# Supplementary information: Monolithically-integrated cytometer for measuring particle diameter in the extracellular vesicle size range using multi-angle scattering

## Additional Figures

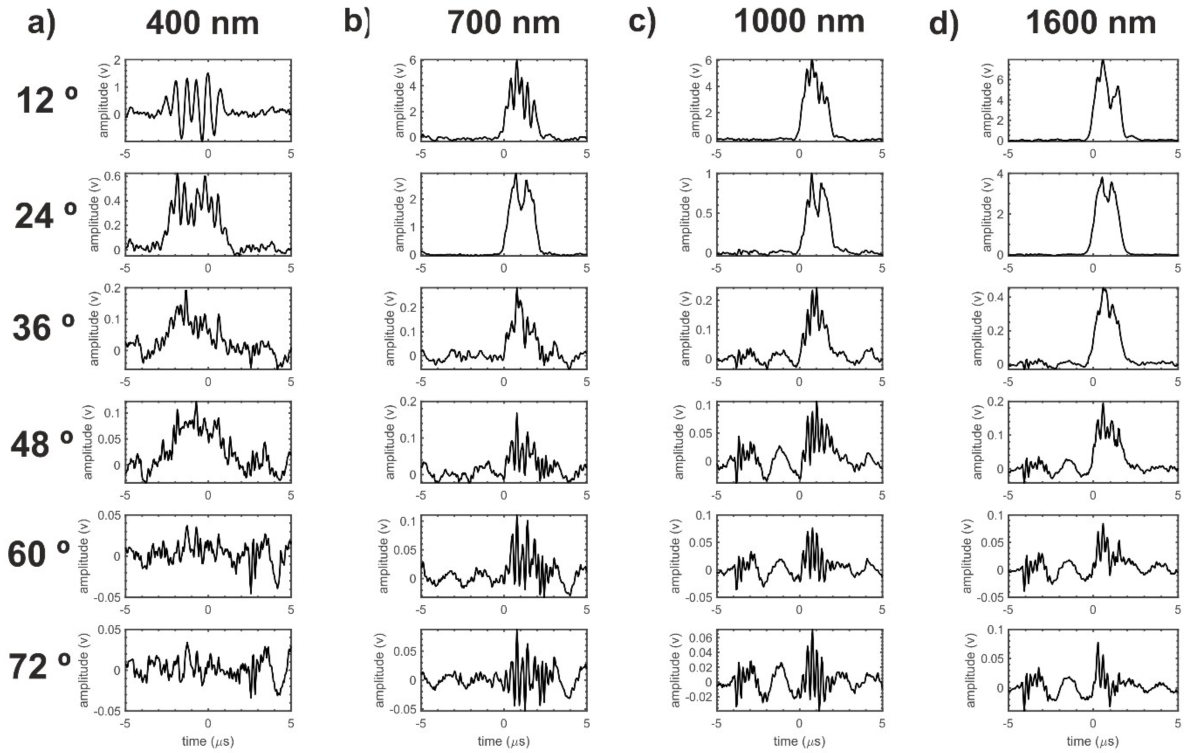


**Figure 1. Example detector pulse shapes on each angular collection channel for size standard samples of (a) 400 nm diameter, (b) 700 nm diameter, (c) 1000 nm diameter and (d) 1600 nm diameter.**

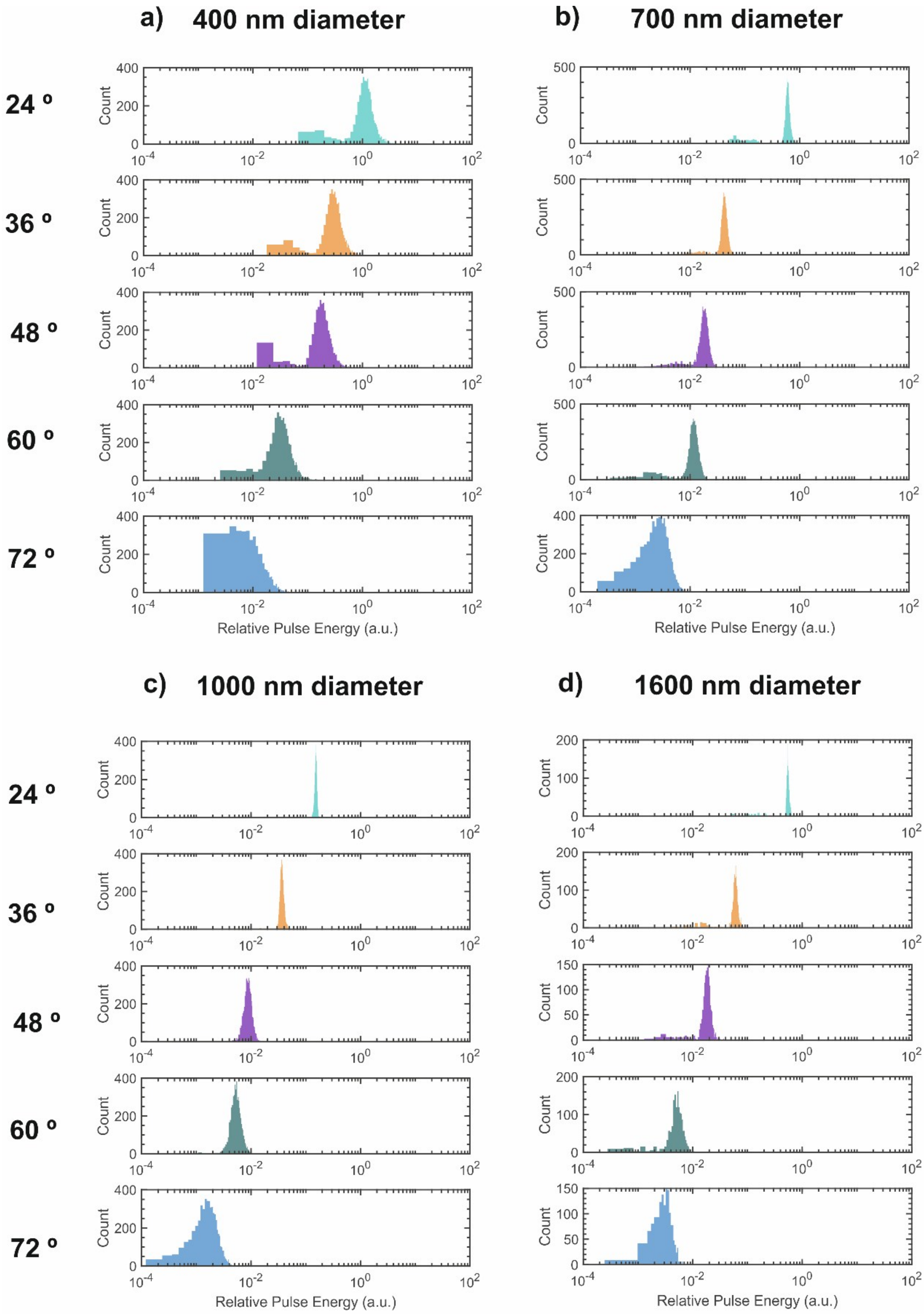


**Figure 2. Histograms of relative pulse energy on each collection channel for samples of (a) 400 nm diameter (N=5000), (b) 700 nm diameter (N=6838), (c) 1000 nm diameter (N=5444) and (d) 1600 nm diameter (N=1569).**

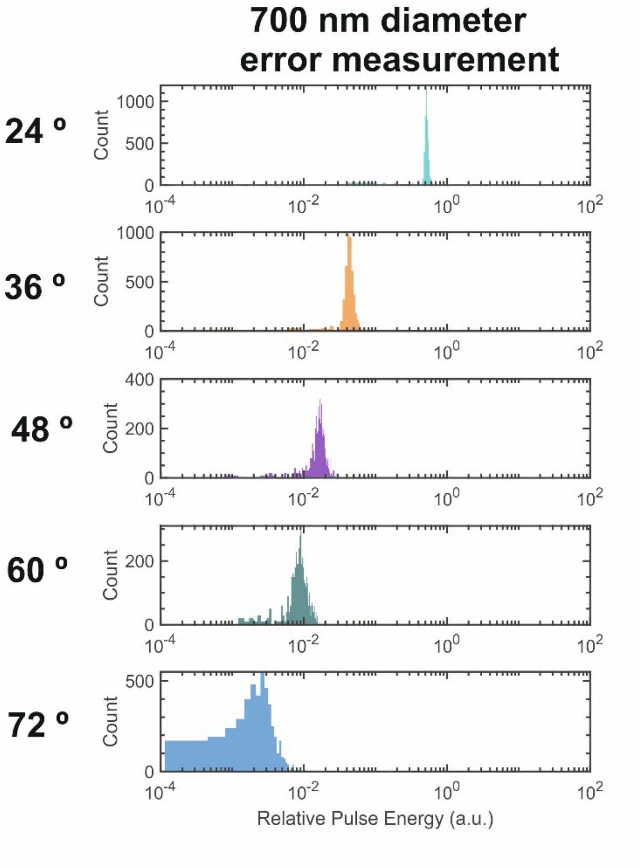


**Figure 3: Histograms of relative pulse energy on each collection channel for a monodisperse sample of 700 nm diameter size standards (N=5000) when the device was initially installed in the apparatus. The variance of these measurements were used to establish measurement uncertainty for weighted least squares fitting.**

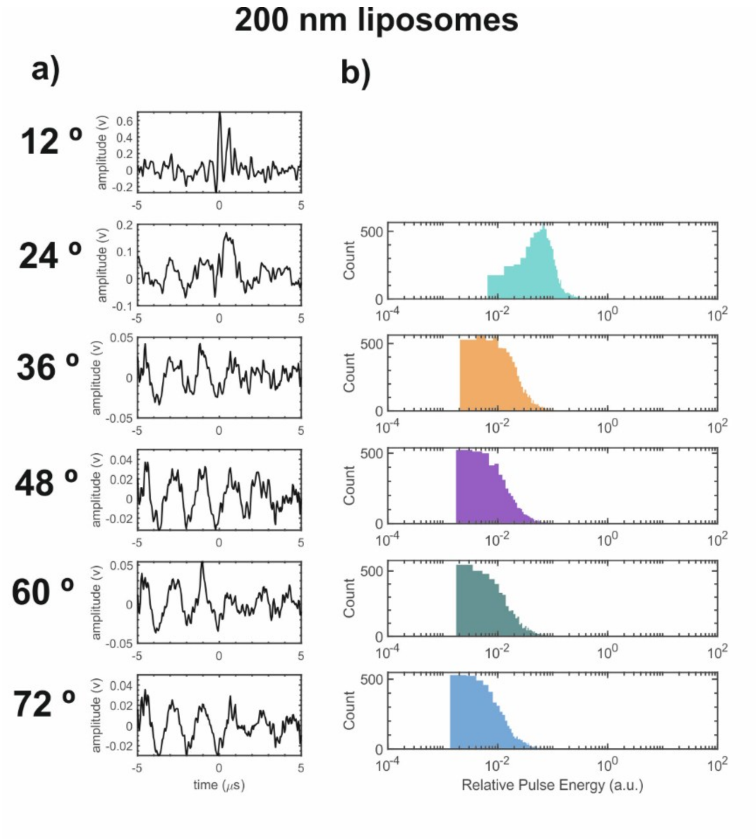


**Figure 4. (a) Example detector pulse shapes on each angular collection channel for a sample of 200 nm nominal diameter DPLC liposomes (N=10000) and (b) histograms of relative pulse energy on each collection channe**

**Table 1: Relative pulse energy statistics measured for polystyrene particle size standard samples and liposomes**

| | | Angular channel relative pulse energy (a.u.) | | | | | |
|---|---|---|---|---|---|---|---|
| **Nominal diameter** | **Channel>>** | **12°** | **24°** | **36°** | **48°** | **60°** | **72°** |
| **400 nm Polystyrene** | **median** | 1.00E+00 | 1.22E+00 | 3.20E-01 | 1.94E-01 | 3.58E-02 | 7.86E-03 |
| | **iqr** | 0.00E+00 | 5.74E-01 | 1.53E-01 | 1.00E-01 | 2.13E-02 | 1.06E-02 |
| | **rCV (%)** | 0.00 | 35.37 | 35.78 | 38.85 | 44.65 | 101.14 |
| | **mean** | 1.00E+00 | 1.47E+00 | 3.86E-01 | 2.34E-01 | 4.45E-02 | 1.02E-02 |
| | **StD** | 0.00E+00 | 5.47E+00 | 1.45E+00 | 9.14E-01 | 1.80E-01 | 5.81E-02 |
| | **CV (%)** | 0 | 373 | 377 | 390 | 405 | 568 |
| **700 nm Polystyrene** | **median** | 1.00E+00 | 6.07E-01 | 4.26E-02 | 1.85E-02 | 1.19E-02 | 2.89E-03 |
| | **iqr** | 0.00E+00 | 8.28E-02 | 7.46E-03 | 4.56E-03 | 3.26E-03 | 1.93E-03 |
| | **rCV (%)** | 0.00 | 10.23 | 13.14 | 18.47 | 20.47 | 49.97 |
| | **mean** | 1.00E+00 | 5.90E-01 | 4.26E-02 | 1.84E-02 | 1.18E-02 | 2.99E-03 |
| | **StD** | 0.00E+00 | 1.45E-01 | 1.71E-02 | 1.12E-02 | 5.26E-03 | 1.66E-03 |
| | **CV (%)** | 0 | 25 | 40 | 61 | 44 | 55 |
| **1000 nm Polystyrene** | **median** | 1.00E+00 | 1.53E-01 | 3.68E-02 | 8.98E-03 | 5.48E-03 | 1.74E-03 |
| | **iqr** | 0.00E+00 | 1.02E-02 | 4.22E-03 | 1.98E-03 | 1.58E-03 | 1.05E-03 |
| | **rCV (%)** | 0.00 | 5.01 | 8.60 | 16.51 | 21.59 | 45.11 |
| | **mean** | 1.00E+00 | 1.55E-01 | 3.72E-02 | 9.13E-03 | 5.64E-03 | 1.80E-03 |
| | **StD** | 0.00E+00 | 2.94E-02 | 4.65E-03 | 1.90E-03 | 1.42E-03 | 8.58E-04 |
| | **CV (%)** | 0 | 19 | 13 | 21 | 25 | 48 |
| **1600 nm Polystyrene** | **median** | 1.00E+00 | 5.47E-01 | 6.04E-02 | 1.84E-02 | 5.32E-03 | 3.07E-03 |
| | **iqr** | 0.00E+00 | 4.19E-02 | 7.95E-03 | 3.77E-03 | 1.59E-03 | 1.43E-03 |
| | **rCV (%)** | 0.00 | 5.75 | 9.87 | 15.36 | 22.48 | 34.89 |
| | **mean** | 1.00E+00 | 5.28E-01 | 5.86E-02 | 1.83E-02 | 5.30E-03 | 3.03E-03 |
| | **StD** | 0.00E+00 | 1.03E-01 | 1.25E-02 | 4.40E-03 | 1.49E-03 | 1.16E-03 |
| | **CV (%)** | 0 | 19 | 21 | 24 | 28 | 38 |
| **200 nm Liposomes** | **median** | 1.00E+00 | 6.69E-02 | 9.63E-03 | 3.03E-03 | 2.71E-03 | 2.51E-03 |
| | **iqr** | 0.00E+00 | 6.97E-02 | 2.21E-02 | 1.87E-02 | 1.89E-02 | 1.47E-02 |
| | **rCV (%)** | 0.00 | 78.16 | 171.94 | 463.01 | 522.20 | 438.71 |
| | **mean** | 1.00E+00 | 1.05E-01 | 1.14E-02 | 3.18E-03 | 4.85E-03 | 2.86E-03 |
| | **StD** | 0.00E+00 | 1.71E+00 | 3.01E-01 | 4.90E-01 | 2.78E-01 | 1.45E-01 |
| | **CV (%)** | 0 | 1637 | 2644 | 15389 | 5720 | 5060 |
| **Device measurement error using 700 nm diameter size standards** | **median** | 1.00E+00 | 5.18E-01 | 4.36E-02 | 1.68E-02 | 9.03E-03 | 2.54E-03 |
| | **iqr** | 0.00E+00 | 3.96E-02 | 8.07E-03 | 4.02E-03 | 3.02E-03 | 1.73E-03 |
| | **rCV (%)** | 0.00 | 5.73 | 13.87 | 17.94 | 25.06 | 50.91 |
| | **mean** | 1.00E+00 | 5.08E-01 | 4.45E-02 | 1.67E-02 | 9.17E-03 | 2.69E-03 |
| | **StD** | 0.00E+00 | 1.04E-01 | 1.40E-02 | 4.10E-03 | 2.63E-03 | 1.75E-03 |
| | **Variance** | 0.00E+00 | 1.08E-02 | 1.96E-04 | 1.68E-05 | 6.92E-06 | 3.08E-06 |
| | **CV (%)** | 0 | 20 | 31 | 25 | 29 | 65 |

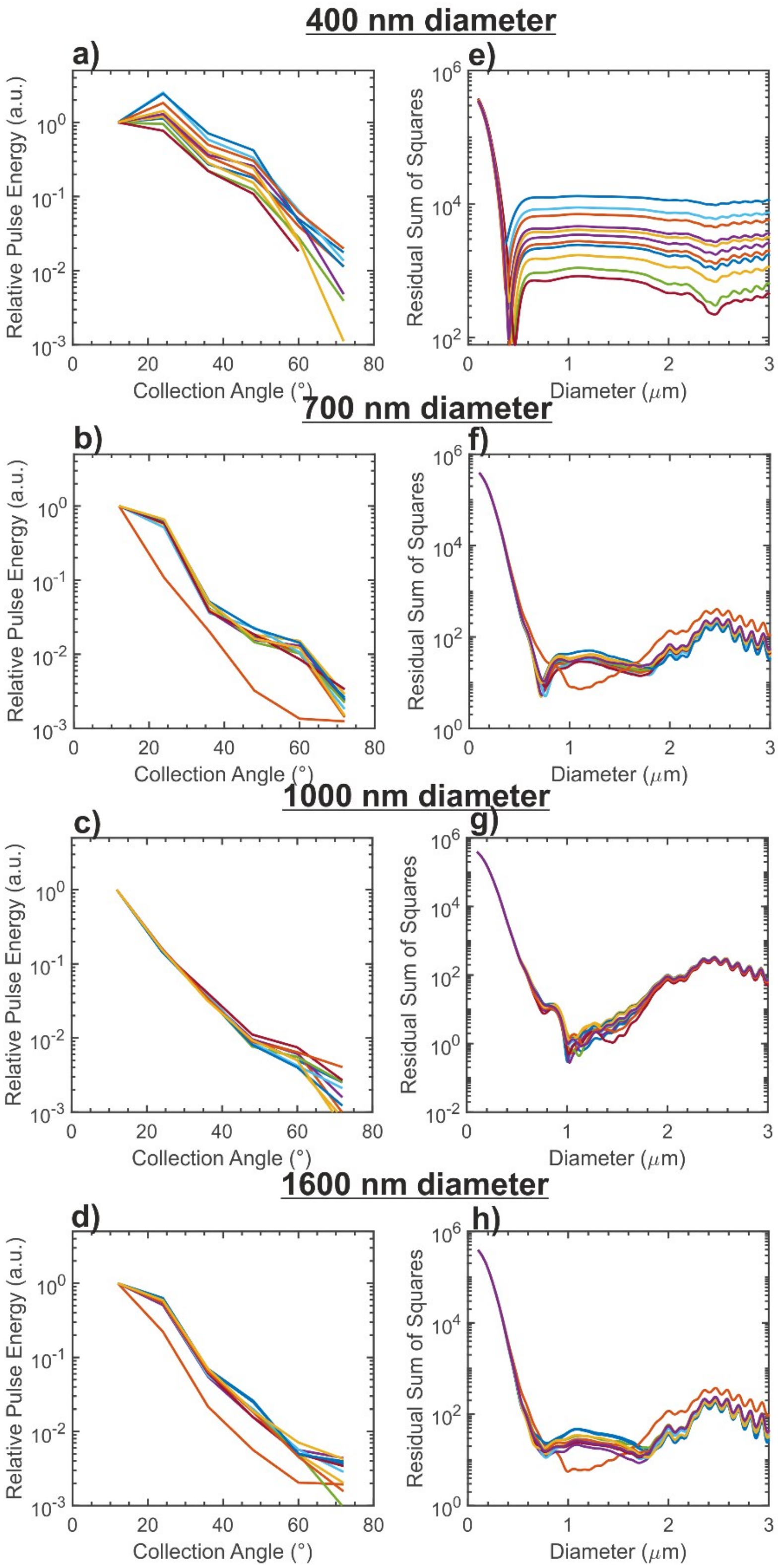


**Figure 5. (a)-(d) Example recorded scattering distributions for 10 individual particles for each nominal diameter. (e)-(h) Weight least squares values for fitting calculated diameter.**

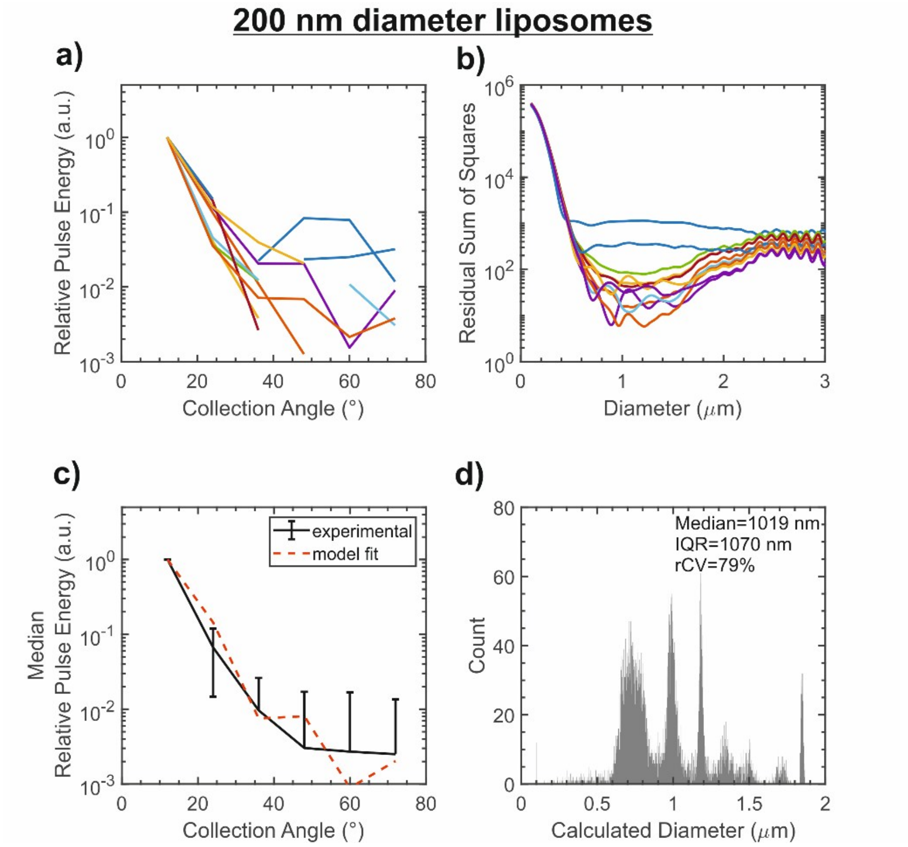


**Figure 6. Measurements from DPLC liposomes of 200 nm nominal diameter. (a) Example recorded scattering distributions for 10 individual liposomes with (b) weighted least squares values for fitting diameter. (c) Experimentally measured median scattering distribution for a monodisperse sample of liposomes with IQR error bars compared to best fit scattering distribution from analytical model. (d) Histograms of particle diameter calculated by fitting of the analytical model to individual measured scattering distributions of 200 nm liposomes with the median calculated diameter shown inset with IQR and rCV.**

## Matlab programme Scripts

### Script 1: Converting DAQ card files to matlab .m

This script reads batches TPC5 files which contain the pulse data on the DAQ card and converts the data into .m files which are then saved.

EVC_trace_analysis_2018_multiblock__multifile_v05_p1000.m

```
% Adapted by: 2018-05-01, Jonathan Butement from Elysy Switzerland script
% Description:
% This example shows how to read and plot traces from a TPC5 file.
%Pulse array now flipped correctly
%Pulse max amplitude now time correlated for all channels
clear variables
close all
% Filename and path to Elsys tpc5 file
tic
DC_filename = 'TM P700 750mms DC 01.tpc5';
% Read number of traces and channel information
nTraces=7;
% loop through each channel
for n = 1:nTraces
    % one single trace
    [x, y, traceInfo] = tpc5Trace(DC_filename, n);

    % build an array with all Y values
    DC_C(n,:)=y;
    DC_opt(n,:)=traceInfo;;
end
DC_offset=mean(DC_C,2);
```

```
DC_SD=std(DC_C,0,2);
DC_CV=100*std(DC_C,0,2)./DC_offset;
for file_num=1:10;
    clear C
    clear C3
filename = sprintf('TM P700 750mms AC t0-5h0-1 %02g.tpc5',file_num); %replace 2 digit
enumerator before .tpc6 with %02g
beamw=10e-6; %Width of beam in metres
SAVE=1; %save all variables with origninalfilename.m
% Read number of traces and channel information
nTraces= tpc5Info(filename);
nTraces=7;
nblocks=1000;
for block=1:nblocks
% loop through each channel
for n = 1:nTraces
    % one single trace
    [x, y, traceInfo] = tpc5Trace_mb(filename, n,block);

    % build an array with all Y values
    C(n,:)=y;
    opt(n,:)=traceInfo;;
end
C3(:,:,block)=C;
triggerTimeSeconds(block)=traceInfo.triggerTimeSeconds;
end
C3=flipud(rot90(C3)); %now flipped with time going correct direction!
t=x; %time vector in sec
SF=traceInfo.sampleRateHertz;
dt=1/traceInfo.sampleRateHertz; %time increment in s
time1reading=toc
%% multiblock analysis
chn=[12:12:12*nTraces];

for i=1:size(C3,3);
    [pulse_max_v(i), pulse_max_t(i)]=max(C3(:,1,i),[],1);
    pulse_max(i,:)=C3(pulse_max_t(i),:,i);
end
pulse_mean=mean(pulse_max,1);
pulse_median=median(pulse_max,1);
pulse_mode=mode(pulse_max,1);
pulse_sd=std(pulse_max,1);
count_rate=size(C3,3)/triggerTimeSeconds(end);
if SAVE==1
    save(strcat('Timecor',filename(1:end-5)))
else
end
end
%% Figures
figure(1)
hold on
for i=1:nTraces-1
histogram(pulse_max(:,i),50);
end
hold off
xlabel('voltage');
ylabel('count');
legend('12 \circ','24 \circ','36 \circ','48 \circ','60 \circ','72 \circ','control');
set(gca,'xScale','log')
figure(103)
histogram(pulse_max(:,3),100);
figure(104)
errorbar(chn(1:6),pulse_mean(1:6),pulse_sd(1:6),'LineWidth',2);
hold on
errorbar(chn(1:6),pulse_median(1:6),pulse_sd(1:6),'LineWidth',2);
errorbar(chn(1:6),pulse_mode(1:6),pulse_sd(1:6),'LineWidth',2);
hold off
set(gca,'yScale','log')
xlabel('Collection angle (degrees)');
ylabel('Pulse max amplitude / V');
title('Scattered power distribution');
threshold_line=refline([0 pulse_mean(end)]);
threshold_line.Color='r';
threshold_line2=refline([0 pulse_mean(end)+pulse_sd(end)]);
threshold_line2.Color='y';
set(gca,'XMinorTick','on','YMinorTick','on','fontsize',14,'Linewidth',1)
grid on
```

```
grid minor
figure(2)
plot(t,C(1,:))
figure(3)
plot(t,C3(:,1,1000))
```

## Script 2: Pulse analysis

Calculates pulse area (energy) for each pulse and channel for a batch of .m files

### EVC_combi_v11_p400_3D_750mms_t0_7m_time_corr.m

```
clear all
close all
SAVE=0;
SAVE2=0;
combi_file='TimecorTM P400 750mms AC t1-5 h0-1 %02g.mat';

legend_entries=["12 \circ","24 \circ","36 \circ","48 \circ","60 \circ","72 \circ","control"];

pulse=[];
count=[];
pulse_shape=[];
loop=0;
for i=1:5
%load(sprintf(combi_file,i));
load(sprintf(combi_file,i),'pulse_max','count_rate','chn','C3','t','DC_offset','DC_SD','DC_CV'
);
%C3=flipud(C3); % FLIPPS columns of FILES CREATED BEFORE 08/10/18 SO TIME IS CORRECT DIRECTION
loop=loop+1;
acq_mean(loop,:)=mean(pulse_max);
pulse=[pulse; pulse_max];
pulse_shape=cat(3,pulse_shape,C3);
count=[count; count_rate];
end

%%
thresh=0; %split data with threshold
area_boundaries=[470:570];
RGB=[146 0 0;0 146 146;219 109 0; 73 0 146;0 73 73;0 109 219]/255;
bin_num1=[40 150 125 170 500 200];
bin_num2=[120 150 200 216 700 200];

pulse_above=find(pulse(:,1)>thresh);
pulse_below=pulse(find(pulse(:,1)<=thresh),:);
pulse=pulse(pulse_above,:);

pulse_shape_above=pulse_shape(:,:,pulse_above);
pulse_shape_mean=mean(pulse_shape_above,3);
pulse_area=sum(pulse_shape_above(area_boundaries,:,:),1);
pulse_area_rel=pulse_area./pulse_area(:,1,:);

DC_offset_rel=DC_offset./DC_offset(1);
DC_offset_rel=DC_offset';
pulse_rel=pulse./pulse(:,1);

area_mean=mean(pulse_area,3);
area_median=median(pulse_area,3);
area_mode=mode(pulse_area,3);
area_sd=std(pulse_area,0,3);
area_iqr=iqr(pulse_area,3);
area_cv=100*area_sd./area_mean;
area_rcv=100*0.75*area_iqr./area_median;
axport_v_area=[area_mean; area_median; area_mode; area_sd; area_cv; area_iqr; area_rcv];

amp_mean=mean(pulse);
amp_median=median(pulse);
amp_mode=mode(pulse);
amp_sd=std(pulse);
amp_iqr=iqr(pulse);
amp_cv=100*amp_sd./amp_mean;
amp_rcv=100*0.75*amp_iqr./amp_median;

amp_mean_below=mean(pulse_below);
amp_median_below=median(pulse_below);
```

```
amp_mode_below=mode(pulse_below);
amp_sd_below=std(pulse_below);
amp_iqr_below=iqr(pulse_below);
amp_cv_below=100*amp_sd_below./amp_mean_below;
amp_rcv_below=100*0.75*amp_iqr_below./amp_median_below;

count_rate_mean=mean(count);
count_rate_sd=std(count);
count_rate_cv=100*count_rate_sd/count_rate_mean;

acq_sd=std(acq_mean(:,1));
acq_cv=100*acq_sd/amp_mean(1);

axport_v=[amp_mean; amp_median; amp_mode; amp_sd; amp_cv; amp_iqr; amp_rcv];
axport_count=[count_rate_mean count_rate_sd acq_cv];
axport_DC=[DC_offset';DC_SD';DC_CV'];

clear 'i'
if SAVE==1
    save(strcat('Combi',combi_file(1:end-8)))
else
end
%%
figure(1)
%errorbar(chn(1:6),amp_mean(1:6),amp_sd(1:6),'LineWidth',2);
hold on
errorbar(chn(1:6),amp_median(1:6),amp_iqr(1:6)./2,'LineWidth',2);
%errorbar(chn(1:6),amp_median_below(1:6),amp_iqr_below(1:6),'LineWidth',2);
%errorbar(chn(1:6),amp_mode(1:6),amp_sd(1:6),'LineWidth',2);
errorbar(chn(1:6),3.*DC_SD(1:6),[],'LineWidth',2);
hold off
set(gca,'yScale','log')
xlabel('Collection angle (degrees)');
ylabel('Pulse max amplitude / V');
title('Scattered power distribution');
threshold_line=refline([0 amp_median(end)]);
threshold_line.Color='r';
threshold_line2=refline([0 amp_median(end)+0.5*amp_iqr(end)]);
threshold_line2.Color='y';
set(gca,'XMinorTick','on','YMinorTick','on','fontsize',14,'Linewidth',1)
legend('median','3sd detection limit');
grid on
grid minor

% figure(11)
% polarplot(chn(1:6)*pi()./180,log10(1000*amp_median(1:6)));

figure(12)
hold on
for i=1:200:1001
plot(chn(1:6),pulse(i,1:6),'LineWidth',2);
end
hold off
set(gca,'yScale','log')
xlabel('Collection angle (degrees)');
ylabel('Pulse max amplitude / V');
title('PULSE AMPLITUDE individual scatter distribution');
% threshold_line=refline([0 amp_median(end)]);
% threshold_line.Color='r';
% threshold_line2=refline([0 amp_median(end)+0.5*amp_iqr(end)]);
% threshold_line2.Color='y';
set(gca,'XMinorTick','on','YMinorTick','on','fontsize',14,'Linewidth',1)
%legend('median','3sd detection limit');
grid on
grid minor

figure(13)
plot(chn(1:6),area_median(1:6),'LineWidth',2);
errorbar(chn(1:6),area_median(1:6),area_iqr(1:6)./2,'LineWidth',2);
set(gca,'yScale','log')
xlabel('Collection angle (degrees)');
ylabel('Pulse area / V.s');
title('PULSE AREA MEDIAN');
% threshold_line=refline([0 amp_median(end)]);
% threshold_line.Color='r';
% threshold_line2=refline([0 amp_median(end)+0.5*amp_iqr(end)]);
% threshold_line2.Color='y';
```

```
set(gca,'XMinorTick','on','YMinorTick','on','fontsize',14,'Linewidth',1)
legend('median','3sd detection limit');
grid on
grid minor

figure(14)
hold on
for i=1:20:1001
plot(chn(1:6),pulse_area(1,1:6,i),'LineWidth',2);
end
hold off
set(gca,'yScale','log')
xlabel('Collection angle (degrees)');
ylabel('Pulse area / V.s');
title('PULSE AREA');
% threshold_line=refline([0 amp_median(end)]);
% threshold_line.Color='r';
% threshold_line2=refline([0 amp_median(end)+0.5*amp_iqr(end)]);
% threshold_line2.Color='y';
set(gca,'XMinorTick','on','YMinorTick','on','fontsize',14,'Linewidth',1)
%legend('median','3sd detection limit');
grid on
grid minor

figure(15)
hold on
for i=1:20:1001
plot(chn(1:6),pulse_area_rel(1,1:6,i),'LineWidth',2);
end
hold off
set(gca,'yScale','log')
xlabel('Collection angle (degrees)');
ylabel('Pulse area / V.s');
title('PULSE AREA');
% threshold_line=refline([0 amp_median(end)]);
% threshold_line.Color='r';
% threshold_line2=refline([0 amp_median(end)+0.5*amp_iqr(end)]);
% threshold_line2.Color='y';
set(gca,'XMinorTick','on','YMinorTick','on','fontsize',14,'Linewidth',1)
%legend('median','3sd detection limit');
grid on
grid minor

figure(2)
hold on
for i=1:6
histogram(pulse(:,i));
end
hold off
xlabel('voltage');
ylabel('count');
legend('12 \circ','24 \circ','36 \circ','48 \circ','60 \circ','72 \circ','control');
set(gca,'xScale','log')

figure(21)
hold on
for i=1:6
histogram(pulse_area(:,i,:),200);
end
hold off
xlabel('voltage.seconds');
ylabel('count');
legend('12 \circ','24 \circ','36 \circ','48 \circ','60 \circ','72 \circ','control');
set(gca,'xScale','log')

figure(3)
hold on
for i=1:7
    subplot(7,1,i)
histogram(pulse(:,i));
set(gca,'xScale','log')
xlim([1e-2 1e2]);
ylabel( legend_entries(i));
end
hold off
xlabel('voltage');
```

```
figure(31)
hold on
for i=1:7
    subplot(7,1,i)
histogram(pulse_area(:,i,:));
set(gca,'xScale','log')
xlim([1e-2 1e3]);
ylabel( legend_entries(i));
end
hold off
xlabel('voltage');

figure(4)
subplot(2,1,1);
plot(t*1e6,pulse_shape(:,1,10));
set(gca,'XMinorTick','on','YMinorTick','on')
grid on
grid minor
ylabel('amplitude (v)');
xlabel('time (\mus)');
xlim([-5 5]);
legend('single pulse')
subplot(2,1,2);
plot(t*1e6,pulse_shape(:,1,10));
hold on
plot(t*1e6,pulse_shape_mean(:,1,1),'LineWidth',1);
set(gca,'XMinorTick','on','YMinorTick','on')
grid on
grid minor
ylabel('amplitude (v)');
xlabel('time (\mus)');
xlim([-5 5]);
legend('single pulse', 'average pulse')
hold off

figure(41)
subplot(2,1,1);
plot(pulse_shape(:,1,10));
set(gca,'XMinorTick','on','YMinorTick','on')
grid on
grid minor
ylabel('amplitude (v)');
xlabel('time (\mus)');
% xlim([-5 5]);
legend('single pulse')
subplot(2,1,2);
plot(pulse_shape(:,1,10));
hold on
plot(pulse_shape_mean(:,1,1),'LineWidth',1);
set(gca,'XMinorTick','on','YMinorTick','on')
grid on
grid minor
ylabel('amplitude (v)');
xlabel('time (\mus)');
% xlim([-5 5]);
legend('single pulse', 'average pulse')
hold off

figure(5)
for fig=1:5
subplot(5,1,fig);
plot(t*1e6,pulse_shape(:,1,fig));
set(gca,'XMinorTick','on','YMinorTick','on')
grid on
grid minor
ylabel('amplitude (v)');
xlabel('time (\mus)');
xlim([-5 5]);
legend('single pulse')
end

figure(6)
for fig=1:6
subplot(6,1,fig);
plot(t*1e6,pulse_shape(:,fig,1));
set(gca,'XMinorTick','on','YMinorTick','on')
grid on
```

```
grid minor
ylabel('amplitude (v)');
ylabel(strcat(legend_entries(fig),'amplitude (V)'))
xlabel('time (\mus)');
xlim([-5 5]);
legend('single pulse')
end

set(gcf,'renderer','Painters')
figure(7)
for fig=1:6
subplot(6,1,fig);
plot(t*1e6,pulse_shape(:,fig,pulse_above(100)),'Linewidth',1.5,'color','k');
set(gca,'XMinorTick','on','YMinorTick','on','fontsize',11,'linewidth',1.5)

ylabel('amplitude (v)');
%ylabel(strcat(legend_entries(fig),'amplitude (V)'))
%xlabel('time (\mus)');
xlim([-5 5]);
end
xlabel('time (\mus)');
set(figure(7), 'Position', [1, 1, 300, 1100]);
print -depsc2 -tiff -r300 -painters 400nm_pulse_shapes.eps

figure(8)
for i=1:6
subplot(6,1,i)
h1=histogram(pulse(:,i),'EdgeAlpha',0,'FaceColor',RGB(i,:));
set(gca,'xScale','log','fontsize',14,'XMinorTick','on','YMinorTick','on','linewidth',1.5)
xlim([1e-3 1e2]);
%ylabel( legend_entries(i));
ylabel('Count');
end
xlabel('Pulse peak amplitude (V)');
set(figure(8), 'Position', [1, 1, 600, 1100]);
if SAVE2==1;
print -depsc2 -tiff -r300 -painters 400nm_pulse_histograms.eps
else
end

figure(81)
for i=1:6
subplot(6,1,i)
h2=histogram(pulse_area(:,i,:),'EdgeAlpha',0,'FaceColor',RGB(i,:));
set(gca,'xScale','log','fontsize',14,'XMinorTick','on','YMinorTick','on','linewidth',1.5)
xlim([1e-2 1e3]);
%ylabel( legend_entries(i));
ylabel('Count');
end
xlabel('Pulse energy (a.u.)');
set(figure(81), 'Position', [1, 1, 600, 1100]);
if SAVE2==1;
print -depsc2 -tiff -r300 -painters 400nm_pulse_area_histograms.eps
else
end
```

## Script 3: Calculation of theoretical relative scattering distributions

Calculates theoretical relative scattering distributions as would be recorded on the cytometer for a range of particle diameters.

### Scatter_distribution_regression_v07_700.m

```
%Modification by Jonathan Butement 18/09/2018

% version _ph: only calculate the relevant radii, not a grid

%Scans sphere radii scattering cross section and then loops through
%through each discrete collection waveguide angle.
%Produces a matrix ,qexample1_wg, with rows=radii columns=waveguide angle
%magnitude=scatter cross section

% adapted from part 3 of mie_test1.m code by Peter Horak, 2018.
% based on Mie_xscan by C. Mätzler, May 2002.
```

```
% define microsphere and medium
clear all
SAVE=1;
lambda = 0.532;       % wavelength [um]

% nsphere = silica_n(lambda);      % refractive index of silica sphere
nsphere=1.598;                     % refractive index of polystyrene sphere at 532 nm
nmedium = 1.3337;         % refractive index of surrounding medium

k0 = 2*pi*nmedium/lambda;    % wave number in surrounding medium

a=[0.05:0.001:1.55];    %radii to plot on graph
[0.380000000000000,0.580000000000000,1.48000000000000,0.620000000000000]
d=2*a;           %diameters to plot

% set parameters
m = nsphere/nmedium;

%%%%%%%%%%% example 1:
%%%%%%%%%%% light scattered into a given range of angles vs sphere radius


xvec = a*k0;
avec = a;        % just for consistency with previous version

% calculate scattering into directions around 45 degrees (+/-5) from laser
% propagation direction (="left/right"), and 90 degrees (+/-5) from laser
% polarisation axis (="up/down")

acc=7.38; % NA 532 0.128 half angle of acceptance of waveguide (based on wg numerical
aperture)
loop=0% for loop counter

for i=12:12:72 %loopes through each waveguide collection angle (phi)

qexample1 = Mie_angle_integral_xscan2(m,xvec,i-acc,i+acc,85.4,94.6);

loop=loop+1
qexample1_wg(:,loop)=qexample1; %creates matrix with data for each collection waveguide in
collumn
end

qexample1_wg_corr=qexample1_wg./(ones(length(avec),1)*sin((12:12:72)*pi/180)); % CORRECTION
divides by 1/sin(theta)

qexample1_wg_rel=qexample1_wg./(max(qexample1_wg,[],2)*ones(1,6)); %converts to relative power
distribution
qexample1_wg_corr_rel=qexample1_wg_corr./(max(qexample1_wg_corr,[],2)*ones(1,6)); %converts to
relative power distribution


%% Figures
close all

row=1:length(avec);    %finds row in matrix or selected radii
%legend_d=strsplit(num2str(d(:)')); % Diameter labels for figure legend
legend_d=num2str(d(:)); % Diameter labels for figure legend

figure (1)
plot(avec,qexample1)
xlabel('Radius a (um)')
ylabel('Scattering cross section')
%title(['Mie efficiencies in angle teta=[' num2str(teta1) ',' num2str(teta2) '], phi=['
num2str(phi1) ',' num2str(phi2) ']'])

figure (2)
surf(qexample1_wg_corr);

figure(3)
plot(12:12:72,qexample1_wg_rel)

xlabel('Waveguide collection angle (\circ)')
ylabel('Relative power (a.u.)')
set(gca,'yScale','log');
legend(legend_d);
title('NOT corrected relative wg measured scatter distribution');
```

```
figure(4)
plot(12:12:72,qexample1_wg_corr_rel)

xlabel('Waveguide collection angle (\circ)')
ylabel('Relative power (a.u.)')
set(gca,'yScale','log');
legend(legend_d);
title('CORRECTED relative wg measured scatter distribution');

max_v=184.824 % max voltage reading to produce normalised data
qexample1_wg_corr_norm_max=max_v*qexample1_wg_corr./qexample1_wg_corr(4,1);

figure(5)
plot(12:12:72,qexample1_wg_corr_norm_max,'linewidth',2)

xlabel('Waveguide collection angle (\circ)')
ylabel('Voltage (V.)')
set(gca,'yScale','log','linewidth',2);
legend(legend_d);
title('CORRECTED absolute wg measured scatter distribution');

figure(6)
hold on
for i=1:6
plot(d,qexample1_wg_corr_norm_max);
end
xlabel('Particle diameter (\circ)')
ylabel('Voltage (V.s.)')
set(gca,'yScale','log','linewidth',2);
legend('12','24','36','48','60','72');
xlim([0 2]);

if SAVE==1
save('EVC_scatter_distribution_jb_r2');
else
end
```

## Script 4: Weighted least squares regression

Calculates diameter of particle from relative pulse energies by weighted least squares regression fitting of the theoretical curves from script 3 to individual scattering distributions measured from particles.

```
%Modification by Jonathan Butement 18/09/2018

% version _ph: only calculate the relevant radii, not a grid

%Scans sphere radii scattering cross section and then loops through
%through each discrete collection waveguide angle.
%Produces a matrix ,qexample1_wg, with rows=radii columns=waveguide angle
%magnitude=scatter cross section

% adapted from part 3 of mie_test1.m code by Peter Horak, 2018.
% based on Mie_xscan by C. Mätzler, May 2002.

% define microsphere and medium
clear all
SAVE=1;
lambda = 0.532;      % wavelength [um]

% nsphere = silica_n(lambda);     % refractive index of silica sphere
nsphere=1.598;                    % refractive index of polystyrene sphere at 532 nm
nmedium = 1.3337;        % refractive index of surrounding medium

k0 = 2*pi*nmedium/lambda;    % wave number in surrounding medium

a=[0.05:0.001:1.55];    %radii to plot on graph
[0.380000000000000,0.580000000000000,1.48000000000000,0.620000000000000]
d=2*a;          %diameters to plot

% set parameters
m = nsphere/nmedium;

%%%%%%%%%%% example 1:
%%%%%%%%%%% light scattered into a given range of angles vs sphere radius
```

```
xvec = a*k0;
avec = a;       % just for consistency with previous version

% calculate scattering into directions around 45 degrees (+/-5) from laser
% propagation direction (="left/right"), and 90 degrees (+/-5) from laser
% polarisation axis (="up/down")

acc=7.38; % NA 532 0.128 half angle of acceptance of waveguide (based on wg numerical
aperture)
loop=0% for loop counter

for i=12:12:72 %loopes through each waveguide collection angle (phi)

qexample1 = Mie_angle_integral_xscan2(m,xvec,i-acc,i+acc,85.4,94.6);

loop=loop+1
qexample1_wg(:,loop)=qexample1; %creates matrix with data for each collection waveguide in
collumn
end

qexample1_wg_corr=qexample1_wg./(ones(length(avec),1)*sin((12:12:72)*pi/180)); % CORRECTION
divides by 1/sin(theta)

qexample1_wg_rel=qexample1_wg./(max(qexample1_wg,[],2)*ones(1,6)); %converts to relative power
distribution
qexample1_wg_corr_rel=qexample1_wg_corr./(max(qexample1_wg_corr,[],2)*ones(1,6)); %converts to
relative power distribution


%% Figures
close all

row=1:length(avec);    %finds row in matrix or selected radii
%legend_d=strsplit(num2str(d(:)')); % Diameter labels for figure legend
legend_d=num2str(d(:)); % Diameter labels for figure legend

figure (1)
plot(avec,qexample1)
xlabel('Radius a (um)')
ylabel('Scattering cross section')
%title(['Mie efficiencies in angle teta=[' num2str(teta1) ',' num2str(teta2) '], phi=['
num2str(phi1) ',' num2str(phi2) ']'])

figure (2)
surf(qexample1_wg_corr);

figure(3)
plot(12:12:72,qexample1_wg_rel)

xlabel('Waveguide collection angle (\circ)')
ylabel('Relative power (a.u.)')
set(gca,'yScale','log');
legend(legend_d);
title('NOT corrected relative wg measured scatter distribution');

figure(4)
plot(12:12:72,qexample1_wg_corr_rel)

xlabel('Waveguide collection angle (\circ)')
ylabel('Relative power (a.u.)')
set(gca,'yScale','log');
legend(legend_d);
title('CORRECTED relative wg measured scatter distribution');

max_v=184.824 % max voltage reading to produce normalised data
qexample1_wg_corr_norm_max=max_v*qexample1_wg_corr./qexample1_wg_corr(4,1);

figure(5)
plot(12:12:72,qexample1_wg_corr_norm_max,'linewidth',2)

xlabel('Waveguide collection angle (\circ)')
ylabel('Voltage (V.)')
set(gca,'yScale','log','linewidth',2);
legend(legend_d);
title('CORRECTED absolute wg measured scatter distribution');
```

```
figure(6)
hold on
for i=1:6
plot(d,qexample1_wg_corr_norm_max);
end
xlabel('Particle diameter (\circ)')
ylabel('Voltage (V.s.)')
set(gca,'yScale','log','linewidth',2);
legend('12','24','36','48','60','72');
xlim([0 2]);

if SAVE==1
save('EVC_scatter_distribution_jb_r2');
else
end
```