APPLICATION NOTE

# Precise Analysis of Cilia Beating Frequency and Drug Delivery: Investigating Mucociliary Clearance Mechanisms on Organ Chips Using High-Content Imaging

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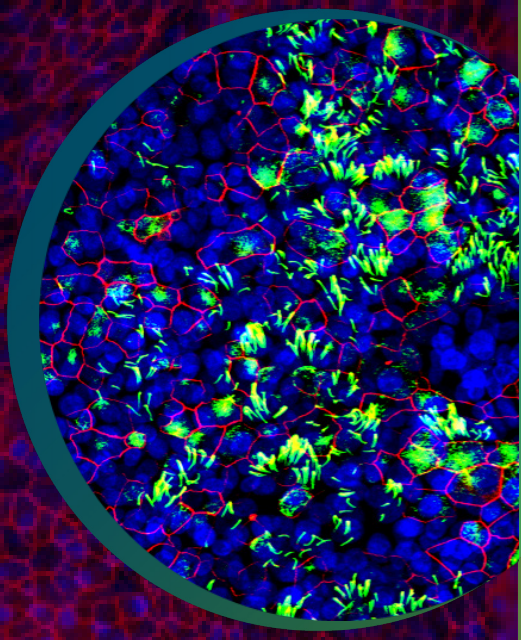

## Introduction

Organ-on-Chip (OoC) systems are emerging as powerful tools for modeling cell and tissue behavior. In pulmonary research, they replicate airway structures with functional cilia and mucus layers, supporting mucociliary clearance and airway defense. With dynamic microfluidics, OoC platforms precisely control the cellular environment. This technology allows the replication of complex physiological responses and increases the relevance of OoC models to diseases such as COPD and pulmonary fibrosis.

As organ-on-chip technology advances, researchers seek imaging solutions for real-time cellular analysis. Molecular Devices' High-Content Imaging System offers a compatible platform to address this demand. It combines high-resolution imaging with temporal analysis of ciliary motion, enabling precise functional assessment. Furthermore, the system maintains physiological culture conditions through accurate temperature and humidity control, ensuring mucociliary clearance stability and improving the reproducibility and reliability of experimental outcomes.

The integration of organ-on-chip platforms with high-content imaging systems establishes a more efficient framework for accurately modeling mucociliary dynamics. This approach further improves the precision of high-throughput image analysis and optimizes workflow for evaluating drug efficacy. The application of this innovative technology significantly enhances the reliability of pulmonary disease models. It also offers new perspectives for investigating respiratory disease mechanisms and advancing the development of inhalation therapies.

### Benefits

- Efficient integration of organ-on-chip technology with high-content imaging systems
- 60% faster imaging workflow with a 20-fold increase in data throughput
- Quantifying ciliary beat frequency reveals temperature effects and supports physiological evaluation models.

To assess clinical relevance, organ-on-chip models using airway cells from healthy and COPD donors were established. Ciliary beat frequency was measured under varying temperatures to explore physiological differences and temperature effects, supporting disease research and inhaled drug development.

Building on this, we established an integrated workflow combining organ-on-chip systems with high-content imaging for quantitative analysis of ciliary beat frequency. The workflow consists of: (1) dynamic microfluidic culture using organ-on-chip technology, (2) high-content imaging-based data acquisition, and (3) analysis of ciliary beat frequency using a proprietary algorithm developed by Anivance AI, with results visualized through data mapping. Our customized chip mount enables automated positioning and focusing, leading to more consistent image acquisition and significantly enhancing throughput and system reliability. This setup exemplifies the integrated application of organ chips and their advantages in high-throughput image analysis and physiological parameter control.

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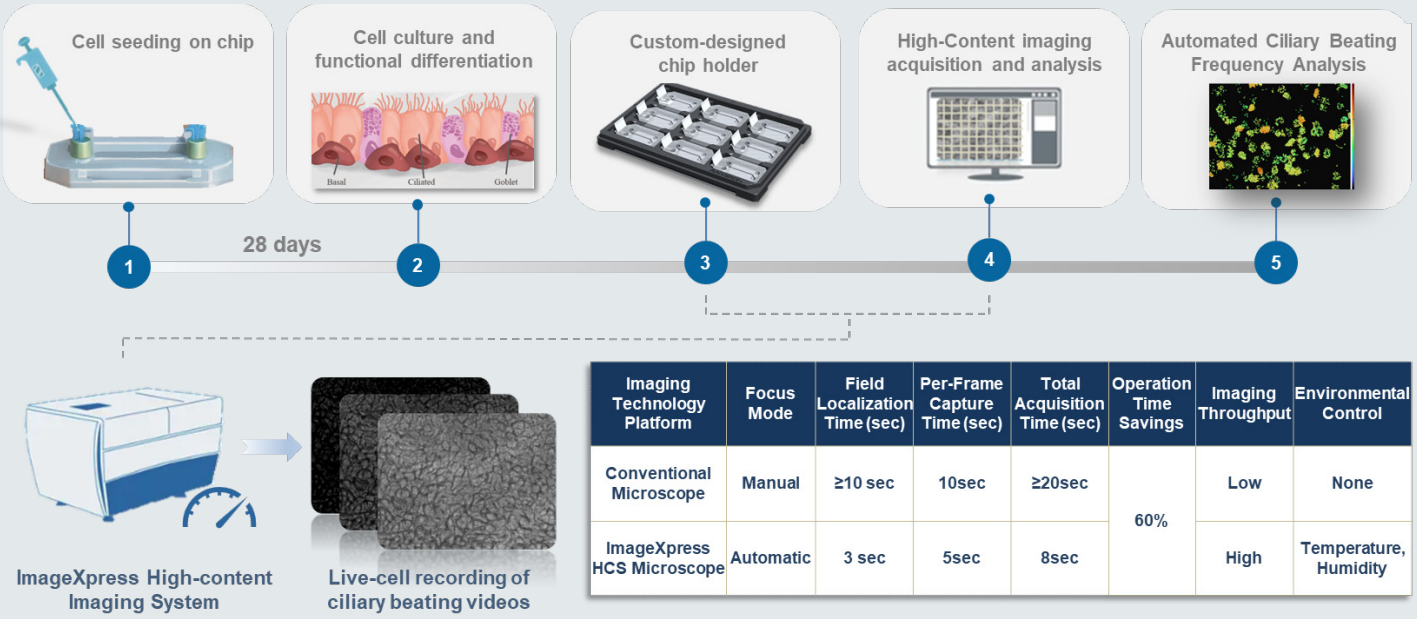


| Imaging Technology Platform | Focus Mode | Field Localization Time (sec) | Per-Frame Capture Time (sec) | Total Acquisition Time (sec) | Operation Time Savings | Imaging Throughput | Environmental Control |
|---|---|---|---|---|---|---|---|
| Conventional Microscope | Manual | ≥10 sec | 10sec | ≥20sec | 60% | Low | None |
| ImageXpress HCS Microscope | Automatic | 3 sec | 5sec | 8sec | | High | Temperature, Humidity |

**Figure 1.** Integration of organ-on-chip with the ImageXpress Micro 4 high-content imaging system enables the establishment of a functional small airway chip platform. This allows automated, high-throughput acquisition and analysis of ciliary motion, enabling precise measurement of ciliary beating frequency.

# Methods

## Establishment of a Small Airway Model on an Organ-on-Chip Platform

The OoC chip is pre-conditioned for immediate use. Diluted collagen solution (Cat. #A1048301, Gibco) is applied to both channels to support cell adhesion. Human primary small airway epithelial cells (HSAECs) from healthy and COPD donors are seeded into the upper channel and initially cultured with Pneumult™-Ex Plus Medium (Cat. #05040, STEMCELL). After 6 hours, the chip is connected to a perfusion system (2 µL/min) and switched to an air-liquid interface (ALI) culture. A functionally ciliated airway epithelium forms after 28 days.

## Cell Immunofluorescence Staining

After the small airway chip model was established, cells were fixed and permeabilized (Cat. #554722, BD Biosciences) at the endpoint. Target-specific antibodies and fluorescent dyes were then applied for immunofluorescence staining.

| Antibody Name | Catalog No. / Supplier |
|---|---|
| Acetylated Tubulin (Ac-tubulin) | Cat. #T7451, Sigma |
| Cytokeratin 5 | Cat. #ab53121, Abcam |
| Ulteroglobin | Cat. #ab40873, Abcam |
| MUC5B | Cat. #HPA008246, Sigma |
| Hoechst | Cat. #H1399, Thermo Fisher |

## High-Content Imaging-Based Acquisition

The ImageXpress Micro 4 High-Content Imaging System (HCS), developed by Molecular Devices, integrates with a custom OoC chip holder to simultaneously process up to 9 chips for high-throughput, automated ciliary motion imaging. The workflow is as follows:

1. Chips and the holder are loaded for automated positioning, and the temperature is maintained at 37°C.
2. A 20× objective captures ciliary motion at 100 fps with 10 ms exposure, recorded for 8–10 seconds per field.
3. Videos are acquired from 5 regions per chip to facilitate downstream analysis.

## Quantitative Imaging of Ciliary Beating Frequency and Mucociliary Clearance

Using a custom-developed image analysis software, both ciliary beating frequency (CBF) and mucociliary clearance (MCC) were quantitatively analyzed. For CBF analysis, dynamic recordings of ciliary motion were imported into the software. Background noise was excluded using automated masking, followed by precise segmentation of ciliated regions for quantitative analysis. Grayscale fluctuations were calculated to generate a CBF heatmap. For MCC, 2 µm fluorescent particles were aerosolized onto the epithelial surface, and their motion was tracked to evaluate clearance speed and direction.

 

# Results

## Characterization of small airway epithelium reconstructed on OoC

After 28 days of air–liquid interface (ALI) culture, the OoC chip successfully established a mature small airway epithelium comprising four major cell types: ciliated cells, goblet cells, club cells, and basal cells (Figure 2A). High-content imaging (HCS) revealed that in the healthy model, the ciliary protein AC-tubulin was expressed uniformly and densely, with clearly defined polarity. In contrast, the COPD model exhibited reduced and disorganized AC-tubulin expression. This pattern is consistent with pathological features commonly observed in clinical cases of ciliary damage and impaired motility (Figure 2B). Additionally, the COPD model exhibited sparse and disorganized ciliary distribution, indicating impaired structural integrity that may compromise mucociliary clearance efficiency.

## Ciliary beating frequency quantified by High-Content imaging

To quantify ciliary motion, a high-content imaging system was used to capture 5–8 second real-time videos of the small airway OoC model. The ciliary beating frequency (CBF) was then calculated using automated image analysis software. Notably, under physiological conditions (37˚C), the healthy model exhibited a significantly higher CBF (11.30 ± 0.63 Hz) compared to room temperature (25˚C, 4.83 ± 0.29 Hz Figure 3). This highlights temperature as a critical factor in maintaining ciliary function. In contrast, the COPD model showed a lower CBF (7.52 ± 0.28 Hz at 37˚C), indicating impaired coordinated motion under disease conditions ($p = 0.0002$). This platform offers advantages such as high throughput, physiological relevance, and standardized workflows. It effectively overcomes the limitations of manual measurement by improving data accuracy and reproducibility.

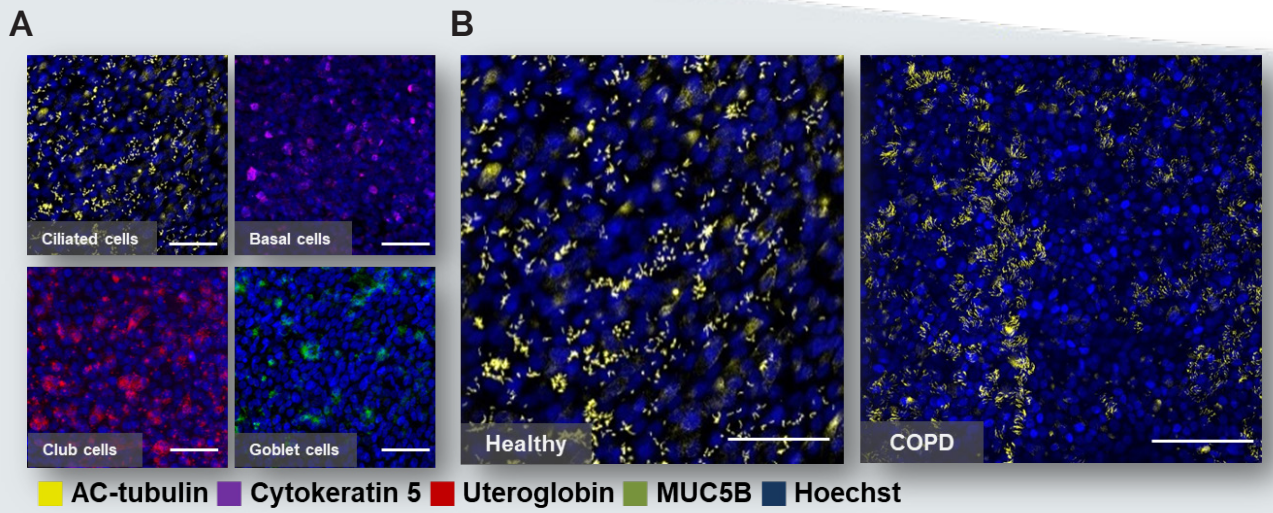


**Figure 2.** Immunofluorescence analysis of epithelial cell types and cilia structure in healthy and COPD airway chip models.(A) After 28 days of ALI culture, the epithelium differentiated into four major cell types: ciliated cells (AC-tubulin, yellow), basal cells (Cytokeratin 5, purple), club cells (Uteroglobin, red), and goblet cells (MUC5B, green), with nuclei stained by Hoechst (blue).(B) In the COPD model, AC-tubulin expression was reduced and cilia appeared sparse and disorganized, indicating ciliary damage compared to the dense, uniform structure in the healthy model.

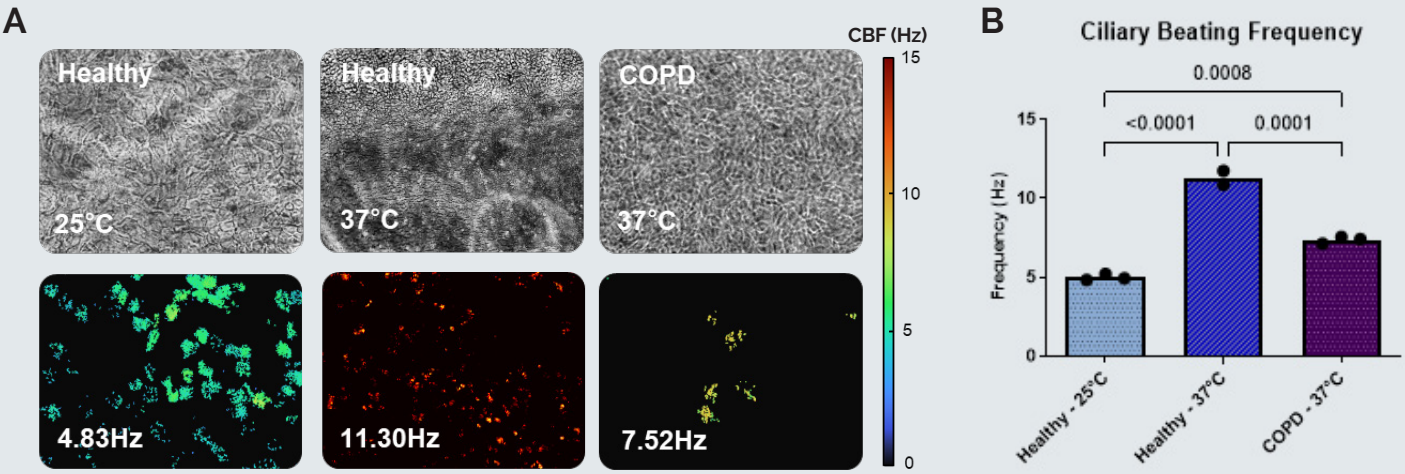


**Figure 3.** Ciliary beating frequency (CBF) analysis using the small airway chip combined with a high-content imaging system. (A) Representative ciliary motion recordings and heatmaps show CBF differences between healthy and COPD models under 25˚C and 37˚C. Color intensity reflects local CBF variations. (B) Quantitative analysis under three conditions indicates temperature-regulated ciliary function and significant differences between healthy and COPD models.

 

## Comparative analysis of ciliary beating function and clinical Data

The ciliary beating frequencies measured on the platform closely matched clinical data from human small airways. The healthy model showed a CBF of 11.30 ± 0.63 Hz at 37˚C, consistent with the clinical value of 11.15 ± 3.37 Hz. Similarly, the COPD model yielded 7.52 ± 0.28 Hz, aligning with the observed patient value of 7.89 ± 3.39 Hz (Table 1). These findings validate the platform's clinical relevance and reliability, while also confirming the critical role of temperature in modulating ciliary function.

**Table 1.** Comparison of CBF between clinical and OoC.

| Data Source | Sample Type | Environmental Temperature | Ciliary Beating Frequency (CBF) |
|---|---|---|---|
| Clinical Data | Healthy | Physiological Temperature (~37°C) | 11.15 ± 3.37 Hz |
| | COPD | | 7.89 ± 3.39 Hz |
| Organ-on-Chip | Healthy | 25°C | 4.83 ± 0.29 Hz |
| | | 37°C | 11.30 ± 0.63 Hz |
| | COPD | 37°C | 7.52 ± 0.28 Hz |

## Small Airway-on-Chip Model for Mucociliary Clearance Simulation

To assess the MCC functionality of the small airway-on-chip model, 2 µm fluorescent particles were applied to the apical surface of the chip. Subsequently, real-time imaging and automated trajectory analysis quantified particle velocity, directionality, and clearance efficiency. At physiological temperature (37 ˚C), the observed directional particle movement reflected effective MCC, and both the mean velocity and vector pattern were in close agreement with clinical human airway data. Furthermore, polar plot analysis confirmed the directional concentration of particle movement, validating the coordinated action of functional cilia. These findings demonstrate the platform's potential for studying respiratory diseases such as COPD, cystic fibrosis, and idiopathic pulmonary fibrosis, while also supporting the evaluation of inhaled drug delivery efficiency and therapeutic efficacy.

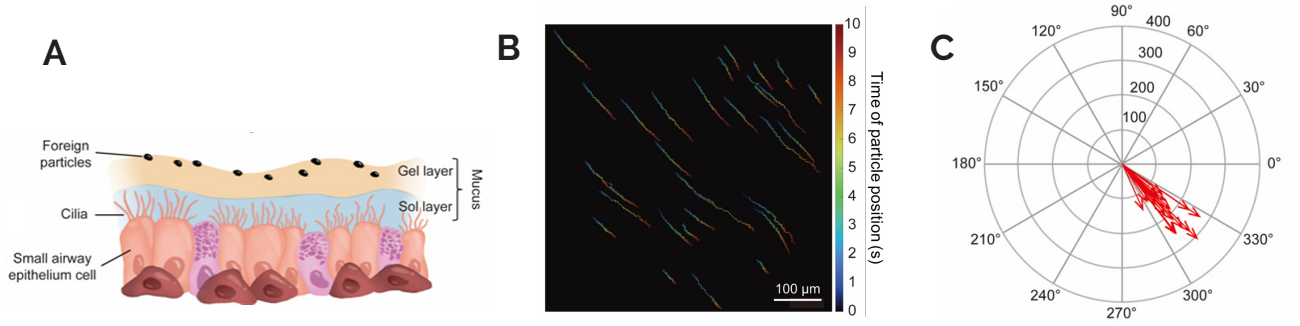


**Figure 4.** Analysis of mucociliary clearance (MCC) in the small airway chip model. (A) Diagram showing ciliary-driven mucus transport for particle removal. (B) Fluorescent particle tracking reveals directional trajectories over time (0–10 s). (C) Polar plot indicates concentrated movement, consistent with physiological ciliary beating.

# Conclusion

- Developed an integrated OoC with HCS workflow enabling real-time analysis of small airway ciliary motion under physiological conditions.
- Reduced data acquisition time by up to 60%, improved imaging throughput and fidelity, and supported parallel multi-sample analysis.
- Data indicate that ciliary function is highly sensitive to temperature, supporting the platform's feasibility for pulmonary disease research and drug evaluation.

## References


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2. Lin, K.-C., et al., Inhalable Mucociliary-On-Chip System Revealing Pulmonary Clearance Dynamics in Nanodrug Delivery. ACS nano, 2025.



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