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High-resolution imaging of nuclear dynamics in live cells under uniaxial tensile strain

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Dr. Ronald Myers
 Science Editor, *Journal of Visualized Experiments*

We are pleased to submit our manuscript, “**High-resolution imaging of nuclear dynamics in live cells under uniaxial tensile strain**” to be considered as an article in *Journal of Visualized Experiments*.

We have previously designed a device¹ to apply extracellular mechanical strain to adherent cells. The design of the elastomeric substratum in that work was sufficient for biochemical assays as well as low-resolution imaging of strained cells. In this work, we redesigned the substratum and introduced a novel imaging configuration that facilitates high resolution subcellular live cell imaging under applied strain. Here, we demonstrate the application of this new apparatus to probe the mechanism by which strain promotes differentiation of oligodendrocytes^{2,3}. The high-resolution time lapse imaging of nuclei in strained and unstrained oligodendrocyte progenitor cells showed that mechanical tensile strain hastens the dampening of nuclear dynamics, consistent with accelerated cell differentiation. Our method has several advantages such as it can be fabricated simply, is small enough to fit inside tissue culture incubators as well as on top of microscope stages, and allows high-resolution optical imaging of any adherent cell type.

We believe that the *The Journal of Visualized Experiments* is an appropriate venue in which to publish this method, and look forward to your and the reviewers’ response.

Sincerely,

Krystyn J. Van Vliet, Ph.D.

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2. Jagielska, A. et al. Mechanical strain promotes oligodendrocyte differentiation by global changes of gene expression. *Front. Cell. Neurosci.* 11, 93 (2017).
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TITLE:

High-resolution Imaging of Nuclear Dynamics in Live Cells under Uniaxial Tensile Strain

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KEYWORDS:

Cell stretching, tensile strain, mechanobiology of differentiation, oligodendrocyte progenitor cells, nuclear dynamics, mechanotransduction

SUMMARY:

Using a previously designed device to apply mechanical strain to adherent cells, this paper describes a redesigned substratum geometry and a customized apparatus for high-resolution single-cell imaging of strained cells with a 100x oil immersion objective.

ABSTRACT:

Extracellular mechanical strain is known to elicit cell phenotypic responses and has physiological relevance in several tissue systems. To capture the effect of applied extracellular tensile strain on cell populations in vitro via biochemical assays, a device has previously been designed which can be fabricated simply and is small enough to fit inside tissue culture incubators, as well as on top of microscope stages. However, the previous design of the polydimethylsiloxane substratum did not allow high-resolution subcellular imaging via oil-immersion objectives. This work describes a redesigned geometry of the polydimethylsiloxane substratum and a customized imaging setup that together can facilitate high-resolution subcellular imaging of live cells while under applied strain. This substratum can be used with the same, earlier designed device and, hence, has the same advantages as listed above, in addition to allowing high-resolution optical imaging. The design of the polydimethylsiloxane substratum can be improved by incorporating a grid which will facilitate tracking the same cell before and after the application of strain. Representative results demonstrate high-resolution time-lapse imaging of fluorescently labeled nuclei within strained cells captured using the method described here. These nuclear dynamics data give insights into the mechanism by which applied tensile strain promotes differentiation of oligodendrocyte progenitor cells.

INTRODUCTION:

Cells and tissues in the body are subjected to various mechanical cues, including tensile strains. However, the effects of these cues on the biology of neural cells have not yet been studied extensively and understood fully. In the central nervous system, sources of mechanical strain include developmental growth¹⁻⁴, physiological processes such as spinal cord bending, blood and cerebrospinal fluid pulsation, and pathological conditions such as trauma, axon swelling, glial scarring, or tumor growth⁵⁻⁸. It is worth investigating how tensile strain affects the differentiation of oligodendrocytes and the subsequent myelination of axons, which is a critical process in the vertebrate central nervous system. Using a custom-designed strain device and elastomeric multiwell plates, previous works^{9,10} have shown that static uniaxial strain can increase oligodendrocyte differentiation via global changes in gene expression¹⁰. To gain further understanding of the mechanisms of strain mechanotransduction in these cells, the previous experimental apparatus must be redesigned as described here, to enable high-resolution fluorescence imaging of nuclear dynamics in living cells under strain. Specifically, a single-well polydimethylsiloxane plate is developed, and the imaging configuration is redesigned to allow for the time-lapse imaging of live cells under strain using a 100x oil immersion lens. To eliminate the negative optical effects of polydimethylsiloxane in the light pathway, cells are imaged not through the polydimethylsiloxane plate, but in the inverted position, through the cover glass covering the cell compartment. Using this new imaging design, hundreds of high-resolution time-lapse movies are recorded, of individual cell nuclei within intact adherent cells, where chromatin is labeled by tagging histone H2B to green fluorescent protein. These movies demonstrate that tensile strain induces changes in chromatin structure and dynamics that are consistent with the progression of oligodendrocyte differentiation.

Live cell imaging under applied strain is technically challenging and requires a device design that is compatible with the microscope system. The custom design described here presents an inexpensive alternative to commercial solutions. Its dimensions enable its installation on microscope stages and live cell imaging at high spatial resolution during applied strain. The imaging setup is designed to facilitate live cell imaging using a 100x oil immersion lens with the highest clarity, through the cover glass, not through the layer of polydimethylsiloxane plate which otherwise decreases the image quality and is common in most imaging setups under strain. The device, with a mounted plate containing cells, can also be stored easily in the incubator. This device is designed to apply uniaxial strain to substrata that facilitate adherent cell culture and maintain a stable and uniform strain over multiple days. The setup described here can be used for the high-resolution imaging of various adherent cell types under strain, making it applicable to mechanotransduction studies in many fields of cell mechanobiology.

PROTOCOL:

1. Design of the single-well polydimethylsiloxane mold for high-resolution imaging

NOTE: The mold for manufacturing polydimethylsiloxane plates is designed with the following features to enable imaging with a 100x oil immersion lens and a correct fit in the custom-build strain device (**Figure 1A,B**).

1.1. Keep the overall plate dimensions such that it fits in the clamps of the strain device.

NOTE: Here, they are 60 mm x 73 mm.

1.2. Make a cell compartment or well that is significantly smaller than the whole plate's lateral dimensions, to avoid arcing (out-of-plane bending) effects that happen at the unclamped edges of the polydimethylsiloxane plate during applied strain.

NOTE: Here, the compartment was designed as a 23 mm x 23 mm square.

1.3. Keep the depth of the cell compartment as minimal as possible (not more than 80 μm), to enable focusing with the 100x oil immersion lens through the cover glass placed on top of the compartment, but make it sufficient enough to contain media and to avoid contacting or squeezing the cells.

1.4. Raise the cell compartment on a square with a height of 3 mm, to bring the cells closer to the lens in the inverted microscope setup to enable easy focusing.

NOTE: Molds with the above features can be manufactured by many techniques including, for example, milled aluminum. Alternatively, master molds can also be assembled by using acrylic sheets cut with a laser cutter, cover glass #0 for the cell compartment, and super glue. This master mold is used to make a polydimethylsiloxane imprint, which is then used to make working molds using a casting resin.

2. Fabrication of single-well polydimethylsiloxane plates and square compartments

2.1. Mix polydimethylsiloxane base and curing agent in a ratio of 20:1 (by weight) in a disposable cup. Weigh a total of 20 g of polydimethylsiloxane per mold (for making one polydimethylsiloxane plate) and 150 g of polydimethylsiloxane per 150 mm-in-diameter plastic dish (for making one batch of square compartments).

2.2. Leave the polydimethylsiloxane mix in a vacuum degasser at -0.8 bar for 30 min (or until all bubbles are removed).

2.3. Pour the polydimethylsiloxane mix into the molds (for plates) or into 150 mm plastic dishes (for square compartments).

2.4. Remove any additional bubbles at this stage, either by blowing air (by mouth) or degassing the polydimethylsiloxane-filled molds/dishes again at -0.8 bar vacuum for 30 min.

2.5. Leave the molds/dishes in an 80 °C oven on a leveling table for a minimum of 2 h and a maximum of 24 h.

2.6. Carefully remove the molds/dishes from the oven and let them cool down to room temperature. Gently peel out the cured polydimethylsiloxane after carefully cutting the edges with a blade (**Figure 1C**).

2.7. On the 150 mm-diameter polydimethylsiloxane, draw a 2 cm x 2 cm grid with a marker. Within each square, draw a 1 cm x 1 cm square, leaving a margin of 0.5 cm on all sides. Using a blade, carefully cut along the lines to obtain square compartments (**Figure 2A**).

2.8. Clean the polydimethylsiloxane plates/square compartments by incubating them in 100% acetone for 4–24 h, followed by 100% ethanol for 4–24 h, followed by autoclaved water for 4–24 h.

2.9. Place the plates/square compartments on paraffin film backing paper (not the paraffin film but the paper) inside a 150 mm diameter plastic dish (**Figure 2C**).

2.10. Leave the polydimethylsiloxane plate in the 80 °C oven to dry for 4–24 h.

2.11. Seal the 150 mm-diameter dishes containing polydimethylsiloxane plates and container squares with paraffin film, and store them in a cold room until further use.

3. Functionalization of polydimethylsiloxane plates

3.1. Remove the paraffin film, remove the cover of the plastic dish, and place the polydimethylsiloxane plates and square compartments under ultraviolet light for 30 min.

3.2. Plasma-treat (at 150 W for 5 min) the polydimethylsiloxane plates (with the cell compartment facing up) without the square compartments, to make the culture surface hydrophilic.

3.3. Immediately place the square compartments onto the plasma-treated surface (**Figure 2B**) and press manually to temporarily stick the two together.

NOTE: Do not plasma-treat the square compartments, or else they will stick strongly to the polydimethylsiloxane plate and will not come off easily when they must be peeled off during imaging.

3.4. Add 200 µL of 100 mM (3-aminopropyl)triethoxysilane to the well for 2 h to introduce –NH₂ groups to the polydimethylsiloxane surface. To make a 100 mM solution, dissolve 234 µL of pure (3-aminopropyl)triethoxysilane in 10 mL of water. After a 2 h incubation, wash the wells 3x with deionized water.

3.5. Weigh 2 mg of the molecular crosslinker bissulfosuccinimidyl suberate and dissolve it in 700 µL of 1 M (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid buffer (pH 8.0) and 2.8 mL of water.

NOTE: This will give a 1 mM bissulfosuccinimidyl suberate solution in 200 mM (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid buffer. Note that a 50 mM concentration buffer also works well.

3.5.1. Add 135 μ L of this solution and 15 μ L of 1 mg/mL fibronectin to the well in each polydimethylsiloxane plate for 4 h at room temperature.

3.6. Wash 3x with phosphate-buffered saline solution. Add 500 μ L of phosphate-buffered saline solution to the well. Cover the 150 mm-diameter dish containing the polydimethylsiloxane with paraffin film and store it in a cold room, until further use.

4. Functionalization of plastic dishes and flasks

4.1. Incubate plastic dishes and flasks with a 5 μ g/mL solution of poly-D-lysine (PDL, in sterile water) for 1 h. Add 1.5 mL of this solution (ligand) per 35 mm-diameter dish, 3 mL of it per 60 mm-diameter dish, and 10 mL per 75 cm² culture flask.

4.2. Wash the dishes and flasks 2x with sterile water.

4.3. Leave them to dry in the biosafety cabinet for 1 h (or until dry) and store them in the cold room at 4 °C until further use.

5. Proliferation and differentiation medium for murine oligodendrocyte progenitor cells

5.1. Prepare 50 mL of 100x solutions of the proliferation and differentiation medium, make aliquots of 2.5 mL, and store them at -80 °C.

NOTE: The composition of proliferation medium is 0.1 mg/mL bovine serum albumin, 62 ng/mL progesterone, 16 μ g/mL putrescine, 5 μ g/mL insulin, 50 μ g/mL holo-transferrin, 5 ng/mL sodium selenite, 1x penicillin-streptomycin, 10 ng/mL platelet-derived growth factor, and 10 ng/mL fibroblast growth factor in Dulbecco's modified Eagle's medium (DMEM) with high glucose and pyruvate. While bovine serum albumin, progesterone, putrescine, and sodium selenite can be constituted and stored at 100x, insulin, holo-transferrin, and penicillin-streptomycin must be added fresh while preparing the 1x solution. The growth factors must be constituted at 10 μ g/mL and must be added fresh to the cells every day (1 μ L/mL of medium). The composition of the differentiation medium is 0.1 mg/mL bovine serum albumin, 62 ng/mL progesterone, 16 μ g/mL putrescine, 5 μ g/mL insulin, 50 μ g/mL holo-transferrin, 5 ng/mL sodium selenite, 1x penicillin-streptomycin, 400 ng/mL triiodothyronine, 400 ng/mL L-thyroxine, and 0.5% fetal bovine serum in DMEM with high glucose and pyruvate. Triiodothyronine and L-thyroxine can be constituted with other reagents and stored at 100x. Insulin, holo-transferrin, and penicillin-streptomycin must be added fresh while preparing the 1x solution.

5.2. For preparing 50 mL of 100x proliferation medium, dissolve 510 mg of bovine serum albumin

in 17 mL of Dulbecco's medium, 310 µg of progesterone in 31 µL of ethanol, 80 mg of putrescine in 500 µL of Dulbecco's medium, 25 µg of sodium selenite in 10 µL of Dulbecco's medium, and full DMEM up to 50 mL

5.3. For preparing 50 mL of 100x solution of differentiation medium, additionally dissolve 2 mg of triiodothyronine in 40 µL of sodium hydroxide and 2 mg of L-thyroxine in 40 µL of sodium hydroxide, then fill up the solution to 50 mL with Dulbecco's medium. Filter the solution through a sterile disposable filter unit, aliquot, and store the aliquots at -80 °C.

5.4. For preparing 250 mL of 1x proliferation/differentiation medium, mix 2.5 mL of 100x solution of proliferation/differentiation medium with 12.5 mg of holo-transferrin, 125 µL of 10 mg/mL insulin, and 2.5 mL of 100x penicillin-streptomycin. Fill up the solution to 250 mL with Dulbecco's medium and filter it through a sterile disposable filter unit.

NOTE: The growth factors must be added fresh and directly to the cell culture, not to the whole batch of reconstituted medium.

6. Cell culture

6.1. Start with oligodendrocyte progenitor cells suspended in proliferation medium. Seed these cells at a density of 35,000 cells/cm² onto PDL-coated plastic surfaces (dishes or flasks, see section 4). Adjust the volume of the proliferation medium to 3 mL per 10 cm² of surface area of plastic substratum; a lower volume of medium may lead to cell clumping arising from surface tension forces, while a higher volume of medium in the dishes may cause spillage.

6.2. Add growth factors (platelet-derived growth factor and fibronectin growth factor) every day, maintaining their concentration at 10 ng/mL and change half of the proliferation medium on alternate days.

6.3. On the third day, detach the cells from the plastic surface using a gentle cell detachment solution (e.g., accutase): remove the medium, wash 1x with phosphate-buffered saline solution, add 1 mL of detachment solution per 20 cm² area, leave the dish/flask in an incubator at 37 °C for 10 min, and gently tap it until all cells have detached (look under a microscope).

6.4. Pipette using a 200 µL tip to break clumps into single cells, transfer them to a 50 mL tube, dilute them in proliferation medium, spin at 0.2 x g for 10 min, discard the supernatant, and resuspend the pellet in proliferation medium to make a total volume of 1 mL. Count the cells using a cytometer.

6.5. Wash the fibronectin-functionalized polydimethylsiloxane plates 2x, 15 min per wash, with proliferation medium.

6.6. Seed 35,000 cells per polydimethylsiloxane plate in 700 µL of proliferation medium.

264 6.7. Add a plasmid construct of H2B-GFP for labeling histone H2B with green fluorescent protein.

265
266 NOTE: Here, 1.4 μ L of CellLight H2B-GFP BacMam2 transfection mix was added to each plate (2
267 μ L per 50,000 cells).

268
269 6.8. After 24 h, mount the polydimethylsiloxane samples with cells to be stretched on the uniaxial
270 strain device (as shown in **Figure 2D**). Change the medium in all samples to differentiation
271 medium.

272
273 6.9. To strain the samples, measure the unstrained cell compartment length (**Figure 3A**) and turn
274 the stage micrometer screw to increase the cell compartment length by the desired amount (e.g.,
275 10%, see **Figure 3B**). Leave the stretched and unstretched polydimethylsiloxane samples in the
276 incubator at 37 °C until imaging.

277 278 7. Imaging

279
280 7.1. Turn on the microscope. Set the microscope incubator temperature to 37 °C, the humidity
281 to 95%, and the carbon dioxide to 5%.

282
283 7.2. Bring the objective to be used (100x oil immersion) to the central position as the objective
284 turret will not be accessible later. Unscrew the objective and screw it back together with an
285 objective ring (**Figure 4A**) so that the objective can be brought closer to the cells. If an oil objective
286 is being used here, add a drop of oil at this step.

287
288 7.3. Synthesize beforehand (via 3D printing or machining) a plastic or metal holder that has two
289 important features—an angled window and a step (**Figure 4B**).

290
291 NOTE: The window supports a thickness #0 glass coverslip that will hold the medium while the
292 strain device is in an inverted state. The angled cut at the window edges (**Figure 4D**) allows the
293 objective to come closer to the glass coverslip. The step in the holder allows us to bring the
294 stretched polydimethylsiloxane further down, closer to the objective.

295
296 7.4. Place a glass coverslip (thickness #0, with dimensions of 25 mm x 25 mm or 35 mm in
297 diameter) onto the top surface of the holder by spreading vacuum grease at the periphery of the
298 window (**Figure 4C**), and tape the plastic window onto the microscope stage (**Figure 4D** and
299 **Figure 5A**).

300
301 7.5. Screw a z-translation stage onto the microscope stage and move it to the topmost z-position
302 (**Figure 5A**).

303
304 7.6. Remove 500 μ L of the medium from the stretched polydimethylsiloxane plate to be imaged
305 and add this medium onto the glass coverslip in the white plastic window.

306
307 7.7. Carefully detach the square compartment from the polydimethylsiloxane plate with sterile

tweezers.

7.8. Hold the strain device in an upright position (cells facing up) above the white plastic window and carefully invert the strain device so as to let any extra medium drop directly in the middle of the glass coverslip (**Figure 5B**) (cells facing down).

7.9. Place the bottom part of the strain device onto the z-translation stage with the double-sided tape (**Figure 5C,D**).

7.10. While looking through the eyepiece under brightfield, slowly bring the strain device down (**Figure 5E**) (by lowering the z-translation stage) and move the objective up to focus on the cells.

7.10.1. Perform this step extremely slowly, step by step. If the strain device presses too much onto the coverslip, the cells can get compressed (causing them to die) and if the objective presses too much onto the coverslip, the coverslip can break (causing medium to leak and spill onto the objective).

7.11. Scan the polydimethylsiloxane plate in *x*- and *y*-directions to find a cell that has a fluorescent nucleus (under epifluorescence with 488 nm of wavelength excitation) and appropriate cell morphology (under brightfield).

7.12. If the lateral displacement of the microscope stage does not affect the vertical focusing too much, select multiple regions of interests using a multipoint feature on the software. Sometimes, the focusing is very sensitive to any lateral displacement of the stage. In such cases, image one cell at a time. Mark the *x*-, *y*-, and *z*-positions for each cell of interest.

7.13. Record wide-field (or open-pinhole) images of the nucleus with 488 nm of wavelength excitation and of the cell with brightfield excitation at intervals of 30 s per frame for a total duration of at least 30 min.

8. Data analysis

8.1. Nuclear fluctuations

8.1.1. Open the sequence of nucleus images (**Figure 6A**) in an image analysis software and threshold the nucleus time-lapse images (for example, use the **Threshold** command in ImageJ or the **Graythresh** command in the software MATLAB).

8.1.2. Get the nucleus area (in pixels) as a function of time, plot it in a data plotting software (**Figure 6B**), and fit a third order polynomial to the data (**Figure 6C**) (for example, use the **Analysis | Fitting | Polynomial Fit** command in Origin or the **Polyfit** command in the software MATLAB).

8.1.3. Subtract the value of the fitted polynomial from the actual area (in pixels) for each time point. This corrected area is known as the residual area.

NOTE: This process removes any increasing or decreasing trend in the data coming from instrumental errors and is called detrending.

8.1.3.1. Calculate the percentage of residual area by dividing the residual area at each time point with the value of the fitted polynomial at that time point (**Figure 6D**).

8.1.4. Calculate the standard deviation of the residual area time series. This standard deviation denotes the amplitude of nuclear fluctuations.

8.2. Data plotting and statistical analysis

8.2.1. Calculate the amplitude of nuclear fluctuations as a mean of at least 20 nuclei per condition.

8.2.2. Conduct a one-way analysis-of-variance statistical test with Bonferroni correction to determine whether there is a significant difference in the amplitude of fluctuations as a function of conditions of interest (for example, with and without applied strain).

REPRESENTATIVE RESULTS:

Recent work aimed at investigating the effect of tensile strain on oligodendrocytes¹⁰ showed that a 10% uniaxial tensile strain promotes the differentiation of oligodendrocyte progenitor cells by global changes in gene expression. The mechanism behind these changes in gene expression can be probed via the imaging of subcellular parameters, such as the cytoskeleton structure, transcription factor localization, nuclear dynamics, and chromatin organization. However, the previous geometry of the polydimethylsiloxane substratum did not allow high-resolution single-cell imaging. As described in this work, the redesigned geometry of the polydimethylsiloxane substratum and the imaging setup minimized the distance between the cells and the objective. This allows capturing time-lapse images of fluorescently labeled cell nuclei using a 100x oil immersion objective (**Figure 6A**).

Fluctuations in the nuclear projected area depend on the differentiation state of the cells^{11,12}. A comparison of the nuclear fluctuations of oligodendrocyte progenitor cells and that of terminally differentiated oligodendrocytes showed that the latter have significantly lower fluctuations (**Figure 6E**). Next, the nuclear fluctuations of oligodendrocyte progenitor cells at 1 h, 24 h, and 48 h post-chemical induction of differentiation with and without a 10% tensile strain were compared. With chemical induction alone, the amplitude of nuclear fluctuations showed a significant decrease at 48 h but not at 24 h (**Figure 6F**). On the other hand, chemical induction together with a 10% tensile strain showed a significant decrease at 24 h, which remained constant, without further reduction at 48 h (**Figure 6G**).

The substratum geometry and the imaging configuration described in this paper enabled the recording of high-resolution movies of strained cells. Subsequent analysis of these movies demonstrated that strain hastens the dampening of nuclear fluctuations, which is a marker of

differentiation. These results give insight into the mechanism by which strain promotes oligodendrocyte differentiation. Further discussion on the interpretation of these results and future experiments are described in Makhija et al.¹³.

FIGURE AND TABLE LEGENDS:

Figure 1: Design and geometry of the polydimethylsiloxane mold. (A) Sketch of the mold showing all dimensions in millimeters (this sketch was generated by Whits Technologies, Singapore). (B) Three-dimensional view of the mold (adapted from Makhija et al.¹¹). Inset shows a photo of the mold. (C) Three-dimensional view of the polydimethylsiloxane plate fabricated using the mold (adapted from Makhija et al.¹¹). Inset shows a photo of the polydimethylsiloxane plate.

Figure 2: Sample preparation. (A) Photo of a polydimethylsiloxane plate and a square compartment. (B) The square compartment is placed on the raised square on the polydimethylsiloxane plate. Its purpose is to contain medium for the cells. (C) Polydimethylsiloxane plates are stored in 150 mm-diameter plastic dishes using parafilm paper to prevent the polydimethylsiloxane from sticking onto the plastic. (D) While mounting the plate onto the strain device, support it from the bottom using two fingers to prevent any sagging of the plate. If the plate still sags a little bit after mounting, increase the distance between the strain device arms by turning the translation stage. At this step, the translation of the stage should not induce strain in the polydimethylsiloxane plate.

Figure 3: Cell stretching. (A) Measure the initial length of the plate between the clamps, using a ruler. (B) Stretch the plate by turning the screw on the translation stage to increase the initial plate length by the desired percentage. X% increase in length corresponds to X% of strain. Note that, to generate the desired strain (X%) in the raised cell culture compartment, the main plate must be strained by a higher amount (approximately 2X%) because of the difference in their thickness in the described plate geometry.

Figure 4: Preparation for imaging. (A) Bring the 100x objective to the central position in the turret, unscrew the objective, and screw it back together with the objective ring. (B) Sketch of the holder showing all dimensions in millimeters. (C) Spread vacuum grease at the periphery of the window in the holder, using a pipette tip, and place a glass coverslip on top. Use a cover glass with thickness #0 or #1, to enable focusing with the 100x oil immersion lens. (D) Place the holder (1) on the microscope stage (2). Bring the oil (6) objective (3) closer to the coverslip (5) that is stuck to the holder, using vacuum grease (4).

Figure 5: Imaging setup on the microscope. (A) Assemble the z-translation stage (yellow arrow) and the holder (red arrow) onto the microscope stage. (B) Tilt the strain device onto the microscope stage so as to let any medium drop onto the glass coverslip of the holder. Stick a double-sided tape (blue arrow) on the strain device that will stick onto the z-translation stage. (C) Place the strain device in an inverted position, supporting it on the z-translation stage. The cells must be aligned with the glass coverslip of the plastic holder. (D) Inverted geometry of the

strain device minimizes the distance between the cells and the objective, thereby facilitating high-resolution imaging (adapted from Makhija et al.¹¹). (E) Side view before bringing the strain device down (1); side view after bringing the strain device down (2); top-view after bringing the strain device down (3).

Figure 6: Representative images and area fluctuations data of the nucleus. This figure is adapted from Makhija et al.¹¹. (A) Typical image of a nucleus labeled with histone H2B tagged to green fluorescent protein, and a differentiating oligodendrocyte progenitor cell. The nucleus is in focus, while the cell processes are out of focus. (B) Typical time series of a nuclear area (in pixels). (C) Third order polynomial fitted to the data. (D) Percentage of the residual area fluctuation time series. (E) Nuclear edge fluctuations of proliferating oligodendrocyte progenitor cells and terminally differentiated oligodendrocytes. (F) Nuclear edge fluctuations at 1 h, 24 h, and 48 h postinduction of chemical differentiation without strain. (G) Nuclear area fluctuations at 1 h, 24 h, and 48 h postinduction of chemical differentiation with 10% tensile strain.

DISCUSSION:

A device has previously¹ been designed for the application of extracellular tensile strain on adherent cells. The design of the polydimethylsiloxane substratum in that work was sufficient for biochemical assays, as well as the low-resolution imaging of stretched cells. In this work, the substratum was redesigned, and a novel imaging configuration that facilitates high-resolution subcellular live cell imaging was introduced. The advantages of this system are numerous: it can be built in-lab using simple components, it is inexpensive compared to commercial strain devices (500 USD per cell strain device), and it is small enough to fit inside tissue culture incubators, as well as onto microscopes. Moreover, although the imaging system has been described here with the inverted microscope setup, it can be readily adjusted for the upright microscope configuration.

There are a few critical steps involved in the sample preparation and imaging. First, the polydimethylsiloxane plates and square compartments have to be thoroughly cleaned before cell seeding (protocol step 2.8) to ensure cell survival (as uncured polydimethylsiloxane is toxic to cells). Second, since the volume of fluid medium that can fit inside the square compartment is less than 1 mL, it may evaporate if the sample is in the incubator for a few days. Hence, the medium must be checked every day and replenished when required. Additionally, the raised area on the polydimethylsiloxane plate can be covered with an inverted 60 mm-diameter plastic dish to minimize evaporation. Third, extreme caution must be exercised while moving the strain device on the microscope down via the z-translation stage to bring the cells closer to the glass coverslip. Compressing the cells (even for a moment) between the polydimethylsiloxane substratum and the glass coverslip may cause cell death. Fourth, the dead cells and cell debris may remain stuck to the glass coverslip after the polydimethylsiloxane sample has been removed. Hence, after imaging, the glass coverslip must be pulled off from the vacuum grease and a fresh coverslip should be attached prior to mounting a new sample.

The limitations of the strain device are that the application of stage lateral displacement cannot be programmed to perform cyclic or ramped strain and that it can only apply uniaxial strain. The

limitation of the polydimethylsiloxane substratum in the described geometry is that a strain higher than 25% may cause a fracture of the polydimethylsiloxane.

A future modification to improve the design of the polydimethylsiloxane substratum could be the incorporation of a grid on the cell culture surface. This would allow for tracking the same cell before and after the application of tensile strain.

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DISCLOSURES:

The authors have nothing to disclose.

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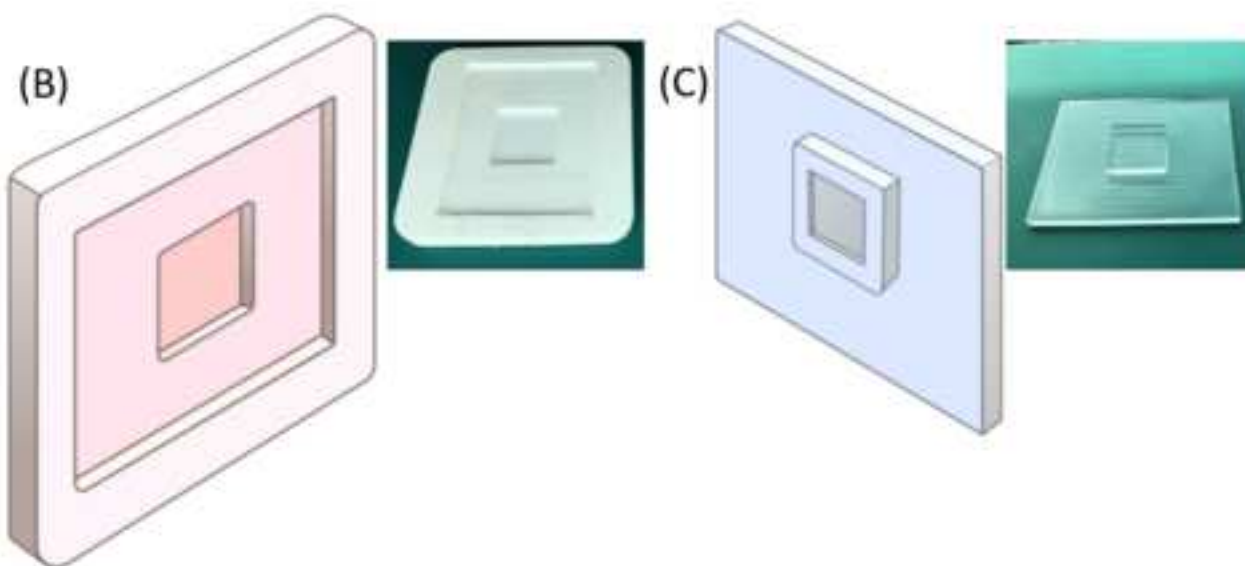
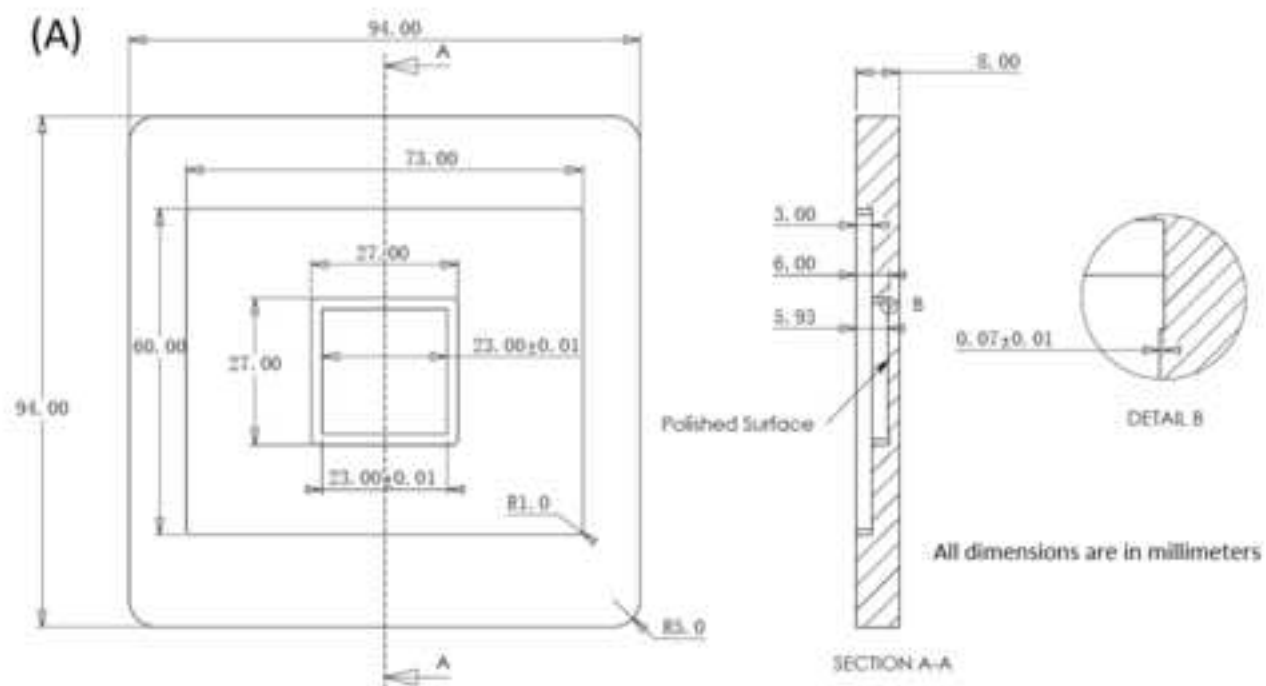
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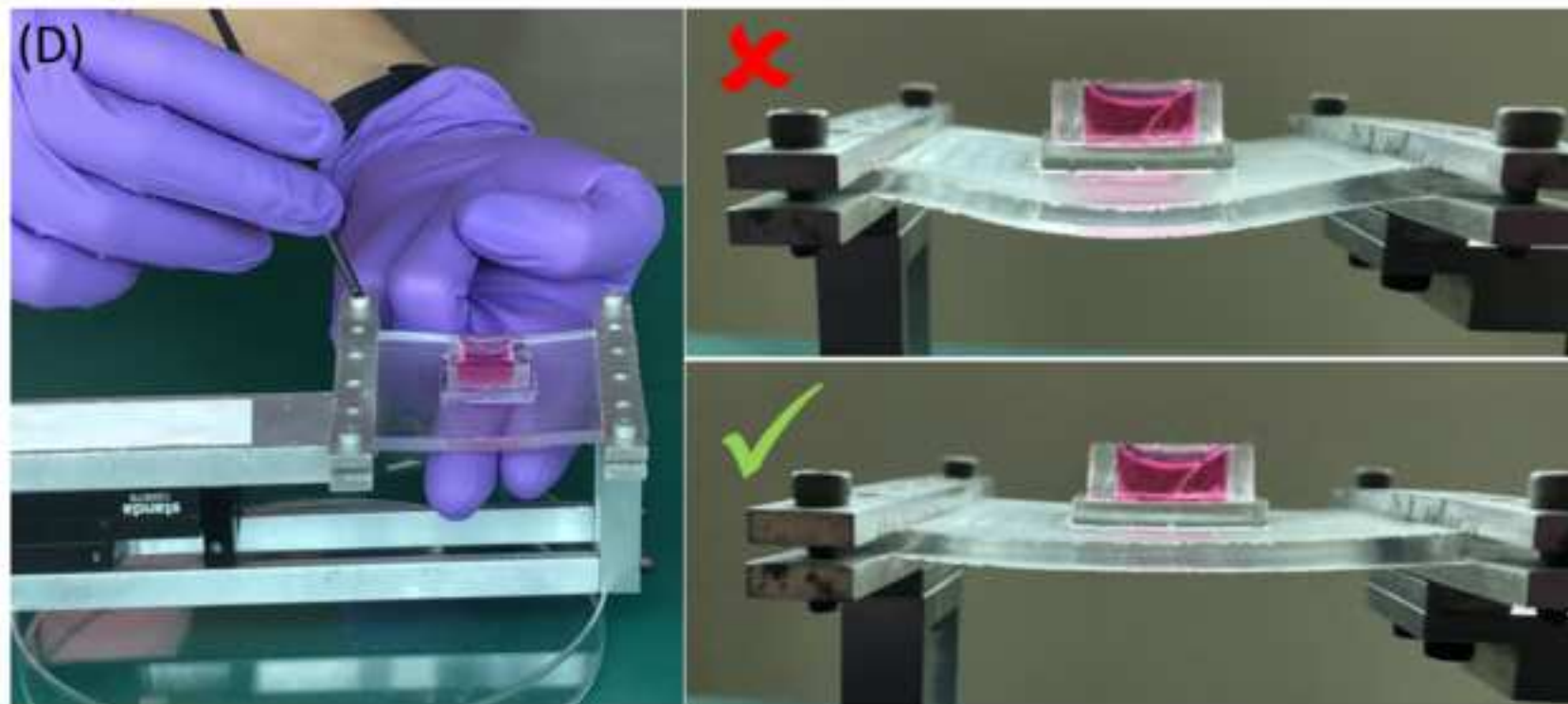
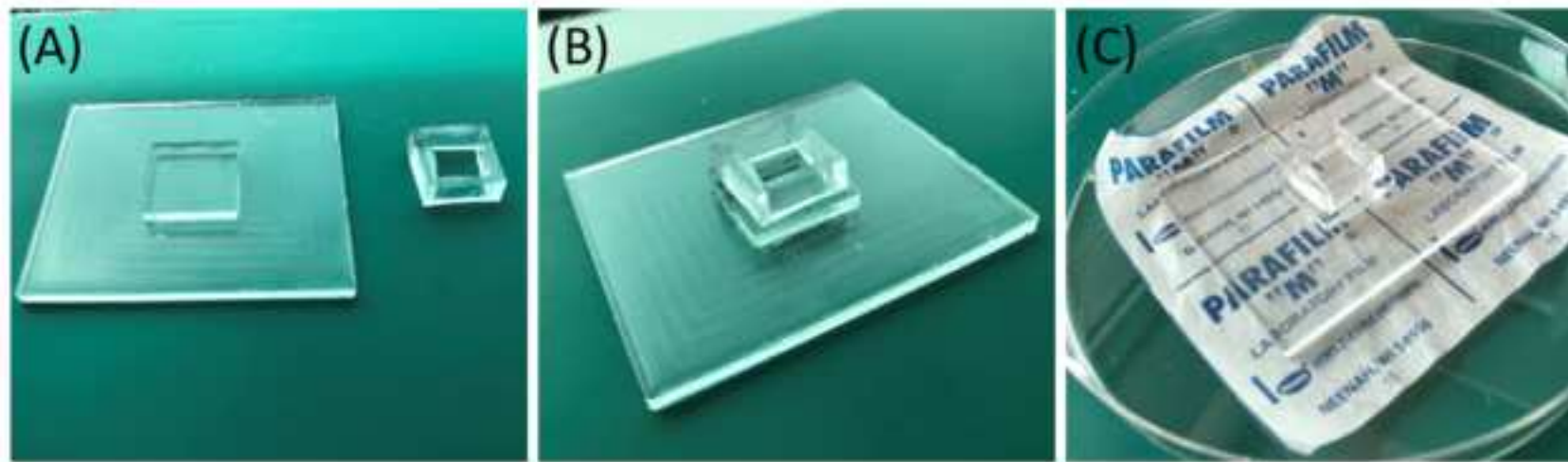
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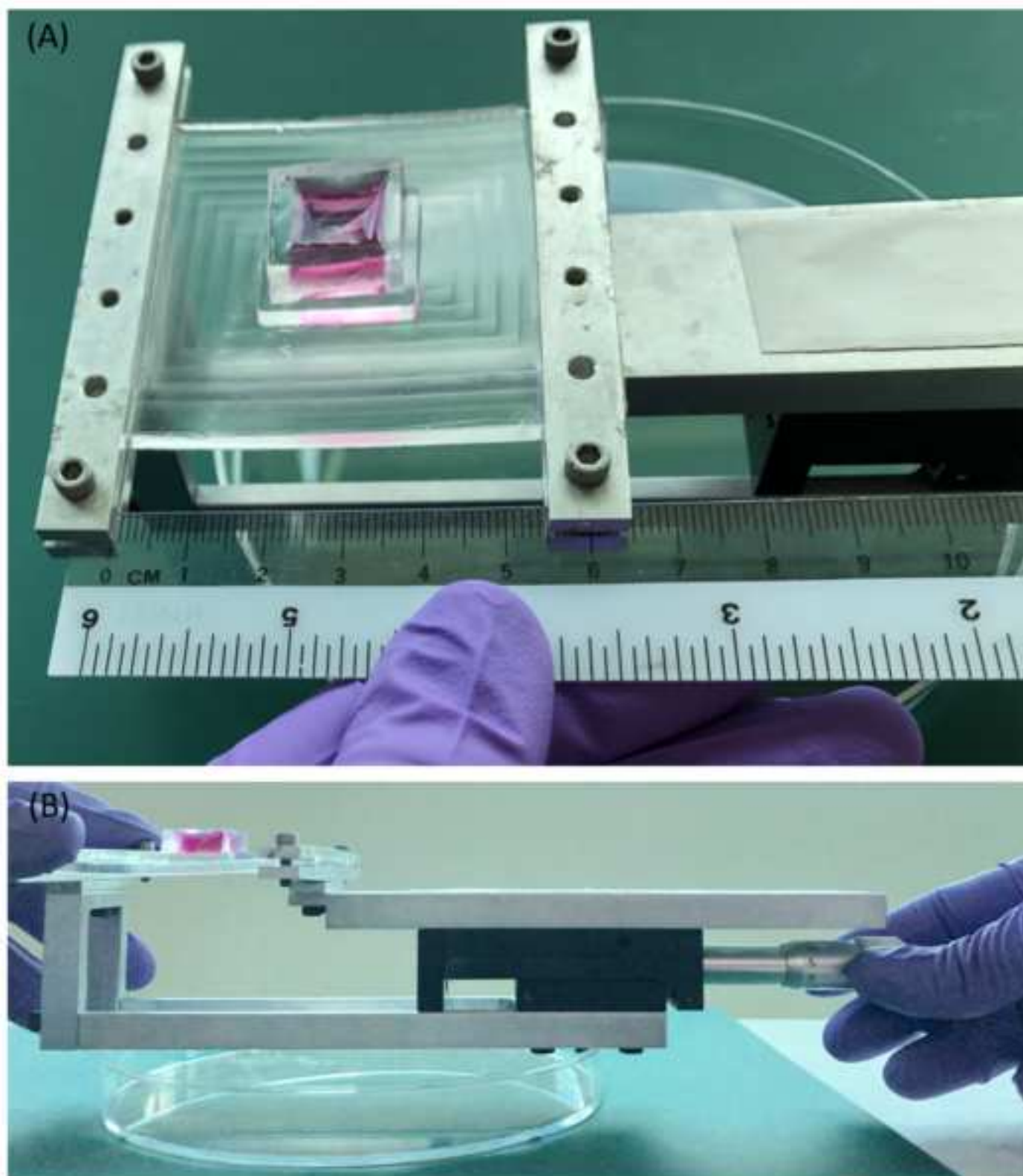
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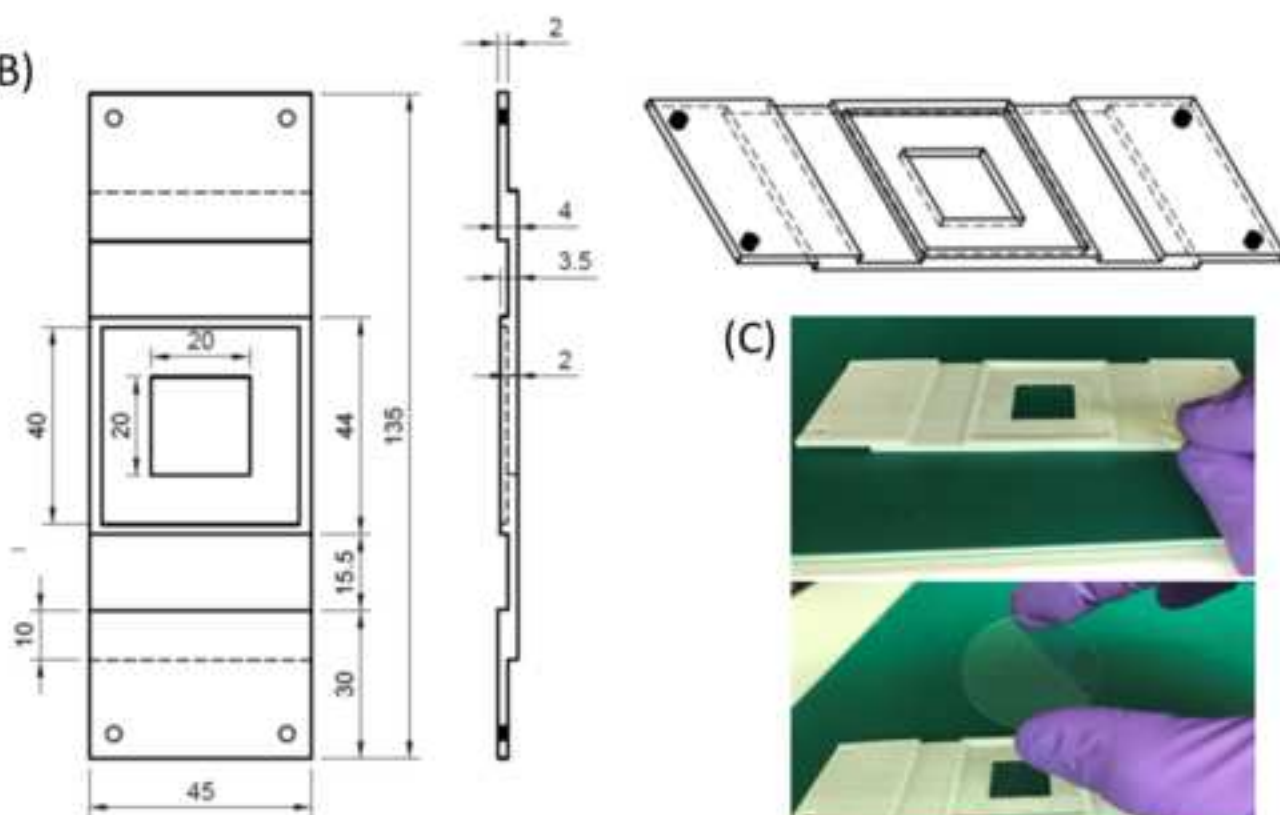




(A)

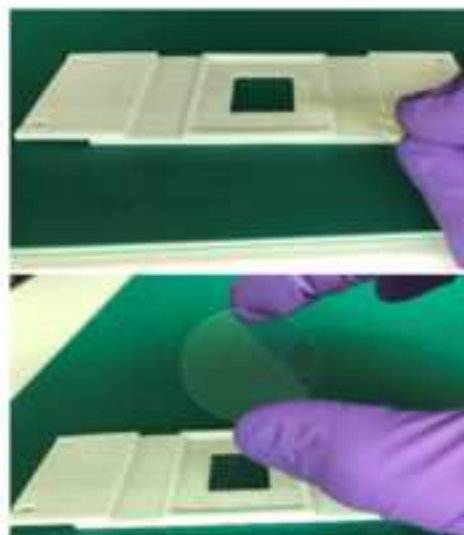


(B)

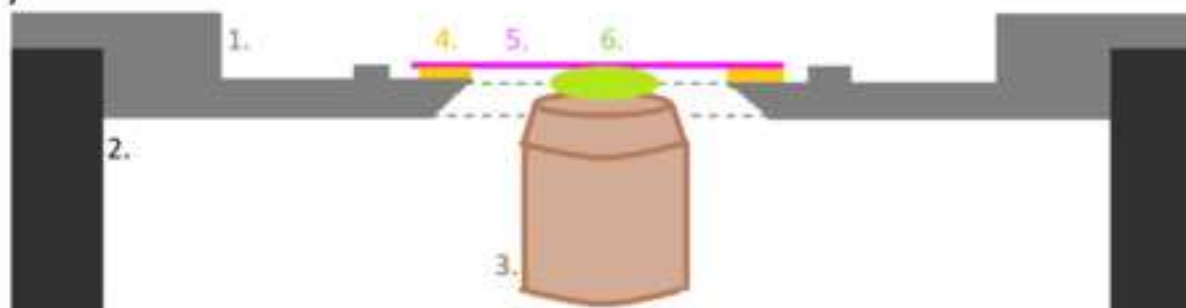


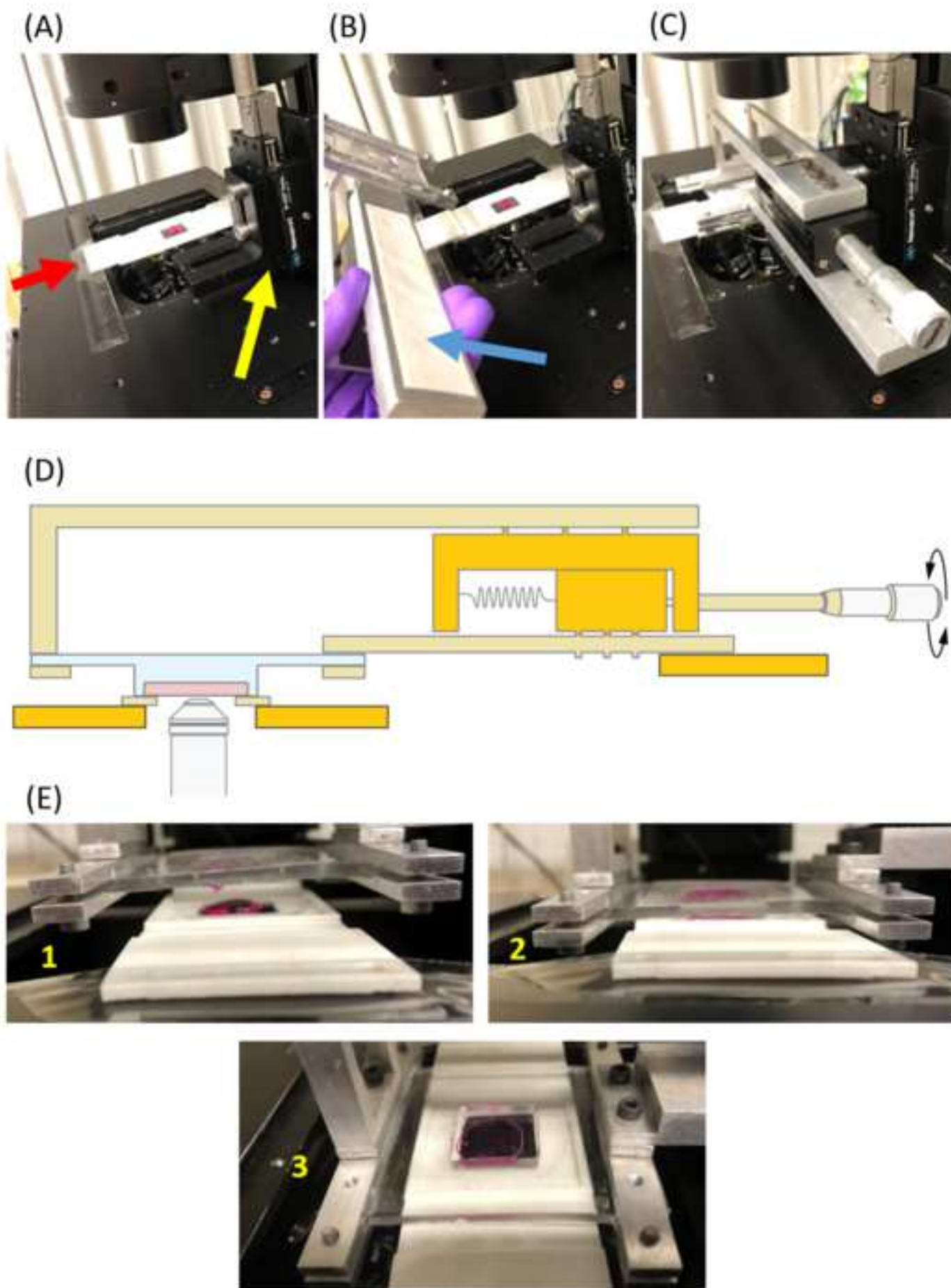
All dimensions are in millimeters

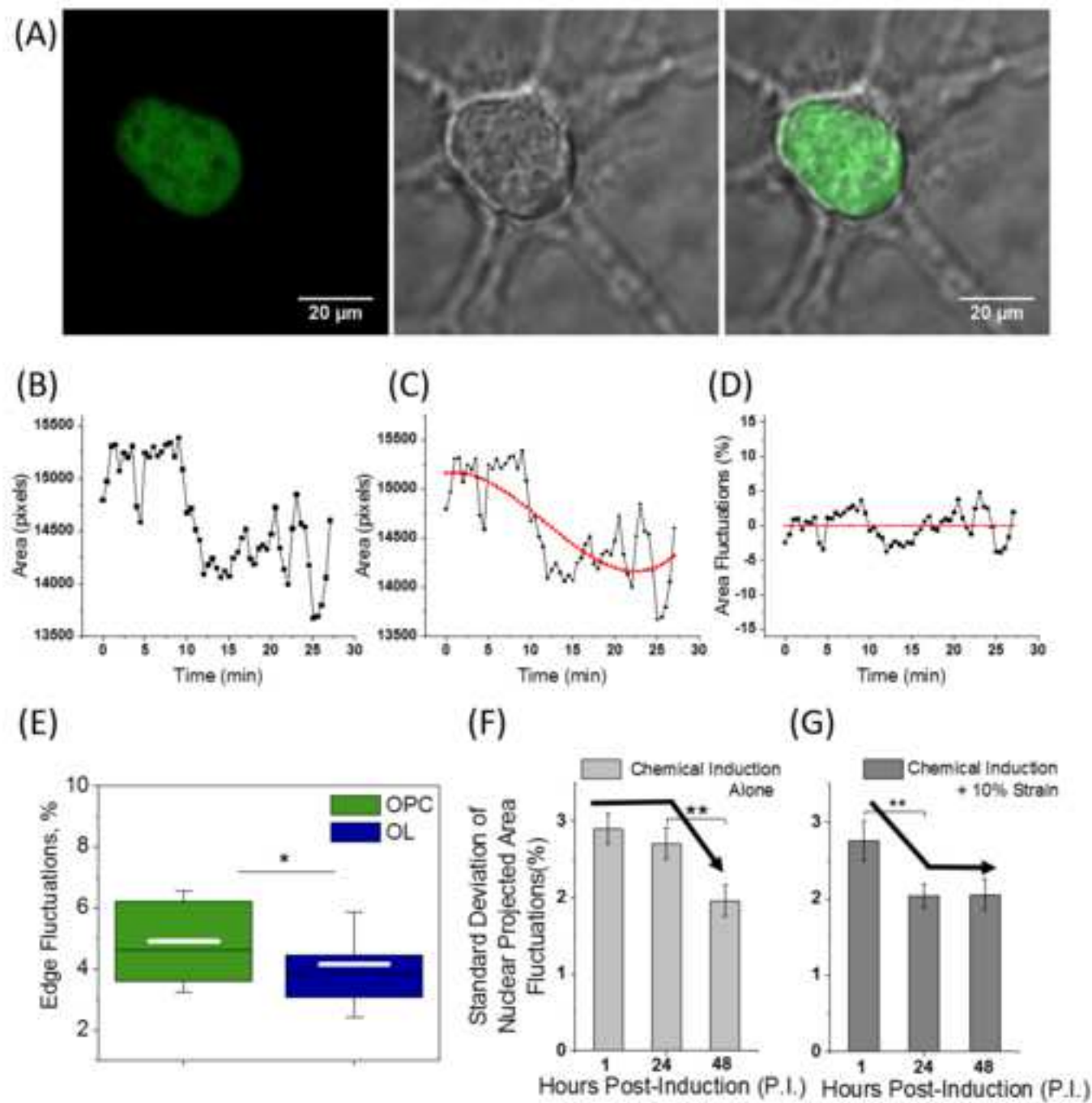
(C)



(D)







Name of Material/ Equipment	Company
Primary cells	
Primary Oligodendrocyte Progenitors were provided by Dr. S. Y. Chew's lab	
Media	
DMEM high glucose	Gibco
Pen/Strep	Gibco
FGF	Prospec
PDGF	Prospec
Media Stock components	
BSA	Sigma
Progesterone	Sigma
Putrescine	Sigma
Sodium Selenite	Sigma
Tri-iodothyronine	Sigma
Thyroxine	Sigma
Holo-Transferrin	Sigma
Insulin	Sigma
Mold and PDMS plate	
PDMS - Dow Corning Sylgard 184	Ellsworth
Mold - Liquid Plastic	Smooth-On
Substrate Coatings	
poly-D-lysine	Sigma

Fibronectin	Sigma
Histone staining	
CellLigt H2B-GFP BacMam	Molecular Probes
Surface functionalization	
APTES	Sigma
BS3	Covachem
HEPES buffer pH 8.0	Alfa Aesar
Cell detachment	
Accutase	Invitrogen

Catalog Number

or Cells isolated from Neonatal Rats at Nanyang Technological University
11996-065-500ml
15070-063-100ml
CYT-608-50ug
CYT-776-10ug
A9418-10G
P8783-1G
P5780-5G
S5261-10G
T6397-100MG
T1775-100MG
T0665-500MG
I9278
184 SIL ELAST KIT 0.5KG
Smooth Cast 310 - Trail Size
P6407-5MG

F1141-2MG
C10594
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13306-100mg
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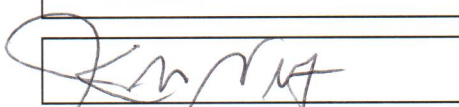
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We thank the editorial for their comment. We have now removed abbreviations for all journal titles under references.
3. *What are the units in Figure 1A and 4B?*
The figure legends for both Fig.1A and Fig.4B already stated that the dimensions are in millimeters. In the revised version, we have also added a line in the figure, saying "All measurements are in millimeters".
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We thank the editorial for this comment. We have now rephrased the short abstract.
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6. *Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).*
We found 27 occurrences of personal pronouns in the manuscript. We have revised the text to remove pronouns from the following 27 lines:
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7. *Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.*

We have added more details to protocol steps 2.8, 2.11, 3.4, 4.1, 5.2 and 5.3. We have also added steps 5.1, 5.4, and 5.5.

8. *3.4: How much is added?*

We thank the editorial for pointing this missing volume. We have now edited protocol step 3.4 in the revised manuscript to read, “Add 200 microliters of 100 millimolar (3-Aminopropyl)triethoxysilane...”

9. *3.5: How much is added?*

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Please let me know if you have any other questions or concerns.

Katie Simpson
Research Integrity Specialist

Research Integrity Manager: [Gearóid Ó Faoleán](#), PhD

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On Fri, Jan 18, 2019 at 2:27 AM Ekta Parshotam Makhija <ekta@smart.mit.edu> wrote:

Dear Editorial Office of Frontiers,

I would like to adapt (with slight modifications) 5 sub-figures (1A, 1C, 2D, 2E, S3C) from my following article for a methods paper that I am submitting to Journal of Visualized Experiments. **Could you please guide me how to proceed with regards to copyright permissions?**

Frontiers paper:

Mechanical Strain Alters Cellular and Nuclear Dynamics at Early Stages of Oligodendrocyte Differentiation

Front. Cell. Neurosci., 06 March 2018 | <https://doi.org/10.3389/fncel.2018.00059>

Journal of Visualized Experiments paper title:

High-resolution imaging of nuclear dynamics in live cells under uniaxial tensile strain

Thanks and Best Regards,

Ekta

MAKHIIJA Ekta (Dr) :: Postdoctoral Associate :: BioSystems and Micromechanics (BioSyM) Inter-Disciplinary Research Group (IRG)
Singapore MIT Alliance for Research and Technology (SMART) :: 1 CREATE WAY, #04-13/14, Enterprise Wing, Singapore 138602

