Supplementary Material for "VolumePeeler: A novel FIJI plugin for geometric tissue peeling to improve visualization and quantification of 3D image stacks"

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Contents

1 Supplementary Note 1. Image acquisition.

Annual killifish embryos (*Austrolebias nigripinnis*) expressing GAP43-EGFP were mounted in a custom-designed chamber filled with dissolved 1% low-melting-point agarose in ERM rearing medium and placed on the microscope stage, between 48 and 72 hours post-fertilization. Zebrafish (*Danio rerio)* wild-type AB embryos injected with 50 pg of gap43-RFP mRNA were mounted in 0.5% low melting point agarose in a custom-designed chamber at 50% epiboly stage. *In vivo* confocal microscopy was performed in a Leica TCS LSI microscope (Leica Microsystems, Germany) using a 5x objective for killifish and zebrafish embryo. *Marchantia* of expressing GFP-MpTUB1 and Lit6b-mCitrin transgenic lines were mounted in a custom chamber with Gambor B5 agar media. Microscopy was performed using a Leica SP8 upright microscope (Leica Microsystems, Germany) equipped with a 40x objective.

2 Supplementary Note 2. Spline projection User Interface.

The interface for spline projection has several features that allow for an efficient and user-friendly experience, as shown in Supplementary Figure 3. To summarize, the interface allows: 3x3, 4x4, or 5x5 control points and channel selection, save/load z-values, repeat z-values, interpolate z-values, selection of peeling behavior (upper, lower, band), and visualization options (preview, process, adjust brightness).
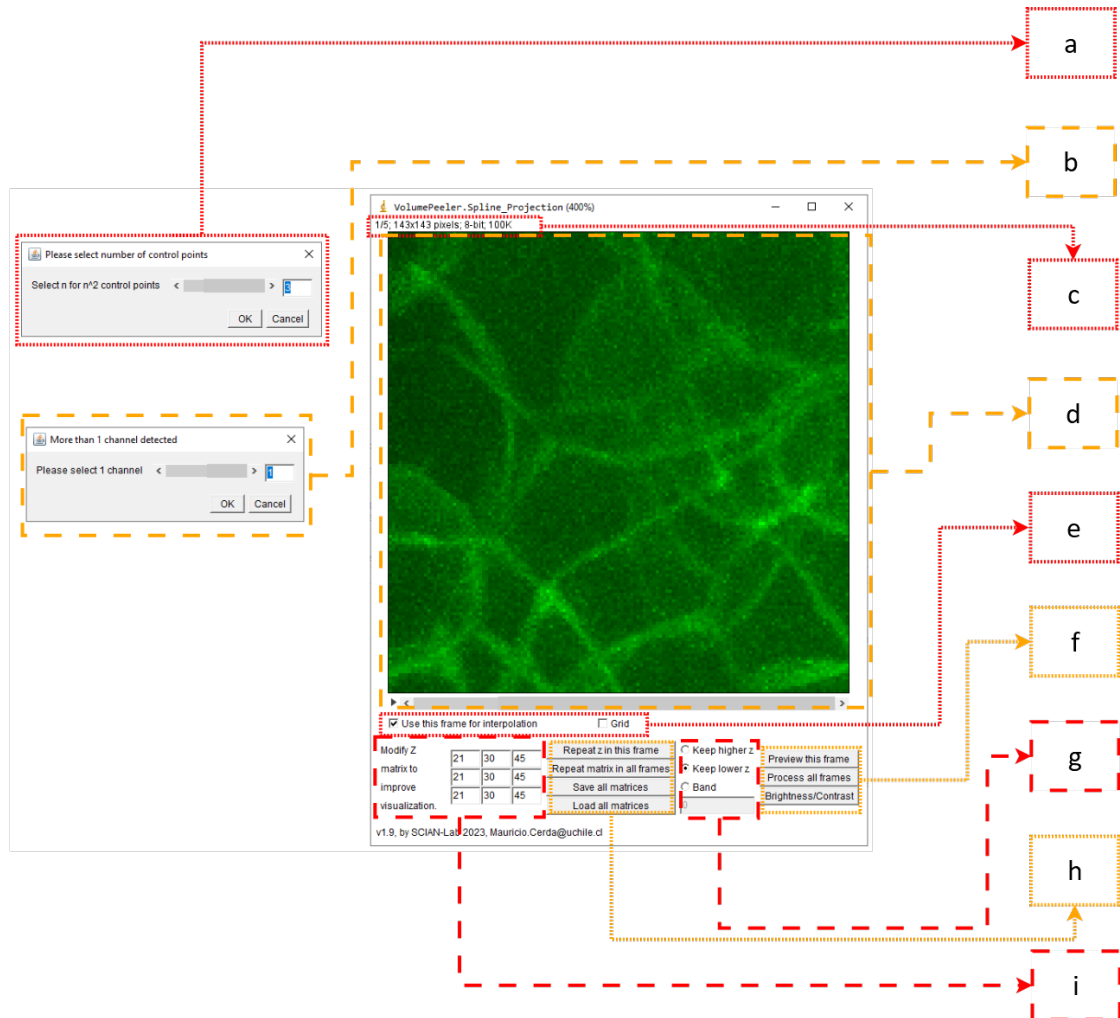
3   Supplementary Figures.
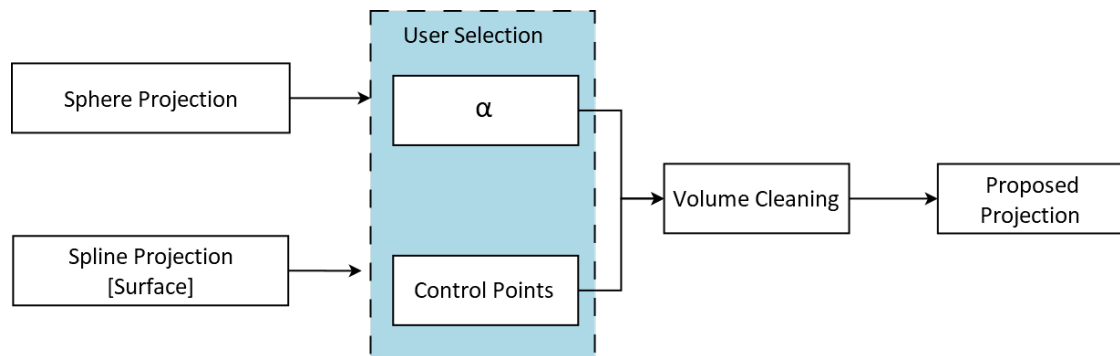
Supplementary Figure 1: Block diagram of the VolumePeeler approach.
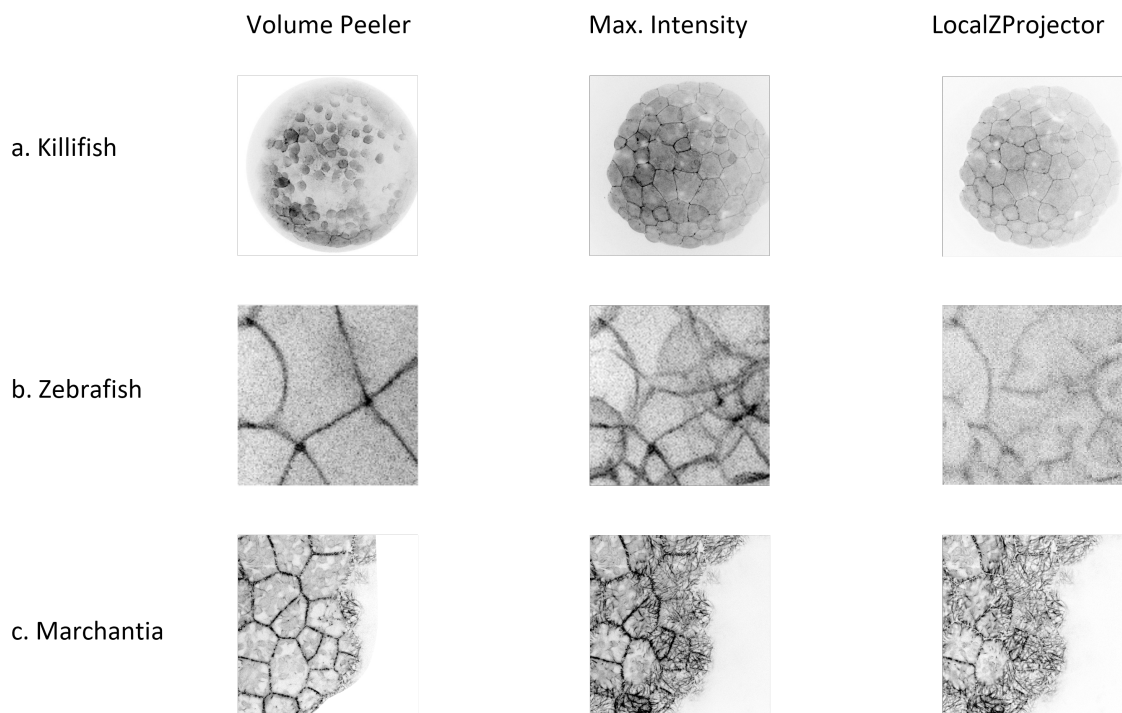
Supplementary Figure 2: Visual comparison of VolumePeeler (proposed approach), Maximum Intensity Projection, and LocalZProjector, for three biological model examples: **a** killifish, **b** zebrafish, **c** *Marchantia*.

Supplementary Figure 3: Spline projection user interface. **a** Selector for number of control points. **b** Selector of working channel. **c** Stack information label. **d** Preview window. **e** Frame selection option for interpolation and grid lines (on/off) control. **f** Preview, process, and brightness/contrast control buttons. **g** Volume peeling behavior selector. **h** Shortcut buttons for cloning and saving/loading values and matrices. **i** Depth matrix control points to define the reference surface.