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Applications Note

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NucleusJ: an ImageJ plugin for quantifying 3D images of interphase nuclei

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Abstract

Summary: *NucleusJ* is a simple and user-friendly ImageJ plugin dedicated to the characterization of nuclear morphology and chromatin organization in 3D. Starting from image stacks, the nuclear boundary is delimited by combining the Otsu segmentation method with optimization of nuclear sphericity. Chromatin domains are segmented by partitioning the nucleus using a 3D watershed algorithm and by thresholding a contrast measure over the resulting regions. As output, *NucleusJ* quantifies 15 parameters including shape and size of nuclei as well as intra-nuclear objects and their position within the nucleus. A step-by-step documentation is available for self-training, together with data sets of nuclei with different nuclear organization.

Availability and implementation: Dataset of nuclei is available at https://www.gred-clermont.fr/media/WorkDirectory.zip. *NucleusJ* is available at http://imagejdocu.tudor.lu/doku.php?id=plugin:stacks:nuclear_analysis_plugin:start.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

The nucleus is a compartmentalized organelle containing distinct chromosomal domains and a variety of nuclear bodies. Organization and positioning of chromatin domains within the nuclear space, such as clusters of heterochromatin termed chromocentres (Fransz *et al.*, 2002), impact gene expression (Bickmore and van Steensel, 2013). To link nuclear structure and function, imaging tools that quantify nuclear morphology, positioning and organization of chromatin domains in three dimensions (3D) are needed. Although proprietary softwares or open source programs such as NEMO and TANGO are available (Iannuccelli *et al.*, 2010; Ollion *et al.*, 2013), they either require financial investment or minimal knowledge of image processing algorithms. Our *NucleusJ* plugin assembles a complete set of methods in a coherent strategy, quantifies 15 different nuclear and chromatin parameters and does not require expertise in image analysis.

2 Methods

The *NucleusJ* plugin processes 3D images of nuclei either acquired by live imaging with fluorescent reporters, from fixed tissues or isolated nuclei stained with DNA dyes. *NucleusJ* uses the *MorphoLibJ* library (https://github.com/ijpb/MorphoLibJ) available for ImageJ and Fiji. The implemented 3D image processing and analysis pipeline can be run either completely or partially, on single images or in batch mode, thus offering flexibility to accommodate the diversity of practical needs and application purposes (Fig. 1). In the course of the analysis, transformed images are generated, automatically organized in sub-directories, and results of quantitative analyses are arranged in four output files (Fig. 1).

Segmentation is an essential procedure in image analysis as it separates objects from each other and from the background. *NucleusJ* includes two segmentation steps that extract first the nucleus and then chromatin domains such as chromocentres. 3D nucleus

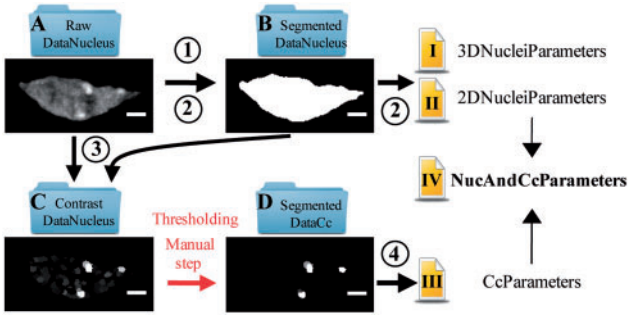

Fig. 1. *NucleusJ* combines tools that can be run in single or batch mode and along different scenarios (1–4). Raw images are stored in A by the user. Transformed images are automatically organized in directories (B–D) and quantitative parameters exported as output files (I–IV). *Nucleus Segmentation* (1) and *Nucleus Segmentation and Analysis* (2) produce segmented images automatically stored in B. Quantitative parameters are exported as files I and II only when (2) is chosen. *Chromocentre Segmentation* (3) applies the 3D watershed transformation to raw images in A using segmented nuclei stored in B. Contrast images are then stored in C. Chromocentres are defined by manual thresholding and images stored in D. Chromocentre parameters are computed by *Chromocentre analysis* (4) and exported as file III. Finally, a compilation of all parameters is exported as file IV. Images of *Arabidopsis* nuclei were acquired with a structured illumination microscope (Leica microsystems) using a water × 63 objective. Resolution: $xy = 0.103\,\mu m$ and $z = 0.200\,\mu m$. Scale bar: 2 μm

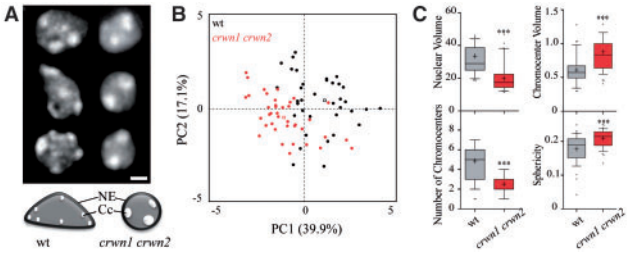

Fig. 2. Application of *NucleusJ* using plant cell nuclei. (**A**) Top: representative epidermis pavement cell nuclei of wild type (wt, left) and mutant (*crwn1 crwn2,* right) cotyledons. Scale bar: 2 μm. Bottom: model from the quantitative data: nucleus in grey is surrounded by the nuclear envelope (NE) in dark gray, chromocentres (Cc) in white. (**B**) Principal component analysis using the 15 parameters on wt (black) and *crwn1 crwn2* (red) nuclei. (**C**) Statistical analysis of four selected parameters (***$P < 0.0001$)

segmentation is performed using an adaptation of the Otsu thresholding method (Otsu, 1979), here combined with optimization of a shape parameter called sphericity ($36\pi \times \text{volume}^2/\text{surface area}^3$). The threshold value provided by the standard Otsu method is used as a starting point to test a range of thresholds, which eventually leads to the selection of the value for which the sphericity is maximal (Fig. 1 and Supplementary Fig. S1 and S2). Chromocentre segmentation is performed using the segmented nucleus image as mask. We implemented the watershed immersion algorithm (Vincent and Soille, 1991) in 3D to partition the nucleus mask into a number of regions. To overcome the low contrast between chromocentres and the background intensity of the nucleus, the resulting image is analysed to create a map of intensity contrasts between neighbouring regions (Andrey *et al.*, 2010). Because semi-automatic or manual approaches are more accurate than fully automatic procedures, we kept the final thresholding of the intensity contrast to obtain the segmented image of chromocentres as a manual step.

3 Results

Following segmentation, a morphometric analysis is applied to measure nuclear volume and 3D shape descriptors (flatness, elongation and sphericity). *NucleusJ* also computes chromatin organization parameters such as number, total volume and intensity of chromocentres relative to the entire nucleus (Tessadori *et al.*, 2007) as well as individual chromocentre parameters including volume and distance to the nuclear periphery. Overall, the nucleus is characterized by 15 quantitative parameters (Fig. 1 and Supplementary Table 1), which are automatically saved as output files for further statistical analysis. *NucleusJ* was validated using an *Arabidopsis* mutant line lacking CROWDED NUCLEI (CRWN) proteins, which are putative components of the plant nucleoskeleton (Wang *et al.*, 2013). Analysis of a small set of 38 (wild type) and 39 (*crwn1 crwn2* mutant) nuclei revealed reduced nuclear volume, increased sphericity and fewer chromocentres of increased size in the mutant compared with WT, in perfect agreement with Wang *et al.* (2013; Fig. 2).

The analysis of the 77 nuclei took about 1 h. The time-limiting step is the manual thresholding of contrast images for chromocentre segmentation, since it involves visual crosschecking with raw images (Fig. 1). The statistical analysis of the data validates our method and opens avenues to phenotype nuclei in other *Arabidopsis* mutants, as well as in different cell types of other organisms.

4 Conclusion

Computational image analysis provides precise, objective and reproducible quantitative data from images. Our plugin generates, within a few steps, 3D quantitative measurements from single images or large data sets, without requiring expertise in image analysis. Nuclear domains visible with DNA dyes, such as chromocentres, are not specific to plants. *NucleusJ* will, therefore, be useful for a large community of users interested in quantifying size and shape of nuclei, nuclear objects or chromatin domains as well as positioning of the latter in nuclear space. In the future, we expect to improve our segmentation methods, to extend *NucleusJ* to quantify fluorescent *in situ* hybridization (FISH) and immunochemistry signals and to define a new method to automatically segment chromocentres.

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