Quantification of Cellular Actin Fibers Alignment from Confocal Microscopic Images

Marcel Philippi Dorta, Carla Luana Dinardo, Alexandre Pereira da Costa, Adriano Mesquita Alencar
USP -SP - Brasil

The cellular rheology has recently undergone a rapid development with particular attention to the cytoskeleton mechanical properties and its main components - actin filaments, intermediate filaments, microtubules and crosslinked proteins. However it is not clear what are the cellular structural changes that directly affect the cell mechanical properties. Thus, in this work, we aimed to quantify the structural rearrangement of these fibers that may emerge in changes in the cell mechanics. We created an image analysis platform to study smooth muscle cells from different arteries: aorta, mammary, renal, carotid and coronary, and processed respectively 31, 29, 31, 30 and 35 cell image obtained by confocal microscopy. The platform was developed in Matlab (MathWorks) and it uses the Sobel operator to determine the actin fiber image orientation of the cell, labeled with phalloidin. The Sobel operator is used as a filter capable of calculating the pixel brightness gradient, point to point, in the image. The operator uses vertical and horizontal convolution kernels to calculate the magnitude and the angle of the pixel intensity gradient. The image analysis followed the sequence: (1) opens a given cells image set to be processed; (2) sets a fix threshold to eliminate noise, based on Otsu's method; (3) detect the fiber edges in the image using the Sobel operator; and (4) quantify the actin fiber orientation. Our first result is the probability distribution $\Pi(\Delta\theta)$ to find a given fiber angle deviation ($\Delta\theta$) from the main cell fiber orientation θ_0. The $\Pi(\Delta\theta)$ follows an exponential decay $\Pi(\Delta\theta) = A\exp(-\alpha\Delta\theta)$ regarding to its θ_0. We defined and determined a misalignment index α of the fibers of each artery kind: coronary $\alpha_{Co} = (1.72 \pm 0.36)\text{rad}^{-1}$; renal $\alpha_{Re} = (1.43 \pm 0.64)\text{rad}^{-1}$; aorta $\alpha_{Ao} = (1.42 \pm 0.43)\text{rad}^{-1}$; mammary $\alpha_{Ma} = (1.12 \pm 0.50)\text{rad}^{-1}$; and carotid $\alpha_{Ca} = (1.01 \pm 0.39)\text{rad}^{-1}$. The α of coronary and carotid are statistically different ($p < 0.05$) among all analyzed cells. We discussed our results correlating the misalignment index data with the experimental cell mechanical properties obtained by using Optical Magnetic Twisting Cytometry with the same group of cells.