

# SPIO MRI Contrast Can Be Enhanced by Off-resonance Saturation

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## Introduction

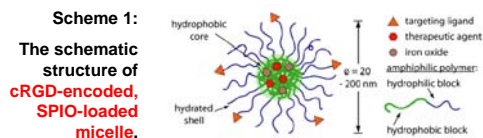
- The growing use of **SPIO particles** as MRI contrast agents has resulted in the development of newer formulations with different coatings and particle size.
- Recent work from our lab has focused on the preparation of SPIO encapsulated in **micelles** prepared from **block copolymers**.
- The efficacy of such agents in shortening water **T<sub>2</sub>** and **T<sub>2</sub>\*** depends upon several parameters including the **magnetic susceptibility** of the SPIO particle and the **number and the distribution** of water molecules residing near the hydrophilic polymeric layer which wraps the SPIO core.
- The MRI method for detecting SPIOs in tissue is generally **T<sub>2w</sub>**, with **SPIO distribution** shown as a dark spot. This method falls short in differentiating SPIO from a **tissue void** with low proton density. In addition it is generally more vulnerable to **motion artifacts**.
- Off-resonance pre-saturation** is used in magnetization transfer (MT) imaging and sequence is widely available on clinical MRI scanners.

We demonstrate here that off-resonance pre-saturation can enhance SPIO MRI contrast by using spin density sequences with short TE.

## Methods

### SPIO-micelle targeting SLK cells

- Sample preparation:** Cultured SLK cells (1 million) were incubated with media containing SPIO-micelles or cRGD-SPIO-micelle for 1h at 37°C, followed by washing, trypsinization and neutralization, and then re-suspended in PBS/para-formaldehyde and incubated for 1h at 4°C. After washing and centrifugation, 200  $\mu$ l of 2% agarose solution was mixed with the cells and the mixture was transferred into 384 micro-well plates (3x3x10mm<sup>3</sup>) and kept overnight at 4°C prior to MRI.
- MRI images** were acquired on a 4.7T animal scanner (Varian).
- Samples were placed in a Litz TR coil (Doty) and scanned using a SE sequence (TR/TE: 6s/9ms) with a pre-saturation pulse (B1 power level: 105 Hz; duration 500 ms).
- The **pre-saturation offset frequency** was either 2.0, 0.4 or 0.2 kHz from water.
- Other imaging parameters:** single 5mm slice, FOV = 30x30 mm, 128x128 data points.
- The **ratio images** (Fig. 1 right) were obtained by ratio of saturation-on image intensity divided by saturation-off image intensity on a pixel-by-pixel basis.



Off-resonance saturation enhances detection of SPIO-micelles in proportion to the SPIO content.

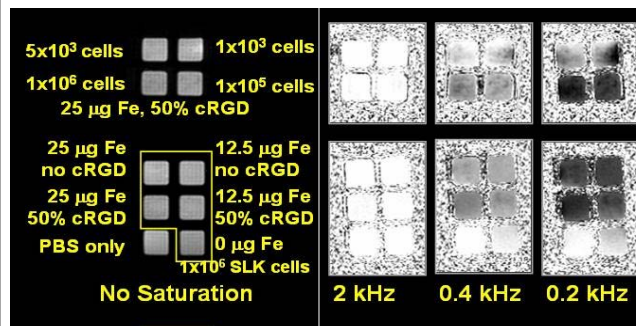


Fig. 1: 4.7T **SDw images** of SLK cancer cells incubated with varying amounts of cRGD-encoded SPIO-micelles. The left panel shows the image with pre-saturation pulse turned-off. The right panel shows ratio images of saturation-on images divided by saturation-off images on a pixel-by-pixel basis. White represents no enhancement (ratio = 1, as illustrated by the PBS sample), while the black represents the largest enhancement (SPIO-labeled cells).

Off-resonance saturation showed sensitivity improvements in detecting SPIO-labeled cells.

### SPIO-micelle injections in mouse brain

- 3 SPIO-micelle injections** (25 ug Fe/ml, 10 $\mu$ l (location 1), 7.5 $\mu$ l (location 2) and 5 $\mu$ l (location 3)) were given to a post-mortem mouse brain at widely-spaced locations but in **one axial plane**.
- SDw images** (TR/TE: 4s/12ms) were acquired at 4.7T in the plane containing the SPIO particles with the **pre-saturation pulse turned-off** (Fig. 2, left) and **-on** (right) at power level of 105Hz (duration 500ms).

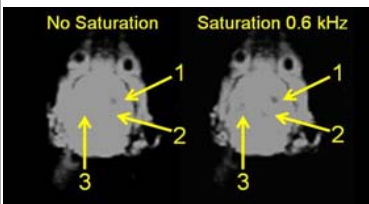


Fig. 2: 4.7T axial **SDw images** of a mouse brain injected with SPIO-micelles, with pre-saturation pulse turned-off (left) and **-on**. Note that the site with the lowest volume of SPIO is not visible on saturation-off image (left) but was detected in saturation-on image (right).

Off-resonance saturation improves the detection of SPIO-micelles in mouse brain.

### SPIO-micelle injections in implanted A459 tumors model

- 5 SPIO-micelle injections** (15  $\mu$ L each) were given to A459 xenografted tumors planted in nude athymic mouse on abdominal area at flanked positions, **3 injection sites in the left tumor** (major) and **2 injection sites in the right tumor** (minor) at well-separated locations but within **one axial plane**.

- MR images of mouse tumor pre- and post- SPIO-micelle injection were acquired on 4.7T scanner using a pre-saturation pulse-on or -off (Fig. 3)
- Other imaging parameters: TR/TE: 2s/12ms; FOV 40x40mm; 128x128 data points; single slice: 3 mm
- The two solid tumors appear bright in the water suppressed axial image (Fig. 3B).
- The SPIO injection sites were barely visible in the SDw images (Fig. 3 C). However, the difference image (saturation-off minus saturation-on) shows clearly all five injection sites. Parameters used: offset frequency 0.2 kHz, B1: 150 Hz, duration 500 ms.

All five SPIO injection sites were clearly detected in the difference image.

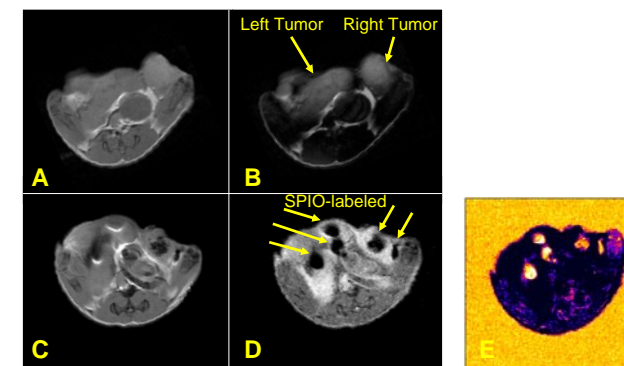


Fig. 3: 4.7T axial **MR images** (TR/TE: 2s/12ms) of a female nude athymic mouse with xenograft tumors (A459) grown in the lower abdominal area. (A) **Pre-injection** image with saturation pulse turned off; (B) **pre-injection** image with water suppression to enhance the tumor contrast; (C) **post-injection** image with saturation pulse-off; (D) difference image, pre-saturation pulse-off minus pulse-on (B1: 105 Hz; duration 500 ms) and (E) inverted color image of (D).

## Conclusions

- Off-resonance saturation can be used to enhance SPIO detection from the surrounding background such as brain and tumor tissue by using short TE pulse sequence (9 -12 ms) and soft saturation pulses (B1: 100-150 Hz; duration 200-500 ms)
- Further experiments will be needed to integrate off-resonance pre-saturation pulses with fast imaging sequences on high-field scanners.

## References:

Al, H.; Flask, C.; Weinberg, B.; Shuai, X.; Pagel, M. D.; Farrell, D.; Duerk, J.; Gao, J. M. *Advanced Materials* 2005, 17, 1949-1952.

## Acknowledgements

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