## Fuzzy clustering-based segmentation of manganese-enhanced neuronal network areas on MR images

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**Introduction**: Manganese-enhanced MRI (MEMRI) allows in vivo tracing of neuronal connections [1] and may be applied to assess neuronal reorganization after experimental stroke [2]. To measure the amount of tracer accumulation within specific neuronal network areas, manganese enhancement is commonly measured in manually selected regions-of-interest (ROIs). However, ROI analyses may be subject to partial-volume effects. Additionally manual outlines are dependent on contrast settings of viewing tools and introduce bias to certain areas of interest. User bias and partial volume artifacts may be minimized by use of automated voxel-by-voxel analysis to depict manganese enhancement on MR images. In this study we retrospectively analyzed a large MEMRI dataset and compared the competence of four fuzzy clustering algorithms to identify areas of manganese accumulation.

**Materials and methods:** We included a total of 28 adult male Wistar rats from a MEMRI-based neuronal tract tracing study in a rat stroke model[2]. All procedures were approved by our institution's animal care committee. Animals were subjected to 90-min unilateral middle cerebral artery (MCA) occlusion [2]. Seven sham-operated rats served as controls. At 2 (n=7), 4 (n=7), and 10 (n=7) weeks post-stroke 0.2 µl 1 mol/L $MnCl_2$ solution was injected in the perilesional sensorimotor cortex. MRI was acquired on a 4.7 T horizontal bore MR spectrometer (Varian Instruments, Palo Alto, CA, USA) with a Helmholtz volume coil (90-mm diameter) and inductively coupled surface coil (25-mm diameter) for signal excitation and detection, respectively. Saturation recovery T1-weighted gradient-echo MRI with seven TRs (TR/TE = 55 to 3,000/18ms; acquisition matrix 128x128; 15 coronal slices; voxel dimensions = 0.19x0.19x1.5mm$^3$; number of averages = 2) was performed 2 days before tracer injection and 4 days after tracer injection. Quantitative pre- and post-manganese R1 (=1/T1) maps were calculated for measurement of manganese-induced ΔR1. Post- and pre-manganese R1 maps were co-registered to a common rat brain template using a semi-automatic co-registration tool (MNI, Autoreg), and subsequently subtracted. Resultant ΔR1 maps were pre-processed by masking out brain surrounding tissue and thresholding between values of -0.5 and 3.0 s$^{-1}$.

Fuzzy clustering was applied to identify regions of manganese enhancement on the pre-processed ΔR1 maps. Fuzzy logic allows for modeling of partial volume by assigning a confidence measure of a voxel 'belonging' to a particular cluster. Four different fuzzy clustering approaches were compared. A conventional fuzzy C-Means (FCM) [3] without any spatial contiguity constraints was tested to a Markov random field (MRF)-based spatial contiguity constrained FCM approach (GFCM) [4], where the MRF incorporated neighboring pixels in assigning confidence measures to each voxel. A third method used a linearly weighting neighborhood homogeneity measure to create a pre-calculated image which was then clustered using a conventional FCM approach (FGFCM) [5]. A fourth method was a combination of the latter two methods (FGFCM-MRF). All methods were pseudo-randomly initialized and conducted with a clustering of 5 clusters, of which one cluster depicted the regions with prominent manganese enhancement. This cluster's confidence map was selected for further processing. Binary manganese-enhanced outlines were obtained after excluding all voxels with confidence level below 0.1 and additional spatial pruning. Resulting outlines were each compared to expert-reviewed manual outlines of areas with unequivocal manganese enhancement, which served as a reference for performance calculation. Dice's similarity index (SI) [6] and the area under the ROC curve (accuracy) were used as indicators of performance. A SI of 0.7 denotes excellent agreement [7]. One-way ANOVA with post-hoc LSD testing was used to compare the methods' performance.

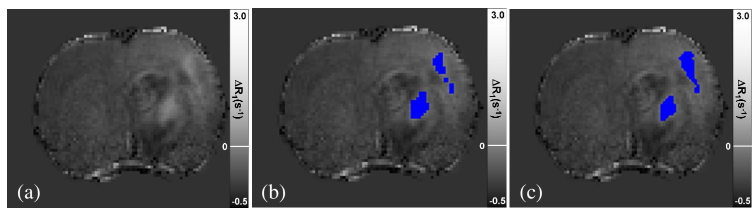


Figure 1: ΔR1 map of rat brain slice 2 weeks after unilateral stroke and 4 days after $MnCl_2$ injection in perilesional sensorimotor cortex. Major manganese-enhanced areas are depicted by binary maps of manual outlines (b) and from FGFCM clustering (c)

**Results:** All 28 datasets were segmented by all four methods to obtain a total of 112 binary outlines. Figure 1 shows an example ΔR1 map of a rat brain 2 weeks after unilateral stroke and 4 days after tracer injection in the perilesional sensorimotor cortex (a), with manual outlines (b) and FGFCM-MRF clustering-based maps of major manganese-enhanced areas (c). Manganese accumulation is characterized by elevated ΔR1. All clustering methods were able to indicate areas of manganese enhancement. The resulting binary maps were each compared to the corresponding manual outline and performance wise evaluated by distinguishing the rightfully and wrongfully classified voxels. Methods with spatial contiguity demonstrated significantly ($p<0.05$) better performance compared to conventional FCM (figure 2). The use of neighborhood homogeneity weighting showed significantly better overlap as compared to FCM; GFCM displayed increased overlap. MRF modeling showed significantly better performance in accuracy compared to FCM. FGFCM-MRF gave best performance with an overlap SI of 0.59 and accuracy of 0.83, both significantly better compared to a conventional FCM approach. FCM showed least performance with 0.50 overlap and 0.76 accuracy.

**Discussion:** Four fuzzy clustering methods were evaluated for identifying regions of manganese enhancement. Spatial contiguity proved to be most valuable, with a highest degree of performance validated against manual outlines. Combining both MRF modeling and neighborhood weighting gave best performance.

Manual outlining is often dependent on contrast settings of the viewing tool, causing differences in perception of manganese enhancement. This could lead to over- or underestimation of areas of manganese accumulation. Voxel-by-voxel analyses evaluate each voxel based on its intensity which is fixed and deprived of user bias.

**Conclusion:** This study compared four fuzzy logic based voxel-based segmentation methods for unbiased delineation of regions of manganese enhancement. Of all methods the use of spatial contiguity weighting in combination with Markov random field modeling showed to be most applicable for depicting areas of manganese accumulation on MR images.

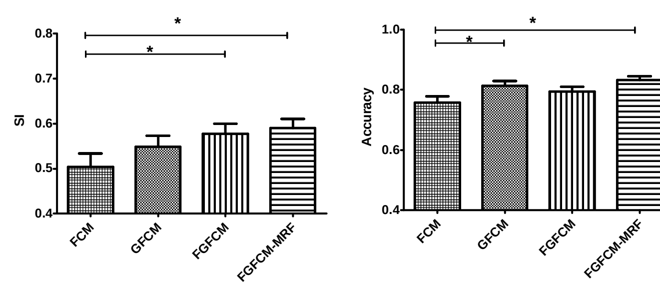


Figure 2: Performance measures after comparing method output with manual outlines. (a) denotes the overlap (Similarity Index (SI)), (b) denotes the accuracy of each method. The error bars indicate standard error of the mean. * $p<0.05$.

**References:** [1] Pautler R, et al. *Magn Reson Med.* 40:740–8,(1998). [2] Van der Zijden J, et al. *Cereb Blood Flow Metab.* 28:832-40,(2008). [3] Bezdek J, et al. *pattern recognition with fuzzy objects*,(1981). [4] Cai W, et al. *Pattern Rec.* 40:825-838,(2007). [5] Feng Y, et al. *LNCS.* 3150,188-196,(2004). [6] Dice L, *Ecology*.26:297-302,(1945). [7] Bartko J, et al. *Schizophrenia*. 17:483-489,(1991).