Supporting Information

Real-time PCR melting analysis with Fiber Optic SPR enables multiplex DNA identification of bacteria

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1. Experimental Section

1.1. Reagents

All chemicals were of analytical reagent grade and purchased from Sigma-Aldrich (Bornem, Belgium), unless stated otherwise. The oligonucleotides were chemically synthesized by Integrated DNA Technologies (IDT, Haasrode, Belgium) and an overview can be found in Supplementary Information (Table S1). Citrate stabilized AuNPs, with a mean diameter of 20 nm, were purchased from BBI international (Cardiff, United Kingdom).

The oligonucleotides were chemically synthesized by Integrated DNA Technologies (IDT, Haasrode, Belgium). Sequences of the DNA targets, primers and the DNA hybridization probes used in this paper are listed in Table S1. Three target sequences were chosen for both *M. Bovis* and *MAP* to test the measuring principal and the sensitivity of the FO-SPR biosensor platform: wild type (WT), mismatch one SNP (MM1) and mismatch three SNPs (MM3). For amplifying a specific region of *M. Bovis,* primers were designed within so-called regions of difference, which contain sequences not present in other Mycobacteriaceae.[1] For *MAP*, primer sequences were adopted from Ravva and Stanker,[2] who selected these primers using the insertion sequence IS900, which is a repetitive element present only in the *MAP* genome. 2 hybridization probes, used to capture the target sequences, are modified with a 3' C3 (probe 1) or 5' C6 thiol modifier (-SH) (probe 2) for their immobilization on the FO-SPR sensor and the AuNPs gold surfaces, respectively. All the hybridization probes were equipped with a poly T spacer in order to improve the hybridization efficiency of the DNA.[3] The free 3' end of the hybridization probes was blocked from extension by the polymerase enzyme using a 3' phosphate modification.

Table S1. Overview of used oligonucleotides for the FO-PCR-MA.

Targets (5'→3')
M. Bovis WT (76 bp)
GCA GAA GCG CAA CAC TCT TGG AGT GGC CTA CAA CGG CGCTCT CCG CGG CGC GGG CGT ACC GGA TAT CTT AGC TGGT
M. Bovis 5' MM1 (76 bp)
GCA GAA GCG CAA CAC TCT TTG AGT GGC CTA CAA CGG CGCTCT CCG CGG CGC GGG CGT ACC GGA TAT CTT AGC TGG T
M. Bovis 5' MM3 (76 bp)
GCA GAA GCG CAA CAC TCT TTG AGC GTC CTA CAA CGG CGCTCT CCG CGG CGC GGG CGT ACC GGATAT CTT AGC TGG T
MAP WT (56 bp)
TGG TCG TCT GCT GGGTTG ATC TGG ACA ATG ACG GTT ACG GAG GTG GTT GTG GC -3
MAP 5' MM1 (56 bp)
TGG TCG TCT GCT GGG TTG ATA TGG ACA ATG ACG GTT ACG GAG GTG GTT GTG GC -3
MAP 5' MM3 (56 bp)
TGG TCG TCT GCT GGG TTG ATA TTA ACA ATG ACG GTT ACG GAG GTG GTT GTG GC
Primers (5'→3')
M. Bovis primer forward
GCA GAA GCG CAA CAC TCT T
M. Bovis primer reverse
ACC AGCTAA GAT ATC CGG TAC G
MAP primer forward
TGG TCG TCT GCT GGG TTG A
MAP primer reverse
GCC ACA ACC ACCTCC GTA AC
Hybridization probes (5'→3')
M. Bovis probe 1 (48 bp)
CGC CGT TGT AGG CCA CTC CAA GAGTGT TGC GCT TCT GCT TTT TTT TTT - SH
M. Bovis probe 2 (48 bp)
SH- TTT TTT TTT TAC CAG CTA AGATAT CCG GTA CGC CCG CGC CGC GGA GAG - Phos
MAP probe 1 (45 bp)
TGT CCA GAT CAA CCC AGC AGA CGA CCATTT TTT TTT TTT TTT TTT - SH
MAP probe 2 (44 bp)
SH - TTT TTT TTT TTT TTT TTT GCC ACA ACC ACCTCC GTA ACC GTC AT -Phos

1.2. Device setup

The FO-SPR setup was made of a HL-2000 tungsten halogen lamp (Ocean optics, Dunedin, USA), a miniature UV-VIS spectrophotometer (USB4000, Ocean Optics, Dunedin, USA) and an FO-SPR sensor, as previously described in Pollet et al.[4] The FO-SPR setup (Figure S1A) was built in order that replaceable and interchangeable FO-SPR sensors can be coupled with a bifurcated optical fiber to guide white light from the tungsten halogen lamp into the FO-SPR sensor. The light passes first the SPR sensitive gold zone, reflecting back at the tip and travels towards the spectrometer (Figure S1B). Binding phenomena at the gold surface results in a shift of the typical spectral resonance SPR-dip recorded by the spectrometer.[5, 6] When the gold layer is coated with DNA, as is the case in the FO-SPR melting assay and the FO-PCR-MA, the FO-SPR sensor can be used to monitor interactions of this immobilized DNA with free DNA in a solution. In order to augment assay sensitivity the complementary hybridization probes were labeled with AuNP.

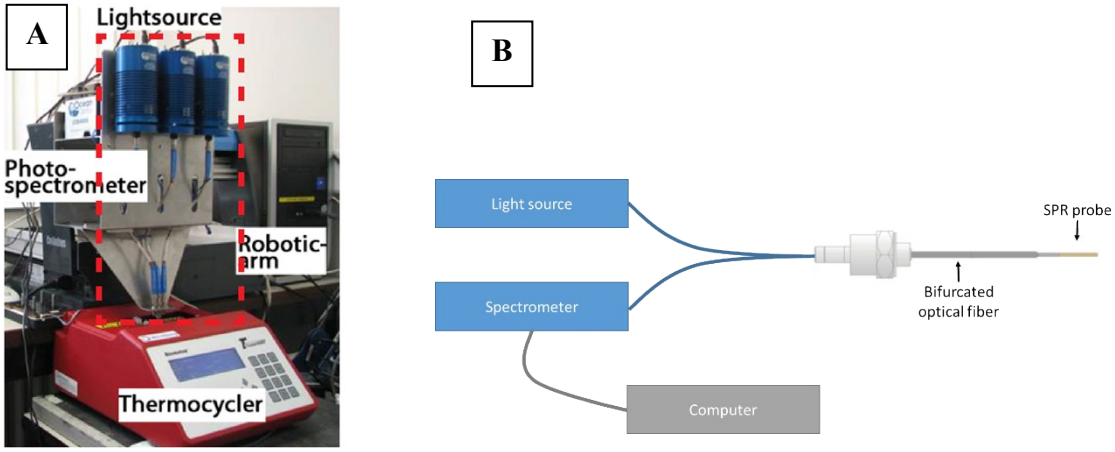

Figure S1. Representation oft he FO-SPR setup. A) Picture of the measuring setup with all components. B) Schematic representation of the FO-SPR platform.

The setup was extended with a T1 thermocycler (Biometra, Goettingen, Germany) to control the sample temperature and a C&C robotic arm (Colinbus, Hulshout, Belgium) to automate handling. The thermocycler temperature was monitored externally using a NI-DAQ (National instruments, Austin, USA) with three highly responsive T-type WK-200 thermocouple threads (Labfacillity, Leads, United Kingdom). This allowed to monitor the exact temperature at the FO-SPR sensor and derive the exact melting temperature by combining temperature data with the SPR sensorgram.

1.3. Data processing

Data acquisition on the two spectrometers and NiDaq coupled thermocouples was done with the in-house developed LabView program (National Instruments, Austin, Texas). Once the SPR data and thermocouple data were combined, a first order derivative was calculated for each PCR melting cycle. The resulting melting peak was fitted using a Gaussian fit in Matlab (the mathworks, Natick, USA) to determine the T_m and evaluate the melting peak quality for each PCR cycle.

2. Results and Discussion

2.1. Real-time multiplex FO-PCR-MA

Since the exact composition of the PCR master mix is not known, a FO-SPR DNA melting assay was first performed to evaluate if a similar resolution can be obtained in this mixture as in the buffers used in the previous experiment. Two peaks, each corresponding to one target, could be resolved, although the peak heights were considerably lower (Figure S2) compared to the previous experiment (Figure 2B). This demonstrates the possibility to perform the melting assay in the PCR master mix. The decreased peak heights can be attributed to a lower ionic strength of the buffer. Also, the ΔT_m between two targets was reduced from 7.0 °C in the previous experiments to 5.3 °C for the analysis in the PCR master mix. These results showed that buffer ionic strength has a strong influence on the resolution of the FO-SPR melting analysis. However, to prevent PCR enzyme inhibition, the ionic strength cannot be substantially altered.

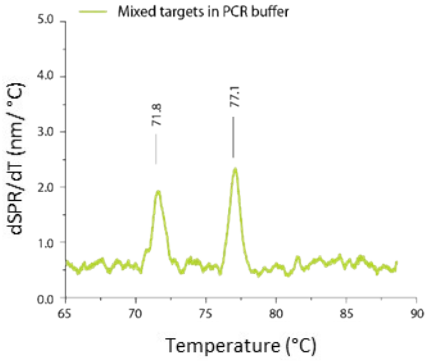

Figure S2. Multiplex FO-SPR melting analysis. Multiplex FO-SPR melting analysis for the *MAP* and *M. Bovis* target sequence performed in PCR buffer.

The amplification of DNA in solution was studied in the presence of the FO-SPR sensor. Initially, the FO-SPR signal of the FO-PCR-MA reaction is the inverse of the temperature change (Figure 3A), which is continuously monitored with a thermocouple (Figure S3, shown for 9 cycles). However, from the moment DNA target is amplified through FO-PCR-MA above the detection threshold of the FO-SPR, the melting signal of the amplified DNA target will superimpose on the refraction index shift of the temperature measured with the FO-SPR sensor.

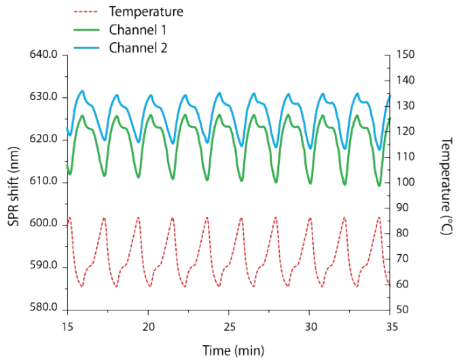

Figure S3. Raw data of an FO-SPR PCR measurement. Two measurement channels (channel 1 and channel 2) measure continually changes in refractive index due to the thermo cycling. As a result, the FO-SPR signal is the inverse of the temperature measured with a thermocouple.

2.2. Real-time mutation analysis with FO-PCR-MA

When the FO-SPR melting peak height is plotted versus the PCR cycle number for both WT and MM targets (Figure S4), it can be seen that melting peaks are substantially lower for both mutated targets in comparison with the melting signal of the WT target. Because mutations were not located in the priming regions and therefore could not influence PCR efficiency (meaning that approximately the same amount of reaction cycles was needed for both *MAP* and *M. Bovis* to reach the FO-SPR threshold), these findings can be explained by a lower hybridization efficiency of the two hybridization probes on the FO-SPR sensor and the AuNPs.

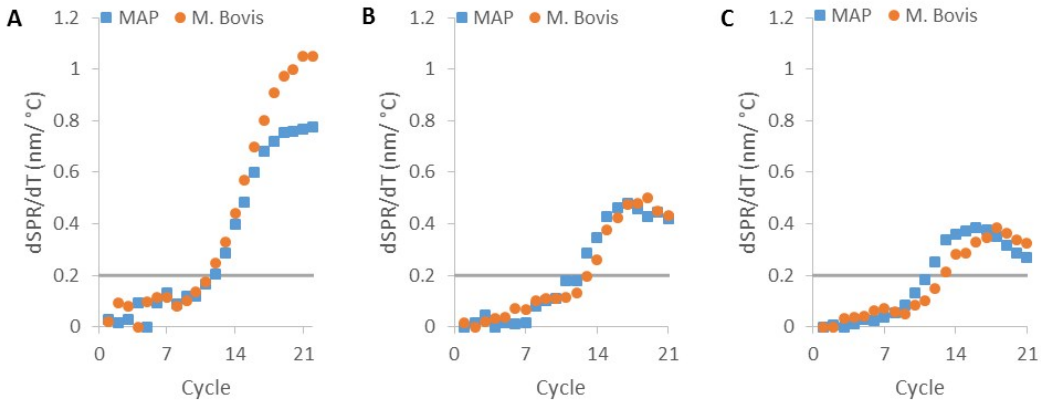

Figure S4. Presentation of the melting peak signals in function of the number of PCR cycles The threshold of 0.2 is shown on the figures. Analysis of the signal height at a concentration of 10 pM is shown for the WT *MAP* and *M. Bovis* target sequence (A), MM1 sequences (B) and MM3 sequences (C).

References

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