Systematizing DNA Replication Patterns Using Dual Label 3D Imaging in Maize

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ABSTRACT: Complete and accurate replication of genetic material is required for cells to grow and divide. Accurate genome replication is essential for plants to achieve optimal productivity, serving as the foundation of the food chain and agriculture. To better understand DNA replication in plant cells, we work with collaborators at NCSU to visualize DNA synthesis using 3D microscopy of nuclei from maize (*Zea mays*, corn) root tip cells. The Bass Lab previously observed unique microscopic patterns of DNA Synthesis (S phase) at different time points within the S phase, for instance, early S phase nuclei show DNA synthesis staining patterns that we classify as "distributed" throughout the entire nucleus. In contrast, late S phase nuclei show DNA synthesis staining patterns that we classify as small "patchy" regions. In our study, we employed sequential DNA staining methods, specifically dual labeling techniques with green and red DNA synthesis dyes, to chronologically organize and identify the various patterns (or pattern pairs) in relation to S phase progression. For each nucleus, three pictures were captured (1) DAPI which stains all DNA. (2) EdU is used to stain the first labeling, and (3) BrdU is used to stain the second labeling. In this way, we gathered image pairs reflecting immediately sequential replication timing patterns and quantified their relative abundance. The pattern categories were Background, Dispersed, Patchy Disbursed, Patchy and Knobby, and finally Knobby, and the pattern pairs quantified were all possible pairs of these. Analysis of over 500 nuclei allowed us to determine how long cells are in each stage of the cell cycle, and to subdivide the patterns beyond the previously known three (early, middle, late) into their proper chronological order.

BACKGROUND: Using the DeltaVision microscope, the lab collects its images with a method called 3D deconvolution microscopy. This approach blends optical and computational techniques to enhance the resolution and clarity of images obtained from biological samples[2]. First, the maize root tip cell nuclei are collected and stained blue by a fluorescent staining agent called DAPI. This is used to identify the amount of nuclei in the sample. Then, assays EdU and BrdU are implemented to measure functional activity as DNA replication occurs. EdU and BrdU are colorless, to see them EdU is coupled to a green dye using Click-it chemistry, and BrdU is visualized using antibodies to BrdU followed by secondary antibodies that are made fluorescent red. Since they fluoresce in different wavelengths, we can use different light sources and filters on the microscope to visualize them one at a time. Maize DNA replication activity exhibits a distinct distribution in the nucleoplasm during both early and middle S phase, differing from patterns observed in mammalian cells[1]. This study, conducted with nuclei isolated from naturally developing plant organs, provides a valuable benchmark for understanding DNA replication in plants and lays a foundation for future genetic, epigenetic, and genomic analyses[1].

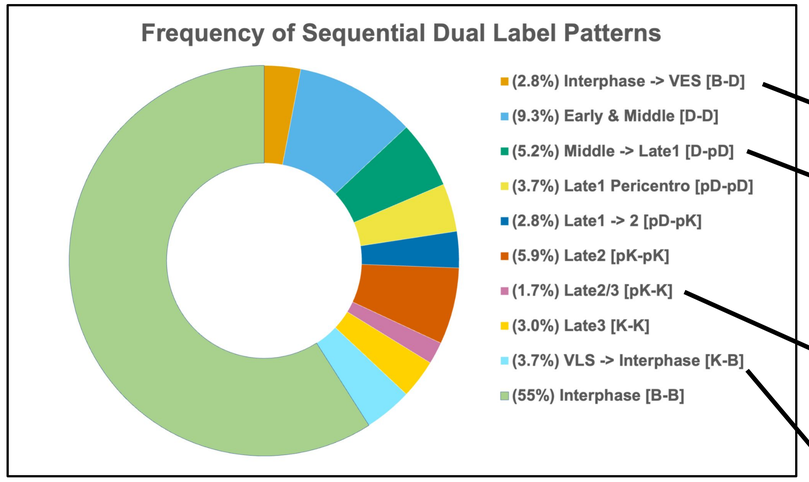


Figure 1. Frequency of Sequential Dual Label Patterns
Nuclei were classified according to the patterns shown in Table 1. Their frequency was calculated and displayed as the pie chart shown.

Acronym	Category	Stage
B-D	Background then Dispersed	Interphase -> VES
D-D	Dispersed then Dispersed	Early & Middle
D-pD	Disbursed then Patchy Dispersed	Middle -> Late 1
pD-pD	Patchy Dispersed then Patchy Dispersed	Late 1 Pericentro
pD-pK	Patchy Dispersed then Patchy and Knobby	Late 1 -> 2
pK-pK	Patchy and Knobby then Patchy and Knobby	Late2
pK-K	Patchy and Knobby then Knobby	Late 2/3
K-K	Knobby then Knobby	Late 3
K-B	Knobby then Background	VLS -> Interphase
B-B	Background then Background	Interphase

Table 1: Systemized DNA Replication Patterns
Includes all important categories of DNA replication and their assigned description.

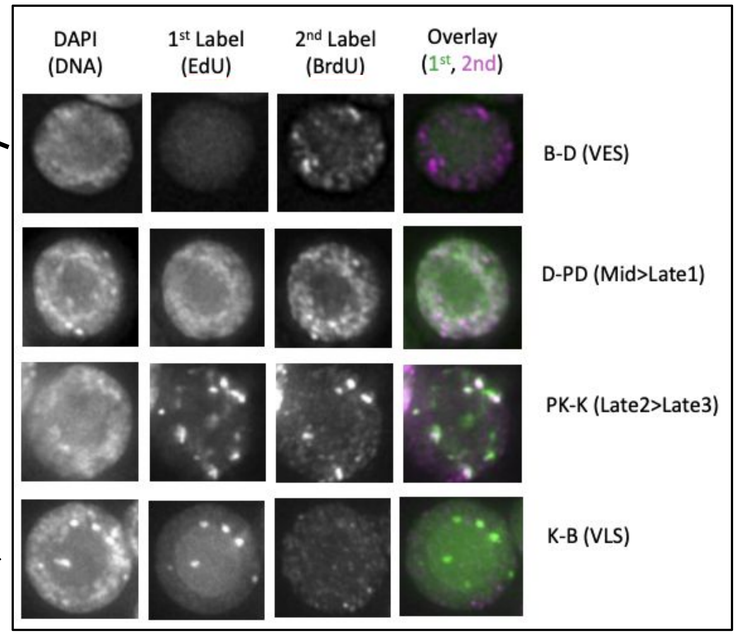
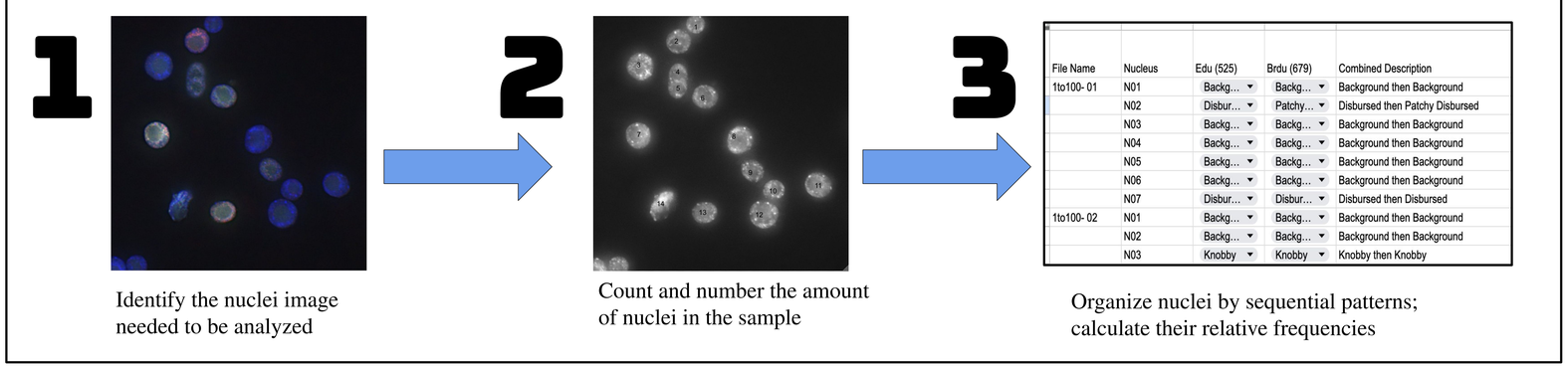


Figure 2: Vizualizing DNA Patterns Using Different Stains
Shows visual examples of what nuclei in the most prevalent stages appear like under the microscope.

CONCLUSION: From the study, we have gained valuable insight into the process of DNA replication within maize root tip cells. Using 3D microscopy and sequential DNA staining techniques, we have observed and categorized patterns of DNA synthesis as it progresses through S phase. When quantifying these patterns we found that fifty-nine percent of the nuclei were found in a "background then background" pattern, this allows us to theorize that cells do not spend most of their time in S phase and are usually among G1, Mitosis, or G2. This would make sense since cells do not replicate continuously. The rest of the classifications have allowed us to infer the length of early, middle, and late S phases. We have found that early and middle S phases occur rather quickly, and nuclei are in late S phase for a much longer period. These new understandings have allowed us to explore the mainly uninvestigated eukaryotic genome. As we continue investigating DNA replication, we aim to gain more insight into why early, middle, and late S phases have different longevities and how they correlate to the rest of the cell cycle.



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