

PyF2F_image_registration

June 7, 2023

PyF2F - Image Registration Notebook

To achieve accurate distance measurements between two fluorophores that emit at different wavelengths, it is crucial to register the positions of the two fluorophores obtained in the two channels. This notebook runs the functionalities of PyF2F to calculate the chromatic shift between the two color positions (corresponding to the the two channels) over the entire field of view at the nanometer scale.

0.0.1 Scientific IPython Setup

Before starting, we need to : 1. Scientific IPython Setup 2. Set parameters to run the image registration workflow 3. Set paths to working directories

1. Scientific IPython Setup

Load some required scientific Python libraries:

```
[1]: %load_ext autoreload
      %autoreload 2
      %matplotlib agg
      import os
      import glob
      import shutil
      import sys
      import time
      import pandas as pd
      import numpy as np
      import trackpy as tp
      import pims
      import matplotlib.pyplot as plt
      import seaborn as sns
      import plotly.express as px
      import plotly.graph_objects as go
      import tkinter as tk
      from tkinter import filedialog
      from skimage import io, util
      from skimage import data
      from scipy import stats
      from scipy import spatial
      from pymicro.view.vol_utils import compute_affine_transform
```

```
from matplotlib import rcParams
```

as well as PyF2F-Ruler's custom functions:

```
[2]: # Append PyF2F scripts path and import functions
sys.path.append(''.join(os.getcwd().split("/")[:-1]) + '/scripts/')
from detect_beads import *
%aimport detect_beads
```

2. Parameters for Image Registration

Now, we have to set the **parameters** to run the image registration workflow. PyF2F calculates the registration map between the two channels using a *piecewise affine transformation*, correcting spot positions locally using nearby fiducial points.

Important parameters here are the **pixel size** of the camera (*nm/px*) and the **spot diameter** (approximate the spot size to the PSF of the microscope, actually the diameter should approximate twice the PSF of the microscope).

The rest of the parameters help to optimize the spot detection based on intensity filters such as the **percentile** (detecting spots above a given percentile), the **mass (integrated brightness)** (avoiding selecting low-intense spots or too high-intense spots (such as bead aggregates)). Moreover, since the local affine correction is based on a nearest-neighbor search, two parameters can influence the registration outcome: minimum number of fiducial points (**min_fiducials** ~ 100) and maximum distance to the coordinate to correct (**search_range** ~ 2 μm).

```
[8]: beads_head = "*.tif"           # Beads_head (pattern in bead images)
px_size = 64.5                     # Zyla camera (64.5 nm/px) // Prime camera
    ↪ (110 nm/px)
db_PSF = 700                       # Double size of the PSF to calculate the spot
    ↪ diameter
if px_size == 64.5:                # spot detection: diameter of spots in px
    spot_diameter = 11             # Spot size Zyla
elif px_size == 110:              # Spot size Prime
    spot_diameter = 7
percentile = 99.8                  # spot detection: sort spots below this
    ↪ percentile of intensity
min_mass = 0.01                   # spot detection: sort spots with a mass above
    ↪ this threshold (range 0-1).
max_mass = 0.95                   # spot detection: sort spots with a mass below
    ↪ this threshold (range 0-1).
max_displacement=1                # linking: link spots from ch1-ch2 channels
    ↪ separated by this cutoff in px
search_range = 2000 // px_size    # local registration: max distance in px for
    ↪ nearest-neighbour search
min_fiducials = 10                # local registration: minimum number of
    ↪ fiducial markers to correct locally
```

```
[9]: spot_diameter
```

```
[9]: 11
```

3. Set paths to your working directories

The last thing to go is setting the paths to the working directories. At least we should have two different directories:

- Directory with beads images to create the registration map (Beads_reg)
- Directory with beads images to calculate the error of registration (Beads_test)
- Directory to output of registration
- Directory to output of test

```
[18]: # Select directories where Beads_reg and Beads_test
root = tk.Tk()
root.withdraw()
path_beads_reg = filedialog.askdirectory(title="Select Directory Beads_
↳Registration") + "/"
path_beads_test = filedialog.askdirectory(title="Select Directory Beads Test")_
↳+ "/"

# Create directories for output
path_output_reg = "/".join(path_beads_reg.split("/")[:-2]) + "/output_reg/"
path_output_test = "/".join(path_beads_reg.split("/")[:-2]) + "/output_test/"
if not os.path.exists(path_output_reg):
    os.mkdir(path_output_reg)

if not os.path.exists(path_output_test):
    os.mkdir(path_output_test)
```

```
[19]: print("Your Paths: \n"
        " -Beads_Reg directory: {}\n"
        " -Beads_Test directory: {}\n"
        " -Output_Reg directory: {}\n"
        " -Output_Test directory: {}\n".format(path_beads_reg,
                                                path_beads_test,
                                                path_output_reg,
                                                path_output_test))
```

Your Paths:

```
-Beads_Reg directory:
/home/altair/PycharmProjects/book_chapter_local_affine/Beads_230523/images_reg/
-Beads_Test directory:
/home/altair/PycharmProjects/book_chapter_local_affine/Beads_230523/images_test/
-Output_Reg directory:
/home/altair/PycharmProjects/book_chapter_local_affine/Beads_230523/output_reg/
-Output_Test directory:
```

/home/altair/PycharmProjects/book_chapter_local_affine/Beads_230523/output_test/

Now, we can calculate the Target Registration Error (TRE).

0.1 Calculate TRE

Step 1

Get two channels (c1-c2) coordinates from the two sets of beads to a) calculate the registration map (REF) and b) to calculate the TRE (TEST). If the coordinates have been previously calculated, then are loaded from the saved CSV files in the “output_reg” and “output_test” directories.

```
[20]: #####
# 1. Get Coordinates
#####
# Check if spots have been previously detected and saved in
# the "path_output_reg" and "path_output_test" directories
if os.path.exists(path_output_reg + "/coords_W1.csv"):
    c1_ref = np.loadtxt(path_output_reg + "/coords_W1.csv", delimiter=",")
    c2_ref = np.loadtxt(path_output_reg + "/coords_W2.csv", delimiter=",")
else:
    # Get coordinates from beads for registration
    c2_ref, c1_ref = get_coords(path_output_reg, path_beads_reg, beads_head,
    ↪diameter=spot_diameter, separation=max_displacement, percentile=percentile,
    ↪min_mass_cutoff=min_mass, max_mass_cutoff=max_mass, px_size=px_size)

if os.path.exists(path_output_test + "/coords_W1.csv") and os.path.
    ↪exists(path_output_test + "/coords_W2.csv"):
    c1_test = np.loadtxt(path_output_test + "/coords_W1.csv", delimiter=",")
    c2_test = np.loadtxt(path_output_test + "/coords_W2.csv", delimiter=",")
else:
    # Get coordinates from beads for registration
    c2_test, c1_test = get_coords(path_output_test, path_beads_test,
    ↪beads_head, diameter=spot_diameter, separation=max_displacement,
    ↪percentile=percentile, min_mass_cutoff=min_mass, max_mass_cutoff=max_mass,
    ↪px_size=px_size)
```

```
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos005_000.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos005_000.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos006_000.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos006_000.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos007_000.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos007_000.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos008_000.ome.tif'
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OME series: failed to read 'T0_10x10_1_MMStack_2-Pos009_000.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos009_000.ome.tif'
```

[illegible]

[illegible]

OME series: failed to read 'T0_10x10_1_MMStack_2-Pos009_008.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos009_008.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos009_009.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos009_009.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos008_009.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos008_009.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos007_009.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos007_009.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos006_009.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos006_009.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos005_009.ome.tif'
OME series: failed to read 'T0_10x10_1_MMStack_2-Pos005_009.ome.tif'
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 92
Number of spots discarded regarding mass: 4
Final number of selected spots: 88

Processing image T0_10x10_1_MMStack_2-Pos000_001

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 92
Number of spots discarded regarding mass: 5
Final number of selected spots: 87

Processing image T0_10x10_1_MMStack_2-Pos000_002

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 93
Number of spots discarded regarding mass: 5

Final number of selected spots: 88

Processing image T0_10x10_1_MMStack_2-Pos000_003

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 98

Number of spots discarded regarding mass: 5

Final number of selected spots: 93

Processing image T0_10x10_1_MMStack_2-Pos000_004

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 104

Number of spots discarded regarding mass: 5

Final number of selected spots: 99

Processing image T0_10x10_1_MMStack_2-Pos000_005

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 96

Number of spots discarded regarding mass: 4

Final number of selected spots: 92

Processing image T0_10x10_1_MMStack_2-Pos000_006

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 90
Number of spots discarded regarding mass: 5
Final number of selected spots: 85

Processing image T0_10x10_1_MMStack_2-Pos000_007

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 90
Number of spots discarded regarding mass: 4
Final number of selected spots: 86

Processing image T0_10x10_1_MMStack_2-Pos000_008

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 96
Number of spots discarded regarding mass: 5
Final number of selected spots: 91

Processing image T0_10x10_1_MMStack_2-Pos000_009

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 90
Number of spots discarded regarding mass: 5
Final number of selected spots: 85

Processing image T0_10x10_1_MMStack_2-Pos001_000

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 96
Number of spots discarded regarding mass: 5
Final number of selected spots: 91

Processing image T0_10x10_1_MMStack_2-Pos001_001

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 100
Number of spots discarded regarding mass: 5
Final number of selected spots: 95

Processing image T0_10x10_1_MMStack_2-Pos001_002

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 102
Number of spots discarded regarding mass: 5
Final number of selected spots: 97

Processing image T0_10x10_1_MMStack_2-Pos001_003

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 108
Number of spots discarded regarding mass: 5
Final number of selected spots: 103

Processing image T0_10x10_1_MMStack_2-Pos001_004

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 112
Number of spots discarded regarding mass: 5
Final number of selected spots: 107

Processing image T0_10x10_1_MMStack_2-Pos001_005

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 104
Number of spots discarded regarding mass: 5
Final number of selected spots: 99

Processing image T0_10x10_1_MMStack_2-Pos001_006

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
Sum pdf = 100

Initial number of spots detected by trackpy: 96
Number of spots discarded regarding mass: 6
Final number of selected spots: 90

Processing image T0_10x10_1_MMStack_2-Pos001_007

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 92
Number of spots discarded regarding mass: 5
Final number of selected spots: 87

Processing image T0_10x10_1_MMStack_2-Pos001_008

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 98
Number of spots discarded regarding mass: 5
Final number of selected spots: 93

Processing image T0_10x10_1_MMStack_2-Pos001_009

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 90
Number of spots discarded regarding mass: 5
Final number of selected spots: 85

Processing image T0_10x10_1_MMStack_2-Pos002_000

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 94
Number of spots discarded regarding mass: 6
Final number of selected spots: 88

Processing image T0_10x10_1_MMStack_2-Pos002_001

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 104
Number of spots discarded regarding mass: 6
Final number of selected spots: 98

Processing image T0_10x10_1_MMStack_2-Pos002_002

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
Sum pdf = 100

Initial number of spots detected by trackpy: 104
Number of spots discarded regarding mass: 5
Final number of selected spots: 99

Processing image T0_10x10_1_MMStack_2-Pos002_003

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
Sum pdf = 100

Initial number of spots detected by trackpy: 114
Number of spots discarded regarding mass: 6
Final number of selected spots: 108

Processing image T0_10x10_1_MMStack_2-Pos002_004

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
Sum pdf = 100

Initial number of spots detected by trackpy: 115
Number of spots discarded regarding mass: 6
Final number of selected spots: 109

Processing image T0_10x10_1_MMStack_2-Pos002_005

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
Sum pdf = 100

Initial number of spots detected by trackpy: 108
Number of spots discarded regarding mass: 6
Final number of selected spots: 102

Processing image T0_10x10_1_MMStack_2-Pos002_006

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
Sum pdf = 100

Initial number of spots detected by trackpy: 101
Number of spots discarded regarding mass: 4
Final number of selected spots: 97

Processing image T0_10x10_1_MMStack_2-Pos002_007

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
Sum pdf = 100

Initial number of spots detected by trackpy: 100
Number of spots discarded regarding mass: 5
Final number of selected spots: 95

Processing image T0_10x10_1_MMStack_2-Pos002_008

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 105
Number of spots discarded regarding mass: 6
Final number of selected spots: 99

Processing image T0_10x10_1_MMStack_2-Pos002_009

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 98
Number of spots discarded regarding mass: 6
Final number of selected spots: 92

Processing image T0_10x10_1_MMStack_2-Pos003_000

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 94
Number of spots discarded regarding mass: 1
Final number of selected spots: 93

Processing image T0_10x10_1_MMStack_2-Pos003_001

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 106
Number of spots discarded regarding mass: 2
Final number of selected spots: 104

Processing image T0_10x10_1_MMStack_2-Pos003_002

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 102
Number of spots discarded regarding mass: 7
Final number of selected spots: 95

Processing image T0_10x10_1_MMStack_2-Pos003_003

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 107
Number of spots discarded regarding mass: 5
Final number of selected spots: 102

Processing image T0_10x10_1_MMStack_2-Pos003_004

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 109
Number of spots discarded regarding mass: 4
Final number of selected spots: 105

Processing image T0_10x10_1_MMStack_2-Pos003_005

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
Sum pdf = 100

Initial number of spots detected by trackpy: 102
Number of spots discarded regarding mass: 6
Final number of selected spots: 96

Processing image T0_10x10_1_MMStack_2-Pos003_006

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
Sum pdf = 100

Initial number of spots detected by trackpy: 96
Number of spots discarded regarding mass: 4
Final number of selected spots: 92

Processing image T0_10x10_1_MMStack_2-Pos003_007

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
Sum pdf = 100

Initial number of spots detected by trackpy: 92
Number of spots discarded regarding mass: 5
Final number of selected spots: 87

Processing image T0_10x10_1_MMStack_2-Pos003_008

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
Sum pdf = 100

Initial number of spots detected by trackpy: 97
Number of spots discarded regarding mass: 6
Final number of selected spots: 91

Processing image T0_10x10_1_MMStack_2-Pos003_009

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
Sum pdf = 100

Initial number of spots detected by trackpy: 90
Number of spots discarded regarding mass: 6
Final number of selected spots: 84

Processing image T0_10x10_1_MMStack_2-Pos004_000

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 86

Number of spots discarded regarding mass: 3
Final number of selected spots: 83

Processing image T0_10x10_1_MMStack_2-Pos004_001

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 96
Number of spots discarded regarding mass: 2
Final number of selected spots: 94

Processing image T0_10x10_1_MMStack_2-Pos004_002

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 92
Number of spots discarded regarding mass: 6
Final number of selected spots: 86

Processing image T0_10x10_1_MMStack_2-Pos004_003

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 100
Number of spots discarded regarding mass: 5
Final number of selected spots: 95

Processing image T0_10x10_1_MMStack_2-Pos004_004

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 102
Number of spots discarded regarding mass: 6
Final number of selected spots: 96

Processing image T0_10x10_1_MMStack_2-Pos004_005

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 94
Number of spots discarded regarding mass: 5
Final number of selected spots: 89

Processing image T0_10x10_1_MMStack_2-Pos004_006

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 94
Number of spots discarded regarding mass: 4
Final number of selected spots: 90

Processing image T0_10x10_1_MMStack_2-Pos004_007

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 94
Number of spots discarded regarding mass: 4
Final number of selected spots: 90

Processing image T0_10x10_1_MMStack_2-Pos004_008

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 98
Number of spots discarded regarding mass: 6
Final number of selected spots: 92

Processing image T0_10x10_1_MMStack_2-Pos004_009

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 96
Number of spots discarded regarding mass: 5
Final number of selected spots: 91

```
#####  
      BEADS REGISTRATION  
#####
```

Processing image T0_10x10_1_MMStack_2-Pos005_000

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 84
Number of spots discarded regarding mass: 2
Final number of selected spots: 82

Processing image T0_10x10_1_MMStack_2-Pos005_001

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 96
Number of spots discarded regarding mass: 4
Final number of selected spots: 92

Processing image T0_10x10_1_MMStack_2-Pos005_002

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 94
Number of spots discarded regarding mass: 2
Final number of selected spots: 92

Processing image T0_10x10_1_MMStack_2-Pos005_003

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 102
Number of spots discarded regarding mass: 5
Final number of selected spots: 97

Processing image T0_10x10_1_MMStack_2-Pos005_004

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 104
Number of spots discarded regarding mass: 4
Final number of selected spots: 100

Processing image T0_10x10_1_MMStack_2-Pos005_005

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 92
Number of spots discarded regarding mass: 4
Final number of selected spots: 88

Processing image T0_10x10_1_MMStack_2-Pos005_006

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 98
Number of spots discarded regarding mass: 3
Final number of selected spots: 95

Processing image T0_10x10_1_MMStack_2-Pos005_007

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 96
Number of spots discarded regarding mass: 4
Final number of selected spots: 92

Processing image T0_10x10_1_MMStack_2-Pos005_008

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 100
Number of spots discarded regarding mass: 5
Final number of selected spots: 95

Processing image T0_10x10_1_MMStack_2-Pos005_009

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 96

Number of spots discarded regarding mass: 6
Final number of selected spots: 90

Processing image T0_10x10_1_MMStack_2-Pos006_000

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 92
Number of spots discarded regarding mass: 3
Final number of selected spots: 89

Processing image T0_10x10_1_MMStack_2-Pos006_001

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 102
Number of spots discarded regarding mass: 5
Final number of selected spots: 97

Processing image T0_10x10_1_MMStack_2-Pos006_002

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 98
Number of spots discarded regarding mass: 5
Final number of selected spots: 93

Processing image T0_10x10_1_MMStack_2-Pos006_003

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 98
Number of spots discarded regarding mass: 4
Final number of selected spots: 94

Processing image T0_10x10_1_MMStack_2-Pos006_004

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 96
Number of spots discarded regarding mass: 4
Final number of selected spots: 92

Processing image T0_10x10_1_MMStack_2-Pos006_005

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 86
Number of spots discarded regarding mass: 3
Final number of selected spots: 83

Processing image T0_10x10_1_MMStack_2-Pos006_006

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 94
Number of spots discarded regarding mass: 3
Final number of selected spots: 91

Processing image T0_10x10_1_MMStack_2-Pos006_007

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 92
Number of spots discarded regarding mass: 3
Final number of selected spots: 89

Processing image T0_10x10_1_MMStack_2-Pos006_008

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 94
Number of spots discarded regarding mass: 3
Final number of selected spots: 91

Processing image T0_10x10_1_MMStack_2-Pos006_009

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 88
Number of spots discarded regarding mass: 4
Final number of selected spots: 84

Processing image T0_10x10_1_MMStack_2-Pos007_000

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 90
Number of spots discarded regarding mass: 3
Final number of selected spots: 87

Processing image T0_10x10_1_MMStack_2-Pos007_001

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 96
Number of spots discarded regarding mass: 4
Final number of selected spots: 92

Processing image T0_10x10_1_MMStack_2-Pos007_002

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 90
Number of spots discarded regarding mass: 2
Final number of selected spots: 88

Processing image T0_10x10_1_MMStack_2-Pos007_003

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 88
Number of spots discarded regarding mass: 3
Final number of selected spots: 85

Processing image T0_10x10_1_MMStack_2-Pos007_004

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 90
Number of spots discarded regarding mass: 3
Final number of selected spots: 87

Processing image T0_10x10_1_MMStack_2-Pos007_005

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 86
Number of spots discarded regarding mass: 3
Final number of selected spots: 83

Processing image T0_10x10_1_MMStack_2-Pos007_006

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 92

Number of spots discarded regarding mass: 2
Final number of selected spots: 90

Processing image T0_10x10_1_MMStack_2-Pos007_007

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 89
Number of spots discarded regarding mass: 1
Final number of selected spots: 88

Processing image T0_10x10_1_MMStack_2-Pos007_008

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 89
Number of spots discarded regarding mass: 3
Final number of selected spots: 86

Processing image T0_10x10_1_MMStack_2-Pos007_009

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 85
Number of spots discarded regarding mass: 4
Final number of selected spots: 81

Processing image T0_10x10_1_MMStack_2-Pos008_000

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 86
Number of spots discarded regarding mass: 3
Final number of selected spots: 83

Processing image T0_10x10_1_MMStack_2-Pos008_001

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 96
Number of spots discarded regarding mass: 4
Final number of selected spots: 92

Processing image T0_10x10_1_MMStack_2-Pos008_002

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 91
Number of spots discarded regarding mass: 3
Final number of selected spots: 88

Processing image T0_10x10_1_MMStack_2-Pos008_003

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 90
Number of spots discarded regarding mass: 4
Final number of selected spots: 86

Processing image T0_10x10_1_MMStack_2-Pos008_004

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 98
Number of spots discarded regarding mass: 4
Final number of selected spots: 94

Processing image T0_10x10_1_MMStack_2-Pos008_005

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 94
Number of spots discarded regarding mass: 4
Final number of selected spots: 90

Processing image T0_10x10_1_MMStack_2-Pos008_006

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 96
Number of spots discarded regarding mass: 2

Final number of selected spots: 94

Processing image T0_10x10_1_MMStack_2-Pos008_007

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 94

Number of spots discarded regarding mass: 2

Final number of selected spots: 92

Processing image T0_10x10_1_MMStack_2-Pos008_008

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 94

Number of spots discarded regarding mass: 3

Final number of selected spots: 91

Processing image T0_10x10_1_MMStack_2-Pos008_009

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 88

Number of spots discarded regarding mass: 4

Final number of selected spots: 84

Processing image T0_10x10_1_MMStack_2-Pos009_000

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 88
Number of spots discarded regarding mass: 3
Final number of selected spots: 85

Processing image T0_10x10_1_MMStack_2-Pos009_001

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 99
Number of spots discarded regarding mass: 5
Final number of selected spots: 94

Processing image T0_10x10_1_MMStack_2-Pos009_002

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 93
Number of spots discarded regarding mass: 3
Final number of selected spots: 90

Processing image T0_10x10_1_MMStack_2-Pos009_003

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 96

Number of spots discarded regarding mass: 5

Final number of selected spots: 91

Processing image T0_10x10_1_MMStack_2-Pos009_004

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 102

Number of spots discarded regarding mass: 6

Final number of selected spots: 96

Processing image T0_10x10_1_MMStack_2-Pos009_005

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 96

Number of spots discarded regarding mass: 5

Final number of selected spots: 91

Processing image T0_10x10_1_MMStack_2-Pos009_006

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

OME series: not an ome-tiff master file

Sum pdf = 100

Initial number of spots detected by trackpy: 98

Number of spots discarded regarding mass: 3
Final number of selected spots: 95

Processing image T0_10x10_1_MMStack_2-Pos009_007

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 98
Number of spots discarded regarding mass: 3
Final number of selected spots: 95

Processing image T0_10x10_1_MMStack_2-Pos009_008

OME series: not an ome-tiff master file
OME series: not an ome-tiff master file
OME series: not an ome-tiff master file

Initial number of spots detected by trackpy: 96
Number of spots discarded regarding mass: 3
Final number of selected spots: 93

Processing image T0_10x10_1_MMStack_2-Pos009_009

OME series: not an ome-tiff master file
Sum pdf = 100

Initial number of spots detected by trackpy: 88
Number of spots discarded regarding mass: 3
Final number of selected spots: 85

```
[21]: plotly_coords(c1_test, c2_test, path=path_output_reg, title=f"TEST\n",
↳N={len(c1_test)})
```

Step 2

Apply local affine (piecewise) transformation to the TEST dataset from the REF dataset

```
[22]: print("Your Search Range Parameters for Local Affine are:\n"
↳f"\t Search Range: {search_range} nm\n"
↳f"\t Minim Fiducials: {min_fiducials}\n"
↳f"\t Pixel Size is: {px_size} nm")
```

Your Search Range Parameters for Local Affine are:

```
Search Range: 31.0 nm
Minim Fiducials: 10
Pixel Size is: 64.5 nm
```

```
[23]: %matplotlib inline
#####
# 2. Piecewise Affine
#####
c1_test_new = np.empty_like(c1_test)
for i in range(len(c1_test)):
    c1_test_new[i] = piecewise_affine(c1_test[i], c1_ref, c2_ref,
↳search_range=search_range, min_candidates=min_fiducials)
    if c1_test_new[i][0] == np.nan:
        sys.stderr.write(f"Coord {c1_test[i]} cannot be locally corrected, no
↳neighbours found!\n")
# Plot and save coordinates
# plotly_coords(c1_test, c2_test, path=path_output_test,
↳c1_corrected=c1_test_new, title=f"TEST\n N={len(c1_test)}")
```

Step 3

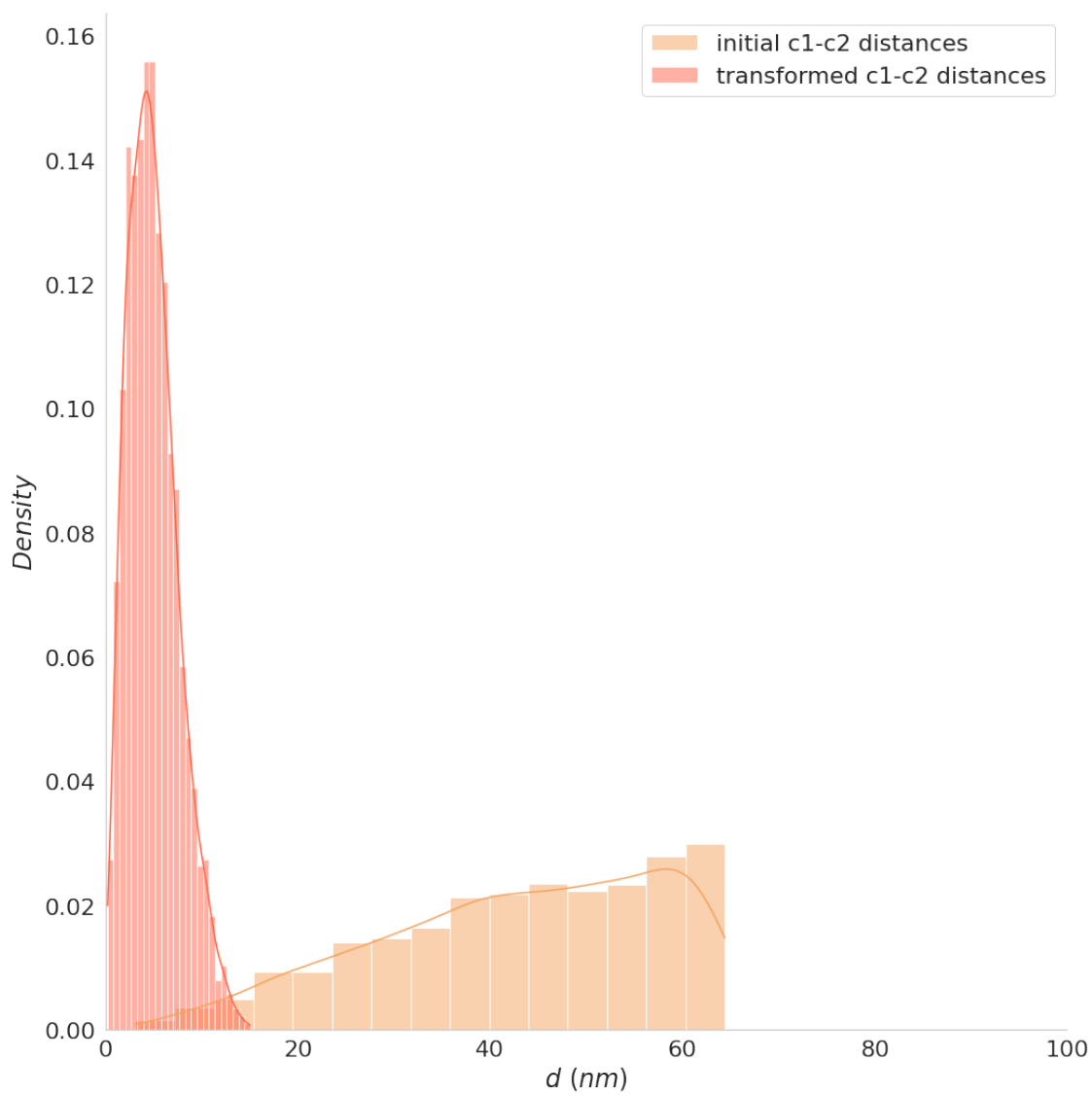
Calculate the Target Registration Error and plot the distances between c1-c2 TEST beads before and after the piecewise affine transformation

```
[24]: #####
# 3. Calculate TRE
#####
plot_distance_distribution(path_output_test, c2_test, c1_test, c1_test_new,
↳px_size=px_size)
calculate_tre(path_output_test, c2_test, c1_test, c1_test_new, px_size=px_size)
```

Mean shift in X axis = 0.569 nm

Mean shift in Y axis = 0.966 nm

Target Registration Error (TRE) = 1.122 nm



<Figure size 1080x1080 with 0 Axes>

<Figure size 1080x1080 with 0 Axes>

0.2 END