

# How-to Guide for CSHS Microscope

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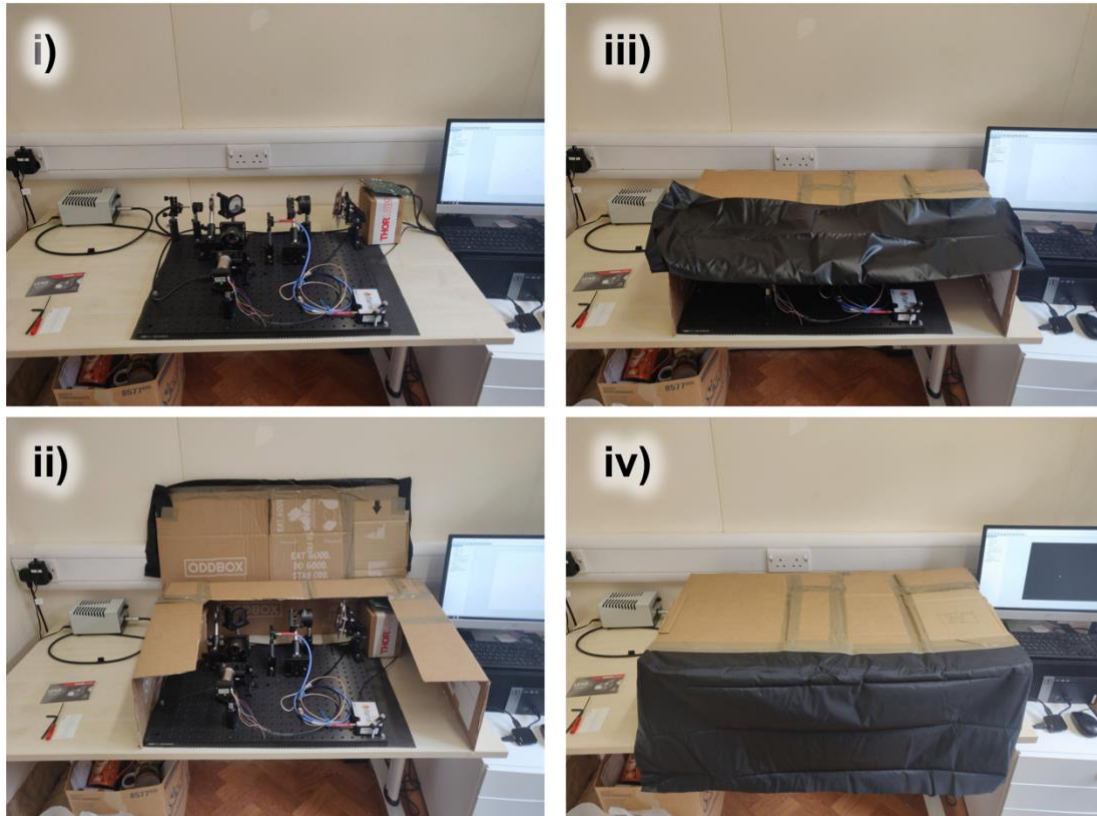
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## 1. Basic use

### 1.1 Turn things on

- Switch DMD on from the wall
- Switch light source on from the wall
- Switch on PC
  - o Login with password 'qcp616BYF'
  - o Anydesk connection: '577 591 155', same password
- Plug in the USB adaptor to the PC (this connects the camera and DMD)
- Start with enclosure lid off



- i) Fully removed:** For optics adjustment
- ii) Lid removed:** For changing samples
- iii) Partial cover:** For focusing on ROI
- iv) Full cover:** For measurements

## 1.2 Sample preparation

- Dropcast particles onto a glass slide.
- Add a coverslip
- Load sample into the sample holder

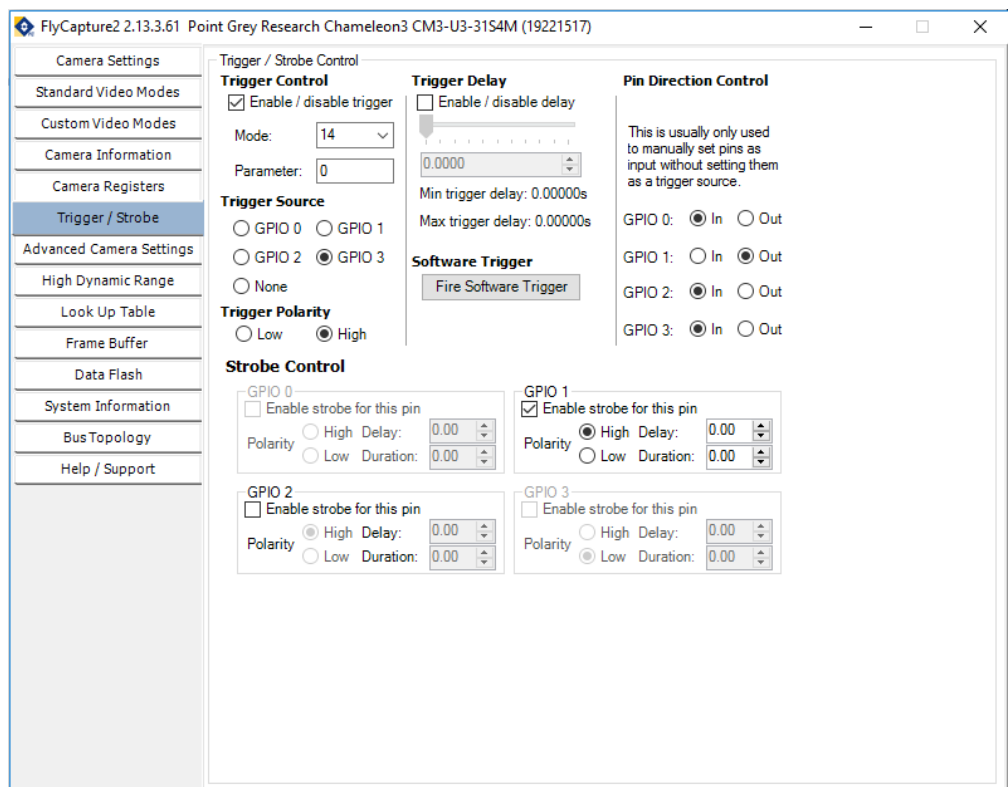
## 1.3 Identify sample region

- Put camera into correct imaging format:
  - o Open Point Grey FlyCap2 – this is used for controlling the camera
  - o Open the camera settings
  - o Go to the trigger tab, and make sure 'trigger mode' is currently deselected
  - o Go to the general settings tab, put contrast/gain/brightness on auto
  - o Now you should be able to track a live feed from the camera
- Direct light to the sample:
  - o Open DLP LCR6500 (Lightcrafter) - this is used for controlling the DMD
  - o Go to 'Capture on the fly' mode
  - o Click 'load a pattern'
  - o Select the 'All white' pattern
  - o Increase number of bits to at least 2
  - o Click 'Update LUT'
  - o Press 'Start' – the mirrors should now all be switched on.

- Focus onto the sample:
  - o If recently aligned, the OL should be lined up with the bright illuminated region coming from the CL, meaning only small focus adjustments are required. If this is not the case, follow section '2.1 Aligning the imaging lenses'.
  - o Adjust the sample holder optic axis translator to adjust sample-CL distance until brightness from particles is at a maximum.
  - o Adjust the OL+camera optic axis translator to adjust sample-OL distance until image is in focus (note, there will be multiple surfaces corresponding to each glass interface, so make sure to find the one that actually has your sample!)
  - o Use the two dials on the side of the sample holder to find the sample ROI

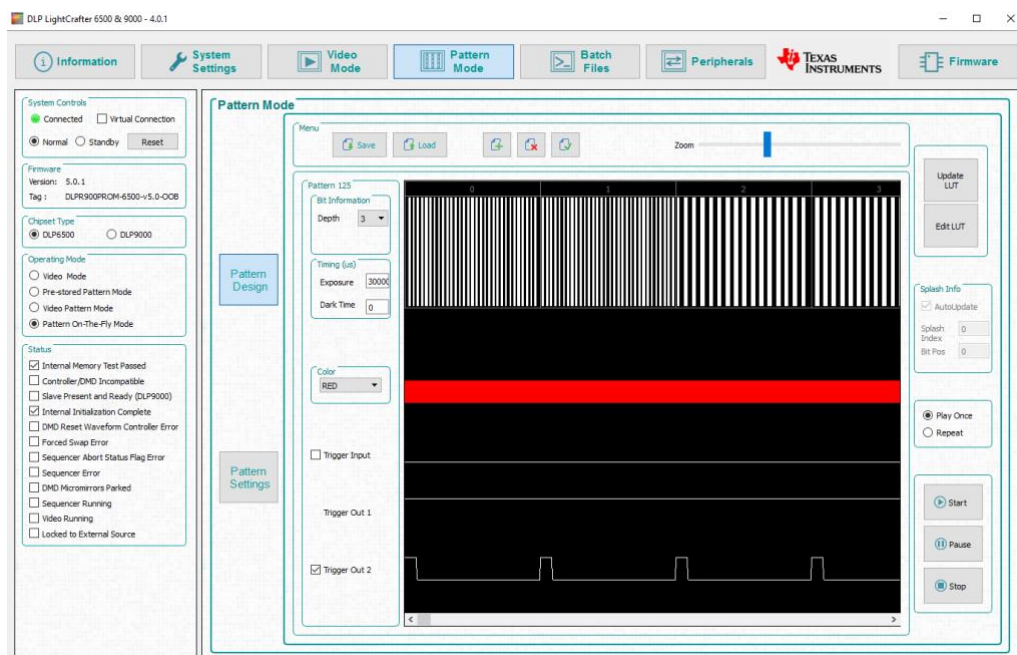
## 1.4 Record dataset

- Put camera into recording format:
  - o Adjust gain to zero and exposure to an appropriate value (should be the fastest possible that still gives full dynamic range. Approx. 1 s is good rule of thumb, though up to ~ 200 ms can be achieved with careful aligning – note the exposure here is not what will be used when recording, but it gives a helpful preview of how images will come out).
  - o In camera settings, go to trigger tab and check 'Enable / disable trigger' on.
  - o (Double check 'Raw 16' format is applied in custom video mode tab)



- Load patterns onto DMD:
  - o Open DLP Lightcrafter, and enter 'pattern on the fly' mode.
  - o If already on, stop any current instruction and clear the current pattern list
  - o Click 'Load' to open a set of DMD patterns. (Presets loaded at 'Dropbox/CS\_George/DMD\_patterns'). 'Inverse patterns' are recommended to achieve best SNR – this will open up 126 patterns.

- Adjust the 'exposure' – this is the duration that each pattern will be displayed for. This should be slightly longer (e.g. 0.1 s) than the camera exposure that will be used to ensure correct triggering behaviour. Note exposure time is in  $\mu\text{s}$ , so for a camera exposure of 1 s, the DMD exposure should be 1100000  $\mu\text{s}$ .
- Adjust the bit depth. If this is too low, patterns won't display properly, but if too high then it won't fit into the DMD memory. A value of 3 often works.
- Change to 'Play once' instead of 'repeat'. (otherwise acquisition will never stop!)
- (Double check 'Trigger out 2' is checked)
- 'Update LUT' (this may take a minute or two to finish),
- Press start and visually check patterns are displaying properly and check that exposure is still approximately correct (intensity will be ~half that when all mirrors are on).



- Set up image record settings:
  - Press the red record button in Flycap
  - Change save folder and name prefix
  - Specify to capture '0' frames (this will let you manually stop acquisition)
  - Save in TIFF format with no compression.

Recording Settings

Save Filename  
 Filename: C:\Users\er407\Desktop\DMD demo\demo  
 A timestamp, sequence number and file extension based on output type will be appended Browse

Saving Options  
☒ Capture  frames\*  
☐ Capture 1 out of every  frames from a run of  frames\*  
☐ Capture for  ms  
☐ Capture once out of every  ms from a run of  ms\*  
 \*Specify zero to capture until manually stopped

Recording Mode  
☒ Buffered: Frames are buffered in physical memory while recording. Recording will stop if physical memory usage hits 95%  
☐ Streaming: Frames are not buffered in system memory while recording. Dropped frames may occur depending on the speed of the PC and the current camera/recording settings.

☐ Only save corrupt frames when capturing

Images | Videos

Image Format: TIFF

Tiff Options  
 Compression Method: None

Good Frames:	126	Available Physical Memory:	4511.00 MB
Corrupt Frames:	0	Total Physical Memory:	8057.00 MB
Buffered Frames:	0	Usage:	44%
Saved Frames:	126		

Hide Recording is stopped Start Recording

- Acquire dataset
  - o When ready, press 'Start recording' in Flycap
  - o Then press 'Start' in Lightcrafter – you should see the number of saved frames start increasing in Flycap and the image should be gently fluctuating in intensity.
  - o When finished (126 images saved), press 'Stop recording' in Flycap. This will take  $1.1 \text{ s} \times 126 = \sim 2 \text{ min } 20 \text{ s}$  at a DMD duration of 1.1 s.
- Close programs, power off light/DMD, and unplug USB.

### 1.5 Reconstruct dataset

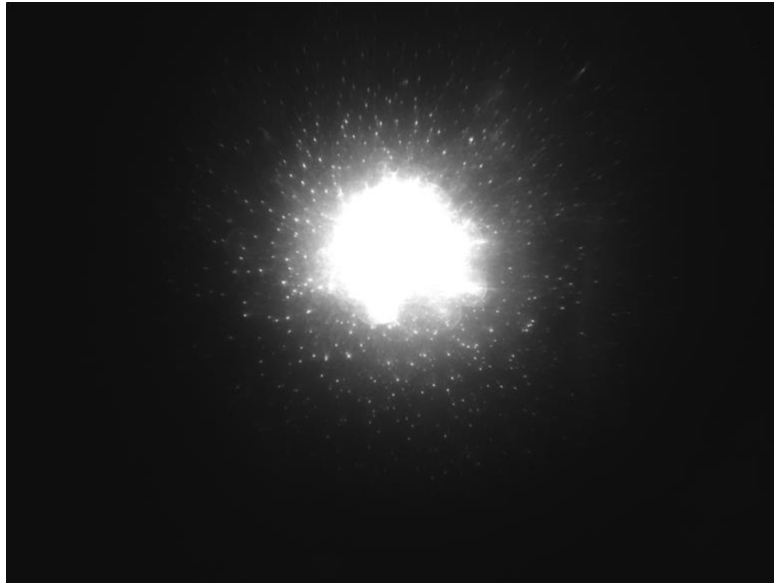
- Decide which computer to use and open 'Datacube\_analysis.ipynb':

- Lab PC: Code is configured on the lab PC, though the PC is quite slow. To use this one:
  - Open the anaconda prompt
  - Type 'conda activate cs\_hs1' and press enter
  - Type 'jupyter lab' and press enter – open link in Chrome
  - Navigate to Desktop/DMD demo/Processing and open Datacube\_analysis.ipynb
- Workstation:
  - Anydesk to s205 lab PC
  - ID: '584 593 978', Password: sheep1016
- Personal:
  - To setup: Download code from github, use conda to configure a python environment with matplotlib, numpy, PIL, scipy, pandas, and jupyterlab
  - <https://github.com/grlewis333/PhD-Public>
- Follow instructions in the notebook to reconstruct a datacube from the images and do some basic analysis.

## 2. Advanced use

### 2.1 Aligning the imaging lenses

- Setup:
  - Switch room lights off, close blinds, switch all DMD mirrors on, and with the enclosure lid off, very roughly move components into position – easiest with a scratch on the sample
  - Acquire a live feed with the camera, use a relatively short exposure (~400 ms) and not too much gain (~8 dB) – this will help with finding the light.
- Now there are three things to achieve:
  - i) Position sample at optimum distance from condenser lens
    - This can be approximately achieved using a card to see where the focal point of the CL is and moving the sample into that position
    - It can later be finetuned by adjusting the sample until maximum brightness is recorded by the camera
  - ii) Direct the OL+camera towards the focused region of light
    - A common error can be to align the system using the camera whilst room lights are on and camera gain is on auto. Like this it will be 'easy' to focus on the sample, but more likely than not you will be focused in a region lit from ambient light, not from light that has passed through the optics, hence when you go to acquire a dataset you'll find very little signal.
    - To avoid this, switch room lights off and turn off the auto adjust settings for camera exposure/gain before trying to align.
    - Move the OL+camera manually with loose screws until you locate the illuminated region of the sample – it should be very bright compared to everything else, and crucially if you place the enclosure lid back on and reduce gain to zero, then it should remain bright (see example below).



- Tighten screws in place.
- iii) Focus onto the sample
  - Now you can finetune the sample position (for maximum brightness) and the OL position (for optimal focus).

## 2.2 Calibrate wavelengths

- Direct light into spectrometer
  - To align things, switch all DMD mirrors on as described previously
  - Open OceanART on the PC
  - Insert the flipup mirror into position
  - Align the mirror until the signal intensity is at a maximum
- Load patterns onto the DMD
  - Load the calibration patterns (saved in Dropbox/CS\_George/DMD\_patterns)
  - Set 1s exposure, depth 2, play once, update LUT, test to see it works.
- Run code
  - Open conda terminal, activate cs\_hs1, open 'wavelengths\_calibration.ipynb'
  - Run through the code to save a new set of calibration wavelengths

## 3. Relevant thesis section

### 3.1 Overview

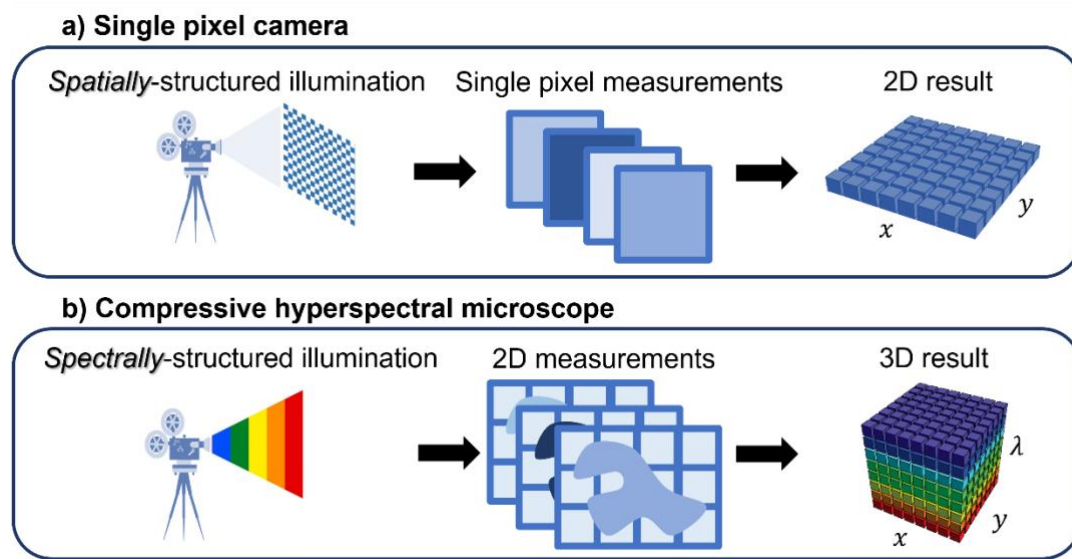
Compressive sensing (CS) has been utilised extensively in the previous chapters as a mathematical framework for incorporating prior knowledge into tomography reconstructions. As described in Section **Error! Reference source not found.**, many natural signals are compressible, and if transformed to the correct domain can be sparsely represented. In ET regularisation, we make the assumption that our signal has *already* been sparsely sampled, and then it is a case of choosing in which domain that sparsity occurs to regularise the reconstruction process. However, the utility of CS extends beyond this regime, and indeed is of great interest when considering how best one might *acquire* a signal in the first place. In this section, I introduce the idea of compressive hyperspectral optical microscopy and detail the development of it towards a proof-of-concept system for studying plasmonic nanomaterials.

Metallic nanoparticles (NPs) capable of sustaining localized surface plasmon resonances (LSPRs) are the key component for many applications ranging from photocatalysis to



biomedical treatment [1]. As we will see in Section **Error! Reference source not found.**, electron tomography is a powerful method for studying their physical properties. However, due to their unique interactions with light, optical microscopy also plays a crucial role. Single-particle scattering spectroscopy techniques like dark-field (DF) hyperspectral imaging have become a key tool for studying the optical properties of plasmonic NPs because of the information that can be obtained from the resulting spectral-spatial datacubes [2]. Unfortunately, the data acquisition process for this technique is notoriously slow, since long integration times are needed at each position to ensure sufficient signal to noise ratio (SNR). A range of parallel acquisition schemes have been suggested to overcome this limitation such as the “push-broom” technique which has been implemented with both point-scan and faster line-scan methodologies [3]. However, recent work demonstrates further speed improvements through the use of a compressive sensing (CS) imaging system [4].

The principle of the hyperspectral CS system builds upon ideas implemented in the ‘single pixel camera’, first demonstrated by Duarte et al. in 2008 [5]; the single pixel camera records single intensity values (1D) from a series of spatially-structured illumination patterns, and uses this to reconstruct a 2D image (**Figure 3-1a**). Extending this concept to 3D, it is possible to use 2D greyscale images acquired with spectrally-structured light to reconstruct a 3D hyperspectral datacube from 2D images (**Figure 3-1b**) (note that a conceptually equivalent 3D imaging mode can also be achieved by recording spatially-structured light into a 1D spectrometer).



**Figure 3-1 | Compressive sampling concept.** a) and b) show the parallels between the concept of the single pixel camera and the compressive hyperspectral microscope respectively.

The key component for achieving spatial/spectral illumination is a digital micromirror device (DMD), an array of individually motorized mirrors (each  $\sim 8 \mu\text{m}$  wide) as shown in **Figure 3-2e**. In a DMD, each mirror can be set to either deflect the beam (‘off’) or to send it to illuminate the sample (‘on’). If a spectrally spread diffraction pattern is focused onto the mirror, then the mirrors will alter the spectrum of the reflected light; else if a collimated beam is incident on the mirror, its reflection will be structured spatially. Depending on the choice of DMD pattern, it is possible to achieve sub-Nyquist sampling rates with this approach and thus improve the speed of the system. DMD pattern options include: raster scans (the most conceptually simple), Hadamard multiplexing (this greatly improves the SNR compared to



raster scans [6]), and random patterns (suitable for CS). It can be demonstrated mathematically that random sampling schemes have a high probability to result in compressed measurements, which can be combined using a sparse reconstruction algorithm to produce a result in fewer measurements than a conventional system [5].

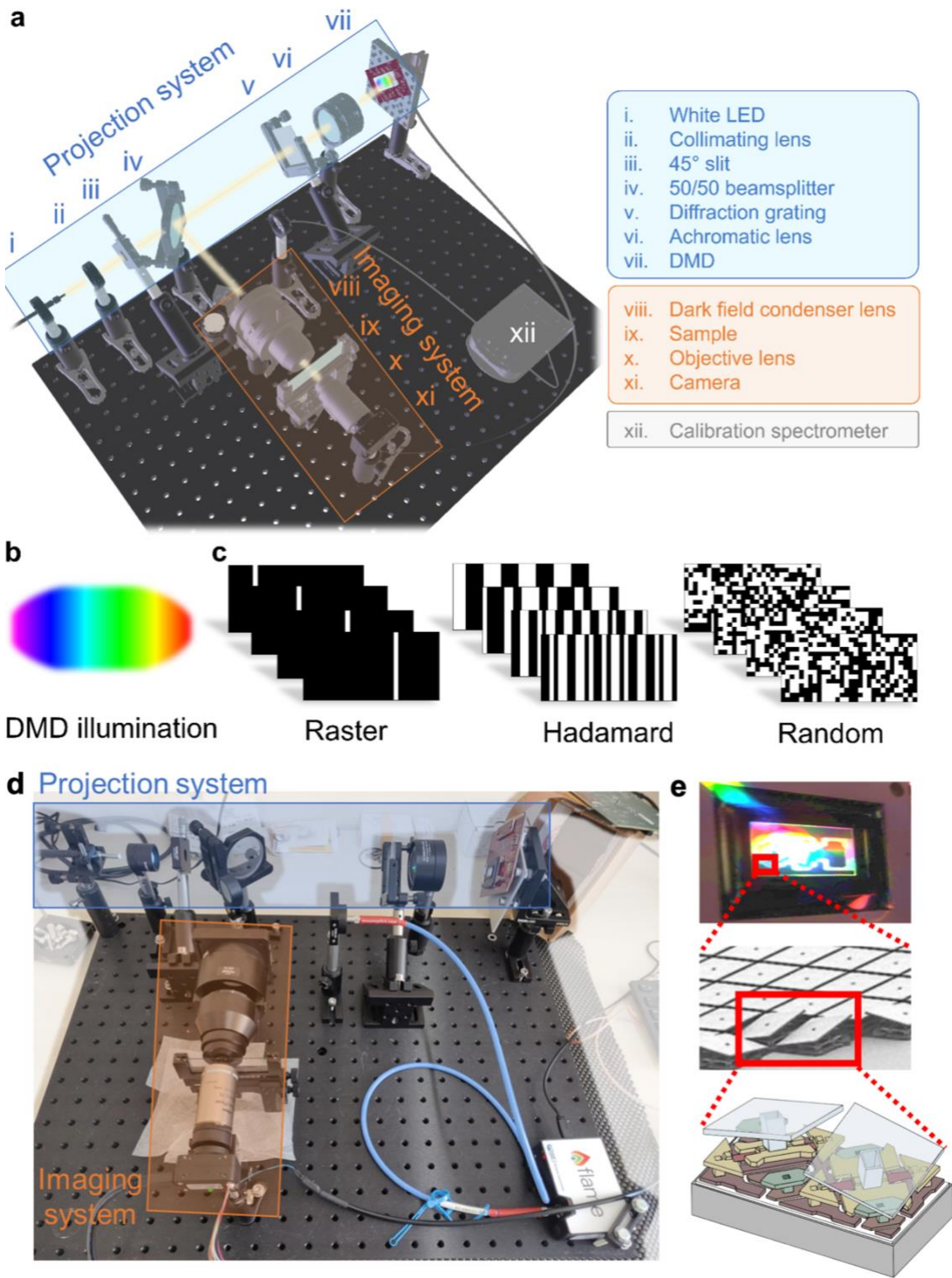
The concept of CS hyperspectral imaging has been relatively well studied for photography/low-resolution imaging [7], but only very recently has it been considered for use in high-resolution optical microscopy [4], [8]. The motivation for designing a CS hyperspectral microscope is two-fold: firstly the system is significantly more affordable than commercial hyperspectral imaging systems,<sup>1</sup> and secondly the ability to multiplex/use CS means that datacubes can potentially be acquired much faster.

### 3.2 Design of experimental setup

The design of the hyperspectral CS microscope can be split into two key sections: the projection system and the imaging system. Kevin Kelly and Anthony Giljum at RICE university provided useful advice throughout the design of the system. The imaging system was designed much like any other optical microscope and in this case consists of a dark-field condenser lens, vertically-mounted sample, an objective lens, and a grayscale CCD camera. The projector system is more complex and as described previously, relies on the use of a DMD to achieve spectral modulation of the light source. In this case, collimated white light is passed through a diffraction grating (300 grooves/mm) and an achromatic lens is used to focus the resulting diffraction pattern on to the DMD which reflects the spectrally modulated light back through the lens and diffraction grating towards a beamsplitter which then sends the light into the imaging system. A diagram of this setup is shown in **Figure 3-2a** with an image of the actual system shown in **Figure 3-2d**. For clarification, an illustration of the diffraction pattern imaged onto the DMD is shown in **Figure 3-2b**, with corresponding potential DMD patterns in **Figure 3-2c**.

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<sup>1</sup> For comparison, a commercial hyperspectral imaging system such as the [ThorLabs Cerna ®](#) is currently priced at £42,817. Meanwhile, a DMD (the most expensive component of a CS hyperspectral microscope), such as the [Texas Instruments DLP ® 7000](#) is priced at £743.92 (January 2023).



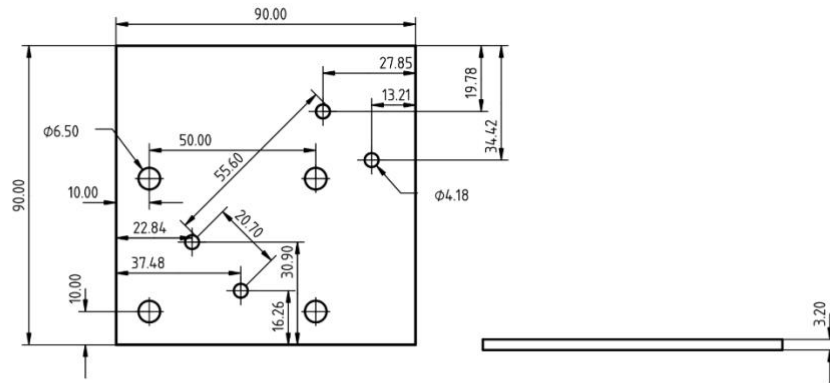
**Figure 3-2 | Compressive sensing hyperspectral microscope overview.** (a) Diagram of the optics, split into projection, imaging, and calibration systems. (b) Illustration of the diffraction pattern focused onto the DMD. (c) DMD patterns for hyperspectral imaging, showcasing raster, Hadamard, and random patterns. (d) Image of the actual experimental setup. (e) Multi-scale view of the DMD (adapted from [Texas Instruments](https://www.ti.com) website).

Several additional factors were considered in the design of the system:

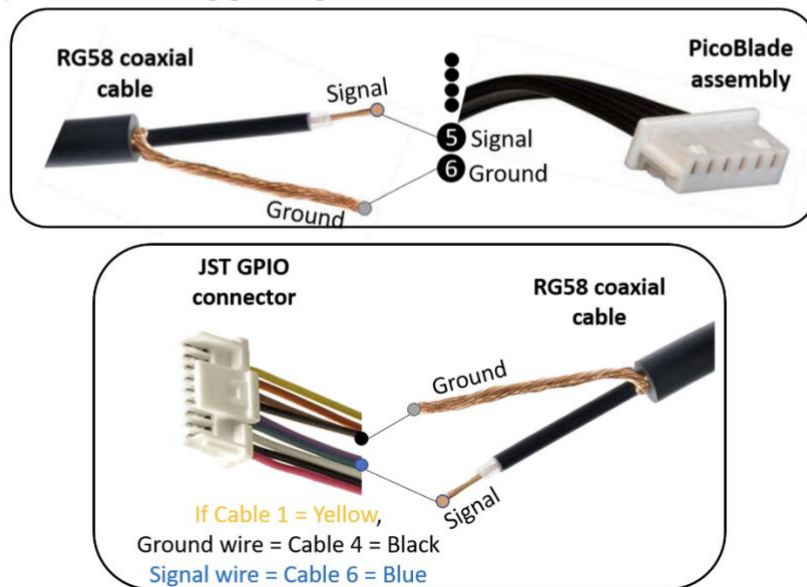
- *Spectral vs spatial modulation*: As mentioned previously, it is possible to build a CS hyperspectral microscope using either spectral or spatial modulation. The decision was made to focus on spectral modulation for a few reasons: i) spatial resolution is given by the resolution of the camera which is higher than what would be achievable through spatial modulation (in which case resolution would depend on spectrometer quality and the DMD array); ii) the spectral resolution is essentially programmable, in that it can be chosen depending on how the mirrors on the DMD are binned; iii) the system is much faster due to the parallel recording of information offered by the camera (e.g. recording a datacube with  $256 \times 256$  spatial resolution and 200 spectral bands, the spectral system will need to take just 200 camera images @ ~1 s exposure, whilst the spatial system would require ~ 65,500 spectrometer readings @ ~25 ms exposure, giving times of ~3 mins vs ~27 mins respectively).
- *Beam shape*: The initial collimated beam was passed through a slit to create an elliptical pattern that could be imaged onto the DMD. This allowed as much as possible of the rectangular  $912 \times 1140$  mirror array to be covered by the diffraction pattern. The spectral resolution of the system is directly impacted by the spread achieved on the DMD, and so it is important to maximise the mirror coverage.
- *DMD mirror rotation axis*: Each individual mirror on the DLP ® 6500 DMD is hinged about an axis of rotation running diagonally across it at  $45^\circ$ , and the mirrors rotate between  $\pm 12^\circ$  when moving between the on/off states. Because of this geometry, the most straightforward way to get reflected light back to the beamsplitter is to have the entire DMD array mounted at  $45^\circ$  (and correspondingly the slit and diffraction grating must also be mounted at  $45^\circ$  to maintain full illumination of the DMD). A knock-on impact of the  $12^\circ$  reflection angle is that it is difficult to reflect light exactly back along the optic axis, hence the use of a high-diameter achromatic lens is necessary to ensure the reflected light can be captured. The specification of the custom designed  $45^\circ$  mount for the DMD is shown in **Figure 3-3a**.
- *Triggering*: For successful operation of the CS hyperspectral microscope, the DMD and camera must be electronically connected to enable accurate triggering. However, the triggering port for the DLP ® 6500 DMD takes a 'PicoBlade' assembly, whilst typical cameras (such as the Chameleon3 USB3, used here) offer a general purpose input/output (GPIO) port for triggering. Therefore, it was necessary to splice a custom cable for the connection, which was achieved by connecting the device specific GPIO/picoblade cables via a generic BNC coaxial cable. From the device specifications, it can be determined that the ground/signal wires are at positions 4 and 6 for the picoblade, and positions 6 and 5 for the GPIO cable (see **Figure 3-3b** for details).
- *Calibration*: Because of the diffraction pattern imaged onto the DMD, each column of mirrors will correspond to a different wavelength. It was found in previous work that the mirrors should be binned together to avoid unwanted diffraction resulting from the small spacing between mirrors [9]. Therefore, the 1140 columns were binned into groups of 10 and rastered whilst recording the reflected light with a spectrometer. Since the spectrometer detection was not triggered (this would require another custom cable), a workaround for determining the wavelength of each column is to raster at a known rate, slower than the spectrometer acquisition (here 1 s per frame), and then

continuously acquire spectra. From each individual spectrum, the peak reflected wavelength can be calculated; a staircase pattern will be observed wherein many columns have more than one spectrum measured for them. The spectra can then be accurately matched by successively assigning each unique wavelength to columns on the DMD.

### a) Custom 45° DMD mount



### b) Custom triggering cable



**Figure 3-3 | Custom parts for CS hyperspectral microscope.** (a) Specification of the 45° mount designed for the DMD (lengths in mm). (b) Illustration of the cable splicing procedure to enable DMD/camera triggers.

### 3.3 Demonstration of basic operation

In this section I demonstrate how the system may be used to take a typical set of measurements, discussing the data acquisition and analysis process.

A sample can be prepared by depositing a suspension of nanoparticles onto a glass slide and placing a coverslip over the top. It can be useful to create a small scratch on the glass; this will both help with region identification, and also produces a strongly scattering region which is useful for alignment.

Several initial measurements should be made prior to searching for particles. Ideally, wavelengths should be calibrated prior to each experiment by rastering through columns on the DMD and directing the reflected light to the spectrometer, though in practice there is only significant change to the wavelengths if the projection system is modified. Next, a bright and dark reference should be acquired. To collect the bright reference, the DMD is set with all mirrors 'on', and an image and spectrometer reading are then taken. For the dark reference, readings should be taken with the mirrors switched to the off position. Corrected spectra can then be achieved by calculating (specimen – dark)/(bright - dark). However, this method is limited in that if using the spectrometer, corrections will be specific to the spectrometer rather than the camera which is actually used for measurements, and if using the camera, the bright measurement corresponds to variation in CCD pixel intensity, which does not easily relate to the emission profile of the lamp. In practice, a good background correction can be obtained by averaging the signal over an empty region in a given dataset and subtracting this profile from the specimen profile.

The hyperspectral dataset is then acquired by stepping through the sequence of programmed DMD patterns. In this case, I validate the system using Hadamard patterns (**Figure 3-2c**); patterns were generated using the rows of a 64 square Hadamard matrix<sup>2</sup>, and discarding the first row where all values are 1 to get  $H$ , the sensing matrix. Signal to noise ratio in the measured spectra can be improved by recording two sets of images; 63 using the as-calculated Hadamard values ( $I_{Reg}$ ), and a further 63 where values of 0 and 1 are flipped ( $I_{Flip}$ ). This method also removes the need to measure a dark reference measurement.

From the acquired images, the reconstructed hyperspectral datacube  $C$  is calculated by simply multiplying the difference between regular and flipped images by the inverse of the Hadamard row corresponding to each pattern;

$$C = H^{-1}(I_{Reg} - I_{Flip}). \quad (3-1)$$

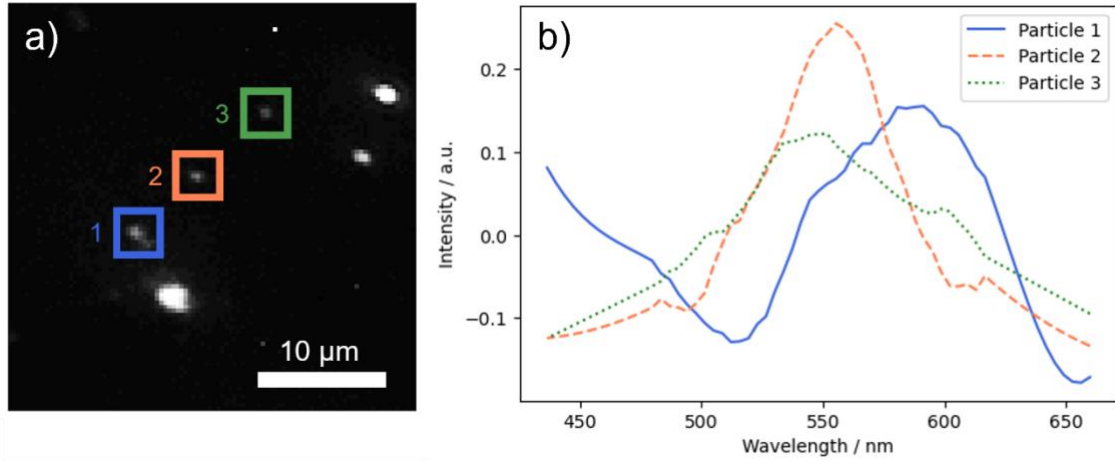
By matching each Hadamard row number to its calibrated wavelength, this results in a datacube with spatial and spectral information.

To demonstrate the microscope's performance, measurements were taken on a set of Au NPs. These particles were prepared by a collaborator (M. Elabbadi) following the method of Seo et al. [10] to produce a wide mixture of polyhedral NP morphologies, with an average size of 90 nm. The system is able to achieve measurements on single particles, with plasmon resonances observed at wavelengths between 550 nm and 600 nm (**Figure 3-4**). According to literature this coincides with the bulk plasmon resonance for gold particles sized between 80 nm and 130 nm [11], suggesting the system is correctly calibrated. Whilst the spectral resolution (~5 nm) is lower than a typical commercial hyperspectral microscope, this system is significantly faster; the dataset in **Figure 3-4** was collected in 126 s, whilst a similar scan may take ~15 minutes conventionally.

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<sup>2</sup> Hadamard matrices contain mutually orthogonal rows with entries of 1 and -1, and are always square, sized as multiples of 2 [12]. Except for the first, each row contains 50% valued 1, and 50% valued -1. The smallest example would be  $\begin{bmatrix} 1 & 1 \\ 1 & -1 \end{bmatrix}$ . In this section I use 0 rather than -1, to indicate an 'off' DMD mirror.

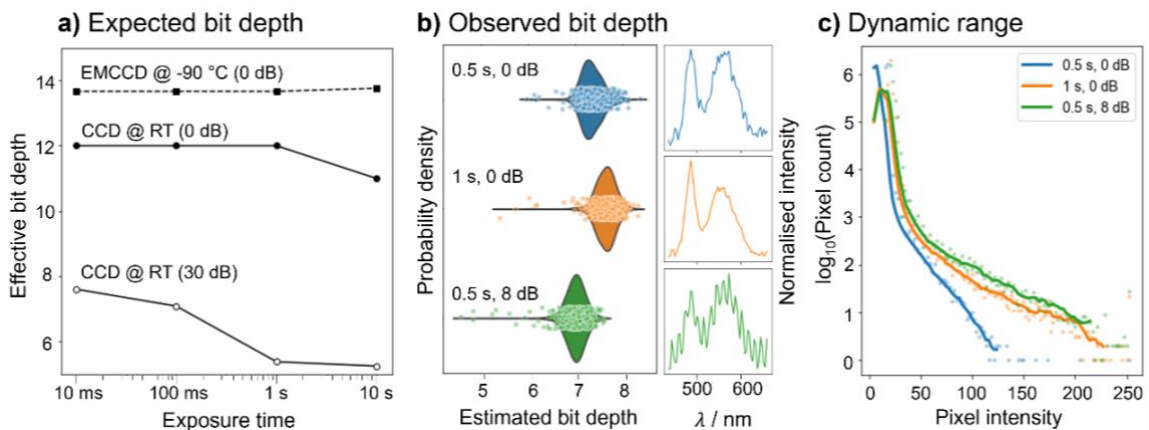




**Figure 3-4 | CS hyperspectral measurement of gold NPs.** (a) Image summed over all wavelengths from a flipped Hadamard acquisition on gold NPs, with ROI indicated by boxes. (b) Spectra reconstructed from ROI in (a).

### 3.4 Analysis of performance limits

In this section I focus on benchmarking the performance of this new microscope. First, by exploring the effective bit depth (EBD) for cooled and room temperature cameras, with and without added gain. The EBD can be estimated from a range of dark, blank images (in this case 10) using  $EBD = \log_2((2^{ADC \text{ precision}} - I_{mean})/I_{range})$ , and essentially tells us what range of intensity values can actually be measured, given the presence of various sources of noise in the detector. For a high quality, cooled scientific camera, a constant EBD is observed over the range of exposure times since the limiting factor is only readout noise (**Figure 3-5a**). However, for a regular camera at room temperature (preferred here for its availability and affordability) the EBD dips significantly for longer exposures since dark shot noise is more prevalent; this is exacerbated if digital gain is added as this amplifies noise in the signal (**Figure 3-5a**).



**Figure 3-5 | Competing impact of exposure/gain settings.** (a) Effective bit depth measured from dark images for a cooled scientific camera (top), and regular camera run without (middle) and with (bottom) gain. (b) Bit depth estimated from spectra acquired with hyperspectral CS microscope at RT – points represent individual measurements and envelope widths correspond to the density of points (left), with a representative spectrum for each series (right). (c) Distribution of intensities in a sample of Au NPs.



As a confirmation of this effect, the EBD was estimated using spectra obtained by the CS microscope of Au nanoparticles. By calculating the mean separation between adjacent data points (normalised by maximum intensity), it is possible to estimate an 'observed' bit depth in reconstructed spectra. Measuring this estimated bit depth over 500 acquired spectra showed the significant degradation when gain is applied (**Figure 3-5b**, green). However, one can also observe that the longer 1 s exposure has a better-quality spectrum than expected (**Figure 3-5b**, orange/blue) from simply noise characteristics alone.

To explain this result, we must also consider the dynamic range which is achieved with different exposure times. By measuring the spread of pixel intensity values in different imaging conditions, it was found that the 1 s exposure utilises the full dynamic range of the camera, whilst at 0.5 s exposure the dynamic range is roughly halved (**Figure 3-5c**).

*These results demonstrate the role of exposure time in the hyperspectral CS microscope – long exposures lead to additional thermal noise in non-cooled cameras, however single-particle scattering experiments typically have low intensities and so longer exposures or digital gain are needed to make use of the full dynamic range. Digital gain should be avoided due to the reduction of effective bit depth, which results in significantly reduced spectral intensity resolution. In the case of Au NPs, it is shown that exposures of 1 s provide a good balance of thermal noise and image intensity when using multiplexed DMD patterns.*

### 3.5 Conclusions

In this section, I have detailed the design of a proof-of-concept compressive sampling hyperspectral microscope. A number of design hurdles were overcome to result in a system which is approximately an order of magnitude less expensive than commercial systems. Through validation on measurements of gold nanoparticles I show that this new microscope is capable of measuring the scattering profiles from single particles down to a spectral resolution of approximately 5 nm in the visible range, at a significantly increased speed compared to 'push-broom' systems. An analysis of the signal quality found that the main barrier to reaching faster speeds on the current system is a combination of camera quality/signal intensity.

There are several changes which could be made to the system to improve performance:

- **Usability:** The ease of operation is currently hindered by the use of a vertically mounted oil-condenser lens. This causes oil to slowly drip away through the experiment which necessitates frequent oil replacement/refocussing. This could be easily avoided by conversion of the imaging system to an upright microscope, or by purchasing a non-oil condenser.
- **Scattering intensity:** As mentioned above, signal intensity is a limiting factor in reaching faster scan times. An easy change would be of course to purchase a brighter light source or a more sensitive camera. However, a four-fold increase in intensity could potentially be achieved by rearranging the projection optics; the beamsplitter could be removed and the diffraction pattern could then fall on the DMD at an angle, and be reflected straight into the imaging system. This would likely require a diffraction grating and lens with different groove spacing/focal length to provide the necessary working distance for operation.

This would potentially enable some interesting experiments to be performed. Some potential future experiments include:

- **Compressed acquisition:** The system has so far been validated using Hadamard multiplexing, but significant improvements to speed could be achieved by switching to

a CS acquisition protocol. This would require altering the setup so that DMD modulation occurs post-interaction with the specimen as described in [4]. With this setup it would be possible to achieve reasonable measurements with only ~25% the amount of data.

- **Automated sample mapping:** A key process in designing plasmonic NPs is the optical characterisation of each new sample batch through hyperspectral imaging. Due to the slow nature of conventional systems, acquiring a statistically significant dataset can be very time consuming. The high speed of this system could enable this data to be rapidly collected, and if the sample stage were replaced with a motorised stage, large-scale area maps could be automatically recorded.
- ***In situ* experiments:** The high-speed of the system could be particularly well-suited to tracking the change of spectral profiles *in situ*. In its current state the system could be used for example to track changes during oxidation. With the incorporation of a heating stage and NIR photomultiplier tube, it would also be possible to track the effects of sample heating.

## 4. References

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