

Supplemental Materials

Vesicle dynamics during plant cytokinesis reveals distinct developmental phases

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Assessment of STICS performance compared with conventional particle tracking

Pollen tube growth conditions

Prior to transformation by DNA (G-RK plasmid (Nelson et al., 2007)) coated particles bombardment tobacco pollen grains (*Nicotiana tabacum*) were prepared as previously described (Zerzour et al., 2009; Brewbaker and Kwack, 1963). Pollen tubes were allowed to grow in Brewbaker and Kwack medium (Brewbaker and Kwack, 1963) supplemented with 9% sucrose for 4 hours on a rotating shaker at 22°C. Upon observations, a drop of pollen tube culture was put on the top of a microscope slide coated with 1% agarose. The pollen tubes were covered with a cover slip and sealed with VALAP (1:1:1 Vaseline:Lanolin:paraffin).

Quantification of organellar movements using STICS and conventional particle tracking

Intracellular traffic is particularly easy to track in cells where it occurs rapidly and in clearly defined spatial patterns. This is the case in pollen tubes which are extremely elongated cells in which organelle movement is largely bidirectional (Bove et al., 2008). We used STICS on image series of pollen tubes expressing the first 49 amino acids of the α -1,2-mannosidase fused to mCherry fluorescent protein (Saint-Jore-Dupas et al., 2006) to mark Golgi bodies (Fig. S2). This G-RK construct (Nelson et al., 2007) was expressed under the pollen specific pLAT52 promoter (Twell et al., 1989). STICS data provide two types of information in form of a vector map: The direction of the movement is represented by the orientation of the vectors and velocity magnitude is coded by the color and the length of the vectors. Based on STICS we determined the average speed of Golgi bodies within the pollen tube to be 29 $\mu\text{m}/\text{min}$ with maximum speeds at 69 $\mu\text{m}/\text{min}$. Consistent with this, manual tracking of individual Golgi bodies yielded velocities between 32 and 54 $\mu\text{m}/\text{min}$. STICS was also able to correctly reproduce the direction of the motion pattern of the Golgi bodies that in pollen tubes occurs in an inverse fountain shaped pattern, demonstrating the reliability of STICS in the cellular context. STICS has also been used successfully to map adhesion and cytoskeletal protein flux in migrating mammalian cells (Toplak et al., 2012).

Next we tested STICS reliability on BY-2 cells and compared the velocity of microtubules forming the mitotic spindle during metaphase with values published by others (Fig. S2a). This microtubular movement is responsible for the alignment of chromosomes in the equatorial plane of the cell. STICS revealed that within the spindle, labeled tubulin p35s::GFP-MBD (Microtubule Binding Domain of the microtubule associated protein 4 (MAP4) (Granger and Cyr, 2000)) moved towards the equatorial plane with a velocity of 7.5 $\mu\text{m}/\text{min}$. This is in agreement with values determined by kymography (Samuels et al., 1995; Seguí-Simarro et al., 2004).

Effect of BDM on the cytoskeleton

STICS was able to detect dynamics of microtubules in control cells (expressing GFP-MBD, Figs. S2b-d) during anaphase, at the ring phragmoplast stage, microtubules are moving mainly towards the cell plate margins with a speed of 1.2 to 2 $\mu\text{m}/\text{min}$ (Figs. S2c,d). After BDM treatment, the movement of these microtubules slowed somewhat (Fig. S2b).

In control cells at the phragmoplast stage, actin filaments labeled with lifeact-GFP (Figs. S2e-g), moved towards the margins of the cell plate with a speed reaching 3.5 $\mu\text{m}/\text{min}$ (Figs. S2f, g). However, upon BDM treatment (Fig. S2e), actin filament movement essentially ceased (compare Fig. S2e and S2f-g). The effect of BDM on vesicle trafficking towards the cell plate is thus much more dramatic than that of LatB-mediated depolymerization of actin filaments on cell plate formation which does not interfere with the initial phases of this process (see below). This likely means that the effect of BDM is not specific to actin-driven vesicle transport, but also interferes with other ATP-dependent vesicle trafficking pathways that could be microtubule-mediated.

Role of microtubules

To assess the role of the microtubule array for the initiation of cell plate formation during IPA we treated a GFP-MBD expressing BY-2 line with 10 μM oryzalin, an inhibitor of microtubule polymerization (Fig. 4b). After 30 min, this treatment prevented the segregation of chromosomes following their separation. Instead, the two sets of chromosomes stayed very close to each other. In GFP-KN expressing cells treated with oryzalin prior to cytokinesis GFP-KN labeled vesicles are present, but they accumulate at the two poles of the cell, and formation of the IPA did not occur (not shown). If oryzalin was administered after the cells passed the anaphase check point on the other hand, a cell plate was initiated. However, the cell plate never entered the PCG stage and did not expand beyond the 5.5 μm of the IPA stage (Fig. 4e). This confirms that microtubules play a major role in the initiation of the cell plate and in the delivery of vesicles to the expanding cell plate (Jürgens, 2005).

After 30 min of treatment with 10 μM oryzalin, cell plate expansion was arrested at the IPA stage and no further expansion occurred (Fig. 4e). A cell plate that had already entered PCG when the drug was applied suspended expansion in the radial direction and instead formed a bend at the margin of the plate that never connected to the parental wall (Fig. 4k-m). During oryzalin treatment, the mobile fraction of GFP-KN labeled structures in the cell plate was only 33.6% compared to 53.3% and 52.8% for untreated or DMSO (0.1%) treated cells, respectively.

Quantification of cell plate growth in *Arabidopsis* meristematic root cells

Multiple published papers show figures of cell plate formation in cells within a tissue context. However, only few articles provide either video footage or time lapse images of extended duration and sufficient resolution. We based our analyses on three articles in which the presented data fulfilled these requirements: Rybak et al., 2014 (Rybak et al., 2014), Kang et al., 2003 (Kang et al., 2003) (movie with frame rate of 2 images/min) and Fendrych et al., 2010 (Fendrych et al., 2010) present image data on cell division in *Arabidopsis thaliana* meristematic root cells. A total of 9 data sets were analyzed (Table 1). To quantify cell plate diameter we used Fiji software (Schindelin et al., 2012), since FluMOS is optimized for high throughput analysis of raw images. The growth rate during PCG used for statistics was based on time points available during the first 10 min of cell

plate expansion (FigS1a, pale grey region), while the SCG growth rate was calculated based on the time points after 10 min (FigS1a, dark grey region). Microsoft Excel for Mac 2011 was used for the statistical analyses (averaging, standard deviation and paired T.TEST function).

Table 1 References used to quantify cell plate diameter in Arabidopsis meristematic root cell

Cell	Paper	Figure	Marker used
1	Rybak et al., 2014	Fig.1b	FM4-64
2	Rybak et al., 2014	Fig.S1k	p35S::EXO70 A1
3	Rybak et al., 2014	Fig.S2a	pUb::CLUB/TRS130-GFP (TRAPP1)
4	Rybak et al., 2014	Fig.S2b	pUb::TRS120-GFP (TRAPP1)
5	Rybak et al., 2014	Fig.S3d	pUb::TRS120-GFP (TRAPP1)
6	Kang et al., 2003	Supplemental Movie	pADL1A::ADL1A-mGFP5
7	Kang et al., 2003	Supplemental Movie	pADL1A::ADL1A-mGFP5
8	Kang et al., 2003	Supplemental Movie	pADL1A::ADL1A-mGFP5
9	Fendrych et al., 2010	Fig.7b	pSEC8::SEC8-GFP

Statistical testing of selected TOI size

To accurately map a flow process that changes over time, there needs to be an optimal TOI length which is long enough to sample sufficient temporal fluorescence fluctuations yet not so long as to average over significant changes during the time evolution. To determine the optimal time window size, we calculate the dispersion in the STICS vector fields as a function of TOI length. This test involves determining the STICS vector maps for a range of TOI lengths and calculating a normalized vector dot product between each TOI in the range, and the empirically determine TOI length to be tested. For our system, TOI=100 empirically appeared to give the best results for the dividing plant cells and TOI 20 for the pollen tube control data. To test this in a non-subjective way, we calculated the following normalized vector dot product for every vector in each ROI analyzed:

$$\text{Eq\#5 : } \frac{\mathbf{V}_{\text{TOI}=j} \cdot \mathbf{V}_{\text{TOI}=test}}{|\mathbf{V}_{\text{TOI}=j}| \cdot |\mathbf{V}_{\text{TOI}=test}|} \text{ for } j = \text{TOI} = 10, 20, 30, \dots, 140, 150 (\text{dividing cells})$$

$$j = \text{TOI} = 15, 20, 25, 30, 35, 40 (\text{pollen tubes})$$

Where $\text{TOI}_{test} = 100$ and 20 respective for the two different data sets.

STICS analysis with ROI size=16x16 pixels, ROI shift=4 pixels). For every ROI there were 15 or 6 different recovered velocities, one for each TOI size used for a data set. We compared how the recovered vector, at a given ROI and TOI, diverged from the vector velocity found for TOI_{test} frames (100 or 20). The normalized vector dot product calculated via Eq. #5 and could vary between 1 (vectors for a given ROI pointing in the

same direction) and -1 (vectors pointing in the opposite directions). For every TOI tested in the range, approximately 2500 vectors were compared with their equivalents at TOI =100 or 20 frames.

We analyzed the variation in the recovered velocities by plotting the dispersion data as box plots as a function of TOI to objectively map the dispersion statistics. The results for experimental data were compiled in a boxplot format as a function of TOI size (see Fig. S9).

Supplemental Figures

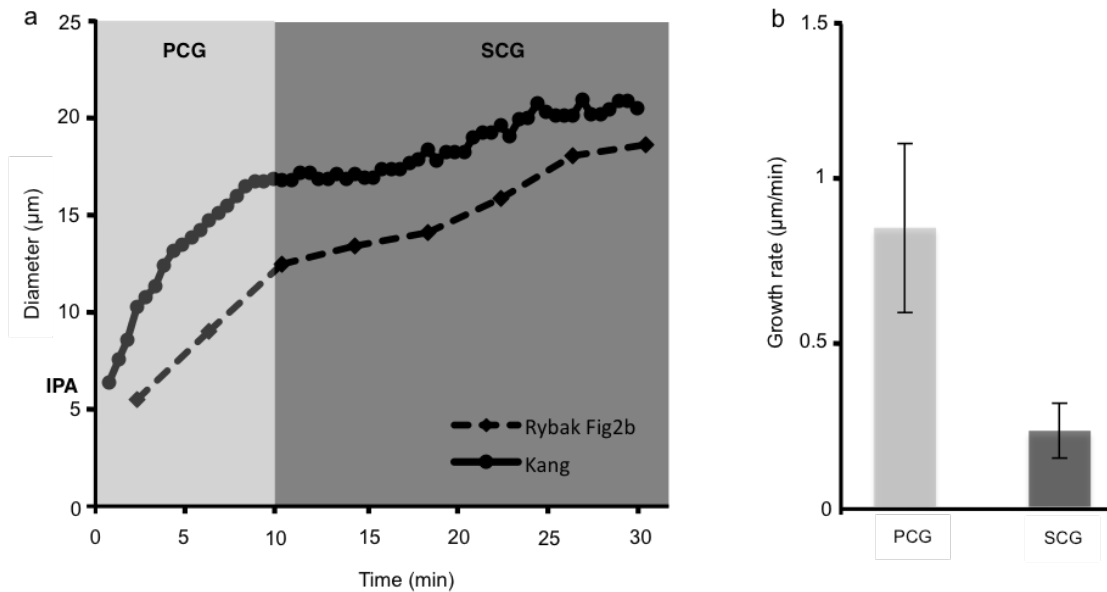


Figure S1 | Cell plate diameter in dividing *A. thaliana* root meristem cells (a) Typical cell plate growth dynamics based on analysis of published image data sets (b) Average radial growth rates during primary centrifugal growth (PCG) and secondary centrifugal growth (SCG) based on a total of 9 data series from 3 published papers ($\pm$ SD, $n=9$, paired, Ttest $p=1,12 \cdot 10^{-4}$).

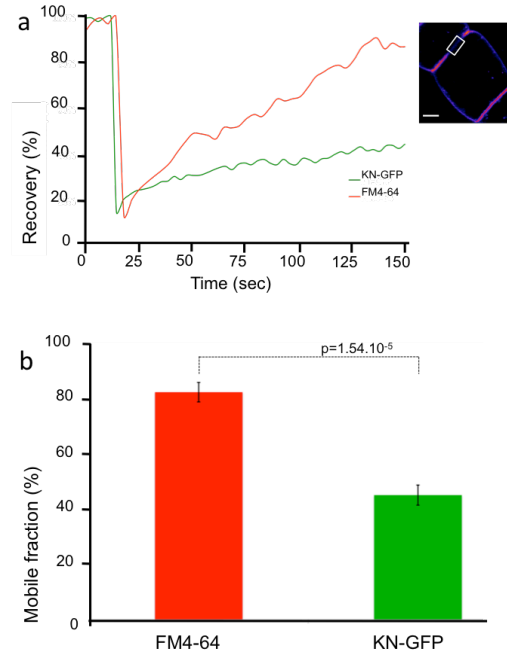


Figure S2 | FRAP analysis at the plasma membrane. (a) Typical FRAP dynamics of GFP-KN and FM4-64 labeled plasma membrane. Fluorescence intensity is normalized to background and pre-bleach values (b) Average of mobile fraction after FRAP on FM4-64 and GFP-KN labeled cell plate (n=6)

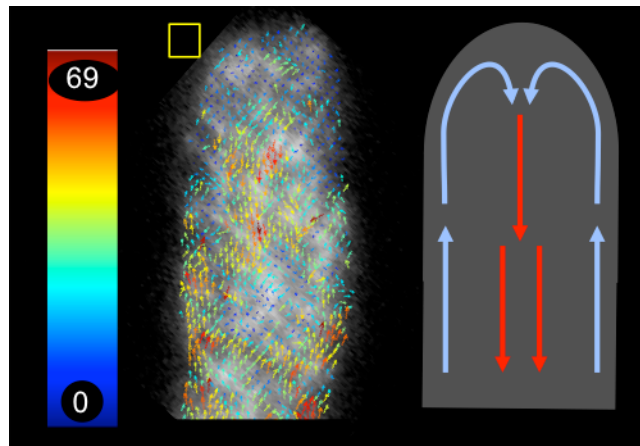


Figure S3 | Golgi dynamics in pollen tube processed with STICS. Velocity maps of Golgi bodies labeled with pLAT52::G-RK plasmid. The vector map overlaying the micrograph reveals the direction and speed of particle motion in the cell as imaged every 190ms over a 3.8 sec period. Flows were averaged every 20 frames TOI. Numbers on color lookup tables are vector magnitudes in $\mu\text{m}/\text{min}$. ROI (yellow square) corresponds to 16 x 16 pixels ($1.6 \mu\text{m} \times 1.6 \mu\text{m}$) with a pixel shift of 4 between adjacent ROIs.

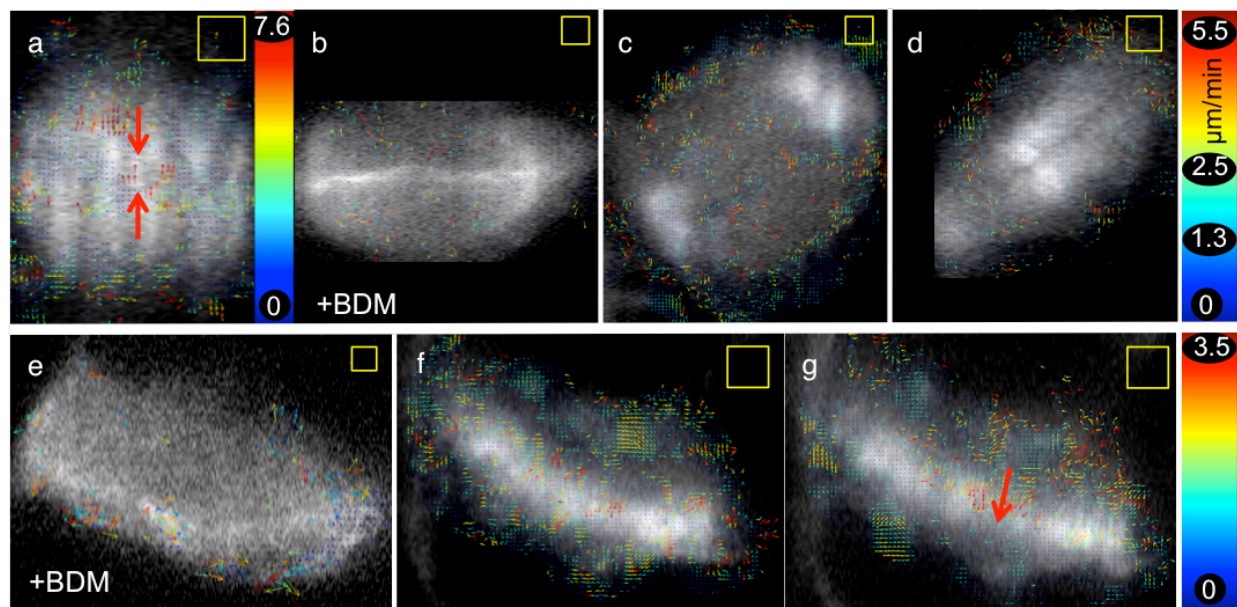


Figure S4 | STICS analysis of the vesicle supply to allow cell plate growth. (a-d) Velocity maps of microtubules labeled with GFP-MBD (a-c: center plane, d: peripheral plane). The vector map overlying the micrograph reveals the direction and speed of microtubules motion in the cell as analyzed over a (a) 50 s period (50 frames) (b) 64 s (100 frames), (c-d) 100 sec (100 frames). (e-g) Velocity maps of actin labeled with lifeact-GFP over a period of (e) 100 s period (100 frames) (f-g) 81 s period (100 frames). Numbers on color lookup tables are vector magnitudes in $\mu\text{m}/\text{min}$. ROI (yellow square) corresponds to 16 x 16 pixels ($2.7 \mu\text{m} \times 2.7 \mu\text{m}$). Adjacent ROI pixel shift was 2, except for b and e, where the pixel shift was 4.

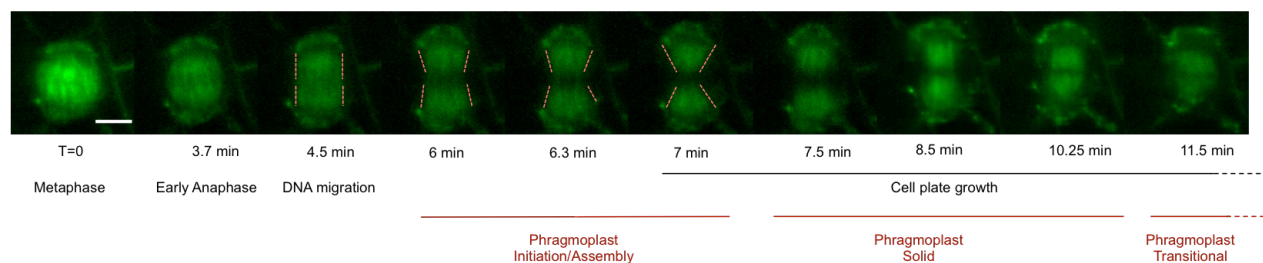


Figure S5 | Microtubule array from metaphase to telophase. During the migration of the chromosomes, the mitotic spindle stays aligned straight along the long axis of the cell. Before the cell plate is initiated, the microtubule spindle becomes narrower at its equator. Scale bar 10 μm

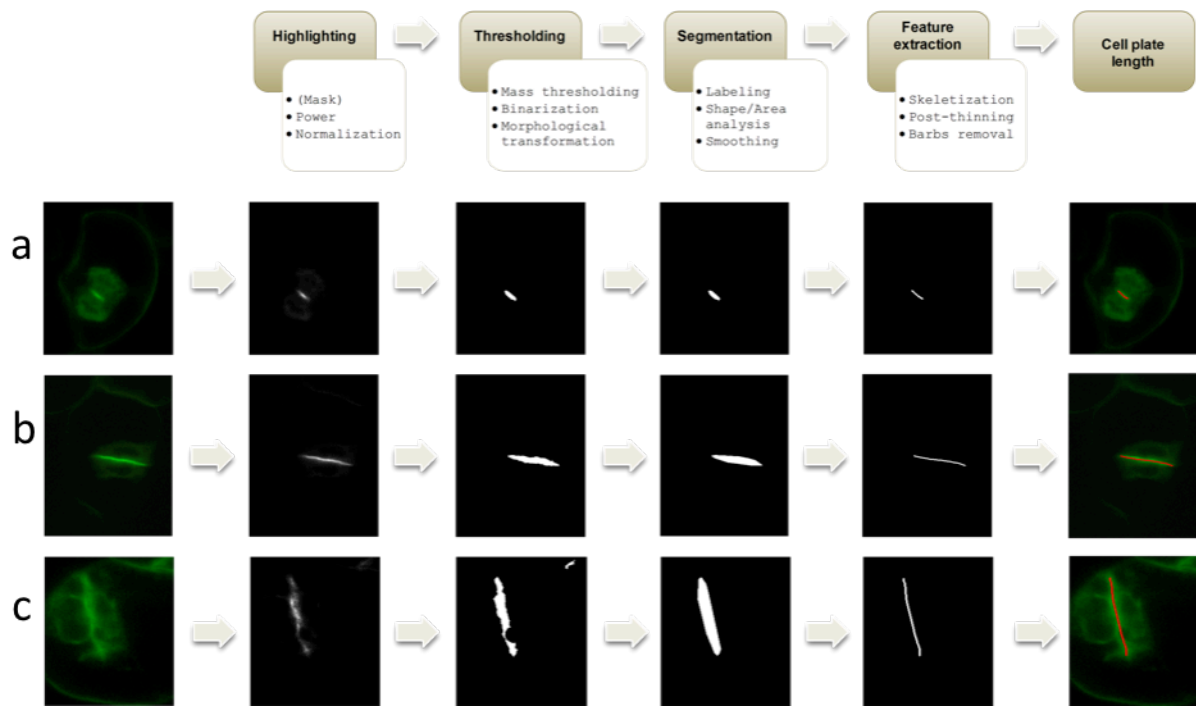


Figure S6 | FLuMOS pipeline

Exemples of FLuMOS application a) IPA stage cell plate, b) SCG stage, c) SCG stage of a cell treated with LatB inhibitor

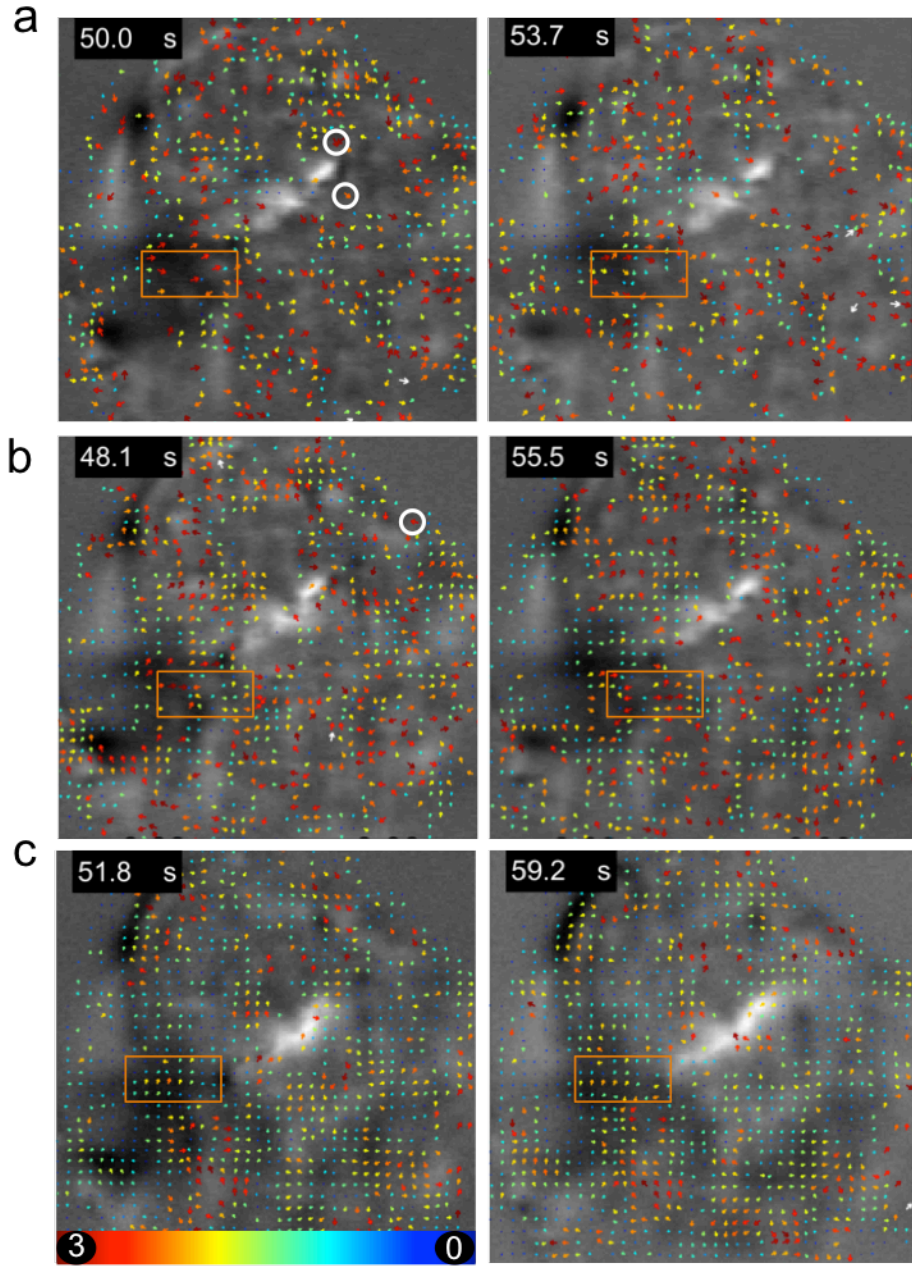


Figure S7 | STICS Vector Maps obtained for TOI 25 50 and 100 frames with time shift oversampling

(a) Two sequential STICS vector maps from analysis of TOI 25 frames with a 5 frame time shift between each analysis (so 80% common input image data between sequential TOIs) (b) Two sequential STICS vector maps from analysis of TOI 50 frames with a 10 frame time shift between each analysis (so 80% common input image data between sequential TOIs). (c) Two sequential STICS vector maps from analysis of TOI 100 frames with a 10 frame time shift between each analysis (so 90% common input image data between sequential TOIs). Vectors which appear or disappear between sequential frame shift analyses (horizontally adjacent vector maps) indicate noisy vectors from failed fits (see white circles for examples for each TOI). The number of noise vectors decreases as the TOI increases. The orange rectangles highlight a region with vesicular

flow that is best resolved with the increased temporal sampling of vesicle transits with TOI 100. All ROI's 16x16 pixels with a 4 pixels shift between adjacent ROI's for all three analyses. Numbers on color lookup tables are movement rates in $\mu\text{m}/\text{min}$.

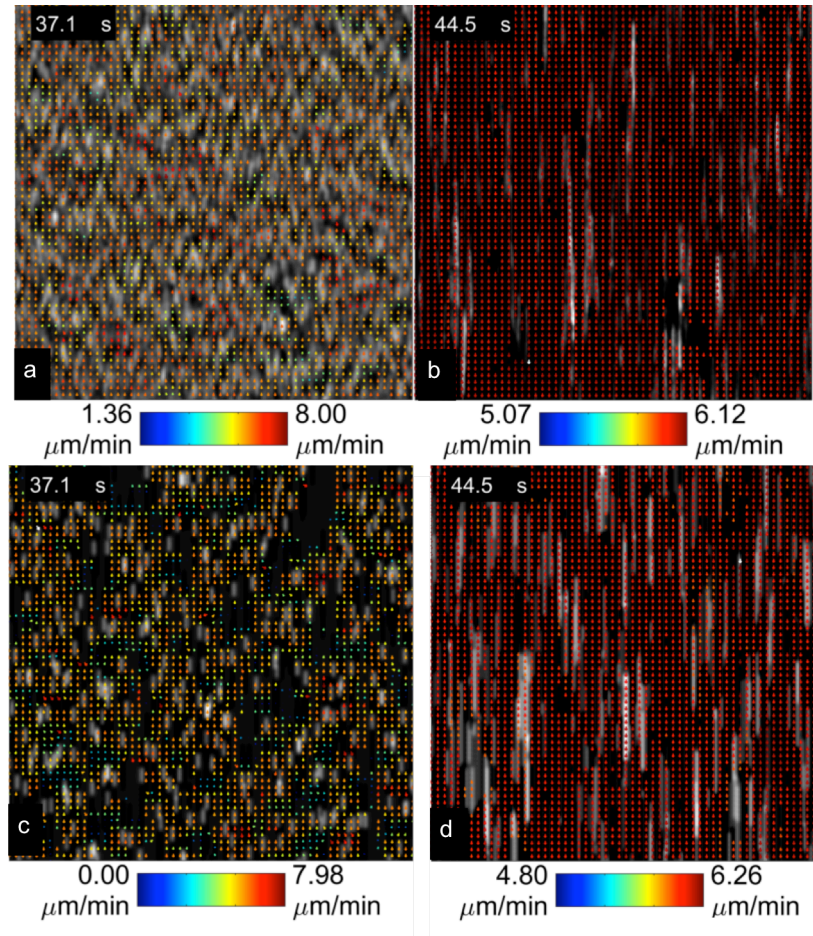


Figure S8 | STICS analysis of computer simulated image series with uniform particle flow with different TOI temporal sampling and different particle densities

STICS TOI (a) and (c) TOI 20 frames; (b) & (d) TOI 120 frames. Computer simulated image series of point particles flowing top to bottom with a uniform set velocity of 6 mm/min with 3 $\text{particles}/\text{mm}^2$ (a) & (b) and 0.3 $\text{particles}/\text{mm}^2$ (c) & (d). All simulations had 120 total frames, 0.1715 mm/pixel and 0.735 s/frame . For each image frame, the point particles were convolved with a 2D Gaussian of 0.4 mm radius to simulate the optical point spread function and acts as the spatial correlator for the STICS analysis. STICS analysis was performed on the full 120 frame TOI (b) & (d) or sequential 20 frame TOIs (a) & (c) (only one vector map displayed for each shorter TOI). All ROI's were 16x16 pixels with a 4 pixels shift between adjacent ROI's for all three analyses. Note that an average particle would cover half the ROI (8 pixels) in 18.7 frames with these settings and would decorrelate. The STICS analysis over 20 frames shows a much greater variation in the calculated vector maps for this uniform perfect flow as compared to the analysis over 120 frames due to the greater statistical sampling of particle transit events in time. A lower density also impacts by reducing the number of particle transit events, but is improved with longer time sampling if the flow does not change.

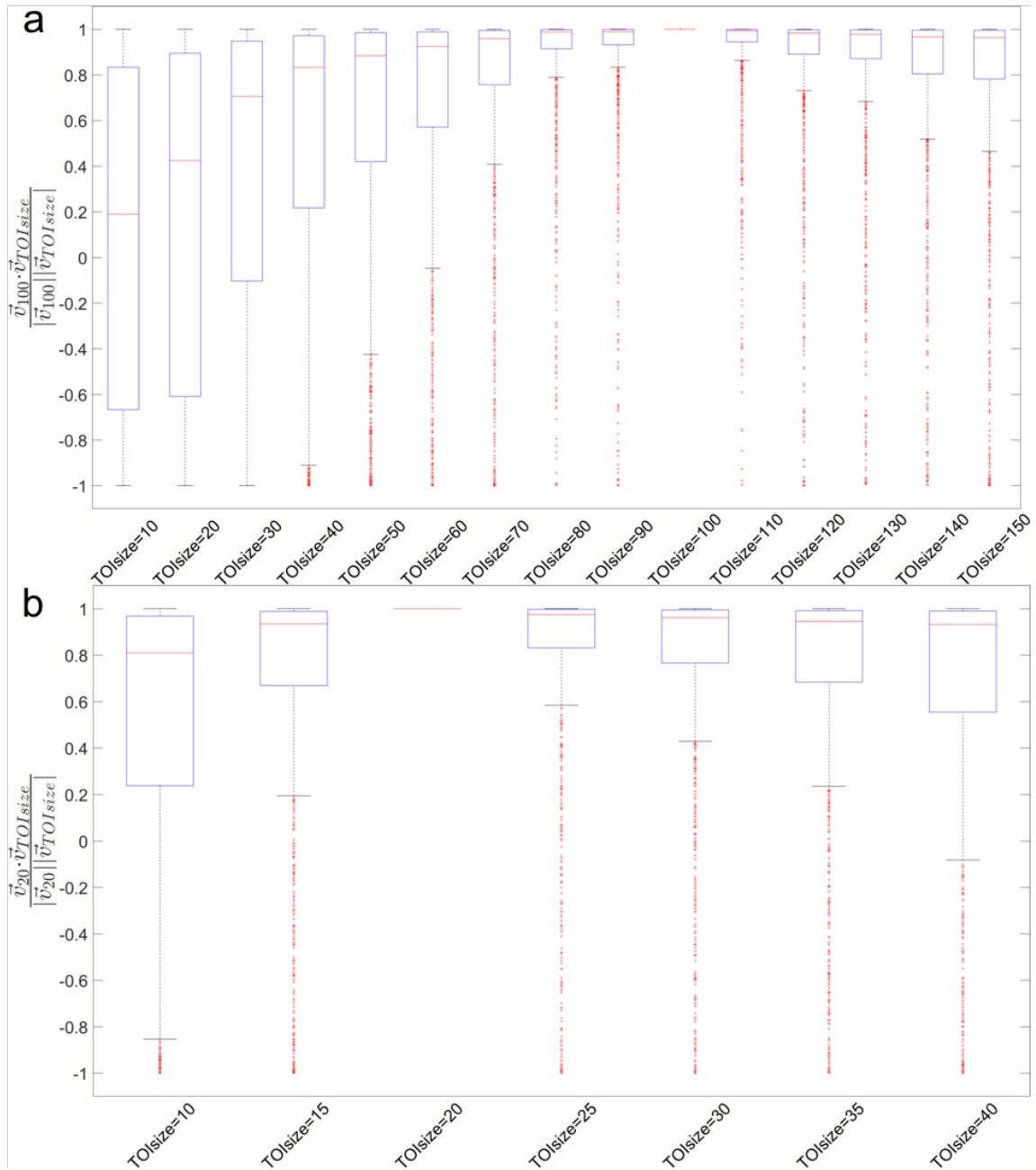


Figure S9 | Statistical testing of selected TOI sizes used in STICS measurements via dispersion analysis

Box plots for dispersion analysis calculated via Eq.5 from STICS vector maps calculated for a) TOI values 10, 20, 30, ..., 140, 150 dotted with $TOI_{test}=100$ for plant cell data set and for b) TOI values 15, 20, 25, 30, 35, 40 dotted with $TOI_{test}=20$ for the pollen tube control data (Figure S3).

Supplemental Movies

Movie 1 | Detection of cell plate growth with FluMOS. Left, pKN::GFP-KN labeled cell plate. Right, merged image of the FluMOS detection (red line) with the fluorescent image. Time series recorded every 3 sec and displayed at 15 fps for a total duration of 45 min with a pixel size of 0.17 μm . Scale bar 10 μm .

Movie 2 | GFP-KN labeled cell plate expansion used for the surface rendering. Time series recorded every 26 sec and displayed at 15 fps for a total duration of 46 min with a pixel size of 0.33 μm . Scale bar 10 μm .

Movie 3 | 4D representation of a cell plate centrifugal growth. The surface rendering tool of Imaris software was used to represent the GFP-KN labeled cell plate expansion: the parameters were so so that the diameter of the largest sphere was 35 μm without smoothing and a surface grain of 1 μm was used. Relative time and dynamic scale bar are included in the video, which is displayed at 5 fps showing the primary and secondary centrifugal growth of a forming cell plate for a total duration of 21 min.

Movie 4 | FRAP on plasma membrane labeled with FM4-64. Bleaching applied after 6 sec (yellow box). Time series recorded every 3 sec and displayed at 5 fps for a total duration of 2 min with a pixel size of 0.11 μm . Scale bar 10 μm .

Movie 5 | FRAP on pKN::GFP-KN labeled plasma membrane. Bleaching applied after 6 sec (yellow box). A photobleaching correction has been applied with Bleach correction plugin for ImageJ. Time series recorded every 3 sec and displayed at 5 fps for a total duration of 1 min 36 sec with a pixel size of 0.11 μm . Scale bar 10 μm .

Movie 6 | FRAP on pKN::GFP-KN labeled cell plate. Bleaching applied after 5 sec (yellow box). A photobleaching correction has been applied with Bleach correction plugin for ImageJ. Time series recorded every 1 sec and displayed at 15 fps for a total duration of 2 min with a pixel size of 0.2 μm . Scale bar 10 μm .

Movie 7 | FM4-64 labeled cell plate-forming vesicles. Time series recorded every 26 sec and displayed at 15 fps for a total duration of 46 min with a pixel size of 0.33 μm . Scale bar 10 μm .

Movie 8 | GFP-KN labeled cell plate expansion used for the STICS analysis. Time series recorded every 735 ms sec and displayed at 60 fps for a total duration of 20 min with a pixel size of 0.17 μm . Scale bar 10 μm .

Movie 9 | Tracking with STICS of the motion of vesicles forming cell plate during IPA and PCG. Left, GFP-KN labeled cell. Right, STICS detection of GFP-KN labeled vesicles. Time series every 1.5 sec for a total duration of 2.5 min and displayed at 8 fps. Scale bar 10 μm .

Movie 10 | Tracking with STICS the motion of vesicles forming cell plate during SCG. Left, GFP-KN labeled cell. Right, STICS detection of GFP-KN labeled vesicles Time series every 1.5 sec for a total duration of 3.7 min and displayed at 8 fps. Scale bar 10 μm .

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