<u>Formulas used:</u>

Relative coordinates x_{rel}, y_{rel} and z_{rel}:

$$x_{rel}(t) = x(t) - x(ref)$$

$$y_{rel}(t) = y(t) - y(ref)$$

$$z_{rel}(t) = z(t) - z(ref)$$

$x(t)$, $y(t)$ and $z(t)$, are the bead's coordinates at frame interval t, $x(ref)$, $y(ref)$ and $z(ref)$ is the bead position at the reference frame (currently the first frame t=0).

Normalized coordinates x_{norm}, y_{norm} and z_{norm}:

$x_{norm}(t) = \frac{x_{rel}(t) - mean_x}{\sigma_x}$ with $mean_x$ and σ_x being the mean and standard deviation of the x relative coordinates across the timelapse.

$y_{norm}(t) = \frac{y_{rel}(t) - mean_y}{\sigma_y}$ with $mean_y$ and σ_y being the mean and standard deviation of the y relative coordinates across the timelapse.

$z_{norm}(t) = \frac{z_{rel}(t) - mean_z}{\sigma_z}$ with $mean_z$ and σ_z being the mean and standard deviation of the z relative coordinates across the timelapse.

Mean Squared Displacement:

$$MSD_X\ (frame) = x_{rel}^2(t)$$

$$MSD_Y\ (frame) = y_{rel}^2(t)$$

$$MSD_Z\ (frame) = z_{rel}^2(t)$$

$$MSD_{3D}\ (frame) = x_{rel}^2(t) + y_{rel}^2(t) + z_{rel}^2(t)$$

Elapsed time:

$$elapsed\ time\ (frame) = frame * frame\ interval$$

1D displacement threshold values:

X and Y displacements between two consecutive frames are compared to the lateral resolution value $res_{x,y}^o$

$$res_{x,y}^o = \frac{0.51 * \lambda_{em}}{NA}$$

Z displacement between two consecutive frames is compared to the axial resolution value res_z^o

$$res_z^o = \frac{\lambda_{em}}{n - \sqrt{n^2 - NA^2}}$$

3D displacement between two consecutive frames t-1 and t is compared to the reference resolution distance $res_{\theta,\varphi}^{o}$

$$res_{\theta,\varphi}^{o} = \frac{res_x^o * res_y^o * res_z^o}{\sqrt{(res_y^o * res_z^o * cos\Phi * sin\Theta)^2 + \ (res_x^o * res_z^o * sin\Phi * sin\Theta)^2 + (res_x^o * res_y^o * cos\Theta)^2}}$$

With

$$\Phi = \arccos \frac{x_t - \ x_{t-1}}{\sqrt{(x_t - \ x_{t-1)}{}^2 + \ (y_t - \ y_{t-1)}{}^2}}$$

$$\Theta = \arccos \frac{z_t - \ z_{t-1}}{\sqrt{(x_t - \ x_{t-1)}{}^2 + \ (y_t - \ y_{t-1)}{}^2 + \ (z_t - \ z_{t-1)}{}^2}}$$