

Quantitation of ER morphology and dynamics

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Abstract

The plant endoplasmic reticulum forms a network of tubules connected by three-way junctions or sheet-like cisternae. Although the network is three-dimensional, in many plant cells, it is constrained to thin volume sandwiched between the vacuole and plasma-membrane, effectively restricting it to a 2-D planar network. The structure of the network, and the morphology of the tubules and cisternae can be automatically extracted following intensity-independent edge-enhancement and various segmentation techniques to give an initial pixel-based skeleton, which is then converted to a graph representation. ER dynamics can be determined using optical flow techniques from computer vision, or persistency analysis. Collectively, this approach yields a wealth of quantitative metrics for ER structure, and can be used to describe the effects of pharmacological treatments or genetic manipulation. The software is publicly available.

Keywords

Quantitative confocal imaging, Endoplasmic reticulum morphology, Endoplasmic reticulum dynamics, Persistency analysis, Network analysis, Phase congruency, Reticulon, Lunapark, ER cisternae.

1 Introduction

The endoplasmic reticulum (ER) forms a complex and dynamic network of tubules and sheet-like cisternae that ramify throughout the cytoplasm (Westrate *et al.*, 2015). In this Chapter we describe AnalyzER v2 ER network analysis program that is designed to quantify:

- (i) The length, width, morphology and protein distribution along ER tubules following extraction of a single-pixel wide skeleton (Fig. 1A,B);

- (ii) The degree and branch angles at three-way junctions (nodes) in the tubular network (Fig. 1A,B);
- (iii) The size, shape, and protein distribution in cisternae, with the latter estimated using gray-level co-occurrence (GLCM) texture metrics (Fig. 1C);
- (iv) The topological organisation of the tubular and cisternal network determined using graph-theoretic metrics (Fig. 1D);
- (v) The distribution of immobile nodes, tubules and cisternae using persistency mapping;
- (vi) The local speed, direction, coherence, divergence and curl of movement of tubules and cisternae using optical flow;
- (vii) The size and shape of the polygonal regions enclosed by the network.
- (viii) Physiological responses measured using ratiometric reporters.

The AnalyzER package is implemented in MatLab® (www.Mathworks.com) and available as a MatLab® app, or as a standalone package. The program was originally designed to quantify ER organisation in plant epidermal cells where the ER is confined to a very thin layer of cytoplasm appressed to the periclinal cell wall as a planar, 2-D network. It is a development of a tubule morphology analysis program (Fricker *et al.*, 2018), that was used to quantify constrictions in ER tubular network caused by reticulon over-expression in single channel (x,y) images (Breeze *et al.*, 2016). AnalyzER extends the analysis to a complete quantitation of all aspects of ER structure and dynamics for multi-channel 4D time-series (Pain *et al.*, 2019). This includes multiple improvements and expansion to the processing, segmentation and graph extraction algorithms, comparison with ground truth data to validate the automated network structure, introduction of time domain metrics for persistency and optical flow, introduction of cisternal substructure analysis, including texture features, perimeter and radial profiles, and extension of the graph-theoretic metrics. All aspects of the analyses are handled through a single graphical user interface (GUI) to provide an integrated platform. A complete manual for the program is available at:

<https://doi.org/10.5281/zenodo.6386982>

The simplest method to identify the ER automatically is an intensity-based segmentation of the fluorescence image to give a binary image, with ones representing the ER structure and zeros for the background. However, the resultant binary image is critically dependent on the value for the threshold used, and it is rare that a single threshold provides adequate segmentation without either losing dimmer structures if it is set too high, or artificially expanding and fusing adjacent regions if it is set too low (see Boučekhima *et al.*, 2009). Thus, the approach adopted here exploits additional intensity-independent information over a range of scales and orientations to enhance the network structure, prior to segmentation as a single-pixel wide skeleton (Fig. 1B). The skeleton is then used as a template to interrogate the image locally to provide an estimate of the relative amount of fluorescent probe present.

The labelling intensity also provides an indication of the tubule width, with a number of assumptions. The expected width of the ER tubule is 50-70 nm (Westrate *et al.*, 2015) or less, which is below the resolution of the confocal microscope, but can just be resolved with super-resolution techniques, such as stimulated emission depletion microscopy (STED, Hein *et al.*, 2008), although this is not routine for dynamic imaging. For most laboratories, access to super-resolution techniques may be limited, necessitating the development of approaches that can be used on a routine basis with existing systems. Nevertheless, with additional assumptions about the distribution of the fluorescent luminal marker and the point spread