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Kinematics and Kinetics of Capacitated and Non-Capacitated Mouse Sperm

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Washington University in St. Louis
JAMES MCKELVEY SCHOOL OF ENGINEERING

Fall 2021 Independent Study

Independent Study Research: Kinematics and Kinetics of Capacitated and Non-Capacitated Mouse Sperm

Lab Instructor: Dr. Philip Bayly

Experiment Date: Fall 2021

Report Submission Date: Tuesday, December 21, 2021

I hereby certify that the lab report herein is my original academic work, completed in accordance with the McKelvey School of Engineering and Undergraduate Student academic integrity policies, and submitted to fulfill the requirements of this assignment:

Alicia Gupte

ABSTRACT: *The differences in kinetic measurements of force, work, power, and torque are quantitativly observed between non-capacitated and capacitated sperm. Isomap, a nonlinear dimensionality reduction technique, is used to get higher resolution imaging data of the sperm videos. This allowed accurate calculations of spatial and temporal derivatives to calculate the kinetics of the flagella. The results showed no statistical significance between the kinetic measurements of non-capacitated and capacitated mouse sperm.*

1 INTRODUCTION

Infertility affects approximately 8-12% of couples worldwide, 50% of which are caused by male infertility [1]. Male infertility is usually a result of deficiencies in semen; one of the most common deficiencies being poor sperm motility. Sperm motility describes the ability of the sperm to move through the female reproductive tract to reach and fertilize an egg. Sperm motility directly depends on the function of its flagellum; therefore, it is important to look at the mechanics of the flagellum.

1.1 Sperm Flagella Mechanics. The flagellum is a long appendage of the sperm cell that moves in a whiplike motion to propel the cell forward. Just as living cells are continuously exposed to forces from their environment and must develop specific mechanisms to accommodate these forces, sperm cells have developed a specific motion where their flagellum moves in an oscillation. Although it is uncertain how the cell modulates these oscillations, it has been observed that eukaryotic flagella exhibit a planar sinusoidal wave motion that propagates from the base to the tip of the cell [2]. The waveform can be estimated as Eq. 1 below,

$$y(s,t) = Asin(ks - wt - \phi) \tag{1}$$

where A is the amplitude [μm], k is the spatial frequency [rad/μm], w is the temporal frequency [rad/s], t is time [s], and is ϕ the phase shift [rad]. This wave equation gives the position of y as a function of space and time (s and t).

1.2 Sperm Flagella Kinetics. At each moment in time, a flagellum's direction of motion can be described by normal and tangential vectors, which can be rotated to reference a cartesian coordinate system. Figure 1 below shows a flagellum's free body diagram along with its normal and tangential components in relation to the cartesian coordinate system by an arbitrary angle θ [3].

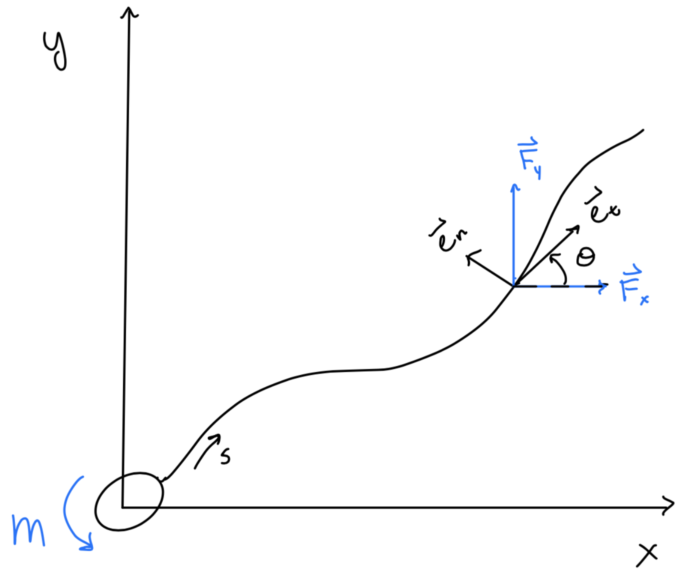

Figure 1   Free body diagram and normal and tangential components of flagellum

The cartesian coordinates of a flagellum can be found using Eq. 2 and Eq. 3.

$$x(s,t) = \int_0^s cos(\theta)(s,t)ds \tag{2}$$

$$y(s,t) = \int_0^s sin(\theta)(s,t)ds \tag{3}$$

where s is the length along the flagellum [μm], and t is time [s].

The forces in the normal and tangential directions can be found using resistive force theory [3]. As the sperm migrates through the female reproductive tract it experiences resistive forces due to the fluid's viscosity. Discovered by Gray and Hancock, resistive force theory neglects long-range hydrodynamic interactions and focuses on anisotropic local hydrodynamic friction between the sperm surface and the adjacent fluid [4]. Using resistive force theory, where frictional forces dominate inertial forces, the forces along the flagellum can be found using Eq. 4 and Eq.5.

$$F_n = -c_n * v_n \tag{4}$$

$$F_t = -c_t * v_t \tag{5}$$

where v_n and v_t are the velocity components in the normal and tangential direction, and c_n and c_t are the resistive friction coefficients in the normal and tangential directions. In this paper, the values of c_n and c_t are 3.4*10^{-3} and 1.7*10^{-3} [5]. These normal and tangential forces can be used to calculate the forces in the cartesian coordinate system as shown in Eq. 6 and Eq. 7.

$$F_x = F_n * -sin(\theta) + F_t * cos(\theta) \tag{6}$$

$$F_y = F_n * cos(\theta) + F_t * sin(\theta) \tag{7}$$

Using these forces, additional kinetic measurements such as the work, power, and torque exerted by the flagellum can be calculated as shown in Eq. 8, Eq. 9, and Eq. 10.

$$Power = F_n * v_n + F_t * v_t = F_x * v_x + F_y * v_y \tag{8}$$

$$Work = \int (Power)dt \tag{9}$$

$$Torque = F_y * X - F_x * Y \tag{10}$$

1.3 Reconstructing the waveform. In order to get accurate measurements for the kinematics of the flagellum motion, the motion has to be smooth as that of a sinusoidal wave. The imaging of flagellar motion is not always at high enough resolution to take accurate spatial and time derivatives so there is a need to sort the flagellar beat. Sorting the beat will make higher resolution images of the wave form. One way this can be done is by isometric mapping or isomap for short. Isomap is a nonlinear dimesnionality reduction method which preservers local structures [6]. Isomap works by taking a data set with high dimensionality and computing the data points geodesic distance to reduce it into a lower dimension. A common example used to explain isomap is the swiss roll shown in Fig.2.

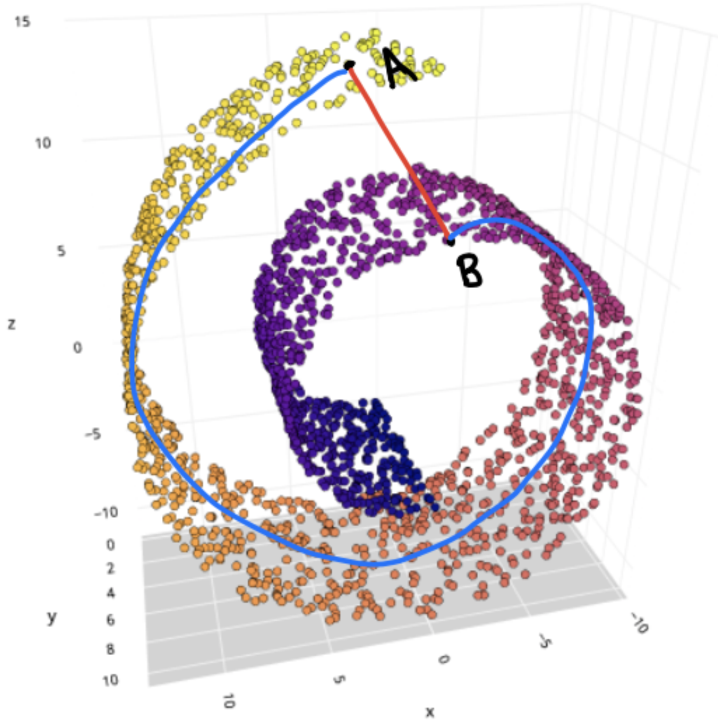

Figure 2 Swiss roll 3D data points [6]

The red line between arbitrary points A and B shows the euclidean distance, commonly used in linear reduction methods, while the blue line shows the geodesic distance. The geodesic distance can be found by specifying parameters of either neighbors, or epsilon. A numeric value for neighbors will connect data points to as many of its neighboring data points as specified by that value. Epsilon will connect data points based on whether its neighboring points fall within an imaginary ball of radius epsilon around that point.

In relation to flagella motion, since flagella are assumed to move in a periodic motion the expected isomap embedding should result in a circle because of the repeating pattern. The values obtained from isomap can be used to sort the flagellar beat such that similar motions are grouped together. This will result in a smoother reconstructed waveform which can be used to calculate time derivatives to get accurate results of kinetic measurements [3].

1.4 Capacitiation. The waveforms of mouse sperm with conditions A and B, non capacitated and capacitated sperm, are analyzed in this paper. Sperm are unable to fertilize an egg immediately after ejaculation and must go through a physiological maturation process known as capacitation [7].

As the sperm swims through the female reproductive tract, capacitation is triggered by the uterine wall secretions. During this process, the sperm head and flagellum both undergo physical changes. The membranes of the sperm head become more fluid while the flagellum becomes hyperactive. Hyperactivation allows the sperm to generate greater propulsive forces to be able to move through the viscous fluids of the fallopian tubes to fertilize the egg [1].

2 METHODS

2.1 Tracing and Isomap. The flagellar waveform of the mouse sperm was traced using autotrace_fun3.m, a MATLAB program created by Professor Louis Woodhams at Washington University in St. Louis. This program recorded qualitative measurements such as the frequency, period, wavelength, curvature, and amplitude as well as the θ values as a function of time and space. The euclidean distance between the θ values was calculated using MATLAB function L2_Distance.m created by Roland Bunschoten at University of Amsterdam. The euclidean distance was then used as an input argument in MATLAB program Isomap.m created by Josh Tenenbaum at Stanford University. The additional input arguments, k, neighbors, or E, epsilon, were selected by trial and error for each video depending on which gave the most clear isomap embedding result. Once the output from Isomap.m was obtained, the waveform was sorted.

2.2 Sorting and reconstructing the waveform. Isomap.m outputs a vector each for the x and y coordinates from the isomap transformation. These values were used to calculate the new θ values using trigonometry. Using MATLAB's sort function, the new θ value indices were found. Using the indices, the polynomial coefficients of the waveform (Out.PP.pArray from autotrace_fun3.m) were sorted. A fast fourier transform (FFT) was taken of the sorted polynomial coefficients in order to get rid of higher harmonics. Next, the inverse FFT was taken to go back to the time domain. Finally, the waveform was reconstructed using the polynomial coefficients and space vector Out.PP.s2 from autotrace_fun3.m.

2.3 Kinetics. The derivatives of the reconstructed wave form with respect to the time step of the reconstructed beat (found from the beat frequency and number of frames) were taken in order to find the velocity components of the flagellum. These values were then used to find the forces, power, torque, and work produced by the flagellum using Eq. 6 through Eq. 10.

This procedure was repeated for each mouse sperm video for conditions A and B.

3 RESULTS

3.1 Autotrace Results. Autotrace_fun3.m provides the input data to plot the flagellum's trace with respect to time and space. Figure 3 shows the trace for video 3b.

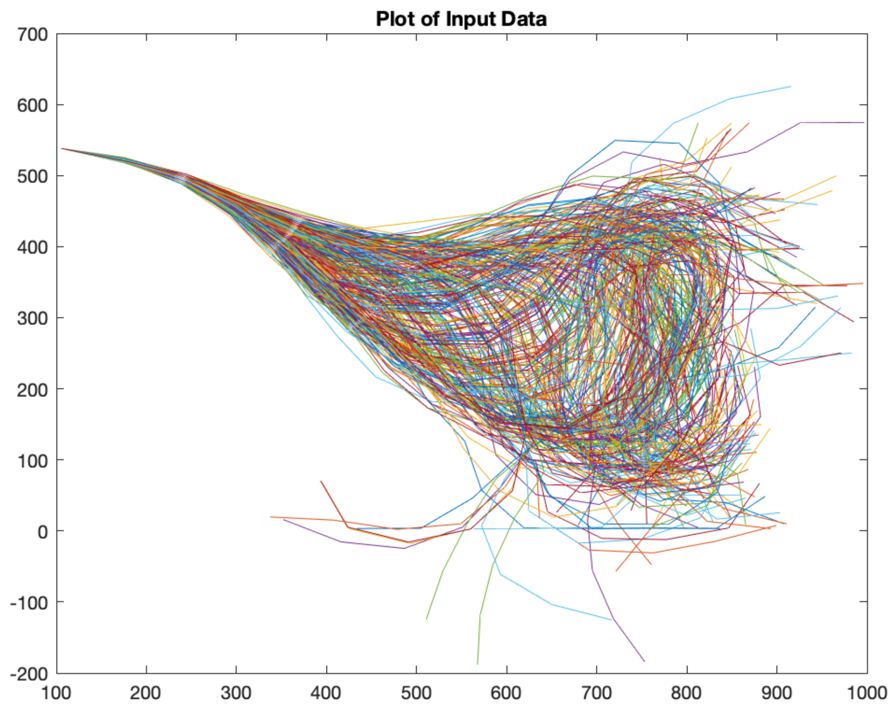

Figure 3 Video 3b trace

3.2 Isomap Results. The output from Isomap.m is a two dimensional isomap embedding shown in Fig 4. Figure 5 is the corresponding residual variance graph, which shows the error of the isomap transformation for each dimension.

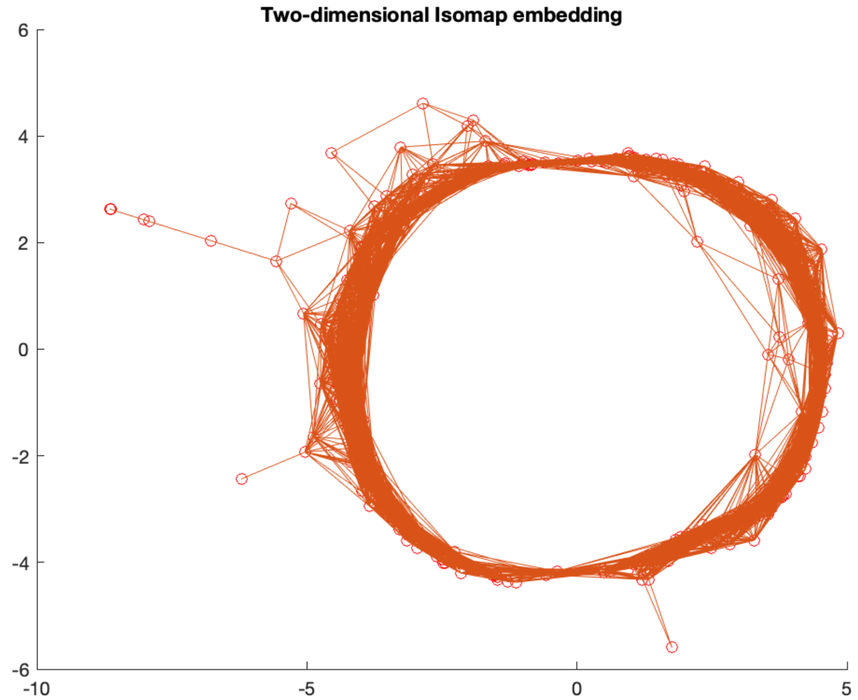

Figure 4 Video 3b isomap embedding

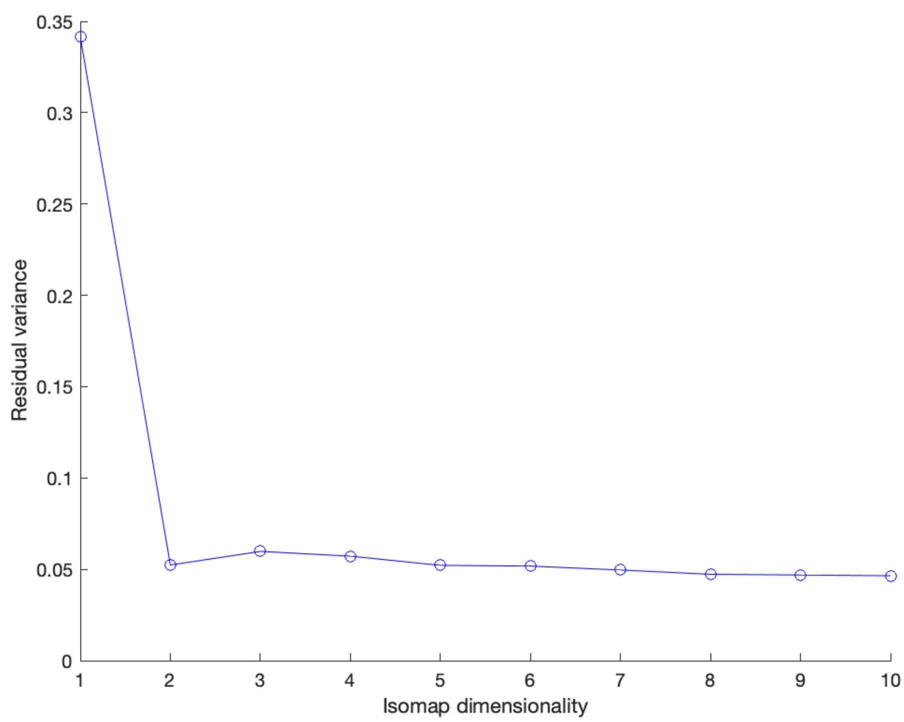

Figure 5 Video 3b residual variance for isomap embedding

3.3 Reconstructed Waveform. Figure 6 shows the process of reconstructing the waveform. The result is a smooth curve of the waveform.

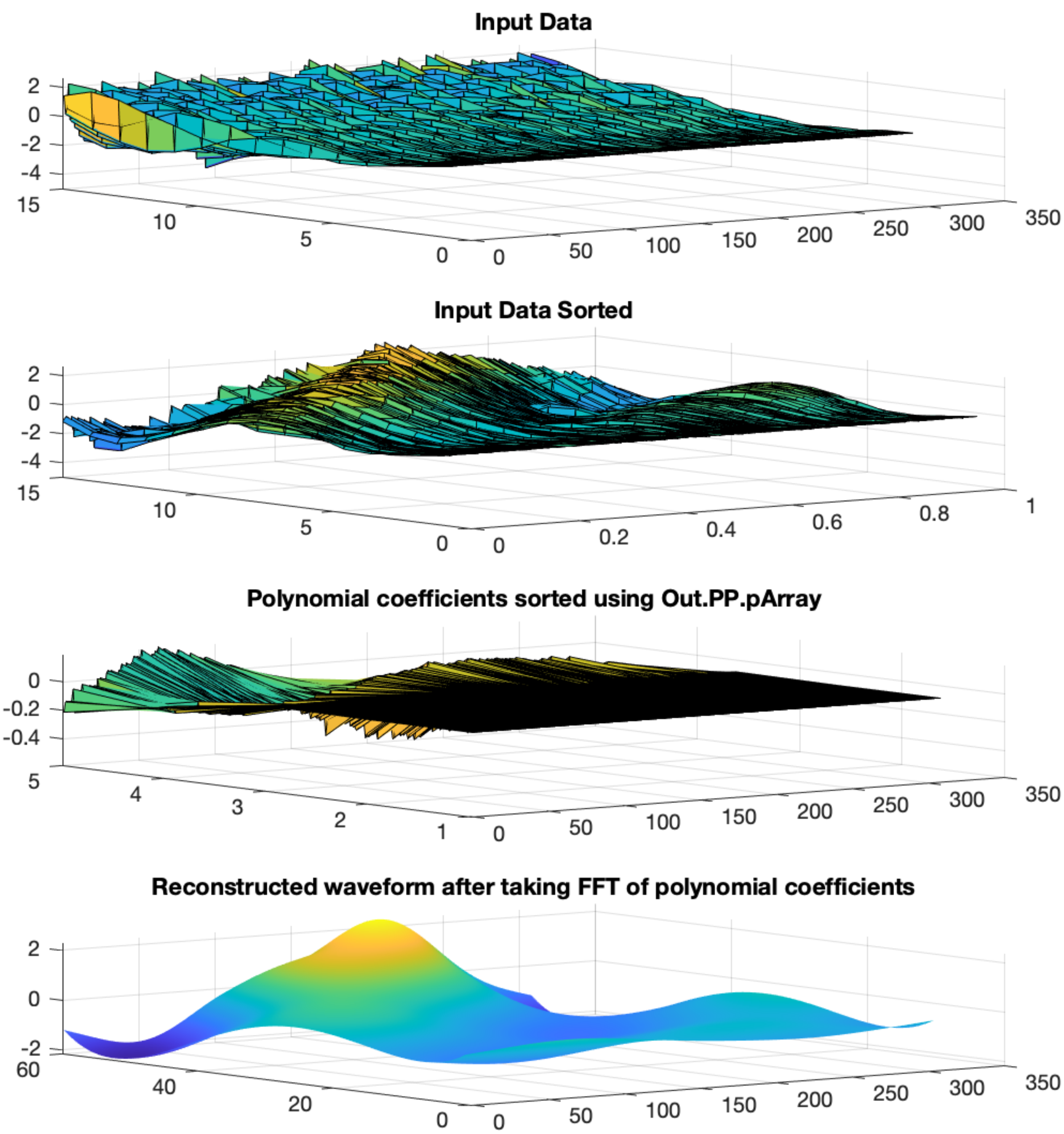

Figure 6 Video 3b smooth reconstructed waveform

3.4 Kinematics. The forces (x and y direction), work, power, and torque are plotted over the time of the flagellum's beat in Fig. 7, 8, 9, 10, and 11.

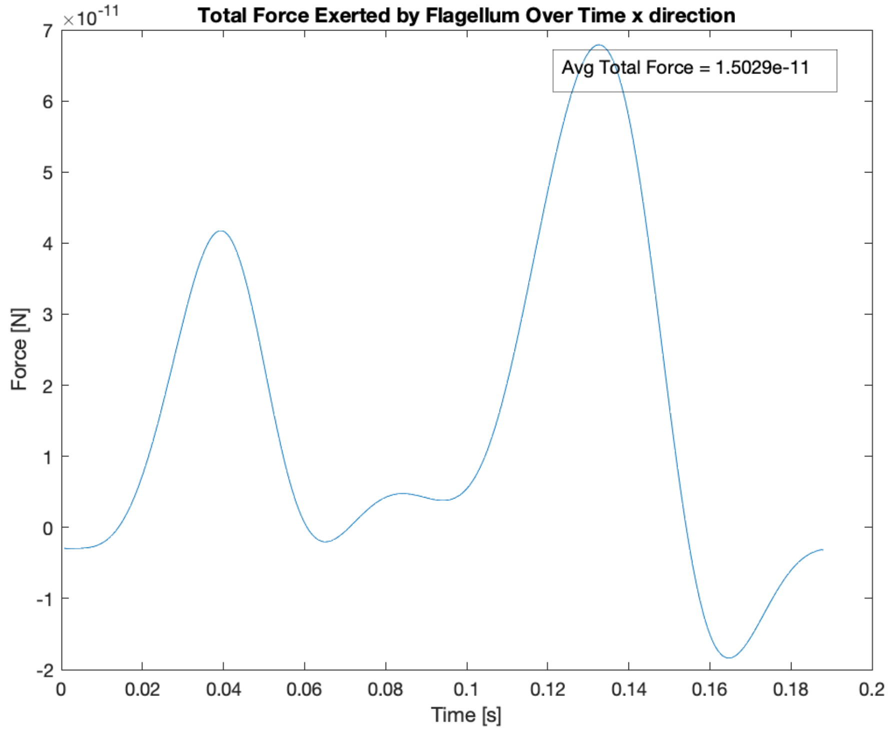

Figure 7 Video 3b force in the x-direction plotted as a function of time

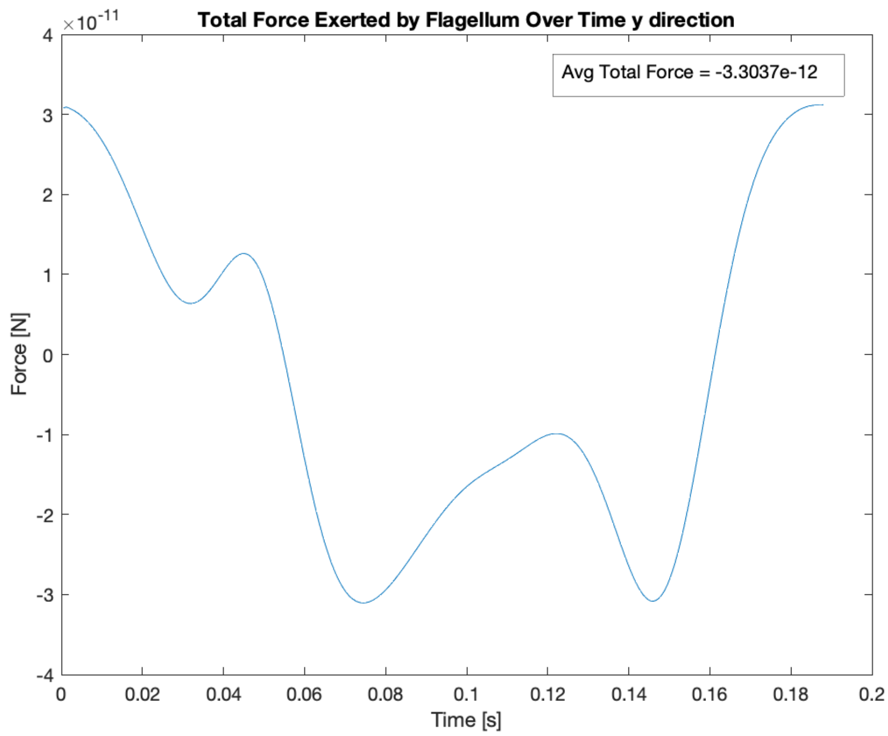

Figure 8 Video 3b force in the y-direction plotted as a function of time

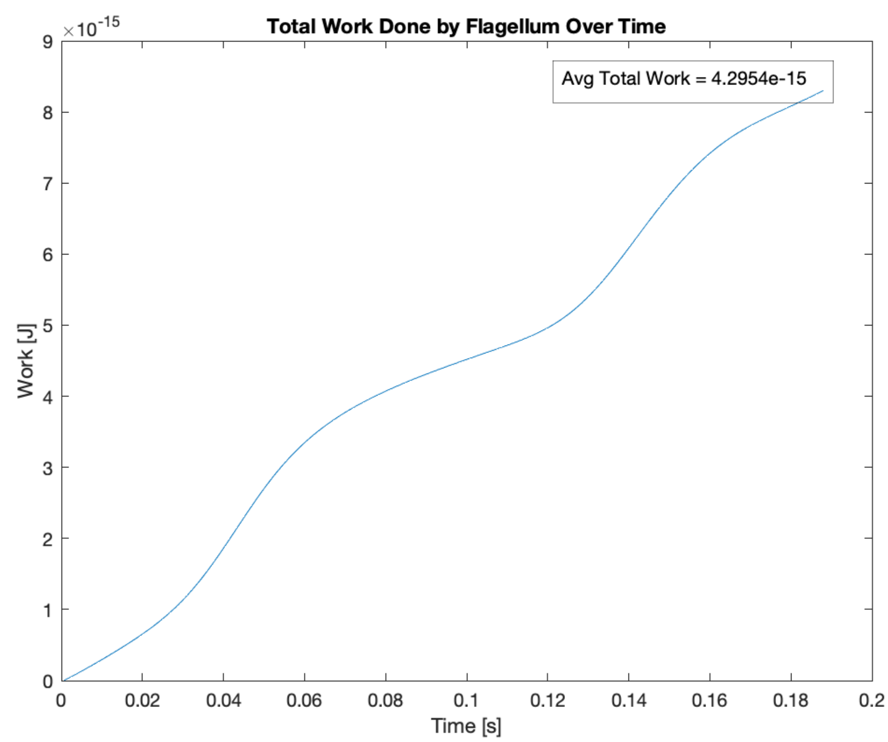

Figure 9 Video 3b work plotted as a function of time

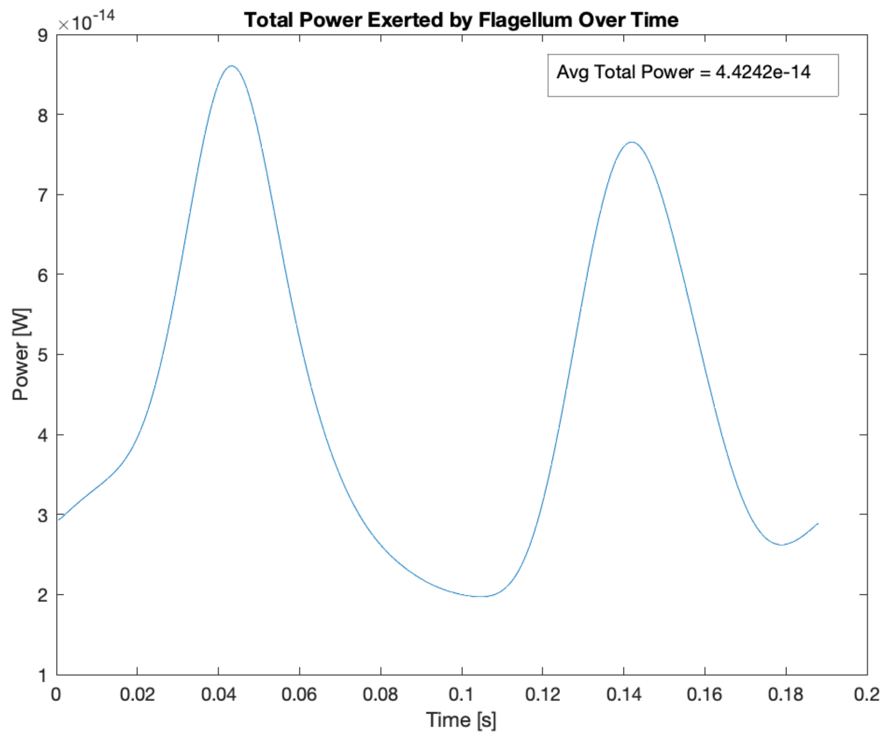

Figure 10 Video 3b power plotted as a function of time

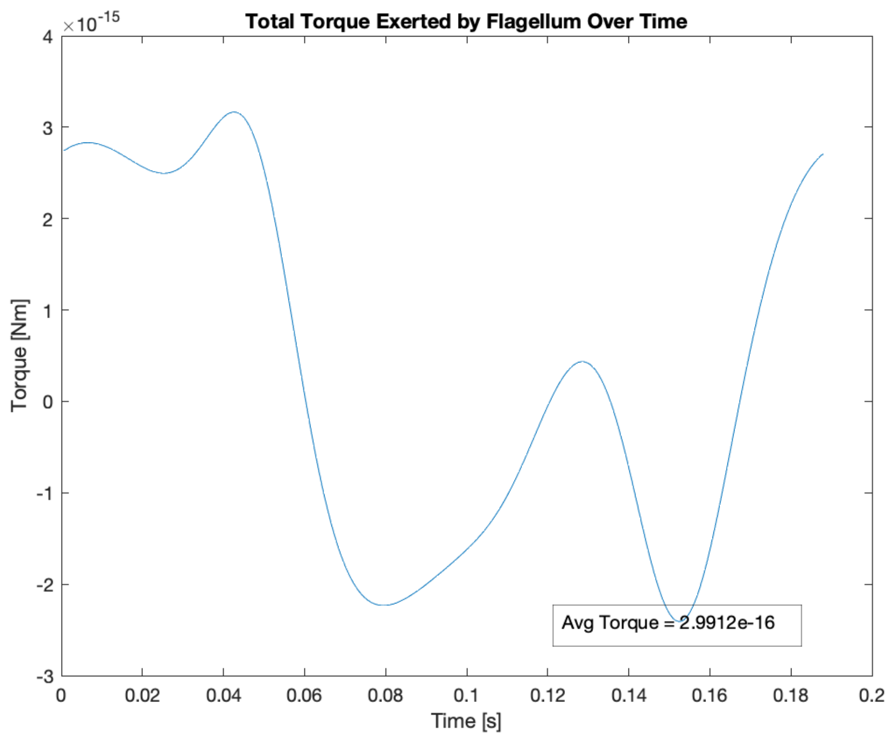

Figure 11 Video 3b torque plotted as a function of time

3.5 Comparing conditions A and B. The kinetic graphs were made for each video, shown in appendix, and a statistical analysis was done comparing the average work, average force magnitude, average power, and average torque between conditions A and B. The results are plotted in Fig. 12.

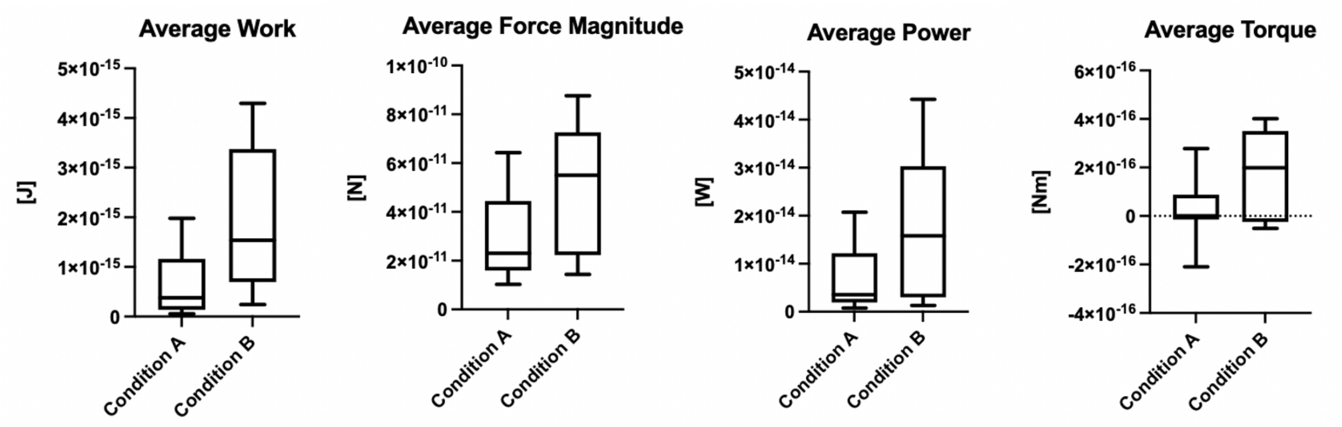

Figure 12 Box plots made for comparing conditions A, non-capacitated sperm, and condtion B, capacitated sperm. 8/9 videos worked using Isomap.m for condition A and 5/7 videos worked for condition B.

A two-tailed T-test assuming unequal variances was performed for each of the kinetic measurements and the resulting p values is shown in Table 1.

Table 1 Video 3a Isomap Output data and reconstructed waveform

Output Data	P-value	Statistically Significant? (P < 0.05)
Average Work	0.2185	No
Average Force Magnitude	0.2762	No
Average Power	0.1459	No
Average Torque	0.2808	No

DISCUSSION

The circular shape of the isomap embedding in Fig. 4 shows that the motion of the flagella is periodic. Figure 5 shows that the residual variance is is low, 0.05, for dimensions 2 and higher which means the isomap transformation is reliable. In Fig. 6 the input data produces a rough curve and becomes smoother as the data is sorted and even smoother after the FFT is taken. The result is a smooth manifold which gives more accurate time derivatives. Even though the videos taken are of a flagellum with a fixed head, the reconstructed waveform shows slight movement at the head of the flagellum which suggests that there is some rotation at the head. The force for both the x and y directions shown in Fig 7 and 8 are in the range of tens of pico-newtons. This is a similar range to other mammalian sperm compared in papers by Kathleen A. Shmidtz et al. [8], and Wesley W Hsiao et al. [9]. The work, power, and torque are also found to be in the expected range. The plots of Fig. 12 show that condition B does have a higher average work, force, power, and torque than condition A. However, the results show that there is no statistical significance between the two conditions. This is unexpected since capacitated sperm are thought to produce more force in order to be able to penetrate the egg.

CONCLUSION

The motion of flagella can be analyzed from tracing the flagellum, using isomap reduction techniques to reconstruct the wave form, and then taking time derivatives to compute kinetic measurements such as forces, work, power, and torque produced by the flagellum. The analysis on mouse sperm showed there was no statistical significance of the kinetic measurements between non-capacitated and capacitated sperm. This was not expected since capacitation cause the sperm to undergo hyperactivation which is believed to increase the flagellum's propulsive forces. The small sample size of videos traced and analyzed could be a reason for maintaining the null hypothesis that there is no statistical significanc between conditions A and B. Researching the capacitation effects on the motion of flagella are important in understanding sperm motility and its role in male fertility.

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A Appendix

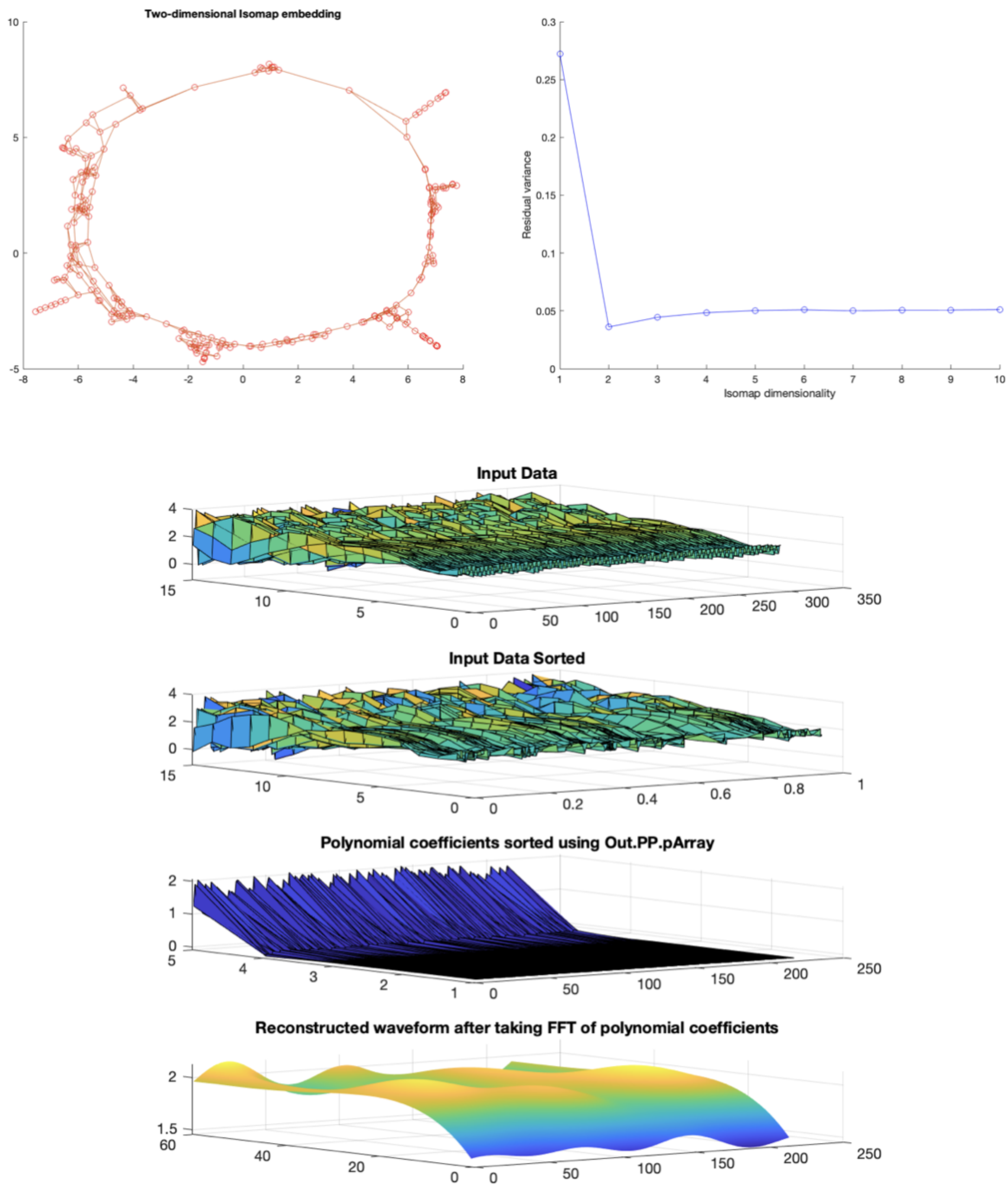

Figure A.1 Video 2a Isomap Output data and reconstructed waveform

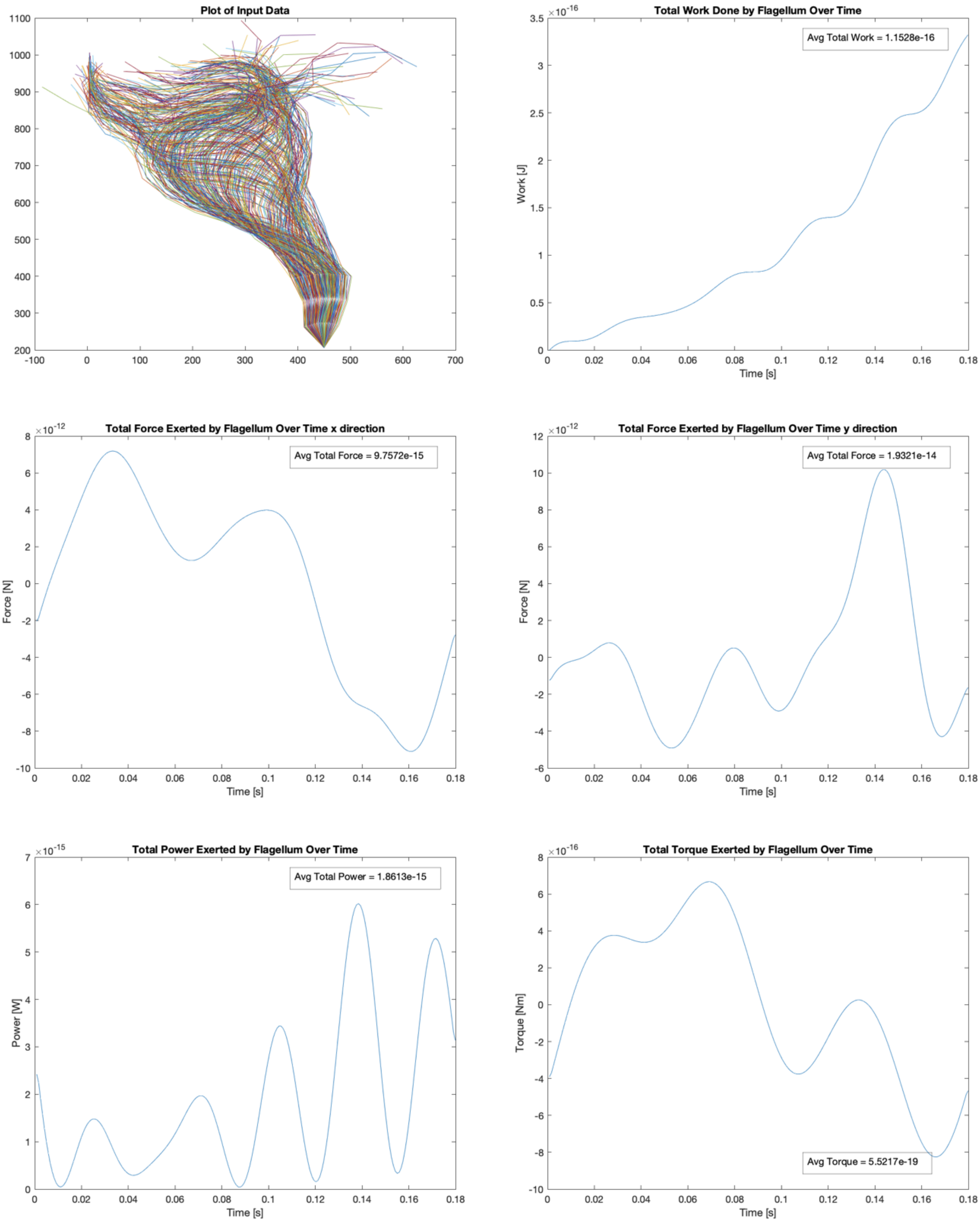

Figure A.2 Video 2a Out put trace and kinematic data graphed

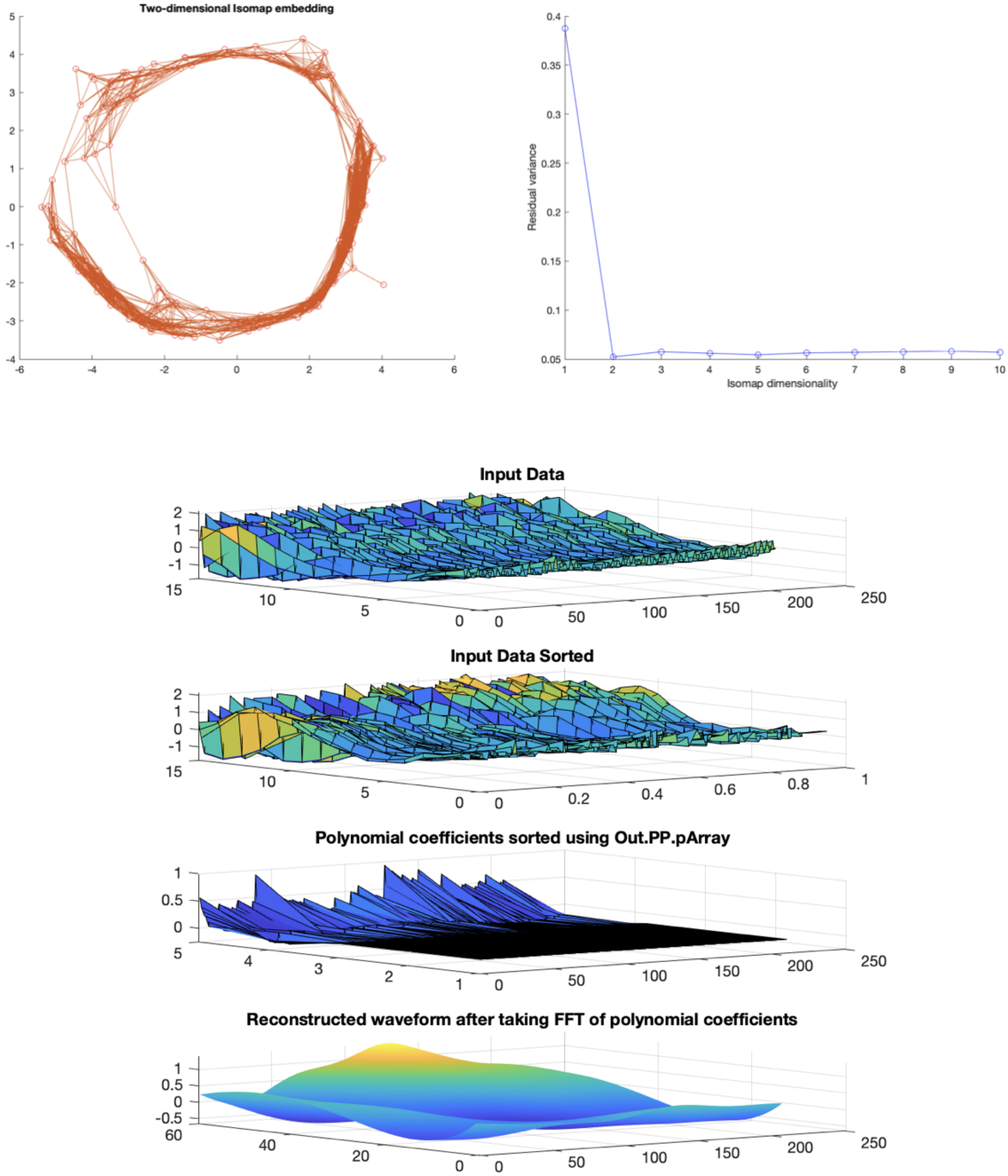

Figure A.3 Video 3a Isomap Output data and reconstructed waveform

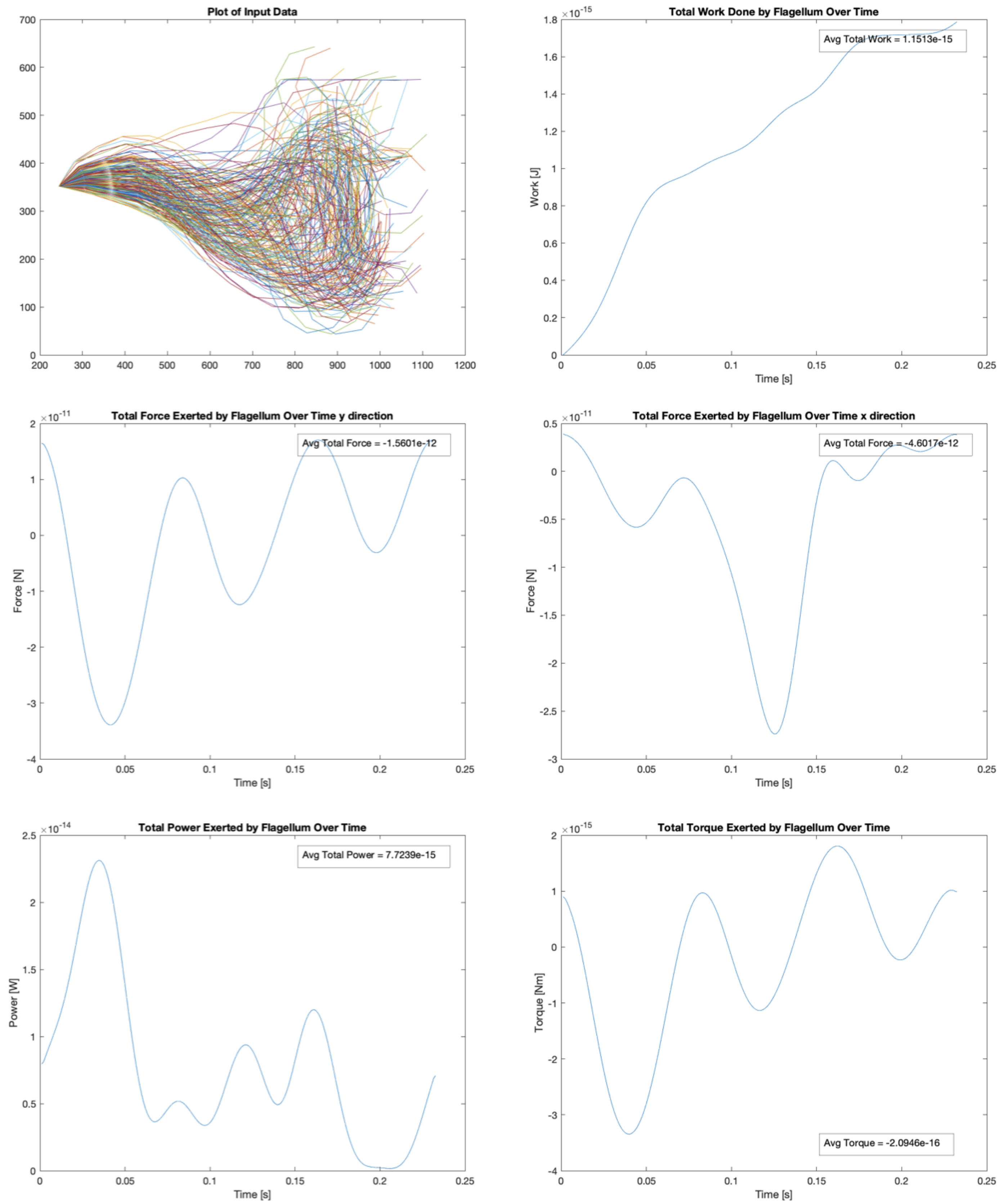

Figure A.4 Sample schematic diagram of test setup.

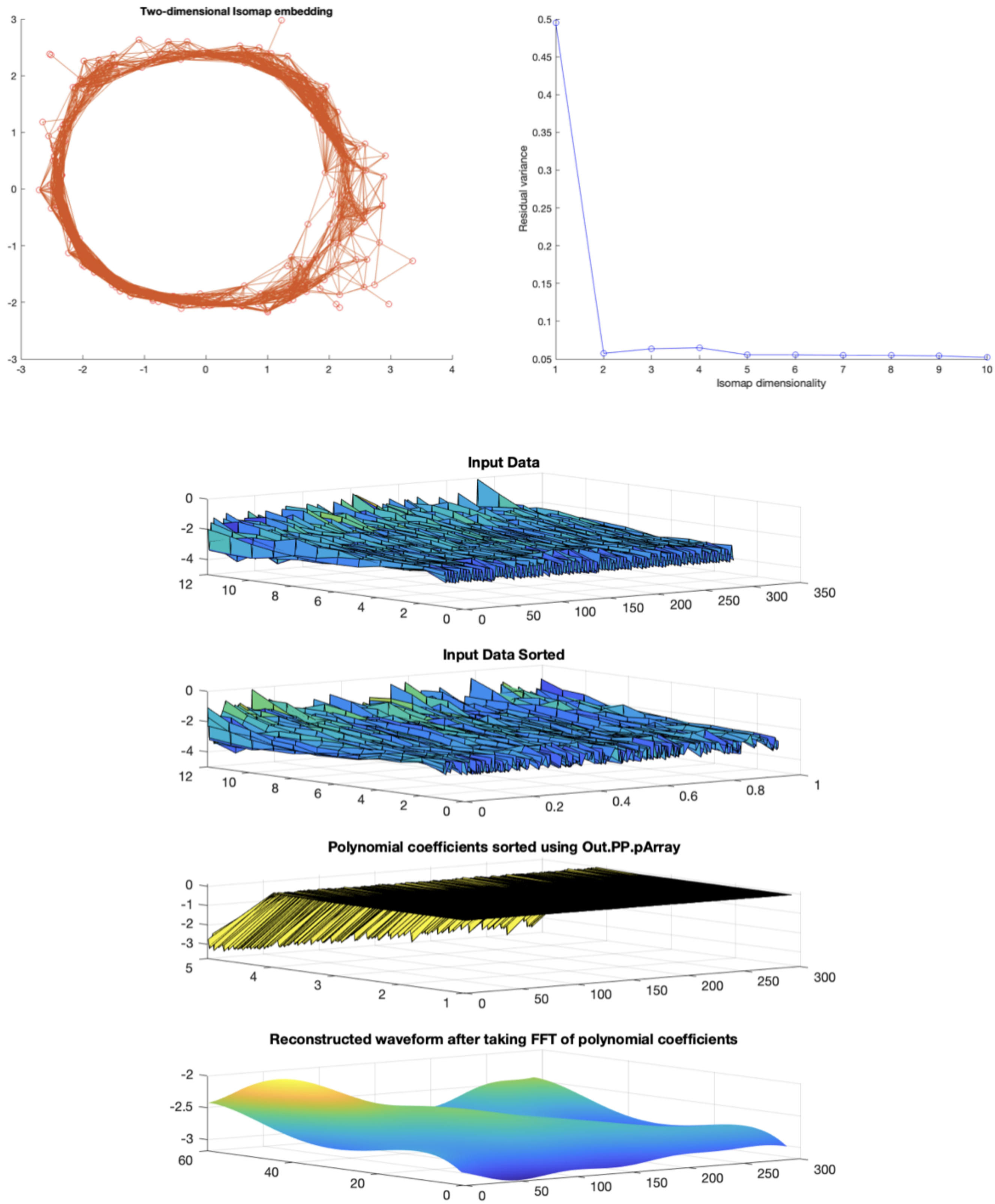

Figure A.5 Video 4a Isomap Output data and reconstructed waveform

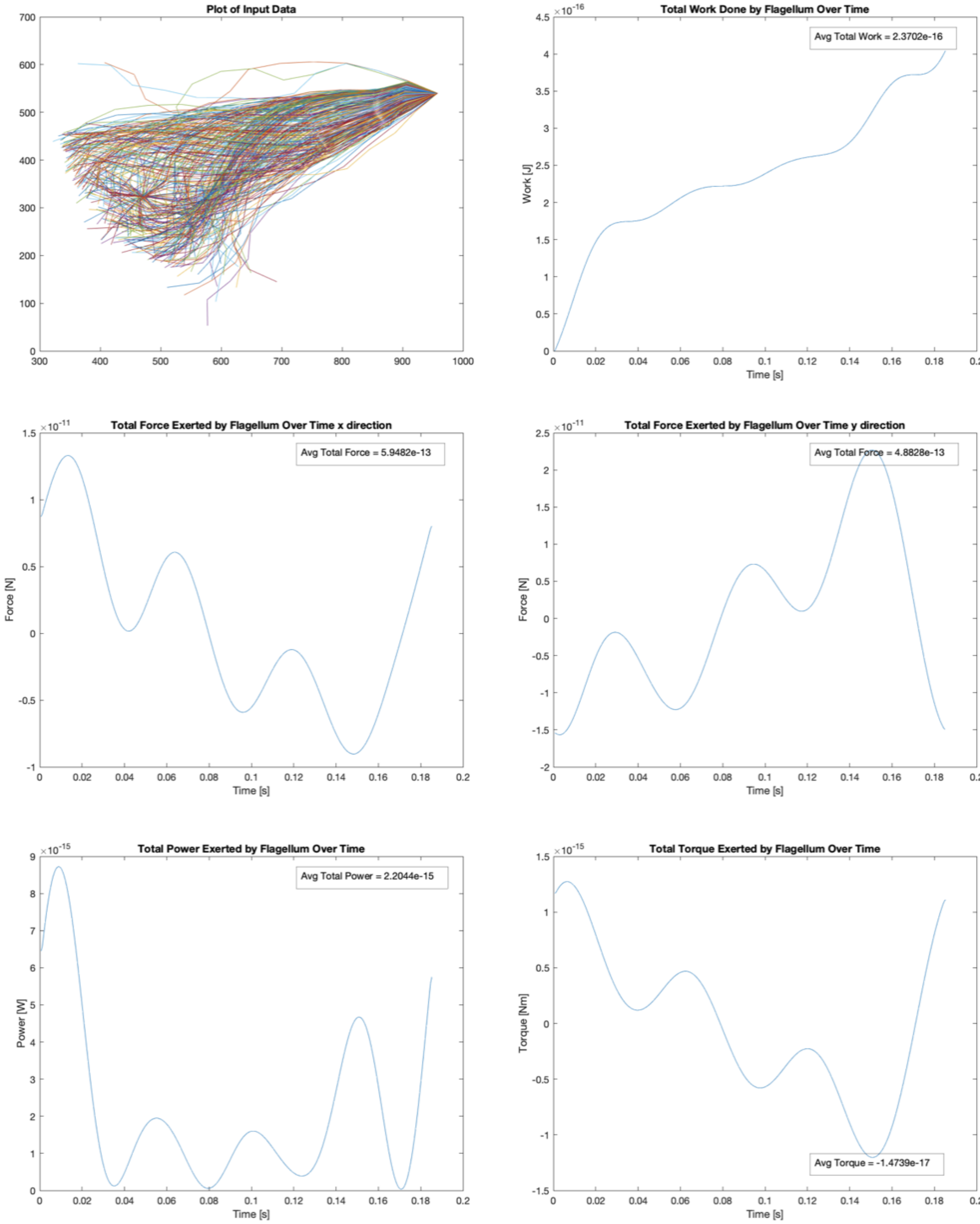

Figure A.6 Sample schematic diagram of test setup.

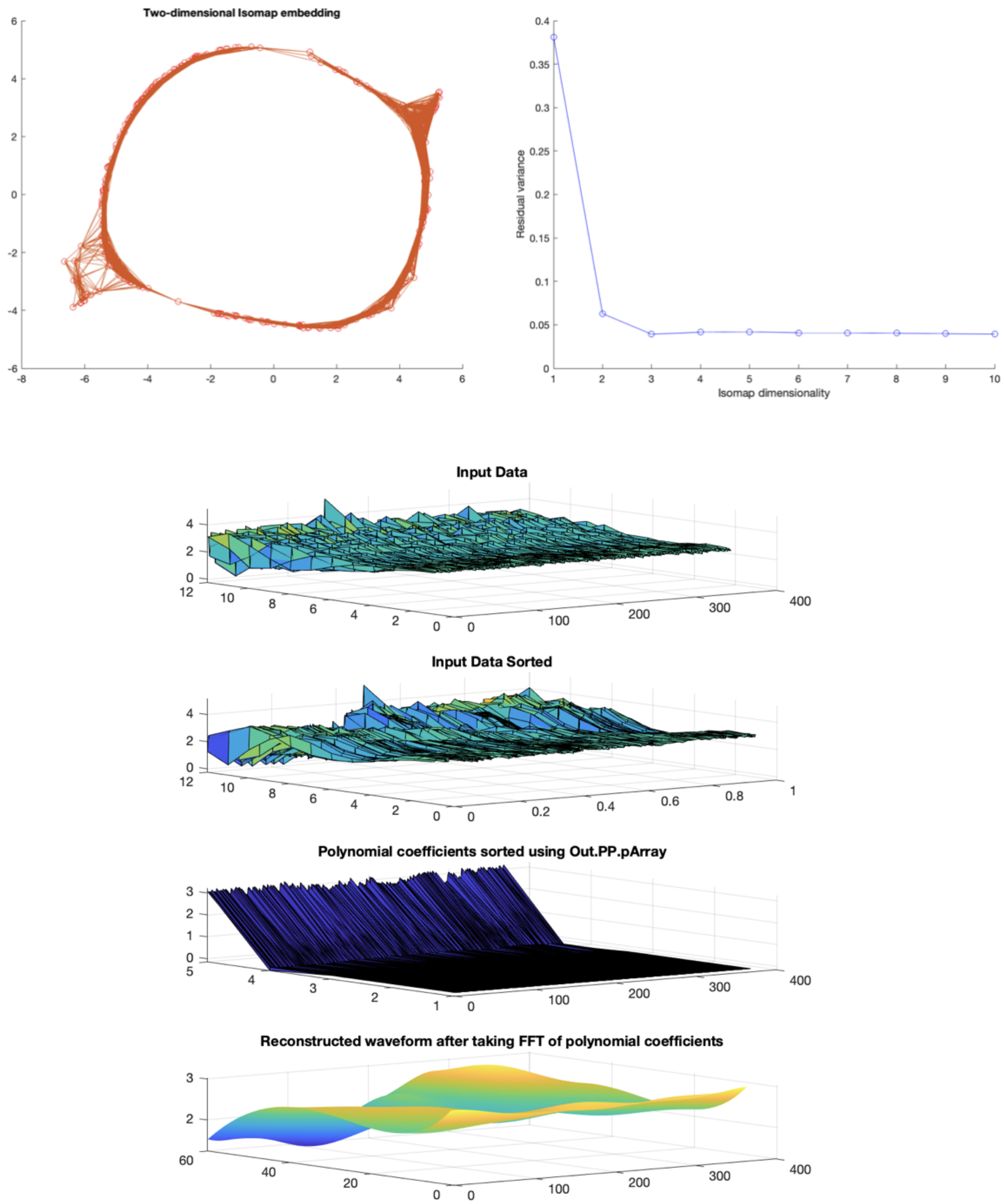

Figure A.7 Video 5a Isomap Output data and reconstructed waveform

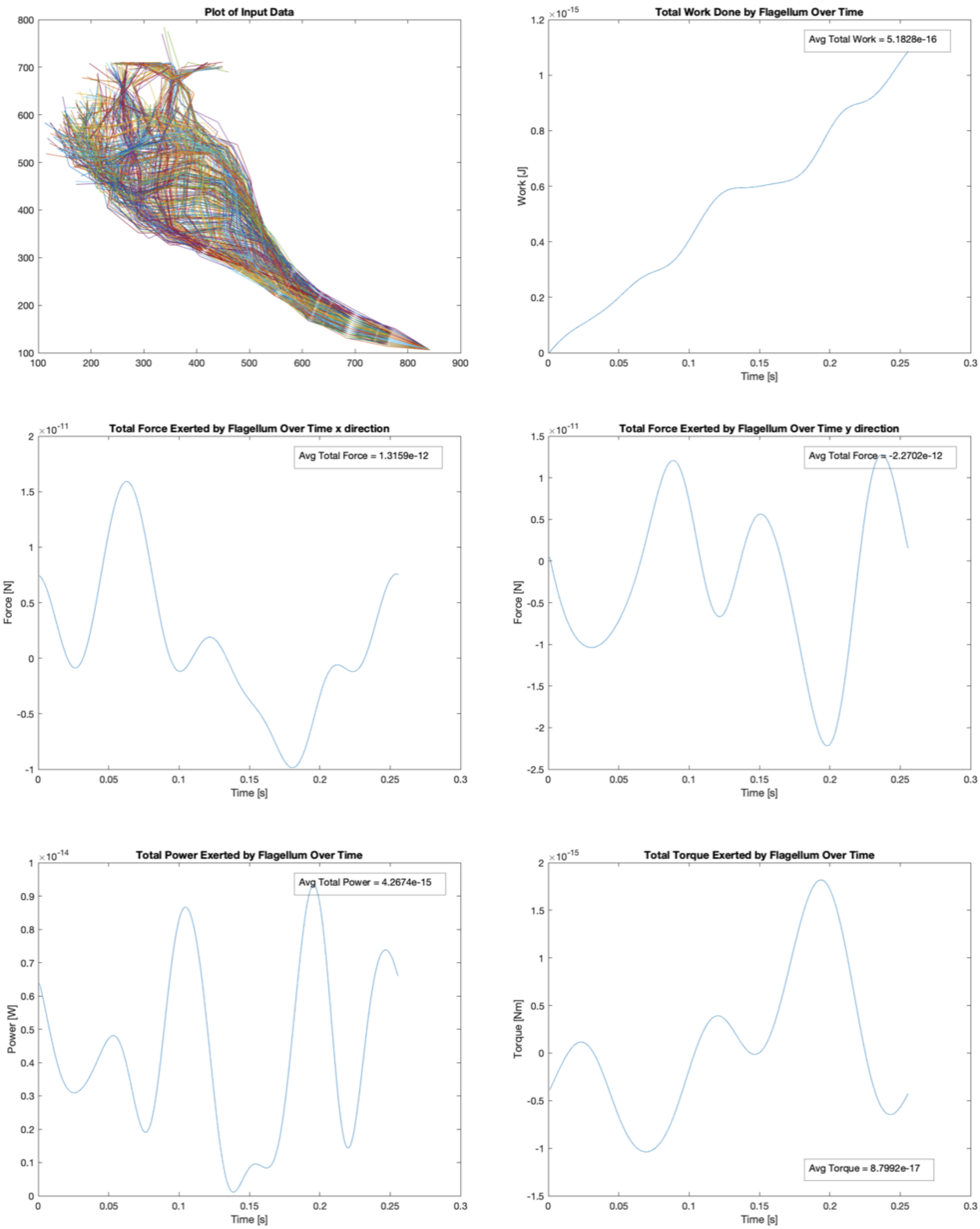

Figure A.8 Sample schematic diagram of test setup.

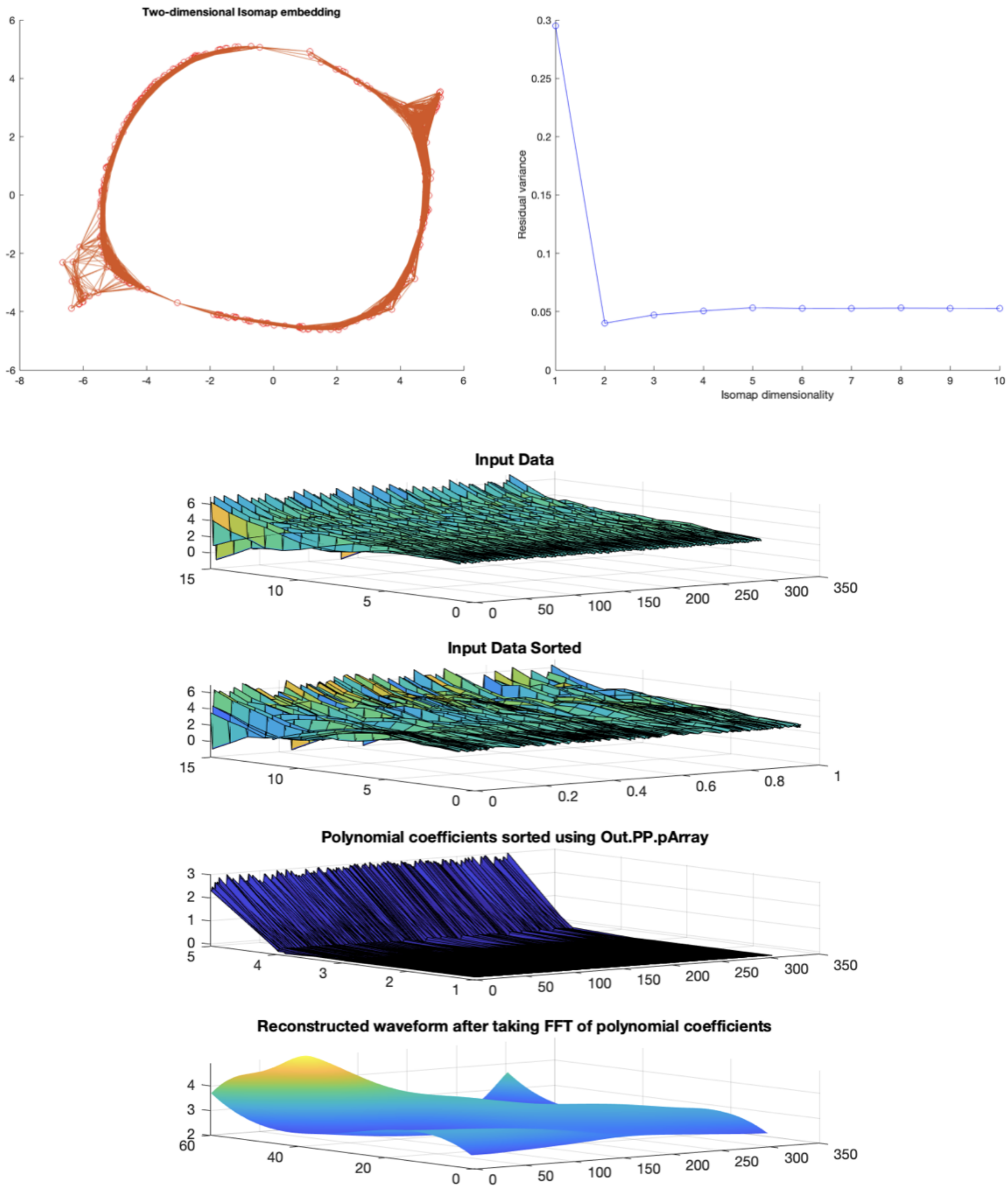

Figure A.9 Video 6a Isomap Output data and reconstructed waveform

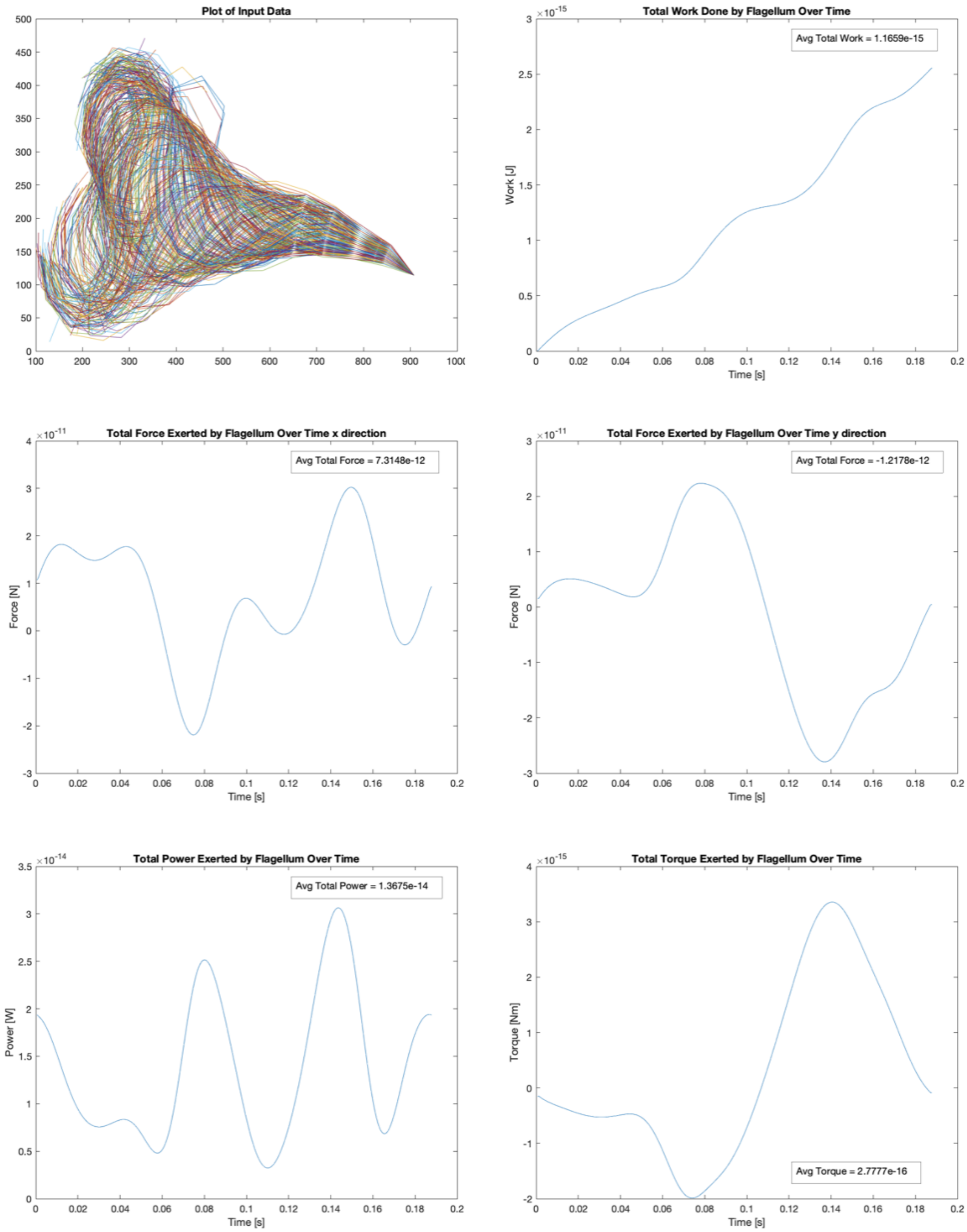

Figure A.10 Sample schematic diagram of test setup.

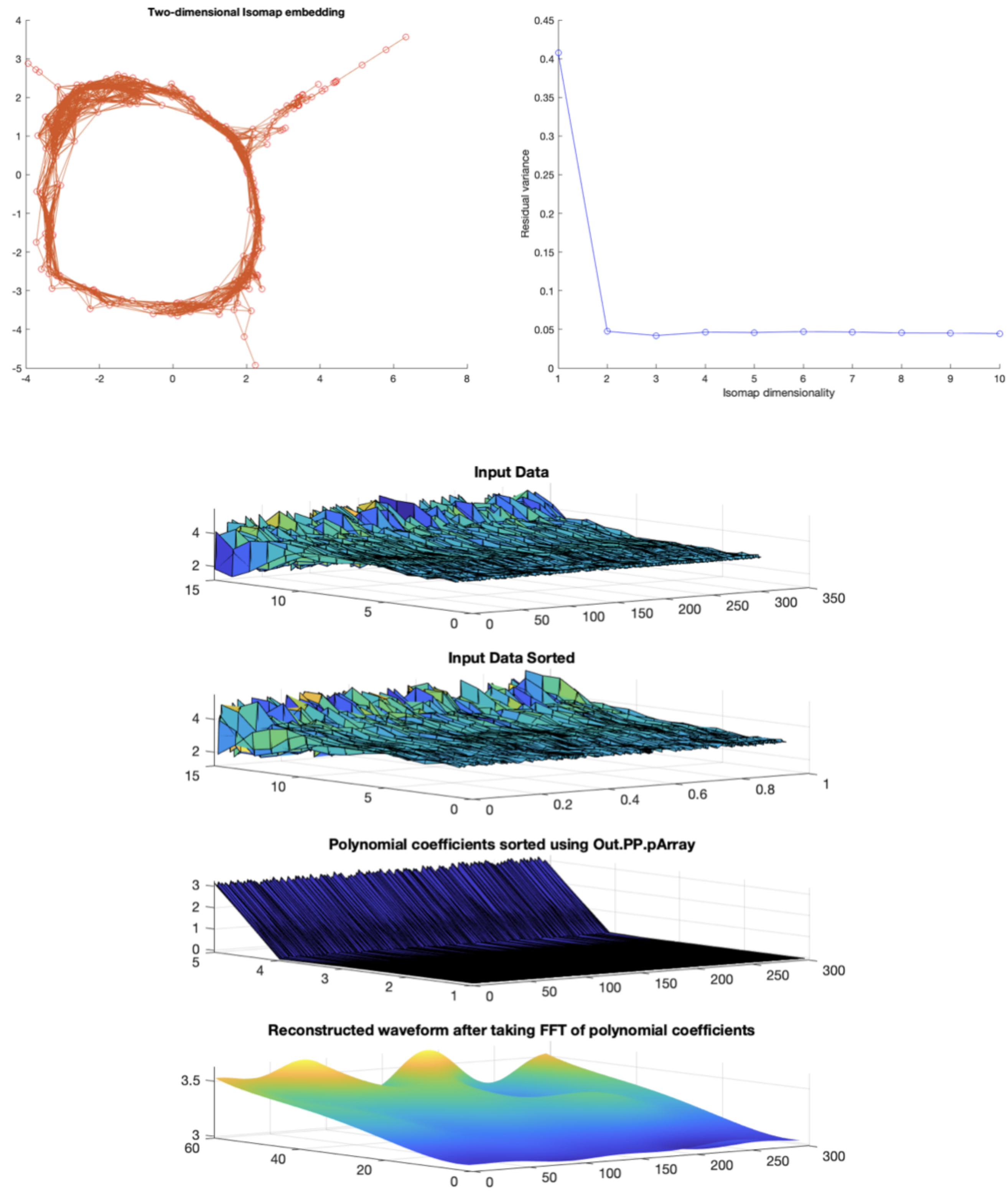

Figure A.11 Video 7a Isomap Output data and reconstructed waveform

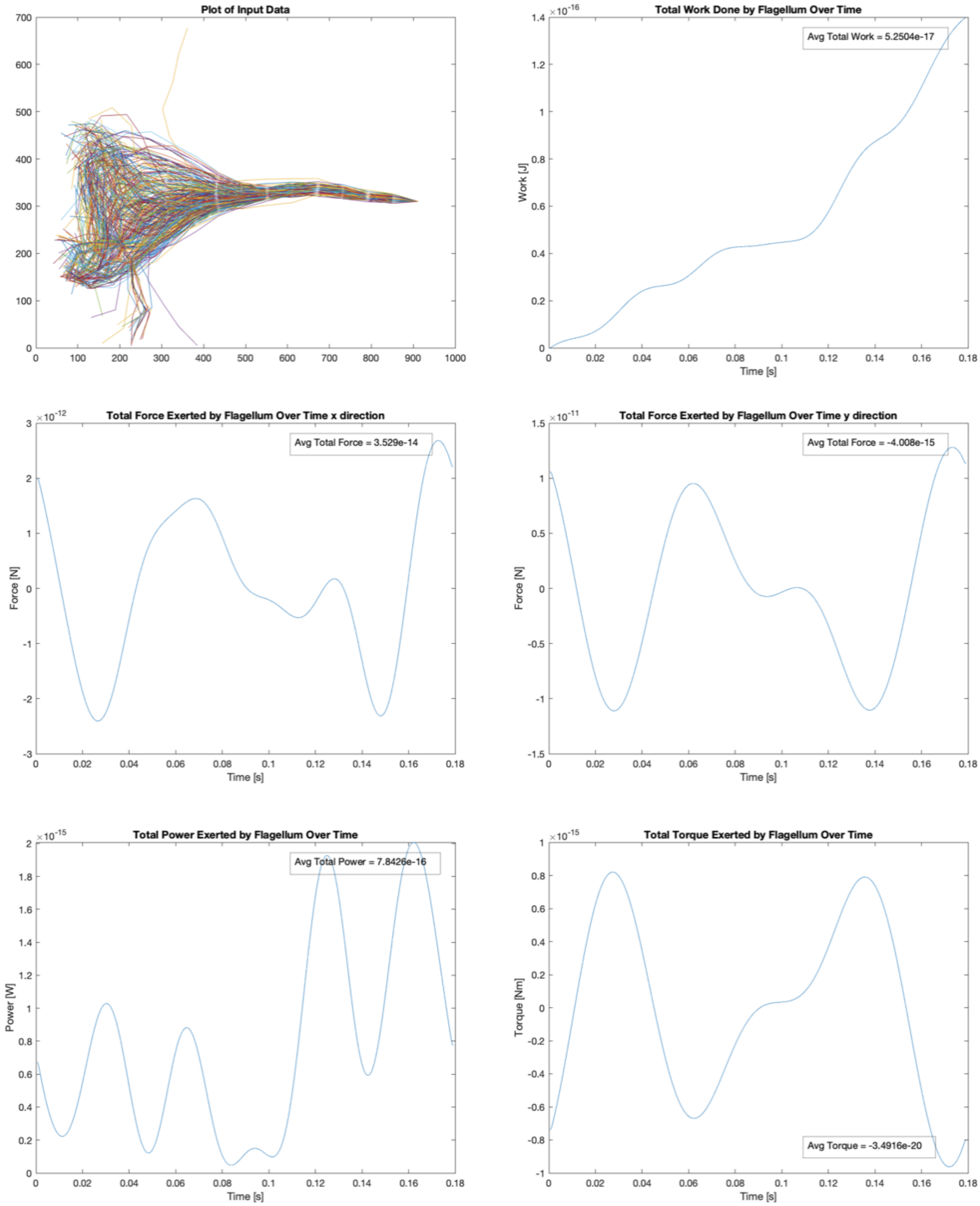

Figure A.12 Sample schematic diagram of test setup.

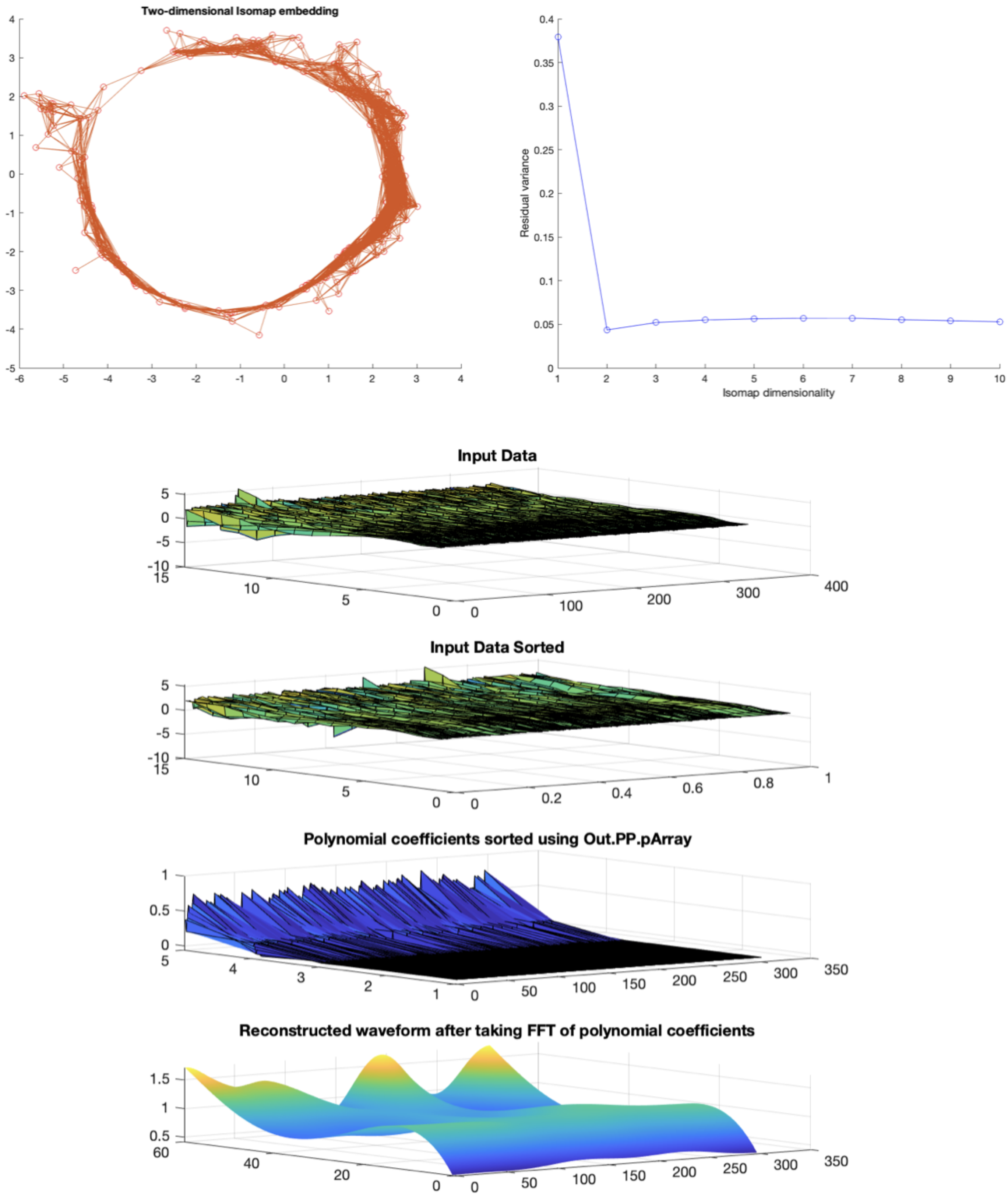

Figure A.13 Video 9a Isomap Output data and reconstructed waveform

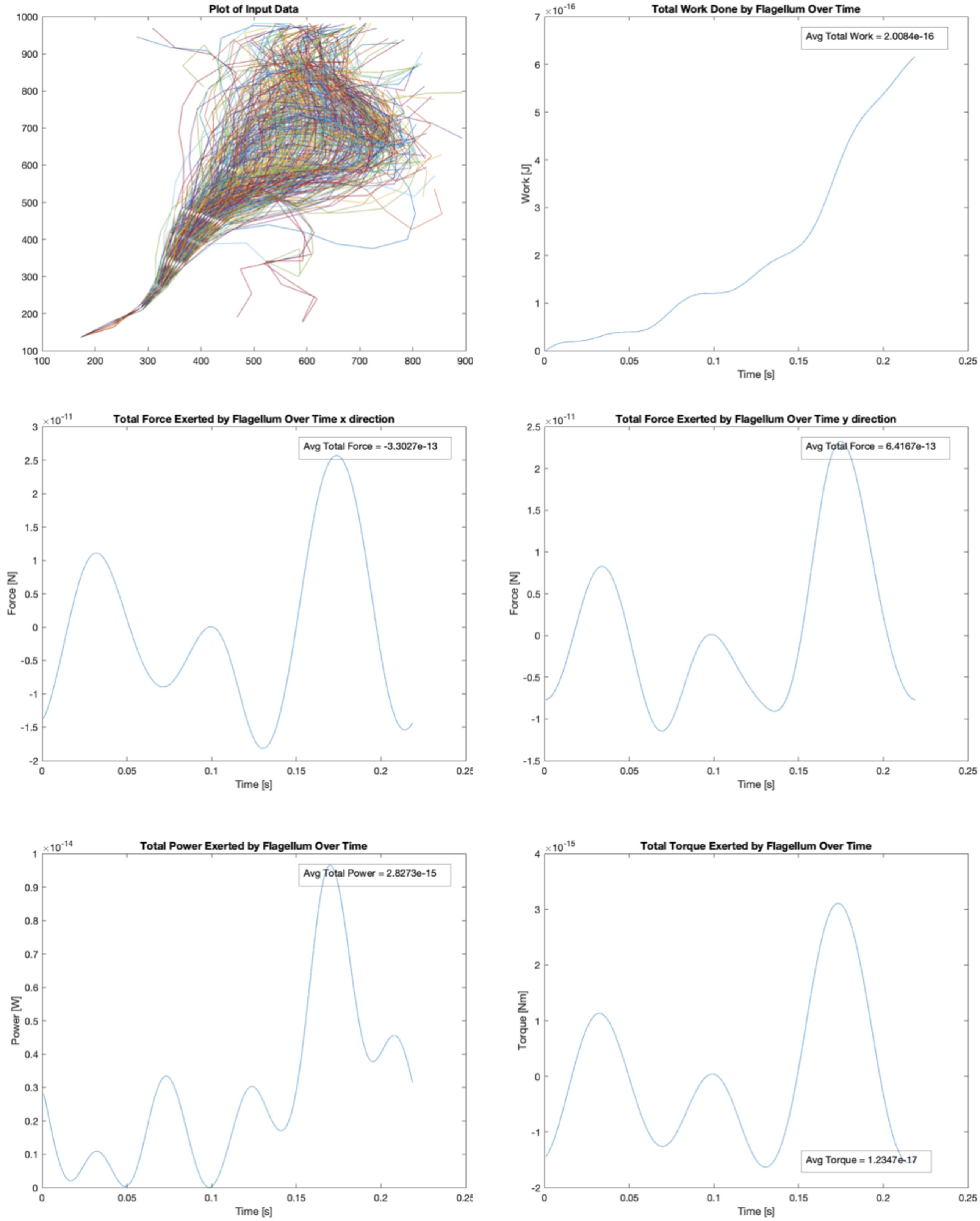

Figure A.14 Sample schematic diagram of test setup.

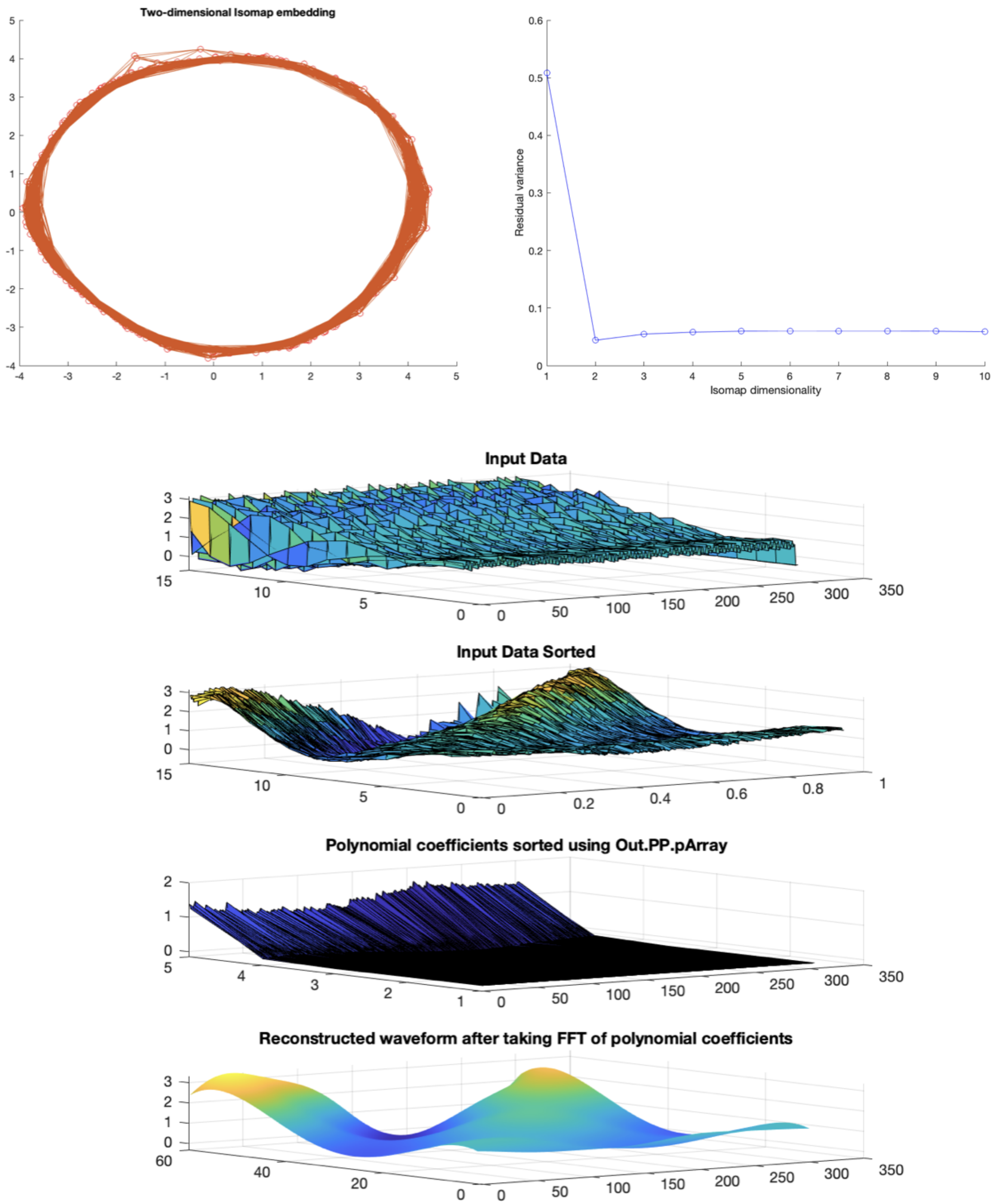

Figure A.15 Video 10a Isomap Output data and reconstructed waveform

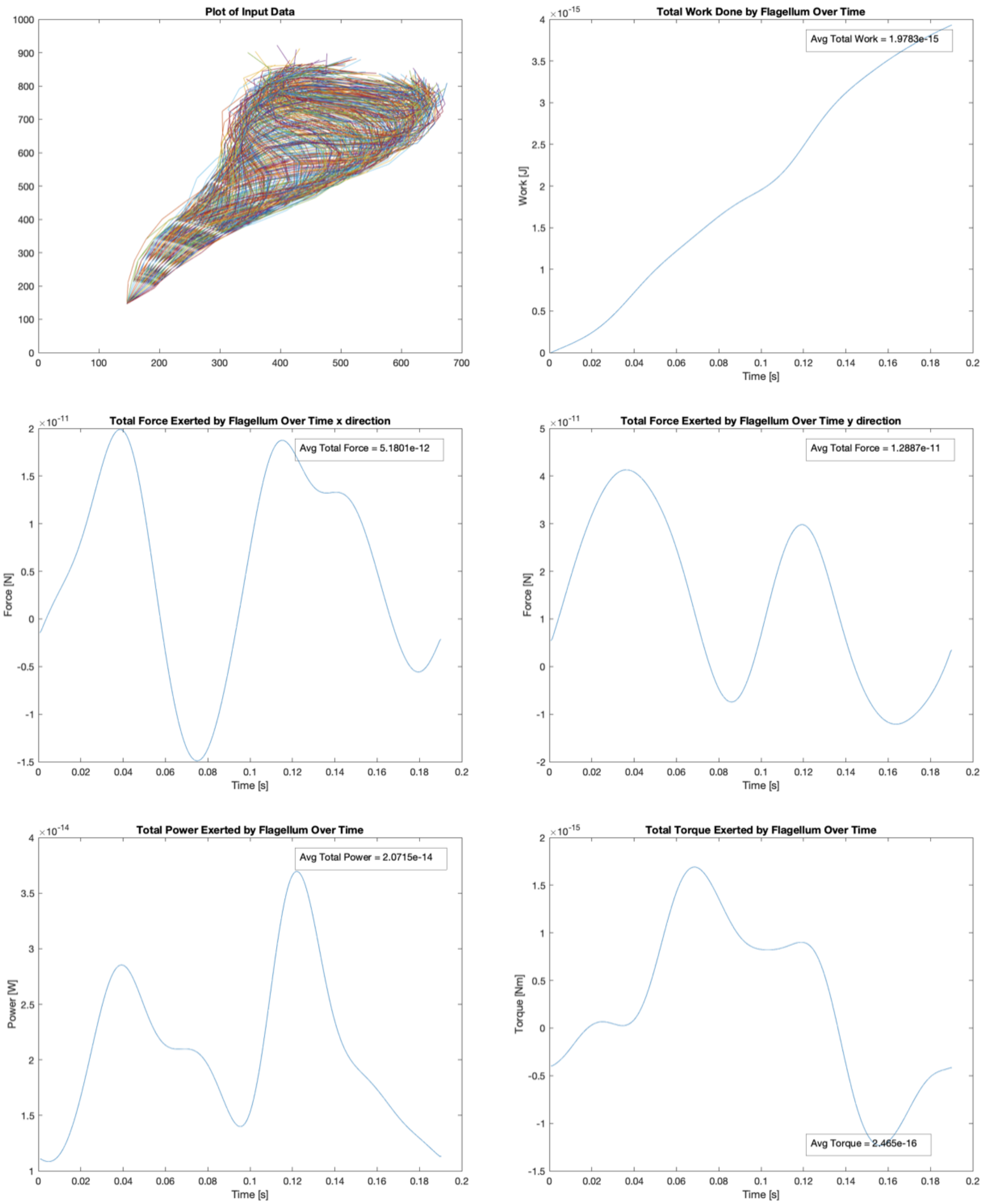

Figure A.16 Sample schematic diagram of test setup.

A.1 Output data for videos of mouse sperm with condition A.

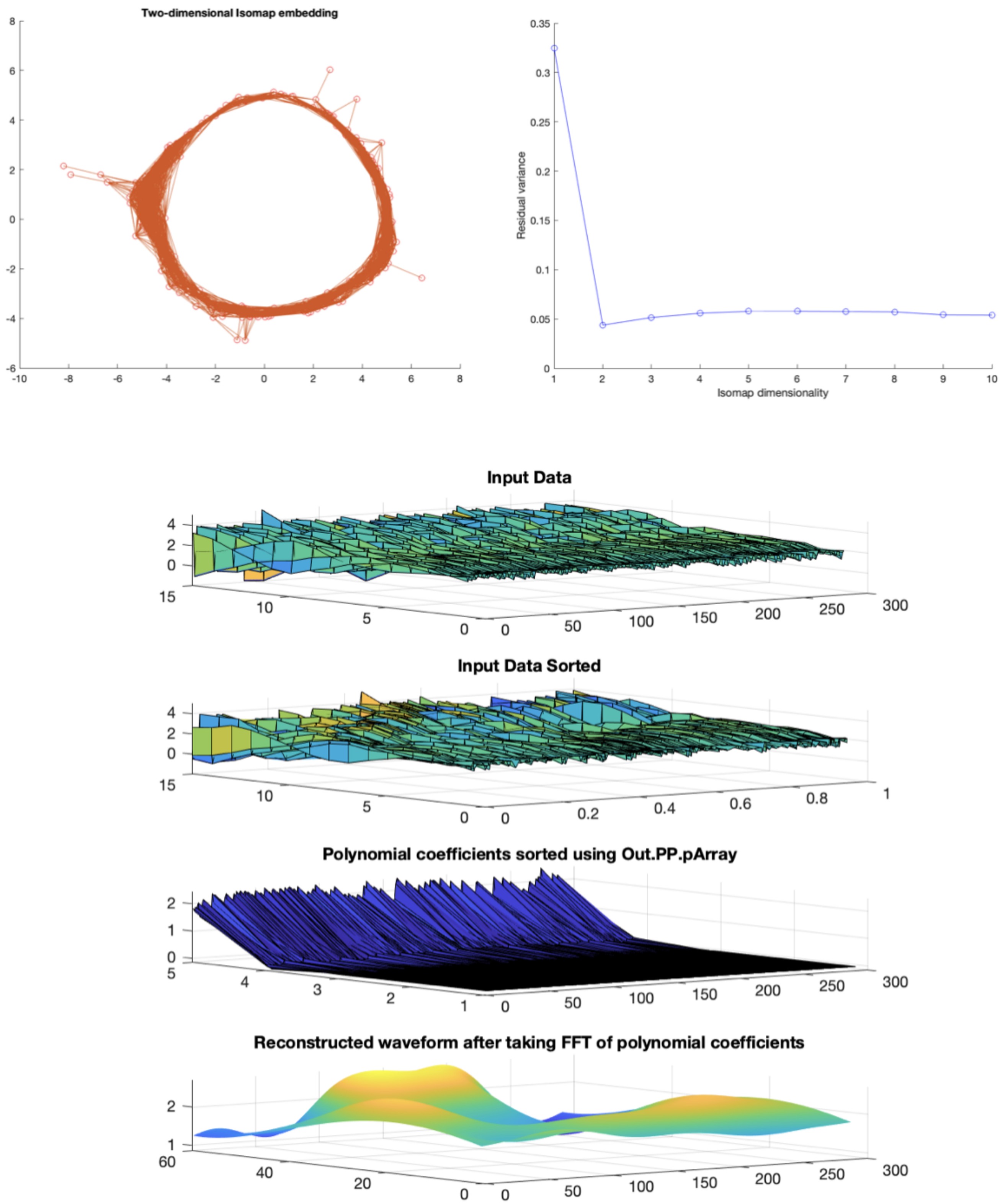

Figure A.17 Video 2b Isomap Output data and reconstructed waveform

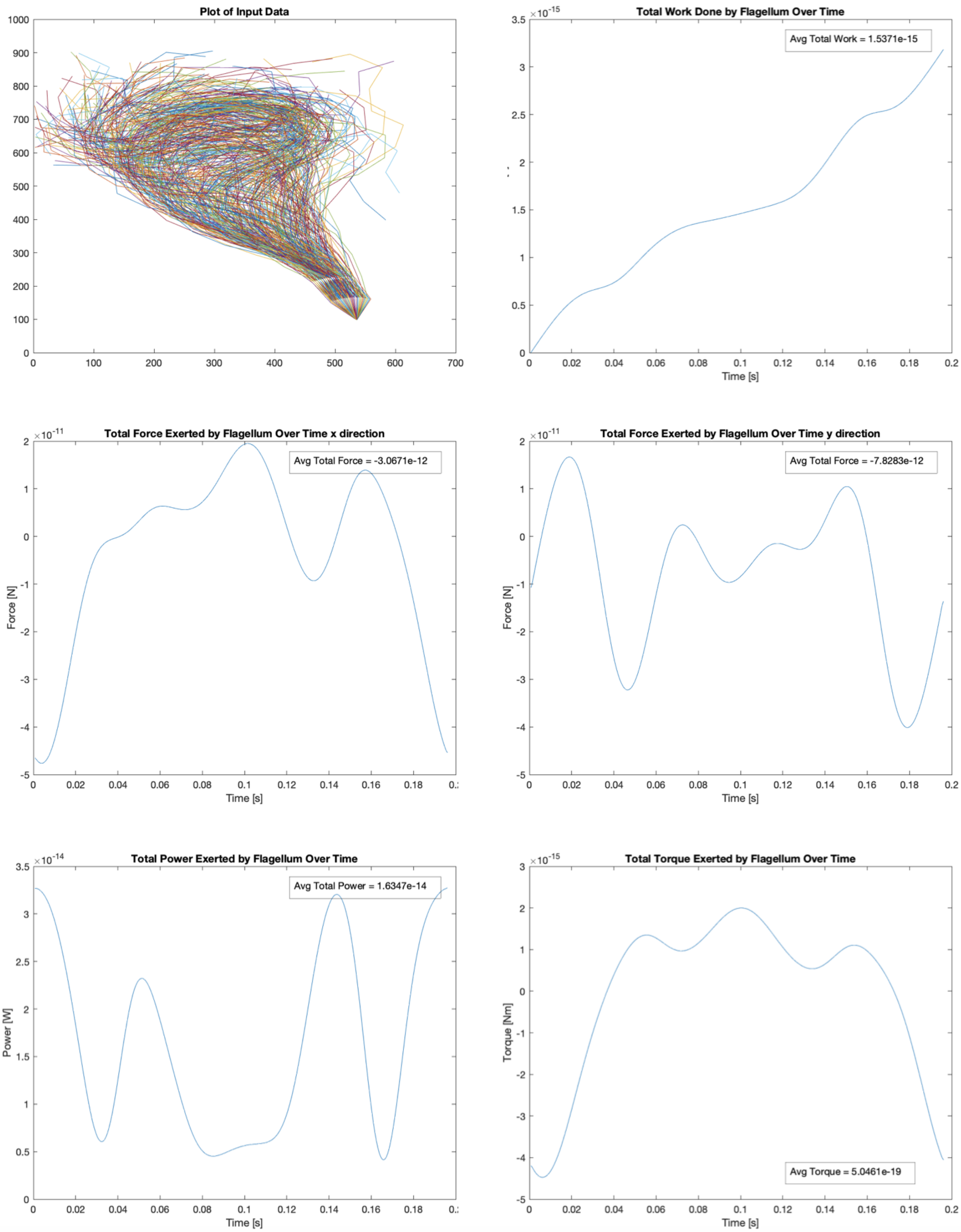

Figure A.18 Sample schematic diagram of test setup.

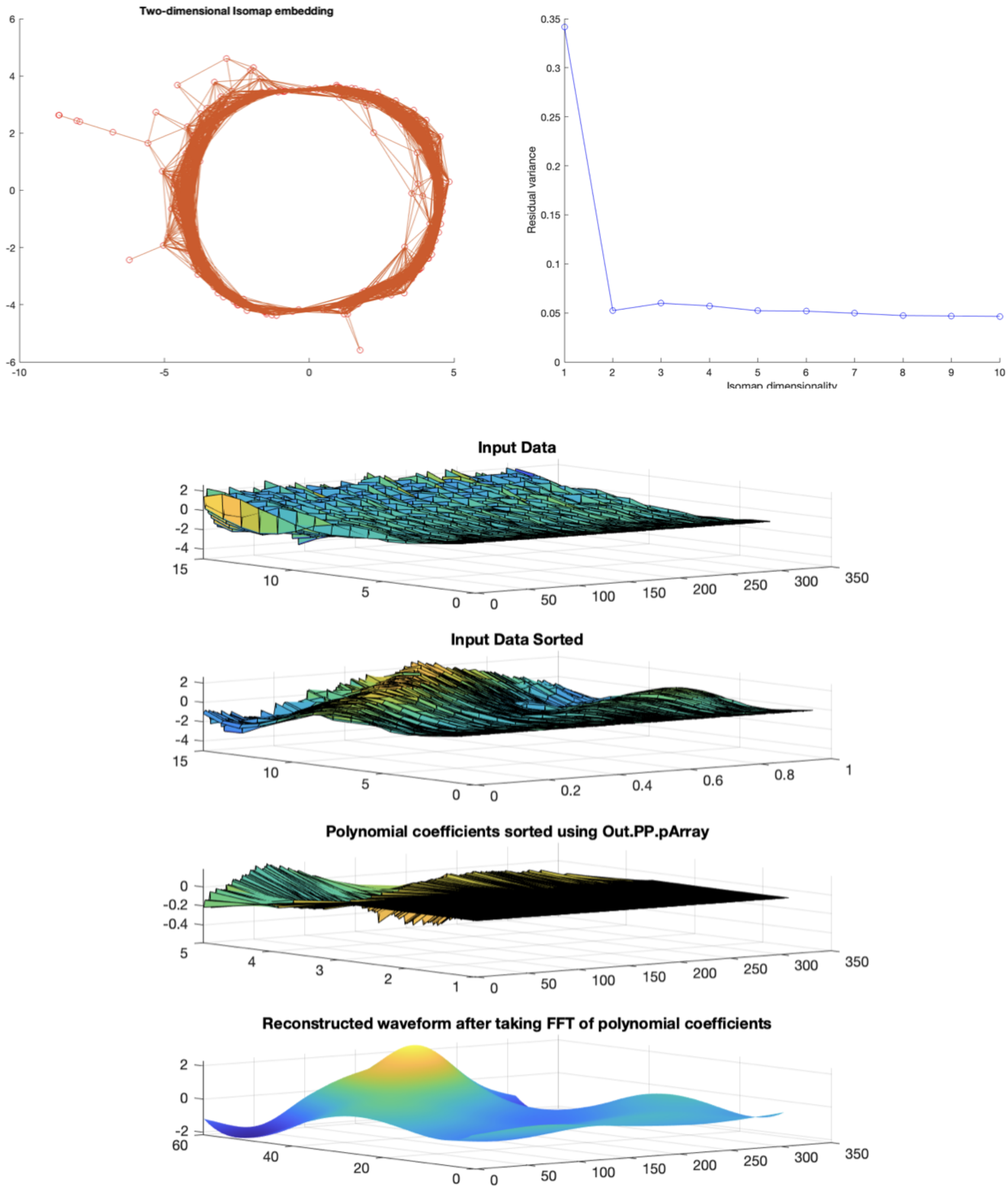

Figure A.19 Video 3b Isomap Output data and reconstructed waveform

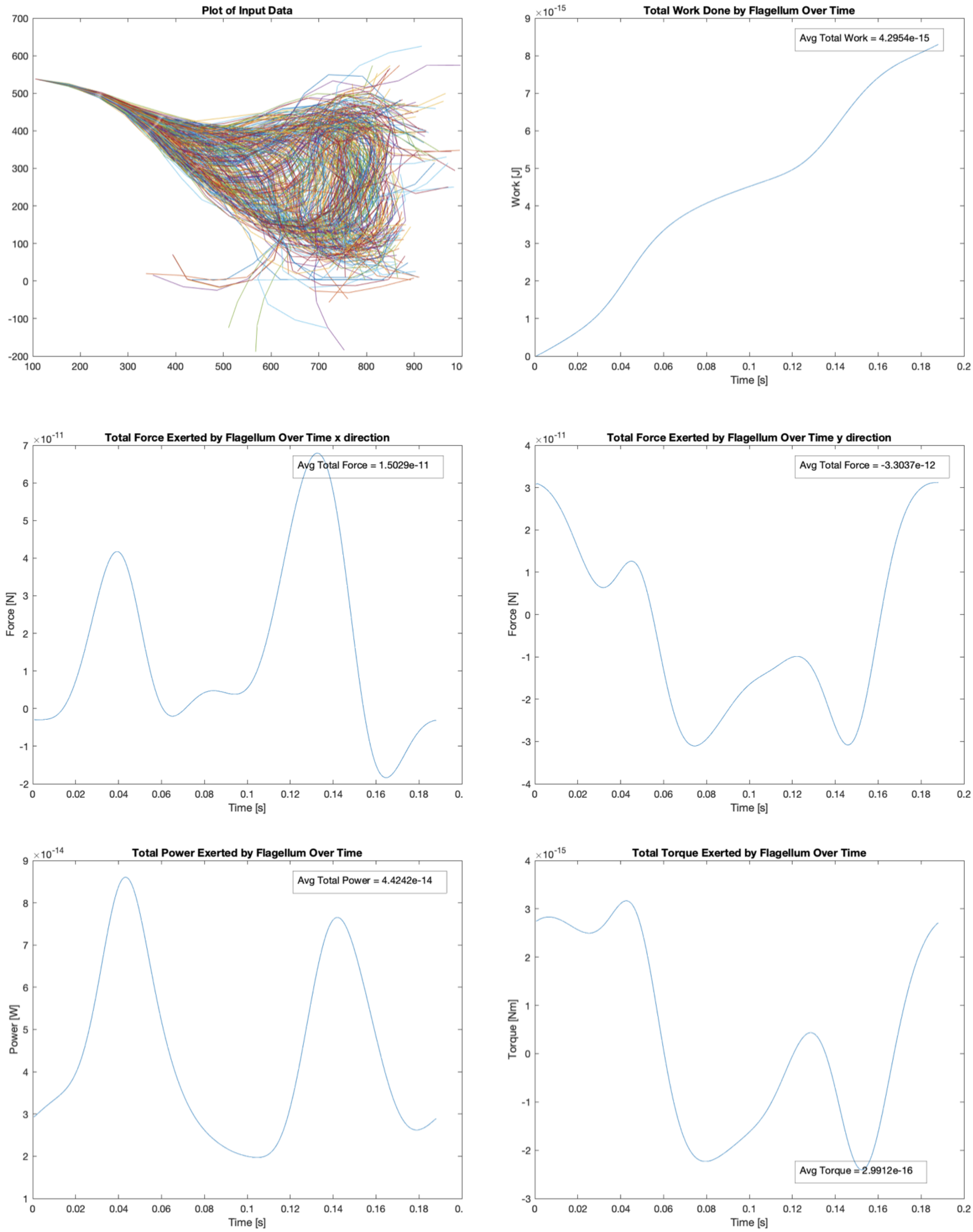

Figure A.20 Video 2b Isomap Output data and reconstructed waveform

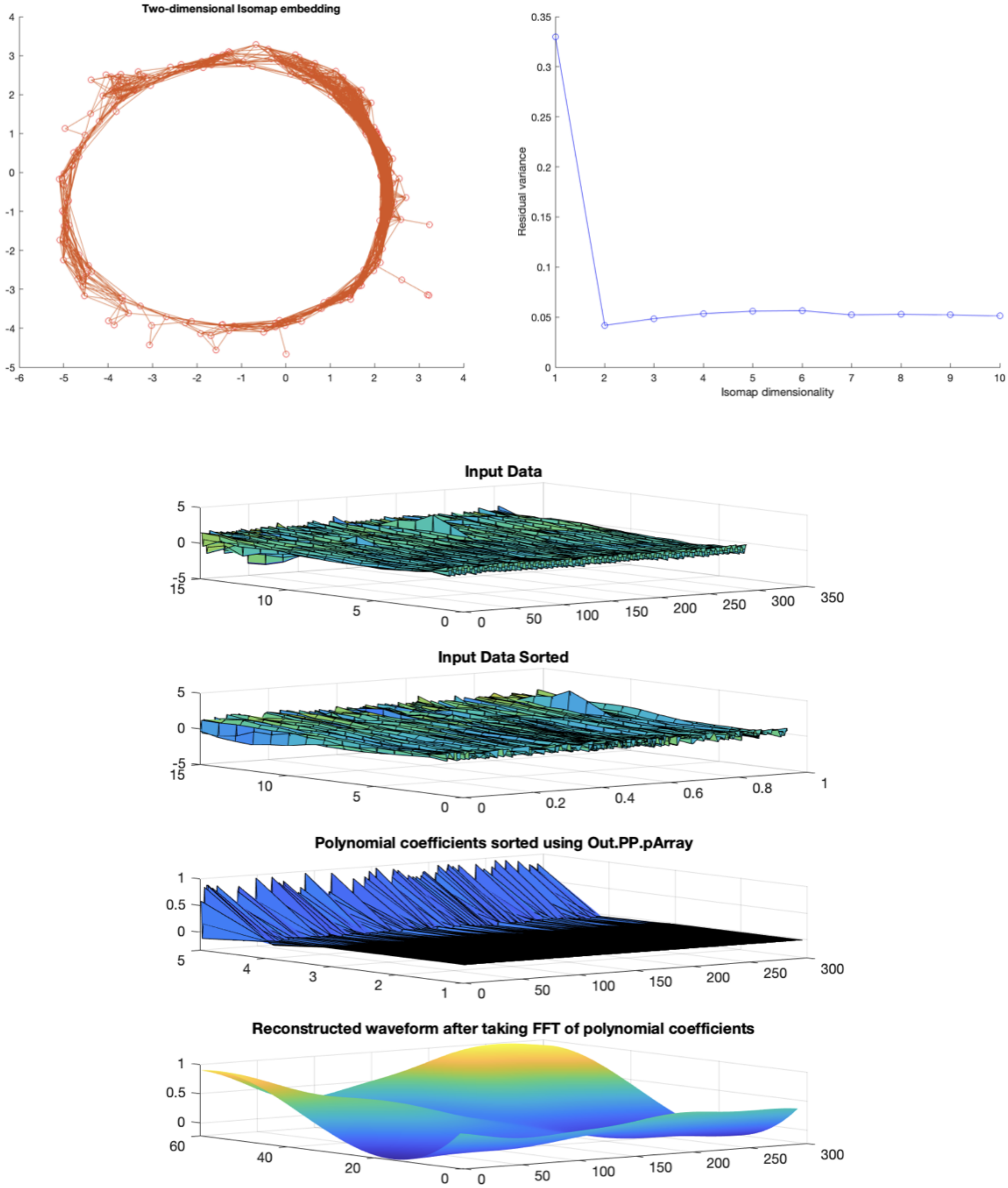

Figure A.21 Video 6b Isomap Output data and reconstructed waveform

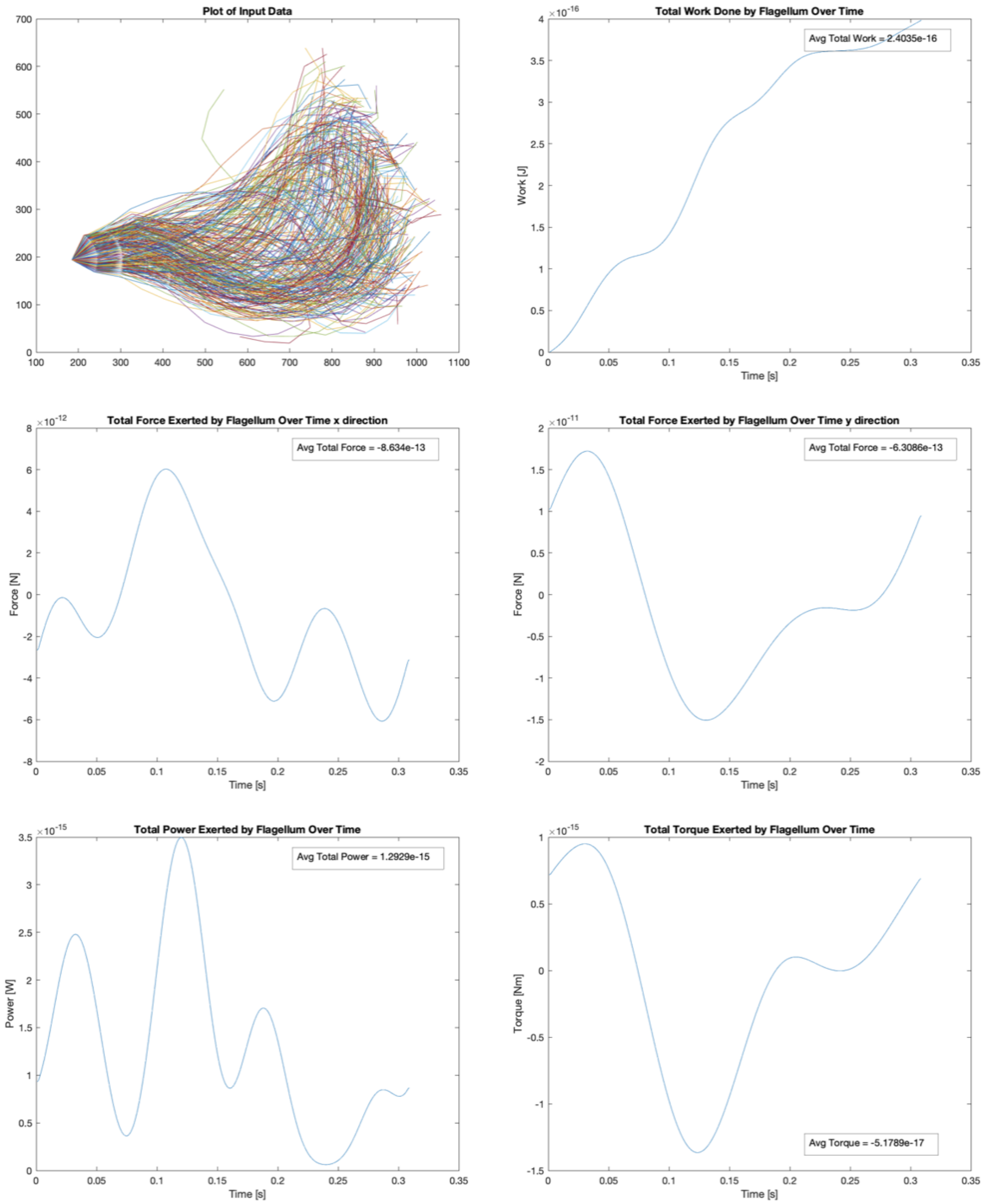

Figure A.22 Sample schematic diagram of test setup.

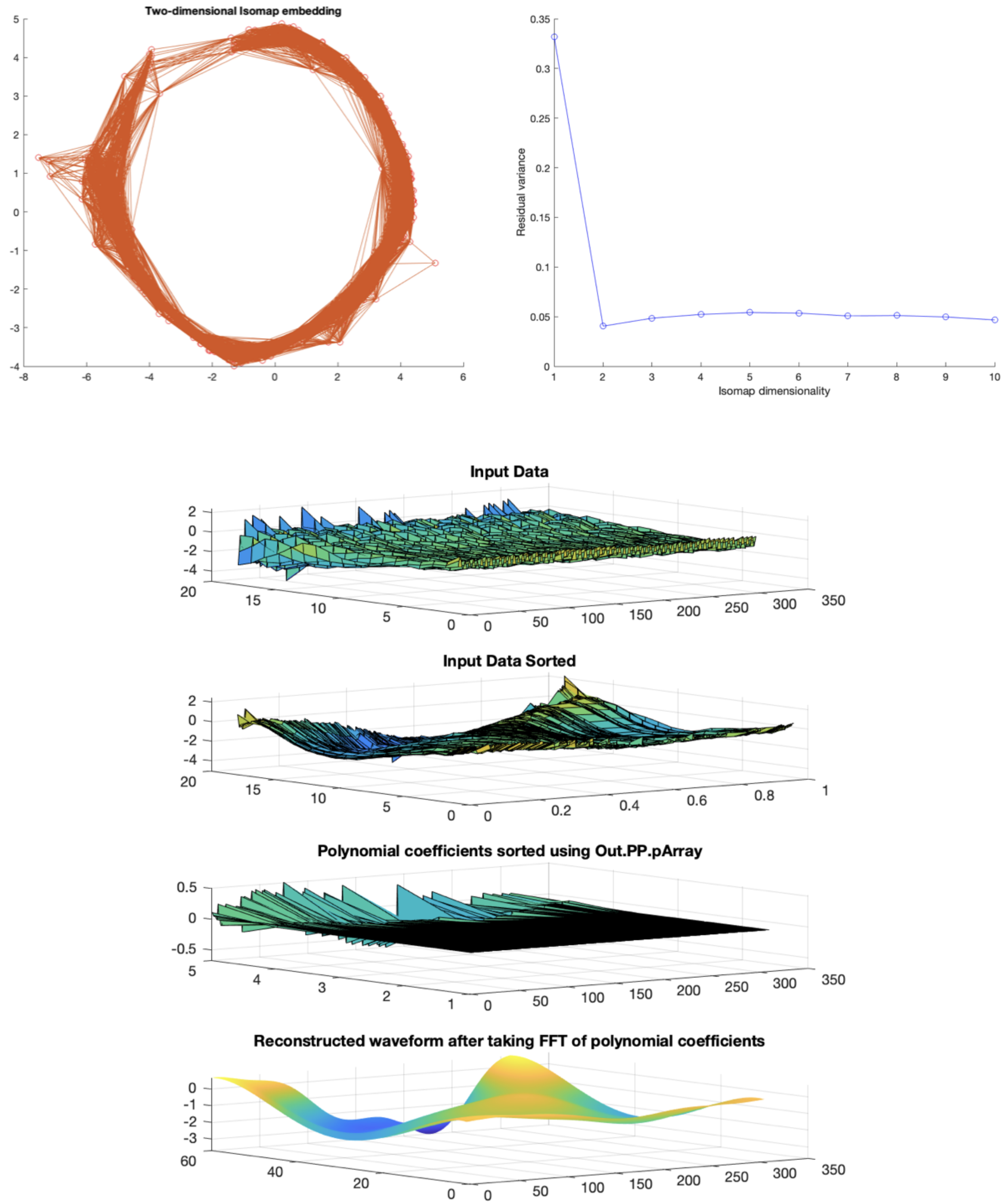

Figure A.23 Video 9b Isomap Output data and reconstructed waveform

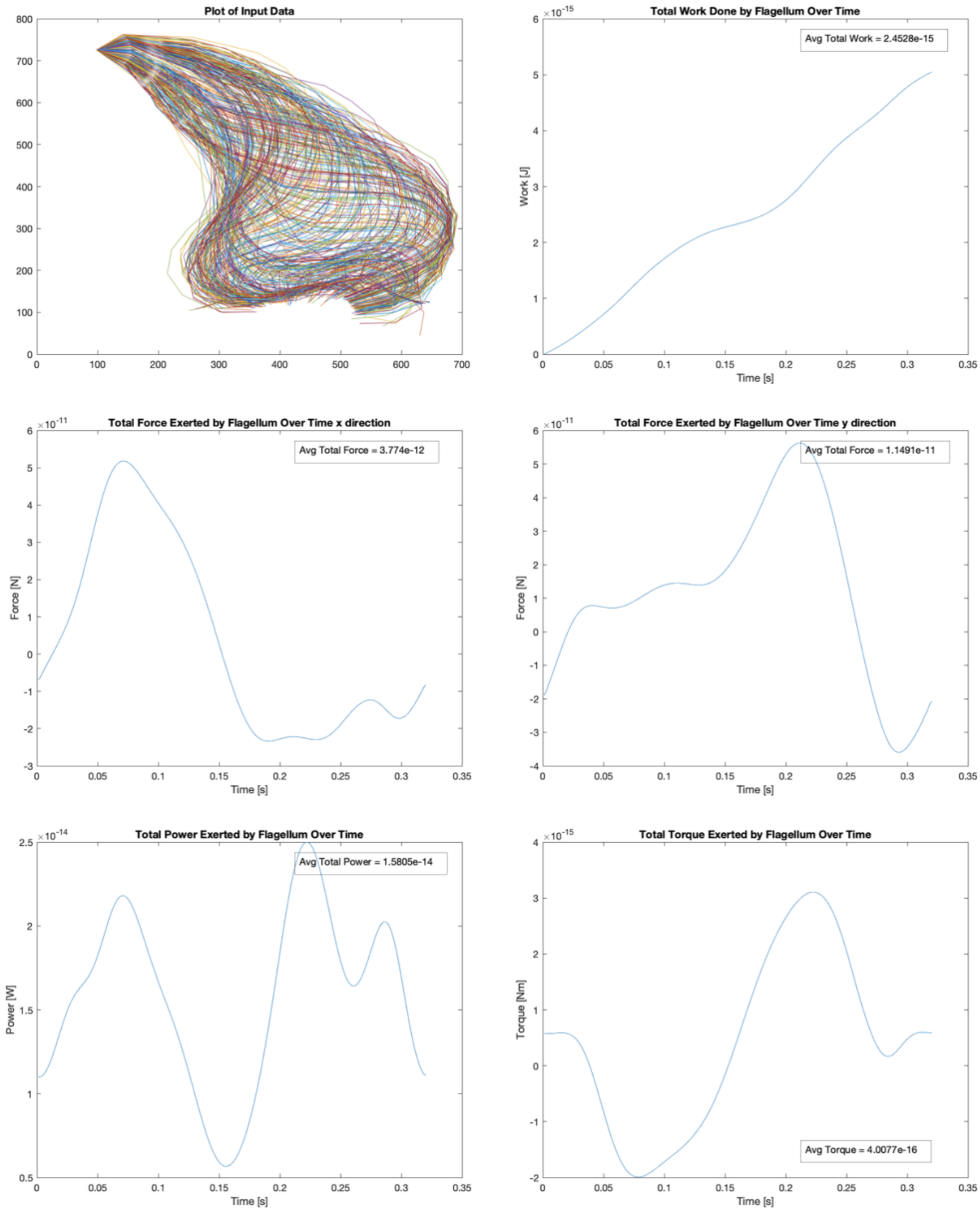

Figure A.24 Sample schematic diagram of test setup.

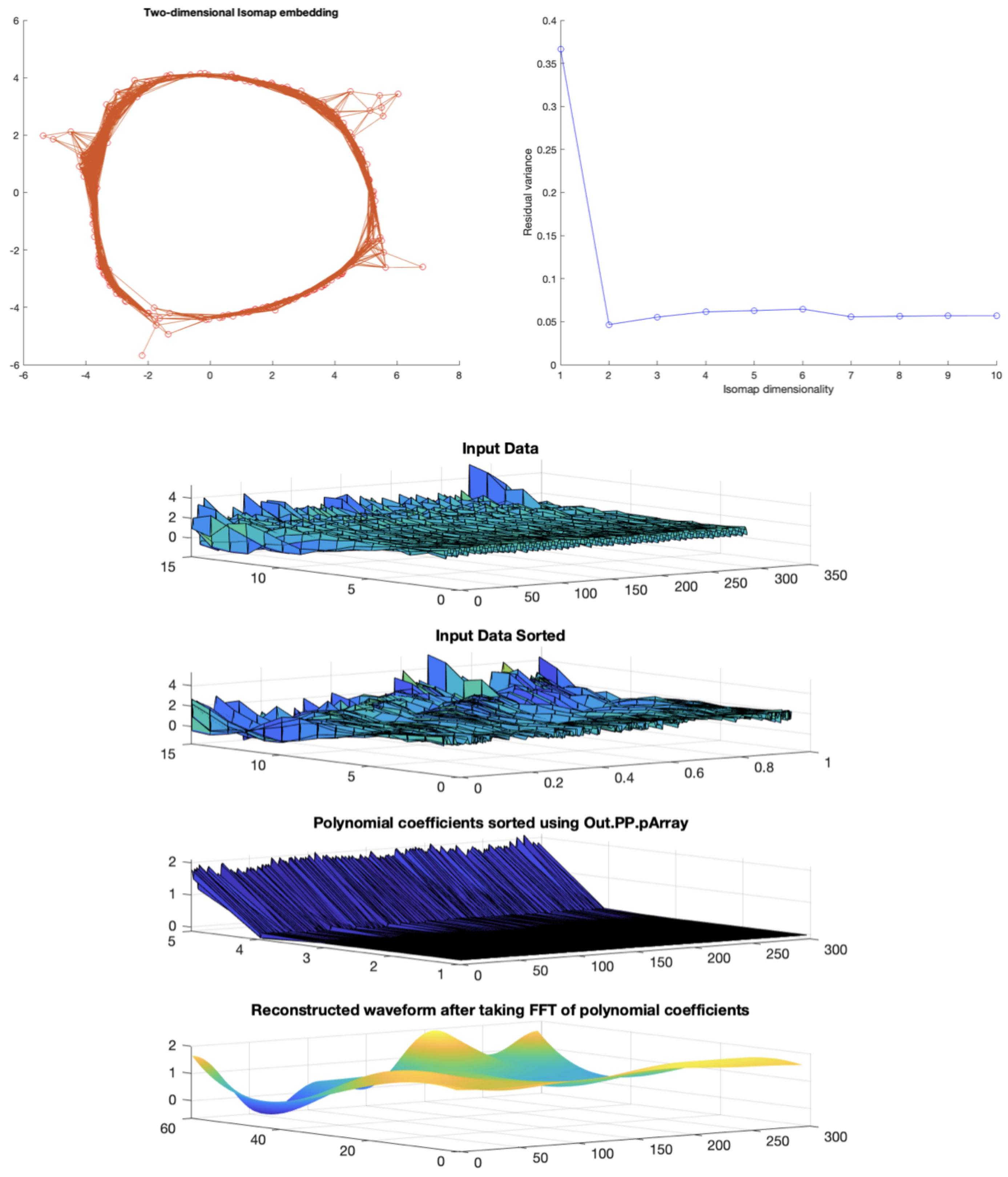

Figure A.25 Video 11b Isomap Output data and reconstructed waveform

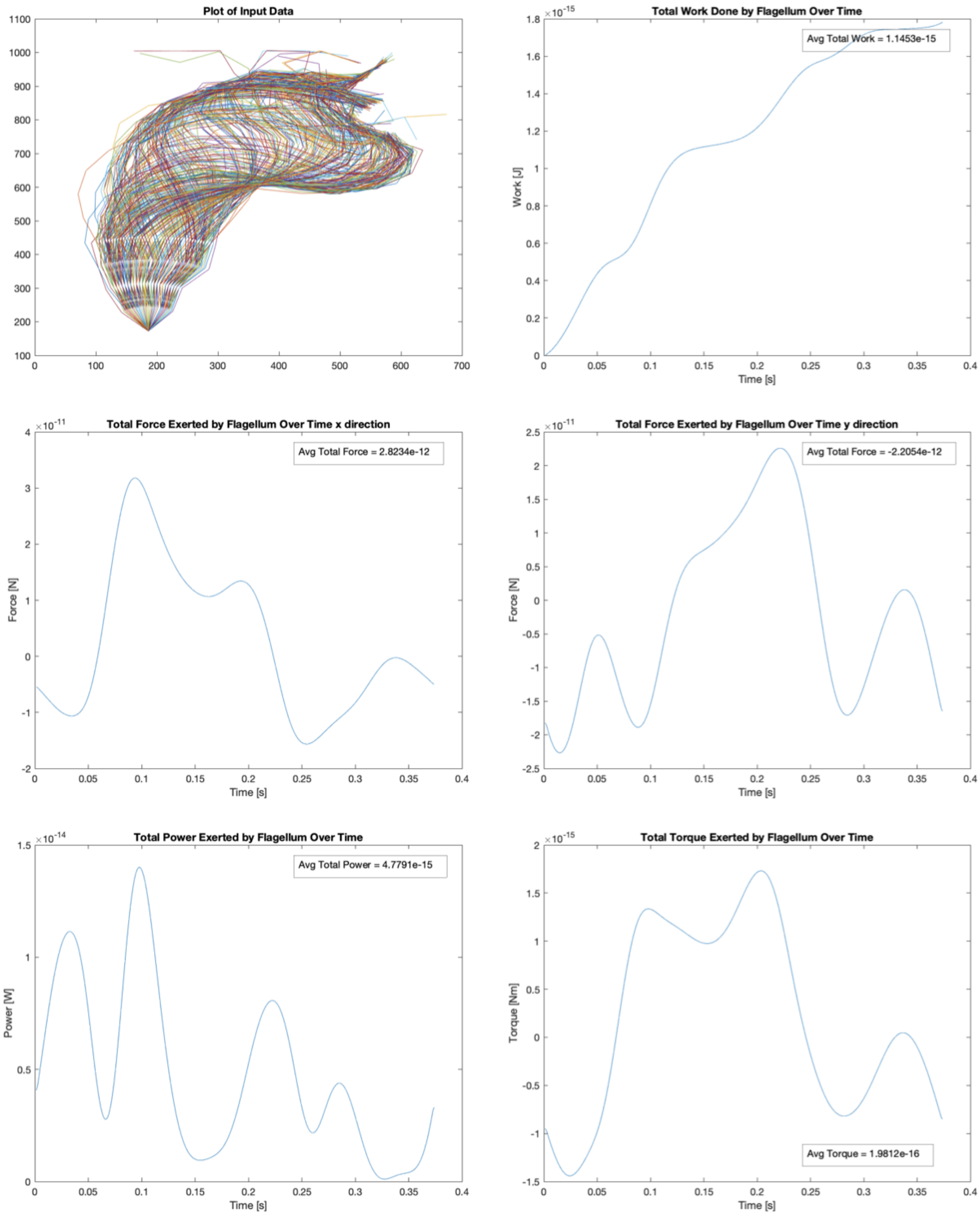

Figure A.26 Sample schematic diagram of test setup.

A.2 Output data for videos of mouse sperm with condition B.

B MATLAB Code

```
% Sort waveforms and compute forces
%  A Gupte and PVB 2021 1207
MOVIEYES = 0;            % set to 1 to show movies
% Get coordinates in low-dimensional projection
coords = Y.coords(2);        % coordinates in embedding space
len = length(Y.index);       % how many time steps

% % LGW Purge:
% twod = find(options.dims==2);
% nc = sum(E);
% outside = nc < 0;
% inside = ¬outside;


% get each component
x = coords{1}(1,:);
y = coords{1}(2,:);
thetaRecon = Out.PP.thetaRecon;
% sort by phase angle in cycle
theta = atan2(y,x);
[thetas, inds] = sort(theta);


% % LGW
% elim = find(outside);
% for ii = elim
%     inds(inds == ii) = [];
% end
len = numel(inds);
tt = (theta(inds)+pi)/(2*pi);
% apply to sort your input
% PVB 2021 1207 check of sorting
% xi=x(inds);
```

```
% yi=y(inds);
% figure(100), plot(xi,yi)

inputsorted = input_data(:,inds);
% subplot(4,1,1)
% imagesc(input_data)
% subplot(4,1,2)
% imagesc(inputsorted)

% next, reconstruct waveform based on the sorted beat
% smooth the sorted beat
% fit a polynomial or sinusoid over time - NEXT
subplot(4,1,1)
surf(input_data)
title('Input Data')

subplot(4,1,2)
surf(tt, 1:num_theta, inputsorted)
title('Input Data Sorted')

% perform FFTs of polynomial coefficients
parraysorted = Out.PP.pArray(:,inds);
subplot(4,1,3)
surf(parraysorted)
title('Polynomial coefficients sorted using Out.PP.pArray')
fp = fft(parraysorted,[],2);
fp0 = fp;

% get rid of higher harmonics ( > nh/period)
nh = 3;                      % number of harmonics
fp0(:,nh+2:len-nh) = 0;     % keep first and last elements of FFT
p0 = ifft(fp0,[],2);        % should be purely real

% reconstruct waveforms
for n = 1:len
    q(:,n) = polyval(p0(:,n),Out.PP.s2);
end

```

```
subplot(4,1,4)
surf(q)
title('Reconstructed waveform after taking FFT of polynomial coefficients')
shading interp

%% %%
% Analysis section

L = Out.length *10^-6;
numpoints = 60;
nframes = Out.Stats.numFrames;
h = (((0:60)./60)*Out.length*10)/Out.length;
h = round(h*100)/100;

% PVB 2021 1207 replace framespersec with freq
% prompt = 'number of frames per sec: ';
% framespersec = input(prompt);
% prompt = 'Beat frequency (Hz): ';
% beatfreq = input(prompt);
beatfreq = Out.PP.freq;

%orientation of hooke on sperm (CW or CCW)
prompt = 'Is it CW? Y/N [Y]: ';
str = input(prompt,'s');
if str == 'Y'
    q = -1*q;
%     %     kappaRecon = -1*kappaRecon;
end
%get x and y coordinates of theta
sinVals = sin(q);
cosVals = cos(q);

% PVB 2021 1207 moved these lines up
numpoints = 60;
leachseg = L/numpoints;
xVals = cumtrapz(cosVals)*leachseg;
yVals = cumtrapz(sinVals)*leachseg;

```

```
% PVB 2021 1207 addition of Dt
%      [dxdt,¬] = gradient(xVals);
%      [dydt,¬] = gradient(yVals);
beatT = (1/beatfreq);                    % period of "reconstructed" beat
Dt = beatT/nframes;                      % Dt - time step of reconstructed beat (sec)
TT = Dt*(1:n);                     % time vector
[dxdt,¬] = gradient(xVals,Dt);           % units are now microns/sect
[dydt,¬] = gradient(yVals,Dt);

Vx = dxdt;
Vy = dydt;

%normal and tangent vectors
Tx = cos(q);
Ty = sin(q);
Nx = -sin(q);
Ny = cos(q);

%finding normal and tangent velocity vectors
Vnormalx = Vx.*Nx;
Vnormaly = Vy.*Ny;
Vtanx = Vx.*Tx;
Vtany = Vy.*Ty;
Vn = Vnormalx+Vnormaly;
Vt = Vtanx+Vtany;

%coefficients
%friction coefficient normal = 3.4*10^-3
%parallel is half of normal
%finding force in normal and tangent directions
Cn = 0.0034;
Ct = Cn/2;
Fn = Vn*Cn;
Ft = Vt*Ct;

%Force
Fx = Fn.*Nx + Ft.*Tx;
Fy = Fn.*Ny + Ft.*Ty;
```

```
fmag = sqrt(Fx.^2+Fy.^2);%get x and y components and add them separately
avgfmag = mean(sum(fmag,1)*leachseg)
% PVB 2021 1207 added segment length (leachseg) to integral
% Forceateachtimex = sum(Fx,1);
Forceateachtimex = sum(Fx,1)*leachseg;
%     Forceateachlengthx = sum(Fx,2);
% PVB 2021 1207 added segment length (leachseg) to integral
% Forceateachtimey = sum(Fy,1);
Forceateachtimey = sum(Fy,1)*leachseg;
%     Forceateachlengthy = sum(Fy,2);

% Average force over time
avgTotalForcethrougtimex = mean(Forceateachtimex);
%   avgTotalForceeachlength = mean(Forceateachlengtx);
avgTotalForcethrougtimey = mean(Forceateachtimey);
%   avgTotalForceeachlengthy = mean(Forceateachlengty);

figure(8)
plot(TT,Forceateachtimex);
title('Total Force Exerted by Flagellum Over Time x direction')
% newLim = get(gca,'XLim');
% newx = linspace(newLim(1), newLim(2),8);
% set(gca,'xticklabel', round(newx*(1/15)*100)/100);
xlabel('Time [s]')
ylabel('Force [N]')
annotation('textbox', [0.60, 0.8, 0.1, 0.1], 'String', "Avg Total Force = " + ...
    avgTotalForcethrougtimex)

%    figure(7)
%     plot(Forceateachlengthx);
%     set(gca,'xticklabel',(h));
%     title('Total Force Exerted Over Normalized Length of Flagellum x direction')
%     xlabel('Normalized Length')
%     ylabel('Force [N]')
%     annotation('textbox', [0.60, 0.1, 0.1, 0.1], 'String', "Avg Total Force ...
    = " + avgTotalForceeachlengthx)

```

```
figure(9)
plot(TT,Forceateachtimey);
title('Total Force Exerted by Flagellum Over Time y direction')
% newLim = get(gca,'XLim');
% newx = linspace(newLim(1), newLim(2),8);
% set(gca,'xticklabel', round(newx*(1/15)*100)/100);
xlabel('Time [s]')
ylabel('Force [N]')
annotation('textbox', [0.60, 0.8, 0.1, 0.1], 'String', "Avg Total Force = " + ...
    avgTotalForcethrougtimey)

%    figure(10)
%     plot(Forceateachlengthy);
%     set(gca,'xticklabel',(h));
%     title('Total Force Exerted Over Normalized Length of Flagellum y direction')
%     xlabel('Normalized Length')
%     ylabel('Force [N]')
%     annotation('textbox', [0.60, 0.1, 0.1, 0.1], 'String', "Avg Total Force ...
    = " + avgTotalForceeachlengthy)

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Power calculation
% PVB 2021 1207 added the lenght element (leachseg) to integral
POWER = (Fn.*Vn+Ft.*Vt);         % Power per unit length at each segment
% Could also used POWER  =  Fx.*Vx + Fy.*Vy; % should be the same

powerateachtime = sum(POWER,1)*leachseg;
%powerateachlength = sum(POWER,2);
TotalavgPowertime = mean(powerateachtime);
%     totalavgPowerlength = mean(powerateachlength);

figure(11)
plot(TT,powerateachtime);
%0.0640
title('Total Power Exerted by Flagellum Over Time');
% newLim = get(gca,'XLim');
% newx = linspace(newLim(1), newLim(2),8);
```

```
% % PVB 2021 1207 not sure but replacing framespersec with beatfreq
% % set(gca,'xticklabel', round(newx*(1/framespersec)*100)/100);
% set(gca,'xticklabel', round(newx*(1/beatfreq)*100)/100);

xlabel('Time [s]'); %frames per second 15.6131575
ylabel('Power [W]');
annotation('textbox', [0.60, 0.8, 0.1, 0.1], 'String', "Avg Total Power = " + ...
    TotalavgPowertime)

%     figure(12)
%     plot(powerateachlength);
%     set(gca,'xticklabel',(h));
%     title('Total Power Exerted Over Normalized Length of Flagellum');
%     xlabel('Normalized Length');
%     ylabel('Power [W]');
%     annotation('textbox', [0.60, 0.1, 0.1, 0.1], 'String', "Avg

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% PVB 2021 1207 moved the length  multiplier to integral
%TORQUE
Torque = (Fy.*xVals - Fx.*yVals);
%     avgTorquelength = sum(Torque,2)
%      avgavgtorquel = mean(avgTorquelength)
avgTorquetime = sum(Torque,1)*leachseg;
avgavgtorquet = mean(avgTorquetime);
%     figure(4)
%     plot(avgTorquelength)
%     set(gca,'xticklabel',(h));
%     title('Total Torque Exerted Over Normalized Length of Flagellum')
%     xlabel('Normalized Length');
%     ylabel('Torque [Nm]');
%     annotation('textbox', [0.60, 0.1, 0.1, 0.1], 'String', "Avg Torque = " + ...
    avgavgtorquel)
%
figure(13)
plot(TT, avgTorquetime)
```

```
% newLim = get(gca,'XLim');
% newx = linspace(newLim(1), newLim(2),8);
% % PVB 2021 1207 not sure but replacing framespersec with beatfreq
% % set(gca,'xticklabel', round(newx*(1/framespersec)*100)/100);
% set(gca,'xticklabel', round(newx*(1/beatfreq)*100)/100);
title('Total Torque Exerted by Flagellum Over Time')
xlabel('Time [s]');
ylabel('Torque [Nm]');
annotation('textbox', [0.60, 0.1, 0.1, 0.1], 'String', "Avg Torque = " + ...
    avgavgtorquet)

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% % WORK
% % t = [1:nframes];
% TotalWork = trapz(TT,powerateachtime);
TotalWork = trapz(powerateachtime)*Dt;

intofpateachtime = cumtrapz(powerateachtime)*Dt;
%intofpateachlength = cumtrapz(powerateachlength);

avgWorkateachtime = mean(intofpateachtime);
%avgWorkateachlength = mean(intofpateachlength);

figure(2)
plot(TT,intofpateachtime)
title('Total Work Done by Flagellum Over Time')
% newLim = get(gca,'XLim');
% newx = linspace(newLim(1), newLim(2),8);
% % PVB 2021 1207 not sure but replacing framespersec with beatfreq
% % set(gca,'xticklabel', round(newx*(1/framespersec)*100)/100);
% set(gca,'xticklabel', round(newx*(1/beatfreq)*100)/100);

xlabel('Time [s]');
ylabel('Work [J]');
annotation('textbox', [0.60, 0.8, 0.1, 0.1], 'String', "Avg Total Work = " + ...
    avgWorkateachtime)
%
```

```
% figure(1)
% plot(intofpateachlength)
% set(gca,'xticklabel',(h));
% title('Total Work Done Over Normalized Length of Flagellum')
% xlabel('Normalized Length');
% ylabel('Work [J]');
% annotation('textbox', [0.60, 0.1, 0.1, 0.1], 'String', "Avg Total Work = " + ...
    avgWorkateachlength);

%%

if MOVIEYES,
    loops = num_frames;
    %loops = 30; %display first 30 frames
    M(loops) = struct('cdata',[],'colormap',[]);

    h = figure;
    h.Visible = 'off';
    for j = 1:loops
        plot(Out.Data.xArray(:,j),Out.Data.yArray(:,j))
        drawnow
        M(j) = getframe;
    end
    h.Visible = 'on';

    movie(M,1,3);
    %%

    loops = num_frames;
    %loops = 30; %display first 30 frames
    M(loops) = struct('cdata',[],'colormap',[]);

    h = figure;
    h.Visible = 'off';
    for j = 1:loops
        plot(xVals(:,j),yVals(:,j))
        drawnow
        M(j) = getframe;
```

```
    end
    h.Visible = 'on';

    movie(M,1,3);

end;
```

```
% RUn isomap to organize data int a closed cycle
% A Gupte PVB 2021 1207
%
clear;
%load Out1b.mat
%load Out2a.mat
%load Out2b.mat
%load Out3a.mat
%load Out3b.mat
%load Out4a.mat
%load Out5a.mat
%load Out5b.mat
%load Out6a.mat
%load Out6b.mat
%load Out7a.mat
%load Out8a.mat
%load Out9a.mat
%load Out9b.mat
%load Out10a.mat
load Out11b.mat

options.dims = 1:10;
options.verbose = false;
options.overlay =true;
num_neighbors =3;  % this is a hyper parameter and changing this gives ...
    different embedding result

[num_points, num_frames] = size(Out.Data.xArray)
[num_theta, num_theta_frames] = size(Out.Data.thetaArray)

```

```
% Using vector of theta

input_data = Out.Data.thetaArray(:,:);
D = L2_distance(input_data(:,:),input_data(:,:),1);

% PVB 2021 1207
% use distance criterion
eps = 1.5;      % choose to make simple closed curve, if periodic beating
[Y,R] = Isomap(D,'epsilon', eps, options);

%alternave us number criterion
 %[Y,R] = Isomap(D,'k', num_neighbors,options);


figure;
plot(Out.Data.xArray,Out.Data.yArray)
title("Plot of Input Data")
```