Supporting Information

Real-time, label-free analysis reveals novel low-affinity binding to blood group antigens by *Helicobacter pylori*

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Text S1:

Scanning optical microscope for label-free glycoconjugate microarray detection. The amount of the bacteria captured by immobilized Lewis glycoconjugates per unit area, N(t), was recorded in real time by a series of OI-RD images taken at two-hour time interval, from when the bacterial solution was injected into the chambers [1]. The images were acquired with a scanning oblique-incidence reflectivity difference microscope as illustrated in Fig. 1. For illustration the image in Fig. 1 is that of 8 BSA microarrays with a footprint of 3 mm × 4.5 mm, each enclosed in a separate fluid chamber, and the regions outside the chambers. When imaging the Lewis glycoconjugate microarray, we only scan the microarray-covered regions as shown in Fig. 2 and 3, and **Fig. S1**.

Though the principal features of this microscope have been described in two earlier publications [2,3], they are described here for completeness. The key feature different from the earlier arrangement is that the single-chamber fluidic assembly that houses large microarrays (consisting of up to 11,000 targets) is replaced with an 8-chamber fluidic assembly that houses 8 smaller microarrays. The scanning microscope has an encoded translation stage for x-scan and an encoded y-scan mirror.

As illustrated in Fig. 1, a *linearly* polarized *He-Ne* laser beam at $\lambda = 632$ nm passes through a photo-elastic modulator (PEM) so that the polarization of the exit beam changes from *p*- to *s*-

polarization at a frequency Ω = 50 kHz. The beam passes through a phase shifter (PS) that adds a static and yet variable phase Φ_{PS} between the *p*- and *s*-polarized components. Using a combination of a scan mirror (RM) and an *f*-theta lens (FTL), we focus the polarization-modulated beam to a 10-μm spot on the microarray-covered glass slide surface at an incident angle $\theta = 34.66^{\circ}$ inside the glass (with optical constant ε_0). The microarray-covered surface is in contact with an aqueous solution (with optical constant ε_s). The reflected beam from the illuminated spot passes through an analyzer (A) with its transmission axis set at $\theta_A = 45°$ in the present study from *s*-polarization and is imaged with an objective lens on a single-element photodiode (PD) and analyzed with an SR830 digital Lock-in Amplifier (Stanford Research Systems, Palo Alto, CA). Let r_p and r_s be the respective reflectivities for *p*- and *s*-polarized components of the He-Ne laser beam. The ellipsometry ratio is defined by convention as

$$r_p/r_s = \tan \Psi \times \exp(i\delta) \tag{S-1}$$

The amplitude of the first-harmonic (in modulation frequency Ω) in the detected light intensity is given by $I(\Omega) = I_{inc}|r_p r_s|\sin(2\theta_A)\sin(\eta_{sys} + \delta + \Phi_{PS}) = I_{inc}|r_p r_s|\sin(\eta_{sys} + \delta + \Phi_{PS})$. The amplitude of the second-harmonic is given by $I(2\Omega) = I_{inc}(|r_p|^2 - |r_s|^2)$. η_{sys} is the phase difference between the *p*- and *s*-polarized components of the laser beam introduced by elements in the beam path *other than* the microarray-covered surface; δ is the phase difference due to reflection from the surface. Let δ_0 be the phase difference for a bare glass surface. We adjust Φ_{PS} so that $\eta_{sys} + \delta_0 + \Phi_{PS} = 0$ and thus $I(\Omega) = 0$ on the bare glass surface (nulling ellipsometry). When the focused beam is scanned from the bare region to the region covered with immobilized targets or probe-target complexes (with optical dielectric constant ε_d), $I(\Omega)$ becomes $I_{inc}|r_p r_s|\sin(\delta - \delta_0)$. By separately measuring $I_{inc}|r_p r_s|$, we extract $\Delta\delta = \delta - \delta_0$ [2,4,5].

When the absorption of the laser beam by targets and captured probes can be neglected and the thickness d of targets and probe-target complexes are much less than the optical wavelength λ, the targets and probe-target complexes only change δ. As a result we detect the Lewis glycoconjugate microarray and its reaction with recombinant BabA by measuring $\Delta\delta$. The latter varies with d as [2,3].

$$\Delta\delta \cong \frac{(-i)4\pi\sqrt{\varepsilon_s}\varepsilon_0}{(\varepsilon_s-\varepsilon_0)(\varepsilon_s/\varepsilon_0-\cot^2\theta)}\frac{(\varepsilon_d-\varepsilon_0)(\varepsilon_d-\varepsilon_s)}{\varepsilon_d}\left(\frac{d}{\lambda}\right)\Theta_{target}. \quad \text{(S-2)}$$

Θ_{target} is the coverage of the targets. Let $\rho_{probe\text{-}target} = 1.33$ g/cm^3 be the volume mass density of the probe-target complex layer. The surface mass density $\Gamma_{probe\text{-}target} = d\ \Theta_{target}\ \rho_{probe\text{-}target}$ of the probe-target complex layer can be then related to the optical signal $\Delta\delta$ as follows [2,6],

$$\Delta\delta \cong \frac{(-i)4\pi\sqrt{\varepsilon_s}\varepsilon_0}{(\varepsilon_s-\varepsilon_0)(\varepsilon_s/\varepsilon_0-\cot^2\theta)}\frac{(\varepsilon_d-\varepsilon_0)(\varepsilon_d-\varepsilon_s)}{\varepsilon_d\ \lambda\ \rho_{probe-target}}\times\Gamma_{probe-target} \quad \text{(S-3)}$$

We obtain An OI-RD image of a microarray, equivalent of a surface mass density map, by scanning the illumination beam along *y*-direction with the y-scan mirror and moving the glass slide assembly on the linear stage along *x*-direction [2]. Fig. 1 shows the OI-RD image in $\Delta\delta$ of 8 BSA microarrays immobilized on a glass slide. Fig. 2A shows the OI-RD image in $\Delta\delta$ of a small Lewis glycoconjugate microarray before reaction with recombinant BabA protein. Fig. 2B shows the change in OI-RD image of the same microarray after it has reacted with recombinant BabA protein at 400 nM in 1×PBS. **Fig. S1** shows the OI-RD image in $\Delta\delta$ of a larger Lewis glycoconjugate microarray before reaction with *H. pylori*.

When the thickness of captured probe is not small compared to the optical wavelength λ, as is the case for *H. pylori*, the corresponding optical signal change due to probe-target binding reaction on a glycoconjugate microarray is mainly in the magnitude of r_p/r_s. In this case we detect

whole cell bacterial binding to the microarray by measuring $I(2\Omega) = I_{inc}(|r_p|^2 - |r_s|^2)$ instead. This is different from the experimental procedures reported earlier. Presently we divide $I(2\Omega)$ by its value before the bacterial solution is introduced to the microarray and express the OI-RD signal in terms of the *fractional oblique-incidence reflectance difference* defined as

$$S = \frac{\left(|r_p|^2 - |r_s|^2\right) - \left(|r_{p0}|^2 - |r_{s0}|^2\right)}{\left(|r_{p0}|^2 - |r_{s0}|^2\right)} \qquad \text{(S-4)}$$

r_{p0} and r_{s0} are the reflectivity for the *p*- and *s*-polarized components of the illuminating laser beam from a bare glass surface. We detect the binding reaction of *H. pylori* with Lewis glycoconjugate microarrays by following *S* as defined by Eq. (S-4). Fig. 3A,B,C shows the change in *S* of a Lewis glycoconjugate microarray after reaction with *H. pylori* J166WT, J166△*babA,* and J99△*babA*, respectively.

Text S2:

Calibration of coverage of immobilized glycoconjugate targets. Due to variations in wetting properties across a functionalized glass slide surface and physiochemical properties of different targets, and in the state of a printing tip, the surface density or coverage of a target microarray even from the same printing concentration varies from spot to spot and within a spot after the excess printed materials are washed off. Such target density variation in a microarray is common. In fluorescence-based detection, the calibration of the target density is generally not possible. As a result fluorescence-based detection of microarrays is typically used for qualitative or semi-quantitative end-point assays. Our present label-free optical detection platform enables us to acquire intermediate images and use these images to determine the target density or surface

coverage, thus making the OI-RD optical microscope a quantitative assay platform. The strategy is as follows; Let Θ_{target} be the coverage of the immobilized targets on a microarray-covered surface with the maximum of unity (when the targets fully cover the glass surface) and the minimum of zero (in unprinted regions). We take an OI-RD image in $\Delta\delta$ of the microarray before BSA blocking treatment (**Fig. S1**). We designate s_{target} to be the optical signal from a surface *fully* covered with the immobilized targets. The OI-RD image in $\Delta\delta$ is proportional to $\Theta_{target}\, s_{target}$,

$$\Delta\delta_{pre\text{-}blocking} = \eta\, \Theta_{target}\, s_{target} \tag{S-5}$$

After the surface is blocked with a solution of BSA, the uncovered part of the surface is now covered with a layer of blocking BSA. We designate $s_{blocking\text{-}BSA}$ to be the optical signal from the unprinted region. The OI-RD image in $\Delta\delta$ after BSA-blocking treatment is then given by

$$\Delta\delta_{BSA\text{-}blocked} = \Theta_{target}\, s_{target} + (1 - \Theta_{target})\, s_{blocking\text{-}BSA} \tag{S-6}$$

The difference of the two images yields

$$(\Delta\delta_{BSA\text{-}blocked} - \Delta\delta_{pre\text{-}blocking}) = (1 - \Theta_{target})\, s_{blocking\text{-}BSA}. \tag{S-7}$$

We separately measure $\Delta\delta_{BSA} \equiv s_{blocking\text{-}BSA}$ using the same microscope.

From $\Delta\delta_{BSA}$ and Eq. (S-7) we extract Θ_{target} as

$$\Theta_{target} = 1 - (\Delta\delta_{BSA\text{-}blocked} - \Delta\delta_{pre\text{-}blocking})/\Delta\delta_{BSA} \tag{S-8}$$

This information is used in the main text to separate the optical signals due to specific bacterial binding to the targets from the signals due to non-specific bacterial binding to blocking BSA.

Text S3:

Cloning and expression of BabA$_{547}$. A partial fragment of *babA* was cloned into plasmid pOPE101 (Genbank #Y14585). pOPE101 was originally designed for the expression of antibody variable regions and contains an IPTG-inducible *lac* promoter/operator region upstream of a pelB leader sequence [7]. The amino acid sequence of BabA (accession number AAC38081) was submitted to the protein fold recognition server LOOPP (**http://cbsuapps.tc.cornell.edu/loopp.aspx**) and PHYRE (**http://www.sbg.bio.ic.ac.uk/~phyre**). The resulting .pdb files viewed in RasMol (**http://www.rasmol.org/**) identified an outer membrane protein A (OMP-A)-like fold, starting at approximately amino acid 550 and spanning the rest of the BabA C-terminus. A *babA* fragment encoding amino acids 1-547 was amplified using genomic DNA prepared from *H. pylori* strain CCUG 17875 (Qiagen DNeasy Blood and Tissue Kit, Hilden, Germany), high-fidelity polymerase (Fermentas, Lithuania), and oligonucleotides that incorporated restriction sites (underlined) recognized by *Nco1* and *BamH1* in the sense (5'-gaatagggccatggcggaagacgacggcttttacac-3') and antisense (5´-tcgtatggatccgttacgccctaattcttggttg-3) primers, respectively. The column-purified PCR-product was digested with *NcoI* and *BamH1*, gel-purified, and cloned into the appropriately digested pOPE101. The expression cassette consisting of the pelB leader sequence and the truncated *babA*-domain was followed by a c-*myc*- and a $(His)_6$ tag for detection and purification, respectively.

The plasmid encoding BabA$_{547}$ was transformed into *E. coli* XL10 Gold (Stratagene, USA) and transformants were selected for on LB agar plates containing 100 µg/ml carbenicillin, 12.5 µg/ml tetracycline and 0.1 M glucose as a repressor. For expression, an overnight culture in selective LB medium (100 µg/ml carbenicillin, 12.5 µg/ml tetracycline and 0.1 M glucose; hereafter called LB$_{CTG}$) was diluted in freshly prepared LB$_{CTG}$ medium and grown at 37°C until

an optical density (at 600 nm) of 0.6 was reached. The cultures were cooled to room temperature and protein expression was induced by addition of IPTG to a final concentration of 75 µM. After 12 hours induction at 24°C (230 rpm), the proteins were harvested from the periplasmatic space. Briefly, the pelleted bacteria were resuspended in 1/10 culture volume of cold Spheroblast solution (50 mM Tris-HCl pH = 8.0, 20% Sucrose, 1 mM EDTA) and gently shaken for 2 hours at 4°C. The suspension was centrifuged at 30,000 g for 1 hour at 4°C and the supernatant, representing the periplasmatic extract, was dialyzed twice overnight against 5 liters of PBS at 4°C. The solution was passed through a 0.44 µm filter. The NaCl and imidazole concentrations were adjusted to 0.5 M and 25 mM, respectively, in order to adjust to the conditions required for Ni-NTA column purification (GE-Healthcare, USA). The $(His)_6$-tagged protein was eluted with elution buffer (PBS adjusted to 0.5 M NaCl and 0.5 M imidazole) and protein-containing fractions were dialyzed twice overnight against 2 liters of PBS at 4°C. $BabA_{547}$ aliquots were adjusted to 1 mg/ml with PBS and stored at - 20°C. If necessary, the protein was concentrated by ultrafiltration columns (Vivaspin 500, Sartorius-stedim Biotech, Goettingen, Germany). Purity of the BabA protein was confirmed by Coomassie-gel staining and Immunoblot using BabA-specific antisera [8], and a mAb directed against the c-myc tag (9E10) or $(His)_6$-tag (AD1.1.10, Serotec, Germany), respectively. The expected molecular weight of the processed $BabA_{547}$ is 58,572 Da including the tags.

Text S4:

Binding of $BabA_{547}$ to Le^b. Specific binding of $BabA_{547}$ to Le^b was tested by ELISA using immobilized Le^b-HSA glycoconjugates. Briefly, flat-bottomed 96-well microtiter plates (MaxiSorp-Nunc, Roskilde, Denmark) were coated with 100 µl of a Le^b-HSA solution (5 µg/ml

diluted in sodium carbonate buffer pH 9.6) at 4°C over night. Plates were washed and blocked with PBS containing 2% skimmed milk for two hours at room temperature. After washing with PBS containing 0.05% Tween 20 (PBS-T), the wells were incubated with $BabA_{547}$ (5 µg/ml diluted in PBS-T) over night at 4°C. Plates were washed four times with PBS-T and 100 µl of biotinylated anti-myc (Clone 9E10, Sigma Aldrich, St. Louis-USA) diluted 1:2000 in PBS-T was added for 2 hours at room temperature. After extensive washing, Streptavidin-peroxidase (Sigma-Aldrich, St. Louis-USA) diluted 1:2000 in PBS-T was added for 1 hour at room temperature. After plates had been washed four times with PBS-T and twice with PBS, 100 µl of TMB (Tetramethylbenzidine) chromogen substrate (Merck, Darmstadt-Germany) dissolved in sodium acetate/citric acid pH 4.9/H_2O_2 was added. The reaction was stopped by addition of H_2SO_4 and the absorbance was measured at 450 nm.

References:

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Supporting figure captions:

Figure S1. OI-RD image in Δδ of a Lewis glycoconjugate microarray in 1×PBS obtained using the scanning microscope as shown in Fig. 1. The microarray has been washed with 1×PBS to remove the excess printed materials from the glass surface.

Figure S2. Binding of FITC-labeled *H. pylori* 17875/Le^b to 1 μg/μl of Le^a-HSA, Le^b-HSA, Le^x-HSA and Le^y-HSA immobilized on epoxy-functionalized glass slide. Approximately 400 bacterial cells were found to bind to Le^b-HSA spot but none to Le^y-HSA spot (the two upper panels). The results were quantified in the lower panel. Strain 17875/Le^b is a spontaneous mutant of *H. pylori* strain CCUG17875 that binds to ABO/Le^b antigens, but not to sialylated antigens [9]. The bacterial adherence was recorded with Zeiss AXIOcam MRm (Carl Zeiss AB, Stockholm, Sweden) at magnification of 400×. Zeiss AxioVision v.4.5 was used to count the captured bacteria. FITC-labeling and bacterial adhesion procedures were described previously [10].

Figure S3. Recombinant BabA$_{547}$ binds specifically to Le^b-HSA in ELISA: Wells were coated either with the conjugate Le^b-HSA, the control conjugates Le^a-HSA, Le^y-HSA or HSA and BSA only. Recombinant, myc-tagged BabA$_{547}$ was incubated over-night and the bound fraction was detected with biotinylated anti-myc mAb followed by HRP-conjugated streptavidin. As a further control, BabA$_{547}$ was omitted and wells were incubated with the detection reagents only. All samples were measured in triplicate and the mean and the standard errors of OD values are shown.

Fig. S1

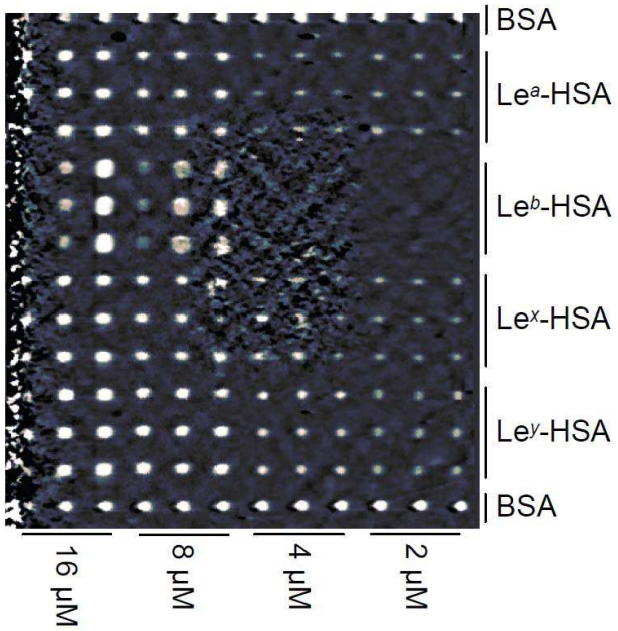


Fig. S2:

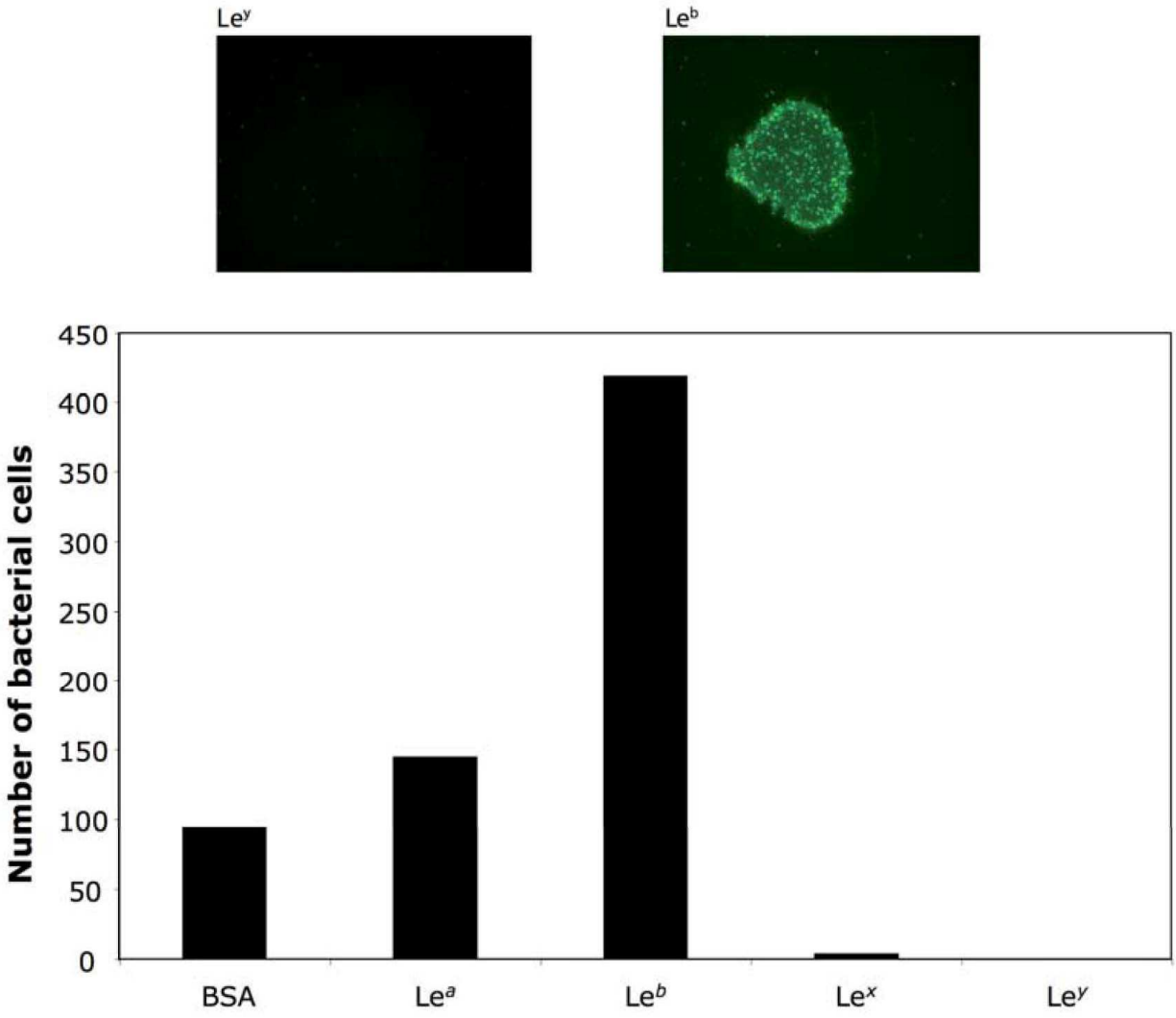
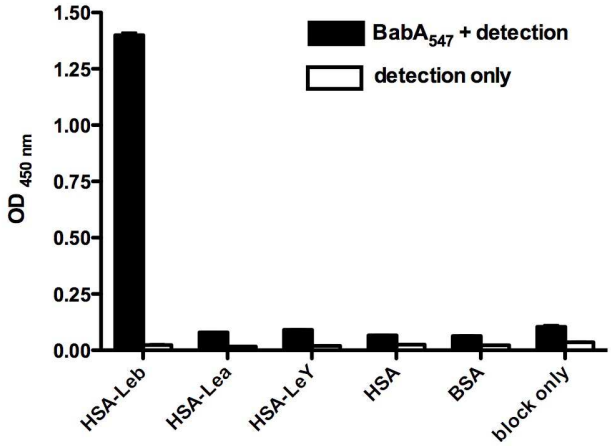

Le^y
Le^b
Number of bacterial cells
450
400
350
300
250
200
150
100
50
0
BSA
Le^a
Le^b
Le^x
Le^y

Fig. S3:


1.50
1.25
1.00
0.75
0.50
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0.00
OD 450 nm
BabA547 + detection
detection only
HSA-Leb
HSA-Lea
HSA-LeY
HSA
BSA
block only