Karlsruhe Institute of Technology

IBCS-FMS Functional-Molecular-Systems

Towards Analysis of Drug Induced Differential Gene Expression on Droplet Microarray in high throughput and nanoliter format

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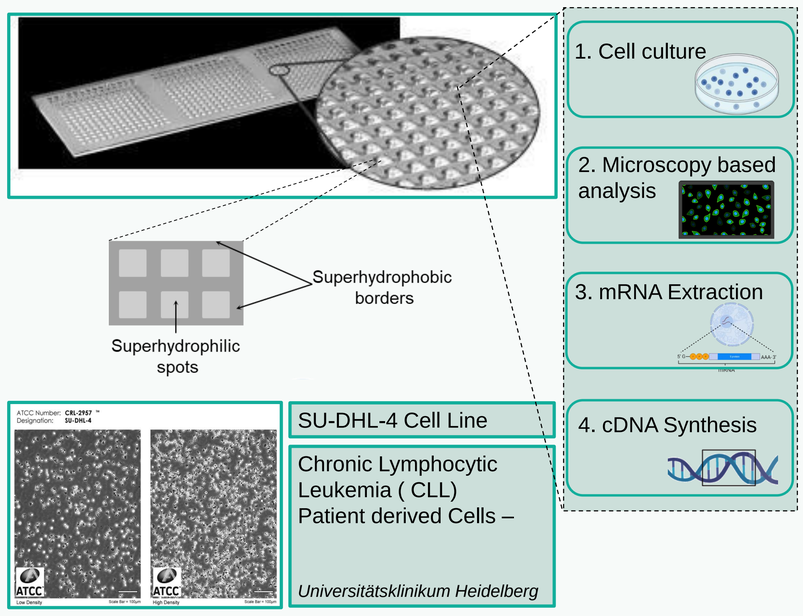
Introduction:

•Differentially expressed gene identification is crucial for understanding phenotypic differences and selecting gene expression targets for further study.

• High-throughput analysis of gene expression is labor-intensive and costly due to multiple steps and large amounts of starting materials required.

• A protocol has been developed for high-throughput mRNA isolation directly on the Droplet-Microarray (DMA) platform.



•The DMA platform enables various steps (culturing, drug treatment, cell lysis, mRNA isolation, cDNA conversion, barcode introduction) in separate droplets on a single chip.

•The Screen-mRNA-to-cDNA protocol on the DMA platform allows differential gene expression analysis on small numbers of patient-derived cells treated with anti-cancer compounds.

•The protocol provides in-depth information on differential gene expression analysis in primary patient-derived cells treated with multiple drugs.

•The approach aims to discover new predictive biomarkers of drug response in patients.

Methodology:

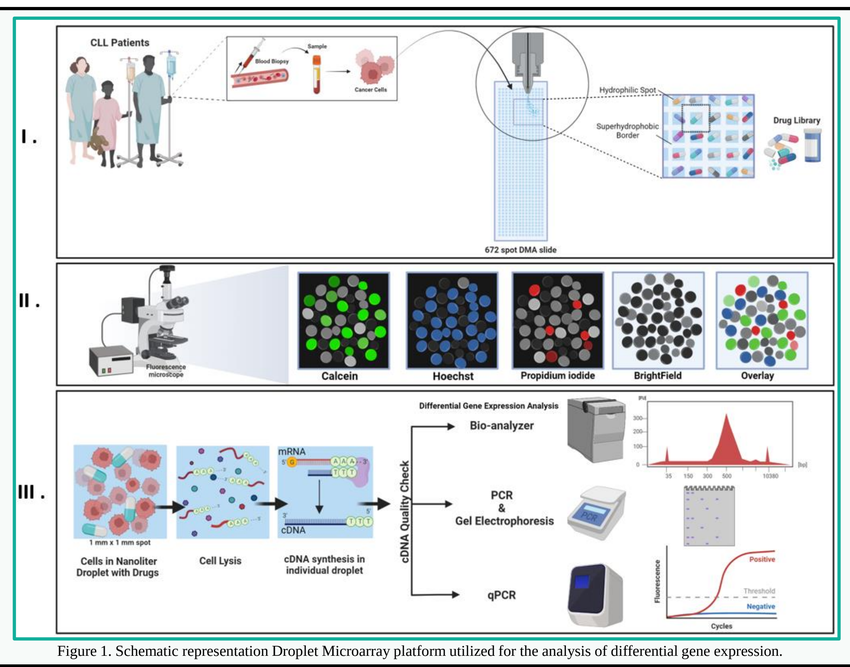


Figure 1. Schematic representation Droplet Microarray platform utilized for the analysis of differential gene expression.

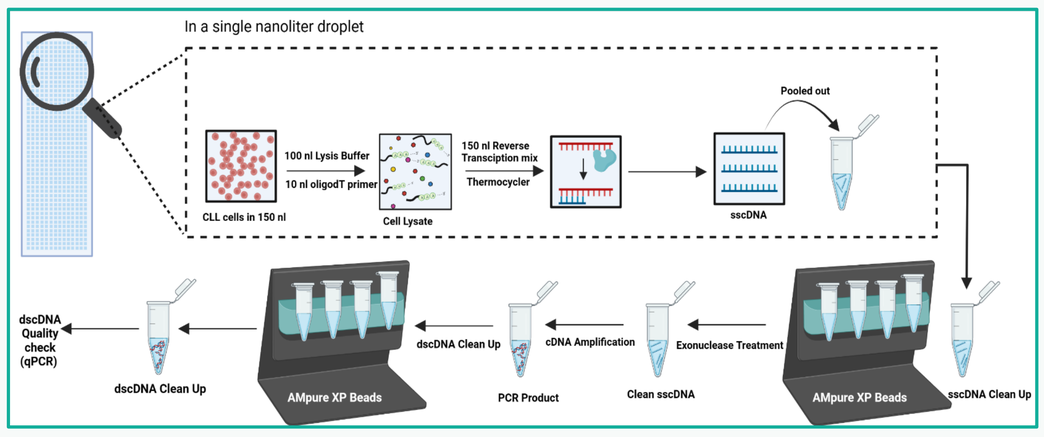


Figure 2. Illustration of the experimental procedure using DMA slide for synthesizing individual droplet-based cDNA.

Results:

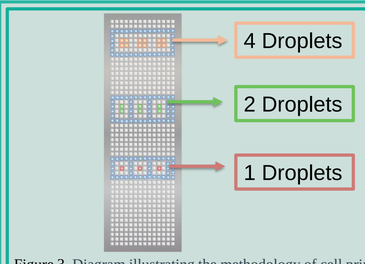


Figure 3. Diagram illustrating the methodology of cell printing, showcasing the arrangement of cells in 4, 2, and individual droplets.

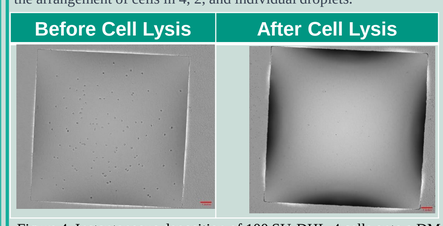


Figure 4. Instantaneous deposition of 100 SU-DHL-4 cells onto a DMA slide single spot followed by complete cell lysis.

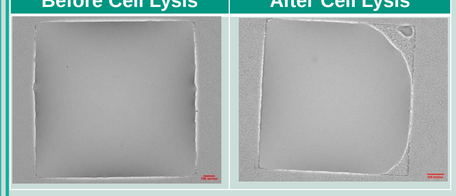


Figure 5. Instantaneous deposition of a single SU-DHL-4 cell onto a DMA slide at a single spot, followed by complete cell lysis.

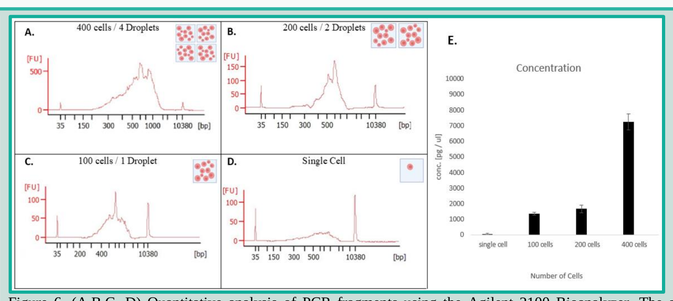
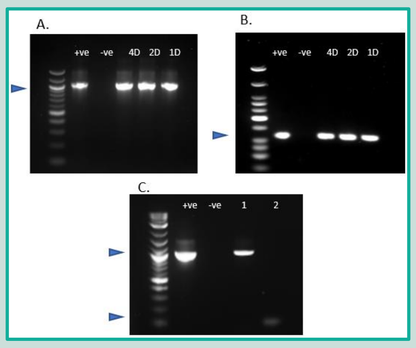


Figure 6. (A,B,C ,D) Quantitative analysis of PCR fragments using the Agilent 2100 Bioanalyzer .The analysis includes samples obtained from 4 droplets, 2 droplets, 1 droplet, and a single cell per droplet on the DMA platform, with three replicates for each condition. (E) Increased cDNA concentration correlates with an increasing number of cells.



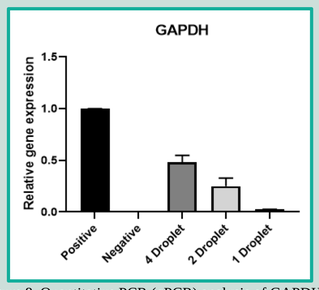


Figure 8. Quantitative PCR (qPCR) analysis of GAPDH in SU-DHL4 samples prepared from 4, 2, and single droplets.

Figure 7. Gel Electrophoresis results for cDNA synthesized from 4, 2, and 1 droplets using ACTB and GAPDH genes. Panel (A) shows the ACTB PCR product, while panel (B) displays the GAPDH PCR product. The "+ve" lane represents the positive control of cDNA synthesized from SU-DHL-4 cells using the standard protocol, while the "-ve" lane serves as the negative control with no template. In panel (C), the single-cell gel electrophoresis is depicted. Lane 1 corresponds to the 1 kb DNA ladder, lane 1 exhibits the ACTB PCR product from single-cell cDNA synthesis, and lane 4 demonstrates the GAPDH PCR product from single-cell cDNA synthesis.

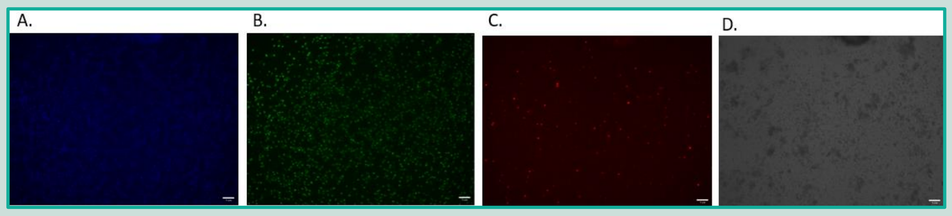


Figure 9. Assessment of cellular viability in Doxorubicin-treated SU-DHL-4 cells after 48 hours. The cells were stained using a live-dead fluorescent dye.
(A) Hoechst staining was employed to visualize all cells. (B) Calcein staining specifically detects live cells. (C) PI staining highlights dead cells. (D) A brightfield image is also included for reference.

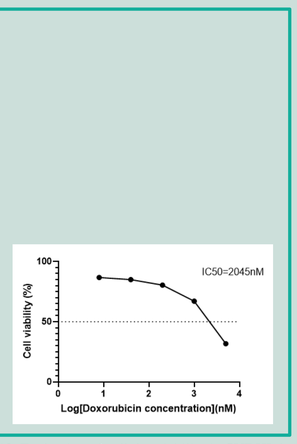


Figure 10. Evaluation of cellular viability in SUDHL4 cells treated with varying concentrations of DOX drug. Determination of the half-maximal inhibitory concentration (IC50).

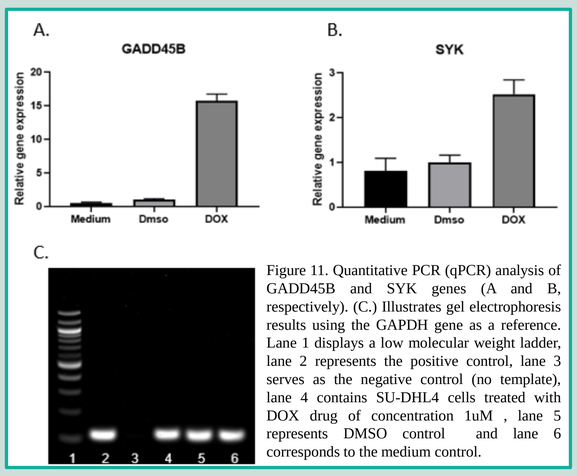


Figure 11. Quantitative PCR (qPCR) analysis of GADD45B and SYK genes (A and B, respectively). (C.) Illustrates gel electrophoresis results using the GAPDH gene as a reference. Lane 1 displays a low molecular weight ladder, lane 2 represents the positive control, lane 3 serves as the negative control (no template), lane 4 contains SU-DHL4 cells treated with DOX drug of concentration 1uM , lane 5 represents DMSO control and lane 6 corresponds to the medium control.

Conclusion:

•The DMA platform shows significant potential for drug screening assays and sample preparation procedures.
•It is versatile and compatible with various applications, including antibacterial compound screening, 3D cell culture, and proteomics.
•The study introduces a miniaturized, high-throughput mRNA isolation and cDNA conversion methodology for sample preparation and sequencing in nanoliter volumes.
•Microscopy-based phenotypic readouts validate the efficacy of the methodology for drug screening and differential gene expression analysis.
•Qualitative and quantitative gene expression analysis was successfully demonstrated using Gel electrophoresis, qPCR, and bioanalyzer techniques.
•Comparison with standard protocols conducted in 384-well plates and tubes was performed.

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