# Quantification of Cell Density of SPIO-Labelled Cell Populations

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## Introduction

In recent years, numerous reports concerning the reduced relaxivity of cell-internalized contrast agents (CA) have been made (e.g. [1], [2]). This reduction is caused by the compartmentalization of CA inside the cells and has already been thoroughly investigated [3]. The aim of this study was to develop a method to correctly quantify the density of cell populations with intracellular CA despite the presence of quenching effects.

## Materials and Methods

Endothelial progenitor cells (EPC) were incubated with 50µg/ml Fe of Resovist, subsequently washed, counted and then prepared using low melting-point agarose gel. Four different concentrations of EPCs have been prepared in a 1% agar phantom: 250, 500 and 1000 Cells/µl. To evaluate the quenching effect each cell concentration has been prepared twice using complete cells for one and lysed cells for the second sample. All in all, 6 samples have been prepared in one phantom which is shown in **Fig. 1**.
All measurements were performed on a clinical 3T scanner. An inversion recovery (IR) turbo spin echo sequence (128x128 Matrix, FOV=80mm, THK=5mm, TE=7.7ms, TR=10000ms, TF=9) for 31 different inversion times TI reaching from 23ms to 7s (100ms steps from 100 to 2000ms and 500ms steps afterwards) was performed for the identification of longitudinal relaxation times $T_1$. To the results of these scans mono- and biexponential fits were applied to investigate changes of $T_1$ with changes of cell concentration.
Cellular iron load quantification was performed evaluating intracellular magnetization using the suszeptometry technique proposed in [4] and fitting the magnetic field profile of a cylindrical sample of loaded cells.

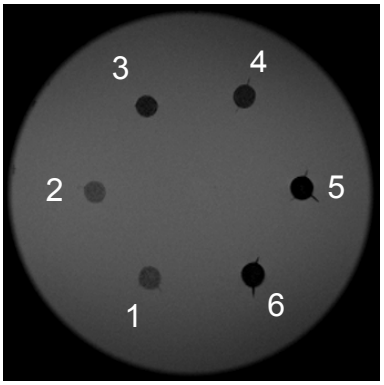


**Fig. 1:** Phantom with complete and lysed cells for different cell concentrations: (1,2) 250 Cells/µl, (3,4) 500 Cells/µl and (5,6) 1000 Cells/µl. Odd numbered areas include complete cells, even numbers mark lysed cells.

## Results

Results of the IR measurements are shown in **Fig. 2**. It can be seen that samples with lysed cells (marked with +) produce shorter $T_1$ times than complete cells for all cell concentrations. This is expected, as the CA in the intact cells is compartmentalized in vesicles and therefore has limited access to surrounding water.

| Area | $M_{0,mono}$ | $T_{1,mono}$ ms | $M_{0A,bi}$ | $T_{1A,bi}$ ms | $M_{0B,bi}$ | $T_{1B,bi}$ ms |
|---|---|---|---|---|---|---|
| 1 | 622 | 2445 | 26 | 360 | 614.2 | 2610 |
| 2 | 663 | 2374 | 27 | 407 | 652 | 2527 |
| 3 | 562 | 2420 | 29 | 330 | 555 | 2630 |
| 4 | 490 | 2222 | 70 | 2967 | 422 | 2130 |
| 5 | 412 | 2298 | 23 | 276 | 407 | 2520 |
| 6 | 412 | 2006 | 25 | 707 | 393 | 2148 |

**Tab. 1**: Results of mono- and biexponential fits to the signal intensities of the IR measurements.

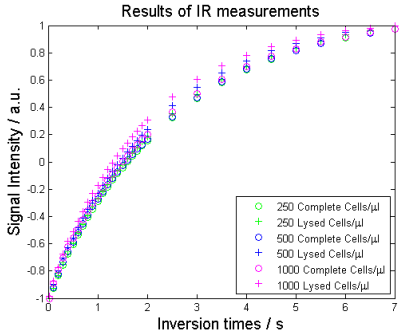


**Fig. 2:** Signal intensities of inversion recovery measurements for areas shown in **Fig. 1**. Intensities are normalized between -1 and 1.

Results of the 3- and 6-parameter fitting routines [6] are shown in **Tab. 1**. As expected, the relaxation rate $R_1=1/T_1$ increases linearly with cell concentration for lysed cells while the rates of intact-cell-populations do not follow a linear trend when fitted monoexponentially. Plotting the relaxation rate of compartment *A* of the biexponential fit against cell concentration, however, provides an almost perfectly linear trend (**Fig. 3**). From this trend a $\Delta R_1$ of $0.280s^{-1}$ per 250 cells can be derived which can be used for quantification. To evaluate the correctness of this factor a quantification of cellular iron load has been performed additionally.
Quantification of iron load per cell provided a value of 12±1.4pg Fe/cell. This results in 3ng per µl for 250 cells, respectively a molar concentration of 0.05mM. Using the linear relationship between relaxation rate and contrast agent concentration, $\Delta R_1=r_1\cdot\Delta[CA]$, and a relaxivity of $r_1=4.6mM^{-1}s^{-1}$ [5] yields a change of the relaxation rate of $\Delta R_1=0.230s^{-1}$ for the contrast agent included in 250 cells.

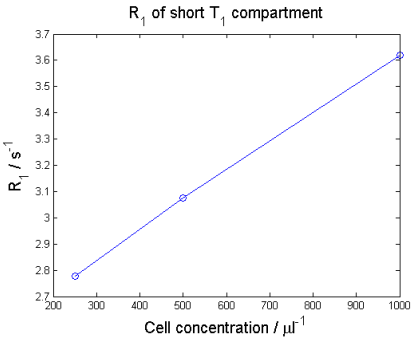


**Fig. 3:** Dependence of the relaxation rate of the small compartment of the biexponential fit on the cell concentration.

## Discussion

The presented results indicate that the effect of cell-internalized CA can be evaluated directly by applying bi-exponential fits to IR data. Furthermore, it is possible to perform quantitative analysis in the investigated range of cell concentrations. Further investigation will involve exact quantification of iron load per cell, as already a value of 13pg Fe/cell would result in a $\Delta R_1$ almost identical to the value derived from the data in **Fig. 3**.
All fits were performed for ROIs as large as possible omitting border pixels. Biexponential fits provided stable results when applied to different regions of the same sample, except for lysed-cell-samples which do not provide useful results as they do not consist of separable compartments. When looking at the $M_{0,A}$ values it is tempting to assume that they may represent the size of the compartment including the CA. However, these values do not scale linearly with cell concentration and represent larger fractions due to exchange processes.

## Acknowlegdements

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## References

[1] Magn Reson Med 2003;49:646–654 [2] Kobayashi et al., Bioconjug Chem 2001;12:587–593. [3] Simon et al., Eur Radiol 2006;16:738–745 [4] Bowen et al., MRM 48:52-61 (2002) [5] Rohrer et al., Invest. Radiol., 40(11):715-724, [6] Ejchart et al., Jour. Magn. Reson. 1984 Mar; 59: 446-451