

Stomatal anatomy influences leaf-level physiology traits including conductance to water vapor. Stomatal anatomy can be visualized in situ by microscopy, but the difficulty of regulating the atmospheric environment of a microscope stage means that the conditions under which imaging is done are rarely physiologically relevant. Alternatively, leaf gas exchange instruments that measure gas fluxes reflect stomatal anatomical characteristics in aggregate, but the relative strengths of anatomical traits to control water use (e.g., size vs density) cannot be firmly established. To reconcile the microscopic stomatal characteristics with leaf-level gas exchange, we describe a tool that combines laser scanning confocal microscopy, gas exchange instruments, and machine-learning image analysis to simultaneously observe anatomical characteristics of many (>40) stomata alongside leaf-level traits like photosynthesis, transpiration, and stomatal conductance.

We demonstrate how the tool has the resolution capable of quantifying aperture sizes and variability in maize (*Zea mays*) leaves under five steady-state light/ $p\text{CO}_2$  treatments while tightly controlling other environmental variables like relative humidity and temperature.

This study demonstrated a technical advance that can reconcile variation in stomatal aperture and stomatal density with variation in operational stomatal conductance ( $g_{sw-gasex}$ ). A model used to calculate stomatal conductance from measured apertures and stomatal density accurately matched stomatal conductance measured by gas exchange.

This technical advancement will provide insight on how stomatal anatomy and function trade off to influence stomatal conductance and leaf-level water use efficiency. The integration of a confocal microscope with a leaf gas exchange system is significant because it has the potential to explore long-standing unknowns about stomatal structure-function relationships in eco-physiology and stomatal behavior, which can aid efforts to engineer stomatal traits for improved WUE in agriculture.

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