SUPPORTING INFORMATION

A new calibration concept for a reproducible quantitative detection based on SERS measurements in a microfluidic device demonstrated on the model analyte adenine†

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Table S1 Ratio of the reagents and analyte concentration in one sample drop

Flow rate [μl/s]				time [min]		step		analyte concentration
colloid	act. agent	water	analyte	start	end	No.	name	in one drop [μM]
0.009	0.0018	0.009	0	0	16	1	analyte	0
		0.0045	0.0045	16	26	2	control	2.3
		0.009	0	26	30		zero	0
		0.006	0.003	30	46	3	analyte	1.5
		0.009	0	46	50		zero	0
		0.0045	0.0045	50	60	4	control	2.3
		0.009	0	60	64		zero	0
		0.003	0.00	64	80	5	analyte	3
		0.009	0	80	84		zero	0
		0.0045	0.0045	84	94	6	control	2.3
		0.009	0	94	98		zero	0
		0	0.009	98	114	7	analyte	4.5
		0.009	0	114	118		zero	0
		0.0045	0.0045	118	128	8	control	2.3
		0.009	0	128	132		zero	0

The flowrates of each aqueous reagent are given in this table. For the carrier and separation medium tetradecane, a flowrate of 0.01 μl/s was chosen. Due to the change of the flowrates over time, a gradient with different steps of concentrations was generated. Next to the flowrates, the duration of each step is also presented in the table. The resulting steps can be distinguished based on the concentration of the analyte. The control steps have a period of 10 minutes and always the same analyte concentration of 2.3 μM. The gradient includes four control steps labeled with even step numbers and the same number of analyte steps labeled with odd step numbers. Each analyte step was measured for 16 minutes. The concentrations of the analyte steps can also be found in the table. In between the analyte and control steps so called zero steps are placed. These steps serve as visual separators between the other step types. Every zero step continued for 4 minutes and contained no analyte molecules in the corresponding droplets.

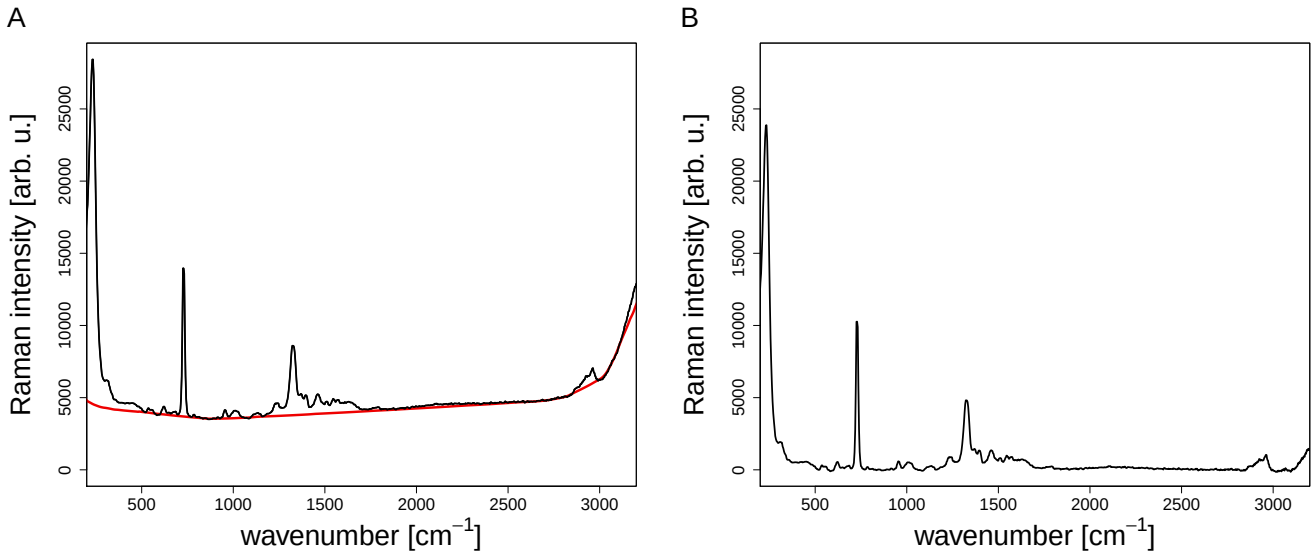

Figure S1 (A) The plot represented a raw spectra of adenine (in black) and the red line illustrates the fit of the spectral background using the SNIP algorithm. (B) The resulting and background-corrected spectrum is shown.

The figure illustrates the correction of the spectral background. For this purpose, the statistics-sensitive nonlinear peak-clipping (SNIP) algorithm[?] implemented in the GnuR[?] package Peaks[?] (function: SpectrumBackground; parameter: smooth = TRUE and order = 2) was used. (A) In the left-hand picture a raw spectrum of the analyte (black) is plotted. The red line represents the fit of the SNIP background estimate of the spectrum that is subtracted from the spectrum. (B) The resulting background-corrected spectrum is shown in the right-hand picture.

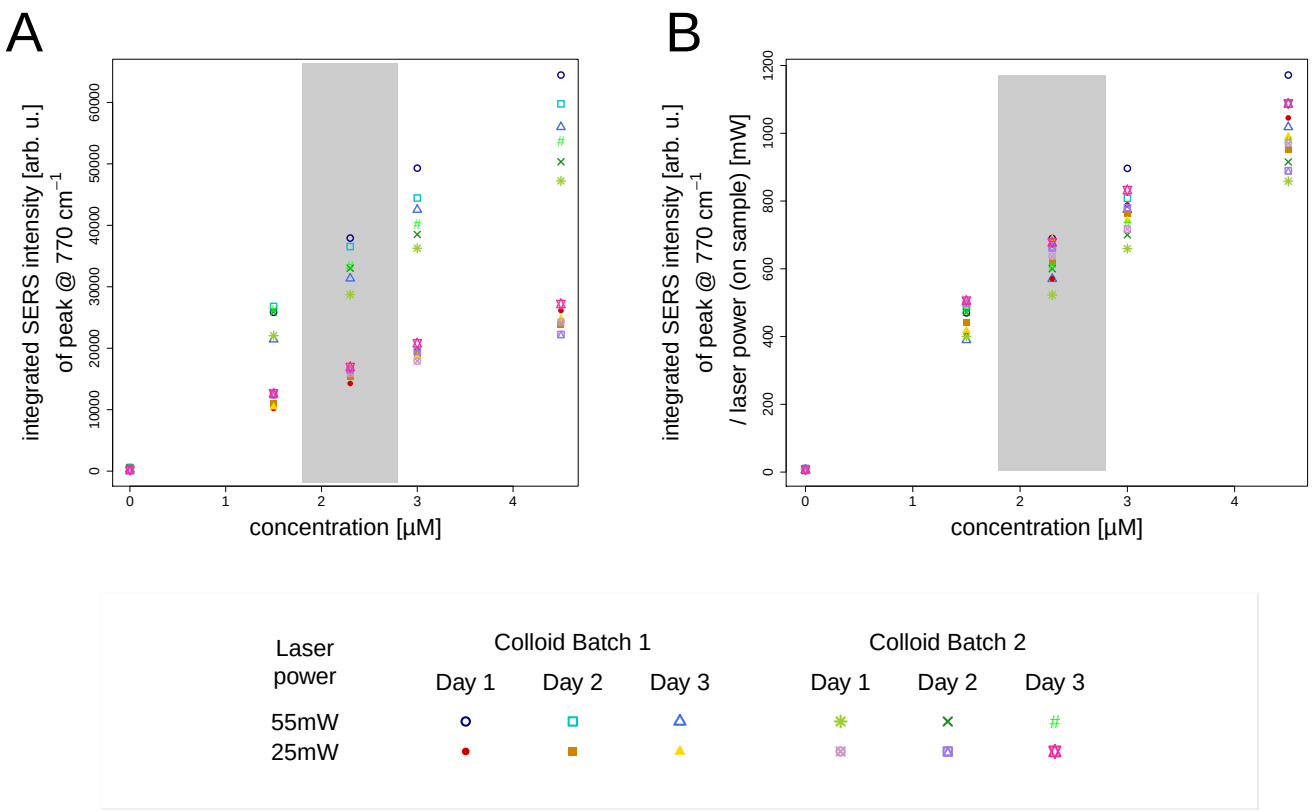

Figure S2 (A) Plot of the integrated SERS intensity of the adenine peak at 770 cm^{-1} against the step-wise concentration. (B) Plot of the integrated SERS intensity in relation to the laser power; the gray area in each plot illustrates the control step. The symbols are explained in the legend below.

The mean integrated SERS intensities of all measurements are presented in Fig. **??**. The blue points refer to the measurement of adenine with batch 1 of the Ag colloid and 55 mW laser power and the green ones to the measurement with the same laser power but with batch 2 of the Ag colloid. In comparison, the mean integrated SERS intensity of the measurements with 25 mW laser power is illustrated through the red to yellow (batch 1) and violet/magenta (batch 2) symbols. These shown mean integrated SERS intensities are not normalized to the control steps. It is obvious that increasing analyte concentrations lead to a higher intensity of the SERS signals. Moreover, the difference in the signal intensities for the measurements with different laser power is clearly recognizable. Hence, the laser power has a huge impact on the resulting SERS intensities. It also has a decisive effect on the quantification of substances with SERS and must be taken into account. Even minimal fluctuations can distort the quantitative results. To minimize this influence, the resulting SERS intensities have to be put in relation to the laser power. Fig. **??**B presents the mean integrated SERS intensities in relation to the used laser power, *i.e.*, the integrated SERS intensities of the adenine peak (at 770 cm^{-1}) in each analyte spectrum is divided by the corresponding laser power. The plot illustrates that the comparability of measurements is improved. However, this simple relation involves some pitfalls, *e.g.*, the probability for the appearance of burning as well as of saturating and other effects in SERS experiments increases with higher laser power which, in turn, must be considered in the quantification of target molecules.

Table S2 Number of spectra per step

step		Ag colloid batch 1						Ag colloid batch 2					
No.	name	Laser power: 55 mW			Laser power: 25 mW			Laser power: 55 mW			Laser power: 25 mW		
		day 1	day 2	day 3	day 1	day 2	day 3	day 1	day 2	day 3	day 1	day 2	day 3
1	analyte	283	261	329	268	126	270	248	258	276	182	218	252
2	control	145	154	125	140	154	136	161	135	151	152	145	141
3	analyte	196	184	186	189	192	196	203	193	189	180	192	185
4	control	98	105	95	104	103	103	104	97	98	102	110	102
5	analyte	163	145	140	149	149	146	151	154	128	138	135	152
6	control	81	75	74	74	80	81	75	85	85	83	82	86
7	analyte	123	140	117	125	125	131	124	133	124	122	133	132
8	control	73	76	75	67	74	70	71	74	69	82	65	76

Table S3 Mean value of the Raman intensity for the raw and normalized data

Ag colloid batch	Laser power [mW]	step		Raman intensity					
		name	No.	raw data			normalized data		
				day 1	day 2	day 3	day 1	day 2	day 3
1	55	analyte	1	466.9 ± 153.4	588.8 ± 163.1	306.9 ± 162.2	0.010 ± 0.003	0.014 ± 0.004	0.009 ± 0.005
		control	2	44287.8 ± 12388.5	42036.1 ± 8951.5	34186.9 ± 7786.4	1.000 ± 0.280	1.000 ± 0.213	1.000 ± 0.228
		analyte	3	25850.4 ± 8739.2	26799.2 ± 6234.2	21427.6 ± 5109.9	0.584 ± 0.180	0.638 ± 0.148	0.627 ± 0.149
		control	4	37923.0 ± 7969.5	36541.9 ± 9765.1	31347.7 ± 7213.3	1.000 ± 0.210	1.000 ± 0.267	1.000 ± 0.230
		analyte	5	49302.6 ± 12581.7	44445.1 ± 10294.1	42532.4 ± 9686.5	1.300 ± 0.332	1.216 ± 0.282	1.357 ± 0.309
		control	6	33407.7 ± 8895.7	34397.2 ± 9214.3	32621.9 ± 7583.3	1.000 ± 0.266	1.000 ± 0.268	1.000 ± 0.232
		analyte	7	64453.9 ± 14902.5	59764.0 ± 16582.9	55990.5 ± 12694.9	1.929 ± 0.446	1.737 ± 0.482	1.716 ± 0.389
		control	8	36088.5 ± 8859.2	34190.8 ± 8567.2	31483.9 ± 6965.6	1.000 ± 0.245	1.000 ± 0.251	1.000 ± 0.221
1	25	analyte	1	219.7 ± 104.0	196.9 ± 114.0	234.5 ± 107.4	0.013 ± 0.006	0.011 ± 0.006	0.015 ± 0.007
		control	2	16357.5 ± 4030.8	17667.3 ± 4731.7	15429.0 ± 3187.6	1.000 ± 0.246	1.000 ± 0.268	1.000 ± 0.207
		analyte	3	10186.6 ± 2716.6	11063.6 ± 3068.6	10442.5 ± 2571.6	0.623 ± 0.166	0.626 ± 0.174	0.677 ± 0.167
		control	4	14267.1 ± 3183.1	15451.1 ± 3896.7	17196.5 ± 7352.7	1.000 ± 0.223	1.000 ± 0.252	1.000 ± 0.428
		analyte	5	19715.5 ± 4579.1	19074.7 ± 3307.5	18566.3 ± 3860.0	1.382 ± 0.321	1.235 ± 0.214	1.080 ± 0.224
		control	6	15086.8 ± 3492.8	14563.2 ± 3046.3	15662.8 ± 2961.7	1.000 ± 0.232	1.000 ± 0.209	1.000 ± 0.189
		analyte	7	26133.4 ± 5548.6	23827.6 ± 4387.1	24751.0 ± 4330.4	1.732 ± 0.368	1.636 ± 0.301	1.580 ± 0.276
		control	8	14007.2 ± 2853.6	15346.3 ± 3396.1	15420.3 ± 3397.9	1.000 ± 0.204	1.000 ± 0.221	1.000 ± 0.220