Supporting Information

Ultra-sensitive sniff-cam for biofluorometric-imaging of breath ethanol caused by metabolism of intestinal flora

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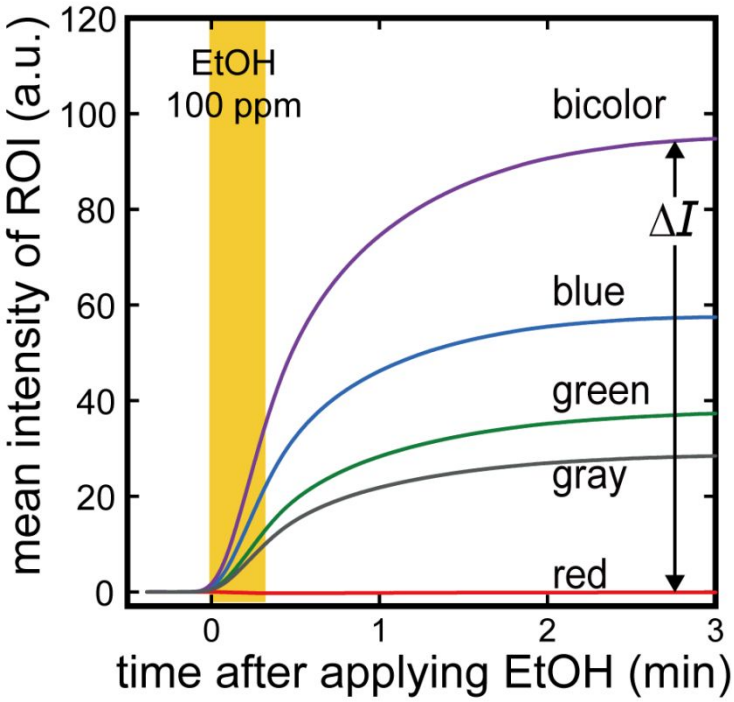

Figure S-1. Time course of the mean intensity of ROI with various image analysis method. ΔI was calculated by averaging a mean intensity of ROI at 180 to 180 s after applying EtOH.

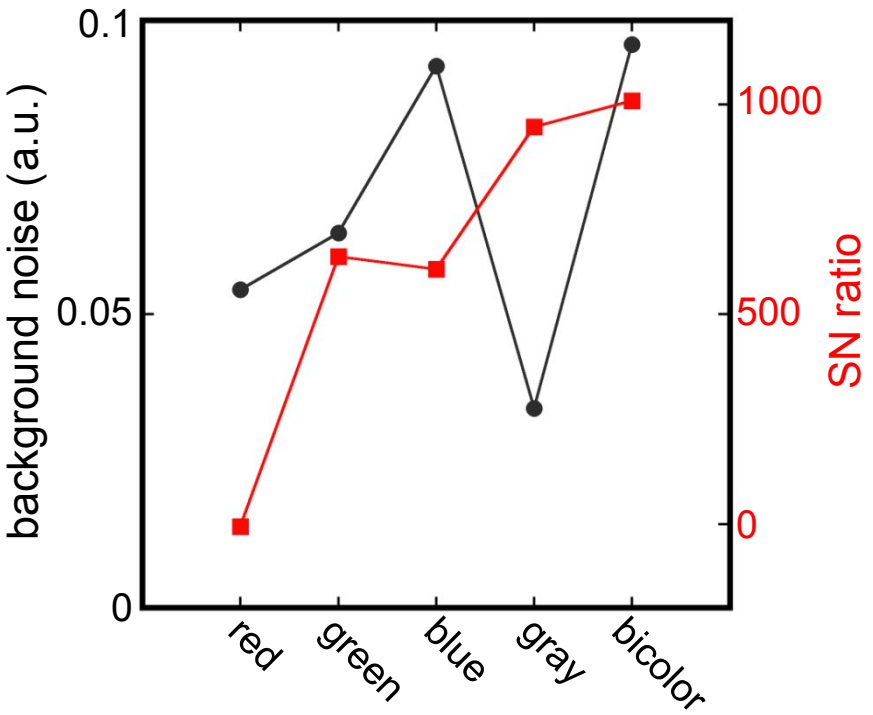

Figure S-2. Comparison of background noise and SN ratio between 5 different image analysis method.

Table S-1. Preparation condition of NAD^+/NADH mixture solution

Molar ratio	NAD^+ (mM)	NADH (mM)
Control	0	0.1
1:1	0.1	0.1
10:1	1	0.1
100:1	10	0.1
1000:1	100	0.1

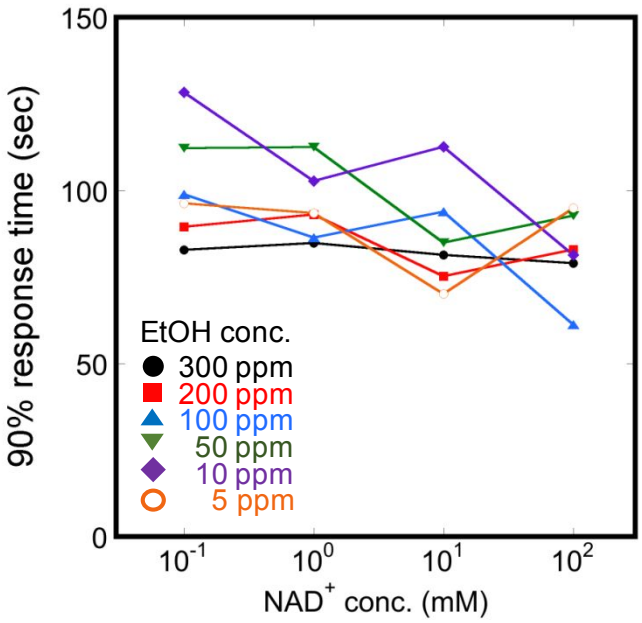

Figure S-3. The relationship between 90% response time and initial NAD^+ concentration.

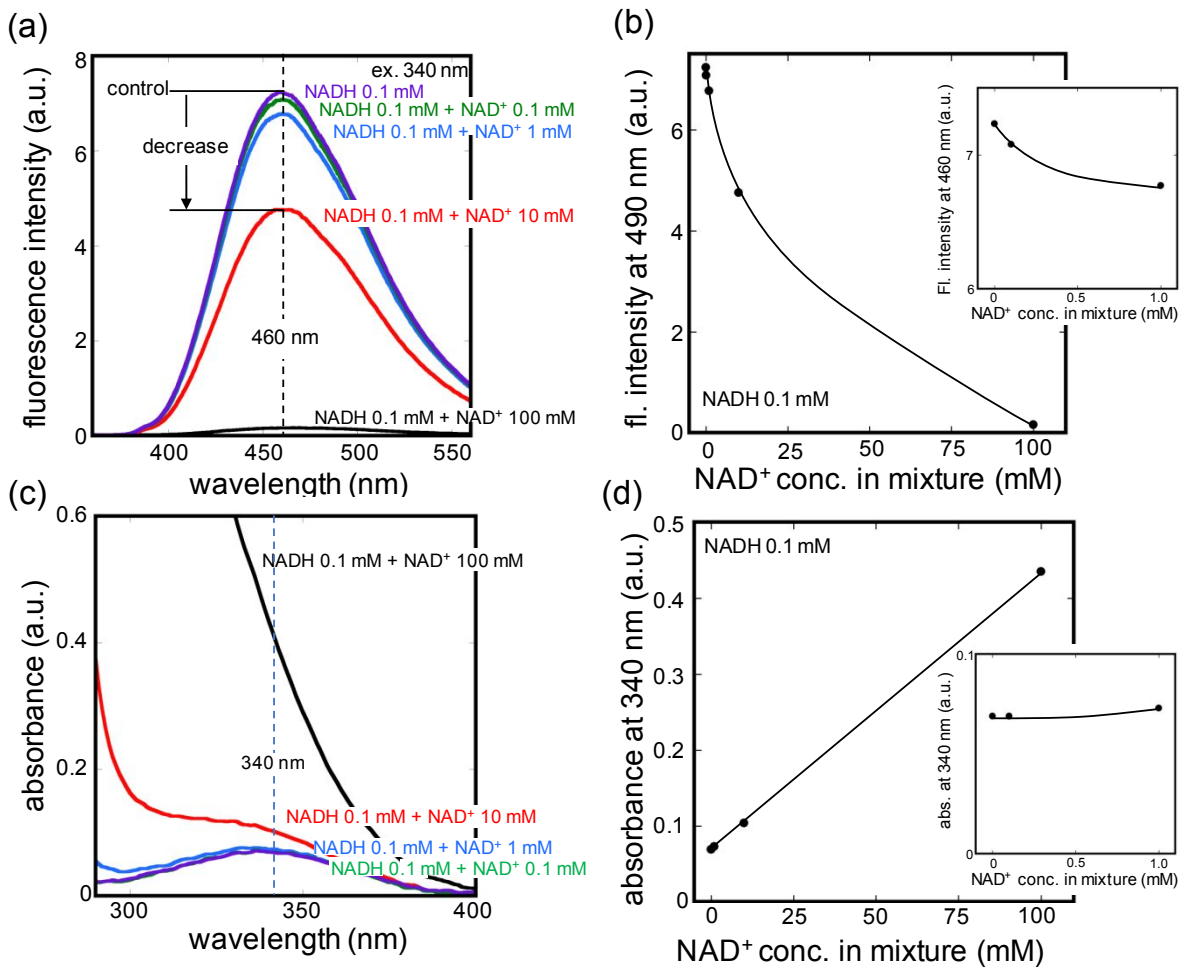

Figure S-4. (a) Fluorescence spectrum of NAD^+/ NADH mixture solutions that were excited by 340 nm of UV light. These samples were prepared as shown in table S-1. (b) Relationship between ΔI_{fl} and NAD+ concentration in NAD^+/NADH mixture solution. (c) The absorbance spectrum of the same sample to (a) and (b). (d) The relationship between absorbance at 340 nm and NAD^+ concentration in NAD^+/NADH mixture solution.

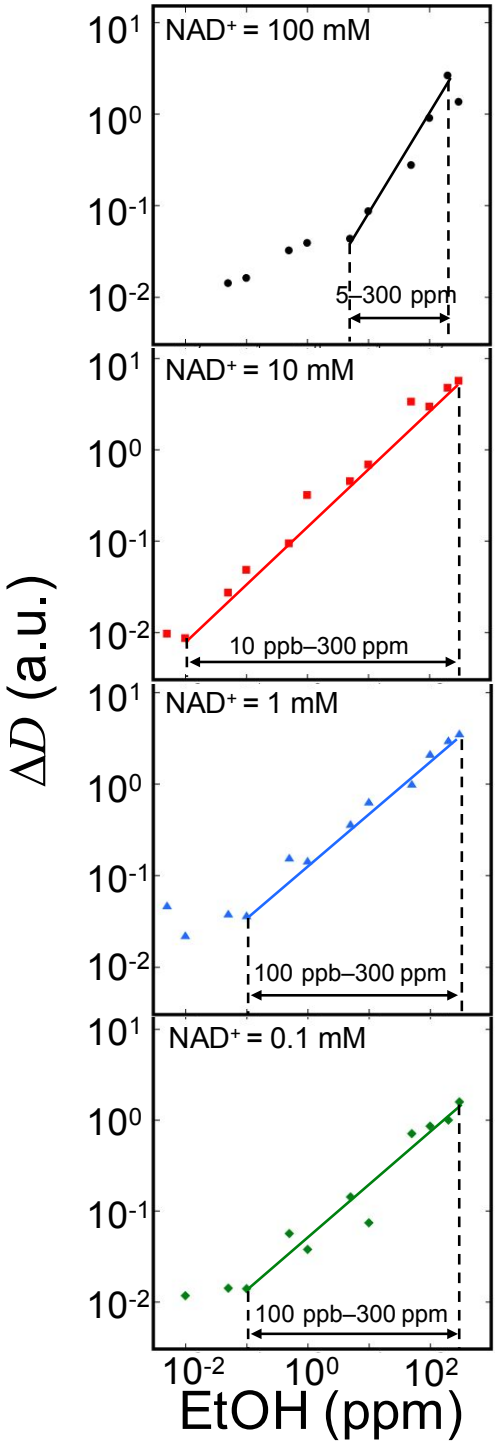

Figure S-5. Calibration curves of EtOH that were obtained by using a different concentration of NAD^+ solutions. Dynamic range was changed depending on the concentration of the NAD^+ solution.

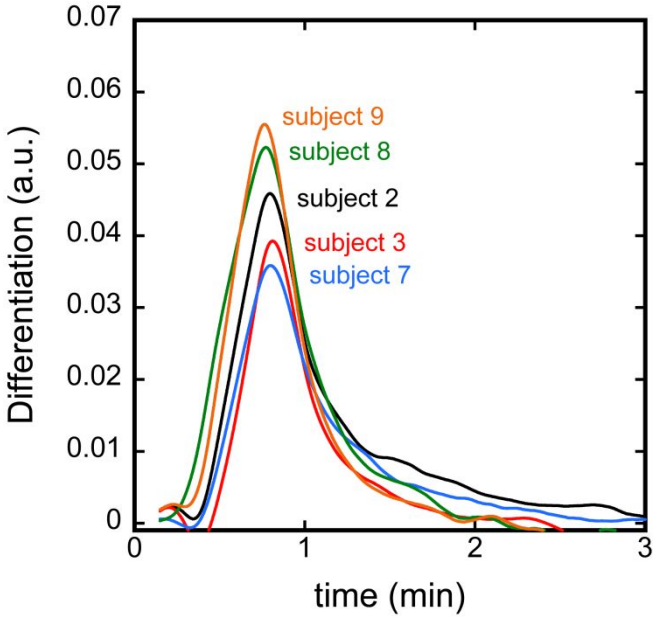

Figure S-6. Typical responses of the differential value in to breath EtOH in the absence of alcohol consumption.

Table S-2. Comparison of previously developed Sniff-cam and newly developed one

	dynamic range (ppm)	EtOH dose (g/kg bw)	peak max conc. in breath	ref.
EtOH sniff-cam	1–150	0.4	127.7 ppm (EtOH, ALDH2[+]) 143.6 ppm (EtOH, ALDH2[-])	1
AcH sniff-cam	0.1–10	0.4	2.75±0.38 ppm (AcH, ALDH2[+]) 8.64±0.32 ppm (AcH, ALDH2[-])	2
switchable sniff-cam	0.1–1000 (EtOH) 0.2–10 (AcH)	0.4	145.3±13.5 ppm (EtOH, ALDH2[+] 1.7±0.2 ppm (AcH, ALDH2[+] 163.28.0 ppm (EtOH, ALDH2[-] 8.4±0.5 ppm (AcH, ALDH2[-])	3
This study	0.02–300	0 (without)	116.2±35.7 ppb	

bw; body weight

References

(1) Arakawa, T.; Sato, T.; Iitani, K.; Toma, K.; Mitsubayashi, K. Fluorometric Biosniffer Camera "Sniff-Cam" for Direct Imaging of Gaseous Ethanol in Breath and Transdermal Vapor. *Anal. Chem.* **2017**, *89* (8), 4495–4501. https://doi.org/10.1021/acs.analchem.6b04676.

(2) Iitani, K.; Sato, T.; Naisierding, M.; Hayakawa, Y.; Toma, K.; Arakawa, T.; Mitsubayashi, K. Fluorometric Sniff-Cam (Gas-Imaging System) Utilizing Alcohol Dehydrogenase for Imaging Concentration Distribution of Acetaldehyde in Breath and Transdermal Vapor after Drinking. *Anal. Chem.* **2018**, *90* (4), 2678–2685. https://doi.org/10.1021/acs.analchem.7b04474.

(3) Iitani, K.; Hayakawa, Y.; Toma, K.; Arakawa, T.; Mitsubayashi, K. Switchable Sniff-Cam (Gas-Imaging System) Based on Redox Reactions of Alcohol Dehydrogenase for Ethanol and Acetaldehyde in Exhaled Breath. *Talanta* **2019**, *197* (December 2018), 249–256. https://doi.org/10.1016/j.talanta.2018.12.070.