**Tracking configuration**

**Filters parameters (detection\_centroids functions):**

Speckle Filter options:

blurred = cv2.GaussianBlur(frame\_gray, (5, 5), 0)

blurred = cv2.bilateralFilter(frame\_gray,9,75,75)

Filters Area: Ensures that only blobs with a minimum or range of sizes (in pixels) are detected.

Circularly Filter: Filters blobs based on how circular they are. **Range:** From 0 (very irregular) to 1 (perfect circle).

Inertia Filter: Measures elongation (aspect ratio). High values favor round shapes; low values accept elongated blobs.

Convexity Filter: Filters based on how convex the shape is. Ratio between the blob area and its convex hull. A perfect convex shape = 1.0. **Use case:** Helps exclude irregular or concave blobs.

Color Filter: Filters blobs based on intensity. Value = 255 to detect *bright* (white) blobs on a dark background. Value = 0 to detect dark blobs on a bright background.

**Recommendation option for star:**

Area Filter = True

Color filter = True

**Filters parameters (Kalman filter class):**

**P - State Covariance Matrix:** Represents the initial uncertainty of the system's state (position and velocity). High values of P, the filter assumes high uncertainty in the initial state and reacts quickly to initial measurements. Low values of P, the filter trusts the initial state and reacts slowly to changes.

**Recommendation**: Use moderately high values (e.g., P = 100 - 400) unless the initial particle positions are known with high certainty.

**Q - Process Noise Covariance Matrix:** Models uncertainty in the system dynamics (e.g., unexpected accelerations or particle interactions). High values of Q, the model allows for rapid or erratic changes in motion and the filter adapts quickly but may produce noisier trajectories. Low values of Q, assume smooth, predictable motion and the filter provides smoother results but may lag if the particle accelerates or changes direction suddenly.

**Recommendation**: Use higher Q when tracking particles in turbulent environments or with irregular motion. And use lower Q for smooth, predictable trajectories.

**R - Measurement Noise Covariance Matrix:** Models the uncertainty of the measurements (e.g., detected centroids). High values of R, the filter trusts the prediction more than the measurement and is useful when detections are noisy, unreliable, or missing. Low values of R, the filter closely follows the measurements and is suitable when detections are accurate and consistent.

**Recommendation**: Increase R if your detection method sometimes produces false positives or misses frames. And decrease R if detections are precise and stable across frames.

**max\_dist (line 152):** Maximum allowed distance between a prediction and detection to consider them a match.

**max\_skips (line 186):** Maximum number of consecutive frames a tracker can go unmatched before being removed.

**Table of ranges to work with**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| P | | | Q | | | R | | |
| 1-50 | 100-400 | >500 | 0.01-1 | 1-10 | >10 | 0.1-2 | 5-10 | >20 |
| Very reliable initial positions and clear detection | Moderate unfamiliarity with the initial state | High uncertainty in the initial position of the particles | Very smooth movement, without abrupt changes | Moderately erratic movement | Unpredictable motion or strong accelerations | Highly accurate detection | Some noise or inconsistent detection | Very unstable detection or frequent false positives |

**Recommendation values (lines 95-97 in the code):**

self.P = np.eye(4, dtype=np.float32) \* 100  # high initial uncertainty

self.Q = np.eye(4, dtype=np.float32) \* 5  # small process noise

self.R = np.eye(2, dtype=np.float32) \* 20    # measurement noise

**Examples of the parameters with videos:**

Simulation 1: video\_phase\_simul\_5.avi

Gaussian Filter

Area Filter = True

Min = 200

Max = 600

P = 100

Q = 1

R = 10

max\_dist = 30

Experimental 1: video\_compo\_corto5.mp4

Gaussian Filter

Area Filter = True

Min = 200

Max = 600

Color Filter = True

Blobcolor = 255

P = 500

Q = 5

R = 20

max\_dist = 50

max\_skipped\_frames = 20

Experimental 2: video\_2compos\_corto.mp4

Gaussian Filter

Area Filter = True

Min = 100

Max = 300

Color Filter = False

P = 100

Q = 5

R = 10

max\_dist = 50

max\_skipped\_frames = 50

Experimental 3: video\_phase\_RBC\_M\_40.avi

Bilateral Filter

Area Filter = True

Min = 600

Inertia Filter = True

Color Filter = False

P = 100

Q = 0.1

R = 1

max\_dist = 45

max\_skipped\_frames = 10

Experimental 4: video\_phase\_Normoxia\_A7\_40x.avi

Gaussean Filter

Area Filter = True

Min = 600

Color Filter = True

Blobcolor = 0

P = 100

Q = 0.1

R = 1

max\_dist = 45

max\_skipped\_frames = 0

Experimental 5: video\_phase\_RBC\_10x.mp4

Gaussean Filter

Area Filter = True

Min = 150

Max = 500

Color Filter = True

Blobcolor = 255

P = 100

Q = 0.1

R = 1

max\_dist = 45

max\_skipped\_frames = 0

Maria, please answer this comment with the next information.  
- What is the format of the input video? RTA/ whatever format the code accepts the video, but I always use .avi or .mp4.  
- What is the spatial resolution (in pixels) min and max, Could it be whatever? RTA/ right now I don’t have a sample with a resolution that the code didn’t work. Finally, with the Area filter you can limit de pixel you want to catch.

* What is the frame rate (frames per second), min and max? RTA/ I thinks its depends of the velocity of the sample, because it’s not the same to have a RBCs at 15fps as it is to have sperm at 15fps.
* What is the bit depth of the images? RTA/ The images used for tracking are 8-bit grayscale (256 intensity levels, from 0 to 255).
* What spatial accuracy is expected or required? RTA/ I'm not sure at the moment, but I can find out in the future.
* What is the maximum allowed particle speed? RTA/ I'm not sure at the moment, but I can find out in the future.  
    
  I understand that you might not have the answer to all of these questions, but please try to answer as many as possible. For the remaining ones, consider what the answers might be in the near future.