Microstructure characterization of muscle tissue by quantitative imaging

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Characterizing the behavior of water diffusion within a voxel provides a means for describing inner microstructure at cellular scale, taking into account experimental diffusion times and apparent diffusion coefficients measured in muscle.The mean-square displacement of water molecules is close to thedimensions of cells of the order of microns.Water diffusion is anisotropic in meat due to its highly fibrillar structure, and can therefore be modeled in three dimensions using tensors (1). First-order tensor fitting followed by diagonalization at each *b*-value of data obtained on meat samples reveals a diffusion reference frame. The first eigenvector of this frame corresponds to the main fiber axis direction, which remains constant, enabling diffusion behavior to be broken down into two decays, one parallel and one perpendicular to this direction. These decays both deviate from the Gaussian diffusion expressed as a mono-exponential decay that is well-fitted by a bi/multi-exponential model, expressing hindered and restricted diffusion of water diffusing out of and into the muscle fibers. Maps of the expressed diffusion parameters (includingeigenvalues or trace of the diffusion tensor, diffusion anisotropy, difference between mono- and multi-exponential fits) were superimposed onto morphological images, *i.e.* histological and high-resolution susceptibility-weighted gradient-echo images (SWI, with 300 × 300 0.05 mm square voxels), revealing structural details that were not visible in SWI. Quantitative parameters related to structural dimensions and derived from diffusion MRI and histology were compared over the same regions-of-interest. At low *b*-values, diffusion parameters seem to be correlated with metabolic characteristics of meat fibers, as highlighted by photomicrography of fibers submitted to histoenzymological ATPase staining. We also have tried to observe the diffraction pattern of echo attenuation as a function of wave vector *q*. Where are now trying to link this diffraction pattern to cellular geometries.

1. Liu *et al.* Characterizing non-Gaussian diffusion by using generalized diffusion tensors, *Magn. Reson. Med.* 51:924-937 (2004)

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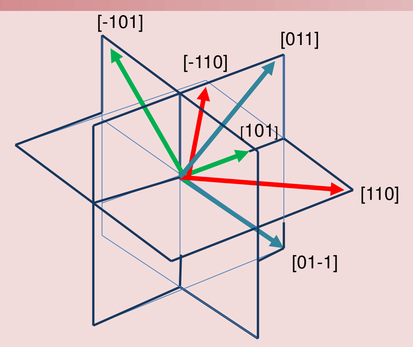
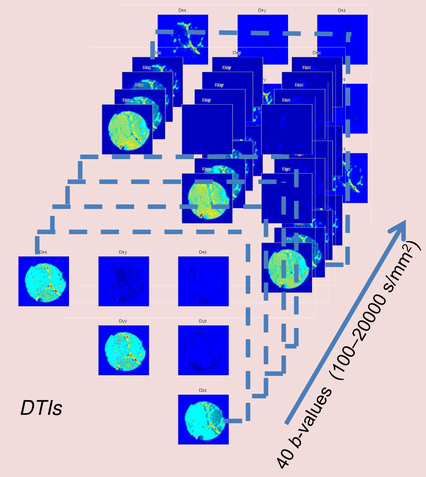
Characterizing the behavior of water diffusion within a voxel provides a means for describing inner microstructure at cellular scale, taking into account experimental diffusion times and apparent diffusion coefficients measured in muscle. The mean-square displacement of water molecules is close to the dimensions of cells of the order of microns. Furthermore water diffusion is anisotropic in meat due to its highly fibrillar structure, and can therefore be modeled in three dimensions using tensors. We used Diffusion Tensor Imaging (DTI) at different b-values to obtain high resolution diffusion parameter mapping of tissue which have been registered to high-resolution susceptibility-weighted gradient-echo images and histological images to put in obvious relationships between meat microstucture and diffusion observed at a meso-scale.

Materials and Methods

Diffusion gradients were applied in six non-collinear directions allowing diffusion tensor imaging (DTI).
Diffusion weighting using a pulsed-gradient spin-echo imaging sequence was applied with increasing b-values.

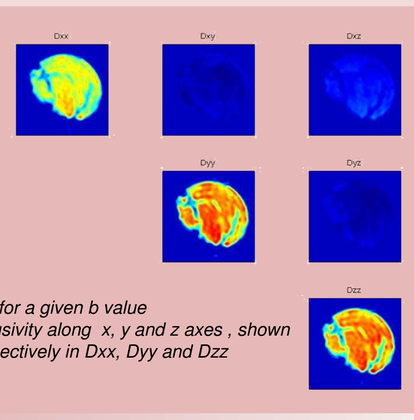




Diffusion decays were measured at a mesoscopic scale (30 × 30 0.5 mm square voxels).
Experiments were conducted at 400 MHz on fresh meat (pork *biceps femoris* samples Ø = 15 mm) at 8°C.

240 diffusion weighted images:
- 6 non-collinear directions
- 40 b-values ranging 100-20000 s/mm2

First order tensor fitting and subsequent diagonalisation at each *b*-value were applied to reveal a diffusion reference frame in an anisotropic medium.
The first eigenvector corresponds to the main fiber axis direction, which has been checked to remain constant.
Hence diffusion behavior was decomposed into two decays, parallel // (Dxx) and perpendicular ⊥ (Dyy and Dzz) to this direction.

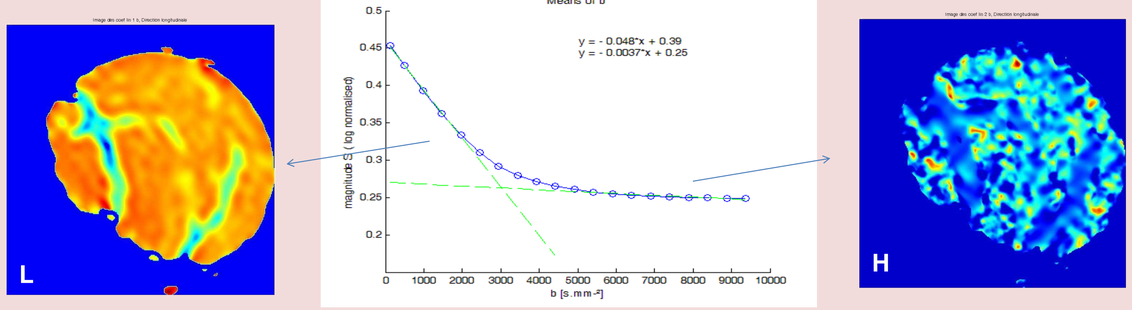


DTI for a given b value
Diffusivity along x, y and z axes , shown respectively in Dxx, Dyy and Dzz

Results

Multi exponential diffusion decay

// and ⊥ decays both deviate from the Gaussian diffusion expressed by a mono-exponential decay.
They are well fitted by a bi/multi-exponential model, expressing hindered and restricted diffusion of water diffusing out of and into muscle fibers.

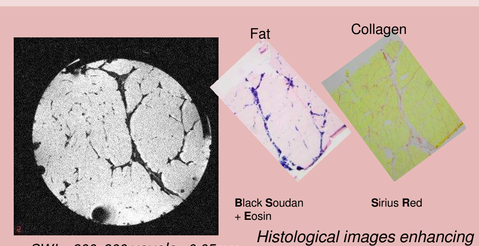


Plot of diffusion-weighted intensity attenuation exhibiting hindered and restricted diffusion, from the trace of diffusion tensor matrix.

Parametric images reveal structural details obtained from the amplitude coefficients of the exponential decays at low (L) and high (H) b values.

MRI/histology registration

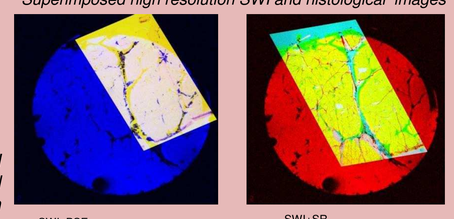
Maps of diffusion parameters expressed (*e.g.* eigenvalues or trace of the diffusion tensor, diffusion anisotropy, difference between mono and multi-exponential fits) were superimposed on morphologic images, *i.e.* histological and high-resolution susceptibility-weighted gradient-echo images (SWI, with 300 × 300 0.05 mm square voxels) revealing structural details not appearing in SWI.
Histological images after spatial registration were superimposed on SWIs to identify morphologic components (fat/collagen network), and subsequently superimposed on parametric images.



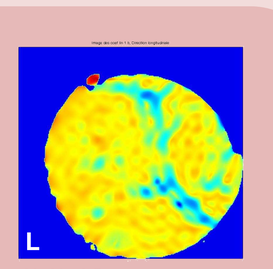
SWI 300x300 voxels 0.05mm

Histological images enhancing fat and collagen

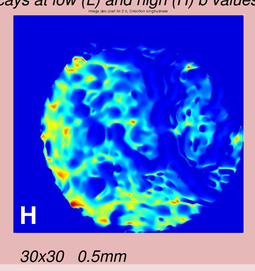
Superimposed high resolution SWI and histological images



Comparison of high resolution SWI morphologic structural images and parametric diffusion images obtained from low resolution pulsed spin echo sequence.



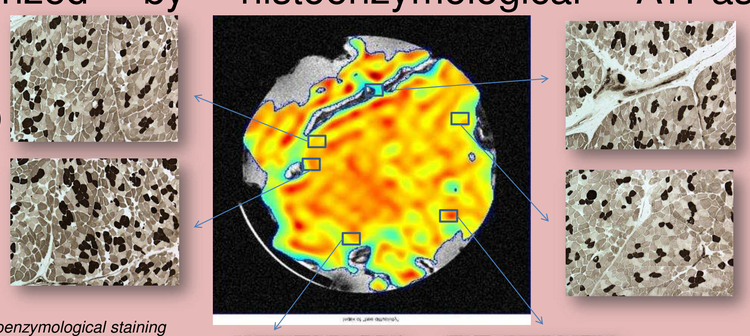
30x30 0.5mm
amplitude coefficients of the exponential decays at low (L) and high (H) b values.



30x30 0.5mm

Relationship between diffusion and muscle fibers types

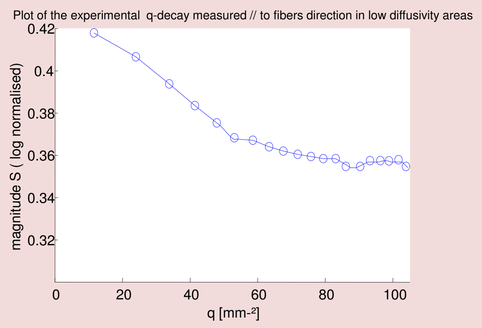
At low *b*-values, diffusion parameters seem to be correlated with metabolic characteristics of meat fibers, as highlighted by photomicrography of areas characterized by histoenzymological ATPase staining.
(pH 4.35 preincubation)



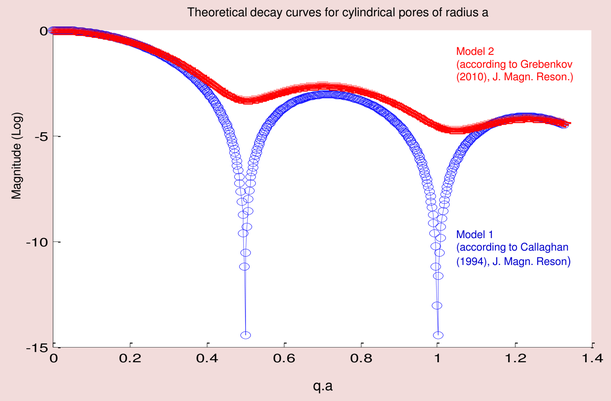
Localization of the histoenzymological staining
Three intensities are black (type I), white (IIA) and grey (IIX plus IIB). Diffusion is mapped with a blue/green to orange/red scale. Type I fibers (blacks) seem to be correlated with regions of high diffusivities as type II fibers (greys and whites) with regions of low diffusivities.

Diffraction pattern of echo attenuation

Minima in the echo decay vs *q* can be observed on acquired data with lower minima amplitudes compared to prediction of models, where meat fibers are assumed to be a simple geometry of an array of filled tubes.



Echo decay attenuation observed on localized area compared to 2 different prediction models



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