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Supplementary Materials for

Label-free quantitation of glycated hemoglobin in single red blood cells by transient absorption microscopy and phasor analysis

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Fig. S1. Fourier transform Raman spectra of HbA1c (0.025 mg/ml) and Hb (0.025 mg/ml). The vibrations of porphyrin rings are pointed in the purple bracket. Excitation wavelength: 1064 nm.



Fig. S2. Schematic of a visible-pump (520 nm), near-infrared probe (780 nm) transient absorption microscope. HWP, half wave plate. PBS, polarization beam splitter. AOM, acousto-optic modulator. BBO, barium borate crystal. SHG, second harmonic generation. B coating: 600 - 1050 nm; A coating: 350 - 700 nm.



Fig. S3. Transient absorption signal of Hb solution from in-phase channel (cosine channel) and quadrature channel (sine channel) at a phase of 180° . (a) In-phase channel signal of hemoglobin solution. The interface between air and sample is pointed by a white arrow. Region of interest (ROI) is highlighted by red dashed line. (b) In-phase channel signal of hemoglobin solution. The interface between air and sample is pointed by a white arrow. ROI is highlighted by red dashed line. (c) The intensity changes versus the pixel number in the ROI of (a). (d) The intensity changes versus the pixel number in the ROI of (b).



Fig. S4. Phasor analysis of time-resolved curves from standard HbA1c solutions (3.3%, **a**), (5.9%, **b**). Scattered plot was acquired from focused 1600 pixels in the time-resolved stacks. The coordinates of most aggregated point (pointed by red cross and red arrow) was obtained through mean-shift theory.



Fig. S5. HbA1c fraction (in single RBC) distribution along with the derived glucose concentration from four new type 2 diabetic whole blood samples.



Fig. S6. HbA1c fraction (in single RBC) distribution along with the derived glucose concentration from four new healthy whole blood samples.

Table S1. Key parameter comparison between type 2 diabetic whole blood and healthy whole blood. SEM stands for standard error of the mean. *N* is the number of red blood cells acquired.

Type 2 diabetic whole blood			Healthy whole blood	
Derived glucose concentration	HPLC approach (HbA1c%)	TA approach (HbA1c%) Mean ± SEM	Derived glucose concentration	TA approach (HbA1c%) Mean ± SEM
258.41 mg/dl	10%	10.63 ± 0.50%, <i>N</i> =150	122.56 mg/dl	5.18 ± 0.40%, <i>N</i> =150
169.54 mg/dl	6.9%	7.53 ± 0.35%, <i>N</i> =150	118.45 mg/dl	5.07 ± 0.41%, <i>N</i> =150
169.19 mg/dl	6.6%	7.46 ± 0.30%, <i>N</i> =150	127.22 mg/dl	5.25 ± 0.32%, <i>N</i> =150
142.97 mg/dl	7.7%	7.54 ± 0.089%, <i>N</i> =596	115.57 mg/dl	5.46 ± 0.12%, <i>N</i> =447
151.78 mg/dl	8.6%	8.61± 0.054%, <i>N</i> =925	106.55 mg/dl	4.48 ± 0.14%, <i>N</i> =476
128.74 mg/dl	5.8%	7.77± 0.062%, <i>N</i> =426	110.97 mg/dl	4.85 ± 0.12%, <i>N</i> =726
135.70 mg/dl	7.0%	7.77± 0.071%, <i>N</i> =431	114.22 mg/dl	5.18 ± 0.13%, <i>N</i> =428