

img2net: automated network-based analysis of imaged phenotypes

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ABSTRACT

Summary: Automated analysis of imaged phenotypes enables fast and reproducible quantification of biologically relevant features. Despite recent developments, recordings of complex networked structures, such as leaf venation patterns, cytoskeletal structures or traffic networks, remain challenging to analyze. Here we illustrate the applicability of img2net to automatically analyze such structures by reconstructing the underlying network, computing relevant network properties and statistically comparing networks of different types or under different conditions. The software can be readily used for analyzing image data of arbitrary 2D and 3D network-like structures.

Availability and Implementation: img2net is open-source software under the GPL and can be downloaded from <http://mathbiol.mpimp-golm.mpg.de/img2net/>, where supplementary information and data-sets for testing are provided.

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1 INTRODUCTION

Biological and man-made systems, ranging from biochemical reactions to neural and social interactions, can often be represented as networks, with nodes and edges representing the components and their interactions, respectively. Network representations facilitate not only intuitive visualization but also quantitative studies of the systems' structure and dynamics.

Spatial networks constitute an import subclass, which includes networks, such as leaf venation (Dodds *et al.*, 2010), cellular cytoskeleton (Volkman *et al.*, 1999) and city streets (Barthélemy, 2011). While some of these networks, like city infrastructure, have been well-characterized, others, like biological spatial networks, remain poorly understood.

Snapshots of spatial networks can be obtained by imaging technologies. The computational challenge is that of extracting the underlying networks from the gathered images in a fast and reliable fashion, and of examining the reconstructions to reveal the underlying organizational principles.

The existing image-based methods for reconstruction of biological networks are typically designed for specific types of networks: tree-like networks for plant root architectures (Pound *et al.*, 2013); fungal or leaf venation networks (Obara *et al.*, 2012); or neuronal topology of the human connectome

(Longair *et al.*, 2011; Meijering, 2010). While most of these approaches require user input, rendering them unfeasible for high-throughput studies, fully automated algorithms are usually tailored to specific image sources and challenged by low signal-to-noise ratios, which may strongly affect the resulting networks.

Here, we present a robust approach to reconstruct 2D and 3D (non-)biological spatial networks from gray-scale image data. By constructing weighted networks and computing their seminal network properties, img2net allows an extensive quantification and statistical comparison of network topologies.

2 METHODS AND FUNCTIONALITY

We extract the networks from image data in two steps: starting from a gray-scale image, where high intensities reflect strong links in the network, we place a (arbitrary) grid, which covers the region of interest. For each grid-edge, we determine a weight by convolving a Gaussian kernel with the original image to capture the edge's capacities to carry certain traffic, the speed of transportation or combinations thereof (cf. Results).

To facilitate comparative analyses, different seminal properties of the resulting weighted network are computed, including degree distribution, path lengths or random-walk-related properties. To evaluate the biological importance of the calculated properties, we developed several null models, which randomize the network while preserving the distribution of edge weights. They provide the basis for revealing the principles underlying the network organization (Breuer *et al.*, 2014).

img2net is written in Python and provides a graphical user interface (GUI; Fig. 1A) for the selection of the image input data and the parameters of the network reconstruction procedure. It operates on folders of .png, .jpg, or .tiff files that display the networked structure of interest, with a gray-scale representation of edge strengths. The directory tree divides the images into different treatments and experiments, e.g.

```
root/treatment_003/experiment_005/image_z001_t001.tiff
```

The usage of rectangular, triangular or hexagonal grids is supported; grid spacings, periodic boundary conditions, the widths of the convolution kernel and the number of layers for 3D image data can be specified by the user. Different null models are available and the number of null model realizations can be set. Finally, img2net can be run on multiple cores.

An output folder is generated in the root directory of the chosen image directory tree: a human-readable document is

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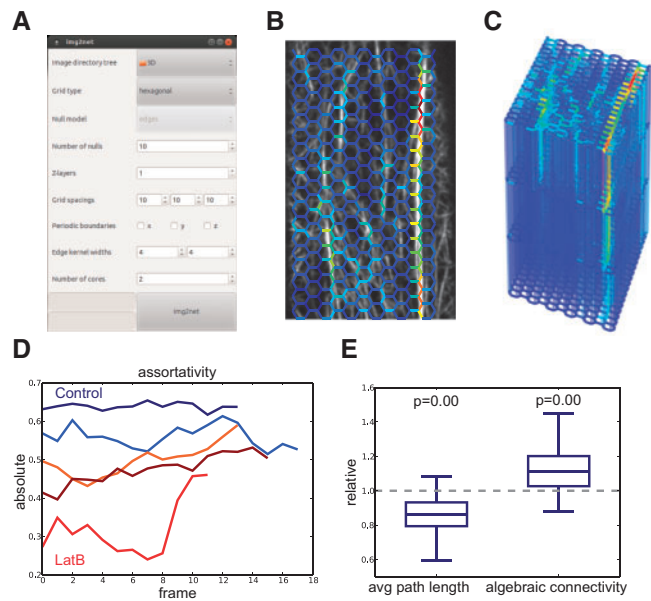


Fig. 1. Img2net: GUI and output visualizations. (A) GUI to set parameters for the network reconstruction. (B) Actin network reconstructed with a hexagonal grid. (C) 3D reconstruction for image data with four z-slices. (D) Time series of the assortativity for multiple untreated (blue) and treated (red) plants. (E) Ratios of average path lengths and algebraic connectivities of observed and null model networks

created, which contains the values of the network properties for the analyzed networks and their null models to allow further analyses and visualizations of the results. img2net generates standard plots as .svg files: for each experiment, a reconstructed network overlaying the original image data is saved. For all analyzed network properties, plots of the resulting time series are generated to facilitate network comparisons.

3 RESULTS

For testing, img2net was used to compare confocal recordings of the actin cytoskeleton (Fig. 1B and C) of untreated plant cells and cells treated with an actin disrupting drug (Breuer *et al.*, 2014). We find that the actin networks of treated cells display a consistently lower assortativity (Fig. 1D), indicating a drug-related filament fragmentation. Further, for untreated cells, the observed networks display significantly smaller average path lengths ('accessibility') and significantly higher algebraic connectivities ('robustness') than expected by chance (P -values estimated from the null model via one-sample t -tests; Fig. 1E), suggesting a biological basis for the maintenance of short and robust transportation routes.

We also used img2net to analyze the network structure of the German autobahn, which was obtained from OpenStreetMap as an image by using a gray-scale coding of the speed limits (Breuer *et al.*, 2014). We find that, similar to the actin cytoskeleton, the

autobahn shows significantly smaller path lengths and a higher algebraic connectivity than expected by chance.

4 CONCLUSION

Because networks provide an intuitively accessible as well as mathematically sound framework for the representation of complex systems, the reconstruction of spatial networks from image data is useful in biological and technical research. img2net implements an automated method for fast and robust reconstruction of arbitrary 2D and 3D (non-)biological spatial networks.

As our approach relies on fixed grid topologies, img2net offers different types of grids to verify that the findings are grid-independent. For small grid spacings, the grid typically approaches the 'true' structure of the underlying network. The main benefit of this approach is its robustness against noise and flaws in the input images. For example, small ruptures in the underlying network do not disrupt the corresponding edges but only weaken them. Hence, our approach does not require sophisticated error-correcting image processing steps. Furthermore, img2net is directly applicable to a wide range of image data from different sources.

The analyses of the reconstructed networks implemented in img2net allow a quantification of structural properties, comparisons of networks, e.g. under different conditions, and an assessment whether the network properties reflect underlying organizational principles.

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