The Search for Mitotic DNA Synthesis (MiDAS) in Maize Root Tips

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Abstract

This ongoing study uses 3D epifluorescence imaging to understand the implications of Mitotic DNA synthesis (MiDAS), a rare and elusive abnormality within DNA synthesis. The study uses both primary and secondary (seminal) root tips of maize (*Zea mays* L.) strains to create multi-nucleic slides that were then observed using fluorescent staining techniques. The Leica Thunder Imaging System then captured 3-D, multi-wavelength (DAPI for DNA, FITC for DNA synthesis) image files that were uploaded to OMERO server (omero.bio.fsu.edu) for visual analysis. Data was derived through observations and quantified by distinguishing mitotic cells from other nuclei. The stains were then cross referenced with each other to detect MiDAS within the mitotic cells. Overall, the study processed imagery gained from multi-nucleic slides to detect and determine MiDAS within root tips of maize. The results so far showcase MiDAS as a rare occurrence in maize root tips.

What is MiDAS?

Experimentation into understanding the abnormalities of DNA synthesis includes Mitotic DNA synthesis (MiDAS), which is a deviation from synthesis in S phase and is instead synthesis in the mitotic phase (prophase, metaphase, anaphase, or telophase) of the cell cycle. Although it is uncommon, other members of the lab have observed a late replicating pattern of nuclei which is shown in the figure. This replicating pattern is similar to MiDAS. Other researchers have observed MiDAS before (Franchet et al. 2020). Certain cell types produce higher levels of observable DNA replication, specifically maize root tips (Bass et al. 2015). Maize root tip cells are thought to contain higher levels of mitotic cells, which increases likelihood MiDAS observation. Considering the scarcity of this pattern in nuclei, a large sample size is be required to observe it.
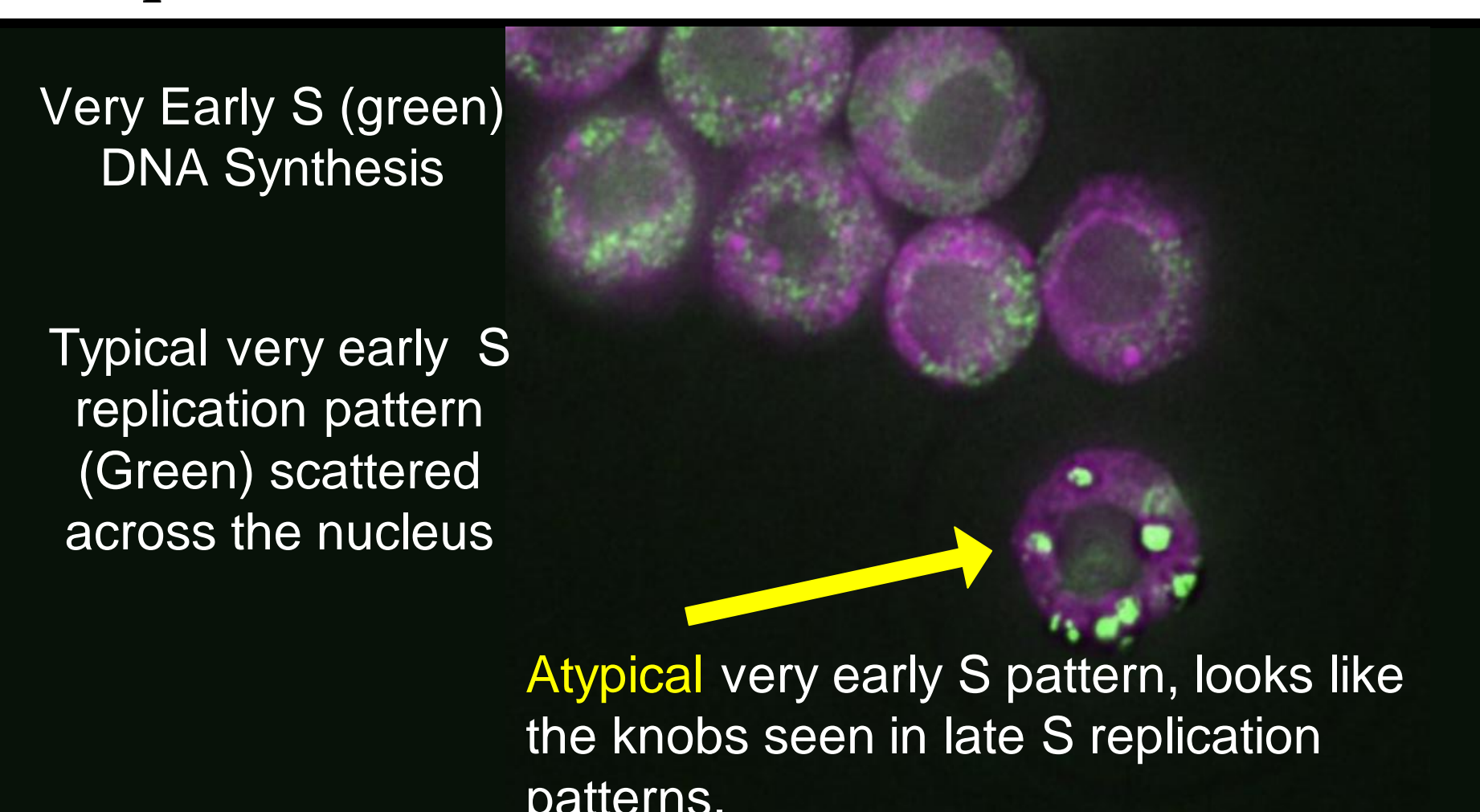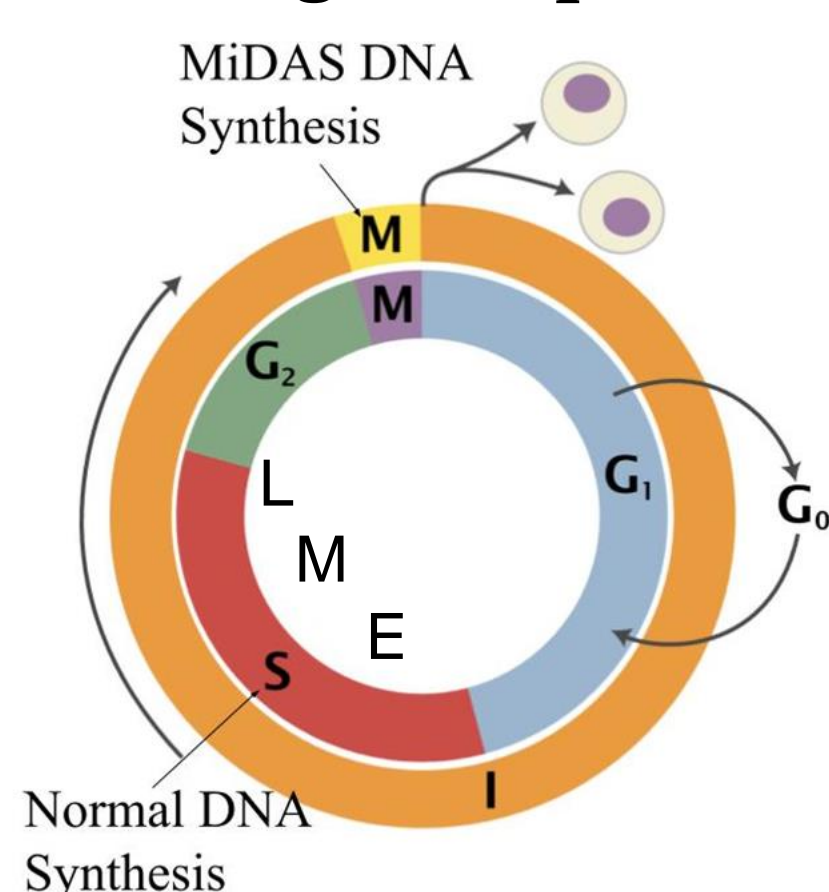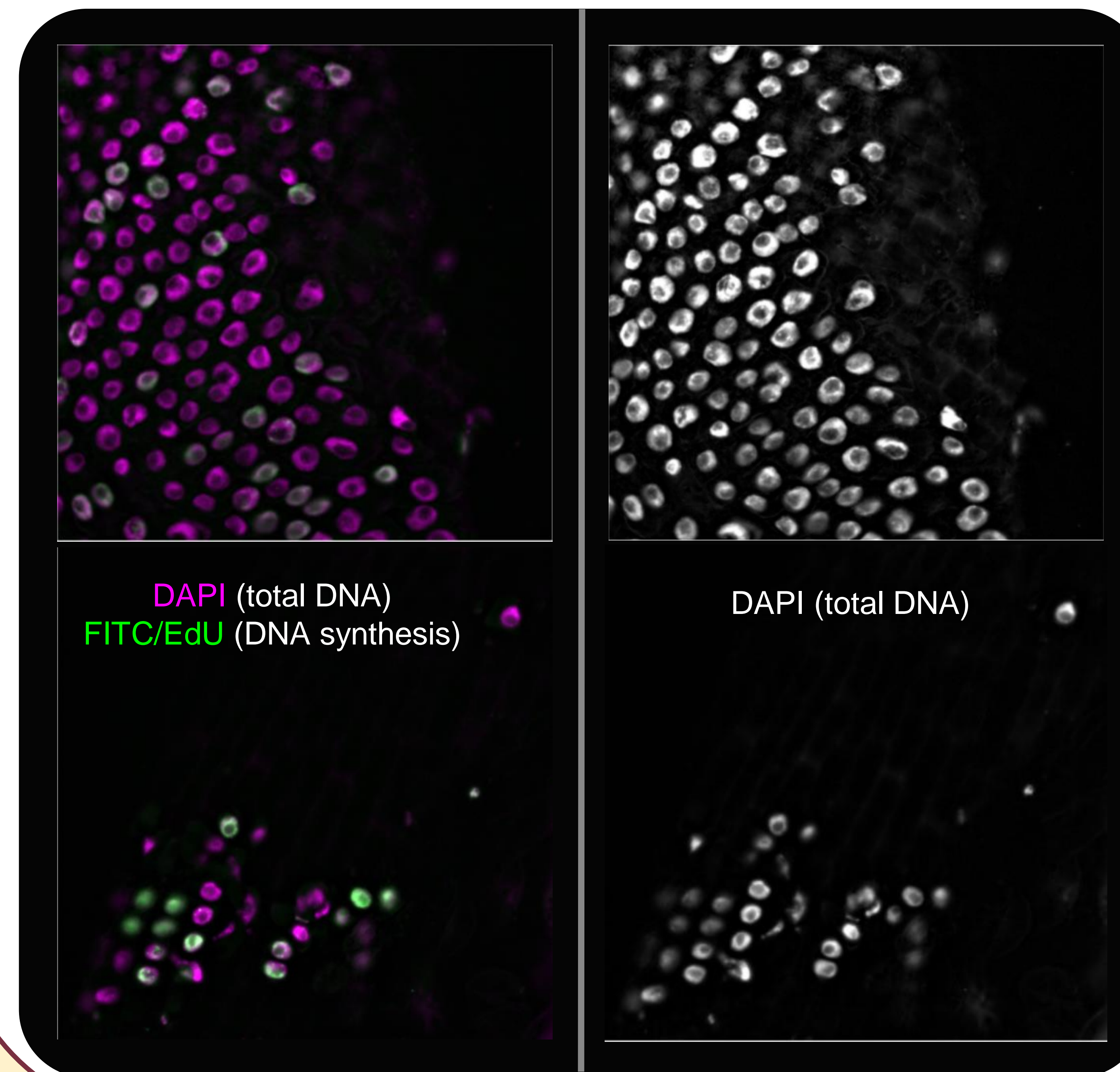
Diagram of Cell Cycle with arrows denoting normal and MiDAS DNA synthesis (Wikipedia).
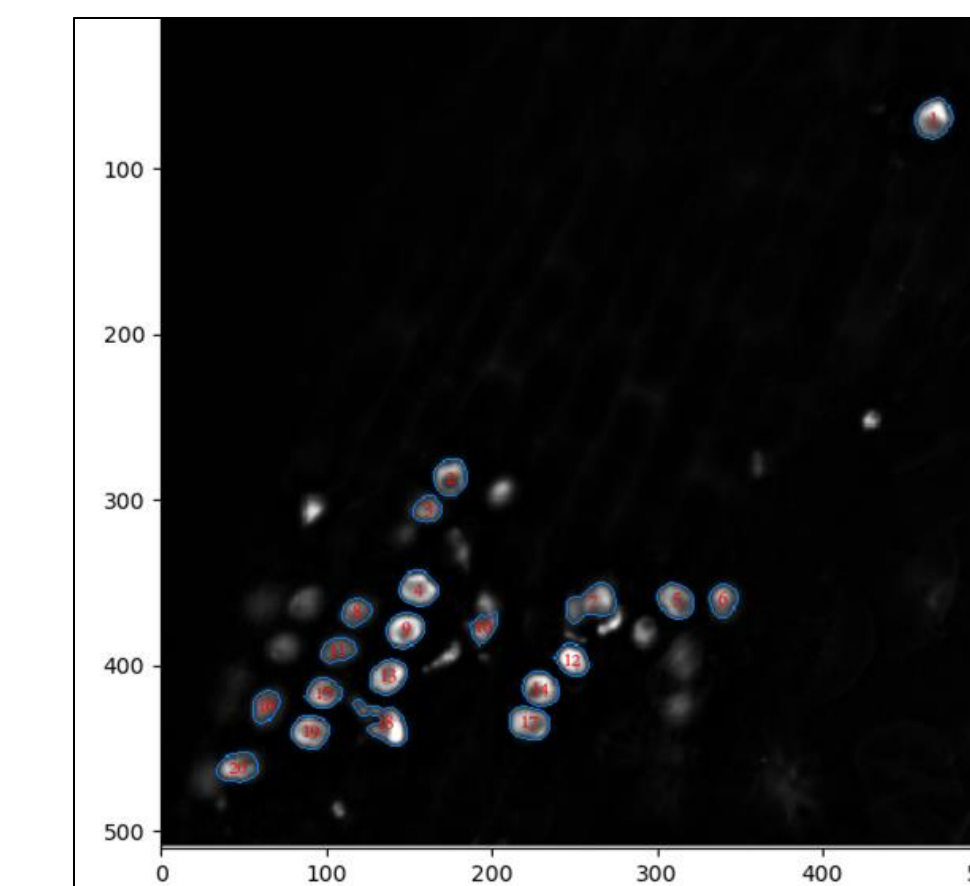
Results and Methods

The root tips were cut longitudinally using a dissection microscope, then treated with Click-it EDU and marked with DAPI and FITC fluorescence to be analyzed. Data analysis included counting and identifying cells between DAPI and FITC channels for any DNA synthesis within mitotic cells. The photos below contain both S-phase (green) and other stages of the cell cycle (no mitotic images in this photo). DAPI stains total DNA. EdU-A488 fluoresces green and marks newly synthesized DNA.
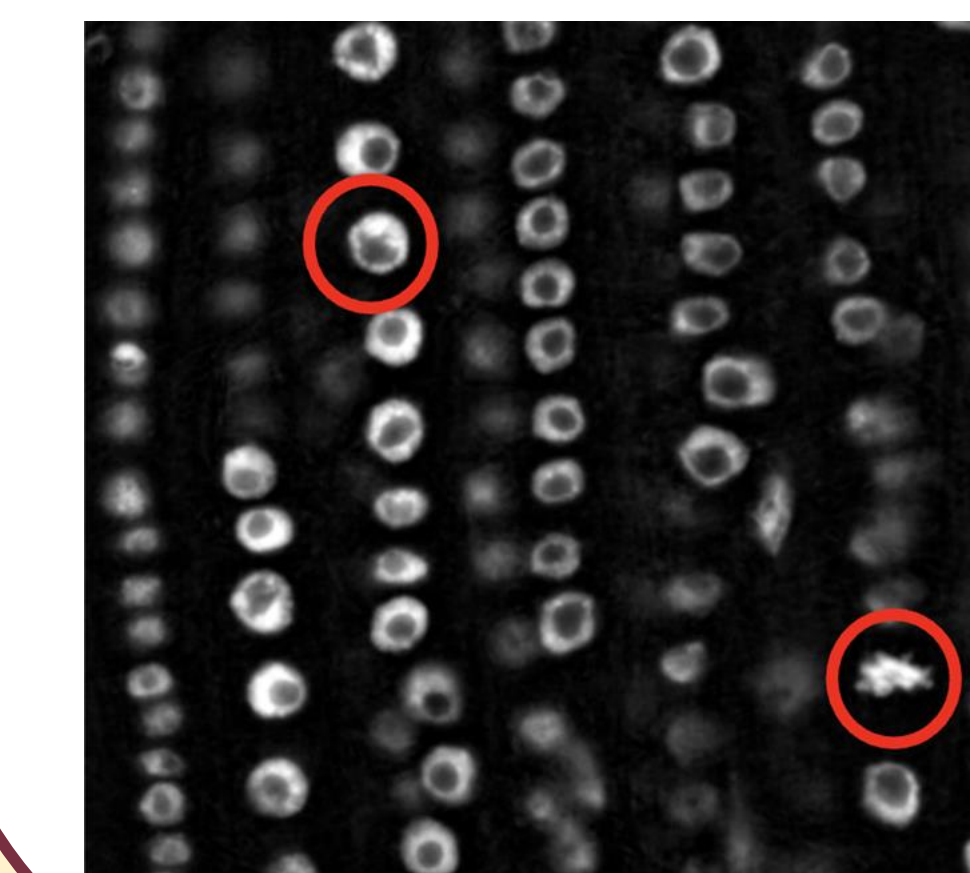
The images show both wavelengths (left) or DAPI only (right).

DAPI (total DNA)
FITC/EdU (DNA synthesis)

DAPI (total DNA)

Root Tip Nuclei Analysis

This image shows the capabilities of *CellProfiler*; a program designed to aid in nuclei recognition and counts. This program allowed for cataloging of nuclei to help aid in the data collection. https://cellprofiler.org/

This image show root tip nuclei. The red circle denotes clear examples of mitotic division, including nuclei in prophase (left circle) and metaphase (right circle).

Acknowledgements

Conclusion

- Over 1,000 cells selected for analysis
- Inspected over 350 nuclei for evidence of MiDAS
- So far, zero clear examples of MiDAS observed
- The frequency of MiDAS is less than 0.29% (< 1/355)

Future Plans:
- Continue to inspect for MiDAS for a total of 1,000
- Explore semi-automated image analysis software to aid in cell count

References

1. Bass, Hank W et al. "Defining multiple, distinct, and shared spatiotemporal patterns of DNA replication and endoreduplication from 3D image analysis of developing maize (*Zea mays* L.) root tip nuclei." *Plant Molecular Biology* vol. 89,4-5 (2015): 339-51. doi:10.1007/s11103-015-0364-4

2. Franchet, C.; Hoffmann, J.-S. When RAD52 Allows Mitosis to Accept Unscheduled DNA Synthesis. *Cancers* **2020**, *12*, 26. https://doi.org/10.3390/cancers12010026

3. "Cell Cycle." *Wikipedia*, Wikimedia Foundation, 27 Oct. 2023, en.wikipedia.org/wiki/Cell_cycle. Accessed 09 Nov. 2023.

MiDAS DNA Synthesis

Normal DNA Synthesis

Very Early S (green) DNA Synthesis

Typical very early S replication pattern (Green) scattered across the nucleus

Atypical very early S pattern, looks like the knobs seen in late S replication patterns.