Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.

Suppl. Information

Highly Sensitive Eight-Channel Light Sensing System for Biomedical Applications

Sung Bae Kim [a,†], Sharon Seiko Hori [b], Negar Sadeghipour [b], Uday Kumar Sukumar [b], Rika Fujii [a], Tarik F Massoud [b], Ramasamy Paulmurugan [b,†]

a. Research Institute for Environmental Management Technology, National Institute of Advanced Industrial Science and Technology (AIST), Onogawa 16-1, Tsukuba, Ibaraki 305-8569, Japan.

b. Molecular Imaging Program at Stanford, Stanford University School of Medicine, Palo Alto, California 94304, United States

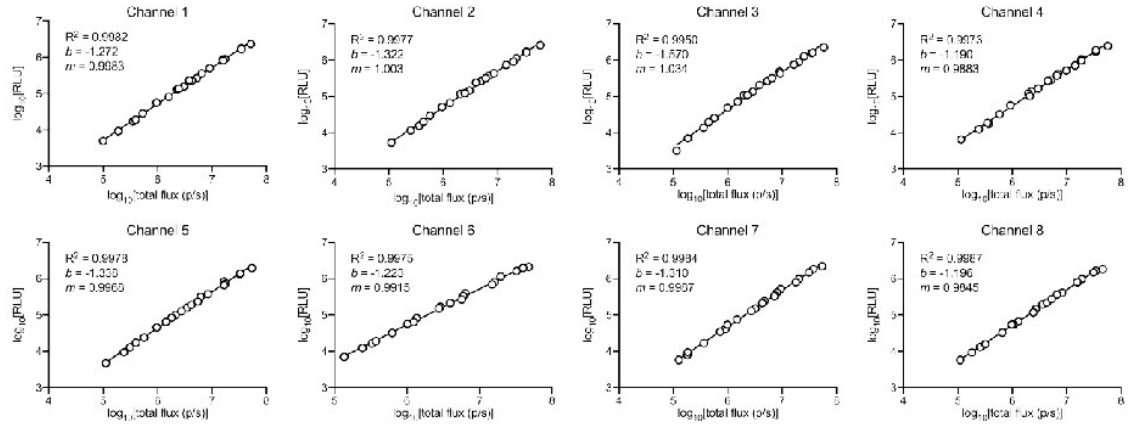
c. Canary Center at Stanford for Cancer Early Detection, Stanford University School of Medicine, Palo Alto, California 94304, United States

† **Corresponding authors:** kimu-sb@aist.go.jp, **paulmur8**@stanford.edu

Running title: BBI system

Suppl. Figure 1. Normalization of the light sensitivities of 8 PMTs of the Black Box I (BBI) in comparison with the dataset of IVIS Lumina II system (abbreviated to IVIS system hereafter, PerkinElmer). (A) Linear correlation between the dataset of PMTs in the BBI system and the data set of the IVIS system. (B) Statistical analysis on the linear correlation between the dataset of 8 PMTs and that of the IVIS system showing linearity slope, standard errors, and correlation coefficients.

(A)

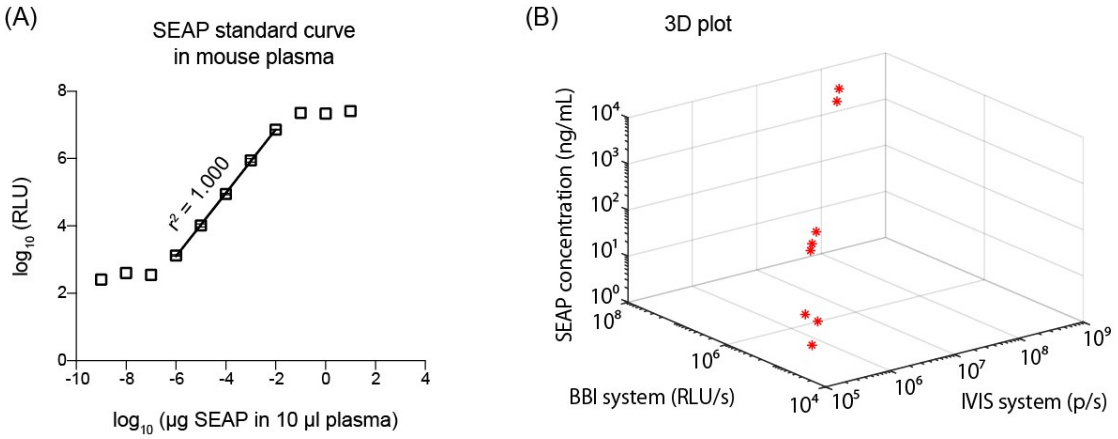


$$\log_{10}[\text{RLU}] = m \times \log_{10}[\text{total flux}] + b$$

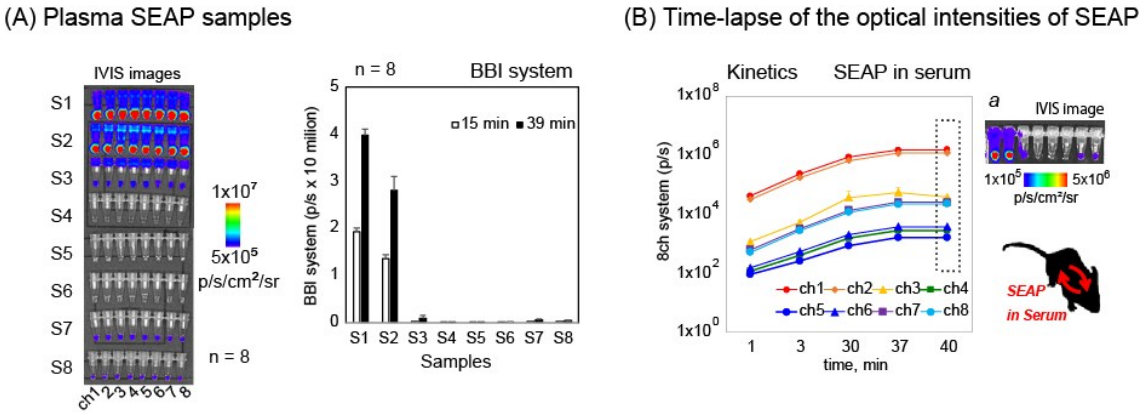
(B)

		Channel 1 (RLU/s)	Channel 2 (RLU/s)	Channel 3 (RLU/s)	Channel 4 (RLU/s)	Channel 5 (RLU/s)	Channel 6 (RLU/s)	Channel 7 (RLU/s)	Channel 8 (RLU/s)
Best-Fit Values	Y-Intercept	-1.272	-1.322	-1.570	-1.190	-1.338	-1.223	-1.310	-1.196
	Slope	0.9983	1.003	1.034	0.9883	0.9968	0.9915	0.9987	0.9845
Standard Error	Y-Intercept	0.06373	0.07257	0.1111	0.07716	0.07085	0.07459	0.05988	0.05363
	Slope	0.009723	0.01103	0.01686	0.01172	0.01083	0.0114	0.009156	0.008224
95% Confidence Interval (Profile Likelihood)	Y-Intercept	-1.405 to -1.139	-1.474 to -1.171	-1.803 to -1.338	-1.352 to -1.029	-1.486 to -1.190	-1.379 to -1.067	-1.436 to -1.185	-1.308 to -1.083
	Slope	0.9780 to 1.019	0.9799 to 1.026	0.9991 to 1.070	0.9638 to 1.013	0.9741 to 1.019	0.9677 to 1.015	0.9796 to 1.018	0.9673 to 1.002
Goodness of Fit	Degrees of Freedom	19	19	19	19	19	19	19	19
	R squared	0.9982	0.9977	0.9950	0.9973	0.9978	0.9975	0.9984	0.9987
	Sum of Squares	0.0216	0.02763	0.0677	0.031	0.02619	0.02823	0.01998	0.01579
	Standard Deviation of Residuals	0.03371	0.03813	0.05969	0.04039	0.03712	0.03855	0.03243	0.02882
Number of Points	# of X values	21	21	21	21	21	21	21	21
	# of Y values	21	21	21	21	21	21	21	21

Suppl. Figure 2. (A) Log-scale standard curve of the SEAP assay that was determined with a single-tube luminometer in mouse plasma. (B) Three dimensional (3D) plot of the data sets of the BBI and IVIS systems showing average radiance vs. SEAP concentration (note axes are plotted on log scale).

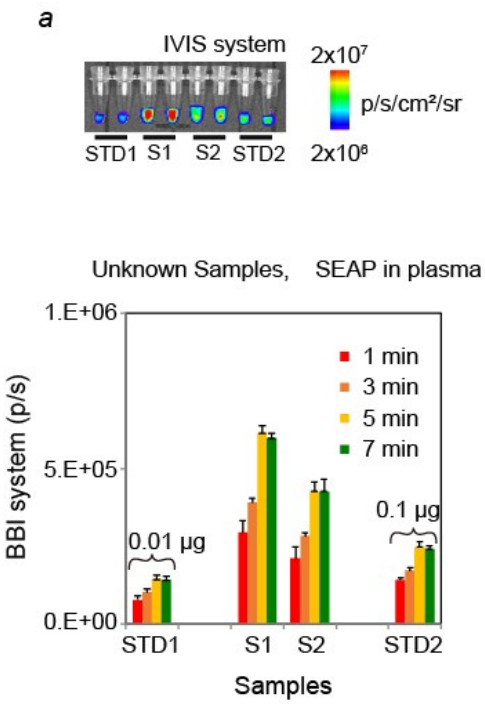


Suppl. Figure 3. (A) Evaluation of the the BBI system using 8 plasma SEAP samples. (B) Time course of the optical intensities of the SEAP plasma samples. **Inset '*a*'** shows the optical image of 8-lane PCR tube. The dotted box highlights the most stable time window after addition of the substrate.



Suppl. Figure 4. (A) Determination of SEAP levels in plasma. **Inset '*a*'** shows the optical image of 8-lane PCR tube, carrying duplicates of two standards (STD1 and STD2) and two unknown SEAP plasma samples (S1 and S2). (B) Simultaneous determination of unknown plasma SEAP samples with the BBI and IVIS systems. **Inset '*b*'** demonstrates the table of results obtained by using the BBI and IVIS systems. The sampling time indicates the light acquisition time after substrate injection.

(A)

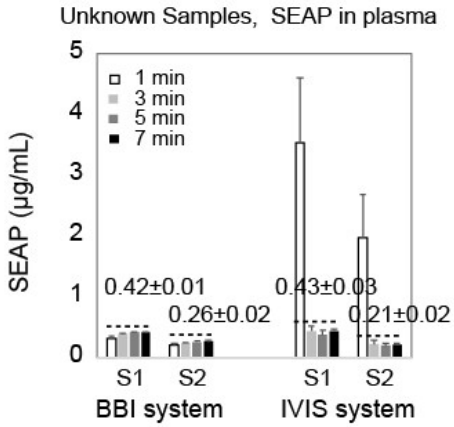


(B)

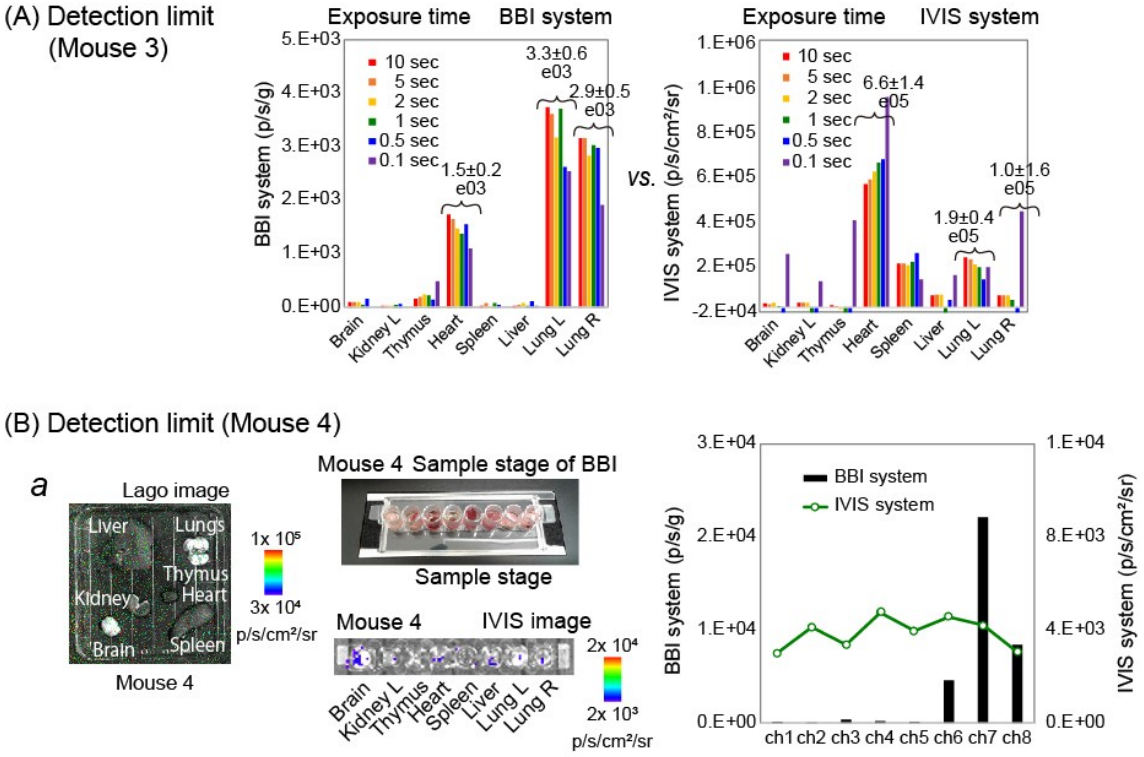
b

SEAP values in plasma (µg)

sampling time (min)	BBI system S1	BBI system S2	IVIS system S1	IVIS system S2
1	0.32±0.04	0.20±0.04	3.54±1.06	1.97±0.71
3	0.38±0.01	0.24±0.01	0.43±0.09	0.22±0.06
5	0.41±0.02	0.25±0.02	0.37±0.08	0.19±0.05
7	0.42±0.01	0.26±0.02	0.43±0.03	0.21±0.02



Suppl. Figure 5. Evaluation of the optical sensitivity of the BBI, Lago and IVIS systems to very fast low light level (LLL) signals from mouse organ tissues. (A) Variance in the dataset according to varying light exposure times the of BBI and IVIS systems. The left and right graphs represent the optical intensities of the BBI and IVIS systems, respectively. The numbers on bars represent the average values of each channel. (B) Determination of organ tissue metastases of cancers. The organ tissues were first placed on a Petri dish and imaged with the Lago system (Spectral Instruments Imaging). The tissues were then transferred to an 8-well microstrip, and the corresponding optical intensities were simultaneously determined using the BBI and IVIS systems. The bar and line graphs represent the optical intensities of the tissue biopsies by the BBI and IVIS systems, respectively. **Inset *a*** shows the optical image of eight different organ tissues from Mouse 4 on a Petri dish.



Suppl. Methods

Mouse imaging. To image the tumors, the mice were anesthetized by standard gas anesthesia (2% isoflurane with oxygen flow of 0.8 to 1 L/min) and were *i.p.* injected with 50 μg of nCTZ in 100 µl saline cocktail supplemented with 35% PEG400 and 10% ethanol (n=3). The corresponding BL images of the tumor xenografts were captured in the prone or supine position with the Lago system (Spectral Instruments Imaging). To quantify the number of emitted photons, regions of interest (ROI) were drawn over the areas of the tumors, and the maximum photons per second per square centimeter per steradian (p/sec/cm^2/sr) generated by Aura (ver 2.2.0) were recorded (Figure 3A).