Supplementary Information

Time-resolved microrheology of actively remodeling actomyosin networks

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Supporting methods: particle tracking software

Video particle tracking was performed using BeadTracker tracking software written in C# (Microsoft .Net Framework 4.0, Redmond, U.S.A.) by Marco Seynen of the Software Engineering department at AMOLF. The software is freely available from the authors on request. The tracking algorithm was based on existing algorithms written originally in IDL (Interactive Data Language, ITT VIS, U.S.A.) by J. Crocker and D. Grier [1]. The software was able to swiftly analyze large data sets (>10000 frames or >15 Gb). The new software has two principal advantages over the original IDL version. First, it can handle larger datasets, since it loads and processes 1 frame at a time, instead of loading all frames at once. Second, it is faster because it calculates boxcar and Gaussian filters during image processing steps (details below) on a graphics-card using CUDA (NVIDIA Corporation, Santa Clara, USA) and using C-sharp (or C#, a third-generation language, 3GL), which is faster than IDL (a fourth generation language, 4GL). Furthermore, BeadTracker has several new post-tracking functions, including the option to connect particle coordinates with selected pixel and frame ranges and the option to correct missing particle coordinates. The latter correction can be executed either by filling missing positions with the last detected position of a segment of a trajectory, or by interpolating the position between the last frame of a segment and the first frame of the following one. Other functions are fast data navigation and inspection, including the ability to directly overlay trajectories on the corresponding raw data, and screening raw images frame by frame accompanied by a dynamic position marker along the trajectory. Also featured is zooming in and out on raw data and trajectories, as well as removal of tracks that have defects caused, for instance, by particles moving out of focus.

The particle tracking algorithm devised by Crocker and Grier comprises several steps. It is optimized for small and relatively well separated spherical particles. The first step consists of a background removal operation using a filter that is a convolution kernel of the original image $A_w(x,y)$, with a boxcar average over a region of $2w+1$ pixels:

$$A_w(x,y) = \frac{1}{(2w+1)^2} \sum_{ij=-w}^{w} A(x+i, y+j), \quad (1)$$

and with a Gaussian surface of revolution $\lambda_n/2$,

$$A_{\lambda_n}(x,y) = \frac{1}{B} \sum_{ij=-w}^{w} A(x+i, y+j) \exp\left(-\frac{i^2+j^2}{4\lambda_n^2}\right), \quad (2)$$
where \( w \) is an integer larger than a single sphere’s apparent radius (in pixels) and \( B \) is a normalization factor. The boxcar average in Eq. (1) removes background contrast gradients, while the Gaussian surface in Eq. (2) reduces random noise due to digitization, which typically has a correlation length \( \lambda_n \sim 1 \) pixel (25). In the BeadTracker interface, \( LP \) (low pass, Gaussian filter) and \( HP \) (high pass, boxcar filter) parameters control the noise removal, and a \( TH \) (threshold) parameter controls the thresholding step.

The center of mass of the particles is determined based on identification of the brightest pixel of a particle within a radial distance \( w \) of a spherical particle. The brightest pixels of the particles must be greater than the set threshold level. The position is then refined to the brightness-weighted centroid of the pixels in a region around \((x, y)\), such that the offset \((\epsilon_x, \epsilon_y)\) from the initial coordinates is

\[
\begin{pmatrix} \epsilon_x \\ \epsilon_y \end{pmatrix} = \frac{1}{m_0} \sum_{i^2+j^2 \leq w^2} A(x+i, y+j),
\]

where \( m_0 \) is the integrated brightness of the sphere’s image, and the refined location is \((x, y) = (x+\epsilon_x, y+\epsilon_y)\). The particle detection step in BeadTracker uses as parameters \( \text{Min} \) (minimum particle size, which is 2-5 pixels depending on the binning of the image and resulting apparent particle size) and \( \text{Max} \) (maximum particle size, which is 5-11 pixels, also depending on the binning), \( \text{Ratio} \) (representing the percentage of roundness of a particle, where a perfect sphere has a \( \text{Ratio} \) of 100%, while elongated elliptical particles have a \( \text{Ratio} \) up to 150%) and \( \text{COM} \) (center of mass, particles which have a center of mass that is off by more than 30-50% from the real center of mass after position refinement will also not be detected).

The final step in the tracking procedure consists of connecting coordinates into linked trajectories by evaluating criteria of proximity in space and time for each position detected. The BeadTracker parameters to connect particle positions include \( \text{Len} \), which determines the number of frames a particle is allowed to be absent, and \( \text{Space} \) is the maximum distance allowed between consecutive particle coordinates. Here, \( \text{Min} \) (not to be confused with the parameter \( \text{Min} \) for minimum particle size) is the minimal length of consecutive frames for a trajectory to be accepted for the output file.

References

1. Crocker JC and Grier DG 1996 Methods of digital video microscopy for colloidal studies *Journal of Colloid and Interface Science* 179 298-310
Supporting figures

Figure S1. Macroscopic shear rheology of active actin networks. (a) Frequency dependence of the elastic shear moduli (solid symbols) and loss shear moduli (open symbols). (b) Elastic modulus (top panel) and loss tangent (bottom panel) at $\omega = 1$ rad/s for different motor densities and with and without biotin-streptavidin crosslinking (see legend). Red inverted triangles represents an active, crosslinked sample prepared with 1 mM blebbistatin to inhibit myosin activity. Networks were polymerized between the cone and plate of a rheometer (Paar Physics MCR501) and the steady-state linear viscoelastic moduli were measured after 30 min by applying a small amplitude oscillatory shear. The viscoelastic properties of the actin networks are minimally affected by the presence of crosslinks and motors at these concentrations.
Figure S2. Myosin-driven contractile coarsening of actin networks. Confocal fluorescence images show actin filaments (red) and myosin filaments (green). A) At low motor density ($R_M = 1:200$), the myosin filaments self-organize into small clusters surrounded by dense actin shells within 30 minutes. B) At high motor density ($R_M = 1:65$), the myosin filaments cluster faster (within 5 minutes) and they coalesce into large superclusters (within 30 min). The final network structure is highly inhomogeneous. Scale bar (same for all images) is 5 µm. Actin filaments were fluorescently labeled by co-polymerizing actin with 5 mole% Alexa488-labeled G-actin and myosin was labeled with DyLight-594 NHS-Ester (Perbio). Samples were observed with a spinning disk confocal scanner (CSU22, Yokogawa Electric Corp.) on a DMIRB Leica inverted microscope using a 100× (1.3 NA) oil immersion objective (PL Fluotar Leica). Images were recorded with a cooled EM-CCD camera (C9100, Hamamatsu Photonics) using an exposure time of 50-100 ms.
Figure S3. Microscopy images showing probe particle distribution in the networks. (a) Bright field image of an actin network at low motor density ($R_M = 1:200$). The particles are homogeneously distributed. (b) Confocal micrograph of a low motor density network after 2 hours of sample preparation showing actin (red) and myosin (green; see white arrow for example of myosin cluster). White dotted circle indicates the position of an unlabeled probe particle, which shows up as a dark circular hole. There is no visible recruitment of actin or myosin to the particle surface. (c) Bright field micrograph of inhomogeneously distributed probe particles in a network at high motor density ($R_M = 1:65$).
Figure S4. Time evolution of particle image velocimetry (PIV) data for all individual datasets. Each row represents one movie (labels on right hand side). Movies are sorted according to motor concentration (high or low) and dynamics regime (slow or fast). (a) Time evolution of frame-averaged velocity magnitude. Each data set (plotted as thick line) is shown for comparison with the other data sets (light grey lines) and with the data of the passive control sample (crossed circles) and for stuck beads (grey circles). (b) Corresponding velocity histograms (dark triangles: \( t = 2 \) min; bright triangles: \( t = 80 \) min). Crossed circles: Passive sample without motors (dark: \( t = 2 \) min; light: \( t = 80 \) min). Gray circles: stuck beads (dark: \( t = 2 \) min; light: \( t = 80 \) min).
Figure S5. Measures of nonequilibrium activity from particle tracking analysis plotted as a function of sample age (see legend on top): diffusive coefficient $\alpha$, Gaussian standard deviation ratio $\beta$, non-Gaussian parameter $\xi$ at a lag time of 10 s, and probability for a particle to undergo directed motion $p_{\text{dir}}$. Data are show for each data set separately (plotted as thick line) together with the other data sets (light grey lines) and (in case of $\alpha$ and $\xi$) with the data of the passive control sample (crossed circles). The data sets are grouped as slow or fast samples based on classification from PIV analysis. Black solid squares show the averaged data.
Figure S6. Automated segmentation of particle trajectories to detect events of active directed tracer motion (see Movie S6). (a) Brightfield micrograph of a polystyrene bead embedded in an actin-myosin gel, with particle track overlaid in blue (non-directed motion) and green/red (directed motions). (b) Instantaneous particle velocity components $v_x(t)$ and $v_y(t)$ were computed from raw particle tracking data as functions of time $t = n \cdot \Delta t$ ($n$: the frame number; $1/\Delta t$: frame rate). Velocity components were smoothed with a moving average filter (window size: 3s) which spaces data equally on either side of a time point of interest. (c) Smoothed velocities $v_{x,avg}(t)$ and $v_{y,avg}(t)$ were transformed into particle translocation directions $\Delta \Theta(t)$. The differential angle $\Delta \Theta(t)$ is a measure of the straightness or directional persistence of particle motion. The differential angle is large if a particle exhibits random fluctuations, and it is small if a particle moves in a directed fashion. We thresholded the differential angle to retrieve time spans of directed motion (“segments”), where $\Delta \Theta(t)$ falls short of a threshold angle $\Delta \Theta_{thr}$. Only segments with a duration exceeding 0.8 s and particle displacements exceeding 0.5 µm are considered to be active segments. These strict criteria were chosen to prevent overly sensitive and hence erroneous detection. The parameters were tested against a passive control network devoid of myosin motors, where zero “segments” were found.
Figure S7. Example kymographs of particles exhibiting contractile fluctuations in active actin-myosin networks. (a) Particle exhibiting multiple translocation-reversal events in a low motor density sample. Scale bars: 2 s (time axis) and 3.2 µm (distance axis). (b) Particle exhibiting multiple translocation-reversal events in a high motor density sample. Scale bars: 4 s (time axis) and 3.2 µm (distance axis). (c) Two particles exhibiting multiple anti-correlated contractile fluctuations (as explained in schematic). (d) Three particles exhibiting a correlated contractile fluctuation (see schematic).
Figure S8. Translocation and reversal speeds as a function of sample age extracted from kymograph analysis. (a) Translocation speeds for low motor density samples. (b) Translocation speeds for high motor density samples. (c) Reversal speeds for low motor density samples. (d) Reversal speeds for high motor density samples. Symbols show individual data points and colored box plots represent 25th to 75th percentile of data with sample age incrementing towards hot colors. Histograms on the right hand side of each graph show lumped distribution of translocation and reversal speeds. Translocation and reversal speeds are higher in high motor density samples than in low motor density samples.