

Thomas Arnold¹, Franz G. Würtz², Raimund Leitner¹

¹ CTR Carinthian Tech Research AG, 9524 Villach/St. Magdalen, Austria

² LKH Klagenfurt, Department of Pathology, 9020 Klagenfurt, Austria

ABSTRACT

Fluorescence microscopy plays an important role in the visualization of bio-nanostructures and the classification of cancerous tissue. Fluorescent in situ hybridization (FISH) is a common method for marking different cell components (e.g. nucleus, cytoplasm, proteins) as well as specific DNA positions or whole DNA sequences. The FISH method/variant used in this paper facilitates a special combination of filters (DAPI/Orange/Green) and corresponding fluorescent dyes to discriminate between the different parts of a cell. FITC marks chromosome 17, Spectrum Orange marks HER-2/neu receptors and DAPI is used to counter stain the cell nucleus. Upon excitation, each marked chromosome emits a fluorescent signal (spot). A common problem with multi-color FISH (M-FISH) preparations is the crosstalk between the channels caused by the overlap of the emission spectra of the different fluorochromes. This crosstalk is one of the reasons that the evaluation and classification of M-FISH preparations is difficult and requires experienced experts. The crosstalk cannot be resolved on the filter level (excitation/emission), and not by specialized fluorochromes (which have different emission spectra).

However, the crosstalk can be eliminated if spectral imaging techniques are used to acquire hyper spectral image data of M-FISH preparations and spectral unmixing methods are employed to "algorithmically reduce" the spectral overlap of the emission spectra. Spectral imaging is the combination of computer vision and spectroscopy. And due to the fact that every object of interest consists of more than one pixel, every pixel is dependent on its neighboring pixels. Thus the spatial context of the image contains useful information for a classification and increase the sensitivity and specificity of a spectral classification. For each cell nucleus the spatial context of the image makes it possible to determine the ratio between HER-2/neu and CEP 17 signals. This method is referred to as "Spot Counting" in the literature. For "Spot Counting" the spatial context of the image is indispensable.

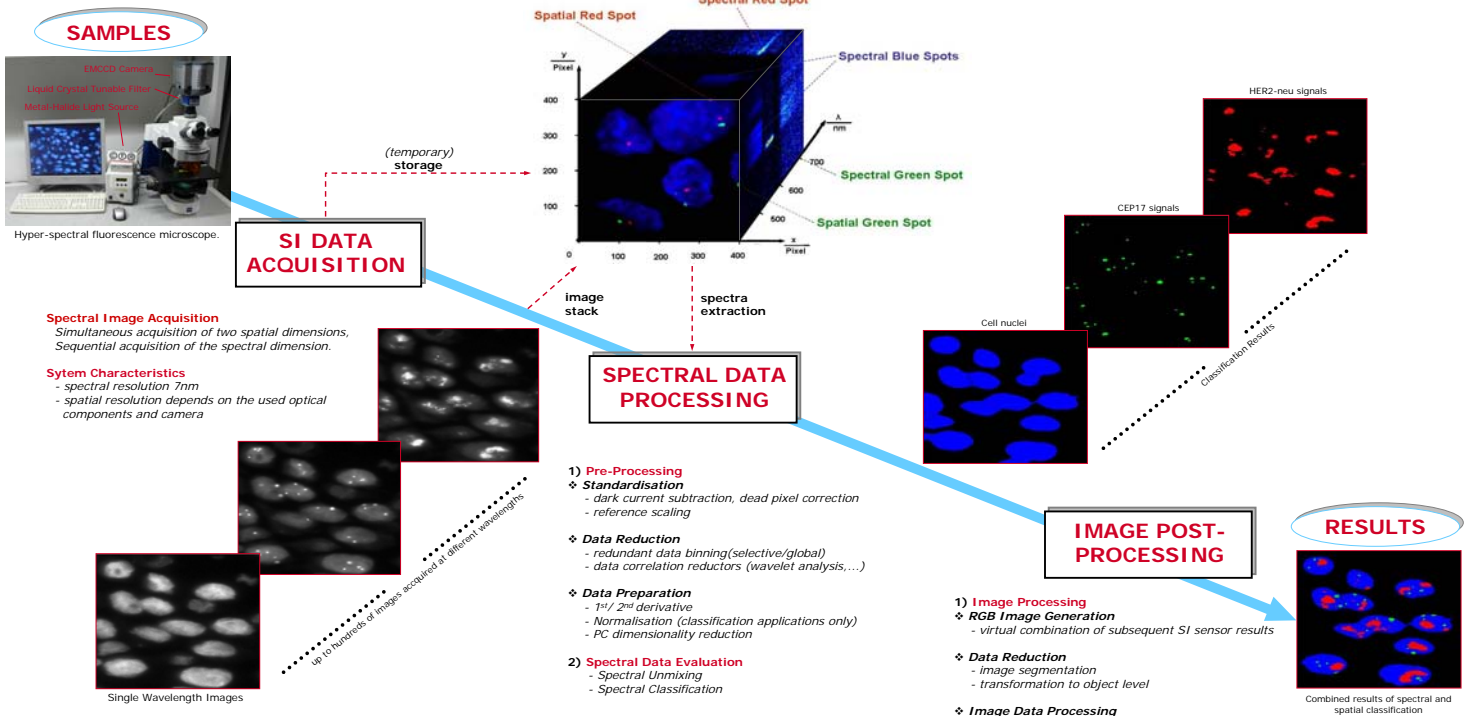
INTRODUCTION

Breast carcinoma is after skin cancer 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 [2].

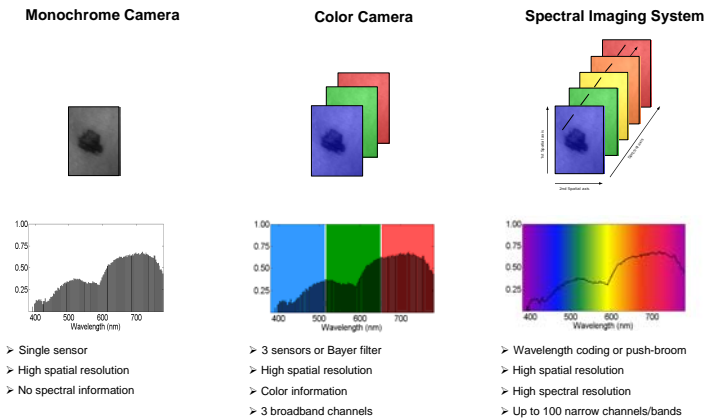
Fluorescence in situ hybridization (FISH) is a well established molecular cytogenetic method for genetic analysis. DNA probes are labeled with fluorescent dyes and hybridized (attached) to chromosomes inside cell nuclei. The probe hybridizes to a defined target nucleotide sequence of DNA in the cell, and the dye emits fluorescence in a particular wavelength (color) when excited by an excitation light source. Typical FISH analysis employs three different fluorochromes. A common problem with these multi-colored FISH (M-FISH) measurements is the overlap of the emission spectra of the different fluorochromes. This overlap increases with the number of fluorochromes used. An additional problem is the auto-fluorescence of the tissue. Auto-fluorescence originates from tissue components as collagen or elastin. The substances show an unspecific broad banded fluorescence emission overlapping the wanted signal and thus causing a decrease in image quality. The overlapping emission spectra and the tissue auto-fluorescence are the two reasons that the evaluation and classification of M-FISH preparations is difficult and requires experienced experts. Currently pathologists use RGB color images of M-FISH samples to make their diagnosis. The quality of these images can be enhanced by acquiring hyper-spectral data and applying spectral unmixing (SU) methods.

A hyper-spectral imaging system measures the spectrum at each pixel in the image. The information content of these hyper-spectral images is higher than in standard color images enabling SU methods to unmix the overlapping emission spectra more effectively and reduce tissue auto-fluorescence by 78% [3]. This allows classification algorithms to count characteristic fluorescent signals more reliably and support experts in their diagnosis.

HYPER-SPECTRAL IMAGING WORKFLOW



SPECTRAL IMAGING



CONCLUSION

Hyper-spectral imaging in combination with spectral unmixing can improve the classification of M-FISH images by reducing the spectral overlap of the used fluorescent dyes by a post-processing of the measurement data. It is also capable of reducing the effects of tissue auto-fluorescence. For an analysis of the spectrally classified data the spatial context of the data is inevitable. Because only cell nuclei which fulfill the predefined properties (e.g. size, shape, position) may be taken into account. With the spatial information it is possible to determine a HER-2/neu to CEP 17 ratio per cell nucleus.

Future work will include the development of a semi-automated method to support pathologists determining the HER-2/neu to CEP 17 ratio. Also will the acquisition of the hyper-spectral data of the whole object slide be automated.

REFERENCES

- N. Harbeck, W. Eiermann, J. Engel, I. Funke, A. Lebeau, W. Parmanetter, and M. Untch. *Prognosefaktoren beim primären Mammakarzinom*, volume 8. Zuckerschwendt, München, Bern, Wien, New York, 2001.
- 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*, 6:135–145, 1995.
- M. De Biasio, R. Leitner, S. Verzakov, F. G. Wuertz, and P. J. Elbischger. Enhancement of M-FISH Images using Spectral Unmixing. *International Conference on Medical Informatics and Biomedical Engineering*, Venice, Italy, 2008.