

Scalable Alignment of Electron Microscope Image Sections

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■ Goals

- Scalable alignment of 3D Electron Microscope sections of mouse brain.
- Integration with CAJAL-3D API for easy image retrieval and upload from database.

■ Difficulties

- Approach must be scalable.
- $1024 \times 1024 \times 2000$ in the smallest data set $\approx 2B$ voxels.
- Most datasets occupy TB of space; infeasible to align all at once.

■ General Method

- 1 Compute transformations for alignment between adjacent pairs of images using cross-correlation.
- 2 Globally align entire image cube using pairwise transformation parameters.

Microscope



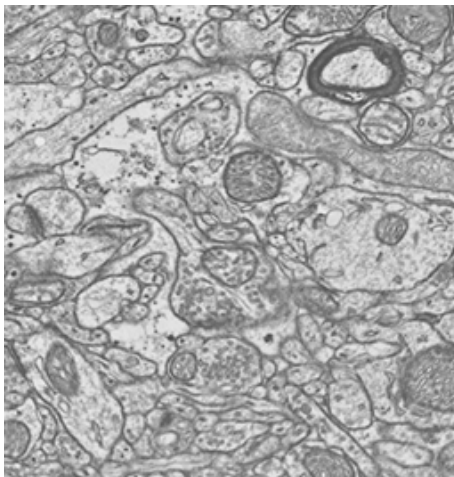


Figure 2 : Dataset

Pairwise Alignment

Overview

Objective: Compute transformations to align adjacent pairs of images.

- 1 Compute pairwise transformation parameters.
- 2 Improve rotation parameter through error minimization.
- 3 Refine transformations using image data outside pairwise images to minimize error.

Pairwise Alignment

Compute Pairwise Transformation Parameters

Objective: Determine transformations to align image pair.

For each pair of images:

- 1 Apply median filtering and histogram equalization.
- 2 Take Discrete Fourier Transform, apply high-pass filter, and resample in log-polar coordinates.
- 3 Find best ρ , θ by correlation and max picking.
- 4 Rotate image, then correlate to find best translation parameters.
- 5 Use Support Vector Machine (SVM) to identify peak in cross-correlation of image pair.
- 6 Save transformation parameters.

Pairwise Alignment

Peak Identification Illustration

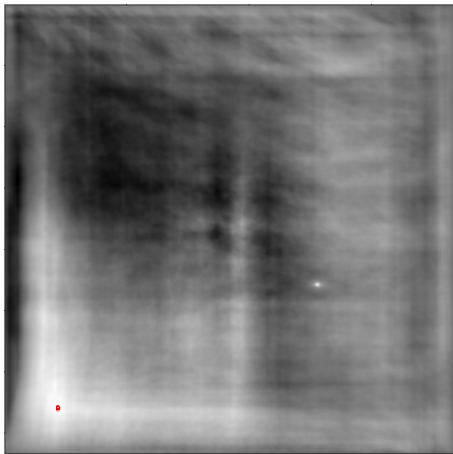


Figure 3 : Motivation for Peak Identification

Pairwise Alignment

Peak Identification

Objective: Determine peaks in cross-correlation of two adjacent images that correspond to translations for correct alignment.

- Support Vector Machine (SVM)

- 1 Train SVM classifier with peak features from aligned images (ground truth).
- 2 Partition cross-correlation of images into 9 equal parts.
- 3 Find point of maximum intensity in each partition.
- 4 Sort coordinates from greatest to least maximum intensity.
- 5 Classify each potential peak until a peak is found.
- 6 If no potential peaks are classified as peaks, then no peaks detected.

- Other Attempted Methods:

- Choose maximum values.
- Correlate pairwise cross-correlation with normal distribution.

Pairwise Alignment

Improve Rotation Parameter

Objective: Evaluate correct alignment rotation with finer level of discretization.

- 1 Given initial estimate of rotation angle θ to align images...
- 2 For each k , iterate over small window $\theta_{new} = [\theta - k\epsilon, \theta + k\epsilon]$ in increments of ϵ .
- 3 Compute alignment error with θ_{new} as rotation angle.
- 4 Update rotation parameter with angle minimizing Mean Squared Error (MSE) for image pair.

Pairwise Alignment

Refine Transformation Parameters

Objective: To align image pair I_2, I_3 , use data from images I_1 and I_4 .

- 1 Calculate pairwise transformation parameters between I_1, I_3 and I_2, I_4 .
- 2 Obtain 2 more estimations of transformation parameters between I_2, I_3 using new information.
- 3 Determine Mean Squared Error between image pair using all estimates of transformations.
- 4 Pick transformation minimizing error.

Pairwise Alignment

Refine Transform Parameter Illustration

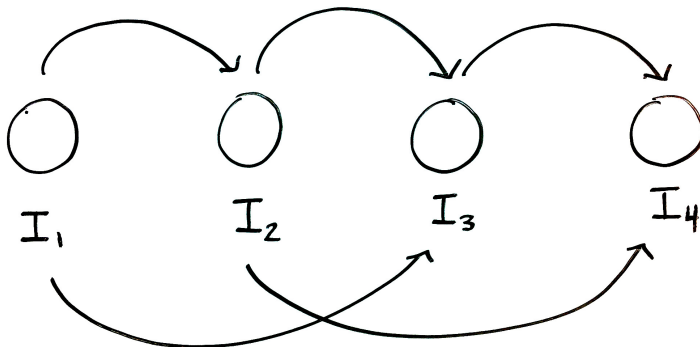


Figure 4 : Refine Transform Parameters

Global Stack Alignment

Objective: Given transformation parameters for all adjacent image pairs, compute transformation for each image in global coordinate frame.

- 1 Set global transformation parameters of previous image to that of current image.
- 2 Find new rotation angle by adding previous rotation parameter to pairwise rotation angle.
- 3 Find new translation parameters using previous rotation angle and pairwise translations.
- 4 Positive translations: shift current image. Negative translations: shift all previous images.
- 5 Iterate through image cube to globally align stack.

Other Attempted Methods

- RANSAC: Detect linear folds
- SURF Feature Matching: Align images
- Superpixels and Earth Mover's Distance: 'Better' error metric for image alignment

RANSAC for Fold Detection

- **Objective:** Given set of points P and inlier distance d , outputs line of 'best' fit.
- Procedure:
 - 1 From P , chooses points and finds line through them.
 - 2 Finds number of inliers within d .
 - 3 Based on percentage of outliers, adaptively computes number of iterations.
 - 4 Use to split image above and below single, linear fold line.
- Did not encounter instances where this method is helpful, so ultimately not included in final pipeline.

RANSAC for Fold Detection

Results

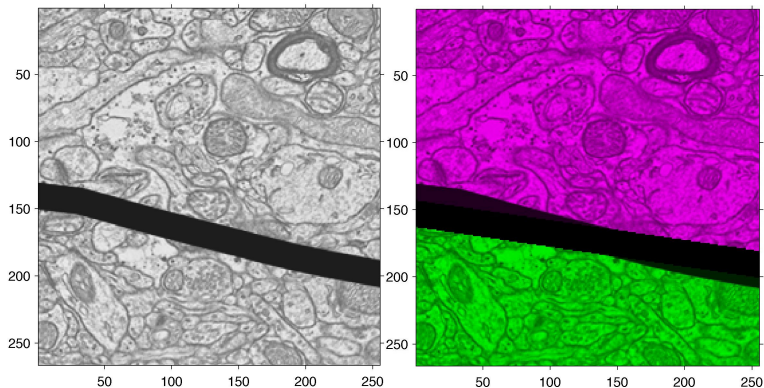


Figure 5 : Fold detection with RANSAC

SURF Feature Detection

- **Objective:** Match image features to correct rotations before cross-correlation step.
- Proposed method:
 - 1 Use Gaussian blur on images, then resize.
 - 2 Detect SURF (Speeded Up Robust Features) features.
 - 3 Match features to detect rotation angle.
 - 4 Rotate image, input for 2D cross-correlation.
 - 5 Determine and save transformation parameters.
- Unfortunately, noise and structural similarities in EM images reduce its effectiveness.

Superpixels and Earth Mover's Distance

- **Objective:** Weigh alignment error at different regions of the image differently.
- Mean Squared Error weighs every part of the image equally.
- Proposed method between two images:
 - 1 SLIC Superpixels to construct superpixels in both images.
 - 2 Each superpixel center is associated with weight related to number of pixels and intensity of pixels.
 - 3 Find the Earth Mover's Distance between the cluster centers in image pair.
- Unfortunately, does not appear to have any advantages compared to MSE.

Earth Mover's Distance

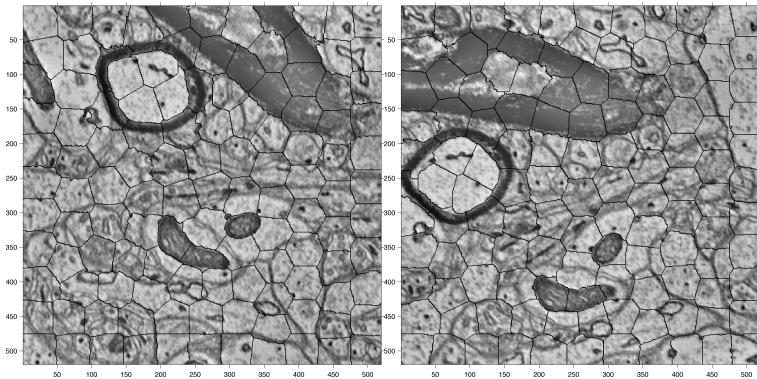


Figure 6 : Earth Mover's Distance

Earth Mover's Distance

$$\text{WORK}(P, Q, \mathbf{F}) = \sum_{i=1}^m \sum_{j=1}^n d_{ij} f_{ij},$$

subject to the following constraints:

$$f_{ij} \geq 0 \quad 1 \leq i \leq m, 1 \leq j \leq n$$

$$\sum_{j=1}^n f_{ij} \leq w_{\mathbf{p}_i} \quad 1 \leq i \leq m \quad \sum_{i=1}^m f_{ij} \leq w_{\mathbf{q}_j} \quad 1 \leq j \leq n$$

$$\sum_{i=1}^m \sum_{j=1}^n f_{ij} = \min \left(\sum_{i=1}^m w_{\mathbf{p}_i}, \sum_{j=1}^n w_{\mathbf{q}_j} \right),$$

Figure 7 : EMD Equations

Superpixels and Earth Mover's Distance

Results

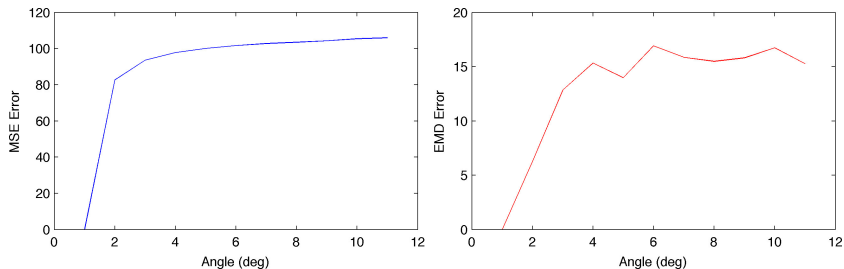


Figure 8 : MSE vs EMD Comparison

CAJAL3D-API Integration

- **Objective:** Perform affine alignment on RAMONVolume.
- Pipeline:
 - 1 Configure settings for pairwise and global alignment, and API data retrieval.
 - 2 Retrieve data from API.
 - 3 Compute transformations for pairwise alignment on sub-cubes.
 - 4 Align cube!
 - 5 Optional: Perform operations on aligned cube (like 3D mitochondria detection).
 - 6 Optional: Unalign cube to original state.

User Functions

- **read_api:** Retrieves data from the API and saves as RAMONVolume.
- **alignRAMONVol:** Given a RAMONVolume, computes transformation parameters and aligns image cube.
- **unalignRAMONVol:** Given an aligned image cube and original transformation parameters, takes inverse to revert image cube back to unaligned state.
- **constructingcubetransforms:** computes transformation parameters for entire data set in manageable sub-cubes.
- **constructingcubealignment:** Uses transformation parameters from constructingcubetransforms, to align image cube that that data set of any specified size.

DEMO!

References

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