Methods: Statistical analysis

Nuclei are usually distributed close to the surface of the fiber. For each muscle fiber, an idealized circular cylinder segment with constant radius was constructed. The length and radius of the cylinder were found by the Imaris software. From these parameters, the distance from each nuclei to its nearest neighbor was compared to the cases of:

- Nuclei spread uniformly at random on the cylinder surface.
- Nuclei spread optimally to minimize transport distances.

In principle, one could use the distances as measured along the surface of the parameterized cylinder in the statistical analysis. However, as this allows for possible errors in the surface parameters due to the uncertainties in the nuclei position, we have here chosen to use the direct, euclidean distances.

For the chosen distance measurement, one can show that the nearest neighbor distance probability density function for the case of N nuclei spread randomly on a cylinder segment of radius R and length L, is given by (See appendix for derivation of this expression):

$$f_{cyl}(r) = \frac{\mathrm{d}}{\mathrm{d}r}(1 - P(\mathrm{NND}(x_i) > r)), \tag{1}$$

where

$$P(\mathrm{NND}(x_i) > r) = 1 - \frac{4}{\pi L^2}\int_0^{\min\{L,r\}}\int_0^{\min\{2R,\sqrt{r^2-r_1^2}\}} \frac{L - r_1}{\sqrt{4R^2 - r_2^2}}\,\mathrm{d}r_2\mathrm{d}r_1, \tag{2}$$

It is difficult to determine the optimal distribution of nuclei to minimize transport distances. Here we follow [Number and spatial distribution of nuclei in the muscle fibers of normal mice studied in vivo, Bruusgaard et al. J Physiol (2003) 551.2], which suggest using a hexagonal grid pattern on the cylinder surface. There are several ways to create such a pattern. The way chosen here was to create a rectangular grid off equispaced points, with N_L points along the length of the cylinder, and N_C points along the circumference, such that $N_C/N_L \approx C/L$ and $N_C N_L \approx N$, where C is the circumference of a circle with radius R, $C = 2\pi R$. Ideally, these would be exactly equal, but this is not possible for all sets of L, R and N. Afterwards, every second line of points either along the circumference a distance $\Delta C = C/(2N_c)$. These points now define the centers of approximately regular hexagons.

In order to measure how ordered the nuclei distribution for a particular fiber is an the mean nearest neighbor distance was calculated for the experimental data, as well as for the random and optimal distribution using parameters from the experiment. We denote the experimental, random and optimal means by M_E, M_R and M_O. An "orderness-score", $g(M_E)$, was then calculated as

$$g(M_E, M_R, M_O) = \frac{M_E - M_R}{M_O - M_R}$$

In the case where the experimental nuclei are clustered, this score may be negative.

Appendix: Mathematical derivation

Here we will derive equation (2).

We begin by considering the nearest neighbor distance (NND) probability density function (PDF) for points on a line. For two points x and y uniformly randomly placed on a line segment of length L, the probability density for the distance between the two points, $r_1 = |x - y|$ is

$$g_l(r_1)\,\mathrm{d}r_1 = \frac{2}{L}(1 - r_1/L)\,\mathrm{d}r_1 \tag{3}$$

For euclidean distances on a circle of radius R, if two points are selected at uniformly random angles, then the probability density of the chord length is

$$g_c(r_2)\,\mathrm{d}r_2 = \frac{2}{\pi\sqrt{4R^2 - r_2^2}}\,\mathrm{d}r_2 \tag{4}$$

for $0 \le r_2 \le 2R$.

For two points on the surface of the complete cylinder, what is the probability that the distance between the points is larger than some value r?

$$\begin{aligned}
P(r_1^2 + r_2^2 > r^2) &= 1 - P(r_1^2 + r_2^2 < r^2) &(5)\\
&= 1 - \int_0^{\min\{L,r\}} \int_0^{\min\{2R,\sqrt{r^2 - r_1^2}\}} g_c(r_2) g_l(r_1)\,\mathrm{d}r_2 \mathrm{d}r_1 &(6)\\
&= 1 - \int_0^{\min\{L,r\}} g_l(r_1) \int_0^{\min\{2R,\sqrt{r^2 - r_1^2}\}} \frac{2}{\pi\sqrt{4R^2 - r_2^2}}\,\mathrm{d}r_2 \mathrm{d}r_1 &(7)
\end{aligned}$$

We can solve part of the integral analytically as

$$\begin{aligned}
P(r_1^2 + r_2^2 > r^2) &= 1 - \int_0^{\min\{L,r\}} g_l(r_1) \frac{2}{\pi} \left[\sin^{-1}(r_2/(2R))\right]_0^{\min\{2R,\sqrt{r^2 - r_1^2}\}}\,\mathrm{d}r_1 &(8)\\
&= 1 - \int_0^{\min\{L,r\}} g_l(r_1) \frac{2}{\pi} \sin^{-1}(\min\left\{2R, \sqrt{r^2 - r_1^2}\right\}/(2R))\,\mathrm{d}r_1 &(9)
\end{aligned}$$

The last integral can be solved analytically.

For a collection of N particles, the probability that none are closer than r is

$$P(\mathrm{NND}(x_i) > r) = P(r_1^2 + r_2^2 > r^2)^{N-1}$$

and the PDF for $NND(x_i)$ is

$$f_{cyl}(r) = \frac{\mathrm{d}}{\mathrm{d}r}(1 - P(\mathrm{NND}(x_i) > r)) \tag{10}$$