Cell Biology | Application Note

Automated Cell Assay Set-Up with the Cell Care STAR: Enhancing Throughput and Reproducibility

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Introduction

Nowadays, biomedical research demands large-scale, cell-based assays for developing novel therapies in a cost-effective manner. Therefore, cell culture automation is essential for meeting high-throughput requirements and reducing costs. Additionally, automation enables seamless traceability of experimental procedures, improved standardization, and precision through automated liquid handling, making cell culture experiments more productive. The Hamilton Cell Care STAR is a state-of-the-art, Assay-Ready Workstation, designed to autonomously manage routine cell culture maintenance and the seeding of cells into culture plates for high-throughput assays. By automating these processes, the system significantly reduces experimental variability while maintaining high levels of precision. The intuitive user interface of the Cell Control software simplifies the management of various cell culture applications simultaneously, while continuously tracking each process to prevent data loss. Here, we demonstrate the advantages of automated cell culture using the "Seeding from Trough" method (work step) within Cell Control, applied to two commonly used cell types: HeLa and T47D.

Seeding cell suspensions into multi-well culture plates can be efficiently automated, via a wide range of adjustable parameters within Cell Control. This is particularly beneficial for high-throughput experiments, as it ensures consistency and significantly reduces the potential for human error. In contrast, manual seeding of different cell types, such as HeLa and colony-forming T47D cells, can be both challenging and tedious. Manual seeding often leads to variability in cell distribution, increased time consumption, and higher risk for contamination, making it less efficient for high-throughput applications.

- The "Seeding from Trough" work step on Cell Care STAR enables fully traceable, high-throughput, cell-based assays with enhanced standardization.
- Automated liquid handling minimizes well-to-well and plate-to-plate variation, increasing assay consistency.
- Integrated imaging and confluency measurements reduce manual intervention, optimizing workflow efficiency.

Figure 1: Hamilton Cell Care STAR.

Workflow

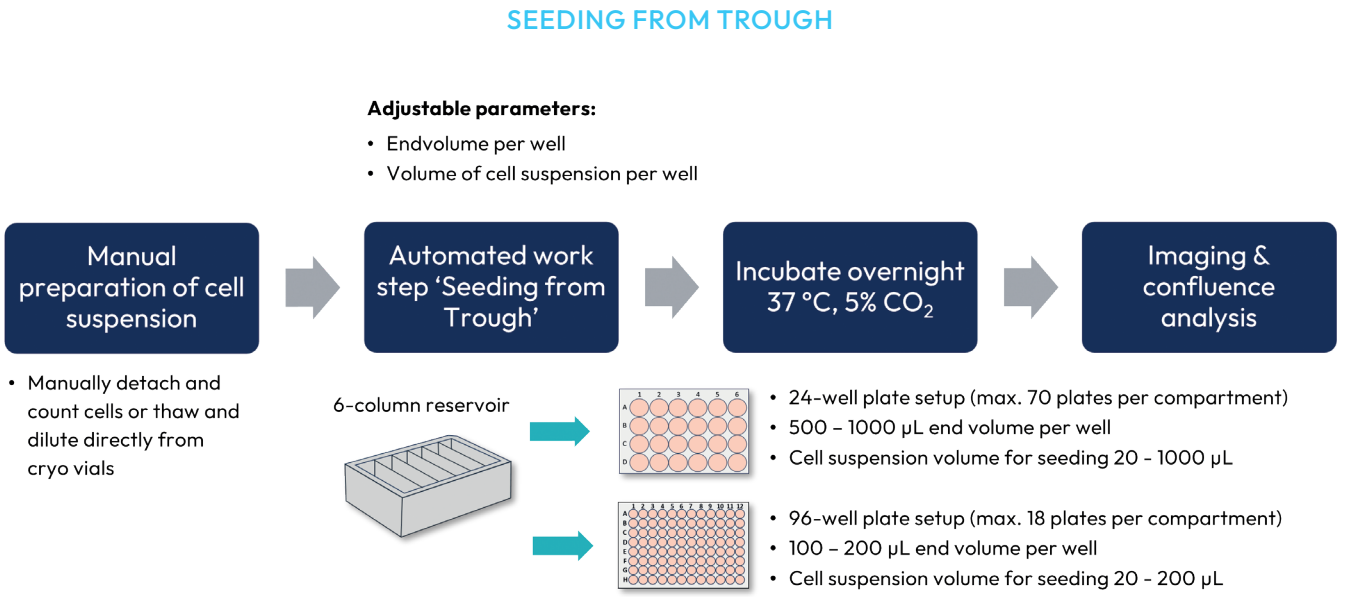

Figure 2: "Seeding from Trough" 24- and 96-well workflow. A fully automated workflow was implemented using Cell Care STAR, which includes cell seeding, incubation, imaging, and confluency analysis.

Step 1: Cell suspension is prepared by the user and transferred into a 6-column reservoir (trough).
Step 2: Cells are automatically seeded into barcoded 24- and 96-well plates with precise volume control.
Step 3: After the work step is completed, plates are automatically transported into the incubator and cultured at +37 °C and 5% CO_2 overnight.
Step 4: Automated confluency analysis is performed via Cytation 1 imager and Gen5 software.
Step 5: Data is compiled and analyzed by the user to assess reproducibility and variation.

Revolutionizing Research: The Cell Care STAR. Enhances Large-Scale Cell Culture with Unmatched Consistency and Minimized Assay Variation.

Cell Culture

Cell Culture Conditions

HeLa cells (Sigma-Aldrich, 93021013) and T47D cells (ECACC, 85102201) were cultured under standard tissue culture conditions in high glucose DMEM (Gibco, L0101-500), supplemented with 10% FBS (Gibco, A5256801).

Workflow

Cell suspension was prepared manually by detaching cells with Trypsin-EDTA 0.05% (Fisher Scientific, 25300104) and adding to a 6-column reservoir (Agilent Technologies, 204284-100) at the desired density. This seeding was performed by either using the Cell Care STAR work step "Seeding from Trough" or manually. Cells were left to settle before transport to the incubator. After 24 hours, cell confluency was determined using the Cytation 1 imager and Cellular Analysis tool of the Gen5 software (Agilent).

Seeding Process

- 24-well plates (500 µL of cell suspension + 500 µL of growth medium per well; HeLa ($1x10^5$cells/mL) and T47D ($3x10^5$ cells/mL).
- 96-well plates (50 µL of cell suspension + 150 µL of growth medium per well; HeLa ($1x10^4$ cells/mL) and T47D ($3x10^4$ cells/mL).
- After 24 hours, confluency was assessed using Cytation 1 imager and Gen5 software (Agilent).
- Biological replicates were generated using cells with different passage numbers to ensure variability and robustness in the experimental results.

System Description / Deck Layout Description
Minimal intervention and maximal safety

1. Microlab STAR
2. H-Box Biosafety Class II Cabinet (Erlab)
3. Cytomat C10 (Thermo Fisher Scientific)
4. R10 Hotel
5. Huber Ministat 125 (Huberlab AG)
6. Cytation 1 Imager (Agilent)
7. Liquid Waste & Disinfectant Containers
8. Solid Waste

Figure 3: Cell Care STAR.

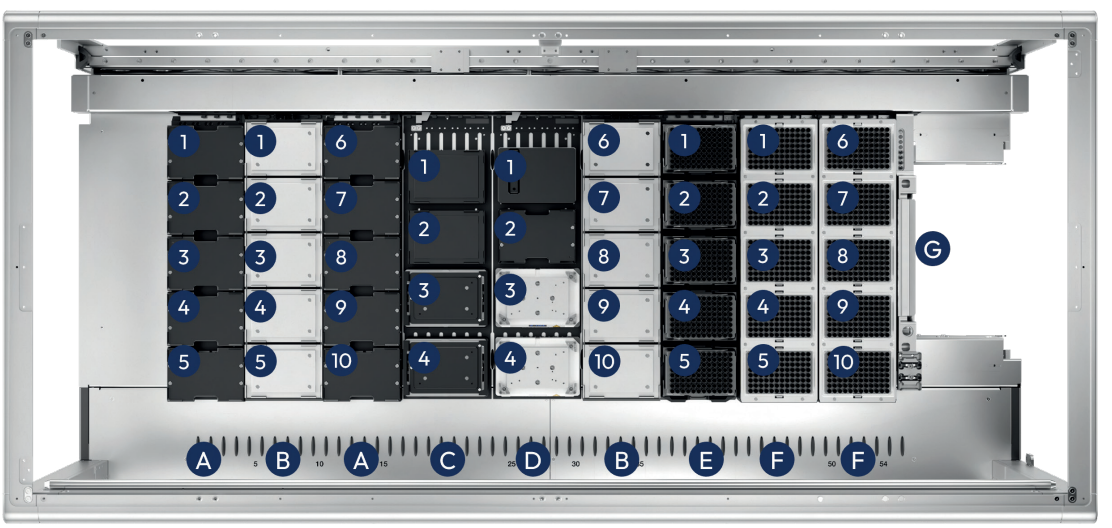

Figure 4: Cell Care STAR Deck Layout.

A MultiFlex Carrier Base
1-5. Lid Parking Positions
6-10. Target Plate Positions

B Cooled Carrier
1-5. Cooled Positions for Samples
6-10. Temperature-regulated Positions for Media Troughs

C Mixed Carrier
1-2. Tilt Module
3-4. Hamilton Heater Shaker

D Mixed Carrier
1. Liquid Dispenser
2. PBS Position
3-4. Hamilton Heater Cooler

E Nested Tip Carrier
1-5. 300 µL Conductive Tips

F Tip Carrier
1-10. 1 mL Conductive Filter Tips

G Solid Waste

Application Software

Cell Control Software

The Hamilton Cell Control software was specifically developed to manage complex cell culture maintenance workflows autonomously over the course of several days or weeks. Its easy-to-use interface allows for the setup of batches with multiple work steps, reloading of reagents and tips, and estimation of consumption; all with just a few clicks (Figure 5). Each work step has a predefined set of adjustable parameters (e.g., incubation time of detachment solution, passaging ratio, volume of medium, etc.) that can be optimized for different cell lines (Figure 6).

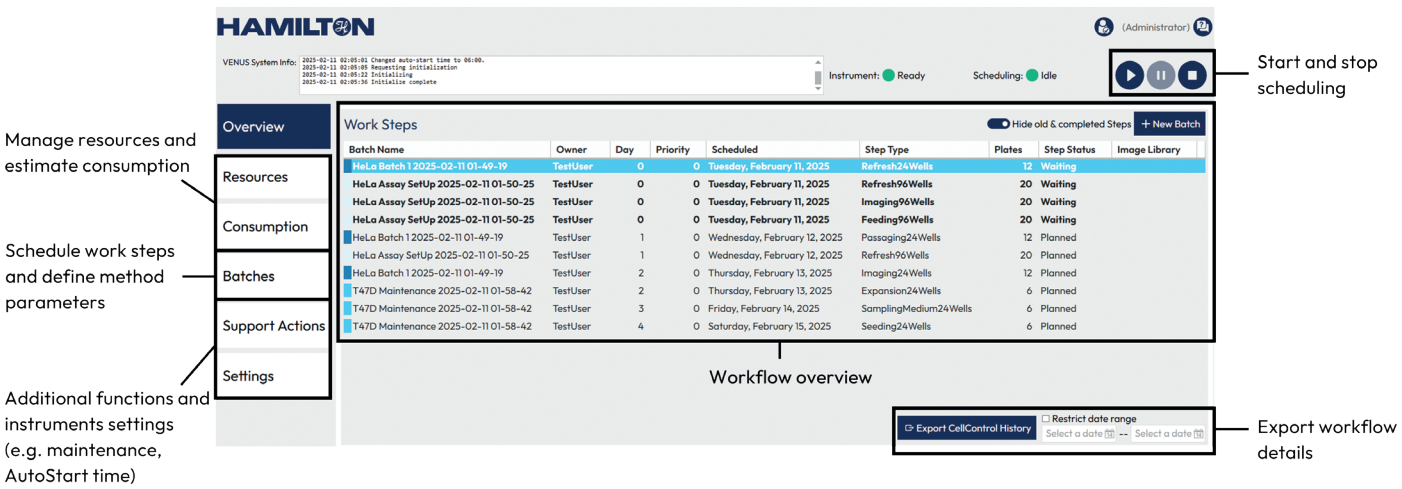

Figure 5: Overview tab of the Hamilton Cell Control software with highlighted main functions.

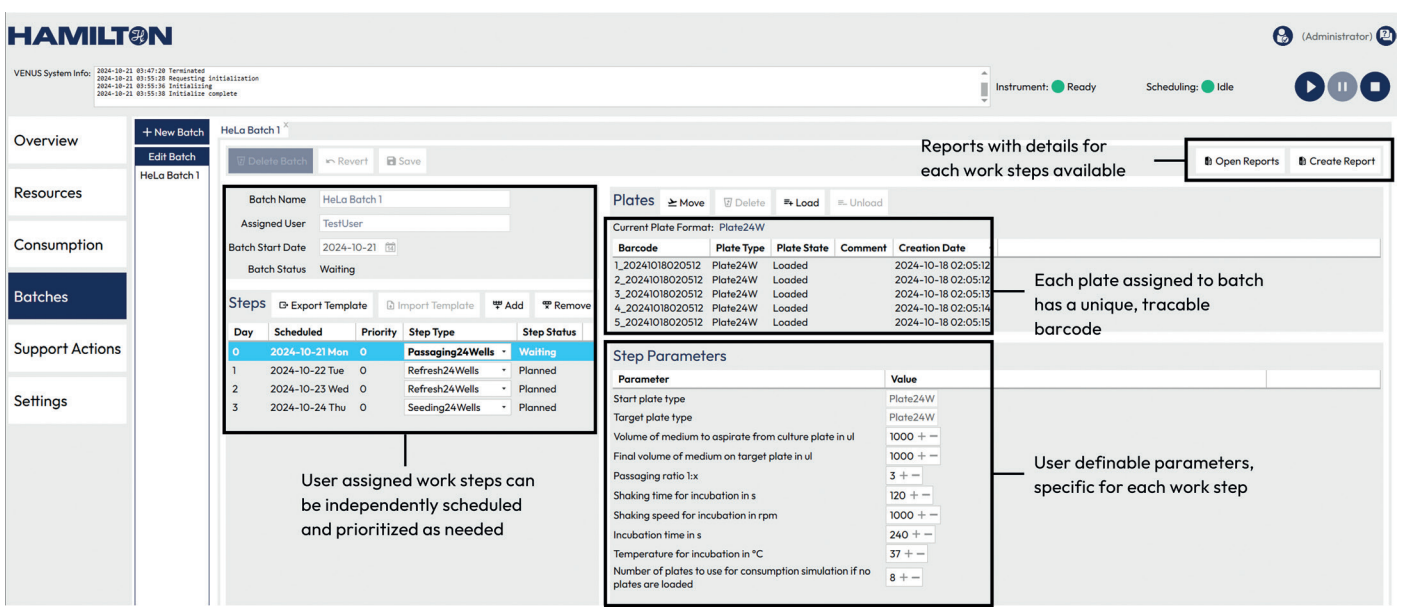

Figure 6: Batch overview tab of the Hamilton Cell Control software. Work steps can be scheduled individually for each batch and each one has a defined set of adjustable parameters

Technology

The Cell Care STAR seamlessly integrates multiple automation technologies:

- Microlab STAR with CO-RE II Technology: Ensures precise liquid handling.
- Tilt Modules and Hamilton Heater Shaker: Enables the cell-friendly processing of two plates in parallel.
- H-Box Biosafety Class II Cabinet: Maintains sample sterility and operator safety.
- Cell Control Software: Enables the direct and seamless management of complex cell culture maintenance workflows.
- Cell Control work steps: comes with 11 adjustable, ready-to-use pre-programmed, tested, and qualified methods for 6-, 24-, and 96-well plate formats and enables the automated tracing of workflows.
- On-Deck Temperature Control: Supports a consistent reagent and sample temperature.
- Cytation 1 Imager: Automates confluency tracking and data analysis.

Cell suspension was prepared for HeLa and T47D cells and seeded into two 24-well or five 96-well plates, either manually or using the Cell Care STAR automated system. Cells were imaged, and cell confluency was measured 24 hours after seeding (Figure 7A, C; 8A, C). To evaluate reproducibility, the Coefficient of Variation (CV) of cell confluency between wells within a plate and the CV of average cell confluency between plates were calculated (Figure 7B, D; 8B, D).

Automated processing with the Cell Care STAR resulted in significantly reduced cell confluency variation in cell culture plates for both cell types. For HeLa cells seeded into 24-well plates, automated seeding resulted in a 41.2% lower CV ($p<0.05$) of cell confluency between wells and a 41.3% lower CV ($p<0.05$) between plates compared to manual seeding (Figure 7A, B, Table 1).

In 96-well plates, automated seeding of HeLa cells led to a 43.6% lower CV ($p<0.001$) between wells and a 75.1% lower CV ($p<0.05$) between plates compared to manual seeding (Figure 7C, D, Table 1).

For T47D cells seeded into 24-well plates, automated seeding resulted in a 33.9% lower CV ($p<0.05$) of cell confluency between wells and a 56% lower CV ($p<0.05$) between plates compared to manual seeding (Figure 8A, B, Table 1).

In 96-well plates, automated seeding of T47D cells showed a 40.2% lower CV ($p<0.05$) of cell confluency between wells and 60.2% lower CV ($p<0.05$) between plates compared to manual processing by an experienced operator (Figure 8C, D, Table 1).

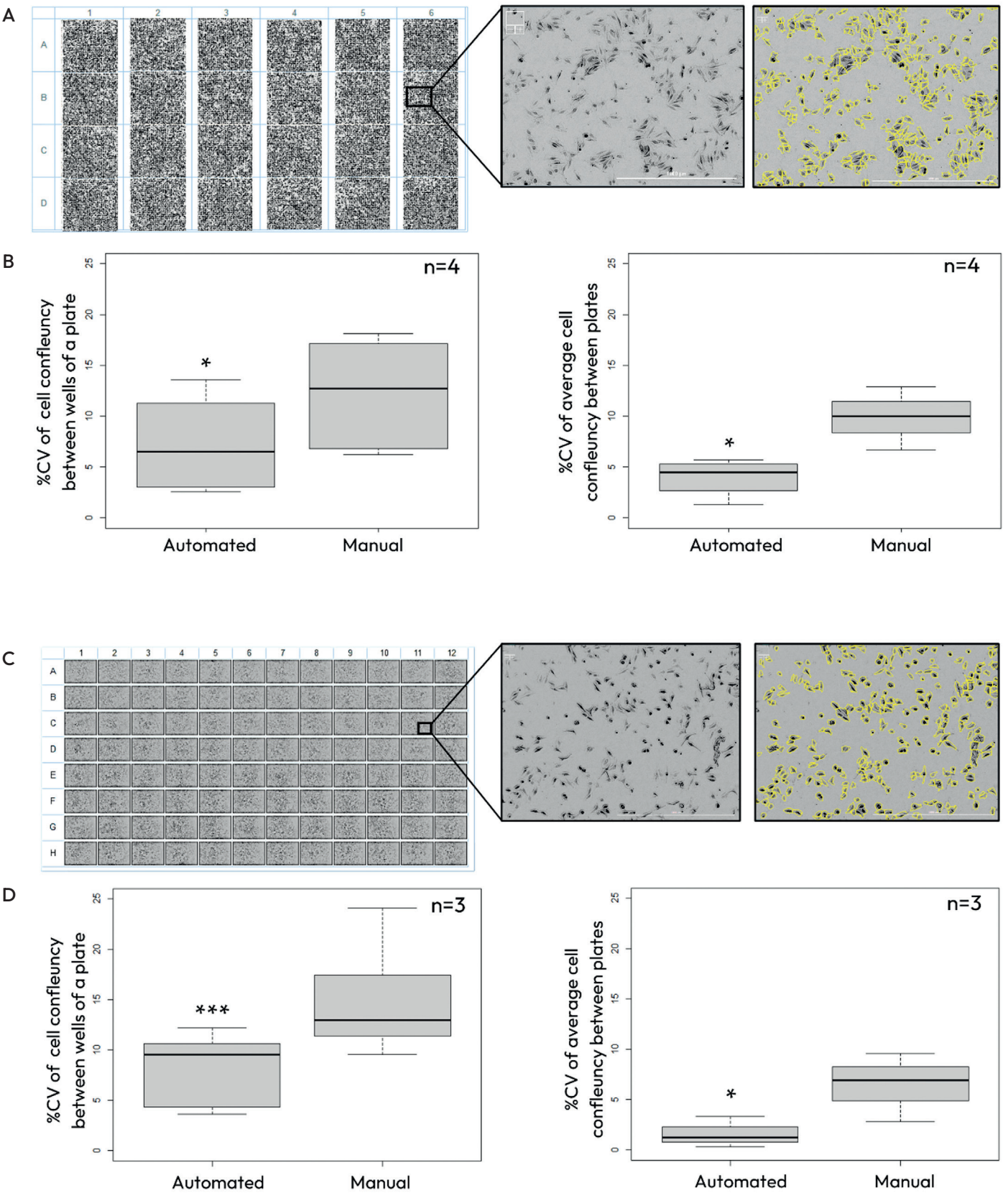

Figure 7: HeLa cells from a 6-column reservoir were transferred to either two 24-well plates or five 96-well plates using Cell Care STAR or manually and imaged after 24 hours. A) Representative image overview of a 24-well plate automatically seeded with Cell Care STAR. Right-hand panel: image zoom-in and segmentation mask for confluency analysis with Gen5. B) Coefficient of Variation of cell confluency between wells of a 24-well plate (left side) and the average cell confluency between plates (right side) of four biological replicates (n=4). C) Representative image overview of a 96-well plate automatically seeded with Cell Care STAR. Right-hand panel: image zoom-in and segmentation mask for confluency analysis with Gen5. D) Coefficient of variance of cell confluency between wells of a 96-well plate (left side) and the average cell confluency between plates (right side) of three biological replicates (n=3). Asterisks indicate significance: * for $p<0.05$ and *** for $p<0.001$ among groups tested with a non-paired Student's t-test.

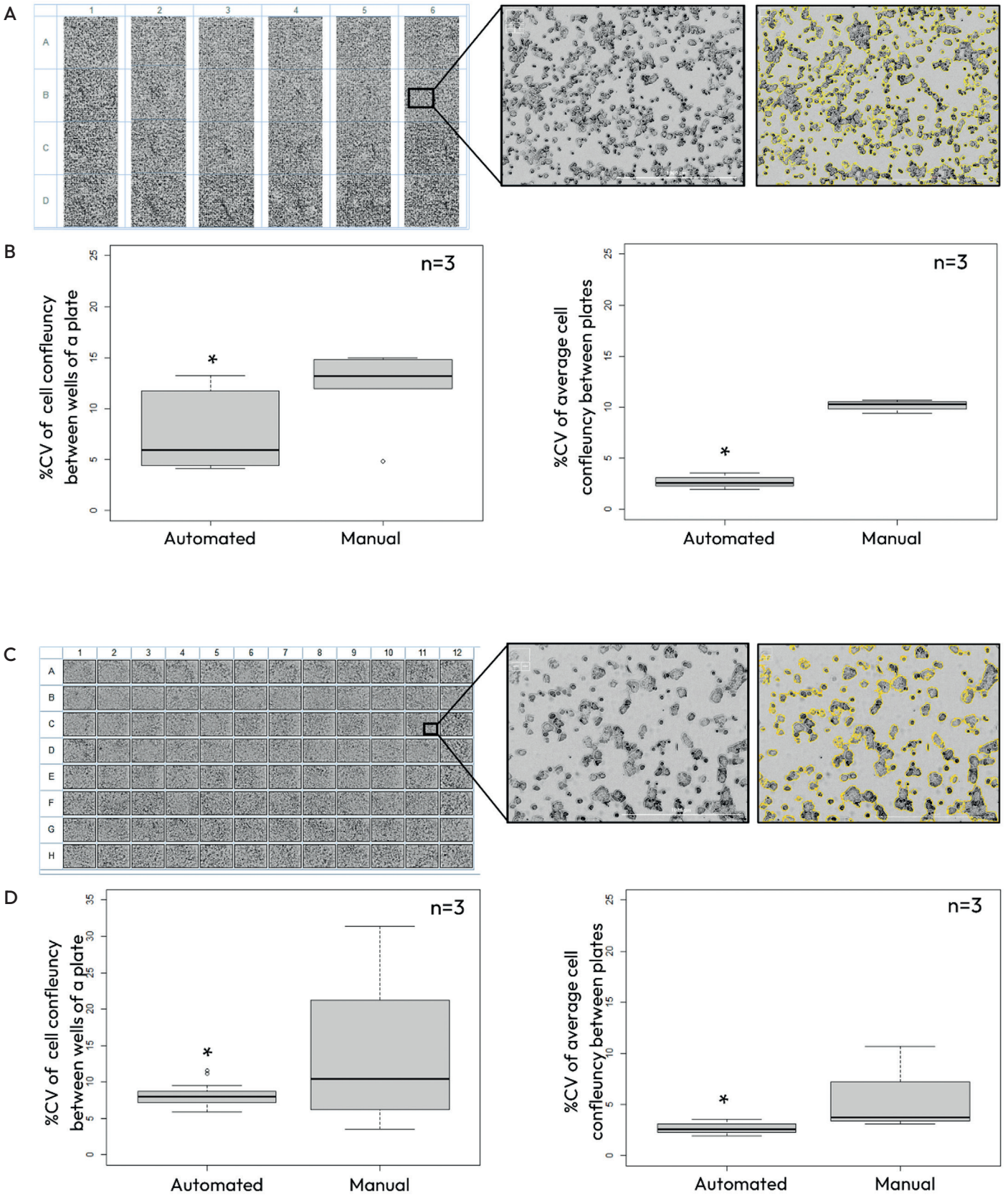

Figure 8: T47D cells from a 6-column reservoir were transferred to either two 24-well plates or five 96-well plates using Cell Care STAR or manually and imaged after 24 hours. A) Representative image overview of a 24-well plate automatically seeded with Cell Care STAR. Right-hand panel: image zoom-in and segmentation mask for confluency analysis with Gen5. B) Coefficient of variance of cell confluency between wells of a 24-well plate (left side) and the average cell confluency between plates (right side) of four biological replicates. C) Representative image overview of a 96-well plate automatically seeded with Cell Care STAR. Right-hand panel: image zoom-in and segmentation mask for confluency analysis with Gen5. D) Coefficient of variance of cell confluency between wells of a 96-well plate (left side) and the average cell confluency between plates (right side) of three biological replicates. Asterisks indicate significance: * for $p<0.05$ and *** for $p<0.001$ among groups tested with a non-paired Student's t-test.

Coefficient of variation of cell confluency (%CV) when comparing automated with manual cell seeding.

Cell Type	Plate Type	%CV well-well		%CV plate-plate	
		automated	manual	automated	manual
HeLa	24-well	7.23	12.29	4.90	8.35
	96-well	8.25	14.62	1.61	6.46
T47D	24-well	8.04	12.16	4.04	10.14
	96-well	8.16	13.65	2.22	5.12

Discussion / Summary

Automating cell culture workflows improves reproducibility, efficiency, and scalability. The Cell Care STAR's "Seeding from Trough" work step enhances experimental reliability by:

- Minimizing human error through precise liquid handling.
- Reducing hands-on time for researchers.
- Providing full traceability of cell culture steps.
- Improving data quality by precise liquid handling with low variability in executing critical cell handling steps, such as pipetting speeds, cell mixing cycles, and cell dispensing parameters.

Hamilton's Cell Care STAR is an off-the-shelf solution designed for large-scale biomedical research and drug discovery, eliminating manual variability while improving data quality and workflow standardization, making scientific breakthroughs more cost-effective. Additionally, the Cell Control software allows for batch scheduling and process optimization, further enhancing lab productivity.

Others

System Requirements	Part Number
Cell Care STAR	870111
Cell Control	10179845
H-Box Erlab Biosafety Class II Cabinet	10183569

System Dimensions

Cell Care STAR	Width: 4749 mm
	Height: 2479 mm
	Depth: 1344 mm
	Weight: app. 1550 kg
Microlab STAR	Width: 1670 mm
	Height: 903 mm
	Depth: 1005 mm

Consumables	Part Number / Provider
Nunc™ MicroWell™ 96-Well, Nunclon Delta-Treated, Flat-Bottom Microplate	167008 / Thermo Fisher Scientific
Cell Culture Multi-Well Plate, PS, 24-Well, BC, Sterile	10183400 / Hamilton Bonaduz AG
300 µL CO-RE® II Nested Sterile Disposable Tips	235985 / Hamilton Bonaduz AG
1000 µL CO-RE® II Filtered Sterile Disposable Tips	235940 / Hamilton Bonaduz AG
Reservoir, single cavity, polypropylene, 300 mL, 96 pyramids base geometry, 44 mm height	204504-100 / Agilent
Reservoir, 6 column, irradiated polypropylene, 47 mL/column, 282 mL maximum, 24 pyramid base geometries, 44 mm height	204284-100 / Agilent

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