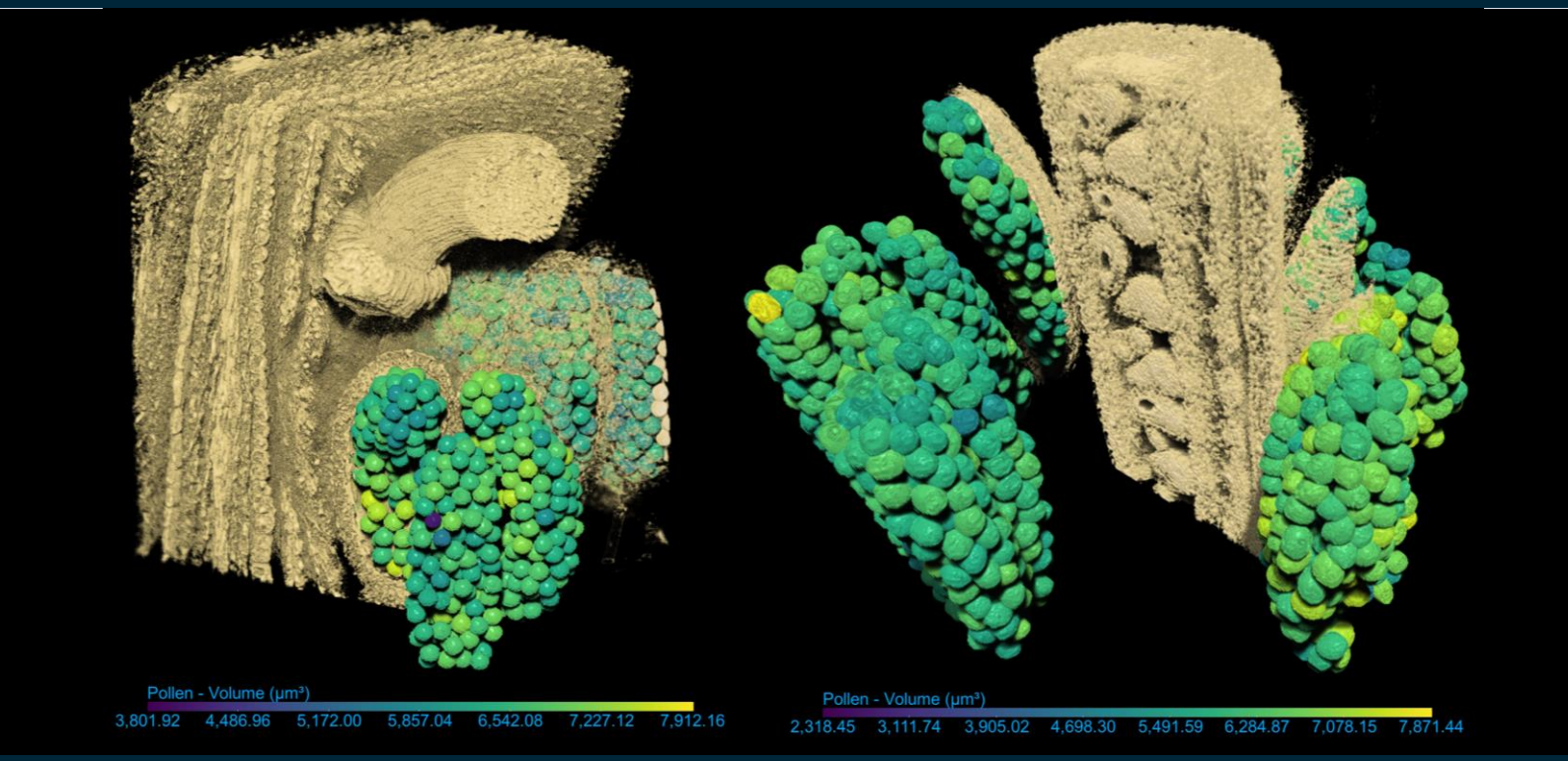
Application Note



Cellpose-SAM 3D and Fast Split: Measuring pollen grains in flowers

Flowers contain complex and delicate internal structures that can be imaged in 3D using X-ray microscopy (XRM). The anthers contain pollen grains whose shape, size, and numerical distribution in 3D space can provide fundamental information about pollination and how plants make grains, seeds, and fruits. The clustered and connected nature of the pollen grains makes digitally separating and quantifying each one complicated. Here, we demonstrate two methods in Dragonfly 3D World to overcome this challenge. The Cellpose-SAM 3D and Fast Split features allow for quick separation and analysis of touching objects in 3D datasets. Individual pollen grains, or other clustered objects, can now be easily segmented, separated, and quantified.

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Keith Duncan (Donald Danforth Plant Science Center)

dragonfly

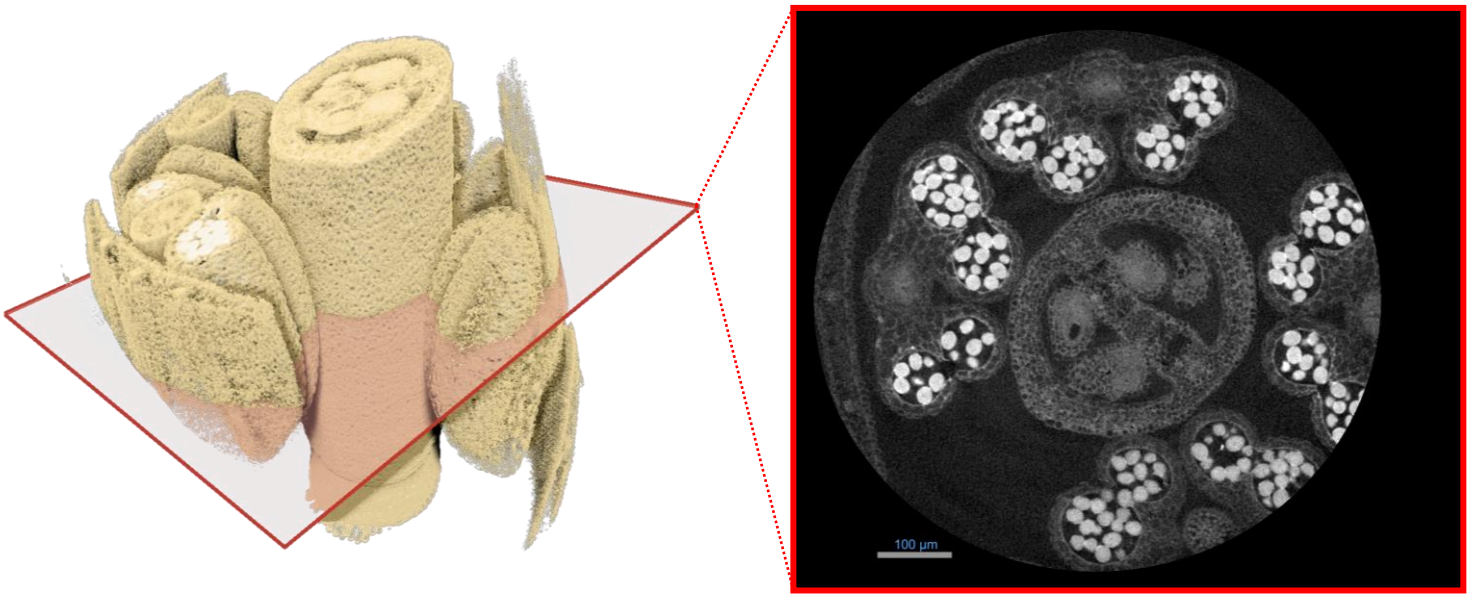
Requirements

A high-quality XRM scan of the flower is required, although conventional X-ray tomography of sufficient resolution can also work on larger samples. To ensure proper sampling, the pollen grains' average diameter should be at least 4 to 5 times larger than the voxel length. Most common industrial XRM file formats can be analyzed using Dragonfly 3D World with a computer meeting system requirements. The Cellpose-SAM 3D and Fast Split features used here are available starting in versions 2025.1 and 2027.1, respectively.

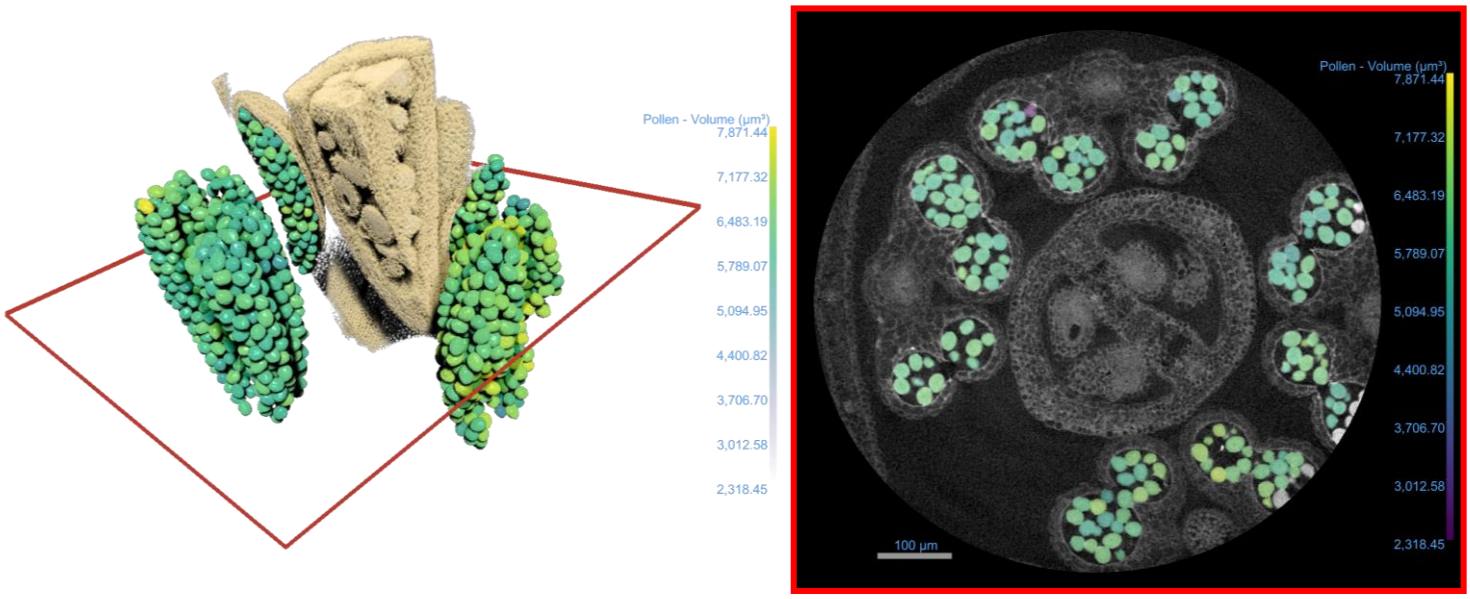
The XRM data in this Application Note were collected by Keith Duncan at the Donald Danforth Plant Science Center using a Zeiss Xradia 520 Versa with a 20X objective lens, resulting in a reconstructed voxel edge length of 0.8 µm for both scans. Zeiss' proprietary .TXM file format can be directly read into the "Zeiss Edition" of Dragonfly 3D World.

Outputs

- 2D cross-sectional slices and 3D volume rendering of the flower structures.
- Measurements of shape, size, and location for each pollen grain color-coded by value



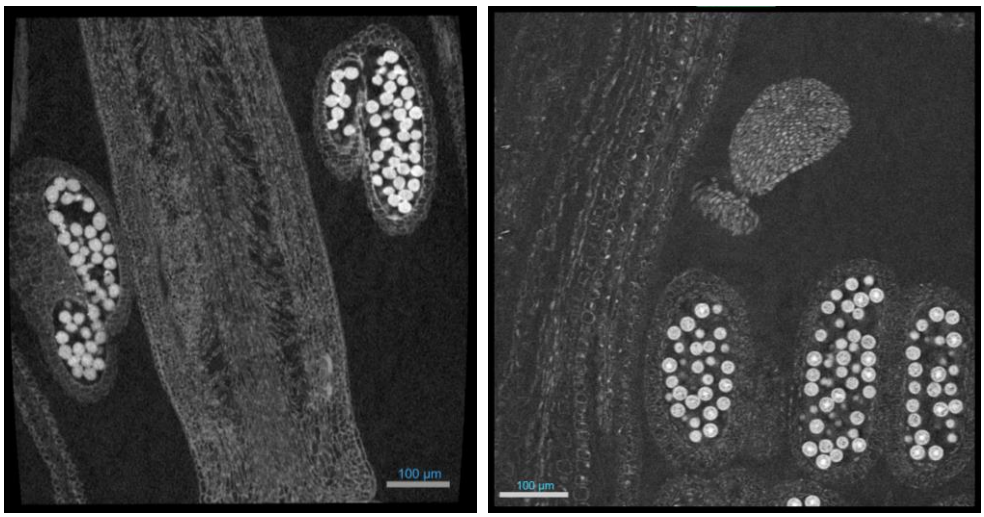
(Left) 3D volume rendering of Arabidopsis flower with red plane showing location of grayscale XRM slice (right). Pollen grains are the clustered ellipsoidal objects with brighter grayscale values. 3D volume dimensions are approximately 750 µm x 750 µm x 770 µm



(Left) Clipped 3D volume rendering of Arabidopsis flower with individual pollen grains segmented and separated using Cellpose-SAM 3D and color-coded by grain volume. The red plane shows the location of the grayscale XRM slice (right) with measured pollen grains overlain. 3D volume dimensions are approximately 750 µm x 750 µm x 770 µm

From visualization to quantification

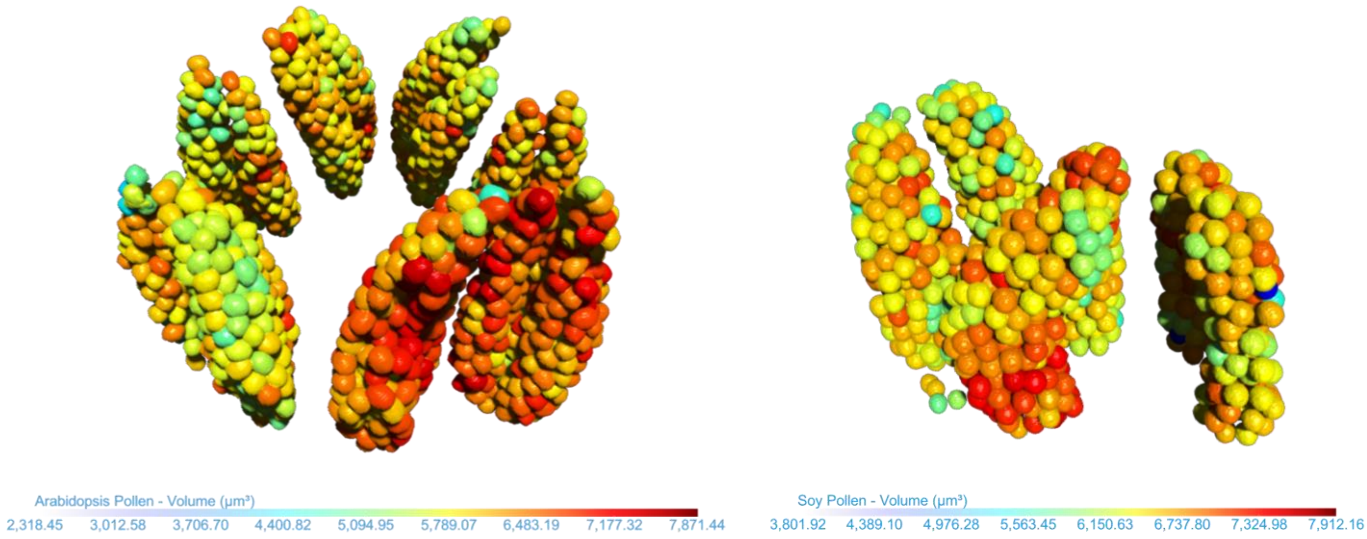
The XRM data show the highly-detailed internal structures of the Arabidopsis and soy flowers. Pollen grains are easily identifiable as the clustered, ellipsoidal objects with higher grayscale values. Digitally isolating the voxels that belong to pollen and assigning them to a region-of-interest (ROI), a process called "segmentation", is relatively straightforward because of their distinct grayscale values. However, measuring each pollen grain requires digitally separating them, which is complicated due to their highly-connected nature. Here, we will use both the Cellpose-SAM 3D and Fast Split tools in Dragonfly 3D World to digitally separate the pollen grains so that each one can be quantified.



2D XRM slices of the Arabidopsis flower (left) and soy flower (right). Pollen grains are ellipsoidal objects with brighter grayscale values.

Cellpose-SAM 3D is a tool created by Pachitariu et al., (2025)[1] as a one-click solution for general purpose instance segmentation of cells and other similarly shaped objects. We applied Cellpose-SAM 3D using the default parameters (Flow threshold = 0.4 and Object Probability = 0) and it took approximately 1 hour to complete for each sample.

Dragonfly 3D World's Fast Split tool works on existing ROIs and separates touching objects by eroding the class a user-defined number of layers and preserving cores with a user-defined number of layers. Cores are then dilated to re-fill the original ROI volume. For this workflow, pollen grains were segmented into an ROI using a 2.5D U-Net Deep Learning model trained with the Segmentation Wizard. A new Deep Learning model was trained for each flower. Pollen grain ROIs were separated with the Fast Split tool using the default parameters (How many layers to erode = 20 and How many layers to reserve = 4 with Non-homogeneous shapes selected). The Fast Split tool took approximately 30 seconds to complete for each sample.



3D volume renderings showing the results of Cellpose-SAM 3D for the Arabidopsis flower pollen (left) and Fast Split for the soy flower pollen (right) color coded by pollen grain volume.

Cellpose-SAM 3D and Fast Split both produce multi-ROIs where each pollen grain is a unique class that can be measured by right-clicking on the multi-ROI and selecting "Measurements and Scalar Values" then "Compute Measurements". Pollen grains touching and truncated by the dataset boundaries were removed using the "Refine Multi-ROI" tools then selecting "Remove objects that touch another ROI or Multi-ROI". A 1-voxel thick boundary ROI was created for each dataset and any pollen grains touching it were discarded.

[1]*Pachitariu, M., Rariden, M. and Stringer, C., 2025. Cellpose-SAM: superhuman generalization for cellular segmentation. BioRxiv, pp.2025-04.*

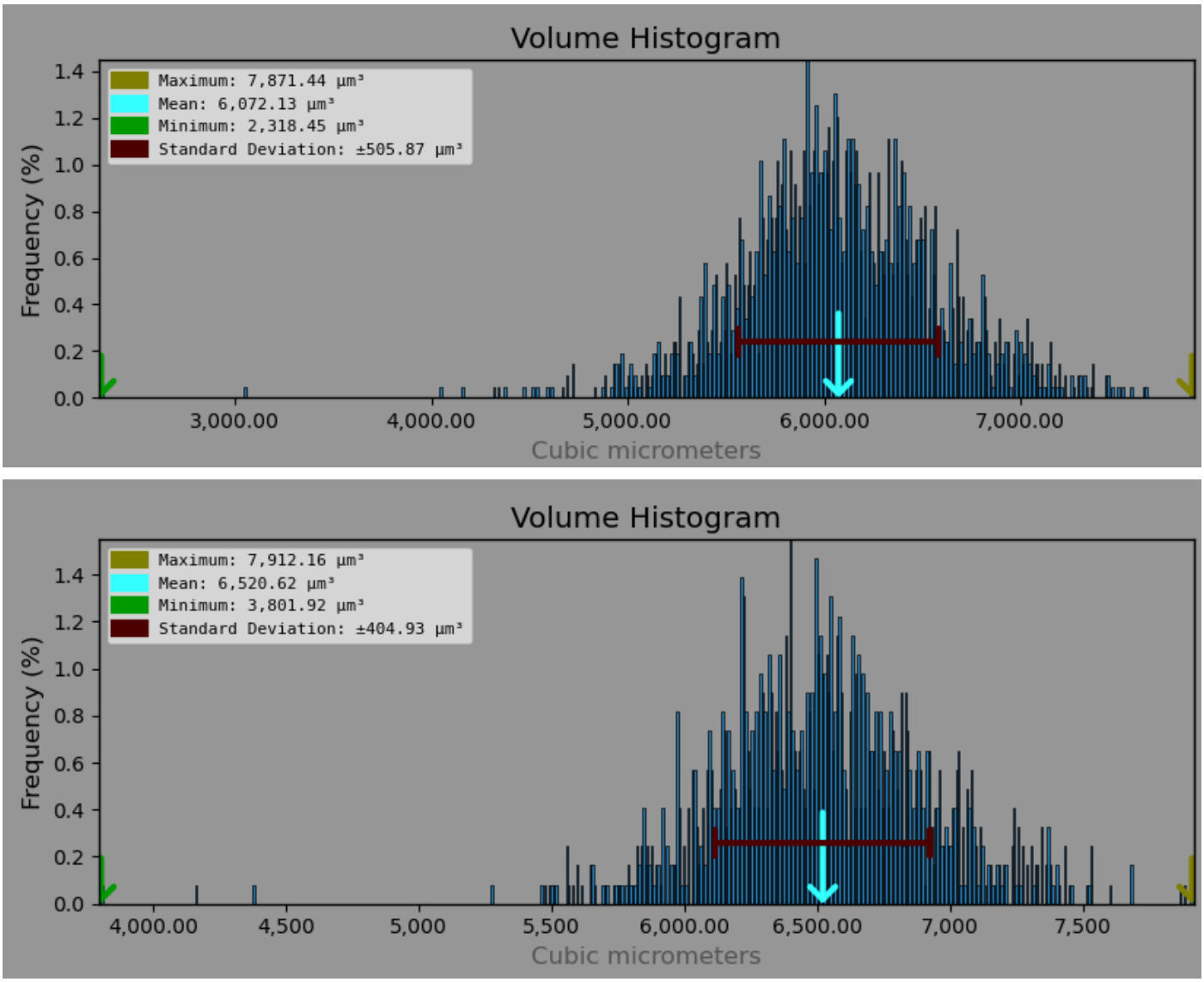
A total of 1,226 pollen grains were measured within the soy flower using the Cellpose-SAM 3D method and 1,233 were measured with Fast Split. Cellpose-SAM 3D and Fast Split measured 2,073 and 2,051 pollen grains, respectively, in the Arabidopsis flower. The tables below contain statistical information describing the pollen grain populations in the soy and Arabidopsis flowers. Volume, sphericity, and Feret max dimensions from Cellpose-SAM 3D and Fast Split methods are compared. The percent differences between the Cellpose-SAM 3D and Fast Split methods for average volume, Feret max, and sphericity are 6.3% or less across both flower types. The average volume and Feret max from Cellpose-SAM 3D are larger than the Fast Split values for the soy flower but smaller for the Arabidopsis flower. Average sphericity with Cellpose-SAM 3D is lower in the soy flower but higher in the Arabidopsis flower.

Soy flower									
	Volume (µm^3)			Feret Max (µm)			Sphericity		
	Cellpose	Fast Split	% difference	Cellpose	Fast Split	% difference	Cellpose	Fast Split	% difference
Mean	6521	6190	5.2	25.8	24.7	4.2	0.97	0.99	2.2
Standard Deviation	405	399	1.4	0.7	0.6	13.4	0.01	0.00	120.7
Minimum	3802	3595	5.6	21.8	21.0	3.6	0.85	0.97	13.7
Maximum	7912	7558	4.6	28.2	28.6	1.4	1.00	1.00	0.5
Number	1226	1233	0.6						

Arabidopsis flower									
	Volume (µm^3)			Feret Max (µm)			Sphericity		
	Cellpose	Fast Split	% difference	Cellpose	Fast Split	% difference	Cellpose	Fast Split	% difference
Mean	6072	6467	6.3	27.7	28.9	4.4	0.96	0.93	3.2
Standard Deviation	506	551	8.5	1.3	1.9	36.6	0.01	0.03	100.0
Minimum	2318	2548	9.4	20.5	24.1	16.2	0.82	0.61	29.4
Maximum	7871	8863	11.9	34.1	44.4	26.3	0.99	0.97	2.0
Number	2073	2051	1.1						

Both methods produce comparable results with subtle differences in the number, average size, and average shape of pollen grains. Cellpose-SAM 3D is a one-click solution with few tunable parameters, whereas the Fast Split tool is based on morphological operations applied to a user-created ROI. For the soy flower, the pollen grain ROI used for Fast Split was slightly under-segmented compared to the Cellpose-SAM 3D results and over-segmented for the Arabidopsis flower. Both methods produce reliable and comparable results, but we urge the analyst to carefully evaluate the quality of the ROI segmentation if using Fast Split and also assess the results of the Cellpose-SAM 3D method.

With minimal manual input and post-processing, the size, shape, and spatial distributions of pollen grain populations in both flowers can now be investigated and quantified in 3D.



Histograms showing the distribution of pollen grain volume for the Arabidopsis flower using Cellpose-SAM 3D (top) and soy flower using Cellpose-SAM 3D (bottom). Statistics for each population are also reported.

Benefits

Extracting meaningful 3D measurements is often a key objective of X-ray tomography investigations, but achieving this can be challenging. Fortunately, Dragonfly 3D World provides multiple easy-to-use workflows for segmenting, separating, and measuring your objects-of-interest. We show that Dragonfly 3D World's Cellpose-SAM 3D and Fast Split tools are simple and accurate for measuring individual pollen grains in different flowers.

For a video workflow demonstration of the above case:
https://youtu.be/1xD2fr1Wc0k

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