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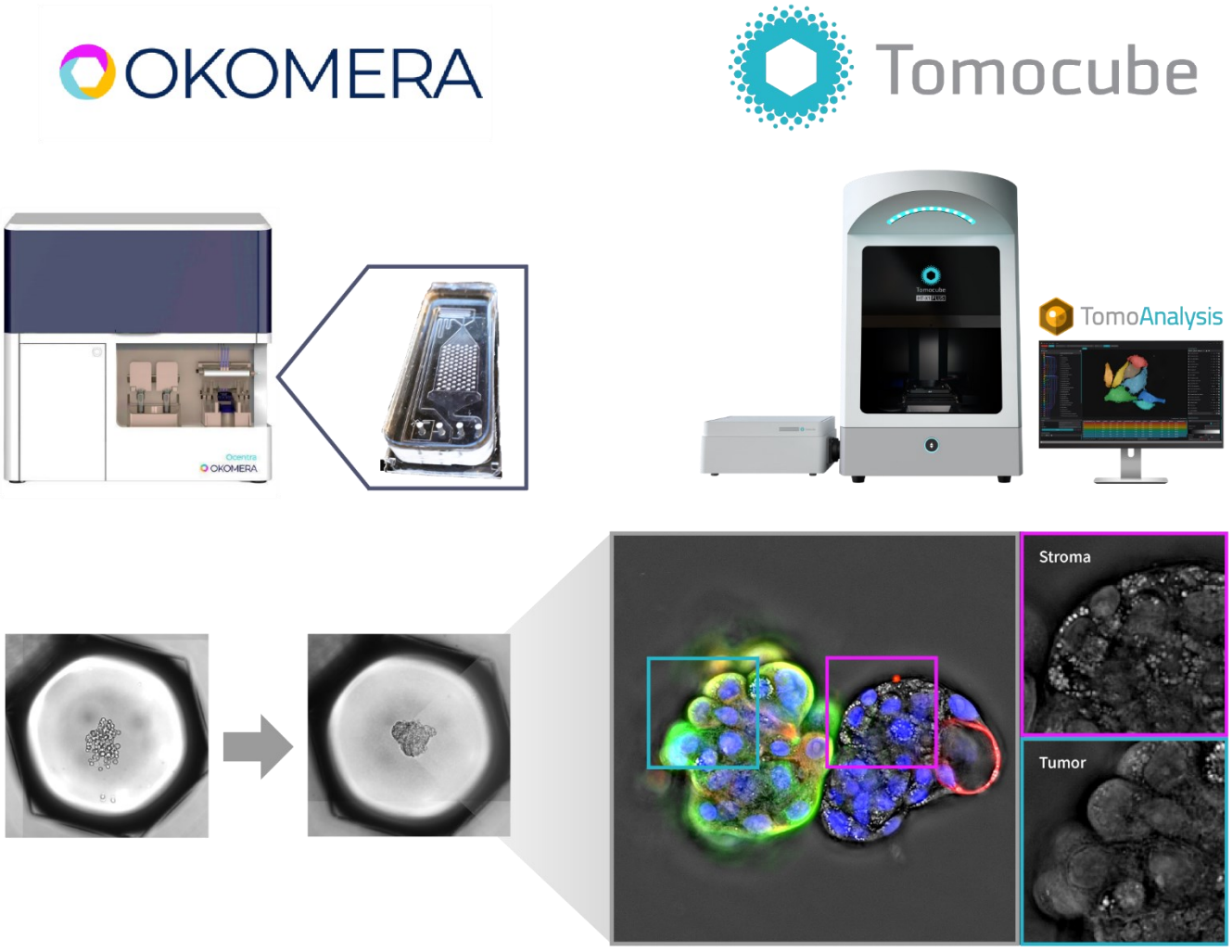

Case Study: Holotomography reveals insights into chemotherapy response of heterogeneous breast cancer patient derived tumoroids

Patient derived tumoroid drug screening by combining Okomera's microfluidic platform and Tomocube holotomography

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Introduction and Background

3D culture of patient biopsies is increasingly recognized as more physiologically relevant models for translational oncology than traditional 2D cell culture. The preservation of tumor heterogeneity and microenvironmental features makes it a valuable tool for functional precision medicine and drug discovery. Their ability to mimic *in vivo* treatment response has raised expectations for more predictive preclinical testing [1], [2], [3]. Yet, conventional workflows face **practical and analytical limitations**: they often require large input material, require several weeks for organoid development, and depend mainly on endpoint viability assays and immunostaining, which cannot fully capture intermediate changes or microenvironmental influences during drug exposure.

Okomera is a biotech startup developing a next-generation organoid screening platform powered by **microfluidics and artificial intelligence**. Its automated benchtop instrument, **Ocentra**, generates uniform droplets containing patient-derived cells, forming single organoids from as few as 30 cells. These droplets are loaded into proprietary **microfluidic chips** within minutes, supporting parallel culture and drug testing. The workflow can be expanded using the **O-Plex module**, which creates barcoded drug libraries and enables multiplexed screening of up to ten conditions per chip. Finally, the **Okomera AI** analysis suite provides automated viability scoring and phenotypic insights across thousands of organoids in a single cycle. This streamlined platform thus facilitates efficient, higher-throughput organoid assays starting directly from fresh biopsy material or established PDO cultures.

Complementing this system, Tomocube's **holotomography (HT)** provides a unique imaging solution that reconstructs three-dimensional refractive index tomograms of living organoids **without labels or dyes**. The **HT-X1 Plus** system employs LED illumination for minimal phototoxicity and supports long-term imaging of large, optically thick specimens in dishes, multiwell plates, or microfluidic chips. Refractive index data can be directly translated into **quantitative biophysical parameters** such as dry mass, protein concentration, and structural compactness. Together with the **TomoAnalysis** software suite, HT offers tailored pipelines and AI-assisted segmentation, enabling reproducible quantification of cells, nuclei, and subcellular organelles. **These features were particularly applied to distinguish tumor and stromal compartments** in biopsy-derived PDOs **without the need for staining.**

In the present study, the combined use of the Okomera platform and Tomocube HT-X1 Plus was applied to fresh

breast cancer patient biopsy-derived organoids treated with carboplatin. This integrated workflow demonstrates how stromal components, preserved from the original patient samples, can alter apparent drug sensitivity. By visualizing these effects in real time, HT adds an analytical dimension that conventional endpoint-only assays cannot provide, supporting more comprehensive and clinically relevant drug screening.

Figure 1 below shows the integrated workflow combining Okomera's droplet-based microfluidic organoid culture with Tomocube's HT and TomoAnalysis. Patient-derived samples are dissociated and loaded into microfluidic chips, where robust organoid formation occurs within a few days. HT provides label-free, high-resolution 3D imaging of organoid morphology and dynamics, while TomoAnalysis enables multiparametric quantitative analysis. This combined platform allows reproducible and quantitative monitoring of drug responses in patient-derived organoid models.

Highlights:

- **High-throughput multiplexed screening:** Robust statistics from hundreds of organoids, from a single patient sample
- **Biopsy-ready:** Miniaturization suitable for the management of small patient tumor samples
- **Fast turnaround:** Chip loading in minutes, compact organoids formed within 48 hours
- **AI analytics:** Automated growth, viability, and dose–response scoring
- **High resolution, label-free 3D imaging**: Non-invasive imaging of organoids over days, Detailed structural and phenotypic characterization
- **TomoAnalysis pipelines:** Reproducible organoid and organelle quantification in PDOs measuring biophysical features—dry mass, protein concentration of organoids
- **Microenvironmental insight:** Reveals stromal influences hidden in endpoint assays

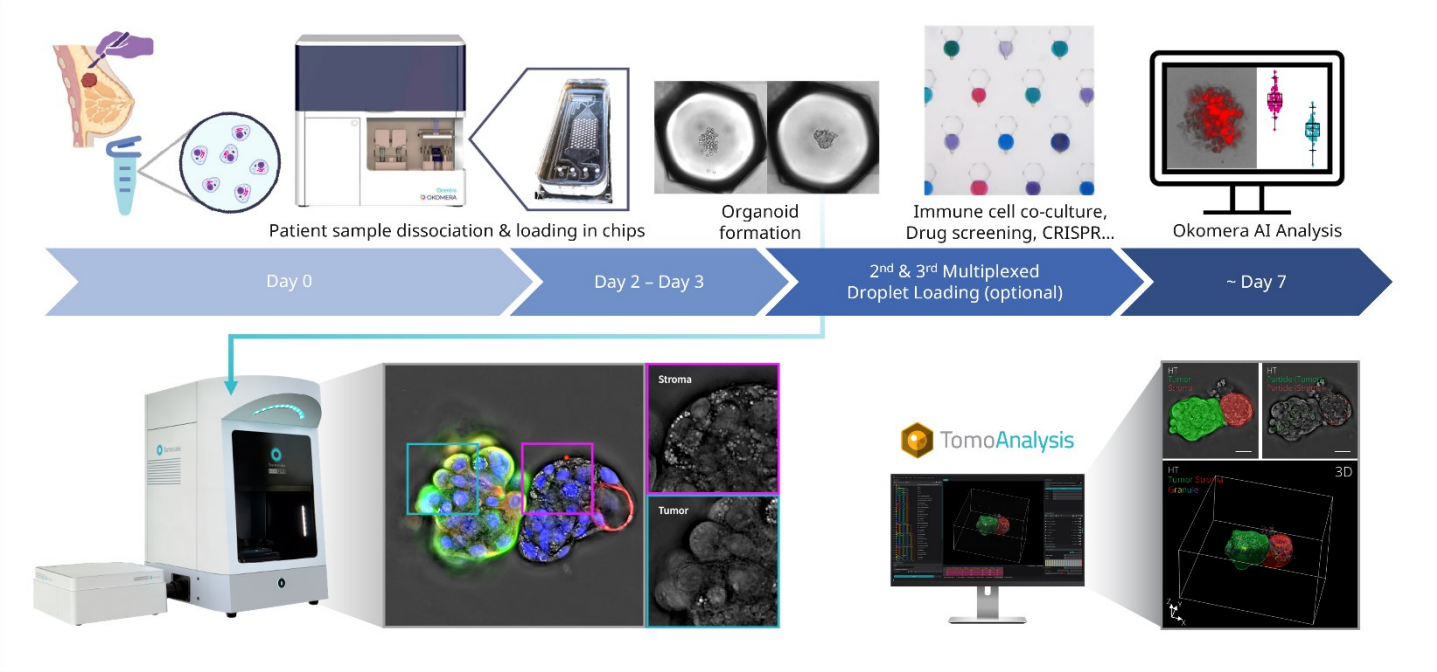

Figure 1: Okomera's droplet-based organoid culture combined with Tomocube HT-X1 Plus holotomography and TomoAnalysis enables label-free, quantitative monitoring of drug responses in patient-derived organoid models.

Materials and Methods

Okomera Tech Platform

- Microfluidic system
- Okomera O-Plex module
- Okomera microfluidic chips and loading oils
- OkoRed and OkoGreen fluorescent dye
- Okomera AI web platform

Tomocube Tech Platform

- Tomocube HT-X1 Plus holotomography system
- FLX 100: widefield fluorescence module with sCMOS
- TomoAnalysis 2.0: AI-powered image analysis platform

Biological Samples and Reagents

- Surgical resection of Luminal B breast tumor (203 mg)
- MACS® Tissue Storage Solution (Miltenyi Biotec 130-100-008)
- Tumor Dissociation Kit, Human (Miltenyi Biotec 130-095-929)
- Red Blood Cell Lysis Buffer (Roche, 11814389001)
- EasySep™ Release Human CD45 Positive Selection Kit (StemCell™ Technologies #100-0105)
- Collagen I (Corning® 354249)
- Breast cancer organoid culture medium
- Propidium Iodide (Invitrogen™ P3566)
- Fixation solution (4% PFA in DPBS)
- Permeabilization solution (0.02% Triton X100 in DPBS)
- Purified Keratin 5 pAb, clone Poly19055 (BioLegend® 905501)
- Purified anti-Cytokeratin 8, clone 1E8 (BioLegend® 904804)
- Goat anti-mouse IgG Alexa Fluor™ Plus 488 (Invitrogen™ A32723TR)
- Goat anti-rabbit IgG Alexa Fluor™ Plus 555 (Invitrogen™ A32732)
- Goat serum (Sigma-Aldrich G9023)
- Triton™ X-100 (Sigma-Aldrich X100)
- Hoechst 33258 (Sigma-Aldrich H6024)

Surgical resection, dissociation and sample preparation

After surgery, a sample of tumor tissue was selected by a pathologist and placed in MACS® Tissue Storage Solution. Tumor samples were then dissociated into a single cell suspension following the protocol established by Corgnac et al.. CD45+ cells were removed from the suspension by positive selection with magnetic beads. The resulting fraction containing cancer and stromal cells was collected and prepared for culture in Breast Tumor organoid culture medium and adjusted to a final cell concentration of 0.6 × 10^6 cells/mL.

Chip loading

Okomera reagent, Propidium Iodide (3 µM), and Collagen I (6 µg/mL) were added to the prepared cell suspension on ice. The solution was loaded into chips and incubated at 37°C with 5% CO_2. 3D Holotomography, 2D brightfield, and Cy5 fluorescence images were acquired on the Tomocube HT-X1 Plus holotomography system with FLX100 module from day 2 to 5.

Drug screening

Once compact organoids were formed on Day 2 after cell loading, a drug library emulsion (0 to 1 mM) was prepared and multiplexed using the OkoRed barcode at defined concentrations. The vehicle of the maximum concentration was associated with a single OkoGreen concentration. Each barcode corresponds to a specific drug dose and is detectable in the Cy5 channel of a fluorescent microscope. The drug libraries were produced using the O-Plex instrument and loaded via the microfluidic system into the chips. Viability was assessed 72 hours later, on Day 5, to evaluate the drug response.

HT Time-lapse Imaging with HT-X1 Plus

After drug library injection and barcode acquisition, microfluidic chips were imaged for 72 hours using the Tomocube HT-X1 Plus Holotomography System. A preview scan function was used to map the Okomera chip, and each well was designated for imaging. Each field of view (FOV) covered 300 µm × 300 µm × 140 µm, sufficient to capture individual organoids without stitching, with axial stacks acquired in less than 10 seconds. Environmental conditions were maintained at 37°C, 5% CO_2, and controlled humidity throughout the experiment. HT images were collected every 2 hours, and Cy3 fluorescence images every 12 hours to detect PI-stained dead cells. At the end of the 72-hour period, fixed and stained organoids were re-imaged with HT and fluorescence (DAPI/GFP/Cy3).

Immunocytochemistry (CK5/CK8/Hoechst)

After 5 days of culture, organoids were fixed and permeabilized in the chip by perfusing it first with fixation followed by permeabilization solution (respectively, PFA 4% in DPBS and Triton 0.02% in DPBS), and incubated for 15 minutes each. Following a wash with DPBS 1X, the organoids were incubated with the solution containing primary antibodies anti-CK5 (BioLegend) and anti-CK8 (BioLegend) in DPBS containing 10% goat serum for 1 hour at room temperature. The microfluidic chambers were then washed with a blocking solution (Tween 0.05% in DPBS 1X), perfused with the solution containing the fluorescent secondary antibodies (goat anti-mouse Alexa Fluor 488, and goat anti-rabbit Alexa Fluor 555 in DPBS with 10% goat serum), and incubated for 1 hour at room temperature protected from light. Finally, nuclei were stained by perfusing Hoechst diluted in DPBS and incubating for 15 minutes at room temperature and protected from light.

Automated Image Analysis with Okomera AI

The viability score was calculated using the proprietary Okomera AI software. Quantitative results were exported, and statistical analyses were performed using GraphPad Prism 10.3.0 for Windows (GraphPad Software, Boston, Massachusetts USA).

HT image analysis with TomoAnalysis

HT datasets were processed using TomoAnalysis (TA) software in combination with the microSAM module. For endpoint imaging, datasets included HT (RI) and fluorescence channels (DAPI/CK5/CK8). Analysis was performed by customizing three pre-designed pipelines provided in TA: (3D) Cystic Organoid Volumetric Analysis, (3D) Lipid Droplet, and (3D) FL 1Ch Segmentation & HT Quantification. Based on these pipelines, RI data were used for granule segmentation, CK5/CK8 signals were used to segment tumor and stromal regions, and DAPI was used for cell counting. Granule measurements were normalized to cell density derived from the DAPI counts.

For time-lapse datasets (HT only), a customized version of the (3D) Lipid Droplet pipeline was applied for Granule segmentation, while microSAM was employed for organoid segmentation. Organoid volume was used to normalize Granularity over time.

All quantitative results were exported, and statistical analyses were performed in Python.

Results and Discussion

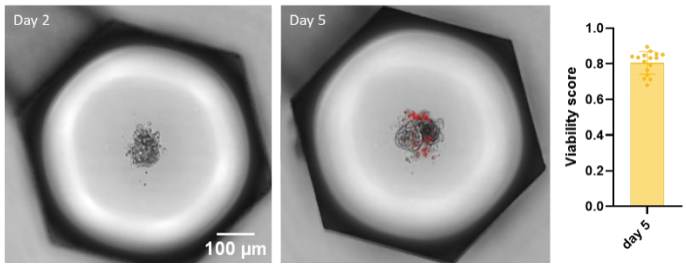

Figure 2: Viability and structural integrity of breast cancer organoids maintained after 5 days of culture in microfluidic droplets.

Generation of heterotypic PDOs in chip and characterization

Heterogeneous organoids, containing tumor and stromal cells, were generated from a dissociated and CD45+ depleted cell suspension. A total of 3 chips were loaded, resulting in 234 organoids produced from a Luminal B breast tumor sample weighing 203 mg. Compact 3D structures were formed after 2 days of culture **(Figure 2).** By using the Okomera AI analysis pipeline, we were able to automatically assess organoid viability. After 5 days of culture in microfluidic droplets, organoid viability was high, and structural integrity was maintained **(Figure 2).**

Assessing drug sensitivity in microfluidic chip

Based on brightfield images and Okomera AI viability score, we were able to generate a drug-response curve after 72 hours of treatment with carboplatin. Multiple metrics were extracted from the analysis, such as IC50 and AUC, Hill coefficient, and maximum cytotoxic efficacy **(Figure 3).** This multiparametric analysis enables a full capture of the sensitivity profile of the organoids: we observed a complete response to carboplatin by reaching a maximum cytotoxic efficacy of 99.18%, associated with a Hill coefficient of -2.712, indicating a rapid drop in viability score when reaching the IC50 value of 134.1 µM, and an AUC value of 113.7. Overall, we can determine that organoids from this patient displayed a sensitive profile to carboplatin based on the results of the multiparametric analysis.

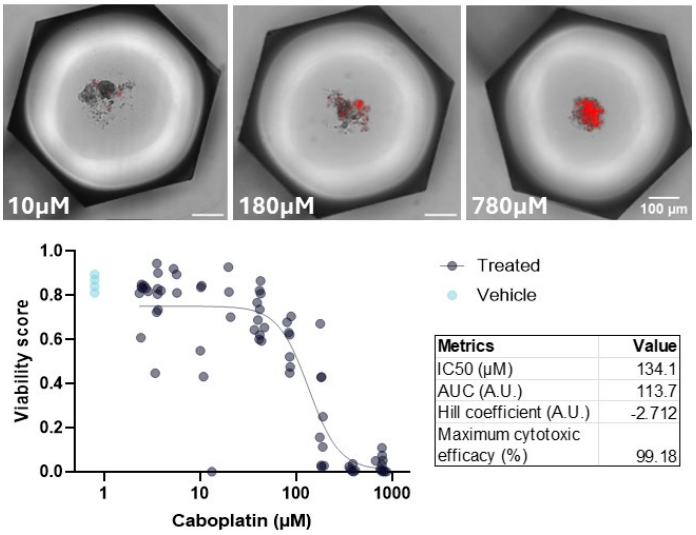

Figure 3: Response profile of the breast cancer organoids to carboplatin on chip. Biopsy-derived organoids were treated with an increasing concentration gradient of Carboplatin for 72 hours in chip. Viability score was calculated using Okomera analysis pipeline based on structural integrity and PI signal.

Characterization of heterotypic PDOs by correlative imaging with HT and immunofluorescence

To characterize the generated organoids, on-chip immunostaining was carried out, followed by correlative imaging with holotomography. Fluorescent images revealed the presence of a heterogeneous tumor cell population (CK8+, CK5+, and CK8+CK5+). In addition, compact structures negative for tumor epithelial markers were observed **(Figure 4, upper panels),** reflecting the heterotypic nature of the structures. Interestingly, in some organoids, a polarized architecture was visible, with a tumoral core characterized by a high density of cells with large, round nuclei, and a non-tumoral core with more

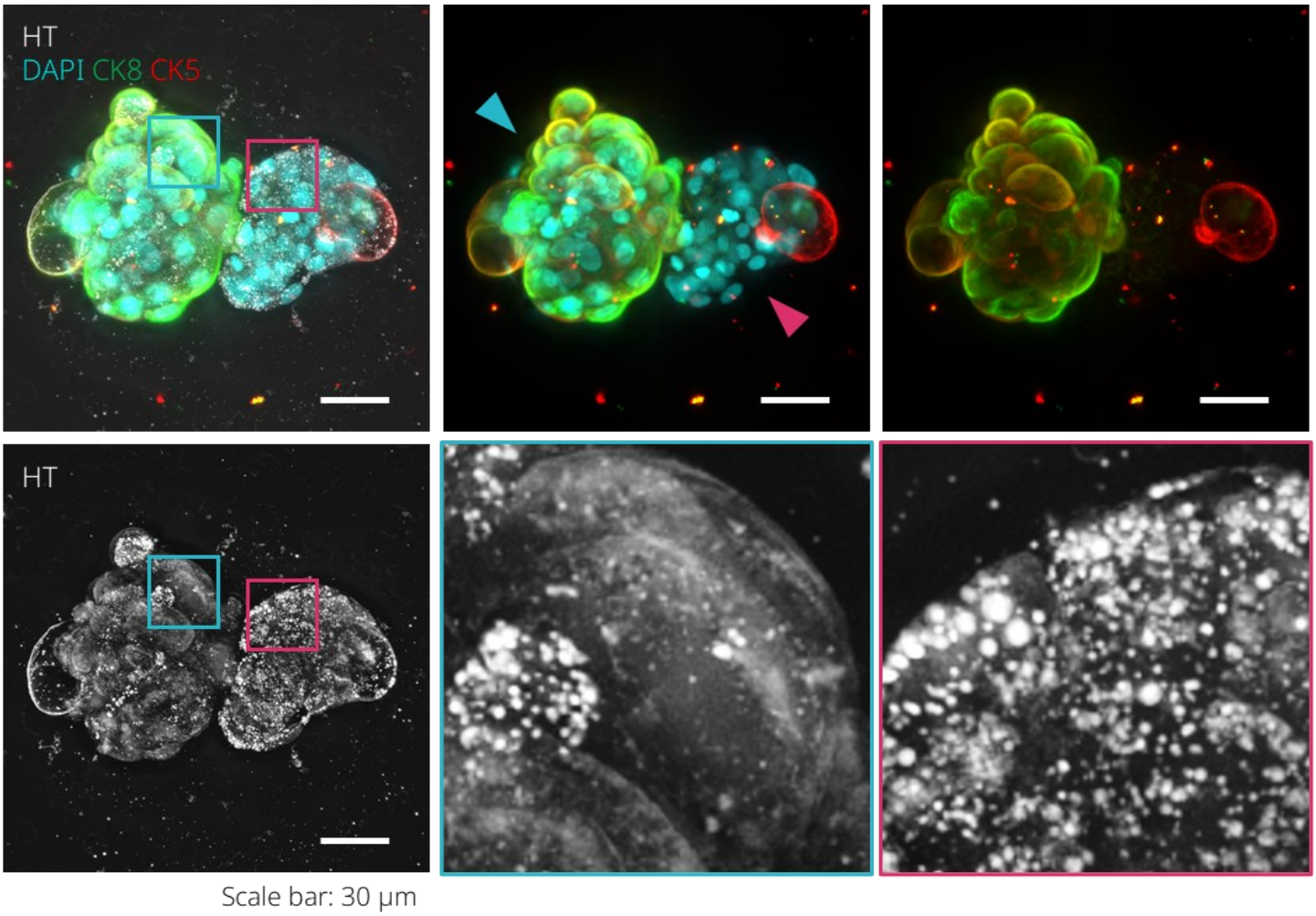

Figure 4: Correlative imaging reveals tumor–stroma heterogeneity in biopsy-derived organoids. Biopsy derived organoids were stained on-chip for cytokeratin markers (CK5: basal, CK8: luminal), after 5 days of culture in chip. Immunostaining revealed heterogeneous tumor cell populations (Cyan box, arrowhead) and stromal-like regions (Magenta box, arrowhead) lacking epithelial markers (upper panels). HT further distinguished compartments, showing higher granularity in stromal-enriched regions (CK5/CK8–) compared with tumor-enriched regions (CK5/CK8+) (lower panels). All images are Maximum Intensity Projections (MIPs) from entire Z-stacks.

elongated nuclei, typically associated with fibroblasts. Complementing these observations, holotomography revealed clear morphological differences between tumor cell–enriched and stromal cell–enriched regions. Granularity, represented by high-RI granules, was consistently lower in tumor cell–enriched regions compared with stromal cell–enriched regions **(Figure 4, lower panels).** These differences could be distinguished directly from HT datasets without additional labeling, highlighting how holotomography provides a label-free biophysical perspective on organoid.

Granularity as a potential marker of stromal regions

These observations were further validated through customized analysis pipelines in TomoAnalysis. By segmenting granules from RI tomograms and classifying regions using CK5/CK8 immunofluorescence, tumor regions were found to consistently exhibit significantly lower granularity than stromal regions **(Figure 5).** Although the exact identity of these granules has not been investigated, this greater granularity in stromal regions may point towards the widely reported secretory activity of Cancer Associated Fibroblasts (CAFs) and their ability to release a diverse array of cytokines, growth factors, enzymes, and extracellular vesicles to shape tumor

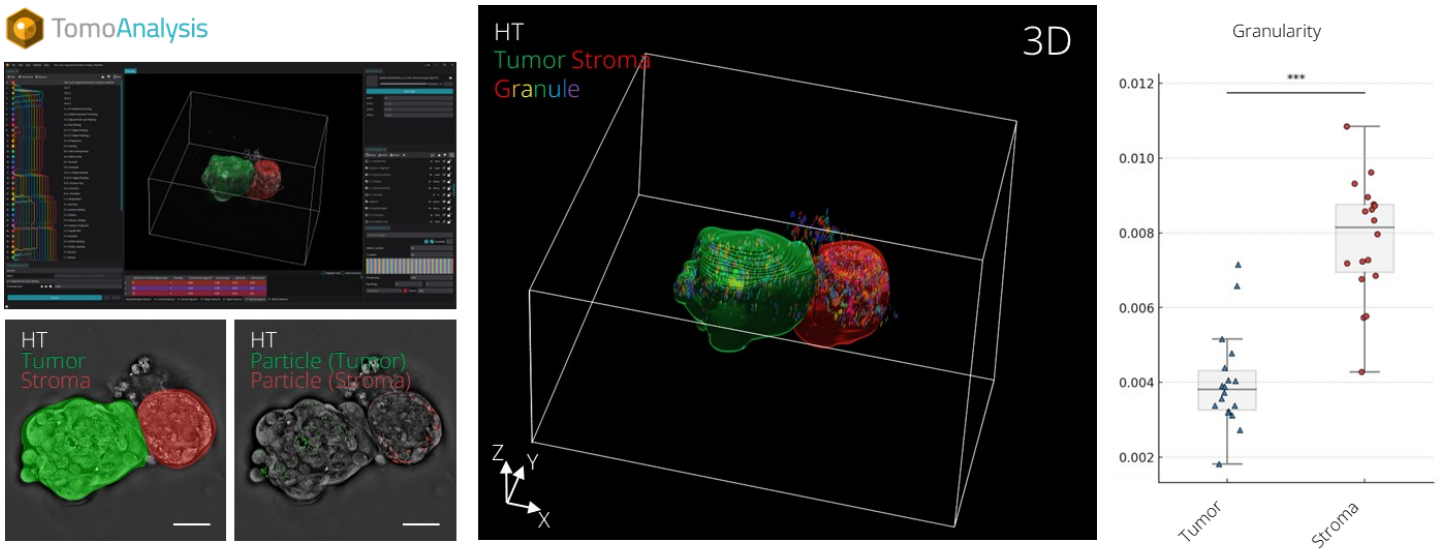

Figure 5: Stromal regions display a greater granularity than the tumoral regions in PDOs. Tumor region: CK5+ or CK8+; Stroma region: CK5-/CK8-. Granularity = Granule count/region volume.

behavior. This establishes granularity as a potential quantitative marker for identifying stromal compartments within biopsy-derived organoids.

Stromal modulation of drug response sensitivity

Considering the critical role of CAFs in shaping therapeutic resistance, we investigated whether the composition of the organoids could impact the drug response. The relationship between stromal composition and drug response was evaluated using time-lapse HT acquired during carboplatin treatment. Granularity at the initial time point was used as a surrogate to estimate the tumor-to-stroma ratio of each organoid. Organoids were stratified into low– and high–granularity groups, and changes in organoid volume from baseline to endpoint were quantified relative to drug concentration determined via OkoRed/OkoGreen barcoding. Tumor cell-rich organoids showed greater sensitivity to carboplatin, with larger reductions in volume compared with stroma-enriched organoids **(Figure 6).** This trend corresponds with the expected pharmacological action of the drug to have a strong effect on highly proliferative tumour cells and illustrates the influence of stromal content on apparent drug response.

Conclusion

This case study demonstrates the powerful combination of the Okomera droplet-based microfluidic platform and HT imaging to decipher drug response in biopsy-derived organoids.

The miniaturization aspect of the Okomera platform enables the generation of hundreds of replicates from a small amount of biological material while retaining the ability to screen a wide range of different conditions in a clinically relevant time frame. The 3D drug screen enables the functional assessment of the drug response profile of a patient's tumor sample, providing useful insights for personalized medicine and clinical applications.

HT imaging adds a complementary dimension by enabling high-resolution, label-free, and quantitative analysis of living organoids throughout the screening process. Refractive index–based measurements provide direct access to biophysical parameters such as granularity, volume, and structural compactness, allowing tumor and stromal compartments to be distinguished without additional staining. HT Time-lapse imaging further captures how stromal composition influences drug sensitivity in real time, offering mechanistic insight beyond the reach of endpoint assays.

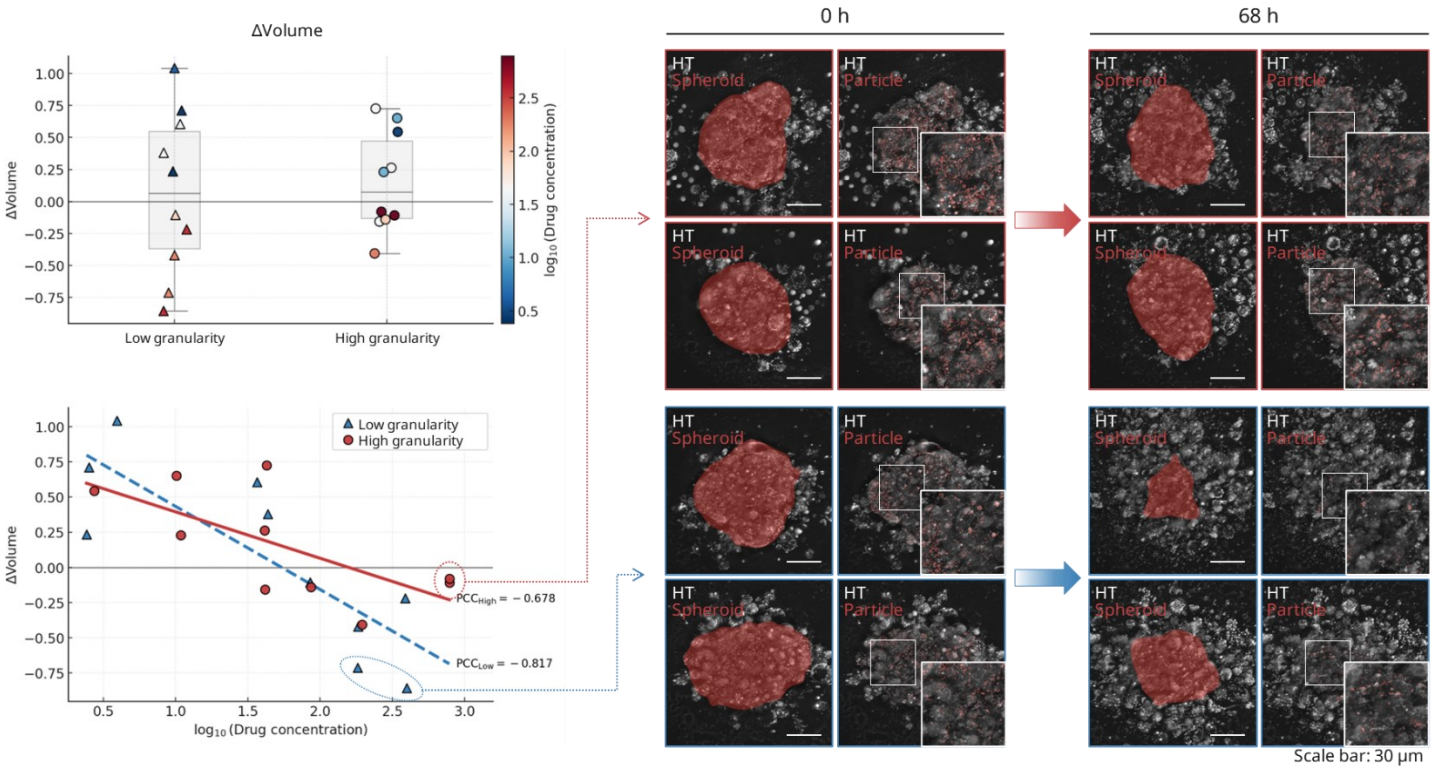

Figure 6: Drug response is influenced by the composition of the PDOs. Organoids stratified by initial granularity showed differential drug sensitivity. Low-granularity organoids exhibited greater volume reduction with increasing carboplatin concentration compared with high-granularity organoids. Representative HT images illustrate these differences at baseline (0 h) and after treatment (68 h).

Reference

1. W. Li et al., “3D biomimetic models to reconstitute tumor microenvironment in vitro: spheroids, organoids, and tumor-on-a-chip,” Adv. Healthc. Mater., vol. 12, no. 18, e2202609, Jul. 2023, doi: 10.1002/adhm.202202609.
2. G. Vlachogiannis et al., “Patient-derived organoids model treatment response of metastatic gastrointestinal cancers,” Science, vol. 359, no. 6378, pp. 920–926, Feb. 2018, doi: 10.1126/science.aao2774.
3. J. A. Taverna et al., “Ex vivo drug testing of patient-derived lung organoids to predict treatment responses for personalized medicine,” Lung Cancer, vol. 190, 107533, 2024, doi: 10.1016/j.lungcan.2024.107533.
4. M. J. Lee et al., “Long-term three-dimensional high-resolution imaging of live unlabeled small intestinal organoids via low-coherence holotomography,” Exp. Mol. Med., vol. 56, no. 10, pp. 2162–2170, Oct. 2024, doi: 10.1038/s12276-024-01312-0.
5. S. Corgnac, Y. Lecluse, and F. Mami-chouaib, “Isolation of tumor-resident CD8+ T cells from human lung tumors,” STAR Protoc., vol. 2, no. 1, 100267, Mar. 2021, doi: 10.1016/j.xpro.2020.100267.

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