# Application Note

## Holographic Functional Live-Cell Imaging of Airway Epithelium


### FEATURED SCIENTISTS

**Cecilia Andersson, Docent, Lund University**
"The HoloMonitor® NG live-cell imaging system provides high-resolution, quantitative insight into how mast cell mediators influence epithelial repair, greatly deepening our understanding of mechanisms relevant to asthma".

**Asger Sverrild, Clinical Research Associate Professor, Bispebjerg-Frederiksberg Hospital**
"HoloMonitor® NG Live cell imaging reveals how airway inflammation, virus-driven exacerbations and the airway epithelium's repair and barrier functions interact- insights essential to understand asthma and COPD ."

## ABSTRACT

Airway epithelial remodeling and dysfunction is a hallmark of asthma, yet the functional behavior of epithelial cells during biologic treatment is not well characterized. Quantitative phase imaging (QPI) enables continuous, label-free assessment of epithelial wound closure, morphology, and migration. This application note presents preliminary observations from an ongoing study investigating epithelial behavior before and after biologic therapy. These exploratory findings highlight the potential of holographic live-cell imaging assays as complement to traditional clinical biomarkers.

## BACKGROUND

Asthma is a heterogeneous airway disease characterized not only by inflammation but also by structural epithelial abnormalities, including impaired wound repair, altered morphology, and dysregulated barrier signaling. Upstream alarmins such as TSLP orchestrate inflammatory responses across phenotypes. Biologics targeting these pathways have demonstrated clinical benefit, yet their functional impact on epithelial repair remains unclear.

Previous collaborative work using HoloMonitor® has shown that mast-cell mediators, including chymase, modulate epithelial migration and wound closure across asthma phenotypes and independently of corticosteroid treatment. These observations underscore the importance of functional epithelial analyses alongside inflammatory markers such as FeNO, blood eosinophils, and bronchial biopsy IHC profiles.

The UPSTREAM clinical study demonstrated reductions in eosinophilic inflammation and shifts in mast-cell phenotypes following treatment with upstream-targeting biologics. Evaluation of epithelial live cell imaging may provide an additional dimension to understand these treatment effects.

## HOLOGRAPHIC MICROSCOPY

HoloMonitor employs quantitative phase imaging to reconstruct and visualize fundamental cellular behavior in live adherent cells without labels and without causing phototoxicity. This allows extraction of morphological and kinetic parameters such as wound gap width, cell coverage, optical thickness and single-cell migration trajectories. The newest generation of the HoloMonitor® system provides higher resolution, broader field of view, and improved segmentation algorithms. Evaluation of AI-assisted workflows is anticipated as part of future analytical development.

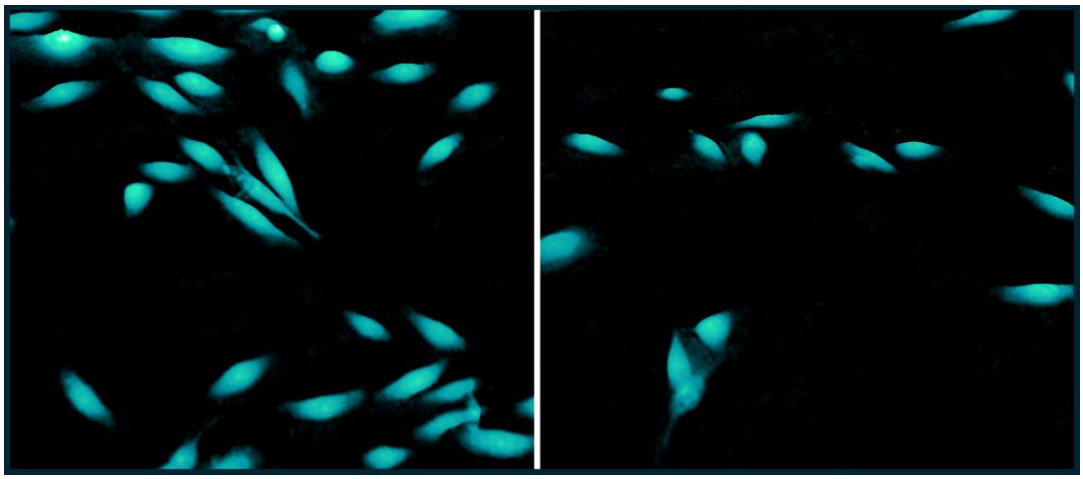

*Fig 1.* Label-free image from HoloMonitor NG revealing morphological heterogeneity in primary bronchial epithelial cell populations.

## METHODS

Primary bronchial epithelial cells (BECs) were collected via bronchoscopy from uncontrolled asthma patients enrolled in upstream biologic treatment study. Cells were cultured under standardized conditions and subjected to wound-healing assays compatible with quantitative phase imaging. Time-lapse recordings for 72 hours were performed using HoloMonitor to assess read outs such as wound closure dynamics, cell confluence, cell morphology and migration behavior.

As the study is ongoing and additional patient samples are pending, the observations presented here reflect early exploratory trends rather than quantitative outcomes. At this stage, the data remains blinded in terms of treatment/placebo and inflammatory phenotypes.

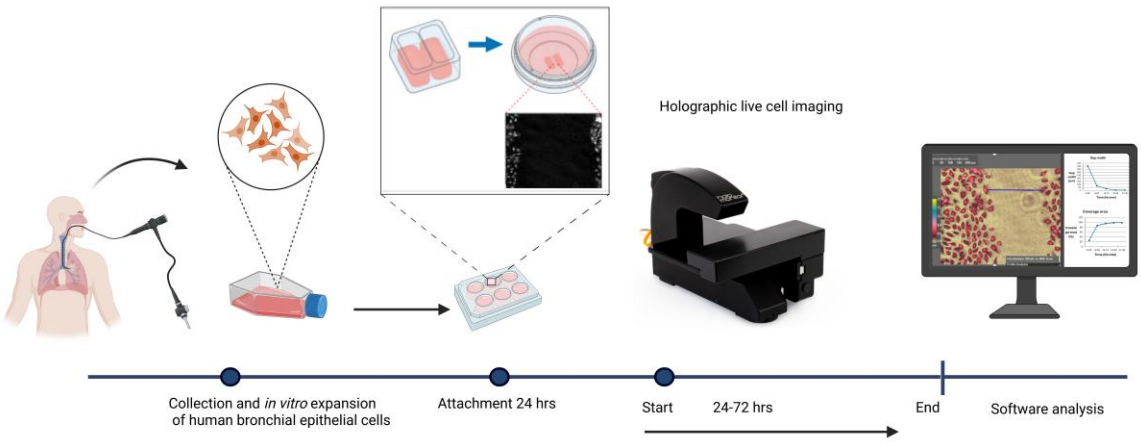


*Fig 2.* Workflow for holographic live-cell imaging of human bronchial epithelial cells, from collection and culture to real-time analysis of wound healing and cell morphology assays.

## RESULTS & DISCUSSION

Below, preliminary exploratory wound healing data are presented to illustrate how quantitative phase imaging can capture epithelial repair dynamics over time. These early trends are not yet conclusive but provide an initial qualitative impression of differences in gap closure between sample groups. As additional datasets are processed, these observations will be refined.

Preliminary observations suggest slight differences in epithelial wound closure between pre- and post-treatment in patient samples, especially at time point earlier than 6 hours. Post-treatment cultures appeared to close wounds more rapidly and develop confluence earlier. Although no conclusions can be drawn yet, these trends may indicate a shift in epithelial repair behavior following biological treatment.

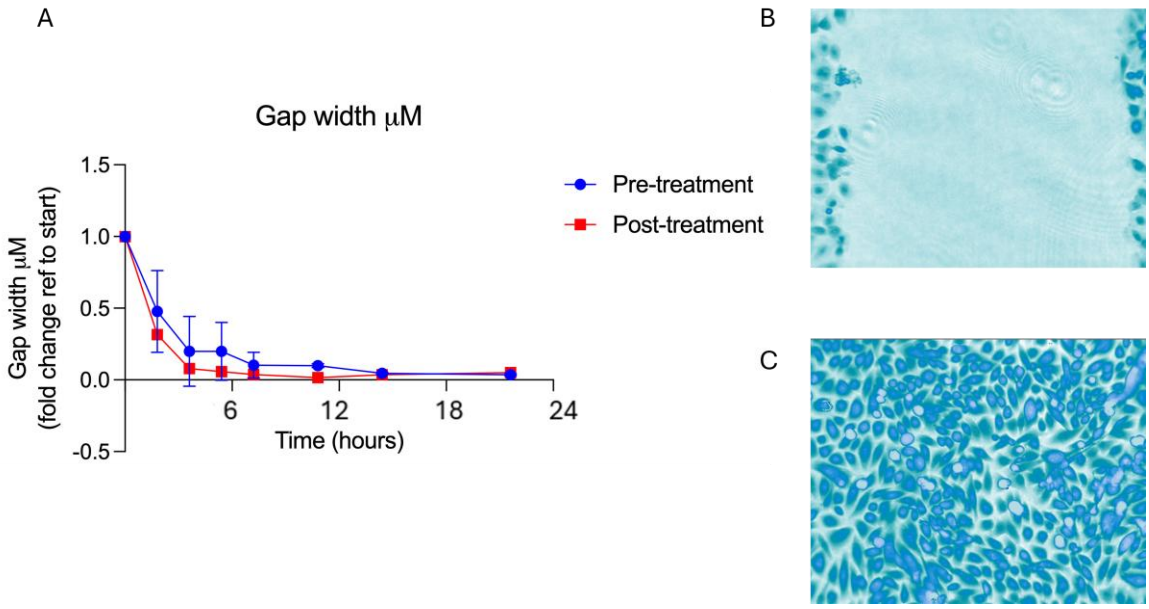


*Fig 3.* Wound healing dynamics over 24 hours illustrating gradual gap width reduction in respiratory epithelial cells from asthma patients pre and post treatment (A). Representative images demonstrating the *in vitro* wound at start (B) and end point (C). n=5

Cell identification is the first step in the analysis of morphological features for individual cells. The automatic segmentation of the phase images allows for extraction of single-cell features. Morphology parameters such as cell area and optical thickness were explored to evaluate potential differences between treatments. These label-free metrics provide an overview of population heterogeneity and allow rapid identification of shifts in cellular phenotype.

Morphological data showed subtle shifts in optical thickness between treatment groups, indicating possible changes in epithelial organization.

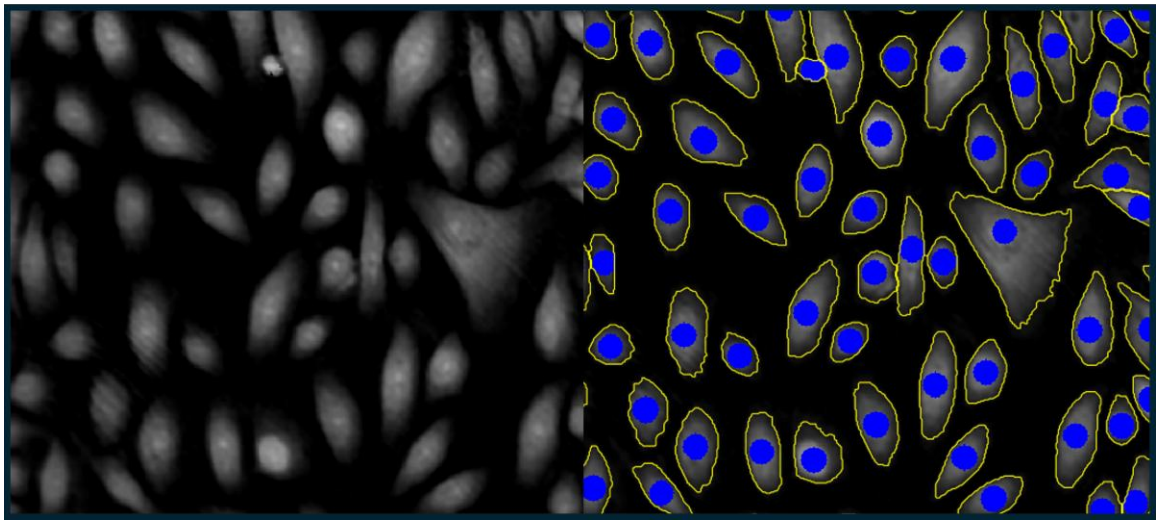

*Fig 4.* Example of label free automatic cell segmentation. Raw phase images (A) and corresponding segmentation outlines (B) illustrate identification of individual epithelial cells for downstream morphological or motility-related analysis.

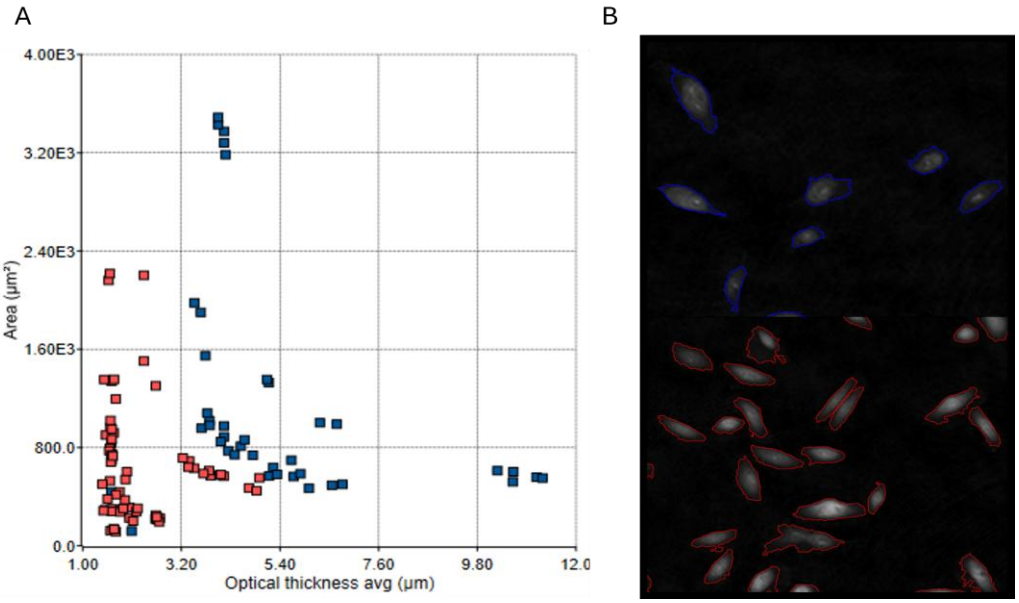


*Fig 4.* Example of label free morphology analysis. Scatter plot (A) illustrates single cell optical thickness and area for comparison between pre (blue) and post (red) treatment. Representative segmented cells showing how cell outlines are identified for downstream morphological quantification (B).

These early exploratory findings demonstrate the sensitivity of holographic live-cell imaging for detecting functional epithelial changes that may complement classical inflammatory biomarkers such as FeNO or blood eosinophils. Ongoing analysis of additional patient samples will help clarify phenotype-specific patterns.

At the image analysis level, HoloMonitor NG provided robust, automated segmentation of heterogenous primary epithelial cells despite variation in cell size, thickness and morphology. The large field of view and high image clarity enable reliable single cell detection across complex cellular cultures. Designed for AI integration, the system is well suited for future advanced studies within the field of translational research.

## CONCLUSION

HoloMonitor NG enables long-time, non-invasive, label-free quantification of primary epithelial wound closure, morphology, and migration. In this ongoing study, functional epithelial readouts show promise as exploratory indicators of treatment response to upstream-targeting biologics. Continued data collection and analysis will determine the robustness and clinical relevance of these observations.

## REFERENCES


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- PHI HoloMonitor® technical/public documentation.