harnesses the power of SPR in an easy-to-use fiber-optic sensor. In this white paper, we demonstrate the potential of FOx BIOSYSTEMS' FO-SPR instrument to detect proteins or antibodies in crude biological samples such as whole blood and dried blood spots. As an example system, we demonstrate the quantification of a therapeutic monoclonal antibody, infliximab (IFX), in whole blood, dried blood spots, serum and plasma.

A 10-minute FO-SPR bioassay has been developed that is capable of detecting IFX in 10 to 100-fold diluted whole blood, serum and plasma down to a limit of detection of 0.75 ng/ml. The detection limit falls well below the predicted ranges of typical IFX concentrations in patient blood and serum of 0.23–4.5 µg/ml and 0.5 – 10 µg/ml, respectively. For other targets, a lower detection limit may be reached but, for this example, the assay design did not require additional sensitivity.

To confirm this detection range, plasma, and serum from five IFX-treated IBD patients were tested with both the 10-minute FO-SPR assay and ELISA, showing excellent agreement between the two methods (Pearson correlations and intraclass correlation coefficients (ICC) greater than 0.99 in all cases). Furthermore, the bioassay developed here demonstrated that extraction from dried blood spots was 99% efficient, indicating that FO-SPR was not affected by the blood drying process.

Therefore, we have shown that, in just 10 minutes, a FO-SPR sandwich-style bioassay can provide accurate analysis of therapeutically relevant protein and antibody concentrations in crude samples with minimal processing, thus showing great potential as an R&D tool.

## Introduction

Crude biological samples often present challenges when quantifying biomolecules of interest during the research and development of diagnostics or therapeutics. The purification steps used to overcome these challenges can themselves introduce a bias in the experimental results, not to mention the extra hands-on time and cost. Therefore, a more direct detection method would be highly desirable.

In this white paper, we use infliximab (IFX) to demonstrate the direct detection of an antibody or protein in whole blood, dried blood spot, serum, and plasma samples. IFX is a therapeutic monoclonal antibody that is used to treat inflammatory bowel disease. Optimal dosing requires the analysis of levels in the patient's blood just prior to administration, therefore a fast and sensitive quantification method is crucial to optimize therapeutic outcomes while minimizing costs.

There are two main challenges for measuring IFX concentrations in whole blood:

1. Red blood cells and platelets typically rupture when handling blood samples, causing clotting and increased nonspecific absorption to the sensor surface that makes it more difficult to detect target molecules.

2. IFX concentrations in whole blood are expected to be in the range of 0.23–4.5 µg/ml and may require a more sensitive assay.

Capillary blood collected as dried blood spots (DBS) from a finger prick presents further difficulties over whole blood:

1. Red blood cells bursting after whole blood is dried can increase nonspecific interactions between the cell fragments and the sensor surface.

2. Structural alterations of analytes during the drying process can strongly reduce the extraction efficiency, inducing increased variation.

ELISA is a commonly used detection technique, but even with the development of a faster IFX-specific ELISA test (2), this still takes two hours and requires a well-equipped lab and is, therefore, not convenient for typical patient appointment times. High-pressure liquid chromatography (HPLC) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) are also frequently used.

Surface plasmon resonance (SPR) exploits electromagnetic waves to measure local refractive index changes due to immobilized biomolecules and analyte binding on the sensor surface. The most commonly used SPR biosensors are sensitive, reliable and precise, but often expensive since they typically use microfluidics and an optical path that require experts for operation and maintenance.

An adapted SPR technique using fiber optics (FO-SPR) has the advantage of using a dip-in sensor instead of microfluidics. FO-SPR has been shown to offer a sensitive, reliable, and precise real-time alternative to ELISA for quantifying IFX in serum samples from patients (3).

This white paper is an evaluation and demonstration of FO-SPR to measure antibodies (or other proteins) in whole blood, and even dried blood spots. This is a reliable method offering a fast time to result in a user-friendly hands-off operation, combined with a significant improvement in convenience for sample collection and preparation.

## Materials and Methods

### What is FO-SPR

FOx BIOSYSTEMS turned an optical fiber into a mass-sensitive sensor using the well-established surface plasmon resonance (SPR) principle for biomolecular interaction analysis.

The SPR effect is achieved by coupling a white light source to the fiber optic sensor probe. In this consumable probe, the light interacts with a gold layer and senses the refractive index up to 200 nm away from the outer surface. At the end of the probe, light is reflected back through the bifurcated fiber to a spectrometer. The resonance condition is monitored by tracking the wavelength at which the least light is reflected.

The fiber-optic surface plasmon resonance (FO-SPR) sensor is coated with bioreceptor molecules which can bind to the target molecules of interest. This binding changes the refractive index, resulting in a wavelength shift that produces a sensor signal as shown in figure 1. The sensor probe is simply dipped into the liquid sample to measure biomolecular interactions directly.

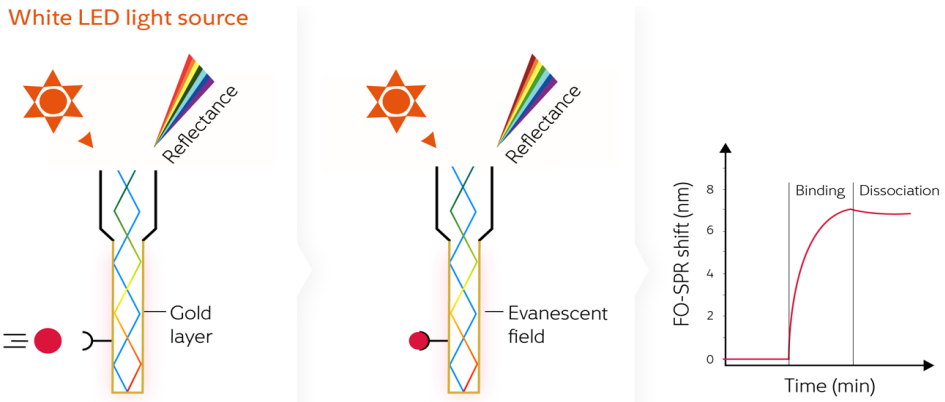


Figure 1: FO-SPR principle

## Tools and reagents

To perform the procedures in this application you will need:

- White FOx instrument with FOx-SPR acquisition software and the FOx data processing tool
- Carboxyl probes for label-free quantification (FOx BIOSYSTEMS product nr: 30.0003)
- Microsoft Excel or other data calculation software for data handling and viewing
- Micro pipettes from 10 to 1000 µl with disposable tips
- On-desk refrigeration for protein solutions
- Vertically rotating shaker
- 903 protein saver card, Whatman
- Microstar 17R, VWR
- Costar high binding 96-well plates (ELISA)
- Shaker (ELISA)
- ELx808 Absorbance Microplate Reader (ELISA)

| Buffer/Reagent | Concentration |
|---|---|
| MES: 2-(N-morpholino) ethanesulfonic acid, pH 6.0 | 50 mM |
| PBS: Phosphate buffer saline, pH 7.4 | 10 mM |
| EDC: 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide | 0.4 M in EDC/NHS |
| NHS: N-hydroxysuccinimide | 0.1 M in EDC/NHS |
| Anti-IFX-C monoclonal capture antibody | 1 mg/ml |
| Sodium acetate buffer, pH 5.5 | 10 mM |
| Regeneration buffer, pH 2 | 10 mM glycine/HCl |
| Blocking buffer: Superblock PBS (Thermo Fisher Scientific) | |
| Anti-IFX-D monoclonal detection antibody | 1 to 2.5 mg/ml stock<br>20 ml, optical density (OD) 1 |
| 20 nm diameter gold nanoparticles EMGC20 | Average size 19.7 nm (CV 8%) |
| Sodium carbonate | 0.2 mM |
| Bovine serum albumin (BSA) | 0.5% w/v |
| Storage buffer: BSA in PBS | 0.5% |
| Whole blood from healthy volunteers (collected in sodium citrate coated tubes) | |
| Plasma obtained from the above whole blood | |
| Serum obtained from the above whole blood | |
| De-identified serum and plasma samples from five IFX-treated IBD patients | |
| Tween-20 (detection buffer) | 0.01% in PBS |
| Infliximab (Remicade) | Received lyophilized<br>Resuspended to 1 mg/ml stock |
| Tween-80 (extraction buffer) | 0.1% in PBS |
| TNF alpha (ELISA) | 240 ng/ml (2) |
| pAB-IFX-HRP or HRP-conjugated MA-IFX (ELISA) | 20 mg/ml (2) |
| o-phenylenediamine and $H_2O_2$ in citrate buffer (ELISA) | 400 mcg/ml - $3x10^{-3}$% (2) |
| $H_2SO_4$: Sulfuric acid (ELISA) | 2 M (2) |

Table 1: Reagents and buffers.
All solutions were prepared with deionized water purified by a Milli-Q Plus system.

**Note:** Special attention is required for EDC/NHS which is a highly reactive and short-lived reagent (EDC user guide, Sigma Aldrich) and should be prepared just prior to use. Separately dissolve NHS and EDC no more than 5 minutes before use, mix in equal quantities, and pipet into the designated wells immediately before use.

Alternatively, you can also prepare pre-dissolved aliquots of EDC and of NHS, respectively, and store them at -20°C until use, as explained in the white paper number 1 about immobilization on carboxyl probes. Thaw one aliquot of each at room temperature for about 20 minutes before use and mix in the designated wells just before placing the reagent in the instrument.

### Surface functionalization of FO probes with capture antibody

Carboxyl probes were treated in MES buffer prior to activation. Carboxyl groups were activated by incubation in 0.4 M EDC/0.1 M NHS in MES buffer for 15 minutes. A 20 µg/ml solution of anti-IFX-C monoclonal capture antibody in a 10 mM sodium acetate buffer at pH 5.5 was covalently immobilized to the activated carboxyl groups for 30 minutes. Two 30-second washes in regeneration buffer (10 mM glycine/HCl pH 2) removed non-covalently bound antibodies. A final immersion in blocking buffer (Superblock PBS) deactivated any remaining unoccupied carboxyl groups.

### Surface functionalization of gold nanoparticles with detection antibody

Detection antibody (anti IFX-D) was conjugated to the gold nanoparticles (AuNPs) via physical adsorption using a modified protocol based on that from Jans et al. (4). The AuNP solution was adjusted to pH 9.2 using 0.2 mM sodium carbonate prior to use. Antibody solution was added to 800 µL of 7 x $10^{11}$ particles/ml AuNP solution to give a final antibody concentration of 5 µg/ml and incubated at room temperature on a vertically rotating shaker for 20 min. 560 µL of bovine serum albumin (BSA) (0.5% w/v) was added to stabilize the particles and the mixture was returned to the shaker for 1 hour at room temperature. The solution was then centrifuged at 7000 rpm for 30 min at 20°C. After discarding the supernatant containing unbound antibodies, the nanoparticle residue was resuspended in PBS with 0.5% BSA. To ensure reproducibility, the final concentration of AuNPs was adjusted to be the same as pre-functionalized AuNPs (7x$10^{11}$ particles/ml, OD of 1). The prepared AuNPs were stored at 4°C until use. 150 µl of functionalized AuNPs were used in each assay.

### Calibration curve generation

A concentration series of spiked IFX (0, 2.5, 5, 10, 20, 40, 80, and 100 ng/ml) was prepared in whole blood diluted 10-fold in PBS with 0.01% Tween 20 and analyzed using a previously developed FO-SPR bioassay for the detection of IFX in serum (3). Functionalized FO probes were immersed in the IFX dilution for 15 minutes, with each concentration measured by an individual functionalized FO-SPR sensor. This was followed by 20 minutes of signal amplification using functionalized AuNPs. These results were used to generate a calibration curve (figure 3) to validate the shorter FO-SPR bioassay developed during this study.

### Preparation and extraction of dried blood spot samples

100-fold diluted dried blood spot (DBS) samples were prepared by first diluting 10 µl of IFX stock solution in 90 µl of whole blood. 45 µl of this dilution was applied to filter paper (903 protein saver card, Whatman) and allowed to dry overnight at room temperature. 6 mm diameter disks were then placed into an Eppendorf tube containing 240 µl extraction buffer (PBS with 0.1% Tween 80) and placed on a shaker at 300 rpm for 1 h at 21°C. The samples were centrifuged for 5 min at 13,000 rpm (16,249 g) and used immediately.

Approximately 10 µl reconstituted blood was obtained from the 6 mm disk which was diluted 25-fold in 240 µl extraction buffer, then further diluted in detection buffer (PBS with 0.01% Tween 20) to reach the final IFX concentrations (0, 2.5, 5, 10, 20, 40, 80, and 100 ng/ml) after a final 4-fold dilution in detection buffer. These samples are referred to as spiked DBS samples for the remainder of this paper.

DBS samples without IFX were prepared to estimate the extraction efficiency by adding 10 µl detection buffer (in place of the IFX) to 90 µl whole blood and extracted as described above, then spiked with IFX to obtain the same series of final concentrations. These samples are referred to as reference DBS samples for the remainder of this paper.

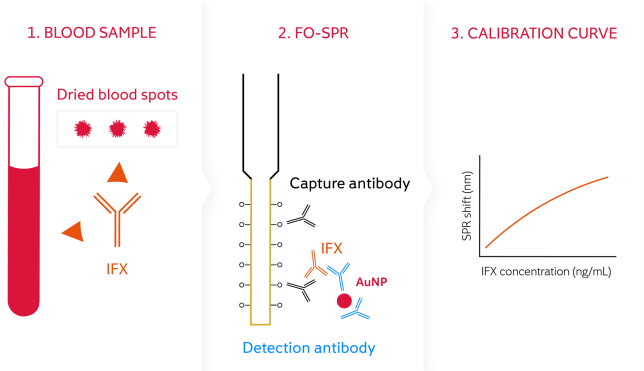


Figure 2: Assay set-up

### Establishing an accelerated FO-SPR bioassay for IFX detection

To establish the bioassay, functionalized FO-SPR sensors were immersed in IFX-spiked samples for 5 min, AuNP storage buffer (PBS with 0.5% BSA) for 3 min, and AuNP solution for 5 min, resulting in a total detection time of 13 min. This assay was used to obtain calibration curves in 10-fold diluted whole blood, 100-fold diluted whole blood, serum, plasma, and DBS extracts, each spiked with final IFX concentrations ranging from 0 to 100 ng/ml. For each calibration curve, four repeats, generated with two batches of independently prepared FO-SPR sensors and AuNPs, were included.

### FO-SPR bioassay validation with serum and plasma from IFX-treated patients

The above FO-SPR bioassay was validated using five IFX-treated patient samples, with matching serum and plasma samples from each patient.
Three samples (S2, S3, and S4) were diluted 100-fold. Due to higher IFX concentrations in the other two samples, they were further diluted; S1 150-fold diluted and S5 300-fold. Three repeats were measured for each sample.

### Accelerated FO-SPR bioassay for detecting IFX in 10-fold diluted whole blood

To further shorten the previously described bioassay time from 40 minutes, the IFX incubation and AuNP amplification steps were shortened from 15 and 20 minutes, respectively, to 5 minutes each. This resulted in a total detection time of 13 minutes from the time the pre-functionalized probe was immersed in the IFX solution. A calibration curve (Figure 3) was created from the SPR shifts obtained from measuring a series of IFX concentrations (1 – 100 ng/ml) spiked in 10-fold diluted whole blood four times in a random order.

### IFX quantification in serum and plasma from IFX-treated patients using ELISA

IFX in serum and plasma samples from IFX-treated IBD patients was also quantified by ELISA using previously optimized conditions for IFX detection (2). Each sample was measured three times on different days. 96-well plates were coated overnight with TNF at 4°C, then blocked with PBS / 1% BSA for 2 hours at room temperature. Samples were diluted 1:150 and 1:300 in PBS / 1% BSA, added to the plate, and incubated for 2 hours at 37°C on a shaker. Plates were then washed and pAb-IFX-HRP or HRP-conjugated MA-IFX was applied for detection of bound IFX and incubated for 1 hour at room temperature. Plates were washed and developed using o-phenylenediamine and H2O2 in citrate buffer, and the reaction was stopped with 2 M H2SO4. The absorbance was measured at 490 nm with an ELx808 Absorbance Microplate Reader.

### Calculation of SPR response

SPR response can be measured in multiple ways.
The first one is wavelength shift, referred to as “SPR shift”, which is simply calculated by subtracting the pre-binding SPR wavelength from the SPR wavelength at a given binding time point.

However, if the binding curves show a linear behavior and sufficient signal increase at their onset, this part of the curve can be used to perform a slope analysis, which can be plotted as a function of target concentrations (Figure 4B). Calculating the SPR response from the slope of the initial binding curve is hereafter referred to as “slope”.

## Results

### FO-SPR detection of IFX in 10-fold diluted whole blood

The limit of detection (LOD) determined from the calibration curve (Figure 3) as blank sample + 3 SD was 1.12 ng/ml with a coefficient of variation (CV) below 10%. Non-specific binding was measured after blocking with Superblock PBS by immersing the FO-SPR sensor functionalized with capture antibody in 10-fold diluted whole blood in the absence of IFX, followed by a AuNP amplification step. This resulted in less than 0.1 nm wavelength shift (n = 4).

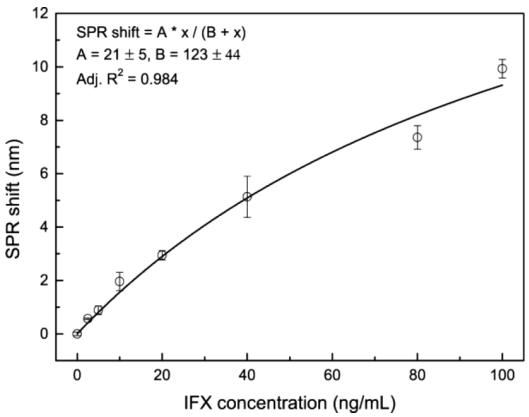


Figure 3: Calibration curve for IFX spiked in 10-fold diluted whole blood using a previously developed FO-SPR protocol (3).

## Accelerated FO-SPR bioassay for detecting IFX in 10-fold diluted whole blood

From calculating SPR shift, the accelerated bioassay time of 13 minutes resulted in a calculated LOD of 0.75 ng/ml with an average CV of less than 12% (figure 4A). However, the AuNP binding curves showed a linear behavior within the first two minutes, therefore, this part of the curve was used to perform a slope analysis, which was further plotted as a function of IFX concentrations. Calculating the SPR response by slope (figure 4B) resulted in an LOD of 0.9 ng/ml and an average CV of less than 18%. This CV is slightly higher than that obtained with SPR shift, but it is still acceptable because it is below 20%. Since the LODs were similar for both methods, it was decided that the detection time could be reduced by 3 minutes, thereby resulting in a total assay time of just 10 minutes.

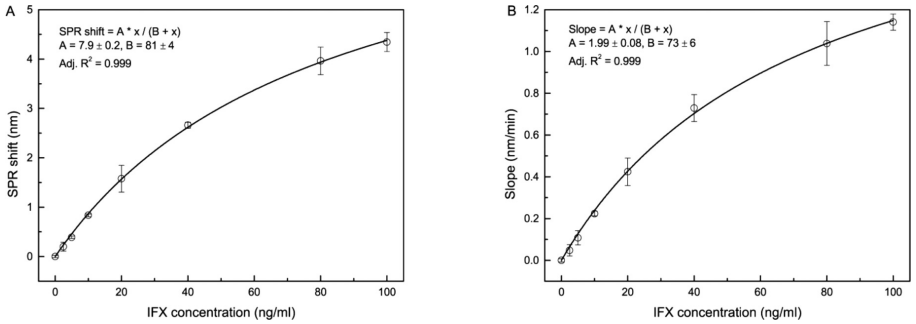


Figure 4: Calibration curves for IFX spiked in 10-fold diluted whole blood using the accelerated FO-SPR protocol. (A) SPR shift. (B) Slope.

## FO-SPR bioassay for IFX detection in 100-fold diluted serum, plasma, and whole blood

To achieve IFX detection in the clinically relevant range (0.5–10 µg/ml in serum samples and 0.23–4.5 µg/ml in whole blood), the samples were further diluted to ensure that measurements fell within the dynamic range of the FO-SPR bioassay. IFX dilution series from 0 to 100 ng/ml were created in 100-fold diluted serum, plasma, and whole blood. These were quantified using FO-SPR and calibration curves generated using both methods one and two (figure 5). The resulting LODs are shown in table 2, with all CVs being below 15% for both methods.

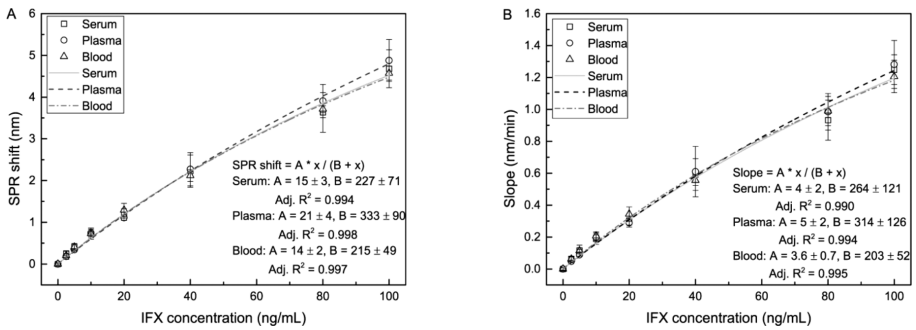


Figure 5: Calibration curves obtained from 100-fold diluted serum, plasma, and whole blood from SPR shift (A) and slope (B) methods using the accelerated FO-SPR bioassay. n=4, error bars are standard deviations.

| | SPR shift method | Slope method |
|---|---|---|
| **Serum** | 1.05 ng/ml | 1.42 ng/ml |
| **Plasma** | 1.00 ng/ml | 1.00 ng/ml |
| **Whole blood** | 1.05 ng/ml | 1.34 ng/ml |

A random block design showed that the differences between batches of FO-SPR sensors and AuNPs prepared on different days were insignificant. This suggests that one control (zero) measurement and one IFX measurement is sufficient to obtain a concentration value from the established calibration curve.

Table 2: LOD of IFX in 100-fold diluted serum, plasma, and whole blood using SPR shift and slope methods.

## FO-SPR bioassay for IFX detection in 100-fold diluted dried blood spots

To assess the extraction efficiency of the protocol, IFX concentrations were measured in 100-fold diluted reference (whole blood spiked with IFX after extraction from the filter paper) and spiked samples (whole blood spiked with IFX before extraction from the filter paper). Using the calibration curves obtained using the accelerated FO-SPR bioassay, LODs and CVs were as shown in table 3.

| | SPR shift | | Slope | |
|---|---|---|---|---|
| | LOD (ng/ml) | CV (%) | LOD (ng/ml) | CV (%) |
| **Spiked** | 1.17 | 18 | 1.83 | 17 |
| **Reference** | 1.39 | 20 | 1.59 | 20 |

Table 3: LODs and CVs of IFX measurements in 100-fold diluted samples. Reference = whole blood spiked with IFX after extraction from the filter paper. Spiked = whole blood spiked with IFX before extraction from the filter paper. n = 4.

The extraction efficiency was measured as a ratio of the analyte concentrations between spiked and reference DBS samples, i.e., the SPR shifts obtained for each IFX concentration. This was done individually for each of the seven concentrations which were then averaged to give 0.98 using SPR shift, and 0.97 using slope calculation. Similar results for the extraction efficiency were also obtained by performing a correlation analysis between calibration curves from the reference and spiked DBS samples: 0.99 (95% CI slope: 0.99 ± 0.02) for SPR shift and 0.98 (95% CI slope: 0.99 ± 0.04) for slope calculation, (Pearson correlations 0.998 and 0.995, respectively).

## Comparison of the accelerated FO-SPR bioassay performance in different matrices

To investigate the impact of different matrices on measuring IFX concentrations with the accelerated FO-SPR bioassay, correlations were calculated between calibration curves obtained for 100-fold diluted whole blood, serum, plasma, and DBS. The linear equation of the fitted curve, 95% CI of the slopes, and Pearson correlations are shown in table 4 using SPR shift (see supplementary data in the original paper (1) for data obtained using slope calculation).

| y-axis → / x-axis ↓ | 100-fold diluted whole blood | 100-fold diluted serum | 100-fold diluted plasma | 100-fold diluted spiked DBS |
|---|---|---|---|---|
| **100-fold diluted whole blood** | | y = 1.01x – 0.02<br>95% CI = 0.04<br>Pearson = 0.999 | y = 1.08x – 0.09<br>95% CI = 0.04<br>Pearson = 0.998 | y = 0.91x – 0.05<br>95% CI = 0.02<br>Pearson = 0.999 |
| **100-fold diluted serum** | y = 0.99x + 0.01<br>95% CI = 0.04<br>Pearson = 0.999 | | y = 1.07x – 0.07<br>95% CI = 0.04<br>Pearson = 0.999 | y = 0.93x – 0.07<br>95% CI = 0.02<br>Pearson = 0.999 |
| **100-fold diluted plasma** | y = 0.93x + 0.07<br>95% CI = 0.04<br>Pearson = 0.999 | y = 1.00x + 0.01<br>95% CI = 0.1<br>Pearson = 0.996 | | y = 0.88x – 0.01<br>95% CI = 0.02<br>Pearson = 0.999 |
| **100-fold diluted DBS** | y = 1.10x + 0.01<br>95% CI = 0.04<br>Pearson = 0.999 | y = 1.10x + 0.06<br>95% CI = 0.06<br>Pearson = 0.998 | y = 1.18x – 0.02<br>95% CI = 0.06<br>Pearson = 0.998 | |

Table 4: Correlations between calibration curves based on SPR shift for 100-fold diluted serum, plasma, whole blood, and DBS. Linear regressions were fitted using calibration curves in the column as x-axis and in the row as y-axis.

For 100-fold diluted serum, plasma, and whole blood, the results were approximately 1 for both SPR shift and slope calculation methods, suggesting that the SPR signal for a given dilution factor is almost identical, i.e., the matrix effect was insignificant. The results of these three matrices with spiked DBS samples gave slope values of approximately 1.1, suggesting a nearly 10% higher signal for non-dried sample matrices.

### Validation of the accelerated FO-SPR bioassay with plasma and serum samples from IFX-treated patients

The accelerated FO-SPR bioassay was validated using matching serum and plasma samples from five IFX-treated IBD patients. These samples were also tested using a clinically validated ELISA test which showed cutoffs and lower limit of quantification were 0.2 and 0.5 µg/ml IFX, respectively, in 150-fold diluted serum and plasma.

IFX concentrations from serum samples, S1, S2, S3, S4, and S5 were calculated by interpolating the obtained SPR signals into the calibration curve, which was made using the series of IFX concentrations spiked in 100-fold diluted serum. Although dilution factors of S1 and S5 were different from the calibration curve, it was demonstrated in our previous white paper (white paper 2: Sensitive protein quantification in crude serum samples: comparison of FO-SPR and ELISA) that this still allows the determination of IFX concentrations.

IFX was also quantified in the five plasma samples (referred to as P1, P2, P3, P4, and P5) from the same patients using the same dilution factors. The only difference is the presence of clotting factors in plasma that are not present in serum and, therefore, similar IFX concentrations are expected in both matrices. Good Pearson correlations of 0.959 and 0.968 were obtained for SPR shift and slope calculations, respectively. Correlation slopes were less than 1 for both methods, indicating that IFX concentrations in the plasma samples were slightly lower than those in the corresponding serum samples. This could be due to the clotting factors in plasma making these samples less stable compared to serum during handling and storage.

Finally, IFX concentrations measured with the accelerated FO-SPR bioassay (slope calculation) were compared to those measured with ELISA (figure 6). This method was used because of its shorter detection time while also giving excellent correlations with results obtained using SPR shift.

ELISA also measured a lower IFX concentration in plasma compared to serum. The FO-SPR bioassay and ELISA had an excellent correlation, with Pearson correlation and intraclass correlation coefficients (ICC) of 0.999 and 0.998, respectively, for serum samples, and 0.997 and 0.992, respectively, for plasma samples. The average CV of the FO-SPR from both serum and plasma samples was approximately 10%.

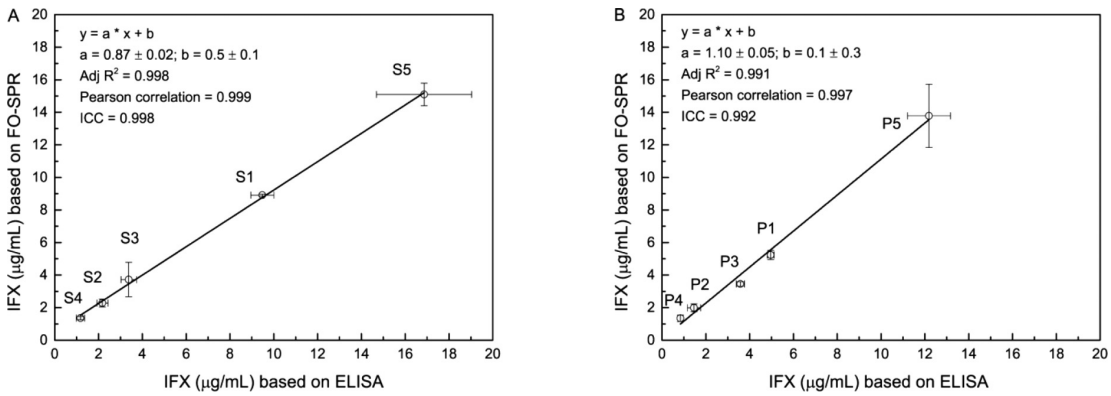


Figure 6. Comparison between the IFX concentrations measured in serum (A) and plasma (B) samples from IBD patients obtained from the accelerated FO-SPR immunoassay and ELISA. Error bars represent standard deviation (n = 3 for both FO-SPR and ELISA).
Note: samples 1 and 5 were outside the calibration curve range and required an extra dilution step to measure.

## Discussion / Conclusion

The advantage of detecting biomolecules directly in whole blood is the ability to study the target molecules in the actual environment at biologically relevant concentrations. This allows the scientist to focus on the molecule of interest rather than on the time-consuming, and potentially biased purification procedures needed to avoid cross-contamination and clogging in microfluidics systems.

The fiber-optic probe setup based on surface plasmon resonance (SPR) investigated here allows signal amplification using a sandwich assay format with gold nanoparticles conjugated to detection antibody. This format allows the detection of concentrations of therapeutic antibodies (here, infliximab (IFX)) down to 0.75 ng/ml within a 10-minute time frame for 100-fold diluted serum, plasma, or whole blood. In addition, the various calibration curves obtained from serum, plasma, and whole blood show excellent correlations, indicating insignificant matrix effects at this dilution.

Furthermore, two effective methods of determining concentration using SPR have been demonstrated here: the SPR shift (a 13-minute assay) and the slope of the obtained binding curves (a 10-minute assay) without compromising on sensitivity or reproducibility.

The results obtained from testing plasma and serum from five IFX-treated IBD patients with FO-SPR show excellent agreement with an IFX-specific ELISA test, with Pearson correlations and intraclass correlation coefficients (ICC) greater than 0.99 in all cases.

The developed method was further assessed using dried blood spot (DBS) samples giving a calculated IFX extraction efficiency of 99%. This demonstrates that (i) the blood drying process did not affect the extraction of IFX or its interaction with the antibodies used in the FO-SPR bioassay and (ii) the distribution of IFX on the DBS paper was uniform since only part of the DBS paper was used for extraction.

This study shows that FO-SPR is a user-friendly technology that combines the speed and accuracy of SPR with the advantage of fluidics-free dip-in sensing. The 10-minute assay provides a much faster and more convenient alternative to ELISA, and even to other SPR technologies that rely on microfluidics.

FO-SPR is also fully automated and can either run individual measurements or with 4 parallel FO sensors. This means it could be used to determine biological drugs in crude lab samples or patient samples both for a limited number of samples as well as in high throughput mode.

## Acknowledgement

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## References

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3. Lu et al. (2016) Fiber optic-SPR platform for fast and sensitive infliximab detection in serum of inflammatory bowel disease patients. Biosensors and Bioelectronics 79, 173–179.
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## Related white papers

How to optimize immobilization to the FO-SPR probes is described in white paper 1: Carboxyl probe immobilization for label-free protein quantification.

A comparison of FO-SPR with ELISA for protein quantification in serum samples is described in white paper 2: Sensitive protein quantification in crude serum samples: comparison of FO-SPR and ELISA.

How to set up a label-free quantification assay starting from an immobilized capture surface is described in white paper 4: Label-free protein quantification.

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