Supporting Information

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SI Materials and Methods

Cell Culture and Image Acquisition. Fluorescence observations were done with NG108-15 cells transfected with a pEGFP (enhanced green fluorescent protein)-actin vector (Clontech) to fluorescently label the actin cytoskeleton. Transfection was performed using Nanofectin (PAA) according to the product manual. Cells were cultured in Dulbecco's Modified Eagle Medium (PAA) supplemented with 10% fetal bovine serum (PAA), 100 U/mL penicillin/streptomycin (Sigma) and 10 mM Hepes (Sigma). To ensure cell viability and pH stability during the observation period, the petri dish was placed in a sealed temperature controlled chamber. Furthermore, the objective was heated to 37 °C to prevent cooling of the samples. Fluorescence image time series were recorded for 5–20 min with 3–6 s time resolution using an oil immersion objective [63× 1.4 NA (numerical aperture), Leica Microsystems] on a confocal laser scanning microscope (TCS SP2 AOBS, Leica Microsystems) with a resolution of 1,024× 1,024 pixel and 4× line average in bidirectional mode.

Measurement of the Viscoelastic Properties. Scanning force microscopy (SFM) measurements were recorded using a NanoWizard SFM (JPK Instruments) placed on an inverted microscope DM IRB (Leica Microsystems). Commercial cantilevers (NANOSENSORS; Nano World) with spring constants of approximately 0.02–0.06 N/m were modified as described previously (1) by gluing polystyrene beads (Seradyn Particle Technology; radius approximately 2.8 μm) to the tip. Oscillatory drive signals necessary to perform frequency-dependent viscoelasticity measurements were generated by a lock-in amplifier (SR850; Stanford Research Systems). Phase and amplitude differences between the applied modulation and the cantilever response signal were recorded. Cells were placed on commercial glass slides (Superfrost-Plus, Menzel–Gläser). A detailed description of rheological SFM measurements on isolated cells has previously been published (1, 2).

For frequency-dependent measurements, the cantilever slowly approached the sample, whereas an oscillation was superimposed on the cantilever. This experiment was done several times with different frequencies (0.3 Hz, 1 Hz, 3 Hz, 10 Hz, 30 Hz). Using a standard rheology approach (1–3), the measured stress and strain signals are expressed in terms of frequencies, and thus a frequency-dependent complex dynamic elasticity modulus $E^* = E' + iE''$ is measured.

The following gives the calculations that allow to extract the viscoelastic properties like relaxation time and the elastic relaxation modulus from frequency-dependent measurements of the storage modulus $E'(\omega)$ and the loss modulus $E''(\omega)$. We use a viscoelasticity model to relate the SFM measurements to the growth cones' viscoelastic properties. Wottawah et al. (3) have shown that the viscoelastic behavior of cells can be described by a viscoelastic three parameter model where elastic and viscous elements are arranged in parallel (Voigt–Model), combined with a viscous element that is in series to the previous pair (Fig. S2). For each of the three elements the stress can be related to the strain:

$$\sigma_1 = \eta_1 * \dot{u} \qquad \textbf{[S1]}$$

$$\sigma_2 = \eta_2 * \dot{u} \qquad \textbf{[S2]}$$

$$\sigma_3 = E_3 * u. \qquad \textbf{[S3]}$$

Furthermore, the geometry constraints the total stress to be $\sigma = \sigma_1 = \sigma_2 + \sigma_3$ and the total displacement to be $u = u_1 + u_2 = u_1 + u_3$. Combining these equations, one finds a general differential equation that describes the behavior of such a model:

$$a_1\dot{u} + a_2\ddot{u} = \sigma + b_1\dot{\sigma}, \qquad \textbf{[S4]}$$

with the parameters expressed in terms of η_1, η_2, and E_3. Eq. **S4** can also be derived more general as the first terms of a series expansion (4). Because the frequency-dependent properties of this differential equation are required, we used the Fourier transformation method to solve this equation further. Expressing $u(t)$ and $\sigma(t)$ as its Fourier transform the time derivatives can be obtained as $\dot{u}(t) = i\omega u(t)$, $\ddot{u}(t) = -\omega^2 u(t)$, and $\dot{\sigma}(t) = i\omega\sigma(t)$. Thus Eq. **S4** is solved to be:

$$\sigma(t) = \Bigg(\underbrace{\frac{\omega^2(a_1b_1 - a_2)}{1 + \omega^2 {b_1}^2}}_{E'(\omega)} + i \underbrace{\frac{\omega a_1 - \omega^3 a_2 b_1}{1 + \omega^2 {b_1}^2}}_{E''(\omega)} \Bigg) u(t). \qquad \textbf{[S5]}$$

This is just in the form to identify the storage modulus $E'(\omega)$ and the loss modulus $E''(\omega)$ with fit parameters a_1, a_2 and b_1:

$$E' = \frac{\omega^2(a_1b_1 - a_2)}{1 + \omega^2 {b_1}^2} \qquad \textbf{[S6]}$$

$$E'' = \frac{\omega a_1 - \omega^3 a_2 b_1}{1 + \omega^2 {b_1}^2}. \qquad \textbf{[S7]}$$

This model is used to fit the measured complex elastic modulus as shown in Fig. 1 of the main text. The fit parameters relate to the viscoelastic steady state parameters by $\tau_R = b_1$,$\eta = a_1$, $E = \frac{{a_1}^2}{a_1 b_1 - a_2}$, where τ_R is the relaxation time, η is the steady state viscosity and E is the Young's modulus.

Transforming the frequency-dependent complex dynamic modulus into the time domain yields the time-dependent relaxation modulus, which is required for the internal force calculation:

$$E(t) = \frac{a_1 b_1 - a_2}{{b_1}^2} e^{(-t/b_1)} + \frac{a_2}{b_1}\delta(t). \qquad \textbf{[S8]}$$

As the SFM measures $E'(\omega)$ and $E''(\omega)$, Eqs. **S6** and **S7** can be used as fit functions to extract the three parameters a_1, a_2, and b_1 that fully describe the model.

Theory of Viscoelasticity to Calculate Internal Forces. To gain the forces that drive retrograde actin flow within the lamellipodium of neuronal growth cones, we extended linear elasticity theory to the viscoelastic regime. Linear elasticity theory (5, 6) connects stress σ and strain u. The simplest application is Hooke's law for a 1D deformation of an elastic:

$$\sigma = E * u, \qquad \textbf{[S9]}$$

with E being the Young's modulus. In the ideal case of pure elastic deformation, the stress does not decrease over time if the deformation persists. The other extreme is a pure viscous deformation, in which the stress in the object relaxes immediately. Here, the stress only depends on the speed of the deformation, represented by the strain rate $\dot{u} = du/dt$:

$$\sigma = \eta\dot{u}, \qquad \textbf{[S10]}$$

where η is the steady state viscosity. Theory of viscoelasticity combines both types of deformation. If the stress resulting from a known deformation is sought, the Boltzmann superposition principle is useful (4). This principle states that the effect of a compound cause is the sum of the effects of the separated individual causes. Thus the strain history is considered as a composition of single deformation pulses:

$$\sigma(t) = \int_{-\infty}^{t} E(t-\tau)\frac{du}{d\tau}d\tau. \qquad \textbf{[S11]}$$

If the time-dependent relaxation modulus $E(t)$ and the strain $u(t)$ are known, the time-dependent stress $\sigma(t)$ can be calculated. To reach a similar description in 3D, it is necessary to change to a tensorial description of strain and stress. The strain tensor u_{ij} is calculated at each point in the object from the deformation vector $\vec{u}$ by

$$u_{ij} = \frac{1}{2}\left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} + \frac{\partial u_i}{\partial x_j}\frac{\partial u_j}{\partial x_i}\right), \qquad \textbf{[S12]}$$

and for small deformations u_{ij}, this equation can be linearized by neglecting the last term: $u_{ij} = 1/2(\partial u_i/\partial x_j + \partial u_j/\partial x_i)$. The linearization is valid in the system of growth cones, because during the relaxation time, the flow deforms the lamellipodium by less than 5%. Deriving Hooke's law in three dimensions leads to an expression in which a fourth order tensor C_{ijkl} is required to connect stress and strain:

$$\sigma_{ij} = C_{ijkl}u_{kl}. \qquad \textbf{[S13]}$$

Here, the Einstein sum convention is used. Using symmetry arguments and assuming a homogeneous, isotropic material, it can be shown (5) that the complicated fourth order elastic modulus tensor C_{ijkl} can be reduced to two nonzero values. These two numbers are called Lamé coefficients λ,μ, and Eq. **S13** reduces to:

$$\sigma_{ij} = \lambda u_{kk}\delta_{ij} + 2\mu u_{ij}, \qquad \textbf{[S14]}$$

where δ_{ij} is the Kronecker Delta. This relation can be converted to use the bulk properties Young's modulus E and Poisson ratio ν to calculate the stress:

$$\sigma_{ij} = \frac{E}{1+\nu}\left(u_{ij} + \frac{\nu}{1-2\nu}u_{kk}\delta_{ij}\right). \qquad \textbf{[S15]}$$

Recalling the Boltzmann superposition principle Eq. **S11**, the time-dependent stress can be calculated by:

$$\sigma_{ij}(t) = \int_{-\infty}^{t} \frac{E(t-\tau)}{1+\nu}\left(\frac{du_{ij}}{d\tau} + \frac{\nu}{1-2\nu}\frac{du_{kk}}{d\tau}\delta_{ij}\right)d\tau. \qquad \textbf{[S16]}$$

Internal Force Calculation. To calculate the time-dependent stress from the measured deformation data, the viscoelastic constitutive equation has to be integrated:

$$\sigma_{ij}(t) = \int_{-\infty}^{t} \frac{E(t-\tau)}{1+\nu}\left(\frac{du_{ij}}{d\tau} + \frac{\nu}{1-2\nu}\frac{du_{kk}}{d\tau}\delta_{ij}\right)d\tau. \qquad \textbf{[S17]}$$

The integral of Eq. **S17** is solved using the measured data for the strain rate, which arises from the deformation between two confocal image recordings (separated by time Δt). One can separate the process in four steps:

1. Detection of the flow fields using previously described correlation algorithms (7).
2. Calculation of the static stress field that is generated by the observed deformation during two successive images. This results in one tensorial stress field for each image pair.
3. Accumulating the stresses from the previous deformations, whereas integrating the decay of these stresses according to the viscous dissipation.
4. Calculation of the internal forces using the local force balance.

Step 1: Detection of the retrograde actin flow. This step has been already extensively discussed previously, and hence we refer the interested reader to previous articles (7).

Step 2: Calculation of the static stress. We assume that the strain rate $\dot{u}_{ij}$ is constant during the time interval between two confocal image recordings (Δt), therefore the integral for this fixed time sequence becomes independent of this constant strain rate. Hence, the integral can be split into subsequent time parts of length Δt.

The solution for the most recent time interval $[0,\Delta t]$ can be calculated to yield:

$$\sigma_{ij}(\Delta t)_{[0,\Delta t]} = \int_{0}^{\Delta t} \frac{E(\Delta t-\tau)}{1+\nu}\left(\frac{du_{ij}}{d\tau} + \frac{\nu}{1-2\nu}\frac{du_{kk}}{d\tau}\delta_{ij}\right)d\tau \qquad \textbf{[S18]}$$

$$= \underbrace{\frac{1}{1+\nu}\left(\frac{\Delta u_{ij}}{\Delta t} + \frac{\nu}{1-2\nu}\frac{\Delta u_{kk}}{\Delta t}\delta_{ij}\right)}_{\alpha_0} \int_{0}^{\Delta t} E(\Delta t-\tau)d\tau \qquad \textbf{[S19]}$$

$$= \alpha_0 \int_{0}^{\Delta t}\left(\frac{a_1 b_1 - a_2}{{b_1}^2}e^{(-(\Delta t-\tau)/b_1)} + \frac{a_2}{b_1}\delta(\Delta t-\tau)\right)d\tau. \qquad \textbf{[S20]}$$

Because the integral goes over half of the delta function, the second term is trivial:

$$\int_{0}^{\Delta t}\frac{a_2}{b_1}\delta(\Delta t-\tau)d\tau = 1/2 * \frac{a_2}{b_1}. \qquad \textbf{[S21]}$$

The first part of the integral can be calculated by:

$$\int_{0}^{\Delta t}\frac{a_1 b_1 - a_2}{{b_1}^2}e^{(-(\Delta t-\tau)/b_1)}d\tau = \frac{a_1 b_1 - a_2}{{b_1}^2}e^{(-\Delta t/b_1)}\int_{0}^{\Delta t}e^{(\tau/b_1)}\delta\tau \qquad \textbf{[S22]}$$

$$= \frac{a_1 b_1 - a_2}{{b_1}^2}e^{(-\Delta t/b_1)}[b_1 e^{\tau/b_1}]_0^{\Delta t} \qquad \textbf{[S23]}$$

$$= \frac{a_1 b_1 - a_2}{b_1}(1 - e^{-\Delta t/b_1}), \qquad \textbf{[S24]}$$

which reveals the result for the first time interval:

$$\sigma_{ij}(\Delta t)_{[0,\Delta t]} = \alpha_0 * \left(\frac{a_1 b_1 - a_2}{b_1}(1 - e^{-\Delta t/b_1}) + \frac{a_2}{2b_1}\right). \qquad \textbf{[S25]}$$

Step 3: The previous equation calculates the stress from the most recent deformation data. Unrelaxed stress from previous deformations also has to be included. We solve this problem by separating the integral of the deformation history into the discrete time spans that we measured, which is motivated by the sketch:

$$\int_{-\infty}^{0} = \sum_{n=1}^{m=t/\Delta t} \int_{-n\times\Delta t}^{-(n-1)\times\Delta t}. \qquad \textbf{[S26]}$$

Hence, we separate the integral into the sum of times. Each integral in this sum represents the time between two images. The goal is to derive a relation for each of these time spans, and then weight them by the prefactor that corresponds to the relaxation up to the current moment. Together with the time interval $[0,\Delta t]$, this yields the full history. Now the problem reduced to calculating for each previous time interval $[-n*\Delta t, -(n-1)\Delta t]$ the integral:

$$\sigma_{ij}(\Delta t)_{[-n\Delta t,-(n-1)\Delta t]} = \int_{-n\Delta t}^{-(n-1)\Delta t} \frac{E(\Delta t-\tau)}{1+\nu}\left(\left\{\frac{du_{ij}}{d\tau}\right\}_n + \frac{\nu}{1-2\nu}\left\{\frac{du_{kk}}{d\tau}\right\}_n \delta_{ij}\right)d\tau. \qquad \textbf{[S27]}$$

Similar as in the second step, we assumed that the deformation rate was constant during the recording of subsequent images.The index n at the curly brackets denoted the average deformation rate in the nth interval. Hence the non-time-dependent parameters can be separated and we combine them in the α_n parameter:

$$\sigma_{ij}(\Delta t)_{[-n\Delta t,-(n-1)\Delta t]} = \underbrace{\frac{1}{1+\nu}\left(\left\{\frac{du_{ij}}{d\tau}\right\}_n + \frac{\nu}{1-2\nu}\left\{\frac{du_{kk}}{d\tau}\right\}_n \delta_{ij}\right)}_{\alpha_n} \times \int_{-n\Delta t}^{-(n-1)\Delta t} E(\Delta t-\tau)d\tau \qquad \textbf{[S28]}$$

$$=\alpha_n \int_{-n\Delta t}^{-(n-1)\Delta t} \frac{a_1 b_1 - a_2}{{b_1}^2} e^{(-(\Delta t-\tau)/b_1)} + \frac{a_2}{b_1}\delta(\Delta t-\tau)d\tau. \qquad \textbf{[S29]}$$

The last term vanishes for $n \geq 1$ and the first term reduces to:

$$\sigma_{ij}(\Delta t)_{[-n\Delta t,-(n-1)\Delta t]} = \alpha_n \int_{-n\Delta t}^{-(n-1)\Delta t} \frac{a_1 b_1 - a_2}{{b_1}^2} e^{(-(\Delta t-\tau)/b_1)} d\tau \qquad \textbf{[S30]}$$

$$=\alpha_n \frac{a_1 b_1 - a_2}{{b_1}^2} e^{(-\Delta t/b_1)} [b_1 e^{\tau/b_1}]_{-n\Delta t}^{-n\Delta t+\Delta t} \qquad \textbf{[S31]}$$

$$=\alpha_n \frac{a_1 b_1 - a_2}{b_1} e^{(-\Delta t/b_1)} e^{(-\Delta t/b_1)n} (e^{\Delta t/b_1} - 1) \qquad \textbf{[S32]}$$

$$=\alpha_n \frac{a_1 b_1 - a_2}{b_1} e^{(-\Delta t/b_1)n} (1 - e^{-\Delta t/b_1}). \qquad \textbf{[S33]}$$

The last expression can be calculated using the measured strain rates at the nth time interval, and the viscoelastic parameters.

For the full stress tensor this calculation has to be done for the previous history, and then values are summed up. It has to be mentioned that the stress field of previous times we need to deform according to the measured deformation rates. This is necessary because a position, which is at a given point in the lab system, was previously at a different position. As the flow is known, this flow of stress can be calculated using the retrograde flow fields.

Step 4: Finally, the stress tensor has to be translate into a force. This is done by recognizing that a gradient in stress will immediately relax, unless it is compensated by an internal force in the system (5):

$$f_i^{\text{int}} = -\frac{\partial \sigma_{ik}}{\partial x_k}. \qquad \textbf{[S34]}$$

Here the Einstein sum convention applies.

1. Lu YB, et al. (2006) Viscoelastic properties of individual glial cells and neurons in the CNS. *Proc Natl Acad Sci USA* 103:17759–17764.
2. Mahaffy RE, Park S, Gerde E, Kas J, Shih CK (2004) Quantitative analysis of the viscoelastic properties of thin regions of fibroblasts using atomic force microscopy. *Biophys J* 86:1777–1793.
3. Wottawah F, et al. (2005) Optical rheology of biological cells. *Phys Rev Lett* 94:098103.
4. Lakes RS (1998) *Viscoelastic Solids* (CRC, Boca Raton).
5. Landau LD, Lifschitz EM (1986) *Theory of Elasticity* (Pergamon, Oxford).
6. Doi M, Edwards SF (1988) *The Theory of Polymer Dynamics* (Oxford University Press, Oxford).
7. Betz T, Koch D, Lim D, Kas JA (2009) Stochastic actin polymerization and steady retrograde flow determine growth cone advancement. *Biophys J* 96:5130–5138.

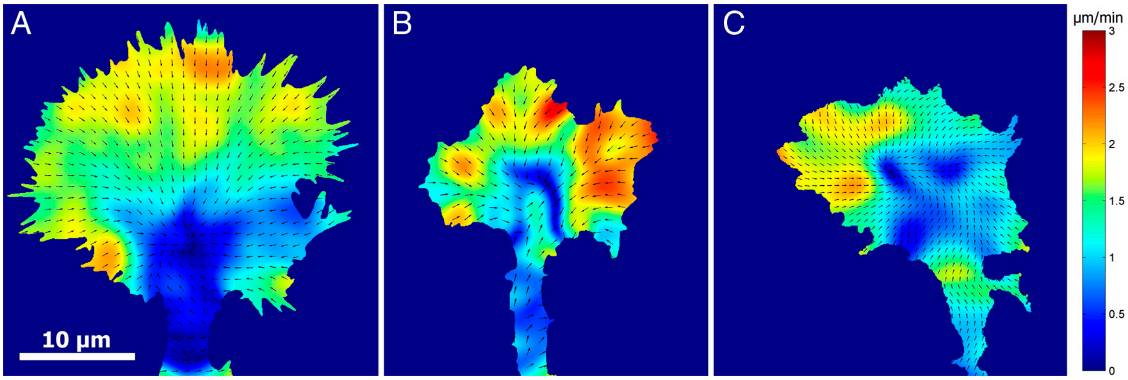


Fig. S1. Retrograde actin flow in neuronal growth cones. The color coded flow field represents the retrograde flow amplitude in µm/ min, the arrows (black) give the direction of flow. (*A*) Example of a very homogeneous retrograde flow field (this data was already used in a previous publication) (7). (*B*) Retrograde flow field of the growth cone presented in Fig. 2 of the main text. (*C*) Retrograde flow field of the growth cone presented in Fig. 3 of the main text.

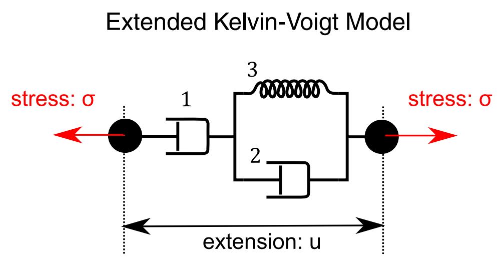
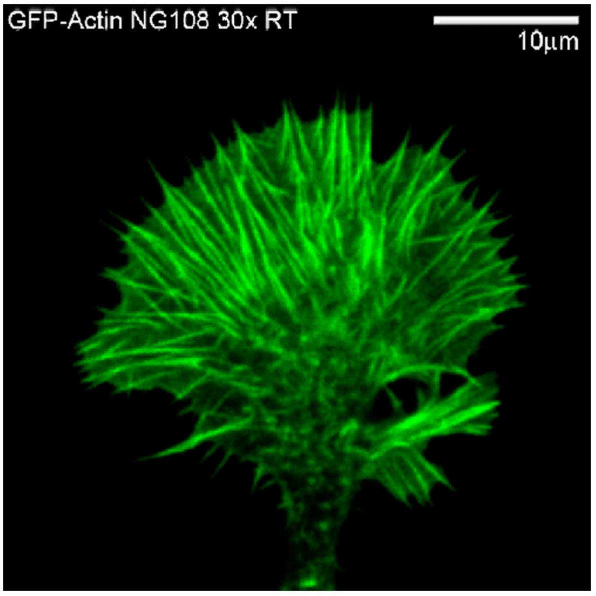
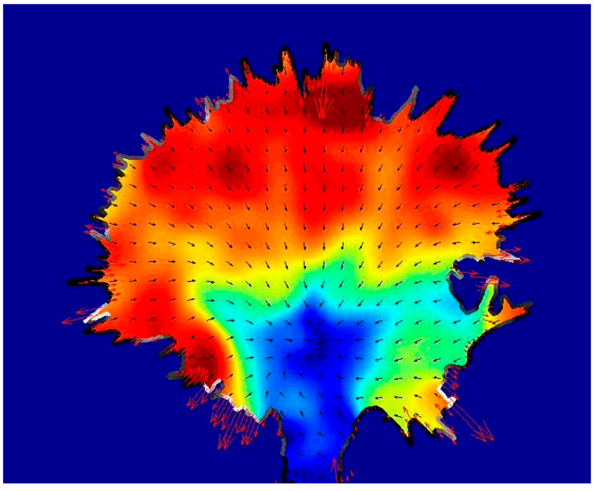


Fig. S2. Extended Kelvin–Voigt Model. Sketch of the viscoelastic model used to fit the SFM data presented in Fig. 1.



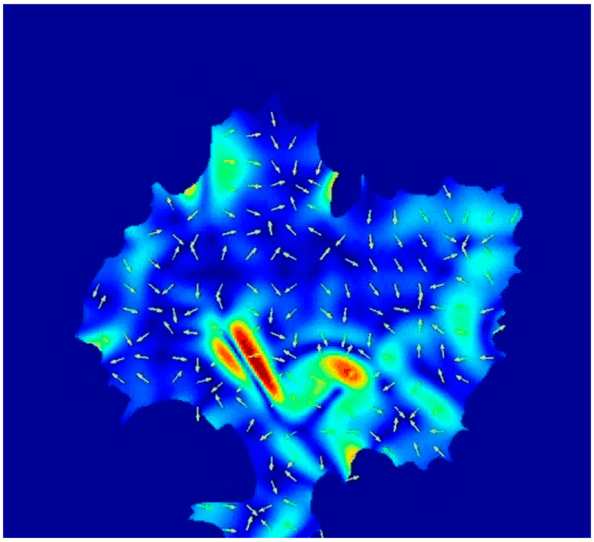
Movie S1. Fluorescence time series a GFP-actin transfected NG108-15 neuronal growth cone.

Movie S1 (AVI)



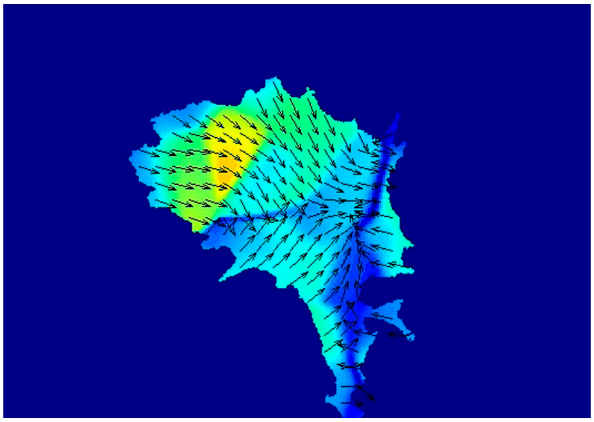
Movie S2. Detected retrograde flow field for a NG108-15 growth cone as presented in Movie S1. The color coding represents the flow velocity in µm/ min, and the black arrows denote the direction of flow.

Movie S2 (AVI)



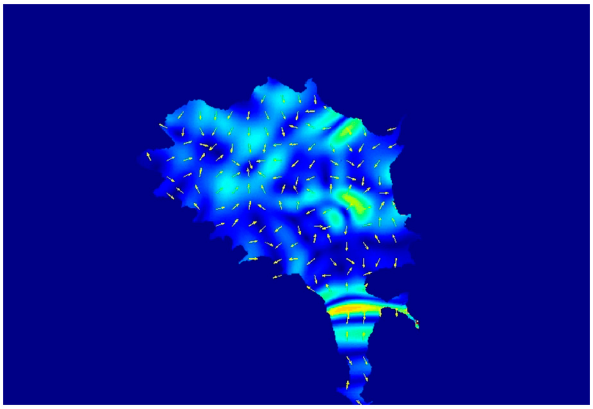
Movie S3. Calculated force field shows distribution and dynamics of internal forces, as shown in Fig. 2 of the main text. Color coding gives the force magnitude in Pa, arrows indicate the direction of the forces.

Movie S3 (AVI)



Movie S4. Traction stress field of a neuronal growth cone exerted onto a laminin coated elastic polyacrylamide gel substrate as shown in Fig. 3 of the main text. Color code gives traction stress magnitude in Pa.

Movie S4 (AVI)



Movie S5. Internal stress field for a growth cone on a laminin coated polyacrylamide substrate as shown in Fig. 3 of the main text. This growth cone corresponds to the growth cone shown in Movie S4, but now the internal stresses are presented. Color code gives internal stress magnitude in Pa.

Movie S5 (AVI)