Microcoil MRI of plants and algae at ultra-high field : an exploration of metabolic imaging

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SUMMARY

This thesis investigates the relations between metabolism and anatomy through the use of Magnetic Resonance Imaging (MRI). Two model systems are studied: *Botryococcus braunii*, a green oleaginous algae and *Medicago truncatula,* a small leguminous plant in symbiosis with *Sinorhizobium meliloti* bacteria. Understanding the processes within the studied model systems may potentially aid in answering challenges facing humanity and its environment, related to energy and food security. In order to map the variation of metabolic profiles across tissues, Magnetic Resonance Imaging (MRI) is used extensively, due to its ability to non-invasively study both anatomy and spectroscopy. Dedicated pulse sequences and home-built microcoils are developed for studying these organisms.

Chapter 1 provides a general introduction to the field of metabolic imaging using MRI, as well as the general principles and theoretical background of MRI. The design and characteristics of solenoid microcoils, used for magnetic resonance microscopy, are discussed. The range of pulse sequences used for magnetic resonance microscopy, localised spectroscopy, and combinations thereof, are covered briefly.

Botryococcus braunii is an oleaginous green alga with the distinctive property of accumulating high quantities of hydrocarbons per dry weight in its colonies. Large variation in colony structure exists, yet its implications and influence of oil distribution and diffusion dynamics are not known and could not be answered due to lack of suitable *in vivo* methods. **Chapter 2** seeks to further the understanding on oil dynamics, by investigating naturally relevant large (700-1500 µm) and extra-large (1500-2500 µm) sized colonies of *Botryococcus braunii* (race B, strain Showa), *in vivo*. A comprehensive approach of chemical shift selective imaging, chemical shift imaging and spin echo diffusion measurements are used to study the algae at high magnetic field (17.6T). Hydrocarbon distribution in large colonies was found to be localised in two concentric oil layers with different thickness and concentration. Extra-large colonies were highly unstructured and oil was spread throughout the colonies, but with large local variations. Interestingly, fluid channels were observed in extra-large colonies. Diffusion-weighted MRI revealed a strong correlation between colony heterogeneity, oil distribution, and diffusion dynamics in different parts of *Botryococcus* colonies.

Dedicated solenoid microcoils are used in this thesis to study small-volume samples at high resolutions. The microcoil design may be adapted and iterated to match the sample of interest optimally. **Chapter 3** describes a method to calibrate such microcoils designed for high-resolution magnetic resonance microscopy (MRM). Calibration of new or home-built microcoils is explained systematically using a reference sample. Steps include a method to determine coil resonant modes and determining the reference pulse power using a nutation curve. Performance characteristics of novel microcoils, including sensitivity and RF homogeneity, are established using standardised pulse sequences. A volume-normalised Signal-to-Noise Ratio is calculated to facilitate coil

performance comparison based on image SNR. Sample preparation and avoiding possible pitfalls are discussed.

Interactions between plants and the microbial & fungal soil flora are crucial for the health of soil ecosystems and food production. Microbe-plant interactions are difficult to investigate *in situ* due to their intertwined relation involving morphology and metabolism. **Chapter 4** describes an approach to overcome this challenge by elucidating anatomy and metabolic profile of *Medicago truncatula* root nodules using Magnetic Resonance Microscopy at 22.3 T. A home-built solenoid MR signal detector with an inner diameter of 1500 µm was used to study individual root nodules. A 3D imaging sequence with an isotropic resolution of $(7\ \mu m)^3$ was able to resolve individual cells and distinguish between cells infected with Rhizobia and uninfected cells. The metabolic profile of cells in different sections of the root nodule was detected using localised spectroscopy and reveals that several metabolites including betaine, asparagine/aspartate and choline, vary across nodule zones. Metabolite distribution was visualised using complementary chemical shift imaging. The technical challenges and outlook towards *in vivo* imaging of nodules and the plant root system are discussed.

Chapter 5 contains the general discussion and outlook for imaging plants and algae using MRM. In addition, initial results for the implementation of Diffusion-Weighted Chemical Shift Imaging (DW-CSI) at high field are discussed. The DW-CSI sequence is an elegant way of combining information derived from diffusion weighting of secondary metabolites in a spatially resolved manner. The feasibility of calculating apparent diffusion coefficients from DW-CSI measurements has been shown for water and hexadecane. However, further research and development, both in sequence and hardware design, is required to utilise this approach to its full potential.