A KNIME-Based Workflow for the Distinction of S-Phase Stages in Cells Immunolabeled for PCNA Detection

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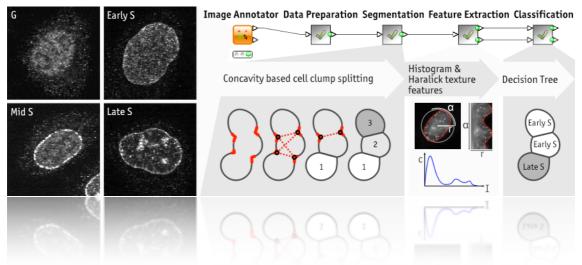
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Abstract

Most physiological processes in eukaryotic cells are regulated in a cell-cycle specific manner. Assigning cellular phenotypes to distinct cell cycle phases enables to detect subtle effects that appear only transiently, and improves the understanding of their regulation. In S-phase, cells duplicate their genome in a spatially and temporally precisely regulated fashion. Open, active regions of the genome are replicated first, while inactive, compacted areas mostly located at the nuclear periphery lag behind. PCNA is an essential factor for eukaryotic DNA replication and a marker of replication activity. Its distribution in the cell nucleus, which is conveniently visualized via immunocytochemistry, reflects the location of actively replicating genomic regions. This PCNA staining pattern yields information about the stage of S-phase the cell has reached at the time point of fixation. Here, we present a KNIME workflow which enables to automatically assign cells to early-mid-or late S-phase in stationary images of PCNA-labeled nuclei obtained by epifluorescence or confocal microscopy. This workflow uses KNIP, the KNIME Image Processing framework along with the KNIME data mining tools. To correctly segment cell agglomerates a cluster splitting method was developed that separates nuclei based on the criterion of maximum contour concavity. The 110-dimensional feature vector is a combination of histogram features and Haralick texture features. Using this feature set the KNIME decision tree is the classifier with the best performance. In sum, we present a general tool for classifying S-phase stages with high accuracy in fluorescence images lacking the temporal information provided by time-lapse recording.

Keywords

KNIME, cell cycle, antibody-labeling, fluorescence microscopy, segmentation, classification, features, clump splitting