

Supplementary Information

Two-dimensional TIRF-SIM - Traction Force Microscopy (2D TIRF-SIM-TFM)

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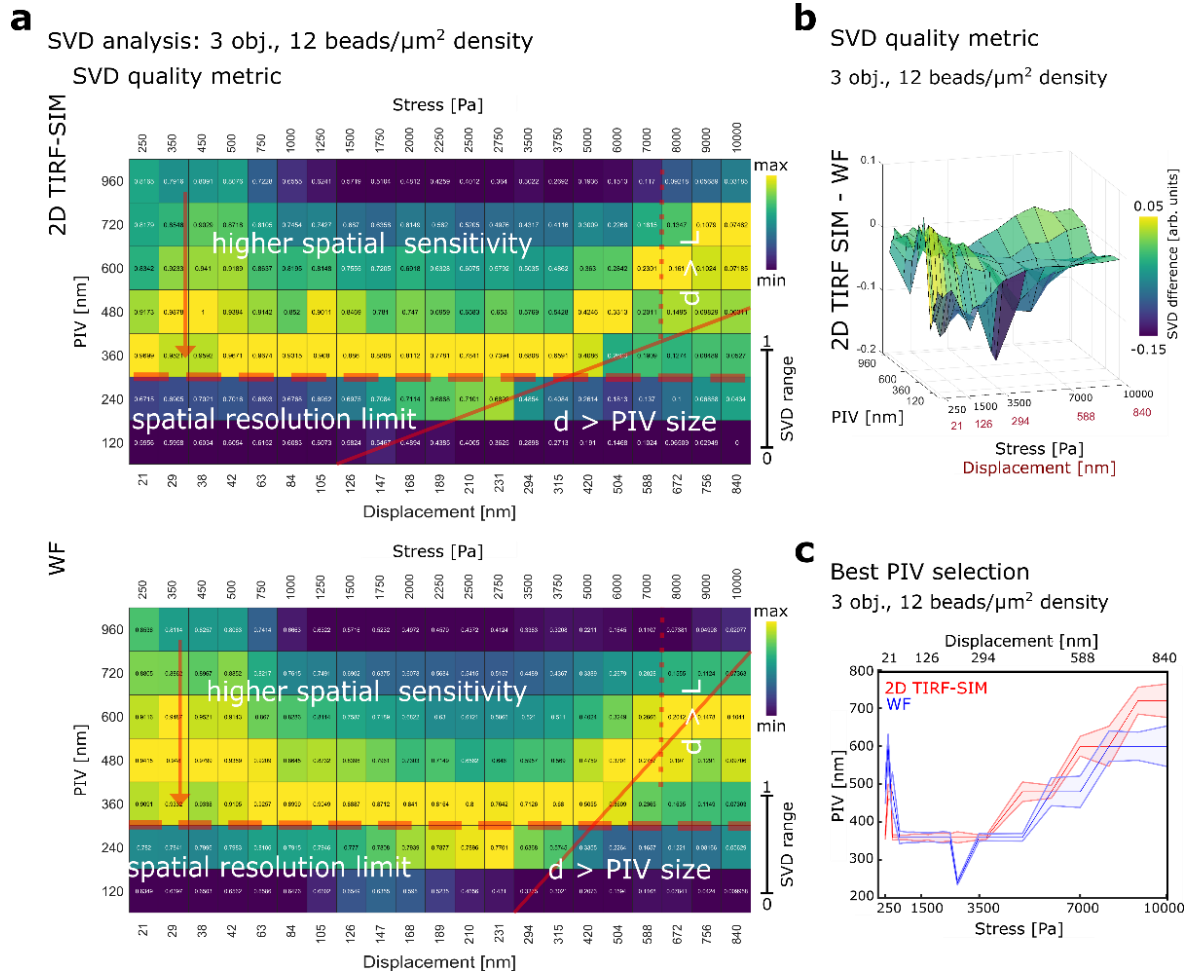
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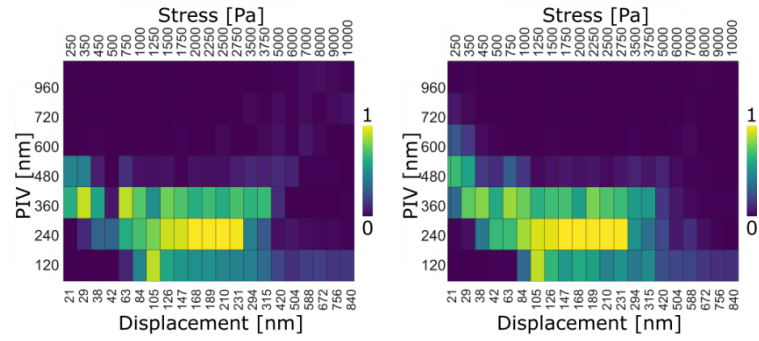
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Supplementary Figures

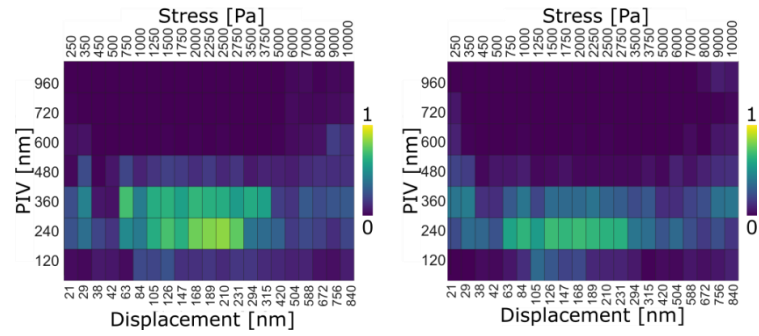


Supplementary Figure 1: SVD analysis for triple square-shaped stress zones with a bead density of 12 beads per μm^2 . (a) Heatmap of the SVD analysis metric quantifying the quality of the displacement estimation compared to the GT for a range of PIV window sizes and displacement magnitudes at bead density of 12 beads/ μm^2 for both TIRF-SIM (left) and WF (right). The overall SVD range of values is from 0 to 1, with 1 representing the best quality of displacement estimation. Each column is colour-scaled to represent the maximum and minimum SVD values. (b) Difference in the SVD quality metric between TIRF-SIM and WF. All plots are normalised to the maximum SVD quality metric for TIRF-SIM, with values close to 1 indicating improved displacement estimation. (c) Plot highlighting the best PIV windows size for a given displacement at a bead density of 12 beads/ μm^2 , based on the minimum SVD quality metric for both TIRF-SIM (red) and WF (blue).

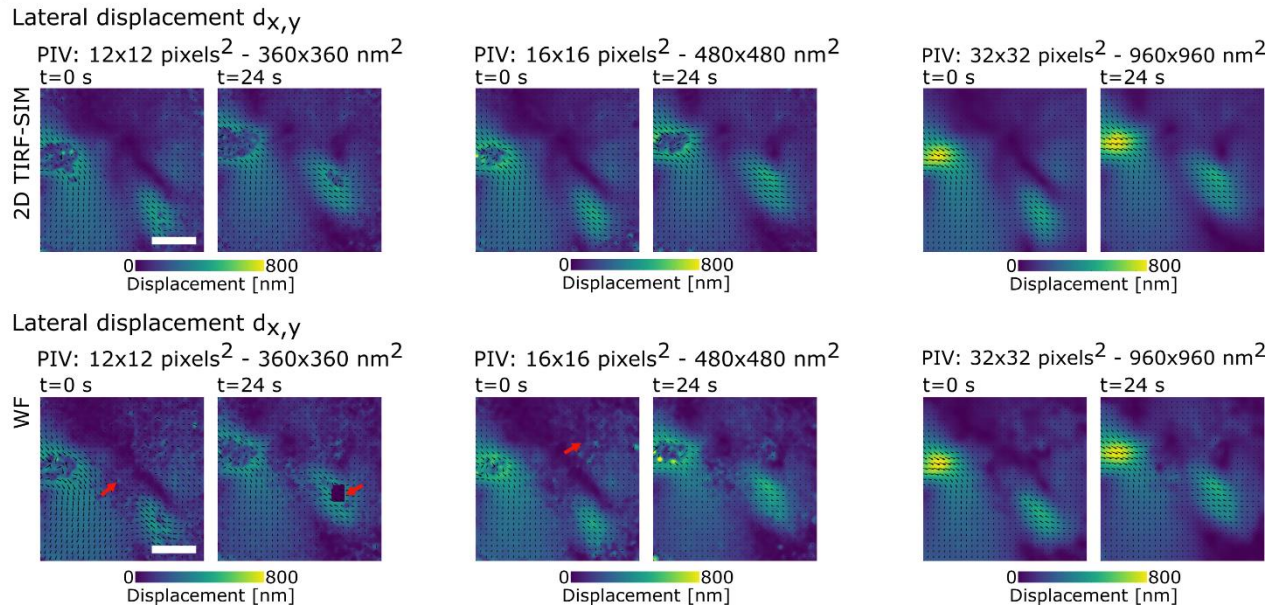
a Precision analysis: 3 obj., 12 beads/ μm^2 density
2D TIRF-SIM WF



b Accuracy analysis: 3 obj., 12 beads/ μm^2 density
2D TIRF-SIM WF



Supplementary Figure 2: Precision and accuracy diagrams for triple square-shaped stress zones with a bead density of 12 beads per μm^2 . (a) Heatmap of the precision diagram for the case of three co-linear positioned square-shaped stress zones at a bead density of 12 beads/ μm^2 for both TIRF-SIM (left) and WF (right). The values show the fraction of success among 100 repeats. (b) Heatmap of corresponding accuracy diagrams for the case of three co-linear positioned square-shaped stress zones at a bead density of 12 beads/ μm^2 for both TIRF-SIM (left) and WF (right).

a

Supplementary Figure 3: Lateral displacement during migration of salmon keratocyte recovered with different PIV window size. (a) Lateral displacement magnitude of the gel generated by the Salmon keratocyte cell on top of the gel at time 0 and 24 sec, recovered with 2D TIRF-SIM-TFM and WF-TFM. The displacement magnitude is shown for PIV window sizes of 12, 16, and 32 px. The maximum estimated displacement was 800 nm. Optimal PIV window is chosen to be 32 px. Scale bar is 10 μm .