Labeling of Dense Light Data in Amira 3D 2022.1 Classic Segmentation Workroom

CellMap Project

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ABSTRACT

This protocol is optimized for densely labeling high dynamic range light data subvolumes requiring unique identification of each expressed structure.

Segmentation Summary

1. Set up Amira project and segmentation.
2. Segment slices across a single cell, interpolate, and add to a unique material.
3. Clean working material through exterior material interpolation across a second plane and universal smoothing.
4. Confirm segmentation boundaries in relation to cell signal, lock current material, and create a new working material.

GUIDELINES

Note: While Amira 3D 2022.1 Segmentation+ workroom supports an arbitrary number of unique label IDs, the Classic Segmentation workroom tools, which only support 8-bit label data, are better suited for this application. Multiple label fields can be merged after subvolume segmentation.

MATERIALS

Amira 3D 2022.1
Amira Project and Segmentation Setup

1. After opening a new Amira project, select File → Open Data, or click and drag the raw data tiff to the empty space under the Project tab. Confirm coordinates and voxel size in the Image Read Parameters window.

   For higher label resolution: Select the arrow of the raw data object in the Project tab. In the window search bar, type ‘resample’. Select the Resample object. With the Resample object selected, adjust Voxel Size: under Properties to half of the original resolution value. Select Apply, and select the generated raw data object as the new working raw data object.

   Select the Segmentation tab to automatically generate a label field. Confirm Image: dropdown under Segmentation Editor is the raw data. Under Materials, ensure the Exterior (Not Assigned) material remains unlocked (1). The existing Inside material may be renamed and used as the first cell segmentation material.

   Note: Once material 256 is reached within a single label field, select New across Label Field: to continue segmentations in a new label field.

   In order to track previously segmented cells in a new label field, select Exterior (Not Assigned) material in the previous label field, click the physical exterior space in the 2D viewer, and select Shift+I to inverse 3D exterior material selection so that all cell materials are now selected. Under Label Field: dropdown, select the new label field and add current selection to the first material. All older segmentations should now be under a single locked material in the new label field.
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Amira User Interface Icon Legend; additional guidance for Amira keystrokes and tools can be found in Amira's main toolbar under Help → User's Guide

### Segment, Interpolate, Add

#### 2
With the first material highlighted under the **Materials** section, select the brush (2) under the **Selection** section. Above the **2D Viewer**, select the XY plane icon (3); initial segmentation and interpolations should be performed down the imaged plane.

#### 3
After adjusting **2D viewer** zoom settings and brush size under the brush (2) window, paint in a single slice of a cell, using either a large brush size or 'F' to fill in the cell slice after painting a signal-inclusive boundary.

#### 4
Scroll ~3-4 slices (~5-7 slices if raw data was resampled) and repeat the painting process from end to end of the cell.

#### 5
Select **Ctrl+I** to interpolate across all painted slices, followed by **Ctrl+W**, which performs a more radial interpolation, eliminating the requirement for multiple interpolations across multiple
planes. With the interpolations selected, select **Selection → Smooth → All Slices**. Add ('A' or +) the selection to the working material.

### Cleaning Interpolations and Universal Smoothing

6. Select the XZ plane icon (4) and locate the working material. Using the **Exterior (Not Assigned)** material, paint and interpolate across any false positive segmentations, one area at a time. Add ('A' or +) the selection to the **Exterior (Not Assigned)** material, which will remove any overlapping, unlocked working material. Continue to ‘clean’ the working material using this process until false positives from XY plane interpolations are removed.

*Note: Interpolated selections can also be removed from the working material by first highlighting the working material under the Materials section, and subtracting ('S' or -) the selection.*

7. With no selection, select **Segmentation → Smooth labels**. Under the **Smooth Labels** window, adjust the **Size:** to ~2-4, and change **Mode:** to **3D volume**. While watching the working material in the **2D** and **3D Viewers**, select **Apply** until the working material is smoothed and inclusive of signal boundaries.

8. If smoothing results in excessive merging with bordering labels, select locked, bordering label, grow the selected label using **Grow Selection** (5) under the **Selection** section. Then, highlight the **Exterior (Not Assigned)** material, and add ('A' or +) the extended selection. Only the portion of the selection that is outside of a locked material will be added to the **Exterior (Not Assigned)** material.

### Confirmation and Locking

9. After confirming working material has included sufficient cell boundary signal in all three planes, lock (6) the working material.

10. Under the **Materials** section, select the **Add** button to create a new working material. After a working material is finalized, it should always be locked before creating and working on a new cell segmentation. While selection-based tools will not impact unlocked materials, universal, volume-based tools will modify any unlocked materials.

11. Repeat earlier steps for each new cell/material, ensuring all non-working materials are locked at all times. See **Amira Project and Segmentation Setup** section for raw data objects that require more than 256 unique ID labels.
Export label fields for merging using Python (see Software link) or direct use by selecting the **Project** tab, selecting the arrow of the label field object, and selecting the **Export Data As** icon.

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<td>David Ackerman</td>
<td><a href="https://gist.github.com/jonesa3/259a64f44eaeef06d2ad1e570d3cc5865">https://gist.github.com/jonesa3/259a64f44eaeef06d2ad1e570d3cc5865</a></td>
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