**Experimental Validation of the Hyperpolarized 129Xe Chemical Shift Saturation Recovery Technique in Healthy Volunteers and Subjects with Interstitial Lung Disease**

*Running Head*: Experimental Validation of HP 129Xe CSSR

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**Abstract**

***Purpose:*** To assess the sensitivity of the hyperpolarized 129Xe chemical shift saturation recovery (CSSR) technique for non-invasive quantification of changes to lung microstructure and function in idiopathic pulmonary fibrosis (IPF) and systemic sclerosis (SSc).

***Methods:*** Ten healthy volunteers, four subjects with SSc and four with IPF were scanned at 1.5 T. A CSSR pulse sequence was implemented using binomial-composite radiofrequency pulses to monitor 129Xe magnetization in tissues and blood plasma (T/P) and red blood cells (RBCs). The dynamics of 129Xe uptake into these compartments were fitted with three existing analytical models of gas diffusion to extract important parameters of lung physiology. These parameters were quantitatively compared between models.

***Results:*** Uptake of xenon into the pulmonary capillaries was impaired in subjects with IPF and SSc. Statistically significant septal thickening was measured by 129Xe CSSR in IPF patients. Preliminary data suggests age-dependent alterations to septal thickness in healthy volunteers. These findings were reproduced using each of the literature models. CSSR-derived parameters were compared with gold-standard indicators of pulmonary function; diffusing capacity of carbon monoxide and pulmonary transit-time.

***Conclusion:*** CSSR with hyperpolarized 129Xe is sensitive to pathology-induced degradation of lung structure/function and shows promise for quantification of disease severity and monitoring treatment response.

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**Keywords:** hyperpolarized gas; xenon-129; lung physiology; gas-exchange; idiopathic pulmonary fibrosis; systemic sclerosis

**Introduction**

MRI with the hyperpolarized (HP) noble gases 3He and 129Xe has facilitated innovative, non-invasive studies of lung structure ([1-3](#_ENREF_1)) and function ([4-7](#_ENREF_4)). Advances in spin exchange optical pumping (SEOP) technologies ([8-10](#_ENREF_8)) have enabled routine achievement of 129Xe nuclear polarizations of up to 40%, allowing the acquisition of pulmonary ventilation images of comparable quality to 3He ([11](#_ENREF_11)). 129Xe possesses attractive properties for in-vivo functional studies of the lungs; for example its solubility in parenchymal tissues and blood ([12](#_ENREF_12)). Also, since the xenon electron cloud is highly polarizable, 129Xe exhibits a wide range of NMR chemical shifts in different chemical environments ([13](#_ENREF_13)). Of particular importance for lung studies are the resonances of 129Xe dissolved in parenchymal tissues and blood plasma (T/P) and 129Xe dissolved in red blood cells (RBCs) – collectively termed “dissolved-phase” 129Xe. These are well separated from the “gaseous-phase” 129Xe resonance, at 197 ppm and 217 ppm downfield, respectively. Since xenon is chemically inert and is not metabolized in the body, the NMR signal measured from dissolved-phase 129Xe in-vivo is governed by diffusive uptake from the lung airspaces and perfusion in the capillaries, allowing quantitative mathematical modeling of the gas-exchange process from 129Xe NMR data.

These properties have been explored in several studies of gas-exchange function in the lungs with different pulse sequences. Direct imaging techniques ([4](#_ENREF_4),[5](#_ENREF_5),[14](#_ENREF_14)) have been used, wherein the gaseous and dissolved 129Xe resonances from the lungs are imaged simultaneously to provide information about pulmonary ventilation and perfusion. The xenon polarization transfer contrast (XTC) method ([15-17](#_ENREF_15)), uses the weak signal from dissolved 129Xe to modulate the higher SNR gaseous 129Xe images via diffusional exchange. The chemical shift saturation recovery (CSSR) spectroscopy method ([18-20](#_ENREF_18)) involves monitoring the time-dependent build-up of dissolved 129Xe magnetization in the T/P and RBC compartments following selective saturation. XTC has been extended recently to allow acquisition of gas-exchange contrast images at multiple exchange times (MXTC) ([21](#_ENREF_21),[22](#_ENREF_22)), however, unlike CSSR, this method has not been shown to provide separation between the two dissolved 129Xe compartments. The ability of the CSSR spectroscopic technique to yield dynamic information about both dissolved 129Xe compartments simultaneously is thus a unique advantage over alternative techniques. The dynamics of xenon exchange measured by 129Xe CSSR can be modeled with standard diffusion equations ([18](#_ENREF_18),[20](#_ENREF_20)) in order to estimate parameters of lung function and structure, including septal thickness, capillary transit-time and surface-area-to-volume ratio. To date, this methodology has been applied in preliminary studies in humans ([19](#_ENREF_19),[20](#_ENREF_20),[23](#_ENREF_23)), which included two subjects with chronic obstructive pulmonary disease (COPD) and two with interstitial lung disease (ILD) ([20](#_ENREF_20)). In addition, it has been utilized to examine lung function in small animals with models of lung disease ([24-27](#_ENREF_24)).

The CSSR technique is particularly suited to quantification of gas-exchange impairment in interstitial pulmonary pathologies, such as idiopathic pulmonary fibrosis (IPF), a condition characterized by fibrosis of parenchymal tissues ([28](#_ENREF_28),[29](#_ENREF_29)). IPF is the most prevalent form of ILD and carries a poor prognosis. Also of interest is systemic sclerosis (SSc), a rare connective-tissue disease in which respiratory problems are the most common cause of death, characterized by varying contributions from alveolar, interstitial and pulmonary-vascular components ([30](#_ENREF_30),[31](#_ENREF_31)). Assessment of the causes of symptomatic limitation in these patients is challenging: pulmonary function tests including measurement of the diffusing capacity of carbon monoxide - DLCO, are frequently reduced, but DLCO provides no information as to the mechanism of the abnormality of gas-exchange. Quantitative high-resolution CT can be used to assess the extent of structural lung disease but is challenging in IPF and is limited by a multitude of co-factors that contribute to X-ray attenuation ([32](#_ENREF_32)). Interestingly, a reduced DLCO is frequently observed in SSc patients with normal CT scans. Novel techniques are therefore required to quantify the functional consequences of structural changes in the lung impacting on gas-exchange and also to assess efficacy of possible treatments.

The purpose of this work was to demonstrate the practicality of the CSSR method for non-invasive quantification of lung microstructure, gas-uptake and pulmonary-vascular function in healthy normals and subjects with SSc and IPF. For 129Xe CSSR to be accepted as a clinical tool for routine application, experimental substantiation with existing gold-standard methods is needed. Here, we compare 129Xe CSSR-derived parameters with DLCO measurements and dynamic contrast enhanced (DCE)-MRI ([33](#_ENREF_33),[34](#_ENREF_34)). Current quantitative models of diffusive uptake of xenon in the lungs ([20](#_ENREF_20),[26](#_ENREF_26),[35](#_ENREF_35)) are reviewed and their application to in-vivo data is appraised in order to determine the most appropriate and informative model for routine clinical use.

**Methods**

*Study Subjects*

Four subjects with SSc and four with IPF were recruited for this preliminary study. Inclusion criteria: patients aged 35-85 years; confirmed diagnosis of IPF or SSc (determined from current clinically-accepted guidelines); resting oxygen hemoglobin saturation level (SaO2) of ≥ 90% on room air, as measured by pulse-oximetry. Patients receiving targeted drug therapies were excluded.

Ten healthy volunteers aged 23-74 years - with no history of respiratory or connective-tissue disease - were also recruited. The study protocol and recruitment procedure were approved by the National Research Ethics Committee. Pulmonary function tests (including whole-lung transfer factor, DLCO, forced expiratory volume in one second, FEV1, and forced vital capacity, FVC) were performed on all subjects and blood samples were taken from all patients.

*CSSR Spectroscopy Details*

All HP 129Xe spectroscopy experiments were performed on a 1.5 T whole-body MRI scanner (GE Healthcare, Milwaukee, WI), with a flexible transmit-receive vest coil (Clinical MR Solutions, Brookfield, WI) tuned to the 129Xe Larmor frequency (17.66 MHz). 129Xe was polarized by spin-exchange optical pumping ([36-38](#_ENREF_36)), using a “freeze-out” accumulation procedure and a home-built xenon polarizer ([10](#_ENREF_10)). This system uses a gas-mixture of 3% isotopically-enriched xenon (86% 129-isotope), 87% helium and 10% nitrogen (Spectra Gases, The Linde Group, UK), and routinely achieves static (in-cell) 129Xe nuclear polarizations of 30-40% and between 10-15% following cryogenic accumulation.

Specially-designed radiofrequency (RF) pulses were employed for excitation of 129Xe nuclei in all experiments. A 14-element, pulse-width modulated, binomial-composite RF pulse (1.13 ms duration) provided extremely selective excitation of dissolved 129Xe ([39](#_ENREF_39)). When centered on the 129Xe gas resonance, the excitation profile of the “balanced” binomial pulse yielded an on-resonant excitation of almost zero and maximum RF power deposition at a frequency ~ 3500 Hz (200 ppm at 1.5 T) downfield from the center frequency. Thus, for CSSR, near-perfect saturation of dissolved-phase 129Xe (flip-angle ~ 90°) in the lung could be attained whilst a small, repeatable excitation of gaseous 129Xe (flip-angle ~ 1°) was ensured by adding a single positive element (length 0.02 ms) to the end of the RF pulse ([39](#_ENREF_39)).

Prior to CSSR, in order to calibrate the flip-angle and gaseous 129Xe center frequency, subjects inhaled 1 L of gas extracted from the cell of the polarizer (3% xenon, 30-40% polarization) from a Tedlar bag (Jensen, Coral Springs, FL). A simple pulse-acquire sequence was utilized for this purpose, with thirty RF pulses and an inter-pulse delay time (TR) of 30 ms (≪ T1 of 129Xe gas in the lungs ([40](#_ENREF_40))), leading to a breath-hold of less than one second. The bandwidth was 12 kHz and the center frequency was chosen such that the RF pulse excitation profile was maximal at the 129Xe gas frequency. The normalized decay in 129Xe gas signal from pulse-to-pulse was fitted to a function of the form, where *α* denotes the flip-angle and *n* the RF pulse number.

The calibrated RF pulse amplitude was then used to determine the equivalent settings required for a 90° excitation of dissolved 129Xe for CSSR experiments. Each subject exhaled to functional residual capacity (FRC) and then inhaled a 50:50 mixture of cryogenically-accumulated, isotopically-enriched xenon gas (10-15% nuclear polarization) and nitrogen gas from a 1 L Tedlar bag. The saturation recovery technique involves varying the time allowed for gas-exchange (TR) after each successive saturation pulse, in order to sensitize the NMR acquisition to gas-uptake (see Figure 1). 25 different TRs were used in the range 20 ms to 1 s; these were sequentially swept through in three repeated cycles during a single breath-hold in order to obtain average signal values for each TR and to estimate the uncertainty in each measurement (standard deviation of each point). Additional pulse sequence parameters were as follows: 12 kHz receiver bandwidth; 64 spectral points; total breath-hold, 15 s.

*CSSR Data Analysis*

CSSR “xenon-uptake curves” were generated by evaluating *F*(*t*), the ratio of dissolved 129Xe signal intensity at time *t* = TR to gaseous 129Xe signal intensity at *t* = 0 (the start of the TR), for each TR. The signal intensities were calculated by integration of the respective NMR peaks in the magnitude spectra. The first acquired free induction decay (FID) was discarded since it was associated with an unknown exchange time during inhalation. The signal intensities from each of the three consecutive TR sweeps were first normalized by the 129Xe gas peak and corrected for differences in flip-angle experienced by gaseous and dissolved-phase 129Xe and then finally, the signals were averaged to represent the mean result. The combined signal from 129Xe in T/P and RBC compartments (i.e. total dissolved 129Xe) was fitted with the model of Patz et al. (hereafter denoted “Patz”) ([18-20](#_ENREF_18)), and individual uptake curves for 129Xe in T/P and RBCs were fitted with the models of Månsson et al. (hereafter denoted “Månsson”) ([26](#_ENREF_26)), and Chang (hereafter denoted “MOXE”; model of xenon exchange) ([35](#_ENREF_35)), using non-linear least squares fitting routines developed in Matlab (MathWorks, Natick, MA).

A complete mathematical description of each model can be found in the respective papers cited above; however, the principal results are reviewed below and a diagrammatical summary of the underlying geometry of each model is provided in Figure 2.

The Patz model ([20](#_ENREF_20)) is based on the solution of the diffusion equation for xenon in the alveolar septum (a slab of thickness *d*, comprising tissue and capillaries) surrounded by alveolar space on both sides. This is analogous to studying heat transfer in a metal rod, with the temperature fixed at both ends, and yields the following result for *F*(*t*):

 (1)

Where: *Mdiss*(*t*⭢*∞*) denotes the dissolved 129Xe magnetization when the septal slab is saturated with xenon; *Mgas*(*t*=0) denotes the gaseous 129Xe magnetization at *t* = 0; *ρ* is the dimensionless dissolved 129Xe magnetization density in the septal slab, *τ* is the capillary transit-time (the average length of time a RBC resides in the gas-exchange region), *SA/VG* is the alveolar surface-area-to-volume ratio, *D* and *λ* are the dissolved-phase 129Xe diffusion coefficient and xenon Ostwald solubility in tissues, respectively, and *f* and *g* are functions defined in the following way:

 (2a)

 (2b)

The MOXE model ([35](#_ENREF_35)) is an extension of this methodology in which the septum is separated into its parenchymal tissue and capillary components. Using the hematocrit (*HCT*) and tissue-barrier-to-septum ratio (*δ/d*) parameters to differentiate the contributions of tissues and blood plasma to the combined T/P peak, two coupled equations describing xenon uptake into T/P and RBCs have been derived (*STP*(*t*) and *SRBC*(*t*) respectively). In this and the Patz model, xenon diffusion is considered along only one direction (*x*) and blood flow is perpendicular to and independent of diffusion.

 (3a)

Where: ; and *η* denotes the fraction of 129Xe in RBCs to 129Xe in blood as a whole.

The parameter *STP*(*t*) is essentially equivalent to *F*(*t*) in the Patz model, minus the RBC contribution. Recasting the terms within curly brackets in Equation 3a as *S*x(*t*), the time-dependence of the 129Xe RBC signal can be written:

 (3b)

Equations 3a and 3b are coupled, such that they can be simultaneously fitted to experimental data, thereby constraining the fit parameters. An estimate of hematocrit can be extracted from the *η* parameter in the following manner:

 (3c)

Finally, Månsson ([26](#_ENREF_26)) considered a circularly-symmetric geometry in order to solve the 129Xe septal diffusion problem. In that work, the T/P and RBC signal dynamics were described by the same equation - an exponential growth function on short timescales (diffusion-dominant) and a linear increase on longer timescales (perfusion-dominant):

 (4)

Where: *S*0 (the y-intercept of *S(t)* at *t* = 0, if there was no exponential component), *S*1 (the linear slope of *S(t)* at large *t*) and *TM* (the exponential time-constant) are fitting parameters to be determined independently for the separate RBC and T/P components. For further details of the relation of these parameters to physiologically meaningful quantities, we refer the reader to ([26](#_ENREF_26)).

A number of interesting parameters related to lung physiology and microstructure can be derived from the application of these models to CSSR data. The alveolar septal thickness (ST), capillary transit-time (CTT) and surface-area-to-volume ratio (S/V) were derived for each subject in this study. The results extracted from each model were compared in order to assess the suitability of each for interpretation of 129Xe CSSR data. The accuracy of the model fit to the data was evaluated by re-fitting each data set with different weightings – i.e. applying the error function, weighted to specific data points, to the residuals before calculating the sum of squares.

Furthermore, as a semi-quantitative measure of gas-exchange efficiency, the ratio of RBC to T/P peak integrals was determined from phased CSSR spectra at a TR of 100 ms, consistent with the recent work of Kaushik et al. ([41](#_ENREF_41)). Lastly, statistical testing was performed using SPSS (IBM SPSS Statistics, V19, Armonk, NY) in order to: (i) identify parameters of lung physiology that were significantly different between subject groups (two-tailed, one-way analysis of variance (ANOVA) with post-hoc contrast); (ii) determine significant correlations between parameters (Pearson’s correlation coefficients).

*DCE-MRI*

For validation of the xenon capillary transit-times measured by CSSR, two measures of pulmonary-vascular output were determined from DCE-MRI: (i) the full-width at half-maximum (FWHM) of the lung parenchyma enhancement and (ii) the pulmonary transit-time. Patients were placed in an 8-channel cardiac-array coil and a 0.05 mL/kg dose of 1 mmol/mL Gd-DPTA solution (Gadovist, Schering, Leverkusen, DE) was injected with a power injector (Spectris, Medrad, Warrendale, PA) at a rate of 4 mL/s into the antecubital vein, followed by a 20 mL saline flush. A time-resolved 3D coronal gradient-recalled echo sequence with: 2x phase acceleration and view sharing ([42](#_ENREF_42)); 24 slices of thickness 10 mm; bandwidth = ±125 kHz; TE/TR = 0.8/2.3 ms; flip-angle = 30°; 200 x 80 matrix; 36 temporal phases at an effective frame rate of 2 volumetric frames per second, was used to image the dynamics of the first pass of Gd through the pulmonary vasculature. In order to calculate the pulmonary transit-time (PTT) from the time series of images, signal enhancement as a function of time was evaluated for regions of interest (ROIs) placed in the left atrium and pulmonary artery ([43](#_ENREF_43)). The PTT was calculated by subtracting the time-to-peak signal of the pulmonary artery from that of the left atrium. For the FWHM of the lung parenchyma enhancement, a ROI was placed on each lung and the FWHMs of the resulting signal enhancement curves were averaged. Due to the positioning of the ROIs, both methods intrinsically provided an estimation of the “whole-lung” Gd transit-time for comparison to CSSR data.

**Results**

NMR spectra typically highlighted a dominant peak due to 129Xe dissolved in parenchymal tissues and blood plasma (T/P) with an accompanying small peak from 129Xe in RBCs, in both SSc and IPF patients when compared to healthy volunteers. At short exchange times, 129Xe spectra from patients exhibited almost no signal from RBCs, and only a moderate peak was observed at very long TRs (~ 1 s) (see Figure 3, right panel). As illustrated in the left panel of Figure 3, 129Xe uptake curves indicated that gas-exchange from the alveoli to capillaries was impaired and delayed in both patient groups compared to volunteers. The xenon uptake (exponential, diffusive part of the curve) was visibly prolonged in patients and the T/P component was observed to reach a greater value of *F*(TR), consistent with the spectra.

The apparent inhibition of gas-exchange in patients suggested thickening of septal tissue, and this was corroborated by fitting the 129Xe uptake curves with the models of Patz, Månsson and Chang (MOXE). Each model exhibited a considerably increased whole-lung alveolar ST in older healthy volunteers and patients with SSc, and an even greater increase in IPF subjects, when compared to younger (< 50 years) volunteers (see Table 1 for a summary of CSSR-derived parameters and pulmonary function test results). The ST values determined from application of the MOXE model to 129Xe CSSR data are shown in Figure 4. Using a dissolved-phase 129Xe diffusion coefficient of *D* = 3.3x10-6 cm2s-1 ([17](#_ENREF_17)), mean ST values derived from this model were 10.0 ± 1.6 μm for healthy volunteers, 13.0 ± 1.5 μm for subjects with SSc and 17.2 ± 1.1 μm for those with IPF. Statistically significant differences between derived ST values for all three subject groups were found upon fitting each of the three models of lung microstructure to HP 129Xe CSSR data. These values are summarized in Table 2, along with the *P* values denoting statistical significance.

A strong, positive correlation was identified between the CSSR-derived ST and healthy volunteer age (correlation statistics: r = 0.74, *P* = 0.015 using MOXE; r = 0.77, *P* = 0.010, Patz; r = 0.75, *P* = 0.012, Månsson), as illustrated in the center panel of Figure 4. This correlation was used to remove the effect of aging from volunteer and patient data (bottom panel, Figure 4). With age-correction, a significant increase in ST in IPF patients was still observed (*P* < 0.05) for all models, however no model exhibited a difference in ST between volunteers and SSc subjects (*P* > 0.05). The results of the age-correction are detailed in Table 2.

Furthermore, a statistically significant correlation was observed between the CSSR-derived ST values and DLCO. Results from fitting CSSR data with MOXE are displayed against %-predicted DLCO in Figure 5. Pearson’s correlation coefficients (r) and *P* values for ST versus %-predicted DLCO were: r = -0.90, *P* < 0.001 for data fitted with the Patz model; r = -0.91, *P* < 0.001, MOXE; r = -0.92, *P* < 0.001, Månsson. The correlations against DLCO in standard units (mL/min/mmHg, i.e. not considering predictions for height, age, sex) were: r = -0.85, *P* < 0.001 for data fitted with the Patz model; r = -0.85, *P* < 0.001, MOXE; r = -0.86, *P* < 0.001, Månsson.

Complementary to the CSSR modeling results, the ratio of RBC-to-T/P peak integrals was found to be significantly different between patients and healthy volunteers, although, unlike the ST parameter, the ratio did not distinguish between the two patient groups. Mean values (*P* values) were: 0.42 ± 0.18 in healthy volunteers; 0.18 ± 0.04 in SSc subjects (*P*(HV-SSc) = 0.040) and 0.13 ± 0.04 in IPF subjects (*P*(HV-IPF) = 0.009, *P*(SSc-IPF) > 0.05). Furthermore, the RBC-to-T/P ratio measurements correlated well with ST values from all models (r = -0.74, *P* < 0.001, MOXE, r = -0.74, *P* < 0.001, Patz, r = -0.77, *P* < 0.001, Månsson).

The mean CSSR CTT was: 2.2 ± 1.0 s, 2.5 ± 0.7 s, 2.5 ± 0.6 s (volunteers); 2.3 ± 0.8 s, 2.5 ± 0.9 s, 2.0 ± 0.5 s (SSc); 2.2 ± 0.2 s, 2.4 ± 0.2 s, 1.5 ± 0.1 s (IPF); respectively for the MOXE, Patz and Månsson models. This parameter was not significantly different between subject groups (*P* > 0.05). The CTTs calculated from CSSR were not found to correlate significantly with the PTT or lung FWHM as determined from DCE-MRI; the highest correlation coefficient was between the CTT (Patz) and the lung FWHM (r = 0.34, *P* = 0.416). In addition, the derived hematocrit from the MOXE and Månsson models was notably lower in IPF patients as compared to healthy volunteers, with values of: 0.23 ± 0.05 and 0.24 ± 0.05 (volunteers); 0.17 ± 0.04 and 0.15 ± 0.01 (SSc); 0.15 ± 0.01 and 0.11 ± 0.01 (IPF). The HCT was significantly different between healthy volunteers and IPF patients (MOXE) and between volunteers and both patient groups (Månsson model) (*P* < 0.05). Finally, as extracted from the MOXE and Patz models, the mean alveolar surface-area-to-volume ratio was: 135 ± 37 cm-1 and 128 ± 36 cm-1 (volunteers); 141 ± 53 cm-1 and 134 ± 51 cm-1 (SSc); 166 ± 53 cm-1 and 156 ± 51 cm-1 (IPF); respectively, with *P* values of significance between groups > 0.05 in all cases.

**Discussion**

*Clinical Findings*

Septal thickness values for healthy volunteers calculated from Patz and MOXE models agreed well with CT observations (10 μm for normals) ([44](#_ENREF_44)). Thickening of alveolar septa in IPF patients is attributable to underlying fibrotic changes in lung microstructure that were confirmed by CT. The significance of apparent septal thickening in SSc subjects is clinically interesting and must be further assessed as these patients had little discernible fibrosis on CT and no evidence of pulmonary hypertension (PH). If validated histologically, this may help explain the reduced DLCO frequently observed in patients with SSc in the absence of PH and ILD. Nevertheless, since some patients were > 50 years old, the measured thickening may be partially attributable to age-dependent changes in the gas-exchange surface, as identified by the apparent increase in ST of healthy volunteers with age. Indeed, with more healthy volunteer data, it may be possible to derive a “%-predicted ST”, in agreement with the clinical standard for DLCO (i.e. normalizing for subject height, sex, age).

Of further potential clinical importance is the fact that the surface-area-to-volume ratio derived from these CSSR models is not significantly different between subject groups. This finding might suggest that the remodeling of lung parenchyma due to fibrosis is not necessarily associated with emphysematous processes. In future work, we endeavor to assess this result with 3He apparent diffusion coefficient mapping ([45](#_ENREF_45)). Finally, we re-iterate that the RBC-to-T/P ratios also exhibit significant differences between subject groups. However, since this parameter is a semi-quantitative measurement of gas-exchange that does not distinguish between diffusive and perfusive components of 129Xe uptake, it is not possible to derive clinically-relevant parameters of lung microstructure.

The demonstration of a distinct relation between whole-lung transfer factor, DLCO, and 129Xe CSSR ST is the first in-vivo validation of the CSSR technique with a clinically-accepted method and has important implications for routine application of this technique. For example, if adapted to enable acquisition of regionally-localized spectra (e.g. with receiver-coil arrays), the CSSR method could provide information about gas-exchange that is not obtainable by standard pulmonary function testing or indeed, CT. The lack of correlation between 129Xe CSSR CTT values and DCE-MRI parameters suggests that the CTT parameter is not sensitive to small changes in pulmonary-vascular output, or, that its meaning has been obscured by other modeling parameters. In all cases, CTT values were significantly lower than the corresponding DCE-MRI PTT values; however, this would be expected due to the different definitions of the parameters. The former describes the average time a RBC resides in the gas-exchange region, whilst the latter represents the complete transit-time of a contrast bolus from the right heart, through the pulmonary-arterial system and lung gas-exchange vasculature and back to the left atrium through the pulmonary-venous system. Although the lung FWHM metric potentially provides a closer representation of the CTT measurement, the MR signal (even with contrast enhancement) was close to the noise level in many patients and thus it was difficult to accurately estimate the FWHM; this may explain the observation of large FWHM values compared to PTT values.

The HCT values derived from the MOXE and Månsson models were in general lower than expected from patient blood samples. The CSSR-HCT was found to be reduced in patients with IPF when compared with healthy volunteers (P < 0.05), although blood samples indicated relatively normal values. As recently highlighted ([23](#_ENREF_23)), the HCT in the narrow capillaries is reduced (to values as low as 0.28) when compared to the whole-body HCT – due to the Fåhraeus effect ([46](#_ENREF_46)). Although this may partially explain the low HCT values for healthy subjects, it does not indicate why the CSSR-derived HCT of patients should be further reduced. However, fixing the HCT to a nominal value in the expected range (0.4-0.5) and repeating the fitting process, tends to yield increased ST values for all subjects. Thus, the apparent reduced HCT values in patients may actually be a further artefact of inhibited gas-exchange / septal thickening and the inter-dependent nature of the model parameters. In fact, the HCT correlates with the ST parameter for both models on the *P* < 0.01 level.

*Modeling*

Based upon the statistical testing, it could be argued that each of the three models assessed are useful for quantification of lung microstructure. A pictorial comparison of the application of each of the three models to 129Xe CSSR data from a single healthy volunteer is shown in the left panel of Figure 6. It can be seen that each of the three models describes the data reasonably well; the goodness-of-fit as determined from R2 and χ2 statistics was typically better for MOXE and Patz models, because the Månsson model fails to follow the middle section of the uptake curves as accurately as the other two models, suggesting that it is not appropriate to only consider a single exponential component of diffusive uptake. Also depicted in Figure 6 (right panel) are Bland-Altman plots highlighting the systematic differences between CSSR parameters extracted from all three models. As expected due to the geometries employed, the Patz and MOXE models perform similarly, whereas the Månsson ST values are consistently lower than those of the MOXE model, with an increased discrepancy in patients. Despite these systematic differences, parameters extracted from all models correlate with each other to the significance level of *P* < 0.01.

Since MOXE is an incremental extension of the Patz model, providing estimates of additional important parameters of lung physiology, it would seem to be the most accurate and beneficial model to use in CSSR studies wherein the dissolved 129Xe resonances can be spectrally resolved. Despite this fact, an increased number of inter-dependent fit parameters may obscure their interpretation; for example, in this study the tissue-barrier-to-septum ratio parameter could not be compared between subject groups due to the fact that in some cases, the parameter tended to unrealistically low and even negative values. Hence in all fitting results shown, *δ/d* was restricted to within a range 0.05 – 0.30 (note, this restriction had negligible effect on the other fitting parameters). Therefore, it may be advantageous to reduce the complexity of the MOXE model, by constraining the fit parameters. To this end, with further data, we may be able to establish a link between the CTT parameter and DCE-MRI data and could utilize this to yield prior knowledge about the CTT. Furthermore, it may be possible to estimate the true tissue-barrier-to-septum ratio from the RBC-to-T/P signal ratio measurements, which arguably provide a better representation of the relative fractions of xenon in parenchymal tissues and blood. In combination with knowledge of the HCT (from blood samples) the MOXE fitting could be reduced to a 2 or 3-parameter problem, though some correction may be required to account for the Fåhraeus effect.

*Experimental Limitations*

Achievement of precise 90° excitations of dissolved-phase 129Xe in-vivo is problematic. The design of the flexible transmit-receive coil used in this work does not deliver a uniform flip-angle across the whole of the lungs (~ 12% variation overall). Of course, the delivered flip-angle is dependent on the position of the flexible coil, and the loading realized by subjects of varying size. The coil transmit inhomogeneity has important implications for CSSR measurements. Since the acquired 129Xe spectra are whole-lung averages, the averaging process takes into account not only the heterogeneity in gas-exchange function across the lungs, but also the variation in delivered flip-angle. Furthermore, we often observed elevated dissolved 129Xe signal amplitudes at the start of the first TR sweep, which may be associated with B1 inhomogeneity and hence imperfect dissolved 129Xe saturation. Indeed, the early data points may be affected not only by the unknown exchange time present during inhalation, but also by 129Xe signal outside the active region of the coil (this effect was not observed in subsequent TR sweeps). More homogeneous transmitter coils (e.g. of birdcage design ([47](#_ENREF_47))) could be utilized; however these would typically be larger and less power-efficient than flexible coils, necessitating increased RF powers to achieve complete saturation of dissolved-phase 129Xe.

**Conclusions**

CSSR spectroscopy with HP 129Xe is capable of detecting alterations in whole-lung structure-function, allowing non-invasive measurement of alveolar septal thickness. We have shown statistically significant septal thickening in subjects with IPF and SSc, the latter with little / no known interstitial involvement. Further data may elucidate whether the technique is sensitive to age-dependent changes in lung microstructure. Previously established models of xenon uptake have been reviewed and each has been shown to distinguish between ILD subjects and healthy volunteers. Additionally, the 129Xe CSSR technique has been compared for the first time against gold-standard clinical methods; DLCO and DCE-MRI. The findings presented in this article highlight the potential of this technique for identification of clinically-important alterations of lung microstructure and pulmonary-vascular function. Its use in a number of novel studies to provide information not obtainable by CT and pulmonary function testing can be envisaged.

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**List of Figure Captions**

Figure 1: The CSSR pulse sequence, as implemented in this work. Pulse-width modulated binomial RF pulses (amplitude modulated shown for display purposes) can produce 90° excitations of dissolved-phase 129Xe, with minimal on-resonance excitation of gaseous 129Xe. These RF pulses were used for both saturation and excitation of NMR signal (generated free induction decays are shown in grey). The inter-pulse delay time (TR) was varied between values of 20 ms and 1000 ms, in order to quantify the dynamics of xenon uptake. Each TR sweep was repeated three times to estimate the same-breath variability in the CSSR measurement.

\*\*\*Double column figure\*\*\*

Figure 2: Comparison of the geometries adopted for diffusional modeling of xenon uptake in the lungs; from the airspace across the alveolar septum, thickness d. The Patz and MOXE models consider solving the diffusion equation with periodic boundary conditions in one dimension, x, created by identical airspaces on either side of the septum. (i) the model of Patz does not distinguish between T/P and RBC compartments; (ii) the model of Chang (MOXE) includes a tissue barrier term (δ) permitting separation of the T/P and RBC compartments, in conjunction with the blood hematocrit; (iii) the Månsson model also considers the two dissolved 129Xe compartments individually, but with a circularly symmetric geometry, with an alveolar radius (RA) and tissue and capillary thicknesses LT and LC, respectively. In the first two models, blood flow is treated orthogonal to and independent of diffusional gas-exchange. In each case D denotes the diffusion coefficient of xenon, in the airspace (D0, xenon self-diffusion coefficient), tissues (DT), blood (DB) or combined dissolved-phase (DD). Note: in the main body of this manuscript, D (instead of DD) is used to represent the dissolved-phase xenon diffusion coefficient, which is assumed to be the same for both tissues and blood in all three models. In practice, these values are likely to be different but are not currently well-known; hence we designate them with different symbols for completeness.

\*\*\*Double column figure\*\*\*

Table 1: Subject demographics and pulmonary function test results. Septal thicknesses (ST), capillary transit-times (CTT), surface-area-to-volume ratios (S/V) and hematocrit (HCT) values, as extracted from fitting the models of Chang (MOXE), Patz and Månsson to HP 129Xe CSSR data, are quoted along with pulmonary transit-times (PTT) and the FWHM of lung parenchyma signal enhancement calculated from the first pass of gadolinium contrast agent through the lung vasculature, and HCT values derived from patient blood samples.

\*\*\*Double column table\*\*\*

Figure 3: Left panel: Dynamics of 129Xe uptake into tissues and blood plasma (T/P), and RBCs, for (a) a typical healthy volunteer, (b) a subject with SSc and (c) a subject with IPF. Uptake curves, denoted by *F*(TR), are displayed with corresponding MOXE model fits (solid black lines). Dashed lines indicate increments in *F*(TR) of 0.005. Right panel: NMR spectra (moderately line-broadened and zero-filled to 128 points) of subjects from the same three groups, acquired at a TR of 1000 ms. The peaks due to 129Xe in RBCs, T/P and the gaseous-phase (G) are clearly indicated.

\*\*\*1.5 column figure\*\*\*

Table 2: Average septal thickness values and corresponding *P* values of statistical significance for healthy volunteers and patients, extracted from the models of Patz, Chang and Månsson, including values corrected for predicted changes due to subject age. Quoted uncertainties represent the standard deviation of the septal thickness values for each group.

\*\*\*Double column table\*\*\*

Figure 4: Top: Mean (whole-lung) alveolar septal thicknesses for healthy volunteers and subjects with SSc and IPF, as derived from fitting the MOXE model to 129Xe CSSR data, displayed in order of subject ID from Table 1. Center: Positive correlation between septal thickness and healthy volunteer age, with corresponding Pearson’s correlation coefficient (r) and *P* value. Bottom: “Age-corrected” alveolar septal thickness values for all subjects.

\*\*\*Single column figure\*\*\*

Figure 5: Validation of HP 129Xe CSSR-derived septal thickness values (from the MOXE model) with the clinical gold-standard measure of pulmonary function, whole-lung transfer factor (DLCO). The Pearson’s correlation coefficient (r) and corresponding *P* value are shown. Error bars in ST were estimated by re-fitting the data with different weightings applied to the residuals before taking the sum of squares, as described in the main text. Note: %-predicted DLCO represents the measured DLCO value as a percentage of an expected value, based on subject height, sex, age etc.

\*\*\*Single column figure\*\*\*

Figure 6: (i) Comparison of the accuracy and applicability of the Patz (a), MOXE (b) and Månsson (c) models in describing in-vivo 129Xe CSSR data from a single healthy volunteer. Blue data points denote the total dissolved 129Xe signal intensities, green the T/P component and red the RBC component. The model fits are shown both with forced weighting to the middle section of each curve (dashed lines), and in the absence of any weighting (solid lines). (ii) Bland-Altman plots of the systematic differences in CSSR-derived parameters (septal thickness (top), capillary transit time (center) and surface-area-to-volume ratio (bottom)) between models, in each case comparing the Patz and Månsson model results to those of the MOXE model.

\*\*\*Double column figure\*\*\*