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ALPS: An Automated Liquid Pipetting System for Spheroid Handling in Nanoliter Droplets

Abstract: Cell spheroids provide physiologically relevant 3D cell models, but the manual handling of individual spheroids remains challenging, particularly in miniaturized, high-density formats such as droplet microarrays (DMAs). Here, we present ALPS, an automated liquid-handling platform that collects spheroid-containing nanoliter droplets and redeposits them to specified target spots within or between DMAs. Using HEK (human embryonic kidney) spheroids, ALPS achieved transfer yields of up to ~80% under optimized conditions with a processing time of ~5 s per sample. Transferred spheroids retained morphology after handling and showed a ~1.25-fold increase in diameter within 24 h, indicating that the transfer procedure did not compromise spheroid structural integrity. Together, these results establish ALPS as a practical interface between high-density on-DMA spheroid generation and standard downstream formats, including microtiter plates and tube racks, enabling scalable 3D culture workflows.

Keywords: Automated Liquid Handling, Spheroids Transfer, Droplet Microarray, Nanoliter droplets, 3D Cell Culture

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1 Introduction

Cell spheroids are widely used as 3D culture models for drug screening, toxicity testing, and disease modeling since they reproduce cell-cell interactions and tissue-like organization better than conventional 2D cultures [1]. Among the available spheroid formation methods, hanging-drop culture is widely

used because it is simple, robust, and well suited for generating isolated spheroids. Droplet microarrays (DMAs) translate this concept into a miniaturized, high-throughput format by patterning hydrophilic spots on a superhydrophobic background, which confines cell suspensions and medium into stable, pinned droplets. When inverted, these droplets function as parallel hanging drops, enabling the formation of hundreds of individual spheroids in nanoliter volumes [2]. This approach substantially reduces reagent consumption while maintaining a standard microscope-slide footprint 75 mm × 25 mm (**Fig. 1a**). However, the downstream handling of individual spheroids on DMAs remains a major limitation. After formation, spheroids are distributed across dense arrays of nanoliter droplets, making manual collection and transfer labor-intensive, difficult to scale, and poorly standardized. As a result, the integration of DMA-based spheroid culture into workflows requiring spheroid relocation, medium exchange, or transfer to downstream assays remains limited [3]. To address this limitation, we developed ALPS, an Automated Liquid Pipetting robot for Spheroid Handling in Nanoliter Droplets (**Fig. 1b**). In this study, we evaluated ALPS using HEK293 cell spheroids generated in ~300 nl droplets on DMAs, demonstrating automated collection and transfer of individual spheroid-containing droplets on dense droplet microarrays and assessing spheroid integrity after transfer.

2 Materials and Method

2.1 ALPS Platform

ALPS is a benchtop modular platform developed for the manipulation of nanoliter droplets on DMAs. The system is designed for droplet collection, transfer, mixing, and deposition onto secondary substrates, including other microarrays, microtiter plates, tube racks, and custom 3D-printed holders (**Fig. 1b**). The platform is built around a *UFactory Lite 6* robotic arm with a repeatability of ±0.5 mm. This robot was selected as an accessible off-the-shelf benchtop platform for modular proof-of-concept implementation of automated droplet transfer on DMAs, prioritizing ease of integration and experimental flexibility over maximal

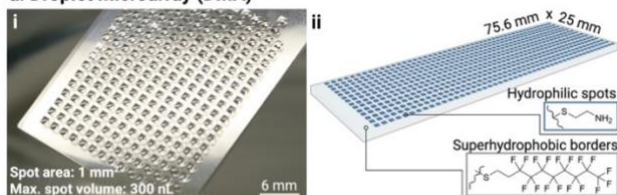
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positioning accuracy. Robotic motion was used to perform all three-dimensional positioning steps, including approach, aspiration, and dispensing routines. A wrist-mounted pen-type syringe pump module served as the nanoliter pipetting unit. The pump was based on a *Takasago SBP 100G* syringe module with a theoretical resolution of 10.5 nl per step over a 9600-step stroke. Pump motion was controlled through Modbus communication implemented on a *Raspberry Pi Pico*, enabling synchronized aspiration, dispensing, purge, and cleaning operations. A Tkinter-based graphical user interface was used for calibration, manual control, and execution of automated transfer workflows. During operation, the DMA was placed in a defined holder within the ALPS workspace to ensure reproducible substrate positioning. Working coordinates for transfer routines were then established through GUI-based calibration before automated handling.

a. Droplet Microarray (DMA)



b. Robotic Platform for Nanoliter Droplet Pipetting

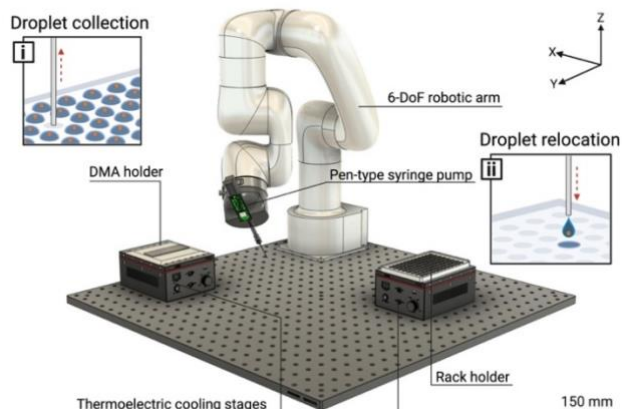


Fig. 1 Droplet Microarray (DMA) and ALPS setup for nanoliter droplet transfer. (a) DMA slide used for spheroid formation. (i) image of the patterned surface (Spot area 1 mm²; maximum spot volume 300 nl); (ii) Schematic of the slide showing hydrophilic spots surrounded by superhydrophobic borders that confine pinned droplets. (b) 3D model of the robotic platform for automated nanoliter droplet pipetting (ALPS), consisting of a 6-DoF robotic arm equipped with a pen-type syringe pump, a temperature-controlled DMA and rack holder, inset schematics illustrate (i) droplet collection and (ii) redeposition/relocation to target spots.

2.2 Spheroid Formation

HEK293 (human embryonic kidney 293) cells were used as the spheroid model. Spheroids were generated on DMAs using a hanging-drop workflow [4]. In the 672-spot format, the array

consisted of 672 hydrophilic spots arranged in 14 columns and 48 rows. Each spot measured 1 × 1 mm² and was separated by a 0.5 mm superhydrophobic gap (**Fig 1a**). To minimize cell adhesion to the substrate, an anti-adherence solution diluted 1:1 in Milli-Q water was dispensed onto the DMA spots and air-dried for 30 min. The cell suspension was then printed onto the array using a *Certus Flex* liquid dispenser. Standard spheroid formation on the 672-spot DMA was performed by dispensing 500 cells in ~300 nl per spot, yielding spheroids of ~120-170 μm in diameter after 48 h. After printing, the DMAs were inverted and incubated in a humidified chamber at 5% CO₂ for 2–3 days to enable spheroid formation by gravity-driven aggregation.

2.3 Automated Transfer Experiments

Transfer experiments were performed on 672-spot DMAs after spheroid formation. ALPS was used to aspirate and dispense individual spheroid-containing droplets from defined source regions to defined target regions either within the same DMA or between DMAs. During optimization, the main operating parameters were nozzle height, intake volume, and needle gauge. Transfer experiments were performed using a 20G *Hamilton* needle (OD 0.9 mm, ID 0.6 mm) and a 24G *Hamilton* needle (OD 0.5 mm, ID 0.3 mm). During the transfer experiments, the DMA was positioned on a cooled stage maintained at ~7-10 °C to reduce evaporation.

2.4 Imaging and Transfer Evaluation

Brightfield images of the source and target regions were acquired before and after transfer using a *Keyence BZ-X810* microscope. Transfer performance was quantified as the fraction of spheroids successfully relocated from the predefined source spots to the intended target spots. Spheroid diameters were measured in *ImageJ* from the brightfield images and visualized in *Python*.

3 Results and Discussion

3.1 Automated Spheroid Transfer on DMAs

To characterize automated spheroid transfer on DMAs, we quantified transfer performance while varying nozzle height, aspiration setting (syringe pump motor steps), and needle gauge (**Tables 1–2**). With the 20G needle, performance was highly height-dependent: at 0.5 mm and 25 steps (~250 nl) no spheroids were transferred (only medium), whereas 0.4 mm and 30 steps (~300 nl) increased transfer to 67%. Further

lowering to 0.3 mm reduced transfer to $12 \pm 5\%$. The 24G needle yielded the highest transfer rates among the tested conditions, reaching a maximum of $81.5 \pm 1.5\%$ at 0.4 mm and 35 steps (~ 350 nl); at 0.5 mm and 30 steps transfer was $6 \pm 2\%$, and at 0.3 mm and 35 steps performance decreased to $42.5 \pm 26.5\%$.

Overall, transfer was sensitive to the combined effects of approach height, aspiration volume, and needle size. Across the tested conditions, 0.4 mm yielded the highest transfer rates, and the 24G needle outperformed the 20G needle. Moreover, the smaller nozzle is advantageous in dense arrays because it is less likely to disturb neighboring droplets, thereby reducing droplet merging and potential cross-contamination. In contrast, the larger 20G needle is comparable to the characteristic spot dimension (1 mm^2) and is more sensitive to positioning errors and mechanical backlash. Under the optimized 24G settings, one collection-and-redeposition cycle required ~ 5 s per spheroid.

Under the limited parameter sets tested here, the 24G needle at 0.4 mm and 35 steps yielded the highest mean transfer rate. However, incomplete transfer events remained a relevant limitation, indicating that the present study should be interpreted as a feasibility demonstration rather than a robustly optimized transfer workflow. Our results further suggest that these incomplete events primarily arise from spheroid capture failures rather than droplet-handling failures, as droplets could still be aspirated even when the spheroid remained at the source spot (Fig 2.ii). Several factors likely contribute to these failures, including spheroid-surface interactions at the hydrophilic spot, partial adhesion during local drying, and variability in spheroid positioning within the droplet relative to the nozzle trajectory. Addressing these failure modes will therefore require targeted optimization of capture physics (e.g., nozzle geometry, approach height, aspiration profiles, and surface/passivation strategies) rather than further refinement of the operating parameters evaluated here. Higher-accuracy robotic systems may also improve transfer robustness in future versions.

Table 1: Transfer rates using a 20G needle. 1 step = $10 \text{ nl} \pm 10\%$. n=2

Nozzle height (mm)	Syringe pump steps	Transfer rate (%)
0.5	25	0
0.4	30	67
0.3	30	12 ± 5

Table 2: Transfer rates using a 24G needle. 1 step = $10 \text{ nl} \pm 10\%$. n=2

Nozzle height (mm)	Syringe pump steps	Transfer rate (%)
0.5	30	6 ± 2
0.4	35	81.5 ± 1.5
0.3	35	42.5 ± 26.5

3.2 Spheroid Integrity After Automated Handling

In addition to transfer efficiency, we assessed whether ALPS enables directed relocation of spheroid-containing droplets while preserving spheroid integrity. Using the optimized 24G settings (Section 3.1), spheroids were relocated in representative 3x3 spot groups (Fig. 2).

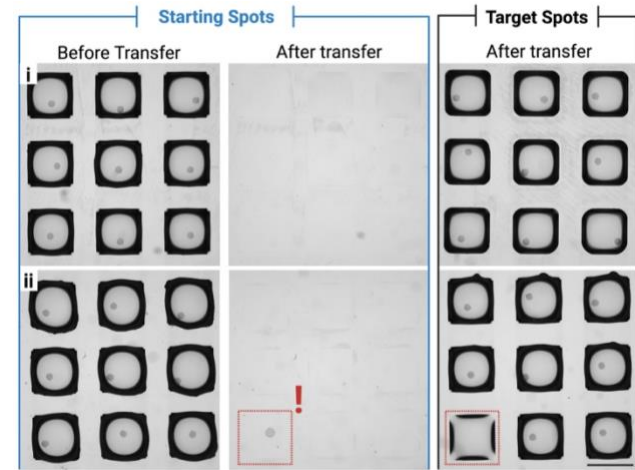


Fig. 2. Spheroid transfer on droplet microarrays. Brightfield images of representative 3x3 spot groups at the source “Starting spots” and destination “Target spots” positions. (i) Successful transfer: spheroid-containing droplets are present at the starting spots before transfer, absent at the starting spots after transfer, and detected at the corresponding target spots after transfer. (ii) Representative incomplete transfer event: the droplet was aspirated from the starting spot, but the spheroid was not captured and remained at the source position (red dashed box and exclamation mark), while the corresponding target spot received only medium (red dashed box). The visible grey circular object in the center of the red box corresponds to the retained spheroid at the source position after incomplete capture. Scale bar 1mm.

Brightfield inspection showed that successfully transferred spheroids retained their overall morphology immediately after handling. To probe post-transfer development, spheroids were monitored for 48 h after relocation (Fig. 3). Mean spheroid diameter increased from $144 \pm 9 \mu\text{m}$ at 0 h to $180 \pm 11 \mu\text{m}$ at 24 h and measured $168 \pm 5 \mu\text{m}$ at 48 h ($n = 9$), corresponding to fold-changes of 1.25 ± 0.10 (24 h) and 1.17 ± 0.08 (48 h) relative to 0 h (Fig. 3.ii).

The diameter increase during the first 24 h suggests that, within the limited sample set analyzed ($n = 9$), the transfer procedure was compatible with short-term spheroid growth and did not cause obvious structural damage immediately after relocation. However, the slight decrease at 48 h is consistent with nutrient limitation in the confined droplet environment (~ 300 nl), where the available medium is insufficient to sustain continued growth over multi-day incubation without medium exchange [5].

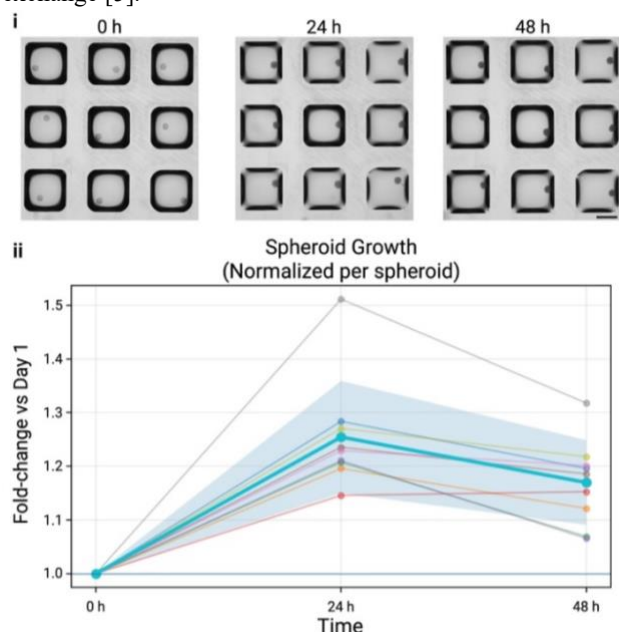


Fig. 3. Post-transfer spheroid growth on DMAs.

(i) Representative brightfield images of transferred spheroids at the target spots immediately after transfer (0 h) and after 24 h and 48 h incubation. (ii) Spheroid diameter changes over time plotted as fold-change relative to 0 h for each spheroid (thin lines), with mean $\pm$ SD shown as a thick line and shaded band ($n = 9$). Scale bar 0.5 mm.

3.3 Conclusion

In this work, we demonstrated the feasibility of ALPS as an automated liquid-handling approach for the collection and relocation of individual spheroid-containing nanoliter droplets on droplet microarrays. Under optimized conditions within the parameter sets tested here, ALPS achieved transfer yields of $\sim 80\%$ with a processing time of ~ 5 s per drop. Importantly, automated handling was compatible with spheroid integrity in the size range studied (~ 120 – 170 μm). Brightfield inspection confirmed preserved morphology immediately after transfer, and post-transfer monitoring showed a clear diameter increase within the first 24 h, indicating that the transfer procedure did not cause structural damage that would prevent short-term growth.

Based on these results, the DMA can serve as a high-density spheroid formation platform, while ALPS provides a basis for selective transfer of spheroids into standard downstream formats (e.g., microtiter plates) for assays workflows such as high-throughput drug screening or functional assays. For sensitive applications, incorporating an optional nozzle cleaning step between samples in ALPS could help mitigate carryover and reduce the risk of cross-contamination.

Overall, ALPS serves as an interface between high-density nanoliter spheroid culture on DMAs and conventional downstream assay formats. Its modular design, based largely on off-the-shelf components with minimal custom hardware, enables a cost-effective implementation of automated spheroid handling. The platform is also readily scalable by integrating multiple independently controlled nozzle–syringe modules on a single robot. In addition, alternative robotic architectures such as parallel kinematic systems may be attractive for future implementations requiring higher positioning accuracy and throughput. Such parallelization would be particularly relevant for next-generation DMAs with thousands of samples per slide, enabling bulk transfer instead of purely sequential handling.

Author Statement

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