bionomous Celvivo

Intelligent Automation for Advanced 3D Models

Application Note

Introduction

Three-dimensional (3D) cell culture models are transforming biomedical research by enabling in vitro physiologically relevant systems that better mimic native tissue structure and function. While constructs generation technologies have advanced significantly, downstream processes such as selection, characterization, and plating often remain time-consuming and variable, relying on manual labor, limiting throughput and experimental standardization.

The Celvivo ClinoStar® system enables the formation of highly reproducible, tissue-like 3D constructs under gentle, low-shear dynamic culture conditions, supporting long-term culture and consistent model generation.

The Bionomous EggSorter enables intelligent automation of downstream construct processing through high-speed imaging, morphometric analysis, and gentle sorting and plating of micro-objects.

Automated measurement of construct parameters such as diameter, roundness and surface area enables objective classification and selection. Processed construct can be directly distributed into ClinoReactors for further culturing or accurately deposited into multi-well plates and be used for downstream high-throughput assay applications while preserving constructs viability and morphology.

Together, ClinoStar® 3D construct generation and Bionomous automated sorting platform create a streamlined workflow from 3D culture to assay-ready samples, enabling standardized, scalable, and high-performance 3D cell culture workflows.

This application note demonstrates automated imaging, characterization, sorting, and plating of ClinoStar® system-derived constructs using the Bionomous EggSorter to enable next-generation 3D cell culture applications.

Application Opportunities

The combination of the ClinoStar® system and the Bionomous EggSorter enables advanced automated, size-controlled 3D culture workflows that are difficult to achieve manually.

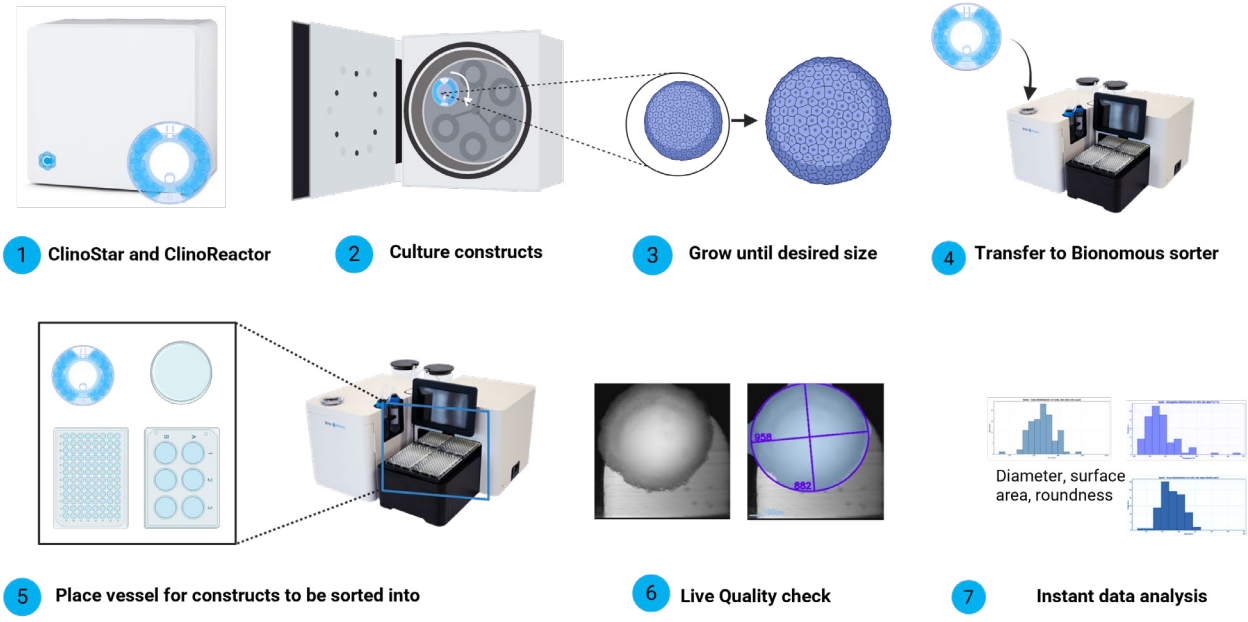
The combined systems workflow where Bionomous EggSorter enables precise selection of constructs within a defined size windows (e.g., 800–900 µm diameter “big” and/or 700-800 µm diameter “small”) and re-dispensing into a ClinoReactor vessel for continued dynamic culture.

This selective size classification and distribution approach allows researchers to:

- Standardize starting populations for longitudinal studies
- Reduce biological variability prior to treatment
- Synchronize construct maturation stages
- Investigation of size-dependent drug penetration
- Evaluation of hypoxia-driven effects in larger construct
- Comparative toxicity or metabolic profiling
- Control and reproduce foundation for drug discovery workflows
- Improved predictability of toxicity responses

Below is an example of how the ClinoStar system and the Bionomous EggSorter can be combined to first create a large number of uniform constructs followed by automated sorting and data analysis with user-defined output (e.g. vessels, petri dishes or 6-384 well plates for easy downstream high throughput drug screening).

Workflow



Key Results

- < 20 minutes to plate and analyze a full 96-well plate of 3D constructs
- > 80% reduction in hands-on time
- Automated morphology analysis and heterogeneity characterisation
- Direct plating into ClinoReactors, 96-well plates or petri dishes
- No loss of viability or change in morphology

Materials

- Cells (e.g. HepG2/C3A (CRL-3581, ATCC))
- Growth media
- Syringe (B.Braun, 4616103)
- Needle (B.Braun, 466512)
- ClinoReactor (Celvivo, 10004-12)
- Ethanol 70%
- ClinoStar® (Celvivo, 30003)
- EggSorter (Bionomous)
- Purair PCR Laminar Flow Cabinet (Air Science)
- MiliQ water
- PBS (e.g. Gibco, 10010023)
- 96-well plate (e,g. Merck, CLS3795)

Methods

The Bionomous EggSorter in configuration with compact laminar flow hood (e.g. Purair PCR Laminar Flow Cabinet (Air Science)) supports sterile operation. Sterility throughout the workflow is maintained using the EggSorter's built-in wash/sterilization program, which should be run before and after each sorting session.

Preparation

1. Hydrate and equilibrate the required number of ClinoReactor vessels according to Celvivo protocol 003[1] or Wrzesinski et al.[2]

2. Subculture cells according to the manufacturer's guidelines and standard laboratory procedures.

Construct setup and maintenance in the ClinoStar® system

1. Initiate construct culture by either single cell setup or microwell plate setup according to Celvivo protocol 008[1] or 009[1], respectively, or Wrzesinski et al.[2].

2. Perform media exchanges according to cell line and experimental design. For HepG2/C3A constructs cultured in the ClinoStar® system, a 48h/48h/72h media change interval is recommended.

3. Maintain constructs until the desired age and/or size is reached, depending on the experimental endpoint.

Sorting constructs from ClinoReactors using the Bionomous Eggsorter

1. Transfer constructs from the ClinoReactor into the EggSorter's input reservoir, under sterile conditions if desired.

2. Adjust/calibrate the imaging view to ensure constructs are in optimal focus in the slot view.

3. Start the sorting run using the selected program.

4. Upon completion, remove the collection plate/vessel/dish and proceed with downstream assays or continued culture.

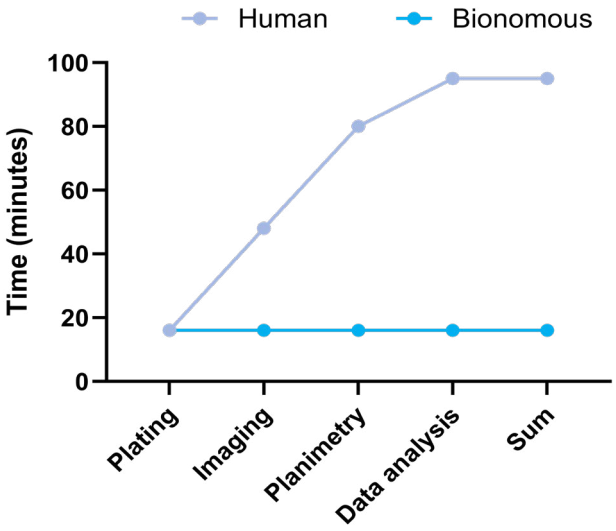
5. Export sorting metadata (e.g., image data, size distribution, event counts, and applied selection criteria) for documentation and downstream analysis.

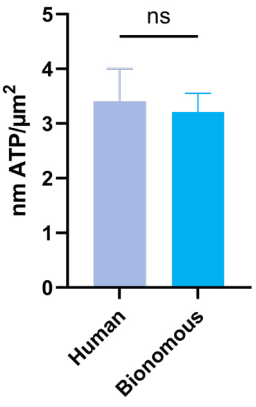
6. Run the EggSorter post-run cleaning cycle to prepare the system for the next sterile sorting session, or empty the instrument and perform surface cleaning using ethanol-based solution followed by a UV-light cycle

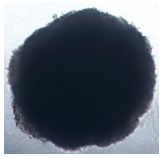
Results & discussion

Fast, Gentle, and Fully Automated Constructs Sorting

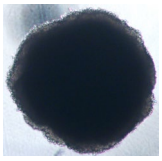
The integration of the ClinoStar® system with the Bionomous EggSorter enables automated individual selection, imaging, measurement, plating and data analysis of a full 96-well plate under 20 minutes with typical run time averaging just 16 minutes. The same workflow performed manually requires approximately 95 minutes and multiple handling and analysis steps. Automation therefore reduces hands-on time by more than 80% while improving standardization and reproducibility.







Before sort



After sort

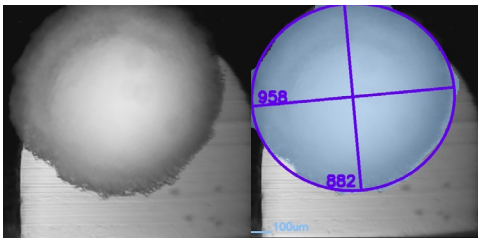
Despite the rapid processing, construct quality remains uncompromised. Brightfield imaging confirmed unchanged morphology after sorting. Intracellular ATP levels, normalized to construct surface area as a measure of viability, showed no significant difference between manually handled constructs and those processed using the Bionomous sorter.

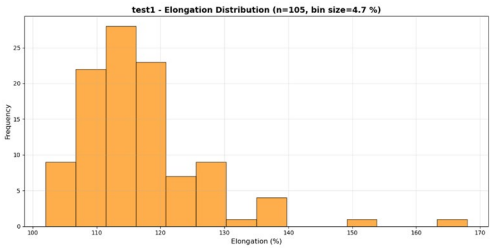
These results confirm that the platform delivers high-speed processing without mechanical stress or loss of viability.

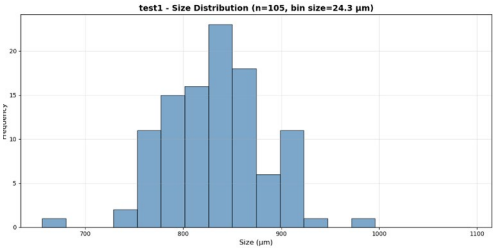
Immediate Quantification and data analysis

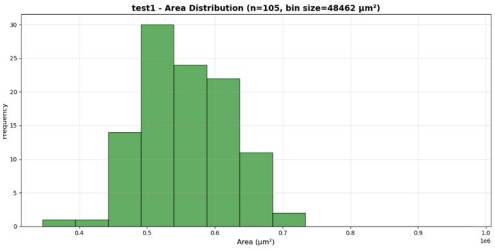
In addition to sorting, the Bionomous platform provides real-time measurement and analysis of:

- Diameter
- Surface area (planimetry)
- Roundness
- Population distribution
- Fluorescence expression (not applied in this application note)









This eliminates separate imaging and analysis steps and provides immediate insight into culture uniformity and experimental readiness. Using the ClinoStar® system, construct size variance as low as 6% was achieved, an exceptional level of uniformity for matrix-free dynamic 3D cultures. Combined with automated size-based analysis, this enables:

- Precise size-normalized drug dosing
- Reliable growth monitoring
- Sensitive detection of drug toxicity
- Predictable middle-throughput screening

Together, Celvivo and Bionomous create a streamlined, data-driven 3D culture workflow that accelerates timelines, reduces variability, and increases confidence in experimental outcomes.

Conclusion

The integration of the ClinoStar® system with the Bionomous EggSorter delivers a fast, gentle, and data-driven solution for 3D construct workflows. Automated sorting, plating, and real-time quantitative analysis reduce hands-on time by more than 80% in comparison with manual handling and data acquisition while preserving construct morphology and viability.

The combination of highly uniform matrix-free constructs production and immediate size-based quality analysis and selection enables improved reproducibility, precise dose normalization, and more predictive toxicity assessment.

Together, Celvivo and Bionomous provide a streamlined platform that enhances efficiency, standardization, and confidence in 3D cell culture experiments, supporting more reliable decision-making in drug development and toxicity studies.

Products Used

Bionomous Eggsorter
(Bionomous)
bionomous.ch/eggsorter/

ClinoStar®
(CelVivo)
celvivo.com/products/clinostar/

References

1. Celvivo online protocols library: https://celvivo.com/community/selected-protocols/

2. Wrzesinski, K., Frandsen, H.S., Calitz, C., Gouws, C., Korzeniowska, B., Fey, S.J. (2021). Clinostat 3D Cell Culture: Protocols for the Preparation and Functional Anaysis of Highly Reproducible, Large, Uniform Spheroids and Organoids. In: Brevini, T.A., Fazeli, A., Turksen, K. (eds) Next Generation Culture Platforms for Reliable In Vitro Models. Methods in Molecular Biology, vol 2273. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-1246-0_2

Celvivo

Celvivo ApS
Phone: +45 70 228 228
info@celvivo.com
celvivo.com

Værløse Office
Ny Vestergårdsvej 25
3500 Værløse
Denmark

Odense Office
Ørbækvej 264
5220 Odense
Denmark

Celvivo, Inc
5425 Wisconsin Ave. Suite 600
Chevy Chase, Maryland 20815, USA
Phone: +1 888-CELVIVO

bionomous

Bionomous SA
Villaz-St-Pierre, Switzerland
info@bionomous.ch
bionomous.ch