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Quantifying Spiral Ganglion Neurite and Schwann Behavior on Micropatterned polymer Substrates

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Abstract

The first successful *in vitro* experiments on the cochlea were conducted in 1928 by Honor Fell [1]. Since then, techniques for culture of this tissue have been refined, and dissociated primary culture of the spiral ganglion has become a widely accepted *in vitro* model for studying nerve damage and regeneration in the cochlea. Additionally, patterned substrates have been developed that facilitate

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¹⁵Neurofilament 200 (NF200) is an excellent marker for neurons, for which multiple antibodies are also available. We have the best results with Invitrogen's mouse anti-NF200, which we typically use at a 1:800 dilution.

²⁰It is important to have the degree of magnification saved into the image so pixel-to-micron conversions are calculated automatically, when opening in another program (such as ImageJ).. This save is crucial for the MATLAB program. If this is not the case, then be sure that a pixels-to-actual measurement conversion factor is known for the magnification of the images taken, so you can convert your measurements to microns.

²¹At high cell densities, neurites may overlap and make tracing difficult. Furthermore, cells at high densities may tend to direct each other's neurite growth, clouding the substrate's influence on neurite alignment. Therefore it is generally easier to use a low-density cell culture.

²²Zoom in to the image in order to improve the outline of your Schwann cells. This approach allows you to more closely follow the outline of the cell, though a perfect outline is usually not critical to fitting the best ellipse.

²⁶We typically use NT-3 and BDNF for trophic support, though it is also possible to use high-potassium MEM to depolarize the cell membrane. Membrane depolarization, however, reduces neurite growth [12].

²⁷Fibroblast count can be reduced by using medium containing only D-valine, an enantiomer of L-valine that Schwann cells metabolize, due to the expression of an enzyme that fibroblasts lack, D-amino acid oxidase [13].

²⁸AraC is used to preferentially inhibit fibroblast growth. However, keep in mind that treating cells with this antimitotic agent also limits Schwann cell growth. Treatment with AraC is a three-day process. Make N2 media with 10% FBS and 20 μ M AraC and incubate cells for 24 h with this solution. After 24 h of incubation, change media to serum-free N2 media and incubate for 48 h.

²⁹When Schwann cells grow to confluence, it can be helpful to split them into containers with larger surface areas. When performing this procedure, you should expect a moderate amount of cell loss, and increased fibroblast growth given the additional room for cell proliferation. To split the cells, first prepare as many containers as desired by plating with poly L-ornithine then laminin as described above. Then, replace the cell culture medium with 0.25% trypsin-EDTA solution and incubate at 37 °C for 5 min or until the cells appear to be free-floating and round in shape when viewed with a light microscope. Using a pipette tip, suck up the trypsin-cell solution and transfer to a 15 mL conical centrifuge tube. Rinse the cell culture container with several mL of N2 medium and transfer this solution to the previous 15 mL conical tube containing the solution of cells. Spin down at 1250 xg for 5 min at 23°C. Remove the supernatant, resuspend the cells in culture medium, and plate onto prepared surfaces as described previously.

and direct neural outgrowth. A number of automated and semi-automated methods for quantifying this neurite outgrowth has been utilized in recent years [2,3].

We describe a method to study the effect of topographical cues on spiral ganglion neurite and Schwann cell alignment. We discuss our microfabrication process, characterization of pattern features, cell culture techniques for both spiral ganglion neurons and spiral ganglion Schwann cells. In addition, we describe protocols for reducing fibroblast count, immunocytochemistry, and methods for quantifying neurite and Schwann cell alignment.

Keywords

Photopolymerization; topographical micropattern; spiral ganglion; primary culture; dissociated culture; explant

1. Introduction

Spiral ganglion neurite and Schwann cell guidance is an important therapeutic aim in the treatment of hearing loss and design of cochlear implants. Microfabrication of patterned substrates to direct cell growth has had a significant impact on understanding Schwann cell and neurite guidance [4]. Imposed topography on engineered substrates can promote cell adhesion, spreading, alignment, and changes in morphology and gene expression [5]. The conventional microfabrication technique, to form topographical micro- and nanopatterns, is photolithography, which most often involves a clean room and numerous coating and washing steps to generate substrates [6]. This approach is often time consuming and expensive, and results in sharp patterns with zero-one transitions and a virtually infinite slope.

In contrast to photolithography, photopolymerization is a simple one-step method to quickly and inexpensively form substrates with smooth topographies and gradually sloping transitions between ridges and grooves. The amplitude of these linear patterns is easily controlled; channel depths may range from 250 nm-10 μ m. These micropatterns guide spiral ganglion neurite pathfinding *in vitro* [7]. Here, we describe the one-step fabrication method to generate parallel groove-ridge topographies. Spiral ganglion cultures can then be plated on the photopatterned substrates and observed for alignment to the pattern.

Both explant and dissociated spiral ganglia may be plated on these micropatterned materials. While neurite behavior can be observed in explant cultures, it is sometimes difficult to quantify, because of high cell densities. However, explants may be advantageous, if large numbers of cells are needed within a small area or when preserving native tissue structure. In contrast, dissociated culture is best for observing single cell morphology. It should be noted that dissociated spiral ganglion cultures have a mixed population of cells with distinct shapes. Spiral ganglion neurons typically have round cell bodies with eccentric nuclei and a variable number of thin hair-like neurites extending out from the soma. These neurons require trophic support for survival, since they will typically die within 48 hours without neurotrophins or other sources of support [8]. Schwann cells typically are spindle shaped, though in some cases the cell edges have a ruffled appearance. Fibroblasts have a variable

morphology, but tend to be multipolar and flattened. Immunocytochemical staining for cell type specific markers is always used to confirm cell identity.

We also describe the system we developed to quantify the effect of patterned substrates on neurite alignment. A number of automated and semi-automated methods for quantifying neurite outgrowth have been developed in recent years [2,3], but most methods focus on measuring neurite length rather than directionality. To determine the alignment of neurites to linear patterns, we calculate an alignment ratio of each neurite relative to the pattern, using the measurement tool in ImageJ (NIH). This measurement is defined as the overall traced neurite length divided by the length of a line parallel to the pattern (end-to-end distance) (Fig. 1). Using this method, we are able to measure neurite alignment to a linear feature, as well as neurite segment angle distribution relative to the linear feature [9,7]. For more complex patterns, we developed a simple custom MATLAB (MathWorks) based algorithm for parsing neurite segments and binning angle measurements [7].

Finally, we describe a simple method for quantifying the alignment of Schwann cells on patterned substrates. An ellipse is fit to the outline of the Schwann cell, and the major axis of this ellipse is used as a measurement of the directionality of the cell relative to the pattern [10].

2. Materials

2.1. Monomer and Silane Coupling Agent Solutions

1. General Monomer Solutions: Weigh 1 wt. % of 2,2-Dimethoxy-1,2-diphenylethane-1-one (Irgacure 651(I-651)), a photoinitiator used for photopolymerization, in a glass vial.
2. Place the vial onto a balance, pipette the appropriate amounts of monomers into the vial (*see* Note 1) and vortex until completely dissolved [10,11].
3. Store the resulting pre-polymer solution in the dark at room temperature until needed. This storage will avoid any unwanted premature polymerization (*see* Note 2).
4. Silane Coupling Agent Solution: Pipette 1 mL of 3-(trimethoxysilyl)propyl methacrylate (silane coupling agent) into a glass bottle containing 100 mL of hexanes. Swirl the solution gently to ensure mixing.

2.2. Micropattern Substrate Fabrication and Characterization

1. Mercury vapor arc lamp equipped with a collimator lens to ensure uniform intensity throughout illuminated region.

¹Although a number of monomer formulations are compatible with this method [11], a 40 wt % hexyl methacrylate/60 wt % hexanediol dimethacrylate system is the typical formulation of choice. For an approximately 1 g solution of this formulation specifically, weigh 0.01 g of I-651 in a glass vial and pipette 0.40 g of hexyl methacrylate and 0.60 g of hexanediol diacrylate monomer into the vial.

²New monomer solutions should be made every 30 days. The solutions will gradually form oligomers, which will affect kinetics of polymerization and polymer micropatterns.

2. Unfrosted glass microscope slides (2.54 cm × 7.62 cm).
 3. Quartz or glass photomasks (10–100 μm periodicity).
 4. Glass cutter.
 5. Glass dish with a sealable lid large enough to store several glass slides without stacking.
 6. Non-sterile Petri dish.
 7. 95% v/v ethanol solution.
 8. Hexanes.
 9. White light interferometer.
- Scanning electron microscope.

2.3 Surgical Instruments

1. Heavy scissors.
2. Tissue scissors (small Iris scissors work well).
3. Fine forceps x2 (size 55 forceps work particularly well).
4. Bucket of ice.
5. Sterile normal saline.
6. 2 sterile Petri dishes.
7. Sterile scalpel blades (11 or 15 blades will work well).

2.4 Cell Culture Reagents

1. Tissue culture hood with UV germicidal lamp.
2. N2 media with 10% fetal bovine serum (FBS): To make 10 mL of media use 8.7 mL 1x Dulbecco's Modified Eagle Medium, 1 mL FBS, 100 μL of 1 mg/mL insulin stock for a final concentration of 10 μg/mL, 200 μL of 100X N-2 Supplement (Gibco, Life Technologies, Grand Island, NY).
3. Hank's Buffered Saline Solution with $\text{Ca}^{2+}/\text{Mg}^{2+}$ (HBSS^{+/+}).
4. Hank's Buffered Saline Solution without $\text{Ca}^{2+}/\text{Mg}^{2+}$ (HBSS^{-/-}).
5. Poly L-Ornithine 0.01% aqueous solution.
6. Laminin at 20 μg/mL in HBSS^{-/-}.
7. Neurotrophin 3 (NT-3): Mix NT-3 in N2 medium for a 50 ng/mL final concentration.
8. Brain-derived neurotrophic factor (BDNF): Mix BDNF in N2 medium for a 50 ng/mL final concentration.

2.4 Immunocytochemistry, Protease and Anti-fibroblastic Reagents

1. 50 mL of Blocking Buffer: Dissolve 1 g of powdered bovine serum albumin (BSA) in 25 mL of PBS and vortex. Add 2.5mL of Normal Goat Serum (NGS), top off to 50 mL with additional PBS, then add 50 uL of Triton-X.
2. PBS: We typically make 10X concentrated stock solution, then dilute it 1:10 with double deionized water as needed. To make 1 L of 10X PBS, weigh out 80 g NaCl, 2 g KCl, 12.5 g Na₂HPO₄, 2 g KH₂PO₄ and add to a 1 L volumetric flask. Add double deionized water to 1 liter and heat to dissolve.
3. 50 mL of 0.8% Triton-X in PBS: Add 0.4 mL of Triton-X to 49.6mL of PBS.
4. 50 mL of 4% Paraformaldehyde (PFA) in PBS: Dissolve 2g of PFA in ~ 48mL of PBS.
5. 0.25% Trypsin solution in HBSS^{-/-} (Gibco by Life Technologies, Grand Island, NY)
6. 0.2% Collagenase solution in HBSS^{-/-}: For 15 mL of solution, weigh 30 mg of collagenase powder (Sigma-Aldrich, St. Louis, MO) and dissolve in 15 mL of HBSS^{-/-}. Sterile filter and store in 0.5–1 mL aliquots at –20°C until needed.
7. Minimum Essential Medium Eagle with D-valine modification (Sigma-Aldrich) (*see* Note 3)
8. Cytosine β-D-arabinofuranoside (AraC, Sigma-Aldrich)

2.5 Microscopy and Software

1. Epifluorescent microscope with digital camera.
2. MetaMorph® Microscopy Automation and Image Analysis Software
3. Computer with a large, high-quality monitor
4. ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://imagej.nih.gov/ij/>, 1997–2014.).
5. MATLAB (R2013b, Natick, Massachusetts: The MathWorks Inc., 2013.).

3. Methods

3.1. Glass slide activation

1. Arrange standard 2.54 cm × 7.62 cm glass microscope slides in an O₂ plasma cleaner with the slides placed side by side. Expose the slides with plasma at 30 W RF for at least 3 min to ensure sufficient surface polarization.
2. Remove the glass slides from the plasma chamber and quickly transfer them to a sealed glass dish for further functionalization.

³Unmodified MEM usually has L-valine at 46 µg/mL whereas MEM with D-valine modification substitutes L-valine with D-valine at 92 µg/mL

3. To this container, pour 100 mL of silane coupling agent solution over the glass slides with the plasma exposed side of the slide face up. Verify that all slides are completely submerged in the coupling agent solution. Cover the glass dish with aluminum foil or parafilm to prevent solvent evaporation overnight (*see Note 4*).
4. After letting the slides react overnight gently remove each slide, using tweezers, and wash in hexanes then let the slides air dry (*see Note 5*). Cut activated slides in half, using a glass cutter, forming two 2.54 cm × 3.81 cm slides, then transfer the activated glass slides into a sealed container.

3.2. Polymer Microfabrication and Characterization

1. Place an activated 2.54 cm × 3.81 cm slide on a small movable platform (an inverted petri dish works well) and pipette 20 µL of monomer solution on the middle of the glass slide.
2. Lower a clean, lint-free, photomask slowly until it contacts the monomer droplet and the solution evenly disperses between the glass slide and the photomask.
3. Without disrupting the position of the photomask on the glass slide, move the inverted petri dish with the slide under a mercury vapor lamp and illuminate the sample (*see Note 6 and 7*). After polymerization, remove the photomask from the polymer by wedging a razorblade between one of the corners of the photomask and the polymer. Once the blade is secured, gently lift and the photomask should release without difficulty (*see Note 8*).
4. Wash the polymer copiously with 95% v/v ethanol. Let the polymer air dry and store in a covered container to avoid dust collection. The resulting polymers have microstructure as represented in Figure 2.
5. Clean the photomask by rinsing with 95% v/v solution.
6. Micropattern periodicity, channel amplitude, and general surface morphology of polymer samples are characterized by standard white light interferometry methods or scanning electron microscopy. Channel amplitudes will vary from 0.25 µm to 10 µm depending on the polymerization conditions (Fig. 3, *see Note 7*).

3.3 Preparing Polymers for Culture

1. Sterilize micropatterned polymers in a tissue culture hood by irradiating with a UV germicidal lamp in the hood for at least 5 min. Be sure that no laminin or

⁴The seal between the container and the aluminum should be as air tight as possible to prevent solvent release. Do not use a plastic lid to cover the dish, as solvent will swell and potentially dissolve the plastic.

⁵When handling glass slides the surface must not be scratched, as it will remove the activation layer. Handle the slides from the edges when possible.

⁶An intensity of 16 mW/cm² at 365 nm is recommended, when using a mercury vapor arc lamp.

⁷For a given photomask bandspacing, the amplitude of the surface features is highly dependent on illumination time, illumination intensity, and photoinitiator concentration. Varying polymerization time is the most practical method to reach target amplitudes. More information regarding channel amplitude as a function of the aforementioned variables is found in [7].

⁸The photomask should release easily for HMA/HDDMA polymer systems, but may strongly adhere for other polymers. A small coating of Rain-X® water repellent or a release film will allow the photomask to be removed from most polymers. If the polymer strongly adheres to the photomask, soak in acetone overnight to soften the polymer and remove the photomask.

poly L-ornithine solutions are in the hood during this process, as the UV germicidal lamp may inactivate proteins.

2. Pour poly L-ornithine to coat the micropatterned polymers for tissue culture and allow to coat at room temperature for 30 min, then remove.
3. Pour 20 µg/mL laminin solution on the poly L-ornithine coated micropatterned polymer and allow to coat at room temperature for 30 min or overnight at 4 °C, then remove.

3.5 Dissection

We recommend perinatal (p3–p6) rats or mice for cell cultures. All protocols should be approved by the Institutional Animal Care and Use Committee under the guidelines set forth by the National Institutes of Health.

1. Decapitate the animal using heavy scissors and bisect the head in the midline with tissue scissors (*see Note 9*). Using a blunt instrument (such as the side of the scissors), gently remove the brain tissue from the cranial cavity, clipping attached cranial nerve roots with scissors as needed.
2. Once the brain is removed, the tentorium cerebelli should be visible as a thin shelf of tissue just posterior to the temporal bone/otic capsule. Cut the area containing the otic capsule away from the rest of the skull and place in a Petri dish of PBS on ice under a dissecting microscope (*see Note 10*).
3. Remove excess tissue and bone around the otic capsule (*see Note 11–12*). Stabilize the bone by grasping the lateral semicircular canal with forceps held in the non-dominant hand.
4. Remove the bony capsule of the cochlea by flicking the bone off the exterior of the cochlea using forceps held in the dominant hand.
5. Scrape off the spiral ligament and Organ of Corti, transecting the radial fibers extending outwards from the modiolus. The modiolus, containing the spiral ganglion neurons, should now look approximately like a pine tree, tapering towards the apex and widening towards the base.
6. Using fine forceps, remove as much of the modiolar connective tissue as possible without damaging the ganglion. Incomplete removal of connective

⁹Spray the rat or mouse pups with 70% EtOH and wipe prior to decapitation. This reduces the bacterial load on the skin and the chance for culture contamination.

¹⁰Dissecting the otic capsule against a black background makes the tissue more visible. In order to perform dissections on ice, we will typically place a black rubber mat over a frozen cold pack. We mount a microscope in the laminar flow hood in order to maintain sterility of the tissue during dissection.

¹¹Rat middle ear anatomy features a tympanic bulla, a thin capsule of bone located on the inferior-lateral side of the skull, posterior to mandibular head. The tympanic bulla contains all the middle ear structures and the cochlea is located in the medial wall of the tympanic cavity. When inserting forceps into the external auditory canal, to remove the lateral wall of the tympanic bulla, avoid damaging the medial wall of the tympanic cavity with the forceps. A clean dissection of the spiral ganglion is difficult, after damaging the bony capsule of the cochlea.

¹²Because the otic capsule is harder than the surrounding bone, the surrounding bone can usually be flicked away from the otic capsule with relative ease. To separate the otic capsule from the remainder of the temporal bone, a quick and easy method is to grip one of the semicircular canals seen from the inside of the skull, then apply ventrally-directed pressure. The otic capsule, including the cochlea, should separate cleanly from the rest of the temporal bone.

tissue is acceptable in the interest of achieving a higher neuron yield, though it may increase fibroblast count.

7. Once cleaning is complete, place the ganglion in a 1.5–2 cc tube of HBSS^{+/+} on ice and continue to harvest as many spiral ganglia as indicated by experimental needs.
8. When you have harvested as many spiral ganglia as needed, process the tissue for explants or dissociated culture.

3.6 Preparation of Explants

1. Place a single ganglion in a Petri dish of PBS on ice under the dissecting microscope. Grasp the base of the ganglion or the stump of the cochlear nerve with fine forceps to stabilize.
2. Using the blunt side of a scalpel blade or a second set of forceps, gently uncurl the ganglion so that it lies as flat as possible.
3. Using a fresh scalpel blade, cut the ganglion into 4 pieces (or as many as desired). Keep track of which pieces are closest to the apex vs the base if these data are important.
4. Using fine forceps, carefully place these explants in the desired position on your prepared substrate. It is best to have a very thin film of N2 medium on the surface of the substrate before putting the explants down, as surface tension and gravity will help keep the explant from floating away.
5. Allow the explants to settle down for 1–2 h and attach to the substrate, then add more N2 medium. Be sure to supplement the N2 medium with NT-3 and BDNF if neuronal survival is desired.

3.7 Preparation of Dissociated Cultures

1. Gently spin the tissue to the bottom of the tube of HBSS^{+/+} or simply allow the tissue to settle to the bottom of the Eppendorf vial. Remove the HBSS^{+/+} and in its place add 500mL of warmed 0.25% trypsin and 0.2% warmed collagenase. Close the vial and incubate at 37 °C for 20 min with intermittent agitation.
2. Add 100 µL of FBS to the Eppendorf vial to inactivate the trypsin. Allow the tissue to settle or spin briefly in a microfuge. Remove the supernatant and add a small amount of N2 medium. Shake lightly to rinse, allow to settle again, then repeat N2 rinse a second time.
3. After the final rinse, add 0.5 mL of N2 medium to the tissue.
4. Using the cut-off 1000 µL pipette tip, triturate by pipetting the digested tissue up and down 5–8 times. The solution should now be turbid.
5. Using an uncut 1000 µL pipette tip, triturate again 5–8 X.
6. Using an uncut 200 µL pipette tip, triturate again 5–8 X. It is acceptable that some small undissociated pieces of tissue remain at this point.

7. Add the cell suspension onto your prepared substrate (*see* Note 13). Add N2 medium, which is now supplemented with NT-3 at 50 ng/mL and BDNF at 50 ng/mL, to the desired final volume and incubate at 37 °C in 6% CO₂.

3.8 Enriching for Spiral Ganglion Schwann cells

1. To treat with D-valine, make serum-free N2 medium using MEM with D-valine modification (*see* Note 3). Change the cell culture medium using this formulation (*see* Note 24). This type of medium may be used constantly and indefinitely.
2. To treat with cytosine arabinoside (AraC) (*see* Note 25), supplement N2 medium with serum with AraC (20 μM, we keep our stock AraC at 200 mM so that it can be diluted 1:1000). Change the cell culture medium using the AraC supplemented medium, and incubate for 24 h. After 24 hours, change to serum-free N2 medium for 48 hours.

3.9 Fixation and Immunocytochemistry

1. Remove your culture from the incubator. Remove medium, then add 4% PFA solution. Incubate at room temperature on shaker for 10–15 minutes.
2. Rinse 3x in PBS.
3. Permeabilize in 0.8% Triton in PBS for 30 minutes at room temperature on shaker.
4. Incubate in blocking buffer for 1 hour at room temperature on shaker.
5. Dilute primary antibodies in blocking buffer as desired (*see* Notes 14–16). Replace blocking buffer with primary antibody and incubate overnight at 4°C or at room temperature for 2 hours..
6. Rinse 3x in PBS.
7. Dilute secondary antibodies in blocking buffer as desired (*see* Note 17). Incubate overnight at 4°C on shaker or at room temperature for 1 hour.
8. Rinse 3x in PBS.
9. Coverslip and proceed to imaging; microscopy instructions vary by manufacturer; we use the scan slide tool in Metamorph to generate an image the

¹³To plate on only a small area of your surface, add a thin layer of N2 to the area of interest, then carefully add the cell suspension. After adding the cells, wait at least 30 min, but preferably 1 h, to allow cells to adhere. Then add the remainder of the medium.

²⁴If the pattern is not horizontally aligned in the image you have taken, you may correct for the difference by measuring the angle of the pattern relative to horizontal in this image, then subtract this measurement from the major-axis angle measurements. Be sure to do this prior to correcting your measurements to the 90° range.

²⁵Because overlapping or adjacent cells can make this method difficult, and also because at higher cell densities Schwann cell alignment is increasingly affected by adjacent cells, it is best to use a lower-density culture

¹⁴S100β is an excellent marker for Schwann cells, for which multiple antibodies are available from vendors. Our lab has the best results with Abcam's rabbit anti-S100β antibody. We typically store this reagent at a dilution of 1:2 in glycerol for better preservation, whereas we use the antibody at a dilution of 1:400 or 1:800 final.

¹⁶Vimentin is a marker for mesenchymal cells including fibroblasts, but it should be noted that not all fibroblasts stain for vimentin.

¹⁷We typically use secondary antibodies conjugated to Alexa Fluor® dyes (Life Technologies, Grand Island, NY). Alexa Fluor® dyes are available in a variety of excitation and emission spectra. Be sure to co-stain with secondary antibodies conjugated to dyes with sufficiently different excitation and emission spectra to avoid bleed-through across stains. It is recommended to use dyes with emission spectra that are at least 100 nm wavelength apart.

entire substrate surface using a 20X objective. This provides sufficient resolution to score neurite and Schwann cell alignment.

3.10 Quantifying neurite alignment

1. Open a scan-slide image in ImageJ (*see* Note 18).
2. In the toolbar, right-click the Line tool and select “Freehand line” option.
3. Click Measure under the Analyze menu to open a window with measurements. Ensure that angle and distance are being measured (*see* Note 19).
4. Select the neurite(s) to be measured in the scan slide image and trace the neurite using the Freehand line tool. Record the length and angle for each segment by pressing Control-M, on a PC, or Command-M, on a Macintosh. Next, trace and measure the end-to-end distance of the pattern from the neuronal soma to the end of the neurite and corresponding distance along the pattern to calculate alignment ratio (Fig. 1). Repeat these measurements as many times as needed within the image. Data can be imported and saved for analysis in Microsoft Excel, Sigmaplot or other data analysis software.

3.11 Quantifying Schwann cell alignment

1. Within ImageJ, open the images of the Schwann cell cultures (*see* Note 18).
2. Select the “Freehand selections” tool and outline the periphery of the cell. Ensure that Major axis, Minor axis, and Angle are being measured (*see* Note 19).
3. Once the cell has been outlined, click Edit > Selection > Fit Ellipse in order to fit an ellipse to the outline of the cell.
4. Press control-M on a PC or command-M on a Macintosh to measure the angle of the major axis to the horizontal plane. A window with measurements (Major axis, Minor axis, Angle) should appear.
5. Once you have measured the desired number of cells in each image, you can copy the measurement data into the data analysis software.
6. After importing the data into a spreadsheet, correct all angles that are greater than 90° by subtracting from 180° (*see* Note 23–24).

¹⁸A scan-slide of the sample is taken and stitched together using MetaMorph software, when quantifying neurite alignment. This approach is taken to avoid a bias towards neurites shorter than the size of the capture window, when only capturing single-frame images. For Schwann cell alignment, randomly taken pictures are sufficient, as they are almost always smaller than a capture window. However, only quantify alignment on Schwann cells that are entirely captured within your image.

¹⁹Go to the “Select Measurements” tab under Measure and ensure that the Angle and Distance boxes are checked, if after selecting Measure or pressing control/command-M, you find that angle and distance are not recorded. Re-open the Measure tool to record measurements.

²³Because we investigate linear patterns where 0 and 180° alignment is equivalent, we correct all Schwann cell alignment measurements to a range of 0–90°. For example, a measurement of 150° is actually equivalent to a measurement of 30°, except that it is left-leaning rather than right-leaning.

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Figure 1.

Method for quantifying neurite alignment to linear patterns. Alignment ratio is defined as the ratio of neurite length to end-to-end distance. As the length of the neurite increases over the end-to-end distance, the alignment ratio increases. A perfectly aligned neurite would have an alignment ratio of 1.

Figure 2.

Schematic of photopatterning process. Monomer is selectively exposed to UV light through transparent bands of the photomask resulting in raised microfeatures across the polymer surface.

Figure 3. Characterization of micropatterned surfaces. A) Representative SEM images showing gradually sloping transitions of a parallel line patterned polymer of 8 μm amplitude and 50 μm periodicity. B) 2 D profile of micropatterned polymer using white light interferometry showing 1 μm amplitude and 50 μm periodicity. Inset: 3 D representation of 100 μm² area of the same polymer (Figures adapted from reference 3. Permission for reproduction granted by Elsevier).