**Application of Principal Component Analysis (PCA) to Multispectral Imaging Microscopy for Malaria Diagnostics**

**Introduction**

- Malaria is a serious, global disease caused by protozoa of the genus Plasmodium.
- Over 247 million people are infected by the disease annually, 1 million of whom succumb to the disease.1
- Infection begins via a bite by a female Anopheles mosquito carrying the malaria parasites.
- The malaria parasites develop inside the human body as merozoites, trophozoites and finally to schizonts or gametocytes, feeding on red blood cells.2
- Intraerythrocytic (trophozoit and schizont) stages are the common diagnostic stages of malaria.

**Materials and Methods**

- In vitro cultures of *Plasmodium falciparum* were used.
- Unstained thin smears were imaged by a modified optical microscope (shown in fig 1) whose broad band white light is replaced with a set of Light Emitting Diodes (LEDs) emitting light centred at 375 nm, 400 nm, 435 nm, 470 nm, 525 nm, 590 nm, 625 nm, 660 nm, 700 nm, 750 nm, 810 nm, 850 nm and 940 nm.
- The LEDs are activated sequentially, and for each LED illumination, a gray level image is captured with a CMOS camera.
- A total of 13 gray level images of the same scene are captured at a different wavelength of light. The 13 images compose a multispectral image.

**Results and Data Analysis**

- Due to a very large number of spectra contained in a multispectral image, meaningful information can be mined by exploiting Chemometric techniques.
- Figure 2 shows three of the 13 gray level images captured at 375 nm, 590 nm and 940 nm to represent UV, visible and NIR regions.
- Principal Component Analysis was applied to a cropped 13 band multispectral image to give score images which describe the main variation in the images. PCA ranks the scores according to importance, the first score being the most important.

![Fig 2: Raw images captured at (a)-375 nm, (b)-590 nm and (c)-940 nm.](image)

- Fig 3 shows the first two score images of a cropped single red blood cell suspected of plasmodium infection. It is clear by visual inspection that the amount of information decreases in higher score images with the first 3 score images accounting for 98% variance.

![Fig 3: Score images for PC1, PC2 and PC3.](image)

Multivariate chemometric tools such as Principal Component Analysis can be used to segment features from multispectral images thus enabling detection of hidden objects such as malaria parasites in human thin blood smear.

**Conclusions**

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**References**