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Petri dish PCR: laser-heated reactions in nanoliter droplet arrays

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Abstract

We report high-speed real-time PCR performed on an unmodified disposable polystyrene Petri dish. The reaction cycle relies solely on an infrared laser for heating; no conventional heater is required. Nanoliter droplets of PCR mixture as water-in-oil emulsions printed in an array format served as individual PCR microreactors. A simple contact printing technique was developed to generate a large array of uniform sized nanoliter droplets using disposable pipette tips. Printed droplets showed variation of less than 10% in volume and the oil/water/polystyrene interface formed a compact droplet microreactor approximately spherical in shape. The uniform droplet array was used to optimize the laser power required for the two heating steps of PCR, annealing/extension and melting, while the ambient conditions were at room temperature. The optical heating allows for an extremely fast heating rate due to the selective absorption of the infrared laser by PCR buffer only and not the oil or polystyrene Petri dish, allowing completion of 40 amplification cycles in ~6 minutes. The quantitative assay capability of the system is also presented and discussed.

Introduction

Aqueous solutions dispersed in an oil phase (inverted emulsions) have been widely tested and adopted as ideal microreactors especially for high-throughput lab-on-a-chip applications.^{1–3} Successful executions of many biochemical reactions have been demonstrated in a single droplet without affecting neighboring droplets^{4–8} including polymerase chain reaction (PCR) in nano to picoliter volume droplets.^{9–14} This broad use of droplet methods covering a range of applications is due to the droplet's high stability, low volume required per droplet, absence of cross talk between droplets when separated by the oil phase, and the straightforward production of a large number of reaction wells or droplets without any microfluidic device.¹⁵ Moreover, recently developed techniques for manipulation of individual droplets on a planar substrate, microfluidics without channels,^{9,16,17} set the stage for the direct application of droplet-based methods to highly parallel assays with microfluidic control methods that can also perform the sample preparation steps such as heating and mixing. Such a high-throughput system naturally requires a suitable droplet generation in a controlled manner. Various channel- or chip-based systems have been developed to meet this requirement.^{6,17–20} However, the microfabrication of microfluidic and/or electrical circuitry on the substrate is often time consuming and expensive. Furthermore, any reuse of a microfluidic substrate runs the risk of cross contamination between samples. For this work on PCR in nanoliter droplets, we prepare droplet arrays using contact printing²¹ as shown in Fig. 1 (a).

An optical heating method was used to drive the heating cycles of PCR of each droplet of the printed array using a 1460 nm infrared laser. This pioneering method is initiated from room temperature without any additional heater. The modulated power of the infrared laser aimed at an aqueous droplet in an oil phase provides the heating cycles of PCR as illustrated in Fig. 1 (b). In this configuration, the only region heated is the selected droplet unlike the conventional heater-based PCR systems, which heat a larger region.

Laser heating²² combined with a droplet-based method provides several significant advantages over other contact or noncontact heating methods including (1) extremely fast heating and cooling due to the selective and direct heating of the PCR buffer not the oil or substrate, (2) no need for fluidic, heating or cooling circuitry on the substrate (a disposable polystyrene Petri dish in this case), and (3) simple and random access selective heating of droplets for PCR achieved through aiming the laser source at the chosen droplets. A few attempts have been made to run the heating cycles of PCR with an infrared laser but all of them were aided by a supplementary heater that maintained the substrate at the temperature required for the annealing/extension step of PCR.^{10,12,23} While the addition of the laser heating to the conventional heater system of PCR may benefit from some of the advantages of the optical heating, the necessity of the contact heating has clear limitations. First, construction of a rather complex reaction chamber incorporating a transparent heater is required. Second, limitations are placed on the composition of the reaction substrate. Here, we present a system that can run real-time PCR on a disposable Petri dish only using an infrared laser heating method, no substrate modification or heater is required.

Materials and methods

Contact printing system

A home built system was constructed for contact or spotting droplet array generation using conventional plastic micropipette tips (10 μ l pipettor tips, VWR Scientific 46620-316) filled with the dispensed liquid. Brief contact (typically less than 100 ms) of the tip on a hydrophobic substrate such as a polystyrene Petri dish is used to generate a large array of nanoliter droplets. Vertical motion of the tip was performed using a linear actuator (850F, Newport). Synchronized horizontal displacement by dual-axis motorized linear stages (404XR, Parker) positions the Petri dish for each array element. A pre-pulled glass pipette tip (TIP5TW1LSO1, World Precision Instruments) also reliably produced droplets with smaller sizes (droplets with a diameter as small as 100 μ m at pitches of 250 μ m or less between droplets). To prevent evaporation of the droplets, a fine water mist was blown on top of the Petri dish using a medical nebulizer driven by compressed air while an array is printed. With this humidity control, no significant evaporation effect on the size of the droplets was found even after several minutes of printing. After printing, the array was permanently protected from evaporation by adding mineral oil (M5310, Sigma-Aldrich) around the array very slowly using a conventional pipette until the array is covered. Mineral oil was used because it is lighter than water (0.82–0.88 g/mL) and its solubility in water is extremely low.²⁴ The slow spreading speed of the oil over the surface rarely disrupted the array as seen in Fig. 2. A syringe pump (NE-1000, New Era Pump Systems) and pressure sensor (PX139, Omega) connected to the back of the pipette tip formed a pressure regulated circuit. A negative back pressure (–300–600 Pa) was used to load the liquid through the tip from a sample reservoir and a positive back pressure (200–1000 Pa) was used while printing. Array printing was fully automated using a Labview program to control the three axes of the translation stages and the syringe pump.

Other methods such as inkjet printing²⁵ or multi-tip deposition can also be used for droplet array preparation. While inkjet methods are faster and deposit smaller droplets, contact printing using single-use tips and substrates allows simple and contamination-free

performance without any washing step and low-cost operation. However, the PCR methods described here can readily be applied to droplet arrays prepared using other printing methods and the contact printing is not an essential part of the method.

PCR protocols

PCR mixture was prepared in 20 μ l volumes that contain 10 μ l of Taqman Fast Universal PCR Master Mix 2X (#4352042, Applied Biosystems), 1 μ l of Taqman gene expression assay of primer/probe set targeting eukaryotic 18S rRNA gene (#4331182, Applied Biosystems) and human genomic DNA (#4312660, Applied Biosystems) diluted in a total volume of 9 μ l with DI water. The starting concentration of the template DNA was 10 ng/ μ l. The length of the amplicon was 187 base pairs.

We have tested various combinations of the temporal duration for each PCR step according to the proposed protocol from the assay manufacturer. Except for the data on the optimization of the extension period, all of the results presented here were acquired with 60 cycles of 2 seconds for melting and 8 seconds for annealing/extension in addition to the initial enzyme activation at the melting temperature for 10 seconds, which is comparable to the protocol used by Neuzil et al.¹¹

A uniform concentration during the reaction was confirmed through the negligible volume change of the droplet by carefully monitoring bright field images taken before and after PCR. Timed image acquisition and laser power control were fully automated with Labview software.

Amplification curves were plotted from the average intensity of the fluorescence image taken at the end of the extension step of each cycle. Conventionally, cycle of threshold (C_t) is determined as a parameter of quantification of each real-time PCR assay. It is defined as the fractional heating cycle where the fluorescence intensity of the amplified sample crossed the background fluorescence level, the detection of threshold. In this work C_t was calculated using the first derivative of the amplification curve as it transitions from the initial linear phase to the exponential phase. A common but arbitrary threshold that is set to roughly the twice the standard deviation of the linear phase was applied universally for all amplification curves.

Assay platform and detection system

A dual filter-turret inverted Nikon microscope (TE2000, Nikon) provides the platform for the optical setup, with the top filter cube used for fluorescence excitation and emission collection and the bottom filter cube for delivery of the infrared laser beam for heating the droplet. The bottom cube has a commercially available dichroic mirror (Q481LP, Chroma) that has high reflectivity at the laser wavelength and broad transmission in the visible range. A set of band pass filters was used for fluorescence detection with a 480/40 nm filter for excitation and a 520/40 nm filter for emission of FAM (HQ480/40X and D520/40M, Chroma). A 530-nm long pass filter (NT46-059, Edmund Optics) was also inserted in the optical emission light path to cut off any stray excitation light. Images were acquired with a cooled CCD camera (Retiga Exi, Qimaging). A regular digital camera (Nikon Coolpix 995) was also added to one of the side ports of the microscope to image a larger field of view and to capture the array images (Figs. 2 and 3).

Laser heating

Laser heating was performed using an infrared diode laser at a wavelength of 1.46 μ m (FOL 1402PLY-617-1457, Furukawa Electric) that was directed through the center of the droplet. The light emitted by the fiber pigtail attached to the diode laser was collimated with a 10 cm

lens and coupled by a high reflecting mirror through the lower back port into the microscope. The laser wavelength corresponds to the first overtone of the O-H stretch vibration of liquid water with a peak absorption coefficient as high as 32.4 cm^{-1} .²⁶ The mineral oil was measured to have about 40 times weaker absorption at this wavelength (data not shown). A low noise current source (LDX-3620, ILX Lightwave, controlled by a computer) supplies the laser current. The laser was defocused at the object plane of the microscope so that the droplet can be heated more uniformly. The beam size was measured to be $\sim 200 \text{ }\mu\text{m}$ at FWHM with the 10X objective lens that was used throughout the optical heating method. The power of the infrared laser was alternated between two power levels to achieve the temperature of the melting and annealing/extending steps respectively.¹⁰

Results and discussion

Droplet printing

A variety of liquids have been tested for contact printing including distilled water with or without dye, PCR mixture and cellular growth medium. All provided uniform sized droplet arrays. Actuation for each droplet took typically less than 2 seconds to print and 3 minutes was enough to print a 10 by 10 array or 100 droplets. The droplets remained very stable without coalescing over a period of days with the array covered with mineral oil. We have not found it necessary to add surfactants²⁷ to improve droplet stability. The hydrophobic polystyrene surface of the Petri dish with the oil/water interface helped to keep the contact area of a droplet very small and the contact angle larger than 150° . Thus the shape of the droplet immersed in the oil roughly followed a compact sphere, which is very suitable for a closely packed array and helped the heating as described in Extensions of method below. Fig. 2 shows bright field and fluorescence images of a typical 10 by 10 array printed with aqueous Rhodamine B dye solution. The standard deviation among droplets in the array was $\sim 3\%$ in diameter and $\sim 9\%$ in volume. The size of the printed droplet depends on many parameters including the back pressure (controlled by the syringe pump), the contact time of the tip on the surface, and the diameter of the pipette tip.

Once the contact printing method was established, we tested PCR reactions with the droplets printed on a Petri dish using the laser heating method. An array of droplets whose diameters are close to the laser's extinction length in water ($\sim 300 \text{ }\mu\text{m}$) was printed with a PCR mixture targeting 18S rRNA gene from human genomic DNA on a Petri dish and covered with mineral oil.

Temperature measurement and optimization

Droplet temperatures were calibrated based on fluorescence measurements.^{10,11,28} Briefly, droplets made of solutions of hydrophilic temperature sensitive fluorescence dyes such as kiton red or LDS 698 (Exciton)^{29–31} were heated on a home-built transparent heater system utilizing an indium-tin-oxide coated conductive glass slide (ITO-0011, Nanocs Inc.) and a hydrophobic disposable reaction substrate (HS22, Grace Bio Labs). The decrease in fluorescence intensity was calibrated against temperatures measured with a thermocouple. The fluorescence quenching was in turn used to calibrate the laser power required to heat the droplet up to annealing/extension temperature ($\sim 60^\circ\text{C}$) and melting temperature ($\sim 95^\circ\text{C}$) with an accuracy of $\pm 3^\circ\text{C}$. Using variation of the laser power between droplets in an array, we further optimized the temperature range in the present work based on the PCR results to achieve the highest amplification efficiency. For a typical droplet with diameter of $\sim 300 \text{ }\mu\text{m}$, 25 and 50 mW of laser power was required for the annealing/extension and melting steps, respectively. Because the power absorbed by a droplet of fixed diameter is directly proportional to its temperature change (see Extensions of method below), the laser power can be used as an indicator for the droplet temperature once this calibration is performed. In

this manner temperature precision of better than 1 °C should be achievable. From temperature-laser power calibrations performed using LDS 698, we have determined the annealing/extension and melting temperatures to be 59 °C and 94 °C, respectively, with an uncertainty of ± 3 °C.

Unlike conventional heater-based PCR, the selective heating scheme with the laser allowed for extremely fast temperature ramping and no delay was necessary between the two thermal steps of PCR. The polystyrene Petri dish is recommended for use below 70 °C because it starts to deform around its glass transition temperature of 80–120 °C. However, the small contact area of the droplet that is heated for only few seconds up to the DNA melting temperature didn't thermally damage the polystyrene Petri dish surface, as was confirmed by bright field images acquired following the PCR reactions.

PCR reactions

To determine the shortest effective PCR melting and annealing/extension times, different periods for each step were tested while the initial enzyme activation was fixed at 10 seconds. We found that a melting time as short as 1 second didn't slow the amplification kinetics or reduce amplification efficiency significantly. However, 2 seconds of melting time was used for all of the reactions presented in this work to ensure the maximum amplification efficiency for every droplet without influence from any variability in conditions between droplets. Several time periods of the annealing/extension step were tested while the amplification kinetics were monitored. As seen in Fig. 4, a significant change occurred when the extension time was reduced below 7 seconds. In particular, an extension time of 6 seconds has a C_t that is more than 5 cycles longer than that for a 7 seconds extension time, even though the C_t 's for extension times of 7 to 10 seconds agree to within approximately 1.5 cycles. This is also shown very clearly in the inset of Fig. 4 presenting a magnification of the data near the cycles of threshold. The extension speed of the Taqman polymerase enzyme is known to be around 30–60 bases per second. Considering the amplicon size of 187 bases, a minimum of 7 seconds for the extension step appears reasonable. Based on this result, the combination of 8 seconds for annealing/extension, 2 seconds for melting and an initial 10 seconds for the enzyme activation was deemed near optimal and used for all the reactions presented below.

Concentration dependence

To check for the quantitative assay performance of the method, PCR mixtures with 4 different target concentrations including a control that contained no target sequence were prepared. Each mixture was separately printed on a Petri dish and one droplet was amplified at a time with the same laser power and time periods for all concentrations as determined above. Fig. 3 shows the bright field and fluorescence images of 20 droplets of the printed array after amplification of 10 droplets from the upper right. Only the amplified droplets showed up in the fluorescence image. The apparent variability in brightness between droplets in Fig. 3 (b) is due to variability in the fluorescence brightness, not volume change. The final fluorescence intensity after PCR reaction varies among droplets even when the C_t varies quite little (see, e.g., Fig 5). For the 4 ng/ μ l droplets, the raw final fluorescence intensity differs by as much as 30% while the C_t is within ± 1 cycle. The dark granular halo around droplets in bright field images may be air bubbles formed during heating and they disappear within minutes as can be seen in Fig. 3 (a), where only the central and most recently heated droplet shows a halo.

The acquired fluorescence images at every tenth cycle of 4 different concentrations are shown in Fig. 6 in addition to the bright field images before and after PCR. In the case of the control sample, the brightness was uniformly raised by ~ 5 times to pronounce the weak but

uniform fluorescence intensities throughout the cycles. While the fluorescence reduction between the 4-fold sequential dilutions in Fig. 6 are not readily distinguishable by eye, the different levels of fluorescence at the 30th cycle gives a qualitative measure for the estimated concentration difference. From the bright field images of Fig. 6, we estimated the typical volume change of the droplets after 60 PCR heating cycles to be ~5% or less, which will have a negligible effect on the assay results. Note that the change in the inner diameter of the black rings in Fig. 6 is due to changes in the contact area, not the droplet volume. The outer circumference of the black ring yields the outer diameter of the droplet, while the inner circumference yields the diameter of the contact area. The change in contact area may be due to contact angle hysteresis during the heat cycling.

Fig. 5 is a quantitative presentation of data that is drawn with the average intensity of the fluorescence images acquired at every cycle. The amplification kinetics slows as the target concentration is reduced as expected. The C_t 's are determined to be 22.6 ± 1.0 (6 runs), 25.5 ± 0.7 (4 runs) and 30.2 ± 1.2 (6 runs) for 4, 1 and 0.25 ng/ μ l target concentrations respectively. For the control sample, no amplification was seen for all 5 droplets we have tested. In addition to the quantitative agreement between the C_t 's and the target concentrations, the consistency within the same concentration is very encouraging. The inset of Fig. 5, a magnified plot of the same curves around the cycles of threshold, shows how closely the curves are distributed for each concentration. Because of the small size of the droplets, the small number of targets contained in each nanoliter droplet may contribute to variation of the C_t 's. Typically the number of human genomes contained in a single droplet is less than 20 as discussed below. Considering that the typical volume of the droplet is ~12 nl and the mass of the haploid human genome 3 pg,³² even the highest concentrated 4 ng/ μ l droplet may have 16 genomes and the lowest concentrated one may have only one genome in average. Since each genome has ~400 copies of 18S rRNA gene,³³ even the lowest concentrated droplet will have only 5% variation in target numbers, which is negligible for the variations of C_t 's measured above.

Extensions of method

The low maximum power of our laser (~70 mW at the exit of the objective lens) allowed only one droplet to be amplified at a time. We require ~50 mW to heat the droplets to the DNA melting point. A simple calculation based on heat loss due to conduction (radiative and convective losses are negligible in comparison to conduction losses) agrees with the measured power within 40%. Our results contrast with the heater-assisted work of Terazono et al.¹² who used roughly 20 times more laser power (around 1 W). The improved power efficiency in our work is mainly due to the longer optical path of the droplet when resting on the hydrophobic substrate. The low power consumption is promising for extension to parallel heating methods as are common with current PCR technologies. With spatial light modulators or a microlens array and high power laser (5–150 W is commercially available), simultaneous array amplification can be performed. In principle, a commercially available 2 μ m laser can be also used more economically with smaller droplets because the conductive heat loss is approximately proportional to the radius of the droplet.

The droplet approach also seems very attractive for many other critical applications such as amplification of small amounts of template DNA¹⁵ and single cell assays.^{4,6,18} The current standard paradigm for performing PCR is simultaneous reactions in large arrays. However, the ability to perform customized reactions on each droplet in an array is also quite useful. For example, changing the reaction conditions for many droplets containing the same target sequence is an ideal method for PCR optimization, as we have done in this work. Similarly, when different targets are present in each droplet, the reaction conditions such as the annealing temperature or extension time may be tailored for each target. When performing studies on cells, reactions can be performed on a cell in one droplet while not impacting

cells in neighboring droplets. For example, one could add different primers and tailor the reaction conditions for different DNA amplification targets depending on the type of cell or organism, which would be very valuable for work on circulating tumor cells, pathogens for food or water quality testing, or forensics. Once the method is extended to reverse transcription PCR, one could perform gene expression analysis on specific cells based on specific time points such as the phase of cell development, during stages of phagocytosis, or during viral attack, while maintaining the integrity of other cells under observation. These and other similar possibilities are not possible with current methods. Note that using a spatial light modulator and a high power laser, it will be possible to perform array amplification while also customizing the reaction in each well, a unique and very exciting possibility.

Currently we are attempting to print an array of droplets that contain a single cell per droplet. With the help of the laser heating, a fully optically controllable system that can lyse the cell, extract DNA or reverse transcribe RNA and run the real-time PCR far faster than the current technology seems feasible based on this work.

Conclusions

We constructed a real-time PCR system with an array of nanoliter droplets printed on a Petri dish surface using an infrared laser at 1.46 μm without help of any conventional heater. We have shown that the newly developed system is capable of quantitative assays.

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References

1. Griffiths AD, Tawfik DS. Trends Biotech. 2006; 24:395–402.
2. Haeberle S, Zengerle R. Lab Chip. 2007; 7:1094–1110. [PubMed: 17713606]
3. Teh SY, Lin R, Hung LH, Lee AP. Lab Chip. 2008; 8:198–220. [PubMed: 18231657]
4. He M, Edgar JS, Jeffries GDM, Lorenz RM, Shelby JP, Chiu DT. Anal. Chem. 2005; 77:1539–1544. [PubMed: 15762555]
5. Holden MA, Needham D, Bayley H. J. Am. Chem. Soc. 2007; 129:8650–8655. [PubMed: 17571891]
6. Köster S, Angilè FE, Duan H, Agresti JJ, Wintner A, Schmitz C, Rowat AC, Merten CA, Pisignano D, Griffiths AD, Weitz DA. Lab Chip. 2008; 8:1110–1115. [PubMed: 18584086]
7. Kotz KT, Gu Y, Faris GW. J. Am. Chem. Soc. 2005; 127:5736–5737. [PubMed: 15839641]
8. Chabert M, Dorfman KD, de Cremoux P, Roeraade J, Viovy JL. Anal. Chem. 2006; 78:7722–7728. [PubMed: 17105164]
9. Guttenberg Z, Müller H, Habermüller H, Geisbauer A, Pipper J, Felbel J, Kielpinski M, Scriba J, Wixforth A. Lab Chip. 2005; 5:308–317. [PubMed: 15726207]
10. Kim, H.; Dixit, S.; Pan, C-P.; Faris, GW. Biomedical Optics/Digital Holography and Three-Dimensional Imaging/Laser Applications to Chemical, Security and Environmental Analysis. St. Petersburg, FL USA: Optical society of America; 2008. p. BMD69
11. Neuzil P, Zhang C, Pipper J, Oh S, Zhuo L. Nucleic Acids Res. 2006; 34:e77. [PubMed: 16807313]
12. Terazono H, Hattori A, Takei H, Takeda K, Yasuda K. Jpn. J. Appl. Phys. 2008; 47:5212–5216.
13. Beer NR, Hindson BJ, Wheeler EK, Hall SB, Rose KA, Kennedy IM, Colston BW. Anal. Chem. 2007; 79:8471–8475. [PubMed: 17929880]

14. Pippert J, Inoue M, Ng LF-P, Neuzil P, Zhang Y, Novak L. *Nature Med.* 2007; 13:1259–1263. [PubMed: 17891145]
15. Williams R, Peisajovich SG, Miller OJ, Magdassi S, Tawfik DS, Griffiths AD. *Nature Methods.* 2006; 3:545–550. [PubMed: 16791213]
16. Kotz KT, Noble KA, Faris GW. *Appl. Phys. Lett.* 2004; 85:2658–2660.
17. Gong J, Kim C-J. *Lab Chip.* 2008; 8:898–906. [PubMed: 18497909]
18. Clausell-Tormos J, Lieber D, Baret JC, El-Harrak A, Miller OJ, Frenz L, Blouwolff J, Humphry KJ, Köster S, Duan H, Holtze C, Weitz DA, Griffiths AD, Merten CA. *Chem. Biol.* 2008; 15:427–437. [PubMed: 18482695]
19. Baroud CN, Delville JP, Gallaire F, Wunenburger R. *Phys. Rev. E.* 2007; 75:046302.
20. Beer NR, Wheeler EK, Lee-Houghton L, Watkins N, Nasarabadi S, Hebert N, Leung P, Arnold DW, Bailey CG, Colston BW. *Anal. Chem.* 2008; 80:1854–1858. [PubMed: 18278951]
21. Hong JM, Ozkeskin FM, Zou J. *J. Micromech. Microeng.* 2008; 18:015003.
22. Reinhardt H, Dittrich PS, Manz A, Franzke J. *Lab Chip.* 2007; 7:1509–1514. [PubMed: 17960279]
23. Braun D, Goddard NL, Libchaber A. *Phys. Rev. Lett.* 2003; 91:158103. [PubMed: 14611502]
24. He M, Sun C, Chiu D. *Anal. Chem.* 2004; 76:1222–1227. [PubMed: 14987074]
25. Boland T, Xu T, Damon B, Cui X. *Biotechnol J.* 2006; 1:910–917. [PubMed: 16941443]
26. Kou L, Labrie D, Chylek P. *Appl. Opt.* 1993; 32:3531–3540. [PubMed: 20829977]
27. Pautot S, Frisken BJ, Cheng J-X, Xie XS, Weitz DA. *Langmuir.* 2003; 19:10281–10287.
28. Kim H, Dixit S, Green CJ, Faris GW. *Opt. Express.* 2009; 17:218–227. [PubMed: 19129891]
29. Coppeta J, Rogers C. *Exp. Fluids.* 1998; 25:1–15.
30. Ross D, Gaitan M, Locascio LE. *Anal. Chem.* 2001; 73:4117–4123. [PubMed: 11569800]
31. Slyadnev MN, Tanaka Y, Tokeshi M, Kitamori T. *Anal. Chem.* 2001; 73:4037–4044. [PubMed: 11534733]
32. Vogelstein B, Kinzler KW. *Proc. Natl. Acad. Sci. USA.* 1999; 96:9236–9241. [PubMed: 10430926]
33. Gonzalez IL, Sylvester JE, Schmickel RD. *Nucleic Acids Res.* 1988; 16:10213–10224. [PubMed: 3194198]

Fig. 1.

(a) Illustration of the contact printing on a Petri dish. A disposable pipette tip is vertically controlled to make a brief contact with the substrate leaving a droplet on the surface. The Petri dish is positioned by the dual-axis motorized linear stages. (b) Illustration of the infrared laser heating of a droplet in the oil phase using an inverted microscope.

Fig. 2. Bright field (left) and fluorescence (right) images of 10 by 10 array of 100 droplets deposited on a Petri dish with contact printing. Droplets contain aqueous Rhodamine solution.

Fig. 3. Bright field (a) and fluorescence (b) images of a 4 by 5 array of PCR mixture droplets. 10 droplets in the upper right were PCR amplified while the rest were not amplified.

Fig. 4. Amplification curves with the extension time varied from 6 to 10 seconds. The inset shows a magnified plot of the same curves around the cycle of threshold region.

Fig. 5. Amplification curves of PCR droplets with 4 different template DNA concentrations. The inset shows a magnified plot of the same curves around the cycle of threshold region, which shows the narrow distribution for curves of the same DNA template concentration.

Fig. 6.

Images of droplets during PCR amplification at four concentrations of the template DNA. At left are bright field images before and after amplification. At right are fluorescence images at every tenth thermal cycle. In case of the control droplet, the brightness of the fluorescence image was increased ~5 times so that the weak intensity can be pronounced. The black bars in the bright field images are 100 μ m long.