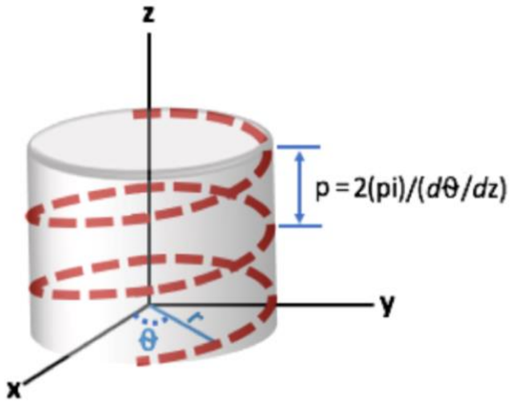
## Determination of centroid paths

The centroid paths of chromosome shapes are defined with an algorithm that uses an intensity-weighted centroid computation.



First, candidate objects in the imaged volume are segmented from adjacent objects and each object is rotated so that its major axis is aligned in the z-direction. The software then segments the object slice-by-slice calculates the centroid for each slice. The unit of distance from slice to slice in the z-direction is the same as the dimension of one pixel in the input image. A minimum Euclidean distance filter then spatially links the centroid across slices.

One end of the centroid path (shown as the "bottom" below) is then assigned as the local origin, and centroid locations are converted to the cylindrical coordinate system, (r,q,z), which provides a convenient way to reference changes in pitch and handedness. In this coordinate system, z is the distance along the major axis, while r and $\theta$ are lateral and angular displacements from the local origin, respectively.

Here, pitch p is defined as the distance in z over which changes in $\theta$ accumulate to $2\pi$ radians. Because the change in angle is in the denominator, the pitch measurement is sensitive to noise which presents itself as small-scale, large magnitude swings in value. A smoothing Gaussian derivative is used when calculating pitch, where the smoothing scale t is equivalent to the variance of the smoothing Gaussian. This means that any changes in pitch larger than $1/\sqrt{t}$ are smoothed away.

Changes in sign/zero-crossings in $d\theta/dz$ provides information about the handedness in the helical tendency of the object centroid. The consequences of using the smoothing factor t means that the algorithm is insensitive to the location of changes in handedness at length scales smaller than $\sqrt{t}$. Since t is measured in pixels, the corresponding insensitivity in length will depend on scale calibration on the axis over which the centroid major axis was aligned. The Gaussian smoothing "smears" out uncertainty in the location of the change in handedness, with the uncertainty being equal to 2*sqrt(t).