Electronic Supplementary Material (ESI) for Lab on a Chip

Supplemental information

S1: Fluorometric estimation of the concentration of LoopAmp® control DNA

The concentration of DNA template contained in the LoopAmp® kit was too low for quantification based on its absorbance at 260nm. We therefore performed an alternative experiment, where the amount of DNA was estimated from the signal change of an intercalating dye added to the solution. A dilution series of a DNA sample of known concentration and length was used as a reference to which the signal from the control DNA was compared.

A five-fold dilution of the LoopAmp® control DNA stock solution was prepared along with a serial dilution of full size λ-phage DNA ranging from 10^9 to 10^3 copies per µl. The concentration of the λ-phage DNA was determined by its absorbance at 260 nm to be 465 µg/ml, or about 8.9×10^9 copies per µl. The serial dilution was then prepared in TE-buffer (10 mM Tris-HCl, 0.1 mM EDTA, adjusted to pH 7.5 and supplemented with 0.02% Nonidet P-40. Two sets of samples were prepared and mixed with different amounts of Evagreen® (0.1× and 1×, respectively, where 1× corresponded to the final dye concentration recommended by the manufacturer). Samples were incubated for 30 minutes, loaded onto a 384 well microplate (Sensoplus, Greiner BioOne, USA) and imaged on the Typhoon™ scanner (excitation with 473nm, detection filter: 510LP). The limited amount of control DNA solution allowed for only 2 wells to be used with the same solution. Upon intercalation into double stranded DNA, Evagreen® undergoes a conformational change, which results in an increase in fluorescence. Figure S1A shows images of a section of a microplate for both dye concentrations. For analysis, a rectangular region of interest was defined for each sample well and the integrated intensity was averaged over all wells containing the same solution. Figure S1B compares the intensities from the control series to the DNA template. The five-fold diluted template DNA showed an intensity comparable to that observed for 10^3 to 10^5 λ-DNA copies per µl. To improve the accuracy in the low DNA concentration range, data from both dye concentrations were combined by first normalizing all λ-DNA intensities to the value observed for the respective LoopAmp® control sample, followed by computation of the average from both dye concentrations. The inset in Figure S1B shows a close-up of the four lowest λ-DNA concentrations after combination of both data sets. Within the errors, the signal from the five-fold diluted DNA template appeared to be lower than that for 10^5 control DNA copies per µl. More accurate determination of the template concentration, however, was not possible due to the size of the error bars. We therefore estimated that the concentration of template DNA in the stock solution was between 10^4 and 10^5 copies per µl.

S2: Determination of the DNA stock concentration from the dLAMP experiment

The distribution of the number of template molecules in each digitized volume in the chamber can be described by Poisson statistics. For a given average of λ molecules per chamber, the probability to observe k copies per chamber is given as

$$P(k,\lambda) = \frac{\lambda^{k} \cdot e^{-\lambda}}{k!}, \quad \text{(S1)}$$

where k! is the factorial of k. The probability of having no template inside the chamber is $P(k=0,\lambda) = e^{-\lambda}$, while the chance of having at least one copy per chamber is given by $P(k>0,\lambda) = 1 - P(k=0,\lambda) = 1-e^{-\lambda}$.

The average number of copies per chamber for each dilution, λ, equals the product of the stock concentration c_0 (in units of molecules per chamber volume) and the dilution factor x_{dil}, $\lambda = c_0 \cdot x_{dil}$. x_{dil} = 1 for the stock solution, while for a 100-fold dilution of the stock solution we have x_{dil} = 0.01, and so forth.

Each chamber containing one or more templates should produce a positive signal; therefore, the observed fraction of LAMP-competent chambers, f_0, on the chip should equal P(k>0,λ). We have

$$f_0 = 1 - e^{-\lambda} = 1 - e^{-c_0 \cdot x_{dil}}, \quad \text{(S2)}$$

which can be rewritten as

$$\ln(1 - f_0) = -c_0 \cdot x_{dil}. \quad \text{(S3)}$$

We determined the initial concentration of the DNA stock solution from a linear regression fit of $\ln(1-f_0)$ against the experimental dilution factor x_{dil}. Supplemental Figure S3 shows a linear regression fit to the lowest three concentrations in the dilution series, which yielded a value of c_0 = (59.24 ± 1.62) copies per chamber volume. For a side chamber volume of 6 nl, this equals to a stock concentration of c_0 = (9.87 ± 0.27) × 10^3 copies per µl.

Supplemental Figures

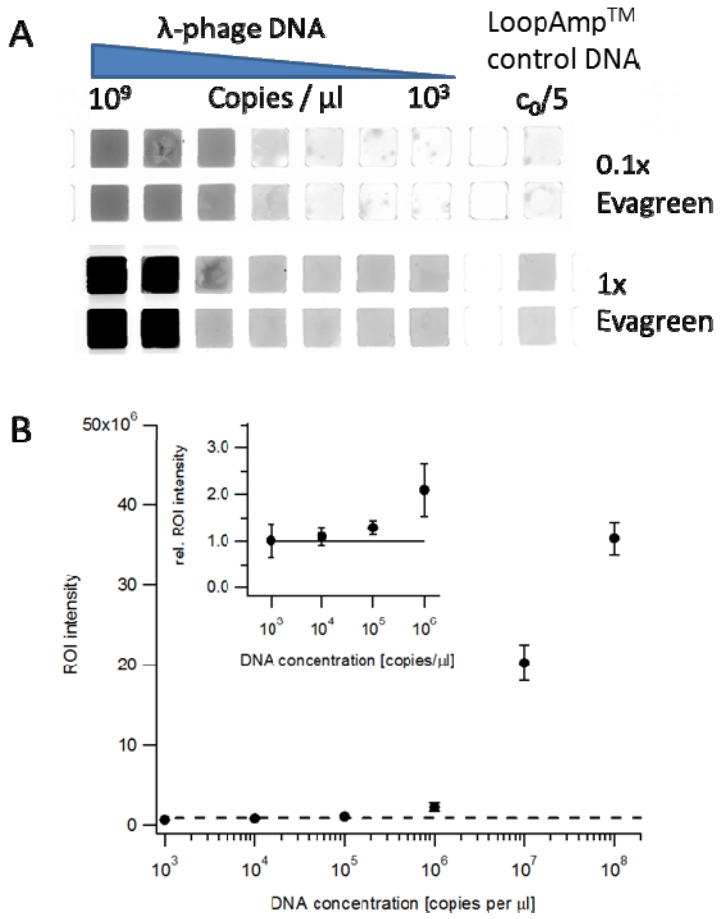

Figure S1: Estimation of the LoopAmp® control DNA concentration with Evagreen®. **(A)** Grey scale fluorescence images of sections of a multiwell plate filled with a serial dilution of λ-phage DNA and a five-fold dilution of control DNA. **(B)** Quantification of fluorescence intensities and estimation of the template DNA concentration. The average intensity from each sample is plotted against the nominal DNA concentration. Data are shown for 0.1× Evagreen®. The dashed line represents the intensity observed for the five-fold dilution of the LoopAmp® control DNA. The inset shows a close-up of the data set combined from both dye concentrations, normalized to the intensity from the unknown control DNA (solid line).

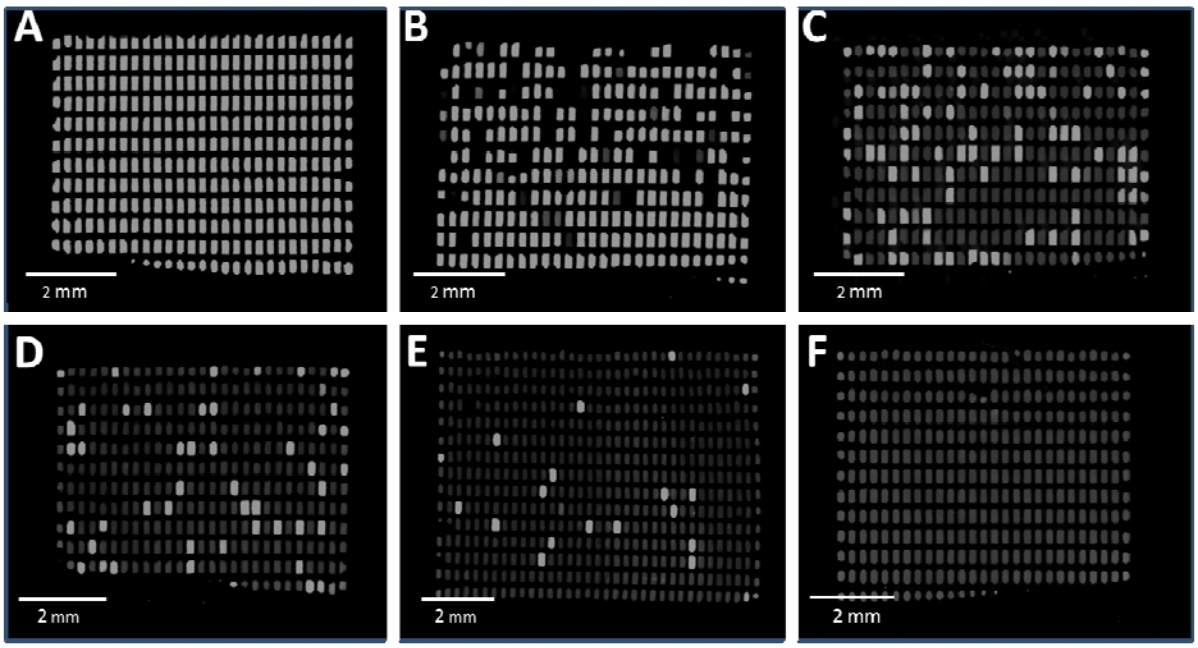

Figure S2: Digital LAMP results for different DNA template concentrations c_i: $c_i = c_0/4$ (**A**), $c_i = c_0/30$ (**B**), $c_i = c_0/150$ (**C**), $c_i = c_0/430$ (**D**) and $c_i = c_0/1300$ (**E**). c_0 is the concentration of the template stock solution. The respective fraction of LAMP-competent chambers is analyzed in Figure 4A of the manuscript. For the experiment at lowest sample concentration ($c_0/1300$), a 535-chamber chip was used to increase the absolute number of positive chambers. (**F**) Control experiment of a LAMP solution with no template added. As expected, none of the chambers showed an increase in fluorescence beyond background.

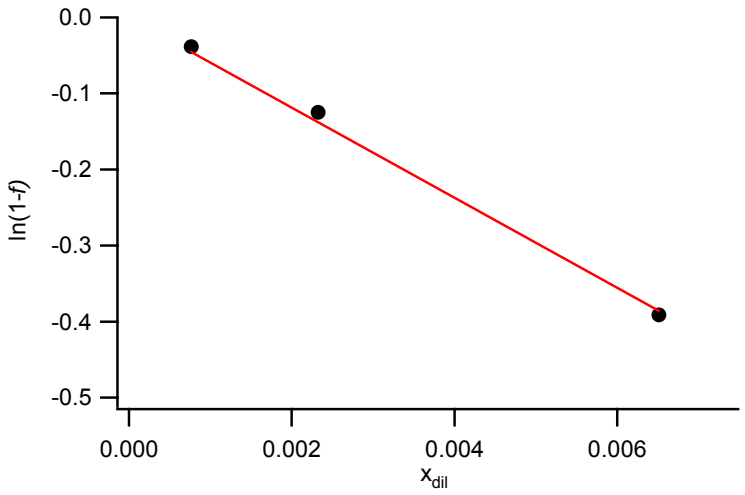

Figure S3: Calculation of the DNA template concentration in the LoopAmp® control solution with dLAMP. For the lowest three dilutions measured we determined the fraction of LAMP-active droplets, f_0, and plotted $\ln(1-f_0)$ against the dilution factor x_{dil}. The data were approximated by a linear regression based on Poisson statistics, see equation SI3. From the slope of the curve and the volume of one side chamber (6 nl) we obtain a stock solution of $c_0 = (9.87 \pm 0.27) \times 10^3$ copies per µl.

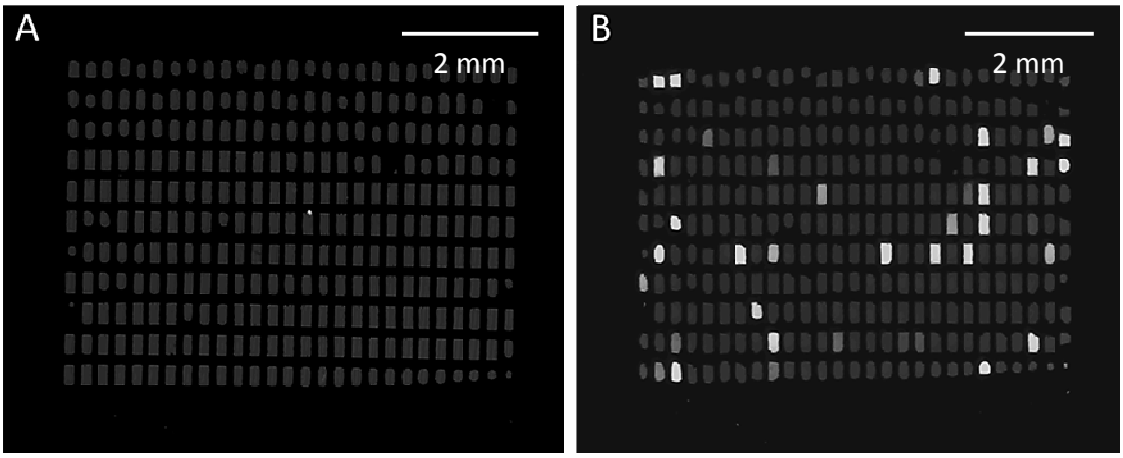

Figure S4: Images of a 351-chamber chip with a nominal λ-phage DNA concentration of 20 copies per µl before and after digital LAMP. The chip was incubated at 65 °C for 70 minutes. Based on the DNA concentration and the volume of the side chamber (6 nl), the fraction of positive events is expected to be 0.12. From the number of LAMP-competent chambers (n_a=32) after incubation and the total number of initial chambers (n_i=294) we compute a relative fraction of f_a=0.107, which is in good agreement with the expectation.