Using Machine Learning Techniques to Denoise NDR Images

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Noise in images is the random grainy effect seen on the picture. This is produced by the random distribution of light (photons) falling on the camera detector and the electronics in the detector. Noise can be produced by the movement of electrons during the process of reading the detector's pixels, from the heat in the detector, or a fixed pattern of noise across the detector due to variations in the pixels.

A novel Non-Destructive Readout (NDR) camera was used to image fluorescent cellular samples by taking rapidly acquired images without reading out electrons, where each sub-frame is the previous sub-frame plus any newly captured photons. This means many sub-frames are taken during a normal camera's exposure time [1]. By subtracting a lower sub-frame from a higher sub-frame, it is possible to produce a normal image of any required sub-frame rate in post-processing - Fig. 1(i). However, the higher the sub-frame rate the higher the noise. It is necessary to remove as much noise as possible to improve image resolution.

The aim of this project was to remove noise from these images to improve their contrast using two machine learning algorithms: Noise2Void (N2V) [2] and CARE [3]. Both have different techniques of using noisy images to train neural networks. After the neural networks are trained, images with different sub-frame rates can be fed into them to be denoised.

These restored images were analysed by comparing the restorations by eye and finding the signal-to-noise ratio (SNR). If the SNR is higher this indicates less noise and a better restoration. This work will help us understand the noise sources within the camera, quantify them and find ways to increase the signal-to-noise ratio in post-processing to improve microscopy image quality and resolution in the future.

Report

In this project, a novel camera architecture based on non-destructive readout (NDR) is used to produce images of fluorescent cellular samples under a microscope. During the exposure time of a regular camera, the NDR camera can rapidly acquire many sub-frames by counting the electrons in the pixel without reading them out. Each subsequent sub-frame is the previous sub-frame with any additional photons captured in the time between them [1]. Over 1000 of these sub-frames can be captured in the time of a standard camera exposure. Manipulating these sub-frames via subtracting lower ones from higher ones, regular images can be produced with a chosen sub-frame rate. Images made with these subtractions which have a higher sub-frame rate have a lower signal-to-noise level, due to the reduced photon count.

To improve the noise levels in the images, two machine learning algorithms, Noise2Void and CARE, were used. The decrease in the noise levels was measured by calculating the SNR of the images before and after they were restored. Increasing the SNR indicates an increase in the resolution of imaging data via the analysis - not hardware. This can be applied for low light imaging where there is lots of noise and low signal to prevent damage and photobleaching of biological samples.

Firstly, to prepare the images for the algorithms, sub-frames within NDR images had to be subtracted to get varied sub-frame rates with different levels of noise - Fig. 1(i). This was done with python code using the tools developed by the research group for analysing the NDR images. These were then saved as multi-frame TIFF images so that they could be fed into the machine learning algorithms.

Noise2Void (N2V) was trained on an image with a sub-frame difference of 50. Contrastingly, CARE was trained on an image with a sub-frame difference of 20 and a ground truth – a subtraction of the first and the 100th sub-frames of the exposure, therefore it has very minimal noise. These noisy images were fed into the algorithms using python code from the GitHub pages of the respective algorithms [4,5].

Both training processes are based on TensorFlow machine learning platform and CSBDeep – a toolset for restoring fluorescent microscopy images. N2V trains its neural network by extracting patches

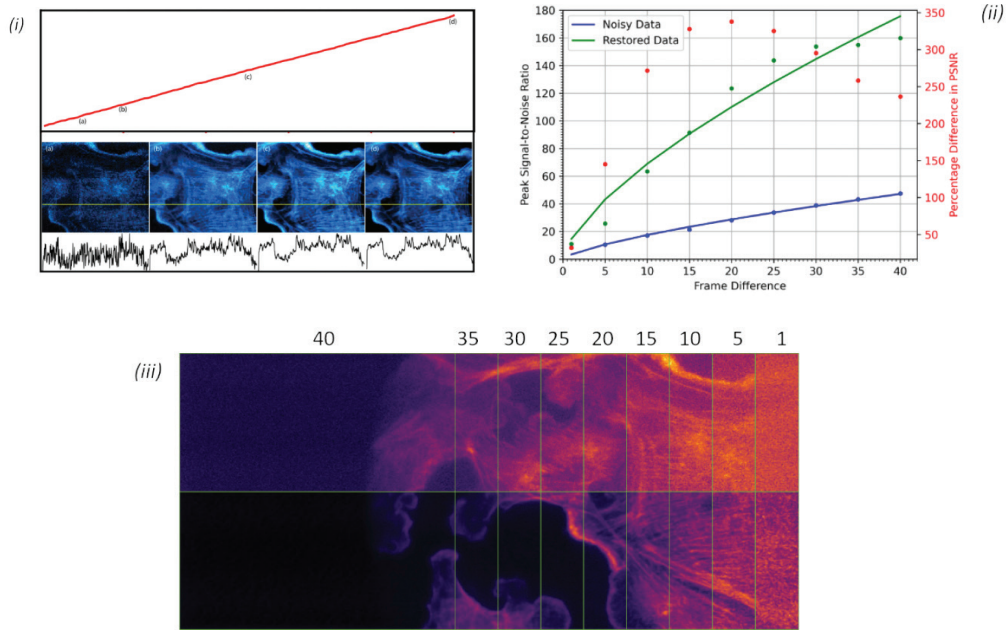

Figure 1 – (i) Top is a graph of the increase in brightness of the sub- frames acquired in one standard exposure by the NDR camera. The sub- frames below the graph labelled from (a) to (d) correspond to the similarly labelled points on the graph. Note the increase in brightness of the sub- frames moving right along the graph. At the bottom are graphs of pixel count along the yellow line seen in all the images. Here you can see the noise decreases as the sub- frames get brighter, as the signal starts to dominate.

(ii) The Peak Signal-to-Noise Ratio (PSNR) of the original noisy images (blue) and CARE network restorations trained on 20 sub- frames (green) against the sub- frame difference of the images along with their power-law lines of best fit. In red is the percentage difference between the noisy and restored PSNRs for their respective sub- frame difference. Axis for this is on the right. Note the peak of the percentage difference at a sub- frame difference of 20 which CARE was trained on.

(iii) A sliced-up image of the one used by the CARE machine learning algorithm. The top half contains the original noisy images with a sub- frame difference of 40 to 1 going left to right (labelled at the top). The bottom half contains the corresponding restored image slices. Note that after a sub- frame difference of 20 (the middle slice), the sub- frame difference CARE was trained on, the quality of the restoration seems to stay constant as sub- frame difference increases (going left).

of pixels from its single noisy training image. The neural network is then trained to predict the true signal of a central obscured pixel of the patch using the pixels surrounding it [2]. This prediction is then compared to a validation patch and the process is repeated many times to minimise the loss. CARE also works by extracting patches, but the central pixel isn't obscured from the neural network. Instead, it predicts a value of this central pixel which is then compared to the same pixel on the ground truth image. The network repeats the process until it gets close to the ground truth value, which is closer to the true signal as it has minimal noise [3].

Images with sub-frame differences of 10, 20, 50, 100 and 250 were restored using the N2V network and for the images inputted into the CARE network the sub-frame differences were 1, 5,10, 15, 20, 25, 30, 35 and 40. It was then possible to slice these images up to see how they compared with each other and to the original noisy images. This can be seen in Fig. 1(iii) for the images restored using the CARE network.

The restorations were also compared analytically using SNRs and Peak SNRs (PSNRs). The former was found by making a python code that extracted dark sections dominated by noise and bright sections dominated by signal. The mean pixel count in the bright section was divided by the standard deviation of the dark section to get the SNR. Alternativity, the PSNR was calculated by finding the highest pixel value and dividing it by the standard deviation of the darkest region of the image.

What was found out was that both neural networks seemed to restore images better if the sub-frame difference was close to that of which it was trained on. This can be seen in the comparisons, as the quality of the restored images which were above the original training sub-frame difference seem to remain constant in their restoration improvement, see Fig. 1(iii) for CARE comparisons. This is also evidenced by the percentage difference graph of the PSNR between the noisy and restored images peaking at the sub-frame difference the network was trained on. This is at a sub-frame difference of 20 for CARE which can be seen in Fig. 1(ii).

It was also found that CARE restored images a lot better than N2V, as it has higher SNRs and PSNRs than N2V and the image restorations looked better. This is likely due to CARE's advantage of having the low noise ground truth image to compare in training. However, in some microscopy techniques, these ground truths may not be obtainable so N2V would be the only option.

Reflections

I am grateful to the Royal Microscopy Society for funding my summer project even though the Covid-19 pandemic meant it couldn't be run as planned. Even though I wasn't able to use the microscope and NDR camera as planned I still found this project enjoyable, as it meant I was able to learn about microscopy - a field I am not familiar with due to being an Astrophysics student. I also learnt about machine learning algorithms (how to train and run them remotely on a Linux based server) and different ways in which images can be manipulated - which I had very little knowledge about beforehand.

The willingness of the RMS to support my project also meant I learnt many new skills during it. Such as learning how to use ImageJ to manipulate the NDR images. This led to expanding my python coding abilities by having to write code to automate some of these processes done in ImageJ to make things quicker. This involved learning how to use the python tools the research group had made for the NDR images, as well as gaining experience with other image manipulating python libraries.

It was also enjoyable collaborating with other members of the research group to get help in their areas of expertise. I also learnt more about fluorescent microscopy and imaging techniques by attending online seminars given by members of the research group and the wider Single Molecule Imaging Group here at Sheffield.

I really enjoyed the collaborative and helpful nature of the research group and it was very rewarding being able to work on this project. Due to this, it has made me more inclined to investigate doing a PhD when my degree is finished. Hopefully, I will be doing more work with microscopy next summer as I should be able to do the project that I was planning for this year which the pandemic didn't allow. I also have the opportunity to do a project on developing machine learning algorithms for the same purpose as used here as one of my university modules this coming academic year, thanks to the lead of the research group Ashley Cadby.

Bibliography

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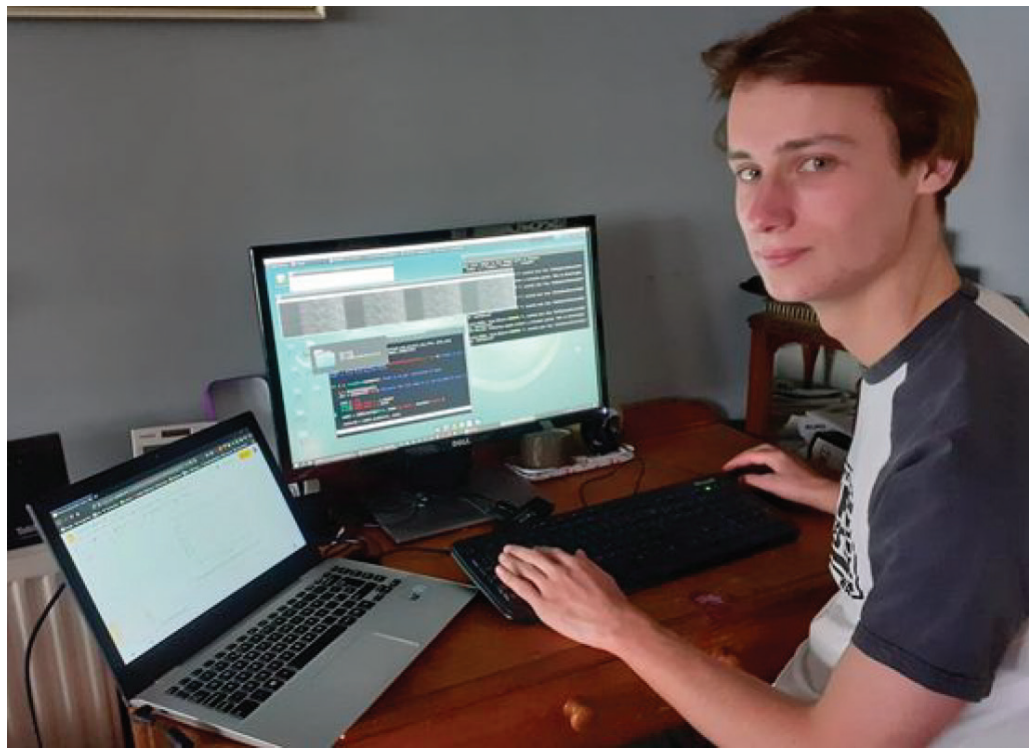

Figure 2 – Me at my desk working on the project. Working remotely on the research group's Linux-based server on the monitor and a PowerPoint about CARE on the laptop to show supervisors.