

adf

Appendix A

Light-controlled *E. coli*

A.1 Plasmid Construction and Transformation

A.1.1 Plasmid Construction

Here I present the plasmid construction protocol using Gibson assembly. This method can ligate the target gene PR with the plasmid carrier pZE. We first use polymerase chain reaction (PCR) to amplify the gene to sufficient concentration for the ligation. PCR can be easily done with a standard thermo cycler, and all we need to do is to prepare a mixture according to the recipe shown in Table. A.1

The sequence of the primers can be found in Table. ??, and are synthesized by Eurofins Scientific. After we obtain the more concentrated DNA from PCR, we use Gibson assembly to ligate the plasmid carrier pZE and the target DNA sequence PR. Gibson assembly is done in the following steps:

- prepare the Gibson assembly mix according to the Table. A.2.
- add the mix into a 200 μ l centrifuge tube, then put the tube into a thermal cycler and run the Gibson assembly program (50-55 °C for 1 hour).

Ingredients	Amount (μ l)
ddH ₂ O	37
5X HF buffer (Phusion)	10
dNTP	1
Primer-forward	0.5
Primer-reverse	0.5
Template	small amount of cell culture / 0.5 μ l plasmid solution
Phusion polymerase	0.5
Sum	50

Table A.1: PCR recipe using Phusion polymerase

- thaw the chemical competent cells in ice (XL10-Gold, 3-4 min).
- mix cells and plasmid solution for 10 min in ice.
- spread the mix on a selective plate.

Ingredients	Amount (μ l)
PCR product	3.3
pZE solution	1.7
2X Gibson assembly mix	5
Sum	10

Table A.2: Gibson assembly recipe.

A.1.2 Extraction and Purification

After growing the chemical competent cells into concentrated liquid cultures, we extract the plasmid from the cells and purify them. We follow the steps in the Zyppy Plasmid Miniprep Kit manual and Zymoclean Gel DNA Recovery Kit manual.

A.1.3 Transformation

Make electrocompetent cells

- Inoculate target cells (*E. coli*, 1 ml) into a test tube and incubate at 37 °C for 16-18 hours.
- Prepare a box of ice and put 10% glycerol on it.
- Place the cell culture on ice for 15 minutes (Note: from this step, the cell culture must be on ice all the time).
- Centrifuge at 4000 rpm for 5 min, at 4 °C (use the low temperature centrifuge)
- Discard the supernatant, aspirate the residual broth and add 1 ml glycerol. Resuspend the cells by pipetting up and down.
- Repeat step 4 and 5 for 2 more times (Note: for the last time, add $\sim 50 \mu\text{l}$ glycerol instead of 1 ml).

Electroporation

- Thaw the cell suspensions prepared in the previous step on ice. Place a 1.5 ml centrifuge tube and a 0.1 cm cuvette on ice.
- Add 40 μl cell suspension and 1 μl DNA solution to the 1.5 ml centrifuge tube.
- Set the electroporator mode to Ec1.
- Transfer the mixture of cells and DNA to the cuvette and tap it to make the cell suspension go to the bottom of the cuvette.
- Put the cuvette into the electroporator, and press the pulse button once.

- Remove the cuvette from the electroporator and spread the cell suspension on a selective plate.

A.2 DNA Sequences

A.2.1 Proteorhodopsin (PR)

```

1 ATGAAGTTGT TGTGATCTT GGGATCTGTG ATTGCACTTC CGACGTTTCG TGCCGGCGGC
61 GGGGATTTGG ACGCATCAGA CTACACAGGG GTTTCGTTTT GGCTGGTCAC AGCCGCGCTG
121 TTAGCGTCCA CCGTCTTTTT TTTCGTTGAA CGGGATAGAG TCTCAGCTAA GTGGAAGACA
181 TCGTTGACCG TCTCAGGCTT GGTACCGGC ATTGCCTTCT GGCATTATAT GTACATGCGT
241 GGTGTCTGGA TTGAAACGGG TGACAGCCCG ACGGTGTTCC GTTATATCGA CTGGCTTTTA
301 ACCGTTCCCC TTCTGATTG TGAGTTTTAT TTAATATTGG CGGCAGCAAC GAATGTGGCC
361 GGTTCACTGT TCAAGAAGCT TCTTGTAGGA AGTTTAGTTA TGTTGGTTTT CGGCTACATG
421 GGAGAGGCAG GGATAATGGC GGCCTGGCCG GCGTTCATAA TTGGTTGCTT GGCTTGGGTG
481 TACATGATCT ACGAGCTGTG GGCAGGAGAA GGCAAGTCTG CGTGCAACAC AGCATCGCCA
541 GCAGTTCAAT CCGCATATAA TACGATGATG TATATAATTA TCTTTGGTTG GGCAATTTAC
601 CCGGTCGGAT ACTTCACCGG CTATCTTATG GGCGACGGGG GCTCTGCCTT GAACTTGAAT
661 CTTATATATA ACCTGGCCGA TTTCGTGAAC AAGATTTTGT TTGGACTTAT AATATGGAAC
721 GTAGCCGTGA AAGAGTCATC GAACGCATAA

```

A.2.2 pZE-PR

```

1 AGGCGTATCA CGAGGCCCTT TCGTCTTCAC CTCGAGAATT GTGAGCGGAT AACAATTGAC
61 ATTGTGAGCG GATAACAAGA TACTGAGCAC ATCAGCAGGA CGCACTGACC GAATTCATTA
121 AAGAGGAGAA AGGTACCATG AAGTTGTTGT TGATCTTGGG ATCTGTGATT GCACTTCCGA
181 CGTTCGCTGC CGGCGGCGGG GATTTGGACG CATCAGACTA CACAGGGGTT TCGTTTTGGC
241 TGGTCACAGC CGCGCTGTTA GCGTCCACCG TCTTTTTTTT CGTTGAACGG GATAGAGTCT

```

301 CAGCTAAGTG GAAGACATCG TTGACCGTCT CAGGCTTGGT TACCGGCATT GCCTTCTGGC
361 ATTATATGTA CATGCGTGGT GTCTGGATTG AAACGGGTGA CAGCCCGACG GTGTTCCGTT
421 ATATCGACTG GCTTTTAACC GTTCCCCTTC TGATTTGTGA GTTTTATTTA ATATTGGCGG
481 CAGCAACGAA TGTGGCCGGT TCACTGTTCA AGAAGCTTCT TGTAGGAAGT TTAGTTATGT
541 TGGTTTTTCG CTACATGGGA GAGGCAGGGA TAATGGCGGC CTGGCCGGCG TTCATAATTG
601 GTTGCTTGGC TTGGGTGTAC ATGATCTACG AGCTGTGGGC AGGAGAAGGC AAGTCTGCGT
661 GCAACACAGC ATCGCCAGCA GTTCAATCCG CATATAATAC GATGATGTAT ATAATTATCT
721 TTGGTTGGGC AATTTACCGG GTCGGATACT TCACCGGCTA TCTTATGGGC GACGGGGGCT
781 CTGCCTTGAA CTTGAATCTT ATATATAACC TGGCCGATTT CGTGAACAAG ATTTTGTGTTG
841 GACTTATAAT ATGGAACGTA GCCGTGAAAG AGTCATCGAA CGCATAATCT AGAGGCATCA
901 AATAAAACGA AAGGCTCAGT CGAAAGACTG GGCCTTTCGT TTTATCTGTT GTTTGTGCGT
961 GAACGCTCTC CTGAGTAGGA CAAATCCGCC GCCCTAGACC TAGGCGTTTCG GCTGCGGCGA
1021 GCGGTATCAG CTCACTCAAA GGCGGTAATA CGGTTATCCA CAGAATCAGG GGATAACGCA
1081 GGAAAGAACA TGTGAGCAAA AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG
1141 CTGGCGTTTT TCCATAGGCT CCGCCCCCT GACGAGCATC AAAAAATCG ACGCTCAAGT
1201 CAGAGGTGGC GAAACCCGAC AGGACTATAA AGATACCAGG CGTTTCCCCC TGGAAGCTCC
1261 CTCGTGCGCT CTCCTGTTCC GACCCTGCCG CTTACCGGAT ACCTGTCCGC CTTTCTCCCT
1321 TCGGGAAGCG TGGCGCTTTC TCAATGCTCA CGCTGTAGGT ATCTCAGTTC GGTGTAGGTC
1381 GTTCGCTCCA AGCTGGGCTG TGTGCACGAA CCCCCGTTC AGCCCGACCG CTGCGCCTTA
1441 TCCGGTAACT ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA
1501 GCCACTGGTA ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG
1561 TGGTGGCCTA ACTACGGCTA CACTAGAAGG ACAGTATTTG GTATCTGCGC TCTGCTGAAG
1621 CCAGTTACCT TCGGAAAAAG AGTTGGTAGC TCTTGATCCG GCAAACAAAC CACCGCTGGT
1681 AGCGGTGGTT TTTTGTGTTG CAAGCAGCAG ATTACGCGCA GAAAAAAGG ATCTCAAGAA
1741 GATCCTTTGA TCTTTTCTAC GGGGTCTGAC GCTCAGTGGA ACGAAAACTC ACGTTAAGGG
1801 ATTTTGGTCA TGA CTAGTGC TTGGATTCTC ACCAATAAAA AACGCCGGC GGCAACCGAG

1861 CGTTCTGAAC AAATCCAGAT GGAGTTCTGA GGTCACTACT GGATCTATCA ACAGGAGTCC
1921 AAGCGAGCTC TCACTGCCCCG CTTTCCAGTC GGGAAACCTG TCGTGCCAGC TGCATTAATG
1981 AATCGGCCAA CGCGCGGGGA GAGGCGGTTT GCGTATTGGG CGCCAGGGTG GTTTTTCTTT
2041 TCACCAGTGA GACGGGCAAC AGCTGATTGC CCTTCACCGC CTGGCCCTGA GAGAGTTGCA
2101 GCAAGCGGTC CACGCTGGTT TGCCCCAGCA GGCGAAAATC CTGTTTGATG GTGGTTAACG
2161 GCGGGATATA ACATGAGCTG TCTTCGGTAT CGTCGTATCC CACTACCGAG ATATCCGCAC
2221 CAACGCGCAG CCCGGACTCG GTAATGGCGC GCATTGCGCC CAGCGCCATC TGATCGTTGG
2281 CAACCAGCAT CGCAGTGGGA ACGATGCCCT CATTGAGCAT TTGCATGGTT TGTGAAAAAC
2341 CGGACATGGC ACTCCAGTCG CCTTCCCGTT CCGCTATCGG CTGAATTTGA TTGCGAGTGA
2401 GATATTTATG CCAGCCAGCC AGACGCAGAC GCGCCGAGAC AGAACTTAAT GGGCCCGCTA
2461 ACAGCGCGAT TTGCTGGTGA CCCAATGCGA CCAGATGCTC CACGCCCAGT CGCGTACCGT
2521 CTTTCATGGGA GAAAATAATA CTGTTGATGG GTGTCTGGTC AGAGACATCA AGAAATAACG
2581 CCGGAACATT AGTGCAGGCA GCTTCCACAG CAATGGCATC CTGGTCATCC AGCGGATAGT
2641 TAATGATCAG CCCACTGACG CGTTGCGCGA GAAGATTGTG CACCGCCGCT TTACAGGCTT
2701 CGACGCCGCT TCGTTCTACC ATCGACACCA CCACGCTGGC ACCCAGTTGA TCGGCGCGAG
2761 ATTTAATCGC CGCGACAATT TGCGACGGCG CGTGCAGGGC CAGACTGGAG GTGGCAACGC
2821 CAATCAGCAA CGACTGTTTG CCCGCCAGTT GTTGTGCCAC GCGGTTGGGA ATGTAATTCA
2881 GCTCCGCCAT CGCCGCTTCC ACTTTTTCCC GCGTTTTCGC AGAAACGTGG CTGGCCTGGT
2941 TCACCACGCG GGAAACGGTC TGATAAGAGA CACCGGCATA CTCTGCGACA TCGTATAACG
3001 TTAATGTTTT CATGGTATAT CTCCTTCGAG CTCGTAAACT TGGTCTGACA GTTACCAATG
3061 CTTAATCAGT GAGGCACCTA TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG
3121 ACTCCCCGTC GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC
3181 AATGATACCG CGAGACCCAC GCTCACCAGC TCCAGATTTA TCAGCAATAA ACCAGCCAGC
3241 CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC GCCTCCATCC AGTCTATTAA
3301 TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC
3361 CATTGCTACA GGCATCGTGG TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG

```

3421 TTCCAACGA TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC
3481 CTTGCGTCCT CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC TCATGGTTAT
3541 GGCAGCACTG CATAATTCTC TTA CTGTCAT GCCATCCGTA AGATGCTTTT CTGTGACTGG
3601 TGAGTACTCA ACCAAGTCAT TCTGAGAATA GTGTATGCGG CGACCGAGTT GCTCTTGCCC
3661 GCGTCAATA CGGGATAATA CCGCGCCACA TAGCAGAACT TAAAAGTGC TCATCATTGG
3721 AAAACGTTCT TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT
3781 GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA GCGTTTCTGG
3841 GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA ATAAGGGCGA CACGAAAATG
3901 TTGAATACTC ATACTCTTCC TTTTCAATA TTATTGAAGC ATTTATCAGG GTTATTGTCT
3961 CATGAGCGGA TACATATTTG AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC
4021 ATTTCCCGA AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAACCTA
4081 TAAAAAT

```

A.2.3 An Illustration of the Plasmid Structure

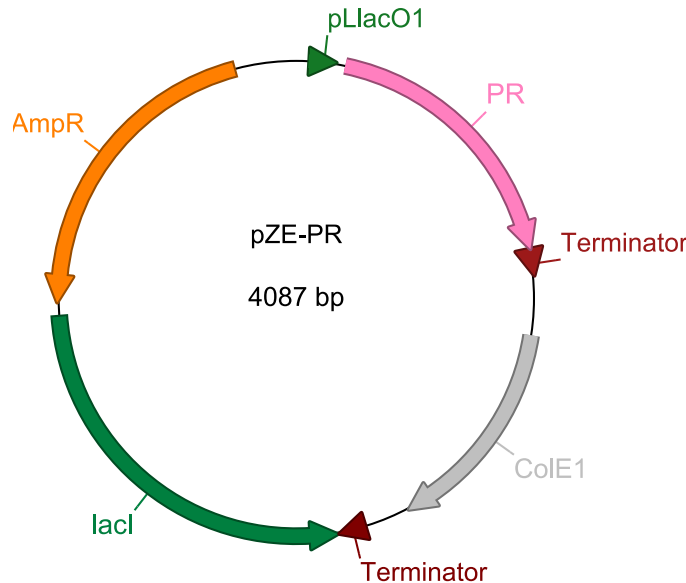


Figure A.1: An illustration of pZE-PR plasmid.

Appendix B

Image Analysis Code

B.1 Code Vectorization

B.1.1 Vectorized Code for Spatial Correlation Function

```
1 def corrS(X, Y, U, V):
2     row, col = X.shape
3     vsqrt = (U ** 2 + V ** 2) ** 0.5
4     U = U - U.mean()
5     V = V - V.mean()
6     Ax = U / vsqrt
7     Ay = V / vsqrt
8     CA = np.ones(X.shape)
9     CV = np.ones(X.shape)
10    for xin in range(0, col):
11        for yin in range(0, row):
12            if xin != 0 or yin != 0:
13                CA[yin, xin] = (Ax[0:row-yin, 0:col-xin] * Ax[yin:row, xin:col] + Ay[0:row-yin, 0:col-xin] * Ay[yin:row, xin:col])
14                CV[yin, xin] = (U[0:row-yin, 0:col-xin] * U[yin:row, xin:col] + V[0:row-yin, 0:col-xin] * V[yin:row, xin:col])
15    return CA, CV
```

B.1.2 Non-vectorized Code for Spatial Correlation Function

```
1 def corrS(X, Y, U, V):
2     row, col = X.shape
3     vsq = 0
4     CA = np.zeros((row, col))
5     CV = np.zeros((row, col))
6     for i in range(0, row):
7         for j in range(0, col):
8             vsq += U[i, j]**2 + V[i, j]**2
9     for xin in range(0, col):
10        for yin in range(0, row):
11            count = 0
12            CA_t = 0
13            CV_t = 0
14            for i in range(0, col-xin):
15                for j in range(0, row-yin):
16                    ua = U[j, i]
17                    va = V[j, i]
18                    ub = U[j+yin, i+xin]
19                    vb = V[j+yin, i+xin]
20                    CA_t += (ua*ub+va*vb)/((ua**2+va**2)*(ub**2+vb**2))**.5
21                    CV_t += ua*ub + va*vb
22                    count += 1
23            CA[yin, xin] = CA_t / count
24            CV[yin, xin] = CV_t / vsq
25    return CA, CV
```

B.1.3 Performance Comparison

We notice that the vectorized code has two less nested `for` loops compared to the non-vectorized code. As a result, the vectorized one runs much faster for the same task. To quantify this performance difference, we perform the spatial correlation function calculation using both code on the same velocity field, shown in Fig. B.1a. The times

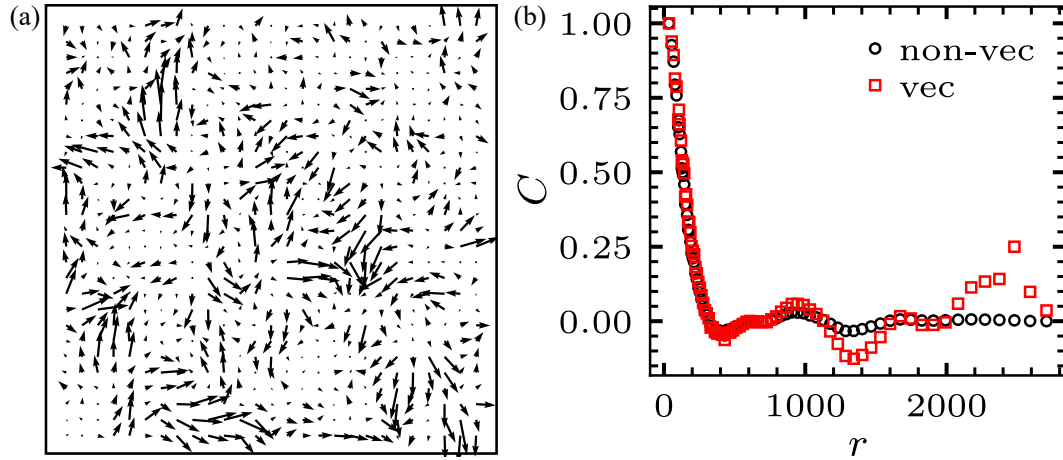


Figure B.1: **Compare the performance of vectorized and non-vectorized code.** (a) Sample velocity field. (b) Velocity correlation functions obtained from the vectorized and non-vectorized code.

taken for the two functions are:

- Vectorized code: 0.84 s
- Non-vectorized code: 52.06 s

The result is shown in Fig. B.1b. Although in the large r regime, two methods show discrepancies, in the meaningful small r regime, two methods give exactly the same results.

B.2 Energy Spectrum Calculation

The following code is used for calculating the energy spectra in Chap. ??.

```

1 def compute_energy_density(pivData, d=25*0.33, MPP=0.33):
2     """
3     Compute kinetic energy density in k space from piv data. The unit of the return val.
4     where [velocity] is the unit of pivData, and [length] is the unit of sample_spacing

```

```

5      Note, the default value of sampling_spacing does not have any significance. It is j
6      and should be set with caution when a different magnification and PIV are used.
7
8      Args:
9      pivData -- piv data
10     d -- sample spacing
11     MPP -- microns per pixel
12
13     Returns:
14     E -- kinetic energy field in k space
15     """
16
17     row = len(pivData.y.drop_duplicates())
18     col = len(pivData.x.drop_duplicates())
19     U = np.array(pivData.u).reshape((row, col)) * MPP
20     V = np.array(pivData.v).reshape((row, col)) * MPP
21
22     u_fft = np.fft.fft2(U) * d * d
23     v_fft = np.fft.fft2(V) * d * d
24
25     E = (u_fft * u_fft.conjugate() + v_fft * v_fft.conjugate()) / 2
26
27     return E
28
29 def compute_wavenumber_field(shape, d):
30     """
31     Compute the wave number field Kx and Ky, and magnitude field k.
32     Note that this function works for even higher dimensional shape.
33
34     Args:
35     shape -- shape of the velocity field and velocity fft field, tuple
36     d -- sample spacing. This is the distance between adjacent samples, for example, ve
37     The resulting frequency space has the unit which is inverse of the unit of d. T
38
39     Returns:
40     k -- wavenumber magnitude field
41     K -- wavenumber fields in given dimensions
42     """

```

```

43
44     for num, length in enumerate(shape):
45         kx = np.fft.fftfreq(length, d=d)
46         if num == 0:
47             k = (kx,)
48         else:
49             k += (kx,)
50
51     K = np.meshgrid(*k, indexing='ij')
52
53     for num, k1 in enumerate(K):
54         if num == 0:
55             ksq = k1 ** 2
56         else:
57             ksq += k1 ** 2
58
59     k_mag = ksq ** 0.5 * 2 * np.pi
60
61     return k_mag, K
62
63 def energy_spectrum(pivData, d=25*0.33):
64     """
65     Compute energy spectrum (E vs k) from pivData.
66
67     Args:
68     pivData -- piv data
69     d -- sample spacing. This is the distance between adjacent samples, for example, ve
70         The resulting frequency space has the unit which is inverse of the unit of d. T
71
72     Returns:
73     es -- energy spectrum, DataFrame (k, E)
74     """
75
76     row = len(pivData.y.drop_duplicates())
77     col = len(pivData.x.drop_duplicates())
78
79     E = compute_energy_density(pivData, d) / (row * d * col * d)
80     k, K = compute_wavenumber_field(E.shape, d)

```

```

81
82     ind = np.argsort(k.flatten())
83     k_plot = k.flatten()[ind]
84     E_plot = E.real.flatten()[ind]
85
86     es = pd.DataFrame(data={'k': k_plot, 'E': E_plot})
87
88     return es

```

B.3 Cross-Correlation Based Particle Detecting Method

This is designed for tracking colloidal chains. We show here the basic working principle and the code here. The advantage of this method is that it does not require the feature to be very bright or dark, like traditional particle detecting methods. As long as the features share similar characteristics, i.e. spatial patterns, this method can work well.

B.3.1 Working principle

From raw images, we crop out a single feature which we are looking for as a target mask, as shown in Fig. B.2a inset. Then, we do a 2D correlation between the target mask and an image (Fig. B.2a). The resulting correlation map is shown in Fig. B.2b, where bright pixels stand for high correlation and dark pixels stand for low correlation. The last step is to look for the pixel intensity peaks in the correlation map, as in traditional particle detecting methods.

B.3.2 Code

```

1  import numpy as np
2  from scipy.signal import medfilt2d, convolve2d, fftconvolve
3  from math import exp
4

```

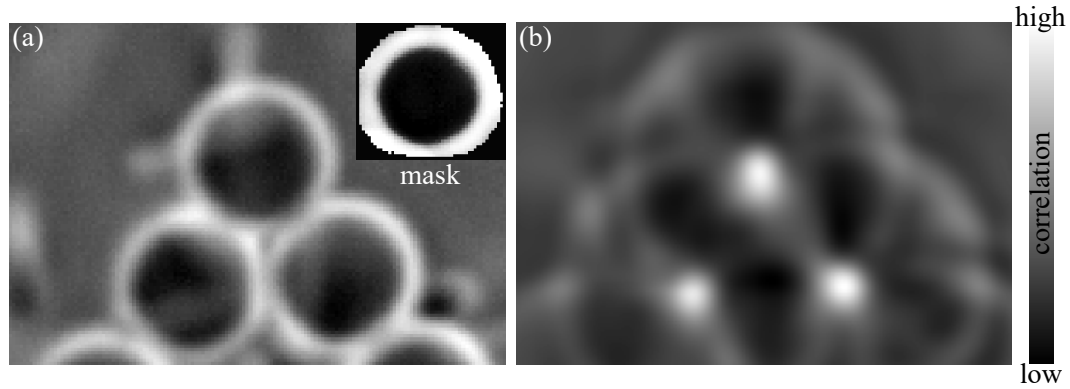


Figure B.2: **Cross-correlation based particle detecting method.** (a) A sample image of colloidal particles. Inset: a mask produced by cropping the raw image and binarize. (b) The cross-correlation map.

```

5 def normxcorr2(template, image, mode="full"):
6     if np.ndim(template) > np.ndim(image) or \
7         len([i for i in range(np.ndim(template)) if template.shape[i] > image.shape
8             print("normxcorr2: TEMPLATE larger than IMG. Arguments may be swapped.")
9     template = template - np.mean(template)
10    image = image - np.mean(image)
11    a1 = np.ones(template.shape)
12    ar = np.flipud(np.fliplr(template))
13    out = fftconvolve(image, ar.conj(), mode=mode)
14    image = fftconvolve(np.square(image), a1, mode=mode) - \
15        np.square(fftconvolve(image, a1, mode=mode)) / (np.prod(template.shape))
16    image[np.where(image < 0)] = 0
17    template = np.sum(np.square(template))
18    out = out / np.sqrt(image * template)
19    out[np.where(np.logical_not(np.isfinite(out)))] = 0
20    return -out
21
22 def matlab_style_gauss2D(shape=(3,3),sigma=0.5):
23     m,n = [(ss-1.)/2. for ss in shape]
24     y,x = np.ogrid[-m:m+1,-n:n+1]
25     h = np.exp( -(x*x + y*y) / (2.*sigma*sigma) )
26     h[ h < np.finfo(h.dtype).eps*h.max() ] = 0

```

```

27     sumh = h.sum()
28     if sumh != 0:
29         h /= sumh
30     return h
31
32 def FastPeakFind(data):
33     if str(data.dtype) != 'float32':
34         data = data.astype('float32')
35     mf = medfilt2d(data, kernel_size=3)
36     mf = mf.astype('float32')
37     thres = max(min(np.amax(mf,axis=0)), min(np.amax(mf,axis=1)))
38     filt = matlab_style_gauss2D()
39     conv = convolve2d(mf, filt, mode='same')
40     w_idx = conv > thres
41     bw = conv.copy()
42     bw[w_idx] = 1
43     bw[~w_idx] = 0
44     thresholded = np.multiply(bw, conv)
45     edg = 3
46     shape = data.shape
47     idx = np.nonzero(thresholded[edg-1: shape[0]-edg-1, edg-1: shape[1]-edg-1])
48     idx = np.transpose(idx)
49     cent = []
50     for xy in idx:
51         x = xy[0]
52         y = xy[1]
53         if thresholded[x, y] >= thresholded[x-1, y-1] and \
54             thresholded[x, y] > thresholded[x-1, y] and \
55             thresholded[x, y] >= thresholded[x-1, y+1] and \
56             thresholded[x, y] > thresholded[x, y-1] and \
57             thresholded[x, y] > thresholded[x, y+1] and \
58             thresholded[x, y] >= thresholded[x+1, y-1] and \
59             thresholded[x, y] > thresholded[x+1, y] and \
60             thresholded[x, y] >= thresholded[x+1, y+1]:
61             cent.append(xy)
62     cent = np.asarray(cent).transpose()
63     return cent
64

```



```

65 def maxk(array, num_max):
66     array = np.asarray(array)
67     length = array.size
68     array = array.reshape((1, length))
69     idx = np.argsort(array)
70     idx2 = np.flip(idx)
71     return idx2[0, 0:num_max]
72
73 def gauss1(x,a,x0,sigma):
74     return a*exp(-(x-x0)**2/(2*sigma**2))
75
76 def track_spheres(img, mask, num_particles):
77     def gauss1(x,a,x0,sigma):
78         return a*exp(-(x-x0)**2/(2*sigma**2))
79     corr = normxcorr2(mask, img, mode='same')
80     cent = FastPeakFind(corr)
81     peaks = corr[cent[0], cent[1]]
82     ind = maxk(peaks, num_particles)
83     max_coor_tmp = cent[:, ind]
84     max_coor = max_coor_tmp.astype('float32')
85     pk_value = peaks[ind]
86     for num in range(0, num_particles):
87         x = max_coor_tmp[0, num]
88         y = max_coor_tmp[1, num]
89         fitx1 = np.asarray(range(x-7, x+8))
90         fity1 = np.asarray(corr[range(x-7, x+8), y])
91         popt, pcov = curve_fit(gauss1, fitx1, fity1, p0=[1, x, 3])
92         max_coor[0, num] = popt[1]
93         fitx2 = np.asarray(range(y-7, y+8))
94         fity2 = np.asarray(corr[x, range(y-7, y+8)])
95         popt,pcov = curve_fit(gauss1, fitx2, fity2, p0=[1, y, 3])
96         max_coor[1, num] = popt[1]
97     return max_coor, pk_value

```

B.4 Fourier Transform Based Orientation Analysis

B.5 Density Fluctuation Calculation