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Compressive Hyperspectral Microscopy of Plasmonic Nanoparticles – Noise Characteristics and Performance Limits

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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]. Single-particle scattering spectroscopy techniques like dark-field (DF) hyperspectral imaging have become a key tool for studying the optical properties of these NPs because of the wealth of 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]. In this work we explore some of the factors limiting performance for a spectrally-modulating hyperspectral CS microscope. Our results show that for applications to single-particle scattering experiments, the hyperspectral CS microscope requires particularly careful selection of exposure and gain settings to balance intensity resolution against SNR.

The principle of the hyperspectral CS system builds upon ideas implemented in the 'single pixel camera' [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. The hyperspectral CS microscope in [4] performs Nyquist spectral sampling in parallel on a spectrometer and compressive spatial sampling, whereas this work performs compressive spectral sampling and Nyquist spatial sampling on a camera (for increased parallelism), allowing us to reconstruct a 3D hyperspectral datacube from 2D images. The key component of our projector system is a digital micromirror device (DMD), a 912 x 1140 array of individually motorized mirrors (~ 8 μm width). A white light source is directed through a diffraction grating and focused onto the surface of the DMD where each mirror can be set to either deflect the beam or direct it back through a beamsplitter into the imaging system (Figure 1a, 1b). Since each micromirror reflects a different portion of the diffraction pattern, the resulting beam is spectrally modulated; this is described in more detail in Xu *et al*. (2020) [6]. Depending on the choice of illumination pattern, it is possible to achieve sub-Nyquist sampling rates with this approach and thus improve the speed of the system, options include: raster scan (the most conceptually simple), Hadamard multiplex (greatly improves SNR compared to raster scan), and random patterns (suitable for CS) (Figure 1c).

In this work we focus on benchmarking the performance of this new microscope. We first explore the effective bit depth (EBD) for cooled and room temperature cameras, with and without added gain (calculated from the mean intensity and intensity range across ten dark, blank images). For a high quality, cooled scientific camera we find constant EBD over the range of exposure times since the limiting factor is readout noise. 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 we attempt to add digital gain as this amplifies noise in the signal (Figure 2a). This behaviour was observed to follow through to affect the quality of the spectra obtained by the CS microscope; by calculating the mean separation between adjacent data points (normalised by maximum intensity), we were able to estimate the bit depth in reconstructed spectra and saw degradation with gain applied (Figure 2b, green). However, we see that the longer 1 s exposure has a better-quality spectrum than expected (Figure 2b, orange/blue).; we show that this is because when imaging NPs, long exposures or added gain is required to utilise the full dynamic range of the camera (Figure 2c).

Our 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 long exposures or digital gain are needed to make use of the full dynamic range. We find that digital gain should be avoided due to the reduction of effective bit depth, which leaves us with reduced spectral intensity resolution. In the case of Au NPs, we show that exposures of 1 s provide a good balance of thermal noise and image intensity when using multiplexed DMD patterns. In future work we hope to push this to faster acquisition times by adjusting the optics to result in brighter illumination, and acquire time-resolved hyperspectral datasets of plasmonic NPs subjected to *in-situ* environmental changes [7].

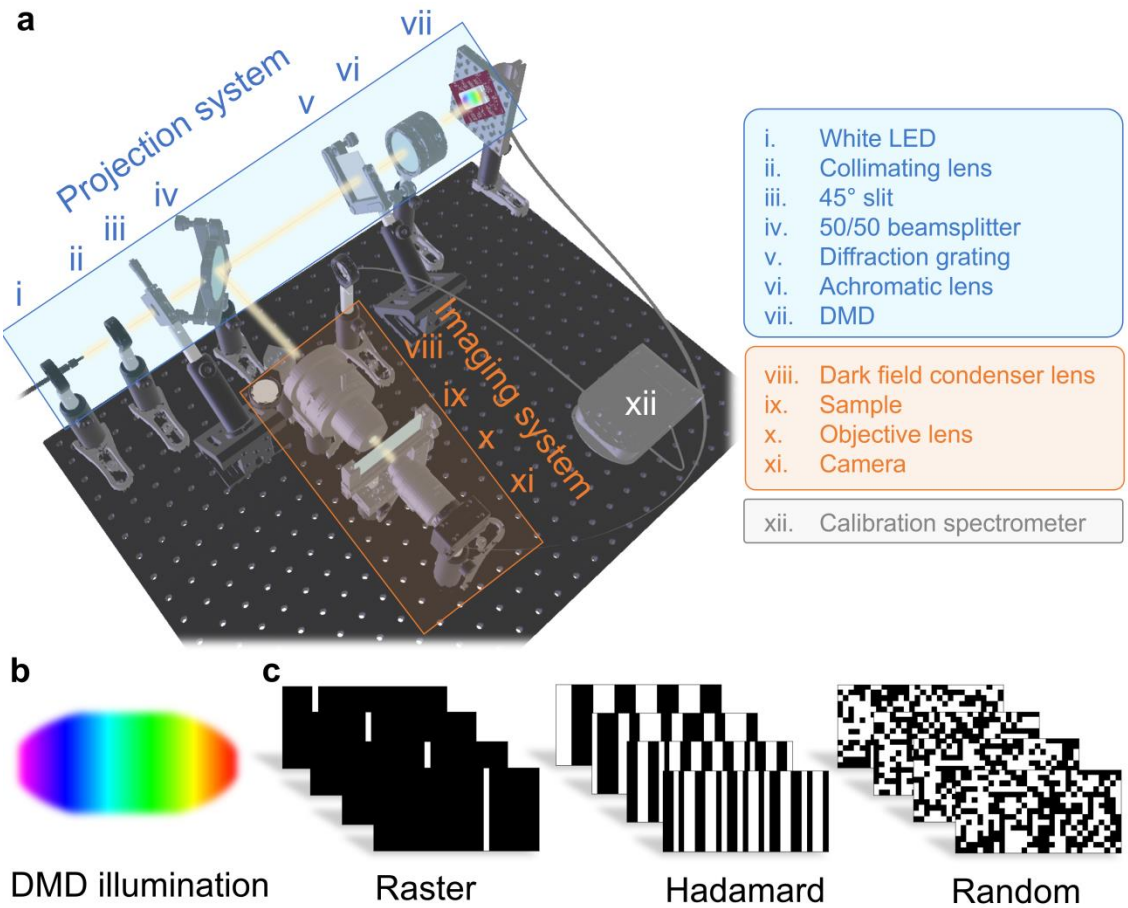

Figure 1. Compressive sensing hyperspectral microscope overview. (a) Diagram of the optics, split into projection, imaging, and calibration systems. (b) Illustration of the diffraction pattern focused onto the DMD. (c) DMD patterns for hyperspectral imaging, showcasing raster, Hadamard, and random patterns.

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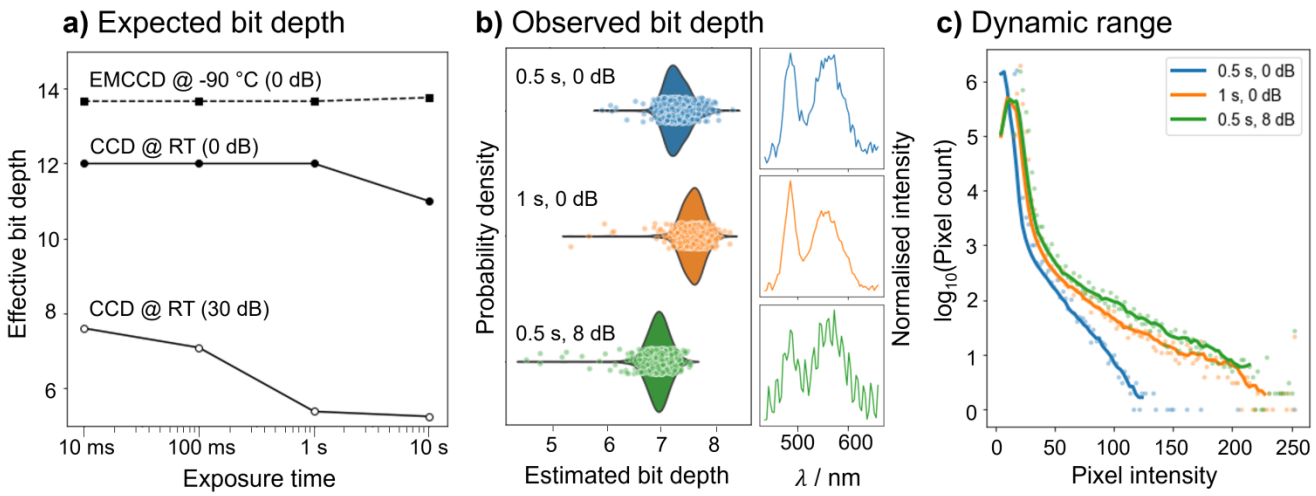

Figure 2. Competing impact of exposure/gain settings. (a) Effective bit depth measured from dark images for a cooled scientific camera (top), and regular camera run without (middle) and with (bottom) gain. (b) Bit depth estimated from spectra acquired with hyperspectral CS microscope at RT – points represent 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.

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