Research Square

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Probabilistic mapping and image clustering for quantitative assessment of fluorescent protein localizations in Arabidopsis guard cells

Takumi Higaki (takumi.higaki@gmail.com)
Graduate School of Frontier Sciences, The University of Tokyo
Natsumaro Kutsuna
Graduate School of Frontier Sciences, The University of Tokyo
Seiichiro Hasezawa
Graduate School of Frontier Sciences, The University of Tokyo

Method Article

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Abstract

The protocol reported here describes a method to quantitatively evaluate fluorescently-tagged protein localizations from fluorescent microscopic images with a combination of probabilistic mapping and image clustering. We demonstrate the use of this protocol using kidney-shaped guard cells of plants.

Introduction

Microscopic assessment of protein localizations with fluorescent protein tagging is an important assay in cell biological studies. We believe that protein localizations should be statistically assessed based on many observations, because the interpretation of a single microscopic image often leads to biased information. As proved in previous magnetic resonance imaging \(MRI) analyses[1,2,3], probabilistic mapping is a simple and powerful method to determine average distributions of visualized objects. Clustering is a kind of unsupervised learning based on distances between each data point. Similarities between microscopic images can be evaluated using clustering with appropriate image metrics[4]. Using the fluorescent image of kidney-shaped guard cells, we recently developed the image processing framework to quantitatively evaluate protein localizations with probabilistic mapping and image clustering[5]. This protocol explains how to evaluate the localization similarity of marker proteins from the 3-D images of Arabidopsis guard cells that are freely available in our "image database LIPS":http://hasezawa.ib.k.u-tokyo.ac.jp/lips/ [5]. We prefer self-organizing mapping \(SOM)[6] as an image clustering method because of its superiority for image inspection.

Equipment

Computer A laptop computer \(CF-J9, Panasonic) with 64-bit Windows operating system, 6 GiB of random access memory \(RAM), and the Intel Core i5 CPU was used here. **ImageJ software** Download and install the ImageJ software \(http://rsbweb.nih.gov/ij/download.html). For general operations, please refer the official user guide[7] "http://rsbweb.nih.gov/ij/docs/guide/userguide.html":http://rsbweb.nih.gov/ij/docs/guide/userguide.html. **Download Kbi plug-ins and Hig macros** 1. Download our in-house KBI JAR file \('kbi_ij_plugins...') and scala JAR file \('scala-library') in the plugin folder of ImageJ "http://hasezawa.ib.k.u-tokyo.ac.jp/zp/Kbi/ImageJKbiPlugins":http://hasezawa.ib.k.u-tokyo.ac.jp/zp/Kbi/ImageJKbiPlugins. 2. Download our in-house HIG IJM files in the macro folder of ImageJ "http://hasezawa.ib.k.u-tokyo.ac.jp/zp/Kbi/HigPDMClustering":http://hasezawa.ib.k.u-tokyo.ac.jp/zp/Kbi/HigPDMClustering. 3. Restart the ImageJ.

Procedure

Image processing for probabilistic mapping 1. Prepare the target stack image. Here, we prepare the 16-bit TIFF stack images \(bright field and fluorescence images) that are freely available in the "LIPS database":http://hasezawa.ib.k.u-tokyo.ac.jp/lips/ \(**Figure 1**). 2. Run the ImageJ software

"http://rsbweb.nih.gov/ij/":http://rsbweb.nih.gov/ij/. 3. Open the target image using the ImageJ menu “File-Open”. "http://rsbweb.nih.gov/ij/docs/guide/userguide-23.html#toc-Subsection-23.2":http://rsbweb.nih.gov/ij/docs/guide/userguide-23.html#toc-Subsection-23.2. 4. Draw the major stomatal pore line on the bright field image using the ImageJ tool bar “Straight Line Selection Tool” "http://rsbweb.nih.gov/ij/docs/guide/userguide-18.html#toc-Subsection-18.2":http://rsbweb.nih.gov/ij/docs/guide/userguide-18.html#toc-Subsection-18.2 \(**Figure 2a**). 5. Copy the segmented straight line from the bright field image to the fluorescence images using the ImageJ menu “Edit-Selection-Restore Selection” "http://rsbweb.nih.gov/ij/docs/guide/userguide-24.html#toc-Subsection-24.12":http://rsbweb.nih.gov/ij/docs/guide/userguide-24.html#toc-Subsection-24.12 \(**Figure 2b**). 6. Rotate the fluorescence image using the ImageJ menu “Plugins-kbi-Kbi_registration \(mode: horizoner)” \(**Figure 2c**). 7. Get the maximum intensity projection image using the ImageJ menu “Image-Stacks-Z project…” "http://rsbweb.nih.gov/ij/docs/guide/userguide-25.html#toc-Subsection-25.6":http://rsbweb.nih.gov/ij/docs/guide/userguide-25.html#toc-Subsection-25.6 \(**Figure 2d**). 8. Put a bounding box around the cell using the ImageJ tool bar menu “Rectangular Selection Tool” "http://rsbweb.nih.gov/ij/docs/guide/userguide-18.html#toc-Subsection-18.1":http://rsbweb.nih.gov/ij/docs/guide/userguide-18.html#toc-Subsection-18.1 \(**Figure 2e**). 9. Crop the boxed cell region using the ImageJ menu “Image-Crop” "http://rsbweb.nih.gov/ij/docs/guide/userguide-25.html#toc-Subsection-25.8":http://rsbweb.nih.gov/ij/docs/guide/userguide-25.html#toc-Subsection-25.8 \(**Figure 2f**). 10. If the is pore located in the upper half of the cropped image, rotate the image 180 degrees using the ImageJ menu “Image-Transform-Rotate…” "http://rsbweb.nih.gov/ij/docs/guide/userguide-25.html#toc-Subsection-25.12":http://rsbweb.nih.gov/ij/docs/guide/userguide-25.html#toc-Subsection-25.12. 11. Resize the images to the same size using the ImageJ menu “Image-Adjust-Size…” "http://rsbweb.nih.gov/ij/docs/guide/userguide-25.html#toc-Subsection-25.2":http://rsbweb.nih.gov/ij/docs/guide/userguide-25.html#toc-Subsection-25.2. Here, we resized all images to 304×119 pixels, which was the mean size of the guard-cell bounding boxes. 12. Make a stack image of the grouped images using the ImageJ menu “Plugins-kbi-Kbi_StkJoin \(mode: all axis: z)”. \(**Figure 2g**). 13. Run the macro hig_Pdmapping.ijm "http://hasezawa.ib.k.u-tokyo.ac.jp/zp/Kbi/HigPDMClustering":http://hasezawa.ib.k.u-tokyo.ac.jp/zp/Kbi/HigPDMClustering. You will see the probability map \(**Figure 2h**). 14. Save the probability map image using the ImageJ menu “File-Save”. **Measurement of raster-scanned intensity profiles** 1. Run the ‘ImageJ’ software. 2. Open the stack image of the probability maps using the ImageJ menu “File-Open”. 3. To reduce the data size, shrink the image size using the ImageJ menu “Image-Adjust-Size…”. Here, we reduced the size to 70×27 pixels. 4. Run the macro hig_Lineprofile.ijm "http://hasezawa.ib.k.u-tokyo.ac.jp/zp/Kbi/HigPDMClustering":http://hasezawa.ib.k.u-tokyo.ac.jp/zp/Kbi/HigPDMClustering. 5. You will see the Results table of the raster-scanned intensity profiles \(**Figure 3**). 6. Uncheck the checkbox ‘Save column headers’ and ‘Save row numbers’ using the Results table menu “Results-Options…”. 7. Save the raster-scanned intensity profiles as a csv file using the Results table menu “File-Save As…”. **SOM clustering with ImageJ** 1. Run the ‘ImageJ’ software. 2. Open the stack image of the probability maps using the ImageJ menu “File-Open”. 3. Run the plug-in using the ImageJ menu “Plugins-

kbi-Kbi_Clustering” and select ‘som’ in the modeClustering drop-down list. 4. Select ‘sampleZ_featureCsv_patchImg’ in the somMode drop-down list. 5. Load the csv file of the raster-scanned intensity profiles. 6. You will see the SOM image \(**Figure 4**). In the case that multiple images were allocated in same node in SOM, the images will be allocated in next slice of the stack image \(SOM output) \(**Figure 4**).

Troubleshooting

Display error message ‘Out of memory’: Allocate more memory using the ImageJ menu “Edit-Options-Memory & Threads…-Maximum memory” "http://rsbweb.nih.gov/ij/docs/guide/userguide-24.html#toc-Subsection-24.13":http://rsbweb.nih.gov/ij/docs/guide/userguide-24.html#toc-Subsection-24.13 **Display error message ‘Stack required’:** The macros hig_Pdmapping.ijm and hig_Lineprofile.ijm do not support single-slice images. Please prepare a stack image. **Display error message ‘This macro does not work with RGB images’:** The macros hig_Pdmapping.ijm and hig_Lineprofile.ijm do not support RGB images. Please prepare gray-scale images.

References

1. Penhune, V.B., Zatorre, R.J., MacDonald, J.D. & Evans, A.C. Interhemispheric anatomical differences in human primary auditory cortex: probabilistic mapping and volume measurement from magnetic resonance scans. _Cereb Cortex_, **6**, 661-672 \(1996). 2. McCarthy, G., Luby, M., Gore, J. & Goldman-Rakic, P. Infrequent events transiently activate human prefrontal and parietal cortex as measured by functional MRI. _J Neurophysiol._ **77**, 1630-1634 \(1997). 3. Jones, D.K. et al. Spatial normalization and averaging of diffusion tensor MRI data sets. _Neuroimage_, **17**, 592-617 \(2002). 4. Higaki, T., Kutsuna, N., Sano, T., Kondo, N., Hasezawa, S. Quantification and cluster analysis of actin cytoskeletal structures in plant cells: role of actin bundling in stomatal movement during diurnal cycles in Arabidopsis guard cells. _Plant J._ **61**, 156-165 \(2010). 5. Higaki, T. et al. Statistical organelle dissection of Arabidopsis guard cells using image database LIPS. _Sci. Rep._ in press. 6. Kohonen, T. Self-Organizing Maps. Springer-Verlag, ISBN 3-540-67921-9, New York, Berlin, Heidelberg \(2001). 7. ImageJ User Guide website. Available: "http://rsbweb.nih.gov/ij/docs/guide/userguide.html":http://rsbweb.nih.gov/ij/docs/guide/userguide.html. Accessed 2012 May 1.

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Figures

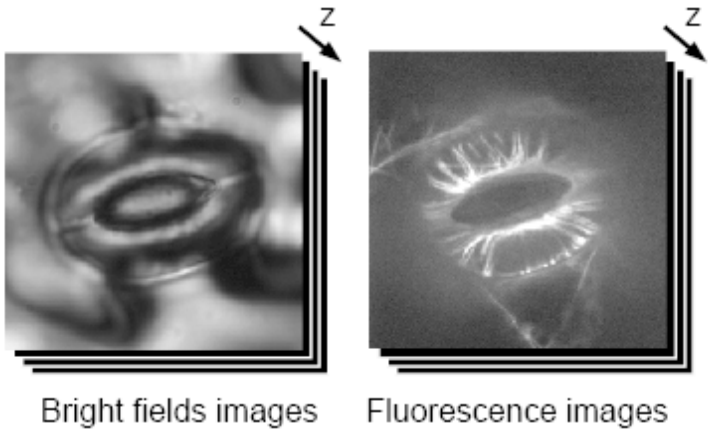


Figure 1

Examples of target images. The 3-D images of Arabidopsis guard cells are freely available from "LIP database":http://hasezawa.ib.k.u-tokyo.ac.jp/lips/ .

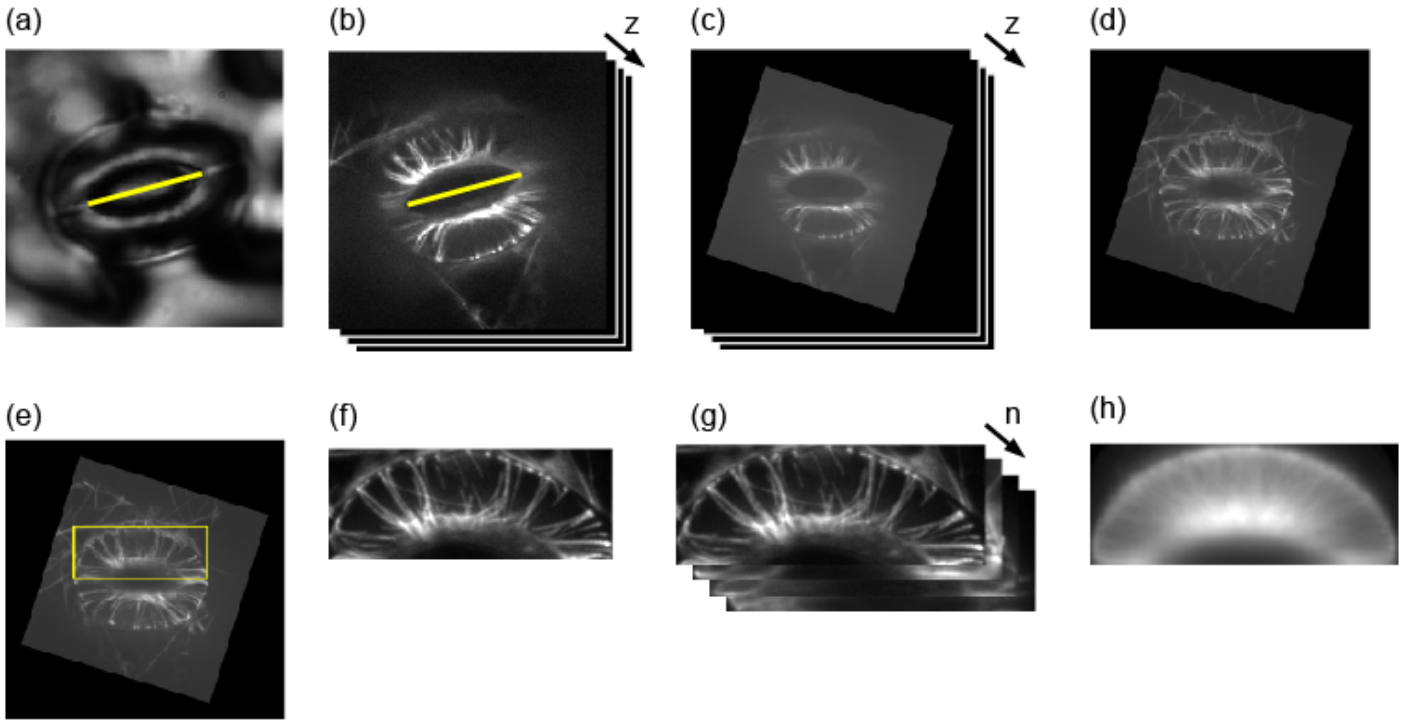
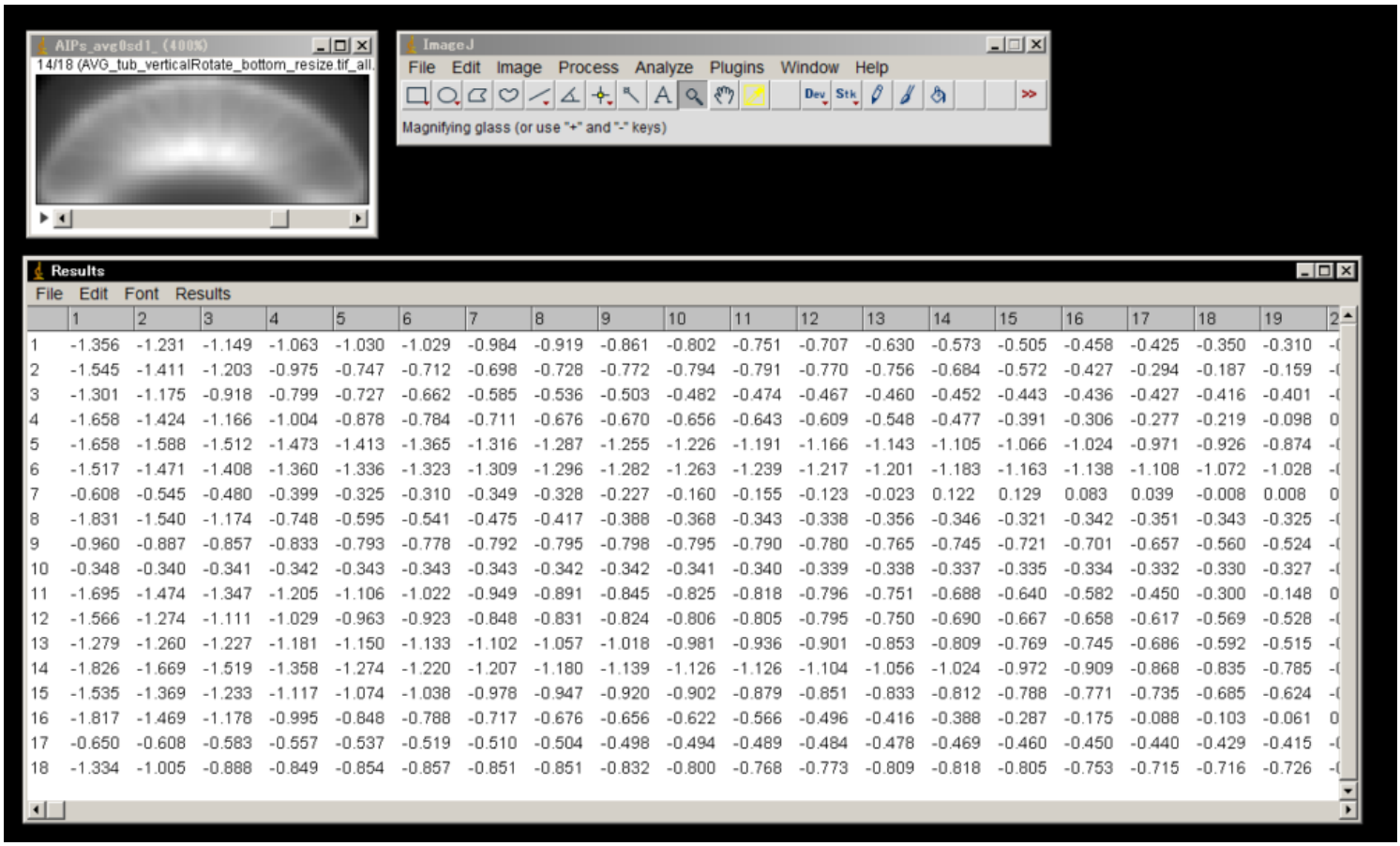


Figure 2

Workflow of image processing for probability mapping. (a) An original bright-field image with a stomatal pore line. (b) An original green-fluorescence protein (GFP) stack image with a stomatal pore line. (c) A rotated GFP stack image. (d) A rotated maximum intensity projection (MIP) image. (e) Selection of the cell region with a bounding box. (f) A cropped guard cell MIP image. (g) Multiple cropped MIP images. Note that all images are the same size. (h) A probabilistic map.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	2
1	-1.356	-1.231	-1.149	-1.063	-1.030	-1.029	-0.984	-0.919	-0.861	-0.802	-0.751	-0.707	-0.630	-0.573	-0.505	-0.458	-0.425	-0.350	-0.310	-(
2	-1.545	-1.411	-1.203	-0.975	-0.747	-0.712	-0.698	-0.728	-0.772	-0.794	-0.791	-0.770	-0.756	-0.684	-0.572	-0.427	-0.294	-0.187	-0.159	-(
3	-1.301	-1.175	-0.918	-0.799	-0.727	-0.662	-0.585	-0.536	-0.503	-0.482	-0.474	-0.467	-0.460	-0.452	-0.443	-0.436	-0.427	-0.416	-0.401	-(
4	-1.658	-1.424	-1.166	-1.004	-0.878	-0.784	-0.711	-0.676	-0.670	-0.656	-0.643	-0.609	-0.548	-0.477	-0.391	-0.306	-0.277	-0.219	-0.098	0
5	-1.658	-1.588	-1.512	-1.473	-1.413	-1.365	-1.316	-1.287	-1.255	-1.226	-1.191	-1.166	-1.143	-1.105	-1.066	-1.024	-0.971	-0.926	-0.874	-(
6	-1.517	-1.471	-1.408	-1.360	-1.336	-1.323	-1.309	-1.296	-1.282	-1.263	-1.239	-1.217	-1.201	-1.183	-1.163	-1.138	-1.108	-1.072	-1.028	-(
7	-0.608	-0.545	-0.480	-0.399	-0.325	-0.310	-0.349	-0.328	-0.227	-0.160	-0.155	-0.123	-0.023	0.122	0.129	0.083	0.039	-0.008	0.008	0
8	-1.831	-1.540	-1.174	-0.748	-0.595	-0.541	-0.475	-0.417	-0.388	-0.368	-0.343	-0.338	-0.356	-0.346	-0.321	-0.342	-0.351	-0.343	-0.325	-(
9	-0.960	-0.887	-0.857	-0.833	-0.793	-0.778	-0.792	-0.795	-0.798	-0.795	-0.790	-0.780	-0.765	-0.745	-0.721	-0.701	-0.657	-0.560	-0.524	-(
10	-0.348	-0.340	-0.341	-0.342	-0.343	-0.343	-0.343	-0.342	-0.342	-0.341	-0.340	-0.339	-0.338	-0.337	-0.335	-0.334	-0.332	-0.330	-0.327	-(
11	-1.695	-1.474	-1.347	-1.205	-1.106	-1.022	-0.949	-0.891	-0.845	-0.825	-0.818	-0.796	-0.751	-0.688	-0.640	-0.582	-0.450	-0.300	-0.148	0
12	-1.566	-1.274	-1.111	-1.029	-0.963	-0.923	-0.848	-0.831	-0.824	-0.806	-0.805	-0.795	-0.750	-0.690	-0.667	-0.658	-0.617	-0.569	-0.528	-(
13	-1.279	-1.260	-1.227	-1.181	-1.150	-1.133	-1.102	-1.057	-1.018	-0.981	-0.936	-0.901	-0.853	-0.809	-0.769	-0.745	-0.686	-0.592	-0.515	-(
14	-1.826	-1.669	-1.519	-1.358	-1.274	-1.220	-1.207	-1.180	-1.139	-1.126	-1.126	-1.104	-1.056	-1.024	-0.972	-0.909	-0.868	-0.835	-0.785	-(
15	-1.535	-1.369	-1.233	-1.117	-1.074	-1.038	-0.978	-0.947	-0.920	-0.902	-0.879	-0.851	-0.833	-0.812	-0.788	-0.771	-0.735	-0.685	-0.624	-(
16	-1.817	-1.469	-1.178	-0.995	-0.848	-0.788	-0.717	-0.676	-0.656	-0.622	-0.566	-0.496	-0.416	-0.388	-0.287	-0.175	-0.088	-0.103	-0.061	0
17	-0.650	-0.608	-0.583	-0.557	-0.537	-0.519	-0.510	-0.504	-0.498	-0.494	-0.489	-0.484	-0.478	-0.469	-0.460	-0.450	-0.440	-0.429	-0.415	-(
18	-1.334	-1.005	-0.888	-0.849	-0.854	-0.857	-0.851	-0.851	-0.832	-0.800	-0.768	-0.773	-0.809	-0.818	-0.805	-0.753	-0.715	-0.716	-0.726	-(

Figure 3

Screenshot of output of the macro hig_Pdmapping.ijm

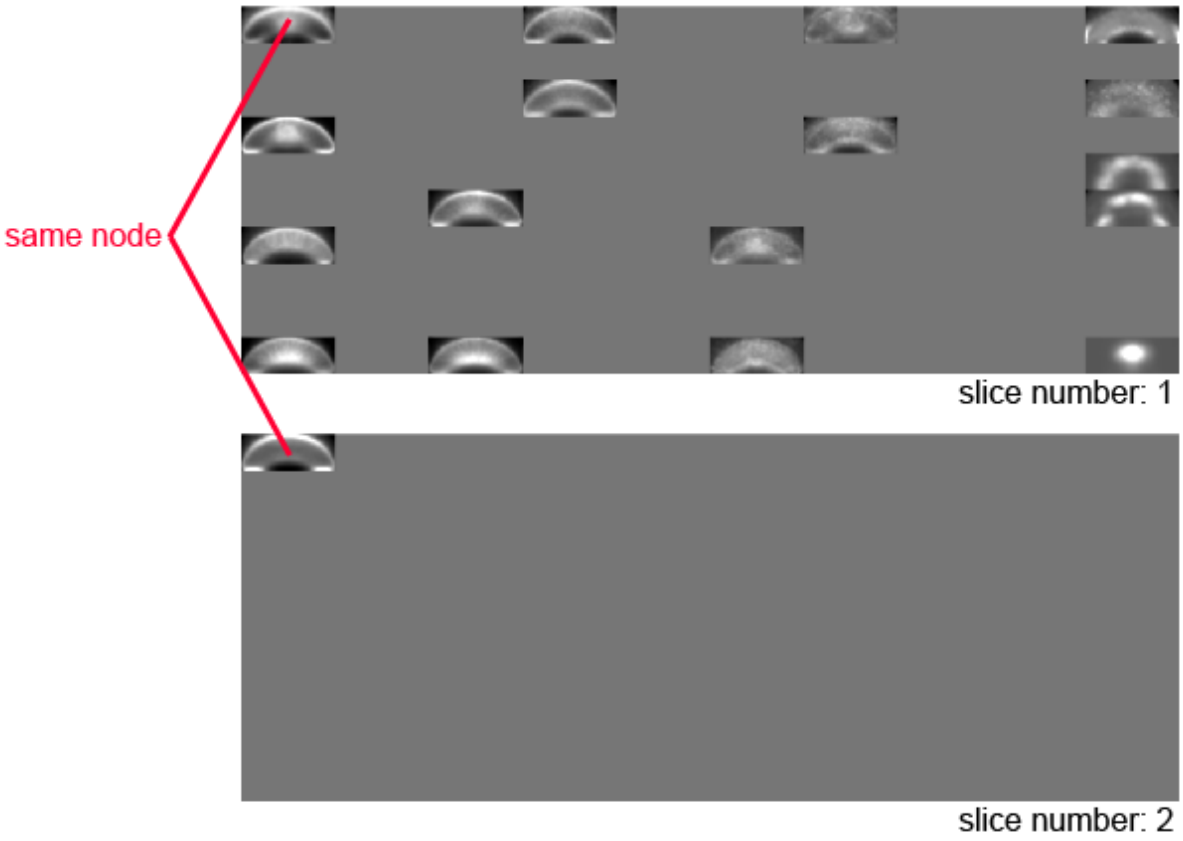


Figure 4

An example of SOM output. The images allocated in the same node are displayed in the same positions of different slices.