Reconstructing the orientation distribution of actin filaments in the lamellipodium of migrating keratocytes from electron microscopy tomography data – Supporting Information

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I. SUPPORTING MOVIES

1. SI movie 1

As explained in the main text, the cell marked as Cell 2 in Fig. 1 slows down significantly just before fixation for EM. To illustrate the dynamic behavior of this cell before fixation, we provide the live-microscopy video clip as supporting movie `SI_movie_1.avi`.

2. SI movie 2

To illustrate the three dimensionality of our EM tomography data shown by a slice plot in Fig. 2, supporting movie `SI_movie_2.avi` zooms through an exemplary image volume of a lamellipodium network from top to bottom.

II. ANALYSIS OF ADDITIONAL EM DATA

To show the applicability of our analysis method, which is discussed in detail in the main text, we provide additional samples at this point. In Fig. S1, the front of the lamellipodium

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of three cells, which moved continuously before fixation, where analyzed. In all cases a dominant criss-cross pattern, peaked at around $\pm 35^\circ$ becomes apparent in the resulting orientation distributions (cf. Fig. S1(c)).

As an alternative, we analyzed actin networks emerging at the lateral side of the lamellipodium of steadily moving cells. At this position protrusion of the leading edge is diminished. The resulting orientation distributions are illustrated in Fig. S2. Similar to the case of a cell which slowed down just before fixation for EM, that was discussed in the main text (cf. Fig. 9.2), here again we find qualitatively different filament orientation distributions. The resulting distributions show a single dominant peak in at filament orientation almost orthogonal to the leading edge.

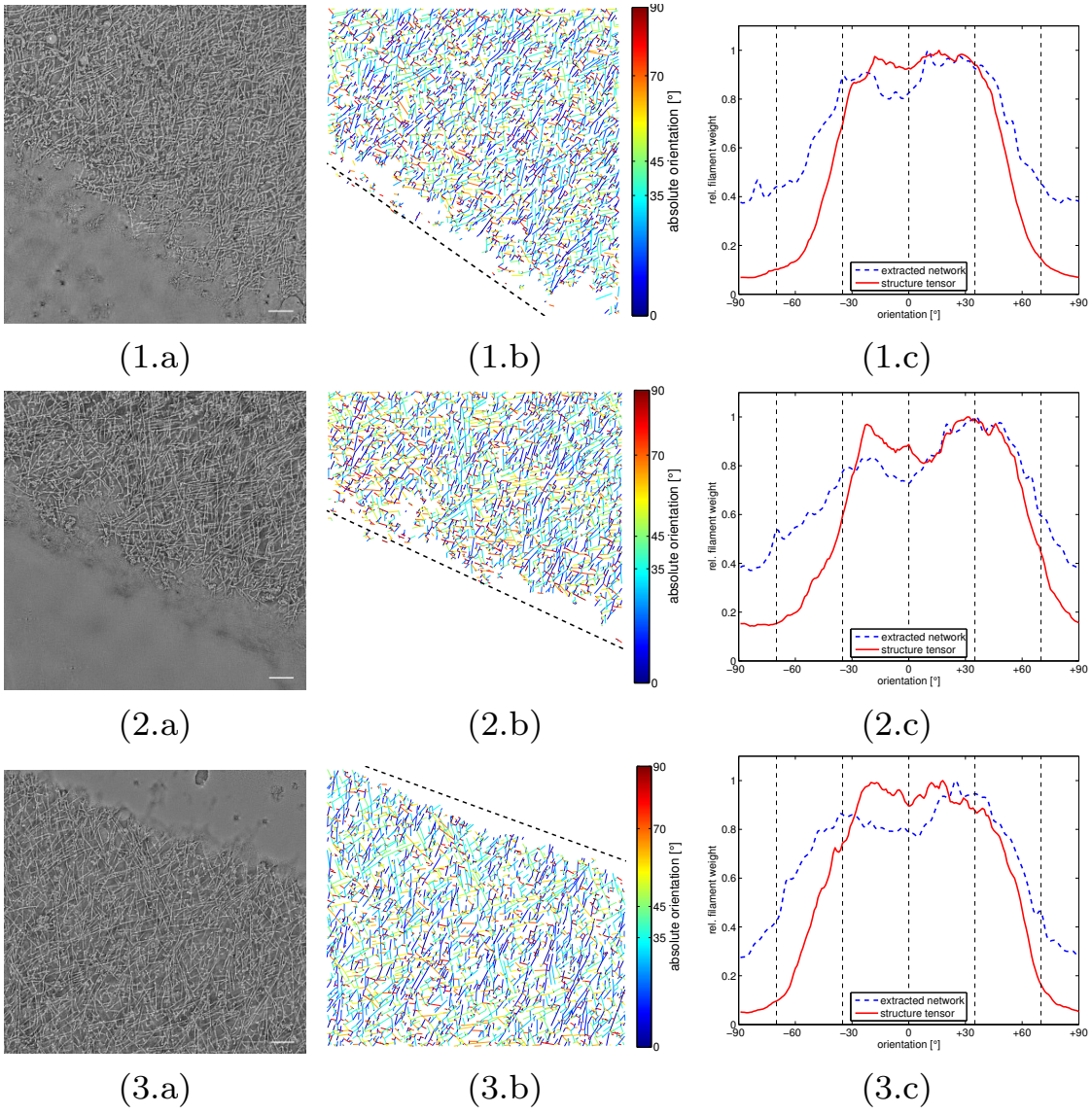

FIG. S1: Orientation analysis of EM data at the front of the lamellipodium from cells moving continuously before fixation. (a) Representative EM image slice. The scale bar corresponds to 0.1 μm. (b) Extracted filaments, color labeled for their orientations. The reference orientation of the leading edge,which has been adjusted manually, is indicated as the black dashed line. (c) Resulting orientation distribution from independent structure tensor analysis and network extraction. In all cases the resulting filament orientation distribution features a criss-cross pattern, which is peaked at around $\pm 35°$.

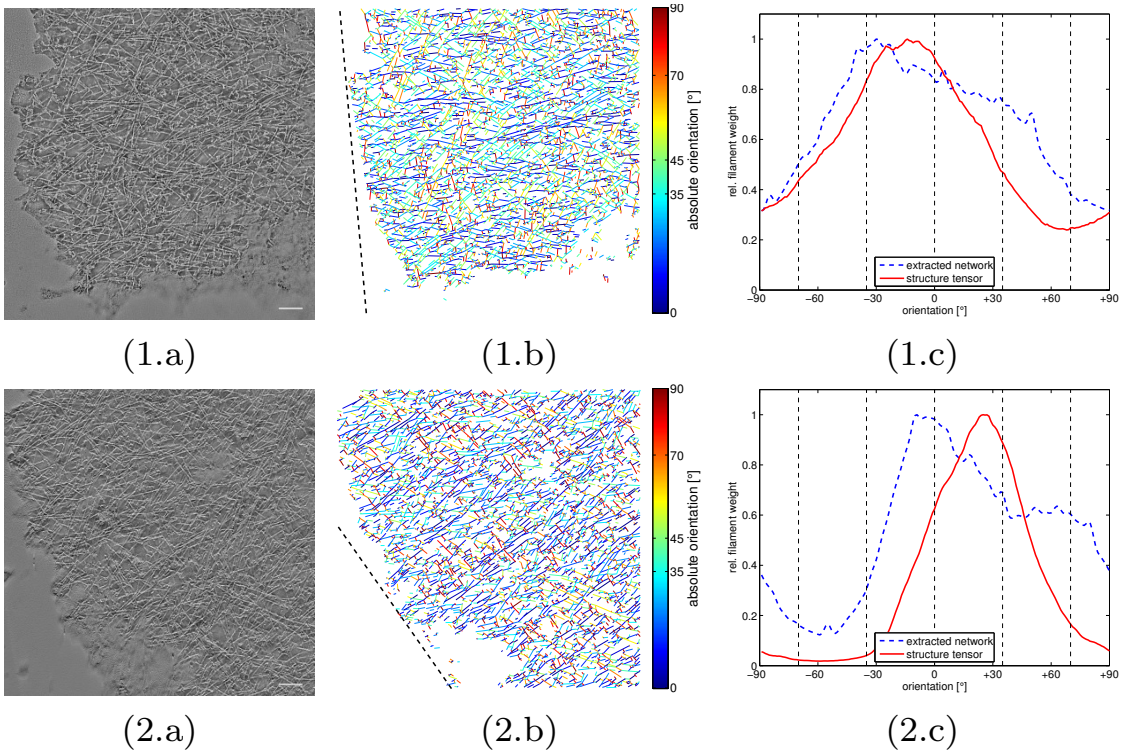

FIG. S2: Orientation analysis of EM data at the side of the lamellipodium. (a) Representative EM image slice. The scale bar corresponds to 0.1 μm. (b) Extracted filaments, color labeled for their orientations. The reference orientation of the leading edge, which has been adjusted manually, is indicated as the black dashed line. (c) Resulting orientation distribution from independent structure tensor analysis and network extraction. The orientation analysis of EM data imaged at the side of the lamellipodium yields qualitatively different results compared to the criss-cross patterns observed at the front of continuously moving cells. The resulting orientation distributions feature a single peak approximately orthogonal to the local orientation of the cell membrane (at around 0°), very similar to a cell, which slowed down just before fixation (cf. Fig. 9.2 in the main text).