Modeling acute and chronic hypoxia using serial images of ^{18}F -FMISO PET

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Purpose: Two types of tumor hypoxia most likely exist in human cancers: Chronic hypoxia due to the paucity of blood capillaries and acute hypoxia due to temporary shutdown of microvasculatures or fluctuation in the red cell flux. In a recent hypoxia imaging study using ^{18}F -FMISO PET, the authors observed variation in tracer uptake in two sequential images and hypothesized that variation in acute hypoxia may be the cause. In this study, they develop an iterative optimization method to delineate chronic and acute hypoxia based on the ^{18}F -FMISO PET serial images.

Methods: They assume that (1) chronic hypoxia is the same in the two scans and can be represented by a *Gaussian* distribution, while (2) acute hypoxia varies in the two scans and can be represented by *Poisson* distributions. For validation, they used *Monte Carlo* simulations to generate pairs of ^{18}F -FMISO PET images with known proportion of chronic and acute hypoxia and then applied the optimization method to the simulated serial images, yielding excellent fit between the input and the fitted results. They then applied this method to the serial ^{18}F -FMISO PET images of 14 patients with head and neck cancers.

Results: The results show good fit of the chronic hypoxia to Gaussian distributions for 13 out of 14 patients (with $R^2 > 0.7$). Similarly, acute hypoxia appears to be well described by the Poisson distribution ($R^2 > 0.7$) with three exceptions. The model calculation yielded the amount of acute hypoxia, which differed among the patients, ranging from $\sim 13\%$ to 52% , with an average of $\sim 34\%$.

Conclusions: This is the first effort to separate acute and chronic hypoxia from serial PET images of cancer patients. © 2009 American Association of Physicists in Medicine.

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I. INTRODUCTION

Tumor hypoxia is of much current interest in cancer research and management. Histological studies, $p\text{O}_2$ probe measurements and nuclear imaging have evinced that regions of hypoxia exist in many human cancers,¹⁻⁴ probably the result of uncontrolled proliferation causing the tumors to outgrow the blood supply. Moreover, it is now established that tumor hypoxia is an important determinant of relapse-free survival and overall clinical outcome for both radiotherapy and surgery.⁵⁻⁷ Because hypoxic cells are three times more resistant than aerobic cells to ionizing radiation, it has been hypothesized that some failures to achieve local control by radiotherapy may be a result of hypoxia-induced radioresistance. Since the presence of hypoxic tumor cells is likely to indicate a poor treatment outcome, it would be useful to identify hypoxic tumor and modify treatment accordingly.

Two types of tumor hypoxia have been suggested by laboratory and clinical studies. Chronic hypoxia is presumably caused by the consumption and depletion of oxygen by tumor cells located between the blood capillaries and the hypoxic regions. Transient or acute hypoxia occurs when there is temporary shutdown of vessels in the highly abnormal microvasculatures in tumors⁸ or fluctuation in the red cell flux.⁹ Reoxygenation after radiation treatment has been reported in laboratory studies¹⁰ and is believed to be an important factor in circumventing hypoxia-induced radioresistance in fractionated radiotherapy.

A number of techniques have been applied to study acute hypoxia in human and animal tumors. Pigott *et al.*¹¹ used laser Doppler flowmetry to detect perfusion fluctuations in human breast cancer. Polarographic (e.g., the Eppendorf) and fluorescence (e.g., the OxyLite) probes were used to evaluate fluctuations of partial oxygen pressure in rodent and canine

tumor models.^{12–15} Several nuclear magnetic resonance (NMR) techniques have also been applied, such as blood-oxygen-level-dependent imaging^{16,17} and dynamic contrast enhanced MRI.¹⁸ In addition, fluorescence immunohistochemistry (IHC) can be used with dual hypoxic tracers to detect changes in tumor hypoxia in tumor sections.¹⁹ Some of these techniques have been used in our laboratory.²⁰

To overcome tumor hypoxia-mediated radioresistance that negatively impacts the local control of human cancers by radiotherapy, a number of strategies have been proposed in the past. These include using intensity-modulated radiotherapy to give a higher dose to the hypoxic volume^{21–23} and the use of toxic agents that preferentially kill hypoxic cells.²⁴

Given the importance of tumor hypoxia in the management and treatment of cancer, much effort is ongoing to develop methods to detect and measure tumor hypoxia, including direct probe measurement, IHC, and noninvasive PET and NMR imaging. For PET scanning, a number of radiotracers for viable hypoxic cells in solid tumors have been developed.⁴ At Memorial Sloan-Kettering Cancer Center (MSKCC), we are evaluating the use of ¹⁸F-FMISO for tumor hypoxia imaging. We recently reported on the study of the first 20 patients with cancers of the head and neck (H/N)^{23,25,26} for whom two ¹⁸F-FMISO PET imaging studies were performed prior to radiotherapy, separated by 3 days. As reported by Nehmeh *et al.*,²⁶ we observed changes in the intensity distribution of tracer uptake in the serial images. As described in that paper, comparative analysis of the two datasets suggested that the observed variations were largely random in nature, leading to the hypothesis that the differences in spatial distributions of tracer uptake were due to the variation in acute or transient hypoxia. This hypothesis underlies our present attempt to develop analytical approaches to quantitatively differentiate acute from chronic hypoxia based on the serial images. This paper describes our basic assumptions, the mathematical methods, and the results obtained.

II. MATERIALS AND METHODS

Twenty male head and neck cancer patients scheduled for radiation therapy were included for this pretreatment hypoxia PET/CT study using the specific study protocol approved by the MSKCC Institutional Review Board. All patients underwent a FDG PET/CT scan (day 1) and two ¹⁸F-FMISO PET/CT scans on day 2 (FMISO1) and day 5 (FMISO2), respectively. Data from 14 patients were included in this analysis, as the images from the others did not have sufficient positive volumes for adequate statistics. The average age for these 14 patients was 58 yr, ranging between 46 and 79 yr. The mean tumor volume of the 14 patients was ~24 cc, ranging between 4 and 55 cc.

Both ¹⁸F-FMISO and FDG were produced at MSKCC Cyclotron-Radiochemistry Core Facility. All PET/CT images were acquired on a GE Discovery ST PET/CT scanner (GE Healthcare Technologies, Waukesha, WI). The CT (Light-Speed 4-slice) has a 50 cm transaxial field of view (FOV),

with slice thickness ranging from 0.63 to 20.0 mm. The PET (Advance NXi) scanner has transaxial and axial FOVs of 55 and 15.2 cm, respectively. All PET imaging were performed exclusively in two-dimensional mode, with intrinsic resolution of 4.2 mm full width at half maximum (FWHM).

Patients were injected intravenously with 10.4 mCi (range of 9.3–11.0 mCi) ¹⁸F-FMISO without fasting. Patients were imaged in supine positions, with head, neck, and shoulders immobilized using Aquaplast mask prepared during the simulation session for radiation therapy. FMISO1 PET/CT scans were acquired 2.7 h (range of 1.95–3.25 h) postinjection, and FMISO2 scans were acquired 2.7 h (range of 2.1–3.25 h) postinjection. FMISO raw image datasets were first corrected for attenuation, scattered, and random events, and then reconstructed using the standard clinical FDG reconstruction protocol (subsets=28, iterations=2, post filter =6.0 mm FWHM, and loop filter=4.3 mm FWHM). Venous blood samples for patients were taken immediately before and after the PET/CT scans and were counted by a CompuGamma CS Gamma Counter (LKB-Wallis, Turku, Finland) to compute the activity concentration (Bq/cc) and blood standard uptake value (SUV).

Image registrations between the different datasets were performed using the software IMGREG developed at MSKCC. The algorithm is based on maximizing the mutual information between the CT image sets of the respective PET/CT exams using the following procedure. The first step of the image registration involved manually aligning the different CT datasets, guided by the fiducial markers in the immobilization mask. The CT_{FDG} and CT_{FMISO2} images were then registered rigidly to the CT_{FMISO1} images by maximizing the mutual information in the respective datasets. The transformation matrices were then applied to the FDG and FMISO2 image sets, thus registering the FDG and FMISO2 target volumes to the FMISO1 target volume. Imaging data initially available in units of μ Ci/ml per pixel were decay corrected to the time of injection and converted into SUV values. The two ¹⁸F-FMISO images were then compared voxel by voxel.

Prior to the analysis of each patient's clinical data, the total number of counts within the two ROIs of the two ¹⁸F-FMISO images was normalized by rescaling the total activity of each ROI to be the same. This procedure reduced the differences between the two images caused by factors such as differences in injected activity and variation in the procedure of the two scanning sessions (e.g., interval between isotope injection and imaging and imaging period). The normalization of the total hypoxia of the two scans, and the fact that chronic hypoxia was taken to be the same in the two scans, resulted in the total acute hypoxia also being same in both scans. However, it was the changes in the spatial distribution of acute hypoxia that resulted in the altered FMISO distribution in the two scans. The above were implicitly assumed in the *Monte Carlo* (MC) simulation discussed later.

III. MATHEMATICAL MODELING AND ITERATIVE OPTIMIZATION TO DELINEATE CHRONIC AND ACUTE HYPOXIA IN SERIAL ^{18}F -FMISO PET IMAGES

Both chronic and acute hypoxia contribute to the ^{18}F -FMISO images. The total uptake H in a voxel is the sum of their contributions,

$$H = H_a + H_c, \quad (1)$$

where H_a is due to acute hypoxia and H_c is due to chronic hypoxia.

Equation (1) applies to both scans, thus the total uptake H_1 and H_2 (in scans 1 and 2) of the same voxel can be written as

$$H_1 = H_{a1} + H_{c1} = H_{a1} + H_c, \quad (2)$$

$$H_2 = H_{a2} + H_{c2} = H_{a2} + H_c.$$

The above assumes that chronic hypoxia is unchanged in the two scans, and therefore $H_{c1} = H_{c2} = H_c$. While there is scanty data on the time course of changes in chronic hypoxia due to tumor vasculature alteration, a recent paper by de Langen *et al.*²⁷ reported that tumor perfusion as measured by ^{15}O -labeled water and PET was unchanged over a seven day period in untreated human NSCLC. This observation is consistent with current views on the process and kinetics of angiogenesis and blood vessel formation, and that vascular architecture of a “mature” human tumor will not change significantly over a few days. Thus our assumption that chronic hypoxia remains approximately constant for the two scans is reasonable.

In Eq. (2), H_1 and H_2 are measured values so that there are two equations with three unknowns H_{a1} , H_{a2} , and H_c . By dividing the entire range of uptake values (from the lowest to the maximum) of each scan into equal bins, we can generate an uptake-volume histogram, i.e., the number of voxels as a function of uptake, as shown in Fig. 3(a). Similarly, histograms could be generated for H_a and H_c once they are derived.

To estimate the acute and chronic hypoxia, we devised the iterative optimization method described below. We observed in the majority of patient data that the frequency distributions of FMISO uptake in patients resembled *Gaussian* distributions. Given that chronic hypoxia is generally considered to be more prevalent relative to acute hypoxia, we assume that the FMISO uptake is primarily due to chronic hypoxia and adopt the hypothesis that chronic hypoxia can be represented by a Gaussian distribution. The validity of this assumption will be tested in the application of our method to the patient data and in Monte Carlo simulation studies.

In Fig. 1, H_h and H_s are the higher and smaller readings in a same voxel of the patient's two ^{18}F -FMISO PET scans, respectively. Denoting ω as the fraction of H_h due to acute hypoxia, we write

FIG. 1. Schematics illustrating the two SUV values in the two scans for the same voxel, and the meaning of the terms H_s , H_h , H_c , H_a , and ΔH are defined in the text.

$$\omega = \frac{H_a}{H_h}, \quad (3)$$

We also define $x = \Delta H / H_h$, where ΔH is $H_h - H_s$ (Fig. 1), and write ω as a function of x , i.e., $\omega = \rho f(x)$, with the value of ρ to be determined later. We assume that the dominant behavior of $f(x)$ is that of a power law with a positive exponent because of its simplicity. As will be shown in the Monte Carlo study, the power law dependence of x was able to generate results that fitted the input data. This functional form behaves sensibly in the limiting case of zero acute hypoxia, but would fail if total hypoxia or chronic hypoxia approaches zero. However, given the size of a PET voxel of approximately $4 \times 4 \times 4 \text{ mm}^3$ and the pattern of tumor hypoxia in human cancer, it is very unlikely that there is zero hypoxia or zero chronic hypoxia in a PET voxel.²⁸ Thus, we write

$$\omega = \rho x^\beta = \rho \frac{\Delta H^\beta}{H_h^\beta}. \quad (4)$$

For the voxel with H_h , the chronic hypoxia is calculated by combining Eqs. (1), (3), and (4),

$$H_c = (1 - \rho x^\beta) H_h. \quad (5)$$

In the MC simulations described below, the power law $f(x) = x^\beta$ yields good agreement between the MC-generated input data and the results of the iterative optimization method with $\beta \sim 0.6$. For application to the patient studies, chronic hypoxia was then written as

$$H_c = (1 - \rho x^{0.6}) H_h, \quad (5a)$$

in which H_c , x , and H_h vary from voxel to voxel, while ρ is constant for the two scans of a patient.

To apply the model, we iteratively varied ρ to obtain the value ρ_0 for which the chronic hypoxia distribution predicted by Eq. (5a) best fits a Gaussian distribution. To find the best fit we used the iterative optimization method provided in MATLAB to maximize *R*-square (R^2), which is defined below. In Eq. (6), f_i is the value predicted by the Gaussian for the i th voxel, y_i is the value predicted by Eq. (5a) for the same voxel, and y_m is the average value of y_i . Then, with

$$SS_{\text{tot}} = \sum_i (y_i - y_m)^2, \quad (6)$$

$$SS_{\text{err}} = \sum_i (y_i - f_i)^2,$$

R^2 is defined as

$$R^2 = 1 - SS_{\text{err}}/SS_{\text{tot}}. \quad (7)$$

The method is identical to a least-squares fit since maximizing R^2 is equivalent to minimizing SS_{err} . R^2 is between 0 and 1, with a value closer to 1, indicating a better fit.

Using Eq. (3) and (5a) and the derived ρ_0 , we calculated the ω and the chronic hypoxia H_c for each voxel. Then using Eq. (2) we compute the contribution of transient hypoxia in the two scans for that patient.

IV. MONTE CARLO SIMULATIONS FOR EVALUATION OF THE OPTIMIZATION METHOD

To evaluate the optimization method, we applied it to simulated serial ^{18}F -FMISO PET scans with known chronic and acute contributions. Specifically, we used Monte Carlo simulations to generate pairs of ^{18}F -FMISO PET images (each consisting of 1000 voxels) with known proportion of chronic and acute hypoxia. We then applied the iterative optimization method to derive fits to each image pair and compared the results to the original MC-generated input data. In the course of this testing, we also scrutinized the utility of the equation $f(x)=x^\beta$ and determined the best value of β . The detailed procedures are described below.

IV.A. Generation of simulated serial ^{18}F -FMISO images with the Monte Carlo method

Chronic hypoxia was approximated by a Gaussian distribution $G(\mu; \sigma)$, with μ as the mean chronic SUV and σ as the standard deviation. For acute hypoxia, the prevailing view is that it is caused by temporary shutdown of tumor vasculatures or a change in red cell flux.^{11–15} The number of blood vessels N in a PET voxel is large (i.e., $N \gg 1$), and they closed with probability p ($0 < p < 1$). With the parameter $\lambda = Np$ representing the number of closed vessels at an instant of time, we approximate acute hypoxia by a Poisson distribution $P(\lambda)$.^{13,14} The assumption of Poisson distribution for acute hypoxia is derived from our reading of literature on the measurement of temporal changes in partial oxygen level in rodent tumors. In particular, analysis of the data from Dewhirst *et al.*²⁹ on the number of events of intermittent hypoxia and the associated durations yielded an approximate Poisson curve. The results obtained by Brurberg *et al.*¹³ were also consistent with the Poisson assumption. We introduce another dimensionless parameter δ , which represents the ratio of acute to chronic hypoxia. Then the product $\delta\mu$ (in SUV) is acute hypoxia.

For each voxel, we first randomly generated an entry X of a standard normal distribution, so the chronic hypoxia in this

voxel for a given μ and σ is $\mu + X\sigma$. We then independently sampled the Poisson distribution $P(\lambda)$ twice and obtained two independent entries y_1 and y_2 to yield the acute hypoxia $(\delta\mu/\lambda)y_1$ and $(\delta\mu/\lambda)y_2$ for the first and second scans, respectively. The total hypoxia-related uptakes in this voxel for the two scans are $(\mu + X\sigma) + (\delta\mu/\lambda)y_1$ and $(\mu + X\sigma) + (\delta\mu/\lambda)y_2$, respectively. This was repeated 1000 times to obtain a pair of simulated ^{18}F -FMISO scans, each of 1000 voxels.

A large number of paired images were generated by varying the values of μ , σ , λ , and δ so as to examine the validity of the optimization method, as described below.

IV.B. Evaluation of the optimization method using the Monte Carlo generated images

We applied the optimization method [Eqs. (3)–(5), (5a), (6), and (7)] to the simulated serial images to determine the best β value. Since $H_h \geq H_c$, it follows that $\rho \geq 0$. For a given β , we increased ρ in steps of 0.01 and used Eqs. (6) and (7) to test how well the H_c distribution obtained from Eq. (5) was fit by a Gaussian. The best fit (with the largest value of R^2) determines ρ_0 as well as the “optimized H_c .” We also compared the optimized H_c with the known input H_c that had been generated with MC simulation. The difference is expressed as $\% \text{Dev}_{\text{av}}$, the percentage deviation averaged over all 1000 voxels,

$$\% \text{Dev}_{\text{av}} = \left(\frac{\text{optimized } H_c}{\text{input } H_c} - 1 \right) 100\%. \quad (8)$$

For example, using $\beta=0.58$, we have $\% \text{Dev}_{\text{av}} = -0.5 \pm 11.7$ when $\mu=0.8$, $\lambda=8$, and $\sigma=0.10$ [Fig. 2(a)].

We first allowed β to vary over a broad range of $0 \leq \beta \leq 2.0$ but found that the model was effective only within the range of $0.4 \leq \beta \leq 1.0$. We then varied β in finer steps within this range to find the best β value.

In addition to varying β value, we also performed the same evaluation for a large number of MC-simulated image pairs obtained for a range of values for each of the variables μ , σ , λ , and δ : μ —0.6, 0.7, 0.8, 0.9, 1.0, 1.1, and 1.2; σ —0.05, 0.1, 0.15, 0.2, and 0.25; λ —4, 8, 12, 16, and 20; δ —0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, and 0.7. The choices for μ and σ , initially estimated from the clinical ^{18}F -FMISO images, were subsequently adjusted to be in the range of the fitted results. The ranges of λ and δ are, in part, based on a literature review of laboratory studies and one clinical investigation^{9,11–15} on acute tumor hypoxia.

To study how each variable in MC simulation affects the performance of the optimization method, we changed the value of 1 variable in one set of simulation, with the other three values kept constant. For instance, to evaluate the influence of μ , we set $\lambda=8$, $\sigma=0.10$, and $\delta=0.50$, and varied μ from 0.6 to 1.2. For each μ , we repeated the simulation 50 times. Therefore, the number of total simulations for this test alone was $50 \times 7 = 350$. In addition, we repeated this for each of eight β values, so the total number of simulation datasets (of paired images) was 2800. The results of this exercise are given in Fig. 2(a) which plots $\% \text{Dev}_{\text{av}}$ versus μ for different

FIG. 2. [Panel (a)] Comparison of the fit of the iterative optimization method to the MC-generated input data; the differences between the fitted and the input values are given as $\%Dev_{av}$. This figure shows the effect of different μ and β values (with $\lambda=8$, $\sigma=0.10$, and $\delta=0.50$) on $\%Dev_{av}$. For each combination of μ and β values, we repeated the simulation 50 times for a total of 2800. The error bars in the figure are the standard deviation of $\%Dev_{av}$ for the 50 simulations. [Panel (b)] Similar to panel (a) except that σ and β values were varied. [Panel (c)] Similar to panel (a) except that λ and β values were varied. [Panel (d)] Similar to panel (a) except that the fraction of acute hypoxia $-\delta/(1+\delta)$, and β were varied.

β values (results from only five out of the eight β values are plotted for clarity). The error bars in all the figures are the standard deviation of $\%Dev_{av}$ for the 50 times of simulations.

Similarly, at fixed values of $\mu=1.0$, $\lambda=8$, and $\delta=0.50$, we varied β (0.46, 0.50, 0.58, 0.60, 0.70, 0.75, 0.80, and 0.85) and σ (0.05, 0.10, 0.15, 0.20, and 0.25) and repeated the simulation 50 times for a total number of 2000 simulations [results given in Fig. 2(b)].

Figure 2(c) plots $\%Dev_{av}$ versus λ for fixed values of $\mu=1.0$, $\sigma=0.10$, and $\delta=0.50$, and combinations of seven β values (0.46, 0.50, 0.58, 0.60, 0.65, 0.70, and 0.75) and five λ values (4, 8, 12, 16, and 20). Again 50 simulations were performed for each set of parameters for a total of 1750.

Similarly, Fig. 2(d) plots $\%Dev_{av}$ versus $\delta/(1+\delta)$ for fixed values of $\mu=1.0$, $\sigma=0.10$, and $\lambda=8$, and combinations of 8 β (0.46, 0.50, 0.58, 0.63, 0.70, 0.75, 0.80, and 0.85) and 9 δ values (0.30, 0.35, 0.40, 0.45, 0.50, 0.55, 0.60, 0.65, and 0.70). A total of 3600 simulations was performed to generate

this data. In this figure, the horizontal axis is $\delta/(1+\delta)$ times 100, which stands for the percentage of acute hypoxia in a whole scan.

In aggregate, the above simulation and validation studies showed that the simple expression of $f(x)=x^\beta$ in the mathematical model sufficed to yield good agreement between the input and fitted H_c values. In addition, β values of 0.58–0.70 yielded the smallest $\%Dev_{av}$, i.e., best agreement between the input and the computed H_c . In terms of the effect of variation in the other parameters on the performance of the optimization method, changes in μ , λ , and σ minimally affected the $\%Dev_{av}$ for all β values. Changes in δ affected $\%Dev_{av}$ with a positive slope, but for $\delta=0.3$ –0.4 and $\beta=0.58$ –0.70, the change in $\%Dev_{av}$ was minimal. We subsequently adopted 0.6 for β .

V. RESULTS

After validating the iterative optimization method with the MC-generated paired images, as described above, we ap-

TABLE I. Results of iterative optimization fitting H_c to Gaussian distributions. The R^2 values (column 2) are all >0.7 (except for patients 13), indicating good approximation of the fitted H_c by Gaussian distributions. Based on the fitted H_c , H_{a1} and H_{a2} were computed and compared to Poisson statistics. With few exceptions, the H_{a1} and H_{a2} distributions are well represented by Poisson curves with R^2 values of 0.7 or higher (last two columns). The percentages of acute hypoxia in the total hypoxia H are listed in column 3.

Patient ID	R^2 chronic	Ave. H_a/H (%)	R^2 acute1	R^2 acute2
1	0.924	13.4	0.789	0.699
2	0.921	40.3	0.911	0.809
3	0.980	25.6	0.766	0.803
4	0.771	25.9	0.955	0.746
5	0.915	25.2	0.834	0.931
6	0.767	38.3	0.838	0.885
7	0.941	24.5	0.800	0.767
8	0.906	45.4	0.932	0.900
9	0.821	41.9	0.793	0.977
10	0.909	30.6	0.905	0.657
11	0.761	51.6	0.846	0.408
12	0.892	24.2	0.957	0.894
13	0.606	42.4	0.706	0.791
14	0.942	43.6	0.953	0.911
Ave	0.861	33.8	0.856	0.798

plied it to the patient data using $\beta=0.6$. Briefly, we iteratively varied ρ in Eq. (5a) to maximize R^2 of Eq. (7) to derive ρ_0 for which the chronic hypoxia distribution predicted by Eq. (5a) best fits a Gaussian distribution.

Table I summarizes the results of applying the mathematical model and iterative optimization method to the clinical PET images for 14 patients. The fit to a Gaussian distribution for H_c was excellent for ten patients ($R^2>0.8$), fair for three patients ($R^2=0.7-0.8$), and poor for the other one patient ($R^2<0.7$). In comparison, the average R^2 for all MC-simulated paired images was 0.93 with a standard deviation of 0.03.

After performing the model fit, we calculated the acute hypoxia for each clinical image using Eq. (2) and computed the R^2 value for the Poisson distribution $P(\lambda)$ fit to the acute hypoxia for each scan. The results from these fits are also given in Table I. Of the 28 values of R^2 , 18 are >0.8 , 7 are between 0.7 and 0.8, and 3 are <0.7 , indicating that most of the distributions are Poisson-like. Figures 3(a) and 3(b) show the original hypoxia distribution and the fitted Gaussian distribution of chronic hypoxia for patient 14. Similarly, Figs. 4(a) and 4(b) show the fitted Poisson distributions for scans 1 and 2 for patient 14. These and similar distributions for other patient datasets support the approximations made in our mathematical model.

As discussed above, ρ is a variable in the iterative process to optimize the fit of the chronic hypoxia distribution to the Gaussian distribution. The value of ρ_0 for the best fit for each patient is plotted in Fig. 5(a). Figure 5(b) shows a plot of the % acute hypoxia and ρ_0 , showing no correlation between the two. While Eqs. (4) and (5) would indicate that in a voxel ρ

FIG. 3. [Panel (a)] Distribution of the measured SUV of the first ^{18}F -FMISO scan of patient 14. The smooth curve is the fitted Gaussian of $\mu=2.01$, $\sigma=0.42$, with $R^2=0.789$. [Panel (b)] The chronic hypoxia SUV derived from the data of panel (a) using the iterative optimization method. The smooth curve is the fitted Gaussian of $\mu=1.78$, $\sigma=0.33$, with $R^2=0.942$. Panel (b) is more Gaussian-like with better R^2 .

is related to the maximum fraction of acute hypoxia, the correlation does not exist across the patient population.

VI. DISCUSSION

To the best of our knowledge, this study is the first attempt to quantify acute and chronic hypoxia in human tumors based on repeated PET images with a hypoxic cell tracer. Because conventional image analysis approaches cannot differentiate the relative contribution of these two types of tumor hypoxia, we develop a mathematic model, using iterative optimization to separate the two components. An important assumption is that the chronic hypoxia remains unchanged between the first and second ^{18}F -FMISO PET studies. We believe that this assumption is reasonable based on the current understanding of the time scale of angiogenesis and on the experimental evidence that tumor perfusion, as measured by ^{15}O -labeled water and PET, remained the same over a seven day period in untreated human NSCLC.²⁷

FIG. 4. [Panel (a)] Acute hypoxia SUV for ^{18}F -FMISO scan 1 of patient 14, which closely match the Poisson distribution with $R^2=0.953$, $\lambda=4.99$ (smooth curve). Panel (b)] Acute hypoxia SUV for ^{18}F -FMISO scan 2 of patient 14, which closely match the Poisson distribution with $R^2=0.911$, $\lambda=5.59$ (smooth curve).

As discussed in our paper by Nehmeh *et al.*,²⁶ uncertainty in spatial registration between FMISO1 and FMISO2 may compromise the voxel-by-voxel correlation of hypoxia images. The accuracy of registration between two serial PET studies performed on a PET/CT scanner depends on two factors: (a) The registration accuracy of CT to CT between the respective studies (used as the basis of the PET to PET registration) and (b) the alignment accuracy between the PET and CT images acquired during the same session. Zhang *et al.*³⁰ studied the accuracy of CT to CT image registration for radiotherapy patients with head and neck (H/N) cancers immobilized with a patient-specific mask, similar to that used for our patients. For the palatine process of the maxilla, the anatomical site most similar to the H/N disease of our study cohort, they reported uncertainties of ~ 2 mm. For PET-CT registration accuracy, we performed a phantom study and demonstrated uncertainties of <0.3 mm. Thus, the combined uncertainty is ~ 2 mm, or approximately half of the

FIG. 5. [Panel (a)] The value of ρ_0 for the best fit for each patient. [Panel (b)] A plot of the % acute hypoxia in a ^{18}F -FMISO PET scan with ρ_0 .

dimension of a voxel, and therefore should not significantly affect the validity of the voxel-to-voxel comparison of this study.

In our model, we assume that chronic hypoxia generate SUV distribution that can be described by Gaussian statistics. Experimental studies on chronic and acute hypoxia provided information on a microscopic scale of $100\ \mu\text{m}$ or less,^{28,31} whereas each PET voxel is approximately 4 mm in each dimension. Thus, the intensity of ^{18}F -FMISO in a PET voxel represents the composite uptake of a spectrum of microscopic regions, ranging from normoxic to hypoxic to anoxic. In this context, the term acute hypoxia in this paper is an operational definition, denoting voxels in which variation in FMISO uptake is observed. Biologically, it may mean that in these voxels the contribution from acute hypoxia is significant or dominant relative to that from chronic hypoxia, but that remains a conjecture.

In contrast to our previous study in which we applied a threshold to denote hypoxic voxel,²⁶ in this analysis we considered the entire frequency distribution of FMISO intensity

for all the voxels within the tumor. This is because each $4 \times 4 \times 4$ mm³ PET voxel represents a large number of microscopic regions of 100 μ m or less, the dimension of tumor hypoxia. Therefore, the FMISO intensity in a PET voxel represents the composite uptake averaged over the spectrum of anoxia, hypoxia, and well-oxygenated regions, and there is always some degree of FMISO uptake due to hypoxia in all the voxels. For that reason we included all the voxels in our analysis. This is mirrored in our use of the Monte Carlo method for generating simulated data of chronic and acute hypoxia.

To validate our approach, we use MC simulation to generate input images and then show that the iterative optimization method can reproduce the input data. We then applied the method to analyze patient data and show that the fitted chronic and acute hypoxia SUV distributions are well represented by Gaussian and Poisson curves [e.g., Figs. 3(b) and 4].

Specifically, Table I shows good fit of the chronic hypoxia to Gaussian distributions for 13 out of 14 patients (with $R^2 > 0.7$). Similarly, acute hypoxia appears to be well described by the Poisson distribution ($R^2 > 0.7$) with three exceptions. Based on the fitted parameters, the average (over all the voxels) acute hypoxia for each patient is also calculated and is given in Table I. The amount of acute hypoxia differed among the patients, ranging from $\sim 14\%$ to 52% , with an average of $\sim 34\%$.

It would be of interest to compare the results of this study to data on acute hypoxia in human cancers; however, the latter data are not available. There exist measured data of acute hypoxia in rodent tumor models,^{13,29} although a direct comparison between those data and our results is subject to questionable interpretation. Specifically, the measured data of acute hypoxia in rodent tumor models are in terms of frequencies, durations and magnitudes of pO_2 changes, whereas our results are derived using mathematical methods based on serial ¹⁸F-FMISO images. Nevertheless, there is an overall consistency between the $\sim 34\%$ acute hypoxia of this study and the measured data of the references.^{13,29} For example, in Ref. 13 "Fluctuations around 3 mm Hg were seen in 44% ... and 37% ..." in the two tumor models, "around 5 mm Hg ... in 39% ... and 24%," and "around 10 mm Hg in 29%...and 14%." Similarly, in Ref. 29 they observed that "The percentages of sites that did not fluctuate across the thresholds were 38% and 61% for the 10 and 5mm Hg values, respectively."

As discussed in Sec. II, our mathematical model involves a number of assumptions and approximations, without which the study cannot be carried out. While some of the assumptions (e.g., Gaussian distribution) were in the main consistent with the patient data, others, although reasonable, are unproven. In addition, in modeling the acute hypoxia, we need to consider complex biological processes. As there is a paucity of human data on this subject, we relied primarily on published experimental data in studies using rodents. Thus the results and conclusions derived must be viewed in the context of these assumptions and approximations. Neverthe-

less, within these limitations and uncertainties we believe that this study provide insights on the proportion of acute vis-à-vis chronic hypoxia in human head and neck tumors.

Our optimization model is perhaps the first step to separate out acute from chronic hypoxia in hypoxia images. Improvement is most likely possible, perhaps by including data from a third hypoxia scan. An optimization model based on three ¹⁸F-FMISO serial scans could be more powerful in differentiating acute and chronic hypoxia, but the cost and logistical difficulties are serious impediments.

Finally, we emphasize that this study involves modeling which requires assumptions and approximations. We believe that we have provided new information and insights about tumor hypoxia, but that interpretation of the results must be in the context of the assumptions and approximations adopted in the model.

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