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## Detecting free radicals in single cells using diamond relaxometry

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# Summary

Magnetic field fluctuations caused by paramagnetic species, such as free radicals, is one type of magnetic signal in the biological system. Free radicals are any molecular species that contain an unpaired electron in an atomic orbit. These free radicals are the major cause of aging and diseases that cause most deaths worldwide.

Diamond magnetometry allows nanoscale magnetic resonance measurements by utilizing fluorescent defects in diamond. This method makes use of a fluorescent defect in diamond which converts magnetic resonance signals into optical signals.

This thesis explores the utilization of relaxometry (also called T1 measurement), which is a specific type of magnetometry, to sense magnetic fields generated by free radicals in single cells.

**Chapter 1** introduces topics that are important in this thesis by explaining the scientific background of free radical detection in biological systems. This chapter gives an overview about free radical generation and some existing methods for detecting free radicals with their limitations, which are low sensitivity and low spatial and temporal resolution. Then, relaxometry was introduced as a solution to these limitations. The properties of fluorescent nanodiamonds and their use as a biosensor were discussed.

Since this technique is relatively new, there was no standard way to interpret the data yet. In **chapter 2**, we aimed to develop a calibration for this system. We performed measurements in controlled environments with known concentrations of gadolinium or radicals. More specifically, we follow photolysis of  $\text{H}_2\text{O}_2$  as well as the Haber-Weiss reaction which are important reactions in biological environments. We also investigate different nanodiamond probes and their ability to sense gadolinium spin

labels. While this was so far only done in a clean environment, we took into account the effect of salts and proteins that are present in a biological environment. Surprisingly, in contrast to single defect measurements, smaller nanodiamonds have better coherence times. This is an important step towards quantifying magnetic resonance signals in a biological environment.

It is more useful to have signals from specific regions in cells. In **chapter 3** we aimed to target mitochondria, the energy factories of the cells. It is relevant to measure in mitochondria because this is also the area where most free radical waste is generated. We coated our diamond sensors using antibodies to target them to mitochondria. This way we could differentiate between free radical generation in mitochondria and somewhere else in the cell. This free radical generation was investigated by triggering or inhibiting certain metabolic processes. It responds to the trigger when the nanodiamond is on the mitochondria and remains unaffected when it is not. We also isolated mitochondria and performed the same measurement there.

Free radicals play a key role in the ageing process. In **chapter 4**, we utilize relaxometry to detect free radical generation in yeast cells during oxidative stress and aging. We are able to follow free radical generation after chemically inducing stress. In addition, we can observe the reduction of the radical load in presence of an antioxidant. We were able to clearly differentiate between mutant strains with an altered metabolism. We found that relaxometry is able to follow the ageing process and differentiate between young and old cells and compare the ageing behavior of mutant strains.

While previously we used relaxometry inside living cells from a cell line, in **chapter 5** we applied this technique in human primary cells. We investigated primary dendritic cells from different donors. These cells play a key role in the human immune system

and have great significance in many diseases. We compared and validated our results with conventional techniques.

We observed free radical concentrations of individual bacteria in **chapter 6**. We found that the amount of free radicals increases in the presence of UV irradiation as well as dependent on the dose of antibiotics the cells were exposed to. This finding is highly valuable, especially for applications in drug resistance.

**Chapter 7** concludes with general discussion and outlines future application of diamond magnetometry.