Appendix 1

The code was uploaded to GitHub: https://github.com/easyCEST/KALE.git.

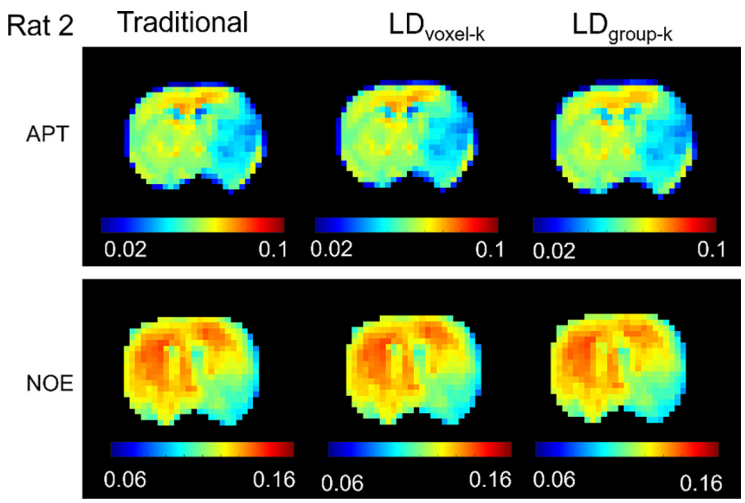
As shown in *Figure S1*, the Z-spectra image stack was clustered into three groups in Layer 1, while only the background was fine-clustered. Then, the other two groups were further clustered with the LF parameters, *Para*, passed to the next layer as the initial values of fitting. Finally, after five layers of clustering, all subclusters were fine-clustered.

Compared to voxelwise LF, which uses fixed initial values and boundaries for all voxels, KALE has varied initial values and boundaries among clusters, and these values are optimized and updated progressively as the cluster size decreases. *Figure S4* illustrates more clearly that the voxelwise LF could have inaccurate fitting in some voxels. In comparison, KALE allows for more adaptive and intelligent fitting through multilevel voxel clustering.

IDEAL produced smoother amplitude maps than the traditional method. The CNR of IDEAL is comparable to that of KALE for the NOE contrast map, ~1.47 lower for amides and ~0.40 higher for amines (*Figure S5E*). Compared to IDEAL, the principle of the SNR improvement through the regional updating of fitting parameters is the same. However, KALE is slightly more intelligent because it clusters the voxels according to their structural correlations, while the smoothness of IDEAL was squarish in some areas. The R^2 values of IDEAL, the traditional LF method and KALE were 0.9605, 0.9924 and 0.9928, respectively. The computation times of IDEAL and KALE were 65.14 s and 20.98 s on data from this rat.

We used the unmasked brain image with muscles and vessels to test the performance of KALE on outlier voxels. As demonstrated in *Figure S6*, the internal carotid artery (circle 1) and the vertebral arteries (circle 2) show high intensity in both the traditional LD APT contrast map and the APT map of KALE. The unmarked side of the internal carotid artery shows lower intensity, probably due to the partial volume effect. For groupwise and voxelwise KALE, clustering was robust to outlier voxels.

LD results on data from all rats (Rat 1 in *Figure 3*).



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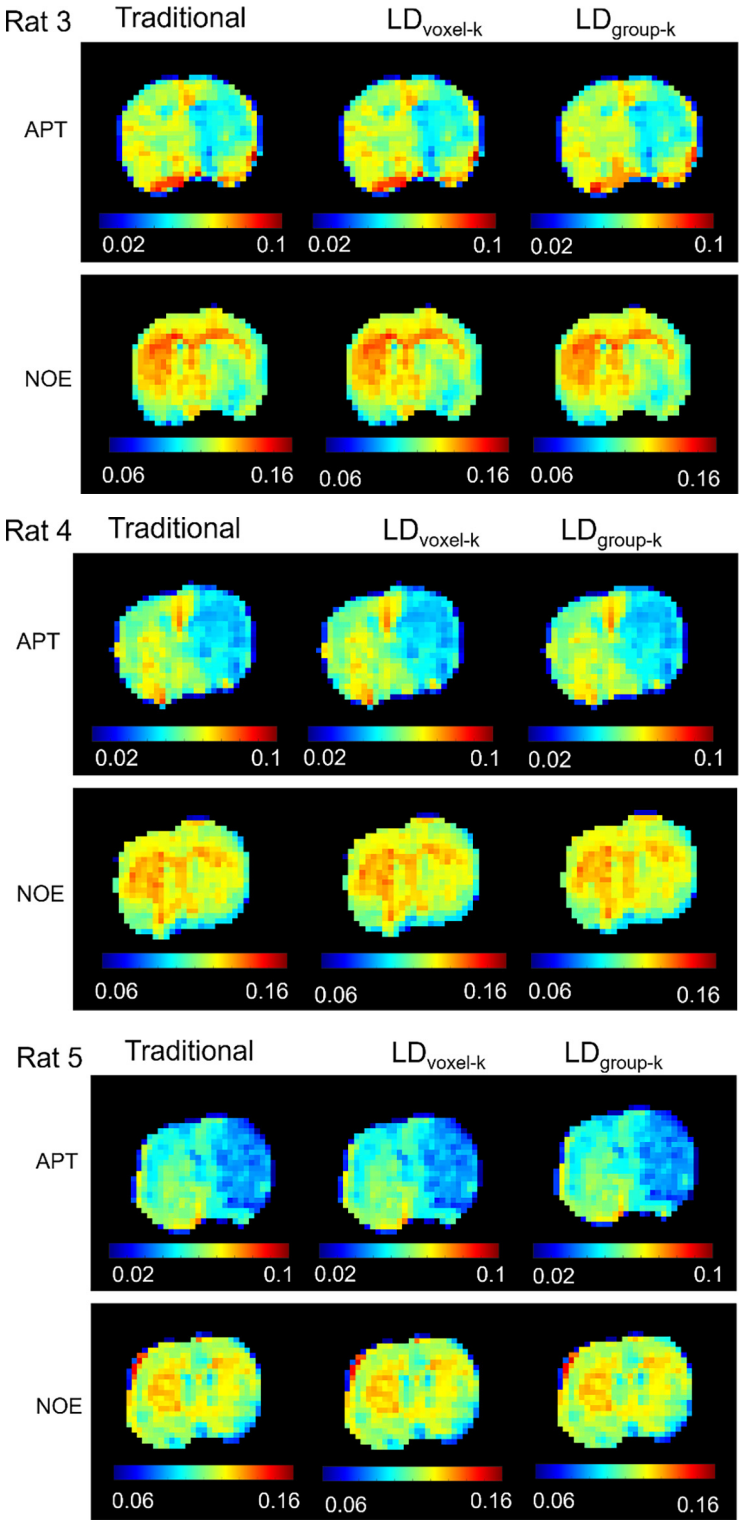

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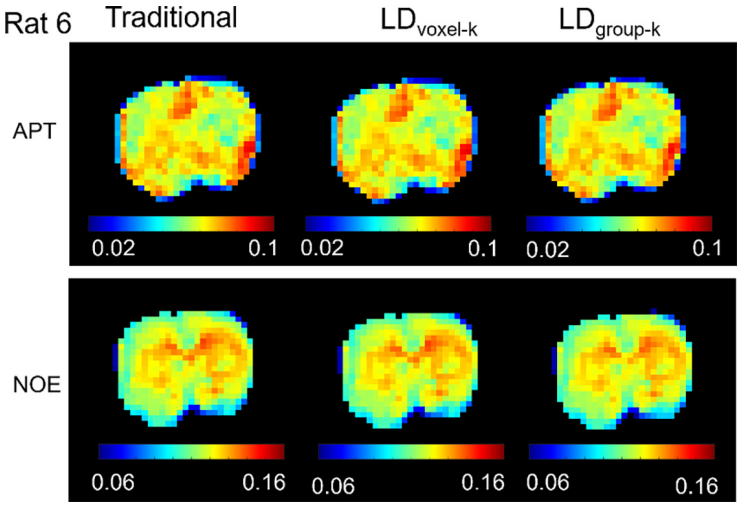
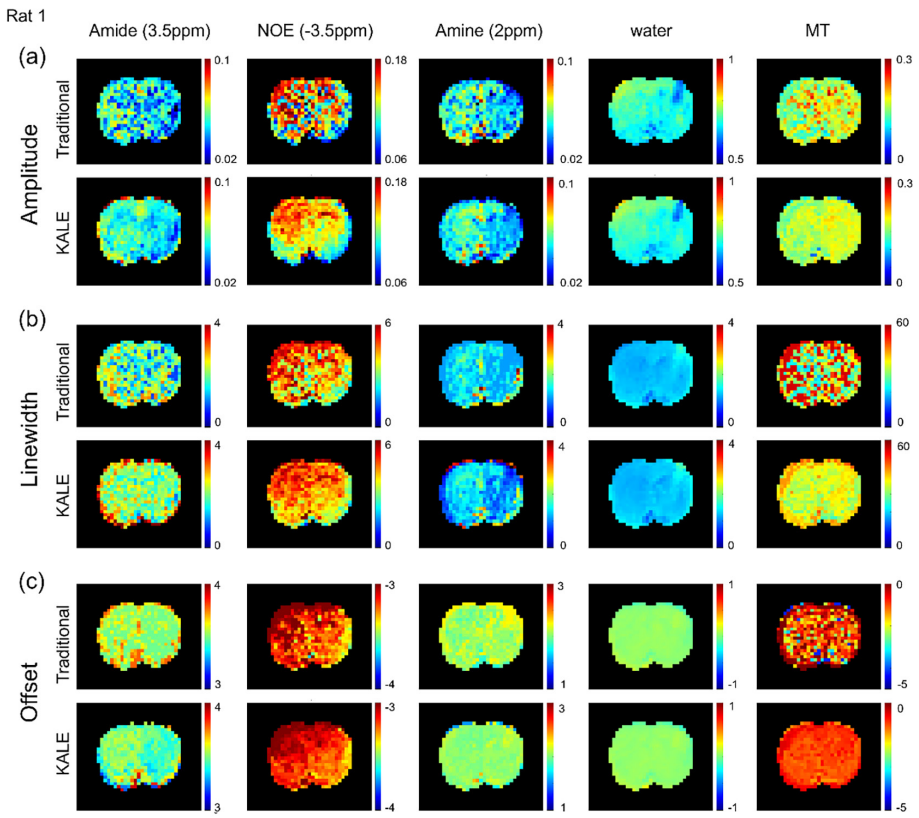


Five-pool Lorentzian fitting results on data from all rats (Rat 2 in *Figure 5*).



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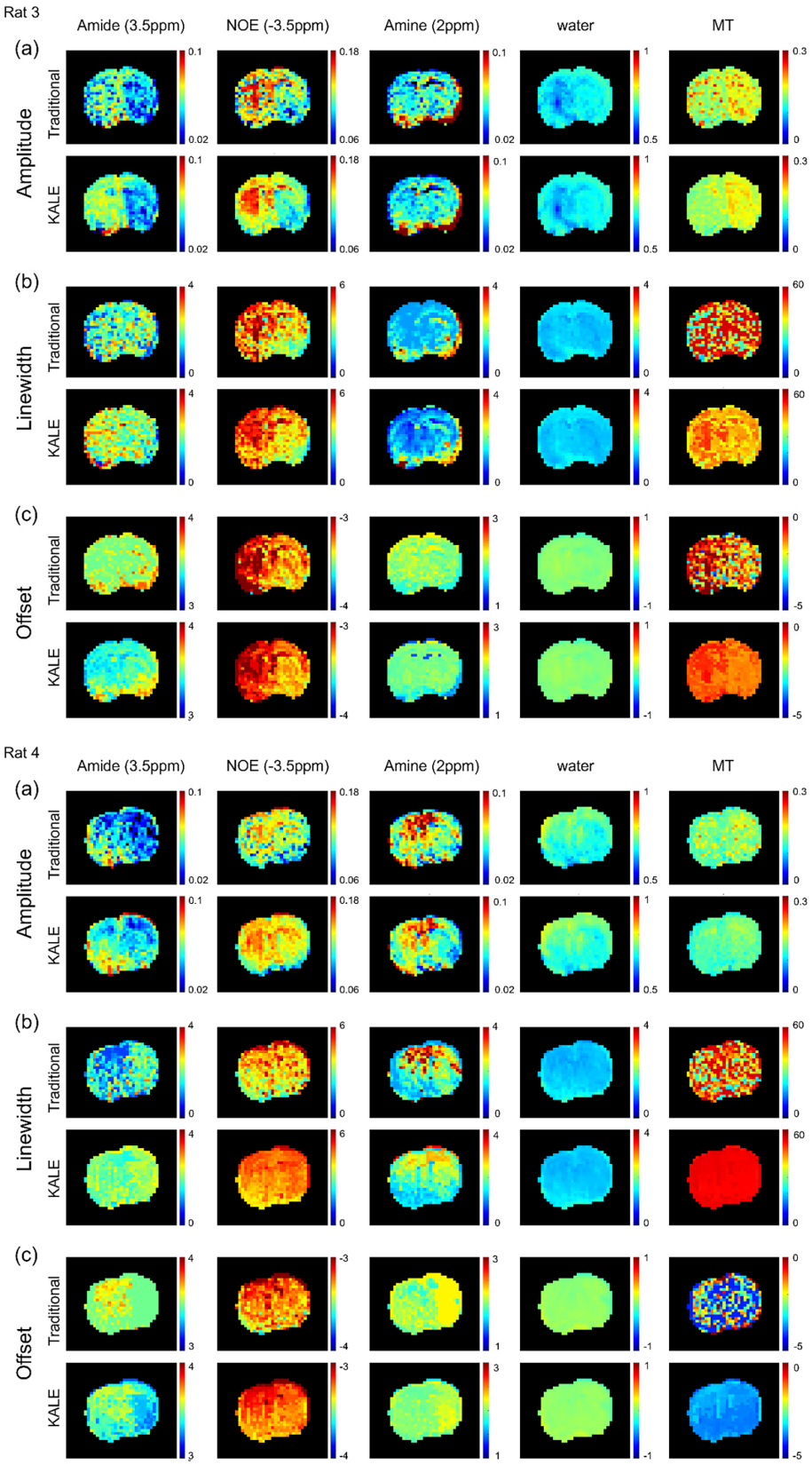
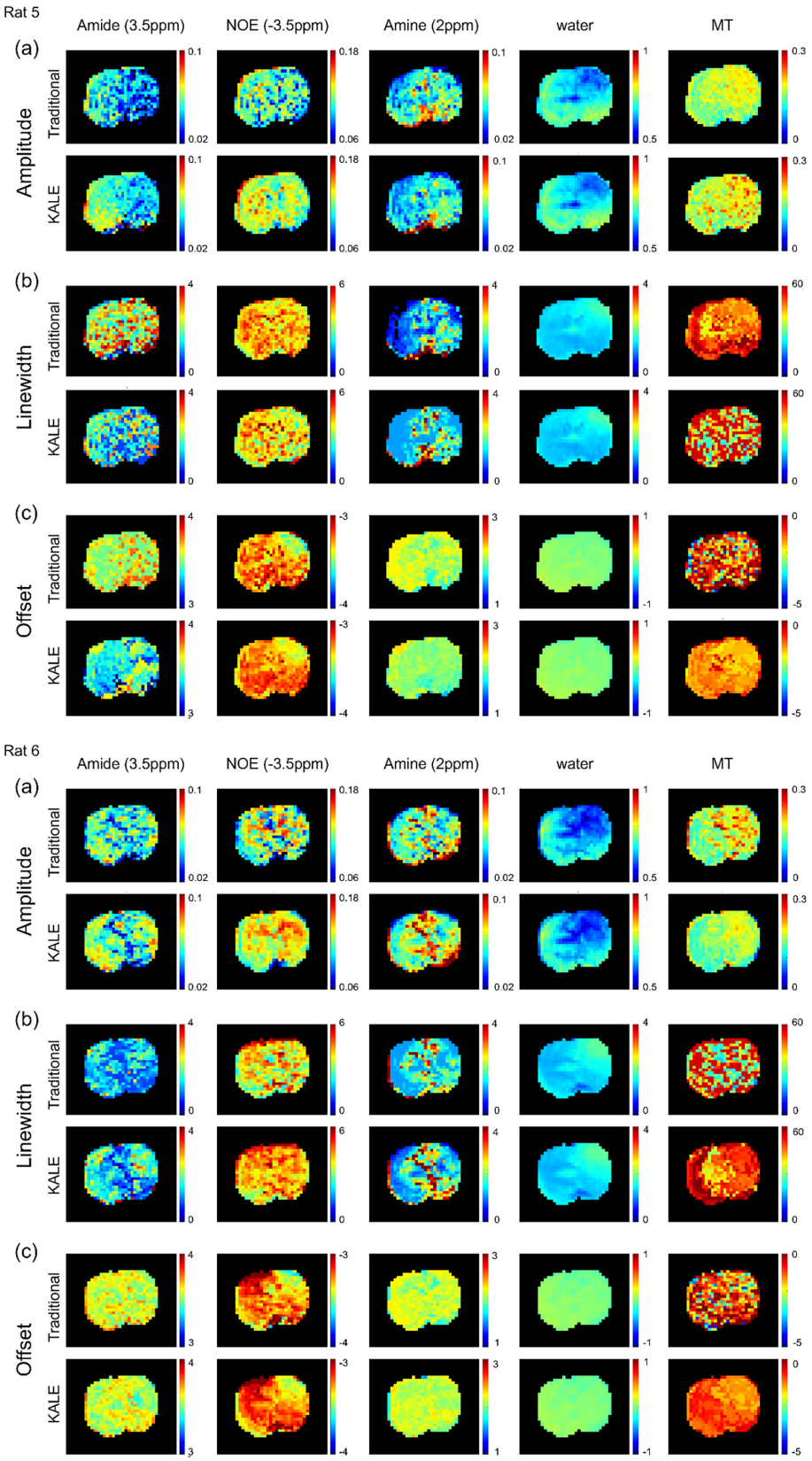


https://dx.doi.org/10.21037/qims-22-1379



https://dx.doi.org/10.21037/qims-22-1379

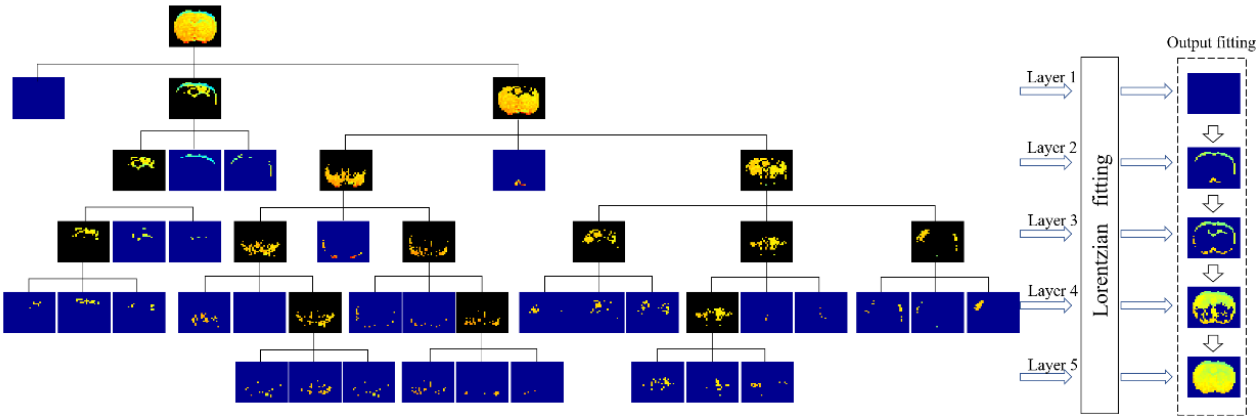


Figure S1 A demonstration of KALE implemented on data from an IR rat brain. The blue background represents fine-clustered groups. The black background denotes groups that need to be further clustered. In the BFS strategy, each layer produces an image merged from fine-clustered groups in the layer and is integrated with the fine-clustered image generated from the previous layers.

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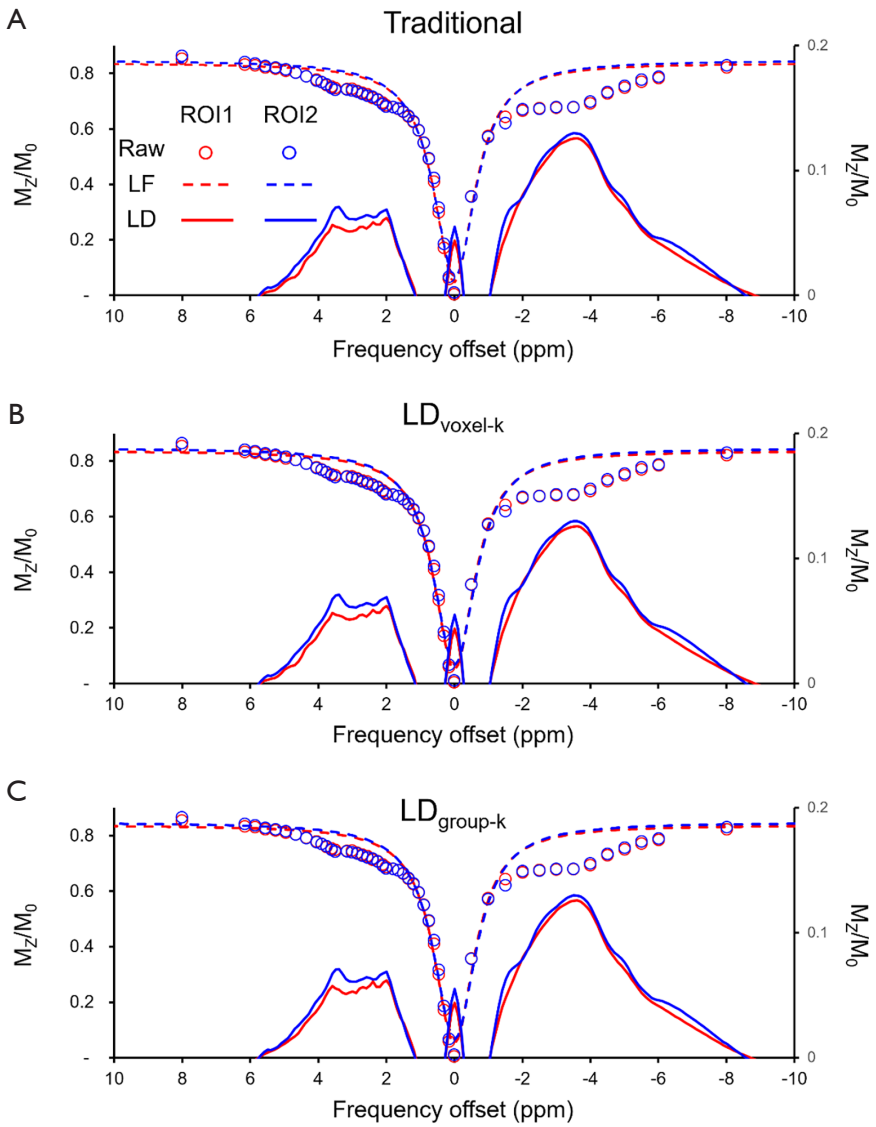

Figure S2 The raw data and the spectra of the two ROIs plotted in *Figure 3A*. (A) The fitted Lorentzian line shape and the corresponding Lorentzian difference spectra by traditional LD. (B) The fitted Lorentzian line shape and the corresponding Lorentzian difference spectra by voxelwise KALE ($LD_{voxel-k}$). (C) The fitted Lorentzian line shape and the corresponding Lorentzian difference spectra by the groupwise KALE ($LD_{group-k}$). The ROI on the lesion side (ROI1)-related data are colored red, and the contralateral ROI (ROI2) is colored blue. The raw data are plotted as circles. The Lorentzian fitting (LF) is plotted as dashed lines. The Lorentzian difference (LD) is plotted as solid lines on the right vertical axis.

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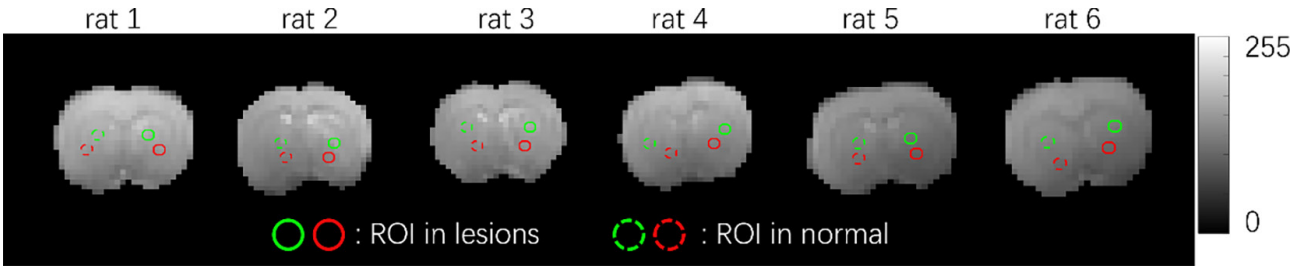

Figure S3 ROIs for CNR calculation. Two sets of ROIs for each rat.

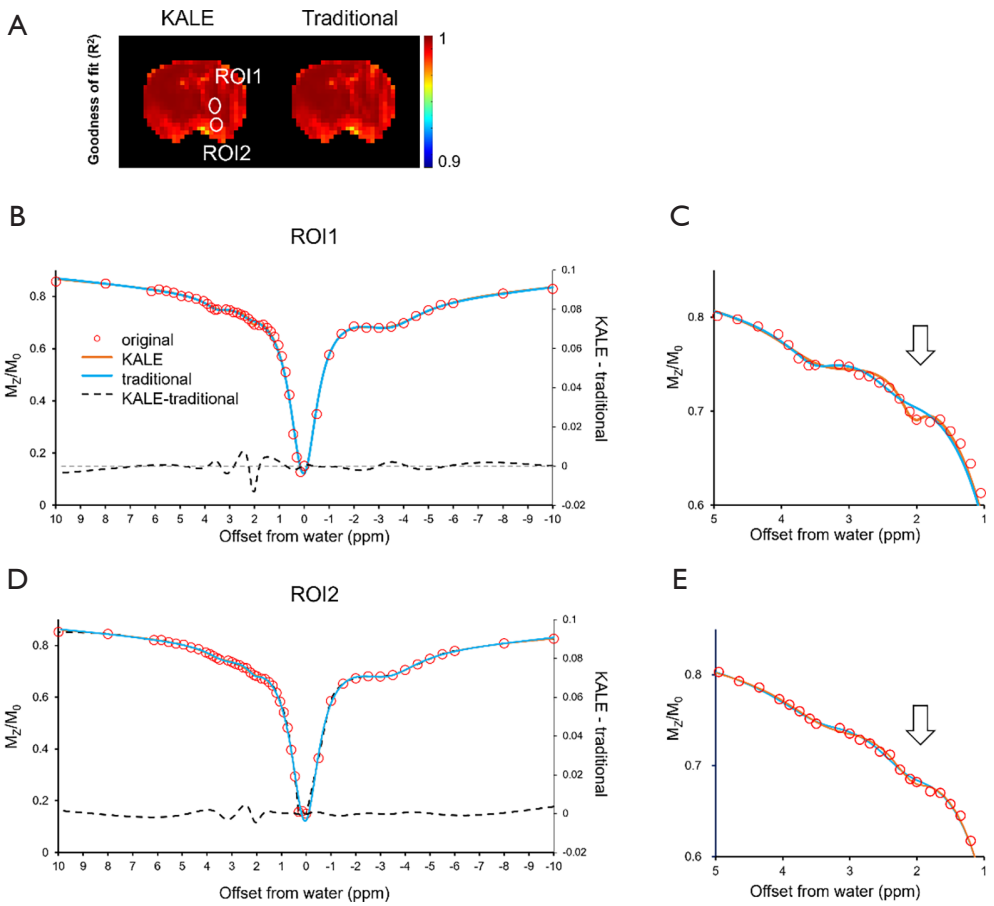

Figure S4 The R^2 maps of the two methods in five-pool fitting and the fitted spectra. (A) The R^2 map of KALE and the traditional method. ROI1 plotted on KALE marked the region with a higher R^2 value on the KALE map. ROI2 plotted on the region with comparable R^2 for the two methods. (B) ROI1: The original data points (red circle), the fitted spectrum by KALE (orange line), the fitted spectrum by the traditional method (blue line), and the difference in the spectrum obtained by the two methods (dash line) plotted by the right vertical axis. (C) Data within 1 to 5 ppm of (B). (D) ROI2: The original data points (red circle), the fitted spectrum by KALE (orange line), the fitted spectrum by the traditional method (blue line), and the difference in the spectrum obtained by the two methods (dash line) plotted by the right vertical axis. (E) Data within 1 to 5 ppm of (D). The arrows in (C,E) indicate the different fitting performance of the traditional method around 2 ppm on different voxels.

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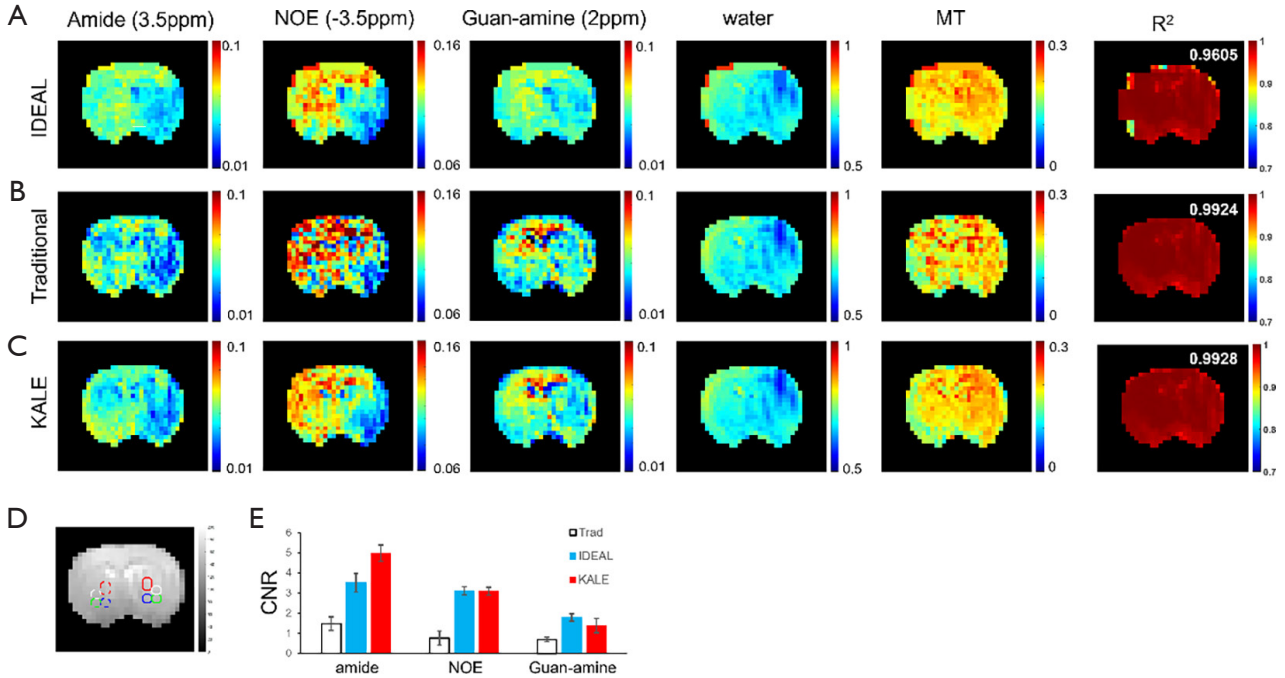

Figure S5 The amplitude maps and the corresponding R^2 maps of the three methods. Each row from left to right, the amide, NOE (-3.5 ppm), amine (2 ppm), water and MT amplitude map, and the R^2 map. (A) Results of IDEAL. (B) Results of the traditional LD method. (C) Results of KALE. (D) Four sets of ROIs plotted on the lesioned (solid lines) and normal (dashed lines) hemispheres. (E) CNR of the three methods on the amide, NOE and amine amplitude contrast maps. Blank bars denote the traditional method, blue bars denote IDEAL and red bars denote KALE.

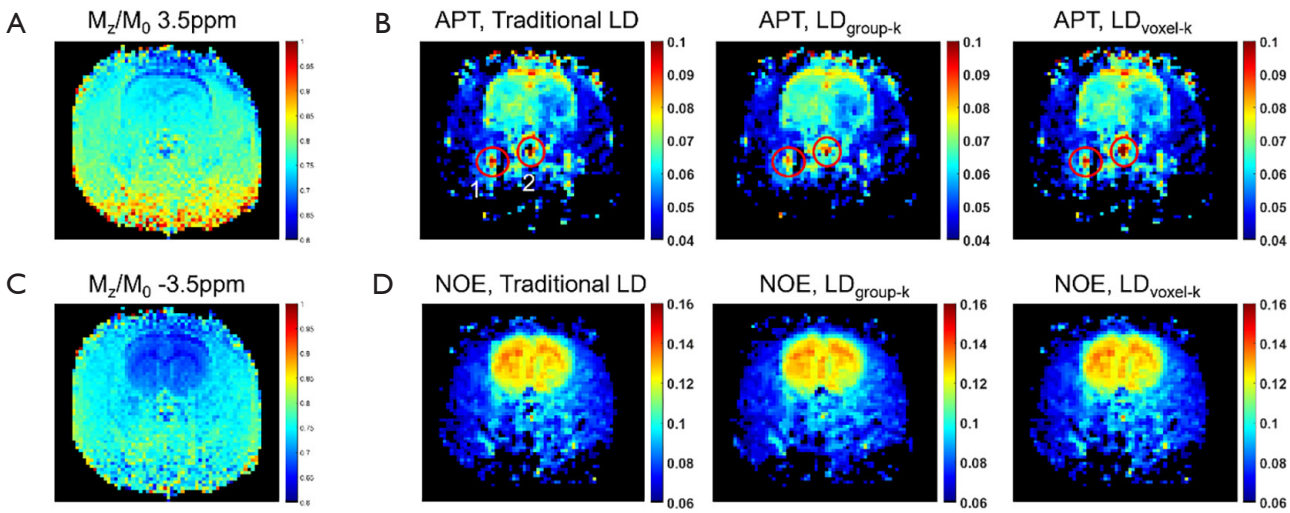

Figure S6 Experiments on the data from the whole brain of a rat. (A) The saturated image at 3.5 ppm. (B) From left to right, APT contrast maps by the traditional LD method, groupwise KALE, and voxelwise KALE. (C) The saturated image at -3.5 ppm. (D) NOE contrast maps with the same layout as in (B).

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