Evaluation of microscopy techniques may help scientists to better understand ancient plants

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In a paper published in *PLoS ONE*, scientists at the University of Illinois released their findings on what microscopy techniques are needed to identify the shape and texture of pollen grains. Understanding pollen morphology is important to classifying ancient vegetation.

Because pollen morphologies often align quite closely to taxonomic groupings, understanding the appearance of ancient pollen allows scientists to better understand prehistoric flora in the context of modern-day ancestors.

The team's research, led by Surangi Punyasena and Mayandi Sivaguru of the Institute for Genomic Biology, focused on comparing how several reflected and transmitted light microscopy techniques image individual pollen grains. By choosing three pollen samples of diverse grain size and texture, they were better able to understand how each technique functions in different situations.

"The accuracy and consistency of pollen analysis relies on our ability to see as much morphology as possible," explains Punyasena. "Images like those produced by this research are the foundation of my lab's quantitative morphological work - work that we hope will allow us to break through the many taxonomic limitations of pollen identification in the very near future."

"The results of this paper have encouraged us to revisit some longstanding classification problems in vegetation science," says Luke Mander, co-author. "For example, there are around 11,000 species of grass on the planet today, but these species produce pollen that looks extraordinarily similar. This means that it is extremely difficult to use fossil pollen grains to reconstruct the diversification history and evolution of this major plant group. Advances in imaging technology, such as the super-resolution technique used in our paper, allow us to image morphological features less than 200 nanometers in size using light."

"In the future, we hope these morphological features might be used to study the diversity and composition of ancient grasslands," he adds.

The team found that no reflected or transmitted light technique provided a completely adequate image. While reflected light techniques capture pollen shape effectively, they remain unable to resolve fine surface textures. Transmitted light techniques, however, are able to resolve even extremely fine textures, but give poor idea of grain shape.

They conclude that to construct an accurate image through conventional microscopy techniques, it is best to use a combination of both transmitted and reflected light imaging.

"Most pollen analysis is currently completed using transmitted light. This paper demonstrates how much more can be seen - and consequently analyzed - with alternative imaging techniques. This paper provides a much needed comparison of the capabilities of existing technologies that should be incorporated into mainstream pollen analysis," says Punyasena.

"Some of the most exciting results of this work are the images that were produced using super-resolution structured illumination (SR-SIM). There

is little existing research on the use of SR-SIM with auto-fluorescent material like pollen," she adds. "Our demonstration of its ability to capture morphology below the diffraction limit of light strongly suggests that SR-SIM is a viable alternative to electron microscopy (EM) and may represent the future of pollen analysis."

More information: "Capturing the Surface Texture and Shape of Pollen: A Comparison of Microscopy Techniques," Mayandi Sivaguru, Luke Mander, Glenn Fried, Surangi W. Punyasena, dx.plos.org/10.1371/journal.pone.0039129

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