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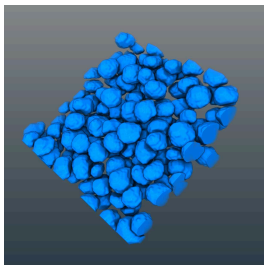
Labeling of Dense Light Data in Amira 3D 2022.1 Classic Segmentation Workroom



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Alyson Petruncio[1], CellMap Project Team[2]

[1]HHMI - Janelia; [2]HHMI - Janelia Research Campus

Alyson Petruncio: https://orcid.org/0000-0002-1093-6182;
CellMap Project Team: https://www.janelia.org/project-team/cellmap

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Protocol status: In development
We are still developing and optimizing this protocol

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Keywords: light annotation, Amira, segmentation, fluorescence annotation, manual segmentation, manual annotation, dense light data in amira 3d, dense light data, light data subvolume, high dynamic range light data subvolume, classic segmentation workroom this protocol, classic segmentation workroom, segmentation summary, segmentation boundary, segmentation, segment slice, segmentation boundaries in relation, amira 3d, labeling high dynamic range, amira project, exterior material interpolation, universal smoothing

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

Troubleshooting

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.

	Section	Function	Icon
1	Materials	Unlock	
2	Selection	Brush	
3	2D Viewer	View XY	
4	2D Viewer	View XZ	
5	Selection	Grow Selection	
6	Materials	Lock	
7	Project Tab	Export Data As	

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.

12 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 (*7*).

Software

combine_labels.py	NAME
David Ackerman	DEVELOPER
https://gist.github.com/jonesa3/259a64f44eaef06d2ad1e570d3cc5865	SOURCE LINK