Apr 18, 2024 Version 2

CODA (part 4): register the deep learning labelled images and Construct 3D tissue matrix | HuBMAP | JHU-TMC V.2

DOI
dx.doi.org/10.17504/protocols.io.yxmvme7eog3p/v2

Kyu Sang Han[1], Pei-Hsun Wu[1], Joel Sunshine[2], Ashley Kiemen[2], Sashank Reddy[2], Denis Wirtz[1,2]

[1]Johns Hopkins University; [2]Johns Hopkins Medicine

Human BioMolecular Atla…

TMC - Johns Hopkins Uni…

Kyu Sang Han
Johns Hopkins University

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.yxmvme7eog3p/v2

Protocol Citation: Kyu Sang Han, Pei-Hsun Wu, Joel Sunshine, Ashley Kiemen, Sashank Reddy, Denis Wirtz 2024. CODA (part 4): register the deep learning labelled images and Construct 3D tissue matrix | HuBMAP | JHU-TMC. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.yxmvme7eog3p/v2**Version created by **Kyu Sang Han**

Manuscript citation:
A.M. Braxton, A.L. Kiemen, M.P. Grahn, A. Forjaz, J. Parksong, J.M. Babu, J. Lai, L. Zheng, N. Niknafs, L. Jiang, H. Cheng, Q. Song, R. Reichel, S. Graham, A.I. Damanakis, C.G. Fischer, S. Mou, C. Metz, J. Granger, X.-D. Liu, N. Bachmann, Y. Zhu, Y.Z. Liu, C. Almagro-Pérez, A.C. Jiang, J. Yoo, B. Kim, S. Du, E. Foster, J.Y. Hsu, P.A. Rivera, L.C. Chu, D. Liu, E.K. Fishman, A. Yuille, N.J. Roberts, E.D. Thompson, R.B. Scharpf, T.C. Cornish, Y. Jiao, R. Karchin, R.H. Hruban, P.-H. Wu, D. Wirtz, and L.D. Wood, “3D genomic mapping reveals multifocality of human pancreatic precancers”, Nature (2024)

A.L. Kiemen, A. Forjaz, R. Sousa, K. Sang Han, R.H. Hruban, L.D. Wood, P.H. Wu, and D. Wirtz, “High-resolution 3D printing of pancreatic ductal microanatomy enabled by serial histology”, Advanced Materials Technologies 9, 2301837 (2024)

T. Yoshizawa, J. W. Lee, S.-M. Hong, D.J. Jung, M. Noe, W. Sbijewski, A. Kiemen, P.H, Wu, D. Wirtz, R.H. Hruban, L.D. Wood, and K. Oshima. “Three-dimensional analysis of ductular reactions and their correlation with liver regeneration and fibrosis”, Virchows Archiv (2023).

A.L. Kiemen, A.I. Damanakis, A.M. Braxton, J. He, D. Laheru, E.K. Fishman, P. Chames, C. Almagro Perez, P.-H. Wu, D. Wirtz, L.D. Wood, and R. Hruban, “Tissue clearing and 3D reconstruction of digitized, serially sectioned slides provide novel insights into pancreatic cancer”, Med 4, 75-91 (2023)

A. Kiemen, Y. Choi, A. Braxton, C. Almagro Perez, S. Graham, M. Grahm, N., N. Roberts, L. Wood, P. Wu, R. Hruban, and D. Wirtz, “Intraparenchymal metastases as a cause for local recurrence of pancreatic cancer”, Histopathology 82: 504-506 (2022)

A.L. Kiemen, A.M. Braxton, M.P. Grahn, K.S. Han, J.M. Babu, R. Reichel, A.C. Jiang, B. Kim, J. Hsu, F. Amoa, S. Reddy, S.-M. Hong, T.C. Cornish, E.D. Thompson, P. Huang, L.D. Wood, R.H. Hruban, D. Wirtz and P.H. Wu, “CODA: quantitative 3D reconstruction of large tissues at cellular resolution”, Nature Methods 19: 1490-1499 (2022)

K.S.Han, I. Sander, J. Kumer, E. Resnick, C. Booth, B. Starich, J. Walston, A.L. Kiemen, S. Reddy, C. Joshu, J. Sunshine, D. Wirtz, P.-H. Wu "The microanatomy of human skin in aging." bioRxiv (2024): 2024-04.

Protocol status: Working
We use this protocol and it's working

Created: April 18, 2024

Last Modified: April 18, 2024

Protocol Integer ID: 98442

Keywords: CODA, deeplearning, semanticsegmentation

Funders Acknowledgement:
Institute of Arthritis and Musculoskeletal and Skin Diseases
Grant ID: U54AR081774
National Cancer Institute
Grant ID: U54CA143868

Abstract

CODA (part 4): register the deep learning labelled images and Construct 3D tissue matrix

Register the deep learning labelled images

1 Using the registration transformations calculated in low-resolution (as described in **CODA-part2**), register the segmentation masks of the high-resolution tif images generated in **CODA-part3**.

Function requirements

2 High-resolution images from **CODA-part3** (**pthclassified**)

3 The path containing the image registration information from **CODA-part2** (**pthdata**),

4 The scale between the high-resolution classified images and the low-resolution registration images (here for registration of 1x images and classification of 10x images, scale=10)

5 The pixel number of the background class in the segmentation model built in **CODA-part3** (here, nwhite=3)

Execution

6 **pthim=pthclassified;**
pthdata=[pth,'1x\registered\elastic registration\save_warps'];
scale=10;
padnum=3;
apply_image_registration(pthim,pthdata,scale,padnum);

7 This function will create an subfolder named 'registeredE' inside the **pthclassified** folder that contains the registered, classified images. **pthclassifiedE=[pthclassified,'registeredE'];**

Construct 3D tissue matrix

8 Next, we will create a 3D quantifiable matrix from the tissue labels using the registered, segmented images we just created above. **vol** will be a 3D matrix containing the registered, labelled data.

9 To call this function, you need to locate the subfolder containing the registered classified images, define a location to save the output matrix, define the desired resolution of the volume matrix (relative to the current resolution of the images, and identify the RGB color map used in the deep learning model in **CODA-part3**

10 **pthclassifiedE** is the subfolder with the registered, classified images created in earlier steps in this protocol:

pthclassifiedE=[pthclassified,'registeredE'];

11 Define a subfolder where you would like to save the volumetric data:
pthvolume=[pth,'lung_data'];

12 Set **sk** to 4 so that 10x image (1 um / pixel resolution) are downsampled to 4 micron / pixel:
sk=4;

13 **nwhite** is the background class from the deep learning model. Here, let's use 6:
nwhite=6;

14 **cmap** is the matrix containing RGB triplets for each class in the deep learning model
cmap=[];

build_tissue_volume(pthclassifiedE,pthvolume,sk,nwhite,cmap);

15 First, the function will display to you a concatenated image containing the first, center, and last classified image in your image stack. Here, you will manually drag a rectangle to surround the tissue in the image, then double-click. This allows you to crop out excess whitespace from your 3D matrix to save RAM.

16 Next, the function will load all serial images, crop them, downsample them using the variable **sk**, and build them into the matrix **vol**. A z-projection will be created and displayed showing a combination of all non-background classes. This z-projection will be saved inside the folder **pthvolume**.

17 Additionally, this function will create a .mat file named **volume.mat** saved inside the subfolder **pthvolume**. Inside this file will be a variable named **vol** containing the volumetric tissue labels, **rr** containing the cropping information defined inside the function, **pthclassifiedE** the folder containing the registered classified images used to create the volume, **imlist** containing the filenames of the images comprising the 3D matrix, and **sk** the downsample factor between the images in **imlist** and the data in **vol**.

Note: When determining what resolution to make your volumetric matrix, there are a few considerations:

18 You may want to make your 3D matrix isometric (same resolution in x, y, and z) to simplify 3D quantifications. In this case, define **sk** such that your classified images will be downsampled to the spacing between adjacent histological images

19 You may want to consider the size of structures you are trying to quantify. If you need to maintain classification of very fine structures (such as thin blood vessels), down sampling too much may eliminate the connectivity between these thin objects.