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Fluorescence-activated droplet sorting of lipolytic microorganisms using a compact optical system†

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Lipases are ubiquitous enzymes of great physiological significance that have been used extensively in multiple industries. Environmental microorganisms are a major source for the discovery of novel lipases with high catalytic efficiency and selectivity. However, current plate-based screening of lipase-producing strains is time consuming, labour intensive and inefficient. In this study, we developed an ultra-high throughput screening pipeline for lipase-producing strains based on fluorescence-activated droplet sorting (FADS) using a compact optical system that could be easily set up in an alignment-free manner. The pipeline includes droplet generation, droplet incubation, picoinjection of the fluorescence probe, and sorting of droplets with a throughput of 2×10^6 drops per h. We applied the pipeline to screen samples collected from different locations, including sediments from a hot spring in Tibet, soils from the Zoige wetland, contaminated soils from an abandoned oilfield, and a Chinese Daqu starter. In total, we obtained 47 lipase-producing bacterial strains belonging to seven genera, including *Staphylococcus*, *Bacillus*, *Enterobacter*, *Serratia*, *Prolinoborus*, *Acinetobacter*, and *Leclercia*. We believe that this FADS-based pipeline could be extended to screen various enzymes from the environment, and may find wide applications in breeding of industrial microorganisms.

Introduction

Microorganisms are a major source for the discovery and production of various enzymes with wide industrial applications.[1] Screening processes are routinely used to obtain new enzymes from natural microorganisms and for selection of target strains from mutant libraries.[2] Currently, microtiter plate-based screening is the most common screening format; however, the throughput is limited to 10^3 to 10^4 per round.[3] Fluorescence-activated cell sorting (FACS), which can screen individual cells or water/oil/water emulsions[4–8] with extremely high throughput ($\sim 10^7$ h^{-1}), has become an appealing alternative. However, the widespread use of the FACS-based method is limited by its high cost and the multiple restrictions on complex double emulsion generation and modification.[9,10] Recently, fluorescence-activated droplet sorting (FADS) using microfluidic technologies has quickly emerged as a powerful tool for single cell screening with ultra-high throughput.[11] Important progress has been made in generating,[12] mixing,[13] dividing,[14] and sorting[15] of picoliter droplets. In the past few years, FADS has been applied to screening of many different enzymes including β-galactosidase,[9] cellulase,[16] α-amylase,[17] and aldolase.[18] Additionally, screening methods have been developed to encapsulate single cells in droplets, incubate droplets under various conditions, set up quantitative assays and reactions in droplets, and sort droplets with extraordinary efficiency. The flexibility of droplet manipulation on microfluidic devices opens the way to designing more complex and versatile screening assays for enzymes, as well as other biomedical applications.[19–22]

Lipases are ubiquitous enzymes that catalyze the hydrolysis of lipids[23] and are broadly used in the food, pharmaceutical, and petroleum industries, as well as for biocatalysis.[24,25] In the past few years, there has been great interest in the application of lipases to the production of biodiesel as an alternative renewable and eco-friendly fuel.[23] Nevertheless, new lipases with distinct substrate specificities and improved stabilities still hold great importance in industrial applications.[23] Recently, *in vitro* compartmentalization-based FACS for directed evolution of lipase in a model strain has been

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realized using a double-emulsion generated by membrane-extrusion.[7] Gel microdroplets (GMD) have also been used to screen metagenomic libraries for lipolytic enzymes based on digital enzymatic activity assays.[26] However, the FADS-based workflow for screening of lipases in droplets has not yet been reported. The feasibility of FADS for sorting environmental microorganisms, which grow much slower than model strains, based on their lipolytic activity remains obscure. Herein, we introduce a high throughput pipeline for screening of environmental lipase-producing bacteria based on the FADS technique. The pipeline allowed single-cell droplet generation, long-term droplet incubation, picoinjection[13] of a fluorogenic substrate, and sorting of droplets with a throughput of up to 2×10^6 drops per h. We applied this pipeline to sorting and recovery of lipolytic microorganisms sampled from various unique environments of great research interest and application potential. Moreover, we developed a compact optical system for FADS that could easily be set up in a common laboratory without the need for complicated optical alignment.

Experimental

Bacteria and samples

The *Pseudoalteromonas lipolytica* S29 strain was discovered with high lipolytic activity in a sediment sample collected from the northwest Indian Ocean (50°51′E, 37°38′S) at a depth of 2500 m. The strain was isolated on marine agar 2216E (Difco, Becton Dickinson) containing 1% olive oil at 16 °C. In this study, we cultivated S29 using marine broth 2216E at 37 °C. The cells were then spun down and suspended in 2216E to 7×10^6 cells per mL to make droplets for evaluation of system performance.

Four samples from different locations were used, a soil sample from the Zoige wetland (33°25′N, 102°52′E, Sichuan, China) provided by Dr. Juanli Yun from the Institute of Microbiology, Chinese Academy of Sciences, a petroleum-polluted soil sample collected from an abandoned oil field in Dongying (38°15′N, 118°5′E, Shandong, China), a sediment sample from the Lalong hot spring in Maizhokunggar, Lhasa (29°24′N, 92°6′E, Tibet, China) provided by Professor Bian Wu from the Institute of Microbiology, Chinese Academy of Sciences, and a Daqu starter sample of Gujing Tribute Liquor from Bozhou (32°51′N, 115°53′E, Anhui Province, China). The samples were stored at −20 °C without cryopreservation. Ten grams of each sample were suspended in 100 ml of sterile 1× PBS (pH = 7.4) and shaken for 30 min (160 rpm, 30 °C). Next, 5 ml of extract was inoculated in a flask with 200 mL of Luria Bertani broth (LB) (peptone 10 g L^{-1}, yeast extract 5 g L^{-1}, NaCl 10 g L^{-1}, pH = 7) supplemented with 1% (v/v) tributyrin. The samples were then cultivated for three days (200 rpm, 37 °C), after which they were diluted to a concentration of $\sim 7 \times 10^7$ cells per mL with LB medium to make droplets. The sample from the Zoige wetland was also tested with FADS without cultivation by direct dilution of the extract with LB medium.

Device fabrication

Microfluidic devices were used for droplet generation, picoinjection, and sorting (Fig. S2†). The devices were designed and fabricated based on previously described procedures,[13] and fabricated in poly(dimethyl-siloxane) (PDMS) using rapid-prototyping soft lithography.[27] The devices had channel heights between 14 and 25 μm and holes that were punched for channel inlets and outlets. The devices for picoinjection and sorting contained electrodes fabricated by filling empty channels in the shape of electrodes with low melting-temperature liquid solder (Indium Co., Clinton, NY, USA). All devices were used without silanization.

Lipase screening

Bacterial suspensions were prepared in culture media with the cell density adjusted to approximately 7×10^7 cells per mL. The flow-focusing device generated 3–4 pL droplets at a rate of 2700 per second. The number of cells per droplet followed a Poisson distribution[28] when the bacterial cells were introduced at a theoretical concentration of 0.3 cells per drop, resulting in 74% of the droplets being empty and 22% containing a single cell. The device was operated with a total aqueous flow rate of 40 μL h^{-1} and 80 μL h^{-1} fluorinated oil with a surfactant (Cat. No.186-4006, BioRad, Hercules, CA, USA). The droplets were incubated in a 20–30 cm long piece of Teflon tubing (Cat. No. AWG30, 0.010 inch i.d., 0.028 inch o.d., Zeus Inc., Orangeburg, SC, USA) at 37 °C, with both ends of the tubing sealed with capillary wax (Hampton Research, Aliso Viejo, CA, USA). The incubation time was 12 h for S29 and 3 days for the environmental samples unless otherwise stated.

After incubation, the droplets were loaded into the picoinjection device to introduce fluorescein dibutyrate (FDB) as a fluorogenic substrate for measurement of lipase activity. A 10 mM FDB stock solution in dimethyl sulfoxide (DMSO) was diluted 1 : 20 with 13 mM Tris–HCl buffer (pH = 7.4), then injected into the droplets. The final FDB concentration in the droplets was about 250 μM. A voltage between 0 and 200 V at a frequency of 30 kHz was applied to the electrodes to trigger the injection at a throughput of 300 droplets per second. The droplets were collected in the receiving Teflon tubing (20–30 cm in length), then incubated at 25 °C for 1 h to allow lipolysis of FDB.

Next, the Teflon tubing with droplets was connected to the sorting device with a channel depth of 25 μm. The sorting process was operated with a flow rate of 10 μL h^{-1} droplets and 100 μL h^{-1} fluorinated oil without a surfactant (Cat. no.186-3004, BioRad, Hercules, CA, USA) to space the droplets. The fluorescence signal of each droplet was collected using a custom-made compact detection module mounted to the side port of an inverted microscope (IX81, Olympus, Japan). By defining the fluorescence threshold, droplets were sorted by dielectrophoresis.[9] The FADS process was controlled using a program written in LabVIEW software (National Instruments, USA). Time series recordings were

analysed using a program written in Matlab (MathWorks, Natick, MA, USA, the Matlab code is provided in the ESI†) to extract the fluorescence signal of each droplet. Before and after sorting, droplets were collected and imaged by fluorescence microscopy (Eclipse Ti, Nikon, Tokyo, Japan). Positive-sorted droplets were then collected in an Eppendorf tube with 100 μL LB medium. Next, 100 μL 1*H*,1*H*,2*H*,2*H*-perfluoro-1-octanol (PFO, Sigma Aldrich, USA) was added and vortexed to break the droplets and recover the bacterial cells in the medium. To validate the sorting efficiency, we also made a binary mixture of positive and negative ~4 pL droplets. The positive droplets contained 25 μM FDB and 400 U mL^{-1} lipase (Aladdin, Shanghai, China), while the negative droplets contained 25 μM FDB without lipase. The mixed droplets were sorted directly after 1 hour incubation.

Lipase activity assays

The suspension containing recovered cells was plated on agar plates with LB medium, then incubated at 37 °C to allow growth of individual colonies. The colonies were selected and inoculated in 2 mL LB broth in culture tubes, then cultivated for 3 d (200 rpm, 37 °C). The lipase activities of the strains were evaluated with a plate assay as previously described.[29] Briefly, holes of 8 mm were punched in agar plates containing LB broth supplemented with tributyrin as the substrate and Rhodamine B as the indicator. Next, 100 μL of cultivated bacteria in LB broth were added into the holes and the samples were then incubated for 2 days. Opaque halos developed around the holes filled with lipase-positive strains, and their sizes were measured for preliminary estimation of lipase activity. Next, selected strains were inoculated in 100 mL LB broth containing 1% tributyrin, then incubated for 60 h at 37 °C and 200 rpm. Following cultivation, the broth of each strain was centrifuged at 5000 rpm for 15 min. The supernatant was then filtered using a 0.2 μm pore size filter, after which it was subjected to ultrafiltration at 5500 rpm for 25 min at 4 °C in a Centricon 50 centrifuge filter with a 10 kDa cut-off membrane (Millipore, Bedford, MA, USA). The concentrated solutes were subsequently collected and adjusted to 1 mL, after which microgram quantities of proteins in the solutes were determined by the Bradford protein assay.[30] Further analysis of extracellular lipase activity was conducted following the colorimetric method using *p*-nitrophenyl palmitate (*p*-NPP) as the substrate as previously described[31] (experimental details can be found in the ESI†).

Results & discussion

Framework for FADS-based lipase screening

To establish a robust lipase screening pipeline, we divided our work into three phases. In the first phase, commercially available lipase was used to evaluate the properties of the fluorogenic substrate, including cytotoxicity, self-hydrolysis, and leakage in droplets. In the second phase, the sorting efficiency of FADS was optimized using commercially available lipase and a lipase-positive S29 strain. In the third phase, the pipeline was used in sorting and recovery of lipolytic microorganisms from different locations to assess the performance of the FADS system.

The optimized lipase screening pipeline was composed of four major steps as outlined in Fig. 1: i) microbial cell suspensions were prepared using samples collected from various sites, and droplets were then prepared using flow-focusing geometry (Fig. 1B); ii) the droplets were incubated for 0.5–3 d for secretion of lipase by the microbial cells; iii) the droplets were loaded into the picoinjection device to introduce FDB into each droplet as the fluorogenic substrate of lipase (Fig. 1C), after which the droplets were incubated for about 1 h to allow hydrolysis of FDB; iv) the droplets were sorted according to their fluorescence intensity using the sorting device[2] (Fig. 1D). Finally, the positive-sorted droplets were demulsified, after which bacterial strains were retrieved and subjected to lipase activity assays.

To simplify the procedure and eliminate the loss of droplets because of extra transfer steps, we used a piece of thin-wall Teflon tubing connected to the outlets of the PDMS devices to receive and store the droplets between different steps. At a length of 30 cm, the Teflon tubing can store more than 2.5×10^6 4 pL droplets, which requires 1 h of sorting time under the current settings. The droplets were then incubated in the Teflon tubing by sealing the two ends with capillary wax. The cells in droplets could receive sufficient oxygen supply because of the excellent gas permeability of Teflon.[32] Following the removal of the sealing wax, the Teflon tubing was simply connected to the picoinjection device or sorting device for direct loading of droplets into the microfluidic channels.

Setup of the compact optical system for FADS

In contrast to the rapid progress regarding the new assays developed based on FADS, only limited work has been conducted to investigate the development of an integrated and portable detection and controlling system for FADS,[33] which is critical to widespread adoption of FADS in common

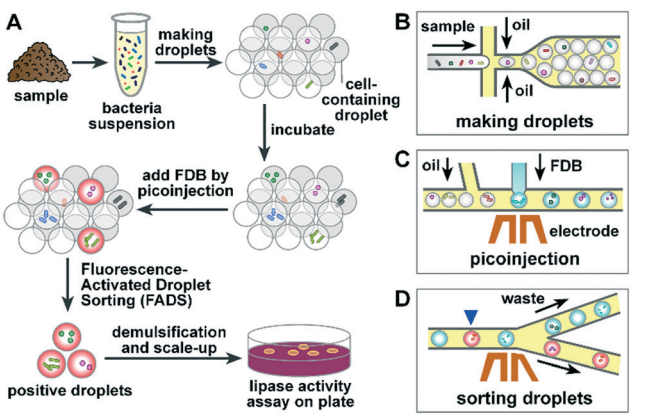

Fig. 1 (A) The fluorescence-activated droplet sorting (FADS) pipeline for lipase discovery. (B–D) Schematics showing microfluidic devices for generation of single cell-encapsulated droplets, picoinjection of the substrate into the droplets, and FADS triggered by dielectrophoresis.

laboratories. Therefore, we designed an integrated optical system to help common users overcome the optical alignment challenges associated with fluorescence-activated droplet sorting[34] (Fig. S1†). The system consisted of a compact optical module to measure the fluorescence of droplets, a high voltage module to deliver a high voltage–high frequency electric field to solder injected electrodes, a high-speed CCD camera to capture images and videos, and a power supply module to control the microscope and FADS system (Fig. S1A and B†).

The detection module (160 mm × 143 mm × 54 mm in size) contained a high-power diode laser, three photomultipliers (PMT) for detection of three fluorescence signals, and a 2 MHz analog-to-digital conversion circuit (Fig. S1C†). The wavelength of the laser and detectors can be flexibly customized to enable simultaneous detection of multiple fluorogenic probes (Table S1†). The V-mount of the detection module allowed easy connection with the side port of a standard inverted microscope. The optical path was aligned and die bonded in the detection module; thus, no further alignment was required. We also made a low-cost and compact high voltage module that was designed specifically for FADS. This optical system is alignment-free and features a compact size, light-weight portability, quick installation, and high robustness.

Measurements of lipase activity in droplets

Lipase can catalyse the hydrolysis of FDB to release fluorescein with green fluorescence (Fig. 2A). However, FADS-based lipase screening has not yet been reported, and the suitability of FDB for FADS-based sorting has not yet been evaluated. Moreover, when compared to previously used model strains such as *Escherichia coli* or *Saccharomyces cerevisiae*,[7,26] a long incubation time for cell-encapsulated droplets is required to screen uncultivated bacteria, which grow much slower in laboratory media. To investigate the long-term cytotoxic effects of FDB, we measured the growth (OD600) of S29 with or without FDB (250 μM) using standard well-plate assays. We found that addition of FDB had no significant effect on the growth of S29 (Fig. 2B). However, the fluorescence data showed that the hydrolysis of FDB occurred spontaneously without S29 cells added (Fig. 2C). Therefore, FDB should not be added with bacterial cells during droplet generation as previously described,[26] and the picoinjection step is essential to the quantitative measurement of the lipase activity of environmental bacteria in the droplets.

The leakage of reagents in water-in-oil droplets[35,36] is a major compromise to the precision of droplet-based screening. To determine whether the measurement of droplet-based lipase activity is prone to leakage in BioRad droplet generation oil, two types of droplets were generated: "positive" droplets containing FDB and commercially-available lipase (40 U mL^{-1}), and "negative" droplets containing FDB only. The dye leakage among droplets at different times was studied by mixing the positive and negative droplets (Fig. 2D). As shown

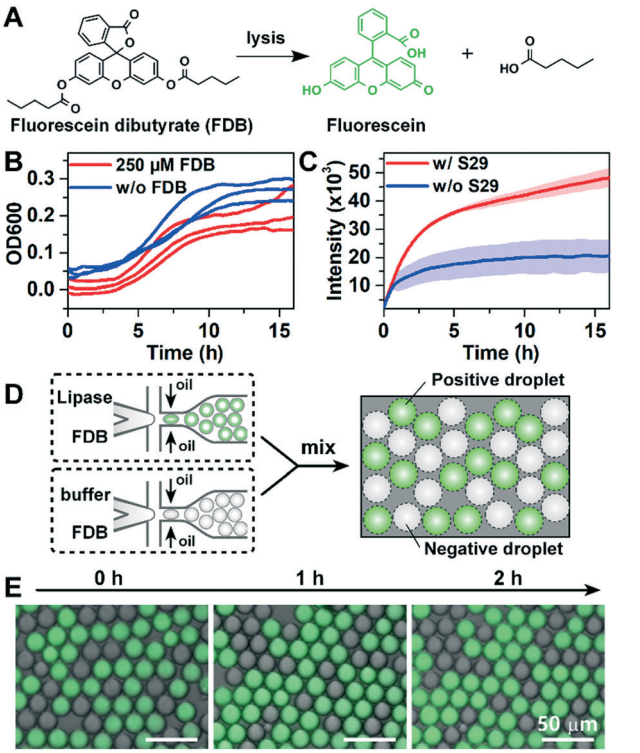

Fig. 2 Lipase activity measurements using fluorescein dibutyrate (FDB). (A) Hydrolysis of FDB releases fluorescein, which renders the droplet flourescent; (B) plate-based OD600 measurements show that the addition of FDB didn't affect the growth of S29 cells. In the test group (red curves), FDB was added at a concentration of 250 μM; in the control group (blue curves), no FDB was added. (C) Plate-based fluorescence measurements indicate spontaneous hydrolysis of FDB (250 μM) without S29 cells added. In the test group (red curve), 100 μL of 500 μM FDB and 100 μL of S29 cells in 2216E medium (OD = 1.25) were added; in the control group (blue curve), 100 μL of 500 μM FDB and 100 μL sterile 2216E were added. (D) Evaluation of "leakage" of FDB using a binary mixture of droplets with or without lipase, the concentration of FDB is 250 μM; (E) time-lapse fluorescence imaging of the binary droplet mixture demonstrates that there is no leakage between neighboring droplets after 2 h incubation.

in Fig. 2E, there was no detectable increase in fluorescence in the negative droplets during 2-h incubation, indicating that the leakage was insignificant (see the detailed method in the ESI†).

Sorting droplets based on lipase activity

To optimize the sorting condition, a binary mixture of positive and negative droplets (~4 pL in volume) with a 20 : 19 mixing ratio was produced using either 400 U mL^{-1} lipase or Tris–HCl and 25 μM FDB. Sorting parameters including flow rates, frequency and magnitude of AC voltage pulses applied on electrodes and trigger delays were investigated and optimized (see Fig. S3 in the ESI† for a typical time series recording of the detected fluorescence signal). The positive and negative sorted droplets were collected separately, imaged and counted by fluorescence microscopy to calculate the sorting

efficiency (Fig. S3†). A high enrichment factor was achieved with a speed of ~2 × 10^6 drops per h, and the optimized sorting condition was adopted in the later sorting experiments for bacterial cells and real samples.

To further optimize the performance of the FADS-based lipase pipeline for bacterial samples, we encapsulated S29 cells in droplets of 2216E medium, and tested the effects of FDB concentration on sorting efficiency in the range of 50–250 μM. We did not test higher concentrations since precipitation occurred when the concentration of FDB exceeded 250 μM (Fig. S4A†). Before sorting, the droplets were incubated for 12 h to allow lipase production and secretion from the S29 cells. FDB was added to the droplets by picoinjection, and then incubated for 1 h before sorting. An optimal FDB concentration of 250 μM for bacterial cell sorting was obtained based on discrimination between droplets that did not contain S29 cells (negatives) and those that did (positives) (Fig. 3, Fig. S4†).

Lipase screening from various sampling sites

We applied the lipase screening method for the discovery of lipase-producing bacteria from various locations (see Fig. 4A for geographical locations), including the Zoige wetland, the Lalong hot spring in Tibet, an abandoned oil field in Dongying, and the Daqu starter of Gujin liquor from Anhui Province. These samples were selected either for their representation of untamed or extreme environments where novel types of lipase-producing microorganisms might exist, or for their great application potential in the liquor and petroleum industries. We extended the incubation time of environmental samples to 3 d, since their growth rates are usually slower and it may take a much longer time for them to adapt to laboratory cultivations. Using the optimized conditions, similar

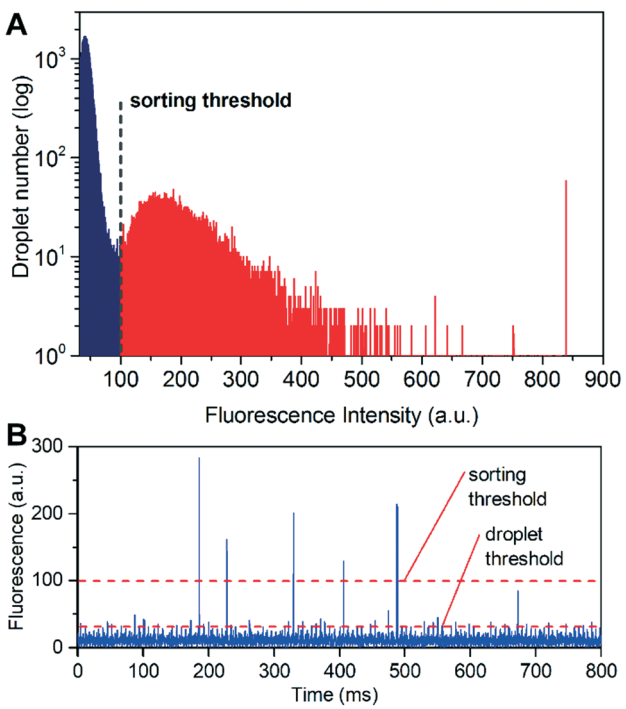

Fig. 3 (A) Histogram showing the distribution of droplet fluorescence intensities for sorting of *P. lipolytica* S29 with 250 μM FDB (final concentration) as the substrate. Fluorescent droplets passing the sorting threshold (gray dashed line) were sorted and collected for cultivation. (B) A typical time series recording of the detected fluorescence signal of S29 sorting. A droplet detection threshold of 32 and a sorting threshold of 100 were used.

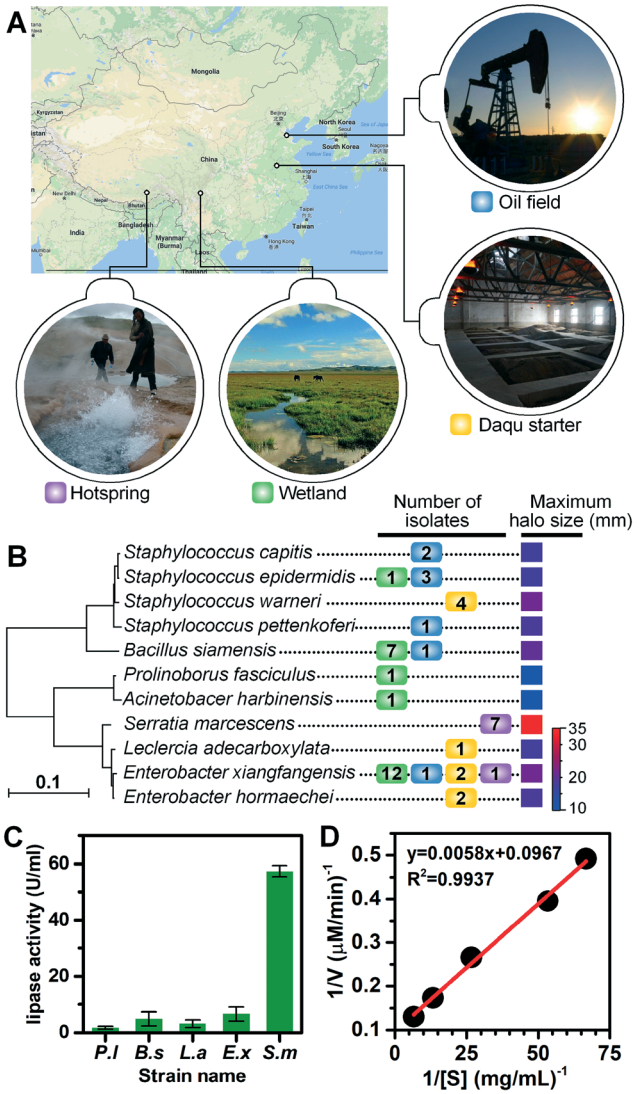

Fig. 4 Lipase screening from environmental samples. (A) The geographical locations of the sampling sites (source: Google map) include: an oil field (blue, Dongying, Shandong), a Daqu starter (orange, Bozhou, Anhui), the Lalong hot spring (magenta, Lhasa, Tibet), and the Zoige wetland (green, Sichuan). (B) The phylogenetic tree shows the members of lipase-producing species we screened and the largest opaque halo sizes from related species. Numbers of isolates are colored corresponding to the color markers of sampling sites as shown in panel A. (C) The activity of extracellular crude lipases of *P. lipolytica* S29 (*P.l*), *Bacillus siamensis* ZF4 (*B.s*), *Leclercia adecarboxylata* J11 (*L.a*), *Enterobacter xiangfangensis* Z1 (*E.x*), and *Serratia marcescens* Y2 (*S.m*), measured using *p*-nitrophenyl palmitate (*p*-NPP) assays. (D) Lineweaver–Burk plot of extracellular crude lipase of *S. marcescens* Y2 obtained by p-NPP assays.

sorting histograms were obtained (Fig. S6D†), and those positive droplets with signals above the threshold were recovered and spread on LB agar plates to allow growth (Fig. S5 and S6†). Colonies were collected and transferred to liquid cultures, after which their lipolytic activities were evaluated *via* the tributyrin plate assays (Fig. S7†). The phylogenetic tree for positive lipolytic species based on 16S rRNA sequences and the number of strains isolated is shown in Fig. 4B. Among the 47 colonies identified with lipase activity, 11 bacterial species belonging to seven genera were identified, including *Staphylococcus*, *Bacillus*, *Enterobacter*, *Serratia*, *Prolinoborus*, *Acinetobacter*, and *Leclercia* (see Table S2† for the full list of sorted strains). These results suggest that our FADS-based lipase screening pipeline is robust and can be successfully applied to various samples from different environments.

Four strains, *Serratia marcescens* strain Y2, *Bacillus siamensis* ZF4, *Leclercia adecarboxylata* J11, and *Enterobacter xiangfangensis* Z1, were selected for further study of lipase activity because they developed large halos on agar plates. The extracellular crude lipase activity of *P. lipolytica* S29 was also measured as the control. S29 cells were cultivated using 2216E culture medium instead of LB. The crude enzymes in culture supernatants were purified and concentrated 100 times by ultrafiltration to measure the lipolytic activity of crude enzymes in supernatants based on the *p*-NPP assay. One unit of enzyme activity (U) was defined as the amount of enzyme releasing 1 μmol *p*-nitrophenol (*p*-NP) from the substrate per min at 37 °C. As shown in Fig. 4C, Y2 exhibited the highest extracellular lipase activity (57.34 U mL^{-1}) among the four strains. The Michaelis constant (K_m) value and the specific activity of Y2 were calculated to be 158.9 μM and 2926.7 U mg^{-1} from the Lineweaver–Burk plot constructed using activity values depending on *p*-NPP concentrations. The linear plot indicated that the hydrolysis of *p*-NPP by the extracellular lipases of Y2 followed Michaelis–Menten kinetics (Fig. 4D). Further study is underway to clone, express and purify lipases from the obtained strains to enable more quantitative analysis of lipase activity.

Conclusions

In summary, we developed a FADS-based pipeline for high throughput screening of lipolytic microorganisms which consisted of droplet generation, incubation, introduction of a substrate using picoinjection, and sorting of droplets based on their fluorescence intensities. We investigated factors that might influence the sorting efficiency and concluded that picoinjection was indispensable for quantitative sorting of environmental samples because it prevented the interference induced by spontaneous hydrolysis of FDB after long-term incubation. We applied the pipeline to samples collected from various sites and successfully obtained 47 lipolytic bacterial strains, confirming the high effectiveness and robustness of the FADS method. Furthermore, we built a compact optical system that consisted of a laser light source, multi-channel fluorescence detectors, and FADS controlling circuits. The optical system can be easily coupled with standard microscopes for FADS without the need for optical alignments, which might significantly promote the wider adoption of FADS.

We believe that the FADS-based lipase screening pipeline can be widely applied to high throughput sorting of various environmental samples or mutant libraries. It will also be a useful tool for functional metagenomic screening based on *in vitro* gene expression systems.[37] Future studies will be directed toward extending the workflow to other enzymes with industrial importance, and providing a highly efficient, low reagent consumption, and low-cost alternative to current screening technologies.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Electronic Supplementary Information

Fluorescence-Activated Droplet Sorting of Lipolytic Microorganisms Using a Compact Optical System

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Chemicals and materials

All reagents were purchased from commercial sources and used as received unless otherwise stated. Silicon wafer was purchased from Qimin Silicon Material Co., Ltd. (Shanghai, China). Photomasks were designed in AutoCAD and ordered from Heheng Optoelectronics (Changsha, China). SU-8 photoresist was purchased from MicroChem Corp. (Newton, MA, USA). Poly-dimethylsiloxane (PDMS) was purchased from Momentive Performance Materials Inc. (Waterford, NY, USA). Syringe pumps (Pump 11 PicoPlus Elite, Harvard Apparatus, Holliston, MA, USA) was used for controlling fluid flow in microfluidic devices. Gastight glass syringes (Agilent, Santa Clara, CA, USA) with volume of 250 μL or 500 μL were used to deliver solutions. LB broth medium and Potassium phosphate were purchased from Sangon (Shanghai, China). Marine broth 2216 was purchased from BD (Franklin Lakes, New Jersey, USA). Peptone was purchased from Aoboxing Bio-Tech (Beijing, China). Magnesium sulfate heptahydrate was purchased from Sinopharm (Shanghai, China). Ammonium sulfate was purchased from Guanghua Sci-Tech (Guangzhou, China). *p*-nitrophenyl palmitate (*p*-NPP) was purchased from Aladdin (Shanghai, China). Tributyrin was purchased from Tokyo Chemical Industry (TCI, Tokyo, Japan).

Construction and operation of the compact optical system for FADS.

The compact optical system for FADS was composed of a detection module to measure the fluorescence of picoliter droplets, a high voltage module to deliver high voltage-high frequency electric field to solder-injected electrodes, an Edgertronic™ high speed camera (SC1 Monochrome version, Sanstreak Corp., San Jose, CA, USA) to capture the images and videos, and a power supply to control the microscope and the FADS system. The detection module had all optical components aligned and die bonded in a compact size (160 mm×143 mm×54 mm), and was connected to the side port of an standard inverted microscope (IX81; Olympus, Tokyo, Japan)

via its V-mount without further optical alignment. The detection module used a high-power diode laser (200 mW, 470 nm, NDB7675, Nichia, Tokushima, Japan), three photomultipliers (R5600U, Hamamatsu Photonics, Hamamatsu, Japan), and a 2-MHz 24bit analog-to-digital conversion circuit. This compact module measured three different fluorescence signals of various fluorophores simultaneously as listed in Table S1. In this work, only one photomultiplier (PMT1) was used for measurement of FDB hydrolysis by lipase. The FADS process was controlled using a program written in LabVIEW software (National Instruments, USA). Time series recordings were analysed using a program written in Matlab (MathWorks, Natick, MA, USA) to extract the fluorescence intensities of all droplets. The histograms were generated to show the distribution of droplet fluorescence intensity, as well as to determine the threshold of sorting. The sorting threshold was defined based on the histogram we obtained for each experiment, and might vary based on PMT gain (controlled by high voltage applied to the photomultiplier).

The leakage test for the fluorogenic substrate FDB in picoliter droplet system

We designed and made a microfluidic device with two flow-focusing junctions which can produce 1:1 proportion of positive droplets containing 25 μM FDB and 40 U mL^{-1} lipase, and negative droplets containing 25 μM FDB without lipase. The droplets were directly mixed and collected in an Eppendorf tube. 10 μL of mixed droplets were pipetted into a Countess™ cell counting chamber (Cat. No. C10228, ThermoFisher, USA), and sealed with capillary wax to avoid evaporation. To investigate the leakage of fluorescence dye between droplets, the monolayer droplet array in the chamber was imaged by fluorescence microscopy (Eclipse Ti, Nikon, Tokyo, Japan).

Amplification, sequencing and phylogenetic analysis of 16S rRNA genes

The 16S rRNA genes of obtained bacterial strains were amplified by PCR with the forward primer 27F and the reverse primer 1492R as described[1]. The obtained sequences were assembled using SeqMan software (DNASTAR). The sequence similarity was analyzed by comparing 16S rRNA gene sequences of obtained strains with known sequences available from the EzTaxon-e database (http://www.ezbiocloud.net/eztaxon)[2], The phylogenetic analysis based on complete 16S rRNA gene sequences indicates that the strains are closely related to reported strains (98.56% to 100%, as listed in Supplementary Table S2). Sequences were then aligned using CLUSTAL W[3], and the phylogenetic tree was established with MEGA 6 (Molecular Evolutionary Genetics Analysis version 6) program[4], using the neighbor-joining[5] and the maximum-likelihood method, with bootstrap values based on 1000 replications[6].

Lipase activity assay.

Analysis of extracellular lipase activity was carried out following the colorimetric method using *p*-NPP as the substrate. First, we mixed 100 μL *p*-NPP (0.15 to 3 mg/mL in isopropanol) with 1.8 mL Tris-HCl (50 mM, pH7.5), and incubated it at 37 °C for 5 min. A concentrated enzyme solution of 100 μL volume obtained by ultrafiltration was then added, and incubated at 37°C for another 10 min. During this step, *p*-nitrophenol was enzymatically released from the substrate *p*-NPP. Then,

we added 500 μL trichloroacetic acid (TCA, 10% w/v in water) into the tube to stop the reaction. We added 500 μL Na_2CO_3 solution (10% w/v in water) as the chromogenic agent, and measured the absorbance of solution at 410 nm.

Quantification of Sorting efficiency.

The efficiency of droplet sorting is defined as enrichment factor as previously described by Jean-Christophe Baret et al.[7] Briefly, the ratio of positive droplets to negative droplets is defined as ε as:

$$\varepsilon = \frac{N_+}{N_-}$$

We obtained ε based on counting of more than 2000 droplets from fluorescence images. The enrichment is defined as the ratio of ε′ after sorting to ε before sorting:

$$\eta = \frac{\varepsilon'}{\varepsilon}$$

In the sorting experiments of a binary mixture of positive and negative droplets made with lipase and FDB, we obtained an 82-fold enrichment of positive droplets after sorting (Fig. S2).

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Table S1. Configuration of fluorescence channels of the compact FADS detection system.

Channel	Laser (nm)	Bandpass Filter (nm)	Dichroic Filter (nm)	Fluorochromes
PMT 1	470	505/40	485	FITC, Alexa Fluor 488,CFSE, Fluo-3
PMT 2	470	600/42	565	PE, PI
PMT 3	470	635/20	610	ECD, PE-Texas Red, PE-CF594, PI, PE-Cy5

Notes: FITC, Fluorescein isothiocyanate; CFSE, Carboxyfluorescein succinimidyl ester; PE, R-phycoerythrin; PI, Propidium iodide; ECD, PE-Texas Red®-x available from Beckman-Coulter; PE-Texas Red, A tandem conjugate of Texas Red with R-phycoerythrin; PE-CF594, a tandem conjugate that combines PE and CF594, available from BD Biosciences; PE-Cy5 a tandem conjugate that combines phycoerythrin and a cyanine dye available from BD Biosciences.

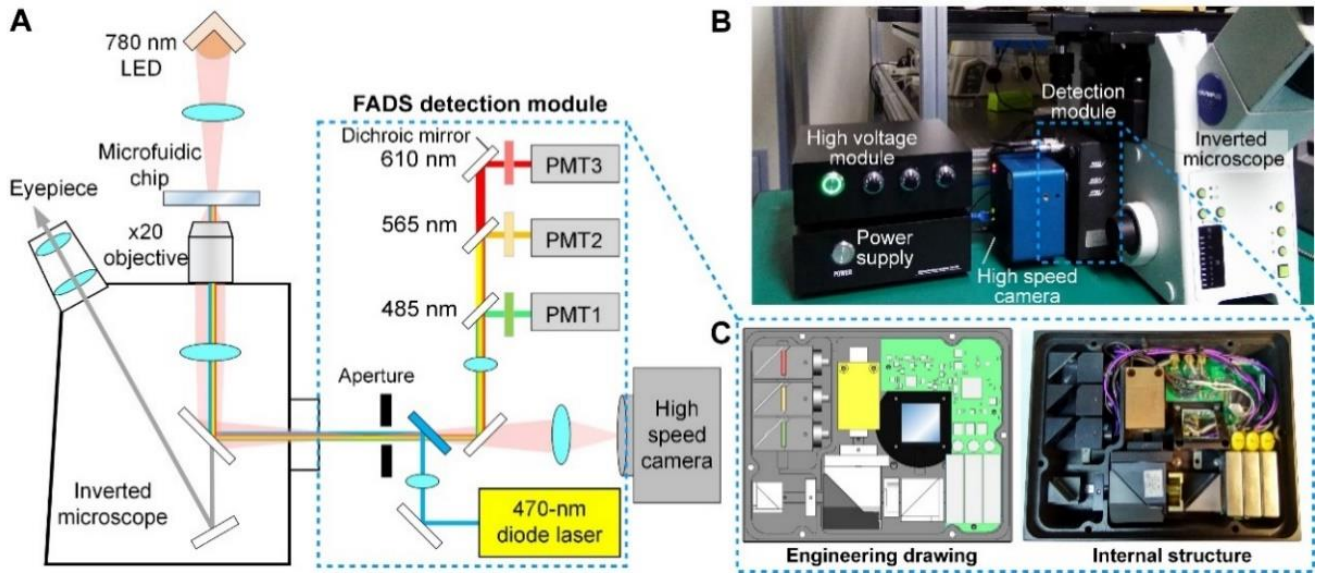

Figure S1. The compact optical system designed for FADS. (A) Diagram of the optical setup. The microscope has a side port, where we place the portable FADS detection module which integrating the laser PMT: photomultiplier; (B) Photograph of the optical experiment setup in panel A; (C) Engineering drawing and photograph of the internal structure of the portable FADS detection module.

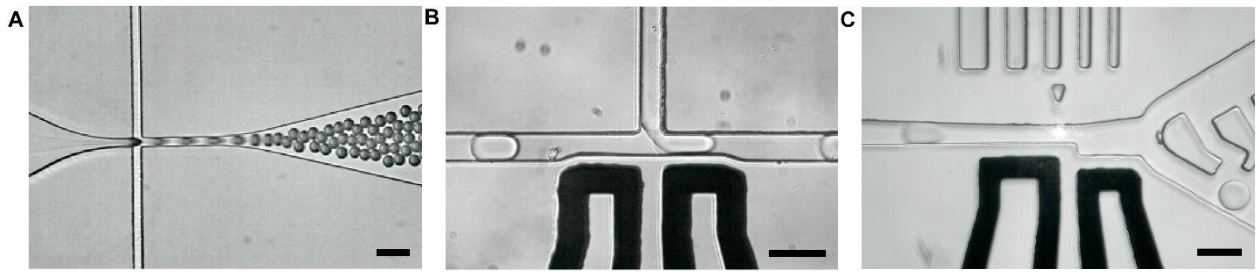

Figure S2. Microscopic images of microfluidic devices for (A) droplet generation, (B) picoinjection of substrate into the droplets, and (C) FADS triggered by dielectrophoresis. Scale bar: 50 μm.

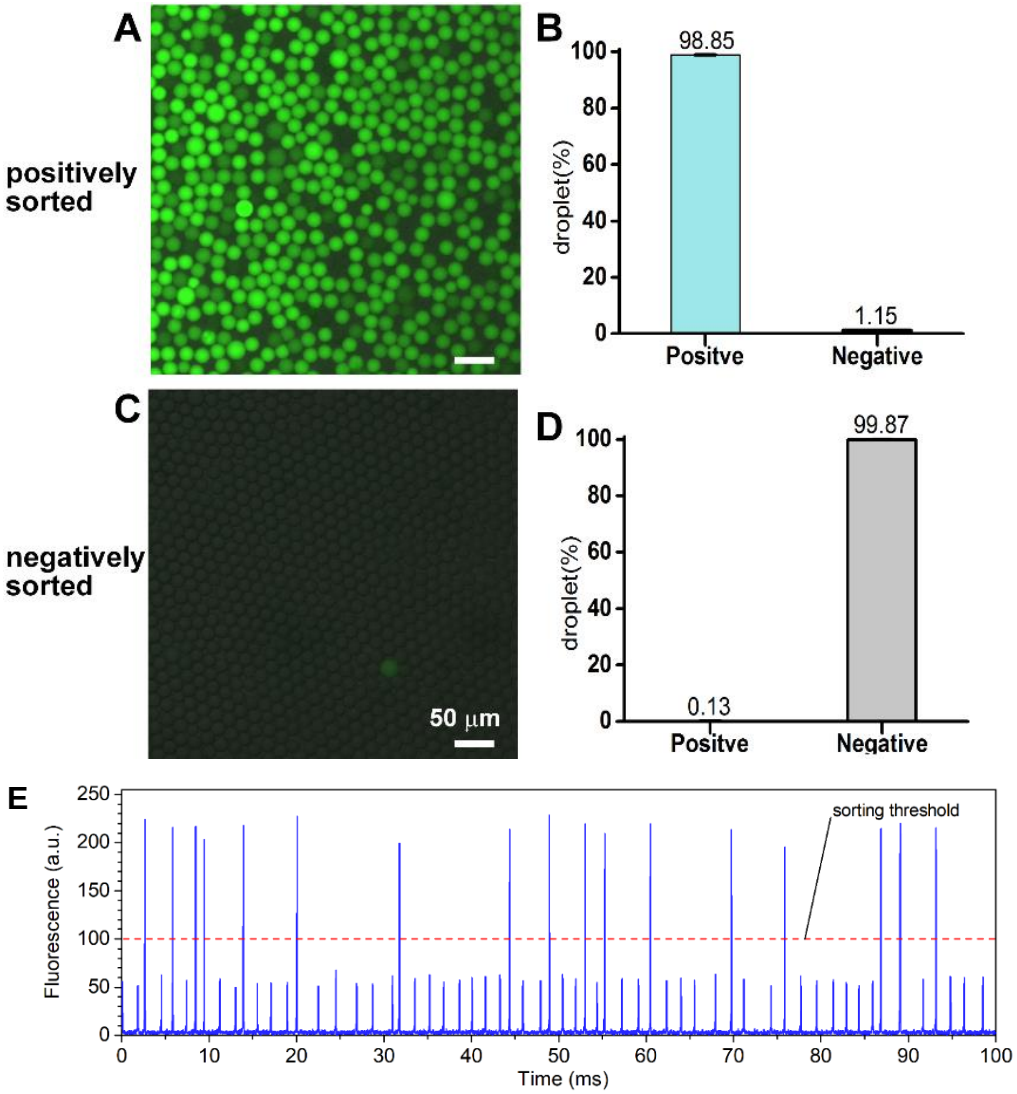

Figure S3. Sorting efficiency of fluorescence-activated droplet sorting (FADS) evaluated by commercial available lipase and its fluorogenic substrate FDB. (A, B) A bright-field and fluorescence merged image of the positive-sorted droplets and the statistical analysis. (C, D) the negative-sorted droplets and the statistical analysis. (E) Time series during a sort showing the detected fluorescence signal (blue). Those peaks about the threshold (red dashed line) was sorted and collected.

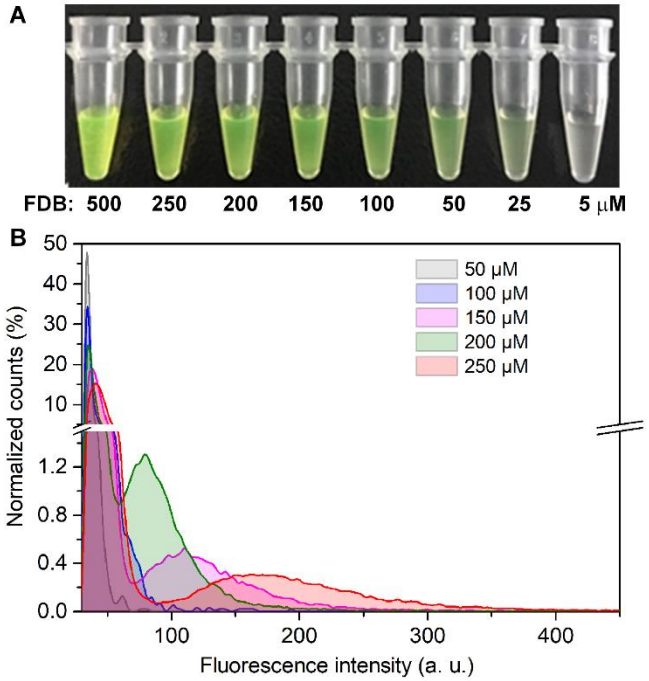

Figure S4. Validation of FDB as the fluorogenic substrate for FADS-based lipase screening. (A) Photograph of the tube assay which mixed *Pseudoalteromonas lipolytica* S29 with FDB of different concentrations. (B) Sorting histogram of droplet fluorescence intensities for S29 cells using FDB of different concentrations. FDB solution was added to droplets by picoinjection. After 1 hour of the picoinjection step, the cells were sorted. The counts were normalized to 100% based on around 200,000 droplets of various FDB concentrations. Intensity threshold of 32 was used to enumerate droplets.

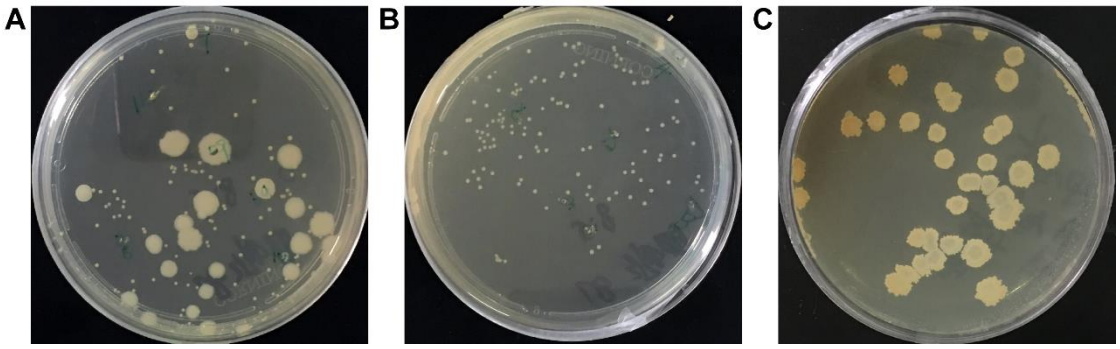

Figure S5. Images of typical agar plates with growth of bacterial cells demulsified from positive-sorted droplets. (A, B) for Daqu starter sample of Gujin liquor from Anhui Province, China, (C) for Lalong hot spring in Tibet, China.

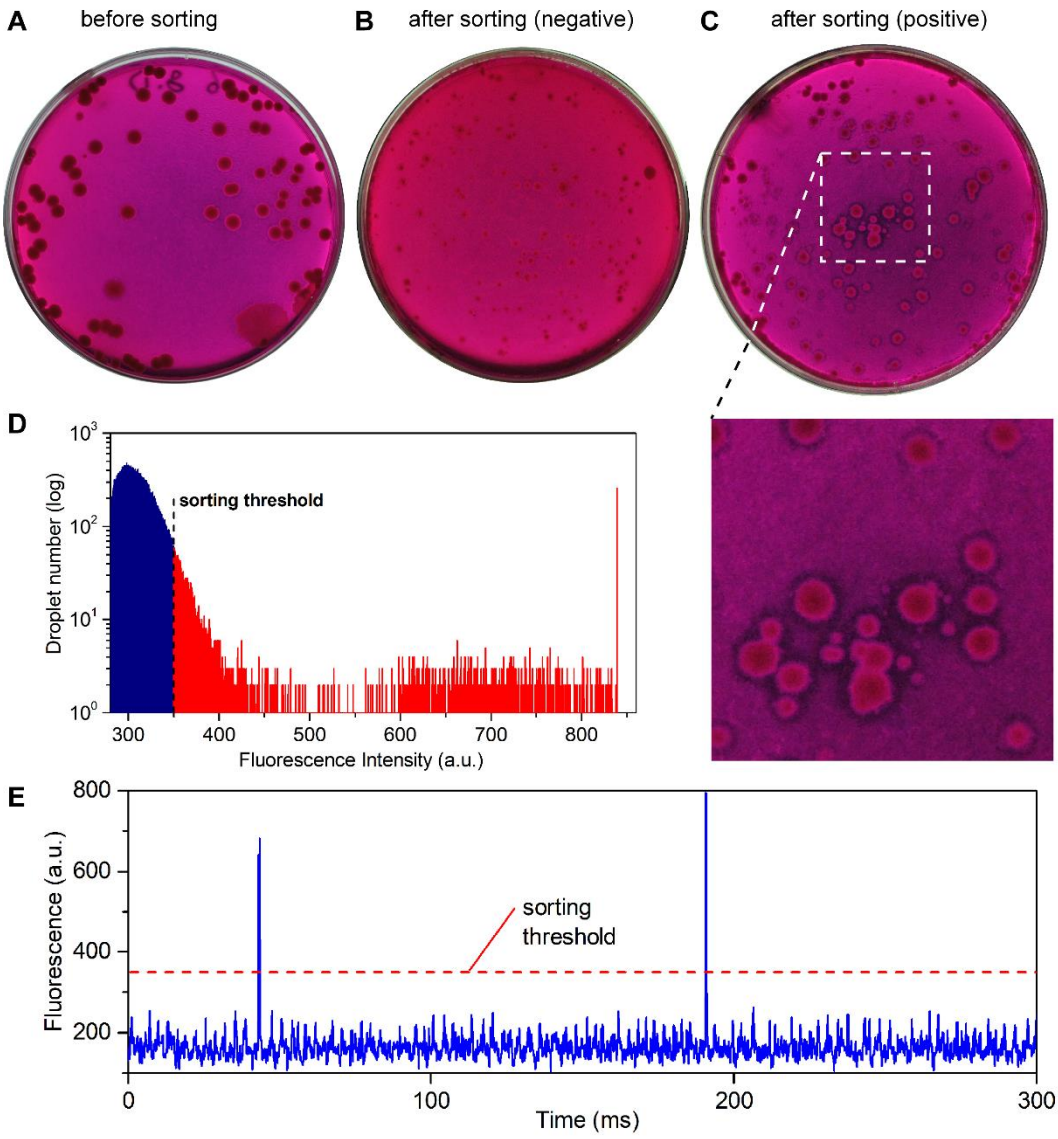

Figure S6. Lipase assays on LB agar plates with 1% (v/v) tributyrin and Rhodamine B (0.5%) for Lalong Hot Spring sample. (A) Before FADS sorting, sample was plated with 10^{-6} X diluted, showing no transparent halos. (B) Negatively sorted colonies showing no transparent halos. (C) Positively sorted colonies showing transparent halos around colonies. The images were taken after 3 d cultivation. Histogram (D) and the time series (E) are showing the distribution of droplet fluorescence intensities for sorting of Lalong Hot Spring sample with 250 μM FDB (final concentration) as the substrate. Droplet detection threshold of 275 and sorting threshold of 350 were used.

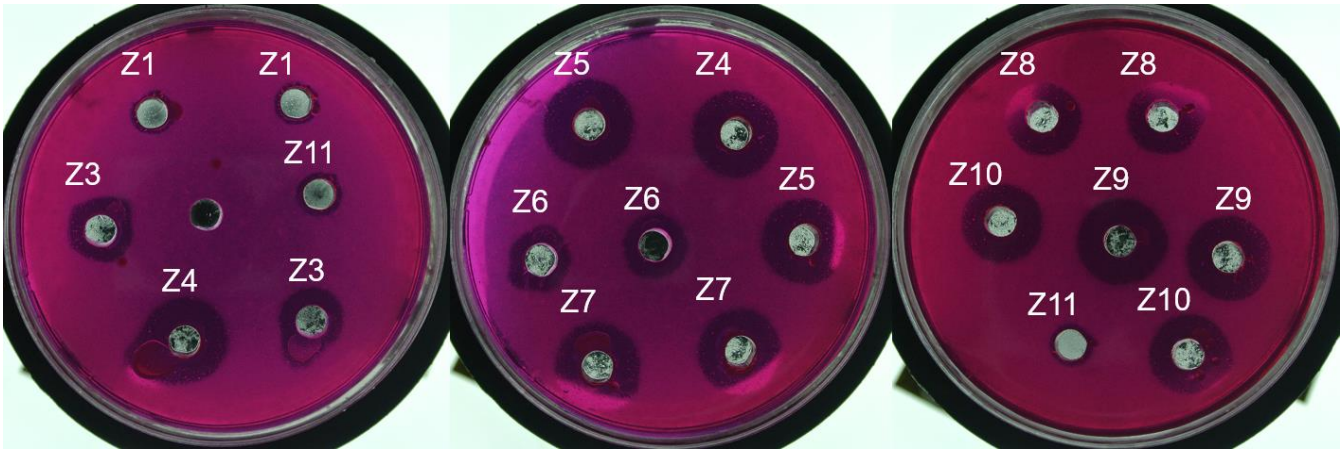

Figure S7. Images show typical results of plate halo assay for strains obtained by FADS (Zoige wetland samples).

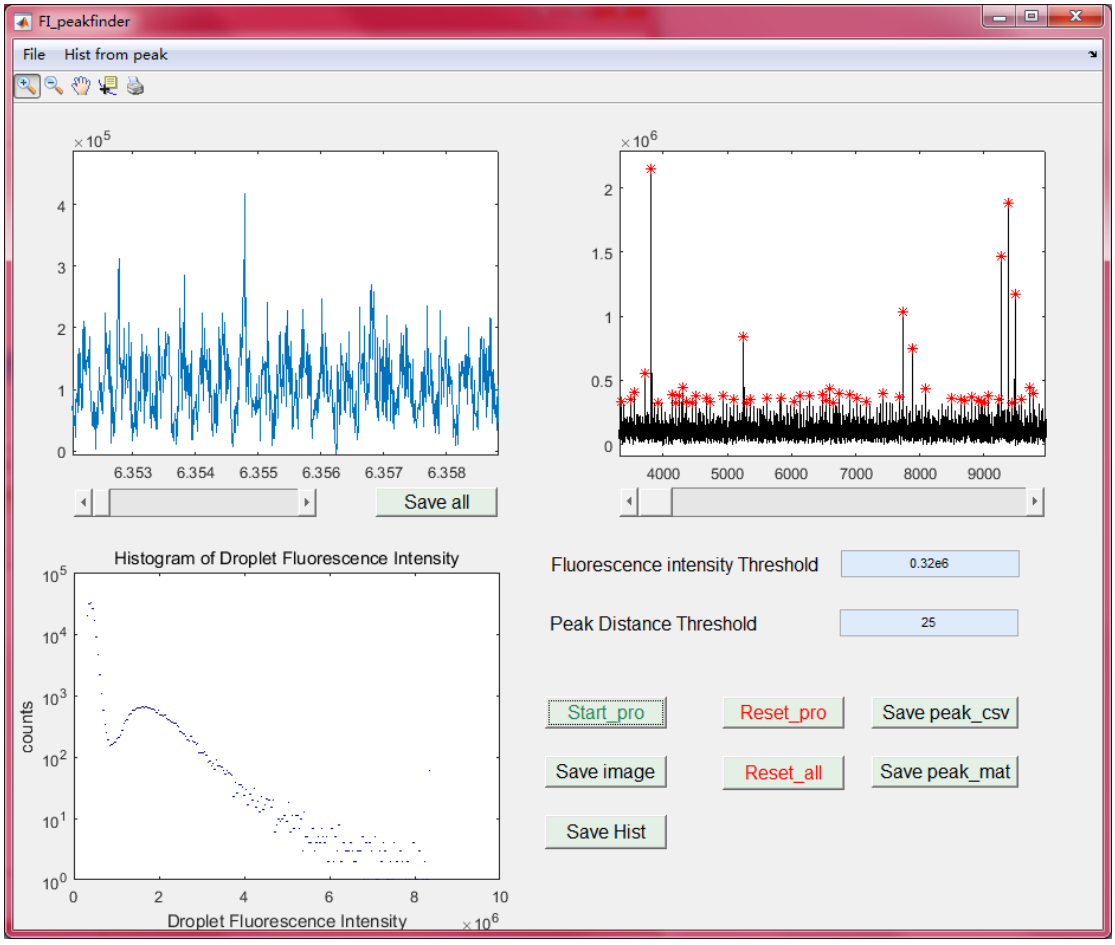

Figure S8. The front panel of Matlab program for data processing of FADS fluorescence signal recording.

Peak analysis program written in Matlab.

```
function varargout = FI_peakfinder(varargin)
% FI_PEAKFINDER MATLAB code for FI_peakfinder.fig
%      FI_PEAKFINDER, by itself, creates a new FI_PEAKFINDER or raises the existing
%      singleton*.
%
%      H = FI_PEAKFINDER returns the handle to a new FI_PEAKFINDER or the handle to
%      the existing singleton*.
%
%      FI_PEAKFINDER('CALLBACK',hObject,eventData,handles,...) calls the local
%      function named CALLBACK in FI_PEAKFINDER.M with the given input arguments.
%
%      FI_PEAKFINDER('Property','Value',...) creates a new FI_PEAKFINDER or raises the
%      existing singleton*.  Starting from the left, property value pairs are
%      applied to the GUI before FI_peakfinder_OpeningFcn gets called.  An
%      unrecognized property name or invalid value makes property application
%      stop.  All inputs are passed to FI_peakfinder_OpeningFcn via varargin.
%
%      *See GUI Options on GUIDE's Tools menu.  Choose "GUI allows only one
%      instance to run (singleton)".
%
% See also: GUIDE, GUIDATA, GUIHANDLES

% Edit the above text to modify the response to help FI_peakfinder

% Last Modified by GUIDE v2.5 15-Nov-2017 17:23:49

% Begin initialization code - DO NOT EDIT
gui_Singleton = 1;
gui_State = struct('gui_Name',       mfilename, ...
   'gui_Singleton',  gui_Singleton, ...
   'gui_OpeningFcn', @FI_peakfinder_OpeningFcn, ...
   'gui_OutputFcn',  @FI_peakfinder_OutputFcn, ...
   'gui_LayoutFcn',  [] , ...
   'gui_Callback',   []);
if nargin && ischar(varargin{1})
   gui_State.gui_Callback = str2func(varargin{1});
end

if nargout
```

```
    [varargout{1:nargout}] = gui_mainfcn(gui_State, varargin{:});
else
    gui_mainfcn(gui_State, varargin{:});
end
% End initialization code - DO NOT EDIT


% --- Executes just before FI_peakfinder is made visible.
function FI_peakfinder_OpeningFcn(hObject, eventdata, handles, varargin)
% This function has no output args, see OutputFcn.
% hObject    handle to figure
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
% varargin   command line arguments to FI_peakfinder (see VARARGIN)

% Choose default command line output for FI_peakfinder
handles.output = hObject;

% Update handles structure
guidata(hObject, handles);

% UIWAIT makes FI_peakfinder wait for user response (see UIRESUME)
% uiwait(handles.figure1);


% --- Outputs from this function are returned to the command line.
function varargout = FI_peakfinder_OutputFcn(hObject, eventdata, handles)
% varargout  cell array for returning output args (see VARARGOUT);
% hObject    handle to figure
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Get default command line output from handles structure
varargout{1} = handles.output;


% --- Executes on slider movement.
function slider1_Callback(hObject, eventdata, handles)
% hObject    handle to slider1 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
```

```
% Hints: get(hObject,'Value') returns position of slider
%        get(hObject,'Min') and get(hObject,'Max') to determine range of slider
global View_length len_data
val_sli=get(handles.slider1,'Value');
val_sli=val_sli*(len_data-View_length);
set(handles.Ori_sig,'Xlim',[val_sli val_sli+View_length-1]);
guidata(hObject,handles);

% --- Executes during object creation, after setting all properties.
function slider1_CreateFcn(hObject, eventdata, handles)
% hObject    handle to slider1 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: slider controls usually have a light gray background.
if isequal(get(hObject,'BackgroundColor'), get(0,'defaultUicontrolBackgroundColor'))
   set(hObject,'BackgroundColor',[.9 .9 .9]);
end


% --- Executes on button press in Save_all.
function Save_all_Callback(hObject, eventdata, handles)
% hObject    handle to Save_all (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
global FIDATA View_length len_data
View_length=4000;
[filename,pathname]=uiputfile({'*.jpg';'*.bmp'},'save current image','untitled.jpg');
fig_count=1;
for nn=1:(floor((len_data)/View_length))
   fidstart_d=(nn-1)*(View_length)+1;
   figend_d=nn*(View_length);
   fig_count = fig_count+1;
   figure(fig_count);
   %        set(gcf,'Position',[50,50,900,600]);
   y1_lim=max(FIDATA(fidstart_d:figend_d));

   plot(fidstart_d:figend_d,FIDATA(fidstart_d:figend_d),'k');
   xlim([fidstart_d figend_d]);
   %        saveas(gcf,[pathname,num2str(nn)],'fig');
```

```
    saveas(gcf,[pathname,num2str(nn)],'jpg');
    close gcf
end

for nn=floor((len_data)/(View_length))+1
    if mod(length(FIDATA),4000)==0
        break
    else
        fidstart_d=(floor((len_data-1)/(View_length)))*(View_length)+1;
        figend_d=len_data;
        figure(floor((len_data-1)/(View_length))+1);
        %         set(gcf,'Position',[50,50,900,600]);
        y1_lim=max(FIDATA(fidstart_d:figend_d));

        plot(fidstart_d:figend_d,FIDATA(fidstart_d:figend_d),'k');
        xlim([fidstart_d figend_d]);

        %        saveas(gcf,[pathname,num2str(nn)],'fig');
        saveas(gcf,[pathname,num2str(nn)],'jpg');
        close gcf
    end
end


% --------------------------------------------------------------------
function File_Callback(hObject, eventdata, handles)
% hObject    handle to File (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)


% --------------------------------------------------------------------
function Load_Callback(hObject, eventdata, handles)
% hObject    handle to Load (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)


% --------------------------------------------------------------------
function Exit_Callback(hObject, eventdata, handles)
% hObject    handle to Exit (see GCBO)
```

```
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
clc;
close all;
close(gcf);

% --------------------------------------------------------------------
function load_xls_Callback(hObject, eventdata, handles)
% hObject    handle to load_xls (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
[name path]=uigetfile({'*.xls'},'load the fluorescence signal');
if isequal(name,0)||isequal(path,0)
   errordlg('Not an effective path','error');
   return;
else
   global FIDATA View_length len_data
   View_length=4000;
   FIDATA=xlsread([path name]);
   FIDATA(FIDATA==-2^23)=2^23;%% fluorescence intensity saturation control
   len_data=length(FIDATA);
   msgbox('Signal successfully loaded','Message');
   axes(handles.Ori_sig);
   plot(FIDATA);
   guidata(hObject,handles)
end

% --------------------------------------------------------------------
function load_csv_Callback(hObject, eventdata, handles)
% hObject    handle to load_csv (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
[name path]=uigetfile({'*.csv'},'load the fluorescence signal');
if isequal(name,0)||isequal(path,0)
   errordlg('Not an effective path','error');
   return;
else
   global FIDATA View_length len_data
   View_length=4000;
   FIDATA=csvread([path name]);
    FIDATA(FIDATA==-2^23)=2^23;%% fluorescence intensity saturation control
```

```
    len_data=length(FIDATA);
    msgbox('Signal successfully loaded','Message');
    axes(handles.Ori_sig);
    plot(FIDATA);
    guidata(hObject,handles)
end


% --- Executes on slider movement.
function slider2_Callback(hObject, eventdata, handles)
% hObject    handle to slider2 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'Value') returns position of slider
%        get(hObject,'Min') and get(hObject,'Max') to determine range of slider
global View_length len_data
val_sli=get(handles.slider2,'Value');
val_sli=val_sli*(len_data-View_length);
set(handles.Processing_part,'Xlim',[val_sli val_sli+View_length-1]);
guidata(hObject,handles);

% --- Executes during object creation, after setting all properties.
function slider2_CreateFcn(hObject, eventdata, handles)
% hObject    handle to slider2 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: slider controls usually have a light gray background.
if isequal(get(hObject,'BackgroundColor'), get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor',[.9 .9 .9]);
end


function thre_num_Callback(hObject, eventdata, handles)
% hObject    handle to thre_num (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of thre_num as text
%        str2double(get(hObject,'String')) returns contents of thre_num as a double
```

```
global thre1
thre11=get(hObject,'String');
thre1=str2double((get(hObject,'String')));
guidata(hObject,handles);


% --- Executes during object creation, after setting all properties.
function thre_num_CreateFcn(hObject, eventdata, handles)
% hObject    handle to thre_num (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
%       See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'), get(0,'defaultUicontrolBackgroundColor'))
   set(hObject,'BackgroundColor','white');
end


% --- Executes on button press in Start_pro.
function Start_pro_Callback(hObject, eventdata, handles)
% hObject    handle to Start_pro (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
global FIDATA  len_data thre1 peak_loc_all peak_num_all peak_value_all PEAK_VALUE cal_count
DistanceThreshold
len_data=length(FIDATA);
if mod(length(FIDATA),1e6)==0%% Process the whole dataset as several sub-datasets
   PEAK_LOC=cell(floor(length(FIDATA)/1e6),1);
   PEAK_VALUE=cell(floor(length(FIDATA)/1e6),1);
else
   PEAK_LOC=cell(floor(length(FIDATA)/1e6)+1,1);
   PEAK_VALUE=cell(floor(length(FIDATA)/1e6)+1,1);
end
for cal_count=1:length(PEAK_VALUE)
   clear FI_NUM point_loc Value_peak FI_DEC diff_pointloc
   if cal_count==length(PEAK_VALUE)
      FI_NUM=FIDATA((cal_count-1)*1e6+1:end);
      FI_NUM(FI_NUM<0)=0;
   else
      FI_NUM=FIDATA((cal_count-1)*1e6+1:cal_count*1e6);
```

```
    FI_NUM(FI_NUM<0)=0;
  end

  len_segment=length(FI_NUM);
  FI_DEC=diff(FI_NUM);%% Get first order difference of intensity

  FI_DEC(FI_DEC<0)=0;
  FI_DEC(FI_DEC>0)=1;

  point_loc=zeros(len_segment-1,1);
  for ii=2:len_segment-2
    if (FI_DEC(ii)==1)&&(FI_DEC(ii+1)==0)%%Get peak position^
      point_loc(ii)=ii;
    else
      point_loc(ii)=0;
    end
  end
  point_loc=point_loc(point_loc~=0);
  point_loc=point_loc+1;
  Value_peak=FI_NUM(point_loc);

  %% peak selection
  for pp=1:length(point_loc)
    if FI_NUM(point_loc(pp))<thre1%% threshold screening
      point_loc(pp)=0;
      Value_peak(pp)=0;
    end
  end

  point_loc=point_loc(point_loc~=0);
  Value_peak=Value_peak(Value_peak~=0);
  diff_pointloc=diff(point_loc);%% Get first order difference of peak position and peak-to-peak distance
  %temp=(mean(diff_pointloc)-2*std(diff_pointloc));
  temp = DistanceThreshold;

  for MM=1:length(point_loc)-1
    if diff_pointloc(MM)>temp
      continue
    else
      array_temp=[Value_peak(MM),Value_peak(MM+1)]; %If two peaks are too close, chose the
larger peak value, and discard the smaller one
```

```
            [num loc]=max(array_temp);
            point_loc(MM+2-loc)=NaN;
            Value_peak(MM+2-loc)=NaN;
        end
    end
    point_loc=point_loc(isnan(point_loc)==0);
    Value_peak=Value_peak(isnan(Value_peak)==0);
    point_loc=point_loc+(cal_count-1)*1e6;
    PEAK_LOC{cal_count,1}=point_loc;
    PEAK_VALUE{cal_count,1}=Value_peak;
end
peak_loc_all=[];
peak_value_all=[];
for MM = 1:length(PEAK_VALUE)
    peak_value_all=[peak_value_all;PEAK_VALUE{MM,1}];
    peak_loc_all=[peak_loc_all;PEAK_LOC{MM,1}];
end
peak_num_all=peak_value_all;
axes(handles.Processing_part)
plot(FIDATA(1:end),'k');
hold on
plot(peak_loc_all(1:end),FIDATA(peak_loc_all(1:end)),'r*');

axes(handles.Hist)
hist(peak_num_all,200)
set(gca,'YScale','log');
title('Histogram of Droplet Fluorescence Intensity');
ylabel('counts');
xlabel('Droplet Fluorescence Intensity');
guidata(hObject,handles)

% --- Executes on button press in Reset_pro.
function Reset_pro_Callback(hObject, eventdata, handles)
% hObject    handle to Reset_pro (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
try
    delete(allchild(handles.Processing_part));
    delete(allchild(handles.slider2));
   %% set(handles.thre_num,'string',' ');
    delete(allchild(handles.Start_pro));
```

```
    delete(allchild(handles.Hist));
    delete(allchild(handles.log_scale));
    clear peak_loc_all peak_num_all thre1
end


% --- Executes on button press in Reset_all.
function Reset_all_Callback(hObject, eventdata, handles)
% hObject    handle to Reset_all (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
global FIDATA View_length len_data thre1 Value_peak point_loc
try
    delete(allchild(handles.Ori_sig));
    delete(allchild(handles.slider1));
    delete(allchild(handles.Save_all));
    delete(allchild(handles.Processing_part));
    delete(allchild(handles.slider2));
    set(handles.thre_num,'string',' ');
    delete(allchild(handles.Start_pro));
    delete(allchild(handles.Hist));
    delete(allchild(handles.log_scale));
    clear FIDATA View_length len_data thre1 peak_loc_all peak_num_all
    clear all
end


% --- Executes on button press in save_hist.
function save_hist_Callback(hObject, eventdata, handles)
% hObject    handle to save_hist (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
global peak_num_all
[filename,pathname]=uiputfile({'*.jpg';'*.bmp'},'save current image','untitled.jpg');
if isequal([filename pathname],[0,0])
    errordlg('image not saved','error')
    return
else
    figure
    hist(peak_num_all,200)
    set(gca,'YScale','log');
```

```
    title('Histogram of Droplet Fluorescence Intensity');
    ylabel('counts');
    xlabel('Droplet Fluorescence Intensity');
    saveas(gcf,[pathname,filename],'jpg');
    close gcf
    guidata(hObject,handles)
end


% --- Executes on button press in save_image.
function save_image_Callback(hObject, eventdata, handles)
% hObject    handle to save_image (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
global FIDATA View_length len_data peak_loc_all peak_num_all
[filename,pathname]=uiputfile({'*.jpg';'*.bmp'},'save current image','untitled.jpg');
fig_count=1;
for nn=1:(floor((len_data)/View_length))
    fidstart_d=(nn-1)*(View_length)+1;
    figend_d=nn*(View_length);
    fig_count = fig_count+1;
    figure(fig_count);
    set(gcf,'Position',[50,50,900,600]);
    y1_lim=max(FIDATA(fidstart_d:figend_d));
    y2_lim=max(FIDATA(fidstart_d:figend_d));

    plot(fidstart_d:figend_d,FIDATA(fidstart_d:figend_d),'k');
    xlim([fidstart_d figend_d]);
    hold on
    plot(peak_loc_all(1:end),FIDATA(peak_loc_all(1:end)),'r*');
    text(figend_d-10,y2_lim,'signal');
    saveas(gcf,[pathname,num2str(nn)],'jpg');
    close gcf
end

for nn=floor((length(FIDATA))/(4000))+1
    if mod(length(FIDATA),4000)==0
        break
    else
        fidstart_d=(floor((length(FIDATA)-1)/(4000)))*(4000)+1;
        figend_d=length(FIDATA);
```

```
        figure(floor((len_data-1)/(4000))+1);
        set(gcf,'Position',[50,50,900,600]);
        y1_lim=max(FIDATA(fidstart_d:figend_d));
        y2_lim=max(FIDATA(fidstart_d:figend_d));

        plot(fidstart_d:figend_d,FIDATA(fidstart_d:figend_d),'k');
        xlim([fidstart_d figend_d]);
        hold on
        plot(peak_loc_all(1:end),FIDATA(peak_loc_all(1:end)),'r*');
        text(figend_d-10,y2_lim,'signal');

        saveas(gcf,[pathname,num2str(nn)],'jpg');
        close all
    end
end

% --- Executes on button press in save_peak_csv.
function save_peak_csv_Callback(hObject, eventdata, handles)
% hObject    handle to save_peak_csv (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
global peak_loc_all peak_value_all
[filename,pathname]=uiputfile('*.csv','save peak information','untitled.csv');
% save([pathname,filename],'peak_value_all','peak_loc_all');
csvwrite([pathname,filename],peak_value_all);
guidata(hObject,handles)


% --------------------------------------------------------------------
function hist_peak_Callback(hObject, eventdata, handles)
% hObject    handle to hist_peak (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
[name path]=uigetfile({'*.mat'},'load the peak information');
if isequal(path,0)
    errordlg('Not an effective path','error');
    return;
else
    global peak_find peak_num_all
    peak_find=[];
    MAT_File=dir([path '*.mat']);
```

```
    len_file=length(MAT_File);
    for file_count=1:len_file
        load([path MAT_File(file_count).name]);
        peak_find=[peak_find;peak_value_all];
    end
    peak_num_all=peak_find;
    msgbox('Peak information successfully loaded','Message');
    axes(handles.Hist);
    hist(peak_num_all,200)
    set(gca,'YScale','log');
    title('Histogram of Droplet Fluorescence Intensity');
    ylabel('counts');
    xlabel('Droplet Fluorescence Intensity');
    guidata(hObject,handles)
end


% --- Executes on button press in save_peak_mat.
function save_peak_mat_Callback(hObject, eventdata, handles)
% hObject    handle to save_peak_mat (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
global peak_loc_all peak_value_all
[filename,pathname]=uiputfile('*.mat','save peak information','untitled.mat');
save([pathname,filename],'peak_value_all','peak_loc_all');
guidata(hObject,handles)



function edit5_Callback(hObject, eventdata, handles)
% hObject    handle to edit5 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of edit5 as text
%        str2double(get(hObject,'String')) returns contents of edit5 as a double
global DistanceThreshold
DistanceThreshold=get(hObject,'String');
DistanceThreshold=str2double((get(hObject,'String')));
guidata(hObject,handles);
```

```
% --- Executes during object creation, after setting all properties.
function edit5_CreateFcn(hObject, eventdata, handles)
% hObject    handle to edit5 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
%       See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'), get(0,'defaultUicontrolBackgroundColor'))
   set(hObject,'BackgroundColor','white');
end
```