Bloch surface wave label-free and fluorescence platform for the detection of VEGF biomarker in biological matrices

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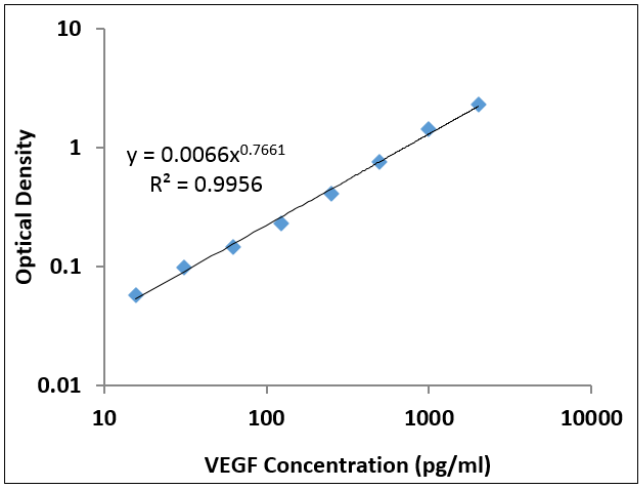
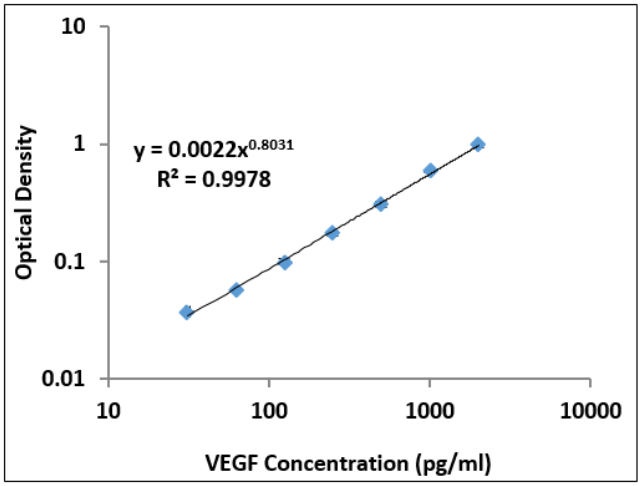
Supplementary Material

Quantification of VEGF concentration by means of ELISA



Cell type	VEGF concentration in 1:10 diluted cell supernatant (pg / ml)
MCF-7 WT	77.0 ± 0.5
MCF-7 $VEGF_{165}$	1553.9 ± 95.9

Fig. S1: ELISA results for the assessment of VEGF concentration in cell culture supernatant. ELISA standard curve (left panel) and measured values (right panel). Mean values ± the standard error of the mean (SEM) of three replicas are presented. 1:10 diluted samples were used to assess VEGF concentration.



Human blood plasma sample	VEGF concentration in undiluted sample (pg / ml)
01	24.3 ± 1.6
02	50.7 ± 0.6
03	17.1 ± 0.5
04	62.6 ± 2.5
05	89.8 ± 2.1
06	40.5 ± 2.3
07	82.3 ± 0.0
08	29.1 ± 1.1
09	64.7 ± 10.9
10	104.4 ± 1.4
11	155.3 ± 8.4
12	182.6 ± 4.7
13	174.0 ± 5.4
14	301.5 ± 10.4

Fig. S2: ELISA results for the assessment of VEGF concentration in human blood plasma. ELISA standard curve (left panel) and measured values (right panel). Mean values ± SEM of three replicas are presented. Undiluted samples were used to assess VEGF concentration.

Optical System

Fig. S3 illustrates the optical read system described in the text of the main article.

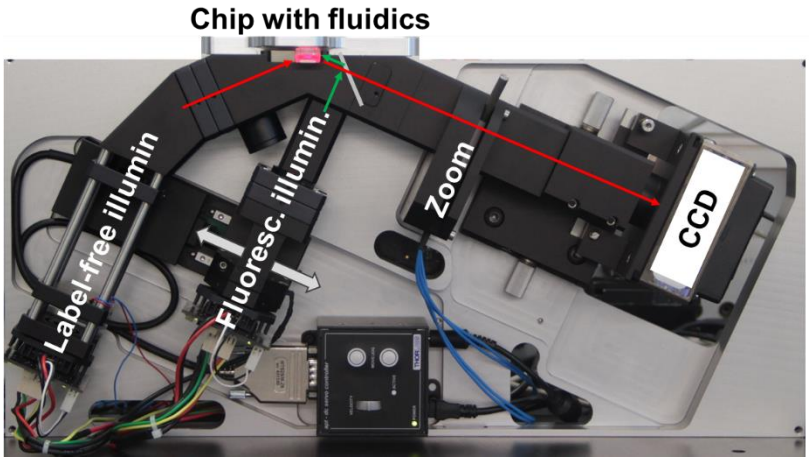


Fig. S3: Photograph of the optical system illustrating the path of the label-free (670 nm) and fluorescence wavelengths (red arrows) along with the fluorescence excitation path (635 nm, green arrow). The fluorescence excitation light source with collimation optics and telescope is mounted on a translation stage for scanning the excitation angle, as indicated by the white arrows.

The two illumination systems for label-free analysis (670 nm) and fluorescence excitation (635 nm) are indicated along with the schematic light path and the dichroic mirror for spectrally

discriminating the fluorescence excitation from the fluorescence emission and label-free detection. In order to scan the angle of fluorescence excitation, the corresponding unit is mounted onto a motorized translation stage. The imaging optical system is completely housed and not accessible; just the zoom wheel for increasing the angular detection range in fluorescence mode is operated by the used during the experiment. On top of the optical system the disposable parts (described below) are mounted.

Surface functionalization

APTES readily reacts with hydroxyl groups and forms a monolayer under carefully controlled conditions. During preparation, we functionalized the biochips in batches of 4. Modification of the surfaces through APTES chemistry results in amino functional groups that can be easily conjugated (e.g. through an imine formation) to a chemical agent that would lead to the crosslinking with the protein of interest. We used glutaraldehyde (GAH) as a bifunctional molecule to couple the APTES-functionalized surface with the specific antibodies we wish to immobilize on the surface.

The 1DPC surface was first cleaned with piranha (H_2SO_4:H_2O_2 = 3:1) for 10 minutes, washed thoroughly using de-ionized water and ethanol, and dried with N_2. This step allows the removal of organic contaminants and exposes hydroxyl groups for the subsequent functionalization. We immersed the cleaned biochips in different concentrations of APTES solution: 2%, 5%, and 10% (v/v) solution in a mixture of ethanol/water (95:5 v/v) for 1 hour at room temperature. The biochips were then washed in pure ethanol and sonicated three times. After drying with N_2 we baked the biochips on a hot plate at 110 °C for 1 hour, and we evaluated APTES functionalization via contact angle measurements. **Fig. S4** shows that the formation of the amine-reactive groups on the surface by APTES can be achieved with a 2% (v/v) solution.

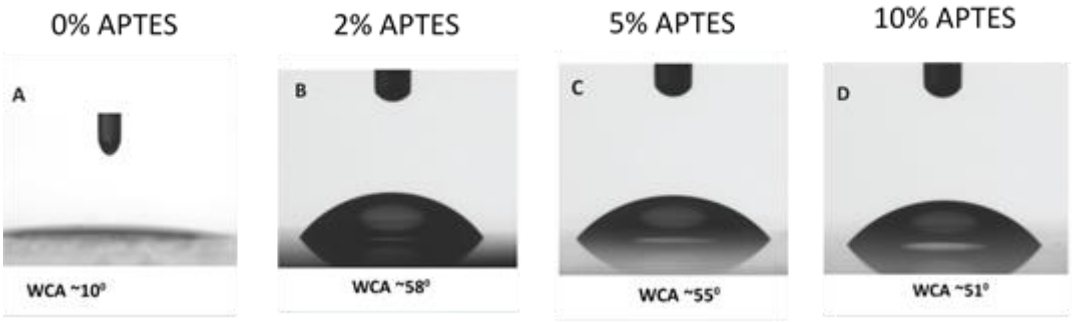


Fig. S4: Water contact angle was measured to evaluate chemical changes of the surface by APTES functionalization. The formation of the amine-reactive groups on the surface by APTES can be achieved with a 2% (v/v) solution.

Next, glutaraldehyde (GAH) was used as a homobifunctional crosslinker to allow conjugation of antibodies onto the APTES-functionalized 1DPC surface. We exposed the functionalized biochips in a solution of 1% (v/v) GAH in 100 mM sodium bicarbonate buffer (pH 8.5) in the presence of 0.1 mM $NaCNBH_3$ for 1 hour, followed by washing and sonication in de-ionized water. $NaCNBH_3$ is a mild reducing agent and its presence leads to an efficient reaction between aldehyde and amine, without causing detrimental effects to other chemical groups in biological samples. **Fig. S5** shows contact angle measurements of the coated chips after GAH functionalization in the presence and absence of $NaCNBH_3$, indicating that the conjugation is more efficient in the presence of $NaCNBH_3$.

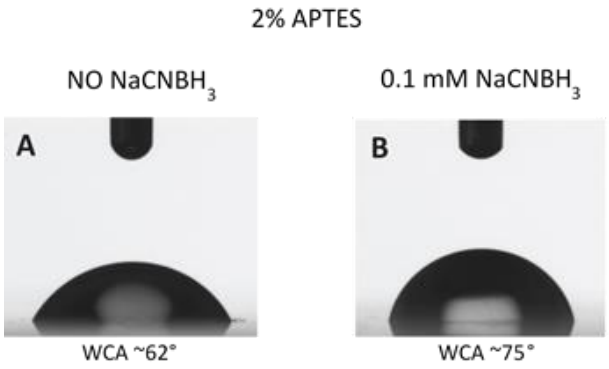


Fig. S5: Water contact angle measurements of GAH-functionalized surface with and without $NaCNBH_3$ (0.1 mM).

Fluidic System

Fig. S6 illustrates a picture and a technical scheme of the temperature controlled support of the fluidic system. This support utilizes springs for the fluidic tight connection of the fluidic system and can be loaded with the flow cells and chips, after they are plugged together. An external, 500 mL syringe pump is used for pumping analyte solutions with very few pulsation and user-selected speed through the flow cell. The external fluidic handling system is coupled via Upchurch connectors that are passively aligned with respect to the injection molded flow cells. The temperature is controlled by an electrical heater (22 Ohm resistor), measured by a thermocouple type PT1000 and regulated via a microcontroller.

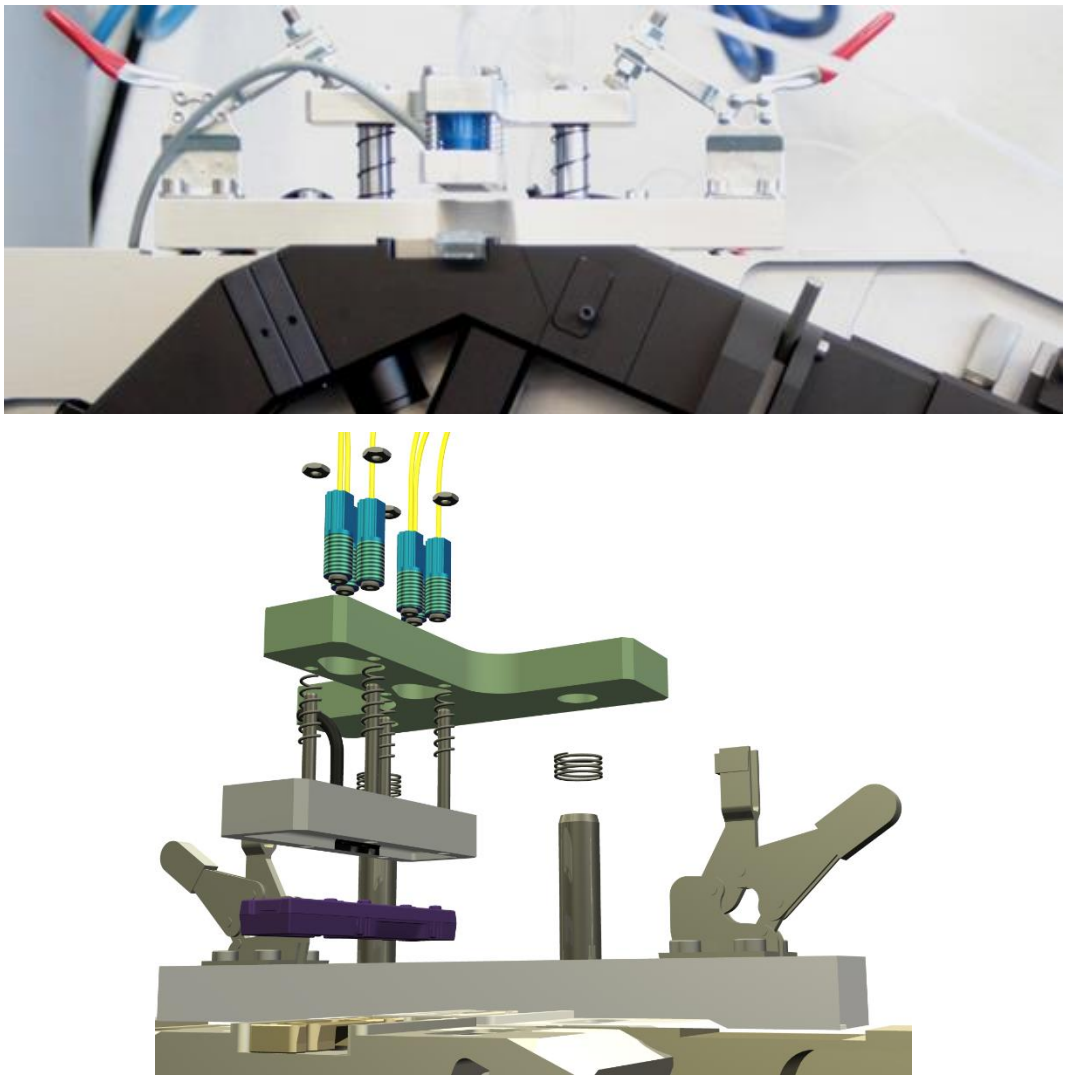


Fig. S6: Photograph and technical scheme of the temperature controlled support of the fluidic system mounted on top of the optical system.

Spotting Tool

Fig. S7 illustrates the spotting tool used for the functionalization of the chips. It is composed of a Polydimethylsiloxane (PDMS) flow cell with five parallel channels (each 200 µm wide and 200 µm high) that is attached to an aluminum plate. For the immobilization of the antibodies, the biochip is loaded into a socket plate and topped with the five-channel flow cell, which is then locked using screws. Thus, a closed fluidic system on the chip is obtained which allows filling the proteins to be immobilized into the channels using a standard microliter pipette.

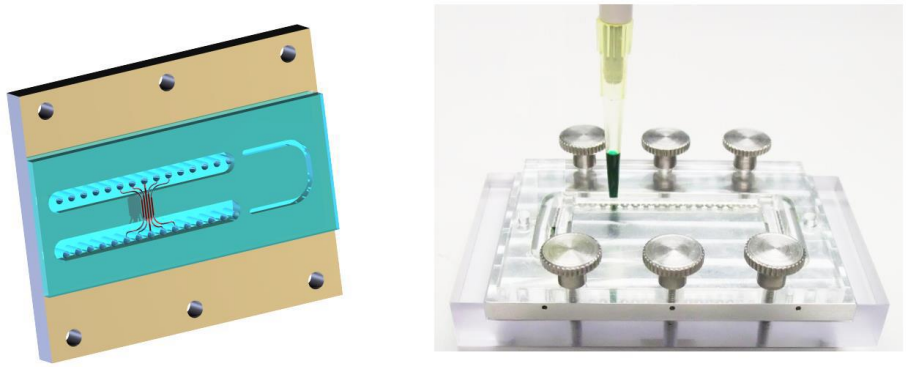


Fig. S7: Pictures of the spotting tool used for the functionalization of the Chips. The Polydimethylsiloxane (PDMS) flow cell (blue) with the five fluidic channels (red) attached to an aluminum plate (left). Filling of a channel flow cell using a standard microliter pipette (right).

Stability of the label-free baseline and of the chemical functionalization of the plastic biochips

Fig. S8 shows the baseline signals recorded in the label-free mode at the beginning of a standard assay on a 15 min time interval. As it can be seen the signals drift in both the reference and signal regions, due to parasitic effects (temperature, illumination laser wavelength, pressure, …). We registered a drift (in both, signal and reference spots) $\Delta\delta$ = 5 pix all over the 15 min temporal range, corresponding to a drift rate of 0.33 pix/min for non-compensated sensorgrams. However the differential signal is very stable along the 15 min, demonstrating the stability of the platform. Moreover, the result demonstrates that here is no degradation of the chemical functionality of the surface of the biochip.

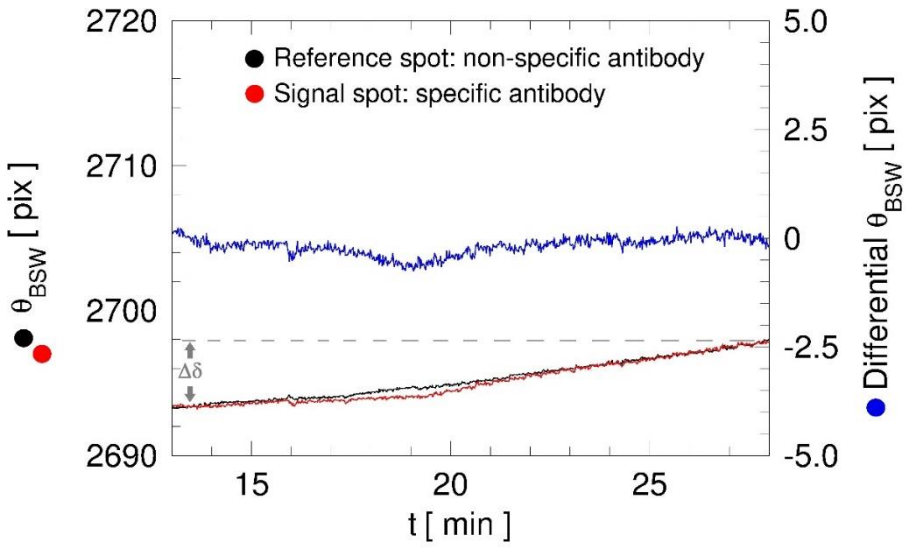


Fig. S8: Label-free sensograms recorded in the reference (black curve) and signal (red curve) spots in D-PBS 1 X in static conditions (15 min). A residual drift can be recorded in both regions ($\Delta\delta$) and easily compensated by subtracting signal to reference regions obtaining a differential sensogram (blue curve, right axis).