Part II Handout: Dynamics in Complex Fluids

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[1] *with thanks to Luigi Feriani, Nicola Pellicciotta, Steven Moseley and H. 'Eric' Hou for all their help in setting up the experiment since 2015, developing the codes and the handout.**

(Dated: Michaelmas Term 2018, version 6.0)

This is an experimental investigation aiming to: (a) measure the motility of micron-sized objects suspended in solutions of both Newtonian and viscoelastic fluids; (b) become familiar with, and test, a fairly new method of processing time-lapse video data, called Differential Dynamic Microscopy (DDM). Compared to traditional approaches such as Dynamic Light Scattering, or considering the optics required to obtain sharp images to perform particle tracking, the experimental setup here is made of very simple elements, and the components of the experiment do not have stringent technical constraints.

SOME RULES

A. Lab Generalities and time management

Bear in mind that the lab is open only at specified times, and you will need to spend most of these hours (or more, by running automated recordings!) on gathering experimental readings. You should certainly check frequently whilst in the lab that deductions from your readings are physically reasonable, but plan so that further computation and report-writing can be done outside the lab.

You can borrow external hard drives to keep personal copies of your data and facilitate data transfer (these should be returned in time for the next session though). The collection of data is separate from its analysis - don't underestimate the time on data analysis in this experiment!

You can analyse data on your own computers (copy over the relevant Matlab scripts) - demonstrator can help with this.

B. Health and Safety

1. Laser beam in DLS rig

The DLS experiment uses a laser of Class 3R. The University Safety Manual for Lasers states "Class 3R lasers are considered safe to the skin. (...) Class 3R lasers are potentially damaging to the eyes". The power density available from this laser is greater than the Maximum Permissible Exposure to the eye. Therefore users must take certain precautions, as follows:

- The laser beam should travel away from your working position at the bench.
- You should complete alignment swiftly; it is safer to perform alignment actions individually (preferably without others' help).
- Use the minimum beam intensity, by adjusting the polarizer. When not in use, switch off.
- Beware of 'secondary sources' produced by reflection from component surfaces. Identify them and minimise where possible by directing to somewhere absorbent near the main beam. Remove any rings and watches.
- Ensure that your main beam and secondaries cannot stray onto other benches - enclose them. If you remove the detector, use a 'beam stop'.
- Whenever you don't need to observe by eye, use the detector and electrical signal.

2. Sharps

In the later part of the experiment you will be working with microscope slides and cover slips. These are somewhat fragile, and it is not impossible you will break some. Dispose of all glass in the yellow 'sharps bin' provided. Promptly pick up and dispose of any stray glass from the floor or bench surfaces. Use wet paper to pick up small fragments. Do not use bare hands to pick up glass. Ask demonstrator (Mr Nicola Pellicciotta) or lab staff (Mr Richard King) if you need help.

INTRODUCTION - A QUICK PERSPECTIVE ON OPTICAL METHODS IN PHYSICS

In the 1970s, experimental condensed matter physics boomed thanks to the invention of laboratory *lasers*, which allowed spectroscopic and diffraction experiments to be carried out. This was the decade of critical phenomena, with beautiful work of both experimental and theoretical nature. Not coincidentally, the field of 'Soft Matter' ('Complex fluids', in the USA) was born. Initially the

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key systems that people investigated were liquid crystals and polymer solutions, where the physics of phase transitions (critical phenomena and universality) is key. Then quantitative and robust physical models expanded to cover and explain all kinds of materials in which entropy is important, such as foams, emulsions, suspensions of particles with finely tuned interactions, etc. This has been and still is a great area of science, with challenging fundamental questions and very important applications in the real world processing of fluids and materials. As well as technological challenges, open fundamental physics questions still remain, for example the kinetic arrest due to high concentration of particles (it is a glass transition) is a really hard problem, not fully understood despite decades of work. When studying these systems, the laser was the ideal light source, and most of the questions revolved around the thermodynamic behaviour, or measuring structure. The experimental efforts focused on averaging the observables across as much of the sample, and over time, so to increase precision and *remove* spurious effects of heterogeneity. Shining coherent light (laser) beams through systems, and studying the properties of the diffracted light allows precisely this kind of 'bulk average'. In classic far-field diffraction experiments, the information is obtained for each Fourier component in the system, i.e. it is well defined by lengthscale, and it is averaged over the illuminated sample volume. These approaches required high quality lasers (good coherence and power stability) and very stable mechanics of the setup.

Come the 2000s, many who could trace their academic roots (supervisors of supervisors...) to those early critical phenomena or Soft Matter days moved to looking at biological systems, for example collections of cells, tissues, or smaller scale structures within cells. In addressing many of these questions, averaging is now *not* good: A lot of information resides in the spatial variability, typically in the variability between cells. This is therefore naturally the realm of 'real space' microscopy, i.e. the art of magnifying in scale, and with various contrast enhancing tricks, to identify structures, whilst maintaining clear spatial information. Optical microscopy has a long and distinguished history. As well as improvements in the manufacture of various microscope components, various things happened around the turn of the century which -combined together- gave a sort of revolution: (a) computing processing power, and hard drive storage, had been increasing exponentially, and overtook the typical rate of "sensible" image acquisition, meaning one could save videos almost indefinitely, and also think of performing image analysis in real time; (b) digital cameras, which had been around from the 90s as expensive, slow and cumbersome devices (saving to tape, or to the PC through expensive and proprietary frame grabber hardware), quite suddenly became mainstream (driven by consumer, industry quality control, and security CCTV markets) and affordable. The sensor technology (size, number of pixels, noise levels, integration with electronics in CMOS sensors) also saw tremendous advances. This led to an explosion of camera-based experiments, opening new areas for physics in biology, and also in the other more traditional Soft Matter Physics systems, exploring that heterogeneity which other methods had been averaging out.

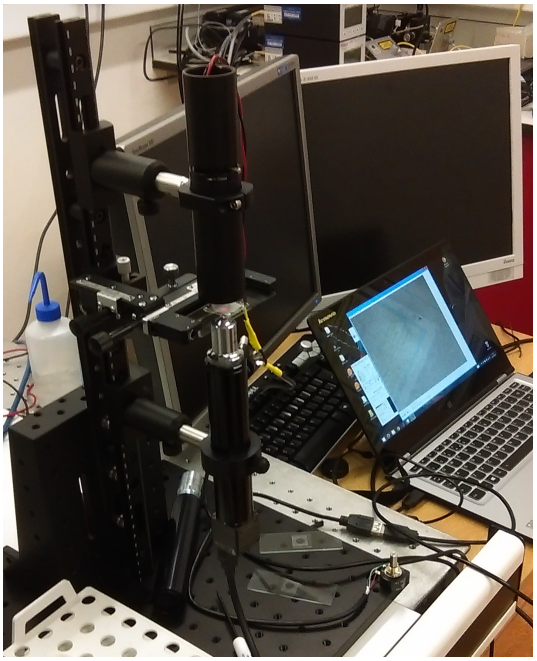


FIG. 1. The experimental setup built by Luigi Feriani in Cambridge, Summer 2015.

The previous paragraphs hopefully give a sense of perspective: it is interesting sometimes to look back and try to understand how certain areas boomed, and clusters of discovery took place, and how these can be traced to one or more of a new technology, some special individual, a particularly fruitful idea, or some strong societal drive. It is difficult to overstate the revolutionary impact of cameras that are now available for (cost to manufacturer) around $1, and provide "video rate" streams of images, with pixel numbers and density such that if you connect these up to an optical system then the camera itself will almost certainly not be the limiting factor in resolution. This means that *there has never been a better time to do imaging experiments*. Expensive cameras are still sometimes required, but only for some very special niche (ultra high speed, or single photon detection) applications, whereas a whole universe is accessible at unprecedented low cost, together with the computing power to process these signals and data streams.

You can now appreciate the beauty of the experiment proposed to you, which fits centrally in this historical moment. We take no credit for the DDM technique as used here [1], it was first described by R.Cerbino and V.Trappe [2], and is explained more fully in [3–5]: It exploits the power of cameras and computer, whilst put-

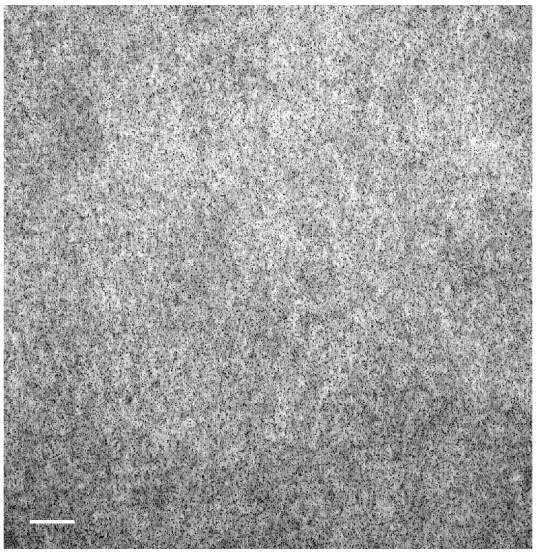


FIG. 2. Typical image of colloidal particles (500 nm in diameter) in solution. Scalebar is 50 μm. Contrast was enhanced for display purposes.

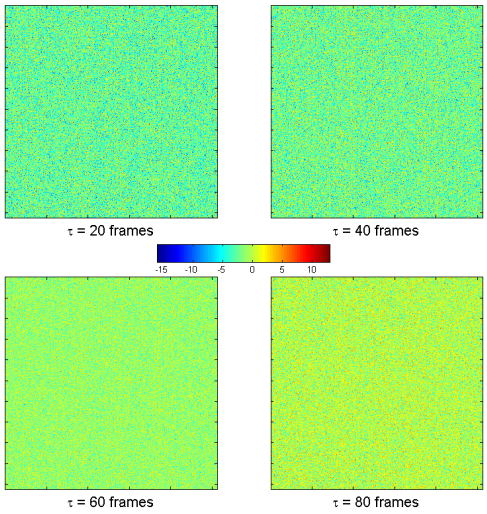


FIG. 3. Difference of frames, at increasing "lag times" τ.

ting really few constraints on the quality (i.e. cost) of all components, see Figure 1.

The systems you will study are spherical particles of colloidal size (colloid range is usually defined to be between 10 nm and a few μm). These scatter enough light to provide some contrast in the image. Looking at a sample containing such particles, if we know their size then the motility is well known (Brownian diffusion) and we can measure viscosity of Newtonian fluids. You will first test this well understood condition. Then you will explore ways of deviating from Brownian motion: It is possible to put particles in viscolatic fluids, which have a degree of elastic response (a 'memory term' in the stress-strain relation) and this typically gives 'sub-diffusive' motion. [6]

I. TO DO: DYNAMIC LIGHT SCATTERING - 2 DAYS

You are provided with a simple home-built dynamic light scattering (DLS) setup. A complete reference for this technique is [7]. Your demonstrator will help you to quickly get the system aligned, and to collect autocorrelation functions of the scattered intensity, from some dilute samples of colloidal particle suspensions.

This is the standard technique for particle sizing in the range from a few 10 nm to 1 μm, used worldwide over the last 50 years.

The purpose of starting your investigation with DLS is: (a) to understand its limits (some of which are related to the simple equipment you have, but some are intrinsic to the method); (b) understand (in relation to various "static" diffraction experiments you did in Part IB) what it means to work with light collected at a certain scattering angle.

You can move on when: (1) you have a few decent plots of autocorrelation functions, at a few (measured) angles, and have fitted the relaxation times τ_q; (2) you understand why in DLS of Brownian motion particles the relation $\tau_q = 1/Dq^2$ holds.

II. TO DO: DDM ON BROWNIAN MOTION - 3 DAYS

A. DDM method

We do not reproduce here the details of this method, for which you should go to the original references. We only summarize the main points. It is quite obvious that if you have a video of things that move around, then if you take a frame minus itself you will get a black result; if you take a frame minus the next frame you will get some signal; then more signal if you wait longer, and so on, until if things have moved enough that they have gone 'everywhere' in the image, there will be no more signal increase even if you wait for longer. Let's call the waiting interval a "lag time", and use the symbol τ.
It is perhaps also intuitive that if you look at the Fourier transforms of these 'difference frames', then the amplitude of each of the Fourier modes grows as a function of τ. What is probably not obvious is that a lot of information is contained in the function that describes the growth of this signal.

Scripts for the DDM analysis run in Matlab on the computers provided, and you are also welcome to copy them elsewhere if you want.

B. A guide to your first DDM based analysis

Let's take this one step at a time. A typical image of colloidal particles in solution looks like the one in Figure 2. The colloidal particles in focus appear as tiny black dots, and the gray background is diffuse light from other particles out of focus; the solution is quite dense (in this case, the volume fraction is high, and also the suspension scatters a lot of light - the two things do not always go together because the difference in index of refraction is also important for diffusing light) . If you look inside the microscope, you will see them move in a diffusive fashion, because of thermal motion. It is known from the work of Stokes and Einstein that indeed these microscopic spheres will diffuse such that their mean square displacement in each direction is

$$\langle x(\tau)^2 \rangle \;=\; 2D\,\tau,$$

and that the diffusion coefficient D is related to the particle radius a, the viscosity of the solvent η, the temperature T, Boltzmann's constant k_B by

$$D \;=\; \frac{k_B T}{6\pi\eta a}.$$

Examples of frame differences are shown in Figure 3: The signal increases in absolute value, with increasing τ. These frame differences are then Fourier transformed. We call q the Fourier mode (look at the units, to see if it is the mode number, or the wavevector). These are 2-dimensional images, so the Fourier transform is done in both dimensions. We square the result of the Fourier transform, and we make a radial average since we expect the dynamics in these systems to be isotropic (if the sample is in equilibrium, and hence has average properties that don't change in time, we can also average over time).

The result of this is shown in Figure 4. Notice a few things: (1) for increasing τ, these signals increase; (2) for high q, the signals decrease; (3) the signals overlap each other at high τ, and they do so more quickly for large q. What does this mean? You will have to think about this, and discuss.

For now, let us press on. If we choose a few q to look at, and plot the intensity like in Figure 4 but now for all the τ we have in our time series, then we get data that looks like the one in Figure 5. What you see here are three signals, each of which reaches a steady state (plateau, saturation) value. Notice that the greater q take less time to reach saturation level. Greater q means of course smaller spatial lengthscale, and what this is telling us is that the diffusion process has had time to take place over a scale $\lambda \simeq 1/q$. Interestingly, and we do not prove it here, these signals are exponentials in τ; so from each trace (each q), by fitting an exponential increase up to the plateau, we get a time constant that we call τ_q.

Now the "final plot" is how τ_q depends on q: you can see in Figure 6 that for this system there is a range of q within which $\tau_q \propto 1/q^2$. Indeed, the prefactor here (not proven, but dimensionally correct) is nothing else than the inverse of the diffusion coefficient D, giving $\tau_q = 1/(Dq^2)$.

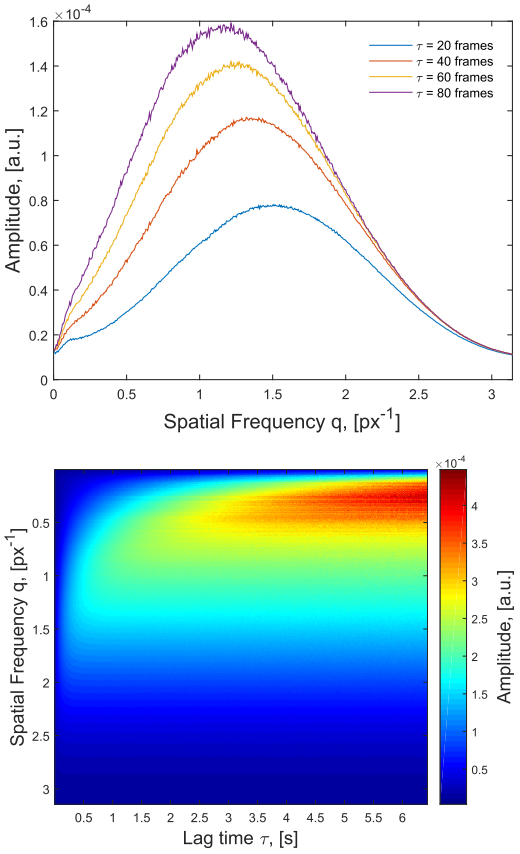


FIG. 4. The Fourier transform, squared, and radially averaged, of the image-differences from Figure 3, give an intensity. This is function of q and τ. **top**: intensity versus q, at some selected values of τ. Notice the increase at fixed q, with τ, up to a saturation intensity. The same information, for the whole array of (q, τ) can be plotted as a heatmap. **bottom**: the color gives the intensity, for each choice of q and τ. The smaller the q, the longer it takes to 'grow' and saturate a signal. Note also that for very high q the signal is always low, and also it would saturate in very short times. The plots at the top are columns (vertical sections) of this heatmap. Rows (horizontal sections) are shown in Figure 5.

These Figures 2 to 6 were taken and analysed by us in the past, using a "real" (commercial) microscope, with a slightly better camera and condensor/objective optics than what you have here.

There are a lot of things you can do.

C. Basic things to do - required

1. Take videos of different colloid sizes; A typical video might be 10 s long, with frame rate 100 frames

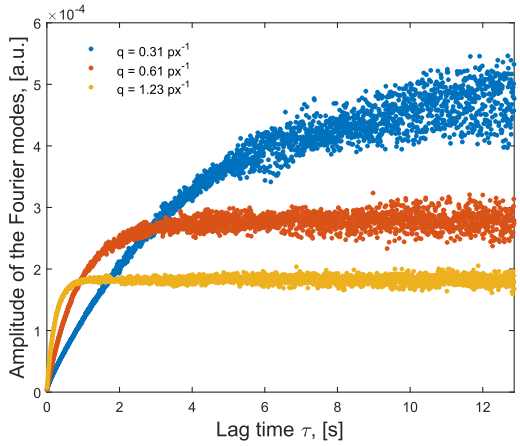


FIG. 5. Intensity of the signal as a function of the lag time τ, at three different values of q (i.e. rows of the matrix in the bottom panel of Figure 4). By fitting these curves with an exponential function we get the time constant τ_q.

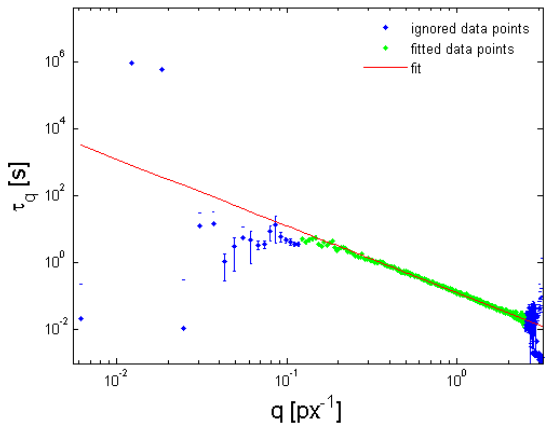


FIG. 6. Fit of the time constant τ_q (obtained by fitting the curves in Figure 5) vs q.

per second (fps), and exposure time on the camera of 1 ms. These are values you can play with and explore consequences.

2. Run the DDM analysis on these videos;

3. Plot your data and extract the diffusion coefficient;

4. Compare where possible to the results of DLS.

Already this requires some care: to be quantitative, you need to know the pixel size (we have a calibration slide), the time between frames, the room temperature.

D. Secondary things to explore - rest of first week

1. With the colloids: change size, and concentration. Does D change? What happens to the other parameters of the DDM analysis? How much light makes it through the sample?

2. Play with the "limits" of the equipment you have, and this DDM approach. Change the objective from the 10x to the 40x, what happens? Can you make a link between the lower colloid size when the approach fails, and the numerical aperture (NA) of these objectives?

3. What are the reasons that the signals become difficult to analyse (or not related to Brownian motion?) at low q? High q?

4. If it is feasible, change the temperature of the colloid system (hot, cold water from taps?); what happens to D? Can you get $\eta(T)$?

5. Other simple experimental ideas?

Proceed to next section once you have some solid graphs of Brownian motion, you have explored as much as possible of the quantitative points above, and you have a solid understanding of what the DDM approach is doing on the video data.

III. SECOND WEEK EXPERIMENTS - 5 DAYS

We intend for the second week to be flexible, and you can choose to explore various things more individually. Most of these suggestions will require you to open the Matlab analysis functions, understand the code, and make some modifications (particularly you will want to fit your own functions on $I_q(\tau)$ data). So we present various ideas, you will not have time (and we don't recommend trying) to do all properly:

A. Particle mixing

A huge challenge in the industrial application of particle sizing is how to cope with polydispersity and measure it robustly. The challenge is (a) mathematical, because signals are convolved (and need to be deconvolved, often this is mathematically ill defined, and assumptions need to be made); (b) physical, because large particles dominate the diffraction (or the contrast in an image) and this can hide the presence of small particles in the actual signal, and thus bias a size distribution if the experimenter is not very careful.

Can you make mixtures of 2 (or 3 ?) particle sizes, in a few different concentration ratios that you control, and run the DDM? Can you get out the separate timescales for each? Can you even find a way to extract back the relative concentrations?

B. Beyond Brownian motion - subdiffusion

You will see in the Part II Soft Condensed Matter course that many materials are viscoelastic. That is, their response to a strain or stress is not purely viscous but also elastic. This is different from just having high viscosity, because these liquids have some 'shape memory', often transient. Polymer solutions (which make up many everyday products like shampoo, liquid detergents) are viscoelastic liquids if the polymers are long and concentrated enough to be entangled. Tracer particles in liquids like this perform random walks with subdiffusive behaviour, often there is a range of observation time intervals for which mean square displacement is a power law of lag time: $\langle x^2(\tau)\rangle \sim \tau^\gamma$.

Your demonstrator can show you how to prepare samples between glass slides. The simplest viscoelastic system we have tried are mixtures of short and long PEG polymers in water. For these, we have a nice table reporting on the viscoelastic properties of various mixtures of water based polymers; These solutions are described and characterised fully in ref. [8].
Another family of viscoelastic systems are polymer gels, and you can try these by making solutions of agarose in water; agarose melts readily on heating (50^oC should be enough), and upon cooling you will get a gel network percolating the sample if the agarose concentration is high enough. You can explore agarose concentration systematically.

Care needs to be taken to make samples with correct concentrations, and to have samples mixed well. Remember that a viscoelastic system can be much more difficult, and take much longer, to mix than a normal liquid. You may want to leave samples mixing overnight, and also once you flow them into microscope slides for imaging they will build up internal stresses that take time to relax.

You still want to add colloid particles, which will be the objects with the strongest optical contrast in the images.

This is a very new area, first publication with DDM on these samples is from 2017 [9]!) - you can:

1. Find appropriate concentration of particles so that contrast in the image is mainly given by them, get videos;
2. Run DDM, comment on how $I_q(\tau)$ is different from that in Brownian motion;
3. Think how you can fit this. If you want to do things rigorously, you will have to learn what was done in [10] and adapt to viscoelastic;
4. What has changed in the DDM output? Have the different material properties led you into a regime where you are seeing limitations of the instrument, or are you seeing the dynamics of the complex liquid?
5. Ideally (but this could take long!): fitting $I_q(\tau)$ appropriately should now give you something related to the stress relaxation modulus $G(t)$. Is it possible to relate back to the elastic and viscous components of the response, as measured by others in bulk rheometers?

Try to analyse the data as in [9]: we have some scripts to get you started.

C. Beyond everything

1. Can you *improve* on the scripts that we have made for DDM? As you explore strange conditions, you will want to change at least fitting functions, but maybe you want to change more. You might want to make some batch/main scripts to automate the analysis, you might want to organise your data systematically into folders, and/or devise some clever naming scheme. Matlab scripts and data structures can be quite powerful, but you are free to explore other "database" formats. Avoid if at all possible using excel.

2. Can you make an Android app to capture video and do DDM?

3. For discussion in report: can you speculate about other areas of video analysis where DDM could be a powerful approach?

YOUR WRITE-UP

The guidelines you were given in 1A and 1B still hold (number the sections, pages, what to put in an abstract, etc.). Your write up can be in the style of a paper, and follow the stages of the investigation as you were guided in this handout, but you are free to change if you like. **Quality of graphics (figures of data), and the captions that go with it, are a central part of any scientific report.** Look carefully at this handout, and open the original references quoted here, as examples. Excel will probably **not** be a good environment to make graphics nor data analysis. Fitting, annotating, etc. should be part of many figures. Matlab can produce publication quality figures, and is the environment in which we can support you. If you have experience working in other environments (various linux or python libraries and programs for scientific work) that's ok but you are on your own... Discuss with demonstrator or head of class.

Note: as of Michaelmas 2018 there is now a 3000 word limit on the report, and 300 words on the abstract. Make use of appendices (which do not count on the word limit) if you want to provide extra information.

Appendix 1. DYNAMIC LIGHT SCATTERING

A. Data collection and processing

Your computer (new in 2018 - tell us of there are teething problems!) controls the Analogue-to-Digital Converter (ADC), for collecting DLS readings and for simple calculations with these readings. You are recommended to create a folder whose name is your usual identifier, to contain your own readings and results. Please keep this folder and any other new file out of the folders named 'Pico ...', and please remove your folder before the last day of the experiment.

To obtain reliable DLS results you need to consider the following queries:

- The time interval for correlation τ_C between samples of data is to be set in the PicoLog program. You should investigate, for each sampling method, how well the sampling interval, as set in the range $1-50$ ms, is met by the values recorded in the spreadsheet. If values are inconsistent, you need to check carefully what to believe did the ADC sample as instructed but record the wrong time, or sample wrongly but report what it did correctly?
- How reliable is the frequency setting on the signal generator (check with separate oscilloscope)?
- To collect data over many tens of seconds at intervals down to 1 ms, which method will be most suitable?

1. Recording data

Experimental readings are collected by an ADC with sampling control (called a PicoScope) and PicoLog software (icon on computer desktop), and are then analyzed by Matlab programs. If you haven't previously used either Picolog or Matlab, please get help from the demonstrator.

Start PicoLog Recorder. First go to File/Preferences/Converter, select 'Use converter specified here' and select 'PicoLog1012/1216', then OK. Then go to Settings/Input channels; the same converter type should be shown. The USB device should be detected and shown in the list box; if not, click Refresh. If this doesn't work, consult your demonstrator. Next, if the window 'PicoLog1012/1216 measurements' appears with a blank list, click 'Add' and give the converter a name. Initial settings for recording should be made with the File menu. To check them or change them subsequently, the Settings menu can be used, after pressing the 're- record' button.

In the 'Recording' window, you can choose between three types of sampling: (1) 'Real time continuous', (2) 'Streaming' and (3) 'Fast block'. With (1), in the 'Sampling Rate' window ensure that 'Readings per sample' is set to Single and specify the sampling interval and number of samples (is there a maximum for either?). The minimum interval that can be achieved depends on how many other jobs the operating system is trying to do, so it may be worth closing any unwanted programs. In the 'Converter details' window, ensure that the converter type is 'PicoLog1012/1216'. (2), (3): Smaller sampling intervals can be obtained by choosing 'Fast block' or 'Streaming' in the 'Recording' window. The converter type is confirmed next. In the window 'Picolog 1216 block', select 'Channel 1 unused', then Edit and rename it. Choose the number of samples and the total sampling time (Note: microseconds for Fast block, ms for Streaming). In these modes, the timing is not affected by operating system activity. Fast Block has a maximum number of samples.

The file specified for recording should be given extension .plw . This file can be re-used to record successive sets of readings. The collected data can be shown in the Graph window of PicoLog. To copy it out for analysis, use the Spreadsheet view. If no data appears, find the 'Select channels' button and select the converter name given in the Recorder window. Then click on the small light blue button. This selects all the data. Now click on the leftmost button, to send the data to a file with a pathname of your choice and extension .prn . You might choose to give different names to successive files that you plan to keep for analysis.

To use the analysis programs, first start Matlab from the desktop icon, then open Histogram.m and/or Autocorr.m from the list of recent files in the Open menu, or otherwise. The name of the data file to be read must have extension .prn . Extending a program may be desirable but please check with the demonstrator.

To carry out analysis away from the lab and for convenience in preparing your lab report, you can file data on a volume that can be seen from elsewhere in the Teaching network, or use the external drives provided.

B. DLS Software

Reference (read-only) versions of the programs Histogram.m and Autocorr.m are available on the Classes desktop of the PC provided, and can be copied to your filespace for modification there. The file from which readings are taken

is set by the file name in the statement 'importdata( )', and can be changed as required. In each program, the number of bins can be set by the user, but the default value should be used initially.

The Histogram program reports also the mean and standard deviation of the data read in. When used with readings from a regulated power supply, such as the file PSU.prn , it shows unexpected detail. A scan shows that all readings are within a few mV of the mean, but the distribution appears to consist of discrete peaks within this range. Why does this happen?

You should inspect the Autocorr program in MATLAB. The calculation cycle is as follows:

1. The digital samples in memory of the signal, A_k , are shifted to A_{k+1} . The earliest sample is lost.

2. A new reading A_1 is entered into bin 1.

3. A_1 is multiplied by each of A_1 to $A_{N_{bins}}$, where N_{bins} is the number of bins, and the products are added to output bins B_1 to $B_{N_{bins}}$.

4. A_1 is added to a separate bin which is later used to calculate $\langle I \rangle$.

After many repetitions the bins contain measures of $g^{(2)}$ for $\tau = \tau_C$ to $N_{bins} * \tau_C$. These may be plotted graphically or listed as numbers.

Separation of the collection and processing of readings limits the speed of investigation but the use of standard procedures has the advantages of low cost and some flexibility. Purpose- built auto-correlators with 1-bit inputs and hard-wired multipliers can run much faster, up to sample times of 1 ns or less.

Appendix 2. COMPONENTS OF THE DDM MICROSCOPE STAND

A. Apparatus

It is self evident that the DDM apparatus is beautifully simple, a full list of components and building instructions is here:
`people.bss.phy.cam.ac.uk/~lf352/out/microscope_instructions.pdf`

The illumination is provided by a LED, whose light is transferred in the Koehler scheme to provide a near flat illumination.[11]

This light is incident on the sample slide, which is held by a stage free to move in 3D; the transmitted and scattered light are collected by an objective lens, which forms an image on the camera's detector. The optical axis is arranged vertically and the system is attached securely to an optical platform.

A computer with a large RAM (currently 16 GB) and HDD provides the storage of the image stacks taken, and also powers the LED (moderated by two variable resistors), and performs the DDM computations and fits.

Pointgray camera operation is described below.[12]

Before any useful data can be taken, the optical system must be aligned and the relation between numbers of pixels and real distance in the imaging plane be determined.

B. Alignment of the DDM

In this setup, alignment means that the light from the condenser, when the diaphragm is closed to form its smallest aperture, forms a focussed image at the centre of the camera's detector, see Fig. A1. Note: Intensity flatness, and absence of 'dirt', don't need to be perfect since the technique relies on subtracted-images.

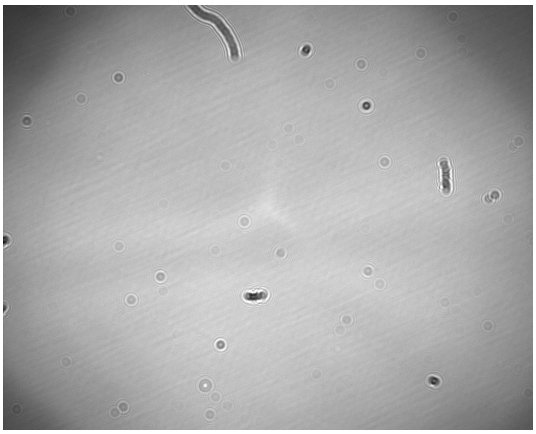
This procedure sounds straightforward but can be fiddly for smaller camera detectors and/or objectives with larger magnifications. It is best done by:

1 aligning both the Koehler condenser and camera tube by eye along the vertical line

2 closing the diaphragm as far as possible while still being able to observe light

3 then adjusting *one* of the two parts further

By repeating the last two steps it is possible to achieve something like Fig A1.



Appendix Figure A1. Successful Alignment of 40x Objective. This is a frame taken from a short framestack that shows how a properly aligned DDM apparatus shows on the live view of the camera with the diaphragm closed to its smallest extent.

C. Scale Calibration

To get a meaningful number out of the DDM analysis, one must know how to convert between numbers of pixels and distances in SI. The current implementation converts from px $\rightarrow$ μm (there is a variable called `px2mum`) so one needs the ratio μm/px. To find this, a calibration slide denominated in units of 10^2 μm was made level, imaged in focus, and displayed in Matlab. Code to do this could be as follows:

```
abc = ometiffreader; % Reads in image stack (folder choice via GUI)
abc1 = abc(:,:,1); % Takes the first image.  N.B. ';'!
imagesc(abc1); colormap jet; shg % Shows first frame in false colour
```

The error in this calibration is important because the ratio is used to give the diffusivity. A number of ways of reducing the error are conceivable like plotting a line for multiple intervals or taking several images of different single intervals.

D. Acquiring images from camera

This assumes you have the PointGrey camera connected to USB3 port [13].

- Open the software "PointGrey FlyCap 2"
- Click ok if the camera on the list is the Chameleon3
- Open the settings (7th icon from the left); you can change the settings in "Camera Settings" as you want. We've been unticking all the "auto" checkbox, and have been taking 100 fps videos with 1 ms shutter time. We know these settings work:
  In 'Custom Video Modes': Mode 0, Raw 8, Image Left 128, width and height 1024, Packet Size 13200 (so that after you click 'APPLY' you get on the right 'Estimated bandwidth $\sim$ 105 MB').
  All other tabs shouldn't really matter (Unless you want to experiment with 'Advanced Camera Settings')
- Click on the 'Record' button and then:
  Filename: `C:\MicroscopeData\%%your_experiment_name%%\frame`
  Capture: take typically 10 s videos for Brownian motion.
  Recording Mode: Buffered (for the sake of stable framerate)
  Images: format PGM, 'save as binary file' ticked
  Start Recording

  If you record like that, then in MatLab you can then use the function pgmreader, as follows.
  This opens the gui and you have to click on the folder `C:\MicroscopeData\%%your_experiment_name%%`:
  `frame_stack = pgmreader;`

This reads all the frames that are saved in the folder:
`frame_stack = pgmreader('C:\MicroscopeData\%%your_experiment_name%%');`

The script for reading isn't very robust, the only check is that if more than a collection of pgms is saved in the same folder it will ask the user which one to load.

We don't suggest just recording in .avi because this format is limited to 2GB of file size (also, frames are skipped and software is more likely to crash).

This is just a guideline, feel free to experiment/change/improve and make suggestions.

Appendix 3. QUICK GUIDE TO DDM SOFTWARE AND SCRIPTS

As described in previous sections, the DDM technique is computationally intensive and there are several different jobs to do:

1. Read the frames, into a usable form;
2. Perform the DDM analysis of Fourier Transforms of differences on the frames;
3. (a) Fit a model function to the saturation functions, $I(\tau)$, and (b) display those fits (interactively);
4. Plot the characteristic times, τ_q, of the saturation functions against wavenumber q in log space and interactively choose the range to fit over;
5. Fit the expected relation between τ_q and wavevector q;
6. Replot τ_q against q with the fits plotted and dynamic parameters, e.g. Diffusion coefficient

Four Matlab functions and scripts have been prepared to do most of this work, summarised in Table A1.

Name	Description
`pgmreader`	function reads in '*.png'. We have similar functions for '.tiff' if needed.
`DDM_core`	function
`fits_Iqtau_colloids`	script
`scroll_through_Iqtau_fits`	function

Appendix Table A1. Initial functions and scripts.

TIPS ON VISCOELASTIC FLUID EXPERIMENTS

1. Preparing the Viscoelastic Samples

The 'recipes' for mixtures of 'PEG' and 'PEO', both of which are terms for the same polymer but with different molecular weights, come from [8]. The polymer in question is Poly(Eth[yl]ene oxide), or $-CH_2-CH_2-O-$, that is available in such a large range of molecular weights (MWt).

Samples can be made up in batches of 10 g, which is not so large as to make the measurements cumbersome, large enough to give four or five 1 mL samples, and also makes the arithmetic easier.

1. First work out how much of PEG, PEO, and water each sample needs
2. Measure out the desired mass of PEG and PEO
3. Dissolve PEO and PEG in equal volumes of water, but leave the PEO solution overnight and start the PEG in the morning[14]
4. Once both are dissolved, mix the two together, put in a sample bottle, and place on roller/shaker overnight

5. Once homogeneous, to samples of 1 mL add 2, 5, 10, 20 μL of particle solution[**?**] and roll/shake overnight again

6. Sonicate before putting onto microscope slides

2. General Considerations

The VE samples are viscous, so the particles' movements and hence dynamics in the image are slow. Hence, it is no longer necessary to have a very short interval; on the contrary, framestacks of longer duration might be required to follow a slow relaxation.

The focus for the VE investigation can be on the form of the saturation functions, and the form of and exponent given by the τ_q against q plots. A systematic analysis of the viscosities could also be done, but is perhaps a bit ambitious in the two weeks.

3. Viscosities of Pure PEG Solutions

The pure PEG samples should probably (check!) fit with exponential saturation functions, and their viscosities are expected to go exponentially with the weight percent of PEG.

[1] In our research lab we are using and modifying this technique to quantify a very complicated system: thousands of motile microscopic filaments (cilia) beating fast (10 Hz) and in synchrony - this is how pathogens and dust are constantly expelled out of our airways.

[2] R. Cerbino and V. Trappe, Physical Review Letters **100**, 188102 (2008).

[3] F. Giavazzi, D. Brogioli, V. Trappe, T. Bellini, and R. Cerbino, Physical Review E **80**, 031403 (2009).

[4] F. Giavazzi and R. Cerbino, Journal of Optics (2014).

[5] R. Cerbino and P. Cicuta, J. Chem. Phys. **147**, 110901 (2017).

[6] It is also possible to 'drive' the particles, e.g. exploit gravitational force, and obtain directed motion (this is "super-diffusive").

[7] B. J. Berne and R. Pecora, *Dynamic Light Scattering* (Wiley, New York, 1976).

[8] P. Dontula, C. W. Macosko, and L. E. Scriven, AIChE **44**, 12471255 (1998).

[9] P. Edera, D. Bergamini, V. Trappe, F. Giavazzi, and R. Cerbino, Phys. Rev. Materials **1**, 073804 (2017).

[10] V. Martinez, R. Besseling, O. A. Croze, J. Tailleur, J. Reufer, Mathias Schwarz-Linek, L. G. Wilson, M. A. Bees, and W. C. K. Poon, Biophys. J. **103**, 525 (2012).

[11] In other words, the 2D light intensity distribution is uniform, for more info see these lecture notes: http://people.bss.phy.cam.ac.uk/courses/ biolectures/Koehler_Illumination.pdf.

[12] Https://www.micro-manager.org/wiki/Micro-Manager.

[13] The PEG dissolves relatively quickly, especially with encouragement from an ultrasonic, but later solidifies out again, while the PEO just takes time, or a lot encouragement from a magnetic stirrer.

[14] Particle Solution info.