Aplo Flow enables high-throughput multiplexed DNA-PAINT imaging with fully automated fluidic exchange

SUMMARY

Successful execution of unsupervised, high-throughput super-resolution imaging with DNA-PAINT requires robust hardware coordination, optimized fluidic design, and an exceptionally reliable total imaging ecosystem.

The highly engineered Aplo Platform integrates fluidics via Aplo Flow with super-resolution imaging on Aplo Scope to enable fully automated multi-lane, multi-cycle DNA-PAINT imaging for a virtually unlimited number of targets using a uniquely compact and reliable setup.

Using Aplo Flow coupled with Aplo Scope enables:

- Fully automated fluid handling and image acquisition for high-throughput multiplexed DNA-PAINT
- Stable repositioning between cycles with a low positioning error (< 250 nm)
- Highly effective lane washout with minimal signal carryover
- Super-resolution reconstruction with nanoscale precision (<6 nm)
- A benchtop, error-proof setup for complex fluidics assays with parallel operation to maximize efficiency

INTRODUCTION

DNA-PAINT (DNA-based Point Accumulation for Imaging in Nanoscale Topography) is a single-molecule localization microscopy (SMLM) technique that enables scientists to visualize several targets within the same sample and study single proteins in cells or tissues with sub-20 nm resolution[1,2]. DNA-PAINT harnesses the transient, reversible binding between short fluorescently labeled "imager" strands and complementary "docking" strands attached to the targets of interest to produce stochastic blinking events for localizing single molecules.

One of the most powerful features of DNA-PAINT is its potential for virtually unlimited multiplexing through the sequential exchange of sequence-specific imager strands. Multiplexed DNA-PAINT uses the cyclic addition and washing of different imager strands to resolve nanoscale protein organization, reveal the ultrastructure of cellular components, and spatially map heterogeneous subcellular distributions[3,4]. In many applications, researchers need to acquire multiple fields of view (FOV) across multiple samples while maintaining stable imaging and reliable cycle-to-cycle performance.

Because the number of fluidic intervention steps scales up with the number of targets, multi-cycle DNA-PAINT quickly becomes infeasible as a manually executed technique, particularly when extended across multiple samples, in terms of both inconsistent fluidics' delivery and labor-intensiveness. In addition, repeatable high-precision positioning is essential to return to the same FOV across cycles and lanes and ensure maximal data retention after positional corrections are performed.

Finally, a highly effective washout between cycles is critical to avoid carryover signals that could result in misinterpretation of the spatial relationship between targets. Traditional workflows and competitive systems are often limited by slower sequential fluid handling, incompatible products or combinations of technology, and the practical difficulty of scaling experiments to prepare and image multiple samples or positions within a single run.

Here we demonstrate automated, multi-sample, multi-cycle DNA-PAINT imaging of microtubules using Aplo Flow. Through a unified script-based framework, the workflow combines automated fluid handling and acquisition to run eight imaging cycles across four FOVs spanning two sample lanes. The system is designed to decouple imaging from fluid handling, enabling simultaneous parallel operation in which one lane can be actively imaged, while other lanes undergo fluid exchange and preparation steps.

METHODS

Aplo Flow integrates programmable, automated fluid exchange with the ONI Aplo Scope SLMLM platform to support multi-lane DNA-PAINT experiments. For the work presented here, Aplo Flow was configured for automated staging (sequential liquid delivery) of blank buffer, imaging solution (containing the imager strand), and wash steps on a per-lane basis.

For sample labeling, the ONI DNA-PAINT kit MASSIVE-sdAB ONI 2-PLEX Kit (Massive Photonics)[5] was used. U2OS cells were cultured in ONI's proprietary Cell Flow Chip for 24-48 hours, and fixed with pre-warmed 4% PFA, 0.25% glutaraldehyde, and 0.1% Triton X-100 at 37°C for 15 min. Cells were incubated for 7 min at room temperature (RT) with 0.1% $NaBH_4$ to quench any autofluorescence background signal, and a further 1 hour at RT with 5% BSA+0.2% Triton-X 100 in 1xPBS to prevent non-specific probe binding and permeabilize cells. Cell microtubules were stained using a primary anti-alpha tubulin antibody (ab18251, Abcam) diluted 1:200 in 5% BSA, 0.1% Triton X 100 in 1xPBS for 1 hour at RT, followed by PBS washing, and a secondary antibody staining using 1:100 sdAb anti-Rabbit F2 in Antibody incubation buffer (Massive Photonics) for 1 hour. After further washing, cells were post-fixed with 4% PFA in PBS for 10 min at RT. 100 nm gold nanoparticles (AAJ67348AC, Fisher Scientific) diluted 1/10 in 1xPBS were added to each channel for 10 min before imaging as fiducial markers for image registration. For each blank cycle, imaging buffer (ONI DNA-PAINT kit, Massive Photonics) was used without imager strand addition, and for each imaging cycle, imaging buffer containing 200 pM F2-Cy3B imager strand was added to the lane via the Aplo Flow system.

Each imaging cycle consisted of an initial blank phase, followed by DNA-PAINT imaging at each FOV, and a subsequent wash step. Aplo Flow's architecture allows simultaneous parallel lane operation, meaning that sample preparation took place in one lane, while imaging occurred in another lane (Figure 1). This sequence was repeated for 8 cycles at 32 °C using fully automated acquisition to demonstrate that our system is capable of multi-cycle exchange-PAINT imaging with high reproducibility and stable sample positioning on the scope. Two chip lanes were imaged using Aplo Scope's 561 nm laser (0.1-0.3 kW/cm2) for 10,000 frames, with two FOVs being acquired per lane (4 total FOVs). The entire experimental workflow, including image acquisition, fluidic control, and hardware synchronization, was executed via a custom Python script integrating the Aplo Flow software interface controlling the fluidics hardware with the Aplo Scope SMLM microscope. Following image acquisition, post-processing and quantification were performed using ONI's CODI software.

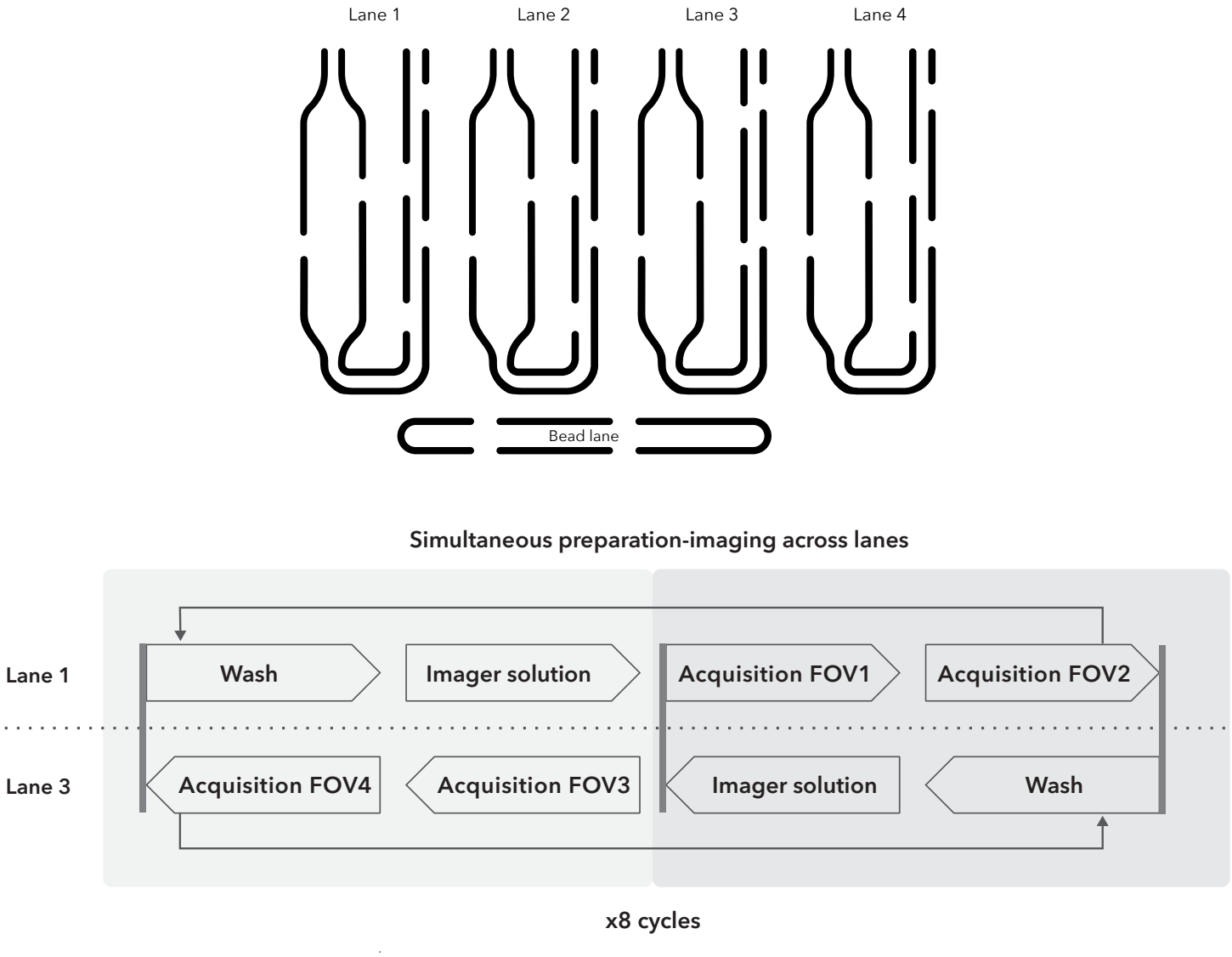

Figure 1 | Top: Outline of the Cell Flow Chip with 4 lanes. Bottom: Schematic of the automated multi-lane and multi-position DNA-PAINT imaging workflow over 8 cycles, which includes simultaneous sample preparation (blank, wash, and imager solution addition) and imaging (acquisition of field of views, FOV). The dark bars represent essential points of synchronization.

RESULTS

Multi-lane microtubule DNA-PAINT imaging with high localization accuracy

Multi-lane, multi-position automated DNA-PAINT imaging was successfully performed within a single experiment, including two lanes and two spatially distinct FOVs acquired per lane, resulting in four total imaging positions. All FOVs were revisited reproducibly across 8 cycles using automated stage positioning, enabling consistent multi-position acquisition without manual intervention.

Representative super-resolution images obtained from each FOV and zoomed-in regions demonstrate comparable image quality and structural detail across lanes and positions (Figure 2). These data illustrate that multi-lane operation does not compromise imaging performance and supports scalable acquisition of multiple samples or regions within a single automated run.

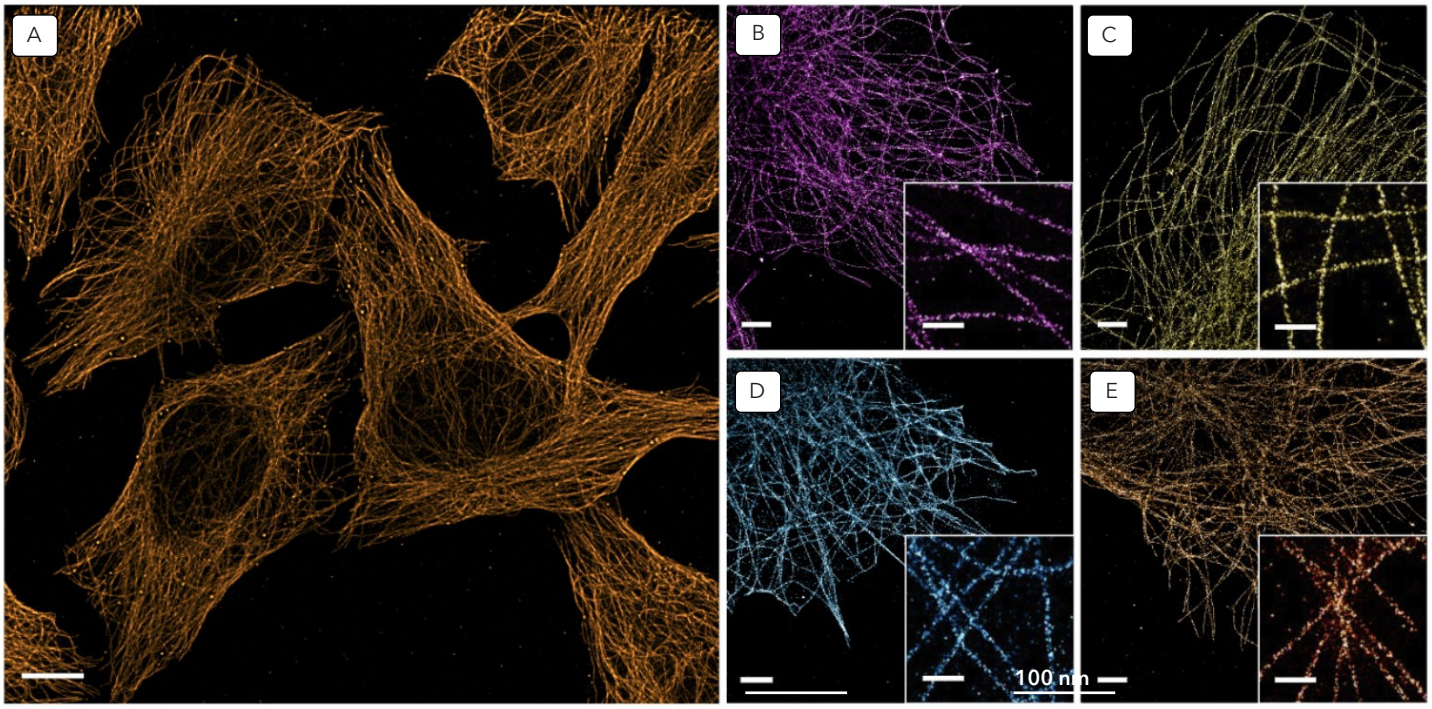

Figure 2 | DNA-PAINT images of microtubules from U2OS cells cultured in the Cell Flow Chip, and acquired using fully automated fluid handling and image acquisition with Aplo Flow and Aplo Scope. Images across lanes and FOV showed high-localization accuracy. A) Sample overview. Scale bar = 10 µm. B-E) FOV1-4 imaged across two separate lanes, scale bar = 2 µm, insets show zoomed-in views, scale bar = 500 nm.

Next, we verified the resolution metrics of the experiment to validate our automated fluid exchange workflow for multi-lane DNA-PAINT imaging using Aplo Flow. Microtubule super-resolution reconstructions achieved a Fourier ring correlation (FRC) resolution estimate of approximately 46 nm, with a median localization accuracy of 5.8 nm (Figure 3). These resolution metrics are consistent with high-quality DNA-PAINT imaging and demonstrate that integrated fluidic automation and multi-lane operation do not compromise achievable spatial resolution. The observed performance was maintained across automated cycles and imaging positions, supporting the use of Aplo Flow for high-resolution, multi-cycle super-resolution experiments.

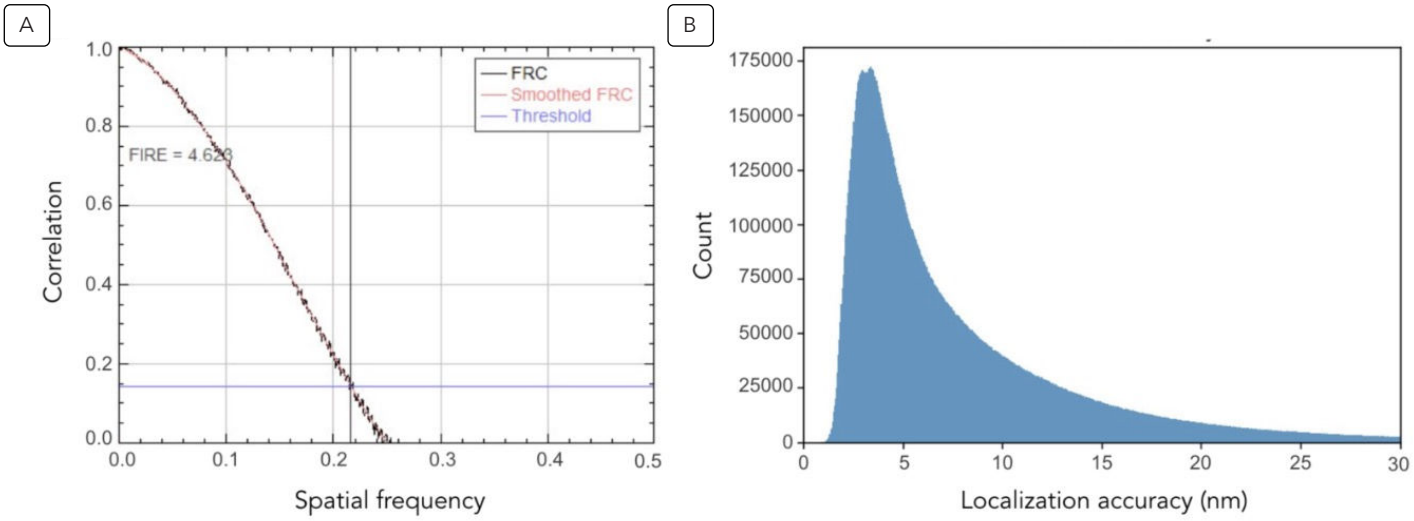

Figure 3 | Resolution metrics of ONI's automated fluid exchange workflow for multi-lane DNA-PAINT imaging using Aplo Flow. A) Graph showing a Fourier ring correlation (FRC)[6] resolution quantification as a Fourier Image REsolution (FIRE) value of 4.623, which means a 46 nm resolution (pixel size is 10 nm). B) Histogram showing the localization accuracy of the counted single-molecule events, with a median value of 5.8 nm.

Automation performance: repositioning and washout across cycles

The experimental sequence was repeated for 8 cycles using fully automated acquisition to demonstrate that our system is capable of multi-cycle exchange-PAINT imaging with high reproducibility and positional stability. Automation performance was evaluated across eight consecutive imaging cycles to assess stage repositioning stability and lane washout efficiency. Across all cycles, automated stage repositioning remained stable, and the median positioning error was 230 nm across all FOV, with maximum deviation from the initial imaging position remaining under 1 µm for all positions. This level of positional consistency supports preserving 99% of spatial data across multiple cycles of imaging once positionally corrected.

Lane wash performance was assessed using intervening blank cycles to quantify residual signal following imaging and wash steps. Blank-cycle localization counts remained low, corresponding to 0.2 to 1.1% of imaging-cycle localizations when using 1.5 mL of wash buffer between cycles. Most detected localizations in the blank session originated from the gold nanoparticle fiducials and cellular background signal. No accumulation of background signal was observed over successive cycles, indicating effective removal of imaging strands and minimal carryover between imaging rounds. Together, these results demonstrate that automated multi-cycle operation can be performed without compromising positional stability or data integrity.

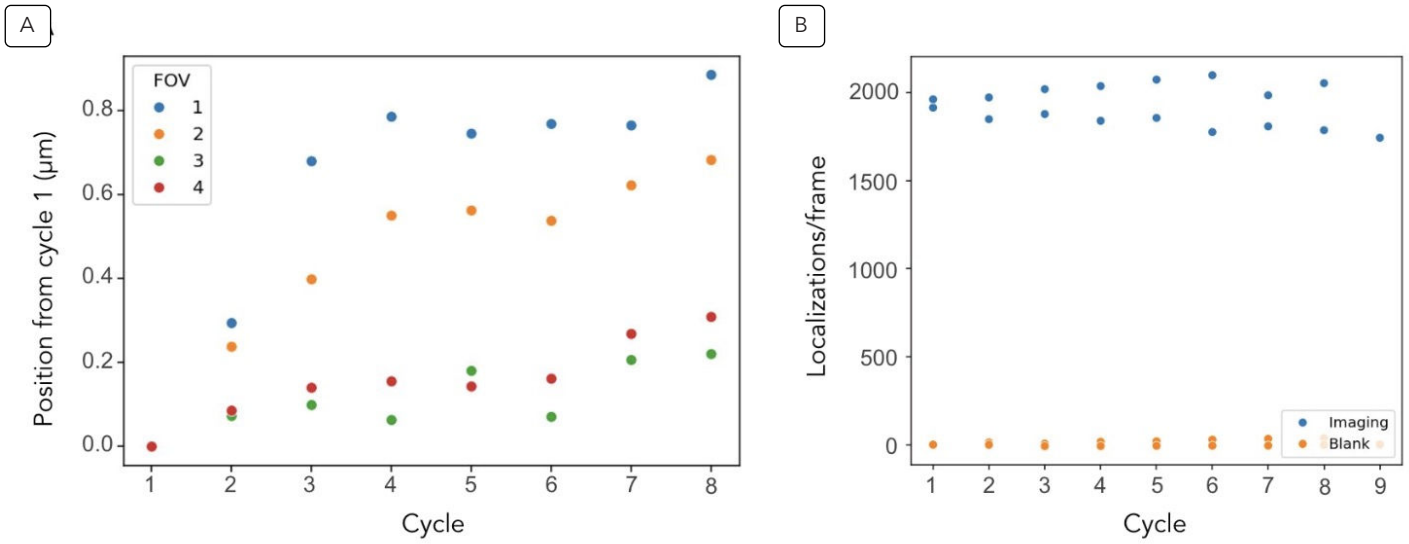

Figure 4 | Quantification of automation performance across cycles. A) Stage positioning errors over 8 cycles plotted relative to the starting position in cycle 1, all below 1 µm and with a median value of 230 nm across FOVs and cycles. B) Graph showing the number of localizations per frame (any gold nanoparticle localizations or system background was removed) to assess lane washing performance between cycles. This did not increase over cycles, with 0.2-1.1% localizations observed in the blank cycle normalized by the number of localizations in the imaging cycle.

CONCLUSION

The results shown here demonstrate that Aplo Flow facilitates automated, high-throughput multiplexed DNA-PAINT workflows with robust cycle-to-cycle performance across lanes and imaging positions within a single run. By decoupling image acquisition from fluid exchange, the Aplo Flow architecture represents a significant advancement in DNA-PAINT productivity, effectively neutralizing the downtime inherent to traditional sequential PAINT cycles. Across an 8-cycle experiment across four imaging positions, stage repositioning remained highly stable, while effective washout minimized residual signal. This ensures that multi-cycle and multi-position datasets are directly comparable and free from carryover artifacts. Ultimately, Aplo Flow resolves the traditional "plexity-vs-resolution" trade-off, transforming DNA-PAINT from a labor-intensive, specialized method into a scalable and reproducible, high-throughput tool. By automating sample transitions while maintaining nanometer-scale accuracy, the Aplo platform can provide the statistical power necessary for large-scale biological discovery.

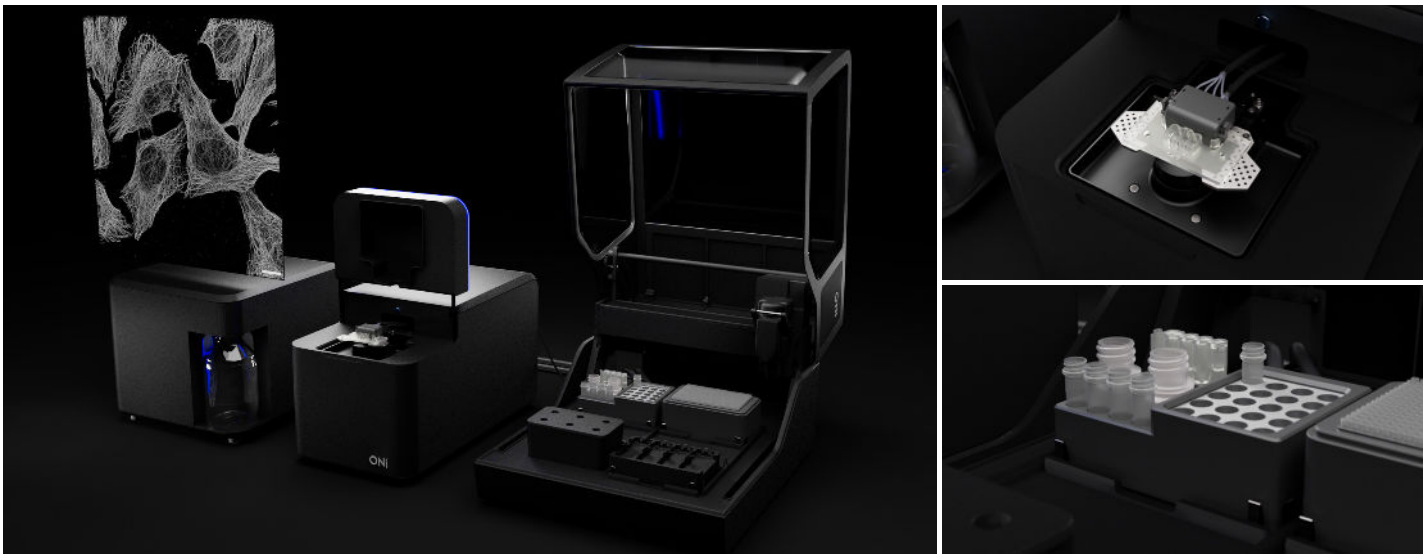

Figure 5 | The highly engineered Aplo Platform integrates fluidics via Aplo Flow with automated imaging on Aplo Scope to enable high-throughput multiplexed DNA-PAINT imaging (left). Component detail image renders show the ONI Cell Flow Chip interface and fluid distributor (right).

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