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## ABSTRACT

Breast carcinoma is the second most common form of cancer among women. Multicolour Fluorescent In-Situ Hybridisation (M-FISH) is a common method for staging breast carcinoma. The interpretation of M-FISH images is complicated by two effects: (i) the emission spectra of the fluorochrome marked DNA probes overlap, and (ii) healthy tissue autofluorescence. In this paper spectral unmixing is applied to hyper-spectral images of M-FISH preparations to produce false colour images with higher contrast and better information content than standard RGB images. Spectral unmixing is realised by combinations of: Orthogonal Projection Analysis (OPA), Altering Least Squares (ALS), Simple-to-use Interactive Self-Modelling Mixture Analysis (SIMPLISMA) and VARIMAX. These are applied to the data to reduce tissue autofluorescence and resolve spectral overlap in the emission spectra. The results show that (i) spectral unmixing reduces the intensity caused by tissue autofluorescence by up to 78% and (ii) the image contrast is enhanced by reducing unwanted intensity caused by the overlap between the emission spectra.

## INTRODUCTION

Breast carcinoma is, after skin carcinoma, the second most common form of cancer among women. Almost 10% of malignancies in women are diagnosed as a breast carcinoma, which represents 22% of all cancer cases in women [1]. 5% to 10% of these breast carcinoma are genetically conditioned. The risk for women, whose mother or sister had a breast carcinoma, is twice that of women without a positive family anamnesis. This risk increases by a factor of between four and six if two family members developed cancer [2]. Hence methods for a reliable diagnosis of breast carcinoma during routine checks are required.

Fluorescent In-Situ Hybridisation (FISH) is a technology that is used to stage breast carcinoma. FISH marks different cell components (e.g. nucleus, cytoplasm, proteins) as well as specific DNA positions or DNA sequences with fluorescently labelled DNA probes. Fluorochromes are substances that emit light when excited by a specific wavelength. The emitted light has a longer wavelength than the excitation light. Fluorescence microscopy can be used to measure fluorescence and acquire images of FISH samples. It uses bandpass filters to measure only the emission of the fluorochromes.

FISH samples marked with multiple fluorescently labelled DNA probes are termed multicolour-FISH (M-FISH). Analysis of M-FISH images is complicated by two problems: (i) spectral overlap of the emission spectra and (ii) tissue autofluorescence. The spectral overlap is caused by the broad emission spectra of the fluorochromes, fig. 1, which cannot be resolved completely by emission filters or fluorochrome selection. Tissue autofluorescence originates from substances such as collagen or elastin that show an intrinsic fluorescence. The substances show an unspecific broad-band fluorescence emission that overlaps the wanted signal and causes a decrease of the image quality.

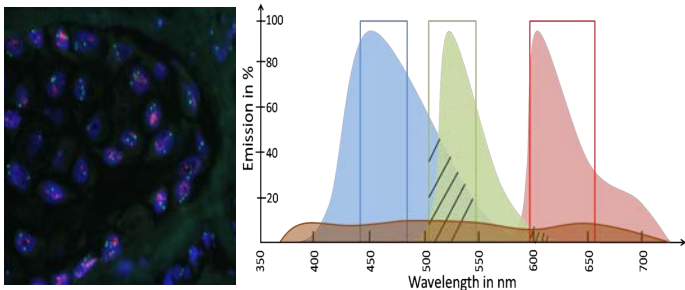


Figure 1: Typical M-FISH image (left). (right) Emission spectra of three common fluorochromes: DAPI (blue), FITC (green) and SpectrumOrange (red). The rectangles represent the ideal filter curves of the emission filters. Even with ideal filters there is a crosstalk between 500nm and 550nm where the fluorochromes DAPI and FITC heavily overlap. The brown emission spectrum represents tissue autofluorescence. This is present over the whole wavelength range and reduces image quality.

Currently, pathologists use RGB colour images of M-FISH preparations acquired by standard colour cameras to make their diagnosis. However, the poor quality of the images makes diagnosis difficult and requires much experience for a reliable diagnosis. The quality of these images can be enhanced with spectral unmixing (SU) methods. A hyper-spectral imaging system, e.g. a tuneable filter mounted on a camera, measures the spectrum at each pixel in an image. The information content of these hyper-spectral images is higher than in standard colour images, enabling SU methods to unmix the overlapping emission spectra efficiently. In this paper the following semi-supervised spectral unmixing methods are applied on hyper-spectral images of M-FISH preparations: Orthogonal Projection Analysis (OPA), Altering Least Squares (ALS), Simple-to-use Interactive Self-Modelling Mixture Analysis (SIMPLISMA) and VARIMAX.

## SPECTRAL IMAGING

Spectral imaging (SI) acquires spatially resolved images of a measurement sample at different wavelengths and combines them into a three dimensional image cube, fig. 2. The two main approaches for the acquisition of hyper-spectral image data are *wavelength scanning* and *spatial scanning*. *Wavelength scanning* methods take images at a certain wavelength range and both spatial axis at once. The spectral information is acquired sequentially. *Spatial scanning* techniques such as imaging spectrographs are prism-grating-prism combinations that disperse incident light of a single line of an object into its spectra and project it onto a two dimensional sensor array. Hyper-spectral image data is generated by scanning the measurement sample line-wise and combining the spectra of each line to a hyper-spectral image cube.

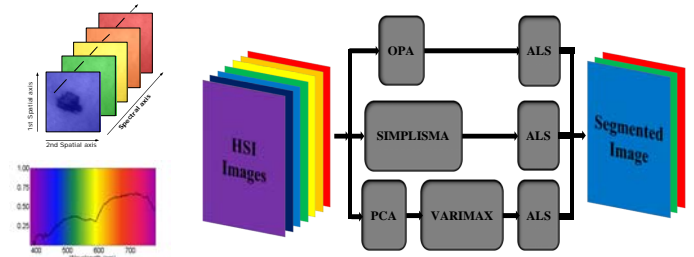


Figure 2: HSI data structure (left), data flow (right)

## RESULTS

SU algorithms were used to reduce tissue autofluorescence and enhance image contrast.

### Tissue autofluorescence

Tissue autofluorescence degrades the quality of fluorescent images. It has a nonspecific emission spectrum that causes an unwanted intensity added to the intensity in every channel and thus degrades the desired signal information.

Table 1: Mean values of six images for each colour channel of user defined ROIs.

|                    | Tissue autofluorescence |                 |               |
|--------------------|-------------------------|-----------------|---------------|
|                    | FITC AF (std)           | CEP-17 AF (std) | DAPI AF (std) |
| Standard RGB Image | 0.15 (0.01)             | 0.14 (0.01)     | 0.1 (0.03)    |
| OPA/ALS            | 0.08 (0.04)             | 0.11 (0.04)     | 0.03 (0.02)   |
| SIMPLISMA/ALS      | 0.11 (0.02)             | 0.11 (0.02)     | 0.02 (0.01)   |
| PCA/VARIMAX/ALS    | 0.03 (0.01)             | 0.05 (0.01)     | 0.01 (0.01)   |

The combination PCA/VARIMAX/ALS reduces tissue autofluorescence most effectively. Compared to a standard RGB image, a reduction of tissue autofluorescence of 80% for HER-2/neu spots, 64% for CEP-17 spots and 90% for cell nuclei spots was achieved, Table 1.

### Reduction of spectral overlap in emission spectra

In a standard RGB image on average 22% of the pixels can not be assigned unambiguously to a single class (HER2/neu, CEP17). By applying SU methods the number of ambiguous pixels can be reduced. The best result is achieved by combining PCA, VARIMAX and ALS. This reduces the percentage of ambiguous pixels to 1.1%.

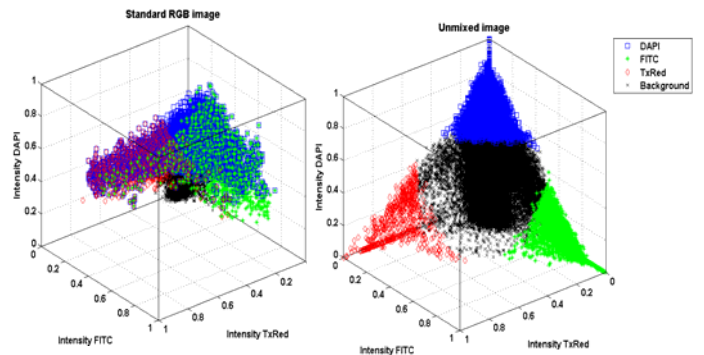


Figure 3: 3D plot of the SU result achieved with the SU method combination PCA/VARIMAX/ALS of a hyper-spectral M-FISH breast carcinoma tissue preparation. There is a heavy overlap of the point clouds in the standard RGB image (left). The right 3D plot shows that the overlap is considerably reduced by SU. The four point clouds in the 3D plot are in the corners of the plot and there is only a minimal overlap.

## CONCLUSIONS

Spectral unmixing methods have been applied to hyper-spectral M-FISH images with the objective to reduce tissue autofluorescence and enhance image contrast. The combination PCA/VARIMAX/ALS reduced tissue autofluorescence by 80% for HER-2/neu spots, 64% for CEP 17 spots and 90% for cell nuclei spots. For the enhancement of image contrast the percentage of ambiguous pixels were compared. In a standard RGB image 21.8% of all pixels could not be assigned unambiguously to either CEP 17 genes, HER-2/neu genes or tissue autofluorescence. This value was reduced to 1.1%. The results show that SU is a powerful pre-processing step to improve the quality of hyperspectral M-FISH images. Subsequent semi-automatic analysis steps would benefit from a pre-processing using spectral unmixing methods.

## REFERENCES

- [1] N. Harbeck, W. Eiermann, J. Engel, I. Funke, A. Lebeau, W. Parmanetter and M. Untch, *Prognosefaktoren beim primären Mammakarzinom*, Tumorzentrum Muenchen: Manual Mammakarzinome: Empfehlungen zur Diagnostik, Therapie und Nachsorge, 8. Aufl., Zuckerschwed, Muenchen, Bern, Wien, New York, pages 39-43, 2001
- [2] R. Lupu, M. Cardillo, L. Harris, M. Hijazi and K. Rosenberg, *Interaction between erbB-receptors and heregulin in breast cancer tumor progression and drug resistance*, Sem Cancer Biol, Vol 6, pages 135-145, 1995