

Imaging-guided platform for real-time intervention in complex in vitro models

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Biological Sciences - Article

Keywords:

Posted Date: August 18th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7179174/v1>

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Additional Declarations: There is **NO** Competing Interest.

Abstract

Complex in vitro models (CIVMs), including organoids, spheroids, and bioprinted constructs, have emerged as powerful platforms for recapitulating human tissue architecture and function. However, their inherent heterogeneity and dynamic nature pose significant challenges for standardization, reproducibility, and real-time manipulation. Here, we present VISIBLE (Versatile Imaging-Guided Sampling and Interactive Bioprinting System), a modular platform that integrates real-time monitoring with automated manipulation and 3D bioprinting that addresses these challenges. VISIBLE employs a unique co-registered dual-axis system, enabling image-guided, closed-loop spatiotemporal interventions within live cultures. We demonstrate its transformative capabilities across diverse applications, including precise morphology- and function-based sampling of organoids and neurospheres, interactive 3D bioprinting with on-the-fly adjustments, and autonomous serial interventions for longitudinal studies. Furthermore, we illustrate its utility in translational pipelines through selective sampling and successful in vivo implantation of barcoded patient-derived cancer organoids for clonal lineage tracing. VISIBLE supports long-term culture within an integrated incubation environment and accommodates interchangeable tool-heads for scalable, high-throughput workflows. By enabling dynamic, feedback-controlled experimentation, VISIBLE addresses critical bottlenecks in current CIVM platforms, offering a versatile and powerful solution for a wide range of biomedical applications. By transforming CIVMs from static cultures into interactive, programmable systems, VISIBLE represents a critical step toward autonomous in vitro experimentation and paves the way for next-generation platforms in tissue engineering, disease modelling, and preclinical research.

Introduction

Complex in vitro models (CIVMs), including organoids^{1, 2}, spheroids³, micro-physiological organ-on-chip systems⁴, and bioprinted tissues^{5, 6} are transforming our ability to recapitulate human specific biology outside the body. These engineered culture platforms are designed to more faithfully recapitulate key structural and functional features of human organs than conventional 2D monolayers. By incorporating multiple cell types within extracellular matrices (ECM) and often integrating precise microfluidic control, CIVMs provide superior insights into human development, disease progression, and drug responses while significantly decreasing reliance on animal models^{7, 8}. Their increasing adoption across academic research, industry, and regulatory science reflects a growing body of validation data and the urgent need for human-relevant test systems in bioscience and drug discovery. Indeed, the past decade has seen a significant increase in the number of studies that uses them as the main or one of the principal platforms for testing⁹. This upward trend underscored a global pivot toward complex human-relevant methodologies in bioscience, as a steady increase in adoption can be seen across industry¹⁰. This is also mirrored by changes in regulatory frameworks, which have recently started to acknowledge patient-derived organoids as acceptable non-clinical testing system¹¹.

The emergence of CIVMs has been accompanied by rapid progress in enabling technologies, such as, stem cell technologies¹², 3D bioprinting¹³, high-content imaging^{14, 15}, and laboratory automation¹⁶. However, as CIVMs become more structurally and functionally sophisticated they also presents new challenges for instance, reproducibility, standardisation, and operator dependence. These models are inherently dynamic and heterogenous, particularly stem cell-derived systems, making culture outcomes variable and difficult to control. Although tools like the SpheroidPicker for 3D cell culture manipulation¹⁷ and imaging cell picker for morphology-based cell separation¹⁸ have attempted to automate selection and sampling, they remain task-specific, lacking iterative feedback or manipulation capabilities. Similarly, 3D bioprinting technology¹⁹ have advanced the construction of functional 3D tissues, yet typically operates as open-loop systems with no imaging-guided manipulation once the printing commences. Microscope-integrated 3D bioprinting²⁰ have begun to bridge this gap, but are still limited in their scope of real-time, interactive control.

A central unmet need in the field is a generalisable, feedback-controlled platform that supports real-time imaging, precise manipulation, and iterative intervention, a true 'closed-loop' system. Existing tools that allow spatial control of cells, such as micromanipulators²¹, acoustic tweezers²² or optical tweezers²³, either lack scalability or require specialised and complex instrumentation. Fluorescence-activated cell or organoid sorting (FACS)²⁴ provides robust population-level sampling but operates on suspended samples with no direct spatial control or downstream reintegration into live cultures. Recent advances in microfluidic with integrated optics coupled with machine learning have enabled simultaneous deposition and monitoring of single-cell²⁵ and achieved higher efficiency in single-cell cloning²⁶, but still do not support in situ, real-time interaction with live, adherent, or spatially structured cultures. Across the CIVM landscape, there remains no unified system capable of handling, analysing, and interacting with complex cultures continuously over time.

Here, we present VISIBLE (Versatile Imaging-Guided Sampling and Interactive Bioprinting System), an integrated, modular platform that couples real-time imaging with bidirectional manipulation to support live feedback-driven spatiotemporal control of complex in vitro experiments. VISIBLE comprises three synergetic modules: a top-mounted, pneumatically-controlled manipulation and bioprinting tool-head; a middle incubation chamber with environmental regulation; and a bottom-mounted motorized microscope stage for in-line imaging. Unlike existing tools, VISIBLE allows independent yet co-registered motion of the imaging and manipulation axes, enabling precise targeting, sampling, and iterative intervention without disturbing the culture or transferring between systems.

We validate the capabilities of VISIBLE across a range of biological applications with increasing complexity. We demonstrate morphology- and fluorescence-based organoid selection from both suspension and adherent cultures; function-based sampling of neurospheres based on calcium activity; real-time, imaging-guided 3D bioprinting with interactive control and iterative analysis; and serial manipulation experiments such as scratch assays with automated recovery and monitoring. Further, we show that the platform supports spatially targeted co-culture deposition, exemplified by the precision

placement of motor neuron spheres on differentiated skeletal myotubes, and can be seamlessly integrated into in vivo pipelines, as shown through pLARRY-barcoded patient-derived cancer organoids injected into mice following eGFP-guided sampling.

Together, these diverse use cases establish VISIBLE as a next-generation platform for CIVM research, one that enables imaging-driven experimentation, supports dynamic biological decisions, and lays the groundwork for self-driving biological laboratory. Its open, modular architecture and compatibility with standard lab components position it as a widely adoptable solution to longstanding bottlenecks in reproducibility, automation, and complex experimental design.

Results

Design and engineering of the VISIBLE platform

The platform is built around two independently motorised and software-synchronised axes: a top-mounted XY gantry carrying custom manipulation tool-heads, and a bottom motorised microscope stage. A schematic of the platform outlines the distinct structural elements, including the top tool-head rail system, enclosed incubator with environmental regulation, and base-mounted microscope for imaging feedback (Fig. 1a, Extended data Fig. 1a). The imaging-guided feedback loop of VISIBLE is schematised in a control diagram (Fig. 1b), wherein each manipulation action is informed by live imaging and spatial metadata, enabling an automated, iterative pipeline of sampling, printing, perturbation, and monitoring. The full experimental workflow includes: (i) load the sample, (ii) top-camera scan to generate a coordinate map of the well-plate, (iii) precise microscope imaging of targets, (iv) feedback-based interaction via the tool-head (e.g., sampling, deposition, or scratch injury), and (v) guided collection into Eppendorf tubes for downstream analysis (Fig. 1c).

To validate long-term live culture compatibility, we maintained hiPSC-derived motor neuron cultures inside VISIBLE and acquired continuous time-lapse imaging. Acquired images revealed progressive extension of neuronal processes over time, indicating stable environmental control and cytocompatibility during extended experiments (Fig. 1d, e). Moreover, readings from a mini-LCD temperature monitor, resistance temperature detector (Pt-100) and forward looking infrared (FLIR) gun also confirm robust temperature retention (37° C) (Extended data Fig. 1c, h) validating the incubator design for live cell experiments.

We engineered modular components to support a wide range of manipulative functions. These include a tool-head housing for controlled aspiration or extrusion and a drop station for automated sample dispensing (Extended data Fig. 1d–g). Tool-head integrates a custom-designed precisely regulated pneumatically-operated syringe extrusion system. Interchangeable syringes accommodate a broad range of nozzles (50–2000 μm), ensuring versatility across diverse applications. Tool-head repeatability for bi-directional movement was assessed via high-resolution calibration experiments, showing lateral targeting accuracy within $\pm 2\mu\text{m}$ (Fig. 1f–h, Extended data Fig. 1i). This was further validated by iterative single-cell sampling from densely cultured skeletal muscle cells, with microscopic images confirming

successful targeting and removal without disturbing neighbouring cells (Fig. 1i). This demonstrates VISIBLE's precise control and pinpoint accuracy essential for single-cell resolution studies.

In addition to precision manipulation, VISIBLE enables high-throughput scalability. The full platform footprint is compact, and the custom-designed incubation chamber can accommodate multi-well plates (6-well up to 96-well format) (Extended data Fig. 1b). Viewed holistically, these features establish VISIBLE as an integrated platform for closed-loop biological experimentation. Its real-time imaging-guided capabilities and single-cell manipulation precision uniquely position it to perform advanced, interactive workflows previously unattainable.

Image-guided precision sampling mitigates organoid heterogeneity

Recent advancements in hiPSC differentiation, organoid technology and synthetic developmental biology have profoundly deepened our understanding of biological processes and disease states^{27, 28}. Despite extensive optimisation, a significant limitation for organoids-based in vitro studies persists: the inherent inter- and intra-experimental organoid heterogeneity, notably in size and shape^{29, 30}. Furthermore, the proliferation of differentiation protocols compounds challenges in replicating results across laboratories, and even within the same research group³¹. To effectively characterise and manage this variability in dynamic cultures, continuous monitoring coupled with iterative, targeted intervention is essential for understanding its origins and ensuring experimental reproducibility. Current approaches, such as manual selection with wide-diameter pipette tips³² or fluorescent-based flow cytometry for cell suspensions³³, are labour-intensive, prone to human error, and often result in compromised cytoarchitecture or cell death. These methods fundamentally impede continuous tracking of variability and precise sampling of organoids necessary for experimental fidelity.

We next evaluated the capability of the VISIBLE platform to resolve heterogeneity within complex organoid cultures by performing real-time, morphology-guided sampling in suspension. We first generated embryoid bodies (EBs) using custom-designed (400x400x200 µm) PDMS microwells³⁴ (Extended Data Fig. 2a, b) and differentiated them into neuroectodermal organoids following a modified protocol³⁵. Next, we generated two populations of neuroectodermal organoids with differential Wnt activation, 'low' and 'high' by varying CHIR concentrations during induction (Fig. 2a). The resulting populations exhibited clear morphological differences, notably in symmetry and compactness, and were mixed in equal proportions to create a heterogeneous CIVM representative of typical experimental variability (Fig. 2b, left panel). This posed an ideal testbed for assessing the VISIBLE's ability to selectively isolate morphologically distinct subpopulations.

The VISIBLE was used to scan the PDMS macrowell containing suspended organoids and generate a stitched top-view image of the entire culture (Fig. 2b, left-most panel). Targeted organoids were then selected in silico based on shape parameters, specifically those displaying symmetry-breaking features (population 2) indicative of higher Wnt activation and physically sampled using an integrated select-lock-pick sequence. The platform achieved precise retrieval of individual organoids without perturbing

neighbouring structures (Fig. 2b, right-most panel, top), highlighting its spatial accuracy and non-disruptive handling. The platform successfully performed iterative, sequential sampling of target organoids across the entire well (Extended Data Fig. 2c). This gentle collection process, involving controlled forces (Extended Data Fig. 2d, Supplementary video 1), was critical to maintaining the structural integrity of the sampled organoids, as evidenced by the intricate details preserved within the collected samples (Fig. 2b, right-most panel, bottom, white arrows: shows preservation of fine architectural features). Crucially, the suction pressure was precisely regulated to prevent damage to the organoid morphology, unlike previously reported Aspiration Assisted Bioprinting (AAB) methods, which often lead to significant structural deformation³⁶.

To confirm that the VISIBLE-sampled organoids remained intact at both structural and molecular levels, we conducted immunocytochemistry (ICC) and qPCR analyses. DAPI staining of sampled Population 1 and 2 organoids revealed remarkably well-preserved organoid cytoarchitecture (Fig. 2c, Extended Data Fig. 2e), confirming the integrity of the collected structures. Parallel RNA extraction from sampled organoids and original cultures for gene expression analysis further validated the precision of VISIBLE's selection (Fig. 2d). Relative gene expression showed that the VISIBLE-picked high Wnt population maintained similar levels of neuroectodermal (*SOX1*, *SOX2*, *PAX6*), posterior identity (*HOXC9*) and somitic and presomitic markers (*TBXT*, *TBX6*) as the original high Wnt population. We also assessed the viability of sampled organoids through re-plating experiments: organoids re-plated into fresh matrix remained viable, preserved their cytoarchitecture, and displayed spontaneous outward migration of cells after 48 hours in culture (Fig. 2e).

Cumulatively, these results demonstrate that VISIBLE enables selective, image-guided organoid sampling based on morphological cues, while preserving sample integrity for downstream analysis. This addresses a major limitation of current CIVM-handling workflows, namely, the inability to interact with specific subpopulations within live heterogeneous cultures and establishes VISIBLE as a high-precision platform for spatially resolved, real-time sampling of complex 3D models.

Precision sampling of adherent organoids using image-guided intervention

While the ability to precisely sample organoids from suspension cultures addresses a key aspect of heterogeneity, many complex in vitro models form or thrive as adherent cultures, presenting distinct challenges for non-destructive, image-guided intervention. Furthermore, the most insightful biological questions often require selecting cells or organoids based on dynamic functional states or specific molecular markers, moving beyond simple morphology. VISIBLE was engineered to extend its precision sampling capabilities to these more complex scenarios.

Following our previous experiment, we plated a mixed population of low and high Wnt activation organoids into a tissue culture plate and cultured them for an additional six days. Owing to their intrinsic developmental differences established during the initial induction phase, these adherent organoids exhibited pronounced morphological distinctions and generated characteristic migratory cell populations in their vicinity making them an ideal testbed for imaging-guided selection and extraction

(Fig. 3a, left-most panel). VISIBLE's imaging capability could then spot specific dynamic features, such as filamentous structures extending from a live organoid. This enabled the targeted selection of that particular organoid for collection (Fig. 3a, middle panel). Crucially, the platform executed the collection with remarkable precision, leaving an empty space exactly where the organoid had been, without disturbing or compromising the integrity of neighbouring adherent structures (Fig. 3a, right-most panel and Supplementary video 2). This delicate interaction highlights VISIBLE's unique ability to navigate and precisely sample within densely packed adherent environments.

Extending beyond visual morphology, VISIBLE enables fluorescence-guided sampling through multi-channel imaging. We demonstrated this by culturing lineage-traced organoids expressing different fluorescent markers and selectively sampling based on protein expression profiles (Fig. 3b). The platform's imaging system supports simultaneous visualization of multiple fluorophores, allowing users to define sampling criteria based on reporter expression, subcellular localization, or dynamic fluorescent signals. This ability to couple functional imaging with targeted manipulation in situ represents a major advance in handling complex cell systems with spatial and phenotypic specificity.

To quantitatively benchmark VISIBLE's performance for this challenging task, we conducted a rigorous comparison against manual handling across a range of organoid sizes: small ($<250\text{ }\mu\text{m}$), medium ($250\text{--}750\text{ }\mu\text{m}$), and large ($>750\text{ }\mu\text{m}$). VISIBLE consistently outperformed manual sampling in terms of purity (defined as retrieval of only the intended organoid), throughput (measured as pure samples per unit time), and error rate (instances of either failed lift-off or disturbance of neighbouring organoids) (Fig. 3c–e). These metrics establish VISIBLE's superiority in executing reproducible and scalable sampling operations, which are particularly critical when working with heterogeneous cultures.

To further characterise the interaction forces during the sampling process, we measured the suction pressure required to lift organoids of varying sizes. The required force scaled linearly with organoid diameter (Fig. 3f), consistent with mechanical predictions and previous literature^{36, 37} and reflective of the varying adhesion forces involved. Crucially, VISIBLE's precise pressure control ensures the gentle detachment and collection of even firmly adhered structures and highlights its adaptive sampling capabilities based on real-time biophysical feedback.

Taken together, these results demonstrate that VISIBLE provides a high degree of spatiotemporal control for sampling adherent organoids based on both structural and molecular features. This level of precision is difficult to achieve with traditional methods and offers a transformative approach for dissecting and perturbing complex CIVMs in real-time.

Function-based sampling via calcium imaging in neuronal cultures

Beyond morphology, functional heterogeneity represents a critical axis of variability in complex in vitro systems, particularly in neuronal models where maturation and network activity vary across seemingly identical structures. To demonstrate that VISIBLE can enable sampling based on physiological features rather than morphology alone, we established an experimental pipeline to target and isolate functionally

mature human iPSC-derived neurospheres based on their calcium dynamics. To achieve this, we generated glutamatergic neurons by doxycycline-inducible overexpression of NGN2 in the KOLF2.1J hiPSC line using the piggyBac transposon system (Fig. 4a). Neurospheres were then formed as previously described³⁴ with modifications, plated on a Matrigel-coated culture plate, and maintained for 16 days to facilitate maturation. Maturing glutamatergic neurons, particularly within complex neurospheres, establish intricate connections and exhibit spontaneous calcium spikes and transients. However, the degree of connectivity and resulting calcium activity, a direct hallmark of neuronal maturity, varies significantly among individual neurospheres within the same culture. This inherent functional heterogeneity makes targeted studies challenging.

To address this, we employed a calcium dye to assess these functional maturation differences, enabling the active selection and isolation of individual neurosphere. We chose this experimental set-up due to its broad applicability across diverse complex in vitro models and experimental pipelines. As a first step, the platform generated a stitched image of the entire well to precisely map and log the position of individual neurospheres (Fig. 4b, Step I). Subsequently, high-resolution videos were captured from identified regions of interest to quantify neuronal activity based on Ca^{2+} fluctuations. For this demonstration, we selected six randomly positioned neurospheres from the stitched image (Fig. 4b, step I). Within each selected neurosphere, multiple positions were identified, and neuronal activity was recorded over a five-minute period with 1-second intervals (488 nm excitation) (Extended Data Fig. 3b-c). Neurospheres with more than five calcium peaks across their ROIs were designated as functionally active (Fig. 4b Step II–III, Extended Data Fig. 3b-c).

These active neurospheres were selected for collection using the VISIBLE's select-lock-collect mechanism. The tool-head was precisely navigated to the target coordinates and lowered via Z-axis control to engage the sphere, which was then gently aspirated using a nozzle of 520 μm diameter. The process was executed with single-neurosphere precision: active neurospheres (1, 2, and 6) were removed cleanly, while nearby, less active structures (3, 4, and 5) remained undisturbed (Fig. 4b Step IV-V, Extended Data Fig. 3a). The precision of this operation guided by live functional imaging highlights a unique capability of VISIBLE. Neither standard cell-sorting technologies such as FACS nor conventional micromanipulators are capable of this type of closed-loop, physiology-driven sampling within dense adherent cultures. Moreover, this process was fully iterative: functional imaging, decision-making, and collection could be repeated across the same well or across multiple wells without sample transfer or human intervention.

Overall, these experiments establish VISIBLE's ability to couple real-time physiological readouts with precise microscale manipulation. This functionality is especially valuable for studies requiring stratification of CIVMs by maturity or activity, for example, in neural development, drug screening, or disease modelling, thereby expanding the experimental possibilities far beyond morphology-based sampling.

Interactive 3D bioprinting for adaptive spatiotemporal experiments

A major bottleneck in advancing complex in vitro cultures is the critical need for dynamic interaction with live cultures based on real-time imaging feedback. Most commercially available 3D bioprinters, while capable of creating structures from millimetres to centimeters^{5, 6}, generally lack real-time feedback. These bioprinters lack in situ imaging capabilities and operate in an open-loop manner, executing predefined instructions without the ability to pause, assess, or intervene based on live culture states. This static approach is fundamentally problematic for complex biological cultures, which are inherently dynamic. To overcome this, we leveraged the VISIBLE's architecture, wherein the top-mounted tool-head and bottom-mounted imaging system operate independently but are spatially co-registered, to enable interactive 3D bioprinting with closed-loop control and facilitates on-the-fly adaptation within live experiments.

We first validated this adaptive bioprinting capability by implementing a print–pause–analyse–print cycle. A target geometry, designed as a segmented hydrogel structure embedded with iPSC-derived cells, was uploaded to the VISIBLE. The target geometry was segmented into 4 parts, allowing us to print each segment sequentially. The platform executed the first segment of the print, followed by immediate imaging to assess construct fidelity. If no discrepancies were detected, printing resumed with the next segment; otherwise, the print protocol was dynamically adjusted to correct for artefacts or deviations (Fig. 5a, b and Supplementary video 3). This iterative printing flow demonstrates the capacity of VISIBLE to intelligently adapt ongoing biofabrication steps, mitigating structural errors, enhancing printing precision, and significantly reducing failure rates associated with static bioprinting.

We further tested the combined power of iterative printing and precise sampling within the same printed construct using the VISIBLE. For this, we generated a sequential array of hydrogel matrix dots and subsequently selected specific regions to be sampled without disturbing nearby structures (Fig. 5c). This experiment conclusively demonstrated that VISIBLE's precise pneumatic sampling mechanism can be seamlessly integrated with its bioprinting capabilities, allowing for complex, iterative spatiotemporal interactions with 3D cultures. Moreover, we validated the viability of cells printed within hydrogel using VISIBLE (Fig. 5d), showing robust cell viability two days post-printing. This confirms that the hydraulic printing mechanism exerts appropriate pressure for hydrogel deposition without compromising cellular integrity.

To demonstrate the spatiotemporal orchestration of co-cultures, we designed an experiment involving hiPSC-derived motor neurons (MNs) and primary human skeletal muscle cells. Briefly, we grew human myoblasts on a Matrigel-coated plate and observed their differentiation pattern over a week within the VISIBLE. Myoblast fusion and differentiation into multinucleated myotubes are triggered by cell-cell contact; consequently, heterogeneity in culture conditions (e.g., density) results in regions with distinct differentiation efficiencies. VISIBLE allowed us to scan the entire well in real-time to identify and map areas with relatively higher differentiation (areas with differentiation >50%) based on the morphology and size of the myotubes (Fig. 5e, f). These highly-differentiated areas were then precisely selected for the targeted deposition hiPSC-derived motor neurospheres. Neurospheres embedded in hydrogel were deposited only on these pre-selected, mature locations, allowing them to interact specifically with the

underlying skeletal muscle cells. After a week of co-culturing, we observed clear evidence of processes extending from motor neurons and establishing contact with the underlying muscle cells (Fig. 5g, h, Extended data Fig. 4 a-c). This example demonstrates that VISIBLE enables spatiotemporally resolved construction of complex, interactive microenvironments, such as neuron–muscle interfaces, using real-time biological cues as the basis for experimental decisions.

Altogether, these experiments demonstrate VISIBLE's interactive 3D bioprinting capabilities, driven by real-time image feedback, enable adaptive construction and precision co-culturing of heterogeneous cell types. This advancement moves beyond static bioprinting, empowering researchers to create complex, functional in vitro models with unprecedented spatial and temporal control, thereby reducing experimental failure rates and enabling far more complex and precisely defined investigations.

Autonomous serial intervention for reproducible longitudinal studies

Longitudinal studies are essential for understanding dynamic biological processes, but their execution is often hampered by the need for repeated, precise human intervention, leading to variability and throughput limitations. VISIBLE addresses this challenge by enabling autonomous serial intervention and monitoring within a controlled environment, facilitating reproducible longitudinal experiments with minimal human involvement.

For this purpose, we selected the well-established scratch assay model in skeletal muscle cells, widely used in wound repair and developmental biology studies³⁸. The entire process, from scratch creation to continuous monitoring of its recovery and subsequent cellular differentiation was performed entirely by VISIBLE with automated image acquisition and analysis (Fig. 6a). This significantly reduces human handling and potential variability inherent in traditional manual workflows. In this set of experiments, the manipulation tool-head, fitted with a ~480 µm nozzle, was precisely controlled to induce a standardised scratch in a confluent monolayer of skeletal muscle cells (Fig. 6a, b). A series of high-resolution images were then acquired at 6-hour intervals by the integrated microscope, demonstrating the consistent and quantifiable recovery of the scratch in situ within the platform (Fig. 6b). In the final step, differentiation medium was precisely introduced into the well via a syringe mounted on the tool-head, showcasing the VISIBLE's ability to perform fluidic exchange. After one week, the final image confirmed a fully recovered and differentiated muscle cell culture following the induced scratch.

The growth dynamics of the muscle cells during recovery phase, quantified as area vs. time and gap width vs. time (Fig. 6 c-d) demonstrated a consistent trend aligning with previous studies performed with manual intervention^{38,39}. Critically, the entire scratch induction and recovery procedure was performed autonomously by the VISIBLE, thereby eliminating the significant experimental variability in scratch size and positioning commonly associated with manual execution. This automation guarantees the unprecedented repeatability and consistency of the scratch assay across multiple timepoints and samples, a feat virtually infeasible through manual methods. Furthermore, VISIBLE's design supports the simultaneous execution of multiplexed experiments, significantly enhancing throughput. To illustrate

this, we autonomously introduced scratches into confluent muscle cell monolayers in two neighbouring wells within the same multi-well plate (Extended Data Fig. 5a). The platform then concurrently monitored the scratch recovery in both wells through a series of time-lapse images, demonstrating the feasibility of parallel, high-throughput longitudinal studies.

These experiments establishes that VISIBLE can manage complex, multi-step experimental protocols spanning days to weeks, with precise control over spatiotemporal perturbations and full integration of imaging and intervention. The ability to perform such serial interventions in a programmable and repeatable manner, while maintaining uninterrupted environmental control, represents a significant leap forward in in vitro experimentation throughput and reproducibility.

Integration into preclinical experimental pipeline via barcoded organoid sampling

To demonstrate the integration of VISIBLE within preclinical experimental pipelines, we evaluated its ability to support complex workflows that span in vitro lineage tracing and organoid selection for downstream in vivo validation. Patient-derived cancer organoid models, for instance, are increasingly critical for preclinical studies, and recent advancements in DNA barcoding enable lineage tracing and single-cell RNA sequencing to track clonal identities through phenotypic space⁴⁰. However, optimizing single-barcode-per-cell conditions can lead to incomplete infection, negatively impacting the comprehensive coverage of lineages within a population.

To address this, we employed a patient-derived xenograft organoid (PDXO) model of triple negative breast cancer⁴¹. Single cells were first infected with the pLARRY barcoding system and then allowed organoids to form (Fig. 7a). The VISIBLE was then used to identify and select successfully barcoded organoids based on eGFP expression, guided by real-time imaging and precise tool-head manipulation (Fig. 7b).

To test the feasibility of using VISIBLE-sampled organoids in downstream in vivo analysis, we collected the eGFP⁺ organoids and transplanted them into immunodeficient mice. After five weeks, tumours were harvested and dissociated (Fig. 7c), followed by single-cell RNA sequencing (Fig. 7d). From these tumours, we identified that 39% of cells contained either eGFP⁺, the LARRY barcode, or the EEF1A promoter with barcodes detected in 1492 cells across 130 distinct clonal identities (Fig. 7e). Consistent with previous observations from this model⁴², we observed a subset of cells exhibiting high FGFR1 expression (Fig. 7e). Intriguingly, we found no significant difference in the Shannon diversity index between these specific subsets and the global population, suggesting that phenotypic space in this human triple-negative breast cancer model is largely independent of clonal origin (Fig. 7f).

Collectively, this experiment provides a proof-of-concept for using VISIBLE to isolate phenotypically defined subpopulations from complex organoid cultures for downstream applications. It also demonstrates that VISIBLE-sampled organoids maintain both functional integrity and clonal identity during in vivo propagation. More broadly, this pipeline establishes VISIBLE as a powerful tool for bridging

imaging-guided selection in vitro with translational experimentation in vivo, expanding the VISIBLE's potential in enabling unprecedented precision during preclinical experiments that ultimately enhance in vivo studies. Our present approach is generalisable to other barcoding systems or genetic engineering strategies.

Discussion

In this study, we introduce the VISIBLE platform, an integrated, feedback-controlled system that synergistically combines real-time imaging-guided automated manipulation and bioprinting. This advanced platform directly addresses critical challenges of reproducibility and heterogeneity prevalent in complex in vitro models. By enabling precise, iterative interventions guided by live image analysis, VISIBLE facilitates an unparalleled range of experimental workflows, from single cell resolution sampling to the dynamic handling of millimetre-scale hydrogel constructs. VISIBLE represents a foundational tool for any laboratory aiming to automate and enhance the precision of their preclinical studies and fundamental biological research.

A key advance is the independent yet co-registered movement of the microscope stage and manipulation tool-head, establishing a direct visual feedback loop that distinguishes VISIBLE from conventional static bioprinters or manual manipulation systems. This allows for continuous, high-resolution monitoring and real-time adaptation of tool-head actions. VISIBLE's enclosed, sterile, and environmentally controlled incubation chamber is crucial for sustaining complex in vitro models over extended periods, validated by the robust viability and outgrowth of hiPSC-derived motor neurons. Furthermore, the demonstrated $\pm 2 \mu\text{m}$ displacement precision underscores its capability for even single-cell resolution interventions. This foundational engineering establishes VISIBLE as a platform designed for dynamic, rather than static, interaction with living biological systems.

Our experiments demonstrate the breadth of biological workflows that VISIBLE can support. Beginning with automated imaging-guided sampling, we used the platform to isolate organoids from heterogeneous suspension cultures based on morphometric and fluorescence criteria, capturing symmetry-breaking events during neuroectodermal differentiation. Crucially, organoids sampled using VISIBLE retained both structural integrity and transcriptional identity, allowing for downstream culture and transcriptomics. We further validated VISIBLE's ability to sample organoids in adherent cultures, which typically present an even greater challenge due to differential adhesion and local cell migration. VISIBLE's precise hydraulic control permitted spatially confined lifting of organoids or even single muscle cells, without perturbing neighbouring structures. These experiments underscore a key differentiator of VISIBLE, its ability to selectively interface with individual structures based on real-time positional and phenotypic imaging, rather than population-based bulk approaches.

Expanding from structure to function-based sampling, we demonstrated that VISIBLE can target hiPSC-derived neurospheres based on Ca^{2+} activity, a hallmark of functional neuronal maturation. By dynamically tracking calcium transients within neurospheres and selecting the most active ones for

isolation, VISIBLE uniquely enables functionally stratified sampling; a critical feature for studying neurodevelopmental heterogeneity, synaptic maturation, and network dynamics. No existing system currently supports this level of live functional phenotyping and intervention in a unified platform.

We next demonstrated interactive 3D bioprinting, guided by iterative imaging and feedback. By decoupling the motion paths of the print-head and microscope stage, VISIBLE supports segmented, real-time-validated printing workflows, a significant advance over conventional bioprinters, which lack feedback loops and often fail due to batch-based, non-adaptive protocols. Moreover, by integrating bioprinting with live sampling, we showcased bidirectional interaction with the printed constructs and spatiotemporal co-culture interaction of neurospheres with differentiated muscle cells expanding the experimental design space for dynamic tissue engineering. To highlight VISIBLE's utility for longitudinal live culture experiments, we implemented a fully automated scratch-recovery assay in skeletal muscle cells. VISIBLE introduced controlled scratch, monitored its recovery over several days, and introduced differentiation media without manual intervention. The high precision and repeatability of this assay underscore VISIBLE's potential to reduce variability in time-course and perturbation studies, especially in applications such as regenerative and developmental biology.

Finally, we demonstrated that VISIBLE can be integrated into preclinical translational pipelines, by sampling pLARRY-barcoded organoids from a patient-derived xenograft model of triple-negative breast cancer. The platform enabled the targeted collection of GFP⁺ barcoded organoids for transplantation into mice, followed by clonal tracing via single-cell RNA sequencing. Barcode retention, lineage mapping, and functional heterogeneity in vivo confirm the utility of VISIBLE-sampled organoids for in vivo clonal fate mapping. This underscores VISIBLE's translational relevance and its compatibility with cutting-edge lineage tracing technologies.

The modular architecture of VISIBLE; featuring interchangeable tool-heads, control electronics, and a customizable incubation chamber; positions it as a versatile, scalable solution adaptable to a broad range of laboratory settings and model systems. While our current hydraulic sampling resolution (~50 μm) already permits single-cell work, future iterations could integrate optogenetic or photothermal actuation to further refine precision. Likewise, integration of computer vision algorithms and machine learning-based decision-making will open avenues for autonomous biological experimentation, transforming CIVM handling into a self-driving biological laboratory paradigm.

Taken together, VISIBLE is a transformational platform that redefines how complex cultures are monitored, manipulated, and interpreted. Its modular and scalable design, compatible with widely available electronic and opto-mechanical components, offers extraordinary flexibility for customizing in vitro experimental workflows. We envision widespread utility across disciplines; from neuroscience, oncology, and regenerative medicine to developmental biology and pharmacological screening, wherever dynamic biological systems must be observed and steered in real-time.

Declarations

Acknowledgments

A.S. wishes to acknowledge the support of the BBSRC (Grant BB/T011572/1 and Grant BB/W006561/1), and of the Dementia Research Institute (UKDRI). **F.S.T.** and **A.S.** acknowledge funding by the European Union (Horizon Europe project no. 101080690 – MAGIC). Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or HADEA. Neither the European Union nor HADEA can be held responsible for them. This work is funded by the UK Research and Innovation (UKRI) under the UK government's Horizon Europe funding guarantee grant no. 10080927, 10079726 and 10078461. The authors are grateful to the Myoline platform of the Institute of Myology (Paris, FR) for providing myoblasts. This research was funded in whole, or in part, by the Wellcome Trust. We would like to acknowledge the Making Lab facility, a Science Technology Platform at the Francis Crick Institute. **A.S.**, **A.I.**, and **F.S.T.** acknowledge support by the Francis Crick Institute, which receives its core funding from Cancer Research UK, the UK Medical Research Council (MRC) and the Wellcome Trust (CC0102). **E.S.** is supported by the Francis Crick Institute, which receives its core funding from Cancer Research UK (CC2040), the UK Medical Research Council (CC2040), and the Wellcome Trust (CC2040) and the European Research Council (ERC Advanced Grant CAN_ORGANISE, Grant agreement number 101019366). **E.S.** reports grants from Novartis, Merck Sharp Dohme, AstraZeneca and personal fees from Phenomic outside the submitted work.

S.J. acknowledges funding support from the BBSRC TRDF Grant (BB/T011572/1) and the Chris Banton Fund of The Francis Crick Institute, London. **S.J.** would like to thank the MedTech Super Connector (MTSC) fellowship and the BBSRC ICUR Explore Programme for funding support towards commercialization of innovation. **S.J.** is the recipient of LUSH Prize in the young researcher category and extends gratitude for generous funding support. **C.D.H.R.** is a recipient of a Bourse postdoctorale from the Fonds de recherche du Québec, Santé (<https://doi.org/10.69777/273104>), as well as an EACR-AstraZeneca Postdoctoral Fellowship. C.D.H.R. is supported by funding from the AstraZeneca-Crick Research Alliance.

The breast cancer patient-derived xenograft organoids PDXO GCRC1915 was obtained from the breast tissue and data bank (Park lab, McGill University) (PMID: 32546838), supported by the Réseau de Recherche sur le Cancer of the Fonds de Recherche du Québec-Santé and the Québec Breast Cancer Foundation, and certified by the Canadian Tumor Repository Network (CTRNet).

CRedit (Contributor Roles Taxonomy) Statement

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Methods

Assembling the VISIBLE platform

The VISIBLE platform is built around a custom-designed manipulation tool-head, mounted on an XY gantry driven by two Bipolar Hybrid Stepper Motors (Nema 17) arranged in a core XY coordination mechanism. Motion along the Z-axis is enabled by a custom-developed, screw-based assembly, engineered in-house to achieve controlled vertical displacement. Accurate Z-axis positioning is essential for safeguarding the integrated microscopic system, as even minor displacement errors could result in contact with and potential damage to the objective lens. To ensure single-cell level precision, Z-axis movements are tightly regulated via integrated limit switches, enabling reliable and reproducible positioning during experimental manipulation.

Microscopic Unit

The VISIBLE platform integrates a dedicated microscopic unit for real-time, high-resolution imaging of tissue culture plates. This unit is centred around a compact and configurable fluorescence microscope (MVR-ENG4131, ZABER). This ZABER module facilitates automated well-plate scanning via its motorized XY stage (X-ASR305B305BSE03D12), precise focus adjustments, and automated filter cube changes. The motorized stage is controlled by a joystick and interfaced with a custom-developed software module utilizing ZABER's stage controller drivers and .dll files. For fluorescence imaging, the platform

incorporates a filter cube equipped with excitation and emission filters optimized for DAPI, FITC, and Texas Red fluorophores (LED-DA/FI/TX-A-ZHE) (Semrock). Illumination is provided by a CoolLED pE-300 light source, with light delivered through a liquid light guide, a collimating adapter, and a lens arrangement. High-resolution images are captured by a Hamamatsu ORCA Spark (2.3 MP global shutter CMOS) scientific camera positioned opposite the objective lens. All control operations for the microscopic unit are managed through a custom-developed, open-source software system, enabling full customization based on specific experimental requirements.

The Integration Hardware

The central controlling unit of the VISIBLE platform is a Smoothieboard V2, a 32-bit open-source firmware board equipped with five stepper drivers offering 1/32 microstepping capability. This unit is powered by a 24 V power supply, enclosed within a polycarbonate casing, and is solely responsible for precise control of the stepper motors and limit switches within the manipulation unit. Communication with a personal computer (PC) is established via an Ethernet cable, with XY movement commands for the print-head encoded in G-code. Additionally, commands can be sent to the manipulation unit using other programming languages such as MATLAB or Python.

The entire gantry system is mounted on a squared solid aluminium optical breadboard (45x45 cm, matte black anodized finish, ThorLabs). A custom-cut rectangular opening (18x14 cm) at the centre of this top breadboard accommodates the objective lens and associated setup for the fluorescence microscope. The bottom microscope assembly is also robustly mounted on a separate squared solid aluminium optical breadboard (45x45 cm, matte black anodized finish, ThorLabs). Four Pedestal Pillar Posts (ThorLabs), each with M4 tapped holes on both ends, support the top breadboard at a height of 32 cm above the bottom assembly. The system integrates a top-mounted object-identifier camera (Ennovor 1920 HD Flexible Rigid Snake Inspection Camera) for automated well plate identification and a bottom-mounted inverted fluorescence microscope for single-cell level resolution imaging.

To maintain optimal physiological conditions, a controlled chamber (The Cube 2, Life Imaging Services) continuously supplies regulated hot air. A metallic sensing element based on Resistance Temperature Detection (RTD) is strategically positioned at the height of the stage to provide accurate temperature feedback in the immediate vicinity of the cell culture environment.

Cell culture

Human induced pluripotent stem cell culture

hiPSC lines were obtained either from commercially available sources: Control 3 (ThermoFisher Scientific Cat No. A18945) and KOLF2.1J (Jackson Laboratory, JIPSC1000) or kindly donated: KOLF2 (Maximiliano Gutierrez, The Francis Crick Institute). Cells were cultured in Matrigel-coated plates (1:100 from stock vial, Corning, 356234) and fed every other day with Essential 8 Flex medium (ThermoFisher Scientific Cat No. A2858501). All cells were maintained in a humidified environment at 5% CO₂ 37° C.

Once confluent, hiPSCs were passaged using a cell dissociation buffer solution (ThermoFisher Scientific Cat No. 13151014) for 3 minutes at 37° C. hiPSCs were regularly tested for mycoplasma and genetic abnormalities.

Neuroectodermal organoid differentiation

hiPSCs were differentiated into neuroectodermal organoids following a modified protocol³⁵. Instead of 2D CHIR pre-treatment in stem cell culture medium, hiPSCs were directly aggregated. Cells were seeded at a density of 700,000 cells/cm² in custom pyramidal-shaped PDMS microwells (400x400x250 µm), as previously described³⁴, in the presence of Y-27632 dihydrochloride (10 µM, Tocris, 1254). After 18 hours, differentiating organoids were transferred to rotatory culture in 35 mm TC-treated petri dishes (Corning Cat No. 430165) for the remainder of the differentiation protocol.

Unlike the original CHIR pre-treatment medium, aggregoids were cultured for the first two days in Differentiating Medium A supplemented with CHIR (Tocris Cat No. 4423) at concentrations of either 0.8 µM (low Wnt), 1.6 µM, or 2 µM (high Wnt). After 48 hours, the medium was changed to Differentiating Medium B, supplemented with CHIR at concentrations of 2 µM (low Wnt), 4 µM, or 6 µM (high Wnt) for the rest of the induction phase. Organoids were fed every other day until transfer to the PDMS organoid sorting device for population sampling using VISIBLE, typically between days 7 and 9, depending on the specific experiment.

VISIBLE-sampled organoid culture

Organoids precisely sampled by the VISIBLE were seeded onto Matrigel-coated 48-well plates using the VISIBLE's deposition capabilities. These cultures were maintained in Differentiating Medium B supplemented with ROCK Inhibitor (Y-27632 dihydrochloride, 10µM, Tocris, 1254) for the initial 24 hours to promote attachment and viability. Plated organoids were subsequently fed every other day with fresh Differentiating Medium B (without ROCK Inhibitor) up until day 13, at which point they were fixed and processed for fluorescence microscopy.

Organoid post-processing after VISIBLE sampling

Live Organoid Staining: Live organoids plated on Matrigel-coated plates were fluorescently labeled by incubation with SPY555-FastAct and SPY650-DNA live dyes (both 1:1,000 dilution from stock solution, Spirochrome) for 1 hour. Staining was performed in a humidified environment at 37° C with 5% CO₂.

Organoid Fixation: For fixation, sampled organoids in suspension were incubated in 4% paraformaldehyde (PFA) (BOSTER, AR1068) for 45 minutes on a shaker. Plated organoids were fixed with 4% PFA for 15 minutes. All fixed cells were washed three times with phosphate-buffered saline (PBS) before long-term storage at 4° C in PBS.

Organoid Staining: Suspension organoids were stained with DAPI (1 µg/mL, Sigma, D9542) for 2 hours on a shaker. Plated organoids were stained with ActinGreen488 (ThermoFisher Cat No. R37110) and

DAPI (1 µg/mL, Sigma, D9542) for 5 minutes. Following staining, all cells were washed three times with PBS to remove excess dye, protected from light, and stored at 4° C in PBS. Unless otherwise stated, all staining and washing procedures were performed at room temperature.

Organoid Imaging: Suspension organoids intended for whole-mount imaging were cleared overnight at room temperature using Rapiclear 1.47 (Sunji Lab, RC149001) and subsequently mounted onto glass coverslips. Whole-mount organoid imaging was performed using an Olympus CSU-W1 SORA spinning-disk system. Three-dimensional rendering and optical slices were visualized and analysed using Imaris software. Both plated fixed and live organoids were imaged using a Nikon Ti2 Eclipse system.

Gene expression analysis

Organoids collected by the VISIBLE, along with control samples from the original Low Wnt and High Wnt mixed populations (4 to 5 organoids per condition), were snap-frozen immediately after collection and stored at -80° C. Total RNA was extracted using the PureLink RNA Micro Kit (Thermo Fisher Scientific Cat No. 12183-016) for the organoid samples and the RNeasy Mini Kit (Qiagen, 74104) for other relevant samples. RNA was then retrotranscribed to cDNA using the High-Capacity RNA-to-cDNA Kit (Thermo Fisher Scientific Cat No. 4387406), strictly following the manufacturer's instructions. Real-time quantitative PCR (RT-qPCR) reactions were performed on a 7500 Real-Time PCR System (Applied Biosystems) utilizing Fast SYBR Green Master Mix (Thermo Fisher Scientific Cat No. 4385612). Relative gene expression analysis was obtained using the $\Delta\Delta C_t$ method, normalized to GAPDH as the housekeeping gene, and with Day 0 hiPSCs serving as the control.

Microfabrication

Stereolithography 3D printing

PDMS microwells (400×400×200 µm) for embryoid body formation, slide spacers (500 µm thickness) for whole-organoid mounting, and PDMS reservoirs (1000×1000×1000 µm at the base) for organoid sorting were fabricated as previously described³⁴. Briefly, device designs were created using Fusion360 (Autodesk) and exported as .stl files. These files were then processed using Chitubox (3D Slicer Software) for 3D printing. Following printing, the moulds were washed in isopropanol for 10 minutes in a sonicator bath and subsequently cured with UV light for 60 minutes.

Device fabrication

For the fabrication of microwell and organoid sorting devices, polydimethylsiloxane (PDMS) was prepared by mixing SYLGARD 184 Silicone Elastomer Kit (Dow) at a 1:10 ratio of silicone base to curing agent. The mixture was thoroughly degassed in a desiccator for approximately 30 minutes to remove air bubbles. Subsequently, the degassed PDMS was poured onto the previously prepared 3D-printed moulds and baked in an oven at 75° C for 15 minutes to initiate curing. The final dimensions of both the moulds and the resulting PDMS devices were precisely measured using a 3D Optical Profiler (Sensofar S Neox). All PDMS devices were then sterilized under UV-light for 15 minutes prior to use.

Medium composition

Differentiating Medium A: 1:1 Advanced DMEM/F12 (Thermofisher Cat No. 12634010) to Neurobasal (Thermofisher Cat No. 21103049), N-2 Supplement (100x, Thermofisher Cat No. 17502048), B-27 Supplement (100x, Thermofisher Cat No. 17504044), Glutamax (100x, Thermofisher Cat No. 35050061), Penicillin-Streptomycin (100x, Thermofisher Cat No. 15140122), MEM Non-Essential Amino Acids (100x, Thermofisher Cat No. 11140050). Low Wnt (0.8µM CHIR), High Wnt (1.6uM, 2uM).

Differentiating Medium B: Advanced DMEM/F12 (Thermofisher Cat No. 12634010), N-2 Supplement (100x, Thermofisher Cat No. 17502048), Glutamax (100x, Thermofisher Cat No. 35050061), Penicillin-Streptomycin (100x, Thermofisher Cat No. 15140122), MEM Non-Essential Amino Acids (100x, Thermofisher Cat No. 11140050), SB431542 (10µM, Tocris, 1614), LDN193189 (0.2µM, Stemolecule, 04-0074).

qPCR Primer List

Target Gene	Primer Forward	Primer Reverse
GAPDH	ACCCACTCCTCCACCTTTGAC	TGTTGCTGTAGCCAAATTCGTT
HOXC9	GCAGCAAGCACAAAGAGGA	CGTCTGGTACTTGGTGTAGGG
PAX3	AGGAGGCCGACTTGGAGA	CTTCATCTGATTGGGGTGCT
PAX6	GCCCTCACAAACACCTACAG	TCATAACTCCGCCCATTCAG
SOX1	GAAGCCCAGATGGAAATACG	GGACAAGGAAGGGTGTTGAG
SOX2	GGGAAATGGGAGGGGTGCAAAGAGG	TTGCGTGAGTGTGGATGGGATTGGTG
TBXT	GCTGTGACAGGTACCCAACC	CATGCAGGTGAGTTGTCAG
TBX6	CAGCTCTGTGGGAACAGAAA	CCGGAATCACATCCAGAAGAA

Neurosphere generation and culture

hiPSC-derived NGN2-induced neurons were generated using a doxycycline-inducible system from piggyBac-mediated stable integrated NGN2 hiPSCs (cell line: KOLF2.1J). Neurospheres were generated following a modified protocol as previously described³⁴. Single hiPSCs were plated onto PDMS-based microwell devices, each with well dimensions of 400 µm width × 400 µm length, placed in a 24-well plate at a density of 1.4 × 10⁶ cells per well. The cells were maintained in induction media, consisting of KnockOut DMEM/F12 (Thermo Fisher Scientific), N2 supplement (Thermo Fisher Scientific), NEAA (Thermo Fisher Scientific), mouse laminin (1 µg/mL, Thermo Fisher Scientific), ROCK inhibitor (10 µM, Tocris), and doxycycline (2 µg/mL, Sigma). Cultures were maintained at 37° C in a 5% CO₂ humidified incubator. After 24 hours, the ROCK inhibitor was removed from the culture medium. Neurospheres were then harvested and transferred to a 10-mm dish with orbital shaking at 60 rpm. On day 3 of induction, the

medium was switched to maturation media, consisting of 50% Neurobasal (Thermo Fisher Scientific) with B27 supplement (Thermo Fisher Scientific), and 50% Advanced DMEM/F12 (Thermo Fisher Scientific) with N2 supplement, along with Glutamax (Thermo Fisher Scientific) and Pen/Strep (Thermo Fisher Scientific). The neurospheres were subsequently plated onto Matrigel (Corning)-coated plates and allowed to mature for 16 days before functional-based selection experiments were conducted.

Neuronal activity analysis

Adherent neurospheres were incubated with the Ca^{2+} dye Fluo-4 AM (5 μM , Thermo Fisher Scientific) for 1 hour at 37° C in a 5% CO_2 humidified incubator. Following incubation, neurospheres were washed once with phosphate-buffered saline (PBS) and then replenished with fresh maturation medium. For calcium image analysis, acquired videos were processed using ImageJ software. Initial processing included bleach correction, followed by the selection of regions of interest (ROIs) and subsequent intensity measurements. After bleach correction, five circular ROIs, each with a 3-pixel radius, were manually drawn on individual cell soma within each neurosphere to measure the mean fluorescence intensity across the recorded image sequence. The extracted intensity data were then input into the Peakcaller⁴³ software to quantify the number of calcium peaks. Representative calcium traces for each ROI were calculated using the formula:

$$\frac{\Delta F}{F} = \frac{(F - F_0)}{F_0}$$

where F represents the current fluorescence value at any given time point, and F_0 is defined as the mean fluorescence value of the first 5 frames, serving as the baseline fluorescence.

Skeletal muscle cell culture and analysis

Myoblast cultures

Human immortalized skeletal myoblasts (AB1167) were kindly provided by the Myoline platform of the Institute of Myology (Paris, France). Cells were stably transfected with a lentivirus encoding a GFP reporter to facilitate live visualization. Myoblasts were expanded in skeletal muscle cell growth medium (Promocell) at 37° C in a 5% CO_2 humidified incubator.

For co-culture and scratch assay experiments, cells were seeded at a density of 21,000 cells/ cm^2 onto Matrigel (Corning)-coated plates. Upon reaching the appropriate confluency (typically 80-90%), the growth medium was replaced with differentiation medium. This differentiation medium consisted of DMEM high glucose (Sigma), supplemented with 1% Glutamax (Thermo Fisher Scientific), 1% penicillin/streptomycin (Thermo Fisher Scientific), and 10 $\mu\text{g}/\text{mL}$ insulin (Gibco). After 4 days of differentiation, mCherry neurospheres (NSs) were precisely deposited onto highly differentiated myoblast areas using the VISIBLE platform. Simultaneously, the skeletal muscle differentiation medium

was diluted at a 1:1 ratio with fresh medium. The resulting co-culture was maintained for a further 3 days, up to a total of 7 days.

Analysis of differentiation areas and neurosphere coverage

Acquired microscopic images were processed using Fiji (ImageJ). For the analysis of myogenic differentiation densities, five regions of interest (ROIs) were manually selected and distributed across identified low- and high-density myogenic areas within each image. Within each ROI, a selection was generated to quantify the area covered by the myogenic cells, and this data was subsequently expressed as a percentage of the total ROI area. For co-culture experiments, the areas covered by deposited neurospheres (NSs) and their processes were calculated following the identical image processing and quantification methodology.

Scratch test and analysis

To perform automated scratch tests using the VISIBLE, a 12-well plate containing 80% confluent skeletal muscle cells was placed within its incubation chamber. A syringe was attached to the print-head to introduce the scratch injury. The VISIBLE first recorded the coordinates of the scratch's starting point (X5, Y20). Subsequently, the print-head moved to the endpoint coordinates (X5, Y30) to introduce a straight, consistent scratch. This automated scratch injury creation process took 2 minutes. Following injury, the plate was continuously incubated within the VISIBLE, and images were acquired at 6-hour intervals until full scratch recovery was observed. Live-captured images were subsequently analysed using Fiji (ImageJ). The cell's ability for recovery was quantitatively measured by tracking changes in both the total empty scratch area and the gap width over various time points. Selections were generated within Fiji to precisely quantify these parameters.

Hydrogel preparation

All weighing measurements and mixing steps for hydrogel constitution were performed under sterile conditions within a biosafety cabinet. Glass beakers and stirring magnets were autoclaved prior to use.

Hydrogel matrix preparation: Preparation began by measuring 4 mL of neuronal culture medium and homogenously mixing it with 1 mL of Matrigel. Hyaluronic acid (5 mg/mL, Sigma-Aldrich) was then added to this solvent and stirred overnight (12 hours) at ambient temperature. This was followed by the addition of Fibrinogen (45 mg/mL, Sigma-Aldrich), and the mixture was stirred for an additional 5 hours at room temperature. As a final step, Alginate (5% w/v, Sigma-Aldrich) was incorporated into the mixture and stirred overnight to obtain a homogenous hydrogel matrix. Freshly prepared hydrogel was used for immediate experimental studies; however, the as-prepared hydrogel matrix can be stored at -20° C for up to 6 months.

Bio-ink preparation and crosslinking: Motor Neuron Progenitor cells (MNPs) were dissociated from a 6-well plate using a solution of EDTA in PBS. These dissociated MNPs were then gently mixed with the as-prepared hydrogel matrix at a final concentration of 4-5 million cells/mL. Homogeneous distribution of

cells throughout the hydrogel was achieved by gentle up-and-down pipetting, leading to the formation of a cell-laden bio-ink. This bio-ink was subsequently crosslinked by immersion in a 50:50 solution consisting of calcium chloride (CaCl_2) (1.5% (w/v)) and thrombin (25 U/mL in 0.1% BSA solution). Bio-ink was crosslinked for 15 minutes at room temperature. After crosslinking, the bio-ink was washed with PBS for 3 times. This was followed by flooding the wells gently through the walls of a cell plate with neuronal culture media supplemented with Compound E and kept in an incubator at 37° C with 5% CO_2 .

LARRY lineage tracing

Detailed patient-derived breast cancer organoid culturing conditions and experimental methods can be found in Ratcliffe et al, BioRxiv 2025. Key steps are summarised below. GCRC1915 PDXO parental organoid lines were generated from the GCRC1915Tc PDX⁴¹ and authenticated, as well as tested for mycoplasma contamination by the Cell Services Platform at the Francis Crick Institute. LARRY Barcode Version 1 library was a gift from Fernando Camargo (Addgene #140024) and was used to prepare lentiviral particles. GCRC1915 PDXO cells were infected with an MOI of 50 in 200 μL culture media lacking Cultrex under culture conditions (37°C, 5% CO_2 , humidified atmosphere) for 1 hour. Cells were then split into 3 and plated. After 7 days of culture, organoids were sampled using VISIBLE.

In vivo experiments

The Francis Crick Institute's Animal Welfare and Ethical Review Body and UK Home Office authority provided by Project License 0736231 approved all animal model procedures. Procedures described in this study were compliant with relevant ethical regulations regarding animal research. Using the VISIBLE, GFP⁺ organoids were sampled, centrifuged and resuspended in Matrigel (Corning Cat No. 354234) prior to injection. After 5 weeks, tumours were collected, dissociated and human tumour cells were enriched using a Miltenyi tumour dissociator according manufacturer's instructions.

Single cell processing, sequencing and analysis

Cell concentration and viability was measured and approximately 120,000 cells per tumour were loaded on a Chromium Chip and processed according to manufacturer's instructions (CG000315 Chromium Single Cell 3' Reagent Kits User Guide (v3.1 - Dual Index)) to generate cDNA libraries using Chromium Next GEM Single Cell library reagents. Final libraries are QC'd using the Agilent TapeStation and sequenced using the Illumina NovaSeq 6000. Sequencing read configuration: 28-10-10-90.

The GFP-LARRY plasmid was added to the reference genome refdata-gex-GRCh38-2020-A and the GTF annotation of the plasmid transcripts was added to the same reference annotation file. Cellranger (version 7.0.1) mkref was run to create a new reference for genome annotation. Cellranger count was used to count the 10x libraries. A whitelist of cell barcodes was obtained with UMI-tools (version 1.1.2)⁴⁴ and the cellranger quantifications were imported into a Seurat (version 4.3.0)⁴⁵ object. Doublets were calculated using scDblFinder (version 1.8.0)⁴⁶ and the LARRY barcode information was added to the Seurat objects. The R programming language was used (version 4.1.2) (R Development Core Team

2008). We investigated key QC parameters, and we removed cells with a low number of detected features or cells with a very high proportion of mitochondrial gene expression. We selected a lower bound filter for both the minimum number of reads per cell and the minimum number of detected features using 3 median absolute deviations (MADs). For the percentage of mitochondrial genes, we set upper bounds using 3 MADs. We use the SCTransform method (version 0.3.5)⁴⁷ for the normalisation and variance stabilisation. The dataset was subsetting according to the lowest 25% and highest 25% FGFR1 expressing cells. In the global population, as well as within subsets, barcodes were used to determine the Shannon diversity index according to:

$$H = - \sum (p_i * \ln(p_i))$$

Where H is the Shannon diversity index, p_i is the proportion of a clone belonging to the barcoded population.

Figures

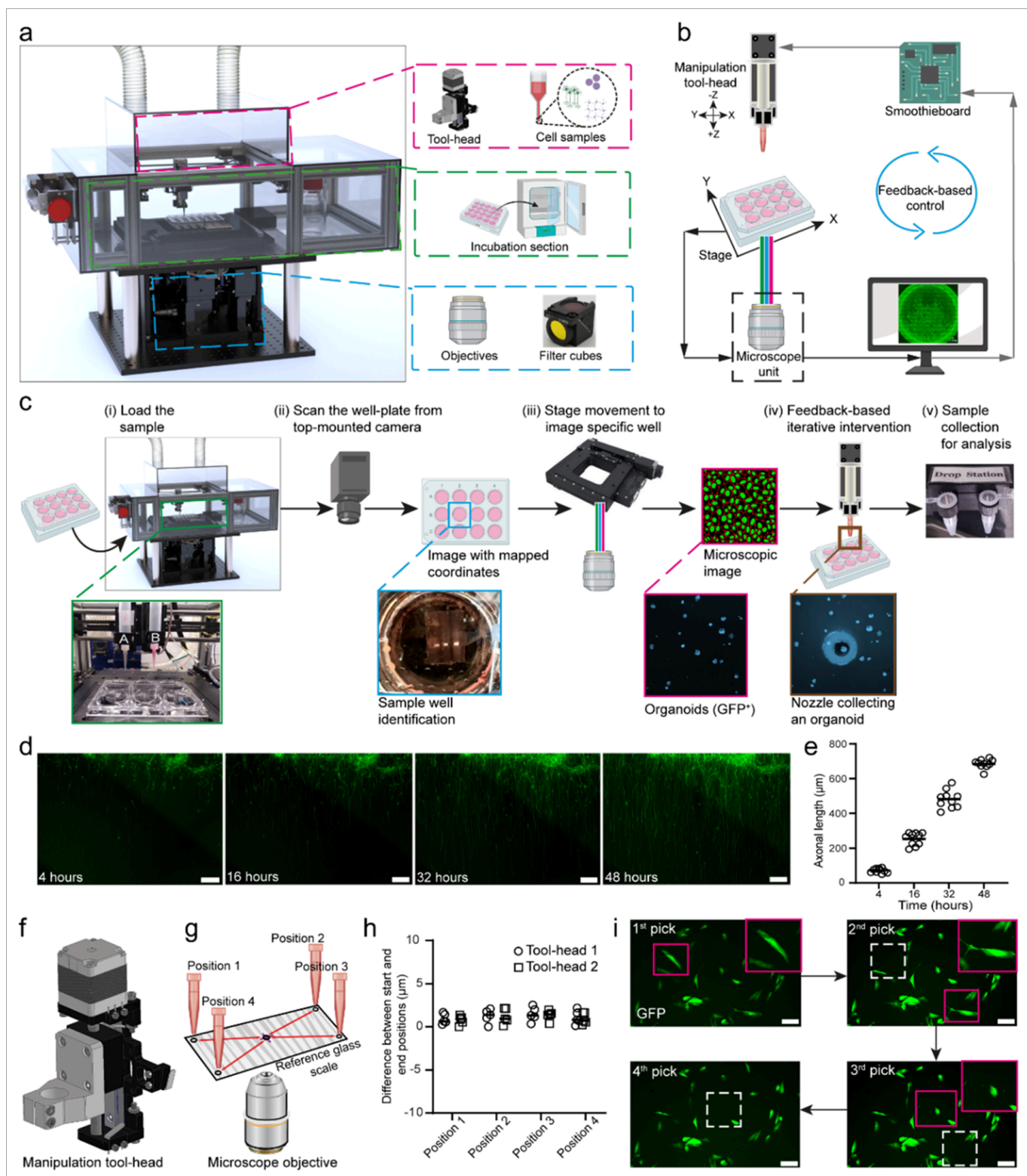


Figure 1

Design and engineering of the VISIBLE platform. (a) Schematic representation of the VISIBLE platform detailing its modular sections and core components. (b) Flow diagram illustrating VISIBLE's feedback-based closed-loop operation, which enables imaging-guided iterative interaction with live biological experiments. (c) Stepwise process flow of a typical experiment: coordinate mapping via top camera, microscope-guided targeting, sample manipulation (e.g., aspiration, deposition), and sample collection

Image-guided precision sampling mitigates organoid heterogeneity. (a) Schematic illustrating the experimental design: Neuroectodermal organoids treated with varying CHIR concentrations generate distinct low and high Wnt activation populations. These are mixed in equal proportions to create a complex heterogeneous culture (CIVM) for VISIBLE's targeted sampling and subsequent downstream analysis. (b) Real-time morphology-based sampling by VISIBLE. Left panel: stitched overview of the PDMS macrowell containing suspended organoid populations. Scale bar: 1 mm. Centre panel: organoids displaying symmetry-breaking morphology (population 2, yellow outline) were identified and targeted. Right panel-top: select-lock-pick sequence illustrating sampling of an individual organoid without disturbing adjacent structures. Right panel-bottom: high-resolution image of sampled organoids shows preservation of fine architectural features (white arrows). Scale bar: 200 μm . (c-d) Immunocytochemistry (ICC) studies of sampled Population 1 and Population 2 organoids confirm their distinct cellular organization (c). Scale bar: 2 μm . Relative gene expression analysis of original and VISIBLE-sampled organoids validate the preservation of molecular identity post-sampling (d). (e) A collected organoid successfully re-plated by the VISIBLE remains viable and shows robust migratory cell populations after two days in culture, affirming functional integrity post-sampling. Scale bar: 1 μm .

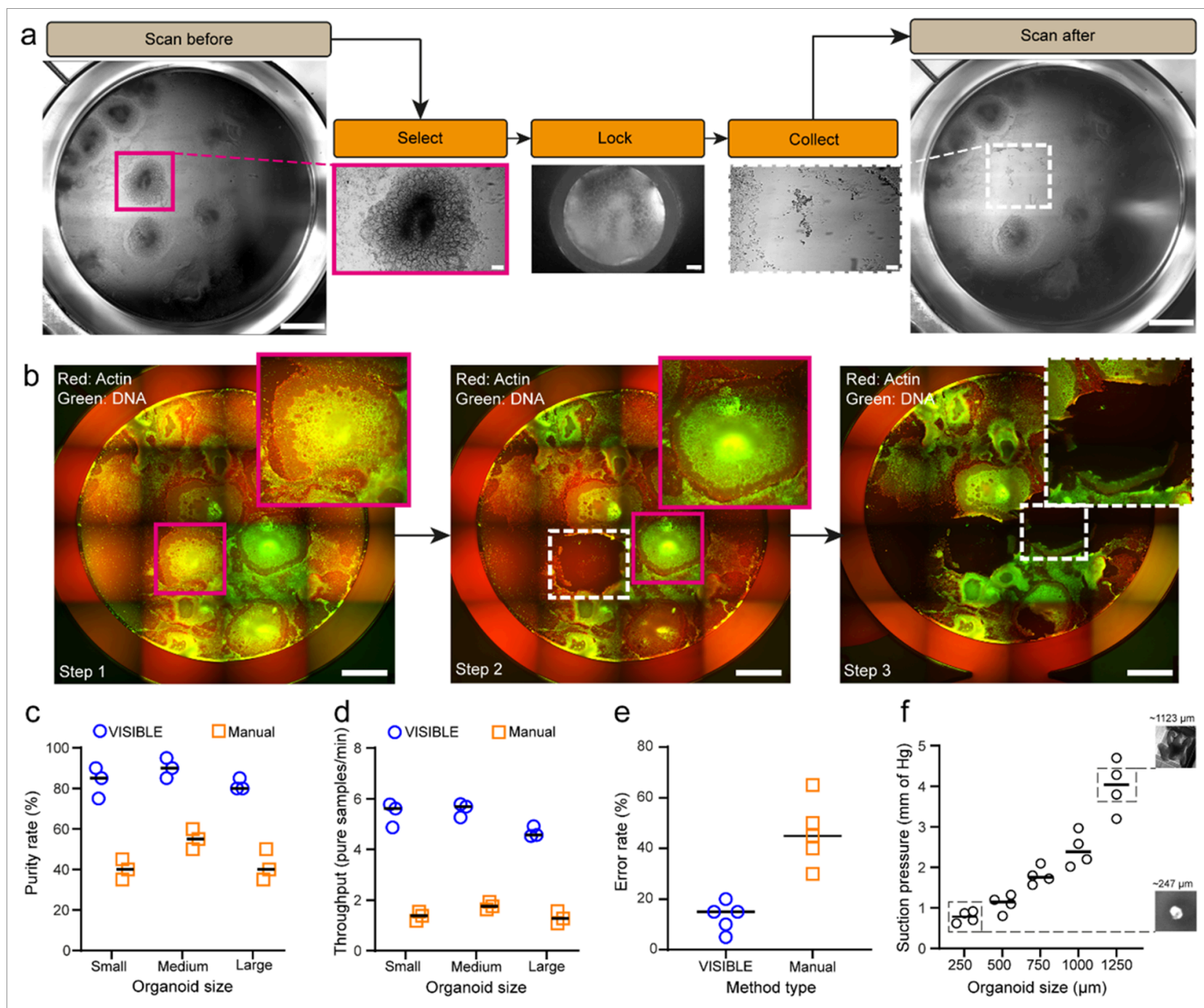


Figure 3

Precision sampling of adherent organoids using image-guided intervention. (a) Stitched image of a well containing live, growing adherent organoids (left-most panel) Scale bar: 1 mm. VISIBLE's image-guided system spots dynamic filamentous structures extending from a live organoid (magenta outline), enabling its precise selection for collection (middle panel). The post-sampling image confirms precise removal of the target organoid (white dashed outline) without disturbing neighbouring structures (right-most panel). Scale bar: 1 mm. (b) Multichannel fluorescence detection using VISIBLE allows for the simultaneous imaging of multiple protein markers, enabling function- and lineage-based identification of adherent organoids for targeted sampling. Scale bar: 1 mm. (c-e) Performance benchmarking of VISIBLE compared to manual handling, quantifying purity rate (c), throughput (d), and error rate (e) across varying organoid sizes: small (<250 μm), medium (250-750 μm), and large (>750 μm). VISIBLE consistently demonstrates superior performance. (f) Suction pressure required for organoid lift-off shows linear

scaling with organoid diameter, underscoring the need for real-time pressure calibration within the platform, crucial for non-destructive, size-specific sampling.

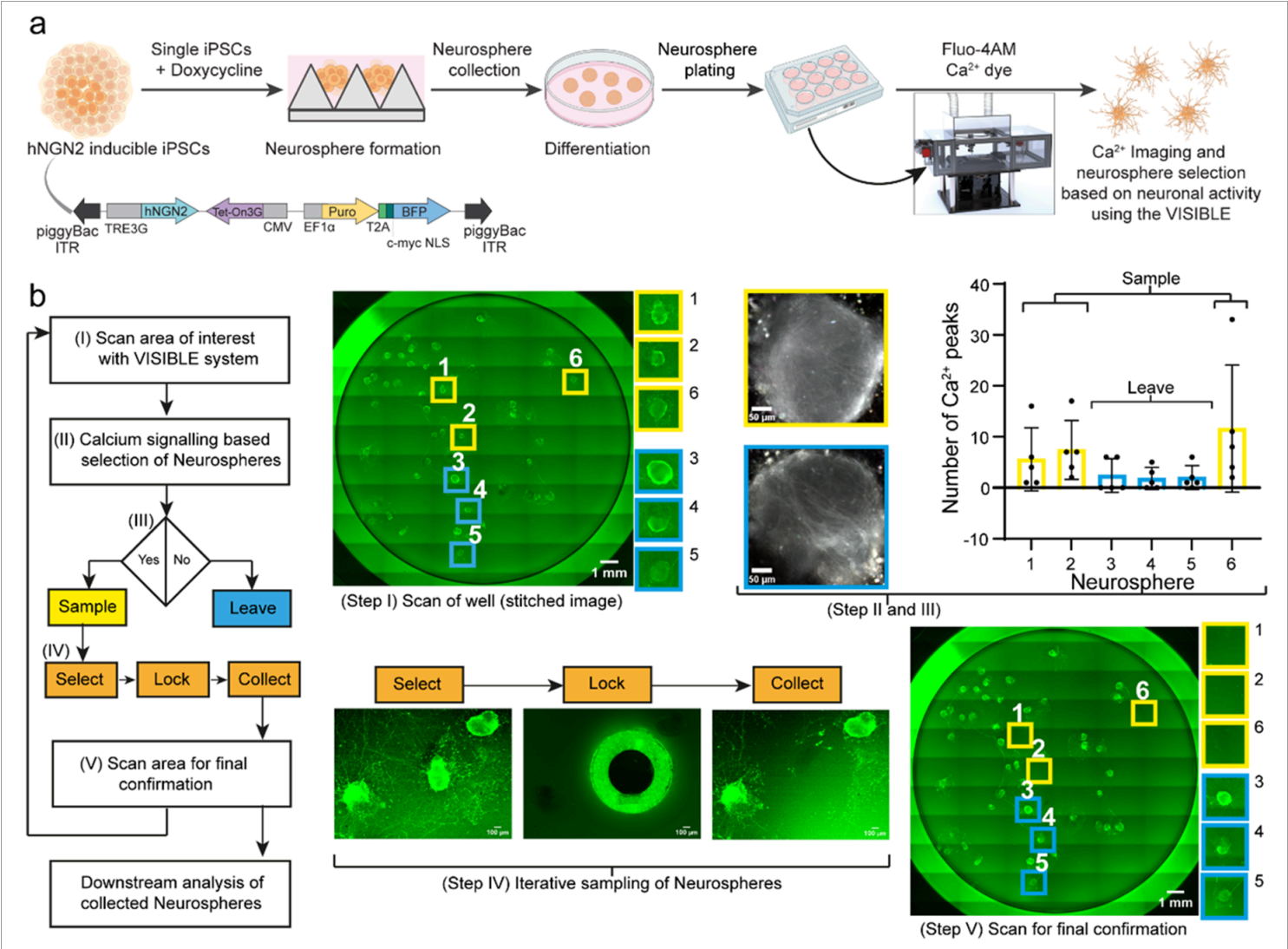


Figure 4

Function-based sampling of adherent neurospheres in complex culture. (a) Schematic illustrating the generation of glutamatergic neurons through doxycycline-inducible expression of the NGN2 gene integrated into a hiPSC line (KOLF2.1J) using the piggyBac transposon system. These neurospheres were subsequently plated in a 12-well plate for interactive calcium signalling experiments with the VISIBLE platform. (b) Process flow chart of VISIBLE's operation for functional sampling: (Step I) The platform performs a comprehensive scan of the entire area, showing adherent neurospheres on a Matrigel-coated plate. Scale bar: 1 mm. (Step II-III) Identification of neurospheres exhibiting active calcium signals, specifically those with more than 5 peaks from 10 defined regions of interest (ROIs). (Step IV) The VISIBLE then executes a precise select, lock, and collect mechanism to extract the targeted neurosphere of interest (exhibiting >5 peaks). (Step V) A final scan of the well showing the successful selective sampling: neurospheres with higher numbers of calcium peaks (numbered 1, 2, and 6) (yellow

outline) were collected, while those with fewer peaks (numbered 3, 4, and 5) (blue outline) remained intact. Scale bar: 1 mm.

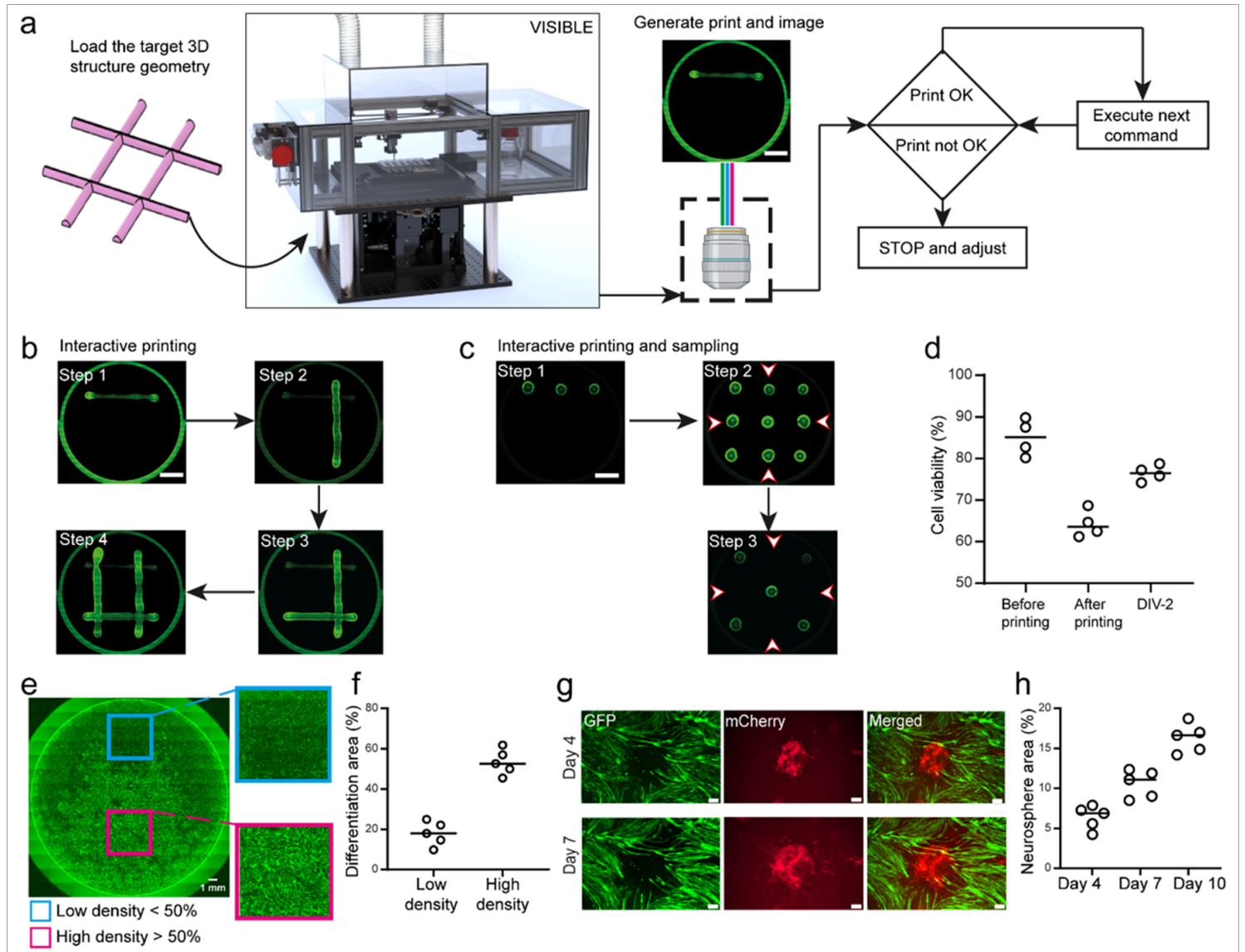


Figure 5

Interactive 3D bioprinting and serial intervention for spatiotemporal experiments. (a) Schematic illustrating the interactive 3D bioprinting process: a desired target geometry is fed to VISIBLE, which executes the first segment, images it, and analyzes for discrepancies. The VISIBLE then either proceeds to the next instruction if the print is optimal or pauses to adjust print parameters for error elimination. (b) Demonstration of serial intervention 3D printing of hydrogel, showcasing a print-pause-analyse-print procedure that enables live, adaptive interaction based on the previous print. Scale bar: 1 mm. (c) Example of 3D printing a dot-patterned hydrogel (top), followed by the precise selection and collection of a specific region of interest (bottom). Scale bar: 1 mm. (d) Quantification of cell viability after the printing process, establishing the biocompatibility of the hydraulic-based extrusion method for cell-embedded hydrogels. Scale bar: 1 mm. (e-f) Scan of a well cultured with differentiated muscle cells (e), followed by the selection of two distinct areas based on their differentiation densities and respective quantification

(f). (g-h) Co-culturing of hiPSC-derived motor neurospheres precisely deposited onto high-density muscle cell areas. After one week in culture, effective interaction between motor neuron processes and the underlying differentiated muscle cells is observed, characterized by increased coverage area. Scale bar: 200 μm .

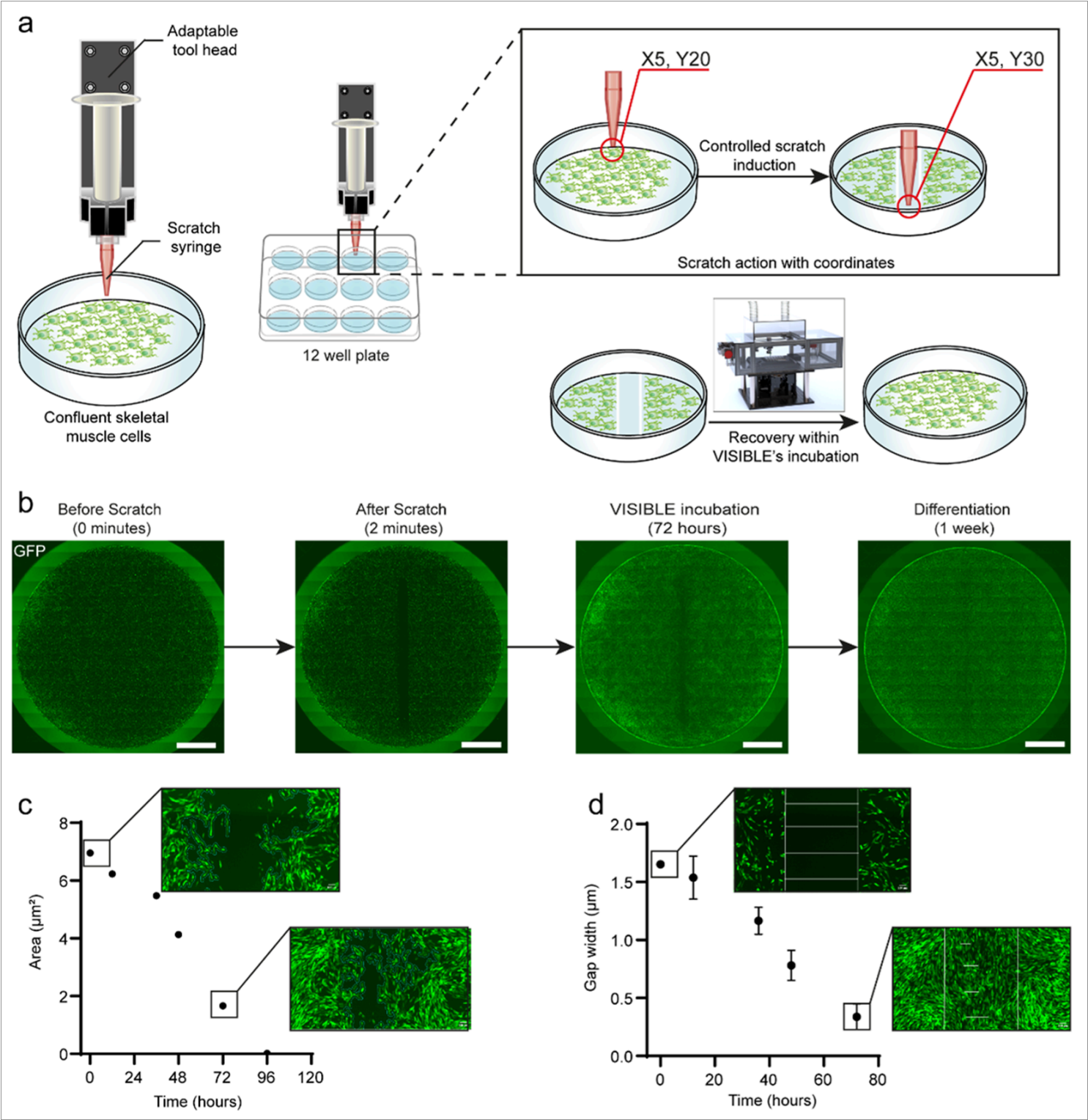


Figure 6

Autonomous serial intervention for reproducible longitudinal studies. (a) Schematic outlining the steps for an autonomous scratch assay, illustrating scratch creation, subsequent recovery, and cellular differentiation were comprehensively monitored via the VISIBLE platform with minimal human involvement. (b) A series of time-lapse images demonstrating the progression of scratch recovery within VISIBLE's integrated incubation environment, confirming its suitability for extended longitudinal experiments. Scale bar: 1 mm. (c-d) Quantification of cell migration during scratch recovery: graphs illustrating the decreasing trend in both scratch area (c) and gap width (d) as time progresses, indicative of robust and reproducible recovery.

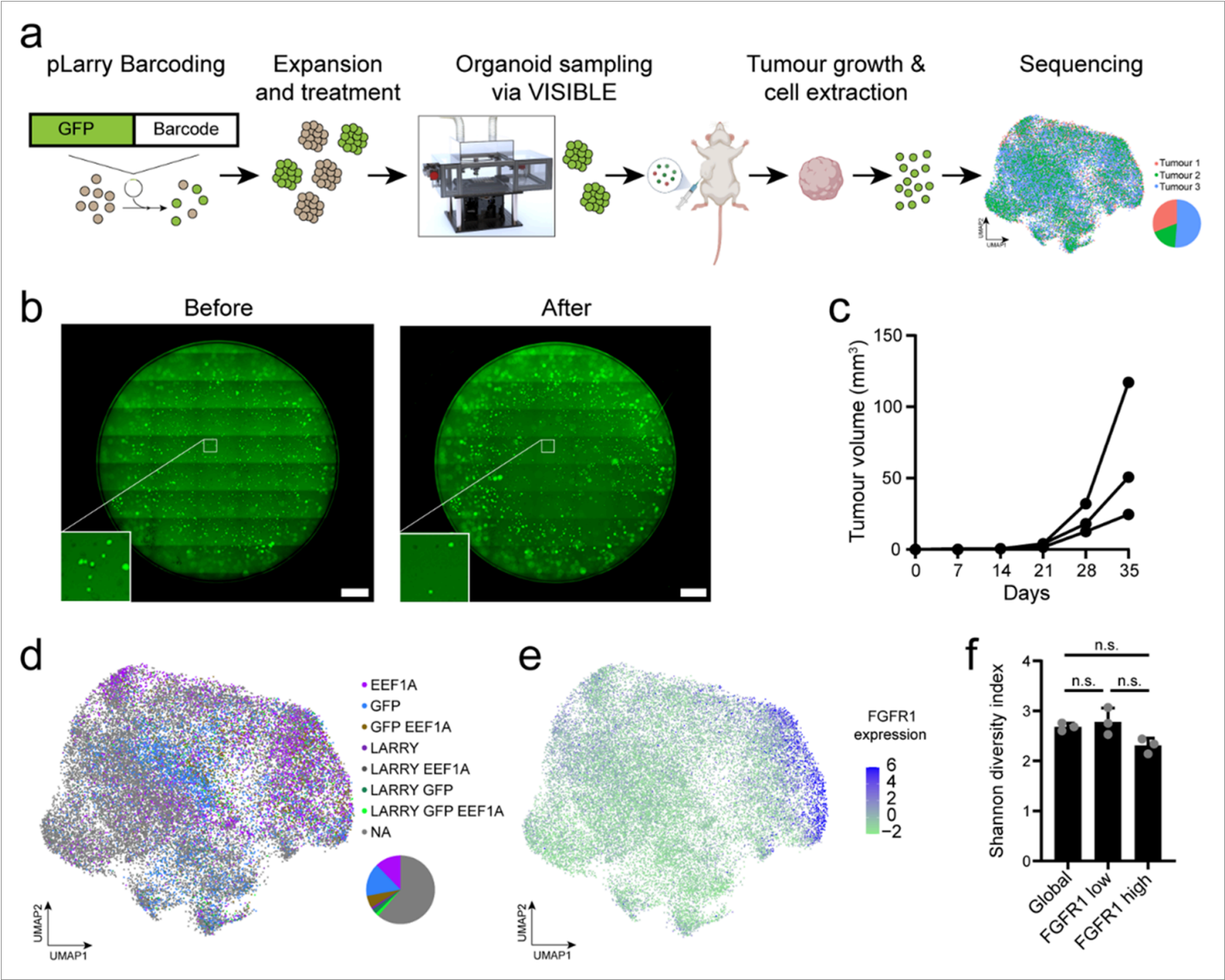


Figure 7

Integration into preclinical experimental pipelines via barcoded organoid sampling. (a) Schematic of the experimental workflow wherein pLARRY barcoded, eGFP-expressing triple-negative breast cancer PDXO organoids were selectively sampled using the VISIBLE platform and subsequently implanted into immunodeficient mice for tumour formation and single-cell RNA sequencing. (b) Representative well

images before and after VISIBLE-mediated sampling of GFP⁺ organoids, demonstrating selective isolation without disturbing surrounding structures. Scale bar: 1 mm. **(c)** Volume quantification of tumours formed from VISIBLE-selected GFP⁺ organoids (n = 3 mice), demonstrating their sustained tumorigenic potential in vivo. **(d-e)** Uniform manifold approximation and projection (UMAP) representation of single cell gene expression data generated from dissociated tumours. Overlay indicates cells where elements of the pLARRY vector were detected **(d)**, and overlay indicates FGFR1 expression **(e)**. **(f)** Shannon diversity indices comparing clonal diversity across the global tumour population, FGFR1-low (bottom 25% quartile), and FGFR1-high (top 25% quartile) populations.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [ExtendedData18072025.docx](#)