Filament reconstruction (April 2015)

1. Tracking filaments

The initial step of filament shape reconstruction finds each filament skeleton as connected pixels within the skeletonized image. A pool of filament end points (ends) is collected by an automated end-finding algorithm or hand-picked by the user. Then, starting from one of two identified ends for each filament, a directional tracking algorithm searches the neighboring pixels for the next point in the filament skeleton until the other end is reached. The filament-tracking algorithm avoids skeletal filaments with branches longer than a pre-specified length (in pixels) because long branches and/or crossovers are likely real and possibly due to overlapping filaments. Skeletal filaments with spurious short branches (generated during image processing as described in the main text of the original paper) shorter than the pre-specified length (in pixels) will be reconstructed. In the latter case, the algorithm reconstructs only the main skeletal filaments and ignores the spurious branches. In addition, users can choose whether or not the algorithm should allow single-pixel gaps during filament-tracking by selecting “yes” or “no” at the beginning of the analysis.

2. Smoothing filament skeletons - Bézier spline

The second step is to smooth the filament skeletons. A smoothing algorithm groups every four contiguous pixels (P1, P2, P3, P4,), i.e. three segments, along a filament. P1 in any group is shared with the previous group as P4. Equivalently, P4 in any group is P1 in the subsequent group. For every group, a third-order Bézier spline is created along the four pixels that represent the filament backbone of the region. By definition, the Bézier spline passes through P1 and P4 of a group, but not necessarily P2 and P3, using the line segment joining P1 and P2 as the tangent line of the spline at P1 and the line segment joining P3 and P4 as the tangent line of the spline at P4 (see figure below). A Bézier spline function is described mathematically as functions $x=x(t)$, $y=y(t)$, where parameter $t \in [0, 1]$ and the Bézier spline goes through P1 when $t=0$ and P4 when $t=1$. Tangent lines along a smoothed filament can be calculated directly from the first order derivatives of the corresponding Bézier spline functions as $\frac{dy}{dx} = \frac{dy}{dt} \Big/ \frac{dx}{dt}$. Replacing groups of pixels in skeletonized filaments with the corresponding Bézier splines smooths filaments locally (every three segments) and allows for a fine-grained shape reconstruction. That reconstruction eliminates the effect of pixelation introduced in the skeletonization procedure and increases the number of tangent vectors along a filament (Figure 2 C & D in the original paper).

3. Digitizing smoothed filaments

The last step in filament shape reconstruction is to re-digitize the smoothed filaments represented by consecutive Bézier splines. Every spline from the original 4 pixel points is re-digitized to 10 position points with the first and last points superimposing with the original P1 and P4 in the corresponding group, i.e. the original 3 segments become 9 segments after smoothing and re-digitizing.

4. <u>Analyzing filaments</u>

The re-digitized position points $(i,j)=(y(\mathrm{t}), x(t))$ and their derivatives ($\left(\frac{di}{dt},\frac{dj}{dt}\right)=\left(-\frac{dy}{dt},\frac{dx}{dt}\right)$) from corresponding Bézier splines for all reconstructed filaments are used by the program for polymer persistence length analysis, and also exported to the file xxx_ Nfils_contours.txt, as Columns 2 through 5 to be used for other purposes as needed. The negative sign in $\frac{dy}{dt}$ in relation to $\frac{di}{dt}$ is due to the algorithm counting i values in the image from top to bottom. The tangent angles at every point are calculated from $\frac{dj}{dt}\Big/\frac{di}{dt}=-\frac{dx}{dt}\Big/\frac{dy}{dt}=-\frac{dx}{dy}$, which is the tangent to the y-axis, and exported to the file xxx_ _Nfils_tangent_angles.txt as column 3. Column 4 in that file is the curvature ($\frac{\Delta\theta}{\Delta s}$) along the filament calculated from the difference of tangent angles (Column 3) over the difference of segment lengths (Column 2) between neighboring points.

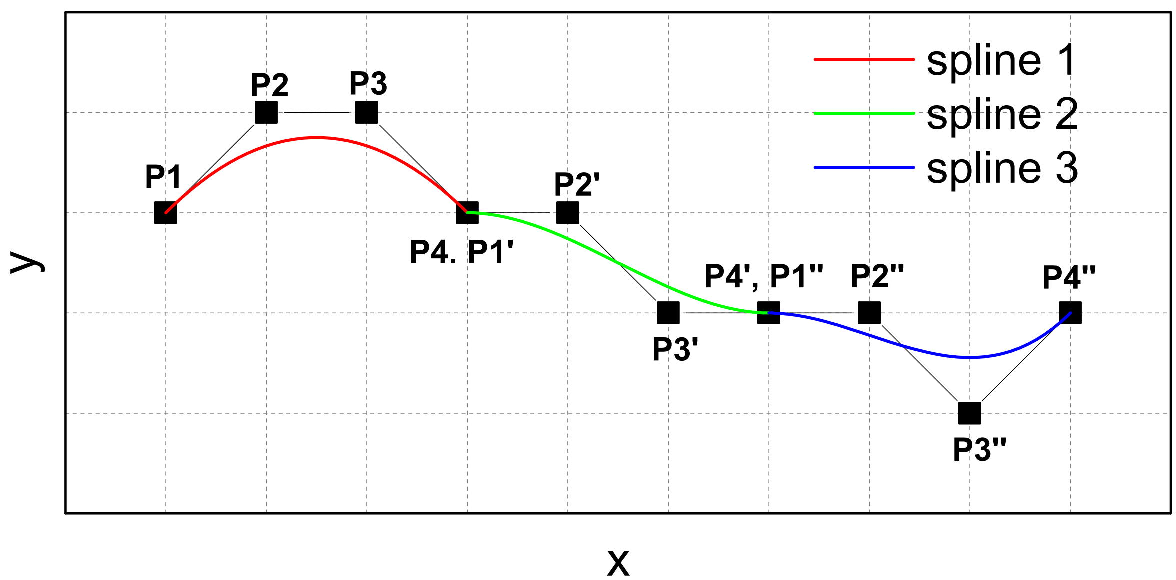

Figure: An example of a filament skeleton smoothed by consecutive third-order Bézier splines.