# Generation of the Probabilistic Template of Default Mode Network Derived from Resting-State fMRI

| | |
|---|---|
| Journal: | *Transactions on Biomedical Engineering* |
| Manuscript ID: | TBME-01499-2013.R1 |
| Manuscript Type: | Paper |
| Date Submitted by the Author: | n/a |
| Complete List of Authors: | Wang, Defeng; The Chinese University of Hong Kong, Department of Imaging and Interventional Radiology<br>Kong, Youyong; The Chinese University of Hong Kong, Department of Imaging and Interventional Radiology<br>Chu, Winnie CW; The Chinese University of Hong Kong, Department of Imaging and Interventional Radiology<br>Tam, Cindy; North District Hospital, Sheung Shui, Department of Psychiatry<br>Lam, Linda; The Chinese University of Hong Kong, Department of Psychiatry<br>Yilong, Wang; Beijing Tiantan Hospital, Capital Medical University, Department of Neurology<br>Northoff, Georg; Institute of Mental Health Research, University of Ottawa, Mind, Brain Imaging and Neuroethics<br>Mok, Vincent; The Chinese University of Hong Kong, Department of Medicine and Therapeutics<br>Wang, Yongjun; Beijing Tiantan Hospital, Capital Medical University, Department of Neurology<br>Shi, Lin; The Chinese University of Hong Kong, Department of Medicine and Therapeutics |
| TIPS: | neuroimaging, resting-state fMRI, Default mode network, template, brain |
| | |

# Generation of the Probabilistic Template of Default Mode Network Derived from Resting-State fMRI

Defeng Wang[1,2,3], Youyong Kong[1,2], Winnie CW Chu[1,2,*], Cindy WC Tam[4], Linda CW Lam[5], Yilong Wang[6], Georg Northoff[7], Vincent CT Mok[8,9], Yongjun Wang[6], Lin Shi[8,9,**]

[1]Department of Imaging and Interventional Radiology, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR, China

[2]Research Center for Medical Image Computing, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR, China

[3]CUHK Shenzhen Research Institute, Shenzhen, China

[4]Department of Psychiatry, North District Hospital, Sheung Shui, NT, Hong Kong

[5]Department of Psychiatry, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR, China

[6]Department of Neurology, Beijing Tiantan Hospital, Capital Medical University, Beijing, PR China

[7]Mind, Brain Imaging and Neuroethics, Institute of Mental Health Research, University of Ottawa, Ottawa, ON, Canada

[8]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China

[9]Lui Che Woo Institute of Innovation Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China

**Corresponding Author:**

* Winnie CW Chu (for clinical issues); Email address: winnie@med.cuhk.edu.hk

** Lin Shi (for technical issues); Email address: shilin@cuhk.edu.hk

**Mailing address:** Department of Imaging and Interventional Radiology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong.

# Generation of the Probabilistic Template of Default Mode Network Derived from Resting-State fMRI


## Abstract

Default-mode network (DMN) has become a prominent network among all large-scale brain networks which can be derived from the resting-state fMRI (rs-fMRI) data. Statistical template labelling the common location of hubs in DMN is favourable in the identification of DMN from tens of components resulted from the independent component analysis (ICA). This paper proposed a novel iterative framework to generate a probabilistic DMN template from a coherent group of 40 healthy subjects. An initial template was visually selected from the independent components derived from group ICA analysis of the concatenated rs-fMRI data of all subjects. An effective similarity measure was designed to choose the best-fit component from all independent components of each subject computed given different component numbers. The selected DMN components for all subjects were averaged to generate an updated DMN template and then used to select the DMN for each subject in the next iteration. This process iterated until the convergence was reached, i.e., overlapping region between the DMN areas of current template and the one generated from the previous stage is more than 95%. By validating the constructed DMN template on the rs-fMRI data from another 40 subjects, the generated probabilistic DMN template and the proposed similarity matching mechanism were demonstrated to be effective in automatic selection of independent components from the ICA analysis results.


**Key words:** Default mode network, resting-state fMRI, template, brain network.

## Introduction

Over the last few decades, there has been growing interest in investigating the large-scale brain networks that exist in the human cognition, perception and emotion. The default-mode network (DMN) is a prominent one among these discovered brain networks. The commonly accepted hubs of DMN include the posterior cingulate cortex (PCC), the medial temporal lobes (MTL), the medial prefrontal cortex (mPFC) and the angular gyrus (AG) [1, 2]. These related brain regions are considered to be active during resting state while deactivated when specific purposeful cognitive tasks are performed. Moreover, the DMN has been suggested to be related to various cognitive functions and be altered in a series of neuropsychological disorders [3, 4].

The identification of DMN has been extensively studied in both healthy individuals and subjects of various pathological conditions from resting state fMRI (rsfMRI) [5]. Plenty of methods have been proposed to derive the DMN from the rsfMRI datasets [6-8]. Among these approaches, the method using independent component analysis (ICA) has been widely utilized to extract networks in previous studies [9, 10]. ICA technique enables the blind-source separation of mixed signals into independent spatial and temporal components, which are considered to represent different functionally connected networks [6, 11]. However, there remains the problem of how to accurately select the component that best represents the DMN from the numerous components. Manual selection may be time consuming as all components should be carefully visualized and analysed. One objective approach is to automatically select the best-fit component by matching with a given DMN template [12, 13].

Therefore, a standardized DMN template representing the statistical location of the hubs is demanding for objective component selections.

Plenty of approaches have been so far proposed to construct the statistical structural brain template. In the early 1990s, Evans, et al. [14] introduced the concept of a statistical MRI template for brain mapping. They constructed the MNI305 template by linearly mapping 305 native MRI volumes to a manually-derived average MRI. In 1998, Holmes, et al. [15] created a new atlas with much higher signal-to-noise ratio. One subject was scanned 27 times, and all the images were linearly registered to compute an average which was finally mapped to MNI305. However, linear registration can't capture the local deformation between different subjects. In 2000, Guimond, et al. [16] developed a method of building a template atlas by nonlinearly registered each subject to an initial template. With all nonlinear transformations, original images were resampled to construct a template with an average unbiased shape and average intensity. Recently, Fonov, et al. [17] proposed an iterative approach to create an unbiased non-linear statistical atlas that were not subject to the vagaries of any single brain. This procedure consisted of 40 iterations, and at each iteration native volumes were non-linearly fitted to the template from the previous iteration. Being different from constructing statistical brain template derived directly from scanned MRI volumes, DMN template cannot be directly computed from fMRI data. Therefore, the proposed structural brain atlas generation algorithms could not be directly extended to the construction of DMN template.

To construct a DMN template, researchers have proposed a lot of approaches, which can be classified into two groups. One strategy is to manually select some regions from a structural brain atlas to generate a template [18, 19]. However, there exist some inconsistencies in determining the neuronal regions to compose a DMN template. The other approach is to perform a group-level ICA analysis to produce a "distributed" template [20, 21]. In this way, the fMRI data from all the subjects is first concatenated to form one 4D dataset. The DMN template is then visually recognized from the independent components that are produced by the ICA analysis on the concatenated 4D dataset. However, the difference among the fMRI data of each subject may not capture the intrinsic functional connectivity [22]. The concatenated data can't well reflect the temporal relationship in the fMRI data of each subject, and thus the resulting template may not fully preserve the information in the original data. Moreover, only a few subjects were utilized to generate the template [23]. Therefore, it is of great importance to construct a statistically representative template for DMN.

This paper presents a novel framework to generate a probabilistic DMN template from resting-state fMRI. Inspired by the structural brain atlas generation, an iterative approach was developed to create an unbiased DMN template. Resting-state fMRI data from 50 healthy subjects were utilized to generate the template. An initial template was visually selected from the independent components, which were obtained by performing ICA analysis on the concatenated fMRI data from all the 50 subjects using FSL melodic software. For each subject, as the selection of component number could affect the ability to capture the resting-state functional connectivity, independent components were obtained on different

component numbers. To find the DMN from the components in each subject, we developed a specific similarity measure. The component with the highest similarity was selected as the DMN component for each subject. All the selected DMN components were then averaged to generate a refined DMN template. The iteration process would stop once overlapping region between the DMN areas of current template and the one generated from the previous stage was more than 95%. All selected components were finally masked with a given z score. By averaging all the masks, a statistical DMN template was achieved. Resting-state data from another forty subjects were utilized to validate the automatic component selection method using the constructed statistical template.

## Materials and Methods

### *Subjects and Image Acquisition*

Ninety healthy subjects (age range, 60 – 89; mean age, 73; 48 females; all right-handed) were scanned at the resting-state. The resting state fMRI series were acquire on a 3T Philips Medical Systems (Achieva, Philips, Germany), using the 8-channel head coil. Subjects were instructed to relax and keep their eyes open. In each scanning sequence, we obtained a series of 40 axial T2-weighted gradient echo-planar images (repetition time (TR) = 3000ms, echo time (TE) = 25ms, flip angle = $90^{o}$, field of view (FOV) = $220\times220mm^2$, matrix = 64×64, in-plane resolution=$2.4\times2.4mm^2$, slice thickness=3mm). Eighty volumes were acquired in the resting-state functional scan for each subject. Three-dimensional T1-weighted images (TR = 18 ms, TE= 2ms, flip angle= $30^{o}$, voxel size = $1.04\times1.04\times0.6\ mm^3$) were acquired in the same

session in order to have high-resolution spatial references for registration and normalization of the functional images.

***Pre-processing of rs-fMRI data***

Among the 90 subjects, 50 subjects were used to generate the template and the other 40 subjects were used for evaluation. Functional MRI data of all the 90 subjects was preprocessed using SPM8 (www.fol.ion.ucl.ac.uk/spm). Time series images were first spatially registered to the MNI space using spatial normalisation. This could minimize the misalignment due to patient motion and correct for slice-time acquisition differences. Images were then resampled into 2×2×2 $mm^3$ and spatially blurred using an 8-mm Gaussian full-width half-maximum filter.

***Generation of Anatomical Brain Template***

The anatomical T1W images of the 50 healthy subjects were nonlinearly registered and transformed to the T1W template using spatial normalization tool provided by SPM8. All the transformed images were finally averaged and spatially blurred using a 6-mm Gaussian full-width half-maximum filter to generate an anatomical brain template.

***Generation of the Initial Template***

Resting-state fMRI data from fifty healthy subjects was utilized to construct the template. To construct a template, an initial template should be first given to guide the template generation process. In structural MRI, the initial template was always a single volume of one subject or an average of native MRI volumes. However, the initial template couldn't be directly

obtained from the fMRI data. An appropriate way to obtain an initial template was to perform a group-level ICA analysis.

For each subject, the smoothed normalized fMRI images were concatenated across time to form a single 4D volumetric dataset. All the preprocessed images from each subject were then concatenated into one set of 4D image. The 4D images with all the subjects were then processed with FSL melodic ICA software (http://www.fmrib.ox.ac.uk/fsl/index.html). The Melodic software utilized probabilistic ICA to estimate the number of relevant noise and signal sources in the 4D data. The number of components was automatically estimated. Afterwards, the fMRI data was decomposed into a set of spatially independent maps. These maps provided intensity values (z scores) and thus a measure of the contribution of the time course of a component to the signal for a given voxel. The initial DMN template was finally selected visually based on their correspondence to the DMN identified by Damoiseaux, et al. [24]. Fig.1 shows the selected initial DMN template from the estimated maps.

***Similarity Measure between DMN Components***

For each subject, ICA analysis was also carried out using FSL Melodic software. For each subject, there were a large number of spatial maps. It was time consuming to select the best-fit component by visually comparing among all the components. To automatically select the most similar component, we developed an effective similarity measurement algorithm. Z scores reflect the degree to which a given voxel's time series was correlated with the time series corresponding to the specific ICA component. Similarity between the independent

components and the template was defined as the absolute value between their z maps derived from the melodic as equation (1),

$$s(z_i, z_t) = \left| \frac{1}{M} \sum_{m=1}^{M} \left[ \left( \frac{z_i(m) - \overline{z_i}}{\delta_{z_i}} \right) \left( \frac{z_t(m) - \overline{z_t}}{\delta_{z_t}} \right) \right] \right|, \quad (1)$$

where $z_i$ and $z_t$ are the z-value of M voxels in the common mask of the z-value maps of the i-th component and the initial template, $\overline{z_i}$ and $\overline{z_t}$ are the mean values of the $z_i$ and $z_t$, $\delta_{z_i}$ and $\delta_{z_t}$ are the corresponding standard deviations.

***Iterative Procedure for Template Refinement***

The selection of the number of components (N) could influence the ability of spatial component to capture the resting state functional connectivity [25]. Setting N too small could squeeze independent networks to the same component. On the other hand, hubs belonging to the same network might be separated into different components if N was set too big. Although some researchers estimated the appropriate number of ICA components for fMRI data, it could vary across different individuals [26]. Therefore, in order to obtain real independent components, multiple types of component numbers were utilized in our approach, i.e., 10, 15, 20, 25, 30, 35, 40, 50, 60 and 70. Best-fit components were selected by matching with the initial DMN template using the proposed similarity measure.

The main procedure of the template generation algorithm is illustrated as Fig. 2. Starting with the initial template, we iteratively selected the best-fit component from the independent components of each subject and reconstructed a refined DMN template. For each subject,

similarity between each component with the current DMN template was computed. Only the component with the highest similarity was selected as the DMN component for that subject. All the selected components were then averaged to generate a refined DMN template. The DMN template was then thresholded with a given z score to extract the DMN area. The termination criterion was that the similarity measurement between the DMN areas of current template and the one generated from the previous stage was more than 95%. Fig. 5 illustrates the similarities between templates generated from adjacent stages during the iterations. The procedure stopped after six iterations in our experiment. All selected components were finally thresholded with a given z score. By averaging all the masks, a statistical DMN template was achieved. The template was further smoothed using a 6-mm Gaussian full-width half-maximum filter.

## Results

### *Templates*

The sagittal, coronal, and axial views of the anatomical template are shown in Fig. 3. Fig. 4 shows the statistical DMN template derived from the 50 healthy subjects. The DMN template, a sample dataset, the DMN selected using the proposed method, and the Matlab code of DMN selection and similarity measurement are downloadable at http://www.droid.cuhk.edu.hk/staff/staff_list/dfwang/dmn.htm. The identified areas were consistent with binary DMN templates generated in previous works [1, 3, 25].

### *Validation of the Statistical Template*

To validate the automatic component selection method using the statistical template, we employed here the resting-state fMRI data from the rest 40 subjects. ICA analysis was performed on the resting-state data for each subject using Melodic software. Independent components were also generated in a range of components numbers: 10, 15, 20, 25, 30, 35, 40, 50, 60 and 70. We first applied the proposed automatic selection approach to choose the best-fit DMN components among numerous components. On the other hand, we visually selected DMN components from the acquired components. The DMN components from automatic and manual selections were all thresholded to achieve the DMN. To evaluate the automatic selection result, the Dice coefficient was used to measure the spatial overlap between the two DMN maps for each subject. With the binary DMN $z_a$ and $z_v$ from the automatic and visual selection, the dice coefficient was defined as

$$d(z_a, z_v) = \frac{2|z_a \cap z_v|}{|z_a| + |z_v|}. \quad (2)$$

Dice coefficients have a restricted range of [0,1], where 0 represents that there is no overlap and 1 denotes that they are perfectly overlapped. Fig. 6 gives the Dice coefficients for all the 40 subjects. Most of the values were equal to 1 and all the other ones were very close to 1. This indicated that the DMN selected with the automatic method was quite similar to the visually selected results.

We further evaluate the superiority of our statistical template by comparing with the traditional binary template. The binary template was obtained by thresholding the recognized DMN component from group-ICA analysis. This was widely used in previous studies [20, 21].

We computed the dice coefficients of the manually selected DMN of each subject with the two templates. Fig. 7 gives the dice coefficients of the DMN of each subject on the two templates. The statistical template obtained higher dice coefficient than initial binary template. This demonstrated that the statistical template was more consistent with the DMN distribution.

**Discussion**

We presented a novel iterative method for creating a statistical DMN template through resting-state fMRI data from a large number of subjects. Our method used iterative refinement with an effective similarity measurement algorithm to select the DMN component from the numerous independent components for each subject. The iteration process terminated until the difference between the templates of the current and previous stage was less than 5%. With all the masks of the DMN areas from each subject being averaged, the statistical DMN template was achieved. For validation, unseen data from another forty healthy subjects were employed. The results demonstrated that the DMN can be accurately selected with the currently constructed statistical template.

Our template-building algorithm is different from the iterative methods for brain structural template in the following ways. In the construction of traditional structural MRI template, data was directly used to generate the template. Here, the template was constructed from the independent components which were derived from ICA analysis on resting-state fMRI data. The initial template was visually selected from the independent components that derived from group ICA analysis on the concatenated data of all subjects. This is quite different from

traditional structural MRI that takes a single subject data or the averaged data as an initial template. Previous methods performed ICA analyses on a specific or an automatically estimated component number. This differs from our method that utilized multiple types of component numbers to better capture the resting-state functional connectivity.

Previous DMN templates were often derived from group-level ICA analysis with binary masks. All fMRI data from all the subjects was concatenated to form one 4D dataset. DMN templates were visually recognized among the components from ICA analysis on the 4D dataset. The templates are binary masks from a few subjects [20, 21, 23]. In our approach, a large number of healthy subjects were employed to generate the probabilistic template. Compared with the binary templates, probabilistic template could better represent the statistical DMN distribution within a population. Limited by the data at hand, present template was constructed from the older subjects only. The connectivity in DMN has been demonstrated to be changed with normal aging [27]. The DMN template may have differences for different age stages. In future, more subjects with a wider age range will be employed to refine the statistical template and construct new age-specific templates. The algorithm can also be extended to create template for other types of brain networks, such as the executive control network, the salience network, and the sensorimotor network, etc.

The probabilistic DMN template and the implementation of the similarity measurement will be available online for free download. The proposed DMN template can serve as an unbiased reference for the identification of DMN from resting-state fMRI, and hopefully can be adopted as a useful tool for further research into the brain network.

**Acknowledgement**

The work described in this paper was supported by grants from the Research Grants Council of the Hong Kong Special Administrative Region, China (Project No.: CUHK 475711, 411910, 411811), a grant from the Science, Industry, Trade and Information Commission of Shenzhen Municipality (Project No. JC201005250030A), and a grant from the National Natural Science Foundation of China (Project No. 81101111).

**Figure Legend**

Fig. 1. Initial DMN template

Fig. 2. Flowchart of the DMN generation process

Fig. 3. Anatomical template

Fig. 4. DMN statistical template mapped onto the anatomical template

Fig. 5. Similarities between templates of adjacent stage during the iterations

Fig. 6. Dice coefficients between automatic and manual DMN selection

Fig. 7. Comparison of dice coefficients of the manually selected DMN with the statistical template and the initial binary template

**Reference**

[1] K. Supekar, L. Q. Uddin, K. Prater, H. Amin, M. D. Greicius, and V. Menon, "Development of functional and structural connectivity within the default mode network in young children," *Neuroimage,* vol. 52, pp. 290-301, Aug 1 2010.

[2] F. De Vogelaere, P. Santens, E. Achten, P. Boon, and G. Vingerhoets, "Altered default-mode network activation in mild cognitive impairment compared with healthy aging," *Neuroradiology,* Apr 12 2012.

[3] M. D. Greicius, G. Srivastava, A. L. Reiss, and V. Menon, "Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI," *Proc Natl Acad Sci U S A,* vol. 101, pp. 4637-42, Mar 30 2004.

[4] G. Mingoia, G. Wagner, K. Langbein, R. Maitra, S. Smesny, M. Dietzek*, et al.*, "Default mode network activity in schizophrenia studied at resting state using probabilistic ICA," *Schizophr Res,* vol. 138, pp. 143-9, Jul 2012.

[5] E. Widjaja, M. Zamyadi, C. Raybaud, O. C. Snead, and M. L. Smith, "Impaired Default Mode Network on Resting-State fMRI in Children with Medically Refractory Epilepsy," *American Journal of Neuroradiology,* vol. 34, pp. 552-557, Mar 2013.

[6] C. F. Beckmann and S. A. Smith, "Probabilistic independent component analysis for functional magnetic resonance imaging," *Ieee Transactions on Medical Imaging,* vol. 23, pp. 137-152, Feb 2004.

[7] M. H. Lee, C. D. Hacker, A. Z. Snyder, M. Corbetta, D. Y. Zhang, E. C. Leuthardt*, et al.*, "Clustering of Resting State Networks," *Plos One,* vol. 7, Jul 9 2012.

[8] R. F. Qi, L. J. Zhang, Q. Xu, J. H. Zhong, S. Y. Wu, Z. Q. Zhang*, et al.*, "Selective Impairments of Resting-State Networks in Minimal Hepatic Encephalopathy," *Plos One,* vol. 7, May 25 2012.

[9] C. Rosazza, L. Minati, F. Ghielmetti, M. L. Mandelli, and M. G. Bruzzone, "Functional Connectivity during Resting-State Functional MR Imaging: Study of the Correspondence between Independent Component Analysis and Region-of-Interest-Based Methods," *American Journal of Neuroradiology,* vol. 33, pp. 180-187, Jan 2012.

[10] G. V. Pendse, D. Borsook, and L. Becerra, "A Simple and Objective Method for Reproducible Resting State Network (RSN) Detection in fMRI," *Plos One,* vol. 6, Dec 12 2011.

[11] Y. Deng, Q. H. Dai, and Z. K. Zhang, "Graph Laplace for Occluded Face Completion and Recognition," *Ieee Transactions on Image Processing,* vol. 20, pp. 2329-2338, Aug 2011.

[12] A. R. Franco, A. Pritchard, V. D. Calhoun, and A. R. Mayer, "Interrater and intermethod reliability of default mode network selection," *Hum Brain Mapp,* vol. 30, pp. 2293-303, Jul 2009.

[13] P. G. Samann, R. Wehrle, D. Hoehn, V. I. Spoormaker, H. Peters, C. Tully*, et al.*, "Development of the Brain's Default Mode Network from Wakefulness to Slow Wave Sleep," *Cerebral Cortex,* vol. 21, pp. 2082-2093, Sep 2011.

[14] A. C. Evans, S. Marrett, P. Neelin, L. Collins, K. Worsley, W. Dai*, et al.*, "Anatomical mapping of functional activation in stereotactic coordinate space," *Neuroimage,* vol. 1, pp. 43-53, Aug 1992.

[15] C. J. Holmes, R. Hoge, L. Collins, R. Woods, A. W. Toga, and A. C. Evans, "Enhancement of MR images using registration for signal averaging," *Journal of Computer Assisted Tomography,* vol. 22, pp. 324-333, Mar-Apr 1998.

[16] A. Guimond, J. Meunier, and J. P. Thirion, "Average brain models: A convergence study," *Computer Vision and Image Understanding,* vol. 77, pp. 192-210, Feb 2000.

[17] V. Fonov, A. C. Evans, K. Botteron, C. R. Almli, R. C. McKinstry, and D. L. Collins, "Unbiased average age-appropriate atlases for pediatric studies," *Neuroimage,* vol. 54, pp. 313-27, Jan 1 2011.

[18] R. P. Dhond, C. Yeh, K. Park, N. Kettner, and V. Napadow, "Acupuncture modulates resting state connectivity in default and sensorimotor brain networks," *Pain,* vol. 136, pp. 407-18, Jun 2008.

[19] V. Napadow, L. LaCount, K. Park, S. As-Sanie, D. J. Clauw, and R. E. Harris, "Intrinsic Brain Connectivity in Fibromyalgia Is Associated With Chronic Pain Intensity," *Arthritis and Rheumatism,* vol. 62, pp. 2545-2555, Aug 2010.

[20] F. Esposito, A. Aragri, I. Pesaresi, S. Cirillo, G. Tedeschi, E. Marciano, *et al.*, "Independent component model of the default-mode brain function: combining individual-level and population-level analyses in resting-state fMRI," *Magn Reson Imaging,* vol. 26, pp. 905-13, Sep 2008.

[21] C. C. Guo, F. Kurth, J. Zhou, E. A. Mayer, S. B. Eickhoff, J. H. Kramer, *et al.*, "One-year test-retest reliability of intrinsic connectivity network fMRI in older adults," *Neuroimage,* vol. 61, pp. 1471-83, Jul 16 2012.

[22] Y. Deng, Y. Y. Zhao, Y. B. Liu, and Q. H. Dai, "Differences Help Recognition: A Probabilistic Interpretation," *Plos One,* vol. 8, Jun 3 2013.

[23] C. Habas, N. Kamdar, D. Nguyen, K. Prater, C. F. Beckmann, V. Menon, *et al.*, "Distinct cerebellar contributions to intrinsic connectivity networks," *J Neurosci,* vol. 29, pp. 8586-94, Jul 1 2009.

[24] J. S. Damoiseaux, S. A. Rombouts, F. Barkhof, P. Scheltens, C. J. Stam, S. M. Smith, *et al.*, "Consistent resting-state networks across healthy subjects," *Proc Natl Acad Sci U S A,* vol. 103, pp. 13848-53, Sep 12 2006.

[25] A. Abou-Elseoud, T. Starck, J. Remes, J. Nikkinen, O. Tervonen, and V. Kiviniemi, "The effect of model order selection in group PICA," *Hum Brain Mapp,* vol. 31, pp. 1207-16, Aug 2010.

[26] L. S. Ma, B. Q. Wang, X. Y. Chen, and J. H. Xiong, "Detecting functional connectivity in the resting brain: a comparison between ICA and CCA," *Magn Reson Imaging,* vol. 25, pp. 47-56, Jan 2007.

[27] L. Geerligs, N. M. Maurits, R. J. Renken, and M. M. Lorist, "Reduced specificity of functional connectivity in the aging brain during task performance," *Hum Brain Mapp,* vol. 35, pp. 319-30, Jan 2014.

Dear Editor and Reviewers,

Thank you very much for your supportive and constructive comments. We have thoroughly checked the manuscript and made the modifications accordingly to your suggestions.

Best regards,

Lin Shi

Reviewer's Comments
==========

Reviewer: 1

Comments to the Author
The manuscript entitled Generation of the Probabilistic Template of Default Mode Network Derived from Resting-State fMRI generated a probabilistic template for DMN using resting state fMRI data. This work is interesting and useful to the brain imaging community.
I have several questions as follows:
1. The dataset used to generate the template only includes old people. Does age make a difference to the template?

Reply: The connectivity in DMN has been demonstrated to be changed with normal aging [1]. The DMN template may have differences for different age stages. In our study, old subjects were utilized to generate template. Our developed approach can be directly utilized generate templates for available datasets from subjects with other age stages. The detailed description has been added in the Discussion section (Page 14, Paragraph 2).

[1] Geerligs L, Maurits NM, Renken RJ, Lorist MM. Reduced specificity of functional connectivity in the aging brain during task performance. Human Brain Mapping. 2014 35(1):319-30.

2. How sensitive is the template to the similarity metric and smoothing kernel size used in the manuscript?

Reply: Different from traditional metric, the defined similarity metric measured the distance between the z maps of the independent components. It was specifically designed to characterize the difference of the distributions in two components. Few other similarity metrics have been proposed for measuring the distance of z maps. Our defined metric was non-parametric. In the metric definition, M means the number of voxels, z represents the mean value of z maps and $\delta$ is the standard deviation of the z values in the z map.

3. I'm curious about how the iteration procedure converges. The authors used a hard threshold (95% overlap with previous iteration), and it took 6 iterations to reach that requirement.

It would be helpful to have a table/figure to show how the overlap ratio changes along iteration times. Also, when the iteration converges, does the z-score map of each subject represent DMN?

Reply: Thank you very much for the kind suggestions. Figure 5 has been added as suggested. As the iteration converges, z-score map indicates the DMN for each subject.

4. I can't open the link to the website: http://www.droid.cuhk.edu.hk/web/staff/staff_list/dfwang/dmn.htm

Reply: Many thanks for pointing out this problem. The correct link to the website is as follows.

http://www.droid.cuhk.edu.hk/staff/staff_list/dfwang/dmn.htm

We have updated the link in the manuscript.

5. Does it make sense to up-sample fMRI images to 2mm isotropic considering the original ones are just 2.4mm*2.4mm*3mm?

Reply: In various studies, fMRI images are always registered to a same template for meaningful comparisons. In general, fMRI images are up-sampled or down-sampled to align to a template with similar spatial resolution [1,2].

[1] Di X, Biswal BB.Identifying the default mode network structure using dynamic causal modeling on resting-state functional magnetic resonance imaging. Neuroimage. 2014 86:53-9.
[2]Cha J, Jo HJ, Kim HJ, Seo SW, Kim HS, Yoon U, Park H, Na DL, Lee JM. Functional alteration patterns of default mode networks: comparisons of normal aging, amnestic mild cognitive impairment and Alzheimer's disease. Eur J Neurosci. 2013;37(12):1916-24.

6. How to generate traditional binary DMN (P13, L30) should be more detailed. Probabilistic template may cover more areas than its binary counterpart. Therefore, the overlapping ratio by the probabilistic template might be theoretically larger than that by binary template using the metric at P13.

Reply: We have added more detailed description of binary DMN generation as suggested (Page 12, paragraph 2). The overlapping ratio may not demonstrate the superiority of the statistical template. We have changed to compute the dice coefficients to make a better demonstration. Figure 7 provides the updated dice coefficients of selected DMN component from the statistical template and binary template.

Reviewer: 2

Comments to the Author
This is a well written manuscript wherein the authors propose an automatic procedure for identifying the DMN from ICA results. I have two minor comments which will improve the manuscript:

1. A figure showing the convergence of the iteration is required. Plot iteration on x axis and similarity index on y axis

Reply: Thanks for the king suggestion. We have added figure 5 as suggested.

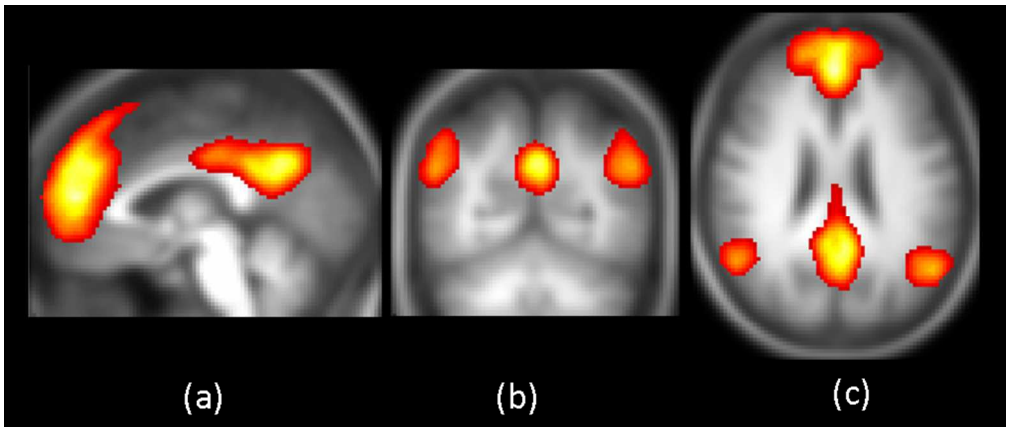
2. The population as well as scan parameters are pretty atypical. The population itself represents only older adults. TR= 3s is pretty long since newer scanners and sequences have made TRs typically lesser than 2 s. Also, 80 volumes is pretty less for a resting state scan. The authors need to comment on the effect of these atypical parameters

Reply: Thank you for the kind suggestion. In our study, only older subjects were included to generate a probabilistic template. The dataset was utilized to evaluate the framework of default mode network template generation. Our approach can also be utilized on datasets from other age stages. The discussion was added to the Discussion section (Page 14, Paragraph 2).

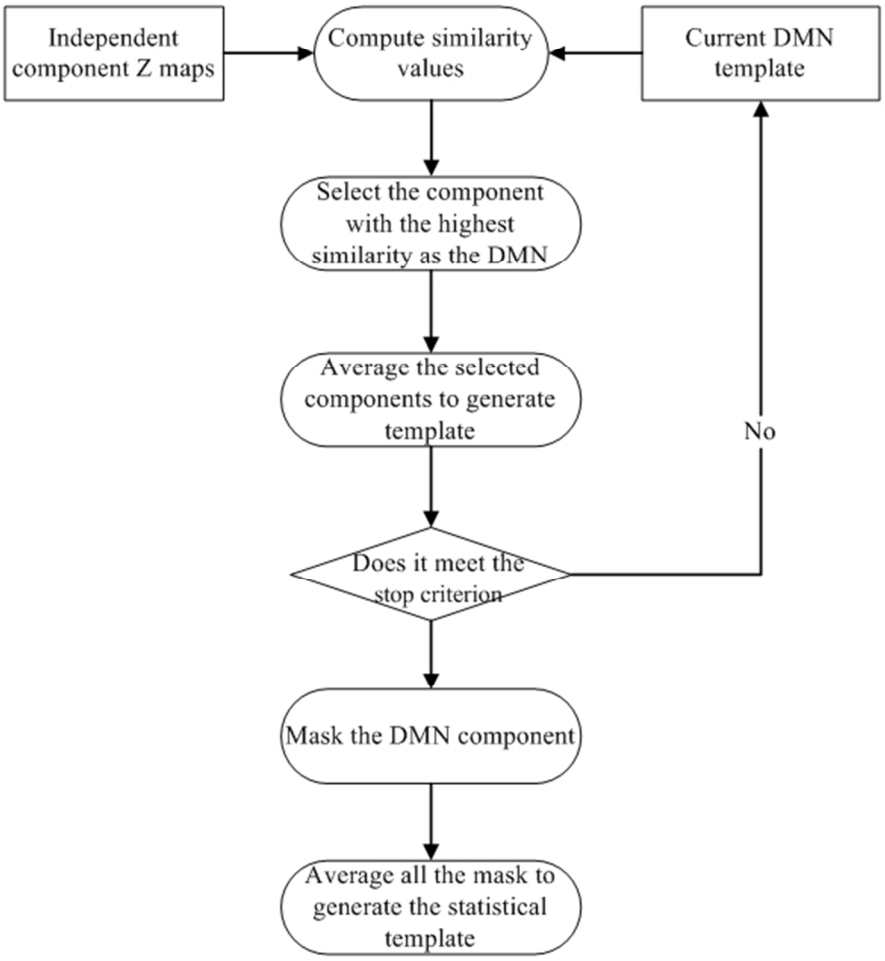
For repetition time (TR), the values range from 2s to 3s in recent studies [1,2]. Two second is a better choice. Thank you for the kind suggestions. We will change our imaging parameters for scanning new subjects.

Eighty volumes are pretty less for a resting state scan. Our experimental results demonstrated that the regions of obtained default mode networks were consistent with previous studies. This may indicate that the acquired volumes were enough for independent component analysis. Some previous studies also utilized 80 volumes rsfMRI for analysis, for instance [3].

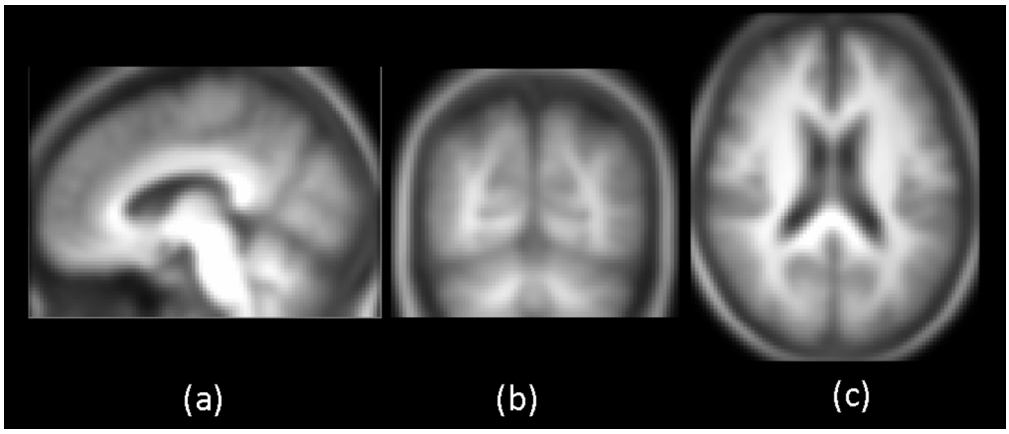
[1] Geerligs L, Maurits NM, Renken RJ, Lorist MM. Reduced specificity of functional connectivity in the aging brain during task performance. Human Brain Mapping. 2014 35(1):319-30.
[2] Cha J, Jo HJ, Kim HJ, Seo SW, Kim HS, Yoon U, Park H, Na DL, Lee JM.Functional alteration patterns of default mode networks: comparisons of normal aging, amnestic mild cognitive impairment and Alzheimer's disease.Eur J Neurosci. 2013 37(12):1916-24.
[3] Alonso-Solís A, Corripio I, de Castro-Manglano P, Duran-Sindreu S, Garcia-Garcia M, Proal E, Nuñez-Marín F, Soutullo C, Alvarez E, Gómez-Ansón B, Kelly C, Castellanos FX. Altered default network resting state functional connectivity in patients with a first episode of psychosis.Schizophr Research. 2012 139(1-3):13-8.



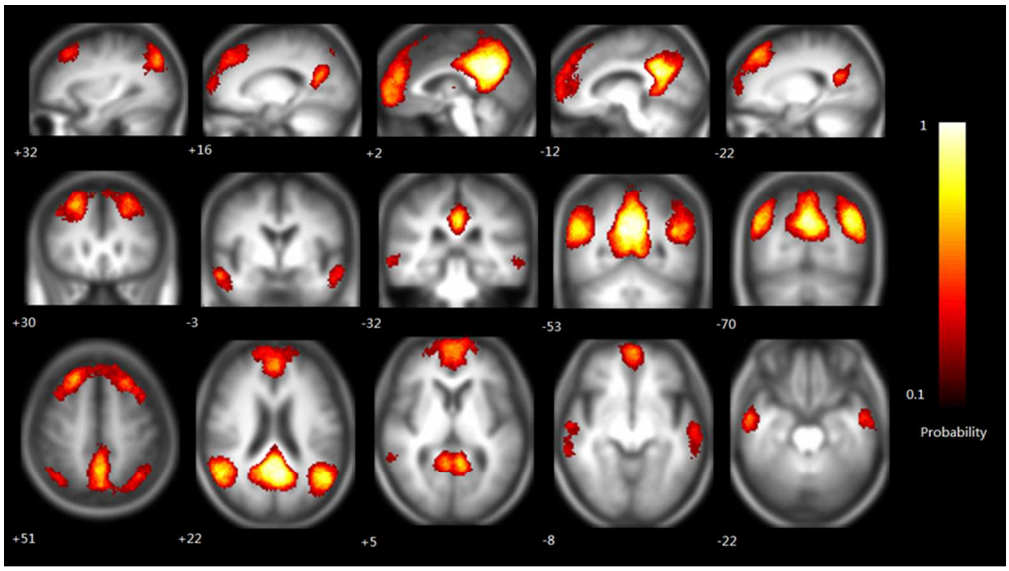

Initial DMN template
85x36mm (300 x 300 DPI)



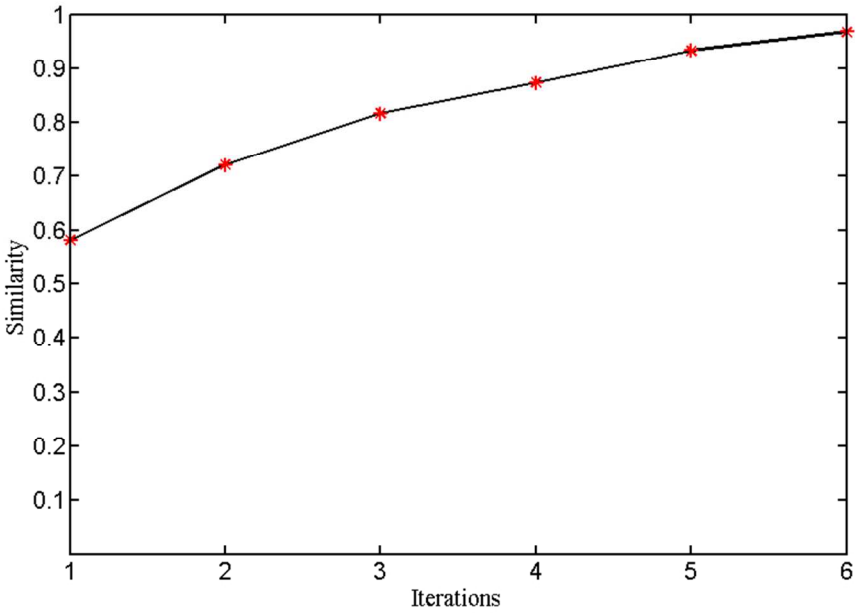

Flowchart of the DMN generation process
175x184mm (96 x 96 DPI)



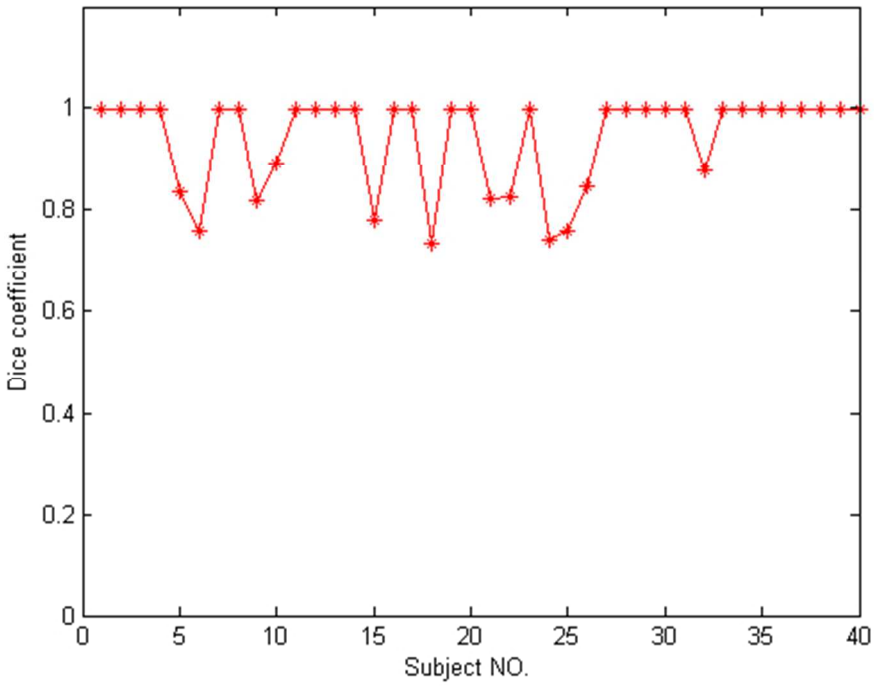

Anatomical template
85x36mm (300 x 300 DPI)



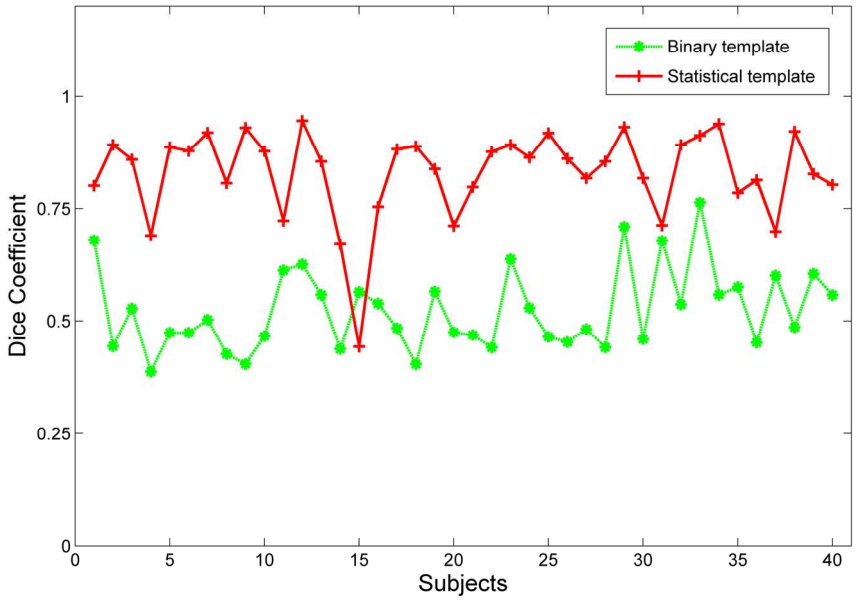

DMN statistical template mapped onto the anatomical template
85x47mm (300 x 300 DPI)




Similarities between templates of adjacent stage during the iterations
241x160mm (96 x 96 DPI)




Dice coefficients between automatic and manual DMN selection
148x111mm (96 x 96 DPI)




Comparison of dice coefficients of the manually selected DMN with the statistical template and the initial binary template
160x106mm (300 x 300 DPI)