Supplementary Information (SI) for Analyst.
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Supporting Information

Bioluminescence Readout Lateral Flow Immunoassay Using Nanobody Targeting Aflatoxin B1

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Table S1 IC_{50} values, limit of detection (LOD; IC_{90} values) and mean relative standard deviations over the 0.01 – 1000 ng/mL AFB1 concentration range obtained for devices without conjugate pads fabricated with nitrocellulose membranes of different flow speeds and bioluminescence signals recorded at different times after furimazine substrate solution application (concentration of nanobody-Nluc 5 µg/mL). The corresponding response curves are shown in Fig. 1 in the main text.

	CN140 (150 s/40 mm)			HF90 (90 s/40 mm)		
Time[a] (min)	IC_{50} (ng/mL)	LOD(IC_{90}) (ng/mL)	RSD_{mean} (%)	IC_{50} (ng/mL)	LOD(IC_{90}) (ng/mL)	RSD_{mean} (%)
0	**3.83**	**0.23**	**9.8**	12.1	1.22	10.2
3	6.20	1.63	19.1	12.2	1.83	13.3
6	6.88	2.74	17.4	13.2	2.17	18.6
9	6.05	1.22	14.8	10.1	1.15	11.4
12	6.19	0.92	15.4	9.94	0.97	14.7
15	6.55	0.97	15.8	10.1	1.02	13.4

[a] A delay of 30 s between substrate solution application and start of signal acquisition applies to all cases.

Table S2 Nanobody-Nluc concentration-dependent IC_{50} values and limit of detection (LOD; IC_{90} values) obtained for devices without conjugate pads.

Nanobody-Nluc (µg/mL)	IC_{50} (ng/mL)	LOD(IC_{90}) (ng/mL)
1.0	4.59	0.54
2.5	7.73	1.29
5.0	**4.44**	**0.26**
10	15.2	3.45

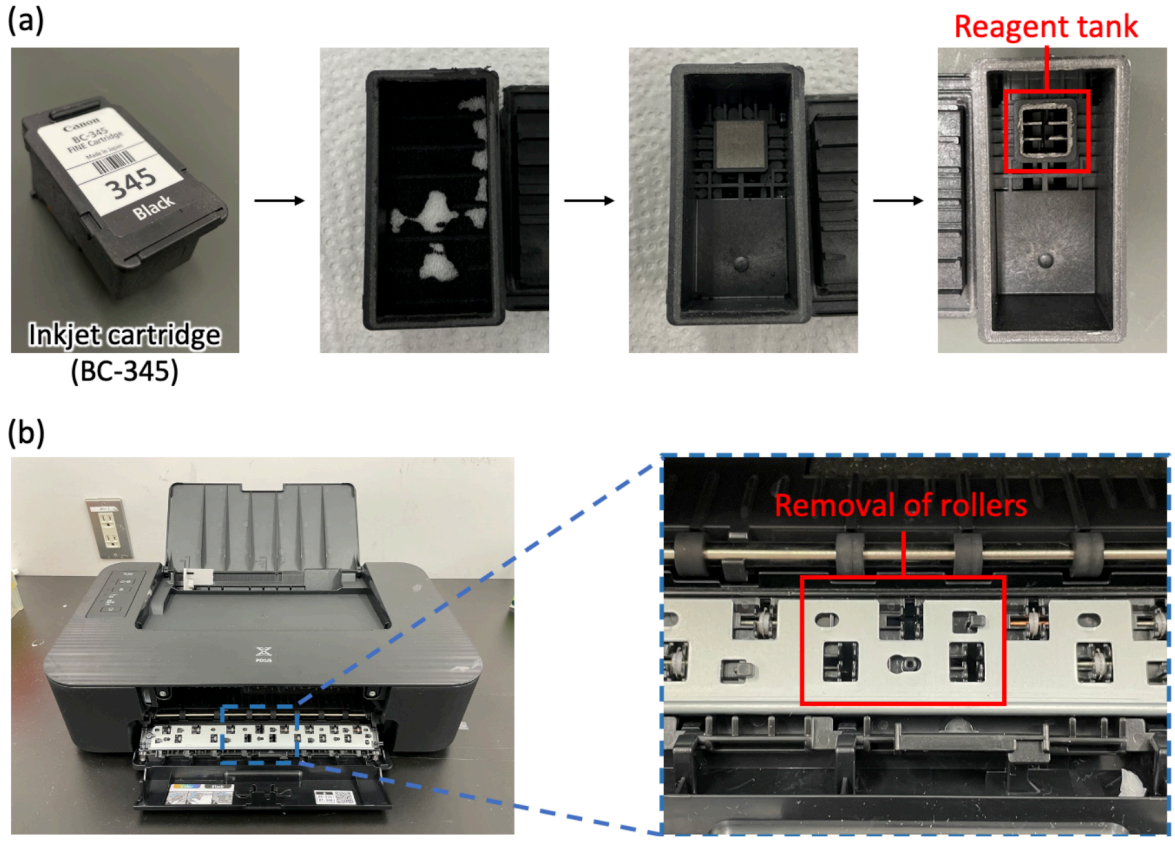
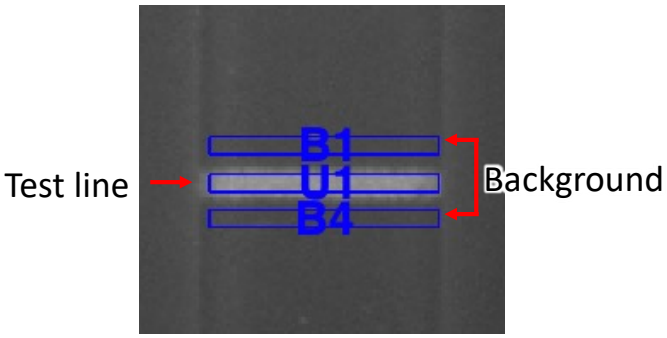


Fig. S1 Preparation of the Canon PIXUS TS203 office inkjet printer for inkjet-based deposition of AFB1-BSA conjugate as test lines on nitrocellulose membranes: (a) black ink cartridges (Canon BC-345) were cut open, the original ink liquid discarded, the sponge removed and washed with copious amounts of pure water; the cover of the ink outlet was removed and the exposed underlying reservoir served as the reagent ink tank; (b) in order to protect the sensitive nitrocellulose membrane from physical damage, paper feed rollers in the central section of the printer were removed.

(a)



(b)

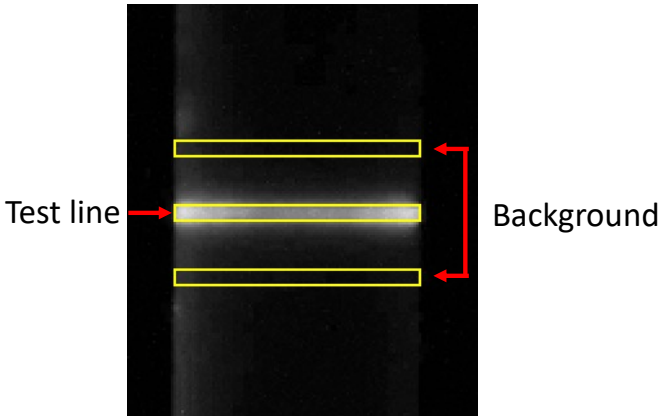
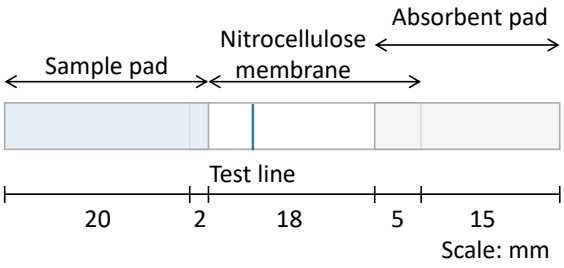


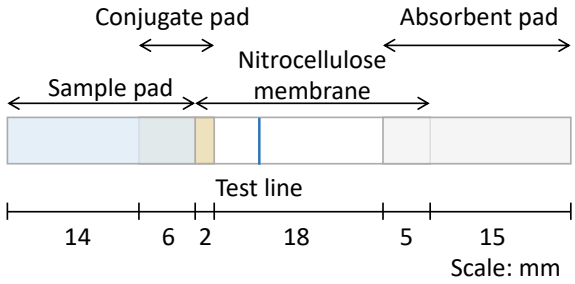
Fig. S2 Positions for collecting test line and background bioluminescence signals on the nitrocellulose membrane on the examples of: (a) signal collected with the ChemiDoc imaging system; background signal was extracted using Image Lab from the marked rectangles (8 x 146 pixels) located 30 pixels above and below the test line; (b) signal collected with the compact digital camera; background signal was extracted using ImageJ from the marked rectangles (25 x 380 pixels) located 100 pixels above and below the test line.

(a)



Sample pad	: Glass fiber (GFDX)
NC membrane	: CN140 or HF90
Absorbent pad	: Cellulose fiber (CF7)
Test line	: 0.01w/v% AFB1-BSA various printing cycles

(b)



Sample pad	: Glass fiber (GFDX)
Conjugate pad	: Glass fiber (GFDX)
NC membrane	: CN140
Absorbent pad	: Cellulose fiber (CF7)
Test line	: 0.01w/v% AFB1-BSA 20 printing cycles

Fig. S3 Dimensions and arrangement of components used to fabricate LFIA test strips: (a) setup without conjugate pad used for system optimization experiments and (b) system with glass fiber conjugate pad containing pre-deposited Nanobody-Nluc used for application proof-of-concept experiments.

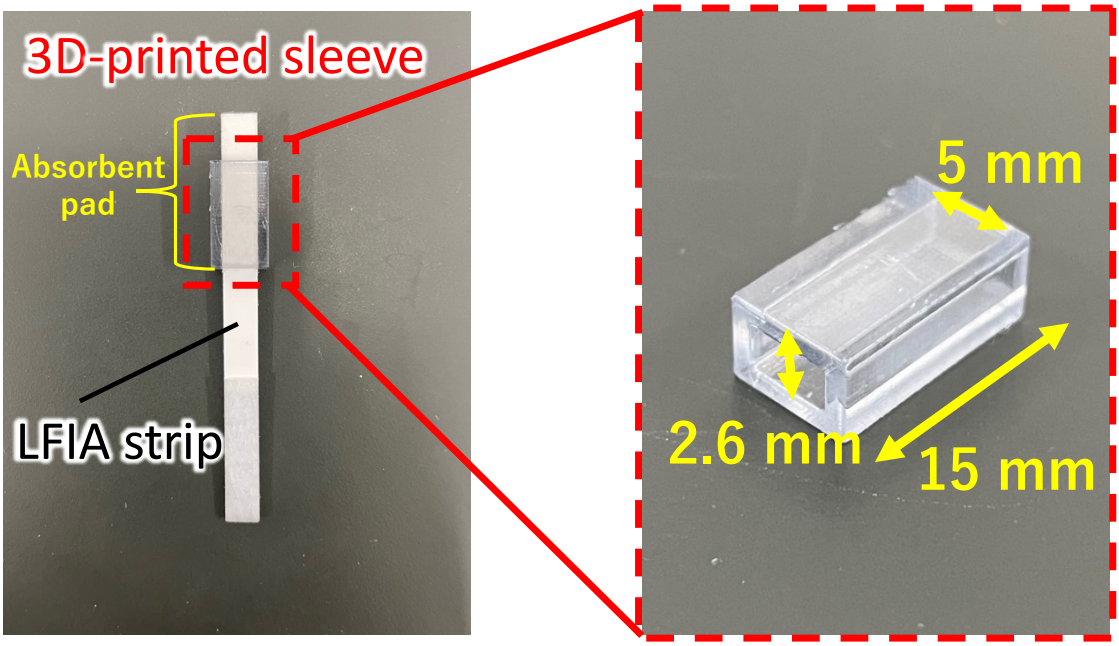


Fig. S4 3D-printed plastic sleeve used to achieve reproducible contact between nitrocellulose membrane and absorbent pad.

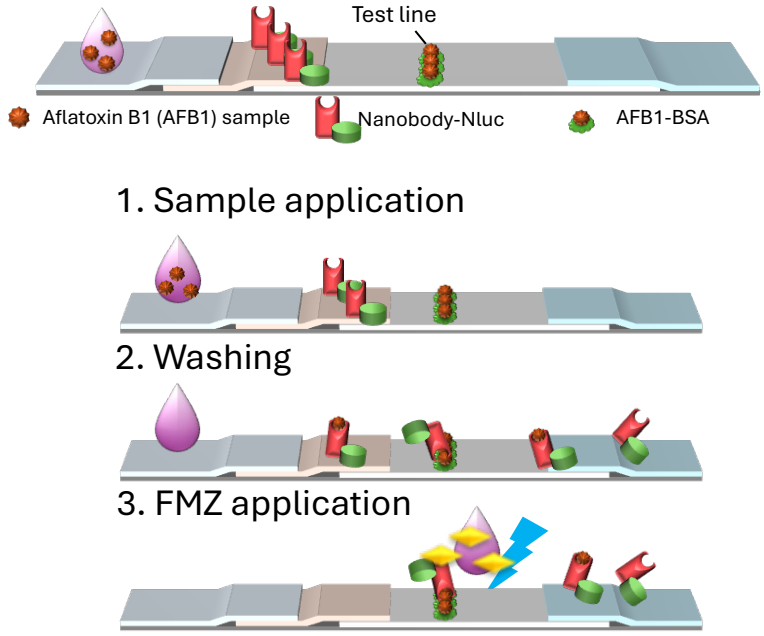


Fig. S5 Principle of the Nanobody-Nluc-based competitive LFIA assay targeting AFB1.

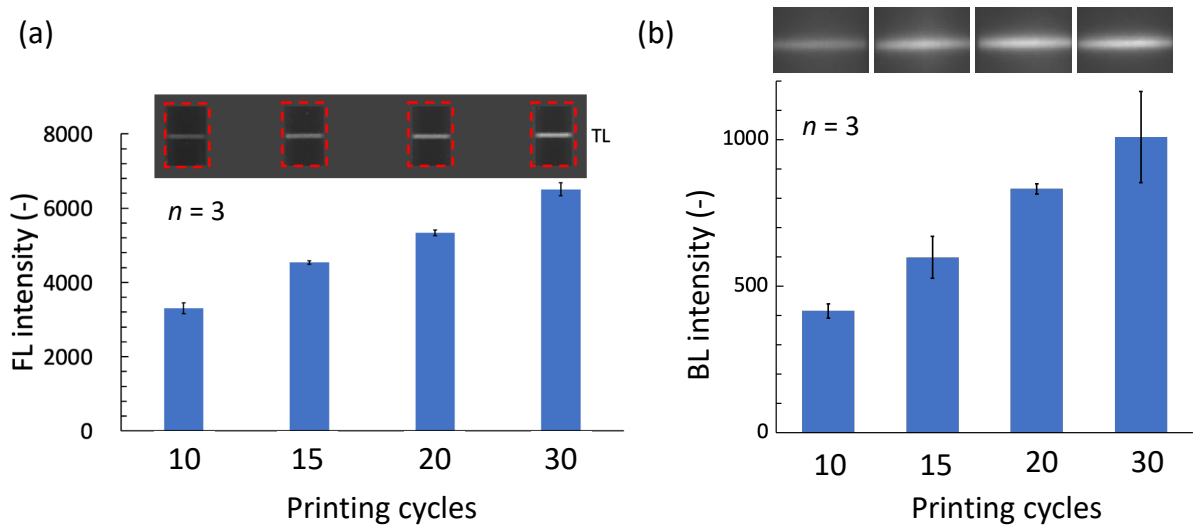


Fig. S6 Optimization of amounts of AFB1-BSA conjugate capture reagent (0.01 w/v% solution) deposited by inkjet printing in multiple printing cycles on the test line: (a) observed by intrinsic fluorescence emission of AFB1, and (b) bioluminescence emission observed after application of a blank sample with 5.0 µg/mL Nanobody-Nluc; error bars represent mean values ±1σ.

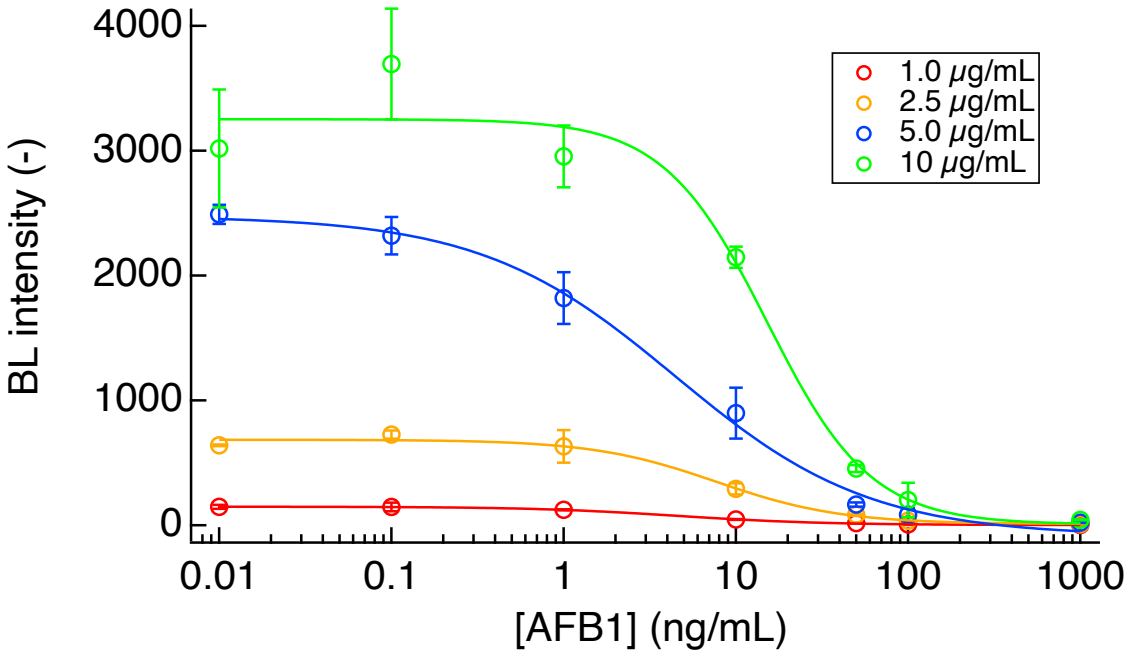


Fig. S7 Optimization of amounts of Nanobody-Nluc (1.0 – 10 µg/mL) added to the running buffer in assays on LFIA devices without conjugate pad.