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THREE-DIMENSIONAL PRINTING OF MITES IMAGED FROM CONFOCAL MICROSCOPY

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A major limitation of 3D printing is the difficulty in creating morphologically accurate models of complex biological specimens that would otherwise require a scientific illustrator months to produce. Here we present a new, rapid method for generating highly detailed morphological accurate models of mites, representing the first application combining confocal laser scanning microscopy with 3D printing to generate large physical models of mites. For this study, we examined eight mites, representing various genera of Acari; including *Cheyletus* sp., *Neoseiulus* sp., *Phyllocoptes* sp., *Brevipalpus* sp., *Varroa* sp., *Tropilaelaps* sp., *Trouessartia* sp., *Daidalotarsonemus* sp.. These mites ranged in size from 300nm to 3mm and exhibited a variety of distinct morphologies. Mites were mounted between two coverslips in glycerin and supported by spacers of equal depth to prevent the coverslip from distorting the mites. Three-dimensional volume data of the entire mite was acquired and captured utilizing a Zeiss LSM710 confocal laser scanning microscopy system attached to a Zeiss Axio Observer inverted microscope with a 40x 1.2 NA Plan-Apochromat objective. Three excitation wavelengths at 405nm (DAPI), 488nm (GFP) and 561nm (DsRed) were used with a pin hole of 33µm, and a broad filter set to capture all emissions from 410nm to 704nm wavelengths. Zeiss Zen 2012 Pro software was utilized to obtain 20-150 z-stack images of the dorsal and ventral side of the mites. FIJI/ImageJ2 was used to convert CZI files into OBJ files, and filters in MeshLab 2016 were used to remove any unwanted background noise or artifacts. AutoDesk Meshmixer was used to combine the dorsal and ventral halves of the mite and further refine the model to ensure all appendages were positioned for optimal printing. Models were cut into two or more pieces to allow the appendages to be directed upwards at an angle of 45 degrees or more for optimal printing. The STL files are then imported into Ultimaker Cura 3.1 and various settings are adjusted as needed to increase the thickness of the outer shell, fill in unwanted holes, add supports and adjust the density of the infill. The model is then sliced, converted into G-code and printed with PLA plastic with an Ultimaker2 3D printer. Depending on the size and complexity of the model the 3D printing process could take from 24 to 72 hours to complete. After printing, support structures are detached from the model and the halves of the mite are glued together using cyanoacrylate glue. These models have numerous benefits and can be used as training aid for students, inspectors at ports of entry and experienced researchers to directly visualize these microscopic organisms.

Keywords: 3D printing, confocal, Acari, mite models.