Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Quantification of Solution-Free Red Blood Cell Staining by Sorption Kinetics of Romanowsky Stains to Agarose Gels

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Supplementary Figures

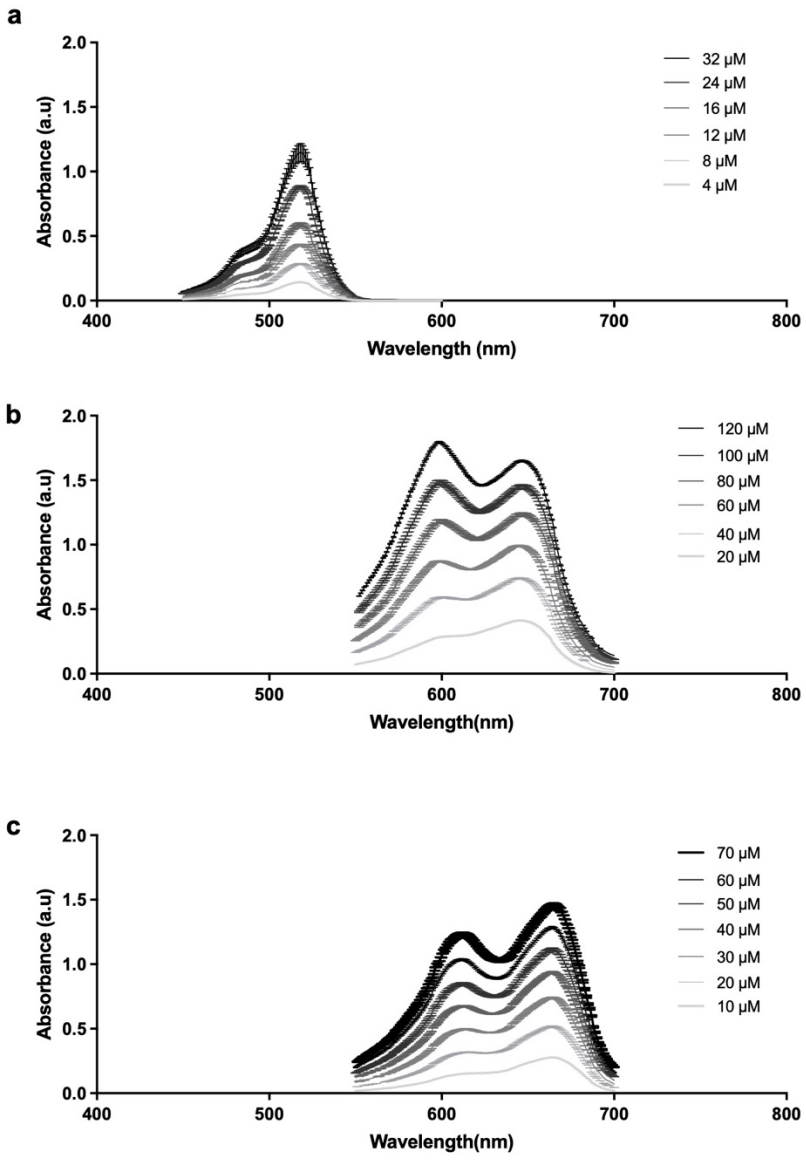

Figure S1. Spectral scanning. Individual Romanowsky dye solutions at different concentrations scanned from 450 to 700 nm. EY (a), AZB (b) and MB (c).

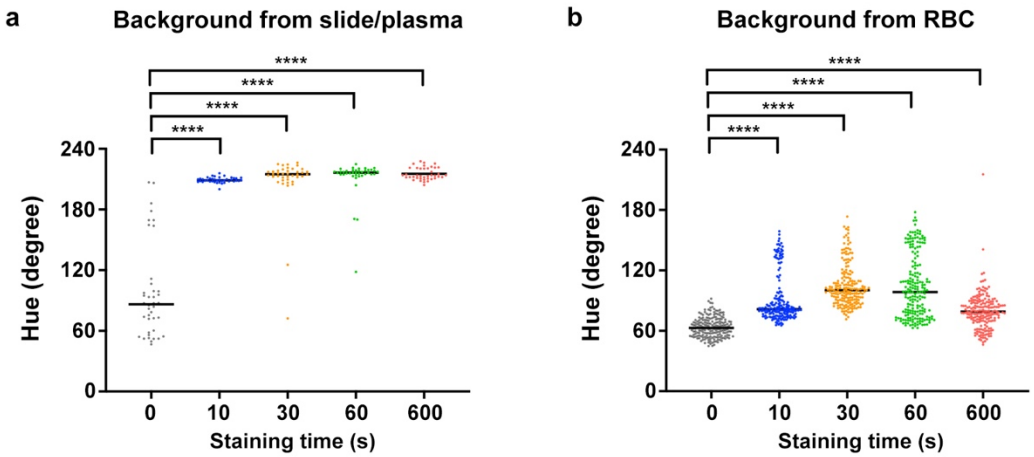

Figure S2. Distribution of values of hue (H) respect to different periods of staining and destaining of blood cells. (a) H of plasma after staining, n=3 samples and 40 randomly selected empty ROIs without RBCs in each column. (b) H of RBCs after staining; n=3 samples and 198 RBCs in each column. One way ANOVA with Dunnett's multiple comparison tests, ****p<0.0001. Scatter dot plot with mean.

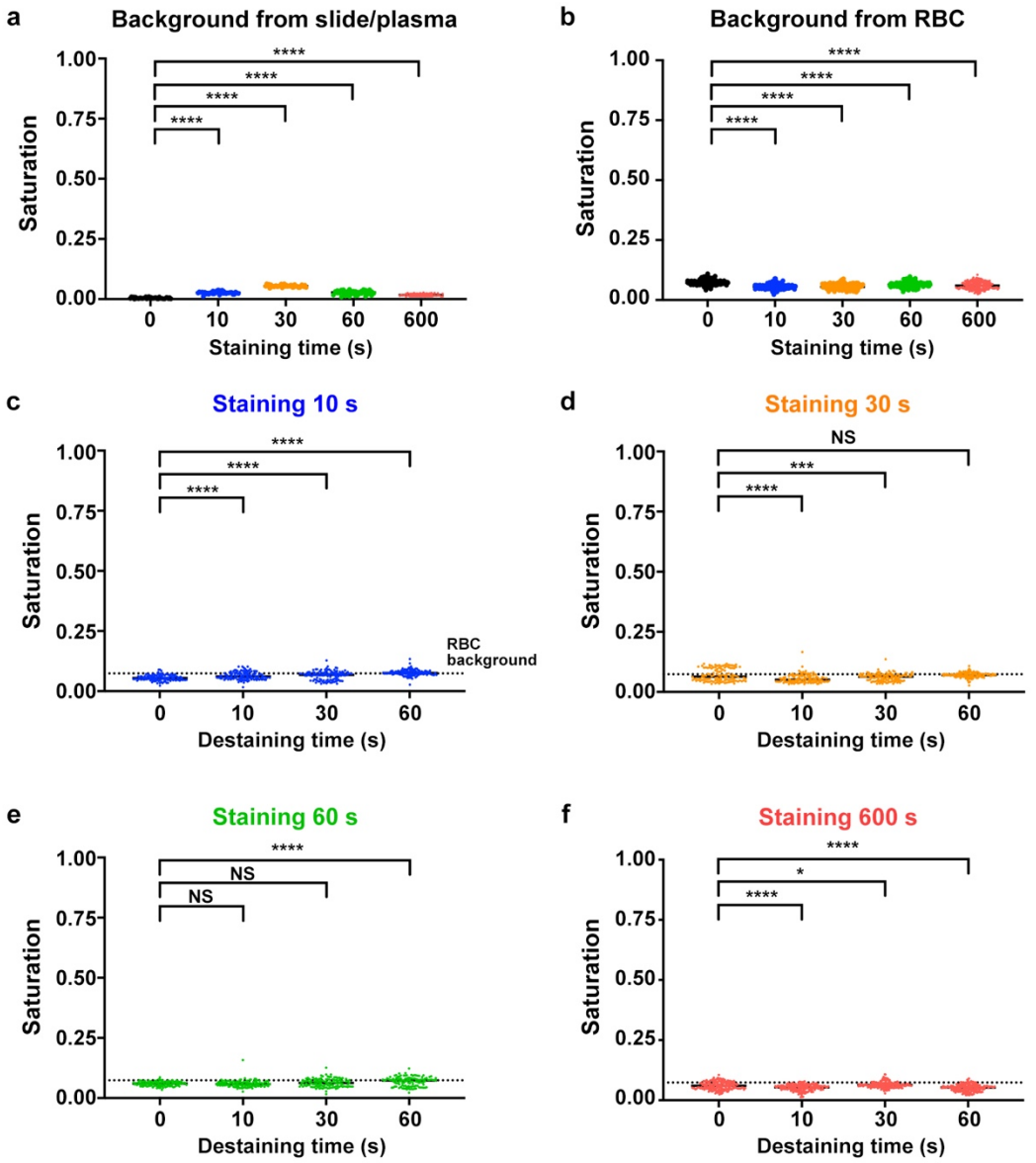

Figure S3. Distribution of values of saturation (S) with respect to different periods of staining and destaining of blood cells. (a) S of plasma after staining, n=3 samples and 40 randomly selected empty ROIs without RBCs in each column. (b) S of RBCs after staining; n=3 samples and 198 RBCs in each column. In (c-f), destaining was performed for 10 s, 30 s and 60 s after 10 s, 30 s, 60 s and 600 s of staining. One way ANOVA with Dunnett's multiple comparison tests, ****p<0.0001, ***p<0.001 and NS (non-significant). Scatter dot plot with mean.

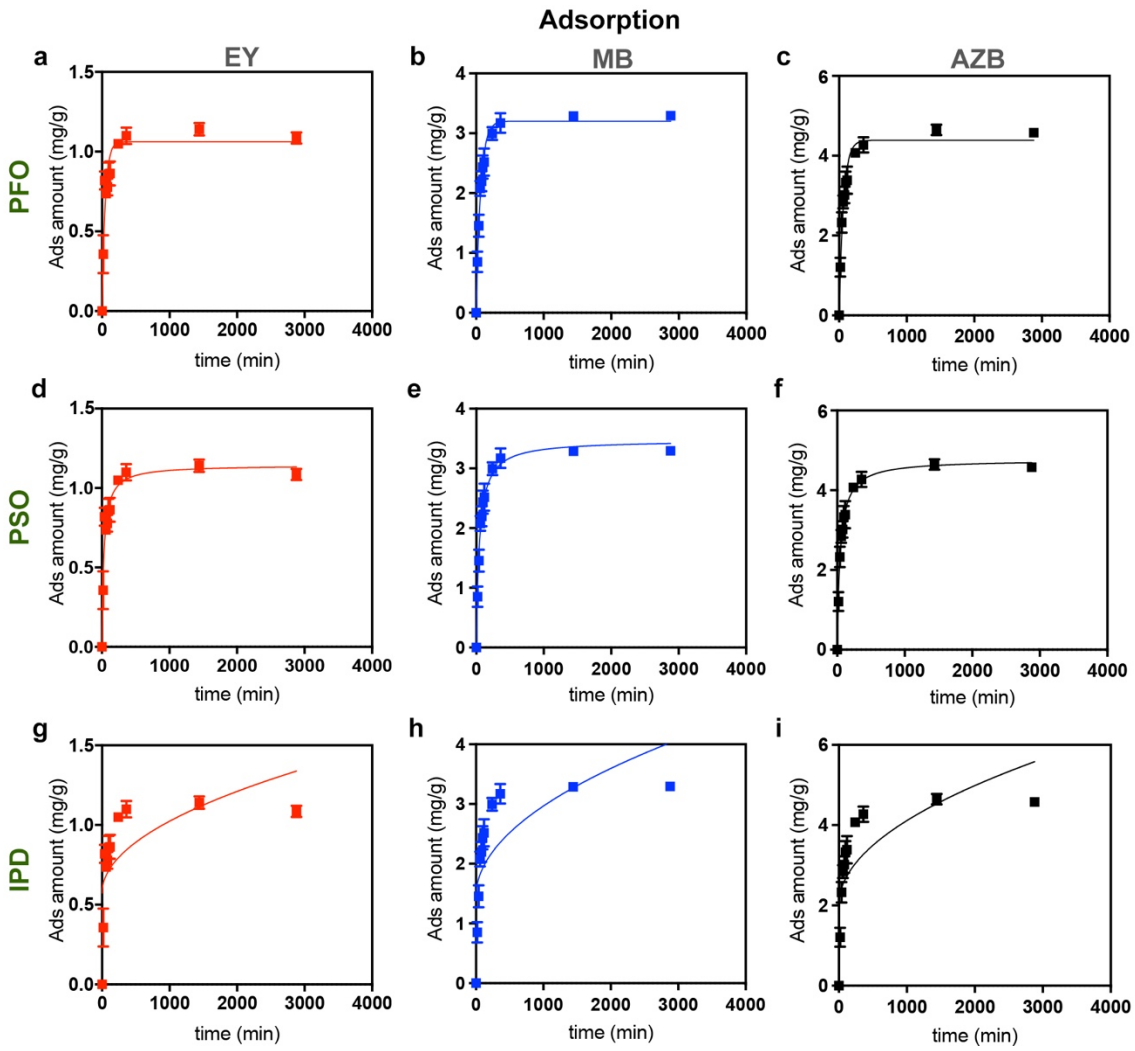

Figure S4. Three different models of adsorption kinetics. At a fixed concentration of the ternary mixture (EY 3.5 μM; MB 70 μM; AZB 140 μM), kinetics of adsorption (destaining) over 48 h was measured and fitted with PFO (pseudo-first order, a-c), PSO (pseudo-second order, d-f) and IPD (intraparticle diffusion, g-i) models; mean±SD, n=5.

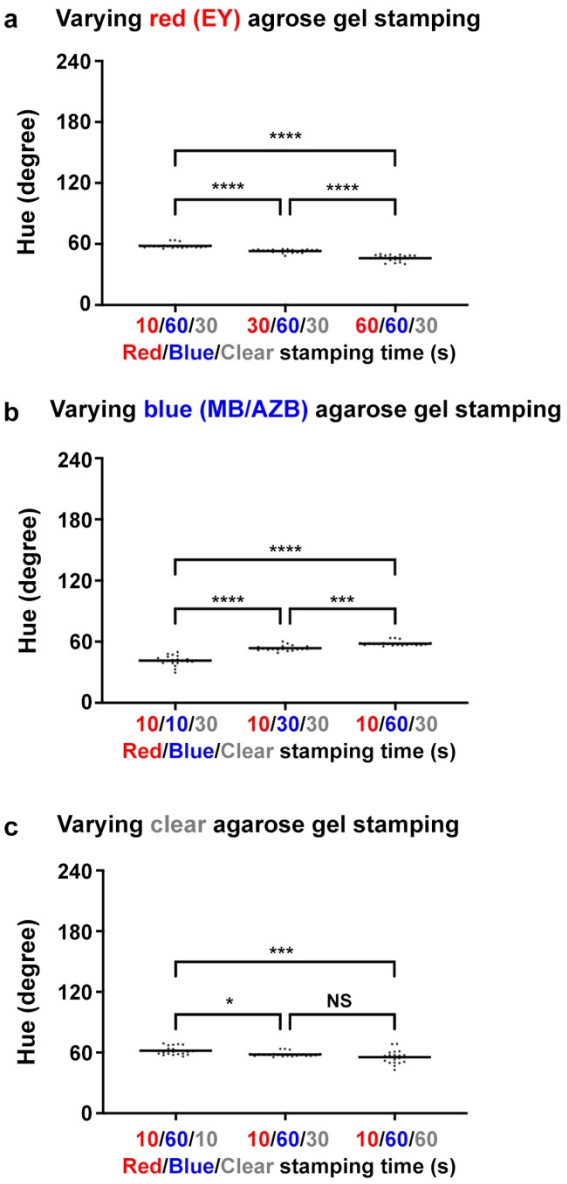

Figure S5. Distribution of background values of hue (H) with respect to different periods of staining and destaining of blood cells with hydrogel stamps. H of slide/plasma after staining and destaining with varying time of (a) red (EY), (b) blue (MB/AZB) and (c) clear agarose gels; n=3 samples and 20 randomly selected empty ROIs without RBCs in each column. Staining and destaining periods are denoted in each column. One way ANOVA with Tukey's *post hoc* tests, ****p<0.0001, ***p<0.001, *p<0.05 and NS (non-significant). Scatter dot plot with mean.

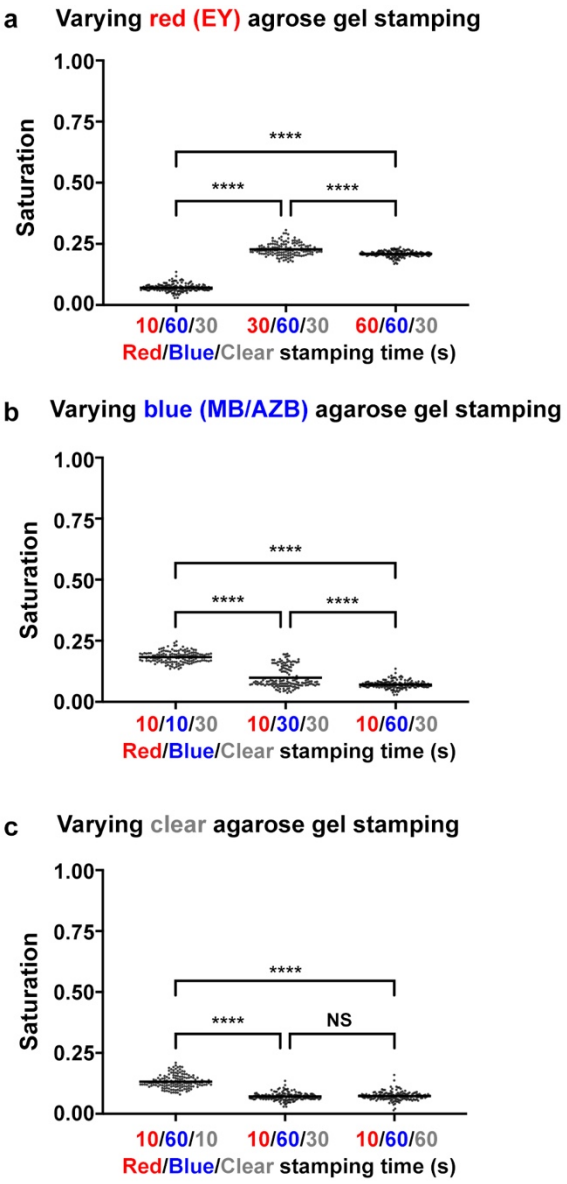

Figure S6. Distribution of values of saturation (S) with respect to different periods of staining and destaining of blood cells with hydrogel stamps. S of RBCs after staining and destaining with varying time of (a) red (EY), (b) blue (MB/AZB) and (c) clear agarose gels; n=3 samples and a total of 150 RBCs in each column. Staining and destaining periods are denoted in each column. One way ANOVA with Tukey's *post hoc* tests, ****p<0.0001 and NS (non-significant). Scatter dot plot with mean.

Supplementary Tables

Table S1. Parameters of kinetics models represented in Figure S4.

Model	Parameters	Adsorption		
		EY	**MB**	**AZB**
Pseudo-1st-order kinetics (**PFO**)	**Q_e (mg/g)**	1.06	3.20	4.39
	k_1 (L min^{-1})	0.0483	0.0342	0.0355
	R^2	0.933	0.992	0.980
Pseudo-2nd-order kinetics (**PSO**)	**Q_e (mg/g)**	1.15	3.47	4.77
	k_2 (g/mg min^{-1})	0.0285	0.00612	0.00465
	R^2	0.959	0.990	0.994
Intraparticle diffusion (**IPD**)	**k_3 (mg/g min$^{-1/2}$)**	0.0143	0.0481	0.0666
	C (mg/g)	0.574	1.45	2.01
	R^2	0.437	0.524	0.547

Table S2. Parameters of the one phase decay kinetics model in Figure 7 up to 240 min.

Model	Parameters	Desorption		
		EY	**MB**	**AZB**
One phase decay	**Q_{max} (mg/g)**	1.06	3.24	4.48
	Q_{min} (mg/g)	0.532	0.783	0.804
	τ (min)	39.3	30.4	30.4
	R^2	0.977	0.984	0.982

Table S3. Parameters of multiple kinetics models in Figure S4 up to 240 min.

Model	Parameters	Adsorption		
		EY	**MB**	**AZB**
Pseudo-1st-order kinetics (**PFO**)	**Q_e (mg/g)**	0.948	2.99	2.89
	k_1 (L min^{-1})	0.0668	0.0396	~91300000
	R^2	0.932	0.994	0.593
Pseudo-2nd-order kinetics (**PSO**)	**Q_e (mg/g)**	1.14	3.79	4.91
	k_2 (g/mg min^{-1})	0.0287	0.00451	0.00416
	R^2	0.944	0.992	0.993
Intraparticle diffusion (**IPD**)	**k_3 (mg/g min$^{-1/2}$)**	0.0677	0.208	0.277
	C (mg/g)	0.142	0.156	0.309
	R^2	0.853	0.941	0.932