3D superresolution EPR imaging of nitroxyl radicals in mice

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INTRODUCTION

One of the most important problems in electron paramagnetic resonance (EPR) imaging is the low spatial resolution of reconstructed images. Recently, a post-processing technique for deblurring EPR images called superresolution EPR imaging has been reported (1,2). However, this technique has been tested with only in vitro phantoms. We applied the superresolution technique to three-dimensional (3D) continuous-wave (CW) EPR imaging of live mice. This post-processing technique could improve the spatial resolution of 3D EPR images.

METHODS

After reconstructing 3D EPR images, a post-processing iterative deconvolution technique was applied to the blurred images. The main reason for the blurring was a lower cut-off frequency of a window function used in image reconstruction. We assumed that point spread function could be estimated from the inverse Fourier transform of the window function. In iterative deconvolution processing, iterative calculation was stopped when a shift in error between an estimated low-resolution image and the initial blurred image reached a value that was less than the given threshold. A multi-coil parallel-gap resonator (24 mm in diameter and 34 mm in length) was used in a 650-MHz home-built CW-EPR imager (3). We employed the uniform distribution of projections and used a one-step image reconstruction (4).

We used anesthetized ICR mice, which were all male, 6 weeks old, and weighed 30 g as subjects for 3D EPR imaging. We administered 0.2–0.25 ml solution of 200 mM 3-hydroxymethyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (hydroxymethyl-PROXYL) spin probes intraperitoneally and measured the lowest field absorption peak of hydroxymethyl-PROXYL. We performed EPR imaging under the following conditions: 46 projections, scan time of 0.5 s, time constant of a lock-in amplifier of 1 ms, microwave power of 32 mW, magnetic field modulation of 0.1 mT, field gradient of 40 mT/m, field scanning of 2 mT, and number of averaging of 2. The image size was 128 x 128 x 128 voxels and the field-of-view was 50 mm. Iterative calculation was stopped at the 9th iteration when a shift in error of computed images was less than the threshold of 10^{-3}. The computation code for image reconstruction and iterative deconvolution was written in FORTRAN language (Pro Fortran 9.2, Absoft Corp., Rochester Hills, MI) on MacOS X. Surface-rendered images were drawn using IDL 6.4 data visualization software (ITT Visual Information Solutions, Boulder, CO).

RESULTS

Figure 1a shows a surface-rendered EPR image of a mouse's head. Since the axial length of the resonator was 34 mm, the section from nose to neck of the subject mouse was visualized. The isosurface level that was used for surface rendering of 3D images was set at half the maximum intensity of the image. In Fig. 1, the dorsal side of the mouse corresponds to the upper side of the surface-rendered images. The image in Fig. 1b was blurred due to a lower cut-off frequency that was chosen to suppress noise in the reconstructed image. We then applied the superresolution technique to this 3D blurred image. Figure 1b shows the superresolution EPR image that was generated from the blurred image in Fig. 1a. The superresolution EPR image shows more realistic profiles of a mouse's head. In the blurred image, we cannot recognize the shape of the eyes. However, those profiles can be recognized in Fig. 1b. Since there are cavities in both ears of the mouse, those places should have lower signals. However, there are no such cavities in Fig. 1a. In contrast, obviously low-signal areas near the neck can be seen in Fig. 1b. This image agrees with the anatomical structure of a mouse's head.

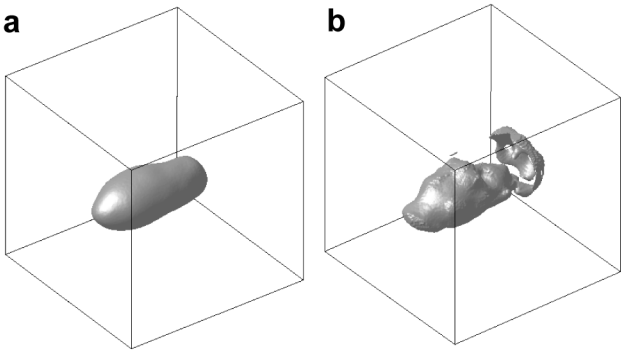

Fig. 1. Surface-rendered EPR images of mouse head. **a:** Blurred image and **b:** superresolution image.

DISCUSSION

Experimental evidence showed that superresolution EPR imaging with administered hydroxymethyl-PROXYL spin probes can create a detailed image of a mouse's head. Our results support that the superresolution EPR imaging technique can be applicable to in vivo 3D animal imaging.

CONCLUSION

We demonstrated 3D superresolution EPR imaging with living mice. This technique can enhance the spatial resolution of 3D blurred images of in vivo animal experiments. This approach helps solve a problem of EPR imaging so that measuring EPR images with broader line-widths, such as NO-adduct complexes, can be more effective.

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