60ND × AXION BIOSYSTEMS

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Seeing the force

Integrating the 60Nd NeoMag® actuation platform with the Axion BioSystems Lux3™ incubator live-cell imaging device.

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Actuation and live-cell imaging have been mutually exclusive in practice: the actuator either needs a hand on it or interferes with the optical path, so the sample leaves the incubator to be imaged. Mounted on the Lux3™ stage inside a standard CO_2 incubator, NeoMag® and the Lux3™ work as one unit that images continuously, in focus, while the sample is under load.

See what you actuate. Actuate what you see.

ACTUATION AREA	CONTINUOUS MONITORING	OPTICAL RESOLUTION
Ø 8 mm	>120 h	0.7 µm/px
DEFORMATION IN 2D	**SUBSTRATE RIGIDITY**	**SYSTEM FOOTPRINT**
~30%	100 Pa–20 kPa	166 × 140 × 135 mm

SUMMARY

Combining mechanical actuation with live-cell imaging remains technically demanding. Generally, the mechanical actuator will either require manual intervention or interfere with the optical path of the imaging device, thus requiring the sample to be removed from the incubator and/or imaged separately. Therefore, most in vitro assays either leave mechanics out or read a single static endpoint and do not capture how cells respond to defined mechanical input over time.[1]

Here we demonstrate the integration of NeoMag®, a remote in vitro mechanical actuation platform, with the Axion Lux3™ incubator-compatible live-cell imaging device as a single functional unit. Using a scratch wound-healing assay on human fibroblasts as the validation case, we show continuous, in-focus live-cell imaging under both sustained and dynamic actuation protocols. The result is a plug-and-play and portable workflow in which researchers can, for the first time, see what they actuate and actuate what they see.

WHAT THIS ENABLES

- Mechanotransduction in real time. Apply cyclic strain and watch the response unfold: migration and proliferation dynamics in disease and drug response, read on a substrate tuned to real tissue (~100 Pa–20 kPa) rather than on rigid plastic.
- Your assay, not just ours. We have seeded at the most demanding focal plane, so an assay at that plane or a lower one fits comfortably: a 2D monolayer, or a 3D scaffold within the MagFlex™ holder.
- No infrastructure overhaul. The actuation assembly mounts on the Lux3™ stage inside your lab's standard CO_2 incubator. No custom enclosure, no dedicated imaging room, no changes to your workflow.

If you already run a Lux3™, this adds the mechanical dimension to assays you already image. If you already run a NeoMag®, it lets you watch, continuously, what you have been measuring at endpoints.

● THE INTEGRATION

Two incubator-resident instruments, one functional unit

The NeoMag® platform (60Nd) addresses the actuation side, delivering controlled, programmable mechanical stimulation to in vitro models at loads of up to ~30% deformation and frequencies of up to 1 Hz.[2] The Axion Lux3™ addresses the imaging side: a compact, fully incubator-compatible inverted brightfield live-cell imager with a 1.45×1.45 mm field of view and 0.7 µm/pixel resolution (2072×2072 px). **Put simply, NeoMag® provides the touch and the Lux3™ provides the eyes.**

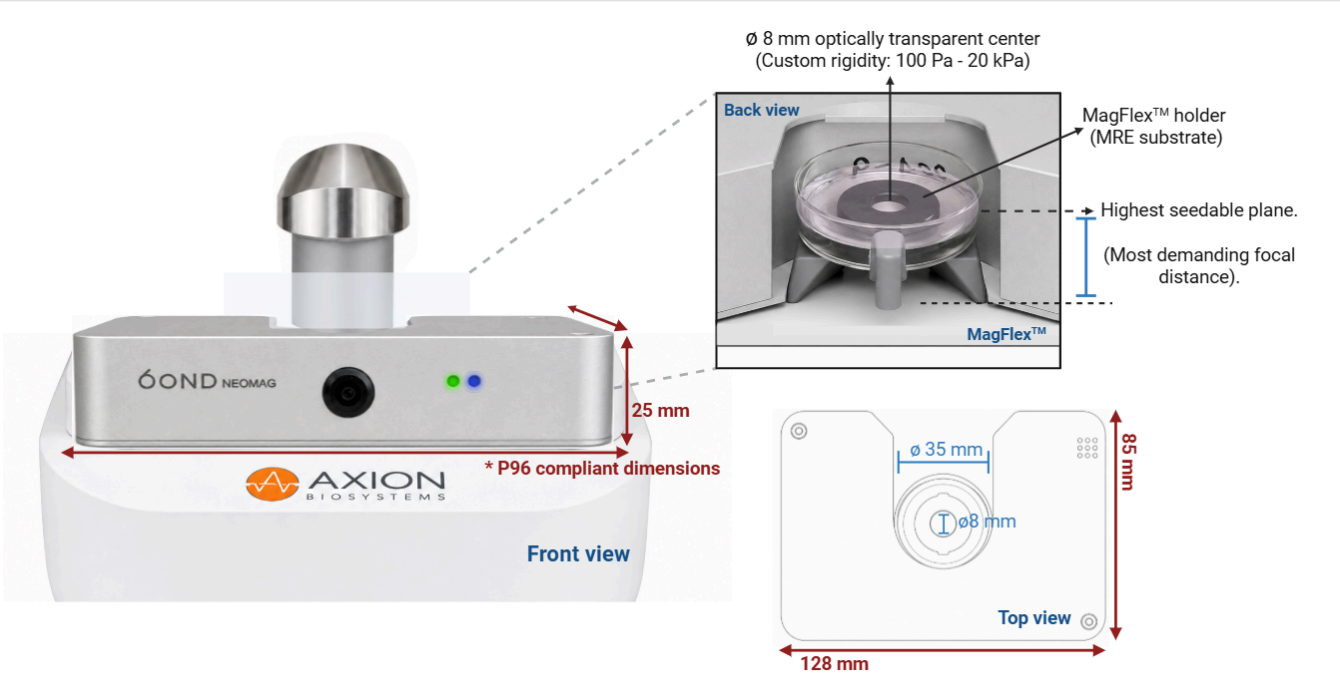

FIGURE 1. HARDWARE INTEGRATION AND MAGFLEX™ SET-UP

P35 dish and active area dimensioned. The Ø 8 mm optically transparent central region of the MagFlex™ is the actuation domain: the magnetically inert, cell-carrying area over which strain is delivered and imaged, 4 mm deep for 3D models. The highest seedable plane sits ~200 µm inside the Lux3™ working distance.

A targeted NeoMag® hardware redesign positions the highest seedable surface within the Lux3™ microscope's working distance, with approximately 200 µm of remaining clearance (about 20% of the working distance). This margin also accommodates assays at lower focal planes, supporting configurations beyond those examined here, including 2D monolayers within the MagFlex™ MRE and 3D scaffolds embedded in ECM-mimicking hydrogel matrices.[3]

Field of view	1.45 × 1.45 mm
Optical resolution	0.7 µm/px (2072 × 2072 px)
Cyclic loading	up to 1 Hz
Capture interval	From 5 min, software-set
System footprint	166 × 140 × 135 mm

FIGURE 2. THE COMPLETE SYSTEM ON ONE INCUBATOR SHELF

● WHAT THE INTEGRATION GIVES YOU

SEE WHAT YOU ACTUATE

Uninterrupted acquisition under mechanical load: no sample removal from the incubator and no focus adjustment during actuation. Focus holds across both quasi-static and cyclic loading, up to ~20% and ~10% deformation, respectively.

DAYS OF DATA, NOT TIMEPOINTS

Multi-day acquisition captures the full time-course of the mechanical response, resolving direction-dependent behavior that a single-endpoint assay cannot see.

ONE PLATFORM, EVERY MODEL

From monolayers to 3D matrices: the Ø 8 mm actuation area, with 4 mm of depth, covers 2D monolayers and 3D scaffolds alike.

● THE OPTICAL PROBLEM

A moving target, captured without refocusing

Combining mechanical actuation with continuous live-cell imaging is still the exception rather than the rule. Most cell-stretching devices are built around the mechanics alone: actuation is paused during image acquisition or only carried out at the endpoint, and the handful of systems that do image under load typically require a stage-top enclosure or a bespoke rig built around one dedicated microscope.[1]

Even where one instrument does both, the actuation works against the optics. Stretching the substrate also moves it vertically, so the cells sit at a slightly lower focal plane when the sample is stretched than when it is relaxed. Under a cyclic or intermittent load, referred to here as dynamic actuation, the sample therefore alternates between two focal planes within every cycle. Most

instruments can hold only one of them in focus, so they must either pause the actuation while imaging or refocus between frames.

VALIDATION CASE

Wound-healing assay, run and imaged under mechanical load

To test this in practice, we ran a scratch wound-healing assay on human fibroblasts end to end under mechanical actuation. The workflow begins with MagFlex™, the proprietary NeoMag consumable, whose Ø 8 mm central region holds the cells over a soft PDMS layer tuned to the firmness of natural skin tissue (~3 kPa; Figure 1). Human fibroblasts were seeded onto this region as a 2D monolayer and showed healthy growth and viability over seven days, growing from 20% to 100% confluency.

Before testing under load, we confirmed that the integrated system reproduces a standard scratch assay when nothing is actuated: continuous incubator imaging with the Lux3™ showed a clean, comparable recreation of the assay on the MagFlex™ consumable under no mechanical actuation, with the initial wound defined by a commercial ibidi insert (ref. 80209, kept for consistency across every condition).

Only once this baseline held did we test the harder case: keeping the sample in focus while it was actually moving. Sustained, quasi-static loading was imaged cleanly up to ~20% deformation. The harder optical challenge is dynamic loading, where the sample alternates between two focal planes on every cycle; here, we confirmed no-refocusing performance at 0.3 Hz between the relaxed state and ~10% deformation, imaging every 15 minutes over 24 hours. The technology's envelope is wider still: NeoMag® reaches deformations of ~30% and frequencies of up to 1 Hz, and the Lux3™ can acquire at intervals as short as 5 minutes continuously during multi-day experiments.

With continuous, in-focus acquisition in hand, we used it to ask a biological question, not only a technical one. Directional mechanical cues are known to steer fibroblast behavior: cells sense and align relative to the axis of an applied strain, a form of contact guidance that can speed up or resist migration depending on whether the force runs with or against the direction of travel.[4,5]

A scratch wound is a natural way to test this, because closing it requires directed migration across a defined gap, so we asked whether the direction and magnitude of an applied strain shifts how fast that gap closes. Six mechanical actuation conditions were tested, varying strain magnitude (10% and 20%) and actuation mode: a relaxed control with no actuation, sustained actuation in parallel and perpendicular orientations at the two load levels, and a uniaxial dynamic protocol at 0.3 Hz. Both deformation magnitudes represent real strains attainable in human skin, so the conditions are physiologically plausible rather than abstract test points.

Perpendicular stretch is pro-closure geometrically, since the wound narrows, but it polarizes fibroblasts against the closure direction. Parallel stretch does the opposite: it widens the wound yet polarizes the cells toward closure. Because the load changes the apparent size of the initial wound, all closure data are normalized to the initial wound area of each condition, which isolates the mechanical effect from the initial wound size.

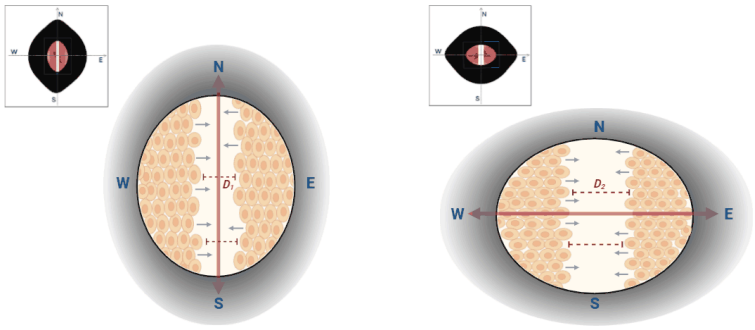

FIGURE 3. WOUND-CLOSURE GEOMETRY UNDER THE TWO LOAD DIRECTIONS

Left, perpendicular load: it narrows the wound but polarizes fibroblasts against the closure axis. Right, parallel load: it widens the wound while polarizing them toward closure. Red arrows mark the traction direction, grey arrows the direction of wound closure.

RESULTS

Direction changes closure speed by a factor of 1.6

Cell-free area was quantified in each frame by delineating the wound front. Normalized to the relaxed control, mean closure speed ran from 1.12 for parallel-high down to 0.70 for perpendicular-high: a 1.6-fold spread driven by the direction of the load alone, with parallel-high closing fastest.

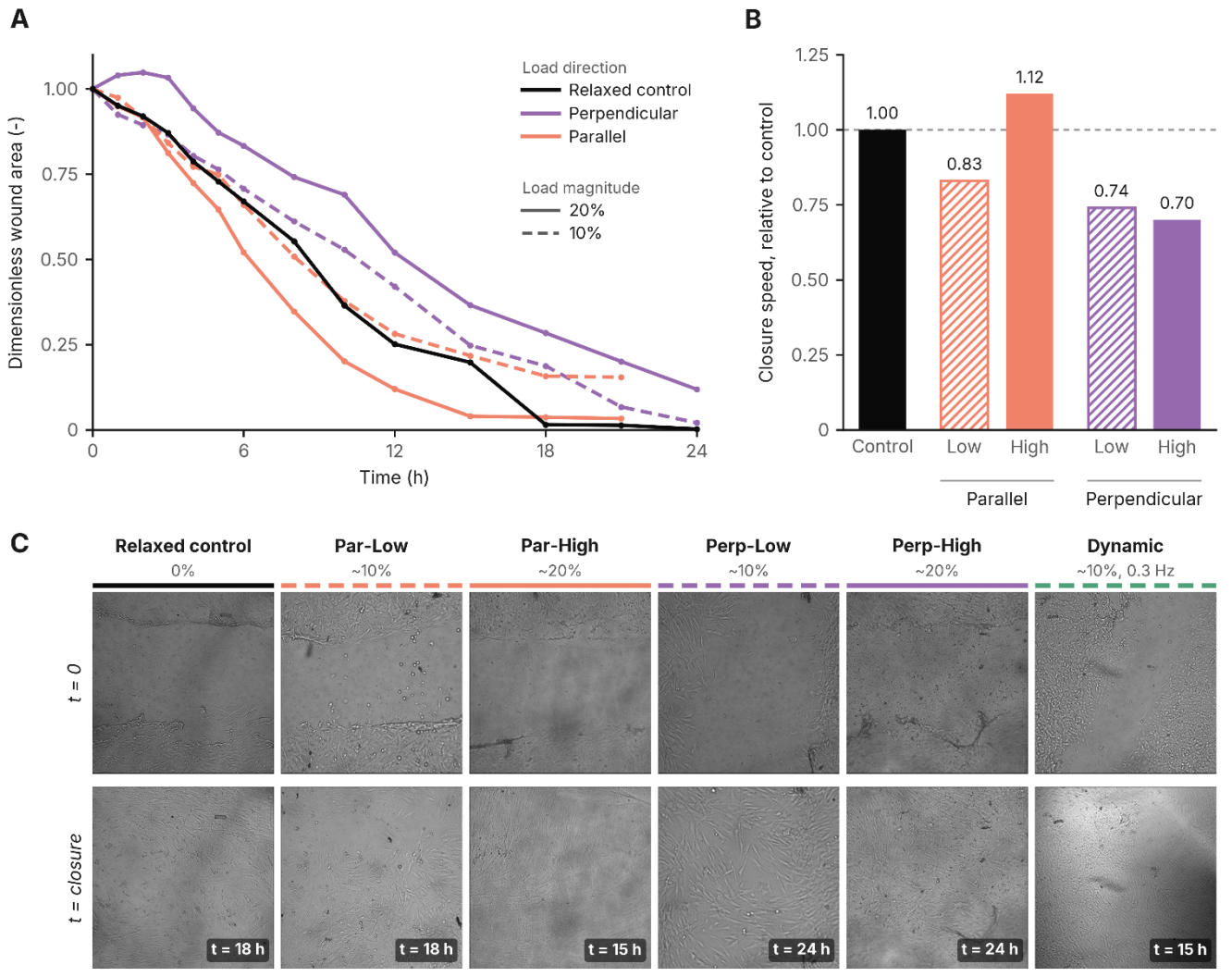

FIGURE 4. DIRECTION AND MAGNITUDE OF MECHANICAL LOAD MODULATE WOUND CLOSURE UNDER CONTINUOUS LUX3™ IMAGING

(A) Wound area over time for each condition, normalized to the initial area of the same wound. Line color indicates load direction relative to the closure axis (black, relaxed control; orange, parallel; purple, perpendicular), and line style indicates load magnitude (solid, ~20% strain; dashed, ~10% strain). (B) Mean closure speed for each condition, normalized to the relaxed control. The dashed line marks the control at 1.00. Solid bars correspond to ~20% strain and hatched bars to ~10% strain, following the line styles in (A). (C) Representative brightfield images of the wound on the MagFlex™, acquired with the Lux3™ inside the incubator. The top row shows t=0, immediately after removal of the insert. The bottom row shows t=closure, the condition-specific time at which the closure rate plateaus (indicated in each image). The dynamic condition was actuated cyclically at 0.3 Hz between the relaxed state and ~10% strain, parallel to the closure axis. Images were acquired every 15 min over 24 h. One replicate per condition (n=1): the data show trends that demonstrate the acquisition-to-quantification pipeline and are not statistically powered comparisons.

This spread follows the geometric logic set out above. Perpendicular loading tenses the cell sheet across the closure axis, so the leading edge treats the stretch as a directional cue and migrates more slowly; parallel loading polarizes cells along the same axis they must travel, so closure proceeds faster.

Perpendicular-low follows the high-load trend, whereas parallel-low does not conform strictly to the hypothesis. As a technology-enabling proof of concept, this study used a single replicate per condition: the data are presented as trends that demonstrate the full acquisition-to-quantification pipeline operating under active actuation, rather than as statistically powered biological conclusions.

WHY IT MATTERS

The mechanical dimension, made visible

The closure-rate differences above are only visible because the system kept imaging while the sample was under strain. **A single endpoint reading would show that every wound eventually closed, not that direction and magnitude change how fast, nor that two opposite geometric effects, a narrower wound that closes more slowly and a wider one that closes faster, can hold at once.**

That distinction matters because it acknowledges the mechanical microenvironment as a primary regulator of cell behavior, whereby strain, stiffness and the polarity of an applied force influence migration, proliferation and tissue remodelling.[4,5] These cues in skin have been demonstrated to shape fibroblast activation and scar formation.[6,7] However, conventional scratch assays have largely ignored them, run instead on rigid plastics that do not reflect the true stiffness of skin.[1]

What has been missing is a bridge between stimulation and measurement: models that reproduce the physiological and mechanical environment of real tissue, and hardware that can capture the cellular response in real time. The FDA Modernization Act 2.0, pushing the field from animal models toward in vitro evidence, makes that combination a requirement rather than a refinement, and the workflow described here is built for it.

Our study also connects to the wider mechanobiology field. Extracellular matrix (ECM) stiffness and mechanotransduction pathways, among them YAP/TAZ, PI3K/Akt and FAK/ERK, are established regulators of proliferation and migration in dermal and scar fibroblasts, and recent reports also show that cyclic stretch can trigger Piezo1-dependent calcium influx in human dermal fibroblasts, elevating not only proliferation and migration but also differentiation and collagen production.[7] Strain direction is not only a migratory cue but a determinant of cell mechanics: in glioblastoma cells, aligned and perpendicular actuation reorganize the actin network differently, producing opposite changes in cell stiffness,[8] the same directional sensitivity we show above for wound closure. Our integrated system provides users with a pipeline that can apply that cyclic stretch or sustained actuation and simultaneously watch the response as it unfolds, which is precisely what is needed to interrogate these pathways in vitro.

CONCLUSION

The integration of NeoMag® with the Lux3™ enables real-time, in-focus imaging of cells while they are mechanically actuated, closing a long-standing gap between mechanical stimulation and live visualization. A targeted hardware redesign keeps the sample within the microscope's working distance even at the most demanding focal plane, leaving ~20% headroom and thereby generalizing the approach to any 2D assay and to 3D scaffolds cultured within the MagFlex™.

Wound closure was tracked end to end under active actuation across sustained and dynamic conditions, demonstrating a complete acquisition-to-quantification workflow. Several extensions are under assessment with Axion, including the Lux3™ fluorescence module and the AxIS Vue analysis algorithms for automated closure quantification, opening disease-relevant directions in fibrosis where directional mechanical cues are central.[6]

MATERIALS AND METHODS

CELLS AND CONSUMABLE

The MagFlex™, NeoMag's proprietary consumable, is 3D-printed as a two-region substrate: an outer magneto-responsive region, a PDMS-based magnetorheological elastomer (MRE) that transmits the applied magnetic field as mechanical strain to a central, magnetically inert and optically transparent region that carries the cells. The soft imaging layer is cast in biocompatible PDMS (DOWSIL CY52-276), tuned to ~3 kPa to approximate the firmness of skin tissue. The central region was surface-treated by oxygen-plasma activation and then coated with 20 µg/mL bovine collagen I. Human fibroblasts were seeded onto it at 100,000 cells, confined to the central area, and allowed to adhere for four hours until confluent.

WOUND STANDARDIZATION

A commercial ibidi scratch insert (ref. 80209, cell-free gap 500 µm) was used to define a reproducible initial gap. Removal of the insert produced a clean, consistent scratch, allowing wound closure to be normalized across mechanical and control conditions.

MECHANICAL ACTUATION CONDITIONS

Mechanical load was set physically by the distance between the actuating magnet and the P35 dish: high load corresponded to ~20% maximum strain, and low load to ~10% strain. Strain was applied either perpendicular or parallel to the axis of wound closure, giving two load levels per direction. Four static loading conditions were compared alongside an individual dynamic protocol at 0.3 Hz, with a maximum load of ~10% deformation.

HARDWARE CONFIGURATION

To ensure compatibility and ease of use with incubator-resident inverted microscopy, the NeoMag® hardware was redesigned to reduce its profile and adapt to the working distance of the Lux3™. The actuation assembly was mounted on the Lux3™ stage inside a standard CO_2 incubator (37°C, 5.0% CO_2).

IMAGING

Brightfield images were captured on the Lux3™ at 15-minute intervals over 24 hours for each condition, under both sustained and dynamic actuation. Wound area was extracted from the image series and cell-free area quantified in each frame by delineating the wound front. Wound area was normalized to its initial value (t=0) for each condition.

TABLE 1. MECHANICAL ACTUATION CONDITIONS

CONDITION	DIRECTION	LOAD / STRAIN	FIBROBLAST POLARIZATION	WOUND GEOMETRY
Relaxed control	None	0%	Unbiased	Baseline
Perp-Low	Across closure axis	~10%	Against closure	Narrows
Perp-High	Across closure axis	~20%	Against closure	Narrows
Par-Low	Along closure axis	~10%	Toward closure	Widens
Par-High	Along closure axis	~20%	Toward closure	Widens
Dynamic	Along closure axis	~10%	Toward closure	Widens

Magnets in direct contact with the MagFlex™ consumable represent the maximal deformation. The dynamic condition was actuated cyclically at 0.3 Hz between the relaxed state and ~10% deformation.

The study is a technology-enabling proof of concept and used one replicate per condition, so the closure data carry no error bars and no statistical test. They demonstrate that the acquisition-to-quantification pipeline runs under active actuation; they are not powered biological conclusions.

The wound front was delineated manually and cell-free area quantified per frame. Every figure quoted for the instruments traces to the product specifications: Ø 8 mm actuation area with 4 mm of depth, deformation to ~30% in 2D, cyclic loading to 1 Hz, substrate rigidity from 100 Pa to 20 kPa.

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