Free radical detection in living cells with relaxometry

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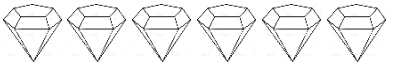
Chapter



Valorization Chapter

Diamond magnetometry is a new technology, which allows nanoscale MRI. We make use of a fluorescent defect, which changes its optical properties based on the magnetic surrounding. While it is already established in physics, we recently pioneered to use the technique in living cells. There it allows measuring free radicals, which play a major role in the natural metabolism of ageing as well as multiple diseases including cancer cardiovascular diseases or viral/bacterial infections. Despite their relevance these radicals are challenging to measure for the state of the art. Potentially, this method could be used to gain fundamental insights into stress in cells but also assess drug efficacy. The purpose of writing this chapter with the goal of finding a company. I will first discuss the applicability of diamond magnetometry for commercial purposes where I will provide an outlook with respect to the experiments discussed in this thesis. Second, I will discuss the pros and cons of this technique compared to the competing methods.

6.1 Diamond Magnetometry

Diamond magnetometry is a new technology, which emerged from the quantum information field and allows nanoscale magnetic resonance measurements. The technique is based on defects in diamonds, which change their optical properties based on their magnetic surrounding. This means that we can read out a magnetic resonance signal with an optical microscope without the need of conventional magnetic resonance equipment. Since optical signals can be read out more sensitively, this method has unprecedented sensitivity.

In physics this technique has already been applied for several applications. These include measurements of the magnetic structures of hard drives, magnetic vortices and nanoparticles. Arguably the most impressive demonstrations of sensitivity are the measurements of the magnetic moment of single electrons or even a small number of nuclear spins.

Our research group led by Prof. Schirhagl, is the first world-wide who is using it successfully to measure free radical metabolism in living cells. Part of our work, a specific way to perform magnetometry, has led to a patent application.

The technique reached a proof of concept for several applications, including:

1. Measurements of cell signalling.
2. The free radical load during ageing (and how it changes in presence of anti-ageing drugs).
3. Free radical generation due to drugs, shear stress, and physiological changes (chapter 4 &5).
4. Virus infection or radiation.

These latest technological developments led to the idea of founding a start-up, which valorises the efforts of the scientific research group by applying diamond magnetometry to biological samples.

6.2 The Problem

Magnetic resonance spectroscopy (NMR or ESR) and magnetic resonance imaging (MRI) are widely used. In chemistry these techniques determine molecule structures and in medicine they resolve and visualize for instance the fine details of the brain. However, magnetic resonance signals are low in energy and thus difficult to detect. As a result, the equipment that is required to detect these low energy signals is costly. For SMEs and start-ups (in for instance the drug discovery field) such costly equipment might not be an option.

NMR/ESR and MRI are not only expensive but also have fundamental limits. Since a large amount of homogeneous sample is needed one cannot measure small samples (like the content of single cells or trace amounts of substances). The same problem arises when a spectrum changes in time for instance when a reaction takes place.

Currently, the lack of techniques to quantify free radicals is obstructing drug developers from optimizing the right compounds, this could be interesting for a field such as healthy ageing. The solution we are offering has clear advantages compared to conventional methods and alternatives.

6.3 The Solution

We are able to sense magnetic resonance signals produced by free radicals in biological samples, using a novel technique called Diamond Magnetometry. This methodology utilizes fluorescent nanodiamonds that host defects in their crystal structure. More specifically, we utilise nitrogen-vacancy centres which are very sensitive to magnetic noise which can be observed via optical microscopic readout as described previously in this thesis.

In other words: we can measure magnetic resonances with a special optical microscope without the need of conventional magnetic resonance machines. Optical signals can be read out easier and this technique is unprecedentedly sensitive.

These characteristics make our tool perfect for sensing and quantifying changes of free radical loads as discussed in chapters 4 & 5. Examples for applications are to measure the efficiency of new antioxidants (e.g. in food) or drugs which cause stress in cells (as discussed in chapter 5 using different drugs to monitor free radical concentrations), cosmetics, monitoring the natural ageing process, or even the effect of shear stress as discussed in chapter 4.

We offer custom made solution including reports of localized concentrations of free radicals in biological systems (in vitro assays). Our test can report real-time fluctuations of free radicals (increase and decrease of free radical concentration) down to nanomolar concentrations. Thus, our measurements allow customers to get information about the free radical load of cells, the working mechanism and potentially the therapeutic effects of developed antioxidants/drugs on a sub-cellular scale.

6.4 Innovative Aspects

Compared to conventional magnetic resonance techniques, this new technology has the following innovative and unique selling points:

1. First, since the detection is optical, only a microscope with a few modifications is needed, in comparison to high field gradient tubes or magnetic fields. These are the most expensive parts in conventional instruments and thus the new approach reduces the equipment costs by two orders of magnitude (to about €30.000). Additionally, optical equipment is less complex than conventional magnetic resonance equipment.
2. Second, optical transitions are higher in energy. As a consequence, an optical signal that is coupled to a magnetic signal can be detected much more sensitively than the magnetic signal itself. The result is that diamond defects can even detect magnetic resonances from single electron spins. For our project this means that we only need sample volumes of some nanoliters instead of milliliters.

When comparing our technology to standard ROS dyes, we have several distinct advantages:

1. Most of these dyes detect reactive oxygen species (ROS, consisting of radicals and other reactive molecules) rather than radicals. Given that non-radicals are more abundant (but much less damaging to cells and thus potentially less relevant) the signals are dominated by them.
2. Conventional dyes can diffuse and thus the location where they are measured is not necessarily where the radical has formed as discussed in chapters 4 & 5 in this thesis.
3. ROS dyes reveal the history of the sample while our technique is the only one which provides the current status in real time.

4. Nanodiamonds are better in terms of biocompatibility (especially since they are only needed in very low quantity).

Our technique allows for real-time detection of free radicals with high spatial resolution and localisation through fluorescence microscopy. This gives information of intra- or extracellular free radicals. The surface of nanodiamonds for targeting specific locations is modifiable and the information that can be acquired is up to single-electron spins.

6.5 Commercial Aspects

6.5.1 Market Analysis

The potential markets of free radical sensing through diamond magnetometry are diverse. Additional end users and market applications could include a broad variety of companies and researchers who are performing organic synthesis and wish to control or better understand the process:

1. The pharmaceutical industry (drug discovery and development, equipment developers);
2. The Cosmetic industry (skin care, anti-ageing, sunscreen protection);
3. Food industry (e.g. supplements & anti-oxidants);
4. Biomedical, biological or chemical research groups (for example in healthy ageing);
5. Diagnostics (many diseases including cancer, cardiovascular disease and bacterial/viral infection are linked to an imbalance in free radical load)

These market segments all investigate formation of ROS. For example, in the pharmaceutical industry, a novel drug has to be tested to discover the effect of the drug in biological systems. However, monitoring drug efficiency with current ROS assays does not measure radicals specifically. Since radicals are more reactive and thus most damaging than ROS, it is plausible that they might reflect a clinical outcome better or at least give a more detailed picture in addition to existing methods. Additionally, existing methods provide less spatial resolution (we have subcellular resolution while usually ensembles of cells are investigated). Finally, due to bleaching it is not possible for conventional techniques to follow the same cell/sample over more than a few seconds. Our technology offers a biocompatible tool, to potentially monitor drugs efficiency in real time from localized source of

radicals within the biological system. Similar arguments apply for other market segments described above.

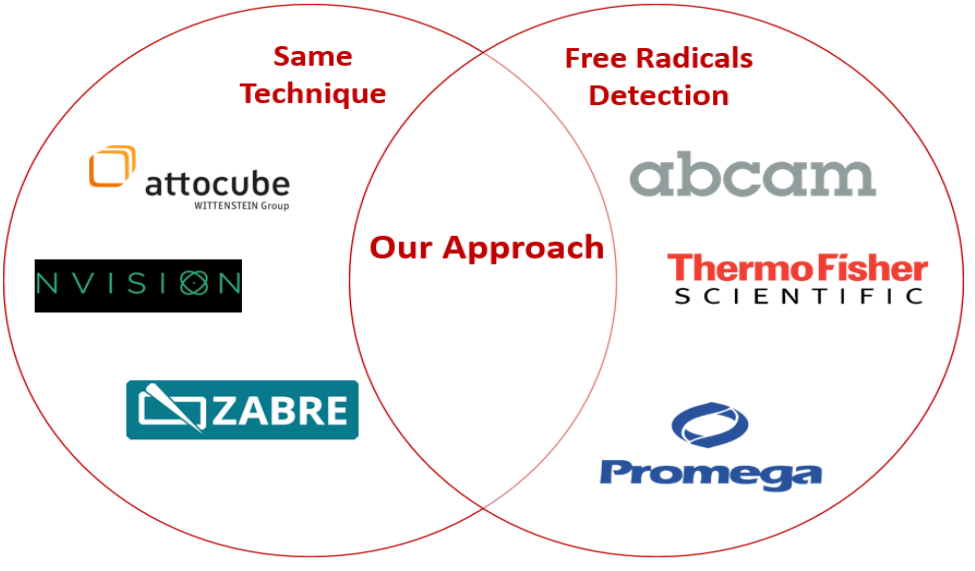
6.5.2 Unique selling points

The main strength of our instrument or service we envision is its ability to detect radicals specifically. Further, our technology works in real-time. Free radicals can be used as read-out to follow the interaction of a drug with biological systems. The second unique selling point is the fact that analysis is unprecedentedly sensitive and can be performed with only small amounts of sample. As a result, we obtain sub-cellular resolution. The technology is optical based and thus cheaper than conventional alternatives. Our equipment is relatively easy to use, and development and user costs are limited.

6.5.3 Competitive Analysis

Competitors can be divided into two segments:

1. The first segment includes companies who develop assays to measure free radicals in laboratories based on technologies different from diamond magnetometry (the ROS dyes), such as Abcam, ThermoFisher Scientific and Promega. However, these kinds of assays do not provide information about radical formation in real-time, they are not sensitive to fluctuations in free radicals (increase and decrease of concentrations), and most of them are not sensitive in the nanomolar scale. Also, these assays detect and quantify reactive oxygen species (ROS) and not free radicals. Additionally, they reveal all the molecules that have been created up to a certain time point (accumulation), while our technique reveals the current state.
2. The second segment includes companies using diamond magnetometry in their assays focusing on other applications, like Attocube, QZABRE, Qnami, NVision, Thales, Bosch. These companies are focused on device development mostly for applications in physics. Their equipment is based on scanning magnetometry. This is more complex than our approach and thus the equipment is about an order of magnitude more expensive (and has substantial running costs). Their approach is superior in terms of sensitivity and spatial resolution but does not work within (living) cells.



COMPANY	INSTRUMENT	INSIDE CELLS?	SPECIFICATION	SPATIAL RESOLUTION	RUNNING COSTS	PRICE €
OUR APPROACH	-	Yes	Purely optical relaxometry	Tens of nm	Maintenance only	TBD below 50k
QNAMI	ProteusQ	No	Scanning magnetometer	nm	Around 20k/year for tips + maintenance	500.000
ATTOCUBE	attoAFM/CFM	No	Scanning magnetometer	nm	Around 20k/year for tips + maintenance	500.000
QZABRES	QSM	No	Scanning magnetometer	nm	Around 20k/year for tips + maintenance	500.000

Table 1 Shows a comparison between our approaches companies who detect ROS

COMPANY	FREE RADICALS	ASSAY PERFORMANCE	PROBE SCALE	SENSITIVITY	DYNAMIC	MEASUREMENTS LEVEL	PRICE€
OUR APPROACH	YES	Real time	Nanomolar	High	Free radicals' fluctuation	Inside cell	TBD
ABCAM	NO Reactive oxygen species	History of free radical's presence	Nanomolar	High	Free radicals' history	Inside cell	270
THERMOFISER SCIENTIFIC	NO Reactive oxygen species	History of free radical's presence	-	High	Free radicals' history	Inside cell	274
PROMEGA	Hydrogen Peroxide (H2O2)	History of H2O2 presence	Micromolar	High	H2O2 history	Inside cell	370

Table 2 Shows a comparison between our approach vs companies who use similar technique

Currently, we could claim we will be first on the market using Diamond Magnetometry for free radical assays and our patent would give us a competitive advantage. Moreover, the competitors identified above could become partners and share customers for service with needs, or even commercialize the technology as a product using them as producers and distributors.

6.5.4 Key Risks

As a biotechnological initiative (start-up) which aims to position into the market with a new product based on an emerging technology, we are aware that there are risks involved.

Concerning the most prominent market, pharmaceutical companies and research laboratories could be used to conventional methods and might not acknowledge the value of the invention. Therefore, we focus on identifying early adopters in order to overcome the barrier to attract first customers. Our current collaborations broadly promoted the technology in scientific meetings (in The Free Radical Society, Material Research Society, EMBO) and publications in scientific journals. These collaborators might be potential customers, or even ambassadors, as they already know the value of our results. We continue to establish scientific collaborations and publications with researchers to validate our technology with different types of samples, which gives us evidence to convince new customers

about the reliability of our method and comparative advantages over our competitors.

Lastly, diamond magnetometry is highly increasing in popularity among scientific fields, and there might be more start-ups and established companies adding diamond magnetometers to their products and portfolio.

6.6 Conclusion

The interesting properties of nanodiamonds could yield to an interesting device application. Optimizing such a technique (Diamond magnetometry) for free radical detection could open the doors for an enormous amount of new knowledge about the origin of free radicals. Although, the potential of the discussed device is high, a full understanding of the novel technique and what it does is required. Based on the analysis we made diamond magnetometry has the potential to be commercialized. However, a long-term business model can be problematic as we are still exploring the possibilities and markets. Additionally, with regard to societal impact this technique needs time to gain publicity among users.