Investigating systemic and tumour-specific fluctuations in tumour R2* measurement with independent component analysis and pulse oximetry

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Target audience: Researchers interested in cancer studies, particularly cycling tumour hypoxia. Also, Independent Component Analysis (ICA) applications.

Introduction: Using blood oxygen level dependent (BOLD) MRI, solid tumours have previously been found to exhibit transient patterns of hypoxia with subsequent reoxygenation and/or reperfusion [1, 2], thought to be caused by vascular instability and raised interstitial fluid pressure [3, 4]. However, the influence of systemic changes in blood oxygenation on these cyclical events has not been investigated. Independent component analysis (ICA) [5] has been used in the brain to identify resting state network patterns of activation [6], by detecting individual features in coherent patterns of oscillations in blood flow and/or oxygenation. As tumour 'resting state' R_2* fluctuations are likely to be composed of a combination of systemic and tumour-specific effects, we present here a method based on ICA to characterise both contributions.

Methods: *Tumour models and MRI:* $5x10^6$ SW1222 (n=6) or LS174T (n=5) colorectal carcinoma cells were injected subcutaneously into MF1 *nu/nu* mice. Two weeks after injection, tumours reached an approximate volume of 500mm^3, and were then imaged on a 9.4T Agilent VNMRS 20cm horizontal-bore system, with a 39mm birdcage coil, using a multi-slice, spoiled multi-gradient echo (GEMS) sequence. Mice were anaesthetised using isoflurane (1.25% in medical air). Respiratory frequency varied between 43-92 breaths/min and temperature was maintained at 36.7 °C. Dynamic, multi-slice gradient echo data were acquired for 60min in each tumour, from which R_2* was calculated. Sequence parameters included: TR=59.62ms, 5 echoes, TE_1=2ms, echo spacing=2ms, 5 slices, 64x64 matrix, voxel volume 312x312x1500µm, flip angle=20°. Arterial hemoglobin O_2 saturation (O_2sat) was simultaneously measured on the thigh, by pulse oximetry (MouseOx®, Starr Life Sciences Corp., Oakmont, PA). *Post-processing:* Voxel-wise post processing was performed in Matlab. Maps of the standard deviation of resting-state R_2* timecourses ($RS_{(SD)}$) were produced for each tumour to identify regions undergoing fluctuations in oxygen saturation and/or perfusion. ICA maps were created from decomposition of R_2* timecourses into independent components (ICs) with ICA [5]. After decomposition, ICA temporal patterns were correlated with the systemic O_2 saturation timecourse and categorised into systemic ($p < 0.01$) or tumour-specific ($p > 0.01$) ICs. Z-score maps were produced from ICA spatial maps, one for each IC, and a z-score threshold of 2.2 was imposed to identify voxels whose R_2* timecourse has similarities with a given IC. Finally, the ICA maps were averaged across ICs into a single map, considering only the z-scores that survived the threshold.

Results: An example $RS_{(SD)}$ map from an SW1222 tumour is shown in Fig.1a, which shows regions with raised temporal fluctuations (blue arrows). Maps of ICA z-scores are also shown, from the same tumour, categorised into components that either significantly correlate with systemic changes in O_2sat (Fig. 1b), or show no correlation with systemic O_2sat (Fig.1c). In this example, the regions identified with raised $RS_{(SD)}$ were not associated with systemic changes in O_2sat, rather were specific to the tumour. Plots of the mean systemic and tumour-specific ICA timecourses from these data are shown in Figs. 1d and 1e, which show clear differences in temporal dynamics. Across the cohort of tumours, the number of pixels with a tumour-specific or systemic mean z-score greater than 2.2 was compared between tumour types (Fig. 2). According to this analysis, the mean percentage of tumour voxels displaying tumour-specific fluctuations was 86.0% and 96.0% for SW1222 and LS174T tumours, respectively (p=0.13, Mann-Whitney U test); the mean percentage of tumour voxels displaying systemic R_2* fluctuations was 69.5% and 73.3% for SW1222 and LS174T, respectively (p=0.66, Mann-Whitney U test).

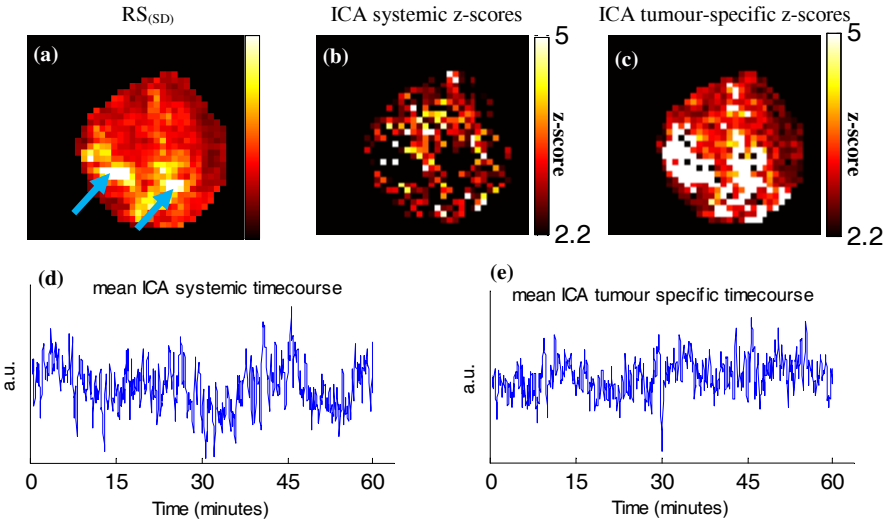

Fig. 1 – SW1222 tumour xenograft. **(a)** Resting State standard deviation – $RS_{(SD)}$ map of R_2* fluctuations. (b, c) ICA maps of systemic and tumour specific z-scores. (d, e) Mean timecourse curves of ICA decomposition for both systemic and tumour specific independent components.

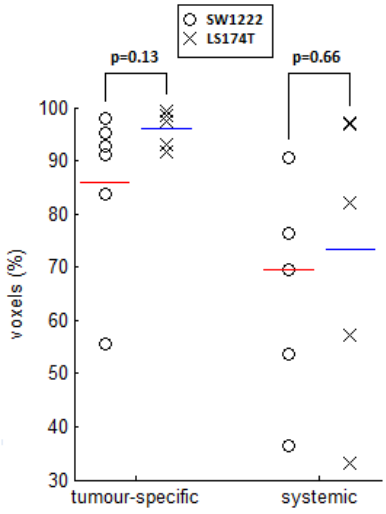

Fig. 2 – Percentage of tumour-specific and systemic voxels for each tumour type.

Discussion & Conclusion: In this study, we have presented a novel method for separating systemic and tumour-specific tumour R_2* fluctuations, based on independent component analysis (ICA) and pulse oximetry. The advantage of this approach over methods that directly correlate R_2* timecourses, and segment voxels into purely systemic or tumour-specific effects, is the ability of ICA to assess the relative contribution of systemic and tumour-specific effects in individual voxels. LS174T tumours revealed a greater proportion of voxels with tumour-specific and systemic fluctuations than SW1222 tumours, although these differences were not statistically significant. The relatively high percentage of voxels in both tumour-specific and systemic groups is possibly due to the ability ICA has to separate between the two effects. Clear differences have previously been observed in the hypoxia and vascular perfusion status of the tumours used in this study, with SW1222 tumours much better perfused and less hypoxic than LS174T tumours [7]. Further work is required to evaluate the details of mechanisms underpinning spontaneous, tumour-specific fluctuations in each tumour type, which we anticipate will be elucidated with the novel methodology presented here.

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References: [1] Baudelet C, *et al., Phys. Med. Biol.* 49: 3389-411. [2] Brown JM, *Br. J. Radiol.* 52: 650-6. [3] Patan S, *et al., Microvasc.* Res. 51:260-72. [4] Mollica F, *et al., Microvasc. Res.* 65:56-60. [5] Hyvärinen A, *IEEE Trans. Neural Netw.* 10:626-34. [6] van de Ven, *et al., Hum. Brain Mapp.* 22: 165-78. [7] Folarin A, *et al., Microvascular Res.* 80: 89-98.