Olivia C. Sehl[1,2] Brice Tiret[3] Paula J. Foster[1,2]

# Region of Interest (ROI) Analysis for Magnetic Particle Imaging

Magnetic particle imaging (MPI) is a quantitative modality that directly detects superparamagnetic iron oxide nanoparticles. Careful and rigorous choice of the region of interest (ROI) is important for quantification. To explore the effect of ROI selection, 10 samples of varying volumes were created, each with identical quantities of iron but differing sample volumes. We then explored three different methods for ROI selection and assessed the linearity of quantitation.

## Experimental Setup

Ten samples of a VivoTrax titration were prepared, each containing 34.4 µg of iron (specified in **Table 1**) in a 1.5 mL Eppendorf tube. Each tube was imaged separately in 2D with a 5.7 T/m gradient and excitation strength of 20 mT and 26 mT in the X and Z channels, respectively. The resulting images are shown in **Figure 1** and line profiles through these images are shown in **Figure 2**.

| Sample # | Total sample volume (µL) | Volume ratio VivoTrax: saline |
|---|---|---|
| 1 | 6.25 (VivoTrax only) | 1:0 |
| 2 | 12.5 | 1:1 |
| 3 | 25.0 | 1:3 |
| 4 | 50.0 | 1:7 |
| 5 | 100.0 | 1:15 |
| 6 | 200.0 | 1:31 |
| 7 | 400.0 | 1:63 |
| 8 | 600.0 | 1:95 |
| 9 | 800.0 | 1:127 |
| 10 | 1200.0 | 1:191 |

**Table 1:** Ten samples created by dilution of VivoTrax in different volumes of saline.

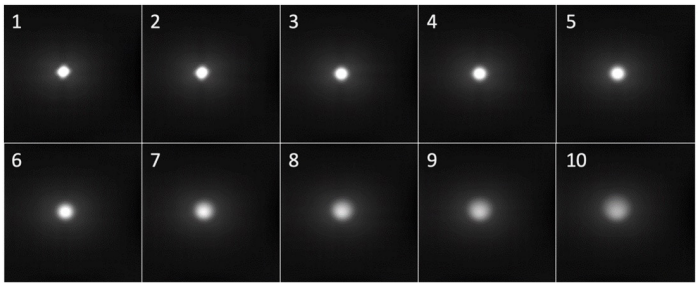


**Figure 1:** Projection images showing the same amount of VivoTrax in increasing volumes of saline.

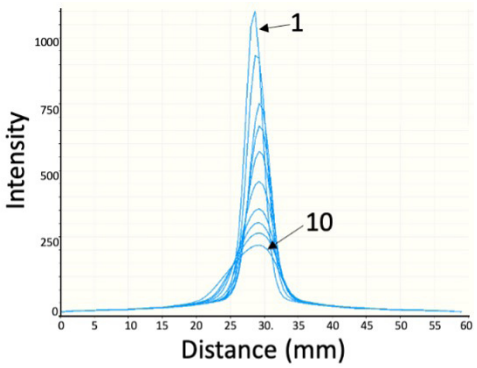


**Figure 2:** Line profiles showing differences in signal intensity for images 1-10. Undiluted VivoTrax (sample 1) produces higher intensity signal as the sample is concentrated at the bottom of the conical tube, while the point source spreads out more as the sample is diluted.

## Region of Interest Analysis

**Method 1: Custom-sized ROI.**

This method is quantitative only for high-SNR and high-resolution images and breaks down for more complex images. In this method, a line profile is drawn over each image and the distance between the edges of the peak is estimated (**Figure 3**). This value is used as the diameter for a circular ROI to delineate the high values (signal) from the lower values (noise). An arbitrary multiplication factor (> 1) is used to enlarge the ROI and mitigate user variability and include signal bleed-over due to poor PSF. The circular ROI

is centered on the peak signal manually. For non-circular objects, automatic image thresholding in the presence of high SNR (like Otsu's method) help identify the ROI.

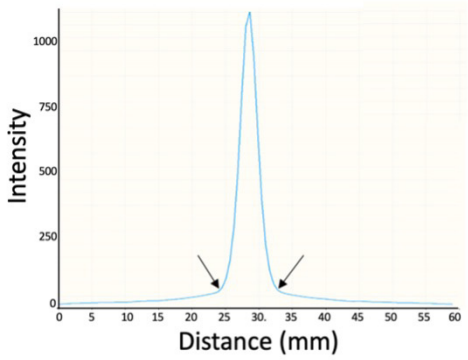


**Figure 3:** The edges of the peak are indicated by the black arrows.

**Method 2: A single, large ROI.**
This method is optimal for image datasets that assume the same physical layout of objects and can be used for quantification of a large range of SNR (high to low). The largest ROI from the dataset (using method 1) is applied to all images. With the delineation tied to the largest signal spread, this method guarantees to measure all actual signal in lower concentrations.

**Method 3: Noise threshold segmentation.**
The standard deviation (Stdev) of background signal is measured by drawing an ROI in an area of background noise or an image of an empty sample holder. A minimum threshold of 3 * Stdev is used to mask lower amplitude signals in all images. This yields a reliable measurement of MPI signal with SNR v. This method assumes that the background noise is similar in each image in the dataset.

## Measurement of Calibration Curve

Samples 1-10 were quantified using methods 1, 2, and 3. The relationship between MPI signal and iron content was established by imaging known quantities of VivoTrax (0.34 – 55 µg in 10 µL). Calibration curves were created by measuring signal from these samples using methods 1, 2, and 3 (**Figure 4**).

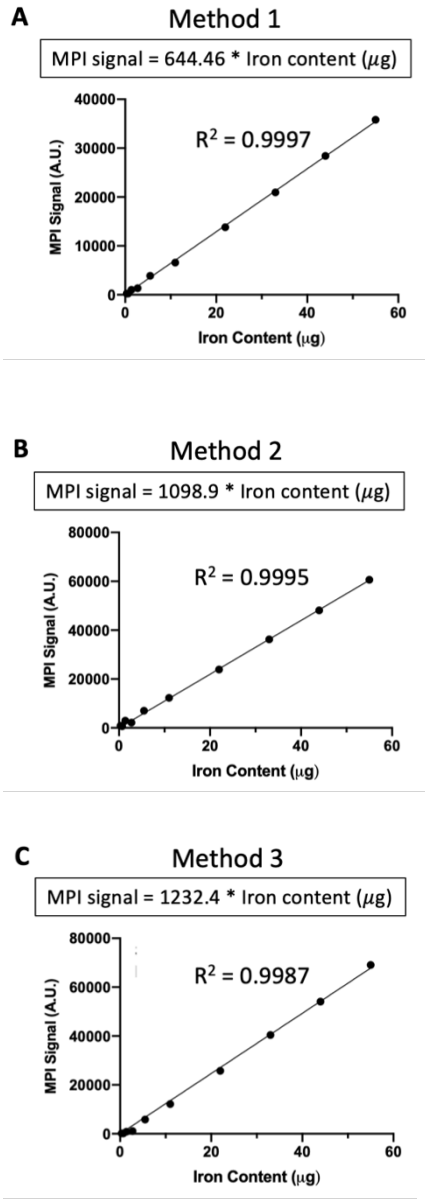


**Figure 4:** MPI signal measured using methods 1 **(A)**, 2 **(B)**, and 3 **(C)** from samples of VivoTrax containing various amounts of iron.

## Measurement of Test Samples

Samples 1-10 were then imaged and the MPI signal measured for each ROI method. The MPI signal was converted into an estimate of iron content using the linear calibration curves.



info@magneticinsight.com
magneticinsight.com

## Results

Iron mass estimated from samples 1-10 using methods 1-3 is shown in **Figure 5**. Method 1 underestimates the total MPI signal for dilute sample values because the ROIs are too small for dilute samples. This translates to an overestimation of iron content by up to 70% (sample 10) over a 4-fold maximum signal change range. Methods 2 and 3 use larger ROIs and provide more accurate (<5% error) estimates of iron mass, regardless of the sample volume.

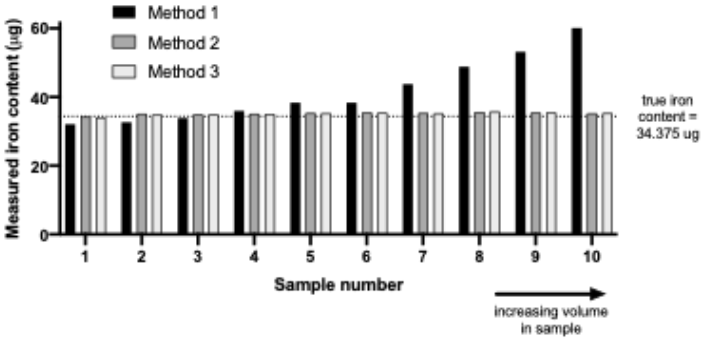


**Figure 5:** Measured iron content (µg) of samples 1-10 using methods 1, 2, and 3.

## Discussion

Methods 1-3 use different sized ROIs to quantify MPI signal (**Figure 6**). Method 1 uses a smaller sized circular ROI that is manually placed on the peak signal. Method 1 requires 3-user inputs per image; therefore, it is time consuming and user variability is expected. Method 2 uses ROIs of the same size on all images in a dataset, using the largest-sized ROI from method 1. This is a faster method, which has reduced user variability (a single manual bias) and the amount of noise introduced to the ROI is consistent for each image. Method 3 uses a threshold-based segmentation with no manual input. This method has both high accuracy and precision and fast analysis. The size of the ROI depends on the standard deviation of background signal and the threshold factor (≥3). For the quantification of VivoTrax in at multiple dilution levels, large ROIs (methods 2, 3) were the most accurate for measuring iron mass. These comparisons are summarized in **Table 2**.

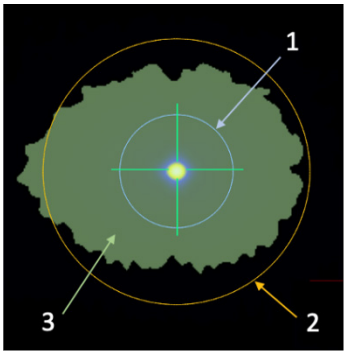


**Figure 6:** Visual comparison of the ROI created by each method. Method 1 is the small, round ROI, Method 2 is the large, round ROI, and Method 3 is the large ROI with an irregular perimeter.

| Criteria | **Method 1** Custom-Sized ROI | **Method 2** A single, large ROI | **Method 3** Noise threshold segmentation |
|---|---|---|---|
| **Size of ROI** | Small Depends on margin extension | Large Depends on margin extension | Medium Depends on Sdev and scaling factor |
| **Speed** | Slow | Medium | Fast |
| **User variability** | High | Medium | Low |
| **Custom shapes** | No (circular) Yes (automatic thresholds) | No (circular) | Yes |
| **Different volume samples** | Poor | Good | Good |

**Table 2:** Comparison of ROI methods 1-3 in terms of the size of the ROI, time spent on analysis, user variability, the ability to create custom shaped ROIs, and the quantification accuracy of iron in different volumes.

[1]Imaging Research Laboratories, Robarts Research Institute, London, Ontario, Canada
[2]Department of Medical Biophysics, The University of Western Ontario, London, Ontario, Canada
[3]Magnetic Insight Inc., Alameda, California, USA