Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information:

1. *Minimum and maximum values of incidence angles at inner/outer bend surface of waveguides:*

Table-1s: Minimum and maximum values of incidence angles at inner/outer bend surface of both U bend surface of embedded *S-bend* waveguides.

Sensing S-Bend Surfaces	Bend Radius (μm)	Φ_1 or δ_1 (degree) Min value	Φ_2 or δ_2 (degree) Max value	ψ_1 (Upper limit)	ψ_1' (Coupled to receiving Fiber)	ψ_2 (Lower limit)	ψ_2' (Coupled to receiving Fiber)
U_1-OUTER	750	51	90	39	39	0	0
U_1-INNER	750	82	90	8	8	0	0
U_2-OUTER	750	41	90	49	39	0	0
U_2-INNER	750	61	90	29	8	0	0

Table-2s: Minimum and maximum values of incidence angles at inner/outer bend surface of both U bend surface of embedded *Spiral-bend* waveguides.

Sensing S-Bend Surfaces	Bend Radius (μm)	Φ_1 or δ_1 (degree) Min value	Φ_2 or δ_2 (degree) Max value	ψ_1 (Upper limit)	ψ_1' (Coupled to receiving Fiber)	ψ_2 (Lower limit)	ψ_2' (Coupled to receiving Fiber)
U_1-OUTER	1000	55	90	35	35	0	0
U_1-INNER	1000	82	90	8	8	0	0
U_2-OUTER	750	89	90	1	1	0	0
U_2-INNER	750	52	90	38	35	0	0

2. *Calculation of Range of angle of incidences for S-bend waveguide:*

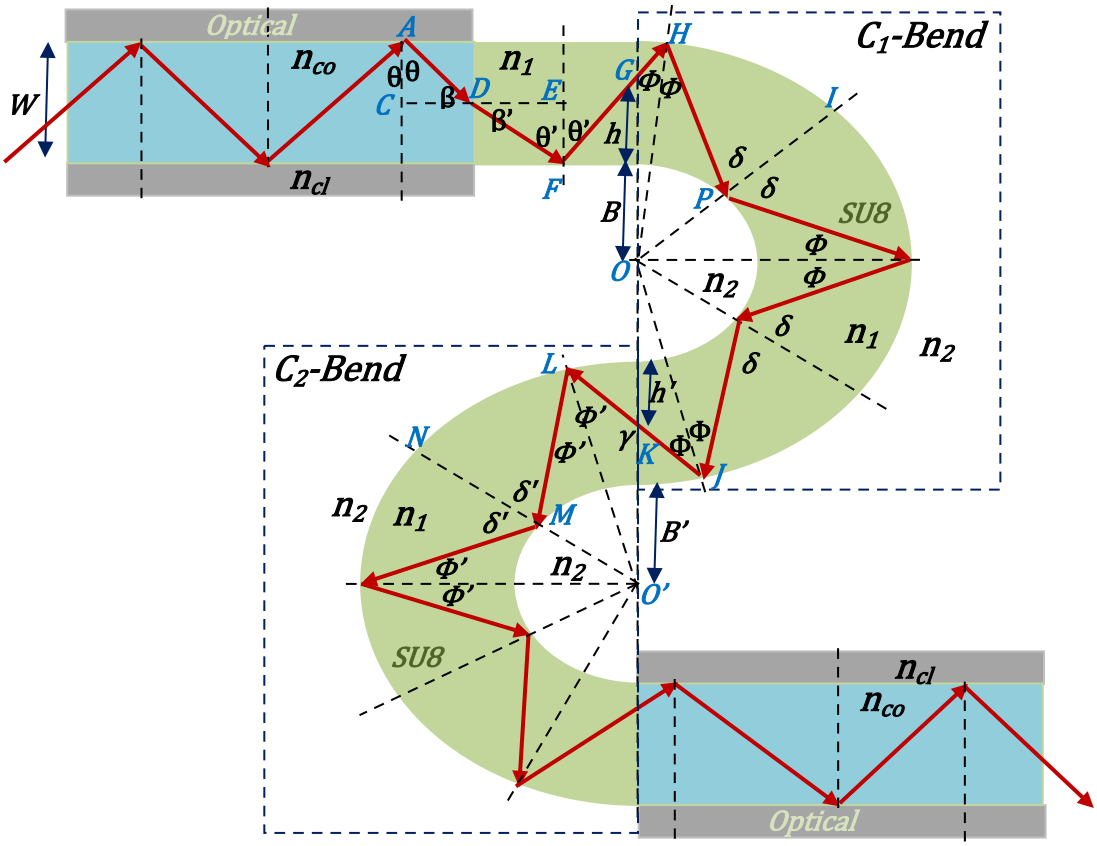


Figure S1: Schematic of ray guidance in two of the U-bends of embedded *S-bend* optical waveguide sensing probe.

Assuming:-

Angle ADC = β; Angle EDF = β'

Angle GHO = Φ; Angle HPI = δ

Angle KLO' = Φ'; Angle LMN = δ'

Angle JKO = Angle LKO' = γ ;

Refractive indices (RI) of Optical fiber Core and cladding are n_{co} and n_{cl} respectively.

Refractive indices (RI) of SU8 waveguide Core and analyte cladding are n_1 and n_2 respectively.

2.1 For C_1 - Bend structure of S-bend waveguide:

For figure S1, in triangle ΔACD and ΔDEF:-

$n_{co} \times Sin\beta = n_1 \times Sin\beta'$ => $Sin\beta' = \frac{n_{co}}{n_1} \times Sin\beta$ =>

$Sin(\frac{\mu}{2} - \theta') = \frac{n_{co}}{n_1} \times Sin(\frac{\mu}{2} - \theta)$

=> $Cos\theta' = \frac{n_{co}}{n_1} \times Cos\theta$ or, $Sin\theta' = \sqrt{1 - (\frac{n_{co}}{n_1} \times Cos\theta)^2}$ .....(1)

If, $\theta = \theta_c$ (For optical fiber)

$$Sin\theta_c = \frac{n_{cl}}{n_{co}} \quad => \quad cos\theta_c = \sqrt{1 - (\frac{n_{cl}}{n_{co}})^2} \quad .....(1a)$$

From equation 1 and 1a:

$$Sin\theta_c' = \sqrt{1 - (\frac{n_{co}}{n_1})^2 + (\frac{n_{cl}}{n_1})^2} \quad .....(2)$$

Considering the ΔOGH:

$$\Phi = \sin^{-1}[\frac{(B+h)}{(B+W)} \times Sin\theta'] .....(3)$$

For, $\theta' = \theta_c'$;

$$\Phi_1 = \sin^{-1}[\frac{(B+h)}{(B+W)} \times \sqrt{1 - \left(\frac{n_{co}}{n_1}\right)^2 + \left(\frac{n_{cl}}{n_1}\right)^2}] .....(4)$$

For, $\theta' = \pi/2$;

$$\Phi_2 = \sin^{-1}[\frac{(B+h)}{(B+W)}] .....(5)$$

Considering the ΔHOP:

$OP/\sin\Phi = OH/\sin(\pi - \delta)$;

$\sin\delta = (OH/OP) * \sin\Phi = (B+W/B) * \sin\Phi = (B+h/B) * \sin\theta'$;

$$\delta_1 = \sin^{-1}[\frac{(B+h)}{(B)} \times \sqrt{1 - \left(\frac{n_{co}}{n_1}\right)^2 + \left(\frac{n_{cl}}{n_1}\right)^2}] .....(6)$$

2.2 For C_2 - Bend structure of S-bend waveguide:

Ideally, the angle of incidences of individual rays at outer or inner bend surface (i.e. Φ and δ) of the light receiving part of 1st bend (C_1) will remain unchanged up to the other end of C_1-bend waveguide, which couples light finally in to the C_2 - bend structure of waveguide.

For Figure S1, considering the ΔOJK:

$OJ/\sin\gamma = OK/\sin\Phi;$

$\text{Sin}\ \gamma = (OJ/OK)^* \sin\Phi = (B+W/B+h')\sin\Phi$.....(A)

Considering the ΔO'KL:

$O'L/\sin\gamma = O'K/\sin\Phi';$

$\sin\Phi' = (O'K/O'L)^* \sin\gamma = [(B'+W\text{-}h)/(B'+W)\ ]^* \sin\gamma$

From equation (A):

$\sin\Phi' = [(B'+W\text{-}h)/(B'+W)\ ]^* (B+W/B+h')\ \sin\Phi;$

Here, after different number of TIR in 1st bend, light rays couple in to the 2nd bend structure. The angle of incidences (Φ) of rays (from 1st bend) which may couple into the 2nd bend will vary between the critical angle of SU8 - analyte surface (i.e. $\theta_c = \sin^{-1} n_2/n_1$) and 90°.

So:

$$\Phi'_1 = \sin^{-1}\left[\frac{(B+W)}{(B'+W)} \times \frac{(B'+W-h')}{(B'+h')} \times \left(\frac{n_2}{n_1}\right)\right] \text{.....(7)}$$

and,

$$\Phi'_2 = \sin^{-1}\left[\frac{(B+W)}{(B'+W)} \times \frac{(B'+W-h')}{(B'+h')}\right] \text{.....(8)}$$

If B=B', then:

$\sin\Phi' = [(B+W\text{-}h)/(\ B+h')\ ]^* \sin\Phi;$

For Figure S1, considering the Δ LO'M:

$O'L/\sin(\pi - \delta') = O'M/\sin\Phi';$

$\sin\delta' = (O'L/O'M)^* \sin\Phi' = [(B'+W)/(B')\ ]^* \sin\Phi'$

So

$$\delta'_1 = \sin^{-1}\left[\frac{(B' + W)}{B'} \times \frac{(B + W)}{(B' + W)} \times \frac{(B' + W - h')}{(B' + h')} \times \left(\frac{n_2}{n_1}\right)\right] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (9)$$

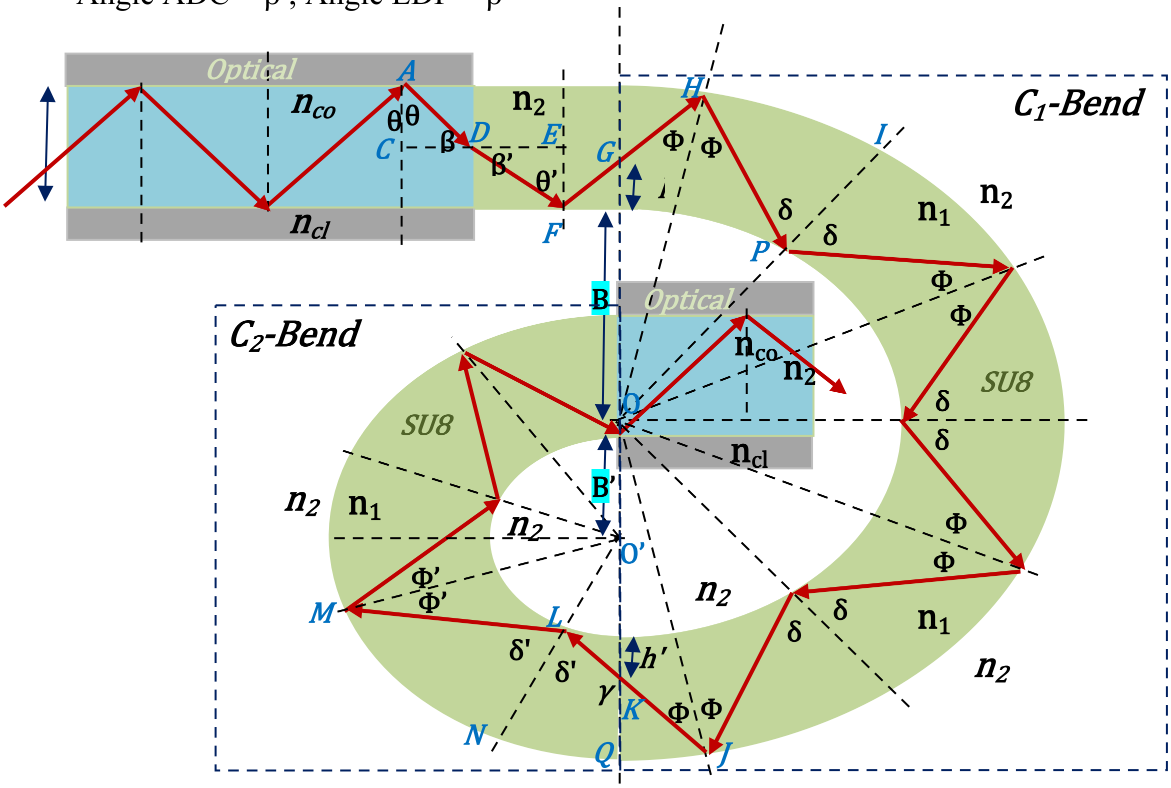
and,

$$\delta'_2 = \sin^{-1}\left[\frac{(B' + W)}{B'} \times \frac{(B + W)}{(B' + W)} \times \frac{(B' + W - h')}{(B' + h')}\right] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (10)$$

3. Calculation of Range of angle of incidences for Spiral waveguide:

Assuming:-

Angle ADC = β ; Angle EDF = β'



Angle GHO = Φ ; Angle HPI = δ

Figure S2: Schematic of ray guidance in two of the U-bends of embedded *Spiral-bend* optical waveguide sensing probe.

Angle LMO' = Φ' ; Angle KLN = δ'

Angle JKO = Angle LKQ = γ

Refractive indices (RI) of Optical fiber Core and cladding are n_{co} and n_{cl} respectively.

Refractive indices (RI) of SU8 waveguide Core and analyte cladding are n_1 and n_2 respectively.

3.1 For C_1 - Bend structure of Spiral waveguide:

It is similar to C_1 Bend structure of S-bend waveguide.

For Figure S2, considering the ΔOGH:

$$\Phi = \sin^{-1}\left[\frac{(B+h)}{(B+W)} \times Sin\theta'\right] \text{..}(3)$$

For, θ' = θ_c' ;

$$\Phi_1 = \sin^{-1}\left[\frac{(B+h)}{(B+W)} \times \sqrt{1-\left(\frac{n_{co}}{n_1}\right)^2+\left(\frac{n_{cl}}{n_1}\right)^2}\right] \text{...}(4)$$

For, θ' = π/2;

$$\Phi_2 = \sin^{-1}\left[\frac{(B+h)}{(B+W)}\right] \quad \text{..........(5)}$$

For figure S2, considering the ΔHOP:

$OP/\sin\Phi = OH/\sin(\pi - \delta)$;

$\sin\delta = (OH/OP)^* \sin\Phi = (B+W/B) \sin\Phi = (B+h/B) \sin\theta'$;

$$\delta_1 = \sin^{-1}\left[\frac{(B+h)}{(B)} \times \sqrt{1 - \left(\frac{n_{co}}{n_1}\right)^2 + \left(\frac{n_{cl}}{n_1}\right)^2}\right] \quad \text{..........(6)}$$

3.2 For C_2 - Bend structure of Spiral waveguide:

Ideally, the angle of incidences of individual rays at outer or inner bend surface (i.e. Φ and δ) of the light receiving part of 1st bend (C_1) will remain unchanged up to the other end of C_1-bend waveguide, which couples light finally in to the C_2 - bend structure of waveguide.

For Figure S2, Considering the ΔOJK:-

$OJ/\sin\gamma = OK/\sin\Phi$;

$\sin\gamma = (OJ/OK)^* \sin\Phi = (B+W/B+h') \sin\Phi$ ....(B)

For Figure S2, Considering the ΔO'KL:-

$O'L/\sin(\pi-\gamma) = O'K/\sin(\pi - \delta')$;

$\sin\delta' = (O'K/O'L)^* \sin\gamma = [(B'+h')/(B')]^* \sin\gamma$;

From equation (B):

$\sin\delta' = [(B'+h')/(B')]^* [(B+W)/(B+h')] \sin\Phi$

Here, after different number of TIR in 1st bend, light rays couple in to the 2nd bend structure. The angle of incidences (Φ) of rays (from 1st bend) which may couple into the 2nd bend, will vary between the critical angle of SU8 - analyte surface (i.e. $\theta_c = \sin^{-1} n_2/n_1$) and 90°.

So:

$$\delta'_1 = \sin^{-1}\left[\frac{(B+W)}{(B+h')} \times \frac{(B'+h')}{(B')} \times \left(\frac{n_2}{n_1}\right)\right] \quad \text{..........(11)}$$

and,

$$\delta'_2 = \sin^{-1}\left[\frac{(B+W)}{(B+h')} \times \frac{(B'+h')}{(B')}\right] \quad (12)$$

For Figure S2, considering the Δ LO'M:

O'M/sin (π - δ') = O'L/sin Φ';

sin Φ' = (O'L/O'M)* sin δ' = [(B')/(B'+W)]* sin δ'

So:

$$\Phi'_1 = \sin^{-1}\left[\frac{B'}{B'+W} \times \frac{(B+W)}{(B+h')} \times \frac{(B'+h')}{(B')} \times \left(\frac{n_2}{n_1}\right)\right] \quad (13)$$

and,

$$\Phi'_2 = \sin^{-1}\left[\frac{B'}{B'+W} \times \frac{(B+W)}{(B+h')} \times \frac{(B'+h')}{(B')}\right] \quad (14)$$

4. Calculated calibration curves of methylene blue at 625 nm for embedded Spiral and S-bend waveguide:

Calculated calibration curves of methylene blue at 625 nm for embedded (1) Spiral and (2) S-bend waveguide has been explained in figure S3.

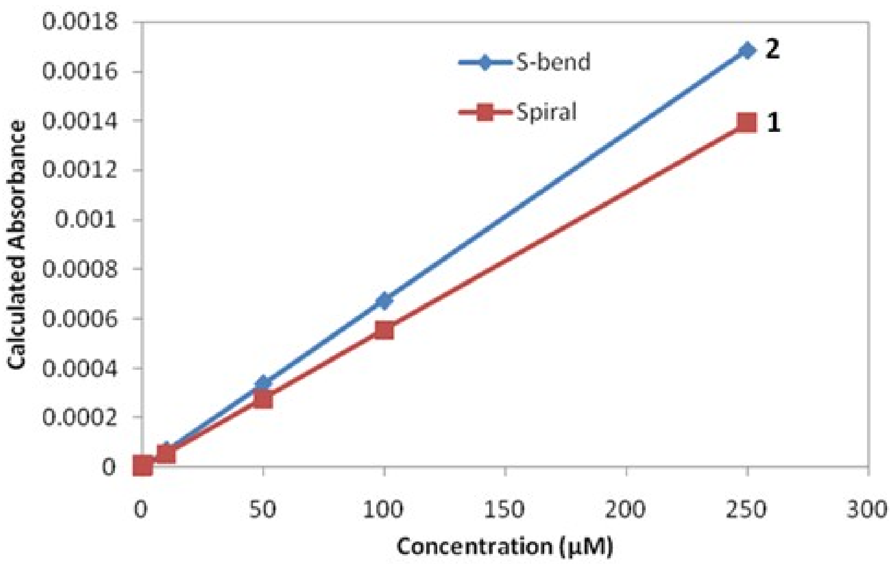


Figure S3: Calculated calibration curves of methylene blue at 625 nm for embedded (1) Spiral and (2) S-bend waveguide.

5. Methylene blue as analyte

Suitability of these devices for analytical purposes has been demonstrated by evanescent wave absorption measurements with Methylene Blue solution as an analyte. The selection of this dye molecule for the experiments is due to the fact that the adsorption of positively charged methylene blue molecules over the negatively charged SU8 surfaces has comparable reaction kinetics to receptor-analyte binding kinetics. The origin of the negative surface charge of SU8 surface could be due to the hexafluoroantimonate complex (SbF_6^-), added as photoinitiator [26]. K. Fujita *et al.,* [27] have reported that the dimerisation or trimerisation of methylene blue molecules occur more extensively over charged surfaces (here SU8 waveguides) rather than its aqueous solution. After the dye solution is introduced into the microchannel, these molecules interact with the waveguide surface and undergo dimerisation and trimerisation as well as adsorb on the surface, thereby increasing the extinction coefficient at the surface between 575 nm to 625 nm. Electrostatic binding phenomenon of positively charged methylene blue molecules on specific negative molecular sites (due to SbF_6^-photoinitiator) of SU8 waveguide surface can be compared with specific binding of FITC tagged GaHIgG analyte with immobilized HIgG molecular sites on waveguide surface (Figure S4), discussed in our earlier work [15, 16]. The forces binding the antigen-antibody complex are not firm covalent bonds but delicate bonds, appropriately named *weak interactions* [28].

Based on chemical composition of SU8 (*Reference: URL1*), surface density of SbF_6^- photoinitiator (causing negative charge on surface) can be estimated as ~10^{-3} moles per unit mole of SU8. Assuming a ~2nm linear span for single SU8 molecules (uncrosslinked), 0.04 nano-moles (nM) of SU8 molecules, and 4×10^{-5} nM of SbF_6^- can be assumed to be existing in 1cm^2 of SU8 surface area. This means that positively charge methylene blue molecules can bind over 4×10^{-5} nM binding sites in 1 cm^2 surface area of SU8. On other hand, HIgG bioreceptor molecules (binding sites for GaHIgG analyte) are immobilized on SU8 surface, based on created hydroxyl group (-OH), caused by acid/alkali based cleavage of SU8 epoxy bond [15, 29]. Under favorable conditions, a single molecule of SU8 (uncrosslinked) may create 16 hydroxyl (-OH) groups. Assuming that only four of these participate in the HIgG binding process, (0.04×4) nM useful hydroxyl groups can be created in 1 cm^2 surface area of alkali/acid treated SU8. Because of higher surface density of -OH group, density of HIgG bioreceptor molecules are expected to be higher than that of methylene blue binding sites on SU8 surface (Figure S4).

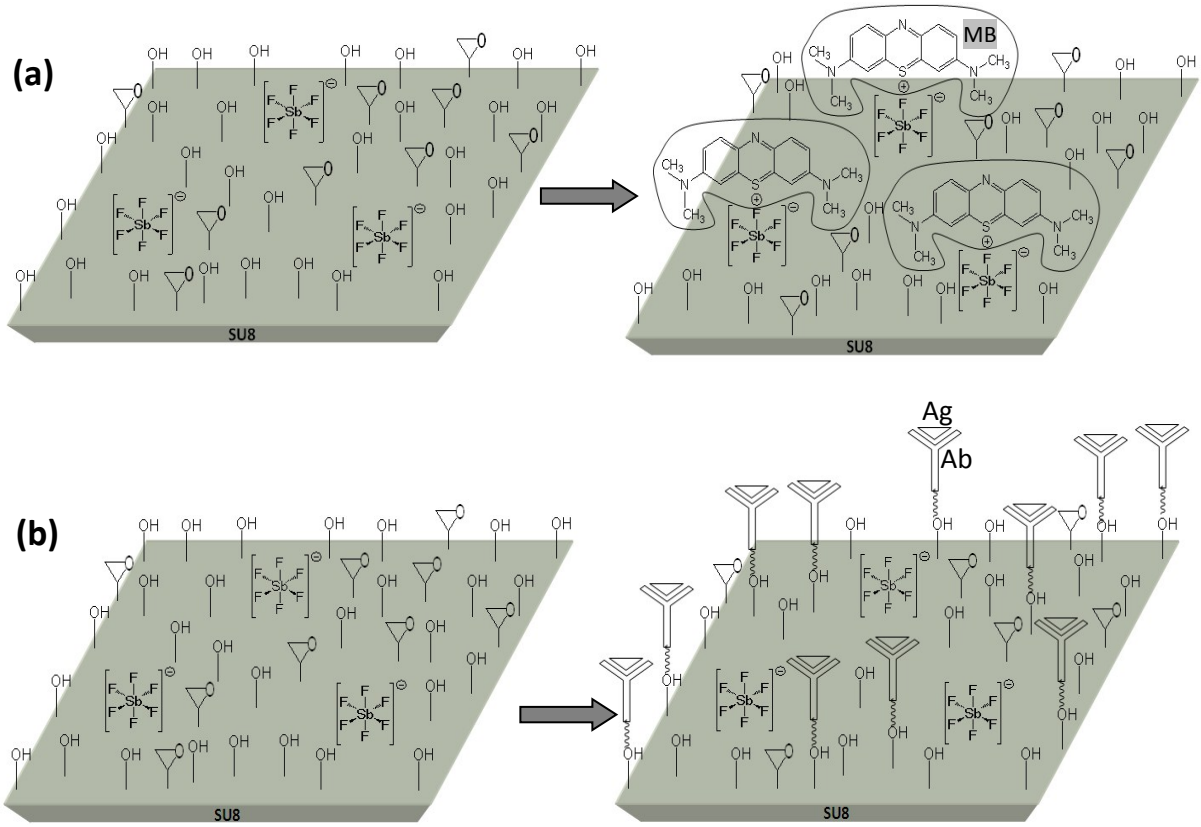


Figure S4: Schematic presentation of distribution of: (a) negative charges (due to SbF_6^- groups) on SU8 surface and binding of positively charged methylene blue molecules on these specific sites, (b) OH groups (due to breaking of epoxy bond by acid/alkali treatment) on SU8 surface and binding of antibody (antigen complex) on these specific groups (via Glycine and EDC/NHS molecules [20]; Ab =Antibody, Ag =Antigen, MB = Methylene Blue

To calculate the number of moles of HIgG and analyte GaHIgG interacting with each other, we assume 50nm^2 binding area of single HIgG molecule, 50% binding efficiency of the immobilization process, an embedded surface of single bend waveguide with 1mm bend radius (surface area = ~4×10^5 μm^2). This can accommodate ~0.7×10^{-5} nanomoles of HIgG bioreceptor molecule. Amount of bio-receptor adsorption density is quite similar to the earlier reported study by Tao *et al.,* [29] for protein adsorption on functionalized SU8 surface. Considering, bivalent (i.e. 2 similar antigen binding sites in single molecule) property of IgG biomolecules, 0.7×10^{-5} nanomoles can bind with ~1.4×10^{-5} nanomoles of GaHIgG molecules. Dissociation constant (k_d) for antigen antibody reaction are usually in range of 10^{-7} M, which indicates that, almost a negligible concentration of free antigen/antibody exist in solution, after the reaction of equimolar concentration of both reactants proceed in to the equilibrium state. In view of ~0.04 microlitre volume of GaHIgG injected in to embedded microchannel

(Microchannel volume = 2mm×200µm×100µm = 0.04 microlitre) and ~40µg/ml of saturation binding concentration, calculation reveals that ~1.3×10^{-5}nanomoles of these molecules were required (experimentally) for saturation binding with ~0.7×10^{-5}nanomoles of HIgG bioreceptor molecule bound on the surface. It reveals a close match between theoretical (~1.4×10^{-5}nanomoles) and experimental (~1.3×10^{-5}nanomoles) values of bound analyte (FITC tagged GaHIgG) molecules over bioreceptor (HIgG) immobilized on SU8 waveguide surface.

Experimental results also suggest that, for similar absorbance changes in single bend embedded waveguide probes, a ~ 70 times lower molar concentration of FITC tagged GaHIgG molecules are required, compared to methylene blue molecules [15] (***view section 6 of supplementary information***). As because the dual bend waveguide is able to resolve 1µM of Methylene blue, so it may be comfortably expected that these waveguide will be able to detect below 2µg/ml concentration of FITC tagged GaHIgG analyte. To experimentally validate these theoretical findings, both *embedded S-bend* and *Spiral waveguides* were designed and microfabricated. Its detail is explained in next section.

6. Comparative sensitivity for Methylene blue and FITC tagged GaHIgG molecule:

Experimental results suggest that, for similar absorbance changes in single bend embedded waveguide probes, a ~ 70 times lower molar concentration of FITC tagged GaHIgG molecules are required, compared to methylene blue molecules. It can be explained by the fact that, extinction coefficient of methylene blue (30,000 M^{-1} cm^{-1} at 600 nm) is ~2.4 times lower compared to FITC (70,000 M^{-1} cm^{-1} at 495 nm). Further, ~5 *molar incorporation ratio* (MIR) of GaHIgG molecules (i.e. the average 5 moles of label FITC molecule covalently bound to one mole of protein GaHIgG molecule) used for experiments (*URL 2*), may cause ~12 times higher extinction coefficient of FITC tagged GaHIgG molecules compared to methylene blue molecules. Furthermore, a much higher density of binding sites (i.e. bioreceptor HIgG) for FITC tagged GaHIgG molecules compared to negatively charged binding sites (i.e. SbF_6^-) for methylene blue molecules may compensate for the rest of the difference.