Kinetic Modelling and Inference of Hyperpolarized $^{13}$C Molecules in Cancer Metabolism

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To Huiqiang Zhao (1967-2013) and Zhanjiang Pu (1928-2012), brave father and grandfather who died of cancer yet achieved numerous miracles and have guided me along my life.
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A year ago at Johns Hopkins, I have had the luck to be introduced to Warburg and Seyfried’s work on cancer metabolism. Last but not the least I must thank my teacher Mrs. Geo Derick for the inspiration and blessing she gave me during the two weeks in Baltimore. I believe that by eliminating the dogma and returning to the respect for patients, we are able to further our understanding towards cancer and ultimately make people free from the disease.

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Abstract

Hyperpolarized $^{13}$C-MRI allows real time observation of metabolism in vivo. Imaging sequences have been developed to follow the metabolism of [1-$^{13}$C] pyruvate and extract reaction kinetics, which can show tumour treatment response. We applied the fitting model and algorithm for the imaging data of mice tumour models and determined error estimates for the parameters of interest. Data was least-squares fitted onto a two-site exchange model in MATLAB, followed by statistic computation to assess model performance. Inference through the application of MCMC was also performed. The modelling and inference process extracted quantitative information satisfactorily and reproducibly, demonstrating metabolic activity and intratumour heterogeneity. Finally, novel fitting methods were evaluated and further recommendations were made.
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List of Abbreviations

AIC  Akaike Information Criterion
AIF  Arterial Input Function (of pyruvate)
CT   Computed Tomography
DNP  Dynamic Nuclear Polarization
ECM  Extracellular Matrix
FDG  Fluorodeoxyglucose
FID  Free Induction Decay
HMC  Hamiltonian Monte Carlo
MCMC Markov Chain Monte Carlo
MH   Metropolis-Hastings Algorithm
MRI  Magnetic Resonance Imaging
MRS  Magnetic Resonance Spectroscopy
(N)MR (Nuclear) Magnetic Resonance
ODE  Ordinary Differential Equation
PET  Positron Emission Tomography
RSS  Residual Sum of Squares
SD   Standard Deviation
SNR  Signal to Noise Ratio
Introduction

Cancer is a complex disease that involves malignant cell growth. It consists of disorders in many distinct aspects, but a number of cancers do share some characteristic features\textsuperscript{1}, among which, notably, is abnormal metabolism\textsuperscript{1,2}. The Warburg effect of cancer cells is the mass production of lactate (‘fermentation’) via glycolysis even in the presence of oxygen\textsuperscript{3}, which is a signature of mitochondria damage. Recent findings suggest that such alteration is not only an adaptation towards hypoxic microenvironment as a result of unlimited replication, but potentially the underlying cause of cancer\textsuperscript{4}.

It is therefore important to visualise the metabolic activity of cancer. Pre-clinically, it helps to advance the understanding of cancer pathophysiology. In clinical practice it contributes to more precise diagnosis and better detection of treatment response\textsuperscript{5}: an example being FDG-PET, where \(^{18}\text{F}-\text{FDG}\) is injected into the patient and its uptake is analyzed and mapped onto a CT image of anatomy\textsuperscript{6} (Fig. 1). However, the imaging technique yields static image, poor spatial resolution and also brings ionizing radiation which may lead to disease progression.

Advances in molecular imaging has provided a novel non-invasive tool for oncology that shows high clinical promise\textsuperscript{5,7}. Among them is dynamic nuclear polarization (DNP), which increases the SNR of \(^{13}\text{C}\) magnetic resonance (\(^{13}\text{C}-\text{MR}\)) by over 10,000 folds\textsuperscript{8}. Hyperpolarized, non-radioactive \([1-^{13}\text{C}]\) pyruvate is injected intravenously, followed by imaging sequences that map the Warburg effect, where \([1-^{13}\text{C}]\) pyruvate converts, mostly, to \([1-^{13}\text{C}]\) lactate\textsuperscript{9}. This allows real-time observation of metabolism \textit{in vivo}: not only can we image the distribution of metabolites down to tissue (voxel) level, we are also able to extract reaction kinetics (e.g. rate of conversion) through quantitative modelling. These observations can further demonstrate intratumour heterogeneity\textsuperscript{10} and indicate early treatment response\textsuperscript{5,9}.

The goal of this project was to determine the best kinetic model and fitting algorithm for imaging data, and to determine error estimates for the parameters of interest. Example models were taken from literature and applied to spectroscopic/imaging datasets acquired, whilst examining fitting performance both qualitatively (by curve fitting) and quantitatively (by information criteria). Markov Chain Monte Carlo (MCMC) analysis was performed to estimate error and increase confidence in modelling results. Finally, suggestions were made on modelling and inference algorithms.

\textbf{Figure 1} An example of FDG-PET/CT. Coronal fused image (a) together with maximum intensity projection (b) showed a lung tumour as arrowed in (a). Other FDG signals (from kidneys, bladder, brain etc.) are physiological. (Taken from Rockall et al.\textsuperscript{6})
Animal Model and Data Acquisition

$5 \times 10^6$ EL4 lymphoma cells were injected into the flank of C57BL/6J mice which were imaged 8 days post-injection when tumour size was approximately 1.5cm$^3$. 44-mg samples of 91% [1-13C] pyruvate solution were polarized, using a clinical hyperpolarizer (GE Healthcare, Chicago, IL, USA), to 20%± 1%, as measured by an NMR polarimeter (Oxford Instruments, Abingdon, UK). Hyperpolarized substrates were made by rapid dissolution of these samples. The mice were anaesthetized and placed in a self-made surface coil, in a 7T animal MR magnet. Substrates were injected intravenously through tail vein, and data collection began shortly before or after injection (normally -8s~2s). The free induction decay (FID) signals in time domain (1s in length) were collected by the coil and Fourier-transformed to a series of 13C-NMR spectra (Fig. 2a&b). For each spectrum, the area under each metabolite peak was calculated after phase correction (which sets the peaks perpendicular to the base line), giving signal intensities of metabolites at that specific time point. A complete set of time course signals of different metabolites was formed (Fig. 2c) and thus became the starting point of kinetic modelling and statistical inference.

Two experiments were performed in Spring 2015 and Summer 2016 respectively. The former was performed with a pulse sequence from literature. It used a uniform 5° flip angle and was able to detect signals from pyruvate, lactate and alanine (see the next section), but the SNR was mixed. As for the 2016 experiment, mice were imaged twice with a 2-day gap during which single etoposide treatment was given (n = 4). Imaging was based on a single-shot spiral pulse sequence developed in 2016 to optimize SNR (Fig. 3), with different flip angles for pyruvate (7°) and lactate (45°) so as to preserve fresh polarization and produce higher lactate signals$^{11}$. It yielded a spatial resolution of $1.25 \times 1.25 \times 2.5$mm and a time resolution of 1s, but sacrificed lactate data in the first 10s as well as signals from other metabolites.

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Figure 2 Data acquisition from hyperpolarized 13C MRS. (a): A typical spectrum showing 1: [2-13C] pyruvate; 2: [1-13C] lactate; 3: [1-13C] pyruvate hydrate; and 4: [1-13C] pyruvate. (b): Sequential time series of spectra (every third is shown for clarity). (c): Integrated time course data and its fit to a two-site exchange model (every fourth data point is shown for clarity). (Taken from Day et al.$^9$)
Kinetic Modelling

Biomarkers associated with pyruvate that demonstrate observable peaks in $^{13}$C-NMR spectra include (but not limited to) lactate (via fermentation), alanine (via reversible transamination), urea (via downstream urea cycle) and bicarbonate (via TCA cycle). In this project, only the first three were considered as signals from urea and bicarbonate were considerably lower and might be ignored. Pyruvate hydrate may also appear as a peak but it does not involve in any metabolic pathways\(^9\).

In order to extract quantitative reaction kinetics, the time course signal intensities can be fitted into the following two-site exchange kinetics model based on modified Bloch equations\(^12\), assuming all metabolites in vivo are able to have direct contact with intracellular enzymes that facilitate exchange (P: pyruvate; L: lactate; A: alanine):

$$\frac{d}{dt} \begin{bmatrix} P(t) \\ L(t) \\ A(t) \end{bmatrix} = \begin{bmatrix} -(k_{pl} + k_{pa} + \rho_p) & k_{lp} & k_{ap} \\ k_{pl} & -(k_{lp} + \rho_L) & 0 \\ k_{pa} & 0 & -(k_{ap} + \rho_A) \end{bmatrix} \begin{bmatrix} P(t) \\ L(t) \\ A(t) \end{bmatrix} + \begin{bmatrix} P_{in}(t) \\ 0 \\ 0 \end{bmatrix}$$

Where $k_{XY}$ is the apparent rate constant of conversion from metabolite X to Y. For a metabolite $M$, $\rho_M = \frac{1}{T_1} + \frac{\ln(\cos \theta_M)}{Tr}$ (T1: spin-lattice relaxation time; TR: repetition time; $\theta_M$: flip angle for M)\(^13\). $P_{in}(t)$ is the arterial input function (AIF) of pyruvate based on a gamma-variate function, which, if not measured\(^14\), would also need to be fitted. Later sections will demonstrate further simplification of this model, however one should note that this model itself is already a simplified one, ignoring the difference between intravascular, extravascular (ECM) and intracellular environments. Nevertheless, being a non-linear ODE model with a number of parameters to be fitted, the robustness and reliability of fitting results are in serious doubt, particularly when many of the parameters may not be measured directly (notably the AIF, since real-time information on circulation is unavailable).

Markov Chain Monte Carlo (MCMC)

MCMC is widely applied in computational biomedicine\(^15,16\), as it significantly increases one’s understanding and certainty towards parameter values via Bayesian statistical analysis\(^17\). Compared with least-squares fitting which offered a single result, MCMC algorithms randomly (hence ‘Monte Carlo’) sample over the entire parameter space, forming a Markov chain whose distribution at equilibrium is the target probability distribution of parameter vectors. Two major algorithms include Metropolis-Hastings (MH) and the Gibbs Sampler\(^17\). Since the latter requires a higher degree of independence between parameters, the MH algorithm was applied in this project, whose flow chart is shown in Fig. 4. This is a simplified description of the algorithm whose mathematical details are beyond the scope of this report, however there are two things one should pay particular attention to.

Firstly, the likelihood $p(D|x)$. Let $d_i$ represent the $i^{th}$ measured data value and $M(X)_i$ be the value predicted by the model. We can thus write:

$$d_i = M(X)_i + \varepsilon_i$$
for the current choice of parameter vector. The principle of maximum entropy leads to the assumption that the error (residual) $\varepsilon_i$ follows a Gaussian distribution with standard deviation $\sigma_e$. A further assumption of datapoint-independence brings us to the likelihood equation shown in the figure.

More importantly, it is critical how to choose (‘tune’) the proposed covariance matrix $\Sigma$. Ideally it is proportional to the target, but since we had no information whatsoever, it seemed the only way was to manually tune the matrix (aka ‘trial and error’), so as to avoid the situation where $\Sigma$ if too large rejects most updates or too small makes the movement
extremely slow (Fig. 5). Fortunately, an adaptive algorithm is developed to automatically update $\Sigma$ during the stochastic process\textsuperscript{18,19}. Proved by mathematical principles, the sampling efficiency tends to its highest when the proposed $\Sigma$ for the $n^{th}$ iteration is given by:

$$\Sigma_n = \frac{2.38^2}{d} \hat{\Sigma}_{n-1}$$

Where $d$ is the dimension of parameter vector and $\hat{\Sigma}_{n-1}$ is the empirical covariance matrix based on vectors of the previous $n-1$ iterations. The optimal acceptance rate of updates is $\sim 44\%$ for $d=1$ and $\sim 24\%$ for $d>5$. Thus, the Adaptive-MH algorithm proposes a multivariate normal distribution for the $n^{th}$ iteration given by:

$$x^* \sim N(x_{n-1}, \Sigma_n)$$

Since it is impossible to estimate target covariance right at the beginning, the algorithm samples with a fixed covariance for the first few ($\sim 10\%$) iterations, usually in the form of $\Sigma = \sigma^2 I_d$ ($I_d$: $d$-dimensional identity matrix), assuming no parameter interdependence. The adaptive process begins afterwards and ends at $\sim 50\%$ of total iterations, in order to prevent the Markov chain from converging into the wrong direction. Finally, the algorithm runs with the last covariance obtained\textsuperscript{19}.

**Results**

The project was performed in MATLAB environment (MathWorks, Natick, MA, USA). The ODE model was coded directly into a script as it was difficult to solve by hand (because of the AIF). Datasets were least-squares fitted onto the model using the *lsqcurvefit* function, whose performance was assessed by Akaike Information Criteria (AIC) quantitatively and by observing plots qualitatively. Surprisingly, *lsqcurvefit* depended highly on initial parameter vector (guess), upper bound and lower bound, and they had to be tuned according to fitting performance so as to yield a near best fit. The resulting parameter vector was passed onto the MCMC function to obtain a final fitting result as well as error estimate (standard deviation, SD). To show the Markov chain converged to the right direction, another chain starting from a distant parameter vector was produced and two chains were compared against each other\textsuperscript{17,20}.
Datasets were fitted onto the full model as in Section 3, but ignored the correction factor for flip angle, essentially uniform the $\rho$ values as reciprocal of T1. Table 1 listed important fitted values for a typical mouse dataset including 4 rate constants and T1. The table also showed the mean AIC value of the model. It can be concluded that the rate constants for the reverse reactions ($k_{ap}$, $k_{lp}$) were 3~30% of forward rate constants, with some $k_{ap}$ values even at the order of $10^{-4}$. Nevertheless, fitting quality would be compromised if those parameters were omitted in the model.

Compared with values in literature\textsuperscript{9,21,22}, the kinetics were consistent with other rodent tumour models, with some fittings have significantly lower AIC values. Time course plots showing both original and fitted values (Fig. 6) demonstrate a good match for many mice datasets, but for others post-peak signals were not well-fitted. For data with low SNRs, the model was able to produce a decent fit showing robustness.

MCMC analysis gave the error estimate (SD) for the fitted values and the adaptive algorithm yielded an acceptance rate of ~50%. Trace plots and histograms (Fig. 7a\&b) indicated good mixing of the chain. Interestingly, however, the two chains from distinct starting points were unable to converge into a uniform range (Fig. 7c). The effect on AIC values differed greatly between datasets, while some lowered the AIC values by less than 10% compared with 45% as in Table 1, which implied no significant improvement as to lsqcurvefit results.

|       | $k_{pl}$ | $k_{lp}$ | $k_{pu}$ | $k_{ap}$ | T1  | AIC(e) |
|-------|----------|----------|----------|----------|-----|--------|
| lsqcurvefit | 0.092    | 0.030    | 0.0026   | 1.0e-4   | 39  | 93.25  |
| MCMC  | 0.094    | 0.032    | 0.0026   | 1.0e-4   | 39  | 51.29  |

Table 1. Fitting and inference results for a typical mouse dataset in the 2015 experiment. MCMC shows mean±SD for parameters except AIC. Rate constants have the unit of s\textsuperscript{-1}, while T1 has the unit of s.

Figure 6 Time course plot for one of the datasets (as that for Table 1). AIC scores for different metabolite: pyruvate: 279.7; lactate: 42.5; alanine: -207.8.

Figure 7 Trace plot (a) and histogram (b) for $k_{pl}$ (s\textsuperscript{-1}) in MCMC (d = 12). Generated after first 50% iterations were discarded as ‘burn-in’. The built-in histfit function gave normal distribution fitting in (b). (c): Failure of two-chain convergence (blue chain started from lsqcurvefit result).
The 2016 Experiment

Several points may be noted when simplifying the model for the latest experiment. First, all alanine terms and reverse rate constants were omitted. Second, the flip-angle correction factor was not negligible as \( \theta_L = 45^\circ \), but was hard-coded to reduce fitting parameters, assuming accurate flip angles for each RF pulse. Finally, the first few data points for lactate were missing. Table 2 showed an example of whole-tumour fitting results (obtained by averaging data in each voxel within the tumour region), with higher \( k_{pl} \) and lower T1.

MCMC analysis gave a stable acceptance rate of around 20%, with trace plots indicating good chain-mixing and successful convergence of two distinct chains (Fig. 8). Notwithstanding these encouraging signs, we had seen strong dependence of fitting results on AIF, as well as greater uncertainty (i.e. SD) towards parameter values. Surprisingly, AIC values stayed the same as that of least-squares fitting (within computer tolerance). Time course plots (Fig. 9) showed failure in fitting pre-peak signals (possibly due to the missing lactate data), but the algorithm nevertheless produced decent fits indicating its robustness.

Voxel-by-voxel least-squares fitting\(^\text{10}\) was performed and fitted \( k_{pl} \) values were mapped onto \(^{1}\text{H} \) anatomical MRI images (Fig. 10). The functional maps were compared with lactate distribution (as pyruvate was only found in aorta for a few seconds) showing those regions emitting higher lactate signals generally overlap with regions with higher \( k_{pl} \). One may thus interpret that metabolic activity was mostly found deep inside the tumour and occasionally on the rim, which implies substantial occurrence of cell death within the tumour. The distinct \( k_{pl} \) values across the tumour show evidence of intratumour heterogeneity. From post-treatment images one may conclude that etoposide lowered \( k_{pl} \) but made intratumour metabolism more chaotic.

Discussion

Validity of Modelling

The strong dependence of fitting results on least-squares parameters (i.e. boundaries and initial guess) as well as AIF puts the robustness and even validity of modelling algorithm in doubt. In fact, when we assigned a boundary
that seemed physiologically improbable (e.g. $k_{pt} > 5 \text{ s}^{-1}$ with $k_{tp} > 0.5 \text{ s}^{-1}$), we were still able to obtain a good fit while other parameters had normal values. This brings us to the possibility of multimodal distribution of parameter vector, which can occur for high-dimensional ODE models\(^\text{16}\). Such multimodal behaviour might also be the reason why two parallel Markov chains could not converge together, regarding the MCMC analysis for 2015 datasets. The chain initiated from a vector distinct from target distribution might converge to a local mode\(^\text{16}\) that was supposed to be rejected due to physiology. Therefore, it became critical to accumulate adequate prior knowledge and carefully select the mode that best fits the metabolic conditions \textit{in vivo}.

A number of articles\(^\text{21,23}\) proposed methodology of analysis where fitting was performed for the time course after signals have reached their highest, discarding data prior to peaks, possibly for the purpose of avoiding incorporation of AIF into modelling. Indeed, in many experiments researchers were unable to, or chose not to, measure AIF directly or indirectly because of timing and costs. Addition of AIF into fitting also increased its difficulty and uncertainly. Notwithstanding that, discarding data puts validity of modelling at risk, particularly when accurate quantitative metabolic information is needed (e.g. for decision-
making in future clinical applications). We therefore suggest considerations to be made when designing experiments and processing data.

There has also been discussion regarding the correct computation of AIC. The value is directly related to the RSS between acquired and fitting data, however as the order-of-magnitude of signal intensities is vastly different between different experiments, it might be important to consider the necessity and feasibility to normalize RSS, so as to provide a fair comparison between fitting performance for different datasets.

**Other Kinetic Models**

The kinetic model presented in this report is shown to be efficient with satisfactory fitting performance, nevertheless the model itself has contributed to several imperfections. First, as suggested in Section 3, a number of assumptions and thus omissions were made, underlying systematic error. Second, the fact that the ODE could not be solved analytically has led to reliance upon built-in ODE solvers (ode15s in this project) which inevitably introduced error and compromised accuracy. Third, the sheer number of parameters in the model (12 in 2015 and 6 in 2016) had increased the difficulty of optimization and made MCMC inference fragile, as the chain could easily go into wrong direction and terminate the whole program subsequently. Such trade-off behaviour between fitting efficiency and accuracy, as witnessed in this project, is of central importance in kinetic modelling and statistical inference.

There have been controversies as to whether higher complexity of model would lead to better fitting results. Whilst some\(^8\) suggested no significant bias caused by model variation, others concluded separately\(^21,24\) that some models do outperform others with lower AIC scores and higher likelihood. Model-free approaches\(^13,23\), though seemed appealing, showed only modest performance\(^21\). We encoded one ‘enhanced’ model with separate intra- and extravascular environments as suggested in the Bankson et al. paper, but were nevertheless unable to obtain a fit for the 2016 datasets we acquired. Future work may be done to examine and improve the repeatability of different kinetic models.

**Other Inference Methods**

As suggested, the MH algorithm may fall short when sampling through high-dimensional or multimodal distributions that demonstrate strong parameter correlations\(^16\). The chains may also converge into wrong regions due to its random-walk behaviour. Therefore, new MCMC methods have been emerging with the progress of machine learning and computational sciences, among which Hamiltonian Monte Carlo (HMC) can a powerful approach targeting multidimensional problems\(^19,25\). Inspired by molecular kinetics, it aims to overcome the inefficiency of MH by walking straight towards the target distribution with a ‘momentum’ vector. In this project the HMC algorithm was coded and trialled on 2016 datasets. Performance was assessed against criteria suggested in literature\(^25\). It did show promise from histograms and trace plots (Fig. 11), as we were able to witness the vector walking along a path around a specific region, and then moving on to seek other possible modes. However, there are some critical problems that need to be solved before further applications. First, HMC requires first order sensitivities\(^16,25\) of the ODE model. In this project
they were computed numerically rather than analytically (again, because of AIF), consuming substantial amount of time whilst losing accuracy. Second, it requires very precise tuning of parameters like the mass matrix, which, if not done automatically, would cost considerable manual effort. In light of future clinical demand of both efficiency and accuracy, MH might still be the first choice for inference, as it is much faster, produces satisfactory convergence and requires less tuning.

**Conclusion**

In this project, kinetic modelling of hyperpolarized $^{13}$C MR spectroscopy and imaging was performed, from which we were able to extract quantitative metabolic information of mice EL4 tumour models. Data was acquired and least-squares fitted onto a two-site exchange model, followed by statistical inference through the application of MCMC. Performance of the modelling process was assessed and compared with those suggested in literature, demonstrating reproducibility of the experiment. From the reaction kinetics, real-time metabolic activity was witnessed, showing Warburg effect and intratumour heterogeneity. Novel modelling and inference approaches were also introduced and examined. Further research may be performed in the following aspects:

1) Better pulse sequences (e.g. flip angles) that improve SNR whilst preserving data;
2) More sophisticated models that are robust, efficient and accurate;
3) Better mathematical description of AIF;
4) Improving MCMC algorithms to target multimodal, high-dimensional and highly-correlated parameter distributions; and
5) Automation of modelling and inference, so as to prepare for clinical applications.

Finally, novel molecular imaging techniques like hyperpolarized $^{13}$C-MRI, glucoCEST and photoacoustic imaging have all shown potential to measure metabolic activity in human tumours. The first clinical trials of hyperpolarized $^{13}$C-MRI have yielded encouraging results. Nevertheless, one needs to bear in mind that these techniques are inherently qualitative and thus requires careful interpretation when trying to extract quantitative information, so as to increase confidence in data and contribute towards a better decision-making for the benefit of cancer patients.
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Optimisation of 4D hyperpolarised $^{13}$C magnetic resonance imaging

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Introduction
Dissolution dynamic nuclear polarisation of $^{13}$C metabolites has enabled real-time investigation of metabolism in vivo. Injection of hyperpolarised [1-$^{13}$C]pyruvate predominantly results in the detection of labelled lactate in tumours that exhibit the Warburg Effect. We have recently demonstrated a fast and highly efficient spectral-spatial imaging technique capable of imaging [1-$^{13}$C]pyruvate and [1-$^{13}$C]lactate in three spatial dimensions and at a temporal resolution of 250 ms.

This poster presents our work on optimising the signal-to-noise ratio, improving the reproducibility of the experiment and using the technique to study in vivo metabolic distribution and tumour heterogeneity at an unprecedented resolution. We also present a comparison of analysis methods including two-site exchange model fitting and area-under-the-curve methods.

Methods and optimisation
5 x $10^7$ EL4 T-cell lymphoma cells suspended in 200 µL PBS were implanted subcutaneously into the flank of C57BL/6j mice. Mice were imaged eight days post-implantation when tumours were ∼1.5 cm$^3$. Mice were anaesthetised and placed in a 7T horizontal bore magnet (Varian) and a 20 mm $^{13}$C surface coil (Rapid Biomedical) placed over the tumour. After localisation of the tumour, 15 ml/kg 75mM hyperpolarised [1-$^{13}$C]pyruvate was injected intravenously. A single spectral-spatial pulse alternately excited [1-$^{13}$C]pyruvate and [1-$^{13}$C]lactate acquiring a stack-of-spirals, obtaining a 3D k-space where each kx-ky plane is filled with a spiral-out trajectory and the spatial information in z-direction is encoded with a blipping gradient train. The spirals are interleaved by a pair of adiabatic inversion pulses to reduce the impact of system imperfections (Fig. 1a and b). Images of each metabolite had an in-plane resolution of 1.25 x 1.25 x 2.5 mm, a spatial resolution of 1 s.

Imaging tumour heterogeneity
Substantial variation in metabolite distribution has been observed in EL4 bearing mice. Generally pyruvate is seen mostly in the great vessels (Figure 3 b & c). Lactate distribution is usually distributed at the deepest edge of the tumour (Fig. 3 a). However, lactate production from the whole tumour rim has also been observed (Fig. 3 c).

Etoposide treatment also alters metabolite distribution (Fig. 3 b & d).

Kinetic modelling
The time course data was fitted to a simplified two-site model based on modified Bloch equations$^{2,3}$:

$$\frac{dP(t)}{dt} = -(k_{ps} + p_{0}P(t) + P_{ps}(S))$$

$$\frac{dS(t)}{dt} = k_{ps}P(t) - p_{ps}S(t)$$

where $k_{ps}$ is the rate constant of pyruvate-lactate conversion and for a metabolite $M$, $p_{M} = \frac{1}{\tau} + \frac{1}{\tau} \ln(\cot\theta)$. To reduce fitting parameters, we assume uniform T1 and T2, as well as accurate flip angles for each metabolite throughout image acquisition ($\theta_{T1} = 75°$, $\theta_{T2} = 45°$). $P_{ps}(S)$ is the Arterial Input Function (AIF) of pyruvate based on a gamma-variate. The lsqcurvefit function in MATLAB (MathWorks, Natick, MA, USA) was applied (Fig. 4a & b), and a self-coded Markov Chain Monte Carlo (MCMC) function$^3$ was implemented to estimate error and increase confidence in fitting results.

Conclusions
This poster presents a spiral acquisition spectral-spatial pulse capable of 4D imaging of [1-$^{13}$C]pyruvate and lactate. In subcutaneous tumours we are able to detect inter- and intratumoral heterogeneity, while demonstrating reproducible kinetic measurements.

References
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Fig. 1. The pulse sequence and (a) its 3D k-space trajectory, (b) the spectral-spatial excitation pulse and (c) the experiment timeline. [1-$^{13}$C]pyruvate is infused over 8s (purple box), pyruvate is excited every two seconds for 10s and alternate pyruvate – lactate acquisitions occur every second from 10s – 90s. Signal from hyperpolarised metabolites is reduced with each excitation. To maximise SNR, minimising unnecessary excitation is desirable. Therefore, no lactate images were acquired for the first 10s post-injection (while the majority of [1-$^{13}$C]pyruvate is still in the circulation). The pyruvate flip angle was reduced to 7° and the lactate flip angle was increased to 45°(Fig. 1c). Instead of delivering a rapid bolus, 15 ml/kg [1-$^{13}$C]pyruvate was infused over 8s. As an infusion is less dependent than a bolus on the quality of cannulation and is less stressful on the circulatory system, this has improved the reproducibility of [1-$^{13}$C]pyruvate delivery (Fig. 2).

Fig. 2. Time courses of $^{13}$C labelled pyruvate (red) and lactate (blue) in EL4 tumours (n = 3). Error bars represent SEM. The pyruvate signal from the tumour was highly reproducible after infusion over 8 seconds.

Fig. 3. Heterogeneity of [1-$^{13}$C]pyruvate and lactate distribution in two EL4 bearing mice, before (a & c) and after (b & d) etoposide treatment. All images are the 6th slice in the z-direction. All pyruvate and lactate images are normalised. Pyruvate images consist of summed spectra between 2 and 12 seconds post-injection. Lactate images are summed spectra between 11 and 21 seconds.

Fig. 4. Curve-fitting of data from a) a pre-treatment EL4 tumour and (b) the same tumour post-treatment.