

Quantitative analysis of plant ER
architecture and dynamics

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Supplementary Information

The screenshot displays the AnalyzER GUI with the following components:

- Directory:** Shows the file path 'GFP-HDELAlrymovie_2_rot300.czi'.
- Setup:** Includes options for channels (R, G, B), norm, 12 bit, and rotation.
- Profile:** Shows FWHM, peak, and time parameters.
- Image processing:** Includes resample, subtract, and guided options.
- Network template:** Includes T, C, and method settings.
- Skeleton extract:** Includes T1 boundary, thres2, and Feature Type settings.
- Optical flow:** Includes levels, iterati, filter, and scale settings.
- Display controls:** Includes image, filtered, merge method, and persistence options.
- Network extract:** Includes Sample, psf, sheet, and int to um settings.
- Tubule analysis:** Includes Analysis, Morphology, Trace, Distance, and Region settings.
- Cisternal analysis:** Includes Statistics, Perimeter, Profile, and Polygonal regions settings.
- Results:** Shows a table of statistics for the tubule network.
- Output:** Includes options for export, save image, save mat, and save data.

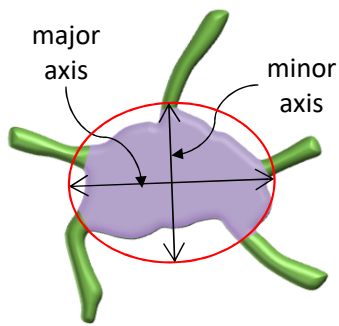
target	grouping	med	mod	var	skw	kut	min	max
graph	channel							
channel 1								
GroupCount_graph		10						
mean_G		1						
mean_nodes		277.7000						
mean_edges		334						
mean_total_length		1.2237						
mean_mean_length		3.6702						
mean_median_length		1.0547						

Supplementary figure 1: AnalyzER: A Graphical User Interface (GUI) for rapid ER analysis

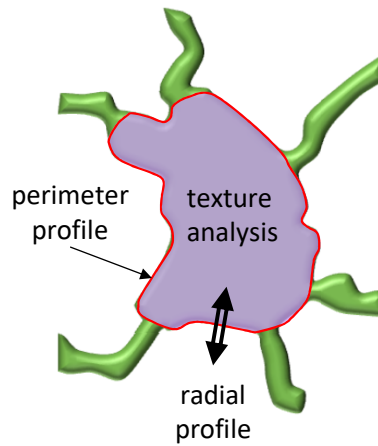
The GUI used for complete analysis of ER structure and dynamics, demonstrating typical settings for the analysis of a 50 frame, single channel time series, with the graph representation of tubule length and cisternal area shown. A complete description of the interface is available in the accompanying manual available from the Oxford Research Archive:

<https://ora.ox.ac.uk/objects/uuid:cb0e2845-2a9c-495a-84f0-4dd2c5164463>

(a) Cisternal measurements



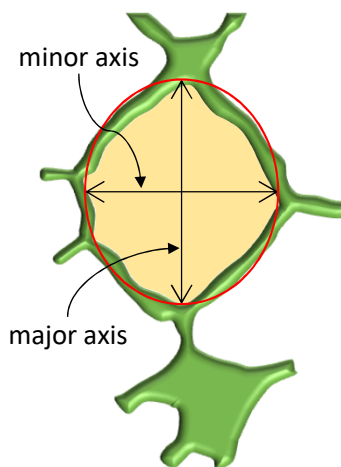
Morphology
area
perimeter
orientation
eccentricity
circularity
roughness
solidity



Texture
intensity
variance
contrast
correlation
energy
homogeneity

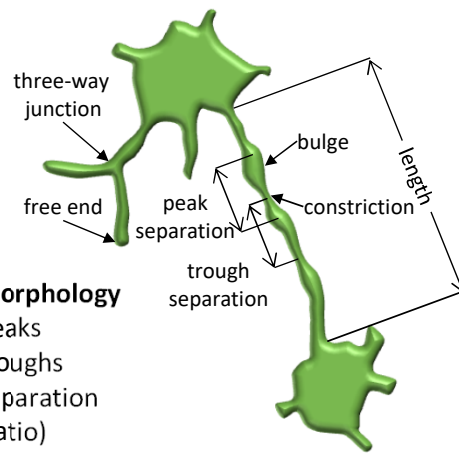
Dynamics
persistence
speed
direction
curl
divergence
coherence

(b) polygonal region measurements



Morphology
area
perimeter
orientation
eccentricity
circularity
roughness
solidity

(c) Tubule measurements

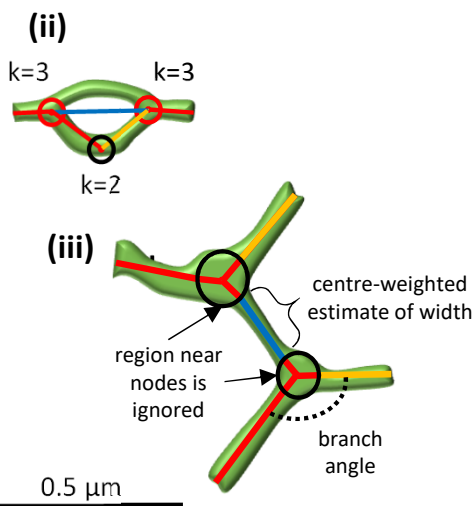
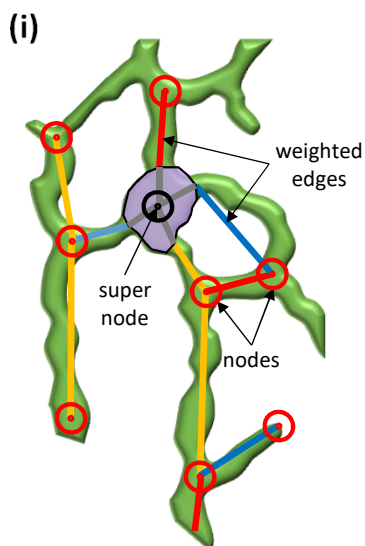


Morphology
peaks
troughs
separation
(ratio)
(covariance)

Morphology
width
surface area
length
volume
Tortuosity

Dynamics
persistence
speed
direction
coherence
curl
divergence

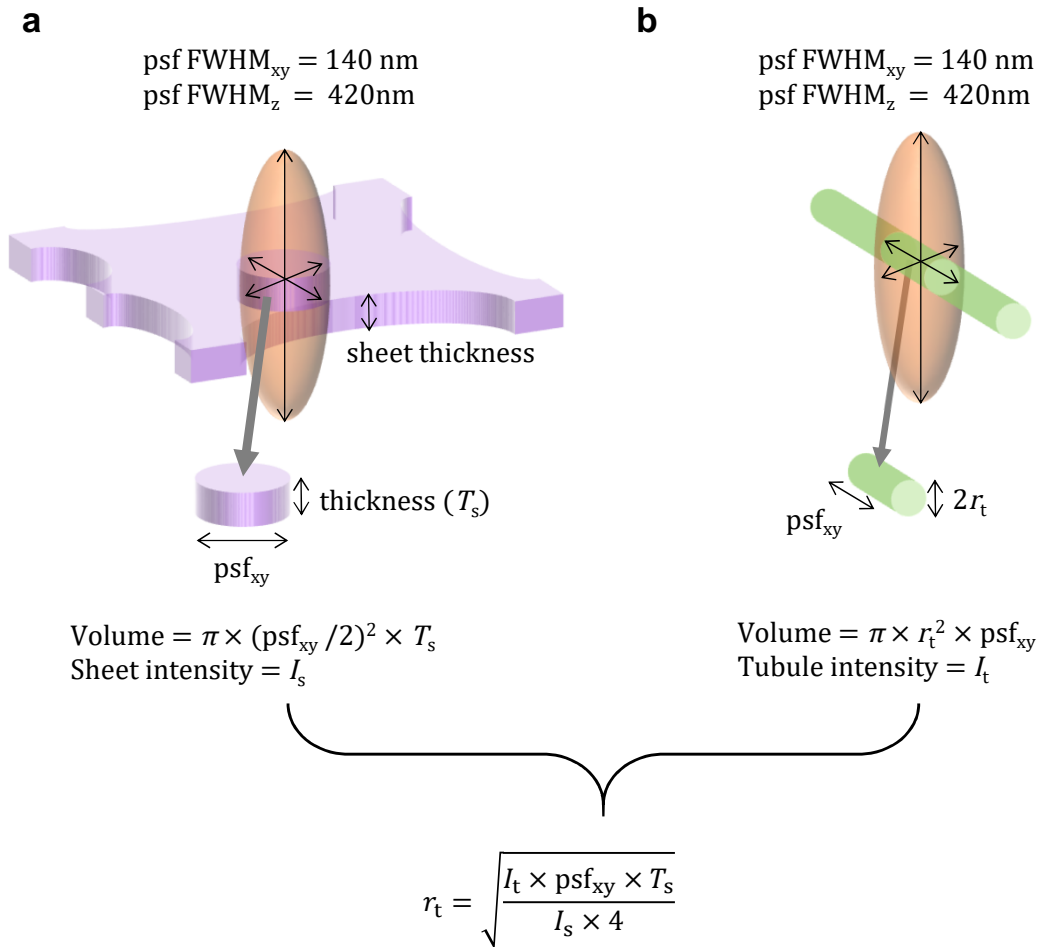
(d) Graph measurements



Graph-theoretic
total length
mean width
total volume
tubule density
node density
cisternal density
average degree
global efficiency
meshedness
free end ratio
betweenness
centrality

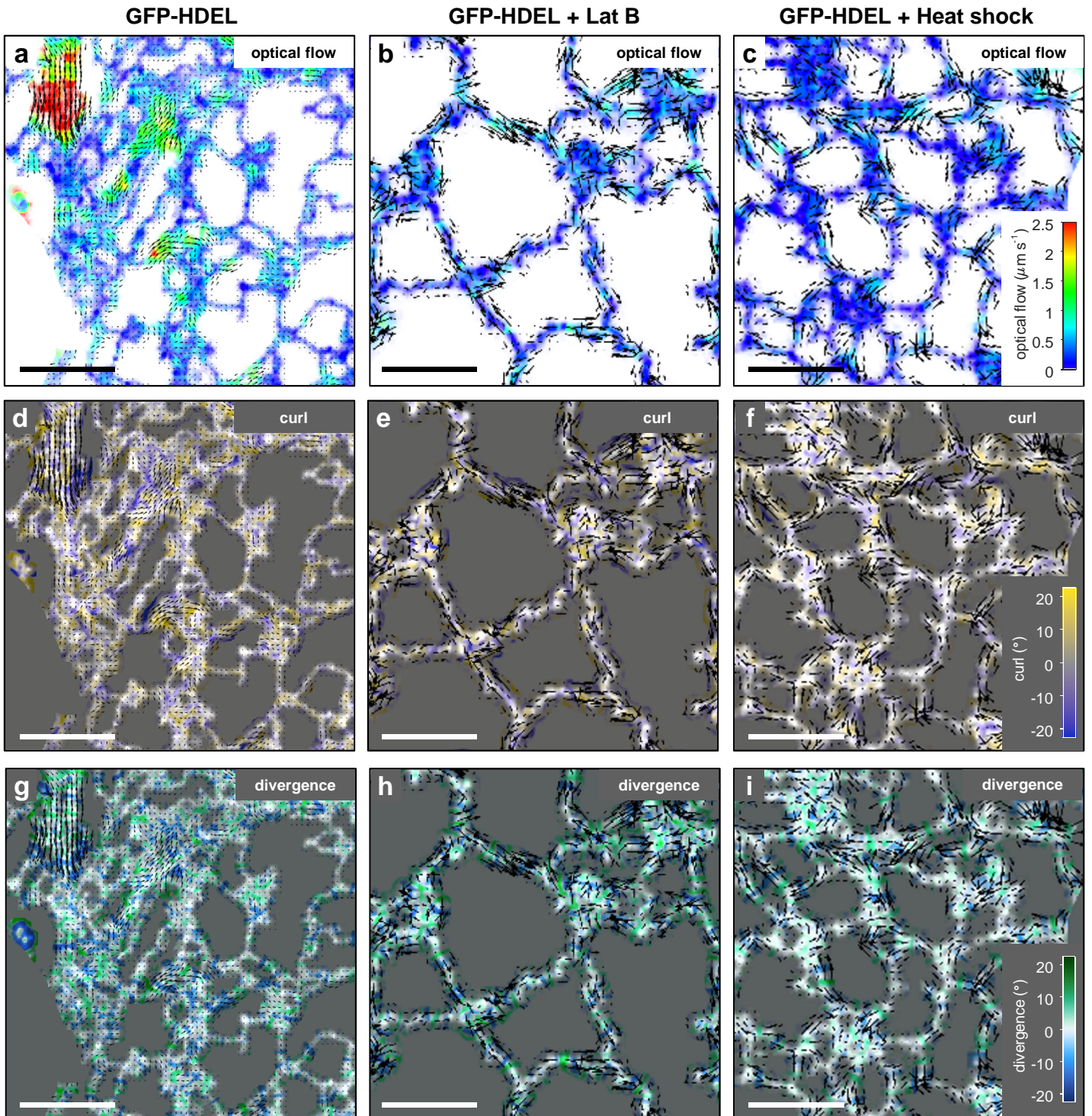
Supplementary figure 2: Schematic illustrations of the main ER metrics automatically measured in the AnalyzER program

Schematic diagrams showing the morphological measurements made for cisternae (a); polygonal regions (b); and tubules (c); along with the conversion to a weighted graph representation where each edge and node was associated with a vector of properties (d(i)). (d(ii)) Two tubules connected to the same junctions were resolved in the graph representation by creating an additional $k=2$ node in the longer arm. (d(iii)) The tubule width was estimated from the central part of the tubule excluding the region near the nodes.



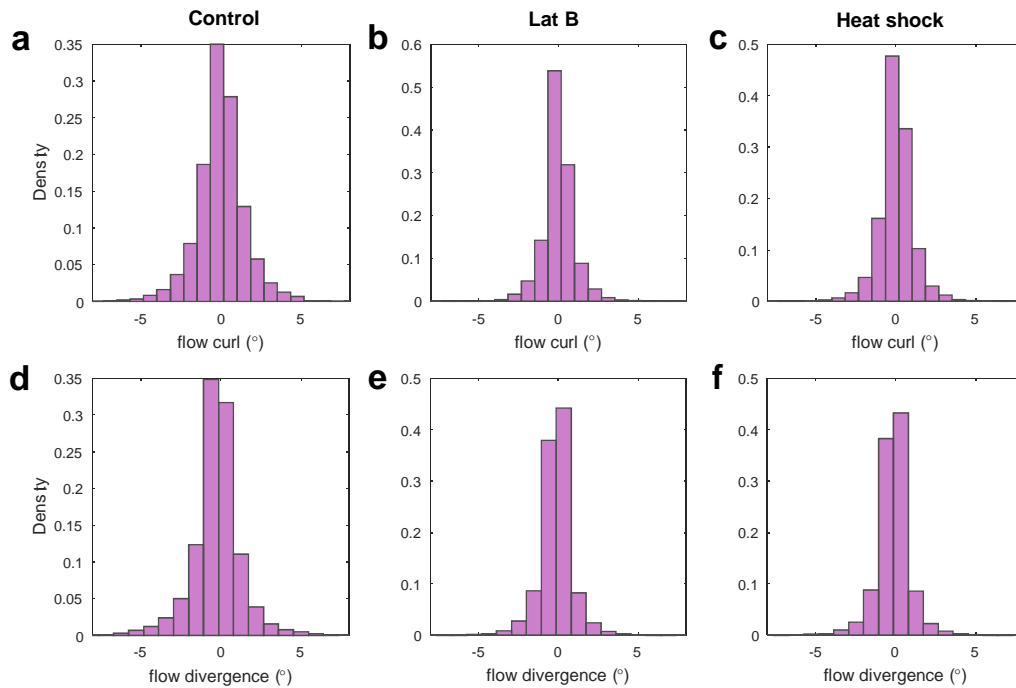
Supplementary figure 3: Estimation of tubule width from intensity measurements

(a) A cysternal sheet provides an internal reference for the amount of fluorescence intensity (I_s) expected from a defined volume of ER when sampled with a stylised point spread function (psf), shown here with dimensions appropriate for the Zeiss Airyscan confocal microscope. The volume approximates to a flattened cylinder with a radius given by half the full-width half-maximum (FWHM) of the psf in the xy plane (psf_{xy}), and the length equivalent to the thickness of the sheet (T_s), independently measured as $40.51 \text{ nm} \pm 0.82$ (mean \pm SD, $n=1$, 25 technical repeats) using SBF-SEM. (b) The intensity (I_t) for a tubule sampled with the same psf would be expected to scale with the relative volume of the tubule, approximated as a horizontal cylinder with length equal to psf_{xy} and the radius (r_t) as the unknown. The radius is estimated as: $r_t = \sqrt{\frac{I_t \times \text{psf}_{xy} \times T_s}{I_s \times 4}}$



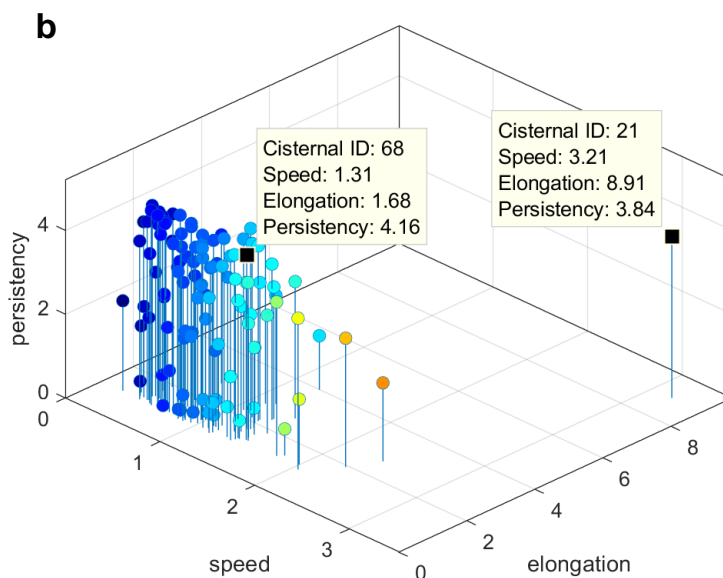
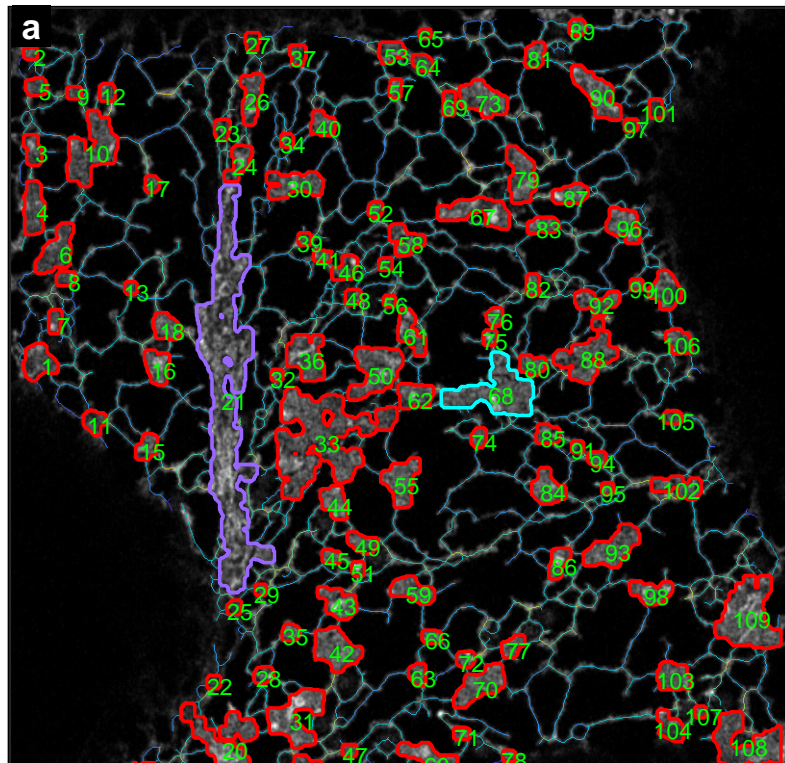
Supplementary Figure 4: Mapping flow curl and divergence

The velocity field calculated from the optical flow (a-c) was analysed for the local curl (d-f) and divergence (g-i) for HDEL controls (a,d,g), and after treatment with Lat B (b,e,h) or heat shock (c,f,i). Images were pseudo-colour coded according to the inset colour scales. Scale bars = 5 μm .



Supplementary figure 5: Quantifying flow curl and flow divergence for cisternae

Density histograms of flow curl (a-c) and flow divergence (d-f) derived from optical flow measurements of cisternae from HDEL controls (a,d), compared to Lat B (b,e) or heat shock (c,f) show a greater spread in the distribution for the controls for both variables, but little difference between Lat B and heat shock treatments (GFP-HDEL, n=6, Lat B, n=8, Heat shock, n=8, where n is the number of independent 50-frame time-series images). Source data are provided as a Source Data file.



Supplementary figure 6: Classification of cisterna

Cisternae are identified as structures remaining after removal of the tubules by image opening. These structures include sheet-like regions of the ER, but also regions where tubules are closely appressed that cannot be segmented without recourse to super-resolution techniques. This is particularly true in the streaming cytoplasm, where segmentation is further complicated by movement. However, it is straightforward to group cisternae into different classes using a range of metrics. (a) shows a single image from a 50 frame time series of a tobacco epidermal cell expressing GFP-HDEL, with the tubule pixel skeleton colour-coded according to average width, and the cisternae outlined in red. An elongated cisterna in the streaming cytoplasm that probably represents a ribbon of appressed tubules and cisternae that cannot be resolved, is outlined in magenta, and a reference cisterna in cyan. (b) a 3D scatter plot of all the cisternae shows the cisterna in the streaming cytoplasm is clearly separated from the other cisternae in terms of elongation and speed.